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Abstract

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Trace Metal Speciation and Bioavailability in Surface Waters of the Black Sea Coastal Area Evaluated by HF-PLM and DGT

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Trace metal speciation in seawater from the Bulgarian Black Sea coast was studied in situ by hollow fiber permeation liquid membrane (HF-PLM) and by diffusion gradients in thin-film gels (DGT). The concentrations of Cd, Cu, Ni, and Pb determined by HF-PLM were lower than those measured by DGT, in agreement with their analytical windows, e.g., free metal ions provided by the HF-PLM and dynamic (mobile and labile) species by the DGT. The obtained suite of data was further used to evaluate the bioavailability of these metals to the microorganisms, which was then compared with experimental results of metal uptake to green microalga *Chlorella salina*. Uptake fluxes of the Cd, Cu, Ni, and Pb to *C. salina*, were predicted from the measured HF-PLM concentrations and laboratory experimentation in artificial seawater, in agreement with theoretical considerations. The HF-PLM and DGT appear to be promising analytical techniques for speciation and bioavailability studies in complex environmental media and allow improved understanding of the role of different chemical species in metal bioavailability (and impact) in seawaters.

Introduction

In natural waters, trace metals exist in many different physical and chemical forms (1, 2) which determine their mobility, bioavailability, and toxicity to aquatic organisms. Therefore, the determination of relevant species of trace metals, in addition to their total metal concentrations, will be of utmost importance in improving our understanding of the processes that govern their fate and ultimate impact on the environment. Recent progress in the development of different speciation techniques (3, 4) capable of providing (often in situ) specific metal species, that may control biological availability (and effect), gave further impetus to the research in this area. Examples of such techniques include diffusion

gradients in thin-film gels (DGT) (5, 6), gel integrated microelectrodes combined with a voltammetric system (GIME) (7) permeation liquid membranes (PLM) (8, 9), the Donnan membrane technique (DMT) (10), competitive ligand-exchange/stripping voltammetry (CLE-SV) (7, 11), stripping chronopotentiometry (SCP) (12), and ion exchange resins (13). The capabilities of DGT, GIME, SCP, PLM, DMT, and CLE-SV were tested for in situ speciation analysis of Cd, Cu, Ni, Pb, and Zn in freshwater systems and compared with respect to their performance and to the metal species detected (4). Similar intercomparisons between different speciation techniques, as well as linking metal speciation with bioavailability to microorganisms in seawater, are currently lacking, even though greater environmental interest is deserved.

The Black Sea is a unique marine ecosystem characterized by a large river inflow and very limited exchange of water with the Mediterranean Sea, which determines its low salinity and particular aquatic chemistry (14). It is the largest permanently anoxic basin with a strong redox gradient across the oxic/anoxic interface and a well distinguished hydrogen sulfide zone. As such, different studies are dedicated to trace element chemistry in anoxic waters as well as in the transition zones between the oxic and anoxic layers, e.g., (14–18). Very limited and contradictory information exists concerning the speciation of trace metals in Black Sea surface water (16, 17, 19). Cu and Zn were reported to be bound to the dissolved organic matter in large proportions, from 93 to 99% of Cu and from 82 to 97% of Zn, in surface waters from the western Black Sea (19). By contrast, 95–99% of uncomplexed Cd, Cu, Pb, Ni, and Zn were found in surface oxic waters by sequential filtration ion-exchange during the U.S.–Turkish Black Sea expedition (16). No information exists concerning the speciation of the trace metals Cd, Cu, Ni and Pb, even though specific species or fractions could control their uptake by biota and represent an ecotoxicological concern in Bulgarian coastal zones. Recently, inorganic trace element speciation in the river–estuary–Black Sea system was calculated, by using measured total dissolved metal concentrations; however, the complexation by dissolved organic matter (DOM) was neglected (20). Alternatively, the amounts of metals accumulated in macroalgae (21, 22) and different marine invertebrates (23, 24) collected from the Black Sea are regularly monitored, albeit rarely supplemented by data on seawater metal concentrations or speciation. In summary, there is a lack of reliable trace metal speciation and bioavailability information necessary for a better understanding of their impact to the marine organisms, as well as for an improved understanding of the biological and dynamical processes in the surface waters of the Black Sea.

In the present study, the HF-PLM and DGT techniques for in situ sampling and preconcentration were employed to explore the speciation of Cd, Cu, Ni, and Pb in surface waters of the Bulgarian Black Sea coastal area. Because of the bioanalytical features of these speciation techniques (25, 26), the obtained suite of data was used to evaluate the potential bioavailability of these metals to organisms, which was then compared with obtained experimental results of metal uptake fluxes to green microalga *Chlorella salina*. To the best of our knowledge, this is the first study that combines simultaneous in situ use of DGT and HF-PLM with bioassays to provide further insight to the relationship between speciation and bioavailability of Cd, Cu, Ni, and Pb in seawater.

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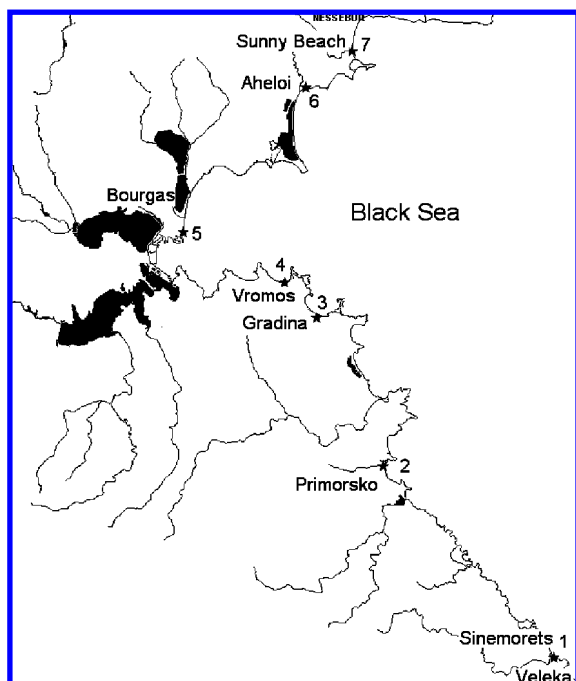


FIGURE 1. Location of the sampling sites on the southern Bulgarian Black Sea coast.

Experimental Section

Sampling Sites and Water Characteristics. Surface seawater samples were taken at the end of September 2007. The sampling area included the southern Bulgarian Black Sea coast (Figure 1). Several locations were explored including sampling sites (Ss) of the Bourgas gulf (Ss 3 to 5), which is largely influenced by the industrial activity in this area. Ss 1 (Sinemorets) and 2 (Primorsko) are situated south of Bourgas gulf, corresponding to a natural reserve, far from known contamination sources. They are near Veleka River (Ss 1) and Devil River (Ss 2); however, the samples were taken about 100 m north of the river mouth. Ss 4 (Vromos) is located near an old, inactive sink of waste waters from copper mines; Ss 5 is in the Bourgas city gulf, a location under anthropological (petrochemical industry and refinery as well as nonferrous mining and processing) influence. Ss 3 (Gradina) and 7 (Sunny Beach) as well as 6 (Aheloi) correspond to the north and south points of the large Bourgas gulf. The main physicochemical characteristics of the seawater samples are given, in Table S2 of the Supporting Information, as well as details about sampling collection and handling.

Determination of Total Cd, Cu, Ni, and Pb Concentrations in Seawater. Total dissolved metal concentrations (e.g., following 0.22 μm filtration) were measured by electrothermal atomic absorption spectrometry (ET-AAS) following liquid/liquid extraction of metal dithiocarbamate complexes into isobutyl methyl ketone. Details on the extraction procedure and ET-AAS measurements are provided in the Supporting Information. Detection limits of the liquid/liquid extraction–ET-AAS procedure were 0.02 nM for Cd, 0.12 nM for Cu, 0.17 nM for Ni, and 0.05 nM for Pb.

HF-PLM Sampling and Handling. Hollow fiber permeation liquid membrane cells were deployed in situ at 1.5 m depth at each sampling site for 2 h, to obtain a preconcentration factor, F , independent of the deployment time (9). After retrieval of the HF-PLM from the water, the strip solution in the lumen was collected and analyzed by ET-AAS. More details about exact HF-PLM setup and procedure can be found in the Supporting Information and refs 9 and 27.

DGT Sampling and Handling. Standard type DGT units (DGT Research Ltd.) were deployed in situ by using a plastic strip simultaneously with an HF-PLM device for 6 days. Metal

concentrations in the disks eluted with HNO_3 were determined by ET-AAS without further dilution. The concentration of metal in the bulk waters was calculated, as given in details in the Supporting Information.

Metal Bioavailability Determination. Metal bioavailability to the marine alga *Chlorella salina* (strain CCAP 211/25, Culture Collection of Algae and Protozoa, The Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Scotland) was quantified by determination of the intracellular metal concentrations and uptake fluxes. Algal cells at the midexponential growth phase were suspended in seawater from sampling sites 1 to 7. After 1 h exposure, algae were filtered, washed with 0.01 M EDTA in 0.24 M NaCl and 0.0053 M KCl, and digested with concentrated HNO_3 (suprapur, Baker). Intracellular metal fractions, $\{M\}_{\text{int}}$ were measured by inductively coupled plasma mass spectrometry (Perkin-Elmer, Elan DRC II). Since the amount of the intracellular metal is an integral of the uptake flux over the exposure time, the uptake flux, J_{int} were determined based on the temporary evolution of $\{M\}_{\text{int}}$. Further details about algal culture cultivation and bioavailability determination can be found in the Supporting Information.

Data Treatment. The normal distribution and variance homogeneity of the test data sets were examined using Kolmogoroff–Smirnov’s test and Bartlett’s test, respectively. Statistical differences in dissolved, HF-PLM, and DGT concentrations determined at different sampling stations were evaluated by one-way ANOVA followed by Dunnett’s test at $p < 0.05$ using STATISTICA 6 (StatSoft).

Results

Total Dissolved Concentrations of Cd, Cu, Ni, and Pb. Total dissolved metal concentrations were very low in the sub- and nanomolar range. The concentrations of Cd, Cu, and Pb, at sampling locations within the Bourgas gulf (Ss 4 and 5), were significantly higher ($p < 0.05$) than those determined in the sampling sites outside the gulf (Ss 1, 2 and 7). High dissolved Cd, Cu, and Pb concentrations found in the Bourgas gulf are consistent with past mining activities in this area (Ss 4) and also industrial activity in the city of Bourgas (Ss 5). No difference in Ni concentrations is found among sampling sites. The averaged dissolved Cd, Cu, Ni, and Pb concentrations determined at all of the studied locations are comparable with the levels published for shelf waters of the northwestern Black Sea (17) and close to the lowest values reported in the northwestern Black Sea water near the Danube River delta (16).

Chemical Speciation of Cd, Cu, Ni, and Pb. In situ deployed HF-PLM and DGT techniques combined with subsequent ET-AAS measurements were used for the speciation analysis of Cd, Cu, Ni, and Pb in seawater. The HF-PLM, based on carrier mediated transport of the metal ion across a hydrophobic membrane, was used in an equilibrium mode (8, 9) and provides free metal ion concentrations. The discrimination of metal species in the DGT gel is based on their mobility (size) and lability (5, 6). Thus, DGT provides the average accumulated amount of dynamic species, including free metal ions, small inorganic and organic complexes, and kinetically labile organic complexes that can diffuse through the open pore gel used in the present study, while excluding kinetically inert species (28).

HF-PLM and DGT determined concentrations of Cd, Cu, Ni, and Pb at each sampling site are given in Figure 2 as a percentage of the total dissolved metal concentration (Table 1).

HF-PLM-obtained concentrations for Cd were in the range of 0.95 to 3.4 pM, representing 19 to 24% of the dissolved Cd (Figure 2a). DGT obtained concentrations for Cd ranged from 65 to 80% of the dissolved Cd and corresponded to 33 to 118 pM. HF-PLM determined that copper concentrations varied

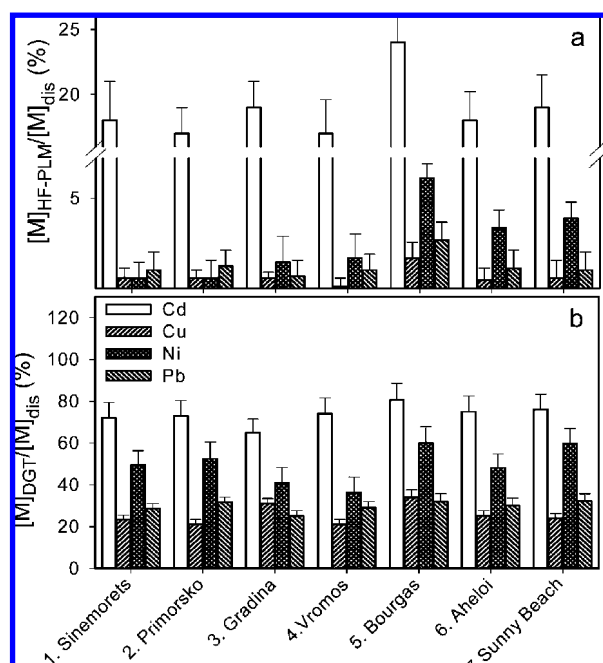


FIGURE 2. HF-PLM (a) and open-pore DGT (b) concentrations determined at sampling sites 1 to 7. The results are presented as a percentage of the total dissolved metal (e.g., following 0.22 μm filtration) and correspond to the deployment time of 2 h for HF-PLM and 6 days for DGT.

TABLE 1. Total Dissolved Metal Concentrations (nM), (e.g., following 0.22 μm filtration), in the Samples from Locations 1 to 7, Determined by Liquid/Liquid Extraction and ET-AAS Detection (mean of three replicated \pm standard deviation)

sampling site	Cd	Cu	Ni	Pb
1. Sinemorets	0.07 \pm 0.01	8.1 \pm 0.2	7.9 \pm 1.2	0.15 \pm 0.02
2. Primorsko	0.06 \pm 0.01	7.8 \pm 0.2	8.2 \pm 1.3	0.13 \pm 0.01
3. Gradina	0.05 \pm 0.01	5.8 \pm 0.2	8.1 \pm 1.3	0.12 \pm 0.02
4. Vromos	0.16 \pm 0.03	12 \pm 1.0	8.9 \pm 1.2	0.21 \pm 0.04
5. Bourgas	0.14 \pm 0.02	18 \pm 2.0	8.3 \pm 1.3	0.19 \pm 0.03
6. Aheloi	0.09 \pm 0.01	10 \pm 1.0	8.4 \pm 1.4	0.17 \pm 0.02
7. Sunny Beach	0.08 \pm 0.01	6.6 \pm 0.1	8.1 \pm 1.2	0.14 \pm 0.01
open seawaters ^{a,b}	0.053–2.58	0.16–2.83	7.67–10.9	NR ^e
surface waters ^{a,c}	0.033–0.161	2.9–28.8	8.6–17.5	0.03–0.61
surface waters ^{a,d}	0.36–13.7	4.7–36.2	NR	NR

^a Range of 0.45- μm filterable metal concentrations.

^b Open sea (16). ^c NW Black Sea shelf (17). ^d Near Danube River delta (18). ^e NR = nonreported.

between 5.8 and 8 pM, which is less than 1% of the dissolved Cu, in agreement with the existing literature stating the significant complexation of Cu in seawater by different organic ligands (29–31). A 5-fold higher $[\text{Cu}^{2+}]_{\text{HF-PLM}}$ than the average of the other sampling sites was determined in waters of the Bourgas gulf (Ss 5), which is consistent with existing industrial activity in this area. DGT Cu concentrations were about 1–2 nM (6 nM for the Ss 5), corresponding to 21–34% of the 0.22 μm filterable Cu. These values are lower than those determined in oligotrophic seawater by cross-flow filtration and ion exchange (32).

