

## Trace metal partitioning and potential mobility in the naturally acidic sediment of Lake Caviahue, Neuquén, Argentina

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**ABSTRACT.** Lake Caviahue, in Patagonia (Argentina), is a very acid water body (Patagonia, Argentina) due to the influx of volcanic fluids. Over the past 18 years, the lake has been progressively alkalinizing and pH is close to the ferric iron precipitation threshold (pH>3,0). Should iron precipitate, water and sediment composition will be altered. To set a baseline, trace metal partitioning (Cu, Cd, Cr, Pb, Zn and Mn) was studied for three sediment cores (0 to 16 cm depth) at three sampling stations using a sequential extraction procedure (SEP) and the acid volatile sulfide/simultaneously extracted metals (AVS/SEM) protocol. The total metals content of the sediments ranged between 0 to 408  $\mu\text{g g}^{-1}$  of dry sediment, with  $\text{Pb} > \text{Cr} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Cd}$ . No Cd was measured above the limit of detection. The sediment was richest in Mn and Cr, two non-toxic metals at the pH / Eh combination of the lake basin. Total Pb was at a moderate contamination level although it is associated with the most recalcitrant fraction and therefore has low mobility. The sediment fractions with higher metal content were the oxidizable and residual, the former commonly associated with labile organic matter and pyrite while the latter are related to recalcitrant organic matter and rock forming minerals. In addition, a high correlation was found between Cr, Pb and Mn, thus these metals may be subject to analogous precipitation processes and possibly to co-variation in the volcano effluents. Furthermore, no metals were detected in the exchangeable/carbonates fraction, which is the most labile of all the sediment fractions. The metal content in (SEM) was likewise below the toxicity thresholds of two international sediment quality guidelines and the ratio AVS/SEM was over one, indicative of non-toxicity. Both results indicate that metal mobility in the acidic sediments of lake Caviahue is very low.

*Keywords:* Naturally acid sediments, Trace metal partitioning, Metal mobility, Sediment toxicity.

**RESUMEN. Fraccionamiento y movilidad potencial de metales traza en el sedimento ácido natural del Lago Caviahue, Neuquén, Argentina.** El Lago Caviahue es un cuerpo de agua extremadamente ácido ubicado en el norte de la Patagonia Argentina. La acidez se debe a que la naciente de uno de sus afluentes principales se encuentra en un manantial de efluentes del volcán Copahue. Durante los últimos 18 años, el pH del lago a aumentado progresivamente hasta hallarse cerca del umbral de precipitación del ion férrico (pH>3,0). Si el hierro precipitara, la composición del agua y del sedimento cambiaría. Para determinar una línea de base previa a este cambio, se realizó un estudio del fraccionamiento de metales traza (Cu, Cd, Cr, Pb, Zn y Mn) en tres testigos de sedimentos (de 0 a 16 cm de profundidad en el sedimento) en tres sitios representativos del lago. Para ello se utilizaron un método de extracción secuencial (SEP) y el protocolo de extracción de sulfuros ácidos volátiles y metales simultáneamente extraídos (AVS/SEM). El contenido total de los metales estudiados en los sedimentos osciló entre 0 y 408  $\mu\text{g g}^{-1}$  de sedimento seco, con  $\text{Pb} > \text{Cr} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Cd}$ . El cadmio se halló siempre por debajo del límite de detección. Dos de los metales más abundantes, Cr y Mn no son tóxicos en la combinación de pH / Eh determinadas en el sedimento de la cuenca. Se determinó que los niveles de Pb hallados se corresponden con un nivel de contaminación moderado, aunque este se halla mayormente asociado a la fracción más recalcitrante del sedimento y por lo tanto su movilidad potencial es baja. Las fracciones de sedimento con mayor contenido de metales fueron la oxidable y la residual, la primera comúnmente asociada con materia orgánica lábil y pirita, mientras que la segunda con materia orgánica recalcitrante y con minerales formadores de rocas. Además, se halló una fuerte correlación entre Cr, Pb y Mn, por lo que estos metales pueden estar sujetos a procesos de precipitación

análogos y posiblemente su contenido en los efluentes volcánicos varíe en la misma proporción. No se detectaron metales asociados a la fracción intercambiable/carbonatos que son las más lábiles del sedimento. Sumado a ello, el contenido de metales hallados en la fracción SEM estuvo en todos los casos por debajo del umbral de toxicidad de dos niveles guía internacionales para sedimentos de cuerpos de agua dulce. En este sentido, el indicador de toxicidad calculado como AVS/SEM no arrojó indicios de toxicidad para ninguno de los sitios y estratos evaluados. Estos últimos resultados indican que la movilidad potencial de los metales estudiados en el sedimento ácido del Lago Caviahue es muy baja.

*Palabras clave:* Sedimentos naturalmente ácidos, Fraccionamiento de metales traza, Movilidad de metales, Toxicidad del sedimento.

## 1. Introduction

The effects of anthropogenically induced acidification of surface water bodies have been extensively studied (Dillon *et al.*, 1984; Geller *et al.*, 1998). In recent years highly acidic lakes and rivers of natural origin were thoroughly studied to better understand nutrient dynamics, limnology and hydrochemistry (Pedrozo *et al.*, 2001; Wendt-Potthoff and Koschorreck, 2002; Pedrozo *et al.*, 2008a; Varekamp, 2008; Cabrera *et al.*, 2016; among many others).

Surface waters may become extremely acid due to the influence of acid mine drainage (AMD), volcanic activity and/or natural weathering of pyrite. In these kind of processes, the contribution of acid

rain or organic acids runoff are negligible. Final pH in strongly acidic water bodies is below 4.0 (Geller *et al.*, 1998; Löhr *et al.*, 2006), creating high concentrations of iron and aluminum in such waters (Stumm and Morgan, 1996; Geller *et al.*, 1998). In addition, potentially toxic concentrations of trace elements such as copper, lead, chromium, zinc and cadmium are found (Gammons *et al.*, 2005; Pedrozo *et al.*, 2008a; Varekamp, 2008).

Lake Caviahue, the subject of the present study, is very acid, deep, oligotrophic with low planktonic diversity and is unique in South America (Pedrozo *et al.*, 2001; Pedrozo *et al.*, 2008a). Located in the north Patagonian Andean Mountains (Fig. 1), the special characteristics that differentiate it from the

