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A novel Cyphos IL 104-based polymer inclusion membrane (PIM) probe to mimic biofilm zinc accumulation

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Abstract

The presence of Zn in surface waters from abandoned mining zones is a critical issue since excess Zn concentrations may affect aquatic life and whole ecosystems. We present, for the first time, a simple tool based on a polymer inclusion membrane (PIM) intended to monitor Zn in river water by mimicking metal accumulation in the biofilm. The PIM-based probe contains a polymeric membrane prepared using cellulose triacetate (CTA, 50% w/w) as the base polymer, nitrophenyloctyl ether (NPOE) as the plasticizer (20% w/w), and the ionic liquid (IL) Cyphos 104 as the extractant (30% w/w). The accumulation of Zn in the acceptor phase (0.01 M HNO_3*) was evaluated for different free metal concentrations at 4 h accumulation time resulting in a good correlation between the free metal concentration and the accumulated one. We also found that the metal accumulated agrees with the free metal fraction upon addition of EDTA in the donor solution. The results for Zn accumulation with the PIM-based probe were found to be comparable to those obtained for a biofilm that was grown in a stream from an abandoned mine area and subsequently translocated to the laboratory and put in contact with Zn polluted stream water, so confirming the effectiveness of this new probe in mimicking Zn accumulation in the biofilm.*

Keywords Zn · Polymer inclusion membrane · Cyphos IL 104 River water · Metal accumulation · Biofilm

1. **Introduction**

The presence of metals in the environmental is an issue of great concern both for the general public and the scientific community. Metals not only affect water quality but can also accumulate in living organisms, having an adverse effect on them. Zinc is a common contaminant of surface freshwater, with no observed toxicity at concentrations below 10 µg L^{-1}. However, it may be toxic at the concentrations found in many fluvial systems which vary from µg to mg [1], where mining activity has been the main source of contamination[2–5]. For instance, Gozzard et al. (2011) [4], reported that zinc is the most commonly encountered metal in the surface waters of metal mining regions in the United Kingdom. High zinc concentrations (above 1000 µg L^{-1}), are common in stream water in the Dalarna region of Sweden, which is known for metal exploitation. Zinc occurrence has motivated environmental regulation in countries such as Portugal (APA, 2016), Canada (ATSDR, 2014), USA (CEPA, 1999) and Spain (BOE, 2011), which set limits in the range of 30-59 µg L^{-1} and 210-500 µg L^{-1} (depending on the water hardness). The toxicity of Zn is associated with an overproduction of reactive oxygen species leading to oxidative stress resulting in lipid peroxidation, protein damage and DNA alterations affecting most aquatic organisms [5]. It can become toxic for fish at increased water levels as the main target is the gills, where the calcium uptake is disrupted, leading to hypocalcaemia and eventually to death [6].

The term toxicity, applied to aquatic ecosystems, is closely related to the internalization process of the pollutant and its specificity. It is broadly accepted that the bioavailability and toxicity of metals cannot be predicted by total concentrations, but rather by the concentration of various chemical species, in particular the free ion [7]. Therefore, the assessment of bioavailability requires either the manipulation of biological entities, such as microorganisms, algae, insects, plants or biofilm in aquatic ecosystems, or a chemical test can be performed if the uptake of the contaminant by an organism can be mimicked by a chemical process [8].

Permeation liquid membranes (PLM) have been proposed as chemical probes to measure bioavailability for inorganic species [9]. The separation of species with PLMs is based on carrier-mediated mechanism. The extractant interacts with the species of interest initially present in the donor, forming a complex at the interface donor solution-PIM. The complex then diffuses to the interface PIM-acceptor solution where the species is back-extracted and finally released into the acceptor. This chemical pumping allows the accumulation of the analyte in the acceptor solution even against the concentration gradient. As a consequence, the efficiency of the PLM is mainly dictated by the extractant used. PLMs incorporating selective extractants have been used as analytical tools to study copper, lead, cadmium, zinc and nickel speciation [10,11]. However, the use of PLM is often hampered due to the solubility of the extractants in the adjacent water media affecting membrane stability and effectivity.

Polymer inclusion membranes (PIM) are advanced liquid extracting membranes that are composed of a base polymer, providing mechanical strength, an extractant, and, in some cases, a plasticizer, which provides elasticity to the membrane [12]. Since the extractant is immobilized within the chains of the base polymer, PIMs are more stable than PLMs, and highly selective when the appropriate extractant is used. As general purpose extractant for cations, the compound di(2-ethylhexyl)phosphoric acid (D2EHPA) has been extensively used. Moreover, D2EHPA has been incorporated in a passive sampler PIM-based device to determine Zn in pond waters [13]. In our previous work, we developed a device incorporating a PIM containing D2EHPA for the measurement of free Zn concentrations in hydroponic solution and its performance has been compared with the fluxes measured in the roots of potato plants [14].

Recently, more lipophilic, less-water soluble, and less toxic extractants have been investigated and implemented in membrane systems, and among them, ionic liquids (IL) have received particular attention. Ionic liquids (ILs) are liquid molten salts at room temperature formed by an organic cation (dialkylimidazolium, tetralkylammonium, pyridinium, and phosphonium) and an organic or inorganic anion, which exhibit certain remarkable characteristics, such as negligible vapour pressure, great thermal and chemical stability, high ion conductivity, and low volatility. They have been exploited in analytical sciences in different application fields (e.g., chromatography, electrochemistry or extraction). Moreover, they have been used as tailor-made extractants exhibiting greater selectivity than other conventional extractant reagents [15]. In particular, the IL trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate (Cyphos IL 104 or Cyphos 104) has been used for Cr(VI) transport using a plasticized PIM [16], Au(III) transport from hydrochloric acid solutions [17], Cd(II) and Cu(II) separation also from hydrochloric acid solutions [18], and ~~Zn(II) transport or for the~~ separation of Zn(II) from Fe(III) from highly acidic solutions [19,20].

In the present work, a new PIM-based probe containing Cyphos IL 104 has been evaluated in natural water for the first time to perform Zn accumulation measurements and to model metal accumulation (Zn) in biofilm. The PIM-based probe was prepared and calibrated in the laboratory using model solutions. The selectivity of the PIM-probe towards the free metal species will also be investigated by the addition of an organic ligand (ethylenediaminetetraacetate, EDTA). The assessment of the new probe in mimicking the Zn accumulation in biological systems has been tested by comparison with fluvial biofilms that were grown on submerged substrata in the Osor Stream, a river polluted with Zn due to past mining activity, and then translocated to the laboratory.

2. Experimental

2.1 Reagents and solutions

A Zn stock solution (500 mg L^{-1}) was prepared from the solid $ZnSO_4.7H_2O$ (Panreac) and a standard solution of 1000 mg L^{-1} Zn (Sigma Aldrich) was used to prepare the calibration standards for ICP analysis. 2-(N-morpholino) ethanesulfonic acid (MES) (Fluka) was used as a buffer. Other solids used were: $Cu(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$, all of them from Panreac.

