Determination of free Zn^{2+} concentration in synthetic and natural samples with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique)

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Abstract

The determination of free Zn^{2+} ion concentration is key in the study of environmental systems like river water and soils, due to its impact on bioavailability and toxicity. AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique) are emerging techniques suited for the determination of free heavy metal concentrations, especially in the case of Zn^{2+}, given that there is no commercial Ion Selective Electrode. In this work, both techniques have been applied to synthetic samples (containing Zn and NTA) and natural samples (Rhine river water and soils), showing good agreement. pH fluctuations in DMT and N$_2$/CO$_2$ purging system used in AGNES did not affect considerably the measurements done in Rhine river water and soil samples. Results of DMT *in situ* of Rhine River water are comparable to those of AGNES in the lab. The comparison of this work provides a cross-validation for both techniques.

Keywords: AGNES, DMT, speciation techniques, zinc, FIAM, environmental analysis.

1. Introduction

31

32   Heavy metals in the environment can lead to negative effects on plants and

33   animals, and consequently on human health (van Straalen and Looke,1997). The

34   reliable determination of both the amount and the chemical speciation of heavy metals

35   in environmental studies is essential for an accurate risk assessment. According to the

36   free ion activity model (FIAM) (Campbell,1995;Parker and Pedler, 1997), the

37   bioavailability of heavy metals to organisms depends mostly on the free metal ion

38   concentration (which is directly linked to activity). Moreover, the free ion concentration

39   is a well-defined thermodynamic entity and its measured value can be compared to

40   calculations done with modern geochemical speciation codes with the proviso that the

41   composition of the sample is well characterized.

42   However, reliable measurement of free ion concentrations in the natural

43   environment (often at trace levels) remains an analytical challenge (Davison et. al.

44   2000;Pesavento et al., 2009). The co-existence of various chemical forms that are hard

45   to distinguish from the free ions complicates the measurement. Nevertheless, free metal

46   ion concentrations can be directly measured by a few techniques such as Ion Selective

47   Electrode (ISE) (Bakker and Pretsch, 2007), Permeation Liquid Membrane (PLM)

48   (Parthasarathy et al., 2001), DMT (Temminghoff et al., 2000) or AGNES (Galceran et

49   al., 2004). Each technique has its advantages and limitations.

50   The element zinc is introduced into soils and surface waters by atmospheric

51   deposition, agrochemicals, manure, and aerial fallout from factories (Charlesworth et

52   al., 2011). It occurs also naturally in the soil and water environment. Zn is an essential

53   micro nutrient for plants and animals. Zn deficiency is considered as a wide-spread

54   malnutrition problem that affects the growth of children (Penland, 2000). At elevated

55   levels, Zn becomes toxic to terrestrial and aquatic organisms.

56   The use of an Ion Selective Electrode would be a simple way to determine the

57   free Zn^{2+} concentration, but, up to date, there is no commercial ISE for Zn.

58   Alternatively, both DMT and AGNES are able to determine free Zn^{2+} concentrations.

59   DMT uses a cation exchange membrane to measure free ion concentrations based on

60   Donnan membrane equilibrium, whereas AGNES is an electrochemical technique.

61   Previous works of DMT and AGNES have addressed their implementation in different

62   systems: synthetic solutions using various ligands (Alberti et al., 2007;Companys et al.,

63   2007;Domingos et al., 2004;Galceran et al., 2010;Kalis et al., 2006a;Oste et al.,

64   2002;Temminghoff et al., 2000), soils (Weng et al., 2001a), natural waters (river and

65   seawater) (Galceran et al., 2007;Kalis et al., 2006b;Zavarise et al., 2010), wine

66   (Companys et al., 2008), multi-metal systems (Chito et al., 2010;Kalis et al.,

67   2007a;Kalis et al., 2007b), etc. However, there is, so far, no direct comparison of

68   measurements using these two techniques (DMT and AGNES), which are based on very

69   different physicochemical properties leading to equilibrium.

70   The aim of this work was to determine free Zn^{2+} concentration in three types of

71   samples under similar conditions with AGNES and DMT and to establish a comparison

72   between both techniques. Firstly, synthetic solutions containing Zn-NTA mixtures were

73   analyzed with DMT and AGNES and compared with the predictions using the

74   speciation code VMinteq. Then, Rhine river water was analysed with DMT *in situ* and

75   AGNES in the lab. Finally, extraction samples from 4 soils were analysed in the lab

76   with both techniques.

77

2. Theory

79   2.1   DMT (Donnan Membrane Technique)

80   This technique is based on the theory of Donnan membrane equilibrium

81   (Donnan, 1925;Helfferich, 1969). Experimentally, a cation exchange membrane

82   separates the sample solution (donor solution) from an acceptor solution. The negative

83   electrostatic potential generated in the membrane by the deprotonated sulphonic acid

84   groups favours transport of cations and strongly retards anion transport and practically

85   only allows cation exchange through the membrane. The technique has been

86   implemented in the lab and *in situ* (Kalis et al., 2006b;Oste et al., 2002;Weng et al.,

87   2001a).