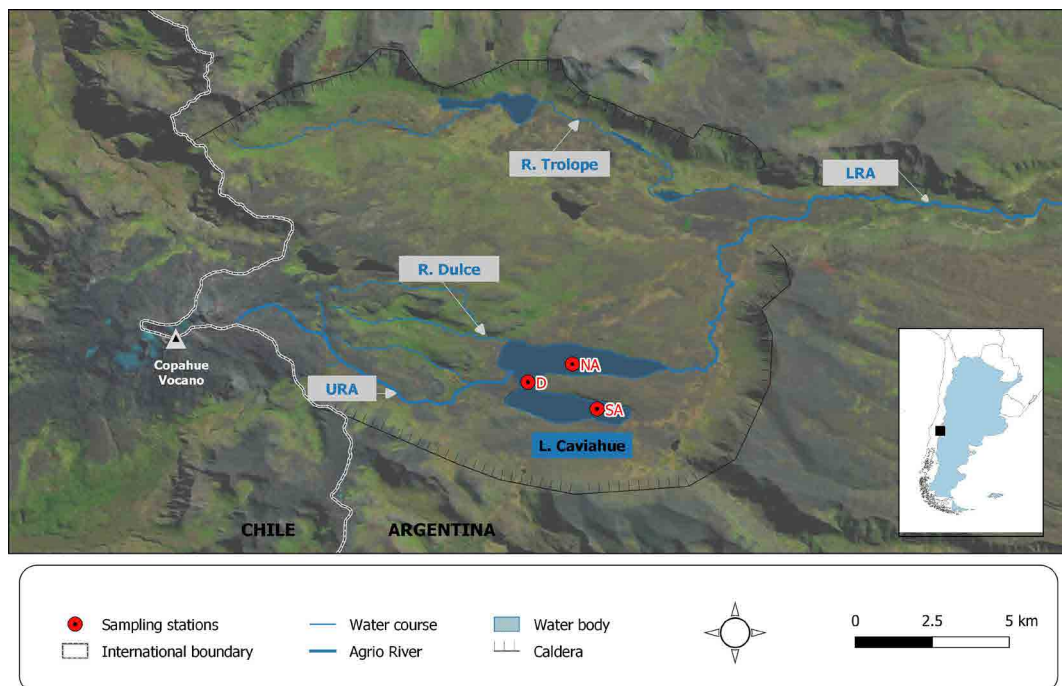


FIG. 1. Copahue Volcano, Caviahue Lake, upper río Agrio (URA), lower río Agrio (LRA), Ríos Dulce and Trolope. Sampling stations at NA, SA and at URA delta (D).

other lakes of the region are primarily due to the activity of the Copahue Volcano (Delpino *et al.*, 1997; Diaz *et al.*, 2007; Varekamp, 2008).

Extractable heavy metals in lake sediments are often found in association with chemical phases such as exchangeable (adsorbed) heavy metals, carbonates, Fe oxides, Al and Mn oxides, monosulfides (MeS), pyrite (FeS<sub>2</sub>) and organic matter (OM). Organic matter related metals are often found in tissue or cell walls of living organisms, detritus, soluble humic substances (HS) with low molecular weight as well as in HS coatings on mineral particles (Tessier *et al.*, 1979). The association of metals in lake sediments is governed by local conditions such as pH and redox potential (Eh; Butler and Rickard, 2000). For example, trace metals mainly co-precipitate with Fe-Mn oxides in oxic sediments while precipitation is related to metal sulfides and pyrite under anoxic conditions. At both Eh ranges carbonates and OM may also be conspicuous metal scavengers.

The most labile fractions considered elsewhere are: exchangeable, monosulfides and carbonates (Allen *et al.*, 1991, 1993; Perin *et al.*, 1985) and thus, the most readily available metal is commonly related to them. Many sediment toxicity indexes are based on the concentration of these fractions, such as the Acid Volatile Sulfide (AVS) to Simultaneously Extracted Metals (SEM) ratio (AVS/SEM ratio) (Allen *et al.*, 1993) or the percentage of heavy metals in the exchangeable/carbonates fraction (Perin *et al.*, 1985). Moreover, international sediment quality guidelines devised by the Canadian Council of Ministers of the Environment (CCME, 2001) or the Australian and New Zealand Conservation Council (ANZECC, 2000) are often used as contamination level indicators. Hübner *et al.* (2009) suggested that these guidelines are composed considering parameters such as local background concentration and should be carefully employed outside their original area of application.

Metal partitioning studies are often performed on acidic sediments from acid mine drainage (AMD). Examples are the Iberian Pyrite Belt, the Mining Lakes District in Germany or massive sulfide deposits in the Yukon Territory, Canada (Geller *et al.*, 1998; Friese *et al.*, 1998; Kwong and Lawrence, 1998; Bachmann *et al.*, 2001; Torres and Auleda, 2013). Moreover, investigations of sediments of naturally acid water bodies mainly analyze total metal content and mineralogy rather than chemical partitioning.

Objectives of the present study are to analyze the partitioning of Mn, Pb, Cr, Cu, Zn, Cd in the highly acidic sediments of Lake Caviahue, to study the potential mobility of the metals and to create the first baseline record of an extremely acid lake. Our findings will allow to predict new scenarios in metal cycling if the pH of lake Caviahue continues to rise.

## 2. Study Area

Lake Caviahue is located in the Copahue-Caviahue Provincial Park (37°53' S; 71°02' W), Neuquén, Argentina, at 1,600 m a.s.l. (Fig. 1). Located on the west side of the northern arm is the town of Caviahue, with almost 1,000 inhabitants and a variable number of incoming tourists that visit Copahue thermal bath complex and Caviahue ski resort both located on the volcano slope. The lake is a glacial water body with a maximum length of 9.7 km, maximum width of 4.7 km, coastline of 22.3 km, total area of 9.2 km<sup>2</sup> and total volume of 0.47 km<sup>3</sup>. The water residence time has been calculated by Varekamp (2003, 2008) to be 42-48 months based on conservative element fluxes (Mg, Cl) in and out of the lake. Lake Caviahue has a horseshoe shape with two basins, northern and southern with a depth of 90 m and 70 m, respectively. It has two major influxes: the río Dulce (average flow of 1.23 m<sup>3</sup>s<sup>-1</sup>) and the upper río Agrio (URA, average flow of 1.10 m<sup>3</sup>s<sup>-1</sup>). The former is a scrubby forested, soil-covered basin with a pH of 6.5 while the latter is born on the east slope of the Copahue Volcano (Pedrozo *et al.*, 2001) and has an initial pH <1.0, temperature of 82 °C, conductivity of 560 mS cm<sup>-1</sup> and flows into the lake with a pH of 0.8 to 2.5. The río Dulce discharge into the northern arm, the URA joins the lake close to the union of the arms (Delta, Fig. 1). Lake Caviahue pH has been rising from 2.2 to 3.4 over the last 18 years (Pedrozo *et al.*, 2008a; Varekamp, 2008; Cabrera *et al.*, 2016). In addition, it has high electric conductivity (560 to 1600 μS cm<sup>-1</sup>) and high concentrations of PO<sub>4</sub><sup>-3</sup>, Fe, Al, Mn, Cl, F, Si, S, Na, K, Ca and trace metals (Pedrozo *et al.*, 2001; Pedrozo *et al.*, 2008a; Varekamp, 2008; Cabrera *et al.*, 2016). Because of these characteristics, Lake Caviahue is unique amongst Andean lakes, which are usually circumneutral and oligotrophic (Diaz *et al.*, 2007).

The lower río Agrio (LRA) exits Lake Caviahue with a pH near 3.0 and joins the río Neuquén approximately 200 km downstream with a pH of 8.0

due to the contribution of several dilute tributaries (Baffico *et al.*, 2004).