To prepare the membranes, cellulose triacetate (CTA) polymer (Acros Organics), the plasticizer 2-nitrophenyloctyl ether (NPOE) (Sigma-Aldrich), and chloroform (Panreac) were used. Trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate (Cyphos IL 104) was kindly donated by Cytec (USA).

The organic ligands trans-1,2-diaminocyclohexan-N,N,N′,N′-tetraacetic acid (CDTA) and ethylenediamine N,N,N′,N′-tetraacetic acid disodium salt (EDTA) were purchased from Sigma-Aldrich. HNO_3 69% (Panreac) and sodium sulphate (Merck) were also employed.

Ultrapure water with resistivity ≥18 µS cm^{-1} was taken from a MilliQ system (Millipore).

2.2 Instruments

A GLP-22 pH-meter (Crison) was used to measure the pH of the samples. Conductivity was monitored with an Ecoscan portable conductometer from Entech Instruments. Total organic carbon was measured with a TOC-V CSH from Shimadzu.

Zn concentrations were determined using an inductively-coupled plasma atomic emission spectrometer, ICP-AES 5100 (Agilent Technologies).

2.3 Water samples

The appropriate volume of Zn stock solution was added to simulated natural water (SNW) containing 2 mM $NaHCO_3$, 0.25 mM Na_2SO_4, 0.75 mM $CaCl_2$ and 2.5 mM MES solution (pH 6.3). The preparation of SNW was adapted from [21] and the chemical parameters of Osor Stream water (Table S1) were also taken into account.

Water samples from the Osor Stream (41°56'51"N, 2°33'25"E), a small tributary of the River Ter (Girona, Spain), were collected during different time periods (July 2017, March-April 2018).

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This stream is influenced by continuous (effluent) and diffuse (run-off) metal inputs from a disused mine [22].

Two sampling points were selected: a reference point (Osor-R), where Zn was not detected by ICP-MS (< 1.5 µg L^{-1}), and a point downstream of the effluent from the mine area (Osor-M), where $[Zn]_{total}$ varied from 200 to 450 µg L^{-1} (depending on the sampling period). The main chemical parameters of these samples were pH 8.2-8.3, conductivity 400-500 µS cm^{-1}, and TOC 2-3 mg C L^{-1} (see Table S1). Additionally, to compare the influence of the metal concentration on both the PIM-probe and the biofilm, another water sample, Osor-d, was obtained by diluting Osor-M with Osor-R (60:40, v/v) in order to ensure the same chemical characteristics.

2.4 Membrane preparation and PIM-probe experiments

A PIM containing 50% CTA: 30% Cyphos 104: 20% NPOE (% in w/w) [19] was prepared by a casting method. First, the ability of the PIM to extract Zn was investigated by contacting a piece of PIM (2 cm x 2cm) with 500 µg L^{-1} Zn in 15 mL of ultrapure water, SNW, or in solutions separately containing 2 mM hydrogen carbonate, 0.25 mM sulphate, or 1.5 mM chloride (the same concentration as in SNW). The contact time was 4 h under rotary agitation. The metal remaining in the solution was determined and the zinc extracted in the PIM was calculated by mass balance. The same procedure was followed to study the selectivity of the PIM using solutions containing Cu(II), Pb(II), Ni(II) or Cd(II) at a concentration 500 µg L^{-1} each.

For the accumulation measurements, the PIM was mounted on a special device [14], which was immersed 1 cm in a vertical position in the donor water sample containing Zn, under agitation. The exposed area was 2.5 cm^2 and the acceptor solution (2.5 mL) was located inside the device. The volume of donor phase was 250 mL and magnetic stirring was applied. Different acceptor phase compositions, 0.01 M HNO_3, 5 $x10^{-4}$ M CDTA and 0.5 M Na_2SO_4, were tested to recover the extracted zinc from the PIM. Calibration was performed, fixing 4 h as the accumulation time, 0.01 M HNO_3 as acceptor solution under magnetic stirring. The same experimental conditions as in the calibration were used when free zinc was measured in spiked SNW and in spiked Osor-R (both at 0.50 mg L^{-1} Zn), and also in Osor-M.

An additional accumulation experiment was performed using Osor-M in 10 L buckets under agitation (teflon blades -Heidolph Hei-TORQUE Value 100) at 150 rpm.

2.5 Metal accumulation in biofilm

Biofilms were grown on submerged artificial substrata (7 cm x 7 cm sand-blasted glass supports) at the colonization site (Osor-R) for 14 days, in April 2018, under optimum conditions of light and water velocity. The substrata colonized with biofilm were then taken to the laboratory and subjected to different treatments (see the experimental lay-out presented in Figure S1): three biofilm samples were immediately separated for analysis; two biofilm samples were exposed to Osor-M, and another two biofilm-colonized glasses were exposed to Osor-d in 10 L buckets under agitation (teflon blades -Heidolph Hei-TORQUE Value 100) at 150 rpm. Experiments for the determination of Zn accumulated in the PIM-based probe were simultaneously run in the 10 L buckets where biofilm was exposed.

After 4 h exposure time, the biofilm was recovered in a Petri dish, and dried in the oven at 50 ºC for 15 h to determine the dry weight (DW). Around 200-400 mg DW biofilm was digested with 4 mL of concentrated HNO_3 (65% suprapure) and 1 mL H_2O_2 (31% suprapure) in a microwave ETHOS SEL High Performance Extraction System (Milestone). The Zn concentration in the acid extract of the biofilm as well as the metal accumulated in the acceptor phase of the PIM-probe were determined by ICP-AES after filtration through a 0.45 µm cellulose acetate filter.

All experiments were run at least in duplicate at 22 ± 2 ºC.

3. Results and discussion

3.1 Performance of the PIM-based probe

The compositions of the PIM, as well as the chemical characteristics of the donor and the acceptor phases, are key factors in the design of an effective transport system. Baczynska et al. studied the effect of the plasticizer on the extraction of anionic zinc chlorocomplexes [19], finding that a PIM composition of 50% CTA: 30% Cyphos 104: 20% NPOE resulted in a higher Zn transport rate. Therefore, we fixed this composition for the PIM-based probe and we focussed in evaluating, for the first time, its effectiveness for the transport of Zn in natural waters. For this, a 500 µg L^{-1} Zn solution in SNW at pH = 6.3 was used as a donor phase, where Zn exists mainly as a free cation (94.5%, according to visual MINTEQ calculations), and it was found that the metal was successfully transported to a 0.01M HNO_3 solution used as the acceptor phase. Some exploratory experiments were performed to better understand the transport mechanism, since, to the best of our knowledge, all published work dealing with extraction of metals with Cyphos 104 has been performed with anionic species present at high hydrochloric acid concentrations [16–20]. The experiments were conducted using 500 µg L^{-1} Zn in both ultrapure water or in solutions separately containing carbonate, sulphate, or chloride (Figure S2). Similar percentages of Zn extraction were found in all cases except for ultrapure water, showing out that Cyphos 104 can extract positive charged Zn species through an ion-pair mechanism that also involves the extraction of an anion. The extraction in SNW was higher (Figure S2), around 85%, probably due to the cumulative effect of the anions that were simultaneously present. Selectivity was also studied. To this end, pieces of the PIM were contacted with Cu(II), Pb(II), Ni(II) or Cd(II) contained in SNW solution at pH 6.3. Only around 20% extraction for Cu(II) was found, and less than 5% in the other cases.

