I - EXECUTIVE SUMMARY
This synthesis, coordinated and edited by Nick S. Fisher, was initiated during the Ancona meeting and prepared by all the workshop participants.

1 - Introduction

The workshop was held from 27 to 30 October 2002 in Ancona, a busy port in the Marches region on the Italian Adriatic coast. The workshop was hosted by the Istituto di Ricerche sulla Pesca Marittima (IRPEM), through the kind assistance of its Director, Dr. Antonio Arregiani, who serves as National Representative of Italy on the CIESM Board. Eighteen scientists (see list at the end of this volume) participated at the invitation of CIESM.

In their opening remarks, Prs Frederic Briand and Nicholas Fisher, Director General of CIESM and Chair of its Marine Biogeochemistry Committee respectively, provided the overall philosophy behind such research workshops. They encouraged the participants to avoid a simple review of their own work or of the field in general, recommending instead that discussions aim to produce a constructive synthesis and critique of the major developments in the area of metal/radionuclide bioaccumulation in marine organisms together with some recommendations for future work in this broad and growing field.

Bioaccumulation (defined here as association of a metal with an organism) is a necessary first step before organisms can manifest a response to metals or influence metal geochemical cycling. Many studies have been conducted in recent years to evaluate the rates and mechanisms of metal bioaccumulation in marine organisms. These studies have been motivated in part by a number of practical issues, such as public health concerns arising from the consumption of contaminated seafood, a need to establish improved water or sediment quality criteria in coastal regions, and risk assessment exercises (for both marine communities and human populations) which require information on bioconcentration factors for contaminants in diverse marine organisms. Risk assessments have been particularly applied to long-lived components of radioactive wastes emanating from the nuclear fuel cycle, most of which are metals. However, there is also considerable interest in understanding metal accumulation in marine organisms as they can greatly influence the cycling, fluxes, and residence times of metals/radionuclides in marine systems. Moreover, the recognition that low concentrations of some essential metals in a bioavailable form can be limiting to some marine organisms, most notably iron to phytoplankton in high nutrient, low chlorophyll (HNLC) areas of the oceans, has spurred many recent studies on relating metal speciation in water to bioavailability and metal accumulation to metabolic responses.

The short papers that follow in this volume consider such matters as (1) relating metal speciation (chemical and physical) to metal bioavailability to marine organisms; (2) environmental (or external) and physiological (internal) controls of metal fluxes through marine organisms; (3) evaluating the significance of metal uptake by organisms for understanding metal fluxes and residence times in oceanic systems; (4) relating biological responses (e.g., growth, toxicity) to pathways of metal uptake; (5) biochemical distributions of metals in marine organisms and their implications for metal toxicity and trophic transfer; and (6) modeling metal bioaccumulation in marine organisms and an evaluation of its appropriateness to understanding metal concentrations in marine organisms.

A number of publications have presented organizing principles by which the fate and effects of broad classes of metals can be understood and predicted in marine systems. These include geochemical considerations (Whittfield and Turner, 1987), general biological interactions (Nieboer
and Richardson, 1980), including bioaccumulation in marine organisms (Williams, 1981; Fisher, 1986) and toxicological effects (Shaw, 1954; Kaiser, 1980; Nieboer and Sanford, 1985). The advantages and limitations of these broad groupings of metals based on properties of the metal ions themselves were discussed by Rainbow (1997a), who focused on metal interactions with marine invertebrates. Despite some limitations, such generalizations were considered useful and sometimes provided penetrating insights into the nature of metal binding to, or effects on, marine organisms. They helped form the framework for the discussions of this Workshop which considered the processes governing metal bioaccumulation in marine organisms including analytical, monitoring, and modeling tools for studying these processes, and the geochemical and biological consequences of this accumulation.

2 - METAL BIOAVAILABILITY

2.1. Speciation effects in solution

Laboratory and field observations clearly indicate that the total concentration of a metal, in solution (“dissolved” metal) or associated with particles (“particulate” metal), is rarely a good predictor of metal bioaccumulation or toxicity in aquatic organisms. On the other hand, environmental geochemists have demonstrated that metals exist in a variety of physical and chemical forms in the water column and in the bottom sediments. Faced with these general observations, environmental scientists and managers have rather uncritically accepted the notion that some fraction of the total metal concentration is “bioavailable”. There is no doubt that this statement is qualitatively correct, but it has proven difficult to develop a universally applicable quantitative definition of “bioavailability”. Two major factors contribute to this dilemma: (i) the diversity of routes by which metals may in fact be bioaccumulated by aquatic organisms, and (ii) the dynamic nature of metal speciation (and the analytical limitations to measuring this speciation).