88   Figure 1 shows the lab and field cells usually employed. In the lab cell, the

89   donor side contains the solution with unknown concentration, while the acceptor side

90   contains the supporting electrolyte ($Ca(NO_3)_2$ or $CaCl_2$) with an ionic strength similar to

91   that of the donor solution. The donor and acceptor solutions are separated by the cation

92   exchange membrane. The cell has been designed so that there is a direct contact of both

93   solutions with the membrane and there is a continuous recirculation at each side. The

94   field cell consists of one acceptor chamber enclosed by two cation exchange membranes

95   and it is immersed in the donor solution.

96   A certain time is required to attain the Donnan membrane equilibrium. After the

97   chosen time, samples are taken from both donor and acceptor side of the DMT and the

98   total element concentrations are determined, for instance with Inductively Coupled

99   Plasma Mass Spectrometry (ICP-MS). The salt difference between the two sides of the

100   DMT can be corrected by measuring the concentration of a reference cation in the donor

101   and acceptor solution. Ideally the reference cation has a total concentration that is

102   approximately equal to the free concentration, because only weak complexes are formed

103   and/or the concentration is high compared to the dissolved ligand. In this work calcium

104   can be used as a reference cation. The correction is based on the Donnan membrane

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105   equilibrium principle that the cationic activities in the donor and acceptor sides (for any

106   given cation) are equal at Donnan membrane equilibrium (Helfferich, 1969). In the case

107   of Zn, it reads:

108

$$\frac{\left[Zn^{2+} \right]_{don}}{\left[Zn^{2+} \right]_{acc}} = \frac{\left[Ca^{2+} \right]_{don}}{\left[Ca^{2+} \right]_{acc}} \qquad (1)$$

109   In this work, the total Ca concentrations in the donor and acceptor have been

110   taken as the free concentrations, given that the expected complexation by the relevant

111   ligands can be considered as negligible.

112   ## 2.2   AGNES (Absence of Gradients and Nernstian

113   Equilibrium Stripping) Technique

114   AGNES is a stripping technique which consists in the application of two stages:

115   i) preconcentration up to equilibrium and ii) stripping. In the first stage, a

116   preconcentration factor or gain Y between the reduced metal concentration in the

117   amalgam, $[Zn^{o}]$, and the free metal concentration in the solution, $[Zn^{2+}]$, is eventually

118   reached. The simplest way to achieve this equilibrium goal consists in applying a

119   deposition potential E_1 during a sufficiently long time, t_1. In the second stage, the

120   stripping takes place when a potential E_2 is applied under diffusion limited conditions

121   (Galceran et al., 2004). In order to reduce the deposition time, the first stage can be split

122   into two different sub-stages (a potential step at diffusion limited conditions $E_{1,a}$ for $t_{1,a}$,

123   followed by a potential step $E_{1,b}$ corresponding to the desired concentration gain Y for

124      $t_{1,b}$) (Companys et al., 2005). The response function can be the intensity current

125   (typically at $t_2=200$ ms) or the total faradaic charge measured in the stripping stage

126   (Galceran et al., 2010;Parat et al., 2011a;Parat et al., 2011b).

127   When the current is the analytical response, the free metal concentration in the

128   solution can be computed from the proportionality factor η (obtained from a

129   calibration), the faradaic current (I) measured at t_2 and the gain Y, through:

130
$$[Zn^{2+}] = \frac{I}{Y\eta} \qquad\qquad (2)$$

131   # 3. Experimental

132   ## 3.1   Reagents

133   A membrane (BDH Laboratory Supplies, Poole, UK) of polystyrene and

134   divinylbenzene with sulphonic acid groups was used as cation exchange membrane in

135   DMT experiments. The ion-exchange capacity is 0.8 meq g^{-1} and the thickness of the

136   membrane is 0.15–0.17 mm. All material used for the experimental set up of DMT was

137   cleaned following the procedure described elsewhere (Kalis et al., 2006b).

138   For DMT and AGNES experiments with synthetic solutions, zinc standard

139   solutions were prepared by proper dilution from a Merck 1000 mg L^{-1} stock solution.

140   Nitrilotriacetic acid (NTA) (Fluka, analytical grade) in the H_3L form was used as ligand.

141   NaOH and HCl titrisol (Merck) were added to adjust the pH to desired values.

142   Calcium chloride was used as inert supporting electrolyte in AGNES

143   experiments and as acceptor solution in DMT experiments and it was prepared from

144   solid $CaCl_2$ (Merck and Prolabo, analytical grade). In all the experiments ultrapure

145   water (Milli-Q, Millipore) was employed.

146   Purified water-saturated nitrogen N_2 (99.999 %) was used for deaeration and

147   blanketing of solutions in AGNES experiments. A mixture of N_2 and CO_2 (99.995 %)

148   was used to adjust the pH in river and soils samples.

