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### Research Article

Heavy Metals in Marine Pollution Perspective—A Mini Review

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#### Abstract

Anthropogenic inputs of pollutants such as heavy metals into the marine environment have increased their levels to large extents within past a few decades. These pollutants tend to accumulate in the bottom sediments. As a result, ecosystems such as seaports or other industrialized coastal areas that have chronic inputs of metals have highly contaminated sediments. This characteristic has led to concerns over the ecological effects that may be associated with sediment quality. Of particular concern are toxic effects and the potential for bioaccumulation of metals in biota exposed to the sediments. The availability of heavy metals to the biomass of a polluted region is the prime concern both in terms of the prediction of the effects of metal pollution on an ecosystem and in terms of possible human health risks. With growing interest on environmental issues, several intriguing questions related to heavy metals are often raised. This review addresses the basic concepts, sources, speciation, mode of action, levels, analytical measurement, bioavailability, bioaccumulation, biological role and toxicity of heavy metals in the marine environment. Lead, Cadmium, Zinc, Copper, Manganese, Iron, Mercury, Arsenic and Barium are selected because these metals are common and are often at measurable levels in marine samples. An attempt has been made to answer the queries presented by the environmentalists working on various aspects of heavy metal pollution in the marine environment.



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## INTRODUCTION

**Heavy metals-definition and significance:** The term “**Heavy metals**” is widely used by environmental scientists and is widely defined so that now-a-days it rarely means what it says. Lapedes<sup>[1]</sup> includes those metals whose specific gravity is approximately 5 or higher, while Anonymous<sup>[2]</sup> allows the inclusion of metals with specific gravities above 4. Lesaca<sup>[3]</sup> adopted the view of metals of Burrell<sup>[4]</sup> who designated the rectangular block of elements in the periodic table flanked by Ti, Hf, As and Bi at its corners as the heavy metals and added two non metals, Se and Te. According to this interpretation, the 'heavy metals' characteristically have specific gravities ranging from 4.5 (Ti) to 22.5 (Os). Venugopal and Luckey<sup>[5]</sup> consider any metal beyond Ca in the periodic table of elements as 'heavy' which gets nearer to today's usage while Nieboer and Richardson<sup>[6]</sup> proposed the replacement of the nondescript term 'heavy metals' by a classification of metal ions into three classes, which would be both chemically and biologically significant. Class A metal ions (oxygen-seeking) include ions of the alkali metals, alkaline earths, lanthanides and actinides and aluminium. All macro-nutrient metals belonged to class A whereas Class B metal ions (nitrogen/sulphur seeking) included Cu<sup>+</sup>, Rh, Pd, Ag<sup>+</sup>, Ir, Pt<sup>2+</sup>, Au<sup>+</sup>, Hg<sup>2+</sup>, Tl<sup>3+</sup>, Pb<sup>IV</sup> and Bi<sup>3+</sup>. Borderline (or intermediate) metal ions comprised the first row of transition metals, in their common oxidation states as well as Ga<sup>3+</sup>, In<sup>3+</sup>, Cd<sup>2+</sup>, Sn<sup>2+</sup> and Pb<sup>2+</sup>. The hydrogen ion and the metalloid ions As<sup>III</sup> and Sb<sup>III</sup> are also included in this category. The micro-nutrient metals belonged to this class.

In general, the expression 'Heavy Metals' is used where there are connotations of toxicity. A number of authors have critically reviewed the usage of the term “Heavy Metals” and have called it hopelessly imprecise and objectionable<sup>[7-9]</sup>. In our opinion, usage of this term will be continued until IUPAC takes initiative to thoroughly examine the issue and then develop a policy on this subject. The metals discussed in this review include lead, cadmium, mercury, arsenic, barium, zinc, copper, manganese and iron. These metals have been selected firstly due to their toxic effects on living organisms and secondly because their levels are often easily measurable in the marine samples.

**Heavy metals** are natural constituents of the marine and freshwater environment, generally found in very low concentrations. Human activity has inevitably increased the levels of metal ions in many of these natural water systems. Mine drainage, offshore oil and gas exploration, industrial (pesticides, paints, leather, textile, fertilizers, pharmaceuticals) and domestic effluents, agricultural runoff, acid rain etc. have all contributed to the increased metal load in these waters being ultimately incorporated into aquatic sediments.

Some heavy metals are essential for life and some are merely beneficial, many are highly toxic. The concentrations at which metals may be considered important vary as some are essential at low levels yet toxic at others. Criteria for establishing whether or not a trace heavy metal is essential for the normal healthy growth of plants and /or animals include: (i) The organism can neither grow nor complete its life cycle without an adequate supply of the element; (ii) The element cannot be wholly replaced by any other element; (iii) The element has a direct influence on the organism and is involved in its metabolism<sup>[10]</sup>. Apart from C, H, O, N, P, K and S, the elements which have been shown to be essential for plants are: Al, B, Br, Ca, Cl, Co, Cu, F, I, K, Mg, Mn, Mo, Na, Ni, Rb, Si, Ti, V, Zn<sup>[11]</sup>. However, although these elements fulfil the criteria for essentiality many are unlikely to cause deficiency problems in agricultural crops. The unequivocally essential trace elements which are most likely to give rise to deficiency problems in plants are: B, Cu, Fe, Mn, Mo, Zn. Cobalt is vitally important for

symbiotic bacteria in the roots of legumes<sup>[11,12]</sup>.

The elements essential for animals are: Ca, Cl, Cr, Co, Cu, F, Fe, I, K, Mg, Mn, Mo, Na, Ni, Se, Si, Sn, V, As and Zn. In addition, surprisingly the elements such as Ba, Cd, Pb and Sr have also been included in the list of essential elements for their probable role at very low concentrations<sup>[10]</sup>. Several of these elements are essential at very low concentrations and are of little practical importance with regard to deficiencies.

**Metal solubility in seawater:** Solubility of heavy metal compounds in marine environment is an interesting query with regards to dissolved metal levels in seawater and route of metal uptake by marine organisms.

Table 1: The major constituents of seawater<sup>[13]</sup>

Ion	% by weight
Cl <sup>-</sup>	55.07
Na <sup>+</sup>	30.62
SO <sub>4</sub> <sup>2-</sup>	7.72
Mg <sup>2+</sup>	3.68
Ca <sup>2+</sup>	1.17
K <sup>+</sup>	1.10

Table 2: Historic trends in concentrations (nM) of some heavy metals in the ocean<sup>[14]</sup>

Element	1963	1975	1983	1992	1995
Fe	180.0	36.0	0.7	0.6	0.02-1
Cu	50.0	8.0	2.0	2.0	0.5-4.5
Ag	3.0	0.3	0.03	0.02	0.001-0.023
Au	0.02	0.02	0.06	0.00015	0.00005-0.00015
Pb	0.2	0.2	0.005	0.005	0.003-0.150
Bi	0.1	0.1	0.05	0.00015	0.00002-0.0025

Table 3: Solubility product data of some metal compounds<sup>[87]</sup>

Metal Compound	K <sub>sp</sub> at 25°C
BaCO <sub>3</sub>	2.58 x 10 <sup>-9</sup>
BaSO <sub>4</sub>	1.07 x 10 <sup>-10</sup>
CdCO <sub>3</sub>	6.18 x 10 <sup>-12</sup>
CD <sub>5</sub>	1.40 x 10 <sup>-29</sup>
CaCO <sub>3</sub>	4.96 x 10 <sup>-9</sup>
CaSO <sub>4</sub>	7.10 x 10 <sup>-5</sup>
Cu <sub>2</sub> S	2.26 x 10 <sup>-48</sup>
CuS	1.27 x 10 <sup>-36</sup>
FeCO <sub>3</sub>	3.07 x 10 <sup>-11</sup>
Fe(OH) <sub>2</sub>	4.87 x 10 <sup>-17</sup>
FeS	1.59 x 10 <sup>-10</sup>
Fe(OH) <sub>3</sub>	2.64 x 10 <sup>-39</sup>
PbCO <sub>3</sub>	1.46 x 10 <sup>-13</sup>
PbSO <sub>4</sub>	1.82 x 10 <sup>-8</sup>
PbS	9.04 x 10 <sup>-29</sup>
MnCO <sub>3</sub>	2.24 x 10 <sup>-11</sup>
MnS	4.65 x 10 <sup>-14</sup>
Hg <sub>2</sub> CO <sub>3</sub>	3.67 x 10 <sup>-17</sup>
Hg <sub>2</sub> SO <sub>4</sub>	7.99 x 10 <sup>-7</sup>
HgS	6.44 x 10 <sup>-53</sup>
NiCO <sub>3</sub>	1.42 x 10 <sup>-7</sup>
NiS	1.07 x 10 <sup>-21</sup>
Su(OH) <sub>2</sub>	5.45 x 10 <sup>-27</sup>
SuS	3.25 x 10 <sup>-28</sup>
ZnCO <sub>3</sub>	1.19 x 10 <sup>-10</sup>
Zn(OH) <sub>2</sub>	6.86 x 10 <sup>-17</sup>
ZnS	2.93 x 10 <sup>-25</sup>

A clear understanding of the chemistry involved in dissolution phenomenon of metal compounds helps to reach some interesting and sound conclusions especially the **bioavailability**, bioaccumulation and toxicity of heavy metals in the marine environment. Seawater contains 3.5% by weight of dissolved salts, the most significant of which is **sodium chloride**. Just six ions account for over 99% of the salinity of seawater ([Table 1](#)) whereas the metals constitute a minor component of seawater.

Comparatively higher metal levels in seawater reported earlier in the literature are questionable. Over the years, the elemental composition in the world's oceans is being updated with highly specialized oceanographic vessels, new analytical instrumentation, contamination-free sampling protocols and large scale data intercomparisons to measure reliable trace concentrations of many dissolved constituents in seawater.

