FINAL REPORT
INCA PROJECT NO. 223
THE BIOLOGICAL IMPORTANCE OF COPPER
A Literature Review
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Preface

In 1973 the International Copper Research Association Incorporated 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 the number of publications concerning copper in the marine environment was large and that an annual review was appropriate.

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 1986-1987 although a number of earlier works are included and, where appropriate, a few appearing early in 1988 have been used. Many of the earlier than 1986 references are from eastern European and Asian workers. This is because this literature takes some 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, has kindly provided the metals section of the Marine Pollution Research Titles as a source of European as well as North American References.

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.

Signature of Grant Recipient

A.G. Lewis, Professor
Department of Oceanography
The University of British Columbia

Executive Summary

The 1986-1987 literature on the biological importance of copper is voluminous and covers a wide array of topics. These include the requirement for copper, exhibited by all organisms, the effects of excess metal, biologically important chemical properties of copper, and the uses of the metal by man. From the estimated 2,940 references that were selected for this review, some of the highlights include:

> The increasing evidence that, under certain conditions, plants and animals need copper supplementation for normal growth to occur. With plants, this is especially true in organic-rich soils such as peat (Adams et al., 1981) in which the copper that is present is often bound by organics and not available for uptake. In animals, Copper deficiency has been Pinked with swayback in sheep, certain types of haemolytic anaemia, and decreased resistance to infection. Copper supplementation may be essential for the production of normal connective tissue (Strause et al., 1987) as well as normal enzyme and hormone balance (e.g. Recant et al., 1986).

Copper has long been used to control the growth of organisms in wood products as well as in aquaculture, agriculture and medicine. Additional references cite the benefits of these uses. As well, a number of copper-containing antiviral, antibacterial and antitumour agents have been tested. Copper in fungicides offers an alternative to a number of organics that promote resistance in fungi (e.g. Olvang, 1987). There is continued use of copper sulfate as a control for noxious weeds and aquatic plants. It also continues to be an economical method of controlling the mollusc hosts of a parasite that causes schistosomiasis in man. Recent references describe the continued beneficial uses of dietary copper in the control of parasites in economically important animals such as pigs and rabbits.

The ability of copper to control the growth of noxious organisms is achieved with high levels of metal. Concern has been expressed about the effects of excess copper released into the environment. The use of copper-containing fungicides can, for example, increase soil copper levels enough to cause a some plant damage (e.g. Johnson et al., 1986). Similar concerns have also been expressed about the effects of copper in municipal and industrial effluents and the release of aerosol metal. Long term exposure of plant communities to high concentrations of copper has been reported to affect the types of organisms found in the communities. Certain species of ferns and plants are restricted to localities where copper is abundant (e.g. Tabbada and Tenorio-Borja, 1986).

Levels of aerosol copper are often used as an indication of industrial activity. As an example, concentrations in the northern hemisphere are estimated to be approximately 50 times those in the southern hemisphere (Delmas, 1986). However, the biological impact of copper is controlled by the chemistry of the metal, the chemistry of the environment and the amount of metal released. Craig (1986) comments that "... the chemical form of an element is vital in the determination of the actual properties of stability, toxicity, and transport ... in the natural environment." Environmental factors such as pH can affect biological impact which is one of the reasons for the concern about acid rain. Campbell and Tessier (1987), for example, report biologically important changes in the chemistry of copper over the pH range from 7 to 4.

It is pleasing to see more and more references pointing out the necessity of understanding metal chemistry to predict biological impact. Bernhard et al. (1986c), for example, comment that "an effort should be made to develop chemical methods suitable for (chemical) species identification and quantification." Geochemical techniques and models are now being proposed that win relate metal speciation to biological effects of copper and other metals (e.g. Cowan et al., 1986). Davies and Wilson (1987) successfully used factor analysis to differentiate anthropogenic metals from naturally occurring metals in soils of a mineralized area. Efforts like this will ultimately allow more realistic environmental standards to be achieved.

Metals often interact so that a change in the concentration of one metal can offset the effect of another. With copper, perhaps the best known metal-metal interaction is with molybdenum. The action of one metal tends to offset the action of the other, especially in ruminants. This interaction is frequently considered in fertilizers, to maintain a proper balance within plants (e.g. Coventry et al., 1987). In sheep, the detrimental effects of excess copper can sometimes be offset by increasing the concentration of dietary molybdenum. However excess molybdenum can produce an apparent copper deficiency.

Literature used in the present review provides information about the basic processes that allow or prevent an organism from obtaining the copper that is essential for life. It also provides information important to a better understanding of the effects of copper when used to control organisms or when present as metal released from man's activities.

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I - THE BIOLOGICAL IMPORTANCE OF COPPER

I.1 COPPER AS A REQUIRED TRACE METAL

Introduction

Copper is considered to be one of the most "familiar metals in our life" (Ochiai, 1986) and is one of the minerals considered to form "... the ultimate basis of life, ..." (Ashmead, 1986). Not only is it essential for life, it has been used by man for thousands of years for objects ranging from copper bells (Palmer et al., 1987) to microelectronics. In fact, the production of copper is and has been a central part of the economy of a number of nations (e.g. Mtambala-Manga, 1970). Copper also plays numerous physiological roles in all organisms (Prohaska, 1987; Zhao, 1986) and is used in the treatment of a wide variety of metabolic disorders (e.g. Munteanu et al., 1985).

The widespread occurrence and use of copper in biological systems is a result of its ability to chemically interact with organic and inorganic substances. This has sponsored widespread examination and discussion of copper chemistry in medicine and biology. (See, for example, abstracts of the Fourth International Workshop on Trace Element Analytical Chemistry in Medicine and Biology, edited by Bratter and Schramel, 1986.) Similar examinations and discussions have been held in environmental science, because of the need for a better understanding of the beneficial and detrimental roles played by copper and other metals in the environment (e.g. Merian, 1984; see also the 1984 report from the German seminar of the Gesellschaft fuer Strahlen-und Umweltforschung MBH Muenchen). These two areas overlap broadly because of the interaction of copper in biological systems with copper in the environment. As an indication of this, Wolf et al. (1986) discuss the "Importance and Determination of Chemical Species in Biological Systems" in the proceedings of a conference entitled "The Importance of Chemical 'Speciation' in Environmental Processes", edited by Bernhard et al. (1986). Recent reviews of copper in biological systems include discussions of the metabolism and effects of metals on domestic animals (Brewer, 1987; Hapke, 1984b) and man (Aaseth and Norseth, 1986; Aggett, 1985; Cooppan, 1987; Mills, 1987) as well as a review of the effect of copper deficiencies on behaviour in animals and humans (Halas and Eberhardt, 1987).

Copper in microorganisms and plants

In a discussion on "Trace Elements in Arable Agriculture", Tinker (1986) points out how very little we know about the basic processes controlling trace metal deficiencies. The availability is governed by the chemistry of the medium - soil or water (e.g. Shah et al., 1986) - as well as chemical reactions that occur in the organism. Atmospheric situations such as acid rain, can affect the chemistry of the medium as well as the input of aerosol metals (e.g. McColl and Firestone, 1987). The effect of agricultural, forestry, and land management practices can affect total metal concentrations as well as copper bioavailability (e.g. Thran and Everett, 1987). Shorrocks (1987) points to the draining of heath and moorland soils in Europe in the 1920s as a practice that produced plant copper deficiency and reduced crop production. In the forword to a book on "Foliar Feeding of Plants with Amino Acid Chelates" (Ashmead et al., 1986), Hess comments on the effect of topsoil erosion on plant availability of nutrients, including trace metals.

Soil organic matter plays an important role in controlling metal availability to plants (Chen and Stevenson, 1986) and can produce copper deficiencies even with adequate total metal levels present. Soil metal extraction procedures have been used to indicate metal bioavailability, deficiencies, and critical levels (Gajbhiye, 1985; Sakal et al., 1986). The application of this is the determination of metal levels critical to agricultural crops as well as livestock (e.g. Youssef and Brathwaite, 1987). Addition of copper to organic-rich soils such as peat soils has been shown to increase vegetable (Adams et al., 1987) and grain production (Lamb, 1986; Selevtsova et al., 1987). Soil copper deficiencies have been shown to be transmitted to livestock (Leech and Thornton, 1987), indicating the importance of knowing the soil copper status in farming. Tejada et al. (1987) evaluate cattle trace mineral status in specific regions of Guatemala, pointing out regions that may be copper deficient and in need of forage metal supplementation. Recommended copper levels are found not only for plants and livestock but also for other animals and humans (e.g. Miller-Ihli and Wolf, 1986) and are considered to be important in the evaluation of food materials for farmed animals (e.g. Plotnikoff et al., 1987).

Copper is usually included in the trace metal mix of growth media and plant fertilizers (e.g. Treiman, 1985). Although some organisms appear to have little need for trace metal supplementation (e.g. Klein and Charles, 1987), continued growth and cell maintenance does require a supply of copper for the production of copper-containing enzymes. Blue and Malik (1986) noted, for example, that with white clover (*Trifolium repens*), there was no yield depression until the third harvest with trace metal omission. Then yields were depressed with deficiencies of sulphur, boron and copper. Delhaize et al. (1986) report a strong correlation between concentration of copper and the copper-containing enzyme diamine oxidase in clover leaves. Copper-binding ligands reduce the concentration of the enzyme (Delhaize and Webb, 1987), presumably by reducing the supply of biologically available copper. In higher plants, copper plays an important role in photosynthesis and respiration (Baszynski and Tukendorf, 1984; Droppa et al., 1987; Miller et al., 1986a; Zinkiewicz et al., 1986). Copper deficiencies are associated with die-back and chlorosis in new growth as well as several other general trace metal deficiency symptoms (Miller et al., 1986b).

Plants vary in their requirements for copper and other trace elements. Krahmer and Podlesak (1985) comment (page 132) that "wheat, barley and oats are much more demanding than rye in terms of the soil copper status. They are therefore considered 'copper-intensive' crops." Soil composition can, however, strongly affect apparent trace metal requirements as can the particular plant genetic strain being used (e.g. Cline et al., 1986). Graham et al. (1987) were able to transfer a genetic "copper efficiency factor" of rye to wheat, in an attempt to develop plant strains capable of growing in copper deficient Australian soils. Radwan and Harrington (1986) related "site index" to foliar chemical concentrations of copper with the western red cedar *Thuja plicata*. Van Praag and Weissen (1986) report dieback in Norway spruce (*Picea abies*) on acid oligotrophic soils that had low levels of copper, calcium, magnesium and boron. Galrao and Sousa (1985) note that with wheat in an organic soil, the absence of copper supplementation reduced yield and increased male sterility. As a result of variations in the plant and a variety of chemical properties of the soil and cultivation practices, apparent copper requirements will vary. Fregoni (1986) notes an annual utilization of 64-910 grams and a loss (erosion, etc.) of 30-60 grams of copper per hectare of vineyard in Italy, an amount which must be made up by copper supplementation. Dubikovskii and Kovalevich (1987) report that 12.5 kg copper per hectare increased pea yields by 9.5% on sod-podzolic sandy loam soil. Recent literature describes the importance of copper supplementation for a number of economically important plants. These include various grains (Gajbhiye, 1986; Kudashkin, 1987; Rymar et al., 1986; Shtefan and Volokitina, 1987), potatoes (Agaev, 1987) and fruit trees and vines (Langenegger and Du Plessis, 1986;. Initial tests of copper fertilization have also been made with the conifer *Pinus radiata* in an attempt to overcome the effects of copper deficiency on wood production and quality (Gonzalez et al., 1987; see also Downes and Turvey, 1986). The mineral nutrient requirements of conifers are of importance in seedlings on tree farms; there is also evidence of species-specific copper requirements (Teasdale et al., 1986).

Copper in Animals

The functions of copper in animals are numerous, as they are in all organisms. Copper plays a general role in enzymes and in a number of oxidation reactions (e.g. Allen et al., 1986; Marino et al., 1986). Goldstein and Czapski (1986a) discuss the somewhat ambivalent nature of copper-containing complexes which function both to enhance or protect the body from the toxicity of O_2^-. Some of the specific roles of copper are unique. The nervous system is an example of this. Prohaska (1987) reviews the functions of trace elements in brain metabolism, commenting that there are two main neurochemical functions of copper, a role in oxygen metabolism and in certain aspects of catecholamine metabolism. Irregular metabolism of copper in some organisms can lead to malfunction of the nervous system (e.g. Bourre et al., 1987; Nalbandyan, 1986). Interestingly, control of tissue copper levels can be beneficially used to control abnormal tissue growth. Zagzag and Brem (1986) and Nicolle et al. (1987) report that copper depletion can reduce the growth of brain tumours. This is because copper is required for the continual proliferation of new blood vessels necessary for growth of brain tumours. However, it may also be due to microsomal membrane damage occurring as a result of copper deficiency (Bartoli et al., 1985). Copper deficiency-induced changes are reported for the fatty acid composition of mitochondrial and microsomal membranes of rat liver (Balevska et al., 1985); deficiencies produced an increase in the relative amounts of linoleic and arachidonic acids. Hassel et al. (1987) find hypercholesterolemia and hyperlipoproteinemia of the rat liver under copper deficiency. Results similar to these are also reported for humans (Reiser et al., 1987). Part of the explanation for a shift in liver chemistry, under copper deficiency, may be a result of improper iron metabolism and its effect on lipid peroxidation (Hammermueller et al., 1987; Lawrence and Jenkinson, 1987). In fact, Willson (1987) reviews the possible involvement of highly reactive free radicals in the development of several diseases, commenting at some length on the involvement of iron and copper. The importance of this topic is the application of the results to a better understanding of heart disease in humans (e.g. Reiser et al., 1987; see also Tuomilehto et al., 1985), copper deficiency has been considered as an important factor in heart disease (e.g. Huttunen and Virtamo, 1986; Klevay, 1986). Klevay (1987) and Medeiros (1987) report hyptertension in rats due to copper deficiency. There is continuing argument, however, on the role played by diet and trace metal deficiencies in cardiovascular disease. Some individuals feel that there is only a weak relationship between cardiac disease and mineral supplementation (e.g. Huttunen and Virtamo, 1986; Pariza et al., 1986) while others believe that cardiac metabolism, and disease, is strongly affected by copper deficiency (King et al., 1986). Blood pressure is reportedly higher in rats fed a high NaCl diet that produces low copper concentrations in the liver, kidney and heart (Clegg et al., 1987). There is an increase in serum nickel and copper during the early phases of myocardial infarction (Dumolard et al., 1986). Masironi (1987), in fact, reports a higher rate of cardiovascular mortality and other forms of cardiovascualr pathology in regions with low levels of soil metals.

Copper plays an important role in the production and maintenance of connective tissue (Bamji, 1987; Kal'nitskii and Kuznetsov, 1987; Mittal et al., 1987; Ziche et al., 1987). Greve et al. (1987) obtained evidence that copper deficiency in the diet of chickens can lead to to scoliosis. Read et al. (1986) found that copper deficiency in young dogs was associated with poor collagen cross-linking. Long-term deficiencies may lead to abnormal bone metabolism, including osteogenesis (Strause et al., 1987). Survival of erythrocytes may be altered by copper deficiency, possibly through modification of erythrocyte membrane proteins. A similar situation appears to exist for lymphocyte membranes (Davis et al., 1987; Korte and Prohaska, 1987; Lucasewycz et al., 1987).

Action of the hormone estrogen appears to be affected by copper (Fishman and Fishman, 1987); divalent copper causes increased estradiol binding to receptors. Barnea and Cho (1987) show that copper amplifies prostaglandin E_2 stimulation of luteinizing hormone-releasing hormone under certain conditions. Copper deficiency has also been found to be associated with aortic (Bielenberg et al., 1986) and kidney lesions in laboratory rats and hormone production by the pancreas (Recant et al., 1986) as well as necrosis and depletion of acinar cells (Rao et al., 1987). One role of copper is in the control of tissue inflammation. Milanino et al. (1986) as well as Freeman and Callaghan (1987) found a significant increase in blood copper during periods of inflammation although Freeman and Callaghan (1987) comment that much of the copper may be unavailable. Related to this is the tendency for levels to increase with rheumatoid arthritis. Munthe et al. (1986) review the role of copper in the disease, particularly with superoxide dismutase. Sadique et al. (1986) discusses the possibility that most of the antiarthritic medicinal plants act by controlling serum trace element levels. A severe copper depletion has been recorded as a result of a major burn (Brian et al., 1987). Production of several organics (ceruloplasmin, Danks et al., 1986; cytochrome c-552, Merchant and Bogorad, 1987b) has been demonstrated to be genetically controlled but the genetic expression is dictated by copper availability to the cells. Even certain nucleoproteins are copper-rich (Bryan et al., 1985) although it is not known if the metal is complexed to protein, to the nucleic acid, or exists in a protein-copper-nucleic acid complex (Bryan et al., 1986). Basile and Barton (1987) present the design of a double-stranded DNA cleaving agent with two polyamine metal-binding arms which they suggest may be able to deliver metal-activated chemistry to one or both DNA strands. (See George et al., 1987, for a discussion of DNA-copper and radiation sensitivity in human lymphocytes.) Cu(II) has a differential reaction with DNA and chromatin which Clark et al. (1987) suggest allow copper to be used as a probe for certain structural changes in chromatin. Interestingly, metalloproteins have been deposited directly onto carbon-formvar-coated electon microscope grids as standards for X-ray microanalysis of biological material (El-Masry and Sigee, 1986).

Recent work on copper-containing organics of importance to animals includes the review on "Interactions of trace elements in enzymes in humans" (Shamberger, 1987) and numerous works on individual organics such as ceruloplasmin (e.g. Danks et al., 1986; Syed and Coombs, 1986) or various oxidases (e.g. Skiba and Mullin, 1987)

Other biologically important, copper-containing organics appearing in the recent literature include a growth factor isolated from human plasma that has superoxide dismutase-like and wound-healing properties (Pickart et al., 1986). Wissler et al. (1986b) discuss a copper-containing polypeptide which plays a role in tissue regeneration. Experimental Nutrition (Nutrition Foundation, 1985) lists and discusses some other organics in an article entitled "Newly Found Roles for Copper".

A number of factors affect tissue metal concentration. Although these will be discussed later in this review, it is appropriate to mention a few of them at this time. Copper levels have been shown to change with age (e.g. Gochfeld and Burger, 1987), size (e.g. White and Rainbow, 1987) and trace metal intake (Henrivaux et al., 1986). Parasite infestations may be responsible for reduced tissue copper levels (Hucker and Yong, 1986) although it is suggested that high levels of infestation may be in part, a result of copper deficiency reducing the resistance of the host (Crocker and Lee, 1986). Certain food materials (e.g. phytates, fructose) can reduce metal uptake and utilization (e.g. Lewis et al., 1987) and thus have the potential to affect tissue copper levels. Hormones may also be important. Fields et al. (1987) report that testosterone may play a role in the severity of copper deficiency expressed in rats fed a fructose-containing copper-deficient diet.

Copper deficiency is responsible for a number of physical and physiological problems in animals (e.g. Johnson et al., 1985). Congenital swayback in sheep (Balbuena et al., 1987; Haughey, 1983), certain types of haemolytic anaemia (Suttle et al., 1987), inflamation in ruminants (Lamand, 1986), foot lesions (Britt, 1984) and decreased resistance to infection (Boyne and Arthur, 1986; Woolliams et al., 1986). As well, growth rate can be reduced (e.g. Kirchgessner et al., 1987; Nutrition Foundation, 1984), partially as a result of reduced synthesis of copper-containing enzymes (e.g. Kuznetsov et al., 1984).

As with plants, the availability of copper to animals is controlled by the nature of the organism as well as the chemistry and concentration of the metal. Some of these are discussed in a review by Suttle (1986) of recent developments in copper deficiency in ruminants. Copper requirements can in part be due to the genetic makeup of the organism (e.g. Woolliams et al., 1986). Part may be due to environmental conditions (e.g. Fenske, 1985). Bain et al. (1986) found a monthly variation in bovine serum copper levels which they could relate to rainfall. They discuss this in terms of factors that affect the concentration of copper in the herbage but it could also be a result of the duration of feeding during wet and dry periods. One of the factors that they discuss is the uptake of molybdenum by plants and how it could affect animal copper levels. Molybdenum has been reported to be antagonistic towards copper metabolism and growth in ruminants (e.g. Boyne and Arthur, 1986; O'Gorman et al., 1987) although Strickland et al. (1987) report that, in horses, an increase in dietary molybdenum is unlikely to interfere with copper metabolism.

Because of the importance of trace metals to human nutrition (e.g. Laspin and Sass-Kortsak, 1981; Fishbein, 1987; Wang, 1986), human trace metal requirements have long been the focus of study, but often with mixed results. Within the last two decades, however, "... remarkable advances have taken place in research on trace elements, metallic nutrients that are vital to human nutrition even though they are present in exceedingly small amounts" (page 5 in Prasad, 1983). Levander (1986), however, points out (page 88) that "clearly, there is considerable uncertainty about copper requirements of humans." This is being addressed by major research programs such as the one initiated by the International Atomic Energy Agency in March 1985 to obtain data on the daily intakes of nutritionally important minor and trace elements (Parr, 1987). Part of the problem is a result of the changing requirements produced in response to changing physiological conditions. This is indicated by changes in tissue copper concentrations, for example during and shortly after pregnancy (e.g. Ferrari et al., 1985) as well as in the first year of an infants life (Kirsten et al., 1985a). Favier (1986) reports that the treatment of pregnant women with the copper-complexing agent penicillamine can be associated with malformed development of the neonate. Newman et al. (1987) review nutritional requirements of pregnancy and comment (abstract) that "use of a low-potency product that contains a wide range of vitamins and minerals appears to be the most prudent approach to prenatal vitamin and mineral supplementation." (Rachman et al., 1987, provide evidence of modified vitamin metabolism with copper deficiency.)

Changes in dietary copper intake are often evidenced during infancy, as a result of the nutrient source (Bratter et al., 1987) as well as changes in copper levels in breast-milk (e.g. Kirsten et al., 1985b) and differences in copper concentrations between milk formula types (Salim et al., 1986). Deficiencies in copper in infants can be a result of deficiencies in formula concentrations. This is also a possibility with humans maintained with intravenous nutrient solutions (Huston et al., 1987; Kadowaki et al., 1987; Shenkin et al., 1987; Sriram et al., 1986).

Physiological imbalances and general poor health can be associated with copper deficiency. Oppenheimer et al. (1987) present details of a patient expressing macrocytic anaemia due to acquired copper deficiency. Ruocco et al. (1986) discuss a case of severe pancytopenia due to copper deficiency. In both of these cases, copper supplementation eliminated the immediate effects of the disorder. The metabolism of copper can be affected by diseases such as diabetes (e.g. Kamei et al., 1985) which may, in turn, affect tissue copper levels and the potential for secondary physiological disorders.

Exercise, especially intense exercise, can have a pronounced effect on copper metabolism and hence tissue copper levels (Campbell and Anderson, 1987; Keen and Hackman, 1986). Keen and Hackman (1986) comment, that "it should be stressed, however, that excessive dietary supplementation of copper is not warranted, and if dietary supplements are to be used, they need not exceed the current RDA level of 2 mg/day." (Note that Turnlund et al., 1987a, suggest that 0.8 mg/d dietary copper is adequate for most normal, healthy young men.) A similar comment is made by McDonald and Saltman (1986). Since copper intake will be associated with levels in food, there can be a correlation of nutritional adequacy with caloric intake. Earll et al. (1987) suggest 100% RDA mineral and vitamin supplementation for individuals consuming less than 1,500 Kcalories per day. Supplementation is also recommended for elderly individuals exhibiting clinical and laboratory evidence of immunologic dysfunction (Hollingsworth et al., 1987). It is important to note that supplementation should be balanced, at the levels recommended by RDA. Abnormal intake of single elements can produce deficiencies in others. Amos et al. (1986), for example, report a case where intake of high levels of zinc produced a copper deficiency. It is also important to recognize the influence of chemical speciation on the absorption and utilization of dietary copper, and other trace metals (e.g. Mills, 1986).

Copper deficiency can lead to malformations. Mieden et al. (1986) report head malformations, reduced protein contents and other irregularities in rat embryos cultured on sera taken from rats fed diets deficient in copper or zinc. Copper and zinc supplementation of the sera produced normal embryos. Impaired antibody production and enhanced susceptibility to endotoxin have been observed in copper-deficient mice (Blakley and Hamilton, 1987; Koller et al., 1987)

I.2 BIOLOGICALLY IMPORTANT USES OF COPPER

Because copper is an essential metal, an adequate supply is necessary for normal metabolism. In cases where deficiencies exist, supplementation is necessary to permit normal metabolic functions. This is true with plants and animals just as it is true for humans. As well, copper is useful as a control mechanism because it is toxic at elevated concentrations of biologically available metal. Uses are itemized in the chapter on Copper and Man while their biological importance is discussed in this section.

Nutrient supplementation

Miller et al. (1986a) discuss the roles played by copper in plants and comment on some of the factors that affect copper bioavailability in soils. Mortvedt (1985) discusses micronutrient fertilizers and fertilization practices, commenting that, for copper, selection of sources should consider residual effectiveness, cost over the long term, and availability to the plant. Selevtsova et al. (1987) report on the effectiveness of copper and zinc fertilizers from a series of trials in Russia. They note copper benefit to grain, tuber and legume quality and yields. Micronutrient problems in different regions of the world have been the focus of research to improve the production of food materials. Kanwar and Youngdahl (1985) point out (page 63) that "micronutrient deficiency is becoming a constaint to crop production in the tropics" and note (table 9) that with increased plant yields, the projected copper removal rate will increase. This has the potential for producing copper deficiencies, especially in areas where they now already occur. In tropical Africa, Kang and Osiname (1985) report low copper levels in a number of soils and the need for copper supplementation. Particular soil types tend to be more of a problem than others, as a result of the chemical parameters that affect both metal concentration and bioavailability. Shorrocks (1987) comments (page 277) that "the clear message coming not only from animal studies, but also from human dietary and physiological studies, is that grassland and many of our arable crops are of low/marginal copper status, not only in their own right but for the animals consuming them. The way ahead must surely be through improving the copper status of soils and crops generally." This is not, however, by simple addition of copper. The nature of the soil and the uptake capability of the plant will dictate the relative concentrations of nutrients and trace metals to be added (e.g. Solov'ev et al., 1987) as well as the chemical form of the fertilizer components (e.g. Ostrovskaya, 1986; Potatueva et al., 1987). Systematic studies of trace element contents of soils (e.g. Archer and Hodgson, 1987) form an index of metal concentrations and soil types while appropriate metal extraction techniques can provide an indication of metal bioavailability.

The chemical nature of nutrients and trace metals, including copper, as well as the method of their application is important in the correction of metal deficiencies. In a book on "Foliar Feeding of Plants with Amino Acid Chelates" (Ashmead et al., 1986), Shazly (1986) comments that foliar applications of amino acid complexed metals may correct some of the metal deficiencies found in fruit trees in Egypt. In another article, Hsu (1986) reports that these complexes, marketed under the trade name "Metalosates" are (page 252) "... better absorbed and translocated by plants than equal amounts of of minerals from sulfates and EDTA and DTPA chelates." The Ashmead et al. (1986) book also provides a discussion of the chemical nature of copper-chelates (Oyler, 1986). Plant tissue analysis is suggested as a test for micronutrient deficiencies and toxicities (e.g. Quinche et al., 1987). Moraghan (1985) comments (summary) that "the relationship between nutrient concentration and yield, when properly used, is a powerful tool for diagnosing the nutritional status of annual crops for B, Cu, Mn, Mo, Zn and occasionally Fe." This should be combined with information on geochemical properties of the soil and nature of the plant (e.g. Dhillon et al., 1986; Fecenko et al., 1986; Kadar, 1987; Kadar et al., 1985; Korcak, 1986a; Lamb, 1986; Smith et al., 1987; Stiles, 1987; Terenko and Obraztsova,

1985) to allow estimation of fertilizer requirements. Additional information of value includes the effect of copper on the quality of the products from the plant. Flynn et al. (1987) examined the effect of copper deficiency on the baking quality and dough properties of wheat flour. They report that a single application of copper resulted in increased grain yield and quality but only a slight improvement in the dough and baking quality. However, a second application of copper, after pollen production, caused a marked improvement in both dough quality and loaf volume. The use of "recommended concentrations" (e.g. Lamb, 1986) is an easy way of making decisions on the quantity of nutrient to be applied. However, they do not adequately consider the variability of the soil and the nature of plant varieties. They can also lead to accumulation of excess metal under various soil conditions, a factor which is of some concern (e.g. Protasova et al., 1986). Of perhaps greater value are techniques such as the "Diagnosis and Recommendation Integrated System" (DRIS) which is based on mineral ratios developed from a range of conditions (see Davee et al., 1986).

A number of patents have been recently obtained for copper-containing trace element fertilizers (e.g. Beranek, 1987; Krahmer and Podlesak, 1986; Kimbro, 1987; Nabiev et al., 1987a; Okumura, 1986, 1987; Ruppe and Podlesak, 1987; Vadasz and Vadasz, 1987). The reaction chemistry of copper oxide was examined by Turaev et al. (1985) but is presented only in abstract form. Application of trace elements in irrigation water has been demonstrated to improve the yield and quality of corn forage (Shtefan and Volokitina, 1987a), soybeans (Shtefan and Volokitina, 1987b) and wheat (Shtefan et al., 1987c). Improvement of cotton roots is reported with copper application (Khallyeva et al., 1986). Residual copper is often reported in soils, however (e.g. Silva et al., 1986). Podlesak and Krause (1987) discuss the use of micronutrients in the German Democratic Republic on the basis of computerized programs. They state that there were 18,000 micronutrient tests run by the Agrochemical testing and Advisory Service of the G.D.R. during 1986 and that, in 1979, copper was used as a micronutrient fertilizer on 130,000 hectares of land. They, and others, point out the increasing need for micronutrient fertilizers, especially with the use of marginal and near-marginal land in certain parts of Europe and South America (e.g. Gembarzewski et al., 1986). This has caused an upturn in the sales of micronutrients - 34% in the case of copper (Richardson, 1986), at a time when fertilizer sales are declining. Copper-containing fertilizers are also used to enhance decomposition of straw and improve its tillage into the soil as a nutrient source (Weichelt, 1986; Weichelt and Gerhardt, 1986; Weichelt et al., 1986).

Recent literature on specific plant copper requirements as well as the effect of copper includes the limiting nutrient study of white clover mentioned earlier (Blue and Malik, 1986). There is continuing trace metal work on rice, with the hope of increasing productivity and plant quality while reducing pests (e.g. Balakrishnan and Nair, 1986). Yan (1985) points out the effect of soil organics, in reducing plant metal bioavailability. The author also reports that copper supplementation increases growth and dry weight of the root system and the plant, increases grain size and viability of the plant, promotes early seed development, and increases the resistance to fungal diseases. Rymar et al. (1986) comment that (translated abstract) "presowing application of Mo, Cu, Mn or V to rice grown on meadow-chernozem soils increased the level of soil exchangeable NH_4 and rice yields and quality." Chavan and Gupta (1986) found that either soil application or root dipping of rice seedlings in a copper-containing micronutrient mix increased grain yield and suggest dipping of roots as an easy method of overcoming micronutrient deficiencies (see also Sheudzhen et al., 1985, 1986). In contrast to these authors, Mian and Eaqub (1986), working in Bangladesh, found no direct or residual effect of copper supplementation on rice growth or quality. In the summary of a study on copper nutrition of cereal crops, Krahmer and Podlesak (1985) point out that wheat, barley and oats require more copper, that the Cu:N ratio can be used to indicate the copper status of a cereal plant, and that early copper fertilization is superior to late application (see also Kuznetsov et al., 1983). Morard and Anne (1985) report a beneficial growth effect of foliar spraying of copper on hard wheat. Kudashkin (1987) found an increase in spring wheat yield after presowing leached chernozem soils with copper, as $CuSO_4$. Presowing is also reported to affect protein composition of spring wheat (Ursu and Pukalov, 1984). Wheat pollen sterility can also be a result of copper deficiency and is improved with supplementation (Galrao and de Sousa, 1985). Chhibba et al. (1985) used soil characteristics as well as nutrient indexing of wheat to characterize reclaimed alkali soils in India. This provides some indication of plant metal bioavailability as well as total soil metal levels.

Dubikovskii and Kovalevich (1987) report a substantial increase in pea yields with copper fertilization of a sod-podzolic sandy loam soil. Similar results have been obtained with yield and quality of urd (Varshney, 1985), potatoes (Agaev, 1987; Sirotkina et al., 1984; Valadz'ko and Makhnach, 1986), sugar beets (Firgany et al., 1981; Milosevic et al., 1984), and melons (Ilamanova, 1987). In contrast, Jones and Leslie (1986) did not find any effect of boron, copper or zinc supplementation with flue-cured tobacco. Copper supplementation has been examined in a variety of fruit types. Mann and Sidhu (1983) found that copper-containing foliar sprays increased copper levels in Kinnow leaves. Copper supplementation increased copper concentrations in cranberries (Sidorovich et al., 1987), strawberries (Albregts and Howard, 1987) and raspberries (Lenartowicz, 1986). Fregoni (1986) points out the apparent need for copper by grape vines, as evidenced by uptake and soil loss. The widespread use of copper-containing fugicidal agents on grapes is a source of nutrient copper but also a source of excess metal which can be detrimental (e.g. Quinche, 1985; Ruhl and Pleninger, 1986) and, as such, will be discussed later in this review. Langenegger and Du Plessis (1986) discuss symptoms of copper deficiency and excess in citrus, consider soil factors which affect metal bioavailability, and suggest supplementation procedures. Deficiency is associated with reduced twig and shoot growth as well as irregular growth and colouration of the fruit.

Copper supplementation in animals and humans is no less important than it is in plants. Fishbein (1987) reviews the importance and discusses some of the metal-metal interactions that have been demonstrated to affect copper metabolism. In agriculture, copper supplementation has been demonstrated to be beneficial to the growth and maintenance of a number of animal types (e.g. Sidorova and Morozova, 1986). The addition of 500 ppm copper as copper sulfate, to the diet of turkey poults, improved body weight, feed efficiency and feed consumption (Harms and Buresh, 1987) although 750 ppm depressed the various parameters measured. Leghorn laying hens and chicks accumulate dietary copper although Goergievskii and Polyakova (1984a,b) as well as Ko et al. (1985) found no measurable benefit at the levels that were being used. Since copper is an essential element, dietary copper or copper supplementation is necessary to provide an adequate supply. It is also useful in controlling coccidiosis infections (e.g. Fox and Southern, 1987). However, in at least some cases, the level of dietary copper has not been properly controlled. MacMillan et al. (1986), for example, describe a case in which a feed manufacturer intentionally set the copper levels above values recommended for sheep. This produced illness and, in some cases death. Proper supplementation in sheep has been demonstrated to reduce excess adipose tissue which can lead to improved carcass value (Sinnett-Smith and Woolliams, 1987). Even more importantly, copper supplementation is essential to reduce the effects of congenital copper deficiency which are found in certain regions (e.g. Haughey, 1983). Ingraham et al. (1987) found improved fertility in Holstein cows with combined copper and magnesium supplementation. Zaderii and Bratenko (1984) note improved milk production with microelement supplementation (including copper). Copper supplementation is not always beneficial. Echevarria et al. (1986), for example, found no increase in liveweight gains in steers when fed a grass or grass-legume mixture in the Peruvian tropics. (It should be noted that high temperatures can affect trace element metabolism in cows (Kume et al., 1986), and may have been a factor in the Echevarria et al. study (1986).) Various methods of supplementation have been evaluated for ruminants, ranging from copper salts (Piva et al., 1986) to soluble glass bullets which dissolve in the ruminant forestomach. Ellis et al. (1987) report weight gain in lambs with the use of a soluble glass bullet containing cobalt, selenium and copper but comment that the copper may limit the use of the bullet to areas with inadequate normal dietary copper. Suttle examined the safety and effectiveness of cupric oxide particles for increasing liver copper stores in sheep (1987a) and cattle (1987b). A dose of 0.1 g/kg liveweight was considered safe for the North Ronaldsay sheep under the conditions of the study. With crossbred steers, Suttle (1987b) found increases in liver copper stores with single dosages of 5 g of cupric oxide particles and no biochemical evidence of toxicity with single dosages as high as 40 g. Gaffarov et al. (1985) report that supplementation of calves with 30 mg $CuSO_4$/day from 4 to 6 months of age increased the average daily weight gain by 19.3% when compared with controls.

Copper sulfate is widely used as a dietary supplement for pigs because of its beneficial effect on growth (e.g. Allee, 1985; Astrup and Lyso, 1986; Eskin, 1984; Hagen et al., 1987; Hamada et al., 1985; Khitrinov and Sirotkina, 1987; Kornegay et al., 1985/86; Ming et al., 1986). Khitrinov and Sirotkina (1987) report that the copper chelate of ethanolamine has the same effect on piglet growth rate as dietary copper sulfate. Copper has also been added to various nutrient mixes to provide a suitable laboratory supply of the metal (e.g. Brokken and Porubcan, 1987). Copper sulfate has been found to improve food utilization when rape seed is used to fatten pigs (Lüdke et al., 1985; Schöne et al., 1986). The copper, along with iodine and possibly iron, reduce the depressive effects of rapeseed meal on food intake in domestic and laboratory animals (e.g. Vermorel and Evrard, 1987). There has been, and still is, concern over the fate of copper released in swine fecal material (e.g. Nutrition Foundation, 1984). As a result, regulations have been established in the European Economic Community which limit nutrient copper to 200 mg Cu per kilogram up to 50 kg body weight and 125 mg Cu per kilogram thereafter (Nutrition Foundation, 1984). A further reduction is being considered which has caused examination of alternative factors to maintain accepted growth rates in pigs (Moreels et al., 1987). (The background for regulating nutrient copper in swine is discussed later in this review.)

During development, infants may require copper supplementation (e.g. Huston et al., 1987). Supplementation can be accomplished either as a direct addition, in some kind of nutritive mix (e.g. Shenkin et al., 1987) or as an indirect addition, in the preparation of a nutrient. In a patent document, Pal et al. (1986) describe the use of microorganisms grown in mineral waters, to produce trace element-rich yogurt. Revici (1987) reports on a copper and animal, vegetable or fish oil mixture for treating copper deficiency. Alkaline salts of copper-chlorophyll complexes are used in human food additives, cosmetics and drugs (Ciurdaru et al., 1986; Zhou and Wang, 1986). They have also been used to combat characteristics of cadmium poisoning (Bordas et al., 1986), presumably through complexation of the cadmium or as a result of copper-cadmium antagonism.

The use of copper to control the growth of organisms

Copper is used to increase or decrease the rate of growth in organisms. It has, for example, been used to improve the storage life of some fresh foods and organics, by reducing the growth of decay organisms. Desai (1984) describes the use of $CuSO_4$ for increasing the storage period of bananas, oranges, apples and chiku. Deo and Gupta (1986) comment that copper sulfate reduces the incidence and severity of mould on stored food-grains. A copper salt of 8-hydroxyquinoline has also been used to protect food products and plants from the effect of microorganisms (Novikov et al., 1983). Copper has been used to rot-proof jute materials (Ghosh, 1986). The addition of ionic copper, cadmium, nickel and cobalt to solutions of the enzyme phosphofructokinase prior to freezing improves the percentage of activity recovered after thawing (Carpenter et al., 1986). Copper has been linked with hair production (Gumenyuk, 1987; Rajendran and Devaraj, 1986) and zinc oxide and copper sulfate have been associated with increased growth of hair in patients with Alopecia areata (Timoshkova et al., 1986). Although not a "growth control" but rather a control of another kind, Lekstrom and Koons (1986) describe a test for copper and nickel that can be used at the scene of a crime, to determine the type of bullet used by a criminal!

Laboratory cultures of organisms or cells often benefit from the addition of copper as a trace metal supplement (e.g. Batyrbekov et al., 1986; Kuang et al., 1985; Soderberg et al., 1987). The metal is used to control of growth of microorganisms in the food and drink industry (e.g. Watanabe and Iino, 1985). In the production of citric acid from brewery wastes by *Aspergillus niger*, Roukas and Kotzekidou (1987) report the addition of copper to improve yield under certain conditions. Copper was one of four trace metals that were needed to cause maximum citric acid production by *A. niger* from tamarind seed powder (Purohit and Daginawala, 1986). Diacetyl, an organic responsible for the buttery aroma of many cultured dairy products, is produced by the microorganism *Streptococcus lactis*. Kaneko et al. (1987) report more than a 5 fold increase in diacetyl production with copper supplementation of the medium. Improved yields of pharmaceutical agents can often be achieved through supplementation with copper (e.g. Sato et al., 1986) or copper-containing agents (Franz et al., 1987; Mertens et al., 1987). Recovery of organics after production may also use copper. Hochuli and Dobeli (1987) used copper in the recovery of certain types of proteins, Evans et al. (1987) note that L-phenylalanine can be precipitated from a biotransformation reaction mixture as an insoluble copper complex. The organic is important in the production of the dipeptide sweetener Aspartame. Copper is frequently used in the isolation of bioreactor-produced enzymes (e.g. Krishnan et al., 1987; Miyata-Asano et al., 1986) or forms an important part of the produced enzymes, such as Cu/Zn superoxide dismutase (e.g. Flohe et al., 1986; Hassan and Lee, 1986) or other organics (Megalla et al., 1987; Mertens et al., 1986).

The use of copper in biologically important chemicals

Although copper is used to preserve chemicals such as cellulose (e.g. Collett, 1987), its reactivity has allowed use in a unique series of chemicals that are designed and produced to handle noxious materials. Menger et al. (1987) describe two long-chained cupric ion complexes that are capable of catalytic activity towards phosphate triesters, diesters, and other phosphorus (V) compounds that include such noxious materials as nerve gases. Copper-containing non-steroidal antiinflammatory agents have been shown to be effective antiinflammatory agents (e.g. Kishore, 1987), perhaps more effective than the parent drugs. This is probably because the complexes activate copper-dependent opioid receptors (Okuyama et al., 1987). Pickart (1987), in a patent document, describes the chemical structure of a copper-containing agent which is purported to possess wound-healing activity. The suggestion (Pickart et al., 1986) is that it is due to a tissue-protective superoxide dismutase-like activity. Copper radiopharmaceuticals are proving to be of value in disease diagnoses and therapy (Green, 1987; Srivastava, 1986). One application is for positron emission tomography (PET) imaging of the brain and heart (Green et al., 1987a) which has the potential to allow measurement of cerebral blood flow (Green et al., 1987b). Crook et al. (1985) measured the radiation dosages that would be aquired by the use of tumour-imaging agents suitable for PET use. Organic compounds containing the metal have also been used as anticonvulsants (Sorenson, 1987), agents useful in the examination of several types of diseases (e.g. Rajan et al., 1986) and as antibacterial agents (e.g. Singh et al., 1986).

Senires and Lim-Sylianco (1984) found antimutagenic activity with copper sulfate and copper chloride. As a result of activity of this type, copper has been and is being widely used in the treatment of tumours and cancers (Kropf-Maier, 1987). Elo and Sunila (1987) noted preferential accumulation of a copper complex by the rat pancreas and suggest that the complex might be of value in the treatment of pancreatic tumours. Treshchalina et al. (1986) found antitumour activity of glycinato-L-serinate complex of copper, a mixed complex compound of divalent copper and α-amino acids. Soderberg et al. (1987) report that copper complexes enhanced the proliferation of splenic stem cells in mice after irradiation. Chemical properties of a number of copper-containing anti-tumour and anti-cancer drugs have appeared in recent literature (e.g. Ames et al., 1986; Basosi et al., 1987; Elo and Luume, 1987; Elo et al., 1987; Ghose et al., 1986; Harrison et al., 1986; Malatesta et al., 1985; Shao et al., 1986; Tamura et al., 1987). Patiashvili et al. (1987) report that copper ions introduced into ascites tumours penetrate the nucleic acid (DNA) and damage it, causing disordering of the chromatin structure. The toxicity of some anticancer drugs is suggested to be associated with lipid peroxidation (McGirr et al., 1985). A copper-phthalocyanine membrane electrode has also been developed for rapid preliminary detection of polycyclic mutagens (Tomoda et al., 1986).

A number of copper-containing antiviral and antibacterial agents have been proposed and reported to be effective (e.g. Chatterjee et al., 1986; Sindelkova and Chaikina, 1986). "Suspensions of herpes simplex virus types 1 and 1, cytomegalovirus, and parainfluenzavirus type 2 were inactivated within 24 h when treated at 37°C with 1 mg (5.05 mM) of copper-catalyzed sodium ascorbate per ml" (abstract, White et al., 1986). Reduced virulence has been found in copper-stressed bacteria (e.g. Singh and McFeters, 1987). In fungicides, copper has proven effective with a wide range of economically important organisms. They also offer an alternative to a number of organic fungicides that promote resistance in fungi. Olvang (1987) comments that copper-containing compounds have some effect on a wheat-infecting fungus, *Gerlachia nivalis*, that developed resistance to benzimidazole fungicides. The incorporation of copper into organic complexes can provide a broad spectrum fungicidal agent that may have less tendency to promote resistance in the fungi. The present and future use of these agents is demonstrated by the large number of descriptions and patent documents that have appeared in recent literature (e.g. Alexandri et al., 1986a-c; Barlett, 1986; Gunther et al., 1987; Hokko Chemical Industry Co., 1985; Kobayashi and Kobayashi, 1987; Kukalenko et al., 1987; Mollin et al., 1986; Teodorescu et al., 1986a-c; Tyeklar et al., 1986; Vasile, 1985). Samus et al. (1985) discuss the antimicrobial activity of coordination compounds of copper and nickel with two β-semicarbazones. Arimoto et al. (1985) examined the biological activity of inorganic copper fungicides and present estimations of inhibitory effects. Copper oxychloride has been reported effective against two soil-borne pathogens (Suseelendra and Hegde, 1986), copper chloride against certain infestations in grapes and apples (Inczedy and Maros, 1984).

Copper-containing fungicides are widely used for crop plants and new fungicides are continually being developed and tested (e.g. Jaitly and Wadhwani, 1986; Keshavan and Janardhan, 1986). Smilanick et al. (1987) report that four applications of copper hydroxide was at least partially successful in the control of Karnal bunt of wheat. Rai and Singh (1986) found that a copper-containing agent gave satisfactory control of bacterial leaf spot and stem canker diseases of pigeon pea. Patil et al. (1986) found effective control of wilt of betelvine by copper oxychloride and Bordeaux mixture. Copper oxychloride continues to be widely used as a fungicidal agent for diseases such as leaf spot (e.g. Colin and Chafik, 1986; Indi et al., 1987; Issa et al., 1985). In the tropics, oil-based copper-containing agents have proven effective for crops such as tomato, when grown during the rainy season (e.g. Mabbett and Phelps, 1985). As a root crop, potatoes are often prone to fungal diseases. In a patent document, Suteu et al. (1985) describe a complex fertilizer with bacterial and fungicidal activity provided by copper and zinc obtained from metal-containing wastes. Elphinstone and Perombelon (1987) describe the use of copper oxychloride as a foliar spray to control airborne *Erwinia carotovora*, a pathogen that causes potato blackleg and is one of the organisms that can cause bacterial soft rot of stored potatoes as well as early seed tuber decay in the field. Damping-off of potato seedlings is being examined as one of the problems in using true seeds rather than seed tubers. The disease is caused by fungal infestation and can be controlled by a variety of fungicides, including those that contain copper (Elango, 1986). Root rot of turnips can also be controlled by copper-containing fungicides (Kagiwata, 1986). Control of peanut leafspot is capable with copper-containing fungicides either acting alone or in combination with other fungicides (Littrell and Heath, 1986). Johnson et al. (1985) provide discussions of peanut leafspot control that include not only the effectiveness of fungicides but also the timing for their use. Using weather-based strategy for timing fungicide applications, they report annual savings ranging from $192.55 to $259.99 per hectare, as compared with costs of using the fungicides without consideration of weather. Kushalappa et al. (1986) comment on the importance of timing in fungicide application for coffee rusts.