Andesite and basaltic andesite are the predominant rock types of the basin (Pesce, 1989; Varekamp *et al.*, 2008; Varekamp, 2015) the latter from the year 2000 eruption of the volcano. The average content of organic matter in the lake sediments is 9.5%. The sediment is also rich in phosphorus (average  $1.1 \text{ mg g}^{-1}$  in association with the organic fraction). The sediments average electric potential (Eh) is  $-120 \text{ mV}$  ( $-20/-180 \text{ mV}$ ), and the pH ranges between 3.0 and 4.0 for both arms of the lake, and between 3.0 and 6.0 at the delta near the mouth of the URA (Temporetti *et al.*, 2013; Cabrera *et al.*, 2016).

The sediments of the deep areas of the basin are covered by a benthic biofilm (Fig. 2) composed mainly by *Keratococcus raphidioides*, bacteria and yeast. The same algal species is also the most abundant (>90%) of the lake phytoplakton (Beamud *et al.*, 2010) and the cell walls are coated with adsorbed Fe and P in similar ratios to those found in the sediments (Cabrera *et al.*, 2016 and data not shown).

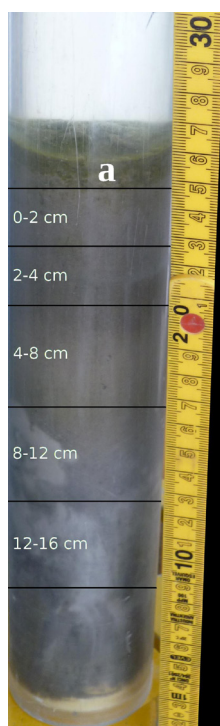


FIG. 2. Sediment core from SA sampling station. The letter “a” corresponds with the green benthic biofilm due to *K. raphidioides*.

### 3. Materials and methods

#### 3.1. Sample collection, processing and *in situ* measurements

Three sediment cores were obtained with a 6 cm diameter, Uwitec-type corer in January of 2013. Sampling was performed at three sites: northern arm (NA; 90 m depth), southern arm (SA; 70 m depth), and at the URA delta (D; 20 m depth) (Fig. 1). Cores were sliced into five strata of 2 cm thick in the upper horizon (0 to 4 cm) and 4 cm thick thereafter (4 to 16 cm), registering pH and Eh (Orion 920A) of the pore water from each stratum. Samples were then stored in polyethylene bags under  $\text{N}_2$  atmosphere at  $4^\circ\text{C}$  for no longer than 24 h before being frozen. All analyses were performed within one week.

In the laboratory, fresh sediment samples were carefully homogenized by hand inside the bag (under  $\text{N}_2$  atmosphere). Aliquots of approximately 3 g (equivalent to 1 g of dry sediment, dw) of each fresh sample was put into polypropylene and glass tubes for metal extraction. All reagents were of analytical grade and deionized water was used. Oxygen in the reagents was removed by  $\text{N}_2$  bubbling before use. The extraction procedures were completed before 48 h after sub-sampling.

#### 3.2. Chemical analysis

Metal partitioning was analyzed by two methods: **1)** Sequential extraction procedure (SEP) proposed by Tessier *et al.* (1979) and **2)** Acid Volatile Sulfides-Simultaneously Extracted Metals (AVS-SEM) method proposed by Allen *et al.* (1991, 1993). Both protocols were performed on three replicates of fresh anoxic sediments under nitrogen atmosphere in order to preserve the metal distribution among the Sediment components. SEP steps were as follows:

1. Ten milliliters of 1.0M NaOAc (adjusted to  $\text{pH}=5.0$  with acetic acid) were added to a fresh aliquot of sediment from each stratum (equivalent to approximately 1 g of dry sediment). The first step was eliminated because, based on previous determinations, all the trace metals studied were below the limit of detection in the pore water and  $\text{MgCl}_2$  extracts. In addition, carbonates are not a conspicuous phase at the sediment pH. In consequence, this step will be referred to as exchangeable and/or F1.

2. Twenty milliliters of 0.5 M Hydroxylamine · HCl were added to the residue of step 1) and the mixture was heated to 96 °C for five hours, softly agitated every 15 minutes. This step dissolves elements bound to Fe-Mn oxides and will be referred to as reducible and/or F2.
  3. The residue of step 2) was treated with 5.0 mL of 8.8M Hydrogen peroxide adjusted to pH 2.0 with HNO<sub>3</sub> and the mixture heated to 85 °C for 2h. At that time, an extra aliquot of solvent of 3.0 mL was added and heated to the same temperature for three more hours. In both steps reaction vessels were agitated every 20 minutes. At the end time, 5.0 mL of 3.2M NH<sub>4</sub>OAc in 20% HNO<sub>3</sub> were added, the mixture was diluted to 20 mL and agitated again for 30 minutes. With concentrated H<sub>2</sub>O<sub>2</sub> labile OM, pyrite and the remaining sulfides are extracted. This step will be referred to as oxidizable and/or F3.
  4. The residue of step 3) was digested with 12 mL of H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub> (1:1) mixture heated to 450 °C (Carter, 1993). This is a pseudo-total extraction that will partially attack the minerals of rocks such as andesite or basaltic andesite, although the hot acid will attack and partially dissolve volcanic glasses, plagioclase, feldspar, piroxene and amphibole. Tessier *et al.* (1979) and others saw that recalcitrant OM is not completely oxidized with F3) and the metals in potential association with that fraction were of interest in this work. This step will be referred to as residual and/or F4.
- Acid volatile sulfides (AVS) and the simultaneously extracted metals [SEM] content were analyzed following the cold-acid purge-and-trap method as done in Allen *et al.* (1993). Briefly, aliquots of 3 g of fresh sediments were put in glass containers (as described above). The extraction apparatus consisted of a N<sub>2</sub> gas tube connected in series with the sample container and two polypropylene tubes with 1.0M NaOH solutions. Samples were purged with N<sub>2</sub> for ten minutes to remove H<sub>2</sub>S with a flow of 40 mL min<sup>-1</sup>. The gas flow was stopped while 10 mL of 1.0M HCl was being introduced with a 20 mL, stainless steel needle, glass syringe. The cold acid reacts with the metal sulfides of the sediments to form volatile H<sub>2</sub>S and dissolved metal chlorides. This hydrogen sulfide (AVS) was carried by the N<sub>2</sub> flow and trapped in the 1.0M NaOH solutions. The acid solution containing the metal chlorides [SEM] was filtered by 0.2 µm acetate membranes from the

acid/sediment mixture and stored at 4 °C until metal content was quantified. The concentration of sulfides in the 1.0M NaOH solutions was determined by the iodometric method (APHA, 1992). The percent recovery was estimated at 90.1±5.1% after the analysis of six replicates of Na<sub>2</sub>S solutions. Allen *et al.* (1993) calculated the ratio AVS to SEM and defined it as an indicator of contamination when the value is of 1.0 or lower, meaning the content of metals leachable with cold acid was higher than the content of metal sulfides.

The sequential extraction procedure was employed to describe metal partitioning amongst the sediment facies and will yield information about how labile or recalcitrant the studied trace metals are. The second extraction was used to assess the content of metal sulfides and to calculate the risk indicator described above. Both methods complement each other and allow a broader description of the sediments.

