Review of literature
2.01 Background

Exposure to metals are currently of much environmental concern due to its effects on health of humans and animals (Zheng et al., 2007). These elements tend to bio-accumulate in food chain as large areas of agricultural land have been observed to be contaminated with metals (Quartacci et al., 2006). Mining activities (Quartacci et al., 2006), agronomic practices (Burgos et al., 2008), application of industrial effluents as water source for irrigation of crop plants (Rattan et al., 2005; Abbas et al., 2007) or sludge as manure are the major sources of metal contamination in agricultural land (Singh et al., 2005; Bose et al., 2008). The threat that some metals pose to human and animal health is aggravated by their long-term persistence in the environment (Gupta et al., 2007). They are widely used in the industry for electroplating and galvanization processes, in the production of pigments, batteries, glass and as components of many alloys (ATSDR, 1993). Effluents from these industries often pollute water, air and food with such waste by-products releasing unacceptable levels of these metals.

Cadmium toxicity, emphasized in this work, is found in natural materials have been analyzed (UNEP, 2006). The kidney and the liver are the major target organs of cadmium accumulation and intoxication (WHO, 1992). Exposure to cadmium leads to renal tubular dysfunction. This is primarily expressed as a renal tubular reabsorption defect, and is now recognized as a cardinal feature of cadmium induced renal damage (Horiguchi et al., 1996). Cadmium is also implicated in the etiology of hypertension (Lall et al., 1997). This metal is currently believed to cause most of its toxic effects by mechanism (s) related to its ability to generate free radicals at a rate high enough to overwhelm the natural antioxidant defense system of the body (Bagchi et al., 1996).

2.02 Cadmium exposure, uses and route of exposure

Cadmium is an important metal with many applications. It is widely used in pigment and plastic materials production, battery factories (Fig. 1). Its most important usage is in galvanization, due to its anti-corrosive characteristics. It is an important pollutant of the
Western world cadmium consumption

Fig. 1: Cadmium exposure and their different sources
environment, mostly spread by industrial effluents. Tobacco smoking is another major source of cadmium exposure. The tobacco leaves accumulate cadmium in a manner similar to certain food from plants. A cigarette may roughly contain 1–2 μg cadmium (varies depending on the type and brand). Roughly 10% of the cadmium content is inhaled with an approximate 50% absorption in the lung. It is estimated that a person smoking 20 cigarettes per day will absorb about 1 μg cadmium daily (Olsson et al., 2002). Although there is some evidence of cadmium exposure from environmental tobacco smoke (ETS) in children (Willers et al., 2005), ETS does not seem to be a source of cadmium exposure in adults (McElroy et al., 2007). Atmospheric cadmium contributes only to less than a few percent of the total absorbed dose of cadmium in the body (Vahter et al., 1991). As in the case of contaminated water, cadmium-polluted air may occur in the vicinity of some metal industries. In areas with contaminated soils, house dust is potentially an important route of exposure to cadmium, even after the closure of the cadmium emitting source (Hogervorst et al., 2007). Cadmium toxicity occurs through various mechanisms of 44 varying species under different experimental conditions (Iscan et al., 1994, Zikic et al., 1996, Waisberg et al., 2003). The production of nickel-cadmium batteries is currently the primary use of cadmium (ATSDR, 1993). Cadmium, a by-product of zinc and sulfide-ore processing, is also used for metal plating, in pigments and plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3672 pounds of cadmium (CARB, 1999). In the 1950s and 1960s industrial exposure to cadmium was high. However, as the toxic effects of cadmium became apparent, industrial limits on cadmium exposure have been reduced in most industrialized nations and many policy makers agree on the need to further reduce its exposure. It has been realized that while working with cadmium, it is important to do so under a fume hood to protect against dangerous fumes. Silver solder, for example, which contains cadmium, should be handled with care. Serious toxicity problems have resulted from long-term exposure to cadmium plating baths. Prolonged exposure to low concentrations of cadmium can increase glutathione levels (Beyersmann and Hechtenberg, 1997; Tully et al., 2000) whereas high cadmium concentrations lead to glutathione depletion in vitro (Sarkar et al., 1998). In vivo (Shaikh et al., 1999) and in...
vitro depletion of glutathione results in increased toxicity following administration of cadmium.

