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VOLTAMMETRIC TRACE ANALYSIS IN ECOLOGICAL CHEMISTRY OF TOXIC METALS

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Abstract - In context with the key significance of reliable trace analysis for the considered ecochemical subject general aspects of the voltammetric approach are treated. The voltammetric modes to be usually applied are differential pulse stripping voltammetry (DPSV) or differential pulse voltammetry (DPV) at suitable working electrodes. The significance of reliable prior stages (sampling and sample pretreatment) of the whole analytical procedure to obtain relevant data is emphasized. Universal analytical procedures for the determination of Cu, Pb, Cd, Zn, Hg, Se, Ni and Co are presented for natural waters, rain and waste water and for biological materials. While for natural waters voltammetry is the method of first choice for toxic trace metal chemistry concerning analysis and speciation, the voltammetric approach has also become for the reliable trace analysis in all types of biomatrices one of the indispensable methods in the basic outfit of corresponding laboratories. The far reaching potentialities of pulse voltammetry are featured by a number of examples from chemical oceanography and limnology, the analysis of atmospheric precipitates and waste water, applications in the control of drinking water and the components of the food basket and in the analysis of body fluids.

INTRODUCTION

A large number of potentially or actually hazardous chemicals are emitted from anthropogenic sources into the environment. This is, within certain limits, an inevitable side effect of modern technology needed for the functioning, if not the survival, of human society. The point is that efficient and comprehensive control and management of the environmental burden caused by hazardous chemicals has become an issue of primary importance for man. In this context ecochemical research on the levels, fate and pathways of environmental chemicals and on their ecotoxicological impacts have gained great significance. Only this research will provide a sound, scientifically founded basis for necessary regulations to keep the environmental burden within tolerable limits. Although many toxic chemicals are emitted in large quantities into the environment, they are frequently rapidly dissipated and occur, fortunately still, in most terrestrial and marine ecosystems at trace levels. An important and urgent task is to assess if they remain below critical thresholds and to improve and expand the knowledge on their environmental behaviour and fate. Therefore, the methods and approaches of trace chemistry and trace analysis have a key function in ecochemistry and ecotoxicology in deepening and extending the knowledge and understanding in these branches of science, in controlling the fulfillment of regulations for environmental protection and in the detection and diagnosis of existing pollution and its hazards. Wrong or unreliable data on environmental pollution levels of hazardous substances, as consequence of unqualified trace chemical work or the application of unsuitable methods, will not only lead to dubious scientific results but will cause inevitably severe social, economical and ecological penalties and damages.

General trace analytical aspects

Modern trace analytical determinations are usually carried out by instrumental methods. Suitable methods have to fulfill certain basic prerequisites. They have to combine sufficient determination *sensitivity* with good *precision* and satisfactory *reliability*. Desirable are with respect to the economy of the required extended analytical work, due to the necessarily in environmental surveillance required substantial numbers of samples, a reasonable rapidity and in context with the considerable variety of environmental chemicals a wide applicability. Furthermore, the dynamic range should be large covering a concentration range from typically several hundred mg/kg, i.e. ppm, to several µg/kg or ppb. For certain chemicals in certain matrix types even an extension of the determination potentialities down to ng/kg or 1o^{-3}ppb is indispensable, e.g. if their environmental base lines are to be established or particular problems are to be treated. With respect to field work, in mobile terrestrial laboratories or on ships and boats, *compactness* of the equipment is a point of practical significance. A very important aspect with respect to budgeting potentialities are, particularly for large scale routine applications in environmental surveillance, moderate investment and operation *costs* for the selected method.

In the context of these general considerations it has to be emphasized that generally the most severe and usually most difficult fundamental problem in trace analysis is *accuracy*. The attainable accuracy by the possible minimisation of systematic errors decides about the reliability of the determined data. There are a number of potential sources for systematic errors leading to corresponding accuracy-deficiencies intrinsic to the various stages of the whole trace analytical procedure, where usually only in the final stage, i.e. the actual determination, the instrumental methods are applied. Thus, it is obvious that procedures which apply instrumental methods with low tendencies for accuracy-deficiencies provide a substantial inherent advantage with respect to the still most critical and particular important aspect of reliability. It is also evident that an especially favourable situation arises, if in connection with a particularly accurate instrumental determination method prior sample pretreatment can be kept simple.

There is a variety of instrumental methods available todate which correspond more or less to the beforementioned basic requirements. Methods with determination capabilities in the lower trace range below 1oo µg/kg are listed in table 1. Among these methods many are restricted either just to the determination of elements or of chemical compounds. However, there exists only a limited number of instrumental methods which fulfills, in a rather comprehensive manner, the basic trace analytical requirements mentioned before. Among these particularly suitable methods the electrochemical approach, represented by advanced modes of *voltammetry*, has gained within recent years a prominent position.

TABLE 1. Characteristics and costs of determination methods for trace metal analysis

Method	Characteriscs of method	Costs 1o^3 $	Determination rate; Samples/day (real time/h)	Remarks
Differential pulse stripping voltammetry (DPSV) (for Ni differential pulse voltammetry after chelate adsorption, DPV)	Oligo-substance	1o	12-15 (8)	suitable for toxic metals; up to 5 metals simultaneously; species sensitive; also applicable to organics
Graphite furnace atomic absorption spectrometry (GFAAS); (for Hg cold vapor-AAS)	single-element	4o-5o	5o-6o (8)	1 metal per determination; determination rate based on 2 standard additions per determination; suitable for 4o metals
Atomic emission spectroscopy with inductive coupled plasma excitation (AES-ICP)	multi element	1oo-15o	1oo (8)	suitable for 6o elements; determ. limit 1-1o µg/kg
Neutron activation analysis (NAA)	multi element	5o-1oo	1o (24)	access to nuclear reactor and special lab required - no Pb - determin.
Spark source mass spectroscopy (SSMS)	multi element	25o	4 (8)	

VOLTAMMETRY

General trace analytical features of voltammetry

The applicability of voltammetry is wide and versatile, because it is essentially a *substance specific* and not just an element specific approach. Many types of *organic* chemicals (Ref.1) with ecotoxicological relevance as well as most of the *toxic metals* (Ref.2) can be therefore studied and determined.

Voltammetric and polarographic methods are based on the Faraday law, according to which 1 mole of substance undergoing an electrode process is equivalent to the enormous electric charge of n x 965oo C, where the number, n, of electrons transferred through the interface electrode/solution in the electrode reaction amounts usually to values of 1 to 4 and equals 2 for most toxic heavy metals. This physicochemical foundation provides excellent determination *sensitivity* combined with satisfactory *precision* and a high inherent *accuracy*. It is particularly this focal point of reliability besides the in many applications unbeaten determination sensitivity which has caused since about a decade the still considerably growing and extending application of voltammetry in environmental chemistry of inorganic and organic pollutants.

Furthermore, the voltammetric approach covers, with its various modes, the whole concentration range from the upper mg/l level to ng/l in the analyte. Robust, reliable and compact commercial instruments are available. As Table 1 shows the investment costs are among the lowest of instrumental methods and the operation costs are also very moderate. Rapidity is not particularly high, but this is frequently compensated to a significant extent by the remarkable reliability. The latter focal property provides a rather complete use of the determination capacity for actual analysis, in contrast to other apparently faster nonelectrochemical methods requiring frequent within-run -tests and a certain amount of test analysis by other independent methods to establish the necessary accuracy. Moreover, the presently going on introduction of partial and full automation into voltammetry will contribute significantly to the enhancement of rapidity.

A comprehensive treatment of the manifold application potentialities of voltammetry in trace analysis and trace chemistry of environmental chemicals will be beyond the scope of this paper. Therefore, these aspects shall be featured by a more detailed discussion of toxic metals, a group of environmental chemicals of particular ecotoxicological significance. They are a significant topic of the ecochemical research program of the author's institute and have been therefore chosen. It has to be emphasized in this context that in the field of organics the voltammetric approach is applicable to a large variety of further pollutants and hazardous substances. For a rather comprehensive description of this still vividly expanding field reference is made to two recent textbooks (Ref.3,4).

Basic methodological aspects

In voltammetry and polarography one records the current-potential relationship due to the electrode process of the studied substance. The adjusted entity is the potential, E, and the measured entity is the resulting current i. The substances to be determined have to be in the dissolved state in a usually aqueous analyte.

Differential pulse modes. With respect to the fact that mostly one has to deal in ecochemical applications with trace levels the voltammetric modes to be applied most frequently are: *differential pulse polarography* (DPP) at the dropping mercury electrode (DME); *differential pulse voltammetry* (DPV) at the hanging mercury drop electrode (HMDE), or solid electrodes, as the gold or carbon paste electrode; and *differential pulse stripping voltammetry* (DPSV) at the beforementioned stationary electrodes or, for certain heavy metals in the ultra trace range below o.5 µg/l at the mercury film electrode (MFE). Pulse polarography and pulse voltammetry are one of the most important achievements of the pioneering methodological work of Barker.(Ref.5). The differential pulse mode is now incorporated in every commercial polarograph and represents for analytical applications the most important function.

In the various versions of differential pulse voltammetry (Ref.6), the working electrode is polarized, not just by a dc-ramp, but by a sequence of rectangular potential pulses superimposed on a linear ramp potential (see fig.5). Only the current flowing during each pulse is recorded. Most frequently. the pulses have a height of 5o mV and a duration of 60 ms. In DPP at the DME one pulse per drop life is applied. At stationary electrodes in DPV and DPSV the clock time of the pulses is frequently o.5 s. In this manner the sensitivity can be significantly enhanced, because an efficient separation between the principally existing two components of the recorded current, i, can be achieved.

The recorded current, i, consists of the faradaic current, i_F, connected with the electron transfer in the electrode process of reduction or oxidation of the studied substance and the charging current, i_C, connected with the alteration of the charge of the interfacial double layer at the electrode when the potential changes. The interfacial double layer resembles, electrically, a condenser.

Thus, one has in principle (1) $i = i_F + i_c$.

As only i_F is proportional to the bulk concentration of the studied substance in the analyte the voltammetric determination sensitivity depends ultimately on the perfectness in satisfying the condition $i_c \ll i_F$ and consequently $i \approx i_F$. This can be very elegantly and efficiently achieved, if pulsed polarization is applied and only the total current i flowing during the pulse is recorded, because then use can be made of the different time laws for i_F and i_c (viz.fig.1).

(2) $i_F \sim t^{-b}$ with $b \leq 0.5$ (3) $i_c \sim \exp -\frac{t}{RC}$

While i_F decreases within the pulse duration for diffusion control of the electrode process according to a square root law with b = o.5, the charging current, i_c, decays much more rapidly according to an exponential time function. The time constant RC is the product of the ohmic resistance of the medium and the double layer capacity. If i is recorded in the final interval of the pulse (fig.1), i_c will become negligible and $i \approx i_F$.

This feature has made voltammetry in the differential pulse mode to one of the most sensitive instrumental methods in trace analysis.

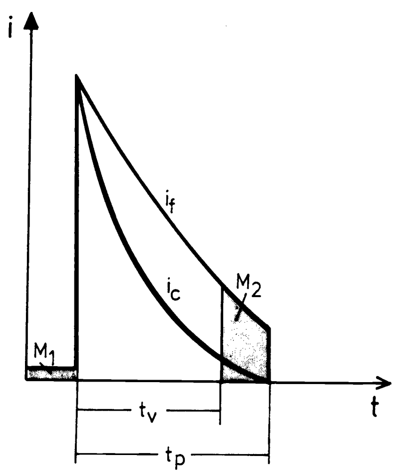

Fig. 1. Time function of faradaic current, i_F, and charging current, i_c during rectangular voltage pulse. t_p pulse duration; t_v delay time until measurement of total current i; M_1, M_2 measuring intervals of i.