Table 4: Summary of analytical performance and the characteristics of the most important instruments used in metal analysis<sup>[15]</sup>

System	Liquid sample	Solid sample	Sample vol (mL)	Max. matrix conc. (g L <sup>-1</sup> )	Limit of Detection (ng mL <sup>-1</sup> )	Limit of Detection (ppm)	Sequential multielement	Simultaneous multielement	Matrix effects	Spectral interferences	Precision % RSD
Spark-AES	a)	Ideal	a)	a)	a)	1-10	Yes	yes	Large	Significant	1
Arc-AES	Possible	Ideal	a)	c)	a)	0.1-1	Yes	Yes	Large	Significant	5-10
Flame-AES	Ideal	a)	5-10	30	1-100	-	Yes	Yes	Large	Significant	0.5-1
ICP-AES	Ideal	Possible	1-10	10-100	0.1-10	-	Yes	Yes	Small	Large	0.5-1
GDL-AES	Possible	Ideal	a)	a)	a)	-	Yes	Yes	Small	Significant	a)
Flame-AAS	Ideal	a)	5-10	30	1-10 <sup>3</sup>	-	Possible	No	Large	Few	0.5-1
GF-AAS	Ideal	Possible	0.01-0.1	200	10 <sup>-2</sup> -0.1	-	Possible	Yes	Moderate	Few	3-10
TIMS	Ideal	a)	0.002	1	d)	-	Yes	Yes	c)	Few	0.05-0.5
ICP-MS	Ideal	Possible	1-10	0.1-0.5	10 <sup>-3</sup> -10 <sup>-2</sup>	-	Yes	Yes	Moderate	Significant	1-3
SSMS	a)	Ideal	a)	a)	d)	10 <sup>-3</sup> -10 <sup>-2</sup>	No	Yes	Large	Moderate	a)
GDMS	a)	Ideal	a)	a)	a)	10 <sup>-3</sup> -10 <sup>-2</sup>	Yes	Yes	Small	Significant	a)
Furnace-RIMS	Ideal	Possible	0.001-1	b)	b)	-	No	No	b)	Negligible	b)
GF-LIFS	Ideal	Possible	0.01-0.1	10	10 <sup>-3</sup> -10 <sup>-2</sup>	-	No	No	Moderate	Negligible	5
WD-XRF	Possible	Ideal	c)	c)	c)	0.1-10 <sup>4</sup>	Yes	Yes	Large		1

Spark-AES Spark Atomic Emission Spectrometry; Arc-AES Arc Atomic Emission Spectrometry; Flame-AES Flame Atomic Emission Spectrometry; ICP-AES Inductively Coupled Plasma Atomic Emission Spectrometry; GDL-AES Glow Discharge Lamp Atomic Emission Spectrometry; Flame-AAS Flame Atomic Absorption Spectrometry; GF-AAS Graphite Furnace Atomic Absorption Spectrometry; TIMS Thermo-ionization Mass Spectrometry; ICP-MS Inductively Coupled Plasma Mass Spectrometry; SSMS Spark-source Mass Spectrometry; GDMS Glow Discharge Mass Spectrometry; Furnace-RIMS ; GF-LIFS Graphite Furnace Laser Induced Fluorescence Spectroscopy; WD-XRF Wavelength Dispersive X-ray Fluorescence Spectrometry; a) not applicable; b) no accepted values because the technique is still at a research stage; c) depends on the analytical problems or the sample preparation d) not used for this purpose

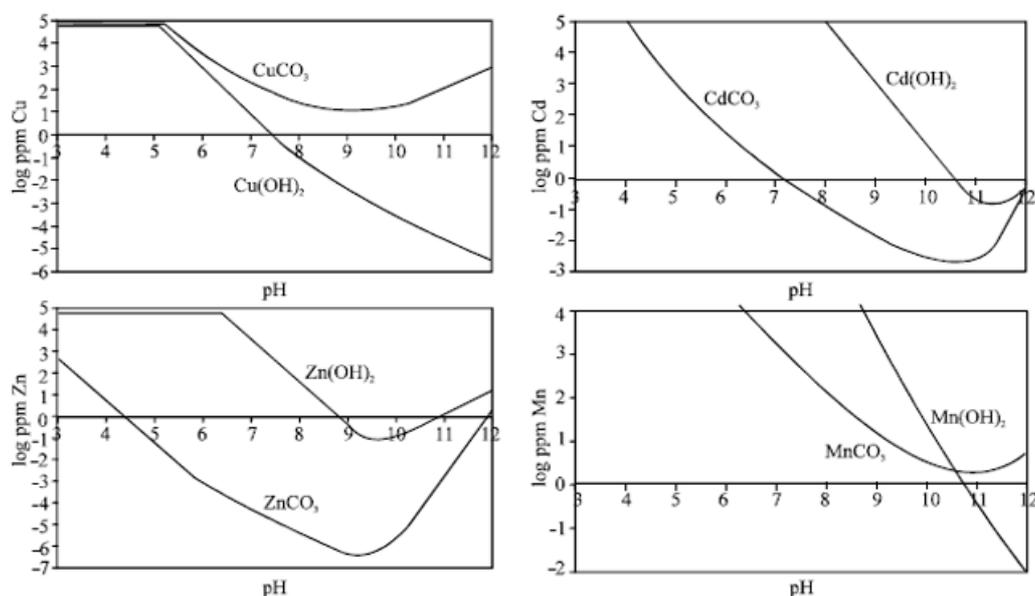


Fig. 1: Solubility of copper, cadmium, zinc and manganese metal ions in seawater at different pHs

An example of historic trends in concentrations of some metals in the ocean is presented in [Table 2](#).

Solubility of heavy metal pollutants in seawater is controlled by several factors such as pH, temperature, salinity, nature of different anions etc. Thermodynamic solubility product data of some metal compounds in water is presented in [Table 3](#).

Metal sulphides show extremely low solubilities in water at 25°C.

The predicted solubilities of various metal ions from their carbonate and hydroxide salts in seawater are not very high considering pH and different side reaction coefficients. [Fig. 1](#) presents the solubility of copper, cadmium, zinc and manganese metal ions from their carbonates and hydroxides in seawater at different pHs calculated from thermodynamic data.

Heavy metal pollutants (either in soluble or particulate form) introduced to the marine environment ultimately settle on to the seabed. The soluble metal cations are precipitated by the anions such as sulphate, chloride, fluoride, bicarbonates/carbonates in seawater. The formed precipitates have extremely low solubilities at the pHs of surface water (8.1-8.3) and deep waters (7.6-8.1) in the marine environment. For example the calculations show that Ba and Pb concentrations resulting from the solubilities of BaSO<sub>4</sub> and PbSO<sub>4</sub> in presence of sulphate i.e. 2725 mg SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup><sup>[115]</sup> in natural seawater do not exceed 3 µg L<sup>-1</sup> and 800 µg L<sup>-1</sup>, respectively. This is supported by the reported concentrations of dissolved **trace metals** in surface and deep waters of the North Atlantic Ocean<sup>[116]</sup>. Concentrations of dissolved **trace**

**metals** in surface and deep waters have been found to be as: Pb (21-31, 4 ng L<sup>-1</sup>), Zn (6.5-13, 105 ng L<sup>-1</sup>), Cu (64-83, 127 ng L<sup>-1</sup>), Hg (0.2-1.4, 0.2 ng L<sup>-1</sup>), Cd (0.1-1.1, 39.3 ng L<sup>-1</sup>), As (1.5, 1.5 µg L<sup>-1</sup>), Mn (55-165, 14-27 ng L<sup>-1</sup>), Fe (3-56, 34-56 ng L<sup>-1</sup>), Cr (182, 234 ng L<sup>-1</sup>) and Ni (117, 352 ng L<sup>-1</sup>). Jeandel *et al.*<sup>[117]</sup> measured dissolved barium levels in the western and southern Indian Ocean during 1985-87 in the French expedition INDIGO. Barium concentrations were found to be 30 nmol kg<sup>-1</sup> at the surface and 100 µmol kg<sup>-1</sup> at depth in the Southern Ocean. At the North of the Polar front, Ba values range from 40 nmol kg<sup>-1</sup> at the surface to 120 nmol kg<sup>-1</sup> in the bottom waters. They reported that Ba was supersaturated with respect to barite (based on thermodynamic calculations) in the upper 3000 m of the southern waters.

It is also accepted that the interaction of dissolved trace elements with particles suspended in seawater is the predominant mechanism of the observed concentration and distribution patterns. Whitfield and Turner<sup>[16]</sup> proposed three principal distribution types of elements on the basis of their biogeochemical interaction with particles. These elements have been distinguished as conservatives, recycled and scavenged. Conservative trace elements include monovalent cations such as Cs<sup>+</sup> or Tl<sup>+</sup> which interact weakly with particles and have long residence time (> 10<sup>5</sup> years). Recycled elements (e.g. Cd, Cr, Ni and Zn) are involved in the internal cycles of biologically derived particulate matter. Their concentrations are depleted in surface waters and increase with depth. Their residence times are in the range of 10<sup>3</sup>-10<sup>5</sup> years. Scavenged elements (e.g. Al, Co, Pb, Hg, As, or Mn) exhibit relatively short residence time (< 10<sup>3</sup> years) due to their strong interaction with particles.

**Analytical techniques for metal analysis:** Analytical techniques have played an important role in solving marine pollution problems and have been extensively applied to measure various heavy metal levels in seawater, marine sediments and biota.

