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Free Zn²⁺ determination in natural freshwaters of the Pyrenees: towards on-site measurements with AGNES

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Environmental context. Knowledge of the speciation of metals, especially of the free metal ion concentration, is essential to understand the fate of these elements in rivers and their effects on living organisms. On-site analyses are preferred for these measurements as they allow problems associated with sample transportation and preservation to be avoided. In this context, an on-site methodology, based on an electrochemical method and screen printed sensors has been developed in the laboratory and validated on-site.

Abstract.

An on-site methodology has been developed for the direct determination of free Zn²⁺ with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) in freshwaters. This implementation includes: i) the use of screen printed electrodes, which provide good limits of detection and easy transportation and deployment; ii) no need for sample purging; iii) a calibration in a synthetic river solution that reproduces well the speciation changes of the natural samples and allows the oxygen interferences to be minimised; iv) the addition of a background electrolyte up to 0.01 mol L⁻¹ in both the calibration and freshwater samples; v) chemical stripping chronopotentiometry as the quantification stage of AGNES. This procedure minimizes the effects of working at low ionic strength and in the presence of dissolved oxygen.

In the laboratory, the methodology was checked with different natural samples taken from rivers Garonne, Gave de Cauterets and Gave de Pau in the Pyrenees. Results appeared in good agreement with theoretical estimations computed from Visual Minteq. On-site measurements were performed for the first time with AGNES in the Gave de Cauterets in Soulom (France) and the results were corroborated with purged measurements performed in the laboratory.

Additional keywords: FIAM, geochemical model, heavy metals, screen-printed electrodes, speciation

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Introduction

The free Zn^{2+} ion concentration in environmental aqueous systems is an important factor in determining Zn deficiency or toxicity to organisms as the element in this form is directly bioavailable. Many analytical techniques like ion exchange methods^[1], Donnan Membrane Technique (DMT)^[2, 3] or Permeation Liquid Membrane (PLM)^[4] have been developed to determine the free metal ion concentrations in natural samples. These methods agree well with speciation models (WHAM or MINTEQ),^[5-8] but require complementary equipment which cannot be moved into the field for on-site measurements. Commercial ion selective electrodes (ISE) are of particular relevance for trace metal studies,^[9, 10] but their commercial versions lack the required sensitivity and cannot measure Zn.

During the last few years, an electroanalytical technique called Absence of Gradients and Nernstian Equilibrium Stripping (AGNES)^[11] has been developed to determine free Zn^{2+} concentration^[12-15]. Typically, it has been implemented with the hanging mercury drop electrode (HMDE) and has been used, amongst other applications, to determine free Zn^{2+} or Pb^{2+} concentrations in sea^[13, 16] and river^[14, 15] waters.

Recent developments of AGNES^[17-19] are making the possibility of on-site measurements realistic which would avoid numerous problems that can potentially occur during sampling and sample handling of natural solutions (contamination, changes in pH and temperature, loss by adsorption, etc). Obviously, the HMDE is poorly adapted for on-site studies. Consequently, the application of AGNES has been undertaken with thin film mercury screen-printed electrodes (SPE) which represent an ideal alternative to the use of the HMDE.^[19, 20] These sensors have a very small size (9.6 mm²) and use a thin mercury film thickness (200 nm), which make them easy to deploy on-site. Unlike the HMDE, SPEs do not need any N_2 pressure and, thus, the transport of N_2 bottles to the field, which could be very problematic, is avoided. A recent work has demonstrated the feasibility of measuring free Zn^{2+} and Cd^{2+} in non-deaerated synthetic solutions provided that the changes in speciation, due to the reduction of the dissolved oxygen, is similar in the calibration and sample solutions^[17]. Another challenge presented by on-site analysis is measurements made in freshwaters of low ionic strength. The analytical signal obtained using AGNES is affected by low ionic strength media^[18]. Finally, the last variant of AGNES, called AGNES-SCP, where the reoxidation stage consists of stripping chronopotentiometry (SCP) (also called potentiometric stripping analysis (PSA)), has shown an important improvement as metal interferences can be avoided^[19, 20].

The objective of this work was to determine free Zn^{2+} concentration in natural freshwaters from some rivers of the Pyrenees (Garonne, Gave de Cauterets and Gave de Pau) by developing a new analytical strategy using AGNES-SCP.

In this work, two aspects limiting the on-site applicability of electrochemical techniques to freshwater samples are tackled: the low ionic strength of the media, and the presence of dissolved oxygen. For the first time using AGNES, the developed methodology has been applied on-site in a highly contaminated natural freshwater sample of the Gave de Cauterets.

This strategy has been validated by comparing results with measurements performed on the same samples by applying the purged classical AGNES methodology in the laboratory. The

laboratory results have been corroborated with theoretical estimations carried out with Visual Minteq modelling software^[21].

Experimental

Reagents

Zn 1000 mg L⁻¹ stock solution was obtained from Merck. KNO₃ (Trace Select), HNO₃ (69-70%, Baker Instra-Analysed for trace metal analysis), NaOH (Baker Analysed), HCl (Baker Instra-Analysed for trace metal analysis) were purchased from Sigma Aldrich. Ultrapure milli-Q water (resistivity 18.2 MΩ cm) was employed in all the experiments. Purified water-saturated nitrogen N₂ (50) was used for deaeration and blanketing of solutions.

Synthetic river water was prepared from sodium hydrogen carbonate (Scharlau), magnesium sulphate (Merck, Pro Analysis), calcium chloride (Merck, Pro analysis) and potassium nitrate (Sigma Aldrich, Trace Select). A stock solution of synthetic river water was prepared as follows: 10⁻² mol L⁻¹ NaHCO₃, 10⁻³ mol L⁻¹ MgSO₄·7H₂O, 2×10⁻³ mol L⁻¹ CaCl₂·2H₂O and 5×10⁻⁴ mol L⁻¹ KNO₃, which corresponds to an ionic strength of 0.0192 mol L⁻¹. For each experiment with synthetic river water, the stock solution was diluted to obtain a solution having a composition close to the river sample (**Erreur ! Source du renvoi introuvable.**). To avoid voltammetric problems derived from the low ionic strengths of the samples^[18], the solutions (both synthetic and freshwater) were spiked with KNO₃ up to 0.01 mol L⁻¹, to give a conductivity close to 1500 μS cm⁻¹. At this ionic strength, no problems derived from low supporting electrolyte are expected. Using Visual Minteq, it has been observed that this addition does not alter the speciation of the river samples. The pH was adjusted with 0.01 mol L⁻¹ NaOH or 0.01 mol L⁻¹ HNO₃ to reach the pH of the natural river sample.