TOXIC METALS

General ecochemical and ecotoxicological aspects

Among the hazardous environmental chemicals certain heavy metals and some metalloids have gained, due to their toxicity (Ref.7,8), particular significance and priority (Ref.2). In the primary focus are the a priori toxic heavy metals Cd, Pb and Hg. But also a number of others, e.g. Cu, Zn, Ni, Cr(VI) etc., which have at low concentrations essential functions for living systems, exert toxicity above respective threshold levels, depending among other parameters, on the type of the exposed organism. Furthermore, the metalloid As, particularly as As(III), and above a threshold level limiting essential functions Se belong to the group of toxic or potentially toxic metals. The beforementioned metals constitute a category of environmental chemicals with rather special common basic properties of ecological impact. They are either a priori or above their respective threshold levels insidious poisons, particularly for man and mammalians, although they exert generally toxic effects at sufficient concentrations on all types of organisms and can therefore perturb ecosystems. One of the particular ecochemical properties of metals is that a considerable amount is continuously emitted from natural sources into the terrestrial and marine environment. Toxic metals occur, therefore, at certain base line levels ubiquitously. However, as consequence of a growing emission from anthropogenic sources (fossil fuel burning, metal ore mining, processing and usage of metals) the general abundance of these toxic metals in most parts of the world exceeds already the natural base lines and in certain regions and locations the toxic metal pollution reaches intolerable and hazardous values. A further special feature of toxic metals is, that they are nearly the only group of environmental chemicals which are not biodegradable. Instead they undergo a biogeochemical cycle with substantially different residence times in the various spheres and compartments of the environment (fig. 2). Within this cycle they will be taken up also by man, predominantly with food and drinking water. In this respect toxic metals constitute a particular risk, because, although a certain fraction of the ingested amount is again excreted, they have a pronounced tendency to accumulate in vital organs of man. Thus, they will exert progressively growing toxic actions over long periods of the life span depending also significantly in its integrated magnitude on the total dose accumulated as function of the long term exposure of the respective individual from the environment.

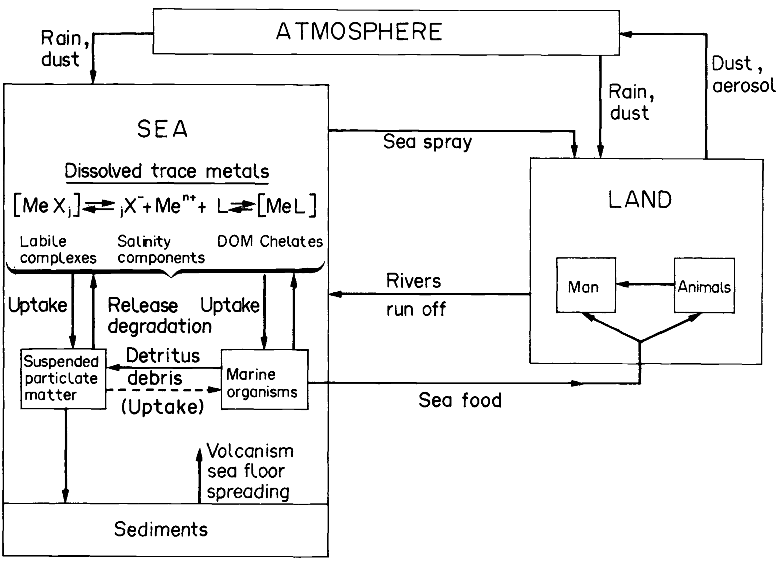

Fig. 2. Biogeochemical cycle of heavy metals

General aspects of voltammetric trace analysis of toxic metals

It just so happens, that nearly all of the metals of particular ecotoxicological significance are well determinable by voltammetry (Ref.2,9) although it has to be stated, that for some of them the voltammetric determination potentialities can and have to be still further developed. This applies to As(III), Cr(VI) and partially Se, while for Cd, Pb, Hg, Cu, Zn and Ni all requirements can be now satisfied.

Reliable analysis of toxic heavy metals has to overcome special problems with respect to the pronounced risks of accuracy deficiencies, due to analytical contamination and possibilities of metal losses either by volatisation during ashing of biological materials or by adsorption at container walls from aqueous samples. Especially problematic is at ultratrace levels the avoidance of artefacts caused by contamination for the ubiquitously abundant metals Pb and Zn.

In this context one has to visualize the total analytical procedure. It consists of three major stages, i.e. *sampling*, *sample digestion* and/or *pretreatment* and *determination* of the metals in the resulting analyte.

Frequently, severe systematic errors occur, perhaps during sampling and spoil the accuracy. Then, even if in the subsequent stages systematic errors can be kept low, the paradox situation arises that meaningless or dubious values are determined with possibly high precision. Reliable sampling requires therefore great care and precautions to avoid intolerable contamination. Contamination can be also a rather critical in the subsequent stages of digestion or other kinds of sample preparation and the determination itself. These problems can be overcome, however, by good laboratory practice scrupulously performed, according to appropriate trace analytical standards.

Avoidance of accuracy deficiencies. Some approved basic rules are as follows. In every stage of the whole analytical procedure exclusively labware scrupulously cleaned according to approved techniques (Ref.1o) is to be used. It should be noted that not for every trace metal the same labware material is not suitable. Thus, for Cu, Pb, Cd, Zn polyethylene flasks are optimal and at least at the ultra trace level (<1 µg/l) voltammetric cells from teflon should be used while Hg requires quartz or glass. The pH of the solution or of aqueous samples, particularly if they have to be stored longer, is an important parameter. To avoid losses by adsorption at container walls or during storage by microbiological metabolisms the milieu should be acidic (pH 2). Of course, necessary separation steps, e.g. filtering off suspended matter from natural water or waste water samples, have to be performed before acidification and therefore within several hours after sampling. All manipulations with the sample and the resulting analyte should be performed either in closed systems, e.g. filtration, or under clean benches with filtered counter current laminar air flow to avoid contamination by dust particles. Trace analytical reagents of Merck, suprapur- grade, with extremely low heavy metal blanks have to be used, also in the final stage of the labware cleaning. Water required for standard solutions and for dilution can be at the ultratrace level a source of problems. The lowest and always satisfactory heavy metal blanks (Cd o.1 ng/l; Pb o.8 ng/l; Cu $<$ 1o ng/l) are provided by deionized water prepared by ion exchange in the Milli-Q-system from Millipore Corp. It is even superior to quartz distilled water. Ashing or digestion procedures tested for their metal yield have to be used, if this preparation step is required.

There remains, of course, possibly still the risk of systematic errors inherent to a certain determination method. In this respect voltammetry is in a particularly favourable position. As a rule accuracy deficiencies in the voltammetric determination stage are negligible, which reduces this problem to the prior stages of pretreatment and sampling. These stages prior to the determination are, however, in most cases a common necessity in connection with any instrumental determination method. On the other hand it has to be noted if one analyses biological material, that voltammetry is demanding concerning digestion, as it requires complete mineralisazion. Therefore, frequently an aftertreatment of the raw analyte by UV-irradiation to achieve photolytic decomposition of dissolved organic matter is necessary. With respect to complete digestion the various modes of atomic absorption spectrophotometry (AAS) certainly have the advantage to be less demanding. Despite the larger digestion effort voltammetry with its superior reliability remains, however, for biomatrices always an important alternative (Ref.9) to the in the past unjustifiably the trace analysis of heavy metals from biomatrices monopolizing AAS.

Analytical procedure for water samples

The trace analysis of toxic heavy metals in all kinds of aqueous environmental samples (fresh water, sea water, rain, drinking water etc.) and certain types of liquid food requires no major operations in sample preparation as digestion. Then the superior potentialities of voltammetry can be fully displayed and this has made, meanwhile, voltammetry definitely the method of first choice for all types of aquatic samples (Ref.2,11-14). An exception might be Hg. For this toxic metal advanced cold vapor AAS and voltammetry provide comparable determination potentialities concerning sensitivity, accuracy and convenience (Ref.15).

The flow chart in fig.3 summarizes the analysis stages for all types of natural water.

Sampling. The respective sampling procedure depends on the natural water type. Usually sample volumes between o.5 and 2 litres are taken.

From the *surface* zone in the sea as well as in rivers and lakes, samples are collected manually with polyethylene flasks fitted to a telescopic fiber gear (length up to 3.5 m). Very important is the avoidance of contamination by the ship which is always surrounded by a plume of contaminated water due to the release of heavy metals from the antifouling paint, exhaustion of the engine and other local pollution emitters. Therefore, surface water samples have to be collected from small boats (preferentially rubber boats) at a safe distance (o.5 to 1 km) from the research vessel. The actual sampling is carried out in front of the bow of the rubber boat while it moves against the wind to prevent contamination of the sampled water by the boat. Of course, the precautions against contamination are most stringent for oceanic surface water as here the lowest heavy metal concentrations of the aquatic environment in the lower ng/kg range occur, while in coastal waters, estuaries, rivers and lakes the toxic trace metal levels are usually already higher (Ref.16,17).