Table 5: Typical instrumental detection limits (µg L<sup>-1</sup>) attainable for aqueous metal samples with various atomic spectrometric methods<sup>[17]</sup> except for NAA (Detection limits are reported in ng, murrwww, 2003)

Metal	FAAS	GF-AAS <sup>a</sup>	ICP-AES	Laser-AFS	ICP-MS	NAA
As	20.0	0.2	45.0	-	7	0.01
Ba	10.0	0.04	0.4	8.0	0.3	1.0
Bi	20.0	0.1	40.0	3.0	0.2	-
Cd	0.5	0.003	2.0	8.0	0.2	1.0
Co	6.0	0.02	7.0	200.0	0.2	0.1
Cr	2.0	0.01	4.0	1.0	0.2	1.0
Cu	1.0	0.02	3.0	1.0	0.1	1.0
Fe	5.0	0.02	3.0	30.0	-	10.0
Ge	100.0	3	40.0	-	1	10.0
Hg	200.0	2	20.0	-	0.4	1.0
Mn	1.0	0.01	0.6	0.4	3.0	0.01
Mo	30.0	0.02	4.0	12.0	-	1.0
Ni	4.0	0.2	8.0	2.0	0.2	10.0
Pb	10.0	0.05	30.0	-	0.3	-
Sb	30.0	0.1	30.0	50.0	-	0.1
Se	100.0	0.5	60.0	-	15.0	0.1
Sn	20.0	0.1	25.0	-	-	1.0
Zn	1.0	0.001	2.0	-	3.0	1.0

<sup>a</sup> 100 µl sample solution; FAAS Flame Atomic Absorption Spectrometry; GF-AAS Graphite Furnace Atomic Absorption Spectrometry; ICP-AES Inductively Coupled Plasma Atomic Emission Spectrometry; Laser-AFS Laser Atomic Fluorescence Spectrometry; ICP-MS Inductively Coupled Plasma Mass Spectrometry; Neutron Activation Analysis

Within past a few decades, many analytical techniques have emerged and have gained acceptance for routine analysis through commercially available systems. These techniques are widely accepted due to several important features such as low detection limits, high sensitivity, high selectivity, wide spread availability, versatility with wide range of metals capable of being detected, modest cost per sample after initial set-up, good precision, acceptable accuracy, automation, ease of operation and interferences in an analysis can be reduced, minimized or eliminated. The analytical performance includes the quality of the results and the quality of the system. The quality of results is related to the accuracy, i.e., both to the precision and trueness. The analytical quality of the system includes the number of elements which can be determined by the method, long term stability, selectivity (absence of spectral interferences), robustness (absence of matrix effects), sensitivity, low limits of detection, linearity and dynamic range. The instrument operation characteristics are related to instrument operation and economic aspects. The instrument operation characteristics include ease of operation, ease of maintenance, full automation, use of any form of the sample, solid, liquid or gas, low sample consumption, small size of the system while the economic aspects include high sample throughput, reliability, safety, low capital investment and low running cost<sup>[15]</sup>. The

summary of the analytical performance and the characteristics of the most commonly used analytical instruments used for metal analysis is given in [Table 4](#).

Table 6: Speciation of some heavy metals in seawater<sup>[18,19]</sup>

Metal	Principal species
As	$\text{HAsO}_4^{2-}$ , $\text{H}_2\text{AsO}_4^-$
Ba	$\text{Ba}^{2+}$
Cd	$\text{CdCl}_2$ , $\text{CdCl}_3^-$ , $\text{CdCl}^+$ , $\text{Cd}^{2+}$
Co	$\text{Co}^{2+}$ , $\text{CoCO}_3$ , $\text{CoCl}^+$
Cr	$\text{CrO}_4^{2-}$ , $\text{NaCrO}_4^-$
Cu	$\text{Cu}_2^+$ , $\text{CuCO}_3$ , $\text{CuOH}^+$
Fe	$\text{Fe}(\text{OH})_3$
Hg	$\text{HgCl}_4^{2-}$ , $\text{HgCl}_3^-$ , $\text{HgCl}_2$
Mn	$\text{Mn}^{2+}$ , $\text{MnCl}^+$
Ni	$\text{Ni}^{2+}$ , $\text{NiCO}_3$ , $\text{NiCl}^+$
Pb	$\text{PbCO}_3$ , $\text{Pb}(\text{CO}_3)_2^{2-}$ , $\text{PbCl}^+$
Zn	$\text{ZnOH}^+$ , $\text{Zn}^{2+}$ , $\text{ZnCO}_3$

Analytical atomic spectrometric techniques in particular have characteristics such as high resolution monochromators which allow separation of very close wavelengths excepts direct overlap, highly efficient and dependable atomization/ excitation sources, reliable and versatile optical components allowing simultaneous or sequential multi-element determination, computerized and automatic control of analysis, computerized collection and reduction of data, excellent, fast and reliable electronic components for detection of transient and small signals buried in noise and stable light sources<sup>[20]</sup>. Detection limits for some toxic heavy metals which can be achieved with various analytical atomic spectrometric techniques are presented in [Table 5](#).

Anodic stripping voltametry (ASV) gives excellent results for a number of heavy metals such as Pb, Cd, Zn, Cu, Bi etc. in seawater samples using glassy carbon electrode. The detection limits can be at the sub  $\mu\text{g l}^{-1}$  level. ASV only applies to the metals which can be reduced at a mercury electrode. Interferences can be minimised by optimisation of the analytical conditions such as degassing, choice of medium, potential range, masking agents, UV photolysis etc<sup>[21,22]</sup>.

**Speciation, bioavailability and accumulation of heavy metals:** Speciation refers to the various physical and chemical forms in which an element may exist in the system. In oceanic waters, it is difficult to determine speciation directly. Whereas some individual species can be analysed, others can only be inferred from thermodynamic equilibrium models. Speciation determines the environmental mobility of an element, especially with respect to partitioning between the water and sediment reservoir<sup>[23]</sup>. Speciation of metal ions in seawater and the main controlling mechanisms are shown in [Fig. 2](#).

Various heavy metals upon dissolution in seawater may give a number of chemical species at different pHs upon interaction with anions available in seawater. [Table 6](#) presents the speciation of some heavy metals in seawater. Heavy metal toxicity is dependant upon chemical speciation.

Bioavailability is an important and interesting aspect in environmental pollution studies. It refers to the portion of the total quantity or concentration of a chemical in the environment or a portion of it that is potentially available for biological action such as uptake by an aquatic organism. Bioavailability incorporates not only characteristics of the chemical and environmental speciation but also the behavior and physiology of the organism<sup>[24]</sup>. Bioconcentration Factor (BCF) represents the ratio of the pollutant (such as heavy metals) in biota and their natural habitat (sediment or water). Bioavailability of heavy metals is an important issue. Any discussion of potential environmental consequences following release or discharge of a toxic substance must be based on a clear understanding of the term **bioavailability**, taken together with relevant and appropriate analytical data.

Peijnenburg *et al.*<sup>[25]</sup> suggested that the current scientific basis for the adequate use of **bioavailability**, in particular for metals, in the assessment of ecological risks at polluted field sites is weak. They proposed that **bioavailability** should be handled as a dynamic process and that the relevant end points relating to **bioavailability** should be taken into consideration explicitly, including the relevant exposure and uptake routes, chemical fluxes for specific biological species, their time dependency and dynamic aspects as well as acclimatisation or redistribution processes within species. They also suggested

that the dynamic approach to **bioavailability** should comprise two distinct and different phases: a **physico-chemically** driven desorption process (also referred to as “environmental availability”) and a physiologically driven uptake process (also referred to as “environmental **bioavailability**”).

Concentrations of heavy metals in sediments usually exceed those in the overlying water by between three and five orders of magnitude. With such high concentrations, **bioavailability** of even a minute fraction of the total sediment metal content assumes considerable importance, especially in benthic organisms. Bryan and Langston<sup>[26]</sup> in a comprehensive review on **bioavailability**, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries, have reported that concentrations and bioavailabilities of metals in estuarine sediments depend on many different processes which include a) mobilisation of metals to the interstitial water and their chemical speciation, b) transformation (e.g. methylation) of metals including As, Hg, Pb and Sn, c) the control exerted by major sediment components (e.g. oxide of Fe and organics) to which metals are preferentially bound, d) competition between sediment metals (e.g. Cu and Ag; Zn and Cd) for uptake sites in organisms, e) the influence of bioturbation, salinity, redox or pH on these processes.

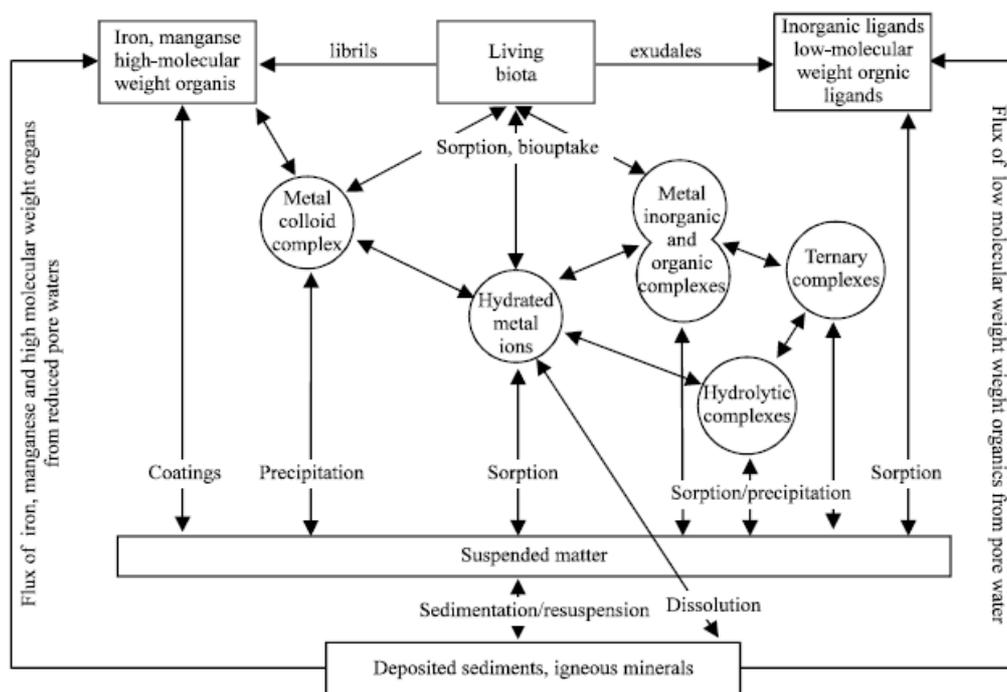


Fig. 2: Speciation of metal ions in seawater and the main controlling mechanisms<sup>[28]</sup>

Strategies adopted by aquatic organisms to counter the toxic impacts of trace elements may be considered to lie on a continuum between ‘ regulators’ which generally exhibit low net uptake rates for metals (because of exclusion mechanisms or efficient excretion) and ‘non regulators’ which may accumulate large concentrations of metals and tend to store them in detoxified forms in the tissues<sup>[27]</sup>. This may occur through binding of metals to metallothioneins and /or in granular form in the tissues<sup>[7]</sup>.

