ICA PROJECT 223

THE BIOLOGICAL IMPORTANCE OF COPPER

A Literature Review

June, 1991

Preface

In 1973 the then 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 became 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 reviews pointed out the broad application of concepts about the biological importance of copper.

The present review includes literature for the period 1988-1989 although a number of earlier works are included and, where appropriate, a few appearing early in 1990 have been used. Many of the earlier references are from eastern Europe and Asia because this literature takes time to appear in the North American data review bases. References were obtained in major part, through literature search programs available through the Woodward Biomedical Library at The University of British Columbia. Mr. Brian Moreton, the European ICA Director, has kindly provided the metals section of the Marine Pollution Research Titles as a source of European as well as North American References.

The 1990 review was written using 3,068 references selected from the literature searches. These references have been catalogued with those used in previous reviews to form the ICA Reference Collection. With financial assistance from ICA, the cross-referencing scheme of all references is being updated to be compatable with the scheme used in the present review. This will better allow computer searches for industry, government and academia. Sharon DeWreede is responsible for the ICA collection which, with the 1990 references, now contains 23,166 references.

It will be apparent to the reader that the background of the reviewer (A.G.L.) is in marine sciences. With this in mind, special effort has been made to cover other areas. Table 2, copper levels in the environment, was prepared by Leslie Chan. Tables 3 and 4, copper levels in plant and animal tissues, were prepared by Sharon DeWreede. Table 5, copper levels in human-related topics, was prepared by Leslie Chan. (The reviewer and Grant Recipient is accountable for all tables.)

Because of the problems of obtaining certain references, particularly manuscript reports, this review should be considered as a "critical review" of the literature. The cross-referencing scheme used in the preparation and writing of the review provides an integration of concepts from all areas covered by the literature search. It is a review that addresses four basic questions:

1. What does copper do to organisms?
2. What are the sources of environmental copper?
3. What happens to copper once it enters the environment?
4. What are the relationships between the chemistry of copper and its biological importance?

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

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Executive Summary

The literature used in this review demonstrates an increasing interest in the value of dietary copper in medicine and agriculture. The importance of metal speciation is also becoming more widely accepted in explanations of the biological effect of copper in the environment as well as in nutrition, medicine and agriculture. Metal chemistry plays a major role in controlling the biological availability of copper. From the 3,068 references used in the review, some of the highlights include:

Soil copper deficiencies are not uncommon and supplementation is used widely to improve the growth of plants (Adams et al., 1989; McIvor et al., 1988) and the animals that feed on them (Calhoun et al., 1987; Chase, 1987). It is important to note that in addition to low soil or food copper concentrations, copper deficiency can occur as a result of metal-metal competition and complexation by organics (Gawthorne, 1987; Sas, 1989).

The importance of nutritional copper to humans is indicated by work being done at several human nutrition centers. (See, for example, the short article by Kinzel, 1989, entitled "Pinning Down Copper's Place in the Diet".) Specifics are discussed in a number of individual references. Copper deficiency has been noted as a potentially serious problem in the chronically ill elderly (Steffee and Teran, 1989). Copper deficiencies continue to be linked to heart disease (e.g. Bhathena et al., 1988b,c; Klevay, 1989, 1990; Watson, 1987).

Bremner (1990) comments (page 1) that "Surprisingly little is known of the speciation of metals in the diet or in tissues, other than through their association with specific enzymes." He continues, commenting on the value of studying the metabolism of copper (and zinc) at the molecular level, not only to an understanding of metal reactions and organism susceptibility but also to the development of improved methods of disease treatment.

The beneficial effects of copper to plants and animals are numerous. The effect of copper on resistance to bacterial and viral infection in ruminants is discussed by Suttle and Jones (1989). Copper appears to play a role in some of the activities of of the central nervous system (Lagercrantz, 1988; Seidel et al., 1989). Tissue inflammation can be more severe without adequate copper (e.g. Milanino et al., 1989a). Elevated activity of the copper-containing enzyme ceruloplasmin has been reported in the aqueous humour of inflamed eyes and is suggested to be associated with protective functions during inflammation (McGahan et al., 1989b,c). Lunec (1989) comments that "During inflammation we are protected from the onslaught of metal-catalysed free radical reactions paradoxically by (copper-containing) antioxidant metalloenzymes such as superoxide dismutase and caeruloplasmin".

Copper can be used as a pesticide as well as a nutrient (Mabbett, 1987). The contradictory capabilities are due to the ability of copper to interact with organics, either to provide the complexes essential for life (nutrient aspect) or to adversely affect the structure and function of existing organics (pesticide; e.g. Novikov et al., 1989). In the latter case it is a result of excessive metal being present in a chemical form that is available for uptake by the organism.

Excess biologically available copper can interact with certain organics to affect the genetic machinery in an organism (Bumgardner et al., 1989a; Levy and Hecht, 1988; Quinlan and Gutteridge, 1988a,b, 1989; Tofigh and Frenkel, 1989; Yamamoto and Kawanishi, 1989). The effect is not all detrimental, however; Apelgot et al. (1989) presents evidence that "... copper atoms bound to DNA (a nucleic acid) are essential for cellular functioning". The repair of damaged nucleic acids can occur when exposed to ascorbic acid in the presence of a catalytic amount of copper (Yanada et al., 1989).

The potential for the interaction of copper with organics indicates its value in chemistry. Francois et al. (1988), for example, describe a technique for cleaving DNA using an oligonucleotide in the presence of ionic copper and a reducing agent.

The literature includes a number of examples where excess copper has been used to control growth. Arnold and Struve (1989a) used $CuCO_3$-treated plastic containers to inhibit (but not kill) root growth of green ash seedlings. They comment that "... $CuCO_3$-treated containers can be used to control undesirable green ash root growth and produce large seedlings in small containers while still maintaining high root regeneration potential". They (Arnold and Struve, 1989b) also found this for red oak and Wenny and Woollen (1989) used the technique for containerized Douglas-fir, ponderosa pine and western white pine.

Copper has long been used as an antifouling agent in aquatic environments. Actually, with the reduced use of tin in antifouling agents, copper is being more widely used. It is also widely used to reduce the rate of wood decomposition in soil. Evidence given in a House of Lords (1989) report states that the Nature Conservancy Council (NCC) "... is not aware that copper-chrome-arsenic based compounds have caused environmental problems in the UK". Recent literature includes discussions of the interaction of copper-coated surfaces with fouling organisms, interactions which can affect the leaching rate of copper (Lindner, 1988). Evans (1988) discusses the sequence of biofouling, commenting on the bacterial biofilm which appears to be a prerequisite to development of marine fouling communities. In ICA-supported work, Geesey et al. (1988) and Jones et al. (1986) describe the immobilization of copper by slime films from marine fouling bacteria and algae. Techniques for examining corrosion in seawater and characterising biofilms have been developed by Chamberlain et al. (1988a) and Castle et al. (1988; ICA-supported work).

From an environmental standpoint, metal speciation is finally being recognized as essential to the understanding the biological effect of copper. In a book on "Mining and the Freshwater Environment", Kelly (1988) comments that "One point that will be repeated over and over again in later chapters is that the speciation of a metal, rather than its total concentration, is the key to understanding its effect on the biota." In a review entitled "Copper toxicity and chemistry in the environment:...", Flemming and Trevors (1989) note that any detrimental effects are dependent upon the biological availability of the metal and the physico-chemical characteristics of the particular environment that influence metal speciation.

Singleton (1987) comments that "In setting water quality objectives for waterbodies where the copper concentration exceeds the criteria as a result of existing discharges, the form of copper stated in the objectives needs to be defined in advance. In view of the dependence of copper toxicity on the complexing capacity of a waterbody, an assessment ... would have to be performed on a site-specific basis to determine if the biota are being harmed."

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I - THE BIOLOGICAL IMPORTANCE OF COPPER

I.1 COPPER AS A REQUIRED TRACE METAL

Introduction

As an essential element, copper plays an important role in all organisms, including man (e.g. Kies, 1989a; Lenihan, 1988; Sorenson, 1989d). It is implicated in the prebiotic evolution of peptides (Schwendinger and Rode, 1989) and is one of the metals that participate in the interactions between the geochemical and biological components of the Earth (in the sense of Hamilton, 1988) to affect ecological relationships (e.g. Steubing, 1987). The effects of deficient and excess levels of copper have stimulated a great deal of work on the metabolism and role of copper in plants, animals, and man (Denis et al., 1987; Howell and Gawthorne, 1987a,b; Meo et al., 1987; Relli et al., 1988). Among other things, this has demonstrated the importance of adequate copper in the diet (e.g. Hale, 1987). This work has also shown that copper deficiency can occur as a result of metal-metal competition (Gawthorne, 1987; Sas, 1989) as well as from copper complexation by organics.

Recent reviews, many of them mentioned elsewhere in this review, include the discussion of availability and roles of copper, in plants by Delhaize et al. (1987), in animals by Fell (1987). Lofstedt et al. (1988) discuss a goat herd with copper deficient feed, and the expression of that deficiency in 2 kids. In an article in German, Cypher (1988) reviews the importance of copper for the health of humans. Nicola (1989) reviews anemia due to copper deficiency and Milanino et al. (1989a) edit a review on copper and zinc in inflammation. Girchev and Tzachev (1988) review copper metabolism while Kinzel (1989) discusses the importance of nutritional copper to humans. Copper deficiency in infancy is reviewed by Paterson and Burns (1988) and Prohaska and Lukasewycz (1989) review the effect of deficiency during perinatal development on the immune response in mice. Paynter (1987) reviews methods to evaluate copper insufficiency in animals. The nutritional importance of several trace metals is discussed in a brief review by Frenk (1988). The treatment of deficiency and excess copper in animals is reviewed by Allen (1987). The effect of copper on resistance to bacterial and viral infection in ruminants is discussed by Suttle and Jones (1989). Suttle (1987) discusses the nutritional requirements for copper in animals and man. Grace (1988) reviews recent work on the use of trace elements in animal production. Virtamo and Huttunen (1988) include a section on copper in their overview on minerals, trace elements and cardiovascular disease. Recent developments in understanding copper transport, absorption and storage are discussed in an excellent review by Camakaris (1987) while Wiener (1987) reviews the genetics of copper metabolism in animals and man, commenting on some of the problems and benefits with selective breeding programs.

The metabolism of copper and other trace metals is often linked to both normal and abnormal growth (e.g. Carpentieri et al., 1988). Abnormal concentrations or abnormal copper metabolism can lead to severe physiological problems or death although, in most instances, there are physiological controls that prevent this. Sourkes (1988), in a review of trace metals and neurochemistry, comments (page 73) that "The copper content of the brain is ... tightly controlled, so that it is resistant to change either as a consequence of experimental copper loading or dietary deficiency." Copper participates in the function of the nervous system (Bhathena, 1989; Sourkes, 1988) and in hormonal regulation (Gross and Prohaska, 1989). Deficiencies are being linked to heart disease (Bhathena et al., 1988b,c; Watson, 1987) and abnormal conditions in the circulatory system in general (Hennig and Stuart, 1988; Lukaski et al., 1988). Copper is involved in connective tissue formation and maintenance (Dollwet and Sorenson, 1988; Orzali et al., 1987)

There has been increasing effort directed towards an understanding of the physiological changes occurring in the elderly. Part of this effort has been with trace elements such as copper (Greger, 1986; Prasad, 1989). Freeland-Graves and Behmardi (1989) discuss trace mineral requirements in the elderly and Greger (1989) examines the potential for deficiencies as well as detrimental effects of excess metal. Copper deficiency in the chronically ill elderly also occurs (Steffee and Teran, 1989) and can be a serious problem, especially with intravenous feeding. Cousins

(1989) examines the role of copper and other trace metals, in the nutritional regulation of host defense systems in the elderly. Trace metal absorption in the aged is reviewed by Solomons (1989).

Copper can be used as a pesticide as well as a nutrient (Mabbett, 1987). Although this use will be more fully reviewed later, the contradictory capabilities are due to the ability of copper to interact with organics, either to provide the complexes essential for life (nutrient aspect) or to adversely affect the structure and function of existing organics (pesticide; e.g. Novikov et al., 1989). In the latter case it is a result of excessive metal being present, in a chemical form that is available for uptake by the organism.

The interaction of copper with man has occurred throughout time (Grupe and Herrmann, 1988). This interact has been both indirect and direct. Indirect, as for example a component in objects and materials used by man (e.g. Ali et al., 1988a; Cojocaru and Manea, 1987; Kuleff et al., 1988; Newton and Fuchs, 1988; Schiegel et al., 1989; Zhang, 1987) and direct, as a metal useful to man (e.g. Chuan and Kuang, 1987; Hua and Fan, 1988; Ma and Han, 1988; Maddin, 1988). El Gayar and Jones (1989), for example, describe Old Kingdom copper smelting artifacts from a town in Upper Egypt. Laub (1988) discusses the history of a medieval copper drossing plant in the Western Harz district and Presslinger et al. (1988) discuss Bronze Age copper smelting plants in the eastern Alps. Bamberger et al. (1988) mathematically modelled late Bronze Age/Iron Age smelting of oxide copper ore in an attempt to evaluate ancient smelting techniques. Fabrizi et al. (1989) discuss a complex copper phosphate found as a corrosion product on fifteenth to twelfth century (B.C.) Egyptian copper alloy objects. (See Robbiola et al., 1988 for a discussion of corrosion of certain archaeological bronze artifacts.) Chikwendu et al. (1989) discuss the Nigerian sources of copper, lead and tin for the tenth century Igbo-Ukwu bronzes.

Copper in microorganisms and plants

Whether in artificial growth medium or in natural soils, copper is required by microorganisms and plants for growth. Lack of adequate copper can cause a variety of defects, such as reduction in nitrogen fixation by legumes (e.g. O'Hara et al., 1988; Ruszkowska et al., 1986), reduction in plant saccharide content (Slusarczyk and Ruszkowska, 1986), shoot dieback in *Eucalyptus* (Dell and Bywaters, 1989) and pollen sterility in wheat (Krähmer and Podlesak, 1987). As a result, supplementation often improves growth (e.g. Adams et al., 1989; Al-Obaidi et al., 1987; Hsieh, 1988; Luyindula, 1988; McIvor et al., 1988; San Valentin et al., 1986). The benefit, however, is dependent upon the availability of the metal to the organism, a factor that can be controlled by certain soil types for example (Rahmatullah et al., 1988). Detecting various types of mineral and nutrient deficiencies in an extensive series of tropical and temperate crops are discussed in a book edited by Plucknett and Sprague (1989). Chapters discuss the copper requirements and the causes and effects of copper deficiencies for commercially important plants such as cucumber (Pike and Jones, 1989), eggplant (Paterson, 1989), wheat (Olson, 1989), sugarbeets (Ulrich and Hills, 1989), walnuts (Uriu and Ramos, 1989), citrus (Koo, 1989), apples (Oberly, 1989), mango (Plucknett, 1989) and peaches (Childers, 1989). Utilization of available copper, by plants, indicates differential allocation to plant parts. Stark et al. (1989a), for example, report that in huckleberries, roots tend to be a sink for copper.

Copper in animals

Animals, like plants, use copper in an array of enzyme systems as well as elsewhere. In a major group of crustaceans (decapods), Depledge (1989b) calculates 82.8 μg CuÀg^{-1} dry weight present in enzymes and the copper-containing blood pigment haemocyanin. Copper deficiency has been reported from both wild and domestic animals. Gogan et al. (1989) report severe copper deficiency in tule elk near Point Reyes, California as an apparent result of low copper levels in the soil and plants. Koen (1988) suggests marginal copper levels in vegetation to be a possible factor in the inability of a remnant Knysa (South Africa) elephant population to increase. Copper deficiency can be a major problem with domestic ruminants (e.g. Anderson, 1987; Coffey, 1986; Gonzalez et al., 1988b; Johnson et al., 1987; Thomas et al., 1987), enough that a good deal of research is directed towards the diagnosis of copper deficiency and the best methods of supplementation (e.g. Calhoun et al., 1987; Chase, 1987; Coffey, 1989; Hidiroglou and Proulx, 1988; McPhee and Cawley, 1988; Naylor et al., 1989; Raghib and Blincoe, 1989). Suttle (1988) points out the importance of comparative pathology in developing an understanding of the problems of nutritional deficiencies of copper, cobalt and other essential elements. The importance of copper to laboratory animals and humans has also sponsored a great deal of nutritional research. Some of this is reviewed by Kies (1989a). Frimpong and Magee (1989), for example, point out that the copper and zinc contents of American diets is often less than the recommended daily intake of 2 mg (copper) and 15 mg (zinc) per day. They, and others, point out the importance of a proper zinc:copper ratio in diets, especially at low levels of copper and zinc. High levels of dietary molybdenum are known to interfere with the uptake and metabolism of copper by ruminants (Wittenberg and Boila, 1988).

The effects of copper deficiency are numerous, many are covered in reviews by Fell (1987) and Smith (1987b). Metal levels in hair have been used to indicate the trace metal status of living animals and humans as well as archaeological specimens (e.g. Dörner, 1988). Waldron (1988) discusses the heavy metal burden in ancient societies, as indicated by bone metal levels; only a brief mention is made of skeletal copper levels. Francalacci and Tarli (1988) point out some of the problems of using metal levels in bones from prehistoric sites, including soil contamination and bone diagenesis. Gooneratne and Christensen (1989) used bovine fetal tissue and adult liver tissue from packing houses in Saskatchewan, a Canadian province, to survey copper concentrations. They found hypocuprosis most commonly in northern Saskatchewan and emphasize the importance of adequate copper nutrition in pregnant cattle to maintain acceptable fetal status. Copper absorption in humans is diet-dependent (e.g. Turnlund et al., 1989) with age-related changes in requirements as well as availability. Lonnerdal (1988) reviews copper requirements in the perinatal period and Paterson and Burns (1988) review copper deficiency in infancy. Lonnerdal (1988) comments on the apparent lack of serious deficiency even if the mother consumes less than the copper currently recommended (2 mgÀday^{-1}).

Copper appears to play an important role in immune responsiveness in animals, including humans (Beach, 1987; Chandra, 1990; Fletcher et al., 1988; Roberts et al., 1987) although the exact nature of that role is not yet fully understood (e.g. Lukasewycz and Prohaska, 1989; Suttle and Jones, 1989). Spallholz and Stewart (1989) list some of the effects of copper deficiency, stating (page 133) that it "... has effects on immunologic functions, including thymus and spleen histology, decreased immunoglobulin synthesis, cellular immunity and possibly decreased microbicidal capacity of neutrophils." A copper(I)-nicotinic acid complex has been identified as an immunopotentiator in chickens vaccinated against Newcastle disease (Musa et al., 1987). Copper deficiency is reported to change regulatory mechanisms governing thrombosis and inflammation (Peretz et al., 1987b; Schuschke et al., 1989a,b). Certain copper-containing enzymes and organics like haemocyanin and Cu-DIPS (Sorenson et al., 1989b; Vuillaume et al., 1989) appear to act as an antioxidant defense system in animals (Bettger and Bray, 1989; Calabrese et al., 1989a; Lewinsohn, 1988; Reiners, 1987; Soderberg, 1989b). As noted by several authors (e.g. Hubbard et al., 1989; Numata et al., 1989), however, the role of copper in enzymes like cytochrome c oxidase is not fully understood although the metal may be essential for enzyme activity (e.g. Yamada et al., 1987). Immune response may also be affected by direct or indirect action of copper on other aspects of metabolism. Copper-deficiency may affect the physiological response of blood platelets to thrombin (Johnson and Dufault, 1988) or correct lipid metabolism (e.g. Lee and Koo, 1988; Lei et al., 1989; Wachnik et al., 1989). It may also be involved in the repair of components of nucleic acids (Yanada et al., 1989) and affect the activity of a human plasma copper-binding growth factor (Pickart and Lovejoy, 1987).

The formation and maintenance of connective tissue (e.g. bone, cartilage) requires an adequate supply of copper (Dollwet and Sorenson, 1988; Greenaway and O'Gara, 1987). Hintz and Schryver (1987) point out that copper deficiency in dogs causes a bone disease similar to rickets in humans. In foals, Knight et al. (1988) obtained evidence suggesting that dietary copper supplementation decreases the incidence of cartilage lesions. Osteoporosis is a disease characterised by a reduction in bone tissue, a disease noted in copper deficient animals (e.g. Read et al., 1989) and with humans, most commonly with post-menopausal women. Strain comments (abstract) "... that more attention should be given to dietary trace elements, especially copper, in the aetiology of post-menopausal osteoporosis." The roles played by copper in connective tissue formation are not well understood. As examples of this, certain copper complexes appear to associate with fibrillar proteins of collagen (Shenyakina, 1987, 1988), the organic "matrix" of bone and yet copper-catalyzed peroxidation may cause degradation of joint tissue in rheumatoid arthritis (Kim, 1987). Copper deficiency has also been related to psychomotor retardation and visual disturbance (in review by Sourkes, 1988) with evidence that copper is important in neurochemistry. Bhathena (1989) and Bhathena and Recant (1990) review the importance of copper in endocrine and neuroendocrine function. Copper is reported to be important in opiate binding in the brain although Bhathena et al. (1988a) found an interaction with dietary fructose suggesting that the action of copper may be dependent on other factors. In a review of infant nutrition and neurotransmitters, Lagercrantz (1988) comments that copper is important to neurotransmitters in the developing fetus and infant. Kardos et al. (1989b) present evidence from studies with the rat brain, that copper may play a role in regulating neuronal excitability. Seidel et al. (1989) note that synthesis of norepinephrine in cardiac nerve endings is sensitive to dietary copper deficiency.

In the abstract of a presentation on the importance of copper in human nutrition and health, Mills (1990) suggests that dietary requirements decline between infancy and adulthood, from about 45 to 15 µg/kg body weight per day. The effects of copper deficiency, such as cardiovascular dysfunction, may also be affected by a variety of other factors, such as dietary fructose and anemia (Fields et al., 1990; Redman et al., 1988) and sex hormones (Bhathena et al., 1988b,c). Genetic effects may be important both from a generic standpoint (e.g. sex hormones) as well as from an individual standpoint. Priev (1990), for example, comments (abstract) that "Copper-loading capacity of the human organism seems to be under genetic control." Nielsen and Milne (1990), studying the effect of copper deprivation in a group of men, suggest that genetic make-up may effect (sic) the response to short-term copper deprivation. Drugs may also have an effect, by complexing tissue copper (e.g. Hasinoff, 1989). In contrast, copper may affect the pharmacokinetics of agents such as salicylic acid (Shetty, 1988).

Severe copper deficiency can produce myocardial and vascular lesions as well as hypercholesterolemia in experimental animals (Ray et al., 1989; reviewed in Virtamo and Huttunen, 1988) as well as humans (e.g. Liu and Chen, 1987). Molteni et al. (1988) note that serum copper concentration can be used as an index of cariopulmonary injury in rats treated with monocrotaline, an alkaloid that produces inflammation and hypertension. Klevay (1989, 1990) suggests (1990) that "... copper deficiency is important in the etiology and pathophysiology of ischemic heart disease." Meissmer (1990) notes that "Copper deficiency causes a rise in serum cholesterol and in the aorta there is a significant inverse correlation of copper content with the area of lipid deposits." A number of other studies support the concept that copper deficiency is associated with atherosclerosis, ischemic heart disease and aortic lesions (e.g. Burns et al., 1989; Kinsman et al., 1990; Radhakrishnamurthy et al., 1989; Riley et al., 1990). This is evidenced by changes in cardiovascular tissue composition (e.g. Burns et al., 1989; Medeiros et al., 1989) as well as altered lipid and fatty acid metabolism (Al-Othman and Lei, 1989; Carr and Lei, 1989a,b; Cunnane et al., 1988; Koo and Lee, 1989). From work on rats, Fields et al. (1987, 1989a,d), however, suggest that (abstract, Fields et al., 1989d) "... heart pathology and mortality in copper deficiency are sex related and not due to high levels of plasma cholesterol, triglycerides, and uric acid or to differences in myocardial fatty acid composition." Carville and Strain (1988, 1989) report results with male and female rats that (abstract, Carville and Strain, 1988) "... demonstrate sex differentiated effects of low copper diets on blood cholesterol and antioxidant defence mechanisms." Copper deficiency produced changes in lipid metabolism can be related to changes in enzyme levels (Kohno and Nakagawa, 1988; Lynch and Strain, 1989) which suggests that any sex-related effect would be secondary, possibly a result of hormone effect.

Copper has been associated with pancreatic secretion although to varying degrees (e.g. Ribero and Tauler, 1988). Dubick et al. (1989) and Majumdar et al. (1989) present evidence that marginal copper deficiency affects exocrine pancreatic structure and its responsiveness to physiological secretagogues. This is enhanced by a high fructose diet in copper deficient male rats (Lewis and

Fields, 1989). Glandular atrophy (Weaver, 1989) can be produced by copper deficiency although this is more evident with severe rather than minor deficiency (e.g. Mylroie et al., 1989). Copper-deficient mice are reported to have small thymus glands even though corticosterone levels are normal (Prohaska et al., 1989). Kidney function can also be reduced with copper deficiency (in Saari et al., 1989, 1990), expressed for example as a reduction in salt tolerance in laboratory rats (Moore et al., 1989a). Copper and zinc balances can be affected by kidney malfunction requiring the use of dialysis (Tamura et al., 1989). Copper balance is important in nutrition, including total parental nutrition (e.g. Davis et al., 1987; Fujita et al., 1989; Matsuda et al., 1989; Saudin et al., 1988). Copper deficiency is reported to alter the physiology of blood platelets under certain conditions (Dufault et al., 1989). Demands for copper appear to change both with physiological status and age (e.g. Breedveld et al., 1988). van Niekerk et al. (1988), for example, obtained evidence with ewes that the need for copper is higher for reproduction than normal body maintenance.

