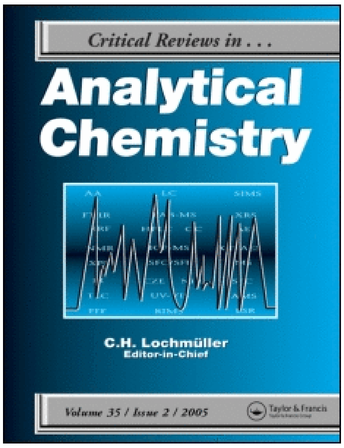

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TRACE ELEMENTS: DATA AND INFORMATION IN THE CHARACTERIZATION OF AN AQUEOUS ECOSYSTEM

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I. INTRODUCTION

Several elements, generally called trace elements, are present in natural waters at very low background concentration levels, as shown in Table 1. There is actually a consistent degree of arbitrariness in the definition of which elements should be considered as trace elements. In addition, several synonyms are used, mainly based on a concept of their concentration level (trace elements, ultratrace metals, trace inorganics) or their toxic effects (toxic metals, toxic elements, ecotoxic heavy metals). In the former case, once an operative definition of trace and ultratrace has been adopted, any difficulty would be overcome. In the latter case, an operative toxicity definition would seem to be more difficult to achieve because it depends too much on the type of toxicity considered (acute, chronic, lethal), on the type of biological species tested, and on the particular experimental conditions used, which might not be perfectly reproducible in different laboratories.

The consequence of the above arbitrariness is that the list of the trace elements considered does vary consistently according to the different environmental agencies or authors involved in this topic.

In the list shown in Table 1, most of the significant toxic elements naturally occurring in the hydrocycle are reported along with their average concentration in open ocean seawater and in freshwater. Some of these elements are essential, i.e., below and above a specific range of concentration, physiological effects can be evidenced, due to deficiency or excess, respectively.

As it is impossible to settle the question of a suitable operative definition, which should be up to international organizations such as IUPAC to decide, this review focuses attention on those elements of Table 1 for which biologists' interest is of prime importance, and which present severe difficulties or contrasting results in the measurement of their content. These elements are essentially arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, tin, and zinc, and constitute a class of high significance in ecotoxicology and are frequently present in anthropogenic pollution.

In spite of the large number of publications dedicated to analytical procedures concerning the accurate determination of the above elements, incorrect results still may be obtained due to the adulteration of the sample prior to the analysis (i.e., during its sampling, storage, and handling) and/or the uncompensated effects arising from matrix and reagent impurities during the analysis.

The authors of this review attempt to present the current state-of-the-art on many aspects of the topic which have been considered pertinent. These include dissolved and particulate matter, general criteria for sample handling, detection limits and figures of merit for some

Table 1
TRACE ELEMENT BACKGROUND CONCENTRATION LEVELS IN OPEN OCEAN SEA WATER AND FRESH WATER

	μg/ℓ	
Element	**Sea water**	**Fresh water**
Ag[a]	0.003—0.01	0.01—3
As	0.3—2	0.1—3
Au[a]	0.01	0.01
Be[a]	0.006	0.01
Cd	0.01—0.05	0.01
Co	0.04	0.05
Cr[b]	0.05—0.08	0.1—10
Cu[b]	0.1	0.1—1.8
Hg	0.001—0.01	0.01
Ni[b]	0.2—0.3	0.1—5
Pb	0.002—0.15	0.2—1
Sb[a]	0.1—0.2	0.05—0.5
Se[b]	0.04—0.1	0.01—0.3
Sn	0.005—0.01	0.01—1
Tl[a]	0.01	0.004
Zn[b]	0.01—0.1	0.5—5

[a] Not considered in the present review.
[b] Essential.

significant procedures proposed; accuracy and precision as they result from interlaboratory quality control measurements; and chemical speciation. The problem of sampling methods has been reviewed extensively by Berman and Yeats[1] and is not further considered here. The final aim of this review is to individuate:

1. The most convenient procedures to be used in order to minimize the bias between experimental and real values (reliability of the analytical results with respect to the sample)
2. The sampling procedure criteria to be used during the surveys (in terms of number of samples, frequency of sampling, location of sampling stations, etc.) in order to obtain as much information as possible (representativity of all the gathered samples with respect to the ecosystem under consideration)

Owing to the extent and complexity of the topic, this review should not be considered exhaustive of all the papers published. In addition, we apologize because some of the significant papers undoubtedly are omitted.

It is likely that the general considerations drawn for the above-mentioned elements will hold for the remaining trace elements of Table 1 which are not considered herein. Besides, due to the lack of research dealing with these latter elements, any discussion on the more reliable procedures for sampling, handling, and analysis would be useless. The reader is therefore kindly requested to refer to standard method books for those elements that have not been mentioned here.

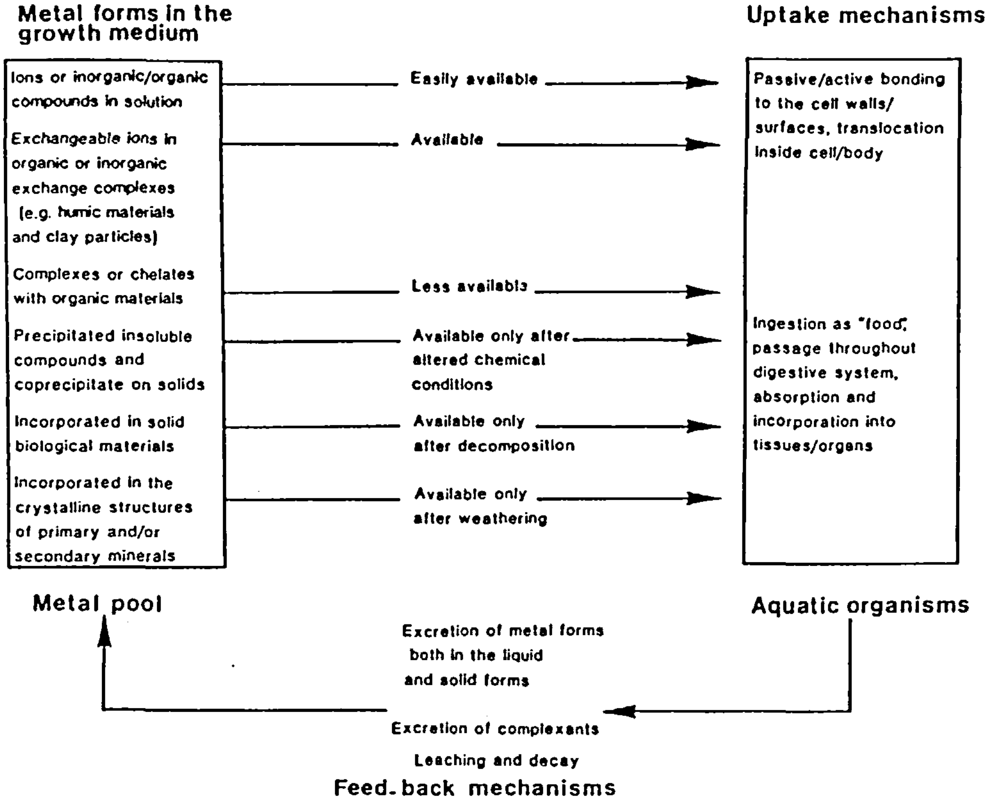

FIGURE 1. Feedback mechanisms of the metal form availability for biological uptake.[5]

II. DISSOLVED VS. PARTICULATED MATTER

According to a widely accepted convention, chemical constituents in natural waters are classified as dissolved (soluble, filtrable), particulate (nonfiltrable, suspended), and sedimented matter. From a biological viewpoint, the uptake of trace metals by organisms chiefly occurs in the aqueous phase, the uptake from suspended solids being confined to the filter feeder organisms. These filter feeder organisms, with the exception of bivalves, generally ingest suspended matter which has a diameter lower than 50 to 100 μm.[2-4] Figure 1 shows the feedback mechanisms of the metal form availability for biological uptake.[5]

The uptake of metals by organisms has been discussed extensively by Salomons and Förstner[6] and by Förstner and Wittmann[7] in their excellent books concerning metals in the hydrocycle. From a geochemical viewpoint, suspended matter does play an important role, particularly in the circulation of trace metals in the hydrocycle. The importance of it is related to two main factors: (1) the suspended matter has a large specific surface area and consequently a large capacity to undergo adsorption/desorption reactions, and (2) the water transport mechanisms of trace metals are strongly influenced by the behavior of suspended solids.[8-12]

Depending on internal conditions (the composition and adsorption capacity of the suspended matter) and external conditions (pH, oxygen, temperature, streams, man-derived organic and inorganic products, etc.), interaction between dissolved, suspended, and sedimented matter takes place with a consequent redistribution of trace elements. The rate of redistribution depends on many factors and it is likely that biological matter acts as a catalyst.

Several researchers have shown that the equilibrium times for adsorption and desorption of heavy metals in sediment/water mixtures generally take days.[13-16] Maximum adsorption usually occurs at pH values above 7.5.[17]

The distinction between dissolved matter and particulate was operatively defined by Goldberg et al.,[18] who used filters with a nominal pore size of 0.5 μm. Currently, the acceptable size cutoff varies in the range of 0.4 to 0.5 μm, depending on the choice of the filter material and manufacturer.

As far as the dissolved matter is concerned, the above operative distinction can be accepted with no reservation provided that all the boundary experimental conditions proposed to avoid contamination or significant interaction between solid and liquid phases during filtration have been checked previously and stated precisely in the procedure. Generally, these procedures suggest filtering the water sample as soon as possible under moderate nitrogen pressure and collecting the desired volume after a sufficient amount of filtered water has passed. In this way, the filtrate only contains colloids and the fraction of very small particles of mineralogic and biologic origin which have passed the pores of the 0.45-μm filter. Usually these particles are a rather minor part of the inorganic and organic matter types suspended in natural waters. All phytoplankton and most bacteria are normally retained.

As for particulate, doubts arise about the practical meaning of the operative definition of suspended matter as "the one which is retained on the 0.45-μm pore size filter". Actually, due to the lack of an assignment of an upper limit of particle size above which suspended matter must be disregarded, gross errors due to suspended matter, such as phytoplankton or solid wastes fortuitously present in the water sample, cannot be avoided and it is likely that in these cases arbitrary decisions might be taken by the operator each time. In addition, since the amount and composition of the particulate may present a variation in different sampling points or at different sampling times larger than the aqueous phase does, there is a risk that the results obtained during a survey might be scarcely representative of the aqueous system under consideration, unless large numbers of samples, each sampling involving a large volume of water (100 to 500 ℓ), are drawn. However, a large volume of the samples implies a long filtration period (on average 1 day per sample). The dilemma is, therefore, whether to collect, filtrate, and analyze 1 sample per day with the consistent risk of getting the correlations among the samples wrong, or to collect, say, 20 samples in 1 day, filtrate and analyze them in 20 days, leaving in the meantime suspended matter in contact with the water phase. In the latter case, particulate-dissolved matter exchanges may occur. Indeed, Campbell et al.[19] observed, in unfiltered low-salinity estuarine water samples, a massive increase in copper-dissolved concentration (140%) over a period of 24 hr. No effects at all were observed during the same experiments for nickel, cadmium, and lead.

A. Phase Separation Techniques

Physical speciation, defined as the determination of the distribution of trace elements among solids of different size separated by using some physical techniques, has been considered by different authors and is reported in specialized papers and reviews.[20-28]

In particulate-dissolved matter separation, which is considered here in some detail, different approaches are used according to whether the form of interest is dissolved or particulate. Owing to the extremely low concentrations of the elements involved and to the complexity of the systems, it should be borne in mind that no physical methods for phase separation have been and can be suggested as essentially free from risks of stimulating *in situ* precipitation or flocculation processes, provoked by changes in pH, redox potential, etc. That is why it is impossible to devise any experiment for phase separation which will be interpreted unambiguously.

1. Deposition

In principle, separation by gravity (deposition) ought to be chosen, both for dissolved and particulate analysis, since any adsorption or contamination due to separating devices can be avoided. Owing to the length of time required to obtain complete deposition (5 days for a 40-cm-high water column, according to Stoke's equation, assuming that the suspended matter is in a state of flocculation),[20,26] adsorption of the metals on the inner surfaces of the container or volatilization of particular compounds may occur with a consequent decrease of metal content as evidenced by some authors.[29,30] For these reasons, desposition is seldom used for phase separation.

2. *Centrifugation*

Centrifugation, even though it requires rather a long manipulation, offers a satisfactory recovery rate (approximately 98%) of particulate and is competitive with filtration in particulate analysis of high turbidity waters.[20,26] Contamination of the solution and sometimes of particulate with Cr, Mn, and Fe as well as with other metals from the bearing has been observed by Van der Sloot and Duinker.[21] No systematic information on this topic has been found in the literature. Yet centrifugation is not the method which should be chosen if, in addition to particulate, the solution has to be analyzed for trace elements as well.[21] In the case of size separation, however, it is likely that the effect of contamination, if any, will be more consistent the more the number of fractions of separated particulate increases.

3. *Filtration*

Filtration can be used successfully for both particulate and dissolved matter analysis. Water filtration can be carried out under negative or positive applied pressure. With pressure filtration, it is essential that pressure differences lower than 20 cm Hg are used in order to avoid rupturing phytoplankton cells as this may lead to an increase in heavy metals as well as organic matter concentrations in the filtrate.[17] Positive pressure filtration in closed systems offers advantages in terms of speed of filtration and reduces the risk of airborne contamination.[31-33,69,71] Positive pressure is preferable with fresh water samples that have a high suspended sediment load, whereas using negative pressure, filtration rates may be as low as 100 mℓ/hr for standard filters (47 mm diameter, 0.45 μm pore size). Furthermore, by using nitrogen gas for positive pressure filtration, the technique eliminates pH variations due to CO_2 evolution caused during negative pressure filtration,[34] and maintains the integrity of anoxic samples with respect to redox conditions.[35]

The main problem, which has not yet been overcome, is the dependence of filtering efficiency of the filter load. In waters with a high colloid load, such as bog, lake, and estuarine waters, it was observed that organic matter, which colloids such as iron hydroxide are bound to, may pass in the filtrate, and its amount depends on the filter load and sharply decreases after a given filter load value.[22] This implies that quite variable concentration values could be obtained by analyzing successive filtrate fractions of the same sample whenever trace elements are initially bound to colloids in the solution or are retained by colloids during the filtration on the filter. In these cases, the conditioning of the filter by filtering a given volume of the sample may facilitate reproducible results, but with a risk of quite unreliable results. Collecting consecutive fractions of filtered solution and then analyzing each for trace elements would give sound information about the consistency of the results, and this should be a task of preliminary experiments for each system being studied.

Different kinds of filter material can be used as reported in Table 2. Nucleopore and particularly Millipore are the most frequently used; this is due to their high selectivity and low content in inorganic salts.