Water samples were filtered on-site with 0.45 μm acetate cellulose filters by using Nalgene polysulfone, a reusable complete filter unit (from VWR) and a Mityvac hand vacuum pump (model MV8010).

Equipment

Major cation concentrations were obtained with an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Horiba Jobin Yvon, Activa-M). Trace elements were obtained with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7500ce). Major anions were measured with an ion chromatograph (Dionex DX-120). Dissolved organic carbon (DOC) was obtained with a Total Organic Carbon Analyzer (Shimadzu TOC-V CSN). Dissolved oxygen was measured with a multi-parameter analyser Consort C535. Temperature, pH and conductivity were measured with a multi-parameter analyser WTW 340i.

Voltammetric measurements were performed with an Eco Chemie μ-Autolab III potentiostat attached to a Metrohm 663 VA Stand and to a computer by means of the GPES 4.9 (Eco Chemie) software package. The auxiliary electrode was a glassy carbon electrode. The reference electrode was a DRIFEF-5 (3 mol L⁻¹ KCl) purchased from World Precision Instrument. All potentials were given versus this reference electrode. Glass jacketed cells, provided by Metrohm, were used to maintain the temperature constant (generally at 20°C).

SPE were prepared using a commercial ink electrodag PF-407A (Acheson Colloids). The electrodes were screen-printed on 1 mm-thick polystyrene support for serigraphy (Sericol) (Figure 1). After a drying step (1 hour at room temperature) and a curing step (1 hour at 60°C), an insulating layer was spread manually over the conductive track, leaving a working disk area of 9.6 mm²[22]. A thin layer of mercury was deposited onto the electrode surface for trace metal detection. The mercury deposition on the SPE was carried out using an acetate buffer solution (0.2 mol L⁻¹, pH = 4.6) prepared from acetic acid (Trace select), sodium acetate trihydrate (Trace select) and 0.83 mmol L⁻¹ Hg(NO₃)₂ (all obtained from J.T. Baker). First, the working surface of the electrode was conditioned by applying 4 cycles of cyclic voltammetry (CV) using the following conditions: potential range from -0.1 V to +0.8 V, scan rate 100 mV/s, step potential 2.4 mV. The Hg film was then deposited at -1.0 V, with stirring until the charge associated with the deposited mercury (Q_{Hg}) reached 25 mC.

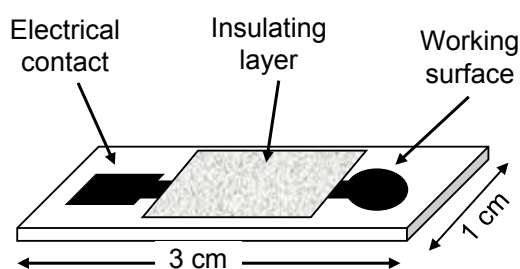


Figure 1. Scheme of a screen-printed electrode

Sampling and on-site analyses

Two rivers in the Pyrenees have been studied in this work (Figure 2): the Garonne river, which flows in Spain downstream from a Zn mining area and enters into France, and the Gave de Pau which is supplied by a stream, the Gave de Cauterets, also located downstream from a Zn mining area, and continues until Pau (France). The sampling campaigns were carried out on two different dates: April 2012 with a high water level in the Gave de Pau (due to the plentiful rainfalls during the month of April^[23]) and in May 2012 with a low water level.

At all sampling places, temperature, pH, conductivity and dissolved oxygen were measured directly in the river. 250 mL of filtered water samples have been acidified with HNO₃ at 2% and stored at 4°C in new polystyrene bottles to measure the concentrations of major cations and trace metals. 250 mL of filtered water sample has been kept without acidification for carbon analysis, anion analysis and AGNES experiments.

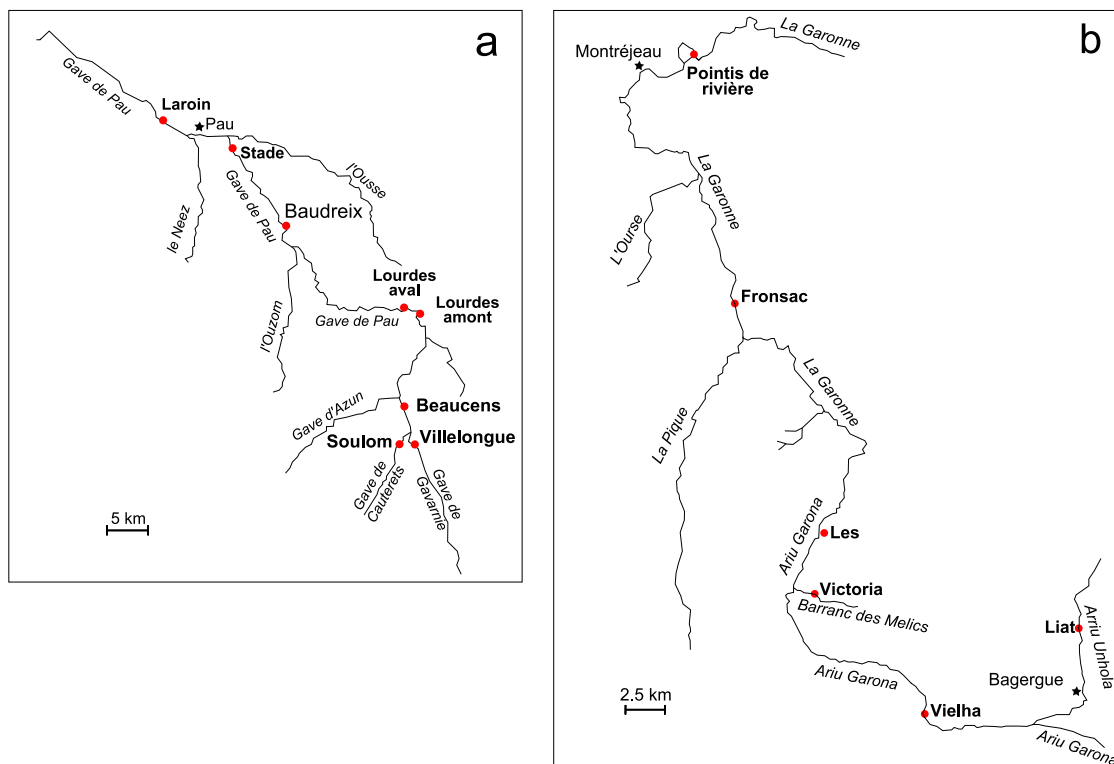


Figure 2. Map of the studied area: a. Gave de Cauterets and Gave de Pau and b. Garonne river. Sampling sites are indicated with a red circle (●).

