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Identification and on-line monitoring of reduced sulphur species (RSS) by voltammetry in oxic waters

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ABSTRACT

Based on automatic on-line measurements on the Deûle River that showed daily variation of a peak around −0.56 V (vs Ag|AgCl 3 M), identification of Reduced Sulphur Species (RSS) in oxic waters was performed applying cathodic stripping voltammetry (CSV) with the hanging mercury drop electrode (HMDE). Pseudopolarographic studies accompanied with increasing concentrations of copper revealed the presence of elemental sulphur S(0), thioacetamide (TA) and reduced glutathione (GSH) as the main sulphur compounds in the Deûle River. In order to resolve these three species, a simple procedure was developed and integrated in an automatic on-line monitoring system. During one week monitoring with hourly measurements, GSH and S(0) exhibited daily cycles whereas no consequential pattern was observed for TA.

1. Introduction

In aquatic environments, the sulphur cycle plays a key role in the fate of trace metals [1–4]. In anoxic media such as muddy sediments, sulphides and poly-sulphides, are able to efficiently scavenge numerous of trace metals (e.g., Cd, Cu, Pb, Zn…), reducing therefore their toxicity [5–7]. Conversely, under hypoxic and oxic conditions, sulphides species tend to be oxidised with various kinetic rates, promoting an increase of the trace metal bioavailability [1,8–10]. Thermodynamically, the three stable species of inorganic S are sulphides, elemental sulphur and sulphate [3] but the disproportionation of the other species is low so that the presence of $S_2O_3^{2-}$ and SO_3^{2-}, for instance, were already detected in natural aquatic systems [11,12]. Furthermore, some metal sulphides were recently found relatively stable when dissolved in oxic waters [13,14]. In parallel, sulphur is also present in various organic compounds in different oxidation states. It is difficult to make an exhaustive list of these compounds that can be found in the environment since S is present in the smallest molecules but also in the biggest ones like humic substances [15].

Numerous studies were dedicated to the determination of sulphur compounds at trace levels in aquatic systems with various techniques: spectrophotometry [16], ion chromatography [17], voltammetry [18–23] and more specifically for organic compounds after an extraction procedure, GC-MS, FTIR, ^{13}C NMR and XANES spectroscopy [24–27]. In all cases, as most of the sulphur species are not stable under oxic conditions and/or may spontaneously disproportionate, the sampling, the treatment of the samples and the measurement timing are three paramount steps that need special care to get accurate and reproducible results. Additionally, several techniques are not selective enough and the clear identification of the sulphur species is not fully achieved so far (e.g. [12,28]).

Recently, several studies have pointed out the interest of high frequency monitoring for better understanding the fate of nutrients and trace metals in riverine aquatic systems, particularly when rapid changes occur at hourly scale [29–31]. The most common portable techniques used for achieving good time resolution and for measuring on line or in-situ are spectrophotometry and electrochemical techniques [32] including potentiometry [33], amperometry [34], voltammetry [35–38] and chronopotentiometry [39]. In oxic overlying water, concentrations of sulphur species that possibly interact with trace metals are generally low, barely exceeding a few hundred of nM [20,22,28,40]. As a consequence, voltammetric methods appear to be the most adapted for this monitoring approach.

Until now, most of the studies on RSS have been performed in seawater. To complete the knowledge on voltammetric techniques for RSS determination, the Deûle River was chosen for this work. The site is located in the town of Auby, close to the city of Douai, France. As the river is intensively used for fluvial traffic, anoxic sediment resuspension during the day and settlement of particles at night occur. Along the water course of the River, several areas

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have been severely polluted by metallurgical activities, especially in the area where the study site was located, just downstream the UMICORE and NYRSTAR factories that produce high quantity of Zn sold as standard zinc cathodes and high quality battery-grade cathodes.

The aim of this study is to provide for the first time a Voltammetric protocol to perform high frequency monitoring of reduced sulphur speciation in a water body. It is indeed a substantial improvement over existing methods because of (i) the evolution of the RSS speciation before analysis is really limited (on line measurements); and (ii) transitional phenomena and daily variations that occur in the river relative to RSS have never been monitored previously. For that purpose, preliminary experiments were carried out in the laboratory on model solutions in order to find the best conditions for partitioning of different sulphur species. The optimised protocol was afterwards validated using real water samples, while its robustness was finally tested on the field by a high frequency monitoring study performed over 8 days in the Deûle River.

2. Experimental

2.1. Field work

During three surveys (from April 5th to 11th, from July 7th to 16th, and from November 23rd to December 3rd 2011), Cathodic Stripping Voltammetry was conducted for detection of total RSS. After developing the new protocol, a new 8-day survey was conducted from September 24th to October 3rd 2012 for sulphur speciation monitoring.

For laboratory experiments, water samples were manually taken in LDPE bottles previously washed with ultrapure HNO_3 and rinsed afterwards with ultra-pure water (Millipore, mQ system, resistivity = 18.2 MΩ cm). They were immediately filtered through 0.45 μm nitrocellulose filter, stored at 3 °C and used within a few days.

