Tillandsia usneoides as biomonitors of trace elements contents in the atmosphere of the mining district of Cartagena-La Unión (Spain): New insights for element transfer and pollution source tracing

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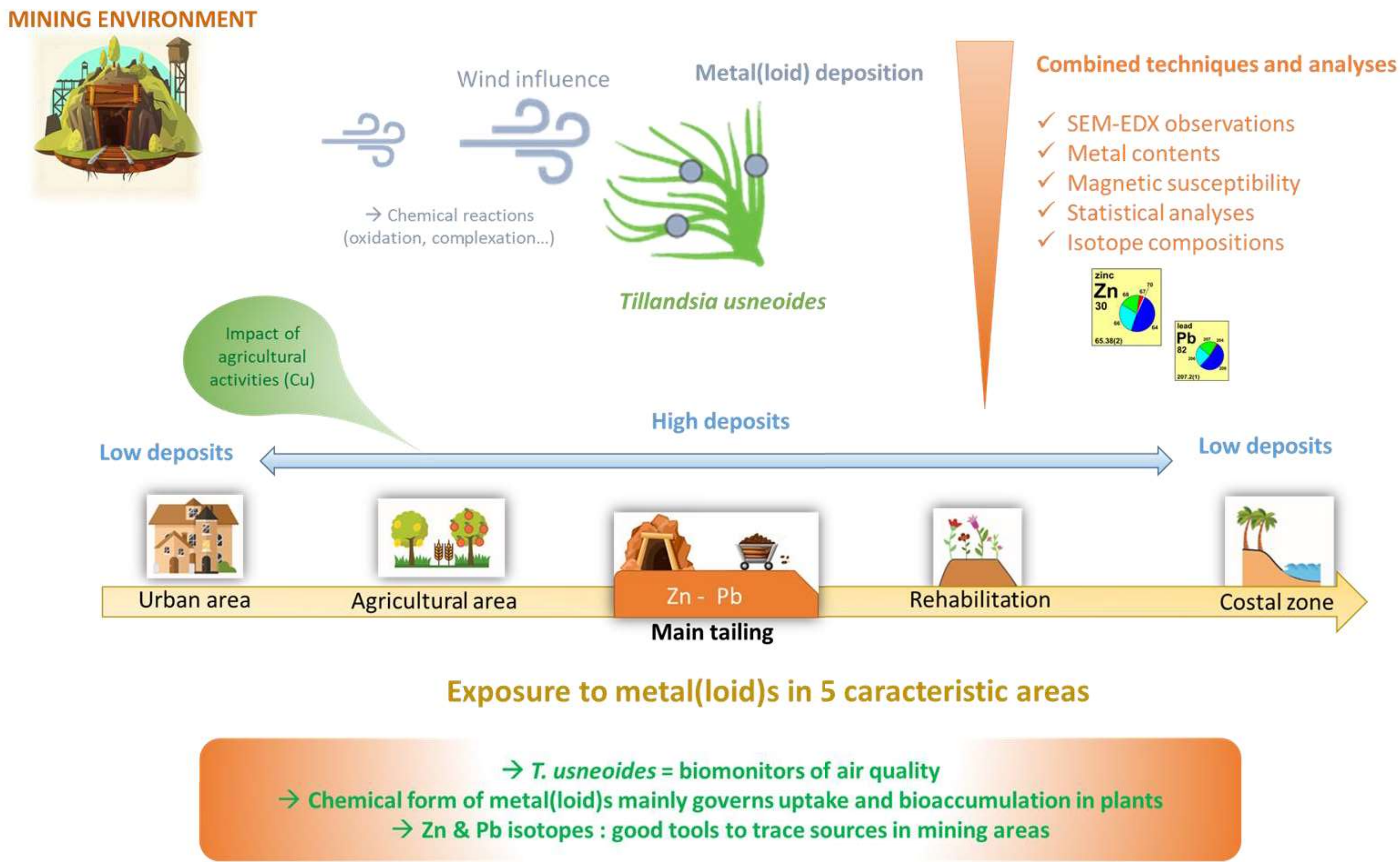
Declarations of interest : none

Graphical Abstract

GRAPHICAL ABSTRACT



***Highlights (3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

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***Tillandsia usneoides* as Biomonitors of Air Quality in the Mining District of Cartagena-La Unión (Spain): New Insights for Element Transfer and Pollution Source Tracing**

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Highlights

- *T. usneoides* are significantly enriched in As, Cd, Pb, Sb and Zn in the mining district.
- Dust deposition is slower in urban and coastal sites compared to mining zones.
- Chemical form of metal in the air seems to govern its uptake and bioaccumulation.
- *T. usneoides* could be a powerful tool to trace element sources in the atmosphere.

Tillandsia usneoides as Biomonitors of Trace Elements Contents in the Atmosphere of the Mining District of Cartagena-La Unión (Spain): New Insights for Element Transfer and Pollution Source Tracing

Eva Schreck[1], Jérôme Viers[1], Isalyne Blondet[1], Yves Auda[1], Mélina Macouin[1], Cyril Zouiten[1], Rémi Freydier[2], Grégory Dufrechou[1], Jérôme Chmeleff[1], José Darrozes[1]

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Abstract

Nowadays, atmospheric pollution has a major impact on the human health and the environment, encouraging the development of biomonitors of the air quality over a wide zone.

In this study, the relevance of the epiphyte plants *Tillandsia usneoides* is studied to estimate the transfer of metal(loid)s from a former Zn and Pb mining zone in the Southeast of Spain (Cartagena-La Unión) to the local atmosphere. Biomonitoring was performed by installing plants in 5 sites along a transect from the main mining area to the urban and the coastal zones. An aliquot of plants was collected in every site every 2 months over 1 year. The *Tillandsia usneoides* have been observed with SEM-EDX, and analysed by ICP-MS to determine trace element concentrations, magnetic susceptibility signals and Zn and Pb isotopes ratios.

Results show that atmospheric particles are distributed homogeneously at the plant surface. By comparing elemental contents in *Tillandsia usneoides* with regard to the values of the geochemical background of the region of Murcia, significant enrichments are observed in the epiphyte plants for Sb, As, Cd, Zn and Pb.

The statistical analyses (decentred PCA and PLS) also suggest that the kinetics of dust deposition is slower for the urban and coastal sites compared to the mining sites and highlight an influence of agricultural activities in Cu deposition. The similarity of isotopic compositions (Zn and Pb) between *Tillandsia usneoides*, soils and atmospheric particles also put in evidence that these plants could be a powerful tool to trace the source of matter in the atmosphere. Finally, this experiment provides new insight to better understand the foliar absorption mechanisms.

Keywords: *Tillandsia usneoides*; air quality biomonitoring; mining activities; isotopes; source tracing; magnetic susceptibility.

1. Introduction

Mining activities are still an important source of contamination by trace elements (TE) worldwide, involving severe damages in the different environmental compartments (Bes et al., 2014). Trace metals are highly toxic and persistent in the environment, and both heavy metals and metalloids pose a very serious and significant threat to the ecosystem health and nearby habitats, including soil, surface water and atmosphere (Boamponsem et al., 2010; Gu et al., 2018).

Among these damaged places, the Cartagena-La Unión mining district (Murcia region, SE Spain) is one of the most notable sites of geochemical pollution and geotechnical instability in Spain's abandoned mining heritage (IGME, 2002, Rodríguez et al., 2011). Metal extraction has been carried out since ancient (Roman) times, with a peak of activities in the 1980s (Navarro et al., 2008). A number of research papers have been published in the last decade regarding soil contamination in this area (Conesa et al., 2006, Conesa et al., 2008) and possible strategies for the remediation or the alleviation of the TE toxicity of these soils (Pardo et al., 2017; Zornoza et al., 2012).

But, there is a notable lack of knowledge concerning air quality in this old mining site and its surroundings, whereas air pollution was reported to be one of the most important environmental problems in the developing countries (Serbula et al., 2017). Sánchez Bisquert et al. (2017) studied metallic contaminations in soluble and insoluble fractions of atmospheric deposits in a large scale of 50 km² from the plain of Campo de Cartagena to the Mar Menor touristic area. Our research team had recently recorded and analysed atmospheric dusts using Owen gauges in the mining district of Cartagena-La Unión, and first results reported high contents of Zn, Pb, As, Cd and Sb (Blondet et al., *2019*). Also, only Querol et al. (2008) have really taken this area into consideration for modelling spatial and temporal variations in airborne particulate matter across Spain during the 1999–2005 period. However, the monitoring of air quality in mining sites has now become really important because TE emitted through mining activities in the atmosphere can inter in the pulmonary system and cause severe damages or chronic inflammatory and respiratory diseases (Boamponsem et al, 2010; Goix et al., 2011; Wise et al., 2017).