Bioavailability is influenced by a complex variety of interrelated factors; both physico-**chemical properties** of metals and sediments and of biological strategies of the organisms involved. There is still a significant lack of understanding of many of these parameters and how they affect **bioavailability** and particularly of how these processes may affect each other. Such is the complexity of biological systems that a simple relationship between concentration of a metal and its **bioavailability** to organism is not to be expected<sup>[29]</sup>. Under field conditions, examples of deleterious effects on the benthic organisms that can be attributed to specific metallic pollutants are comparatively rare<sup>[26]</sup>. Very little is known about the synergistic or antagonistic effects of groups of even two metals. Interpretation of such effects would be more difficult in the cases where a variety of metals are present such as those found in harytes<sup>[30]</sup>. A thorough knowledge of the processes affecting the **bioavailability** of metals is necessary in order to understand the toxicity and bioaccumulation of metals in the natural ecosystem where variation in the surrounding environmental parameters, the sediment and organisms present is much greater than in a controlled laboratory experiment.

The estimation of **bioavailability** of heavy metals from polluted marine sediments is an important aspect from an ecotoxicological viewpoint since a fraction of these pollutants may be absorbed and accumulated by marine organisms leading to adverse effects. A biological indicator (an organism which may be used to quantify relative levels of pollution by the measurement of the toxicant concentration in its tissues) may be used for this purpose. Either the entire organism, or a part of it, or a single tissue (which may sequester metals from the rest of the organism) may be used<sup>[114, 31]</sup>.

**Effect of route of transport on metal bioavailability:** The route of transport of heavy metals from polluted marine sediment to biota, i.e. as soluble or as suspended particulate form, is a very important if not the most important aspect of metal **bioavailability**. Page *et al.*<sup>[32]</sup> studied the **bioavailability** of toxic constituents of used drilling muds. Four used drilling muds were analysed for Cr, Cd and Pb and petroleum residues to document potentially toxic substances present in the muds. The results showed that the mussels gained the most chromium from Cr<sup>3+</sup> solution. The form of chromium available has a major effect on its uptake by organisms and should be taken into account in assessing potentially harmful effects of discharges of used drilling muds at sea. McLeay *et al.*<sup>[33]</sup> studied the bioaccumulation of metals, Cd, Cu, Pb, Mo and Zn by two species of marine bivalves (filter feeding mussels, *Mytilus edulis*; and detrital feeding *Yoldia limatula*) exposed to Alice Arm (Canada) sediments contaminated with Amax / Kitsault mine tailings in the laboratory. The elevated tissue metal concentrations in the mussels were attributed to dissolved metal released from the Alice arm sediment whereas those in *Yoldia* species were thought to reflect direct uptake from the sediment ingested as well as that from the dissolved metals.

Langston<sup>[34]</sup> determined twelve metals in sediments and benthic organisms in the Mersey estuary, all moderately contaminated with the majority of the metals analysed. Occasionally high levels of Cd, Mn and Ni in sediments and Zn, Cr, Mn and Fe in organisms were detected. Mercury in the estuary is mainly particulate and associated with organic material in sediments. The biological availability of arsenic in the Mersey sediments is similarly influenced by complexation with iron oxyhydroxides. King and Davies<sup>[35]</sup> used laboratory and field experiments to indicate the relative importance of Hg in both dissolved and particulate phases as sources to mussels in a contaminated estuary. Laboratory experiments have demonstrated that mussels can accumulate inorganic Hg from seawater, phytoplankton and suspended sediments. The relative bioavailabilities of inorganic Hg in water, phytoplankton and sediments are estimated to be 10:5:1.

Rule and Alden<sup>[36]</sup> studied the effects of sediment characteristics and geochemical fractions on the biological availability of cadmium to estuarine animals. Sediment cadmium was extracted sequentially to determine the exchangeable, easily reducible, organic sulphides, moderately reducible and acid extractable geochemical phases. The exchangeable and easily reducible fractions of all sediments were directly related to the Cd body burdens of the test species. Despite the fact that the major portion of added Cd entered the organically bound sulphide fraction, the exchangeable and easily reducible fractions that characterised the sands appeared to be most biologically available. They suggested that analytical protocols focussing upon these easily extractable fractions would provide a more ecologically realistic picture of the significance of metal contamination than would bulk sediment analyses and those using strong extraction techniques. Loring and Asmund<sup>[37]</sup> reported on the carriers, transport mode, the dispersal pattern and potential **bioavailability** of metal-rich mine tailings in a fjord system. The metal particles artificially introduced into the system have unique weakly bound (non-detrital) and strongly bound (detrital) characteristics quite different from the natural particles because of the formation of various sulphide/sulphate/carbonate reaction products with seawater. The data suggested that the metal-rich particles containing high proportions of non-detrital lead were responsible for the high lead contamination in mussels. Bourgoin *et al.*<sup>[38]</sup> investigated the factors influencing Pb **bioavailability** to *Mytilus edulis* collected near a lead smelter. Sediment samples were sequentially extracted. Significant correlations occurred between tissue Pb concentration and sediment fractions of exchangeable phase and easily reducible phases. Results indicated that the total sulphur content in the sediment played an important role in defining the Pb accumulation in the mussel tissues. Campbell and Tessier<sup>[39]</sup> studied the partitioning of metals in sediments and tried to find possible links between partitioning and the **bioavailability** of metals. Emphasis has been placed in anoxic sediments in freshwater environments and suggested that the coupling of metal partitioning models with relatively useful geochemical measurements would provide useful predictions of metal concentrations in sediment-

dwelling organisms.

Lobel *et al.*<sup>[40]</sup> explained a major factor contributing to the high degree of variability of some element concentrations in biological tissues. They found that elements which are stored primarily in an insoluble form showed much higher degrees of variability than those stored in a soluble form. Elements such as heavy metals, lanthanides and actinides found primarily in an insoluble form are often isolated from cellular metabolism including any regulatory processes and may build up to high levels in some individuals. The elements of the kidney generally have higher levels of residual variability than those of any other organ probably because of the kidney's ability to store high concentration of elements in insoluble granules. Burbidge *et al.*<sup>[41]</sup> compared the uptake of particulate (elemental) and soluble zinc in whole animal and individual organs in *Mytilus edulis*. Greater whole body concentrations were obtained with particulate zinc than with soluble zinc. The results indicate that soluble zinc is both absorbed and taken up by the tissues far more readily than is particulate zinc.

**Factors affecting the bioaccumulation of heavy metals:** An array of factors, **physico-chemical**, environmental and biological, have been reported to affect the uptake and retention of heavy metals by marine organisms. In general, the whole soft tissue concentration of metals in marine organisms is many times greater than the concentration of metals in the surrounding seawater. Often, the metal level in the organism is proportional to the metal level in seawater so that the organism can be used as a biological indicator of metal pollution. In a bioindicator programme, many variables must be taken into account. For example, the rate of uptake of metal by an organism may depend on the chemical form of the metal which may vary from site to site. Other chemical factors such as pH, temperature, hardness and salinity may affect the rate of metal uptake as well. Indeed, it is even possible for metal pollutants to interact with each other so that one metal inhibits the uptake of another metal by a particular organism.

Possibly the best known factors influencing metal concentration is the size of the organism. It has been observed that in many organisms, the metal concentration is correlated with soft tissue weight of the organism. Negative correlations are more commonly observed than positive ones. No satisfactory explanation of these association has been given. Size effects may show themselves in various ways. For example, differences in metal concentrations between intertidal organisms from different shore levels have been observed and may be partly related to weight differences between these organisms. Age and growth rate, position of mussels in the water column also contributes important roles.

Similarly, **seasonal variations** of the metal concentrations at a given site may often be due to seasonal changes of the organism's tissue weight rather than to any variability in the absolute metal content of the organism. Such weight changes may be gametogenic in origin so that the reproductive cycle of the organism might in some cases significantly influence the whole soft tissue metal concentrations. In a few cases, the metal concentration has been found to be partly dependent on the sex of the organism. Even when organisms are collected extremely cautiously so as to eliminate all known sources of variation, a considerable amount of variation may be present between individuals of the same species from the same site<sup>[42]</sup>. All of these variables may combine to obscure the interpretation of studies on variation in metal accumulation: namely the assessment of relative degrees of environmental contamination.

George and Coombs<sup>[43]</sup> studied the effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Complexation of cadmium with either EDTA, humic and alginic acids or pectin doubles both rate of accumulation and the final tissue concentration. Borchardt *et al.*<sup>[44]</sup> determined trace metal (Hg, Cd, Cu, Ag, Zn and Pb) concentrations in mussels from the estuaries were compared with those of coastal and offshore regions in the south eastern North Sea. *Mytilus edulis* from offshore area contained metal concentrations as high as specimens from estuaries. They suggested that the unexpected high cadmium and lead levels in mussels from the central North Sea may be due to: (i) changes in trace metal speciation-an increasing **bioavailability** counteracts declining concentrations in seawater towards the open sea; (ii) the pattern of residual currents-a great part of the pollutants brought into the sea around the British Isles settles and accumulates in the Central North Sea; (iii) oxygen deficiencies-in certain regions this leads to pronounced changes in redox conditions in sediments thus allowing remobilization.