Free Zn²⁺ determination

The determination of the free Zn²⁺ using AGNES-SCP procedure^[19] consists of two conceptual stages. Along the first (deposition) stage, the metal ion Zn²⁺ from the solution is reduced to Zn⁰ until attainment of a special situation of Nernstian equilibrium and absence of gradients in the concentration profiles^[11]. This equilibrium is achieved by applying a potential E_1 more negative than the standard formal potential of the couple Zn²⁺/Zn⁰, E^0 , for a sufficiently long time t_1 . These conditions imply that identical analytical signals (in the second stage) are obtained for deposition times longer than a certain minimum t_1 .

The Nernst equation allows the concentration ratio at each side of the mercury electrode, also called gain Y , to be calculated as (Eq. 1):

$$Y = \frac{[M^0]}{[M^{n+}]} = \exp\left[\frac{-nF}{RT}(E_1 - E^0)\right] \quad \text{Eq. 1}$$

where n is the number of exchanged electrons, F is the faraday constant, R the gas constant and T the temperature.

The deposition potential E_1 corresponding to a given gain Y can be determined from the peak potential of a differential pulse polarogram (DPP)^[11]. The higher the gain Y , the higher the sensitivity of the AGNES measurement. However, higher gains will require longer

electrodeposition times to reach Nernstian equilibrium. Consequently, the chosen deposition potential will correspond to the lowest gain that allows obtainment of a well-shaped peak. The goal of the second stage of AGNES-SCP is to measure the concentration of the reduced metal M^0 inside the mercury amalgam, e.g. by applying a constant oxidizing stripping current, generally between 1 and 10 μA ^[19]. The analytical signal is the time taken for the analyte reoxidation (transition time, τ). SCP conditions of full depletion allow measuring the total faradaic charge Q which is proportional to the free metal ion concentration $[M^{2+}]$ according to Eq. 2.

$$Q = nFV_{\text{Hg}} [M^0] = nFV_{\text{Hg}} Y [M^{2+}] = h_Q [M^{2+}] \quad \text{Eq. 2}$$

where V_{Hg} is the volume of deposited mercury and h_Q is the slope of the calibration plots.

Speciation models

The geochemical modelling program Visual MINTEQ, version 3.0^[21] (using the standard databases) was used to predict metal speciation in the investigated rivers. The following inputs were provided: pH, temperature, and total concentrations for Ca^{2+} , Na^+ , K^+ , Mg^{2+} , Mn^{2+} , NO_3^- , SO_4^{2-} , Cl^- , CO_3^{2-} , total Zn and DOC. The NICA-Donnan model was used to describe the formation of metal-DOM (dissolved organic matter) complexes. It was assumed that the ratio of active DOM to DOC (dissolved organic C) was 1.65 and that 100% of the active DOM was fulvic acids (FA)^[24].

Results and discussion

General physicochemical analyses of river waters

As shown in Table 1, physicochemical properties show little difference between both rivers. The pH of the samples were between 7.25 and 7.77 in the Garonne, with a slight increase observed from upstream in the Gave de Pau (7.35 in Soulom) to downstream (8.16 in Laroïn). Dissolved oxygen does not show strong variations with an average concentration of 13 mg L^{-1} , except in Pointis de Rivière in the Garonne (6.25 mg L^{-1}). This low value can be explained by the presence of stagnant water. Conductivity recorded at the different places appeared relatively low with values between 78 and 226 $\mu\text{S cm}^{-1}$. An increase is observed from upstream to downstream related to the simultaneous increase of the concentrations of cations (Ca^{2+} , K^+ , Na^+ , Mg^{2+}) and anions (Cl^- , NO_3^- , SO_4^{2-}). The DOC concentrations vary from 0.7 to 3.7 mg L^{-1} except in Baudreix where the value is higher (6.8 mg L^{-1}), probably due to human activity in the leisure area.

Table 1. Geochemical characteristics of rivers Garonne and Gave de Pau

	pH	T °C	Conductivity $\mu S cm^{-1}$	O ₂	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	CO ₃ ²⁻	DOC	Ca ²⁺	K ⁺	Na ⁺	Mg ²⁺	
	$mg L^{-1}$													
Garonne	Liat	7.25	8.6	111	12.05	0.42	0.86	9.05	45.1	2	21.44	0.14	0.63	0.21
	Vielha	7.77	11	142	13.15	nd*	nd*	nd*	nd*	nd*	23.93	0.44	1.18	2.48
	Victoria	7.68	12	226	11.1	1.2	1.08	40.82	74.1	2.7	39.61	1.15	1.96	1.83
	Les	7.44	10	116	12.85	2.24	0.91	6.86	46.2	2.4	19.94	0.3	1.73	1.58
	Fronsac	7.51	12	122	11.65	2.09	1.21	7.95	47.5	3	20.49	0.4	0.95	1.63
	Pointis de rivière	7.55	14	136	6.25	2.15	1.46	8.83	55.5	2.5	23.3	0.42	1.17	1.57
Gave de Pau	Soulom	7.35	8.8	78	14.15	0.67	1.78	4.95	29.2	3.7	14.05	0.19	0.26	0.72
	Villelongue	7.66	8	125	13.35	0.65	1.15	6.82	56.6	0.7	23.1	0.22	0.97	0.69
	Beaucens	7.66	9.1	125	13.4	0.72	1.38	6.78	57.2	2	23.07	0.25	0.9	0.74
	Lourdes amont	7.67	11	137	13.65	1.06	1.58	7.68	61.9	3.2	25.06	0.26	1.16	0.85
	Lourdes aval	7.69	11	142	12.5	1.1	1.45	7.51	66.6	2.9	27.13	0.27	1.18	0.94
	Baudreix	8.08	12	152	12.9	1.32	1.65	8.31	69.5	6.8	27.39	0.31	1.65	1.07
	Stade	8.05	14	166	13.25	1.67	2.35	8.57	77.2	2.8	29.68	0.38	1.83	1.24
	Laroin	8.16	14	185	12.5	2.55	2.84	8.8	87.1	1	34.11	0.53	1.95	1.76