Using a particular device, Etcheber and Jouanneau[20] were able to filter large amounts of water (200 ℓ) and collect particulate also. They concluded that filtration proves to be the best method as far as the recovery rate of suspended matter is concerned. These authors

Table 2
THE MOST FREQUENTLY USED FILTER MATERIALS AND FILTER BRANDS IN NATURAL WATER ANALYSIS

Brand name	Material	Pore diameter (μm)
Millipore HA	Mixed cellulose esters	0.45
Membranfilters Ges.	Cellulose acetate	0.45
Nucleopore	Polycarbonate	0.5
Gelman A	Borosilicate glass fiber	0.3
Sartorious SM	Cellulose nitrate or acetate	0.45

Table 3
SUSPENDED MATTER: COMPARISON OF METAL CONTENTS IN WATERS OF HIGH TURBIDITY AS OBTAINED USING DIFFERENT PHYSICAL SEPARATION TECHNIQUES[20]

Sample sites			Cu (parts 10^{-6})			Pb (parts 10^{-6})			Ni (parts 10^{-6})			Zn (parts 10^{-6})		
			D	F	C	D	F	C	D	F	C	D	F	C
PK_{35}	April	77	33	24	22	59	55	64	33	30	42	260	276	269
	June	77	20	22	14	41	45	44	35	30	38	248	256	237
	October	77	22	20	17	45	51	55	42	34	40	273	284	271
Bordeaux	April	78	36	31	29	73	69	78	40	42	52			

Note: D, deposition; F, filtration; C, centrifugation. Number of samples analyzed each time = 8.

criticize its high cost and the considerable length of time which is required to collect just a few grams of solid matter from samples of low turbidity. In the latter case, many hundred liters of water must be treated. Since half a day might be required for 100 ℓ, the separation of one single sample may take more than 24 hr.[20]

B. Bias in Particulate Matter Analysis

In order to have an idea of the bias in deposition, filtration, and centrifugation methods on suspended matter analysis, the results obtained by Etcheber and Jouanneau[20] are commented on. In spite of the high concentrations involved — several tens or hundreds of milligrams per liter — consistent variations were found among the average concentrations obtained by using deposition, filtration, and centrifugation as separating methods. Centrifugation generally presented the lowest values of concentration in the case of copper and the highest ones in the case of lead and nickel; deposition presented the highest values in the case of copper; and filtration presented the lowest values in the case of nickel (Table 3). It is likely, but not certain, that higher concentrations of trace elements found in the particulate are caused by contamination of the sample during separation.

From all these findings and those relating to other data of the same authors, it is possible to state that using different physical separating methods, the maximal bias in particulate analyses may range between 13 and 60% and the relative standard deviation (r.s.d.) between 10 and 16%; both are consistently high considering that the bias and the r.s.d. due to the analytical procedure alone should be better than 1% at these concentration levels. There is no sound evidence that one method has general validity regardless of the nature of the sample and of the analyte being analyzed. Yet this level of bias can be accepted as far as the total content of particulate is concerned, as in the above case. When distribution among solids of different size is required for trace elements, contamination during the phase separation is likely to play a significant role. Additional systematic studies are needed, therefore, to gain more information about the contamination-free procedures to be used and to avoid gross errors. In the case of centrifugation, particularly, it would be worth verifying which tangential rate corresponds to the pressure effect during filtration whereby the phytoplankton cell rupture occurs.

As for the procedures for particulate analysis, X-ray fluorescence spectrometry and neutron activation analysis seem to be particularly suitable for sensitivity, accuracy, and multielement determination features.[36,37]

C. Bias in Dissolved Matter Analysis

In the case of particulate, the bias in trace element analysis can be attributed essentially to phase separation effects. However, in the case of the analysis of the filtrate, several sources of artifacts contribute to the overall bias — from filtering to instrumental determination — depending on the procedures selected each time for executing the determination.

Many valuable papers have been published and proposals suggested in order to get acceptable analytical data in water analysis.[38-42]

Several interlaboratory tests, relevant to trace elements, coordinated by the Water Research Centre (United Kingdom) in the framework of an Analytical Quality Control (AQC) program, have been performed.[43-46] As a rule, one would expect that the selected laboratories accepting to participate in AQC tests consider themselves able to cope with the task and that they will take special care to achieve the greatest accuracy and precision possible. In addition, it should be borne in mind that each result in any AQC test is not less than the average of four replicates.

In spite of careful attention to details during AQC experiments, large biases have been observed throughout, coupled in some cases with large standard deviations. Some significant results are reported here in order to make a comparison with the ones relating to the particulate. In the case of mercury after a 3-year trial, only 4 out of 9 laboratories gave acceptable values within a 10% bias at the concentration level of 0.36 μg/ℓ, and 8 laboratories met this criterium at the 1.62-μg/ℓ level.[44] Note that one laboratory which found good results at the 0.36-μg/ℓ level failed the test at the 1.62-μg/ℓ level. Having observed that the concentration of mercury in unpolluted natural waters does not exceed 0.01 μg/ℓ, the questions arise as to what the bias could be within AQC tests at concentration levels between 0.36 and 0.01 μg/ℓ and how these concentrations can be handled and determined reliably.

The AQC interlaboratory results for cadmium, copper, lead, nickel, and zinc in river water also bring to light the need for further improvements in the analytical procedures used by the participants to achieve higher accuracy. Three different samples were prepared for each analyte and each sample was analyzed once for each metal for 4 days.[43,45] In Table 4, some significant data are reported.

As in the previous case of mercury, it should be pointed out that the lowest concentrations of the elements in Table 4 are from 25 to 13,000 times higher than in natural unpolluted waters. When the maximal variation between laboratories was considered for each concentration of each analyte, this variation was found to range between 19 and 238%, on average 40%, which is really too high after a 3-year trial. Note that the maximum variation is for cadmium and it drops from 238 to 37% when the concentration value varies from 0.25 to 1 μg/ℓ.

Trace metals in filtered sea water were also considered in another intercalibration exercise.[47] It was carried out with 63 participants after 3 previous experiments. Once the outliers had been excluded on the basis of Chauvenet's criterion,[48] the following were found:

Table 4
INTERLABORATORY MAXIMAL VARIATION FOR Cd, Cu, Pb, Ni, AND Zn AT VARYING CONCENTRATIONS

WRC values	Concentration found: Min	Concentration found: Max	Variation % (max/min)	Ref.
Cd				
0.255[a]	0.189 (9)	0.450 (8)	238	45
1.000[b]	0.866 (9)	1.191 (11)	37	45
1.817[a]	1.425 (7)	2.234 (11)	56	45
Cu				
24.6[c]	20.85 (4)	27.17 (6)[d]	30	43
40[b]	31.60 (4)	41.20 (9)[d]	30	43
58.0[c]	48.48 (4)	58.70 (8)[d]	21	43
Pb				
25.5[c]	21.63 (3)	27.90 (6)[d]	29	43
40[b]	33.75 (11)	42.70 (9)	27	43
55.1[c]	46.00 (11)	56.20 (9)[d]	22	43
Ni				
25.6[c]	20.63 (3)	26.13 (2)[d]	27	43
40.00[b]	31.75 (4)	39.92 (1)[d]	26	43
66.0[c]	51.50 (3)	70.00 (9)[d]	36	43
Zn				
20.9[c]	18.98 (5)	25.55 (6)	35	43
40[b]	33.88 (4)	41.20 (6)[d]	22	43
55.2[c]	45.88 (3)	54.65 (8)[d]	19	43

Note: All values are expressed in μg/ℓ. Numbers in parentheses refer to the code of the laboratories which performed the analyses. WRC, Water Research Centre.

[a] Spiked river water.
[b] Standard solution.
[c] River water.
[d] For these values, $\frac{\text{Cfound} - \text{CWRC}}{\text{CWRC}}$ 100 does not exceed 10%.

1. The average interlaboratory values for preacidified samples were, with respect to frozen samples, 43% higher for cadmium, 230% for copper, 23% for lead, and 177% for zinc, with no statistical evidence for iron, manganese, and nickel.
2. Large differences between the results: in Figure 2, the spread of the results relevant to the dissolved copper determination for preacidified samples is shown.
3. Very large standard deviations relevant to mean interlaboratory values (% r.s.d.): 87(59) for cadmium, 78(115) for copper, 70(95) for lead, 30(38) for zinc, 39(89) for manganese, 80(65) for nickel, and 70(112) for iron (the values in parentheses refer to frozen samples).
4. Very poor spike recovery, which can be considered a measure of the accuracy reached by the participants, between 36 and 96%, depending on the analyte.
5. Significant improvements, although the above remarks still hold under conditions where fewer participants have been selected and the number of individual analyses has been increased up to between 100 and 130.

In a subsequent round,[49] for most metals the range of values reported and the coefficient of variation remained largely as shown in Table 5.

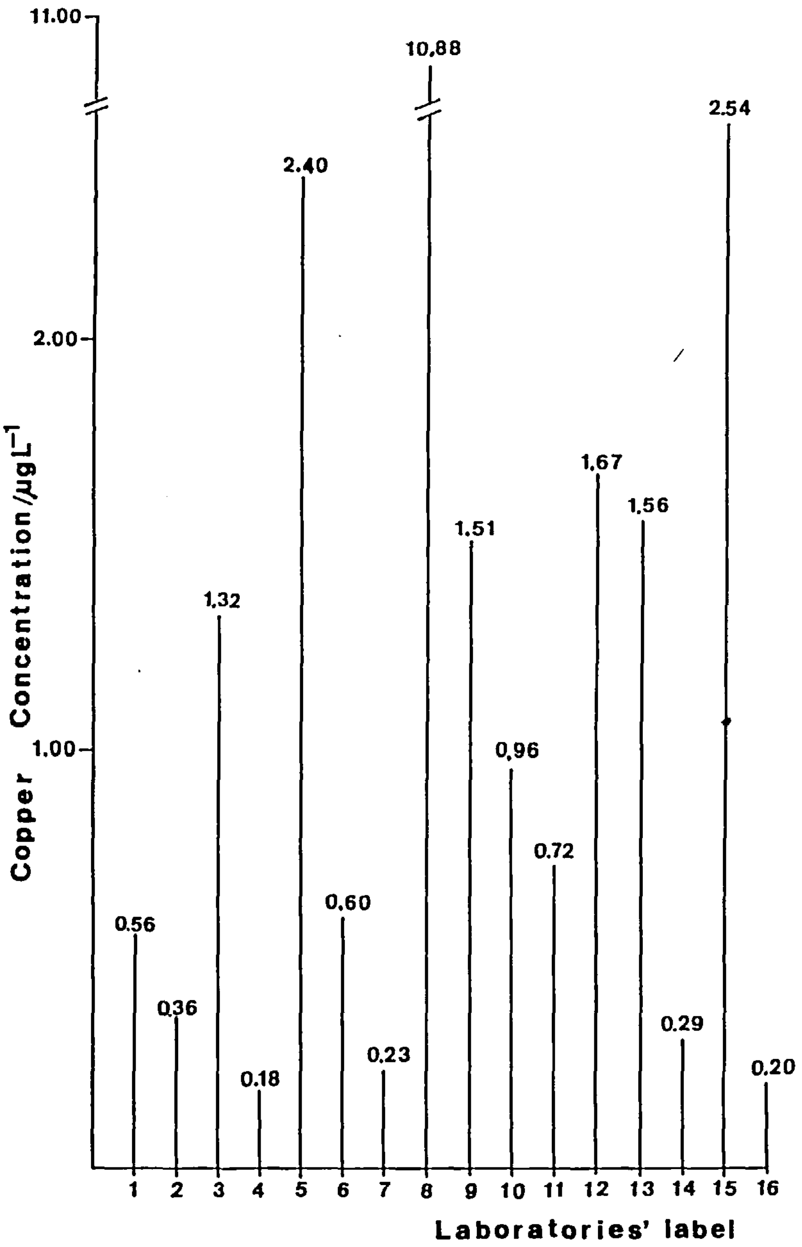

FIGURE 2. Interlaboratory exercise results relevant to the dissolved copper determination in preacidified samples.[47]

Besides water, a large scattering of data in interlaboratory collaborative tests seems to be very frequent whenever low analyte concentrations are involved regardless of the type of matrix. In Figure 3, the results collected by Sherlock et al.[50] for lead content in food are reported. Again, the spread of results is unexpectedly large.

The dependence of mean bias size ($\overline{B}$) on the concentration (C) in absence of random errors, i.e., when a large number of data are available, may assume the form:

$$\overline{B} = a + bC \qquad (1)$$

Table 5
INTERCALIBRATION FOR TRACE METALS DISSOLVED IN TWO SEA WATER SAMPLES (A AND B) FROM THE SOUTHERN NORTH SEA

	Sample A				Sample B			
Metal	Mean (μg/ℓ)	No. of observations	Range (μg/ℓ)	Coefficient of variation (%)	Mean (μg/ℓ)	No. of observations	Range (μg/ℓ)	Coefficient of variation (%)
Co	0.70	4	0.13—2.5	156	0.92	4	0.13—3.0	151
Fe	4.53	11	0.9—15	96	5.99	13	0.7—35	158
Ni	1.74	13	0.19—64	135	3.43	18	0.7—24.2	184
Cu	0.93	40	0.2—31	83	1.16	44	0.2—3.1	113
Mn	1.60	11	0.4—3.9	77	10.75	14	7.2—16	22
Zn	7.98	41	1.7—22.2	62	11.93	41	3.43—37	57
Pb	1.16	30	0.06—5.6	121	1.92	33	0.09—23	228
Cd	0.21	37	0.02—1.5	148	0.40	42	0.03—4.4	197
Cr	0.72	6	0.09—2.3	110	1.06	6	0.11—3.3	113

From Jones, P. G. W., *Anal. Proc.*, 19, 565, 1982. With permission.

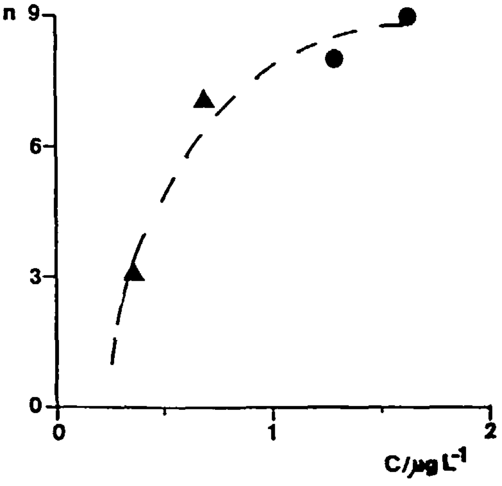

FIGURE 4. Correlation between the number of laboratories (n) which obtained the correct value within the accepted bias and the concentration of mercury (C). (▲) Standard solutions. (●) Real samples.[44]

on the condition that (a) and (b) are independent of concentration in the observed range. This condition may hold in practice, for example, in the case where the loss or uptake of analyte throughout the physical and chemical steps involved, from sampling up to the analysis, is independent of (C) and the only cause of error in the analysis lies in the standardization factor used. In collaborative studies with a sufficient number of participants, the mean bias will have as limit a finite value of (a) different from zero, at any concentration, whether the systematic errors due to the procedures used are the same in the different laboratories. A relationship also can be found between (n) (where n is the number of laboratories that obtained the correct value within the accepted bias) and (C). In the case of the previously mentioned sets of data concerning mercury,[44] selecting the values relevant to the third-year interlaboratory tests, the plot of Figure 4 is obtained where the number (n) of laboratories that performed the analyses within the 10% maximum bias is reported vs. the nominal concentration value (C).

Apparently, the minimum concentration that can be measured for the most accurate laboratory ranges between 0.2 and 0.3 µg/ℓ. Below this value, which is closer to the natural concentration, not one of those laboratories, even after 3 years of collaborative studies, seems to have been able to get accurate results.

The precision characteristics of 18 analytical methods for metals and other elements subjected to interlaboratory collaborative studies over the last 10 years by the Association of Official Analytical Chemists have been examined by Boyer et al.[51] The authors claim that the interlaboratory r.s.d. relevant to a variety of analytes, matrixes, and measuring techniques can be expressed by the unique equation:

$$\%\ \text{r.s.d.} = 2^{1-0.5\ \text{Log}\ c} \tag{2}$$

where (c) is the concentration expressed as a decimal fraction, e.g., 1 ppm = 10^{-6} g/ℓ. The lower limit depends on the detection limit for each analyte and measurement technique. As a matter of fact, in the case of fluorescence atomic spectroscopy by D'Ulivo and Papoff,[52] it was found that the r.s.d. can be expressed according to the error propagation theory as

$$\% \text{ r.s.d.} = \frac{100(a^2 + b^2c^2)^{1/2}}{c} \qquad (3)$$

where (a) is the average peak-to-peak noise variation in the absence of analyte and (b), which ranges between 2 and 4%, is independent of concentration.