Hall and Anderson<sup>[45]</sup> reviewed the influence of salinity on the toxicity of various classes of chemicals to aquatic biota. The toxicity of most metals such as cadmium, Cr, Cu, Hg, Ni and Zn was reported to increase with decreasing salinity. This finding is likely related to the greater **bioavailability** of free metal ion (toxic form) at lower salinity conditions. Naimo<sup>[46]</sup> reviewed the effects of heavy metals on freshwater mussels and highlighted some areas to be further investigated such as mode of metal uptake by the mussels in order to design contaminant monitoring programmes and to develop water quality criteria. Boyden<sup>[47]</sup> has demonstrated that heavy metals show increasing contents in bivalves with increasing size whereas the concentrations are uniform or possess modest positive and negative regressions with size. Simpson<sup>[48]</sup> studied uptake and loss of zinc by mussels (*Mytilus edulis*) and relationships with body weight and reproduction cycle. He found that uptake and loss were greatly affected by changing body weights and suggested that phases of reproduction cycle and condition have to be closely considered when reporting on levels of metals in mussels especially in terms of concentration.

Cossa *et al.*<sup>[49]</sup> studied geographical and **seasonal variations** in the relationship between trace metal content and body weight in *Mytilus edulis*. The relationships between trace metal contents (Cd, Cu, Fe, Mn, Ni and Zn) and body weight of *Mytilus edulis* show that smaller mussels are richer in **trace metals** than larger ones. Multivariate analyses showed that the weight accounted for most of the variance in metal content while age and gonad maturation explained little of the variance observed. Latouche and Mix<sup>[51]</sup> studied **seasonal variation** in soft tissue weights and trace metal burdens in *Mytilus edulis* and suggested that gonadal and somatic tissues should be analysed separately. More meaningful results would be provided if tissue weights were recorded since predictive relationships between tissue weights and metal contents could then be developed.

Table 7: Apparent stability constants for mercury, cadmium and lead for different biochemical groups<sup>[50]</sup>

Group	Log K (pH = 7)		
	Hg	Cd	Pb
Carboxyl	5.6	1.8	1.9
Amino	6.0	0.3	-0.5
Imidazole	3.7	2.7	2.2
Thiol	10.2	5.6	4.9
Phosphate	-	2.7	3.1
Chloride	7.3	2.0	1.6

Popham and D'Auria<sup>[52]</sup> studied the combined effect of body size, season and location on trace element levels in mussels (*Mytilus edulis*). Janssen and Scholz<sup>[53]</sup> studied cadmium uptake in starved and fed specimens of *Mytilus edulis* and suggested that fairly elevated cadmium contents in fed mussels are not due to contaminated food but to increased pumping rate when food is available. Highest concentration and main body burden are found in the mid-gut. Transport via haemolymph and selective discrimination at the basement lamina of the mid-gut gland tubuli are regarded as mainly responsible for accumulation. In the tubuli metals are immobilised in membrane-bound vesicles which are finally defecated. George *et al.*<sup>[54]</sup> investigated subcellular localisation of heavy metals in polluted marine shellfish by electron microscope X-ray analysis and revealed that bivalve shellfish tolerate the extremely high concentrations of heavy metals within the tissue by isolating the potentially toxic metal within a membrane thereby immobilising and detoxifying it. Popham and D'Auria<sup>[55]</sup> developed statistical models for estimating seawater metal concentrations from metal concentrations in *Mytilus edulis*. Szefer<sup>[56]</sup> investigated inter-elemental relationships in organisms and bottom sediments of the Southern Baltic. The correlation coefficients computed for Baltic organisms are not in principle comparable to those for bottom sediments. This indicated that post-depositional antagonistic and synergistic processes play an important role in the redistribution of some metals in detritus from dead marine organisms forming a biogenic fraction of the bottom sediments. Cross correlation coefficients computed for all metal pairs in sediment cores showed highly significant association of Ni, Ti, Co, Th, K and partly Mg with Al and Fe.

Nott<sup>[57]</sup> reviewed the applications of microanalytical techniques to the cytology of pollutant metals in marine invertebrates. The applications of X-ray microanalysis (XRMA) to pollution research is providing information on the effects of metals on marine organisms and is demonstrating that detoxification of metals by a particular species can reduce the availability and

toxicity of metals to other organisms.

Chou and Uthe<sup>[58]</sup> studied the effect of starvation on trace metal levels in *Mytilus edulis* and reported that starvation has no effect on either shell length or total weight, though a substantial decrease (43.3%) in soft tissue weight was observed. Tay *et al.*<sup>[59]</sup> assessed the biological effects of heavy metals and organic contaminants in Halifax harbour sediments by a series of toxicity tests. Extraction of metals from the sediments and tissue samples was carried out by acid digestion procedures. Samples were totally decomposed by HF to analyse Cd, Pb, Zn and Cu whereas samples were digested with H<sub>2</sub>SO<sub>4</sub>/HNO<sub>3</sub> for As. Mortality of the biological indicator *Macoma* was increased, although no significant uptake of contaminant was detected in the bioaccumulation test.

Robinson *et al.*<sup>[60]</sup> confirmed the presence of particulate metals in the gut of *Mytilus edulis* having a marked effect on the reported concentration of mussels' whole body' Al, Cr, Fe, Ni concentrations. Wang *et al.*<sup>[61]</sup> studied the assimilation of trace elements ingested by the mussel and found that feeding components of the mussel (*i.e.* gut passage time, digestive partitioning and metal chemistry (*i.e.* metal release at lowered pH within the bivalve gut) are responsible for the difference in the assimilation of **trace metals** at different food quantities observed in mussels.

**Toxicity of heavy metals:** The concept that the toxicity of a material resides in the quantity to which an individual is exposed is generally attributed to the philosopher-physician Aureolus Phillipus Theophrastus Bombast also known as Paracelsus the Great: 'All substances are poisons [he wrote]; there is none which is not a poison. The right dose differentiates a poison from a remedy'. [Fig. 3](#) shows the relationship between health response and the concentrations of the elements.

Heavy metal toxicity can be influenced by a number of factors such as its geochemical behaviour and the physiology of the target species considered. The following have been included among the most important of these factors:

- Chemical speciation of metals in the aquatic environment: These may be found in the form of free ions or as organometallic molecules and be transported in dissolved or particulate phase. The distribution of dissolved and particulate fractions must be taken into account when examining pathways into the organism.
- Presence of other metals or toxicants: This can reduce (antagonism) or increase (synergism) the additive toxicity of each element.

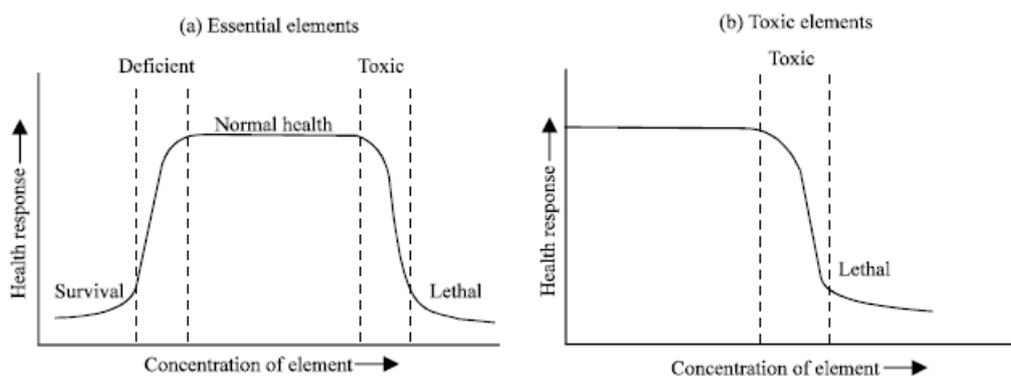


Fig. 3: The relationship between health response and the concentrations of the elements, (a) essential elements, (b) toxic elements<sup>[62]</sup>

- Environmental conditions: Temperature, pH, salinity and dissolved oxygen are parameters which condition the physiological activity and the metabolism of aquatic organisms thus rendering them more or less susceptible to the effects of toxicants.
- Condition of the organisms tested: The sensitivity of the organisms varies according to a number of factors such as the age of the individuals (larvae/juveniles/adults), their sex, food (starvation), reproduction etc.
- Adaptation of the organism to the absorption of metals: The study of the bioaccumulation of metals in marine organisms has clearly demonstrated the existence of detoxification mechanisms. Thus metals can be: (i) stored in specialised cells, as the case for oyster amibocytes with copper and zinc; (ii) blocked by complexation with thio-proteins of low molecular weight (metallothioneins) whose biosynthesis they provoke, as is the case of the elements in groups I B and II B, Cu, Ag, Au, Zn, Cd and Hg for molluscs, crustaceans and mammals and (iii) immobilised by the formation of stable compounds based on antagonistic elements which is the case for mercury and selenium which form mercuric selenide in the tuna fish. These mechanisms all achieve the aim of limiting the effects of chronic exposure<sup>[63]</sup>.

A number of heavy metals are toxic because of their interaction with sulphur-containing biochemicals, such as enzymes and proteins. Some of the metals are soft acids and interact strongly with soft bases of which sulphur is an example. Softness is related to the size of the atoms. Larger atoms, which have a lot of electrons, are polarizable and are therefore able to form strong interactions with other polarizable atoms. The interaction also has stereochemical requirements. Another factor to consider is the kinetics of toxic reactions. Some factors affecting the kinetics are solubility of the metallic species, bond energies and accessibility of the metal to a nucleophile<sup>[64]</sup>. The stability constants for metal organic systems are a useful guide to the biological influence of a metal. The apparent and approximate stability constants at a pH of 7 for the three metals mercury, lead and cadmium bonded to different groups are given in the [Table 7](#).

