

REVIEW ARTICLE

**Microalgae, Vitamins and Multi-Mineral Supplementation-Promising Protective Effects against Cadmium Toxicity**

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**ABSTRACT**

Heavy metals like Cd, Pb and Hg have no known biological functions and consequently detrimental to essential life processes. These metals in the form of inorganic compounds from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose a serious threat because of their toxicity. Pollution of the aquatic environment with metals has become a serious health concern in recent years. Although environmental concentrations of metals are rarely directly dangerous for fish survival, are known to accumulate in fish tissues reaching concentrations of up to thousands of times higher than in the surrounding water environment and becoming extremely harmful. Cadmium (Cd) is widely distributed in aquatic environments and is an extremely toxic metal commonly found in industrial settings. It has been demonstrated that the feed is the principal source of contamination by metals such as Cd that tends to accumulate in tissues, which in the end are ingested by consumers. However, it has been demonstrated that toxicological effects of Cd exposure to aquatic organisms can be altered by some others parameters: water hardness, water minerals concentration (calcium, Zinc and Selenium), protective effects of supplements (microalgae, vitamins). In this way, this mini-review aimed to bring the main work on the promising effects of microalgae (e.g Spirulina), Vitamines (C and E) and multi-mineral supplementation against the toxicity of cadmium.

**Key words:** Cadmium, Heavy metal toxicity, protective effects.

**1. INTRODUCTION**

Water is one of the most valuable natural resources and a major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil and water. A wide range of inorganic and organic compounds cause contamination includes heavy metals, combustible substances and hazardous wastes. Major component of inorganic contaminates are heavy metals. They have some different problems than organic contaminants (Ghosh *et al.*, 2005; Jadhav *et al.*, 2010).

Metal pollution of the sea is less visible and direct than other types of marine pollution but its effects on marine ecosystems and humans are very extensive. The presence of metals varies between fish species; depend on age, developmental stage and other physiological factors. Additionally, fish are the largest sources of contaminants (e.g heavy metal) for man. The bioaccumulation of metals e.g

Cadmium, Arsenic, Manganese, Mercury, and Lead in the trophic food chain is cause of concern since they can have deleterious effects on human health (Ersoy and Celik, 2009; Jarup and Akesson, 2009). Heavy metals normally occur in nature and are essential to life but can become toxic through accumulation in organisms.

Arsenic, cadmium, chromium, copper, nickel, lead and mercury are the most common heavy metals which can pollute the environment. Mercury, lead and cadmium are of greatest concern because of their ability to travel long distances in the atmosphere. Cadmium is also toxic to plants and micro-organisms. Cd accumulates mainly in the kidney and liver of vertebrates and in aquatic invertebrates and algae. Acute toxic effects on fish, birds and other animals may include death or fetal malformations. Aquatic organisms, such as fish, accumulate pollutants directly from

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contaminated water and indirectly via the food chain. Heavy metals in water are particularly dangerous for fish juveniles and may considerably reduce the size of fish populations or even cause extinction of entire fish population in polluted reservoirs (Stomińska and Jezierska, 2000). Heavy metals have the tendency to accumulate in various organs of aquatic organisms, which in turn may enter into the human metabolism through consumption causing serious health hazards (Raja *et al.*, 2009). It's known that fish tissue accumulate large amounts of toxic contaminants from their living environment (Suhaimi *et al.*, 2005). Sub-lethal effects of heavy metals are of concern as they accumulate and are transferred through food-chains to humans (Yılmaz and Yılmaz, 2007). Every pollution in the aquatic environment which impacts physiology, development, growth or survival of fish, affects human that, at the top of the food chain, consume fish. Cadmium as a toxic element might be a stressor agent for fish. Cadmium exposure may lead to the results of some patho-physiological damages including growth rate reduction in fish (Kaviraj and Ghosal, 1997; Hansen *et al.*, 2002) and also in other aquatic organisms (Das and Khangarot, 2010). For this purpose, one of the aims of aquatic toxicology is to elucidate the subtler and most pronounced alterations induced by pollutants on aquatic organisms and their environment. Environmental stress is further accentuated by a concomitant increase in metal toxicity. In this way, it has been reported that acute toxicological and physiological effects to aquatic organisms following waterborne Cd exposure can be altered by some others parameters: water hardness (Davies *et al.*, 1993), water metal concentration e.g Calcium, Zinc and Selenium (Brando-Netro *et al.*, 1995; Meyers, 1999; Lin and Shiau, 2007; Abdel-Tawwab *et al.*, 2007; Messaoudi *et al.*, 2009, 2010). In this way, the antioxidant therapy is considered an important approach to intervention of heavy metal toxicity.