149   Rhine river water and soil extracts were filtered through 0.45 μm membranes

150   (Millipore), except for the *in situ* determinations in Rhine river with DMT.

151   All laboratory ware and equipment for sampling and filtering were extensively

152   washed using the procedure detailed in (Galceran et al., 2007).

153   Nitric acid (Fluka, TraceSelect) was used to acidify the samples to analyse the

154   total metal and DOC content.

155   ## 3.2   Instrumentation

156   Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Spectro

157   Analytical Instruments and Activa-S, Horiba Scientific) and Inductively Coupled

158   Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, Elan 6000) were used respectively

159   to determine total Ca and total Zn content in all samples filtered through 0.45 μm

160   (Aquatron 30, Schleicher and Schuell) and acidified to 0.63% HNO_3.

161   A handheld meter pH 340 (WTW) and a LF330 type conductivity meter

162   combined with a conductivity electrode TetraCon 325 (WTW) were used to measure *in*

163   *situ* pH and electric conductivity.

164   DOC concentration was measured in river waters and soil samples filtered and

165   acidified to pH around 2.20 to remove inorganic carbonate by means of a DOC/TOC

166   analyser (Sievers 900 portable).

167   VMINTEQ database has been used to predict the metal speciation (Gustafsson,

168   2010).

169   ### 3.2.1   Instrumentation for DMT

170   DMT cells made of Perspex were used (see Figure 1 and ref. (Temminghoff et

171   al., 2000)). In the lab DMT cell (see Fig. 1.a), a donor chamber and an acceptor

172   chamber are separated by a negatively charged membrane held by two O-rings. The

173   membrane surface area is ca. 7 cm^2. Donor and acceptor reservoirs are connected

174 respectively to the donor and acceptor side of the lab DMT cell via PTFE pump tubes

175 and the solutions were circulated with a peristaltic pump (Gilson Minipuls III) (flow

176 rate=2 mL min^{-1}).

177 The field DMT cell (used for *in situ* measurement of river water) consists of one

178 chamber (the acceptor) enclosed between two membranes which separate the acceptor

179 solution from the donor solution (river water) (see Fig. 1.b). The surface area of the two

180 membranes is ca. 19 cm^2. To hold the field cells inside the river, they were attached to a

181 floating life buoy which was fixed with a rope to the corner of a bridge.

182 DMT measurements in the lab were run at room temperature.

183   3.2.2   Instrumentation for AGNES

184 Voltammetric measurements were carried out with an Eco Chemie Autolab

185 PGSTAT12 potentiostat attached to a Metrohm 663VA Stand and to a computer by

186 means of the GPES 4.9 (Eco Chemie) software package. The working electrode was a

187 Metrohm multimode mercury drop electrode. The smallest drop in our stand was chosen

188 for AGNES experiments (r_0=1.41×10^{-4} m). The auxiliary electrode was a glassy carbon

189 electrode and the reference electrode was Ag/AgCl/3 mol L^{-1} KCl, encased in a 0.1 mol

190 L^{-1} KNO$_3$ jacket. A glass combined electrode (Orion 9103) was attached to an Ion

191 check 45 Radiometer analytical ion analyser and introduced in the cell to control the

192 pH. A glass jacketed cell thermostated (at 25.0 ºC for synthetic and soil samples

193 analysis and at 12.8ºC for Rhine river analysis) was used in all measurements, which

194 were performed under a purified nitrogen atmosphere. A Peristaltic pump (Gilson

195 Minipuls III) was used in the N$_2$/CO$_2$ purging system.

196

197

198 ## 3.1   Samples

199 ### 3.1.1   Synthetic samples

200   Two different solutions were prepared: (1) 0.71 µM Zn, 2.00 µM NTA, 10 mM

201 $CaCl_2$, pH around 5.5; (2) 2.23 µM Zn, 2.00 µM NTA, 10 mM $CaCl_2$, pH 6.20.

202 ### 3.1.2   Rhine River water

203   For AGNES experiments, sampling from Rhine took place on 28th March 2011

204 in Wageningen (The Netherlands). Water was collected at 50 cm depth and at about 0.3

205 m from the riverbank with a clean polyethylene bottle. River water samples were

206 immediately transferred to the laboratory where they were filtered and stored in the

207 fridge at 3°C for AGNES analysis the following day.

208   The field DMT cells were installed on 13th April 2011 in the Rhine river at the

209 same place of the previous sampling. pH, temperature and conductivity were measured

210 *in situ*.

