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## Review Article

# Metals from cell to environment: Connecting Metallomics with other omics

## Abstract

Research activities and data collection of metals present in living organisms are called as “metallomics”. In metallomics, biomolecules incorporating metal ions viz. metalloenzymes and metalloproteins, are known as “metallomes”. Metallomics aims to identify metallomes of living organisms and to annotate the physiological significance as well as the biological functions. However, in order to ascertain metallomics to be the part of biometal science, recent analytical technologies like chemical speciation are required to analyze the metallomes. Environmental applications like bioleaching, phytoremediation of soil by using microbes, and to deal with the uptake, transport, storage of trace metals necessary for protein functions and biomarkers identification under ecotoxicological studies really require metallomics involvement. As an interdisciplinary research area, metallomics cover plant and animal physiology, nutrition and become a potential candidate in pharmacology, biogeochemistry and clinical chemistry. Metallomics uses analytical and spectroscopic methods to find the quantitative and qualitative information about metal ions that are present as ligands a well multifaceted biological matrix in trace amounts or occur as non-covalent complexes in order to perform different biological processes. Latest spectroscopic methods along with in-silico approaches including bioinformatics are the important tools needed for research activities in metallomics. The present review highlights the basics of metallomics in biological sciences and its emergence as a novel omics era in relation to other fields. Besides the above aspects, applications and future prospects of metallomics have been highlighted.

## Introduction from Metals to Omics

Biological systems utilize metal ions for their fundamental processes like signaling, catalysis and gene expression. Few metals due to their metallic properties cause various diseases like Pt, Cr, as are carcinogenic whereas Pt, Co, Cr, Ni, Au cause immunological diseases. Mercury is teratogenic and causes diseases related to embryos. Nephrological disorders are caused by Cd and U whereas Al, Hg, and Mn are neurotoxic [1]. The essential transition metals e.g. Zn, Fe, Se, Cu are generally used by cells as cofactors of enzymes, can also potentially catalyze cytotoxic reactions [2]. Further, metals ions govern important properties like the folding of proteins, their conformation, assembly, stability, and catalysis. Many metals mostly transition metals (Mo, Fe, Zn, Cu, Mn) are required as a cofactor of various enzymes for their specific activities [3]. Metal ions also up- and down-regulate protein expression in the cells. Metallothionein proteins play a crucial role in cellular homeostasis and detoxification responses [4]. Therefore, metal ion concentration in the cell, their integration in metalloproteins and their allocation to different cell organelles has been considered to be under tight regulation [5]. The DNA, RNA,

and biosynthesis of protein require various metalloenzymes and related metal ions [6]. In this line, nowadays with the help of the high-throughput approaches the complete genetic blueprint of various organisms has been studied which resulted in various “omics”; the disciplines aiming at the annotation of a particular class of components of a living organism in its ensemble [7]. Therefore, metallomics is a very needful area to be focused scientifically among researchers along with other omics such as genomics and proteomics.

Other than “metallome” and “metallomics” many related definitions have emerged, for instance, ionomics [8], hetero-atom tagged proteomics [9], or elementomics. It is very important to define the terms and render their use systematically [10]. Among various omics, metallomics is defined to apprehend the metal-dependent life processes [11], or the assemblage of metals related research in the living organisms. However, the word “metallome” was first given by Williams who referred to it as an element distribution, metal ion concentration, or as free elements present in the cellular organelles, cell or organism [12]. Under metallomics, “metallomes” is a general annotation for metalloenzymes,

metalloproteins, and many other biomolecules containing metal ions which are similar to genomes for genomics (the study of nucleic acids) and proteomes for proteomics (the study of proteins and amino acids). The main research objectives of metallomics involve the study of physiological and biological functions of living systems and identification of metallomes, however, chemical speciation, the important technology required for the establishment of metallomics is an integral branch of biometal science [13]. Now metallomics has become the hot topic of various reviews [14-17], and special issues of many reputed journals [18,19].

Recently, the advanced technologies make platforms for acquiring the “omics” data generated through numerous experimental sources to come-up with the system level broad perspective of organisms. The genomics and proteomics data helped a lot in knowing cell molecules and their interrelations at the molecular level, so one can determine the biological activity of those components [20]. In this direction, the initial approach is to construct the dataset for metalloproteins that can be used with other proteomics data for the modulation of multifaceted cellular processes integrating metals for which “omics” data could be extremely useful. In the present review, we discuss the recent scientific achievements on metallomics and related fields advocating the uptake, role, upkeep, and movement of metal ions crucial for living beings. Compared to other omics such as genomics and proteomics, metallomics is a relatively new field; however, they have constructed a tremendous data that can be used to study metallomics. Metallomics must be the core focus of scientific investigation because functional genomics and proteomics cannot be completed without the role of metalloenzymes and metal ions [21].

In order to understand the science of “metallomics”, it is essential to use a novel way to look at the relevance of metals in organisms and as the fraction of ecosystem thus bringing together the environment and cellular life (Singh et al. 2011). The molecular basis for many metal-dependent biochemical processes even now remains ambiguous [3]. Mechanism of metal sensing, metal uptake and storing or incorporation of metals as a cofactor in the cell is few of the thrust areas to work or to meet the coming challenges in metallomics.

Hence, it is crucial to interpret the significance of metals in governing various biological processes and stress responses by systematic evaluation of metal ions, speciation, and localization in various tissues or under particular conditions. In this report, “metallomics” is suggested as a novel scientific area emphasizing the importance of research in biometals.