So, this study aims to develop a biomonitoring of metals and metalloids present in the air in the mining district of La Unión-Cartagena, using epiphyte plants in order to propose efficient but easy-to-perform and not expensive tools to follow contamination dispersion in the atmosphere and later develop the right remediation techniques. In several sites, standard techniques for air monitoring were prohibitive in terms of cost (Carnevale et al., 2012; Schreck et al., 2016; Xing et al., 2019). Thus, biomonitoring methods using mosses, lichens and epiphytic bromeliads (i.e., *Tillandsia spp.*) have been largely developed (Ares et al., 2012, Bermudez et al., 2009, Szczepaniak and Biziuk, 2003, Grangeon et al., 2012; Schreck et al., 2016). Among these plants, *Tillandsia spp.* are reported to be efficient due to their epidermal trichomes allowing the uptake of atmospheric water, minerals and organic nutrients (Martin et al., 2013; Schreck et al., 2016) and are therefore independent of the soil (Rodriguez et al., 2011; Martínez-Reséndiz et al., 2015).

The epiphyte *T. usneoides* has largely been studied as a good bioindicator of metallic contamination in several areas (Martínez-Carrillo et al., 2010; Pellegrini et al., 2014; Zheng et al., 2016). These *Bromeliaceae* plants are mainly found in the neotropic ecotone (from southern North America to southern South America, Techato et al. 2014). Their particularity comes from the fact that they adapt to arid and semi-arid climates (such as the Cartagena zone) and are resistant to water stress. Zheng et al. (2016) demonstrated that *Tillandsia usneoides* are excellent bioindicators that can deal with the abiotic stress associated with a long period of exposure to strontium. In addition, *T. usneoides* are recognised as indicators of effective atmospheric contamination for elements such as As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Sn and Zn (Zheng et al., 2016), suggesting their promising role in mining environments. Moreover, Pellegrini et al. (2014) also approve the use of *T. usneoides* as biomonitor of TE in urban areas of the Mediterranean basin, particularly in Italy.

However, even if the biomonitoring role of *Tillandsia usneoides* has been relatively well studied, to our knowledge, nothing has been reported concerning their use as tools for sources tracing and pollution dispersion through the air. In environmental studies, metal pollution is often assessed by comparing stable metal isotope ratios as signatures of point sources (Rosca et al., 2019). Their use allows us to improve our understanding of contaminant sources and pathways in the environment and especially in anthropogenic emissions from mines, industries, metallurgy (Cloquet et al., 2006; Araújo et al., 2018). Of special interest are the elements Pb and Zn, since they are important constituents of several man-made materials and especially extracted in this mining district of Cartagena-La Unión, and whose cycles are drastically altered by anthropogenic activities (Guéguen et al., 2012).

Thus, focusing on the area of the Cartagena-La Unión mining district, this study aims to investigate the potential use of *T. usneoides* as new and easy-to-perform tools to monitor atmospheric contents of metals and metalloids in mining environments. Experimentations will be performed in an area of 25 km^2 on 5 strategic sites including mining sources (the main tailing and a rehabilitated zone) but also agricultural (Las Matildes) and inhabitant or touristic zones, such as La Union city or the Gorguel beach, located in the vicinity of the main tailing. We plan to use combined techniques (microscopic observations using SEM-EDX and elemental content determination by ICP-MS coupled to magnetic susceptibility measurements) in view of determining the kinetics of the atmospheric deposition and the potential contribution of abiotic factors such as pluviometry, agricultural inputs, etc. We also aim to better understand the mechanisms of transfer of TE in the environmental compartments and later through the plant. Finally, by investigating isotope ratios of Zn and Pb in epiphyte plants, we propose a source tracing in mining environments and to better understand the fate of these TE in mining environments and especially in the atmosphere.

2. Materials and Methods

2.1 Study area description

The investigated area of the mining district of Cartagena-La Unión is located in the Sierra Minera, an area of approximately 50 km^2 (SIGES, 2017), at 10 km from the city of Cartagena in South-Eastern Spain. Polymetallic ore deposits were created during the post-orogenic magmatic activity associated with a hydrothermal circulation (Garcia et al., 2008). Exploited ores consisted of silver galena (PbS, Ag), sphalerite (ZnS) and pyrite (FeS), as underlined by Alcolea-Rubio (2015) and Rico et al. (2009). This exploitation led then to the mobilisation of the following elements: As, Cu, Cd, Sb, Sn, Co, Ni and Cr (Alcolea-Rubio, 2015).

Five strategic sites that have been selected for air biomonitoring over an area of 25 km^2 in the mining district of La Unión (Figure 1 and Blondet et al., 2019): the urban area of La Unión, the main mining tailing zone called "Avenque Tailing", the coastal area corresponding to "Playa del Gorguel" and located near the Mediterranean Sea, an area of soil remediation by organic amendments “Santa Antonieta” and finally the agricultural area (corresponding to an old mining area) of "Las Matildes".

Atmospheric depositions (dry and wet) were also sampled using Owen gauges (NFX43-014 standard, Navel et al., 2015) exposed for 1 year and collected every 6 months (from May 2017 to April 2018). This experiment was entirely described in Blondet et al. (2019).

In this area, climate is reported to be semi-arid, with a pluviometry of about 250 to 400 mm per year (Los Belones station), essentially concentrated during the spring and autumn seasons, with a mean annual temperature of 18°C (SIAM, 2017). Prevailing winds are facing South/Southwest directions, with a 190–200° azimuth (Figure 1).

2.2 Experimental set-up of biomonitoring by *T. usneoides*

Several batches of *T. usneoides* (from the same initial plant) were installed at the 5 sites (Figure 1) in May 2017, using trunks or palm trees with small plastic cable ties, at 2.5 meters in height to better catch air pollution (see Figure SI-1 for more details). Each batch corresponds to 3 g of fresh material, placed in a nylon mesh bag, as already described in Schreck et al. (2016). In May 2017, 3 mesh bags of *T. usneoides* were kept as control (no exposure in the mining district of Cartagena-La Unión), they were cultivated in a clean greenhouse during all the exposure experiment, and later analysed as well as the exposed ones. For exposed *T. usneoides*, one batch was taken every 2 months, between July 2017 and April 2018, to follow the kinetics of deposition and potential absorption of atmospheric particles.

A washing procedure was also applied for several plants to determine the effects of the washing process and remove potential adherent particles present on the plant surfaces (Goix et al., 2013; Smodiš et al., 2004). Then, each plant was placed in ultrapure water, sonicated for 3 min and rinsed again. Then plants were dried at 40°C for 48 h and ground in an agate mortar in liquid N_2. Ground plant samples were then dried again for 48 h and stored in polyethylene vials (Goix et al., 2013). The washing procedure allowed us to take off about 20% of the deposited particles (results not shown).

2.3 Soil sampling

In parallel to *T. usneoides* exposure, soil samples were collected in the vicinity of bioindicators installation. A composite sample of topsoil was collected, air-dried and sieved at 2 mm and ground with an agate bench ring mill.

2.4 Morphological observations by SEM-EDX

Environmental SEM-EDX measurements using a (FEG) JSM-7800F Prime (Jeol®) instrument equipped with EDX Detector were carried out to investigate the epiphyte morphology, trichome conformation and potential elemental distribution of the plant surface. Before observation analysis, *T. usneoides* plants were dried and fixed on a carbon substrate without any further preparation before analysis. The apparatus was operated in low-vacuum mode (~133 Pa) at 25 kV. Observations were performed on plant fragments exposed for 6 months, before and after washing process, to determine its effect on deposit lost.

2.5 Elemental contents in plants and soils

Dried samples (0.1 g of soil or plant) were digested using a mixture of bi-distilled and suprapur quality reagents. A solution of 5 mL bi-distilled HNO_3, 1 mL H_2O_2 and 200 µL HF was prepared and added to each sample and then left overnight. After addition of 5 mL bi-distilled HNO_3, acid mineralisation was conducted on a Discover micro-waves apparatus from CEM® using 35 mL pressure vials in quartz tubes and adapted Teflon PFA liners and caps (during 10 min, 3 cycles until 180°C). Afterwards, solutions were completely evaporated at 60°C on a hotplate and the dry residue was resuspended by adding 0.5 ml of HNO_3.

After mineralisation, samples were diluted with bi-distillated water and filtered through a 0.22 µm cellulose acetate membrane. Filter membranes were checked to not have any influence on metal contents in samples (results not shown).

All diluted samples (HNO_3 0.37 N) were analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analyses (Agilent® 7500 CE and iCAP Q Thermo Scientific®) in order to determine trace metals concentration. Each batch of samples included a blank acid, a certified reference material and some replicates. Limits of detection (LD) and quantification (LQ) were calculated as 3 and 10 times the standard deviation (SD) of laboratory blank samples. There were 3 certified reference materials (from the National Institute of Standards and Technology and Institute for Reference Materials and Measurements)—SRM 2709 "San Joaquin soil", SRM 1515 "Apple leaves" and BCR482 "Lichen"—used to validate the mineralisation methods. Recoveries of 79 to 100% were obtained for the different analysed elements.