The accumulation of Zn in the PIM-based probe was then studied. Acceptor phases of different composition such as 0.01 M HNO_3, as was used in previous works [11,13], a complexing agent, CDTA (5 x10^{-4} M) and a 0.5 M Na_2SO_4 solution [19] were tested. The results are shown in Figure 1 (a) as the time dependence accumulation of Zn. As it can be observed, a 0.01 M HNO_3 solution provided a more effective transport of metal, resulting in a linear relationship between the Zn accumulated and the accumulation time until 17 h of exposure. The Zn accumulation in the acceptor phase is in agreement with a steady-state regime, and the average flux calculated is 15.5 ± 1.8 nmol cm^{-2} h^{-1} [23]. However, experiments over longer periods of time showed a decrease of Zn in the acceptor phase due to the deployment of the metal in the donor solution. For this reason, and fixing 0.01 M HNO_3 for further studies, the same type of experiment was carried out using 10 L of Osor-M, which naturally contained 314 µg L^{-1} Zn, as a donor phase (see Figure 1 (b)). The trend observed is similar to that in Figure 1(a) for 0.01 M HNO_3, with a linear accumulation at shorter times and a maximum value at 17 h. The higher volume of the donor solution ensured that no depletion of the metal occurred, reaching an equilibrium state.

Figure 1

Membrane stability in SNW was checked by measuring the weight difference of the PIM at different accumulation times *versus* the initial weight. After 6 h contact time, the mass loss of the Cyphos 104-based PIM was 3%, being 13-15% for a PIM prepared with D2EHPA (50% CTA: 30% D2EHPA: 20% NPOE (w/w)). Moreover, the reuse of the PIM was also checked and we obtained similar fluxes when a new membrane was compared with a PIM which has previously been in contact for 7 hours with the Osor-R, being 12.2 ± 0.2 and 9.8 ± 0.5 nmol cm^{-2} h^{-1}, respectively (fluxes obtained at 28 h accumulation time, n=2). The stability and the long-term performance are clear advantages for the application of the probe for extended time periods.

3.2 Calibration and speciation measurements

A calibration curve was obtained for an exposure time of 4 h affording an intermediate accumulation of Zn within steady state conditions and without depletion of the metal even when 250 mL of donor solution was used. The results obtained are depicted in Figure 2, together with the equation that relates the accumulated Zn to the free concentration of metal in the donor solution. The good correlation between the two variables is particularly noteworthy.

Figure 2

To test whether the PIM-probe can be applied to measure free Zn, different experiments were performed using SNW as a donor solution, with and without the EDTA ligand, and Osor-R and Osor-M, which contain natural ligands. The total Zn concentration (measured with ICP-AES) was between 0.48-0.51 mg L^{-1}, in agreement with the amount added. The accumulated Zn in the acceptor phase and the free Zn measured with the PIM-probe in the different water samples are presented in Table 1, and compared with Zn free values calculated by MINTEQ.

Table 1

The addition of EDTA in SNW diminishes the value of free Zn to 31.7% as calculated by MINTEQ, which is in good agreement, within the experimental error, with the 29% obtained with the PIM-based probe. Therefore, the addition of EDTA results in the formation of ~~non-labile~~ complexes with Zn that cannot be transported through the PIM (non-reactive species). For both Osor-R (with added Zn) and Osor-M water samples, around 72-87% of the total metal is measured, meaning that Zn is either in the free form, ~~or~~ forming labile complexes (i.e. complexes that dissociate at the membrane interface), or forming neutral lipophilic complexes soluble in the PIM phase. The remaining metal forms stable complexes, probably with organic ligands, or complexes that are kinetically inert ~~from the kinetic point of view~~. The results obtained highlight the good ability of the developed PIM-based probe for Zn speciation measurements in natural waters to determine the PIM concentration of Zn (Zn_{PIM} in analogy to the C_{DGT} defined for the diffusion gradients in thin film technique or DGT [23]).

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3.3 Comparison with Zn accumulated in biofilm

The developed PIM-based probe was investigated as a tool to mimic metal accumulation in living organisms. We selected biofilm communities, formed by benthic microbial communities in a structure of highly complex extracellular polymeric substances, which were previously tested for metal accumulation [22]. It was found that biofilm accumulated 150–225 µg Zn g DW^{-1} in microcosms, and 200–500 µg Zn g DW^{-1} in the Osor-M Stream (acute toxicity, 6-24h) and that the metal in biofilm continued to accumulate until the end of the experiment (5 weeks). This exposure was found to affect the structure and function of the biofilm communities [22,24].

We have compared Zn accumulation in the PIM-based probe with that in the biofilm translocated to the laboratory (see experimental section for details and Figure S1). Laboratory conditions were selected in order to exercise a tight control over the experiments. The results obtained are shown in Figure 3. The background Zn concentration in the biofilm was determined to be 110 µg Zn g DW^{-1}.

Figure 3

The high capacity of the biofilm to accumulate Zn is observed in Figure 3: the values found are in good agreement with the concentration values reported by Corcoll et al. [22]. Moreover, the Zn accumulation in both the biofilm and the PIM-probe respond in concordance with the amount of metal present in the water samples. The trend that is observed constitutes a promising result for the application of the PIM-based probe for Zn bioavailability measurements and to predict metal accumulation in biofilm. Thus, the use of this probe can avoid the long and tedious biofilm manipulation steps (collection, transportation to the laboratory, biofilm drying and acid digestion) needed to investigate metal contamination. Moreover, the PIM-probe can be easily prepared at a low cost as the estimated costs for a PIM is less than 1 euro. It is worth mentioning that this is the first study to present a direct comparison between the accumulation of a PIM-based probe and biofilm grown under natural conditions.

Conclusions

A novel ionic liquid-based PIM probe has been designed for Zn bioavailability monitoring in surface waters. The accumulation of Zn from both simulated natural water or water samples from a polluted stream was possible using a membrane containing 50% CTA: 30% Cyphos 104: 20% NPOE (w/w) and a 0.01 M HNO_3 solution as the acceptor phase. The calibration of the PIM-based probe was successfully performed and by the addition of EDTA to the donor solution, it has been demonstrated that the PIM-probe can be used for speciation studies as only a fraction of the total ~~the free~~ metal ~~and the labile complexes are~~ is measured by the PIM. Finally, the comparison of the metal measured by the PIM-probe and the metal accumulated by biofilm, showed the ability of the developed tool to perform metal bioavailability studies.