I.2 USES OF COPPER

Copper is used in a wide range of products, including those that help or control the growth of organisms. Since it is required by all organisms, including bacteria (see Beveridge and Doyle, 1989), it is considered an essential metal. This section of the review will use recent literature to discuss the uses of copper by man.

Copper as a nutrient supplement in plant production

Copper is an essential microorganism and plant micronutrient. It can be deficient in some (e.g. Blue, 1988; Unno et al., 1984) although not in all soil types (e.g. Gupta, 1989b). This is true for both heavily farmed (e.g. Mathur et al., 1989) and acidic soils. Deficient soil copper levels means either supplementation or the use of plants that can survive at low concentrations of soil copper (e.g. Melendez et al., 1986). The latter is not advisable if plants are to be used as a food source for herbivores, often causing copper deficiencies. Copper is also useful as a fungicide for improved plant growth. As Mabbett (1987) states (page 143), "Copper deficiencies can only be corrected by application of copper salts or copper chelates but there are hundreds of alternative fungicides available to farmers." Kuduk (1988) points out that low copper sulfate doses increase wheat growth but high concentrations cause a reduction. Reclaimed peat soils, or soils with high humic acid content, are often improved by application of fertilizers that include copper (Laszkiewicz et al., 1987a; Mamaeva et al., 1989). Part of this is a result of copper complexation by the humic materials, with the tendency of producing a copper-deficient soil. A number of copper-containing fertilizers have been prepared from a variety of agents, including industrial byproducts (e.g. Chernyi and Strel'tsov, 1988; Laszkiewicz et al., 1987b; Ovchinnikova et al., 1988). These can include metal-processing wastes that include copper (e.g. Lubis et al., 1988). Copper has been used to improve the granule size and caking factor for a nitrogen-containing fertilizer (Komarov et al., 1988).

The use of copper-containing fertilizers has the potential to increase plant yield (e.g. Belanger et al., 1987; Cheng, 1987; Lungu and Toma, 1988; Mamedkhanov, 1989; Singh and Misra, 1986; Szakal and Tolgyesi, 1989) along with the increase in tissue copper levels (e.g. Lasztity, 1988a). The latter is, of course, beneficial in a deficiency condition but is considered potentially harmful with excess copper (discussed later in this review). The increase in tissue copper levels may be minor and affected by seasonal changes in the plant. Morris et al. (1987) note, for example, that although copper levels in ryegrass forage increased slightly with copper supplementation there was also a seasonal change in concentration. Soil pH may also affect metal uptake (e.g. Jokinen and Tahtinen, 1987a), due to its effect on metal bioavailability as well as plant physiology. Metal-metal and metal-nutrient interaction is also an important consideration in the use of copper-containing fertilizers. Zinc-copper interaction has, for example, been found to decrease the yield of rice under certain conditions (Gangwar et al., 1988). Javadi (1988) reports (Ph.D. thesis) that in wheat, soil phosphorus and copper interacted to increase leaf plastocyanin and phosphorus.

Other uses of copper with plants are discussed in a number of recent publications. Dfaz et al. (1987), for example, use it to improved bud breaking in peach and apple under warm climates in Mexico. In a patent document, Feng et al. (1988) describe the use of ammonium bicarbonate and copper sulfate-containing germicides to reduce crop withering. Copper-containing defoliants are used to facilitate harvesting of vegetables and root and tuber crops (Nakajima and Sumi, 1988). Copper coatings can help reduce root deformation in container-grown planting stock (Arnold and Struve, 1989a,b; Mauer, 1987; Wenny and Woollen, 1989). Preservation of plant materials can often be improved by copper. This has been recently reported for pollen in archaeological deposits (Greig, 1989) and is routinely reported for agricultural products (e.g. Hayakawa, 1988; Matsumura and Honda, 1987; Yokota, 1989). Krotyuk (19887) describes the use of cobalt-copper catalysts for hydrogenation of sunflower oil.

Copper in fermentation

The addition of copper can benefit the growth and fermentative capacity of yeasts, as for example in the production of ethanol (Hajdu and Kiraly, 1988) and aflatoxin (Park et al., 1988). It has also been used in a growth medium with the bacterium *Escherichia coli* to produce Cu-Zn superoxide dismutase (Tomita et al., 1988). Excess copper can, however, be detrimental to fermentation as well as the quality of the product (Cejka et al., 1989).

Copper as a nutrient supplement in animal production

Omarkozhaev (1988) reports that supplementation of feed for livestock and poultry, with several metals (Co, Cu, Zn, Mn) and iodine improved metabolism and performance in areas where deficiencies occurred. Pimental and Cook (1989) found that copper supplementation increased chick body weight and improved hematocrit levels. In a patent document, Ruszbach et al. (1988) describe the manufacture of a copper sulfate monohydrate feed additive. Along with methionine supplementation, added copper improves growth in female broiler chicks (Jensen et al., 1989). (The authors did not find copper to be helpful with male chicks.) Copper is also used as a fungal inhibitor in feeds and has been found to overcome the growth depression in chicks due to mouldy food (Johri et al., 1986). Incidentally, it is also used with human foods, to reduce microbial activity (e.g. Ando et al., 1988).

Limited copper supplementation is beneficial and routinely done in swine production (e.g. Komegay et al., 1989; Menten et al., 1987; Polasek et al., 1987; Ruda et al., 1988). This is a result of increased feed efficiency (e.g. Heitman et al., 1989), a factor which can be affected by the chemistry of the environment (e.g. Hennig et al., 1988). Copper acts as an antimicrobial agent in the gut (Shurson et al., 1987b) and the increased feed efficiency may, in part, be a result of decreased ammonia production in the lower intestine (Menten, 1988). Organ weights and intestinal characteristics may be altered by swine microorganisms killed by copper (Shurson et al., 1987c). Effects of copper supplementation can but do not always include higher plasma copper levels as well as increased plasma glutathione peroxidase activity (Zhang et al., 1985). Hamada et al. (1988b) did not find higher plasma copper levels although they report accumulation in the liver of supplemented animals.

With sheep, Boev and Karbo (1988) comment (abstract) that "The addition of copper and iodine to grass meal consisting of clover/timothy mixture grown with the application of 33 t/ha liquid manure contributed to normalization of trace element metabolism in the bodies of sheep and improved the quality of their meat." Valdes et al. (1988) note the importance of copper supplementation to cattle, for animal health and productivity. (Kleczkowski et al., 1990, report some of the histopathological and histochemical effects of copper deficiency on bulls.) As with other animals, plasma copper levels of calves can be raised with supplementation (e.g. Stabel et al., 1989). This, however, can be affected by hormones (e.g. House et al., 1989). Copper supplementation of ruminants can be accomplished by several methods, including copper oxide particles (Langlands et al., 1989) and needles (Cameron et al., 1989). A number of other uses for copper with animals and animal materials are described in the recent literature.

Copper in human diets

Kinzel (1989) discusses the place of copper in the diet, commenting on the functions of the metal. Copper requirements vary between 2-3 mg/day although both requirements, and intake, tend to change with age, being less in elderly individuals (Gregar, 1986).

Physiological benefits to humans from copper and copper complexes

Because of its affinity for other molecules, ionic copper combines with organics and occurs only at very low levels (ca. 10^{18}) in the human body (Sorenson, 1989c). Girchev and Tzachev (1988) comment that the body of a normal adult contains approximately 100-150 mg of copper. Tomita (1990) is the editor of a series of articles on trace elements in clinical medicine, including a number of articles dealing with copper. Sorenson (1987a) discusses the physiological basis for pharmacological activities of copper complexes and (Sorenson, 1988b, 1990) discusses the antiarthritic, antiulcer, and analgesic activities of copper complexes. Inflammation is considered to be essential to wound healing (e.g. Rao et al., 1988a). Copper-containing organics such as superoxide dismutase and caeruloplasmin have been studied because of their importance as anti-inflammatory agents to both wound-healing and arthritis (e.g. Hoey, 1987), through their antioxidant properties (Cuthbert et al., 1989; Lasheras et al., 1988; Lunec, 1989). They have also been studied because of the lack of information on the specific nature of the anti-inflammatory action (e.g. Bressan et al., 1989). Copper devices such as bracelets, have long been used for anti-inflammatory and anti-arthritic purposes and may serve as a source of copper taken into the body with cupriphores from sweat (e.g. Beveridge, 1989; Fernandez-Madrid, 1989). In a review, Denko (1989) comments that (page 4) "... endogenous copper, especially serum caeruloplasmin, rises during the acute phase of inflammation and falls during the chronic phase." Korolkiewicz et al. (1989) report that copper complexes with salicylates or amminopyrine were more effective than the parent ligands as anti-inflammatory agents suggesting a specific action of copper. Copper and aspirin increase the concentration of anti-inflammatory agent(s) in plasma (McGahan, 1990). Several new copper-containing anti-inflammatory and wound-healing agents have been developed (e.g. Garuti et al., 1988; Pickart, 1987; Ueno et al., 1988). Grider and Cousins (1989) provide some evidence suggesting that (page 29) "... metallothionein has a relationship, perhaps functional in nature, to the inflammatory process. ... (It) may act as an inducible free radical scavenger to help cells handle the elevated amounts of active oxygen species, particularly hydroxyl radicals, produced during inflammation."

Microbial cidal agents

Thurman and Gerba (1988) review the molecular mechanisms of copper and silver ion disinfection of bacteria and viruses, pointing out that the reactions can occur in a variety of ways. Recent literature includes discussion of a number of viricidal and bactericidal agents (e.g. Al-Mashikhi and Nakai, 1988; Kawale et al., 1989; Mollin et al., 1989; Schuster et al., 1989; Singh and Singh, 1988), used for a wide range of purposes. Copper, with or without other agents, is often able to inactivate virus and bacteria (Landeen et al., 1989a; Yaha et al., 1989). Recently developed fungicidal, microbicidal and herbicidal compounds have been patented by Miki and Ueda (1988, 1989), Nakajima (1988), Paul and Cairns (1988), Shiroshita et al. (1989), Kleemann and Claus (1989), Hess et al. (1988), Goetzschel et al. (1988) and Arki et al., (1985). Environmental conditions affect the efficiency and behaviour of bactericidal agents, factors such as the pH and organic nature of the gut with gastrointestinal bacteria (e.g. Shurson et al., 1987a).

A variety of copper-containing fungicides are used in agriculture, medicine and elsewhere. Wisniewski and Ziemba-Zoltowska (1988) provide a brief review of copper fungicides manufactured in Poland. These are agents which limit growth and/or reproduction (e.g. Aggarwal and Mehrotra, 1988; Chauhan and Singh, 1988). The synthesis, effects and efficiency of various cidal agents have been widely examined (e.g. Abbaiah and Reddy, 1989; Aggarwal and Mehrotra, 1987; Ali et al., 1988b; Chauhan and Singh, 1988; Gorska-Poczopka et al., 1986; Pradhan et al., 1988; Revankar and Mahale, 1989). It often varies depending on the nature of the agent (e.g. Baicu, 1987) and the tolerance of the organism. Since fungi are found on structures (e.g. Katircioglu and Gurcan, 1987) as well as plants (e.g. Baicu, 1987) and animals, conditions of the environment can also play an important role. Side effects, discussed elsewhere, can affect beneficial as well as harmful organisms, for example in the soil (e.g. Torstensson, 1988)

Copper-containing bactericides and fungicides have been used for a wide variety of plants. Recent literature includes work on wheat (Cook and Culshaw, 1989; Forster and Schaad, 1988), barley (Forster and Olson, 1988; Sarhan and Jalal, 1989; Singh, 1988; Singh and Dwivedi, 1987), Blackgram (Gupta and Khare, 1988), Sesame (Song et al., 1987). The efficacy of copper-containing fungicide treatment of cotton (Padule and Shinde, 1989), beans (Garrett and Schwartz, 1988), cabbage (Onsando, 1988), cucumber (Gupta and Bhardwaj, 1988) and tomatoes (Dillard, 1987; Stachewicz et al., 1987) and has also been examined. Root crops such as potatoes (De and Sengupta, 1988; Mantecon, 1989; Redl and Purkhauser, 1988; van Bruggen et al., 1988; Shukla et al., 1987), onions (Gupta and Srivastava, 1988), parsnip (Cerkauskas and McGarvey, 1987) and carrots (Muniz and da Ponte, 1989) are often infested with fungi and, as such, have formed subjects of examination for fungicides, including those that contain copper. With citrus, a number of publications describe various uses of copper-containing cidal agents. These include Garza Lopez (1988), McGuire (1988), Orozco Santos (1987) and Utikar and Shinde (1987). Work with other fruit includes that on peach (Mandoki, 1988), plum (du Plessis, 1987), grape (Gadoury and Pearson, 1988; Ramanathan and Sivapalan, 1986; Sfintichi, 1988), date palm (Mehta et al., 1989b), avocado (Darvas et al., 1987) and guava (Rawal and Ullasa, 1988a,b). Csutak et al. (1988) describe a new complex (aluminum-ethyl-phosphonate and copper-oxychloride as active agents) for grape vines hops, and possibly other crops. Teviotdale et al. (1989a,b) discusses the use of copper-containing fungicides on olive leaf spot. Recent work on copper-containing fungicide treatment of nuts includes that on almonds (Teviotdale et al., 1989c) and walnuts (Pinto de Torres and Carreno I, 1988). Problems with the use of a number of these herbicides are provided by Haag et al. (1988), Macek (1987). Details of these problems are discussed later in this review.

Work on fungicide use, including copper-containing agents, with other plants includes that on Makhana (Haidar and Nath, 1987), cocoa (Jollands and Jollands, 1989; Jollands et al., 1989), betelvine (Das Gupta et al., 1988), groundnut (Murugesan and Mahadevan, 1987, 1988), rubber (Albuquerque et al., 1987; Joseph et al., 1987; Pereira et al., 1988), gourd (Ullasa and Amin, 1988) and tobacco (Patel et al., 1988; Shenoi and Abdul Wajid, 1988). Lepp and Dickinson (1987) provide an excellent review of the partitioning and transport of copper in Kenyan coffee stands (*Coffea arabica*) pointing out some of the problems of copper accumulation. This review is discussed several times in later sections of this report. The efficacy of treatment of garden plants such as lovage (Grzybowska, 1986) and roses (Wittmann and Fickert, 1988a) has also been examined. Moorman et al. (1989) present an easily-understood review of some of the problems associated with fungicide treatment of *Botrytis* in Pennsylvania greenouses. Wittmann and Fickert (1988b) describe sycamore leaf blight and a number of treatments, including what they term "Kupferpräparate".

Copper as a herbicide

Copper can be an effective herbicide when the concentration of biologically available metal is excessive. Anderson and Dechoretz (1988) comment that, for control of aquatic weeds, ethylenediamine copper "... is a contact herbicide which generally results in rapid control after relatively short periods of exposure.". Copper sulfate is also used to control aquatic weeds (e.g. Pal and Chatterjee, 1989) and Raman and Cook (1988) provide guidelines for its use based on a study at lake Loami in Illinois. Swain et al. (1986) provide information on the impact of copper sulfate treatment of lakes.

Copper as a cidal agent for animal and human pests

Copper sulfate and other copper-containing compounds have long been used as a molluscicide to kill the intermediate host of several important human parasites (Abdel-Rahman et al., 1988). Copper has been effectively used against yeast isolates causing thrush in poultry (Lin et al., 1989b). Cupric chloride has been used to control another mollusc, *Lymnaea truncatula* (Rondelaud, 1988b). Copper sulfate has also been unsuccessfully tested on the snail *Pomacea lineata* introduced into Taiwan (Cheng, 1989), a result of dilution by muddy water in the paddy and concern expressed by environmentally concerned individuals. The author comments, however, that copper sulphate could be used at lower concentrations by treating only the shoreline rather than the entire paddy. Electrolitically generated copper and silver ions have been proposed as a control for pathogenic bacteria in swimming pools, hot tubs and cooling towers (Landeen et al., 1989b). Copper is also used by hobbyists and aquarists for specific aquarium disease problems (Cardeilhac and Whitaker, 1988). Nectoux et al. (1988) successfully used cupric sulfate to treat what they term a parasite of the honeybee (varroatosis) with very little loss of honeybees. Choudary et al. (1989) advocate the use of pesticide-metal(Cu/Co)-montmorillonite complexes for controlled release of pesticides. They report satisfactory results with the housefly *Musca domestica*. Sheet copper acts as an effective barrier for snails with plants and may be a useful material for containing snails in snail farming (Moens et al., 1986).

Copper in disease-control agents

A number of copper-containing agents have been found to combat human diseases (e.g. Sorenson, 1989a), including secondary metabolites from a variety of organisms (e.g. Zahner et al., 1987). As a result, the nature and actions of these agents have become important research topics. Recent literature on the nature and action of these, and related agents, includes work with:

anticancer and antitumour drugs (Apelgot et al., 1989; Berners-Price and Sadler, 1988a,b; Chang et al., 1989; Crispens and Sorenson, 1987b, 1989; De Pauw-Gillet et al., 1987; Egner et al., 1987; Kasemeier et al., 1987; Kovacic et al., 1988; Morphy et al., 1989; Patil et al., 1989; Pickart, 1988b; Soderberg et al., 1987a,b; Solaiman, 1988; Takamura et al., 1989b; Tsipis et al., 1988; Von Muenchhausen and Sulkowski, 1988).

ischemia injury treatment agents (Hernandez et al., 1987; see also Yoon et al., 1989).

drugs to treat cardiovascular defects from copper deficiency (Saari, 1989, 1990).

copper complexes for radiation-protection and therapy (Das Gupta et al., 1989; Foye and Ghosh, 1988a; Salari et al., 1987; Soderberg et al., 1987a,b; Sorenson, 1989b).

antimycoplasmal agents (de Zwart et al., 1989; Van der Goot et al., 1987)

antiasthmatic agents (Badawi et al., 1987).

wound-healing agents (Pickart, 1988c).

analgesic drugs (Okuyama et al., 1987)

Uses of copper in dentistry

Copper is able to inhibit oral bacteria (e.g. Drake and Waerhaug, 1989) and reduce plaque (Moore et al., 1989b). There is some evidence that, with palladium (Cu_3Pd), it may improve the corrosion properties of amalgam (Mante et al., 1989). Recent patent documents (e.g. Parker, 1989) indicate the continued potential for use for copper in dental amalgam alloys.

Miscellaneous medical/physiological uses and capabilities of copper

Mathias et al. (1989) suggest using copper-labeled (generator-produced Cu-62)-PTSM (pyruvaldehyde bis (N^4-methylthiosemicarbazone) as a tracer for cerebral blood flow. Cu-62-PTSM has also been shown to serve as a tracer for regional blood flow in the heart and kidney (Barnhart et al., 1989) as well as the brain (e.g. Green and John, 1989). It has also been proposed as a means of labeling leucocytes with positron-emitting copper (Yu et al., 1989b). Other copper agents have also been proposed for tracing blood flow (e.g. Chauhan et al., 1989). Labeling with Cu-64 is suggested for antibodies by McPherson et al. (1989). Saracoglu and Eryilmaz (1987) report that measurement of vitamin B_1 concentration can be accomplished by the thiochrome reaction, using Cu^2. To better understand the distribution of lipophilic $Cu(II)_2$(3,5-DIPS) in the body, Sorenson et al. (1989a) used a ^{67}Cu-^{14}C complex as a tag. Hirschberg and Hofferberth (1988) used intravasally placed copper coils to produce *in situ* thrombosis of the middle cerebral artery in dogs. Copper vapor laser light has been used to estimate the activities of several plasma enzymes (Matyushicev et al., 1987), to liquify and remove thrombusa-included arteries (Wei et al., 1988, 1989) and to treat "port wine stains" in humans (Walker ete al., 1989).

Velasco (1989) describes a copper-peroxide-silver histological method for the selective demonstration of Alzheimer's disease. Khan (1987) discusses the preparation and characterization of a copper complex for the estimation of thiols or sulphydryl groups in clinical studies of rheumatoid arthritis patients. They also have been used for isostachophoresis of amino acids (Stover, 1989). Copper salts have been used as histological stains for bone and collagenous tissue (Harder, 1988). Cohenford et al. (1989) describe a copper-using technique for the assay of free and bound L-Fucose. A chromatographic technique using copper (II) modified silica gell is proposed for ligand exchange work, at least with amino acids and peptides (Foucault and Rosset, 1987). This also includes Copper-containing eluent has also been used to isolate specific organic agents (e.g. Arai et al., 1988). Copper complexes as tools for intermolecular cross-linking or bridging of proteins has been proposed by Moriya et al. (1989b).

Bacterial exopolymers have been reported to biodeteriorate copper-covered surfaces (Ford et al., 1988)as well as cause metal biodeterioration (Ford et al., 1988). Part of this may be a result of metal-organic interactions. Copper iodide staining for proteins can, for example, detect protein levels as low as 100-150 pg/mm^2 (Root and Reisler, 1989).

The use of copper in contraceptives

Copper is routinely used as a means of reducing the viability of sperm and the opportunity for fertilization to occur. Recent literature includes a discussion about copper as a male contraceptive (e.g. Skandhan, 1988) although most of the literature concerns the nature and efficiency of various uterine devices. Examples of this literature include discussions of the following devices:

Cu-Fix - Wildemeersch et al., 1988.

Cu-7 - Apelo et al. (1989).

MLCu250 - Batar (1988), Bratt et al. (1988).

MLCu375 - Bratt et al. (1988).

Nova-T - Bratt et al. (1988).

Copper-catalyzed degradations of toxic compounds

Dependent on the concentration and the nature of the organic, copper can either hasten (Jones and Bradshaw, 1989) or inhibit (Jardim and Campos, 1988) the degradation of organic compounds. Dechlorination/hydrogenation of polychlorinated compounds can be catalyzed by copper (Govindaraj et al., 1987; Hagenmaier et al., 1987). Copper, as an oxide with various solid materials, has been evaluated for desulfurizing reactions (Flytzani-Stephanopoulos et al., 1987; Kyotani et al., 1989; Melson, 1988; Shah and Leshock, 1988) with flue gases and coal gases. Copper oxide has generally been found to be an efficient reaction agent for removing selected aerosol and particulate contaminants in industry (e.g. Stelman, 1988; Stelman et al., 1987). Yahata et al. (1988) describe the use of copper monoxide as a catalyst in the incineration of ion exchange resins.

Copper in materials, implements and money

Directly and indirectly, man has always been exposed to copper. Trace element analysis of ancient Pueblo pottery, for example, indicates the presence of copper in clays important to maintenance and survival (Foust et al., 1989). It also occurs in pigments used in printing during the 16th century (Cojocaru and Manea, 1987). Copper is also found in copper alloys from Egypt (1500-1200 B.C.; Fabrizi et al., 1989), coins of the Roman Republic (217-31 B.C.; Carter and Razi, 1989) and bronze artifacts from the tenth centuray A.D. (Chikwendu et al., 1989). Hua and Fan (1988) describe 2,000 year-old copper artifacts from China, that indicate a metallurgical ability at that time, including welding. Bronze and copper implements are important enough in today's life that articles have been written on their care (e.g. Keller, 1979). The ability to react with other agents makes the metal useful in the commercial degradation of wool keratin (Fukatsu, 1989). The use in superconductors provides a new dimension for the use of copper, both in superconductors and in experiments with supercondductors (e.g. Jacob et al., 1988). Copper is now being used in storage containers for nuclear waste (Ivarsson and Oesterberg, 1988; McClanahan and Bradley, 1988).

Copper as a wood preservative

The rate of decay of wood can be reduced by copper, in various formulations, in both terrestrial and aquatic environments. However, pressure treatment of posts and poles, with the agent, needs to be done carefully in order to obtain a suitable distribution (e.g. Leightley, 1987). Wood type is also important, *Eucalyptus* is, for example, more resistant to termites than *Pinus radiata* (Johnson et al., 1988a). There is also concern about the most commonly-used preservative, copper chrome arsenic (CCA), and efforts have recently been made to replace it with phenol-based compounds (e.g. Schnippenkoetter et al., 1988). In spite of these problems, CCA is widely used and provides an effective wood preservative (e.g. Pugel, 1987). Recent discussions and evaluations of CCA, and other copper-containing preservatives can be found in Barnes (1987), Briscoe (1987); Collett (1988), Ostmeyer et al. (1989), Raghu-Kumar et al. (1988), Shaler et al. (1988), Sharma et al. (1988b) and Suzuki and Higaki (1988). Eaton et al. (1989) present the results of an international collaborative marine trial of CCA and CCB (B = boron) preservatives. The study underlined the importance of copper and chromium in timber protection in the marine environment. In a patent document, Reed (1988) describes a copper-based agent for controlling growth of organisms on masonry, Hein a water-soluble copper salt of carboxylic acids for wood preservation and Maksimenko et al. (1988) a copper sulfate-based wood preservative. Ando et al. (1988) describes microbicide-containing sheets and films for building materials. Metzner and Seepe (1988), again in a patent document, report improved fixation of wood preservatives with copper sulfate.