D. Conclusions

These findings show that it is quite probable that a large amount of published data concerning environmental analyses is inaccurate with the consequence that, in characterizing natural water or waste water discharge systems, the concentrations of some toxic compounds are underestimated and others are overestimated. This may be caused both by the inadequacy of the overall analytical procedures used, which are not sufficiently tested in each laboratory environment, and by the inexperience of the analysts. Trace analyses should really be performed only by experienced environmental chemists in specific laboratories. Owing to the economical cost arising from these conditions, it would be advisable that only central laboratories be devoted to this task. General criteria have been suggested for organizing such a laboratory for environmental toxic pollutant analyses.[53]

The correct approach to obtain accurate final results, as some laboratories do, is to avoid as much as possible any risk of contamination in each step of the sample treatment procedure, no matter how tedious it may seem. The risk of incorrect information relating to a survey would be somewhat lower if one did not try to save time and money by disregarding some procedural steps. Besides, accurate data are not sufficient whenever the number of sampling and the relevant sites chosen are not suitable for representing the complexity of the aqueous system being considered. Automation of the overall procedure — from sampling, filtering, and conditioning up to analysis — would get more reproducible data, but would not necessarily reduce bias.

As far as the particulate-dissolved matter correlation is concerned, one can sum up as follows:

1. Particulate trace analysis requires the collection of a very large volume (approximately 100 ℓ) of sample. Sampling and phase separation are time consuming so that in the characterization of an aqueous ecosystem, a severe cutoff of the number of samples becomes necessary with a real risk that data are not representative of the system particularly in the case of rivers and estuaries.
2. Trace element distribution according to the size and origin of particulate (size speciation) is not, nowadays, accurately possible at an acceptable cost and time.
3. The organization and accomplishment of research on particulate physical speciation in real samples at present remain a task for specialized laboratories.
4. Dissolved trace element analysis requires small volumes of sample (approximately 1 ℓ) and a relatively short time for sampling and phase separation so that by using suitable criteria for sampling and a large number of samples, dissolved matter analyses may produce sound information on an aqueous system. It must be stressed that whenever mass balance is the goal; particulate as well as dissolved matter must be analyzed.

For these reasons, the next sections are devoted to dissolved trace elements only.

III. ANALYTICAL PROCEDURES FOR DISSOLVED TRACE ELEMENTS

Great care must be taken to avoid contamination by airborne particulate, especially during filtration, acid digestion, and sample transfer. Sample and labware must be stored in precleaned polyethylene bags. Clean-air laboratories are recommended, even though they are not essential.

A. Cleaning of Sample Bottles and Other Labware

FEP teflon has been recommended as a superior material for bottles, as well as for beakers, transfer pipettes, separatory funnels, etc.[62] Conventional linear polyethylene bottles are widely used for heavy metal solution storage. Exchanges with environment through the walls of the containers of volatile compounds, such as organometallic compounds,[57,58,63,65,83] mercury,[63] and selenium,[59-61] are avoided by using pyrex or quartz bottles. Thick polyethylene containers are also suggested to avoid mercury loss.[64]

Whatever the material of the containers and the ware, they are cleaned in exactly the same way except that HCl is substituted by HNO_3 for teflon in acid treatments (see the schemes below). In order to remove grease and other organic substances that may interact with the sample or interfere in the instrumental analysis, rinsing the inside of containers with chloroform,[62] *n*-hexane,[65] or detergent[71] has been suggested. For its superior solvent ability and chemical inertia, *n*-hexane is preferable particularly with respect to detergents when electrochemical methods are used in the analytical procedure.

After organic solvent or detergent rinsing, different reliable schemes are available to minimize leaching from the walls or adsorption on the walls of the container:

1. HNO_3 rinse; A.R. concentrated HNO_3 fill and leave in a 70°C bath of A.R. concentrated HNO_3 for 3 days; water rinse; 0.05% HNO_3 fill on hot plate for 1 day; water rinse; 0.05% HNO_3 fill on hot plate for 5 days; water rinse; 0.5% HNO_3 fill and leave at room temperature until ready for sample collection.[62]
2. 10% HNO_3, soak 24 hr; water rinse; for sea water: 25 g of NaCl + 2 g of $CaSO_4$ + 3 g of $MgSO_4/\ell$ of water; for inland water: 1 g of $CaSO_4$ + 1 g of $MgSO_4/\ell$ of water minimum soak 24 hr; water rinse before sample collecting.[66]
3. 15% HCl, minimum soak 3 days; water rinse before use.[17]
4. 10% HNO_3, soak 2 weeks; water rinse; 0.2% "Aristar" HNO_3, soak 1 week; water rinse; water minimum soak 1 week with changes of water.[67]
5. 0.01 *M* EDTA solution (pH = 8), soak 3 hr; 1:1 reagent-grade HCl fill and minimum 2 days leave before the next step; 10^{-2} *M* suprapur HCl, soak 3 days before sampling; buffer solution (pH slightly alkaline) rinse; then bidistilled water rinse until the rinsing water pH is 7; sample water conditioning before retaining a sample.[65]
6. 1.5 *M* reagent-grade HNO_3 soak, minimum 1 week; empty the acid before the sample is collected; bottle fill and empty with the sample water at least 4 times before retaining a sample.[68]

The use of calcium and magnesium sulfates (scheme 2) or of slightly alkaline buffer solution (scheme 5) is effective in neutralizing adsorption sites created on the walls of the container during the acid washing treatments. These sites are responsible for metal loss[66] or decomposition of organometallic compounds.[65]

It must be stressed that when organometallic compounds such as tetraalkyllead are assumed to be present, owing to their tendency to be adsorbed by solid surfaces, preconditioning with sample fractions must be avoided. The whole of the sample volume is used up and the glass container walls are washed with several aliquots of an organic solvent (for instance, *n*-heptane) which will be analyzed in the same manner as the sample. In fact, this procedural step should be carried out each time as a precaution.

B. Cleaning of Filters and Filtration Devices

Filtration must be performed as soon as possible before any chemical treatment. Trace metal adsorption onto or leaching from filters is circumvented by soaking the filters in acid for several days, then rinsing them in the precleaned filtering equipment with pure water and large amounts of sample before collecting the filtrate.[69-73] When using cellulose acetate filters, shorter soaking times have to be observed in order to avoid pulp. Wallace et al.,[74] for instance, proposed soaking in 2 *M* reagent-grade HCl or an equivolume mixture of 1 *M* quartz-distilled HNO_3 and 1 *M* suprapur HF for 15 to 20 hr in polyethylene containers. Filter rinsing was then accomplished by soaking the filters in deionized water for several 2- to 3-hr periods until the pH of the rinsing water showed no change.

In our experience, the preconditioning of the filters is carried out satisfactorily by soaking in renewed solutions of 1:1 reagent-grade HCl for 2 periods of 3 to 4 hr and then in 10^{-2} *M* suprapur HCl for 2 periods of 5 to 6 hr. Filter rinsing is accomplished by soaking the filters in deionized-bidistilled water until the rinsing water pH was near neutrality. Conditioning filters and apparatus with 0.1 *M* $Ca(NO_3)_2$ prior to use have also been suggested,[77] since it was found that the presence of group 1 and group 2 metal cations reduces the adsorption of trace metals.[66,78]

Some filtration devices are commercially available, such as the one proposed by Mart,[71] made in polycarbonate (Sartorius, SM 16511). Gardner and Hunt[75] found that in the range of pH between 6 to 9, the adsorption of lead on the walls of this device was always below 10% and, in many tests, not statistically significant. By contrast, very large losses of lead (greater than 80% at pH 9) have been evidenced by the same authors using a borosilicate glass system (Millipore).

The cleaning of the filtration device may be performed by means of the same procedure used for the sample containers. For inorganic mercury determination, the use of glass-fiber filters, previously decontaminated by ignition (T = 500°C), along with a glass filtration device is preferable.[24]

C. Sample Preservation

The extent of exchange between sample solution and the container walls depends on the material of the container, the cleaning procedure used, the type and the concentration level of the elements dissolved, and the nature of the sample as well. For instance, the presence of iron and organic substances in the sample may induce loss of heavy metals by coprecipitation/adsorption mechanisms; the presence of complexing agents may counteract this phenomenon. Temperature also plays an important role in controlling the extent of exchanges between container and sample.

At room temperature, the following findings were obtained. In a study performed on synthetic and prefiltered natural water river samples, Subramanian et al.[76] found that linear polyethylene (Nalgene) is preferable to a pirex glass container, owing to the minor adsorption of metals from the solution. With polyethylene, no loss was observed over a 30-day period for chromium, cadmium, nickel, cobalt (pH 1.5 to 8.0), lead (pH < 1.5), and copper (pH < 4). From estuarine low-salinity (1%) water samples, loss of lead (30%) at natural pH as also verified by Campbell et al.[19] over a time period of approximately 24 hr. For all the elements considered (Cd, Cr, Ni, Pb, Zn, Co, Mn, Fe, and Sr) by Subramanian et al.,[76] the amount lost increased when the pH was increased, despite the nature of the water sample and the type of container used. At a given pH, the loss of elements from natural river water samples was always higher than that from synthetic water samples, probably due to the greater biological activity in the natural water. This activity can be hindered by storing the samples at temperatures such as 3°C,[66] 4°C,[70,73,79,80,168] and 5°C.[71]

Nürnberg et al.,[66] performing long-term stability studies at 3°C, found no concentration changes, at a nanogram level, larger than ±20% for cadmium and lead, and a slow trend for cadmium to disappear and for lead to increase after 75 days of storage, using filtered sea water. These results are essentially confirmed by Scarponi et al.[73] for cadmium, copper, and lead. In the light of all these findings, for preservation of the filtered samples for long periods of time, storage at 3 to 4°C appears to be the safest method for both fresh and sea water samples either acidified at pH 2 or maintained at their natural pH.

Storage of mercury, chromium hexavalent, and tin requires special procedures. In the case of mercury, both acidification and addition to the sample of an oxidant or complexing agent such as H_2O_2, $K_2Cr_2O_7$, or cysteine are necessary.[17] The results obtained by Knechtel[64] indicate that samples containing mercury at a microgram level can be stored safely for a long time (3 months) in the presence of sodium chloride (3% *M*/vol) and nitric acid (0.1 *M*) by using either glass containers or plastic bottles which must be thicker than the usual ones.

As for chromium hexavalent, the best long-term stability was obtained at pH 9 at 5°C in the presence of a bicarbonate-carbonate buffer and EDTA: the final buffer concentration was 0.05 *M*, and the final EDTA concentration was 0.01 *M*.[81] For tin, which is strongly adsorbed onto glass surfaces, the use of polyethylene containers along with acidification of the solution with HCl (pH 1) or H_2SO_4 has been suggested.[82] For organotin compounds, in order to avoid their decomposition, storing the sample in a glass container in the dark and at low temperatures as well as performing the analysis as quickly as possible are recommended.[83]

Deep freezing of the sample at $-20°C$ has also been proposed.[71] Moreover, in the case of fresh water, whenever speciation is the goal, deep freezing of the unacidified sample is not permissible. In fact, as a water sample freezes, metal ions are continuously concentrated in the unfrozen liquid remaining in the center of the container. At these relatively high transient concentrations, irreversible hydrolysis and polymerization of iron and other metal ions may take place so that heating the thawed sample with acid may be necessary to return them to the solution. With sea water, freezing could be an acceptable method of preservation since the high chloride concentration may prevent hydrolysis reactions from taking place.[68] It must be remembered that freezing may be the cause of variations of heavy metal concentrations (see Section II.C).

D. Preconcentration Methods for Dissolved Trace Element Determination

The most generally used preconcentration methods for the determination of trace elements in natural waters are water evaporation, ion exchange (column and batch mode), precipitation and coprecipitation, chelation and solvent extraction or adsorption on silica-immobilized chelating agents (i.e., 8-hydroxyquinoline[84]). Some reviews and papers have been published on this matter.[85-95] Comparisons of preconcentration procedures for trace elements in natural waters have been reported in several papers.[96-99]

In the case of ion-exchange resins or chelation-solvent extraction, the collection efficiency of trace elements depends on the bulk composition of the real sample. The presence in the sample of antagonistic complexing agents (among which the trace element is shared), of colloids and of ions such as calcium and magnesium (which can be bound to chelant agents poured on the solution), is determinant both from a thermodynamic and a kinetical viewpoint.

Florence and Batley[100] using Chelex-100 (ionic resin) measured very low recoveries of zinc from sea water, although freshly added radioactive ionic spikes were fully recovered, Abdullah et al.[101] found that only the dissolved and electroreducible species of copper, zinc, cadmium, and lead may be retained by Chelex-100, not the ones present in colloidal form or adsorbed on particulates, which could be separated from the sample by centrifugation. In general, ion exchangers will recover hydrated ions and most complexed ions. The recovery percentage depends on the stability constants of the complexes in the solution and on the exchange kinetics.[97,101]

Solvent extraction requires the formation of uncharged complexes in the solution which are competitive with the charged ones. This technique, even though less selective than ion-exchange resins in that unwanted complexes of calcium and magnesium will pass nearly quantitatively into the organic phase, is preferable particularly when organometallic compounds are to be determined as well.

Lyophilization (freeze drying) might also provide a method for the preconcentration of water samples for total metal analysis. Performing an evaluation of the lyophilization for the preconcentration of natural water samples, Harrison et al.[102] found that, with the exception of mercury, more than 95% of a large number of heavy metals was retained in the residue. It would be worth finding out if volatile inorganic compounds and metallorganics are quantitatively retained. Filby et al.[103] applied lyophilization to river water samples for trace metal analysis by neutron activation analysis.

The principal advantages offered by this method (even though it is more time consuming) are the low temperature at which lyophilization occurs and the reduced contamination during the freeze-drying process (the leaching of trace elements from the container walls is accelerated at elevated temperatures).

The commercially available lyophilizers, built above all for freeze-drying biological fluids, tissue, and food, as well as pharmaceutical products (materials for which lyophilization has nowadays been widely employed), present some drawbacks for trace element analysis. The primary disadvantages are bulky stainless-steel cabinets or drying chambers; vessels for liquids constructed from unsuitable soft glass material; and the frequent presence of vacuum seals that depend on the use of stopcock grease or rubber O-rings.[104] Hopefully, more suitable lyophilizers will be built for trace element analysis in natural waters.

E. Instrumental Techniques for Dissolved Trace Element Determination

Direct determination of trace elements at concentration levels that occur in natural water as well as the determination of an analyte between its different molecular states (chemical speciation), without needing a preconcentration step, have been the concern of many publications over the past few years. This has been made possible owing to the consistent improvements achieved in the field of instrumentation and to the related procedures. The coupling of chromatographic separation techniques to optical or mass spectroscopic detectors is assuming particular importance in speciation of trace-element containing molecular species directly on the sample or after derivatization.

The most sensitive and commonly used instrumental techniques in trace element analysis are electrothermal atomization atomic absorption spectrometry (ETA-AAS), fluorescence atomic absorption spectrometry (FAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and polarographic, voltammetric, and potentiometric methods.

With regard to ETA-AAS, particularly when graphite tube is employed, many problems concerning accuracy are present. The main instrumental causes of error are carbide formation owing to interacations of the analyte with the graphite tube walls; incomplete compensation for nonspecific absorption effects; and preatomization losses of volatile compounds.

The judicious choice of the temperature profile, the measurement of the peak area instead of height, as well as the use of spikes may in large part overcome these kinds of errors with the exception of those concerning uncompensated nonspecific absorption and preatomization losses.