Recent literature discusses the use of copper in the control of fungal diseases of berries (e.g. Washington, 1987), grape vines (Rao, 1986; Thiolliere, 1985), citrus trees (Andersen and Lindow, 1986; Bornemisza et al., 1985b; Davis, 1985; Eger et al., 1986; Feichtenberger et al., 1985; Oliveira et al., 1987), banana plants (Lukade, 1985) and coffee plants (Galvez and Javed, 1986; Kushalappa et al., 1986). Goldweber (1986) reports that red algae can infect citrus trees, causing bark splitting which opens the tree to fungal infection. Metallic copper is recommended as a preventative spray for the algal infestations. Grimm (1987) reports good control of crown gall in Swiss apple nurseries, with ammoniacal cupric sulphate and copper oxychloride, either in clay slurries or mixed with glue. Control of a fungal infection called "pink disease" on ecalyptus trees in Kerala, India, has been possible with application of Emulsicop, a fungicide containing copper oxychloride (Thankamma et al., 1986).

Both organic and inorganic copper-containing compounds have been used to control noxious algae and terrestrial plants (e.g. Hillebrand and De Vries, 1986; Ludyanskiy and Solonin, 1986; Pal and Chatterjee, 1987; Sarim and Ali, 1986; Stillman, 1987; Sugino et al., 1986; Tewari, 1987). Anderson and Dechoretz (1987a,b) provide evidence of Eurasian watermilfoil and hydrilla control with the ethylenediamine complex of copper but also find that the phytotoxic activity is significantly greater with fluridone, another herbicide, suggesting a synergistic effect. (Synergistic interactions are of potential importance in fungus control as well - Das and Mohanty, 1985; Thiolliere, 1985). In a third paper (Anderson et al., 1987), they report that inhibition of growth in *Hydrilla verticillata* is related to the amount of copper associated with the shoots. Working with three copper formulations, their results suggest that formulation differences are important because they appear to affect copper uptake. Copper sulfate has been tested as a foliar spray, for purple nutsedge, a troublesome perennial weed in agriculture (Shiam et al., 1987). The authors report that low concentrations produced an increase in growth while higher concentrations inhibited growth. Copper has proven to be an effective fungicide, disinfectant, antifouling agent and insecticide (e.g. Kanda and Mizusuchi, 1986; Leightley, 1986; Sawashita, 1986; Zarundaya and Minkevich, 1986).

Successful use of any cidal agent is dependent upon the tolerance of the organism, the biological availability of the metal (e.g. Raman and Cook, 1986) and environmental conditions which can affect metal bioavailability and concentration as well as the impact of the disease

(Jardine and Stephens, 1987). Rapid replacement of water can, for example, dilute an algicide to an ineffective level (e.g. Hawkins and Griffiths, 1987). Copper-resistant strains of microorganisms do develop and will affect the success of fungicidal agents. Cooksey (1987) characterizes the copper resistance plasmid in copper resistant strains of a bacteria that is found on tomatoes. An understanding of some of these factors allow wider application of copper-based agents. Dong and Burdett (1986), for example, used cupric sulfide impregnated kraft paper containers to control root growth in Chinese pine seedlings.

In the control of animals, copper sulfate has been found to be an effective molluscicide. Perhaps the widest use has been in the control of the snail host of blood flukes that cause schistosomiasis (e.g. Parashar and Rao, 1986). Helaly and Nosseir (1987a,b) report that the controlled release of copper from a rubber formulation containing copper sulfate was adequate for killing snails over an extended period of time. Copper has also been tested for the control of slugs with crops such as strawberries. Prystupa et al. (1987) report the effectiveness to be limited under the conditions that they used. They also found that effectiveness of molluscicides may vary as a result of the physiological state of the animal, appearing to be least effective when applied during the reproductive period. Copper sulfate is used in the control of various fish diseases. White spot disease of catfish, appropriately called "Ich", is a protozoan parasite that can seriously affect freshwater fish in commercial production ponds. MacMillan (1984) comments that, of the three chemicals that can be legally used in fish ponds, "copper sulfate is probably the most effective, ...".

Evans and Hoagland (1985) are the editors of a series of articles on algal biofouling while Christie and Dalley (1987) discuss marine fouling and its control in terms of the life cycle of barnacles. Since organotin compounds are no longer allowed in many countries, the use of copper-containing antifouling agents is increasing. Use of copper as an antifoulant is for the control of algae as well as animals. Ludyanskiy and Solonin (1986) report its usefulness with algae in industrial water supply systems. It is also used to control micoorganisms in circulating water (Kurdish and Khenkina, 1987). Sawashita (1987) and Bews (1986) describe a metal-containing plastic for use in marine environments as well as in fishing and mariculture and Yamamoto et al. (1987) developed copper-containing water soluble glass for similar uses. The use of copper alloy mesh for pens and shellfish trays provides a durable, long lasting antifouling surface for use in aquaculture (INCRA Project 168B, Woods Hole Engineering Associates, Inc., 1984).

Dietary copper sulfate is one of the agents used to control ureolytic bacteria found in the pig large intestine (Varel et al., 1987). As a complex, copper is also used to increase the resistance of rabbits to the cysticercus stage of a parasitic worm (Mosina, 1983). Copper intrauterine devices have been developed and used (e.g. Rob et al., 1987) for an extended period of time, as contraceptive mechanisms. These are discussed later in the review. Copper wire has been used in an electrothrombosis technique, to treat giant aneurysms in man (Fujita et al., 1987). Copper is also being used in laser photodynamic therapy of experimental tumours (Straight et al., 1987) and is being used in the development of techniques for laser treatment of retinal pathology (Kremer et al., 1987). In an INCRA-supported project, Sorenson (1987) used organic copper complexes as radioprotectants, to protect mammalian cells from damage caused by gamma- or X-irradiation. ^{64}Cu citrate has been recently used as a new myocardial imaging agent which is easily prepared and requires a minimum amount of preinjection preparation. The development was by the Health Sciences division of Oak Ridge as well as associated universities and is described in a 1987 U.S. Department of Energy release.

Copper in dental amalgams and mouth rinses

Afseth et al. (1986) comment (page 300) that "the metal content of food and drinking water may have an important effect on dental caries. They may ... influence plaque formation, composition and metabolism." The authors report that a combination of copper and fluorine exhibited higher caries inhibition than either element alone. Evans (1986), in the abstract of a Ph.D. thesis, comments that reduced copper would be detrimental to *Streptococcus mutans*, the primary pathogen found in dental plaque. In the evaluation of antiplaque capabilities of a copper-containing prophylaxis paste, Moore et al. (1987) found a diminishing effect with time and suggest that it may be appropriate to apply ionic copper daily for best anti-plaque results. Grytten et al. (1987) suggest that copper, and an organic (hexetidine), may have a combined effect on plaque, apparently as a result of the organic causing greater uptake of copper by the plaque or organisms associated with it. Histocompatability of implanted copper-containing amalgams has been studied by several workers (e.g. Marshall et al., 1987; McKinney et al., 1987; Naylor, 1986; Niemi, 1986; Welder et al., 1985) as has the effect of preparation (e.g. Ben-Amar et al., 1987; Brackett et al., 1987; Lucas et al., 1987). Smales and Gerke (1986), for example, report that surface discolouration of a high copper amalgam was dissatisfactory but could be removed with light polishing. Surface discolouration or tarnishing appears to be a major drawback of many amalgams (Hero and Niemi, 1986; Niemi, 1986).

Copper in wood preservatives

Copper is widely used in the chemical protection of wood against biological degradation in both terrestrial and aquatic environments. Copper-chromium-arsenic (CCA) preservatives are one of the most well known; Coggins (1985) reviews their development and future. Pizzi and Conradie (1986) discuss the importance of the conditions of treatment, for soft-rot control with hardwoods. Recent patent documents include descriptions of new preservatives (e.g. Hager, 1987a,b) as well as techniques (e.g. Nishimoto et al., 1987). Smith (1986) discusses the treatability of several U.S. northeastern tree species and comments that, with some woods, incising improves the preservative retention level. Pressure treatment of fast-grown loblolly pine is suggested to produce a more uniform product than regular growth pine, possibly because of its lower specific gravity and heartwood content (Dietrich and Levi, 1984). One problem with preservative treatment of utility poles is an increase in surface hardness (Williams, 1986) which increases the difficulty of climbing poles by linemen.

In examining wood decay in a laboratory type fungus cellar, Walace (1986) comments that mathematical modelling (abstract) "... is a powerful tool to discriminate biological performance of a series of experimental preservative systems subjected to the accelerated decay hazard of the fungus cellar." Evaluations of wood preservation treatments in recent literature include the remedial treatment of soft rot attacked CCA-treated eucalyptus poles (Ziobro et al., 1987) in which a groundline bandage of either Osmoplastic® or Patox® provided additional protection. Stake test results are found in the Madison Wisconsin Forest Products Laboratory report of Gjovik and Gutzmer (1986) and, for composite lumber stakes, in the report by Gaby (1986) which indicates good performance for both CCA and ACA waterborne preservatives. Waferboard and particleboard with preservative treatment have been examined by Schmidt et al. (1987) and Hall et al. (1987) who comment that ground contact in both cases can produce failure over a long-term period. The replacement of arsenic with zinc, in copper-chrome-arsenic or similar preservatives, has been advocated because of public concern about wood treated with waterborne preservatives. Wilcox (1987) reports that (abstract) "... the vast majority of the fungitoxic components of the ACZA formulation are tightly bound in the wood and not subject to leaching under the ..." the test conditions. Kim et al. (1985) examine the relationship between fixation and surface electrochemical potential of wood preserved with CCA preservatives. They report a number of differences with different preservatives.

The effect of various preservatives on organisms is important in the evaluation of preservative potential. It is also important in evaluating the interaction of decay organisms. Tanaka et al. (1987), for example, found that (abstract) "wood destructive basidiomycetes failed to degrade ... wood blocks pre-colonized by each of (the) deuteromycetes including *Trichoderma* sp. and *Gliocladium* sp. which were incapable of degrading wood." In an examination of the effect of crustacean marine wood borers belonging to the genus *Limnoria*, Srinivasan and Vallabhan (1986) found a higher CCA protection to certain nondurable timbers than lower creosote protection although, under other conditions, creosote protection was better. With durable timbers, both CCA and creosote provided protection against *Limnoria* attack in waters near Madras, India. Two severe cases of human effect of CCA preservative are given in by Peters et al. (1986). In the first case, a family used CCA-treated lumber as stove firewood, In the second, two people sawed and drilled lumber for picnic tables in a non-ventilated room heated with an overhead space heater. In both cases, the air was impregnated with fumes or sawdust adequate to produce health hazards. The situations in both cases compromise normal, logical health etiquette!

All agents used in the control of organisms may not always work and can have deleterious side effects. Copper-containing agents are no exception (e.g. Hawkins and Griffiths, 1987; Kumar and Singh, 1986; Nanjo and Watanabe, 1986; Sapegin and Neuimina, 1987). Singh et al. (1986), for example, report that copper oxychloride was not an effective fungicide for foliar diseases of mungbean (*Vigna radiata*). Accumulation of copper in soils (e.g. Helweg, 1986; Terenko and Obraztsova, 1985) or in water (e.g. Frenet-Piron and Alliot (1987) has been cause for concern. Copper corrosion products in drinking water may be high enough to be of concern (e.g. Eife et al., 1987; Ohanian, 1986), enough to warrant water treatment to reduce corrosion rates (Cohen and Myers, 1987; Pisigan and Singley, 1987). Alonso and Ocon (1987) discuss the kinetics of corrosion as a function of pH and oxygen. Side effects of copper agents that are discussed in recent literature are presented later in this review.

Copper participates in a number of reactions that have environmental importance. Degradation of carbamate herbicides has been reported for various metal-containing (including copper) clays (Sabadie and Coste, 1986). Boyd and Mortland (1986) discuss radical formation and polymerization of chlorophenols and chloroanisole on copper(II)-smectite and comment (abstract) that this "... may provide the basis for a new detoxication technology in which recalcitrant halogenated aromatic molecules are converted to less toxic products ... ." Stelman et al. (1987) describe a copper oxide absorber-catalyst that is used in the removal of sulfur dioxide and nitrite from flue gas. This is further discussed in a 1986 U.S. Department of Energy Technical Progress Report. The copper-organic complex copper hexacyanoferrate is also used to adsorb cesium (Ganzerli Valentini et al., 1986).

Chemical work on copper-containing compounds useful in both medicine and agriculture is discussed in greater detail later in this review. A few examples are given in this section to provide some indication of the variety of chemicals being examined. In the development of fertilizers, Ugai et al. (1984) examined the cocrystallization of copper salts with ammonium nitrate and sulfate. In agriculture, Kane (1987) discusses the use of CuSO4/TiO2 as catalysts in the Kjeldahl determination of crude proteins in animal feeds. In the field of antimicrobial, antiviral and anti-tumour agents, Bell et al. (1987) discuss copper complexes of two hetereocyclic thiosemicarbazones.

I.3 COPPER IN ORGANISMS

The requirement for copper, exhibited by all organisms, means an uptake from the environment or from food and maintenance of suitable levels within the body. Uptake under conditions of metal deficiency or excess can produce low or high levels of metal as can abnormal physiological conditions within the organism. This section presents recent literature dealing with levels of copper in tissues. The reader is referred to the later section on metal uptake for a discussion of the processes that affect the uptake, maintenance and loss of copper from the organism. Specific concentration values are presented in tables 3-5.

The relationship between metal flux and tissue metal levels is complex and involves both the organism and the environment (e.g. Berrow and Burridge, 1984). In aquatic environments, for example, metal levels in tissues can be affected by hydrographic processes which affect metal levels and metal speciation (e.g. Wachs, 1985). The relationship between metal levels in the environment and metal levels in the organism is dependent on the chemistry of the organism as well as the chemistry of the metal in the environment. Various analytical approaches have been used in an attempt to establish relationships between organism tissue metal levels and the environment. Voulgaropoulos et al. (1986), for example, discuss the use of cluster analysis to examine temporal differences in metal levels in organisms from the Aegean Sea.

I.3.1 METAL LEVELS IN NORMAL TISSUES

Surprisingly, there is little on metal concentrations in microorganisms in the literature examined for this year's review. In plants, however, concentrations of metal have been measured in various parts as well as in the entire organism. Burdin et al. (1987) report an increase in copper concentration with age, in the alga *Sargassum pallidum*, with average values ranging from 14.9-26.1 mg/g dry weight. Alzueta et al. (1986) measured copper, at 3.97 mg/kg, for the protein concentrate of a vegetable, *Sophora japonica*. Copper concentrations in certain plants have been measured with the intent of using the plants as biomonitoring agents. Wallner et al. (1986) report 11 ug/g in the macroalga *Enteromorpha* sp in the Santa Cruz Canal. They comment that this is low and report that, of the metals examined, only lead levels were above normal. Raunemaa et al. (1987) tested bark of the Scots pine, *Pinus sylvestris* as a material for long-term analysis of forest growth changes. Using the concentrations of ten elements, including copper, no obvious long-term trends were seen in old bark samples and, only with calcium and iron, were trends evident in samples of younger bark. Plants have also been used to measure the effect of metals from roads and paving materials. Dueck et al. (1987a), for example, report that roadside soils and plant materials next to sinter-paved roads have elevated levels of metals. These, at least in part, are a result of the source of the sinters, from zinc smelters.

Levels of essential trace metals in plants provide an indication of trace metal availability. They also indicate differences between plant types and tissues as well as seasonal changes. Whitehead (1987) found concentrations of copper higher in clover shoots than grass shoots, Joshi et al. (1987) report differences between salt marsh angiosperms (see also Tait et al., 1986). Rakicevic (1985) found higher copper levels in seedling leaves of pear trees than quince trees and reports a seasonal decrease in levels (May through August). Stephenson and Cull (1986) note differences in leaf nutrients and trace metals between Hawaiian and south east Queensland macadamia trees. They also note some differences between two cultivars. Khadi et al. (1987) presents evidence of tissue copper differences between varieties of chilli (*Capsicum annuum*). Vigor in knnow mandarin rootstock has been associated with leaf composition, including copper concentration (Tayde et al., 1984). Wentworth and Davidson (1987) examined foliar mineral elements in native plants on contrasting rock types in southeastern Arizona. They report that foliar copper concentrations appeared to reflect relative availability in the soil. Fisher and Bates (1986) found a relationship between copper concentration in forages and the percent of acid detergent fiber. The relationship was positive with corn silage and negative with hay. There was also a positive relationship with protein content in the plants. Soil pH controls metal availability although the effect on plant tissue levels is not clear (e.g. Blojic and Veljovic, 1986). In an examination of copper nutrition of greenouse roses, Rey and Tsujita (1987) found no influence of supplementary irradiation on foliar copper content. They did note an effect of soil type on copper availability, higher with sand than with soil, and an iron deficiency at high levels of copper. Like Rey and Tsujita (1987), Burton et al. (1987) did not find a significant effect of different light intensity on foliar copper levels but did with field-grown tree seedlings of *Metrosideros polymorpha*. Ozone has been reported to alter the concentrations of nutrients in some plants. Tingey et al. (1986) found this to be true with some foliar nutrients (Ca, Mg, Fe, Mn, K, P, Mo) but not with copper, at least in bean tissue (*Phaseolus vulgaris*).

Kudrev et al. (1986) note an effect of nitrogen sources on copper content in young sunflower plants. Copper content of the leaves was lowest with a 1:1 ratio of NO_3:NH_4 and highest with only NH_4 in the growth medium. In the stems, highest copper was found with only NO_3 and least with only NH_4. Copper is a metal that is frequently measured when there is evidence of disease or environmental change (e.g. Smith and Hallmark, 1987; Van Praag and Weissen, 1986), to estimate the effect on plant or animal health. Rapid uptake of copper has been noted in a variety of plants after fertilization. Gonzalez et al.(1987), working with *Pinus radiata* in Chile, note this in an examination of growth requirements for the species. But Quinche et al. (1987) did not find an increase in copper with a higher rate of fertilization, in either wheat grains or potato tubers. Frequently, however, concentrations of copper, and other metals, change with the age of the plant as well as metal bioavailability. Menzel et al. (1987) found that the levels of several nutrients and metals declined with leaf age in non-fruiting litchii. Copper, however varied more with availability than with age. Leaf age is a factor in copper levels in the seagrass *Posidonia australis* (Ward, 1987) and in the brown alga *Hizikia fusiforme* (Ishikawa et al., 1987). It is most certainly responsible for seasonal changes noted by Joshi et al. (1987) for salt marsh angiosperms and for changes during the vegetative period for winter rye and triticale by Lasztity (1986). Many of these are, however, a result of physiological requirements by different plant tissues during phases of growth as well as changes occurring during senescence. Injuk et al. (1987) found that the Zn/Cu ratio had a constant value in individual growth rings of a 32-year-old pine tree near a coal-burning power plant in Yugoslavia. They suggest that this is caused by "... heterogeneous tree tissue structure." Stephenson et al. (1986) report no consistent seasonal trends in leaf copper levels in macadamia in southeast Queensland. Similarly, Sanchez-Alonzo and Lachica (1987a) found no seasonal change for leaf copper levels in sweet cherry. They (Sanchez-Alonso and Lachica, 1987b) did, however, find a seasonal accumulation of copper in the leaves of Golden Japan plum trees.

Metal-metal interactions play a role in plant tissue metal levels. Reddy et al. (1987) note that manganese application, in general, increased leaf copper concentrations in soybean. There was, however, no significant change in seed copper concentration. They also affect metal levels in animals. Gendron and Vicente (1986), for example, found a reduction in tissue copper levels in the oyster *Crassostrea gigas* when exposed to tributyl-tin antifouling paint. At the same time, the organism accumulated tin.

In animals as in plants, tissue copper levels are a result of the nature of the environment, the concentration of metal present, and the nature of the organism. Khristoiorova and Kavun (1987) found higher levels of metals, including copper, in near-surface cultured blue mussels (*Mytilus edulis*) than in sub-surface specimens. Trace metal levels in *Mytilus* are influenced by season, size and body condition (Borchardt et al., 1988) as well as location and metal bioavailability. Hackney et al. (1987) notes that levels of potassium, copper and chromium were related to pH in the oyster *Crassostrea virginica*. In Western Australia, the intertidal rock oyster *Saccostrea cuccullata* exhibits highest copper and zinc concentrations during the spawning period suggesting seasonal variation due to physiological condition. Sexual differences in copper concentrations are indicated by higher levels in the ovary than in the spermary in the scallop *Patinopecten yessoensis* (Ikuta, 1985a). This has also been reported for the herbivorous gastropod *Batillus cornutus* (Ikuta, 1985b) but not for the abalone *Haliotis discus* (Ikuta, 1985d) or the whelk *Volutharpa ampullacea perryi* (Ikuta and Abe, 1986). Working with the wedge clam *Latona cuneata*, Ikuta suggested tissue copper levels to be influenced by food type as well as species specificity (Ikuta, 1985c).

Nakamura (1986) comments (abstract) that "... plotting metal contents (including copper) against three body weights (soft body, conch and whole body) of the wedge clams on a double logarithmic diagram produced regressive relationships with two or three straight lines." Ishii et al. (1987) also notes an influence of body size on metal concentrations. Kidney concentrations of copper, and several other metals, increased with shell length in the clam *Cyclosunetta menstrualis*. This implies the ability to regulate metal uptake and retention, a feature which Amiard-Triquet et al. (1987b) note for grazing gastropod molluscs, at least with uncontaminated and moderately contaminated water and food. Howard (1984) notes a possible copper regulating ability in the marine polychaete worm *Nereis diversicolor*. Seasonal variations in tissue metal levels in this organism have been reported (Gillet, 1987; Howard and Brown, 1983) suggesting the potential for change in requirements or regulating ability.

In comparing tissue copper and zinc concentrations in a series of amphipod crustaceans, Moore and Rainbow (1987) found that copper concentrations (summary) "... reflected their ecological zonation from sea to land closely, with species living proximal to the sea having lowest Cu concentrations." With many crustaceans, the copper concentration is affected by the amount of haemocyanin, a copper-containing pigment. White and Rainbow (1987), for example, suggest that the low copper concentration found in a mesopelagic crustacean may be a result of the low body content of haemocyanin. Seasonal variation in copper content of many crustaceans is reported (e.g. Shrestha and Morales, 1987) and may, in part, be a result of changes in haemocyanin content (Moore and Rainbow, 1987). However, haemocyanin content as well as copper and zinc have both been found to decrease during moulting, at least in the blue crab *Callinectes sapidus* (Engel, 1987). Engel, however, found that the amount of copper bound by metallothionein varied during the moult cycle and suggests that metallothioneins are naturally involved in the synthesis of haemocyanin and the regulation of copper and zinc. If this is the case, it could influence the seasonal changes in tissue copper levels.

In an examination of termite mounds, Prasad and Sankaranna (1987) found appreciably higher levels of trace metals in mound material than in adjacent soils. This is a result of translocation of metals from plant materials and is an indication of the importance of termites in the biogeochemical cycling of metals. The unique trace metal composition of food would influence the concentration of metal in mound material and possibly the termite tissue concentration. Herbert and Miller-Ihli (1987) note seasonal variation in minerals and trace metals, including copper, in honey bee-collected pollen which they attribute to floral source and soil conditions under which the pollen was grown. Du Toit et al. (1987) report that copper concentration in house flies is a means of identifying their breeding sites. Copper concentrations in flies bred in pig manure were twice as high as those in flies bred in chicken manure. Copper concentrations in the aquatic larvae of some insects, such as the midges, can be elevated if they live in metal-rich sediments (e.g. Mudroch and Rao, 1987).

Misra and Uthe (1987) discuss methods of time trend analysis for tissue metal and organic contaminants in Canadian Atlantic cod (*Gadus morhua*). They report (abstract) "significant linear time trends ... although these did not explain all variations between years."

Satoh et al. (1987) found changes in tissue mineral compositions in rainbow trout during growth although there is little information on changes in copper. With the white sucker *Catostomus commersoni*, pH-related trends have been noted in bone for some minerals although not for copper (Bendell-Young and Harvey, 1986). Sexual differences in copper have been noted occasionally, female gonads may contain higher concentrations of copper than male gonads; the reverse is true for liver copper concentrations (Protasowicki, 1987). Different organs tend to have different copper concentrations. Nemcsok et al. (1987), working with pesticide accumulation in the organs of carp, found that the order of concentration was skeletal muscle > liver > gills > intestine > kidney > heart > brain. An increase in metallothionein has been noted, in rainbow trout, during the reproductive cycle (Olsson et al., 1987) which has been suggested to ensure an adequate supply of available zinc. It may also play a role in regulating copper availability. The relatively consistent copper and zinc levels found in fish in both normal and metal-enriched areas (e.g. Johnson, 1987) suggests that metallothionein, or some analogue of it, is an operational factor, maintaining metal concentrations.

In birds, as in other organisms, metal levels tend to vary from one tissue to the next (e.g. Osborn et al., 1987; Tholey et al., 1987). In the common tern, *Sterna hirundo*, Gochfeld and Burger (1987a) found significant differences in copper concentration between the liver of the young and the egg. In an examination of metal concentrations in the liver of three species of ducks, Gochfeld and Burger (1987b) found higher levels of copper in greater scaup (*Aythya marila*) than in black duck (*Anas rubripes*) or mallard (*A. platyrhynchos*). In black duck, males had significantly higher levels of copper, manganese and zinc than did females. Metal-metal and metal-metalloid interactions also occur in birds. Combs et al. (1986) report that selenium deficiency resulted in decreased levels of copper, zinc and molybdenum in the pancreas but not the liver and plasma of chicks. Khan et al. (1987), however, note that chick plasma zinc levels were reduced by dietary copper supplementation but at adequate protein levels, not at low protein levels. In turkey embryos, increased concentrations of serum copper were obtained after an endotoxin injection (Klasing et al., 1987), presumably as a result of the mobilization of available copper from storage sites.

Similarities to other groups exist in tissue copper levels in mammals. Honda et al. (1987a) note higher liver copper levels in males than females in the Japanese serow (*Capricornis crispus*), the only wild bovine ruminant in Japan. Kluczek and Kluczek (1985) report a relationship between serum copper, ceruloplasm, temperature and humidity in mares. Serum copper was lowest in the spring and highest in the autumn, ceruloplasm lowest in the winter and highest in the summer. At least part of this may be a result of forage quality at different times of the year (e.g. Fisher and Bates, 1986; Freudenberger et al., 1987; Tait et al., 1986), while part may be due to physiological changes occurring in the dams. Monthly variations in bovine serum copper levels have also been related to rainfall (Bain et al., 1986), the higher the rainfall the lower the copper level. Since forage copper concentrations are affected by soil conditions, livestock tissue levels can also vary in response to location (e.g. Morton, 1986). This would suggest that seasonal influences on tissue copper would be reduced with constant management practices. Limited evidence to support this is given by Alvarez et al. (1986), who did not find a seasonal influence on blood copper in sire bulls kept under similar feed and management conditions in Cuba. They did, however, find differences in serum copper levels between different breeds of bulls (Alvarez et al., 1987). Breed differences in cattle are known as are differences within breeds (Gibson et al., 1986). Levels of copper in the blood of cattle have been reported to increase in response to transport of the animals (Locatelli et al., 1985). Changes occur in serum copper and ceruloplasmin as a result of growth. Working with foals, Bell et al. (1987) found that serum copper and ceruloplasmin concentrations increased in a linear fashion from day 0 to 28 after birth, then levelled off. In addition to serum copper and ceruloplasm, erythrocyte copper-zinc superoxide dismutase activity has also been related to available dietary copper levels (Sharma and Prasad, 1985). With many livestock, molybdenum and sulfur are known to affect copper uptake and mobilization (Robinson et al., 1987).

Laboratory examinations of the effects of copper have provided a broad spectrum of tissue metal concentrations and the factors affecting them. This includes tissue distribution of metal under normal, reduced, and elevated concentrations of dietary copper (Inoue and Maitani, 1985; Kawamura and Hamada, 1985; Lee et al., 1985; Malvis Stracciari et al., 1985) and the effect of both internal and external factors on tissue distribution. Sodium chloride ("table salt") is reported to increase plasma copper levels in laboratory rats (Clegg et al., 1987). Markov et al. (1986), for example, found that blood copper levels could be affected by exposure of young rats to a constant magnetic field. Teraki and Maemura (1986) present evidence that the metabolism of zinc, copper and iron in the rat changes during pregnancy. Mas et al. (1985) note that (abstract) "copper levels increased slightly at midpregnancy but returned to control levels at the end of gestation." They suggest that this is explained "... on the basis of equilibrium between assimilation and fetal needs for copper, ...". Romeu et al. (1987a) suggest (abstract) that "... metal retention is an adaptative element in maintaining the pregnant rat's metal supply, as the considerable metal retention in the first half of pregnancy is modulated in the second half according to the actual needs of the maternofetal unit."

Metal-metal interactions also occur in the rat. Keen et al. (1985) found that increasing levels of zinc lower tissue copper concentrations in male rats, especially those maintained on a copper deficient diet. Comparable results were obtained by Song et al. (1986) and Son and Magee (1987). Cossack (1987) notes that iron overload in rats was not associated with a significant change in copper concentrations in the liver, kidneys, heart, brain and whole blood. Lead acetate administered subcutaneously to rats caused an initial increase in copper, iron and zinc in the kidney, liver and spleen although levels were reduced after 96 hours (Gasiorowski et al., 1987). (Note, the changes could be a result of the acetate as well as the lead.)

Certain organics are capable of affecting tissue copper concentrtations in laboratory animals. Son and Magee (1987) noted increased rat liver copper concentrations with increases in dietary vitamin B-6. Although fructose has been associated with changes in copper metabolism, the ingestion of fruit is not always associated with changes in tissue copper concentrations. Sable-Amplis et al. (1987) found no significant changes in rat tissue copper levels with an apple-enriched diet. Phenobarbitol treatment is reported to cause increases in hepatic copper concentrations while colchicine causes decreases (Asano and Hokari, 1987a,b). Jasim et al. (1985) note that injection of diethyldithiocarbamate, a strong chelating agent, causes an increase in hepatic copper in both male and pregnant female mice, the latter at all stages of pregnancy.

The measurement of trace metals in humans provides estimates of physiological well being as well as information on natural variability. A variety of techniques have been used in these measurements (e.g. Shizuma et al., 1987). Stauber and Florence (1987b), however, point out that non-invasive sampling techniques are becoming more and more important because of the increase of blood-related communicable diseases. They measured zinc, cadmium, lead and copper in human sweat and report higher copper levels in males than females although the use of oral contraceptives may increase levels in females. Heese et al. (1987), for example, found higher mean serum copper levels in women using oral contraceptives. Various methods have been used to measure tisue copper levels in humans. Gutteridge (1987) discusses problems with the use of the phenanthroline asay for non-caeruloplasmin copper. He points out that phenanthroline-detectable copper in synovial fluid samples may very well be due to an artifact associated with tissue storage. Iyengar (1987), of the U.S. National Bureau of Standards, provides reference values in selected human tissues and body fluids. In an examination of metal levels in bones from an ancient Egyptian mummy and from a contemporary man, Cholewa et al. (1987) report consistently higher values in contemporary man. Copper may be localized in various organs or even in certain parts of structures. Uitti et al. (1987) reports uneven distribution in the brain and Anttila and Anttila (1987) found surface copper concentrations in childrens teeth to be more than ten times the concentration for whole teeth. Variations in serum copper levels have been reported, Kong et al. (1986) reporting higher levels in the morning than in the evening, higher levels in female than male subjects. Lehto and Mussalo-Rauhamaa (1987) also found daily changes in serum copper concentrations. These conditions were not found in whole blood and erythrocytes. Variation also occurs as a result of environmental background, nutrition and physiological state. Panessa-Warren et al. (1987) found copper in association with melanin in human ocular tissues. They comment that (page 92) this "... is consistent with the presence of the metalloenzyme O-diphenyloxidase, the essential bioacatalyst in natural melanin formation ...". Elderly individuals with high cholesterol levels are reported to have higher fasting serum copper levels than elderly individuals with low cholesterol levels (Lopez et al., 1987).

Changes in trace metal concentrations during pregnancy and, for children, after birth have been primary subjects for research (e.g. Kohrs et al., 1986). Plasma copper levels increase during pregnancy (Habib and Abdulla, 1986; Yamashita et al., 1985) although Ferrari et al. (1985) found no significant correlation between neonatal birth weight and copper, zinc and magnesium concentrations in maternal and cord blood. However, Donzelli et al. (1987) records reduced erythrocyte superoxide dismutase activity and zinc and copper concentrations in low birthweight infants. Based on tabular values in Yamashita et al. (1987), maternal plasma copper is significantly different from nonpregnant controls but there appears to be little change in concentrations during and after labour. Lembrych et al. (1986) found mean copper concentrations in breast milk to be 0.55 mg/L but Kirsten et al. (1985b) found levels to decline after delivery, from 0.57 mg/L at 3 days to 0.28 mg/L at 36 weeks. Huston et al. (1986) point out that conventional parenteral nutrition formulas do not maintain serum zinc and selenium values in low birthweight infants but do not suggest that this is the case for copper. In neonates, lower serum copper levels have been reported for twins than singletons (Dincsoy et al., 1987) while concentrations tend to be higher in newborn males (0.08 mg/g) than females (0.06 mg/g; Miyasaki et al., 1987). During the first year of life, serum copper levels are initially low but reach levels similar to those of the adult at 4 months of age (Kirsten et al., 1985a). Hepatic copper distribution is high but reported to be uneven in the human newborn (Faa et al., 1987) although variability makes it difficult to draw any systematic conclusions about causal factors. Houot et al. (1986) measured plasma copper in 10,000 subjects and comment that "the copper level regularly drops between the ages of 4 and 15, and this fall does not vary between sexes". Tsugane (1985) used principal components analysis to examine data on trace element concentrations in scalp hair of growing children and adolescents. Although copper is included in a group which the author states correlates positively with age, examination of tabular data (table 2) indicates enough variation to warrant closer examination. Certain genetic disorders are associated with changes in blood and tissue copper levels (e.g. Down's Syndrome, Groner et al., 1986a).

Trace metal concentrations have been related to exercise and athletic activity. Maughan et al. (1987), for example, found a slight increase in plasma copper shortly after endurance cycling although the concentration stabilized within 24 hours. Singh (1986) reports higher plasma and lower erythrocyte copper values in female marathon runners than in sedentary individuals. Keen and Hackman (1986) review trace elements in athletic performance. Greater emphasis on trace elements in athletes has come about partly in response to the effect of high level performance on trace metal requirements, but also because of the increasing concern about the need for greater awareness of mineral nutrition. The flux of copper, and other metals, through the human system has also been examined in individuals exposed to high levels of metal. Araki et al. (1986), for example, examined concentrations and clearance of metals and organic substance in metal workers. They report that the major renal excretory mechanism for copper is glomerular filtration and net tubular secreation.

Chemicals such as chelating agents have also been shown to affect copper levels, Waters (1985) noting altered excretion of copper after administration of EDTA (ethylenediaminetetraacetic acid). Stauber and Florence (1987b) found evidence of an increase in copper concentrations in female sweat. Powell-Beard et al. (1987), however, found no significant changes in plasma copper levels after terminating oral contraceptive agent therapy. Oral zinc supplementation in healthy human volunteers did not change plasma copper levels significantly (Samman and Roberts, 1987b).

I.3.2 LEVELS IN ABNORMAL TISSUES -

Levels of copper are affected by physiological imbalance as well as by inherited genetic disturbances. Aspin and Sass-Kortsak review abnormal copper metabolism in a book on Disorders of Mineral Metabolism by Bronner and Coburn (1981). Danks (1985) comments that "genetic disorders of trace element transport are now known in humans, mice, dogs and cattle. Those involving copper have been known longest and are best known clinically." Disease frequently brings with it abnormal distributions of trace metals, especially copper. This occurs in a wide variety of organisms ranging from plants (e.g. Berneike et al., 1987) to humans. Inskeep and Bloom (1987), for example, report increases in plant tissue copper, and other metals with increases in soybean chlorosis in calcareous soils of the upper U.S. Midwest.

Excess soil copper is related to elevated levels of copper in the roots of a metal-tolerant fern (*Athyrium yokoscense*; Nishizono et al., 1987a). They note however (Nishizono et al., 1987b), that the root cell wall of the fern is a metal storage site as well as the fern having some other mechanism of metal tolerance. Excess soil copper does not, however, mean that plant metal levels will always increase. Tissue metal levels in corn grown in copper-enriched soils did not increase in proportion to the enrichment (Perera, 1986). Neither were levels related to soil organic matter or pH. In spite of this, soil properties such as pH and organic matter often play important roles in the control of plant metal bioavailability. Pokatilov (1984), for example, found that sulfur contamination of soils could produce reduced copper levels in elm leaves and elevated levels in the stems. In addition to soil enrichment, foliar copper sprays increase plant metal levels (e.g. Mann and Sidhu, 1983). This may be important as it can reduce the effect of soil chemical properties on metal bioavailability.

With invertebrates, environmental and physiological stress can affect tissue metal levels (e.g. Feng, 1986). The relationships between stress and levels are not always obvious. Taylor et al. (1987) note an inability to maintain the volume of copper-containing blood pigment in crayfish during extended dehydration. Although not stated, it is expected that this would be associated with a reduction in total copper level unless the metal was stored, presumably in the hepatopancreas. Rao et al. (1985) note that copper-enriched water caused an increase in haemolymph and hepatopancreas copper in a species of crab.

Levels of copper producing producing either a deficiency or an increase in tissue copper have been examined in a number of domestic and laboratory animals. These data have been used to better understand supplementation for deficiencies and treatment of excess metal (e.g. Salih et al., 1987). This is particularly true for parenteral feeding (e.g. Poryadkov et al., 1986; Shulman, 1987). Sharma and Prasad (1986) found that in goats, blood copper levels were not increased by supplementation with 5.5 ppm copper but were with 20 ppm. Kadrabova et al. (1986) report an increase in serum and organ copper in rats following chronic immobilization stress. The relationship between stress and copper can, however, be a two-way street, copper deficiencies can produce physiological responses in many organisms.

Recant et al. (1986) note that copper deficiency causes an increase in pankreatic enkephalin-containing peptides and insulin. Insulin, and diabetes has been associated with abnormal copper levels, at least in laboratory animals. Donaldson et al. (1987) report that diabetic rats had significantly increased hepatic and renal zinc and copper concentrations. Elevated copper (Al, Mg and Rb) levels have been reported in the beta cells of the pancreas of diabetic Chinese hamsters (Juntti-Berggren et al., 1987). These are the cells that secrete insulin although the authors point out that the elemental changes can either be a result of or cause of the disease. Serum copper levels in humans with diabetes are also elevated (Qian et al., 1986). In contrast, levels in human striated muscle tissue are reported to be lower than normal (Sjogren et al., 1986b). Rats with induced diabetes have reduced activity of the copper-containing enzyme superoxide dismutase in the liver (Simonyan et al., 1987). Arai et al. (1987), working with patients with diabetes, found an increased percentage of glucosylated superoxide dismutase in erythrocytes. This is the first demonstration that the enzyme is glucosylated in erythrocytes and that glucosylation leads to inactivation of superoxide dismutase. However, the superoxide dismutase story is contradicted by the work of Kamei et al. (1985), who report that (abstract) "serum Cu, Zn-SOD in the diabetics showed a significantly high level of 64±45 ng/mL compared with 33±9 ng/mL in the controls ...." They also found increases in patients with certain side effects of long term diabetes, suggesting the role that the enzyme plays as a scavenger of active oxygen which acts against tissue injuries due to insulin-dependent diabetes.

Parasites can produce a tissue challenge for the host, which affects tissue copper concentrations. Spicarova (1985), working with aphid-infested leaves, comments (page 203) that "... the highest quantity of Cu is contained in waste substances from the aphides ...". Insect galls also contained more copper than leaf stalks and nearly the same quantity as in the leaf blades. Increased plasma copper has been found in chicks, during one phase of coccidial infections (Turk, 1986). With chicks fed excess copper, plasma increases are greater in coccidial-infected than uninfected chicks (Fox et al., 1987). Gadzhiev and Garaev (1986) report that, with sheep, changes in liver copper occur as a result of parasitization by liver flukes. They attribute this to the use of host copper by the fluke. Decreases in both liver and plasma copper as well as ceruloplasmin activity were found to be enhanced in copper deficient sheep with an induced nematode infection (Hucker and Yong, 1986). The authors comment (abstract) that "although the results support the contention that gastro-intestinal nematodiasis can significantly exacerbate an existing Cu deficiency in sheep, there was no evidence that hypocuprosis would predispose sheep to higher nematode burdens." Fettman et al. (1987) used *Escherichia coli* infection-produced enteritis in calves as a model to study trace element responses to inflammatory diseases. The onset of fever, diarrhea and hypoglycemia were associated with decreases in serum zinc and copper concentrations. Boulos et al. (1985) found reduced serum copper levels in human patients infected with the round worms that produce trichinosis. From these reports, and others, there is a general tendency for parasite infection to reduce tissue copper levels in the host. This can be a result of the requirement for copper exhibited by the parasite, the infection response of the host, or both.

The liver plays important roles in the control of copper concentrations in animals (e.g. Nederbragt et al., 1987). As a result, infection or damage to the liver can affect tissue copper metabolism (e.g. Al-Othman et al., 1986; Dashti et al., 1986; Lai et al., 1985; Srinivas et al., 1986a,b). The copper:zinc ratio in jaundice patients is, for example, much higher than in normal patients (Rao et al., 1986). Cirrhosis of the liver is a challenge that has been associated with changes in tissue trace metal concentrations. Wakiyama et al. (1986), however, note no significant change in liver copper concentrations in rats with experimentally-induced cirrhosis. They did, however, note an increase in copper in liver superoxide dismutase, ceruloplasmin and metallothionein. This suggests that although the total concentration did not change, the distribution amongst the organic fractions was affected. Tissue damage to any tissue or organ involved in the regulation of copper can affect tissue metal concentrations. Honda and Nogawa (1987) note that kidney copper concentrations were lower in humans exposed to environmental cadmium than in controls. They attribute this to renal damage produced by the cadmium.

There are several inherited diseases that seriously affect copper metabolism and liver copper concentrations. The "Lethal Milk Mutant Mouse", for example, is a genetic strain of mice which has an increased uptake and/or retention of zinc and copper in the tissues (Piletz et al., 1987). One of the more pronounced diseases is copper toxicosis in dogs, a "heritable autosomal recessive trait that results in hepatitis, cirrhosis and early death ..." (Thornburg et al., 1985a, page 41). In these animals, accumulation of copper occurs in the liver, to exceptionally high levels (Lucke and Herrtage, 1987; Thornburg et al., 1985b). Inherited copper toxicosis is often compared with, and used as a model for Wilson's disease in humans. (Discussed in Dorney et al., 1986.) This disease is associated with the accumulation of copper in the liver, to levels where copper granules are formed (Noda et al., 1986; Sato, 1986). It is also associated with low levels of copper elsewhere in the body (e.g. Goto et al., 1986; Zhang et al., 1985), an indication of improper copper metabolism. The antagonism between zinc and copper has led to the use of zinc supplementation as a mechanism for treating Wilson's disease patients (Lee et al., 1986; Sha et al., 1987). Brewer et al. (1987) discuss this and the measurement of urine and plasma copper levels as monitoring tools in treatment of the disease. (See also Weisner et al., 1987 for another monitoring technique.) Treatment with specific copper complexing agents is also used, to improve copper metabolism in Wilson's disease patients (e.g. Sasa et al., 1986). Although the effects of the disease are known, the cause is not well understood. Bingle et al. (1986) point out that neonatal mammals have a copper profile similar to Wilson's disease but that a change occurs shortly after birth. They, and others, suggest that the cause of the disease is the inability to change from a normal neonatal metabolism to the normal postnatal metabolism. Indian childhood cirrhosis is a disease of improper copper metabolism in with elevated tissue copper concentrations (e.g. Sharda and Bhandrai, 1986; Sharda, 1987; Tanner, 1987).

Age, illness, and environmental conditions all affect copper concentrations in humans (e.g. Alayash et al., 1987; Marlowe et al., 1987; Oroszlan and Szabo, 1987; Petrescu et al., 1985; Sommer and Massonnet, 1987). Bunker et al. (1987) found significant differences in plasma and whole blood copper concentrations, between healthy and housebound elderly individuals. Plasma copper levels in infants increase shortly after birth, normally as well as with metal supplementation (Friel et al., 1987b). Makela et al. (1986) note a continuous decrease in serum copper and selenium concentrations in children undergoing plasma exchange. They also note that concentrations returned to near normal after three weeks.

Changes in tissue copper concentrations may be either causal factors or a result of disease. Mussalo-Rauhamaa et al. (1986) note no significant difference in urine and hair copper concentrations between normal and alopecia patients in Finland. They did find significant differences in serum copper, between patients with different types of alopecia. No significant difference in serum copper concentrations is apparent between patients that are normal and those that have anorexia nervosa (Dinsmore et al., 1986). In children with asthma or eczema or psoriasis, mean hair and serum copper concentrations are higher than normal (Bichonski et al., 1985; Di Toro et al., 1987; Hinks et al., 1987). This has also been found for rheumatic patients (reviewed in Munthe et al., 1986; see also Hyora et al., 1986; Mussalo-Rauhamaa et al., 1987) although the metabolism of copper in these patients is not well understood (e.g. Abella et al., 1987; Winyard et al., 1987b). Meyle et al. (1987) reported elevated cellular blood copper levels for some patients with pereiodontitis. Indeed, elevated copper levels are generally characteristic of patients with acute infections (e.g. Arnbjornsson and Abdulla, 1986; Heck et al., 1987; Mamatkulov and Valiev, 1985; Srinivas et al., 1986; Taggart et al., 1986; Tsareva, 1985; Valiev and Mamatkulov, 1986) or ulcerated tissues (Agren et al., 1986). In rats (Oliva et al., 1987) and in humans (Beguin et al., 1987; Hallgren et al., 1987), chronic inflammation is associated with elevated blood copper concentrations. Hallgren et al. (1987) comment (abstract) that "... data obtained are compatible with the hypothesis that major redistribution of mineral and trace elements occurs in peripheral blood cells as a physiological response to chronic inflammation." Many anti-arthritic medicinal drugs appear to operate through the metabolic pathway of copper in patients (Sadique et al., 1986).

Depressed serum copper and serum ceruloplasmin levels have been reported for thermal injury patients (Boosalis et al., 1986; Brian et al., 1987). Lowest levels were found in patients with more than 40% of total body surface area burns (Boosalis et al., 1986) and Brian et al.

(1987) comment that (page 336) "... it appears that depression of serum copper levels following thermal injury is directly proportional to burn size." Alrowaih et al. (1986) report changes in plasma copper concentrations as a result of traumatic bone injury, partly as a result of the inflammation, partly because of the role of copper in collagen formation during bone repair. Interestingly, serum copper values for patients with osteoporosis do not differ significantly from normal patients (Stephens-Newsham et al., 1987).

Elevated serum copper concentrations have also been reported for patients receiving numerous (> 600) laser photocoagulation burns to the retinal pigment epithelium (Silverstone et al., 1985). Patients with retinitis pigmentosa reportedly have elevated plasma copper concentrations (Tsata-Voyatzoglou et al., 1986). Abnormal zinc and copper metabolism are also associated with pigmentary retinopathies in the eye (Silverstone et al., 1986a). These authors found supporting evidence for an earlier observation that albinism is a possible primary abnormality of zinc and copper metabolism. They (Silverstone et al., 1986b) also report signifiantly elevated urine copper concentrations in high myopic patients with retinal detachment. Changes in blood copper concentrations and ceruloplasmin activity occur in response to abortion (Ozgunes et al., 1987) and as a result of abnormalities of the uterine cervix (Grail and Norval, 1986). In patients with renal insufficiency treated with continuous ambulatory peritoneal dialysis, Wallaeys et al. (1986) report no deviation from normal in either serum or packed cell copper concentrations. Similar results are reported by Floyd-Gimon et al. (1986) although elevated whole blood copper concentrations are reported by Danielson et al. (1986), who also found higher levels of whole blood copper in uraemic patients and in patients undergoing hemodialysis and hemofiltration. The latter authors note, however, that normal levels were restored in transplanted patients. Use of desferrioxamine to treat renal patients suffering from the effects of aluminum overload is reported to have long term effects on reducing bone copper although not on serum copper levels (Hewitt et al., 1986).