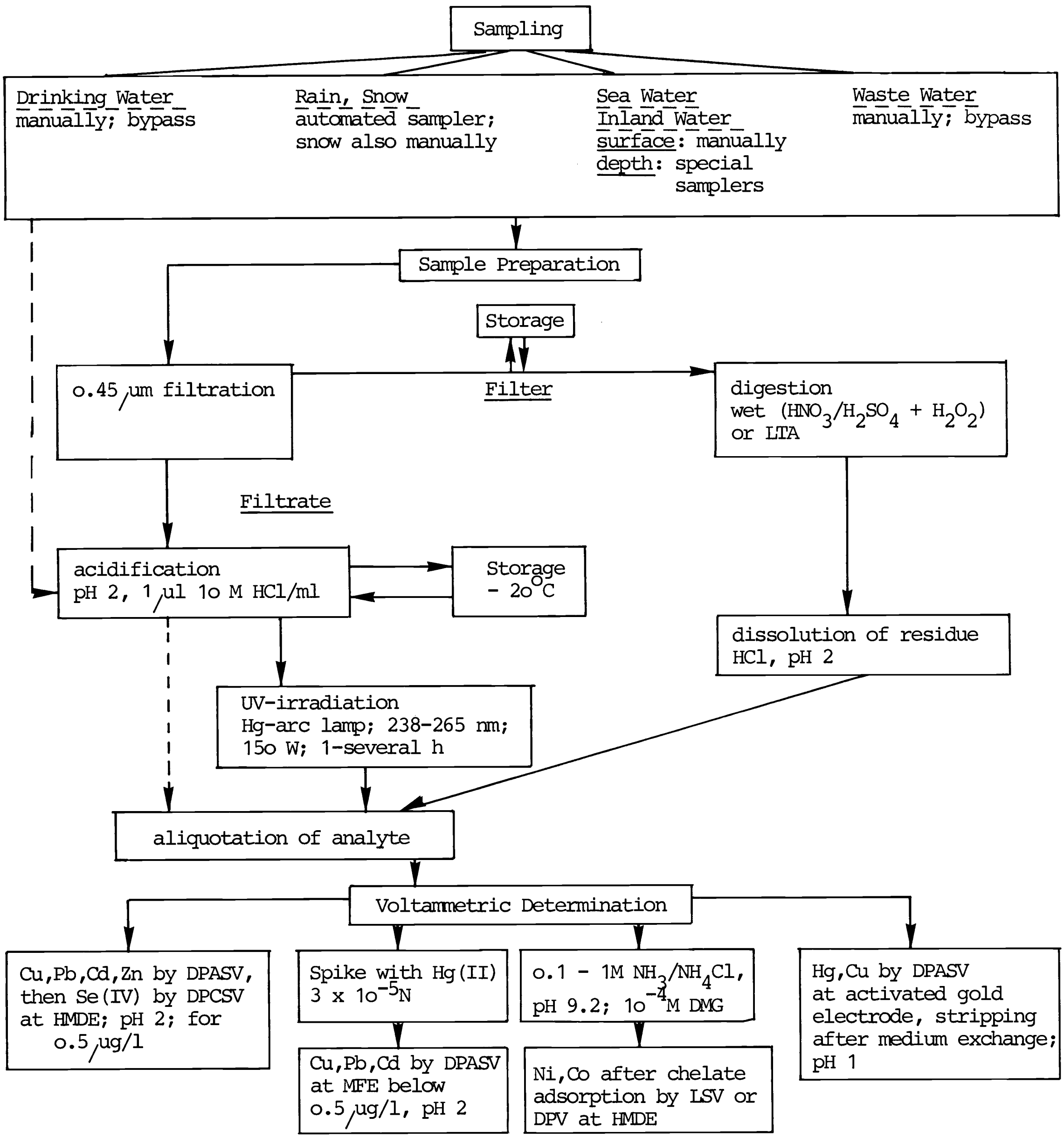

Fig. 3. Analytical procedure for various water types

The collection of samples from *deeper* waters, to obtain the vertical profiles of the trace metal levels, requires for contamination-exclusion substantial technical efforts and very special samplers. Only with their introduction during the last five years meaningful data on heavy metals, particularly for such a difficult case as Pb, have been obtained for oceanic deep waters. Problems to be overcome are primarily connected with the fact that deep water samplers have to be always based on the research vessel itself. One can use, particularly for intermediate depths (<1oo m), the new version of Go-Flo-samplers fabricated completely from plastic material. A satisfactory performance can be obtained with a new special sampler based on a concept (CIT-sampler) originating from Schaule and Patterson (Ref.18). With this new Juel-sampler (Ref. 17), termed "Moonlander", contamination free water samples can be taken in one haul, from 1oo m downwards, at three predetermined depths. The beforementioned types of deep water samplers are all lowered from the ship with a hydrowire.

An interesting alternative is a free fall sampler also developed by us (Ref. 19). It is mainly used to collect water samples 1 to 2 m above the sea bottom. By contact with the ground the closing of the water sampling bottle is triggered before the plume of upwhirled sand and mud reaches the bottle, a clamp grabs eventually available sediment specimens, and weights are released. This enables the sampler, which contains a lift body, to return by its buoyancy back to the surface where it is collected.

The collection of *atmospheric precipitates*, rain and snow, is hitherto widely carried out by, from trace analytical aspects, completely inadequate devices. Reliable sampling of rain and snow is, however, achieved with an automated sampler (Ref.2o). As its operation is controlled by a humidity sensor, it opens only during the rain or snow fall and closes after its termination. In this manner a reliable discrimination from dry dust deposition is ensured.

Municipal waste water samples are taken manually into precleaned polyethylene flasks with o.5 l volume.

It is obvious, that all sampling flasks have to be subjected before use to scrupulous *cleaning procedures* (Ref.1o,21). These have to be followed by *conditioning* with clean sea water or other pure aqueous solutions containing appropriate concentrations of Ca and Mg. These divalent alkaline earth ions occupy the adsorption sites for metals at the walls and in this manner losses of heavy metal traces by wall adsorption are prohibited. Also the membrane filters are to be purified and conditioned in this way.

Sample pretreatment. The first stage of sample pretreatment consists usually in the separation from suspended particulate matter by *filtration* through o.45 µm pore size membrane filters. The filtration is to be performed in a close device under slight N_2-pressure (1 bar) to exclude contamination by dust from the ambient air (Ref.1o). The heavy metal traces in the filtrate are regarded as being in the dissolved state. This is an operational convention, although a rather relevant one, because almost all the suspended inorganic and organic particles are retained on the filter. This is not the case for certain types of colloids. Yet the use of filters with substantially smaller pore sizes has as a rule not turned out recommendable, because then the filtration time will increase substantially, particularly if higher amounts of suspended matter are present in the sample. This creates the risk that ion exchange processes during filtration become significant and would introduce artefacts in the metal distribution between the dissolved and suspended material phase.

In the treatment of the *filtrate* the next step is the *acidification* to pH 2 with a small volume of ultrapure HCl. An aliquot for Hg-determinations should be transferred before into quartz or glass flasks. By the acidification the filtrate is stabilized in several aspects (Ref.1o,22). The tendency for adsorption at container walls becomes, even over longer times, negligible. Any further possible microbiological activities are inhibited. According to the analytical facilities during field missions, if necessary, the sample filtrates can be now safely stored, for longer times, preferably at -2o °C.

By acidification, also from a number of nonlabile complex species, the heavy metals are released and transformed into labile species, e.g. as chlorocomplexes or free hydrated cations, species which undergo readily a reversible electrode process (Ref.21). Nevertheless, in a number of natural water types, frequently already in coastal waters, but almost usually in estuarine, river and lake waters and certainly in waste water, the water contains a certain amount of dissolved organic matter (DOM) of which a fraction has the capability to bind heavy metals in a nonlabile manner. This heavy metal amount would be not or incompletely accessible to voltammetric determination. Therefore, the corresponding nonlabile species have to be decomposed.

UV-irradiation with a 15o W mercury arc lamp has turned out to be a completely efficient and optimal method for this purpose (Ref.17,23-25). The nonlabile species are decomposed by photolysis. To support and speed up this process a small amount of H_2O_2 is added. In this manner, even in the filtrate of municipal waste water, the contained nonlabile organic heavy metal species are decomposed completely within several hours irradiation time (Ref.14,59). Water samples, relatively low in DOM-levels, as sea water require smaller irradiation durations up to 1 h typically. If oceanic and coastal water samples have to be subjected to UV-irradiation no H_2O_2 is added to avoid contamination risks with respect to the very low heavy metal levels in these water types (Ref.23,24). In this context it is to be emphasized, that the UV-irradiation, carried out with a mercury arc lamp, placed outside the sample container, transmitting the light through a quartz cover onto the water sample, provides the necessary sample pretreatment without any enhancement of the contamination risk. Special precautions with respect to the dense closure of the flasks are taken for the subsamples in which subsequently Hg is to be determined (Ref.25).

Treatment of filtered off suspended matter. The filters can be *stored* in pre-cleaned polyethylene boxes which are put preferably into a refrigerator. The filters with the suspended particulate matter have to be subjected to *digestion*. In the study of natural waters, with somewhat higher heavy metal levels an appropriately performed wet digestion will always yield satisfactory results. But in samples from sea water, digestion by low temperature ashing (LTA) in an oxygen plasma is the safer route (Ref.12,13,21). In this context it is to be emphasized, that one is interested in the metal content of organic particles and the metal amount in the organic films on the surface of inorganic particles (clay, silica etc.) as well as in the heavy metals bound to the actual inorganic surface of inorganic particles. Although these inorganic particles are not ashed by LTA, the heavy metals at their surface will be anyway leached off during the dissolution stage of the digestion residue. The heavy metals transformed into dissolved species by this somewhat selective procedures are those which are possibly involved in the interactions with the dissolved state or which can be leached off, at least, to a certain extent by filter feeders, e.g. mussels, oysters, and may thus become of certain relevance for the marine food chain. Hg, Se, As always require pressurized wet digestion (viz.fig.14).
The last pretreatment step is the *aliquotation* of the obtained analyte, because only certain groupings of heavy metal traces can be determined simultaneously in one run by an appropriate voltammetric mode and at a suitable working electrode as indicated in fig.3.

Simplifications. The described analytical procedure for toxic trace metals in natural waters and waste water can be significantly simplified for certain water types. Very often waters from the open ocean will not even require filtration nor any other pretreatment except acidification and aliquotation (Ref.75). The reason is that in vast parts of the oceans the amount of suspended particulate matter is almost negligible in contrast to most coastal areas and estuaries. Moreover, major parts of the open oceans, except upwelling areas and some others, are essentially marine deserts, e.g. the central gyres, and have a very low biological productivity (Ref. 26) which is anyway consisting predominantly in the respective abundance of phytoplankton. Therefore, the DOM-levels will be very small (Ref. 27) at low phytoplankton abundance.

Rain water and snow also require usually only filtration, acidification and aliquotation. However, there are rain water samples from regions with rather substantial air pollution by organics which demand prior UV-treatment. Frequently, as with oceanic water also in drinking water control no pretreatment except acidification and aliquotation is usually required. In general it is to be remarked already here, that in the investigation of biological material after the matrix type adapted digestion the remaining relevant pretreatment steps for the resulting aqueous analyte and the subsequently discussed voltammetric determination are similar to the analysis of waters.

Voltammetric determination stage. At first always the analyte is deaerated with 99.999 % N_2 for 1o to 2o min. In routine work simultaneously several cells are operated. According to a working scheme one measures in one cell while other ones are deaerated and further cells are rinsed (Ref.24). In the future full automation with microprocessor control will even permit simultaneous measurements in several cells. Usually the heavy metal traces occur at concentration levels in natural waters, including the content in suspended particles, which make preconcentration mandatory before voltammetric determination becomes possible. A particular advantage of the voltammetric approach is that this preconcentration can be performed without any additional contamination risk, because the preconcentration is carried out electrochemically in situ during the first stage of the voltammetric determination.

For most heavy metals a voltammetric approach, termed inverse voltammetry or stripping voltammetry, is applied and the interfacial accumulation is achieved electrolytically, e.g. for Cu, Pb, Cd, Zn by cathodic deposition at stationary mercury working electrodes (HMDE, MFE) and for Hg and Cu at the gold electrode. At the mercury electrodes the mentioned metals are deposited as amalgams. The concentration of the to be determined metals are considerably increased by accumulation in the interface electrode/solution, i.e. at the location where the electrode reaction is actually going on (Ref.9,28). To speed up mass transfer from the bulk of the solution, the solution is stirred with a magnetic bar, when the HMDE is used, while the MFE or gold electrode cause convective mass transfer by rotation. As in the recording stage of the voltammogram the principle of pulse voltammetry, being as outlined before, inherently highly sensitive, is applied, the cathodic deposition times for preconcentration can be kept relatively small, between 3 and about 1o min. according to the heavy metal concentration range in the analyte solution µg/l and ng/l. These small deposition times contribute significantly to the economy of the overall time for the voltammetric determination. A further advantage of small deposition times is, that intermetallic compound formation reactions between the metal amalgams and the resulting interferences remain regligible. After the preconcentration time, t_d, at the deposition potential, E_d, the rotation or stirring is stopped and after a quiescent interval of 3o s the recording of the voltammogram is carried out in the differential pulse mode. For this purpose the electrode potential is scanned in the anodic direction and at their respective redoxpotential values the deposited heavy metals are again reoxidized to their ionic state and redissoluted. Therefore one speaks of *inverse* or *stripping voltammetry*, in the considered case of *differential pulse anodic stripping voltammetry* (DPASV). The principle is depicted in fig.4. As the heavy metals have different redox potentials several metals can be determined simultaneously in one run. The height of the peaks in the obtained voltammogram (fig.5) is proportional to the concentration of the respective heavy metal. The evaluation of the concentration is performed by standard additions of which usually two are sufficient (Ref.21, 24,29).