## 2. MICROALGAE: IMPLICATION IN HEAVY METAL DETOX

Land plants, aquatic plants and algae have all attracted considerable attention for the capacity to eliminate heavy metal. Indeed, some species and ecotypes of algae can live in the presence of toxic metal concentrations in the environment that are lethal for other species. Previous researchers demonstrate (Aziz and Ng, 1993; Oswald, 1995) that microalgae play an important role during the tertiary treatment of domestic wastewater in

maturation ponds and/or in the treatment of small–middle-scale municipal wastewater in facultative or aerobic ponds. Microalgae enhance the removal of nutrients, organic contaminants, heavy metals, and pathogens from domestic wastewater and can furnish an interesting raw material e.g. algae metabolites and biogas for the production of high-value chemicals.

Adverse effects of heavy metals on the environment and their accretion through the food chain have lead to research in the development of efficient, low cost techniques for wastewater treatment (Pan *et al.*, 2009; Sahan *et al.*, 2010; Singh *et al.*, 2007), with methods using algae biomass receiving a great deal of attention (Mehta and Gaur, 2005; Singh *et al.*, 2007; Tuzen and Sari, 2010). To their ubiquity in aquatic ecosystems, the use of algae to monitor heavy metal toxicity is increasing due to their involvement in the most aquatic processes.

For example, *Spirulina* and *Chlorella* are two separate micro-algae organisms which have existed on earth since the dawn of time. Both were revered as powerful super foods in many traditional societies, and today are more relevant than ever for achieving overall health and well-being. Many studies have reported that *Spirogyra* and *Cladophora* spp. have a very high capacity for binding with metals due to the presence of polysaccharides, proteins, or lipids on the surface of cell walls (Alimohamadi *et al.*, 2005; Deng *et al.*, 2007; Gupta and Rastogi, 2008; Tuzen and Sari, 2010).

McLean *et al.* (1972) first reported the presence of Cd-binding material in a freshwater blue green algae, *Anacystis nidulans*, but no attempt was made further characterization. Indeed, metal-binding proteins have been reported for different algae, i.e. *Chlorella ellipsoidea* (Nagano *et al.*, 1982), *C. pyrenoidosa* (Hart and Bertram, 1980), *Dunaliella bioculata* (Heuillet *et al.*, 1988), *Synechococcus* sp. (Olafson *et al.*, 1979), *Euglena gracilis* (Gingrich *et al.*, 1986), *Scenedesmus acutiformis* (Stokes *et al.*, 1977) and *S. quadricauda* (Reddy and Prasad, 1989). All such proteins are within a mol. wt range of 8000-10,000. Photosynthetic aeration is therefore especially interesting to reduce operation costs and limit the risks for pollutant volatilization under mechanical aeration and recent studies have shown that microalgae can indeed support the aerobic degradation of various hazardous

contaminants (Munoz *et al.*, 2004; Safonova *et al.*, 2004).

It has been reported that microalgae are aquatic organisms presenting molecular mechanisms that allow them to discriminate non-essential heavy metals from those essential ones for their growth. Stokes *et al.* (1977) first discovered MtIII complex synthesis in the microalga *Scenedesmus acutiformis*. The different detoxification processes executed by algae are reviewed with special emphasis on those involving the peptides metallothioneins, mainly the post transcriptionally synthesized class III metallothioneins or phytochelatin (Gekeler *et al.*, 1988).

Heavy metals represent an important group of polluted and hazardous contaminants often found in industrial wastewater (Kratochvil and Volesky, 1998; Volesky, 2001). Microalgae, related eukaryotic photosynthetic organisms, and some fungi have preferentially developed the production of peptides capable to bind heavy metals. These molecules, as organometallic complexes, are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect (Cobbett and Goldsbrough, 2002). In contrast to this mechanism used by eukaryotes, prokaryotic cells employ ATP consuming efflux of heavy metals or enzymatic change of speciation to achieve detoxification (Nies, 1999).

For example, Cd is believed to have no nutritive value for algae. Canizares-Villanueva (2000) have reported that the microalgae can be efficiently use to remove heavy metals and a special uptake of  $15\text{mg g}^{-1}\text{Biomass}$  at 99% removal efficiency has been reported. This data showed that the process is competitive compared to other treatment methods. Stokes *et al.* (1977) have discovered MtIII complex synthesis in the microalga *Scenedesmus acutiformis*.