The concentration of metals in the extractants was determined by atomic absorption spectrometry (FAAS, PE Analyst 100) with deuterium background correction and quantified with matrix-matched standards. The limit of detection (LOD) varied between metals, being 0.10 µg g dw<sup>-1</sup> for Zn, 0.9 µg g dw<sup>-1</sup> for Cu, 5.0 µg g dw<sup>-1</sup> for Pb, 0.5 µg g dw<sup>-1</sup> for Cd, 4.0 µg g dw<sup>-1</sup> for Cr and 0.9 µg g dw<sup>-1</sup> for Mn in accordance with the mass of sediment and volume of extractant employed in the extractions. Iron and humic acid concentration in the sediments was taken from the dataset of Cabrera *et al.* (2016).

### 3.3. Statistical analysis

Due to the lack of homogeneity of variances, Kruskal Wallis (KW) test was used to search for significant differences in metal concentrations among sites and strata. The degree of correlation between the studied variables was determined using a Pearson's correlation test. The level of significance was fixed at  $\alpha=0.05$  and Infostat 2017 software was used to perform all analyses.

## 4. Results

### 4.1. Sediment characterization

The textural characteristics of Lake Caviahue sediment cores were described in Temporetti *et al.* (2013). These authors found that the sediment

cores in NA y SA sampling stations were dominated mainly by medium to coarse texture fractions (20 to 250  $\mu\text{m}$ ), the clay fraction in both sites represented <5% of the total while the silt and fine sand fractions predominated in all groups, representing between 10 and 20% of the total. In this sense, the sediments of the Lake were classified as sandy-loam. Moreover, the element abundance in sediments determined with EDS was  $\text{Si} \gg \text{Al} > \text{S} > \text{Na} > \text{Fe} > \text{P} > \text{Ca} > \text{K} > \text{Mg}$  in both arms with minor differences in composition ratios. Expressed as oxides, the most abundant was  $\text{SiO}_2$  with an average 73% in all layers,  $\text{Al}_2\text{O}_3$  ranged between 11% and 12% while  $\text{SO}_3$  accounted for 3.4% to 6.1% of the total oxides (Temporetti *et al.*, 2013). Likewise, these authors analyzed the mineralogy of lake sediments and found that the predominant minerals were: Andesine ((Na, Ca) (Si, Al) $_4\text{O}_8$ ), Albite (Na AlSi $_3\text{O}_8$ ), Anorthite (CaAl $_2\text{Si}_2\text{O}_8$ ) and Cristobalite ( $\text{SiO}_2$ ), indicating that the sediments are of volcanic origin supplied mainly by the URA and reflect the volcanic features of the basin.

In figure 2, the photograph of a sediment core from SA sampling station is shown. The letter "a" marks the benthic biofilm green layer on the sediment surface. The basaltic andesite and volcanic ash derived main components of the sediment, decomposing OM and to some extent iron sulfides such as pyrite are responsible for the grey color. In addition, no red/orange oxidized iron species can be seen in the vertical profile of the core.

Eh, pH and OM content in the sediments was presented in Cabrera *et al.* (2016). Briefly, interstitial water pH rose towards the lower strata (3.0 to 4.0) at a similar rate for both NA and SA sampling

stations. At D the same trend was observed but final pH was 6.0 (Fig. 3A). Eh also becomes more negative downwards at all three sites. Measurements on sediments from sampling station D yielded less negative values from 0 to 12 cm although it drops to values ca. -100 mv at the bottom stratum (Fig. 3A). Eh for the water column above the sediments was +680 mV and average pH 2.9.

Organic matter (OM) content varied from 5 to 13% on average (Fig. 3B). Humic acid (HA) ranged from 0.5 to 3.5% and no correlation was observed with total OM. While total HA at NA sampling station dropped by 75% between upper and lower strata (2.2 to 0.5% average content, Fig. 3C), total OM fell by only 50% (10 to 5%). Organic matter had the same profile at SA sampling station though with higher values while HA concentration increased from 2.5 to 3.5% before it finally dropped to 1.5% (Fig. 3B). Lastly, at D sampling station organic matter rose from 7.5 to 11% as HA dropped from 1 to 0.5%.

## 4.2. Trace metal partitioning by SEP

Overall trace metal content analyzed by SEP was:  $\text{Pb} > \text{Cr} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Cd}$ .

### 4.2.1. Manganese

Eighty nine percent of Mn was found significantly associated with the F4 residual fraction ( $p < 0.0001$ ). Its total content (expressed as the sum of all fractions) ranged between 43-87  $\mu\text{g g}^{-1}$  at NA sampling station, 24-40  $\mu\text{g g}^{-1}$  at D sampling station and 40-74  $\mu\text{g g}^{-1}$  at SA sampling station (Fig. 4A-C). The reducible (F2) and oxidizable (F3) fractions were significantly

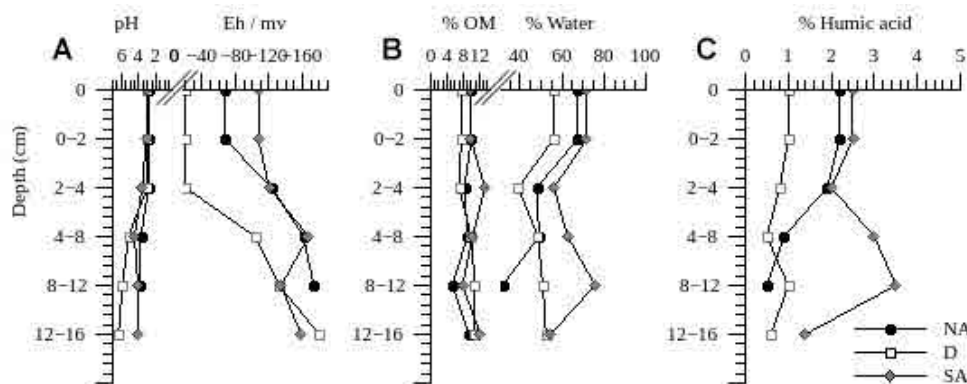


FIG 3. pH, Eh, OM, Water and humic acid (HA) content (from Cabrera *et al.*, 2016)