With respect to the first factor, one can make a useful first distinction between those organisms that are exposed only to dissolved metals, e.g. bacterioplankton, phytoplankton cells, or macrophytes, and those that are exposed both to dissolved metals and to metals that are incorporated into their ingested food, e.g. protozoa, zooplankton and higher herbivores / carnivores. However, even within these classes there will exist a wide variety of routes of metal uptake. For example, even for a simple unicellular alga (Fig. 1), metals may enter the cell by facilitated cation transport, by facilitated anion transport and by passive diffusion of lipophilic metal forms; the relative importance of these uptake pathways will vary from one algal cell to another. Similarly, while similar transport systems will exist at gill and intestinal epithelia, the relative importance of the different uptake

1 - Facilitated transport \( M \)
2 - Facilitated transport \( L \)
3 - Passive transport \( ML_n^0 \)

![Fig. 1. General scheme showing how metals may enter living cells: (1) carrier-mediated cation transport and/or cation-channel transport; (2) carrier-mediated transport of metal-ligand anionic complexes; (3) passive transport of lipophilic metal forms.](image-url)
routes will vary from one biological interface to another. In addition, in a given aquatic environment the indigenous organisms will tend to occupy different (micro)habitats; if the chemical conditions (e.g., \([O_2]\), pH, pCl, [dissolved organic matter]) in these microhabitats differ, then metal speciation and bioavailability will also vary from one microhabitat to another within the given (macro)environment. For both these reasons (different routes of exposure to metals and different physiologies; different microhabitats), it follows that even in a defined aquatic environment, metal “bioavailability” will not be a constant property of the system but will tend to vary from one organism to another.

The influence of the second factor, i.e. the dynamic nature of metal speciation, is perhaps a little more subtle. In most cases, the distribution of a metal among its various chemical forms (see Fig. 2) is very rapid. Under such circumstances, if one particular metal form is taken up from the exposure solution (e.g., the free metal ion), its concentration will decrease only transiently; the external equilibria will immediately adjust to replenish the metal form that has been taken up. Laboratory experiments suggest that this general scenario is often played out in just this manner. If this is the case, the free metal ion activity will be the best predictor of metal uptake from solution, but it cannot legitimately be described as the “bioavailable form” of the metal because, in fact, all of the forms in equilibrium with the free metal ion are contributing to the uptake flux by maintaining the external free ion concentration/activity.

![Fig. 2. Examples of metal "species" found in natural waters.](image)

Trace metals are taken up by aquatic organisms from solution across the cell membrane of permeable surfaces by one or more transport routes (Blust; Campbell; Rainbow, this volume), including:

a) carrier-mediated transport whereby a metal ion binds with a membrane protein;
b) a membrane channel consisting of a protein with a hydrophilic core through which metal ions are transported, perhaps to be considered as a variant of the carrier protein route;
c) passive diffusion of lipid-soluble (non-polar) metal forms which dissolve in the lipid bilayer, including alkyl-metal compounds and neutral, lipophilic, inorganically complexed metal species (e.g., \(HgCl_2\));
d) endocytosis when a region of the cell membrane invaginates to engulf a metalliferous particle for transfer into an intracellular vesicle.

Once trace metals cross the cell membrane, their binding with non-membrane permeable cytosolic proteins of higher affinity for the metal ensures that they continue to enter passively, even though the internal concentration of total metal in the cell is higher than the external dissolved metal concentration. Major ions like Na\(^+\) and K\(^+\) do not have such high affinities for complexing agents,
including proteins, and remain relatively uncomplexed in the intracellular environment; maintenance of the concentration gradients for these metal ions may require the use of energy, e.g., involvement of membrane-bound ATP-ases.

2.2. Metal bioavailability from food sources

As mentioned above, it is generally considered that the free metal ion is available for transport across the cell membrane via a carrier protein or through a membrane channel, and therefore the free metal ion will often be the best predictor of uptake of metal from the dissolved phase (Campbell, this volume). Whereas organisms positioned at the bottom of the food web such as unicellular algae take up metals exclusively (or predominantly) from the dissolved phase (with the exception of rooted plants), organisms positioned at higher trophic levels are exposed via other routes including suspended matter, sediment particles and different food sources. This creates a more complex situation so that the relationship between the free metal ion concentration and metal uptake may be lost.

Inside an organism, metals are distributed among different compartments, of which some are strong accumulators (e.g., liver and kidney) and others are much weaker accumulators (e.g., muscle tissue). Inside the cells of the tissues the metals are bound to different types of ligands and partitioned into exchangeable (or labile) and non-exchangeable (or inert) metal pools. Thus, inside an organism metals occur in various compartments and forms, the chemical speciation of which is very different from that in the external environment. Feeding involves the ingestion and digestion of food items (which may include suspended or deposited sediment particles). Depending on their physiology and position in the food web, organisms have developed different feeding and digestion strategies. This also means that the way in which the metals are processed during the digestion process may be very different, resulting in potentially large differences in metal assimilation efficiencies. Several studies have indeed shown that assimilation efficiencies of metals from food strongly depend on food preference, feeding strategy, and digestive physiology. Hence, a direct relationship between the free metal ion concentration in the exposure solution and metal uptake by an animal will only be found when (i) uptake via water is the predominant exposure route, or (ii) the concentrations of the metals present in the food items are proportional to the ambient free metal ion activity, and assimilation efficiency is invariant across different milieux.

In addition, aquatic organisms have developed different strategies to deal with metals. Some metals are essential nutrients for which the body or tissue concentrations are more or less under homeostatic control (e.g., copper or zinc), whereas others are not essential and do not appear to be regulated very strongly (e.g., cadmium or mercury). Regulation can occur in two different ways, either by matching metal excretion to metal uptake so that the internal body concentration of the metal remains constant, or by storing part of the metal in a physiologically inactive pool. Aquatic organisms employ both strategies and in many cases effective regulation and/or detoxification is achieved by combining the two methods. Together with the relative importance of different exposure routes, these differences in internal processing explain why there can be important differences in body metal concentrations and internal metal speciation among organisms from the same environment.