2.2. Instrumentation and chemicals

All voltammetric measurements were carried out with a potentiostat-galvanostat μAutolab III (Metrohm, Switzerland) controlled by GPES 4.9.007 software (Eco Chemie) coupled with the three electrode system of 663 VA Stand (Metrohm, Switzerland). The working electrode was a Hanging Mercury Drop Electrode (HMDE) (size 3, 0.52 mm^2 of area). Potentials were given versus Ag|AgCl (3 M KCl) reference electrode and a platinum wire was used as a counter electrode. Electrochemical laboratory experiments were done in a glass cell for RSS and in a Teflon cell for metals. A glass cell was used on the field. The solution was stirred with a Teflon rotating stirrer at 1500 rpm (position 3 on the 663 VA Stand). When needed, prior to analysis, dissolved oxygen was removed from the solution by purging with ultra-pure nitrogen, for at least 5 min. pH and temperature were monitored using a PHM210 pH-metre (MeterLab, Radiometer) after calibration of the combined glass electrode (Crison 52-21) with NBS standard solutions (pH 7 and 9). Turbidity was measured with the turbidimeter Hach Lange (TSS portable) with its LXG321.99.00001 probe. All the used vessels and the tubing were cleaned with 10% nitric acid (Fisher Scientific, analytical grade) and rinsed with ultra-pure water before use.

Concentrated HNO_3 (Fisher, *Optima*) was used for acidification to pH 2. Readjustment of pH at 8 was done with a borate buffer prepared from a mixture of H_3BO_3 and NaOH (Merck, *suprapur*). For UV digestion, 100 mL of river sample was acidified with 100 μL of concentrated HNO_3. The sample was then irradiated for 6 h with 6 UV lamps Philips (PL-L 55W TUV UV-C). 1 g L^{-1} metal standards (Merck) were diluted for addition in the samples and calibration. 3 M KCl used in the reference electrode is from KCl (Merck, *suprapur*). GSH calibration was done by dissolving L-Glutathione reduced (Sigma-Aldrich, ≥98% purity) in ultra-pure water purged with nitrogen for 1 h. The standard was then maintained in anoxic conditions with a N_2 flow and used within 1 h. Elemental sulphur standard was prepared by saturation of methanol with 99.5% pure sulphur (Sigma-Aldrich). Intermediate solutions diluted 100 times were then prepared in ultra-pure water every day. Their concentrations were determined by ICP-AES (Vista-Pro, axial View, Varian) at 180.669 nm (detection limit of 1 ppm). Thioacetamide (TA) (Sigma-Aldrich, ACS reagent, ≥99%) was used for TA calibration.

The calibration curves were conducted in a spring water (St Amand) located in the vicinity of the sampling site with approximately the same concentrations of major elements, but with low trace metals (a voltammetric analysis revealed the following concentration: Zn = 460 pM, Cd = 90 pM, Pb = 390 pM, Cu = 30 pM) and negligible organic matter contents.

2.3. Voltammetric detection of RSS and metals

In the experiments, anodic and cathodic stripping voltammetry was used for the determination of metals and RSS, respectively. In all cases, the technique used for the stripping process was the Square Wave Voltammetry with the following parameters: 2 mV potential step increment, 12.5 mV pulse amplitude and 10 Hz frequency. Equilibration time was fixed at 5 s.

Voltammogram treatment (automatic smoothing, baseline correction, peak height or area measurement) was performed with a home written software ECDSOFT [41] whose latest version is available at http://gss.srce.hr/pithos/rest/omanovic@irb.hr/files/Software/.

For most of the RSS, the mechanism of their detection involves the oxidation of the metallic mercury from the drop. The mercury is oxidised to Hg(I) or Hg(II) in the potential range from −0.5 to +0.1 V and adsorbed as RSS-complexes onto the mercury drop surface. Summaries of the reactions of sulphide (Eq. (1)) [18], elemental sulphur (Eq. (2)) [42] and thiols (Eq. (3)) [12] are as follows:

$$HS^- + Hg^0 \leftrightarrow HgS_{(s)} + H^+ + 2e^- \tag{1}$$

$$S^0 + Hg^0 \leftrightarrow HgS_{(s)} \tag{2}$$

$$2RSH + 2Hg \leftrightarrow (RS)_2Hg_2 + 2H^+ + 2e^- \tag{3}$$

After the accumulation step, the oxidised mercury is then stripped cathodically leading to a peak around −0.6 V (potential depending on the RSS). During the deposition, the mercury cation can be in competition with other metallic cations such as Cu, Zn or Pb if their concentrations are high enough. For some RSS (e.g. S(0), HS^-) this competition leads to a decrease of the peak [28], for some other (e.g. GSH, GSSG), the presence of metal enhances the detection [22]. As the Deûle River contains relatively high concentrations of Zn and Pb, such interference has been carefully studied and if necessary, corrections have been made.

2.4. Automatic Trace Metal Monitoring System

The Automatic Trace Metal Monitoring System (ATMS) used in this study is a derivation from that developed by [43,30]. The whole system has been already thoroughly described in [31]. Briefly, it consists of a flow-through, three-way voltammetric cell where water samples to be measured (in batch mode using HMDE) were supplied by the three on line peristaltic pumps. Three attached automatic micro-burettes allow the precise addition of solutions in the range of 5 to 500 μL. For this study, 2 solutions were used: (1) a 10% HNO_3 solution and (2) a 10 ppm Cu standard solution.

