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METAL SPECIATION STUDIES IN A BRACKISH / MARINE INTERFACE SYSTEM

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ABSTRACT

The paper presents some interesting results of metal speciation studies employing Anodic Stripping Voltammetry (ASV) applied in a dynamic natural system such as the brakish – marine interface formed between the polluted waters discharged by the Koumoundouros lake and the marine ones of the Gulf of Elefsis.

The distributions and behaviour of various forms of Pb, Cd and Zn have been followed seasonally. Four categories of dissolved species were identified (very labile, moderately labile, slowly labile and inert), as well as particulate species. It was found that their relative contribution and patterns are determined by three main factors: salinity, pH, and the presence of dissolved organic carbon. Increasing salinity stimulates formation of very labile chloro-complexes, while increasing pH stimulates the formation of inert species and particulates. A considerable part of slowly labile species seems to be metal complexes with organic ligands.

KEYWORDS: Metal speciation, Koumoundouros lake, (A.S.V.), lead, zinc, cadmium

INTRODUCTION

In the centre of interest of all environmental sciences lies the question about the relationship between the forms, properties and quantities of specific physical and chemical forms of an element in the environment and the effects of these forms on the biota and geochemical cycles.

The toxicity, bioavailability, bioaccumulation, biodegradability, persistence, mobility, solubility, extractability and many other critical properties depend on the form and nature of the chemical species (Florence, 1986, Scoullos and Pavlidou, 1997, Lores and Pennock, 1998). "Speciation analysis" or simply "speciation" is in fact the determination of these distinct species.

Speciation has gained gradually a very wide recognition as vital component of environmental chemistry, since the knowledge of the total concentration of an element in specific environmental compartment is often inadequate to explain its various different roles and properties. The term "speciation" encompasses usually two slightly different connotations of speciation: The functional one and the operational one. These two may overlap but they are not at all identical.

Functionally we may identify and distinguish between species that are, for example, available to plants or ecotoxic; species of an element that are more easily exchangeable in mineral surfaces than others, etc. The International Union of Pure and Applied Chemistry (IUPAC) has tried to dissociate speciation from functionality by defining speciation as “the process yielding evidence of the atomic or molecular form of an analyte”. However even if this might be the objective of all environmental studies, operationally speciation is determined by the physicochemical properties of the real natural entities which are the so called «species” and from the analytical techniques and means which are available to the researchers to determine them. So, speciation refers, basically, to the determination of the concentration of various “fractions”, which are clusters of species which having different physicochemical properties and such as are “extractable” or “detectable” under specific conditions and with specific means. Then, according to our models (either experimental or theoretical) we assume that these fractions correspond to specific atomic or molecular forms and we attribute to them specific biochemical or geochemical characteristics.

In inorganic analytical chemistry speciation was always a vital component of its development, if not its starting point. The determination of the various nitrogenous species (NO_3^-,NO_2^-,NH_4^+) offers valuable example of the early achievements where functional and operational definitions coincide. However, in the field of environmental and marine chemistry dealing with metals the developments were more recent and perhaps, less impressive due to the inherent difficulties of the exercise.

An important part of our previous work was focusing on the impact of factors such as pH, salinity and redox conditions on the speciation of trace metals (e.g. Scoullos, 1983; Scoullos and Pavlidou, 1997; Pavlidou, 1998). A large number of our studies are being carried in the Gulf of Elefsis which offers an ideal natural laboratory with a variety of extremely interesting microenvironments suitable for the study of metal speciation under different natural conditions (Scoullos and Oldfield, 1984; Scoullos, 1986; Scoullos and Zeri, 1992; Scoullos *et al.*, 1994a, 1994b; 1995; Scoullos and Pavlidou 1997). Koumoundouros lake is one of these interesting subsystems of the Gulf of Elefsis (Scoullos *et al.*, 1995; Scoullos and Pavlidou, 1997; Pavlidou 1998).

Classifying trace metals in waters into different categories of species is a very difficult and unsafe task, particularly when the total concentration is at the level of microgram per litre or below and when we wish to group species according to similar functional or behavioural characters.

Electroanalysis and especially anodic stripping voltammetry (ASV) which is used in the present work, in combination with other techniques is considered, perhaps as the most powerful technique available for speciation studies (Florence, 1986). Until now most of the applications of ASV are in the simple identification of the relative «lability» of various ions, ion pairs and inorganic or organic complexes, such as the ones given in Table 1, determined in natural waters.