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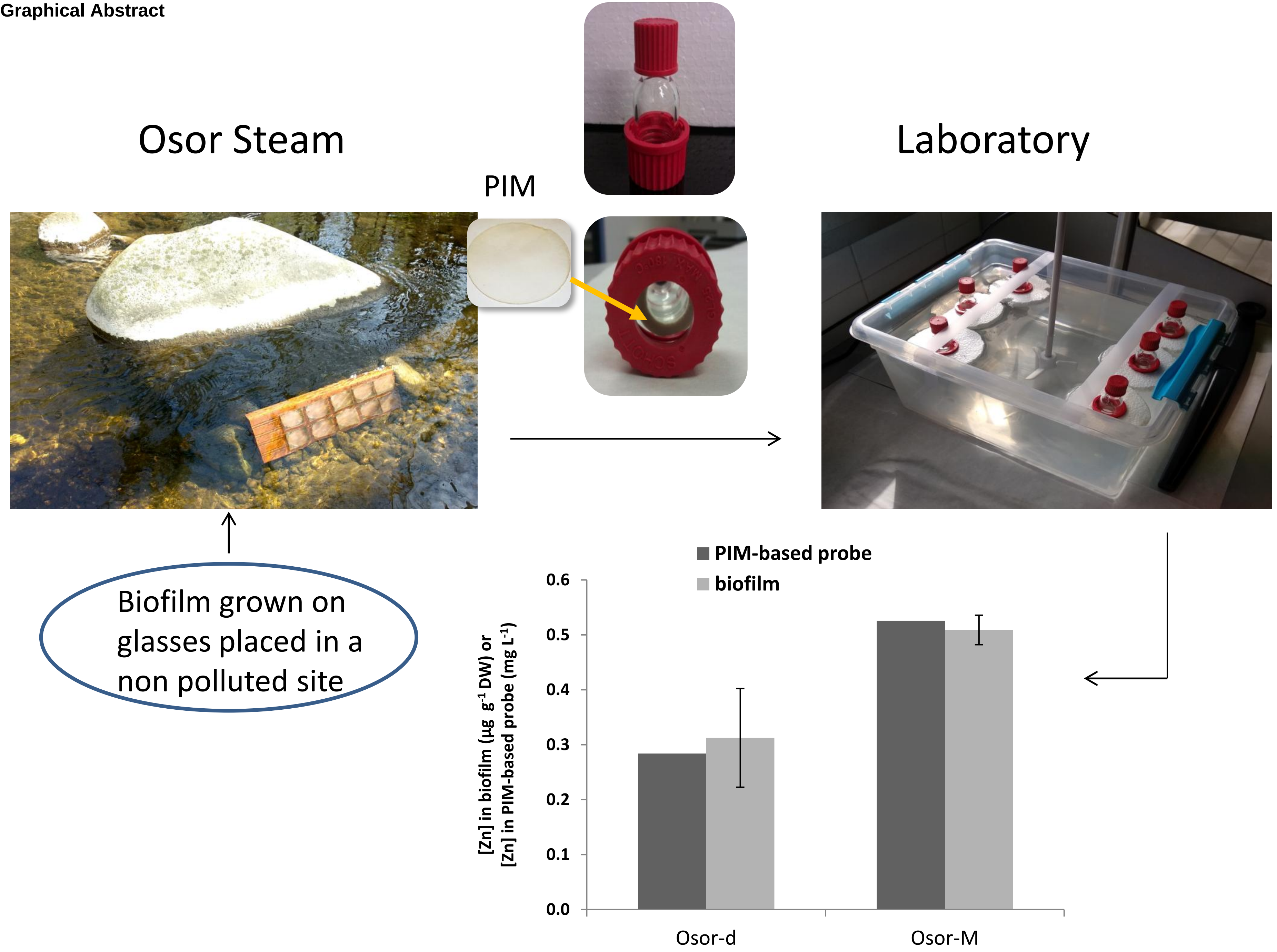
Figure captions

Figure 1. Zn accumulated at different exposure times. (a) Donor solution (250 mL): 500 µg L^{-1} Zn in SNW; (b) Donor solution: Osor-M (314 µg L^{-1} Zn), 10 L. Acceptor phase 0.01 M HNO_3.

Figure 2. Relationship between Zn accumulated in the acceptor phase and free Zn in SNW (250 mL). Acceptor phase 0.01 M HNO_3. Exposure time: 4 h.

Figure 3. Comparison of Zn accumulated in PIM-based probe (acceptor phase 0.01 M HNO_3) vs. Zn in the biofilm. Donor solution: Osor-M and Osor-d, exposure time: 4 h.

*Graphical Abstract



Osor Steam

PIM

Laboratory

Biofilm grown on glasses placed in a non polluted site

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

Highlights

-Design of a new probe containing a polymer inclusion membrane for Zn measurements

-PIM prepared with the green ionic liquid Cyphos 104 with improved stability

- Only 3% mass loss after 6 h contact time for the Cyphos 104-based PIM

-The probe measures free Zn in simulated water and water from an abandoned mine site

-The new probe is effective in mimicking Zn accumulation in biofilm

A novel Cyphos IL 104-based polymer inclusion membrane (PIM) probe to mimic biofilm zinc accumulation

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Abstract

The presence of Zn in surface waters from abandoned mining zones is a critical issue since excess Zn concentrations may affect aquatic life and whole ecosystems. We present, for the first time, a simple tool based on a polymer inclusion membrane (PIM) intended to monitor Zn in river water by mimicking metal accumulation in the biofilm. The PIM-based probe contains a polymeric membrane prepared using cellulose triacetate (CTA, 50% w/w) as the base polymer, nitrophenyloctyl ether (NPOE) as the plasticizer (20% w/w), and the ionic liquid (IL) Cyphos 104 as the extractant (30% w/w). The accumulation of Zn in the acceptor phase (0.01 M HNO_3*) was evaluated for different free metal concentrations at 4 h accumulation time resulting in a good correlation between the free metal concentration and the accumulated one. We also found that the metal accumulated agrees with the free metal fraction upon addition of EDTA in the donor solution. The results for Zn accumulation with the PIM-based probe were found to be comparable to those obtained for a biofilm that was grown in a stream from an abandoned mine area and subsequently translocated to the laboratory and put in contact with Zn polluted stream water, so confirming the effectiveness of this new probe in mimicking Zn accumulation in the biofilm.*

Keywords Zn · Polymer inclusion membrane · Cyphos IL 104 River water · Metal accumulation · Biofilm

1. **Introduction**

The presence of metals in the environmental is an issue of great concern both for the general public and the scientific community. Metals not only affect water quality but can also accumulate in living organisms, having an adverse effect on them. Zinc is a common contaminant of surface freshwater, with no observed toxicity at concentrations below 10 µg L^{-1}. However, it may be toxic at the concentrations found in many fluvial systems which vary from µg to mg [1], where mining activity has been the main source of contamination[2–5]. For instance, Gozzard et al. (2011) [4], reported that zinc is the most commonly encountered metal in the surface waters of metal mining regions in the United Kingdom. High zinc concentrations (above 1000 µg L^{-1}), are common in stream water in the Dalarna region of Sweden, which is known for metal exploitation. Zinc occurrence has motivated environmental regulation in countries such as Portugal (APA, 2016), Canada (ATSDR, 2014), USA (CEPA, 1999) and Spain (BOE, 2011), which set limits in the range of 30-59 µg L^{-1} and 210-500 µg L^{-1} (depending on the water hardness). The toxicity of Zn is associated with an overproduction of reactive oxygen species leading to oxidative stress resulting in lipid peroxidation, protein damage and DNA alterations affecting most aquatic organisms [5]. It can become toxic for fish at increased water levels as the main target is the gills, where the calcium uptake is disrupted, leading to hypocalcaemia and eventually to death [6].