3. Results and discussion

3.1. Monitoring of total RSS

In natural waters (marine and/or freshwaters), CSV scans performed with a HMDE often exhibit a peak whose potential can vary from −0.5 to −0.6 V, depending on the measurement procedure and the sample composition. Typical example of SWCSV stripping scan obtained in sample of Deûle River is presented in Fig. 1a. This voltammogram was obtained from untreated sample (pH=8.3) using 120 s of deposition at −0.2 V and stripping from −0.2 to −1.6 V. According to the literature, this peak is usually attributed to a single or a mixture of several RSS (sulphide, polysulfide, elemental sulphur, (Co, Fe, Mn, Ni) bisulfides, dimethyl sulphide (DMS), dimethyl disulphide (DMDS), carbonyl sulphide (COS), oxidised glutathione (GSSG), reduced glutathione (GSH), thioacetamide (TA), thiourea (TU) [21,23,28]) and can be easily detected in both anoxic and oxic waters. Although the peak is due to a mixture of RSS, it could be used as a marker (or indicator) of sulphur species content in a particular aquatic environment, evidencing their temporal dynamics in relation to chemical and physical changes of the water body. In order to examine variation of total RSS concentrations in the Deûle River, three short term campaigns were performed (in April, July and November, 2011) using an automated monitoring system. In Fig. 1b, the RSS peak heights for the three time series are presented. Unlike the July campaign, where an increasing trend over the examined time period was observed, the April and November campaigns showed a clear dial cycle: an increase in the peak during the day and a decrease in the peak during the night. The daytime increase of the peaks was ascribed to the sulphur species released from the sediment due to its resuspension caused by the intensive large boats traffic. Authorised boat operating periods, from 8 a.m. to 6 p.m. and only during the week days, correspond to observed intervals having increased voltammetric signal (April and November campaigns). Average peak heights over the monitored period for the three campaigns (around 3.5 nA in April and July, and 6.5 nA in November) refer to a potential seasonal cycle.

3.2. Identification of the RSS

3.2.1. Pseudopolarography and copper additions

A step forward towards understanding of the sulphur role in the aquatic systems, particularly for the trace metals speciation studies, is identification and determination of its chemical species. As a direct deconvolution of the coalesced stripping peak could not provide adequate results, an indirect method of sulphur species decomposition, based on their different chemical reactivity and/or redox properties, should be applied. Laglera and Tovar-Sanchez [23] showed that by careful selection of the deposition potential, combined with the addition of copper and/or EDTA, a satisfactory discrimination of model sulphur species such as TA, TU, GSH and GSSG in real seawater is achievable. Here we applied similar methodology but in fresh water media. Initial laboratory experiments were conducted in order to find the best experimental conditions, and thereafter an optimised measurement protocol was used for on-line monitoring and sulphur speciation in real river water by automated monitoring system.

The method for discrimination of sulphur species originates from the pseudopolarographic measurements: several stripping voltammetric analysis (cathodic in the case of RSS) are performed with different deposition potential and the stripping peak currents are then plotted as a function of the applied deposition potential [44,45]. A set of such pseudopolarograms measured in Deûle river sample under different experimental conditions are presented in Fig. 2. Pseudopolarogram obtained without copper addition (◆) showed two distinct waves: a first with the half-wave potential around −0.48 V, and a second wave starting at $E_{dep}=-0.15$ V but without well defined plateau (more positive deposition potentials than −0.05 V were not scanned because of the interference of the mercury oxidation which starts at that potential range). This pseudopolarogram is basically a cumulative response composed of different sulphur species having different properties toward oxidised-mercury complexation. More insight into the species distribution was gained by the addition of copper, which competes with the mercury for sulphur species. A sequential copper additions up to the 90 nM (●) (initial concentration of Cu is negligible) produced uniform decrease of pseudopolarogram intensities in the

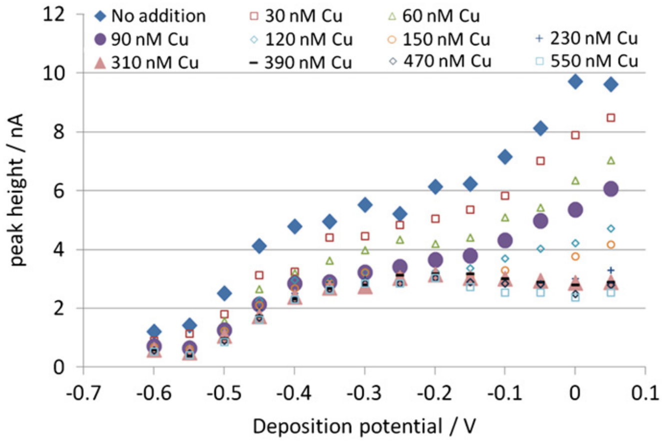

Fig. 2. Pseudopolarograms of RSS measured without and with increasing concentrations of added Cu.