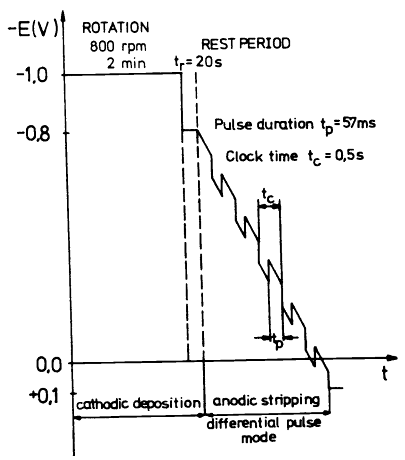

Fig. 4. Principle of differential pulse anodic stripping voltammetry (DPASV).

The frequently applied *working electrode* is the commercially available HMDE. It permits readily, even at pH 2, the simultaneous determination of Cu, Pb, Cd and Zn. In addition Se(IV) can be determined subsequently in the same run (Ref. 2o,3o). In this case after the anodic stripping of the other metals Se(IV) is preconcentrated at the interface by formation of a HgSe film applying, under stirring, a deposition potential of -o.2 V for 3 min. Then *differential pulse cathodic stripping (DPCSV)* is performed by scanning the potential to more negative values in the differential pulse mode.

While the HMDE is suitable for the trace metal levels in many water types, sea water has usually very low heavy metal concentrations below 5oo ng/l amounting, in unpolluted sea water, for certain heavy metals as Pb and particularly Cd even to only several ng/l. Then the higher preconcentration values providing MFE is to be applied (Ref. 21,23,24). It is formed in situ during the cathodic preconcentration step by depositing a mercury film on a specially polished glassy carbon support. Therefore the analyte has to be spiked before with $Hg(NO_3)$ adjusting a Hg^{2+}-concentration of about 3 x $1o^{-5}$M in the solution. A thin mercury film with a thickness of 2oo to 1ooo Å forms in which at the

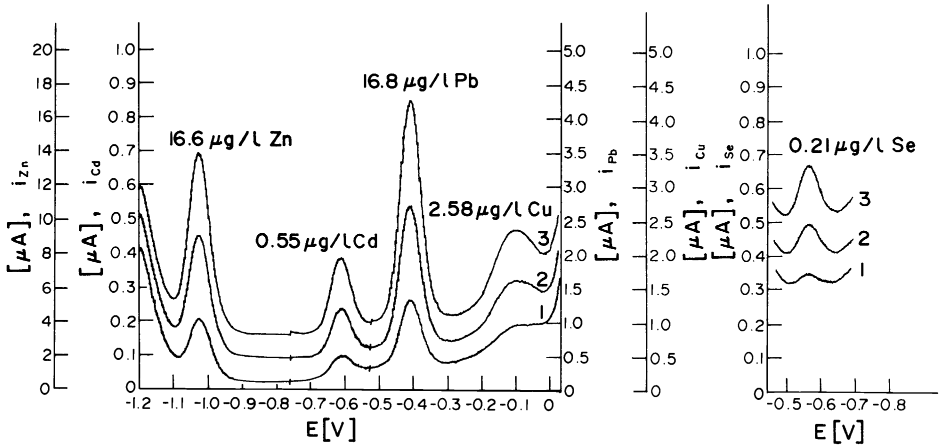

Fig. 5. Voltammogram of simultaneous DPASV-determination of Zn, Cd, Pb, Cu in rain water; E_d −1.2 V; t_d 3 min and subsequent DPCSV-determination of Se(IV) E_d −o,2 V; t_d 5 min. 1 sample, 2 and 3 first and second standard addition

same time the heavy metals are deposited as amalgams. The determination of Zn at the MFE requires a higher pH of 4.5, to be adjusted e.g. with acetate buffer. Otherwise the hydrogen discharge will interfere with the Zn-deposition at the MFE. The glassy carbon electrode covered by the mercury film behaves practically similar to a genuine mercury electrode, although in reality the smooth appearing film consists microscopically of a dense layer of Hg-microdroplets of similar average diameter. After the last standard addition the Hg-film is wiped off, the glassy carbon surface is rinsed and is then ready for the analysis of a new sample. The construction and polishing of a reliable and suitable glassy carbon electrode as support for a satisfactory in-situ formed MFE is a delicate procedure outlined in detail elsewhere (Ref.23,24). If, however, one follows precisely this procedure, a MFE with very good performance is obtained. The glassy carbon support can be used without repolishing for a long time, typically over 1 year, provided the glassy carbon surface is kept intact. Therefore, if not in use, the electrode has to be stored with the glassy carbon surface immersed into mercury. For the optimalisation of the pulse parameters and the pulse clock time in applications of the MFE, ref. 31 should be consulted.

A particular valuable practical advantage during oceanic expeditions is the insensibility of the MFE against sea motion and vibrations originating from the ship engines. While frequently purified glass or quartz cells suffice, for the determination of the low Cd and Pb concentrations in relatively unpolluted sea water, cells from teflon are preferable (Ref. 24)

Obviously the determination of Hg-traces requires a different working electrode. A very good performance is provided by a common gold disc electrode down to about 5o ng Hg/l (Ref.25). At the same time the electrode is very suitable for the simultaneous determination of Cu. Higher levels of Cu can produce as consequence of the limited solubility of Cu-amalgam problems at the MFE. Then the use of the gold electrode is preferable. After the preconcentration, to avoid damage of the gold surface by oxidation by excess chloride, medium exchange to o.1 M $HClO_4$ plus 2.5 x $1o^{-3}$M HCl is necessary before stripping. For details including necessary activation of the gold surface before each measurement, Ref. 25 is to be consulted.

For ultra trace levels of Hg, below 3o to 5o ng/l, as they occur in sea water without Hg-pollution, the voltammetric determination becomes much more complicated. It can be still performed down to 1 ng/l if *subtractive* differential pulse anodic stripping voltammetry (SDPAV) is applied at a special twin gold electrode (Ref.32). It consists of two halves of a splitted gold disc seperated by an insulator layer. The application of this mode demands special expertise. Therefore, in routine ultra trace determinations of Hg the application of cold vapor AAS is more convenient (Ref.15).

The *determination limits and precision* of the discussed differential pulse stripping methods are summarized in Table 2. In this context it should be noted, that until a few years ago mostly simple stripping voltammetry with conventional linear potential scan in anodic direction (ASV), instead of

DPASV, has been applied. This is now as a rule anachronistic and sacrifices determination sensitivity, precision and analysis time.

TABLE 2. Determination limits and precision of differential pulse voltammetric methods in natural waters and aqueous analytes

Method	Determination limits at RSD ± 20 % (µg/l)					Concentrations determinable with RSD ≤ 10 % (µg/l)				
	Cd	Pb	Cu	Ni	Hg	Cd	Pb	Cu	Ni	Hg
DPASV/HMDE	0.01	0.01	0.05	-	-	0.1	0.3	1.0	-	-
DPASV/gold	-	-	0.02	-	0.02	-	-	0.1	-	0.2
DPASV/MFE	0.0003	0.001	0.007	-	-	0.0015	0.0015	0.05	-	-
SDPASV/twin gold	-	-	0.001	-	0.001	-	-	-	-	-
DPV/HMDE after chelate adsorption	-	-	-	0.001	-	-	-	-	0.02	-

There are heavy metals of particular environmental interest, as Ni and Co, which are not able to form amalgams. For their preconcentration at the electrode interface a different approach has to be applied (Ref.33) using adsorption of a suitable heavy metal chelate at the surface of the HMDE. For Ni and Co dimethylglyoxime (DMG) is used as chelator. Therefore, after aliquotation (viz.Fig.3) pH (9.2) is adjusted with ammonia buffer and the analyte is spiked with DMG to obtain a DMG-concentration of about 10^{-4}M. Then a potential of -0.74 V, close to the electrocapillary zero value which is most favourable for adsorption, is applied for an adsorption time t_a of 2 min. In the lower ng/l range t_a is extended to 5 or even 10 min. Subsequently the adsorbed $Ni(DMG)_2$ and $Co(DMG)_2$ are reduced by scanning with the potential into negative direction. The peaks for Ni and Co appear at -0.9 V and -1.15 V, respectively. Their height is proportional to the metal concentration in the analyte via the adsorption equilibrium as long as one works sufficiently below full coverage, i.e. in the quasi-linear part of the adsorption isotherm. Above 1 µg/l simple linear scan voltammetry suffices while down to 1 ng/l DPV has to be applied. This very efficient and versatile alternative to stripping voltammetry can be extended to many other substances in voltammetric trace analysis. An example is a very recently developed procedure (Ref.34) for the trace determination of dissolved silica in natural waters and boiler water via its previously formed and then adsorbed silicomolybdate complex.

Analysis of water samples according to the respectively relevant version of the outlined procedure, depicted as flow chart in fig.3, will yield reliable data on the level of toxic metals in the dissolved state and in suspended particulate matter. It should be noted that also the metal content of suspended particulate matter is usually reported as volume concentration per l or kg natural water and not as concentration in the various particulate matrices. In this manner one obtains also immediately the total trace metal concentration in a given natural water in µg/l or µg/kg water by adding to the dissolved value the value obtained for the filtered off suspended particulate matter expressed as volume concentration. The different trace metals have different affinities to the dissolved or the particulate matter phases. As a rule one observes the following sequence for the preference of the particulate phase in natural waters: Pb $\gg$ Cu $>$ Zn, Cd.

Heavy metal levels in natural waters

The systematic application of voltammetry in all branches of aquatic trace metal chemistry has yielded in recent years already a substantial amount of reliable data and extended greatly the knowledge on the levels, fate and behaviour of important toxic metals in the aquatic environment. The subsequent, necessarily rather sporadic, survey on a variety of studies intends to give an impression on our achievements.

Chemical oceanography and limnology. This is a very important research field for which the voltammetric approach, due to its inherent properties and potentialities, has opened new dimensions in various aspects (Ref.11-13). Among them have particular significance the elucidation of the toxic trace metal *distribution* in the oceans, coastal waters, estuaries and in inland waters and in a later section, discussion of *speciation* of dissolved heavy metals in different water types. The remarkable accuracy even at the ultra trace levels encountered in oceanic waters is well featured by Table 3, particularly by the data for the most critical metal Pb (Ref.24). Completed are those studies on aquatic ecosystems by the investigation of typical organisms (plankton, fish, molluscs and crustaceans) applying voltammetry as well as suitable AAS-modes (Ref.65).

TABLE 3. Accuracy test by interlaboratory comparison with different independent trace analytical procedures

Laboratory	Method	Cd	Pb µg/l	Cu
C.C. Patterson, Caltech, Pasadena, Calif.	CH_3Cl-dithizone extraction - isotope dilution mass spectroscopy	-	3.3 ± o.2	-
K.Bruland, Santa Cruz, Univ. Calif.	APDC-DDDC extraction - GFAAS	o.1o5	-	1o1
L.Mart, this laboratory, Juelich	pH 2 - DPASV/MFE	o.1o1	2.9 ± o.2	11o

Sea water sample taken 12o km off California coast from 1ooo m depth with CIT sampler

Within the biogeochemical cycle the sea has the important function to act as a pseudo-sink with a continuous input by rivers, continental run off, and via the atmosphere by wet and dry deposition. Particularly vulnerable to toxic metal pollution are the coastal zones and estuaries with frequently relatively shallow waters. They are, however, the areas from which a substantial part of marine food originates and where a future improved sea food production by large scale aqua culture and fish farming is to take place. In the more shallow coastal waters and in rivers the sediments act as the reservoir of toxic metals. The important function of the dissolved state is that it is the state of exchange, uptake and release with respect to suspended particulates and organisms. In the open oceans the sediments are usually only of significance for the bottom water which comes, however, also to the surface in upwelling zones.