Simultaneous multielement analysis and improved detection limits have been obtained recently by using FAAS coupled with hydride generation. In this case, it was possible to distinguish between some classes of compounds by the thermal stripping of hydrides previously frozen or by changes in the chemical procedure.[105,106] Interference effects mainly due to foreign substances which hinder the trace element hydride formation may play a significant role.[107]

As for anodic stripping voltammetry (ASV), once the matrix effects, which may play a major role, have been eliminated, the problems which need to be solved are noise filtering and baseline subtraction. Extremely low concentrations, such as 0.01 $\mu g/\ell$ can be measured with confidence by using either a rotating disk electrode of a large area or a hanging drop mercury electrode. In the latter case, sophisticated filtering and background subtraction algorithms are required (Figure 5).[108]

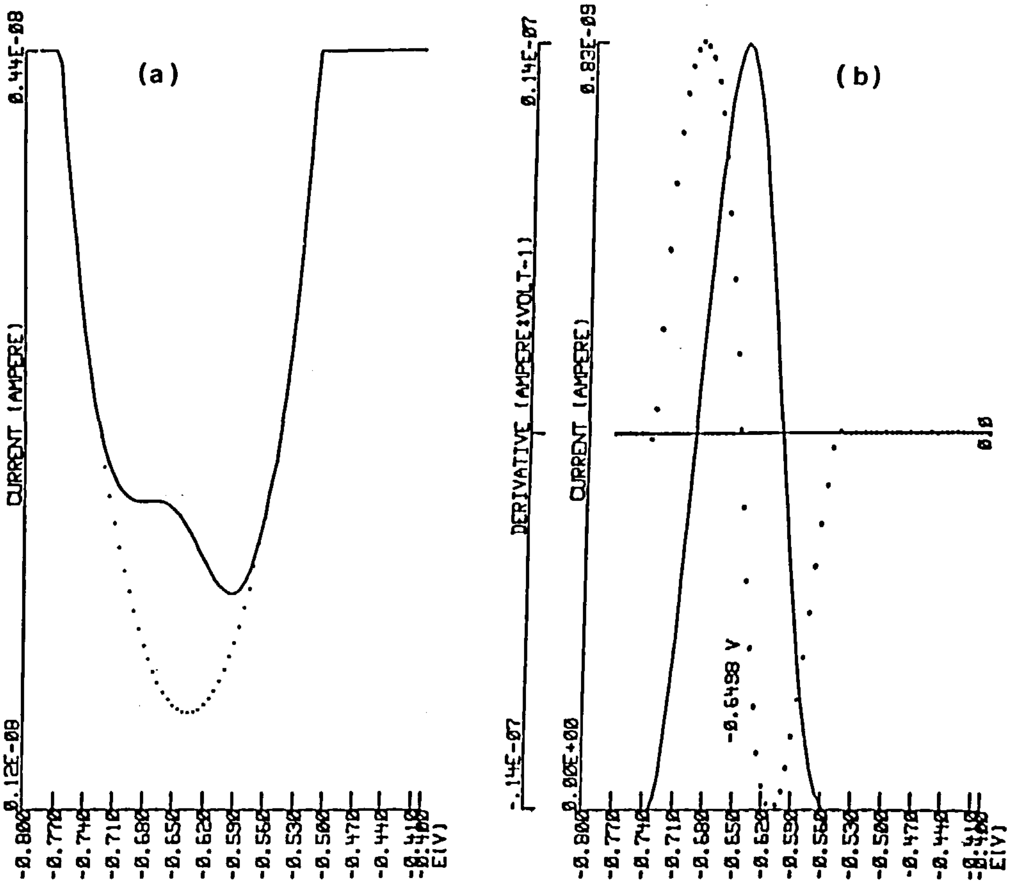

FIGURE 5. Differential pulse anodic stripping voltammogram of 1.8 10^{-10} *M* Cd^{2+} in 0.5 *M* NaCl solution (pH = 3); hanging mercury electrode. (a) Smoothed voltammogram with its computer-calculated baseline. (b) Same as Figure 3a after baseline subtraction (continuous line) and its derivative (dotted line). Plating potential: −800 mV; pulse amplitude: 50 mV; step of staircase sweep: 5 mV; plating time: 4 min; rest time: 30 sec. (From Fanelli, N., Fuoco, R., Guidarini, D., and Papoff, P., *Anal. Chim. Acta*, 185, 33, 1986. With permission.)

Potentiometric stripping analysis (PSA)[109] is based on the preconcentration of the trace metal analytes, by means of potentiostatic reduction on a thin layer of mercury plated onto a glassy carbon electrode, followed by injection of an oxidant solution. The shape of the potential (E) vs. time curve depends on the oxidant concentration, and on the plating time (the detection limit is inversely proportional to the plating time).[110-112] Using 32 min of plating time, Jagner et al.[113] were able to measure metal concentrations as low as 0.01 μg/ℓ in sea water samples with well-defined and separated signals. This is particularly important for copper whose signal at low concentration is badly separated in ASV by the background due to the oxidation of the mercury in ASV. This computer-controlled technique, with the use of fast analogic-to-digital converters and direct memory access, is likely to become increasingly popular.

Adsorptive stripping voltammetry (AdSV) has increasingly been applied in the area of trace element analysis in the last 4 years. It is based, as in ASV, on a preconcentration step on the electrode followed by an electrochemical step. Unlike ASV, the preconcentration step does not involve redox reaction at the interface but adsorption of the element via a specific compound which, added to the solution, selectively complexes the element and is adsorbed on the electrode at the selected potential. In the subsequent step, the surface-bound element undergoes electrochemical reduction or oxidation, and the relevant peak current is proportional to the initial concentration of the element in the solution at a constant preconcentration time. Competitive adsorption of foreign substances may affect the sensitivity of the method or simulate the presence of the analyte as well. Ultraviolet (UV) irradiation prior to adding the complexing ligand in question should be suggested as a standard procedural pretreatment step.[114-116] AdSV analysis has been applied successfully to the simultaneous determination in natural waters of traces of nickel and cobalt (surface-active complexes with dimethylglyoxime[117,118]), copper (surface-active complexes with cathecol[119] or thiourea[120]), and chromium (surface-active complexes with diethylenetriaminepentaacetic acid[121]).

Chronopotentiometry, preceded by an adsorptive step of dimethylglyoximate complexes on a glassy carbon electrode, was also used for nickel and cobalt determination in sea water.[122]

High-performance ionic chromatography (HPIC) recently has been proposed for trace element determination.[123,124] Systematic comparisons, in order to elucidate potentiality, advantages, and drawbacks of HPIC in trace element determination in natural waters, are in progress; the authors of this review are among those who are working on these comparisons. For its versatility and its superior ability in species separation, HPIC appears highly likely to play an increasing role in trace environmental analysis.

In Table 6, some significant spectrometric and electrochemical detection limit values related to trace element determination are summarized.

F. Total Concentration Determination in Filtered Samples

Just as the origin of water samples, with its peculiar bulk concentration and matrix effects, affects the results in the preconcentration step, so too does it affect total dissolved concentration determination. In the latter case, depending on the instrumental technique used, surfactants (normally present at 0.8- to 5-mg/ℓ levels) as well as occasional traces of oils or grease may contribute in causing significant errors or a large decrease in sensitivity. Surfactant concentrations can be measured electrochemically by using a polargraphic method based on the reduction wave of Hg(II) at −0.28 V (SCE).[152] By this way Hunter and Liss[146] found that surface-active substrates are present in sea water at 20.4 mg/ℓ concentration level (expressed in Triton-X100®).

In AAS, coupled with electrothermal vaporization and atomization steps, all the above classes of compounds may affect the mechanisms of vaporization and the yield of atom formation giving rise to negative errors, the magnitude of which depends on the matrix characteristics of the water sample.

In ASV, surface-active substances and oils may affect heavily the reduction-reoxidation mechanisms, while some colloids or soluble compounds present in the filtrate may be electroinactive.

In PSA, where the oxidant step is chemically achieved, organic material appears to have a lower interference effect than in ASV.[147]

Sample decomposition for total concentration determination therefore appears to be the recommended procedure on every occasion. Unfortunately, decomposition procedures may give rise to systematic errors too through: (1) incomplete decomposition; (2) contamination by reagent, vessel material, work environment; (3) loss of analyte by volatilization, adsorption on/reaction with the vessel walls. Of the numerous decomposition methods that have been published, only a few, based on UV irradiation, meet the requirement of being both efficient and error free.[148-150] In these methods, a small amount of hydrogen peroxide, as a source of oxygen-free radicals, has to be added to the acidified sample. Depending on the organic content of the water,[150] boiling from 1 to 4 hr, in order to destroy organic matter without producing perceptible errors, was found necessary.

With the photodigestion device proposed by Dorten et al.,[151] the organic matter in water

Table 6
SPECTROMETRIC AND ELECTROCHEMICAL SIGNIFICANT DETECTION LIMIT VALUES RELEVANT TO TRACE ELEMENT DETERMINATION

Analyte	Technique	d.l. (μg/ℓ)	Sample[a] (mℓ)	r.s.d. (%)	Ref.
As	AFS	2 10^{-2}	5	7 (0.2 μg/ℓ)	125
	AAS	5 10^{-4}	100	9 (0.05 μg/ℓ)	126
	AE-ICP	2 10^{-2}	—	n.r.	127
	DPASV (RGE)	2 10^{-1}	—	6 (2 μg/ℓ)	135
Cd	AFS	1 10^{-3}	1 10^{-3}	n.r.	134
	AAS	2 10^{-2}	5 10^{-3}	n.r.	134
	AAS	1.3 10^{-2}	1.2 10^{-2}	n.r.	139
	AFS-ICP	1 10^{-1}	—	n.r.	140
	DPASV (HMDE)	5.6 10^{-3}	—	n.r.	108
	DPASV (MFE)	3 10^{-4}	—	10 (0.0015 μg/ℓ)	144
	PSA (MFE)	4.5 10^{-3}	—	n.r.	113
Co	AAS	2 10^{-1}	2 10^{-2}	n.r.	137
	PSA (MFE)	3.3 10^{-2}	—	n.r.	122
	AdSV (HDME)	2 10^{-3}	—	7 (2 μg/ℓ)	138
	AdSV (HDME)	1 10^{-3}	—	10 (0.02 μg/ℓ)	144
Cr	AAS	2 10^{-1}	2 10^{-2}	n.r.	137
	DPP	4	—	n.r.	186
	AdSV (HDME)	2 10^{-2}	—	5 (0.2 μg/ℓ)	121
Cu	AFS	2.9 10^{-1}	1 10^{-3}	n.r.	134
	AAS	1.4	5 10^{-3}	n.r.	134
	DPASV (HMDE)	6 10^{-2}	—	n.r.	108
	DPASV (MFE)	7 10^{-3}	—	10 (0.0015 μg/ℓ)	144
	AdSV (RDE)	4 10^{-3}	—	n.r.	119
	PSA (MFE)	6 10^{-3}	—	n.r.	113
Hg	AFS	5 10^{-3}	1	5 (0.05 μg/ℓ)	129
	AAS	1 10^{-3}	1	3 (0.05 μg/ℓ)	130
	AE-MIP	1 10^{-6}	100	n.r.	131
	AFS-ICP	2 10^{-1}	—	n.r.	142
	PSA (AuE)	4 10^{-1}	—	n.r.	143
Ni	AAS	4 10^{-1}	2 10^{-2}	n.r.	137
	PSA (MFE)	2.7 10^{-3}	—	n.r.	122
	AdSV (HDME)	1 10^{-3}	—	10 (0.02 μg/ℓ)	144
Pb	AFS	6 10^{-2}	5	6 (0.35 μg/ℓ)	52
	AAS	1.1	—	n.r.	132
	AE-ICP	1	—	n.r.	133
	AFS	10	1 10^{-3}	n.r.	134
	AAS	1	5 10^{-3}	n.r.	134
	DPASV (HDME)	1 10^{-2}	—	n.r.	108
	DPASV (MFE)	1 10^{-3}	—	10 (0.0015 μg/ℓ)	144
	PSA (MFE)	1.2 10^{-2}	—	n.r.	113
Se	AFS	1 10^{-2}	5	11 (0.03 μg/ℓ)	125
	AAS	8 10^{-1}	2 10^{-2}	n.r.	137
	AAS	2 10^{-2}	—	n.r.	128
	AE-ICP	3 10^{-2}	100	n.r.	127
	DCPS	1.5	—	n.r.	136
Sn	AFS	1 10^{-1}	1	6.2 (0.6 μg/ℓ)	125
	AFS-ICP	3	—	n.r.	141
	DPASV (HDME)	1 10^{-1}	—	n.r.	145
Zn	AFS	1.9 10^{-2}	1 10^{-3}	n.r.	134
	AAS	1.9 10^{-2}	5 10^{-3}	n.r.	134
	PSA (MFE)	1.6 10^{-2}	—	n.r.	113
	DPASV (HDME)	2 10^{-2}	—	10 (0.5 μg/ℓ)	144

Table 6 (continued)
SPECTROMETRIC AND ELECTROCHEMICAL SIGNIFICANT DETECTION LIMIT VALUES RELEVANT TO TRACE ELEMENT DETERMINATION

Note: HMDE, hanging mercury drop electrode; MFE, mercury film electrode; RGE, rotating gold electrode; AuE: gold electrode; DCPS, direct current plasma spectrometry; PSA, potentiometric stripping analysis; AdSV, adsorptive stripping voltammetry; AAS, atomic absorption spectrometry; ICP, inductively coupled plasma; MIP, microwave induced plasma.

[a] Only in the case where volume is a significant parameter of the procedure.

was reduced practically to zero at temperatures not above 65°C. In general, the use of closed reflux apparatus is recommended to avoid the loss of volatile compounds during the initial step of the operation. It is likely that this risk still holds in part when ozone is flowing through the sample instead of when the H_2O_2-UV procedure is used. Apart from this, ozone oxidation at pH 2 seems to be effective in destroying EDTA, APDC, humic acids, and tannic acids at 3-, 25-, 25-, and 100-mg/ℓ levels, respectively, in less than 1 hr.[153]

H. Evaluation of the Reliability of the Laboratory Procedures by the Use of Standard Reference Materials

Standard reference materials (SRMs), of known matrix and certified analyte concentration, are widely accepted in analytical chemistry research.[154-167,169]

SRMs are generally applied for quality control of analytical data by their use in the direct calibration of methods and instruments, verification of the precision, accuracy and sensitivity of the methods used in control analyses, and the development and evaluation of new reference methods. Reference methods can be used to develop and/or assess field methods, to evaluate secondary reference materials, and control the accuracy of quality-assurance procedures.

As for natural waters, synthetic samples usually differ from authentic ones because of the presence of a unique dissolved organic matter (DOM) species, generally a humic substance. For this reason, authentic samples should be preferred, as much as possible, to synthetic reference materials. In the case of open sea waters owing to their nearly constant background composition, the relevant SRM can be considered universally reliable. This is not so true for nearshore or riverine water SRMs.

Authentic open ocean sea water (NASS-1), nearshore sea water (CASS-1), and riverine water (SLRS-1) reference materials are only provided, as far as we known, by the National Research Council of Canada (NCR) in their Marine Analytical Chemistry Standards Program. In the framework of this program, Berman et al.[168] reported the procedure for gathering a large volume of an open ocean water sample and for ensuring the integrity of its metal content in a prolonged storage. The metal concentrations of unopened NASS-1 bottles are warranted for a 2-year period from the shipping date of label. Besides, the trace metal storage is not affected by the ratio of air-to-sea-water volume in a bottle.[168]

The drawbacks of using noncertified reference materials (RMs) or nonmarine SRM in marine water analysis standardization have been pointed out by Topping.[171] According to this author, when using spiked samples or noncertified RMs [normally prepared by agencies such as International Council for the Exploration of the Sea (ICES) and International Atomic Energy Association (IAEA) for specific intercomparison exercises],[170,172-174] problems arise in interpreting analytical performance in both these cases. The relevant conclusions, which are later reported herein, concur with the conculsions drawn in Section II.C as far as the parts related to it are concerned:[171]

The complete recovery of a spike does not necessarily mean that the analytical method has no bias, since the spike (or added analyte) may behave differently to that present naturally. The consensus concentration of each analyte in an RM is based on the range of concentrations reported in the respective intercomparison exercise; these consensus values do not, in all cases, represent the true concentration of the analyte in the matrix. The usefulness or value of an RM increases in direct proportion to the amount of effort put into its preparation by the organizer of the exercise (i.e., ensuring that the sample is homogeneous and properly stored prior to its use and the number of expert laboratories that are persuaded to participate (i.e., to ensure that a good consensus value for each analyte is obtained). Once a method has been validated through the use of one or more SRMs (which cover the range of matrixes and/or range of analyte concentration encountered in the monitoring study), appropriate RMs may subsequently be used, at periodic intervals, to monitor any changes in bias of the analytical procedure.