### Metals in an environment and their biological significance

Metals in the environment are generally beneficial but can become a source of toxicity if they exceed a certain limit [22]. With increasing pollution, metal toxicity in the water and soil is constantly growing. The environmental pollutants can be the inorganic, organic or heterogeneous combination of both. Inorganic pollutants from anthropogenic activities include carcinogenic heavy metals for instance arsenic

(As), mercury (Hg), nickel (Ni) and many others [23]. The sources of environmental pollution include extermination and improvisation activities, mineral processing and mining, agriculture-related activities, wind-blown dust, sea spray, transportation activities and automobiles and other anthropogenic operations [24]. The environmental contamination by inhabitation of deleterious metals pose threat to living beings and ecological community through direct consumption or contact with polluted soil, the food web, imbibition of infected groundwater, attrition in food quality, decrement in land adoption for agriculture thus eliciting food insecurity, and land occupancy disagreements [25]. Although metals are deleterious when present in excess amount but are also essential in order to perform important processes of life [22]. Since the harmful consequences of metals are widely known, the present review emphasizes the significant and crucial aspects of metals in the living system.

### Evolution of metal biology from prokaryotes to eukaryotes

Millions of years ago, environmental conditions were reductive and therefore drastically different from today [26]. The primitive cells being anaerobic could not utilize all the elements accessible due to the presence of a substantial volume of hydrogen sulphide ( $H_2S$ ) in the environment [27]. The archean ocean which was reductive and anoxic was rich in iron (Fe), manganese (Mn), and cobalt (Co), thus far low in copper (Cu), zinc (Zn) and molybdenum (Mo) [28]. Therefore, the earliest organisms utilized metals that were abundant in the earliest ocean, Mn, Fe, and Co. The composition of the primitive ocean determined the metal-binding architectures evolution and the organisms' choice of elements [29]. However, as organisms evolved the presence of metals determined the metals inside the cell which further became available to perform the biological functions. Anaerobes utilize part of  $Co(B_{12})$  and Ni as reducing catalysts and Fe as the electron transferring agent while Mg ions are used in catalysis of weak acids. Initially, aerobic bacteria developed utilizing Mo and Cu due to sulfides oxidation. The cell utilized copper ions in the periplasmic space where they operated on novel substrates like Nitric Oxide produced by oxygen [30]. The prokaryotic cells largely utilized Fe and Mg ions in various metabolic pathways and assisted transcription factors as messengers. However, there was no involvement of metal ions such as Ca, Zn and Cu [31]. Reductive environment supported the evolution of prokaryotic organisms when certain transition metals became available. During the advancement of evolution, this condition, additionally with the redox chemical activity of transition metals culminated in the small free cytosolic compartments in prokaryotes [32]. In order to maintain the moderate metal ion accumulation in the cytosol and to defend the cell against cytotoxic augmentation effects of these intracellular metal ions, modern prokaryotic cells evolved new metal binding proteins [33]. This state of proportionately low metal ions in the cytosol is observed in the present day eukaryotes. However, increased abundance and availability of metal ions due to increased oxygen availability was conducive for eukaryotic organism's evolution [34]. For messenger systems, catalysts,

structure etc. both metals and non-metals are crucial but their introduction into cellular activity at the comparable amount at the same period of evolution is an uncommon phenomenon. The changing environment leads to this evolution [32].

The change was slow as Oxygen was removed at first by the ferrous and sulfide in the sea. The need was for organisms to acclimatize to the new environment and they were able to adapt due to the multi-chambered bigger, gradual reproducing, cell system, and the eukaryotes, simultaneously with fast-reproducing prokaryotes [35]. The main applicability of additional compartments is the isolation of reductive reactions from the oxidative reactions of the cytoplasm and ions storage vesicles of the cytoplasm was not allowed. The bigger cells possessed filaments, had an extensible membrane and integrated aerobic bacteria and other symbiotic chambers. An elementary and beneficial modulation of messenger inter-communication was necessary among the non-flexible compartments to provide homeostasis and from the environment to provide an elevated sensitivity to cellular surroundings [32]. The Ca ions were utilized in essential messengers, Zn ions in transcription agents and Cu ions generally in vesicles [36]. The Zn and Cu became more accessible as their sulphides can be partially oxidized.

Anaerobic cells acquired defensive mechanisms so as to survive in the oxidizing environment. In addition, cell mastered to use oxygen for respiratory metabolism, thereby augmenting the efficacy of ATP synthesis [37]. For instance, the role of superoxide dismutase and catalase is well demonstrated in protecting the cell against the toxic residues of oxygen metabolism. Cells made use of at least some metals that today are called the “essential” metals. Due to the adequacy of essential metals at the time of prokaryotic and subsequently eukaryotic organism’s evolution many metals integrated with biochemical functions as elementary factors [38] (Figure 1).

### Emergence of novel omics: Metallomics

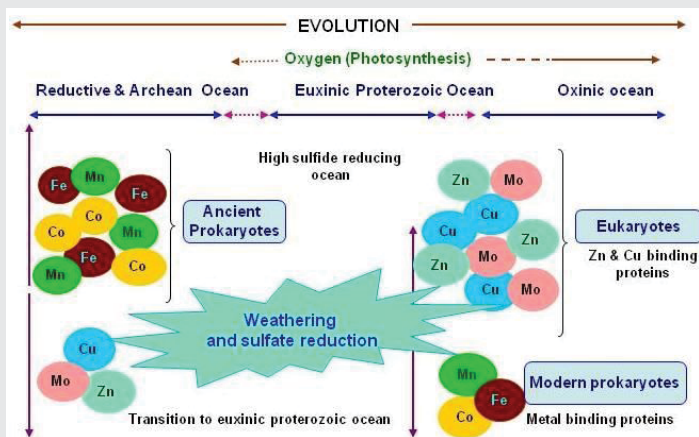
The fields like genomics, proteomics, and metabolomics

have collectively known as “omics” had been subjected to tremendous improvement in the past. The first to develop was genomics, which produces complete genomic DNA sequences of living creatures. The next “omics” includes proteomics i.e. the interpretation of the structure, stability, localization, and interaction of cellular or organism’s proteins [39]. Further, metabolomics annotates the complete metabolites of an organism [40]. The techniques used in proteomics and metabolomics have usually evaded the metals present in proteins and metabolites. However, the cell chemistry should be characterized not only by its nucleic acids, proteins, and metabolites but also with its metallome [16]. Therefore, metallomics should be recognized as emerging omics that characterize the metallomes and find out the interactions and functional associations of metals with proteins, genes, and metabolites [11]. The high-throughput technologies provided tremendous data about the genome. “Omics” data are accessible for many cell constituents and interactions, in addition to, for instance, genomics, proteomics, transcriptomics and protein-protein interaction maps (interactomics) [41]. The availability of large-scale information has largely helped researchers in interpreting functions of organisms but due to inadequate metallome data, the understanding remains partial. However, to explore any biological function/signaling pathway/cellular mechanism, metallomics should be linked with other omics and so in this review further we explain how other omics require metallomics to retrieve any information.