Extended investigations on the *oceanic distribution* of some toxic metals (viz.Tab. 4) have recently established that the distribution pattern shows, for various geographical areas, significant differences for the surface water and also the deeper waters (Ref.17,35,36,75). One concludes also that hitherto the major toxic metal input into the open oceans occurs from the atmosphere while there is no indication for significant spreading of the coastal water and estuarine pollution, particularly caused by fluvial input, into the core of oceanic waters. The significantly higher levels of Cu, Pb and Cd in the North Atlantic, compared with the Pacific, correspond to global trends in atmospheric pollution transport. The westerlies prevailing in the Pacific transport from less industrialized Asia smaller metal freights while into the North Atlantic parts of the substantial emissions from most heavy industrialized regions in Europe and North America are brought. Along the water column the nutrient-like metals Cd, Cu and Ni show well defined correlations with the depth profiles of the nutrients, nitrate and phosphate, and also with dissolved silica (see fig.6). Typically the metal and nutrient levels are low in the surface zone, due to metal uptake and nutrient consumption by phytoplankton. As its growth is mainly confined to the upper part of the euphotic zone, metal and nutrient levels increase with depth in parallel. Although this type of pattern is quite general, it shows in quantity significant differences in different parts of the oceans. In contrast to the nutrient-like heavy metals, Pb has a completely different behaviour and decreases to quite small levels down to about 1ooo m. Special oceanographic conditions can lead to very low heavy metal levels in surface waters. A striking example are the hitherto found lowest Cd- and Pb-values in oceanic surface waters from the Aitutaki Passage (Cook Islands, South Pacific). A typical example for the input of upwelling waters richer in metals, except Pb, and nutrients reflect the data from the northern Weddell Sea (see table 4).

Examples for extended investigations in *coastal waters* show fig.7 and Table 5. The profiles in fig.7 reflect the dissolved Pb- and Cd-content in Ligurian and Tyrrhenian shore water (Ref. 23,37,38). They are based on 225 stations.

TABLE 4. Ranges (R) and average values ($\bar{x}$) of heavy metal concentrations in various parts of the oceans

Area	Depth (m)		Cd	Pb	Cu (ng/l = 10^{-3} µg/l)	Ni	Co
North Atlantic 40°-20°N; 20°-70°W	0.5	R	3-10	40-60	70-150	-	-
	500-1500	R	20-35	-	70-200	-	-
Western Mediterranean 40°-37°N; 0°-10°E	0.5	$\bar{x}$	19	62	140	-	-
	500-3000	$\bar{x}$	13	46	100	-	-
Cariboic	0.5	$\bar{x}$	3	16	94	-	-
Pacific 10°N-10°S; 130°-125°W	0.5	R	2-5	5-15	60-90	-	-
	4000-5000	R	40-90	-	160-300	330-530	20
Aitutaki Passage, Cook Islands, 20°S 158°N	0.5	R	0.7-2	4-5	40-60	-	-
Australian shelf 28°-33°S; 152°-153°E	0.5	R	3-6	8-20	60-100	-	-
Peru Basin 5°-10°S; 90°W	0.5	$\bar{x}$	7	10	95	230	10
	4000-5000	$\bar{x}$	80	3	265	620	20
Arctic Ocean 78°-82°N; 10°W-40°E	0.5	R	5-18	4-26	50-185	80-130	-
	400-1400	$\bar{x}$	21	4	180	220	-
Northern Weddell Sea 62°-67°S; 44°-53°W	0.5	R	17-54	3-13	100-200	-	-

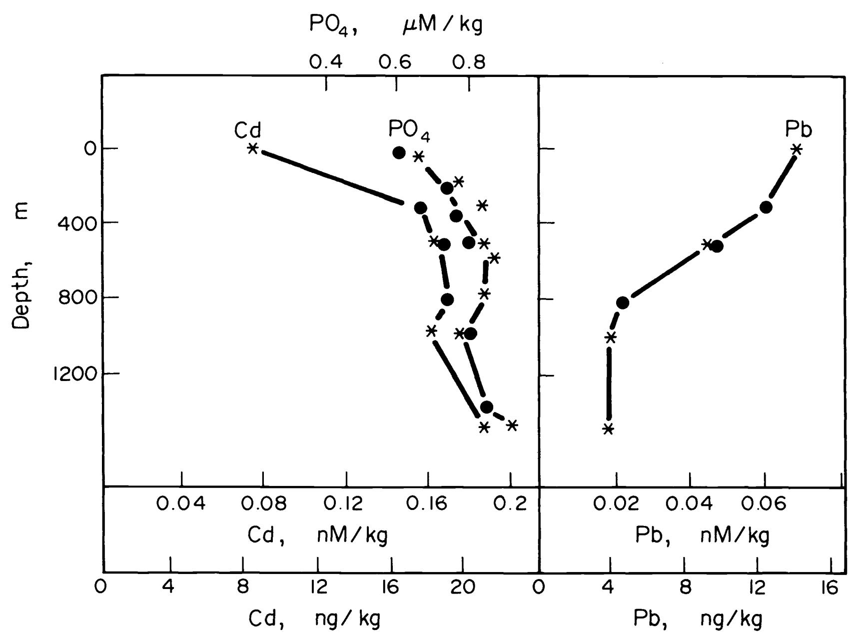

Fig. 6. Depth profiles for Cd and Pb and correlation of the nutrient like metal Cd with phosphate at two stations in Arctic Ocean from "Ymer-80" expedition.
81°43'N; 8°51'W ✱ and 81°41'N; 9°05'W ●

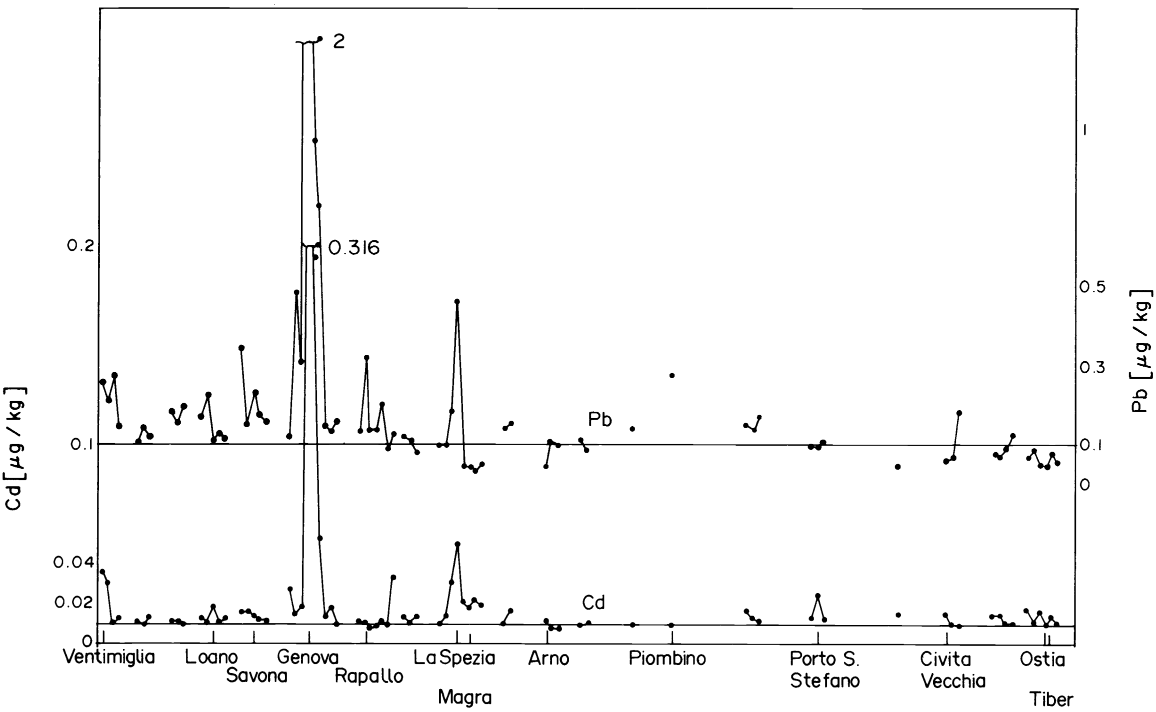

Fig. 7. Dissolved levels of Pb and Cd in Ligurian and Tyrrhenian shore waters sampled at o.5 m within 3 km from the shore at 225 stations in summer 1976. Each point corresponds to the average values from sampling stations within 4 km long intervals along the coast line.

TABLE 5. Heavy metal trace levels in Mediterranean and North Sea coastal waters. (Ranges or average values in the dissolved state (d) and at suspended particulate matter (sp))

Area	Cd		Pb		Hg	
	(ng/l = 1o^{-3}/ug/l)					
Ligurian and Tyrrhenian coast	5 - 3o 1o	(d) (sp)	5o - 3oo 5 - 15o	(d) (sp)	-	
Belgian and Dutch coast, southern North Sea Bight	15 - 1oo 8 - 5o	(d) (sp)	5o - 35o 25o-35oo	(d) (sp)	-	
German Bight	15 - 3o 15 - 3o	(d) (sp)	53 1oo- 4oo	(d) (sp)	6 - 3o	(d)
Elbe Estuary	33 17o	(d) (sp)	112 43oo	(d) (sp)	65	(d)
Wadden Sea, German coast, behind Frisian Islands	17 3o	(d) (sp)	14o 1ooo	(d) (sp)	-	

Repetition of 1oo stations two years later yielded about the same levels and indicated that the situation for both toxic metals remains rather constant in this coastal zone. The various enhancements usually reflect local anthropogenic pollution. This is particularly pronounced for the larger port entrances in Genova and La Spezia with relatively busy ship traffic. But this pollution remains, on the other hand, rather localized to the area immediately before and around those ports. Interesting is the extended elevation of the Pb-level in the region of Piombino which is to be attributed to Pb-input from natural geological sources. Similar investigations have been carried out along the Belgian, Dutch and German North Sea coast and in the Wadden Sea (Ref.17,23, 38). The data in Table 5 show that in the German Bight the burden by Pb and Cd is significantly higher than in the studied Western Mediterranean coastal area. This is also the case along the the Belgian and Dutch coast. A characteristic feature of the North Sea coastal waters is further, that they contain generally much more suspended particulate matter which reaches especially high values in the Wadden Sea and the estuaries of larger rivers. While Cd is distributed in about equal portions between the dissolved and particle phase, the latter contains the major amount of Pb.

An example for the application in the investigation of *inland waters* is the profiles of Cd and Pb from an extended study of the River Rhine (fig.8 and 9) and its major tributaries. While for the Cd-contents about a 1:1 ratio between dissolved state and suspended particulate matter is observed, the ratio for Pb is 1:2.8 in the upper Rhine, 7:1 in the middle Rhine and 9.5:1 in the lower Rhine. In the tributaries, for both metals the ratio is even more in favour of suspended particulate matter (Ref.39).

