FINAL REPORT

INCA PROJECT NO. 223

THE BIOLOGICAL IMPORTANCE OF COPPER

A Literature Review

June, 1990

INCA PROJECT 223

Preface

In 1973 the International Copper Research Association initiated a grant to review the literature dealing with the biological importance of copper in marine and estuarine environments. This was followed by a second review in 1978. It was then apparent that there was a very large number of publications concerning copper in the marine environment. As a result, an annual review was initiated.

Reviews prior to 1984 considered copper only in marine and estuarine environments. However, events occurring on land and in freshwater were often mentioned because chemical and biological factors and processes pertinent to one environment could often be applied to the others. As a result, the review became larger, covering not only freshwater, saltwater and terrestrial environments but also agriculture and medicine. These broad reviews pointed out the broad application of concepts about the biological importance of copper.

The present review includes literature for the period 1987-1988 although a number of earlier references are included and, where appropriate, a few appearing in 1989 have been used. Many of the earlier references are from Eastern Europe and Asia because this literature takes time to appear in the North American data review bases. References were obtained in major part through literature search programs available through the Woodward Biomedical Library at the University of British Columbia. Mr. Brian Moreton, the European INCA Director, kindly provided the metals section of the Marine Pollution Research Titles as a source of European as well as North American References.

The 1989 review was written using 3,766 references selected from the literature searches. The appropriate sections of each reference have been catalogued with the references used in previous reviews. This collection now contains appropriate sections of 20,106 references which have been indexed for search purposes. Sharon DeWreede is responsible for this outstanding collection.

It will be apparent to the reader that the background of the reviewer is in marine science. With the reviewer aware of this, special effort has been made to cover all aspects of the biological importance of copper. Because of the problems of obtaining certain references, particularly manuscript reports, this review should be considered as a "critical review" of the literature. The cross-referencing scheme used in the preparation and writing of the review provides an integration of concepts from all areas covered by the literature search. It is a review that addresses four basic questions:

1. What does copper do to organisms?

2. What are the sources of environmental copper?

3. What happens to copper once it enters the environment?

4. What are the relationships between the chemistry of copper and its biological importance?

These questions translate into a series of topics that form the chapters of this review.

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EXECUTIVE SUMMARY

The literature used in this review covers a wide range of topics. These include the importance of copper to man, the requirements for copper exhibited by organisms and the various functions or roles of copper in organisms. They include the biological effects of excess metal and the chemical conditions that help to determine the availability of copper to organisms. Metal chemistry is also a factor in the biologically important changes that occur when copper is introduced naturally or from a man-made source. From the 3,766 references used in the review, some of the highlights include:

Copper plays a number of essential roles in organisms. It is found in several enzymes and Galiazzo et al. (1988) present evidence that copper acts as a regulator of the expression of major enzyme activities involved in biological oxygen activation. Wissler et al. (1987) describe a copper-containing organic that can regulate cell division and act as what the authors term a “novel type of ‘Wound-Hormone’.”

Because it is a required element, copper deficiency produces physiological problems in plants and animals. Chlorosis has been reported under copper deficient conditions, for leaves of young oil palm plants (Pacheco et al., 1986; Pacheco and Tailliez, 1986) and soybean (Casanova and de Valls, 1987). Agricultural studies of alfalfa (Isaev and Khalileva, 1987), bermudagrass (Angel and Feagley, 1987), bromegrass (Horvath, 1986), clover (Nikolaeva, 1988) and a number of cereals (Anke et al., 1986; Coventry et al., 1987; Gordetskaya et al., 1987; Razuvanov, 1985; Saad et al., 1984) have shown direct benefit from copper supplementation in certain soils. Barley pollen development is highly irregular in copper-deficient plants, resulting in low and variable pollen fertility (Jewell et al., 1988).

In lambs in flocks, a combination of cobalt and copper deficiency produces poor growth, depressed appetite, poor general condition, anaemia, loss of wool, serious lacrimal secretion and ataxia (Schwan et al., 1987). Tanner et al. (1988) report some of these problems in copper-deficient cattle; poor growth and anaemia appear to be common expressions of low copper status (e.g. Suttle and Jones, 1987), factors that can often be corrected with supplementation (e.g. Wittenberg and Boila, 1987).

In humans, Danks (1988) comments (page 236) that "there is no longer any debate about the essential role of Cu in humans and the main effects of severe deficiency are well established, even though not all these effects can be explained adequately." However, the U.S. Food and Nutrition Board (Anonymous, 1986) questions the adequacy of data from metabolic balance studies, to establish a recommended daily allowance (RDA). In an article by Raloff (1989) it is pointed out that the new RDA guidelines by the U.S. National Research Council loosely advocate levels up to 3 mg daily. However, the author comments (page 277) that "... less than half the U.S. population consumes even 1.5 mg ... and one-third ... eat less than I mg daily, a level studies indicate can foster dozens of changes linked with heart disease, including elevated cholesterol and blood pressure." Until copper gets an RDA, Klevay (quoted in Raloff, 1989) "... argues, consumers and the food industry 'will continue to ignore copper' in labeling, research and their menus."

The effect of copper deficiency on cartilage and other connective tissue is detrimental (e.g. Allen et al., 1988) and congenital copper deficiency has been associated with Sudden Infant Death Syndrome (SIDS; Reid, 1987). Deficiencies are not uncommon in older people (e.g. Gershwin and Hurley, 1987), either as a result of physiological stress or simply improper diet. Even in healthy adults, copper has been shown to affect behavioral and sleep patterns (Penland, 1988). Deficiencies can also be produced by food components that actively scavenge copper (e.g. Emsley, 1989).

There is continuing work on the chemistry and biochemistry of copper-containing drugs, not only to elucidate their structure but also to better understand the mechanism of their action and the reasons for unwanted side effects. These include drugs for blood pressure and cardiovascular problems (Adachi et al., 1988; Balman et al., 1988; Christie et al., 1988; Gross and Prohaska, 1988; Hammond et al., 1988; Peters et al., 1988; Sugiyama et al., 1986), strong metal chelating agents used to treat Wilson's disease patients (Trombetta et al., 1988), drugs used to treat inflammation (e.g. Roch-Arveiller et al., 1987; Shetty and Melethil, 1987), anticonvulsant and emetic drugs (Palm et al., 1986; Ueno et al., 1987), antitumour and antineoplastic drugs (Harrison et al., 1987; Hasinoff and Davey, 1988; Litterst, 1988) and antimicrobial agents (Ali et al., 1985; Chatterjee et al., 1988; Lambs and Berthon, 1988; Tumanov et al., 1983).

The use of copper to control organism growth is important in pesticides, wood preservatives and antifouling agents. A wide variety of copper complexes have been developed as bacteriostatic agents (e.g. Fang et al., 1987) and a number of fungicides contain copper. Many of these are used to protect important plant crops or their products (Iino et al., 1987).

Metal released into the environment from these agents is of concern. Copper-containing fungicides have been suggested to cause chromosomal aberrations (Osiecka, 1987) and may act as mutagenic agents. In bivalve molluscs, like the mussel *Mytilus,* excess copper has been shown to reduce or stop water filtration (Abel and Papathanassiou, 1986; Redpath and Davenport, 1988) and o associated with a decline in growth potential (Widdows and Johnson, 1988). One of the detrimental effects of excess copper is due to its ability to affect lipids. Excess copper may be associated with the deterioration of the lipid portion of the cell membrane and cause a change in membrane permeability (de Vos et al., 1988).

The biological effect of excess copper can be reduced by organics which bind the metal. A number of organics are capable of doing this. Microorganisms and algae produce polysaccharides, many of which have the ability to bind copper (Kaplan et al., 1987b). In fact, Geesey et al. (1987) suggest that bacteria-produced acidic polysaccharides can promote deterioration of copper in aquatic environments. Many organisms produce an organic or group of organics that can bind excess metal once within the organism. The ability of organisms to adapt to changes in copper concentration is suggested by the increase in organics such as metallothionein after exposure (e.g. Steinert and Pickwell, 1988), a feature which tends to stabilize tissue metal concentrations.

Metal-metal interactions can change the biological effect of copper. Carson (1988) comments that chronic copper poisoning is a problem in the sheep industry as a result of excess copper or low molybdenum in commercial feeds. It can often be rectified by increasing the concentration of molybdenum. But not too much, excess molybdenum can produce an apparent copper deficiency, even in cases where there is enough copper.

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I - THE BIOLOGICAL IMPORTANCE OF COPPER

I.1 COPPER AS A REQUIRED TRACE METAL

Introduction

Throughout the geological history of the Earth, metals have played key roles in the evolution and maintenance of life (e.g. Boichenko, 1987). Their interaction with clay minerals may, for example, have provided a catalyst for the formation of organic compounds (Ferris et al., 1988; Lawless, 1986). Because of its ability to react with both inorganics and organics, copper is one of the more important metals. It is required by all organisms and is essential for a number of metabolic reactions. Because of its nature and abundance, copper has also played an important role in the development of man. Movements and migrations of both ancient and modern man have, for example, been affected by major deposits of copper (e.g. Bahr, 1985). Copper-containing paints have long been used by man (Duang et al., 1897). Copper-containing coins were in use at least in the late Neolithic (McGeehan-Liritzis and Gale, 1988) and recently-developed equipment and techniques such as ion beam analysis have provided important information on the metallurgical nature of copper artifacts (Beauchesne et al., 1988; Frontier et al., 1988; Gale and Stos-Bale, 1986; Holtta and Rosenberg, 1987; Rao et al., 1988; see also Ganorkar et al., 1988 for preservation techniques). Using some of these, Notis et al. (1988) comment on the character of copper smelting in the third or fourth millenium B.C.. As a reactive chemical, copper has been widely used in both industry and academia (e.g. Cooke and Speckhard, 1988; Schlbeci and Carlsen, 1988). It has been widely used in medicinal agents for thousands of years. Dollwet and Sorenson (1985) comment that the first Egyptian record of its use is in a papyrus, an ancient medical text, written between 2600 and 2200 B.C., which records the use of copper to treat chest wounds and to sterilize drinking water. Davis and Mertz (1987) note that copper and copper-containing compounds have been used medicinally at least as early as 400 B.C..

Because copper is an essential element to all organisms (e.g. Prohaska, 1988a; Siegemund et al., 1988a; Tandon, 1985) it is considered as a micronutrient. Lastra et al. (1987) review its nutritional role in higher plants. Ruth et al. (1988) comment (page 9) that in *Nautilus* (a unique group of cephalopods, related to the squid), "The element copper undoubtedly plays a crucial role in the synthesis of the respiratory pigment hemocyanin". In a paper on copper in human pathology, Flipo et al. (1987) comment that (summary) "Copper is a key element in the biological processes of life and an essential constituent of a great number of enzymatic systems." Publications on metal-organic interactions are numerous (e.g. review by Sarkar, 1987, on metal protein interactions) and are discussed elsewhere in this review. Klevay (1987b) notes an average daily requirement, by humans, of slightly less than 2 mg/day, to replace copper lost by urinary and fecal elimination and an additional 0.3 mg to replace daily loss from the body surface. He points out that dietary copper deficiency is common. Copper deficiency in plants, animals, and humans can lead to a variety of diseases (e.g. Danks, 1988; Triplett, 1985), which will be discussed later in this review. Reviews of copper as an essential nutrient are included in several publications dealing with trace metals (e.g. Mertz, 1987; Prasad, 1988) and include an excellent article by Solomons (1988). Takahashi and Kikunaga (1988) review the nutritional value of copper, molybdenum, manganese and silicon and the diseases associated with deficiencies.

Interactions between copper and other metals have been demonstrated to be important to normal metabolism. Nasolodin et al. (1986) comment (translation of page 84) that "The metabolism of Fe and Cu is interrelated with protein metabolism and the provision of vitamin-C by the organism, and ceruloplasm could possibly affect the quantity of haemoglobin in the blood and Fe content in the blood plasma." Copper, iron and magnesium status have been linked with mortality in the elderly (Tsui et al., 1986). Toxic effects of cadmium in buffalo calves have been reportedly reduced by copper (Zheng, 1988). Copper can act as a catalyst for certain oxidation reactions (Wolff et al., 1987).

Copper in microorganisms and plants

The availability of copper to plants is controlled by the nature of the soil (e.g. Ivashov, 1985; Sillanpää, 1987; Sinclair et al., 1988; Spiva, 1987), or other growth medium, as well as the nature of the plant. Stoddard (1987), for example, notes that phosphorus, in combination with deficiencies in iron or copper, may limit phytoplankton growth in an alpine lake in the Sierra Nevada mountains of California. Eun and Sedberry (1986) note that addition of copper caused an increase in rice plant dry matter production in a muck soil but not in a sandy loam. Koo (1988) points out that copper is one of the micronutrients required by citrus trees in Florida, to correct leaf-deficiency symptoms and maintain healthy trees. Locascio and Fiskell (1988) discuss similar requirements by vegetables, commenting on the importance of soil type in controlling metal availability.

As a required nutrient (e.g. Lastra et al., 1987), copper plays a number of roles in microorganisms and plants. Sharma and Sharma (1987) report that deficiencies of iron and copper are associated with plant water relationships, producing an increase in water potential, transpiration rate and water loss. Root growth in sunflower plants has been reported to be reduced with copper deficiency (Ozoliiya et al., 1983). Leaf and root chemistry as well as leaf appearance appears to be affected by deficiencies of copper and several other metals and nutrients (Abadia et al., 1988; Baez et al., 1987; Romero, 1987). Chlorosis has been reported under copper deficient conditions, for leaves of young oil palm plants (Pacheco et al., 1986; Pacheco and Tailliez, 1986) and soybean (Casanova and de Valls, 1987). (Note that either or both iron and copper deficiency can produce chlorosis (e.g. Fang et al., 1986).) Photosynthetic dysfunction is reported for wheat and barley with deficiencies of copper as well as manganese (Kriedemann and Anderson, 1988). Plant respiration can also be affected by copper deficiency (e.g. Ayala and Sandmann, 1988). Ozolina (1986) reviews the content and functional activity of copper-containing proteins in the leaves of crop plants, especially those receiving copper supplementation. This is important since copper deficiency can affect the activity of enzymes (Ayala and Sandmann, 1988b; Wachnik, 1987b; note that enzymes are one group of proteins). Deficiency may be detectable by analysis of the activity of specific plant enzymes (e.g. Kamala, 1986; Marziah and Lam, 1987; Marziah and Sekitar, 1986). This is true in all organisms. Greco (1987), in a Ph.D. dissertation, reports a decrease in superoxide dismutase activity with copper deficiency in the yeast *Saccharomyces cerevisiae* while Dameron (1987), also in a Ph.D. dissertation, reports the same thing in chick aortic SOD activity.

Copper is involved in plant reproduction. Barley pollen development is highly irregular in copper-deficient plants, resulting in low and variable pollen fertility (Jewell et al., 1988). Similar results have been obtained with wheat (Morard, 1984). Adams (1984) reports that omission of copper from a micronutrient mix, in a peat soil, severely reduced the yield and quality of cucumber fruit. Gabal et al. (1985) report that supplementation of a clay loam soil and spraying bean plants with a copper-containing supplement increased the number of flowers and fruit.

Copper in animals and humans

Copper is found in a variety of organics, such as blood pigments (e.g. Standley et al., 1988), and deficiency is associated with a number of diseases (e.g. Atukorala, 1985; Resnick, 1988; Prasad, 1988; Sternlieb, 1988). Tufts University diet and nutrition letter (1987) discusses some of these in an article entitled "Tracing the facts about trace minerals". In a review of "Metals as nutritional factors", Klevay (1987b) points out that copper deficiency is a substantial agricultural problem. He also reviews literature on the numerous diseases resulting from copper deficiency, stressing the effects of deficiency on cardiovascular problems. This is also discussed in his article on "Dietary copper and human health" (Klevay, 1987a).

Mills (1987) discusses the responses of livestock to copper deficiencies and comments on the importance of adequate knowledge about metabolic responses to deficiencies, to the control of trace element problems in agriculture. In the summary of an article entitled "Copper deficit pathophysiology", Jendryczko et al. (1986) comment that copper deficit affects a number of enzymes and the formation of a number of essential organics such as collagen (see also Jendryczko et al., 1987). Schwan et al. (1987) note, in lambs in flocks, that a combination of cobalt and copper deficiency produced poor growth, depressed appetite, poor general condition, anaemia, loss of wool, serous lacrimal secretion and ataxia. Tanner et al. (1988) report some of these problems in copper-deficient cattle; poor growth and anaemia appear to be common expressions of low copper status (e.g. Suttle and Jones, 1987), factors that can often be corrected with supplementation (e.g. Wittenberg and Boila, 1987). However, some of the diseases reported in cattle may be caused by factors other than nutritional copper deficiency (e.g Sager et al., 1988). Some may also be a result of metal-metal interactions (e.g. molybdenum and copper, zinc and copper), producing an apparent deficiency in the presence of an optimum or even adequate copper supply (e.g. Cinto et al., 1986; Hoffman et al., 1988; Simon et al., 1988; Wittenberg and Boila, 1987).

In laboratory animals as well as humans, copper is required in feeds and requirements have been shown to be affected by physiological status (e.g. Higuchi et al., 1988). Deficiencies have also been related to metal bioavailability as affected by food type. Fields et al. (1988), found a mortality of rat pups in dams fed a low copper diet with fructose but not in those fed the same copper concentration with starch. Castillo-Duran et al. (1988), working with infants, found that acute diarrhea lead to copper and zinc depletion. Deficiencies are not uncommon in older people (e.g. Gershwin and Hurley, 1987), either as a result of physiological stress or simply improper diet. Even in healthy adults, copper has been shown to affect behavioural and sleep patterns (Penland, 1988). (Copper deficiency is associated with decreased activity in rats (Root et al., 1988).)

Copper-containing superoxide dismutase is an important anti-inflammatory agent (Jadot et al., 1986a,b) whose activity can be reduced by copper deficiency (Lynch and Strain, 1988; Mitchell et al., 1988; Taylor et al., 1988). As in plants, copper deficiency can cause a reduction in the activity of copper-containing enzymes (e.g. Bartoli et al., 1988b; Kays and Johnson, 1988; Prohaska, 1988b,c). Lewis et al. (1988c) found that high dietary fructose and low dietary copper in combination interact to produce severe reductions in pancreatic amylase, lipase, chymotripsinotgen and trypsinogen activities which could not be explained by either copper deficiency or the dietary fructose alone. Deficiencies can also produce reductions in the concentrations of tissue copper and copper-containing agents like caeruloplasmin (Coppen and Davies, 1988). A direct relationship between copper deficiency and anemia has been demonstrated (Jain and Williams, 1988). This has also been shown for the nature of and metabolic activity in certain cell types (e.g. Babu and Failla, 1988; Johnson and Dufault, 1988; Kramer et al., 1988a,b; Lukasewycz et al., 1988; Prohaska et al., 1988a; Ralston and Milne, 1988; Seidel and Failla, 1988; Shafit-Zagardo et al., 1988) and for levels of membrane lipids (Lei et al., 1988a) and cholesterol (e.g. Bettger and Bray, 1988). A number of copper-containing agents have been isolated and characterized (e.g. Whittaker and Whittaker, 1988), some of which have been found to have anticancer activity (Willingham and Sorenson, 1986).

Danks (1988) discusses the question "does copper deficiency occur in humans?" The answer is given by a statement (page 236) that "there is no longer any debate about the essential role of Cu in humans and the main effects of severe deficiency are well established, even though not all these effects can be explained adequately." However, copper deficiency symptoms are not frequently observed in humans (Turnlund, 1988). In a review of copper as a nutrient, Solomons (1988) discusses uptake and utilization in the body. He discusses a variety of diseases caused by copper deficiency as well as diseases caused by improper utilization of available metal. Copper deficiency has, for example, been shown to cause fluid retention and edema formation in rats fed a high sodium chloride diet (Moore et al., 1988d). In adult brindle mice, Everett et al. (1987) demonstrated a relationship between proper spermatogenesis, available copper, and testosterone.

Copper deficiencies are often associated with a reduction in immune response capability and an increased risk of infection (e.g. Anonymous, 1987a; Brooke, 1986; Chandra, 1987, 1988a,b; Christie et al., 1988; Crocker and Lee, 1987; Eason et al., 1989; Gutteridge, 1987; Mulhern et al., 1987; Mulhern and Koller, 1988; Prohaska, 1988b). This may be a result of modifications in cell metabolism or reduced activity of superoxide dismutase, with its protective roles. However, it also includes effect from a number of other copper-containing organics such as ceruloplasmin (Stabel et al., 1988). This points out the importance of an adequate trace metal balance for individuals under physiological stress (e.g. Wu et al., 1988b). As an example, trace metal supplementation for total parenteral nutrition (TPN) is important in humans and includes an adequate supply of copper (e.g. Fujita et al., 1988a; Salis et al., 1986; Shike, 1988; Takagi et al., 1988a; Triplett, 1985) as well as other metals. Shulman (1987) reports that the recommended parenteral copper intake of 10 µg/kg/day was adequate for most of the infants studied.

The production of bone and cartilage requires copper and copper deficiency is often associated with either poor development or bone metabolism. This has been shown in laboratory animals (Read et al., 1988) as well as humans. Reduced copper intake may be one of the factors involved in the loss of bone that occurs with age, especially in postmenopausal women (Johnston, 1987). Brittle bone disease and reduced collagen have been associated with copper deficiency in animals (Bridges and Harris, 1988). Case histories of children with brittle bone disease have been shown to be a result of dietary copper deficiency (e.g. Paterson, 1988). In an article entitled "Brittle or battered", Carty (1988) discusses the difference between nutritional deficiency and child abuse and points out that the effects of deficiency are found in all bones whereas fractures due to child abuse affect specific bones. The effect of copper deficiency on cartilage and other connective tissue is detrimental (e.g. Allen et al., 1988) and congenital copper deficiency has been associated with Sudden Infant Death Syndrome (SIDS; Reid, 1987).

The relationships between fat metabolism and copper are numerous, often complicated and, under certain conditions, play important roles in the physiological well being of the cardiovascular system. Copper deficiency has been associated with improper dietary fat metabolism resulting in abnormal levels of certain tissue fats (e.g. Carr and Lei, 1988; Fields et al., 1988; Koo et al., 1988a,b; Lei et al., 1988; Lynch et al., 1988; Ovecka et al., 1988; Radhakrishnamurthy et al., 1988; Stacy et al., 1988; Valsala and Kurup, 1987; Zhang et al., 1988c) as well as byproducts of protein metabolism (e.g. Allen et al., 1988a,b). Although copper deficiency impairs intestinal transport of cholesterol (Koo et al., 1988a,b), plasma cholesterol levels are frequently elevated (Bestervelt and Hill, 1988; Hassel et al., 1988a,b; Jiang et al., 1987a; Lee and Koo, 1988). Klevay (1987c) comments on the ability of dietary copper to reduce plasma cholesterol levels. He (Klevay, 1988) reports that dietary cholesterol caused a decrease in liver copper levels in rabbits, with an increase in plasma copper levels. The relationship between copper deficiency and cardiac abnormalities have been demonstrated although the factors causing this relationship are as yet, poorly understood (e.g. Anonymous, 1986; Farquharson and Robins, 1988; Gross and Prohaska, 1988; Moore and Klevay, 1988; Raloff, 1989; Saari and Klevay, 1988). Nutritional inadequacies as well as nutrient composition have been inferred both in terms of copper adequacy and bioavailability (Anonymous, 1987c; Strain, 1988).

A relationship has been shown, between copper deficiency and abnormalities of the pancreas. Some of these are as yet unproven, such as the hypothesis by Paterson and Wormsley (1988) that there is a relationship between neonatal copper deficiency and Schwachman's syndrome, the second most common cause of exocrine pancreatic insufficiency in childhood. (Second after cystic fibrosis.) Morphological and biochemical changes have been shown to occur in the pancreas of copper-deficient rats (Kimura et al., 1987; Weaver et al., 1988), changes which affect enzyme synthesis amongst other things. The effect of deficiency on enzyme synthesis is described in the recent literature by Mylroie et al. (1987a,b) and others. Moretti et al. (1987) report a possible relationship between chronic pancreatitis and hypocupremia in rats and Rao et al. (1988d) note that diet manipulation, using copper deficiency, caused an almost total conversion of pancreas to liver in the adult rat.

Uno and Arya (1987) comment that "The metabolic disorder caused by copper deficiency induces severe neuronal degeneration that is apparently exaggerated by extensive and progressive vascular abnormality." This was in the abstract of a description of a child with Menkes syndrome, a genetic, sex-linked defect of copper metabolism. The importance of copper in normal nerve function is becoming more apparent with increasing work. Hartter and Barnea (1988b) provide evidence for the release of copper in the brain. Copper is also required for several copper-dependent enzyme systems in the brain as well as for normal receptor function (Essman, 1987). Miller and O'Dell (1987), however, point out that copper probably does not effect the only control in many of these functions, however, possibly interacting with genetic or environmental factors.

I.2 BIOLOGICALLY IMPORTANT USES OF COPPER

Nutrient supplementation - *plants*

Copper is introduced in a range of nutrient supplements to offset potential nutrient deficiencies (see review by Mortvedt, 1988). The nature of the soil, for example, may reduce the concentration or biological availability of copper and other trace metals (e.g. Ivashov, 1985; Khalileva et al., 1986; Rahmatullah and Zia, 1987; Tyksinski, 1986). Gembarzewski and Stanislawska (1987) note that copper-deficient fields formed 20-30% of the fields used as controls in a fertilization study in Poland. Copper deficiency is also a problem in greenhouse soils (Chuprikova et al., 1985), especially when using specialized soil materials such as sawdust (Cheng, 1987). Copper supplementation has also been reported to reduce the effect of radioactivity on plants (Fedotov and Tikhomirov, 1987; Rakosy-Tican and Trifu, 1986). Recent patent documents describing new copper-containing plant fertilizers includes Fedun et al. (1988), Mateescu et al. (1988), Szakal et al. (1988), Wagner (1988), Yao et al. (1987), Zhao (1987). A number of anthropogenic "waste" materials have also been tested as fertilizers (e.g. Vovkotrub, 1988) and will be reviewed in a later chapter.

Recent literature on the use of copper as a plant nutrient supplement is varied and listed in later sections of this review. The literature does indicate that the need for supplementation is dependent upon soil type (Eun and Sedberry, 1986; Golovina et al., 1988), past history of fertilization (Szwonek and Nowosielski, 1984), the interaction of micronutrients (Shuman, 1988), plant type (Koo, 1988; Locascio and Fiskell, 1988), and plant condition (e.g. Dell, 1987; Pacheco and Tailliez, 1986). The nature and effects of a wide variety of copper-containing fertilizers has been recently examined (e.g. Anderson, 1988; Arora et al., 1985; El-Hady et al., 1986; Kovalevich & Dubikovskii, 1988a; Lotfy et al., 1987; Matveev and Kozlava, 1984; Potatueva et al., 1985).

Determinations of supplementary copper needs have been found for cultures of yeasts (Kamra and Madan, 1987; Ricci et al., 1987) and plant cells (Morimoto et al., 1988b). The growth of both wild and domestic rice has been found limiting without copper, at least under certain conditions (Das and Mandal, 1986; Keenan and Lee, 1988; Muhammed et al., 1987). Agricultural studies of alfalfa (Isaev and Khalileva, 1987), bermudagrass (Angel and Feagley, 1987), bromegrass (Horvath, 1986), clover (Nikolaeva, 1988) and a number of cereals (Anke et al., 1986; Coventry et al., 1987; Gordetskaya et al., 1987; Razuvanov, 1985; Saad et al., 1984) have shown direct benefit from copper supplementation in certain soils. Prasad and Ram (1988) suggest that the lack of response of tropical legumes to fertilizers may be a result of trace element deficiency to the symbiotic, nitrogen-fixing bacteria found in nodules on the plants. They point out that copper and zinc improve nodulation and chlorophyll content of the host plant (greengram - *Vigna radiata*). The use of copper has also been found to be beneficial in other agricultural crops such as apples (Stiles, 1987), cardamom (Hernandez and Pacheco, 1986), cassava (Gomes and Lemos de Carvalho, 1986), citrus fruits (El-Sherif et al., 1983; Fawzi et al., 1986), cotton (Constable et al., 1988; Iagodin et al., 1984; Khodzhaev and Stesnyagina, 1987; Yusupov et al., 1986), melon (Ilamanova, 1982), peas (Kovalevich and Dubikovskii, 1988b), potatoes (Sharma and Grewal, 1988), sunflower (Salama and Buzas, 1987), and tobacco (Littlemore et al., 1987; Ryabchuk, 1987). The growth of some coniferous trees (e.g. *Picea abies*; Santanen and Simola, 1988) can benefit from the use of copper-containing as can the foliage and oils of certain plants used for perfumes (e.g. *Ocimum gratissimum*; Choudhury et al., 1986). Wenny et al. (1988) report better root growth from seedlings containerized with cupric carbonate soil supplementation.

Fertilizer components often mutually interact with the plant, affecting the response in a manner different from that predicted by single nutrient fertilization. Marks and Kazakova (1987) note that fertilizers stimulated copper uptake over the entire growth period of spring wheat. Kadar and Shalaby (1984) report that increasing nitrogen doses caused a decrease in the shoot copper concentration in spring barley and millet although root copper was increased by nitrogen fertilization in barley. Mamo and Parsons (1987), working with the teff plant, report that phosphorus depressed shoot copper concentrations. Molybdenum and copper sprays increased total nitrogen in broad bean root nodules and increased total nitrogen fixation. Interactions do not always occur, however; Raju and Deshpande (1987) found no interaction between copper and zinc in coffee seedlings, interaction which had been expected on the basis of other plants. This points out the species-specific nature of plant response to various fertilizers and the importance of understanding the relationship between the requirements of the plant, the nature of the soil, and the nature of the fertilizer.

Nutrient supplementation - *animals*

The importance of copper supplementation to animals is discussed by Mills (1987). It can reduce the impact of physiological stress (e.g. Wu et al., 1988b) and improve the growth and feed conversion efficiency in a variety of animals, including poultry, sheep, pigs (Bolz et al., 1987) and cattle (de Souza et al., 1986; Wittenberg and Boila, 1987). The need for supplementation usually arises as a result of inadequate dietary copper. With grazing animals, this is often a result of soil deficiency resulting in low plant copper values. Since the chemistry of the soil is often responsible for copper concentration as well as metal bioavailability (Anke et al., 1987), deficiencies are quite frequently of a regional nature. Copper supplementation must thus be based on regional needs as well as general animal needs. In feed mixtures, supplements should be designed to suit the requirements of the animal (Kossaczka, 1986) as well as the biological availability of the metal in the mixtures.

Various types of supplements are available. Egorov et al. (1985), for example, found that high-dispersion powders of zinc, iron or copper increased broiler chick weight gain rate by 3.8-8.1% and feed conversion efficiency by 3-8% when compared to results with metal sulfates. In comparing various meal types or meal components, trace metal levels are either kept constant (e.g. Nelson et al., 1988) to evaluate nutritional value, or are varied (e.g. Wang et al., 1987a) to determine effect on trace metal expression in the organism.

In sheep, Podshibyakin et al. (1988) report that vitamin and trace metal supplementation improves milk and wool yield as well as growth of lambs. Since both copper defficiency and toxicity occur in sheep, with potentially disastrous results, there is a need to monitor feed copper levels and organics that can affect metal bioavailability (e.g. Sappington et al., 1987). The use of soluble glass is one mechanism used to introduce trace metals and metalloids to sheep, goats and cattle, independently from food (Judson et al., 1988; Zervas, 1986, 1988a,b; Zervas et al., 1988).

Copper is widely used with pigs, as a nutrient supplement, a means of improving food utilization (e.g. Dove and Ewan, 1987; 1988), and a control of internal parasites (e.g. Izquierdo et al., 1987). Availability of naturally-occurring copper, in food materials, has been examined (Kal'nitskii et al., 1986) as has the use of copper supplements (e.g. Southern et al., 1987). Thacker (1987) provides evidence that (page 1209) "... feeding high levels of copper or dichlorvos during late gestation and lactation may be useful as a means of reducing preweaning mortality in baby pigs." Similar results have been obtained by Ruda et al. (1988). With weanling pigs, the addition of copper, as copper sulfate has been found to increase growth rate in a variety of diets (Burnell et al., 1987, 1988; Walker and Danielson, 1988). This has also been found with weaned and growing pigs (Moser et al., 1988b; Pond et al., 1987, 1988) as well as "finishing" pigs (Cromwell et al., 1987). However, feeding excess copper has not always been proven beneficial (e.g. Borg et al., 1988; Hamada et al., 1986) although Menten et al. (1988) comment (abstract) that, with starter pigs, "Feeding high levels of copper (tends) to reduce ammonia concentration in the intestinal contents, reduce absorption of ammonia from the gut and reduce the turnover rate of intestinal mucosa."

The low copper concentration of natural food materials has been associated with copper deficiency in cattle in a number of areas (Bialkowski, 1988; Binnerts and Viets, 1986; Ogwang, 1988; Turner et al., 1988a). This can be offset by copper supplementation fertilization of grazing areas (Rokicki et al., 1986) or mineral and ionophore supplementation of feed materials (e.g. Bineev et al., 1983; Falkowska and Iwanska, 1987; Harvey et al., 1988). de Souza et al. (1986), for example, note that cattle with periodontal disease showed an improvement when supplemented with cottonseed meal and high concentrations of copper. Trace copper supplementation has been reported to improve reproductive function in breeding bulls (Petryankin and Tukmakov, 1987) and supplementation to cows improves calf trace metal status (Gooneratne et al., 1987a). Copper has been recently used to improve trace metal status in calves, heifers, and steers (Boila, 1987; Buckley et al., 1987; Edmiston and Bull, 1988; Givens et al., 1988; Knebusch et al., 1988; Messina and Nocchi, 1986; Sankoh and Boila, 1987), through injection, food supplementation, or supplemention in soluble glass.

Copper supplementation has also been used for humans (e.g. Klevay, 1987a,c), especially in situations associated with physiological stress (e.g. Baumann, 1987; Bogden et al., 1988; Takagi et al., 1988a). Campbell (1988) reviews trace element requirements in pregnancy, commenting on the increase in plasma copper levels. However, Allen (1986), in a review of "trace minerals and outcome of human pregnancy, reports that "Low maternal intakes of copper, manganese, and selenium have not been associated with adverse outcomes of pregnancy, ...". Brown (1988b), in a discussion of physiologic anemia of infants, comments on the occurrence of copper deficiency and the importance of copper in superoxide dismutase. However, the author points out (page 283), that "... copper deficiency does not seem to be a factor producing hemolysis or contributing to the development of anemia of prematurity ...". Copper has also been used with unique food agents (e.g. peanut skins, Badresingh et al., 1988) to maintain appropriate tissue copper concentrations.

<u>The use of copper to control the growth of organisms</u>

Organisms can cause damage to a wide variety of substances and media. Although growth of both plants and animals requires copper, growth can also be reduced or stopped by excess copper. As a result, one or more chemical forms of copper are frequently incorporated into biocidal agents. (Some of these agents are discussed under wood preservatives and antifouling compounds.) Bailey (1988) discusses fungal diseases of important plants and their control, in a series entitled "Fungal Foes in Your Garden" (e.g. 1988). Bebee (1988) provides a bibliography of papers on the protection of leaf vegetables from fungal and bactericidal diseases. The "Pesticide Fact Sheet Number 89: Copper Sulfate" of the U.S. Environmental Protection Agency (1986) provides chemical information on the nature and use of one widely used agent. The sheet also gives a summary of the regulatory position and rationale of the E.P.A..

Growth control can be beneficial in managing commercially important crop plants. As an example, Bergmann et al. (1987) describe a recently patented, copper-containing desiccant and defoliant for potatoes. However, most of the uses of copper in growth control are to control the growth of unwanted organisms. A wide variety of copper complexes have, for instance, been developed as bacteriostatic agents (e.g. Fang et al., 1987). A number of fungicides contain copper. Many of these are used to protect important plant crops or their products (Iino et al., 1987). Descriptions of the control and side effects of their use include Santos and Medeiros (1981). Fungicides of recent formulation are described in patent documents such as that of Frey et al. (1988) and Kleemann and Claus (1988). Nakahara (1987) patented the formulation and use of a cupric acetate-containing medicine for treating athlete's foot. Copper-containing fungicides have been used to control diseases of alfalfa (Broscious and Kirby, 1988; Gray and Fernandez, 1987) and barley (Krutova and Srkza, 1981). They are useful for controlling leaf spot on sesame plants (Rao et al., 1988c), ringspot on brussel sprouts (Wicks and Vogelzang, 1988) and scab disease of potatoes (Ramsey et al., 1988). Recent applications for fungal diseases of other vegetables and fruits are discussed for cucumbers (Sagi and Sipos, 1987), tomatoes (Mathur and Shekhawat, 1986), pigeonpea (Agrawal, 1987), soybeans (Singh and Agarwal, 1987), mangos (Hussain et al., 1987), citrus fruits (Eskes et al., 1985), and chilies (Eswaramurthy et al., 1988). Copper-containing agents are widely used for the control of shoot rot disorders of tea plants (Yano et al., 1986) and coffee rust (Almeida et al., 1983a,b; Campacci and Chiba, 1983; Carneiro Filho et al., 1983; dos Reis et al., 1983; Matiello and Mansk, 1983a,b; Mansk and Matiello, 1983; Miguel et al., 1983). Other commercially important plants in which fungal diseases have been successfully treated with copper-containing agents include sunflower (Krishnappa and Shetty, 1987), safflower (Lukade et al., 1985), groundnut (Gangawane and Saler, 1988), cotton (Miller and Bekker, 1987) and bottlegourd (Amin and Ullasa, 1985). Nursery diseases of forest trees include leaf spot, "damping off" and blight, which have been treated with copper (Gupta, 1987; Singh et al., 1985). Karadzic (1987) describes the successful use of copper-containing agents in the control of pine needle blight in Australian pine plantations. Side effects of fungicide use include effects on non-target fungi (e.g. El-Sharouny et al., 1988; Singh et al., 1987c), biological control organisms (Reddy, 1987), plants treated for fungal diseases (e.g. Price and Lenne, 1987), and food products from treated plants (Leoni et al., 1985).

Copper also serves as an effective bactericidal agent by itself or with organics (e.g. Bakola-Christianopoulou et al., 1988; Khadikar et al., 1986; Sharma and Parashar, 1988; Singh et al., 1988a; Wilkins and Moore, 1988). Copper-silver disinfection units have been developed and used to reduce chlorine concentrations needed in swimming pools and hot water baths (Yaha et al., 1988). Copper bactericides have been used to control *Xanthomonas* infection of bell pepper leaves (Simons, 1988) and tomato leaves (e.g. Jones et al., 1987c) although there is some evidence of increased copper tolerance by members of this genus and other genera infecting tomato plants (Cooksey, 1988). Cupric hydroxide is used as a control for *Pseudomonas* infection of beans (Legard and Schwartz, 1987) and copper oxychloride and sulfate as controls for Mexican lime bacteriosis (Medina Urrutia and Stapleton, 1986). However the causative agent of citrus canker (*Xanthomonas campestris* pv *citri*) may be copper tolerant (Zubrzycki and de Zubrzycki, 1986). Copper tolerance in *Xanthomonas* has also been recorded by Bender et al. (1988) for tomato spot disease agents. Part of this may be a result of excess magnesium, reducing the toxicity of copper (Woltz et al., 1988). Obata et al. (1988) describe the use of copper salt and ascorbic acid to control the ice-nucleating activity of *Pseudomonas fluorescens* which causes freezing injury to corn and other, sensitive vegetables.

Copper-containing agents are used as herbicides (e.g. Natchev, 1988) although a cuprix hydroxide fungicide has been shown to reduce the herbicidal effects of paraquat, at least with the common nightshade *Solanum americanum* (Stall and Kostewicz, 1987). Agents have also been used to control growth of spoilage organisms and thus to preserve animal feed materials such as hay (Tomes, 1988a) as well as human food materials. Control of animal parasites has been effected with copper. Ramadan and Salam (1986) describes the effective use of formalin and copper sulfate to control ectoparasitic ciliates of grass carp. Continuing development and use of controlled release copper is being made in eliminating the snail intermediate host of schistosomiasis. African snails form an agricultural pest that can be controlled by copper sulfate as well as a number of other pesticides. The greening of nonmetallic porous materials such as building materials, can occur as a result of algae, mosses and lichens. In a patent document abstract, Albenque et al. (1988) briefly discuss a copper-sulfate-containing compound which is reported to control greening.

The use of copper in biologically important chemicals

Because of its biological activity, and compatability at normal concentrations, copper has been used in a number of medications throughout the history of present day man (Dollwet and Sorenson, 1985). Galiazzo et al. (1988) present evidence that copper acts as a regulator of the expression of major enzyme activities involved in biological oxygen activation. In association with one or more organics, copper has potential as well as proven applications in diagnostic imaging and therapy (Chauhan et al., 1987b; Fujibayashi et al., 1988; Green et al., 1988b; John et al., 1988; Mertens et al., 1988; Roberts et al., 1987; U.S. Department of Health and Human Service, 1988). Crook (1988), for example, has recently patented a method of imaging the heart using copper-64 citrate. It is also used to examine biologically important interactions in other organisms, such as that between chlorophyll and plant chloroplast thylakoid lipids (Evans et al., 1988).

The ability to react with other organics makes copper useful in medical, chemical and biological research (e.g. Depreux et al., 1988; Donato et al., 1988). Issopoulos (1988) describes the use of copper acetate as a complexing agent in the investigation of β-lactam antibiotics. Gupta and Jha (1987) discusss the physico-chemical characteristics of copper complexes of sulfadrugs. Copper is one of the few metals whose activity makes it invaluable to biochemists examining metal-drug complexes (e.g. Chatterjees et al., 1986; Eberhardt et al., 1988; Parashar et al., 1987; Real et al., 1987) as well as biological activity (e.g. Adachi et al., 1988a; Roch-Arveiller et al., 1987).

A number of copper complexes have antibiotic activities (e.g. Bartmann et al., 1988; Berners-Price et al., 1988; Dahlund and Olin, 1987; de Zwart et al., 1988; Joshi et al., 1988; Kim et al., 1987). Others act as anti-inflammatory agents (Abe et al., 1986; Andronescu et al., 1986; Arnaud et al., 1987;

Hac and Gagalo, 1987; Kishore, 1988a; Milanino et al., 1988c; Wiener, 1987). This includes some naturally-occurring agents (e.g. ceruloplasmin) which are transported to inflamed regions, causing an increase in copper concentration in association with inflammation (McGahan and Fleisher, 1986). Jackson and Kelly (1988) used a computer model of blood plasma to aid in designing copper anti-inflammatory drugs for rheumatoid arthritis. Possibly as a result of its antiinflammatory effect, copper-based drugs have been used for a variety of aches and pains (e.g. Pratt et al., 1985). Wissler et al. (1987a) describe a copper-containing organic that can regulate cell division and act as what the authors term a "novel type of 'Wound-Hormone'." Li et al. (1986a) report that zinc and copper can exert protective effects on cardiac lesions that are induced by adriamycin.

Iqbal et al. (1987) report that a copper complex of an oral antidiabetic drug exhibited more activity than the original drug. Sorenson (1987a-c), in association with the International Copper Association, reports the treatment of epilepsy and convulsions with organic copper compounds. With Sorenson and others, Dollwet et al. (1987) note superoxide dismutase-mimetic activity of antiepileptic drug copper complexes. They suggest that (abstract) "... superoxide or the accumulation of its more reactive oxyradical products are a cause of seizures and that the superoxide disproportionating reactivity of copper complexes or their possible role in *de novo* synthesis of SOD in brain tissue merit consideration as plausible mechanisms of action of antiepileptic drugs." Copper-containing liposomal SOD is also suggested as a possible treatment of coma states and brain traumas (Michelson et al., 1988).

Willingham and Sorenson (1986) review the physiological role of copper complexes as anticancer agents. Copper-containing organics are more and more frequently, being found useful in controlling the types of irregular cell growth found in diseases like cancer (e.g. Basosi et al., 1987; DeNardo et al., 1986; Diplock, 1988; Elo, 1987; Lepri et al., 1988; Mohan et al., 1988; Morishige et al., 1986; Rabinovitz and Fisher, 1988a; Reiners and Colby, 1988; Sakai and Yamane, 1986) or at least to inhibit the mutagenic activity of some organics (e.g. Francis et al., 1988a,b). Part of this is its ability to associate with or affect the division of genetic material in the cell (e.g. Chrisey and Hecht, 1988; Sideris et al., 1988; Young and Hanson, 1987). As a result, a number of copper-containing agents are being examined both for their structure and composition as well as their ability to control cell growth (Asaturyan, 1988; Basosi, 1988; Brown et al., 1988b; Deshpande et al., 1988; Fiallo and Garnier-Suillerot, 1987). As well, work continues on a better understanding of the interaction between copper and cell products (e.g. Bogush et al., 1987; Cerutti, 1988; Melnyk et al., 1987), in an attempt to explain the action of anticancer agents and allow improved treatment of the various types of irregular cell growth. Some of this is reviewed in "Chemical reactions occurring between metal-containing antineoplastic drugs and biological molecules" by Litterst (1988). Many anticancer agents have undesirable side effects such as disturbed renal function. Syrkin and Chlenova (1987) report that copper sulfate, injected subcutaneously into mice with Ehrlich's tumour, reduces the disturbed renal function side effect of cisplatin, an important antitumour agent. Copper sulfate also has an inhibitory effect on growth of Ehrlich's type of tumours as shown by Syrkin and Chlenova (1987) and Kramhoft et al. (1988). Some copper-containing agents are also useful in reducing the effects of irradiation, used for cancer treatment (Soderberg et al., 1987, 1988a) although the reason for this is not well understood (e.g. Westman and Marklund, 1987; see also Tahsildar et al., 1988 and Wu et al., 1988b).

Copper in dental amalgams and mouth rinses

Copper, in combination with organics such as hexetidine, is used to control bacteria causing dental plaque (Grytten et al., 1987, 1988a,b). Maltz and Emilson (1988), for example, report that CuF_2 provided the best caries reduction in hamsters infected with dental plaque bacteria. Copper alloys have been used in dental castings (Bumgardner and Lucas, 1988; Craig and Hanks, 1988;) although there is some concern about biocompatability, in part a result of corrosion-released metal (Burns et al., 1988; Filler et al., 1988; Johansson and Mjor, 1988; Lucas et al., 1988a; O'Neal et al., 1988). Bumgardner and Lucas (1988), note that three commercial copper alloys did not cause atrophy in cultured cells immediately adjacent to the alloy although there were "... changes in cell morphology ... in selected areas." Lemons et al. (1988) note that (abstract) "Overall biocompatability profiles do not support clinical applications of some of these copper base alloys."

Copper in wood preservatives and antifouling compounds

Copper is widely used in wood preservatives and antifouling compounds because of its toxicity at high concentrations. Reports by both government and industry, for example, indicate that treating with a combination of copper-containing preservatives (ammoniacal copper arsenate and chromated copper arsenate) provides adequate protection for marine pilings (Bultman and Webb, 1985). These same preservatives are successfully used on utility poles on land although Morrell et al. (1988) note decay fungal infection if the poles are stored for extended periods of time before use. Jewell et al. (1985) reports no decay of ACA-treated 2x4 stakes after 13 years, but substantial decay in pentachlorophenol-treated stakes.

New preservatives are continuously being developed. Recent patents for copper-containing preservatives include Goettsche (1988), Kanda et al., (1988), Mowius et al. (1988). Gorshin and Maksimenko (1987) describe a copper-containing wood preservative, developed at the Senezhskoe Laboratory of Wood Preservation in Russia, that utilizes a fluoride stabilizer. In addition to new preservatives, decay resistance of various wood types continues to be examined. Mitchoff and Morrell (1988), for example, discuss the resistance of red alder after treatment with various preservatives (CCA treatment provided the best protection). Leightley discusses preservative-penetration problems with softwood timbers in Queensland, Australia. Different methods of applying preservatives are also being tested. Bergervoet (1984) discusses a method for treating partially seasoned pine, in New Zealand, with CCA preservatives. Ostmeyer et al. (1988) used X-ray photoelectron spectroscopy to examine CCA-treated southern pine, in the U.S.. They report indications that the preservative reacted with wood through aromatic and possibly alkene substitution. Ruddick (1985) found beter CCA penetration into spruce and lodgepole pine lumber with needle incising, when compared with conventional North American incising processes. Keith and Chauret (1988) obtained similar results, reporting much greater wood damage by conventional tooth incising techniques. Hosli and Ruddick (1988) found much better penetration of CCA into white spruce with high pressure pulsation than regular pressure penetration processes. Ultrasonic treatment also increases absorption of CCA by spruce, Douglas-fir and ponderosa pine (Avramidis, 1988). Testing of undamaged CCA-treated southern pine indicates little if any effect of treatment on mechanical properties (Ostmeyer and Elder, 1986). Winandy and Boone (1988), however, found CCA treatment to reduce the strength of southern pine lumber containing pith to a greater extent than pith-free lumber. They also report that this is a result of the strength ratio, lumber having a strength ratio of ≥ 0.65 and containing pith was not affected by CCA treatment.

Fouling of ships and structures is a continuing problems in both marine and freshwater environments (e.g. Arias et al., 1986; Igic, 1982). Antifouling techniques are numerous, many utilizing copper-based paints or coatings. Czimmek and Sandor (1985) present test results on the Cu-Ni sheathing test panels installed on the tanker Arco Texas, they propose new cost-effective welding techniques for Cu-Ni sheathing of ship hulls. McKay et al. (1987) discuss the copper-holding ability of chitosan, from chitin which they obtained from prawn waste. They report, however (abstract), "... that copper-chitosan complexes are not as effective as the cuprous oxide anti-fouling agent currently used in marine paint formulations." A great deal of concern has been expressed about antifouling agents in general, causing damage to nontarget organisms (e.g. Minichev and Seravin, 1988). This concern has been primarily focused on the use of tin. Toxic effects of copper are much less severe although they have been reported when organisms are exposed to high levels of metal (e.g. Bondon et al., 1988). This will be discussed in a later section of the review.

Miscellaneous uses

Metallic copper has a wide number of uses. With its physical properties, the metal makes excellent pots and pans for cooking (Anonymous, 1985). Gabay et al. (1988a,b) describe the use of copper vapor laser exposure for opthalmology. Moens (1984) describes the use of copper screens to line the inner walls of open breeding boxes for economically valuable snails. In this case, the screening acts as a deterrant to the movement of the snails. As an ion, the ability to control organism growth with high levels of copper makes the metal useful in controlling the growth of coliform bacteria in aquatic environments (e.g. Jana and Bhattacharya, 1988). Adhesion of bacteria is reduced on copper surfaces (Branting et al., 1988), when compared with many other commercially-available surfaces. Pedersen et al. (1986) note that, with municipal drinking water systems, polyethylene tubing exhibited much higher bacterial attachment than did copper tubing and inert glass surfaces. Schoenen and Wehse (1988) and Schoenen et al. (1988) examined microbial colonization (including growth of *Legionella pneumophila*) of water pipes and hoses and found little if any colonization on glass, high grade steel, copper and PTFE when compared with PVC, PE, PA, silicon and rubber tubing. Inhibitory effects of copper plumbing systems, on growth of *Legionella*, were found by Habicht and Muller (1988) in warm water systems of hospitals and hotels in Lower Saxony.