Low plasma copper concentrations are recorded for mentally retarded dwarfs and male microcephalic subjects (Bruhl et al., 1987). Tada et al. (1986) presents evidence of decreased hair copper concentrations in schizophrenics although points out that the findings contradict several earlier studies. In patients with Parkinson's disease, cerebrospinal-fluid copper concentrations were higher than in normal patients (Pall et al., 1987a) as also were serum copper concentrations in epileptics (Lue, 1986). Lithium treatment is an established practice with mania although it reportedly has detrimental long term effects due to the induction of a collagen-like syndrome (Jendryczko and Drozdz, 1987). These authors report significantly lower activity of superoxide dismutase, the antioxidative copper-zinc dependent enzyme, in rats treated with lithium salt

Changes in serum copper levels are associated with cardiovascular disease. Increased concentrations are, for example, found in patients with hyptertension (Berthelot et al., 1987) and in hyperlipoproteinemic patients with or without atherosclerosis (Uza et al., 1985). In contrast, Anderson (1986) comments (abstract) that "insufficient dietary copper ... leads to elevated lipid levels and impaired heart function." There is controversy about the role of both zinc and copper in cardiovascular disease. Tiber et al. (1986) state (abstract) that "although dietary zinc and/or copper may influence the plasma levels of these trace metals, our studies show that there was no association between plasma zinc or copper and the serum levels of lipids or lipoproteins; we believe that this indicates that these trace metals are of doubtful value as markers for coronary atherosclerosis." With myocardial infarction, serum copper increases are reported for the first four days (Dumolard et al., 1986). Survivors of myocardial infarction characteristically have significantly higher hair Zn/Cu ratios than control individuals (Bialkowska et al., 1987). During open heart surgery, there is a drop in serum copper, followed by a gradual return to normal concentrations (Sjogren et al., 1986a). Fuhrer et al. (1986) reports that, with cardiopulmonary bypass operations (page 354), "plasma copper levels increased significantly after the beginning of ECC like the other trace elements. But 40 minutes ofter the start of bypass plasma concentrations below the initial values were found ...."

Abnormal growth, as with tumours, is often paralleled by abnormal concentrations of copper (e.g. Beguin et al., 1986; Carpentieri et al., 1986; Drozoz et al., 1987; Ebadi, 1987; Haratake et al., 1987a; Johansson et al., 1987; Maeda et al., 1987; Romeu et al., 1987b; Tapper et al., 1987; Uda et al., 1987; Zeng et al., 1987). Owen et al. (1986) found blood copper concentrations of 0.2 and 0.21 mg/100 mL in a mare with renal adenocarcinoma while here stablemates had concentrations between 0.09-0.11 mg/100 mL. Invasive cells of basal and squamous cell carcinomas contain detectable copper (Bedrick et al., 1986), serum copper concentrations are significantly reduced in patients with oral cancer and oral submucous fibrosis (Varghese et al., 1987). In contrast, serum copper levels increased with tumour size in laboratory mice with mammary adenocarcinomas (Fuchs et al., 1986). Tumours and estrogens are reported to reduce the rate at which copper is excreted from the body, in the rat, and to increase levels of plasma ceruloplasmin (Illowsky Karp et al., 1986). Margalioth et al. (1987) reports that, with gynecologic malignancies, "serum copper level correlated well with stage of cancer disease ... except for ovarian carcinoma, in which serum copper level was already significantly elevated in Stages I and II." They continue, "our data imply that the addition of serum copper level determination to other screening tests could increase their sensitivity." Gao et al. (1986) report elevated serum copper levels in children with acute leukemia. Hu (1985) found elevated serum but reduced tumour copper concentrations in patients with esophageal cancer and suggests (abstract) that " ... the variations in Cu represent the result rather than the cause of cancer ... ." Elevated serum copper levels have also been reported for patients with various cancers, including those of the larynx (Drozoz et al., 1986) and liver (Liu, 1985). Kwiatek et al. (1986; 1987), however, found no significant differences in hair trace element concentrations between control and colon cancer patients. As well, Poukkula et al. (1987) found no significant differences in serum copper and the histological nature of the tumour in patients with lung cancer. They comment (abstract) that "it is concluded that SCu (serum copper), SZn and SCP (serum ceruloplasmin) determinations are of no help in distinguishing malignant from nonmalignant lung disease and are only of limited importance for estimating the extent of the disease or the prognosis of a patient with lung cancer."

The treatment and preparation of food as well as the nature of food can have an impact on the trace metal status of an organism. Concern has, for example, been stated about the trace element status of humans after consumption of irradiated foods. With copper, at least, Huang and Zhou (1986) and Huang et al. (1987) found no evidence of effect of food irradiation either with rats or humans. Long-standing concern has also been raised with the use of coal tar dyes. Gautam et al. (1986) fed "a permissible dose" to rats and noted an effect on iron but not liver and faecal copper concentrations. The effect of alcohol on trace metal concentrations is also an active area of study, in part due to the interest in its effect on the developing fetus. Schmidt and Hultcrantz (1986) report a depletion of liver copper and zinc in alcoholics without liver disease. With rats, ethanol administration is associated with decreased copper concentrations in certain parts of the brain but increased levels in the spinal cord (Shafiq-Ur-Rehman, 1986). Tissue concentrations of copper were not affected by ethanol administration to "elderly" rats (McGinty et al., 1986). Halmesmaki et al. (1986) report an effect of drinking on maternal and/or fetal zinc and selenium balance in women, but no apparent effect on copper.

Prescribed and unprescribed drugs can affect the trace metal composition of the body, an effect which is often drug specific. Michel (1984) reviews the changes of trace element concentrations of human tissues and body fluids due to therapeutic and diagnostic treatment. Citrate (but not heparin), an anticoagulant, is stated to have a diluting effect on various trace metals when present at hyperosmolar concentrations (Smith et al., 1987). Hydralazine, a prescribed drug for the treatment of hypertension, has been found to give rise to statistically significant incidences of lung tumours with a concomitant decrease in copper concentration and superoxide dismutase activity (Drozdz et al., 1987a). Hurd et al. (1987) found an increase in liver and spleen copper and zinc in rats treated with anticonvulsants. Cimetidine is used in the treatment of gastric and duodenal ulcers. In female rats, the drug has been reported to cause an increase of copper levels in the heart, kidney, liver, jejunum, ileum and uterus (Naveh et al., 1987b). Administration of the immunosuppressive agent cyclosporin to rats caused an increase in Mg, Cu and Zn in the thymus (Allain and Leblondel, 1986). Acute treatment of rats with a strong metal chelating agent, sodium diethyldithiocarbamate, causes a significant increase in copper level in the hypothalmus and hippocampus portions of the rat brain (Szerdahelyi and Kasa, 1987).

I.3.3 COPPER AND THE RESPONSE OF THE ORGANISM

Dose-response relationships are reviewed in Aaseth and Norseth (1986), Hapke (1984b), Hellawell and Nor (1987). Petering and Antholine (1988) provide an excellent, readily understood discussion of copper speciation and biological effect, commenting that in order to understand the roles of copper it is necessary to understand how the reactions of copper can be modulated between the extremes of deficiency and excess. Many of these relate to geochemical properties of soil (Aggett and Rose, 1987) and water as well as direct and indirect anthropogenic effects both on the chemistry of the environment as well as metal emissions (Hall and Anderson, 1985; Helweg, 1986; Tamm and Andersson, 1985). Reisman et al. (1987) review health effects associated with copper, emphasizing aerosol metal. This document provides the scientific supporting basis for U.S. Environmental Protection Agency decision-making concerning retulation of copper under the clean air act.

Trace metals are known to affect microbial activity in natural (e.g. Terzieva et al., 1985), laboratory (Klein and Charles, 1987; Powell and Prosser, 1986) and industrial situations (Jones, 1986). The addition of 0.5-50 ppm Cu^{2+} to spent grain liquor, for example, is reported to reduce the citric acid production by *Aspergillus niger* (Roukas and Kotzekidou, 1987). The authors note, however, that an increase has been reported by others, under certain conditions. The production of "exotoxin A" by *Pseudomonas aeruginosa* has also been reported to be inhibited by copper (Blumentals et al., 1987). There is obviously species specificity in the response to copper as at least one microorganism, *Thiobacillus ferrooxidans*, is used in microbial leaching of copper from flotation tailings, producing solutions of leachate containing 290-470 mg/L copper (Grudev, 1985). Sugio et al. (1986), however, note that copper, as Cu^{2+}, can inhibit the ferric-ion reducing system in this organism. Certain strains of wine yeasts are capable of tolerating relatively high levels of copper sulfate (Watanabe and Iino, 1985). The toxic effect of copper on microbial activity can also be beneficial, the coliform bacteria *Escherichia coli* exhibits reduced oxygen utilization when exposed to copper in carbonate buffer that simulates a drinking water environment (Domek et al., 1987). *E. coli* has also been used to evaluate herbicide toxicity (Kohen and Chevion, 1985). Kohen et al. (1986) found that paraquat toxicity is mediated by iron or copper. Copper may also increase membrane permeability in *E. coli* (Lebedev et al., 1987).

In aquatic and terrestrial environments, reduction in microbial activity by copper is capable of causing a long term effect on the biodegradation of organic matter (Benmoussa et al., 1986; Brookes and McGrath, 1986; Burton et al., 1987; Falkner et al., 1985; Terzieva et al., 1985). The nature of the receiving environment is capable of affecting the effect of copper on microbial activity. Vedy et al. (1986) report that the toxic effects of metals in composted sludge change with the nature of the substrate. The inhibition of microbial respiration by copper is greater in a granite than a sandstone substrate. As well, metal-complexing ability of sewage sludge can protect decomposing organisms, such as methanogens, against heavy metal toxicity (Jarrell et al., 1987).

With brown rot wood fungi, copper inhibits cellulase enzymes but only at high concentrations (25 mg/mL assay volume; Collett, 1987). The nature of the substrate, as well as the nature of the organism is reported to be important in metal uptake by fungi (e.g. Dietl, 1987; Shaw, 1987) and would presumably play an important role in controlling metal bioavailability. Organics can, for example, combine with copper to form an organic-buffered pesticide (e.g. Kurdish and Khenkina, 1987) and can buffer the availability of copper in the environment. Metal-metal relationships are also important, Kapul'tsevich and Parshina (1986) note a copper-molybdenum relationship within which maximum productivity of an ethanol-assimilating yeast occurred. Copper-iron interactions can lead to glycogen deposition in *Neurospora crassa* (Subramanyam and Gupta, 1986). These factors affect growth both in the laboratory and in the field. Microbial enzyme activity has, for example, been used as a bioassay of copper availability in natural environments such as streams (Burton et al., 1987). A number of microbial organisms as well as fungi, mosses, and higher plants exhibit either a tolerance to high concentrations of copper or an ability to evolve the tolerance. One of the best known of these is the group called "copper mosses" which are generally restricted to copper-enriched substrates or substrates with high concentrations of iron (Shaw, 1987a). The tolerance is genetically determined although can be affected by environmental factors (Shaw, 1987b).

High levels of environmental metals have been related to water and sediment toxicity (Munawar et al., 1985, 1987). Copper plays a role in photosynthesis (e.g. Horvath et al., 1987; Sibbald and Green, 1987) but, in excess, has been associated with growth inhibition and reduced photosynthesis in plants ranging from bluegreen "algae" to fruit trees (Azeez and Banerjee, 1987; Baszynski and Tukendorf, 1984; Dueck and Duijff, 1987; Gupta, 1986; Haley et al., 1986; Li and Cai, 1985; Oliveira et al., 1985; Sarkar and Jana, 1987; Starodub et al., 1987a; Stiborova et al., 1986a,b; Zolotukhina et al., 1987). As a result, the metal has been used as an algicide (e.g. Hawkins and Griffiths, 1987) and antifoulant (e.g. Ludyanskiy and Solonin, 1986). Nakamura et al. (1986) report that growth inhibition in a red tide flagellate (*Chattonella antiqua*) is a function of cupric ion activity. This is supported by some of the work of Fuse (1987). Cytological changes in the green algae *Chaetomorpha brachygona* (Chan and Wong, 1987) and *Chara* spp. (Pal and Chatterjee, 1987) have been related to high levels of metals although tolerance, occurring in at least two green alga, *Pithophora oedogonia* and *Scenedesmus quadricauda*, (Balasubrahmanyuam et al., 1987; Osokina et al., 1986; Perlmutter and Lembi, 1986), may affect these changes. With copper-containing antifouling surfaces, Pyne et al. (1986) note that a number of diatoms are associated with the biofilm covering the surface but that only a few actually attach. These are usually associated with a slime. With the dominant genus, *Amphora*, copper does inhibit photosynthesis but only at high concentrations (Rao and Sivasubramanian, 1985a). With *Amphora coffeaeformis*, these authors note decreasing metal toxicity in the order Hg > Cd > Cu > Cr > Pb = Zn. Thomas and Robinson (1987a), working with the same species, note a reduction in silicate uptake although no obvious effect on nitrate and phosphate uptake. Valente et al. (1987) note an interaction of silica and copper in the planktonic diatom *Chaetoceros protuberans*, inhibition of resting spore formation by copper being associated with the concentration of available silicic acid. With another species, *Chaetoceros muelleri*, Zhenfen (1986) notes a reduction in growth with several metals, in the order Cu > Pb > Zn > Cd. Metal-metal and metal-metalloid interactions have been reported in recent literature on algae. Damyanova and Tyankova (1985), for example note that the macroalga *Ulva lactuca* exhibits decreased selenium uptake with added copper. They also note that copper, as $CuSO_4$, reduces the chlorophyll and carotenoid levels in the alga. The effect of excess ionic copper is reported to be ameliorated by trivalent metal ions in two marine diatoms and one freshwater green alga (Stauber and Florence, 1987a).

The use of copper-containing plant fungicides increases soil copper levels (e.g. Kunisch and Hurle, 1986; Quinche, 1985) and can produce phytotoxic effects. With mustard, Kumas and Singh (1986) report that blue copper caused poor germination and seedling growth. With certain varietal grapevines,, chlorosis may occur at high copper levels (3 ppm) although this appears to be transitory, vigorous growth occurring subsequently (Ruhl and Pleninger, 1986). Defoliation occurred on peach trees receiving copper-containing fungicides (Johnson et al., 1986).

Defoliation, preceded by a chlorotic condition, has also been noted as a result of excess copper with other plants (e.g. Russelle and McGraw, 1986). Chlorophyll fluorescence is inhibited by excess copper in the Chinese cabbage (Park and Kwon, 1986); reduced Hill activity is also reported (Jana et al., 1987; Sarkar and Jana, 1987; Singh and Singh, 1987). With a roadside weed, *Argemone mexicana*, Gopal et al. (1986) report that copper and certain other metal salts retard both pollen germination and pollen tube growth. At the concentrations tested, the order of effect was Ni > Bi,Pb > Cu. Seedling growth in oat and sunflower is inhibited by high concentrations of copper although these levels (25 and 250 ppm Cu) did not diminish the activity of ascorbate oxidase and monophenol monooxygenase in the seedlings (Lyszcz, 1985). Wheat is considered to be "sensitive" to copper, with supplementation required in deficient soils (Taureau, 1985). However, the relationships between soil components and added copper can be complex (e.g. De Haan et al., 1985). Copper is, for example, reported to have an antagonistic effect on leaf iron concentration in wheat (Galrao and de Sousa, 1985). Levels of copper acquired by cereal plants, from the soil is one of several factors that has been used in estimating soil contamination (e.g. Nemenko et al., 1987).

In an examination of plants growing on metal-enriched slag heaps, Chronopoulos and Chronopoulou-Sereli (1986) found decreased growth, early flowering and elevated tissue metal concentrations. Zinc- and copper-tolerant populations of the flowering plant *Silene cucubalus* had higher flower porduction when grown in soil supplemented with their specific metal (Dueck et al., 1987b). The fern *Pteris melanocaulon* reportedly does not grow in soils with less than 300 ppm copper (Tabbada and Tenorio-Borja, 1986). High levels of copper are toxic to some forest tree species (e.g. Teasdale et al. (1986) and reduced seed germination has been considered as a possible effect of acid rain acting on copper and other trace metal bioavailability. Scherbatskoy et al. (1987), however, found no evidence of this with seeds of two conifers and two deciduous forest trees.

Copper can affect a number of metabolic processes in invertebrate animals. In the single-celled *Tetrahymena pyriformis*, excess copper (0.1 mM) is reported to inhibit oxygen uptake, possibly by inhibition of one or more of the respiratory-related enzymes (Wakatsuki et al., 1986). Copper sulfate is used to control the snail intermediate host of bilharzia. It is also reported to reduce the invasive abilities of the liver fluke *Fasciola hepatica* (Bielecki, 1985), suggesting its usefulness in the control of a variety of parasites. With the polychaete worm *Arenicola marina*, copper is reported to affect the functioning of the blood by inhibiting oxygen binding to the haemoglobin in the respiratory area and oxygen delivery in the tissues (Everaarts, 1986). Using a variety of metal salts, Neuhauser et al. (1985b) found reduction in survival of the earthworm *Eisenia fetida* in the order Cu>Zn>Ni~Cd>Pb. There was no obvious difference between the acetate, chloride, nitrate and sulfate salts of each metal. Jethon et al. (1987) note an effect on oxygen consumption in *Lumbricus terrestris*, of metal-containing dust from a zinc smelter. Whether copper had any effect is not made apparent in this publication.

A number of publications deal with the effects of metals on molluscs. Havlik (1987) reviews literature on the effects of contaminants on naiads, a group of freshwater molluscs. Copper sulfate is a commonly used molluscicide which is reported to inhibit enzyme activity (Babu and Rao, 1987). In the terrestrial slug *Arion ater*, feeding activity is affected by copper and zinc although acclimation may occur over time (Marigomez et al., 1986). In many molluscs, the requirement for copper in enzymes and in the blood pigment haemocyanin obviates detrimental effects of low levels of environmental copper (e.g. Reddy and Rao, 1987). Suresh and Mohandas (1987) found increases in haemolymph lactic acid in a clam, with sublethal concentrations of mercury and copper. Wolmarans and Van Aardt (1986) do note an overall increase in tissue copper in a freshwater snail when exposed to copper sulfate although increased levels were not exhibited in the haemolymph and shells. Uptake appears variable in a number of molluscs (e.g. Catsiki and Arnoux, 1987) and may be a result of environmental properties or seasonal changes in the organism. Storage of metals, including copper, can occur in the kidneys of some molluscs (e.g. Reid and Brand, 1985). A copper-oxide-containing fouling paint was not found to affect growth of the oyster *Crassostrea gigas* or viability of embryos or larvae (His and Robert, 1987). It did, however, reduce the "condition factor" in the adult oyster. In contrast, organotin paint reduced growth rate in the adult oyster. Gendron and Vicente (1986) note that organotin paint reduces the quantity of copper and zinc in the oyster. In an excellent paper, Beaumont et al. (1987) note that the veliger larvae of both *Mytilus edulis* and *Pecten maximus* were less sensitve to copper than the juveniles or adults, a finding that contradicts the usual belief that tolerance increases with stage of development. Conditions of both tests and organisms must be considered, Phelps and Hetzel (1987) noting differences in oyster size, age, and copper and zinc accumulation in populations within Chesapeake Bay. Increased copper concentration has been associated with decreased filtration rate in the clam *Villorita cyprinoides* (Abraham et al., 1986). At 20 μg/L, it has also been associated with histological changes in the kidney of the scallop *Placopecten magellanicus* (Fowler and Gould, 1988) and, at 10 and 20 μg/L, inhibited gamete production and maturation in the scallop (Gould et al., 1988).

The effects of copper on arthropods has been of longstanding interest, in part because of the presence of a copper-containing blood pigment in many representatives of the phylum (e.g. Naich and Alikhan, 1987). Price (1978) discusses the effects of excess copper, cadmium and zinc on the brown shrimp *Crangon crangon*, with respect to both life history and moult as well as salinity and temperature. For the freshwater shrimp *Macrobrachium carcinus*, Correa (1987) noted a reduction in respiration and ammonia excretion rates with increasing concentrations of zinc and copper. Johnson (1987) found an effect of high levels of copper and zinc only under hypoxia, with *Crangon crangon* and *Carcinus maenas*. Copper-containing ricefield pesticides are reportedly toxic to crayfish living in the flooded fields (Andreu-Moliner et al., 1986). In the crab *Scylla serrata*, Arumugam and Ravindranath (1987) found no mortality with elevated levels (0.1-100 mg/L) of copper as carbonate but 40, 80 and 100% mortality with 10, 50 and 100 mg of copper as copper sulfate. Malyarevskaya et al. (1984) comment that 24-hour exposure of larval stages of the insect *Chironomus plumosus* to 100 mg/L copper sulfate caused irreversible changes in the concentrations of ATP (adenosine triphosphate) and two respiratory enzymes. Cohen et al. (1985) related tissue concentrations of eight trace minerals to location and age of the insect pest *Lygus hesperus*. They found wide variations that were age, diet, sex and location related. Starfish are echinoderms that are sometimes predatory on economically important molluscs. Levin (1986) became interested in the motor responses of starfishes to various solutions in the hope of developing a starfish repellent. With copper sulfate, as with many agents however, the response was inconsistent even when the agents were at toxic levels.

Fish have formed an important study group because of their economic and recreational importance as well as their physiological nature. References published between 1977 and 1987 that deal with metals and fish are cited in the 1987 National Technical Information Service publication PB87-866810. Evans (1987) provides a good review of selected copper literature when he discusses the fish gill as the site of action for effects environmental pollutants. Trace metal loadings to streams, lakes and marine waters have been examined in terms of biological impact on fish and effect on tissue metal concentrations (Johnson, 1987; Palm, 1985; Spehar and Fiandt, 1986). The results of these studies are varied and depend to some extent on the chemical nature of the receiving water and the concentration of metal as well as the nature of the organism. Palm (1985), for example, comments that in the Gulf of Finland, concentrations of copper are sufficiently low that no effect is expected on the embryonic development of the Baltic herring. Mukhopadhyay and Konar (1985) found depression of fish (*Tilapia mossambica*) growth and respiratory rates after exposure to high levels of a mixture of copper, zinc and iron. Other work on fish toxicity and the physiological effect of copper, includes Aloj Totaro et al. (1986), Ansari (1987), Benger et al. (1986), Collin (1985), Gupta and Rajbanshi (1986), Ou et al. (1986), Tort et al. (1987) and Wani (1986). In the abstract of a talk, Heath (1986) points out that the freshwater bluegill (*Lepomis macrochirus*) can osmoregulate in hyperosmotic water better after exposure to excess copper. This, they suggest, is a result of the copper reducing gill permeability to salt as well as possible alterations to kidney function. (Trace metal concentrations in the kidney of some vertebrates can also be affected by certain organics (e.g. Furniss et al., 1985).) Doimi et al. (1985) discuss a dieoff of cultured sea bass (*Dicentrarchus labrax*) in Italy and conclude that this was a result of excess metals in the feed (lead, zinc, chromium and copper) plus a deficiency in vitamin C which would act as a metal-buffering agent as well as a vitamin. Lauren and McDonald (1987) examined the biochemical events occurring during acclimation of rainbow trout (*Salmo gairdneri*) to excess copper. They report an increase in a metallothionein-like agent as well as changes in enzyme activity. Metallothionein-like agents are important to metal flux and metal effects in both invertebrates (e.g. Fowler and Gould, 1988) and vertebrates (e.g. Delval, 1984; Sone et al., 1987) and will be discussed later in this review. The effect of copper-containing pesticides on fish tissues has been examined although primarily with carp (Asztalos, 1986; Benedeczky et al., 1986; Vig et al., 1987).

The use of copper in feeds and medicines for domestic animals has been of benefit to both the animals and man. It has, however, raised concern about the effects of excess copper. Georgievskii and Polyakova (1984) found no detrimental effect with Leghorn layers and chicks given a compound feed containing 15-20 grams $\mathrm{CuSO_4}$È$\mathrm{5H_2O}$ per ton. Ledoux et al. (1987) note a growth depression of female Cobb chicks given 400 mg Cu per kg bird weight. This could be reduced by feeding methionine although not at higher levels of copper (800 mg/kg). They provide evidence suggesting that the reduced feeding associated with high levels of the metal may be responsible for a number of copper-associated changes occurring in the birds. In turkey meat, copper along with iron acts as an oxidant for lipids, increasing the rate of oxidative rancidity and decreasing the storage time (Salih, 1986).

With ruminants, excess biologically available copper can occur in pastures and in feed under certain conditions although so can copper deficiency (Balbuena et al., 1987; Caple and McDonald, 1983). Reagor and Eugster (1986) point out that the use of cattle feed for lambs can produce a toxic condition as a result of the higher copper:molybdenum ratio. (Molybdenum is antagonistic to copper in a number of ruminants and is used to reduce the toxic effects of excess dietary copper.) Fuentealba (1985) reviews copper toxicity in sheep, discussing the copper-molybdenum relationship and the phases of copper poisoning. Other work on the effects of excess copper to sheep includes Anke and Groppel (1987), Clegg et al. (1986), Gooneratne (1986), Humphries et al. (1986, 1987), Kumaratilake and Howell (1987a). In many cases, this is a result of an imbalance of copper and molybdenum, either in natural or prepared feeds (e.g. Niederman et al., 1987). Correction of the condition can be achieved either with a change in food composition (Niederman et al., 1987) or use of tetrathiomolybdate (Humphries et al., 1986; Kumaratilake and Howell, 1987b). High concentrations of copper can cause liver necrosis and death in cows (Bohman et al., 1987). As well, neonatal diarrhea in beef calves can be produced in cases of excess minerals, including copper (Johnson et al., 1985). In pigs, dietary copper sulfate reduces gut streptocci and urease activity, a feature which is beneficial to weight gain (Varel et al., 1987). Side effects from the copper include increases in hepatic copper concentrations and "softening" of the fat in certain areas (Astrup and Lyso, 1986). It may also cause loss of potassium and gain of sodium in erythrocytes as well as cross-linking of membrane proteins (Asano and Hokari, 1987).

Copper control of *Salmonella typhimurium* has been tested in rabbits although treatment with excess copper plus desferioxamine can be toxic (Garcia Penarrubia et al., 1986). Rabbits and rats have been used as laboratory animals for a variety of studies on the effects of copper (e.g. Bito and Baroody, 1987). Detrimental effects of copper-containing fungicides have been reported for rats (e.g. Sapegin et al., 1987) and Inoue and Maitani (1985) report suppression of body weight gain and increase in tissue copper levels in rats receiving high levels of oral copper. Body weight loss, decreased ability to maintain body temperature, and an inflammatory response in the rat lung has been reported as effects of rats inhaling a Cu-Zn alloy powder (Snipes et al., 1986). Health hazard evaluations (e.g. Pryor, 1987) have, at times, shown exposure to aerosol copper in the workplace in excess of the Occupational Safety and Health standard of 25 mg/m^3. Effects of this are not adequately understood although, in male rats, inhalation of 0.0075-0.020 mg $CuCl_2$/m^3 for 3 months induced testis and prostrate weight reduction, increased RNA concentration in testis, caused sclerosis of Leydig cells and increased the number of abnormal spermatozoa (Ermachenko et al., 1987). Grin et al. (1987) used rats exposed to elevated concentrations of atmospheric $CuCl_2$ to obtain an estimate of the maximum permissible concentration of atmospheric $CuCl_2$ (3 µg/m^3). Detrimental effects of excess aerosol copper on humans have also been noted with individuals who work with chromium-copper-arsenic-treated lumber without adequate safeguards (Peters et al., 1986). Concerns have also been expressed about copper in drinking water (see citations in the 1986 and 1987 National Technical Information Service bulletins PB87-851960, PB-87-852356 and PB87-853305; see also Anan'ev, 1987). In an abstract, Eife et al. (1987) relate features of Indian Childhood Cirrhosis to hepatic copper overload in a Lower Bavarian farm family which they suggest was due to excess copper in tap-water. Levels in the well forming the source of the water are given as 12 µg/L while levels in the drinking water were 3400 µg/L. The authors do not state whether the latter value was obtained from running water or water that had been in copper tubing for an extended period of time. Clegg et al. (1986) report a case of waterborne copper toxicity in sheep resulting from the use of copper piping.

Results from experiments with rats exposed to subtoxic doses of lead, copper and humic acids lead Gaede and Kuhnert (1986) to suggest that environmental conditions can control metal impact but that (abstract) "... long-time exposure of warm-blooded animals to heavy metals may impair the therapeutic effectiveness of pharmaceuticals ... ." Even the magnetic field may affect copper uptake and mobilization (Markov et al., 1986). Frimpong and Magee (1987b) examined the effects of dietary copper and zinc on serum lipid parameters in young male rats. They note that increases in dietary copper levels were associated with decreases in serum total cholesterol and HDL-cholesterol levels. High levels of oral copper can be toxic. Ochiai et al. (1985) note that basic cupric carbonate is toxic to rats at levels of 2,000 ppm. High concentrations of copper can be identified by taste, Kasahara et al. (1987) report taste selection against $CuCl_2$ by mice. Copper is also one of the plasma constituents that has been considered to possibly affect taste capability (Greeley et al., 1986; Suzuki et al., 1987b). Janjua and Ali (1986) suggest that, when present at high levels in food, copper may replace zinc from the gustin protein present in saliva. This would reportedly produce a saliva zinc deficiency associated with a temporary loss of taste acuity. Cupric ions are also reportedly able to inhibit sugar (galactose) transport across the rat jejunum (Rodriguez-Yoldi and Ponz, 1987). Kumar and Sharma (1987) report decreases in percent haemoglobin, number of red blood corpuscles, plasma corpuscular volume and mean corpuscular volume in rats after 30 days oral administration of copper sulfate (0.1 g/kg body weight).

The virulence of the coliform bacteria *Yersinia enterocolitica* can be reduced by a combination of copper and chlorine. Rapid passage of a treated innoculum through the mouse stomach, with its acid nature, has not been demonstrated to further reduce the virulence (Singh and McFeters, 1987a,b). Within the organism, a number of factors interact with copper, affecting both tissue concentration and metabolic function of the metal. Milanino et al. (1986) present evidence that during acute inflammation, there is an increase in requirements for both copper and zinc. Copper complexes are known to act as analgesics and possibly activate copper-dependent opioid receptors (Okuyama et al., 1987). Copper deficiency is associated with increased lipid peroxidation (Hammermueller et al., 1987; Lawrence and Jenkinson, 1987; see also Belyaev and Shmeleva, 1987; Homer, 1986; Walter et al., 1987). Deficiency is also associated with the tendency towards hypertension (Klevay, 1987; Medeiros, 1987). The effect of metal deficiency and excess on cell ultrastructure is sufficient to suggest that animal cells may be useful in monitoring both environmental and nutritional conditions (Storch, 1986). In effect, the difference in response of various cell types is the basis for use of antineoplastic copper chelates (e.g. Elo et al., 1987).

The use of copper in dental amalgams has sponsored work to define any detrimental side effects from the metal. Cox and Eley (1987b) note a mild early inflammatory response in guinea pigs, from subcutaneously implanted conventional and high-copper dental amalgam powders. Hero and Niemi (1986) discuss tarnishing in *in vivo* Ag-Pd-Cu-Zn alloys. They suggest that this is a result of local microgalvanic cells due to chemical inhomogeneities. Release of copper also occurs from amalgams although the rate of release appears to be related to the concentration of paladium in the amalgum (Chung et al., 1987) as well as the chemistry of the medium (e.g. Sutow et al., 1987). Implanted metals have also been examined for their effect on enzyme kinetics. Copper, for example, has been demonstrated to inhibit lactate dehydrogenase activity (Williams and Crowley, 1986). Immune cells have been shown to be inhibited by copper levels occurring with the use of copper intrauterine devices. Copper associates with DNA at higher copper concentrations (Marion et al., 1987) and simultaneous changes of Ca, Mg, Fe, Cu and Zn concentrations in cultured human lymphocytes affected thymidine incorporation and surface antigens of human T and B lymphocytes (Carpentieri, 1987; Carpentieri et al., 1987). Copper has been related to the integrity of the fetal membrane although Remohi et al. (1985) found no evidence that deficiency is associated with premature membrane rupture. During pregnancy in the rat at least, there is evidence of homeostasis with the essential metals (Cu, Zn, Fe), with normal fetal concentrations being maintained even with maternal exposure to excess metal (Tsuchiya et al., 1987). Pleban et al. (1985) review trace element metabolism in the fetus and neonate. The effects of excess copper and other metals are often difficult to relate back to the causal factors in natural environments. Van Thiel (1986) discusses some of the problems faced by environmental toxicologists in recognizing, much less quantifying industrial hepatotoxicity. Prociv (1987, page 345) recalls a "... mysterious outbreak of hepato-enteritis on Palm Island, north Queensland, in November 1979..." and hypothesizes that this could have been due to copper intoxication.

I.3.4 THE USE OF COPPER IN CONTRACEPTIVE DEVICES

The use of contraceptive devices is of ever increasing importance not only because of the need to control the size of the human population but also to reduce the spread of sexually-transmitted diseases (e.g. World Federation of Contraception & Health, 1987). Copper-containing intrauterine contraceptive devices are widely used (e.g. Sheldyaeva and Kadyrova, 1986) and copper injection into the male reproductive tract is reported to have a potential value as a contraceptive (e.g. Gabuchyan, 1987; Skandhan and Skandhan, 1986; Zhang et al., 1987). The variety of intrauterine devices available has been responsible for studies evaluating existing and new mechanisms (Audebert et al., 1985; Batar et al., 1987; Bratt et al., 1987; Diaz et al., 1985; Fylling, 1987; McCarthy et al., 1986; Sivin et al., 1987) and the medium in which these devices operate (e.g. Sekulovic and Primorac, 1985). Thiery and Kosonen (1987), discuss the effect of long-term use on corrosion and dissolution of copper in a copper-containing device. They comment that calcareous deposits reduce the dissolution and corrosion of copper but not the contraceptive effectiveness of the device that they examined. Without deposits they record a 23 µg/day loss of copper from the device. Goluda and Lembas (1985) note that copper loss from a similar device is proportional to the length of time it is used.

Sivin and Schmidt (1987) review the effectiveness of various types of intrauterine devices. Failures (pregnancies) are associated with the type of device but, perhaps more importantly, with the size of the device and the size of the uterine cavity (Larsson and Lindhe, 1986). The mechanisms of action of the devices vary from one type of device to another. With copper-containing devices the effect is believed to be a result of ionic copper released into the reproductive tract and its effect either on the physiology of the wearer or on sperm activity (Goldstuck, 1987; Johannisson, 1987). However, evidence is accumulating that the level of copper produced by devices does not produce major changes in the biochemical nature of cervical mucus (Bull et al., 1987) or markedly affect the ability of the sperm to swim through cervical mucus (Robert et al., 1986). Li and Hutchens (1987) do suggest that estrogen receptor proteins function as metal complexing agents and thus could affect metal bioavailability in the genital tract.

Recovery of fertility after removal of an intrauterine device has been of concern to users although evidence suggests that pregnancy can be achieved in most cases (Belhadj et al., 1986; Diaz et al., 1987). Side effects of released copper include possible inhibition of immune cell function (Bugbee and Fives-Taylor, 1987) and autoerythrocyte sensitization (Grossman, 1987). Side effects of intrauterine devices are due to the physical presence of the device and the response of the body to it. These include an increased risk of pelvic inflammatory disease (Grimes, 1987; Kryzaniak et al., 1986) and perforation of the uterus (Goldstuck, 1987).

Oral contraceptives can reportedly affect trace metal levels. Stauber and Florence (1987b), for example, present evidence suggesting that oral contraceptives increase sweat copper concentration in women. Blood copper levels may also be increased (Phillips, 1982) although Powell-Beard et al. (1987) present evidence that this is only while oral contraceptives are being taken.

I.3.5 PHYSIOLOGY AND THE EFFECTS OF COPPER

Copper interacts with the organism, playing important roles in enzymes, blood pigments in certain invertebrates, and other organics. These all contribute to the physiological well being, or ill health of the organism. Inborn errors of copper metabolism (see Danks, 1985) can, for example, lead to death. Potential and real anthropogenic effects to the environment have been responsible for numerous studies of what could be termed "pollution physiology" (e.g. Vernberg et al., 1987), often without adequate consideration of appropriate techniques and protocol. Wong et al. (1986), for example, note the necessity of using ultra-clean techniques to assess the effects of metals on phytoplankton. something that has been all to frequently forgotten.

This section considers the effect of copper on the physiology of organisms, from bacteria to humans. Because of the interaction of organics with copper, the reader will find 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.

Copper and the liver

One of the better organs to demonstrate organism-copper relationships is the liver. Liver diseases are often associated with irregular copper metabolism and copper levels (e.g. Lai et al., 1985). Robertson et al. (1987), for example, discuss "Gilles de la Tourette" syndrome in which there is a very rapid disappearance of copper from the plasma and an abnormally slow uptake by the liver. Danks (1985) provides an excellent review of inherited diseases that affect trace element metabolism. In normal humans, values of liver copper are usually less than 50 μg/g dry weight while in certain inborn errors of copper metabolism, the value can be five times that much or more (Ritland and Aaseth, 1986, 1987). In addition, a number of changes can occur with faulty copper storage in the liver. Leevy et al. (1986), for example, suggest that Mallory bodies play a role in the development of clinical and laboratory abnormalities in the late phases of certain liver disorders. (Copper may also play a role, although minor if at all, in testicular development (Kennedy et al., 1987).) The use of high specific ^{64}Cu activity, by the Szilard-Chalmers reaction, has been proposed for medical diagnosis of irregular as well as regular copper metabolism (Hetherington et al., 1986). Other techniques have been proposed for measuring effects of improper copper storage (e.g. Gunther et al., 1987) or modelling genetic, physiological and pathological changes resulting from the disease (Farrer et al., 1986; Messripour and Haddady, 1987).

In dogs, copper-associated liver disease was first recognized in the Bedlington Terrier. It is inherited and characterized by impaired biliary excretion of copper that can result in hepatitis, cirrhosis and early death (Lucke and Herrtage, 1987; Thornburg et al., 1985a,b, 1986). Defective copper storage is also characteristic of a breed of mice called the brindled mouse (Garnica et al., 1987b), an organism that serves as a model for Menkes syndrome found in humans (e.g. Packman et al., 1987). Barrow et al. (1987), however, caution against widespread use of the rat as a model for at least two copper-associated liver diseases, Wilson's disease and Indian Childhood Cirrhosis. Danks (1983) reviews Wilson's disease and Menkes' disease, two widely reviewed inherited disorders of copper metabolism. In a "plea for increased awareness", Dorney et al. (1986) point out that Wilson's disease in childhood is treatable if diagnosed before severe complications occur. Accumulation of liver copper is high enough to be evident as changes in the color and surface of the liver, as seen by laparoscope techniques (Noda et al., 1986). Plasma copper and ceruloplasmin levels are, however, decreased (e.g. Goto, 1986). Sato (1986) presents evidence of copper accumulation in fibroblasts from patients with Wilson's disease and suggests that metallothionein synthesis is involved in higher copper accumulation rates. This is supported by evidence of metallothionein-like organic material in the liver (Sasa et al., 1986). Janssens et al. (1986), however, obtained evidence suggesting (abstract) that the "... inability to synthesize MT (metallothionein) upon storage of copper is the cause of WD (Wilson's disease)." Treatment includes restriction of dietary copper intake (e.g. McGuiness et al., 1987), the use of copper chelating agents, and oral zinc treatment, the zinc competing with copper for binding sites (e.g. Brewer et al., 1987a,b; Cossack, 1987; Cossack and Bouquet, 1986; Hoogenraad et al., 1987; Lee et al., 1986). There is evidence that Wilsons' disease is a perpetuation of the fetal mode of copper metabolism into childhood (Bingle et al., 1986; Srai et al., 1986b), that normal biliary excretion of ceruloplasmin-bound copper is not operational as it is in normal humans (Iyengar et al., 1986). Indian childhood cirrhosis (ICC) has many of the characteristics of Wilson's disease, with some but not convincing evidence of heritability (Sharda, 1987). Tanner (1987) reviews the disease and its prevention and treatment. As with Wilson's disease, the chelating agent D-penicillamine is used in the treatment of ICC (e.g. Bhave and Pandit, 1987).

The physiological roles of copper in organics

Metals, including copper, are required for the formation of a wide variety of proteins such as enzymes and hormones or hormone-like agents (e.g. Shoham et al., 1987). They can also reduce formation and activity when in excess. Hurst et al. (1987) report 50% inhibition of the synthesis of rabbit reticulocyte lysate protein by copper concentrations of 40 µm. With enzymes, excess biologically available metal can affect both formation and activity rate. Increasing concentrations of copper inhibited pectic enzyme activities of the fungus *Alternaria alternata* (Vazquez et al., 1986), glutamine synthetase and nitrogenase activity in the blue green alga *Nostoc calcicola* (Singh et al., 1987), rice bran lipoxygenase activity (Jhon and Lee, 1985) and carboxylase activity in barley (Stiborova et al., 1987). Copper-associated reduction in sunflower leaf peroxidase activity has also been reported (El-Kadousy and Alexandrescu, 1987) as has aspartate transaminase activity in tobacco tissue culture (Gaal et al., 1985).

The enzyme laminarinase occurs in a number of marine invertebrates feeding on phytoplankton or brown algae. Roche-Mayzaud and Mayzaud (1987) report that copper (20 mM, as $CuSO_4$) causes a 97% inhibition of the enzyme in the planktonic copepod *Acartia clausi*. The activity of other enzymes can be lowered by excess metal (e.g. Ibrahim et al., 1986; Lu and Combs, 1987; Williams and Crowley, 1986). In contrast, copper is one of the metals that enhances hyaluronidase activity in the anchovy *Engraulis encrassicholus ponticus* (Rosoiu et al., 1987). Wess et al. (1986) provide evidence that after inhibition of argenine aminopeptidase with 1, 19-phenanthroline, some (9-11%) of the activity could be restored with copper. Copper also plays important roles in the synthesis of hormones. Fujimoto et al. (1987) suggest that Cu^{2+} has the potential to modulate prostaglandin synthesis while Colombani-Vidal and Barnea (1986a,b, 1987) discuss the interaction of "chelated" copper on the stimulation of luteinizing hormone-releasing hormone. In the latter, the role of copper is complex, appearing to amplify the action of an organic (prostaglandin E_2) that stimulates the release of luteinizing hormone-releasing hormone. As discussed earlier in this review, deficiencies of copper are associated with a wide range of physical and physiological malfunctions in domestic animals (e.g. Greve et al., 1987; Suttle et al., 1987) and in humans. Many of these malfunctions are directly or indirectly related to copper-enzyme associations (see review by Shamberger, 1987).

Copper-zinc superoxide dismutase (S.O.D.) is an enzyme that catalyzes the dismutation of the superoxide ion to molecular oxygen and hydrogen peroxide in the manner:

$$2H^+ + 2O_2^- \text{ --superoxide dismutase--> } H_2O_2 + O_2$$

The enzyme is found in a wide variety of organisms (e.g. Natvig et al., 1987; Petrovic et al., 1986) and is affected by metal-metal interactions (Chung et al., 1987; Mylroie et al., 1985) as well as the physiological state of the organism (e.g. Drozdz et al., 1987a; Elroy-Stein et al., 1986; Groner et al., 1986a; Kamei et al., 1985; Simonyan et al., 1987). It is also used as an antioxidant in the food industry, produced by genetically engineered microorganisms. Lee and Hassan (1985, 1986), examined the production of S.O.D. by *Saccharomyces cerevisiae* and found that the addition of copper to the growth medium elicited an increase in production.

A number of studies have examined the ability of copper complexes, like superoxide dismutase, to act as active oxygen species scavengers (e.g. Liochev et al., 1987). Copper deficiencies have been associated with abnormal oxidation of organics such as lipids (Hammermueller et al., 1987) and chronic copper loading with disturbances of antioxidative enzyme function (Russanov et al., 1986). The interaction of ascorbates with copper-containing organics (e.g. Machoy et al., 1985) suggests that the ascorbate may act as an antioxidant while the copper is involved with its oxidation. The interaction plays an important role in a number of physiological/biochemical processes occurring in humans (e.g. Fong et al., 1987).

Elevated plasma copper levels are often associated with inflammation and increased synthesis of ceruloplasmin which binds the copper and acts as an antioxidant (e.g. McGahan and Fleisher, 1987; Milanino et al., 1986; Pall et al., 1987b; Winyard et al., 1987a). Rheumatoid arthritis has been considered as "a disturbance in copper homeostasis" by Rafter (1987) which can be treated by chelation therapy (D-penicillamine et al.). The relationship between arthritis and copper may not be simple (Bergstrom et al., 1987; Matsubara et al., 1987; Williams et al., 1987), possibly involving the role of copper in the production of connective tissue and maintenance of a competent immune system (Freeman and O'Callaghan, 1987). (Vascularization is also affected by copper and copper-containing organics (Fenselau and Adams, 1987; Nicolle et al., 1987; Wissler et al., 1986b; Ziche et al., 1987).) Additionally, certain organic copper complexes can initiate joint degradation in laboratory animals (Cashin, 1987) suggesting that it may not be the copper by itself that causes inflammation. Copper aspirinate has been recommended over regular aspirin as an analgesic because of reduced damage to the gastric mucosa. Wittmers et al. (1987), however, report that the copper aspirinate caused more mucosal damage to rats than did regular aspirin. However, decreased serum metal levels have been associated with the use of regular aspirin (Andres et al., 1987).

Physiological responses to copper

Workers exposed to lead, zinc and copper have been reported to develop central and peripheral nervous system dysfunction (Araki et al., 1987). It is known that copper levels vary in different parts of the nervous system and can be affected by the physiological nature of the organism (e.g. Bourre et al., 1987; Hartter and Barnea, 1987). As well, under some conditions, copper can affect polarity and activity in nerves (Boiko, 1984; Niijima et al., 1987; Weinreich and Wonderlin, 1987). Saxena et al. (1986) found that intracerebroventricular injection of copper into the rat produced a dose-dependent hyperthermia. With the eye, copper at high levels (100+ μg Cu^{++}/L) has been shown to produce lesions in the eyes of fish (Bodammer, 1987). Postic-Grujin et al. (1987) obtained evidence suggesting a decrease in energy potential in the lens of rats exposed to copper. With the bullfrog corneal epithelium, Scheide et al. (1987) found that a serosal addition of 10^{-5} M $CuSO_4$ resulted in a transient increase of the short circuit current. Copper particles in the vitreous body of the rat caused a decrease in the amplitude of the retinal signal (Schmidt et al., 1987) while, in the vitreous body of the rabbit eye caused a decrease in glycogen and pyruvic acid content (Zygulska-Mach et al., 1986a). These same authors (Zygulska-Mach et al., 1986b) report that copper wires in the lenses of rabbits caused an increase in free fatty acids and cholesteral and a decrease in lens triglycerides. There is some evidence that once released into the vitreous body, copper is bound and retained although uptake by portions of the eye is possible (Biro and Baroody, 1987). An effect by metals, on ciliary activity of mouse trachea organ cultures, has been reported by Lag and Helgeland (1987), with a decrease in activity exhibited both with time and with concentration for copper.