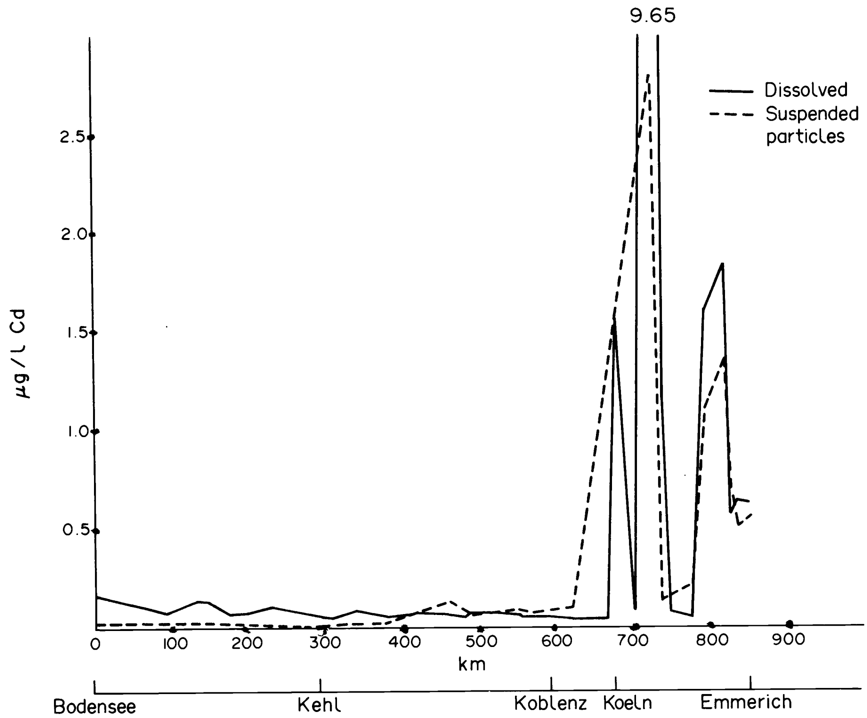

Fig. 8. Profile of Cd contents in the dissolved state and at suspended particulate matter in the River Rhine

Investigations on heavy metal speciation in natural waters

For the uptake by organisms and the interactions with suspended particles and sediments, the speciation of dissolved heavy metals in natural waters is of great significance. Therefore the elucidation of the prerequisites for the formation of certain species and of the distribution of the total dissolved heavy metal concentration over the major species have become topics of great interest and priority in aquatic chemistry. Voltammetry offers, due to its substance-specificity, particular potentialities for investigations on speciation (Ref.2,11-13, 4o-42).

Complexation capacity. This is an important empirical diagnostic parameter (Ref. 42,43) of a natural water type for a given heavy metal, e.g. Cu or Pb or Cd. The complexation capacity corresponds to the equivalence point of the titration of the natural water with a standard solution of a certain heavy metal (viz.fig.1o). One records the reversible peak after each addition of heavy metal titrant solution to obtain the titration graph. In the first branch with the smaller slope a fraction of the added heavy metal amount is bound by organic ligands forming nonlabile species. The reversible voltammetric peak, e.g. measured by DPASV, is produced by that amount of heavy metal which is not bound. When the whole amount of those components of dissolved organic matter (DOM) able to bind the respective titrant heavy metal in nonlabile species has been consumed, one comes into the second branch with the steeper slope. Now all further heavy metal added will remain present as labile species, yielding the reversible peak. The crossing point of the first and second branch is the equivalence point. From the corresponding value of added heavy metal titrant

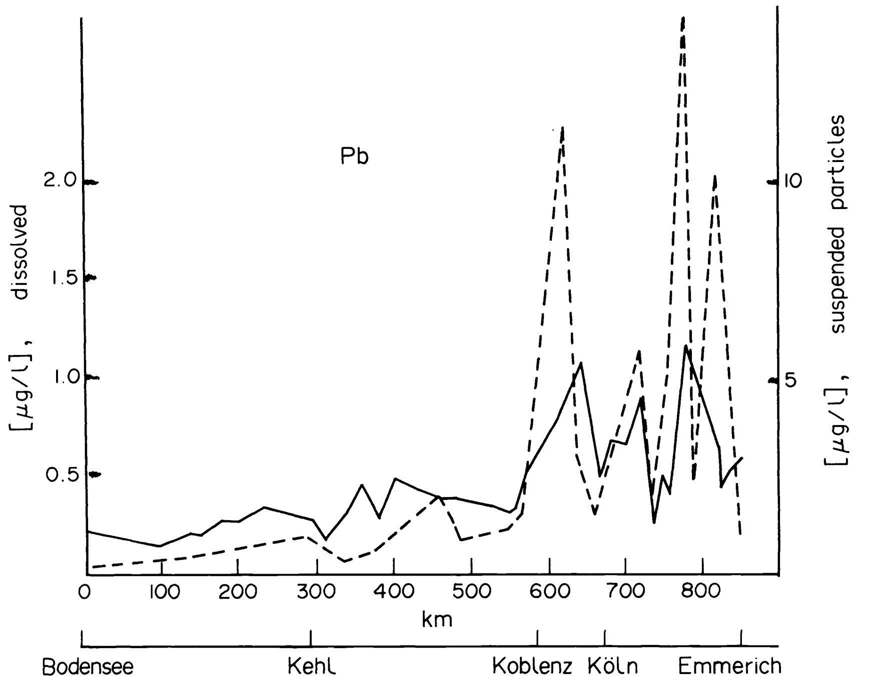

Fig. 9. Profile of Pb contents in the dissolved state and at suspended particulate matter in the River Rhine

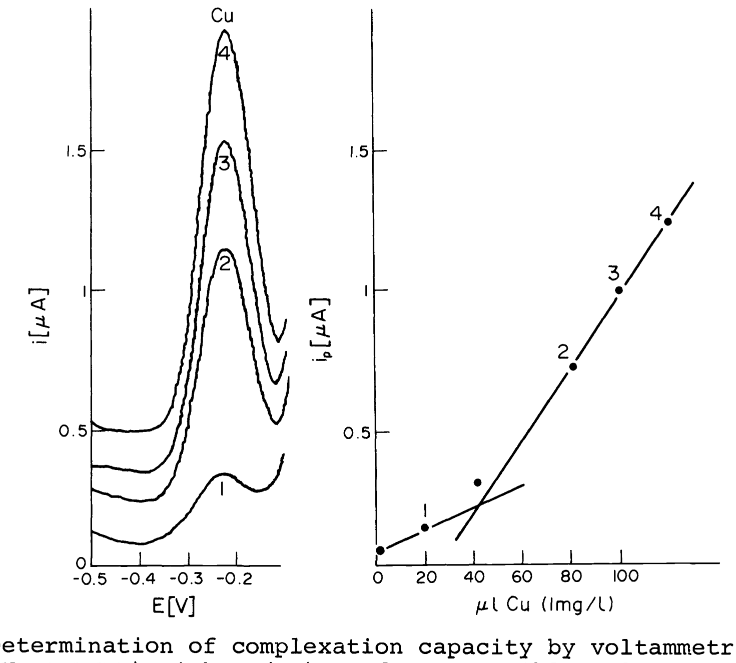

Fig. 1o. Determination of complexation capacity by voltammetric titration (DPASV at HMDE) with Cu(II). Left: reversible voltammetric peak corresponding to Cu remaining present as labile species. Right: titration graph with titrant solution of 1 mg/l Cu(II), sample volume 11,6ml. Recorded during expedition with RV "Sonne" 198o at subtropical convergence in southern Pacific (41°55'S; 144°58'W) in deep sea water sample above sea bottom, collected from 55oo m with free fall sampler. Complexation capacity for Cu is 3.6 μg/l.

solution follows the complexation capacity of the natural water type studied for the applied heavy metal, e.g. Cu or Pb. The complexation capacity equals the overall concentration of that DOM-fraction which is able to form nonlabile species with the heavy metal. It is therefore an important diagnostic parameter for the water type studied. The complexation capacities of the same water sample will be different for different titrant heavy metals. Consequently the information will be increased if it is determined with various heavy metals.

One observes the following sequence for the complexation capacities: Cu > Pb >> Cd. In this context, the literature tabulations (Ref.47) indicate at least the magnitude of the stability constants of organic complexes and chelates, formed by corresponding components of DOM, e.g. carbohydrates amino acids, proteins, humates, and based on the elucidated general basic prerequisites for nonlabile species formation from below mentioned results of fundamental investigations (Ref. 41,42,45-48), important conclusions on the contribution of DOM-components to heavy metal speciation emerge. Obviously the determination of the complexation capacity has to be carried out in water samples which have not been subjected to any pretreatment except the o.45 µm filtration. As untreated water samples are delicate in their stability and can therefore not be stored for longer times, particularly as deep freezing is prohibited to preserve the original speciation, the complexation capacity should be determined within hours after sampling, i.e. during oceanic expeditions on board.

Nonlabile complexes. Voltammetry has been also very successfully applied in fundamental investigations with defined organic ligands (Ref.43-48). Studies with the moderately strong chelator NTA have revealed for Cd, Pb and Zn in sea water the respective *ligand concentrations required* for a significant contribution of a chelate with a respective stability constant to the speciation of those heavy metals. Also the significant specific side effects have been clarified in this manner. The most important side effect is in sea water and inland waters of higher hardness the pronounced competition of Ca and Mg for the organic ligand which leads to a substantial enhancement in the required organic ligand concentration. The chelation degrees of the studied heavy metal obtainable with NTA-concentrations adjusted in sea water have been determined by measuring with DPASV the corresponding decrease of the reversible peak corresponding to the trace metal amount remaining unchelated (Fig.11). Here use is made of the fact that the heavy metal amount bound in nonlabile NTA-chelate is only reducible with a several hundred mV more negative overvoltage. This discrimination potentiality between the reversible response of labile species and the irreversible peaks of nonlabile species is also exploited in the measurements of the complexation capacity. From the NTA-studies emerged general prognostic predictions on the required ligand concentrations as function of the stability constant of the nonlabile complex formed for its significant contribution to the speciation of various heavy metals (Ref.42). It could be concluded that with respect to the usually abundant open sea concentrations of humates and amino acids their contribution to the speciation of Cd, Pb and Zn remains negligible. These prognostic conclusions have been meanwhile confirmed by similar direct investigations in sea water for Zn with amino acids (Ref. 49,5o) and for Cd, Pb and Zn with marine humates (Ref.51).

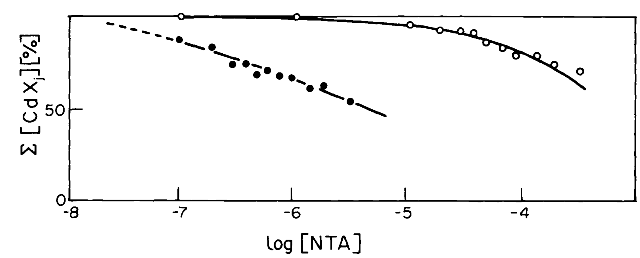

Fig. 11. Relation between percentage of unchelated Cd (present as labile complexes CdX_j) decreasing with added NTA concentration due to formation of the nonlabile chelate $CdNTA^-$. The unchelated Cd is determined via its reversible DPASV-peak. Total Cd-concentration $3x1o^{-9}$M. ● o.59 M NaCl model solution (sea water ionic strength); o Adriatic sea water.

If to a natural water sample containing a given trace concentration of a heavy metal an equal amount of a model chelator, e.g. EDTA, is added and the decrease of the reversible DPASV-peak, due to the unchelated remaining heavy metal concentration, is followed as function of time, one can determine the *rate constant* k_f of chelate formation and elucidate the *mechanism* (Ref.52,53). In sea water and also in inland waters having sufficient hardness, e.g. Lake Ontario (Ref. 47), again the Ca-excess is of dominating influence. The consequence is that for chelate formation of heavy metals, present at the trace level, a ligand exchange mechanism prevails according to:

$$MgL \rightleftharpoons CaL + MeX_j \xrightarrow{k_f} MeL + Ca^{2+} + jX^-$$
with $X \equiv Cl^-, OH^-, CO_3^{2-}, HCO_3^-$ etc.