Metal reactivity combined with metallurgical properties has led to the use of copper in environmental control equipment and technology. Melson (1987, 1988) describes the use of copper and iron absorbents for sulfur dioxide removal from flue gasses. A copper chromite catalyst has been developed and tested for automobile emission control (Laine et al., 1986). This same type of reactivity allows copper to act as a catalyst in a variety of industrial processes including hydrogenation of rapeseed oils (Drozdowski and Szukalska, 1983) and synthesis of certain organics (e.g. Nugent and Waller, 1988; Reddy and Subrahmanyam, 1988). Copper has also been used as a protein stain (Lee et al., 1987a) and a reagent for use in the analysis of a number of organics (Hara et al., 1988; Ishiyama et al., 1985; Jovanovic and Sankovic, 1988; Shing, 1987, 1988; Ugalde et al., 1988; Verma et al., 1986).

I.3 COPPER IN ORGANISMS

The concentration of copper in organisms varies in response to inherited and environmental factors. Uptake under conditions of metal deficiency or excess can produce low or high concentrations within the organism as can abnormal physiological conditions. This section deals with recent literature that discusses factors associated with tissue metal concentrations. Metal uptake is discussed in a later section while specific concentrations are presented in tables 3-5.

In preparing this and previous reviews it has become obvious that there needs to be more effort spent on determining the nature of the factors that affect tissue metal concentrations. A large number of papers provide some information on concentrations and requirements, especially for food organisms (e.g. Herrera, 1987; Stiles, 1987) however, very few attempt to relate these data to other factors. It is these relatively few papers that form the basis for this section.

I.3.1 METAL LEVELS IN NORMAL TISSUES

Microorganisms and plants

In an examination of two species of cyanobacteria (blue-green "algae"), Weckesser et al. (1988) note that the outer sheath (abstract) "... bound heavy metals (up to at least 0.7% of sheath dry weight) with the effectiveness ... Fe>Zn>Cu>Ni>Mn>Mo>Co. Ni, Cu, Zn and Fe were highly enriched relative to their concentration in the culture medium." The mechanism causing binding was not examined although it could have been either adsorption or complexation. Brongersma-Sanders (1988) discusses a possible relationship between cyanobacterial (blue-green algae) mats and the trapping and deposition of metals to form stratiform deposits. Uptake and accumulation of copper is noted in soil fungi by Letunova et al. (1988), *Sphagnum* mosses and lichens. In the latter two cases, uptake can be an expression of aerosol input (Malmer, 1988; Thompson et al., 1987).

Plant metal concentrations are controlled by the nature of the plant and soil geology, metal concentration and metal bioavailability (Anke et al., 1987; Bonneau, 1988; Krizek and Foy, 1988a,b; Szentmihalyi et al., 1986). Concentrations can vary as a result of the genetic nature of the plant as well as by the interaction with the environment (Clark and Gourley, 1988). The effectiveness of copper supplementation to soils is also dependent on the nature of the soil as well the nature of the plant and the supplementation (e.g. Gorlach and Jasewicz, 1987; Solovlev et al., 1987; Tropea et al., 1984). In an examination of trace-element fertilization of peas, Kovalevich and Dubikovskii (1988b) found accumulation in seeds and straw to decrease in the order Mo>B>Zn>Cu. Horvath and Szodfridtne (1986) report that addition of high levels of synthetic nitrogen fertilizers was associated with an increase of copper and zinc in awnless bromegrass. In contrast, Mazur et al. (1988) found a copper concentration decrease in hay with high amounts of nitrogen-containing fertilizer (120 kg N/ha). Copper supplementation (10 kg Cu/ha) alleviated the problem. Salama and Buzas (1987) found that addition of nitrogen (with phosphorus and potassium) caused a decrease in sunflower copper content in one soil type although, in both soil types tested, there was an interaction between growth regulators, the fertilizer used and the copper content in sunflowers. Working with Bermudagrass grown on lignite overburdens, Angel and Feagley (1987) noted that plant yield and nutrient uptake (including copper) increased with fertilizer addition. Tissue copper concentrations in broccoli were generally increased by ammonium more than by nitrate supplementation (Shelp, 1987) although there was evidence for cultivar differences in metal translocation after uptake. With corn, Sawyer et al. (1987) note that the use of copper-rich swine manure as a fertilizer did not increase tissue metal levels.

Supplementation with phosphorus can also have an effect on tissue copper levels in plants. Addition of 100 µg/g P to a highly saline-sodic soil in most instances decreased accumulation of copper in guayule, an arid-land shrub (Pfeiffer and Bloss,1988). Tomar et al. (1986) note copper and iron deficiency symptoms in wheat, in manure-treated soil, when phosphorus concentrations exceeded 3700 ppm. The use of sewage sludge can increase tissue metal concentrations, especially when sludge metal concentrations are high (e.g. Bell et al., 1988b; Morel et al., 1988b). Treatment of sludge is capable of reducing metal concentrations (Tyagi and Couillard, 1987) to produce a more suitable plant nutrient.

Metal concentrations in native plants are often used as an expression of metal availability and stress. In rooted aquatic plants, copper concentrations can be a result of uptake from the sediments, the water, or both. Barnett et al. (1989) note a general decrease in tissue copper concentrations down the Humber Estuary (U.K.) in the the alga *Fucus vesiculosus*, which they attribute to saltwater dilution of copper inputs by tidal rivers. Increased tissue copper was noted in six sunflower cultivars exposed to excess aluminum (Krizek and Foy, 1988b). Spraying lettuce and bean plants with two pesticides (Methomyl and Pyridaphenthion) reduced tissue copper concentrations (El-Sherif et al., 1986). Lobersli and Steinnes (1988) found elevated levels of tissue copper in four tree species near a copper smelter, decreasing away from the site. Lukaszewski et al. (1988) report a high correlation between soil metal concentration and metal levels in recently produced xylem rings in a species of pine. In a study of growth rate and elemental composition of tree rings in the Great Smoky Mountains National Park, Baes and McLaughlin (1986) report that although there is some indication of anthropogenic input, it is not enough to separate anthropogenic effect from localized effect due to site factors.

Changes can occur in nutrient and metal utilization during plant germination (e.g. Bittencourt et al., 1987; Harmuth-Hoene et al., 1987) as well as growth and fruiting (Evert et al., 1988; Izonfuo and Omuaru, 1988). In boysenberry, fruit development is associated with a net influx of K, Mg, Fe, Cu, Zn, P and S (Monro and Lee, 1987). A similar increase in fruit copper (and other nutrients) has been reported in wine grapes (Navarro et al., 1987). Kermasha et al. (1987a) reports an increase in Fe, B, and Zn but not copper, in development of the Kew cultivar of Indian pineapple. In beech trees, fruiting was not associated with overall tissue increase (Staeva and Petkov, 1986). Gomez Castro et al. (1987), with laudanum (*Cistus ladanifer*), note a decrease in ether extractable P, K, Mg, Cu and Zn with increasing size. A decrease in leaf concentrations of N, P, K, Cu, Mn and Zn, with increasing age has been reported for the groundnut (*Arachis hypogaea*) by Sahrawat et al. (1987). Changes in trace metal uptake and accumulation during development implies differences in tissue concentration in different plant parts (e.g. Frolich and Nyman, 1988).

Metal levels in animals

Tissue metal levels in animals, as in plants, are controlled by the nature of the organism and the concentration and bioavailability of the metal. As well, incorrect methods of metal analysis or data treatment can reduce or negate the accuracy and value of the work (Uthe and Chou, 1988). Variability in tissue metal levels of a species occurs routinely, between sites (e.g. Abdullah and Steffenak, 1988; Borchard, 1986; Ikuta, 1988d; Khrustalev et al., 1988), with age and size (e.g. Ikuta, 1988d; Okuda et al., 1987), and on a seasonal basis. In an examination of variability in tissue copper and zinc levels in several inshore species of invertebrates, Catsiki (1986) comments (page 32) that "The bioaccumulation of copper and zinc ions varied with the species and the stations of study. A wide change was also observed in relation to time." As an excellent example of this, Pavlova (1988), working with cultured mussels (*Mytilus galloprovincialis*), records average (and standard deviation) copper levels of 8.40 (1.30) ppm live weight for May and 3.77 (0.66) ppm for August, for specimens from the same area. (See also Pavlova, 1987.) With sedentary organisms, such as clams and oysters, variation between sites can be a result of anthropogenic effect. Adair (1987) reports higher tissue copper and zinc concentrations in oysters (*Crassostrea virginica*) from heavily used marinas and suggests this to be a result of metal from marine antifouling paints. Uthe and Chou (1986), working with the mussel *Mytilus edulis*, reports an increase in copper with starvation, probably a result of retaining the same burden with decreasing soft tissue weight. It is sometimes difficult to separate anthropogenic effect from the nature of the organism. Phelps and Hetzel (1987) report a copper-zinc ratio of one group of Chesapeake Bay oysters that is twice that of another group. They comment (page 69) that "... this ... cannot be considered indicative of estuarine copper enrichment, as with normal oysters ... . A high copper-zinc ratio may be related to differences in accumulation, storage or excretion mechanisms for the two metals."

Tissue copper levels often vary within the organism, tending to be concentrated in areas of mobilization, transformation or excretion. Ikuta (1986b) notes that in bivalve molluscs that form attachment threads (byssus), there is a tendency for higher copper concentrations in these threads than in the soft body tissues. The hepatopancreas of crustaceans is often copper-rich, as a result of metal storage and mobilization (Hilmy et al., 1988b). Copper concentrations in the arms of a starfish species (*Asterias rubens*) were higher than in the disck (Brugmann and Lange, 1988). Mucus-rich burrows of a crustacean (*Callichirus* sp.) may act as a reservoir for some trace metals, possibly through metal complexation by the mucus (Abu-Hilal et al., 1988). Robinson et al. (1985), working with the clam *Mercenaria mercenaria*, comment (page 83) that "Different metals have different subcellular distributions within the kidneys. For example, Fe and Zn are primarily associated with kidney granules and other subcellular organelles ... (while) Cd and Cu are predominately associated with a soluble metal-binding protein(s) ... ." Rainbow (1988) comments, for decapod crustaceans, that (synopsis) "Any interpretation of the significance of a trace metal concentration in a decapod crustacean requires an appreciation of the metabolic requirements (if any) for that metal and an understanding of the pattern of its accumulation with or without associated detoxification." Copper concentrations in crustaceans may be elevated since many crustaceans use copper in a blood pigment, as well as for other metabolic functions. Since arthropods moult, there can be major changes in the location and use of metals during various phases of the moult cycle. Al-Mohanna and Nott (1985) note accumulation of metals in the hepatopancreas of a shrimp species (*Penaeus semisulcatus*) during the moult. They suggest that this is an indication of the ability of the hepatopancreas to act as a detoxification agent with excess cvopper. Similar results have been obtained for a crab (*Ocypoda macrocera*) by Rao et al., 1986b. Metal accumulation may, however, be more a result of sorption than uptake and accumulation. Krantzberg and Stokes (1988), report evidence for copper adsorption in chironomid larvae and provide some evidence of pH control.

Dark muscle of yellowtail, a saltwater fish (*Seriola quinqueradiata*) are reported to have higher concentrations of copper than in other parts of the body (Date and Yamamoto, 1988). In contrast, Meili and Wills (1985) report copper concentrations to be similar in all tissues of the roach, a freshwater fish (*Rutilus rutilus*). Ishikawa et al. (1987) used a proton microprobe to examine metal concentrations in the vertebra of a flat-fish (*Paralichthys olivaceus*). Concentrations of copper increased toward the inside of the centrum. Ashizawa et al. (1988) used a microprobe to examine changes in elemental concentration during sperm maturation in fowl. They found increasing copper concentrations during maturation, reaching a maximum at ejaculation, and a decrease with *in vitro* storage. Ozawa et al. (1987b) used the technique to examine changes in elemental concentration in the rat sperm head region during maturation in the male genital gract. Copper concentrations were below detection limits in this study.

In comparison with wild pigs, Hecht (1987) found that domestic pigs had higher copper contents in the liver as a result of copper supplementation (wild pigs had highest concentrations in the kidneys). Positive relationships between feed and tissue copper concentrations are found in pigs (Zhang, 1986), cattle (e.g. Cordoba Vital, etc., 1988) and sheep (Kabaija and Smith, 1988a) as well although soil ingestion has been shown to reduce uptake of copper in lambs (Garcia-Bojalil et al., 1988). Part of this may be a result of the protozoan gut infauna which has been shown to decrease the availability of copper in sheep fed a corn silage-soybean meal diet (Ivan, 1988). Metal complexing agents in the food or natural complexing agents in the body can affect metal uptake, tissue copper levels, or biological effect. Dietary fructose decreases the uptake and utilization of copper in rats (Failla et al., 1988; Lewis et al., 1988). Greger and Lyle (1988) note higher liver copper levels in rats fed instant or black tea. Messripur and Haddady (1988) present results suggesting that ascorbic acid reduces the effects of excessive copper deposition in the rat brain hypothalamus. Serum cholesterol levels reportedly parallel serum copper concentrations in rats (Brenner and Koo, 1988; Ohchi et al., 1987). Hormonal and enzyme activities within the organism may also affect uptake and tissue metal levels (Tholey et al., 1988). Mehta et al. (1988) report changes in rat tissue coppper concentrations with estrogen treatment. Serum and brain copper increased as did ceruloplasmin while hepatic copper decreased. Kidney copper levels rose after a short time but later declined.

Copper concentrations may vary on a seasonal basis. With Antarctic krill (*Euphausia superba*), Yamamoto et al. (1987b) found higher concentrations in January to mid-February (summer). Meili and Wills (1985), in early summer, found higher copper concentrations in juvenile than adult roach. Cohen et al. (1987) reports an increase in plasma copper throughout the grazing season in beef cattle. Reproduction also causes a change in tissue copper levels. Eltohamy et al. (1986), for example, reports peak copper levels at mid-pregnancy in the she-camel. Provision of copper to the developing fetus can be at the expense of copper levels in the dam, in cases of starvation. Romeu and Arola (1988) found that, in starved laboratory rats, the total copper content of the conceptus was not affected.

Potrokhov and Vovk (1988) measured concentrations of Mg, Cu, Zn and Fe in the developing embryo and larvae of two plant-eating fish, the grass carp (*Ctenopharngodon idella* and silver carp (*Hypophthapmichthys molitrix*), up to 6 days of age. They report two concentration peaks, in the period when the organs are being formed and in the early larva at age 1-2 days, and two minima, during hatching and before the early larvae start to feed. In foetal sheep, liver copper concentrations have been reported to change little during gestation (Rallis and Papasteriadis, 1987). In buffalo calves (Setia et al., 1987) and in Holstein Friesian calves (de Postiglione, 1986), blood copper levels have been reported to increase after birth. Dubina and Pakrowskaya (1983) report that tissue copper concentration decreases with age in the female rat aorta.

Changes in fish tissue copper concentrations have been related to size (Windom et al., 1987) although any relationship should take life history, food habits and metal bioavailability into account. Seasonal and seral differences in nutrient and metal concentration in deer forage has been evaluated for nutrient and trace metal availability (Van Horne et al., 1988). Uptake varies with food type in animals, with seasonal changes accounting for both metal availability and concentration in food (e.g. Evans et al., 1987). In grazing animals such as sheep and cattle, copper deficiency can occur as a result of excess sulfur or molybdenum, even though there is an adequate supply of forage copper (Gooneratne et al., 1987b; Phillippo et al., 1987a; Wittenberg and Devlin, 1987). In rabbits, excess oral lead intake reportedly inhibits intestinal absorption (El-Waseef, 1987) and mobilization (Sinkina et al., 1987) of copper. Excess cadmium has been found to elevate plasma copper in monkeys (in Nomiyama et al., 1987). In weanling rats, activity of a copper-containing enzyme (superoxide dismutase) was reduced by ingestion of moderate or excess zinc but restored by supplemental copper (Standal et al., 1988). In all of these studies, however, it is important to relate variability in the organism to strain differences (e.g. Nederbragt and van Zutphen, 1987) and to the sample size used to examine the factor (Tanner et al., 1988).

Specimens near sources of anthropogenic metal often have higher concentrations than those that live away from the sources. Heliovaara and Vaisanen (1987) report elevated copper concentrations in two biennial pine insects found near a factory complex, and reduced (normal) concentrations away from the complex. In the pelage of red squirrels living near ore smelters at Sudbury, Ontario, Canada, Lepage and Parker (1988) found relative increases of nickel, copper and iron that coincided with atmospheric loading ratios reported for the smelters. With urban environments, Ikemi et al. (1987) note that long-term exposure of rats did not provide any clear-cut indication of metal uptake. Concentrations of tissue metals have generally been found higher in fish in lakes with lower pH (Haines et al., 1987) although the story is complex, involving sediment chemistry, metal concentrations, and water chemistry.

Metal levels in humans

In a discussion on "The assessment of zinc and copper nutritional status in man", Milne (1987) points out that we do not have an adequate method of assessment even though the requirement for copper are well known and there have been numerous recent technological advances in analytical equipment. Human serum contains approximately 1 µg copper per ml, most of which is associated with ceruloplasmin, the rest with a wide variety of organics (Sarkar, 1988). The involvement of these copper-containing organics with the body allow normal metabolism to occur. Some of the recent and past technological advances have improved our ability to examine the organics and the effects of their involvement. Scanning electron microscopy and energy dispersive X-ray fluorescence have, for example, been used to examine the shape and chemical nature of particles in lungs (Vanoeteren et al., 1985). Particle-induced X-ray emission (PIXE) has been used to measure the concentrations of trace elements (including copper) in body tissues such as portions of the brain (Duflou et al., 1987) or glandular tissue. Thorlacius-Ussing et al. (1988), for example, used PIXE to obtain evidence of age-related differences in copper concentrations in the anterior pituitary gland. The problem with these values, obtained with equipment that permits closer scrutiny of tissue metal levels, is that background data is inadequate to evaluate the data now becoming available. As Iyengar and Woittiez (1988) comment (page 474), "Well-founded reference values or baseline data for trace elements in clinical specimens are needed if one is to interpret results generated in clinical chemistry laboratories." Variations in tissue copper concentrations need to be evaluated in terms of tissue origin (e.g. Kosugi et al., 1986) as well as the role of the metal in the tissue.

In an evaluation of copper and zinc requirements for extremely low birthweight infants, Halliday and McMaster (1988) suggest that 0.3 µmol copper/kg body weight daily is adequate for intravenously-fed individuals. This is more than the approximately 0.16 µmol currently recommended for infants receiving total parental nutrition (e.g. Shulman, 1987). Plasma copper concentrations in infants is characteristically lower than in adults (Jendryczko and Drozdz, 1986) although an adequate copper supply is essential for body maintenance and growth (e.g. Brown, 1988b). Concentrations do, however, increase during early life (Lockitch et al., 1988), sometimes dramatically. Tisue metal concentrations are variable, however, reducing the value of a general statement on requirements. Hair zinc concentration has, for example been associated with hair colour in boys but not in girls, either for zinc or copper (Laitinen et al., 1988). McKenzie-Parnell and Thomson (1987) did not find a relationship between serum copper and height, weight, body mass index, socioeconomic status or iron status in 11-year-old children from Dunedin, New Zealand. Nasolodin and Suvorov (1987), however, in healthy young people, found a direct positive correlation between concentrations of iron and copper in blood corpuscles and the lysozyme and serum complement titres, indicating the dependence of immune resistance on iron and copper metabolism. In healthy adult males (19-54 years), Krebs et al. (1988) found no change in copper balance as a result of extended bed rest while they did find an increase in urinary and fecal zinc loss. Plasma copper levels in obese black females were significantly higher than in non-obese black females although both were within the normal range (Moak et al., 1988).

With exercise, Aruoma et al. (1988) report effects on plasma metal concentrations (Fe, Cu, Zn) varied from subject to subject although there was some evidence of raised copper in the plasma of athletes. Tiedt et al. (1988a) note differences between male and female athletes in serum copper concentrations. Lukaski et al. (1988) report increases in a copper-dependent enzyme (superoxide dismutase) in competitive swimmers which they comment (abstract) indicates "... a unique biochemical adaptation of Cu metabolism to swim training." They, and others (Gladkikh, 1985; Naslodin et al., 1983; Weight et al., 1988) provide evidence that copper supplementation is not necessary, that normal or even slightly less than normal copper intake is adequate for metabolic processes during heavy physical training associated with athletic competition. Gladkikh (1985), however, comments that the higher blood levels of trace elements, combined with any inadequate dietary intake, could produce a trace element deficiency, especially in younger athletes. This has been demonstrated in intensively trained dogs, especially with iron although to some extent with copper (Rusin and Vorobev, 1985).

Kant et al. (1988) report similar copper intakes and profiles and Turnlund et al. (1988) similar copper absorption, for both young and elderly men. Bunker et al. (1987) review zinc, copper, manganese and chromium trace element nutrition in the elderly, commenting (page 118) that "There is no evidence to suggest that the elderly are particularly at risk of consuming suboptimal amounts of copper ... ." However Johnston (1987) reports that (abstract of Ph.D. thesis) "Copper intake was significantly lower in women over 80 years of age compared to younger women. Superoxide dismutase activity was significantly lower in women over 80 years of age." As well, Thomas et al. (1988a) report that copper intake was low in a group of elderly inpatients, in comparison with official recommended levels of intake in healthy elderly people. Bunker et al. (1987) point out that disturbances of copper metabolism in the elderly are often associated with diseases of old age (neoplastic, rheumatic and arthritic diseases).

The requirements for vitamins and minerals during pregnancy, to provide an adequate supply to both the mother and the fetus, are complex (e.g. Allen, 1986; Ballabriga, 1988; Nowacka et al., 1987). Serum copper concentrations incrase significantly during pregnancy and decrease during the first postpartal month as well as between the first and sixth months of lactation (Anttila et al., 1988; Simms, 1988). Most copper accumulated by the fetus occurs during the last trimester of pregnancy. Thus, (page 310 in Kirksey and Rahmanifar, 1988) if the preterm infant "... is not provided adequate Cu in a bioavailable form during early postnatal life, Cu deficiency develops." An adequate supply of copper must also be present in breast milk or metal supplementation is necessary to prevent symptoms of deficiency in the newborn. Copper concentrations in human milk are presented in the tables at the end of this report; reuirements are discussed by Burguera et al. (1988), De Maeyer (1988), Howell et al., 1986, Schramel et al. (1988). Howell et al. (1986) comments that there is no obvious change in milk copper levels after birth, in contrast to the statements in Anttila et al. (1988) and Burguera et al. (1988) which indicate that there is a change.

Copper levels in humans are affected by a number of factors. Uptake, for example, is affected by food phytates and fiber (e.g. Moak et al., 1987) while mobilization is controlled by metal complexing agents such as ascorbic acid (Held et al., 1988). The use of oral contraceptives has been associated with elevated blood copper concentrations (Liukko et al., 1988). Some factors, such as alcohol however, do not appear to affect blood or tissue copper levels (e.g. Louis-Charles and Frimpong, 1988). Elevated oral zinc uptake can reduce plasma copper concentrations in females (but apparently not males; Samman and Roberts, 1988)

Tissue copper levels can be affected by exposure to high copper levels in the workplace (e.g. Aono and Araki, 1988; Linscheid, 1985) although the levels of copper appar relatively stable. Copper levels in hair of people living near a copper mine in New Guinea do not appear to be as elevated as concentrations of iron and cadmium (Jones et al., 1987a).

I.3.2 METAL LEVELS IN ABNORMAL TISSUES

In plants, the interaction of copper with organics such as plastocyanin (e.g. Moore et al., 1988a) can be affected in mutants (Roshchina et al., 1987), producing a change in the concentration of the organometallic compound (Roshchina et al., 1988), a shift in the composition of metal-containing organics, and a change in the distribution of copper within the organism. Similar changes can occur as a result of physiological disturbances or exposure to high levels of biologically available copper. High concentrations of copper have been reported for chlorotic leaves in two varieties of peach (Potalia et al., 1986). Burns and Parker (1988) report high metal burdens in two species of fiddlehead ferns growing near the ore smelters at Sudbury, Canada. Fungal infections are known to enhance nutrient uptake and affect metal uptake in plants like the soybean (Pacovsky and Fuller, 1988) and sorghum (Raju et al., 1987). This relationship is proving to be of immense benefit in utilizing marginal land. Ames and Bethlenfalvay (1987) comment (page 1313) that "The involvement of mycorrhizal fungi in plant growth and nutrition is among the most exciting and challenging areas of agricultural research today: It is exciting because of the number of directions from which one can approach it; and it is challenging because of its potential for maximizing fertilizer use efficiency."

In animals, deficient diets (e.g. Coppen and Davies, 1988; Heng et al., 1987) and genetic and physiological abnormalities can also affect tissue metal concentrations as well as metal uptake, storage and mobilization. Rowlands (1986) describes an unsuccessful attempt to use blood profiles (including copper) of Friesian bulls to indicate potential for siring animals with good growth rate and milk production. Higher concentrations of copper have been reported for infertile than for fertile hens eggs (Szymkiewicz and Niemiec, 1988). Metal concentrations have been measured in commercial animals in an attempt to determine the effects (e.g. Frangenberg, 1986) and causes (e.g. Sawadogo et al., 1988) of disease. Doster et al. (1986) reports high (toxic) amounts of copper and low (deficient) selenium values in kidneys of a number of stillborn and neonate bovines dying within 24 hours of birth. Nutritional haemoglobinuria is responsible for high mortality in female buffaloes and is often associated with a marked decrease in serum copper and inorganic phosphate (Pandey and Misra, 1987). Metal-metal and metal-metalloid imbalances can result from improper nutrition, genetic and environmental mineral imbalance as well as physiological abnormalities. These can have important physiological effects as well as affect tissue copper concentrations in a wide range of organisms (Bires, 1987; Chen et al., 1987b; Dinkova et al., 1987; Elfant and Keen, 1987; El-Waseef and El-Naggar, 1986; Gruen et al., 1986; Kunifuji et al., 1987; Liang et al., 1988; Srivastava et al., 1988; Tiffany-Castiglioni et al., 1987; Tulasi and Rao, 1988).

Although secular changes in human tissue metal concentrations have been recorded, Yoshimura (1987) did not find this with copper in forensic autopsy materials. Genetic abnormalities of copper metabolism occur in humans in diseases such as hepatolenticular degeneration (HLD; Li et al., 1988) and the more commonly known Wilson's disease. The latter is an inherited disorder affecting somewhere between 1 in 50,000 to 1 in 100,000 live births. Garnica et al. (1985) and Marsden (1987) provide reviews of the disease and its expressions. Accumulation of very high concentrations of copper occur in the liver and other storage tissues with reduced concentrations elsewhere, indicating an inability to adequately mobilize and transport copper. Other symptoms of the disease include acute haemolysis (Lehr et al., 1988). Other diseases similar to Wilson's disease have also been reported but without the same diagnostic characteristics (e.g. Heckmann and Saffer, 1988; Ono and Kurisaki, 1988). Indian childhood cirrhosis is another abnormality characterized by high concentrations of liver copper and hair copper (Patel et al., 1988) which may be a result of early exposure to high concentrations of copper (Bhave et al., 1987). (This will be discussed later in the review.) Menkes disease is a third inherited condition of improper copper metabolism. The condition is characterized by increased copper accumulation in several cell types and can often be identified before birth (Tonnesen et al., 1987). Menkes disease is characterized by an inability to properly mobilize copper with a resultant deficiency in the developing brain. This characteristic is also found in quaking mice, an animal model of the disease used for laboratory studies of Menkes disease (Cloëz and Bourre, 1987). Shiraishi et al. (1987, 1988b) discuss copper and metallothionein-copper levels in the "macular mouse", another model of the disease.

In an examination of copper and very low birthweight babies, Soo et al. (1988) found serum copper concentrations high in those weighing less than 1000 g and critically ill or receiving intravenous nutrition. Levels were normal in those low birthweight babies with bone disease, neutropenia or oedema. They point out the necessity of maintaining adequate nutrient copper for the very low birthweight baby. Matthew et al. (1988) also report elevated meconium (first fecal discharge after birth) copper levels in preterm infants suggesting an early loss of metal and the need for appropriate supplementation. Friel et al. (1988) found an increase in serum copper with a commercial trace element supplement, given to very low birthweight preterm infants although the general effect on trace metal balance was judged unsatisfactory. Koo and Hambidge (1988) measured copper concentrations in very low birthweight babies with rickets and fractures, for a year after birth. Compared to healthy term infants, copper levels were much lower at 3 months of age but had increased to near normal levels by 12 months. This is important because copper plays an important role in the development of connective tissue and bone.

Although the evidence is not conclusive (e.g. Vega-Franco et al., 1987), children with malnutrition frequently have low plasma copper levels as do those with asthma (Akinkugbe and Ette, 1987). The latter authors, as well as Adedeji et al. (1988) also report a relationship between the occurrence of sickle cell disease and serum zinc and copper concentrations; they point out the importance of maintaining proper nutrient metal levels either in food or through supplementation. Acute diarrhea in infants and children has been associated with a fecal loss of trace elements, including copper, (Ruz and Solomons, 1987) with a resultant reduction in plasma copper (Castillo-Duran et al., 1988). Low serum copper levels are also reported for humans with intestinal parasites (El Hawy et al., 1988; Shield et al., 1986) as they are for other animals (e.g. Giraldo and Southern, 1988; Watkins et al., 1988). Gabrashanska et al. (1987), however, note that the concentrations of Cu, Co, Fe and Mo in a nematode parasite (*Ascaridia galli*) are higher than for the chick host.

Tissue copper levels frequently change with age, both dependent and independent of physiological status (Tsui, 1986). Abnormal metal levels have been associated with a wide variety of human disorders ranging from skin diseases to the effects of anthropogenic materials (e.g. Galietti et al., 1988; Shinmura et al., 1986). Zou et al. (1986) report low zinc and normal copper levels in blood and hair of obese patients. Deng et al. (1986) found some evidence (not significant) of lower copper levels in hair and higher levels in blood of obese patients when compared to normal patients. (Niiro (1987) reports low hair copper levels in copper-deficient rats.) In an examination of aldrenalectomized genetically obese mice, Prohaska et al. (1988b) report liver and kidney copper-zinc superoxide dismutase activity to be lower than in lean littermates, 30 and 20% respectively. The activity is suggested to be due to the hyperphagia associated with the genetic mutation. Increased plasma, urinary and erythrocyte copper levels have been reported for patients with hyperthyroidism (Nishi et al., 1987, 1988). Raz et al. (1988) point out that a deficiency of certain trace metals in the diet can produce a diabetes-like condition. Teraki and Maemura (1988) found abnormal metal metabolism in rats with chemically-induced diabetes, with increased levels of urinary copper as one expression of the condition. Working with the same type of rat, Tengrup et al. (1988) found that zinc and copper were accumulated in the kidney of rats with uncontrolled, insulin dependent diabetes mellitus. Increased kidney copper levels is also a characteristic of diabetic rats (Oster et al., 1988; Uriu-Hare et al., 1988); the latter authors also report increased liver and kidney metallothionein in diabetic rats. They suggest that the change in metal metabolism is a result of the hormonal status rather than increased metal uptake. In an analysis of metals in blood sera and nails of patients with diabetes, Okazaki et al. (1985) report copper levels that were statistically lower than in normal patients. In contrast, D'Ocon et al. (1987) found higher serum copper levels in diabetics. They also found a positive correlation between zinc and copper levels and comment (abstract) that "... zinc and copper levels seem to be directly correlated and in diabetic patients these levels are increased mainly in the case of obesity." Kofinis et al. (1987) and Schlienger et al. (1988) report an increase in plasma copper concentrations in patients with diabetes. Plasma copper levels in humans with cataracts have been reported to be lower than those in patients without cataracts (Bhat, 1988). In contrast, Broglia et al. (1987) found no change in blood copper concentration in patients with optical neuritis. In a review of copper status in thermal injury (Anonymous, 1987b) it is pointed out that there is a depression of serum levels of both copper and ceruloplasmin while urinary excretion of copper is elevated. Skin diseases that are inheritable (e.g. epidermolysis bullosa) may be associated with elevated plasma copper levels (Cunnane et al., 1987). Psoriasis is often associated with elevated plasma copper levels (e.g. Furfaro et al., 1987); Donadini et al. (1987) note a reduction of plasma copper levels in psoriasis patients treated with a chemical (PUVA). Increased serum copper levels are reported for patients with leprosy (Narang et al., 1988; Rao and Saha, 1986).

Patients with kidney failure frequently undergo dialysis treatments. Long term dialysis patients often exhibit abnormal serum copper levels as a result of metal loss (deficiency) through dialysis or metal gain (excess) from copper in the dialyzer membranes and the use of copper tubing in the dialysis machine (e.g. Chen et al., 1986c; Parra and Romero, 1987). Chen et al. (1986c) however, found (abstract) "... no significant change of copper status in our nondialyzed and dialyzed uremic patients" although the concentrations in the priming normal saline were initially elevated, dropping to very low levels at the end of priming. Ohnishi (1986), however, reports elevated serum copper levels in patients with long-term hemodialysis. In an examination of plasma copper levels in patients - 1) on hemodialysis, 2) on peritoneal dialysis, and 3) uremic patients not yet on dialysis - Sondheimer et al. (1988) found that (abstract) "Cu is elevated in uremia regardless of dialysis status and this elevation is not accounted for by an increase in plasma ceruloplasmin." (Ito and Ito, 1987, found elevated kidney copper levels in adriamycin-induced nephrotic rats.) Metal complexing agents have been used in an attempt to maintain appropriate serum metal levels in dialysis patients (e.g. Gunther et al., 1986) although more for aluminum than copper. However, their use in any situation can have side effects such as metal imbalances (Frackelton and Cranton, 1986) and retinal abnormalities (De Virgiliis et al., 1988). In an examination of trace element status in patients undergoing plasma exchange, Makela et al. (1986) found an initial decrease and then a return to normal during the next 12 hours.

Plantin et al. (1987) comment that the central nervous system should be sensitive to disturbances in trace element concentrations because of its high metabolic rate and low capacity for regeneration.

This can be directly or indirectly translated into changes in copper in other parts of the body. Serum concentrations can change as a result of mental stress or even between awareness and sleep (Reinhardt et al., 1986). Serum copper levels of children with Down syndrome are higher than levels in normal children (Anneren and Gebre-Medhin, 1987). Copper-zinc superoxide dismutase activity in patients with Down syndrome may (Portsmann et al., 1988) or may not (Jeziorowska et al., 1988) be elevated. Zhai et al. (1986) found no significant difference in serum copper levels between normal and schizophrenic patients although Pfeiffer and Mailloux (1987) suggest that this may occur. Significant differences in hair copper have been reported for epileptics (Shrestha and Oswaldo, 1987). The use of anticonvulsant drugs such as phenytoin can, however, have an effect on copper metabolism (Palm et al., 1986).

Corrigan et al. (1987) reports that (page 142) serum "copper levels differentiate untreated depressives from treated depressives and recovered manics, as well as from schizophrenics and alcoholics but not from controls." Serum levels can also differentiate patients with various neurological disorders although, interestingly, copper levels in the cerebrospinal fluid apparently do not (Bourrier-Guerin et al., 1985). However, Kapaki et al. (1988) notes an increase in cerebrospinal fluid copper levels in patients with increased cerebrospinal fluid protein levels. However, they suggest that is is prbably a result of a breakdown in the blood-brain barrier, allowing entry to metal-containing proteins or amino acids. Brazdes et al. (1986) found a "... statistically significant decrease in Cu ($p<0.01$) and K ($p<0.05$) concentrations in (the brain tissue of) Alzheimer's senile dementia." Rheumatoid patients normally exhibit elevated plasma copper levels which Moretti et al. (1988) suggest is attributable to a parallel increase of ceruloplasmin.

Serum copper levels have been correlated with alcohol intake. Karkkainen et al. (1988) state (abstract) that "Among males (and females) alcohol intake per drinking day correlated positively with serum copper ($r = 0.50$; $P<0.001$) and ...". Mussalo-Rauhamaa et al. (1987) found a somewhat higher serum copper level in drunken arrestees than in controls although the difference was not appreciable. Conri et al. (1988a,b) report lower average serum copper levels in heavy drinkers than in controls although the difference is not significant. Navarro Rodriguez et al. (1986) obtained similar results, with serum copper decreasing with increasing liver disease. The latter authors also report elevated urinary copper values indicating a loss of metal through excretory processes. (Neiko, 1985, comments that in chronically persisting hepatitis and liver cirrhosis one is more likely to observe hypercupremia which is in contrast to the observation by Navarro Rodriguez et al., 1986.) Red cell Cu-Zn superoxide dismutase activity has also been reported to be lower in alcoholics than in controls (Emerit et al., 1988). Sulsky et al. (1988) examined the effect of smoking on biochemical markers of nutritional state. They report higher blood copper levels in smokers than in non smokers.

In chemical-induced chronic hepatitis, rats exhibited increased copper levels in serum, liver, kidney and nails (Okazaki et al., 1986; see also Wakiyama, 1987). However, there appears to be variability in tissue copper with different liver problems and treatments for them (e.g. Abdel-Maguid et al., 1986; Andres et al., 1987). As indicated earlier, high liver copper values are found in individuals with Wilson's disease; they also occur in dogs with hepatitis (Thornburg et al., 1984) and in humans with diseases such as extrahepatic biliary atresia (Bayliss et al., 1988). The use of metal complexing agents such as tetramine has been advocated to reduce liver copper levels in dogs (Allen et al., 1987; Twedt et al., 1988).

Disease and irregular cell growth - tumours, cancerous tissue of all kinds - are associated with changes in tissue copper levels and the ratios of various copper-containing compounds (Ciuti et al., 1987; Crawford et al., 1988; Czernik et al., 1987; Drake and Howard, 1988; Ebadi and Swanson, 1988; Grant, 1987; Guigui et al., 1988; Hansen et al., 1988; Kelly et al., 1987; Liu et al., 1987b; Porciani et al., 1987; Romeu et al., 1986; Sun et al., 1988a,b). (See also Nistor et al., 1987 for a computer visual representation scheme for Cu, Zn, Fe variations in cancer.) In a paper on "Serum copper and zinc and the risk of death from cancer and cardiovascular disease", Kok et al. (1988) found excess mortality in subjects with low copper status and an adjusted risk of death from cancer and cardiovascular disease in subjects with excessively high serum copper. However, the relationships between illness, death and metal status are not as meaningful as desired. Ascite-sarcoma cells have been shown to affect the concentration of cell zinc and copper as well as the type of metallothionein (a strong metal-chelating agent) present (Hau and Hsu, 1987; Kobayashi and Sayato-Suzuki, 1988; Rodriguez Velez and Rosiles Martinez, 1988). Part of the change in tissue metal levels can be associated with tumour-promoting agents (Csermely et al., 1987) as well as the effect of the irregular growth itself. Treatment of cancer with chemicals as well as radiation can, likewise, affect tissue metal levels. Slavik et al. (1988) reports significant increases in rabbit serum copper levels with the use of the anticancer drug procarbazine.

Patients with chronic lymphocytic leukemia have been reported to have elevated serum copper concentrations (Beguin et al., 1986, 1987), a condition which becomes more apparent as the disease progresses. In an examination of the effect of low metal (Fe, Cu, Zn) concentrations on leukemic cell differentiation, Carpentieri and Thorpe (1987) suggest "... that regulation of intracellular levels of the metals may induce some differentiation of leukemic cells". Elevated serum copper levels have also been reported for patients with head and neck cancer (Shehin and Zemel, 1988) and cancer of the larynx (Sengas et al., 1987). In contrast to the normal serum copper increase found in cancer patients is the report by Shen et al. (1987b) of reduced lung tissue copper levels in autopsy tissue of miners with coal miners' lung carcinoma. Reduced blood copper levels are also reported for the early stages of stomach cancer although they increase during the development of the disease (in Saito et al., 1987). Elevated serum copper is also reported for patients with gastrointestinal tract cancer (Narang et al., 1987) and elevated serum and erythrocyte copper in patients with rectal cancer (Collecchi et al., 1987). Breast cancer is another disease (or group of diseases) that is associated with elevated serum copper levels (e.g. Nistor et al., 1986). Wong and Chang (1987) examined plasma levels of copper and zinc in patients with cervical neoplasia, reporting no difference in copper levels from controls although zinc levels were lower. Some, but not all, ovarian carcinoma patients are been reported to have elevated serum copper concentrations but the changes in levels do not correlate with the development of the disease (Jacobs et al., 1988).

Dietary cholesterol can produce atherosclerosis but, in so doing, causes a decrease in liver copper in rats and rabbits (Klevay, 1988a,b). Klevay (1988a) comments (abstract) that "Results are consonant with the theoretical implication of copper metabolism and copper deficiency in the etiology and pathogenesis of ischemic heart disease." (Decreased copper is also reported to be related to cholesterol levels in humans; Costello et al., 1988.) Hypertension is associated with an increase in plasma copper in rats (LeBlondel and Allain, 1988a) while some (not all) of the drugs used to treat the disease also cause decreased plasma copper levels (McLean et al., 1985; Peters et al., 1988a; see also Verho et al., 1987). Vivoli et al. (1987) report urinary concentrations of copper in hypertensive patients to be significantly higher than in normotensives suggesting the excretion of body copper as a possible result of hypertension. However, Vardanian and Chukhadjian (1986) report a "significant" increase in copper levels in the vascular walls of hypertensive patients with atherosclerosis. Decreased hair copper levels have been used as an indication of cardiovascular risk (Liang et al., 1986; Smith, 1987a) as has a variety of other factors (L'Abbe et al., 1988). Like hypertensives, plasma copper levels are often higher in heart disease patients (Li and Nan, 1986). However, serum copper levels are reportedly lower in patients with acute myocardial infarction, at least in the first 72 hours after hospital admission (Bakos et al., 1988). Penttila et al. (1986) report a significantly lower tissue copper levels in the right atrial appendage of humans with ischemic heart disease. The tissue was obtained during open heart surgery which has an effect on serum copper levels (Zamparelli et al., 1986).

Tissue copper levels can be affected by chemicals, especially drugs. Some pesticides such as organophosphorous esters have been suggested to affect trace metal status in animals although Lotti et al. (1988) conclude that copper homeostasis is not affected and is not involved in the pathogenesis of polyneuropathy, a side effect of the pesticides. Injections of endogenous pyrogen, prostaglandin and arachidonic acid have been used to examine acute-phase response in rabbbits. Morimoto et al. (1988a) report that injection of endogenous pyrogen increased plasma copper concentrations. Morimoto et al. (1987a), however, found increased plasma copper concentrations as a result of restraint stress which must be taken into account in the interpretation of results with the use of endogenous pyrogen. Pentylenetetrazol-induced convulsions in rabbits caused an increase in copper levels in cerebrospinal fluid (Shirasu, 1987).

Copper is involved in inflammatory diseases and, as a result, tissue concentrations can be affected in diseases such as arthritis (e.g. Kishore, 1988b; Neve et al., 1988). The breakdown of cell membrane permeability during inflammation allows penetration of plasma proteins such as copper-containing ceruloplasmin. This is responsible for an increase in copper levels in inflamed tissue (e.g. McGahan and Fleisher, 1986; see also McGahan et al., 1988); plasma levels also increase as a result of inflammation (Marrella et al., 1988; Milanino et al., 1988a,b). Laboratory inflamation is produced by endotoxin; repeated injections of endotoxin or of immunogenic agents can produce somewhat different animal responses than a single injection (e.g. Laurin and Klasing, 1987). Since inflammation is a result of tissue insult, elevated copper concentrations are often found as a result of injury (e.g. Ganey et al., 1987; Ward et al., 1988b). Surgery can, for example, be associated with increased serum copper although the nature of the surgery and the medication will dictate both the nature and extent of the change in tissue copper (e.g. LeBlondel and Allain, 1988a,b; Ochs et al., 1988; Turk et al., 1988).

I.3.3 COPPER AND THE RESPONSE OF THE ORGANISM

The response of the organism to copper is dependent on metal speciation, the requirements of the organism and the physiological state of the organism. Speciation controls the availability of copper to organisms. Organism requirements dictate what levels of biologically available metal are required or are excessive. Physiological state of the organism tends to act as a modifier of response, deficient or excess levels of metal being less damaging to a healthy than a sick organism (e.g. Danks, 1988). It is important to consider these factors when applying generalized information such as that in the U.S. Environmental Protection Agency Pesticide Fact Sheet Number 87: Copper Sulfate. It is also important to keep in mind that anthropogenic input of metals into the air, water and soils is now high enough to affect trace metal cycles and trace elements in food, on a world-wide scale (Nriagu and Pacyna, 1988).

The response of microorganisms to copper can be both beneficial and detrimental to man. Reduced microbial activity resulting from excess metal can, for example, inhibit biological production of gas in the breakdown of municipal sewage sludges by thermophilic bacteria. Macleod and Forster (1988) report that of the metals tested, toxicity was in the order Pb > Cu ~ Ni > Cd > Zn. Similarly, Chiesa et al. (1987) report that copper inhibits microorganism removal of phosphorus from sewage sludges. Interactions of copper with organic components in the sludge will affect the availability of sludge metals just as they do in other systems. Vasseur et al. (1988b) report increased toxicity of copper to a bacterium and a protozoan when introduced as a copper-pesticide (Zineb, Maneb, Carbaryl) than when introduced by itself.

The beneficial effects of using excess copper as a microorganism control agent have been discussed earlier. They range from the control of ice-nucleating bacteria that increase plant frost damage (Obata et al., 1988) to the inhibition of *Legionella* by copper in plumbing systems (Habicht and Muller, 1988) or from other sources (e.g. Yahya et al., 1988). In a general sense, control is exerted primarily by reduced growth and death of the organism. Jana and Bhattacharya (1988) note effects on growth of the fecal coliform bacterium *Escherichia coli*, by several heavy metals, in the order Cd > Pb > Cu > As > Hg > Cr. However, the response to copper or copper-containing agents, is not always the same, many microorganisms exhibit a tendency towards resistance with repeated exposure (e.g. Kuzovnikova et al., 1987; Shaw et al., 1987). Cotter et al. (1987) report that copper resistance in a strain of *Escherichia coli* was due to decreased cupric ion uptake, a result of a plasmid-encoded characteristic of the strain. Metal speciation also affects growth rate. Sato et al. (1986) found a decrease of growth rate in a microorganism (*Nitrosomonas europaea*) that correlated with the logarithmic activities of Cu(II)-amine species regardless of the total cupric ion activity in the medium. Tumanov et al. (1983) note the dependence of antimicrobial activity of copper with alpha-amino acids. Cystein-rich polypeptides as well as a number of proteins can be produced by organisms as metal complexing agents, agents that often function in metal transport but can also reduce toxicity (e.g. Mehra et al., 1988; Thrower et al., 1988).

As a result of its use as a controlling agent, excess copper can be released into the environment (e.g. Santos and Medeiros, 1981). Here, the effects of metal bioavailability can be expressed, along with other factors, on the species composition and chemical makeup of bacteria and fungi in soils and water. Booth et al. (1987) report seasonal changes in lake bacterial populations that relate to phytoplankton declines but they note changes in cation levels (including copper) that appear to relate more to lake chemistry and hydrography than nutrient availability. Leppard and Rao (1988) relate ultrastructure and physiology to copper and pH stress in lake bacteria. Metal concentrations may affect microbial populations in aquatic sediments (e.g. Chen et al., 1986a) although the relationship will be affected by metal speciation. Wilke (1987) notes long-term inhibition of microbial activity by copper and zinc in a humic, loamy sand, an inhibition which is reportedly due to the high mobility of the metals in the sand.

As with bacteria, the response of fungal microorganisms to copper is a result of the metal availability and organism nature. Sharma et al. (1985) found moderate levels of copper to improve the metabolism of fungi that enhance forest tree growth. However, high levels of copper were not beneficial, a feature which has been reported to decrease the growth of fungi (e.g. Somashekar and Sreenath, 1988). El-Sharouny et al. (1988) note a decrease in population levels and change in species composition of soil mycoflora after treatment with high levels of metal (Hg, Zn, Pb, Cu, Ni, Cd). In an interesting discussion, Casella et al. (1988d) point out that a fungal species can circumvent exposure to heavy metal effects by entering plant roots, presumably using the plant either as a barrier to metal entry or its metabolism to buffer metal effects. The metal-produced decline in microorganism populations is a result of a number of factors. In the yeast *Saccharomyces cerevisiae*, Greco (1987) found evidence suggesting that molecular oxygen may contribute to the deleterious effects associated with excess copper. Changes in cell membrane permeability have also been related to excess copper (Ohsumi et al., 1988). Leakage can be induced by high concentrations and prevented by low concentrations (Bashford et al., 1988). Copper may increase the rate of "ageing" in fungi (and other organisms), as evidenced by the concentration of age pigments (Cuomo et al., 1987). Maturation of fungus reproductive structures can be inhibited by copper (Marinkovic and Karadzic, 1987). Copper-containing fungicides (e.g. Cynkomiedzian and Miedzian) have been suggested to cause chromosomal aberrations (Osiecka, 1987) and may act as mutagenic agents. Copper, by itself may also do this at high concentrations (e.g. Wong, 1988).

The response of freshwater algae to excess copper is discussed in a general review by Munawar et al. (1988). They point out that the use of algae as bioindicators of natural conditions and as laboratory bioassay organisms is a result of their metal sensitivity. Sarosiek et al. (1987) used the liverwort *Ricciocarpus natans* as a bioindicator of excess metal, commenting that (abstract) "... under the influence of Cu thalli deform and reduced rhizoids develop, ...". Species composition of algae in freshwater environments can be used as an indication of metal bioavailability in combination with other environmental factors. Fukushima et al. (1988) note that the number of species decreased with increasing copper, in rivers with high levels of metal. In examining the response of three species of diatoms to copper in simulation experiments, Wu et al. (1987b) note that growth was stimulated at concentrations of approximately 10 ppb Cu and inhibited above that concentration. Crossey and La Point (1988) compared community structural and functional responses of periphyton (organisms attached to projections from the bottom) to heavy metals (Cd, Cu, Ni, Zn) in a natural system. Although they note that variability may limit the general use of community function measures, production and respiration can be useful in providing an indication of functional response to excess metal. Wangberg and Blanck (1988) comment on the variable response of different algae to chemicals (including copper sulfate) and conclude that the phylogeny (ancestral relationships) of the algae is toxicologically relevant. In other words, the response to chemicals tends to vary between different groups of algae.