Copper as an antifoulant

Fouling on and in aquatic facilities is expensive in terms of equipment loss and power use. Copper has long been used as an antifoulant. Manfredi et al. (1987), for example, discuss the selection of copper base alloys for use in polluted seawater. Gaffoglio (1987), in a Copper Development Assciation article, discusses the benefit of copper-nickel alloys in reducing construction and maintenance costs on offshore platforms. Dowd (1988) discusses the effectiveness of tributyltin and copper antifoulants on U.S. Naval ships. Recent work on the use of copper includes that of Tadros (1989) on the chemistry of certain marine coatings, Ohsugi et al. (1989) on novel copper-containing copolymers, and Diprose et al. (1989) on electrically-generated cupric ions in the presence of chloride ions. Concern has been expressed about the possible accumulation of copper from antifouling compounds (e.g. Fingerman, 1988; Henderson, 1988). Henderson (1988) reports that (abstract) "Toxic responses of corals indicated that, on a molar basis, TBTO-SN was at least 10 times more toxic than copper." Alziieu et al. (1987) evaluate changes in tissue metal levels in Arcachon (France) bay oysters after the ban of tributyl tin. They comment (abstract) that after the ban, copper use increased but "... the statistical analysis of monitoring data (1979-1985) show a relative stability in the copper levels in Arcachon bay oysters." Mellouki et al. (1989) report good results with quaternary ammoniium salts bonded to a vinyl copolymer and used as a paint with a copper oxide additive. The advantage of this material is its low solubility, with the retention of the biofouling agent. Recent patents of copper-based antifouling agents include Kamimoto et al. (1989), Maeda et al. (1989) and Moraru (1983). In a patent document, Sunami (1988) describes a copper-containing coating material for fouling control on pearl oyster shells.

Evans (1988) and Pyne (1987) discuss species succession in fouling communities, the latter author considering situations with and without antifouling agents, including both tin and copper. Woods et al. (1987) evaluated a range of antifouling agents under ship trial conditions, in terms of microbial film composition and surface roughness. They report that the use of CuSCN was improved with the higher tributyl tin fluoride concentrations. They also report different tolerance levels by different species of fouling diatoms. In an ICA-supported study, immobilization of copper by bacteria in primary films has been found on both copper and copper-nickel alloys (Blunn and Gareth-Jones, 1988). Iron content of alloys is also important with higher fouling of 90/10 copper-nickel alloy containing iron (Chamberlain and Garner, 1988a). At least part of the fouling rate is dependent on leaching rate of copper from antifouling agents. de la Court (1988) provides "critical leaching rates" for copper to prevent algal fouling (22 µg Cu/cm^2/day) and barnacle settlement (16 µg Cu/cm^2/day), rates which the author says are higher than previously used.

I.3 COPPER AND ORGANISMS

Plants, animals and humans acquire copper from the environment and from food (in the case of animals and humans). The acquisition is a result of the metal being in a physical state and a chemical species that allows uptake and subsequent incorporation into the workings of the organism. Thus, even though there may be a flux of metal through a biological or geographical system, the latter in the sense of Windom et al.(1989a), it is the chemistry of the metal that dictates biological availability. This is why sample collection and analysis is so very important in determining bioavailability. Concentration of a metal is very important (Braithwaite et al., 1987) but so also is metal speciation. Amiard et al. (1989) comment on the increasing analytical variation with increasing metal concentration, a result which can be interpreted in terms of the variation in metal availability as well as the change in total metal concentration. This will be a constant theme in this part of the review because of its importance in relating metal concentration to metal levels in organisms.

I.3.1 TISSUE COPPER LEVELS UNDER NORMAL CONDITIONS

Microorganisms and plants

In a discussion of the influence of microorganisms on contaminant transport, Knowles (1987) comments on the ability to transport metals, including copper, as a result of metal bound by the cells. With three species of bacteria there is a range of 0.09-2.99 μmol of copper per mg dry weight of bacterial walls (From Beveridge, 1985), an indication that movement of various bacterial species will cause movement of different amounts of the metal. High concentrations of microorganisms in aquatic environments, such as blooms of microflagellates, can affect metal concentrations. This is a result of metal uptake by the organisms combined with transport and subsequent death of the organisms (e.g. Kurata, 1989). The effect is first to reduce "dissolved" and increase particulate metal concentration and subsequently, through death, to transport metals to the sediments.

In terrestrial, and laboratory environments, medium or soil metal speciation becomes important in determining plant concentration (e.g. Leinonen, 1989). Jacobsen (1986), working with wort, comments that (summary) "... the concentration of soluble minerals (i.e., a laboratory wort) must be used instead of the total mineral content." In marine and terrestrial fungi and plants, copper concentration varies throughout the plant, a result of differential uptake, transportation and storage of metal (e.g. Gadd et al., 1988; Lyngby and Brix, 1989; Markert, 1989; Ryczkowski and Reczynski, 1988; Stark et al., 1989a). This also implies organism control of tissue concentrations, a feature appearing to occur in the salt-marsh plant Spartina laterniflora (Alberts et al., 1990). However, the control appears to be partial, tissue concentrations are reportedly higher in areas with elevated metal concentrations (e.g. Teraoka, 1989) or metal and metal availability (Tendel and Wolf, 1988). Natural changes occur; seasonal changes in plant tissue copper accumulation have been suggested for the halophyte Halimione portucaloides by Reboredo et al. (1988).

Ylaranta and Sillanpaa (1984) report that although micronutrient concentrations vary from one crop to another, copper concentrations varied the least. The seasonal changes noted in aquatic plant tissue copper concentrations (e.g. Reboredo et al., 1988) have also been reported for terrestrial plants (Clancy et al., 1988; Clark et al., 1988, 1989; Krzeminski and Jakutowicz, 1987; Zhai et al., 1987). Part of this is due to the changes occurring in plant metabolism and the resultant changes in copper requirements, as indicated for grains (Lasztity, 1988b; Lasztity and Biczok, 1988) as well as other plants. However, some of the differences and changes in tissue metal concentration are a result of soil metal concentration and metal availability (e.g. Szakmany, 1986). Lasztity (1988b, 1989), for example, noted higher copper concentrations in winter cereals as a result of fertilizer addition. This has been reported for other plants by a number of authors (e.g. Dhopte, 1987; Gonzalez and Sanchez, 1988; Ivanova, 1988), especially in peat soils where metal availability is reduced (Adams et al., 1989). However, the use of metal-containing supplements is not always beneficial (e.g. Bligh et al., 1986), the benefit being derived most frequently when soil metal bioavailability is limited. It has also been reported with the use of copper-containing fungicides (Teviotdale et al., 1989b). The importance of environmental factors, as opposed to genetic factors, is in the control of metal bioavailability. This is suggested, for example, by the report that, in crested wheatgrass forage, strain diffences in copper are not significant when averaged over locations (Vogel et al., 1989). Tissue copper concentrations can also be affected by the supply of other metals (e.g. Biddappa et al., 1988), in part a result of metal-metal competition for uptake sites.

Animals

Concentrations of metals in animals has been a focus of both regional surveys (e.g. McCrea et al., 1984) and research on metal uptake and storage. As with plants, changes in metal concentration can occur from species to species, within a species and on a seasonal basis (e.g. Cain et al., 1987; Chan, 1989; El-Shakaa and Shahein, 1987; Greville and Morgan, 1989b; Hernando Campos, 1988; Lobel et al., 1989; Novak, 1985; Ridout et al., 1989; Sivadasan and Nambisan, 1989; Tuncer and Uysal, 1988; see also Hare et al., 1989). With many invertebrates, tissue copper is directly related to body weight (Vale and Cortesao, 1988; V.-Balogh et al., 1988; Wu et al., 1988c). This is, in part, a result of the relationship between body size and metabolic requirements for the metal (Depledge, 1989b). It may also be due to the relationship between surface area and sorption (Kowalski et al., 1989). Tissue metal concentrations are often associated with environment type, as a result of metal bioavailability or physiological condition. As an example, concentrations of tissue copper have been reported to be higher in riverine and estuarine than in coastal benthic invertebrates (Everaarts et al., 1989). Kavun and Khristoforova (1987), however, report that the nature of the substrate had no effect on accumulations of copper in tissues of mussels taken from coastal regions of the Far East. Tissue copper concentrations can be elevated in areas of anthropogenic metal (Novak and Mensik, 1987). V.-Balogh (1988b) reports elevated tissue copper concentrations in freshwater mussels (*Unio pictorum*) from a boat harbour on Lake Balaton, Hungary. Animals will frequently isolate or "store" excess copper either for later use or for elimination. Prosi and Dallinger (1988) note that the terrestrial isopod ("pillbug") *Porcellio scaber* deposits lead, copper, zinc and possibly cadmium in membrane-limited vesicles (lysosomes). These are especially common at elevated soil metal levels. It is important to remember, however, that the metal must be biologically available for uptake to occur and that temporal changes can occur in metal uptake (Bromenshenk et al., 1988; Phillips et al., 1986).

There can be changes in metal accumulation in fish, as shown by work on otoliths (ear bones) of the cod *Gadus morhua* (Protasowicki and Kosior, 1987). Copper levels fluctuated over the sixteen years of their study, with general decreases apparent with fish age. Environmental factors play a role in metal accumulation, as shown by the higher liver copper levels in farmed salmon than in wild salmon (Craik and Harvey, 1988). These factors are often difficult to relate to tissue metal concentrations in natural environments, as shown by Smith (1987a) for levels of metals in fish of the Columbia River. Young and Harvey (1989) note that, with the white sucker *Catastomus commersoni* (page 354), "Tissue concentrations of Cu did not correlate with either lake or total sediment Cu concentrations." (Neither did they correlate with lake pH.) They also point out that "A knowledge of the chemical speciation of Cu in lake sediments as well as concentrations of Cu in diet items might have provided a more complete understanding of possible factors determining Cu levels in these fish populations." With rainbow trout, Julshamn et al. (1988a) found a linear relationship between liver copper concentration and dietary copper. As shown by Young and Harvey (1989), the role of pH in tissue metal levels in freshwater fish is difficult to assess. This appears to be true with other animals. In a study of liver metal levels in the young (non-fledged) of a species of duck (Goldeneye - *Bucephala clangula*), Eriksson et al. (1989) report (abstract) "No indications of significantly different concentrations in samples from acidic lakes in comparison with circumneutral lakes were detected for any metal, ..." Feathers have been used as biomonitors, Hahn et al. (1989) reporting a strong correlation between the cadmium lead and copper content of Goshawk feathers and wet deposition rate.

The relationship between dietary copper and liver copper levels found in fish (e.g. Julshamn et al., 1988a) has also been reported for chicks (Ammerman et al., 1990; Ledoux et al., 1989a). The authors note, however (Ammerman et al., 1990) that (abstract) "Liver was ... the only tissue sufficiently sensitive to dietary Cu to be used as a bioassay criterion." There are also changes in liver copper concentrations with physiological status (e.g. Allain et al., 1989). Richards (1989c) reports that liver copper concentrations declined with the onset of egg production in the turkey hen. Polansky et al. (1989), however, comment that with various tissues of turkey hens (including liver tissues), tissue copper levels do not reflect the effects of egg production. Incidentally, copper in turkey eggs is not evenly distributed, being higher in the yolk (102 µg) than the albumin (16 µg; Richards, 1989b). This provides an indication of the source of copper for growth and development of the embryo (Richards, 1989b). Likewise, the distribution of copper-containing enzymes in the developing chick embryo is not evently distributed (e.g. Tholey et al., 1988) as a result of metal use and enzyme function.

Evidence from work with domestic animals suggests an increased need for copper during pregnancy (e.g. van Niekerk et al., 1988). Malinowska (1986) notes that in sows and their foetuses, transfer of copper and ceruloplasmin are maximal between 21 and 35 days gestation. Changes in both plasma copper and superoxide dismutase activity have been noted during pregnancy by some authors (Konstantinova and Russanov, 1988; van Niekerk et al., 1988). Auer et al. (1988b), however, report (page 62) that "... pregnancy in the mare is unaccompanied by changes in either the plasma copper or zinc concentrations". Konstantinova and Russanov (1988) suggest that the changes in both CuZn SOD (superoxide dismutase) activity are associated with changes in copper metabolism. (They may also be associated with the age of the animal, as noted for rats by Radojicic et al., 1987.) Metal-metal interactions may occur in domestic animals (e.g. Favretto et al., 1989). Wittenberg and Devlin (1988), however, note that with copper and molybdenum, there was no apparent influence on plasma copper levels in lactating ewes even though molybdenum can be used to reduce copper uptake and accumulation in sheep (e.g. Vrzgula et al., 1988). With horses, dietary deficiencies have not been found to cause the production of low copper milk (in Hintz, 1987b). However, milk copper levels can change over time, at least in some domestic animals (e.g. De Maria Ghionna et al., 1987; Vahcic et al., 1988) suggesting that the evaluation should be made over the duration of milking. Breedveld et al. (1988) found no change in copper levels in mare serum and milk or foal serum as a result of two different levels of dietary copper-zinc supplementations. They did notice lowest foal serum copper levels at parturition. Kluczek and Kluczek (1988) report an increase in serum copper and ceruloplasmin content in the sixth to seventh month of pregnancy in Arden Type mares.

Kleczkowski (1988b) found no relationship between copper levels in the soil, activities of copper-containing enzymes and liver copper levels in cattle grazing copper deficient sites. The author suggests that there are other factors than soil copper levels that influence copper metabolism (and probably copper bioavailability). One factor could be metallothionein, an organic which binds metals and is involved in metal transport. Kleczkowski (1988a) reports highest metallothionein concentrations in cattle from copper-deficient areas with high cadmium and zinc levels. Kleczkowski and Barej (1989), however, note an association of bull copper levels with food, a reduction in food supply was associated with a reduction with copper deposition in the liver. With growing pigs exogenous growth hormone appears to have only minor effect on tissue copper concentrations (Caperna et al., 1989) as did supplemental folic acid on serum copper (Tremblay et al., 1989).

In laboratory rats, Koh et al. (1989b) obtained evidence suggesting that ingestion of fructose compared to starch increases the requirement for copper, possibly a result of reduced uptake of dietary copper. Fields et al. (1989c) found mortality of rat fetus and neonate with consumption of a copper-deficient diet containing fructose but not with a copper-deficient diet containing starch. They attribute this to the differing effect of the two carbohydrates on copper deficiency. Dietary sulfur amino acids can affect copper metabolism, Shuler and Nielsen (1989) report that dietary arginine and cysteine moderate the effect of copper deficiency on liver and kidney copper concentrations. Nielsen (1988) suggests that some of the difference between male and femal rats in the response to copper deficiency is the result of differences in the sulfur amino acid metabolism. Dietary molybdenum at low levels (0.1 mg Mo/L in drinking water) is reported to increase hepatic copper concentrations (Yang and Yang, 1989) while dietary tin decreases tissue copper concentrations (Rader et al., 1989). In pregnant rats, salicylic acid has been found to transfer copper from the dam to the fetus (Gunther and Vormann, 1989). Requirements for and metabolism of copper vary as a result of physiological condition, Rusin et al. (1989) report a decrease in tissue copper levels with heavy physical exertion in dogs. Lewis et al. (1989b) note with pregnant rats that the interaction of pregnancy and physical training results in lower plasma copper values than pregnancy alone. Hormones can also produce changes in tissue copper levels; Mehta et al. (1989c) note decreased hepatic copper concentrations in estrogen-treated female rats.

In humans, tissue metal concentrations can vary with age (e.g. Ettinger and Colman, 1989; Girchev and Tzachev, 1988; Kant et al., 1989; Paschal et al., 1989) and stature (Laitenen et al., 1989), physiological condition (Panda et al., 1989; Suzuki et al., 1988a) and exercise. Anderson et al. (1989) found increased serum copper immediately following acute exercise. Copper-containing superoxide dismutase activity is reportedly higher in competitive swimmers than in individuals that do not train (Lukaski, 1989; Lukaski et al., 1989). This is in spite of the evidence that dietary copper intakes and body copper losses are similar for both inactive and physically active individuals (e.g. Lukaski et al., 1989l; Singh et al., 1989a). (Umoren (1989) notes higher intakes of copper by physically active individuals.) Copper levels of human oocytes cannot be used as an indication of fertilizing and developmental potential (Simon et al., 1987). However, during pregnancy, blood copper concentrations reportedly increase (Nagra et al., 1989; Okonofua et al., 1989; Suzuki et al., 1988a; Tsachev et al., 1987). From measurements of copper in human milk samples (Casey et al., 1989; Nagra, 1989), it appears that copper levels decline over the first five or six months of lactation and then remain fairly constant. Meconium copper concentrations in preterm infants tend to be less than those from full-term infants (Friel et al., 1989). In healthy infants, postpartum plasma copper and ceruloplasmin concentrations increase (Salmenpera et al., 1989). For humans in general, dietary intake of copper is dependent on the nature of the diet. Copper absorption from preterm human milk is higher than from formula (Ehrenkranz, 1989). Dougherty et al. (1988) note an increase in copper intake when dietary fat decreased. Turnlund et al. (1989) examined copper absorption and retention in men, at three levels of dietary copper, and report that copper absorption is strongly dependent on dietary copper level. This has also been supported by Johnston et al. (1988) who report dietary copper intakes in postmenopausal vegetarian women to be correlated with plasma copper levels. Seasonal variations in urinary excretion of copper has been recorded for individuals on meat-based diets, as a result of variation in food intake (Iyengar, 1989).

I.3.2 TISSUE COPPER LEVELS UNDER ABNORMAL CONDITIONS

Metal levels will change with environmental copper levels (e.g. Höllwarth, 1988a; Radhakrishnaiah, 1988). However, they will also change under physiologically abnormal conditions as a result of changes in metal requirements and the ability to metabolize metal (e.g. Higashi and Itani, 1987). Even the effects of exercise, physical trauma, drug treatment of diseases, anaesthetic and surgery and high sugar intake will do this (Alarcón et al., 1988; Anderson, 1990; Cabalska et al., 1986). These changes are often an attempt by the tissues to correct the abnormal condition. Since, among other things, the physiological activities of copper include counteraction of inflammation it is not surprising that copper complexes offer a means of treating a number of diseases (Sorenson, 1989d).

Copper levels in plant tissues can be affected by disease. With alfalfa, and a number of other plants, metal uptake can be affected by fungal colonization of plant roots. El-Kherbawy et al. (1989), however, comment that the influence of the colonization is partly a function of the availability of metals in the soil. Isawa and Kobayashi (1987), working with stem rust-infected orchardgrass, note 7.3 ppm copper in cuttings from diseased and 10.0 ppm copper in cuttings from "less diseased" plants. Spicarova (1987) reports copper concentrations in leaf tissues and wasp galls of willow to be similar. Ruszkowska and Lyszcz (1988) point out that the copper content in the aerial part of a plant is less indicative of condition than the level of copper accumulation in the roots. Plant tissue copper levels also vary, from species to species as well as within one species, from area to area (e.g. Youssef, 1988). This makes difficult the interpretation of disease effects on tissue copper levels.

As with plants, parasite infestation of animals can produce abnormal tissue copper concentrations. Tufft et al. (1988) found elevated serum and bursal copper concentrations in chicks with a bacterial infection. Similarly, Richards and Augustine (1988) note elevated serum copper and ceruloplasmin levels in chicks six days after infection with a protozoan parasite. Mishra et al. (1987) suggest that toxins from fungi may influence tissue copper levels although the data do not indicate a significant change. Banga et al. (1989) note an increase in copper levels in sheep udder tissue during experimental mycoplasmal mastitis. They also report an increase in milk copper levels, something that Lappalainen et al. (1988) also found with endotoxin-induced mastitis in cows. Kidney copper levels have been reported to be elevated in endotoxin-shocked rats (Abe et al., 1985). In addition to parasite infections, changes in tissue copper levels can be produced by other diseases. Mehta et al. (1989a) note increased muscle copper concentrations in Labrador Retrievers with hereditary muscular dystrophy. However, not all diseases produce changes in tissue copper levels. Dill et al. (1989), for example found no significant change in plasma and liver copper values in horses with equine degenerative myeloencephalopathy. Surgical removal of glands can cause a change in tissue copper levels. LeBlondel and Allain (1989) note this in rats after removal of the thyroid and parathyroid, copper levels increased in whole blood, plasma and liver and decreased in muscle.

Excessive intake of alcohol has been suggested to affect tissue copper levels. With Wistar male rats, Yamaoka (1989) found decreased liver and elevated brain copper levels with chronic ethanol administration. Louis-Charles and Frimpong (1989) report that nondrinkers had significantly higher serum copper and zinc concentrations than those that drank on a variable basis. The report is confusing, however, because the authors comment that "... moderate ETOH consumption was associated with a rise in serum concentration of copper, ...". Excessive alcohol intake during pregnancy can cause birth defects which may be related to trace metal metabolism, possibly through zinc metabolism effects on CuZnSOD activity (e.g. Zidenberg-Cherr et al., 1988). There may also be an effect of cigarette smoking on serum ceruloplasmin and copper and zinc levels although the relationship is not obvious (Nowak et al., 1988) and may be indirect, due to the effects of smoking on other disease factors (e.g. Uza et al., 1986). Exposure to high levels of environmental cadmium has been related to reduced liver and kidney copper levels (Kido et al., 1988). Excesses of other metals, at least with laboratory animals, have also been related to decreased tissue metal concentrations (Rosenberg and Kappas, 1989a.

Infectious diseases in humans can produce changes in tissue copper, normally an increase in plasma levels as noted by Srinivas et al. (1988) for septicaemia, pneumonia and meningitis but not for erysipelas. Alarcón et al. (1989) report levels of copper to be elevated in saliva and blood serum of patients with various otorhinolaryngological ailments. Heise et al. (1989) report normal serum copper levels for patients with acquired immuno-deficiency syndrome. However, Fordyce-Baum et al. (1989) found a significant decrease in plasma copper levels in early HIV-1 infection. In a study of a cohort of HIV-2 positive gay men, Mantero-Atienza et al. (1989) report below normal plasma copper and selenium levels in 50% of the subjects. Vitiligo, a skin disease, has been associated with elevated copper in lymphocytes and chromatin (Nagy-Vezekenyi et al., 1987). Both serum ceruloplasmin activity and copper levels have been reported to be elevated in people with psoriasis (Dogan et al., 1989). Copper levels are abnormal in patients with a variety of respiratory diseases although they can be either elevated or depressed (Dang, 1988).

Although copper deficiency is a factor producing abnormal physiological conditions (Nicola, 1989), the conditions may subsequently cause a change in tissue metal concentrations. Ischemic heart disease is the leading cause of death in industrialized countries and copper deficiency has been proposed as a significant risk factor for vascular disease. Keen et al. (1987) present evidence that hypertension, a serious heart disease risk factor, can alter the metabolism of copper and zinc, producing increases in plasma coppper and reductions in soft tissue copper concentrations. However, changes in serum zinc and copper concentrations are at least partially dependent on the nature of the stress (Reinhardt et al., 1987a). In rats, Klevay and Halas (1989) demonstrated the hypertensive effects of copper deficiency and the increased blood pressure of stress from physical restraint. Serum copper levels increase shortly after acute myocardial infarction (Thiele et al., 1988). They are also elevated in patients with chronic cardiac failure (Riley et al., 1990). Oster et al. (1989b), however, report subnormal serum copper concentrations in patients with coronary heart disease. Attempts have also been made to use serum copper and zinc levels as rejection markers in heart transplant patients, unfortunately without success (Dominguez et al., 1989). Burguera et al. (1988) report elevated serum copper levels in myocardiopathic chagasic patients.

In patients with atherosclerosis and thromboangiitis obliterans, serum copper values are elevated, possibly a result of cigarette smoking and inflamation (Uza and Vlaicu, 1989). Increased cerebrospinal fluid copper levels and serum copper levels have been related to certain types of neurological disorders (Kapaki et al., 1989). In patients with Parkinson's disease, total copper levels were reduced in part of the brain (substantia nigra) but not in the other parts (Dexter et al., 1989).

However, Riederer et al. (1989) did not find any changes in copper levels in brains of individuals who had mild Parkinson's disease. The use of the anticonvulsant drug phenobarbital has been associated with increased serum copper and ceruloplasmin in epileptic children (Domizio et al., 1988). In an evaluation of hair trace elements in mentally retarded children, Shrestha and Carrera (1988) found lower levels of copper. Purice et al. (1988), working with Down's syndrome children, found elevated copper levels in erythrocytes. In contrast, Justice et al. (1988) provide blood level values for Down's syndrome children which suggest no major change in plasma copper.

Ceballos et al. (1988) found localized high concentrations of copper-zinc-superoxide dismutase in the injured hippocampal neurons of an Alzheimer's patient. They suggest that this may be due to biochemical pathways in neurons that are susceptible to degenerative processes of the disease. Mossakowski and Renkawek (1987) discuss the low ceruloplasmin and serum copper levels found in a patient first exhibiting Wilson's disease symptoms but later amyotrophic lateral sclerosis with complications. Erythrocyte copper concentrations are below normal in patients with multiple sclerosis, possibly a result of the disease altering copper (and zinc) homeostasis and possibly an effect of the corticosteroid therapy (Smith et al., 1989a).

