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Review Paper

Oxidative Stress and Heavy Metals: An Appraisal with Reference to Environmental Biology

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Abstract

Rapid developmental activities and other anthropogenic influences have made heavy metals (HM) ubiquitous contaminants of the environment. Investigations on pollution due to HM and their possible effects on living world have increasingly become a prime focus of the environmental biology. An increasing body of evidence shows that excessive presence of essential metals as well as minute presence of non-essential metals can cause many patho-physiological complications in the living organisms via generation of reactive species. This article discusses functional role of some HM in inducing oxidative stress along with the instruments involved viz. oxidants and antioxidative defence systems. Some recent evidences of HM induced oxidative stress in plants and animals, including human beings, have also been examined in the text.

Keywords: Ascorbate-glutathione cycle, catalase, lipid peroxidation, reactive nitrogen species, reactive oxygen species, superoxide dismutase.

Introduction

The advent of industrialization, urbanization, change in land use pattern, application of chemical fertilizers in agricultural fields etc. have caused massive alterations in the quality of air, water and soil. The study of environmental biology has received immense attention in recent decades because pollution emerged as a serious and challenging side effect of the increased developmental activities[1-3]. The release of both inorganic and organic hazardous substances is causing degradation of natural resources, making them unsuitable for use. Heavy metals (HM) form one such group of materials that are capable of causing many patho-physiological conditions in living organisms[4,5]. Although essential metals like iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), etc. are natural part of enzymes and other bio-molecules, and, thus, are needed for normal biochemical and physiological functioning of the body, at higher concentrations they can be detrimental. On the other hand, non-essential metals like lead (Pb), cadmium (Cd) and mercury (Hg) are toxic even at minute concentrations. These metals are widely spread in air, water and soil and find easy entrance into the living organisms. One of the most extensively investigated mechanisms via which HM can produce patho-physiological affairs is the generation of radicals, like reactive oxygen species (ROS), potent to induce oxidative stress. The accumulation of reactive species like hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) disturbs the oxidant-antioxidant balance bringing about a change called oxidative stress. These active species react with bio-molecules like lipids, proteins and DNA impairing their functional properties which in turn brings about alterations in the normal activities of cells, tissues, organs and ultimately organisms evident as disease symptoms, figure-1, and other pathological conditions[6]. Involvement of ROS in metal induced cell death is widely reported[7]. However, to counteract the menace of reactive species the antioxidant defence machinery is activated as radical scavenging system[6]. Antioxidant defence system comprises both enzymatic biomarkers like catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and non-enzymatic biomarkers like β-carotene, glutathione (GSH), Vitamin C (ascorbic acid), Vitamin E (α-tocopherol) etc. The body tries to maintain homeostasis between the presence of oxidants and the antioxidants. Oxidative stress happens when either the amount of reactive species is enhanced significantly high or the antioxidant defence system becomes weak or both.

Oxidative and antioxidative perspectives

Oxidants and reactive species: Chemical reactions can be heterolytic as well as homolytic. Heterolytic reactions produce positive or negative ions as they involve transfer of electron pairs. On the other hand, homolytic reactions, which involve one electron transfer, generate radicals containing an unpaired electron[6]. Free radicals are characterized having one or more unpaired electrons and are competent to exist independently[8]. They are highly reactive and unstable species capable of generating new radicals via chain-reactions with consequences to cause injuries and damages to healthier cells and cellular responses[6,9]. Some common examples of free radicals include: alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$), hydroxyl ($HO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), nitric oxide ($NO^{\bullet}$), superoxide ($O_2^{\bullet-}$) etc. Free radicals, primarily, can undergo four types of chemical reactions viz. hydrogen abstraction (free radical reacts with a hydrogen donor → the radical becomes stable while hydrogen donor

becomes a new free radical); addition (binding occurs between a free radical and a stable molecule which generates a new combined radical species); termination (reaction occurs between two radicals and a stable compound is formed); and disproportionation (reaction occurs between two identical radicals where one radical transfers its unpaired electron to the other → two different stable molecules are formed ultimately)[10].

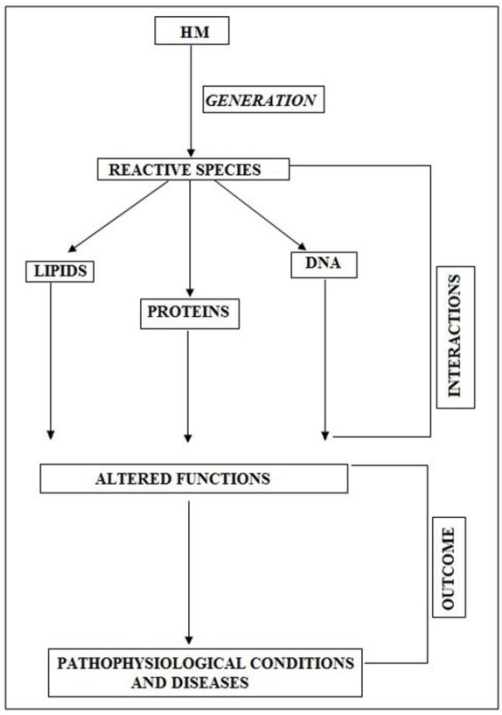

Figure-1
Outline of HM induced oxidative damage

Oxidative and reductive reactions, combined as redox reactions, form the basis of the study of biological oxidative stress. Loss of electrons demonstrate oxidation, whereas, reduction is simply the gain of electrons. An oxidant is an oxidizing agent or a substance that during the reaction process gets reduced by accepting electrons and simultaneously causing other reactant to get oxidized[11]. In biological systems oxidants are commonly referred as pro-oxidants and as synonyms for reactive species. A pro-oxidant in general term is an oxidant of pathological significance and can be defined as a toxic substance capable of causing oxidative injury and damage to a variety of biological entities like lipids, nucleic acids and proteins resulting in alterations of their functional properties[11]. Reactive species comprise both free radicals (mentioned in above paragraph) as well as non-radicals. Common examples of non-radicals include hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), hydroperoxides (e.g. alkyl hydroperoxides, ROOH), hypochlorous acid (HOCl) etc. Though, there are many forms of reactive species, three reactive groups viz. reactive oxygen species (ROS; examples include $HOO^\bullet$, $O_2^{\bullet-}$, H_2O_2, $HO^\bullet$), reactive nitrogen species (RNS; examples include $NO^\bullet$, $NO_2^\bullet$, $ONOO^-$) and reactive halogen species (RHS; examples include HOCl, HOBr) are the most described. A synopsis of major reactive species frequently encountered in environmental biology reports is provided in table-1.

Table-1
A list of some species involved in oxidative stress

Species	Representation
Alkyl hydroperoxide	ROOH
Alkoxyl radical	$RO^\bullet$
Alkyl peroxynitrite	ROONO
Peroxyl radical	$ROO^\bullet$
Ferryl ion	$Fe^{(4+)}O$
Hydrogen peroxide	H_2O_2
Hydroperoxyl radical	$HOO^\bullet$
Hydroxyl radical	$HO^\bullet$
Hypobromous acid	HOBr
Hypochlorous acid	HOCl
Nitric oxide radical	$NO^\bullet$
Nitrogen dioxide radical	$NO_2^\bullet$
Nitronium cation	NO_2^+
Nitrosyl cation	NO^+
Nitroxyl anion	NO^-
Periferryl ion	$Fe^{(5+)}O$
Peroxynitrite	$ONOO^-$
Peroxynitrous acid	ONOOH
Singlet oxygen	$^1O_2$
Superoxide radical	$O_2^{\bullet-}$

Many phenomena can explain the origin of reactive species. The electronic structure of atomic oxygen, characterized by the occurrence of two unpaired electrons in two 2p orbitals ($2p_y$ and $2p_z$) in its external shell, makes it prone to the production of radicals. The sequential and ultimate reduction of oxygen molecule to H_2O produces a series of ROS via stepwise addition of electrons as depicted in figure-2. Ions of metals like Fe and Cu can react with H_2O_2 producing extremely reactive $HO^\bullet$ radical. Of the three major ROS species viz. $O_2^{\bullet-}$, H_2O_2 and $HO^\bullet$, radical $HO^\bullet$ is the most toxic and highly reactive. H_2O_2 and $O_2^{\bullet-}$ are considered as destructive ROS mainly for their involvement in $HO^\bullet$ generation[12]. $NO^\bullet$ in combination with $O_2^{\bullet-}$ can generate a strong oxidant $ONOO^-$, detrimental to many bio-molecules, which at acidic pH can decompose into $HO^\bullet$, the most toxic radical. RHS like HOCl can form, figure-2, in the activated phagocytic cells like neutrophils via myeloperoxidase mediated reaction involving H_2O_2 and chloride ions[8]. Formation of lipid peroxyl radicals can occur via involvement of initiator free radical like $HO^\bullet$ which abstracts hydrogen atom from lipids or polyunsaturated fatty acids. In the initiation step a lipid radical is formed which subsequently undergoes internal rearrangement and O_2 addition in stepwise manner to generate lipid peroxyl radical[13]. Figure-3 illustrates the general mechanisms of formation of some radicals from unsaturated lipids.

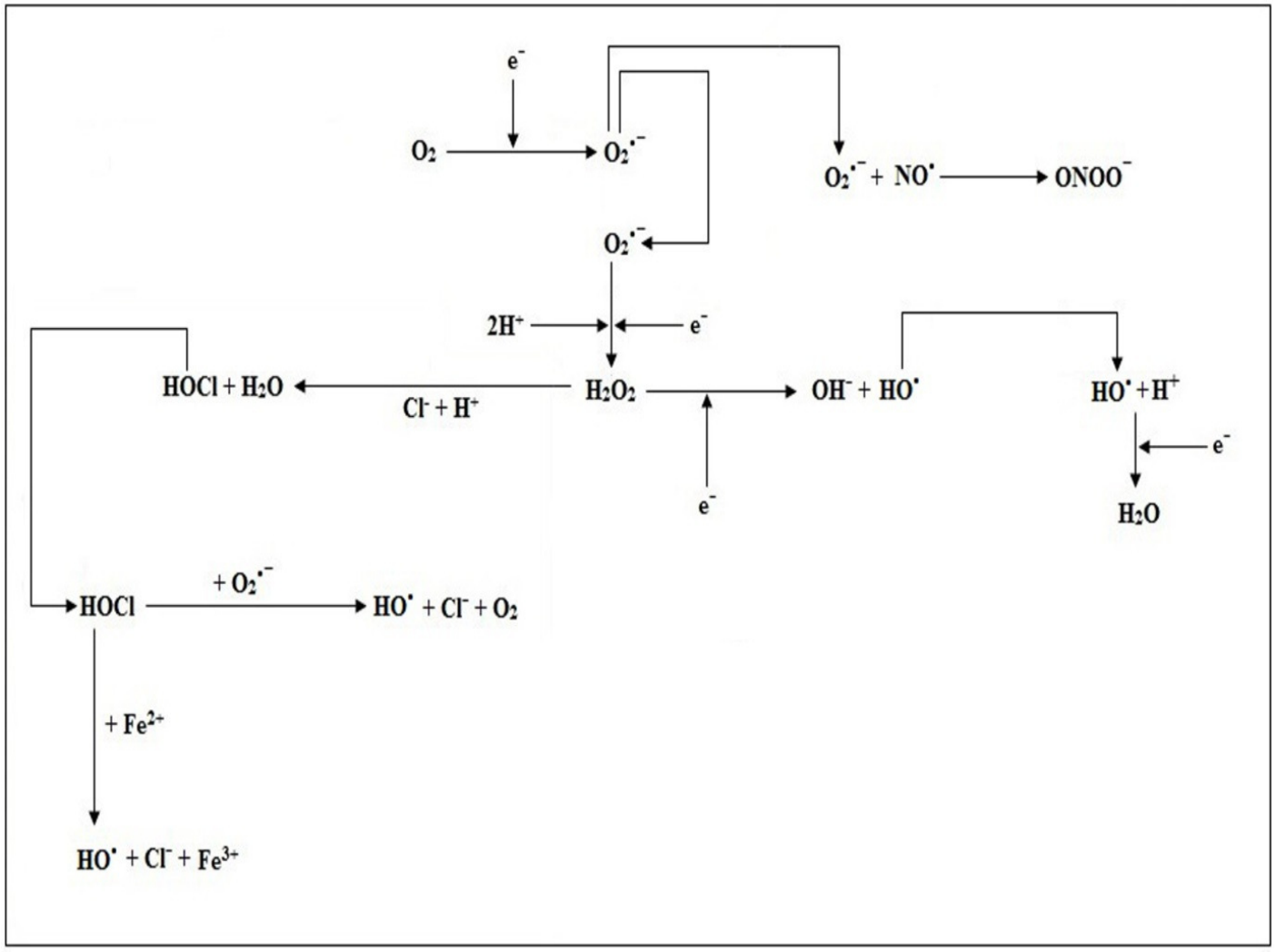

Figure-2
Mechanisms of generation of some reactive species (modified from elsewhere[8])

Oxidative stress and outcomes: Oxidative stress happens when the balance (or equilibrium) between oxidants (free radicals and non radicals both) and antioxidative machinery is disturbed causing malfunctioning of bio-molecules like proteins, lipids and nucleic acids which ultimately leads to various patho-physiological conditions and diseases to occur. In some reported literatures the term "redox stress" has been preferred over oxidative stress, since, oxidation is always accompanied by its chemical partner reduction[16]. As antioxidant defence machinery is a coordinated system involving many components, deficiencies in the functional properties of one component may impair the efficient working of other constituents[17]. Many reports suggest that ROS (particularly H_2O_2) can specifically alter reactive amino acid cysteine residues within proteins, converting them from S—H (thiol) to S—OH (sulphenic) derivatives. This makes normal function of the proteins altered and corresponding damage to the signal transduction pathway. Role of RNS (for example, $NO^{\bullet}$) in alteration of reactive cysteine residues has also been documented[18]. DNA damage because of oxidative reactions may range from injuries at abasic DNA sites, oxidation of purine and pyrimidine bases, to single and double strand breaks in DNA molecules[19]. $HO^{\bullet}$ radical is considered as the most potent agent responsible for oxidative DNA damage which can give rise to many base lesions like 8-oxo-7,8-dihydroguanine; 5,6-dihydroxy-5,6-dihydrocytosine and 5,6-dihydroxy-5,6-dihydrothymine and apart from attacking deoxyribose sugar and forming sugar radicals[19,20]. Loss of integrity of biological membranes can damage the structure and function of cells. Lipids are fundamental components of cellular membranes and are also targets of oxidative damage. Radicals such as hydroxyl, alkoxyl and alkylperoxyl, etc. are capable of causing lipid peroxidation (LP) and extensive damage to membrane structure[21]. In human beings oxidative stress phenomenon can give rise to several patho-physiological states, such as cardiovascular diseases, cancer, diabetes mellitus, neurodegenerative diseases like Alzheimer's and Parkinson's diseases, atherosclerosis, inflammatory diseases, and aging etc.[16,22].

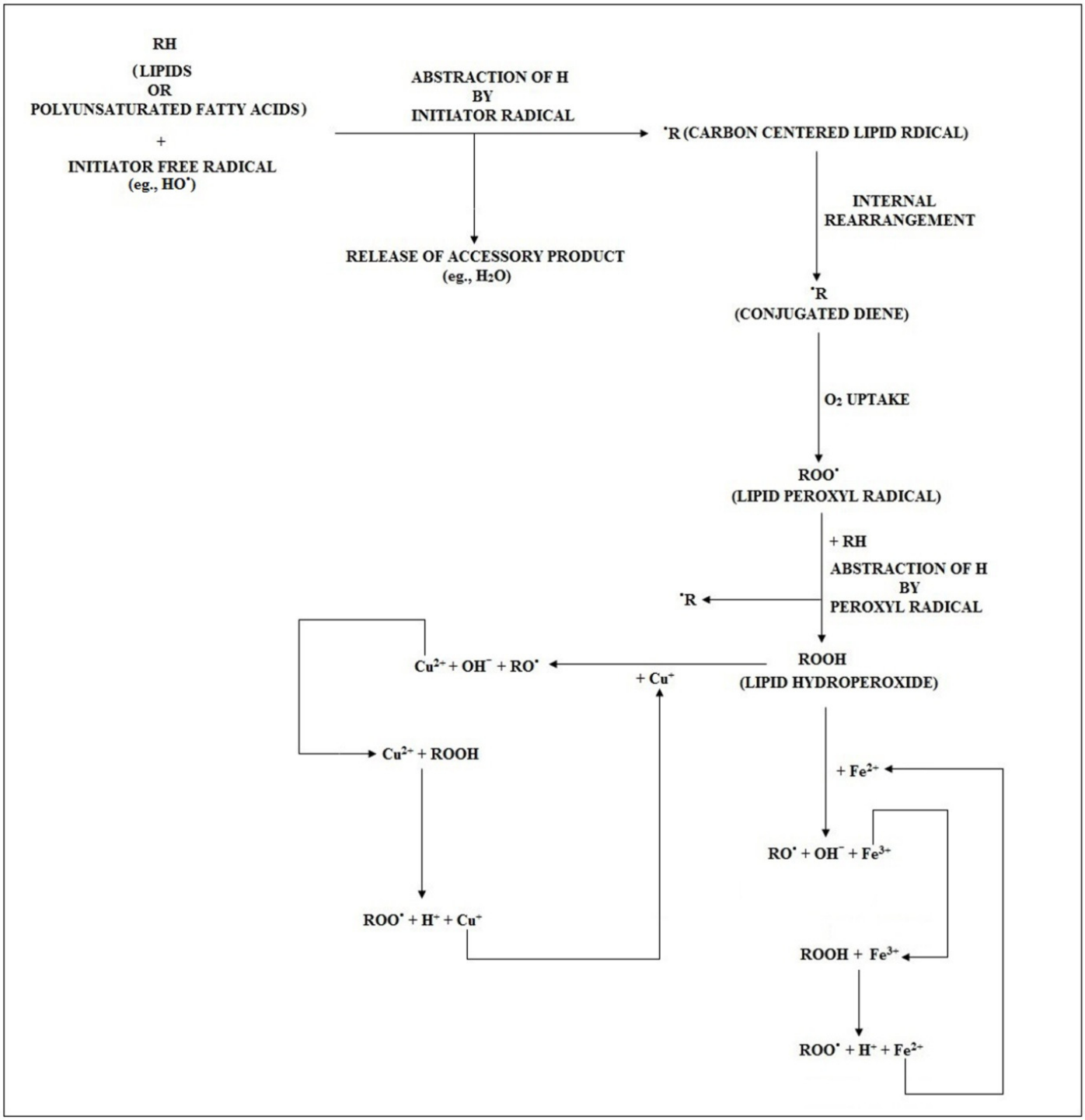

Figure-3
Peroxidation of lipids and involvement of some HM ions (modified from elsewhere[14,15])

Antioxidant defence machinery: Antioxidant in its widest possible meaning has been defined by Gutteridge, in the literature entitled 'lipid peroxidation and antioxidants as biomarkers of tissue damage' as "any substance that, when present at low concentrations compared with those of the oxidizable substrate, considerably delays or inhibits oxidation of the substrate"[8]. The oxidizable substrate in biological systems refers to the bio-molecules like lipids, proteins, carbohydrates and nucleic acids[23]. Antioxidant defence machinery can be categorized into enzymatic and non-enzymatic systems.

Antioxidant biological markers commonly employed to study oxidative stress are presented in table-2. Proteins, capable of binding metals, like albumin, ferritin, lactoferrin, and ceruloplasmin can sequester excess metals (like Fe and Cu) that can cause oxidative reactions when present freely in the body[24]. SOD is the frontline enzyme in detoxification of $O_2^{\bullet-}$ radical which dismutates $O_2^{\bullet-}$ into H_2O_2 and O_2. The enzyme requires specific metal(s) as cofactor for its normal function and is variously classified into Cu/Zn—SOD, Fe—SOD, Mn—SOD and most recently Ni—SOD. Another class of enzymes designated as superoxide reductase also act on $O_2^{\bullet-}$ radical, but they only reduce $O_2^{\bullet-}$ to hydrogen peroxide[25]. CAT is another class of oxidoreductase enzymes which work synergistically with SOD and converts H_2O_2 into H_2O and O_2. It is Fe (in heme groups) containing enzyme having four molecules of firmly bound NADPH[26]. GPX, a selenoenzyme and having functional similarity to CAT, also detoxifies H_2O_2 in the presence of GSH. In addition, selenoprotein GPX also reduces detrimental organic peroxides (hydroperoxides) using GSH and protects cellular membranes against oxidative injuries[27]. The basic mechanisms of working of SOD, CAT and GPX are illustrated in figure-4. Another mechanism of detoxification of H_2O_2 (operating in plant cells) is via the ascorbate-glutathione cycle. The cycle involves four enzymes and ascorbate as the reducing substrate for the reduction of H_2O_2 to H_2O. The first reaction of the cycle is catalyzed by ascorbate peroxidase (APX) where H_2O_2 is reduced to H_2O involving two molecules of ascorbate and corresponding formation of two molecules of monodehydroascorbate (MDHA). MDHA can undergoe enzymatic reduction to regenerate ascorbate as well as non-enzymatic disproportionation forming ascorbate and also dehydroascorbate (DHA). Ascorbate is again regenerated from DHA via the reaction involving DHA reductase and GSH. Glutathione disulphide (oxidized glutathione, GSSG) produced as a byproduct of this reaction is reduced back to GSH by GSH reductase[28]. A schematic representation of ascorbate-glutathione cycle is given in figure-4. Since, LP is an underlying reason for the patho-physiology of many diseases, scavenging of lipid peroxyl radicals is necessary to minimize oxidative damage. Vitamin E, a chain-breaking antioxidant, effectively scavenges free radical peroxyl produced during oxidation of polyunsaturated lipids and protects them from further damage[14]. Reduced form of Vitamin E transfers one hydrogen atom to the peroxyl radical generating lipid hydroperoxide and in the process becomes a radical itself. Another chain breaking antioxidant Vitamin C regenerates reduced Vitamin E from its radical form. This step also generates Vitamin C radical which is converted back to the Vitamin C by dihydrolipoic acid (DHLA) forming α-lipoic acid. This α-lipoic acid is subsequently reduced to DHLA in the presence of NADPH. Moreover, GSH can also reduce Vitamin E radical back to its active form. The reaction generates GSSG (oxidized glutathione) which is reduced back to GSH by DHLA and becomes itself α-lipoic acid. As mentioned above the α-lipoic acid is subsequently converted to DHLA by NADPH[29]. The sequential steps showing involvement of various antioxidative biomarkers in inhibition of peroxidation of polyunsaturated lipids is shown in figure-5.

Table-2
Some non-enzymatic and enzymatic biomarkers for oxidative stress study

Non-enzymatic biomarkers	Enzymatic biomarkers
Albumin	Ascorbate peroxidase (EC 1.11.1.11)
Ascorbic acid	Catalase (EC 1.11.1.6)
Bilirubin	Cytochrome oxidase (EC 1.9.3.1)
β-carotene	Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)
Ceruloplasmin	Glutathione reductase (EC 1.8.1.7)
Coenzyme Q	Glutathione-S-transferase (EC 2.5.1.18)
Dihydrolipoic acid	Peroxidase (Guaiacol) (1.11.1.7)
Flavonoids	Selenium dependent glutathione peroxidase (EC 1.11.1.9)
Glutathione (GSH)	Superoxide dismutase (EC 1.15.1.1)
GSH/GSSG (Oxidized glutathione)	
Lactoferin	
α-lipoic acid	
Lycopene	
Metallothioneins	
Transferrin	
α-tocopherol	
Uric acid	

Heavy metals and oxidative stress/damage

Many HM present in the environment can generate reactive species in the biological systems. Although both natural and anthropogenic activities contribute to their occurrence in the environment, the rapid and extensive developmental activities have elevated their concentrations in air, water and soil. These metals enter living organisms through inhalation, food, drinking, absorption etc. resulting in many drastic biochemical and physiological alterations in the body. A generalized scheme of release of HM in the environment and their entry into the living world (especially with reference to human beings) is illustrated in figure-6. Fe, Cu, nickel (Ni), chromium (Cr), Cd, Arsenic (As), mercury and, Pb are some of the most widely studied HM for their roles in causing oxidative stress. Redox-active metals (e.g. Cu, Cr, Fe etc.) undergo reactions of redox cycling, whereas, redox-inactive metals (e.g. Cd, Hg, Pb etc.) diminish cells' key antioxidant defence constituents, particularly thiol-containing antioxidants and enzymes[30]. Redox-active metals can utilize Fenton chemistry/Haber-Weiss reaction, figure-7, to directly generate reactive species leading to oxidative damage of the structure and functions of the cellular machinery. The Haber-Weiss reaction illustrates that the interaction of less

reactive $O_2^{\bullet-}$ and H_2O_2 radicals can generate more toxic and extremely reactive $HO^{\bullet}$ species. However, to drive the reaction in biological systems a metal ion as a catalyst is needed. The most researched mechanism via which Haber-Weiss reaction produces $HO^{\bullet}$ radicals utilizing a metal ion is the Fenton chemistry, which is a net combination of two reactions[31] — Fe^{3+} interacts with $O_2^{\bullet-}$ to generate Fe^{2+} and O_2; Fe^{2+} reacts with H_2O_2 to produce $HO^{\bullet}$, OH^- and regenerate Fe^{3+} (This reaction is also called Fenton reaction). Apart from Fe^{2+}, other metal ions like Cu^{2+} and Cr^{3+} can also produce $HO^{\bullet}$ radicals via the Fenton reaction or more specifically Fenton-like reaction[15]. A general reaction mechanism of Fenton chemistry with examples of two metals is demonstrated below (the reaction involving Fe is displayed in figure-7).

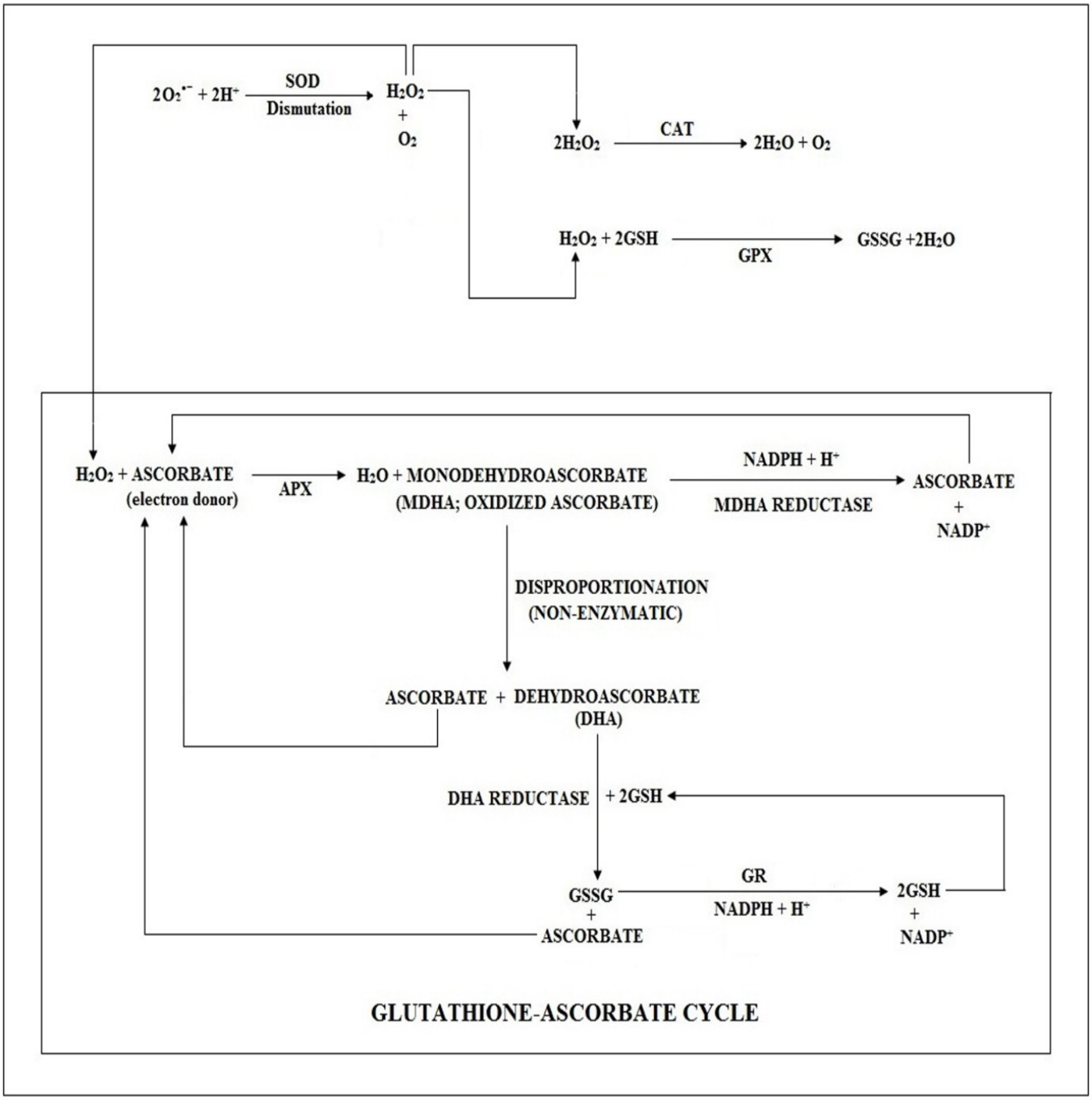

Figure-4
Functions of SOD, CAT, GPX and the glutathione-ascorbate cycle (lower part modified from elsewhere[28])

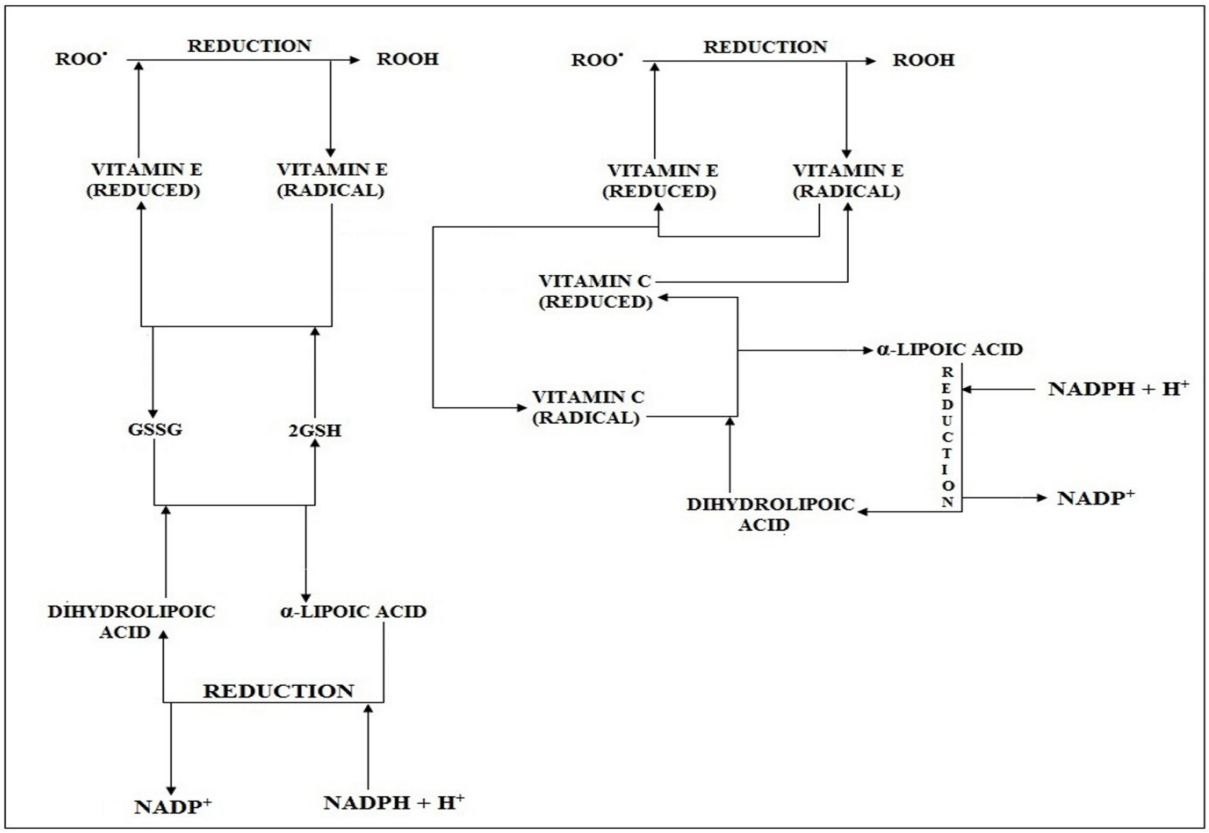

Figure-5
Role of VITAMIN E in inhibition of LP (ROO• = lipid peroxyl radical; modified from elsewhere[29])

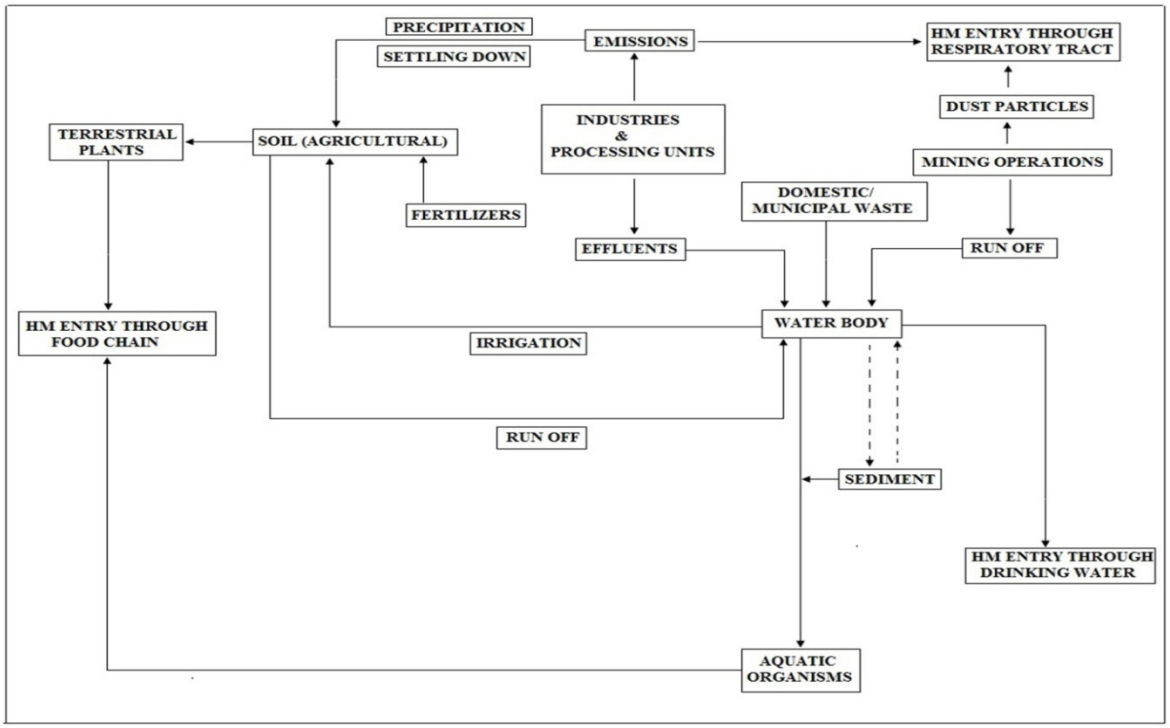

Figure-6
Possible entrance routes of HM with special reference to human beings

$HM^{(n)+} + O_2^{\bullet -} \rightarrow HM^{(n-1)+} + O_2$
$HM^{(n-1)+} + H_2O_2 \rightarrow HM^{(n)+} + OH^- + HO^{\bullet}$
$Cu^{2+} + O_2^{\bullet -} \rightarrow Cu^{+} + O_2$
$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + HO^{\bullet}$
$Cr^{3+} + O_2^{\bullet -} \rightarrow Cr^{2+} + O_2$
$Cr^{2+} + H_2O_2 \rightarrow Cr^{3+} + OH^- + HO^{\bullet}$

Direct role of Cd in generating free radicals is absent; however, they can produce various radicals via some indirect mechanisms. One such mechanism describes Cd can displace Cu and Fe from their bound forms, like ferritin and apoferritin, and making them available to generate reactive radicals via the Fenton chemistry[32]. Cd is also capable of depleting Mn and Zn in SOD altering the antioxidant property of the enzyme. Ercal and co-authors[30] reviewed the mechanisms of Pb induced oxidative damage through its effects on antioxidant defence constituents. Pb interferes with the disulphide bond present in the active site of the enzyme glutathione reductase (GR) and inhibits its activity. GR, an essential constituent of the antioxidant system, is responsible for catalyzing GSSG (oxidized glutathione) back to GSH (reduced form). The decrease in concentrations of GSH makes the cells more prone to oxidative injuries. Pb can also inhibit functional properties of CAT and GPX by interfering with heme synthesis and forming a complex with metal Selenium respectively. Moreover, it can also interfere with the working of SOD enzyme[30]. Though, at higher concentrations zinc can also be designated as a pollutant, chemically it is inert and does not engage in oxidation-reduction reactions. Presence of Zn is essential for the normal functions of many cellular constituents including antioxidant enzyme Cu/Zn—SOD. However, at elevated cellular concentrations it can be toxic causing cell death due to necrosis[32]. Excess Zn can exacerbate oxidative stress through enhanced production of reactive oxygen radicals[33]. For example, Zn^{2+} has been implicated in causing mitochondrial dysfunctions especially its electron transport system (ETS). The study describes Zn^{2+} capable of binding to bc_1 of complex III of ETS and obstructing the electron flow. The consequences of this Zn^{2+} induced inhibition of complex III include phenomena like decline in O_2 consumption and enhanced production of ROS[34]. Mitochondrial origin of Zn^{2+} induced oxidative stress is also supported by one recent work. The study reports that intracellular H_2O_2 accumulation in response to Zn^{2+} exposure is linked with two phenomena viz. declining mitochondrial reducing redox potential and mitochondrial membrane depolarization[35].

HM induced oxidative stress study in environmental biology

This section discusses a few studies undertaken to investigate HM exposures and oxidative stress in both plants and animals.

Oxidative stress in germinating seeds due to HM exposures has been investigated[36-38]. In one such report, *in vitro* germinated seeds of *Brassica juncea* L. were exposed to different concentration grades of Cd (0, 50, 100 and 200 mg/l) and oxidative stress determined. GSH showed elevated content with increasing Cd concentrations which at higher concentrations accumulated significantly. Though, GST activity increased with time at 50 and 100 mg/l concentrations, significant increase in activity was noted only at the highest Cd exposure suggesting that higher concentrations are needed for enhanced induction of the enzyme. The study also demonstrated a positive correlation between accumulation of GSH and GST activity and their elevated levels explained the lower amount of LP in the metal exposed seeds. The study augmented the role of GSH and GST as non-enzymatic and enzymatic antioxidant biomarker systems respectively[36]. Impact assessment of trivalent (III) and hexavalent (VI) Cr species on some antioxidant enzyme activities in an aquatic bryophyte has been carried out. *Fontinalis antipyretica* Hedw. was exposed to different concentrations of Cr salts viz. chromium nitrate ($Cr(NO_3)_3$), chromium chloride ($CrCl_3$) and potassium dichromate ($K_2Cr_2O_7$) and activities of enzymatic biomarkers namely SOD, CAT, APX, guaiacol peroxidase and GR and one non-enzymatic biomarker GSH were recorded. All studied parameters including GSSG/GSH ratio responded to Cr as its exposure disturbed the cellular redox status by generating ROS inside plant cells, substantiating that Cr could induce oxidative stress in the moss leading to impairment of cellular activities. However, nitrate salt of Cr(III) was more damaging as considerable responses were recorded even at the lowest exposure level, and was comparable to harmful effects of Cr(VI). In addition to the Ascorbate-glutathione cycle, SOD and CAT are often considered as the key metal detoxification systems in plants. The study conducted on *F. antipyretica* Hedw. showed increased SOD and CAT enzymes at low Cr exposure levels which decreased at high exposures. The two enzymes also displayed high correlation between them suggesting that higher Cr concentrations induced elevated generation of ROS which disturbed the cellular machinery causing cytotoxic responses. According to the authors (quoted references) two possible explanations could describe the above mentioned trend: first, disturbance of the signal transduction pathway that causes expression of genes of various antioxidative enzymes like SOD by the excess $O_2^{\bullet -}$ radical; for example, inhibition of cellular metabolism and decline of the isoforms expression of Mn—SOD; and second, excess Cr ions getting bound to the active centre of the enzymes. Similar patterns were noted for both GSH and GSSG contents and two hypotheses were suggested to explain these trends: first, increased concentrations of the two unidentified thiolic compounds (and GSH incorporation with them), possibly secondary metabolites, at high Cr exposures; and second, rise in GPX activity or over-expenditure of GSH for reduced ascorbate regeneration via glutathione-ascorbate cycle[39]. In other experiment same species of bryophyte was subjected to different concentrations of four HM i.e. Cd, Cu, Pb and Zn. The exposures caused enhanced LP and significant modifications in the enzymatic activities of SOD, CAT, GR, APX and guaiacol peroxidase. SOD and malondialdehyde (MDA, a product of LP) levels displayed monotonous and linear relationships suggesting their use as environmental bio-markers for bio-monitoring of pollutions involving HM[40].

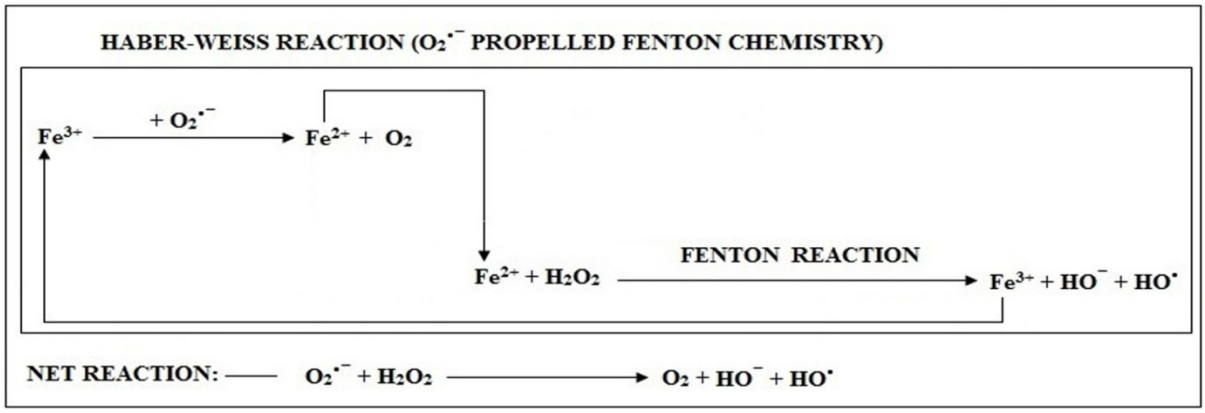

Figure-7
Haber-Weiss reaction and Fenton chemistry (modified from elsewhere[31])

LP and GPX activity have been suggested as suitable biomarkers with respect to multiple HM stress. A study was conducted where leaves and roots of seedlings of mangrove plant *Kandelia candel* and *Bruguiera gymnorrhiza* were exposed to different levels of multiple HM stress involving Pb, Cd and Hg. The study strengthened the view that HM toxicity to plants is via free radical generation mechanism. For example, the plants displayed production of MDA which showed enhanced free radical formation under HM induced stress conditions. Though, at medium concentrations SOD showed increased activity in both leaves and roots, the activity sharply declined at the highest concentration. This observation illustrated the phenomenon that at higher concentrations HM can impair the functioning of SOD. Furthermore, the study also indicated peroxidase as more efficient and important enzyme for scavenging of H_2O_2 than CAT[41].

Profile evaluation of oxidative insult biomarkers in the blood of directly and indirectly exposed individuals to fly ashes revealed enhanced concentrations of thiobarbituric acid reactive substances (TBARS assay is used to quantify degree of LP) and protein carbonyls levels in both groups. Moreover, exposed groups also showed decline in blood GSH levels and noticeably increased activities of SOD and CAT. The reduction of GSH level was possibly due to its increased antioxidant activity and conjugation action with the xenobiotics present within fly ashes propelled by GST. Marked increase in SOD and CAT indicated enhanced production of $O_2^{\bullet -}$ and H_2O_2 on being exposed to fly ashes. The study highlighted that inhalation of particulate matter (PM) or fly ashes generated through coal combustion can produce reactive species due their high HM content capable of causing oxidative damage to both protein and lipid bio-molecules in parallel manner[42].

In another study impact of contaminated river water with special reference to the presence of HM on the oxidative stress of three cichlid species namely *Oreochromis niloticus*, *Tilapia rendalli* and *Geophagus brasiliensis* was investigated, though, other water quality parameters like nitrate, nitrite, ammonical nitrogen, silicate, conductivity etc were also taken into account. Most of the parameters including HM displayed higher concentrations in polluted site as compared to the unpolluted reference site. Species present in the polluted site showed lower haemoglobin levels, higher LP intensity and considerable differences in the activities of SOD, CAT and GPX compared to the unpolluted site. Marked increase in the activity of SOD and LP/CAT + GPX ratio were noted in response to chronic exposure to contaminants in all species exhibiting oxidative stress complications. Enhanced LP/CAT + GPX ratio confirmed ROS induced cell damage, as it suggested that higher levels of peroxide were produced in species from the polluted site and radical scavenging antioxidants like CAT and GPX were unable to neutralize the reactive species. The study implicated higher environmental presence of Zn, Mn, Fe and Cu (along with ammonia) as major culprits for inducing oxidative stress[43].

Bivalves are scientifically advocated as suitable bio-indicators for bio-monitoring water pollution. An assessment of HM pollution in the Saronikos Gulf of Greece was conducted through evaluation of oxidative stress in *Mytilus galloprovincialis* mussels. Gills and mantle of mussels from the polluted sites (Elefsis Bay present in the Saronikos Gulf) recorded two to three times concentrations of most metals involving Cd, Cr, Ni, Pb, Cu and Fe than the unpolluted reference site (Stylida in Malaikos Gulf). Moreover, Gills recorded elevated metal concentrations than the mantle. A noticeable correlation was obtained between the metal concentrations and the activities of antioxidants SOD and CAT as their activities were markedly higher in mussels collected from the Elefsis Bay than the reference site. Moreover, gills also demonstrated higher activities of the antioxidant enzymes than the mantle. However, increased antioxidative actions were unable to decrease elevated levels of LP in mussels from the pollute sites. Higher LP levels were also recorded in gills as compared to the mantle. The investigation clearly highlighted the role of metals in causing oxidative damage and also demonstrated that presence of metals at elevated concentrations overcome the actions of antioxidant enzymes like CAT[44]. Cu and Pb have been shown to accumulate in the mantle of the pearl oyster *Pinctada fucata* and altering the activities of GPX and SOD in similar way. The study also demonstrated that

presence of metals at lower concentrations stimulated SOD activities which gradually reduced at higher concentrations[45].

The studies mentioned above clearly suggest that elevated concentrations of HM are able to generate higher levels of free radicals as well as non-radicals which antioxidant system fails to compete with resulting in pronounced oxidative damage.

Conclusion

The balance between oxidants and antioxidants is essential to maintain homeostasis. Environmental presence of HM and their subsequent exposure can lead to disruption of this balance leading to oxidative stress. Volumes of reported literature confirm the involvement of oxidative stress in generation of many patho-physiological conditions especially in human beings. One of the consequences of increased developmental activities is the rise in environmental presence of HM beyond their recommended or regulatory standards. Therefore, study of HM induced oxidative stress should be one of the central themes of environmental and investigational biology in order to find proper remedies to this quandary.

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