Due to their excess, the alkaline earth ions, particularly Ca, chelate at first with virtually all the available organic ligands. If these chelate species of Ca and Mg meet a labile heavy metal species MeX_j ligand exchange takes place with the rate constant k_f, if the corresponding heavy metal chelate has a higher stability constant than the Ca-chelate as it is often the case.

Labile Complexes: At any rate, in sea water the most abundant heavy metal species are labile inorganic complexes with anionic salinity components X e.g. Cl^-, CO_3^{2-}, HCO_3^- and due to the pH around 8.o, also OH^-; for a given heavy metal they all undergo a reversible electrode process virtually at the same potential. Their stability constants and ligand numbers can be determined from the respective shift of the half wave potential $E_{1/2}$ with increasing concentration of the respective ligand X according to the DeFord-Hume evaluation (Ref. 54) for consecutive complex formation. If the investigations have to be performed at low trace levels of the total heavy metal concentration (e.g. 10^{-9}M) the pseudopolarogram approach, in which the polarogram is constructed from ASV-measurements, has to be applied (Ref.55). This method has been automated meanwhile (Ref.21,56,). In this manner the inorganic speciation distribution in sea water has been established for Pb and Cd (Ref. 57). As in the evaluation the ion pairing effects between the cationic and anionic salinity components have been taken into account, the distribution data have gained significantly in relevance and precision. They are of general validity for the speciation of Pb and Cd in all areas of the oceans where contributions from chelating DOM-components can be regarded as negligible. The net results are that Cd is speciated to 97 % in mono-, di- and trichlorocomplexes existing at rather equal quantities with a slight predominance of $CdCl_2$. Pb is with 43 % predominantly abundant as $PbCO_3$ followed by $PbOH^+$ with 3o % and only 21 % Pb-chlorocomplexes altogether. Only minute amounts of both heavy metals (< 2 %) are present in the sea as free hydrated cations.

Rain and snow

An important pathway for toxic metals emitted from anthropogenic sources (fossil fuel burning, steel production, ore mining, metallurgy, and for Pb automobile traffic) goes via the atmosphere by wet or dry deposition into terrestrial and marine ecosystems. The input of toxic metals with rain and snow is the most significant and efficient route and deserves therefore particular attention.

The hitherto commonly applied methods were in several aspects inadequate and not reliable enough. Again from the viewpoint of trace analysis the basic problem was to combine reliable contamination-free sampling of well defined samples with an efficient determination method suitable for large scale routine application at moderate costs (Ref.11,2o,31). For sampling an automated sampler was introduced which is controlled by a humidity sensor and opens at the start of a rainfall and closes within a short interval (o.5 - 2 min adjustable) after the termination of the precipitation. In this manner interferences by dry dust deposition are excluded. The sampler contains an easily purifiable polyethylene flask with a built-in o.45 µm filtration device. An external temperature controlled heating system ensures the operation down to -3o°C even under alpine conditions in winter time. Wind direction and strength are simultaneously recorded.

In the sampled rain water or molten snow, after digestion of the filtered off particulate matter, the toxic trace metals are voltammetrically determined according to the analytical procedure in fig.3. In an aliquot of the sample the pH also is measured.

From the analysis of several thousand samples collected with a network of the aforementioned samplers in the various regions of the Federal Republic of Germany, covering the different types of ecosystems (alpine, rural, urban, industrial and coastal areas), the following results of general significance emerged, besides a still increasing large set of data on the toxic metal input to the vegetation blanket, reflecting seasonal, meteorological and local ecological influences (Ref. 58); e.g. Cd, as shown in Table 6. In areas with substantial emission, due to heavy industry or even metallurgical smelters, the Cd-input is substantially higher, by a factor 6 to 17, compared with rural regions. In this context it should be noted, that according to the German regulations the total average daily Cd-input (wet and dry deposition) on land used for agriculture should not exceed 2.5 µg/m^2/day.

TABLE 6. Cd-input with rain during 198o at various locations with different ecological conditions in the Federal Republic of Germany

Location	Ecological type	Annual rain fall l/m^2	Average daily Cd-input (µg/m^2/day)	Total amount Cd-input (µg/m^2/y)
Juelich	rural	654	o.55	2o1
Deuselbach	rural	926	o.44	161
Yerseke, NL	Rhine/Scheldt Estuary	755	o.38	139
Hamburg	urban	1o53	o.96	35o
Braunschweig	urban	661	o.74	27o
Frankfurt	urban	761	1.28	467
Essen	urban, heavy industry	988	2.34	854
Dortmund	urban, steel production	998	2.2o	8o3
Stolberg	metallurgical smelters	836	1.65	6o2
Goslar	metallurgical smelters	721	6.47	2362

Usually more than 9o % of the total toxic metal content exists, in the rain, in the *dissolved* state. This indicates that efficient leaching of toxic metals from the surface of dust particles and dissolution of aerosols occurs in the atmosphere during rain fall. This fact is of particular ecotoxicological significance, because the toxic metals dissolved in rain water are thus offered to the vegetation in a most favourable form for uptake by the leaves and by the roots. Moreover, the rain water will also leach in addition toxic metals from the dust particles deposited on the foils of plants and grass. With respect to the aquatic environment also toxic metals are readily brought with the run off water into rivers and lakes besides the direct input. The small sample volumes of only several ml required, in voltammetry, made it easy to follow as a function of time the toxic metal concentrations in rain over precipitation periods (Ref.2o). Always a pattern of the type shown in fig.12 has been observed. In the initial phase of rain fall the toxic metal concentrations are up to one order of magnitude higher than the stationary values, which are approached gradually over 1 to 2 h and remain then in their average rather constant for the rest of the rainfall. This observation reflects the efficient wash out of the atmospheric toxic metal content in the initial phase of rain or snow falls.

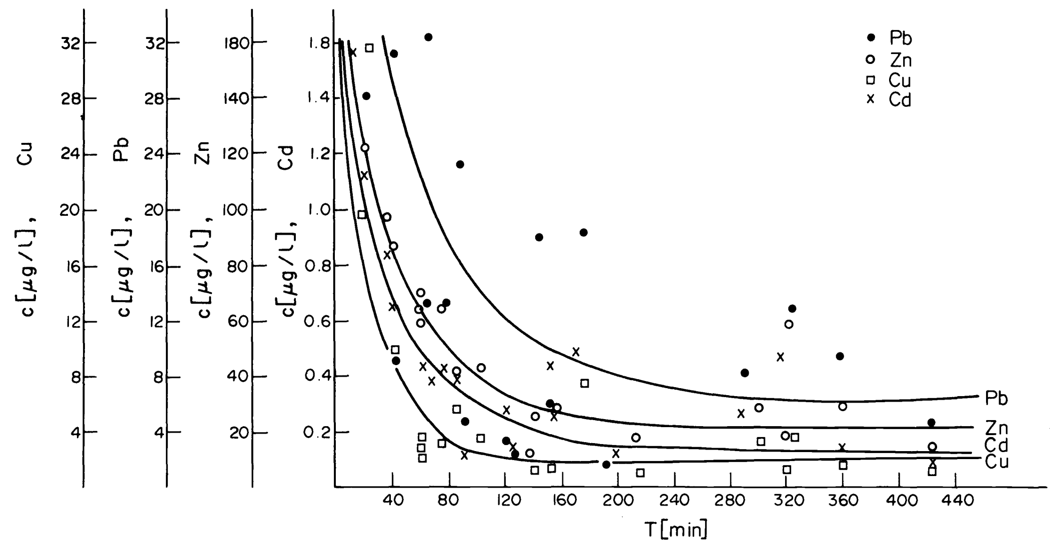

Fig. 12. Typical time pattern of heavy metal concentrations during rain fall.

In *polar* regions the precipitation is always snow. Then manual sampling under special precautions into wide mouth polyethylene bottles is a very good approach as recent experiences during the polar expedition "Ymer-8o" have shown (Ref.17). On the ice cover in the Arctic Ocean north of Greenland and Spitsbergen, for the first time, samples of fresh fallen snow were taken in arctic summer and also samples of agglomerated snow from the previous winter. The data show significant differences for both kind of samples. Typically the values are a factor 1o higher in the agglomerated snow from previous seasons while fresh fallen snow from arctic summer contains extremely low contents. The Cd-values go down to 1oo pg/kg and are from the methodological aspect the lowest values determined until now by voltammetry in environmental samples. From the ecochemical aspect the results of this study furnish the first experimental evidence on the toxic metal base lines in the remote northern Arctic region; they correlate in general well with the expectations on atmospheric toxic metal transport from anthropogenic sources in North America and Europe, which occurs due to the prevailing meteorological situation during winter and spring.

Waste Water

A further very significant pathway of toxic metals into natural waters is represented by municipal waste water. While special industrial waste waters with high toxic metal freights are usually known, controlled and treated before inlet into rivers and lakes, the toxic metal burdens in municipal waste waters originating from a large variety of diversive anthropogenic sources are frequently still underestimated. A certain contribution to the toxic metal burden will be also produced by the run off of polluted rain water as mentioned before. Control of waste water before entering biological treatment plants and in their outlet is therefore of great significance. This can be readily carried out by voltammetry (Ref. 59). The result of UV-irradiation to decompose interfering DOM-components is reflected by the example in fig. 13. The efficiency of biological treatment plants for the elimination of toxic metals is generally limited and quite different for various toxic metals. Moreover, it is to be noted, that although the total toxic metal content is decreased, the concentrations in the dissolved state may be unaltered or even higher in the outlet water going after passage in the treatment plant into the natural water system. This is connected with the solubilization of toxic metals bound to suspended particles and sludge in the treatment plant, due to the action of organic chelators.

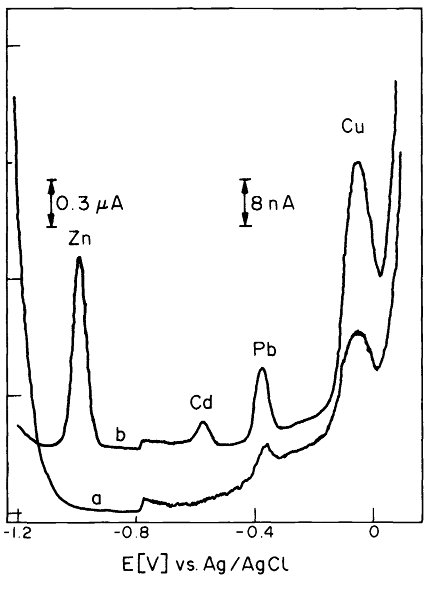

Fig. 13. Voltammogram of simultaneous DPASV-determination in municipal waste water containing 5.8 µg/l Zn, o.35 µg/l Cd, 1.8 µg/l Pb and 1o.7 µg/l Cu. E_d -1.2 V; t_d 12o s. a) without prior UV-irradiation of analyte. b) after 4 h UV-irradiation under addition of o.o3 % H_2O_2.

Drinking water and food control

Drinking water and food are the most significant pathway by which toxic metals are ingested by man. Reliable trace analytical procedures for the respective control of food and water are therefore of particular significance for society. Toxic metals come into food by uptake from the environment and during the processing of food.

Obviously voltammetry is the predestined method for toxic metal control of *drinking water* (Ref.14,6o,61). Due to its high determination sensitivity the voltammetric method enables not only to ensure that the tolerances of the drinking water regulations are not exceeded but yield reliable data on the usually significantly lower actual toxic metal levels. For the amalgams forming heavy metals the HMDE is applied. The obtained data will be also of great value in manifold future ecotoxicological studies on the impact of toxic metal burdens on the population.

Drinking water requires usually only very limited sample pretreatment consisting as a rule just in pH-adjustment and aliquotation. This makes rather easy the usage of fully automated voltammetric analyzers, which can be connected via a bypass to the main output tube of water works, for automated on-line control of drinking water (Ref.61).