The data clearly shows the affinity of the metals for sulphur compared with other donor systems (-O, -N, PO<sub>4</sub><sup>3-</sup> and Cl<sup>-</sup>). The low molecular weight protein metallothionein (MW ~ 600-7000) which contains 61 or 62 **amino acids** is produced in the liver and is an important protection against some heavy metals such as cadmium, mercury and lead. One third of the residues in the proteins are cysteine, giving a high sulphur donor atom content to the protein. The cysteine residues are so arranged that when a metal is coordinated, cage structures are produced such as M<sub>3</sub>S<sub>3</sub> and M<sub>4</sub>S<sub>6</sub><sup>[10,65,66]</sup>.

Low levels of dissolved metals in seawater may or may not present a hazard to marine biota but some species have shown a tendency to accumulate the metals from the water. Sediment bioassays involving benthic organism (*Corophium volutator* Pallas) and barytes (naturally occurring BaSO<sub>4</sub>) carried out at the University of Aberdeen have shown the bioaccumulation of toxic heavy metals such as Ba, Pb, Zn and Mn from barytes spiked marine sediments<sup>[30]</sup>.

**Assessment of heavy metal toxicity and bioavailability from polluted marine sediments:** Assessment of sediment quality has often been limited to chemical characterization. However, quantifying contaminant concentration alone cannot provide enough information to evaluate adequately the potential adverse effects, interactions among chemicals, or time dependant availability of these materials to aquatic organisms<sup>[67]</sup>. Because relationships between concentrations of contaminants in sediment and their **bioavailability** are poorly understood, the investigation of contaminated sediment effects on aquatic organisms requires controlled toxicity and bioaccumulation tests.

Sediment has been defined as particulate material normally lying below water. For experimental purposes a sediment could be formulated from particulate material<sup>[68]</sup>. Whole sediment is typically heterogeneous in physical, chemical and biological characteristics and may include **organic matter** in various stages of decomposition or synthesis, particulate mineral matter and **inorganic matter** of biogenic origin<sup>[69]</sup>. Four main components of sediment have been identified. The largest component is interstitial water, which surrounds sediment particles and is typically 50% by volume of sediment. The second largest component consists of inorganic phases including rock fragments and minerals. The inorganic phases control the **bioavailability** of many divalent metals. The third component is **organic matter** which occupies a small proportion of the sediment. Organic matter is an important component because it controls the sorption and **bioavailability** of many non-ionic organic contaminants. The fourth component consists of anthropogenically derived materials including contaminants<sup>[70-72]</sup>.

Grain size of sediment is generally classified in the following categories: clay (< 2 µm), silt (2 to < 50µm), sand (50 to 2000 µm) and cobble and gravel (>2000 µm). The coarse fractions are composed primarily of stable inorganic materials and are not generally associated with chemical contamination<sup>[73]</sup>. Problem contaminants are most often associated with fine sediment because this fraction consists of particles with relatively large ratios of surface to volume which increase the sorptive capacity for contaminants<sup>[70]</sup>.

Sediments provide habitats for many aquatic organisms and also a major repository for many of the more persistent chemicals that are introduced into surface waters. In the aquatic environment, most anthropogenic chemicals and waste materials including toxic organic and inorganic chemicals eventually accumulate in sediments. Concentrations of contaminants in sediments may be several orders of magnitude higher than in the overlying water. However, bulk sediment concentrations are not highly correlated to **bioavailability**<sup>[74]</sup>.

Partition or sorption of a compound between water and sediment depends on many factors, including aqueous solubility, pH, redox, affinity for sediment organic carbon and dissolved organic carbon, grain size of the sediment, sediment mineral constituents (oxide of iron, manganese and aluminium) and the quantity of acid volatile sulphide in the sediment<sup>[71,75]</sup>. Although certain chemicals are highly sorbed to sediment, these compounds may still be available to biota. Contaminated sediments may be directly toxic to aquatic life<sup>[76,77]</sup> or can be a source of contaminants for bioaccumulation in the food chain<sup>[78]</sup>.

Sediment toxicity may be defined as: 'the ecological and biological changes that are caused by contaminated sediments' or 'an adverse response observed in a test organism exposed to a contaminated sediment<sup>[79]</sup>. Toxicity and **bioavailability** of contaminated sediments can be assessed in a number of ways, *i.e.* **chemical analysis**, ecological studies and sediment bioassays. Each method has its strengths and limitations<sup>[80]</sup>. Chemical analysis is the most widely used method for the identification and quantification of contaminants in sediments. Although analytical technology is ever expanding and is highly recommended for any toxicity study, it still has some serious limitations. Many methods may be equipment limited, inadequately developed or too expensive for routine applications. Even where contaminant concentrations can be measured accurately and precisely (as in case of metal concentration measurements), **chemical analysis** does not provide information on their availability to the resident biota or their potential for adverse effects. Particular contaminants are less bioavailable than others. For instance, many metals are commonly bound to sediments or particulates in water to such an extent that for all intents and purposes they are inert. Thus, high measured levels of certain metals, although very frightening, may be of minimal concern. In contrast, certain other compounds (e.g. chlorophenols) may be present in only relatively small concentrations, but may have a large impact on the resident biota. Consequently, bulk chemical measurements alone are not sufficient to assess the potential for environmental damage<sup>[81]</sup>.

Direct determination of ecological changes caused by contaminated sediment is another approach to assessing contaminant's toxicity and **bioavailability**. Some studies have successfully correlated marine sediment contamination and community changes<sup>[80]</sup> but more often ecological change is extremely difficult to demonstrate. Contaminant effects are difficult to separate from responses to other environmental influences unless ecological processes are well understood. Measurement of community structure is also not enough to demonstrate contaminant effects. The presence/absence of flora or fauna may be due to pollution, but may also be due to factors such as environmental fluctuations (e.g. temperature, salinity, **dissolved oxygen**, pH), physical parameters (e.g. sediment texture) or biotic factors (e.g. reproductive cycles, competition, predation)<sup>[81]</sup>. Contaminant effects will also interact with the effects of natural stresses<sup>[79]</sup>.

Chemical and ecological surveys can be conducted simultaneously and correlations made between the two data sets to test the hypothesis that high levels of particular contaminants and the absence of sensitive fauna are correlated. However, the interpretation of these data is not always clear. Effects of contaminants in mixtures associated with real world sediments are rarely driven by a single factor. Synergistic/antagonistic effects with other contaminants and/or **environmental factors** are probably significant and prevent determination of simple cause-and-effect relationships<sup>[81]</sup>.

Bioassay tests are defined as "estimations of the amount of a biologically active substance by the level of its effect on a test organism". Bioassay (tests with field collected sediments and water) as a significant monitoring and assessment tool has gained importance in recent years. Bioassays of field-collected samples (sediment bioassays) fall into several classes, including acute lethal, sublethal and genotoxic. These bioassays are performed in the laboratory following exposure of the organisms either to the sediments directly or to an extract of the sediments<sup>[81]</sup>. Bioassays address the problems of interactions in contaminant mixtures and measure directly a biological response to the sediment contamination. They therefore improve on many of the problems of **chemical analysis** and overcome the problems of predicting **bioavailability**<sup>[79]</sup>. However, bioassays do have limitations, particularly the limits of ecological extrapolations. Persoone *et al.*<sup>[82]</sup> pointed out that most bioassays are performed under test conditions which are close to optimal, yet in nature, aquatic organisms must cope with fluctuating environmental conditions. Depending on the test involved, some of these limitations

can be improved by continued development of the technology and by greater standardisation of particular tests. However, it is doubtful whether any type of laboratory test can be made to reproduce successfully *in situ* sediment conditions, especially with regard to water movements<sup>[83]</sup>. Single-species bioassays have been particularly criticised in this context. Sensitivities to contaminants in toxicity tests differ widely among species and the causes of those differences are not well understood. Species may vary widely in ecological, physiological and life-history characteristics which affect survival in nature. The specific influences of contaminated sediments on those processes are often not well understood.

Keeping in view the limitations of each method, an integration of these methods would seem to offer an optimal approach for the assessment of toxicity and **bioavailability** of sediment-bound contaminants. This integration of techniques has been pioneered by Chapman, particularly with the use of the Sediment Quality Triad<sup>[84]</sup>. It involves three types of measurements; (1) concentrations of toxic chemicals (2) toxicity of environmental samples and (3) evidence of modified resident biota. Long and Chapman<sup>[84]</sup> recommended that once the technology and significance of sediment bioassays had been established, the studies of *in situ* community ecology could be phased out. According to Chapman<sup>[85]</sup>, sediment bioassays can be used in two separate ways to develop sediment quality criteria: (a) sediment bioassay and chemical analyses can be conducted with sediments collected from contaminated and reference areas. The bioassay responses can be compared quantitatively to identify whether problems exist and the levels of contaminants in sediments can be related to the bioassay responses, (b) dose-dependent relationships can be developed in the laboratory by spiking sediments with individual and mixed contaminants and then carrying out bioassays on these sediments. A number of test methods have been developed by the American Society for Testing and materials<sup>[68]</sup>, the U.S. Environmental Protection Agency and the U.S. Army Corps of Engineers of Materials<sup>[86]</sup>.

Chapman and Long<sup>[81]</sup> suggested that the use of a comprehensive, integrated approach to marine pollution assessment including bioassays would provide the strongest evidence of gradients in contaminant levels and adverse effects resulting from these contaminations. They strongly recommended that bioassays should be a central and integral part of any marine pollution assessment.

**Biological role of heavy metals and human health hazards:** In aquatic systems, the heavy metals of greatest concern are copper, zinc, cadmium, mercury and lead. These elements are toxic to organisms above specific threshold concentrations but many of them (e.g. copper and zinc) are essential for metabolism at lower concentrations. Lead and cadmium have no biological function. Other elements of concern are aluminium, chromium, selenium, silver, arsenic and antimony which have contributed to serious problems in freshwater, estuarine and coastal ecosystems.