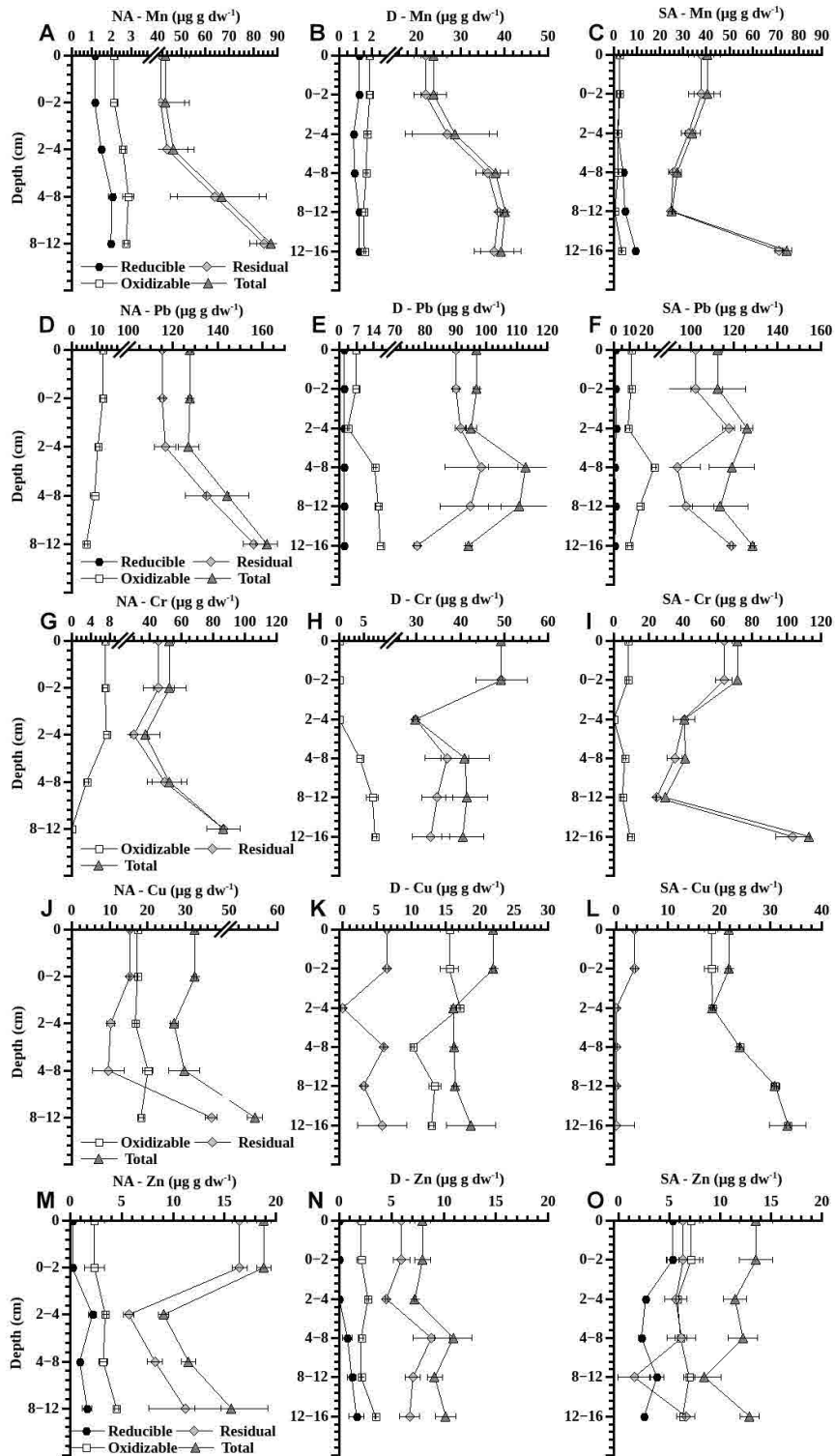


FIG. 4. Trace metal partitioning in reducible, oxidizable and residual phases at NA, D and SA sampling stations (expressed as  $\mu\text{g g}^{-1}$  of sediment; error bars indicate standard deviation with  $n=3$ ). Total metal is the sum of all fractions.

lower than the residual (F4) metal fraction between sampling stations ( $p < 0.0001$ ; 95%). The former is commonly associated to Mn oxides (Tessier *et al.*, 1979) and it was higher at SA sampling station than at the remainder of the lake. The latter is usually associated with labile OM and pyrite. The residual fraction (F4), was comparable between SA and D sampling stations whereas at NA sampling station it was significantly higher ( $p < 0.045$ ). Residual Mn increased towards the bottom sediments at NA and D sampling stations. At SA its content decreased from the first to four strata and it reached the maximum value at the fifth (Fig. 4C). The differences between strata were statistically significant ( $p < 0.026$ ) for all sites, although no statistical groups were formed.

#### 4.2.2. Lead

Residual Pb was significantly higher than the other fractions ( $p < 0.0001$ ; 90% on average). Its total content ranged between 127-162  $\mu\text{g g}^{-1}$  at NA sampling station, 97-112  $\mu\text{g g}^{-1}$  at D sampling station and 112-128  $\mu\text{g g}^{-1}$  at SA sampling station (Fig 4D-F). With regard to F4 residual fraction, a tendency to rise towards the lower strata was observed exclusively at NA sampling station, with the third and fourth strata being significantly richer than the remainder of the profile ( $p = 0.023$ ). At D sampling station, lead content rose 5% between the first and second stratum and then decreased 10% towards the bottom whereas at SA sampling station the content oscillated between 90 and 110  $\mu\text{g g}^{-1}$  (Fig. 4E-F). Reducible (F2) Pb was not detectable at NA sampling station while it was slightly over the LOD at D and SA sampling stations, being higher at D. Finally, oxidizable lead decreased significantly towards the bottom at NA sampling station from 17 to 7  $\mu\text{g g}^{-1}$  ( $p = 0.023$ ). The opposite trend was observed at D sampling station, where it rose downwards and at SA sampling station where it rose from 9 to 24  $\mu\text{g g}^{-1}$  and then it decreases.

#### 4.2.3. Chromium

Chromium, as well as Mn and Pb, was significantly higher in F4 residual fraction (91%;  $p < 0.0001$ ). Its total content ranged between 37-84  $\mu\text{g g}^{-1}$  at NA sampling station, 29-49  $\mu\text{g g}^{-1}$  at D and 29-113  $\mu\text{g g}^{-1}$  at SA (Fig. 4G-I). Differences were significant between strata at each site but not among sites, and no trends were identified. Reducible Cr was below LOD and F3 (oxidizable) pattern was similar to that of Pb. Moreover, at NA and SA sampling stations residual Cr

was correlated with residual Mn ( $r = 0.88$ ;  $p < 0.009$ ), the similarity of the profiles can be seen in figure 4 (A-G and C-I). Finally, F4 residual fraction at D (Fig. 4 H) was nearly constant for the sub-superficial strata (below 4-8 cm).

#### 4.2.4. Copper

Copper was detected only in F3 oxidizable and F4 residual fractions. Its content was significantly different ( $p < 0.0001$ ) at the three stations for both fractions. The order for the former fraction concentrations was: SA > NA > D, while for the latter it was: NA > D > SA. The total content ranged between 27-55  $\mu\text{g g}^{-1}$  at NA, 16-22  $\mu\text{g g}^{-1}$  at D and 10-24  $\mu\text{g g}^{-1}$  at SA (Fig. 4J-L) and it was significantly higher at NA than at the remainder sites ( $p = 0.024$ ). In addition, total Cu rose downwards at NA and SA sampling stations while the opposite tendency was observed at D. Moreover, at SA and D sampling stations, 89% of Cu was detected in the oxidizable fraction whereas the differences at NA sampling station were not significant. At SA the residual metal was negligible, implying that most of the metal was distributed among the most reactive fractions.