Finally, enrichment factors (EF) can be calculated for elemental contents in *T. usneoides* from the main Avenque Tailing site compared to the values of the geochemical background of the Region of Murcia region (Martínez-Sánchez & Pérez-Sirvent, 2007; Alcolea-Rubio, 2015) according to the Equation 1:

Eq (1) $$EF = \frac{[TE]T.usneoides}{[TE]geochemicalbackgroundoftheRegionofMurcia}$$

2.6 Magnetic susceptibility

Magnetic susceptibility was measured in the *T. usneoides* samples, as well as in soils collected in the same areas, in order to estimate the concentration in magnetic particles. Magnetic susceptibility measurements were performed with a KLY5 Kappabridge (AGICO) (400 A/m, 1220 Hz) with a sensitivity of 2.10^{-8} SI. In parallel of geochemical analyses, samples were put into 30 ml tubes and their susceptibility was normalised by mass.

2.7 Zn and Pb isotopes study

Zinc and Pb isotopes determination was performed on 4 different samples of *T. usneoides* and the associated atmospheric fallouts, collected from 4 of the investigated areas (Avenque Tailing, Playa del Gorguel, La Unión and Las Matildes) and also on 2 soils from the main tailing (Avenque tailing). All these analysed samples were collected in November 2017 to avoid all interferences due to temporal variations, according to the different environmental compartments. One more sample of *T. usneoides* collected in January 2018 from Avenque Tailing was also analysed to have a first idea of a potential seasonal effect.

2.7.1 Zn isotopes

To determine isotope fingerprints, Zn must be isolated from the rest of the sample matrix. A chromatography separation was carried out twice on aliquots of digested samples using AG MP-1 resin with Biorad® column and according to the protocol adapted from Maréchal et al. (1999) and Viers et al. (2015). In order to obtain great quality measures, the aliquots containing Zn (collected in the last step of the protocol and diluted in HNO_3 0.05 N to obtain a final content of 300 µg L^{-1}) were analysed by ICP-MS to check that the other elements are missing and that the mineralisation and separation processes were well-performed. Recoveries were between 96 and 106% and then considered as good. Purified solutions were spiked with Cu standard (NIST 976) for measurements on MC-ICP-MS (Neptune plus, Thermo Finnigan® at the Observatoire Midi-Pyrénées in Toulouse). Zn isotope ratios are expressed in δ^{66}Zn (‰) relative to NIST 976 Cu standard, according to the Equation (2):

Eq (2)
$$\delta^{66}Zn = \left[\frac{\left(\frac{^{66}Zn}{^{64}Zn}\right)sample}{\left(\frac{^{66}Zn}{^{64}Zn}\right)NIST976} - 1\right] * 1000$$

If the internal precision on one measure is close to 10 ppm, a triplicate analysis of our samples would give an absolute error of 0.07‰.

2.7.2 Pb isotopes

Lead isotope ratios were directly measured by Element™ Series HR-ICP-MS from Thermo Fisher Scientific® at the Observatoire Midi-Pyrénées in Toulouse. Solutions were analysed at the concentration of 0.5 µg L^{-1}. Standard SRM 981 was periodically measured (every 5 samples) to systematically correct for mass bias and instrument drift with time on both isotope ratios (Goix et al., 2015). Also, corrections were performed using the following ratios: $^{208}Pb/^{206}Pb = 2.168 \pm 0.001$ and $^{207}Pb/^{206}Pb = 0.915 \pm 0.001$. Standard deviations of 0.12 and 0.08% were then obtained for the ratios $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$, respectively.

2.8 Statistical analyses

Two types of statistical analyses were carried out. The first consisted of studying the temporal evolution of the concentration of chemical elements from the reference point constituted by the month of May 2017, the date on which the plants were installed. A decentred principal component analysis was adapted to this type of data (Noy-Meir 1973). The centring was not performed as in a conventional PCA with respect to the average but with respect to a reference common to all samples, corresponding in this experiment to the first date. The missing data encountered for magnetic susceptibility were not completed by the mean of the variables but by its reference value, i.e. its value taken at the beginning of the experiment.

The second analysis compared the chemical element concentrations of plants in April 2018 with the concentrations of elements stored in the soil and accumulated in the air during the experiment. A PLS2 (Partial Least Square) was used to explain plant chemical element concentrations from soil concentrations or those accumulated in the air (Geladi and Kowlaski 1986). The missing data (Santa Antonieta station in January 2018 and La Union magnetic susceptibility in September 2017 and January 2018) were replaced by the average of the corresponding variables.

Calculations were performed using ADE-4 and plsdepot, CRAN packages (Chessel et al. 2004, Gaston Sanchez 2012).

3. Results

3.1 SEM-EDX observations: atmospheric deposits on *T. usneoides*

The SEM-EDX observation (Figure 2) of a sample of a washed *Tillandsia usneoides* (collected in November 2017 in the Avenque Tailing area) has shown the plant morphology and especially the trichome's structure in shields, in order to better uptake nutrients from the atmosphere (Figure 2-A). Deposited particles from the mining site are homogenously distributed on the plant surface. Chemical spectra analyses, combined to our previous results of X-ray diffraction (XRD) on atmospheric particles collected by Owen gauges (Blondet et al., 2019), suggest that the main minerals observed are calcite ($CaCO_3$) and gypsum ($CaSO_4$), but also halite (NaCl), clay minerals (Al, Si, K) and quartz (SiO_2). Other spectra, composed by Fe and S peaks, also seem to highlight the presence of pyrite (FeS_2), also observed in the corresponding atmospheric fallouts (Blondet et al., 2019). Moreover, some spectra show the presence of particles of Zn, Cu, C and O that could correspond to smithsonite minerals ((Zn, Cu) CO_3). Finally, traces of Pb and Ti are also observed in other spectra (not shown), highlighting the contamination by mining deposits.

3.2 Elemental contents in soils and plants

3.2.1 Soils

Elemental contents in soils of the different areas are reported in Table 1. Results showed that some elements, such as Cr, Co and Ni have concentrations in the mining site that are perfectly in accordance with the geochemical background of the region (Martínez-Sánchez and Pérez-Sirvent, 2007). By contrast, the other ones can be directly linked to an anthropogenic contamination of the site, especially Zn, Pb, As, Cd, Sb and Cu that show abnormally high concentrations in the sampled soils (Table 1). Globally, the 5 investigated sites of the mining district of Cartagena-La Unión are contaminated in different elements (Cu, Zn, As, Cd, Sn, Sb and Pb), with a level of contamination following the order: Avenque tailing > Las Matildes ≈ Playa del Gorguel > Santa Antonieta > La Unión for the main considered elements.

High contents of TE are reported in the topsoil of Avenque tailing (the main tailing of this old mine) that are largely higher than the geochemical background of the Region of Murcia in Spain (Martínez-Sánchez and Pérez-Sirvent, 2007). For example, in this site, the concentrations of Pb and Zn reached, respectively, 14,770.39 ± 659.23 and 10,345.08 ± 336.68 ppm, whereas these values are reported to be about 3–10 and 16–55 ppm for Pb and Zn, respectively, in the geochemical background of the Region of Murcia (Table 1). Another site that appeared as widely contaminated is the agricultural zone of Las Matildes showing high contents in Pb, Zn but also Cu (respectively 36,019.35 ± 801.12, 4,352.43 ±. 15.23 and 232.78 ± 4.65). By contrast, in this area, As contents are in accordance with the geochemical background of the site (Table 1). In the remediated site of Santa Antonieta, concentrations seemed to be attenuated in comparison with the main tailing but are still high compared to the geochemical background (Table 1).

3.2.2 Plants

Figure 3 showed the elemental contents of Zn, Pb, As and Cd in *T. usneoides* from the various investigated sites. Only these 4 elements were represented here because they were the most significant in terms of plant contamination, but also because they were the most toxic emitted by mining extraction activities. All the results correspond to the mean value (± standard error SE) of 3 replicates. High concentrations in TE are observed, mainly for the *T. usneoides* from the old mining sites of Avenque Tailing, Santa Antonieta and Las Matildes. Their concentrations recorded significantly exceed the geochemical background of soils in the Region of Murcia (Martínez-Sánchez & Pérez-Sirvent, 2007; Alcolea-Rubio, 2015) and globally increase over time. Results of mean enrichment factors (EF) for Avenque Tailing show that epiphyte plants are significantly enriched in Sb (EF=1.7), As (EF=2.4), Cd (EF= 7.9), Zn (EF=20.5) and Pb (EF= 33.6). Only Cu does not exhibit a significant enrichment factor (EF=0.51). In July 2017, we observed a strong increase in Zn and Cd contents in *T. usneoides* from Avenque Tailing, maybe in relation with the lack of precipitations (*see Discussion section for more information*). The sites of Playa del Gorguel and La Unión have concentrations that are generally constant over time, and very close to the geochemical backgrounds. The area of Las Matildes, an old mining site with large agricultural zones, showed high contents in Cu.

3.3 Magnetic susceptibility of soils and epiphyte plants

Results concerning magnetic susceptibility of soil and *T. usneoides* samples are respectively reported in Table 1 and Figure 3.

