Chapter 1: Introduction

Heavy metals are defined as metals of a density higher than 5 g/cm³. They occur as pure elements, as ions and complexes. The indiscriminate human activities and exploitation of the natural resources have resulted in the release of heavy metals directly or indirectly in the environment causing deleterious effects, thereby, disturbing natural cycles. Heavy metals and their salts, are very important environment pollutants being potent metabolic inhibitors. The inherent toxicity of a metal depends upon its capacity to distribute the dynamic life processes in a biological system by combination of cell organelles, macromolecules and metabolites. Heavy metals such as lead, mercury, chromium, cadmium, zinc, etc. are neither created nor destroyed in significant amount in the environment so they persist indefinitely. These heavy metals become toxic when they are not metabolized by the body and they get accumulated in the soft tissues. Most of the metals present in environment enter the biological system on exposure mainly through air, drinking water, pesticides, batteries, piping, paints, petrochemicals, articles etc. They are present in plastics, paints, inks, body care products, medicines and household pesticides (Chandra et al., 2009). Consequently, composts derived from source-segregated waste streams or green wastes are generally reported to contain small amounts of heavy metals compared to mechanically-sorted products (Epstein et al., 1992). In relation to the application of composted residuals to soil, the main elements generally of concern include: Zn, Cu, Ni, Cd, Pb, Cr and Hg (Wang et al., 2008) because they are potentially present in compost in amounts that may be greater than the values in the receiving soil. The concentrations of conservative elements like heavy metals increase during the composting process (Garcia et al., 1990; Ciavatta et al., 1993) due to the microbial degradation of part of the organic matter and loss of volatile solids. In 1975, U.S. Environmental Protection Agency (USEPA), Occupational Safety and Health Administration (OSHA), Consumer Product Safety Commission (CPSE) listed 24 extremely hazardous substances. Out of these hazardous substances, cadmium is one of the significant environmental pollutant and humans are exposed to it through food, water, air and heavy smoking. Cadmium (Cd) exposure occurs widely in the general
population, especially low-level chronic exposure through smoking and dietary sources, but it is known as one of the most toxic environmental and industrial pollutants (Alfen et al., 1997). The pattern of cadmium uses has changed in recent years. In the past cadmium was mainly used in the electroplating of metals and in pigments or stabilizers for plastics. In 1960, the engineering coatings and plating sectors accounted for over half the cadmium consumed worldwide, but in 1990 this had declined to less than 8%. Now days, cadmium nickel battery manufacture consumes 55% of the cadmium output and it is expected that this application will expand with the increasing use of rechargeable batteries and their potential use for electric vehicles (Yates, 1992).

Cadmium once absorbed gets accumulated in major organs of the living system. Cadmium exert its toxic effects on tissues like red blood cells (Hubskyi et al., 2002), liver (Dudley et al., 1982), kidney and heart (Chan et al., 1993). It has been reported that 50-70 percentage (%) of the body burden accumulates in the liver and kidney (Gubrealy et al., 2004), although the concentration in the latter is more. Cadmium burden especially in the kidney tends to increase in a linear fashion with age up to 50 to 60 years, thereafter, kidney levels remain almost constant (Manca et al., 1991). It also accumulates in the placenta (Kuriwaki et al., 2005). Dermal absorption has also been noticed, however, it is relatively less important (ATSDR Report, 1993). Liver has a capacity to synthesize metallothionein, which traps cadmium efficiently forming metallothionein complex (Muller, 1986; Rikan et al., 2000). Cadmium metallothionein (CdMT) is slowly released from liver into the blood stream and taken up by the kidney (Chan et al., 1992). Cadmium after binding to metallothionein is filtered in the kidney through the glomerulus into the primary urine and then re-absorbed in the proximal tubular cells, where it is catabolized. The unbound cadmium in the renal tubular cells, thereby, preventing the toxic effects of free cadmium. However, kidney seems to have a limited capacity to synthesize metallothionein and therefore, the excess of metal remains unbound, which seems to be responsible for the toxic effects. Damage to proximal tubular cell occurs, the first sign of proteinuria appears if the metallothionein producing capacity is somehow exceeded, (Obrien et al., 1998).

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 (Waalkes, 2003). Cd accumulates in the body because of slow excretion (Satarug et al., 2000). Cd causes toxicity in different organs. Acute and chronic Cd exposure mostly results in hepatotoxicity, renal and haematotoxicity (Ognjanovic et al., 2008; Kanter et al., 2009). It seems that the level of damage depends on the dosage and duration of Cd application. Exposure of cells to toxic chemicals lead to up-regulate the expression of a number of stress proteins and results in activation of apoptotic pathways and consequently cellular damage. In vivo and in vitro studies showed that inflammation and oxidative damage are main mechanisms of Cd induced toxicity (Kanter et al., 2009). The particular factor in the absorption, distribution and retention of cadmium is the formation of protein called thionein which has the capacity to bind certain metals to form metallothionein. The induction of biosynthesis of thionein by administration of low initial levels of cadmium shows its critical role in cadmium toxicity.