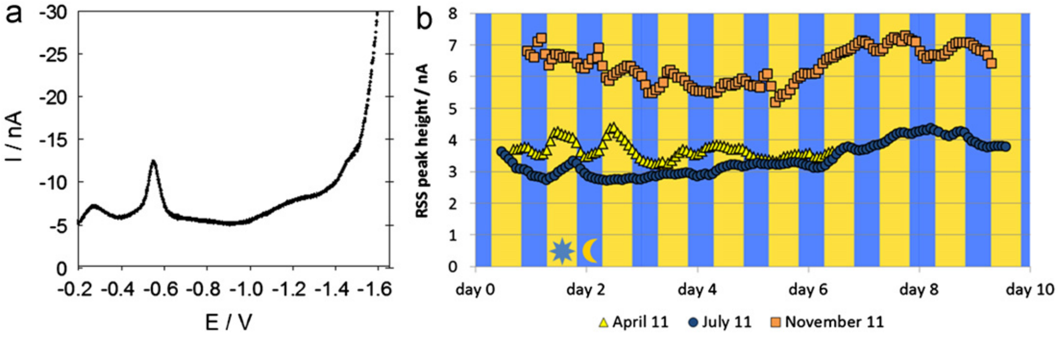

Fig. 1 (a) Typical voltammogram recorded—raw data; (b) variation of the RSS peak height detected by SWCSV (120 s of deposition at −0.2 V. Scan from −0.2 V to -1.6 V) in the Deûle River from April 5th to 12th, July 7th to 16th and November 23rd to December 3rd 2011.

scanned potential range, keeping the two initial present waves still well defined. The drop of peak currents is attributed to the elimination of elemental sulphur from the solution by complexation with copper. This assumption was verified by additional experiments conducted with individual model sulphur compounds. Namely, among the studied compounds, the pseudopolarogram of elemental sulphur exhibited a single wave starting at $E_{dep}=-0.50$ V with a constant plateau up to the most positive deposition potential applied (see Appendix A, Fig. A.1). Furthermore, additions of Cu caused uniform decrease of the pseudopolarographic response until total extinction. Further additions of copper up to the 230 nM (▲) caused decrease of the more positive pseudopolarographic wave. Disappearance of this wave in seawater by added copper was attributed to removal of sulphur components exhibiting behaviour similar to TA and/or TU [23]. Our model experiments in freshwater samples confirmed such behaviour (see Appendix A, Fig. A.2). Higher copper addition (up to 550 nM) did not produce any visible change in the shape of pseudopolarographic waves, indicating absence of further copper influence on the redox active sulphur components present in real river water sample. Similar pseudopolarographic profile and behaviour with copper addition was described by Laglera and Tovar-Sanchez for oxidised glutathione (GSSG) in seawater.

Taking into account our pseudopolarographic measurements, and the information extracted from the available literature (details provided in Appendix A), three compounds or compound mixtures with similar behaviour were distinguished in the samples: (1) RSS sensitive to low copper additions, (2) Cu-sensitive RSS detected only for a deposition potential more positive than −0.15 V and (3) RSS insensitive to copper. They were identified as (1) S(0), (2) TA and TU and (3) GSSG. The additional presence of the unstable GSH species will be discussed thereafter.

3.2.2. *Acidification and UV irradiation*

In order to detect the presence of volatile sulphur species in acidic conditions, the evolution of the RSS pseudopolarogram after acidification was studied. 30 mL of sample was acidified with *suprapur* HNO_3 to pH=2 and purged with N_2 for 600 s. The pH was then readjusted to the initial pH of 8.3 and a pseudopolarogram profile was recorded. No significant change was observed. Thus, the non-volatility of the compounds at acidic pH indicates that none of the peak is due to sulphide, polysulphide, Dimethyl Sulphide (DMS), Dimethyl Disuldide (DMDS), Carbonyl Sulphide (COS) or Co, Fe, Mn and Ni-bisulfides. Other Metal sulphides such as Zn–S, Cu–S or Pb–S should be dismissed as well since they cannot be detected in the applied potential range because of their high stabilities at natural pH [21].

Pseudopolarograms were also recorded after UV digestion in acidic medium. Before analyses, the pH was readjusted to the initial pH of 8.3. In this case, no peak was detected at any deposition potential, suggesting that the RSS signal is exclusively constituted of organic S species. However, additional UV-irradiation tests conducted on a S(0) standard solution demonstrated that it is also efficiently oxidised, that means UV-irradiation cannot be used here to partition organic and mineral sulphur species.

3.3. *Monitoring of RSS*

3.3.1. *Basic principle*

The identification of the compounds inside the RSS peak by pseudopolarography and copper additions has permitted to go further in the monitoring of sulphur species. For that purpose, and taking into account that high frequency measurements are required, a short method has been developed based on four scans:

- Procedure A: deposition at $E=-0.1$ V. Detection of GSSG, TA and S(0). TU is purposely left out since it is deposited for $E_{dep}>0$ V.
- Procedure B: deposition at $E=-0.3$ V. Detection of GSSG and S(0).
- Procedure C: after addition of 500 nM of Cu, deposition at −0.1 V. Detection of GSSG.
- Procedure D: after addition of 500 nM of Cu, deposition at −0.3 V. Detection of GSSG.