Nickel concentrations determined by HF-PLM were in the sub-nanomolar range, representing between 1 and 3% of the total dissolved Ni and varied significantly between sampling sites. A clear increase of $[\text{Ni}^{2+}]_{\text{HF-PLM}}$ concentrations from 0.08 nM $[\text{Ni}^{2+}]$ at Ss 1 to 0.42 nM at Ss 5 and 0.33 nM

$[\text{Ni}^{2+}]_{\text{HF-PLM}}$ at Ss 7 was found. This observation contrasts with the dissolved Ni and the DGT measurements (Figure 2b, Table 1), which gave similar concentrations for all the sampled sites. DGT determined Ni species represented between 36 and 60% of the dissolved Ni. Since the amount of DOC is comparable among the sampling locations (Table S2, Supporting Information), the difference in Ni complexation is probably due to the differences of DOM quality and reactivity. Indeed, sampling sites 1 and 2 are in the areas with river input, which can affect DOM quality.

HF-PLM-determined lead concentrations were also in the sub-nanomolar range and correspond to 1–1.5% of the total dissolved Pb. $[\text{Pb}^{2+}]_{\text{HF-PLM}}$ determined at Ss 4 and 5 were about twice as high as those found in the other sampling locations. DGT Pb concentrations were much higher than the HF-PLM measured and varied from 25 to 32% of the dissolved Pb.

Metal Bioavailability to the Green Microalga *Chlorella salina*. Cd, Cu, Ni, and Pb uptake fluxes to *Chlorella salina*, exposed for 1 h to seawater at sampling stations 1 to 7 increased in the order $J_{\text{int,Cd}} < J_{\text{int,Pb}} \sim J_{\text{int,Ni}} < J_{\text{int,Cu}}$ for all the studied locations. The intracellular metal content (and flux) was higher in algae exposed to seawater from sampling sites 5 for Cu, and Ss 4 and 5 for Cd and Pb, corresponding to higher dissolved metal concentrations of seawater from these sampling sites. Furthermore, no clear relationship was found between uptake fluxes and dissolved metal concentrations for Ni and Pb (Figure 3 and Table 1). For example, for a similar 0.22 μm filterable Ni or Pb concentrations, ten- and two-fold increases in Ni or Pb uptake were found at Ss 5 in comparison to Ss 1.

Discussion

Recent theoretical developments demonstrated that the understanding of metal uptake by microorganisms (and biological response) can be improved by considering the dynamics of speciation and bioavailability (26, 33, 34). According to the current concept of the metal–organism interaction (35), the availability of chemical species to organisms is determined by their chemical reactivity, the supply of these species to the surface, and their internalization by the organisms. There is a conceptual similarity between the bioavailability to the organisms and the process-determined response of the HF-PLM and DGT sensors (26). Hence, the HF-PLM and DGT speciation analysis can be used to predict 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. More specifically, the extent to which complex species in the medium contribute to uptake depends on the interplay and the relative magnitudes of the flux of the unsupported free metal ion relative to the internalization flux (or permeability) (eq S1, Supporting Information). When the uptake flux, J_{int} , is much lower than the diffusion flux, J_{diff} , then the complexes are not called upon to satisfy the organism. Hence, if these conditions are met, a measure of the free metal ion concentration suffices for the purposes. In contrast, when $J_{\text{int}} \gg J_{\text{diff}}$, complexes will contribute to the metal uptake depending on their lability (33). In such a case, measurements of the free metal ion concentration are insufficient, and dynamic techniques, such as DGT, will be more relevant in predicting metal bioavailability. The larger the internalization flux, the larger will be the fraction of dynamic species consumed by the organism.

Furthermore, theoretical estimations demonstrated that for the green microalga *C. salina*, studied in the present work, the diffusion process was much faster than the metal transport across the cell membrane (Table S3, Supporting Information). Therefore, the diffusion flux of the free metal ion is more than enough to supply all the internalized metal;

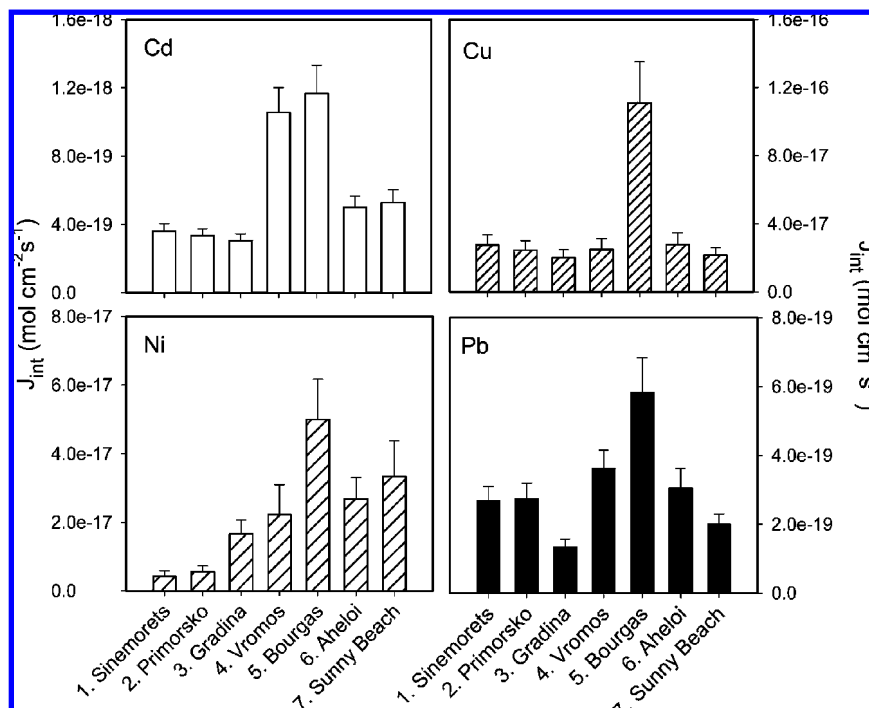


FIGURE 3. Uptake flux of Cd, Cu, Ni, and Pb to alga *Chlorella salina* exposed for 1 h to seawater from different sampling sites.