The effect of excess copper on algae can vary. Several recent studies demonstrate an effect on one or more biochemical processes in photosynthesis (Dmirtrieva, 1985; Grabowski and Leszczynske, 1986; Gupta, 1988; Kosakowska et al., 1988; Samson et al., 1988). In a motile phytoplankter, *Chlamydomonas reinhardtii*, Bean et al. (1987) found that 7-100 μM Cu^{2+} altered the ability to respond normally to photic stimuli, swimming speeds diminished. Ladogina and Osokina (1987) report that, with the bluegreen alga *Synechocystis aquatilis*, 0.2 mg/L copper reduced the amount of change in medium pH normally produced by exposure to light. Copper has also been shown to affect the membrane potential of leaf cells in *Elodea*, altering ion transport (Novak and Ivankina, 1987). Karaush et al. (1988) found changes in cell size with 0.1 and 0.3 mg Cu/L added to the growth medium of the green alga *Scenedesmus quadricauda*. An increase in sinking rate was recorded for *Scenedesmus acuminatus* with added copper (Pekkala and Koopman, 1987). In a Ph.D. dissertation, Alexander (1987) reports copper causes a loss of colour and an inhibition of reproduction and growth in *Enteromorpha intestinalis*. However, Blanco (1987) reports an increase in cyst production with increasing copper, in the flagellate *Scrippsiella trochoidea*. In a microcosm containing the herbivorous crustacean *Daphnia magna*, Halcy et al. (1988) report an increase in green algae with increasing brass dust innoculation. The increase was suggested to be a result of the reduction in grazing due to loss of *Daphnia*. Bluegreen fixation of nitrogen is affected by excess copper (Rueter and Petersen, 1987).

A number of plant responses to various types of stress are discussed in "Cellular and Molecular Biology of Plant Stress", edited by Key and Kosuge (1985). Especially in agriculture, the influence of copper in soils is important, in terms of plant deficiency and toxicity. The influence, and the plant response, varies as a result of soil type as well as plant requirements (e.g. Berdnikova et al., 1986) and plant condition (e.g. Wenny et al., 1988). Varietal-specific inluences of herbicides, on metal (Cu, Fe, Zn) concentrations in wheat have been reported by Kostowska et al. (1986). Aerosol copper can form an important source of soil copper as well as a direct input to the plant in industrial areas. Excess aerosol copper has been shown to affect soil enzyme activity (Kasiak et al., 1986) and reduce photosynthesis (e.g. Fabiszewski et al., 1987) and pollen germination (Cox, 1988a). In the latter case, the effect was in association with reduced pH (Cox 1988b) which may have affected copper bioavailability as well as general plant physiology. Long term exposure to high copper emissions, combined with soil metal accumulation, has been associated with a general loss of plant cover or the restriction of cover to tolerant plant species (Banasova et al., 1987; Fabiszewski, 1987). With any species, however, metal tolerance requires energy and reduces plant efficiency and thus net productivity (Wilson, 1988). Vergnano Gambi and Gabrielli (1987) discuss plant response to heavy metals and various mechanisms of metal tolerance.

Excessive use of copper-containing fungicides can be detrimental to plants (e.g. Hussain et al., 1987; Mohr, 1986; Rezk et al., 1986) or cause soil copper accumulation to high levels (e.g. Simons, 1988). Interactions between copper fungicides and the herbicide paraquat are reported to reduce the ability of the latter to control common nightshade (*Solanum americanum*; Stall et al., 1987, 1988). Interactions between fertilizer components and trace metals also occur (Perez and Bornemisza, 1986), with the potential to affect both soil trace metal levels and plant uptake of metals (Szwonek and Nowosielski, 1984). Excess soil copper, by itself or with other agents, can affect soil nutrient status (Barabasz, 1986) and plant growth or yield (Arnold et al., 1988; Grigoryan and Karakeshishyan, 1988; Kuduk, 1987; McGrath et al., 1988a; Meyer and Heath, 1988; Ratsch and Johndro, 1987; Rousos and Harrison, 1987; Tikhomirov et al., 1988). Singh and Rakipov (1988) note a 6-17% reduction in barley yield when copper was added to the soil (40-120 mg CuÀkg^{-1} soil). Excess soil copper has also been associated with qualitative and quantitative changes in root exudates in corn (Mench et al., 1988). Adalsteinsson and Jensen (1988) report that copper concentrations of 10-500 µM in winter wheat water-culture medium affected root membrane transport, expressed by reduced uptake of certain cations. Comparable results have also been reported by Korner et al. (1987) with nickel uptake by excised barley roots although they attribute this to metal inhibition rather than a copper effect on the root membrane. Primary roots of corn have been found to grow towards high (1-5 millimolar) and away from low (0.1 millimolar) concentrations of Cu^{2+} (Hasenstein et al., 1988).

Excess copper in leaves has been associated with chlorosis (e.g. Ivanova, 1987) which Sahu et al. (1988) suggest is due to interference with metabolic translocation of iron. Copper-induced changes in leaf metabolism have also been indicated by Pennazio and Roggero (1988) who report a rapid increase in ethylene production when asparagus bean cuttings are exposed to Cu^{2+} treatment. The detrimental effects of copper have been related to changes in enzyme activity (Bakyrdzhieva, 1983; Karataglis et al., 1988; Stiborova et al., 1987; Stiborova et al., 1987; Sun et al., 1988d). Its effect on photosynthesis appears to be through metabolic events related to photosystem II (Baszynski et al., 1988; Hsu and Lee, 1988).

In a review, "Physiological effects of toxicants on aquatic animals", Stagg (1986) discusses mechanisms of uptake of metals and a few of their physiological effects, pointing out the importance of understanding trace metal responses and the difficulty produced by natural fluctuations and changes in populations. Differences between sites and even within sites have been used as justification for enclosure experiments to evaluate the effect of metals on aquatic animals (e.g. Abdulla and Steffenak, 1988; Kerrison et al., 1988; Word et al., 1987). However, there are certain drawbacks (e.g. Bakke, 1988); field studies examining metal uptake in relation to anthropogenic sources (e.g. Bondon et al., 1988; Chassard-Bouchaud et al., 1986) can provide evidence of cause and effect in natural systems.

Physiological changes in aquatic plants and animals has been demonstrated as a result of season and the nature of the organism. In a small, shallow, fertile lake, Kerrison et al. (1988) report (abstract) that "Cadmium, copper and mercury additions of between 10 and 100 μgÀL^{-1} had little immediate effect upon phytoplankton biomass in summer. In winter, marked reductions occurred in the first 2 days of the experiment. Zooplankton was more sensitive than phytoplankton to the heavy metals in summer, ..."

Working with the freshwater sponge *Ephydatia fluviatilis,* Francis and Harrison (1988) report copper requirements range between 10^{-9} and 10^{-8} M, with detrimental effects at concentrations in excess of 10^{-7} M. Excess copper caused deterioration of tissues. With the planula larvae of a reef coral, Esquivel (1986) found excess copper to cause increased mucus secretion and signs of contraction, among other things. In long-term (0.5-1.5 yr) bioassay tests with freshwater oligochaete worms exposed to metal-rich (including copper) bulk sediment, Wiederholm et al. (1987) found effects on growth and reproduction as well as survival. There was also a metal bioavailability effect indicated by the level of eutrophication, with reduced sensitivity occurring in eutrophic lakes. This probably was a result of increased metal complexation in lakes with higher levels of organics. Ozoh and Jones (1988) report high levels of copper (100 μgÀL^{-1}) caused inhibition of fertilization and 5-100 μgÀL^{-1} inhibited subsequent embryological development in a marine polychaete worm (*Hediste diversicolor*).

In bivalve molluscs, mainly the mussel *Mytilus*, excess copper has been shown to reduce or stop water filtration (Abel and Papathanassiou, 1986; Redpath and Davenport, 1988) and be associated with a decline in growth potential (Widdows and Johnson, 1988). It has also been related to gill inflammation (Sunila, 1988), altered cellular structure (Lowe, 1988) and biochemical changes (Moore, 1988; Taneeva, 1986). There is some evidence of a change in gill calcium homeostasis, produced by excess copper (Viarengo et al., 1988b). Tissue protein nature and content has been found to change (Shapiro and Zvezdovskaya, 1986) along with an increase in the rate of production of metallothionein, a metal-binding agent (Steinert and Pickwell, 1988). Cellular and biochemical changes have also been found in the oyster *Crassostrea virginica* (Cheng, 1988a; Farley, 1988), the clams *Abra alba* (Martoja et al., 1988) and *Villorita cyprinoides* (Sathyanathan et al., 1988), the "sea scallop" (Fowler et al., 1988) and two marine snails (*Cyclope neritea*, *Littorina littorea*) when exposed to high levels of metal in natural or laboratory situations (Minniti, 1987; Moore, 1988). Cheng (1988b) relates the effects of copper on the oyster to a reduced ability to phagocytize bacteria and thus a reduced defense mechanism capability. The reduced filtration rate noted in bivalves, by excess copper, has been suggested to trigger an increased accumulation of lactic acid in the blood, a result of reduced oxygen uptake (Suresh and Mohandas, 1987). The ability of organisms to adapt to changes in copper concentration is suggested by the increase in metallothionein production found by Steinert and Pickwell (1988), which would tend to stabilize tissue metal concentrations. This is also suggested by relatively consistent tissue copper levels in oysters in Arcachon Bay, an important mariculture site, where increased use of cuprous oxide occured as a result of the ban on tributyltin (Alzieu et al., 1987). Copper sulfate has also been suggested to affect osmoregulation in a freshwater snail (*Lymnaea natalensis*; Wolmarans and Yssel, 1988) and may affect haemolymph properties in the land slug *Lymnaea stagnalis* (Misechko and Stadnichenko, 1988; Stadnichenko et al., 1987). It also produces hyperglycaemia in another freshwater snail *(Lymnaea stagnalis*; Wijsman et al., 1988). This is an elevated blood sugar condition which might provide mobilization of stored nutrients during periods of stress and reduced feeding.

With crustaceans, excess copper has been shown to cause decreased reproductive success in *Daphnia magna* (Presing, 1987) and growth depression in the lobster *Homarus americanus* (Kean et al., 1985). (Hatakeyama (1988) noted these in an insect with freshwater larvae and Islam et al. (1987) noted a reduction in *Drosophila* egg hatch with copper sulfate.) Mortality is also increased with excess metal (e.g. Oganesyan et al., 1988). This is one reason why copper has been used to control nuisance populations of crayfish (e.g. Bills and Marking, 1988). In *Artemia*, respiration rate is increased by copper, as copper sulfate (Verriopoulos et al., 1988). Acclimatization to excess copper can, however affect respiration rate (Moraitou-Apostolopoulou and Verrioppoulos, 1986). Biochemical and physiological changes have been noted in the crab *Portunus pelagicus* with excess copper (Hilmy et al., 1988a). Enzyme activity has been used as an indication of the effect of excess copper. Luzgin and Akhmedov (1982) report a decrease in succinate dehydrogenase activity of the crustacean *Daphnia magna* with added copper, as cupric chloride; Liu et al. (1988a) reported an increase and then decrease in the specific activity of cytochrome oxidase in the prawn *Panaeus orientalis.* Acidic freshwater appears to be able to affect trace metal balances in crustaceans like the crayfish *Orconectes virilis* (France, 1987).

In their review of "Water Quality Criteria for Freshwater Fish", Alabaster and Lloyd (1982) provide an evaluation of copper as a pollutant. Although they fail to adequately recognize that copper is also a required metal, they do discuss the chemistry of copper in fresh water, the factors affecting direct and indirect lethal actions on fish, various sublethal effects, and some of the effects on freshwater invertebrates. They also briefly consider some of the problems associated with water quality criteria. High concentrations of metals as well as organics have been associated with the sea-surface microlayer (Cross et al., 1987), especially in areas of high input of atmospheric aerosol metal and organics. This is of interest because a number of organisms are either restricted to the microlayer or spend part of their life there. Survival of embryos of a number of fish species have, for example, been reduced in metal- or organic-rich microlayer water (Cross et al., 1987; Hardy et al., 1987; von Westernhagen et al., 1987). Fish tissue copper levels have been reported to be elevated at a site where copper-containing wood preservatives were being prepared (Winchester, 1988).

The response of fish to excess copper is varied. Munkittrick and Dixon (1986) note reduced growth and fecundity in white suckers (*Catostomus commersoni*) from copper- and zinc-enriched lakes. Tilapia from a polluted lake (copper concentration = 1.30 mg/L) were found to concentrate some of the agents (including copper) in their livers (Saleh and Hamza, 1986). Water-borne copper (8.17 mg/L) caused a change in the architecture of the gills of a freshwater fish (*Heteropneustes fossilis*; Rajbanshi and Gupta, 1988) and Heath (1987) obtained evidence suggesting added copper caused a reduced gill permeability in the freshwater bluegill (*Lepomis macrochirus*). This latter suggestion was based on an increased survival in salt-containing water, when compared with control fish. Trout olfactory receptors may degenerate in copper-enriched water (Moran et al., 1987).

The effect of excess copper on fish physiology and biochemistry is also varied. Aloj Totaro and Pisanti (1987) found a direct relationship between the concentration of copper in the water and the quantity of age pigments (lipofuscin) in the electric lobe of the electric ray *Torpedo marmorata*. There may also be an effect of copper on the immune response of certain fish (El-Domiaty, 1987; Khangarot et al., 1988). A change in protein content of some tissues may occur when fish are exposed to copper (Jana and Bandyopadhyaya, 1987; Jana and Sahana, 1988; Parrott et al., 1987; Xie et al., 1986). Nemcsok and Hughes (1988) found tissue necrosis and inhibition of acetylcholinersterase activity in rainbow trout exposed to copper sulfate. Enzyme activity can be affected by excess copper (e.g.Akhmedov, 1984; El-Domiaty, 1987) which can produce a depression of activity.

In domestic animals, although copper supplementation is frequently beneficial (e.g. Gooneratne et al., 1987a; Shurson et al., 1987), excess copper may be detrimental or, at times, high levels can be a result of some other physiological imbalance. Szymkiewicz and Niemiec (1988), for example, report much higher copper levels in unfertile hens eggs (22.2 ppm) than fertile eggs (9.5 ppm). Copper toxicity is found in ruminants (Dieckhoff, 1986), frequently occurring as a result of mineral imbalance (Cu:Mo ratio primarily) in natural or synthetic feed materials (e.g. Garcia Escamilla and Rosiles Martines, 1986). Treatment is often achieved by addition of molybdenum to the diet (Vrzgula et al., 1987). Excess copper can cause liver damage in sheep (West et al., 1987) and cattle (Bohman et al., 1987) as well as a plasma mineral imbalance (Bohman et al., 1987; Hayashi et al., 1987). (Changes in tissue mineral concentrations are readily achieved by other means - e.g. Falkowska and Iwanska, 1987.) Doster et al. (1986) report that excess kidney copper was often associated with bovine abortions and perinatal mortality in Nebraska.

A number of studies have used laboratory animals or cell cultures to examine the effects of deficient and excess concentrations of copper and copper-containing agents (e.g. Grin and Govorunova, 1987). Angerhofer and Taylor (1988) report skin and eye irritation in laboratory animals, with copper naphthenate and a wood preservative formulation containing the agent. They also found some toxicity with ingestion of the agents and high toxicity to one fish species. They comment (abstract) "... that disposal of excess preservative material should be done in an environmentally acceptable manner." Combinations of inorganic salts of Cr, As and Cu (as sulfate) are used as wood preservatives and have been demonstrated to be more toxic than the individual salts, when injected into pregnant rats (Mason et al., 1987). Excess copper in drinking water is of concern (e.g. Petersen et al., 1988). Vodichenska (1987) and Vodichenska and Dinoeva (1988), using laboratory rats, found chronic effects of copper in drinking water, at doses of 1 mg/kg or, to a lesser extent, at 0.1 mg/kg, chronic effects relating to oxidation-reduction processes and tissue respiration. Saichenko (1985) notes "embryo instability" in offspring of male rats given drinking water with Mn and Cu levels of 0.05 mg/kg. High levels of copper in wine are considered to be cytotoxic, in part a result of the effect of copper on oxidative reactions (Fernandes et al., 1988). (A disagreeable taste has been ascribed to wines with excess copper (Gurarda, 1985).) Verheesen and Nederbragt (1988) note differences in dietary copper sensitivity with different strains of laboratory rats indicating a variability that must be considered with the application of laboratory studies to humans.

Oxidation reactions can often be enhanced by copper (e.g. Czapski and Goldstein, 1987; Di Cola et al., 1988; Fernandes et al., 1988) although action of the metal can be affected by agents such as tannins (Fujita et al., 1988a) and bilirubin (Stocker and Ames, 1987). This information is important in examining the effects of copper on metabolic processes because it demonstrates modification of copper effect by natural organics. The effect on oxidation reactions is also important with food materials, copper enhancement of oxidation reactions can reduce storage time of certain food materials (Abdel-aal and Abdel-Rahman, 1986). Copper has been associated with lipid peroxidation. However, the mechanism is not well understood (e.g. Piriou et al., 1987). Beckman et al. (1988) report that cuprous ions may potentiate lipid peroxidation by a metal-metal reaction, reducing ferric ions rather than by promoting propagation reactions. Cupric ion administration subcutaneously to male rats was associated with a notable increase in total and free cholesterol (Tanaka et al., 1987), supporting a major effect on overall lipid metabolism. Through the use of metal complexing agents, Tanaka et al. (1988) obtained evidence suggesting cupric ion-dependent inhibition of lysosomal acid cholesteryl ester hydrolase, further evidence of the association of copper with cholesterol metabolism. Klevay (1987c) comments (abstract) that "Dietary copper may be a powerful determinant of cholesterolemia." There is also an association between copper and connective tissue formation (Kaji et al., 1988; see also Carty, 1988 and Shaw, 1988 for application of this relationship). Copper deficiency occurs in both animals and humans (Danks, 1988) and adversely affects the response of cardiovascular connective tissue to injury (e.g. Radhakrishnamurthy et al., 1988). Carville and Strain (1987) provide results with rats suggesting that copper deficiency could lead to increased oxygen free radical-mediated tissue damage in atherosclerosiss.

Copper is associated with the immune system in laboratory animals as well as fish (Prohaska, 1988b). Kucharz and Sierakowski (1988) report that copper ($\geq 10^{-9}$ M) caused a depression of interleukin 2 which has a key function in activation of immune mechanisms. It has also been used as an antiinflammatory agent, often in association with aspirin. Once again, the nature of the reaction is not well known (e.g. Williams et al., 1988a). Kishore (1988a) demonstrated that, with adjuvant arthritic rats, copper aspirinate has an agonist action at lower doses and an antagonist action at higher doses. Part of the action may be associated with the relationship between copper and oxidation reactions, part with the effect on enzyme activity. Elliott et al. (1987), for example report copper modulation of the synthesis of one or more macrophage oxygen enzymes. As indicated in the discussion of fungi, and apparently true for a range of cell types, changes in cell membrane permeability can be produced by copper (Ohsumi et al., 1988; see also Gabriels et al., 1988). Leakage can be induced by high concentrations and prevented by low concentrations (Bashford et al., 1988).

Copper affects growth in a number of ways. In the abstracts of two talks, Carpentieri and Thorpe (1987) and Carpentieri et al. (1988a) discuss the importance of trace metal balance to maintain normal growth in cultures of human lymphocytes. They (Carpentieri and Thorpe, 1987) suggest that regulation of intracellular Fe, Cu and Zn levels may induce some differentiation of leukemic cells. (Keeping in mind that there is a difference in metabolism between normal and abnormal patients (e.g. Sokovnina et al., 1987).) Copper, as $CuSO_4$, has been used to stimulate the growth of new blood vessels in the rabbit cornea, used as a model for anti-growth agents (Parke et al., 1988). The rabbit eye has also been used to assess the effect of copper-containing agents that could act as irritants (e.g. Boiarinov et al., 1986) and the presence of intraocular copper foreign bodies that could arise as a result of irregular copper metabolism (Schachat, 1988). Schmidt (1988a) evaluated the effects of various copper alloys implanted in the rat eye. Deleterious effects (electroretinogram and inflammation) decreased in the order - 99.9% copper + 0.1% silver > specially purified copper > two copper-zinc alloys.

Copper complexes that are cytotoxic have been used to control growth of tumor cells. Pezeshk et al. (1987) examine the response of two of these agents with Ehrlich ascites tumour cells. Rabinovitz and Fisher (1988a) report an agent that reduces growth of a leukemia cell clone. Francis et al. (1988b) present results which suggest that nutritional copper may have an anticarcinogenic activity against aflatoxin B1. However, Fourcade et al. (1988) report that nutritional copper deficiency seems to have little or no effect on granuloma formation in the rat. There appears to be a relationship between copper and cell growth, inflammation, lipids and connective tissue although the relationships are tenuous, multidimensional and difficult to understand. As one indication of this, Hammond et al. (1988) obtained evidence suggesting that (abstract) "... drugs with a reactive thiol group can interact with copper to generate H2O2, which can be toxic to neutrophilic progenitor cells." They postulate that this may be an important mechanism for drug-associated neutropenia and a general mechanism for drug-induced marrow cell injury. Copper, as sodium copper chlorophyllin, is able to inhibit formation, growth and aggregation of calcium oxalate crystals, as in kidney stones (Suzuki et al., 1987a.

Copper has been directly and indirectly related to genotoxicity. Bhunya and Pati (1987) found mutagenic properties of $CuSO_4$ in laboratory studies with mice. Fragmentation of DNA (deoxyribonucleic acid) in the presence of ascorbate and hydrogen peroxide has been reported to be accelerated with the addition of Cu^{2+} (Astashkina et al., 1988; see also Kornilova et al., 1988 and Prutz and Monig, 1987). In laboratory studies, the short-lived ^{64}Cu is reported to have a lethal effect as a consequence of decay when incorporated in cellular DNA (Apelgot and Guille, 1987). There is also some indirect evidence that the translation of genetic information to the control of physical properties may be affected by aerosol-borne metals, including copper (Orsi et al., 1988). This suggestion is, however, very speculative and without adequate laboratory or field evidence.

The exposure of individuals to high levels of dust is often associated with increased frequency of respiratory problems and lung cancer. This is true for those associated with mining and metallurgical processes (Borisov et al., 1987; Tokudome et al., 1988). Although the exposure to dust can include exposure to high levels of aerosol copper (Linscheid, 1985), the literature usually fails to draw the relationship between metal and disease. This is not to suggest that aerosol metal exposure is not a problem, it is to suggest that the association between metal and disease is not well established. The potential and real impact of aerosol metal is suggested in an assessment of worldwide contamination by trace metals (Nriagu and Pacyna, 1988). The authors point out that human activities are now affecting global trace metal cycles and there is accelerating accumulation of metals in human food chains.

Exposure to pesticides, including copper-containing agents, can produce irritation and allergic reactions although it may be to metal-containing agents rather than the metal (Lisi et al., 1987). van Joost et al. (1988), for example, comment that (page 101) "Allergic contact dermatitis apparently due to copper has rarely been reported ... . Copper sensitization as the cause of occupational dermatitis has been described ... but the interpretation of positive patch tests to this metal ($CuSO_4$) can be difficult and has not always been reported convincingly ... ." The authors did find some sensitization to copper sulfate as well as copper-containing agents. Contact allergy is reported for metal-containing coins (Nigam and Saxena, 1988) and Yelin et al. (1987) describe an abnormal death of a mentally disturbed individual, occurring as a result of copper toxicity following massive ingestion of coins. (Gastric contents included 275 partially corroded coins!) Müller-Höcker et al. (1988) discuss the possibility of copper accumulation from copper-rich drinking water and suggest that Indian Childhood Cirrhosis is due to an environmental rather than a genetic disorder. The use of copper in dental amalgams and castings has been examined in terms of possible side effects from excess copper (Bumgardner and Lucas, 1988; Burns, 1988; Jowett et al., 1988; Kaga et al., 1988a,b; Lemons et al., 1988; Lentz et al., 1988; O'Neal et al., 1988).

I.3.4 COPPER IN DENTAL AMALGAMS

Side effects from the use of copper arise from the release of biologically available, ionic metal, at levels that could be detrimental (Burns et al., 1988). In a study of the cytotoxicity of amalgams, alloys and their elements and phases, Kaga et al. (1988b) report (abstract) "The results suggest that the major cause of the cytotoxicity of alloys for amalgam is Cu, while that for amalgam is Zn." Amalgam cytotoxicity decreased with aging time which Kaga et al. (1988a) suggest could be due to the combined effects of surface oxidation and further amalgamation. Since corrosion rate controls copper release, and pH affects corrosion rate as well as dental plaque formation, the chemistry of the oral cavity becomes important in evaluating the effects of copper amalgams (e.g. Grytten et al., 1988).

Uptake of amalgam metal by teeth may occur although Lentz et al. (1988), working with canine teeth, report no significant differences in pulp metal content between amalgam-bearing and control teeth, both taken from the same animals. O'Neal et al. (1988) report marginal redness in gingival tissues adjacent to copper alloy crowned teeth. Lemons et al. (1988), using laboratory, tissue culture and *in vivo* crowns of copper base alloys in dogs, found toxicity in tissue culture and minimal to significant reactions with skin and mucosal contact as well as a range of *in vivo* tissue responses. Both O'Neal et al. (1988) and Lemons et al. (1988) conclude that copper alloys have the potential to produce problems if used in dentistry. Jowett et al. (1988) recommend caution in interpreting data from *in vitro* biocompatability studies although they appear to accept the use of *in vivo* techniques.

I.3.5 COPPER IN CONTRACEPTIVE DEVICES

Both medication and intrauterine devices (IUD) are used for contraception (e.g. Liukko et al., 1988; Luukkainen et al., 1987). Copper is one of the more common materials used in intrauterine devices. This is because it effectively reduces fertilization, in part as a result of the effect of high concentrations of biologically available metal on sperm (e.g. Shoham et al., 1987), fertilization and possibly hormonal regulation. Although the use of oral contraceptives has increased, the IUD is often recommended (Klitsch, 1988). Gupta et al. (1988b) comment that the copper IUD is not only an effective contraceptive but is inexpensive and a "one time method" to reduce the chances of conception.

It is also an effective method of treating certain types of birth canal irregularities (e.g. Asherman's syndrome; Sivanesaratnam, 1986) and, in most cases, causes few problems for the user (Trutko, 1986; Videla-Rivero et al., 1987) and allows return to fertility after removal (Soeprono, 1988).

A variety of copper intrauterine devices have recently been evaluated (Cohen, 1987; de Castro and Anguiano, 1986; de Castro et al., 1986b, 1987; Fochi et al., 1986; McCarthy et al., 1986; Thiery and Kosonen, 1986; Thiery et al., 1987; Van der Pas et al., 1987). Rates of copper release from devices vary with the nature of the device, chemistry of the uterine canal (Sekulovic and Primorac, 1985; Sekulovic and Tufegdzic, 1986), and length of time worn. Drost et al. (1987), for example, report an average daily loss of 18 µg and 30 µg copper for two devices (MLCu250, MLCu375). If pregnancy does occur in a woman wearing an IUD, the general rule is to remove the device if a normal pregnancy is desired. However, removal may be difficult, especially if part of the device has been lost (Assaf et al., 1988). Most of the problems associated with copper IUD use relate to menstrual disorders, pain and pelvic inflammatory disease (Trutko, 1986). Cases of copper sensitivity have been associated with the use of a copper IUD device (Dukes, 1987). Calcium deposits can also accumulate on the device, reducing effectiveness, increasing the chance for breakage, and providing a site for bacterial activity (Grafeille et al., 1987; Huacuja et al., 1987; Yuan et al., 1986). Elhag et al. (1988) report that a copper IUD can affect the nature of the bacterial flora of the genital tract, a change which may increase the chances of pelvic inflammatory disease. However, Ulstein et al. (1987) report no significant changes in cervical or vaginal microflora in women using copper- or norgestrel-releasing IUD's. Histological changes have been reported in the birth canal in device-fitted laboratory animals (Blok, 1985) and in women using copper IUD's (Badrawi et al., 1988; Chiarelli et al., 1986), changes that de Castro et al. (1986a,b) was able to relate to the amount of copper in the device. Based on studies with laboratory animals, there may also be an effect on blood circulation in the uterus (Zhang et al., 1987a). Kirshon et al. (1988) found no evidence of increased pelvic adhesions in IUD users. Vanlancker et al. (1987) report a higher incidense of oviduct inflammation in IUD users and, of those, the response was greater among women using copper devices than non copper devices. Although increased menstrual bleeding is reported for copper IUD users (e.g. Andrade et al., 1988; Pedron et al., 1987), Kleinhout et al. (1987) found no difference in women using one of two copper- or an estradiol-releasing device. The concern about increased menstrual bleeding includes possible iron deficiency anemia, a condition which Faundes et al. (1988) reports can be reduced by using the levonorgestral IUD rather than copper-containing devices. Batra et al. (1986) note no significant change in serum copper and ceruloplasmin in women using a copper-containing IUD device.

I.3.6 PHYSIOLOGICAL EFFECTS OF COPPER

The response of an organism to deficient as well as excess biologically available copper involves metabolic processes. These processes are biochemically driven and physiologically expressed - on the health and well being of the organism. Flipo et al. (1987) note that "Copper is a key element in the biologic processes of life and an essential constituent of a great number of enzymatic systems". Since these enzymes are organometallic compounds, the relationship between organic and metal is important. Deviations from these relationships can exist as a result of excess or deficient levels of copper or the genetic makeup of the organism. As well as enzymes, other organics are involved in the transport, storage, and metabolism of copper. Like enzymes, maintenance of the proper metal-organic relationship is critical to the physiology of the organism. This section considers the physiological response of the organism to copper. Because of the importance of organics, the reader will find some references to recent literature on organics here as well as in the following section which deals more exclusively with organics. Where possible, physiological terms have not been used.

General

In plants, although iron chlorosis is a result of an inadequate iron, it may also be due to excess phosphorous and copper in leaves, interfering with metabolic translocation of iron and rendering the iron inactive for chlorophyll synthesis (Sahu et al., 1988; see also Tomimura et al., 1986). Excess copper can also inhibit photosynthetic reactions. Gupta (1988), Samson et al. (1988), and Sibbald and Green (1987) report inhibition of photosystem II activity in plants. Copper-asparagine complexes are reported to elicit the release of pisatin, an antifungal stress metabolite in the garden pea (Cruickshank et al., 1987). Lowe et al. (1987) found reduction in synthesis of a red pigment (prodigiosin) produced by *Serratia marcescens.* Copper has been used to examine exchange of genetic information in plants (Juarranz et al., 1988) and, in fact, may induce certain exchanges (Hazen et al., 1988) and affect expressions of the genome (e.g. Verkleij et al., 1987).

In certain animals, copper-containing hemocyanin forms an important blood pigment for gas exchange (Avinc and Senozan, 1986; Salvato and Beltramini, 1987; Toulmond et al., 1987. Copper may also be found in association with hemoglobin (Standley et al., 1988). In other animals, there can be a tendency towards incorrect metabolism of copper, especially when it is in excess or when balancing factors are inadequate. Carson (1988) comments that chronic copper poisoning is a continuing problem in the sheep industry, as a result of excess copper or low molybdenum in commercial feeds. In a discussion of copper toxicosis in sheep, Van Saun (1988) discusses the susceptibility of sheep and the clinical signs that are a result of hemolytic crisis and liver cell damage. Plasma bile acid concentrations have been reported to increase as a result of copper-induced liver damage in sheep (West et al., 1987).

Human - inherited copper imbalance

In humans, improper regulation of copper balance can be inherited (Buzo, 1985; Ettinger, 1987; Garnica et al., 1985; Ghishan, 1987; Sternlieb, 1987a,b) or result from excess biologically available copper (Müller-Höcker et al., 1988; Shibuya, 1987). Brewer et al. (1988) discuss the symptoms and effects of some of the diseases resulting from inherited imbalance. Liver damage can occur in these cases, as a result of cell accumulation of copper and possibly metallothionein (Shibuya, 1987). Liver cell uptake of excess copper in Indian childhood cirrhosis has been associated with copper contamination of animal milk at an early age in childhood (Bhave et al., 1987).

Menkes and Wilsons diseases are the two best-known inherited copper disorders. Among other things, Menkes disease is characterized by low plasma copper levels (Uno and Arya, 1987; see also Inagaki et al., 1988; Leone and Hamer, 1987; Tonnesen et al., 1987c). The disease is also fatal. Gupta et al. (1988a) describes a child with Menkes disease, commenting on the infrequency of the disease in India. Sander et al. (1988) discuss the treatment of a child with the disease during his 13.5 years of life. Tonnesen et al. (1987a,b) state that the incidence is in the range of 1 in 50,000 to 1 in 100,000 live births and discuss possible prenatal diagnosis. Hamer (1987) notes that, in cultures of human fibroblast cells, low extracellular copper concentrations can induce synthesis of the metal-binding protein metallothionein in Menkes' cells but not in normal cells. Various genetic lines of mice have been used as models of Menkes disease that is used to better understand and treat the disease in humans (Katsura et al., 1987; Onaga et al., 1987; Packman, 1987; Prohaska, 1988c; Shiraishi et al., 1988b). Results from the blotchy mouse suggest that the disease is not due to a change in the role of metallothionein (Packman, 1987).

Animal models of Wilson's disease include the Bedlington terrier. Recent descriptions of case histories include Herrtage et al. (1987a,b) which (1987b) include comparisons of conditions in normal and affected animals. Twedt et al. (1988) discuss the use of a metal complexing agent to reduce the elevated hepatic copper levels found with inherited copper hepatotoxicosis. Sternlieb (1985) reports the isolation of a copper-thionein from copper-rich granules in the livers of affected Bedlington terriers. Carpenter et al. (1988), using a copper-laden rat model of Wilson's disease, report an altered vitamin D metabolism which they suggest (abstract) "... could be a potential factor in the development of bone and mineral abnormalities in Wilson's disease". Fuentealba et al. (1987) used a similar model to quantify stainable liver copper, a feature which can be related to hepatic copper concentrations in the Bedlington terrier but is often more difficult to relate in the presymptomatic Wilson's disease patient. Variations in the binding of copper and its localisation were noted, even with high hepatic copper concentrations. Myers et al. (1988) report changes in the membrane of hepatic lysosomes during experimental copper overloading. These changes suggested that copper overload is associated with disturbances in the functions of these cells.

The nature and heritability of Wilson's disease is discussed by Marsden (1987). With a frequency of approximately 30 per million individuals, it is not a common disease. It is also difficult to identify in early stages. Walshe (1988) discusses the diagnosis and treatment of presymptomatic Wilson's disease. The most obvious manifestation is the high hepatic copper concentration due to an inability to excrete copper through the bile. However other expressions of the disease do occur (e.g. Bahemuka et al., 1988; Lehr et al., 1988). And, by itself, the high hepatic copper levels are not diagnostic (e.g. Heckmann and Saffer, 1988). The elevated copper has been considered to be associated with disturbed metal uptake and transport, possibly as a result of abnormal metal metabolism associated with the copper-transport protein metallothionein (see, for example, Nartey et al., 1987; Sato and Arima, 1986) or ceruloplasmin (Iyengar et al., 1988a). Rodo et al. (1985), however, provides evidence against the metallothionein hypothesis. Bingle et al. (1987) suggest that Wilson's disease is a result of the failure to switch from the fetal to adult mode of copper metabolism. Treatment is by a metal-complexing agent such as D-penicillamine (Ohnishi et al., 1987) and 2,3-dimercaptopropane-l-sulphonate (but see Aaseth et al., 1987) or removal of copper through hemodialysis and exchange transfusion (Arnold et al., 1988). Use of oral zinc, in a metal-metal antagonism treatment is also advocated, especially in patients exhibiting sensitivity to the metal complexing agents normally used (Kaur et al., 1988).

Metabolic effects

Copper has been associated with red blood cell hemolysis (e.g. Hinberg, 1987), red blood cell proteolysis (Di Cola et al., 1988), platelet activating factor (Hanahan et al., 1988), and lipid peroxidation (e.g. Heinecke and Chait, 1987; Ivancheva et al., 1988; Viarengo et al., 1988). However, in a laboratory situation, copper at low levels (1-3 µM) has been suggested to act as a protective agent, with vitamin E, against detergent-induced hemolysis of pig red blood cells (Hamada et al., 1988). In contrast, copper levels over 15 µM caused hemolysis. The hemolytic effect seen in human red blood cells appears to be a result of copper causing increased rates of membrane lipid peroxidation (Fernandes et al., 1988a,b). Jain and Williams (1988) note an increase in cholesterol and a phospholipid in rats on a low copper diet and suggest that (abstract) "Increased cell viscosity by lipid loading and crosslinking of membrane components caused by lipid peroxidation may have a role in the reduced RBC survival and anemia in Cu deficiency". This would be, at least in part, a result of changed membrane permeability (e.g. Sneddon, 1987). Copper effects on RBC hemolysis have been countered by increasing manganese suggesting a metal-metal relationship (Tsujimoto et al., 1988a). It becomes apparent that, when considering the effect of copper on blood properties, it is important to examine more than just trace metal levels. Zhang (1986), for example, notes that addition of 250 ppm Cu to the basal diet of growing pigs increased plasma copper levels but also had a differential effect on enzyme activity. The interaction of copper with amino acids has also been suggested to have an effect on the mechanical aggregation of immunoglobulin and, through this, possibly the amount of inflammation in rheumatoid arthritis (Gerber and Fuksbrumer, 1988). Hac and Gagalo (1987) discuss the antipyretic activity of copper salicylate, another copper-organic used in treating arthritis.

Copper promotion of lipid peroxidation not only occurs in red blood cells, it also occurs in liver cells (Beckman et al., 1988). Tanaka et al. (1987) found a major increase of total and free serum cholesterol after administration of cupric ions to male rats. They point out that excessive cupric ion concentration may cause irregular lipid metabolism. In contrast, Hassel et al. (1988b) report that copper deficiency raises rat plasma cholesterol concentrations while reducing liver cholesterol. Copper deficiency also impairs intestinal transport of cholesterol (Koo et al., 1988b), at least in rats. Thuillier-Juteau (1987) examined copper-cholesterol relationships in hypercholesterolemic patients and found a negative relationship between serum copper and HDL cholesterol. Whether these kinds of relationships are associated with the relationships between copper deficiency and cardiovascular abnormalities that have been reported (e.g. Farquharson and Robins, 1988; Moore & Klevay, 1988) remains to be determined. Excess copper, under laboratory conditions and in an isolated rat heart, has been associated with some cell damage (Balogh et al., 1987).

Proper formation of connective tissue requires an adequate supply of copper (e.g. Bridges and Harris, 1988) and yet, at elevated levels, copper can damage formation (e.g. Kaji et al., 1987, 1988). The role of the metal is varied and not adequately understood. Hartmanova et al. (1986) note the formation of desmosines in elastin, from copper-lysine complexes. Maquart et al. (1988) report a stimulating effect of the copper complex of glycyl-L-histidyl-L-lysine on collagen synthesis by fibroblast cells in culture. And yet copper sulfate, in combination with D-penicillamine, can supress human fibroblast growth, a feature which may be of importance in tissue repair and tissue inflammation (Matsubara and Hirohata, 1988).

Copper and enzymes

Copper can enter into a variety of reactions with organics, ranging from interaction with organics that can inhibit (Hyun and Seu, 1987) or enhance (Kosugi et al., 1987) snake venom activity, through various oxidation reactions (e.g. Winkler, 1987), to the enhancement of double-strand breaks by ascorbate/copper (Aronovitch et al., 1987; Chevion, 1988). As a result, it is not surprising that enzyme composition and activity can be affected by copper. As will be evident from the discussion about enzymes, regular levels of biologically available metal are required and frequently enhance enzyme activity while high levels of available copper characteristically reduce activity. (Note, however, that enzyme activation may be increased as a result of tissue damage by copper (e.g. Dixon et al., 1987) and enzyme activity has been suggested as a mechanism to detect river pollutants (Harrison and Flint, 1987).)

A number of recent papers discuss the chemical role of copper in enzyme systems (e.g. Bilinski and Liczmanski, 1988; Chang et al., 1988b; Chung et al., 1988; Dameron, 1987; Dameron and Harris, 1987a,b; Gorren et al., 1987; Lyutakova et al., 1986; Ming et al., 1988b; Morpurgo, 1987; Schneider and Denaro, 1988; Shibata and Ogita, 1988; Standal et al., 1988; Thompson, 1988; Rigo et al., 1988). These are supplemented by papers on the effect of copper on enzyme kinetics (e.g. Bayati et al., 1988; Cook et al., 1988; Sakoguchi et al., 1986; Tsen and Lin, 1986), and the interaction of copper or copper complexing agents on enzyme activity (Feig et al., 1988; Rusanov et al., 1986; Takahashi et al., 1987; Tsuchiya and Suzuke, 1986; Willingham and Sorenson, 1988). Methods to isolate and determine enzyme concentration have also been published (e.g. Lickl, 1987; Sun et al., 1988d).

The effect of copper deficiency on enzyme concentration or activity has provided an indication of the importance of copper to organisms (e.g. Dubick et al., 1988; Lampi et al., 1988; Mitchell et al., 1988). Continuing interest is evidenced in the distribution of the enzymes both between species (e.g. Sulochana and Venkaiah, 1988) as well as within the organism (Davis et al., 1988a,b; Del Maestro and McDonald, 1987; Odlind et al., 1988; Radi et al., 1986; Strange et al., 1988) and the nucleotide sequence or expression of genes involved in the production of enzymes such as Cu-,Zn-superoxide dismutase (Fleming and Gitlin, 1988; Gralla et al., 1988; Ogino et al., 1987a; Puga and Oates, 1987). The importance of copper to enzymes related to metabolism of oxygen and oxygen derivatives is indicated by the number of authors working in this general area (e.g. Artico et al., 1988; Chang et al., 1988a; Crawford et al., 1988; Esaka et al., 1988; Gaisser et al., 1987; Galiazzo et al., 1988; Gulyaeva, 1987; Marziah and Lam, 1987; Megrabyan and Nalbandyan, 1987; Mondovi and Avigliano, 1987; Munday, 1988a,b; O'Neill et al., 1988; Simpson et al., 1988; Takahashi, 1986; Tomita, 1988; Whittaker and Whittaker, 1988).

Copper participates in the activity of reductases (e.g. Brenner et al., 1988; Casella et al., 1988e; Ishikura et al., 1988; Jones and Morel, 1988; Perez Mateos and Gonzalez Carcedo, 1988) and some oxidases (e.g. Baron Ayala and Sandmann, 1988; Rich et al., 1988). Vezitskii and Shcherbakov (1987, 1988) provide evidence that copper can affect the enzyme system involved in the esterification of chlorophyllides in plants. Deficiencies of copper have been associated with a reduction in the activity of two oxidases in peanuts and soybeans (Marziah et al., 1986). Activation of a carboxylase from barley could be achieved by copper although long-term exposure to the metal was associated with inhibition (Stiborova et al., 1988). In excess, it has been found to inhibit the activity of an oxidase from water hyacinth (Yanagisawa et al., 1987), is suggested to affect isoperoxidase activity in wheat (Karataglis et al., 1987), a reductase in garden pea (Sun et al., 1988d) and a carboxylase in corn (Stiborova, 1988) and in *Crassula argentea* (Walker et al., 1988; Nguyen et al., 1988). (Note that Ilamanova (1985) found that presowing melon seeds with Mn and Cu increased peroxidase activity.) Clarke and Adams (1987) report copper inhibition of a cellulose-digesting enzyme produced by a microorganism, inhibition of glucosidase has been reported (Sada et al., 1988), while Citharel and Garreau (1987) report inhibition of a peptide-digesting enzyme in dry and germinating seeds of bean plants. Bar Nun et al. (1988) provide evidence that copper is necessary for the formation of laccase and that, in plants, laccase formation is necessary for infection by *Botrytis*. The activity of amylases from microorganisms can be reduced by copper, in a concentration-dependent manner (Koch et al., 1987; Mikami et al., 1987). Datta et al. (1988) report that copper reduced acetylcholinesterase activity in scorpion hemolymph and Maroni et al. (1987) discuss metal tolerance in natural populations of the fruit fly *Drosophila melanogaster*and the some of their work on the genetics of metallothionein production. Ueno et al. (1988a,b) found copper inhibition of a protease in mackerel white muscle.

Urease activity in plants can be reduced by copper (Antonelli et al., 1986, 1987; Rai and Singh, 1987). In animals, respiratory enzyme activity can be reduced by copper (e.g. Malyarevskaya and Karasina, 1985) as can the activity of certain hydrolases (Tanaka et al., 1988). However, copper enhances reactivation of dried rabbit skeletal muscle phosphofructokinase (Carpenter et al., 1987). As well, nutritional copper deficiency has been linked with reduction in cellular antioxidant protection through the enzyme Cu-, Zn-superoxide dismutase (e.g. Allen et al., 1988b; Radi and Matkovics, 1988), an antioxidant defense enzyme (Arias and Walter, 1988; Carraro and Pathak, 1988; Hassan, 1988) whose concentration can usually be increased in response to physiological stress (e.g. Bilinski and Litwinska, 1987; Das and Fanburg, 1988; Hass and Massaro, 1988b; Keizer et al., 1988; Simonian et al., 1987; Szelenyi and Brune, 1988; but see Chan et al., 1988c). It does, for example, exhibit anti-inflammatory activity (Aruoma and Halliwell, 1987; DiSilvestro, 1988a,b; Jadot and Michelson, 1987; Jadot et al., 1986a; Michelson et al., 1986). The enzyme comes in basically two forms, one with manganese, the second with copper and zinc. Originally called haemocuprein, Bannister (1988) and Bannister et al. (1987) present reviews of this most important enzyme. In both plants and animals, the activity and beneficial effects of the copper-containing superoxide dismutase is dependent upon an adequate supply of copper (Baron Ayala and Sandmann, 1988; Lynch and Strain, 1988; Michelson et al., 1986; Mylroie et al., 1987b; Ogino et al., 1987b; Taylor et al., 1988). Any disease that affects the concentration and distribution of the enzyme will, as a result, have an effect on cellular antioxidant protection. Bartoli et al. (1988a,b) comment from work on rats, that as a result of liver cancer there can be a reduction of superoxide dismutase which results in oxidative stress affecting the lipid nature of cells. Delacourte et al. (1988) found preferential localization of Cu-, Zn-superoxide dismutase in the vulnerable cortical neurons in Alzheimer's disease. Mongolism, or Down's syndrome is an inborn pathology due to trisomy of chromosome 21, the same gene for Cu-, Zn-superoxide dismutase (Ceballos et al., 1988; Kedziora and Bartosz, 1988). Possibly as a result, activity of the enzyme is linked with the disorder in animal models of the disease (Avraham et al., 1988; Epstein et al., 1987) as well as in humans (Elroy-Stein and Groner, 1988 but see Zitron, 1988). Activity of the enzyme has also been found to increase in response to renal failure (Kato et al., 1987). Vongai et al. (1988) report on a group of copper-containing complexes (cryptand complexes) which exhibit a superoxide dismutase-like activity. Antioxidant activity has also been reported for carnosine, homocarnosine and anserine which are found in the muscle and brain of many animals and humans (Kohen et al., 1988).

Copper is reported to accelerate the heat inactivation of pyrophosphatases from yeasts and the bacteria *Escherichia coli* (Ichiba et al., 1988) while Tu et al. (1988) report that copper stimulated activity of phosphatase from the cell wall of potato tubers. It is also reported to stimulate or be required for the activity of a variety of other enzymes (Fawzy et al., 1987; Korte et al., 1988; Movaghar and Hunt, 1987; Vaeroy et al., 1987) and to inhibit others at the concentrations used (Bovykin et al., 1985; Ibrahim et al., 1988; Kaniike, 1986; Krupyanko, 1988; Morton, 1987a,b; Scicchitano and Pegg, 1987). El-Waseef et al. (1986) note that what they term "low" copper concentrations (≤ 20 ppm) activated a lead-inhibited enzyme (delta-aminolevulinic acid dehydralase; ALAD) while high copper levels (> 20 ppm) inhibited the activity. They conclude (abstract) "... that dietary copper concentrations up to 20 ppm can lessen the severity of lead toxicity while concentrations higher than this limit may exaggerate lead toxicity in rats". (Note that copper is one of the metals reported to be able to either activate or inhibit ALAD (Bernard and Lauwerys, 1987; see also Salgado et al., 1985).) The close interaction of copper with adenosine triphosphate (Onori, 1987) is another indication of the importance of copper to physiological processes in organisms. This occurs both in terms of normal and abnormal concentrations of copper (e.g. Beckman and Zaugg, 1988), including an effect of copper sulfate on blood glucose levels in trout (Nemcsok and Hughes, 1988).

Miscellaneous effects

Dietary copper deficiency has been related to atrophy of the pancreas in rats. Rao et al. (1988d) note that extreme copper deficiency can cause almost total conversion of pancrease to liver in the adult rat. Mylroie et al. (1987a,c) found copper deficiency to be associated with a decrease in pancreatic amylase and copper superoxide dismutase. With a copper-poor sucrose diet, rats exhibited an impaired glucose tolerance suggesting a role of copper on insulin release in the pancreas (Cohen and Miller, 1986). The role with insulin may be due to the action of copper with insulin binding, Monreal et al. (1987) note that Zn^{2+} or Cu^{2+} resulted in an increase of specific insulin binding when added to rat plasma membrane preparations or purified insulin receptors. However, Vicario et al. (1988) note that inclusion of $CuCl_2$ the insulin receptor tyrosine kinase inhibits the activity of the enzyme. Copper has been found to stimulate the secretion of luteinizing hormone in cultured hen pituitary cells (Hu, 1987) and to increase binding of another hormone, estradiol, in rat uterine cells (Fishman and Fishman, 1988).

Copper plays a major role in prostaglandin E_2 which stimulates the release of a hormone involved in the female reproductive cycle (Barnea et al., 1988a,b; Bhasker and Barnea, 1988). Wissler et al. (1987a) discuss the production of a morphogen which acts as a "wound hormone", from cultures of porcine monocytes. Copper-zinc relationships appear to be affected in irregular growth (Grant, 1987). In radiation treatment for abnormal growth, copper-containing agents (e.g. Cu-diisopropylsalicylate) are able to provide some tissue protection against the damaging effects of radiation (Soderberg et al., 1988b). However, Westman and Marklund (1987) report little protection from radiation, by polyethylene-glycol substituted copper- and zinc-containing superoxide dismutase.

Microorganism cell surfaces can be affected by a number of heavy metals, causing a change in the electrokinetic properties (Collins and Stotzky, 1988). de Vos et al. (1988) report copper-mediated changes in the membrane permeability of certain plant cells, in part as a result of lipid peroxidation (see above). Watanabe et al. (1988) discusses the properties of an electrochemically prepared copper-DNA membrane, reporting changes in membrane potential with changes in temperature as well as ionization tendency of divalent metals. In rainbow trout, Reid and McDonald (1988) found that copper altered net sodium flux through the gills, another indication of changes in membrane permeability and nature as a result of increased copper. Nagel et al. (1988) found minimal effect of added copper (0.1 mmol/L) on chloride movement across amphibian skin. A variety of agents can affect membrane permeability and thus tissue metal levels. Morimoto et al. (1987b) found that large doses of bacterial endotoxin could affect serum copper levels in rabbits. In humans, changes in membrane permeability become important in a number of situations. Two examples are -1) the effect of dialysis on serum metal levels in uremic patients (e.g. Chen et al., 1986c) and -2) the possibility of copper effecting a toxic response if dialysate is delivered in copper tubing (Lindeman, 1987). It should be noted, however, that serum copper levels may already be affected in uremic patients (e.g. Beknazarov, 1986).

I.3.7. THE INTERACTION OF COPPER WITH ORGANICS

Interactions of copper with organics occur outside the organism, at the boundary between the organism and the environment, and within the organism. They help to control the biological availability of metal as well as uptake by the organism and transport and storage within the body. Most importantly of all, copper reacts with certain organics to form essential organometallic compounds. Conversely, when in excess, copper can interfere with the normal functioning of organic compounds. Many of the interactions are with proteins, reviewed by Sarkar (1987), and the coordination chemistry of copper-containing metalloproteins forms an important area of research (see reviews by Dooley, 1987 and Solomon et al., 1987b). Extensive work has been done on copper-containing enzymes, particularly Cu-, Zn-superoxide dismutase (e.g. Kwiatowski, 1987; Lindenbaum et al., 1987). Organometallics in food are also important as they affect metal uptake (see review by Kratzer and Vohra, 1986). In marine environments, Hirose and Sugimura (1985) suggest that dissolved organics are considered to act as metal buffering agents helping to maintain a relatively constant level of biologically available metal (e.g. Rueter and Petersen, 1987). Many of these agents are derived from the decomposition of organics (Higgins and Mackey, 1987) and vary as a result of location (e.g. Mackey and Higgins, 1988) or season. An important area of research concerns the organics used to transport and store metal and isolate excess metal. In this latter case, tolerance to excess metal is often dictated by the ability of an organism to produce and utilize these organics (e.g. Liaw et al., 1988).

Certain organics, such as polyacrylamides, can be combined with metal mixtures to produce plant fertilizers. El-Hady et al. (1986) describe the use of these to minimize nutrient and trace metal losses from sandy soils. They suggest that (page 129) "The possible mode of action for micronutrients especially Cu and Fe in sandy soils is either: the activation of sand neutral surfaces, thereby improving their electrostatic interaction with PAM (polyacrylamide), or polymer crosslinking by reaction with hydrated di and polyvalent metal ions." The use of manures and sewage sludge as fertilizer requires an understanding of metal-organic interactions to estimate plant metal availability and uptake (Dudley et al., 1987; Fletcher and Beckett, 1987a,b; Relan et al., 1986). Relan et al. (1986) provide stability constants of Cu, Pb, Zn, Mn, Fe and Cd complexes with humic acid from farmyard manure. Humic and fulvic acids are degradation products of organic material. They play major roles in trace metal chemistry and plant metal bioavailability in both natural and cultivated soils (Grace et al., 1985; Karlsson et al., 1987a; McGrath et al., 1988b; Sanders and McGrath, 1988; Sposito et al., 1988) as well as in aquatic environments (Apte et al., 1988; Jones and Thomas, 1988; Sun et al., 1988c). Breakdown of organic material to produce these organic acids, as well as the final release of complexed metals from the breakdown products is by microorganisms (Aristovskaya et al., 1986).

Metallothionein-like organics

Once copper is taken into the organism, organics are associated with transport and utilization. They are also involved with buffering excess metal. Metallothionein and other, metallothionein-like, organics are involved in the transport, utilization and buffering of a number of metals in a wide range of organisms. Metallothionein, *per se*, is characterized as an organic with a specific structure. Widely-varying, metallothionein-like, organics have different structures but similar functions (see Hartmann et al., 1987). This latter "group" of organics includes a wide variety of low molecular weight agents ranging from amino acids to proteins which can complex metals and affect the tolerance of organisms (e.g. Dunham et al., 1986).

Because of their importance to physiological processes, these organics have received a great deal of attention in the past decade. The 1985 international meeting on metallothionein and other low molecular weight metal-binding proteins is an indication of this (Kagi and Kojima, 1987). General discussions of the nature and functions of these organics have been given by a number of authors (Bremner, 1987; Hamer, 1988; Mehra et al., 1986; Winge et al., 1988a). Gordon et al. (1988) describe copper-binding proteins produced by a microorganism (*Vibrio alginolyticus*) that are excreted into the medium to reduce the detrimental effects of excess metal. Calmodulin is a metal-complexing agent primarily associated with calcium metabolism (MacNeil et al., 1988) but which may complex other metals (Permyakov et al., 1988). All of these agents, along with metallothionein, can enhance tolerance to excess metal. Tolerance can also be achieved by a reduced response to metal (e.g. Camakaris et al., 1988a) and by agents such as vitamin B_{12} (Glubokova, 1986).

Santhanagopalan and Jayaraman (1988) found a number of yeast strains to be metal tolerant, with the metal frequently associated with high molecular weight organics (not metallothioneins). However, metallothionein is often reported to be produced by microorganisms, as a result of exposure to excess metal (e.g. Munger and Lerch, 1987). A good deal of effort has been devoted to studying the molecular structure and genetics of copper metallothionein in microorganisms (e.g. Bergman and Timblin, 1988; Camakaris et al., 1988b; Diels et al., 1987; Fogel et al., 1988; Higham et al., 1987; Mellano and Cooksey, 1987; Munger et al., 1987, 1988; Naika, 1987; Richter and Weser, 1988; Shen et al., 1987c; Thiele et al., 1987; Thrower et al., 1988; Tomsett et al., 1988; Welch et al., 1987; Winge et al., 1987; Wright et al., 1988). In plants, uptake of excess metal has been associated with a metallothionein-like agent (Leblova and Spirhanzlova, 1987). Increased copper tolerance has been reported as a result of several organics (e.g. Jackson et al., 1988; Verkleij et al., 1988). Zhou and Wangersky (1989) note that, in the diatom *Phaeodactylum tricornutum*, copper-complexing ligand production increased with growth rate and was higher during the day, in a continuous culture. Gekeler et al. (1988) have found Cu-, Cd-, Hg-, Ag-, Zn-, and Pb-induced production of phytochelatins, peptides, by the alga *Chlorella fusca*. Grill (1987a, 1987b, 1988) discusses the nature and chemistry of phytochelatins, produced in a variety of plant types. Scheller et al. (1987), for example, report phytochelatin synthesis in cultured tomato cells as a result of cadmium exposure and suggest that glutathione is a substrate for phytochelatin synthesis. Thurman et al. (1988) found copper-inducible, phytochelatin-like proteins in a copper-tolerant plant (*Mimulus guttatus*) and Grill et al. (1988) identified phytochelatins in roots of both a metal-sensitive and metal-resistant plant species grown in zinc-rich soil of a mine dump. They did not find phytochelatins in plants from a metal-uncontaminated area. Kubota et al. (1988) report a copper-binding protein (mol. wt. of 9,500) from the root and rhizome of a plant growing in copper-enriched soil.

In invertebrate animals, metallothionein-like agents are reported (e.g. Rainbow et al., 1987), and can act as a metal buffering agents at high metal concentrations (Fowler et al., 1988b; Jenkins and Sanders, 1987; Legrand et al., 1987). Jenkins (1986 - final project report), however, notes a decrease in growth rate of crab larvae with elevated levels of copper even though it appeared to be in association with metal-binding proteins. Metallothionein-like agents may also be directly involved in copper sequestration for blood pigment synthesis (Engel, 1988). Tournie and Mednaoui (1986) report that the marine snail *Hydrobia ulvae* produces and releases into the environment enough copper-complexing agent that it may regulate copper bioavailability locally. Metal-containing granules have been reported from bivalve mollusc kidneys (Robinson et al., 1985; Sullivan et al., 1988) and a spider (Ludwig and Alberti, 1988). These may be produced as a result of metal metabolism, possibly through metallothione-like agents. Enhanced metal tolerance has been associated with apparent induction of metal-binding proteins in marine mussels (Roesijadi, 1987; Viarengo et al., 1988a). Viarengo et al. (1988c,d) characterized a copper-thionein in mussel digestive gland lysosomes as metallothionein-like. Brouwer and Winge (1988), in the abstract of a talk, discuss the structure and function of copper metallothionein from the American lobster. (See also Wegnez et al., 1988, for a discussion of the metallothionein gene in the fruit fly *D. melanogaster*.) Roesijadi et al. (1988) used immunochemical techniques to estimate production of metal-binding proteins in the bay mussel *Mytilus edulis*. They report an increase in metal-binding protein levels after exposure to mercury, cadmium, or copper for 28 days but did not find an increase with exposure to zinc. Harrison et al. (1988), working *M. edulis*, obtained evidence that (abstract) "... the capacity for production of metallothioneins is limited and that the quantities present differ greatly with seasonal changes in the environment."

In fish, metallothionein-like agents have been reported to occur as a natural mechanism for copper metabolism (Krezoski et al., 1988) as well as a means of buffering excess metal. Delval et al. (1988) report that in the flounder *Platichthys flessus*, with excess zinc, copper is chelated while cadmium complexation is related to zinc release. Overnell and Abdullah (1988), also working with *P. flessus*, report kidney metallothionein levels were elevated at polluted field sites (elevated concentrations of metal and diesel fuel). However, in 4 copper-enriched mesocosm basins, liver and kidney metallothionein was not elevated and copper was not accumulated in the livers. Stegeman et al. (1988), working with the same fish from the field sites, found an apparent increase in induction of the cytochrome P-450 and suggest that this, along with an enzyme, may form a suitable assay for environmental condition. (Cytochrome P-450 from two molluscs has also been recommended as an assay by Livingstone, 1988.) Bonham et al. (1987) and Zafarullah and Gedamu (1988) briefly discuss the structure of rainbow trout metallothionein-B gene. Olsson et al. (1987) note a seasonal change in metallothionein production in rainbow trout. Increased amounts were found from November through February with a maximum in January (640 nmoles/liver), which they suggest is a result of the reproductive phases

Suzuki (1987) quantified and characterized metallothioneins in a range of animal groups, including earthworms, crustaceans, insects, fish, amphibians, reptiles and birds. Although most of the metallothionein induction was by cadmium, some application of the results can be made to copper and is indicative of the broad spectrum of organisms that use metallothionein to buffer excess metal. Van Beek and Baars (1988) isolated and characterized metalothioneins from cattle. However, Durnam and Palmiter (1987) note that not all metals which induce metallothionein are detoxified by the protein. Kwohn et al. (1987) noted metallothionein in striped dolphin kidney, in association with cadmium. Fleet et al. (1988) note that metallothionein productuion could be induced in the chick liver by copper. Mercer et al. (1988) examined copper homeostasis in sheep in relation to metallothionein, noting that the gene sequence have some similarity to those of mouse and human metallothionein genes. Zinc may compete with copper for metallothionein sites in both sheep (Mercer et al., 1988) and cattle (Whanger and Deagen, 1985). Molybdenum supplementation is often used to reduce the effects of excess copper in sheep. Allen and Gawthorne (1987) note that tetrathiomolybdate caused a shift in the association of copper with organics, from low molecular weight proteins to higher molecular weight proteins. This was due to the formation of protein-tetrathiomolybdate complexes that had a strong affinity for copper. Metal-metal competition for metallothionein sites has been demonstrated with cadmium, zinc and copper, in rabbit kidney metallothionein (Elinder et al., 1987a,b). Blaloch et al. (1988) obtained evidence suggesting that copper and zinc content of renal metallothionein was directly related to the dietary levels of copper and zinc. Increased production of metallothionein may occur in response to metal-containing drugs. Taylor et al. (1987) found what they suggest is a gold-induced accumulation of copper in rats given aurothiomalate. They believe the accumulation of copper may be a result of increased production of metallothionein to handle the gold. The alternative (Mcvety and Shaikh, 1987) is that the increased production of metallothionein is a result of increased accumulation of copper and zinc. Metallothionein can also be introduced from another source and still function. Cismowski et al. (1988), for example, produced increased metal resistance in yeast with metallothionein from the Chinese hamster.

Factors affecting production of metallothionein in the fetus and newborn are not well understood. Cherian et al. (1987) comment that, in the rat, metallothionein levels rise sharply during gestation and then continue into the neonatal period. Metallothionein is present in the nucleus of liver cells in neonates and then in the cytoplasm during postnatal development. Munoz et al. (1988) note metallothionein in limb buds, brains and livers from mouse fetuses and comment that the concentration increases with age of the fetus. As with serum transferrin (Feng et al., 1987b), metallothionein may play a role in the transfer of metal ions from mother to fetus. Like other organisms, the roles of metallothionein are to regulate copper and several other trace elements in humans (e.g. Bem et al., 1988; Chiu et al., 1988; Kurisaki et al., 1988; Sugawara and Sugawara, 1987). As indicated in laboratory animals, this may include regulation of copper-containing metalloenzymes (Arthur et al., 1987; Kambadur et al., 1987; Markossian et al., 1988; Matsubara, 1987; Ravi et al., 1988). The levels of the organic are often affected by disease as, for example, in the insulin-deficient diabetic rat in which the rate of metallothionein synthesis is elevated in both the liver and kidney (Chen and Failla, 1988). Immune response effects by metals may be associated with metallothionein (e.g. Lynes et al., 1988). Mahy et al. (1987) discuss the use of metallothionein levels as a diagnostic aid in liver disease with copper retention. Tissue copper and metallothionein levels are affected by inherited diseases of abnormal copper metabolism, such as Menke's kinky hair disease (Leone and Hamer, 1987; Shiraishi et al., 1987; Sone et al., 1987), models of Wilson's disease (Hunt et al., 1987; Sternlieb, 1985) and Wilson's disease itself. There is increasing evidence of metal and metallothionein imbalance in abnormally growing tissues, especially malignant tissues (Ebadi and Swanson, 1988; Guigi et al., 1988; Kobayashi and Sayato, 1985; Kobayashi and Sayato-Suzuki, 1988; Leone and Remondelli, 1988).

Because of their widespread use as metal buffering agents, levels of metallothionein have been considered as an indicator of metal (including copper) bioavailability (Chan et al., 1989; Engel and Roesijadi, 1987; Mahyaoui and Saghi, 1987; Pavicic et al., 1987). However, as stated in Engel and Roesijadi (1987) (abstract) "Before metallothionein can be used effectively as an indicator of environmental degradation, its physiological and biochemical function under normal conditions must be demonstrated." Lipofuscins have also been suggested as monitoring agents (Gutteridge, 1988; Pisanti et al., 1988a,b). These are "age pigments" apparently produced in response not only to age but also environmental stress. Elsasser et al. (1986) found copper to affect a chemiluminescent response in rainbow trout phagocytes wich they suggest is dose dependent and offers a third environmental assay.

Other copper-containing proteins, such as ceruloplasmin, also change in concentration as a result of disease (Hansen et al., 1988). Vasil'ev and Kononova (1987) report differences in the catalytic properties of ceruloplasmin molecules suggesting the catalytic properties of the organic. As a result of its important role in the well being of man, more and more work is being done on ceruloplasmin (e.g. Barber and Cousins, 1988; Kim and Combs, 1988).

Other organics

Ascorbic acid and copper are often used, or examined, together in the evaluation of nutrient status (e.g. Milne et al., 1988) or of particular metabolic systems. This is because copper-catalyzed oxidation processes often involve ascorbic acid (Aihara et al., 1988; Harrison, 1986; Messripour and Haddady, 1988; Morishita et al., 1987; Nye et al., 1987; Uchida and Kawakishi, 1988; see also Jacob et al., 1987). This can include degradation or scission of nucleic acids (e.g. Aronovitch et al., 1987; Kazakov et al., 1988; Lickl et al., 1988; Stoewe and Pruetz, 1987; Vodolazkin et al., 1987) by copper-catalyzed reactions with ascorbic acid as well as other organics (e.g. Quinlan and Gutteridge, 1987). Tajmir-Riahi et al. (1988) used laser Raman spectroscopy to demonstrate the destabilizing effect of ionic copper on DNA and Matzeu et al. (1988) discuss the enhanced sensitivity of DNA to UV irradiation in the presence of ionic copper. Damage to DNA by ascorbate, in the presence of ionic copper, has been reported to produce mutations (Kobayashi et al., 1988; see also Young and Hanson, 1987). However, the interaction of metal complexing agents such as tannins and flavonoids can complex copper (Eller and Weber, 1987) with resultant inhibition of the oxidation processes (e.g. Fujita et al., 1987). Fazekas et al. (1987), working with liver mytochondrial myosins, note that cupric ion treatment of the myosin caused the release of phosphorous with the retention of a small amount of the copper. Oligonucleotide formation can also be stimulated by a number of divalent metal ions including copper (Sawai, 1988).

The interaction of copper with organic compounds forms a consideration in the preparation and storage of foods (e.g. De Stefanis et al., 1988; Nath and Murthy, 1987) and in nutritional studies. Kratzer and Vohra (1986) examine some of these interactions in their review "Chelates in Nutrition".

Consideration of different types of carbohydrates becomes important in metal bioavailability since fructose is known to reduce the uptake and utilization of copper (Babu and Failla, 1988; Johnson, 1988; Yamauchi et al., 1988). Copper may be involved in glucose autoxidation damage to proteins in poorly-controlled diabetics (Hunt et al., 1988). Various copper-metabolite relationships are known to be nutritionally important, including those with pectinates (Jorge and Chagas, 1988), amino acids and peptides (Gottlieb and Swinehart, 1988; Pearce and Friedman, 1988; Wang et al., 1987a), lipids (Jones and Harwood, 1988; Rao and Murthy, 1987; Stacy and Medeiros, 1988), and fiber and phytates (Champagne, 1987; Frolich and Nyman, 1988; Kabaija and Smith, 1988b; Lee et al., 1988c; Morris et al., 1988; Nandy et al., 1987; Nolan et al., 1987; Persson et al., 1987). Geesey et al. (1987) reports polysaccharide sorption to a copper film with subsequent deterioration of the film. These polysaccharides can be produced by microalgae and released into the environment (Kaplan et al., 1987b).

Amino acids often react with copper, in environmental as well as nutritional and physiological situations. Liedberg et al. (1987) used infrared reflection-absorption spectroscopy to elucidate the molecular orientation and coordination of amino acid-copper complexes formed when the acids sorbed onto copper films. Chemical studies of metal-amino acid complexes include Grecu et al. (1986) and Nair et al. (1988), and also include the examination of physiologically active complexes (Dickinson et al., 1988; Wu et al., 1987a). Amino acids free in seawater can act as metal-complexing agents and have been shown to be able to reduce the bioavailability of copper (Kosakowska et al., 1988). One amino acid (L-α-amino-β-methylaminopropionic acid or "MeDAP") found in the nuts of the false sago palm (*Cycas circinalis*) strongly complexes copper and zinc. When the nuts are used as a food source, this amino acid is believed to scavenge copper and zinc in the body, causing a degeneration of part of the central nervous system (in Emsley, 1989).

Products of metabolism that react with copper are numerous. Rosell and Srivastava provide information on the amount of various minerals and trace metals in kelp residues. Poulicek (1986) discusses some of the chemistry of chitin and Kawano et al. (1986) examine metal uptake by chitosan, a derivative of chitin. Copper is one of the transition metals that has been demonstrated to modify the structure of melanins (Palumbo et al., 1988). Dzyuba et al. (1987) discuss the synthesis and biological activity of hydroxamic acids, strong iron-chelating organics that also bind copper. The three-dimensional solution structure of plastocyanin, a "blue" copper protein, was examined with nuclear magnetic resonance (NMR) spectroscopy by Moore et al. (1988a). Morphogens and enkephalins often interact with copper (Wissler et al., 1987b; Yatsimirskii et al., 1988). Stimulation of synthesis of organics can be accomplished with copper, for ethylene in asparagus bean leaves (Pennazio and Roggero, 1988), phytoelaxins in pea (Cruickshank et al., 1987) and other plants (Threlfall and Whitehead, 1988). (Phytoelaxins are antimicrobial secondary products of plants produced in response to exposure to micro-organisms.) Separation of copper complexes produced by organisms has been studied, to improve analysis or enhance production. Studies include the analysis of copper and magnesium complexes in duckweed (Mo et al., 1988b), copper-flavonoid complexes from vegetables (Wedepohl and Schwedt, 1987), the lectin concanavalin A from the jack bean (El Rassi et al., 1988), ovotransferrin from egg white (Al-Mashikhi and Nakai, 1987). Gardea-Torresdey et al. (1988) describe an "alga-modified carbon paste electrode" for bioaccumulation and subsequent voltammetric quantitation of metal ions.

There is continuing work on the chemistry and biochemistry of metal-complexing drugs, not only to elucidate their structure but also to better understand the mechanism of their action and the reasons for unwanted side effects. These include drugs for blood pressure and cardiovascular problems (Adachi et al., 1988a; Balman et al., 1988; Christie et al., 1988; Gross and Prohaska, 1988; Hammond et al., 1988; Peters et al., 1988b; Sugiyama et al., 1986), strong metal chelating agents used to treat Wilson's disease patients (Trombetta et al., 1988), drugs used to treat inflammation (e.g. Roch-Arveiller et al., 1987; Shetty and Melethil, 1987), anticonvulsant and emetic drugs (Palm et al., 1986; Ueno et al., 1987), antitumour and antineoplastic drugs (Harrison et al., 1987; Hasinoff and Davey, 1988; Litterst, 1988) and antimicrobial agents (Ali et al., 1985; Chatterjee et al., 1988; Lambs and Berthon, 1988; Tumanov et al., 1983). They also include various chemicals used to examine metabolic processes (e.g. Sandy et al., 1987). The chemistry of metal-binding agents used as fungicides (e.g. Gergely and Garaj, 1988a) and anthropogenic agents (e.g. nitrilotriacetic acid or NTA - Wendt et al., 1988; trichlorophenolate - Zainutdinov and Ikramov, 1988) is also important to provide an understanding of the effect of the organics, such as their ability to affect metal bioavailability. Even inorganic materials, such as clay minerals must be considered in evaluating the interaction of metals and organics as these may participate in biologically important reactions (e.g. Ferris et al., 1988).

I.3.8 THE EFFECTS OF COPPER ON GROWTH

Growth is a result of constructive metabolic processes exceeding destructive metabolic processes (i.e., anabolism exceeds catabolism). Since copper is a required trace element for many metabolic processes, a deficiency can affect metabolism and thereby growth. In excess, biologically available copper can react with the wrong organics, reducing the level of constructive metabolic processes and thereby growth. The chemical balance required for growth can also be affected by metal-metal interactions which give an "apparent" deficiency or excess of copper.

Growth of microorganisms is no different than growth of plants and animals, copper is required and excess copper can be detrimental. Organic-copper associations can affect the growth and copper retention by growing bacteria (e.g. Grytten et al., 1987, 1988), cause the production of age pigments in fungi (Pisanti et al., 1988a) as well as their growth and ultimate population levels (Cinto et al., 1986; Wang et al., 1986b). It is this latter effect that allows copper to act as a fungicide (e.g. Barman and Sarma, 1987; Silowiecki et al., 1986).

Growth reduction in plants can be apparent in plant roots with deficient copper. Ozoliiya et al. (1983) comment (translation) that, in cereal sprouts "In our experiments there was a clear description of the reaction of growth in the roots, in the absence of copper on the first day of growth ...". Growth reduction can also be apparent in plants with increasing copper. Lepp and Dickinson (1986), for example, note that when copper was applied to pollen, "Increasing copper concentration inhibited pollen germination, with complete inhibition above 150 ppm Cu." Karataglis (1987) found copper inhibition of root elongation in *Triticum aestivum* and Mench et al. (1988) reports that copper (10^{-4} M) causes a change in the nature of root exudates from corn. Growth of a number of vegetables has been reported to be reduced by excess copper (e.g. Hashimoto et al., 1987). Plant growth may affect the distribution of trace metals within the plant. Szalay and Szeleczky (1986), working with a clone of commercial potatoes, found that tuber Mn was mobilized into the sprout during sprouting while Zn and Cu appeared to be unaffected. Paralkar et al. (1987) provide data suggesting that, during fruiting, fruit copper levels increase in a variety of Sapota (*Manilkara achras*).

Anthropogenic metal is considered as a metal source which could affect the growth of commercial as well as native plants. Long-term use of sewage sludge may introduce metals in amounts sufficient to affect plant growth (e.g. McGrath et al., 1988a) although it is difficult if not impossible to isolate the specific effects of copper. Similar comments can be made about aerosol metal (Ernst and Dueck, 1987). However, Cox (1988a) has demonstrated that, under experimental conditions, wet deposited acidity and copper can inhibit pollen germination and thus reproduction in the poplar *Populus tremuloides*. As well, Folkeson and Andersson-Bringmark (1988) associated copper and zinc levels with impoverished vegetation near an old and a new foundry suggesting a detrimental effect of aerosol metal on plant growth.

In invertebrate animals, copper is often found in structural materials such as the skeleton. Since these can be preserved, they are often useful in examining biological processes throughout geological history. Hickey (1987), for example, discusses the distribution of copper in the skeletal processes of a group of fossil bryozoans from the Ordovician Period, some 450+ million years ago. Excess copper can be used to control undesirable organisms such as fish parasites (e.g. Jana and Ghosh, 1987). With a number of invertebrates, excess biologically available copper has been found to affect survival early in the life history. Ozoh and Jones (1988) note that copper levels of 5 to 100 µg/L inhibited cell division in the embryo of a polychaete worm. Copper, as $CuCl_2$, in this range has also been reported to inhibit normal embryonic development in a marine snail (Contrad, 1988). In the brine shrimp *Artemia salina*, egg hatching is reported to decrease with increasing copper (Liu and Chen, 1987) although the data suggest that the tests should have been redone, perhaps with a flow system similar to that described by van Leeuwen et al. (1988). With the fruit fly *Drosophila melanogaster*, Islam et al. (1987) report that copper sulphate causes a decrease in egg hatchability. Gerasimov (1987) notes that copper inhibited postembryonic growth in the cladoceran *Daphnia magna*, at concentrations of 0.06 mg/L or greater. De Nicola Giudici et al. (1988) found in the isopod *Asellus aquaticus*, that embryonic development was more sensitive to cadmium while juvenile development was more sensitive to copper. There is some evidence that tissue copper levels may increase during the life of a mollusc (Ikuta, 1988) although this does not occur with all organisms. Darlington et al. (1987) note that, in the larval stages of a caddis fly (*Plectrocnema conspersa*), there was an exponential decrease in copper concentration with increasing body weight. They also note a tendency towards seasonality in larval copper concentrations. Changes in metal levels occur in crusaceans as a result of moulting. Copper is sequestered by metallothionein which also assists in the regulation of tissue levels and the use of copper in blood pigment synthesis (Engel and Brouwer, 1988). Metallothionein also helps regulate copper levels in areas of excess metal availability. Jenkins (1986) found metallothionein accumulation of copper in the larval stages of a mud crab (*Rhithropanopeus harrisii*) although they note that (abstract) "Larval growth rates were ... significantly reduced when Cu accumulation in the MT pool exceed(ed) the half saturation constant ...".

Excess copper has been reported to be detrimental to young fish (Gorovaya and Borisova, 1983) and Scudder et al. (1988) found a decrease in hatching time with increasing total copper concentration in the fathead minnow *Pimephales promelas.* They also found an increase in the number of abnormalities with increasing copper. Munkittrick and Dixon (1987) examined reproduction and development of white suckers (*Catostomus commersoni*) in copper- and zinc-contaminated lakes and in a control lake in Ontario, Canada. Fish from the contaminated (15.3 and 13.3 µg/L copper; 253 and 209 µg/L zinc) and control (< 2.0 µg/L copper, 26.1 µg/L zinc) lakes exhibited different tolerance and morphological characteristics. Fecundity was higher in fish from the control lake, larvae from eggs taken from the contaminated lakes were shorter but had a higher tolerance to copper which the authors (1988b) suggest could be due to a maternal yolk factor. The authors comment (Munkittrick and Dixon, 1988a) that (abstract) "Most of the alterations in sucker growth and reproduction appear to be related to nutritional deficiencies as a result of the chronic effects of elevated sediment metals on the food base of the sucker".

Most of the few references concerning the effect of age or physiological condition, on copper in domestic animals, deal with blood copper levels. Blood copper levels in camels has been reported to change during pregnancy (Eltohamy et al., 1986) and, in cows, as a result of lactation (Sampath et al., 1987). Robinson and Blair (1987) obtained evidence that blood copper levels in ram sheep may be controlled by some gonadal property. Rallis and Papasteriadis (1987) report that sheep fetal copper concentrations did not change appreciably during embryonic development.

From work on both laboratory animals and humans it has become apparent that copper can have an effect on reproduction and implantation of the fertilized egg (Peereboom-Steegeman, 1986). Hurley and Keen (1988) discuss fetal and neonatal development in relation to maternal trace element nutrition, considering manganese, zinc and copper. As an example, copper plays an important role in brain development in mammals (Cloez and Bourre, 1987). From cell culture work it is suggested that metal-metal interactions may play an important role in cell development (Carpentieri et al., 1988b). It is not surprising, therefore, that pre-term infants are at risk for copper deficiencies, a conclusion that is supported by the demonstration of trace element loss during fetal development (Matthew et al., 1988). This could be confounded by the action of metal-complexing agents such as metallothionein, before and after birth (e.g. Cherian et al., 1987). Soo et al. (1988), however, point out it is important to maintain adequate trace element concentrations without toxicity. A major factor in neonate nutritional adequacy is the composition of human milk (Kirksey and Rahmanifar, 1988) and a number of studies have examined the trace element status of breast milk at various stages of lactation (DeMaeyer, 1988). Trace element deficiencies are most commonly found at both ends of life, a number of papers have addressed the deficiencies of zinc and possibly copper in the elderly (e.g. Gershwin and Hurley, 1987). As an overview of the effect of copper on growth, in humans as in other organisms, it is important to consider not only the total concentration of the metal but also its chemical state and thus biological availability. Of equal importance is the physiological state of the organism which dictates its requirements for and ability to handle copper as well as other metals.

I.3.9 THE EFFECTS OF COPPER ON COMMUNITIES

The term community, when applied to organisms, means an assemblage of populations living in a prescribed area or physical habitat. There is normally interaction between these populations, normally for food or nutrients. Since trace metals are required for growth and, in excess, can be detrimental, they can affect the members of a community and, through population interactions, the entire community. The composition of a community can be dictated by metal bioavailability which, in turn, can be affected by the metabolites of community members. As an example, available copper has been reported to be greater in soils having perennial grasses than in soils with other forage crops (Roy and Srivastava, 1988). Under conditions of excess copper, the community will be comprised of metal-tolerant species or phenotypes of a species (e.g. Levin et al., 1988; Munkittrick and Dixon, 1988a). In attempting to relate the effect of metal to members of a community, toxicity studies (e.g. Baker and Walden, 1984; Haley et al., 1988) can be of much greater value if the conditions of the study approximate those of the natural environment. Arts and Leuven (1988), for example, note floristic changes in 28 fresh water bodies in The Netherlands which they attribute to the effects of acidification and eutrophication, as well as the metal content.

Kerrison et al. (1988) examined the effect of metals on plankton community dynamics in a small, shallow, fertile lake. Using additions of between 10 and 100 μg/L, cadmium, copper and mercury had little effect on phytoplankton biomass in the summer but caused marked reductions in the winter. They were reductions in certain zooplankton species during the summer, causing reduced grazing and increased phytoplankton biomass. Deniseger et al. (1988) report a change in phytoplankton species composition with decreasing metal concentrations (including copper) in a lake formerly receiving tailings from a copper-lead-zinc mine. Lake Orta in North Italy formerly received copper- and ammonia-laden effluent from a rayon factory which ultimately eliminated or reduced the biota of the lake to very low levels. Since the reduction in effluent input (1980-1982), there has been evidence of recovery. Bonacina et al. (1987) discuss population dynamics of an annelid worm (*Tubifex tubifex*), the first benthic organism to establish sizeable populations in the deeper parts of the lake. Fukushima et al. (1988) examined attached algal flora in four regions of two Japanese rivers that received metal-rich (copper and zinc) runoff from abandoned copper mines. Species diversity as well as abundance was related to copper concentration. In a similar study, Satake et al. (1988) found comparable results with macroinvertebrate communities in three Japanese rivers receiving mine drainage. Effluent from disused mines has been blamed for the absence of both vertebrate and invertebrate species (e.g. Mason and Macdonald, 1988). Roline (1988) notes selection towards metal-tolerant macroinvertebrates in a metal-rich portion of the Arkansas River (Colorado) and Reash and Berra (1987) found a reduction in fish species diversity and abundance in a metal-rich (Cr, Cu, Fe, Ni, Zn) Ohio stream when compared with an adjacent clean-water stream. Clements et al. (1988) used simulated streams in a laboratory to examine the effect of copper on aquatic insect communities. 96-h exposure to $CuSO_4$ (doses = 0, 15-32, 135-178 μg/L) reduced both the total number of individuals and the diversity.

Copper is reported to be acutely toxic to an estuarine microbial community although it is a function of cupric ion concentration rather than total copper (Jonas, 1989). A pCu of 10.1 reduced the bacterial abundance by 60%. Franz and Harris (1988) suggest possible trace metal (Cd, Pb, Cu, Hg) effects on diversity in macrobenthos communities in Jamaica Bay, New York. In an examination of the impacts of trace elements in San Francisco Bay, Luoma and Phillips (1988) suggest that copper is one of the elements of greatest concern. They remark, however (abstract), that "Broad scale impacts will be difficult to determine without a fundamental understanding of ecological processes and a systematic description of the frequency of patches of metal disturbance in the Bay". Work by Roper et al. (1988), in New Zealand, suggests the same thing. Schwinghamer (1988) reports differences in community size-structure that could be related, in part, to proximity to pollution sources in a Norwegian fjord system. However, artificial communities (mesocosm communities) exhibited no significant differences in size-structure that could be attributed to added levels of copper and diesel oil. Warwick et al. (1988) examined the effect of copper and diesel oil on the diversity and abundance of nematode worms and copepod crustaceans living in the interstitial spaces of the mesocosm sediment (meiobenthos). The nematode diversity was unaffected but copepods showed a graded response of decreasing diversity with increasing dose level. Merilainen (1988) points to the need for considering environmental factors other than simply metal concentrations when examining responses in estuarine meiobenthic communities.

Bengtsson et al. (1988), using soil microcosms with indigenous microorganisms, found a reduction in decomposition rate with soil near a brass mill, indicating an effect on microorganism activity. Bengtsson and Rundgren (1988), however, found no linear correlation between soil metal levels near the mill and soil species numbers, diversity or abundance, of a group of soil organisms (Collembola). They did, however, find a relationship with vertical distribution and soil metal concentrations and suggest that it is metal bioavailability rather than concentration and an effect of metal availability on food rather than directly on the organism. Morrey et al. (1988) point out the importance of soil pH as well as soil metal concentration (Pb and Zn primarily) in affecting floristic variation in plant communities on metalliferous mining residues in the northern and southern Pennines, England. Babalonas and Reeves (1988) report an inverse relationship between soil copper concentration and the number of plant species, in a meadow developed on metal-rich soil. However, metal-tolerant species frequently occur in abnormally high densities in metal-rich soils (e.g. Hutchinson and Barrett, 1987) may have special methods of handling or using the copper (e.g. Casida, 1988). Folkeson and Andersson-Bringmark (1988) reports impoverishment of vegetation in a coniferous forest near an old and a new foundry emitting Cu and Zn but virtually no SO_2. Read et al. (1987), working with carabid beetles of six woodlands at varying distances from a source of metal (Zn, Pb, Cd, Cu), found no significant effect of metal concentration on species abundance and species numbers but did on the range of species present.

The interaction of species within a community can be of a more intimate nature than simply a prey-predator relationship. Some species live in beneficial partnership with other species. This symbiotic relationship affects the nature of the community and may dictate the nature of the environment in which the components can occur. Ames and Bethlenfalvay (1987) comment that (page 1313) "The involvement of mycorrhizal fungi in plant growth and nutrition is among the most exciting and challenging areas of agricultural research today: ...". They and others (Pacovsky and Fuller, 1988; Pfeiffer and Bloss, 1988; Raju et al., 1987) point out that these fungi are believed to be involved in plant nutrient uptake, including some metals like copper (e.g. Kucey and Janzen, 1987; Manjunath and Habte, 1988). There is also some evidence that they can ameliorate the effects of excess available metal, including copper, on plants (Burt et al., 1986; see also Bell et al., 1988c). Dixon and Buschena (1988) obtained evidence that of protection with an ectomycorrhizal fungus (*Suillus luteus*) for conifer seedlings (*Pinus banksiana* and *Picea glauca*) in metal-enriched soils. Bell et al. (1988) and Wasserman et al. (1987) found that high levels of metal (Cu, Pb, Zn) is associated with reduced mycorrhizal growth indicating that their metal tolerance is not unlimited. Jones (1987), however, found that growth of copper-treated birch seedlings was depressed by inoculation with ectomycorrhizal fungi suggesting little if any benefit, at least for copper. However, the effect of the fungi varies with the fungal species. The least tolerant species are the most detrimental to copper-treated seedlings (Jones and Hutchinson, 1988). Kucey (1988) concludes that *Penicillium bilaji*, when inoculated in wheat improves wheat growth but indirectly, by solubilizing relatively insoluble forms of Cu, Fe and Zn in soils.

Copper can affect the nature and quantity of metabolites produced by a species and, through this, the nature of the community. Cruickshank et al. (1987) state (introduction) that "Pisatin, a phytoalexin and antifungal stress metabolite of the garden pea ..., can be elicitated also by the application of solutions containing copper or other heavy metals to pod endocarp tissue ...". This metabolite would have an effect on fungal components of the community. Infection of cucumber fruit by *Botrytis cinerea* appears to require formation of laccase, which is a copper-requiring enzyme. The use of a strong complexing agent, like EDTA, represses laccase formation and reduces or prevents infection (Bar Nun et al., 1988). Copper is one of the metals used to combat parasite infection (e.g. Jana and Ghosh, 1987) which improves the health of the host organism. In sap-sucking and gall-forming insects, copper and other metals from the host may be returned to the host or isolated and then stored in the insect (Heliovaara et al., 1987). If copper is returned to the host, there may be elevated levels in the galls produced by the parasite (Brewer et al., 1987). Rumen fauna in sheep have been reported to decrease the availability of copper with a corn silage-soybean meal (Ivan, 1988). With cows, copper supplementation may increase rumen fauna (Bjneev et al., 1983). Copper in intra-uterine contraceptive devices is reported to alter the bacterial flora of the female genital tract which, in turn, plays an important role in the development of pelvic inflammatory disease (Elhag et al., 1988).

I.3.10 COPPER, NUTRITION AND FOOD CHAIN EFFECTS

Because of its essential nature, an adequate supply of copper is needed for normal metabolism. With plants and some animals, this is usually obtained by direct uptake, from the water or soil. With humans and many animals, copper is obtained through food. Tuft's University (1987) published a special report in which they review some of the more basic concepts about the nature and use of trace minerals in the body. Other reviews of the role and nutritional sources of copper include Chandra (1988a), Davis and Mertz (in Mertz, 1987), Klevay (1987), Solomons (1988), Takahashi and Kikunga (1988) and Wachnik (1987a). Davies and Jones (1988) discuss copper in an article entitled "Micronutrients and toxic elements". The effect that trace metals in general and copper in particular have on animals can be indicated by the work of McNaughton who obtained evidence that the mineral content of foods is an important determinant of the spatial distribution of animals within the Serengeti National Park in Tanzania. Copper was one of the minerals although the author suggests that magnesium, sodium and phosphorus are particularly important.

Assessment of metal flux, from food and the environment, through the oyster *Crassostrea gigas*, is discussed by Amiard (1987). In the mussel *Mytilus edulis*, tissue burdens of copper may be maintained during starvation (Uthe and Chou, 1986) suggesting a continuing need for the metal during stress. In examining the relationship between copper toxicity and weight loss in the crustacean *Daphnia magna*, Lazorchak (1987) found that dry weight of the animal was a better predictor of LC-50 values for copper carbonate than total copper. His Ph.D. work suggests greater tolerance with adequate nutrient status and, most importantly, that toxicity is dictated more by metal speciation than total copper. The presence of metal-complexing agents such as humus can affect metal bioavailability and thus toxicity. Hargeby and Petersen (1988), however, also suggest that humus can interact in other ways with the well-being of the organism, possibly related to food conversion. The interaction of metal bioavailability and food is complex and includes the physiological state of both the predator and the prey (e.g. Munkittrick and Dixon, 1988a).

Certain food supplements as well as normal components affect copper uptake and utilization. Dietary EDTA (ethylenediaminetetraacetic acid - a chelating agent) can increase serum copper in chicks (Tufft and Nockels, 1988). Wang et al. (1987a) report that in chicks (abstract), "The primary antagonism between methionine and copper involves the homocysteine moiety, ...". Immunologic stress can decrease methionine in chicks, thereby affecting nutrient copper availability (Klasing and Barnes, 1988). In ruminants, soil ingestion can not only provide an additional source of copper but also elements such as phosphorus which may affect nutrient metal uptake (Arthur and Gates, 1988; Bohra et al., 1988; Garcia-Bojalil et al., 1988). Iron competes with copper absorption (Kumar and Kaur, 1987) affecting mineral utilization. The presence of ruminal fauna so necessary to a ruminant, does change mineral availability. Ivan (1988) reports that faunation decreased the availability of copper in sheep fed a corn silage-soybean meal diet. Since mineral concentrations vary in various feed types as well as the different stages of growth, forage metal supplementation is often advised (e.g. Kabaija and Smith, 1988a). The occurrence of this variation in natural forages is reported to affect southeastern Alaska deer forage (Van Horne et al., 1988) and could thus be an important factor in wildlife success.

Copper is widely used as a supplement for pigs because it stimulates growth and feed efficiency (e.g. Dove and Ewan, 1988; van Heugten et al., 1988; Walker and Danielson, 1988) although not all work has indicated benefit (e.g. Borg et al., 1988). Moser et al. (1988b) found that addition of copper to the diet linearly improved performance of starter pigs but not grower or finisher pigs. There is also a relationship between the efficient use of supplemental copper and other supplemental materials such as vitamins (e.g. Hamada et al., 1986). Shurson et al. (1987), for example, provide evidence that high copper feeding may increase glucose absorption. Part of this may be the effect of the metal on the chemistry of the intesinal tract (e.g. Ivanska and Pysera, 1987; Ivanska et al., 1987; Pysera and Ivanska, 1988). Copper amounts and availability are also affected by the type of feed materials (Kal'nitskii et al., 1986, 1988; Kuznetsov et al., 1986; Raszyk, 1986).

Because of the importance of copper in the diet of economically important animals, evaluations of copper concentrations and availability are routinely made with forage materials (e.g. Bialkowski, 1988; Cohen et al., 1987; Hrdlicka, 1986; Little et al., 1988). Tissue metal concentrations are also of importance in evaluating the effect of forage types and management practices (e.g. Knebusch et al., 1988; Lamand, 1987; Nour and Thonney, 1988). This is especially true with the use of animal byproducts as food materials (Cibulka et al., 1987; Pres et al., 1987) or where anthropogenic metals may be excessive (e.g. Parada et al., 1987). Mills (1987) discusses trace element requirements in animals in terms of biochemical and physiological indicators of copper, cobalt and zinc status. Grace (1986) provides estimations of dietary allowances for copper, for single- and twin-bearing ewes. Metal-metal interactions in feeds have been widely considered with ruminants. Bremner et al. (1987) note that iron and molybdenum are antagonistic to copper metabolism in weaned calves. The effects of molybdenum have long been known, interacting with copper (and perhaps iron) to reduce copper uptake and having the potential to produce an apparent copper deficiency even with adequate total copper levels and copper bioavailability (e.g. Phillippo et al., 1987a; Wittenberg and Devlin, 1987). With ruminants, as with swine, organics in the feed materials can also affect copper availability. Metal complexing agents such as lysocellin, for example, can facilitate metal transport across biological membranes (discussed in Harvey et al., 1988; see also Sappington et al., 1987). Sulphur supplementation has been reported to decrease tissue copper levels in vital organis except the kidney, lungs and skin of lambs (Yadav and Mandokhot, 1988).

Recent reviews of metals in human food materials include a discussion of the metabolic and nutritional requirements of zinc and copper in premature and term infants and children (Denis et al., 1987), iron and copper absorption by men (Nowacka et al., 1986), and trace element nutrition in the elderly (Bunker et al., 1988). In a brief statement, the Nutrition Advisory Committee (1983) of New Zealand provides estimates of the minimum daily requirements for copper - 80 μg/kg/d in infants and (very?) young children, 50 in young children, 40 in older children and 30 μg/kg/d in adults. In an anonymous (1986) article by the Food and Nutrition Board, U.S. National Research Council, serious questions are raised about the adequacy of information on the recommended dietary allowances for copper. The statement is made (page 85) that "In thje absence of evidence of copper deficiency in the population, how can the discrepancy between the data on average copper intake (which is low) and physiological indicators of copper status (which suggest a significantly higher requirement) be reconciled?" This is still a question - as indicated by Klevay (in Raloff, 1989) who points out that one-third of Americans eat less than 1 mg of copper daily, a level studies indicate can foster dozens of changes linked with heart disease, including elevated cholesterol and blood pressure. Most studies indicate that the daily intake of copper is less than the recommended level, especially in groups consuming diets in the lower energy range (Southon et al., 1988). As pointed out by Turnlund (1988) and others, however, the dietary factors that affect bioavailability can affect the efficiency of metal utilization.

The daily intake of copper by Japanese has been estimated at 1.1 mg/d (Santo et al., 1985). In an evaluation of daily copper intake by 6 Japanese housewives, Ikebe et al. (1988) note a level approximately 50% of the recommended allowance. Rivai et al. (1988) found intakes by Java men (2.5 mg/d) and women (1.7 mg/d) to be higher than the daily intake recommended by the World Health Organization. Metal intakes in the past have been estimated for several groups. Rathbun (1987) used skeletal remains to suggest relatively low zinc and copper status in slaves at a South Carolina plantation between 1840 and 1870. Bone specimens from Middle Woodland Amerindians (New Jersey) provided little information on dietary copper status (Byrne and Parris, 1987).

In a discussion of trace element needs in human pregnancy, Campbell (1988) points out that plasma copper levels rise with some 90% of the metal in caeruloplasmin which increasers in pregnancy as a result of oestrogen stimulation. Copper levels of human milk are frequently used as an indication of postnatal nutrient status (e.g. Burguera et al., 1988; Matsuda and Higashi, 1988; Moser et al., 1988a). Element concentrations in various types of infant formulas have been used as an indication of metal availability (e.g. Allegrini et al., 1985). Cooke et al. (1988) comment on the problems of estimating nutrient balances in the preterm infant, pointing out (abstract) "... that factors other than birth weight, gestation, and the type of formula fed have a significant effect on absorption and retention of nutrients and should be considered as confounding variables ..." when interpreting the effects of nutrient supplementation. The method of feeding should also be considered, bolus feeding of human milk supplements has not been found to allow complete uptake of the supplements (Bhatia and Rassin, 1988). Dietary intake of copper in infants 2-12 months of age is considered marginal by Wallace et al. (1988). Copper deficiency can impair growth, especially in infants recovering from malnutrition (Castillo-Duran and Uauy, 1988). Trace element absorption experiments with laboratory animals and infant diet components indicates that copper bioavailability is highest from human milk (25%), followed by cow milk-based formula (23%), cow milk (18%) and soy-based formula (10.3%; Bell, 1987). In spite of the availability of copper in soy-based formula, Chan et al. (1988a) consider the overall mineral status to provide (abstract) "... similar growth, biochemical, and bone mineralization status as solely breast fed infants during (their) first three months of life."

Turnlund et al. (1988) suggest that a dietary copper intake of 0.785 mg/d provides a copper balance for healthy young men, based on individuals kept in a metabolic research unit. Copper intake values for various population groups are given in tabular form later in this review. These include a range of age groups (Shiraishi et al., 1988a; Turnlund et al., 1988), children (e.g. Vanderkooy and Gibson, 1987) and seniors (e.g. Baghurst and Record, 1987; Kling-Steines et al., 1986). Trace element status in athletes is considered by Gladkikh (1985), Nasolodin et al. (1988) and Weight et al. (1988). Umoren (1988) found overall level of copper and zinc intake paralleled that of caloric intake in both physically active and sedentary elderly men and women. However, total cholesterol levels tended to be lower in the physically active individual. In contrast to the initial findings of Umoren (1988), Powers et al. (1987) report that there was considerable variability in micronutrient levels despite adequate nutrient intake in free-living elderly people. Chauhan et al. (1987a) reviews the literature on age-related olfactory and taste changes and comments on the effect of copper in influencing taste function.

Besides monitoring metals in foods (e.g. Andrey et al., 1988), measurements of tissue metal levels need to be evaluated to anticipate potential problems. Nicklas et al. (1987), for example, note that racial differences in hemoglobin-associated metal levels (including copper) may exist independent of racial differences in intake of nutrients and maturational changes. Diet counseling can also be important, especially in cases of trace metal-associated diseases (e.g. Imes et al., 1987). Various techniques are available for monitoring copper uptake and metabolism, including metal budgets (food, fecal material, selected tissue metal levels), stable isotope use (^{65}Cu) and radiotracer (^{64}Cu) use. All have their uses (e.g. Whitley et al., 1987) and problems (Schwartz, 1986).

Copper concentrations in low and very low birthweight babies have been found to be abnormal, either low and requiring supplementation (Halliday and McMaster, 1988) or high, possibly as a result of oversupplementation (Soo et al., 1988). The use of total parenteral nutrition (TPN) is advocated for maintaining adequate copper and other mineral levels as well as semi-synthetic diets for children and older patients with various diseases (Baumann et al., 1987; Pagni et al., 1986; Salis et al., 1986; Shulman, 1987; Takagi et al., 1988a). The importance of an adequate trace metal mix in these situations is obvious, as it is in any prepared food (Fujita et al., 1988a; Shike, 1988). Gropper et al. (1988) comment that (abstract) "Manufacturers of chemically defined medical foods should evaluate composition, specifically molar ratios between minerals, as a basis for product formulation." A number of food additives are designed to provide supplementary copper (e.g. Schubert, 1988). Dietary supplementation of elderly patients has problems analogous with those of the very young. The person is more prone to disease, the capability for uptake and mobilization of trace elements such as copper is often reduced, and quite often a self-chosen menu does not provide adequate levels of essential trace metals. Bogden et al. (1988) noted some of this in their work on the effects of supplentation of the elderly on plasma and cellular concentrations of trace elements required by antioxidant enzymes. Nutritional copper deficiency has also been noted in severely handicapped patients under institutional care (Higuchi et al., 1988).

The composition of food plays an important role in controlling copper bioavailability. Metal-complexing and metal-sorbing agents occur naturally as well as in food supplements (Jorge and Chagas, 1988; see reviews in Kratzer and Vohra, 1986; Schwedt, 1988). Excess vitamin C can decrease tissue copper concentrations and may be associated with a copper-deficient condition (Lonnerdal, 1988). Amino acids are known to affect metal fractionation in the rumen of bovines (e.g. Bineev et al., 1983) and may affect uptake and distribution of copper in pigs (Bell et al., 1988a) and laboratory rats (Nielsen, 1988). In at least one case, the copper-scavenging ability of a natural amino acid found in the sago palm nut is enough to cause a serious effect to humans (Emsley, 1989). Ascorbic acid can affect the uptake of some metals and the average molar ratios of metals, including copper, in invertebrates (Chou et al., 1987) as well as vertebrates. Hsieh and Harris (1987) report that sucrose can enhance the copper-catalyzed oxidation of ascorbic acid. Food preparation and handling can produce changes in total metal, metal chemistry and bioavailability. Transfer of metals can occur from utensils (e.g. Blanco et al., 1986). Nakazawa et al. (1987) found that several trace metals were in "almost" colloidal phase in the cooking solution of cow's milk and potatoes. Copper and some other metals have the ability to enhance oxidation of lipids. If the metal is added as a phosphatidic acid salt, however, it will have lower prooxidant activity than if added as a chloride (Pokorny et al., 1986). EDTA, added as a metal complexing agent, will also prolong the storage ability of oil-containing salad dressing (Warner et al., 1986).

Strain (1988) points out that milk is a poor source of copper and postulates that this is a reason for the assocciation between consumption of cow's milk and the incidence of ischaemic heart disease. Metal bioavailability is greater in human milk than cow's milk (Glazier and Lonnerdal) suggesting the need for greater supplementation with cow's milk infant formulas. Gibson et al. (1988) advocate substitution of soy beverage for 2% cow's milk for hypercholesterolemic men, a modification which decreases serum cholesterol levels without compromising zinc, copper and iron status. Dietary lipids may influence the enzymic antioxidant defence mechanisms which involve copper; in the extreme, copper-deficiency may lead to increased oxygen free radical-mediated tissue damage in atherosclerosis (Carville and Strain, 1987).

Ingestion of instant or black tea has been reported to consistently elevate liver copper levels in rats (Greger and Lyle, 1988). Karkkainen et al. (1988) note a positive relationship between serum copper and alcohol intake. However, there is some indication that moderate alcohol intake may decrease serum copper levels in non-alcoholic elderly people using diuretics (Jacques et al., 1988).

Based to a major extent on work with laboratory rats, there is also some indication that copper deficiency can increase the sensitivity to alcohol (Rosenbaum et al., 1988).

The association between carbohydrates and copper metabolism/effect is complex. Brynhildsen et al. (1988) report that copper (10^{-5} M) was toxic to a microorganism (*Klebsiella* sp.) when glucose carbon concentrations were 10-40 mg/L but not toxic at 0.01-0.1 mg/L. Ingestion of fructose has been directly and indirectly associated with greater dietary copper requirements (Babu and Failla, 1988; Beal et al., 1988; Failla et al., 1988; Fields et al., 1988a,b,c; Lewis et al., 1988a,b,c; Yang et al., 1988) as has the severity of copper deficiency (Bhathena et al., 1988; Fields, 1988). However, at least in laboratory rats, Johnson et al. (1988b) report no significant differences in copper absorption due to different carbohydrates in a single meal. Bowman and Johnson (1986) provide evidence that copper absorption is not affected by fructose, suggesting that the sugar affects the rate of turnover of the metal. The type of dietary carbohydrate has also been reported to have minimal effects on copper-iron interactions in young male rats (Osborne et al., 1988b) although Johnson (1988) obtained evidence (abstract) "... that even small amounts of fructose may cause changes in iron metabolism during Cu deficiency" (in male weanling Sprague-Dawley rats). Lactose may have some of the same copper-associated properties of fructose (Eason et al., 1988).

Dietary fiber can bind copper and reduce its bioavailability. Tissue copper content levels are frequently used to demonstrate the effect of feeding bran or other fiber-containing foods (e.g. Bell et al., 1987; Kabaija and Smith, 1988b; Rockway et al., 1987) as are fecal levels (e.g. Moak et al., 1987). Much of this is due to phytate found primarily in the soluble fibre fractions of grains (Frolich and Nyman, 1988; Nolan et al., 1987); metal-binding ability of the insoluble fibre cellulose is negligible (Persson et al., 1987). Other, actively binding fibre materials include lignin and guar gum (Platt and Clydesdale, 1987) and pectin (Zechalko et al., 1987). Although concern about trace element deficiencies arise from knowledge of the effects of dietary fiber, Morris et al. (1988) found that whole wheat bran did not have a deleterious effect on mineral nutriture of adult men. Others have obtained similar results (e.g. Behall et al., 1988; Held et al., 1988; Johnson and Lykken, 1988; Paulini et al., 1988). Excess amounts of fiber (e.g. 40 grams/day) or fiber-like agents may, however, cause nutrient imbalance (Taper et al., 1988). The action of phytic acid is pH dependent and involves major ions such as calcium as well as minor ions including copper, zinc and iron. Since the chemistry of phytic acid changes with pH as well as ion concentration, the effects on copper bioavailability may also change (e.g. Champagne, 1987).

Copper occurs in drinking water, as a result of natural levels in the water plus metal from water treatment facilities and plumbing. The National Technical Information Service (1988ab,c d) provides several bibliographies on trace metals (including copper) from drinking water. Other drinks can also provide measurable amounts of copper. Other drinks and foods that provide copper include juices (e.g. Dul'neva et al., 1988), beer (Jawad et al., 1987) and wine (Lay and Lieb, 1988, Postel et al., 1986; Tromp and de Klerk, 1988). Stored and preserved foods contain extra copper from the handling of foods (e.g. Ajello et al., 1987; Aldini et al., 1987; Braddock et al., 1988; Leoni et al., 1985; Le Graet and Brule, 1988; Mesallam, 1987; Tamura et al., 1987). Anthropogenic input prior to harvest can also increase nutrient trace metal loads (e.g. Jorgensen, 1987; McKenzie-Parnell et al., 1988).

The input of anthropogenic metals into food chains is of concern because of the possibility that one stage in the chain will concentrate the metal to a level deleterious to humans. Grodzinska et al. (1987) reports a gradual decrease in tissue copper concentration with increasing levels in the food chain of a forested region in Poland. In an examination of uptake and accumulation of copper in a coastal food chain, the body burden increased slightly in the oyster (*Crassostrea gigas*) but not in the oyster drill (Ocenebra erinacea; Amiard-Triquet et al., 1988). In a good review of metal transfer along the marine aquatic food chain, Baudo (1985) found accumulation in the oyster but not in the oyster drill suggesting some control, such as accumulation by metallothionein. Dallinger (1986) discusses heavy metals in limnic food chains, pointing out the effect of polluted habitats on species diversity. High levels of metals, including copper, have been reported from some phases of limnic food chains, which the authors (Dallinger and Kautzky, 1985) comment may cause transfer to higher metal levels. The levels of copper in any food chain will be dictated by the requirements of the organism and the bioavailability (Berdnikov and Dombrovskii, 1987).

I.3.11 ORGANISMS AS INDICATORS OF COPPER BIOAVAILABILITY

Indicator organisms can be used to test for both deficiencies or excesses of metals such as copper. There is increasing use of indicators in an attempt to indicate detrimental effect of anthropogenic materials. This is demonstrated by the number of recent publications describing the use of indicators and by the appearance of summary work such as the book on "Marine Organisms as Indicators" edited by Soule and Kleppel (1988). Other summary work includes Brown (1988a), Reish (1988), Ahlf and Forstner (1988), Levine (1988), Orlando (1985), Ford (1988), Marquenie and Tent (1988), a specific use of plankton communities in Kerrison et al. (1988) and the book on "Ecotoxicology: Problems and Approaches" edited by Levin et al. (1988). The Food and Agricultural Organization of the United Nations meeting on toxicity and bioacumulation of selected substances in marine organisms (1984 but published in 1985) discussed monitoring of toxic effects in natural populations. The National Research Council of Canada's manuscript "Biologically Available Metals in Sediments" (Campabell et al., 1988) discusses the use of indicator organisms. A number of reports discuss the use of equipment and techniques to better evaluate not only the impact of pollutants but also environmental and tissue metal levels. Microcosm and mesocosm use involves contained environment in which manipulations can be made and results evaluated. Their use in marine environments is discussed by Bakke et al. (1988) and Gearing (1988) and in terrestrial environments by Gillett (1988). Taub et al. (1988) report on the interlaboratory testing of a "Standardized Aquatic Microcosm". Abbasi et al. (1988) describes a hydroponic system for studying the impact of water quality on germination and early growth of angiosperms. Elnabarawy et al. (1988) compare the results of three rapid toxicity test procedures to determine which best evaluates the impact of anthropogenic material. Burton and Stemmer (1988) evaluate a battery of environmental impact assessment tests including the use of enzymes. This is interesting because there is increasing use of proteins to indicate environmental conditions (e.g. Petering et al., 1988) but this will be discussed later in this section. Multispecies (community) indication of conditions is also being used more and more (e.g. Kerrison et al., 1988; Seeliger and Sallner, 1988).

Bacteria have been advocated as indicator organisms because of their fast growth rate and their occurrence in most if not all environmental systems. Dutton et al. (1986) report a metal-sensitive bioassay test for toxicity in wastewater systems where copper and other metals can occur at high levels. Anderson et al. (1988) used estimates of maximum growth rate and oxygen depletion rate as measures of microbial toxicity to a number of agents, including $CuSO_4$. Martinez and Vives-Rego (1988) discuss the use of a marine bacteria for ecotoxicological assessment. Using radiolabelled substrates and enzyme-specific substrates, Lee et al. (1988d) describe a bacterial bioassay to predict the potential impact of dumping contaminated sediments in the ocean. Prediction of permissible concentrations of copper in aquatic systems may be possible with data from protozoan communities (Pratt et al., 1987). Measurement of bioluminescence from the luminescent bacterium *Photobacterium phosphoreum* is often used for estimating toxicity although, with freshwater, the ionic strength must be adjusted with possible changes in metal bioavailability (Hinwood and McCormick, 1987). Interactions with metal complexing agents in the water must also be consider as they will affect metal bioavailability (e.g. Vasseur et al., 1988b). In fact, Morel et al. (1988a) used the luminescent bacterial technique to estimate copper complexation by organic compounds.

Fungi and yeasts are used as indicators, of heavy metal effects (Gadd et al., 1986; Morrell, 1987; Newby and Gadd, 1985; White and Gadd, 1987). Pisanti et al. (1988b) found that copper enhanced growth of a marine fungus but also increased production of an age pigment, lipofuscin. Lichens have been used to monitor heavy metals in automobile exhaust fumes (Mankovska and Kyselova, 1987), aerosol metals from other sources (Ammann et al., 1987; Bargagli et al., 1987a,b; Garty and Hagemeyer, 1988; Thompson et al., 1987). Bargagli et al. (1987b) discuss the distribution of metals .within lichens in terms of their use in monitoring programs. Richardson (1988) reports on the mechanisms leading to the accumulation of elements by lichens and Gough et al. (1988a,b) provide baseline elemental compositions of lichens collected at different sites. Garty et al. (1988) found an inverse correlation between lead, copper and adenosine tri phosphate concentrations in a lichen used in an Israeli monitoring program. Mosses have been found to accumulate metals in a manner useful for indicating anthropogenic input. As a result, mosses and moss-containing systems like the "Moss-Bag" monitoring technique are widely used for monitoring aerosol metal input (Gignac, 1987; Lepneva et al., 1987; Makinen, 1987a,b). Low and Lee (1987) report that a moss (*Calymperes delessertii*) could sorb more than 99% of the copper ions in a 400 µM solution in less than 5 minutes, sorption appears affected by metal availability as well as total concentration. Other primitive plants used for monitoring include liverworts, used by (Sarosiek et al. (1987) to indicate water quality.

Algae are primarily aquatic plants and, as such, can often provide an indication of water quality, including the biological availability of copper. Since they occur as part of the attached micro- and macroflora and are important components of the plankton, they also provide an array of organism types for evaluating a range of environmental conditions. Crossey and La Point (1988) evaluated periphyton community structural and functional responses to heavy metals, commenting that inherent variability in the multispecies community complex may place some limits on their use. In an overview of the effect of contaminants on algae, Munawar et al. (1988) suggest that changes in the community structure as well as the physiological activity can provide an indication of effect. In contrast, Wangberg and Blanck (1988) comment (abstract) that "Species-dependent variations in algal sensitivity make predictions from conventional algal growth tests uncertain" in the evalution of the effects of chemicals. They conclude that the phylogeny of the algae may be of importance. Kangas and Autio (1986), in a review of macroalgae as indicators of pollution, conclude that filamentous algae cannot be recommended as indicators of metals in water, in part due to metal bioavailability, in part a result of variability.

Walsh (1988) provides a manual for laboratory toxicity testing with marine unicellular algae. Blaise et al. (1986) developed a simple microplate algal assay technique using the green alga *Selenastrum capricornutum.* In the discussion of an algal assay of heavy metals in wastewater eflluent, Bermingham et al. (1987) used accumulation potential of a series of metals to indicate metal availability. This work is also discussed in Bisson et al. (1989). Anderson and Hunt (0713) give bioassay methods for evaluating toxicity of heavy metals, biocides and sewage effluent using the microscopic stages of the giant kelp *Macrocystis pyrifera.* (The latter authors used zinc for evaluation purposes.) Forsberg et al. (1988) consider metal concentration in the macroalga *Fucus vesiculosus* to be an excellent bioindicator of excess metal availability - if epiphytes, exposure time, alga body part all analysed, etc. are all considered! Vasseur et al. (1988a) note an increase in EC_{50} with increasing metal for *S. capricornutum,* a factor which they did not adequately analyze but suggest is a result of changes in metal speciation. Ahlf (1988b) used the alga *Stichococcus bacillaris* to determine uptake of selected metals, including copper, from suspended sediments in an estuarine water medium. Metal distribution between water, particulates and algae was influenced by salinity.

Using a series of plants, Ruszkowska and Lyszcz (1986) examined the relationship between tissue copper content and plant copper status. They comment (English summary) that "Cu content in the aerial parts is not always an adequate criterion to evaluate copper supply. That index should be supplemented by determining the amount of Cu taken up by plants or by estimating the ration of Cu content to some other nutrient contents (N:Cu, Fe:Cu and Zn:Cu)." Abadia et al. (1988) found that changes in light scattering of leaves were associated with deficiencies of nutrients and some metals (Mn, Fe, Cu) and suggest light scattering as a diagnostic tool for deficiencies. Deficiencies in iron and copper have been reported to cause increases in water potential, transpiration rate and water loss which may be expressed in leaf morphology and metabolism (Sharma and Sharma, 1987).

Tissue metal concentrations in aquatic plants have been suggested as a mechanism to indicate water quality if the unique characteristics of the species are considered (Aulio, 1986). Similar uses have been made of tissue metal concentration in terrestrial plants (e.g. Chrenekova et al., 1987). Seed germination and nature of growth of plants has also been used to indicate metal impact (Chernen'kova and Sizov, 1986; Fiskesjo, 1988; Karatablis, 1987; Ratsch and Johndro, 1987; Zavadil, 1988) as well as plant tolerance (e.g. Mitchell et al., 1987). Growth and tree trunk-base condition have also been used as an indication of environmental suitability (Koenies, 1982; Werner et al., 1987). Cox (1988b) reports pollen mortality in response to air pollution, commenting that although copper by itself did not affect pollen or was stimulatory, in combination with low pH there was an increase in pollen mortality. Fabiszewski et al. (1987) found photosynthesis intensity the most useful in estimating the effect of aerosol metal and SO_2 from a copper smelter.

Animals, like plants and microorganisms, have been widely used to indicate the quality of the environment as well as tolerance to copper. Esquivel (1986), for example, reports that the dispersal life history stage (planula) of a reef coral (*Pocillopora dasicornis*) is more tolerant than other life history stages. Nematode worms have been suggested to be good indicators of anthropogenic effects, including metals (Peretti and Zullini, 1985). They have even been used to predict mammalian acute lethality to metal salts, suggesting that their LC_{50} values are similar to those of mammals (Williams and Dusenberry, 1988). Snell and Persoone (1989) found that the rotifer *Brachionus plicatilis* was a good indicator of excess trace metal levels, including copper. Tissue metal levels of the brittle star *Ophiothrix fragilis* have been used to indicate contamination of the marine environment (Chassard-Bouchaud et al., 1988). The species is an echinoderm commonly found in the bottom fauna of the eastern English Channel, at a depth of about 30 m. Earthworms have been suggested as ideal bioindicators of soil conditions not only because they live within the soils but also obtain nutrients from ingested soils. Stafford (1986) reports a significant correlation between tissue metal concentration in the earthworm *Eisenia foetida* and in the soil. She also used the species to estimate metal bioavailability in dredge spoils (Stafford, 1988). Freshwater tubificid worms, relatives of earthworms, have been used to biologically characterize sediments (Milbrink, 1987). There is some indication that earthworm metal bioassays may replace rodents in determining acute metal toxicity (Serda and Furst, 1987) although the effects with copper have not been adequately evaluated. Ma (1987), for example, notes that heavy metal (Cd, Pb, Zn) accumulation in moles approximates those in earthworms although the accumulation of metals affects normal tissue copper level, interestingly in both the earthworm and the mole.

Molluscs are widely used as indicators, as a result of the sessile (e.g. oysters) or sedentary (e.g. snails; Munzinger, 1987) nature and widespread distribution of certain groups. Phillips and Rainbow (1988) report that the tissue copper concentrations of a mussel, *Perna viridis*, and three species of barnacles (also sessile) were similar and that both groups could serve as biomonitors of metal bioavailability. Metal tolerance and toxicity has been determined for a number of molluscs, particularly bivalves (e.g. Nelson et al., 1988). The National Technical Information Service (1988f) Shellfish: Toxicology Studies bibliography cites references published between October 1982 and May 1988 that concern the effects of a range of toxic substances, on "shellfish" (molluscs and crustaceans). Tissue metal concentrations in molluscs have been used to indicate the biological effects of metals at a variety of locations (e.g. Borchardt, 1986; Campos, 1988; Chen et al., 1986e; Duncan et al., 1987; Green and Hinch, 1986; Miller, 1986; Romeo and Gnassia-Barelli, 1988; Taranaki Catchment Commission, 1986; V.-Balogh and Salanki, 1987). The use of these values must be tempered with the fact that seasonal variation (Marquenie et al., 1985) can occur either as a result of changes in the organism (e.g. Amiard-Triquet et al., 1988) or changes in metal bioavailability (e.g. Kurihara and Suzuki, 1987).

Leglize and Crochard (1987) discuss the use of a filter-feeding, freshwater clam (*Dreissena polymorpha*) to assess metal bioavailability in a stream affected by iron industries. They wisely use transplanted organisms and estimate metal uptake and loss as well as ultrastructural localization, allowing calculation of the kinetics of metal flux through the organism. Widdows and Johnson (1988) report a decline in scope for growth in mussels (*Mytilus edulis*) exposed to increasing concentrations of copper and diesel oil in both a natural and experimental situation. The decline in growth is related to a decline in feeding rate, something that Abel and Papathanassiou (1986) suggest could be measured and used to indicate water quality. (Bakke, 1988, did not find similar tendencies in a marine snail under similar conditions to Widdows and Johnson, 1988.) The success of egg development and the amount of tissue damage has also been suggested as mechanisms to evaluate water quality (Kim and Lee, 1988b). Suresh and Mohandas (1987) suggest that lactic acid levels in the hemolymph of bivalves could provide an indicator of metabolic response in organisms under stress. Farris et al. (1988) used cellulolytic activity in a freshwater clam (*Corbicula* sp.) as an indication of stress in monitoring copper- and zinc-containing power plant effluents. Farley (1988) found preliminary evidence of (abstract) "... relationship between a heavy metal contaminant (most probably copper) and inflammatory lesions in the oyster" (*Crassostrea virginica*). The concentration of metallothion-like organics in the mussel *Mytilus edulis* may be an indication of metal bioavailability (Maryland University, 1987).

Wenner (1988) discusses a number of indicator organisms, including crustaceans, in an article entitled "Crustacean and other invertebrates as indicators of beach pollution". She points out that the vast majority of the World's beaches are sandy yet (introduction) they "... have received little attention in pollution studies, due in part to their seemingly uniform appearance and in part to the difficulty of studying that dynamic habitat." Suter and Rosen (1988) used crustacean and fish LC_{50} and chronic maximum acceptable toxicant concentrations to evaluate their predictive power for assessing risks to marine resources. With respect to the crustaceans, (abstract) "the responses of marine crustaceans are so highly diverse that the concept of a representative crustacean is questionable." Similar problems affect the use of aquatic larval stages of insects, which are often used as indicators of metal bioavailability (e.g. Darlington et al., 1987; Hooftman and Adema, 1988). Insects used as indicators include ants (Stary and Kubiznakova, 1987) and bees as well as their products (Jedruszuk, 1987). Sensitivities of various crustaceans have been measured (e.g. Frankovic, 1986; Moraitou-Apostolopoulou and Verriopoulos, 1986; Thybaud et al., 1987) and have been compared with other groups (e.g. V.-Balogh, 1988). Verriopoulos and Dimas (1988) used proteins to indicate environmental conditions (see also Petering et al., 1988). Multispecies composition of an area is being used more and more as an indication of conditions (e.g. Kerrison et al., 1988; Seeliger and Sallner, 1988).

Fish have been used as aquatic indicator organisms because of their economic importance and the widespread distribution of certain species such as the rainbow trout. Alabaster and Lloyd (1982) discuss lethal and sublethal effects of copper on fish in their book "Water Quality for Freshwater Fish". Copper tolerance has been examined for a number of fish species in the recent literature (e.g. Miyashita, 1988) as has the apparent effect of metal- and organic-contaminated environments (e.g. Cross et al., 1987; Hall et al., 1988b; Hardy et al., 1986). In a study of acidification (copper not included), Hutchinson (1987) found that laboratory tests provided explanation of the conditions affecting fish survival at low toxicant concentrations, partial explanation at high concentrations and poor explanation at intermediate levels. Part of this is due to the varied nature of the response. Moran and Rowley (1987), for example, found an unexpected degeneration of trout olfactory receptors with elevated metal concentrations (Cd, Co, Cu, Zn). Lydy and Wissing (1988) report that sublethal concentrations of copper caused a decrease in the upper temperature tolerance of two freshwater darters. Cytogenetic effects have been reported in fish exposed to elevated concentrations of copper (Chen et al., 1986f) and Segner (1987) suggests that metal-induced changes in liver parenchyma cells can be used to indicate water quality.

Hahn et al. (1985) and Kooiker (1986) discuss the use of magpie feathers as a good bioindicator of heavy metal availability. Holm (1988) presents information on wildlife as an indicator of environmental contaminants, concluding that (page 153) "... the liver might be the organ from roe deer to be sampled and stored in the Specimen Bank." This is because it has levels of metals, including copper, that are high enough to allow ICP detection. Sawicka-Kapusta et al. (1988) used metal concentrations in rodents to detect contamination in forests in southern Poland. Levels in laboratory and agricultural animals have been used as well, to detect variations in natural as well as anthropogenic input (e.g. Hitz et al., 1987; Raszyk et al., 1988) and trace metal speciation (Turner et al., 1988b). Even humans are used as "indicators" of metal concentrations. Boulos and Smolinski (1988) discuss the use of deciduous teeth as indicators of environmental exposure. Lauter (1987) talks about the problems associated with patch testing for allergies such as "brass allergy".

Indicators include organics and physiological processes that respond to excess or deficient copper in a particular manner. Although these are normally within the organism, Jones (1987) discusses the use of peat copper deposition records to reconstruct pollution history. Luminescence is a process which is affected by metals in bacteria (Surowitz et al., 1987), plants (Burger and Schmidt, 1988) and animals (Elsasser et al., 1986). The production of a fluorescent pigment (lipofuscin) known as "age pigment" is increased by excess copper in a microorganism (*Corollospora maritima*; Cuomo et al., 1987; Pisanti et al., 1988a,b) and in the electric ray *Torpedo marmorata* (Aloj Totaro and Pisanti, 1987). The activity of a number of copper-dependent enzymes have been tested as a means to indicate copper deficiencies in plants (Marziah, 1986; Marziah and Lam, 1987) or the effect of pollutants in general (Harrison and Flint, 1987; Lee, 1988b; Livingstone, 1988; Suteau et al., 1988) or specifically, excess metal (Chagnon, 1987; Dutton et al., 1988). Adachi et al. (1988b) discusses an enzyme immunoassay for Cu-, Zn-superoxide dismutase using monoclonal antibodies. Other recently discussed proteins that have been used include certain haemo-proteins (cytochrome P-450; Livingstone, 1988; Stegeman et al., 1988). Continued interest in the metal-binding protein metallothionein and metallothionein-like organics is strongly evidenced by the literature for this year's review (Benson et al., 1988b; Chan et al., 1989; Engel and Roesijadi, 1987; Garvey, 1988; Harrison et al., 1988; Knezovich et al., 1988; Overnell et al., 1988; Viarengo et al., 1988a). Metallothionein is also being used as a diagnostic aid in liver disease with copper retention since much of the copper is metallothionein-bound (Mahy et al., 1987).

The measurement of copper in tissues and the measurement of organically complexed copper is difficult. The problems have led to a need for reference materials of a standardized nature. This need is discussed by Sturgeon and Berman (1988a), in an article about the marine biological reference materials developed at the National Research Council of Canada. Iyengar et al. (1988b) discuss the development of multi-purpose dietary reference materials. Other reference materials discussed in the recent literature include cabbage leaves (Holynska et al., 1987), a mixed human diet reference material (Parr, 1988), human serum (Versieck et al., 1988), a marine reference material of lobster hepatopancreas (Ridout et al., 1988). Finally, in the development of copper "tags" to examine disease situations, Gielow et al. (1988) discuss the production of the positron emitter Cu-64 for radiopharmaceutical use.

I.3.12 TOXICITY

The toxicity of copper is discussed in a review of zinc and copper by Solomons (1988), the review of trace metal speciation and toxicity to aquatic organisms by Hunt (1987), and the discussion of water quality criteria for fish by Alabaster and Lloyd (1982). References concerning specific toxic effects are cited in the "Literature Review of the Effects of Persistent Toxic Substances on Great Lakes Biota" by Fitchko (1986) and National Technical Information Service citations on "Water Pollution Effects of Metals on Fresh Water Fish" (NTIS, 1987b) and "Shellfish: Toxicology Studies" (NTIS, 1988f). Etnier et al. (1987) provide acute and chronic aquatic toxicity data for heavy metals and organic chemicals found at hazardous waste sites. General discussions on toxicity testing are provided by Larsson et al. (1986) and Reish (1988). Ryan and Windom (1988) discuss a geochemical and statistical approach for assessing metal pollution. The latter is of utmost importance since conditions in the field must be considered in evaluating the toxic effects of any agent, a situation pointed out by Alabster and Lloyd (1982).

The toxicity of metals is affected by a number of environmental factors that control metal concentration and speciation (Wang, 1987; see also Hardy et al., 1987; von Westernhagen et al., 1987). Of these, pH is one of the more important (e.g. Payne et al., 1988b). Starodub and Wong (1987), for example, report that in general, the toxicity of individual and multiple metals (Cu, Zn, Pb) to a green alga (*Scenedesmus quadricauda*) increased with increased acidity. Khangarot and Ray (1987) reports a similar response with a freshwater crustacean (*Cypris subglobosa*). However, Blust et al. (1988a) report that with the brine shrimp *Artemia franciscana*, there is a decrease in the biological availability of copper with a decrease in pH of the salt solution. They suggest that this is due to changes in the buffering capability of the medium. Stokes and Campbell (1986) note that a change in pH can affect both metal speciation and biological sensitivity at the level of the cell surface. They point out (page 76) that the two effects "... are antagonistic and will tend to cancel each other; the observed overall response at the organism level to a pH change from 7 to 4, at constant total metal concentration, may well be positive, negative or null." Collins and Stotzky (1988), for example, point out that (abstract) "The toxicity of heavy metals to microbes varies with pH apparently because the specific adsorption of hydrolyzed species of some metals, that occurs at higher pH values, alters the net charge, which affects various physiological functions and interactions with other cells and inanimate surfaces." These comments all indicate the importance of understanding the interaction between metal species and biological effect, they underline how very little we know about copper bioavailability.

In terms of biological effect, a factor that affects the physiology of an organism may also have an effect on the toxic response to copper. Oscillating temperatures are reported to induce metal fluxes and enhance bioaccumulation in cells (Simkiss and Watkins, 1988). Balode (1982) reports that with lead and copper, increased temperatures caused a decrease in the toxic resistance of Baltic Sea diatoms. Thampuran et al. (1982) found little effect of changing salinities (20-30§) on the response of a clam (*Sunetta scripta*) τo ≤6ppm copper. Nutrient limitation has been reported to affect metal toxicity in bacteria (Brynhildsen et al., 1988). It reportedly enhances copper toxicity to the freshwater alga *Chlorella* sp., with phosphorus limitation being more effective than nitrogen limitation (Hall, 1987). With animals, Lazorchak (1987) reports poor food conditions associated with higher copper-induced mortality in the freshwater crustacean *Daphnia magna*. Toxic response can also be affected by interacting chemicals (e.g. Aoyama et al., 1987). Food may also affect metal speciation, metabolites and even food products can complex ionic copper thereby changing bioavailability (e.g. Marquenie et al., 1985). Certain amino acids, for example, are effective copper-complexing agents and reduce the toxicity of copper to phytoplankton (Kosakowska et al., 1988). The biological availability of high levels of copper in swine manure is buffered by the complexing agents in the manure and by diagenetic processes occurring in the soil (e.g. Payne et al., 1988a). However, some anthropogenic materials in municipal wastes and sewage can form bioavailable complexes that may affect human drinkin water as a result of introduction through ground water. Suleimanov and Galiev (1984) note that maximum concentrations of nitrilo tri methyl phosphonic-acid and its copper-containing complex should be 1 mg/L in drinking water. This concept can be of value in bacterial control, copper-carbamates can, for example, be more effective bactericidal agents than copper by itself (Vasseur et al., 1988b).

Toxic effects of copper can be modified by a second metal. With a pepper/tomato leaf spot bacteria, magnesium is reported to either induce copper deficiency or correct copper toxicity (Woltz et al., 1988). As discussed elsewhere in this review, several other metals can have comparable effects. However, metal-metal relationships can also be affected by environmental factors such as pH which are able to control the amounts of ionic metal available for interaction. This is one of the reasons for reduced bioavailability, and biological impact of brass at high pH levels (Haley et al., 1988; Hardy et al., 1988).

A major portion of the toxicity literature deals with effects on specific groups of organisms or specific organisms. Many of these references have been used elsewhere in this review.

1. Microorganisms - Garrett and Schwartz, 1987; Jana and Bhatacharya, 1988; Jonas, 1989; Jones et al., 1987c; Kuti and Bean, 1988; Ladogina and Osokina, 1987; Modamio and Mallo, 1986; Sato et al., 1986; Vacek et al., 1988; Wong, 1988.

2. Plants -
    A. Communities - Mitchell et al., 1987
    B. Algae - Aoyama et al., 1987; Bisson et al., 1989; Dmitrieva, 1985; Favali Hedayat et al., 1987; Vasseur et al., 1988a
    C. Fungi - Newby and Gadd, 1985
    D. Liverworts - Sarosiek et al., 1987
    E. Terrestrial plants - Schmidt, 1988; Tikhomirov et al., 1988

3. Animals -
    A. Sponges - Francis and Harrison, 1988
    B. Corals - Esquivel, 1986; Howard et al., 1986
    C. Nematodes - Williams and Dusenbery, 1988
    D. Rotifers - Snell and Persoone, 1989
    E. Earthworms and polychaetes - Bouche et al., 1987a,b; Clement et al., 1987; Ozoh and Jones, 1988; Varshney and Abidi, 1988
    F. Molluscs -
        1. Clams & Mussels - El-Domiaty, 1987; Kaitala, 1988; Krishnakumar et al., 1987; Nelson et al., 1988
        2. Snails - Khangarot and Ray, 1988; Rani and Ramamurthi, 1987; Shakhmaev, 1984; Singh, 1988

    G. Crustaceans -
        1. Artemia - Liu and Chen, 1987; Moraitou-Apostolopoulou and Verriopoulos, 1986; Verriopoulos et al., 1988
        2. Daphnia and other cladocerans - Lalande and Pinel-Alloul, 1983; Presing, 1987; Wakabayashi, 1988
        3. Ostracods - Khangarot and Ray, 1987;
        4. Copepods - Moraitou-Apostolopoulou and Verriopoulos, 1986; O'Brien et al., 1988; Verriopoulos and Dimas, 1988
        5. Amphipods and isopods - Ahsanullah et al., 1988; de March, 1988; De Nicola Giudici, 1988; Oganesyan et al., 1988; Thybaud et al., 1987
        6. Shrimps and crabs - Chung, 1980; El-Rayis and Ezzat, 1986b; El-Rayis et al., 1985; Liu et al., 1988a; Macdonald et al., 1988; Rani and Ramamurthi, 1987; Rao et al., 1988

    H. Insects - Kuribayashi, 1988
    I. Fish -

1. Freshwater - Baker and Walden, 1984; Guo et al., 1987; Gurovaya and Borisova, 1983; Jangchudjai et al., 1987; Lydy and Wissing, 1988; Miyashita, 1988; Mumtaz and Chari, 1987; Munkittrick and Dixon, 1987; Nishiuchi and Hashimoto, 1988; Rani and Ramamurthi, 1987; Scudder et al., 1988; Shivaraj and Patil, 1988; Zhang et al., 1988a
2. Saltwater - El-Rayis and Ezzat, 1986b; El-Rayis et al., 1985; Wright, 1988

J. Amphibians - Rao and Madhyastha, 1987
K. Cattle, sheep, goats - Bohman et al., 1987; Carson, 1988; Hayashi et al., 1987; Humphries et al., 1988; Solaiman et al., 1988a,b; Mercer et al., 1988; Van Saun, 1988
L. Laboratory mammals - Angerhofer and Taylor, 1988; Bhunya and Pati, 1987; Gulyas and Gercken, 1988; Mason et al., 1987

Resistance to the toxic effects of copper and other metals is not uncommon in organisms. Metal tolerance is most frequently found in areas of adverse habitats, whether due to natural or anthropogenic causes. It has been recently reported for bacteria (Burton, 1987; Casida, 1988), fungi (Arnebrant et al., 1987; Colpaert, 1987), mosses (Beckett, 1986a), algae (Peterson and Swanson, 1988; Nakatsu and Hutchinson, 1988; Twiss, 1988), terrestrial plants (Banasova et al., 1987; Hutchinson and Barrett, 1987), protozoans (Piccinni et al., 1987) and animals (e.g. Jana and Bandyopadhyaya, 1987; Munkittrick and Dixon, 1988b). The ability of organisms to develop metal tolerance is a major problem in agriculture where copper-containing sprays are used as bactericides and fungicides (e.g. Ercolani, 1985; Gangawane and Saler, 1988). However, it provides an ability for colonization and recolonization of metal-rich soils (Alekseeva-Popova et al., 1984; Babalonas and Reeves, 1988).

One of the most interesting cases of apparent copper tolerance is the so-called "copper moss" *Scopelophila ligulata* which is reportedly rare in part due to the absence of high levels of available copper. And yet, Shaw and Anderson (1988) note that is is found at relatively low concentrations of copper in the Appalachian Mountains of North Carolina. Metal tolerance is not a universal trait. Certain species tend to be intolerant, others more tolerant. However, within a species there is often a variable expression of tolerance (Alekseeva-Popova and Igoshina, 1985; Hicks and Rowbury, 1988; Rousos and Harrison, 1987; Shaw et al., 1987). This has been linked to the genetic makeup of some organisms (e.g. Bender et al., 1988; Camakaris et al., 1988b; Fogel et al., 1988; Mellano and Cooksey, 1987, 1988a; Shaw, 1988). One of the mechanisms of tolerance is the ability to bind excess metal. Brown et al. (1988b), for example suggest that binding at the cell surface, to mucilage, may be an important factor in the copper tolerance of the ship-fouling diatom *Amphora coffeaeformis*. Another is the presence of plasmids which have the ability to bind or otherwise tie up excess metal (e.g. Cotter et al., 1987; Diels et al., 1987). Taylor (1987) proposes metal exclusion as a mechanism of metal tolerance in higher plants. This is suggested to be due to immobilization of metal at the cell wall, complexation by root exudates, formation of a redox barrier at the plasma membrane and formation of a pH barrier at the plasma membrane. The copper tolerance of some fungi is reported to be a result of presence of a mycorrhizal "partner" (Burt et al., 1986). Isolation of metal has been suggested as a means of metal tolerance (e.g. Mullins et al., 1985). Organic acids have been reported from plants, in response to excess metals (Vergnano Gambi and Gabbrielli, 1987). Metal-binding polypeptides and proteins are routinely proposed as tolerance mechanisms (e.g. Jackson et al., 1988; Kubota et al., 1988; Mehra et al., 1988; Thurman et al., 1988; Tomasett et al., 1988; Verkleij et al., 1988). Glubokov (1986) reports that (translation) "A comparative analysis of the data for salmon and coho (kisutch) shows that vitamin B_{12} increases the toxic resistance of salmon to phenol and Cu."

Metallothionein and metallothionein-like proteins are considered important metal-binding agents. These are discussed in a large number of recent publications (e.g. Armitage et al., 1988; Camakaris et al., 1988a; Delval et al., 1988; Freedman et al., 1988; Maroni et al., 1987; Munger et al., 1988; Palma et al., 1987; Steinert and Pickwell, 1988) and elsewhere in this review. They are also important in metal metabolism under normal conditions (e.g. Engel, 1988). Plant phytochelatins play a role analogous to metallothioneins (Grill, 1988; Grill et al., 1988). In all cases of metal buffering, however, there is a cost to the organism (Wilson, 1988). The redirection of energy from growth or reproduction to the production of metal complexing agents or cell isolation requires currency, nutritional currency, and diminishes the amount available for other body functions.

The evaluation of toxicity assay results requires an understanding of normal conditions and the metabolic requirements of the organism (e.g. Rainbow, 1988). AS Wangberg and Blanck (1988) point out (abstract), "Species-dependent variations in algal sensitivity make predictions from conventional algal growth tests uncertain." To be of use in field evaluations, laboratory test conditions must be maintained and should simulate field conditions (e.g. Bakke, 1988; Pratt et al., 1987; van Leeuwen et al., 1988).

Numerous toxicity tests have been developed or evaluated in the recent literature. These include plate techniques for fungi and heasts (Gadd et al., 1986) as well as algae (Blaise et al., 1986), the commercial "Microtox™" test for water and soils (Morel et al., 1988a) with its drawbacks (e.g. Hinwood and McCormick, 1987), various tests to measure respiratory activity (Elnabarawy et al., 1988; Reteuna et al., 1986), wastewater (e.g. Dutton et al., 1986) and soil (Ahlf and Forstner, 1988) assay techniques. The use of assay organisms like the onion (Fiskesjo, 1988) and various algae (Walsh, 1988) are considered suitable for relative toxicity measurements. Evaluation of physiological tests methods have been made for several groups of aquatic organisms (de March, 1988; Larsson et al., 1986; Suter and Rosen, 1988) as well as laboratory animals (Turner et al., 1988b). Various techniques for interpretation of results have also been formulated and evaluated (Anderson et al., 1988; Breck, 1988; Devillers et al., 1988; Howard et al., 1986).

II - COPPER AND MAN

II.1 USES OF COPPER

Copper is used for a variety of purposes. Some of these relate to agriculture, others to medicine, still others to biological control of undesirable organisms. Recent references concerning the uses and importance, as well as problems, of copper are numerous and are used elsewhere in this review. To provide an indication of the value and importance of copper to man, some of the recent references and patents are given below, in table 1.

Table 1 - Recent references and patents on the uses of copper

Agriculture - fertilizers, yeast and plant nutrients - Adams, 1984; Anderson, 1988; Anke et al., 1986; Aranbaev and Charyev, 1980; Bailey and Hammer, 1988; Boselli, 1987; Brandt and Houghton, 1987; Chakhovskii et al., 1987; Chaplin, 1987; Cheng, 1987; Chumachenko et al., 1988; Chuprikova et al., 1985; Constable et al., 1988; Coventry et al., 1987; Das and Mandal, 1986; El-Hady et al., 1986; El-Sherif et al., 1983; Eun and Sedberry, 1986; Fawzi et al., 1986; Fedun et al., 1988; Firgany and Traulsen, 1983; Gabal et al., 1985; Gardner and Flynn, 1988; Gembarzewski and Stanislawska, 1987; Gordetskaya et al., 1987; Hernandez and Pacheco, 1986; Horvath and Szodfridtne, 1986; Iagodin et al., 1984; Ilamanova, 1982; Isaev and Khalileva, 1987; Ivashov, 1985; Jeon et al., 1986; Kadar and Shalaby, 1984; Kamra and Madan, 1987; Karlinger, 1987; Khodzhaev and Stesnyagina, 1987; Koo, 1988; Kovalevich and Dubikovskii, 1988a,b; Kraehmer et al., 1987; Kudriashov, 1980; Littlemore et al., 1987; Locascio and Fiskell, 1988; Lotfy et al., 1987; Mamo and Parsons, 1987; Marziah, 1986; Mateescu et al., 1988; Matoba, 1988; Matsunaga, 1987; Matveev and Kozlava, 1984; Miller and Bekker, 1987; Morimoto et al., 1988b; Mortvedt, 1988; Moskvichev et al., 1988; Mozheiko et al., 1988; Nikolaeva, 1988; Pacheco and Tailliez, 1986; Pacheco et al., 1986; Panasin and Shirokov, 1988; Pandit and Jafri, 1986; Ponomarev et al., 1987; Potatueva et al., 1985; Prasad and Ram, 1988; Rahmatullah et al., 1987; Raju, 1985; Raju and Deshpande, 1987; Rakosy-Tican and Trifu, 1986; Razuvanov, 1985; Ricci et al., 1987; Ryabchuk, 1987; Saad et al., 1984; Salama and Buzas, 1987; Santanen and Simola, 1988; Sawyer et al., 1987/1988; Sharma and Grewal, 1988; Shuman, 1988; Spiva, 1987; Szakal et al., 1988; Szwonek and Nowosielski, 1984; Teicher et al., 1987; Tyksinski, 1986; Wagner, 1988; Weichelt, 1987; Wenny et al., 1988; Will, 1987; Yao et al., 1987; Yu et al., 1987; Yusupov et al., 1986; Zhao, 1987

Agriculture - livestock - Badresingh et al., 1988; Beranek, 1987; Bialkowiski, 1988; Bineev et al., 1983; Binnerts and Viets, 1986; Boila, 1987; Bolze et al., 1987; Buckley et al., 1987; Burnell et al., 1987, 1988; de Souza et al., 1986; Cromwell et al., 1987; Dove and Ewan, 1987; Egorov et al., 1985; Falkowska and Iwanska, 1987; Givens et al., 1988; Gooneratne et al., 1987a; Hamada et al., 1986; Harvey et al., 1988; Judson et al., 1988; Kal'nitskii et al., 1986; Kureneva et al., 1987; Menten et al., 1988; Moser et al., 1988; Nelson et al., 1988; Petryankin et al., 1987; Podshibyakin et al., 1988; Pond et al., 1987, 1988; Pysera and Ivanjska, 1988; Reid and Shannon, 1987; Rogers and Poole, 1988; Rokicki et al., 1986; Ruda et al., 1988; Safar et al., 1987; Sankoh and Boila, 1987; Schubert, 1988; Southern et al., 1987; Thacker, 1987; Tomes, 1988a,b; Walker and Danielson, 1988; Wang et al., 1987a; Zervas, 1986, 1988a,b; Zervas et al., 1987, 1988; Zheng, 1988

Agriculture - miscellaneous - Becius et al., 1988; Bergmann et al., 1987; Blumenkrantz and Blomstdt, 1987; Khodzhaev and Stesnyagina, 1983; Moens, 1984; Tomes, 1988a,b

Biofouling - Adair, 1987; Alzieu et al., 1987; Arias et al., 1986; Bondon et al., 1988; Bultman and Webb, 1985; Czimmek and Sandor, 1985; Dick, 1987; Goettsche et al., 1988; Igic, 1982; Imamura, 1987; Kanda et al., 1988; Kawabe, 1984; Marcus et al., 1988; Matracka and Uhacz, 1985; McKay et al., 1987; Minichev and Seravin, 1988; Nichols, 1988; Sawashita, 1987a; Sullivan and Liebert, 1985

Dentistry - Bumgardner and Lucas, 1988; Craig and Hanks, 1988; Filler et al., 1988; Grytten et al., 1987, 1988a,b; Lemons et al., 1988; Lucas et al., 1988; Maltz and Emilson, 1988

Industry - food and water quality - Drozdowski and Szukalska, 1983; Iino et al., 1987; Pedersen et al., 1986; Schoenen and Wehse, 1988; Schoenen et al., 1988; Valota, 1987

Industry and Environment - Duncan, 1986; Laine et al., 1986; Melson, 1987, 1988

Medicine - contraceptives - de Castro et al., 1987; Drost et al., 1987; Klitsch, 1988; Soeprono, 1988; Trutko, 1986

Medicine - nutrient supplementation - Brown, 1988b; Shike, 1988;

Medicine - pharmacology - Abe et al., 1986; Andronescu et al., 1986; Arnaud et al., 1987; Bakola-Christianopoulou et al., 1988; Bartmann et al., 1988; Basosi, 1988; Basosi et al., 1987; Berners-Price et al., 1988; Bogush et al., 1987; Brown et al., 1988b; Chatterjee et al., 1986; Chrisey and Hecht, 1988; Dahlund and Olin, 1987; DeNardo et al., 1986; Depreux et al., 1988; Deshpande et al., 1988; de Zwart et al., 1988; Dollwet et al., 1987; Eberhardt et al., 1988; Elo, 1987; Elo and Lumme, 1986; Fang et al., 1987; Francis et al., 1988a,b,c; Green et al., 1988b; Gupta and Jha, 1987; Hac and Gagalo, 1987; Iqbal et al., 1987; Irth et al., 1988; Ishiyama et al., 1985; Issopoulos, 1988; Jackson and Kelly, 1988; John, 1988; Kim et al., 1987; Kishore, 1988a; Kramhoft et al., 1988; Lepri et al., 1988; Litterst, 1988; Melnyk et al., 1987; Mohan et al., 1988; Morishige et al., 1986; Nakahara, 1987; Parashar et al., 1987; Politzer et al., 1988; Rabinovitz and Fisher, 1988; Real et al., 1987; Reddy and Subrahmanyam, 1988; Roberts et al., 1987; Roch-Arveiller et al., 1987; Sakai and Yamane, 1986; Soderberg et al., 1987; Sorenson, 1987a*,b*,c*; Syrkin and Chlenova, 1987; Tahsildar et al., 1988; Wiener et al., 1987; Wilkins and Moore, 1988; Willingham and Sorenson, 1986; Wissler et al., 1987; Wu et al., 1988a; Zvershkhanovsky et al., 1987.

Medicine - miscellaneous - Dollwet and Sorenson, 1985; Fujibayashi et al., 1988; Gabay et al., 1988a,b; Green et al., 1988a; Lee et al., 1987; Sideris et al., 1988

Pest Control - Ackermann et al., 1987; Aggarwal and Mehrotra, 1987; Agrawal, 1987; Albenque et al., 1988; Almeida et al., 1983a,b; Amin and Ullasa, 1985; Anandaraj and Saraswathy, 1986; Bailey, 1988; Bal et al., 1987; Balasubrahmanyam et al., 1988; Bebee, 1988; Bender et al., 1988; Bihari et al., 1987; Bovykin et al., 1987; Broscious and Kirby, 1988; Campacci and Chiba, 1983; Carneiro Filho et al., 1983; Cooksey, 1988; Cox and Kasimani, 1988; Doster and Bostock, 1988; dos Reis et al., 1983; Eswaramurthy et al., 1988; Frey and Wagner, 1988; Gadzhie and Garaev, 1987; Gargie and Roy, 1988; Grimm and Vogelsanger, 1987; Gupta, 1987; Gupta and Verma, 1988; Eskes et al., 1985; Gupta et al., 1987a; Habicht and Muller, 1988; Hammer and Marois, 1988; Helaly and Badran, 1988; Hussain et al., 1987; Igarashi, 1987; Jones et al., 1987c; Joshi et al., 1988; Kale et al., 1988); Karadzic, 1987; Khadikar et al., 1986; Krutova, 1981; Kumar et al., 1987; Legard and Schwartz, 1987; Leite, Jr. et al., 1987; Lukade et al., 1985; Majumdar and Som, 1987; Manoussakis et al., 1987; Mansk and Matiello, 1983; Maringoni and Kimati, 1987; Mathur and Shekhawat, 1986; Matiello and Mansk, 1983a,b; McInnes et al., 1988; Medina Urrutia and Stapleton, 1986; Miguel et al., 1983; Mishra and Rath, 1988; Moriya, 1987; Narashimhudu et al., 1987; Natchev, 1988; Nugent and Waller, 1988; Obata et al., 1988; Piening et al., 1987; Plensak and Kovbasiuk, 1987; Price and Lenne, 1987; Prior and Ryder, 1987; Rajak and Pandey, 1987; Ramadan and Abdel Salam, 1986; Ramos et al., 1987; Ramsey et al., 1988; Rao et al., 1988; Reddy, 1987; Rodrigues et al., 1987; Ruppersberger and Ley, 1987; Sagi and Sipos, 1987; Santos and Medeiros, 1981; Sawashita, 1987b; Secor et al., 1988; Sharma, 1987a,b; Sharma and Parashar, 1988; Silowiecki et al., 1986; Simons, 1988; Singh, 1988; Singh and Agarwal, 1987; Singh et al., 1985, 1987c, 1988a; Synowiec et al., 1987; Szabo et al., 1988; Tao et al., 1987b; Thind and Jhooty, 1987; Tromp and de Klerk, 1988; Tumanov et al, 1983; United States Environmental Protection Agency, 1986, 1987a; Verma and Singh, 1987; Wicks and Vogelzang, 1988; Yahya et al., 1988; Yamaguchi, 1987; Yano et al., 1986; Yasuyoshi and Kurashige, 1987; Zubrzycki and de Zubrzycki, 1986

Technology - science and medicine - Crook, 1988; Donato et al., 1988; Hara et al., 1988; Jovanovic and Stankovic, 1988; Kitamoto et al., 1988; Mertens et al., 1988; Shing, 1987; Ugalde et al., 1988; U.S. Dept. of Health and Human Service, 1988

Wood Preservation - Avramidis, 1988; Bergervoet, 1984; Bultman and Webb, 1985; Egerton et al., 1987; Goettsche et al., 1987; Gorshin and Maksimenko, 1987; Goettsche and Marx, 1987; Hosli and Ruddick, 1988; Imsgard and Jensen, 1987; Jewell et al., 1985; Keith and Chauret, 1988; Kleemann and Guenter, 1988; Leightley, 1985; Marx et al., 1987; Mitchoff and Morrell, 1988; Morrell et al., 1988; Mowius et al., 1988; Ostmeyer and Elder, 1986; Ostmeyer et al., 1988; Ruddick, 1985; Winandy and Boone, 1988

* Patent document from INCRA-supported research - treatment of convulsions and epilepsy with organic copper compounds.