Changes in trace metal concentrations that normally occur with age can be affected by disease. Abnormally high tissue copper concentrations have, for example, been found with obesity at all ages in one line of laboratory rats (Serfass et al., 1988). Although varying with age, high copper concentrations have been reported in cataractous human lenses (Racz and Erdohelyi, 1988; Srivastava et al., 1989). Preterm infants have significantly lower serum copper concentrations than normal infants (e.g. Bro et al., 1988). Diseases occurring during pregnancy may or may not affect copper levels in the mother and/or fetus. Significantly higher levels of copper are reported, at least during pregnancy, for hyptertensive women (Lopez et al., 1990). El Tabbakh et al. (1989) found no significant correlation between trace elements (including copper) and the occurrence of hyperemesis gravidarum during pregnancy. Adetoro et al. (1988) found (page 29) "... no statistically significant difference between the plasma levels of the trace elements measured (Zn, Cu, Mg, Ca, Fe) in normal pregnancy compared with preeclampsia and eclampsia". In an evaluation of the zinc and copper uptake by premature infants, copper uptake was greater from preterm human milk than from premature formula and term formula (Ehrenkranz et al., 1989a). With infants receiving total parenteral nutrition (TPN), Shulman (1989) reports positive 24-h Zn and Cu balances in infants with diarrhea and negative balances in postoperative infants. He suggests that the latter may be a result of the high zinc and copper concentration in gastrointestinal fluid loss associated with surgery. With patients receiving home parenteral nutrition (primarily older patients), Davis et al. (1987) report that plasma copper concentrations were positive (exceeding the normal range). (Jeejeebhoy, 1990 reviews trace elements in total parenteral nutrition.) Copper and other trace mineral uptake in the elderly are of increasing concern as the average age of the industrialized World population increases (e.g. Greger, 1989; Steffee and Teran, 1989; Tebi et al., 1988).

Lindeman (1989) reviews trace elements and the kidney, commenting on the roles of copper in human metabolism and the effect of kidney malfunction, and treatment, on copper levels. Excessive urinary excretion of copper has been proposed as a mechanism causing the hypocrupremia noted in patients with nephrotic syndrome although evidence for this is not always supportive (e.g. Yamatani and Okada, 1988). Tsukamoto et al. (1990) report an increase in copper in fingernails of nondialyzed patients with uremic poisoning. Plasma concentrations of copper also increase although benefit can be obtained by hemodialysis (Hyun and Bang, 1988). Tamura et al. (1989) found that with zinc and copper supplementation, children on continuous ambulatory peritoneal dialysis absorbed zinc and lost copper in significant amounts.

Serum copper concentrations in children with acute diarrhea are elevated while they are lower than controls in children with chronic diarrhea (Sachdev et al., 1989). The authors conclude that elevation of serum levels in acute diarrhea is a nonspecific response to infection while depletion in the chronic condition is a result of continued stress. Inflammation is often associated with changes in serum copper and ceruloplasmin (and a number of other proteins) although there is considerable variation in the responses. DiSilvestro (1987) comments that "Dietary makeup has been shown to influence the rise in serum ceruloplasmin concentrations during inflammation". Serum copper levels are elevated in patients with Behçets disease, a condition characterized by oral and genital ulcers and eye inflammation. Large increases in liver and pancreas copper, as well as zinc and iron, have been reported for rats with adjuvant arthritis (Kishore, 1989). Elevated plasma copper levels are also reported for patients with rheumatoid arthritis (Peretz et al., 1989). As a result of the involvement of copper in the metabolic processes associated with combating inflammation, copper complexes are used in the treatment of inflammatory conditions (Sorenson, 1989c). Like inflammation, serum copper levels tend to increase after a wound although this may be reversed with infection, copper levels decreasing, at least initially (Mamedov et al., 1988). The changes in trace metal concentrations produced as a result of a wound can be diagnostic, allowing diffentiation between vital wounds from postmortem wounds under certain conditions (Girela et al., 1989). Brizio-Molteni et al. (1989) report an increase in lung lymph copper and a decrease in serum and urine copper levels in sheep exposed to thermal injury. Smoke inhalation produced an increase in serum copper in comparison to thermal injury. A similar finding is reported by Olson et al. (1989) for burn patients. When severely burned children were provided with total parenteral nutrition, plasma copper levels rose but ceruloplasmin levels did not (Cunningham et al., 1988). With guinea pigs, Takeuchi et al. (1989) found increased copper values in scalded skin. In the presence of adequate copper, serum levels will also increase following whole body gamma irradiation (Mansour et al., 1986). Thoracic radiotherapy for cancer is accompanied by increases in serum copper concentrations which Molteni et al. (1988) suggest might be used to predict clinical outcome. With infrared laser irradiation, skin copper levels have been found to increase while muscle and submandibular gland concentrations decrease (Delilbasi et al., 1988).

Serum copper levels have been found to be elevated as a result of goiter, at least in a population in northeastern Turkey (Mocan et al., 1989). The authors also found high soil copper levels in the area which they suggest could be a source for the metal. They comment that the elevated copper might play a role, as an additive effect to iodine deficiency, in the formation of endemic goiter in the region. In contrast, Alekperov and Osmanov (1989) found copper insufficiency in association with endemic thyroid enlargment in a district of Azerbaijan (USSR) and suggest that the addition of copper might reduce the frequency of goiter. Ward et al. (1989) suggest the use of serum copper concentration may provide an index of lung injury. In an examination of trace elements and emphysema, Engstrom et al. (1988, 1989) note slightly higher (not significant) plasma copper concentrations in smokers with emphysema when compared to smokers without emphysema. It is important to note, however, that smokers characteristically have elevated lung tissue concentrations of copper (Paako et al., 1987; see also Kalliomaki et al., 1989). Elevated copper levels are reported by Avolio et al. (1988) for bronchoalveolar lavage fluid and blood of patients with talcosis, an occupational lung disease whose pathogenesis is gradually being defined. In contrast to the trend in emphysema and talcosis, Sarkar et al. (1988b) note reduced levels of plasma copper and ceruloplasmin in adult male rats with experimental pulmonary edema.

In an examination of maternal and fetal zinc and copper metalloprotein in the diabetic rat (diabetes mellitus), Uriu-Hare et al. (1988) found elevated levels of metallothionein-associated copper and zinc in dams and lower zinc-metallothionein in fetuses. This is also reported for humans with diabetes (e.g. Zhou and Wang, 1989) and supports the indication of alterations in the metabolism of copper and zinc in humans as a result of diabetes. Elevated kidney and liver copper levels have also been reported in recently induced alloxan-diabetic and streptozotocin-diabetic rats, two models for human diabetes (Agren et al., 1987; Fushimi et al., 1988). The zinc-copper ratio may also be affected in diabetic rats (Deebaj et al., 1989). Long term effects on streptozotocin-diabetic rats have been reported to be somewhat different, kidney levels approaching normal but elevated levels found in the liver, femur erythrocyte and lymphocyte (Raz and Havivi, 1988). Oster et al. (1989a) report that copper accumulation in tissue from diabetic rats is, however, due to hyperphagia and independent of insulin. In an evaluation of zinc and copper levels in malnutrition-related diabetes, Srivastav et al. (1988) found no effect on serum copper levels. Serum copper levels in underweight diabetic patients were similar to those in underweight non diabetics. Rohn et al. (1989) report no statistical difference between normal children and those with Type I diabetes (insulin-dependent), in serum and blood cell copper levels. However, Kinoshita et al. (1988) have found a higher percentage of what is termed "glycated" copper-zinc superoxide dismutase in diabetic patients. This suggests that although levels of total copper may be the same in the diabetic and non-diabetic, the function may be altered by diabetes. Elevated serum, liver and urine copper levels have been associated with obesity (Bhattacharya et al., 1988), a condition often associated with Type II diabetes (non-insulin-dependent).

The storage of copper by the liver can be affected by a number of diseases. Liver disorders and malignancies can, for example, affect the distribution of copper among serum proteins (Barrow and Tanner, 1988). With the white perch (*Morone americana*), Bunton et al. (1987) report (abstract) "Age-related, progressive accumulation of hepatic copper in levels often exceeding 1,000 µg/g wet weight ... (in association with) peribiliary fibrosis and inflammation, bile duct hyperplasia, prominent, enlarged melanomacrophage centers, and disruption of hepatic architecture in older fish".. Suzuki et al. (1987) note increased serum copper concentrations in rats during liver regeneration after partial hepatectomy. Sheep are particularly susceptible to effects from excess biologically available copper, a factor which is caused by their limited ability to excrete excess copper. Tetrathiomolybdate not only moderates the effects of excess copper but can change the distribution of copper in bile fractions (Gooneratne et al., 1989c) as well as in the distribution in the sheep kidney and in the soluble fraction in the kidney (Gooneratne et al., 1989e,b). Oral zinc therapy has been used to decrease liver and serum copper concentrations in patients with a type of liver fibrosis caused by bilharzia (Hamada et al., 1988a). (Note, however, that considerable variability in liver copper values can be caused by inadequate sampling techniques (Faa et al., 1988).) McArdle et al. (1989a) found differing effects of different copper chelators on copper uptake and metabolism in the mouse. As an example, they comment that (abstract) "Penicillamine, a clinically important chelator, does not block the uptake of copper or remove copper from hepatocytes. Two other copper chelators, sar and diamsar, which form very stable and kinetically inert Cu^{2+} complexes by encapsulating the metal ion in an organic cage were shown to block copper accumulation by the cells and to remove up to 80% of cell-associated copper".

Several liver diseases are genetically controlled. In a study of copper and canine liver disease, Thornburg (1988) comments that (page 6) "... increased hepatic copper is familial in occurrence". Eriksson and Peura (1989) used needle biopsy liver samples to diagnose copper toxicosis in Bedlington terriers. Haywood et al. (1988) describe the characteristics of hepatitis in Skye terriers and note that excess copper (801-2,257 µg/g) was related to the severity of cholestasis. In an examination of the distribution of copper in livers of copper-loaded rats, Fuentealba et al. (1989b) found accumulation almost exclusively in the periportal and mid-zones of the liver lobules. Bingle et al. (1987) used a guinea pig model of Wilson's disease to examine developmental changes in hepatic copper-binding proteins. They comment that (page 221) "The appearance of metallothionein as the major cytosolic copper binding protein around the time of birth confirms what has previously been reported in many other mammals including man". A similar model has been used by Chesta et al. (1989) and others to examine the effects of copper overload. Wilson's disease is often difficult to identify, primarily because other liver diseases (e.g. steatosis) have similar characteristics and the classical characteristics of Wilson's disease are not always apparent (e.g. Geubel et al., 1988). Changes can also occur in serum copper levels with advances of liver diseases, as for example from chronic hepatitis to liver cirrhosis (Arakawa et al., 1990) making identification of liver diseases dependent on the state of the disease. Other commonly examined diseases include Menke's disease and Indian childhood cirrhosis. The brindled mouse and macular mouse (e.g. Tachiiri et al., 1988) have been routinely used as animal models for Menke's disease, the gene for the "brindled" condition resulting in lower liver and milk liver content of the mouse (Prohaska, 1989). Reduced copper levels are discussed in a brief review of the clinical aspects of Menke's disease providied by Kolb and Guthoff (1987) and the case history of an individual with a mild form of the disease by Danks (1988). The supplementation with copper, as a histidinate (Danks, 1988) or sebacate (Williams et al., 1987) provides some relief from the disease, depending on its severity. Indian childhood cirrhosis is a lethal liver copper storage disease that is reportedly inherited (Adamson et al., 1989a,b) or at least the tendency towards the disease is inherited. The heritability is in contrast to the belief that the disease is "... associated with the ingestion of infant milk feeds contaminated with copper from brass utensils ..". (Tanner and Mattocks, 1987). Barrow and Tanner (1989) discuss the possibility that Indian childhood cirrhosis is a result of more than one hepatic insult.

Irregular growth of cells is often associated with increased tissue copper levels. Chilar et al. (1989) found some evidence for this with serum levels in cattle affected with squamous cell carcinoma of horn. Miu and Song (1989) found a copper-zinc relationship in plasmacytoma-bearing mice, organ copper concentrations decreasing with excess dietary zinc. Trace element profiles (Lal et al., 1989) and, more specifically, the ratio of copper to zinc are often regarded as a marker of neoplastic disease (e.g. Diez et al., 1989a; Gimenez Martin and Martin Mateo, 1985; Wasowicz et al., 1989) as well as nutritional status and age (Hisaki et al., 1988). Saito (1990) reviews the kinetics of trace elements in cancer patients. Although copper concentrations frequently increase with irregular tissue growth (e.g. Alarcón et al., 1983; Cai et al., 1988; Diez et al., 1989b; Lian et al., 1989; Nagasue et al., 1989; Saito, 1990; Saxena et al., 1988; Wu, 1989), they are affected by the type of growth (e.g. Müller et al., 1988), as indicated by the elements found most important in distinguishing between malignant and normal tissues. Drake and Sky-Peck (1989) report that Fe, Mn and Cu concentrations can be used with lung samples, Ca, Zn and Fe in colon samples and Ca, Rb and Zn in breast samples. Coates et al. (1989) comment that (abstract) "... the presence of cancer may increase serum copper levels several years prior to its diagnosis. They are less supportive of the hypothesis that serum copper levels affect cancer risk". Evidence supporting this includes the finding that 50-60% of total tissue copper is associated with metallothionein in melanoma patients; metallothionein is a normal metal transporting and metal regulating agent (Krauter et al., 1989). As well, plasma copper concentrations do not always increase, as for example in patients with invasive carcinoma of the cervix (Wong and Arumanayagam, 1988). Blood copper values frequently change during the course of cancer, Saito et al. (1987) noting low blood copper levels in stages I and II of stomach cancer, higher values in stage IV. (Fuchs and de Lustig (1989a,b) suggest that changes in tumour tissue copper levels could be used as an indication of tumour differentiation.) Dawczynski et al. (1988) comment (summary) that "The analysis of the elements iron, zinc, copper, and magnesium in the serum allows no assertion on diagnosis and course of the adeno- and squamous cell carcinoma in the lung to the present date. Copper-zinc superoxide dismutase levels, on the other hand, are elevated in patients with various digestive cancers (Oka et al., 1989). Changes in serum copper levels occur during treatment with anticancer drugs (Slavik et al., 1989). Zinc, selenium and copper concentrations in soils, as well as soil pH, have been reported to be associated with the incidence of gastric cancer in a region (Li et al., 1987).

I.3.3 COPPER AND THE RESPONSE OF THE ORGANISM

There is an increasing awareness of the importance of metal-organism interactions at all levels of biological development (e.g. Beveridge and Doyle, 1989). The nature of these interactions range widely. However, it is important to remember that an organism will respond to copper only when the metal is biologically available or has previously been acquired and is now metabolically active. This realization is widely accepted but with the awareness that we have a great deal to learn about metal speciation to fully understand metal bioavailability. Bremner (1990), for example, comments (page 1) that "Surprisingly little is known of the speciation of metals in the diet or in tissues, other than through their association with specific enzymes." He continues, commenting on the value of studying the metabolism of copper (and zinc) at the molecular level, not only to an understanding of metal reactions and organism susceptibility but also to the development of improved methods of disease treatment. In a book on Mining and the Freshwater Environment, Kelly (1988) comments (page 16) that "One point that will be repeated over and over again in later chapters is that the speciation of a metal, rather than its total concentration, is the key to understanding its effect on the biota." In a review entitled "Copper toxicity and chemistry in the environment:...", Flemming and Trevors (1989) note that any detrimental effects are dependent upon the biological availability of the metal and the physico-chemical characteristics of the particular environment that influence metal speciation. This section of the review will use recent literature in discussions of the response of organisms to copper. However, it is with the underlying assumption that the reader will recognize that a response will not occur unless the metal is in a biologically available state.

Microorganisms and plants

Tissue copper levels in plants are often useful in estimating copper status. As well, there is some evidence that at least in plants such as ryegrass, increasing soil metal concentration (Cd, Ni, Cu) alters the carbohydrate status, particularly of fructan (Frossard et al., 1989). Copper can be benefical in the process of melanization in some nitrogen-fixing soil bacteria, a process that may have enhanced adaptation to an aerobic existance (Shivprasad and Page, 1989). Quite in contrast, stem melanosis in wheat can occur as a result of copper deficiency in soils and can be reduced by copper supplementation (Malhi et al., 1989). Karpinski (1989) presents evidence that the rate of melanin formation is affected by Cu(II). This is presumably due to the requirement for copper by the enzyme tyrosinase which has been implicated in melanogenesis (in de Pauw-Gillet et al., 1987 and Platen and Kutzner, 1987).

Excess copper can be beneficial in the control of plant diseases, decay organisms or unwanted plants. However, this can be associated with elevated residual soil copper levels (e.g. Dickinson et al., 1988; Hyuuga, 1987; Lepp and Dickinson, 1987; Teviotdale et al., 1989a) and the possibility of elevated plant tissue levels (e.g. Thornton, 1988). This is especially true when large amounts of copper are needed for disease control (e.g. Macek and Jozko, 1989) or treatment with copper is not effective (e.g. Nazif, 1988). Excessive use of copper fungicides has been associated with decreased soil microorganism activity (Torstensson, 1988) and foliar disorders and reduced growth in the treated plants (Dickinson and Lepp, 1986). With wine grapes, certain pesticides have been associated with reduced malolactic fermentation (Haag et al., 1988). However, proper application of copper-containing fungicides has been noted to improve organoleptic properties of wines (Lemperle, 1989). Jollands and Jollands (1989) and Jollands et al. (1989) comment on the advisability of using appropriate techniques for dispensing copper fungicide in the control of cocoa black pod disease. This philosophy would seem appropriate wherever cidal agents are used and could reduce the chances of excess soil copper. As an example of this, based on existing and anticipated residual copper levels in Kenyan coffee plantations, Dickinson et al. (1988) conclude (abstract) "... that the continued use of copper fungicides (in Kenyan coffee plantations) is not a viable management option". It must also be realized that the use of any type of pesticide can have undesired side effects. Glyphosate, a herbicide commonly known as "Roundup", and a similar agent (Glufosinate) both form insoluble complexes with iron, copper, calcium and magnesium ions in groundwater (Ambrose and Hoggard, 1989; Subramaniam and Hoggard, 1988) which may affect metal availability.

In aquatic environments, copper is one of the metals most often considered as a contaminant (e.g. Kowalewska, 1988). Sanders (1987), for example includes it in a review of "Contaminant effects on primary producers in Chesapeake Bay". Rao and Mudroch (1986) report a relationship between bacterial densities and trace element concentrations (including copper). Rybak et al. (1989) discuss the impact of atmospheric deposition on lake productivity, noting an increase in a number of metals in nearsurface sediments which they suggest, for Newfoundland, could be more important in controlling primary production than is water aciditiy. Flemming and Trevors (1988a) demonstrate a long-term change in freshwater sediment oxygen consumption with high levels of added copper (up to 5 mg/g sediment). They (Flemming and Trevors, 1988b) note an inhibitory effect on nitrous oxide reduction by sediment copper but only at the highest concentration used. Frond multiplication of the duckweed (*Lemna paucicostata*) is inhibited about 50% by 0.1 ppm Cu^{2+} (Nasu et al., 1988). Pal and Chatterjee (1989) note changes caused by copper sulfate, in the growth and cell division of two aquatic plants belonging to the genus *Chara*. With another aquatic plant (*Azolla pinnata*), high levels of copper (2-20 ppm), as copper sulfate, inhibited growth (Rajarathinam et al., 1989). Swain et al. (1986) recommend the use of enclosures to assess the impact of copper sulfate before whole-lake treatments. Raman and Cook (1988) provide "Guidelines for applying copper sulfate as an algicide ..." based on a field study. They advocate the use of citric acid as a complexing agent to enhance the solubility of copper and mantain it in a dissolved state for longer periods of time. Fungicidal effect is associated with a reduction in growth of the fungal agent whether on living organisms or as decay-causing agents. The latter is especially true with the economic use of wood, where copper and copper-chrome-arsenate formulations are widely used (e.g. Collett, 1988). Hale and Eaton (1988), however, point out that, with the use of CCA, there appears to be differential penetration of the components, better penetration of chromium than copper.

Microorganisms and plants can be adversely affected by excess biologically available copper. This is seen in an array of effects that includes reduction in growth of filamentous bacteria (Shuttleworth and Unz, 1989), yeasts (Rao and Venkateswerlu, 1989), blue-green algae (Gupta, 1989a), a water fern (Sela et al., 1989) and higher plants (e.g. El-Shourbagy et al., 1989; Gorlach and Gambus, 1988; Maroti and Bognar, 1988). It also includes morphological changes in a yeast (Venkateswerlu et al., 1989), enhancement and then inhibition of oxygen uptake by *Nitrobacter agilis* (Tsai and Tuovinen, 1989), reduced salt tolerance in a blue-green alga (Khasanova and Wagner, 1988) and inhibition of gas production in sludge digestion (Tomlin and Forster, 1988). Copper is reported to inhibit nitrifying bacteria (Ibrahim, 1989) and, as $CuCl_2$, has been reported to exhibit genotoxic mutagenic activity in a bioluminescent bacterium (Ulitzur and Barak, 1988). The authors comment, however, that the activity was unexpected since the compound is not known as a mutagen or carcinogen in other systems. Blakely (1988), for example, notes no effect of copper on the size and number of urethane-induced tumors in mice. Rossman (1989) and Rossman et al. (1989), however, note a comutagenic effect of Cu(II) with ultraviolet light. Paleolimnological evidence suggests that increases in metal concentrations (including copper) can cause major changes in phytoplankton species composition in soft-water lakes (Dixit et al., 1989). Evidence of excess copper effect on terrestrial plant species composition as well as growth is given by Rühling (1986), in a discussion of the effects of copper ore concentrates on the growth of trees. In eelgrass, a true grass that grows in certain saltwater environments, growth was inhibited by excess copper and a number of other metals, in the order $Hg \geq Cu > Cd \geq Zn > Cr(III), Pb$ (Lyngby and Brix, 1987). Twiss et al. (1989a) describe an algal bioassay technique (*Scenedesmus acutus*) to determine the effect of copper on photosynthesis. However, at least one species of the genus (*S. quadricauda*) is reported to adapt to added copper (Gapochka et al., 1988). (Adaptation would reduce the value of a bioassay.) Species-specific differences are also found in the response and tolerance of algae to excess metal (e.g. Harstedt-Romeo and Gnassia-Barelli, 1988; Mann et al., 1989b; Takamura et al., 1989a). Since metal is taken up by plants (e.g. Drbal et al., 1985; Mikryakova, 1987; Sela et al., 1989), the response may be affected by decreasing metal concentrations due to biological uptake .

Specific effects of excess copper vary, depending on the nature of the plant. In the white mushroom *Phanerochaete chrysosporium,* Rovel and Metche (1986) found 70% inhibition of ligninolytic activity with 0.4 mM copper. Duke and Mitchell (1989), for example, report that a copper concentration of 10 mg/L will reduce the chemotactic response of a unicellular alga (*Dunaliella tertiolecta*) towards the ammonium ion. Long term exposure of the phytoplankton *Chlamydomonas reinhardtii* to high cupric ion activities is reported to increase protein synthesis (Cote and Bastien, 1988), possibly for the production of metal complexing agents. With duckweed (*Spirodela polyrhiza*), stress sponsors the increased production of anthocyanins and the formation of storage organs or "turions" (Augsten and Gebhard, 1988). However, the authors note that turion formation is inhibited by excess heavy metal, including copper. Copper inhibition of photosynthesis is reported by several workers (Eloranta et al., 1988; Pascal et al., 1988; Renganathan and Bose, 1989; Sastry and Chaudhary, 1989; Singh et al., 1989b). With barley, El-Shourbagy et al. (1989) report that excess copper affected the leaf stomata and the concentrations of plant sugars. With oats, Jurkowska and Wojciechowicz (1988) report increased yield with 6 and 60 mg/kg soil copper supplementation but a decrease with 1000 mg/kg. They (and Jurkowska and Rogoz, 1988) also report a change in macronutrient translocation in plants after copper supplementation. Rousos et al. (1989) report increased root branching in cabbage cultivars exposed to 1.2-2.5 mg/L copper, with colour changes and tip browning also occurring. In contrast, with wicker plants, Baluk and Bukiewicz (1988) found decreased root production and shorter withes with soil enrichment of copper (150 mg/kg). Copper (100 ppm) has been associated with increased cell permeability in cotton (Dhopte and Wankhade, 1988). Huber et al. (1989) treated pine seedlings with copper sulfate or lead acetate and found evidence of detrimental effect on energy homeostasis and oleoresin production in seedlings. Subsequent inoculation of treated seedlings with a pine nematode parasite indicated increased susceptibility caused by the treatment.

Smith et al. (1986) provide an overview of the effects of atmospheric deposition of heavy metals on forest health. Using a New Hampshire northern hardwood forest they report net gains for cadmium, copper, and lead via atmospheric deposition and list three effects of heavy metal interference with nutrient cycling. Schultz et al. (1987) report that 10-15% of soil cadmium and copper were taken up and translocated to the canopy in a beech and spruce stand in Central Germany. Metal-related reduction in soil microbial activity has been demonstrated under laboratory conditions (Gusev et al., 1988; Wilke, 1988) and could affect nutrient recycling in natural systems (e.g. Schäfer, 1987).