* not determined

Table 2 presents the total dissolved trace metal concentrations measured in acidified samples from both rivers. Total dissolved Pb and Cd concentrations are lower than $0.1 \mu g L^{-1}$ except for Cd in Victoria, which is the most contaminated site. Total dissolved Cu concentrations are relatively low at all sampling sites, without obvious impact from human activity, suggesting these values correspond to the natural geochemical concentrations. Regarding Zn, the highest total dissolved concentrations are observed in places near a mining area: Victoria ($549 \mu g L^{-1}$) and Liat ($73 \mu g L^{-1}$) in the Garonne, and Soulom ($136 \mu g L^{-1}$) in the Gave de Pau. In both rivers, a dilution phenomenon is clearly observed downstream from the mines due to many tributaries. Stable values around $5 \mu g L^{-1}$ are reached in the most downstream places, probably close to the geochemical background concentration.

Table 2. Total dissolved metal, and free metal ion analysis results of rivers Garonne and Gave de Pau

	Total Cd	Total Pb	Total Cu	Total Zn	Free Zn	
	$\mu g L^{-1}$					
Garonne	Liat	LOD*	LOD*	0.24 ± 0.01	73.0 ± 0.3	66
	Vielha	LOD*	LOD*	0.59 ± 0.02	13.4 ± 0.3	nd**
	Victoria	1.43 ± 0.02	LOD*	0.49 ± 0.01	549 ± 2	451
	Les	LOD*	LOD*	0.20 ± 0.02	8.7 ± 0.1	8.1
	Fronsac	LOD*	LOD*	0.18 ± 0.03	4.5 ± 0.1	3.5
	Pointis de rivière	LOD*	LOD*	0.19 ± 0.02	2.3 ± 0.1	2
Gave de Pau	Soulom	LOD*	LOD*	0.25 ± 0.03	136 ± 3	136
	Villelongue	LOD*	LOD*	0.23 ± 0.02	LOD*	LOD*
	Beaucens	LOD*	LOD*	0.07 ± 0.03	14.1 ± 0.4	11.7
	Lourdes amont	LOD*	LOD*	0.08 ± 0.02	10.1 ± 0.6	9.4
	Lourdes aval	LOD*	LOD*	0.21 ± 0.01	9.5 ± 0.5	7.2
	Baudreix	LOD*	LOD*	0.15 ± 0.02	6.8 ± 0.6	5
	Stade Eaux Vives	LOD*	LOD*	0.29 ± 0.01	5.7 ± 0.6	4.1
	Laroin	LOD*	LOD*	0.27 ± 0.01	4.1 ± 0.7	3.6

* Limit of detection; ** not determined

Optimization of AGNES-SCP experimental conditions for on-site analyses

Since total Cd and Pb concentrations were found to be lower than the LOD, only free Zn^{2+} was determined. The optimization of the technique was performed in the laboratory with synthetic river water samples before being applied to natural samples in the laboratory and on-site.

Low ionic strength effects on AGNES

The low ionic strength reflected by the conductivity values found in the river (between 78 and 226 $\mu S\ cm^{-1}$) can be problematic when working with electrochemical techniques. As shown in Figure 3, for a common fixed deposition potential ($E_1 = -1.08V$) applied to the purged solution, the analytical signal decreases when increasing the sample conductivity. This can be rationalized from Nernst equation, eqn. 1 written^[18] as:

$$[Zn^0] = \gamma_{Zn^{2+}} [Zn^{2+}] \exp\left[\frac{-nF}{RT} (E_1 - E^0)\right] \quad \text{Eq. 3}$$

where E^0 is the standard redox potential (which is constant at a given temperature). When the ionic strength increases, the activity coefficient decreases, so the number of accumulated moles of Zn^0 and the analytical signal decrease.

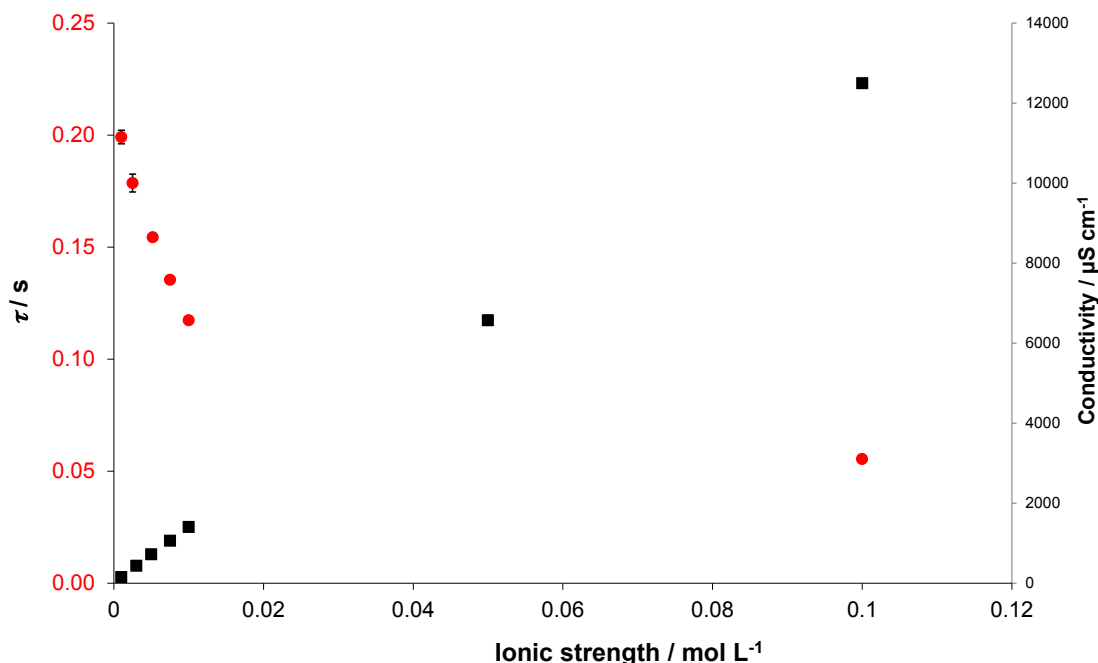


Figure 3. Zn analytical signals obtained in AGNES-SCP at different ionic strengths with purging (red bullets referred to left axis) for a total Zn concentration of $5 \times 10^{-7} \text{ mol L}^{-1}$. Black squares stand for conductivity (referred to right axis). Deposition time between 300 and 800s at $-1.08V$, Stripping current $I_s = 10\mu A$. Error bars of the 2 replicates indicated when larger than the marker symbol.