### Integration of metallomics with genomics

The tremendous advancement in omics technology has led to the growth of genomics to such an extent that, it is divided into structural genomics and functional genomics. Structural genomics procreates high-resolution, 3D structural models of DNA whereas functional genomics tries to decipher functions of various genes through RNA analysis [42]. The metallomics and metalloproteomics are comparatively novel areas of study; however, data generated by genomics can be utilized to rapidly enhance our perceptive of metalloids and metalloproteomics [43].

Metals are important for maintaining the structural integrity of DNA and RNA which are polyanionic in nature. Besides being required for replication, metals are also utilized in structure formation, folding and in catalytic mechanisms e.g. in ribozymes. In prokaryotic DNA replication divalent cations assist the 3’OH to commence a nucleophilic encounter on alpha phosphate of deoxyribonucleotide stabilizing the negative charges of triphosphate [44]. The thermodynamic equalization of tertiary structures of RNA is implemented by monovalent ( $K^+$ ), divalent ( $Mg^{2+}$ ) or trivalent metal cations in various ways. To shield unfavorable electrostatic interactions monovalent ions affiliate peripherally with the RNA backbone. The distinct components of the tertiary structure of RNA are stabilized by site-bound ions. For example, in primary groove magnesium hexahydrate and guanosine interaction stabilizes definite motifs of the tertiary structure of RNA [45]. Similarly, various cations stabilize the multiple phases of RNA folding. The RNA secondary structure is stabilized when the



**Figure 1:** Utilization of metal by organisms through various periods of evolution. In reductive archean ocean, Fe, Mn, Co were more and Cu, Zn, Mo concentration was less. With the progressive increase in oxygen level, Cu, Zn, Mo increased and Fe, Mn, Co declines. Metal ion abundance was influenced by the environmental conditions and metal selection by organisms for various biochemical processes along with other factors.



polyanionic RNA backbone is neutralized by mono or divalent cations and amines. Hence, metal cations stabilize the RNA backbone when the regions of heavy negative charge develop due to the stacking of phosphates. Several motifs in RNA duplexes are identified where metal ions bind. For example, in the group I introns of *Tetrahymena thermophila* the successive adenosines in the RNA strand form a non-conical pseudo base pair in the ion binding motif [46]. The lack of cations in several of such motifs leads to damaged or more flexible tertiary structure [47]. Additionally, to stabilize the junctions in DNA such as transitional Holliday junction in genetic recombination, the divalent ions mainly magnesium is crucial. The magnesium ions in the junction shield the negatively charged phosphate groups. This helps to position the phosphate groups adjacent to one another conceding stacked conformation [48].

The several metalloenzymes and metal ions assist in the synthesis and the metabolic roles of genes and proteins. The amplest transition metal, Zn, aids in carrying out the activity of over three hundred enzymes, stabilization of DNA and expression of genes [49]. The zinc in the eggs of salmon probably assists in the synthesis of DNA/RNA, processes such as the production of energy adenosine triphosphatase (ATPase) and regulation of cell division. Further, calcium ions associate with the carbonyl terminals in the proteins so as to stabilize the structure. Therefore, the imaging of metallomics will concede co-localization of gene expression as well as localization of protein patterns with metals thus providing gene, protein, and metal function linkage [8]. Unlike the correlation of metallo-proteome and genome, the correlation of metallo-metabolome and genome is not so distinct and may be missing. With the molecular cloning methods the metallo-metabolome interaction can be established by investigating metal resistance genes in organisms [11]. Therefore, the three -omics are correlative, and the aggregation of authenticated metallomic data with the transcriptome and proteome knowledge of a cell is the largest challenges for future investigation in this area. One way forward is that better established -omics (transcriptomics and proteomics) incorporate metallomics in their studies on model organisms whose gene/protein sequences are well annotated in databases.

### Metallomics interaction with proteomics

The metalloproteins three-dimensional structure incorporates inorganic ions and comprises nearly 30% of the proteins [50]. Metalloproteins catalyze significant processes of water oxidation and photosynthesis [51]. The subclass of metalloproteins i.e. metalloenzymes performs specific catalytic functions. Metalloenzymes act on the molecule termed as the substrate which undergoes a net chemical transformation. Biological pathways require metalloenzymes to preserve life [52], and consequently use metal ions specifically K, Fe, Ca, Zn, Mn, Mg [26]. Extensive protein networks comprising metal sensing proteins and transporters retain the proper subcellular concentration of elements within the cell. Metalloenzymes are required inside the cell for various metabolic pathways (Table 1). The protein networks shuttle each metal relevant metalloprotein in the cellular compartment as metals compete

for protein binding sites [53]. Metalloproteins containing complex metal clusters metabolize small glucose molecules, assemble proteins containing iron-sulfur clusters, and enzymes incorporating metal ions catalyze the halogenation of organic molecules [54]. The metalloenzymes/ metalloproteins evolution explains the reasons for this diversity. Iron-sulfur proteins, containing FeS clusters exist in many metalloproteins, viz. ferredoxins, hydrogenases, succinate-coenzyme Q reductase, nitrogenase, coenzyme Q-cytochrome c reductase, NADH dehydrogenase [55]. Iron-sulfur clusters play a significant role in the oxidation-reduction process in mitochondria and others coordinate gene expression. They also perform other activities, for instance, catalysis by aconitase, donors of sulfur during biotin and lipoic acid biosynthesis etc. [56]. Metalloproteins also carry out some crucial functions of dioxygen transportation in respiration. In three known classes of proteins for transportation of dioxygen i.e. haemoglobin-myoglobin, hemocyanins, and hemerythrins, a sensitive equilibrium exists for oxygen binding to metal center without irreversible oxidation of metal [57] (Table 1).