211 ### 3.1.3   Soil samples

212   Four types of soils labelled rivier clay, loam, cover sand and reclaimed peat were

213 used. They were sampled in 2008 from grassland fields in the Netherlands at 0-15 cm

214 depth. The soil samples were air dried, sieved through 2 mm and stored in plastic

215 bottles. The most relevant physicochemical parameters of these soils were determined

216 following classical approaches and the results are shown in Table 1. To analyze free

217 Zn^{2+} concentration in the soil samples with AGNES and DMT, an extraction with $CaCl_2$

218 was made. 60 grams of soil were added to 600 mL of 10 mM $CaCl_2$ (soil solution ratio

219 1:10). After 24 h end-over-end shaking at 20 °C, the extract was filtered and used for

220 DMT and AGNES analysis.

3.2   Procedures

221

222   AGNES and DMT experiments for synthetic and soil samples were carried out

223   with the same samples derived from the preparation of Zn-NTA solutions and soil

224   extraction (*i.e.* each final volume was split into 2). Rhine river sample was analysed

225   with DMT *in situ* and with AGNES in the lab. All treatments were done in duplicate.

3.2.1   Set up of the DMT

226

227   Zn-NTA synthetic samples and soil samples were analysed using the lab DMT

228   cell, while Rhine river sample was analysed *in situ* using the field DMT cell. The

229   construction of the cells is extensively described elsewhere (Kalis et al., 2006b). In the

230   experiments with lab DMT cells, 20 mL of 10 mM $CaCl_2$ was employed as acceptor

231   solution. For synthetic samples, a volume of 350 mL Zn-NTA solution was used as the

232   donor solution. For the soil samples, 300 mL of 10 mM $CaCl_2$ extract were taken to be

233   used as donor solution. Aliquots of 5.5 mL from the acceptor and donor solutions were

234   taken at 0 h, 24 h and 48 h and stored for further analysis. After each sampling, the

235   acceptor solution was refilled with fresh acceptor solution (10 mM $CaCl_2$).

236   Field DMT cells were used to analyse Rhine river water *in situ*. 12 mL of

237   synthetic Rhine river water was used as acceptor solution, which was prepared based on

238   the major ion composition found in previous analysis (Ca: 1.90 mM; K: 0.120 mM; Mg:

239   0.480 mM; Na: 2.04 mM; Cl: 6.91 mM) and was adjusted to pH 7.80. In this case, the

240   donor solution was the river Rhine. Two cells were taken out of the river after 48 and 72

241   h, respectively. The cells were transported to the lab in metal-free boxes to minimize

242   contamination. The acceptor solution was sucked out of the cell through an outlet using

243   a syringe and stored for further analysis. In all cases, the pH was measured at each

244   sampling time.

245   ### 3.2.2   Set up of AGNES

246   To determine free Zn^{2+} concentration, AGNES 1P (one potential pulse in the

247   deposition stage) and AGNES 2P (two-pulses) was implemented on 50 mL of sample.

248   The algorithm described in (Pernet-Coudrier et al., 2011) was followed to find the

249   optimal settings. The pH in the solution was adjusted employing the system of N_2/CO_2

250   purging described in (Zavarise et al., 2010) for the experiments with natural samples

251   (for river water see table 7 and for soils see table 8). The gain Y was adjusted to produce

252   currents around 5×10^{-8} A. Thus, AGNES parameters used to determine the free Zn^{2+}

253   concentration in all samples analysed are listed in Table 2.

254   # 4. Results and discussion

255   ## 4.1   Determination of free Zn^{2+} in synthetic samples

256   Zn and NTA is a well-known system that has been previously studied with DMT

257   and AGNES (Alberti et al., 2007;Kalis et al., 2007b), so it can be considered as suitable

258   to perform the comparison between both techniques. Two solutions with a common

259   NTA concentration of 2.00 μM and total Zn concentrations of 0.71 and 2.23 μM were

260   analysed by lab DMT cell and AGNES. Each replicate of DMT and AGNES

261   experiment (for a given total Zn concentration) is labelled as replicate I, II, III or IV

262   (Table 3). Previous experiments have shown that 48 h is a suitable time using DMT in

263   lab for metal ions to reach the Donnan membrane equilibrium (Kalis et al., 2006b).

264   Table 3 gathers the free Zn^{2+} concentrations retrieved by DMT (using eqn. 1

265   and Ca concentrations reported in Table 4) and by AGNES (using eqn. 2) highlighted

266   as bold numbers. A good agreement between the free Zn^{2+} concentrations measured

267   with both techniques and that calculated from VMINTEQ (in between brackets), at the

268   measured pH and temperature, can be seen at all levels of total Zn. Speciation

269   calculations with VMINTEQ indicate that a temperature difference (around 5ºC) leads

270   to negligible variations in the free Zn concentration in these conditions. However, a

271   perfect comparison between replicates of DMT and AGNES in Table 3 is not possible,

272   because the pH value was not exactly the same in all the experiments. A correction of

273   the pH fluctuations on measurements can be applied by taking into account the small

274   impact of pH variation on VMINTEQ calculations of speciation:

275    $$[Zn^{2+}]_{corrected} = [Zn^{2+}]_{measured} \times \frac{[Zn^{2+}]_{theoretical\ at\ pH\ 5.29\ or\ 6.30}}{[Zn^{2+}]_{theoretical,\ pH\ measured}} \qquad (3)$$

276   The average pH 5.29 has been selected as reference pH in the case of low Zn

277   concentration experiments and 6.30 was chosen for the high Zn concentration ones. The

278   application of this correction (see details of computation in Table 5) yields less than 2%

279   of difference between average results of both techniques at a total Zn concentration of

280   0.71 µM and below 15% for total Zn concentration of 2.23 µM. These differences can

281   be attributed to the experimental error of each technique.

282   We conclude that DMT and AGNES agree very well in the measurement of free

283   Zn^{2+} in the sample (which is around 40% of the total). From the implementation of both

284   techniques, we can compare the time scales of the techniques. Figure 2 shows the

285   evolution of the system with the higher total Zn concentration until equilibrium with the

286   Zn–NTA solution is achieved. In this case, the AGNES procedure requires 640 s ($t_{1,a}$ +

287    $t_{1,b}$= 160s + 480s) to reach equilibrium in the first stage, whilst for DMT 24 h have been

288   required. A similar observation can be done with the solution at a lower Zn

289   concentration. This difference in equilibration times can be attributed to the slow

290   permeation across the DMT membrane and the relatively large volume of acceptor

291   solution.

292   4.2   Determination of free Zn^{2+} in Rhine river water

293   Taking into account the low free metal concentrations in natural waters and the

294   required handling for DMT in lab, DMT *in situ* has been employed to avoid

295   contamination and to use larger sample volumes of river as donor solution for better

296   buffering. Table 6 shows the temperature, pH, conductivity and DOC of Rhine river

297   water on three different days (in Spring 2011), at the same sampling place (*i.e.* where

298   the water for AGNES experiments was taken and where the set-up of DMT *in situ* was

299   deployed) in the Rhine river as in (Kalis et al., 2006b). From one day to the other, the

300   retrieved physicochemical parameters are, in general, not very different (Table 6). The

301   main variations affecting speciation are in pH and DOC.

302   AGNES determination was carried out with the N_2/CO_2 purging system

303   (Zavarise et al., 2010) to adjust and fix the pH to the one measured *in situ* in the river

304   water. The standard procedure in AGNES is to deaerate with N_2, but this removes gases

305   such as CO_2, yielding changes in pH and, therefore, modifying the speciation. The

306   N_2/CO_2 system allows removing O_2 while keeping the original pH.

307   Table 7 gathers the total Zn concentration measured with ICP-MS and free Zn^{2+}

308   concentration measured with DMT (considering Ca concentrations reported in Table 4)

309   and AGNES in Rhine water.

310   The total concentration of Zn in both samples differs by less than 18%. Free

311   Zn^{2+} measured using DMT at 48h shows reproducible results between the duplicates

312   and the DMT average falls in the range of the average +/- the standard deviation of

313   AGNES measurement using eight replicates. Free Zn^{2+} determined with AGNES is, on

314   average, 13% below the one measured with DMT. This could, perhaps, be explained by

315   the DOC concentration, which is higher in the water sample analysed with AGNES than

316   with DMT, and therefore, more Zn could be complexed.

317   DMT technique had been previously applied to determine the free Zn^{2+}

318   concentration in Rhine river water at the same location in different seasons, in Summer

319   of 2003 and Winter of 2003/2004 (Kalis et al., 2006b). Despite this paucity of data, we

320   proceed to a tentative comparison of the existing results. The total Zn concentration

321   measured in this work, in Spring of 2011 (0.14 to 0.17 μM, Table 7) is lower than that

322   measured in Summer of 2003 and Winter of 2003/2004 (0.28 and 0.20 μM,

323   respectively). However, the free Zn^{2+} measured in this work (0.084 to 0.097 μM, Table

324   7) is higher than both measurements in 2003/2004 (0.035 and 0.008 μM). Thus, the

325   percentage of free Zn^{2+} over total Zn is significantly higher in this study of 2011 (69 and

326   49%) than in 2003/2004 (Summer 12.6%, Winter 4.3%).

327   Because no significant change in pH and DOC was found between 2003/2004 and 2011,

328   it is unclear to us which reason has caused this change in free Zn^{2+} fraction besides

329   some uncertainties in the analysis. One possible reason is the presence in 2003/2004 of

330   Zn-containing colloidal particles that can pass the 0.45μm filter. This fraction, if

331   present, would have contributed to the total soluble Zn measured and therefore led to a

332   lower free Zn^{2+} to total Zn ratio. Another possibility is the change of composition and

333   binding affinity of DOC. Presence of synthetic ligands like EDTA, which has a high

334   affinity for metals like Zn, will contribute to Zn complexation in the river. Indeed,

335   monitoring data show that EDTA concentration in river Rhine has decreased over the

336   past years (RIWA, 2011;Schmidt et al., 2004). From 2003 to 2010, EDTA concentration

337   in river Rhine is about halved (RIWA, 2011). The strong decrease of EDTA may partly

338   explain the higher free to total ratio of Zn in this study than measured in 2003/2004

339   (Kalis et al., 2006b). It was also reported that EDTA concentration in river Rhine is in

340   general higher in Summer than in Winter time (Schmidt et al., 2004). This may also

341   partly explain the lower free Zn^{2+} concentration measured in Summer than in Winter in

342   2003/2004 by Kalis *et al.* (Kalis et al., 2006b). However, if all Zn measured in the

343   solution is soluble, the complexation with EDTA cannot fully explain the variations of

344   free Zn^{2+} fraction, because total Zn in the river is of the order of 10^{-7} M, whereas EDTA

345   in the 10^{-8} M range (Schmidt et al., 2004). Further research, such as detailed time series

346   measurements of speciation to evaluate its temporal variability, is needed to understand

347   this.