The analysis of low ionic strength by electrochemical techniques can be hindered by the ohmic drop in the solution. At high ionic strength, the application of a potential difference between the electrodes results in this potential drop being spatially localized between the working electrode and the adjacent solution. At low ionic strength, the applied potential accounts for the drop of potential at the electrode interface plus the ohmic drop across the solution due to the non-negligible resistance of the solution. This impacts most electroanalytical techniques, because the potential drop at the electrode interface does not coincide with the nominal applied potential^[18]. Notice, however, that the ohmic drop at the end of the deposition stage of AGNES in purged solutions is negligible due to the low current at equilibrium (arising from the traces of oxygen), but can be important in non-deaerated solutions.

The impact of changing ionic strength (due to changes in the activity coefficients) is pronounced for a KNO_3 concentration lower than $10^{-2} \text{ mol L}^{-1}$ which corresponds to a conductivity lower than $1200 \mu\text{S cm}^{-1}$. This means that AGNES-SCP will be particularly sensitive in the river samples where the conductivity measured in the rivers is around $100 \mu\text{S cm}^{-1}$ (because the ionic strength is not accurately known). For this reason, the conductivity of the natural freshwater sample has been fixed to avoid voltammetric problems in AGNES-SCP derived from the low ionic strengths. Thus, both solutions, synthetic and natural freshwaters, were spiked with KNO_3 up to $10^{-2} \text{ mol L}^{-1}$ where no problems derived from low supporting electrolyte are expected. Simulations made with Visual Minteq and the estimated water composition have shown that the free Zn^{2+} concentration was not affected when adding this amount of KNO_3 (data not shown).

Oxygen influence during AGNES deposition step

In non-purged solutions, the pH at the surface of the electrode is higher than that of the bulk solution, due to the important reduction of O_2 ^[17]. The presence of oxygen hinders the standard AGNES application by preventing the achievement of absence of gradients in the solution concentration profiles by the end of the preconcentration step. Instead, a steady-state situation is reached where the metal concentration at the surface of the electrode (due to the speciation at a higher pH) is depleted with respect to the bulk concentration. Thus, the aimed preconcentration gain Y (referred to bulk concentrations) might not be achieved, but rather be far smaller^[17].

Additionally, the steady-state consumption of O_2 implies an increase of the residual current and a concomitant decrease of the actual applied potential due to ohmic drop. pH effects derived from the oxygen interference can be avoided with an effective buffer that compensates for the OH^- production at the electrode surface without any change in the metal speciation. Alternatively, calibration solutions in which the steady-state depletion of the metal is similar to the depletion of the samples may be used. The latter is the strategy followed in this work. A calibration has been performed in a synthetic river solution in which the chemical composition is close to the one of the river sample in terms of pH and geochemical composition. In this way, we ensure that changes in calibration and measuring samples due to the presence of oxygen are similar.

The determination of the required potential for a given gain has been obtained empirically searching for a sufficient analytical signal. Thus, using E_1 -values between -1.165 and -1.190 V, the gain was enough for a reliable measurement of Zn in Pyrenees river samples.

Oxygen influence during stripping step

In SCP, the analytical signal is the time taken for the analyte reoxidation (transition time, τ) while a constant oxidizing stripping current is applied^[25]. However, this current corresponds to the sum of the imposed stripping current (I_s) and the oxidants current (I_{ox}) due to oxidizing agents (e.g. oxygen) present in the solution^[17]. In classical analyses with purging, I_{ox} is negligible in front of the I_s (10 μ A) usually imposed with AGNES-SCP^[19]. In non-purged solutions, it has been shown that interferences due to oxygen during the stripping stage could be minimized by using specific stripping techniques such as square wave anodic stripping voltammetry (SWASV) with high frequency^[26] or stripping chronopotentiometry (SCP) with sufficiently high stripping currents^[19]. However, in AGNES-SCP, a large oxidizing current can induce a fast stripping step which can lead to a truncated signal. To avoid this, a chemical stripping (SCP_{chem}) was applied where the reoxidation is only performed through oxidants current I_{ox} (mainly due to oxygen) present in the solution without imposing any external current (i.e. $I_s=0$)^[27, 28]. The accumulated charge Q , in this case, can be computed as (Eq. 3):

$$Q = -I_{ox}\tau \quad \text{Eq. 4}$$

I_{ox} , obtained at the end of the deposition stage with the solution in quiescent conditions, appeared to be stable with time, indicating that a steady state for the entire system, including the transport of O_2 towards the electrode, was reached. It has been shown that either Q or τ were proportional to the free metal concentrations at the electrode surface^[20].

AGNES-SCP implementation in the present work

When working with non-deaerated solutions, the deposition potential needs to be very negative, because of the decrease in metal concentration at the electrode surface. This is due to the reaction of the free metal with the OH^- species formed from the O_2 reduction at the electrode surface^[17]. Using E_1 -values between -1.165 and -1.190 V, the gain was enough for a reliable measurement of the metal as explained below.

Chemical stripping has been used during the second stage of AGNES-SCP to obtain a well-shaped peak. In order to avoid confusion with classical SCP using a constant stripping current, this new method will be called AGNES-SCP_{chem}.

As discussed below, calibration of AGNES was performed using synthetic river waters whose characteristics were close to the ones of the Gave de Pau river samples in terms of pH and geochemical composition. In this way, we ensure that changes in calibration and measuring samples due to the presence of oxygen are similar. A typical calibration curve is presented in Figure 4.