The term toxicity, applied to aquatic ecosystems, is closely related to the internalization process of the pollutant and its specificity. It is broadly accepted that the bioavailability and toxicity of metals cannot be predicted by total concentrations, but rather by the concentration of various chemical species, in particular the free ion [7]. Therefore, the assessment of bioavailability requires either the manipulation of biological entities, such as microorganisms, algae, insects, plants or biofilm in aquatic ecosystems, or a chemical test can be performed if the uptake of the contaminant by an organism can be mimicked by a chemical process [8].

Permeation liquid membranes (PLM) have been proposed as chemical probes to measure bioavailability for inorganic species [9]. The separation of species with PLMs is based on carrier-mediated mechanism. The extractant interacts with the species of interest initially present in the donor, forming a complex at the interface donor solution-PIM. The complex then diffuses to the interface PIM-acceptor solution where the species is back-extracted and finally released into the acceptor. This chemical pumping allows the accumulation of the analyte in the acceptor solution even against the concentration gradient. As a consequence, the efficiency of the PLM is mainly dictated by the extractant used. PLMs incorporating selective extractants have been used as analytical tools to study copper, lead, cadmium, zinc and nickel speciation [10,11]. However, the use of PLM is often hampered due to the solubility of the extractants in the adjacent water media affecting membrane stability and effectivity.

Polymer inclusion membranes (PIM) are advanced liquid extracting membranes that are composed of a base polymer, providing mechanical strength, an extractant, and, in some cases, a plasticizer, which provides elasticity to the membrane [12]. Since the extractant is immobilized within the chains of the base polymer, PIMs are more stable than PLMs, and highly selective when the appropriate extractant is used. As general purpose extractant for cations, the compound di(2-ethylhexyl)phosphoric acid (D2EHPA) has been extensively used. Moreover, D2EHPA has been incorporated in a passive sampler PIM-based device to determine Zn in pond waters [13]. In our previous work, we developed a device incorporating a PIM containing D2EHPA for the measurement of free Zn concentrations in hydroponic solution and its performance has been compared with the fluxes measured in the roots of potato plants [14].

Recently, more lipophilic, less-water soluble, and less toxic extractants have been investigated and implemented in membrane systems, and among them, ionic liquids (IL) have received particular attention. Ionic liquids (ILs) are liquid molten salts at room temperature formed by an organic cation (dialkylimidazolium, tetralkylammonium, pyridinium, and phosphonium) and an organic or inorganic anion, which exhibit certain remarkable characteristics, such as negligible vapour pressure, great thermal and chemical stability, high ion conductivity, and low volatility. They have been exploited in analytical sciences in different application fields (e.g., chromatography, electrochemistry or extraction). Moreover, they have been used as tailor-made extractants exhibiting greater selectivity than other conventional extractant reagents [15]. In particular, the IL trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate (Cyphos IL 104 or Cyphos 104) has been used for Cr(VI) transport using a plasticized PIM [16], Au(III) transport from hydrochloric acid solutions [17], Cd(II) and Cu(II) separation also from hydrochloric acid solutions [18], and separation of Zn(II) from Fe(III) from highly acidic solutions [19,20].

In the present work, a new PIM-based probe containing Cyphos IL 104 has been evaluated in natural water for the first time to perform Zn accumulation measurements and to model metal accumulation (Zn) in biofilm. The PIM-based probe was prepared and calibrated in the laboratory using model solutions. The selectivity of the PIM-probe towards the free metal species will also be investigated by the addition of an organic ligand (ethylenediaminetetraacetate, EDTA). The assessment of the new probe in mimicking the Zn accumulation in biological systems has been tested by comparison with fluvial biofilms that were grown on submerged substrata in the Osor Stream, a river polluted with Zn due to past mining activity, and then translocated to the laboratory.

2. Experimental

2.1 Reagents and solutions

A Zn stock solution (500 mg L^{-1}) was prepared from the solid $ZnSO_4.7H_2O$ (Panreac) and a standard solution of 1000 mg L^{-1} Zn (Sigma Aldrich) was used to prepare the calibration standards for ICP analysis. 2-(N-morpholino) ethanesulfonic acid (MES) (Fluka) was used as a buffer. Other solids used were: $Cu(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$, all of them from Panreac.

To prepare the membranes, cellulose triacetate (CTA) polymer (Acros Organics), the plasticizer 2-nitrophenyloctyl ether (NPOE) (Sigma-Aldrich), and chloroform (Panreac) were used. Trihexyl(tetradecyl)phosphonium bis-2,4,4-(trimethylpentyl) phosphinate (Cyphos IL 104) was kindly donated by Cytec (USA).

The organic ligands trans-1,2-diaminocyclohexan-N,N,N′,N′-tetraacetic acid (CDTA) and ethylenediamine N,N,N′,N′-tetraacetic acid disodium salt (EDTA) were purchased from Sigma-Aldrich. HNO_3 69% (Panreac) and sodium sulphate (Merck) were also employed.

Ultrapure water with resistivity ≥18 µS cm^{-1} was taken from a MilliQ system (Millipore).

2.2 Instruments

A GLP-22 pH-meter (Crison) was used to measure the pH of the samples. Conductivity was monitored with an Ecoscan portable conductometer from Entech Instruments. Total organic carbon was measured with a TOC-V CSH from Shimadzu.

Zn concentrations were determined using an inductively-coupled plasma atomic emission spectrometer, ICP-AES 5100 (Agilent Technologies).

2.3 Water samples

The appropriate volume of Zn stock solution was added to simulated natural water (SNW) containing 2 mM $NaHCO_3$, 0.25 mM Na_2SO_4, 0.75 mM $CaCl_2$ and 2.5 mM MES solution (pH 6.3). The preparation of SNW was adapted from [21] and the chemical parameters of Osor Stream water (Table S1) were also taken into account.

Water samples from the Osor Stream (41°56'51"N, 2°33'25"E), a small tributary of the River Ter (Girona, Spain), were collected during different time periods (July 2017, March-April 2018).

This stream is influenced by continuous (effluent) and diffuse (run-off) metal inputs from a disused mine [22].