Animals and humans

The effects of copper on organisms are varied, dependent upon the requirement and nature of the organism and the availability of the metal. They range from the beneficial effects of copper as an essential metal to the detrimental effects of excess metal. The detrimental effects include direct effects, on organisms and humans, and indirect effects, for example on the oxidizing action of copper on paper (Banik, 1989). Reish et al. (1989) comment that ionic copper is "more toxic" than chemical forms bound to organic compounds. (The term "more available" is more correct than "more toxic".) The unique nature of each species allows tissue levels or behavioural responses of some species to be used as indicators of conditions (e.g. Kramer et al., 1989; Wang, 1986). It also provides an explanation for the differences in tolerance between species (e.g. Knowlton, 1988a) and the reasons for some of the changes seen in species composition in metal-enriched sites. With reef corals, for example, tolerance to metals is affected by sedimentation as well as metal bioavailability (e.g. Brown, 1987; Glynn et al., 1989). Sunda et al. (1990) present evidence that naturally occurring concentrations of copper and zinc may inhibit survival of the larval stages of a planktonic copepod crustacean (*Acartia tonsa*). Sullivan and Ritacco (1988) note (page 354) that "the effects of copper on toxicity to copepods (crustaceans) and recovery of zooplankton populations are likely to be more pronounced in systems where either food availability is low (...) or food quality is poor (...) than in moderately nutrient-rich systems". Miller (1988b) notes that the ribbed mussel *Geukensia demissa* synthesizes greater amounts of a protective metal-complexing agent under conditions of high salinity than under low salinity. With elevated metal (Cu, Zn) levels and hypoxic conditions in two crustacean species, Johnson (1988a) notes more evident decreases in blood ion levels at low salinity than high salinity. Effects may also be indirect, acting on factors such as immunity (Cheng et al., 1987) or food-collecting ability. Enesco et al. (1989b) note that long-term exposure of the rotifer *Asplanchna brightwelli* (a small aquatic organism) to high concentrations of copper sulfate (60 µg/L) decreases the lifespan because of enhanced lipid peroxidation. Metal speciation and metal concentration must also be considered in any examination of the effects of copper. Sources of copper, such as brass dust can provide metal adequate to affect species composition (Landis et al., 1988a) if other factors, such as pH (e.g. Ma, 1988) and alkalinity (e.g. Lebedev, 1989) do not affect metal bioavailability.

Responses to copper have been the focus of a number of studies on vertebrate and invertebrate organisms (National Technical Information Service, 1989g). Copper uptake has been reported for various soil invertebrates in vineyard soils exposed to 60 years of continuous use of copper-containing fungicides (Wittassek, 1987a). Cluzeau and Fayolle (1988) found that earthworms were almost absent in an old vineyard with high levels of copper. Fungicides can also affect species composition; Duse (1988) comments that (page 257) "Fungicide programmes influence strongly the possibilities of biological control of spider mites; ..".. Nishiuchi (1988) reviews the effect of pH on the toxicity of pesticides to the toad *Bufo japonicus*. Boitel and Truchot (1988, 1989) found that waterborne copper caused metabolic acidosis in the shore crab *Carcinus maenas.* Tsai et al. (1988) report green oysters in an area of Taiwan, which they attribute to the accumulation of particulate copper. Filenko and Lazareva (1989) found that excess copper produced a change in the mitotic index and chromosomal aberrations in adult specimens of the freshwater crustacean *Daphnia*. Vranken et al. (1986) used development rate, fecundity and mortality in a marine nematode worm as indications of effect of excess metal (including copper) and chemicals. Differences in response can however, be a factor both between species and within a species. de Nicola Giudici and Maria Guarino (1989) report differences in acute toxicity of copper and cadmium between females, males and juveniles in the marine crustacean *Idothea baltica*. Tolerance was in the order females > males > juveniles. Tolerance can also increase with continued exposure, as indicated for a number of organisms, such as fiddler crabs (Uma Devi and Prabhakara Rao, 1989) and the larvae of mayflies (Suzuki et al., 1988b).

High levels of copper have been associated with increased fish tissue metal levels (e.g. Beeke, 1987; Szerow and Milian, 1987) and changes in swimming behaviour and physiology of a number of fishes, including the carp (Huang et al., 1987c), the cichlid fish *Oreochromis niloticus* (Al-Akel, 1987) and the dogfish *Scyliorhinus canicula* (Torres et al., 1987). Cyriac et al. (1989) report increased haemoglobin levels in a similar species of fish (*Oreochromis mossambicus*) after short term exposure to copper or mercury. This should increase oxygen uptake capability unless copper causes haemoglobin degradation. Shivaraj et al. (1989), however, note decreased oxygen consumption in a fish species (*Puntius arulius*) following exposure to copper. Benedetti et al. (1989) describe morphological changes of skin, liver and gills of the bullhead *Ictalurus nebulosus* after exposure to copper. Exposure of the electric ray *Torpedo marmorata* to high levels of copper (4 mg/L) for 60 days has been associated with changes in the ultrastructure of the nerve cells (Enesco et al., 1989a). It should be noted that effects can be modified by other water quality parameters. Hughes and Nemcsok (1988), for example, noted slight tissue damage to rainbow trout by of 200 µg/L copper sulfate at background pH (8.34) but much greater effect at pH 6.5.

In laboratory animals, high levels of copper are reported to produce anatomical, pathological and functional changes in tissues and organs such as the liver and kidneys (e.g. Monkiewicz et al., 1985). Cirrhosis of the rat liver can, for example, be produced by injection of cupric ntrilotriacetate (Toyokuni et al., 1989). This can also be found with dietary copper in certain types of mutated laboratory mice which are unable to maintain normal copper homeostasis (e.g. Biempica et al., 1988). Under certain conditions, copper hepatotoxicity from dietary copper can be reduced by vitamin E (Sokol et al., 1987) suggesting the importance of diet composition. Intestinal absorption of an amino acid (L-histidine) can be depressed in the rat by excess copper although this is modulated by the amount of histidine present (Kiyozumi et al., 1988). In contrast, dietary methionine has been reported to check the accumulation of copper in the kidneys of copper-fed rats (Kumar et al., 1987). Pati and Bhunya (1987) report chromosome-damaging effects in mice, from injection of copper sulphate.

The copper status of an animal can often be estimated from a combination of measurements, including serum and tissue copper levels (e.g. Hamada et al., 1988b; Ledoux et al., 1989b; Pond, 1989; Stevenson, 1981) as well as the activities of several copper-containing proteins (e.g. ceruloplasmin, red cell copper superoxide dismutase; Paynter, 1987). Copper plays a role in the formation of connective tissue and the maintenance of bone in animals (e.g. Dollwet and Sorenson, 1988). However, in excess, it has been reported to inhibit collagen synthesis in embryonic chick bone tissue culture (Kaji et al., 1988). Reader et al. (1989) note metal impairment of calcium uptake in yolk-sac fry of brown trout. In contrast, copper deficiency may be an important factor in osteoporosis, a disease characterized by a reduction of bone in bone tissue (Strain, 1988). Copper-containing enzymes play a number of important neurochemical roles in organisms (e.g. Bhathena, 1989; Sourkes, 1988) although, unlike iron and zinc, copper does not appear to play any role in "intelligence development" in children (Liu et al., 1988b). It may, however, affect the status of glands as indicated by the reduction in goiterogenesis produced in broiler fowl by copper-supplemented rapeseed cake (Schöne et al., 1989). Xin et al. (1989) present evidence that copper status may have some effect on pituitary secretion of luteinizing hormone in dairy steers. With weanling pigs, Kornegay et al. (1989) found that appropriate amounts of supplementary dietary copper can improve growth rate although they also found a depression of immune response to lysozyme and phytohemagglutinin.

There is a genetic factor that can influence the response of an organism to both copper deficiency and excess. This is suggested, for example, as a possible factor in the effect of copper deprivation on glucose metabolism (Nielsen and Milne, 1990). Work on the effects of dietary copper deficiency on the rat pancreas suggest the importance of assessing the copper status of an organism on a copper-deficient diet (Mylroie et al., 1989). Deficiency combined with carbohydrate-enriched diets can influence blood antioxidant enzymes and increase serum cholesterol levels, at least in rats (Carville and Strain, 1989). Carr and Lei (1989a), however, point out that although there is a definite effect of copper deficiency on the concentration of high density lipoproteins, the role of dietary copper in cholesterol metabolism remains unclear. (Cholesterol gallstones may contain metals, including copper although Qian and Xiao, 1988, comment that metals probably play a greater role in the formation of pigment gallstones.) Hunt and Dean (1989) did find an effect of copper and liposomal membranes on protein degradation. There is a relationship between copper and cardiovascular condition, possibly due to the hydroxyl free radical resulting from tissue peroxidation when copper deficiency reduces antioxidant defenses (Saari, 1989; Saari and Johnson, 1989). Fields et al. (1989d), however, provide some evidence of sex-related effect, copper deficiency causing greater mortality and heart pathology in male than in female rats. Dietary carbohydrate types may also play a role, severely copper-deficient male rats (not female) die of sudden rupture of the heart when fed fructose as opposed to starch (Bhathena and Recant, 1987; Bhathena et al., 1988c). Copper deficiency affects the extracellular matrix of arterial walls, leading to cardiovascular lesions (e.g. Radhakrishnamurthy et al., 1989). In the aorta there is an inverse relationship between copper content and the area of lipid deposits (Meissner, 1990). Mild copper depletion has been related to functional alteration of human blood pressure regulation (Lukaski et al., 1988).

Tissue inflammation can be more severe without adequate copper (e.g. Milanino et al., 1989a). Elevated activity of the copper-containing enzyme ceruloplasmin (a ferroxidase) has been reported in the aqueous humour of inflamed eyes and is suggested to be associated with protective functions during inflammation (McGahan et al., 1989b,c). In a review of copper proteins and their role in inflammation, Lunec (1989) comments that (page 17) "During inflammation we are protected from the onslaught of metal-catalysed free radical reactions paradoxically by antioxidant metalloenzymes such as superoxide dismutase and caeruloplasmin". A number of copper-containing pharmacological compounds are active in the treatment of inflammation (e.g. Korolkiewicz et al., 1989; McGahan et al., 1989c; Sorenson, 1989c). Fernandez-Madrid (1989) reviews literature on the use of copper for inflammatory disorders in man, with special reference to rheumatoid arthritis. Wolintz and Gerber (1989) notes that, in patients with rheumatoid arthritis, large particles of immunoglobulin G are formed in response to low levels of a hystidine-cystine-copper complex. These particles may contribute to inflammation or could be an immunogen for rheumatoid factor.

Although supplementary copper is often necessary to prevent deficiencies (e.g. Lofstedt et al., 1988), excess dietary copper has been associated with a detrimental effects. This includes effects on growth in young turkeys (Potter and Potchanakorn, 1989) and on the reproductive capacity of hens (Stevenson, 1981). Brooks (1988) reports a reduction of enzyme activity (NADPH-cytochrome c reductase) in laboratory rats with injection of 1.44 mg copper/kg, as copper sulfate. Auer et al. (1989) describes a suspected case of copper toxicity in a horse and Hintz (1987a) discusses the effects of excess dietary copper on horses. He comments (page 18) that "... the feeding of grain mixtures containing 50 ppm or more of copper, or the proper use of supplements containing high concentrations of copper should not cause copper toxicosis in horses". He points out that 800 ppm is tolerated but 2800 ppm may cause toxicosis. Sheep are often susceptible to excess copper (e.g. Howell and Gooneratne, 1987), particularly under confinement. Examples of this are found in the literature, most with characteristic copper-loading of the liver (e.g. Kumaratilake and McC. Howell, 1989a,c; Martin et al., 1988). Treatment often includes the use of molybdenum, which does not appear to affect normal copper metabolism (Kumaratilake and McC. Howell, 1989b; Wittenberg and Devlin, 1988).

Copper is widely used in the treatment of animal and human deficiencies, pests and diseases (e.g. Landeen et al., 1989b; Lin et al., 1989b; Rondelaud, 1988a; Thurman and Gerba, 1988). It is also an effective birth control mechanism (e.g. Holland and White, 1988). As with plants, however, excess use of copper can be detrimental to the organisms being treated (Cardeilhac and Whitaker, 1988). As well, effective use requires an understanding of the biology of the disease or pest (e.g. Cheng, 1989) as well as the nature of the host, food crop, or agent being protected. Dowd (1988), for example, discusses the use of antifouling coatings and comments on the need for coatings that can best serve the maritime industry without endangering the marine environment or mankind. Rascio et al. (1988) reviews research and development of soluble matrix antifouling paints for aquatic facilities. The use of copper coatings and copper-nickel alloys for marine facilities not only reduces maintenance and operating costs (Gaffoglio, 1987) but eliminates need for the more hazardous coatings of tributyl tin. Continued work with copper-nickel alloys and coatings is needed, to minimize problems such as the corosion due to turbulence (e.g. Clayton, 1987) and the impact of sulfate-reducing bacteria on welded copper-nickel piping systems in seawater (Little et al., 1988). The International Copper Association-supported work on corrosion (e.g. Castle et al., 1988) is an example of this type of work. Fingerman (1988) discusses possible biological effects from copper- and tin-based antifouling compounds although most of the discussion concerns laboratory-based results on the metal rather than when used as an antifouling agent. Environmental concern about the use of antifouling agents is evidenced by a number of publications which deal with the impact of marinas on water quality (e.g. McMahon, 1989) and by government reports such as the "House of Lords" (U.K.) report on anti-fouling paints, which deals mainly with tributyl tin.

Improper use of copper in algicides and fungicides can have detrimental effects on humans. Blanc et al. (1988), for example, describes a case of "green hair" produced by the improper use of algicide in a swimming pool. Green hair has also been noted in a rumbler operator who removed brass parts from the rumbler with ungloved hands and then ran his hands over his hair (Wright and Auger, 1988)! In an article entitled "Copper Sulfate - not a harmless chemical", Lamont and Duflou (1988) report a case of fatal copper sulfate poisoning of a woman after treatment by a witch doctor; treatment included ingestion of a copper sulfate-containing concoction. Excessive exposure to the wood preservative copper-chrome-arsenate (CCA) is reported to be a health hazard (reviewed in Mason and Edwards, 1989a,b). Intake of aerosol copper occurs during exposure to metal-containing dust in certain industrial operations. Hirano and Suzuki (1989) review some of the more recent work on pulmonary clearance and toxicity of a range of heavy metals. They comment on the importance of understanding the species of elements that are present in the environmental and work place atmosphere to understand the availability of aerosol metal. In a study of the effects of inhaled copper-zinc alloy powder, Snipes et al. (1988b) note that with rats, lesions were produced if exposure was at or above 3.2 mg Cu-Zn/m^3, 1.5 hours/day, 4 days/week for 13 weeks. They (Snipes et al. (1988a) also noted an inflammatory response and decreased pulmonary capacity with expsure to 120 and 240 mg hr Cu-Zn/m^3. Johansson and Camner (1986) report that exposure of rabbits to low levels of copper chloride dust produced no effects other than a slight increase in the volume density of one type of alveolar cell. Labedzka et al. (1989) comments on the importance of alveolar macrophages in defense against bacteria and note that Cu(II) decreased oxygen release from rabbit alveolar macrophages.

The effects of copper deficiency as well as excess are seen at both the cellular and subcellular level, often as a result of direct and indirect metal interactions with organics. Sternlieb (1990) comments, with respect to copper overload in the liver, that "excess copper affects a remarkably diverse array of cellular targets". Kardos et al. (1989a) note that ionic copper inhibits GABA-mediated chloride uptake into membrane vesicles from the rat cerebral cortex. Agarwal et al. (1989) comment that (summary) "Both deficiency and excess of copper induce toxic effects on mammalian cell systems in vivo and in vitro. The effects can be related to the affinities of Cu(II) ions for specific cell components". As an example, in metal overload situations such as Wilson's Disease or models of similar diseases, excess metal is incorporated into large, distorted bodies (lysosomes) in liver cells (Myers et al., 1987). Copper also exhibits affinities for particular molecular "groups" such as sulphydryl and amino groups (e.g. Kolinkoeva et al., 1988). There may also be changes in the "state" of cells; Savluk et al. (1988) note that additions of either copper or silver caused a change in the electrokinetic cell potential of the microorganism *Escherichia coli*.

Effects of copper on erythrocytes are discussed in a review (in Polish) prepared by Lewczuk and Smolik (1988). Ribarov and Benchev (1988) report changes in red blood cell membrane microviscosity caused by Cu(II), probably in association with copper-induced lipid peroxidation within the cell membrane. Tallineau et al. (1988), however, report red blood cell hemolysis under a nitrogen atmosphere to the same extent as under an air atmosphere suggesting a lack of correlation between lipid peroxidation and hemolysis. As well, Aykac et al. (1989) report that added copper caused no significant change in rat erythrocyte lipid peroxidation. Copper is linked with lipid oxidation in lymphocyte function (Lipsky, 1987). Excess copper can affect protein synthesis, possibly a direct effect on the cellular translational machinery (Schilsky et al., 1987). Copper can apparently interact with nucleic acid components (e.g. Denizeau and Marion, 1989). Thiols and dithiols have been found to be active as DNA (desoxyribonucleic acid) cleavers in the presence of cupric ions (Reed and Douglas, 1989). Certain pesticide combinations have been reported to induce chromosome aberrations in the Chinese hamster (Wang et al., 1987a). Copper salts in the presence of tetracycline antibiotics have been reported to cause substantial damage to linear duplex DNA, damage involving hydroxyl radicals (Quinlan and Gutteridge, 1988b). Copper(II) also facilitates bleomycin-mediated unwinding of plasmid DNA (Levy and Hecht, 1988). Bumgardner et al. (1989a) report, with cell cultures, exposure to three copper alloys (alloy nature not given in abstract of talk) provided sufficient copper to produce changes in DNA synthesis of the cells. Apelgot et al. (1989), however, used copper isotopes to obtain evidence (abstract) that "... coper atoms bound to DNA are essential for cellular functioning". The potential interaction of copper with DNA (and possibly RNA) potentiates its use as a growth-controlling agent if the metal occurs in excess. Copper sulfate has, for example, been shown to inhibit proliferation in certain melanoma cells (de Pauw-Gillet et al., 1987) and Ehrlich ascites tumour cells (Takamura et al., 1989b). It has also been demonstrated to be one of the metals that can modulate growth of human normal and leukemic lymphocytes (Carpentieri et al., 1988).

Interactions of copper with organics, and microorganisms, can occur in water and soil (e.g. Kozarac et al., 1989) but often occur in "films" whether it is in the laboratory or in natural environments (e.g. Lion et al., 1987). Geesey et al. (1988) report binding of copper by extracellular polymers of biofilm bacteria on copper surfaces and suggest that these polymers can enhance corrosion of the surfaces. Films also form on marine structures coated with antifouling paints although the characteristics and biological components of the film can be influenced by the paint formulation (Woods et al., 1987). Golab and Orlowska (1988b) report high efficiency of copper metal leaching, by tartaric and citric acids produced by the fungus *Aspergillus niger*. Jolley et al. (1989) note biocorrosion of copper by several polymers, including bacterial culture supernatant.

I.3.4 COPPER AS AN ANTIFOULANT

There is widespread use of copper against biofouling in aquatic environments and biodeterioration in terrestrial environments. Major collections of papers on these topics are found in symposium collections such as "Biodeterioration 7", papers from the 7th International Biodeterioration Symposium held at Cambridge, U.K. (Houghton et al., 1988) or the "6th International Congress on Marine Corrosion and Fouling" held in Athens, Greece in 1984.

In evaluating the effect of copper on biofouling organisms it is important to know the amount of metal necessary to eliminate the organism, the effect of the environment on metal bioavailability and, of utmost importance, the dissolution rate of copper. Giudice et al. (1984a) report that the dissolution rate of cuprous oxide in artificial sea water is directly proportional to temperture and chloride ion concentration and inversely proportional to pH. The authors (Giudice et al., 1984b) also report that leaching rate of cuprous oxide is inversely related to particle size of the metal. However, interaction of the copper with biofouling agents and water affects the leaching rate. Lindner (1988) points out (abstract) that "... a slime layer develops on the coating surface and traps the dissolved copper ions. These ions react with the chloride and hydroxide ions in the seawater to form less soluble complexes which precipitate on the surface in a dense bluish-green layer underneath the slime layer. The leaching rate decreases during the exposure because of the accumulated insoluble green copper compounds which block the dissolution of the red Cu_2O". Evans (1988) discusses the sequence of biofouling, commenting on the bacterial biofilm which appears to be a prerequisite to development of marine fouling communities. Chamberlain and Garner (1988b) discuss microbial fouling and corrosion of 90/10 copper-nickel alloys. They comment on the passivating corrosion film which first develops, followed by copper-tolerant bacteria and diatoms which stabilize the corrosion products and act as a second barrier to ion release. This then allows colonization by less tolerant organisms. Chamberlain et al. (1988b) examine the potential role of sulfate-reducing bacteria on corrosion of CA 706, a 90/10 copper-nickel alloy. Techniques for examining corrosion in seawater and characterising biofilms have been developed by Chamberlain et al. (1988a) and Castle et al. (1988; INCRA-support).

I.3.5 COPPER IN DENTAL PRACTICES

Plaque bacteria can produce acids that initiate the dental caries process. Copper salts are reported to inhibit acid production (Eisenberg et al., 1989). Copper (as a citrate) has been used in a prophylaxis paste to reduce plaque. Moore et al. (1989b) report a significant ($p < 0.001$) decrease in plaque, at least over a 48-hour period after application. The combination of chlorhexidine gluconate and cupric acetate has been reported to exert a synergistic growth inhibitory effect on oral bacteria associated with gingivitis (Drake and Waerhaug, 1989). Copper has been used in amalgams for a considerable period of time with the first commercial high copper alloy developed in the mid 1960's (from Osborne et al., 1989). Although there are short-term losses of restorations (Osborne et al., 1989), Laswell et al. (1989) note that copper amalgam restorations have a 34-year half-life. In fixed prosthetic construction, Lappalainen et al. (1989) report release of metal (Zn, Cu, Ag) from solders and suggest reduction in the use of soldered joints.

In an evaluation of the effect of copper-based crowns on dog dental tissue, Hao and Lemons (1989) report more severe tissue (gingiva) reactions with copper-based alloys than gold-based alloys. Similar results are reported by Lemons et al. (1989). Using a high-copper amalgam (ANA 2000^R), Torstenson and Brannstrom (1989) found no obvious damaging effect to the tooth pulp except, possibly in very deep cavities or when the amalgam is placed directly on the pulp. Even then, they suggest the reaction might be attributed to the content of zinc. Biocompatibility of copper dental alloys is discussed briefly by Bumgardner et al. (1989a,b,c), in the abstract of a talk. They point out that several parameters must be tested before a material is considered to be biocompatible. Craig and Hanks (1988) found casting alloys containing 50-60% copper (by weight) were not biocompatible with cultured fibroblast cells.

I.3.6 COPPER IN CONTRACEPTIVE DEVICES

Copper, as either the cupric or cuprous ion, has been demonstrated to be toxic to spermatozoa (Holland and White, 1988). As a result, copper-containing agents have been used as intrauterine devices and, more recently, as implants in the male reproductive tract (Skandhan, 1988). There is widespread use of copper-free and copper-containing intrauterine devices (IUD) with a variety of device types available. Baveja et al. (1989) provides results from a clinical trial with four types of devices. They comment that the CuT 200 device which is now used in the National Programme in India appears suitable for continued use. A number of other recent studies have examined and compared the effects of various intrauterine devices (Apelo et al., 1985, 1989; Champion et al., 1988; Chi et al., 1989a; Wilson, 1989b; Wright and Aisien, 1989) and new types of devices continue to be developed (e.g. Wildemeersch et al., 1988). Van Os and Edelman (1988) review the 15 years of experience with the Multiload IUD which has an exposed copper surface area of 250 mm^2. They present some of the problems as well as the benefits, commenting (abstract) that "On balance, the benefits of IUD usage far exceed the associated risks".

The risks of IUD usage include failure of the device to always prevent pregnancies, damage to the female reproductive tract and the concern about potential loss of fertility once the device has been removed. Skjeldestad et al. (1988) note that women who become pregnant with an IUD in place have a greater chance for spontaneous abortion even if the IUD is removed during the first trimester of pregnancy. IUD use can cause damage to or perforation of the lining of the uterus (Glew and Singh, 1989; Kiilholma et al., 1990; Sheppard and Bonnar, 1987) with the need for IUD removal. Laceration of the cervix can rarely occur at IUD insertion (Chi et al., 1989b) and misplacement of an IUD can cause perforation of adjacent organs (Kiilholma et al., 1989). Breakage of the IUD can occur, rarely, with localized effects until removal or expulsion of the device (Blaauwhoe and Goldstuck, 1988). Pelvic inflammatory disease is higher among IUD users (e.g. Kleinman et al., 1989). Menstrual bleeding is also reported to be increased with IUD use (Pedron, 1988) although post-partum bleeding is not affected by post-partum IUD insertion (Pedron et al., 1987). Chao (1988) comments (page 551) "... that Cu-medicated IUD users showed lower rates of removal due to side effects and pregnancy" (than did non-medicated IUD users). With regard to potential loss of fertility once the device is removed, Skjeldestad and Bratt (1987, 1988) present evidence that the use of copper-containing IUDs does not affect subsequent fertility. Wilson (1989a) obtained similar evidence. Release of copper ions from copper-containing intrauterine devices is considered to be a potential problem, with possible localized tissue injury (Patai et al., 1989a,b,c). Badrawi et al. (1988), however, note return of the uterine lining tissue to normal within a month after IUD removal. Kleinman et al. (1989) discuss the effect of copper on inhibiting the growth of *Chlamydia trachomatis*, a common sexually-transmitted organism that infects the reproductive tract and can cause pelvic inflammatory disease. They note that (abstract) "... it is possible that copper ions released from the copper-containing IUD may partialy protect against chlamydial infection".