A number of interactions of copper with various blood components have been discussed in the recent literature. The rate of activation of blood coagulation can be increased by both zinc and copper suggesting a role of the metals in coagulation (Shore et al., 1987). Excess copper has been demonstrated to be detrimental to fish blood cells (Ou et al., 1986), to decrease the percent hemoglobin, number of red blood corpuscles and plasma corpuscular volume in rats (Kumar and Sharma, 1987), and to affect at least part of the normal function of human lymphocytes (Nagashima, 1986). At least some of these are due to the interaction of copper with cell membrane components and processes, something that is not unique to just blood cells (e.g. Van Huynh and Decleire, 1987). Deformability and haemolysis of blood cells can be induced by excess copper (Asano and Hokari, 1986; Caffrey et al., 1986; Dimitrova et al., 1987; Ito and Kon, 1987). In contrast, copper deficiency may lead to modification in the organization of erythrocyte membrane proteins (Johnson and Kramer, 1987a,b). Chronic dietary copper deficiency alters lymphocyte membrane protein and lipid composition and decreases lymphocyte reactivity and division, at least in laboratory rodents (Davis et al., 1987; Korte and Prohaska, 1987; Lukasewycz et al., 1987). Some sex-related variability in response has, however, been noted, at least for copper deficiency (Fields et al., 1987) and for Con-A stimulated spleen lymphoid cells (Kramer et al., 1987). Deficiency is reported to affect thymocyte activity in mice (Lukasewycz and Prohaska, 1987)

Cell response to deficient and excess concentrations of copper are widely distributed, not limited to erythrocytes and lymphocytes (e.g. Vacario et al., 1987). Merchant and Bogorad (1987b) found that copper deficiency in cells of the green alga *Chlamydomonas reinhardtii* was necessary for expression of the single nuclear gene for a cytochrome, c-552. Santoro (1986) reports cation-dependent mechanism for adhesion of blood platelets to collagen. Copper was one of the cations. (See also Rana and Prakash, 1986.) It is also one of the metal ions that, in excess, can alter lipid metabolism in human neutrophils (Turner et al., 1986). Lei et al. (1987) and Hassel et al. (1987) report data that suggest the hypercholesterolemia and hyperlipoproteinemia observed in copper deficiency may be a result of a change in binding capability of of certain, but not all lipoproteins (e.g. Carr et al., 1987) by liver membrane cells. Dietary copper deficiency has also been reported to reduce the amounts of linoleic and arachidonic acids and increase that of docosahexaenoic acid in both mitochondrial and microsomal membranes of rat liver cells (Balevska et al., 1985). Copper deficiency is also associated with a degeneration of pancreatic acinar cells although some, limited regeneration is reported with supplemental dietary copper (Rao et al., 1987). There is a suggested relationship between copper and nucleoproteins (e.g. Bryan et al., 1985) and copper-related changes in DNA cleavage have been reported (Ehrenfeld et al, 1987). Excess copper, as ionic copper, reportedly produces DNA damage when introduced into ascite tumours (Patiashvili et al., 1987).

The relationship between the cardiovascular system and copper has been mentioned earlier but is also considered in this subsection because it is a "response" of the organism to copper. Diet is important and dietary copper deficiencies have been associated with hypercholesteremia and increased atherosclerosis (Huttunen and Virtamo, 1986; Pariza et al., 1986). Contradictory evidence is presented by Uza et al. (1985) who did not find the expected high Zn:Cu ratio in hyperlipoproteinemic patients with or without atherosclerosis. Tuomilehto et al. (1985) suggest that trace metals play only a minor role in hypertension although the role of copper is not clear (see Borgman, 1985; Schedl et al., 1986). Lukaski et al. (1987), for example, report evidence that low copper status results in elevated diastolic blood pressure during an isometric grip test. Klevay (1987) presents evidence that copper deficiency is an important factor in hypertension in laboratory rats. Copper deficiency is also associated with dilated ventricles and aortic lesions in rats (Bielenberg et al., 1986; King et al., 1986) and deterioration of cardiac morphology in weanling pigs, especially in diets containing fructose (Steele et al., 1987). (Fructose has also been demonstrated to alter cell concentrations of minerals by Beal et al., 1987.) Based on data from patients with aneurysmal and occlusive disease, Dubick et al. (1987) suggest that copper deficiency affects the lipid metabolism associated with aneurysms and occlusive disease of the aorta. Reiser et al. (1987) present evidence that dietary copper deficiency is associated with increased levels of cholesterol. Earlier data from laboratory rats supports this suggestion (e.g. Carville et al., 1986) although Klevay et al. (1987) report a reduction in HDL cholesterol levels in women given a low copper diet. Kramer et al. (1986) suggest the importance of a number of metals in both renal and cardiovascular damage. This, they infer, is due to the ability of metals such as copper, to inhibit the activity of Na-K-ATPase, an enzyme which affects membrane transport of sodium and potassium. Copper deficiency-related physiological stress (e.g. Schmidt and Hultcrantz, 1986) may also be contributing factors to cardiovascular problems.

Singh et al. (1985) report a rise in serum copper levels in patients with acute myocardial infarction. This, they suggest may be associated with collagen status, among other things, and infer that serum copper levels could be useful for both diagnosis and prognosis. (Low levels of arterial elastin have been reported with copper deficiency - e.g. Tinker et al., 1987.) Bialkoska et al. (1987) found elevated Zn:Cu ratios in the hair of survivors of myocardial infarction suggesting a reduced copper, or elevated zinc, condition in the patient.

Since copper participates in the formation of new blood vessels, it has been linked with the formation of a variety of human tumours (e.g. Greene et al., 1987; Zagzag and Brem, 1986). Organometallic complexes of copper are also suggested as possible carcinogenic-affecting factors (Huong et al., 1985; Ogawa et al., 1987) as are some of the relationships between copper and organics (e.g. Schaaper et al., 1987). Blank et al. (1986) reports zinc inhibition of copper toxicity in human hepatoblastoma cells indicating that metal-metal interactions can play a role in metal modulated cell growth whether growth is at normal or abnormal rates.

I.3.6 THE INTERACTION OF COPPER WITH ORGANICS

Biologically important interactions of copper and organics occur both inside and outside the body. Some of these interactions have already been discussed and others will be mentioned in the review of literature on metal speciation. The present section concerns literature which deals primarily with the associations of copper and organics that form biologically important agents. Some of this literature has been reviewed by Reedijk (1987) in an article on bioinorganic chemistryBrewer (1987) in an article on "Comparative Metabolism of Copper", and some in an overview of "Toxic Trace Metals and Trace-metal Binding Proteins in Marine Organisms: ..." by Brouwer et al. (1986) although the latter discusses essential copper-containing organics as trace metal carriers rather than as "toxic" agents. Wood et al. (1986) provide an interesting examination of "Chemical Species in Systems Under Stress" in the report of a symposium on "The Importance of Chemical Speciation in Environmental Processes" (Bernhard et al., eds., 1986). Petering and Antholine (1988) present an excellent review of the interactions of copper with organisms, from the standpoint of detrimental effects but concerning processes more than just effects.

Organocopper compounds have been formulated for the control of pest organisms (e.g. Thiolliere, 1985) and occur as components of economically important produced (e.g. Kintlerova and Ruzichkova, 1985; Mndzhoyan et al., 1985) as well as natural materials. Copper is, for example, capable of combining with blood pigments either in a detrimental fashion (e.g. Everaarts, 1986) or as an essential component, as in the blood pigment haemocyanin of some molluscs (Drexel et al., 1986; Rogener et al., 1987) and some crustaceans (Naich and Alikhan, 1987; Vijayakumari et al., 1987; Zatta, 1985).

Copper and organics are intimately linked in the physiology of all organisms (e.g. Merchant and Bogorad, 1987a). Neurocuprein, for example, is the major copper-containing protein found in the brain and has been related to the etiology of schizophrenia (Nalbandyan, 1986). The rate of biochemical reactions within the organism can be affected by the concentration of biologically available copper such that the metal may naturally, or unnaturally act as a controlling mechanism (e.g. Braestrup and Andersen, 1987). This is of important value in understanding the effect of certain drugs (Carson and Wasson, 1987; Ghose et al., 1986; Jande and Sharma, 1986; Kanoh and Maeda, 1987; Naveh et al., 1987a,b; Petersen, 1987; Rae et al., 1986; Rajan et al., 1987; Reiners et al., 1986; Roberts and Robinson, 1986; Samarskii et al., 1986; Soderberg et al., 1987). It also provides a basis for explaining some of the direct and indirect effects of copper deficiency (e.g. Prohaska et al., 1987) and excess (e.g. Gabrielsson et al., 1986). Changes in the chemistry of the metal and the organic or the organometallic compound dictate, at least to some degree, the nature of the association between the two (e.g. Bell et al., 1987; George et al., 1986; Goldstein and Czapski, 1987b; Sayenko et al., 1987; Takahashi et al., 1987b).

All of these relationships require access to copper in a form or state that is chemically suitable for reactions to occur. Bernhard and George (1986), for example, discuss the importance of chemical speciation in uptake, loss and toxicity of elements for marine organisms. Hsieh and Harris (1987) comment (introduction) that "... measurement of copper activity in solution is critically important when one addresses the effect of copper's catalytic effect." In a Ph.D. thesis, Buckley (1985) examines organic speciation of copper, zinc and lead in seawater. Copper bioavailability is of importance in relating metal load to metal impact, in such diverse media as pig feces (Izquierdo and Baken, 1986), water (Morrison, 1987), hydroponic solutions (Checkai et al., 1987a,b) and plant fluids (e.g. Mullins et al., 1986). The latter authors use computer programs to examine metal speciation in xylem and phloem exudates of plants and point out that amino acids are important metal-binding agents for the transport of metals within plants. Similar results have been obtained for the transport of copper in aqueous and animal fluids (e.g.

Berthon et al., 1986; Haider et al., 1986) and laboratory work demonstrates the effect of metal speciation on tissue uptake of copper (e.g. Barnea and Hartter, 1987). Phlorotannins are also metal binding agents in brown algae (Ragan and Glombitza, 1987), agents that not only are important to the producing organisms but which could affect metal bioavailability in aquatic enviroments.

Hsieh and Harris (1987) report that copper activity increases in the presence of sucrose which would increase its bioavailability. Glucose causes an increase in copper flux across the membrane of yeast cells (Martinez and Connelly, 1987. However, the type of dietary carbohydrate is known to affect copper uptake. Johnson and Gratzek (1986) found that (abstract) "the apparent absorption of copper, but not iron, was significantly lower when rats deficient in both copper and iron were fed sucrose rather than starch". Fructose is reported to specifically affect copper and selenium deficiencies (Smith et al., 1987). Disposition of copper within the organism is affected by drugs and metabolites that associate with the metal. Landis and Graves (1987) note a 27% increase in kidney copper in gerbils in response to caffeine feeding and Haydel et al. (1986) report an effect of caffeine on copper concentration in the rat brain.

Uptake of copper from environmental sources can be strongly affected by organics resulting from the degradation of plant and animal material or by metabolites produced by bacterial microfloras causing the degradation. These agents can mobilize metals from mineral sources (Munier-Lamy and Berthelin, 1987), can provide sources of metal complexes of biological importance (Vadasz and Vadasz, 1987), or can bind ionic metal (Senesi et al., 1987; Teasdale) and act as a metal buffering agent (Andrzejewski and Doregowska, 1986). Iwasaki et al. (1987) note humic substances cause a reduction in metal availability to Italian ryegrass and red clover. Hutchinson and Sprague (1987) found marked reduction in fish toxicity of Al/Zn/Cu mixtures by humic substances in fresh water. Recent literature on humic substances indicates a variable copper complexing capacity dependent upon the nature of the substance and the pH of the soil (Yamada et al., 1987a,b), which allows some modelling of natural materials (Blaser and Sposito, 1987). Work in sea water indicates that copper complexation by humic substances is rapid (Sun, 1985) which supports previous work on the importance of these organics to copper bioavailability.

In a discussion of copper bioavailability, Nor (1985) points out the hazards of using chemical stability constants for humic substances to indicate copper availability to plants. One of the problems is that the organism may take up some organometallic complexes, another is the potential for adsorption of various copper complexes. Thompson et al. (1986), for example, found reduction in apparent copper bioavailability in the presence of both a complexing agent (humic acid) and an adsorbent (cellulose fibers). This provides continuing support for the concept that the availability of copper in the environment is related to the concentration of metal complexing and adsorbing agents as well as the concentration and speciation of the metal (e.g. Kyle, 1987; Mackey et al., 1987; Sunda, 1987; van den Berg and Rebello, 1986; van den Berg et al., 1987). The impact of the available metal is, however, controlled by the tolerance of the organism which is often a result of organic complexing agents produced either naturally or in response to exposure to high levels of metal (e.g. Baker and Czarnecki-Maulden, 1987; Krotz and Wagner, 1987; Palma et al., 1987; Tukendorf, 1987). Sutter and Jones (1985) discuss some of the mechanisms used by copper tolerant wood degrading fungi. Robinson and Thurman (1986) isolated a copper complex from roots of the copper-tolerant plant *Mimulus guttatus*. A wide variety of natural and synthetic organic complexing agents affect metal bioavailability (e.g. Clarke et al., 1987; Henze and Umland, 1987a; Lag and Helgeland, 1987; Mench et al., 1987; Suhayda and Haug, 1987). Many are organics produced and used routinely by the organism while others are either metabolites that affect metal availability to other organisms (e.g. Gadd and Edwards, 1986; Jones and Wilson, 1986; Purvis et al., 1987; Thomas and Robinson, 1987b) or anthropogenic materials such as some of the components of sewage (e.g. Buckley and

Yoshida, 1987; Frimmel and Geywitz, 1987b). (Some of these are agents useful to industry (e.g. Frimmel and Geywitz, 1987; Spiess et al., 1987).) Nutrients and food may provide some of these agents or affect their production and thus the tolerance of the cell or organism (e.g. Erardi and Falkinham, 1987; Reese and Wagner, 1987; Stewart and Olson, 1987).

Pectin and pectin-like compounds are reported to be copper complexing agents in certain foods (Khan et al., 1987; Kohn et al., 1986a). Phytate, and phytic acid, and fiber affect copper metabolism (Astuti et al., 1987; Champagne and Hinojosa, 1987; Farrahi-Aschtiani et al., 1987; Honig and Wolf, 1987; Reddy and Pierson, 1987; Umoren et al., 1987). Casein, is another food component capable of complexing copper and affecting its uptake and metabolism (Mannino et al., 1987). Food supplementation with metal chelates is used as a mechanism to introduce metals to livestock (e.g. Gorobets, 1986). Simple carbohydrates are reported to reduce copper uptake in humans under certain conditions (Hallfrisch et al., 1987). The relationships between food materials, metal availability and organism physiology are not simple (e.g. Naveh et al., 1987b). Platt and Clydesdale (1987), for example, found an interaction of gastrointestinal pH, phytic acid solubility, iron and copper that affected the chemistry of the iron-phytic acid relationship, amongst other things. In examining the availability of copper, zinc and selenium from fishery products, Luten et al. (1987) comment (page 518) that "the in-vitro availability of zinc, copper and selenium in fishery products depends on the type of fishery product and digestion conditions".

A wide variety of organics directly or indirectly affect the distribution and role of copper when it occurs at natural levels. The role of copper in estrogen action may, for example, be affected by an endogenous substance found in the rat uterine cytosol (Fishman and Fishman, 1987). This substance has also been reported from human breast tissue and breast tumours. Long-term ethanol ingestion has been reported to decrease the concentration of a natural metal complexing agent in the liver but not the kidneys of rats (Hopf et al., 1986). Cremades et al. (1986) examined the effect of bacterial endotoxin on serum iron, zinc and copper levels in the rabbit. Copper levels were elevated although the authors question whether this is a direct or indirect effect of the endotoxin. Synthetic metal complexing agents have also been used to control copper bioavailability in commercially important organisms ranging from tomatoes grown in hydroponic solutions (Checkai et al., 1987a) to silkworm larvae (Masui et al., 1986). They have also been used to treat humans with high levels of metal and laboratory animals to examine metal pathways (e.g. Jasim et al., 1985; Jasim, 1986; Szerdahelyi and Kasa, 1987). Aono and Araki (1986) discuss the body burden of chelatable lead, zinc and copper in metal workers, after injection of calcium form of disodium ethylenediamene tetraacetate (CaEDTA). They found that little of the "unchelatable" copper was transformed to chelatable forms over a 24-hour period. Thomas and Chisolm (1986) report similar results in the EDTA treatment of lead-poisoned children. Jacobs et al. (1987), however, note that in cow's milk, the addition of EDTA increased the soluble zinc and copper.

Tolerance to high levels of metal occurs in many organisms. The mechanisms that allow this can be related to growth responses or metal complexing agents produced and/or accumulated within the organism (e.g. Foster and Tessmer, 1987). (They may also be associated with metal-metal realtionships (e.g. Schilsky et al., 1987.) In the tobacco plant *Nicotiana plumbaginifolia* Kishinami and Widholm (1987) obtained evidence suggesting that resistance to copper and zinc was achieved by accumulating citrate and malate. Amino acids are able to reduce copper toxicity to the crustacean *Daphnia magna* (Khangarot et al., 1987b).

Metallothionein and metallothionein-like organic compounds are extensively-studied agents involved in trace metal transport in a wide variety of organisms. Recent reviews of the nature and function of metallothionein are found in Bremner (1987), Hamer (1986), Heilmaier (1984) and Heilmaier et al. (1987b). Bremner (1987) reviews the role of metallothionein in the hepatic metabolism of copper and, in an abstract of a talk, Summer et al. (1986) summarize the significance of metallothionein for the kinetics of metals in the organism. In comparing human and rat levels of metallothionein, Heilmaier et al. (1987a) comment (abstract) that "in most tissues human MT levels were high as compared to rats; particularly in liver and kidney cortex human MT levels exceeded those of rats about 25- and 10-fold respectively". Bremner and Morrison (1986) discuss the assay of extracellular metallothionein levels as an indication of zinc, copper and cadmium status in animals and Lobel and Payne (1987) report the interference of copper and other agents in the mercury-203 method for evaluating metallothioneins (see also Heilmaier, 1986).

When reviewing the plant and animal kingdoms, it becomes apparent that metallothionein and metallothionein-like agents are widespread. Grill et al. (1987) discuss phytochelatins in plants - small, cysteine-rich peptides functionally analogous to metallothioneins. Copper-metallothionein is reported in the fungus *Neurospora crassa* (Germann and Lerch, 1987) and the yeast *Saccharomyces cerevisiae* (Thiele and Hamer, 1987; Winge et al., 1987). In the mussel *Mytilus edulis*, Roesijadi (1986, 1987) reports a metallothionein-like organic at levels which increase in response to excess mercury, cadmium or copper. Regulation and storage of copper in the terrestrial isopod ("pill bug") *Armadillidium vulgare* (Witkus et al., 1987) and the blue crab *Callinectes sapidus* (Engel, 1987; Engel and Brouwer, 1987) may be associated with a metallothionein-like orgnic. Other crustaceans, such as barnacles (Rainbow, 1987) also store metal in granules or "bodies" with a chemistry suggesting an origin from metallothionein-like organics. Acey et al. (1987) present evidence for changes in a metallothionein-like agent during the growth of brine shrimp (*Artemia salina*) and Engel (1987) report data supporting the contention that metallothioneins are involved in the synthesis of the blood pigment hemocyanin in the blue crab. Capasso et al. (1987) suggest that the metallothionein content changes during the embryonic development of sea urchins.

In fish, various forms of metallothionein are important metal-regulating agents (e.g. Suzuki et al., 1987). Copper-rich granules have been reported in rainbow trout reared on copper-rich food (Lanno et al., 1987) suggesting a mechanism for excretion of excess metal which may use metallothionein-like agents. These agents are also important in the metabolism of several metals in both domestic and laboratory animals (Blalock et al., 1987; Bremner et al., 1987; Dielhof et al., 1987; Elinder et al., 1987; Gallant and Cherian, 1986; Klasing et al., 1987; Srai et al., 1986a) which has sponsored work on mechanisms of operation and induction (e.g. Foster et al., 1987). The widespread use of metallothionein-like agents in plants and animals is an indication of their widespread use in the metabolism of copper and other metals. Lefebvre et al. (1987) note that a mammalian metallothionein could function in plants which supports the concept of metal regulation by similar agents in diverse groups of organisms. Changes in the nature or concentration of these agents have been found in humans under conditions of physiological stress (Ebadi, 1987; Haratake et al., 1987a) suggesting their importance in normal metabolism. However, other changes occurring in copper uptake and metabolism during physiological stress (e.g. Sokol, 1987; Sokol et al., 1987; Tumanova and Nalbandyan, 1986) suggest that the changes in metal metabolism may be a result rather than a cause of the stress.

Trace metals such as copper most frequently are found in enzymes where they play key roles in both structure and function (e.g. Chatfield and Armstrong, 1987; Kondo and Mori, 1981; Shinohara and Terada, 1987; Skiba and Mullin, 1987; Zumft et al., 1987b). The reactivity of metals in enzymes is a result of the interplay between the physiology of the organism, the chemistry of the environment and the chemistry of the metal and organic that forms the enzyme (Agarwal and Henkin, 1987; Clain et al., 1986; Khandelwal et al., 1987; Kim et al., 1987; Shavlakadze et al., 1987; Zeppezauer and Maret, 1986). Delhaize et al. (1986) note biosynthesis of diamine oxidase in clover only in young leaves and only in the presence of an adequate supply of copper. Copper is essential for the activity of this enzyme, the apoenzyme being reactivated specifically by copper (Delhaize and Webb, 1987). The effect of metal-metal interactions and excess metal must also be considered (e.g. Asokan et al., 1985; Fujimoto et al., 1987; Fujita et al., 1987; Johnson and Murphy, 1987; Kelleher and Ivan, 1987; Kono et al., 1987; Lee et al., 1985a,b,c; Palumbo et al., 1987; Russanov et al., 1986; Scheuhammer, 1987; Stiborova et al., 1986). van Hooydonk et al. (1986), for example, comment that zinc and copper markedly decrease the rate of enzyme reactions in the renneting of milk whereas calcium, barium and manganese do not. Other organics, such as metallothionein, may be involved in controlling the availability of copper for the production of enzymes within the organism (e.g. Huber and Lerch, 1987; Tarnawski et al., 1987). Even the nature of nutrients may be important (e.g. Fields et al., 1987). Babu et al. (1987) note that fructose attenuates humoral immunity in rats with moderate copper deficiency and Lewis et al. (1987a,b) report severe reductions in several enzymes in rats with a high fructose, low copper diet. Similar results are reported by Henderson and Johnson (1987).

Copper is involved in a number of oxidation processes (e.g. Buettner, 1986; Homer, 1986; Pyo et al., 1985; Uchida and Kawakishi, 1986). Many of these are mediated or moderated by enzymes (e.g.Coleman and Taylor, 1980; Lovstad, 1987) or metal complexing agents (e.g. Nishikimi and Ozawa, 1987). In the introduction to his 1987 paper, Lovstad points out that "copper ions catalyze the oxidation of ascorbate. During reactions with ascorbate, the cupric ions are reduced to cuprous ions, which react with molecular oxygen and produce superoxide radicals. The latter can, by dismutation, form hydrogen peroxide, which may generate the harmful hydroxyl radicals interacting with superoxide radicals or with cuprous ions." An indication of the importance of this is the report by Fong et al. (1987) that ascorbate oxidation in ocular tissues can be inhibited by the addition of a chelating agent (ethylenediaminetetraacetic acid, EDTA). Several recent publications discuss the various factors which influence whether metal complexes protect or enhance the effect of superoxide radicals on biological systems (Agostini et al., 1986; Aust et al., 1985; Czapski and Goldstein, 1986b; Liochev et al., 1987). A number of publications deal with the chemistry of copper-zinc superoxide dismutase and ceruloplasmin, or analogues of these two (Asano and Hokari, 1986; Barra et al., 1986; Czapski and Goldstein, 1986a; Flohe et al., 1986; Gerdin et al., 1986; Goldstein and Czapski, 1986; Gutteridge, 1986; Hallewell et al., 1986; Jewett, 1986; Lu et al., 1985; Manohar and Balasubramanian, 1986; Marmocchi et al., 1986; Pickart et al., 1986; Plonka et al., 1986; Simonyan, 1986). A number of recent publications also deal with factors associated with SOD production (e.g. Delabar et al., 1987; DiSilvestro, 1987; Dubick et al., 1987; Groner et al., 1986b; Hartmann et al., 1987; Hass et al., 1987; Hass and Massaro, 1987a,b; Hassan and Lee, 1986; Jeon et al., 1986; Parker et al., 1986; Steinman, 1987).

The importance of superoxide and superoxide dismutase is indicated in the Proceedings of the 4th International Conference on Superoxide and Superoxide Dismutase (Rotilio, 1986). Dameron and Harris (1987) point out the usefulness of copper-zinc superoxide dismutase levels in assessing dietary responses to copper at the cellular level. Changes occur in the concentrations of superoxide dismutase as a result of physiological malfunctions of the body. Bartoli et al. (1985) present evidence of a relationship between the lack of defensive enzymes such as SOD and the presence of tumour cells. However, Abella et al. (1987) did not find a relationship between the occurrence of rheumatoid polyarthritis and the concentration of SOD. Kamei et al. (1985) found an increase in SOD levels in primary diabetes patients exhibiting signs of poor diabetic control. In contrast, reduced SOD activity has been reported for rats with experimentally-produced diabetes (Simonyan et al., 1987). The difference in results may be due to changes which occur in the nature of the enzyme as a result of the disease (e.g. Arai et al., 1987). Jardim et al. (1986) report the production of superoxide ions in natural and polluted water bodies through the action of cupric ions, and discuss the possibility that this forms a mechanism responsible for copper toxicity. Superoxide is also used in the reduction of ferricyanide; Simonyan and Nalbandyan (1986) note accelerated reduction of metals in the compound by superoxide radicals.

Caeruloplasmin is a copper-containing organic that is important in the oxidation of ferrous iron (Kurdowska and Bereta, 1985) and, like SOD, may play an important role as a scavenger of oxygen free radicals (Agostoni et al., 1986). It is also used as an indicator of the copper status of animals (e.g. Klein et al., 1985) and is involved with a number of organics and responds to a variety of metals (e.g. Sugawara and Sugawara, 1987). Copper and ceruloplasmin levels have also been shown to be regulated by dietary copper, vitamins and metals (Astrup and Lyso, 1985; Barber and Cousins, 1987; Kawamura and Hamada, 1985; Milne et al., 1987). (Vitamin A is suggested to influence copper metabolism, possibly by a decrease in secretion of transport proteins by the liver (Sklan et al., 1987).)

Copper is frequently involved in lipid peroxidation, the oxidative decomposiiton of lipids (Kadiiska et al., 1986; Sunderman, 1986). The involvement can be affected by a number of natural and synthetic agents, many of which are able to complex the copper (e.g. Baker et al., 1987; Bratkowska and Zwierzykowski, 1986; Lu and Baker, 1987). The effect of oxidation can be both detrimental as well as beneficial. Preservation of lipid-containing food materials is often limited by copper-related oxidation. Lipid peroxidation can affect membranes (e.g. Cornatzer et al., 1986) and may thus be a factor in certain diseases (e.g. Dreith et al., 1987). Copper, in association with organics, has been linked to effects on several diseases. Andronescu et al. (1987a,b) found that copper chlorophyllin caused a reduction in blood sugar levels in normal and experimentally induced diabetic rats. (Recant et al. (1986) note that copper deficiency increases insulin in rats.) Cloez et al. (1987) note an unexpected augmentation of lipid synthesis by copper, manganese and nickel, in the sciatic nerve of the trembler mutant mouse. Human brain gangliosides have been suggested to be involved in copper complexation and have the ability to modulate the activity of some copper-sensitive kinase-phosphate systems in peripheral nerve membranes (Yates et al., 1987). The metal also is found in certain compounds important to neurotransmission, at least one of which (dopa) is used as a drug in the treatment of Parkinson's disease (Emanual and Bhattacharya, 1987). Copper is also able to decrease the number of receptors in the bovine adrenal medulla (Yamanaka et al., 1987). It is involved in the formation of connective tissue and can affect the morphology of muscle filaments (Stromer et al., 1987).

I.3.7 THE EFFECTS OF COPPER ON GROWTH

The development of an organism can be affected by copper, whether the organism is a plant or an animal. With copper deficiency, growth is irregular or incomplete. With excess bioavailable copper, growth either does not occur or is irregular. There is frequently a stage in growth where the organism exhibits an enhanced sensitivity to either a deficiency or excess in copper. Although not well understood, this is probably a result of critical changes in the physiology of the organism which, for example, can occur when an organism starts to feed.

For fouling organisms, a critical period occurs during the formation of a biofilm. Ford et al. (1987), however, report that in an Arctic river, biofilm formation did not occur on copper alloy surfaces, even with copper resistant bacterial populations with nutrient enrichment. This suggests some controlling environmental factor. Growth inhibition of a red tide flagellate is reported by Nakamura et al. (1986). They suggest that cupric ion activity in the Seto Inland Sea is adequate to control natural growth of the organism in the absence of organic complexation of ionic copper. Metal-metal interactions are important to the growth of plants as well as animals. With the macroalga *Fucus vesiculosus*, Munda and Hudnik (1986) report that manganese and cobalt could reduce the detrimental effects of 2.5 ppm copper on growth. Synergistic detrimental effects of copper and zinc have been reported with rice plant growth (Hino et al., 1987a) but, intriguingly, not with elongation of rice plant roots (Hino et al., 1987b). Mattoo et al. (1987) report that high levels of copper sulfate (2 X 10^{-4} M) cause senescence in an aquatic angiosperm.

Metal bioavailability is a critical factor that is often difficult to measure. It is important to the growth and development of both plants and animals because it dictates how much of the total metal that is present can actually be taken up by the organism and enter into its metabolism. Gough (1984), for example, discusses soil metal availability and plant uptake and the problems of using soil extraction techniques to estimate availability. Korcak (1986) comments on the effect of soil quality in controlling leaf metal levels and growth in blueberry plants. Copper uptake also changes during growth, often on a seasonal basis as shown by Smith et al. (1987) for kiwifruit vines. The effect of copper on plant growth is varied and often dictated by the sensitivity of the plant (e.g. Taureau, 1985) as well as the growth-regulating ability of the metal complex (e.g. Kokhanovich et al., 1985) as well as by metal availability (e.g. Lasztity, 1987b). Copper has, for example, been reported to stimulates RNA synthesis in germinating wheat seeds (Khan and Fizza, 1986) while pollen germination and pollen tube growth can be reduced by excess copper in other plants (Gopal et al., 1986).

Excess copper has been demonstrated to reduce or terminate growth in animals ranging from worms to sheep. Bielecki (1985) notes a reduced viability of the infective stage of the liver fluke *Fasciola hepatica*. Cupric chloride causes reduced survival of larvae and reduced settlement in the rock oyster *Saccostrea commercialis* at concentrations of 3.14 X 10^{-3} mM (Nell and Holliday, 1986). However, the use of a copper oxide antifouling paint did not affect the viability of embryos or larval and adult growth in the oyster *Crassostrea gigas* although it did reduce the "condition factor" of the adult (His and Robert, 1987). Varying tolerance to copper has been shown by the growth of other molluscs (e.g. Beaumont et al., 1987) suggesting a species-specific growth response to excess bioavailable copper. Phelps and Hetzel (1987) also demonstrated a response control by the general suitability of the environment, with respect to oyster size, age, and copper and zinc accumulation. In an evaluation of chronic toxicity tests, with the cladoceran *Daphnia pulex* and excess copper, Meyer et al. (1987) point out the change in sensitivity during development and the variability exhibited between different types of responses. In crustaceans which have copper-containing blood pigment, metal regulation appears to be an important factor in molting and can involve metallothionein (Engel, 1987; Engel and Brouwer, 1987). Slight increases in copper have been shown to enhance fertilization in sea urchins (Pagano et al., 1986) and there is some evidence that metallothionein-controlled metal regulation increases during development (Capasso et al., 1987).

With economically important animals, there is continuing concern about the long term effects of low levels of anthropogenic materials (e.g. Susani, 1986). Teleost reproduction has, for example, been shown to be affected by sublethal concentrations of copper, as copper sulfate (Shakila and Wagh, 1985). Tolerance to copper has been shown to increase during the prelarva-larva-fry stages of development in coho salmon (Glubokov and Sokolova, 1986). Interaction of copper and agents such as antibiotics, in chicks, has been reported to produce little if any change in certain tissue copper concentrations (Henry et al., 1987).

Laboratory animals provide valuable information on the effects of copper deficiency and excess on animal as well as human reproduction and development. Copper deficiency has been shown to cause impaired sperm development and a decrease in motility in a genetic line of mice (Everett et al., 1986). In excess, as an aerosol, copper chloride inhalation has been found to have moderate gonadotoxic and allergy-inducing effects in rats (Ermachenko et al., 1987). In line with this, but with humans, Wu et al. (1986) report a negative relationship between sperm motility and copper concentration in the seminal plasma. Copper may also play a role in ovulation, at least in rabbits (Erokhin and Rastimeshin, 1985) although the results are difficult to interpret.

Trace metal adequacy during pregnancy and early life is discussed in a number of recent references on both laboratory animals and humans. Mas et al. (1985), working with rats, report that copper levels increased slightly at midpregnancy but later returned to near normal levels. Even with exposure to excess metal, there appears to be an ability of the pregnant dam to maintain copper homeostasis (Tsuchiya et al., 1987) although this may be affected by factors such as long term exposure to ethanol (Hopf et al., 1986). During lactation, the laboratory rat appears able to acquire, mobilize and provide the excess copper required by the offspring, at least under ideal conditions (Segues et al., 1987). Trace metals are important to the developing organism, for enzymes as well as structural proteins (e.g. Cloez and Bourre, 1987; Donzelli et al., 1987; Hass and Massaro, 1987a). Copper is, for example, important for growth and maturation of the nervous system (e.g. Prohaska, 1987; Sandstead, 1986). Copper depletion and repletion during development may also play a role in cell differentiation, both in normal (Scarpelli and Reddy, 1985) and abnormal (De Pauw-Gillet et al., 1985) cell types. Irregular metabolism of copper, as for example in Wilson's disease, will affect normal development (e.g. Srai et al., 1986b).

In humans, there is some change in copper status during pregnancy (Pleban et al., 1985; Yamashita et al., 1985). However, there appears to be little relationship between neonatal birth weight and copper levels in maternal and cord blood (Ferrari et al., 1985). Copper levels also change in breast-milk, Kirsten et al. (1985b) reporting that the low foremilk copper levels found at 3 days of lactation gradually declined to very low levels after 36 weeks of lactation. This is associated with a decrease in copper uptake by breast-fed infants (Butte et al., 1987) which may or may not be associated with changes in infant copper levels (e.g. Kirsten et al., 1985a; Miyasaki et al., 1987). However, several authors (Dahlstrom et al., 1986; Oroszlan and Szabo, 1987; Shulman, 1987) point out the abnormal trace metal changes that occur as a result of physiological stress.

I.3.**8** THE EFFECT OF COPPER ON BEHAVIOUR

When one realizes that copper is essential for life processes (e.g. Caple and McDonald, 1983; Kieffer, 1984; Strause and Saltman, 1984) and that deficiencies produce physiological irregularities, it is easy to see how irregularities in copper can affect behaviour. Halas and Eberhardt (1987) provide "a behavioral review of trace element deficiencies in animals and humans" which relates the physiological and biochemical effects of copper deficiencies and irregularities to behaviour.

A decline in activity has been related to increases in copper in the intertidal gastropod mollusc *Polinices incei* (Kitching et al., 1987). However, Leland (1985) found evidence of an increase in activity with an elevated level of copper, in a freshwater drift insect. Levin (1986) discusses some motor reactions of starfishes to copper sulfate. The results indicate a response although it is difficult to relate the nature of the response to the effect of copper. Atchinson et al. (1987) review the effects of metals on fish behaviour, commenting that although effects have been noted, (abstract) "no behavioral tests have been standardized and few have been verified in the field". One of the authors of this review found a series of behavioural changes in bluegills exposed to copper, in cough, yawn and fin-flick frequencies (Henry and Atchison, 1986). Hartwell et al. (1987a,b) evaluated avoidance responses of fathead minnows, noting acclimation with reduced avoidance under both laboratory and "field" conditions. With the freshwater perch *Perca fluviatilis*, excess copper reduces growth which is associated with decreased swimming activity (Collvin, 1985). It is also associated with an increase in prey-finding time at sub-optimal prey densities.

Health and behaviour in laboratory animals and humans have been related to trace metal status. The presence of physiological disorders, such as malnutrition, anemia (e.g. Johnson and Kramer, 1987a), and protracted diarrhea directly or indirectly affect behaviour. The effect of copper deficiency in producing irregularities of connective tissue (e.g. Tinker et al., 1987; Williams et al., 1987) will also affect behaviour although indirectly. The identification of some of these disorders has been made on the basis of trace metal levels (e.g. Coello-Ramirez et al., 1985). Delves (1985), however, points out the variability in levels and suggests that plasma copper concentrations can only be of use in the diagnosis of copper-related disorders when compared to a matched control group. Activity levels in rats have been related to prolonged sub-clinical trace metal deficiencies and are suggested to compare favourably with aged or brain-damaged rats (Longenecker et al., 1987). Dietary copper deficiency has also ben related to marginal short-term taste depression in rats (Greeley and Gniecko, 1986). Trace metals have been inferred as a possible causative factor in hypteractivity although Barlow and Sidani (1986) did not find evidence of an effect from copper. Hypertension is reported to induce alterations in copper and zinc metabolism in rats, a factor which Clegg et al. (1987) suggest may be an underlying factor for tissue damage. It is known that abnormal copper metabolism is able to affect both physiology and behaviour (Danks, 1985; Pleban et al., 1985; Sandstead, 1986). In an interesting article, Chapman (1987) discusses the effect of copper deficiency in connective tissue in the light of a court decision to not allow copper deficiency to be used as a defense argument for a child abuse. The author points out the dissimilarities of an abused versus a copper-deficient child and notes the relatively few cases where copper deficiency is a serious problem.

I.3.9 THE EFFECTS OF COPPER ON COMMUNITIES

The response of a community of organisms is a result of the direct and indirect response of individual organisms to a situation. With both deficient and excess copper, the response is either indirect, for example on the food web (Amiard-Triquet et al., 1987b; Tamm and Andersson, 1985), or direct, on the organisms themselves. As pointed out by Rice and Whitlow (1985) and others, however, the response of a community is not to the total concentration of one or more metals but rather to the concentration of the biologically available fraction. In this sense, then, the community as well as the component species can provide a mechanism to estimate biological impact of excess metal (e.g. Burton et al., 1987; Cairns, 1986; Dean-Ross, 1987; Leland and Carter, 1986; Mudroch and Rao, 1987; Munawar et al., 1985; Nakamura et al., 1986; Oliveira et al., 1985; Wickham et al., 1987). Bryan et al. (1987) comment (abstract) that "evidence of long-term metal pollution in the Fal Estuary (England) is provided by analyses of Cu in oyster (*Ostrea edulis*) communities extending over more than 120 yr and by analyses of Cu and Zn in sediments dating back to 1921".

The species composition of plant communities are controlled by a variety of factors, including metal bioavailability. Farago et al. (19870, for example, comment that a copper bog in North Wales is "occupied by anomalous plant communities characterised by metal indicator species; ...". Certain plant types are thus useful as indicators of the availability of copper, whether in the soil or as an aerosol, from industry (e.g. Onianwa and Ajayi, 1987). They are often useful to indicate interactions. Soybeans, for example, require copper (Payne and Martens, 1986) and yet Pacofsky (1986) found higher copper levels in soybeans innoculated with ectomycorrhizal fungi than in controls, without the fungi. The author points out (page 384) that fungal "... colonization is advantageous to plant growth by providing immobile limiting nutrients, such as P, Zn, and Cu ...". Fungal associations may also affect metal bioavailability in polluted soils (Jones et al., 1986). However, the relationship between copper uptake by plants, in the presence or absence of ectomycorrhizal fungi, is not well understood and difficult to separate from the effect of soil quality (Sidle and Shaw, 1987).

Aquatic communities include those that occur on solid substrates such as pilings and boat hulls. The composition of these communities is affected by the use of antifouling compounds such as copper. These agents tend to select for copper-tolerant bacterial and algal species (Callow, 1986; French and Evans, 1986; Pyne et al., 1986) although they may also be selected by high levels of metals in the water (e.g. Lucey et al., 1986). However, this may not always be the case. Ford et al. (1987) noted the failure of copper-resistant bacterial species to grow on copper alloy surfaces in an Arctic river, even after nutrient enrichment. Copper can affect planktonic as well as benthic community structure (Kaitala and Maximov, 1986; Sheehan et al., 1986) although the effect varies. The role of metal complexation, whether in benthic or planktonic communities, is important (e.g. Jessie and Smith, 1987). Comparing oceanic and inshore waters, Sunda (1987) notes less effect of copper in inshore water, in part as a result of presumed higher complexing ability. The nature of the community is also important in evaluating metal impact. Munawar et al. (1987) report greater impact by metals, on smaller organisms. This may be, in part, a result of the higher surface area to volume ratio in smaller organisms. Deniseger et al. (1986) report the continued dominance of a metal-tolerant phytoplankton species (*Rhizosolenia eriensis*) in a lake, even after metal input was reduced. This is attributed to the competitive advantage achieved during the period of excess metal. Doelman (1986) notes a shift to metal-resistant bacterial strains in metal-polluted soils.

Effects of aerosol metals from industrial sources is to increase the soil metal concentrations. With acid rain, this is associated with a reduced pH and a greater mobility and bioavailability of soil metals. These are detectable by the species composition of affected forests (e.g. Evans and Bell, 1987; Vaisanen, 1986) as well as by the soil and plant metal concentrations. Nohrstedt (1987), however, found no statistically significant effect on forest floor respiration although this could be a result of a shift towards metal tolerant microorganisms in the sense of Doelman (1986).

Changes occur in animal communities as a result of deficient or excess levels of copper (e.g. Bonacina et al., 1986). In an examination of drift response of aquatic insects to copper, Leland (1985) found that introduced copper produced declines in densities of most, but not all insects with drifting life history stages. Energy reserves of three lake zooplankton species have been related to metal concentrations, lower at higher levels of metal (Arts and Sprules, 1987). Zooplankton and fish metal concentrations are reported to be related to metal concentrations in a lake receiving mining effluent (Nordin et al., 1986). Associations between animals can also affect metal concentrations, as it may between plants and fungi. Chassard-Bouchaud et al. (1985, 1986) report an increase in the concentration of metals in the crab *Carcinus maenas* when it is parasitized by the barnacle *Sacculina carcini*. Plasma copper concentration in coccidiosis-infected chicks is reportedly higher than in uninfected controls (Turk, 1986). The same is true for liver copper in infected chicks fed excess copper (Fox et al., 1987). Splenic copper was higher in chicks infected with *Escherichia coli* than in controls (Nockels et al., 1987). However, at least in sheep, copper deficiency is exacerbated by parasite infestation (Hucker and Yong, 1986). As suggested by laboratory rats infected by a trypanosome, copper deficiency can reduce the antibody-forming ability of the host (Crocker and Lee, 1986). As a final comment on the interactions of organisms, the presence of the copper-containing enzyme superoxide dismutase in a bacterium that is symbiotic with a fish has been suggested to be a result of gene transfer from the fish to the bacterium. Leunissen and de Jong (1986), however, suggest this to be unlikely.

I.3.10 COPPER, NUTRITION AND FOOD CHAIN EFFECTS

Copper is an essential trace metal, required for life in plants, animals and humans (Fishbein, 1987; Kieffer, 1984; Prasad, 1983). It is obtained either directly from the environment or in food. Although deficiencies are not common (e.g. Strause and Saltman, 1984), they do occur frequently enough to be a problem and have sponsored a number of studies on dietary requirements for copper, primarily in humans and in laboratory animals (e.g. Ovecka et al., 1987; Vandenhaute et al., 1987) where application to human problems is possible. They also include the evaluation of metal levels in food organisms (Dallinger et al., 1987; Perdicaro and Sequi, 1984; Satoh et al., 1987). These studies include the examination of physiological responses to both copper deficiency and excess. They also include evaluations of metal bioavailability as it relates to uptake and copper metabolism (Massey et al., 1986; Mills, 1986; Robb et al., 1986a).

Evaluation of metal bioavailability is critical to an understanding of the role played by various food materials in providing an adequate supply of copper to the organism. This has led to the use of various chemical agents, such as metal complexing agents to maintain adequate levels of metal, in animal and human nutrition (e.g. Leporati, 1987). It has also evaluated the use of specific chemical agents in fertilizers (e.g. Gorlach and Gorlach, 1984) and the composition of food products (Kononko et al., 1986). A number of recent references discuss metal binding agents in foods, agents such as phytic acid which is found in plants (e.g. Honig et al., 1987; Nwokolo, 1987a). Others examine the uptake of metals by plants grown in sewage-ammended soils or sewage-enriched water (e.g. Wong and Chan, 1985).

High levels of copper in food have been shown to be accumulated by and detrimental to some freshwater organisms (Hatakeyama, 1986). Accumulation has also been demonstrated for houseflies bred in manure (Du Toit et al., 1987). Copper concentrations in flies bred in pig manure were twice as high as those in flies bred in chicken manure. In both of these studies, it is difficult to separate accumulation through food from accumulation by other uptake mechanisms. This is a factor in many studies of the effect of excess metal in environments affected by man. Pesch et al. (1986) point out some of the problems of isolating food metal effects, in an examination of the effect of diet on copper toxicity to a polychaete worm. Hunter et al. (1987a,b) studied copper and cadmium levels in invertebrates and small mammals in a contaminated grassland ecosystem. They demonstrated elevated concentrations of metal, control by some organisms over metal concentrations and, as a result, differences in metal concentrations between species. They comment (Summary, Hunter et al., 1987b) that "... copper was mobile through the soil-plant-invertebrate pathway but accumulation was effectively regulated in small mammals at all levels of dietary intake". Wong and Cheung (1986) found that catepillars of the white butterfly (*Pieris canidia*) had higher levels of metal (Pb, Cu, Zn) when fed plants grown on sewage sludge than when fed plants grown on animal manure. Accumulation of metals was lower with plants grown on pig manure than with plants grown on chicken manure. Chicken litter has been reported to cause copper toxicosis in cattle when used as a cattle feed, as a result of using copper sulfate in broiler chicken feed (Banton et al., 1987). The authors point out, however, that this can be minimized by sufficient supplementation with molybdenum and thiosulfate.

Prahalad and Seenayya (1986) examined copper and cadmium concentrations in food-chain components in an industrially polluted lake in India. They comment (abstract) that "the concentrations of these metals decreased with increasing trophic level: nanoplankton -> phytoplankton -> zooplankton -> fish, except for Cu in phytoplankton, illustrating no food chain enrichment in the classic sense ...". Hernandez et al. (1987) found no evidence of biomagnification of copper in a study of metal concentrations in the eggs of four species of wading birds. Szefer and Falandysz (1987), however, provide some evidence of accumulation from food organisms in scaup ducks. Baars et al. (1985, 1986) report that metal contamination of a salt marsh did not have any detrimental effect on the health of locally grazing sheep. This is possibly because of metal bioavailability, sheep grazing habits or the use made of the marsh. In a laboratory study of metal uptake by clams from mine tailings, McLeay et al. (1986) report uptake from both the water and the sediments, the latter at least during feeding. Excess metal bioavailability can increase tissue metal levels and may affect energy levels in organisms (e.g. Arts and Sprules, 1987). Seasonal changes may occur in metal uptake, or tissue metal levels, as a result of changes in feeding regime, diet or changes in organism physiology (e.g. Bain et al., 1986; Schryver et al., 1986; Staaland, 1985). Bain et al. (1986), for example, found a monthly variation in bovine serum copper levels which was correlated with rainfall, the higher the rainfall the lower the copper level.