2.03 Toxicological effects of cadmium

In experimental animals, cadmium can produce acute toxic effects on various organs, such as the kidney, liver, pancreas, testes and lung. It has been reported that cadmium causes morphological damage in hepatic (Dehn et al., 2004) and renal tissues (El-Sharaky et al., 2007), testicular necrosis (Lorico et al., 2002), morphological and biochemical changes in lungs and gastrointestinal tract. Furthermore, cadmium exposure was shown to alter carbohydrate metabolism in liver and hepatic microsomal drug metabolism (Gadeholt et al., 1980).

2.04 Effects on Renal tissues

Kidney damage is progressive and leads to glomerular lesions and lithiasis (Horiguchi et al., 1996). Urinary excretion of MT increases after chronic exposure to Cd and is shown to be a good indicator of the severity of the nephropathy induced by Cd exposure (Shaikh et al., 1999). The selective accumulation of both CdMT and HgMT in the kidney may be because the low molecular weight MT is easily filtered and reabsorbed in the kidney. Injection of CdMT and both forms of Hg resulted in nephrotoxicity as shown by the increase in blood urea nitrogen, total urinary protein concentrations and urinary MT excretion (Thomas et al., 2009). It is well established that exposure to high level of Cd may cause kidney damage leading to renal failure (Jarup et al. 1998). Disorder of glomerulus (glomerulopathies) may either be due to degeneration without inflammatory changes (glomerulonephrosis) or due to inflammatory reactions (glomerulonephritis). They are also caused by immunological complexes. Moreover, blockage of tubular lumen (Kobayashi et al., 2009) causes urinary stasis in nephron, distension of Bowman’s capsule and atrophy and sclerosis of the glomerulus. In renal amyloidosis (glomerular), the kidney is enlarged and contains hyaline material called amyloid. The hydropic changes and tubular necrosis are the important disorders in proximal tubules resulting in necrosis (Satarug et al., 2000). Renal papillary necrosis and position of crystals occur in the distal tubules. The initial sign of Cd-induced renal lesions is tubular proteinuria,
usually detected as an increased excretion of low-molecular-weight proteins, such as α-1-microglobulin (A1M), retinol-binding protein (RBP), and the enzyme N-acetyl-β-d-glucosaminidase (NAG). There is increasing evidence that early tubular damage may develop at low cumulative Cd doses (U-Cd = 1–3 nmol/mmol) resulting from environmental Cd exposure (Jarup et al., 2000). Tubular damage may progress to glomerular damage and eventually to renal failure if Cd exposure is prolonged (Jarup et al., 1998; Hellstrom et al., 2001; Akesson et al., 2005; Jeyanti et al., 2009).