In soils, values of magnetic susceptibility range 5.4283E^{-06} to 4.1046E^{-05} $m^3.kg^{-1}$ for the sites of Avenque tailing and Playa del Gorguel, respectively. It is interesting to notice that the lowest values are recorded for the mining site (Avenque Tailing) and the coastal area corresponding to "Playa del Gorguel", whose magnetic susceptibility values are 5.4283E^{-06} and 1.6519E^{-05} $m^3.kg^{-1}$, respectively (Table 1). By contrast, the highest values were measured in the rehabilitated and agricultural areas (Santa Antonieta and Las Matildes).

The epiphyte plants display magnetic susceptibility values that increase over time, from 2.6766E^{-08} (for playa del Gorguel, in July 2017) to 2.4902E^{-07} $m^3.kg^{-1}$ (for Santa Antonieta in November

2017) (Figure 3). As already observed for soil samples, the highest values are reported for Santa Antonieta and Las Matildes sites. In these two sites, variations according to time of exposure are not linear but quite similar, and show a pic in magnetic susceptibility observed in November 2017. For the 3 other sites (Avenque Tailing, Playa del Gorguel and la Unión), magnetic susceptibility values increase slowly with time of exposure (Figure 3).

3.4 Zn and Pb isotope ratios in environmental compartments

Results of isotope ratios (for Zn and Pb) are reported in Table 2. Figure 4 also showed the mean value of δ^{66}Zn from various samples of plants (*T. usneoides*), soils and atmospheric fallouts in relation with Zn concentration. The mean δ^{66}Zn value obtained by Sonke et al. (2008) for the analysis of 29 sphalerites [(Zn, Fe)S] from 10 different mining sites is also reported in Figure 4. Results highlighted that the 2 analysed soils show values (-0.35 and -0.40 ‰) far lower than those of Zn sulphurs. Atmospheric fallouts from Owen gauges evidenced high isotope variability (from -0.23 to -0.56‰). *Tillandsia usneoides* samples also showed a same variability in δ^{66}Zn value (from -0.25 to -0.50‰) for the 4 analysed samples.

Table 2 clearly shows that δ^{66}Zn values are quite the same between the 2 plants from Avenque Tailing, collected in November 2017 (-0.25‰) and in January 2018 (-0.21‰). Isotopes ratios of Pb vary between 2.084 and 2.091 and, 1.187 and 1.192 for the ^{208}Pb/^{206}Pb and ^{206}Pb/^{207}Pb ratios, respectively (Table 2). Since Pb isotope ratios are not modified by high temperature processes, they must have the fingerprint of Pb extracted during the industrial history of this mining site of Cartagena-La Unión.

4. Discussion

4.1 *T. usneoides* as bioindicators of atmospheric contents of TE in the mining district of Cartagena- La Unión

By comparing the metal and metalloid contents in *T. usneoides* for the different investigated areas, it appears that the urban zone of La Unión and the beach "Playa del Gorguel" shows low contents in toxic metallic compounds. Their metal content values are closed to the geochemical background values reported for each element [from Martínez-Sánchez & Pérez-Sirvent (2007) and Alcolea-Rubio (2015)], suggesting that the atmosphere is not enriched in TE. Moreover, the distribution of TE among the various studied sites are globally in accordance with the results previously obtained for atmospheric dust deposition in Owen gauges (Blondet et al., 2019), even if no strict correlation could be calculated between the results of Owen gauges and those of *T. usneoides* for TE contents. There are some differences in the involved processes by the 2 methods of monitoring: gauges collect the general fallouts, whereas plants react with selective accumulation of cations as highlighted by Impens et al. (1980). By the way, the similitudes in contamination profiles between the different sites for TE content in *T. usneoides* and TE accumulated in atmospheric dusts from Owen gauges suggest that *T. usneoides* could be relevant bioindicators of atmospheric contents of TE in this old mining area. This statement was already highlighted by Techado et al. (2014) for various contaminated sites over the world. *Tillandsia* spp., and especially *T. usneoides*, has proved to be an efficient atmospheric accumulator of Hg, Cd, Pb, Ni, Cu, Cr, C and Zn by the mechanism of phytoremediation, as suggested by Brighigna et al. (1997) in Costa Rica, Vianna et al. (2011) in 2 Brazilian metropolitan areas or Malm et al. (1998) in the gold trade centre city, Amazon, Brazil.

The TE enrichment factors calculated in *T. usneoides* from the main Avenque Tailing site are consistent with those reported in soils of mine tailings, as demonstrated by Alcolea-Rubio (2015) in this area. Therefore *T. usneoides* records the atmospheric contamination brought by mine tailings in their vicinity. Only Cu does not exhibit a significant enrichment factor (EF=0.51), and it is also not highly concentrated in atmospheric dusts collected by Owen gauges. A strong enrichment in Zn, As and Sb is also noted by comparing the concentrations obtained in our *T. usneoides* with those of Figueiredo et al. (2004) placed in industrial sites and under high traffic in Sao Paulo (Brazil), but also with those of Sánchez-Chardi (2016) who exposed different *Tillandsia* spp. in the urban environment of Asunción (Paraguay). Indeed, the concentration in Zn (1024 ppm) of *T. usneoide*s collected in November in Avenque Tailing widely exceeds those from the high traffic zones (54 to 102 ppm) and industrial sites (58 to 134 ppm) described by Figueiredo et al. (2004). However, those collected at the same time in La Unión (81 ppm) are in the same order of magnitude. The same observation goes for Sb (with 0.44 to 0.81, 0.2 to 0.59, 0.51 and 3.24 ppm at high traffic sites, industrial sites, La Unión and Avenque Tailing, respectively). The concentrations recorded for As in industrial sites and high traffic areas in Sao Paulo (between 0.11 and 0.28 ppm) are lower than those measured at La Unión (2 ppm) and Avenque Tailing (22 ppm), suggesting really high contents in As in our studied area. Finally, by comparing these results with those obtained recently by our research team in the urban mining area of Oruro in Bolivia (Goix et al., 2013; Schreck et al., 2016), we noticed that the epiphyte plants exposed in the old mining site of Cartagena-La Unión recorded a high level of TE in their tissues, especially for Cd, Zn and Pb (that are almost 10 times higher than in Oruro), suggesting a strong influence of the mining area but also differences bring by plant species. Only As is accumulated in the plants at quite the same range (Schreck et al., 2016).

Figure 5, a graphical summary of the decentred PCA, shows that only the first 2 factors are interpretable. The first factor is a size factor: When a station has a high concentration for 1 chemical element, it also has a high concentration for all others. A concentration gradient was observed from the Avenque Tailing station, the most polluted, to La Union, the least polluted, as already suggested by TE contents in epiphyte plants.

The representation of factor 1 as a time curve (Figure SI-2) clearly summarises the evolution of concentrations for each station. The factor map of the samples, labelled by station, shows a higher sensitivity to contamination at the Avenque Amont, Santa Antonieta and Las Matildes stations (sites of former mining activities) than at the Avenque Aval and La Unión stations. The stations, Avenque

Tailing and Santa Antonieta, located on heaps, regularly accumulated chemical elements throughout the experiment. The Union appears as not polluted by the studied metal(loid)s. Playa del Gorgel is only slightly so at the end of the experiment. The pollution of Las Matildes is fluctuating. Thus, contaminant concentration appears to be directly related to wind erosion of slag heaps in the vicinity of *T. usneoides*. These results confirm that *T. usneoides* correctly records variations in concentrations due to the remobilisation of dusts in the vicinity of mining tailings, even old ones.

Contrary to wind, rainfall seems to add no impact on TE contents in *T. usneoides* as suggested by statistical analyses (results not shown), even if an influence could be suspected at first sight. Nevertheless, very low cumulative rainfall (about 185 mm) was observed during all the exposure period of one year.

4.2 From site contamination to air quality: suggested mechanisms of TE transfer in the environment and their uptake by *T. usneoides*

Environmental magnetism could be an effective tool to monitor a polluted area (see Maher, 2009; Mariyanto et al., 2019; Martin et al., 2018; Petrovsky et al., 2000), being a fast and cost-effective method. Here, results of magnetic susceptibility in soils and in plants have shown that there was no correlation between Pb or Fe concentrations and magnetic susceptibility (R^2=0.41 for magnetic susceptibility = f ([Fe]) in *T. usneoides* of the experiment, Figure SI-3 in Supplementary Information).

In this mining district, exploited ores consisted of silver galena, sphalerite and pyrite (Alcolea-Rubio, 2015; Rico et al., 2009), suggesting that sulphurs are the main compounds. Fe in the form of pyrite induces only a weak magnetisation (Dunlop and Ozdemir, 1991). Then, in Avenque Tailing, magnetic susceptibility is very low in soils due to high contents of pyrite. By contrast, the higher levels of magnetic susceptibility recorded in the old mining site of Las Matildes (agricultural area) and the rehabilitated site of Santa Antonieta suggests either a process of mineral re-oxidation of pyrite into magnetite or the presence of primary magnetite in the exploited deposits (Oen et al., 1975; Manteca Martínez and Ovejero Zapino, 1992). Near the Avenque area, Gómez-García et al. (2015) have studied the infilling process of the Portman Bay (Murcia, Spain) due to direct mine waste discharge into the sea. They acquired rock magnetic parameters, including magnetic susceptibility on both minerals deposits and the sand filling the bay. Magnetic parameters indicated high concentrations (up to 28%) of magnetite in the sand. Moreover, this sand displayed a direct relationship between magnetite content and the heavy metals Pb, Cu and As. Samples taken at the mining site gave lower magnetic susceptibility values between -6.64 $x10^{-7}$ and 6.35 $x10^{-5}$ $m^3.kg^{-1}$, indicating a magnetite concentration significantly lower than in the sand filling the Portman Bay. This indicates that oxidation affected the primary mineralogy.

In the Las Matildes and Santa Antonieta, higher magnetic susceptibility values, hence higher contents in magnetite could be explained by the rehabilitation of the old tailings in these 2 areas by the use of organic matter amendments, irrigation, soil alteration and plant culture (Moreno-Barriga et al., 2017). These processes induce an oxidation of the primary pyrite.

Magnetic measurements of *Tillandsia spp.* have already been reported by 2 studies. Castaneda-Miranda et al. (2016) and Mejía-Echeverry et al. (2018) used *T. recurvata* growing naturally in trees to monitor air pollution in Mexico and Columbia. On unwashed plants, they obtained values between -6.64 $x10^{-7}$ and 372 $x10^{-8}$ $m^3.kg^{-1}$ corresponding to residential and heavy traffic area, respectively. Since the time integrated is unknown in these studies, it is not possible to directly compare these values with those obtained here, the time being certainly longer than the 6 months sampled in our case. Moreover, in our study, *T. usneoides* were washed prior the measurements. Then, we can assume that our values of magnetic susceptibilities up to 2.49 $x10^{-7}$, especially after 6 months of exposure, are significant.

As for the soil samples, the variability in magnetic susceptibility in epiphyte plants could certainly be explained by mineral transformations (changes in chemical forms of TE, redox state, organic matter complexation, etc.) when they are transported by soil erosion and dust remobilisation into the atmosphere. Pyrite is altered and reoxidised into the atmosphere (Blondet et al., 2019). The high signals of magnetic susceptibility in *T. usneoides* from Las Matildes and Santa Antonieta are certainly explained by wind erosion of these restored and cultivated environment. In the same way, the magnetic signal for the site "playa del Gorguel" (very low) show that the main influence is from Avenque Tailing, due to wind erosion of pyrite, as previously suggested by the wind rose and the main wind directions shown in the Figure 1. By contrast, the urban area of La Unión seems to be rather protected from wind erosion from Avenque Tailing, as suggested results of TE concentrations and magnetic susceptibility of *T. usneoides*, as well as the human risk assessment proposed by Blondet et al. (2019).

Copper pollution mainly affects the Las Matildes station, with a peak of contamination in November (211 ppm). The presence of citrus fruits that are treated with Cu-based plant protection products could explain this pollution. Indeed, one of the hypotheses put forward to explain this observation is that the agricultural site could be subject to a Cu fungicide treatment or land preparation at this time. Copper is widely applied in the Region of Murcia according to Dqagro (2015) for the treatment of fruits (e.g. citrus fruits in particular but also peaches, nectarines, plums, melons, apricots) and vegetables (e.g. zucchinis, onions, olives, artichokes, cauliflower, broccoli and potatoes). There are 2 treatments applied, in spring and autumn.

The time curve of factor 2 shows a pollution of Las Maltides by Cu in November 2017 and April 2018, in agreement with Dqagro (2015), and a pollution of Playa del Gorguel in April 2018.

On figure 6, graphics of the PLS2, the explained percentages of variance, indicate that only one factor is sufficient to interpret the structure. There is no simple relationship between the concentrations of chemical elements in plants and those in the soil. On the other hand, plant concentrations can be explained by those of Owen gauges [also discussed in Blondet et al. (2019)], with the exception of Cu and Ni. Avenque Tailing plants have the highest concentrations of TE, except for Cu and to a lesser extent Ni, although these 2 elements are present in Owen gauges. Then, we can ask if these last 2 elements could be subject to preferential accumulation or exclusion, linked to an active mechanism performed by the epiphyte plants. This hypothesis was already suggested by Wannaz et al. (2011) highlighting that concentrations obtained in the studied plants (*T. capillaris*) are higher than those found in comparative passive biomonitoring studies, showing then, a selectivity in the way of metals accumulation. This phenomenon of high contents in elements such as Cu and Zn can also result according to Wannaz et al. (2011) in growth inhibition and toxicity symptoms. Moreover, as previously reported by Shahid et al. (2017), various mechanisms of metal uptake could occur through the plant, depending on plant species and morphological characteristics (stomatal index, leaf morphology, cuticular structure, leaf surface area, trichome density) of course (Barber et al., 2004, Schreck et al., 2012) but also metal chemical form, content, granulometry and solubility (Natasha et al., 2019; Rossi et al., 2019). Several authors (Schreck et al., 2012; Shahid et al., 2017) highlight the fact that metals such as Pb, Co, Mn but mostly Cu could cross the cuticle of aerial organs after dissolution or penetrate by the stomatal pathways in case of small particles, whereas larger particles (1.1 µm) were unable to penetrate through stomata openings (Eichert et al., 2008; Rai et al., 2019). But metal speciation is considered as the main parameter governing biogeochemical behaviour of metals and metalloids at the plant surface (Shahid et al., 2017). Thus, some explanations could be given in our study case for Cu uptake and accumulation, which is variable depending on the different studied sites in the mining district of Cartagena-La Unión. In Avenque Tailing, an active mechanism of Cu inclusion through the plant by the stomatal pathway could be envisaged: Cu-enriched particles from mineral ores and tailings are deposited on the leaf surface, as observed in the SEM-EDX images (Figure 2 C&D). They can enter via the stomata openings. In case of an arid climate, as in summer and autumn 2017, the *T. usneoides* closes its stomata and Cu bioaccumulation is limited by this active process. But, by contrast, in the Las Matildes area, Cu contents in the atmosphere are higher than in the other sites and also relatively high in comparison to other elements [see results of Cu contents in Owen gauges—Figure 5 and data in Blondet et al.

(2019)]. Moreover, its speciation is certainly more soluble due to fungicide application in the cultivated areas at the vicinity of the *T. usneoides* exposure site. Copper uptake through the plant could then be envisaged by another mechanism involving metal dissolution and cuticle crossing (passive mechanism). Finally, our results for Cu could enhance the hypothesis that uptake mechanism occurring for plants in the atmosphere-plant pathways are highly governed by metal chemical form, redox state and maybe complexation, and can vary depending on abiotic conditions and human activities.

4.3 Isotopy and air biomonitoring: new insights for source tracing and mapping

The atmospheric fallouts from Owen gauges were collected after 6 months of deposit (from 05/2017 to 11/2017). We can then assume that they deliver an average value (without granulometric separation) of this period. In the same way, the values of Zn isotopes ratios for *T. usneoides* come from the plant samples collected in November 2017 and also exposed over 6 months.

Firstly, we can observe that Zn isotopes ratios of the soils from the Avenque main tailing are significantly more negative than those of the Zn primary ores (Sonke et al., 2008, Gelly et al., 2019). Due to the history of mining and the industrial processes operated in this site over more than 150 years, it is difficult to provide a direct explanation for this difference in isotope composition. Nevertheless, it is well known that high temperature metallurgical processes induce Zn isotope fractionation. Recent works included in the review of Yin et al. (2016) have shown that during these processes, an enrichment of atmospheric emissions in light isotopes can occur (-0.66 to 0.21 ‰), whereas smelting and slag residues can report heavier isotope signatures (0.13 to 1.49 ‰). The negative isotope values obtained for the 2 Avenque tailing soils and their very high concentrations in Zn suggest that these soils were certainly impacted by the fallouts coming from high temperature processes. This hypothesis is also supported by the recent work of Gelly et al. (2019) on soils sampled around the old smelter of Escalette (South-East, France). Topsoils around this site have negative signatures because of the impact of the smelter activity. They show values at -0.70 ± 0.04 ‰, whereas the geological background is about -0.15 ± 0.02 ‰.

Another important result is the similarity of Zn isotope compositions existing between atmospheric particles (-0.23 to -0.56 ‰) and *T. usneoides* (-0.25 to -0.50 ‰). Considering the analytical errors, we find that Pb isotope ratios are also quite similar for *T. usneoides* and atmospheric fallouts from Owen gauges (Table 2). Moreover, these observations are consistent with the literature data for ores from this Cartagena mining area (Stos-Gale et al., 1995, Rosman et al., 1997). Indeed, this result suggests that the epiphyte plants signature is certainly imposed by deposition of atmospheric particles on these plants and maybe their assimilation in plant tissues after soil erosion, mineral lixiviation and oxidation, dust mobilisation (due to wind) and then atmospheric deposition and contamination. It appears thus that at first order Zn (and Pb in a lesser extend) isotope signatures of *T. usneoides* are good tracers of the origin of the contamination. This observation provides then new insights using Zn isotope ratios for a better pollution distribution characterisation in various and contrasted sites and could also allow a better determination in priorities for mine rehabilitation (in terms of pollution dispersion and the risks involved for natural protected zones and obviously for human health, etc.).

Finally, to go deeper, some differences can be observed in Zn isotope compositions for the same exposure area between atmospheric particles and the associated epiphyte plants. Given our quite limited data, it seems premature to offer solid explanations for these differences. But some hypotheses can nevertheless be proposed: effect of particles granulometry, metal solubility and speciation, and also mechanisms of particle uptake within the *Tillandsia usneoides,* as previously suggested in the section 4.2 for Cu for example and already reported by Schreck et al. (2016).

5. Conclusion

Focusing on the area of the Cartagena-La Unión mining district, this study investigated the role of *T. usneoides* as a new and easy-to-perform tool to monitor TE contents in the air from environments influenced by anthropogenic activities.

Main results highlighted the influence of local and abiotic factors (TE contents and chemical forms with regards to the geochemical background of the region of Murcia, wind direction, anthropogenic activities in the vicinity) on kinetic of the atmospheric depositions at the leaf surface. Isotope ratios of Zn and Pb on epiphyte plants allow us to propose new insights for sources tracing and help, as well as magnetic susceptibility and statistical analyses, to better understand the TE uptake by plants through atmospheric pathways.

The chemical form of TE in the atmosphere seems to highly govern its uptake and bioaccumulation in plant tissues, certainly by acting on bioavailability properties. Then it may induce severe consequences on environment contamination but also risks for human health. Further studies on TE speciation and isotope composition in the different environmental compartments are in progress to better understand the biogeochemical transformations and the mechanisms occurring in mining environments and especially in the epiphyte plants phyllosphere.

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FIGURES

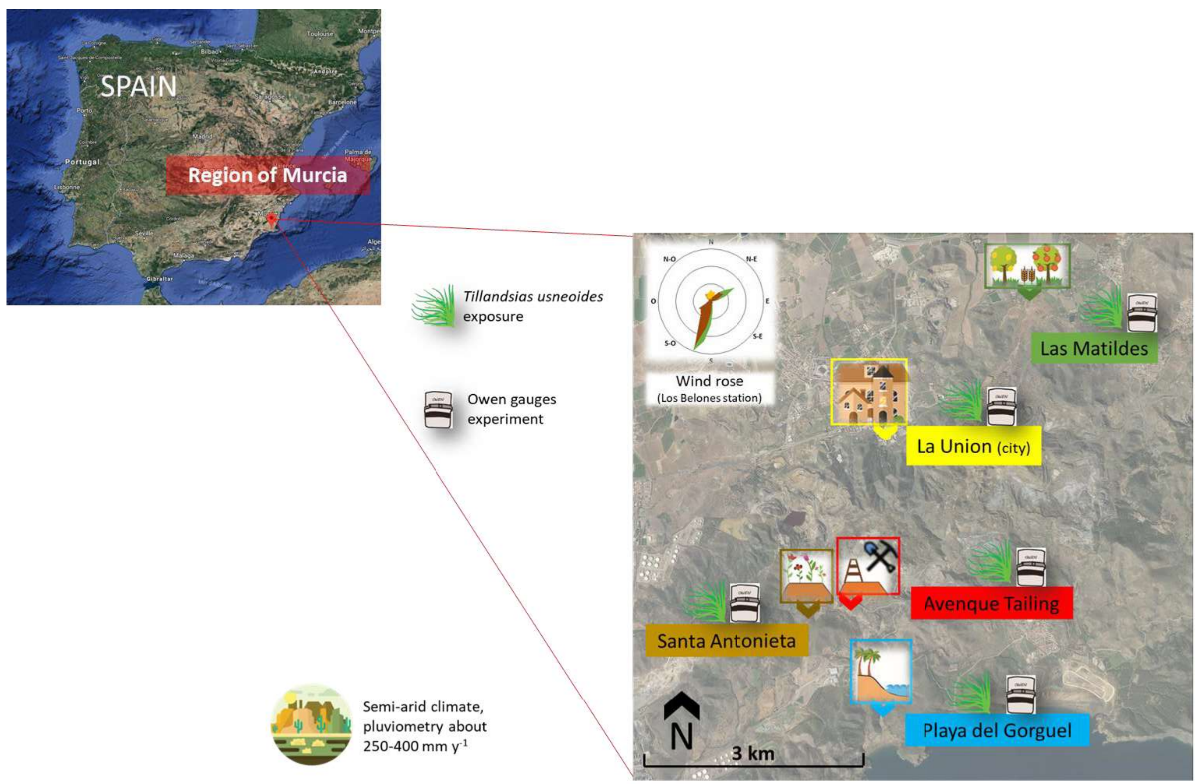

Figure 1. Air quality biomonitoring in 5 strategic sites of the mining district of Cartagena-La Unión (map from Google Earth, 2018)

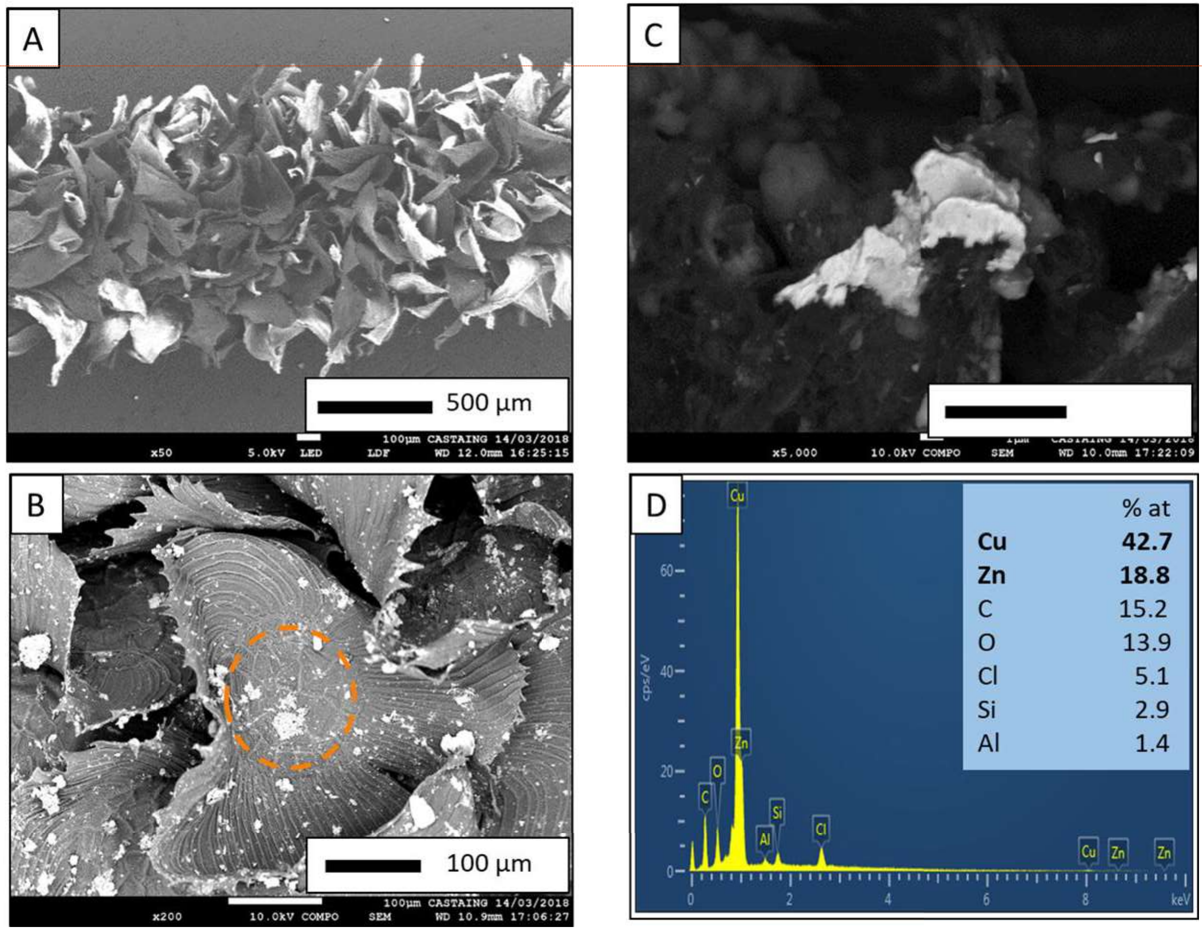

Figure 2. ***Tillandsia usneoides*** **from Avenque tailing area observed by SEM-EDX (after washing).**
A) General observation of the plant structure; B) Atmospheric deposits on ***T. usneoides*** **surface and stomata structure in the middle of a trichome shield (surrounded in orange); C&D) Atmospheric particle (enriched in Zn and Cu) observed in the plant surface and its EDX spectra.**