II.2 ANTHROPOGENIC COPPER - NATURE AND EFFECTS

Like other metals, copper can be introduced into natural environments as a result of man's activities. Aerosol metal emissions are, for example, considered to be a major source of anthropogenic metal (e.g. Lean, 1985). Nriagu and Pacyna (1988) list major emission sources of trace metal release into the atmosphere and indicate the greatest release of copper to occur from metalurgical sources. This includes metal from industrial sources such as smelters (e.g. Bravo-A. et al., 1988a) and may be linked to events producing acid rain (e.g. Baedecker, 1988; Wellburn, 1988).

Biological effects of metal contaminants in aerosol, soil and aquatic systems are discussed in a variety of publications (e.g. CEP Consultants, Ltd., 1986; Clarkson et al., 1988; Coughtrey et al., 1987; Davis and Denbow, 1988; Dumont, 1987; Feind, 1984; Hakanson, 1988; Hoelscher and Walther, 1986; International Council for the Exploration of the Sea, 1988; Kimura, 1988; Lakshminarayana, 1987; Mathy, 1988; Narbonne et al., 1988; Seeliger and Lacerda, 1988; Seeliger et al., 1988a,b; University of Maryland, 1985; Wolf et al., 1988a,b). Many of these articles consider the nature and importance of metal transport (e.g. Coughtrey et al., 1987; van der Zee et al., 1988) and may include the factors affecting mobilization as well as movement of copper in various natural environments. Hellawell (1988), for example, comments on the importance of water chemistry in determining metal leaching and transport in rivers and streams. Hellmann (1987) discusses the use of metal load at different river discharge levels, to estimate long-term trends in metal transport. Anikiev (1987) comments on the physical and geochemical parameters associated with dispersion of anthropogenic metals and hydrocarbons in the ocean.

In a discussion of the maximum permissible concentrations of heavy metals in soils, Il'in (1985) comments on the need to consider soil both as a physical substance, with chemical and mechanical properties, and as a medium for growing crops. This is of importance, especially if anthropogenic material is used as a fertilizer (e.g. Kapetanios et al., 1988). Hortensius and Meinardi (1988) review some of the steps being taken to develop a world-wide standardized approach to soil problems, commenting on the formation of a technical committee in the International Organization for Standardization, to formulate general sampling and analytical techniques. A comparable approach has been made with marine pollution problems, by the United Nations (FAO; Joint Group of Experts on the Scientific Aspects of Marine Pollution, 1986). In general, strategies for control of water and soil quality (e.g. Economopoulos, 1986; Williamson, 1988) are becoming more widely and frequently discussed.

Discussions of copper and other metals are included in the four-volume proceedings from the 1986 World Conference on Large Lakes, edited by Schmidtke (1988a-d). Terytze (1987) provides a brief review of metal input and dispersion in freshwater systems, commenting on various pollutant pathways. Any evaluation of the effect of copper in aquatic systems must include an evaluation of the physical and chemical characteristics of the environment (e.g. Burgan and Grieb, 1988; Campbell et al., 1988; Hem, 1985; Ryan and Windom, 1988; Salomons et al., 1988a,c; van Donk, 1987) as well as metal fluxes (e.g. Environmental Research Laboratory - Duluth, 1988; Lum et al., 1988). Metal concentrations and water and sediment parameters form the basis for the evaluation of water quality (Kasza and Winohradnik, 1987; Myllymaa, 1987; Myllymaa and Murtoniemi, 1986; Ross et al., 1988; Squires, 1987). The management of water quality, whether for copper or any other natural or anthropogenic agent, thus integrates environmental chemistry with toxicology (Nriagu and Lakshminarayana, 1989). Rehabilitation of freshwater environments such as parts of the Great Lakes requires consideration of geochemical and hydrographic situations (Harlow and Hodson, 1988; Holmes, 1988; Mudroch et al., 1988; Thomas et al., 1988c) as well as biological impact (Fitchko, 1986). Management of soil or sediment quality requires considerations analogous to those of water quality (e.g. Forstner, 1988; Kaes-Hoppe, 1987; Marquenie and Tent, 1988; Mogollon et al., 1987). An understanding of soil metal chemistry may also be useful in reconstructing anthropogenic metal sources in the present (Engineering-Science, Inc., 1986; Malmer, 1988) and input in the past (Jones, 1987).

A great deal of effort has been expended to evaluate the level of anthropogenic input (metals and organics) and their biological impact in the North Sea (Huiskes and Rozema, 1988; Kersten et al., 1988; Salomons et al., 1988a,c) and Baltic Sea. Extensive hydrographic and chemical measurements in the Baltic and North Sea were undertaken in 1986 and 1987 by the Deutsches Hydrographisches Institute. Concepts and data appear in a series of papers and reports by the Institute (1985, 1986a-d) as well as by some of its individual members (e.g. Konig and Becker, 1988). Krostoffersson et al. (1986) review previously-published data on metal concentrations in the Gulf of Bothnia. Nolting and Eisma (1988) report elevated particulate copper levels in the western central and northern North Sea and along the Norwegian Channel. Leatherland (1987a) comments on metal concentrations in the "Report of the North Sea Forum", pointing out that except in the Southern Bight, metal levels in the offshore areas are only slightly above "natural" levels. In a report by the Baltic Marine Environment Protection Commission (1987), it is reported that (page 102) "It is unlikely and not to be expected that copper and zinc will affect the human organism through the marine environment." In contrast, localized areas of anthropogenic input are noted to cause an increase in tissue copper concentration and a decrease in survival of the mussel *Mytilus edulis*. Krell and Roeckner (1988) used an atmospheric transport model to simulate deposition of cadmium and lead into the North Sea (not copper).

In considering copper as well as other metals, estuaries and marshes provide unique environments as well as unique hydrographic and sedimentary conditions (Beeftink and Rozema, 1988). A number of recent references deal with characteristics and metal concentrations in particular estuaries. These include the Rhine (Van Urk and Kerkum, 1988), Elbe (Irmer et al., 1986) and Scheldt estuaries (Wollast, 1988) in Europe and the Humber and Thames estuaries (Morris, 1988) in the U.K.. Allen (1988) uses a "pollutant-based" model for dating sediments in the Severn Estuary (U.K.). Pravdic and Juracic (1988) discuss the "environmental capacity" of the Krka River estuary (Yugoslavia) for copper and suggest that (abstract) "Restrictions should be imposed on new activities such as the use of copper-based antifouling paint on boats and yachts entering and mooring in the Prokljan basin" (near the town of Sibenik). Nichols (1988) suggests that the best way to reduce antifouling paint impact is to train people not to repaint until absolutely necessary. Dorten (1986) presents metal concentrations (Zn, Cd, Pb, Cu, Ni, Co, Hg) in estuaries of the Rhone, Ebro, Po and Arno rivers, commenting on some of the effects of estuarine processes. McCain et al. (1988) review metals in a discussion of marine pollution problems along the North American West Coast. A similar approach is taken by Dethlefsen (1988) on aquatic pollution problems in Europe although he does not provide specific information on copper.

The results of geochemical studies of soils have appeared in several recent publications (Fleischhauer, 1988; Nowlan et al., 1987). Anthropogenic copper and other metals in soils is of concern, especially if biologically available metal levels become high enough to cause physiological problems to plants and animals. Although much of this literature is covered later in this section, a brief review of the topic is given by Konig (1986). Restoration of industrial sites (e.g. Finnern, 1988), like restoration of freshwater, requires an understanding of the geochemistry of the soil in addition to knowledge of the levels of metals and other anthropogenic materials.

The toxicity of anthropogenic metals, including copper, is a result of metal bioavailability which is controlled by metal speciation as well as metal concentration. A variety of meetings, papers and manuscript reports present information on both metal toxicity and bioaccumulation in estuarine and marine organisms (Amiard, 1988b; Dorigan and Harrison, 1987; Else et al., 1987; FAO, 1984, 1986; Munawar et al., 1988; Parker, 1988; Pearce, 1987; Reijnders, 1988; Reish et al., 1988), freshwater organisms (Alabaster and Lloyd, 1982; Cosson et al., 1988; Hallett and McNaught, 1988; Schreier et al., 1987; see also NTIS literature citations, 1987b) and soils (Diels et al., 1988; Sun et al., 1987a) as well as aerosol-affected mosses and lichens (Ammann et al., 1987; Makinen, 1987c). Several recent publications address the use (e.g. U.S. Environmental Protection Agency, 1987a) and discharge (e.g. Saliba, 1986) of metal-containing agents as well as soil quality standards (Ruzhitskaya and Torchinskaya, 1988; Vegter et al., 1988; see also Sondermann, 1988 - addressing the legal aspects of soil protection) and research needed to provide adequate assessment (e.g. Ahlf and Forstner, 1988; Pratt et al., 1987; Strachan, 1988).

Mining, Smelting and Metal-Working

The extraction of copper and its subsequent working into economically useful materials and items has had an effect on the nature of man (Bahr, 1985) as well as having a potential effect on the environment. Some of the environmental problems are discussed in "Chemistry and Biology of Solid Waste", edited by Salomons and Forstner (1988), which considers mine tailings and dredged material. König (1986) and others have reported elevated metal concentrations in soils near mining and smelting operations. Biological impacts of aerosols from smelting and industrial regions are considered by Wellburn (1988) and risk assessment for humans is now being considered (Mazumdar et al., 1988).

Deposition of mine tailings and their biological impact has been examined in several recent publications. Ellis (1987, 1988) comments on the importance of monitoring to determine and ultimately reduce biological impact of marine and coastal mines discharging into the oceans. Winsby (1986) comments on the need for adequate analytical methods and review procedures for assessing the impact of mines on aquatic resources in British Columbia. Wastes discharged on land provide leachate sources for introduction into aquatic environments (e.g. Jeffery et al., 1988). Allard et al. (1987) discuss the environmental impacts of an old mine tailings deposit in Sweden, commenting on the distribution and neutralization of acids and the distribution and chemical speciation of metals. They give average background copper levels in the groundwater and surface water as 0.3 and 0.6 μmol/L and leachate concentration as 132 μmol/L. In a discussion of metal concentrations and water pathways for leachate from the same mine, Sanden et al. (1987) conclude that (page 311) "The direct and indirect influence of hydrology on the variations in metal concentrations have to be taken into account when management of the metal pollution from the deposit are considered." Similar problems occur in a number of abandoned mines (e.g. ERickson and Deniseger, 1987). In an assessment of metal mobility in dredged material and mine waste, Forstner and Kersten (1988) point out the tendency for elements introduced with solid waste material to be less stably bound than those in natural systems. Microorganisms are known to extract minerals from mineral-bearing substrate (e.g. Chaschina and Kucharchuk, 1988) which can enhance leaching of spoil piles. Plant growth on mine spoils has been examined in terms of plant tolerance (Fabiszewski, 1986) and replanting (Ernst, 1988). In most instances, there is a selection for plants tolerant to high levels of the metal elevated in the soil, a feature which suggests the use of certain species in revegetation (e.g. Fabiszewski, 1986; Sopper and McMahon, 1988).

Mine spoils released into aquatic systems can undergo diagenesis. However, the nature of the changes are dependent on the nature of the tailings, the receiving waters, and the rate of burial (Pedersen and Losher, 1988). Release of metal, including copper, from coal strip-mine lakes, for example, is often driven by the acidic conditions of the medium (e.g. Brugam et al., 1988). Copper release into a New Guinea river from a gold-copper mine is reportedly greater than will occur when gold production stops and only copper concentrate is produced (Salomons et al., 1988d). Since elevated copper concentrations and low pH have been associated with reduced biological diversity (e.g. Satake et al., 1988; Yasuno and Fukushima, 1987), it is important to understand the changes occurring in the tailings, or effluent and aerosols from mines and smelters (see, for example MacIsaac et al., 1987; Roline, 1988). However, tissue metal accumulation may not be a reliable indicator of environmental conditions (e.g. Arway, 1988; Bowlby et al., 1988) because of the effect of metal-buffering agents within the organism. Deniseger et al. (1988), for example, found interspecific differences in the response of three salmonids to anthropogenic metals (including copper) in a British Columbia lake. They comment (abstract) that "... trends in rainbow trout data should not be generalized to include all three groups."

Asami (1988) lists nine anthropogenic sources of metals that can increase soil concentrations. Several of these introduce material into the air. Aerosol introduction from copper smelters is primarily sulphur dioxide and particulates (Bravo-A. et al., 1988b; see also Blanchard and Stromberg, 1987), which may contain copper. Other metal-using or metal-manufacturing industries may also introduce copper into the atmosphere (e.g. Williams and Bridges, 1984). In certain areas, copper smelters can account for a major portion of the aerosol sulfur dioxide (e.g. White, 1987) and enough metal to produce elevated tissue levels in plants (Andruszczak et al., 1986; Burns and Parker, 1988; Fabiszewski et al., 1987; Lobersli and Steinnes, 1988; Parada et al., 1987) and peat (Zoltai, 1988) downwind from the sites. However, there are interspecific differences in metal accumulation, sufficient that Bowers and Melhuish (1988) recommend sampling either of several species or of plants known to accumulate the specific element under study. In at least certain cases there also appears to be an effect of emissions from smelters and metal working plants on plant and animal abundance as well as species composition (e.g. Banasova et al., 1987; Bengtsson et al., 1988; Gignac, 1987; Heliovaara et al., 1987; Morrey et al., 1988; Nordgren, 1986; Read et al., 1987). Elevated copper concentrations have been found in the wool of sheep bred in the vicinity of a copper processing plant (Bires and Vrzgula, 1983) and in the livers and kidneys of cattle near a copper smelter (Parada et al., 1987). Metal (including copper) uptake occurs in laboratory animals (Dinkova et al., 1987) exposed to dust from ore deposits and respiratory diseases are suggested to increase in humans working in smelters (Borisov et al., 1987; Lipatov et al., 1988). Increases in metal levels are not always found in humans living in the vicinity of smelters; Jones et al. (1987a) noted hair copper levels within the normal range in inhabitants near the OK Tedi mine in New Guinea. Occupational hygiene improvement and technological changes can be of benefit to workers in metal-working industries (Agzamova, 1987; Chaschin and Rocheva, 1987; Lahiri et al., 1986; Lipatova et al., 1988) although they may not eliminate all the effects of inhaling aerosol material (Lipatova et al., 1988).

The disposal of metal finishing and processing wastes is being restricted to a greater and greater degree (e.g. Economopoulos, 1986; Higgins and Desher, 1988). Rather than simple discharge into the environment, metal-containing wastes from metal processing plants are now being used in a variety of ways. Mozheiko et al. (1988) describes the use of electroplating wastewaters to improve a fertilizer (see also Szakal et al., 1988). Metal-rich recycled spent acids are being used as nutrient sources with only a slight increase in tissue metal levels, at least in corn (Budzynski and Fuller, 1986). Wastewaters from the etching of printed electrical circuits have been used in bactericides and fungicides (Szabo et al., 1988) as well as fertilizers (Kraehmer et al., 1987). Recovery of metals from wastewaters is also being examined (e.g. Nordqvist et al., 1988). Vovkotrub (1988) and Vovkotrub et al. (1988) report increased yield in buckwheat with acid-extracted metal-rich induction-furnace slag supplemented with a microfertilizer. Steel-furnace slags have been used as liming agents without apparent increase in plant tissue metal levels (Yagodin et al., 1988). (Ospanov et al. (1987) describe a copper-containing ammophos produced from copper-rich slag and having both oil-absorption and fertilizer properties.)

Industry

Industrial input of copper into the environment can be of several types and environmental effects are of either short-term or long-term duration. In the case of the latter, the effect is most often near the source. Records of government intervention, after the fact, appear in the literature in documents such as the "Superfund Records of Decision" (e.g. U.S. Environmental Protection Agency, 1987d). The types of industrial input include aerosol as well as dry and wet discharges. Soils often receive industrial materials either directly or secondarily, from aerosol fall-out (Abrahams et al., 1988; Freise et al., 1987;

Tang et al., 1986) as do aquatic environments (Alhonen, 1986; Bonacipa et al., 1988; Davies, 1987; Guilizzoni and Lami, 1986; Hardy et al., 1986; Harlow and Hodson, 1988; Iavloshevskii et al., 1986; Ontario Ministry of the Environment, 1983; Schults et al., 1987; Shariatpanaahi and Anderson, 1987; Varnavas et al., 1987; see also the discussion in van Aalst, 1988). Although industrial input into the atmosphere is frequently considered from the standpoint of metals, byproducts from the same industries, (e.g. sulfates) may have a more pronounced biological effect (e.g. Bravo-A. et al., 1988b). Williams and Bridges report organic carbon as well as a range of metals in airborne emissions associated with kiln-drying of CCA-treated wood. The effect of metal-containing particles can play a number of roles in atmospheric chemistry (e.g. Gomes et al., 1988; Vermette et al., 1988). Leaching of metals from the particles occurs and can increase the metal concentration of rainwater (Williams et al., 1988b). However, leaching of aerosol copper does not appear to be as important as some of the more abundant metals (Fe, Mn). Anderson et al. (1988) note changes in particle concentration and particulate metal values with changes in wind direction indicating a change in the source of the particles. Changes in both aerosol and soil metals can change over long time periods as a result of changes in industry. Millan et al. (1987), for example, note a decrease in metal concentration of aerosol particles at a steel factory site after closure of the factory. The biological effect of aerosol metals is often difficult to determine (e.g. Lepage and Parker, 1988). Uptake by both plants and animals may occur but, when compared with natural uptake and changes, is frequently apparent only in close proximity to the metal source.

Metal concentrations in soils vary with respect to proximity of the metal source, wind direction and intensity and the nature of the soil. Asami et al. (1984), for example, note average street dust copper concentrations in four Japanese cities, ranging from 27.2 to 725 ppm (dry weight). Nielsen and Lokke (1987) provide Danish background soil copper levels ranging from 70-450 mg/m^2. Szerszen et al. (1986) report soil copper levels from 379 to 1920 ppm in the vicinity of two Polish copper works. Finnern (1988) found soil copper levels ranging from 0.7 - >10,000 ppm at a former shipyard (northern Germany). Entry et al. (1987) report that forest soil litter can act as a nutrient and metal sink and suggest that a minimum of disturbance during tree harvesting will retain the greatest amount of litter. Soil humus is known to take up copper (e.g. Folkeson and Andersson-Bringmark, 1988).

Konig (1986) relates soil metal contamination with elevated plant tissue concentrations but comments that pH as well as soil metal concentrations are responsible for plant metal levels. Folkesson and Andersson-Bringmark (1988) found elevated copper concentrations in mosses collected in a coniferous forest near foundries emitting copper and zinc. Working with metal uptake (Zn, Cd, Cu) by a species of pine, Lukaszewski et al. (1988) suggest an association between soil metal levels and recently produced xylem. However, they indicate that it takes some time for this relationship to develop, initial industrial input appears to be buffered to some extent. Tissue metal levels in trees can also be affected by management practices. Vihnanek and Ballard (1988) report an increase in certain metals (but not copper) in Douglas-fir foliage as a result of slash burning.

Whatever the interaction between soil levels, plant management practices or metal-metal interactions, effects of excess metal can occur and can have a biological impact. Metal-containing industrial wastes have been linked with reduced nitrogen fixation in soils (Fayez and Shahin, 1987). Fritze (1987) related reduced forest soil respiration to urban impact near a large city (Helsinki) and comments that heavy metals (including copper) may have played a role in this. Transfer of industry-released copper through the food chain is dependent upon a variety of factors, not the least of which is the ability of many organisms to buffer metal uptake (e.g. Wren et al., 1988; see also Hellawell, 1988). Kolczak (1988), for example, did not find high bone tissue copper levels in hens raised near what the authors call a contaminated district near a steelworks. Uptake of excess metal may occur in animals, especially in organisms tightly bound to some food source, like in an insect which parasitizes a particular group of plant species (e.g. Heliovaara & Vaisanen, 1987). It may also occur in severely-affected habitats (Darlington et al., 1987; Schwinghamer, 1988; Warwick et al., 1988). There may, however, be problems in interpreting which of the industry-released agents is causing the problem. Water quality studies and even environmental workplace surveys often include a variety of contaminants which, individually or collectively, can have a biological impact (e.g. Hall et al., 1988b; Krishnamurthy et al., 1987; Little et al., 1987; Winchester, 1988). Organisms, especially microorganisms, can often interact with metals, affecting both metal chemistry (e.g. Ferris et al., 1987) and metal bioavailability. Reduced biological processing of metal-contaminated soils (e.g. Pop, 1987) may change the nature and rate of soil metal diagenesis. There can be secondary problems occurring to animals as a result of industry-released agents. Schalsa et al. (1987) note the potential for copper deficiency as a result of high aerosol molybdenum input to grasslands near a molybdenum ore-processing plant.

Sewage, Sludges and Wastewater

Sewage, sludges and wastewaters form major sources of anthropogenic metal, especially near population centers (e.g. Azevedo et al., 1988; Byrne and DeLeon, 1987; El-Rafei et al., 1987; Hung, 1987). As a result, there is increasing demand for evaluation of metal levels and geochemical processes associated with their discharge (e.g. Qian et al., 1988). There are also increasing restrictions on disposal of metal-rich wastes (e.g. Yapijakis and Papamichael, 1987). Metal levels are routinely measured in sewage and sewage sludges in many countries (e.g. Samhan and Ghobrial, 1987). Gibbs and Angelidis (1988) report higher metal levels in an anaerobic digested sludge than in an undigested sludge. They suggest that the microbial activity and physicochemical changes occuring during digestion were responsible for the differences. (Since the two sludges were from different plants it could also be the source of the sewage.) Extraction techniques allow estimation of the metal forms in sewage sludge (Rudd et al., 1988a,b). Using some of these techniques, Angelidis and Gibbs (1989) found that organic matter and sulfides are the most important carriers of metals (> 85% with copper) in sludge dumped in the New York Bight. Fletcher and Beckett (1987a,b) examined metal binding by digested sewage sludge and, with copper (1987a), report a cation exchange capacity of 8.86 m-equiv/g at pH 6.5.

An indication of the importance of sewage sludge in metal budgets is the statement that 6.5% of the copper entering the North Sea is introduced in sewage sludge (Parker, 1988). Species diversity of plants and animals in sewage-affected waters are frequently less when compared to comparable unaffected waters (e.g. Reash and Berra, 1987). Nolte (1988) discusses methods to link high concentrations of anthropogenic materials, such as copper, to their sources and Atasi and Rodgers (1986) use a detailed hydrodynamic and water quality model to trace wastewater effluent in the Detroit River. Sewage sludge is frequently dumped in the ocean (Gunnerson, 1988; Parker, 1988; Rowlatt and Limpenny, 1987). Degradation of sewage as well as other anthropogenic material occurs in sea water (Hoppe, 1986). Sediment metal concentrations are frequently elevated but decrease downstream and away from a dump site or sewage outfall (Sultanov and Grigoryan, 1983). Dumped sewage and sewage sludge can also be ingested by marine organisms which can increase tissue metal levels as well as introduce metals into marine food chains (Chapman et al., 1988b; Favretto et al., 1988). Sewage sludge can be stabilized into block form by using fly ash, gypsum lime and Portland cement (Shieh and Roethel, 1989). This type of treatment has the potential of better allowing disposal in seawater.

Techniques and efficiency of metal removal from sewage and wastewaters continues to be important. This is not only because excess metal can reduce sewage sludge digestion (Ademoroti, 1988; El-Gohary et al., 1985; Kouzeli-Katsiri et al., 1988; Macleod and Forster, 1988; McKinney et al., 1988) but also because of the increasing need for uncontaminated water (Azcue et al., 1988; Brown, 1988c). In addition to metals, there are continuing attempts to reduce nitrilotriacetic acid (NTA), a strong metal-chelating agent found in laundry detergents and entering municipal waste systems from home use (Wendt et al., 1988). Brown (1988c) reviews some of the techniques for reducing metal concentrations. Dunbabin et al. (1988) discusses one aspect of the use of a metal-bioaccumulating plant (*Typha domingensis*) in removing metal (including copper) from wastewaters. Kurihara and Suzuki (1987) describe the use of a mud snail to remove metal from reed-sewage sludge compost on paddy soils. The report very high concentrations of zinc and copper in the snail flesh (301 µg Cu/g dry weight) as compared with paddy soil (20.1 µg Cu/g dry weight). Recently reviewed techniques include a discussion of the performance of chemical and microbiological methods in heavy metal removal from anaerobically digested sludge (Tyagi et al., 1988). They comment that pH is an important factor and that chemical techniques allow reduction of the copper concentration to an acceptable level at pH 1.5 or lower but that microbial processes allow similar results at somewhat higher pH levels.

The fate of metals in disposed refuse varies both with the nature of the refuse and the nature of the disposal site or technique. In a discussion of mixed domestic and industrial refuse in U.K. landfill sites, Gregson et al. (1988) give copper levels for domestic, co-disposal, and normal soils of 174, 1329 and 2-100 mg/kg. They comment, however (abstract) that "Whilst each metal is individual in character, results show that co-disposal, if properly carried out, is not as unsound environmentally as commonly portrayed." Land application of sewage sludge and other wastes is widely used but with mixed emotions. Some of these are discussed below but all include the concern for accumulation of excess soil metal (e.g. Goodrich et al., 1988; Sommers and Barbarick, 1986; Vermes, 1988; Whitsel et al., 1988). One alternative to landfill disposal of municipal solid waste is incineration, a technique that would, however, have the potential for major atmospheric input of metal-containing aerosol (Lisk, 1988). References concerning the use of industrial, municipal and domestic wastewaters are given in the National Technical Information Service abstract series for August, 1988. Disposal of industrial wastes includes a variety of materials that may be considered hazardous. Etnier et al. (1987) provide acute and chronic aquatic toxicity data for 36 of the chemicals and metals most commonly found in ground and surface water at hazardous waste disposal sites in the U.S.. Herndon and Moerlins (1988a-e) provide extensive data on the use of sanitary landfills for hazardous waste disposal in Florida. The U.S. Environmental Protection Agency "Superfund Record(s) of Decision ..." discuss hazardous waste and waste sites (1987b,c,e,f,g) or sites containing hazardous wastes.

Municipal sludge is used as a nutrient source in agriculture (Webber, 1988). Because of its nature and metal load, there is often a concern about soil pollution (e.g. Nemenko et al., 1987). For this reason a number of recent papers have addressed the topic of soil metal levels with sewage and wastewater treatment (e.g. Campbell and Beckett, 1988; Davis et al., 1988c; Du and Shen, 1987; Dudley et al., 1987; Genevini et al., 1987; Hue, 1988; Hue et al., 1988; Juarez et al., 1987; Kabata-Pendias et al., 1986; King, 1988b; Metz et al., 1987; Mulchi et al., 1987a; Rappaport et al., 1988; Sanders et al., 1986; Strafelda et al., 1987; Verloo and Willaert, 1986; Waly et al., 1987; Webber, 1983; Webber and Shamess, 1987; Yamada and Imaizumi, 1986). In most instances, however, soil metal concentrations are not reported to cause major increases in plants. Municipal refuse compost is used to ammend soils (e.g. Giusquiani et al., 1988) but is reported to have a pH-affected increase in copper bioavailability to plants grown in compost-treated soils (Gallardo-Lara et al., 1984). van Roosmalen et al. (1987) discuss metal sources and contamination mechanisms in compost production. Rossel (1987) points out that sewage sludge metal levels can be reduced to a level more suitable for agricultural use. Resin-buffering is reported to control tissue metal levels in tomato hydroponic cultures (Combs, 1987). The age of activated sludge also has an important effect on copper uptake (Tijero et al., 1988) as does the nature of any wastewater or sewage sludge treatment (e.g. Kaplan et al., 1987a). Kowal (1986) points out health considerations in using minimum-treated wastewater, primarily from the standpoint of disease organisms rather than metals.

With raw sewage water, Ikram-Ul-Haque et al. (1986) found metal uptake in *Coriandrum sativum* in the order K>Na>Fe>Cu>Pb>Cr>Ni. A comparable accumulation is also reported for vegetables, by Naheed et al. (1988). Waste water is reported to increase soil and plant copper concentrations but to have no effect on the copper levels of young bulls receiving copper-enriched hay (Kosla, 1987). The response to sludge-fertilization of soils has been evaluated for a range of plants. With the tall fescue, a hardy grass species, sludge-amended mine soils are superior to both native topsoil and inorganically fertilized spoils in their ability to sustain long-term production without periodic nutrient supplementation (Roberts et al., 1988). The authors also note that metal uptake was not a problem, even in very high sludge treatments. Comparable results have been obtained with two animal manures used to supplement colliery spoils (Tam, 1987). With tomato shoots, Elliott and Singer (1988) found sludge amendment reduced metal (Cd, Zn, Cu, Ni) uptake, even at the highest rates of sludge use. Hue et al. (1988) note that, with lettuce, tissue copper concentrations were not significantly affected by sludge fertilization. Even with application of high levels of copper, minimal effect to corn tissue metal levels are reported (Payne et al., 1988b). In contrast, Chakrabarti and Chakrabarti (1988) note that with wheat plants primary settled sewage and sludge caused was an increase in tissue concentrations of Cu, Zn, Cr and Mn and a decrease in activities of certain enzymes. MacLean et al. (1987) report increases in soil and tissue copper concentration with legumes and grass in sludge-treated soils. Concentrations were, however, within those normally found. With bermudagrass forage, Lane (1988) found copper concentratins twice as high with sludge-treatment than with conventional fertilization. However, he comments that (abstract) "In no case did the metal concentration pose a threat to grazing animals." McGrath et al. (1988a) report better growth of nitrogen-fixing clover (*Trifolium repens*) on 20-year old farmyard manured soil than on sewage sludge treated soil of the same age. They conclude that this was due to reduced bacterial activity in the sludge-treated soil rather than a direct effect on the clover. Bell et al. (1988b) reports increased tobacco leaf concentrations of Zn, Cu, Mn, Ni and Cd with sludge fertilization although possibly a result of reduced soil pH. Ito (1987) also notes a pH effect on metal uptake, greater uptake by Italian ryegrass and rice plants at lower pH levels. The effect of higher pH on metal availability is used by Chu and Wong (1987) to explain the lower metal content of refuse compost-treated vegetables than sludge-treated plants. Petruzzelli et al. (1986) note that pH, the nature of the soil and the nature of any supplement can be important in controlling mobility and bioavailability of soil Cu, Zn, Cr and Ni. Golovina et al. (1988) report that fertilizers increased the removal of copper by approximately 1.5, when applied to chernozem soil in a "... 10-field sugar beet-cereal-legume" experiment. Working with sludge leachate in a forest soil, Zabowski and Zasoski (1987) obtained evidence of metal-metal and metal-organic competition from the leachate, competition that appeared to lower metal absorption by the forest soil. Prolonged sewage use (10 to 60 years) is reported to be associated with increased tissue concentration of metals (including copper) in navel orange trees (Omran et al., 1988).

Concern about excess soil metal levels has been expressed with the use of animal manures, particularly swine manure. The latter because of the use of copper to benefit growth and reduce parasite infection in developing swine (e.g. Dueck et al., 1987). In an examination of the use of piggery wastes as fertilizers for grass, Dueck et al. (1987) found plant uptake of copper to increase with nutrient level. They comment (summary) that "Copper toxicity also increased until the concentration of macronutrients in the rooting medium itself became toxic, exceeding the copper effect. It is concluded that excessive application of piggery wastes to grasslands will be likely to increase copper toxicity to a problem level for plants and sheep but not for cattle." Klessa et al. (1985) also found higher copper, and zinc, levels in herbage treated with pig slurry. Payne et al. (1988a) report that after eight annual applications of copper-enriched swine manure, there was increased copper in corn leaf tissue but not in grain tissue. They also obtained evidence that a substantial portion of the soil copper had changed to chemical forms not available to the plants. Sawyer et al. (1987/1988) found no adverse effects on corn growth, of ten annual applications of copper-enriched swine manure. They comment (summary) that "Copper levels in corn grain and leaf tissue were not increased by the 10 annual applications ... . Copper concentrations in all plant tissues were well within normal ranges for all treatment on all sites. Corn grain yields were not adversely affected by copper application on the three soils." Ritzl and Reiml (1988) review the potential effects of continued use of liquid manure, commenting on the potential for increased soil copper concentrations. Raju and Deshpande (1985) report no perceptible variations in coffee leaf copper with the use of farmyard manures. Similar results are found in Sharma and Arora (1986) for potatoes. Juste and Tauzin (1986) comment that with farmyard manure, added metals were concentrated in the subsoil as a result of metal-organic complex formation. Sundstrom et al. (1988) comment on the use of catfish pond sediment as a growing medium for bell peppers. No abnormality was reported for copper levels. Chicken manure has been reported to cause copper toxicosis when used as an ingredient in sheep feeds (Garcia Escamilla and Martinez, 1986). Fouda (1988) reports no effect of compost manure on copper content of a sandy soil. With sugarcane trash, Yadav et al. (1987) found that soil metals (K, Zn, Fe, Mn, Cu) increased and pH decreased.

Dredged Materials

Dredged materials are essentially aquatic sediments exposed to reworking and oxidation. If the source of the dredged material includes metal-rich anthropogenic material (e.g. sewage) then the reworking and exposure may cause the release of metals. That is if the metals are in a chemical state available for mobilization. Monitoring dredged sediments, like the monitoring of any other material, has a number of problems associated with it, not the least is the problem of chemical state of the metals.

The design of any monitoring scheme thus requires prior consideration of the nature of the problem, something that is all too infrequently done (e.g. Beanlands, 1987).

General discussions of dredged materials and their composition and effects can be found in the proceedings of the seventh "International Ocean Disposal Symposium" (1988) and in Salomons and Forstner (1988). As Robbe (1988) comments, harbour activity includes processes such as vessel maintenance and effluent discharge which allows accumulation of metal-containing agents in sediments that may be dredged. Metal mobility and diagenetic processes are two of the critical aspects of dredge sediments as well as mine tailings in aquatic environments (e.g. Forstner and Kersten, 1988). Bourg (1988) discusses sorption, speciation and metal mobilization from dredged materials and mine tailings. Boelens (1988) discusses the jurisdictional bodies involved in regulating ocean dumping of wastes, including dredged materials. van Veen and Stortelder (1988) discuss research on contaminated sediments in the Netherlands and comments on the potential long-term effect of disposal of dredged material. Dredged materials have long been used for landfill, often with the accumulation of metals both in the soils and in leachate (e.g. Huybregts et al., 1988; Palamarchuk et al., 1983) as well as in plants growing on the soils (van Driel and Nijssen, 1988; Ernst, 1988; Rhett and Richards, 1986). In a comparison of marsh soils and dredged material from Hamburg harbour, Herms et al. (1988) reports a copper enrichment of nine times for dredged material. Fuhrer (1986) presents values for extractable metals (Hg, Cu, Pb, Zn) in sediments in the Columbia River estuary and attempts to relate these to the potential effects of dredging. He found extractable copper values ranging from 2.6 to 26.7 µg/g and comments (page 35) that "... that concentrations of copper do not appear to be elevated, falling within the range observed in uncontaminated estuaries." Forstner et al. (1987) comment on the tendency of metals in dredge sediments to form sparingly soluble sulfides in the marine environment. Steneker et al. (1988) point out that, at least with dredged material from Rotterdam harbour, leaching studies indicate that mobilized copper is largely in the form of soluble organic complexes. This would tend to reduce bioavailability. As well, ageing of dredged materials appears to reduce their environmental impact (Simmers et al., 1987), as a result of changes in metal speciation. This tends to reduce the environmental impact of sediments when properly used for artificial islands in foreshore areas (Taat et al., 1988). Stabilization is also proposed for dredged sludge (Khorasani et al., 1988).

Word et al. (1987) used microcosm laboratory experiments to assess the accumulation and toxicity of contaminants in material proposed for dredging in Everett Harbor, Washington. They report that, as a result of floatable materials in the sediments, levels of certain metals, including copper, can exceed EPA water quality criteria limits. Munawar and Munawar (1987) discuss the use of phytoplankton to bioassay *in situ* sediment contaminants, including metals. Plants bioassays have been used to evaluate effects of dredge-sediment landfill (Lee et al., 1987). A variety of invertebrates have also been used, both with dredge spoils in aquatic environments and in landfill (e.g. Zhangcheng et al., 1988). Using insect larvae (chironomids), Hooftman and Adema (1988) found little sensitivity to metal-enriched (including copper) freshwater sediments; they do report that bacteria provide a more sensitive evaluation although they experienced difficulty in identifying the toxic agent(s). Willford et al. (1987) comment that (abstract) "... the direct measurement of significant bioaccumulation of toxic substances from the sediments remains the most useful index in a decision-making process."

Anthropogenic Copper from the Production and Use of Fossil Fuels

Because of its ubiquitous nature, copper is found in fossil fuels. Use of these fuels can cause the release of copper, and other trace metals, as aerosols and in oil films on roadways. Aerosol metal emissions and acid rain often go together from the use of metal-containing high-sulfur coals. Disposal of byproducts from the use of fossil fuels has been of concern because of the possible leaching of metals. These kinds of concern have been addressed by a number of individuals and agencies, in discussions ranging from land management at industrial sites (e.g. Hinsenveld and Assink, 1988) to discussions of air quality over large land areas (e.g. Lahmann et al., 1985).

Attempts have been made to monitor the environmental and biological effects of metal emissions from fossil fuels, or exploration for fossil fuels (e.g. Bothner et al., 1987; French, 1985; Ho and Tai, 1988; Tuttle et al., 1986; Woodward et al., 1988; Werner et al., 1987). Lichens and mosses have been used to monitor background levels as well as changes in aerosol metal occurring as a result of power plants (Garty, 1988; Makinen, 1987a; Rope et al., 1988). At least in these three publications, copper enrichments were not reported to occur within the affected areas. In contrast, Arp and Manasc (1988) note an increase in copper and several other trace metals in the xylem of red spruce (*Picea rubens*) downwind from a coal-burning power generator. Kimoto et al. (1986) report no significant effect of a coal-burning power plant, on metal levels in marine water, sediments or biological tissues. Lower (1987) reports no significant offsite effect on trace metal concentrations, from cooling water from the Savannah River nuclear power plant facility. Boehm et al. (1987) report no evidence that oil and gas exploratory activities in the Beaufort Sea have affected trace metal concentrations in sediments and biota.

The combustion of fossil fuels for energy production can lead to the formation of large amounts of fly ash and sulfur waste. Metals in fly ash can be leached by environmental as well as biological and anthropogenic processes (e.g. Gissel-Nielsen and Bertelsen, 1988; Gulyas and Gercken, 1988; Harris and Silberman, 1988). Biological availability of metals from ash, or comparable combustion products, appears to be low (Chrenekova et al., 1987; Garcez and Tittlebaum, 1987; Gissel-Nielsen and Bertelsen, 1988; Korcak, 1988) although may be elevated at low pH values (e.g. Wahlstroem and Pohjola, 1987). Sandhu and Mills (1987) note higher elemental translocation in old ash basins rather than new ones and an association of mobile metal fractions with iron-manganese oxides.

The use of fossil fuels, particularly high-sulfur coals, is associated with acid rain. In terms of copper chemistry, the effect of acid rain is to increase metal leaching rates (e.g. Wang et al., 1988c), the concentration of labile metal (e.g. Gruhn, 1986) and, as a result, metal bioavailability. Wellburn (1988) reviews some of the effects of acid rain in a book entitled "Air Pollution and Acid Rain: The Biological Impact". Legge and Crowther (1987) provide a literature review on "Acidic Deposition and the Environment". McCleneghan et al. (1985) evaluated the water quality of 50 California lakes and streams and comment on their sensitivity to acid deposition and acidification. In a report on water quality near Monroe Harbor and adjacent Lake Erie, Smith et al. (1988) note highest metal concentrations near a power plant discharge.

Determining the causal factors behind the biological impact of acid rain is often difficult simply because of the number of factors that can be affected (e.g. Stuanes et al., 1988). In bacteria, combined acid/copper stress can cause changes in ultrastructure and physiology (Leppard and Rao, 1988). The chemistry of aluminum is often used to explain the negative impact of acid rain (e.g. Merilainen, 1988) although higher tissue levels of other metals, including copper, are often noted in organisms from acid-sensitive lakes (Hinch and Stephenson, 1987). pH is considered a controlling factor for species composition as well as abundance (e.g. Kettle et al., 1987; MacIsaac et al., 1987). However, it acts by affecting the chemistry of the organism and the environment rather than by itself (France, 1987; Rothe, 1986; Schier, 1987). Reducing acid rain effects is desirable but expensive (e.g. Emmel et al., 1988). It requires a change in the operations of emitting industries, a correction of existing acid conditions in the environment, and a better understanding of the chemical effects of acid rain and their impact on biological processes.

Road runoff contains metals as well as hydrocarbons from petroleum products (e.g. Jäger and Cordt, 1988). As a result, soil levels in adjacent areas can become metal enriched (Jacques, 1985). The use of deicing salt on roadways increases this enrichment of copper and other metals (Kelsey and Hootman, 1986). High metal levels in urban stormwater runoff is, in part, a result of road runoff (e.g. Byrne and DeLeon, 1987); the metals are often complexed by natural as well as anthropogenic organics (e.g. Morrison and Diaz-Diaz, 1988). Detention-retention basins are often used in an attempt to reduce the impact of runoff, primarily through sedimentation of metal-containing particles (Meyer, 1985).

Copper Deposits

In the computer search for and personal evaluation of references about the biological importance of copper, a few references on sources of copper were included. With several exceptions, these references concern copper mineralizations produced as a result of hydrothermal and anoxic events as well as copper in manganese nodules. Rowley et al. (1988) discuss hydrothermally-altered porphyry-type copper deposits of Cretaceous age, in Antarctica. Ayuso (1987) describes a hydrothermally-altered porphyry copper deposit in Maine. Skei et al. (1988) found high concentrations several metals (Cd, Cu, Pb, Zn) in the bottom sediments of a deep basin in Norway. They suggest that this is a result of metal sulphide precipitation in super-anoxic water. Sulphide precipitation is involved in a number of mineral deposits (e.g. Hoffman, 1986; Rimoli and Mosetti, 1985). Biogenic sulfate reduction is suggested as one of the formative processes for the middle Tertiary San Bartolo copper deposit in Chile (Flint, 1986). A lagoon-type Proterozoic copper deposit is discussed by Davydov and Chiryaev (1986); a possible origin of a similar deposit is discussed by Brongersma-Sanders (1988). Strong et al. (1987) discuss a metal-rich site in New Zealand, associated with the Cretaceous-Tertiary boundary and presumed to be associated with asteroid impact ejecta.

Copper-containing manganese nodules occur in certain regions of the deep sea (see Gross and McLeod, 1987 and Teleki et al., 1987). The potential value as well as the problems of delineating recovery or "mine" sites in the deep sea is discussed in volume 4 of the Seabed Minerals Series, published by the United Nations Ocean Economics and Technology Branch (1987). Jauhari (1987) provides some general information (but no tabular data) on the classification and inter-element relationships of ferromanganese nodules from the Central Indian Ocean Basin. Concentrations of copper and nickel tend to covary and to increase with increasing Mn:Fe ratios. A ferro-managnese encrustation on the tympanic bulla of a minke whale collected in the same area exhibited Cu:Ni characteristics similar to the nodules (Banakar, 1987).

Recovery - metal and environment

The maintenance of environmental condition and the recovery of metals from process byproducts is of increasing concern. Since problems tend to be of a regional or site-specific nature (Bonacina et al., 1988; Gardner, 1987; Romero et al., 1986), there is a need for regionally standardized protocols for environmental studies (e.g. Becker and Armstrong, 1988; Matheson, 1986). It is also important to examine metal-metal interactions in evaluating the biological impact of copper and other metals (e.g. Bois et al., 1988).

Monitoring of metal-production (e.g. Keller et al., 1987) and monitoring and treatment of metal-containing process water is increasing (Hunt, 1988; Kaminski, 1988; Ridgeway et al., 1988; Versar, Inc., 1987; Yapijakis and Papamichael, 1987) and the use of process byproducts is now widespread. With the copper industry, this includes using process waters in agriculture, for fertilization (Kraehmer et al., 1987; Mozheiko et al., 1988; Szabo et al., 1988). A number of techniques are used to handle or treat aqueous as well as solid wastes, techniques that include metal-recovery (Ali et al., 1987; Archibold et al., 1988; Corapcioglu and Huang, 1987; Dannenberg and Gardner, 1987; Dudley et al., 1988; Gabler and Jones, 1986; Galun and Galun, 1988; Glazunov and Ilyaletdinov, 1982; Harless, 1988; Hunt, 1988; Jansen, 1988; Jurdi et al., 1987; Khorasani et al., 1988; Kocornik, 1985; Krause, 1988; Krofta et al., 1985; Kuyucak and Volesky, 1988; Lefers et al., 1987; Lewis and Kiff, 1988; Monick and Blake, 1988; Monte et al., 1987; Morel et al., 1988b; Moriya et al., 1988; Nordqvist et al., 1988; Pahmeier and Edwards, 1988; Panday et al., 1986; Peschen et al., 1986; Schlossel, 1988; Tan, 1985; Tels, 1987; Ueki et al., 1988; Vorstman et al., 1987; Wikoff et al., 1988). Removal techniques include those used to remove copper from liquors (Jankovic, 1987), from drinking water (Nelson, 1987) and from reverse osmosis membranes used in seawater desalination (Peplow and Vernon, 1987). Reduced copper corrosion in potable water systems can be achieved by the addition of a small amount of phosphate (Miller et al., 1988). They may even include the "recovery" of excess metal within humans (e.g. Pitt et al., 1987). Recovery of waste acids for reuse also includes metal recovery (Stewart and Brouns, 1986). Much of this literature appears in the U.S. National Technical Information Service citation lists on "Wastewater Treatment" (NTIS 1987a, 1988g). Removal of metals and organics from highway runoff is of concern because of possible direct introduction of contaminants into waterways (Jager and Cordt, 1988; Krofta et al., 1984).

Some of the metal-containing industrial byproducts may release anthropogenic metal when exposed to the environment. Metz and Trefry (1988), for example, note an increase in dissolved copper and nickel in parts of an artificial reef constructed from stabilized oil ash waste. (And yet Trefry et al. (1988) comment that dissolved metal concentrations in and around the reefs are at background levels!) Lechich and Roethel (1988) advocate stabilized metal processing waste as reef-building material for use in the ocean. The disposal of various sludges without treatment has the potential to introduce metal, including copper (Albert and Eberlei, 1988; Brown, 1988b; Glindemann and Hamburg, 1988). Information is now available on the chemical properties of the wastes (Fletcher and Beckett, 1987a,b; Shieh and Roethel, 1988) and techniques now exist to reduce the concentration of metals (e.g. McKinney et al., 1988; Rossel, 1987; Tyagi et al., 1988) which will reduce the amount of anthropogenic metal available for dissolution. Alternatives to disposal also exist, such as incineration (e.g. Lisk, 1988), although each as its own unique problems. Incineration has the potential to introduce metal into the atmosphere (Feldman et al., 1988) although considerable effort is being made to minimize this effect (e.g. Finkelstein et al., 1988; Klicius et al., 1988; Lee, 1988a; Teller, 1988) as well as the disposal of incineration residue in a stabilized condition (Breslin et al., 1988).

Reclamation of mine tips and other metal-rich land requires a covering that reduces or eliminates metal seepage into ground water (Gunther, 1988). This is also to reduce the contamination of adjacent soils (Diels et al., 1988). Hearn and Hoye (1988) discuss copper dump leaching and management practices that minimize the potential for environmental release of metal. Soil properties must be considered to reduce the metal availability to plants (e.g. King, 1988b; Zolotov and Svintukhovskii, 1988). The use of organic-rich supplements, such as municipal sludge, can often decrease metal availability as well as provide nutrients to the soil (e.g. Sopper, 1988; Sopper and McMahon, 1988). Mixing with sand is recommended as a technique to reduce metal bioavailability in dredged sludge (Kuntze and Bartels, 1988). Adsorption of metals by sand and sandstone may occur naturally, with groundwaters (Mimides and Lloyd, 1987). Treatment of landfill leachate with peat is reported to remove metals, including copper, although it should be used as a pretreatment, in conjunction with other techniques (McLellan and Rock, 1988). Peat is also reported to enhance plant growth in smelter dust (Kuduk, 1985). Controlled microbiological leaching of copper dumps and copper ores is a well known method of recovering metal (e.g. Grudeva and Grudev, 1986, 1987; Puhakka and Tuovinen, 1986). It is also used to recover metal from other solid wastes (Francis and Dodge, 1987, 1988; Francis et al., 1987). It has also been used with coal fly ash (Wilczok et al., 1986) and digested sewage sludge (Tyagi and Couillard, 1987). Mangroves are reported to remove metals from sediment (Wu et al., 1988a) although input of metal-rich organic material from the trees (i.e., leaves) would occur. Roehlly et al. (1987) discuss the use of a brown seaweed (Cysteoseira barbata) to accumulate metals (Cd, Cu, Pb, Zn) from contaminated marine waters.