### 3. VITAMINS

Dietary micronutrients such as vitamins E and C as well as carotenoids have also been regarded as antioxidant defenses e.g in fish (Wilhelm-Filho, 1996; Hamre, 2010). Vitamin C (ascorbic acid) and vitamin E (d-alpha-tocopherol) are recognized as essential nutrients for all species of animals. In other words, these vitamins have been shown to have protective effect against metal induced toxicity (Rao and Sharma, 2001; Jiraungkoorskul *et al.* 2007). Vitamin C is an electron donor, and this property accounts for all its known functions.

Antioxidant effects of vitamin C have been demonstrated in many experiments in vitro. Vitamin C is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. For this purpose, Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (Padayatty *et al.*, 2003). Alpha-tocopherol (vitamin E) is a naturally occurring antioxidant in biological systems and is present in the cell membrane of various tissues, including the intestine and stomach. Vitamin E prevents free radical-induced injury by blocking the free radical chain reaction (Solar, 1959).

Layachi and Kechrid (2012), have reported that the supplementation of Vit-C and Vit- E or there combination in rat exposed to Cd significantly increase of the glutathione level in comparison to groups exposed cadmium. Additionally, the normalization of glutathione levels (GSH), glutathione peroxidase (GSH-Px) and catalase (CAT) activities following vitamin C or vitamin E treatment could be explained by the fact that these vitamins caused a decline in lipid peroxidation (LPO) accompanied by an increase in the activities/level of, GSH, GSH-Px and CAT in liver rat. In addition, it has been reported that the body weights decrease significantly while the liver weight of rats exposed to Cd significantly increase as compared to the control group. However, in the group with vitamin C, vitamin E and vitamin C + vitamin E supplies, the body weight gain became significantly greater and the liver weight decreased than in rats exposed to cadmium (Layachi and Kechrid, 2012). The same authors reported that , the activities of serum hepato specific enzymes serum alanine aminotransaminase ( GOT), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were generally significantly decreased in rat groups treated with vitamin C and vitamin E either alone or in combination. Moreover, the combination of vitamin C and E showed more efficacy than vitamin C or vitamin E alone when comparing to group Cd-vit C and Cd-vit E with Cd-vit C-vit E animals. Erdogan *et al.*, 2005 reported that the reduction in weight gain could have been due to the decrease in food intake, or could be related to the overall increased of lipids and proteins degeneration as a result of cadmium toxicity. Amelioration of blood glucose concentration in cadmium animals treated was observed after addition of vitamin E or vitamin C

alone or in combination (Layachi and Kechrid, 2012). Cadmium is one of the heavy metals which induce membrane damage. Cadmium toxicity was attenuated by the pretreatment with vitamin E even at the highest concentrations tested (Mattie and Freedman, 2001). The co-administration of vitamin C and/or vitamin E to the cadmium treated animals improved body and liver weights. The protective effect of vitamin-C (ascorbic acid) on heavy metal toxicity via its free radical scavenging mechanism and detoxification effect is well reported (Suzuki, 1990). However, according to Sodhi *et al.*, (2008), vitamin E due to its solubility in lipids, plays an important role in protecting lipid-rich structure like hepatic tissue from free radicals damage and an effective inhibitor of autocatalytic process of lipid peroxidation (LPO).

#### 4. MINERAL SUPPLEMENTATION

One of the major mechanisms behind metal toxicity has been attributed to oxidative stress. The biological activity of heavy metals can be markedly affected by the presence of metal chelators which may reverse their toxicity. Much interest has recently been focused on the uptake of cadmium by marine and estuarine organisms. Many of these studies have been prompted by a concern over public health hazards caused by accumulation of this trace metal in food.

Several studies indicated that calcium (Ca<sup>2+</sup>) can reduce heavy metal toxicity by forming complex compounds with them which are then, either eliminated or unable to cross biological membranes. The mechanism of acute Cd toxicity appears to be interference with whole-body calcium regulation resulting in hypocalcaemia, but this interference only occurs at industrial-type concentrations. Furthermore, during such exposures, Cd accumulates in the whole body of the organism to levels (>50  $\mu\text{mol g}^{-1}$ ) as much as 5-fold greater than normal whole-body Ca concentration (10–15  $\mu\text{mol g}^{-1}$ ). This raises interesting questions about the interaction of Ca and Cd uptake in this animal.