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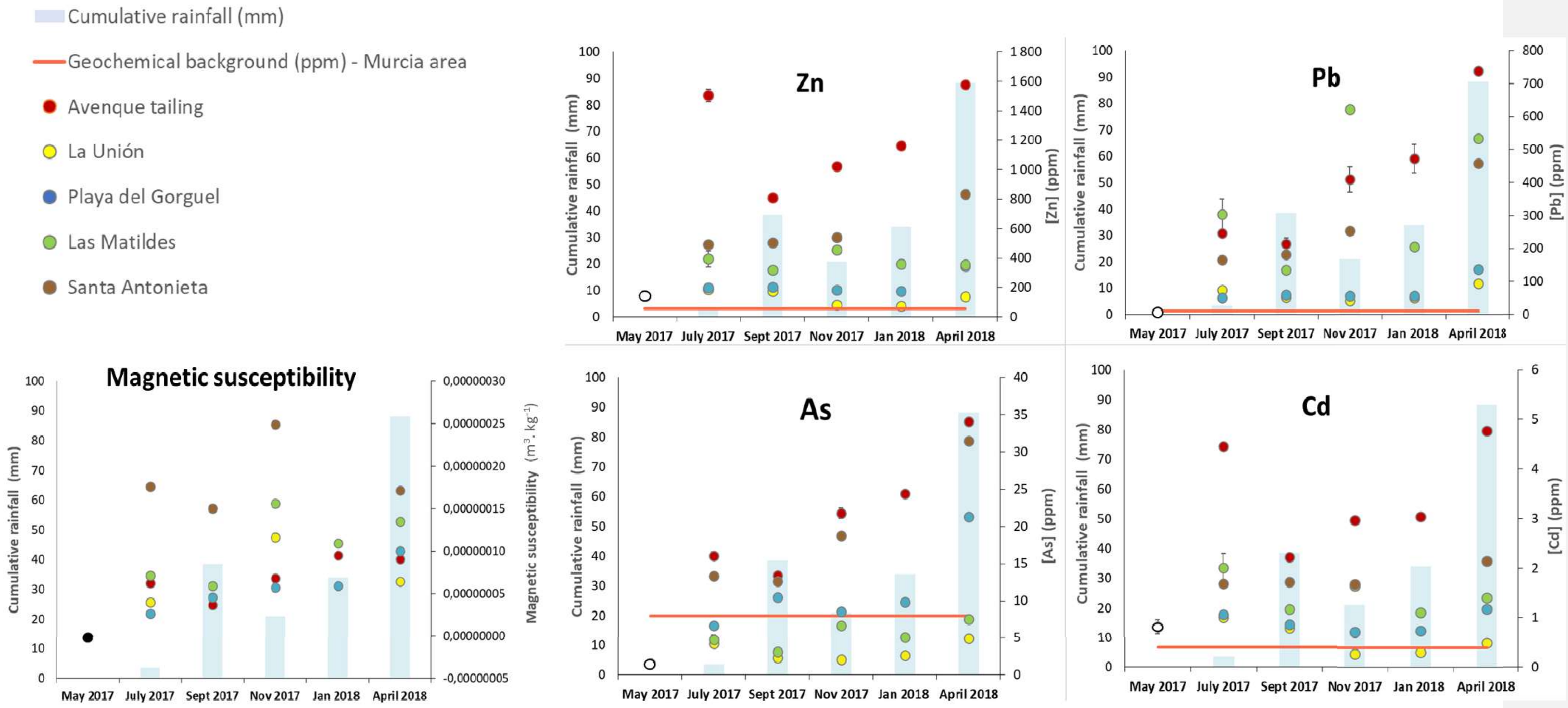

Figure 3. Element (Zn, Pb, As and Cd) contents (ppm) and magnetic susceptibility (m^3 kg^{-1}) of *T. usneoides* tissues for the five monitored sites and according to cumulative rainfall (mm) during the experiment (may 2017 -in black whatever the site- to April 2018). Geochemical background values are reported for each element (orange line) and come from *Martínez-Sánchez & Pérez-Sirvent (2007)* and *Alcolea (2015)*.

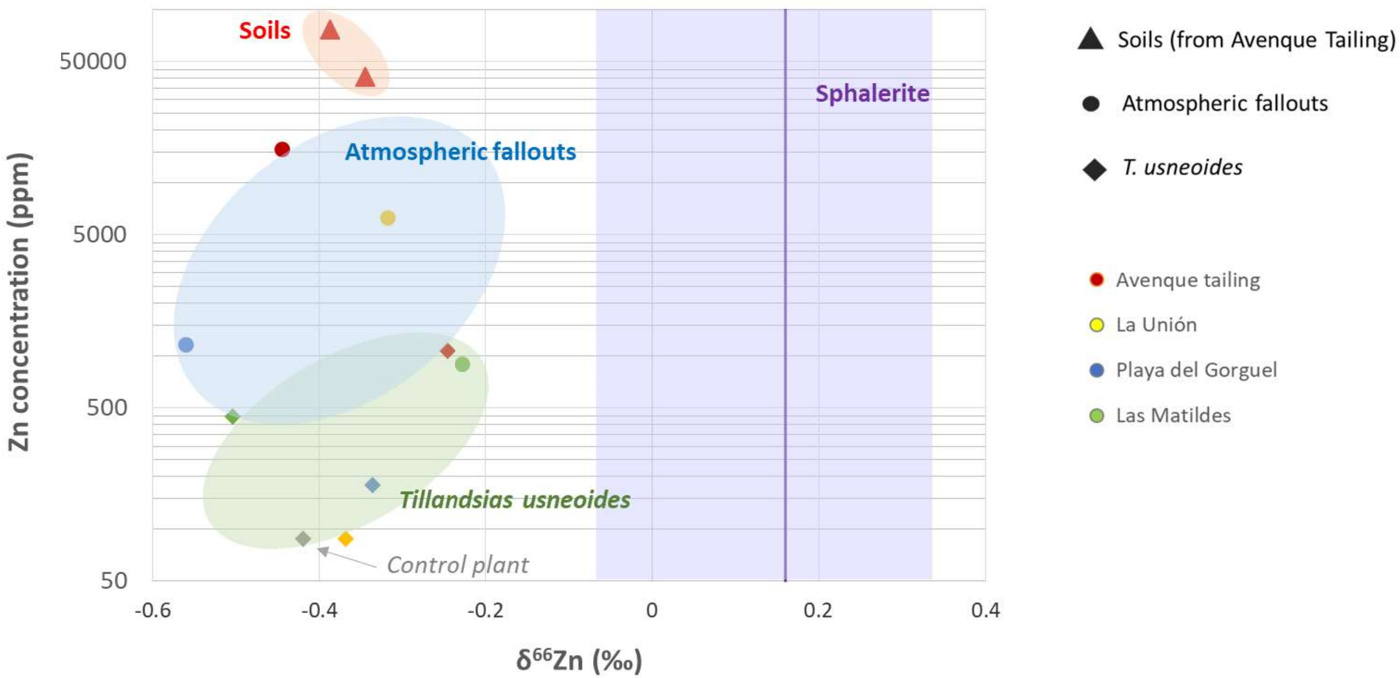

Figure 4. Isotope composition ($\delta^{66}Zn$) and Zn concentrations of Tillandsia usneoides, atmospheric fallouts and soils from the *Avenque Tailing*, compared to 29 sphalerites [(Zn, Fe)S] from 10 different mining sites from Sonke et al. (2008). The control epiphyte plant (from May 2017) is reported in grey in the diagram.