#### 4.2.5. Zinc

Zinc was the least abundant among the quantified metals. The differences in its distribution between chemical fractions or sampling sites (Fig. 4M-O) are not statistically significant. Its total content ranged between 7-18  $\mu\text{g g}^{-1}$ . Traces of reducible metal were found at the three stations, being higher at SA. The richest fraction at NA and D sampling stations was the residual, whereas at SA sampling station the content of residual and oxidizable Zn was the same.

#### 4.2.6. Cadmium

Cadmium concentration values were below the LOD and thus, will not be considered for quantitative analysis.

### 4.3. AVS and SEM

Acid volatile sulfide and cold-acid extractable Fe are shown in figure 5 (A-C). Although AVS will only be used for qualitative analysis, a strong and significant correlation was observed between the two variables ( $r = 0.988$ ,  $p = 0.001$ ). AVS content was higher than Fe in most of the strata, consistent with the work of Koschorreck *et al.* (2003), and the average Fe content



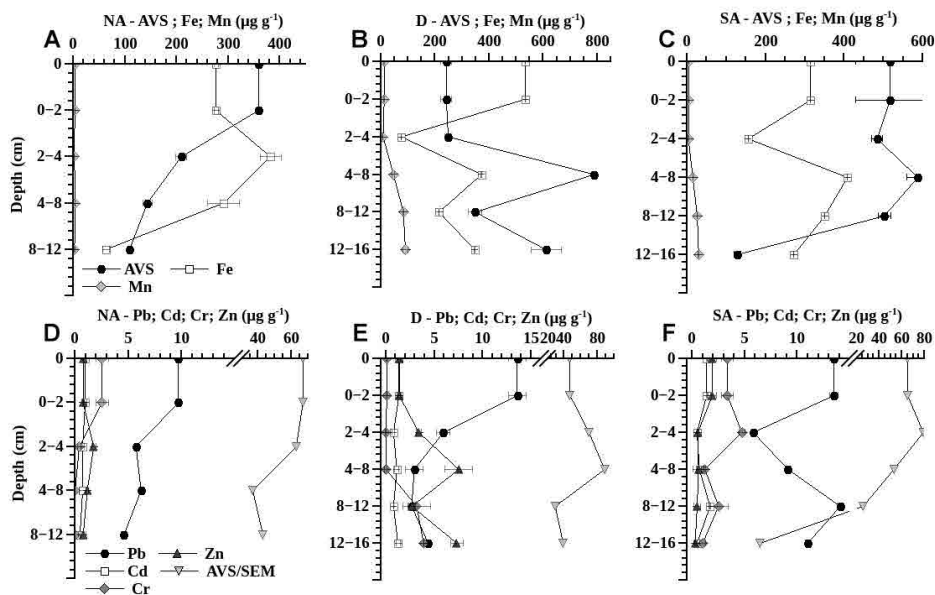


FIG. 5. AVS, Fe, Mn and SEM (Pb, Zn, Cd, Cr) (expressed as  $\mu\text{g g dw}^{-1}$ ; error bars indicate standard deviation with n=3) and the ratio AVS/SEM.

was  $264 \mu\text{g g}^{-1}$  at NA,  $308 \mu\text{g g}^{-1}$  at D and  $300 \mu\text{g g}^{-1}$  at SA, comparable with  $457 \mu\text{g g}^{-1}$  reported by Cabrera *et al.* (2016) in the most labile F1+F2 fractions of SEP and significantly lower than the average total content of  $5,190 \mu\text{g g}^{-1}$  (Cabrera *et al.*, 2016).

Overall trace metal content in the acid extracts were ten times lower than SEP values, the same trend observed for Fe. Zinc had high correlation with AVS ( $r=0.931$ ;  $p=0.020$ ) at the three stations. Lead and Cr had significant association with sulfides exclusively at NA ( $r=0.953$ ;  $p=0.045$  and  $r=0.977$ ;  $p=0.029$ , respectively). Cadmium was slightly over the LOD and not considered for further analysis. Copper was detected exclusively at D, being present only in the superficial stratum (0-2 cm;  $0.10 \mu\text{mol g}^{-1}$ ), although it was expected because  $\text{CuS}$  is not solubilized in cold 1M HCl (Allen *et al.*, 1991). Lastly, the ratio AVS/[SEM] was over 1.0 throughout all the sampled stations of the lake, thus indicating an excess of sulfide over trace metals.

## 5. Discussion

### 5.1. Sequential extraction

Metals associated with pyrite and HCl extracts (carbonates, sulfides, Fe-Mn oxides) have been

previously characterized as reactive because they can dissolve, react to form other mineral phases or become incorporated by the benthic biota (Huerta-Díaz *et al.*, 2014). A number of single reagents and multiple combinations of them have been used to solubilize the mineral phases mentioned above, such as the SEP protocol employed in this work (Tessier *et al.*, 1979). Therefore, extractable and reactive metal are considered synonyms henceforth.

With regard to SEP results, no trace metals were found in NaOAc supernatant (exchangeable and carbonates fraction), the fraction that represents the more readily available metals. Sediments with less than 1% of the total metal content in this fraction, such as Caviahue, are not to be considered toxic to the aquatic environment (Perin *et al.*, 1985).

The metal content in the oxides of the reducible fraction (F2) was slightly above the limit of detection for Mn, Pb, Zn and not detectable for Cu and Cr. Oxides were not expected to be a conspicuous fraction in Caviahue sediment due to the low pH-Eh combination (for a more detailed discussion about iron cycling in this particular environment see Cabrera *et al.*, 2016). Metal content in this fraction rose towards the lower strata, coincidentally with the increase of pH.

On the contrary, in the oxidizable fraction (F3) all the metals were above LOD. Chromium, Pb and Mn average content in this fraction was lower than ten percent. On the other hand, Ox-Cu accounted for 54%, 78% and 97% at NA, D and SA, respectively, of the total Cu, while Oxidizable Zn was 27% at NA and D, and 57% at SA (although it should be noted that the differences for the latter were found to be not significant). In addition, these two metals in this fraction were significantly correlated ( $r=0.73$ ;  $p=0.0029$ ) and richest in SA. A hypothesis to explain the association of these two metals in this lake is that both are intimately related with metalloproteins in eukaryotes (Dupont *et al.*, 2010) and the benthic microbial biofilm on the lakes basin is composed mainly by the sedimented algae *Keratococcus raphidioides*. In this regard, other authors have thoroughly described the positive correlation of Cu, Pb and Zn and the OM fraction (Nissenbaum and Swaine, 1976; Tessier *et al.*, 1979; Rendina *et al.*, 2001a; Rendina *et al.*, 2001b; Lesven *et al.*, 2010; Torres and Auleda, 2013). Moreover, Huerta-Díaz and Morse (1990, 1992) studied anoxic-sulfidic sediments such as Cavihue (although probably with higher pH) and found that Cu and Mn had high tendency to be incorporated to pyrite, another possible phase in this fraction, in contrast with Cr and class “b” metals Pb and Zn which in Cavihue were almost exclusively associated with the residual fraction.