**Heavy metals** are often cumulative toxins and have strong effects when consumed by human beings. Cumulative toxic substances continue to increase in concentration and may be found most abundantly in a single tissue. The accumulation of toxic substances may result in food chain magnification of concentrations in animals at higher trophic levels. A brief account of accumulation and biological role of trace heavy metals in marine animals and the possible human health hazards of these metals is given below:

**Lead:** Lead is not an essential element. Sub-lethal effects of low lead concentrations include a depression of the growth of *Cristigera* (a ciliate protozoan) by 8.5% at 0.15 mg L<sup>-1</sup> and 11.8% at 0.3 mg L<sup>-1</sup>. The growth of the crustacean *Artemia* is significantly reduced at 5-10 mg L<sup>-1</sup> and the mortality rate of the mussel *Mytilus edulis* is increased by prolonged exposure to 10 mg L<sup>-1</sup> or less of lead salts<sup>[88]</sup>. Nevertheless, high concentrations of lead can be accumulated by some animals without apparent harm. The limpet *Acmaea digitalis* may contain about 100 µg g<sup>-1</sup> on the Californian coast near San Francisco where evidently the contamination is from aerial fall-out. In the contaminated Sorfjord in Norway, sea weeds and animals contain high levels, the content in mussels rising to 3000 µg g<sup>-1</sup>. Fish contain little lead and the content in commercial species in the North Sea generally ranges from 0.05-0.15 µg g<sup>-1</sup> (wet weight). A rare instance of lead poisoning occurred among shore birds wintering on the Mersey estuary and a large number of birds died. The dead birds contained more than

10  $\mu\text{g g}^{-1}$  (dry weight) of lead in the liver, 30-70% of which was in the form of trialkyl lead which originated from a factory manufacturing tetraethyl lead additives for petrol. Investigations showed that birds with 0.5  $\mu\text{g g}^{-1}$  trialkyl lead in the liver were at risk and some birds showed neuromuscular disorders symptomatic of lead poisoning. With regard to organolead compounds, there is some evidence concerning their acute toxicities to benthic organisms<sup>[89]</sup> but a few studies have been reported on their long term effects. Lead is toxic and a major hazard to man and animals. Lead has two quite distinct toxic effects on human beings, physiological and neurological. The relatively immediate effects of acute lead poisoning are ill defined symptoms, which include nausea, vomiting, abdominal pains, anorexia, constipation, insomnia, anaemia, irritability, mood disturbances and coordination loss. In more severe situations neurological effects such as restlessness, hyperactivity, confusion and impairment of memory can result as well as coma and death<sup>[90-92]</sup>.

**Mercury:** Mercury is considered as a non-essential element for living organisms and has been studied in connection with mercury poisoning. Organic forms of mercury are highly toxic to marine biota. A number of mercury compounds show toxicity to the red algae *Plumaria elegans*, larvae of the barnacle *Elminius* and brine shrimp *Artemia*. As with other metals, bivalve molluscs take up mercury from the surrounding very quickly. Most species of fish in oceanic waters contain 150  $\mu\text{g kg}^{-1}$  mercury in muscles, though much higher values are found in fish from contaminated waters. Cod taken from the Sound between Denmark and Sweden, which is heavily contaminated with mercury contains up to 1.29  $\mu\text{g g}^{-1}$ ; those from North Sea 0.15-0.20  $\mu\text{g g}^{-1}$ ; and those from the Greenland only 0.01-0.04  $\mu\text{g g}^{-1}$ . Some species notably tunny (*Thunnus* sp.), swordfish (*Xiphias gladius*), marlin (*Makaira indica*) and some other large, oceanic pelagic fish, naturally contain high concentrations of mercury. Marine mammals accumulate large quantities of mercury from their food apparently without coming to harm. Selenium antagonises the toxic effects of mercury and in seals, sea lions and dolphins, the selenium keeps pace with the amount of mercury. Mercury selenide found in the connective tissue of the liver of dolphins is apparently a product of a detoxifying mechanism for the methyl mercury acquired from their food<sup>[88]</sup>.

Mercury is among the most toxic elements to man and many higher animals. All chemical compounds of Hg are toxic to humans<sup>[93]</sup>, although  $\text{Hg}^0$  may have to be oxidised to ionic forms in order to show toxic effects. Mercury salts show a high **acute toxicity**, with a variety of symptoms and damages. Mercury ions and fumes may cause mercury poisoning which injures skin and the kidneys. However, the victim recovers quickly if the exposure ceases, since the inorganic mercury compounds are removable from the body.

Some organomercurials, in particular low molecular-weight alkyl mercury compounds are considered even more hazardous to humans because of their high chronic toxicity with respect to various, largely irreversible, defects of the nervous system. The organic part of the molecule to which the mercury is attached, makes it easily soluble in fats. Therefore it may dissolve in the skin fat layer surrounding the nerve cord. Methyl mercury is particularly highly toxic. It appears to show strong teratogenic effects and carcinogenic and mutagenic activity has also been implied. The investigations initiated by these events and carried out during subsequent decade showed that elevated methyl mercury levels in fish were widespread globally. Thus methyl mercury is the dominant toxic Hg species in the environment, consumption of fish and crustacea being the main hazard to humans and higher animals. Mercury has well characterised toxic effects on both the physiological and the neurological systems of the human body. The mercury vapour is a serious toxin especially in the occupational environment. The critical organs are the lungs, kidneys and the brain. The vapour crosses the blood-brain barrier and the metal is oxidised in the brain and in the blood stream. Once oxidised the mercury remains in the brain whereas free metal may move out again<sup>[94,95]</sup>. The effect of mercury vapour on the respiratory tract are coughing, acute bronchial inflammation, chest pains and in severe cases respiratory arrest. Acute ingestion of inorganic mercury can cause precipitation of protein in the gastrointestinal tract and produce gastric pain, vomiting and bloody diarrhoea. Renal damage can occur, including oliguria, severe anuria with azotemia and in severe cases renal failure<sup>[96]</sup>. Inorganic mercury (II) also has an adverse effect on the central nervous system. The most serious mercury toxin is methylmercury. It has serious neurological health effects. Methylmercury readily crosses the placental and blood brain barriers and it causes disintegration (lysis) of cells within the brain. This may involve a  $\text{CH}_3\text{Hg-S}$  interaction. The effects observed on human beings are, initially loss of sensation at the

extremities and around the mouth (paresthesia) followed by loss of coordination in movement e.g., walking, slurred speech, loss of hearing, restricted visual blindness, coma and death<sup>[91,97]</sup>. Pregnant women and unborn children, are most sensitive to methylmercury, especially developing brain system of the child. Infants born to mothers with high intakes of mercury have had serious mental disturbances, including retardation of mental and physical development<sup>[98]</sup>.

**Cadmium:** Cadmium is not an essential element for any organism although, for unknown reasons, it has been reported to enhance phytoplankton photosynthesis and growth at concentrations up to 100 mg L<sup>-1</sup>. Because of its associations with phosphates, cadmium is assumed to be taken up by phytoplankton but, except in two instances, it does not appear to accumulate in the food chain. The cuphausiid *Meganyctiphanes norvegica* for example, feeding on phytoplankton containing 2.1 µg g<sup>-1</sup> (dry weight) of cadmium produces faecal pellets containing 9.6 µg g<sup>-1</sup> (dry weight), but has a whole-body concentrations of only 0.7 µg g<sup>-1</sup> (dry weight). At higher levels in the food web, fishes and sea mammals have low concentrations of cadmium, at the most a few ppm. Stored chiefly in the kidney and they are able to detoxify it by the production of metalloprotein. Molluscs accumulate large concentrations of cadmium. This is particularly so in the bivalve Pectinidae: *Pecten novae-zeelandiac* has been found with 2000 µg g<sup>-1</sup> (dry weight) in the liver, but 1900 µg g<sup>-1</sup> (dry weight) has been found in oceanic squid *Symplctoteuthis oualaniensis* and oysters, limpets (*Patella vulgata*) and dog whelk (*Nucella lapillus*) also acquire high concentrations of cadmium<sup>[88]</sup>.

The common limpet along the Bristol Channel-Severn estuary was studied by Shore *et al.*<sup>[99]</sup> and a correlation was shown between increasing levels of cadmium and a reduced ability to utilise glucose. Dissolved concentrations similar to those found in this estuary were found to be toxic to animals under experimental conditions. The toxicity of low sediment-cadmium concentrations was suggested by observations showing that in San Francisco Bay, the clams declined as sediment cadmium concentration increased from about 0.1 to 0.4 µg g<sup>-1</sup><sup>[100]</sup>. Cadmium is a very toxic metal and has been responsible for a number of deaths. The most serious situation being the disease called *Itai Itai* disease. The major effects of cadmium poisoning are experienced in the lungs, kidneys and bones. Acute effects of inhalation are bronchitis and pneumonitis and toxemia in the liver. Chronic inhalation of cadmium compounds as fumes or dust produce pulmonary emphysema, where the small air sacs of the lungs become distended and eventually destroyed reducing lung capacity. Both the chronic inhalation and oral intakes of cadmium affect the kidneys producing in the first instance proteinuria similar to proximal tubule proteinuria<sup>[101, 102]</sup>.