A metal species is in general characterized as *“labile”* in the aquatic environment if it can be reduced in the surface of the mercury electrode.

Table 1. Main complexes in the Marine Environment

Inorganic		**Organic**	
		M_xL_y where L organic ligands:	
		Carbohydrates	e.g. Glycol, cellulose
$M(aq)^{+n}$	where M metal ion	Hydrocarbons	e.g. CH_4, C_6H_6
$MCl_x^{+(n-x)}$	x=1 to 4	Fatty Acids	e.g. $CH_3(CH_2)_{16}COOH$
$M(OH)_x^{+(n-x)}$	x=1 to 4	Humic Acids	
$M(SO_4)_x^{+(n-2x)}$	x=1 ή 2	Fulvic Acids	
$M(CO_3)^0$, $M(HCO_3)^+$		Alcohols	e.g. CH_3OH, C_6H_6OH
		Porphyrins	e.g. Chlorophyll
		Sulfur compounds	e.g. CH_3SCH_3, RSH

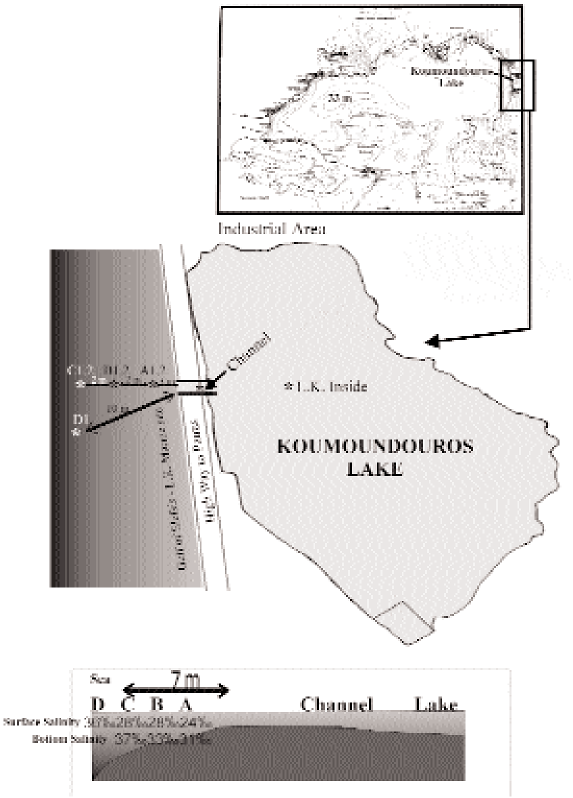

Figure 1. The stations sampled at the intermixing zone of Koumoundouros Lake.

This is affected by the dissociation kinetics and electrochemical criteria such as the effective measurement time.

The *"labile" fraction* includes the free metal ion and those metal complex which can dissociate immediately in the diffusion layer giving the free metal ion and producing an ASV signal (see Fig. 2). The *"non labile"* fraction includes metal complexes or fine colloidals which dissociate very slowly, compared to the appropriate diffusion time.

The *"diffusion time"* is the time that a complex needs to dissociate in the diffusion layer. This time for the ASV is 2 msec (Figura and McDuffie, 1980; Scoullos and Pavlidou 1997).

SAMPLING AND METHODS

Sampling – The area studied

In the present study, we attempt to demonstrate the changes occurring during one of the most common and basic processes in nature, namely the discharge of brackish water into the marine environment and the rapid intermixing taking place in the interface zone where both salinity and pH change rapidly.

The specific natural system chosen for this study is Koumoundouros lake and its connecting system to the Gulf of Elefsis (Fig. 1).

Koumoundouros lake is a coastal lagoon -like shallow, brackish lake located some 20 Km west of Athens on the north-eastern corner of the Gulf of Elefsis near the high way to Patras. Being in the middle of a heavily industrialised area is being exposed since the 1950's to pollution and anthropogenic alterations of its size and depth as well as to reduction of the natural freshwater inputs it receives.

The system has been studied in the course of the present research regularly between August 1994 and December 1995.

Surface and bottom samples were collected with great care at 15-20 cm below the surface and 25-50 cm from the bottom. The stations sampled are shown in Figure 1. When in the text and figures (e.g. 4,5 etc.) "L.K. Marine site" is mentioned this corresponds to station A.