From the four peak height measured on these voltammograms the concentrations of S(0), GSSG and TA can then be calculated: S(0) by subtracting D from B, GSSG from C or D and TA by subtracting S(0)+GSSG from A (cf. Fig. 3).

3.3.2. *Calibrations and corrections*

The last analytical step to quantify the RSS species in the Deûle River is to calibrate each signal obtained from the adapted method for on line monitoring. This task was not trivial because the relative high contents of Zn and Pb in the Deûle River may interfere, like Cu, with Hg. In addition, the variation of the water temperature observed during the monitoring period may also modify sensitivities by influencing viscosity of the water and diffusion coefficient of the studied species.

3.3.2.1. GSH and GSSG. By looking at the peak potential from procedure C and procedure D recorded on line ($E_{peak}=-0.56$ V and $E_{peak}=-0.59$ V respectively), the remaining species after copper addition seemed to fit GSH behaviour rather than GSSG. GSH being unstable in oxic conditions—half-life of GSH in oxic water of ca. 3 h was calculated from [46]—laboratory experiments shown in Fig. 2 might have been done too late after sampling to detect GSH. On the other hand, the laboratory study was not done at the same period as the on line monitoring, so that one cannot exclude an evolution of the Deûle water redox properties: for instance, a lower oxygen content due to the mineralisation of organic matter would result in the stabilisation of GSH whereas high primary production during Spring would favour oxidised species like GSSG [46]. Thus, the simplified procedure for making on-line RSS speciation at high frequency was validated directly in the field at the beginning of the monitoring period by doing detailed pseudopolarograms with 10 successive additions of 30 nM of copper. So GSH presence was confirmed for all on site manipulations instead of GSSG.

To obtain the S(0) response, we need to subtract the GSH response (i.e. i_D) from the GSH+S(0) response (i.e. i_B). But since GSH sensitivity is changing with Cu additions [22], it is necessary

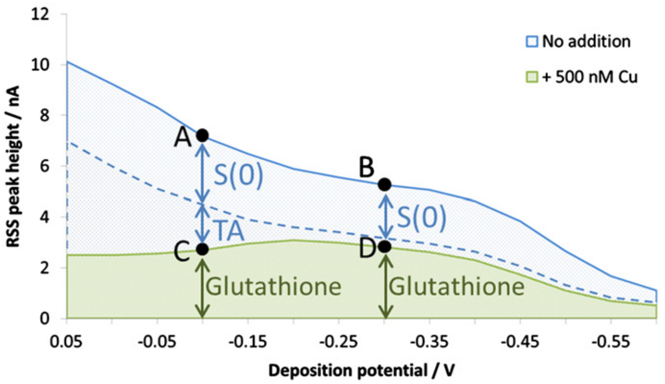

Fig. 3. Principle of the shorten pseudopolarograms for the RSS determination.

to correct i_D before subtracting it from i_B. The correction coefficient α_{GSH}, given in Table 1, was defined as follows:

$$\alpha_{GSH} = \frac{i_{GSHcu}}{i_{GSH}} = \frac{S_{GSHcu}}{S_{GSH}} \tag{4}$$

Thus, the determination of α necessitates two calibrations for GSH: one without copper and one with 500 nM of copper in the solution. In both cases, 6 additions of 30 nM of GSH were performed with 120 s of deposition time at E_{dep}=−0.1 V and −0.3 V. Results are displayed in Table 1.

3.3.2.2. Elemental sulphur. Elemental sulphur was calibrated for E_{dep}=−0.1 V with 10 additions of 1.5 nM of S(0). The results are presented in Table 1.

Elemental sulphur was not found sensitive only to Cu addition but to other metallic cations as well. Zn or Pb additions within the natural concentration range have been found to have an significant impact on the response of S(0). For laboratory analysis, a simple solution is to add EDTA in order to remove any interference from metals [23]. However, during the monitoring procedure established to record the 3 RSS, the presence of EDTA would make the addition of Cu ineffective. Instead, the metal interference was corrected by calculation. Correction curves were performed by adding metals in a solution spiked with S(0). 2 nM of Pb were added 6 times in a 3 nM S(0) solution and the decrease of the S(0) peak was measured. The same experiment was conducted with 6 additions of 5 nM of zinc. In both cases, the attenuation of the S(0) peak was found linear within the studied range. The attenuation factors β_{Me}, expressed in S(0) signal attenuation percentage per nM of metal are displayed (Table 1).

The metals (Pb and Zn) were then monitored as well on the field. As only the most electrolabile part of the metals was considered to be in competition with Hg for RSS complexation onto the mercury electrode, a pseudopolarogram based on SWASV procedure was built in order to find the best deposition potentials for determining the labile fraction of Pb and Zn (data not shown). Calibrations were conducted with 6 additions of 0.5 nM of lead and 6 additions of 5 nM of Zn. The obtained sensitivities are displayed (Table 1).