It's well known that Ca plays diverse role in the living organisms. Indeed, in the vertebrate's organisms it represents a major component of the skeleton but in addition, it also has vital functions in the body fluids and soft tissues. For enzymatic processes, Ca acts as a cofactor in various and couples stimulus excitation reactions (e.g in muscle contraction or the secretion of glands). Both Ca and Cd are divalents and they use the

same transitional channel interacting with each other antagonistically. For example, it have been reported that high concentrations of Ca either in water or diet clearly envisages ameliorating protective effects on water borne Cd toxicity in fish *Oreochromis mossambicus* (Zohouri *et al.*, 2001).

In teleostean fish *Oreochromis mossambicus*, Cd induces significant alterations in the levels of lipid peroxidation (LPO) and certain enzymatic status of antioxidant enzymes (superoxide dismutase and catalase) in liver and kidney tissues. However, these activities were progressively reversed after using trace element supplements like Ca and/or Zn (Jamakala and Rani, 2012).

Ca and/or Zn supplementation significantly counteracted the enhancement of LPO caused by Cd exposure. This finding is in a perfect agreement with the findings of Ng *et al.*, (2009) who reported that elevated Ca protects against Cd induced toxicity in rainbow trout. It has been reported that a lower level of LPO means a lower degree of membrane damage, and Ca and Zn might have alleviated the Cd- induced membrane damage and aids protect the cell (Jamakala and Rani, 2012).

Therefore, the enhanced LPO in the liver, kidney and other tissues might result from the reduction of their SOD activity. The decrease in SOD activity could be due to its inhibition by the excess production of ROS as evidenced by LPO following Cd exposure. The supplementation of Ca and/or Zn in fish (*Oreochromis Mossambicus*) exposed to Cd causes a significant increase in SOD activity in both liver and kidney tissue (Jamakala and Rani, 2012). Similar findings were reported rainbow trout (Baldisserotto *et al.*, 2004) and rats (Patra *et al.*, 2001) exposed to Cd and subjected to Ca and Zn supplementation. Several study reported that Cd exposure decrease CAT activity in *Oreochromis Mossambicus*, *Heteropneustes fossilis* and *Sparus aurata* (Jamakala and Rani, 2012; Radhakrishnan *et al.*, 1999; Vaglio and Landriscina, 2009). However, after supplementation of Ca and/or Zn, CAT activity levels were significantly increased especially in liver and kidney tissue in fish.

Several elements have been shown to have a protective effect against Cd-induced injury. Accordingly, it was reported that selenium (Se) is considered one of the most efficient and an important nutritional trace element (Shilo *et al.*, 2010), which contributes significantly to host

immune responses and antioxidant protection (Brigelius-Flohe and Flohe, 2003). Among the other antioxidants, selenium (Se) is an essential trace mineral in animal nutrition obtained partly from the surrounding water (Lall and Bishop, 1977), but mostly from the diet (Halver, 2002). The importance of Se to oxidative stress involves its presence at the active site of the antioxidant enzyme GPX (Felton *et al.*, 1996), which reduces  $H_2O_2$  at the expense of reduced glutathione (Arteel and Sies, 2001). Se represents one of the important nonenzymatic antioxidant defense systems, which may modulate its toxicity by an antioxidative mechanism. Se acts as a component of the unusual amino acids selenocysteine (Se-Cys) and selenomethionine (Se-Met).

Many proteins contain selenium, among them plasma glutathione peroxidase (GSH-Px), cytoplasm GSH-Px, mitochondrial GSH-Px, cell membrane hydroperoxide phospholipid glutathione peroxidase (PHGSH-Px) and iodothyronine deiodinase (Sies, 1991). Glutathione peroxidase (PHGSH-Px), a selenoenzyme, reduces *in situ* the oxidized phospholipid polyunsaturated fatty acids, restoring cellular membranes to normal (Bock *et al.*, 1991). Combs *et al.*, (2009) reported that Se acts as an antioxidant in the body as an antioxidant, and is also involved in thyroid hormone metabolism, redox reactions, reproduction, and immune function. Se plays a role in protecting cells against free radicals and oxidative stress (Bansal and Kaur, 2005). Additionally, numerous studies have shown that Se can protect against Cd toxicity in mammals *in vitro* and *in vivo* (El-Sharaky *et al.*, 2007, Lazarus *et al.*, 2009; Messaoudi *et al.*, 2009, 2010). In addition, Se acts as a co-factor for the reduction of antioxidant enzymes, including glutathione peroxidases and certain forms of thioredoxin reductase (Chen *et al.*, 2012). Moreover, the protective effect of Se against the toxicity of Cd might, at least partially, be attributed to stimulate the level of heat shock protein (HSPs). Therefore, Se can be considered a potential therapeutic nutrient to protect against toxicity induced by Cd.

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