Metal concentrations in humans and domestic animals can be affected by food composition. As a result, chemical analysis of food and organisms is important and provides information useful in determining metal accumulation values or the requirement for dietary changes (e.g. Fisher and Bates, 1986; Freudenberger et al., 1987; Harms and Buresh, 1987; Hapke, 1984a; Henry et al., 1987; Kalnitskii et al., 1986; Khan et al., 1987; Kirchgessner et al., 1986; Kuznetsov et al., 1985; Ludke et al., 1985; Piva et al., 1985, 1986; Plotnikoff et al., 1987; Richards et al., 1987; Rowan and Lawrence, 1986; Schone et al., 1986; Tait et al., 1986; Tejada et al., 1987; Youssef and Brathwaite, 1987). This is especially important with metal-binding food components (e.g. Samman and Roberts, 1987a) or in evaluating the effect of minerals and other metals (Schryver et al., 1987; Spears et al., 1986; Strickland et al., 1987; Weaver, 1984). Copper supplementation in sheep and cattle has become of major importance in areas of deficiency, with a number of techniques and supplements used (Ellis et al., 1987; Gooneratne et al., 1986c; Petersen et al., 1987; Suttle, 1987a,b).

The physiological condition of the organism is important. Inflammation is, for example, associated with an increase in a copper-containing enzyme (DiSilvestro, 1987). Several studies have demonstrated that coccidial infection of chicks is associated with an increase in some tissue copper concentrations (Fox et al., 1987; Turk, 1986). In fact, the use of copper in animal feeds is often to reduce the viability of bacteria and parasites (e.g. Hagen et al., 1987; Shurson, 1987).

Adequate diet as well as dietary copper levels are important for the maintenance of normal physiological condition. Lee et al. (1985) found that diet restriction caused an increase in liver copper in rats. Changes in mineral composition may be important to growth (e.g. Greger et al., 1987; Theall et al., 1987) although it most probably occurs in response to long term changes in mineral ratios rather than absolute values (e.g. Friel et al., 1987b; Storey and Greger, 1987; Vodicenska and Razbojnikova, 1987). Meal composition is important to livestock and laboratory animals. Some chemical constituents can be toxic when present in excess (e.g. Finot and furniss, 1986; Rao, 1987; Vermorel and Evrard, 1987). Others, such as fructose, may inhibit absorption of copper (Henderson and Johnson, 1987). Fruit-sugars and carbohydrates in general have been examined for their ability to affect copper uptake and metabolism (Babu et al., 1987; Johnson and Flagg, 1986; Johnson and Gratzek, 1986; Lewis et al., 1987a,b,c; Nutrition Foundation, 1986; Sable-Amplis et al., 1987; Smith et al., 1987; Suzuki et al., 1986; Wirth et al., 1987). Vitamin A transport from the liver to the blood may be linked with copper metabolism (Rachman et al., 1987). As well, vitamin B-6 supplementation has been associated with increased liver copper in rats (Son and Magee, 1987). Both suggest a possible interaction of vitamins and copper uptake and metabolism. Other food components examined include egg powder (Uehara et al., 1986) as well as milk (e.g. Greger et al., 1987). Metal complexing agents such as those found in coffee may affect copper uptake and mobilization (Homma et al., 1986). Landis and Graves (1987) found that chronic caffeine consumption may affect metal distribution in the gerbil, increasing kidney copper concentrations. Greger and Emery (1987) report that rats fed coffee had elevated liver copper levels. Coffee is reported to affect iron but not copper metabolism in lactating women (Munoz et al., 1987). Alcohol is also suggested to affect maternal mineral status (e.g. Hopf et al., 1986) although Derr et al. (1987) suggest that this may be, at least in part, a result of inadequate nutrition.

In humans, the uptake and mobilization of copper changes with age, sex, physiology and environmental conditions as well as the content and availability of nutrient copper (Takacs and Tatar, 1987). Pleban et al. (1985) note a rapid uptake of copper by the fetus during the last 4-6 weeks of gestation. "Preterm infants are at risk for copper and zinc depletion if sufficient quantities of these nutrients are not provided in a bioavailable form in postnatal life" (abstract, Tyrala, 1986). This stresses the importance of adequate copper in infants, whether on natural or formula feeding regimes (see Bratter et al., 1987). The World Health Organization provides a recommended daily minimum of 60 μg/kg although Salim et al. (1986) suggest that this is too high for artificially fed infants. Physiological stress during early life may affect copper concentrations although it will be dependent on the nature of the stress (e.g. Di Toro et al., 1987; Henrivaux et al., 1986; Stepnick-Gropper et al., 1987) as well as the chemical nature of the nutrient source (e.g. Dahlstrom et al., 1986). For teen-agers, Nagy (1987) gives an average daily intake of 1.23 mg copper with normal diets while Murphy and Calloway (1986) estimate daily intake to be 1.16 mg for young women between 18-24 years of age. Hill et al. (1987) give 1.0 mg/day for adult black Americans consuming self-selected diets. It should be noted that all of these levels are below the approximately 2 mg/day recommended daily minimum given by the World Health Organization. Trace metal levels in athletes have been monitored to evaluate the effect of physical activity and the requirements of normal as well as world class athletes (Deuster et al., 1986; McDonald and Saltman, 1986; Vorob'ev, 1983). Dietary studies have been used throughout the world to indicate daily average intake of nutrients such as copper (Evans and Sherlock, 1987; Wyttenbach et al., 1987) as well as nutrient levels in special meals (e.g. "Meals-on-Wheels", Bunker et al., 1986) or special groups (e.g. housebound elderly people, Bunker et al., 1987; institutionalized elderly, Dreosti et al., 1984; Thomas et al., 1986). In all of these, as well, daily intake of copper is less than the WHO recommended daily minimum. Copper supplementation is also known to reduce or eliminate certain types of disorders (e.g. Ruocco et al., 1986). The question, of course, is whether these disorders would be as common with adequate daily intake of copper.

Nutritional quality of various foods has long been of interest (e.g. Ishimatsu, 1985; Luten et al., 1987; Nwokolo, 1987b), especially those grown on sewage sludge-amended soil (e.g. Muntau et al., 1987) or affected by other anthropogenic metal loading (Hagel, 1986; Nicola et al., 1987; Schreiber, 1986). The quality and preparation of food materials for human consumption can also affect metal levels (e.g. Albrecht et al., 1987; Amarowicz et al., 1986; Le Graet et al., 1986; Leland et al., 1987; Mazur and Lewandowska-Malinowska, 1986). (Incidentally, Flynn et al., 1987, note that copper deficiency affects the baking quality of wheat flour.) Metal intake also occurs from drinking water, as does intake of certain organics. This situation has sponsored a number of studies of water quality (e.g. Hargis and Associates, 1986; Reiber et al., 1987a,b; Robinson and Knab, 1987). References relating to heavy metals in drinking water are provided by the U.S. National Technical Information Service (1986a). Copper levels in beverages become important in evaluating total daily intake as well as beverage quality (e.g. Houdayer et al., 1987). (Louser and Sanderson, 1986, discuss removal of copper and iron from wine, using a complexing agent.) Darret et al. (1986) estimates 0.46 mg/day for copper intake in beverages by the adult in France. The intake and use of copper in foods such as wild mushrooms (Rohmer, 1984) and spices and medicinal plants (Jawad et al., 1986), although not major sources of metal, must be considered in examining total intake of nutrient copper. However, accuracy as well as variability in metal values is important (Aleshko-Ozhevsky et al., 1986) and should be considered in estimations of total intake. Iyengar et al. (1987), Miller-Ihli and Wolf (1986), and Wolf and Miller-Ihli (1987) characterize mixed diet reference materials of value in determining analytical accuracy. Kumpulainen and Paakki (1987) discuss an analytical quality control program for trace elements in foods and diets, which is used by the FAO

European cooperative network. Parr (1987) presents an international collaborative research programme on minor and trace elements in total diets.

Even with an accurate assesment of nutritional metal levels, metal bioavailability controls uptake and utilization. Several food components affect this. Phytate and fiber, for example, are known to affect copper uptake (Behall et al., 1987; Hallfrisch et al., 1987; Honig and Wolf, 1987; Kohn et al., 1986b; Laszlo, 1987; Martin and Evans, 1987; Moak et al., 1987; Platt and Clydesdale, 1987; Prather et al., 1987; Youssef et al., 1987). Changes in food pH can also affect metal solubility (e.g. Nelson et al., 1987) and bioavailability. Techniques to evaluate metal bioavailability in foods also have problems associated with their use. Radiolabelling, for example, requires that the radioisotope be as bioavailable as the stable isotope (e.g. Lonnerdal, 1987). Uptake of metal does not necessarily mean incorporation into the active metabolism, agents such as metallothionein may act as storage sites (e.g. Funk et al., 1987) as well as transport organics

I.3.11 ORGANISMS AS INDICATORS OF COPPER BIOAVAILABILITY

Organisms can acquire copper from the environment or food only when it is in a biologically available chemical state. This implies that organisms can be used to indicate metal bioavailability. There has been widespread application of this belief and a number of meetings and recent publications devoted in whole or in part to an examination of the problems associated with the use of biological assays of natural and anthropogenic materials. The U.S. Environmental Protection Agency (1980) hosted a seminar on the methods and uses of biomonitoring and its application to setting limits in permits. Herricks (1987) edited a volume on "The Effects of Contaminants on Ecological Systems" in which biomonitoring is a major consideration.

Various types of bioassays have been proposed. Sheehan et al. (1986) evaluated "simple generic aquatic ecosystem tests" to examine the ecological impacts of pesticides, including copper. They used laboratory microcosms containing a variety of orgnism types and report consistancy in ranking pesticide impacts on community metabolic activities. Kovacs and Podani (1986) review the use of plants as indicators of heavy metals while Ramelow et al. (1987) used periphton to monitor heavy metal pollution in a Louisiana estuary. In comparison to sediment metal values, periphyton on the sediments were not enriched in copper. Arrays of organisms have been used, to provide information of wider application than to just one species or group (e.g. Ross and Sloterdijk, 1986). Davis and George (1987) discuss the use of benthic invertebrates to indicate the effects of organics and metals in urban and motorway discharges. Laboratory microcosms are useful in predicting potential water quality in a proposed retention facility (e.g. Craft, 1985). Craft (1985) and Shannon et al. (1986), as well as others, point out that although useful, microcosms do not adequately simulate most real life situations, being affected by their small size and resultant problems with metal chemistry. Chemical indicators of metal bioavailability have also been used, metal-containing enzymes such as Cu-Zn-superoxide dismutase have been used to indicate metal deficiencies or excess (e.g. Dameron and Harris, 1987). Concentrations of tissue metal complexing agents such as calmodulin and metallothionein have been used to indicate excess bioavailable metal (Bremner and Morrison, 1986; Lobel and Payne, 1987; Williams et al., 1986).

In using bioassays it is often important to accurately measure tissue metal levels. To assist in this, the U.S. National Bureau of Standards (Rasberry, 1987) and the National Research Council of Canada (Berman and Sturgeon, 1987) have recently provided additional Standard Reference Materials. These include plant and animal as well as human tissues. In evaluating bioassay results it is essential to differentiate between normal background variability and the effect of metal deficiency or excess (e.g. Berthet et al., 1986; Schramel et al., 1984). Thus the importance of adequate and appropriate control organisms whether working with plants or with humans (e.g. Robins and Blevins, 1987). Jankovski et al. (1987b) point out some of the advantages and disadvantages of bioassay use. They present a good case for the value of envirnonmental measurements, including metal analysis, in place of bioassay measurements when estimating major inputs of anthropogenic metals.

King (1984) and Walker (1987) review a variety of microorganism bioassay procedures for analyzing various chemicals, including trace metals. Coziahr and Vidaver (1986), in a brief abstract, point out that "there has been little *in vitro* standardized testing of various chemicals (particularly copper compounds) for their effectiveness as bacterial disease control agents. They continue, to briefly describe a rapid bioassay for screening chemicals but do not give any results for their tests with the variety of agents tested. Slabbert describes an "improved bacterial growth test for rapid water toxicity screening" while Burton et al. (1987) used microbial enzyme activity to measure water and sediment quality of a stream in South Dakota. Yupina et al. (1986) used wood destroying fungi as bioindicators of heavy metals but restricted their work to nickel and lead concentrations. Cuomo et al. (1985, 1987) suggest the use of age pigments in fungi as a biological marker for excess biologically available metals in the sea. Lichens and mosses have been extensively used to estimate the impact of aerosol metals (Gailey and Lloyd, 1986; Gailey et al., 1985; Garty, 1987; Gignac, 1987; Glooschenko et al., 1986; Onianwa and Ajayi, 1987; Steinnes, 1984).

Various aquatic microalgae have been evaluated and used as estimators of the impact of heavy metals on natural systems (e.g. Lucey et al., 1986; Pfeiffer et al., 1986; Thursby and Steele, 1986; Vladimirov and Skulyari, 1986; Wallner et al., 1986). Munawar et al. (1985, 1987), for example, examined the differential sensitivity of natural phytoplankton size assemblages to metal mixture toxicity. They suggest that the technique is a suitable way of evaluating the impact of anthropogenic activity on lower levels of the food chain. Higgins and Mackey (1987), working with a kelp (*Ecklonia radiata*) report a relationship between oceanic residence times and metal concentration factors. However, they state that the "apparent free space" in the plant allows a rapid exchange to occur which makes the species (abstract) "... not generally useful as a sentinel accumulator species in pollution studies for assessing long term integrated changes of metals in the water column".

Terrestrial as well as marsh plant tissues have been used as indicators of copper deficiencies as well as excess copper (e.g. Peters and Maurer, 1985; Raunemaa et al., 1987). Moraghan (1985) comments that (summary) "the relationship between nutrient concentration and yield, when properly used, is a powerful tool for diagnosing the nutritional status of annual crops for B, Cu, Mn, Mo, Zn and occasionally Fe". Root elongation, in lettuce, cucumber and millet, has been used to assay the impact of excess metal (Wang, 1987) as has tissue metal concentration (e.g. Lyngby and Brix, 1987; Romero et al., 1987a). Frequently, soil metal extraction techniques are used to provide an indication of metal availability (e.g. Kaplunova and Bolshakov, 1987) which can then be compared with tissue metal uptake (e.g. Nemenko et al., 1987). These, combined with estimates of soil microorganism activity (e.g. Skujins et al., 1986), can provide an estimate of the interaction of soil chemical properties and anthropogenic metal input. Terrestrial plants have, however, also been used in metal prospecting, the presence of certain metal-tolerant plants being used as an indication of high levels of metal (e.g. Aery and Tiagi, 1986).

Invertebrate animals are widely used as bioassay organisms. In soils, metal concentrations in earthworms have been used to indicate metal availability (Beyer and Cromartie, 1987; Beyer et al., 1987; Marquenie et al., 1987; Neuhauser et al., 1985b). Several workers (e.g. Beyer and Cromartie, 1987; Stafford and McGrath, 1986) point out some of the problems, and solutions to the problems, of using earthworms, not the least of which is the retention of soil in the gut of the organisms. Polychaete worms are aquatic analogues of earthworms and have been used to evaluate metal problems, primarily in marine sediments (e.g.

Bryan and Gibbs, 1987). Howard (1984) points out some of the problems of using one species (*Nereis diversicolor*). Phelps (1986) found that burrowing speed of the clam *Mya arenaria* could be affected by copper but that this inhibition was reduced or lost within three days. Part (1987) reviews some of the bioassay techniques for evaluating metal bioavailability in marine environments and Farrington et al. (1987) discuss the "Mussel Watch" program which attempts to use bivalves, primarily marine mussels, as indicators of coastal environmental quality. The program is widely used in marine environments (e.g. de Kock, 1986; Feng, 1986; Kennedy, 1986; Morozov et al., 1986) and has been used in fresh water situations (e.g. Czarneski, 1987) although the latter publication does not consider copper. Oysters have also been used as assay organisms (Phelps and Hetzel, 1987; Talbot, 1986) as have the veliger larvae of certain bivalve molluscs (Beaumont et al., 1987). Metal bioavailability has also been examined with suites of organism species (e.g. Simmers et al., 1986), a technique which is far more indicative of natural conditions because it considers variability between species. Kitching et al. (1987) discuss the use of activity levels to indicate sublethal impacts of copper and salinity to the intertidal snail *Polinices incei*.

Carlson et al (1986a) used two crustaceans, *Ceriodaphnia dubia* and *Scapholeberis* sp. and the fathead minnow *Pimephales promelas* to calculate copper LC50 values for water from various sites. These were then used, with total copper, to estimate metal bioavailbility as well as toxicity. *Daphnia magna* continues to be used as a crustacean assay organism for fresh water (e.g. Khangarot et al., 1987a). de Nicola Giudici et al. (1987) examined the copper sensitivity of the life history stages of two isopod crustaceans. Storch (1986) discusses the use of animal cells as monitors for environmental factors that include heavy metals. The relationship between nutrition and metal effect is discussed in the brief description of the work. Cohen et al (1985), working with the sap sucking insect *Lygus hesperus*, found metal profiles that were age, diet, sex and location related, again showing the nutrition-metal relationship but in an insect. The bee has been used as a bioassay organism, for pesticides (Celli et al., 1985) and Jones (1987) discusses the value of honey as an indicator in mineral prospecting and environmental contamination studies. The author concludes that it is not a suitable indicator. Fertilization success in sea urchin eggs has been used as an indicator of environmental conditions, including the bioavailability of copper (Kobayashi, 1985; Pagano et al., 1986). Tissue metal levels in ascidians (sea squirts) are suggested by Monniot et al. (1986) to be useful in assaying water and sediment quality in the marine environment.

In a review of the effects of metals on fish behaviour, Atchison et al. (1987) comment (synopsis) that "behavioral toxicity tests, if properly designed, can be used in conjunction with standard ... tests to add ecological realism to toxicant assessments and the regulations made as an outgrowth of these assessments." Behavioural changes have been noted in "social" groups of fishes exposed to copper (Henry and Atchison, 1986) as has avoidance of point sources of metals (Hartwell et al., 1987b). Moran et al. (1986) note that trout olfactory receptors degenerate when exposed to elevated levels of copper and suggest that this could be used to assay for excess bioavailable metal. Fish tissue metal levels are still used in an attempt to determine the effects of anthropogenic metals as well as the suitability of the flesh for human consumption (e.g. Amiard-Triquet et al., 1987a; Miettinen et al., 1985; Segner, 1987).

Nutrient copper availability to domestic and laboratory animals has been assayed by physiological changes as well as tissue metal levels (e.g. Malvis Stracciari et al., 1985; Sharma and Prasad, 1985). Mieden et al. (1986) describe a "whole rat embryo" assay for detecting deficiencies of copper and zinc. Contaminants in prepared diets are of concern in these animals not only for the health of the animal but, especially for laboratory animals, the accuracy of research work (e.g. Rao and Knapka). Holst et al. (1986) discuss the use of abattoirs for determining the mineral status of range cattle in New South Wales. Veillon et al. (1986) describe a bovine serum reference material for use in calibrating instrumentation and evaluating the reliability of analytical methods for certain biological fluids. Bovine teeth and meat powder are also available as reference materials (Dirscherl et al., 1987). Certain human tissues (hair, whole blood, blood serum, urine, milk, liver) have also been used as reference materials for specific projects (Iyengar, 1987). Some of them (hair, urine) are also useful for monitoring certain diseases (e.g. Weisner et al., 1987) as well as the efficacy and safety of certain disease treatment programs. An example is the use of oral zinc in the treatment of Wilson's disease (Brewer et al., 1987a). For human foods, the Commission of the European Communities has a Community Bureau of Reference which provides a range of reference materials to meet the principal needs for food analysis and measurement (Wagstaffe, 1987).

The use of cell cultures to assay for cytotoxicity is described briefly by Babich et al. (1985). They comment that their *in vitro* assay is useful for the screening of the effect of environmental pollutants, including metals and organics. This type of assay is also useful for examining the action of certain drugs (e.g. Roberts and Robinson, 1986).

I.3.12 TOXICITY

A number of recent summary publications are concerned with toxic effects of metals, including copper. These include a review by Nor (1987b) on the "Ecotoxicity of Copper to Aquatic Biota: ...", "Significance of Heavy Metals in Human Toxicology" by Mallinckrodt (1984), "Pollution Threat of Heavy Metals in Aquatic Environments" (Mance, 1987), "Effects of Pollution on Freshwater Organisms" (Kline et al., 1987), and the ninth volume of "Aquatic Toxicology and Environmental Fate ...", the results of a 1985 symposium, edited by Poston and Purdy (1986). As is evident from these titles, there has been a good deal of evaluation of conditions in aquatic environments, in part a result of increasing knowledge as well as concern about water quality. This is also evidenced by publications which include metals but deal with indicators and techniques for environmental monitoring and management (e.g. Cairns, 1986; Friberg et al., 1986; Geen and Woodward, 1986; Hellawell, 1986; Ozburn, 1986; Reish et al., 1987; Walker, 1987). Other general references deal with the physiological and biochemical aspects of toxicology (Chambers et al., 1986; Hodgson et al., 1988). Exposure of man to copper is discussed by Aaseth and Norseth in the "Handbook on the Toxicology of Metals" edited by Friberg et al. (1986) and the potential health effects of copper in water is evaluated in several publications (e.g. Jardim et al., 1986; Ohanian, 1986), including the development of site-specific water quality criteria for copper (Carlson et al., 1986b) and the evaluation of specific sites (e.g. Munawar et al., 1985).

Metal speciation not only dictates the chemical nature of environmental copper (e.g. Kester et al., 1986; van den Berg et al., 1987), it also controls its biological availability. The chemistry of copper, combined with the chemistry of the organism, controls the nature of any detrimental effects (Bernhard and George, 1986; Evans, 1986; Pagenkopf, 1986; Turner et al., 1985; see also Piscator, 1986 for other metals) as well as the fate of copper once within the organism (e.g. Petering and Antholine, 1988; Wolf et al., 1986). It becomes readily apparent then, why there has been so much effort on the chemistry of metals both in the environment (e.g. Cowan et al., 1986; Stokes and Campbell, 1986) and within the organism.

Beaubien and Jolicoeur (1984) used flow microcalorimeter techniques to examine the heat flux generated by microorganisms exposed to various heavy metal salts. The authors comment, however (page 269) that "the sharp loss of acitivy at pH values in the range 3-5 suggests careful consideration of pH effects in the interpretation of the toxicity of heavy metal salts." (pH control of toxicity has been reported for other microorganisms (e.g. Singh and McFeters, 1987a).) Copper is widely used as a pesticide (e.g. Galvez and Javed, 1986) and antifouling agent (e.g. Miller, 1985). It also may act synergistically, to enhance the toxic effects of pesticides such as cuprosan (Thiolliere, 1985) and paraquat (Kohen and Chevion, 1985). However, levels of copper sensitivity, or tolerance, have been reported for various microorganisms (Bender, 1986; Bertru et al., 1986; Casida, 1987; Desjardins et al., 1986; Doelman, 1986; Garcia et al., 1987; Lynch et al., 1987; Morozzi et al., 1986; Nieto et al., 1987; Sikka et al., 1987; Trevors, 1987). In these cases, copper-containing pesticides may select for the more tolerant species (Andersen and Lindow, 1986). Since the effects of excess copper vary widely, from perturbation of cell metabolism to regulation of immunity (Aliev and Dontsov, 1985; Eife et al., 1987), the reasons for organism tolerance or sensitivity are often difficult to isolate. Mechanisms of copper resistance include precipitation or deposition of excess metal (Erardi and Falkinham, 1987; Sutter and Jones, 1985), favourable nutrient conditions (Stewart, 1987), cell wall impermeability (Gadd et al., 1987) and isolation within the cell by chemical binding (Cooksey, 1986). At least some of the resistance is genetically controlled (Bender and Cooksey, 1986). Microorganisms are also used as bioassay organisms to evaluate metal bioavailability and environmental quality (Burton et al., 1987; King, 1984; Slabbert, 1986)

Copper is used as an algicide, in the control of micro and macroalgae (e.g. Fuse, 1987; Nakamura et al., 1986; Tewari, 1987). Recent reviews of metal toxicity to algae include Kuwabara (1986) Rao and Sivasubramanian (1985b) and Sunda (1987). Stauber and Florence (1987a) discuss mechanisms of toxicity of ionic copper and copper complexes to algae. However Haley et al. (1986) point out that much of the copper entering aquatic environments is in particulate form. These authors report on the toxicity of brass dust to two species of microalgae, commenting that at least part of the toxicity is a result of ionized copper derived from the dust. In a document from the same group, Johnson et al. (1986) present evidence that the toxicity of brass particles is a result of particle ingestion rather than dissociation in the water. Goudey (1987) examines the factors that need to be considered in modeling the inhibitory effects of metals on phytoplankton growth and suggests advantages in using models to examine interactions among physicochemical and biological processes. These processes include the effects of complexation and adsorption (Thompson et al., 1986) as well as pH (Starodub et al., 1987b). The effects of excess copper are discussed elsewhere in this review. Several references report that, of the metals tested, copper is one of the more toxic - at the levels used in their studies (Starodub et al., 1987a; Zhenfen, 1986). This may be a direct result of the excess copper or an indicrect result, copper acting to affect some other variable (e.g. Anderson and Dechoretz, 1987a,b; Rueter et al., 1987; Suhayda and Haug, 1987; Valente et al., 1987). It may also be a result of laboratory procedure enhancing any toxic effects (e.g. Wong et al., 1986). Differential response to excess biologically available metal can produce a change in plant community structure (e.g. Callow, 1986; French and Evans, 1986; Kaitala and Maximov, 1986; Pyne et al., 1986). Copper tolerance has been reported in a number of algae (e.g. Balasubrahmanyam et al., 1987; Hillebrand and De Vries, 1986; Lees, 1985; Osokina et al., 1986; Vladimirov and Skulyari, 1986) and, in the green alga (*Pithophora oedogonia*), has been associated with cell surface and volume, metabolic activity, and cell wall copper-binding components (Pearlmutter and Lembi, 1986). Internally, metal-binding agents such as some of the tannins (Ragan and Glombitza, 1987) can reduce copper toxicity. Metal speciation is important not only to algae but other plants as well. Mosses have been used as bioassay organisms to evaluate metal bioavailability (e.g. Mouvet and Bourg, 1987). However, the metal tolerance of some organisms (Cooley et al., 1986; Nishizono et al., 1987a; Shaw, 1987a,b; Tanaka et al., 1987), including mosses (Shaw, 1987c), and the ability to produce metal-complexing agents after metal exposure (Morselt et al., 1986) may restrict the usefulness of plant biomonitors.

Plants require acceptable levels of required metals. However, soil characteristics will regulate the availability of both natural and fertilizer-applied metals. These same characteristics will also regulate availability in soils or medium with anthropogenic metal (De Haan et al., 1985; Hino et al., 1987a,b; Krtkova and Tichy, 1985; Vedy et al., 1986). This is important in predicting the biological effect of copper and other metals with the use of sewage and sludge as fertilizer. As with other organisms, however, tolerance is due to properties of the plant, metal availability is controlled by the soil. Tolerance has been reported for a number of plant varieties and species (Chronopoulos and Chronopoulou-Sereli, 1986; Hertstein and Jager, 1986; Jana et al., 1987; Palma et al., 1987; Rousos, 1986; Williams et al., 1986; Wu et al., 1983) and tolerant plants are often selected for use in metal-enriched soils (Hutchinson, 1984). The tolerance may be a result of reduced uptake or isolation of the metal by reduced transport or complexation with plant-produced organics (e.g. Kishinami and Widholm, 1987).

A number of recent references deal with the toxicity of metals to animals. The majority of these concern aquatic organisms. They cover both the general effects of anthropogenic copper, on communities or organisms in communities (e.g. Bryan et al., 1987; Nordin et al., 1986), and consideration of particular organisms. Organisms examined include:

1. Coelenterates - Karlsen and Marfenin, 1987.

2. Nematode parasites of plants - Alphey and Brown, 1987.

3. Earthworms - Martin, 1986; Neuhauser et al., 1985b.

4. Molluscs -

    a. Clams, oysters and mussels - Beaumont et al., 1987; Phelps and Hetzel, 1987; Wright and Zamuda, 1987.

    b. Snails (including shistosomiases vectors) and abalone - Babu and Rao, 1987; Gopal and Rao, 1985; Helaly and Nosseir, 1987b; Ikuta, 1987a; Kitching et al., 1987; Parashar and Rao, 1986; Reddy and Rao, 1987

5. Arthropods (primarily crustaceans) -

    a. *Daphnia* ("water fleas") and related species - Carlson et al., 1986a; Elnabarawy et al., 1986; Hall and Anderson, 1985; Johnson et al., 1986; Khangarot and Ray, 1987a; Khangarot et al., 1987a; Meyer et al., 1987.

    b. Copepods - Lalande and Pinel-Alloul, 1986; Sunda et al., 1987.

    c. Barnacles - Rao et al., 1986.

    d. Amphipods and isopods - de Nicola Giudici et al., 1987; Maich and Alikhan, 1987; Vincent et al., 1986.

    e. Shrimps and prawns - Correa, 1987; Patil and Kaliwal, 1986; Price, 1979 (Ph.D. thesis).

    f. Hermit crabs and true crabs - Ajmalkhan et al., 1986; Arumugam and Ravindranath, 1987; Depledge, 1987; Devi, 1987; Nagabhushanam et al., 1986.

    g. Insects (chironomid larvae of midges) - Kosalwat and Knight, 1987a,b; Malyarevskaya et al., 1984.

Work with molluscs has demonstrated that, for some organisms, copper uptake is salinity affected and that this effect is, at least in part, independent of cupric ion activity (Wright and Zamuda, 1987). Phelps and Hetzel (1987) report that copper concentrations in oysters can be affected by size as well as environment metal concentrations. Beaumont et al. (1987) examined copper sensitivity in several stages of the life history of the mussel *Mytilus edulis* and the scallop *Pecten maximus.* They report that veliger larvae of *M. edulis* are 7-10 times more tolerant of copper than either juveniles or adults. With invertebrates, tolerance during the life history may vary (e.g. Kosalwat and Knight, 1987a) or may not vary (e.g. de Nicola Giudici et al., 1987). Developent time can be affected (Ajmalkhan et al., 1986; Meyer et al., 1987). As with other organisms, some species exhibit a greater copper tolerance than others (e.g. Elnabarawy et al., 1986). This may reduce the value of water quality criteria established with bioassay organisms (e.g. Carlson et al., 1986a) although we are often assured that this is not the case (e.g. Khangarot et al., 1987a). Toxic effects may also be related to the food as well as the specific nature of the environment (e.g. Bryan and Gibbs, 1987; Khangarot et al., 1987b; Kosalwat and Knight, 1987b; Pesch et al., 1986).

In an evaluation of the toxicity of a copper-containing "contaminant mixture" to young striped bass, Mehrle et al. (1987) conclude that the age of the larvae, concentration of the contaminants and salinity of the environment play important roles in the impact of the mixture. Cui et al. (1987) note the importance of chemical processes in controlling the effects of several heavy metals on hatching of two species of marine fishes. The toxicity of copper to fish has recently been examined for a number of fish species. Of these, the carp has formed the focus of a number of studies (Benedeczky et al., 1986; Gupta and Rajbanshi, 1986; Khangarot et al., 1984; Svobodova et al., 1985; Vig et al., 1987; Wani, 1986) as have Rainbow trout (Svobodova et al., 1985) and freshwater perch (Collvin, 1985). Others include the guppy (Khangarot and Ray, 1987c), mosquitofish (Zhou and Tang, 1984), fathead minnows (Erickson et al., 1987), and several other freshwater fishes (Bengeri et al., 1986; Devi and Gopal, 1986). Evans (1987) discusses the fish gill as a site of action, including transfer, of metals such as copper. Buckler et al. (1987) reviews the importance of pH in affecting trace metal bioavailability to striped bass on the east coast of North America and Cherry et al. (1987) note higher mortality in rainbow trout than bluegill sunfish when both were exposed to metal-rich coal ash. Acidification of fresh water, in general, increases total dissolved metal and is considered to increase metal bioavailability. However, Cusimano et al. (1986) note that low pH decreases toxicity of cadmium, copper and zinc to trout, possibly due to hydrogen ion interferenece with metal uptake. The presence of organic acids can reduce metal bioavailability through metal complexation (Hutchinson and Sprague, 1987). Metal-metal interactions have also been noted, the acute toxicity to juvenile *Clarias lazera*, of a mixture of copper and zinc was less than that of either metal alone (Hilmy et al., 1987). Nutrition is also a factor in uptake, and possibly toxicity, liver copper accumulates to greater levels in starved roach (*Rutilus rutilus*) than in fed roach. Acclimation to elevated copper levels has been recently examined in rainbow trout by Lauren and McDonald (1987a,b) and coho salmon by Glubokov and Sokolova (1986). The effects of excess metal, including copper, on amphibians have also been examined (de Zwart and Slooff, 1987). Of particular interest is their effect on amphibian larval stages which have been used as indicators of environmental quality (Khangarot and Ray, 1987b; Rao and Madhyastha, 1987).

The potential for detrimental effects of excess copper in terrestrial animals has sponsored veterinary, environmental and medical research on metal uptake, fate and function in a wide variety of organisms. In a review of "Toxic actions of essential trace elements (molybdenum, copper, zinc, iron, manganese)", Anke and Groppel (1987) comment on the sensitivity of sheep to copper intoxication and the role that a suitable molybdenum:copper balance has in maintaining normal levels of tissue copper. A number of recent references discuss copper toxicity in sheep and goats (e.g. Humphries et al., 1987). MacMillan et al. (1986) give the 6:1 copper:molybdenum ratio that is accepted as a standard for feeds. Clegg et al. (1986) report a case of waterborne copper toxicity in sheep due to copper piping used to distribute drinking water to pens from a storage tank. Fuentealba (1985) reviews the three phases of chronic copper poisoning in sheep, describes the clinical and histological changes that occur, and discusses the treatment of copper toxicity in sheep. One of these treatments is the use of injected molybdenum to achieve a copper:molybdenum balance within the organism (Humphries et al., 1986). The activity of certain enzymes has been reported to change as a result of chronic copper poisoning in sheep (Kelleher and Ivan, 1987). In cattle, elevated copper is reported to cause a decrease in superoxide dismutase activity (Asano and Hokari, 1986). Activity of this enzyme is associated with the reaction of copper in other situations (e.g. Czapski and Goldstein, 1986a). Strain-dependent differences in sensitivity to copper intoxication have been reported for the rat and suggested to be due to differences in copper excretion and metallothionein accumulation of copper (Nederbragt et al., 1987). Cysteine is reported to ameliorate copper toxicity in chicks and rats (Baker and Czarnecki-Maulden, 1987; Freedman, 1986) and vitamin E supplementation reported to restore resistance to copper toxicity in vitamin E-deficient isolated rat hepatocytes (Sokol and Devereaux, 1987; Sokol et al., 1987). Zinc supplementation has been reported to inhibit copper toxicity in human hepatoblastoma cells (Blank and Stockert, 1986) and is used as a treatment in Wilson's disease.

The resistance to toxic effects of excess copper is a trait that has been found in a wide variety of organisms. In a review of "Genetic adaptations to heavy metals in aquatic organisms

...", Klerks and Weis (1987) suggest that this could arise from long exposure to high levels of metals. Tolerance has been reported in plants (e.g. Bednarova and Habrnalova, 1986) and animals (e.g. Grodowitz et al., 1987). It can occur as a result of metal-metal interactions in the environment (e.g. Kazumi et al., 1987) or an ability to isolate the metal by metal complexation and subsequent precipitation (Grodowitz et al., 1987; Jones and Wilson, 1986; Purvis et al., 1987; Rainbow, 1987). In certain microorganisms, copper resistance is plasmid-conserved (Bender and Cooksey, 1987; Cooksey, 1987) or plasmid-encoded and involves sulfate-dependent copper precipitation (Erardi et al., 1987). Organics play a major role in copper tolerance. The fouling diatom *Amphora coffeaeformis* has an increased tolerance to copper and tin in the presence of bacteria (Thomas and Robinson, 1987b). Siderophores produced in response to iron starvation is capable of moderating copper toxicity in certain blue-green algae (Clarke et al., 1987). Cysteine-rich agents capable of complexing copper are produced by both plants and animals (e.g. Robinson et al., 1987). These, and similar agents (e.g. Robinson and Thurman, 1986), frequently occur in peptides such as phytochelatins (Grill et al., 1987) or metallothioneins (Acey et al., 1987; Capasso et al., 1987; Delval, 1984; Germann and Lerch, 1987; Roch and McCarter, 1986) which are excellent metal chelators acting either naturally as metal transport agents or produced in response to exposure to excess metal.

II - COPPER AND MAN

II.1 USES OF COPPER

Copper is beneficial to man and is widely used for a variety of purposes ranging from nutrients to antifoulants. Recent references concerning the uses and importance of copper are numerous and are discussed in section I of this review. However, to provide an indication of its wide use and numerous benefits 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, plant nutrient supplementation - Adams et al., 1986; Agaev, 1987; Ashmead, 1986; Ashmead et al., 1986; Bekbanov and Al'zhanov, 1985; Blue and Malik, 1986; Chavan and Gupta, 1986; Chhibba et al., 1985; Cline et al., 1986; Dankiewicz et al., 1985; Davee et al., 1986; Dong and Burdett, 1986; Dubikovskii et al., 1987; Farrahi-Aschtiani et al., 1987; Fecenko et al., 1986; Firgany et al., 1981; Fregoni, 1986; Galrao and de Sousa, 1985; Gorlach and Gorlach, 1984; Hsu, 1986; Jones and Leslie, 1986; Kadar, 1987; Kadar et al., 1985; Kang and Osiname, 1985; Kanwar and Youngdahl, 1985; Khallyeva et al., 1986; Kimbro et al., 1987; Krahmer and Podlesak, 1985, 1986; Kudashkin, 1987; Kuznetsov et al., 1983; Lamb, 1986; Langenegger and Du Plessis, 1986; Mabbett and Phelps, 1985; Mann and Sidhu, 1983; Mian and Eaqub, 1986; Miller et al., 1986a; Milosevic et al., 1984; Morard and Anne, 1985; Mortvedt, 1985; Nabiev et al., 1987a,b; Okumura, 1986, 1987; Perera, 1986; Podlesak and Krause, 1987; Potatueva et al., 1987; Protasova et al., 1986; Quinche et al., 1987; Richardson, 1986; Ruppe and Podlesak, 1987; Rymar et al., 1986; Selevtsova et al., 1987; Shazly, 1986; Sheudzhen, 1986; Shorrocks, 1987; Shtefan and Volokitina, 1987a,b; Shtefan et al., 1987; Sidrovich et al., 1987; Silva et al., 1986; Sirotkina et al., 1984; Solov'ev et al., 1987; Tinker, 1986; Turaev et al., 1985; Ugai et al., 1984; Ursu and Pukalov, 1984; Valadz'ko and Makhnach, 1986; Varshney, 1985; Weichelt, 1986; Weichelt and Gerhardt, 1986; Weichelt et al., 1986; Yan, 1985.

Agriculture - livestock - Allee, 1985; Beranek, 1987; Binnerts et al., 1986; Brokken and Porubcan, 1987; Caple and McDonald, 1983; Ellis et al., 1987; Eskin, 1984; Gaffarov et al., 1985; Hagen et al., 1987; Hamada et al., 1985; Harms and Buresh, 1987; Hassel, 1986; Haughey, 1983; Ingraham et al., 1987; Khitrinov and Sirotkina, 1987a,b; Ko et al., 1985; Kornegay et al., 1985/86; Leporati, 1987b; Ludke et al., 1985; Ming et al., 1986; Moreels et al., 1987; Mosina, 1983; Nutrition Foundation, 1984; Piva et al., 1986; Poliakova, 1984b; Schone et al., 1986; Sinnett-Smith and Woolliams, 1987; Varel et al., 1987; Zaderii, 1984.

Biofouling - Bews, 1986; Evans and Hoagland, 1986; Ludyanskiy and Solonin, 1986; Sawashita, 1986, 1987; Woods Hole Engineering Associations, Inc., 1984 (INCRA Project 268B); Yamamoto et al., 1987.

Dentistry - Afseth et al., 1986; Ben-Amar et al., 1987; Marshall et al., 1987; Moore et al., 1987; Naylor, 1986; Smales and Gerke, 1986.

Medicine - contraceptives - Audebert et a., 1985; Batar et al., 1987; Belhadj et al., 1986; Diaz et al., 1985; Fylling, 1987; Goluda and Lembas, 1985; Grimes, 1987; Rob et al., 1987; Sheldyaeva and Kadyrova, 1986; Sivin and Schmidt, 1987; Sivin et al., 1987; Thiery and Kosonen, 1987.

Medicine - nutrient supplementation - Huston et al., 1987; Kadowaki et al., 1987; Shenkin et al., 1987; Vadasz and Vadasz, 1987.

Medicine - pharmacology - Ames and Kovacic, 1986; Bakola-Christianopoulou et al., 1987; Basile and Barton, 1987; Basosi et al., 1987; Braestrup and Andersen, 1987; Ciurdaru et al., 1986; Cole et al., 1986; Elo and Lumme, 1987; Elo et al., 1987; Flohe et al., 1986; Franz et al., 1987; Freeman and O'Callaghan, 1987; Fujita et al., 1987; Ghose et al., 1986; Green, 1987; Hassan and Lee, 1986; Hochuli and Dobeli, 1987; Hinojosa et al., 1987; Kopf-Maier, 1987; Kremer et al., 1987; Krishnan and Vijayalakshmi, 1987; Malatesta et al., 1985; McGirr and O'Brien, 1985; Mertens et al., 1986a,b, 1987; Mikaelyan et al., 1987; Miyata-Asano et al., 1986; Okuyama et al., 1987; Pezeshk, 1986; Pickart, 1987; Pickart et al., 1986; Rajan et al., 1986, 1987; Rajendran et al., 1986; Rao et al., 1987; Revici, 1987; Sato et al., 1986; Sedergran et al., 1987; Senires and Lim-Sylianco, 1984; Shao et al., 1986; Sorenson, 1987a,b; Srivastava, 1986; Straight et al., 1987; Tachibana and Iwaizumi, 1987; Tachibana et al., 1987; Taguchi et al., 1986; Tamura et al., 1987; Timoshkova et al., 1986; Treshchalina et al., 1986; U.S. Department of Energy, 1987; Wallace, 1986; White et al., 1986; Woods and Mason, 1987; Zhou and Wang, 1986.

Industry - adsorbent use - Ganzerli Valentini et al., 1986; Melson, 1987a,b; Mote, 1986; Stelman et al., 1987; U.S. Department of Energy, 1986.

Industry - food quality - Carpenter et al., 1986; Deo and Gupta, 1986; Desai, 1984; Flynn et al., 1987; Pal et al., 1986.

Industry - microbial production of organics - Evans et al., 1987; Kaneko et al., 1987; Kuang et al., 1985; Megalla et al., 1987; Purohit and Daginawala, 1986.

Industry and Environment - Boyd and Mortland, 1986; Menger et al., 1987; van Staden et al., 1986; Veuthey et al., 1987; Vultier et al., 1987.

Pest Control - bacteria and fungi - Alexandri et al., 1986a-c; Andersen and Lindow, 1986; Arimoto et al., 1985; Barlett, 1986; Blahova et al., 1986; Chatterjee et al., 1986; Colin and Chafik, 1986; Cooksey, 1987; Das and Mohanty, 1985; Davis, 1985; Eger et al., 1986; Elango, 1986; Elphinstone and Perombelon, 1987; Feichtenberger et al., 1985; Galvez and Javed, 1986; Grimm, 1987; Gunther et al., 1987; Hokko Chemical Industry Co., Ltd., 1985; Inczedy and Maros, 1984; Indi et al., 1987; Issa et al., 1985; Jaitly and Wadhwani, 1986; Jardine and Stephens, 1987; Johnson et al., 1985; Johnson et al., 1986; Kagiwata, 1986; Keshavan and Janardhan, 1986; Khadikar et al., 1985; Kobayashi and Kobayashi, 1987; Kukalenko et al., 1987a,b; Kumar and Singh, 1986; Kumar et al., 1987; Kurdish and Khenkina, 1987; Kushalappa et al., 1986; Littrell and Heath, 1986; Lukade, 1985; Mollin et al., 1986; Nandi et al., 1987; Nanjo and Watanabe, 1986; Oliveira et al., 1987; Olvang, 1987; Parashar et al., 1987; Patil et al., 1986; Rai and Singh, 1986; Rao, 1986; Saha and Adak, 1986; Samus et al., 1985, 1987; Sindelkova and Chaikina, 1986; Singh et al., 1986; Singh et al., 1986; Smilanick et al., 1987; Suseelendra and Hegde, 1986; Suteu et al., 1985; Teodorescu et al., 1986a-c; Thankamma et al., 1986; Thiolliere, 1985; Tyeklar et al., 1986; Vasile, 1985; Washington, 1987.

Pest Control - herbicides, insecticides, molluscicides, miscellaneous - Anderson and Dechoretz, 1987a,b; Anderson et al., 1987; Goldweber, 1986; Helaly and Nosseir, 1987a,b; Kanda and Mizuguchi, 1986; Kennard et al., 1986; MacMillan, 1984; Muralidharan et al., 1987; Pal and Chatterjee, 1987; Prystupa et al., 1987; Raman and Cook, 1986; Sabadie and Coste, 1986; Shiam et al., 1987; Stillman, 1987; Sugino et al., 1986.

Wood Preservation - Coggins, 1985; Collett, 1987; Dietrich and Levi, 1984; Gaby, 1986; Ghosh, 1986; Gjovik and Gutzmer, 1986; Hager, 1987a,b; Hall et al., 1987; Kim et al., 1985; Leightley, 1986; Nishimoto et al., 1987; Pizzi and Conradie, 1986; Schmidt et al., 1987; Smith, 1986; Srinivasan and Vallabhan, 1986; Tanaka et al., 1987; Wallace, 1986; Wilcox, 1987; Williams, 1986; Zarudnaya et al., 1986; Ziobro et al., 1987.

II.2 ANTHROPOGENIC COPPER - NATURE AND EFFECTS

As a result of its widespread use by man, copper is introduced into the environment as anthropogenic metal. Although copper is essential for all cells, in excess it can be detrimental (e.g. Brewer, 1987). Because of the anthropogenic input and the potential for detrimental effect, copper is one of the metals often measured in surveys to determine the impact of man on the environment (e.g. Petrovic et al., 1987). Sediment metal concentrations have also been used to indicate anthropogenic effects over extended periods of time (e.g. Ashwood and Olsen, 1988; Pavoni et al., 1987b). Reviews and discussions of environmental conditions (e.g. Bowman et al., 1987; Pritchard, 1986; Taqui Khan, 1986; Vicente, 1987) and discussions of international law (Soni, 1985; United Nations, 1985) often consider the real or potential impacts of anthropogenic metals, including copper. This is also true when considering the management or restoration of aquatic and terrestrial environments (e.g. Breteler, 1984; Lerman and Hull, 1987; Ralston, 1986). A number of symposia and workshops have examined the distribution, analysis, and biological and human effects of introduced metals (Australian Water Resources Council, 1984; Bewers et al., 1986a; Breckle and Kahle, 1985; Herricks, 1987; Lijklema et al., 1987; Merian, 1984; Ozburn, 1986). Fortunately, more and more emphasis is being placed on understanding the dynamics of the environment (e.g. Bird, 1987; Nriagu and Wong, 1986; Strachan, 1986; Wang et al., 1986) and the speciation of metals rather than just total metal concentration. Evidence of the growing interest in more appropriate sampling and analysis of metals, including copper, is provided in several publications (e.g. Allan, 1986; Boyle, 1987; Environmental Protection Agency (U.S.), 1987; Ewers, 1984; Fresenius and Luderwald, 1984; Giam and Dou, 1986; Griepink, 1984; Literathy et al., 1987; Morrison and Revitt, 1987; Raab et al., 1987). Bioassay and tissue metal measurement techniques have also been updated (e.g. Favretto et al., 1987; Ropes, 1987; Spehar and Fiandt, 1986) and suggestions made for improved routine biological effect monitoring (e.g. de Kock, 1986). Carlson et al. (1986a) used comparative acute copper toxicity values for three organisms (*Ceriodaphnia dubia, Scaphrolebaris* sp., and *Pimephales promelas*) to calculate LC_{50} values in both reference and test water. The ratios of these values (site/reference) were then used to modify U.S. Environmental Protection agency ambient aquatic life criteria for copper to permit the use of site-specific values.

Hamilton et al. (1987) list copper as one of the elements of "environmental concern" in their discussion of sources, distribution, and effects of major aquatic contaminants. In a book entitled "Pollution Threat of Heavy Metals in Aquatic Environments", Mance (1987) addresses the detrimental effects of copper to a wide variety of organisms. Hellawell (1986) discusses detrimental effects of excess copper in a book on "Biological Indicators of Freshwater Pollution and Environmental Management". Nor (1987b) reviews literature on "Ecotoxicity of copper to aquatic biota: ...". Lawrence (1986) discusses the placement of copper on the Grey List of elements by the EEC, suggesting that copper is more serious than cadmium. He does not, however appear to recognize that copper is an essential trace element while cadmium is not! In a 1984 "Small Business Administration" report, consequences of the 1983 U.S. EPA effluent limitations guidelines are discussed. The effect of these limitations is given as a percentage of product price although the report does not provide any suggested changes to the limitation guidelines. Records of EPA Superfund Decisions (e.g. Environmental Protection Agency, 1986a,b) for improvement of contaminated sites also provide an indication of financial involvement dictated by government control of industry.

Environmental evaluations have been prepared and, in some cases, published for a number of marine areas, including:

Northeast Pacific (Puget Sound) - Battelle, Marine Research Laboratory, 1986.

Beaufort Sea - Boehm et al., 1986

North Sea and Wadden Sea - Beukema et al., 1986; de Kock, 1986; Kersten and Forstner, 1985

Mediterranean region - GESAMP, 1985; Perin et al., 1987; Persian Gulf - Samhan et al., 1987

Southeast Asia - Hungspreugs, 1985

Similar studies have appeared for freshwater and terrestrial areas:

Erlangen in West Germany - Reichel, 1982

Scandinavia - El-Daoushy, 1986

Northern Greece - Fytianos et al., 1987).

These publications include comparisons of metal levels between areas (e.g. snow in Antarctica and Greenland - Delmas, 1986). They also include examinations of specific agents or groups of agents (Linzon, 1984; Muirhead-Thomson, 1987; Thomas, 1984; Woods, 1986)

Industry

In examining the nature and effect of anthropogenic copper from industrial sources it is important to keep in mind that "... the chemical form of an element is vital in the determination of the actual properties of stability, toxicity, and transport of that element in the natural environment" (Craig, 1986, page 463). Using the mussel *Mytilus edulis* as an assay organism to examine the bioavailability of copper in industrial effluents, Comber et al. (1987) report that the accumulation of copper was related to the "free" and not the total copper present. This does not mean that the copper in industrial effluents and aerosols, it does mean that the overall effect must be related to metal speciation and other factors in the industrial emission. Fenske (1985), for example, reports that sulfur dioxide and dust were responsible for a copper deficiency in cattle feeding in impact areas of lignite power stations. However, effects of metals are frequently associated with changes in other important environmental parameters (e.g. Petrovic et al., 1987).

Although metal levels decrease rapidly away from the immediate source, anthropogenic input into the environment is adequate to be detected in many areas of the world (e.g. Murphy, 1985). Lax et al. (1986) discusses some of the sources of aerosol metal in Perth, Australia. Studies on the deposition rates of metals in a number of forests have been related to aerosol input from industry (Kovacs et al., 1986; Kues, 1984). This has also been demonstrated for uptake by lichens (Gailey, 1985) and food crops grown near industrial point sources (Larsen and Lykke, 1986). Lahiri et al. (1987) provides aerosol metal levels in a plant producing copper salts, ranging from more than 2600 $\mu g/m^3$ to less than 30 $\mu g/m^3$.

Refuse dumps form minor sources of aerosol copper, through volatilisation (Lodenius and Braunschweiler, 1986). Incineration of refuse increases the output of aerosol metal. Measurement of suspended particle, heavy metal and selected organics have been made at several municipal refuse incinerators in England (Clayton and Scott, 1985a,b; Scott et al., 1986). Similar measurements have been made near a number of metal-working facilities in England (Davis and Clayton, 1985a-d). Burnett and Minden (1986) review particulate emission factors for nonferrous industries.

Soil metal levels were found to be elevated near scrap metal yards and car breakers yards (Blake et al., 1987). The authors report, however, that soil metal concentration decreased with distance from the yards. The chemistry of copper in soils affects its mobility and biological availability. Elliott and Linn (1987) report enhanced zinc and copper desorption from metal-enriched soil by a roadway deicing chemical (calcium magnesium acetate - CMA). pH of the soil is of key importance, acid rain decreasing pH and mobilizing soil metal. Acid rain is also a corrosive agent that causes deterioration of metal-containing materials, releasing metal to the environment (e.g. Flinn et al., 1985; Yunker, 1986). The biological impact of acid rain varies with the nature of the organism and the environment. Nohrstedt (1987) reports that irrigation with artificial acid rain over a period of 5 months reduced the pH of a forest floor but there appeared to be some buffering action by the soil. Simulated acid rain treatment effects on seedlings of several conifers included notable decreases in exchangeable cations and soil pH as well as increases in soil Al, Mn, Fe, and Zn but not obvious increases in Cu (McColl and Firestone, 1987).

Definite copper enrichment of wastewater has been noted in front of a chlor-alkali plant in Italy, 96.7 µg/L vs. 0.13 µg/L for background seawater value (Seritti et al., 1987; see also Baluja et al., 1985). This has also been demonstrated for a small treatment plant in Chile (Valle, 1984), sewage and industrial input estuaries (e.g. Haekel et al., 1985; Rule, 1986) and for industrial input into a number of rivers (e.g. Pfeiffer et al., 1986; Reddy and Venkateswarlu, 1985). Nriagu and Rao (1987) note that lake sediments form a sensitive indicator to metal emissions from Sudbury, Ontario, Canada. Provini and Gaggino (1985) used sediments as a record of copper input into Lake Orta (Italy), a lake that has received copper-rich discharges from a cuproammonium rayon factory since 1927. A great deal of work has been done on the geochemistry of anthropogenic metals in sediments of the Niagara River (Mudroch and Duncan, 1986), Great Lakes (Chau et al., 1985; Lum and Gammon, 1985; Poulton, 1986, 1987; Sly, 1984), Detroit River (Fallon and Horvath, 1985; Hamdy and Post, 1985; Lum and Gammon, 1985; Mudroch, 1985). Madsen and Larsen (1986) estimate that anthropogenic input of copper into the Kattegat and Belt Sea approximates 200 tonnes per year. Hoshika and Shiozawa (1986) estimate a total input of 630 tons of copper into the Seto Inland Sea, of which an estimated 310 tons comes from anthropogenic sources.

Mining, Smelting and Metal-Working

Mining, smelting and metal-working all have the potential to release metal into the environment. Release of copper occurs not only with its extraction but with the extraction of other materials such as fossil fuels. Acid mine drainage has received considerable attention as has the technology developed for reclamation (Tyre and Barton, 1986). Citations dealing with control and treatment of acid mine drainage are listed in the National Technical Information Service (U.S., 1987a) and cover the period January 1977 through April 1987. Regulatory aspects of metal finishing and processing industries are given by Higgins et al. (1987) who also discuss metal recovery techniques.

The effect of mining and smelting, on soil and sediment metal levels has recently been examined by several authors (e.g. Arafat, 1985; Xian, 1987; see also Chaika, 1986 and Legorburu and Millan, 1986). Harper et al. (1987), for example, report aerosol transport of metals from smelter waste tips to garden soils. Long distance atomspheric transport from mine and smelter complexes is also suggested by Vermette and Bingham (1987) as an explanation for the elevated rain copper levels in Churchill, Manitoba (Canada). In estimating impact, however, it is important to relate metal levels to soil parent materials (in the sense of Archer and Hodgson, 1987) as well as limitations of the extraction techniques (e.g. Gajbhiye, 1985). Wong et al. (1986) report no substantial elevation in copper resulting from the addition of copper mine tailings to seawater-containing plastic enclosures. Jones (1986) examines the distribution and partitioning of silver, copper and several other metals in sediments associated with an acid mine drainage stream, noting the sorption effect of precipitated ferric oxides and counselling for consideration of results because of the extraction techniques. Johnson and Thornton (1987) report increased metal concentrations in the Carnon River (England) as a result of acid mine drainage during the winter. They also estimate that 45% of the riverine copper originates from mine waters. Johnson (1986) and Johnson and Thornton (1987), as well as Jones (1986) note the importance of iron oxyhydroxides in controlling trace element concentrations in river and estuarine waters containing acid mine drainage.

Although metal uptake by plants grown in soils from metal-bearing sites has been suggested to be a result of soil metal concentration (Babalonas et al., 1987; Kong et al., 1986; see also Handy et al., 1986a,b), metal availability will ultimately dictate the amount of metal taken up by the plant (e.g. Gough, 1984). Contamination can occur as a result of particulates adhering to the plant surface which can bias metal concentration values. Hunter et al. (1987d), for example, found marked seasonal variation in vegetation levels of copper near a metal refinery, as a result of increased winter accumulation of particulates adhering to external leaf surfaces as well as root absorption. Their work (Hunter et al., 1987a,b) also showed the need for adequate sampling during the year and the difference in metal accumulation with the nature of the organism, whether plant or animal.

Nordin et al. (1986) report an effect of increased concentrations of zinc, copper, lead and cadmium, from a mining operation in western Canada, on the aquatic biota of lakes in the watershed. They comment (abstract) that "... more severe effects have occurred in phytoplankton and zooplankton than the fish community ...", with phytoplankton primary production depressed near the minesite. Wotton et al. (1986) report aerosol nickel and copper deposition in soils in Manitoba, up to 35 km from an Inco nickel smelter. Nickel and copper both accumulated in cones of the jack pine *Pinus banksiana* and black spruce *Picea mariana* near the smelter. In Quebec, Canada, Wickham et al. (1987) found increased copper and aluminum accumulations in the benthic biota of an acid and an alkali tailing pond, in comparison to a control; insects dominated the benthic fauna in the tailing ponds, crustacea in the control pond. Other references support the concept that, in general, higher tissue metal levels and detrimental effect occur in organisms associated with metal-rich tailings and leachate (Bagatto and Alikhan, 1987b; Besser and Rabeni, 1987; Gignac, 1987; Hunter et al., 1987d; Ward et al., 1984). However, the effects and concentrations appear to be affected by the nature of the organism (e.g. Howard and Brown, 1987; Hunter et al., 1987d) and certainly by the chemistry of the environment. Gignac (1987), for example, notes a relationship between metal accumulation in the capitula of three species of *Sphagnum* and the partially humified peat below the living moss. Adaptation also occurs with metal and potential metal tolerance in some organisms (e.g. Bednarova and Habrnalova, 1986; Chronopoulos and Chronopoulou-Sereli, 1986). This is, perhaps best exemplified by the use of aciophilic chemolithotrophic bacteria to leach copper from flotation tailings (Grudev, 1985).

Aerosol introduction of metals and sulfides is reported to affect forest floor vegetation in Kokkola, Finland (Vaisanen, 1986). Living bog vegetation is reported to have elevated concentrations of Cu, Pb and Zn out to approximately 50 km from a Quebec copper smelter and from industrialized areas in eastern Canada. Accumulation of metals by "moss bags" is reported for an industrial town, Armadale, in central Scotland (Gailey and Lloyd, 1986). Monkiewicz et al. (1986) examined the influence of emissions from a copper foundry, on the content of Pb, Cu and Zn in fodders, blood and organs of cows. The level of fodder metals was high and the level of lead in cow blood was high near the foundry. However, the level of copper and zinc was not related to the distance from the foundry suggesting the ability of the animals to exert internal control over metal accumulation.

Sewage, Sludges and Wastewater

The processing of sewage, sludges and wastewater is a universal problem of ever increasing magnitude. L'Hermite (1986) comments (preface) that "It is the aim in disposing of sludge to protect health and provide a good environment at realistic cost by utilising best practice based on sound scientific knowledge." In disposal or when used as a fertilizer (e.g. Rappaport et al., 1987; Tackett et al., 1987; Wakefield and Sawyer, 1986), one of the problems is the elevated metal concentration that can occur with sewage, sludges and wastewaters (e.g. Haeni and Kloetzli, 1984; Jackson and Roberts, 1985; Jarrell and Saulnier, 1987; Kouzeli-Katsiri and Kartsonas, 1986; Leonhard and Hegemann, 1985; Mininni and Santori, 1987; Tonkopii et al., 1987). Reviews of some of the use and problems associated with the use of sludges are given by L'Hermite (1986) and Oliveira and Almeida (1987). Fleming and Davis (1986) note the importance of controlled usage and the need for monitoring when sludges are used as fertilizers. Quality control monitoring such as that described by Greenberg et al. (1986) will reduce the impact of sludge use on land. Tjell (1986) reports that sludge metal concentrations in certain countries are diminishing which may allow greater land use; soil conditions will dictate sludge metal availability.

The biological impact of other types of organic wastes has also been examined. Dyer and Razvi (1987), for example, assess the risks of solid waste compost. Problems with the occurrence of excess levels of synthetic organic compounds include toxic effects on biological processes in waste treatment (Bewtra, 1985). Incineration of wastes is receiving increasing use for community solid waste (Candreva and Dams, 1987) and for anthropogenic materials such as metal-containing synthetic organic chemicals (Oppelt, 1986). Travis et al. (1987) discuss the potential health risk of incineration and comment that "Preliminary results indicate that both the carcinogenic and noncarcinogenic risks to populations living near incinerators are small." Candreva and Dams (1987), however, found that most of the heavy metals from a municipal refuse incinerator were associated with fine particles that were poorly retained by an electrostatic precipitator. Bridle et al. (1987, abstract) found "... that incineration of sewage sludge produces a benign ash with most of the metals speciated as insoluble oxides or silicates." However, with incineration of municipal solid waste or hazardous wastes, incineration produces fly ashes in which certain metals (Cd, Zn, Ni, Cu) are readily leachable and probably speciate as water soluble chloride salts. Temperature of incineration is obviously important, Kistler et al. (1986, 1987) note retention of copper in solid residue of sewage sludge at temperatures up to 750òC.

The effects of metals from marine disposal of sewage and wastewater have been widely discussed in individual publications (e.g. Ashwood et al., 1986; Castaing et al., 1986; Haekel et al., 1985; Kouadio and Trefry, 1987) as well as in symposia and seminars (e.g. Ludwig and Almeida, 1986). Brown et al. (1987) examined the cytosolic distribution of metals in livers of fishes from municipal wastewater sites in southern California. They comment that (abstract) "Concentrations of Cd, Cu and Zn were frequently lower in cytosolic pools of ... (fishes) from highly contaminated Palos Verdes (PV) relative to those from less contaminated Santa Monica Bay (SMB) despite much higher concentrations of these metals in sediments at PV." They also note a greater difference in cytosolic patterns between metals than between species of organisms or sampling sites suggesting a metal-specific effect.

To predict the biological effect of copper in sewage, sludges and wastewaters requires an understanding of the chemistry of the metal (e.g. Bhowal et al., 1987; Singh and Kansal, 1985).

Anaerobic waste treatment can, for example, be inhibited by excess metal, thereby reducing the efficiency of metal removal (Jarrell and Saulnier, 1987; Kouzeli-Katsiri and Kartsonas, 1986). However, knowledge of metal chemistry should be augmented by information about the chemical changes that occur when sludges or wastewater are disposed or used as soil supplements. Senesi and Sposito (1987), for example, report that copper complexes with anionic surfactants found in sewage sludge (e.g. sewage sludge fulvic acid) and acts as a complex rather than a metal. This would reduce the biological availability of the copper (see also Petruzzelli et al., 1986). De Haan et al. (1985) discuss acceptable levels of metals (Cd, Cr, Cu, Ni, Pb, Zn) in soils depending on their clay and humus content as well as cation-exchange capacity. Hani and Gupta (1986) discuss chemical methods for the biological characterization of metal in sludge and soil. Minnich and McBride (1987) measured copper activity in soil solutions and comment that increased total copper resulted in higher cupric ion activities and, conversely, increased total sludge resulted in decreased cupric ion activities. Short-term effects of metal-rich sludge application to soils have been noted (e.g. Vedy et al., 1986). Long term effects of sewage sludge application are important (e.g. Sauerbeck and Styperek, 1986; Schmitt and Sticher, 1986) and have been obtained to provide some indication of changes occurring in soil properties as a result of the application. Brookes and McGrath (1986) found elevated metal concentrations and reduced soil microbial biomass, soil ATP and biological nitrogen fixation 20 years after the application of metal-contaminated sewage sludge. Jones et al. (1987) measured metal concentrations in an archived soil collection stored from the mid-1800's. They report that soils treated with farmyard manure had elevated copper concentrations. Heckman et al. (1987) obtained evidence of long term (9 years) effect on metal uptake by soybean, dictated by sludge composition and soil pH, both of which affect metal mobility and biological availability (e.g. Sharma and Kansal, 1986). Long-term effects are also noted by Sarkis (1987) although the author comments that the concentrations are not abnormally high and there is no evidence of adverse impact on the crops or soils that were monitored. Williams et al. (1987) did not find evidence of significant vertical movement of metals in a soil amended with sludge over an 8-year period. Kinkle et al. (1987) did not find a long-term detrimental effect on soil rhizobial numbers 11 years after application of a metal-rich sludge.

Uptake of metals from sewage and sewage sludge has been examined for a variety of organisms. Wong and Chan (1985) report that metal uptake by the algal species *Chlorella pyrenoidosa* varied depending on the organic waste used. Metal (Mn, Fe, Cu, Zn) uptake from aqueous extracts of sewage sludge was higher than from pig and chicken manure as well as refuse compost. Barbarick and Workman (1987) examined the effects of two soil extractants on estimating bioavailable metals in sludge-amended soils. They comment that the concentrations of Cd, Cu, and Ni in the sludge applied did not result in plant uptake at levels that were significantly different from those in the untreated control. Differences in metal tolerance have been found between plant species grown on the same sewage source-containing medium (Bernhart and Salvatori,1986). They, and others (e.g. Manrique et al., 1986), also note different metal retention in different parts of the plant. Greatest retention appears to be in the roots. With soybean, shoot copper concentration increased linearly with increasing digested sludge concentration (Heckman et al., 1987). Minnich et al. (1987), however, report nonlinear relationships of sludge copper activity and copper accumulation by the snapbean *Phaseolus vulgaris*. They suggest that this is a result of the sludge organics buffering the copper and yet helping to maintain a relatively high soil solution copper concentration. They also recognize a physiological control of copper translocation within the plant. Nitrilotriacetic acid (NTA) in wastewaters is known to mobilize copper and other metals from municipal sludge (Elliott and Linn, 1986). Rappaport et al., (1987b) found that copper in sludge-amended soil was not toxic to either corn or barley, even at copper supplementation in excess of 280 kg/hectare. Copper levels did increase in corn but not in barley. Williams et al. (1986) report variability in tolerance and metal uptake in cereals grown in sewage-treated soil. Korcak (1986b) report elevated tissue metal (Mn, Zn, Cu, Ni) levels and reduced growth in apple seeds (York Imperial) grown for 7 months in sand supplemented with composted sludge. In contrast three sludge materials enhanced growth of the seedlings.

Neuhauser et al. (1985a) examined uptake and loss of metal in earthworms kept in sludge-amended soils. They found increased metal levels in metal-enriched soils but comment that when placed in metal-poor soil, the copper body burden was rapidly lost. Wong and Cheung (1986) found elevated tissue concentrations of Pb, Cu, Zn and Mn in vegetables grown in soil amended with sewage sludge and animal manure. However, tissue levels of Pb, Cu and Zn in caterpillars (*Pieris canidia*) fed the vegetables was below that of the food. This is due to some control mechanism which they suggest could be metal loss in fecal material but which might also be a result of metal binding by organics within the caterpillar. Du Toit et al. (1987) report that copper concentrations in the common housefly *Musca domestica* were twice as high if the larvae were grown in pig manure than in chicken manure. In an examination of copper and cadmium bioavailability to juvenile salmon, Buckley and Yoshida (1987) obtained evidence that organic ligands in sewage treatment plant effluent complexes the metals and reduces their bioavailability.

The use of swine manure for soil supplementation has been criticized because of high metal concentrations, particularly copper (e.g. Burns et al., 1987; Dueck and Duijff, 1987; Miller et al., 1987; Westerman et al., 1987). Similar concerns have been expressed about other animal manures (e.g. Krieger et al., 1987). Payne (1986), in a Ph.D. thesis, and Payne et al. (1985/86) report that the application of copper-enriched swine manure had no adverse effects on corn grain yields or on copper concentrations in corn ear leaves or grain. (See also Gettier, 1986.) Meeus-Verdinne et al (1986) report that (abstract) "Large, repeated applications of liq. swine manure did not change metal concns. in crops in comparison with mineral fertilization, and only minimal amts. of added metals were leached downward." In an examination of bioavailability of copper in pig feces, Izquierdo and Baken (1986) fed dried pig feces to chicks and then examined liver copper concentrations. Dietary addition of 748 mg Cu/kg diet, as pig feces, increased liver copper concentration threefold but had no effect on growth. In comparison, dietary addition of 500 mg Cu/kg diet as $CuSO_4$ù5H_2O depressed chick growth and increased liver copper concentration 42-fold.

Earlier in this section, properties of sewage sludge were said to be important in controlling metal bioavailability. pH, for example, will affect metal lability (e.g. Sanders and McM. Adams, 1987; Sanders et al., 1987) as will hydrological conditions (e.g. Melanen et al., 1985). However Tackett et al. (1986) report no significant change in copper leaching ability as the pH of composted sewage sludge was lowered from 7.0 to 2.5. Liming of sludge is still advisable as a means of maintaining higher pH values. Leaching is also affected by the nature of the leaching agent (e.g. Yamaguchi et al., 1986) as well as the source and treatment of the sewage sludge (Legret et al., 1987). Wastewaters from industry are considered a major source of metals, including copper, to sewage treatment plants (e.g. Piccone et al., 1986; Zhao et al., 1987) as well as to receiving waters (Rule, 1986). O'Riordan et al. (1986), for example, reports generally lower levels of metals in Irish sewage sludges and comments that this is a reflection of the level of industry in the country. Rossi et al. (1986) provide the chemical composition of sewage from different-sized cities in the Emilia Romagna region of Italy (tabular copper values presented elsewhere in this review). They comment that "With the exception of Zn for some samples, the heavy metal contents are below those proposed by other coutries of the EEC as limiting values acceptable for agricultural use of sludges." (See Griepink and Muntau, 1987, Krauss and Hagenmaier, 1984, and Schwedt and Hockendorf, 1986, for comments on some of the problems in determining the heavy metal content of municipal wastes.)

Removal of metals from sewage occurs in the primary sedimentation process and through activated sludge treatment (e.g. Tijero et al., 1987). Kempton et al. (1987a,b) and Stephenson and Lester (1987b) note that metal removal in this process is influenced by metal solubility and the settleability of insuoluble forms. Effective removal of soluble copper is achieved by the activated sludge process (Stephenson and Lester, 1987a). Garnett et al. (1987) indicate that the preferential complexation of nitrilotriacetic acid (NTA) with copper in soluble forms produces a change in metal speciation without a corresponding increase in solubility. Elliott and Linn (1986) and Frimmel and Geywitz (1987b), however, note a solubilization of copper from the particulate fraction suggesting an overall increase in soluble copper. Municipal wastes are often disposed through sanitary landfill operations although leachate may contain high levels of metals (Cyr et al., 1987). Schmitt and Sticher (1986) conclude that leakage of heavy metals through soil cannot be prevented. However, variability in the nature of the sludge and the nature of the environment will affect treatment of leachate from domestic waste landfill sites. Kelly (1987, page 261) recommends "... the need to evaluate combined treatment for each specific wastewater and leachate case." Encapsulation is a mechanism that has been considered and, in some cases used for handling hazardous sludges (Mishuck et al., 1985).

Anthropogenic Copper From the Production and Use of Fossil Fuels

Copper is found not only as an element of fossil fuels but also in the machinery and equipment and materials to recover, partition, and use these fuels. The effect of this is to introduce copper into soils and water as well as the atmosphere, along with other components of the fuels and handling which can affect the flux rate, chemistry and biological availability of copper (e.g. Smith, 1986). Although there has been concern about metal concentrations in drilling muds used for offshore petroleum drilling, there is no conclusive evidence that water, sediment and organism copper levels are or will be abnormally high (Boothe and Presley, 1987; Bothner et al., 1987b; Phillips et al., 1987; Thomas et al., 1986). The recovery of oil from oil shales is also of concern because of the potential for introducing metals into adjacent environments either through the processing or subsequent leaching of spent shales (e.g. Essington and Sorini, 1986; Sullivan and Carroll, 1985). Patterson et al. (1987) found partitioning of copper with the oil from the shale and comment that their results with trace elements in general, "... have important implications for shale oil refining and for the disposal of retort waters." Reddy and Lindsay (1987) examined metal uptake by plants grown on recarbonated retorted shale and suggests that recarbonation be used to adjust shale pH. They also suggest that revegetation of recarbonated spent shales can be done directly, without a soil cover.

Frequently, there are increased metal concentrations in soils adjacent to refineries (e.g. Biernacka and Liwski, 1986). Higher concentrations of soil metals are also associated with atmospheric emissions of coal-fired power plants although plant uptake of metals does not always indicate metal bioavailability (e.g. Long and Davis, 1987). With power plants, biological effects are often attributed to factors such as thermal pollution or ash deposits as well as to the effects of metals (Jiang and Cao, 1984).

Drainage through coal and coke piles has been shown to increase metal levels in the water. In a report by the Dearborn Environmental Consulting Service (1982), the factors governing drainage characteristics are stated to be the type and properties of stored coal, meteorological conditions and coal pile management practices. Coals from Western Canada, for example, contain less sulfur than coals from Eastern Canada and produce discharge samples that contain lower levels of dissolved metals. A great deal of this is believed to be due to the more neutral pH of the western coal. The biological impact of leachate metals released into streams will be controlled by chemical conditions in the streams. Tan and Coler (1986), for example, note an effect of stream fulvic acids in complexing metals thus reducing metal bioavailability.

Atmospheric input of metals occurs as a result of industry, fossil fuel utilization, sludge incineration, and natural processes such as volcanoes. As well, environmental metal levels can be increased as a result of effluents and leaching from byproducts of fossil fuel use. Banin et al. (1987) note increases in the concentration of certain metals, but not copper, in soil adjacent to a roadside in Israel. Copper does not appear to be a major problem in exhaust emissions, at least from diesel fuel (Millson and Hull, 1982). However, Szefer and Szefer (1986) recognize an anthropogenic source for aerosol copper, measured in rain water off the southern Baltic coast. Bridle et al. (1987) discuss the possible leaching of copper and other metals, from fly ash produced by incineration of municipal and hazardous wastes. Forstner (1986) discusses the chemical forms and environmental effects of metals in combustion residues, especially coal fly-ash particles. Breslin and Duedall (1987) found metal release from oil ash from oil-fired electric power plants. In 1:100 oil ash-seawater mixtures, 40% of the copper was leached from the ash. The migration of leached metal in soil is a result of metal speciation (Houle et al., 1987; see also Patel and Pandey, 1987) as is metal bioavailability. This means that, in soil, the bioavailability of copper leached from ash will be controlled by factors such as soil pH. Petruzzelli et al. (1987), for example, suggest that metal uptake from coal fly ash, by wheat seedlings, is a function of soil pH. Rainbow trout sensitivity to fly ash is related to dissolved metal availability, from the ash (Cherry et al., 1987). As well, Harris and Shifrine (1987) found that canine serum extracts of eight different fly ashes inhibited canine whole-blood lymphocyte activity and that the inhibition was related to metal concentration. Fenske (1985) reports that coal ash caused a drastic reduction in blood plasma copper concentrations in heifers fed ash-containing food. This is attributed to the high sulfur content as well as metals in the ash and metal-metal interactions.

The burning of sulfur-containing fossil fuels is responsible for acid rain. With repeated exposure, acid rain not only causes corrosion (e.g. Flinn et al., 1985) but reduces the ability of upland soils and lake sediments to retain atmospherically deposited trace metals (e.g. Bergkvist, 1986; McColl and Pohlman, 1986; Ulrich, 1984). The degree to which they are affected is open to debate (e.g. LaZerte, 1986), certainly at low levels of acidification. Arafat and Nriagu (1986) found the release of several metals (Cu, Ni, Zn, Cd, Fe) in lake sediments to increase exponentially below a threshold pH value of about 4.0. Soil pH adjustment, by liming can reduce metal (including copper) bioavailability (Markovic et al., 1987). In most cases, the metals that appear to be causing mortality are aluminum and posibly iron and manganese, released by increasing acidity (Borg, 1986).

The biological effects of acid rain have been extensively studied. In terms of copper, there is relatively little information other than that suggesting a possible increase in water metal levels as a result of copper extraction by the acid, from soils and sediments. However, in areas receiving anthropogenic copper and acid rain, additional copper may be made available to organisms (e.g. Bagatto and Alikhan, 1987a). In a study of bone metal concentration in white sucker (*Catastomus commersoni*) in relation to lake pH, Bendell-Young and Harvey (1986) found no obvious evidence of pH effect. Buckler et al. (1987) report that, with young striped bass (*Morone saxatilis*) in soft fresh water, toxicity of a copper-containing mixture of inorganic contaminants increased with decreasing pH.

Runoff from highway surfaces is often metal-enriched as a result of metal-containing materials in the roadbed and in oil-based products from vehicles (e.g. Ellis et al., 1987). Soil and plant copper concentrations tend to decrease away from a heavily used roadway (Ho and Tai, 1985). Tam et al. (1987) report a relationship between soil and plant metal levels and traffic volume although plant metal concentrations were in part a result of metal-enriched dust. Copper concentration has been shown to vary with particle size of the dust (Fergusson, 1987). Land use is also important. The type of industry present can, for example, affect the release of metal and thus, soil metal levels (e.g. Blake et al., 1987). Hall and Anderson (1985) note a major difference in copper concentrations between street surface contaminants in open or green space regions (117 mg/kg dry weight) and industrial regions (780 mg/kg dry weight). They also note that interval time between rainfall events, as well as land use, affected metal concentration and toxicity. The use of retention/detention ponds for runoff has been shown to reduce ultimate metal concentrations in the water (Nightingale, 1987; Yousef et al., 1986) although soil metal accumulation in the pond regions can increase substantially (e.g. Rowney et al., 1986). Krzysztofiak (1986) reports an effect of elevated levels of copper and lead on the numbers of pupae in ant colonies on roadside lawns and suggests that elevated metal concentrations could affect overall population size.

Contaminated Sediments and Dredging

The rate of accumulation of metals, including copper, in sediments is associated with natural and anthropogenic events (e.g. Smith-Briggs, 1983). Cause for concern arises because the rate tends to be higher with anthropogenic activity and dredging is usually in an area affected by anthropogenic activity. This is especially true with dredging of sediments from regions receiving sewage sludge or industrial effluents. The disposition of dredge spoils runs the risk of increasing biologically available metal. Vellinga (1987), for example, points out that approximately 20 million m^3 of sediments are dredged annually in and near the port of Rotterdam. The disposal of these dredge spoils is a problem not only because of the condition of the sediments but also the nature of the receiving environments, on land or at sea. Vellinga (1987) comments that a classification of the dredged material is being developed for the Rotterdam area, based on the ratio of contaminated fluvial silt and lightly contaminated marine silt. Disposal of dredge spoils in an upland environment is reported to "... allow physicochemical changes to occur that would significantly increase the solubility of Cd, Cu, Ni, Zn, and Mn" (abstract, Skogerboe et al., 1987). The authors continue, pointing out that release of metals could exceed the U.S. EPA maximum criteria for the protection of aquatic life. Capping contaminated dredged material with clean material has been used in aquatic environments to reduce exchange with adjacent sediments and the water column. Brannon et al. (1987) report that clay and silt appeared more effective than sand in preventing contaminant transfer to biota. Although non-burrowing, filter-feeding organisms did not evidence any increase in tissue metal concentration, burrowing organisms did. Burrowing organisms would also, over time, increase the availability of metals in the capped sediments.

To evaluate the potential for release of excess metal from dredged sediments it is first necessary to evaluate the concentration and chemical speciation of sediment-associated metal. Allan (1986), for example, comments that systematic collection and analysis of lake bottom sediments has been of benefit in determining the pollution status, trends and sources of pollutants in the Great Lakes. Saad (1987) comments on the seasonal variations of trace metals in a small shallow lake receiving polluted Nile water. The rate of sediment and sediment metal accumulation should then be monitored to estimate potential biological impact if dredging were to occur at particular times of the year. Simmers et al. (1986) used a suite of indicator organisms to evaluate the bioavailability of metals from contaminated dredged material and suggest that this provides an indication of natural conditions.

Van Coillie et al. (1986) used bacteria, a phytoplankton, and *Mytilus edulis* to evaluate sediments dredged mostly from Halifax harbour (Canada). They report little bioavailability of contaminants in the sediments although some indication of sublethal long-term effect. This seems to be representative of a number of dredged sediments, metal bioavailability being less than expected, possibly as a result of sediment metal complexing agents, probably a result of the combination of chemical and physical features of the sediment.

Sources of Copper for Man

In obtaining references about the biological importance of copper, the search program also identified a few references that consider sources of copper for man. The references deal with copper in manganese nodules, byproducts of their handling (e.g. Schein et al., 1987) and estimates of potential enviornmental damage from mineral extraction from the oceans (Waldichuk, 1987). Chen and Xu (1986) report copper concentrations ranging from 0.19 to 1.11% for nodules from the Northern Central Pacific. The nodules have a copper-scavenging capability which causes metal acquisition from nearsurface sediments (e.g. Ingri and Ponter, 1986). However, Tikhomirov and Shakhova (1987) report that physicochemical differences in the sediments and water affect the uptake and concentration of copper in the nodules.

Metal Recovery

The recovery of copper from both natural and anthropogenic systems is important not only because of the value of the metal but also because of potential detrimental effects with excess biologically available copper. A good deal of work has been recently done on the recovery of metals, from media ranging from apple juice to soils. As an example, clarification or other treatment of beverages (e.g. fruit juices, wines) is often to remove excess mineral elements, including copper (e.g. Dul'neva et al., 1987; Langhans and Schlotter, 1987; Loubser and Sanderson, 1986; Schlemmer, 1986). Leaching of metal from ores (e.g. Malakhova and Mukhamedova, 1986) continues to be of economic as well as environmental importance.

References on acid mine drainage have been previously discussed in this section. Reclamation of land such as mined areas and tailings or waste dumps is discussed by Tyre and Barton (1986) and Wong (1986). Although this is not metal recovery, *per se*, it often includes recovery of metals from industrial and mining wastes. In a report to the U.S. Bureau of Mines, Lapakko et al. (1986a,b), review and techniques and feasibility of removing several metals (Cu, Ni, Co, Zn) from stockpile effluent. The importance of trace metal removal from aqueous solutions is indicated by the symposium organised by the Industrial Division of the Royal Society of Chemistry, held in 1986 (Thompson, 1986). Clifford et al. (1986) review the problems and processes of removing dissolved inorganic contaminants from water. Numerous methods and techniques have been proposed. Coordinating copolymers form a mechanism for recovery of metals from both aqueous and non-aqueous media (Hudson, 1986). Chemical removal includes the use of sodium borohydride (Ulman, 1986), precipitation with carbonates (Elfine, 1987) or other coagulants and flocculants (e.g. Begovich et al., 1986; Milton, 1987; Patterson et al., 1987; Terashima et al., 1986), sorption (e.g. Flytzani-Stephanopoulos et al., 1984) and ion-exchange techniques (Gupta et al., 1985; Sokolova et al., 1986). Biological mechanisms include complexation-sorption-precipitation techniques (DeVoe and Holbein, 1986; Maree et al., 1987), uptake by bacteria (e.g. Francis and Dodge, 1987; Hancock, 1986; Lambda Group Inc., 1986; Morper et al., 1987), algae (Darnall, 1985, 1986; Greene et al., 1987), aquatic plants (Devi and Gopal, 1986; Nor and Cheng, 1986), and byproducts of the fishing industry (chitosan and fish scales; Bell et al., 1987; Muzzarelli and Rocchetti, 1986; Yang, 1984). The August 1987 report by the U.S. National Technical Information Service (1987d) provides a number of references on the uptake of metals from liquid wastes. Huff and Huff (1986) examine the feasibility of a central recovery facility for the metal finishing industry in Cook County, Illinois. This arose from the potential plant closures that would occur with implementation of U.S. pretreatment standards.

McLean et al. (1986) examine various ways to reduce the metal leaching from soil systems impacted by metals from industrial sources. Bewley et al. (1987) discuss the use of a microbial system for land decontamination of a gasworks site in England; they are primarily concerned with organic decontaminants. The industrial use of microorganisms in the leaching of copper from copper-bearing ores (Belyi et al., 1986; Espejo and Ruiz, 1987; Munier-Lamy and

Berthelin, 1987) indicates the ability of biological recovery systems. Hutchins et al. (1986) provide a good review on the use of microorganisms in reclamation of metals. Other organisms that concentrate metals (e.g. mosses - Onianwa and Ajayi, 1987) may also serve to reclaim metals from metal-rich sites.

Metals and as well as other materials, recovered from metal-working facilities have been used in fertilizers having bactericidal and fungicidal activities (e.g. Airapetyan et al., 1985; Nabiev et al., 1987b; Suteu et al., 1985). Uptake by plants has been of some concern (Tailkov and Mamedkhanov, 1983). Recovery of metals from household refuse is discussed by Bidlingmaier et al. (1984) and treatment of leachate from domestic waste sites is discussed in Kelly (1987) and Maloney (1986) and has been mentioned earlier in this review. Leaching of metals from metal-rich sites has been and will be of continuing concern in the use of sites for sludge and wastewater treatment as well as industry (see Desjardins et al., 1986).

III - COPPER SPECIATION AND ITS BIOLOGICAL IMPORTANCE

The occurrence and concentration of copper in the environment and in organisms continues to be of interest to those that are tracing the geochemical or biological fate of the metal (e.g. Baeyens et al., 1987a; Bewers et al., 1986a; Hites and Eisenreich, 1987; Merian, 1984). Knowledge of the properties and reactivities of metals is important to the metallurgist and chemist (e.g. Belew et al., 1987; Forstner, 1984; Jardim et al., 1986; Sermon, 1986). This information is of value in studies ranging from the effect of environmental variables on copper corrosion (Cohen and Myers, 1987; Reiber et al., 1987a,b; Stone, 1987) to the interaction of cells with various copper species (e.g. Jones et al., 1987) to the control of algae in municipal waterworks (Raman and Cook, 1986). With environmental work, more and more effort is being directed towards an understanding of metal speciation. As Landner (1987) comments (preface) "It is to-day generally recognized by environmental scientists that the particular behaviour of trace metals in the environment is determined by their specific physico-chemical forms rather than by their total concentration." Metal speciation thus becomes important (e.g. Bernhard et al., 1986a,b; Stumm and Keller, 1984) and, as stated in Bernhard et al. (1986c, page 8), "An effort should be made to develop chemical methods suitable for species identification and quantification". Kester et al. (1986) discuss the nature of chemical species in marine and estuarine systems and some of the environmental factors that influence metal speciation. Carlson et al. (1986a) provide an excellent discussion on the development and validation of site-specific water quality criteria for copper.

Techniques for the analysis of copper in both medicine and biology are discussed in the report by the "Fourth International Workshop on Trace Element Analytical Chemistry in Medicine and Biology (1986). They also appear in a workshop report edited by Bratter and Schramel (1987) and, for metals in the environment, by Stoeppler and Nurnberg (1984). Although mentioned elsewhere in this review, the nature and accuracy of various analytical techniques are important and dictate not only the ability to measure total copper but, especially, to estimate concentrations of different copper species (e.g. Lieser et al., 1985; Lund, 1986; Moffett and Zika, 1987a; Sweileh et al., 1987). Mendine and Bicknell (1986) discuss a metals exposure analysis modeling system which they suggest "... should help discriminate between the fraction of the metal that is dissolved and in bioavailable form and the fraction that is complexed and is relatively nontoxic". Another area of interest is effect of metal-metal interactions on copper accumulation and toxicity (e.g. Kazumi et al., 1987) as well as the effect of metal speciation on biologically important metal-metal interactions (e.g. Jessie and Smith, 1987).

The approaches to metal speciation analysis form the major topic in at least two recent publications, one concerning all natural waters (Morrison, 1987b), the second marine waters (Kramer, 1986). Motekaitis and Martell (1987; see also Martell et al., 1987) examined metal speciation in surface waters of the open ocean, using critical stability constants of inorganic complexes while Turner and Whitfield (1987) derived an equilibrium speciation model for copper, glycine, EDTA and NTA in estuarine waters. The model was for (abstract) "... use in the calibration of complexation capacity methods, and in the interpretation of bioassay measurements." Andreae (1986) discusses chemical species in seawater and marine particulates. Whitfield and Turner (1986) point out some of the shortcomings in what they term "the traditional equilibrium approach", due in part to the uncertainties in the constants for complexation, hydrolysis and protonation equilibria. As well, equilibrium may not be achieved in seawater, limiting the application of equilibrium models (Kester, 1986). Andreae (1986) comments (page 315) that "Despite all the work on Cu-organic binding, the ligands are characterized only in a very indirect way. Most studies have not even attempted to identify the chemical nature of the ligands but simply treat them as a 'black box'." These problems must be addressed because of the importance of understanding, and determining metal speciation in environmental and biological systemsd (Wolf, 1986; Wolf et al., 1986). As Bernhard and

George (1986) point out, metal bioavailability, uptake, loss and toxicity, as well as deficiency, are controlled by metal speciation as well as metal concentration. This can be taken one step further - even the metal ion activity in an organometallic complex is affected by the oxidation state and geometry of the complex (e.g. Kau et al., 1986).

A number of references deal with the effects of natural or anthropogenic systems on metal speciation in water and sediments. Hall and Anderson (1985) discuss urban runoff from industrial and commercial land use sites. Tessier and Campbell (1987) relate metal partitioning in sediments to metal bioavailability while Rice and Whitlow (1985) relate the early diagenesis of transition metals to metal partitioning between macrofaunal populations and shallow sediments. Munawar et al. (1985) report that the toxicity of Detroit River sediment-bound contaminants was due to metal bioavailability, as indicated by water extraction capability. Moffett and Zika (1987b) discuss the importance of hydrogen peroxide in natural surface waters and examine its effect on the oxidation of copper and iron. Millero et al. (1987) examine the importance of ionic interactions on the rates of oxidation of iron and copper in natural waters. Kumar (1987) estimated cation hydrolysis for a number of elements (including copper) in seawater, commenting on the effect of elevated temperatures, as would be found near active hydrothermal vent systems. The effect of environmental acidification on metal speciation, physicochemical form and toxicity has also been examined (Arafat and Nriagu, 1986; Campbell and Tessier, 1987; Cusimano et al., 1986; Servos et al., 1987; Sprenger, 1987; Stokes and Campbell, 1986). The chemistry of copper, and several other metals, in anoxic basins has been examined by a number of workers, both in terms of metal speciation with sulphur and general metal speciation and concentration (Dyrssen, 1986; Jacobs et al., 1987). Balls (1985) reports "trace metal fronts" in Scottish coastal waters, commenting on the relatively sharp changes in copper concentrations occurring as a result of changes in hydrographic conditions. Metal distribution in aquatic sediments is controlled by sediment nature and chemistry as well as metal flux rates (Cauwet, 1987; De Souza et al., 1986; Forstner et al., 1985; Lee, 1985; Mudroch, 1985; Radovskaya et al., 1982; Salomons et al., 1987a; Schoer and Eggersgluess, 1982; Thornton and Seyfried, 1987). Metals can also be mobilized from sediments (Forstner and Salomons, 1984). Heggie et al. (1987), for example, report manganese and copper fluxes from continental margin sediments, estimating a 2 nmole/cm^2/year flux that could be a source of copper to the deep sea. Sediment interstitial water may contain high concentrations of copper, especially in the presence of organic metal complexing agents (e.g. Douglas, 1987)

In soils, as in sediments, the distribution and chemical as well as biological availability of copper and other trace elements is affected by the chemical characteristics of the soil (e.g. Chen and Stevenson, 1986; Cruz Canadas et al., 1986; Fischer, 1987; Gembarzewski et al., 1986; Gworek, 1986; Kaplunova and Bolshakov, 1987; Linder and Voye, 1987; Minnich and McBride, 1987; Nielsen, 1986; Sanders and Adams, 1987; Shukla and Rattan, 1985; Singh and Mishra, 1987). It may also be affected by the chemistry of any introduced copper (e.g. Yamaguchi et al., 1986). Comparisons of soil and sludge-ammended soil extraction procedures have been made by a number of authors (e.g. Barbarick and Workman, 1987; Burridge and Hewitt, 1987; Hani and Gupta, 1986). With oil-containing soils or spent oil shales, metal extraction and metal effect is controlled by the chemistry of the medium (El-Lebondi et al., 1985; Sullivan and Carroll, 1985). These characteristics also affect metal concentrations in pore water (Bohn and Bohn, 1987). Soluble organic acids can increase metal release from soils although McColl and Pohlman (1986) found little correlation between the initial pH of the organic acid and the rate of metal dissolution. Bergkvist (1986) reports that the mobility of several metals (Fe, Al, Cu, Pb) was regulated mainly by the formation of water-soluble organic compounds rather than directly by pH variations. Copper availability (both chemical and biological) apparently changes over time. Miller et al. (1987) found increases in short-term availability and mobility in soils with copper supplementation although they suggest that longer-term reversion processes may reduce metal bioavailability. This may be one reason why Bradley and Cox (1987) found only low concentrations of easily-extracted copper in North Staffordshire (U.K.) soils affected by historical copper and lead mining activities.

A number of references discuss copper speciation as well as the association of copper and organics in environmental media (e.g. Anikiyev et al., 1985; Frevert, 1985; Mackey et al., 1987; Nor, 1985; van den Berg et al., 1987). The effect of this interaction is of importance both to the isolation and measurement of copper (e.g. Mannino et al., 1987; Plavsic and Branica, 1986; Rostan and Mouvet, 1986; Turner et al., 1987). In a Ph.D. thesis, Buckley (1985) discusses organic speciation of copper, zinc and lead in seawater and comments (page 200) that "... copper is complexed to an extent in excess of 95%." Craft provides a controlled flow-rate Chelex technique which he reports indicates trace metal lability in mine drainage water. Florence (1987) and Zhang and Florence (1987) used an aluminum hydroxide-coated cation exchange resin to estimate copper bioavailability in natural waters. Zorkin et al. (1986) report an ion-exchange procedure for quantifying biologically active copper in sea water. Morrison (1987) and Morrison and Revitt (1987) used a metal-chelating resin in a dialysis membrane to estimate bioavailable metal uptake in polluted fresh waters. Jardim and Gimenez (1987a,b) used a computer model to estimate cupric ion activities in natural waters.

Evidence continues to indicate that accumulation as well as toxicity of copper is a function of metal speciation rather than the concentration of copper (Bernhard and George, 1986; Comber et al., 1987; Haley et al., 1986; Mannino et al., 1987; Mouvet and Bourg, 1987; Nor and Cheng, 1986; Pagenkopf, 1986; Starodub et al., 1987b; Stauber and Florence, 1987a; Sunda et al., 1987; Turner et al., 1985; see also Petering and Antholine, 198, in Hodgson et al., 1988). Khangarot et al. (1987b), for example, report that several amino acids can reduce copper toxicity to *Daphnia magna*. Wood et al. (1986) discuss "chemical species in systems under stress", relating to the physical as well as chemical conditions in physically and chemically stressed systems. Cowan et al. (1986) discuss the use of a geochemical model with toxicity measurements, to relate metal speciation to biological effect. In the examination of the effects of copper on cell growth in freshwater phytoplankton, Thompson et al. (1986) suggest that humic acids as well as cellulose fibers from paper mill effluent may affect copper bioavailability. In aquatic media, the ability to affect bioavailability appears to vary in response to the concentration of organic ligands as well as the concentration of copper (e.g. Sunda, 1987).

The total concentration of copper in soils has been, and still is, measured as an indicator of metal bioavailability (e.g. Singh, 1987; Smirnova and Motuzova, 1985; Zaiko and Lobach, 1986). Total metal concentrations are frequently compared to concentrations obtained with various extractants to obtain estimates of bioavailability (Shah et al., 1986). Soil properties have been widely examined to explain the physico-chemical behaviour and biological availability of copper as well as other metals (Amacher et al., 1986; Jasiewicz and Gambus, 1986; Minnich et al., 1987; Sakal et al., 1986; Scokart and Meeus-Verdinne, 1986; Tonkopill et al., 1987). Brown et al. (1987) report an increase in soil metal cation mobility with reduced pH values and increased chloride ion concentration. (Verloo and Cottenie, 1985, report a similar effect of pH in river sediments.) Soil pH has also been related to metal uptake by plants (Iwasaki et al., 1987) and metal concentrations in earthworms (Beyer et al., 1987). A roadway deicing salt, calcium magnesium acetate, was examined by Elliott and Linn (1987) for its effect on soil metal mobility. They report its use is associated with a temporary increase in metal translocation. Ruszkowska et al. (1984) report that the kind of soil and method of use as well as soil manipulation practises affects metal concentrations as well as bioavailability. Organic matter in soils has a strong effect on metal bioavailability (Chen and Stevenson, 1986; Iwasaki et al., 1987; Terenko and Obraztsova, 1985). Metal complexing agents are, in general, associated with a decrease in plant copper uptake (e.g. Checkai et al., 1987b; Nwokolo, 1987a) and ion exchange and metal chelating resins can be used to control the activities of free metal ions, at least in hydroponic solutions (Checkai et al., 1987a). Copper bioavailability and uptake can also be affected by nutrients such as phosphorous (Al-Showk et al., 1987) as well as excesses of metals such as molybdenum (Gorlach and Gorlach, 1984; Leech and Thornton, 1987) and soil pH (e.g. Bouchard and Gagnon, 1987).

Without metal supplementation, deficiencies will occur in copper-deficient soils (Blue and Malik, 1986); differing mineral requirements dictate impact of deficiencies on plant growth (e.g. Teasdale et al., 1986). However, even within a species of plant, there are differences in requirements as well as tissue metal concentrations, as a result of genetic variability (e.g. Korcak, 1986a). As well, crop management can affect metal concentration as well as bioavailability (e.g. Fairey, 1986; Hammel and Mahler, 1986). Soil liming affects pH and the chemical availability of many metals, copper perhaps less than most (Jahiruddin et al., 1986). The use of fertilizers or sewage sludges as nutrient sources at least has the potential to increase soil metal concentrations as well as metal bioavailability although this will be controlled by soil chemical properties and metal concentrations (e.g. Sanders et al., 1987; Solov'ev et al., 1987). Sewage and sewage treatment plant effluent also contains metal complexing agents that can affect both availability and toxicity of copper (Buckley and Yoshida, 1987). In an examination of soil copper availability as a result of copper-enriched swine manure, Payne (1986) comments that "... the distribution of applied Cu among soil fractions was dependent on soil pH, length of time following Cu additions, and to some degree on the source of applied Cu."

The Nutrition Foundation (1986) discusses the assessment of copper bioavailability in humans and some of the extrinsic factors that affect availability. Chemical speciation is important both in the absorption by and distribution of the metal within the organism (Mills, 1986; Poole et al., 1987; Stetsenko et al., 1985). With grazing animals such as sheep and cattle, the availability of copper to plants affects the uptake (e.g. Morton, 1986). So also can the ability of the plant to concentrate metals. As well, the presence of fiber and metal complexing agents within the plant may reduce the metal available for uptake (e.g. Honig and Wolf, 1987; Honig et al., 1987; Laszlo, 1987). Differences in trace element bioavailability, between human and cow's milk are suggested to be a result of reduced digestability of casein in cow's milk (Lonnerdal, 1987). Nelson et al. (1987) report that, in decaseinated cow's milk at least, coprecipitation of copper and other trace metals, occurs with calcium phosphate and may affect trace metal uptake. Uptake of copper from food may also be affected by the type of dietary carbohydrate, at least in copper-deficient rats (Johnson and Gratzek, 1986). Metal distribution within the organism can be affected by the physiological status of the animal. Freeman and O'Callaghan (1987) demonstrate an increase in ceruloplasmin-bound copper and report a decrease in bioavailable copper in arthritic patients.

The old saying that every action has a reaction is applicable to the interaction between metal speciation and metal uptake by organisms. Organisms can affect metal speciation. Francis and Dodge (1987) comment that in subsurface soils, anaerobic microbial activity can have an effect on the dissolution of metals. The degree of binding of copper by bacteria is dependent upon the nature of the surface ligands, binding can reduce the interaction with hydrous oxides. Surface uptake of metals by macroalgae can buffer the availability of non-sediment metals to coastal marine organisms (Higgins and Mackey, 1987). Fungi have been suggested to biodegrade copper-containing organics, thereby releasing metal (Shavlakadze et al., 1987). Reduction of Cu(II) complexes can occur on the cell surface of some phytoplankton, through plasmalemma redox enzymes (Jones et al., 1987). Izquierdo and Baker (1986) report that, with copper in pig feces, organic agents reduce metal bioavailability. (This is characteristic of sewage and sewage effluent.) Within the organism, metal-complexing agents will affect metal speciation (e.g. Takahashi et al., 1987b), affecting not only the fate of the metal in the organism but also the availability of the metal to higher levels in the food chain (e.g. Honig and Wolf, 1987).

III.1 ORGANIC COPPER COMPLEXING AGENTS

Buffle and Altmann (1987) make the comment (abstract) that "Our ability to measure and interpret the cation complexation equilibria of (complexing)... substances is ... severely limited by their being inherently ill-defined and by their common possession of certain complex qualities." Measurement of certain organic complexing agents is now possible (e.g. Gaisser et al., 1985; Lam and Malikin, 1986) and greater understanding of their chemistry is being achieved (e.g. Collman et al., 1987; Hanson and Pilbrow, 1987). However, knowledge about the relationships in natural environments as well as in organisms still leaves a great deal to be desired. This is true for foods (e.g. Homma et al., 1986; Kohn et al., 1986a) as well as environmental agents. Brouwer et al. (1986) examine trace-metal binding proteins in marine organisms, with respect to cadmium rather than copper, and comment (abstract) thata "The research findings from this project have improved our understanding of the processes involved in the metabolism of trace metals and their effects on marine fish and shellfish under normal and stressed conditions. Such information is mandatory before use can be made of these proteins as indicators of trace-metal pollution." In foods, there may also be an interaction of organics, metals and taste (Greeley et al., 1986).

Hayase et al. (1986) describe a method for determining organically-associated trace metals in estuarine sea-water and Pellenbarg (1985) discusses the geochemistry of organic litter from the plant *Spartina alterniflora* in water and sediments of a salt marsh. The litter is shown to be able to scavenge copper and other metals from marsh tidal waters. Equilibrium models with known and unknown organics in seawater have been developed by Turner and Whitfield (1987) as well as others. Equilibrium models also exist for chemical speciation in digested food (Robb and Williams, 1986). The relationships between organics and sediments have been discussed by Lin et al. (1984), for the East China Sea, and Reboredo and Pais (1984) for a salt marsh in the Sado Estuary in Portugal.

Major groups of organic complexing agents are considered separately in this review. Each has a brief introduction and then a list of the references selected for the section. Because of their nature, some references not listed here are discussed elsewhere in the INCRA review.

Complexing agents in natural and anthropogenic environments

References are numerous and deal with a variety of natural and anthropogenic agents that affect the biological availability of copper. Examples of this literature are given here but numerous examples are discussed elsewhere in this review.

Coastal environments: Mackey et al., 1987; Mills et al., 1987; Moffett and Zika, 1987a; van den Berg and Rebello, 1986.

Oceanic and near-oceanic environments: Hanson et al., 1988; Osteroht et al., 1988.

Freshwater environments: Kyle, 1987

Soils - agents other than humic substances: Linder and Voye, 1987.

Sewage and sewage sludge: Garnett et al., 1987; Jarrell et al., 1987.

Humic Substances

A diverse group of organics found in soils and sediments as well as in water. Many of these organics have the ability to regulate copper bioavailability and hence uptake by plants. Because of their chemical nature, they can produce major changes in metal chemistry and metal concentrations in estuaries.

Reviews: Chen and Stevenson, 1986; Hine and Bursill, 1985.

Occurrences: Il'in and Baidina, 1986; Shanmukhappa et al., 1986; Sigleo, 1987; Wu et al., 1985.

Complexing capacity: Giesy et al., 1986; Mingyi, 1985; Ram and Raman, 1986; Sapek, 1986; Steinbrich and Turski, 1986; Yamada et al., 1987a,b,c.

Chemistry: Blaser and Sposito, 1987; Buffle et al., 1987; Frimmel and Hopp, 1986; Lamy et al., 1987; Lapin and Krasyukov, 1986; Linder, and Murray, 1987; Osterroht and Wenck, 1983; Ryan et al., 1987; Senesi and Sposito, 1987; Senesi et al., 1986, 1987; Sohn and Weese, 1986.

Biological importance: Andrzejewski and Doregowska, 1986; Giannissis and Martin, 1984; Hutchinson and Sprague, 1987; Iwasaki et al., 1987; Krtkova and Tichy, 1985; McCarthy, 1987; Saleh et al., 1987; Teasdale, 1987; Thompson et al., 1986.

Sugars, amino acids, peptides and proteins other than enzymes

A variety of sugars, amino acids, peptides and proteins contain copper and serve important functions in organisms. One example of this is the blood pigment haemocyanin found in some molluscs and crustaceans. Others include the blue copper proteins, some of which are enzymes. Many of these are of interest to the chemist because of their unique structures or reactions. Others are of interest because of an ability to affect organisms or cell growth. Many of the references listed below concern the involvement of copper in the chemical synthesis of biologically active organic compounds. As such, although they often do not "fit" into a general review.

Sugars and sugar-derivatives: Baker, 1987; Hsieh and Harris, 1987

Amino acids: Antolini et al., 1986; Armani et al., 1986; Arnold et al., 1987; Berthon et al., 1986; Bhaskare and Kulkarni, 1986; Buss et al., 1987; Cheng and Pang, 1986; Danielle et al., 1986; Devi and Reddy, 1986; Formicka-Kozlowska, 1985a; Haider et al., 1986; Hitchman et al., 1987; Ishida et al., 1986; Jezierska, 1987; Kala and Reddy, 1986; Kowalik et al., 1987; Livera et al., 1987; M'Boungou et al., 1987; Sigel, 1985; Sovago and Petocz, 1987; Strange et al., 1987; Szabo-Planka, 1985; Vishnumurthy and Lingaiah, 1986;

Blue copper proteins: Ainscough et al., 1987; Bacci and Cannistraro, 1987; Feiters et al., 1986; Goodwin et al., 1987; Gray, 1986 (review); Jackman et al., 1987; Suzuki et al., 1987; Tollin et al., 1986; Trost et al., 1987; Wright and Chazin, 1987;

Haemocyanin and haemocyanin-like compounds: Bak et al., 1986; Beltramini, 1985; Beltramini et al., 1986; Cruse, 1986; Drexel et al., 1986, 1987; Karlin et al., 1987; Naich and Alikhan, 1987; Pate et al., 1987; Ricchelli et al., 1986; Rogener et al., 1987; Telfer and Massey, 1987; Wang et al., 1986;

Globin-copper interactions: Eguchi and Saltman, 1987; Hegetschweiler et al., 1987; Kaivarainen and Rozhkov, 1987

Hydroxamic acids: Leporati, 1986, 1987a,b.

Miscellaneous organics, including proteins and peptides: Abu-Gharib et al., 1986; Anson et al., 1987; Arber et al., 1986; Asaturian, 1986, Asaturian et al., 1986; Bartmann et al., 1987; Bates and Farina, 1987; Bell et al., 1987; Bencini et al., 1987; Boring and Sindelar, 1987; Bratkowska and Zwierzykowski, 1986; Buchanan and Dismukes, 1987; Caswell and Spiro, 1986; Cazorla et al., 1986; Champagne and Hinojosa, 1987; Chondhekar and Khanolkar, 1986; Chukwumerije and Nash, 1987; Clark et al., 1986; Colombo et al., 1987; Couture-Tosi et al., 1986; Crabbe et al., 1985; Decock-Le Reverend et al., 1987; Driscoll and Kosman, 1987; Dubler et al., 1987; Emanuel and Bhattacharya, 1987; Ferrari and Marzona, 1987; Formicka-Kozlowwska, 1985b; Foster and Tessmer, 1987; Fritschi et al., 1986; Funahashi et al., 1986; Garcia et al., 1987; Gewirth et al., 1987; Guss et al., 1986; Harayama et al., 1987; Harrington and Jones, 1987; Hatano, 1987; Ibuka et al., 1986; Inoue and Hirobe, 1987; Jimenez et al., 1987; Kalbag and Voelker, 1987; Kanoh and Maeda, 1987; Kavlentis, 1987; Kiss and Szucs, 1986; Kobayashi and Nishiyama, 1986; Koch et al., 1987; Kohn et al., 1986b; Lehtonen, 1987; McCrindle et al., 1986; Martin and Evans, 1987; Martnovich et al., 1986; Merchant and Bogorad, 1987b; Micskei and Nagypal, 1986; Nair et al., 1987; Naruta et al., 1987; Ogino et al., 1987; Palumbo et al., 1987; Pesek et al., 1986; Pettit et al., 1987; Rebello and Reddy, 1986; Sakurai and Nakahara, 1986; Saxena and Agarwal, 1986; Scholes et al., 1987; Speier, 1986; Subczynski et al., 1987; Suzuki et al., 1986; Syed and Coombs, 1986; Tokita and Morita, 1987; Toussaint et al., 1987; Valent et al., 1987; Vanni and Gastaldi, 1986; Veselinovic and Kapetanovic, 1986; Weser, 1986; Wissler et al., 1986a, 1987; Woon et al., 1986; Yokoi et al., 1986;

Nucleic acids: Babii et al., 1986; Banville et al., 1986; Basile and Barton, 1987; Basile et al., 1987; Blagoi et al., 1987; Chen and Sigman, 1986; Divakar et al., 1987; Ehrenfeld et al., 1987; Fujimoto et al., 1986; Galy et al., 1987; George et al., 1986; Goldstein and Czapski, 1987b; Kuwabara et al., 1986; Laundon and Griffity, 1987; Lee and Roschenthaler, 1987; Lehn et al., 1987; Mochizuki et al., 1987; Morii et al., 1986; Patiashvili et al., 1987; Pawlowski et al., 1987; Prasal, 1986; Sari et al., 1986; Sayenko et al., 1987; Sorokin et al., 1986, 1987; Takahashi et al., 1987a,b; Theophanides and Tajmir-Riahi, 1986; Wedrychowski et al., 1986; Wissler et al., 1987a,b.

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 with copper deficiency. Zeppezauer and Maret (1986) and Khandelwal et al. (1987) discuss the relationship between the chemistry and reactivity of selected enzymes.

Amylase - Agarwal and Henkin, 1987;

ATPase - Shinohara and Terada, 1987;

Cu,Zn-superoxide dismutase (SOD) - an extremely "... important antioxidant enzyme because of its ability to convert superoxide to the less reactive H_2O_2, ..." (Hass and Massaro, 1987a, page 697).

Reviews - Rotilio, 1986.

Origin, evolution - Parker et al., 1986; Steffens et al., 1986a.

Activity (concentration) - Barra et al., 1986; Bast et al., 1986 (activity of SOD analogues); Dameron and Harris, 1987; DiSilvestro, 1987; Dubinina et al., 1986; Hass and Massaro, 1987; Sharma and Prasad, 1985.

Abnormal activity (concentration) - Arai et al., 1987; Czapski and Goldstein, 1986a; Kamei et al., 1985; Roberts and Robinson, 1986; Shiki et al., 1987.

Biochemistry, chemistry, genetics - Argese et al., 1987; Beyer et al., 1987; Cabelli et al., 1987; Cannon and Scandalios, 1987; Czapski and Goldstein, 1986; Delabar et al., 1987; DuElroy-Stein et al., 1986; Flohe et al., 1986; Goldstein and Czapski, 1986b; Groner et al., 1986b; Gulyaeva, 1987; Hallewell et al., 1986, 1987; Hasnain et al., 1986; Janecki, 1986; Jewett, 1986; Kitagawa et al., 1987; Klapper et al., 1986; Marmocchi et al., 1986; Natvig et al., 1987; Reed-Scioli and Zilinskas, 1987; Schinina et al., 1986; Simonyan, 1986; Steffens et al., 1986b; Steinman, 1987; Thaete et al., 1986; Uda et al., 1986, 1987; Wispe and Burhans, 1987; Yano and Kikkawa, 1987.

Functions - Gerdin et al., 1986; Gutteridge, 1986; Manohar and Balasubramanian, 1986; Plonka et al., 1986; Reiners et al., 1986; Simonyan and Nalbandyan, 1986.

Hydroxylases - Martinez et al., 1987; Obata et al., 1987; Pember et al., 1987;

Laccase - Germann and Lerch, 1986; Huber and Lerch, 1987; Kau et al., 1987; Morpurgo et al., 1986;

Miscellaneous enzymes - Arthur et al., 1987; Bayliss and Prescott, 1986; Blackburn et al., 1987; Boschi et al., 1987; Bryan et al., 1985; Carpenter et al., 1987; Flaksaite et al., 1987a,b; Goyne and Sigman, 1987; Hiraoka et al., 1987; Huber and Lerch, 1986; Kono et al., 1987; Maret and Kozlowski, 1987; Rhodes, 1987; Rosoiu et al., 1987; Sellin et al., 1987; Tyagi and Wu, 1987.

Oxidases - Bombelka et al., 1986; Brenner and Klinman, 1987; Chatfield and Armstrong, 1987; Delhaize and Webb, 1987; El-Kadousy and Alexandrescu, 1987; Ellis, 1986; Fujii et al., 1987; Gelles, 1986; Kitajima et al., 1986; Li et al., 1987; McCracken et al., 1987; Malmstrom, 1986; Marsh, 1986; Mondovi et al., 1987; Morpurgo et al., 1987; Nilsson et al., 1987; Powers and Ching, 1987a,b; Powers et al., 1987a-c; Roche-Mayzaud and Mayzaud, 1987; Sakurai et al., 1987; Syvertsen et al., 1987; Steffens et al., 1987; Thomson et al., 1986; van Iersel et al., 1986; Waage et al., 1985; Young and Caughey, 1987;

Reductases - Dooley et al., 1987; Kashem et al., 1987; Machoy et al., 1985; Snyder and Hollocher, 1987; Zumft et al., 1987a,b;

Transferases - Singh et al., 1987; Tarnawski et al., 1987

Metallothionein

Metallothionein or metallothionein-like organics such as phytochelatin play major roles in the transport of copper in organisms ranging from yeast to humans. They have also been considered important in reducing the biological availability of excess metal within the organism.

Reviews: Azeez et al., 1985; Hamer, 1986.

Occurrences: Chan et al., 1987; Doherty et al., 1987; Grill et al., 1987 (phytochelatins); Haratake et al., 1987a; Lanno et al., 1987; Purvis et al., 1987 (norstictic acid); Robinson and Thurman, 1986; Robinson et al., 1987 (tris glycines from copper-tolerant plant species); Witkus et al., 1987.

Induction: Blalock et al., 1987; Clarke et al., 1987 (considers induction of siderophore activity and its moderation of copper toxocity); Foster et al., 1987; Germann and Lerch, 1987; Heilmaier, 1986; Heilmaier et al., 1986; Thiele and Hamer, 1987.

Tissue levels: Bremner and Morrison, 1986; Bremner et al., 1987; Gallant and Cherian, 1986; Heilmaier et al., 1987a,b; Hopf et al., 1986.

Chemistry: Byrd and Winge, 1986; Capasso et al., 1987; Dielhof et al., 1987; Funk et al., 1987; Gulati et al., 1987; Hartmann et al., 1987; Heilmaier, 1984; Lobel and Payne, 1987; Munger et al., 1985; Winge et al., 1987.

Function: Bremner, 1987a,b; Engel, 1987; Engel and Brouwer, 1987; Freedman, 1986; Hamer, 1986; Heilmaier et al., 1987a,b; Klasing et al., 1987; Lefebvre et al., 1987 (does not consider copper); Mench et al., 1987 (exudates from maize, not metallothuionein); Sugawara and Sugawara, 1987; Summer et al., 1986; Suzuki et al., 1987.

Detoxification: Delval, 1984; Elinder et al., 1987; Roesijadi, 1986; Roesijadi and Fellingham, 1987.

III.2 ADSORBING AGENTS

Metal sorption by particles plays an important role in speciation and the fate of metals in all environments (Adediran, 1985; Allard et al., 1987; Leckie, 1986; Whitfield and Turner, 1987). (See review by Alvarez Gonzalez, 1985, on adsorption to colloidal surfaces.) It also affects our ability to accurately measure concentrations of dissolved metals (Salim, 1987a) and has been responsible for the development and evaluation of techniques to extract and measure sorbed metals (e.g. Bartoli et al., 1987; Chang et al., 1987; Frimmel and Geywitz, 1987a; Okazaki et al., 1986; Plavsic et al., 1987; Slavek and Pickering, 1987). Morel and Gschwend (1987) note the potential importance of colloidal-sized particles in sorption and the lack of specifics about their role. In a discussion of river and estuarine waters containing acid mine drainage, Johnson (1986) comments (abstract) that "... binding to the surfaces of amorphous Fe oxyhydroxides regulates Cu and Zn concentrations in solution, in both fresh and saline waters. The sorptive process is pH dependent and is in general agreement with laboratory studies of trace metal adsorption on amorphous Fe oxyhydroxides."

Sorption of copper occurs with particulate iron and manganese (e.g. Chen et al., 1987a; Nozaki and Zhou, 1987). At the sediment-water inteface, ferromanganese nodules have been associated with copper sorption (Ingri and Ponter, 1986). Albert and Yates (1987), Stumm (1987) and Schindler and Stumm (1987) review the surface chemistry of oxides, hydroxides and oxide minerals in solutions and Stone and Morgan (1987) discuss the effect of changes in oxidation state of metal oxides that are capable of metal sorption. Other references pertinent to an understanding of metal sorption include Aualiitia and Pickering (1987b), on sorption of Cu, Pb and Cd by inorganic particulates, Liu et al. (1986) and Zhang et al. (1987), on metal partitioning on clay minerals in seawater, Patterson et al. (1987), on the kinetics of cadmium and copper hydrolysis, and Helios-Rybicka and Forstner (1986) on the effect of oxyhydrate coatings on the binding energy of metals to clay minerals. Modelling trace metal sorption and particle-water interactions has been attempted (Bourg, 1987) although so little is known about certain components (e.g. colloids, Morrel and Gschwend, 1987) that it seems premature to use modelling for most aquatic systems in any other than an academic sense.

Organic metal-complexing agents interact with adsorbants and metals to affect the partitioning of copper and other metals. Frimmel and Geywitz (1987b) report that nitrilotriacetic acid (NTA) causes a decrease in copper sorption to iron hydroxide particles. Wu et al. (1985) report a similar situation with fulvic and humic acids. Organics associated with the particle surface may increase metal uptake however. Lin et al. (1986) comment (abstact) that "... organoclay complexes may be one of the important forms of existence of metallic elements concentrated in the sediments, ..." Colloidal-sized organic particles (e.g. certain humic substances) may interact with dissolved metals both in response to sorption and complexation phenomena (Aualiitia and Pickering, 1986; Tillekeratne et al., 1985).

An understanding of sorption and desorption in soils is important to an understanding of the factors that affect metal bioavailability. Jopony (1986) and Jopony and Young (1987) discuss desorption and a mechanism for measuring copper desorption from soil and clay minerals. Bohn and Bohn (1987) discuss some of the assumptions and factors necessary to predict metal relationships. Soil sorption values have been collected by a number of authors to provide background information on trace metal availability (e.g. Pombo and Klamt, 1986) as has information on fractionation and translocation of copper in soils (Kong et al., 1987; Tkachenko, 1986). Metal-metal interaction appears to be a factor that must be considered in soil sorption of metals (Christensen, 1987)

Sorption also occurs on biological surfaces. Simoes Goncalves et al. (1987) reports this for the bacterium *Klebsiella pneumonia* while de Rome and Gadd (1987b) report copper sorption by three filamentous fungi. Sorption also occurs on certain dietary fibers (e.g. Nair et al., 1987) as well as other fibers (e.g. wool - Carr et al., 1987). Sorbing agents such as clay minerals, when used in food treatment such as apple juice clarification, remove trace metals (Dul'neva et al., 1987) which may be either detrimental or beneficial depending on the metal concentrations of the food. The use of sorbents by man also includes removal of toxic or undesired agents in industrial processes (e.g. Flytzani-Stephanopoulos et al., 1984; Hedges et al., 1985).

IV. METAL-METAL INTERACTIONS IN ORGANISMS

Copper is required by organisms because it interacts with organics to form specific organometallic compounds essential for life. Other metals operate in a similar manner which indicates that metal-organic interaction is a common theme amongst trace metals in biological systems. But the interaction is not always metal specific, metals compete with each other for binding sites on the organic. Zinc and copper, for example, can be antagonistic in this respect (Fishbein, 1987). Antagonistic interaction also occurs outside the organism, with the potential to affect metal bioavailability. The chemistry of a metal in an organism can also be affected by an organometallic compound of another metal. Willson (1987) and others have, for example, examined the effect of zinc- and copper-containing superoxide dismutase in free radical protection against ill-placed iron. Metal-metal interactions can also affected by levels of one of the metals that are either deficient (e.g. Davee et al., 1986; Vandenhaute and Maenhaut, 1987) or in excess (e.g. Vodicenska and Razbojnikova, 1987).

This section of the review uses recent publications to discuss the interactions of several metals as well as metalloids and nutrients which can act in an antagonistic or synergistic manner.

Copper-zinc interactions

Recent reviews which include discussions of the physiological effects of copper-zinc antagonism include Fishbein (1987), Pleban et al. (1985) and Shamberger (1987). Storey and Greger (1987) examined several methodologies to study iron, zinc and copper interactions and have some concern for the use of "... unadjusted humans or animals given a single dose of test substances because acute responses do not reflect all the changes induced by chronic feeding" (abstract).

The application of fertilizer to soils can affect metal ratios and metal bioavailability. Kukushkin et al. (1987) report that not only does labile soil zinc increase with higher doses of phosphorus and copper but this inhibits plant uptake of zinc. Gupta and Gupta (1985) found that copper uptake by soybean increased with increasing zinc in a zinc-deficient soil. Similar results have been found by Ruano et al. (1987) although they also found that toxic levels of zinc inhibit translocation of copper from the roots of hydroponically grown bush bean plants. In contrast, Kumar et al. (1986) found no effect of zinc on the copper content of stems and roots of pearl millet. Copper has been found in association with zinc-containing organics in lettuce (Walker and Welch, 1987) indicating possible Zn-Cu antagonism for organic binding sites.

In an examination of copper and zinc concentrations in artificially-spawned oysters, Phelps and Hetzel (1987) report that one population (stunted oysters) preferentially accumulated copper and had twice the copper:zinc ratio of another set of normal-sized oysters. Environmental factors are important in copper-zinc interactions as indicated by Johnson (1987) for two euryhaline crustaceans. The author reports that (abstract) "In certain instances (the) interactions between Cu or Zn and hypoxia were synergistic ...". In an examination of acute toxicity to a species of fish (*Clarias lazera*), Hilmy et al. (1987) found that copper and zinc potentiated each others lethal action although uptake of copper was decreased at elevated concentrations of zinc.

In sheep, Rosa et al. (1986) report a Cu-Zn interaction that affected kidney Fe and average daily weight gain. High levels of both dietary zinc and copper have been associated with increased weight gain in rats (Frimpong and Magee, 1987a). Copper supplementation can reduce serum zinc concentrations in rats (Koh et al., 1987). Likewise, zinc supplementation has been shown to affect copper status in a number of animals, including laboratory rats (Keen et al., 1985), Cynomolgus monkeys (Fischer and Giroux, 1987), and humans (Amos et al., 1986;

Yadrick, 1986). However, Hackman and Keen (1986) and Samman and Roberts (1987b) found no deleterious effect of zinc supplementation, on human serum copper levels, at least at the supplementation levels used. Fisher and Giroux (1987) note that, in the monkeys, this relationship was moderated by dietary phytate (Fischer and Giroux, 1987) suggesting the importance of metal bioavailability in metal-metal interactions. Higher tissue levels of copper have also been associated with dietary zinc deficiency in rats (Piletz et al., 1987; Song et al., 1986). Metal levels can also be affected by the physiological status of the organism (Lopez et al, 1987; Uza et al., 1985). Rao et al. (1986), for example, found a higher than normal Cu:Zn ratio in jaundice patients. One of the best treatments of Wilson's disease is oral zinc supplementation, which decreases copper uptake and apparently improves urinary copper excretion (Brewer et al., 1987b, Hoogenraad et al., 1987; Lee et al., 1986). Copper toxicity to human hepatoblastoma cells has reportedly been inhibited by zinc (Blank and Stockert, 1986; Schilsky et al., 1987). This is associated with the apparent increase of serum copper and decrease of serum zinc levels reported for patients with liver cancer (e.g. Liu, 1985) and suggests a loss of ability to regulate metal levels by cancerous cells.

Copper-molybdenum interactions

The interaction between copper and molybdenum is frequently considered in fertilizers (e.g. Coventry et al., 1987) because of its importance to most ruminant animals (Suttle, 1986; Weaver, 1984; but also see Strickland et al., 1987). Elevated molybdenum levels can produce a copper deficiency (e.g. Boyne and Arthur, 1986; Mills, 1986a; O'Gorman et al., 1987) although molybdenum excretion has also been noted with elevated copper levels (Bibr, 1979). The metal-metal interaction often involves sulfur either directly or indirectly (Mills, 1986b). Leech and Thornton (1987), for example, found that dietary sulfur plays a critical role in the incidence of bovine hypocupraemia in industrialized Britain. Jessie and Smith (1987), however, note that both metals are able to inhibit sulfate reduction by bacteria although metal complexation by organics and precipitation by sulfides may affect the inhibition. Kapul'tsevich and Parshina (1986) report an interaction of molybdenum and copper on the growth of the yeast *Candida krusei*. Maximum growth at high $CuSO_4$ concentrations could be achieved by low concentrations of Na_2MoO_4 while maximum growth with low $CuSO_4$ concentrations could only be achieved by high concentrations of Na_2MoO_4.

In sheep, excess copper can be toxic without adequate concentrations of molybdenum (Fuentealba, 1985; Humphries et al., 1987; Reagor and Eugster, 1986). Treatment with ammonium tetrathiomolybdenate and sodium sulfate are often recommended (Gooneratne, 1986; Humphries et al., 1986; Kumaratilake and Howell, 1987b; Niederman et al., 1987) although frequently not successful (Reagor and Eugster, 1986). Thiomolybdates are formed in the rumen from molybdenum, and cause a decrease in the availability of copper due to the formation of insoluble copper thiomolybdates (e.g. Allen and Gawthorne, 1987) by rumen bacteria (Mulryan and Mason, 1987). Some suggestion of breed difference has been given (Robinson et al., 1987) although not distinctly shown (e.g. Harrison et al., 1987). When given intravenously to sheep, thiomolybdates increase copper excretion in the bile (Gooneratne et al., 1986b) with possibly some accumulation of Mo-Cu complexes in the kidney (Kincaid and White, 1987). Thiomolybdates have also been shown to inhibit bovine serum amine oxidase activity in cattle (Mulryan and Mason, 1987) and may also affect hormone activity in female rats (Fungwe et al., 1987). There is also evidence of molybdenum uptake by connective tissue (Lener et al., 1986) which may produce an apparent copper deficiency affecting bone development (Read et al., 1986). Thiomolybdates have also been used to treat Wilson's disease patients because of the ability to reduce copper uptake and accumulation (in Woods and Mason, 1987).

Copper-manganese interactions

Copper and manganese interact both in the environment and within the organism. Copper adsorption to manganese oxides occurs and is one of the mechanisms responsible for the relatively high levels of copper in some manganese nodules (e.g. Chen et al., 1987b). A competitive interaction has been reported for the growth of marine phytoplankton, reducing the detrimental effects of excess copper (Kazumi et al., 1987). Similar effects have also been found for certain macroalgae (Munda and Hudnik, 1986). In soil, Mathur and Belanger (1987) report that copper additions may have increased the availability of soil manganese to carrots. Reddy et al. (1987) note that manganese supplementation of sandy loam soil tended to increase leaf copper concentrations. Since soil pH and nitrogen may interact with both copper and manganese (Khatiashvili et al., 1985; Kudrev et al., 1986; Reddy et al., 1987), the metal-metal interactions may be indirect as well as direct. The Cu-Mn interaction appears to be most pronounced with plants. There is evidence that both copper and manganese can affect the physiology of animals (e.g. Strause et al., 1987) although it is difficult to separate individual from combined effects of the two metals.

Copper-iron interactions

As with manganese, copper interacts with iron both in the environment and within the organism. Copper can be sorbed by iron oxides. Copper is one of the metals that can inhibit iron uptake by bacteria (Bezkorovainy et al., 1987). Metal complexing agents are important both in nature and the organism, affecting metal bioavailability and metal-metal interactions, by metal-metal competition for binding sites (e.g. Eguchi and Saltman, 1987; Nishikimi and Ozawa, 1987). In wheat, Galrao and de Sousa (1985) found the application of supplemental soil copper had an antagonistic effect on leaf iron concentration. Similar results have been obtained with greenhouse roses (Rey and Tsujita, 1987). Interactions in both plants and animals can be affected by chemical as well as physiological conditions, including metal levels (e.g. Kolb et al., 1987; Stahl et al., 1987). In rats, Sumati and Kapoor (1986) note an antagonistic relationship between high levels of dietary iron and copper uptake. This is supported by Johnson and Murphy (1987) who found that high dietary iron levels decreased apparent absorption of copper in copper-deficient rats. Cossack (1987) found that iron-overload in mice did not affect the distributions of zinc and copper in liver, kidneys, heart, brain and whole blood.

Copper-cadmium interactions

Cadmium, by itself, is considered to be toxic. However, cadmium interacts with other metals, including copper. Soil sorption of cadmium can, for example, be reduced by copper as well as other metals (Christensen, 1987). Copper levels in plants can be affected by cadmium (Babalakova and Kudrev, 1986; Garmash, 1987; Moser et al., 1986; Wong et al., 1986). In animals, cadmium may have an antagonistic (Honda and Nogawa, 1987), synergistic (e.g. Gould et al., 1988; Kaji et al., 1986) or little effect on the action of copper (e.g. Ikeda et al., 1986). Smith et al. (1982) report a decrease in serum copper in cows and calves after exposure of the cows to elevated dietary cadmium throughout gestation. Similar reductions have been noted in tissue copper concentrations in fetuses from rat dams given excess cadmium in their drinking water (Baranski, 1987). Tanaka et al. (1986) note a cadmium inhibition of copper absorption in the rat small intestine. Liver copper levels may be decreased by dietary cadmium (Friel et al., 1987a) although Gotz and Friedberg (1986) report the level to be unchanged after cadmium treatment. However, heart copper levels are increased in rats by dietary cadmium treatment (Jamall and Sprowls, 1987). Cadmium exposure to diabetic rats produces a change in the blood copper concentration (Chandra et al., 1985).

Cadmium uptake by cultured cells is reported to be inhibited by copper (Meshitsuka et al., 1987). Cadmium reduces absorption of radioactive iron-59 although Hristic and

Krsmanovic, (1984) found indications that the presence of copper or zinc can reduce this effect. Cadmium may compete with copper for sites on metal-complexing organics such as metallothionein and may interact with other metal-containing organics (e.g. Chung et al., 1987; Elinder et al., 1987; Mitane et al., 1987; Nomiyama and Nomiyama, 1986). However, Funk et al. (1987) report that found that cadmium treatment did not alter copper levels in (Zn, Cu)-metallothionein. Alteration of organometallic compounds can have major impacts. Kaji et al. (1986) found an interactive inhibition of bone metabolism by both Cd and Cu in cultured bone tissue. Longterm urinary cadmium excretion is associated with renal dysfunction and changes in urinary copper concentrations (Honda and Nogawa, 1987; Ohmori et al., 1985) and can occur in metal workers.

Interactions of copper with other metals, metalloids and nutrients

Calcium - interaction with copper may occur in bone formation although this may be either a direct or indirect action. Copper does affect calcium deposition in eggshell formation in domestic fowl (Lundholm and Mathson, 1986). In rats, increasing dietary calcium has been associated with an elevation of plasma copper (Chin et al., 1987) although ingestion of calcium was not associated with changes in copper absorption (Behling et al., 1987). Changes in rat tissue copper have been noted as a result of supplemental dietary calcium although the changes may not be a direct result of the increased calcium (Greger et al., 1987).

Lead - addition of copper to a lead-amended medium inhibited 3 fungal strains found in forest soils with metal-metal interaction suggested for only one of the strains (Wargo et al., 1987). Metal-organic interactions occur with both plants and animals, affecting metal bioavailability (e.g. Umoren et al., 1987) and ultimate metal-metal interactions. The role of pH and other modifying factors must, however, be considered in interpretation of results (e.g. Farant and Wigfield, 1987). Addition of lead to rats causes changes in tissue copper levels, as well as levels of other metals (Gasiorowski et al., 1987; Mylroie et al., 1985). Copper and zinc have been reported to antagonize the subclinical effects of lead which reduces peripheral nerve conduction velocities in metal workers (Murata et al., 1987). Calcium disodium EDTA, is a metal chelating agent used to treat lead poisoning. Treatment, however, also causes loss of other metals from the body although copper less than zinc (Thomas and Chisolm, 1986).

Mercury - Singh and Singh (1987) report that copper acted antagonistically against the mercury inhibition of photosynthesis in the cyanobacterium *Nostoc calcicola*. In the mussel *Mytilus edulis*, prior exposure to copper conferred increased tolerance to the toxicity of inorganic mercury, possibly as a result of copper-induced metallothionein production (Roesijadi and Fellingham, 1987). (Mercury and copper may compete for sites on the metallothionein molecule (Lobel and Payne, 1987).)

Real and possible relationships of copper, with other metals, have been described. Nickel - Rafter (1987) and Spears et al. (1986). Boron - Shelp and Shattuck (1987) and Shelp et al. (1987). Metalloids such as selenium can also interact with copper, affecting tissue levels in rats (Wei et al., 1987), chicks (Combs et al., 1986) but not in dairy cows (Buckley et al., 1986), under the conditions of the studies. Uptake of selenium by the alga *Ulva lactuca* is reduced by copper, as $CuSO_4$ (Damyanova and Tyankova, 1985). Increased interaction of aluminum and copper is possible under acid rain conditions. Rueter et al. (1987) found indirect aluminum toxicity to a green alga as a result of increased cupric ion activity. Osteosclerosis is reported to be a result of ingestion of excess fluoride and is associated with copper deficiency in areas of endemic fluorosis in India (Mittal et al., 1987). The effect of sulfur on copper availability and uptake has been mentioned earlier, and is important to the mineral status of cattle (e.g. Gooneratne et al., 1986c; Smart et al., 1986). Interaction of sodium with copper may affect intestinal absorption of copper (Wapnir and Stiel, 1987). Phorphorus fertilization has been reported to influence micronutrient availability and, possibly as a result of pH change, change in soil copper availability (Al-Showk et al., 1987). The relationship between phosphorus and copper has also been related to changes in plant uptake of both elements (Singh et al., 1986; Verma and Tripathi, 1986) and the chemical fate of copper within the plant (Jensen et al., 1986).

V. UPTAKE AND ACCUMULATION OF COPPER BY ORGANISMS

Uptake and accumulation of metals can occur from food, the environment, or both (Beyer, 1986). Uptake is affected by the chemistry of the metal and the nature of the cell membrane (e.g. Melhorn, 1986). In contrast, accumulation is affected by metabolic processes controlling metal distribution, storage and excretion, many of which are metal specific (Mallinckrodt, 1984). Reviews and discussions of the importance of metal speciation to uptake include Bernhard and George (1986), Kester et al. (1986) and Wolf et al. (1986) for aquatic environments and Robb and Williams (1986) for digested food. Petering and Antholine (1988) provide an excellent review of speciation and reactions of copper in biological systems, with particular concern for reactions that produce cell injury.

Reviews of metal uptake in organisms include Berrow and Burridge (1984) for plants and Aspin and Sass-Kortsak (1981) review normal and abnormal copper uptake and accumulation in humans. The latter is in a volume on disorders of mineral metabolism (Bronner and Coburn, 1981) that covers a variety of metals. Aggett (1985) discusses the metabolism of copper, as one of the essential metals. Problems associated with the measurement of metal uptake are discussed by Morrison (1987a), for polluted waters, and Van Barneveld and Van den Hamer (1986) for food. In the former, the problem deals with metal speciation and bioavailability while, in the latter, the problem is related to metal retention after uptake.

Uptake of environmental copper occurs in both water and soil. Maeda (1986), for example, notes that nearly 30% of the copper in near surface Bering Sea water was assimilated by marine plankton or removed during long-range transport. Plants and plant roots in metal-rich soil, ground water and sediments can remove part of the metal load, including copper (Anderson et al., 1980; Azpiazu et al., 1987; Babalakova and Velikov, 1985). This can lead to differences in plant metal concentrations on different soil types (e.g. Prasad and Sankaranna, 1987) and differences in plant distribution and growth characteristics on the basis of soil type (e.g. Wentworth and Davidson, 1987). It is also one of the reasons why tillage practices can affect plant metal concentrations (Hammel and Mahler, 1986). Soil pH and organic matter are primary factors controlling metal uptake by plants (Gough, 1984; Iwasaki et al., 1987b) as may metal-metal interactions (e.g. Iwasaki et al., 1987a; Kukushkin et al., 1987; Mathur and Belanger, 1987). Natural metal-complexing agents form part of the soil organic matter and both natural and synthetic metal-complexing agents will affect metal uptake (Checkai et al., 1987a,b; Farago et al., 1987; Linder and Voye, 1987; Nor, 1985). This is because of competition for the metal, by both the complexing agent and the plant and is one of the reasons why certain metal-complexing agents can be used to estimate soil metal bioavailability (e.g. Jasiewicz and Gambus, 1986).

Uptake of copper by has been reported for both free-living and symbiotic microorganisms (Boulegue et al., 1987; De Rome and Gadd, 1987a; Giaccio and Cichelli, 1986), including bacteria, fungi and microalgae. Beveridge and Fyfe (1985) found cell wall polymers responsible for metal binding in bacterial cell walls. An understanding of uptake is important in the growth and use of these organisms to commercially produce organics (De Rome and Gadd, 1987a; Galun et al., 1987; Lopes et al., 1986; Martinez and Connelly, 1987; Townsley and Ross, 1985, 1986). Under certain conditions, metal concentrations in fungi and algae can be used to indicate metal bioavailability (Dietl, 1987). Copper uptake is reported to be linear in the alga *Ceramium brasiliense*, tissue concentration increasing with metal concentration (Seeliger and Lacerda, 1986). However, metal resistance does occur in certain microorganisms and indicates an ability either to reduce metal uptake or to minimize the effect of excess metal once within the cell (Clarke et al., 1987; Erardi et al., 1987; Gadd et al., 1987; Sutter and Gareth Jones, 1985). This often varies with the nature of the organism, as a result of its biochemistry (Germann and Lerch, 1987). Mineral acids are effective for desorption of copper under certain conditions (de

Rome and Gadd, 1987b). Jones and Wilson (1986) report biomineralization in crustose lichens, possibly as a result of organic acid-secreting activity of the fungal portion.

In algae as well as in vascular plants, there is a relationship between metal uptake, mineral concentration, and nutrients (Jensen et al., 1986; Kennedy and Gonsalves, 1987; Kudrev and Kovacheva, 1986). Part of this may be a result of the chemistry of the nutrients and minerals as well as the metals (e.g. Anderson et al., 1987; Darnall et al., 1986; Lee and Hardy, 1987; Nor, 1987a; Nor and Cheng, 1986; Wong and Chan, 1985). Part is due to metal-metal interactions (e.g. Fuse, 1987) and physical or atmospheric factors (Schwartz et al., 1987; Tingey et al., 1986). Part is also due to the nature of the organism (e.g. Bowen, 1987; Goudey, 1987; Graham et al., 1987; Jamil et al., 1987; Lee and Hardy, 1987; Nishizono et al., 1987a,b). Relationships with mycorrhizal fungi have been demonstrated to affect micronutrient uptake (Pacovsky, 1986; Sidle and Shaw, 1987). There is evidence of chemical changes occurring in the metal at the cell membrane that make the role of metal transport agents questionable in terms of metal bioavailability. Jones et al. (1987) report the reduction of copper(II) and iron(III) at the cell surface in certain phytoplankton. They provide evidence suggesting that (abstract) "... trace metal complexes are not the main electron acceptor in natural waters." Translocation of metal within the plant is also important both in terms of providing an adequate supply of copper and isolating excess metal (e.g. Reay, 1987). Drude de Lacerda and Eduardo de Resende (1986) note that, during one growing season of the seagrass *Halodule wrightii*, the plant exhibited appreciable translocation capacity for copper, from roots to leaves and rhizomes. In the salt-marsh plant *Puccinellia maritima*, second harvest growth shoots characteristically had higher levels of copper than first harvest growth shoots.

Competition for metals, between plant species, is recognized as one of the most important forms of damage caused by weeds to economically important plants (Malicki and Berbeciowa, 1986). Nutrient supplementation, through fertilizers, is one method of providing plants with copper. Gorlach and Olkusnik (1986), however, found litle effect of fertilizers on plant levels of copper and zinc in meadow plants. In greenhouse roses, Rey and Tsujita (1987) report foliar and root copper concentration increase relative to copper concentrations in irrigating solutions. Uptake may also occur as a result of aerosol deposition on leaves, especially with high levels of aerosol metal (Godt et al., 1986; Kazda and Glatzel, 1986). Washoff from leaves also forms a source of metal for root uptake.

A number of recent references address uptake of copper and other nutrients in economically important plants. These include:

Grains and grasses - Fairey, 1986; Kadar et al., 1985; Kavalevich, 1987; Krahmer and Podlesak, 1985; Kumar et al., 1986; Lasztity, 1987b; Lasztity et al., 1985; Payne et al., 1985/86; Perera, 1986; Rappaport et al., 1987b; Singh et al., 1986; Verma and Tripathi, 1986; Whitehead, 1987.

Vegetables - Babalakova et al., 1986; Ibrahim et al., 1986; Minnich et al., 1987; Shariatpanahi et al., 1986; Scholz et al., 1987; Wong et al., 1986.

Fruits and berries - Bouchard and Gagnon, 1987; Lenartowicz, 1986; Sanchez-Alonzo and Lachica, 1987a,b; Sidorovich et al., 1987; Smith et al., 1987; Stephenson and Cull, 1986; Stephenson et al., 1986

Miscellaneous - Gonzales and Escobar, 1987; Gupta and Gupta, 1985; Heckman et al., 1987; Reddy et al., 1987; Tailakov and Mamedkhanov, 1983.

Patterns of accumulation of copper have been widely studied in animals, as indicated by the citations on fish in the National Technical Information Service list (1987b). Amiard et al. (1987a) report that, under certain conditions and for some estuarine and coastal organisms, bioaccumulation of copper (Y) is related to copper concentration in the water (X) by the equation $Y = a X^b$. Copper accumulation rate often differs from that of other metals (e.g. Wang and Yin, 1987) as do concentration thresholds necessary for uptake (Ikuta, 1987a).

Copper uptake and tissue concentrations are controlled by the physiological and biochemical roles of different parts of the organism. Copper content in the mussel *Mytilus galloprovincialis* is related to weight as well as gametogenetic activity (Martincic et al., 1987a). Distribution of copper between organs varies (Ikuta, 1987b; Nemcsok et al., 1987; Wolmarans and Van Aardt, 1986) often as a result of metal regulation and excretion (e.g. Arumugam and Ravindranath, 1987; Robinson and Morse, 1985). Excretory organs frequently have high copper levels because they serve to excrete excess metal (Grodowitz et al., 1987; Marcaillou et al., 1986; Reid and Brand, 1985; Witkus et al., 1987) as do insoluble granules formed to isolate excess metal in a biologically unavailable state (Lanno et al., 1987; Rainbow, 1987). However, the ability to regulate tissue metal levels is not ubiquitous, some organisms appear to have little ability to either eliminate or isolate excess metal (e.g. Spicarova, 1986).

Copper uptake as well as tissue metal concentrations have been found to vary within as well as between organisms (Catsiki and Arnoux, 1987). Part of this has been attributed to the age (Gochfeld and Burger, 1987a) or size of the organism (e.g. McLeay et al., 1986) although evidence continues to suggest that, at least with copper, this is not always the case (e.g. Lobel and Wu, 1982). When it does occur it is most frequently as a result of a requirement for copper, as for example in haemocyanin in growing crustaceans (White and Rainbow, 1987). Certain phases of the life cycle also appear to be less tolerant than others to detrimental effects (Price, 1979), suggesting a reduced ability to control metal uptake or tissue metal concentrations. There is also an influence by the nature of the substrate, uptake from metal-rich substrates is greater than from metal-poor substrates (Hunter et al., 1987d; Johnson, 1987; Kavun and Khristoforova, 1987; Neuhauser et al., 1985a). Substrate pH has been linked to metal uptake, greater uptake occurring at lower pH (Broberg and Lindgren, 1987). This is directly related to metal speciation which directly affects metal bioavailability. With zinc, Watkins and Simkiss (1988) note an increase in rate of uptake with increasing temperature in the mussel *Mytilus edulis.* Copper accumulation in two estuarine molluscs has been related to salinity and, at least in part, suggested to be independent of changes in copper speciation (Wright and Zamuda, 1987). Metal-metal interaction may also affect copper uptake (Gould et al., 1988) and the uptake of other metals (e.g. Bibr and Lener, 1985; Meshitsuka et al., 1987). The nutritional status may also affect metal uptake. Segner (1987) notes increased liver copper concentrations with starvation in a fish species but a reduction in excess liver copper with feeding.

Copper binding by specialized organics occurs within organisms. These organics have been studied as mechanisms important for metal uptake and translocation as well as isolation of excess metal. Tukendorf (1987), for example, found copper-binding proteins in spinach roots and comments on their role in protection against excess metal. Other references that describe similar organics in plants include Mench et al. (1987) and Robinson and Thurman (1986). Phytochelatins are a group of heavy-metal-binding peptides which affect the availability of metal within certain plants (Grill et al., 1987). Phytic acid is a well known metal-binding agent (e.g. Martin and Evans, 1987) which, when ingested with plant foods, can affect the uptake of dietary copper in humans (Champagne and Hinojosa, 1987). Metallothionein and metallothionein-like metal chelating agents have been reported in a wide variety of animals, including crustaceans (Acey et al., 1987; Engel, 1987; Engel and Brouwer, 1987) and mammals (e.g. Sugawara and Sugawara, 1987). A number of recent references have also examined the role of metallothionein in metal speciation and the interaction of copper-thionein with other metal complexes within animals (Bremner, 1987b; Heilmaier et al., 1987b; Sugawara and Sugawara, 1987; Summer et al., 1986; Weser, 1986). Brouwer et al. (1986) provide an overview of various metal-binding agents in marine organisms which are important for internal metal transfer and metal detoxification, with regard to cadmium detoxification. Other organics are discussed elsewhere in this review but not with regard to metal uptake and metal transport within the organism, references include Ettinger et al. (1986), Fowler (1987), Gordon et al. (1987), Krotz and Wagner (1987) and McArdle et al. (1987).

A number of recent references address copper availability, uptake and metabolism in economically important animals as well as laboratory animals. These include a number of references discussed elsewhere in this review. Three reviews (Mills, 1986a,b and Suttle, 1986) examine various aspects of uptake and metal metabolism as do individual papers and abstracts concerning:

Poultry - Gorobets, 1986; Izquierdo and Baken, 1986; Khan et al., 1987; Kirgessner et al., 1986; Richards et al., 1987; Southern and Giraldo, 1987.

Pigs - Kornegay et al., 1985/86; Shurson, 1987; Stetsenko et al., 1985.

Sheep - Allen and Gawthorne, 1987; Clegg et al., 1986; Freudenberger et al., 1987; Kumaratilake and Howell, 1987a; Lemarie et al., 1987; Morton, 1986; Suttle, 1987a; Turner et al., 1987; van Ryssen and Barrowman, 1987; White et al., 1986; Woolliams et al., 1986a,b.

Cattle - Bain et al., 1986; Binnerts et al., 1986; Kume et al., 1986; Poole et al., 1987; Smart et al., 1986; Spears and Harvey, 1987; Suttle, 1987b.

Miscellaneous agricultural animals - Schryver et al., 1986, 1987; Staaland, 1985.

Laboratory animals - Asano and Hokari, 1987a,b; Astuti et al., 1987; Baranski, 1987; Bingle et al., 1986; Cox and Eley, 1987a; Everett, 1985; Fields et al., 1986a-c; Garnica et al., 1987a,b; Greger et al., 1987; Holt et al., 1987; Illowsky Karp et al., 1986; Jasim et al., 1985; Johnson and Gratzek, 1986; Linder and Roboz, 1986; Lui, 1987; Milanino et al., 1986; Nederbragt et al., 1987; Packman et al., 1987; Piletz et al., 1987; Rajan et al., 1987a,b; Rodriguez-Yoldi and Ponz, 1987; Sable-Amplis et al., 1987; Segues et al., 1987; Smith et al., 1987; Srai et al., 1986b; Suzuki et al., 1986; Szerdahelyi and Kasa, 1987; Tanaka et al., 1986; Wapnir and Stiel, 1987.

A great deal of work has been done on trace metals in food and metal bioavailability in food. Much of this has been with laboratory animals although more and more work is being done on humans, especially with stable isotope techniques (Turnlund, 1987). Parr (1987) describes an "International collaborative research programme on minor and trace elements in total diets" initiated by the International Atomic Energy Agency. Mills (1987) discusses models for trace element metabolism in humans. Metal speciation studies have examined digests of human foodstuffs (Massey et al., 1986; Robb et al., 1986b). Turnlund et al. (1987b) suggest that 0.8 mg/d dietary copper is sufficient to maintain adequate copper status in most normal, healthy young men although 2-3 mg/d is recommended by the U.S. National Academy of Sciences (in Parr, 1987). However, these general levels must be modified according to the nature of the individual. Increased copper losses occur as a result of extensive aerobic exercise (Campbell and Anderson, 1987) and supplementation is often advisable (e.g. Deuster et al., 1986). Changes occur in copper levels during early development (Habib and Abdulla, 1986; Pleban et al., 1985) which necessitates knowledge about the requirements for and availability of copper from maternal or external sources (e.g. Bratter et al., 1987). Concerns continue to be focused on zinc and copper levels in the elderly, either as a result of inadequate nutritional standards or physiological imbalance (Bunker et al., 1987). The latter is certainly the case with metabolic malfunctions or injury in humans (e.g. Brian et al., 1987). In these cases, adequate metal levels can only be maintained with an understanding of the metal flux in the body (e.g. Araki et al., 1986b; Ishihara and Matsushiro, 1986). A number of publications concern copper uptake and disposition in Menkes' disease (Herd et al., 1987; Sone et al., 1987), Wilson's disease (e.g. Brewer et al., 1987b; Gunther et al., 1987; Iyengar et al., 1986; Lee et al., 1986; Leevy et al., 1986; Ritland and Aaseth, 1987), rheumatoid arthritis (Winyard et al., 1987b) and diabetes (Sjogren et al., 1986b). Trace element uptake and flux also becomes important in individuals such as vegetarians, who have particular life or nutrient styles that can affect tissue copper concentrations (e.g. Bhattacharya, 1986).

Metal levels have been measured in a variety of foods, ranging from seaweeds (e.g. Yamamoto et al., 1985) and other fishery products (Luten et al., 1987) to vegetables. Concentrations and availability of copper and other trace elements have been examined in the "average" diet of individuals in different countries (e.g. Wyttenbach et al., 1987) or in components of the diet (e.g. Darret et al., 1986). As with other organisms, various factors affect metal concentration (e.g. Moak et al., 1987) and availability in human food materials. pH changes in milk can affect soluble metal concentrations, including copper (Nelson et al., 1987). Samman and Roberts (1987b) report no metal-metal effect of zinc on plasma copper levels in humans. The use of metal complexing agents to alleviate metal poisoning has been found to produce a metal imbalance in humans as well as laboratory animals (Aono and Araki, 1986; Honda and Nogawa, 1987; Murata et al., 1987). Food components such as phytates and fiber may affect metal availability (Platt and Clydesdale, 1987; Turnlund, 1987) although other work suggests that this may not be an important factor (Behall et al., 1987; Dreosti et al., 1984; Hallfrisch et al., 1987).

VI. CHANGES OCCURRING IN COPPER AFTER INTRODUCTION INTO NATURAL ENVIRONMENTS

Copper is introduced into natural environments from a number of natural and anthropogenic sources. The nature of these sources and the nature of the receiving environments vary widely. As a result, changes that occur in the chemistry of copper can vary widely. Understanding the nature of these changes requuires an understanding of the Earth, in the sense of Wedepohl (1984). A knowledge of factors affecting chemical speciation in natural environments is of critical importance (e.g. Buat-Menard, 1986a) because that dictates the form and species of copper and its availability to organisms as well as to man (Bernhard et al., 1986a; Burton et al., 1986). The interaction between organisms and the environment plays a role not just in looking at metal impact but also, the effect that organisms have on metal speciation (Bothner et al., 1987a). Water quality information (e.g. Hargis and Associates, 1986; Hites and Eisenreich, 1987; Nor, 1987b) thus becomes useful in predicting fates and fluxes of metals as well as biological impacts.

Aerosol Copper

Aerosol copper includes natural materials as well as anthropogenic agents. Heaton (1987) found enrichment in the 200 to 500 range for aerosol copper in the Sargasso Sea and the Peru upwelling areas. Miller et al. (1987) report a range of 1.7 to 388 ppb ionic copper in fogwater from the rural northern San Joaquin Valley of California. At least some of this is of natural origin although, certainly in the California example, a good deal of it would originate from vehicle exhaust gases. The major anthropogenic copper source is the variety of combustion residues such as coal fly-ash (Forstner, 1986).

Snow layers in Antarctica and Greenland have been used to provide information on aerosol input over time. They provide a comparison between northern and southern hemisphere atmosphere loading. With copper, levels in the northern hemisphere are approximately 50 times those in the southern hemisphere (in Delmas, 1986). Romero et al. (1987b) provides the composition of atmospheric deposition, apparently within Spain. Rainfall in urban-industrial areas contains an average of 46 μg/L copper, 39 μg/L in rural areas; dry deposition contains an average of 1,000 ppm copper in urban-industrial areas, 330 ppm in rural areas. The problems of assessing atmospheric pollutant input into the Mediterranean are discussed in a 1985 report by GESAMP (Group of Experts on the Scientific Aspects of Marine Pollution). Buat-Menard (1986) discusses the modelling of pollutant transport into the Mediterranean Sea and provides sampling strategies for obtaining atmospheric samples. Using trace element emission factors and industrial consumption of raw materials, Pacyna (1986) found reasonable agreement between estimates and measurements of long range transport of copper in northern Europe. For the Federal Republic of Germany, Nurnberg et al. (1984; see also Valenta et al., 1986) give an estimated 842 tons per year annual wet deposition. Dongmann et al. (1987a,b) provide information on modelled wet deposition in the Stolberg area of the Federal Republic of Germany. Schneider (1987) estimates that anthropogenic emissions account for 100% of the atmospheric copper over Kiel Bight. The arithmetic mean atmospheric concentration for the region is given as 7.7 ng m^{-3} (Schneider, 1987). Szefer and Szefer (1986) estimate rainfall fluxes of 0.29-0.61 μg/m^{-2}/hour copper from the atmosphere to the sea in three locations on the Baltic coast. Variability is a factor, as indicated by the 6.5-51.5 ng/m^{-3} range given for coastal aerosols by Flament et al. (1987) for the northern English Channel. In Armadale (central Scotland), Gailey et al. (1985) used lichen transplants to act as biological filters and monitor airborne metals.

Samhan et al. (1986) provide estimates of aeolian contributions of copper and several other metals, to marine sediments of Kuwait. Dust fallout in Kuwait has an average copper enrichment of 2.3 when compared with continental crustal values. In air samples in Perth, Western Australia, Lax et al. (1986) report "0" to 0.67 $\mu g/m^3$ copper. Ambe and Nishikawa (1986) used selected rainfall events at Tsukuba, Japan, to examine temporal variation of trace elements. They report ranges of copper from below detection limits to 46 ppb. In a discussion of spatial and temporal trends in the chemistry of atmospheric deposition in New England, Hanson and Norton (1987) note that atmospheric deposition of heavy metals, including copper, began increasing in the 19th century in New England and may have started to level out in the past 5-10 years. Part of this may be an "apparent" dropoff due to sample contamination in the past. Barrie et al. (1987) comment (Abstract) that "Recent precipitation chemistry data of known reliability indicate that typical rural concentrations of Cd, Cu, Pb, Ni, V and Zn are lower than those indicated by older measurements owing to sample contamination." Dillon et al. (1986) comment that the concentrations of Al, Cu, Ni and Zn have decreased in lakes near Sudbury, Ontario, Canada, probably from the reduction in metal emissions from smelters in the area and possibly, in part, from the pH changes in the lakes.

Soil Copper

A number of geological processes are involved in soil formation. Some of these "... affect the concentration of major elements and especially trace elements in rocks and sediments, and the availability of these elements as nutrients ..." (page 130 in Koljonen et al., 1986). Land clearing, soil fertilization, tillage practice and crop growth will affect the level of soil copper as will aeolian input (Banin et al., 1987; Golikov, 1984; Hammel and Mahler, 1986; Jones et al., 1987). Microorganisms in the soil and biogenic material introduced into the soil will affect the chemistry and biological availability of soil copper (Francis and Dodge, 1987; Jones and Wilson, 1986; Munier-Lamy and Berthelin, 1987; Shavlakadze et al., 1987) as will any organism closely associated with the soil (e.g. Prasad and Sankaranna, 1987). Mobility of copper, whether in natural soils or anthropogenic systems, is affected by a number of factors that include climate, pH, metal chemistry and soil type (e.g. Cepeda, 1987; Newman and Elzerman, 1987; Singh et al., 1987). These may have obvious or subtle effects on plant metal uptake (e.g. Gough et al., 1986) as well as metal levels in grazing animals (e.g. Bain et al., 1986; Singh and Mishra, 1987).

Scokart and Meeus-Verdinne (1986) report a low mobility of copper in polluted sandy and loamy soils which they attribute to adsorption and organic complexation. Association of copper with organics appears to reduce metal mobility, even in metal-rich soils such as in North Staffordshire in the U.K. (Bradley and Cox, 1987), reduces its mobility, and may produce trace element deficiency. Cover type can affect soil metal levels. Kufel and Kufel (1985) note copper accumulation by *Phragmites australis* and *Typha angustifolia* with loss occurring primarily by leaching both during and after growth. Moyse and Fernandez (1987) report that, with trace metals in a forest floor, (abstract) "... metal levels were strongly related to forest stand type and forest floor properties". This has also been demonstrated for other forest soils (Bergkvist, 1987; Foster and Nicolson, 1986; Morrison and Hogan, 1986; Raisch, 1983). Plant uptake of aerosol metals may be important in the tree canopy although evidence from Godt et al. (1986) and Morrison and Hogan (1986) indicate that this is probably unimportant for copper.

An interaction of nutrient input, copper concentration and plant growth has been demonstrated to be important (e.g. Ilvanova, 1985; Ruszkowska et al., 1984). Harvest practices, whether with annual or multiyear crops, has also been shown to affect levels and availability of soil copper. Thran and Everett (1987) comment that the effects of singleleaf pinyon tree harvest on soil copper levels were site specific. The concentration of soil organics, such as humic substances, has a major effect on the bioavailability of copper (Chen and Stevenson, 1986; Senesi et al., 1987) as does sorption by particles (Aualiitia and Pickering, 1987b). Amacher et al. (1986) discuss several models for the retention and release of metals by soils and their use to evaluate metal transport through soils. Predicting metal speciation and bioavailability is important, not only to estimate the ability of a soil to fulfill copper requirements of a plant (e.g. Fregoni, 1986; Gembarzewski et al., 1986) but also to predict what type and amount of fertilizer needs to be added and the residual effects of using the fertilizer (e.g. Gorlach and Gorlach, 1984).

The use of sewage and swine manure as fertilizer introduces metals into soils (e.g. Leonhard and dHegemann, 1984). Its effects on organisms is discussed elsewhere in this review. Since copper is almost exclusively associated with the organic fraction in manures and sewage, soil mobility and plant availability will be dependent upon the decomposition of organic and the release of the metal in a more labile form. The rate of mobilization depends on the chemistry of the fertilizer, the rate of organic decomposition and the rate of metal leaching (e.g. Elliott and Linn, 1986; Melanen et al., 1985; Westerman et al., 1987). Pretreatment of sludge, as for example by pyrolysis, will modify the chemistry and affect copper speciation (Kistler and Widner, 1987). The occurrence of synthetic organics, such as nitrilotriacetic acid, in sludges also must be considered in examining metal bioavailability (Garnett et al., 1987).

Large amounts of copper are also introduced into soils and streams from highway and urban runoff (Zanoni, 1986). This is a result of vehicular traffic, metal in the surface material used (Dueck et al., 1987a) and agents used for deicing (Elliott and Linn, 1987). Retention-detention ponds will retain the particulate fractions to a certain extent (Harper, 1986; Nightingale, 1987; Yousef et al., 1986) but the finer fractions will escape as will most of the dissolved fractions. Ellis et al. (1986; 1987) provide estimates of the impact of runoff to receiving streams, and (1987) suggest a 78% increase in total copper. Resuspension may also occur, of both runoff-associated metal and metal in dust (Hamilton et al., 1987; see also Fergusson, 1987). Runoff from industrial areas also introduces anthropogenic copper into soils and streams (Blake et al., 1987; Houle et al., 1987). Davies and Wixson (1987) successfully used factor analysis to differentiate anthropogenic metals from naturally occurring metals in soils of a mineralized area of Madison County, Missouri. Fate of anthropogenic is dependent upon the nature of the source (e.g. Sullivan and Carroll, 1985) although, with copper, much of it becomes associated with organics in the soil (Xian, 1987). The fate is also dependent upon the nature of the receiving soil, with pH playing a major role in dictating metal mobilization (Pertsovskaya et al., 1987). The effect of acid rain can thus be the introduction of aerosol metal and, as well, the mobilization of existing soil metal whether of anthropogenic or natural origin. However, buffering capability varies from one soil type to another, which makes the biological impact site specific (see, for example, Nohrstedt, 1987 and Ulrich, 1984).

Copper in Freshwater Environments

Mechanisms controlling the input, distribution, and chemistry of copper in freshwater are reviewed by Murray (1987) and Konovalov et al. (1983). The latter also considers the discharge of trace elements into the sea. Medine and Bicknell (1986) use a metals exposure analysis modeling system to examine metal speciation with the intention of estimating the concentration of bioavailable metal. The importance of metal speciation is becoming more apparent in evaluating the effect of metals in freshwater, especially in waterways receiving appreciable anthropogenic metal input or in bodies of water being restored (e.g. Chau et al., 1985; Lerman and Hull, 1987; Morrison, 1987a; Petrovic et al., 1987).

The geological nature of the drainage basin is responsible for the background levels of filterable and particulate metal. Nriagu (1986) reports average total concentrations of 140 ng/L in River Niger water, a river whose drainage basin is, in major part, Precambrian Shield gneisses, granites and migmatites. Changes in copper concentration and chemistry often occur along a body of water, as a result of natural changes (e.g. chemistry of feeder streams; presence of a dam - Reczynska-Dutka, 1987), periodic events (e.g. McCahon et al., 1987) or anthropogenic input (Furch, 1985; Johnson and Thornton, 1987; Reichel, 1982). With Magela Creek in northern Australia, Hart et al. (1987) report a change in water quality and metal concentration as a result of seasonal changes in flow. Seasonal changes in both dissolved and particulate copper are apparent in a number of rivers, as a result of changes in drainage (e.g. Paez-Osuna et al., 1987). Particulates, including colloids, can provide a major source of copper in rivers, lakes and oceans, a source which can vary seasonally as well as over longer time periods (Chang et al., 1987; Sigleo, 1987; Tillekeratne et al., 1985). Since particulates often adsorb metals, they have been used to indicate the pollution status of a river (Hart and Beckett, 1986; Hellmann, 1986; Linnik and Timchenko, 1986; Malle, 1985). In lakes, as well as oceans, settling particles can account for a major flux of metals to the sediments (Sigg et al., 1987), especially when anoxic conditions occur (Frevert, 1987; Jacobs et al., 1987). Winter overturns have been associated with inputs of sediment copper into a lake (Frevert, 1985). Blachford and Ongley (1984) comment that metal concentrations are not useful in identifying spatial and temporal trends, in part as a result of concentrations of filterable metal being below detectable levels. This is not true, adequate sample procedures do exist that will permit measurement.

In a discussion of the fate of metal ions in lakes, Sigg (1987) comments on the importance of biological particles in removing copper from the water column and regulating its concentration. Khatri (1984) discusses the distribution of four metals (Fe, Zn, Cu, Mn) in Lakhotia Lake in India and reports an inverse relationship of concentration with phytoplankton abundance suggesting the effects of excess metal. Morrison and Revitt (1987) discuss metal speciation methods which identify potentially bioavailable, chemically labile metal fractions in polluted waters. Many of these waters contain large amounts of organics, such as humic substances, which are able to complex metals (e.g. Aualiitia and Pickering, 1986; Buffle et al., 1987; Giesy et al., 1986; Kyle, 1987; Varshall et al., 1982) and reduce metal bioavailability (Hine and Bursill, 1985; Hutchinson and Sprague, 1987).

Metal speciation in lakes and streams is influenced by acidification. Campbell and Tessier (1987) report important changes in both inorganic and organic copper speciation over the pH range from 7 to 4. Changes in pH, and other water quality parameters, can also affect corrosion rates of copper (Stone et al., 1987). The introduction of synthetic metal complexing agents, such as nitrilotriacetate, will also affect metal speciation (Frimmel and Geywitz, 1987b). Schnoor et al. (1987) discuss processes, coefficients and models for simulating heavy metals in surface waters.

Copper in Estuaries and Salt Water

A number of processes affect the concentration, speciation and fate of metals in estuaries and salt water. Some of these are discussed by Whitfield and Turner (1986) along with some of the problems in extrapolating from the equilibrium approach of estimating metal speciation to natural systems. Natural systems can be variable or almost steady state. Harbison (1986), for example, discusses major diurnal changes in the chemistry of shallow tidal inlets in South Australian Gulf waters. Seawater temperatures can range from near freezing to well above 100òC near hydrothermal vents, a factor which Kumar (1987) relates to cation hydrolysis and the regulation of trace metal composition in seawater. Hydes et al. (1986), however, found copper concentrations similar to those of surrounding water, near the median valley of the Mid Atlantic Ridge. The reactivity of organic-rich sediment near the vents or near thermomineral springs may affect the mobility of sediment-associated copper (e.g. Frevert and Sollmann, 1987;

Thornton and Seyfried, 1987). Millero et al. (1987) discuss the effect of pH, temperature, salinity and ionic composition on the oxidation of Cu(I). The presence of hydrogen peroxide in natural surface waters has caused a reexamination of the oxidation of copper and iron in seawater (Moffett and Zika, 1987b).

Particles influence the dissolved concentrations of copper and other trace metals in seawater as they do in freshwater (Lin et al., 1987; Whitfield and Turner, 1987). Bourg (1987) provides a brief review of empirical and conceptual models for metal sorption although with little discussion of copper. Ion exchange reactions with copper and clay minerals have been examined (Liu et al., 1986) and are important, especially in estuarine situations. Particle transport of metals not only includes sedimentation processes but also horizontal movement. Balls (1986) reports enrichment of Fe, Mn, Zn, Pb, Cu and Cd in the Firth of Clyde and (Balls and Topping, 1987) in the Firth of Forth, with dilution and sedimentation causing a decrease in metal concentration away from the area. Similar results have been recently shown in and near the Scheldt estuary (Baeyens et al., 1987b), Carnon River and Restronguet Creek (England; Johnson, 1986), Chesapeake Bay (Rule, 1986) and the Mississippi (Trefry, 1987). However, Bloom and Crecelius (1988) found sediment metal accumulation in Puget Sound to be controlled by sediment grain size rather than proximity to source. Work in an enclosed seawater system (Wong et al., 1986) suggests that mine tailings cause little increase in copper in seawater. Juracic et al. (1986, 1987) discuss the Adige River estuary in the Northern Adriatic Sea, and comment that most of the metal-containing riverborne material is deposited within the estuary. Kersten and Forstner (1985) examine trace metal partitioning in the Southeastern North Sea, finding most of the copper associated with the reducible fraction of the particulates and not with the organic fraction. Biological particles have been associated with trace metal cycling (e.g. Demina et al., 1985).

Since copper forms a large number of inorganic and organic complexes in seawater, there is continuing work on the nature and lability of these complexes. Motekaitis and Martell (1987) examine inorganic complexes in seawater and the effect of metal chelating agents on their equilibria. Turner and Whitfield (1987) provide an equilibrium speciation model for copper with includes complexation with a natural and two synthetic chelating agents. In a Ph.D. thesis, Buckley (1985) examines the organic speciation of copper, zinc and lead in seawater, using electrochemical and ion exchange methods. He reports that, with Atlantic samples, ligand concentrations are high at the surface but tend to decrease with depth. In the Scheldt estuary, van den Berg et al. (1987) found evidence of interactions of copper and zinc with "dissolved" organic complexing agents that they suggest determine the geochemical pathways of the metals. Elevated concentrations of "dissolved" organic copper have been found to be variable in the Baltic Sea (Osterroht et al., 1988) suggesting the presence of some factors to concentrate or dilute the organics or the source of the organics. High ligand concentrations have been related to primary productivity in a tropical bay (van den Berg and Rebello, 1986) and some evidence indicates the association in warm-core ring waters (Hanson et al., 1988), and in coral reef waters (Mackey et al., 1987).

An estuary forms the interface between a river and the ocean. Transport of copper in particulate and dissolved forms, from the land to the sea, occurs in effect in the estuary. Geochemical mass-balance calculations have been done for some rivers, such as the Mackenzie River (Thomas et al., 1986) and the Seine (Avoine et al., 1986). Nixon and Lee (1986), however, point out that few mass balance studies have been conducted on wetlands and are badly needed to understand the fate of natural and anthropogenic metals. With the Mississippi River, Trefry (1987) reports that copper levels are up to 20 times lower in the northern Gulf of Mexico than in the river, as a result of dilution of river water with salt water. Adediran (1985), in a Ph.D. thesis, comments that adsorption of copper decreased drastically in the low salinity regime developed in a laboratory experiment. This suggested (abstract) ".. a dilution phenomena of Cu along an estuarine profile." Duinker (1986), Kester et al. (1986) and Salomons et al. (1987b) discuss the mechanisms involved in the formation and transformation of element species in estuaries. They discuss in generalities, some of the equilibrium concepts for both organic and inorganic species of copper. Valenta et al. (1986b) comment that, for Cu, Cd and Pb, (page 74) "... reversible exchange processes between the dissolved and the particulate matter phase will regulate their fate in estuaries to a significant extent." Bewers et al. (1986a) edited the results of the Nantes Symposium on Contaminant Fluxes through the Coastal Zone. In a discussion of trace element distribution in the Arno river estuary (Italy), Betti et al. (1986) report a relationship with depth, supporting the concept of particulate metal sedimentation in or near an estuary. Organically-associated copper has been described from a number of estuaries (e.g. Demina and Artem'ev, 1984; Hayase et al., 1986). Flocculation of organically bound riverine copper may also occur with the introduction of major ions in saltwater. This causes sedimentation at relatively low salinities. This would cause a fractionation of metal species in the estuary, with inorganic labile copper becoming more predominate at higher salinities. This is discussed by Lapin and Krasyukov (1986) and has been reported for the Razdol'naya River entering the Sea of Japan and the Saigon River entering the South China Sea (Anikiyev et al., 1985; Artem'ev et al., 1982).

Trace metals have been used as oceanic tracers which implies a certain chemical stability (Abdullah, 1985). This is contrary to what is found in estuaries where changes in salinity and other chemical factors occur over a short distance and in a relatively short time period. The greater stability in the oceanic environment maintains a greater chemical stability that allows, for example, examination of the transport of copper from the Bering Sea to the northwestern North Pacific (Maeda, 1986). In general, higher concentrations of copper are found in inshore waters (e.g. Akagi et al., 1986; Baeyens et al., 1987a), ultimately as a result of river input (Kremling et al., 1987; Windom and Smith, 1985; see also Windom, 1986), anthropogenic input (e.g. Balls, 1987) or sediment mobilization (Jickells and Burton, 1988; Kremling and Hydes, 1988). Balls (1985) reports average copper copper levels of 60 ng/dm^3 for oceanic waters and 170 ng/dm^3 for coastal waters around Scotland. Balls (1987) obtained evidence that, in the mixing of Irish Sea and Scottish coastal waters, copper behaves in a conservative manner. There may also be seasonal variations, occurring as a result of biological uptake, decomposition and sedimentation. In the Red Sea, Beltagy (1982) notes two copper maxima, during the winter and summer, possibly associated with biological cycling.

Copper in Sediments

The recent literature includes a number of discussions about the concentrations of metals in sediments of different areas (e.g. Kosov and Demidova, 1985; Madsen and Larsen, 1986; Shankar et al., 1987). Aquatic sediments form the ultimate receiving medium for particulate copper whether from a natural or anthropogenic source (Davis et al., 1987). Sediments thus can form a record of events occurring in the past. Sediment cores have, for example, been used to reconstruct the history of contamination in Venice lagoon (Pavoni et al., 1987). Nriagu and Wong (1986) note problems in the use of pollutant metal profiles to assess human contribution to the metal flux of many lake basins because of sediment diagenesis. Knowledge of sediment-metal chemistry is also insufficient to permit adequate modelling of events (Forstner et al., 1985). An array of processes affect the seawater-sediment interactions (see Cauwet, 1987 and Rumohr et al., 1987), some of which can cause a mobilization of sediment-bound metals (Forstner and Salomons, 1984; Li et al., 1987a; Salomons et al., 1987a). The chemical nature of these processes is controlled by natural as well as anthropogenic events (e.g. Fukushima et al., 1985). Wang et al. (1986), for example, use a change in copper, zinc and lead concentrations of a core to indicate an increase in anthropogenic input to Lake Geneva. Surficial sediment enrichment of copper in coastal waters has been suggested to be a result of anthropogenic input although Ridgway and Price (1987) suggest that it is, at least in part, a result of diagenetic processes. This is partly due to the nature of depositional environments which thus become important in the explanation of trace element distributions today as well as in the geological past (e.g. Walters et al., 1987).

The chemistry of the sediment-water interface is important in the events following metal deposition (Balzer et al., 1987). Heggie et al. (1987) report copper remobilization and fluxes from continental margin sediments of the eastern Bering Sea. They suggest that this flux may be one "source" of copper to the deep sea. Metz (1986) suggests that copper lost from Mississippi River delta sediments can be traced to the deep Gulf of Mexico. This is a result of metal scavenging by particles. This is in contrast to the desorption of copper from particles suggested by work in the Tamar River Estuary (Ackroyd et al., 1987). Sediment particle size affects metal concentration and metal transport, smaller particles accumulate higher levels of copper and other metals and transport them further than larger particles (e.g. Bloom and Crecilius, 1987; Lee, 1985; Mudroch and Duncan, 1986; Schoer et al., 1982; Sly, 1984). Tidal action and the resultant exposure and submersion of anoxic sediments has been reported to mobilize cadmium (Kerner et al., 1986). Similar mobilization could occur with copper. In anoxic sediments, chemically labile copper can react with H_2S to form insoluble copper sulfides. However, organically complexed copper may not be sufficiently labile to react with H_2S (Douglas, 1987). Sorption, by ferromanganese nodules, will scavenge copper from the water and increase benthic metal levels (Chen et al., 1987b; Denton, 1986; Ingri and Ponter, 1986; Tikhomirov and Shakhova, 1987). However, the effect of ferromanganese deposits on copper levels is not well understood (Myllymaa et al., 1985) although coprecipitation processes of the iron and manganese hydroxides/oxides are known to occur (e.g. Prohic and Kniewald, 1987; Schoer and Eggerslgluess, 1982).

Anthropogenic input of copper to sediment environments includes transport of fertilizer and pesticide copper from agricultural fields (Wauchope, 1987). Sewage is considered to be a major source of sediment copper in heavily populated areas (e.g. Ashwood and Olsen, 1988; Ashwood et al., 1986). Because of the importance of organic material in binding copper (Reboredo and Pais, 1984), the proportion of organically bound copper should be higher in sewage-associated metal. Sediment copper levels also reflect the effects of industrial emissions (El-Daoushy, 1986; Fallon and Horvath, 1985; Lum and Gammon, 1985; Mudroch, 1985; Mudroch and Duncan, 1986).

Effects of anthropogenic input, on metal chemistry and concentrations, have been examined for a variety of sediments and tailings (e.g. De Souza et al., 1986; Quevauviller et al., 1986; Smith-Briggs, 1983). Concentrations of several metals, including copper were elevated in the leachate from seawater leaching of processed ferromanganese nodule wastes (Schein et al., 1987). Jones (1986) reports oxidative release of copper from sediments associated with an acid mine drainiage stream. Reduction in lake and stream acidity may be as important to reducing metal bioavailability as reduction in metal input (Nriagu and Rao, 1987). Besser and Rabeni (1987) obtained evidence that vegetative cover over mine tailings might enhance sediment metal mobilization as a result of organic complexing agents from the vegetation.

Relationships exist between benthic organisms and the sediment which can include copper as well as other trace metals. Rice and Whitlow (1985), for example, found an inverse relationship between the biomass of a species of benthic polychaete worm and its copper content. This and other examples indicate that the fate of copper in the sediments is at least partially controlled by the sediment biota as well as its chemistry. Since the partitioning of copper in sediments affects its bioavailability (Tessier and Campbell, 1987), the sediment-metal-organism interaction becomes interactive - metal chemistry affected by organisms, metal bioavailability affected by chemistry. Metal binding by bacterial cell walls is reported by Beveridge and Fyfe (1985). They comment (abstract) that "The anionic cell walls of bacteria are remarkable in their ability to fix metals and provide sites for nucleation and growth of minerals." Lopes et al. (1986) tested the hypothesis that copper absorption by benthic blue-green algal mats could allow mineralogenesis. They comment (page 62) that "Assuming a mineralizing solution (groundwater) containing 1mg Cu/L, and a loss of 90% of the mass of organic material during diagenesis, the introduction of sufficient copper to produce a 1% ore would require about 2000 years. This is a short time, geologically, but may not be in terms of algal mat lifetime." (Blue-green algae are noted for their production of metal complexing agents which may enhance the retention of metal.) Breakdown products of organisms and organics must also be included in the factors that can affect the accumulation of copper in sediments. This would include riverine humic substances that flocculate in estuaries, with resultant sedimentation of organics and metal, including copper (e.g. Giannissis and Martin, 1984). Shanmukhappa et al. (1986) note a seasonal change in humic acid, copper and iron in sediments near Porto Novo, India. They associate this with seasonal abundance of the mangrove source of the humics and runoff which transports it to the ocean. Litter from *Spartina alterniflora*, a salt marsh plant, is reported to be enriched with copper (Pellenbarg, 1985) and would form a source of sediment metal.

VII - COPPER CONCENTRATIONS IN THE ENVIRONMENT

A number of private and govenmental agencies are interested in the levels of copper in the environment and have undertaken examinations of both large and small areas, usually affected or to be affected by industry. Examples include Reczynska-Dutka (1985), who provides metal levels in surface fresh waters of the Upper Silesian Industrial Region of Poland, and Drude de Lacerda (1987) who evaluated heavy metal distribution, availability and fate in a bay near Rio de Janeiro (Brazil) receiving anthropogenic metals from a single point source. Kitano (1987) reviews factors controlling the chemistry of metals in coral reef water and the analysis of these water. The ability of natural organics to complex metals has become of interest (e.g. Hine and Bursill, 1985) as organics become involved in the transport and affect the biological availability of metals such as copper. Liang (1985) discusses the effects of organics and inorganic on metal speciation in natural waters, commenting on the relationship between speciation and toxicity and van den Berg et al. (1987) discuss organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt Estuary in The Netherlands. This is of importance because of the effect organics have on metal bioavailability (e.g. Frimmel and Hopp, 1986; Simonova and Granda, 1987).

Techniques of metal analysis are of major importance not only because of the necessity of evaluating trace metal levels in terms of contamination problems but also because of the necessity of comparing metal values over wide geographic areas. General discussions of the routines of metal sampling and analysis are provided by Ashton and Chan (1985, 1987), Pavoni et al. (1987a), Stoeppler and Nurnberg (1984) and Tramontano et al., 1987. Analysis of samples has been widely discussed, with recent discussions given by Hoffmann and Lieser (1987) and Irgolic (1987). Recent citations on the analysis of heavy metals are provided by the U.S. National Technical Information Service (1987e) as are citations on heavy metals in drinking water (N.T.I.S., 1986a).

New devices for collecting samples for metal analysis are described in Brugmann et al. (1987) and Harper (1987). Resin pre-concentration of metals is being used more and more in sample treatment (e.g. Alam et al., 1986; Landing et al., 1986; Marina et al., 1987; Plavsic and Branica, 1986; Samara and Kouimtzis, 1987). Metal level determination still makes broad use of spectrophotometric techniques (Abe et al., 1987; de Pablos et al., 1986; Gustavsson and Hansson, 1984; Hayase et al., 1986; Notzaki et al., 1987) as well as a variety of other techniques (e.g. Akagi et al., 1987; Beauchemin et al., 1987; Grossmann et al., 1985; Mills et al., 1987; Reddy et al., 1986; Riekkola and Juntto, 1986; Schwedt and Hockendorf, 1986; Themelis and Vasilikiotis, 1987). Anodic stripping voltammetry is widely used and Yang et al. (1987) describe a technique to reduce or eliminate the interference from humic acids or surfactants. Turner et al. (1987) used amperometric techniques to measure copper and lead complexation capacities of fulvic acid extracted from the River Tamar in southwestern England. They report problems suggesting that amperometric titrations, under the conditions used, do not provide a reliable means of estimating complexation capacity. Sweileh et al. (1987) compared estimates of free copper (II) concentrations in freshwaters, obtained by ion exchange-atomic absorption techniques , with those obtained with a cupric ion selective electrode. Although the ion exchange technique was found to be more sensitive than the ion selective electrode, it is subject to interference from cationic and neutral copper complexes as well as from filterable colloid and colloidal copper-hydroxo species at higher pH values. Accurate values were obtained by both methods in the presence of anionic copper-ligand complexes. Other recent references to electrochemical techniques include (Aualiitia and Pickering, 1986, 1987a; Frimmel and Geywitz, 1987a; Newton and van den Berg, 1987; Ostapczuk et al., 1987; Prabhu and Baldwin, 1987; Pyshcheva et al., 1985). Lund (1986) reviews electrochemical methods and discusses their limitations for the determination of metal species in natural waters. Stoessel and Prange (1986) describe an X-ray fluorescence technique for measuring trace elements in rainwater.

Raab et al. (1987) used a portable X-ray fluorescence system developed by the U.S. Environmental Protection Agency and NASA for on-site evaluation of hazardous wastes. They report precision and accuracy within 10% of the values obtained in the laboratory. The general trace metal analytical capabilities of X-ray techniques are discussed by Arber et al. (1988).

The estimation of biologically available copper in soils is important for plant and animal nutrition as well as the provision of copper in fertilizers. Jackson et al. (1987) discuss a stratified sampling protocol for monitoring trace metal concentrations in soil while Kheboian and Bauer (1987) examine extraction procedures for metal speciation in model aquatic sediments. Gajbhiye (1985) reports that a particular extractant (DTPA) appeared to be suitable as a common soil extractant for available Fe, Mn, Zn and Cu in soils. Other references on soil extraction include Barbarick and Workman (1987), Burridge and Hewitt (1987), Nikolaeva et al., 1985, Slavek and Pickering (1987), and Zabel et al. (1987).

Griepink (1984) comments on the quality of environmental trace metal analysis and points out the principal sources of error. Chemical events occurring in both natural and anthropogenic systems will affect the nature of the sampling routine and the quality of the results. Verloo and Cottenie (1985), for example, discuss the influence of redoxpotential and pH on the transfer of heavy metals from the solid to the liquid phase in river sediments. Knowledge of these types of processes is important in the design of sampling programs. The effect of adsorption (e.g. Salim, 1987a) and particle size (e.g. Fergusson, 1987) are important in affecting metal concentrations and speciation and thus sampling programs. Salim (1987b) as well as Towner et al. (1986) discuss the effect of storage on metal distribution in a sample. The problems of trace metal analysis in environmental samples are numerous. As a result, there is increasing use of certified reference materials (e.g. Griepink and Muntau, 1987; MacDonald and O'Brien, 1985) and intercalibration of samples between laboratories (e.g. Berman, 1986; Berman et al., 1986; Bewers et al., 1986b; Loring, 1986, 1987; Palmork, 1986).

Concentrations of metals for various environmental situations are presented in table 2. In using this table the reader is urged to keep in mind the many problems that are encountered in the collection and analysis of samples from natural environments.

VIII- COPPER CONCENTRATIONS IN ORGANISMS

Since copper is essential to an organism, the concentration of copper present can be an indication of a deficient condition. Likewise, excessively high concentrations in an organism may indicate exposure to and uptake of excess copper in the environment. However, the use of metal concentrations to indicate these conditions is fraught with problems, problems due to contamination during collection, erroneous methods of analysis, and improper interpretation of organism metal concentrations. Knowledge and techniques are, however, improving for chemical measurement of organisms and the environment (e.g. Zirino, 1985) which suggests that the relationship between organism metal concentrations and the environment will be better understood. This is especially important when trying to relate metal measurements from one geographic region to those in another (Boniforti and Maouro, 1982).

A number of techniques for measuring copper levels in biological material have appeared in the recent literature (e.g. Hoffmann and Lieser, 1987; Irgolic, 1987; Mehnert, 1986; Schramel et al., 1987; Shravah and Sindhwani, 1986; Themelis and Vasilikiotis, 1987). The use of metal levels in plants has been used as a monitoring tool (e.g. Wallner et al., 1986) although Houba et al. (1986) comment that, with copper, comparison of values may be difficult because of variability. With animals, there is also a suggestion that metal concentrations may vary both seasonally and with size (e.g. Ikuta and Nakamura, 1986) which increases the difficulty of comparisons between populations. Techniques have been described for measuring metal concentrations in human tissues (Aalbers et al., 1987; Baumgardt, 1985; Bregadze and Galagutashvili, 1986; Gutteridge, 1987; Kuchinskii, 1987; Wheeler et al., 1987). Vanoeteren et al. (1986a), however, point out the inconsistencies in at least lung tissue values as a result of improper handling or analysis. In an intriguing study, Sandford et al. (1987) compared metal levels in human bone and soil samples from 6th century Carthage, pointing out the effect of bone metal contamination from the soil. Continuing interest in trace metal levels in foods and food materials has produced a number of new analytical techniques as well as changes in older techniques (e.g. Saito et al., 1984; Stryjewska et al., 1987; Suzuki et al., 1987a; Ushiyama et al., 1986)

A variety of equipment types are used for metal analysis in biological material. Recent literature on uses of equipment include:

Atomic absorption spectometry - Amiard et al., 1987b; Chou et al., 1986; Ebdon and Evans, 1987; Ebdon et al., 1987; Halls et al., 1987; Hutchinson et al., 1986; Ihnat, 1987; Jin and Ni, 1987; Kozak et al., 1985; Nyarku et al., 1986; Oikawa et al., 1987; Petrucci and Van Loon, 1987; Van Beek et al., 1987; Zuehlke and Kester, 1985

Inductively coupled plasma spectrometry - Wandt and Pougnet, 1986; Zarcinas et al., 1987

Polarography - Kalvoda, 1987; Wu and Yang, 1986

Ion-selective electrode - Maslowska and Szmich, 1985

Voltammetry - Cai and Zhang, 1986; Gemmer-Colos and Neeb, 1987; Hoyer and Florence, 1987; Wahdat and Neeb, 1987

X-ray fluorescence - Havranek et al., 1986; von Bohlen et al., 1987

PIXE analysis - Hertel and Thorlacius-Ussing, 1987; Tanaka et al., 1987

Neutron activation - Cunningham and Stroube, 1987; Jayawickreme and Chatt, 1987; Rajadhyaksha and Turel, 1986; Sabbioni et al., 1987; Zhuang et al., 1986

Miscellaneous - Arber et al., 1988; Berneike et al., 1986; Comber, 1986; Gotz and Heumann, 1987; Ichinoki et al., 1987; Lorch et al., 1987

Besides natural trace metal variability (e.g. Injuk et al., 1987 but see also Aleshko-Ozhevsky et al., 1986), there are sources of error in the collection and analysis of biological materials. Narayanan et al. (1986) comment that (page 598), for blood, "the cleanliness of the specimen container is paramount in minimizing preanalytical errors. The specimen container should be a closed system to minimize contamination and also sample evaporation which can lead to an apparent increase in trace element level." Extraction techniques may also introduce errors due to contamination or to the use of an improper extracting agent (e.g. Walder et al., 1987). Sample preparation has also been found to be a potential source of metal contamination to biological tissues (Schmitt and Finger, 1987). Keen and Feldman (1988) comment that the use of EDTA (ethylenediaminetetraacetic acid) as a blood coagulant can produce changes in the concentration of metals such as copper in the blood. Variability in analytical results has sponsored a number of trace metal intercalibration tests (e.g. Berman and Boyko, 1986; De Ruig, 1986; Hendzel et al., 1986; International Laboratory of Marine Radioactivity, 1986a,b; Kumpulainen and Paakki, 1987; Sterrett et al., 1987). It has also been the major reason for developing biological reference materials for trace metal analysis (e.g. Berman and Sturgeon, 1987; Dirscherl et al., 1987; Rasberry, 1987; Veillon et al., 1986; Wagstaffe, 1986, 1987; Wolf and Miller-Ihli, 1987) as well as the provision of reference data (e.g. Iyengar, 1987). At least one attempt has been made to present metal concentration in terms of nutritional variables (Romeo et al., 1987). Treatment of data subsequent to analysis requires careful selection of methods (e.g. Misra and Uthe, 1987) to obtain the desired evaluation.

The following tables present metal levels in plants, animals and humans. Scientific names are normally used because local or common names are so frequently misused and can be very misleading to the individual attempting to relate tissue metal levels from one organism or one region to another. In using the tables, the reader should be concerned with the methods of sample collection, preservation and preparation for analysis. These can often be obtained from the cited publications. Levels of metals are often presented for organisms existing under abnormal conditions. This does not provide a realistic appraisal of normal levels of metals.