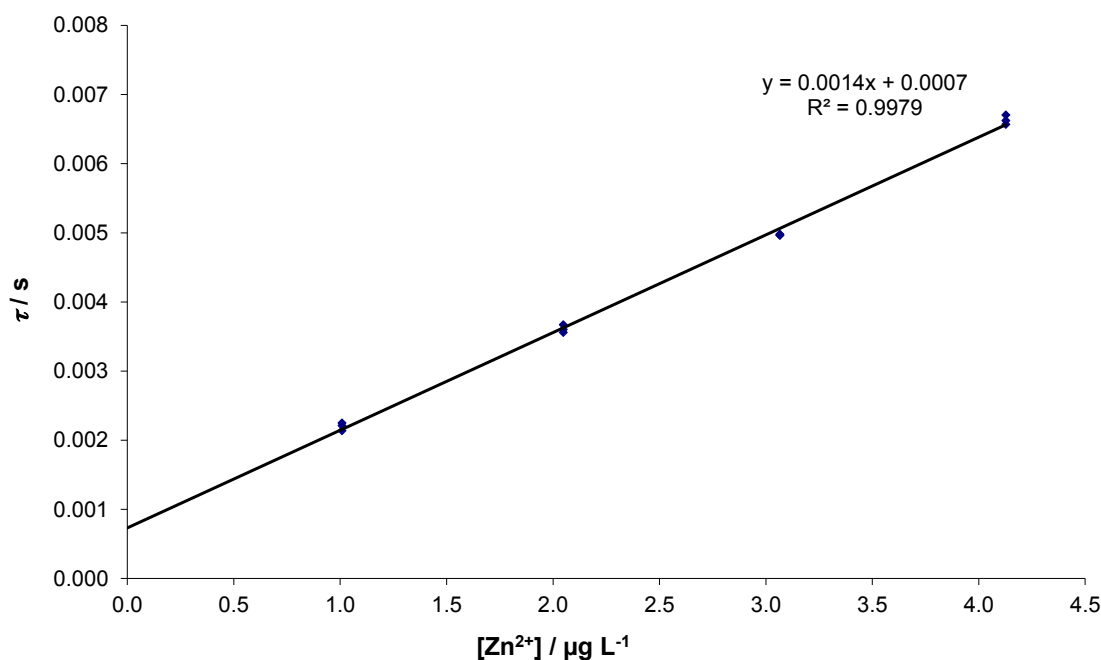


Figure 4. Calibration curve obtained with AGNES-SCP_{chem} in a synthetic river spiked with KNO₃ 0.01M, pH 8. Electrochemical conditions: $E_1 = -1.165V$, $t_1 = 400s$ with stirring, Chemical stripping without stirring.

Temperature effect on AGNES-SCP_{chem} method

The influence of temperature on AGNES-SCP_{chem} was evaluated in a non-purged solution (Figure 5). The analytical signal (τ) increased around 3.7 ms with each increase of 1 °C. This evolution results from: i) the decrease of the dissolved oxygen concentration when temperature increases (Table 1). Lower I_{Ox} values correspond to slower stripping processes, leading to longer τ , ii) the dependence of the $E^{0'}$ potential with temperature. For a fixed deposition potential, the increase in temperature in this system leads to higher accumulations and analytical signals as expected from the endothermic nature of Zn²⁺ reduction^[29]. So, the temperature has to be carefully controlled when performing AGNES with Zn. All the experiments in the laboratory were performed with a thermostated bath at 20 °C, except the on-site measurements and on-site calibrations, which were performed at the natural river temperature.

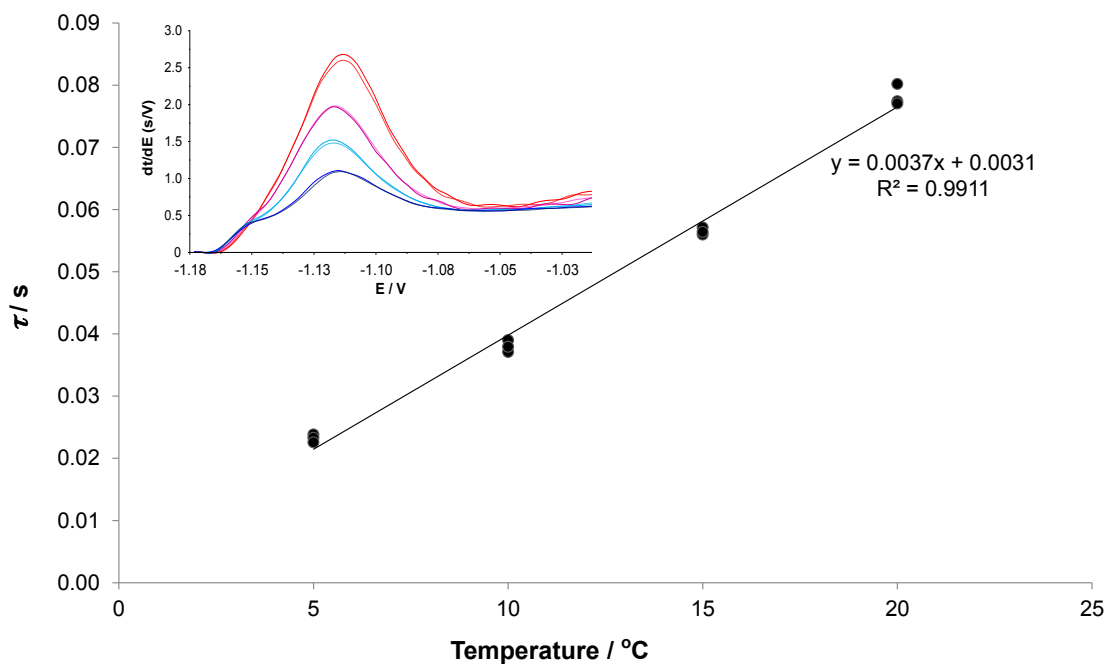


Figure 5. Variation of Zn analytical signals in a non-purged solution of KNO_3 0.01 mol L^{-1} with temperature. Inset: stripping signal at different temperatures: 5°C (—), 10°C (—), 15°C (—) and 20°C (—). Zn concentration $65 \mu\text{g L}^{-1}$, $E_1 = -1.175\text{V}$, $t_1 = 400$ and 500s , chemical stripping.

Interferences of Cu with Zn

In multicontaminated systems, a high concentration of metals can be accumulated in the mercury amalgam, so that intermetallic compounds may form^[30, 31]. This phenomenon arises when Cu and Zn concentrations are sufficiently high so that the solubility of the intermetallic compounds in mercury is exceeded. Figure 6 shows how the analytical signal was not affected by the presence of Cu in the solution up to a concentration of $2 \mu\text{g L}^{-1}$ (well above Cu concentration in the river samples).