4.3   Determination of free Zn^{2+} in soil samples

349   The determination of free Zn^{2+} in soils is quite important (Jansen et al., 2001),

350   since free metal and labile metal concentrations have been correlated with metal uptake

351   by plants (Kalis et al., 2007a;Kalis et al., 2006a;Temminghoff et al., 2008). Free Zn^{2+}

352   concentrations were measured in duplicate with DMT and AGNES in 10 mM $CaCl_2$

353   extractions of four different soils to establish a comparison between AGNES and DMT

354   determinations. The 10 mM $CaCl_2$ extraction corresponds to a standardised lab

355   procedure which resembles the soil solution in pH and ionic strength (van Erp et al.,

356   1998).

357   Table 1 gathers some basic soil characteristics measured for these soils. The pH

358   measured at the end of DMT experiments had varied from the initial one, but it was

359   constant in AGNES (where the N_2/CO_2 system was used). Table 8 gathers the total Zn

360   concentration measured with ICP-MS and free Zn^{2+} concentration measured with DMT

361   (using Ca concentrations reported in Table 4) and AGNES.

362   The free Zn^{2+} concentrations measured between replicates (I and II) were

363   reproducible in clay, sand and loam soil extractions. However, close to 50% of

364   difference was found between the replicate I and II of reclaimed peat soil extraction.

365   Perhaps, it could be due to the heterogeneity of the soil sub-samples or differences in

366   the extraction procedure, given that AGNES and DMT show similar values for a given

367   replicate.

368   In most of the extractions, differences between DMT and AGNES range

369   between 0.6 and 17%. Only, the replicate I of loam soil showed a difference of 26%.

370   The total Zn measured confirms the different origins of the soils. The total Zn content is

371   around three times higher in sand and reclaimed peat extractions, than in clay and loam.

372   However, the ratio of free to total Zn varies in a much narrower range between about

373   40-80%. The results are in agreement with earlier measurement using DMT (Weng et

374   al., 2001b) and confirm that, contrary to metal ions like Cu, which are present in soil

375   solution mainly as complexed with DOC, a large fraction of Zn in soil solution is

376   present as free ion.

377   By assuming that 2 times DOC equals the total amount of dissolved organic

378   matter (DOM), and 60% of DOM behaves as humic acid (HA) in terms of metal

379   binding, we have calculated the theoretical free Zn^{2+} concentration in the soil

380   extractions, from total Zn measured and considering Zn adsorption to DOM using

381   ECOSAT (Keizer and van Riemsdijk, 1994). The adsorption was calculated using

382   NICA-Donnan model (Kinniburgh et al., 1999) with model parameters for generic HA

383   (Milne et al., 2001). In this calculation, we used DOC concentration in the donor

384   measured at the end of the DMT measurement (72 h). The model prediction is given in

385   Table 8. The predicted free Zn^{2+} agrees well with those measured.

5. Conclusions

386

387   AGNES and DMT are reliable techniques to determine free Zn^{2+} concentrations.

388   The analyses of synthetic Zn-NTA solutions and soils with AGNES and DMT have

389   shown good agreement. Alike correlation was found between the results obtained with

390   samples of Rhine river water analysed with DMT *in situ* and AGNES. A previous study

391 (Sigg et al., 2006) measuring free metal concentrations with DMT and PLM in the same

392 surface waters yielded similar results. The agreement between various techniques

393 provides mutual validation in the application of these techniques.

394   The implementation of AGNES and DMT for the systems analysed in this work

395 (with free Zn concentrations above 80 nM) allows a first comparison of some aspects of

396 these techniques. DMT can already be applied *in situ*, while AGNES requires future

397 work, possibly exploiting the capabilities of the Screen Printed Electrodes (Parat et al.,

398 2011a;Parat et al., 2011b). DMT can accumulate several cations in one deployment,

399 which can then be quantified with ICP-MS or similar; AGNES requires specific

400 potential programs for each metal in a mixture (Parat et al., 2011b) and published work

401 on AGNES only reports determinations on three elements: Zn, Cd and Pb. DMT does

402 not require any purging of dissolved oxygen in the aqueous solution; AGNES can

403 achieve this removal of O_2 while buffering the pH with a mixture of N_2 and CO_2