3.3.2.3. Thioacetamide. Thioacetamide was calibrated for E_{dep}= −0.1 V with 6 additions of 25 nM. The results are presented in Table 1. In part 3.2.2, we have seen than the signal of TA was not affected by metal additions before all the S(0) signal was extinguished. In the river, S(0) being still detected, we consider that metals do not influence the signal of TA. No correction relative to the sensitivity of this RSS species is then needed.

Table 1
(a) Sensitivities and (b) correction factors for identified sulphur species and detected metals.

	E_{dep}		Units
(a) *Sensitivities*			
GSH without Cu	−0.1 V	0.069 ± 0.002	nA nM^{-1}
	−0.3 V	0.067 ± 0.002	nA nM^{-1}
GSH with 500 nM Cu	−0.1 V	0.086 ± 0.001	nA nM^{-1}
	−0.3 V	0.093 ± 0.002	nA nM^{-1}
S(0)	−0.1 V	0.283 ± 0.008	nA nM^{-1}
TA	−0.1 V	0.238 ± 0.005	nA nM^{-1}
Zn	−1.4 V	0.329 ± 0.006	nA nM^{-1}
Pb	−1 V	0.501 ± 0.008	nA nM^{-1}
(b) *Correction factors*			
S(0) attenuation	β_{Zn}	0.77 ± 0.03	% nM^{-1}
	β_{Pb}	4.1 ± 0.4	% nM^{-1}
α_{GSH}	E_{dep}=−0.1 V	1.25 ± 0.05	Dimensionless
	E_{dep}=−0.3 V	1.39 ± 0.07	Dimensionless

3.3.2.4. Temperature, pH and chloride interferences. The response of the RSS seems to be very temperature dependant. Experiments conducted on Deûle river samples indicated a linear variation of the peak height of 2.7% per degree Celsius in the range of 5–25 °C, at E_{dep}=0.05 V and −0.3 V. However, separate calibration curves for the three species have not been performed and temperature corrections on the raw data were not applied. The temperature evolution during the survey was rather limited (between 14.7 and 15.5 °C) and should not change significantly the variations of the species responses. This correction will be nevertheless essential when RSS species behaviour will be studied as a function of the seasons.

The monitored river being relatively well carbonated [47], its pH was found well buffered and not significantly changing over the days (average dial peak-to-peak amplitude of 0.1 unit within the range from 8.15 to 8.35 over the 8 days). In this range, GSH signal change is around 3% [22]. S(0) signal decrease from pH 5 to pH 9 amounts to 15% [48], that means a variation of 0.75% for 0.2 pH unit. As for TA, the impact of pH variation in the range of 8–8.5 on TA voltammetric response was not found relevant: after calculation, the correlation between TA and pH was found non-existent (R=0.05) so the two parameters were considered independent. Thus, no pH corrections were found useful in the measured range.

Although chloride interferences have not been examined in this study (because of their low concentrations in the Deûle River), the recorded pseudopolarographic profiles were very similar to those from previous work in seawater [23,28]. Consequently, the presence of chloride should not influence significantly the detection of RSS. However, if CSV with deposition potential above 0.05 V were to be used, e.g. for detection of TU, variations of chloride concentrations should be considered: competition between chloride and RSS in the process of mercury oxidation cannot be dismissed. It would particularly influence for the study of an estuary where salinity variations can be significant.

3.3.3. *Method setup for on-line monitoring*

The following procedure was then developed and used on site (Fig. 4). After pumping the sample into a 22 mL cell, the solution is purged with N_2 for 300 s. A first SWCSV is performed with a deposition time of 120 s at −0.1 V and a cathodic scan from 0 to −1.5 V (peak A). The deposition potential is then switched to −0.3 V for a second SWCSV (peak B). Electrolabile Zn and Pb are measured in the two following scans with the following parameters: for Zn, deposition time of 180 s at -1.4 V and anodic redissolution from -1.3 to −0.7 V; for Pb, deposition time of 300 s at -1 V and anodic redissolution from −0.8 to 0 V. After that, 500 nM of Cu is added and the solution is stirred for 300 s. The two SWCSV procedures are repeated again (peak C for E_{dep}= −0.1 V and peak D for E_{dep}=−0.3 V). Each scan is repeated 3 times.

The cell is then flushed and refilled 3 times with river water. 200 μL of 1.5 M HNO_3 are added and the solution is stirred for 600 s to remove Cu traces. Another cycle of three flushing and refilling is done before starting again the analyses. This loop allows a measurement every one hour and a half.

This whole procedure was applied for eight days of monitoring in the Deûle River and the resulting data are displayed in Fig. 5. The average relative standard deviation values were 5%, 5%, 14% and 9% for A, B, C and D respectively.

Zn and Pb concentrations were measured only during 6 days (from 26/09/12 to 02/10/12. cf. Appendix A, Fig. A.3). The attenuation of S(0) signal due to the presence of electrolabile Pb and Zn ranged between 6 and 15% with regular dial cycles. These findings indicate clearly the importance of taking into account such an interference to treat the raw data before evidencing S(0) dial cycles.