These are mostly two-electron redox mechanisms and involve atom or group transfers. The oxygen atom is added to the substrate in many important and common reactions.

**Table 1:** Essential metal-containing enzymes (metalloenzymes) utilized in metabolic pathways.

Metalloenzymes	Metals imparted	Reference
Carbonic anhydrase	1 Zn	Bagder and Price (1994) (127)
Carboxypeptidase A	1 Zn	Schaller (2004) (128)
Alcohol dehydrogenase	2 Zn or 4 Zn	Goffner et al. (1998) (129)
Superoxide dismutase	2 Zn+ 2 Cu	Alscher et al. (2002) (130)
DNA polymerase	2 Zn	Wu and Wu (1987) (131)
RNA polymerase	2 Zn	Scrutton et al. (1971) (132)
Alkaline phosphatase	3.5 Zn	Bashkin (1999) (56)
Pyruvate carboxylase	3-4 Mn	Attwood (1995) (133)
Superoxide dismutase	2 Mn	Christianson (1997) (134)
Superoxide dismutase	(2 Cu +2 Zn)	Bowler et al. (1994) (135)
Cytochrome oxidase	(2 Cu +2 Fe)	Herrmann and Funes (2005) (136)
Amine oxidase	3 Cu	Angelini et al. (2010) (137)
Ascorbic acid oxidase	4 Cu	Hazid et al. (2011) (138)
NADH dehydrogenase	4 Fe	Nakumar et al. (2010) (139)
Succinate dehydrogenase	8 Fe	Figueroa et al. (2001) (140)
Aldehyde oxidase	8 Fe +2 Mo	Omarov et al. (1999) (141)
Sulphite oxidase	2 Fe +2 Mo	Brychkora et al. (2007) (142)
Cytochrome oxidase	2 Fe +2 Cu	Herrmann and Funes (2005) (136)
Nitrogenase	Fe-Mo	Rubio and Ludden (2008) (143)
Catalase	4 iron groups	Purwar et al. (2011) (144)
Hydrogenase	(Fe-Fe)	Das et al. (2006) (145)
Vitamin B12-dependent enzymes	Co(II)	Kung et al. (2012) (146)
Arginase	Mn	Costanzo et al. (2007) (147)
Aconitase	Fe-S cluster	Menachem (2011) (148)
Urease	10 Ni	Dixon et al. (1975) (149)

Examples include iron porphyrin centers in cytochrome P-450 enzymes catalyzing the oxidation process of formation of alcohols from hydrocarbons [58], tyrosinase which contains a dinuclear-Cu active site catalyzes the ortho-hydroxylation of phenolic substrates [59], and sulfite to sulfate oxidation by sulfite oxidase which contains a molybdenum atom [60]. These enzymes differ in the source of oxygen added to substrate and metal ions identity present. Dehydrogenations constitute another class redox processes involving two electrons. The removal of electrons and protons is equivalent to the loss of dihydrogen from a substrate. For example, the liver alcohol dehydrogenase active sites contain a Zn (II) ion and catalyze the synthesis of acetaldehyde from ethanol. One of the molecules of hydrogen out of the two hydrogens released is added to the organic cofactor NAD<sup>+</sup> to form NADH [61].

However, metalloenzymes which catalyze redox reactions with several electron pairs involve redox reactions with more than two electrons in one reaction and are crucial in living organisms. The reversible conversion of two H<sub>2</sub>O molecules to one O<sub>2</sub> molecule with an exchange of four electrons is a significant reaction of this type. The production of H<sub>2</sub>O from O<sub>2</sub> reduction is significant for the phosphorylation of ADP to ATP and requires cytochrome c oxidase that contains two Cu and two Fe-heme centers [62]. The reverse reaction, involving four Mn ions is water splitting to oxygen which has an important function in photosynthesis [63].

Metalloenzymes in rearrangement reactions that occur without a change in oxidation involve the enzymes like chorismate mutase (EC5.4.99.5) that catalyzes the production of prephenate ion from chorismate ion via Claisen rearrangement [64]. Vitamin B12, an alkyl-cobalt (III) complex of a substituted corrin, is a cofactor of enzymes catalyzing 1, 2-carbon rearrangement [65]. Many hydrolytic enzymes contain Zn<sup>2+</sup> ion. Besides Zn<sup>2+</sup>; Mn<sup>2+</sup>, Ni<sup>2+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> are the metal ions encountered in hydrolytic enzymes. Hydrolytic enzymes catalyze the disruption of proteins, carbohydrates, and fats to their monomer units. The covalent bond attaching monomers is cleaved due to hydrolysis as a water molecule adds to the polymer. Examples include carbonic anhydrase (Zn prosthetic group) that promotes hydrolysis of carbon dioxide, peptidases (a majority of the metallopeptidases contain zinc atom), esterases that catalytically hydrolyze carbonyl compounds, and phosphatases (Mg<sup>2+</sup> or Mn<sup>2+</sup>), catalyzing the splitting of phosphate esters. Another hydrolytic metalloenzyme, thiocyanate hydrolase, purified from the bacteria *Thiobacillus thioparus* TH115 contain cobalt and hydrolyzes thiocyanate to ammonia and carbonyl sulfide [66].