404 (Zavarise et al., 2010). DMT equilibrium requires longer times (of the order of 48 h)

405 than AGNES (of the order of 15 minutes) for the systems considered in this work (see

406 section 4.1). The lowest free concentrations that can be achieved in both techniques

407 depends on a number of factors, such as type of electrode, acceptable deposition time

408 and composition of the sample in the case of AGNES and such as acceptor composition,

409 acceptable equilibration time and ICP-MS limit of detection in the case of DMT;

410 nanomolar concentrations of Zn in seawater had been reached with AGNES (Galceran

411 et al., 2007); a few tens of picomolar concentrations had been found for Cd with DMT

412 in lake water (Kalis et al., 2006b). Both techniques can be considered as low cost in

413 their application (i.e. once the equipment has been acquired). Both techniques are

414 extremely easy to interpret and provide the free metal ion concentration directly.

415

416

Acknowledgments

423

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Figures

(a)   (b)

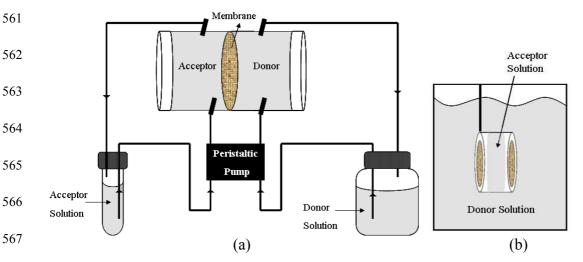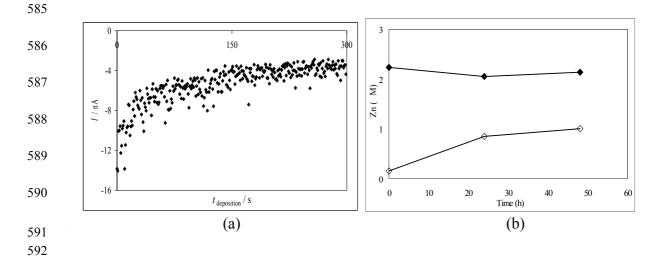Figure 1. Schematic representation of the DMT cell. (a) Lab cell. (b) Field cell.

(a)   (b)

Figure 2. Equilibrium situation achieved in AGNES and DMT procedures in a solution containing $c_{T,Zn}$=2.23 µM, $c_{T,NTA}$=2.00 µM and concentration of CaCl$_2$=0.01 M (a) First stage of AGNES one pulse: Y=10, t_1=300 s. (b) Evolution of total Zn concentration in (♦) donor and (◊) acceptor solution during DMT analysis.

600 **Tables**

601

602 Table 1. pH, cation exchange capacity (CEC), organic matter content, clay and

603 dissolved organic content (measured after the extraction and filtering process) of the 4

604 analysed soils.

Soil	pH	CEC (meq/100g)	Organic Matter (%)	Clay (%)	Initial DOC (mg / L)
Rivier clay	5.0	206	8.5	18	20.2
Cover sand	4.5	47	4.8	-	17.8
Loam	6.2	122	5.2	17	18.7
Reclaimed peat	5.2	126	8.3	-	21.4

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607

608   Table 2. AGNES parameters used to determine the free Zn concentration in synthetic

609   and natural samples. $t_{1,b}$ in some cases was greater than the standard recommended time

610   ($t_{1,b} = 3 \times t_{1,a}$ (Companys et al., 2005)) in order to be on the safe side.

Sample	$Y_{1,a}$	$t_{1,a}$ / s	$Y_{1,b}$	$t_{1,b}$ / s	t_w / s	Y_2	t_2 / s
NTA-low Zn concentration	10^{10}	160	100	480	50	10^{-8}	50
NTA-high Zn concentration	-	-	10	200 300			
Rhine River	10^{10}	450	500	1400			
		900	1000	2700			
Rivier clay	10^{10}	30	40	50 150			
	-	-	20	300 700			
Cover sand	10^{10}	50	40	50 150			
		40	40	50 150			
Loam	10^{10}	50	40	50 150			
Reclaimed peat	-	-	40	800 1500			
	-	-	20	400 700			
	10^{10}	30	40	50 150			

611

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612   Table 3.   Total and free Zn^{2+} concentrations measured and predicted in Zn-NTA

613   solutions with $c_{T,NTA}$=2.00 μM and 10 mM concentration of $CaCl_2$. For each total

614   concentration, replicates are distinguished as I and II (DMT), III and IV (AGNES).

$c_{T,Zn}$ / μM	DMT (t=48h)				AGNES			
	I		II		III		IV	
	pH	[Zn^{2+}]/μM	pH	[Zn^{2+}]/μM	pH	[Zn^{2+}]/μM	pH	[Zn^{2+}]/μM
0.71	5.33	**0.27** (0.30)	5.11	**0.30** (0.29)	5.35	**0.29** (0.30)	5.38	**0.28** (0.28)
2.23	6.43	**0.95** (1.05)	6.20	**0.93** (1.04)	6.37	**1.09** (1.07)	6.20	**1.11** (1.04)

615   Values in brackets correspond to the free Zn^{2+} concentration predicted by VMINTEQ speciation program.

616

617

618   Table 4. Total Ca^{2+} concentrations measured with ICP-OES in donor and acceptor

619   solutions at 48h of DMT experiments.