The lack of relevant SRMs for marine measurements has undoubtedly contributed to the production of poor data and, regrettably, many analysts have resorted to nonmarine SRMs for validating analytical methods. For example, the use of NBS orchard leaves, bovine liver, and spinach (NBS 1571, 1577, and 1570, respectively) for metals in marine tissue. Differences in matrix and analyte concentration between these SRMs and marine tissue have not permitted a true evaluation of bias in the latter samples. For example, orchard leaves containing 40 ppm Pb should not be used for samples such as fish muscle or shellfish soft tissue, which contain a concentration of 0.005 and 0.5 ppb, respectively.

IV. DISSOLVED TRACE ELEMENT CONCENTRATION AND RELEVANT TOXIC EFFECTS

A. General Considerations

The trace element total concentration in the 0.45-μm filtered sample (C_f) can be subdivided as follows:

$$C_f = \underbrace{C_l}_{1} + \underbrace{C_{MX}}_{2} + \underbrace{C_{M_{adsorbed}}}_{3} + \underbrace{C_{M_{englobed}}}_{4} \tag{4}$$

The C_l group represents all the complexes that accept thermodynamic equilibrium with the solvated free ion M^{n+} according to

$$C_l = C_M n+ + C_{ML_1} + \cdots + C_{ML_n} \tag{5}$$

(For the sake of simplicity the charge of the ML_i species and the stoichiometric indexes have been omitted and mixed complexes ignored.) Equation 5 can be expressed in terms of the formal total equilibrium formation constants β_{ML_i} and the actual concentrations of the L_i inorganic or organic ligands:

$$C_l = C_M n+ (1 + \beta_{ML_1} C_{L_1} + \cdots + \beta_{ML_n} C_{L_n}^{n}) = \sum_{i=0}^{n} C_{ML_i} \tag{6}$$

The MX group represents the compounds which release M^{n+} after chemical decomposition. The $M_{adsorbed}$ and $M_{englobed}$ groups represent the element adsorbed on or englobed in colloidal particles, respectively.

Under suitable monitoring programs, C_f profiles may provide information about the interaction among dissolved, suspended, and sedimented matter in terms of the total concentration of the element exchanged. In this connection, C_f may lead to some high-quality information about the dynamic characteristics of the aqueous ecosystem as well as the degree of pollution of anthropogenic origin. Despite this, C_f completely fails in giving any indication about the actual extent of biotoxicity effects induced on the system, which is one of the most important goals in aqueous ecosystem studies. These effects are not related to total concentration but to the individual concentrations of any element-containing chemical forms which are specifically able to be take up by aquatic organisms.

The correct approach to the solution of the biotoxicity problem can be summarized in the following steps:

1. The selection of the aqueous system which must be considered above all for its importance and homogeneity
2. The detection of the main classes of bulk substances which are present as dissolved, adsorbed, or colloidal
3. The study of the physicochemical interaction mechanisms of each trace element with the different substances in the natural system
4. The study of the individual toxic effect of any element-containing chemical forms on some aquatic organisms which have been properly selected as significant biological indicators
5. The individuation of suitable chemical instrumental procedures to analyze single compounds or classes of compounds of toxic relevance

Sea water has been studied extensively because of the importance of the marine environment for human beings and because this system is basically the same all around the world.

Nowadays, sound information only has been obtained for trace element interaction with inorganic compounds. In fact, despite numerous very important studies on DOM, there is a lack of information on the molecular nature of approximately 90% of the DOM.[175,176] Even though trace elements in natural waters appear to be associated mainly with a DOM fraction whose molecular weight is lower than 10,000 daltons,[177] the ability of the DOM components to sequester or release trace elements remains unknown. The topics relating to metal sorption and association with organic substances have been reviewed by Förstner and Wittmann.[178]

Because of the numerous variables which influence the results and the experimental difficulties which have to be overcome, until now biotoxicological tests have been confined mainly to some particular classes of compounds, especially a few high-priority toxicological class elements, such as cadmium, copper, lead, mercury, selenium, and zinc. Even in these cases, the quality of information still needs improving.

These tests are normally performed using synthetic aqueous samples with components as similar as possible to those relating to the aqueous system in question. The closer the natural habitat reproduced and the more correct the selection of aquatic organisms to be used as biological indicators are will determine how high the quality of the results will be. Otherwise, misleading information will be obtained.

Bioassays performed in natural water cannot be used for speciation since the measurable toxic effects are related to all of the toxic components present.

B. Chemical Speciation of Individual Species

Remarkable and praiseworthy endeavors have been made in the direction of chemical speciation in order to evaluate toxicological effects.

Individual species determination is possible whenever analytical procedures do not involve element exchanges between molecular forms during the separation time. This is possible for organometallic compounds and for molecular species containing the element in different valence states. As far as we know, Chau[179-181] was the first to explore the possibility of coupling chromatographic techniques to an atomic absorption (AA) detector in the speciation of organo-lead and organo-tin compounds. Derivatization through Grignard's reaction of the ionic forms made the measurement of each distinct compound present in water, sediment, and fish samples possible. By successive improvements in the original procedure, very low detection limits, 0.1 to 0.3 μg/ℓ are now obtainable for organo-lead compounds and inorganic lead as well. Propylation, rather than butylation, is claimed to offer several advantages,

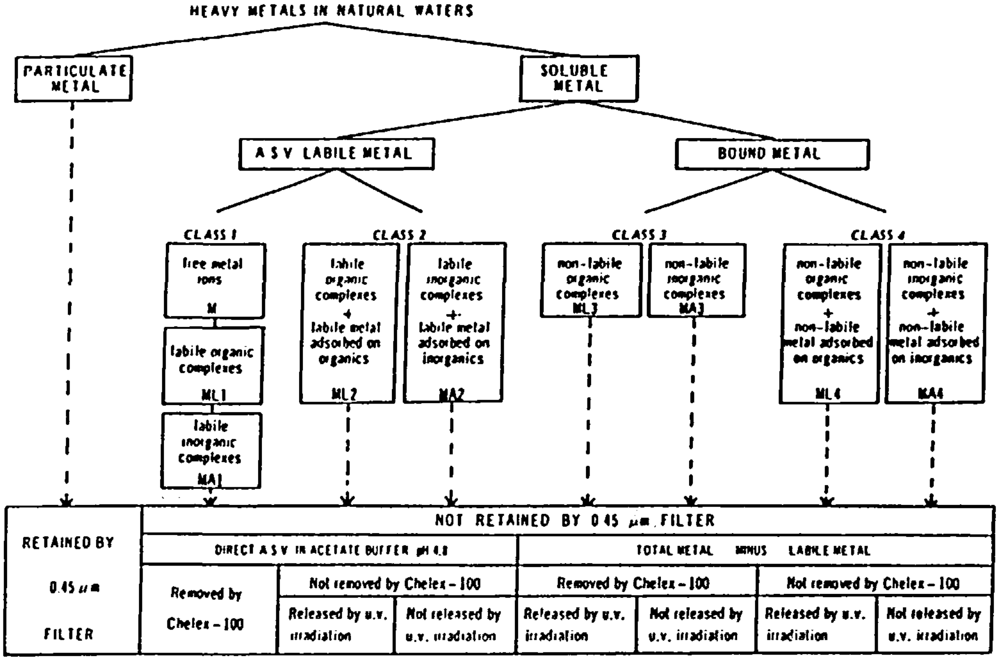

FIGURE 6. Analytical scheme for chemical speciation of trace metals in natural waters. (From Batley, G. E. and Florence, T. M., *Anal. Lett.*, 9, 379, 1976. With permission.)

such as a better recovery of dialkyllead species, a shorter analysis time, and a better resolution of some peaks.[182] Using HPLC with an AA detector good separation of Sn(II), Sn(IV), and tributyltin was obtained at microgram-per-liter levels.[183]

Differential pulsed electrochemical techniques coupled with selective organic phase extraction have been proposed by Colombini et al.[65,184] for consecutive determination of Me_4Pb, Et_4Pb, Me_3Pb^+, Et_3Pb^+, Me_2Pb^{2+} + Et_2PB^{2+}, inorganic Pb^{2+} in natural water samples. At microgram-per-liter levels, the bias in the determination of the above mixture was less than 8%, and typically 4%. Using differential pulse anodic stripping voltammetry, Kenis and Zirino[185] were able to distinguish between inorganic tin and bis(tris-*N*-butyltin) at microgram-per-liter levels. Cr(III)/Cr(VI) separation was obtained by Fuoco and Papoff[186] using differential pulse polarography at microgram-per-liter levels.

C. Chemical Speciation of Classes of Compounds

When individual species determination is not possible, some operative and arbitrary classification has to be introduced.

Based on Batley and Florence's original scheme, the groups of Equation 4 have been classified into seven distinct groups (Figure 6) according to their behavior in the following physicochemical treatments: percolation through Chelex-100 column, UV irradiation, and acid digestion.[187,188] ASV was used for measuring concentrations prior to and after each treatment (Table 7). From a general viewpoint, it is a difficult, if not an impossible task to directly follow the seven classes of compounds through eight different procedures, each potentially involving severe contaminations and large biases. If the previously mentioned interlaboratory tests concerning total concentration determination are used for comparative purposes, one may wonder what kind of discouraging results might be expected when different fractions of C_f have to be analyzed as well.

Table 7
SEPARATION OF HEAVY METAL SPECIES IN SEA WATER

Treatment	Effect of treatment	Measured in solution after treatment: Labile metal species (direct ASV in acetate buffer pH 4.8)	Measured in solution after treatment: Total metal species (ASV after heating with 0.16 *M* HNO_3)
Filtration through 0.45-μm membrane filter	Removes particulate metal	M, ML1, ML2, MA1, MA2	M, ML1, ML2, ML3, ML4, MA1, MA2, MA3, MA4
Passage through Chelex-100	Removes M, ML1, ML3, MA1, MA3	ML2, MA2	ML2, ML4, MA2, MA4
UV irradiation	Releases metal from ML1, ML2, ML3, ML4	M, ML1, ML2, ML3, ML4, MA1, MA2	M, ML1, ML2, ML3, ML4, MA1, MA2, MA3, MA4
Passage through Chelex-100 after UV irradiation	Removes metal released from ML1, ML2, ML3, ML4	MA2	MA2, MA4

Note: M, free metal ion; ML1, ASV-labile organic complexes dissociated by Chelex-100; MA1, ASV-labile inorganic complexes by Chelex-100; ML2, ASV-labile organic complexes and labile metal adsorbed on organic species not dissociated by Chelex-100; MA2, inorganic complexes and labile metal adsorbed on inorganic species not dissociated by Chelex-100; ML3, non-ASV-labile organic complexes dissociated by Chelex-100; MA3, non-ASV-labile inorganic complexes dissociated by Chelex-100; ML4, non-ASV-labile organic complexes and nonlabile metal adsorbed on organic species not dissociated by Chelex-100; MA4, non-ASV-labile inorganic complexes and nonlabile metal adsorbed on inorganic species not dissociated by Chelex-100.

From Batley, G. E. and Florence, T. M., *Anal. Lett.*, 9, 379, 1976. With permission.

1. ASV Signal Features in Real Sample Speciation

Because of the generalized use of ASV in speciation measurements of nondigested real samples, the features and the relevant sources of error peculiar to this technique are considered here in some detail.

It is worth remembering that with this technique the shape as well as the diagnostic parameters i_p and E_p of the anodic peak, in the presence of ligands, are strongly influenced by the electrochemical mechanisms involved in both the reduction and reoxidation steps.

a. The Reduction Step

In the reduction step, assuming that Nernst's equation always holds true and only the C_l group (see Equation 4) is electroactive at the plating potential E_{pl} used, two different electrochemical processes are possible (with two distinct half-wave potentials $E_{1/2}$) depending on whether or not the reduction happens via the free metal ion:

$$ML \rightarrow M^{n+} + L \xrightarrow{e^-} M_{red} + L \qquad (7a)$$

$$ML + e^- \rightarrow M_{red} + L \qquad (7b)$$

where M_{red} is the metal analyte in the reduced form. In Equation 7a, if the rate of M^{n+} ion release from the ML_i complexes is high or low compared to the electron uptake one, the overall electrochemical process will be either mass transport or kinetically controlled, respectively. Thus, at a sufficiently negative potential E_{pl}, all the C_l species (see Equation 5) will undergo reduction and will have zero or higher than zero concentration levels at the electrode interface, depending on the actual rate-determining process.

The minimum plating potential required to obtain $C_i \simeq 0$ (say, 0.05% of the bulk concentration) at the interface can be calculated, at room temperature and constant ionic strength, by the relevant half-wave potential obtained in unstirred solution:

$$E_{pl} \propto (E_{1/2})_c - 6.9 \frac{RT}{nF} \tag{8}$$

where

$$(E_{1/2})_c = (E_{1/2})_s - \frac{RT}{nF} \ln \sum_{i=0}^{n} \beta_{ML_i} C^{i}_{L_i} + \frac{RT}{nF} \ln \left(\frac{\overline{D}_i}{D_s}\right)^{1/2} + E_j \tag{9}$$

$$\overline{D}_i^{1/2} = \frac{1}{C_f} (D^{1/2}_{ML_1} C_{ML_1} + \cdots + D^{1/2}_{ML_n} C_{ML_n}) \tag{10}$$

$(E_{1/2})_s$ and D_s (the diffusion coefficient) being measured at the same ionic strength in the absence of ligands.

Using more negative E_{pl}, there is a greater possibility of also involving the complexes of Equation 7b in the reduction step, so that E_{pl} may play a role in rough separation of Equation 7a and Equation 7b classes of complexes. To some extent, MXs (see Equation 4) and adsorbed metal ion species (group C_3, see Equation 4) may undergo reduction as well.

Two facts arise when the mercury film carbon rod electrode is used and the mercury film is realized *in situ* by means of the addition of high concentrations of Hg(II) to the sample solution: (1) the displacement of the trace element from complexes or from adsorbing colloids, owing to the mass competitive effect of Hg(II) in bulk solution, and (2) the possibility of simultaneous irreversible deposition of the trace element on the carbon rod surface and the inherent decrease of the peak current. The practice of mercury plating *in situ* must be avoided, therefore, particularly in speciation studies.

b. The Oxidation Step

In the oxidation step, only one peak is generally observed regardless of whether Equation 7a and/or Equation 7b mechanism(s) is (are) followed in the preceding reduction step.

For the hanging mercury drop electrode (HMDE) at a constant stirring rate, i_p relating to the ML_i reduction will be proportional to bulk concentrations according to[190]

$$i_p = t_{pl}\left(a \sum_{i=0}^{n} D^{2/3}_{ML_i} C_{ML_i} + b \sum_{i=0}^{n} D_{ML_i} C_{ML_i}\right) \tag{11}$$

where t_{pl} is the plating time, and (a) and (b) are constant, provided that the mass transport is the rate-determing step. When, as is the case for some complexes, the homogeneous dissociation rate is the determining step, the relevant $(i_p)_k$ will be lower than the diffusion-controlled one $(i_p)_d$. In turn, E_p may assume any value between two extreme situations:

$$E_p \propto (E_{1/2})_s \tag{12}$$

$$E_p = (E_{1/2})_s - 1.1 \frac{RT}{nF} \ln \sum_{i=0}^{n} \beta_{ML_i} C^{i}_{L_i} + \frac{RT}{nF} \ln \left(\frac{\overline{D}_i}{D_s}\right)^{1/2} \tag{13}$$

In Equation 12, the free M^{n+} ions, obtained by oxidation, do not undergo complex formation at the electrode interface, the relevant rates being very low compared with the electron transfer according to

$$M_{red} \xrightarrow[-e]{\text{fast}} M^{n+} \xrightarrow[+L]{\text{slow}} ML$$

$$M_{red} \xrightarrow[-e]{\text{fast}} M^{n+} \xrightarrow[+M_1L]{\text{slow}} ML + M_1^{2+}$$

where M_1 = non electroactive metal such as Ca and Mg, competitive in complex formation.