Metallochaperones guide the ions and defend it from the over-chelation potentiality of the cell [67]. Metallochaperones sort specific metal co-factor to the precise metalloenzymes and transport them to diverse location. Many metal ions present in the enzymes acting as co-factors remain located intracellularly or transported extracellularly [68]. For instance, copper is toxic element yet crucial for the living systems. Cells acquire many mechanisms so as to sustain copper homeostasis such as; the protein-mediated intracellular delivery of copper

to target proteins. This is consummated by a group of proteins, the copper chaperones, which conserved proteins present in prokaryotes and eukaryotes. Three different classes of Cu-metallochaperones namely cytochrome oxidase (COX17), antioxidant (ATX1/ HAH1) and copper chaperones of SOD1 (CCS) are known [69]. The copper ion incorporation into SOD1 requires a Cu metallochaperone [67]. Metallochaperones incorporating metals other than copper involve similar cofactor trafficking pathways. However, the chaperones integrating metals besides copper are not present in eukaryotes. The ArsD, arsenic chaperone present in *Escherichia coli* has been established to transfer trivalent arsenic to ArsA. ArsA is the subunit embodied in As(III)/Sb(III) pump for efflux and performs catalytic function. The affinity for arsenite increases as ArsA and ArsD interact. This enhances arsenite extrusion even at lower arsenite concentrations as the activity of ATPase increases. Thus, to prevent intracellular toxicity of arsenite, cells exploit arsenic chaperones [70]. Additionally, proteins which bind nickel is described which may promote metal insertion to the enzymes requiring nickel such as urease, Co-dehydrogenase, Ni-superoxide dismutase, glyoxidentalase etc.

Prokaryotes do not need metal carriers such as COX17 as intracellular compartmentalization is not present in prokaryotes as in eukaryotes. However, in the periplasm of prokaryotes and in gram-negative bacteria cupro-proteins are present. In the gram-negative bacteria, the porins are located in the peripheral membrane through which metals diffuse from the external medium [71]. The proteins like soluble periplasm protein CucA acquire the appropriate metals and export them in unfurled form through the secretory pathway. The most copious Cu<sup>2+</sup> and Mn<sup>2+</sup> proteins, CucA and MncA, were diagnosed in the cyanobacterium *Synechocystis* PCC 6803 [68]. Further, Zn SOD and Cu SOD are expressed in bacteria, but no CCS homolog has been established in prokaryotes. However, for enterobacteria, ATX1 (CopZ) homolog has been defined. The isolated CopZ has potential to contribute copper to CopY for a transcription factor. A model proposes that this transfer of copper displaces the Zn ion required for CopY binding to DNA [72]. Several biological processes require metal-ion binding proteins. Metallothionein is rich in cysteine, low molecular weight protein family in animals, angiosperms, eukaryotes and few prokaryotes. Metallothioneins contain metal ions viz. Cd (II), Zn(II) and Cu(I) in metal clusters [73]. Through the thiols present in cysteine residues, MTs bind the physiological (Cu, Zn Se) and xenobiotic (Ag, As, Cd, Hg) heavy metals [74]. The metal clusters Me(II)<sub>3</sub>(Cys)<sub>9</sub> and Me(II)<sub>4</sub>(Cys)<sub>11</sub> are present in mammalian MTs. These clusters bind heptad of metal ions (Me) through thiolate coordination [75]. Biosynthesis of MTs is induced by several agents and is coordinated at the level of transcription. MTs sequester mainly metals which are non-essential [76].

### Metalloomics role in metabolomics

Metabolomics, a rapidly developing technology deals with metabolome which is described as the metabolites compilation of the cell [77]. Metabolic fingerprinting, targeted analysis and metabolite profiling are the major approaches presently

adopted in metabolomics related research [78]. The three components are significant in metabolomics in order to carry out such processes: (1) antioxidants (2) stress by-products due to disruption of homeostasis; and (3) molecule involved in signal transduction responsible for adaptation response. The molecules involved in signal transduction are either recurrently synthesized or compounds liberated from conjugated forms such as salicylic acid [79]. The metals and ligands interaction is a significant area of metallomics research. The defensive mechanisms of numerous organisms against toxic metals comprise of many organic acids such as succinate, malate, oxalate etc. [16], metallophores [80], and peptides binding metals [81]. Hyper accumulating plants developing metal homeostasis are of particular interest. They can live and reproduce in metal-rich environments [82]. In such plant cells, the complexity of metals leads to many relatively poorly characterized metal complexes.

The study of detoxification controlling mechanisms can benefit from the establishment of the species formed. The metabolite and gene networks can be deciphered through assimilation of the data of metabolomics and transcriptomics [83]. For example, the proteomics and the metabolomics were integrated to establish the response of Arabidopsis to cesium stress [84]. However, in view of the problem of data integration, the collective study of the data from metabolomics and other omics is actually challenging [85]. Though other targets for approaches related to genomics are attainable, to identify metabolites produced in streptomycetes grown in growth media with 0.2 mM Ni or Cd indicates huge probability for revealing strains from cultures. This would help to decipher cluster of genes during metal stress [86]. Metals could be used as inducers of new metabolites such as antibiotics i.e. another area of metallo-metabolomics used in biotechnology [14].

A good approach to metallo-metabolomics should help in element identification in the species or identifies at least some and account clearly for the non-identified ones (quantitative speciation blueprint). In contrast to metallo-proteomics, the de novo recognition of metabolites is performed by using leading spectrometric techniques. Mass spectrometry (MS) technology and a purification method are a comprehensive approach to metallo-metabolomics [11]. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) ideally monitors the separation of metallo-metabolites. The sensitivity of ICP-MS is autonomous of the metalloid surroundings of the molecule, concomitant compounds, and buffer used for chromatography and the matrix. In addition, ICP-MS monitors the quantifiable restoration of an analyte. This is an important attribute which is not possible to measure through electrospray ionization mass spectrometry (ESI-MS). Note that ICP-MS cannot monitor the nature of separation per se but it is considered that analytic purity increases with each separation step when clean volatile buffers are used [16]. The metallo-metabolites are highly polar and less hydrophobic which make hydrophilic interaction chromatography (HILIC) [87], an optimal approach for sample addition by ESI-MS in metallo-metabolomics. The HILIC is a significant technique to investigate seleno-metabolome of yeast [88], and few organic molecules metal complexes

(Weber et al. 2008) and some organic acids (Ni malate, citrate, histidine, EDTA, and nicotianamine) [89].