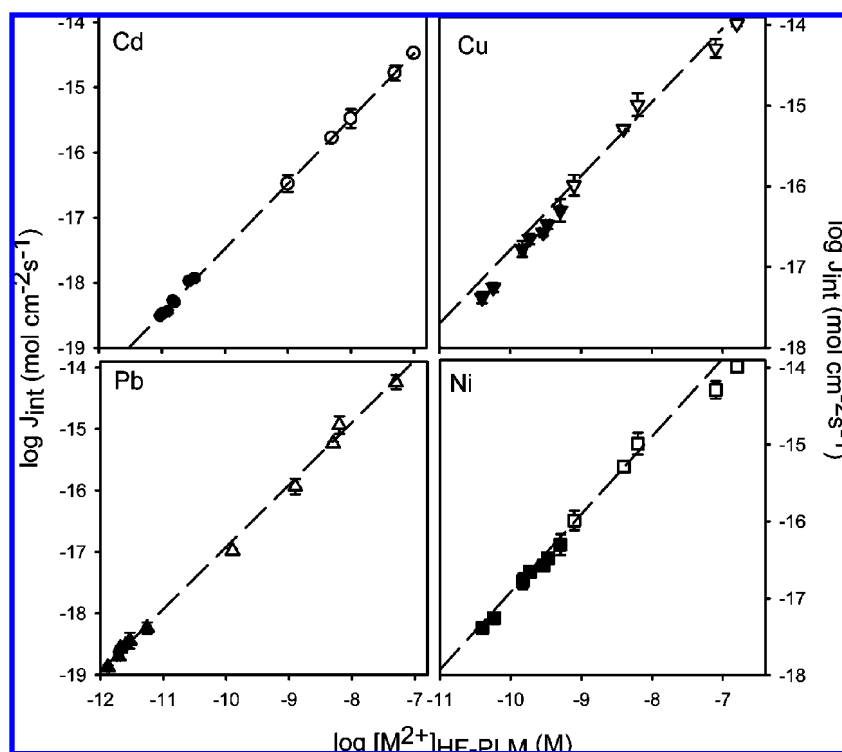


FIGURE 4. Log–log plot of the metal uptake fluxes by alga *Chlorella salina* determined in Black Sea waters from sampling sites 1 to 7 (full symbols) and artificial seawater (open symbols), as a function of HF-PLM measured concentrations. The solid lines correspond to the linear part of the Michaelis–Menten equation, corresponding to concentrations that are much lower than the apparent half-saturation constant. The artificial seawater, matching the Black Sea water composition, was spiked with increasing concentrations of metal in the absence and presence of nitrilotriacetic acid (NTA).

therefore, no increase in the metal uptake flux through dissociation of different complexes can be expected to occur. Indeed, the uptake fluxes seem to be directly correlated (slope of 1) to the free metal ion concentrations determined by HF-PLM in seawater at sampling sites 1 to 7 (Figure 4). Furthermore, the metal uptake fluxes obtained in seawater were superimposed on the experimental points correspond-

ing to equivalent HF-PLM concentrations, obtained in spiked artificial seawater.

Overall, the present study provides pioneering results on the application of HF-PLM and DGT techniques in determining the speciation of Cd, Cu, Ni, and Pb in the surface seawaters of the Black Sea coastal area. For all studied elements and for all sampling points, the concentrations

determined by HF-PLM were lower than those measured by DGT, in agreement with the analytical window of these techniques, i.e., free ions were measured by HF-PLM (8) and dynamic (mobile and labile) species by DGT (5). Indeed, HF-PLM used in the equilibrium mode was shown to measure free metal ion concentrations with a large number of ligands and under different conditions (9). Moreover, comparable concentrations of Pb^{2+} and Cu^{2+} were found by HF-PLM and by a complexing gel integrated microelectrode for direct measurement in the Gulljmar fjord (36). Since the open-pore gels were used, the dynamic DGT species also include metal complexes with dissolved organic matter, that was shown to be DGT labile (5, 37) but with lower diffusion coefficients than that of the free metal ion (38).

In addition to their bioanalytical characteristics (26), these techniques have the advantage to combine high preconcentration and matrix separation with in situ deployment. These are extremely important features, because of the very low trace element concentrations and very complex high salt-content matrix. In addition, in situ deployment of HF-PLM and DGT combined with subsequent elemental detection allowed for the speciation analysis of Cd, Cu, Ni, and Pb with minimal sample handling and pretreatment, thus minimizing possible contamination problems and artifact introduction. Furthermore, combined use of the DGT and HF-PLM techniques provides a measurement of the different metal species in seawater, which allows the identification of the domain of validity of equilibrium models such as the free ion activity model, FIAM (39), or the biotic ligand model, BLM (40, 41). In agreement with theoretical estimations, uptake fluxes of Cd, Cu, Ni, and Pb by the green alga *C. salina* in seawater can be predicted from the measured HF-PLM concentrations and laboratory experimentation in artificial seawater. Overall, the HF-PLM and DGT appear to be promising analytical techniques for speciation and bioavailability studies in complex environmental media such as seawaters. Their combined use allows improved understanding of the role of different chemical species in metal bioavailability and accounts for the chemical speciation in environmental quality regulations and monitoring programs, which traditionally are based on the total or dissolved metal concentrations.

Acknowledgments

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Supporting Information Available

Details of the sample handling and trace metal clean conditions, electrothermal atomic absorption determination of total metal concentrations, HF-PLM, DGT, and uptake experiments. Data about physicochemical characteristics of the Black Sea waters. Comparison of the HF-PLM and DGT concentrations with uptake fluxes by alga *Chlorella salina*. Calculations about the fluxes limiting the algal uptake. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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