In Equation 13, the ML_i formation rates are assumed to be very high compared to the electrochemical metal oxidation rate, and the C_{L_i} concentrations at the interface to be the same as in the bulk solution.

The first condition (Equation 12) is verified to some extent, e.g., in the case of the system cadmium-ethylenediamine in sea water. Plotting the half-wave potential $(E_{1/2})_*$, obtained by measuring the ASV peak heights at different plating potential and constant plating time, vs. the concentration of the ethylenediamine, Kounaves and Zirino[191] found that the first measurable shift in the half-wave potential is obtained for bulk ethylenediamine concentrations higher than 10^{-4} *M*. As a matter of fact, considering the stepwise dissociation constants $pK_1 = 5.7$; $pK_2 = 4.7$; $pK_3 = 2.4$,[192] one would expect a shift of $(E_{1/2})_*$ toward more negative potentials of about 70 mV for a 10^{-4} *M* concentration of ethylenediamine. The dependence of $(E_{1/2})_*$ on the plating time has been studied by Zirino and Kounaves.[189]

When one or more ligands L_i are present at trace level, owing to the large accumulation factor inherent in ASV during the plating time, the interface concentration of M^{n+} ions in the electrochemical oxidation step can progressively become so high that the $(L_i + ML_i)$ concentrations, if not in large excess in the bulk of the solution, become mass transport controlled and more anodic peaks or one broad peak may occur, no matter how high the kinetic and equilibrium formation constants of the complexes involved are. For instance, two peaks have been observed at -0.1 and -0.3 V, respectively (vs. Ag/AgCl), for Cu^{2+} in sea water, with i_{p1}/i_{p2} strongly influenced by pH and plating potential.[193]

Complexing capacity — i.e., the capacity of the actual system to bind toxic metals in an active complex — also suffers from several drawbacks when measured by ASV. In principle, using a suitable metal ion as a titrant, one should be able to calculate the complexing capacity of the system from the anodic peak height vs. the metal concentration plot. In practice, as pointed out by Plavsic et al.,[193] quite different results are obtained depending on the nature of the metal ion used as a titrant. Excellent reviews about different biological or chemical approaches to cope with this problem, along with pertinent criticisms of each approach, have been published by Hart[177] and Neubecker and Allen.[209]

c. Surface-Active Substance Effects

Surfactants as well as many organic substances are known to affect both diagnostic parameters (i_p and E_p) selectively.[194-202] Sagberg and Lund[198] have studied extensively the effect that pH and the concentration of several surfactants (SAS) as well as humic substances (HUMIC SUB) play on the i_p of copper, cadmium, and lead in synthetic sea water (SS). The surfactants considered were sodium dodecyl sulfate (SDS), dodecylamine (DDA), and Triton X-100®.

Figure 7 refers to the effect of pH on copper, lead, and cadmium i_p values, respectively, at a constant SAS (10 mg/ℓ) and HUMIC SUB (4%; organic carbon 18 mg/ℓ) concentrations. As can be seen, whenever the formation of hydroxo-complexes is not significant, i_p is not

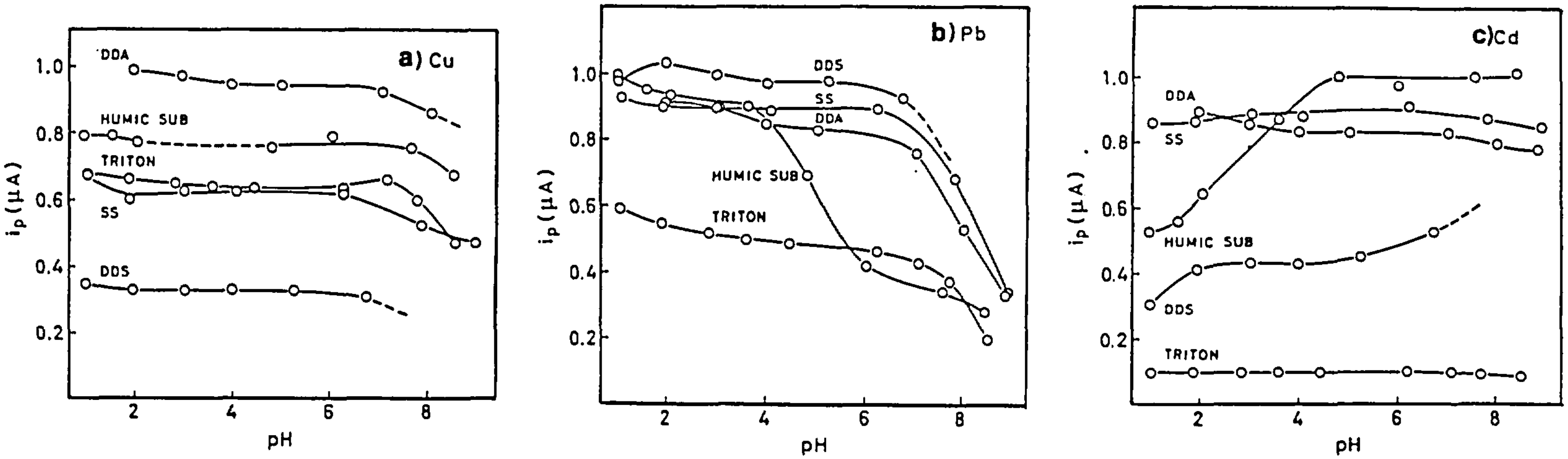

FIGURE 7. Effect of pH on i_p values of copper (a), lead (b), and cadmium (c) at a constant surface-active substance (10 mg/l) and humic substance (4%) concentration. SS, synthetic sea water; DDA, dodecylamine; DDS, sodium dodecyl sulfate. (From Sageberg, P. and Lund, W., *Talanta*, 29, 457, 1982. With permission.)

appreciably affected by pH, with the exception of cadmium and lead in the presence of humic substances. The authors noted that the decrease in peak heights was always accompanied by a cathodic shift in the peak potentials, which may indicate a complex formation.

In Figure 8, the profiles of i_p vs. SAS concentration are shown for copper, lead, and cadmium, respectively, at a pH 2.3 level which is close to the working pH normally used in ASV analysis of natural waters. These profiles strongly depend on the element/surfactant couple considered. In general, they present a large i_p variation in the SAS concentration range which usually occurs in natural water samples.

In Figure 9, the variation of the i_p with the concentration of HUMIC SUB is shown. It can be seen that i_p values were unaffected by concentrations lower than 1% of HUMIC SUB, whereas at high concentration, i_p decreased for copper and cadmium, but not for lead.

It is interesting to note that the same authors using the standard addition method always obtained linear i_p vs. trace element concentration plots, the slopes being controlled by the concentration of surfactant.

These findings show that surfactant presence can be accepted provided that the shift of E_p is not too large to hinder identification, that linear plots are obtained by standard additions, and that sensitivity is still sufficient for analytical determination. In fact, in nondigested natural samples, nonlinear plots often occur as a consequence of the artifacts or of the type and concentration of ligands naturally present. A typical example is shown in Figure 10.[203]

Inter alia, the linear extrapolation at $i_p = 0$ of the points relevant to the highest concentrations of metal ion added leads to an apparent complexity capacity of the sample about 20 times higher when copper rather than lead is used as titrant. In these conditions, destroying the matrix effect is a good practice.

D. Conclusions

From a general viewpoint, one may conclude that, in principle, ASV is an unsurpassable tool for measuring very low total concentrations of analyte provided matrix effects can be overcome. However, when many species concur in giving M_{red} only one anodic peak is generally, but not always, observed. At constant C_t, depending on the relative concentration of each ML_i complex, a large variation of i_p may be caused by changes in the diffusion or kinetic mechanisms. That is, when the mass transfer is the rate-determining step, the i_p value will depend on each D_{ML_i} according to Equation 11. When a kinetic mechanism is the rate-determining step, the i_p value will depend on the dissociation rates of the ML_i species; for extremely low dissociation rates, i_p will be proportional to $C_M{}^{n+}$, the concentration of the free metal ion. Apart from in this latter case (which is extreme), the standard addition method will give the correct value of C_t concentration, provided that L_i concentrations are in excess and sufficient time has elapsed between the addition and measurement in order to guarantee that equilibrium conditions are reached in the bulk of the solution.

As for E_p, when the formation rate for each ML_i complex at the electrode interface is very large or very small compared to the metal oxidation rate, E_p will be relatable, respectively, to the polarographic $(E_{1/2})_c$ or $(E_{1/2})_s$ which should have been obtained for the same ligand-containing or ligand-free solution. Between these extreme conditions, E_p may assume any value.

In the absence of diagnostic tests to investigate kinetic contributions during reduction and/or oxidation steps at a very low concentration level, ASV is not a tool to be used for distinguishing between different classes of complexes in terms of their thermodynamic or kinetic effects. To some extent and under suitable conditions, information may be obtained about some compounds and/or complexes which undergo reduction according to Equation 7b by using different E_{pl}.[184] However, situations as shown in Figure 11, where the i_p varies irregularly with the plating potential, are representative of most real samples and are not easily interpretable.

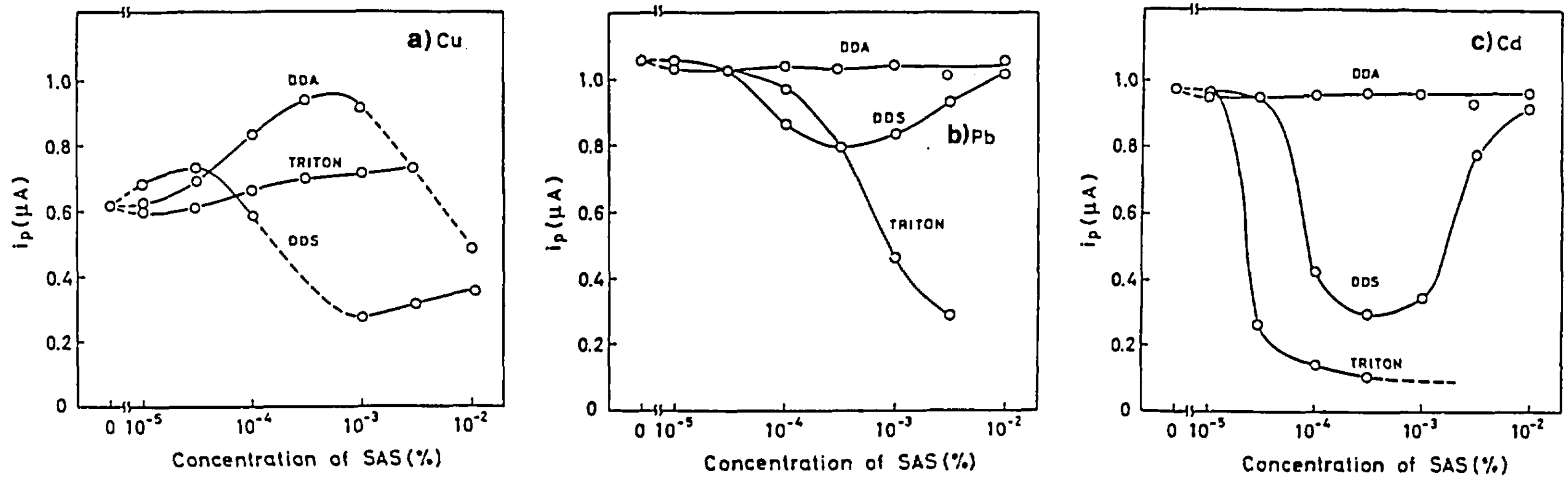

FIGURE 8. Effect of surface-active substance (SAS) concentration on the peak heights of copper (a), lead (b), and cadmium (c) at pH = 2.3. (From Sageberg, P. and Lund, W., *Talanta*, 29, 457, 1982. With permission.)

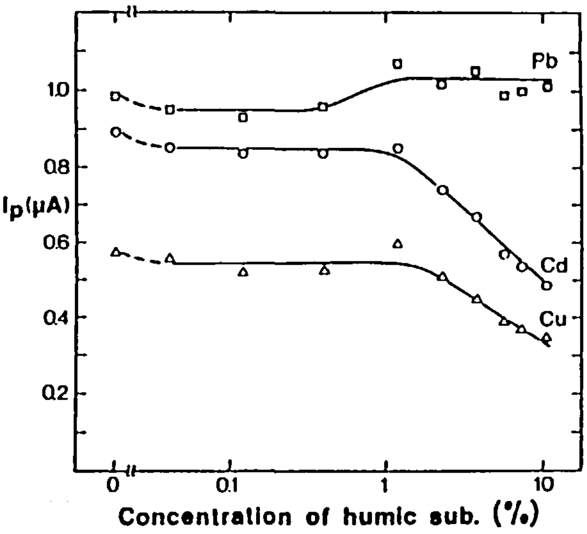

FIGURE 9. Effect of humic substance concentration on the peak heights of copper, lead, and cadmium. (From Sageberg, P. and Lund, W., *Talanta*, 29, 457, 1982. With permission.)

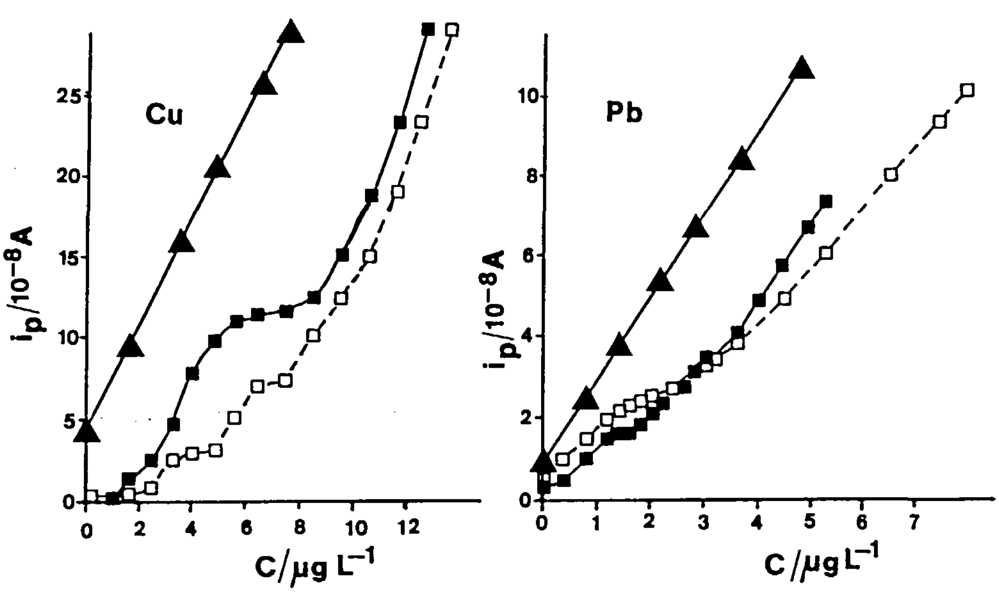

FIGURE 10. Titration of a Baltic coastal water sample with copper and lead solutions, respectively. (□—□) Unfiltered. (■—■) Filtered (0.45 μm). (▲—▲) After UV irradiation. (From Brügmann, L., *Sci. Tot. Environ.*, 37, 41, 1984. With permission.)

As for the use of ASV in the speciation scheme proposed by Batley and Florence[187,188] for Cu, Pb, and Cd apart from artifacts and experimental difficulties, the ASV peak currents (measured in the filtered sample acidified at pH 4.8, under a unique plating potential, −0.9 V) cannot be unequivocally related to distinct classes of compounds with defined toxicological effects in the original sample. Besides, it would be advisable to use different plating potentials in order to test the quality of the results obtained and, in the case of ill-defined

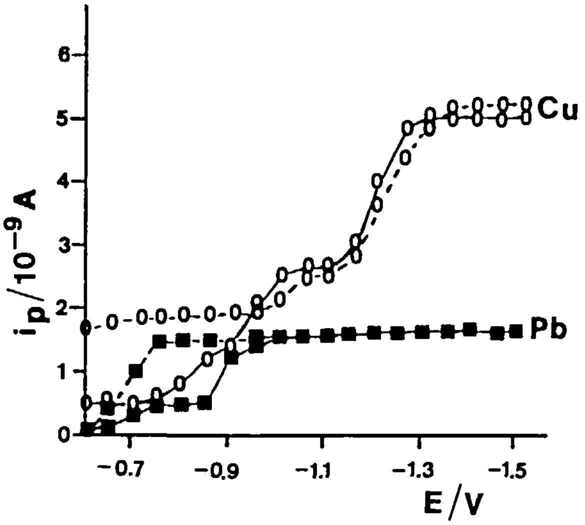

FIGURE 11. Relationship between peak current (i_p) and plating potential (E) for copper (○) and lead (■) in filtered Baltic Sea water. Dotted line refers to the results after UV irradiation. (From Brügmann, L., *Sci. Tot. Environ.*, 37, 41, 1984. With permission.)

anodic peaks, to use medium-exchange procedures.[204,205] The medium-exchange procedure involves a deposition of the metals from the sample solution, followed by their stripping into a more suitable electrolyte. This approach is very efficient in minimizing interferences which arise from various sample components during the oxidation step.

If the solvated metal ion is the main cause of the toxic effects, Batley and Florence's speciation scheme fails to give sound information. In fact, considerable evidence now exists that the free ionic forms of several trace metals (e.g., copper, cadmium, lead, and zinc) are the most toxic forms for phytoplankton, invertebrates, and fish. Complexed forms appear to be nontoxic, or at least are considerably less toxic than the free metal ion.[206,207]

Guidelines have been issued by the U.S. Environmental Protection Agency[208] to derive criteria for the protection of aquatic life. The following are the most important principles to be considered for defining the substances for which the criteria are to be derived, and are reported here in the original form:[208]

A. Each separate chemical which would not ionize significantly in most natural bodies of water should usually be considered a separate substance, except possibly for structurally similar organic compounds that only differ in the number and location of atoms of a specific halogen, and only exist in large quantities as commercial mixtures of the various compounds, and apparently have similar chemical, biological, and toxicological properties.

B. For chemicals, which would ionize significantly in most natural bodies of water, such as inorganic salts, organic acids, and phenols, all forms that would be in chemical equilibrium should usually be considered one substance. For metals, each different valence and each different covalently bonded organometallic compound should usually be considered a separate substance.

C. The definition of the substance may also need to take into account the analytical chemistry and fate of the substance.

The overall salt effect in reducing the biotoxicity of some trace elements has been measured by using biological tests. For fresh water, the maximum concentration value (m.c.), which should not be exceeded at any time, was related to the hardness by a relationship of the type:

Table 8
CRITERIA FOR AQUATIC LIFE PROTECTION: TRACE ELEMENT MAXIMUM CONCENTRATION VALUES IN FRESH WATER (AS FUNCTION OF HARDNESS: mg/ℓ OF $CaCO_3$) AND IN SEA WATER[208]

	Hardness (mg/ℓ $CaCO_3$)	Trace element concentration (μg/ℓ)				
		Cd	Cu	Ni	Pb	Zn
Fresh water	50	1.5	12	56	0.75	180
	100	3.0	22	96	3.8	320
	200	6.3	43	160	20	570
Sea water		59	23	140	—	170

$$\text{m.c.} = e^{\{a[\ln(\text{hardness})] \pm b\}} \tag{14}$$

where a and b are specific constants for each element.[208]

Some m.c. values are reported for cadmium, copper, nickel, lead, and zinc in Table 8. In the case of cadmium, for instance, assuming pH does not change significantly and chloride ion is the only complexing agent in sea water, the percentage of free ion should decrease about 8000 times when the sea water ($\beta_2 = 10^{4.4}$ for $CdCl_2$ complex) is considered rather than the fresh water containing nearly the same hardness (200 mg/ℓ of $CaCO_3$). If only the solvated ion is responsible for the toxic effect, one should expect an increase in m.c. of 6.3 × 8000 mg/ℓ. In fact, the increase is only 10 times, which shows that to some extent the cadmium complexes are toxicologically active or ionic strength plays a significant role. Unfortunately, this behavior cannot be predicted, even in principle, by any of the electrochemical methods available nowadays.

The most frequently used techniques which may allow reliable results to be obtained in the best experimental conditions are summarized in Table 9 for different classes of compounds.

V. AQUEOUS ECOSYSTEM CHARACTERIZATION

In the preceding sections, the different aspects considered have concerned the reliability of analytical data in the characterization of the sample. In the present section, the correlation between the trace element contents of the samples and the ecosystem is briefly considered.

When characterizing a specific water system, a proper monitoring program involving different stations and depths of sampling is required in order to take into proper account the effects due to vertical and horizontal movements of watermasses. Furthermore, in the case of estuarine or coastal waters, the characteristics of effluents entering the water system, and the hydrodynamic mixing conditions as well as the seasonal and tide effects, must be considered.

Several interesting studies have been carried out to ascertain trace element distribution in the oceans, coastal environments, and estuaries. All the relevant findings were considered in detail by Salomons and Förstner[210] in *Metals in the Hydrocycle*. The following findings are related to dissolved matter.

Trace element distribution in the Pacific Ocean is the best known nowadays. Cadmium, copper, nickel, and zinc concentrations have been shown to be depleted in the surface layer, as are the nutrients. In particular, Bruland[212] found that cadmium concentration increased 5 times (from about 0.2 to 1.0 nmol/kg) with an increasing depth for the first 1000 m, and slightly decreased up to a depth of 5000 m. On the other hand, zinc and nickel concentrations

Table 9
SUMMARY OF THE EXPERIMENTAL CONDITIONS REQUIRED FOR SPECIATION OF CLASSES OF COMPOUNDS IN 0.45-μm FILTERED NATURAL WATERS

Distribution of a Trace Element Between Different Chemical Species and Different Phases

$$C_t = \underbrace{C_M n + \left(1 + \sum_{i=0}^{n} \beta_i C^i_{L_i}\right)}_{\sum_{i=0}^{n} C_{ML_i}} + \sum_{j=0}^{m} C_{MX_j} + C_{M_{absorbed}} + C_{M_{englobed}}$$

Reliable Information Obtainable through the Most Frequently Used Instrumental Techniques Under the Specified Conditions

	Detectable species					
Technique	C_t	$C_M n+$	$\sum_{i=0}^{n} C_{ML_i}$	$\sum_{j=0}^{m} C_{MX_j}$	$C_{M_{ads.}}$	$C_{M_{engl.}}$
Atomic spectroscopy	X[a]					
Potentiometry		X[b]				
DPP	X[c]	X[d]	X[e]			
DPASV	X[c]					
Chromatography			X[f]	X[f]		
Ultracentrifugation					X	X

[a] Chemical pretreatment for mineralization of volatile compounds and solvent extraction recommended.
[b] Not suitable at natural concentration.
[c] After matrix effect elimination by physicochemical treatment.
[d] By the E_p shift, in absence of matrix effects.
[e] By i_p, provided that a linear plot is obtained by the standard addition method, and the contribution of MX_j species is negligible at the applied E_{pl}.
[f] Whenever derivatization and solvent extraction procedures prior to separation are available.

increased between 8 and 10 times (from about 0 to 7 and about 2 to 10 nmol/kg), respectively, for the first 1000 m, and afterwards remained practically constant up to a depth of 5000 m. The vertical distribution profile of copper differed from those of the above three metals. Its concentration showed a minimum value at the surface (about 1.0 nmol/kg) and increased fairly linearly with depth.

The vertical distribution of mercury in the waters of the Northwest Pacific was investigated by Miyake and Suzuki.[213] The highest mercury total concentration (about 10 to 20 ng/ℓ) was alwys always found up to a depth of 100 m. Between 100 to 500 m it decreased with depth. Then the Hg level remained constant up to a depth of 5000 m and increased from 5500 to 6000 m.

As for the vertical distribution of lead, Schaule and Patterson[214] found that in the first 2500 m, lead concentration decreased with depth and afterwards remained fairly constant up to 5000 m. These authors[214] found the same behavior in the Atlantic Ocean, but the concentration values were significantly higher than those found in the Pacific Ocean. This does not happen for all trace metals. As a matter of fact, Bruland and Franks'[215] studies show that cadmium, copper, and zinc concentrations in the Atlantic were 2 to 4 times lower than in the Pacific.

Table 10
METAL BALANCE IN THE SCHELDT ESTUARY (tons/year)[239]

Metal	Total inflow	Total outflow	Outflow in % of inflow
Zinc	1271	111	9
Copper	311	33	11
Lead	353	26	7
Cadmium	48	5	10
Nickel	128	53	41

Table 11
MEAN ANNUAL METAL BALANCE IN THE GIRONDE ESTUARY (tons/year)[240]

	Inflow		Outflow		
Metal	Dissolved	Particulate	Dissolved	Particulate	Outflow in % of inflow
Zinc	202	1528	1211	230	83
Copper	82	148	202	22	97
Lead	61	371	326	48	86
Nickel	114	96	141	34	83

Comparative studies on cadmium levels have been performed in the North Sea, Norwegian Sea, Barents Sea, and Eastern Arctic Ocean by Mart et al.[216] From these investigations, covering a period of 7 years (1975 to 1982), the coastal zones of Belgium, The Netherlands, and Germany were shown to be significantly more polluted than open ocean areas. In the open sea, trace metal levels decreased from south to north, yielding the lowest results in the Eastern Arctic Ocean with about 10 ng/kg of cadmium.

The Mediterranean Sea has been studied by different authors.[217,219-225,227,258] Laumond et al.[217] found that vertical distributions of cadmium and copper were quite homogeneous. Up to a depth of 400 m, cadmium concentration oscillated between 5 to 10 ng/ℓ, decreasing slightly with depth up to 3000 m. For copper, concentration varied between 60 to 100 ng/ℓ only in the first 100 m. Lead surface concentrations were always higher than those in deep waters; a concentration decrease occurred between 100 to 700 m, then levels remained practically constant at 25 ng/ℓ up to 3000 m deep.

Several studies concerning transport mechanisms of trace elements in estuaries have been performed.[211,226-241] When measuring the inflow-outflow balance of trace elements in an estuary, total concentrations must be considered in both the particulate and the aqueous phase over extended time intervals. In the case of the Scheldt[239] and St. Lawrence[241] estuaries, the estuarine system was found to act as a filter hindering the transport of particulate in the sea waters. The opposite was found in the case of the Gironde estuary.[240] Here, the particulate was mostly remobilized and discharged into the sea. Some of the above findings are summarized in Tables 10 and 11.

In the interpretation of the distribution mechanisms by which the fresh water is diluted with sea water, salinity is practically the most significant reference parameter. Sound information can be obtained provided that the composition of the end members remains constant during the water flushing time.[218] Samplings should therefore only concern the zone downstream of any fresh water inlets in the river under consideration.

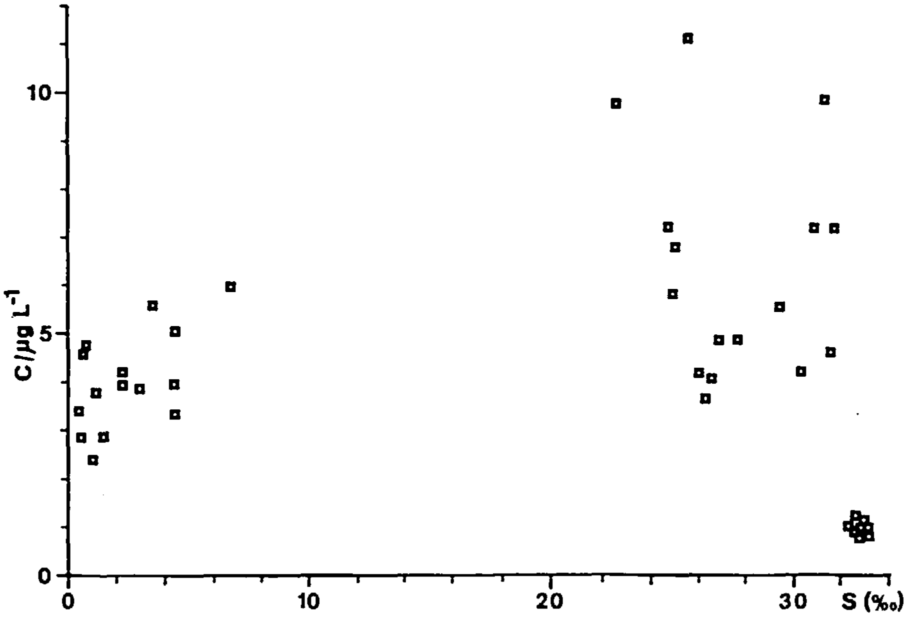

FIGURE 12. Dissolved copper concentration vs. salinity for surface samples collected along the river Ems and its estuary. (From Duinker, J. C., Hillebrand, M. T. J., Nolting, R. F., and Wellershaus, S., *Neth. J. Sea Res.*, 19, 19, 1985. With permission.)

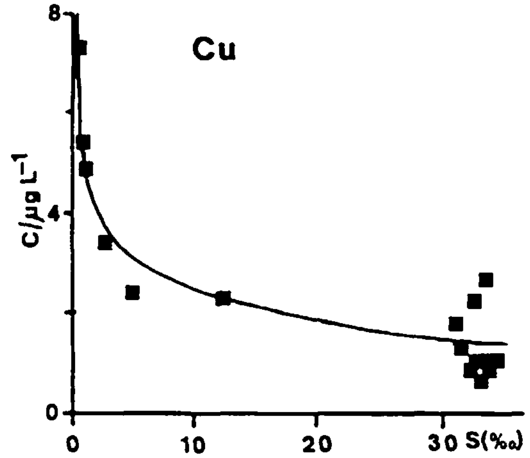

FIGURE 13. Dissolved copper concentration vs. salinity in samples collected in the Rhine estuary. (From Duinker, J. C. and Nolting, R. F., *Mar. Pollut. Bull.*, 8, 65, 1977. With permission.)

Often the particulate or dissolved concentration vs. salinity plots present quite an irregular shape and can hardly be considered controlled by dilution. In Figures 12 and 13, two typical dissolved concentration vs. salinity plots are shown: one relevant to the Ems river[227] and the other to the Rhine estuary,[226] respectively.

The correlation between particulate and dissolved matter at a constant depth is also a difficult task. In Figure 14, the concentrations of cadmium in particulate and dissolved matter are reported vs. distance from the spring (and vs. salinity), as measured at the surface for

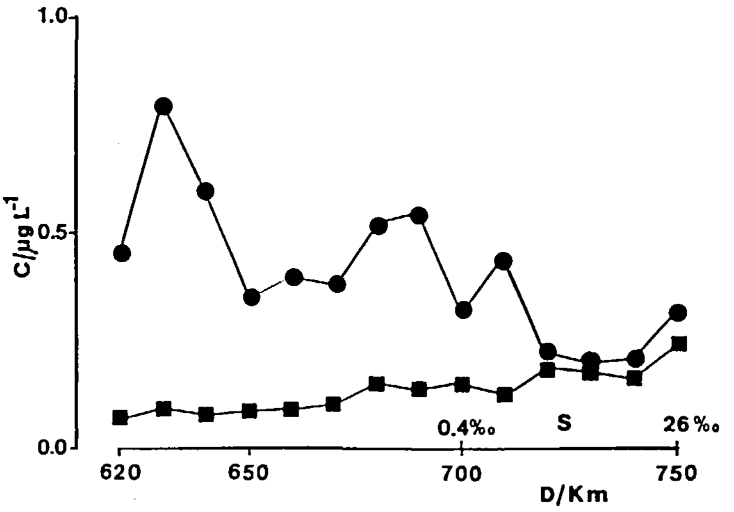

FIGURE 14. Concentration of cadmium in dissolved (■—■) and particulate (●—●) matter vs. distance (and salinity) for surface samples collected in the Elbe estuary. (From Ahlf, W., *Environ. Technol. Lett.*, 4, 405, 1983. With permission.)

the Elbe estuary.[259] As can be seen, suspended matter content is much more affected than that dissolved by pollution sources. No simple explanation can be given about the apparent lack of effect in dilution between 700 and 750 km where the salinity rises from 0.4 to 26%.

Owing to the complexity that the estuarine systems present, no particular attention is generally devoted to explaining why these plots occur in practice. In fact, local changes in temperature and pH, along with changes in organic and inorganic matter due to vertical mixing between layers of different depths in the water column, may be responsible for this behavior. The discordance with respect to the conservative model will be greater the more the other variables in addition to dilution are effective.

The effect of the variation of hydrological regimes on trace element distribution between river and sea water in the estuaries has been investigated by several authors.[211,242-250] Studies have also been carried out in the laboratory to simulate the actual estuarine mixing conditions.[54-56,246,251-256]

Investigations have been carried out extensively by Betti et al.[257,258] in order to evaluate the transport and dilution mechanisms of copper, lead, cadmium, and chromium along the North Tyrrhenian Sea coast. This study, covering a 2-year period, involved 5 surveys along the coast on 13 selected sampling stations, 2 surveys following the floats along the river Arno and its plume, and 5 surveys along its estuary on 6 different stations between 2.5 km inland and 0.4 km offshore at several different depths.

From the analysis of the dissolved matter, it was found that the lowest concentrations of copper and lead were always on the river bed whatever the hydrodynamic regime was. For these elements, the concentration vs. distance plots, at a constant depth along the horizontal slipping axis of the river, were found linear as shown in Figures 15 and 16. At the constant depth condition, a linear relationship between salinity and distance was always found too.

Evidence that dilution is not the only controlling factor was drawn plotting all the experimental data obtained at different depths vs. salinity. From these plots, shown in Figure 17 for copper only, it was possible to evidence that in the case of river in flood, data obtained at constant salinity regardless of depth are statistically indistinguishable (true conservative behavior). In the cases of flood- and ebb-tide, the data relating to the lowest layers (flood-tide) or the surface (ebb-tide) diverge from the average behavior.

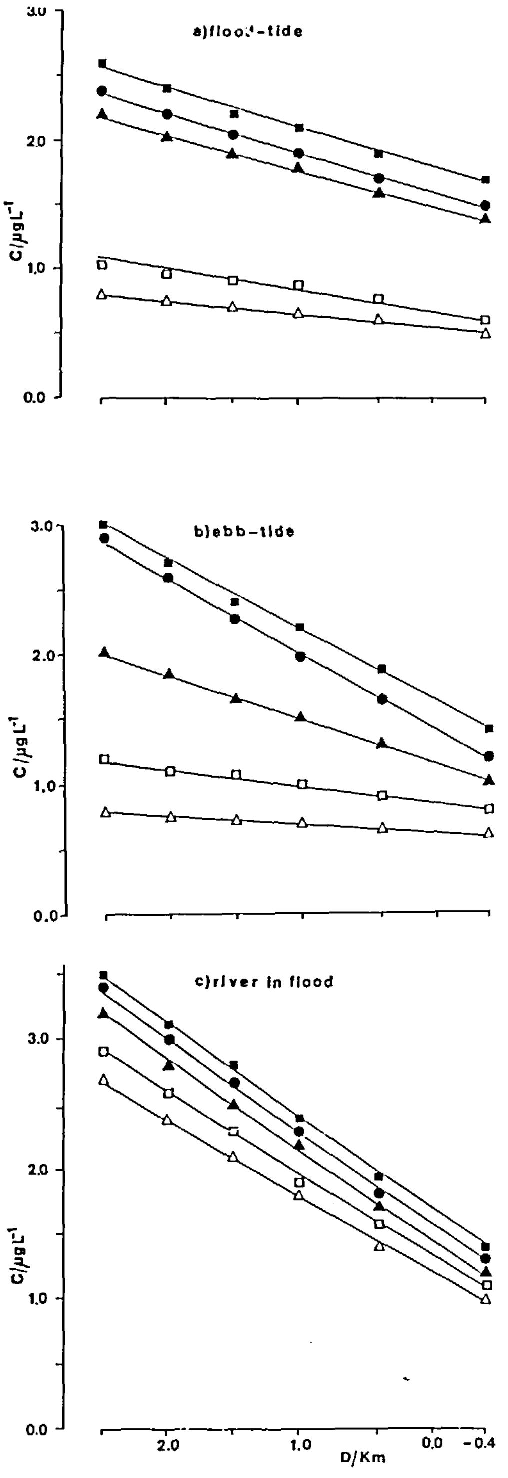

FIGURE 15. Effect of hydrological regimes (a: flood-time; b: ebb-tide; c: river in flood) on the copper concentration vs. distance plots at different depths: (■) 0.1 m; (●) 1 m; (▲) 2 m; (□) 3 m; (△) river bed. Distance as measured from the river mouth. The negative value refers to the offshore sampling station.[258]

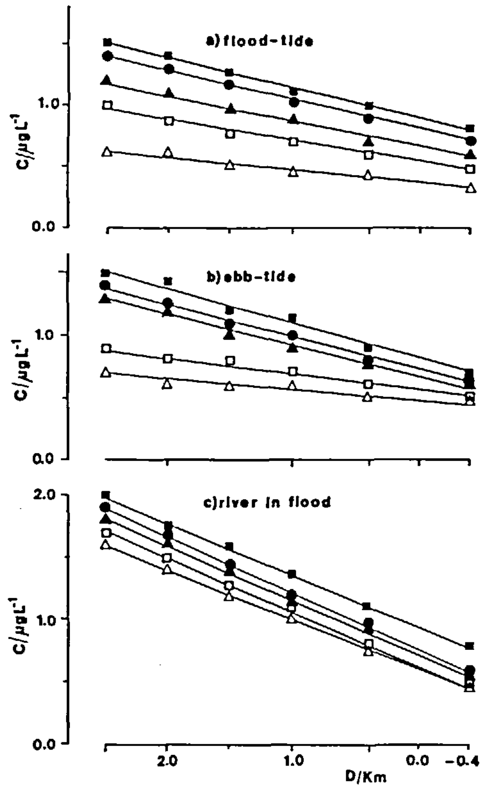

FIGURE 16. Effect of hydrological regimes (a: flood-tide; b: ebb-tide; c: river in flood) on the lead concentration vs. distance plots at different depths: (■) 0.1 m; (●) 1 m; (▲) 2 m; (□) 3 m; (△) river bed. Distance as measured from the river mouth. Negative value refers to the offshore sampling station.[258]

As for cadmium, the highest rather than the lowest concentrations were always found on the river bed, as shown in Figure 18a, for flood- and ebb-tide, respectively. The distinct curves obtained for different depths can be explained in terms of concurrent effect of particulate redissolution. In fact, whenever the amount of particulate in each point is controlled by dilution and depth, the overall effect leads to a family of linear plots of concentration vs. salinity (Figures 18a, b). In regime of high turbulence as in the case of the river in flood, the cadmium concentration was shown to be linearly dependent on salinity (correlation factor: 0.996; standard deviation: $4\ 10^{-3}$ µg/ℓ) regardless of the distance from the mouth and depth (Figure 18c). The positive slope of the curve reveals that the dilution effect was largely counteracted by the cadmium release from particulate, which was in turn strictly controlled by chloride content. Note that the cadmium concentration in open sea, outside the river stream effect, was 0.02 µg/ℓ.

The solubilization effect of cadmium from particulate has been explained by considering the effect of chloride ions present in the sea water layers. This effect, which may take place via formation of chloro-complexes, salt effect, and substitution of Cd^{2+} with Na^{+} and Ca^{2+} ions in particulate sites of adsorption, has been reported by other authors.[253,259-261]

VI. GENERAL CONCLUSIONS

Contamination of the sample during the sampling, storage, and solid separation phases, as well as matrix effects during the analysis, may introduce a large bias in the results. The

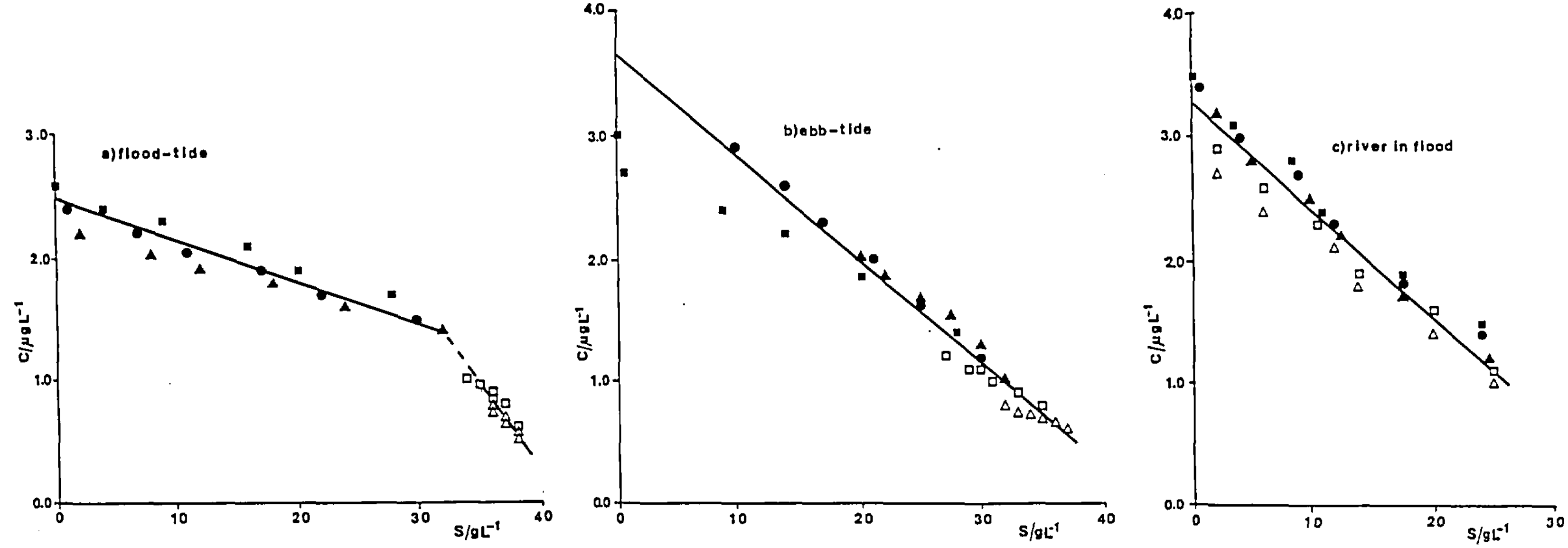

FIGURE 17. Copper concentration vs. salinity plots in different hydrological regimes: (a) flood-tide; (b) ebb-tide: (c) river in flood. Sampling depths: (■) 0.1 m; (●) 1 m; (▲) 2 m; (□) 3 m; (△) river bed.[258]

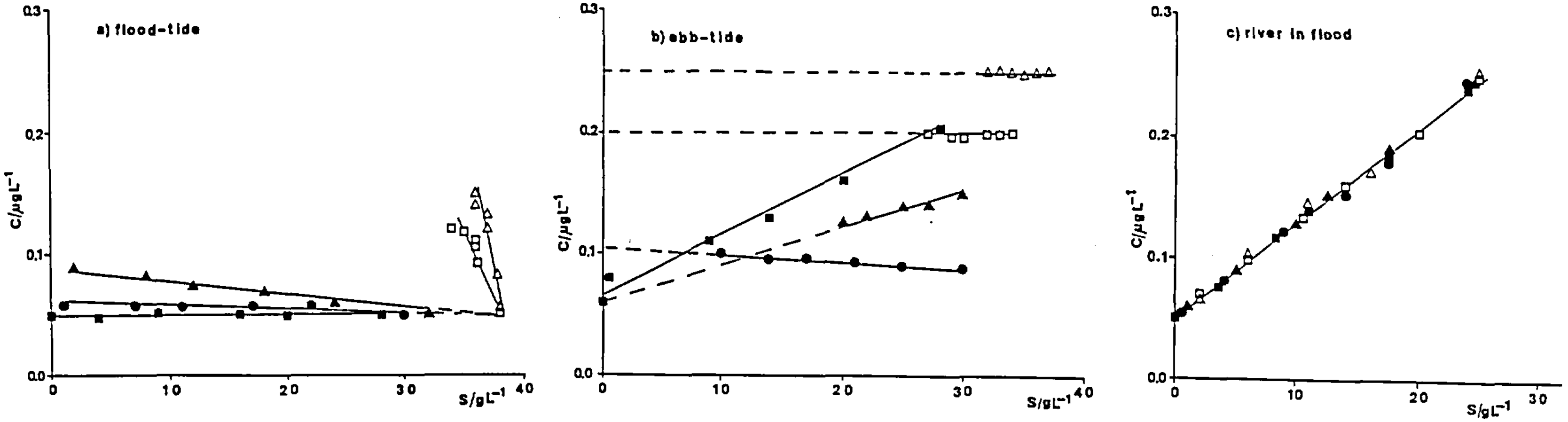

FIGURE 18. Cadmium concentration vs. salinity plots in different hydrological regimes: (a) flood-tide; (b) ebb-tide; (c) river in flood. Sampling depths: (■) 0.1 m; (●) 1 m; (▲) 2 m; (□) 3 m; (△) river bed.[258]

observation of precise guidelines consistently reduces the bias, but does not completely eliminate it.

In the case of dissolved matter analysis, owing to the very low concentration of trace elements in natural waters, solvent extraction or the destruction of interfering substances is recommended in order to eliminate matrix effects. Under these conditions, the total concentration of trace elements can be measured with an acceptable accuracy by skilled people in suitable laboratories.

The fact that in many interlaboratory exercises a very large spread of results is still possible reveals the difficulties inherent in the analysis at micro- and submicrogram-per-liter concentration levels.

Size speciation of particulate is very important in understanding solid/liquid exchange mechanisms. In spite of the large concentration involved in particulate (levels of tens of hundreds of milligrams per kilogram), great care must be taken in order to avoid contamination during separation.

Chemical speciation is possible at the present state-of-the-art for those classes of trace-element-containing compounds which do not decompose or do not undergo rearrangement during the separation step. Many metallorganic compounds present these characteristics and have been separated successfully with or without previous derivatization.

At present, no analytical procedures permit an exact prediction of the correlation between chemical data and toxic effects for trace element forms in chemical equilibrium. It is likely that some new instrumental techniques, which have been under experimentation during the last few years, may be able to cope with this very important task.

In the interpretation of transport and distribution mechanisms of trace elements in an aqueous ecosystem, the following points must be considered:

1. Interaction between dissolved and particulate forms has a decisive influence on trace element transport in the estuaries and oceans.
2. To obtain results which are representative of the essentially evolutive phenomena relevant to any aqueous system, surveys in different sampling stations under different metereological conditions must be performed.
3. In principle, the determination of both dissolved and particulate fractions of trace elements as well as the measure of the interface characteristics of all the solids involved are required.

GLOSSARY OF SYMBOLS

C_f	Trace total concentration in 0.45-μm filtered sample
β_{ML_i}	Formal total equilibrium formation constants
i_p	Peak current
$(i_p)_k$	Kinetic peak current
$(i_p)_d$	Diffusion peak current
E_p	Peak potential
E_{pl}	Plating potential
$E_{1/2}$	Half-wave potential in d.c. polarography
$(E_{1/2})_c$	Half-wave potential in d.c. polarography of complexed ion
$(E_{1/2})_s$	Half-wave potential in d.c. polarography of solvated ion
$(E_{1/2})_*$	Half-wave potential of the polarographic curve obtained by plotting the peak stripping currents as a function of the plating potentials
E_j	Junction potential difference between the solution with and without complexing agents
D_i	Diffusion coefficient

D_s	Diffusion coefficient of solvated ion
t_{pl}	Plating potential
R	Gas constant
T	Absolute temperature
n	Number of electrons involved in the electrode reaction
F	Faraday constant

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