2.3. Biogeochemical oceanographic considerations

To this point we have emphasized the influence of chemical speciation of trace metals on metal bioavailability. The physical speciation of metals can also strongly influence their fate and behavior in marine ecosystems. Thus, metals that show little binding to particulate matter in marine systems (i.e., conservative metals such as uranium or technetium) will be relatively unavailable for uptake by marine herbivores through trophic transfer since there will be little or no uptake of these metals from the dissolved phase into the phytoplankton food. Further, the cycling and fluxes of these metals will not be affected by particles, and hence their oceanic residence times approach that of the water itself.

Many metals do however associate to varying extents with particulate matter and these metals can be accumulated in animals both from ingested particulate matter (i.e., food) as well as from the dissolved phase. The fluxes of these metals are strongly influenced by particle flux, including biogenic particles such as sinking fecal pellets or phytodetritus, which are highly enriched in
metals (Fowler, 1982; Fowler and Knauer, 1986), and abiotic particulate matter. The most particle-reactive metals have oceanic residence times approaching those of the particles themselves. Their residence times in the ocean, and most particularly in surface waters, tend to be very short and have been shown to be closely related to the metal concentration factor in sinking biodetritus (Cherry et al., 1978). Many particle-reactive metals are Class A metals, which have a high affinity for oxygen ligands; examples include thorium, aluminium, plutonium, and many of the lanthanides (Whitfield and Turner, 1987). By size-fractionating metals in surface seawater, it can be shown that some metals presumed to be “dissolved” (i.e., they would pass through a 0.2 or 0.45 µm filter) are, in fact, bound to colloidal particles, operationally defined as suspended material that behaves like particles but is smaller than the common “cut-off” for particulate matter (either 0.2 or 0.45 µm). Many of these Class A, particle-reactive metals show appreciable and rapid binding to colloidal particulate matter, probably because of the very high surface to volume ratios of these very small particles and possibly because, as well, there are numerous oxygen-containing binding groups on the surfaces of these colloids to which the metals can bind. Subsequently, these collooidally-bound metals can associate with larger particles such as phytoplankton cells via particle-particle interactions (Honeyman, 1991). Such collooidally delivered metals cannot easily be transported into the cytoplasm of cells, either by carrier proteins or enzymatic action, and as well would have relatively little chance of diffusing across the cell membranes into the cytoplasm because these metals are not, for the most part, bound directly to the cell surface but rather to colloidal matter which in turn is bound to cell surfaces. Thus, these metals are often found associated with the surfaces of microbial cells, generally showing little penetration into the cytoplasm of these cells. Consequently, when these cells are eaten by herbivores, these surface-bound metals display little assimilation into the tissues of the herbivores and get released from the animals packaged into fecal pellets. Cytoplasmically distributed metals tend to be assimilated into the tissues of herbivores; generally, a very tight relationship has been observed between assimilation efficiencies of ingested trace metals and the cytoplasmic distribution of these elements for herbivores (e.g., copepods, bivalve larvae) with relatively simple guts and short gut transit times (Fig. 3). Note that the slope of the regression shown in Figure 3 is
not significantly different from 1 and the intercept not different from 0. The relationship displayed in Fig. 3 between assimilation efficiency of ingested metals in zooplankton and the cytoplasmic distribution of metals in the phytoplankton food is remarkably robust and holds for both essential and non-essential elements. The data for this Figure, compiled for this report from various recent studies, clearly indicate that the assimilation of ingested metals by zooplankton can be predicted based on the distribution of metal in the food. This correlation between cellular distribution in the food and assimilation efficiency in the herbivore is still apparent but considerably less robust for animals with longer gut residence times and more complicated guts (e.g., adult mussels). Fecal pellets produced by pelagic zooplankton can often sink at rates of 50-150 m day$^{-1}$ (Turner, 2002); hence these metals get transferred rapidly into deeper waters or even sediments. Further, because these particle-reactive metals typically display little assimilation into animal tissues, they show little build-up in marine food chains. Since the resulting concentrations of these metals in marine animals are relatively low due to this low assimilation, these metals would not be expected to exert any toxic action on marine animals nor would they likely pose a public-health problem for human consumers of seafood.

It is noteworthy that even the most particle-reactive metals are predominantly in the dissolved (or colloidal) phase in most marine waters (Fig. 4). This observation simply reflects the fact that suspended particle loads in seawater tend to be low, rarely exceeding 10 mg L$^{-1}$ in surface waters, and often in the 0.1-2 mg L$^{-1}$ range. In turbid estuaries, however, particle loads can be quite high (this loading varies enormously among estuaries and with season), and in these systems a concomitantly greater proportion of the total metal will be associated with particulate matter.

In contrast to the Class A cations, many of the transition metals show intermediate or even high reactivity for particulate matter but negligibly small association with colloidal matter in seawater. These metals may bind to cell surfaces directly, and therefore can get transported to a greater extent into the cytoplasm of phytoplankton cells. When these cells are eaten by herbivores, these

![Graph showing metal partitioning](image)

**Fig. 4.** Calculated partitioning of metals with Kds (equal to dry wt. concentration factors) ranging from $10^3$ to $10^6$ on to particles over a suspended particulate load range of 0 to 4 mg L$^{-1}$. Note that for typical surface seawater particle loads ($\leq 1$ mg L$^{-1}$), even the most particle-reactive metals are predominantly in the dissolved phase. Most metals have Kds $< 10^5$ (Fisher, 1986).
Cytoplasmically associated metals get assimilated into the animal tissue, have a chance to build up to appreciable concentrations in the tissues of marine animals, and thus may pose a threat either to these animals (toxicity) or to human consumers. Because these metals become more closely associated with the carbon cycle, they get recycled biologically by organisms, and consequently display longer oceanic residence times (Fig. 5). Thus, metals display a positive relationship between assimilation efficiencies in zooplankton and oceanic residence times and an inverse relationship between concentration factors in phytoplankton (or any particles) and oceanic residence times.