Two sampling points were selected: a reference point (Osor-R), where Zn was not detected by ICP-MS (< 1.5 µg L^{-1}), and a point downstream of the effluent from the mine area (Osor-M), where $[Zn]_{total}$ varied from 200 to 450 µg L^{-1} (depending on the sampling period). The main chemical parameters of these samples were pH 8.2-8.3, conductivity 400-500 µS cm^{-1}, and TOC 2-3 mg C L^{-1} (see Table S1). Additionally, to compare the influence of the metal concentration on both the PIM-probe and the biofilm, another water sample, Osor-d, was obtained by diluting Osor-M with Osor-R (60:40, v/v) in order to ensure the same chemical characteristics.

2.4 Membrane preparation and PIM-probe experiments

A PIM containing 50% CTA: 30% Cyphos 104: 20% NPOE (% in w/w) [19] was prepared by a casting method. First, the ability of the PIM to extract Zn was investigated by contacting a piece of PIM (2 cm x 2cm) with 500 µg L^{-1} Zn in 15 mL of ultrapure water, SNW, or in solutions separately containing 2 mM hydrogen carbonate, 0.25 mM sulphate, or 1.5 mM chloride (the same concentration as in SNW). The contact time was 4 h under rotary agitation. The metal remaining in the solution was determined and the zinc extracted in the PIM was calculated by mass balance. The same procedure was followed to study the selectivity of the PIM using solutions containing Cu(II), Pb(II), Ni(II) or Cd(II) at a concentration 500 µg L^{-1} each.

For the accumulation measurements, the PIM was mounted on a special device [14], which was immersed 1 cm in a vertical position in the donor water sample containing Zn, under agitation. The exposed area was 2.5 cm^2 and the acceptor solution (2.5 mL) was located inside the device. The volume of donor phase was 250 mL and magnetic stirring was applied. Different acceptor phase compositions, 0.01 M HNO_3, 5 x10^{-4} M CDTA and 0.5 M Na_2SO_4, were tested to recover the extracted zinc from the PIM. Calibration was performed, fixing 4 h as the accumulation time, 0.01 M HNO_3 as acceptor solution under magnetic stirring. The same experimental conditions as in the calibration were used when free zinc was measured in spiked SNW and in spiked Osor-R (both at 0.50 mg L^{-1} Zn), and also in Osor-M.

An additional accumulation experiment was performed using Osor-M in 10 L buckets under agitation (teflon blades -Heidolph Hei-TORQUE Value 100) at 150 rpm.

2.5 Metal accumulation in biofilm

Biofilms were grown on submerged artificial substrata (7 cm x 7 cm sand-blasted glass supports) at the colonization site (Osor-R) for 14 days, in April 2018, under optimum conditions of light and water velocity. The substrata colonized with biofilm were then taken to the laboratory and subjected to different treatments (see the experimental lay-out presented in Figure S1): three biofilm samples were immediately separated for analysis; two biofilm samples were exposed to Osor-M, and another two biofilm-colonized glasses were exposed to Osor-d in 10 L buckets under agitation (teflon blades -Heidolph Hei-TORQUE Value 100) at 150 rpm. Experiments for the determination of Zn accumulated in the PIM-based probe were simultaneously run in the 10 L buckets where biofilm was exposed.

After 4 h exposure time, the biofilm was recovered in a Petri dish, and dried in the oven at 50 ºC for 15 h to determine the dry weight (DW). Around 200-400 mg DW biofilm was digested with 4 mL of concentrated HNO_3 (65% suprapure) and 1 mL H_2O_2 (31% suprapure) in a microwave ETHOS SEL High Performance Extraction System (Milestone). The Zn concentration in the acid extract of the biofilm as well as the metal accumulated in the acceptor phase of the PIM-probe were determined by ICP-AES after filtration through a 0.45 µm cellulose acetate filter.

All experiments were run at least in duplicate at 22 ± 2 ºC.

3. Results and discussion

3.1 Performance of the PIM-based probe

The compositions of the PIM, as well as the chemical characteristics of the donor and the acceptor phases, are key factors in the design of an effective transport system. Baczynska et al. studied the effect of the plasticizer on the extraction of anionic zinc chlorocomplexes [19], finding that a PIM composition of 50% CTA: 30% Cyphos 104: 20% NPOE resulted in a higher Zn transport rate. Therefore, we fixed this composition for the PIM-based probe and we focussed in evaluating, for the first time, its effectiveness for the transport of Zn in natural waters. For this, a 500 µg L^{-1} Zn solution in SNW at pH = 6.3 was used as a donor phase, where Zn exists mainly as a free cation (94.5%, according to visual MINTEQ calculations), and it was found that the metal was successfully transported to a 0.01M HNO_3 solution used as the acceptor phase. Some exploratory experiments were performed to better understand the transport mechanism, since, to the best of our knowledge, all published work dealing with extraction of metals with Cyphos 104 has been performed with anionic species present at high hydrochloric acid concentrations [16–20]. The experiments were conducted using 500 µg L^{-1} Zn in both ultrapure water or in solutions separately containing carbonate, sulphate, or chloride (Figure S2). Similar percentages of Zn extraction were found in all cases except for ultrapure water, showing out that Cyphos 104 can extract positive charged Zn species through an ion-pair mechanism that also involves the extraction of an anion. The extraction in SNW was higher (Figure S2), around 85%, probably due to the cumulative effect of the anions that were simultaneously present. Selectivity was also studied. To this end, pieces of the PIM were contacted with Cu(II), Pb(II), Ni(II) or Cd(II) contained in SNW solution at pH 6.3. Only around 20% extraction for Cu(II) was found, and less than 5% in the other cases.

The accumulation of Zn in the PIM-based probe was then studied. Acceptor phases of different composition such as 0.01 M HNO_3, as was used in previous works [11,13], a complexing agent, CDTA (5 x10^{-4} M) and a 0.5 M Na_2SO_4 solution [19] were tested. The results are shown in Figure 1 (a) as the time dependence accumulation of Zn. As it can be observed, a 0.01 M HNO_3 solution provided a more effective transport of metal, resulting in a linear relationship between the Zn accumulated and the accumulation time until 17 h of exposure. The Zn accumulation in the acceptor phase is in agreement with a steady-state regime, and the average flux calculated is 15.5 ± 1.8 nmol cm^{-2} h^{-1} [23]. However, experiments over longer periods of time showed a decrease of Zn in the acceptor phase due to the deployment of the metal in the donor solution. For this reason, and fixing 0.01 M HNO_3 for further studies, the same type of experiment was carried out using 10 L of Osor-M, which naturally contained 314 µg L^{-1} Zn, as a donor phase (see Figure 1 (b)). The trend observed is similar to that in Figure 1(a) for 0.01 M HNO_3, with a linear accumulation at shorter times and a maximum value at 17 h. The higher volume of the donor solution ensured that no depletion of the metal occurred, reaching an equilibrium state.