The absence of well-depicted metabolome of plants is a great challenge in metabolomics of plants. Approximately 90000–200000 distinct metabolites are produced in plants [90], but the exact statistic of metabolites in any individual plant type is still unknown. The microbes have better-understood metabolism, even then the total metabolites present in microbes is not deciphered. The metabolome characterization of the plants is essential in order to uncover the gene function.

### Metallomics in environment-related studies

Metals generally exist in the atmosphere but their level is increasing due to industrial revolution via technological advancements. The increase in a number of metals in ecosystem occurs in metropolitan areas, metalliferous mines, major road systems, vehicle emissions, areas characterized by automobile activities etc. [91]. The increased level of metals in the biosphere is the cause of slow environmental poisoning. Thus, it necessitates studies on environmental pollution and biogeochemistry. Further, the knowledge of how living creatures sense, adapt and use metals in dynamic ecosystem or biome is fundamental for the precise view of the metabolism of transition metals [92].

Metallomics comprise various types of information from identification of metals (qualitative metallomics) to finding out their level (quantitative metallomics) [14]. Metallomics may be important in studies relevant to plant metal tolerance and homeostasis [93], biogeochemical metal cycles [94], plant proteome annotation engaged in toxicity of metals [95]. Metallomics may be supportive to the improvement of applications for optimized strategies in metal contaminated soils, including microbial-assisted phytoremediation of polluted land, reclamation of soil and biomarker recognition for eco-toxicological studies [94].

For phytoremediation studies, phytochelatin build-up in the plants is significant phenomenon due to their role in detoxification of heavy metals [96]. The study of plant and soil amount of heavy metals in *Opuntia ficus* predicated a complex interplay of heavy metals in the environment and phytochelatin production [97]. Many advanced approaches such as HPLC-ICP-MS is utilized in phytoremediation studies for the establishment of seleno-compounds in the plant extracts from *Brassica juncea*, *Allium sativum*, [98], and *Bertholletia excelsa* [99]. In *Brassica juncea* and *Sesuvium portulacastrum*, complexes with bio-ligands have been investigated after exposure to different amounts of  $Pb(NO_3)_2$ . Studies have revealed that due to the presence of the isoform phytochelatin<sub>3</sub> both the plants are crucial in phytoremediation [96]. Moreover, the metalloproteins have a role as environmental stress biomarkers. The metal and metalloids play a significant function in organisms as large biomolecules (nucleic acids, polysaccharides, and proteins) and organic molecules (oxalate, citrate, tartrate, amino acids, oligopeptides) can be bound to metals, to generate different chemical species [3]. The biomolecules binding metals constitute a considerable quantity of molecules engaged



in the metabolism of the cell and assist in identification of metal cofactor present in the protein. This helps to find out the function of the protein in cellular pathways [100]. Many metalloproteins act as organism's markers, exposure to metals and indicators of their status. For example, metallothioneins are metal binding proteins rich in Cys and are utilized as biomarkers for some organisms exposed to metals in the environment. Metallothioneins in the organisms bind metals and thus act as a detoxifying mechanism [101]. Metallothioneins are utilized to find out the effect of heavy metals in marine organisms [102]. The method of determination of hepatic metallothioneins is confirmed biomarker for determination of biological implication of metal contamination. Other metalloproteins have been established as markers such as metal superoxide dismutase in biological creatures in contact with xenobiotics. Metal superoxide dismutase (SOD) is studied upon drought stress [103], in rice and ozone [104], action. The probability of deciphering more metalloproteins that can act as biomarkers for pollution is potentially high in fish. The metalloproteins which are being studied as environmental biomarkers in fish are matrix metalloproteinases, iron-binding metalloproteins, selenoproteins, Hg-, Se- and As- containing proteins and superoxide dismutases. The research of the biomolecules using metallomic approach simplifies the significance of complex biological matrices. The interpretation of the structure, elucidation of the pathways, and characterization of the metalloproteins are the areas which require further investigation.

### Metallomics in communication biology

Metals not only perform an important function in various crucial processes of life but they are also used as magnetic compasses in living organisms. The various animals such as bees, birds, sea turtles etc. possess compasses inside them. With their compasses, some species navigate entire oceans [105]. Magnetoreception or magnetoception allows the animals which show biomagnetism (magnetic field production by organisms) to investigate magnetic field so as to perceive directions. Magnetoception illustrates the navigational skills of many animal species [106]. The phenomenon of magnetoception is noticed in bacteria, birds, fungi, insects and many other animals. Such cellular communication roles by metals are a very exciting frontier in biology. Magnetotactic bacteria incorporate magnetosomes (magnetite or greigite nanocrystals) which help bacteria navigate geomagnetic field to avoid the higher oxygen concentrations of surface waters, which are toxic to them [107]. They orient on the earth's magnetic pole, and, when transported to the opposite hemisphere, disorient and swim upward.

The *Columba livia* commonly known as homing pigeon provides the best example of magnetoreception. Pigeons have magnetic minerals in the beak that remain in contact with the nerve and react to alteration in a magnetic field [105]. Likewise, based on behavioral evidence of *Apis mellifera* or honeybee characteristic of magnetoreception has been provided. Free flying honeybees can perceive static intensity fluctuations as weak as 26nT against the magnetic field [108]. Biomagnitites

(Fe<sub>3</sub>O<sub>4</sub>) are present as magnetoreceptor and play an important role to transduce information of magnetic field. Honeybees go through iron biomineralization which causes magnetoreception [109].

Some ions, especially Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup>, trigger cellular response. For example, calcium-binding proteins control functions of the cell and the sodium ions influx into cell membrane causes the neurons to respond. Calcium controls many significant cell activities starting with new life creation at fertilization to ending with apoptosis [110]. Likewise, Zinc fingers are metal ion classes in biology that regulate transcription. These pieces of evidence suggest the importance of metal ions in communication biology.