2.4. Metal biomagnification

“Biomagnification” of metals and radionuclides in aquatic organisms refers to an increase in tissue concentration of a given element in higher trophic levels of a specific food chain. The underlying assumption is that the transfer pathway for element bioaccumulation and eventual biomagnification is through ingestion of contaminated prey. This phenomenon occurs when the assimilation efficiency of the element (i.e., transfer of the element from ingested food into the gut cells or beyond) is very high, and the corresponding element excretion rate is very low. In most aquatic food chains, the food chain transfer and resultant biomagnification pertain to element concentrations in whole organisms since rarely are only specific animal organs or tissues consumed in nature. Problems arise in the use of the term biomagnification when element concentrations in different tissues of organisms from different trophic levels are compared and found to “increase” along a specified food chain (Gray, 2002). Tissues are quite specific in their ability to accumulate metals and radionuclides, and many tissues or organs also incorporate their element burdens through direct absorption from water or translocation from other tissues. Hence, certain observed biomagnified element concentrations based on whole body or individual tissue concent-

Fig. 5. Relationship between assimilation efficiency (%) of ingested metals in marine copepods (left y-axis) and mean oceanic residence times of the metals (log years) (triangles connected by solid line, \( y = 18.15 \pm 8.48 \) \( x - 22.4 \pm 27.9 \); \( r^2 = 0.621 \)); relationship between log volume concentration factor (VCF, right y-axis) of metals in phytoplankton and mean oceanic residence time of the metals (circles connected by dashed line, \( y = -1.014 \pm 0.114 \) \( x + 7.276 \pm 0.474 \); \( r^2 = 0.835 \)). Note: elements denoted by “x” associate appreciably with colloidal matter in seawater. Adapted from Fisher (1986) and Fisher and Reinfelder (1995).
trations of higher trophic level organisms may not at all be due to food chain transfer. An example where knowledge of uptake pathways is critical in determining the existence of metal biomagnification is plutonium transfer in the mussel-starfish food chain. Greatly enhanced plutonium concentrations observed in whole starfish feeding on mussels can lead to the conclusion that plutonium, a radionuclide that is generally poorly assimilated in invertebrates, is biomagnified. However, carefully-controlled radiotracer experiments showed that when starfish accumulated plutonium from water, the resulting radiotracer tissue distribution closely matched the natural distribution of $^{239,240}$Pu in tissues of starfish contaminated by fallout and released nuclear wastes; such was not the case when plutonium tracer was incorporated in starfish fed contaminated mussels (Guary et al., 1982).

The general public often holds the view that biomagnification of contaminants in aquatic organisms is a common occurrence in nature. In reality, for trace metals and radionuclides the phenomenon is rare and at present is only known to occur regularly for methyl mercury, radiocesium, and perhaps polonium. Methyl Hg is known to display a high assimilation efficiency and a biological half-life on the order of months to years in many marine species (Meili, this volume). This, in combination with a declining growth efficiency, may lead to an overall accumulation of Hg with age and therefore high concentrations of Hg in older, top-level predator fish and mammals. Biomagnification of $^{137}$Cs has been observed in both freshwater and marine fish food chains (Rowan and Rasmussen, 1994; Zhao et al., 2001) and is thought to result from the longer biological half-life of $^{137}$Cs relative to its analogue element potassium in fish. Correspondingly, the biomagnification of methyl-Hg, typically threefold with each trophic transfer, can be explained by a three-fold slower elimination rate relative to the turnover of proteins, the dominant binding matrix (Meili, 1997). Other elements should also be examined for their potential to biomagnify in specific food chains. For example, very high total Cd concentrations are known to occur in certain tissues of cephalopods and their marine mammal predators, and recent experimental data demonstrate high assimilation from food and virtually no subsequent excretion of ingested Cd in cephalopods (Bustamante and Caurant, this volume). Hence, there is a clear potential for Cd to biomagnify in cephalopods and a more rigorous test of the biomagnification hypothesis in this food chain would be of considerable interest. Further, there is evidence that $^{210}$Po, a naturally occurring radionuclide of considerable interest from the radiological protection standpoint, displays biomagnification in pelagic food webs (Heyraud and Cherry, 1979), consistent with recent observations that it displays high assimilation efficiencies and low afflux rates in crustacean zooplankton (Steward and Fisher, unpubl.).

2.5. Subcellular metal partitioning

As described earlier, a metal's speciation in the external (exposure) environment will affect its availability to aquatic organisms. From a chemical point of view, one might expect this influence of metal speciation to extend from the extracellular to the intracellular environment. It has in fact been hypothesised that the manifestation of metal toxicity is associated with the binding of an “inappropriate” metal to a physiologically important molecule (Mason and Jenkins, 1995); the goal of metal detoxification would then be to “protect” these physiologically important molecules by sequestering the incoming toxic metal and minimizing its intracellular bioavailability. It follows that determination of the subcellular partitioning of a metal in a particular target tissue might be a useful indicator of whether or not the host organism had been able to detoxify the metal successfully.