III - COPPER SPECIATION AND ITS BIOLOGICAL IMPORTANCE

Copper is one of the more labile metals, occurring in a wide array of inorganic and organic complexes. This causes a problem when discussing metal bioavailability as not all metal species are available for uptake by the organism (e.g. Cross and Sunda, 1985). And yet this is not adequately considered when deriving soil and water quality standards even though the importance of metal speciation may be discussed in justifying the use of "total" or "filterable" metal concentrations (e.g. Williamson, 1988). Information about the nature of copper chemistry occurs in a number of general publications (e.g. Alabaster and Lloyd, 1982; Buffle, 1988; Hem, 1985), specifics of chemical processes and the anlysis of metal species are found in journal as well as symposium publications such as that edited by Landner (1987) and Nriagu and Lakshminarayana (1989) or Oxidases and Related Redox Systems Symposium edited by King et al., 1988. Hunt (1987) provides an excellent review of trace metal speciation and toxicity to aquatic organisms. Martell et al. (1988) discuss structural-stability relationships of metals in environmental solutions and Conklin and Hoffmann (1988a,b) the structure and thermodynamics of transient copper(II)-sulphur(IV) complexes. Honeyman and Santschi (1988) point out some of the problems of predicting metal scavenging in both natural and anthropogenic systems. Tarazona (1988) presents a computer program which is described as a "... simple computer program for both total and ion copper speciation ..." for use in toxicological bioassays. Computer simulation has also been used for estimating copper complex formation in soils (Sanders and McGrath, 1988) and in evaluating random error effects in trace metal analysis (Apte et al., 1988). These publications and those considering the effect of chemical and environmental factors on metal speciation (Byrne et al., 1988; Chester et al., 1988; Demina, 1984; Fanning et al., 1988; Guegueniat et al., 1986; Johnson et al., 1987b; Lin et al., 1987; Osichkina and Tillyakhodzhayev, 1986; Panov and Naumova, 1988; Paulson et al., 1988a; Perovic, 1987; Sakal et al., 1988; Sharma and Millero, 1988a; Singh et al., 1988b; Smyrl et al., 1986; Yamamoto, 1986) and its effect on organism growth (e.g. Folscher and Barnard, 1985) are indications of the importance of metal speciation in examining the biological importance of copper in natural environments.

Metal speciation for anthropogenic metal sources is of importance in determining biological impact. Lee (1988a), for example, discusses a model for the analysis of metal partitioning occurring during solid waste incineration. Iavloshevskii et al. (1986) examine metal lability for several metals (Cd, Cu, Pb, Zn) in water in the area of an industrial complex. Maddock and Lopes (1988) discuss partitioning of anthropogenic copper in an organic rich sediment from a hypersaline coastal lagoon. In an evaluation of copper in the Bay of Brest, Courtot et al. (1988) found a linear relationship between copper and dissolved organic carbon concentrations, approximately 25% of the total copper was (summary) "... chelated by organic matter". The importance of natural metal complexing agents such as humic substances is now, fortunately, being considered in the transport and biological availability of copper (e.g Perttila, 1986). As well, the impact of synthetic metal complexing agents in natural as well as laboratory systems is being evaluated in terms of metal impact (e.g. Linder and Voye, 1988; Willingham and Sorenson, 1988). Hirose and Sugimura (1985) discuss the role of metal-organic complexes in the marine environment. Higgins and Mackey (1987) note strong copper complexing capability in the leachate of the kelp *Ecklonia radiata* during detrital decomposition. The metal chemistry of soil in the vicinity of viable roots is often affected by root exudates (e.g. Youssef and Chino, 1987). Agents such as phytic acid, in plants, can reduce copper bioavailability in laboratory and human foods (e.g. Rockway et al., 1987); Mahajan and Chauhan (1988) report HCl-extractability of metals in millet can be improved by natural fermentation. Blancher and McNicol (1987) discuss the chemistry of copper and other metals, in peatland waters affected by acid deposition. They point out the importance of organic carbon in controlling hydrogen ion concentrations and, as a result, metal speciation. Demina and Belyayeva (1986) discuss an ultraviolet irradiation technique for breaking down metallo-organic suspensions in the ocean. In Indian Ocean samples, they report that an average of 12% of the copper was organically bound. In an examination of metal chemistry in surface waters of the Sevan Lake Basin (Armenia), Oganesyan and Babayan (1987) comment (translation) that "Approximately 90% of the copper and 95% of the iron is found in complexes. ... The dominating Cu complex is (a series) of complexes with molecular mass of 1000-10,000." In North Pacific waters, Coale and Bruland (1988) found greater than 99% of the total dissolved copper was associated with organic ligands at depths less than 200 m. Below 200 m, increasing proportions of inorganic or labile copper species. Mackey and Higgins (1988), using chromatographic techniques, found higher concentrations of organically complexed copper in south-east Australian waters than in waters from the Fiji Basin. Release of metal-complexing agents by the deposit-feeding snail *Hydrobia ulvae* is suggested to provide active regulation of cation activity in seawater environments containing sizable populations of the species (Tournie and Mednaoui, 1986).

Examination of the changes occurring in metal chemistry in nearsurface waters has been expanded to include the effect of photooxidation (e.g. Waite, 1988). Moffett and Zika (1987) provide an excellent discussion of photochemistry of copper complexes in sea water. The direct and indirect effects of photooxidation formed some of the reasons for Sharma and Millero's (1988b) evaluation of the oxidation of copper(I) in sea water. With sulfidic seawater, Dyrssen (1988) found reduction of sulfide complexed Cu(II) only at low values of pE. The solubility of copper in sulfidic waters is reported to be strongly dependent on the activity of solid sulfur (Shea and Helz, 1988). Thus, in an anoxic basin (Saanich Inlet, Canada), Zhan et al. (1987) obtained evidence that the solubility of copper is controlled by bisulfide and/or polysulfide complexes. As demonstrated by Francois (1988), however, sedimentation of copper in Saanich Inlet is probaly associated with a number of chemical processes, including sulfide precipitation.

Copper complexation capability of natural waters continues to be examined as an important factor in estimating natural and anthropogenic metal availability (e.g. Pandya et al., 1987; Tan et al., 1988). Campbell et al. (1988) discuss metal speciation and biological availability of metals in marine and freshwater sediments. Luoma (1986) comments that the impacts of metals in aquatic environments cannot be assessed realistically without a better understanding of the factors controlling metal bioavailability in sediments. In an examination of metal speciation in dated lacustrine sediments, Thomas et al. (1985) comment (summary) that "Good correlation between organic matter, iron, and heavy metals (including copper) seems to indicate that organic matter and some iron compounds should play an important part in the accumulation of heavy metals of non atmospheric origin." Extraction techniques are routinely used to examine the chemistry of metal distribution in sediments and soils (e.g. Ahlf, 1988a; Boniforti et al., 1988; Bradley and Cox, 1988; El Ghobary and Latouche, 1982; Hazra and Mandal, 1988; Joshi and Dhir, 1988; Mashhady, 1984; Poulton and Lum, 1986; Raghupathi, 1989; Samanidou and Fytianos, 1987; Versluijs et al., 1988; Vuynovich and Muller, 1986). Models based on copper partitioning in sediments (e.g. Chen et al., 1987a) and various organics (e.g. Decock and Sarkar, 1987) provide an indication of sediment metal speciation. The effect of dredging activities on sediment metal mobilization has been estimated by elutriating sediments with water and measuring metal concentrations (e.g. Mudroch and Davies, 1985) as well as the organic and inorganic fractions (Steneker et al., 1988).

Forstner (1988) discusses the analysis and prognosis of metal mobility in soils and wastes, commenting (page 8) that "Users of predictive models must be aware of the limitations which are the results of the experimental and theoretical conditions ... used in the development of equations ... ." These concerns also apply to models used to estimate metal mobility in dredged sediments or ocean-dumped sewage sludge. Measurement must be done to verify the results from models (e.g. Sanchez-Andreu et al., 1987; Sanders and McGrath, 1988). Angelidis and Gibbs (1989) report from measurements that, with anaerobically-digested sludge, more than 85% of the copper is associated with organic matter and sulfides. Predictions of copper mobility in soils has been attempted to provide an indication of speciation and bioavailability of anthropogenic metal (e.g. Berdnikova et al., 1986; Dietze and Konig, 1988; Dostal and Vaculik, 1986; Wang et al., 1985; Zou et al., 1988a). Fanelli (1986) discusses the importance of soil chemistry in predicting the mobility of copper and cadmium in ground water. Bergkvist (1987) points out some of the effects of tree species and land management on soil metal speciation in forest soils. Copper cycling in forest stands occurs as a result of throughfall and tree stemflow with the amount of metal cycled dependent, to some extent, on tree type (Mahendrappa, 1987). Agricultural and soil management practices such as liming have been repeatedly shown to affect metal speciation and bioavailability (e.g. Anderson, 1987b; Cordero and Chavarria, 1987; Coventry et al., 1987; Naylor, 1988; Rebowska and Kusio, 1985). The use of copper-containing pesticides also affects the concentration of bioavailable metal as well as total concentration of soil copper (Myrlyan, 1987). The commercial use of metal complexing agents such as nitrilotriacetic acid (NTA) has increased its concentration in sewage-based fertilizers. This, in turn, may have increased the mobility of metal in soils (e.g. Linn and Elliott, 1988). Clean-up of anthropogenic metals in soils may be done although Van Gestel et al. (1988) suggests that this is difficult and often inefficient. Aerosol transport of metal-containing particles is important in the introduction of both natural and anthropogenic copper (e.g. Anderson et al., 1988).

The nature of the organism is also of importance in determining metal deficiency, uptake and tissue metal concentrations (e.g. Johnston and Vestal, 1988; Mahendrappa, 1987; Nakatsu and Hutchinson, 1988). Clark and Gourley (1987), for example, found variation in leaf metal concencentrations (including copper) in sorghum plants as a result of leaf position and genotype. Raghupathi (1989), in a study published by the Indian Copper Development Centre, found that foliar application of copper may be necessary in soils with high metal-binding ability. The chemical form of copper applied, whether to plants or soil, also affects metal uptake by plants and metal retention by soils. Kiekens et al. (1984), working with cucumber plants, note strong accumulation of ionic copper by the roots; transfer within the plant appears to have been enhanced by a positively-charged complex. Interactions of metals with protons has been suggested to affect sorption of metals with algal surfaces (Crist et al., 1988). Hyuga (1985a) reports that the chemical forms of added copper retained by soils decreased in the order chelate > organic > exchangeable > water-soluble. He (Hyuga, 1985b) also found that soil copper bioavailability was increased with certain ions and decreased with others. Diffusion of metals into soil minerals has been shown to occur and appears to reduce metal bioavailability (Bruemmer et al., 1986). Nimara et al. (1987) provide an extraction method which reportedly allows determination of chemical form and metal bioavailability in soils treated with complex microelement fertilizers.

The effects of soil and chemical agents, on metal bioavailability, must be considered in establishing concentration guidelines or even considering soil fertility (e.g. Savich et al., 1988). Even information on pH can be informative in evaluating metal solubility and bioavailability (e.g. Adams et al., 1985; Blust et al., 1988a; Khangarot and Ray, 1987; Mulchi et al., 1987a; Starodub and Wong, 1987; Stokes and Campbell, 1986; Verma and Neue, 1988; see also Simkiss and Watkins, 1988). In an INCRA-supported study, Payne et al. (1988a) found little effect of copper-enriched swine manure on metal concentrations in corn. They (Payne et al., 1988b) note that application of 135 kg Cu and 337 kg Zn/hectare in excess of the limits set forth by USEPA guidelines was not detrimental to corn production on a Davidson soil with a pH > 6.5. They comment (abstract) that "The reason for the lack of phytotoxicity of the metals include the relatively high soil pH maintained at the site, which limits the availability and the conversion of the applied Cu and Zn to plant-unavailable forms over time." What is needed is a technique to measure metal availability before application. Checkai et al. (1987), for example, used exchange resins to impose nutrient treatments in a hydroponic situation.

III.1 ORGANIC COPPER COMPLEXING AGENTS

In the preface to Buffle (1988 "Complexation Reactions in Aquatic Systems: ..."), Werner Stumm comments on the complexity of natural environments and the importance of understanding the chemistry. Hirose and Sugimura (1985), in a discussion of the role of metal-organic complexes in the marine environment, comment that (abstract) "... the ecological significance of metal-organic complexes is essentially in the metal buffering action." Linn and Elliott (1988) provide evidence of the ability of a synthetic metal complexing agent to enhance copper release from metal-contaminated soil; Hering and Morel (1988b) discuss some of the effects of alkaline-Earth metals on the reaction kinetics of a copper-synthetic chelating agent combination. Metal-organic complexes are involved in a very large number of biochemical processes, a knowledge of which requires information on the detailed structural arrangement of biomolecules, the ligands they provide, the metals they bind, and the modulation effect of other organics (de Meijere and kDieck, 1987; Hasnain and Garner, 1987; Kawaguchi, 1988; Tandon, 1985) and chemical conditions (e.g. Crabtree, 1988; King et al., 1988; Likhtenshtein, 1988; Lukehart, 1985). The acquisition of this information is of major importance, requires techniques such as paramagnetic resonance (e.g. Blumberg and Peisach, 1987), and is of interest to workers in both theoretical and applied areas of research (e.g. Fish, 1988; Le Graet and Brule, 1988). As Thayer (1988) states (preface), "Organometallic chemistry has become one of the most exciting and active areas in the chemical sciences."

The chemistry of copper-containing metalloproteins plays an important role in organometallic chemistry. Barlow et al. (1988) examines computer-aided modelling of the structures and metal-ion affinities of chelating agents. Metal-protein interactions are discussed in Sarkar (1987). Dooley (1987) points out the extensive effort that has been directed towards understanding protein copper sites. Solomon et al. (1987b) review chemical and spectroscopic work on copper clusters in proteins. Agents such as the copper-containing blood pigment hemocyanin have been widely used in work on structure-reactivity correlations (Solomon, 1988b). Reversible binding and reactions with dioxygen are involved in a number of binuclear copper proteins (Kitagawa, 1985; Solomon, 1988b; Tyeklar et al., 1988).

Major groups of organic complexing agents are given below. Selected references are given here with others used elsewhere in the review.

Humic substances

A diverse group of organics derived from the breakdown of biological material, primarily plant material. Many of these have the ability to regulate copper bioavailability and probably play a very important role in natural environments as well as those affected by man.

Occurrences: Gueguenïat, 1986; McLellan and Rock, 1988

Complexing capacity: Cabaniss and Shuman, 1988; Castlebon et al., 1986; Fischer, 1986; Haworth et al., 1987; Pitluck et al., 1987; Relan et al., 1986; Tao et al., 1987a

Chemistry: Aplincourt et al., 1987; Bartoli et al., 1988a; Fujita et al., 1987; Grace et al., 1985; Hering and Morel, 1988a; Jones and Thomas, 1988; Lesourd-Moulin, 1986; Liu et al., 1985a; Maslennikov et al., 1985; Mudroch et al., 1984; Piemontesi and Baccini, 1986; Pilipenko et al., 1986; Sposito et al., 1988; Sun et al., 1988c.

Biological importance: Aristovskaya et al., 1986; Gedziorowska and Plinski, 1986; Hargeby and Petersen, 1988; Perttila, 1986

Sugars, amino acids, peptides and proteins other than enzymes and metallothionein-like proteins

Copper is broadly involved with the chemistry of organisms. This is either a primary involvement, the metal is required for proper configuration of the molecule, or secondary involvement, complexation occurs in the presence of the organic but is not essential to the organometallic compound. However, the involvement produces a unique organometallic structure with potential chemical usefulness. Since a number of these compounds have been shown to have nutritional (e.g. Kratzer and Vohra, 1986) and medical application, the chemistry of copper and organic ligands has become almost a field of organometallic chemistry unto itself. Many of the references given below concern the search for copper-containing compounds that will ultimately prove to have beneficial biological activity.

Sugars and sugar derivatives: Angyal, 1988; Geesey et al., 1987

Amino acids: Abdel-Mawgound and Abdel-Hamid, 1987; Anjaneyulu et al., 1988; Armani et al., 1988; Barbucci et al., 1988; Daniele et al., 1988; Gotsis and Fiat, 1987a,b; Konig et al., 1987; Liedberg et al., 1987; Mukherjee and Sarkar, 1988; Pearce and Friedman, 1988; Radomska et al., 1988; Rao (B.K.) et al., 1987; Rao (B.V.) et al., 1988a; Szabo-Planka et al., 1988; Verma et al., 1985; Winge et al., 1988b; Zelano et al., 1988

Ceruloplasmin: Barber and Cousins, 1988; Calabrese et al., 1988a; Hilewicz-Grabska et al., 1988; Moshkov et al., 1987; Schechinger et al., 1988; Vassos and Newman, 1988

Miscellaneous blue copper proteins: Farver and Pecht, 1988; Feiters et al., 1988; Gewirth, 1987; Jackman et al., 1987; Lommen et al., 1988; McGinnis et al., 1988; Mino et al., 1987; Nishida, 1987; Pavlishchuk et al., 1988; Ryden, 1988; Schmidt et al., 1988; Sharma et al., 1988a; Solomon et al., 1987a; Yamamoto et al., 1987a

Haemocyanin and haemocyanin-like compounds: Basu et al., 1987; Borovik, 1986; Karlin et al., 1988; Kitajima et al., 1988; Lerch and Germann, 1988; Pate, 1987; Romandini et al., 1987; Salvato and Beltramini, 1987; Sorrell and Borovik, 1987; Toulmond et al., 1987; Van Hoof et al., 1988; Volbeda and Wim, 1988; Wang et al., 1987c

Miscellaneous organic and inorganic metal complexes: Aaseth et al., 1987; Abu-El-Wafa et al., 1985; Ali et al., 1985; Allen et al., 1987; Antonelli et al., 1988; Antolini et al., 1988; Arora et al., 1986; Asaturyan, 1988; Asaturyan et al., 1987, 1988; Bacci and Cannistraro, 1987; Bakola-Christianopoulou et al., 1988; Backvall et al., 1988; Bailey et al., 1988; Balman et al., 1988; Banci et al., 1988b; Barton et al., 1988; Basosi, 1988; Bellosta and Czernecki, 1987; Bertini et al., 1988b; Bianco et al., 1986; Bouwman et al., 1988; Brown et al., 1988b; Buszman et al., 1988; Champagne, 1987; Chazin and Wright, 1988; Christie et al., 1988; Cowan and Sanders, 1987; Cox et al., 1988; Cruse et al., 1988a,b; Cseh and Lienhard, 1988; Dahlund and Olin, 1987; Dale et al., 1988; Debongnie et al., 1987; Decock-Le Reverend et al., 1985; de Zwart et al., 1988; Diez et al., 1988; Drew and Yates, 1987; Duchstein et al., 1988; Durell, 1988; Elo, 1987; El Rassi et al., 1988; El-Ries et al., 1985; Evans and Martin, 1987; Evans et al., 1988; Fan et al., 1988b; Farooq, 1987; Fenton et al., 1987; Fiallo and Garnier-Suillerot, 1987; Gaggelli et al., 1988; Gelling et al., 1988; Ghosh et al., 1987; Groeneveld et al., 1987; Gupta and Jha, 1987; Halevy and Sklan, 1988; Harayama et al., 1987; Herzyk et al., 1987; Hochuli et al., 1987; Holwerda, 1988; Howe, 1988; Huang et al., 1988b; Hunt et al., 1988; Hyde et al., 1986; Jackson and Kelly, 1988; Jacobson et al., 1988; Jayawickreme and Chatt, 1988; Jeffery, 1988; Johari et al., 1987; John, 1988; Joyeau et al., 1987; Julve et al., 1987; Kano et al., 1987; Kato et al., 1988; Katz, 1988; Kavlentis, 1988; Kawano et al., 1986; Kenani et al., 1987; Kwik et al., 1988; Kuroboshi and Ishihara, 1987; Lambs and Berthon, 1988; Latos-Grazynski et al., 1987; Leporati, 1988a,b; Lie Ken Jie and Lam, 1987; Linderman and Lonikar, 1987; Livera et al., 1988; Lucas et al., 1988b; Mahapatra and Das, 1987; Maiya and Krishnan, 1987; Maquart et al., 1988; Masarwa et al., 1988; Matsumoto et al., 1988; Matsushima and Nagata, 1987; Mayadeo and Nalgirkar, 1988; Melnyk et al., 1987; Milanino et al., 1988c; Miller et al., 1988; Miyazawa et al., 1988; Mohan et al., 1988; Mohanakrishnan and Rabenstein, 1988; Mohanty et al., 1987; Moore et al., 1988a; Morphy et al., 1988; Muhoberac et al., 1988; Munday, 1988b; Nair et al., 1988; Nandi and Debnath, 1987; Narayana et al., 1988; Natchev, 1988; Nishida and Takahashi, 1988; Nosonovich et al., 1987; Obozova and Krymova, 1988; Onori, 1987; Ozawa et al., 1987a; Palumbo et al., 1988; Parashar et al., 1987; Permyakov et al., 1988; Persson et al., 1987; Pezeshk et al., 1987; Pierson and Evenson, 1988; Poulicek, 1986; Rabinovitz and Fisher, 1988b; Rai et al., 1988; Rao (P.V.) et al., 1988e,f; Real et al., 1987; Reddy and Subrahmanyam, 1988; Reese et al., 1988; Reid et al., 1987; Reisch and Bathe, 1988; Sahadev et al., 1988; Salata et al., 1988; Sanni et al., 1988; Sarma, 1987; Scaife and Masterson, 1988; Sharma and Parashar, 1988; Sheldrick and Bell, 1987; Shetty and Melethil, 1987; Shirane and Tokimoto, 1988; Sibbald and Green, 1987; Simpson et al., 1988; Singh et al., 1988a; Soderberg et al., 1988b; Solomon et al., 1987a,b; Soto et al., 1988; Standley et al., 1988; Stepien et al., 1987a,b; Strange et al., 1987; Suh et al., 1987; Sun and Cheng, 1988; Trost et al., 1988; van Rijn et al., 1987; Wang et al., 1988a; Wedepohl and Schwedt, 1987; Wilkins and Moore, 1988; Winkler, 1987; Xue et al., 1988a; Yatsimirskii et al., 1988; Zani et al., 1987; Zelichowicz et al., 1988

Hormones: Barnea et al., 1988b; Bhasker and Barnea, 1988; Decock-Le Reverend et al., 1988; Fishman and Fishman, 1988; Gerega et al., 1988; Maskos et al., 1987

Nucleic acids: Bailly et al., 1987, 1988; Carmona et al., 1988; Chen and Sigman, 1988; Cullis et al., 1987; Czarnecka et al., 1988; Foster et al., 1988; Francois et al., 1988; Gelagutashvili and Bregadze, 1987; Hazen et al., 1988; Ito and Sasaki, 1988; Kazakov et al., 1988; Kulikov et al., 1987; Kuwabara and Sigman, 1987; Mellano and Cooksey, 1988a,b; Spassky et al., 1988a,b; Tajmir-Riahi et al., 1988; Veal and Randolph, 1988; Wissler et al., 1987c,d

Copper-containing enzymes

Enzymes are proteins that control the rate at which reactions occur in all organisms. As such, they are often considered the most important group of organics in the body. A number of enzymes contain copper as a "nucleus" and fail to function correctly under copper deficiency.

Amylase: Bovykin et al., 1985

Cu,Zn-superoxide dismutase (SOD) - an extremely important antioxidant enzyme because of its ability to convert superoxide to the less reactive H_2O_2.

Reviews - Bannister et al., 1987; Kwiatowski, 1987

Activity (concentration) - Bilinski and Liczmanski, 1988; Dameron and Harris, 1987a,b; David and DiSilvestro, 1988; Huber et al., 1987; Jadot and Michelson, 1987; Jadot et al., 1986a,b; O'Neill et al., 1988; Viglino et al., 1988; Yamakura et al., 1988

Activity (concentration) under abnormal or deficient conditions - Arias and Walter, 1988; Chang et al., 1988a; DiSilvestro, 1988a,b; Dubick et al., 1988; Groner et al., 1988; Horton et al., 1988; Jendryczko et al., 1987; Kelner et al., 1988; Natvig, 1988

Biochemistry, chemistry, genetics - Arai et al., 1987; Aruoma and Halliwell, 1987; Banci et al., 1988a; Bermingham-McDonogh, 1987; Bermingham-McDonogh et al., 1988; Bertini et al., 1988a; Beyer and Fridovich, 1988; Biliaderis et al., 1987; Carraro and Pathak, 1988; Chang et al., 1988b; Dameron, 1987; Davis et al., 1988a,b; de Jesus et al., 1988; Desideri et al., 1988a,b; Elroy-Stein and Groner, 1988; Frank et al., 1988; Gralla et al., 1988; Hass and Massaro, 1988a,b; Hirose et al., 1988; Ho and Crapo, 1987; Iabal et al., 1988; Ischiropoulos et al., 1988; Lee and Hassan, 1987; Kumahara, 1987; Licki, 1987; Linss and Weser, 1987; Lyutakova et al., 1986; Makita et al., 1988; Marklund et al., 1988; Ming and Valentine, 1987; Ming et al., 1988a,b; Morpeth, 1987; Oka et al., 1987a; Ozaki et al., 1988; Puga and Oates, 1987; Radi et al., 1986; Reddy and Venkaiah, 1988; Reinecke et al., 1988; Rigo et al., 1988; Salin and Oesterhelt, 1988; Schneider and Denaro, 1988; Seto et al., 1987a,b; Shibata and Ogita, 1988; Sulochana and Venkaiah, 1988; Taniguchi et al., 1988; Westerbeek-Marres et al., 1988

Functions - Bielski, 1987; Chung et al., 1988; Hassan, 1988; Krall et al., 1988; Michelson et al., 1986; Mohan et al., 1987; Willingham and Sorenson, 1988

Hydroxylases: Blackburn et al., 1988; Fitzpatrick and Villafranca, 1987; Klinman and Brenner, 1988; McCracken et al., 1988a,b; Stewart and Klinman, 1988

Laccases: Hanna et al., 1988; Koudelka and Ettinger, 1988; Morpurgo, 1987; Spira-Solomon and Solomon, 1987; Wrigley and Gibson, 1987

Nucleases: Feig et al., 1988

Oxidases: Artico et al., 1988; Artzatbanov et al., 1987; Bogush et al., 1987; Calabrese et al., 1988; Casella et al., 1988a,b,c; Chan et al., 1988d; Copeland et al., 1987, 1988; Driscoll, 1987; Duine et al., 1987; Esaka et al., 1988; Fan et al., 1988a; Gorren et al., 1987; Guentherberg et al., 1987; Hall et al., 1988a; Hiraoka et al., 1988; Lamkin et al., 1988; Margerum et al., 1988; Markosyan et al., 1988; Martin et al., 1988; Megrabyan and Nalbandyan, 1987; Merli et al., 1988; Mitchell, 1987; Mondovi and Avigliano, 1987; Morpurgo et al., 1987, 1988; Muller et al., 1988; Nilsson et al., 1988a,b; Peczynska-Czoch, 1987; Rich et al., 1988; Sakharov and Skibida, 1988a,b; Sakurai et al., 1988; Scholes et al., 1988; Scott et al., 1988; Serr et al., 1988; Shah et al., 1987; Steffens and Buse, 1988; Steinrucke et al., 1987; Suzuki et al., 1988; Toussaint and Lerch, 1987; Ukisu and Kazusaka, 1987; Whitakker and Whittaker, 1988; Wikstrom, 1988; Williams, 1987; Yewey and Caughey, 1987, 1988; Yoshikawa et al., 1988; Zimmermann et al., 1988a,b

Phytase: Eskin and Johnson, 1987; Youssef et al., 1987

Reductases: Arunakumari et al., 1988; Casella et al., 1988e; Coyle et al., 1987; Dooley et al., 1988; Hulse et al., 1988; Liu et al., 1988b; Sano and Matsubara, 1988; Sun et al., 1988d; Tomlinson and Hochstein, 1988; Turley et al., 1988

Miscellaneous enzymes: Abduragimov et al., 1987; Bertini et al., 1987; Clarke and Adams, 1987; Huang et al., 1988a; Huber and Lerch, 1988; Ichiba et al., 1988; Karataglis et al., 1988; Lin and Van Wart, 1988; Lynch et al., 1988; Morton, 1987a; Murakami et al., 1988; Nguyen et al., 1988; Stiborova et al., 1988; Thederahn and Sigman, 1988; Tsang and Cho, 1988; Vaeroy et al., 1987; Walker et al., 1988; Wood and Schallreuter, 1988

Metallothionein-like organics

Metallothionein and metallothionein-like organics transport copper within the organism. They can also bind excess metal, assisting the organism either in its elimination or storage in an innocuous state.

General, including reviews: Kagi and Kojima, 1987 (Proceedings of the second international meeting on metallothionein and other low molecular weight metal-binding proteins)

Occurrences: Berka et al., 1988; Chen and Failla, 1988; Gordon et al., 1988; Grill, 1987a,b, 1987, 1988 (phytochelatins); Grill et al., 1988 (phytochelatins); Harrison et al., 1988; Krezoski et al., 1988; Munoz et al., 1988; Naiki, 1987; Santhanagopalan and Jayaraman, 1988; Suzuki, 1987; Thurman et al., 1988; Tomsett et al., 1988

Chemistry and/or function: Allen and Gawthorne, 1987; Arthur et al., 1987; Backowski et al., 1987; Beltramini et al., 1987; Bremner, 1987; Brouwer and Winge, 1988; Byrd et al., 1988; Chen and Douglas, 1987; Deagen and Whanger, 1987; Ebadi and Swanson, 1987; Elinder et al., 1987a; Felix and Weser, 1988; Fleet et al., 1988; Hartmann et al., 1987; Higham et al., 1987; Hunt et al., 1987; Kambadur et al., 1987; Kaplan et al., 1987b (chelating polysaccharides); Kobayashi and Sayato, 1987; Lynes et al., 1988; Mahy et al., 1987; Markossian et al., 1988; Mehra and Winge, 1988; Mehra et al., 1986; Mercer et al., 1988; Munger and Lerch, 1987; Naiki, 1986; Olafson, 1988; Richter and Weser, 1988; Rodo et al., 1985; Roesijadi, 1987; Roesijadi et al., 1988; Sato and Arima, 1986; Shen et al., 1987c; Shiraishi et al., 1987; Stillman et al., 1987; Thrower et al., 1988; Van Beek and Baars, 1988; Wang and Mason, 1988; Wei and Andrews, 1988; Weser and Hartmann, 1988; Winge, 1987; Winge et al., 1988a;

Structure: Armitage et al., 1988; Bonham et al., 1987; Brouwer and Winge, 1988; Freedman et al., 1988; George et al., 1988; Hasnain, 1986; Olafson et al., 1988; Weser, 1987; Zafarullah and Gedamu, 1988

Detoxification: Durnam and Palmiter, 1987; Engel and Roesijadi, 1987 (use as a monitoring tool); Gekeler et al., 1988 (phytochelatin); Jenkins and Sanders, 1987; Kubota et al., 1988; Maroni et al., 1987; Pavicic et al., 1987 (monitoring tool - with cadmium, not copper).

Genetics: Bergman and Timblin, 1988; Blalock et al., 1988; Bunch et al., 1988; Camakaris et al., 1988b; Cismowski et al., 1988; Etcheverry and Forrester, 1988; Fogel et al., 1988; Gedamu et al., 1987; Maroni et al., 1987; Munger et al., 1987, 1988; Thiele et al., 1987; Wegnez et al., 1988; Wright et al., 1988

Miscellaneous metal-complexing organics.

Chemistry: Attia et al., 1985 (antimicrobial activity of an organic ligand, not the metal complex); Baumy and Brule, 1988 (binding of bivalent cations to α-lactalbumin and β-lactoglobulin); Gergely and Garaj, 1988b (metal-containing ethylenebisdithiocarbamic acid); MacNeil et al., 1988 (calmodulin).

III.2 ADSORBING AGENTS

The mobility and bioavailability of copper in soils and aquatic systems can be affected by metal sorption to inorganic as well as organic surfaces (e.g. Bruemmer et al., 1986; Calmano et al., 1988; Gonet, 1988; Pinskii et al., 1986). In essence, metal mobility "... is governed by the competition between the reactions of sorption ... and of solubilization (dissolved complexation)" (Bourg, 1988, page 27). Adediran (1983) reviews the literature on controls and models for trace metal adsorption in estuaries.

Sorption by suspended as well as benthic sediments is affected not only by the nature of the surface but also by the chemistry of the environment (e.g. Konovalov et al., 1983; Young et al., 1987). Saikia et al. (1987) with the Ganges and Theis et al. (1988) with the Detroit River, comment that pH is an influential factor in sorption of trace metals such as copper, zinc and lead by bed sediments. pH plays an important role both in the interaction of the inorganic metal-particulate reactions (Gambus, 1987; Schindler et al., 1987) as well as the effect of organics on the reaction (e.g. Liu et al., 1985a). Particle concentration as well as size is also important in aquatic environments (e.g. Honeyman et al., 1988). The zone of maxiumum turbidity in a river estuary is reported to contain high concentrations of active particles which play an important role in the sorption of natural and anthropogenic metals (e.g. Glegg et al., 1988). With copper, however, changes in particulate metal concentration are considered to be easier to detect in areas of low suspended metal loading (Balls, 1988). Seasonal changes in particle size and nature also occur in estuaries (Kawara, 1988), affecting the amount of sorbed metal and the ratio between the dissolved and particulate fractions of copper.

Characteristics of soils and sediments help to define their physical properties (e.g. Adhikari et al., 1986). As part of this, understanding the interaction of copper with organics and particulate surfaces is of importance not only in determining the fate of the metal but also the nature of sorption events. And this is true for natural soils and sediments (e.g. Jorge and Chagas, 1988; Mimides, 1987; Mimides and Lloyd, 1987; Osichkina and Tillyakhodzhayev, 1986; Swartjes et al., 1988; Zhang et al., 1987b) as well as those affected by industry (e.g. Dudley et al., 1988; Karlsson et al., 1987b; Liedberg et al., 1987; Mote et al., 1987a; Nashikkar and Chakrabarti, 1987; Nordqvist et al., 1988). The reactions of copper with certain clay minerals can, for example, enhance sorption of organics such as amino acids (Tsvetkov and Mingelgrin, 1987) or serve as catalysts for the formation of organics (e.g. Ferris et al., 1988). Sorption of pesticides on copper-containing clay minerals (montmorillonite especially) in soils can affect their persistence and movement in a soil profile (Aochi and Farmer, 1988; Micera et al., 1988b). The clay mineral bentonite is routinely used in the fining of metal-containing juices and wine, including the removal of excess metal (Dul'neva et al., 1988; Enkelmann, 1988). Activated carbon "particles" are widely used to remove metals in wastewaters (Corapicoglu and Huang, 1987).

Sorption of copper to surfaces is dependent on the nature of the surface and the chemical nature of the copper or copper-containing compound (e.g. Veniale, 1985). Sorption can either be enhanced or inhibited by organics. In a discussion of metal sorption in soils of Croatia, Bertic et al. (1988) found highest uptake of Mn, Cu and Co in chernozems with high pH and humus content. Nielsen (1987) concludes that although copper binding in soil fractions is related to the inorganic part, in silt it is related to the humic fraction. Bruemmer et al. (1986) and Rahmatullah et al. (1987) note that sorption of copper on soil particles can occur both on the surface and in the interior of soil minerals. Micera et al. (1987, 1988a) found that sorption behaviour of Cu(II) to amorphous aluminum hydroxides can be accounted for by the interaction with organic ligands. The reactions are, however, affected by the pH of the medium. They are also affected by the concentrations of metals competing for uptake sites (e.g. Christensen, 1985).

Putilina and Varentsov (1986) found that citrate can affect Cu(II) sorption to manganese oxide. The initial effect was a decrease in copper sorption, followed by an increase. Liu et al. (1985b) present evidence that the interaction between organics (fulvic and amino acids) and Cu(II) sorption to clays in seawater is due to the coating of active sites on the clay mineral surfaces. This implies a direct competition with metal, for sites, rather than a complete coating of the particle. However, Wang et al. (1987d) report no effect of seawater amino acids on Cu(II)-goethite ion exchange, at least under experimental conditions.

Sorption of copper can also occur on organisms or particulate byproducts of metabolism. Gordon and Millero (1987) report sorption of copper by living as well as dead bacteria (*Vibrio alginolyticus*) and Ferris et al. (1987) found evidence that individual bacterial cells and their remains can provide nucleation sites for metal sulfides in sediment. The surfaces of at least some algal cells have a high affinity for Cu(II) (Xue et al., 1988b) as do some terrestrial plant cells (e.g. Allan, 1987). However, it is difficult to separate adsorption from complexation by organics on the cell surface (e.g. Tan, 1985). Chitosan, a high molecular weight polymer from arthropod or fungus chitin, can absorb copper and form chitosan-metal complexes (McKay et al., 1987). Copper is also sorbed by lignin (Wieber et al., 1988) and keratin (Fukatsu, 1988). Fiber in food has the ability to reduce copper bioavailability to varying degrees, depending on the nature of the fiber (Platt and Clydesdale, 1987; Rockway et al., 1987). Persson et al. (1987) suggest that it is the phytate fraction of soluble fiber that binds a major portion of the metal.

IV. METAL-METAL INTERACTIONS IN ORGANISMS

Metals interact both in the environment and within the organism. In the environment, some of the interaction is a result of biological processes (e.g. Fanning et al., 1988), affecting the bioavailability of copper and several other metals. In organisms, although there may be a similar interelement relationship (Markert, 1987), metal interactions do occur and can be of either a complementary or antagonistic nature. In this sense, a change in the concentration of one metal can have an impact on the biological effect of a second. Bois et al. (1988), for example, examine optimal pollution control strategies and comment that, with chromium, copper and zinc, it is most appropriate to first reduce the concentration of zinc and chromium. Elsenhans et al. (1988) comment (abstract) that for the rat, "Changes in tissue accumulation of one metal, ..., do not necessarily indicate environmental concentration changes of the corresponding metal alone, but have to be carefully analysed for interference by other metals." The physiological state of the organism must be considered (e.g. Nadeenko et al., 1987) as physiological changes are often expressed as changes in metal requirements or tissue metal concentrations (e.g. Saito et al., 1987).

Copper-iron interactions

In at least some plants, an increase in iron has been related to a decrease in foliar uptake of copper (Tropea et al., 1984). In piglets and rats, tissue copper concentration can be increased by increased availability of iron (Scholz et al., 1988a; Sinha et al., 1987). With piglets at least, this also appears to be true of blood copper concentrations (Scholz et al., 1988b). Iron deficiency in rats can also be induced by copper deficiency (Kramer et al., 1988b) although fructose can affect iron metabolism during copper deficiency (Johnson, 1988 but also see Osborne et al., 1988a,b). Chick haemoglobin concentrations can be increased by both copper and iron (in Hill, 1988). And yet, at least in weaned calves, iron is suggested to be a potent antagonist of copper metabolism (Bremner et al., 1987). Iron has also been suggested to increase fecal copper loss (Beltran et al., 1988). Both tissue and serum iron and copper concentrations appear to be affected by natural metal complexing agents such as amino acids (Batista de Assis et al., 1986) and ascorbic acid (Harrison, 1986; Harrison and Edwards, 1988; Johnson and Murphy, 1988). They may also be affected by the interaction with zinc (Stahl et al., 1988) as well as other metals. Transferrin transport of ionic iron may be blocked by a variety of metals, including copper (Kaplan et al., 1988; Sahu et al., 1988). This may be one of the reasons for plant iron deficiency produced by excess use of copper in the control of bacterial leaf spot (Simons, 1988). Copper sulfate potentiates ferrous ion-induced lipid peroxidation with adequate amounts of reduced glutathione (Beckman et al., 1988).

Copper-manganese interactions

Manganese and copper appear to be antagonistic in terms of manganese uptake by plants (e.g. Lastra et al., 1988). The physiological effect of this is more difficult to determine. It may, for example, be associated with a reduction in iron uptake or chlorosis under low iron conditions (e.g. Jeon et al., 1986). In animals, tissue copper may affect manganese concentrations (e.g. Kaitala, 1988) although both copper and manganese concentrations can be affected by other metals (Reichlmayer-Lais and Kirchgessner, 1988). Manganese can inhibit benzoyl peroxide/copper-dependent lipid peroxidation (Tsujimoto et al., 1988a,b) suggesting a more intimate role than simply the control of tissue metal concentrations.

Copper-molybdenum interactions

Copper and molybdenum are both essential elements but they can interact, especially when one of the two occurs in excess (e.g. Schalscha et al., 1987). In ruminants, a dietary excess of molybdenum can cause a deficiency of copper with resultant physiological problems (Gooneratne et al., 1987b; Neuman et al., 1987; Phillippo et al., 1987a,b; Wittenberg and Devlin, 1987; Zheng, 1988). Likewise, a deficiency of molybdenum may increase the chance for copper toxicity with excess biologically available copper (Mills and Davis, 1987). Proper levels of molybdenum can thus act as a buffer against the effects of excess copper although there tend to be tissue-specific responses (e.g. Humphries et al., 1988; Robinson and Blair, 1987; Vrzgula et al., 1987). Copper supplementation is often carried out in areas with excess soil and plant tissue molybdenum (e.g. Wittenberg and Boila, 1987). Although Cu-Mo interactions are most frequently seen in ruminents, they may also occur in non-ruminant animals (see Clarke et al., 1987). Sulfur can play a major role in the interaction, in the production of thiomolybdates, which affects the speciation and bioavailability of molybdenum (Allen and Gawthorne, 1987; Lamand et al., 1988; Mason et al., 1988; Price et al., 1987; Wang and Mason, 1988). Wang et al. (1987e) suggest that the presence of protein-bound thiomolybdates in the liver of steers gives rise to new ligands which alter the equilibrium of copper between different metal-binding proteins.

Copper-zinc interactions

Interactions between copper and zinc occur in the soil and can affect the bioavailability of one or both metals (e.g. Kukushkin et al., 1988). Zinc fertilization has, for example, been found to increase the level of tissue zinc and copper in cotton plants (Alikhanova and Tursunov, 1988), maize (Bansal and Zyrin, 1988), and coffee (Raju and Deshpande, 1987). Gupta et al. (1987b), however, note a decrease in pigeonpea shoot tissue copper with added zinc. They (Gupta et al., 1987c) do, however, report that (abstract) "Phosphorus and zinc fertilization depressed the Fe, Mn and Cu concentration and enhanced their uptake in shoot and leaves" of pigeonpea. Elevated levels of copper can also cause a decrease in plant tissue zinc concentrations (Singh and Bhobria, 1987). Cantos et al. (1986) found optimal growth in a peach x almond hybrid at background Zn:Cu ratios but ten times background concentrations.

Copper-zinc relationships are also of importance in the physiology of animals and humans (e.g. Costello et al., 1988; Czupryn et al., 1988; El-Waseef, 1987; Fox et al., 1988; Garrott et al., 1988; Hogue et al., 1988; Ikuta, 1988c; Ito, 1986; Kaitala, 1988; Kobayashi and Sayato-Suzuki, 1988; Lockitch et al., 1988; Naveh et al., 1988; Samman and Roberts, 1988; Sodhi and Mehta, 1987; Yamaguchi et al., 1986). In domestic fowl, liver copper can decline as a result of increased dietary zinc oxide concentration. Changes in sheep serum copper may change in response to changes in serum zinc concentration (Bires, 1987). The zinc status of bovines appears to affect the deposition of copper in cytosolic proteins and may be a factor in controlling metal uptake by metallothioneins (Whanger and Deagen, 1987). Differences have also been observed in tissue copper levels in mice, as a result of zinc addition although the significance of the differences and the relationship to zinc metabolism is not clear (Reis et al., 1988). Antagonism is reported between copper and zinc levels in cultured human lymphocytes (Carpentieri et al., 1988b; Ebadi and Swanson, 1988). Altered copper metabolism in diabetic rats and in cancer patients has been suggested to be at least partially related to zinc metabolism (Deebaj et al., 1988). In general, there is an antagonistic effect of zinc on the gastrointestinal absorption of copper, at least in laboratory animals and humans, a feature that is now being used to reduce copper uptake in patients with Wilson's disease (Cossack and van den Hamer, 1987; Kaur et al., 1988; Siegemund et al., 1988b). It is the reason for copper deficiency occurring in laboratory animals and patients receiving excessive daily oral zinc doses (Grant, 1987; Hoffman et al., 1988; Simon et al., 1988; Sohler and Pfeiffer, 1987).

In terms of acute toxic effects, potential Cu-Zn synergism has been suggested for the marine harpacticoid copepod *Tisbe holothuriae* (Verriopoulos and Dimas, 1988) while antagonism has been suggested for the marine amphipod *Allorchestes compressa* (Ahsanullah et al., 1988) and the chick (Hill, 1988; Stahl et al., 1988). However, in an examination of the toxicity of brass powder to a crustacean (*Daphnia magna*), Hardy et al. (1988) report that toxicity appeared to result from concentrations of both Cu^{2+} and Zn^{2+} rather than the interaction of the two ion types. Chmielnicka et al. (1988) note that, with the rat, the injection of copper increased the body retention of zinc.

Copper-cadmium interactions

Although cadmium is toxic, it can interact with copper and other metals as either a synergist or antagonist. Since metal resistance in certain organisms can be a result of exposure to elevated metals (e.g. Casella et al., 1988), some of the metal-metal interactions may be controlled by the nature of the organism as well as the metal. Potential Cu-Cd synergism has been reported for the marine harpacticoid copepod *Tisbe holothuriae* (Verriopoulos and Dimas, 1988), the amphipod crustacean *Allorchstes compressa* (Ahsanullah et al., 1988) and rat liver cells (Denizeau and Marion, 1986), possible antagonism in the mouse (Cho and Lim, 1986) and rat (Bordas and Gabor, 1987). Dietary complexing agents such as ascorbic acid can have an effect on uptake of metals, other than copper, thereby affecting both tissue levels (Chou et al., 1987) and potential impact. Chmielnicka et al. (1988) note an increase in metallothionein-like proteins after metal exposure in the rat. Metallothionein, and other organics like it, would also affect tissue metal concentrations as well as metal metabolism(e.g. Balter et al., 1987; Durnam and Palmiter, 1987; Fowler et al., 1988). Even with the modifications of complexing agents and the variability due to the nature of the organism, there are still some general trends in the interaction of copper and cadmium. There is, for example, a trend for change in tissue copper concentrations as a result of exposure to excess cadmium (e.g. Baranski, 1986, 1987; Kitazume, 1987; Kunifuji et al., 1987; Nomiyama et al., 1987 but see also Komsta-Szumska and Miller, 1986; Muller and Stacey; Olsson et al., 1988; Yamamoto, 1987 for cases where this is not apparent).

Interactions of copper with other metals, metalloids and nutrients

Arsenic - Schmolke and Forth (1988) indicate that in the rat kidney, low levels of dietary arsenic may cause the renal accumulation of metallothionein-bound metals like copper and cadmium.

Calcium - Calcium carbonate is often included as a pH buffer and nutrient supplement to plants (e.g. Patel and Golakiya, 1986). Its use can decrease metal availability to plants, primarily as a result of increasing pH (Halder and Mandal, 1987; Korner et al., 1987). Sadamatsu et al. (1987) note that the effect of copper-containing agents on the control of citrus canker can be enhanced by $CaCO_3$ preparations. Calcium is found in a variety of normal and abnormal tissues, often in association with copper (e.g. Alvisi et al., 1988). This association seems natural since both copper and calcium are active participants in the formation of connective tissue (e.g. Farrell et al., 1988). Calcium supplements are often given to laboratory animals; with aging female rats, Behling and Greger (1988) found no significant effect on liver copper levels.

Cyanide - Nordqvist et al. (1988) note reduction of Cu^{2+} to Cu^{+} by cyanide in simulated mixed-metal plating wastes, with the formation of Cu^{+}-CN complexes. They comment, however, that (abstract) "The effects of metal, chromate and CN concentrations do not appear to be major impediments to the utilization of adsorption as a treatment process." Cyanide is an appropriate substrate analogue for Cu,Zn superoxide dismutase (Paci et al., 1988).

Lead - Mo et al. (1988a) found that the uptake of Cu^{2+} by duckweed could be suppressed by the presence of $Pb^{2+}$and Al^{3+}.With the freshwater crab *Barytelphusa guerini*, Tulasi and Rao (1988) note that exposure to lead can elevate haemolymph and decrease hepatopancreas copper levels, possibly a result of haemocyanin synthesis. Quarterman (1986) reviews the interaction of lead and copper, commenting that (page 293) "The level of dietary copper has little effect on tissue lead concentration or lead retention." However, El-Waseef et al. (1986) conclude that increased dietary copper (up to 20 ppm) can lessen the severity of lead toxicity in the rat. Davydova et al. (1988) conclude (translation) that there is a competitive interaction (antagonism) between copper and lead. Working with cultured rat astroglia, Tiffany-Castiglioni et al. (1986, 1987) comment that intracellular copper levels were increased as a result of exposure to lead. However, they (Tiffany-Castiglioni et al., 1988) found that with rat glioma cells, lead did not affect intracellular copper concentrations.

Nickel - Elsenhans et al. (1987), in a review of metal-metal interactions in the rat, notes that renal and intestinal copper concentrations were dose-dependently affected by dietary nickel.

Phosphorus - Phosphorus or phosphorus-containing compounds at high levels, as in manure-treated soils, can reduce the biological availability of copper (Tomar et al., 1986). Stoddard (1987) noted that algal biomass was limited by phosphorus in combination with iron or copper in a Sierra Nevada lake. Addition of only one of the three, by itself, did not increase biomass significantly. Plant growth regulators may operate through phosphorus-containing fertilizers and trace metals under certain conditions (e.g. Mamo and Parsons, 1987; Prasad and Ram, 1986; Salama and Buzas, 1987). The alga *Chlorella* sp. was found to be more sensitve to Cu^{2+} toxicity under phosphorus limitation than under nitrogen limitation (Hall, 1987).

Selenium - The interactions of this metalloid are reviewed by Marier and Jaworski (1983) in a National Research Council of Canada manuscript report. They point out that there is some evidence indicating a possible copper-selenium antagonism although there is uncertainty as to selenium can prevent copper toxicity. Selenium toxicity is associated with a marked increase in blood, heart, liver, and kidney levels of copper (Chen et al., 1987b). Kezhou et al. (1987) report (abstract) "... that the Cu^{++} ion is much more effective in ameliorating the effects of selenite toxicity in rats than SO_4^{--} ion, that selenite increases the hepatic concentration of Cu, ..."

Silver - Competition between silver and copper has been reported in microorganisms (e.g. Ghandour et al., 1988) and experimental animals (Yatomi, 1986). In the rat, competition by added copper, in the gut, may inhibit the uptake and metabolism of silver (Yatomi, 1986).

Vanadium - In a discussion of the effects of intoxication with vanadium compounds on copper metabolism in the rat, Witkowska et al. (1988) suggest that vanadium may interfere with the normal kinetics and macromolecular binding of other essential metals. Elfant and Keen (1987) did not find an effect of vanadate on tissue copper concentrations in adult and developing rats.

Others - The addition of fluoride to soils has been found to increase the solubility of some elements although, with copper, only slightly (Elrashidi and Lindsay, 1987). Magnesium is suggested to be able to induce an apparent copper deficiency in tomato leaf spot bacteria (Woltz et al., 1988). The same authors comment (Jones et al., 1987b) that "Mg, when applied as a foliar spray, may reduce the efficacy of copper sprays." Electron transfer between ruthenium-modified plastocyanins and copper has been demonstrated (Jackman et al., 1988).