Recently, it has been suggested that Cd may induce oxidative damage in various tissues by enhancing peroxidation of membrane lipids and by inhibition of enzymes involved in the removal of certain reactive oxygen species (ROS). Cd induces the increase of ROS production, including superoxide anion radical, hydrogen peroxide, hydroxyl radical and lipid peroxidation. A variety of accompanied changes in antioxidant defense system were reported (Tandon et al., 2003). Studies of Flora et al., (2008) have shown that free radical scavenger and antioxidants or dietary nutrients are useful in protecting against cadmium toxicity. Various nutritional factors have a significant influence on metal toxicity. Apart from the normal nutritional requirements, sulphur is required for the biosynthesis of detoxicating enzymes, i.e., glutathione peroxidase and superoxide dismutase. Vitamins such as ascorbic acid, thiamine, α-tocopherol and retinoids are essential as protective agents and can act as antioxidants in removing free radicals. Nutrients can affect the toxicity of a metal by interacting at its primary site of action. These nutrients are also expected to modify the body’s response to a toxic metal by altering its metabolism and transport.
Recently, it has been realized that certain essential metals, amino acids, vitamins and antioxidants can play a significant protective role in the treatment of metal induced oxidative stress or damage. Dietary nutrients can also behave as efficient chelators. Therefore, the oxidizing property together with the chelating capacity of antioxidants makes them a strong candidate for decontamination of toxic metals. The compounds like essential metals (zinc and selenium), amino acids (methionine and cystine), vitamins (Vit-B1 and Vit-E) and antioxidants (N-acetyl cysteine (NAC) and melatonin) possess strong antioxidizing properties and are likely to be proved as a novel approach for long term and effective treatment of heavy metal poisoning. Moreover, *Spirulina platensis*, a microscopic blue-green algae, has a property of reducing heavy metals and nephrotoxic substances from the body. It is not only a whole food, but seems to be an ideal therapeutic supplement. So far, no other natural food is found with such a combination and amazing concentration of so many unusual nutrients like proteins, amino acids, iron, beta-carotene, phycocyanin, gamma lenolenic acid, vitamins B₃, B₆, B₉, B₁₂, essential fatty acids etc. In fact it is the highest known source of protein, beta-carotene which is a precursor of vitamin A and only vegetable source of vitamin B₁₂. Beta-carotene concentration of *Spirulina platensis* is ten times higher than carrot. It was evident that food rich in beta carotene can reduce the risk of cancer (Peto et al., 1981). It was found in preclinical studies that the natural carotene of *Spirulina platensis* could inhibit, shrink and destroy oral cancer cells. Phycocyanin of *Spirulina platensis* also prevents cancer and its growth (Peto et al., 1981; Shekelle et al., 1981). In *Spirulina platensis* extract plus zinc-treated group, the clinical scores for keratosis before and after treatment was statistically significant (p<0.05) (Misbahuddin et al., 2006). The beta-carotene in algae and leafy green vegetables has greater anti-oxidant effects than synthetic beta-carotene.

In the present study, treatment with dietary nutrients mentioned above during cadmium exposure has demonstrated to have protective effects on cadmium-induced toxicity in various tissues and vital organs of albino rats. Further, the objective was to examine whether cadmium-induced toxicity is due to oxidative stress, and if so, whether depression of the antioxidant defense system will enhance the toxicity of cadmium and
conversely, whether augmentation of antioxidant levels will suppress the toxicity of cadmium.

No doubt, some work has been carried out in a single dietary nutrient on individual system, however, little attention has been paid towards combinatory effects of dietary nutrients. Therefore, the present study was aimed to investigate the possible beneficial role of dietary nutrients in individual and combinational studies caused by cadmium-induced toxicity in albino rats.

1.01 Aim and Objectives

The present work was planned to study the possible protective role of dietary nutrients against cadmium-induced toxicity, with following specific aims:

- To study the effect of acute and sub chronic Cd exposure on the accumulation of cadmium in blood, liver and kidney.
- To evaluate the physiological response of Cd intoxication towards haematotoxicity (blood) hepatotoxicity (liver) and nephrotoxicity (kidney) based on various biochemical and histological aspects.
- To evaluate the preventive effect of various dietary constituents viz: Essential metals (Zn and Se), Vitamins (Thiamine and Vit-E) Amino acids (Methionine and Cystine) and antioxidants (N-acetyl-cysteine and α-melatonin), on absorption of cadmium in blood and soft organs.
- To evaluate the effects of the individual and combined administration of dietary nutrients on haematological, serum and biochemical variables
- To evaluate the protective effects of *Spirulina platensis* pretreatment against Cd induced nephro-, hepato- and haematotoxicity in male rats.