Rather immediate voltammetric measurements (Ref.33,62) are also possible in certain types of liquid food, as many *wines* and certain *fruit juices*. A better performance of the voltammetric responses is obtained, however, if the wine or juice sample is subjected to prior UV-irradiation for oxidative photolysis of dissolved organic substances with binding capacity for heavy metals. Tests with the alternative of wet digestion ($HNO_3/HClO_4$) have confirmed, that the more conventient UV-irradiation yields complete decomposition of metal binding organics. Thus, the wet digestion can be restricted to very sweet wines with sugar contents above 1o %. The required sample volume is, with o.5 ml wine or juice, very small. To minimize contamination risks it is advisable to perform the whole analytical procedure from UV-irradiation to voltammetric determination in the same quartz vessel which serves in the last stage after immersion of the electrodes as voltammetric cell. It should be noted that frequently the Cd-levels in wines and juices are well below 1 μg/l. Then the MFE is the best choice. Otherwise the simultaneous determination of Cu, Pb, Zn can be performed at the HMDE. Although Cu can be determined simultaneously with Cd and Pb at the MFE, it is preferable to use in view of the limited solubility of Cu-amalgam, for Cu the normal gold disc electrode. A large scale study on the toxic metal content in German and European wines has featured the special suitability of the voltammetric approach in toxic metal control of wines (Ref. 63,64). On average the Cd-level was between o.1 and 1 μg/l. For Pb most values are around 12o μg/l, which corresponds to a factor 3 of the tolerance value for drinking water (4o μg Pb/l). The Ni-content is in the range of 3o to 6o μg/l in the younger vintages and seems to be connected with stainless steel fermentation tanks becoming usual in modern wine cellars for the major amount of ordinary wines. The Cu-content covers a wide range between 2o and 2ooo μg/l with 15o to 3oo μg/l in the majority of the specimens.

Most types of *food* have, however, to be subjected to the analytical procedure depicted as flow chart in fig.14. Dissection of fish, meat etc. or portioning has to be performed with great caution under a clean bench (Ref.65.66). Subsequently one has the choice between two rather universally applicable wet digestion versions (Ref 67,68) to all food types (meat, fish, mussels, milk, fats, flour, bread, vegetables, lever, etc.). The normal wet digestion (Ref 67) is complete, but is to be performed due to the $HClO_4$ content with appropriate precautions. Safety precautions demand also the alternative of the pressurized digestion (Ref. 68,69) which is to be carried out under remote control in a small concrete box outside the laboratory building. It yields not an immediately suitable analyte for voltammetry, because the solution of the digestion residue still contains soluble organic substances, which have to be decomposed photolytically by UV-irradiation. A clean and efficient but in equipment costs somewhat expensive alternative is low temperature ashing (LTA) in an oxygen plasma (Ref.28). Obviously it can, however, not be used for Hg. After the aliquotation of the resulting aqueous analyte follows the voltammetric determination by the same modes as described for natural water samples. Table 7 summarizes some typical results in various components of the food basket (Ref.67). In the muscle meat of fish usually only Hg, present to over 9o % as the very toxic methylmercury, plays in toxicological terms a significant role. Particularly high is the burden of large prey fishes on top of the food chain. An example : Hg-levels between 2 and 3 mg/kg FW in tuna meat from Mediterranean specimens (Ref. 69). For Hg, pressurized digestion is to be applied.

Body fluids

Blood and urine constitute important sample types for large scale ecotoxicological studies on the toxic metal burden of larger groups of the population or for tests in occupational medicine. They are easily available sample types and provide valuable informations on the ingestion of toxic metals (*blood*) and on the excretion after exposure (*urine*).

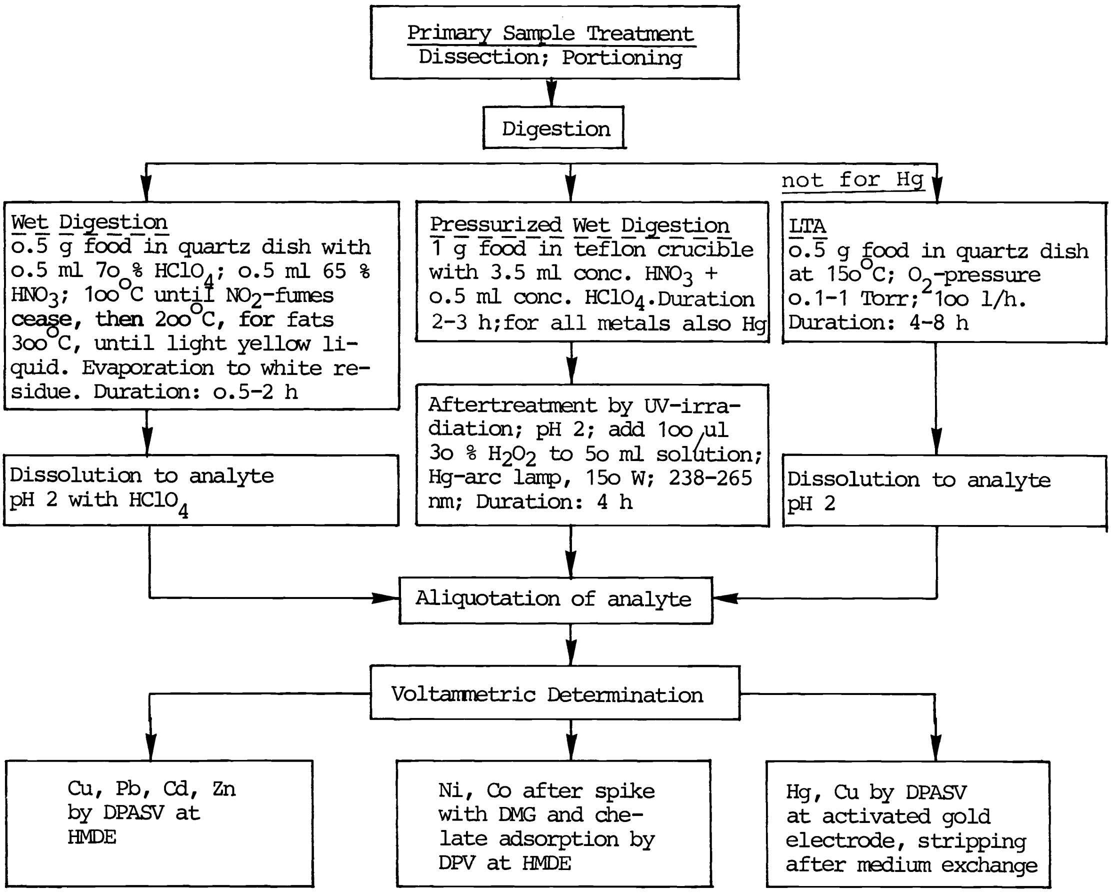

Fig. 14. Analytical procedure for food and biological materials

TABLE 7. Heavy metal content in food

	Cu	Pb	Cd	Zn	Ni	(µg/kg)
Wine	335	117	o.29	338	42	
Orange juice	183	o.5	o.84	198	1o.6	
Beer	18	o.9	o.72	52	7.6	
Whole Milk (3.5 % fat)	4o	1.o	o.28	373o	4.2	
Skim Milk	5o	5.3	o.3o	392o	1o.7	
Wheat Flour	822	22	39	386o	44	
Bread (Wheat and Rye)	2o7o	42	3o	1863o	88	
Butter	97	7.4	o.5o	154o	25	
Margarine	58	75.5	42	127o	313	
Krill Meat	38o	5o	48	-	-	
Krill Meal	32ooo	61o	12oo	-	-	
Relative Standard Deviation	2 %	5 %	4 %	1 %	7 %	

For *blood* the analytical procedure (Ref. 28) is analogous to that for food. Most vulnerable to contamination is the sampling. Samples of 1 ml whole blood are taken with polystyrene syringes equipped with stainless steel tips to be used only once. The syringe has e.g. a Cd-blank of only o.1 µg/l. Either pressurized wet digestion with following aftertreatment by UV-irradiation or LTA is applied. Usually blanks can be kept in LTA below o.1 µg/l Cd, 2 µg/l Pb and 3 µg/l Cu. The with a reasonable precision of 2o % RSD obtainable realistic determination limits in blood applying DPASV at the HMDE are 4 µg/l Pb, o.1 µg/l Cd, 5 µg/l Cu and 1 µg/l Ni. These practical determination limits are not set by voltammetry but depend only on the attainable blank values during sampling and digestion.

The most important feature of the described procedure is its high reliability. A disadvantage is, however, the required prior complete ashing of the blood matrix. Therefore, in the necessary sample pretreatment rather unpretentious and consequently more rapid procedures with subsequent determination by graphite furnace atomic absorption spectrophotometry (GFAAS) are widely applied in common routine analysis (Ref. 7o,71). They need, however, due to their higher inherent accuracy risks at any rate accuracy confirmation by test analyses with an independent method (Ref. 72). This is a further important application field for the outlined high performance procedure with DPASV determination. An interesting rapid routine procedure with voltammetric determination of Pb provides the commercial analyzer from ESA, Bedford, Mass., USA. The simple and very rapid sample pretreatment consists just in addition of 1oo µl blood to a reagent solution containing 1.43 % Ca-acetate and 1.o7 % $CrCl_3$ as main components and a spike of Hg(II). During swirling for some minutes all Pb is decomplexed due to an ion exchange reaction with Ca. Again one has here an example for the competition between Ca and heavy metals for organic chelators as it also exists with DOM-components in natural waters. For blood this competition could be utilized for an elegant and rapid substitution of digestion. The voltammetric Pb-determination is then performed immediately by ASV at a large area mercury film electrode formed on a tubular pyrolytic electrode. To ensure good performance staircase polarisation is applied during stripping.

Also in *urine* the content of Pb and Cd can be determined simultaneously with high accuracy down to o.o5 µg/l Cd and o.7 µg/l Pb. Again the determination potentialities are not limited by voltammetry but by the blank values attainable in the prior sample pretreatment stage. An efficient and rapid method (Ref.73) works with overnight freeze dried urine samples. They are subjected to a rapid wet digestion ($HClO_4/HNO_3$) within 15 min in a quartz flask and subsequent DPASV at the MFE in acetate buffer of pH 4.5. To keep the contamination as low as possible the same quartz flask is used also as voltammetric cell. It has to be emphasized, that although possible, direct determinations in urine without prior digestion are not recommendable, because there are always cases where proteins are contained in urine samples which trap a certain amount of the heavy metals. Besides applications in routine again an important feature of this method is to serve as test procedure for accuracy confirmation of alternatively frequently applied methods with GFAAS determination (Ref. 71,72).

Doubtless for both body fluids, procedures based on GFAAS are very powerful and suitable, for blood and urine, due to the especially unpretentious pretreatment requirements (Ref. 74). Nevertheless, voltammetry is to be regarded with respect to its high inherent reliability as an indispensable second method for laboratories charged with high performance trace analysis of heavy metals in body fluids.

CONCLUDING REMARKS

The treated examples and numerous further applications reported in the recent literature emphasize the manifold and significant potentialities of the voltammetric approach in the ecochemistry and ecotoxicology of heavy metal traces. In the domain of aquatic chemistry voltammetry has opend hitherto unaccessible dimensions of investigation in a convenient and reliable manner. But also for all types of biological matrices the outlined properties make voltammetry one of the important alternatives for trace metal analysis. For all laboratories concerned with research or control of toxic heavy metals in the environment, in food or body fluids voltammetry is to be counted as one of the mandatory trace analytical methods among their basic methodological outfit.

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