V. UPTAKE AND ACCUMULATION OF COPPER BY ORGANISMS

Copper uptake can occur in a number of ways and can be affected by a variety of factors (Wang, 1987). Since the metal is essential to life, details about uptake as well as transport within the organism are important. They have been addressed in a number of reviews, written from the standpoint of animal or human nutrition (Milne, 1987; Nowacka et al., 1986), physiological or biochemical defects in copper metabolism (Ettinger, 1987; Flipo et al., 1987) or toxicity (Wang, 1987) and tolerance (Taylor, 1987). A number of recent references deal with the form or the biological availability of the metal during uptake (e.g. Luoma, 1986; Vana et al., 1986). Discussions of transfer of metal within a food chain and food web frequently include valuable information on the uptake and accumulation of copper (e.g. Amiard, 1988a). The U.S. National Technical Information Service (1988h) provides 281 recent citations on the uptake of heavy metals by shellfish and marine plants.

Uptake of copper is possible only if the metal is in a biologically available chemical state. In a number of studies of plant copper uptake from soils, the controlling factor appears to be the mobility of the metal (El-Kherbawy et al., 1987; Hyuga, 1985a,b). The nature of the plant is also important, plant-specific requirements and capabilities may indicate copper amounts, source and availability (e.g. Bisson et al., 1989; Clark and Gourley, 1987; Mahendrappa, 1987; Pandey and Thakur, 1988; Szentmihalyi et al., 1986; Varro and Gyori, 1987). The chemistry of the environment often dictates the mobility of metals, whether from anthropogenic (e.g. Thomas et al., 1988b) or natural sources. Information on soil chemistry and plant nature can be used to understand plant-soil-atmosphere interactions (e.g. Leininger and Winner, 1988) and to improve plant growth under conditions such as acid subsoil (Farina and Channon, 1988) or in clay and calcareous soils (Constable et al., 1988; Rokba, 1985). Plant-soil-atmosphere interactions become especially important in interpreting the effects of acid rain (Reich et al., 1988) or of specific industries (e.g. Lobersli and Steinnes, 1988). They are also important in predicting the effects of crop management practices whether in forestry (Entry et al., 1987) or agriculture (Karlen et al., 1988; Romero, 1987; Roy and Srivastava, 1988). Similar statements can be made about aquatic environments (e.g. Ahlf, 1988b; Higgins and Mackey, 1987; Hirose and Sugimura, 1985; Moffett and Zika, 1987). The use of aquatic or near-aquatic plants to remove metals is an example of this, whether in freshwater (e.g. Mo et al., 1988a), estuaries (Wu et al., 1988c) or wastewater systems (Roehlly et al., 1987).

Soil organic matter often limits metal bioavailability and thus uptake (e.g. McGrath et al., 1988b). High levels of phosphorous appear to limit copper availability (Gembarzewski et al., 1986), a condition often found in association with high levels of organic matter (Tomar et al., 1986). However, Sharma and Arora (1987) report increased metal uptake with added phosphorous although this was a result of increased plant growth (dry matter production). In contrast, elevated concentrations of soil zinc and copper may limit plant phosphorus uptake (Prasad and Ram, 1986). Copper uptake may also be affected by the amount and nature of soil nitrogen (Kadar and Shalaby, 1984; Lasztity, 1988; Lasztity and Biczok, 1988) although the details of this relationship are not well established and metal uptake may be more a result of pH than the amount of nitrogen (or phosphorus) in the soil (Pal et al., 1987; Rebowska and Kusio, 1985)

Copper uptake by animals differs from that by plants because ingested food acts as a source material. Whether from the environment or from food, however, uptake is still dependent upon the chemistry of the copper-containing material and the nature of the organism. Rainbow (1988) states this quite well when he comments (synopsis) that "Any interpretation of the significance of a trace metal concentration in a decapod crustacean requires an appreciation of the metabolic requirements (if any) for that metal and an understanding of the pattern of its accumulation with or without associated detoxification." As with plants, knowledge of the nature of the organism may permit its use as an indicator of metal bioavailability (e.g. Clark, 1986; Stafford, 1988). With soil organisms such as earthworms, soil pH often dictates metal availability, at least in metalliferous soils (Morgan, 1985). With aquatic organisms this is more difficult as a result of the mobility of the environment and the resultant change in chemical properties over short time periods (e.g. Catsiki, 1986). Within the organism, the association of metals with metal-binding proteins will affect metal concentrations within specific tissues (e.g. Tessier et al., 1985). This can produce both inter- and intra-specific variability since the biochemical factors associated with metal uptake and tissue concentration can vary from species to species and, within a species, over the life history. Amiard-Triquet et al. (1988), for example, report that oysters (*Crassostrea gigas*) accumulated copper from their food source but that the oyster drill (*Ocenebra erinacea*) did not accumulate copper from copper-enriched oysters. Wolmarans and Yssel (1988) suggest that snails with the copper-containing blood pigment hemocyanin may handle external copper differently from those species with the iron-containing blood pigment hemoglobin. Metal-metal interactions may also affect metal uptake and accumulation (e.g. Kaitala, 1988) although Chen and Liu (1987) note that the accumulation of copper by the naupliar life history stages of the brine shrimp *Artemia* was the same for single and multiple metal exposures. There is, however, strong indication of changes in metal uptake and tissue metal concentrations in an organism both over the year and during the life history. With the barnacle *Balanus amphitrite*, Anil and Wagh (1988) note the concentration factor for copper and zinc baried during different periods of the year and with the size of the animal. With crustaceans, the exoskeleton often contains chitin which adsorbs metals from the environment (e.g. Neugebauer et al., 1986). The process of moulting (shedding the old exoskeleton) can cause a shift as well as some loss of metals and a change in rate of metal uptake (including copper; e.g. Al-Mohanna and Nott, 1985; Engel, 1988). Since feathers contain copper and other metals (e.g. Cosson et al., 1988), comparable changes may occur in birds during moulting.

In an evaluation of element uptake by two breeds of cows, Chavez et al. (1988) found evidence that different breeds of cattle absorb minerals at different efficiencies. Thus calculated dietary allowances may vary between breeds of animals as well as during their life. Kume et al. (1987) found that trace element intake and excretion decreased with higher temperatures in lactating cows although this was not true for copper. Medeiros et al. (1988) report that musslce tissue-levels of copper and zinc were greater in concentrate-feed steers than from younger, range-grazed steers, an indication of effect from either or both feed type and age. With ruminants, rumen fauna (bacteria and protozoa) affect metal bioavailability from food (Georgievskii and Ruklova, 1987; Ivan, 1988). Parasite infestation can also affect the copper concentration of certain tissues, such as the liver (Giraldo and Southern, 1988).

The process of metal uptake by plants can involve sorption, especially in an aquatic medium (Gordon and Millero, 1987; Hart et al., 1984; Low and Lee, 1987; Xue et al., 1988b). The plant cuticle may act as a barrier or possibly as a "source" of metal from the environment (Chamel, 1988). Yeasts (Kihn et al., 1987), yeast cell wall materials by themselves (Wakatsuki et al., 1988), especially soil fungi (e.g. Vasilevskaya et al., 1988), and plant roots (Sohabel, 1986) adsorb copper and may provide a sink or a source of metal. Wakatsuki et al. (1988) obtained evidence suggesting that yeast cell wall material not only supplies sorption sites but also is associated with the chemical reduction of copper, possibly a result of copper-reducing proteins in the cell wall. Copper uptake by plants may, in fact, be strongly affected by the microorganisms associated with the surface of the roots or even the aboveground portions of the plant (e.g. Ames and Bethlenfalvay, 1987; Kucey, 1988; Kucey and Janzen, 1987; Raju et al., 1987). Under conditions of copper deficiency, however, uptake of copper by microorganisms may decrease the available copper, thereby increasing the deficiency (e.g. Manjunath and Habte, 1988). Copper uptake may also be affected by the sorption capability of iron and possibly manganese oxides on the plant (Johns, 1988).

Uptake probably involves a series of steps or phases. First, a source of available metal is needed, as for example the ionic metal (Kiekens et al., 1984). This is where factors such as soil (or sediment) pH become important, controlling the chemical form of the metal (Adams et al., 1985; Ahlf, 1985; Demon et al., 1988; Verma and Neue, 1988). Sorption of copper to the cell is likely to occur at chemical "sites" produced by agents such as pectic polysaccharides (Allan, 1987; Crist et al., 1988). At the cell surface the ionic metal may or may not be reduced and/or bound by an organic. In a discussion of some detrimental effects of copper, Gaisser et al. (1987) comments that copper complexes enter the cell and then dissociate, freeing the copper for internal cell use or effects. The complex as well as organics within the cell, will have properties controlling their binding to copper (Katz, 1988). As well, transport across the cell membrane may be affected by metal-metal interaction as, for example, with manganese (e.g. Lastra et al., 1988). This and accumulation, will also be affected by metabolites (Fayed and Abdel-Shafy, 1986) as well as the nature of the organism (e.g. Sela et al., 1988; Suhs, 1987). Certain polymers/resins are being used for the slow release of metals from fertilizers (Checkai et al., 1987; Lotfy et al., 1987).

With animals, uptake follows many of the same steps as with plants. pH is, for example, very important in controlling metal speciation and bioavailability (e.g. Krantzberg and Stokes, 1988). The nature of organic ligands associated with the copper control metal availability (e.g. Hoadley and Cousins, 1988). Once within the organism, rate of copper accumulation can be affected by temperature (Rao et al., 1988) and the nature of ligands associated with the fate of the metal (Hartter and Barnea, 1988a; Stagg, 1986). Several authors (e.g. Harris and Percival, 1988; Linder and Goode, 1988), for example, suggest that copper dissociates from the transport protein ceruloplasmin before it is taken up by the individual cells. Hartter and Barnea (1988b), working with the rat brain, introduces evidence of neuron depolarization releasing newly taken-up copper. Both rate of accumulation and metal concentrations in different tissues can be affected by the physiological state of the organism (e.g. Oster et al., 1988).

The amount of copper taken up by the organism varies depending on the nature of the organism as well as the availability of the metal. With one species of mushroom (*Lentinus edodes*) grown in a sawdust medium, Matsumoto and Tokimoto (1987) report a transfer of 62-95% of the medium nitrogen, potassium and copper to the organism. In the pileus of the oyster mushroom (*Pleurotus ostreatus*), Yasui et al. (1988) found a copper concentration factor of 1.08 when compared with the medium concentration. With the chayote plant (*Sechium edule*), Valverde et al. (1987) report that over a 195 day growth period, 100 grams of copper were accumulated from each hectare of soil. The fate of the metal can vary over time, as a result of requirements by the developing plant (or animal). Hiroce et al. (1985), for example, report that the concentration of copper in the leaves of ramie plants (*Boehmeria nivea*) decreased with plant age up to 90 days (the oldest stage examined). Khallyeva et al. (1985) found that with fine-fibered cotton, copper is localized in the roots but increases with growth in the leaves. The authors provide the biochemical mechanisms involved in the use of copper during growth and flowering.

In animals as in plants, there tend to be tissue-specific copper concentrations whether it is within the brain (e.g. Saito et al., 1988b), specific tissues, or fluids (e.g. Hickey, 1987; Ikuta, 1986a; Ikuta and Morikawa, 1988). Okoshi et al. (1988), working with the Japanese oyster (*Crassostrea gigas*), comment that the high accumulation of several metals (including copper) in certain tissues is probably a result of changes in physiological activity associated with sexual maturation and glycogen storage. Copper is one of the metals (Mg, Zn, Cu) that Ashizawa et al. (1988) found to increase during maturation of rooster sperm and to decrease after storage. Changes in temperature and food availability, among other things, can also affect rate of metal accumulation and tissue concentrations (e.g. Nemcsok et al., 1986; Uthe and Chou, 1986). Hinch and Stephenson (1987), working with freshwater clams, found that (abstract) "Clam size and (or) age successfully predicted tissue metal concentrations, but in a metal-specific and tissue-specific manner." Similar conclusions are drawn for the crab *Portunus pelagicus* (Hilmy et al., 1988b) and Pavel (1986) notes a possible increase in copper with age in a frog although there were differences among localities and experimental years. During moulting, the shrimp *Penaeus semisulcatus* concentrates copper in membrane-bound granules in the hepatopancreas (Al-Mohanna and Nott, 1985). The authors suggest that the reduction in granule number between moults is a way of eliminating excess metal. An alternative suggestion is that the shrimp stores the metal during the moult period and then utilizes it for normal metabolic processes during the intermoult. In the developing bird embryo, the uptake and metabolism of trace elements are mediated by the "egg" membranes while partitioning of the metal is an intracellular process (Richards and Steele, 1987). These authors provide two excellent models that describe the uptake and metabolism of zinc, copper, and iron in the egg and developing embryo.

A number of chemicals affect copper speciation and bioavailability in the environment as well as uptake, transport and storage once the metal has been taken up by an organism. Linder and Voye (1988) discuss the influence of synthetic chelating agents on copper speciation and bioavailability in plant nutrient solutions for cultivated bush beans. They comment on the inability to obtain clear conclusions on the importance of dissolved copper(II) species as bioavailable forms. Zeller and Larsen

(1988) obtained incrased mineral nutrient accumulations of Ca, Mg, N, P, Al, B, Cu, Fe, Mn and Zn in a variety of golden delicious apple with soil-drench applications of two growth inhibitors. No comment is made as to whether this was a result of metal mobilization or increased rate of metal uptake.

Plants produce copper-complexing organics that can affect metal bioavailability in aquatic (Zhou and Wangersky, 1989) as well as soil environments. Metal-metal interactions also control metal uptake as well as tissue distribution in both plants and animals. The molybdenum-copper antagonism discussed in an earlier section is an example of this in sheep and some laboratory animals (Allen and Gawthorne, 1987; Mason et al., 1988) as is the use of zinc overload to reduce copper uptake in patients with Wilson's disease (Cossack and van den Hamer, 1987). Organics are involved in metal transport within the organism. Robinson et al. (1988), for example, suggest that plasma proteins play a role in metal (including copper) transport in bivalve molluscs. Richards (1988), working with turkey laying hens, concludes that vitallogenin (a yolk precursor protein) mediates transport of Zn, Cu and Fe to the yolk of the newly-formed egg. Feng et al. (1987b) obtained evidence that human serum transferrin may play an important role in the transport of metal ions (including copper) from mother to fetus. Storage of copper and other metals within the organism often involves one or more proteins called metallothioneins or "metallothionein-like". These small molecular weight, cysteine-rich chemicals are suggested to play important roles in metal detoxification as well as storage in organisms ranging from bacteria to humans (e.g. Berka et al., 1988; Fujita and Nakano, 1988; Hamer, 1988; Jenkins, 1986; Kobayashi and Sayato, 1987; Krezoski et al., 1988; Kurisaki et al., 1988; Markossian et al., 1988; Overnell and Abdullah, 1988; Viarengo et al., 1988a). This group of proteins, discussed earlier in this review, are perhaps too widely implicated in copper transport, storage and elimination.

Ascorbic acid may be involved in copper metabolism in laboratory animals (Messripour and Haddady, 1988) as well as humans (Jacob et al., 1987; Milne et al., 1988) although the evidence for this is not obvious (e.g. Held et al., 1988). Milne et al. (1988) point out that ceruloplasmin and cytochrome-c oxidase activity of platelets and white cells may be sensitive indicators of copper status in women on a diet marginal in copper. Dietary cholesterol reduces liver copper levels in rabbits (Klevay, 1988a) and biliary copper of rats (Klevay, 1988b). Kuipers et al., (1988) note that glutathione may be involved in the rapid secretion of excess copper during copper overload in the rat liver. Phytic acid is involved in copper bioavailability with or without fiber (Frolich and Nyman, 1988; Nolan et al., 1987; Paulini et al., 1988; Persson et al., 1987). Phytate may enhance copper utilization by its ability to bind other dietary components such as zinc that compete with copper for uptake (Lee et al., 1988c). However, at low phytate:copper ratios, calcium may compete with copper ions for binding by precipitated phytate species (Champagne, 1987). Evans and Klevay (1988) point out that copper absorption is unaffected by a dose of phytate that impairs zinc absorption. Fiber, by itself, may be important as a sorption site and reduce the uptake of copper (e.g. Nandy et al., 1987). Moak et al. (1987) report an effect of bran fiber on increasing fecal metal loss and a greater effect of oat-bran than wheat-bran. Morris et al. (1988) found no overall deleterious effects on copper and general mineral nutriture of adult men with either whole or dephytinized wheat bran. Comparable results were obtained by Behall et al. (1988). Taper et al. (1988) comment that a liquid-formula diet supplemented with 40 g soy polysaccharide could have a deleterious effect on mineral retentions in individuals consuming the diet as a sole nutritional source. Bell et al. (1987, abstract) suggest that "Depending on the feeding practices employed during the weaning period, it is apparent that infant cereals may compromise utilization of zinc and copper from milk diets during weaning." It is apparent that there is controversy about the use - and misuse - of fiber and phytate in normal as well as specialized diets. About the only thing that is obvious is that judicious use of fiber is not detrimental to the copper uptake of a healthy adult human using a well balanced diet.

Carbohydrates are involved in nutrition of organisms. Various types of carbohydrates affect metal sorption by bacteria (Branting et al., 1988) and metal uptake by laboratory animals and perhaps humans. Evidence from a series of studies (reviewed in Fields, 1988) suggests that with rats under copper deficient conditions, copper uptake is reduced in diets containing fructose but ameliorated by diets containing starch. Beal et al. (1988) report an inverse linear relationship between rat liver and heart copper concentrations and the amount of fructose in the diet. Failla et al. (1988) used immunoresponsiveness to demonstrate that the dietary requirement for copper in young rats is greater with dietary fructose than dietary starch. In corpulent rats, fructose consumption produced lower pancreatic copper and iron concentrations, and zinc concentration was elevated (Lewis et al., 1988b). Other work (e.g. Lewis et al., 1988a; Yang et al., 1988) supports the general observation that fructose consumption tends to reduce the copper status. However, Bowman and Johnson (1986) provide evidence that this is not the result of alterations in copper absorption or endogenous excretion. Johnson et al. (1988b) also provide evidence that during a single meal, copper absorption is not affected by the type of carbohydrate in the diet.

Changes in copper distribution have been attributed to changes in the genetic makeup of the organism (e.g. Freedman and Peisach, 1987; Lee et al., 1988a; Roshchina et al., 1988). In genetically copper-deficient mice Cunnane et al. (1988) found evidence of differences in tissue long chain fatty acid as well as brain phospholipid composition. Changes in copper uptake by the cell can also be affected by physiological state (e.g. Cole et al., 1988a) as well as by the response of the cell to the chemical state of the copper (Wilson et al., 1987). There is also an effect of copper storage, liver stores of copper and iron can prolong the period of physiological normalcy even under conditions of copper deficiency (Colville and Johnson, 1988).

Much of the work on metal uptake, accumulation and tissue concentrations is to better understand the fate of metals such as copper in humans (e.g. Southon et al., 1988). Part of this is achieved by understanding the intake of metal with different diets (Cooke et al., 1988; Santo et al., 1985; Shiraishi et al., 1988a; Thomas et al., 1988a), part through the use of isotopic tracers in examining mineral metabolism (e.g. Garrot et al., 1988; Johnson et al., 1988c; Turnlund and Keyes, 1988; Turnlund et al., 1988a,b), part through an understanding of the effects of disease on metal levels (e.g. Joost and Tessadri, 1987; Navarro-Rodriguez et al., 1986; Uriu-Hare et al., 1988). Whitley et al. (1987) point out that radioactive isotopes can be used to investigate mineral metabolism in adults but not in children and pregnant women. For these two cases, the use of stable isotope techniques is adequate although not ideal. One of the problems faced in obtaining tissue or nutrient metal levels is the variability that occurs. Manci and Blackburn (1987), for example, found a coefficient of variation of greater than 10% in copper in fetal membranes of term human placentae. Allegrini et al. (1985) report a wide variation in Italian infant formulas, something that is probably a feature of commercial formulas throughout much of the world. Variation also occur over time, even short time periods. Kanabrocki et al. (1988) note a circadian variation in urinary excretion of human subjects. With copper this was from 20-80 μg/day for 15 subjects.

For individuals with copper-associated physiological problems, a knowledge of the fate of copper once absorbed better allows control of metal intake and excretion. This requires knowledge of the physiological problems since the fate of the metal will be a function of the nature of the disease as well as the nature of the metal (e.g. Kishore, 1988b). Copper deficiency in patients receiving parenteral nutrition (Friel et al., 1988; Shike, 1988) can be controlled with this type of knowledge. The copper depletion in infants that is caused by acute diarrhea is treatable with appropriate supplementation. Siegemund et al. discusses pharmacological regulation of copper bioavailability for patients with Wilson's disease. Removal or at least regulation of copper, zinc and iron during hemodialysis is advocated for patients with acute hepatic necrosis from Wilson's disease (Arnold et al., 1988). Palida et al. (1988) discuss a role for intracellular copper-binding proteins with an intention of better understanding Menkes disease. Recent work on copper in human serum suggests a much larger amount of copper in an excahngeable form than what had previously been estimated (Sarkar, 1988). This indicates a much greater importance for transport ligands in normal as well as pathological sera.

VI. CHANGES OCCURRING IN COPPER AFTER INTRODUCTION INTO NATURAL ENVIRONMENTS

The effect of metal speciation on the biological availability of copper has been a continuing topic in this review. It is important to recognize that changes in speciation occur as a result of the introduction of copper into an environment. This section is a discussion of the changes that occur in copper after introduction into aquatic and terrestrial environments. It begins with a review of references concerning metal in aerosols because atmospheric transport accounts for much of the copper introduced into terrestrial and aquatic environments.

On a worldwide basis, Nriagu and Pacyna (1988) calculate loading rates of trace metals into air, water and soils. Using reference sources, they estimate a 1983 worldwide emission of between 19,860-50,870 x 10^3 kg of anthropogenic copper. Coughtrey et al. (1987) edit a series of papers that examine the transport and "fate" of anthropogenic materials in various ecosystems. Consideration of anthropogenic metal input appears in reviews dealing with specific areas (Baltic Marine Environment Protection Commission, 1987; Hung et al., 1987; Hungspreugs, 1988; Salomons et al., 1988a; Strain, 1988). General reviews of metal speciation in aquatic environments are provided in Hem (1985) and Hunt (1987) and Cross and Sunda (1985) discuss the relationship between metal speciation and bioavailability with respect to marine organisms. Ryan and Windom (1988) provide a geochemical and statistical approach for assessing metal pollution in coastal sediments while the environmental capacity to accomodate anthropogenic materials is considered by the "Joint Group of Experts on the Scientific Aspects of Marine Pollution" (1986).

Aerosol Copper

van Aalst (1988) points out that atmospheric inputs into oceans, seas and lakes have been studied extensively in recent times. So also have inputs into terrestrial environments, especially in or near industrialized regions. In his review, van Aalst (1988) lists aerosol emissions of copper for some European countries, ranging from 10 (Luxembourg) to 1600 (West Germany) tons per year. In estimates of atmospheric input from mines and metal production in Sweden, Ross (1987) gives values of 280 tons per year for 1978 and 100 tons per year for 1984-1985. Ross (1987) notes a regionalization within Sweden, highest input coming from the more industrialized areas. The same pattern is usually the case elsewhere for both emissions and wet and dry deposition (e.g. Blanchard and Stromberg, 1987; Freise et al., 1987; Gomes et al., 1988; Tang et al., 1986; Vermette et al., 1988; see table 2 for amounts). As an indication of the regionalization, Schroeder et al. (1987) give literature values of aerosol particulate copper in remote (0.029-12 ng/m^3), rural (3-280 ng/m^3) and urban (2-6810 ng/m^3) environments. Atmospheric inputs of copper into oceanic environment have been estimated by van Aalst and others although lack of measurement over large oceanic areas reduces the value of the estimates (Krell and Roeckner, 1988; see also Stossel, 1987). (e.g. in 1983 van Aalst estimated atmospheric input of 1400-10000 tons copper per year into the North Sea (data from van Aalst, 1988).) In estimates of the aerosol metals which penetrate into the soils in Central-East Europe, Bublinec and Vosko (1987) list copper at 0.1 kg/hectare/year. Although deposition amounts are related to input amounts, soil metal retention can be affected by the nature of the soil as well as topographic features (Ditter et al., 1984). Plant absorption also plays an important role in what could be termed metal extraction from wet deposition (Stachurski, 1987)

Emission of metals occurs both from natural and anthropogenic sources, from volcanism, vegetation, soil erosion, pollution and air-water transfer through bubbles and spray (Dedeurwaerder et al., 1986). They include copper-containing anthropogenic material ranging from airborne emissions of kiln-dried CCA-treated wood (Williams and Bridges, 1984) to smoke from "mosquito coil", a mosquito repellant (Liu et al., 1987a). Anikiiev et al. (1986) estimate an atmospheric input of 1.7 x 10^8 tons per year of copper from the World's oceans. In an examination of the chemistry of metal dissolution from aerosol particles, Williams et al. (1988b) comment on the importance of leaching to trace metal concentrations in rainwater. They state (abstract) that "The rate of dissolution, the final concentration and the fraction of metal leached increased with decreasing pH over the range of pH 2.5->5." As has been discussed in an earlier section, acid rain-associated factors are often associated with elevated metal concentrations in waters and soils (e.g. Blancher and McNicol, 1987; Stuanes et al., 1988) although this is not always the case (Handa, 1987). The chemical nature of the particles can also provide a clue as to their source (Anderson et al., 1988) and the ability to react with gaseous or solid materials in the atmosphere (e.g. Peoples et al., 1988).

Biological impact of aerosol metal deposition ranges from effect on soil and water trace metal composition (e.g. Stepanok and Golenetskii, 1981) to metal concentrations in wood ant colonies (Stary and Kubiznakova, 1987). Monitoring aerosol emissions is done in a variety of ways including simply, the amount of corrosion of alloys in various dry areas (Al-Kharafi and Ismail, 1988). Environmental impact is often achieved through simple techniques such as the amount of metal accumulated by lichens or mosses (e.g. Ammann et al., 1987; Makinen, 1987b).

Copper in Freshwater Environments

Information on copper levels in surface and ground waters appears in a range of manuscript and published reports (Eckel and Jacob, 1988). All too infrequently, metal levels in bodies of fresh water are related to sources and loadings and, especially to chemical properties of the environment. One example where this is done is the series of publications on the Maarsseveen Lake system near Utrecht in The Netherlands. Swain et al. (1987) characterize the lake system, provide the annual atmospheric loading to the two lakes in the system (values in table 2 of this review), and discuss some of the unique properties. van Donk (1987) discusses water quality in the two lakes in relation to their hydrodynamics, commenting on the potential effect of metallurgical industries on zinc, cadmium and copper levels. Other work that relates metal concentrations to geological and geochemical characteristics includes the study of Hart et al. (1987) on the Magela Creek system in northern Australia. Iavloshevskii et al. (1986) discuss the source and speciation of metals in waters of the "Atommash" industrial complex. They point out that with copper (translation), "stable metalloorganic combinations form 23-25.9% of the total copper". In the surface waters of the Sevan Lake Basin of Armenia, Oganesyan and Babayan (1987) found that "approximately 90% of the copper was complexed, 40% of it in compounds with a molecular weight of 9,000 or more" (translation). Harlow and Hodson (1988) provide a review of the chemical contamination of Hamilton Harbour (Canada), including heavy metals.

Improved analytical techniques combined with cleaner sample handling techniques are continuously being used in environmental work. Huynh-Ngoc et al. (1988) comment on the low levels of copper in the Rhone, which passes through industrial regions. They comment (abstract) that, based on their values, "Tabulated values of background levels of Cu and Pb in the literature may need some re-examination." Shiller and Boyle (1987) report lower than "frequently reported" trace metal levels in the lower Mississippi River with "trace metal sampling and analysis techniques demonstrated to be reliable". Copper levels ranged from 18.3-30.9 nmol/kg water. Malle (1988) comments on the importance of background metal concentrations in evaluating metal flux in the Rhine. Using the metal speciation model "Geochem", Mudroch et al. (1984) comment on the importance of fulvic acid and hydroxide (in calcium hydroxide) to copper speciation in Quebec lakes. Results for other freshwater systems include the effects of humic substances and hydroxides (e.g. Cabaniss and Shuman, 1988). Morfett et al. (1988) report increased concentrations of aqueous dissolved copper in a seasonally anoxic lake. They attribute this to release from the sediments, from decomposing algal material in the summer and, in the winter, from reduced mixing associatd with an ice cover.

The transport and fate of natural and anthropogenic copper in freshwater systems is discussed in a number of recent references (e.g. Abrahams et al., 1988; Chapman et al., 1988a; Kardanova, 1983; Konovalov et al., 1983; Lum elt al., 1988; Reczynska-Dutka, 1987; Smith et al., 1988; Terytze, 1987; Wachs, 1986). The geochemical nature of introduced metal and its biological effect is of importance to industry as well as to the public. An example of this is the program carried out by Bougainville Copper Limited on the mine waste disposal system in a high rainfall area. Date from the system have been combined with hydrological and published chemical data to develop a conceptual and then a numerical model to examine certain aspects of the disposal system and to assist in environmental management. Salomons et al. (1988d) evaluated the impact of a gold-copper mine on the Fly river in New Guinea. High copper concentrations near the mine (several hundred μg/g suspended sediment) decreased downstream as a result of dilution. The total metal concentration in a river or lake system, or even urban runoff, is often controlled by the suspended sediment nature and concentration as well as water flow rate (e.g. Amano and Fukushima, 1988; Streigl, 1987). A reduction in flow rate in a freshwater system will allow sedimentation of sediments with a reduction in total copper in the water but an accumulation of copper in benthic sediments. However, a change in water pH can affect the mobility of the surficial metal on the sediments. Other factors that can affect metal mobility include oxygen, alkalinity and conductivity (e.g. Myllmaa, 1985). All of these have to be considered in management programs as well as programs for the recovery of industry-affected sites. The recovery of Lake Orta in Italy is an excellent example of this (Bonacina et al., 1988). A lake affected by a rayon factory with effluent containing high amounts of copper and ammonium sulfate, new waste treatment facilities have reduced the input of wastes from the plant. This combined with normal water replacement has allowed initial recovery, with the reappearance of a benthic fauna.

The biological availability of copper in freshwater systems is controlled by the chemistry of the water and sediments. One expression of this is the ability of the water or sediments to bind or complex copper, a feature which is ammenable to measurement. Tan et al. (1988), for example, used anodic-stripping voltammetry to estimate metal complexing capacity in water from a river in Malaysia. Waite (1988) discusses some of the nearsurface photochemical reactions that can affect metal complexing ability, breaking down copper-containing organometallic compounds and releasing ionic copper. Copper uptake by algae (freshwater and marine) will also affect metal speciation and the biological availability of copper (e.g. Mann et al., 1988; Peterson and Swanson, 1988). Another expression of availability is the amount of metal extracted from the particulate fraction, or bottom sediments, by various extracting agents. Fuhrer (1986) used this method in a laboratory study to evaluate the potential interaction between bottom material and water as a result of dredging in the lower Columbia River estuary. Results suggested that the amount of copper extracted by 1N HCl was not elevated but within the range observed in uncontaminated estuaries. Bierman et al. (1988) compared methods of estimating tributary loading rates of anthropogenic materials but comment that (abstract) "No single estimation approach was found to be superior for all of the constituents and hydrologic conditions tested." Riverbank filtration has been examined in terms of the introduction of metal-containing water into groundwater (Foerstner et al., 1986a,b; Schoettler, 1985a,b). Metal speciation and availability in groundwater is, as in other aquatic systems, controlled by the chemistry of the water and the activity of the particulate fraction (e.g. Foerstner et al., 1986b). Burger et al. (1988) discuss a computer program to simulate flow patterns and pollutant migration in saturated ground water. Water quality criteria are considered for fresh water, especially for economically important important organisms like fish (Alabaster and Lloyd, 1982; Philippart et al., 1988). Honeyman and Santschi (1988) discuss some of the problems related to the prediction of metal residence times in natural systems. They comment, for example, on the major effect produced in metal speciation by minor changes in pH. Holmes (1988) provides summary values for copper loadings in the Hamilton Harbour area of Lake Ontario and discusses the potential for fisheries rehabilitation.

Copper in Estuarine Environments

Estuaries are transition zones, usually between fresh water and salt water. The addition of the major ions from the salt water combined with changes in flow rate and a number of other parameters can affect metal transport (e.g. Fanger et al., 1987) and produce major changes in the speciation of copper. Since major population centres frequently occur adjacent to estuaries of large rivers, estuarine regions are often areas of anthropogenic metal input (Huiskes and Rozema, 1988). Salomons et al. (1988c) review some of these changes and discuss their importance in the assessment of heavy metal impact in estuarine and coastal zones. Yeats and Bewers (1987) discuss approaches to estimating trace metal transport through coastal zones and apply this to some of the work that has been done in the Gulf of St. Lawrence. Paulson et al. (1988a,b, 1989) determined sources and sinks of dissolved and particulate Pb, Cu and Zn in Puget Sound (Washington), commenting that approximately 40% of the copper and zinc originated from advective inputs and 30% from riverine sources. Pravdic and Juracic (1988) calculate the "environmental capacity" for copper, of a river estuary in Yugoslavia. They consider the biogeochemical fate of copper and suggest restrictions on new activities such as the use of copper-based antifouling paints on boats and yachts.

Recent literature includes discussions of copper flux and speciation in a number of major estuaries. These include the Scheldt (Wollast, 1988), Elbe (Irmer et al., 1986; Salomons et al., 1988b), Seine (Baeyens et al., 1986b; Fischer and Wartel, 1986; Gandon et al., 1986; Guegueniat, 1986; Guegueniat et al., 1986), Rhone (Aubert, 1988), Dnieper-Bug (Linnik and Nabivanets, 1987), Humber (Barnett et al., 1989; Morris, 1988), Thames (Morris, 1988), Tay (Hubbard and Hashim, 1987), Forth (Leatherland, 1987b; see also Davies, 1987), Hanjiang River (Lin et al., 1988), Saigon River (Anikiiev et al., 1988a), Bang Pakong River (Windom et al., 1988), estuaries of western Taiwan (Hung, 1987), Purna River (Zingde et al., 1987), St. Lawrence River (Hamblin et al., 1986), and Milford Haven estuary (see also Drude de Lacerda et al., 1988. Metal levels in a number of these can be found in Table 2.

Geochemical changes in metal speciation have been found to occur at low levels of salinity. Lukashin et al. (1987), for example, note major changes in copper and zinc between 0-7§ in the mixing zone between the Courland Gulf and Baltic Sea. Flocculation of 76 % of the dissolved river copper is reported to occur in the estuary of the Zhujiang River (Lin et al., 1987). Although humics play a major role in flocculation, their role varies depending on whether they are of riverine or marine origin (Jones and Thomas, 1988). In the fjord-type estuary of the Pettaquamscutt River (Rhode Island), dissolved organic copper ranged from 0.29-0.001 µg/kg going from the oxic surface to anoxic deep waters. (Mills et al., 1989). Trace metal adsorption in estuaries is also important (Adediran, 1983; Balls, 1988; Glegg et al., 1988). As a final note, Evans et al. (1987) suggest that shorebirds act as effective recyclers with trace metals, through their ingestion of large proportions of the annual production of benthic intertidal invertebrates in temperate estuaries. They also suggest they may play an important role in the transfer of metals between estuaries.

Copper in Saltwater Environments

Kremling (1988) discusses metal cycles in coastal environments, commenting that for elements which show conservative behaviour and have long residence times (including copper), the influence of anthropogenic discharge on the global ocean is likely to be negligible. The changes which occur in coastal waters tend to be greater than those occurring in the open ocean whether it is seasonal changes in copper or the fractionation between particulate and dissolved copper (e.g. Hong et al., 1988). Especially in shallow seas, the accumulation of copper in the sediments can be rapid. Hoshika et al. (1988), for example, found mean residence time of water column copper to be approximately 0.3 years in the Seto Inland Sea, Japan. This was suggested to be from rapid accumulation in the bottom sediments. In deeper waters, metal concentration can be water mass specific (e.g. Bordin et al., 1987; Yeats, 1988). This is a result of the source of the metal and the chemical properties of the water although often under at least partial influence of the byproducts of plant and animal metabolism (e.g. Carruesco et al., 1982; Demina, 1984).

In a review of the distribution of trace metals in ocean waters, Yeats (1988) points out (page 145) that "... unlike the nutrient-like elements, copper concentrations tend to increase towards the bottom with little or no evidence of intermediate depth maxima." Windom (1987) discusses the data base for a study on trace element geochemistry of the South Atlantic Bight. In a discussion of the suspended particulate matter in the North Sea, Nolting and Eisma (1988) comment on the mostly high copper levels (>100 µg/g) in the western central and northern North Sea and lower levels in the southern and eastern central North Sea. Kersten et al. (1988) point out the increase in dissolved metal concentrations in the North Sea, including copper. Leatherland (1987a) comments on the adoption by the U.K. of environmental quality standards for copper discharge into the North Sea. High levels of copper are found in Baltic Sea sediments, particularly in fine-grained soft sediments below anoxic deep waters (Brugmann, 1988). Gienapp (1984) correlates metal concentrations in the German Bight with optical parameters of the water. Highest correlation coefficients (0.853) were with absorption at 261 nm. Sorption plays an important role in copper associations, whether with organic or inorganic particles, sea water or fresh water (e.g. Gordon and Millero, 1987; Jones et al., 1982; Swartjes et al., 1988; Xue et al., 1988b; see also Negebauer et al., 1986 and Poulicek, 1985 on chitin). Johnson et al. (1988a) point out that both cobalt and copper are removed from the oceans by scavenging. Working in the Southern California Bight, they report a sharp increase in near-bottom cobalt concentrations but not in copper concentrations. Paulson et al. (1988a) point out that in Puget Sound, advection removes about 60% of the total copper and zinc added to the basin while 40% sediments to the bottom on particles. The water-sediment interface can be an area of high metal concentration, especially with refluxing of metals from the sediments (e.g. Hardy et al., 1986). Both the sediment-water interface and the air-water interface (sea-surface microlayer) can be metal-enriched and exhibit elevated toxicity (Cross et al., 1987; Hardy et al., 1987; von Westernhagen et al., 1987).

The importance of organics to copper speciation and biological availability is evidenced in a number of recent references. Hirose and Sugimura (1985) discuss the chemical and ecological role of copper-organic complexes in the marine environment. Detrital decomposition of biological material releases both metal and organic ligands, the latter often capable of forming strong complexes (Higgins and Mackey, 1987). Humic substances, as in estuaries and freshwater environments, play important roles in the distribution of copper (e.g. Courtot et al., 1988; Hering and Morel, 1988a; Sun et al., 1988) In the Northeast Pacific, Coale and Bruland (1988) report greater than 99.7% of the copper was complexed in surface waters, 50-70% at middepths (1,000 m). As a result, copper complexation gives rise to extremely low cupric ion activities in surface waters and higher activities at middepth, at least in the Northeast Pacific (Coale and Bruland, 1988).

Chester et al. (1988) point out (abstract) that "To a first approximation, the preservation of Cu_5 in the sediments mimics that of primary production in the overlying waters, and so 'fingerprints' the operation of the global ocean carbon flux in oceanic deposits." Accumulations of copper also occur in semi-confined basins where sediment transport is reduced (e.g. Haraldsson and Westerlund, 1988; Paez-Osuna and Osuna-Lopez, 1987). Sedimentation plus continual input of copper-containing water, as for example with tidal action, provides a mechanism for enrichment of metal in certain of these areas (Rimoli and Mosetti, 1985). Zeng and Wu (1987) present a transport model for copper and lead in Xiamen Bay, a model in which particulates and biological processes are important.

Changes occurring in the Cu(I)-Cu(II) ratio in oxic waters appear to be more common than originally thought and are a result of photochemistry as well as ionic interactions (Moffett and Zika, 1987, 1988; Sharma and Millero, 1988a). There is a characteristic surface maximum of Cu(I) (5-10% of total copper) and then a rapid decrease with depth, to the bottom of the mixed layer (Moffett and Zika, 1988). Dyrssen (1988) reports that reduction of Cu(II) to Cu(I) in sulfidic seawater only occurs at low values of pE. However, in low oxygen-containing sulfidic waters, bisulfides and/or polysulfides play important roles in copper solubilities and thus sedimentation and the rate of accumulation in bottom sediments (Shea and Helz, 1988; Zhan et al.,1987)

Copper in Sediments

Since metals tend to accumulate in sediments it would seem that sediments could form a record of trace metal flux. This would be true if it weren't for chemical changes occurring within the sediments once they are deposited, changes that not only affect the chemistry of the metal but also its concentration and its bioavailability (Beeftink and Rozema, 1988; Campbell et al., 1988; Van Urk and

Kerkum, 1988). However, sediment copper accumulations have been used to indicate rates of accumulation as well as sediment chemistry. Myllmaa and Murtoniemi (1986) used nutrient and metal levels in the sediments of some small lakes in Finland to indicate natural conditions. They suggest that copper, along with zinc, mercury and cobalt, may be mobilised from sulfide minerals in at least some lakes. This work and the work of Myllmaa (1987) was used to indicate anthropogenic effect in the Kuusamo uplands of Northeast Finland. Siebers (1985) used the patterns of phosphate and heavy metal accumulation in sediments of two shallow lakes in The Netherlands to indicate natural as well as anthropogenic input, the latter primarily from sewage. Similar work has been done in other areas using sediments in lakes and rivers (e.g. Bradley, 1988; Byrne and DeLeon, 1987; Guilizzoni and Lami, 1986; Mudroch et al., 1988; Vuynovich and Mueller, 1986) as well as estuaries and coastal regions (e.g. Allen, 1988; Araujo et al., 1988; Azevedo et al., 1988; Bodur and Ergin, 1988; Couillard, 1987; Donazzolo et al., 1982; El Ghobary and Latouche, 1982; El-Sayed et al., 1988; Finney and Huh, 1989; Luoma and Phillips, 1988; Marcus et al., 1988; Nadeau and Hall, 1988; Schults et al., 1987; Sinex and Wright, 1988; Skei et al., 1988; Szefer and Skwarzec, 1988; Talbot, 1988; Varnavas et al., 1987).

In a study of speciation of heavy metals in lacustrine sediments, Thomas et al. (1985) found an atmospheric flux of approximately 13 $\mu g/m^2$/day as well as an indication that organic matter and iron was associated with the accumulation of metal (Cu, Zn, Pb, Cd) of non atmospheric origin. Rauret et al. (1988) note that copper in the "non-residual" fraction was bonded mainly to organic matter in sediments from the River Tenes (Spain). Metals accumulated by marsh plants can reduce sediment metal levels initially (Wu et al., 1988c) although they ultimately form a major source of metals introduced into aquatic environments (e.g. Feutel et al., 1988; Pulich, 1987). Samanidou and Fytianos (1987) obtained evidence that an important portion of the anthropogenic metal (Pb, Cd, Cu, Fe, Mn, Zn, Cr) introduced into the Axios river in northern Greece is in relatively unstable chemical forms. This suggests that the metal would be labile, available for both biological and chemical reactions. Steffen (1986) and others have found a strong relationship between particle size and particulate metal levels, metal concentration increasing with decreasing grain size. Thus, sediment copper levels depend on a range of physical and chemical factors ranging from the adsorption partition coefficients (e.g. Young et al., 1987) to the concentration and nature of the organics in the sediments (Piemontesi and Baccini, 1986). The nature of the particle is, of course important, dictating the nature of the copper-particle association. With biogenic particles there can be a sorption as well as a complexation (e.g. Ferris et al., 1989). These factors also affect metal partitioning (e.g. Dehnad et al., 1987; Moore et al., 1985b), especially during resuspension (Mudroch, 1985; Theis et al., 1988) as, for example, during dredging (Word et al., 1987). They indicate the potential for geochemical control of anthropogenic metal (Ferretti and Maffei, 1985; Mogollon et al., 1987).

Particle-copper associations may change in the transition from fresh water to salt water as a result of the increase in major ions. Seralathan (1987), for example, notes a sharp decrease in sediment copper concentrations in the mouth of the Cauvery River (India). They also change as a result of remobilization at the sediment-water interface (Baeyens et al., 1986a), or as a result of anoxic environments (Francois, 1987,1988). In the deep sea, copper is often associated with iron hydroxides, oxyhydroxides and oxides (Yamamoto, 1986); the seasonal variation which occurs on marshes (e.g. Baars et al., 1988) or in shallow waters (e.g. Kawara, 1988) is reduced or nonexistent.

Copper in Soil Environments

The movement of copper within a soil type is controlled by the geological and geochemical properties of the soil (Bansal et al., 1986; Bruemmer et al., 1986; Davydov and Chiryaev, 1986; Kotuby-Amacher and Gambrell, 1988; Lopez-Hernandez et al., 1986; Lu and Wu, 1987; Tang, 1987). The importance of this is apparent in agriculture as well as in a diverse array of other topics, including archaeology (Thomas et al., 1988). Soil properties are affected by vegetation (e.g. Jones et al., 1988; Nohrstedt, 1988; Ragsdale and Berish, 1988; Winger, 1986) as well as by soil management practices (Bergkvist, 1987; Entry et al., 1987; Follett and Peterson, 1988; Juste and Tauz, 1986; Mulchi et al., 1987b; Payne et al., 1988b), factors which affect the flux of metals into the soil (e.g. Leininger and Winner, 1988) as well as subsequent chemistry and movement through the soil.

Information about soil properties can be used to estimate uptake and movement of natural as well as anthropogenic metal (Fanelli, 1986; King, 1988b; Pratt et al., 1988; van der Zee et al., 1988; Wang et al., 1985). Mimides and Lloyd (1987), for example, demonstrated uptake of heavy metals in groundwater in a Triassic sandstone. They described the adsorption and developed a mathematical model to fit the adsorption capability (see also Mimides, 1987). In a discussion of copper in ombotrophic peat, Jones (1987) comments (abstract) "... that peat copper deposition records may be used for reconstruction of pollution history." Background metal concentrations in various soil types have also been used to estimate the impact of industry on soil metal levels (Lux et al., 1988; Zoltai, 1988). Myrlyan (1987) discusses the fate of Bordeaux liquor in a vineyard treated for more than 40 years. The author comments (abstract) that "Percolating downward in the soils Cu hydrate oxide of Bordeaux liquor reacts with CO_2 to form radial globules of malachite at the bottom of arable layer, on the surface of $CaCO_3$ deposits, and other barriers." In an evaluation of the degradation of insecticides within plants, Petrova and Blinova (1986) point out that with the exception of copper sulfate, there is an interaction with other agricultural chemicals. They do not, however, relate this to events occuring within the soil.

The distribution of copper in different soil types is important to an understanding of metal levels, availability and plant nutrient satisfaction (Singh et al., 1988). Information about soil properties can be related to plant micronutrient requirements. Based on a range of soil and plant samples, Sillenpää (1987) notes the types of soils in which copper deficiency would be most likely. These are acidic, coarse textured soils with low electrical conductivity. These conditions, combined with low copper values, were found in sample materials from Sierra Leone, Zambia, Ghana and Ethiopia. Fertilization with copper will be affected by soil type as well as the type of fertilizer (e.g. Gorlach and Jasewicz, 1987). Shuman (1988) found that high soil surface phosphorus levels caused an increased lability of copper in fine-textured soils. In research supported by the Indian Copper Development Centre, Raghupathi (1989) reports that inorganically bound soil copper formed the major plant-available fraction. Soil organic matter plays a major role in retaining copper within the soils as well as affecting its availability to plants (e.g. McGrath et al., 1988b; Kovda and Uchvatov, 1988) as does soil pH and acid rain (Gruhn, 1986). Soil water properties are important to metal flux (e.g. Vasil'evskaya and Shibaeva, 1988). Submergence (flooding, irrigation) can affect copper levels (leaching) or lability, a result of the interaction with mineral changes (Hazra and Mandal, 1988).

Copper in Anthropogenic Materials

Discussions of anthropogenic materials are found in the chapter on "Copper and Man" and elsewhere in the present chapter.. The short discussion in this subsection considers some of the changes that can occur in selected materials after their introduction into natural environments.

Anthropogenic materials include everything from house dust to dredge spoils and byproducts of food producing and processing (e.g. Chen et al., 1986; Skurlatov et al., 1983). Since many of these materials contain copper, their introduction provides a source of copper. In many cases, the amount of copper is slight - Kapetanios et al. (1988) report that copper levels in compost from Athens household refuse are usually low and allows use of the compost as plant fertilizer. Lorber (1987) reviews hosehold waste metal levels, reporting 2500 μg/g dry weight, considerably higher than the range of 109-586 μg/g (dry weight) reported by Kapetanios et al. (1988) for compost. Disposal of dredge spoils is of concern because of the metals present. Recent efforts to use spoils as landfill include isolation of what are termed "toxic agents" (Glindemann, 1988; Huybregts et al., 1988), including excess metals. Forstner et al. (1987) suggest stabilization of dredged sludges on land or nearshore subsediment deposit, the latter to produce sparingly soluble sulfide phases of metals. Highway runoff often contains measurable copper levels (e.g. McComas and Sefton, 1985). Seasonal changes also occur as a result of changes in runoff and the use of deicing salt in temperate areas, changes which will affect metal input into receiving waters (Kelsey and Hootman, 1986). However, the chemical changes which occur in the copper speciation will be controlled by the chemistry of the water, particularly pH, and of the receiving medium.

The use of sewage, sewage water aand sewage sludge for irrigation can reduce soil pH and introduce metals (Waly et al., 1987). Stabilization ponds can improve this although not completely (e.g. Kaplan et al., 1987a; Yapijakis and Papamichael, 1987). Although the increased metal and reduced pH, by themselves, could increase copper bioavailability, particle sorption and organic complexation both act as buffering agents. One of the options available for large scale disposal of sewage wastes, particularly sludges, is ocean disposal. Introduction of copper through sewage disposal is slight when considered as a percentage of the total entering the oceans. In the North Sea, the amount of copper introduced in sewage sludge is approximately 5.5-6.5% of the total (from Parker, 1988). Association with organic material means that some buffering of direct metal effect will occur. Another disposal option is on land. The problems of metal "behaviour" in solid wastes on land are discussed by Schoer and Forstner (1988) and include potential introduction of metal into groundwater. A third option is incineration although leaching of metals occurs from incinerator ash (e.g. Francis and White, 1987) is only one of the problems in this option (see Lisk, 1988).

Similar problems concern wastes from metal mining and milling operations or certain metal-containing wastes from industry and the armed forces (e.g. Lee et al., 1988b). The effects of natural sorption (Karlsson et al., 1987b) and metal complexation, on reducing metal bioavailability are often not considered. Dudley et al. (1988) discuss an industrial application, the sorption of copper and cadmium from the water-soluble fraction of an acid mine waste by calcareous soils. Marine disposal after the stabilization of metal processing wastes has been considered as an option for disposal of potentially hazardous waste materials (Lechich and Roethel, 1988). Recovery of metals in both freshwater and saltwater systems is also possible with algae (biological uptake) which offers another option (e.g. Mann et al., 1988).