### Metallomics in pharmacy / medicine

Metal complexes are important to carry out biological and biomedical processes. Metal complexes consist of metal atoms surrounded by ligands. Organic compounds in medicine have different modes of action; some are biotransformed by metalloenzymes (Holm and Solomon 1996) others have an effect on the metabolism of metals. Metal complexes are used as drugs for curing various human diseases like lymphomas, carcinomas, infection control, anti-inflammatory, diabetes, and olfactory disorder. The transition metals upon interaction with molecules having negative charges display different oxidation states. This phenomenon of transition metals is significant for the advancement of drugs based on metals having therapeutic values and pharmacological applications [111]. The metal complexes significance in pharmacy and the recent developments are indexed in table 2.

Novel therapeutics with metal complexes offers real possibilities to pharmaceutical industries. The smooth encounter of the metal complexes in ligand substitution and redox reactions presumably indicate active species to be the biotransformation products of the administered complex [112].

Therefore, metal compounds could be used more effectively as drugs following the active species identification. The metal complexes identification and their biotransformation still require investigation. Further, the toxicity and metal compounds are correlated therefore targeting metal-based drugs to specific locations (cells, tissues or receptors) where they are needed, could lower the toxicity to a significant extent [113]. Thus, to understand bio-coordination chemistries of drugs having metal ions, thermodynamics and kinetics of metal complex reactions, mainly under biologically relevant conditions is significant [114]. Hence, the study of elemental medicine would help to decipher diagnostic and therapeutic approaches and contribute to our insight of natural biological processes.

### Contribution of bioinformatics in exploration of metallomics

The huge repository of genome sequence data is responsible for the boom in bioinformatics. Although the information of metalloproteome is required for the comprehensive understanding of life processes, the presently available

Table 2:

Metal/ Metal complexes	Therapeutic use	Reference
<b>MRI contrast agents</b>		
Gd <sup>III</sup> complexes	Detection of abnormalities of the blood-brain barrier	Sadler and Guo (1998) (112)
<b>Radiopharmaceuticals</b>		
γ-ray emitters e.g. <sup>99m</sup> Tc, <sup>201</sup> Tl, <sup>111</sup> In, <sup>67</sup> Ga, <sup>51</sup> Co, <sup>51</sup> Cr, <sup>169</sup> Yb	Diagnostic imaging	Maguire et al. (1993) (150)
β-emitters e.g. <sup>89</sup> Sr, <sup>153</sup> Sm, <sup>186</sup> Re	For therapy	Maguire et al. (1993) (150)
Tc complexes	Cerebral perfusion imaging of stroke, Myocardial perfusion imaging	Sadler (1991) (151)
<sup>111</sup> In Complex conjugated with mAbs	Diagnosis of colorectal and ovarian cancer	Maguire et al. (1993) (150)
<b>Anti-infective agents</b>		
Ag and its compounds	Antimicrobial agents in medicine	Fricker (1994) (152)
Sb complexes	Treatment of leishmaniasis	Cantos et al. (1993) (153)
Fe chelator desferrioxamine	Treatment of malaria	
MnII and MnII macrocycle complexes	protect regionally ischemic and reperfused myocardium from injury	La Bonte et al. (2008) (154)
Mn <sup>III</sup> porphyrin e.g. (MnTBAP)	Protect against neurodegeneration. Useful in treating Parkinson's and Alzheimer's diseases	Hachmeister et al. (2006) (155)
<b>Cardiovascular system</b>		
Fe <sup>II</sup> complex sodium nitroprusside	Useful in cases of emergency hypertension, heart attacks and surgery to lower down blood pressure	Guo and Saddler (1999) (156)
Ruthenium complexes such as K[Ru(Hedta)Cl]	Nitric oxide scavengers, reverse the poor response of the artery	Fricker et al. (1995) (157)
<b>Insulin mimetic</b>		
Vanadate (V <sup>V</sup> ) Vanadyl complexes (V <sup>IV</sup> )	Mimic some of the effects of insulin	Kadota et al. (1987) (158)
Low molecular weight Cr-binding substance (LMWCr), naturally occurring oligopeptide	Activate the insulin-dependent tyrosine kinase activity of the insulin receptor protein	Davis et al. (1997)
<b>Photodynamic Therapy</b>		
Complexes of texaphyrins (expanded porphyrins) with Cd <sup>II</sup> , La <sup>III</sup> and Lu <sup>III</sup>	Effective photosensitizers (used in treatment of diseased tissues and cells)	Hom and Katzenellenbogen (1997)
<b>Anticancer agents</b>		
Platinum complexes	Effective in combination chemotherapy	Uchida et al. (1998)
Metallocenes (V, Nb, Mo, Fe, Ge, Sn)	Anticancer agents	Guo and Sadler (156)
<b>Gold Antiarthritic drug</b>		
Injectable Au(I) thiolato complexes	Treatment of rheumatoid arthritis	Shaw (1999)
<b>Bismuth antiulcer drugs</b>		
Bismuth (Bi) compounds	Treatment of gastrointestinal disorders	Tillman et al. (1996)

experimental methods or techniques are not capable of achieving such a demanding task. At this point, bioinformatics provides a considerable contribution to subjugate the limitation of empirical methods by using predictive tools and information technology [115]. The metal binding domains can be deciphered with the help of protein sequence data available in Pfam and other libraries [116]. Bertini and Cavallaro [117], have reviewed the bioinformatics approaches dedicated to bimetal. Many metalloprotein databases using primary sequence information have been generated utilizing various bioinformatics methods [118].