2.05 Effects on Hepatic tissues

Despite the predominant role of liver in drug detoxification, this organ frequently is the site of drug-induced toxicity. The liver plays an astonishing array of vital function in the maintenance performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways for growth, and defense against invading pathogens nutrient supply, energy provision and reproduction (Horiguchi et al., 2000). Unfortunately, the liver is often abused by environmental toxins, prescription and over-the-counter drug use, which can damage the liver and lead to hepatitis, cirrhosis and liver disease. Hepatocellular permeability may be altered by metal and represents a toxicant induced perturbation of membrane function. Therefore, elevated blood levels of many proteins and other endogeneous substances has been associated with the changes in the membrane permeability of hepatocytes (Theocharis et al., 1994; Koizumi et al., 1996). Necrosis leads to high levels of certain enzymes in the blood released from the damaged liver. In most clinical circumstances, this is demonstrated by elevated levels of serum transferase (glutamate oxalate transaminase and glutamate pyruvate transaminase) which may be increased from 10 to 100 fold depending upon the degree of damage. Similarly many other enzymes are released from the hepatocytes as a result of a variety of hepatocellular injury. These enzymes including alkaline and acid phosphatase, lactic dehyrogenase isozymes, isocitric dehyrogenase, sirbitol dehydrogenase and many other (Lasfer et al., 2008). Much of the cadmium in the kidney and in other tissues is bound to metallothionein, which is thought to sequester cadmium, prevent damages to cellular constitutes, but which also retains cadmium in the cell. Metallothionein is thought to function in the storage of the essential metals zinc and copper and to serve as an
antioxidant. The free cadmium may bind to MT intracellularly in the kidney. The CdMT that reaches the kidney through the circulation is filtered by the glomerulus, is directly toxic to the brush border membrane of the proximal convoluted tubules. The reabsorption by the proximal convoluted tubules, is indirectly toxic through degradation of the metallothionein and release of free cadmium intracellularly, which may cause tissues damage unless the capacity of the kidney to produce sufficient intracellular Cd binding metallothionein (ATSDR, 1993).

2.06 Effects on Blood
Bone marrow is highly sensitive to the toxic chemicals which cause dysplasia (abnormal production of cells), hypoplasia (decreased production of cells), aplasia (inhibition of cell production) and malignancy of the cells. RBCs membrane is rich in polyunsaturated fatty acids which are very susceptible to free radical mediated peroxidation. Cadmium cause hemolysis or an iron deficiency, hemolysis either in the bloodstream or in the reticuloendothelial system resulting in disturbed haemoglobin production, abnormal membrane structure and deficient glycolytic enzymes. Toxic metal reduces the oxygen-carrying capacity of the RBCs resulting in methaemoglobin. Cadmium enters the blood where, it binds to the erythrocyte membranes and plasma albumin (Bauman et al., 1993). In the blood and tissues, Cd stimulates the formation of metallothioneins (Sarkar et al., 1998) and reactive oxygen species (ROS) thus causing oxidative damage in erythrocytes and in various tissues, which results in a loss of membrane function (Sarkar et al., 1995). Cadmium also induces the onset of anemia, decreases the red blood cell count, hemoglobin concentration and hematocrit values as well as reduced blood iron levels (Kostic et al., 1993a). Functional impairment of red blood cell membrane as expressed by marked alteration in membrane enzymes like acetylcholinesterase, NADH dehydrogenase, Mg$^{++}$ ATPase and p-nitrophenyl phosphatase.

2.07 Effects on other tissues
In humans, acute Cd exposure via inhalation results in pulmonary edema and respiratory tract irritation, while chronic exposure often leads to renal dysfunction, anemia, osteoporosis and bone fracture (Friberg et al., 1986). Cd is also a potent carcinogen in a
number of tissues and is classified by IARC as a human carcinogen (MacIntosh et al., 1996; Thomas et al., 1999; Larison et al., 2000; Mochizuki et al., 2002; Waalkes, 2003; Nordberg et al., 2007). Cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities, interferes with antioxidant defense mechanisms and inhibition of oxidative DNA repair systems and stimulates the production of reactive oxygen species which may act as signaling molecules in the induction of gene expression and apoptosis (Waisberg et al., 2003; Filipic et al., 2006). Cadmium causes pulmonary emphysema, bronchitis and the alveoli of the lungs become dilated with distention of thin walls (Berglund et al., 1994; Larsen et al., 2002; Llobet et al., 2003; Egan et al., 2007). Cadmium treatment has been reported to affect overall carbohydrate metabolism, reduce liver glycogen and increase the concentration of blood glucose in male rats. Cadmium has been a profound effect on sex organ weight, in the primary indication of a possible alteration in androgen status. Cadmium can inhibit primary Leydig cell testosterone levels (Yang et al., 2003).