The subcellular partitioning of a metal may also affect its availability for trophic transfer. A prey organism with a high concentration of a particular trace metal represents a potential opportunity for the trophic transfer of the metal from an enriched source to a predator at the next trophic level. The form of detoxified storage of that accumulated trace metal in the prey species has a significant effect on the potential assimilation of that metal by the predator. For example, Nott and Nicolaidou (1990) have shown that the bioavailability to neogastropod mollusc predators of metals present in detoxified metalliferous granules in prey varies among metals and with type of granule; thus the zinc-rich pyrophosphate granules accumulated in barnacles are not digested in the digestive tract of the predator Nucella lapillus and are therefore not bioavailable to that predator. Similarly the physico-chemical form of accumulated cadmium in the oligochaete Limnodrilus hoffmeisteri is
critical in the assimilation of cadmium by a predator, in this case the decapod *Palaemonetes pugio* (Wallace and Lopez, 1997).

### 2.6. Modeling considerations

A mechanistic understanding of the processes involved in the uptake and accumulation of metals by aquatic organisms requires coupling of the different processes in a dynamic manner. To do this, accumulation models can be constructed that link exposure to uptake, compartmentalization and excretion. These models are powerful tools to analyze the separate events and predict the combined result of the processes in a space- and time- resolved manner. Important model input, usually determined from laboratory experiments under well-defined conditions, includes information concerning the concentrations and speciation of the metal present in the exposure media (e.g., water, sediment, food), and data on the kinetics of uptake and elimination of the metal by the organism. This requirement includes information on the feeding rates of the animals under consideration and assimilation efficiencies of the metals from food in the animals. However, once this information is obtained, a model can be used to predict uptake and accumulation under conditions and scenarios that deviate from the experimentally studied cases.

When model predictions for metal accumulation in aquatic animals have been made on a site-specific basis, they have generally matched independent field measurements for metal concentrations in those animals, as shown for marine (Wang *et al.*, 1996a; Griscom *et al.*, 2002) and freshwater bivalves (Roditi *et al.*, 2000), marine copepods (Fisher *et al.*, 2000), and fish (Baines *et al.*, 2002). The close match between model-predicted and measured metal concentration in animals suggests that (1) the laboratory-derived measurements of metal uptake/release parameters are applicable to natural waters, and (2) we can now quantitatively account for the dominant processes governing metal concentrations in marine organisms. Further, models can be used to determine the relative importance of different exposure routes for metal accumulation, the transfer of metals from one trophic level to another, and the effect of changes in exposure conditions and time on the accumulation processes (Blust, this volume).

Different types of dynamic models can be constructed, ranging from very simple one-compartment models to highly complex multi-compartment models. One-compartment models consider the organism as a single homogeneous pool with an input (uptake) and an output (elimination). More complex models incorporate more compartments so that metal uptake and internal compartmentalization are described with more detail and realism. However, the more complex a model becomes, the more information is required to parameterize it. This step requires long-term and detailed experiments and in many cases that information is not available or is difficult to obtain experimentally. Therefore it is important to consider the purpose of the models carefully and decide on that basis what degree of sophistication and resolution is required to answer the questions being addressed. In any case, it has to be realized that models are inherently (over)simplifications of the processes being simulated and that a model is only as good as the quality of the data on which the parameterizations are based and on the soundness and completeness of the assumptions underlying the model structure.

Metals can be eliminated from metabolic pathways either by excretion or by immobilization/sequestration in bones, shells, exoskeletons, or internal precipitates (e.g., Rainbow, 2002). Both uptake rates and elimination rates vary widely among metals, organisms, and environmental conditions. Elimination rates may be particularly slow if accumulation is not limited to specific organs (e.g., liver or kidney) but rather evenly distributed in a large tissue volume. Metabolic processes apparently exert a significant influence on the turnover of metals, in particular those that are predominantly stored in soft tissues, such as methylmercury and radiocesium. Relationships between the metabolism of trace substances and basic environmental parameters may thus assist in the search of general biokinetic models. Quantitative predictions of physiological and ecological kinetics can be generated from simple scaling models and basic physical data, at least to within an order of magnitude (Meili, this volume).

### Recommendations

- Given the potential importance of the free metal ion as a predictor of metal bioavailability in aquatic environments, it would be helpful to compile a critical review of those approaches that
can be used either to measure the free metal ion activity or to calculate it. The review could also address the challenge of collecting marine samples without introducing inadvertent contamination.

- Information regarding the role of microorganisms (e.g., protozoa, bacteria, viruses) in the biogeochemical cycling of metals in coastal and pelagic marine systems is deficient. Metal residence times in the water column are inherently sensitive to the size, settling velocity and trophic fate of the particles with which they initially interact – in this context there is a need to quantify the importance of bacteria and viruses in controlling trace metal concentrations and fate in marine systems.

- Since net bioaccumulation of metals reflects the balance between uptake and elimination, the assessment of metal elimination is just as important as that of metal uptake for understanding variability in metal concentrations among biota. Since metal elimination is controlled by internal (physiological) processes, the link between metal turnover (pathways as well as rates) and general biological turnover patterns related to size, activity, growth, and reproduction deserves particular attention (Wang, 2002).

- The partitioning of a metal in a prey organism will influence the trophic transfer of the metal to the consuming organism. For consumers with very simple digestive strategies, the assimilation efficiency of a metal can be predicted from the physical partitioning of the metal in the prey item (i.e., the proportion of the metal that is present in the cytosol). There is a need to extend this knowledge base to include consumers with more intensive digestive strategies, and to take into account the chemical speciation of the metal in the prey.