Should only the reactive facies be considered, Cr is described elsewhere chelated with the reducible and oxidizable fractions (Tokalioğlu *et al.*, 2000; Cuong and Obbard, 2006; Rendina *et al.*, 2006; among others) because it is found adsorbed to oxides and chelated with OM, both labile and recalcitrant. In this study, the former was negligible and the latter only found significantly related to labile OM (F3 fraction).

The residual fraction was richest in Mn, Cr and Pb, containing 90% of the extracted metals and poorest in Cu. Moreover, strong and significant correlations were found between the three metals: **1**) between Mn and Cr at the arms ( $r=0.78$ ;  $p=0.0011$ ; Fig. 4A, C, G, I); **2**) between Mn and Pb ( $r=0.83$ ;  $p=0.0002$ ) at NA and D (Fig 4A, B, D, E) and **3**) between Cr and Pb ( $r=0.77$ ;  $p=0.0148$ ; Fig4D, G) specifically at NA. Chromium and lead are present in the lake water in a similar concentration range, however, Mn is one hundred times more concentrated. The same

tendency was observed for Cu and Zn although to a lesser extent.

The Cavihue lake sediments consist mainly of volcanic ash and other volcanic particles. The strong acid used to obtain residual fraction would partially dissolve volcanic glasses, plagioclase, feldspar, piroxene and amphiboles. In this regard, Ruggieri *et al.* (2011) characterized the ashes from Copahue volcano eruption of the year 2000. After SEM and XRD studies, they found that glass is the main constituent of this ash. They leached the ashes with concentrated nitric acid and found similar contents of Mn, Cu and Zn while the ashes were depleted in Cr and Pb, contrasting with the values found in this work for the Cavihue lake sediments. In contrast, J. Varekamp, written communication, 2016, found that the ashes of the eruption of 2012 were richer in Zn and Mn, with similar values of Cu and with deficit of Cr and Pb compared to the sediment analysis presented here. It was mentioned above that chromium and lead have high affinity for humic substances and therefore the precipitation of Cr and Pb-humate complexes would enrich the sediments in these metals compared to the volcanic ash. Furthermore, Ruggieri *et al.* (2011) also found that Zn was one of the most mobile elements, therefore leaching of the ash particles at the low pH of the URA or the lake water (pH~1.0 and 3.0, respectively) could explain its deficit with respect to 2012 ashes.

In addition, annual and inter-annual fluctuations in the concentrations of these metals in the Upper Agrio River and the lake, that were previously described by Varekamp (2008), Pedrozo *et al.* (2008a) and Cabrera *et al.* (2016), could also contribute to the irregularity of the vertical patterns and differences in metal content with respect to the volcanic ash. These variations mean different concentrations of precursors for hydrogenic precipitates.

In this study, no statistical correlation nor visible association was found between any of the metals (or the sum of them) and HA, although the content of the latter is 1,000 times higher. However, the interaction *in situ* cannot be ruled out because at the pH of the lake sediments Pb, Cr and Cu were previously shown to have high binding parameters for the HA extracted from SA sampling station. On this subject, the differential affinity of the studied metals for humic/fulvic ligands by fluorescence titration has been established elsewhere (Christl *et al.*, 2001; Plaza *et al.*, 2006; Kostić *et al.*, 2013).

## 5.2. AVS and SEM

AVS was very high in Caviahue, equivalent to the content found in the sediments of the highly contaminated urban river Matanza-Riachuelo, in Buenos Aires, Argentina (Rendina *et al.*, 2001a) and higher than most reports of contaminated sites. Caviahue lake has a very high content of dissolved sulfur species (Koschorreck *et al.*, 2003) and therefore, high content of AVS was expected, although it is not impacted due to anthropic activities.

The extraction with cold HCl was performed to evaluate specifically the sediments potential toxicity (after Allen *et al.*, 1993). The molar ratio AVS/SEM was over 1.0 in all stations and strata (values were between 5.4 and 88.5) thus indicating trace metal partitioning associated with this labile phase poses no risk to the algae and microorganisms in the water column and the sediments pore water (Allen *et al.*, 1993). This result is in agreement with the low trace metal content in SEP most labile fractions, the exchangeable (F1) and reducible (F2).

Of all the metals detected in SEM extracts, Fe and Zn were shown to have high significant

correlation at all sampling stations and thus, the presence of low amounts of FeS and ZnS species is suggested although pH is below 5.0 at the arms. Lead and Cr had likewise high correlation with AVS but only at NA sampling station. In addition, Mn was higher than the sum of the readily extractable fractions (F2+F3; Fig. 6) in the lower strata of D and SA sampling stations, which implies that some of the residual metal was recovered with cold acid, possibly due to leaching of the weakly bound metal to HA during this step. With the exception of Zn in two strata at D (Fig. 6), for the remainder of the metals the amount recovered in (F2+F3) was always higher.

In addition, the average AVS/Fe molar ratios were calculated to evaluate the presence of different iron sulfide species. Only at NA the value was 1.7, close to greigite, while at D and SA it was of 2.5, thus indicating excess of sulfides, probably due to an excess of H<sub>2</sub>S that was not purged with N<sub>2</sub> during the 10 minutes at the beginning of the extraction. A ratio of 2.0 was not expected because the cold HCl does not dissolve pyrite, this method was designed to evaluate the presence of labile iron sulfide species.

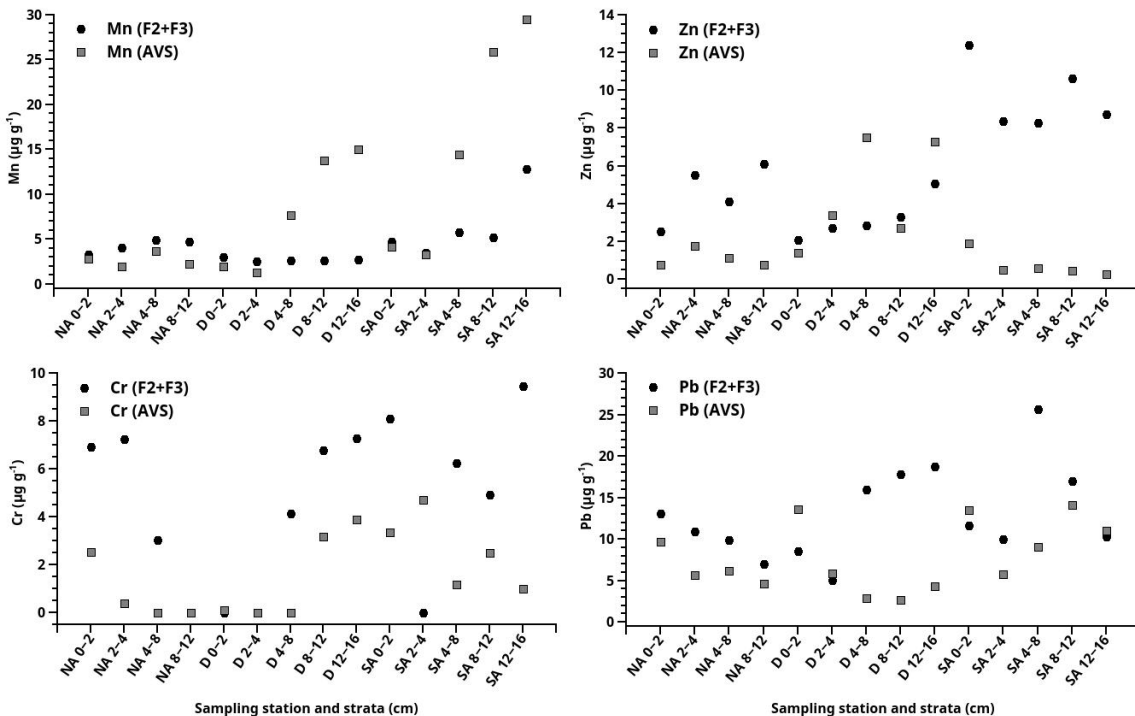


FIG. 6. Comparison between SEM metals (squares) and F2+F3 (reducible and oxidizable; dots) fractions of SEP.