1.3.7 PHYSIOLOGICAL EFFECTS OF COPPER

Deficient and excess copper can affect the well-being of all organisms. Although the effects can be due to the interaction of copper with organics, the interaction with the cell, or some other interaction, all effects will ultimately have some impact on the physiology of the organism. That means that copper has some effect on the functions and activities of the organism. Many of these are discussed in articles presented in "Copper in Animals and Man" edited by McC. Howell and Gawthorne (1987). The nature of physiological effects from copper deficiency are numerous and include anemia (e.g. Nicola, 1989), immunity and infection (Chandra, 1990; Cousins, 1989), loss of organ function (e.g. Ribera and Tauler, 1988) and bone disorders (e.g. Hintz and Schryver, 1987). Many of these have been discussed earlier in this review. Some of them will be mentioned in this section along with discussion of the effects of excess biologically available copper.

Cardiovascular System

Severe copper deficiency can produce myocardial and vascular lesions as well as hypercholesterolemia and cardiac enlargement in experimental animals (Burns et al., 1989; Johnson and Saari, 1989; Lawrence and Farquharson, 1988; Virtamo and Huttunen, 1988). However, antioxidants may be able to reduce the cardiovascular effects of dietary copper deficiency (Saari and Johnson, 1989; Saari et al., 1990). Work on microvascular effects of deficiency indicates marked alterations of the regulatory mechanisms governing inflammation and thrombosis (Schuschke et al., 1989a,b). Certain metals (Fe, Cu, Mn) are also associated with restricted blood flow (ischemia; Yoon et al., 1989) as is dietary fat (e.g. Lynch and Strain, 1989). (In contrast to the effects of deficiency, Patai and Balogh (1988) report copper chloride-induced lesions in isolated rat hearts.) Molteni et al. (1988) found that serum copper concentration could be used as an index of cardiopulmonary injury in monocrataline-treated rats. Copper deficiency is believed to be an important factor in lipid metabolism (Johnson et al., 1989c; Lee and Koo, 1989; Quehenberger et al., 1988; Ray et al., 1989). It is also considered as a possible factor in ischemic heart disease (Klevay, 1989), possibly due to lipid and fatty acid changes occurring with low dietary copper (Cunnane et al., 1988; Medeiros et al., 1986). Allen and Allen (1989) found an association of rat aorta endothelium damage with marginal copper intake which they suggest could be enhanced with hypercholesterolemia. There is also an association between zinc and copper as indicated by Chang et al. (1988) for rabbits with coronary atherosclerosis lesions. Konovalova et al. (1989) advocate the use of coper-containing preparations to improve antioxidative enzyme activity in the cardiovascular system. Hennig and Stuart (1988), commenting on nutrients that may protect against atherosclerotic lesion formation, suggest that copper is one of a number of dietary elements that must be considered. They comment (page 18) that "The key issue in the prevention or reduction of vessel wall injury is maintaining an adequate tissue concentration of those vitamins, minerals and nutrients essential to membrane integrity". In a review of trace elements in the cardiovascular patient, Liu and Chen (1987) note that, with a number of other metals, copper may have some potential for the treatment or prevention of cardiovascular diseases. Cholesterol metabolism may also be affected by trace elements such as copper (Meissner and Klemm, 1987). Since copper metabolism may be affected by such things as hypertension (Garrow et al., 1989), the physiology of the organism must be considered in any treatment routine. This is suggested by the decreased level of cardiac antioxidants reported for endurance-trained rats (Kihlstrom et al., 1989), A number of other factors also affect copper metabolism (e.g. Anderson, 1990; Brandao-Neto et al., 1988a,b; Takeuchi, 1990).

In evaluating the relationship between copper deficiency and blood cholesterol and enzymic antioxidant defence mechanisms, Carville and Strain (1988) found sex differentiated effects. Only copper deficient males showed increased serum cholesterol levels when compared with controls. (Nielsen, 1989, notes a sex effect on signs of copper deficiency but this can be moderated by the amino acid composition of the diet and physiological strain.) In male weanling rats, Mehta et al. (1989d) found that total plasma cholesterol significantly decreased as the dietary copper levels increased, at a constant level of zinc. However, the clearance of low density lipoproteins is faster in copper-deficient than in copper-adequate rats (Koo et al., 1989). Glycolipid transfer protein facilitates the transfer of glycolipids between lipid bilayers; copper sulfate has been shown to enhance glycolipid transfer protein oxidation (Abe and Sasaki, 1989).

Enzymes

Copper plays a key role in the structure and function of certain proteins (e.g. Bogorad, 1987; Vuillaume et al., 1989), including enzymes (Esaka et al., 1988; Hubbard et al., 1989; Platen and Kutzner, 1987). One of the more well-known enzymes (or enzyme "groups") is cytochrome oxidase, with its multiple copper centers (Beinert, 1988; Chan, 1988b). A great deal of work has been done on Cu- Zn-superoxide dismutase (SOD). SOD concentration and activity varies as a result of changes in cell nature and organism physiology (e.g. Coffey, 1988; Dameron and Harris, 1987; David and DiSilvestro, 1989; DiSilvestro and Marten, 1989; Fuchs et al., 1987; Herbert et al., 1990; Kinoshita et al., 1988; Konstantinova and Russanov, 1988; Mussalo-Rauhamaa et al., 1988; Oka et al., 1989; Paoletti and Mocali, 1988; Saito et al., 1989; Taniguchi et al., 1988; Thomas, 1987). SOD is a unique copper-containing enzyme found in tissues of aerobic organisms (e.g. Ohkuma et al., 1987), which catalyzes dismutation of O_2^- to H_2O_2 (Czapski and Goldstein, 1988; Kelner et al., 1989; Phillips et al., 1989). Although SOD is unique, analogues have been proposed and examined (e.g. Ischiropoulos et al.; Luo et al., 1987, 1988; Obrenovic and Spasic, 1988; Shen et al., 1987). SOD plays an important role in inflammation (see review by Garrett and Whitehouse, 1987) and, as such, is considered an important factor in organism defence (e.g. Lee et al., 1988). SOD-activating drugs (e.g. Sorenson, 1988b) have been used against inflammation for diseases such as rheumatoid arthritis (Peretz et al., 1987b) and Crohn's disease (Emerit et al., 1989). Foye and Ghosh (1988a) describe copper complexes which they suggest mimic SOD in the treatment of radiation. Loven (1987) reviews the use of copper complexes with SOD activity for cancer therapy. Mechanisms of action of SOD and SOD-mimetic compounds form an important research aspect (e.g. Harrison, 1987). Excess CuZnSOD has a detrimental effect which may produce premature aging and has been related to characteristics found in Down's syndrome (Elroy-Stein et al., 1987; Schickler et al., 1989; Schwaiger et al., 1989; Yarom et al., 1988).

Plasma antioxidant activity is reportedly due to the ferroxidase activity of the copper transport protein ceruloplasmin (McGahan et al., 1989a). McGahan (1990) reports that copper and aspirin increase the antioxident activity of plasma although it is not simply due to an increase in ceruloplasmin (or SOD). The observation that serum copper levels tend to increase with many physiological events such as pregnancy (e.g. Lewinsohn et al., 1988) and disorders such as cancer (Coates et al., 1989) suggests that the body is responding with copper-containing agents such as ceruloplasmin. Saito (1990), for example, reports higher ceruloplasmin levels in the advanced stages of stomach cancer. The normal equilibrium between various copper-containing enzymes and transport proteins is disturbed by disease, especially those such as Wilson's disease which affect copper storage (e.g. Evans et al., 189). Inadequate copper status can affect the equilibrium although it is often difficult to separate cause from effect (e.g. Reeves et al., 1989).

For copper-requiring proteins such as enzymes, activity is dependent on an adequate supply of the metal (e.g. Mahmood et al., 1987). Thus, α-lactalbumin has several Cu^{2+} per molecule (Permyakov et al., 1988) and forms one of the two components of the lactose synthase enzyme system. However, since copper can interact with proteins to affect both structure and function, excess amounts can be detrimental, affecting the structure and function of the wrong proteins. Thus the detrimental effect of excess copper on photosynthesis (e.g. Gangwar et al., 1989; Mohanty et al., 1989) or protein metabolism (e.g. Venkataramana and Radhakrishnaiah, 1987) is an effect on one or more proteins associated with the process. The activity of a number of enzymes can be inhibited by excess copper (e.g. Cook and Ternai, 1989; Goh and White, 1988; Hasinoff et al., 1989; Iyengar and Mendiratta, 1988; Kennedy and Gonsalves, 1989; Kondakova et al., 1988; Laycock et al., 1989; Leblova and Zatloukalova, 1988; Leblova and Tichy, 1989; Memon et al., 1987; Mizrahi and Achituv, 1989;

Morton, 1989; Speizer et al., 1989). Although the concentration of copper is an important consideration in evaluating copper inhibition, the effect will occur more readily at levels higher than normally found than under realistic levels (e.g. Serafini et al., 1989). It is also important to recognize that ion-metal and metal-metal interactions may affect not only the nature of organics and physiological activity (e.g. Pastor et al., 1989; Sandborg and Smolen, 1989) but also inhibition of enzyme activity. Ginalska et al. (1989), for example, report that the activity of an enzyme (endopolygalacturonase) immobilized by NH_2 groups could be increased by Cu, Mg or Cd ions. Tichy and Leblova (1989) discusses demetallization of zinc-containing maize alcohol dehydrogenase with subsequent reactivation by one of a series of metals, including copper. Minagawa and Zumft (1988) note the nature of a cadmium-copper antagonism in the activation of nitrous oxide reductase in copper deficient cells of the bacterium *Pseudomonas stutzeri.*

Copper and hormones

Copper can enter into the production and action of hormones hormone-like agents. Barnea and Bhasker (1989), for example, use copper to amplify the production of prostaglandin E_2 under laboratory conditions. The biological activity of thymulin, a thymic hormone, is zinc-dependent although copper can enhance its activity (Laussac et al., 1989). Copper inhibition has been recently noted, of histamine release (Sharma and Jande, 1989) and estradiol hydorxylation (Jellinck and Newcombe, 1988). Copper can also affect processes that may be related to hormone production, processes such as the regulation of neuronal excitability (Kardos et al., 1989b) or the modulation of peptide (luteinizing hormone releasing hormone) release in the brain (Barnea, 1987). There is evidence that hormone receptors are metal-binding proteins (e.g. Medici et al., 1989; Suzuki et al., 1988c)

Copper and cells

It is difficult, and perhaps incorrect, to isolate cells from processes since cells are packages of enzymes and other proteins and form sites for much of the metabolic activity of the body. Copper metabolism within the cell is also difficult to evaluate because of the changing nature of the cell, the effect of external agents (e.g. hormones), transport proteins (e.g. Lau et al., 1989; Rauch and Wells, 1989) and agents such as tetrathiomolybdates (e.g. McArdle et al., 1989b). However, there are some aspects of copper importance and effect which can best be discussed in terms of the cell. Excess ionic copper can, for example, cause lysis of vertebrate red blood cells (Smith et al., 1988), possibly a result of the oxidative effect of copper on the cell membrane (Bochev and Ribarov, 1987). Sokol et al. (1989), however suggest otherwise, that the effect is on certain cellular proteins rather than the cell membrane. Copper-catalyzed peroxidation can attack collagen, a component of connective tissue, and change its structure and functional capability (Kim, 1987). Copper uptake by cells has been related to agents such as metallothionein which act as metal-transporting organics (Rauch and wells, 1989). Palida et al. (1989), however, discuss the possible role of intracellular proteins other than metallothionein, in copper uptake. The interaction of copper with organics within the cell can be critical to survival, as in the case of copper-containing enzymes. But it can also cause toxicity, as in the case of the metal-mediated (Cu or Fe) toxicity of the herbicide paraquat to bacteria (Kohen and Chevion, 1988). The interaction of copper (as $CuSO_4$) with paraquat or ultracide (another herbicide) is reported to affect the cytochrome levels in carp liver cells (Simon et al., 1984). Copper can also interact with DNA within the cell, as shown by the isotopic labeling study of Grisvard et al. (1989).

The ability to enter into the cell and react with organics within the cell can be either detrimental or beneficial. Copper is often discussed as a carcinogen (see Lin and Solodar, 1988; Merian et al., 1988) but it is also widely used in medicine. As Sorenson (1990) states (abstract), "...

the pharmacological use of copper complexes represents a physiological approach to treatment of arthritic diseases, ulcers, and pain". Drugs such as copper(II)$_2$ (3,5-diisopropylsalicylate)$_4$ or "Cu-DIPS" is an example of a drug used to minimize cell/organism immunodeficiency (Soderberg et al., 1989a). Morazzoni et al. (1988) provide some evidence that copper in anthracycline antitumour drugs reduces their cardiotoxic effects. A variety of organics, such as some halogenated hydrocarbons, can affect the distribution of copper within cells (e.g. Wahba et al., 1988). Finally, the response of cells/organisms can vary depending on copper status, copper-sufficient and copper-deficient mice respond differently to adriamycin, a strong antibiotic used in chemotherapy (Zidenberg-Cherr et al., 1989). Bogush et al. (1989) found reduced adriamycin toxicity if presented with ionic copper, apparently a result of reduced toxicity of the copper complex.

Copper-storage diseases

In a review on the genetics of copper metabolism in animals and man, Wiener (1987) states (page 55) that "It is clear ... that heredity profoundly affects copper metabolism in a number of species". Although other factors can also affect metal metabolism to varying degrees (e.g. Aaseth et al., 1990), the tendency towards atypical copper metabolism is frequently an inherited tendency although physiological conditions are important in any explanation (e.g. Barrow et al., 1989). Recent reviews of metal-storage diseases include Cossack and Gutteridge (1987), Danks (1990), Denis et al. (1987), Hatta et al. (1985), Matsuoka and Okumura (1985) and Seymour (1987). In a short article (in Polish), Skalski (1986) comments (translation) that " 'kinky hair syndrome' (is a) genetically inherited disease, which is recessive. Wilson's disease is the second, main disease caused by perturbations of copper metabolism". Thornburg (1988), in a review of canine hepatobiliary diseases, reviews copper and liver disease with a discussion of the genetic basis for inheritence of abnormal copper metabolism.

In copper-storage diseases and other forms of copper overload, the distribution of copper between various ligands in normal and abnormal livers as well as blood, provides an indication of the mechanisms for copper retention (e.g. Elmes et al., 1989) or retention failure. Changes in lipid peroxidation can occur with copper overload (Sokol et al., 1988). When compared with ultrastructural changes in the cells of the liver of affected animals (Fuentealba and Haywood, 1988; Fuentealba et al., 1989b), these factors provide a "profile" of abnormal conditions (Sawa and Okita, 1990).

Sheep and sheep-like animals can exhibit what is termed "chronic copper poisoning" (reviewed in Salyi and Banhidi, 1989), an inability to excrete excess dietary copper (e.g. Wu, 1988a). Ionophores such as monensin can increase the accumulation of copper in the liver of sheep (Calhoun et al., 1987). In ruminants in general, the metabolism of copper is linked to that of molybdenum and sulfur and the toxic condition often occurs as a result of an imbalance between these three (Junge and Thornburg, 1989) as well as the inherited tendency towards reduced metabolic capability to handle copper. (Tungstates have also been shown to affect copper metabolism in sheep (Mason et al., 1989a).) Certain breeds of dogs exhibit a tendency towards chronic hepatitis and copper accumulation in the liver (Eriksson and Peura, 1989; Haywood et al., 1988).

Of the copper-storage diseases in man, Menkes' kinky hair syndrome and Wilson's disease are the most well known. Danks (1987) comments that copper deficiency is (page 29) "... seen most dramatically in Menkes' disease, in which severe deficiency persists for a long period of time". Kolb and Guthoff (1987) discuss clinical aspects of this X-linked recessive disease, pointing out the difficulties of early diagnosis. Gautier et al. (1988) and Anton Jiminez et al. (1989) discuss characteristics of the disease in terms of new patients. The mottled mouse and the macular mouse are used as models of Menkes' disease (e.g. Katsura et al., 1987). Kondoh et al. (1988) examined metallothionein and copper levels in the macular mouse. Using the mottled mouse, Kasama and Tanaka (1988) report that a combination of prenatal copper supplementation and intraperitoneal copper injections after birth can be used to minimize the effects of copper deficiency. Maternal administration of zinc, vitamin E and copper are also reported to be beneficial in fetal and neonatal mice (Tanaka et al., 1990a). Yamano et al. (1988) and Kawasaki et al. (1988) used injections of cupric chloride into neonatal macular mice to reduce the effect of copper deficiency on the brain. Nadal and Baerlocher (1988) discuss the use of copper-histidine and D-penicillamine to treat a mild form of Menke's disease in an 8.5 year-old male child. Copper histidinate and cuprous sebacate treatment have also been used (Sherwood et al., 1989; Williams et al., 1987).

Wilson's disease "... is an inborn error of copper metabolism that is associated with cirrhosis of the liver and degenerative changes in the basal ganglia" (Menkes, 1989, page 85). Diagnosis can be difficult because of certain similarities exhibited by other diseases (e.g. Geubel et al., 1988). There is, as expected, inadequate knowledge on the genetics of the disease (e.g. Bowcock et al., 1988). Dening et al. (1988) discuss the relationship between Wilson's disease and epilepsy, which frequently occurs in patients with the disease. They suggest that the seizures are more likely a direct effect of copper deposition than to penicillamine which is used to treat Wilson's disease patients. Friedman and Pearce (1989) suggest the use of lysinoalanine instead of penicillamine because of the side effects of the latter. Recent work suggests that the copper profiles of neonatal mammals are similar to those found in people with Wilson's disease (Bingle et al., 1988; Chesta et al., 1989). As a result, the guinea pig neonate has been used as a model to examine certain aspects of the disease. Copper, as ^{64}Cu, is used both to diagnose and monitor the treatment of Wilson's disease (Archambaud et al., 1988; Van den Hamer et al., 1990; Warwick, 1988a). Disease treatment includes the use of D-penicillamine as well as oral zinc (Brewer et al., 1990; Gretter, 1989; Horoupian et al., 1988; Maurer et al., 1988; Milanino et al., 1989b; Rossaro et al., 1987). Van Thiel et al. (1987) report on the success of liver transplantation for Wilson's disease noting that it also resolves "all the biochemical parameters characteristic ..." of the disease. Brewer and Yuzbasiyan-Gurkan (1989) discuss various means of treating Wilson's disease.

Indian childhood cirrhosis "... is a lethal liver disease characterized by childhood onset and massive hepatic accumulation of copper ..." (Adamson et al., 1989b). The authors further comment that "It is not known whether ICC results from environmental copper toxicity or is a genetic defect". As with other copper-storage diseases, the nature and cause of ICC is not adequately understood (e.g. Gahl et al., 1988; Maggiore et al., 1987). Tanner and Mattocks (1987) note (summary) that "Plant and fungal biocidal agents may be hepatotoxic, may increase hepatic copper concentration, and may be secreted in milk of lactating animals". They hypothesize that ICC is caused by the interaction of copper with metabolic byproducts of certain microorganisms found as contaminants of animal feeds in rural India. The copper may come via bovine milk, from brass vessels (O'Neill and Tanner, 1989).

I.3.8 THE INTERACTION OF COPPER WITH BIOLOGICALLY IMPORTANT ORGANICS

Organics are the primary mechanisms through which copper acts on organisms. They bind with copper to affect metal bioavailability in the environment, they appear to be intimately linked with the uptake of metal into the organism, and they form organometallic complexes within the organism (Mazzucotelli et al., 1988; Mikaelyan and Nalbandyan, 1988; Mikaelyan et al., 1988) that can either be beneficial or detrimental. A great deal of information has been accumulated on organometallic compounds like enzymes (Liebman and Greenberg, 1988), that are of importance to humans. These include compounds involved in metal ion homeostasis (e.g. Hamer and Winge, 1989) as well as the effect of excess copper on essential organics (e.g. Wui and Lee, 1987) or organometallics on organs (e.g. Toyokuni et al., 1989) and metabolism (e.g. Morris et al., 1989a). In terms of beneficial associations, copper complexes are widely used to treat chronic diseases (Sorenson, 1989d) and copper-containing secondary metabolites such as bleomycins occupy an important place in tumour chemotherapy (Zähner et al., 1987). Because copper will react with many organics, recent work also includes discussions of copper in the control of fermentation (e.g. Wang et al., 1988a) and copper as a contaminant in commercial chemicals (e.g. Lund et al., 1988) and its involvement in the formation of unwanted agents in industrial processes like brewing (Riffkin et al., 1989a,b).

Information on copper suggests that the metal can either enhance or inhibit degradation of organic material (e.g. Brynhildsen and Rosswall, 1989; Chin and Jubian, 1989; Jardim and Campos, 1988). Metal-organic ligand interaction has been used in the modelling of copper complexation in seawater (Hirose, 1988). Since organisms produce organic material, complexing capacity can be associated with growth and productivity (e.g. Wangersky et al., 1989). As a result of metal-organic interactions, the uptake of copper from the environment is not always related directly to metal concentration (e.g. Schnabel and Bunke, 1987). It is often affected by organics as well as the interactions with other metals (e.g. Gawthorne, 1987; Iwasaki and Takahashi, 1989; Jenkins, 1987). Residence time of copper in an environment can also be controlled by organisms, through uptake and subsequent release or sedimentation, the latter in aquatic environments (Lin and Li, 1988; Wangersky et al., 1989).

Literature covered in this subsection concerns major groups of organics that either affect metal bioavailability, in the environment, or affect the organism, within the organism.

Organics in blood, serum and cells found in the blood and lymphatic tissue

One of the more intensively studied copper-containing organics is the respiratory protein haemocyanin found in the haemolymph of some molluscs and arthropods (Senozan et al., 1988). As Depledge and Bjerregaard (1989) point out, haemocyanin accounts for more than 60% of the haemolymph and varies with the physiological condition of the organism. Mazur and Gondko (1988) review the structure and spectroscopic properties of haemocyanins.

Changes in human and rat plasma copper levels have been related to plasma variation in free fatty acids and albumin (Brandao-Neto et al., 1989b). The effect of external agents such as polychlorinated biphenyls (Katayama et al., 1989; Kato et al., 1988) or physiological changes can affect the requirements for copper as well as the levels of copper and ceruloplasmin. Copper in mouse hepatocytes is reported to occur in two different "pools", an easily accesible one and a residual pool (McArdle et al., 1989a). Interactions of copper with blood serum analytes is known to occur (Abu-Farsakh et al., 1989; Chikvaidze, 1988). Separation of proteins from vertebrate blood has allowed identifification and description of copper-transport proteins such as ceruloplasmin and transferrin (Al-Mashikhi and Nakai, 1988; Hewitt and Day, 1987). However, there is very little known about the dynamics of the transfer, poor correlation between plasma copper and copper-containing enzymes often being the case (e.g. Solaiman et al., 1989). Transfer of copper from ceruloplasmin to superoxide dismutase has also been examined (e.g. Percival and Harris, 1989; Percival et al., 1989). Goode et al. (1988) suggest that, in the human and rat, blood plasma contains ceruloplasmin species of variable copper content. Ceruloplasmin is one of several enzymes which mediate the reduction of oxygen, apparently as a result of a trinuclear copper cluster, at least in mammalian ceruloplasmin (Calabrese et al., 1989a). Copper and ceruloplasmin are able to affect the growth and function of lymphocytes and macrophages under certain conditions (Elliott et al., 1987; Lipsky, 1987) which can be of benefit in the treatment of diseases causing inflammation.

Organics involved in metal homeostasis

A wide variety of organics can bind copper, ranging from amino acids to nicotianamine (Anderegg and Ripperger, 1989), ovalbumin (Verma et al., 1989) and phosvitin (Kozlowski et al., 1988). In some cases any benefit to the organism seems almost spurious, in others the organic may act as a carrier for the metal ion (e.g. nicotianamine in plants; Anderegg and Ripperger, 1989). A number of organisms are reported to release copper-complexing organics in response to increases in metal. These include bacteria (Gordon, 1989a; Murgel et al., 1989; Schreiber et al., 1990), plants (Morelli et al., 1989; Vieira and Nascimento, 1988) and animals (e.g. Richards, 1989d). Some of these metabolites are released in response to the stress itself (e.g. Tahara et al., 1988), others in response to the metal. Tahara et al. (1988) describe a fungitoxin inducibly produced by dandelion leaves treated with cupric chloride, an organic that does not bind copper.