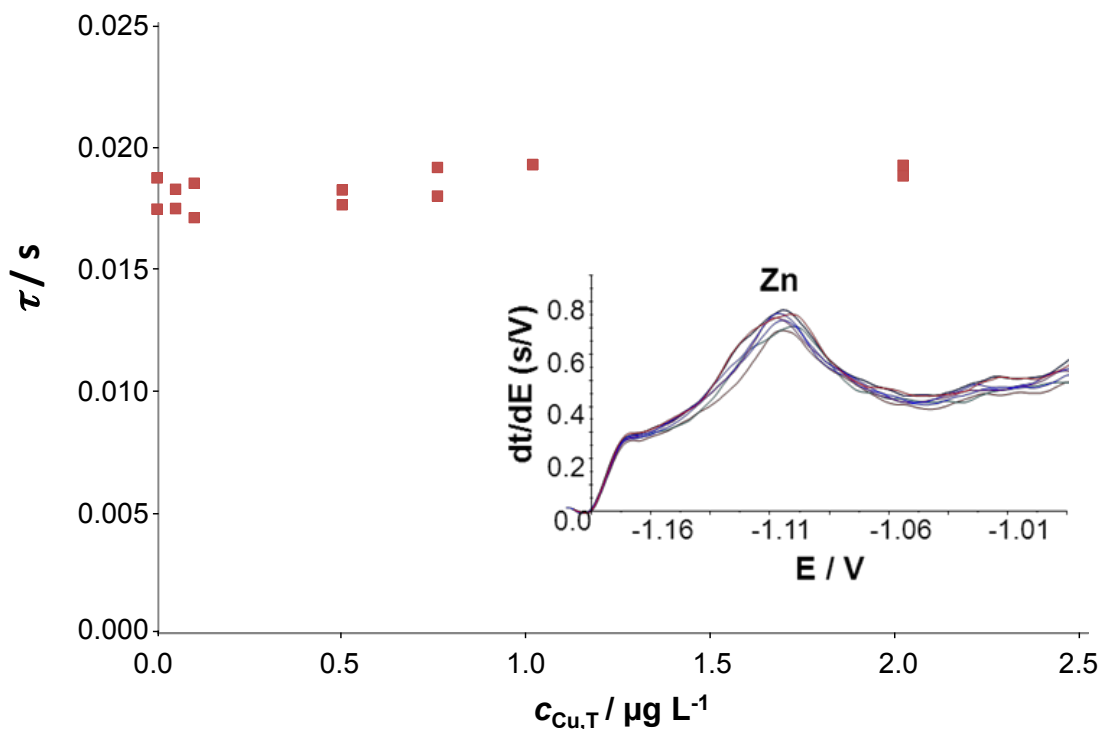


Figure 6. Zn analytical signals obtained with AGNES-SCP_{chem} in a synthetic river solution with 10 μg L⁻¹ total Zn concentration spiked with KNO₃ 0.01 mol L⁻¹ and increasing Cu concentrations from 0 to 2 μg L⁻¹. AGNES-SCP_{chem} conditions: $E_1 = -1.190$ V, $t_1 = 300$ s and 400 s, chemical stripping.

Limit of detection and limit of quantification

The limit of detection (LOD) has been statistically determined from the calibration curve obtained from 12 calibration points in a synthetic river solution at pH 7.9 with AGNES-SCP_{chem} without purging according to Eq. 4^[32]:

$$\text{LOD} = (b + k S_b) / m \quad \text{Eq. 5}$$

where $k = 3$, b is the intercept of the regression line, S_b is the intercept standard deviation of the regression line and m is the slope of the calibration graph. The quantification limit (LOQ) was calculated with the same equation as for the detection limit, but with $k = 10$.

For each Zn concentration, three replicates were performed at three different deposition times to be sure that AGNES conditions are fulfilled. Thus, by using a deposition potential of -1.165V, a LOD of 0.6 μg L⁻¹ and a LOQ of 0.9 μg L⁻¹ have been obtained. If necessary, more negative deposition potentials (which require longer deposition times) could be used to detect lower free Zn²⁺ concentrations.

In lab determination of free Zn²⁺ concentration in freshwaters

The free Zn²⁺ concentrations were determined in the laboratory in different samples taken from the Gave de Pau and Garonne rivers in May 2012 following the non-purged methodology previously described. Calibration was performed in a synthetic river water whose characteristics were close to the ones of the considered river sample^[17].

Table 2 shows the retrieved free Zn²⁺ concentrations applying $E_1 = -1.165$ V, $t_1 = 300-400$ s at 20°C. The proportion of free Zn²⁺ determined in the river is very high for all the locations, ranging from 72 to 100 %, in comparison to previous studies carried out with river waters^[15, 33, 34]. These results are consistent with the low levels of dissolved organic matter found (around 3 mg L⁻¹) and low pH (pH < 8). Calculations of metal speciation show that as pH and carbonates increase, the concentrations of free ions decrease because of the increased complexation with carbonate species and fulvic acids^[35].

Using the geochemical data measured for each sample, an estimation of the free concentration was performed with Visual Minteq. Figure 7 shows that a good correlation is observed between the experimental results obtained with AGNES-SCP_{chem} and the ones computed from Visual Minteq which validates the AGNES-SCP_{chem} method applied in natural water sample without purging. These results confirm the absence of Cu interferences (consistent with the low Cu concentration, Table 2) and agree well with the low conductivity (<200 μS cm⁻¹) and low organic matter contents indicative of low Zn complexation capacity (Table 1).