2.08 Cadmium toxicity and reactive oxygen species (ROS)

Reactive oxygen species may react with cellular components, with resultant degradation and/or inactivation of essential cellular constituents. The products derived from the reaction with reactive oxygen species may be more or less reactive or harmful. ROS are often implicated in Cd toxicology, either in a variety of cell culture systems, or in intact animals through all route of exposure (Amara et al., 2006). It has been suggested that the mechanism of acute Cd toxicity involve the depletion of glutathione and protein bound sulphydryl group, resulting in enhancing production of ROS such as superoxide ion, hydrogen peroxide and hydroxyl radicals. Cd-increased ROS in turn produces lipid peroxidation and results in DNA damage (Gupta et al., 2004; Gupta et al., 2005).

These reactive radical species include a wide variety of oxygen-, carbon-, sulfur- and nitrogen- radicals, originating not only from superoxide radical, hydrogen peroxide, and lipid peroxides but also in chelates of amino-acids, peptides, and proteins complexed with the toxic metals (Fig. 2). These metals generate reactive species, which in turn may cause neurotoxicity, hepatotoxicity and nephrotoxicity in humans and animals (Stohs et al., 2001).
Fig. 2: Formation of free radicals and fenton reaction.
In the figure 3 antioxidant enzymes SOD, CAT and GPx are potential targets of cadmium. Selenium is essential for GPx activity and Cd forms a complex with selenium, thereby, decreases its activity (Konar et al., 2007). Inhibition of heme synthesis by Cd is well reported and since CAT is heme-containing enzymes, its activity decrease (Jemai et al., 2007). SOD requires Zn and Cu for its activity. Copper ions play functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme. All the metal ions are replaced by cadmium, which decreases the activity of SOD.

Overall, these inhibitory effects of Cd on various enzymes would probably result in impaired antioxidant defense by cells and render cells more vulnerable to oxidative attacks. Cadmium, unlike other heavy metals is unable to generate free radicals by itself, however, superoxide radical, hydroxyl radical and nitric oxide radicals could be generated indirectly (Galan et al., 2001; Patrick, 2003). Watanabe et al., (2003) showed generation of non-radical hydrogen peroxide which by itself became a significant source of free radicals via the Fenton chemistry. Cadmium could replace iron and copper from a number of cytoplasmic and membrane proteins like ferritin, which in turn would release and increase the concentration of unbound iron or copper ions. These free ions participate in causing oxidative stress (Casalino et al., 1997; Waisberg et al., 2003). Recently, Watjen et al., (2004) showed evidence in support of the proposed mechanism. They showed that copper and iron ions displaced by cadmium, were able to catalyze the breakdown of hydrogen peroxide. Casalino et al., (2002) proposed that cadmium binds to the imidazole group of the His-74 in SOD which is vital for the breakdown of hydrogen peroxide, thus causing its toxic effects. Cadmium inhibition of liver mitochondrial MnSOD activity was completely removed by Mn (II) ions, suggesting that the reduced effectiveness of this enzyme is probably due to the substitution of cadmium for manganese. Antioxidant capacity of Mn (II) ions, were able to normalize the increased TBARS levels occurring when liver mitochondria were exposed to cadmium. Numerous reports in animal model have depicted that cadmium intoxication significantly increase the activity of malondialdehyde (Yang et al., 2003; Cosic et al., 2007). Free radicals generated by cadmium were scavenged by GSH directly or via the glutathione peroxidase/GSH system (Ognjanovic et al., 2003).
Fig. 3: Effect of cadmium on various antioxidant enzymes and their cofactors leading to inactivation of enzymes activity.
2.09 Cadmium and oxidative stress

Apart from oxidative stress mediated toxicity, cadmium is also known to cause its deleterious effect by deactivating DNA repair activity. Although, there are a number of mechanism that exists to prevent DNA mismatch like direct damage reversal, base excision repair, nucleotide excision repair, double stand break repair and mismatch repair (MMR) but cadmium inhibits only MMR mode (Jin et al., 2003).

