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Evaluation of the phenyl-bonded silica-based sorbent for pre-concentration of the booster antifouling biocides Zinc Pyrithione, Zineb and Ziram using solid-phase extraction technique and Inductively Coupled Plasma Mass Spectrometry

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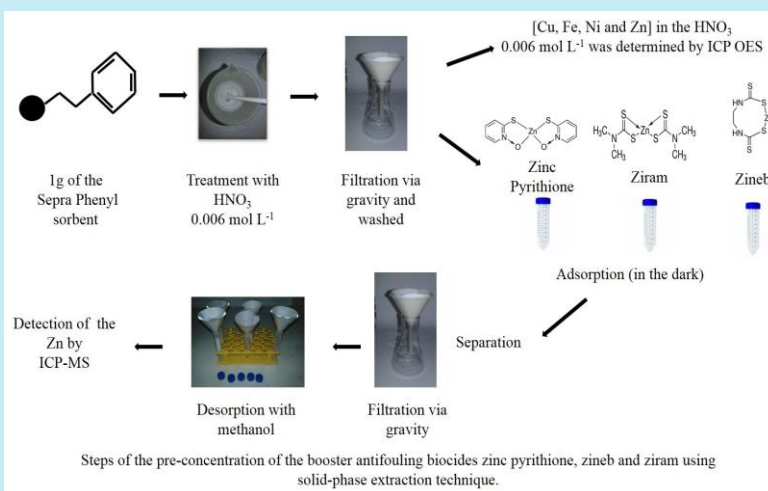
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ABSTRACT: A robust method of solid-phase extraction technique with use of the phenyl-bonded silica-based sorbent (Si-PH sorbent) for pre-concentration of three booster antifouling biocides: zinc pyrithione, Zineb and Ziram in ultrapure water fortified and estuarine water sample was evaluated for zinc determination by collision cell technology-inductively coupled plasma-mass spectrometry (CCT-ICP-MS). Decontamination process to remove metals and prevent (trans-) metallization of the Si-PH sorbent with 20 mL of nitric acid 0.006 mol L⁻¹ was performed. This proposed solid-phase extraction efficiency of three booster antifouling biocides by the phenyl-bonded silica-based sorbent (Si-PH sorbent) was evaluated in 40 mL of ultrapure water fortified sample (40 µg L⁻¹ of the zinc biocides). The adsorption of zinc pyrithione, Zineb and Ziram were 94.2 ± 0.1%, 85.13 ± 0.04% and 93.35 ± 0.09%, respectively. The limit of detection and limit of quantification values obtained were 0.66 µg L⁻¹ and 2.19 µg L⁻¹, respectively. Good recoveries of zinc pyrithione (85 ± 2%), Zineb (89 ± 5%) and Ziram (111 ± 2%) in the elution step for booster antifouling biocides from the fortified estuarine water were obtained.



1. Introduction

The contamination of the marine environment by metals is mainly due to impact of anthropic activities. In areas where the nautical activity is intense, such as ports, marinas and docks, risk of contamination is significant due to circulation of ships and boats. To avoid biological fouling, ships and vessels treat the hull with antifouling paint. Consequently, application of antifouling paints helps to prevent the settlement and growth of

algae, mussels, and other marine organisms in the hulls of vessels and small ship. However, antifouling particles are released into the seawater gradually from these antifouling paints that were applied on the hulls of vessels and small ship representing risk to marine organisms. In 2001, International Maritime Organization (IMO) prohibited tributyltin (TBT) in antifouling paints applied on ships¹. So, a new generation of booster antifouling biocides has been used: chlorothalonil, dichlofluanid, Irgarol 1051, TCMS pyridine,

thiocyanatomethylthio-benzothiazole (TCMTB), diuron, dichloro-octylisothiazolin (DCOIT, Sea Nine 211), zinc and copper pyrithione (zinc and copper omadine), Zineb (zinc ethylenebisdithiocarbamate) and Ziram (zinc dimethyldithiocarbamate)²⁻⁴. Recently, there is a considerable increase in the use of zinc pyrithione (Zn(PT)_2) and copper pyrithione (Cu(PT)_2), in freshwater and seawater booster antifouling biocides, because their antimicrobial and antifungal activity is highly effective⁵.