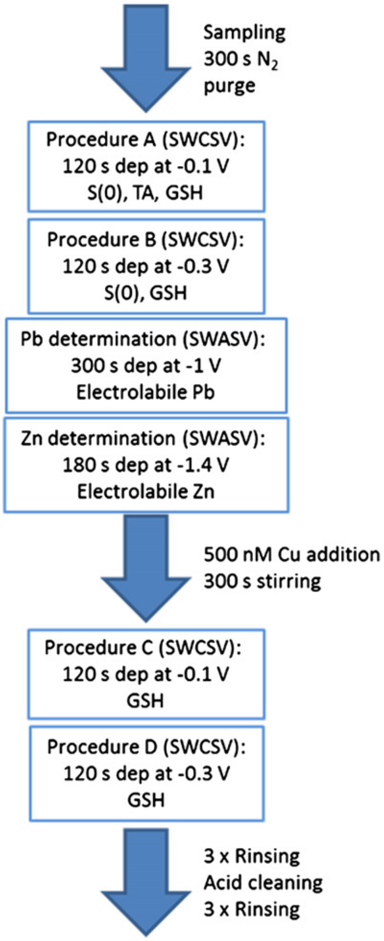

Fig. 4. Summary of the analytical steps optimised to perform automatic RSS speciation on line. For each step, measurements were systematically repeated 3 times.

3.3.4. *Speciation of sulphur during a high frequency monitoring experiment*

To obtain the concentrations of the 3 species from the raw data, the following formulas were applied:

$$[\mathrm{S(0)}] = \frac{1}{S_{\mathrm{S(0)}}} \times \frac{1}{1-\beta_{\mathrm{Zn}}[\mathrm{Zn}]-\beta_{\mathrm{Pb}}[\mathrm{Pb}]} \times (i_{\mathrm{B}}-\alpha_{\mathrm{GSH-0.3\ V}}\, i_{\mathrm{D}}) \tag{5}$$

$$[\mathrm{GSH}] = \frac{1}{S_{\mathrm{GSH}_{\mathrm{Cu-0.3\ V}}}} \times i_{\mathrm{D}} \tag{6}$$

$$[\mathrm{TA}] = \frac{1}{S_{\mathrm{TA}}}[i_{\mathrm{A}}-(i_{\mathrm{B}}-\alpha_{\mathrm{GSH-0.3\ V}}\, i_{\mathrm{D}})-\alpha_{\mathrm{GSH-0.1\ V}}\, i_{\mathrm{C}}] \tag{7}$$

With i_X the peak height of procedure X (X=A, B, C, D), S_j the sensitivity of the RSS species j [j=S(0), GSH, TA], β_{Me} the attenuation factor of Me (Zn or Pb) on S(0) and α_{GSH} the correction factor as defined hereinabove.

The variation of these three species can be seen in Fig. 6. S(0) is only displayed from September 26th since it is only from that day that Pb and Zn contents have been measured. The relative standard deviation of the RSS species concentrations induced by the calibration and the corrections to take into account chemical interferences was also calculated. We found 22%, 11% and 26% for S(0), GSH and TA respectively (see Fig. 6)

According to the concentrations depicted in Fig. 6 and by taking into account the high RSD values, no significant daily variation for TA can be extracted from these data. The concentration of S(0) seems to increase during the day and decrease at night, in accordance with sediment resuspension. Its correlation with turbidity was indeed relatively good (R=0.57 with an R critical of 0.18 for a 95% confidence interval). Production of S(0) in the sediment could be induced by the partial reoxidation of AVS (Acid Volatile Sulphides) in contact with oxidant like dioxygen [49,50]; it can also be consumed and produced biologically by chemoorganotrophs that use oxidised S species as electron acceptors, chemoautotrophs that use reduced S as an energy source and phototrophs that use reduced S as an electron donor [4]. Highly reactive and of low solubility, its concentration represents in the Deûle sediment only a few percentage of the pyritic compounds (unpublished data). GSH variations seem to be linked with biological activity: they are well negatively correlated with pH