Three groups of enzymes play significant roles in protecting cells from oxidative stress: Superoxide dismutases (SOD), that catalyze the conversion of two superoxides into hydrogen peroxide and oxygen. The benefit here is that hydrogen peroxide is substantially less toxic than superoxide. SOD accelerates this detoxifying reaction roughly 10,000-fold over the non-catalyzed reaction.

\[
O_2^- + O_2^- \xrightarrow{\text{SOD}} O_2 + H_2O_2
\]

SODs are metal-containing enzymes that depend on bound manganese, copper or zinc for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms predominant in cytoplasm. Catalase is found in peroxisomes in eukaryotic cells. It degrades hydrogen peroxide to water and oxygen, and hence finishes the detoxification reaction started by SOD. Glutathione peroxidase is a group of enzymes, the most abundant of which contain selenium. These enzymes, like SOD, degrade hydrogen peroxide. They also reduce organic peroxides to alcohols, providing another route for eliminating toxic oxidants. In addition to these enzymes, glutathione transferase, ceruloplasmin, hemoxygenase and possibly several other enzymes may participate in enzymatic control of oxygen radicals and their products.

2.10 Cadmium and antioxidants

Among dietary constituents viz, essential metals, amino acids, vitamins and antioxidants, which play an important role in a number of biological processes in humans and other
species. Deficiency of these elements induces some pathological conditions, such as cancer, coronary heart diseases, liver necrosis and renal failure (Saito et al., 2003; Wu and Huang 2004; Agay et al., 2005). Antioxidants are the defending army. They “quench” free radicals by donating an electron to them. The antioxidant systems including antioxidants and antioxidant enzymes can ameliorate the deleterious effects of ROS in vivo and in vitro. Antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) function by catalyzing the decomposition of oxidants and free radicals. Interventions to increase antioxidant capacity and reduce oxidative damage have been suggested as a potentially useful strategy to prevent or retard the adverse actions of ROS. A regular consumption of antioxidants, in the diet or as supplements appears to be very crucial in the protection against oxidant insults. A large number of reports have assimilated to indicate that a major part of the protection ability of fruits and vegetables was derived is due to their content of the antioxidant nutrients such as Vit- E, thiamine, melatonin, methionine, zinc, selenium, N-acetyl-cysteine and cystine etc. Enzymes are important component of defense system, which play an important role for decomposing radical compounds. Protein that binds, transport and store metallic ions (transferring, ferritin, haptoglobin, hemopexin, ceruplasmin, metallothionein, β-albumin and albumin) can also be considered as defensive mechanism.

2.11 Dietary nutrients as antioxidants

It is becoming increasingly recognized that the composition of a person’s diet is an important determinant in resistance to many adverse factor, including metal intake. Today a wide variety of nutrient supplements are recommended for overcoming environmental stresses. It is important therefore, to evaluate present levels of metal exposure and the quantities of dietary nutrients that may be augment protection against adverse effects of metal. The problem of prevention and therapeutic intervention in cadmium intoxication may be approached in two ways: (i) chelation of cadmium that has been localized intracellularly bound to metallothionein mainly in liver and kidney after the exposure (ii) free radical scavenging by antioxidants and enzymatic defense system.
2.12 Methionine