The effects and toxicity of booster antifouling biocides on different species of fish, crustaceans, invertebrates, and algae were studied⁶. Studies with sea urchin eggs and embryos demonstrated that Zn(PT)_2 (0.01 fg L^{-1}) is more toxic than Cu(PT)_2 ($1,000 \text{ fg L}^{-1}$)⁷. The Zn(PT)_2 showed similar toxicity to TBT for ascidian *Botryllus schlosseri* cultured hemocytes in extremely low concentrations $31.7 \text{ } \mu\text{g L}^{-1}$ and $158 \text{ } \mu\text{g L}^{-1}$, respectively⁸. The sublethal exposure of mussels in the 14-day period to Zn(PT)_2 (0.2 or 2 mmol L^{-1}), along with inorganic Zn and seawater controls) was considered genotoxic for mussel haemocytes⁹. The authors showed in the acute toxicity study of Zn(PT)_2 in fish *Carassius sp.* that the concentration required to kill half the members of a tested population after a specified test duration - 96 h (Lethal Concentration 50% - LC50 96 h) of Zn(PT)_2 in *Carassius sp.* cultivated in freshwater or water with 1.5 or 3% salinity was 0.163, 0.126 and 0.113 mg L^{-1} , respectively¹⁰. Acute toxicity of booster antifouling biocides was determined for phytoplankton *Nitzschia pungens* and zooplankton *Artemia* larvae showed a half maximal effective concentration (EC50 96 h) for Zineb ($232 \text{ } \mu\text{g L}^{-1}$), Zn(PT)_2 ($5.5 \text{ } \mu\text{g L}^{-1}$), Ziram ($5.4 \text{ } \mu\text{g L}^{-1}$), Cu(PT)_2 ($4.9 \text{ } \mu\text{g L}^{-1}$) in phytoplankton *Nitzschia pungens*. In zooplankton *Artemia* larvae, the booster antifouling biocides present LC50 (48 h, for larval survivals) for Zineb (41 mg L^{-1}), Ziram (4.8 mg L^{-1}), Zn(PT)_2 (3.2 mg L^{-1}) and Cu(PT)_2 (0.3 mg L^{-1})¹¹.

The development of analytical methods to determine environmental concentrations of these booster antifouling biocides (e.g. zinc pyrithione - Zn(PT)_2 , Zineb and Ziram) in complex matrices (e.g. estuarine water and seawater samples) is of great relevance due to the mechanisms of the oxidation and (trans-) metallization that currently complicate chromatographic analysis and other studies¹². For the quantification of trace level analytes ($\mu\text{g L}^{-1}$ or ng L^{-1}) in complex matrices, it

is necessary to use a sample preparation step. This step aims for the pre-concentration of analytes and the removal of interferents¹³⁻¹⁵. Solid-Phase Extraction (SPE) has a great potential to provide proper pre-concentration for ultra-trace analysis of booster antifouling biocides. In addition, SPE avoids coelution of the matrix interferents along with the compounds of interest when High Performance Liquid Chromatography (HPLC) is used and an important advantage of SPE is the possibility of extracting a wide range of analytes (from non-polar to very polar analytes) from a wide variety of samples¹⁶. SPE as an aqueous sample preparation step should consider the characteristics of the sorbent, matrix solvents used in the conditioning and elution steps, analyte of interest and detector used to quantify the analyte.

Several methods have been used to pre-concentrate the booster antifouling biocides by using, pre-column cartridge^{17,18}, adsorbent column¹⁹ commercial cartridges^{20,21}, extraction disks²² and C18-functionalized paramagnetic nanoparticles²³. Different hyphenated instrumental methods have been used to determinate the booster antifouling biocides over the years, e.g. HPLC coupled with: i) Ultraviolet-Visible (UV-VIS)^{10,12,17,20,23-26}, ii) Diode-Array (DAD)¹², iii) Electrospray Ionization Mass Spectrometry (ESI-MS)¹², iv) Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS)^{18,25}, v) Tandem-Mass Spectrometry (MS/MS)^{26,27}, vi) Atomic Absorption Spectrometry²⁸, vii) fluorescence²⁹.

Specifically, for Ziram and Zineb, the amount of zinc present in booster antifouling biocide was determined by Flame Atomic Absorption Spectrometry (Flame-AAS) and spectrophotometry after separation using HPLC³⁰ and for residue analysis of Zineb 68% + Hexaconazole 4% mixture was used Gas Chromatography Coupled with Triple Quadrupole Mass Spectrometry (GC-MS/MS)³¹.

The aim of this work was to evaluate the phenyl-bonded silica-based sorbent (Septra Phenyl, Phenomenex) for pre-concentration booster antifouling biocides zinc pyrithione, Zineb and Ziram from the estuarine water sample using SPE technique. For the first time this sorbent was used to pre-concentrate the zinc-based booster antifouling biocides zinc pyrithione, Zineb and Ziram from the ultrapure water fortified sample ($40 \text{ } \mu\text{g L}^{-1}$) and estuarine water sample with metal removal to prevent (trans-) metallization.