With the advancement in high throughput techniques the characterization of metallome would be easy and thus the huge amount of data will be obtained. Therefore, bioinformatics approaches would be required to retrieve the data from the tremendous data set. The computational program utilizes sequence information by taking into count position-specific evolutionary profiles and aspects such as protein length and

amino acid composition. This method provides information on transition metal binding site components and is highly complementary to the high-throughput techniques based on X-ray absorption spectroscopy (HT-XAS), which identifies the metal binding to protein [119]. Further, comparative structure modeling is a very powerful tool in driving protein function. MODBASE is structural models data [120], established on template-target alignments accessible at the website (<http://www.al-to.compbio.ucsf.edu/modbase-cgi/index.cgi>). In general, the accuracy of constructed models is based on sequence similarity of model sequence and the templates. Many applications such as functional analysis and screening of small-molecule databases for potential drug discovery utilizes models based on 30% sequence identity. Databases devoted to metal binding comprise of metalloprotein database (MDB, <http://metallo.scrip-ps.edu>) [121], which consists of quantitative data of all the metal consisting sites accessible from PDB, and PROMISE, which provides information about metalloproteins and is now discontinued [122]. Other related databases include



BIND (<http://bind.ca>), which is the compilation of complete information of interactions with ligands, including metals. It is built up from peer-reviewed literature and only published results are accepted. In addition, databases from the large genomics centers usually include a biophysical depiction of targets and may contain metal binding information. These servers and databases are very beneficial in providing the broad scientific communities easy connection to the existing data on the characterization of metalloproteins.

The availability of genomic data of a large number of organisms spurs enthusiasm for the advancement of bioinformatics methods allowing the prediction (in silico identification) of trace-element containing proteins [123]. A pioneering study reported the progress of bioinformatics tools to identify almost all the genes in many organisms which encode proteins containing selenocysteine, including humans. The introductory algorithms utilized the recognized SECIS (selenocysteine insertion sequence) element (AUGA\_AA\_GA) as an endorsement for mammalian selenoproteins [124], and subsequently searched for SECIS-containing genes within the frame of UGA codon [125]. SECIS element is harbored by mRNAs encoding selenoproteins in their 30 untranslated regions and triggers the UGA codon (normally stop codon) recoding into selenocysteine. Note that whereas the SECIS elements of the eukaryotes concurrence are well characterized and can be determined in data bases, conservation of bacterial SECIS element was inadequate for the computational description [126]. The characterization of the seleno proteomes and metalloproteomes via experimental approaches is very tedious, this leads to the search for in silico methods in order decipher the role of metalloproteins. Recently, a series of bioinformatics studies reported the disposal of iron-, copper- and Zn-binding proteins in several organisms such as eukaryotes, bacteria, and archaea [123–158].

## Conclusion and Future Prospective

Metallomics is projected as the interdisciplinary research field for the promotion of biometal science. Metal ions conserve the physiological functions in living organisms, thus keep us healthy under stable conditions. Paradoxically, some metals are needed in trace amounts and cause toxicity at elevated levels whereas living cells tolerate other metals at extremely high levels. The biometallics has not received significant attention as a systemic scientific field as the researches on biometals are performed independently or distinctly in various scientific fields. The advancement in metal technologies would expedite the course of identification, characterization of novel metalloproteins, metal folds and establish the usage of metal ions in living organisms. Hence, in near future, metallomics and metalloproteomics could be utilized to understand complex biological systems. The various biochemical processes such as respiration, biological nitrogen fixation, sulfur and carbon cycle are dependent on metalloproteins. Thus, the knowledge of metalloproteins is essential to many research areas from climate change, carbon capture, and bioenergy to plant biology and medicine. The applications of metal-technologies would elaborate the understanding of metal

function in the physiology of microbes, ecology, and human disease, and would impact various aspects of biological science in the coming years. The metal ions significance in bioactive macro-molecules present in various organisms makes necessary the advancement of novel analytical tools which, under a multi-dimensional approach, contribute the comprehensive characterization of metal-macromolecules. The breakthrough in spectrometry especially tandem (MS), makes the study of metallomics feasible. This would lead to the estimation of biomarkers, quality of food, diagnosis of diseases and designing of metallo drugs and their action. Metallomics combines separation, detection of metal ions and elucidation of the structure and thus has a multidisciplinary basis. For the rapid process in metallomics the interface among analytical chemists, biologists and biochemists is required. Further, metallomics data of the cell should be analyzed with the genomic and proteomic data. This would aid in perceiving the role of metalloproteins. The metallomics shows integration with different omics and addresses the important role of metal ions in various disciplines of science (Figure 2).

The study of metallomics in line with other -omics spots the emerging pieces of the zigzag puzzle of systems biology; contributes to different fields such as molecular biology, pharmacology, toxicology, medicinal chemistry, nutrition, environmental chemistry, and animal and plant physiology. The progressive technological advancement in analytical instrumentation and interdisciplinary collaborations will link pure and applied parts for a correlative approach and accelerate the pace of studies in metallomics which will positively reflect on different areas of life.

Metallomics should be established as a methodological field of study to demonstrate the functions and chemical evolution of organisms. Thus, though metallomics is a post-genomic and post-proteomic field but needs to be developed in cooperation with genomics, proteomics, and metabolomics with the assistance of advanced techniques for quantization and quality analysis.

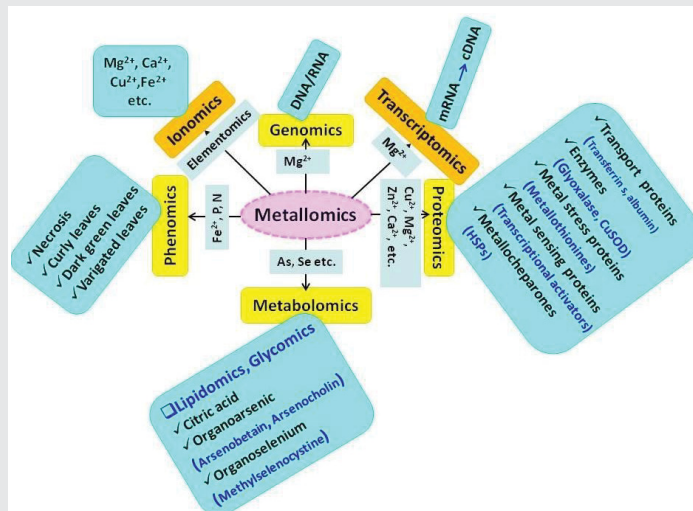


Figure 2: Schematic model of the biological system showing integration among different omics with metallomics.

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