Methionine is an essential amino acid involved in protein synthesis (Poirson-Bichat et al., 1997). It is one of the main sources of glutathione but is also metabolized to S-adenosylmethionine (SAM) that mediates most biochemical methylation reactions (Griffith, 1987). Methionine must be either synthesized endogenously by methylation of homocysteine or supplied from nutrition, which is the main source for humans (Poirson-Bichat et al., 1997). Methionine level helps to determine the liver's concentration of sulfur-containing compounds and SAM improves and normalizes liver function. SAM is used in Europe in the treatment of cirrhosis and damage caused by alcohol. It is essential for the production of glutathione. Methionine itself has a protective effect on glutathione and prevents depletion during toxic overload, which can protect the liver from the damaging effects of toxic compounds. Methionine is an essential amino acid that helps the body process and eliminates fat (Tandon et al., 1994, Tandon et al., 1997). It contains sulfur that is required for the production of a natural antioxidant, glutathione. The body also needs plenty of methionine to produce two other sulfur-containing amino acids, cysteine and taurine, which help the body to eliminate toxins, build strong, healthy tissues, and promote cardiovascular health.

Methionine is a lipotropic, or a chemical substance that helps the liver process fats (lipids). Other lipotropics include choline, inositol, and betaine (trimethylglycine), all of which help to prevent the accumulation of fat in the liver and thus ensure normal liver function, which is essential for the elimination of toxins from the body. Methionine also supports liver function by regulating glutathione supplies; glutathione is needed to help neutralize toxins in the liver.

\[
\begin{align*}
\text{H}_3\text{C}-\text{S} & \text{-} \text{NH}_2 \\
\text{O} & \text{-} \text{OH}
\end{align*}
\]

Methionine
2.13 Vitamin B₁

Thiamin (also spelled "thiamine") is a water-soluble B-complex vitamin, previously known as vitamin B₁ or aneurine. Thiamin was isolated and characterized in the 1920s, and thus was one of the first organic compounds to be recognized as a vitamin. Thiamin is involved in numerous body functions, including: nervous system and muscle functioning, flow of electrolytes in and out of nerve and muscle cells (through ion channels), multiple enzyme processes (via the coenzyme thiamin pyrophosphate); carbohydrate metabolism and production of hydrochloric acid (which is necessary for proper digestion). Because there is very little thiamin stored in the body, depletion can occur as quickly as within 14 days. Thiamine, or thiamin, sometimes called aneurin, is a water-soluble vitamin of the B complex (vitamin B₁), whose phosphate derivatives are involved in many cellular processes. The best characterized form is thiamine diphosphate (ThDP), a coenzyme in the catabolism of sugars and amino acids. Thiamine is synthesized in bacteria, fungi and plants (Flora et al., 1994). Bratton et al., (1981) evaluated the use of thiamine as a protective agent against short term lead intoxication in claves and observed that thiamine administered subcutaneously at a dose of 100 mg/calf, decreased mortality and lead accumulation in different organs of claves. Sasser et al., (1984) observed that in rats which were thiamine deficient when supplemented with thiamine diet for 5 weeks before exposing them to lead acetate. Recorded that thiamine initially facilitated absorption and increase of lead concentration in tissues thus thiamine may also promote a rapid release of lead from tissues.

\[\text{Vit-B}_1\text{ or thiamine}\]
2.14 Selenium

Selenium (Se) is an essential dietary trace element, which plays an important role in a number of biological processes in humans and other species. Deficiency of this element induces some pathological conditions, such as cancer, coronary heart disease, and liver necrosis (Saito et al., 2003; Wu and Huang, 2004; Agay et al., 2005). Se taken in the form of selenite, selenate, selenocysteine, and selenomethionine is most absorbed in the duodenum. After the absorption, increased levels of Se have been detected in the blood plasma (Ersteniuk, 2004) proteins and from there it can be distributed into the tissues where it is incorporated in newly synthesized seleno-proteins. Considerable Se uptake by erythrocytes was described by Loyke et al., 2007. Se is an essential component of several enzymes such as glutathione peroxidase (GPx), thioredoxin reductase (TR) and selenoprotein P (SeP), which contains Se as selenocysteine. It is also well known that Se is essential for cell culture when a serum-free medium is used (Burk, 2002; Saito et al., 2003). Se has a certain protective role against the toxic actions of Cd and other heavy metals (Ognjanovic et al., 2008; Zikic et al., 1996). This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (Tandon et al., 2003). Selenium activates a glutathione peroxidase, which may help to protect the body from cancer. Antioxidant effects of selenium can also be accounted for by its role in the selenium-dependent thio redox in reductases. These enzymes reduce intramolecular disulfide bonds and regenerate ascorbic acid from dehydro ascorbic acid. Selenium is essential for normal thyroid function and thyroid hormone homeostasis (Beckett et al., 2005). This trace element acts as an antioxidant in the thyroid and a regulator of triiodothyronine (T3) production.

2.15 N-acetyl cysteine (NAC) and Cystine

N-acetyl-cysteine (NAC) is commonly used in critical care medicine, toxicology, and pulmonary medicine. It has been the most researched for the GSH-promoting modalities, and newer clinical applications are being developed. NAC is produced in living organisms from the amino acid cysteine. Thus, NAC is a natural sulfur-containing amino
acid derivative found naturally in foods. Its dual properties help repair oxidative damage in the body.

\[
\text{H}_3\text{C} \quad \text{O} \quad \text{NH} \quad \text{H} \\
\text{N-C} \quad \text{H} \quad \text{N} \\
\text{O} \quad \text{H}
\]

**N-acetyl cysteine**

\[
\text{OH} \quad \text{NH}_2 \\
\text{O} \quad \text{C} \quad \text{S} \\
\text{H} \quad \text{S} \\
\text{H}_2\text{N} \quad \text{C} \quad \text{O}
\]

**Cystine**

Cystine that derives from the metabolism of cysteine is a disulfide whose bonds stabilize the tertiary structure of proteins (Griffith, 1987). It participates in a number of biochemical processes that depend on the reactivity of thiols and can act as a scavenger of electrophiles and free radicals (Griffith, 1987). Glutamate and cystine share the same amino acid transport and compete for transport into cells. Elevated glutamate levels, resulting in inhibition of cystine and depleted cellular GSH level, therefore this depletion resulted in increased susceptibility of oxidative stress. Thiol antioxidants such as NAC, cysteine and lipoic acid protect the cell from glutamate induced cell death and free radical toxicity (Varela et al., 2001; Kim et al., 2005).

### 2.16 Zinc

Zinc is an essential trace element for all forms of life. The significance of zinc in human nutrition and public health was recognized relatively recently. Zinc has never been shown to interact directly with an oxidant species but rather prefers to exert its effects in an indirect manner. The past twenty years has seen the generation of a large amount of information on the potential role of Zn as a cellular antioxidant. Zinc is an essential nutrient in humans and animals that is necessary for the function of a large number of
metalloenzymes (Elizabeth et al., 2002; Messaoudi et al., 2009). These enzymes include alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, superoxide dismutase, and deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerase. As such, zinc is required for normal nucleic acid, protein, and membrane metabolism, as well as cell growth and division. Zinc also plays an essential role in the maintenance of nucleic acid structure of genes (zinc finger phenomenon). More than 300 enzymes require Zn for their activity. It also plays an important role in the DNA replication, transcription and protein synthesis, influencing cell division and differentiation. Zinc is probably the most important nutrient that protects the body against toxic metals. The most compelling reason for the protection effects of zinc against cadmium is that zinc induced the production of the metal binding protein, metallothionein (Jihen et al., 2008). Chronic cadmium exposure can cause renal proximal tubular dysfunction resulting from the release of cadmium metallothionein (CdMT) from the liver and its accumulation and degradation in the renal tubular epithelial cells. Pretreatment with zinc can protect against acute CdMT nephrotoxicity. Zinc enhances protection through metallothionein (MT) and non-metallothionein mechanism. Cadmium has inhibitory effects on a number of zinc-containing enzymes. Cadmium is known to replace zinc in metallothionein. It is believed that zinc may reduce lipid peroxidation by increasing the activities of enzymes such as glutathione peroxidase to ameliorate the sign of metal induced neurotoxicity (Jihen et al., 2009).

2.17 Melatonin

Melatonin, the pineal hormone, has potent antioxidative activity. Free radicals are chemical constituents that have an unpaired electron. If an electron is added to O\(_2\) then the superoxide anion radical O\(_2^-\) is formed. O\(_2^-\) is reduced by superoxide dismutase to H\(_2\)O\(_2\) which is toxic at high concentrations and can be reduced to OH. The hydroxyl radical (OH) damages cells. Melatonin is an efficient neutralizer of OH. The cataract formation due to GSH depletion in lens can be inhibited by melatonin administration suggesting that melatonin is capable of stimulatory effect on GSH production (Abe et al., 1994; Longoni et al., 1998; Tan et al., 2002; Chwelatuik et al., 2006).
Vitamin E (α-tocopherol) is a naturally occurring antioxidant that plays an important role in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stress causing agents. The antioxidant function of these micronutrients could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells. Vit-E a known antioxidant is studied extensively to found solution for the health problems induced by any pollutant (Beytut et al., 2003). Vit-E becomes a radical, but is regenerated through the activity of the antioxidants vitamin C and glutathione. Vit-E stops free radical chain reactions protect fat from free radical destruction throughout the body and helps in producing superoxide dismutase. Vit-E plays an important role in termination of chain reaction of lipid peroxidation in membrane and lipoprotein. Vit-E is a family of lipid-soluble vitamins, of which α-tocopherol is the most potent. Vit-E has been shown to act as an antioxidant in cells, interrupting the propagation of LPO in the plasma membrane to preserve membrane integrity (Chow, 1991; Warren et al., 2000). Vit-E is a fat-soluble vitamin that exists in eight different forms. α-tocopherol is the most active form of Vit-E in humans and is a powerful biological antioxidant which is considered to be the major membrane bound antioxidant employed by the cell (Burton et al., 1989). Its main antioxidant function is protection against lipid peroxidation (Chow et al., 1991; Pryor et al., 2000). Vit E protects critical cellular structures against damage caused by oxygen free radicals and reactive products of lipid peroxidation. It has been reported that lipid
peroxidation is prevented by Vit E (Meydani, 1995). Vit E inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into a tocopheroxyl radical. In fact, α-tocopherylquinone may act as a potent anticoagulant and as an antioxidant through its reduction to hydroquinone (Arita et al., 1998; Pillai et al., 2005). Also, Tandon et al., (1995) reported that the protective role of vit E against the toxicity of oxidants may be due to the quenching of hydroxyl radicals.

![Vitamin E Tocopherol](image)

2.19 *Spirulina platensis*

*Spirulina platensis*, (SP) popularly known as the Blue Green Algae (Cyanobacterium), the most powerful food on earth, is rich in all the three types of micronutrients, proteins, lipids and carbohydrates and some more vital elements like zinc, magnesium, manganese, selenium and vitamins like beta-carotene, riboflavin cyanocobalamin, α-tocopherol and α-linoleic acids (Sheshadri et al., 1992). Besides, *Spirulina* is also known to have a protective antioxidant effect. In addition, several studies showed that *Spirulina* species exhibit various biological activities such as anti-inflammatory, antitumor, hepatoprotective, radio protective, antimicrobial, strenghtening immune system, metalloprotective and antioxidant effects (Khan et al., 2005).

Early interest in *Spirulina* focused mainly on its rich content of protein, vitamins, essential amino acids, minerals, and essential fatty acids. *Spirulina* has 60-70% of protein by weight and contains a rich source of vitamins, especially vitamin B₁₂ and provitamin a (β-carotene), and minerals, especially iron. One of the few sources of dietary γ-linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits.

Thus it can concluded from the review of literature that further investigation are needed to find out the toxicity impact of heavy metals along with healing effect of
common antioxidants, so that the probabilities of environment pollution and health hazards can be tackled successfully.