Figure 1

Membrane stability in SNW was checked by measuring the weight difference of the PIM at different accumulation times *versus* the initial weight. After 6 h contact time, the mass loss of the Cyphos 104-based PIM was 3%, being 13-15% for a PIM prepared with D2EHPA (50% CTA: 30% D2EHPA: 20% NPOE (w/w)). Moreover, the reuse of the PIM was also checked and we obtained similar fluxes when a new membrane was compared with a PIM which has previously been in contact for 7 hours with the Osor-R, being 12.2 ± 0.2 and 9.8 ± 0.5 nmol cm^{-2} h^{-1}, respectively (fluxes obtained at 28 h accumulation time, n=2). The stability and the long-term performance are clear advantages for the application of the probe for extended time periods.

3.2 Calibration and speciation measurements

A calibration curve was obtained for an exposure time of 4 h affording an intermediate accumulation of Zn within steady state conditions and without depletion of the metal even when 250 mL of donor solution was used. The results obtained are depicted in Figure 2, together with the equation that relates the accumulated Zn to the free concentration of metal in the donor solution. The good correlation between the two variables is particularly noteworthy.

Figure 2

To test whether the PIM-probe can be applied to measure free Zn, different experiments were performed using SNW as a donor solution, with and without the EDTA ligand, and Osor-R and Osor-M, which contain natural ligands. The total Zn concentration (measured with ICP-AES) was between 0.48-0.51 mg L^{-1}, in agreement with the amount added. The accumulated Zn in the acceptor phase and the free Zn measured with the PIM-probe in the different water samples are presented in Table 1, and compared with Zn free values calculated by MINTEQ.

Table 1

The addition of EDTA in SNW diminishes the value of free Zn to 31.7% as calculated by MINTEQ, which is in good agreement, within the experimental error, with the 29% obtained with the PIM-based probe. Therefore, the addition of EDTA results in the formation of complexes with Zn that cannot be transported through the PIM (non-reactive species). For both Osor-R (with added Zn) and Osor-M water samples, around 72-87% of the total metal is measured, meaning that Zn is either in the free form, forming labile complexes (i.e. complexes that dissociate at the membrane interface), or forming neutral lipophilic complexes soluble in the PIM phase. The remaining metal forms stable complexes, probably with organic ligands, or complexes that are kinetically inert. The results obtained highlight the good ability of the developed PIM-based probe for Zn speciation measurements in natural waters to determine the PIM concentration of Zn (Zn_{PIM} in analogy to the C_{DGT} defined for the diffusion gradients in thin film technique or DGT [23]).

3.3 Comparison with Zn accumulated in biofilm

The developed PIM-based probe was investigated as a tool to mimic metal accumulation in living organisms. We selected biofilm communities, formed by benthic microbial communities in a structure of highly complex extracellular polymeric substances, which were previously tested for metal accumulation [22]. It was found that biofilm accumulated 150–225 µg Zn g DW^{-1} in microcosms, and 200–500 µg Zn g DW^{-1} in the Osor-M Stream (acute toxicity, 6-24h) and that the metal in biofilm continued to accumulate until the end of the experiment (5 weeks). This exposure was found to affect the structure and function of the biofilm communities [22,24].

We have compared Zn accumulation in the PIM-based probe with that in the biofilm translocated to the laboratory (see experimental section for details and Figure S1). Laboratory conditions were selected in order to exercise a tight control over the experiments. The results obtained are shown in Figure 3. The background Zn concentration in the biofilm was determined to be 110 µg Zn g DW^{-1}.

Figure 3

The high capacity of the biofilm to accumulate Zn is observed in Figure 3: the values found are in good agreement with the concentration values reported by Corcoll et al. [22]. Moreover, the Zn accumulation in both the biofilm and the PIM-probe respond in concordance with the amount of metal present in the water samples. The trend that is observed constitutes a promising result for the application of the PIM-based probe for Zn bioavailability measurements and to predict metal accumulation in biofilm. Thus, the use of this probe can avoid the long and tedious biofilm manipulation steps (collection, transportation to the laboratory, biofilm drying and acid digestion) needed to investigate metal contamination. Moreover, the PIM-probe can be easily prepared at a low cost as the estimated costs for a PIM is less than 1 euro. It is worth mentioning that this is the first study to present a direct comparison between the accumulation of a PIM-based probe and biofilm grown under natural conditions.

Conclusions

A novel ionic liquid-based PIM probe has been designed for Zn bioavailability monitoring in surface waters. The accumulation of Zn from both simulated natural water or water samples from a polluted stream was possible using a membrane containing 50% CTA: 30% Cyphos 104: 20% NPOE (w/w) and a 0.01 M HNO_3 solution as the acceptor phase. The calibration of the PIM-based probe was successfully performed and by the addition of EDTA to the donor solution, it has been demonstrated that the PIM-probe can be used for speciation studies as only a fraction of the total metal is measured by the PIM. Finally, the comparison of the metal measured by the PIM-probe and the metal accumulated by biofilm, showed the ability of the developed tool to perform metal bioavailability studies.

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Figure captions

Figure 1. Zn accumulated at different exposure times. (a) Donor solution (250 mL): 500 µg L^{-1} Zn in SNW; (b) Donor solution: Osor-M (314 µg L^{-1} Zn), 10 L. Acceptor phase 0.01 M HNO_3.

Figure 2. Relationship between Zn accumulated in the acceptor phase and free Zn in SNW (250 mL). Acceptor phase 0.01 M HNO_3. Exposure time: 4 h.

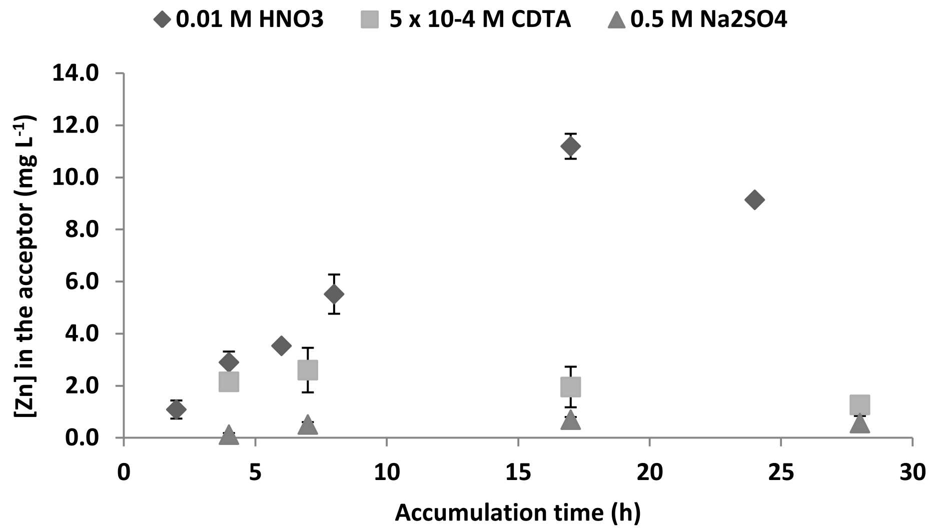
Figure 3. Comparison of Zn accumulated in PIM-based probe (acceptor phase 0.01 M HNO_3) vs. Zn in the biofilm. Donor solution: Osor-M and Osor-d, exposure time: 4 h.