Samples of the "surface microlayer" were also collected, whenever the conditions allowed, by applying carefully a wide perspex plate on the surface of the location sampled and by collecting the water attached on the surface of the plate in polyethylene bottles (Mantoura, 1985). This procedure was repeated several times in order to collect adequate volume of sample.

In Figure 1, the subscript 1 and 2 at the letters of the stations, refer to the surface and the bottom samples at the station.

All samples were filtered through preconditioned 0.45 μm Nuclepore filters. The filtered samples were stored at +4°C until analysis (Florence, 1986).

Analysis

Analytical grade Chelex-100 of 200-400 mesh and concentrated HNO_3 and $HClO_4$ 70% were used. To ensure low trace metal blanks, a Milli-Q water purification system was used. A buffer of 2.5 M CH_3COONa/CH_3COOH/pH 6.3, which has been pretreated with Chelex-100, was used for the determination with different pulse anodic stripping voltammetry (DPASV). Na-Chelex-100 was slurred into 0.8 cm I.D. metal free glass column containing styrofoam fritz and converted to the Ca form with $CaCl_2$ 1M. The $CaCl_2$ solution has been pretreated with Chelex-100 to low trace

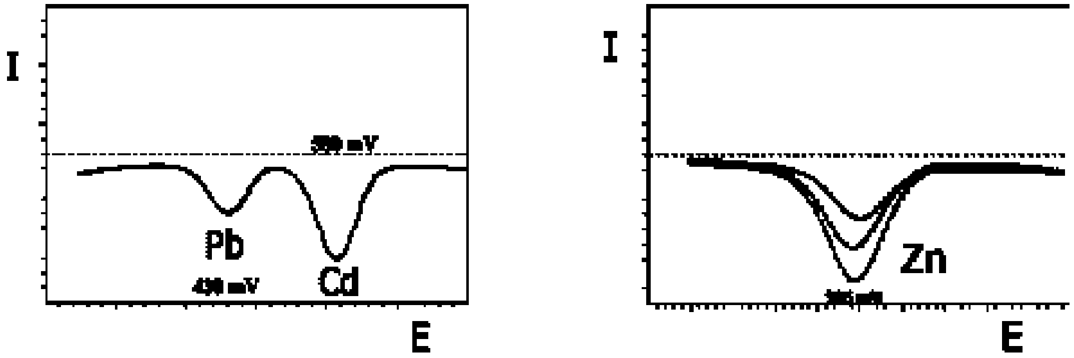

Figure 2. DPASV Polarograms of Pb, Cd and Zn with AMEL 433 Polarographic Analyzer.

metal blanks. A flow rate of 3.5-4.0 ml min^{-1} was used throughout, giving a contact time of 9-10 sec (Figura and McDuffie, 1979). The whole procedure was carried out under laminar flow hood with caution to avoid contamination.

For the DPASV an AMEL model 433 Polarographic Analyzer was employed, using hanging mercury electrode (HMDE) (Pavlidou, 1998). Each metal was determined separately using deposition potential of: -600 mV for Pb, -800 mV for Cd and –1200 mV for Zn (Pavlidou, 1998). The deposition time was 300 seconds for Pb and Cd and 60 sec for Zn. The half potential for the three metals are shown in Figure 2. All ASV measurements were repeated at least three times for each metal.

All glassware and polyethylene bottles and apparatus were pretreated with 10% solution of HNO_3 for two days. To minimize the adsorptive losses of trace metals from water samples, all equipment were equilibrated with purified 2 mM $CaCl_2$ in 2 mM tris(hydroxymethyl)amino methane/HCl overnight before use (Figura and McDuffie, 1977).

Particulate mater was determined after the filtration of known volumes of samples through preweighted and preconditioned 0.45 μM Nucleopore membrane filters. The filters holding the known weight of particulate matter were digested in specially designed PTFE containers placed on a hot plate by using con. HNO_3.

Speciation Procedure

The speciation scheme applied (Fig. 3) is determined by the chosen time scale of measurements, the different contact times with Chelex-100 (in column or batch experiments), the manner of sample stirring and the thickness of diffusion layer in DPASV measurement. This scheme allows, according to Figura and McDuffie (Florence, 1986; Figura and McDuffie, 1980) to classify the soluble metal species, into four operationally defined fractions as:

a) *Very Labile fraction:* This corresponds to what is determined with direct ASV analysis of the sample.

b) *Moderately Labile fraction*: The sample is passed with a contact time of 9 - 10 sec through Ca-

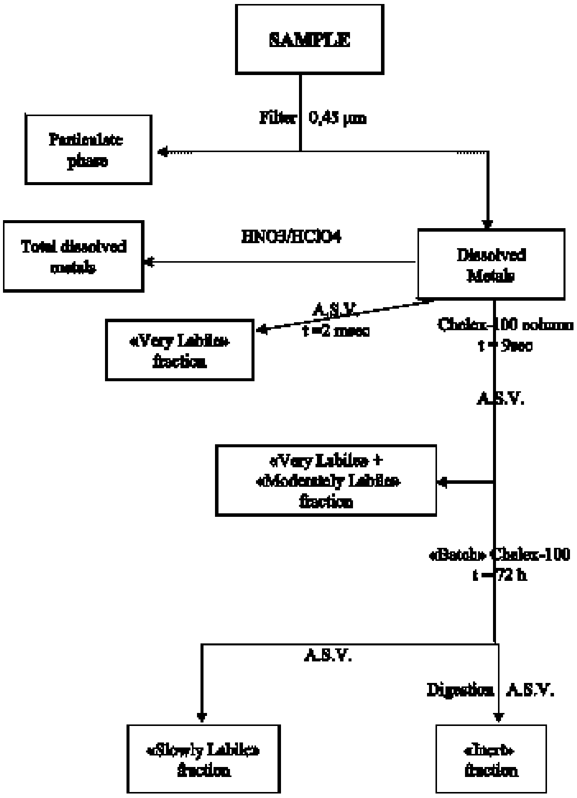

Figure 3. Flow chart of the trace metal speciation scheme used

Table 2. Correlation coefficients of salinity, pH, and % contribution of fractions of Cd, Zn and Pb throughout the period (Aug. 1994 - Dec. 1995) and the area studied.

	pH	Salinity (‰)	"Very Labile"	"Moderately Labile"	"Slowly Labile"	"Inert"	Particulate (μg l^{-1})	
Salinity (‰)	0,36							**Cd**
"Very Labile"	0,32	0,92						
"Moderately Labile"	-0,69	-0,73	-0,67					
"Slowly Labile"	0,53	-0,27	-0,33	-0,34				
"Inert"	0,45	-0,03	-0,22	-0,54	0,64			
Particulate (μg l^{-1})	0,17	0,46	0,13	-0,32	0,14	0,30		
Particulate (μg g^{-1})	0,09	0,12	-0,15	0,14	-0,04	0,01	0,78	
Salinity (‰)	0,36							**Zn**
"Very Labile"	0,02	0,27						
"Moderately Labile"	-0,32	0,26	-0,16					
"Slowly Labile"	0,32	-0,08	-0,57	-0,38				
"Inert"	-0,02	-0,51	-0,64	-0,36	0,18			
Particulate (μg l^{-1})	0,34	-0,30	-0,43	-0,45	0,23	0,75		
Particulate (μg g^{-1})	-0,91	-0,53	-0,02	0,14	-0,15	0,03	-0,22	
Salinity (‰)	0,36							**Pb**
"Very Labile"	-0,21	0,57						
"Moderately Labile"	-0,04	0,20	-0,40					
"Slowly Labile"	-0,09	-0,75	-0,42	-0,41				
"Inert"	0,40	-0,23	-0,22	-0,55	0,09			
Particulate (μg l^{-1})	0,27	0,39	0,60	-0,62	-0,33	0,50		
Particulate (μg g^{-1})	-0,06	-0,06	0,18	-0,55	-0,14	0,73	0,60	

Chelex column to collect the moderately labile fraction together with the very labile one. The difference between retained and very labile fraction gives what is defined as moderately labile fraction.
c) *Slowly Labile fraction*: The effluent from treatment (b) was stirred 72 hours with Ca-Chelex resin. The resin retains the slowly labile fraction.
d) *Inert fraction*: The effluent from (c) containes the inert fraction which is treated with con. HNO_3 and $HClO_4$ 70%.
The *total dissolved metal* is determined after treatment of the sample with con. HNO_3 and $HClO_4$ 70%.

RESULTS AND DISCUSSION

The dynamic nature of the system is indicated by the prevailing physicochemical parameters in its various parts. Inside the lake salinity fluctuates between 12.7 ‰ and 17.2 ‰ whereas outside between 28.0 ‰ and 39.9 ‰. In the channel station some impact of the increasing salinity is usually observed. In all stations, full analyses were carried out for particulate and dissolved species (of all four fractions of lability) as well as for organic carbon. The overall levels of metals determined in the system are high as one could expect from a system located in the center of an industrial zone. The cadmium levels in particular are considerably higher than the average determined offshore even within the gulf of Elefsis. The results of all analyses were intercorrelated (employing the "*statistica*" computing system) in order to identify if some apparent relationships exist.

In Table 2, the statistically significant interparametrical correlations are shaded. From these "shaded" coefficients, it becomes clear that relatively few interparametrical correlations seem statistically significant. Namely the positive and negative correlations between salinity and “very labile”, or “slowly” and “moderately labile”, species of cadmium and lead respectively, as well as some positive and negative correlations between pH and the “slowly” or “moderately labile” species or particulate forms. It is noteworthy the observed very strong positive correlation between the “very labile” fraction and salinity for cadmium, whereas for lead there is a medium positive correlation and a very week one for zinc (Kebork, 1981).

These correlations are statistically important despite the high complexity of the system, being a two layer intermixing zone in a heavily polluted area. However it is obvious that, through such a “gross” treatment of the bulk of data one looses a great part of the detailed, very valuable information (Kebork, 1981). This exercise, also demonstrated that speciation results are difficult, and eventually meaningless, to be generalized even if they concern the same natural system, if the comparison is made under different conditions, particularly if the system is a very dynamic one. Speciation studies, by their nature, could provide the tool to understand the “fine structure” and inter-relationship between species provided that the data obtained are discussed and interpreted in a detailed and careful way.

In this work we have made an attempt to complete another piece of the puzzle which describes the impact of mixing processes upon metal speciation in the study system, presenting the results of all samplings during the period August 1994 – December 1995.

Figure 4 shows the concentrations of zinc, cadmium and lead inside and outside Koumoundouros Lake during August 1994.

It is apparent that all three metals exhibit a decrease of the total concentration of their dissolved species towards the sea. This is attributed to the combined effect of dilution, on one hand and removal by precipitation, on the other.

Three important information is provided by this Figure:

(1) There is a marked increase of the percentage but also the concentration of the very labile species of cadmium and lead outside the lake in comparison to the levels inside it (from 4.69 % to 35.9 % and from 18.6 % to 58.8 % respectively). This is attributed, mainly, to the formation of very labile chloro-complexes.

(2) The percentage contribution of the inert fraction for zinc and cadmium was increased outside the lake (from 21.0 % inside to 25.4 % outside for zinc and from 12.7% to 34.7% for cadmium, respectively). This is attributed mainly to the adsorption of metals on inert organic rich colloidals and iron and aluminum hydroxy-oxides under the prevailing condi-

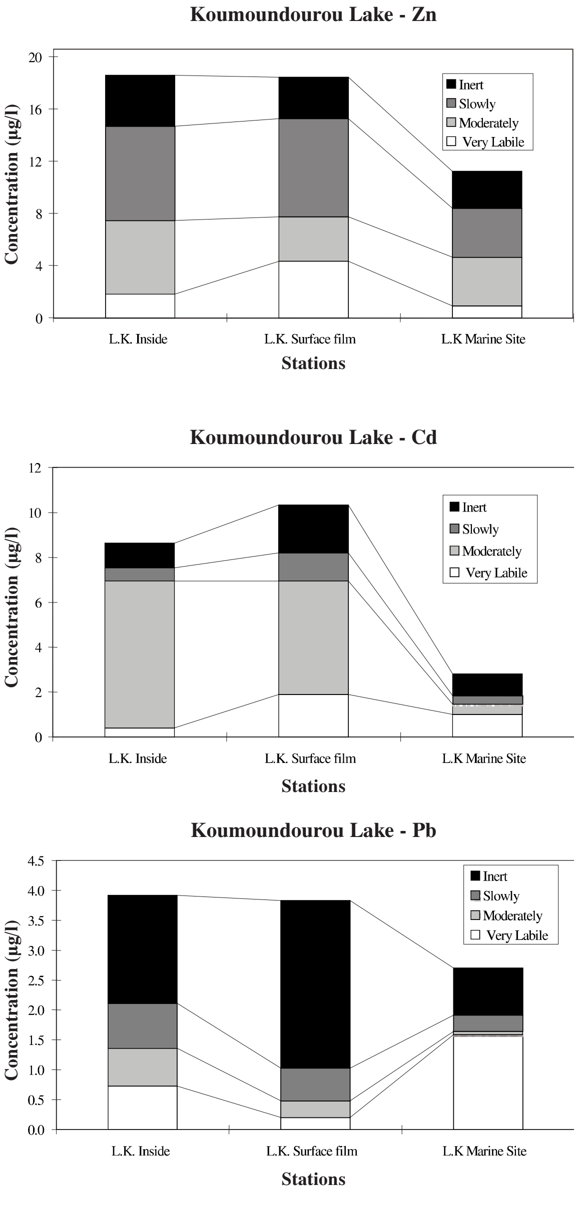

Figure 4. Metal species inside, at the surface film and outside the Koumoundouros lake in August 1994

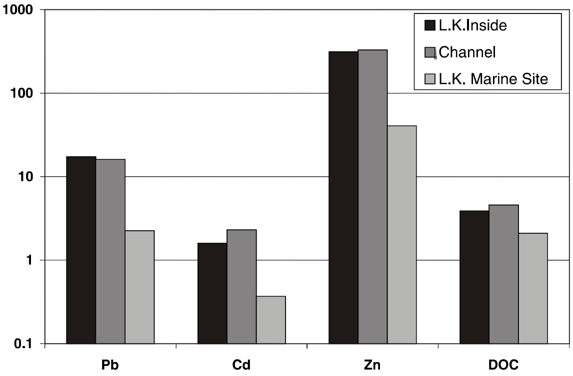

Figure 5. Particulate metals (µg g^{-1}) in the Koumoundouros lake intermixing zone during February 1995.

tions. Both phenomena (1) and (2) were developed at the expense of moderately and slowly labile species.

(3) The extremely high enrichment of the surface film of the lake with inert lead species (73 % of the total dissolved lead), which could be attributed to both inorganic (e.g. , , etc) and organic complexes. The former could be easily found in the emissions of internal combustion engines of the vehicles using the high way, which is located in small distance from the lake (Scoullos and Pavlidou, 1997).

Figure 5 refers to the February 1995, presenting the particulate fraction of the three metals studied (in µg g^{-1}) and the dissolved organic carbon (in ppm). It shows that the metal content of particulate matter is gradually decreasing when approaching the sea with increasing salinity and pH. This reflects regular structure typical at similar systems with minimum disturbances from winds and other phenomena frequently influencing such shallow dynamic systems making their study difficult and the results more difficult to be safely interpreted. In the present case the marked decline of DOC also supports the regularity of the systems structure. The drastic decrease of DOC indicates an increasing co-precipitation and flocculation of the study metals with other substances both inorganic and organic.

Figure 6 presents the actual concentrations of the different species of zinc along the salinity gradients of the two-layer (non turbulent), “salt-wedge-type” intermixing zone of the Koumoundouros lake which is typical of many similar small intermixing systems of the Mediterranean, as it concerns both its structure (stratified, shallow system with strong salinity gradients) and the extend and nature of the pollution occurring in it. The same feature is also observed for cadmium and to a somewhat lesser extend for lead.

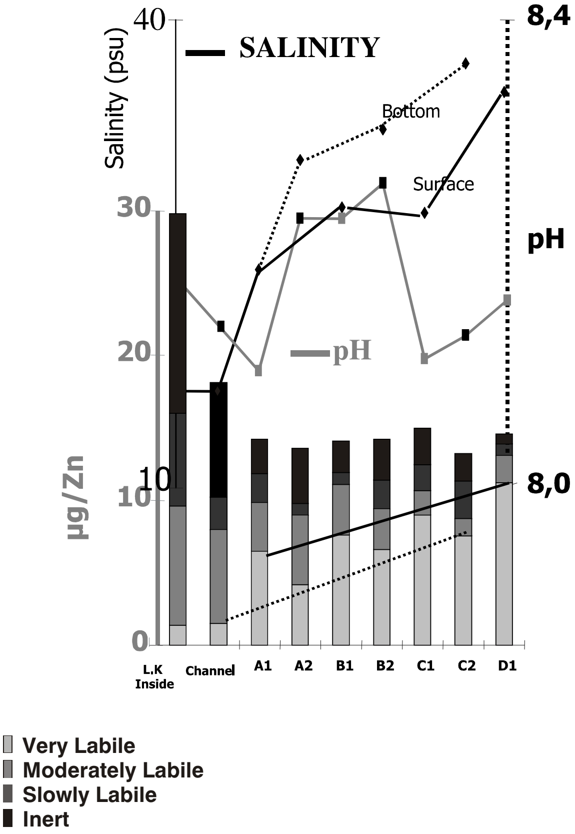

Figure 6. Zinc species along the salinity and pH gradients in Koumoundouros lake intermixing zone, during July 1995.

What is striking for the metals studied is the linear, positive correlation between the salinity and the concentration of the “labile” fractions determined (see Table 2) appeared to be strong for cadmium, medium for lead and week for zinc, probably due to the unequal efficiency of each of the three metals to form stable, non labile complexes with organic ligands, or to flocculate and precipitate with inorganic or organic substances (Figura and McDuffie, 1980). This correlation is attributed to the formation of chloro-complexes, which are formed when chlorides are abundant and act antagonistically to other ligands (such as OH$^-$, CO$_3^{2-}$, HCO$_3^-$, SO$_4^{2-}$) which form less labile species (Nurnberg, 1989; Roux, 1998; Scoullos and Pavlidou 1997).

It is noteworthy that the dissolved organic carbon

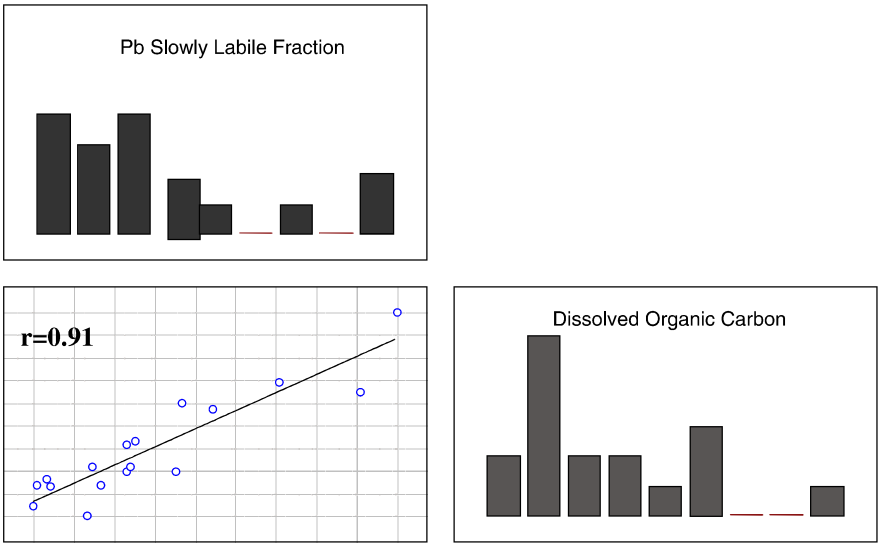

Figure 7. Correlation between the "slowly labile" fraction of Pb and Dissolved Organic Carbon, in the entire Koumoundouros lake system during December 1995.

in the Koumoundouros lake system correlates often quite well with the slowly labile fraction of metals (Figures 7 & 8) indicating that, at least a considerable part of the slowly labile complexes of lead determined are complexes with organic ligands. The even less labile "inert" dissolved metal fraction apparently approaches in nature the very fine, non-retained by 0.45 μM membrane filters, colloidal forms which, by their nature, relate easily to particulate forms. This is supported by Figure 9, which presents the correlation between the "inert" dissolved zinc species and the particulate zinc for the entire period and area studied.

CONCLUSIONS

The information obtained from the present study indicate that application of ASV, in combination

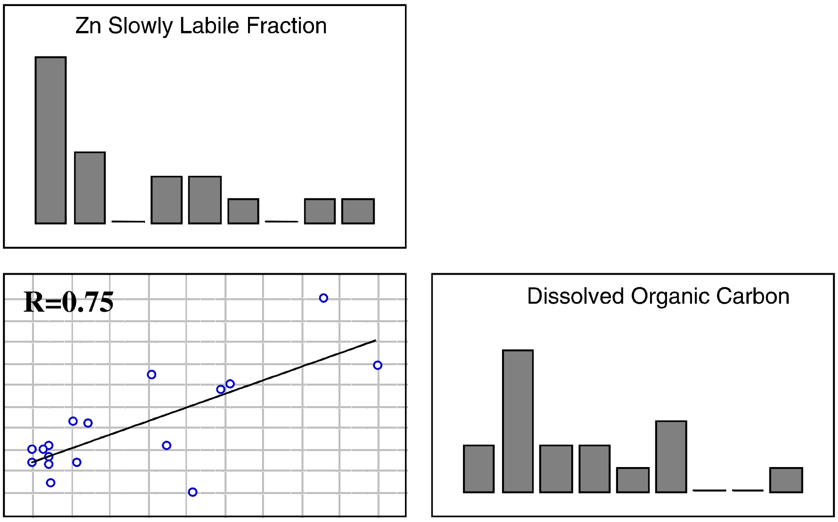

Figure 8. Correlation between the "slowly labile" fraction of Zn and Dissolved Organic Carbon, in the entire Koumoundouros lake system during December 1995.

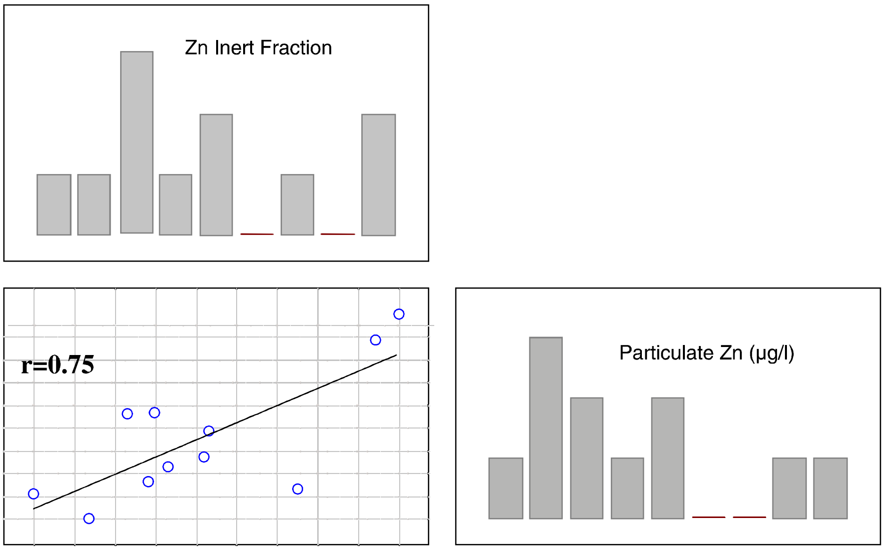

Figure 9. Correlation between the "inert" fraction of Zn and particulate Zn, in the entire Koumoundouros lake system during December 1995.

with the use of Chelex-100 in columns and batches provides a powerful technique for the investigation of metal speciation in natural systems.

Such a dynamic natural system of particular interest is the interface between brackish waters of a coastal lake (in this case of Koumoundouros lake) and the marine waters in a non tidal, "salt-wedge"-type intermixing zone. This system is characterized by rapidly changing percentages of metal species of different lability.

Four "operationally" defined classes of labile species were determined: the "very labile", the "moderately labile", the "slowly labile" and "inert" ones.

The major factors identified influencing speciation are salinity, pH and availability of organic ligands.

Strong correlations between salinity and the "very labile" species of cadmium and lead and were detected, whereas zinc showed a weaker positive correlation with salinity. In some cases the inert or slowly labile species may also increase with salinity. Increasing pH acts to a certain extend antagonistically to salinity increasing the less labile or inert metal species.

Dissolved organic carbon correlates well with "slowly labile" complexes, particularly of lead and zinc and plays an antagonistic role to inorganic ions.

With increasing pH and salinity, thus moving away from the lake outlet few parallel phenomena take place such as:

a) Decrease of the "moderately labile" and "slowly labile" species of all metals with parallel increase of the "very labile" fraction for cadmium and lead.
b) Precipitation of metals and formation of particulate matter. This leads to a significant reduction of the "very labile" species in the bottom waters of each station, when compared to its surface ones (see Figure 6). The decrease of the metal content of the particulate matter with increasing distance from the outlet particularly outside the channel (see Figure 6) is attributed to the gradually increasing pH, which stimulates co-precipitation and flocculation of inorganic and organic species of other elements.
c) The surface film was found to be particularly enriched in metals, in comparison with the underlying water. Though in most cases the concentration of particulate metal is considerably elevated, most probably due to the atmospheric inputs, the presence of other fractions and especially of the "very labile" one is also significant. Its good correlation with salinity applies also to the surface film.

The present research shows that detailed studies of small natural systems with well defined boundary conditions may contribute substantially to our understanding of basic processes of global interest.

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