**Arsenic:** The chemistry of arsenic in marine development is complex and fascinating. Although arsenate is the dominant species in the photic zone, arsenite, MeAsO(OH)<sub>2</sub> and Me<sub>2</sub> AsOOH are found in significant concentration<sup>[103]</sup>. Arsenic is concentrated by aquatic organisms but not a great deal is known about accumulation ratios for organic arsenic compounds. Algae accumulate more than do fish, with crustaceae accumulating intermediate amounts. The chemical forms of arsenic are important as regards its toxicity. The toxicity of the element decreases in the order, As (III) > As (V) > organoarsenic. The gas arsine AsH<sub>3</sub> is particularly toxic. It appears that one of the bodies defences against arsenic is alkylation, producing the less toxic alkylarsenic (V) compounds CH<sub>3</sub>AsO(OH)<sub>2</sub> and (CH<sub>3</sub>)<sub>2</sub>AsO(OH), which are then excreted from the body<sup>[104]</sup>. The acute effects of arsenic poisoning by oral intake are, intense abdominal pains, nausea, vomiting, diarrhoea from damage to the gastrointestinal tract, finally coma and death. Respiratory effects from inhalation include irritation in the nose and throat. Also neurological effects occur such as headache, vertigo, restlessness and irritability<sup>[91, 105]</sup>. Arsenic and selenium are antagonistic to each other in the body and each counteracts the toxicity of the other. Whereas As (III) is regarded the more toxic form of the element, As (V), as arsenate can be disruptive by competing with phosphate and thus uncouples oxidative phosphorylation. Arsenic may also replace the phosphorus in DNA and this appears to inhibit the DNA repair mechanism<sup>[91]</sup>. Arsenic affects the skin and in its most severe form causes skin cancer. The signs of arsenic toxicity on the skin are hyperpigmentation, especially in the areas not exposed to the sun. Arsenic has also been implicated in lung cancer, especially when the arsenic compound inhaled is of low solubility. However, the evidence for this effect is less strong. Arsenic has an effect on the liver producing cirrhosis of the liver and a rare form of cancer called haemangioendothelioma. A

number of other health effects of arsenic have been reported. It can affect the blood system and interfere with porphyrin biosynthesis and affect the white blood cells. Effects on the reproductive system such as spontaneous abortions have been attributed to arsenic. Loss of hearing has also been associated with large scale arsenic poisoning. Incidences, such as contamination of milk in Japan and emissions from copper smelters have caused hearing loss<sup>[98]</sup>. Arsenic also affects the renal system, particularly the reabsorption process. The peripheral nervous system is reported to be influenced by arsenic, such as peripheral neuritis and motor sensory paralysis<sup>[105]</sup>.

**Barium:** There is no conclusive evidence for any essential function of barium in animals or plants. Barium has no biological role. The British Pharmaceutical Codex from 1907 indicates that barium chloride ["barii chloridum",  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ] has a stimulant action on the heart and other muscles. It was said that it "raises **blood pressure** by constricting the vessels and tends to empty the intestines, bladder and gall bladder". Its poisonous nature was also pointed out. Barium sulphide ( $\text{BaS}$ ) was used as a depilatory agent (removes hair). Barium sulphate ( $\text{BaSO}_4$ ) is insoluble and used for body imaging (barium meal). Barium compounds are encountered rarely by most people. All barium compounds should be regarded as highly toxic although initial evidence would appear to suggest the danger is limited. Barium compounds are toxic to humans and other mammals, but the toxicity depends on the solubility and on the nature of the counterion. Thus for barytes (the naturally occurring  $\text{BaSO}_4$ ) the OSHA (occupational safety and health authority) permitted TWA (time weighted average) level in air in the workplace is  $15 \text{ mg m}^{-3}$ , of which not more than  $5 \text{ mg m}^{-3}$  may be in the respirable fraction, 10-100  $\mu\text{m}$  particle size. The  $\text{TDL}_0$  (lowest dose known to have caused a toxic reaction) for rats is reported as  $200 \text{ mg kg}^{-1}$  body weight. For barium chloride, in contrast,  $\text{LDL}_0$  (lowest dose recorded as having been fatal) for humans is  $11 \text{ mg kg}^{-1}$  body weight, as this salt is readily soluble in water and body fluids<sup>[106]</sup>. Baritosis is one form of pneumoconiosis. It is excessive inhalation of barite and barium dust. This disease has been reported to occur in workers exposed to crushed and ground insoluble barium salts<sup>[107]</sup>.

**Zinc:** Zinc is regularly present in plants and animals in concentrations often comparable with those of iron and usually much higher than those of most other trace elements. Zinc has been proved to be essential for bacteria, fungi, blue-green and green algae. Although zinc is not regarded as being especially toxic, it is sometimes released into sea in appreciable quantities. A few examples point to its toxicity to some species at concentrations commonly observed in seawater and sediment interstitial water in estuaries. Stauber and Florence<sup>[108]</sup> observed that growth of cultural diatoms was inhibited by  $20 \mu\text{g L}^{-1}$  of zinc. Effects on fertilisation and embryonic development in Baltic spring-spawning herring at low salinity were detected at only  $5 \mu\text{g L}^{-1}$ . However most examples of zinc toxicity refer to water concentrations approaching and often far exceeding  $100 \mu\text{g L}^{-1}$ . Zinc has a broad and significant role *in vivo* and often linked to the toxicity of cadmium as they tend to occur in the environment together.

Zinc has a multitude of biological functions in human body. It is an important constituent of over 100 enzymes involved in a variety of fundamental metabolic processes. It is major constituent of the choroid region of the eye, a dark layer involved in absorbing excess light within the eye. It is involved in the production and function of several hormones. It plays an important role in reproduction and sexual maturation. Zinc is present in the plasma and in erythrocytes, leukocytes and platelets. The zinc content changes in various diseases; it accumulates in granulation tissue and in and around healing wounds. Zinc deficiency has grave consequences and is reflected in growth depression, sexual immaturity, skin lesions, depression of immunocompetence, taste acuity etc<sup>[109]</sup>. Most common zinc compounds are not particularly toxic but some zinc salts may be carcinogens.

**Copper:** Copper is probably a functional constituent of all cells. Copper is an essential element for animals and the highest concentrations are found in decapod crustaceans, gastropods and cephalopods in which the respiratory pigment haemocyanin contains copper. Excess copper is usually stored in liver ( $4800 \mu\text{g g}^{-1}$ ). Copper has been detected in the liver of *Octopus vulgaris* and  $2000 \mu\text{g g}^{-1}$  in the hepatopancreas of a lobster, *Homarus gammarus*. Oysters may acquire very high concentrations of copper, stored mostly in the wandering leucocytes and these blood cells may contain  $20,000 \mu\text{g g}^{-1}$  copper

and 60,000  $\mu\text{g g}^{-1}$  zinc. Despite the existence of a number of detoxifying and storage systems for copper, it is the most toxic metal, after mercury and silver to a wide spectrum of marine life. There is an experimental evidence that a considerable number of aquatic species are sensitive to copper in the concentration range 1-10  $\mu\text{g L}^{-1}$ [88]. Nelson *et al.*[110] observed that 2  $\mu\text{g L}^{-1}$  had significant effects on young bay scallops and surf clams. Rygg[111] studied the species diversity in benthic communities from Norwegian fjords and concluded that the most sensitive animals were missing from sites where sediment-Cu level exceeded 200  $\mu\text{g g}^{-1}$ . Increased tolerance in some bivalves including *Mytilus edulis* appears to lie in the induction of Cu-binding metallothioneins[112]. Copper is essential in mammalian physiology which has been demonstrated by several observations, especially in animals which were made copper-deficient, either experimentally or naturally as a result of their environment. Numerous proteins and enzymes containing copper have been reported. Mammalian copper proteins include ceruloplasmin, erythrocyte ceruloplasmin, haemocyanin etc. An important feature of copper enzymes is their ability to use molecular oxygen directly. It has been reported to be essential for cross-linking of elastin (e.g. connective tissue). Copper deficiency results in anaemia and changes in ossification and is widespread in malnourished populations and in patients receiving total parenteral nutrition. In large doses, copper is toxic and excessive ingestion results in accumulation in the liver leading to haemolysis, the destruction of red blood cells[109].

**Manganese:** Manganese is an essential element for plants and animals. The biologically active form of manganese is the  $\text{Mn}^{2+}$  ion. Many enzymes have been shown to be activated by manganese. The human body manganese content is estimated to be 12-20 mg. It is widely distributed throughout the body tissues and fluids. Manganese participates in mucopolysaccharide metabolism and is connected with **superoxide dismutase**. Pyruvate carboxylase is the only known manganese containing metalloprotein. Manganese deficiency has been demonstrated in many animals but not in man. Manganese deficiency causes skeletal abnormalities and it is among the least toxic of all the trace elements to animals. Chronic manganese poisoning has been known to occur among miners. Manganese is toxic if inhaled and excess results in neurological disorders [109].

**Iron:** Iron is a vital constituent of plant and animal life. Most of the human body iron exists in complex forms bound to protein, either as porphyrin or haeme compounds, particularly haemoglobin and myoglobin or as non-haeme protein-bound compounds such as ferritin and transferrin. Haemoglobin iron occupies a dominant role in all animals. In humans, it binds oxygen in reversible way and thus assures the transport of oxygen from the lungs to the tissues. The highest concentrations of iron are in the liver, spleen, then kidney and heart. Iron deficiency leads to a sort of anaemia, causing fatigue, headache and anorexia. On the other hand, intake of more than 0.5 g of soluble iron salts may cause grave injury to the alimentary canal followed by a series of serious effects such as hepatitis. Continued intake of excessively high amounts of iron causes haemochromatosis which eventually leads to liver cirrhosis[109,113].

In general, the term 'Heavy Metals' is used by the environmentalists and biologists where there are connotations of toxicity. Solubility of heavy metals in seawater is controlled by factors such as pH, salinity, temperature and presence of different anions. A number of highly sensitive analytical techniques are available for accurate and reliable measurements of toxic heavy metals in seawater, marine sediments and marine biota. Some heavy metals (except Cd, Pb and Hg) have important biological role at low concentrations but show toxic effects at higher concentrations. Toxicity of heavy metals depends upon the metal concentration and speciation and physiology of the target species. A number of metals are toxic because of their strong interaction with sulphur containing biochemicals. **Heavy metals** may be accumulated by marine organisms from the polluted seawater and marine sediments. **Heavy metals** can cause human health hazards upon entering the human food chain.

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Heavy metal pollution, because of its high toxicity, non-biodegradability and biological enrichment, has seriously threatened the health of human beings and the stability of ecological system. The immobilization, removal and detoxification of active heavy metal ions in natural environment can be achieved through microbial activities. However, the relationship of microbial resistance system and their remediation ability towards heavy metal ions is still unclear. To uncover the underneath, we summarized heavy metal bioremediation technologies as well as their detoxification pathways in this review, which will contribute to find their interconnections and develop more efficient bioremediation technologies.