In I.C.A.-supported work, Jones et al. (1986) describe the immobilization of copper by slime films from marine fouling bacteria and algae. They report, however, that the films slough off revealing clean, non-fouled areas. "Exopolymers which anchor sessile bacteria to metallic surfaces exhibit the capacity to bind copper ions with high affinity" (Geesey et al., 1988; an I.C.A.-assisted study). Some plant root exudates have the ability to bind copper and other metals (Guckert and Morel, 1988; Mench et al., 1988; Morel et al., 1987), affecting metal speciation in the soil as well as metal uptake by the plant. Many plants produce one or more metal-binding peptides (e.g. Nishizono et al., 1989b). Rauser comments that (abstract) "In roots of young plants up to half of the metal is bound by phytochelatins", one group of these peptides. Mehra et al. (1988) reports metal-specific synthesis of two metallothioneins (cysteine-rich polypeptides) and γ-glutamyl peptides by a fungus (*Candida glabrata*). In a review of phytochelatins in plants, Grill (1989) notes that (abstract) they "... are ubiquitous in plants from algae to orchids". Gekeler et al. (1989) comment that (abstract) "The ability to form phytochelatins for metal homeostasis and metal detoxification is a principal feature of plant metabolism". The production and nature of phytochelatin-like organics is discussed by Grill et al. (1988), Salt et al. (1989) and Verkleij et al. (1989).

"Metallothionein is a copper- and zinc-binding protein present in most, if not all, tissues of higher eukaryotic species of animals" (abstract, Richards, 1989d). It, or similar compounds, are also found in some fungi and plants (e.g. Beltramini et al., 1989; Tanaka et al., 1990b). (Andersen et al. (1988) note that at least four species of coelenterates lack metallothionein or other metal-binding proteins.) Metal-binding proteins are reported from two polychaete worms (Eriksen et al., 1988) and the mussel *Mytilus galloprovincialis* (Viarengo et al., 1989). Roesijadi and Morris (1988; see also Roesijadi, 1987, 1988) discuss an assay for metal-binding proteins in another mussel (*M. edulis*). Metallothionein-like agents have also recently been reported for the fruit fly *Drosophila melanogaster* (Erraiss et al., 1989), the terrestrial isopod *Porcellio scaber* (Dallinger and Prosi, 1988), fish (Misra et al., 1989; Overnell and McIntosh, 1988), chicks (McCormick and Fleet, 1989), horses (Koizumi et al., 1989), and humans (Drasch et al., 1989).

Engel and Brouwer (1989) present evidence that metallothionein (page 70) "... is a constitutive protein that is directly involved in the synthesis of copper and zinc metalloproteins at many levels in phylogeny, as well as a protein that is induced to high concentration by metal exposure". Metallothionein can act in the transport of copper within the organism and can also play an important role in metal homeostasis. Brouwer and Brouwer-Hoexum (1989) indicate a role of metallothionein in transferring copper to apohemocyanin. There is also evidence that metallothionein plays a role in inflammation, by regulating copper and zinc metabolism (Grider and Cousins, 1989). Wakiyama et al. (1987) found induction of metallothionein during experimental hepatic fibrosis and Krauter et al. (1989) note elevated copper-thionein concentration in melanoma tumor tissue. (50-60% of the total tissue copper was associated with metallothionein.) Deposition of copper granules can occur in the liver of patients with various liver abnormalities other than Wilson's disease and biliary diseases (Miyamura et al., 1988). The authors do not, however, discuss the possible link of deposition with the metabolism of copper-metallothionein.

Metallothionein-like organics are considered to play important roles in metal tolerance with tolerant organisms capable of increased production of metallothionein under metal stress. This may not be completely true, Schultz and Hutchinson (1988), for example, finding that non-tolerant clones of a grass species (*Deschampsia cespitosa*), in the presence of sulfur and excess copper, produced more thiol-rich proteins than tolerant clones. In contrast, work on laboratory rats strongly indicates a role for metallothionein in the sequestration and excretion of copper, at least in acutely-loaded animals (e.g. Sato et al., 1989; Williams et al., 1989). The roles played by metallothionein suggest that it could be used to monitor the health of organisms in terms of metal concentration and possibly effects (e.g. Kleczkowski, 1988a; Miller, 1988b). In an excellent piece of work, Engel (1988) examines some of the problems of doing this. Using three organisms - the oyster *Crassostrea virginica*, the blue crab *Callinectes sapidus* and the beaked whale *Mesoplodon europaeus* - Engel notes how natural environmental and physiological factors can affect the mobilization and partitioning of metals and the variability in metallothionein levels. These are discussed later in this review, in a discussion of bioassay organisms. Physiology and life style may affect metallothionein levels (e.g. Drasch et al., 1989). Food and food composition has been identified as a factor affecting metallothionein production (Engel, 1988), metal and metallothionein levels (Kleczkowski and Barej, 1989) and copper toxicity (Kumar et al., 1987).

The relationship between cells, cell metal levels and metallothionein is poorly understood. Laurin and Klasing (1989) report that chick macrophages may play a role in trace-element metabolism by accumulating large amounts of metallothionein with a high rate of turnover. Exposure of human hepatoma cells for ten hours to high concentrations of copper, zinc and cadmium induced synthesis of several proteins, including metallothioneins (Remondelli et al., 1989). Similar effects have been reported for copper-resistant hepatoma cells, by Freedman et al. (1988; see also Freedman and Peisach, 1989b) who comment (abstract) that "The increased synthesis of MT by Cu-resistant cells, the chelation of Cu by MT, and its sequestration by lysosomes, mimic the events that have been postulated for Cu detoxification and resistance in hepatocytes". Freedman et al. (1989a), however, suggest that copper is taken up by reduced glutathione first then transferred to metallothionein. There is also evidence that copper bound to metallothionein is not permanently bound but can be incorporated into other copper proteins such as superoxide dismutase (Freedman and Peisach, 1989a). In liver disease with copper retention (e.g. Wilson's disease), necrotic hepatocytes are reported to be metallothionein-rich (Elmes et al., 1989). Zinc treatment of certain hepatocells (and Wilson's disease) has been shown to be effective in treating copper overload, a situation which Schilsky et al. (1988) suggest is due to zinc-induced metallothionein which sequesters copper and reduces the apparent metal load.

The levels of metallothionein appear to be controlled by the amount of inducing agent and the genetic makeup of the organism. Farr and Hunt (1989) report genetic differences in zinc and copper induction of liver metallothionein in inbred strains of the mouse. In yeast, however, Fürst et al. (1988a,b) found that copper activates metallothionein gene transcription by altering the conformation of a specific DNA-binding protein (see also Skroch et al., 1990). Czaja et al. (1988) suggest that the molecular basis of copper resistance in copper-resistant hepatoma cells involves gene amplification of metallothionein genes.

Naturally-occurring organics

Major deposits of copper can be associated with degradation products of organic material (e.g. Mehrtens, 1986). These products, as well as existing environmental conditions, can affect metal leachability (e.g. Krueger et al., 1989) and bioavailability. Mathur (1983), for example, notes a bactericidal effect of copper in a mineral soil and a lack of effect in organic soils, a result of copper being bound by the organics.

Metal bioavailability, and the potential for metal uptake, can be increased by the release of certain metal-binding metabolites. These include agents like siderophores (Treeby et al., 1989). Similar agents can reduce the toxicity of copper (Kosakowska et al., 1988). The ambiguous nature of these organics is presumably due in part at least, to the stereochemistry of the compounds, and how it affects the rate of uptake. As well, different organics have different effects on the environmental geochemistry of copper. Dehnad and Förstner (1988) report decreased sorption of metal on sediment with NTA and EDTA but no change with glycine, citric acid and humic acid.

Bezborodov (1989) evaluated the interaction of trace metals with organic substances in ocean water, commenting that the average conditional stability constant for copper was $10^{8.12}$. Moffett et al. (1990) note that copper speciation was dominated by organic complexation in the Sargasso Sea. Mackey and Higgins (1988) provide an excellent discussion of the copper-complexing capacity of seawater, noting that it can vary widely, with high values found in areas of high phytoplankton biomass (see also Coale and Bruland, 1990). This is one reason why variation can also occur on a seasonal basis (Mackey and Szymczak, 1988). Sunda and Gessner (1989) present evidence of extracellular production of strong copper-complexing organics by fungi. Kozarac et al. (1989) examined the interaction of cadmium and copper with surface-active organic matter and complexing ligands released by a species of marine phytoplankton, the chlorophyte *Dunaliella tertiolecta.* They found a strong interaction of (abstract) "... excreted organic substances with copper in the bulk phase ...". A strong reaction was also found by Ogiwara and Kodaira (1989), from extracellular compounds produced by the marine alga *Microcystis aeruginosa.* They report changes in the nature of the exudates with different growth stages. In a series of papers, Xianliang Zhou and others examined the production of a copper-complexing organic during a diatom bloom (Zhou et al., 1989), the distribution of copper-complexing organic ligands in western North Atlantic waters (Zhou and Wangersky, 1989b), changes that occurred during a spring bloom in a coastal basin (Zhou and Wangersky, 1989a), and the isolation and preliminary characterization of copper-complexing agents in seawater (Zhou and Wangersky, 1989c).

A variety of techniques have been used to examine copper complexation in the environment. Morrison and Florence (1989) compare some of the techniques used in aquatic environments. Sun et al. (1988) used electroanalytical techniques to measure metal-complexing (Cu, Cd, Pb) capacities of estuarine waters. van den Berg et al. (1987) used cathodic stripping voltammetry to measure copper-complexing capacity in the Scheldt Estuary. An ion exchange resin technique is discussed by Liu and Ingle (1989a). One strongly-binding resin, Chelex 100®, has been used to estimate copper complexing capacity in estuarine waters (Nourredin et al., 1988a). Uchiyama et al. (1988) used ultrafiltration to obtain and provide a preliminary characterization of copper-complexing agents in a freshwater body. The nature and molecular size of metabolites and decomposition products of aquatic and terrestrial organisms is important in examining metal speciation. In an evaluation of dialysis as a size-based separation method in natural waters, Apte et al. (1989) found that up to 62% of the filterable copper was non-dialysable in estuarine water samples from South Wales.

"Humic substances are ubiquitous components of naturally occurring organic matter in all environments" (page 77 in Alberts et al., 1989). In aquatic environments they can occur in the "dissolved" (= filterable) or particulate state. In the latter, they can be strongly sorbed by hydrated iron oxides where they may be active in complexing copper (Hiraide et al., 1988). They play important roles in the chemical fate of anthropogenic metals (reviewed in Suffet and MacCarthy, 1989) and the availability of copper and other metals to organisms (e.g. Granéli et al., 1989). Soil organic material reacts with copper and other metals (e.g. Boluda Hernandez, 1988). As stated by Aplincourt et al. (p. 167), "Humic and fulvic acids play a significant role in the migration, the accumulation and the solubilization of metal cations in soils". Humic substances can also interact with other organics, such as glycine, affecting metal-catalyzed processes such as decarboxylation (Dolidze, 1988; Ugrekhelidze and Dolidze, 1987). In examining chemical interactions of any kind, biological action must also be considered both with humic precursors (e.g. Hedges et al., 1988) and humic substances. As would be expected, the intimate association of soil humics and copper affects metal bioavailability (Piccolo, 1989) to the organism although humic acids have little if any physiological effect as an organic chemical (e.g. Gaede et al., 1987). It seems likely that ingestion of humic-metal complexes could affect metal uptake since it can be affected by a number of organics associated with dietary fiber (Godron, 1989b; Katseva et al., 1988; Martin and Evans, 1988; Schlemmer, 1989). This is discussed in greater detail later in this review.

Copper and carbohydrates

The relationship between copper and carbohydrates is like a two-edged sword. On the one hand, copper can reportedly affect the content and composition of saccharides in oat plants (Slusarczyk and Ruszkowska, 1986). On the other, at least two simple sugars can affect copper uptake and action, at least in humans and laboratory rats. Fructose is able to alter many biochemical parameters and has been shown to worsen copper deficiency (Bhathena et al., 1988; Henderson and Johnson, 1989). With the rat, Koh et al. (1989) comment (abstract) that "... the fructose-copper interaction occurs either during intestinal digestion and absorption, hepatic uptake of copper via the portal blood or its hepatic utilization". Under copper deficiency, fructose and sucrose are reportedly more damaging to certain organs and/or physiological processes than starch (Beal et al., 1989; Fields, 1987; Lewis et al., 1989a). The effects of these two sugars do not modify all effects of copper deficiency; Scholfield et al. (1989) report that copper deficiency but not the nature of dietary carbohydrates affects the concentration of norepinephrine and epinephrine in rat cardiac tissue (see also Gross and Prohaska, 1989 and Seidel et al., 1989).

Miscellaneous

Ascorbic acid. Intake of excessive amounts of ascorbic acid may reduce copper levels in some laboratory animals (Tsao and Young, 1989). With copper deficiency, dietary ascorbic acid may reduce iron uptake and utilization (Johnson, 1989a). However, the oxidation of ascorbic acid can be enhanced by copper (Smith and Gore, 1988). Together, ascorbic acid and copper can affect the activity of organics such as soybean trypsin inhibitors (Sessa et al., 1989).

Nucleic acids. The repair of damaged nucleic acids can occur when exposed to ascorbic acid in the presence of a catalytic amount of copper (Yanada et al., 1989). In contrast, breakage of calf thymus DNA occurred with the flavonoid quercetin, in the presence of Cu(II) (Shahabuddin et al., 1989). Copper salts, with β-lactam antibiotics, have been shown to be effective in damaging DNA (Quinlan and Gutteridge, 1988a, 1989). Ionic copper is also reported to facilitate bleomycin-mediated unwinding of plasmid DNA (Levy and Hecht, 1988). The effects of ionizing radiation, on DNA damage, can be enhanced with copper (Tofigh and Frenkel, 1989). Yamamoto and Kawanishi (1989) present evidence suggesting that copper-induced damage to DNA is due to copper-peroxide complexes rather than the hydroxyl free radical. Francois et al. (1988) describe a technique for cleaving DNA, using an oligonucleotide in the presence of ionic copper and a reducing agent.

I.3.9 THE EFFECTS OF COPPER ON GROWTH

Copper affects growth in the same way it does any metabolic event, through involvement with organics in normal or abnormal metabolic processes. However, growth is a result of constructive metabolic processes exceeding destructive metabolic processes so the action of copper is in the provision of a required agent or, when in excess, an agent that will reduce the rate of the metabolic processes. And this is the case for all organisms, from bacteria to humans. This subsection looks at the relatively few papers that examine the real or potential requirements for and effects of copper on the growth of organisms.

For bacteria, copper is considered essential for the growth of most species. Bruyneel et al. (1989), however, report growth without copper and iron for several lactic acid bacteria. They used a chelating agent (2,2'-dipyridyl) to tie up the metal in the growth medium, a technique that may provide some metal through equilibrium exchange processes. Mineral nutrient deficiencies are reported to play a major role with nitrogen-fixing bacteria found in legumes. Nitrogen fixation may be specifically limited by low availability of Ca, Co, Cu and Fe, to bacteria in the nodules of the plants (O'Hara et al., 1988). Bacterial strains can be developed to grow on a variety of organic substrates, provided suitable organic and trace metal requirements are met. Auton and Anthony (1989), for example, found greater copper requirements of methylotroph bacteria when grown on methylamine than when grown on methanol. Bacterial growth can be limited by excess bologically available metal, including copper. Abbas and Edwards (1989) note an order of toxicity of Hg > Cd > Co > Zn > Ni > Cu > Cr > Mn for a range of soil bacteria (*Streptomyces* spp.).

Growth of algae and fungi can be affected by the concentration of biologically available copper, optimum growth being achieved with an optimum supply of metal. The equilibrium between available and non-available metal is affected by the concentration and nature of metal complexing agents. Nakashima (1988) notes that the lag phase in growth of a common marine phytoplankton species can be affected by metal complexing agents. The lag phase in deep water was much shorter when chelating agents or metabolites from the species were added. However, tolerance of organisms tends to change with growth. Gapochka et al. (1989), for example, note two distinct "stages of growth" in the green alga *Scenedesmus quadricauda*, with respect to tolerance of copper sulfate. Tolerance varies from species to species which can affect growth and abundance relative to other species (e.g. Sengar and Sharma, 1987), a situation which can explain changes in species composition under metal-enriched conditions (Korsak and Lifshits, 1989). The situation may not be a direct effect of metal concentration, however. Jones and Hutchinson (l985), working with growth of birch seedlings with and without fungal infection, note that infection caused an increased uptake of nickel but not of copper. In a similar study, with corn and soybean, Chao and Wang (1988) note that the effects of metal on infected corn plants depended on the type of metal but the effects on soybean growth seemed to be dependent on the effects of the fungal infection. In other words, there is a unique response of each species to stress. Some plant species have exceptional tolerance, capable of growth in natural or anthropogenic environments with extremes in pH and metal bioavailability (Albertano and Pinto, 1986). Similar comments can be made about changes in tissue metal concentrations as a result of stress. With the poplar, attack by leaf miner beetles causes a significant decrease in foliar K, Mo, Cu and Zn but only a slight decrease in Fe, Na and Mg.

Proper plant growth is dependent on the interaction of a suite of chemicals (e.g. San Valentin et al., 1986; Sangai, 1988). Copper supplementation is frequently used to correct for soil deficiencies in agronomic crops (Blue, 1988). Copper is also one of the soil chemical properties that can influence the plant availability of soil potassium (Yang and Skogley, 1989). As with algae and fungi, however, it is the nature of the plant that dictates the response to environmental conditions. Germination in saltmarsh cordgrass (*Spartina alterniflora*), is not affected by copper (at least up to 100 mg/L) but subsequent growth is affected (Waddell and Kraus, 1990). Copper has been used successfully in improving germination and/or growth in barley (Kuduk, 1986; Rashal et al., 1987), cotton (Dhopte, 1987), eucalyptus (Dell and Bywaters, 1989), rice (Sheudzhen and Rymar, 1984), winter wheat (Lasztity, 1988c; Linke, 1988), rye (Lasztity, 1988c) and triticale (Laszity, 1988c). Under some conditions, copper pesticide treatment improved growth of pepper infected with bacterial spot

(McCarter, 1989) and grape fungal-infected grape plants (Duso et al., 1987). Luchsinger (1987) found no benefit of supplementing corn with several microelements (zinc, copper, iron, manganese) and sulfur. The effect of excess copper on growth is discussed by Fabian and Dezsi-Devay (1987) for early developmental stages of winter wheat; Pusztai (1987) found no mutagenic effect of copper on barley seeds but presumes (abstract) "... that copper ion is an inhibitor for repair enzymes, ...". Luyindula (1988) examines growth of the bean *Phaseolus vulgaris* under metal deficiency. Additive and synergistic relationships exist between excess copper and sodium chloride (with a pH effect thrown in) on growth of this plant (Wallace, 1989a). Zinc-copper interactions affect growth and grain yield of rice (Gangwar et al., 1988) as well as a number of other plants. (Note that Taylor, 1989, cautions against the use of primary growth data and derived root weight index to detect possible interactions between phytotoxic metals.)

Requirements for and effects of copper change throughout the growth of a plant. In leaves of tamarillo trees (*Cyphomandra betacea*, a small tree that produces exotic fruit), Clark et al. (1989) note that the concentrations of copper declined within the first 10 weeks of growth and then remained constant until harvest. In the pistachio, Bobodzhanova et al. (1988) report that copper decreased in the seed and pericarp during maturation. With excess soil copper, plant emergence can be delayed and seedling growth can be affected (Mitchell et al., 1988). The effects of excess copper in reducing growth can, however, be put to good use. Arnold and Struve (1989a) used $CuCO_3$-treated plastic containers to inhibit (but not kill) root growth of green ash seedlings. They comment (page 264) that "... $CuCO_3$-treated containers can be used to control undesirable green ash root growth and produce large seedlings in small containers while still maintaining high root regeneration potential". They (Arnold and Struve, 1989b) also found this for red oak and Wenny and Woollen (1989) used the technique for containerized Douglas-fir, ponderosa pine and western white pine.

In animals, as in plants, the concentrations and effects of copper vary with the nature of the organism as well as its physiology and environment. With the clam *Macoma balthica*, Cain et al. (1987) note an effect of differential growth on spatial comparisons of copper content. Seasonal variations in tissue copper concentrations, in *M. balthica*, have been related to seasonal variations in soft tissue weight (Cain and Luoma, 1990). (Copper concentrations can vary in different parts of an organism (e.g. Liao and Hsieh, 1988).) With domestic animals, changes have been noted in dam and fetus tissue copper levels during pregnancy (Malinowska, 1986). There is often an increased need for copper during early development, as indicated by the beneficial effect of copper supplementation of the dam on reducing the incidence of cartilage lesions in foals (Knight et al., 1988). Evidence also exists for the beneficial effect of copper supplementation early in life, as shown by work with laboratory animals and humans (e.g. Lagercrantz, 1988; Lünnerdal, 1988; Orzali et al., 1987; Stevanovic and Sevkovic, 1987). (Similar information is also accumulating on the importance of copper for the elderly (Cousins, 1989).) Prohaska and Lukasewycz (1989) note a relationship between the severity of perinatal copper deficiency and the immune response of mice. (But note that antigen-antibody reactions can be affected by excess copper (Kolinkoeva et al., 1988).)

Excess copper has been associated with a decrease in lifespan of the rotifer *Asplanchna brightwelli*, possibly due to enhanced lipid peroxidation, especially in older organisms (Enesco et al., 1989b). With the polychaete worm *Hediste diversicolor*, added copper (5-100 µg/L) embryonic development is inhibited as is the motility of the larvae after hatching (Ozoh, 1986). Quite frequently it is the young stages that are affected more than the near adult or adult stages, as shown by Sullivan and Ritacco (1988) for copepod crustaceans, de Nicola Giudici and Migliore (1988) for an isopod crustacean, Sun and Chen (1987b) for a fish (the black porgy), and Berisha et al. (1987) for a frog. However, Bodar et al. (1989) report higher copper tolerance by the early life history stages, when compared with the later stages, of the crustacean *Daphnia magna*. Although Marchal-Ségault (1989) found no effect of a copper-sulfate containing fungicide (Bouillie bordelaise) on eggs of the fruit fly *Drosophila*, there was a dose-related post-hatching mortality. However, a similar fungicide has been found to be genotoxic; high concentrations in drinking water have been associated with abnormal sperm in mice (Rahiman et al., 1988). Cytotoxicity of certain copper-containing compounds can be useful, however, and has been shown to be beneficial in controlling abnormal cell growth, as for example with tumours (Torregrosa et al., 1987).

Resistance to excess copper during growth can be achieved in several ways. Some organisms are tolerant during their entire life cycle (e.g. Bonacina et al., 1987). The structure and chemical characteristics of the egg have been associated with increased survival (e.g. Bodar et al., 1989; Munkittrick and Dixon, 1989). At least in some laboratory animals, and possibly humans, there is a change in the metabolism of copper early in life (Bingle et al., 1987). Although uptake and effect of excess copper can occur either from metal in the medium or in food (e.g. Hatakeyama, 1989), Rao and Latheef (1989) report greater accumulation from the medium for the brine shrimp *Artemia salina*.

I.3.10 THE EFFECTS OF COPPER ON BEHAVIOUR

Changes in behaviour can be an expression of the physiological nature of the organism. Burrowing behaviour in the clam *Mya arenaria* can, for example, be reduced by relatively high levels of sediment copper (51.4 μg/g). Powell and White (1989) report that in two species of barnacles, exposure to excess copper (80 ppb or more) caused a reduction in the rate of movement of the feeding appendages. Changes in the swimming pattern of fish have also been related to added copper. Al-Akel (1987) notes that exposure to 500 or more μg/L copper causes a loss of schooling behaviour and irregularity in movement of the cichlid *Oreochromis niloticus.* Ellgaard and Guillot (1988) noted a reduced locomotor response with exposure of bluegill sunfish to 40 ppb or more copper. Steele (1989) notes hyperactivity and a loss of normal diel activity in the sea catfish (*Arius felis*) immediately after exposure to 0.1 and 0.2 mg/L copper. Hartwell et al. (1988) found avoidance by schools of fathead minnows, of a blend of metals (Cu, Cr, As, Se) in an artificial and natural stream. They did, however, find that acclimation increased the metal concentration causing the response. They (Hartwell et al., 1989) also report avoidance thresholds for golden shiners. In an evaluation of a common soil nematode as a bioassay organism, Williams and Dusenbery (1987) found that copper caused no behavioral changes until lethal concentrations were approached. In that case the rate of movement could be related to general debilitation.

I.3.11 THE EFFECTS OF COPPER ON COMMUNITIES

There is an interaction or a feedback between groups of organisms and chemical properties of the environment in which they live. Some of the interactions affect the chemistry of what could be called the biological medium, as for example the reduction in foliar copper produced by attack of leafminer beetles on poplar leaves (Bouyaiche and Nef, 1987). Other changes affect species composition, for example the longterm changes in chemistry and species composition occurring in naturally acidic lakes (e.g. Gibson and Smol, 1987). Species interactions can be important in these effects. Aerial transport of metals is reported to affect the growth of organisms in certain deciduous forests (e.g. Glavac, 1988). The interaction of two forest components have been examined in terms of the effect of excess copper. Mycorrhizal fungus-infected birch seedlings exposed to copper treatment are reportedly lighter than non-mycorrhizal infected seedlings (Jones and Hutchinson, 1985). Work by El-Kherbawy et al. (1989) with alfalfa and vesicular-arbuscular mycorrhizae suggests that the effect of the fungus on metal uptake is a result of the available soil heavy metal content. Nitrogen fixation by bacteria enhances growth in field peas and yellow lupine plants. However, copper deficiencies reduce the nitrogen fixation and reduce the growth of the two plants (Ruszkowska et al., 1986). Harland and Nganro (1990) describe a situation with the sea anemone *Anemonia viridis* which grows with a group of algae in a mutually beneficial manner. Their results suggest that when the anemone is exposed to 0.05 and 0.2 mg/L coper, the algae accumulate some of the copper and are expelled, effectively regulating the copper. (They note that other mechanisms may also be involved in metal regulation.)