- The knowledge base for those organisms selected for biomonitoring purposes in the Mediterranean Sea should be completed (e.g., dynamics of metal uptake and elimination, from water and food; influence of organism physiology on metal uptake and elimination; influence of seasonal physiological cycles; influence of changing environmental parameters). This has largely been done for Mytilus galloprovincialis, which is virtually identical to Mytilus edulis; and, to a lesser extent, for the sea urchin Paracentrotus lividus and the seagrass Posidonia oceanica (Warnau, this volume). However, similar data bases for other key organisms are not available.

- Modeling rates and routes of metal accumulation in marine organisms can provide a useful framework for understanding key processes and should be incorporated into designing appropriate laboratory and field studies. However, sensitivity analyses should be developed by the modelers to determine how finely measured some of the key uptake parameters (including assimilation efficiencies of metals from ingested food, absorption from the dissolved phase, and efflux rates) need to be so that laboratory experiments can become more efficient and more applicable to field conditions.

### 3 - ANALYTICAL CHEMISTRY CONSIDERATIONS

In natural aquatic systems, trace metals exist in different chemical forms such as the free hydrated ions, inorganic and organic complexes and metals associated with colloidal particles. The proportion of these different forms may vary continuously with space and time due to concurrently occurring physical, chemical and biological processes. As noted earlier, any variation in the speciation of an element will affect its bioavailability and its overall mobility in the aquatic system. Thus, not only total metal concentration, but also reliable measurements of trace metal speciation in the aquatic environment are essential for studies of trace metal cycling and metal bioavailability. Most of the techniques involved in such measurements (ASV, ligand-exchange/CSV, extraction on C-18 cartridges, etc.) require that analyses be completed on samples brought to the laboratory. The results obtained with these techniques will thus be affected by uncontrolled changes in temperature, pressure, pH, $P_{CO_2}$ and $P_{H_2S}$ during sample collection, storage or treatment. Also, many of these techniques require the addition of reagents to the sample that modify the metal speciation. The development of in situ analytical techniques to determine the concentration of bioavailable trace substances, in particular free or labile species at high spatial and temporal resolution, could overcome such problems. Since these are often transient products of rapid transformation and equilibration processes, quantification easily leads to manipulation artifacts (i.e., contamination, losses by adsorption, change of speciation due to coagulation of colloids or microbial activity, etc.).
Most of the techniques developed or in the process of being developed for in situ measurements of trace metal speciation in aquatic environment could be divided into two main groups:

a - Diffusional techniques, which include (i) diffusional equilibration samplers [e.g. dialysis (Hesslein, 1976) and diffusive equilibrium in thin-films – DET (Davison et al., 1991)] and (ii) diffusional preconcentration samplers [diffusion gradients in thin-films - DGT (Davison and Zhang, 1994), and supported liquid membranes - SLM] (Parthasarathy and Buffle, 1994).

b - Voltammetric techniques, which include the voltammetric in situ profiling system (VIP System) using either square wave anodic stripping voltammetry (SWASV) or square wave cathodic sweep voltammetry (SWCSV) – see Tercier et al. (1998).

All these techniques and others not mentioned here have certain advantages and limitations. All of them, typically operationally defined to at least some degree, need to be standardized and compared with each other under a wide variety of conditions. Only such well-defined techniques could help in understanding the biogeochemical cycling of trace metals, including transport, bioaccumulation, and toxicity.

Another emerging technology that may be applied as an analytical tool for better understanding metal bioaccumulation and possibly toxicity in marine protists makes use of synchrotron radiation in which target cells are bombarded with hard X-rays. The trace elements in the cells give off characteristic fluorescence patterns when exposed to the X-rays, and software is currently being developed to quantify the amount of metal in individual cells and its site of cellular deposition (Twining et al., in press).

### 4 - Monitoring Implications for Understanding Bioaccumulation Processes

There was also considerable discussion during the Workshop on biomonitoring programs that consider the accumulation of metals by indicator organisms, usually in coastal waters. Indeed, many monitoring programs are based on bioindicators, using the properties of marine organisms to accumulate contaminants and rendering their measurements technically simpler than in water or sediment. There is also the assumption that levels measured in bioindicators represent an integrated value of the mean ambient load that is biologically available. The objectives of monitoring programs are oriented to provide reliable information on specific issues like human health protection, compliance to regulation standards or risk assessment. In most cases, monitoring programs are not primarily designed to investigate bioaccumulation processes per se but to discern spatial and temporal patterns in contaminant concentrations in the environment. However the group tried to identify information gathered from monitoring studies that could be useful in understanding bioaccumulation processes and resulting effects. The group recognizes that there is a need to include both experimentation and observation in any pursuit of understanding bioaccumulation processes. Experimentation controls confounding factors and is the best approach for studying cause-effect relationships, but always simplifies environmental conditions. Observational approaches (including monitoring and field studies) do not control simultaneous variability of many factors (including co-occurrence of a wide range of different contaminants), so cannot ascertain cause-effect relationships. It can be considered that field investigations are the best way to raise questions and draw inferences derived from the complex, natural systems.