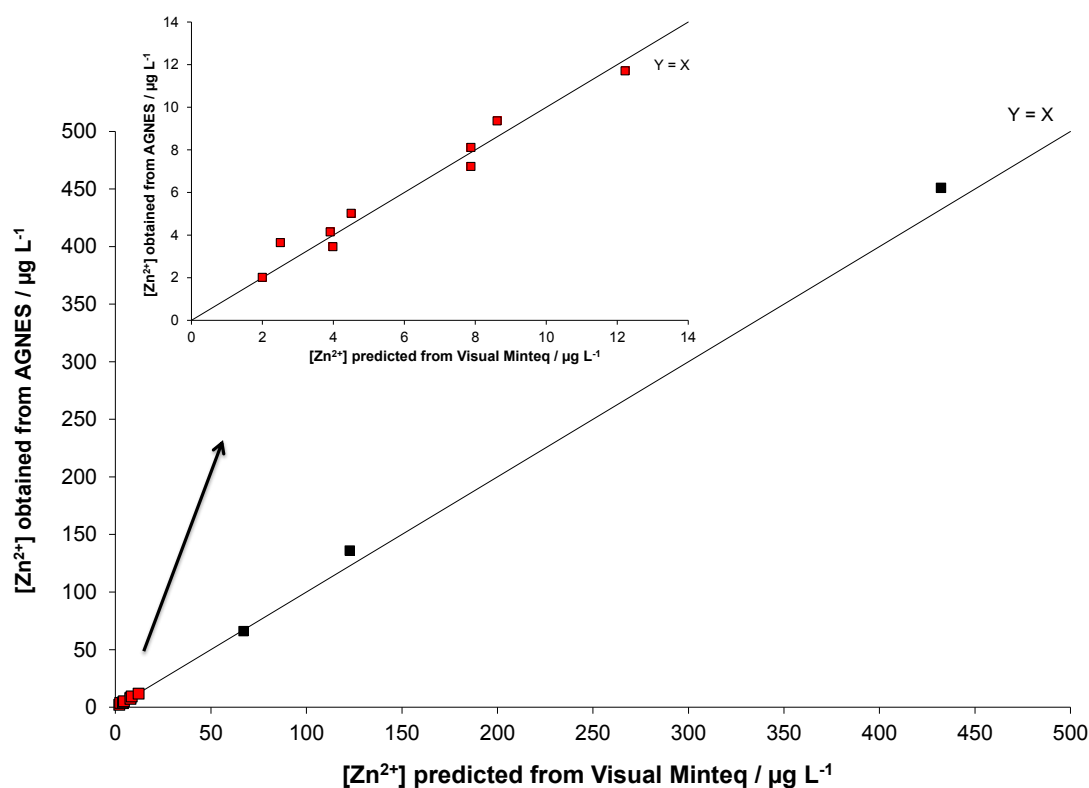


Figure 7. Comparison of [Zn²⁺] determined from AGNES-SCP_{chem} with [Zn²⁺] computed from Visual Minteq in the natural freshwater samples collected in Garonne, Gave de Pau and Gave de Caunterets. Electrochemical conditions were $E_1 = -1.165$ V, $t_1 = 300$ s with stirring, $T = 20^\circ\text{C}$, chemical stripping without stirring

On-site free Zn²⁺ measurements

On-site measurements were performed in the Gave de Cauterets at Soulom (France). In April 2012, the pH of the river was 7.8, $T = 6^{\circ}\text{C}$ and conductivity $154 \mu\text{S cm}^{-1}$. All the material was deployed in the field and plugged into a portable battery if necessary. The collected sample was filtered on-site at $0.45 \mu\text{m}$. An aliquot of the sample was acidified at pH 1.5 for ICP-AES analysis in laboratory. Another fraction of the sample was spiked with KNO_3 up to 0.01 mol L^{-1} for electrochemical analyses. The resulting free Zn^{2+} concentration found on site was $374 \pm 54 \mu\text{g L}^{-1}$. In order to corroborate these results, further classical AGNES-SCP were performed in the laboratory, purging with a mixture of N_2/CO_2 in order to fix the pH in the most natural way possible without altering the speciation of the sample^[14]. Since there is no interference from oxygen (no pH shift close to the electrode), a more positive potential could be used ($E_1 = -1.065 \text{ V}$, $t_1 = 300\text{-}500 \text{ s}$) and a $10 \mu\text{A}$ stripping current was applied. The calibration was performed in a KNO_3 0.01 mol L^{-1} solution at $T = 6^{\circ}\text{C}$. Given that, in purged samples, there is no need for the calibration to be at the same pH as the sample, pH 5 was selected to minimize Zn hydrolysis. The free Zn^{2+} concentration retrieved was $345 \pm 37 \mu\text{g L}^{-1}$ and appeared in good agreement with the previous results obtained on-site.

The results measured in Soulom show huge differences in total Zn concentrations between April 2012 ($647 \mu\text{g L}^{-1}$) and May 2012 ($136 \mu\text{g L}^{-1}$), but also the free Zn^{2+} proportion: in May, all Zn was free, whereas only 58 % of the total Zn was free in April 2012. These differences could be explained by the plentiful rainfalls during the month of April^[23] which induced a higher water level in the Gave de Pau. These events led to a flooding of the old mines and, thus, to a high release of other components able to complex Zn, such as carbonates and organic matter^[35]. This is supported by the highest conductivity being recorded in April ($154 \mu\text{S cm}^{-1}$), relative to that in May ($78 \mu\text{S cm}^{-1}$).

Conclusions

A new methodology has been developed to measure on-site free Zn^{2+} in non-purged river samples of the Pyrenees with AGNES-SCP_{chem}. The use of screen-printed electrodes appeared very suitable for this purpose as they could be easily deployed on site. Difficulties related to these measurements were removed by taking some precautions: i) a calibration in a synthetic solution where the composition is similar to the one of the sample; ii) calibration and sample analyses performed in the same conditions of pH and temperature; iii) an ionic strength fixed by an addition of KNO_3 0.01 mol L^{-1} to avoid anomalous voltammetric responses and ionic strength uncertainties.

Free Zn^{2+} measurements of samples taken from different locations in the Pyrenees rivers have been performed in the laboratory to check the suitability of the AGNES-SCP_{chem} methodology. Observed free Zn^{2+} concentrations appeared well correlated with the estimated ones using Visual Minteq. Finally, AGNES experiments were carried out on-site in the Gave de Cauterets in Soulom (France). The on-site measured free Zn^{2+} appear in concordance with

further measurements performed in the laboratory using the classical N₂/CO₂ purge, validating the proposed methodology.

This study showed that the total and free Zn concentrations depend on the geographical characteristics of the site (i.e. proximity to mines) and that an increase of the total Zn concentration does not systematically lead to a proportional increase of the free Zn concentration. This kind of approach is therefore of great interest in risk assessments, given the different levels of toxicity of different metal species.

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