### 5.3. Toxicity thresholds and mobility

The observed influxes and lake concentrations for the time frame of the study were as follows: Mn>Zn>Cr~Pb>Cu (Varekamp, 2008, Cabrera *et al.*, 2016; data not shown), albeit its content in the sediments was: Cr>Mn>Pb>Cu>Zn. Compared to two international guidelines (Table 1) Zn was at least one order of magnitude below the lowest CCME (2001) threshold and is the least toxic of all. Chromium is within the margins of CCME (2001) values for NA, at D is mostly below and at SA is slightly above the upper limit, which overlaps with the ANZECC-ARMCANZ (2000) lower boundary. In addition, it is expected to be soluble as Cr<sup>+3</sup> and CrOH<sup>+2</sup> rather than as the hexavalent harmful forms (Richard and Bourg, 1991). Copper was below the lower value of the more conservative CCME (2001) for all the measurements with the exception of the lowest stratum of NA and SA. Finally, Pb was over the highest value of the Canadian guidelines and in between the New Zealand/Australian.

According with the mobility index ( $MI=M_R/M_T$  with  $M_R$  residual metal and  $M_T$  total metal) proposed by Lesmes (1996) the most mobile metals are Cu and Zn, these metals have higher proportion of the metals in the reducible and/or oxidizable fractions than Cr, Pb and Mn.

Caviahue lake sediments has higher concentrations of these metals (this work) than most unpolluted natural water bodies and comparable with moderately contaminated environments, rivers and lakes such as the Matanza-Riachuelo (Buenos Aires, Argentina; Magdaleno *et al.*, 2001; Rendina *et al.*, 2001a) a

highly contaminated urban plain river; the Sancho reservoir (Torres and Auleda, 2013) or the Tinto and Odiel rivers (only for Cr; Galán *et al.*, 2003) at the Iberian Pyrite Belt. Moreover, highly contaminated sediments after mining activity have concentrations of these metals up to 5 mg g<sup>-1</sup> of dry sediment (Galán *et al.*, 2003; Kirschbaum *et al.*, 2012; among others). In Caviahue, the metals over the toxicity thresholds are mostly associated with the residual less labile fraction of the sediments, thus implying low risk for the hypolimnion and benthos.

In addition, the lake water pH has been rising since September 1998 to May 2018, with periods of temporal acidification due to an increase in the volcanic activity (*e.g.*, years 2000, 2004 and 2013 after Varekamp 2008, 2015). It went from approximately 2.44 to 3.26 in the epilimnion and from 2.43 to 3.40 in the hypolimnion (G. Baffico, personal communication, 2018). In this regard, the rocks from the coast have recently got coated by orange/red iron oxyhydroxides, similar to the schwertmannite found in the lower Rio Agrio bed where pH >3.0 and iron precipitation occurs (Varekamp, 2015). Should pH continue to rise, the precipitation of iron can change the chemical characteristics of the lake. The amorphous iron oxyhydroxides such as schwertmannite or ferrihydrite may drag to the sediments adsorbed oxyanions of P, V and As (as it occurs in the Agrio effluent; Varekamp, 2015). In addition, trace metals such as Cu, Pb, Zn, Cr or Cd (among others) will be sorbed by the hydrous Fe oxides at pH values above 3.0 for cationic Cr and Cu (Davis and Leckie, 1978; Benjamin *et al.*, 1982) and above 4.0 for the others (Dzombak and Morel, 1986), although the metals

**TABLE 1. COMPARISON OF THE HEAVY METALS CONCENTRATIONS FOUND IN THE THREE SAMPLING STATIONS OF CAVIAHUE LAKE AND CCME (2001) AND ANZECC-ARMCANZ (2000) GUIDELINES FOR THE HM STUDIED.**

Guideline / Metal	Metal concentration (µg g <sup>-1</sup> )						
	CCME	ANZECC-ARMCANZ			Caviahue (sampling stations)		
	ISQG	PEL	ISQG-Low	ISQG-High	NA	D	SA
Zn	123	315	200	410		7-18	
Cr	37	90	80	370	37-84	29-49	29-113
Cu	36	198	65	270	27-55	16-22	10-24
Pb	35	91	50	220	127-162	97-112	112-128

**ISQG:** Interim Sediment Quality Guidelines. **PEL:** Probable Effect Level. The values found for Zn in this work had no significant differences and therefore the average values were informed for all sampling stations.

incorporated to the Fe oxides will increase with pH (Benjamin *et al.*, 1982). Finally, this scenario will additionally imply a change in the pH-buffer system, from Fe to Al (Geller *et al.*, 1998; Pedrozo *et al.*, 2008b; Cabrera *et al.*, 2016).

## 6. Conclusions

The content of trace metals in the sediments of Lake Caviahue was comparable to other aquatic environments (acid or neutral) with low to moderate contamination levels for Cu, Cr and Pb. In contrast, Zn and Cd were below the toxicity threshold and limit of detection, respectively.

The content of trace metals in the exchangeable/carbonates fraction (F1) of SEP extraction was below the limit of detection and SEM metal content was below the toxicity thresholds of two international sediment quality guidelines. Besides, the AVS/SEM ratio, used often as a risk indicator, was higher than one, the threshold value. Moreover, after SEP extraction, Cr and Pb, are not readily available and are the only metals above the toxicity thresholds. Copper was mostly associated with the oxidizable fraction (F3) and is not readily available either. Should pH continue to rise, the present concentration and partitioning of heavy metals in the sediments poses no risk to the aquatic biota.

Finally, no unique pattern was found that encompasses the distribution of all metals. However, Cr, Pb and Mn were highly correlated and almost exclusively associated with the residual fraction (F4) of the SEP extraction, thus suggesting that these metals precipitate due to similar processes and/or co-variations of these metals in the volcano effluents.

## Acknowledgements

Funding for this study was provided by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT Argentina; PICT 2008-1105; PICT 2012-1389), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina; PIP 0013) and Universidad Nacional del Comahue (UNCo, Argentina; Program 04/B166). The authors thank Dr. M. Díaz and Dr. G. Baffico (CONICET, Argentina) for the help during the sampling trips; MSE. A. Ferrari for his review and comments on the English language and A. Fantozzi for the help with the map. The authors also want to thank Professor Varekamp, the anonymous reviewer and Dr. W. Vivallo for their helpful comments.

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