Some aspects that have been learned from long-term monitoring programs have been characterized by on-going data interpretations:

1. Variability of pollution inputs (time and spatial scales of effects/exposure conditions).
2. Variability in important aspects of an organism’s life cycle that influence bioaccumulation, e.g., condition index, protein synthesis, enzyme activity, reproductive cycle or seasonal growth dynamics in plants, and application of such information in designing/interpreting bioaccumulation experiments.
3. Relationship between bioaccumulation and biological effects along gradients (e.g., enzymatic biomarkers, fertilization capacity, reproductive maturity, immune defenses, population dynamics, etc.).
4. Relationships between inputs to the coastal zone and temporal trends in indicator organisms on a broad scale (e.g., Pb, $^{137}$Cs, $^{60}$Co, $^{106}$Ru).
5. Flux measurements in complicated circumstances and inferring significance of proposed phenomena in the field (fecal transport from mussel feces to sediment; metal depletion from the water column).

Monitoring programs provide the following opportunities to learn about bioaccumulation through:

1. Understanding dynamic interactions between natural environmental changes (e.g., season) and bioaccumulation and biomarkers.
2. Observation of responses to cumulative effects or responses to multiple pollutants; spectral and multivariate analyses (e.g., multiple regression or principal component analyses) can help draw inferences from such complex data sets (Sanchiz et al., this volume); analyses of single variable plots can be used to better understand responses that are characteristic of each group of variables.
3. Evaluation of simultaneous responses of batteries of monitoring variables (both in terms of bioaccumulation and biological effects through biomarkers; Odzak, this volume; Moukrim, this volume) in different types of circumstances to identify natural variability (both dynamically stable and stochastic characteristics).
4. Monitoring observations in space (along gradients) and time (after a pulse contamination event) provide excellent opportunities to study/understand bioaccumulation processes, to link bioaccumulation to effects or to test conceptual and quantitative bioaccumulation models (e.g., response to the Chernobyl accident).
5. Monitoring over a long period of time can equate to a controlled experiment, if one variable (e.g., contaminant concentration) changes unidirectionally and characteristics of the natural system do not (e.g., observations over time of a “hot spot” during remediation processes; Hornberger et al., 2000); it then allows inferring effects at any level of biological organization, including those that cannot be realistically (time-scale) investigated in the laboratory (e.g., biological cycles such as reproduction, population and community levels) (Luoma, this volume).
6. Validation of theories developed from laboratory experiments under chronic exposure and complex environmental conditions.
7. Demonstration of differences in bioaccumulation processes and sensitivity to metal contaminants among species; this can lead to understanding which species are most valuable as biomonitors of bioavailable metal concentrations in the environment (i.e., which are most responsive to metal bioavailability), and which species (not necessarily the same) are the best bioindicators of contaminant effects (Luoma, this volume).
8. Test of relationships between proposed measures of bioavailability and actual bioaccumulation responses under complex environmental conditions, including using species difficult to study in the laboratory.
9. Use of conservative tissues to assess long-term trends and/or supplement real time monitoring data (through dating invertebrate skeletons or fish otoliths, lepidochoronolgy in the seagrass Posidonia oceanica; Warnau, this volume).

**Recommendations**

The group suggested a number of recommendations that would improve future research and interpretation of bioaccumulation/monitoring data:

1. There should be a better standardization in the use of monitoring organisms and biomarkers (e.g., homogeneity in sampling and methodologies, quality control of analytical procedures).
2. It was considered that a multi-species approach would be useful to cover a wider range of bioindicative information related to the different compartments of the environment (dissolved phase, suspended particulate matter, sediments); selection of species should take into account their geographical distribution and abundance as well as their ability to accumulate the contaminants of concern (Luoma, this volume; Warnau, this volume).
3. Better advantage should be taken of field studies, in particular along gradients or in “hot spots,” to relate metal concentrations with biomarkers in indicator species used in monitoring studies.
4. Monitoring programs could be designed to improve the interpretative power of the data collected (e.g., addition of relevant ancillary parameters, selection of bioindicators representing well identified trophic levels, etc).

5. Monitoring programs could focus on a battery of valuable biomarkers of exposure and effects and need to develop an algorithm to calculate an integrated stress index (Viarengo, this volume).

6. Monitoring programs should, where possible, be combined with field experiments; such efforts specifically can give better insights into bioaccumulation mechanisms or different problems recognized from the monitoring set of data or laboratory experiments. An example of such experiments is the use of transplanted organisms or in situ mesocosms where accumulation is performed under more controlled but still complex environmental conditions (Thébault, this volume).

7. New conceptual models should be developed to consider complex situations, taking into account interactions among different contaminants, including non-metal contaminants in bioaccumulation processes, and subsequent biological effects.

5 - TOXICOLOGICAL CONSIDERATIONS

Research in recent years has shown clear evidence for interactions between trace metal accumulation, homeostatic and detoxification mechanisms, and metal interference with specific metabolic pathways and cell functions.