2. Experimental

2.1 Materials and reagents

The booster antifouling biocides zinc pyrithione (purity $\geq 95\%$, CAS 13463-41-7), Zineb Pestanal[®] (purity 97%, CAS 12122-67-7) and Ziram Pestanal[®] (purity 97%, CAS 137-30-4) were obtained from Sigma-Aldrich (Brazil). Dimethylsulfoxide (DMSO) was supplied by LABSYNTH (Brazil); HPLC grade methanol (MeOH) was purchased from Panreac (Spain); nitric acid (HNO₃) was obtained from Merck (Germany); resin Chelex[®]-100 (Na form, 100-200 wet mesh) was supplied by Bio-Rad (Canada). The Septra Phenyl Sorbent (endcapped silica-based phenyl, 50 μm , 65 Å, ≤ 10 kDa) were obtained from Phenomenex (USA) and commonly applied to reversed phase, hydrophobic and aromatic, small molecule selectivity from aqueous samples. Quantitative filter paper C42 blue strip, diameter 12.5 cm was supplied Unifil (Germany) and 0.22 μm nylon filter (disk) Allcrom (Brazil).

Ultrapure water (18 M Ω cm resistivity) obtained with a Millipore system (Millipore,

Bedford, MA) was used exclusively. The stock solutions of 200 mg L⁻¹ each booster antifouling biocide (Zn(PT)₂, Zineb and Ziram) was prepared in DMSO monthly and stored in the refrigerator at 4 °C (in dark).

2.2 Instrumentation

The Inductively Coupled Plasma Optical Emission Spectrometer (ICP OES) model iCAP 6000 Series (Thermo Scientific, Germany) was used to determine the higher concentrations of zinc and other metals presented in solution of HNO₃ 0.006 mol L⁻¹ and 1.6 mol L⁻¹ used in decontamination processes. An Inductively Coupled Plasma Mass Spectrometry (ICP-MS) model X Series II (Thermo Scientific, Germany) was used to zinc determination in the ultrapure water fortified sample, estuarine water sample of the Santos/SP Estuary (Brazil) and methanol of the elution step. [Tab. 1](#) reports the instrumental and analytical parameters. The instrument was optimized before each reading with a 10 ng mL⁻¹ standard tune solution (Ba, Be, Bi, Ce, Co, In, Li, Ni, Pb and U).

Table 1. Instrumental and Analytical Parameters of ICP-MS.

Parameter	Configuration Used
RF power	1,350 W
Nebulizer	Mira Mist [®]
Spray chamber	Glass Conical Impact Bead
Torch	1-Piece, 1.5 mm injector diameter
Interface Cones	Ni sampler and skimmer
Sample uptake rate	1 mL min ⁻¹ , approx., pumped
Quadrupole resolution	Standard resolution mode: peak width 0.70 amu at 5 % height
Nebulizer gas	0.75 L min ⁻¹
Plasma gas	13 L min ⁻¹
Auxiliary gas	0.7 L min ⁻¹
Monitored mass	Zn m/z 64
Dwell time	10 ms
CCT gas (He 5.0 - analytical)	8.3 mL min ⁻¹
Acquisition mode	Peak jumping
Internal Standard Isotopes	⁴⁵ Sc and ⁸⁹ Y

2.3 Decontamination processes

2.3.1 Glassware and plastic materials, quantitative filter paper and 0.22 μm nylon filter

All glassware and plastic materials were submerged in HNO₃ 3.2 mol L⁻¹ for 4 h, rinsed with ultrapure water and posteriorly, dried in

laminar flow hood. The quantitative filter paper (used in the gravity filtration of Si-PH sorbent) and 0.22 μm nylon filters (used in vacuum filtration of MeOH) was decontaminated by placing in HNO₃ 1.6 mol L⁻¹ for 4 h. Then they were washed with ultrapure water until pH = 6.0 and dried in a laminar flow hood.

The decontamination process on the glass or

plastic materials, the quantitative filter paper, 0.22 μm nylon filter and the Si-PH sorbent was performed for Zn removal (possibly presented in these materials with overestimation risk of the real Zn concentration in estuarine water samples). Further Zn, the metal removal (Cr, Cu, Fe and Ni, for example) was also performed to avoid the possible (trans-) metallization of these metals with the zinc-based antifouling biocides¹². Therefore, chromium, copper, iron, nickel and zinc concentrations present in HNO_3 used for decontamination were determined by ICP OES. This determination was performed by ICP OES due to the possible presence of these metals in higher concentrations, without a requirement for the detection technique with a higher sensibility as ICP-MS.

2.3.2 Zinc removal in methanol with Chelex®-100 resin

The zinc amount in the treated and not treated MeOH with Chelex®-100 resin (200-400 mesh, sodium form) was evaluated. For the treated MeOH, 0.1 g of Chelex®-100 resin was added in 25 mL of MeOH. This mixture remained for 10 min under constant stirring and 10 min at rest. The zinc amount was determined by ICP-MS from 10 mL of a MeOH solution 5% (v v^{-1}) treated with the Chelex®-100 resin and another MeOH solution 5% (v v^{-1}) not treated with the Chelex®-100 resin. Posteriorly, for the Zn removal present in the MeOH (used in extraction of the booster antifouling biocides), 2.0 g of Chelex®-100 resin were placed in 500 mL of MeOH under constant stirring for 10 min followed by 10 min at rest. Then the MeOH was vacuum filtered using 0.22 μm nylon filter decontaminated and stored in a 500 mL glass bottle.

2.3.3 Removal of the metals present in phenyl-bonded silica based sorbent

To metal removal of the Si-PH sorbent, 1.0 g of the Si-PH sorbent in 20 mL of HNO_3 0.006 mol L^{-1} was placed under constant stirring for 20 min. The Si-PH sorbent was then filtered using the gravity filtration technique and washed with 150 mL of ultrapure water until reach $\text{pH} = 6.0$. The chromium, copper, iron, nickel and zinc present in HNO_3 0.006 mol L^{-1} solution were determined by ICP OES.

2.4 Adsorption and recovery of Zn(PT)_2 , Zineb and Ziram by the Si-PH sorbent in the ultrapure water fortified sample

In order to evaluate the adsorption and elution of Zn(PT)_2 , Zineb and Ziram by the Si-PH sorbent, tests were firstly performed with ultrapure water fortified sample. Since no data of booster antifouling biocides was available for the study area and to prevent possible analyte losses in the experiment, the accuracy of the developed method was carried out at a higher level of fortification (40 $\mu\text{g L}^{-1}$). Using three falcon tubes, 50 mL of the ultrapure water was placed in each tube and was added Zn(PT)_2 in the first tube, Zineb in the second tube and Ziram in the third tube for final concentration of 40 $\mu\text{g L}^{-1}$ of the each biocide ($\text{pH} = 6.5$ and $T = 21.5^\circ\text{C}$). This procedure was performed in triplicate for each booster antifouling biocide.

A 10 mL aliquot of the solution was withdrawn before and after the experiment with Si-PH sorbent. Determination of zinc in each solution containing Zn(PT)_2 , Zineb and Ziram by ICP-MS was performed using 10 mL of each solution and acidified with HNO_3 3.2 mol L^{-1} (1.2 mL) and added ultrapure water (0.8 mL) to a final concentration of HNO_3 0.32 mol L^{-1} . This test was performed in duplicate for each booster antifouling biocide.

The conditioning and equilibration step was performed using 1 g of decontaminated Si-PH sorbent. To condition and equilibrate the Si-PH sorbent the gravity filtration technique was used and 10 mL of methanol and 10 mL of ultrapure water were added, respectively. A mass equal 1 g of Si-PH sorbent (conditioned and equilibrated) was transferred to a falcon tube containing 40 mL of Zn(PT)_2 , Zineb and Ziram solution (40 $\mu\text{g L}^{-1}$). The falcon tube containing the solution and Si-PH sorbent was kept under constant stirring for 1 h in the dark to prevent photodegradation. After stirring, the falcon tube was remained at rest for 10 min to decant the Si-PH sorbent before removing the final aliquot. Zinc determination by ICP-MS in the initial and final aliquots allowed to calculate the adsorbed zinc mass and adsorption percentage of the Zn(PT)_2 , Zineb and Ziram booster antifouling biocides in 1 g of Si-PH sorbent. The adsorbed zinc mass was calculated by the concentration obtained by ICP-MS multiplied by the dilution factor (1.2) and the volume of the solution (40). The percentage (%)

of adsorption was calculated using the values of initial concentration (C_i) and final concentration (C_f) of the solution by means of Eq. 1:

$$\% \text{ Adsorption} = (C_i - C_f / C_i) \times 100. \quad (1)$$

2.5 Adsorption and recovery of Zn(PT)_2 , Zineb and Ziram by the Si-PH sorbent in the water sample of the Santos/SP Estuary (Brazil)

The estuarine water sample was collected in the Estuary of Santos/SP (Brazil) downstream at latitude $\text{S}22^\circ51'30''$ and longitude $\text{W}46^\circ18'29''$. A 6 L plastic bottle of mineral water was washed three times with ultrapure water and three times with the estuarine water. The non-filterable water sample was not acid preserved but the following experiments were performed at maximum 14 days after the sampling (as preconized EPA SW-846 recommendations)³².

To evaluate the adsorption and recovery of the booster antifouling biocides by the Si-PH sorbent using water sample of the Santos Estuary/SP, 500 mL of the estuarine water was removed from the refrigerator one day prior to the experiment, transferred to a beaker and held at room temperature to decant the sediment.

In order to determine the zinc concentrations in the estuarine water sample prior to fortification with booster antifouling biocides Zn(PT)_2 , Zineb and Ziram, the analysis were performed by Collision Cell Technology (CCT)-ICP-MS. The collision cell technology (CCT) features a cell introduced before the mass analyzer which was used in ICP-MS to provide an effective elimination of spectral interferences mainly, polyatomic interferences (i.e. $^{36}\text{Ar}^{14}\text{N}_2$).

Using three falcon tubes, 50 mL of the estuarine water was placed in each tube and the sample was added Zn(PT)_2 in the first tube, Zineb in the second tube and Ziram in the third tube for final concentration of the $100 \mu\text{g L}^{-1}$ ($\text{pH} = 7.8$ and $T = 21.5^\circ\text{C}$). This procedure was performed in triplicate for each booster antifouling biocide.

Before adding the Si-PH sorbent and after end experiment, a 10 mL aliquot of the fortified estuarine water sample was removed. Determination of zinc in each sample containing Zn(PT)_2 , Zineb and Ziram by CCT-ICP-MS was performed using only 0.5 mL of each solution and acidified with HNO_3 3.2 mol L^{-1} (1 mL) and added ultrapure water (8.5 mL) to a final concentration of HNO_3 0.32 mol L^{-1} .

A mass equal 1 g of decontaminated, conditioned and equilibrated Si-PH sorbent was transferred to each falcon tube containing 40 mL of Zn(PT)_2 , Zineb and Ziram. The falcon tube containing fortified estuarine water sample and the Si-PH sorbent was under constant stirring for 1 h in the dark to prevent photodegradation.

After stirring, the falcon tube was held to decant the Si-PH sorbent before removing the final aliquot. Determination of zinc by CCT-ICP-MS in the initial and final aliquots allowed the calculation of the adsorbed zinc mass and the percentage adsorption of the Zn(PT)_2 , Zineb and Ziram biocides in 1 g of Si-PH sorbent in the estuarine water sample. The adsorbed zinc mass was calculated by the concentration obtained by ICP-MS multiplied by the dilution factor (20) and the volume of the solution (40).

Then, gravity filtration of the remainder of the sample (estuarine water and Si-PH sorbent) was performed on the falcon tube. The Si-PH sorbent that remained on the filter paper was washed with 5 mL of ultrapure water and transferred to another decontaminated quantitative filter paper. The sorbent transfer to another filter paper was performed to avoid that residues of the booster antifouling biocides that were not adsorbed by the sorbent and remained on the filter paper were eluted and consequently would overestimate the recovery values.

Initially, 1 mL of 100% MeOH (v v^{-1}) to elute the Zn(PT)_2 , Zineb and Ziram from the Si-PH sorbent was used. However, this volume was not enough to cover the mass of 1 g of Si-PH. So, 5 aliquots of 1 mL (total = 5 mL) of 100% MeOH (v v^{-1}) previously treated with Chelex®-100 were added under Si-PH sorbent using the gravity filtration technique with an elution rate of the 2 mL min^{-1} .

For the Zn determination in ICP-MS, 0.5 mL eluate (MeOH 100% (v v^{-1})) each booster antifouling biocide was diluted with 8.5 mL ultrapure water and acidified with 1 mL HNO_3 3.2 mol L^{-1} . The eluate dilution was 20-fold, resulting in a final concentration of 5% (v v^{-1}) for MeOH and 2% (v v^{-1}) for HNO_3 . This dilution was necessary because the introduction of organic solvents in the ICP-MS can cause signal suppression, as the intensity the signal is directly related to the concentration of methanol³³.

A solution containing MeOH 5% (v v^{-1}) and HNO_3 2% (v v^{-1}) was nebulized for 15 minutes to stabilize the plasma before analysis of calibration

curve and samples. The calibration curve of 0, 5, 10, 25, 50 and 75 $\mu\text{g L}^{-1}$ was prepared using the same MeOH and HNO_3 concentrations of the eluate and the stabilization solution.

2.6 Limit of detection and quantification

The procedure for determining the limit of detection and quantification of the proposed method was performed in the elution step (triplicate). The limit of detection and quantification of the SPE method developed was calculated by multiplying the standard deviation of zinc obtained in the eluate by 3 and by 10, respectively.

The limit of detection and quantification of this SPE method proposed was estimated according IUPAC recommendations by multiplying the standard deviation of zinc obtained in the eluate (blanks) by 3 and by 10, respectively. Subsequently, the zinc concentration obtained in the eluate (blanks) in this experimental procedure was subtracted from the eluent of the biocides in the $\text{Zn}(\text{PT})_2$, Zineb and Ziram recovery test by the Si-PH sorbent in the water sample from the Santos/SP Estuary³⁴.

3. Results and Discussion

3.1 Decontamination processes

3.1.1 Removal of zinc present in methanol with Chelex[®]-100 resin

The Zn amount present in the not treated MeOH solution decreases from $18 \pm 1 \mu\text{g L}^{-1}$ to $3 \pm 0.1 \mu\text{g L}^{-1}$ compared with the treated MeOH solution indicating a reduction of approximately

83%. Therefore, the methanol treated with Chelex[®]-100 resin was used in the SPE method development.

3.1.2 Removal of the metals present in phenyl-bonded silica based sorbent

The concentrations of the Cr, Cu, Fe, Ni, and Zn metals were determined quantitatively in a volume of 20 mL of HNO_3 0.006 mol L^{-1} used in the Si-PH sorbent decontamination process by and the results are presented in Tab. 2.

It was observed that Zn has the highest concentration ($174 \pm 1 \mu\text{g L}^{-1}$) followed by Fe ($28 \pm 1 \mu\text{g L}^{-1}$) and Ni ($15.9 \pm 0.8 \mu\text{g L}^{-1}$). The concentrations of Cu and Cr metals were below $8 \mu\text{g L}^{-1}$. Based on these results, it is demonstrated the need of carrying out the Si-PH sorbent decontamination process for subsequent use in SPE of the booster antifouling biocides. This is justified by the fact that the booster antifouling biocides have Zn in their composition and this metal showed the highest concentration in the Si-PH sorbent decontamination process. It should be noted that the decontamination process avoided the (trans-) metallization of the booster antifouling biocides with the other metals present in the Si-PH sorbent.

The choice of HNO_3 concentration 0.006 mol L^{-1} used in the Si-PH sorbent decontamination process was based on the fact that the Si-PH sorbent is the silica base and its pH working range is between 2 and 8 as recommended by the manufacturer³⁵. Silica becomes unstable and may contain residual silanols resulting in irreversible adsorption of basic compounds outside this pH working range³⁶.

Table 2. Metal concentrations quantified in the Si-PH decontamination process of by ICP OES (axial view).

Solution	[Cr]/ $\mu\text{g L}^{-1}$	[Cu]/ $\mu\text{g L}^{-1}$	[Fe]/ $\mu\text{g L}^{-1}$	[Ni]/ $\mu\text{g L}^{-1}$	[Zn]/ $\mu\text{g L}^{-1}$
1	3.9 ± 0.3	8.5 ± 0.4	< LOQ	< LOQ	202 ± 2
2	3.1 ± 0.5	9.8 ± 0.7	< LOQ	15 ± 6	< LOQ
3	3.4 ± 0.1	8.1 ± 0.1	38.5 ± 0.4	< LOQ	< LOQ
4	3.4 ± 0.1	8.7 ± 0.9	< LOQ	18.60 ± 0.09	177.0 ± 0.4
5	< LOQ	7.5 ± 0.1	< LOQ	< LOQ	149.2 ± 0.7
6	3.1 ± 0.3	10.4 ± 0.4	23.9 ± 0.4	15.1 ± 0.6	192.3 ± 1.4
7	2.9 ± 0.3	7.2 ± 0.4	33.4 ± 0.4	12.5 ± 0.4	155.8 ± 0.2
8	3.1 ± 0.1	7.0 ± 0.4	30.40 ± 0.02	18.0 ± 0.4	164 ± 2
9	2.1 ± 0.9	6.8 ± 0.8	25.7 ± 1.1	14 ± 3	164.5 ± 0.6
10	2.0 ± 0.6	5.5 ± 0.4	30.8 ± 0.3	15.0 ± 0.1	< LOQ
11	< LOQ	6.3 ± 0.9	19.6 ± 1.7	13.6 ± 0.1	152.6 ± 0.6
12	< LOQ	6.7 ± 0.3	16.5 ± 0.3	14.100 ± 0.003	164 ± 2

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13	2.4 ± 0.6	7.9 ± 0.2	30 ± 3	15.0 ± 0.3	175.9 ± 0.8
14	2 ± 1	10.9 ± 0.4	27 ± 2	15.2 ± 0.2	190.9 ± 0.9
15	3.4 ± 0.4	8.6 ± 0.5	27.6 ± 0.6	15.84 ± 0.04	180.7 ± 0.9
16	2.7 ± 0.4	8.4 ± 0.2	40.9 ± 0.8	18.8 ± 0.1	17 ± 1
17	3 ± 1	7 ± 1.4	26 ± 4	19.9 ± 0.8	176 ± 7
18	2.3 ± 0.2	7.2 ± 0.5	18 ± 1	18.3 ± 0.1	197.7 ± 0.9
Mean ± SD	2.9 ± 0.2	7.9 ± 0.5	27.7 ± 1.1	15.9 ± 0.8	174 ± 1
LOD	0.54	1.01	1.73	0.49	0.77
LOQ	1.78	3.38	2.44	1.64	2.58
λ (nm)	283.5	324.7	259.9	231.6	213.8

3.1.3 Quantitative filter paper

Table 3 reports the concentration of the metals found in the quantitative filter paper and a zinc concentration of $156 \pm 2 \mu\text{g L}^{-1}$. Based on these results, it was concluded that it would occur overestimation of levels in the elution step of

biocides. In addition, the presence of Fe and Ni metals may contribute to (trans-) metallization with the booster antifouling biocides. Therefore, the decontaminated filter paper was used in all stages of the gravity filtration technique and especially in the elution step.

Table 3. Metal concentrations quantified in the filter paper decontamination process by ICP OES (axial view) (n = 15).

Solution of HNO ₃	[Cr]/ $\mu\text{g L}^{-1}$	[Cu]/ $\mu\text{g L}^{-1}$	[Fe]/ $\mu\text{g L}^{-1}$	[Ni]/ $\mu\text{g L}^{-1}$	[Zn]/ $\mu\text{g L}^{-1}$
Mean ± SD	< LOQ	< LOQ	194 ± 10	22 ± 3	156 ± 2
LOD	0.82	0.56	1.13	0.40	0.34
LOQ	2.74	1.86	3.76	1.32	1.12
λ (nm)	267.7	324.7	238.2	221.6	213.8

3.1.4 Adsorption and recovery of Zn(PT)₂, Zineb and Ziram by the Si-PH sorbent in the ultrapure water fortified sample

Usually, 100-200 mL of water sample is used to percolate the cartridge containing silica with a flow rate above 2 mL min^{-1} by use of the manifold³⁷. However, the developed method used only 40 mL of sample and the direct contact and constant stirring of the Si-PH sorbent contributed to a good adsorption of the booster antifouling biocides as showed in Tab. 4. The stock solution dissolutions of the booster antifouling biocides resulted a pH value of 6.5 in the ultrapure water fortified sample which is close to that found in tropical estuarine environment water^{38,39}.

The adsorbed mass and adsorption of the booster antifouling biocides by the Si-PH sorbent

in the ultrapure water fortified solution in the SPE procedure as showed in Tab. 4.

The results evidenced that Zn(PT)₂ demonstrated good adsorption, indicating the occurrence of π - π interactions between the phenyl group present in both Si-PH sorbent and Zn(PT)₂⁴⁰. This feature is advantageous because it allows its application in the reverse phase SPE for extraction of Zn(PT)₂ from the estuarine water.

It is suggested that hydrophobic interactions could occur between the Si-PH sorbent and the Zineb and Ziram, once both have nonpolar groups at their endings and were eluted with methanol, which is used for the extraction of nonpolar analyte and moderately nonpolar analyte by use of reverse phase SPE technique.

Table 4. Adsorption of Zn(PT)₂, Zineb and Ziram by the Si-PH sorbent in SPE in ultrapure water fortified with - booster antifouling biocides (n=3).

Analyte	[Zn] In. Sol. / $\mu\text{g L}^{-1}$	[Zn] Final Sol. / $\mu\text{g L}^{-1}$	Adsorbed Mass / $\mu\text{g (\% RSD)}$	Adsorption / %
Zn(PT) ₂	36.6 ± 0.5	2.11 ± 0.05	1.3 ± 0.1 (9.07)	94.2 ± 0.1
Zineb	43.3 ± 0.3	6.40 ± 0.07	1.47 ± 0.02 (1.36)	85.13 ± 0.04
Ziram	49.1 ± 0.4	3.2 ± 0.1	1.83 ± 0.01 (0.73)	93.35 ± 0.09

3.1.5 Adsorption and recovery of Zn(PT)₂, Zineb and Ziram by the Si-PH sorbent in the water sample of the Santos/SP Estuary (Brazil)

The adsorbed and eluted mass and the % recovery of Zn(PT)₂, Zineb and Ziram by the Si-PH sorbent in the sample (Santos/SP estuary water) fortified with the booster antifouling biocides in the SPE procedure are presented in Tab. 5.

The type and volume of conditioning and elution solvent (methanol) used in SPE of booster antifouling biocides from estuarine water demonstrated good extraction efficiency (recoveries between ~85% and 111%) due to the fact that methanol is more polar than Si-PH sorbent and used for the extraction of nonpolar analyte and moderately nonpolar analyte⁴¹. In addition, the extraction efficiency was favored by dividing the volume of 5 mL of methanol used in the elution into 5 times of 1 mL.

The concentration factor obtained by the ratio of the initial sample volume (40 mL) to the final volume of the concentrated solution (5 mL) was 8. This concentration factor was sufficient to

quantify the zinc by ICP-MS in the eluate after the 20-fold dilution.

The recovery of Zn(PT)₂ in estuarine water (85 ± 2%) in the developed method is very close to that obtained by Grunnet and Dahllöf²⁰, where they obtained 85% recovery for Zn(PT)₂ when using cartridge Strata X in the SPE technique to pre-concentrate Zn(PT)₂ from seawater. This fact demonstrates that the use of the gravity filtration technique used in this research did not negatively influence the results obtained in the elution step.

Good recoveries for zinc pyriothione (85 ± 2%), Zineb (89 ± 5%) and Ziram (111 ± 2%) in the elution step for booster antifouling biocides from the fortified estuarine water were obtained. These results are in accordance with the recommendation of the US Environmental Protection Agency (EPA), because they accept to validate extraction methods with recovery in the range of 70% to 130%³⁷. In addition, it is emphasized that the Si-PH sorbent (phenyl-bonded) was first evaluated to adsorb the dithiocarbamates Zineb and Ziram from estuarine water.

Table 5. Zinc recovery in the Santos/SP Estuary water sample fortified with Zn(PT)₂, Zineb and Ziram (n=3).

Analyte	Adsorbed Mass / µg ± SD (% RSD)	Eluted Mass / µg ± SD (% RSD)	Recovery / %
Zn(PT) ₂	1.85 ± 0.08 (4.59)	1.5 ± 0.1 (6.27)	85 ± 2
Zineb	2.43 ± 0.08 (3.49)	2.1 ± 0.1 (7.78)	89 ± 5
Ziram	2.20 ± 0.08 (3.86)	2.45 ± 0.06 (2.60)	111 ± 2

3.1.6 Limit of detection and quantification

Based on the experimental procedure for the determination of the limit of detection and quantification of the SPE method developed, the concentration of zinc obtained in the eluate (triplicate) of this procedure was 0.9 ± 0.2 µg L⁻¹.

Therefore, the limit of detection (LOD) of the SPE analytical method using the fortified sample of estuarine water was calculated by multiplying of the standard deviation (0.2) by 3 and the limit of quantification (LOQ) by multiplying of the standard deviation (0.22) by 10. So, the LOD and LOQ values obtained for SPE were 0.66 µg L⁻¹ and 2.19 µg L⁻¹, respectively.

4. Conclusions

The metals removal procedure of the phenyl-bonded silica-based sorbent was efficient, once it

eliminated the possibility of overestimation of the values during the determination of zinc by ICP-MS and avoided the (trans-) metallization of the biocides with others metals Cr, Cu, Fe, Ni present in the Si-PH sorbent.

The results obtained in this research allow to conclude that the proposed analytical method is efficient, considering the LOD obtained of 0.66 µg L⁻¹.

Good recoveries were obtained between ~85% and 111% in the elution step of the Zn(PT)₂, Zineb and Ziram biocides in the SPE by zinc determination via ICP-MS from the fortified estuarine water without altering the pH, filtering or diluting the sample in ultrapure water.

Additionally, the proposed approach it may be useful in the sample preparation step for speciation studies of the booster antifouling biocides Zn(PT)₂, Zineb and Ziram in estuarine water, such as, in the HPLC-ICP-MS coupling

because the proposed approach pre-concentrated the sample and it eliminates the (trans-) metallization that could occur with the metals present in the evaluated sorbent.

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