Sample	Replicate	Total Ca^{2+} concentration (mM)	
		Donor	Acceptor
NTA-low Zn concentration	I	9.96	10.1
	II	10.0	10.1
NTA-high Zn concentration	I	9.66	9.51
	II	9.32	10.1
Rhine river	I	2.15	2.30
	II	2.15	2.35
River clay	I	16.70	16.8
	II	16.5	17.2
Cover sand	I	16.3	16.9
	II	16.6	17.2
Loam	I	16.5	17.1
	II	17.0	17.2
Reclaimed peat	I	16.7	16.7
	II	16.7	17.1

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622

623   Table 5. Correction of the concentrations of Zn^{2+} in Zn+NTA solutions (see Table 3)

624   to a common pH value in order to facilitate the comparison between AGNES and DMT.

625

| Correction to pH | $c_{T,Zn}$ / μM | Corrected concentration/ μM | | | | Average | | % Difference (AGNES-DMT) /AGNES |
| | | DMT | | AGNES | | DMT | AGNES | |
		I	II	III	IV			
5.29	0.71	0.27	0.31	0.29	0.30	0.29	0.30	1.64
6.30	2.23	0.96	0.93	1.09	1.13	0.94	1.11	14.96

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629   Table 6. pH, temperature, electric conductivity (EC), ionic strength (*I*) and dissolved

630   organic carbon (DOC) in the Rhine river water. The measurements were performed *in*

631   *situ* at the day of sampling for AGNES (March 28th 2011), at the day that DMT was

632   placed *in situ* (April 13th 2011) and when collecting the device, i.e. 48 h after DMT

633   started.

Technique	pH	Temperature (°C)	EC (μS cm^{-1})	*I* (mM)[a]	DOC (ppm)
DMT *in situ* (*t*=0)	7.88	16.1	731	12.1	3.80
DMT *in situ* (*t*=48 h)	7.95	16.0	-	-	3.20
AGNES	7.94	12.8	720	11.5	4.80

634   [a] It was calculated by $I = 1.6 \times 10^{-5}$ C × EC (see Table 2330 of Ref (APHA,1998)).

635   Table 7. pH, total Zn, and free Zn concentrations in Rhine river water. Free Zn^{2+} was

636   measured with DMT (t=48h) *in situ* and AGNES purged with N_2/CO_2. pH in DMT

637   measurement corresponds to the measurement performed in the river when collecting

638   the device (i.e. 48 h); pH of AGNES is the pH of the sample during the analysis.

Technique	pH	$c_{T,Zn}$ / μM	[Zn^{2+}] /μM	
			I	II
DMT *in situ*	7.95	0.14	0.097	0.094
AGNES (n=8)	7.91	0.17	0.084 (0.012)[a]	

639   [a] Number in brackets is the standard deviation between replicates (n=8)

640

641

642   Table 8.   pH, total Zn and free Zn^{2+} concentrations measured in 10 mM $CaCl_2$

643   extractions made in duplicate with 4 soil samples collected from grassland in The

644   Netherlands. Free Zn^{2+} concentration was determined with AGNES and DMT (t=48h)

645   techniques and total Zn with ICP-MS. Free Zn predicted through NICA-Donnan model

646   (see section 4.3) is derived from measured DOC values.

Soil	Replicate	$c_{T,Zn}$ / μM	AGNES			DMT					% Difference (AGNES-DMT) /DMT
			$[Zn^{2+}]$ / μM	%Free Zn	pH	$[Zn^{2+}]$ / μM	%Free Zn	pH	DOC 72 h	predicted $[Zn^{2+}]$ / μM	
Rivier	I	1.43	**0.990**	69.2	5.00	**1.19**	83.2	5.37	19.2	1.19	16.8
clay	II	1.48	**0.961**	64.9	5.00	**1.10**	74.3	5.40	19.7	1.18	12.6
Cover	I	3.19	**2.38**	74.6	4.93	**2.23**	69.9	5.04	15.3	2.76	6.73
sand	II	3.14	**2.25**	71.7	4.91	**2.33**	74.2	5.05	16.0	2.70	3.43
Loam	I	1.18	**0.808**	68.5	6.23	**0.640**	54.2	6.81	16.2	0.71	-26.3
	II	1.07	**0.785**	73.4	6.24	**0.790**	73.8	6.67	13.5	0.71	0.63
Reclaimed	I	2.67	**1.91**	71.5	5.40	**1.93**	72.3	6.09	14.7	2.14	1.04
peat	II	3.08	**1.16**	37.7	5.40	**1.36**	44.2	5.76	18.9	2.56	14.7

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