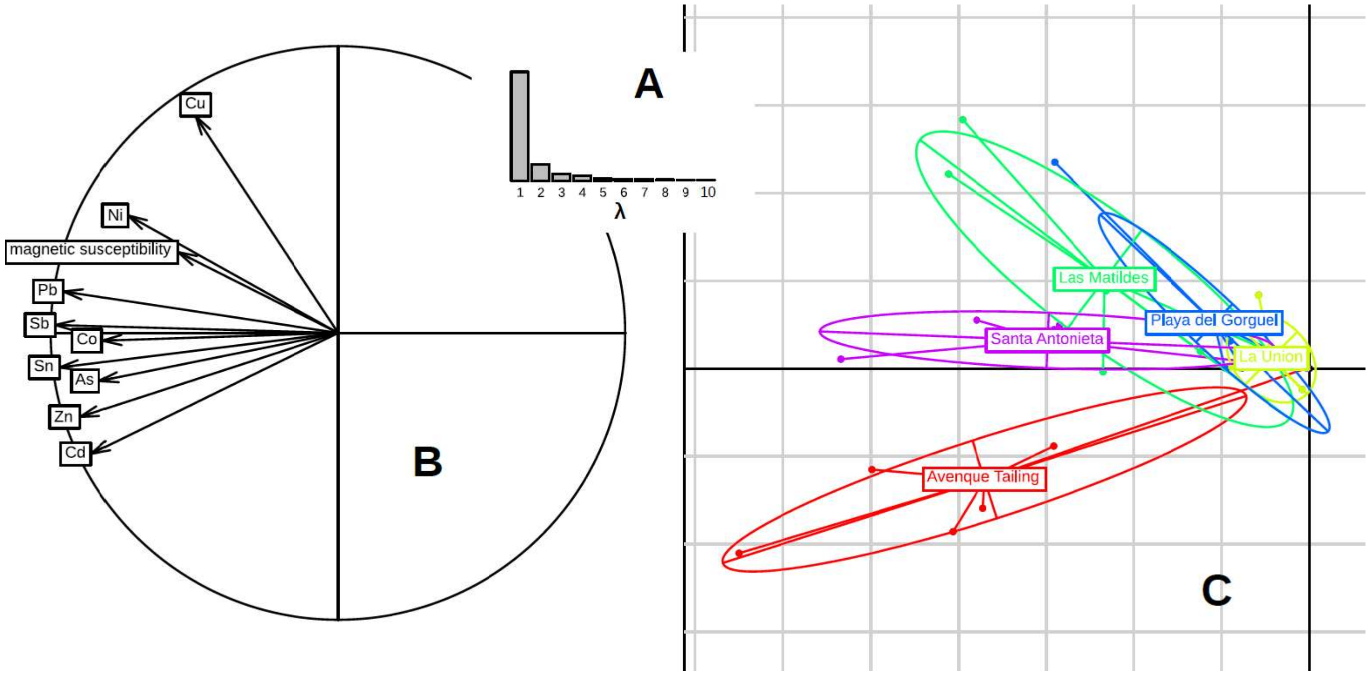

Figure 5. Decentred PCA.

A. Eigen values graph. B. Correlation circle. C. Samples factor map. Inertia ellipses group sample by sites.

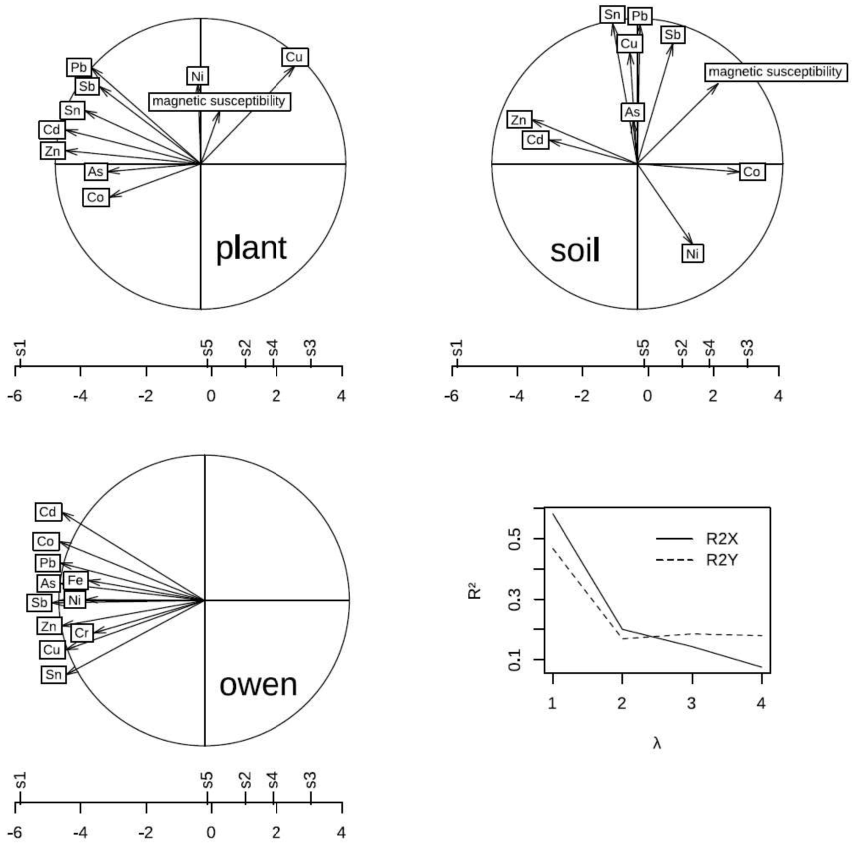

Figure 6. Graph of PLS2. A. Y, dependent variables corresponding to the concentrations measured in plants at the end of the experiment (April 2018). X, explanatory variables grouping the measurements carried out on OWEN gauges and on soil samples.

A. Circle of correlations between Y and scores of X for component 1 and 2.; B, C Circle of correlations between X and scores of X for component 1 and 2, dividing explanatory variables into two groups in different graphs B: OWEN gauge, C: soil samples. At the bottom of A,B,C are represented scores of X. S1, S2, S3, S4, S5 corresponding to Avenque Tailing, La Union, Playa del Gorguel, Las Maltildes, Santa Antonieta. D. R2X explains variance of X by score of X. R2Y explains variance of X by score of Y.

TABLES

Table 1. Trace element concentrations (expressed in ppm – and compared to geochemical background for the Region of Murcia) and magnetic susceptibility for the topsoils (0-20 cm) sampled in the different investigated areas (Avenque tailing, La Unión, Playa del Gorguel, Las Matildes, Santa Antonieta) from the mining district of Cartagena - La Unión, Spain.

Topsoil (0-20 cm)	Elemental contents (ppm)											Magnetic susceptibility
	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Sn	Sb	Pb	
Geochemical background (Martínez-Sánchez & Pérez-Sirvent, 2007)	24-45	n.d.	5-9	17-25	12-23	16-55	5-8	0.1-0.4	n.d.	0.5-1.6	3-10	n.d.
Avenque Tailing	17.79 ± 1.61	1798.13 ± 12.95	6.27 ± 0.23	15.50 ± 1.45	180.70 ± 3.68	10345.08 ± 336.68	973.72 ± 36.79	18.74 ± 6.22	30.07 ± 7.51	120.61 ± 8.59	14770.39 ± 659.23	5.4283E-06
La Union	18.75 ± 1.33	2133.12 ± 11.62	8.21 ± 0.56	17.12 ± 1.63	125.13 ± 2.99	2833.26 ± 39.69	199.16 ± 27.45	4.65 ± 1.80	17.32 ± 3.66	44.89 ± 1.26	857.63 ± 99.24	n.d.
Playa del Gorguel	18.77 ± 0.07	2434.10 ± 1.78	9.01 ± 2.01	18.65 ± 2.02	146.12 ± 3.02	5835.99 ± 83.56	1389.86 ± 98.65	13.61 ± 1.81	23.73 ± 5.27	175.10 ± 11.81	7066.40 ± 263.41	1.6519E-05
Las Matildes	25.14 ± 1.99	3742.21 ± 21.64	7.64 ± 1.64	14.33 ± 0.12	232.78 ± 4.65	4352.43 ± 15.23	569.20 ± 36.71	6.98 ± 0.36	41.40 ± 6.89	196.72 ± 13.96	36019.35 ± 801.12	4.1046E-05
Santa Antonieta	33.39 ± 1.98	2160.76 ± 9.59	5.80 ± 1.12	15.13 ± 0.98	49.28 ± 2.51	2641.08 ± 55.26	257.33 ± 29.86	5.81 ± 1.29	18.10 ± 2.41	45.80 ± 3.32	1028.05 ± 52.86	2.4623E-05

Notes: n.d.= not determined; Soil geochemical background was determined by the "Grupo de Investigación en Contaminación de Suelos. Universidad de Murcia" and reported in Martínez-Sánchez and Pérez-Sirvent (2007).

Table 2. Zn and Pb isotope compositions ($\delta^{66}Zn$ (‰), $\delta^{68}Zn$ (‰), $^{208}Pb/^{206}Pb$, $^{206}Pb/^{207}Pb$) measured in the epiphyte plants, soils and atmospheric fallouts from the mining district of Cartagena - La Unión, Spain.

		$\delta^{66}Zn$ (‰)	$\delta^{68}Zn$ (‰)	$^{208}Pb/^{206}Pb$	$^{206}Pb/^{207}Pb$
Tillandsia usneoides	Control (May 2017)	-0.42	-0.76	2.091	1.186
	Avenque Tailing (November 2017)	-0.25	-0.55	2.085	1.190
	Avenque Tailing (January 2018)	-0.21	-0.39	2.085	1.189
	La Unión (November 2017)	-0.37	-0.64	2.086	1.188
	Playa del Gorguel (November 2017)	-0.34	-0.75	2.086	1.190
	Las Matildes (November 2017)	-0.50	-0.97	2.088	1.187
Soil (from Avenque Tailing)	Soil 1	-0.39	-0.69	2.088	1.190
	Soil 2	-0.35	-0.67	2.087	1.190
Atmospheric fallouts (from Owen gauge, collected in November 2017)	Avenque Tailing	-0.44	-0.82	2.084	1.192
	La Unión	-0.32	-0.64	2.088	1.187
	Playa del Gorguel	-0.56	-1.02	2.084	1.191
	Las Matildes	-0.23	-0.39	2.085	1.189