It is generally accepted that when minimal or physiological amounts of metal cations penetrate into the cells, they are rapidly bound by specific cytosolic ligands such as reduced glutathione (GSH), metallothionein or phytochelatin; metals can also be compartmentalized in lysosomes and granules. Only when the metal uptake rate exceeds the ability of the homeostasis / detoxification systems to process these metals is toxicity evident (Fig. 6). Under such circumstances, these excess metal cations react with cellular components such as enzymes and interfere with metabolic function. The relative importance of specific ligands and detoxification pathways and occur-

Fig. 6. Hypothetical relationship between toxicity of different metals (M1...M4) in an organism as a function of ambient bioavailable metal concentration. The solid lines depict the uptake rates of the metals into the organism, the dotted lines indicate the tolerance limit of the organism for each metal, and the dashed lines indicate toxicity of the metal when the tolerance limits are exceeded for each metal. Note that the tolerance limit is achieved when the rate of metal uptake just equals the maximum detoxification (sequestration plus excretion) rate; when the uptake rate exceeds the detoxification rate, toxicity is exerted. In the figure shown, M1 would be the most toxic metal, M4 the least toxic. The relative toxicities (LC50 or EC50) of the metals would be expected to correlate with their affinities for sulfur ligands (Shaw, 1954), with M1 having the strongest affinity and M4 the weakest affinity.
rence of toxicity responses vary in different cells, tissues and species. Further, both intrinsic and extrinsic factors such as changes in the physiological status in relation to season, age, phase of reproductive cycle, etc., all can affect toxic response. Further complicating matters, it is now recognized that synergistic and antagonistic effects can occur with other metals or classes of pollutants. Notwithstanding the complexity of such phenomena, research developed in the past years has elicited some critical aspects of biological effects of pollutants, allowing the characterization of several cellular responses (biomarkers) that can be used to reveal both the exposure and the deleterious effects caused by these contaminants.

As an important pathway of cellular perturbation induced by trace metals, the imbalance between pro-oxidant forces and anti-oxidant defenses leading to enhanced reactive oxygen species (ROS) production and oxidative stress has been pointed out. Different mechanisms and cell interactions can induce similar alterations, including (a) the binding of some metals to sulfhydryl groups of amino acids and proteins, thus altering the redox cellular potential; (b) the impaired functioning of molecules involved in maintaining the redox potential such as the alterations of enzymatic activities related to ROS removal; (c) elements like Fe and Cu also enhance the intracellular generation of oxyradicals acting as catalysts of Fenton-like reactions. Thus, Cu has been shown to induce ROS-mediated damage at the cytoplasmic and at the nuclear level; in the latter case, a clear effect of DNA oxidative damage was demonstrated.

In addition, data are now available showing the effect of trace metals on different aspects of signal transduction pathways, such as the tyrosine kinase cascade, calcium and cAMP, which can explain numerous cellular responses related both to detoxification and appearance of toxic effects at different cellular levels. In this regard, it has been demonstrated that the mechanisms of destabilization of lysosomal membrane, an early event in the activation of lysosomal macromolecular catabolism, is dependent on the free calcium levels and on the activation of Ca-dependent phospholipase A2. This finding may represent the basis for the interpretation of heavy metal effects on the lysosomal vacuolar system, taking into account that it was demonstrated that Hg, Cd and Cu are able to increase free cytosolic calcium concentration in the cells of different marine organisms when present in seawater at nanomolar concentrations.

New tools to better study the biological responses to bioaccumulated metals are emerging in the field of molecular ecotoxicology. Among these, genomics and proteomics play a key role in the evaluation of mRNA and protein profiles that can characterize the functional state of cells and tissues. The possibility to detect variations induced by trace metals on these cellular processes would represent a fundamental step toward the assessment of metal molecular interactions and their involvement in detoxification and toxicity pathways. In this regard, a mini microarray (containing 25 genes related to stress response) has been specifically developed for marine mussels, and will be soon available for both basic research and application in field biomonitoring programs (Viarengo, this volume).

Monitoring programs using so-called sentinel organisms to monitor the marine environment may provide the opportunity to relate metal co ncentrations to the appearance of potentially deleterious biological effects using biomarkers. A battery of biomarkers (both of stress and exposure) suitable to identify the biological effects attributable to metals is needed. International biomonitoring / research programs, such as MEDPOL and RAMOGE, have found common agreement in the selection of biomarkers to be used for early detection of toxic responses. These core biomarkers should be reviewed in the coming years, taking into account the results of a European-scale project (BEEP, Biological Effects of Environmental Pollutants) that is primarily devoted to this issue.

**Recommendations**

Given the difficulties in evaluating simultaneous variations of different biomarkers in large biomonitoring programs, it would be appropriate to develop in the near future an algorithm suitable to rank the stress syndrome of monitoring organisms on the basis of changes observed in analyzed biomarkers It is also recommended that the choice and analysis of biomarkers be standardized among the various biomonitoring programs established for assessing the quality of coastal waters.

**6. CLOSING COMMENTS - FURTHER DIRECTIONS FOR RESEARCH**

In the course of the workshop discussions, the group noted that important advances have been made in understanding (a) the relationship of metal speciation with bioavailability and passage
across biological membranes, (b) the trophic transfer of metals in marine food webs; (c) the resulting fluxes of metals mediated by organisms in ocean systems; (d) the resulting patterns of toxic responses of organisms to accumulated metals; and (e) the application of biomonitoring programs toward extending the conclusions of laboratory-based studies of metal bioaccumulation toward “real-world” field situations. However, it was felt that more work was needed in areas that have only just begun to receive attention. In addition to the recommended research directions noted above, other priorities include:

1. gaining a better understanding of the mechanisms and rates by which atmospherically delivered metals become bioavailable to marine phytoplankton;
2. examining the interactions of metals with so-called “microbial loop” food webs in general and bacterioplankton and heterotrophic nanoflagellates in particular;
3. evaluating the influence of metal uptake routes on subsequent toxicity in animals; and
4. assessing the effects of multiple metals and/or metals and other contaminants on toxicity in marine organisms.