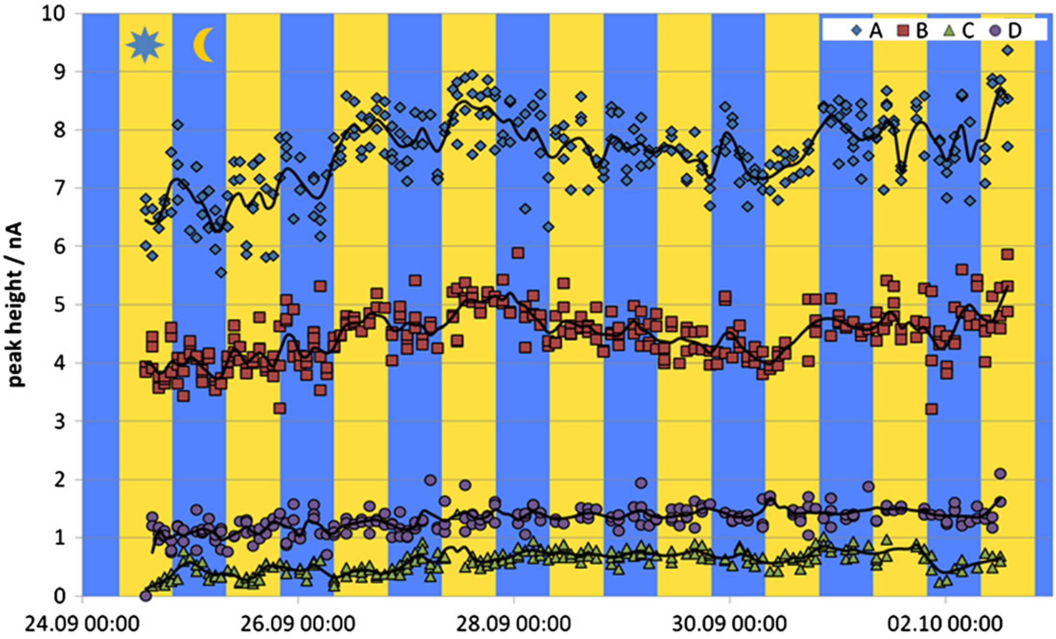

Fig. 5. Variations of A, B, C and D peak height from September 24th to October 3rd 2012 (see the text for explanation of the legend).

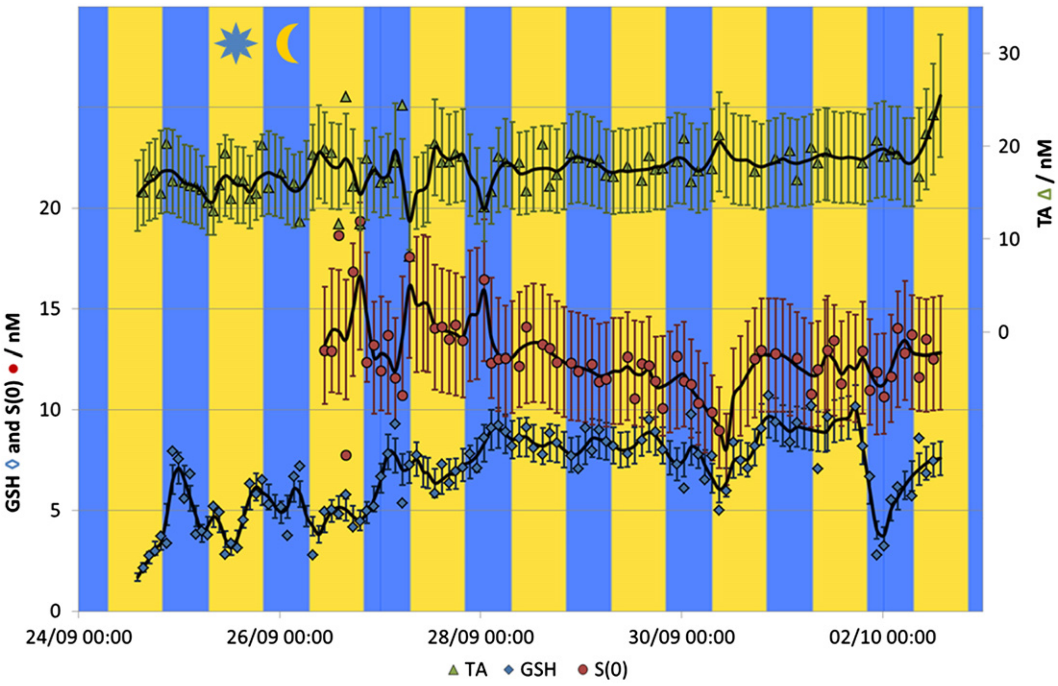

Fig. 6. Variation of GSH, S(0) and TA concentrations from September 24th to October 3rd 2012.

variations ($R = -0.56$) which tend to increase during the day (consumption of CO_2 by the phytoplankton during the primary production) and decrease during the night (mainly due to the respiration). GSH is indeed an important intermediate in the anaerobic decomposition of organic matter in soils, sediments and sewage [51].

4. Conclusion

Monitoring water body at high frequency is of paramount interest to understand precisely the biogeochemistry of a system. Huge variations have been evidenced on the daily scale that can be as important as or even more important than annual variations. This is particularly true for trace metals and the parameters influencing their speciation (e.g. pH, temperature, ligand concentration …). However, no studies have yet been dedicated to reduced sulphur species (RSS), playing an important role as strong ligands. Our findings propose for the first time an on line voltammetric method that allows to monitor sulphur speciation. The main highlights of this work are:

- The development of an identification procedure of RSS based on pseudopolarogram construction along with copper additions;
- The implementation of a simplified procedure for on line monitoring of these identified RSS;
- The application of these procedures to the Deûle River, identifying three species (elemental sulphur, thioacetamide and reduced glutathione) and monitoring them during an 8-day survey with a 90 min temporal resolution.

Although the calibration and interference corrections resulted in relatively high standard deviation of the concentration values, the evolution of these RSS was interpretable: the results underline daily cycles of (i) elemental sulphur probably bound to sediment resuspension events during daytime and (ii) glutathione possibly linked to decomposition of organic matter.

The next step of this work will now be to prove the robustness of the procedure by applying it to water bodies with different matrix and RSS. Overall, the main target will be the monitoring of trace metals and RSS over longer periods in order to really demonstrate the impact of short events (such as flooding, storms, accidental pollutions, dredging), as well as seasonal effects.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.03.045.

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