Table 1. Zn measured with the PIM-based probe (n = 2)) and calculated with visual MINTEQ. The donor volume was 250 mL. The exposure time was 4 h and the acceptor phase was 0.01 M HNO_3. Standard deviation is shown in between parenthesis.

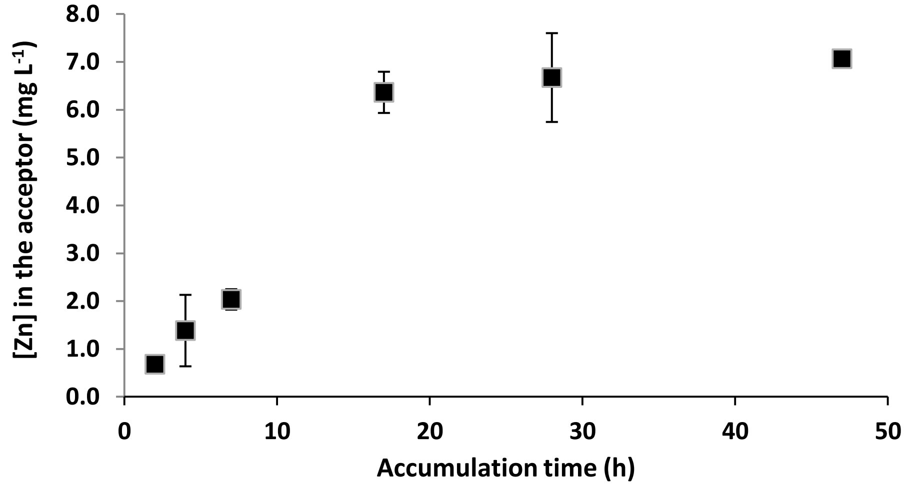
Sample	Added	Zn total content in sample (mg L^{-1})[a]	Total Zn in PIM acceptor (mg L^{-1})	Zn free measured (PIM) (mg L^{-1})[c]	Zn free (PIM) (%)[d]	Zn free (MINTEQ) (%)
SNW	Zn	0.50	2.9(0.4)[b]	0.59(0.09)	119	94.5
	Zn +EDTA	0.50	0.7 (0.2)	0.15(0.04)	29	31.7
Osor-R	-	<LOQ	0.07 (0.07)	<LOQ	n.d.	-
	Zn	0.50	1.74 (0.00)	0.36(0.00)	72	-
Osor-M 2017	-	0.45	1.9 (0.2)	0.39(0.04)	87	-
Osor-M 2018	-	0.31	1.3 (0.1)	0.27(0.02)	86	-

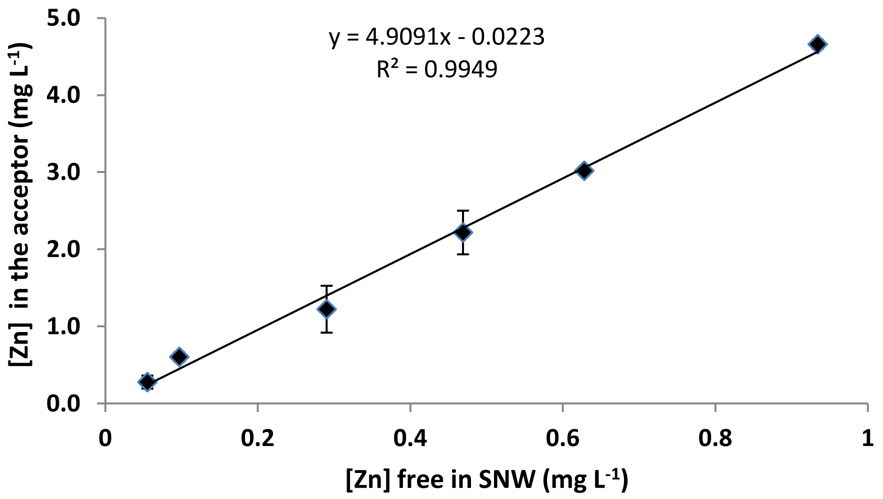
[a] measured with ICP-AES; [b] n = 5; [c] calculated taking into account the measured free Zn and the total present in the donor sample, [d] Using the calibration curve shown in Fig. 2

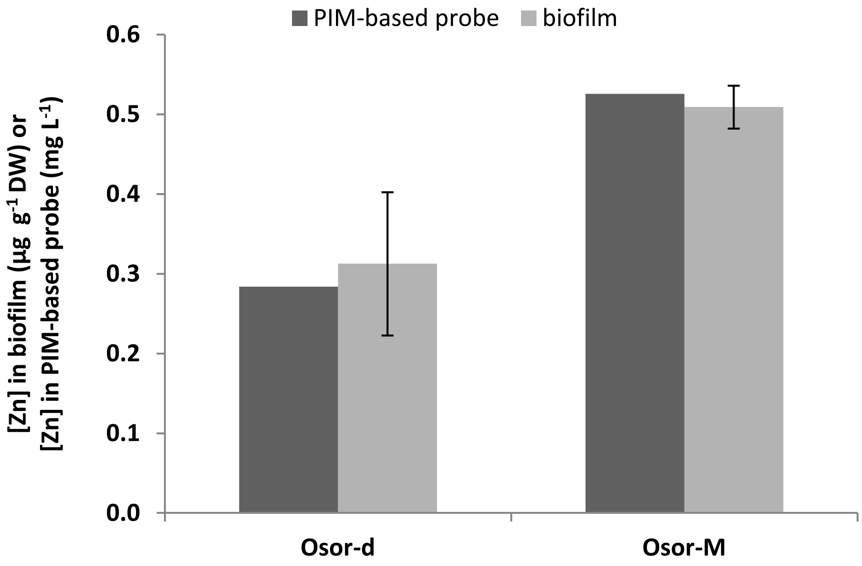
(a)


◆ 0.01 M HNO3
■ 5 x 10-4 M CDTA
▲ 0.5 M Na2SO4
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
[Zn] in the acceptor (mg L-1)
0
5
10
15
20
25
30
Accumulation time (h)

(b)


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
[Zn] in the acceptor (mg L-1)
0
10
20
30
40
50
Accumulation time (h)


y = 4.9091x - 0.0223
R² = 0.9949
5.0
4.0
3.0
2.0
1.0
0.0
0
0.2
0.4
0.6
0.8
1
[Zn] in the acceptor (mg L-1)
[Zn] free in SNW (mg L-1)


PIM-based probe
biofilm
[Zn] in biofilm (µg g-1 DW) or
[Zn] in PIM-based probe (mg L-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Osor-d
Osor-M

***conflict of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: