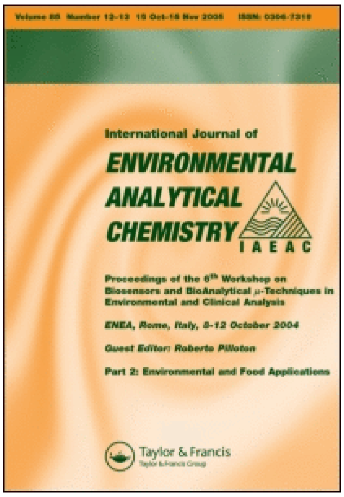

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Solid phase extraction and diffusive gradients in thin films techniques for determination of total and labile concentrations of Cd(II), Cu(II), Ni(II) and Pb(II) in Black Sea water

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Total dissolved and labile concentrations of Cd(II), Cu(II), Ni(II) and Pb(II) were determined at six locations of the Bourgas Gulf of the Bulgarian Black Sea coast. Solid phase extraction procedure based on monodisperse, submicrometer silica spheres modified with 3-aminopropyltrimethoxysilane followed by the electrothermal atomic absorption spectrometry (ETAAS) was developed and applied to quantify the total dissolved metal concentrations in sea water. Quantitative sorption of Cd, Cu, Ni and Pb was achieved in the pH range 7.5–8, for 30 min, adsorbed elements were easily eluted with 2 mL 2 mol L^{-1} HNO_3. Since the optimal pH for quantitative sorption coincides with typical pH of Black Sea water (7.9–8.2), on-site pre-concentration of the analytes without any additional treatment was possible. Detection limits achieved for total dissolved metal quantification were: Cd 0.002 µg L^{-1}, Cu 0.005 µg L^{-1}, Ni 0.03 µg L^{-1}, Pb 0.02 µg L^{-1} and relative standard deviations varied from 5–13% for all studied elements (for typical Cd, Cu, Ni and Pb concentrations in Black Sea water). Open pore diffusive gradients in thin films (DGT) technique was employed for *in-situ* sampling and pre-concentration of the sea water and in combination with ETAAS was used to determine the proportion of dynamic (mobile and kinetically labile) species of Cd(II), Cu(II), Ni(II) and Pb(II) in the sea water. Obtained results showed strong complexation for Cu and Pb with sea water dissolved organic matter. The ratios between DGT-labile and total dissolved concentrations found for Cu(II) and Pb(II) were in the range 0.2–0.4. For Cd and Ni, these ratios varied from 0.6 to 0.8, suggesting higher degree of free and kinetically labile species of these metals in sea water.

Keywords: solid phase extraction; DGT; speciation; trace elements; Black Sea

1. Introduction

The toxicity and bioavailabilty of trace metals in aquatic environment, and therefore the ultimate impact of metals on the ecosystems, depends on both their concentrations and chemical speciation [1,2]. Analytical methods providing accurate and reliable determination of both total dissolved metal concentrations and the proportions of various metal

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species received increasing attention [3], as they can set the basis of the effective environmental monitoring and management. Total dissolved metal concentrations can be determined directly by a number of modern analytical methods such as ICP-MS or ETAAS; however, their application to the complex systems such as sea water is restricted by insufficient sensitivity ETAAS or by matrix interferences, ICP-MS [4]. Therefore analytical procedures for preliminary separation and pre-concentration still have to be used for trace analyte quantification in marine systems. Column solid phase extraction (SPE) is a preferable procedure for this purpose since it can be performed on site immediately after sampling thus minimising any transformations, losses and contamination of trace elements. SPE allows high separation and pre-concentration factors and thus ensures interference-free and sensitive instrumental determination. The extraction efficiency of these procedures depends mostly on the properties of the sorbent used [5]. Several very important features of the sorbent defined its applicability in field solid phase extraction procedures – high pre-concentration factors, fast complex formation and high stability constants of the complexes formed, as well as high sorbent stability during storage. In recent years, silica gel chemically modified with various ligands like amino group [6–8], 8-hydroxyquinoline [9], dithizone [10], thiourea [11], glycerol [12], thioacetamide [13], N-(3-propyl)-*o*-phenylenediamine [14], alizarin violet [15], 2-aninothiazole [16], and *p*-toluenesulfonylamide [17] has been widely used in various SPE procedures. Colloidal silica spheres are the new generation high purity synthetic spherical silica sorbents. The surface of the synthesised silica colloids is covered with reactive functional groups (silanol groups), which act as active sites for subsequent functionalisation. Therefore the reactivity of silica colloidal spheres can be easily modified by chemical or thermal treatments and they are characterised with high mechanical and chemical stability.

In addition, trace metals and their speciation measurements often are preceded by sample transport from the sampling site to the laboratory, which may induce potential contaminations or losses of trace elements, as well as transformation of their chemical species. Therefore on-site and *in situ* procedures for both total metal concentrations and speciation determination are highly sought after. Recently developed analytical techniques that are capable to provide (often *in situ*) specific metal species and fractions include: diffusion gradients in thin film gels (DGT), gel integrated microelectrodes combined to voltammetric system (GIME), permeation liquid membrane (PLM), Donnan membrane technique (DMT), competitive ligand-exchange/stripping voltammetry (CLE-SV), stripping chronopotentiometry and ion exchange resins [3]. In the present work, we explore the capabilities of the technique of diffusive gradients in thin films (DGT) that provides the amount of the dynamic metal species such as free metal ions, small organic and inorganic kinetically labile metal complexes, able to diffuse through the open pore gel and to adsorb to the ion exchange resin [18,19]. Because of the existing conceptual analogy between the processes determining the metal bioavailability to aquatic biota [19] and the response of the DGT, the fraction of chemical species of trace metals trapped in the DGT can be used to evaluate trace metal bioavailability by considering the corresponding effective spatial and time scales for the organism of interest and the nature of the rate-limiting step in the uptake of the target metal [20,21].

The present study explores the capabilities of a new sorbent based on modified 3-aminopropyltrimethoxysilane submicrometer silica spheres (Si-APS) for the determination of total dissolved Cd(II), Cu(II), Ni(II) and Pb(II) concentrations in sea water. More specifically, the optimal parameters for quantitative sorption and elution of trace elements as well as their determination by ETAAS are defined. The developed analytical methodology was applied on site to quantify total dissolved concentrations of Cd(II), Cu(II), Ni(II) and Pb(II) in Black Sea water at six locations situated in the Bourgas Gulf. In addition, the obtained results were complemented by those obtained with the DGT technique for *in situ* sampling and pre-concentration. The total dissolved Cd(II), Cu(II), Ni(II) and Pb(II) concentrations and their kinetically labile species were chosen to be measured since these metals are priority contaminants and under consideration for the Bourgas Gulf.

2. Experimental

2.1 *Reagents*

All reagents used were of analytical reagent grade. The stock standard solutions of Cd(II), Cu(II), Ni(II) and Pb(II), ($1000\,\mu g\,mL^{-1}$) Titrisol, Merck (Darmstadt, Germany, www.merck.de) in 2% HNO_3 were used. Working standard solutions were prepared daily by appropriate dilution with doubly distilled water. Tetraethoxysilane (TEOS, 99%, Fluka, Germany) was used as silica precursor, absolute ethanol (EtOH, 99.6%) and ammonia solution (25%) (Laboratory of High Purity Substances, University of Sofia) were used as solvents and as a reaction and morphological catalyst, respectively. 3-Aminopropyltrimethoxysilane (APS, Fluka, Germany) was used to prepare the amino-functionalises spherical silica sorbent (Si-APS). The following buffer solutions were used for pH adjustment: citrate/HCl for pH 3; CH_3COONa/CH_3COOH for pH 4–6; KH_2PO_4/NaOH for pH 7 and 8. Doubly distilled water was used in all of the experiments.

2.2 *Apparatus*

Flame atomic absorption spectrometry (FAAS) measurements were carried out on a Perkin-Elmer Zeeman 1100 B Spectrometer (Überlingen, Germany) with an air/acetylene flame. The instrumental parameters were optimised in order to obtain maximum signal-to-noise ratio. Electrothermal AAS measurements were carried out on a Perkin-Elmer (Norwalk, CT, USA) Zeeman 3030 Spectrometer with an HGA-600 graphite furnace. The light sources used were hollow cathode lamps for Cu, Ni and Pb and electrodeless discharge lamps for Cd. The spectral bandpass for all analytes was 0.7 nm. Pyrolytic graphite coated tubes with integrated platforms were used as atomisers. Sample aliquots of 20 µL were injected into the graphite furnace using autosampler AS-70. All measurements were carried out with at least three replicates and based on integrated absorbance. Optimal instrumental parameters for ETAAS measurements were defined according to the manufacturer's recommendations.

A microprocessor pH-meter (Hanna Instruments, Portugal, www.hannacom.pt) was used for pH measurements.

2.3 *Characterisation of the sorbent and general solid phase extraction procedures*

2.3.1 *Synthesis of the Si-APS*

As a first step, bare silica particles with uniform size and shape were prepared using TEOS, and then their surface was modified with aminopropyl groups using APS (3-aminopropyltrimethoxysilane). Synthesis of bare silica spheres was carried out following the modified Stöber sol-gel process [22]. Their size is precisely controlled by the seeded-growth technique proposed by Bogush *et al.* [23,24]. First generation silica spheres were prepared after mixing of two reagent solutions TEOS in EtOH and NH_3 in EtOH/H_2O at constant temperature (30°C), under vigorous stirring, so that the final composition was 0.25 M TEOS, 0.5 M NH_3, 5 M H_2O. The growth reaction conditions were studied in order to prevent the formation of new silica seeds and thus to prepare larger monodisperse silica particles. Under magnetic stirring and constant temperature (20°C), TEOS and water were added sequentially to the original seed solution in a 1 : 2 mole ratio with a time interval of 8 h. After the growth reaction was completed, the prepared silica particles were centrifuged out of the reaction solution, washed 5–6 times with both ethanol and distilled water, and finally dried at 100°C. These submicrometer silica spheres were modified with APS according to the described procedure [25]. The methoxy groups of APS were readily hydrolysed and react with silanol groups on the silica particle surface. The yield was 85.2%.

2.3.2 *Characterisation of the particles*

The particle morphology and the size distribution of silica particles were obtained with a scanning electron microscope (SEM, JEOL JSM 5510). The mean diameter (D_{mean}) and relative standard deviation (σ) were evaluated by measuring 200 particles from the micrographs using Image J software. Results shown in Figure 1 indicate the formation of spherical particles, the average diameter (d_{mean}) of which is 344 nm ± 14 nm with a monodisperse size distribution (relative standard deviation σ is 4.2%). The density of particles (ρ) measured in aqueous suspension by picnometry (ASTM specification D792-00 [26]) was around $2.04\,g\,cm^{-3}$, which is close to reported density of Stöber silica in the range $1.7–2.0\,g\,cm^{-3}$ and agrees with the density of non-porous amorphous silica. Assuming non-porous particles, the geometrical specific surface area (a_G) of the particles was calculated based on the SEM particle diameter by the relation $a_G = 6000/(\rho \times D_{mean})$. The calculated specific surface area (a_G) of the particles is $8.5\,m^2\,g^{-1}$. The measured specific surface area (a_{BET}) of the particles is $18\,m^2\,g^{-1}$ and this value is approximately 50% higher than the geometrical specific surface area (a_G). The difference in the calculated

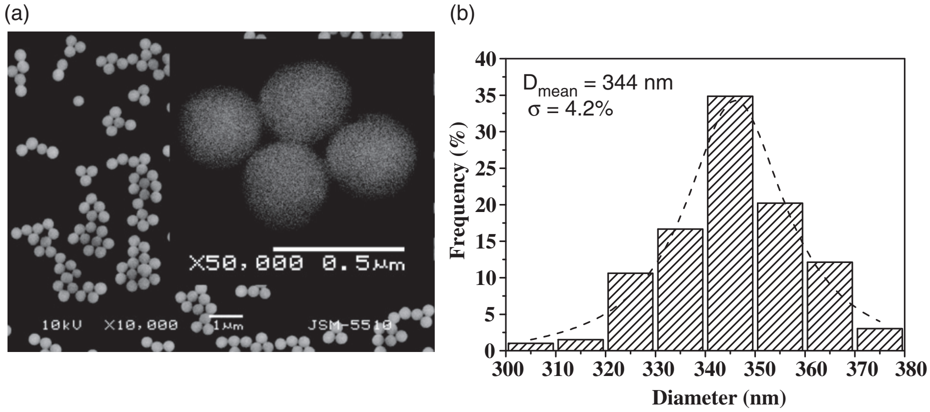

Figure 1. Scanning electron micrograph (a) and particle size distribution (b) of silica particles.

and measured specific surface area of bare silica particles could be explained with some internal microporosity of the particles (see inset in Figure 1).

2.3.3 *General sorption/desorption procedure for sorbent characterisation*

Sorbent particles (50 mg) were added to an aqueous solution (20 mL) containing 1–100 μg L^{-1} Cd, Cu, Ni and Pb. After stirring for 30 min, the solution was filtered through a cellulose membrane filter (Millipore, 0.22 μm pore size) using a peristaltic pump. The sorbent was washed with 5 mL doubly distilled water and eluted with 2 mL 2 M HNO_3. The amounts of trace elements in the efluate and eluate solutions were determined by FAAS or ETAAS depending on the concentration range.

The degree of sorption (R, %) was defined as: $R, \% = (C_0 - C_{eff}) \cdot 100/C_0$, where C_0 (μg) is the initial element amount and C_{eff} (μg) is the element amount in the efluate solution.

The sorption capacity of Si-APS is determined after saturation of the sorbent with the studied cations under optimum conditions (pH = 8). For this purpose, increasing amounts of each metal ion was stirred with 50 mg Si-APS for 30 min. The amount of cation remaining in the solution was determined by FAAS. The process was carried out until the sorbent was saturated and the metal ion content becomes measurable in the efluate solution.

2.4 ***Sampling and analytical procedures for sea water analysis***

2.4.1 *Sampling*

Sea water samples were collected from six sample sites in the Bourgas Gulf (Figure 2) at 1.5 m depth in the surface layer using Teflon samplers (0.5 L). Samples for the determination of total dissolved trace elements using laboratory procedures (SPE or liquid/liquid extraction) were filtered through 0.22 μm cellulose membrane filter

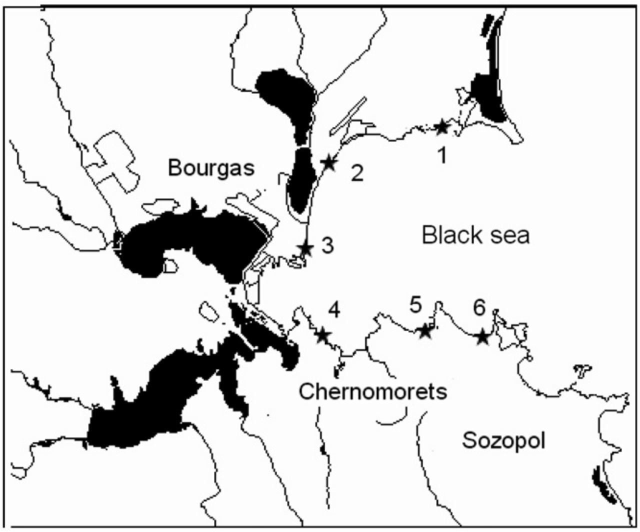

Figure 2. Sampling sites.

(Millipore) and acidified with 1.0 mL c. HNO_3. All samplers, filters and filtration devices were pre-cleaned with 2% nitric acid and Milli-Q water and kept in plastic bags and plastic boxes.

2.4.2 *On site solid phase extraction procedure*

This procedure took place using synthesised silica spheres (Si-APS). The enrichment procedure for total trace element quantification was performed at the sampling site without any previous conservation or acidification of the sample, thus avoiding any artifacts and contamination. Sea water samples of 50 mL (original pH 8.0–8.1) were passed first through 0.22 μm pore size cellulose membrane filter (Millipore) and collected in 50 mL plastic containers containing 50 mg Si-APS sorbent. After 30 min the colloid solution was filtered through a membrane filter, washed with 5 mL doubly distilled water and eluted with 2 mL 2 M HNO_3. Eluates from different samples were collected and later analysed in the laboratory by ETAAS.

2.4.3 *DGT sampling and handling*

Standard type DGT units with 3.14 cm^2 window, 0.8 mm open-pore diffusive gel and polysulfone filter membrane with pore size 0.45 μm were used throughout this study. Chelex 100 resin gel discs (Naform, 200–400 mesh), 0.4 mm, were used as binding agents. The DGT units were deployed at 1.5 m depth using plastic strips. After six days, the DGT units were retrieved and dismounted for analysis. The Chelex resin layer was placed in 1.5 mL Ependorff vessel, and 1.00 mL 1 mol L^{-1} HNO_3 was added, the solution was shaken and then left to stand overnight for quantitative elution of metal ions. Trace elements were determined in this solution without further dilution by ETAAS under optimised instrumental parameters and matrix matched standards. The concentration of metal ions (c_m) in the bulk waters was calculated using the equation:

$$c_m = m\Delta g/Dat$$

where m is the mass of metal accumulated in the resin, Δg is the thickness of hydrogel and membrane filter, D is the diffusion coefficient of metal ion in the gel, a is the exposure area (3.14 cm^2), t is the deployment time.

2.4.4 *Liquid/liquid extraction*

Liquid/liquid extraction of metal dithiocarbamate complexes into isobutylmethyl ketone (IBMK) followed by trace element measurement by ETAAS in the organic phase under optimised instrumental parameters. Extraction was performed with 50 mL acidified sea water samples obtained after sampling and with the same sea water samples previously digested in MW oven (EPA method 200.2 [27], 45 mL sea water with 5 mL c. HNO_3) for complete decomposition of organic matter. Ammonium pyrolidine dithiocarbamate was used as extraction reagent: Extraction was performed for 3 min in 2 mL IBMK.

3. Results and discussion

3.1 ***Sorption of metal ions as a function of pH***

The pH value of the medium is one of the most important factors controlling the degree of sorption of metal ions due to the competitive reactions between chelate forming group

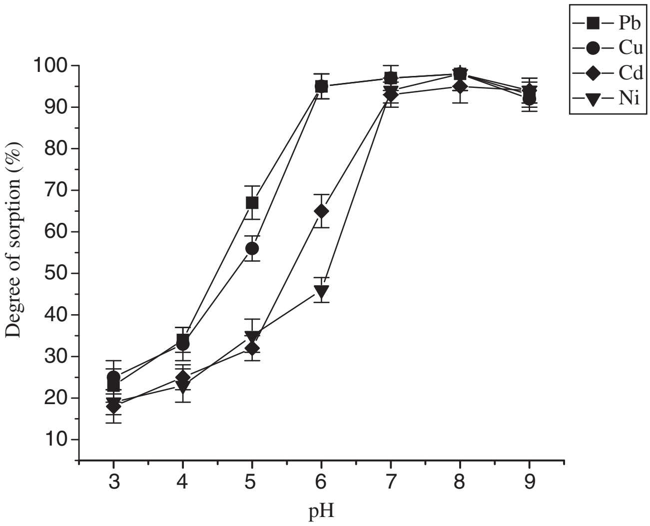

Figure 3. Effect of pH on Cd(II), Cu(II), Ni(II) and Pb(II) sorption on the Si-APS. 50 mg sorbent Si-APS, 30 min contact time, $n = 3$.

of the sorbent and hydrogen ions in solution. The degree of sorption of Cd(II), Cu(II), Ni(II) and Pb(II) on the Si-APS was determined in the pH range 3–9 by batch equilibrium procedure (Figure 3). An increased sorption for different metal ion with increasing pH of the solution was observed. At pH around 4, the degree of sorption was generally low for all metal ions which might be explained with the protonation of the amino groups thus eliminating their ability to form complexes with metal ions. The degree of sorption was >90% for Pb(II) or Cu(II) over a pH range from pH 6 to 8. For Cd and Ni, the maximum degree of sorption was obtained in the pH range 7–8. The obtained trends are consistent with the ability of these cations to form stable complexes with nitrogen-containing ligands [28]. At higher pH values (~9), the decrease in the degree of sorption can be attributed partly to the formation of metal hydroxide complexes or to the degradation of the Si-APS sorbent in strong alkali. Overall, a pH of 7.5–8 was determined as optimal for simultaneous sorption of all studied elements on Si-APS sorbent.

3.2 *Estimation of the sorption capacity of Si-APS and sorption/desorption kinetics*

The average sorption capacity of 62 µmol Cd, 92 µmol Cu, 73 µmol Ni and 31 µmol Pb per gram Si-APS was determined following the addition of increasing concentrations of metals to the Si-APS. The sorption capacity decreased in the order Cu(II) > Ni(II) > Cd(II) ≫ Pb(II) as expected from metal ion size, degree of hydration and affinity toward the functional group immobilised onto the Si-APS particles. The capacities achieved are close to the average values reported in the literature for silica gel modified with APS (non-imprinted sorbent [25]), salicylaldoxime [29] or gallic acid [30]; however, they are lower than those reported for chitosan-modified ordered mesoporous silica [31].

Experiments performed on the rate of sorption revealed that 30 min is optimal time for quantitative sorption of all studied elements. It is worth mentioning that sorption kinetics

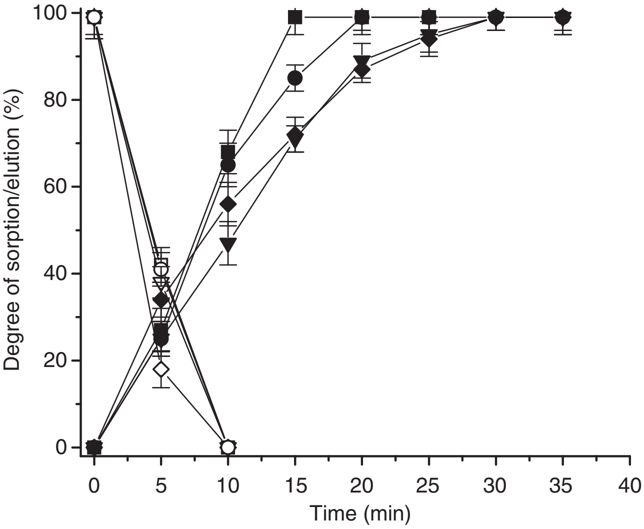

Figure 4. Kinetics of sorption: Cu(●), Cd(◆), Ni (▼), Pb (■)/elution: Cu(○), Cd(◇), Ni (▽), Pb (□). Conditions: sea water sample 50 mL, 0.12 μg L^{-1} Cd, 11.2 μg L^{-1} Cu, 8.1 μg L^{-1} Ni and 0.15 μg L^{-1} Pb, 50 mg sorbent Si-APS, $n = 3$.

was investigated for model solutions (aqueous standards) and for sea waters. Slightly slower sorption was found for all studied elements in sea water in comparison with aqueous standards. Sorption kinetics for sea water is shown in Figure 4. Various concentrations of HNO_3 (1–2 M), HCl (2–4 M) and EDTA (0.2–0.5 M) were studied for rapid desorption of Cd(II), Cu(II), Ni(II) and Pb(II) adsorbed on the Si-APS. Quantitative elution was achieved using 2 M HNO_3 and 0.5 M EDTA. However, the elution process using 2 M HNO_3 was slightly faster and this eluent was chosen as optimal. The kinetics of desorption in 2 mL 2 M HNO_3 is presented in Figure 4.

3.3 *Validation of the SPE procedure*

The SPE procedure developed by using new Si-APS sorbent was further validated for the determination of total dissolved Cd, Cu, Ni and Pb in Black Sea water. Recovery was determined by enriching of the sea water samples with trace metals with concentrations between 20 μg L^{-1} and 100 μg L^{-1}. Experiments performed showed that 50 mL is an optimal volume for sea water samples. Obtained recoveries for the metals of interest varied from 94 to 98% for the whole investigated concentration range.

To verify to what extent the dissolved organic matter from the sea water could influence the degree of sorption of trace metals, the concentrations determined by on-site SPE were compared to those obtained by SPE following sea water digestion. Very good agreement was found between the concentrations determined for digested and undigested samples (Table 1, one way ANOVA, *t*-test, 95% confidence level). This suggests that newly synthesised sorbent allows quantitative sorption of trace elements in the presence of dissolved sea organic matter. Additional measurements by TOC analyser showed, that for these sea water samples the mean concentration of dissolved organic matter was 2.9 mg L^{-1}.

Table 1. Comparative results (mean ± s), $\mu g\,L^{-1}$ for total dissolved Cd, Cu, Ni and Pb concentrations in sea water, $n = 3$.

Sample	Element	Solid phase extraction	Solid phase extraction*	Liquid/liquid extraction
1	Cd	0.008 ± 0.002	0.009 ± 0.003	0.009 ± 0.002
	Cu	3.9 ± 0.2	3.9 ± 0.3	3.7 ± 0.1
	Ni	8.5 ± 0.4	8.6 ± 0.4	8.4 ± 0.2
	Pb	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01
2	Cd	0.023 ± 0.002	0.025 ± 0.003	0.026 ± 0.002
	Cu	6.3 ± 0.01	6.2 ± 0.02	6.4 ± 0.01
	Ni	7.9 ± 0.3	8.1 ± 0.3	8.1 ± 0.2
	Pb	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.01

Note: *Sea water sample digested according to EPA method 200.02.

The accuracy of the SPE procedure was further evidenced by the good agreement of the concentration determined by the SPE procedure and those found by the liquid/liquid extraction by using ammonium pyrolidine dithiocarbamate as an extraction reagent. Results presented in Table 1 illustrate this good agreement, thus confirming the reliability of the proposed pre-concentration and separation procedure for total dissolved Cd, Cu, Ni and Pb determination in sea water using Si-APS sorbent.

Detection limits (3σ criteria) calculated on the basis of repeated analysis of a blank sample (50 mL doubly distilled water passed through the whole proposed SPE procedure) using ETAAS measurement were: Cd $0.002\,\mu g\,L^{-1}$, Cu $0.005\,\mu g\,L^{-1}$, Ni $0.03\,\mu g\,L^{-1}$, Pb $0.02\,\mu g\,L^{-1}$. Relative standard deviations for the typical levels of Cd ($0.08–0.15\,\mu g\,L^{-1}$), Cu ($5–15\,\mu g\,L^{-1}$), Pb ($0.06–0.15\,\mu g\,L^{-1}$) and Ni ($7–9\,\mu g\,L^{-1}$) in tested sea water samples varied in the range 5–13% for all studied elements.

3.4 *Application of the on site SPE procedure for determination of total Cd, Cu, Ni and Pb content in sea water*

The concentrations of dissolved metals for the sampling sites from the Bourgas region obtained by the on site SPE procedure were in the range: $0.08–0.17\,\mu g\,L^{-1}$ for Cd; $5.2–16.8\,\mu g\,L^{-1}$ for Cu; $8.1–8.9\,\mu g\,L^{-1}$ for Ni; and $0.08–0.16\,\mu g\,L^{-1}$ for Pb (Figure 5). Average metal concentrations decreased in the order Ni(II) > Cu(II) > Pb(II) > Cd(II) for all sampling sites. Furthermore, total dissolved Cd, Cu, and Pb concentrations were significantly higher ($P < 0.05$) at sampling sites 3, 5 and 6, while those for Ni were comparable among the sampling locations. The above observations are consistent with the past or existing activities in the region. For example, sampling site 3 is located near the city of Bourgas, which could explain the higher total dissolved concentrations obtained for all studied elements. Sampling sites 5 and 6 in the vicinity of Atia and Vromos are influenced by past Cu mining activities and elevated natural concentrations of Cu in the region. Nonetheless, for all sampling sites, the total dissolved Cd, Cu, Ni and Pb concentrations are lower than permissible levels according to Bulgarian legislation. The results of the present study are comparable with the previous measurements of the Cd, Cu, Ni and Pb, published for shelf waters of the north-western Black Sea [32], and close to the lowest values reported in the north-western Black Sea water near the Danube River delta [33].

3.5 *Determination of DGT labile concentrations in sea waters*

DGT measured concentrations at different sampling sites of the Bourgas Gulf are shown in Figure 5. Since the discrimination of metal species in the DGT gels is based on their size and lability [19,34], measured DGT concentrations provide an integrated time response including free metal ions and small inorganic and organic complexes that diffuse through the gel and accumulate on the Chelex 100 resin. Since DGTs with open-pore gel were applied, the determined concentrations include both inorganic and organic kinetically labile complexes, but excluded kinetically inert species with low diffusion coefficients [34,35].

The ratios between DGT labile Cd or Ni and total dissolved metal concentrations $[M]_{DGT}/[M]_{tot}$ were relatively high from 0.6 to 0.8, suggesting that the complexation of these metals by inorganic complexes or kinetically labile organic complexes is prevailing. Much lower were these ratios for Cu and Pb: 0.2 to 0.38 (Cu) and 0.24 to 0.4 (Pb). These observations are consistent with higher complexation of Cu(II) and Pb(II) by the dissolved organic matter and suspended particles. Such strong complexation of Cu and Pb in sea water was also found using voltammetry [36,37].

The determination of total dissolved Cd, Cu, Ni and Pb concentrations in complex matrices such as sea water and the evaluation of their speciation is a difficult task, taking into account the great variety of existing chemical species and complexes. Therefore new reliable procedures for *in situ* or on site sampling and pre-concentration are highly sought. In the present work, the combined application of the SPE using a newly synthesised sorbent for on site pre-concentration and separation of analytes and *in situ* deployed DGT allowed one to obtain in parallel the total dissolved metal concentrations and labile metal species in sea water. This knowledge can be helpful for an improved understanding

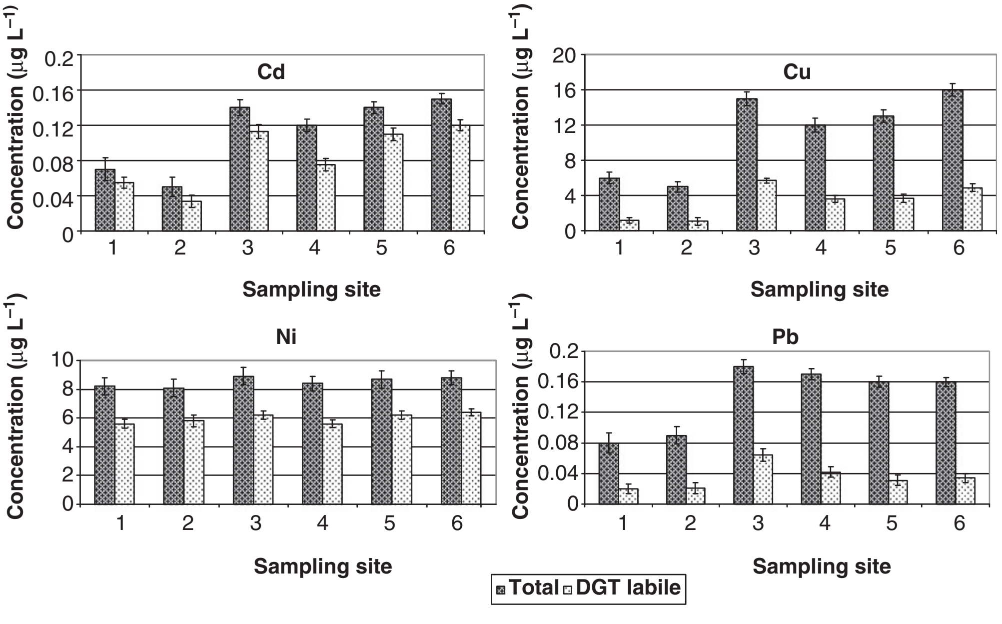

Figure 5. Total dissolved and DGT concentrations of Cd(II), Cu(II), Ni(II) and Pb(II) at sampling sites in the Bourgas Gulf, $n = 3$.

of the biological and dynamic processes in the surface waters of the Black Sea as well as for setting the basis of efficient environmental monitoring and sound management.

4. Conclusion

The column SPE procedure based on chemically modified monodispersed silica spheres for pre-concentration and matrix removal, in combination with ETAAS detection were proposed and tested for determination of the total dissolved Cd(II), Cu(II), Ni(II) and Pb(II) concentrations in sea water. The sorption of the studied metals on the newly synthesised sorbent was unaffected by the dissolved sea organic matter present in the sea water, which avoids the acid digestion of the samples before extraction. Furthermore, the whole procedure is relatively fast and could be easily performed on site immediately after sampling, thus reducing potential transformations and contamination of the samples. The obtained total dissolved Cd(II), Cu(II), Ni(II) and Pb(II) concentrations in the Bourgas Gulf were slightly higher that for the other sampling location in the southern Black Sea coast area, but much lower than legislative limits. The above set of data was complemented by the labile metal concentrations determined by *in situ* deployed DGT. Parallel determination of the total dissolved metal concentrations and kinetically labile concentrations can improve the understanding of fate and impacts of these trace metals in the sea waters.

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