Colonization of aquatic systems has been used as a measure of community response to environmental stress produced by hydrocarbons and metals, including copper (e.g. Hyland et al., 1989). Pratt and Cairns (1988) discuss this for colonization by microorganisms. Dean-Ross and Mills (1989) use bacterial community structure and function as indicators of stress along a heavy metal gradient (Pb, Cu, Cd, Ni, Zn) in a river. Hart et al. (1983) used community structure response of Lake Ontario phytoplankton to examine the effects of metal mixtures. They found blue-green algae dominant at low levels of toxicity although cryptophytes and dinoflagellates were most susceptible to metal addition; green algae were most persistent. Duzzin et al. (1988) used macroinvertebrate communities and sediments as pollution indicators for heavy metals in the River Adige (Italy). Rygg and Skei (1984) found a strong negative correlation between faunal diversity and sediment copper concentration in marine soft-bottom faunal communities. Colonization of aquatic environments includes fouling and fouling communities, species succession and the immobilization of copper coatings. Blunn and Gareth-Jones, in an I.C.A.-supported study, report the species succession occurring on 90/10 copper-nickel alloy and discuss the observation that sloughing off of the biological film can leave a new antifouling surface. One of the competitors for the antifouling market is a group of "non-toxic" (Mellouki et al., 1989) paints containing insolubilized quaternary ammonium salts grafted onto a vinyl copolymer (Mellouki et al., 1989). The authors note that the long period of time taken to develop the first stage in the fouling succession (microbial cover) means an extended life for paints.

Leland et al. (1989) examined the effects of copper on species composition of benthic insects in a Sierra Nevada (California) stream. Between 2.5-15 μg/L total copper (approximately 12-75 ng/L Cu^{2+}), species richness and percentage similarity were sensitive indices of effect. Clements et al. (1988) compared insect communities in experimental streams with impacted field sites and obtained similar results. They suggest that (abstract) "... outdoor stream mesocosms may be employed to predict macroinvertebrate community responses to heavy metals". However, in a later paper (Clements et al., 1989b) they found differences between rivers in macroinvertebrate responses to copper as a result of differences in water chemistry. As a result, they point to the importance of "... accounting for both water quality and composition of the resident fauna for establishing site-specific water quality criteria". Sullivan and Ritacco (1988) comment that the detrimental effects of copper can be affected by food quality and abundance. Predator-prey interactions can be affected by excess copper; Clements et al. (1989a) note increased vulnerability of two species of net-spinning caddisflies to predation by the stonefly when exposed to approximately 6 μg/L added copper in an experimental stream. Sugaya et al. (1988) could relate the distribution of metal tolerant species of larval chironomid insects to the section of a Japanese river that had the highest metal levels. Similar results were obtained in another river (Hatakeyama et al., 1988).

Selection for metal-tolerant organisms occurs in copper-rich soils (Anagnostidis and Roussomoustakaki, 1988; Casioda, 1988). There is some evidence that at least one group of tolerant plants can evolve rapidly. Macnair et al. (1989) describes a recently evolved species of *Mimulus* and its presumed progenitor, both species found on old copper mine tailings piles. Animals such as slugs may also occur in the region of disused mine sites although their distribution and metal accumulation capability tends to be species specific (Greville and Morgan, 1989a). Fungicide effects on predator-prey relationships have been examined by Mani and Thontadarya (1988) for the grape mealybug and two of its natural enemies. The authors note (abstract) that "... copper oxychloride, mancozeb, sulphur, carbendazim, Bordeaux mixture and dicofol were safe to both the natural enemies".

I.3.12 COPPER, NUTRITION AND FOOD CHAIN EFFECTS

In a discussion of mineral metabolism, Kies (1989) and Stamp (1988) note the essential role of copper in the metabolism of organisms. The intake of copper is essential and occurs either from the environment or from food. Cypher (1988) reviews the benefits, and uses, of copper and its importance in nutrition. Man's nutritional requirements for copper have existed through time as indicated by trace metal analysis of skeletons from prehistoric sites (Francalacci and Tarli, 1988; Waldron, 1988). Francalacci and Tarli (1988) comment (page 49) that "... trace element analysis is a reliable dietary indicator and, ... , a valuable tool for the reconstruction of subsistence patterns of ancient human populations". Reviews of the metabolism and homeostasis of copper include the excellent paper by Girchev and Tzachev (1988) while reviews of the nutritional significance of the metal include that in Frank (1988).

In general, terrestrial animals, as well as humans, acquire copper primarily from food. Indices for assessing copper nutriture are discussed by Smith (1987b). The recognition of nutritional deficiencies, including copper, in bovines is discussed by Coffey (1989). Castro Sánchez et al. (1988) were able to use metal levels in serum, coagulum and scalp hair to classify children into 3 groups - urban lower middle class, urban middle class, and rural lower middle class. In a paper entitled "The nutritional requirements for copper in animals and man", Suttle (1987) notes the importance of distinguishing between minimal requirements and recommended daily requirements (RDA). He comments that minimum requirements do not include the safety factor provided by RDA's, for adverse conditions. Another consideration is that copper ingested does not necessarily mean copper absorbed. Absorption from solid foods is, for example, reportedly greater than from liquid foods (in Kinzel, 1989). Allen (1987) discusses some of the techniques for dealing with copper deficiency in animals. Effects of excess copper are also discussed as are some of the techiques for minimizing the effects. Addressing food supplements and fortified foods for humans, Greger (1987) comments on the importance of considering the biological availability of nutrients (including metals) as well as potentially detrimental effects.

In plants, copper deficiencies affect both growth rate and chemical composition. Plucknett and Sprague (1989) comment on the importance of detecting mineral nutrient deficiencies in tropical and temperate crops before they limit crop yields. Medium interactions can also affect both the availability and effects of copper, whether in terrestrial or aquatic environments (Delhaize et al., 1987; Hall et al., 1989b). High Mn:Cu ratios can, for example, lead to an apparent copper deficiency (Karamanos et al., 1989). Soil properties are of prime importance, certain soils can have low levels of soil copper (e.g. quartzeous soil; Saur, 1989). Plant characteristics and physiological condition are also important. Stark et al. (1989a) report, for examle that in huckleberries, roots are sinks for copper, nutrient concentrations in leaves of young plants differed from those of old plants, and mature plants producing berries differed from those that were not producing.

Delhaize et al. (1987) provide a good review of copper availability to plants, the associations of copper and organics within plants, and the availability of plant tissue copper to animals. Dai and Zhang (1988) examined the uptake of metals by the water hyacinth from waste water and the transfer of plant tissue metals to fish feeding on water hyacinth. The hyacinth is able to take up metal from waste water but the authors report no obvious accumulation by fish feeding on the plant. Nutritional composition of useful metal bioaccumulators as well as nuisance species is being examined to determine the usefulness of the species as an animal food source (e.g. Dillon et al., 1988a). The same is true for economically important crops such as fruits and vegetables (Tiemann et al., 1989).

Metal uptake varies in aquatic animals as it does in all other organisms. Depledge (1989a), for example, notes variation in nutritional state in the marine crab *Carcinus maenas.* The effect of high levels of metal on members of aquatic and terrestrial food chains has long been of concern (e.g. Rainbow, 1989). Although evidence indicates that copper is not biomagnified (Campbell et al., 1988; Novak and Mensik, 1987; but see Madden et al., 1988), there is still concern about potential loss of important organisms with excess copper (e.g. Julshamn et al., 1988a; Vardia et al., 1988). In commercially grown organisms such as fish, dietary copper levels can be controlled. This has led to examinations of the effect of varying levels of copper, and other metals, on growth and performance (Gatlin et al., 1989; Maage et al., 1989). Recent studies include examination of the ability of terrestrial wild animals to obtain adequate copper and other metal levels during the entire year (Wlostowski et al., 1988). High levels of copper can impair or cause death when in the food of organisms such as the aquatic life history stages of some insects (Hatakeyama, 1989). Concern about metal levels in human foods includes concern about copper. Phillips et al. (1986), for example, carried out a two-year study of trace metal concentrations in oysters marketed in Hong Kong. Comparisons of their data with previous studies indicate a decrease in average concentrations of copper in marketed oysters. Gnusowski and Zygmunt (1985) describe a technique for determining copper oxychloride residues in medicinal plants and seasoning herbs. They comment (abstract) that "... 55.8% of copper oxychloride contained in parent raw material gets into tinctures while 14.8% gets into infusions and decoctions".

In domestic animals, adequate tissue copper concentrations are important for good health. There is also the concern about the effects of excess copper, especially in sheep (Howell and Gooneratne, 1987; Smith, 1989). Coffey (1986) points out that (summary) "Trace elements are an important part of immunocompetence in that they are a component of enzymes controlling the human response". He points to the importance of detemining serum and liver copper levels and trace element levels in feed materials. Grace (1988) reviews recent developments in trace elements in animal production including a review of the benefit of copper supplementation, particularly to young animals. Copper and other feed additives are used as growth promoters in several animals (e.g. Fekete et al., 1988; Pond et al., 1989). In a Ph.D. thesis, Stevenson (1981) examined the effects of copper added to the normal diet, noting that there was some detrimental effect on reproductive capacity of hens and accumulation of copper in the liver. Bond et al. (1989) found that copper sulfate supplementation improved turkey feed efficiency but did not affect weight gain, bone elasticity or maximum stress. Ledoux (Ph.D. thesis, 1987; also Ledoux et al., 1989a,b) found that broiler chick tissue copper concentrations can be used as a bioassay criterion for determining metal bioavailability with dietary copper. This is useful when combined with information on the chemical composition of dietary sources of copper (e.g. Querubin et al., 1986). Jensen et al. (1989), for example, note a significant copper x methionine interaction with methionine supplementation improving broiler chicken feed efficiency more in the presence of copper than in its absence.

Changes in copper intake and absorption have been examined in young lambs, as they age and change food types. Grace and Watkinson (1988) found a decrease in the fraction of ingested copper that was absorbed when lambs were changed from milk replacer diet (0.92) to pasture diet (0.10). 1.1 mg copper was associated with each kg gain in fleece-free empty body weight. Several studies have evaluated metal concentration in natural and synthetic feed materials for sheep (e.g. Cavalheiro et al., 1989) and their effect on tissue copper levels (Charmley and Ivan, 1989; Ishii et al., 1985; Prasad and Ramachandra, 1989). Alekseev et al. (1989) found copper deficiency in sheep feeds in the Celinograd region of Kazakhstan. Baars et al. (1989) found no obvious increase in tissue metal levels in sheep grazing on metal-rich salt marsh pasture. Copper toxicity has been reported in sheep and llamas (e.g. Junge and Thornburg, 1989). However, it is important to realize that, like many grazing animals, copper absorption in sheep can be affected by the competitive interaction between copper and other agents (e.g. Ochrimenko et al., 1987; Schwarz and Werner, 1987). This points to the importance of information on feed concentrations of interacting chemicals such as molybdenum and sulfur (Moshtaghi-Nia et al., 1989).

In pigs, tissue copper levels can be affected by growth hormones (Caperna et al., 1989). Feed use efficiency can be increased by copper supplementation in pigs (e.g. Heitman et al., 1989; Kornegay et al., 1989; Lüdke and Schöne, 1988; Schöne et al., 1988), possibly as a result of increased activity of certain enzymes, such as glutathione peroxidase (Zhang et al., 1985). In cattle, copper supplementation has been shown to have an effect on tissue copper levels (e.g. Kleezkowski, 1990) but Hidiroglou (1989) reports no effect, at least on the growth of heifers fed barley silage. O'Kiely et al. (1989a,b,c), however, examined the effect of silage preservatives and note that sulphuric acid-treated silage reduced liver copper levels more than formic acid-treated silage. Supplementation of cows with modest amounts of copper has not been associated with significant increases in milk (Hintz, 1987b). Anke (1989) states that the "minimum" copper requirement of ruminants is 8 mg/kg although this can be changed with regard to other soil components. Copper deficiency in cattle can be a result of metal-metal interaction, primarily with molybdenum, as well as the chemical and physical nature of the diet (Cymbaluk and Christison, 1989; House et al., 1989; Kume et al., 1983; Sas, 1987, 1989; Wadsworth et al., 1988; Wittenberg and Boila, 1988). Feeding relationships can sometimes be estimated from tissue metal levels in both domestic and wild animals (e.g. Grupe and Krüger, 1990). At least in certain areas, copper supplementation is advisable to offset deficiencies in natural feeds for both cattle and wild animals (Babinska-Werka and Czarnowska, 1988; Ellis et al., 1988; Gonzalez et al., 1988b; Gooneratne and Christensen, 1989; Koen, 1988; Laredo et al., 1989; Regiusné Möcsényi et al., 1988; Valdes et al., 1988). Jenkins and Hidiroglou (1989) recommend that copper levels not exceed 50 ppm in calf milk replacer. The NRC-NAS (National Academy of Science) maximum tolerable level of dietary copper during growth of cattle is 100 ppm (from Hintz, 1987a). Several copper-containing preparations have been used for supplementation in cattle. These include injected copper EDTA

(Edmiston and Bull, 1988), copper oxide powder (Langlands et al., 1989), copper oxide needles (Cameron et al., 1989) and copper in soluble glass (Hidiroglou and Proulx, 1988).

Kienzle (1988) calculated 0.1 mg/kg copper as the daily maintenance requirement of a healthy dog. This can vary with both the type of dog and any physiological demands placed on the animal. The effect of heavy physical activity has, for example, been associated with a reduction in tissue copper levels in dogs (Rusin et al., 1989). In rats, the severity of copper deficiency is increased by dietary fructose, when compared with dietary starch (Babu et al., 1989; Bhathena et al., 1988b; Fields et al., 1988, 1989b,c; Henderson and Johnson, 1989; Johnson, 1988b, 1989b; Lewis and Fields, 1989; Redman et al., 1988; see also the review by Reiser and Hallfrisch, 1987). "... the fructose-copper interaction occurs either during intestinal digestion and absorption, hepatic uptake of copper via the portal blood or its hepatic utilization" (abstract, Koh et al., 1989). This interaction, and effect may be a result of copper complexation by fructose, as suggested for certain microorganisms (Menkissoglu et al., 1988). However, Failla and Seidel (1988) comment that, with respect to total body content of copper, the copper-carbohydrate interaction is not a result of carbohydrate-dependent effects on the retention of dietary copper and other essential minerals (see also Schoenemann at al., 1989). Holbrook et al. (1989) report that, with humans, dietary fructose enhances mineral balance. Dietary zinc:copper ratios are important, especially when diets contain low levels of copper and zinc supplements (Frimpong and Magee, 1989; see also Rao, 1988). Bonomi et al. (1988) note a zinc deficiency with increasing copper levels in cows. However, Warren et al. (1989) did not find any influence by zinc supplementation, on copper and iron levels in whole human milk and milk fractions. Nielsen (1988; 1989) presents evidence that male/female differences in sulfur amino acid metabolism affect the response to copper deficiency in the rat. A number of chemical agents can affect uptake or effect of ingested copper, including aluminum hydroxide (Nouri et al., 1989), vitamin E (Barrow et al., 1989; Sokol et al., 1987). Jackson and Lee (1988) note that, with rats, the solubility of zinc and copper increased with increasing levels of tea. Beer is reported to improve copper metabolism and increase longevity in copper-deficient rats (Moore and Klevay, 1989) although excess alcohol can cause an increase in activity of a copper-containing enzyme (Cu-Zn superoxide dismutase; Rosenbaum et al., 1989; Zidenberg-Cherr et al., 1988). Addition of PCB to the diet of rats caused a gradual increase in tissue copper levels and ceruloplasmin activity (Kato et al., 1988). Nutrient deficiency of copper is also associated with a number of physiological problems, including kidney failure and fluid retention (Moore et al., 1989a). The brindled gene in mice, a model for Menke's disease, produces lower milk copper even when the dietary supplement is adequate (Prohaska, 1989).

For humans, the recommended daily dietary intake of copper is 1 mg (for a 60 kg person; Parr, 1990). However, Parr (1990) points out that the value is for "groups" of people, not individuals. In other words, it is an average value to used only as a general guideline. Ikebe et al. (1988) give the World Health Organization value of 2-3 mg/day as the recommended intake but point out that in Japan, the average is less (1.1±0.3 mg/day). Pennington et al. (1989) report daily intake of 0.45-1.23 mg copper in the diets of eight age-sex groups in the U.S. with lowest values in the diets of infants. Mareschi et al. (1987) evaluate dietary mineral element intake by "... the French population ..." in terms of the requirements for a balanced diet. Krause et al. (1990) related copper content in foodstuffs to copper intake of adults in central Europe. They report an average of 0.66 mg/day for women and 0.83 mg/day for men but note that the copper concentration in food materials was strongly influenced by the region. An average dietary copper intake of 23 (children), 21 (adults) and 20 (elderly) µmol/day is given by Abdulla et al. (1988) for people in Sweden. Cocchioni et al. (1988) measured metal levels in 70 different Italian diets and calculated an average daily intake of 2.2 mg copper. For people in Czechoslovakia, Hejda et al. (1988) found dietary copper intake averages to range from 1.26-3.08 mg/day for males and 1.61-2.37 mg/day for females. Copper levels in various food materials have been measured (see table 5) as well as in specialized food units such as ration packs (e.g. James et al., 1988) and hospital meals (Sinisalo et al., 1989a,b). The mineral adequacy of certain types of diets has also come under scrutiny (e.g. Freeland-Graves, 1988) as has uptake from various foods (Johnson et al., 1988c; Kelsay et al., 1988). Lönnerdal and Glazier (1989) discuss some of the problems in assessing trace element bioavailability from milk (see also Wapnir and Balkman, 1989). The binding of copper to casein, an important constituent of cow's milk, is affected by pH and ionic strength (Baumy and Brule, 1988). Luten et al. (1989) note that the solubility of copper in fishery products, by simulated gastric and intestinal juices appears to be affected by the high fat content or the methods of preparation. The effect of moderate alcohol consumption on mineral metabolism is to produce an increase in serum copper (Frimpong and Louis-Charles, 1989; Louis-Charles and Frimpong, 1989) with the possibility of at least short-term copper deficiency.

Dietary copper requirements as well as intake can change with age and physiological status (e.g. DiSilvestro, 1987). Mills (1990) discusses the significance of copper deficiency in human nutrition and health, pointing out that there is an indication of declining copper intake for older children, adolescents and adults. In an article entitled "Good nutrition for your growing child", Hale (1987) comments on the importance of copper. Supplementation is important in the infant but, as pointed out by Salmenperä et al. (1989), plasma copper and ceruloplasmin concentrations can be resistant to dietary supplementation. There is also controversy about methods of estimating nutrient uptake (e.g. Cook et al., 1989; Ehrenkranz et al., 1989b), the effect of supplementation (e.g. Venkataraman and Blick, 1988) and some evidence that the genetic make-up of the individual may play a role, at least in the response to short-term copper deprivation (Milne et al., 1990). Uptake and availability of dietary copper can also be affected, at all ages, by dietary agents such as fat (Dougherty et al., 1988; Luten et al., 1989) and fiber (Behall et al., 1989a,b; Churella and Vivian, 1989; Gordon, 1989b; Katseva et al., 1988; Laitinen et al., 1988; Schlemmer, 1989; but see Stedman et al., 1989). In a study of copper intake in young schoolchildren from a highly industrialized area in Germany, Laryea et al. (1990) found a median copper intake in boys of 0.6 mg/day and in girls of 0.7 mg/day. Approximately 70% of the children ingested less than the 1-2 mg copper/day recommended by the German Association of Nutrition. For "high-risk adolescents", Graesser et al. (1989) found 10-20% of the males and 25-75% of the females consumed less than 50% of the recommended daily amount of copper. They comment that "Dietary intervention combined with physical fitness programs are needed to address the health & nutrition problems of (this) socially disadvantaged group ...". In an evaluation of mineral intake by women between the ages of 23-43, Gallagher et al. (1989) report average daily copper intake to be 0.9 mg/day for individuals not living under controlled conditions. In a group of young adult males living under controlled conditions, Rawson and Medeiros (1989) report an overall average copper intake of 0.75 mg/day. Turnlund et al. (1989), using data from young men living under controlled conditions, suggest that copper balance can be achieved by most young men from a diet of 0.8 mg Cu/day (see also Turnlund, 1989a).

The effect of physical exercise on copper requirements has been examined by a number of workers. In an examination of copper, zinc and iron status of female swimmers at the start and end of a competitive season, Lukaski et al. (1989) found that dietary copper did not change and plasma copper and ceruloplasmin were unchanged. Red blood cell superoxide dismutase activity did, however, increase. They comment that (abstract) " ... trace element nutriture is not adversely affected by physical training when dietary intakes are adequate, and that increases in superoxide dismutase activity are a functional adaptation of copper metabolism to aerobic training". The magnesium, zinc and copper status of U.S. Navy SEAL (Sea, Air, and Land) trainees were determined to examine the effects of intensive physical exercise (Singh et al., 1989a). Average intakes exceeded the recommended dietary intake values although there was no obvious association between dietary intakes and plasma concentrations of the minerals. In a study of nutrient intake and dietary supplementation in body-builders, Faber and Benadé (1987) found that (abstract) "The food supplied adequate amounts of nutrients according to the US Recommended Dietary Allowances, and the use of dietary supplements can therefore not be justified". The necessity of an appropriate food supply can be demonstrated by the results of a study of humans on a partial fasting diet; Reinhardt et al. (1987b) report that serum copper and serum ceruloplasmin decreased in both men and women. Determining the copper status in an individual can be difficult although Cunnane (1988) suggests that the profile of long-chain fatty acids in serum phospholipids may be of some value.

Continuing effort is being made to evaluate and improve the understanding of trace mineral requirements for the elderly (e.g. Freeland-Graves and Behmardi, 1989; Greger, 1986, 1989). As stated by Powers et al. (1989, abstract), "Aging may modify both the availability and needs for certain nutrients". Prasad (1989) reviews the uses and requirements for copper in youth and in the elderly. Solomons (1989) discusses the physiological uses of copper and changes that occur with age. He comments that, due to the well established pathway for excretion in the body, copper unlike iron and zinc, shows no trend towards differential absorption with age. However, copper intake is often below the Recommended Daily Intake level (Dowdy et al., 1989; Horwath, 1989; Kemp et al., 1989). Even with this, tissue levels of copper often increase with age (Powers et al., 1989). The effects of disease are more apparent in a population of elderly people than they are in a younger population. These effects include abnormal tissue copper levels (Steffee and Teran, 1989). Copper requirements and the necessity of supplementation are of importance in patients receiving total parenteral nutrition (Fleming, 1989; Fujita et al., 1989; Jeejeebhoy, 1990; Saudin et al., 1988). The effects of various diseases to copper nutriture, at all ages, is important (e.g. Cabalska et al., 1986; Ihanainen et al., 1989; Liu and Chen, 1987).

Copper in drinking water and food

Copper occurs naturally in food and drinking water. It can also occur as a result of anthropogenic effects, for example through pesticide treatment of crops. Handling and transport of food and water in copper-containing vessels is an additional source of copper. This subsection concerns these aspects as well as chemicals that affect the biological availability of copper found in food or water.

Although copper in drinking water can be an effective bactericidal agent (e.g. Landeen et al., 1989a; Yaha et al., 1989), concern has been expressed about the effects of excess copper. The U.S. National Technical Information Service (1989b) provides a bibliography containing citations that discuss heavy metals in drinking water and water quality standards. This bibliography covers the period January 1970 through July 1989. A second bibliography (NTIS, 1989c) includes citations concerning public health aspects of drinking water and covers the period January 1978-September 1989. One of the more important documents is the review of the 1987 "Drinking Water Health Criteria Document for Copper", the review was done by the Metals Subcommittee of the Science Advisory Board's Environmental Health Committee for the U.S. Environmental Protection Agency. In the letter of the review transmittal (United States, 1988a) the review committee recommends a copper level of 1 mg/L for drinking water, as measured at the tap. They comment that "Copper has the unique characteristic that its presence in drinking water comes from the water pipes themselves". This is incorrect since source water will contain some copper, however small, not all copper is derived from the water pipe! Some drinking water treatment processes will remove metal, a factor considered by the U.S. Environmental Protection Agency (United States, 1986) in evaluating water treatment processes. Desalination, a method of obtaining fresh water from salt water, is an example of this. Alam and Sadiq (1989) measured metal levels in drinking water from a desalination plant in Dhahran (Saudi Arabia). Copper levels at taps in various types of buildings receiving the water ranged from less than measurable to more than 2.5 mg/L. Since water at the desalination plant had less than measurable copper levels the increase would be a result of leaching from distribution pipes.

Brass valves and fittings have been implicated as a potential source of lead, copper and zinc in drinking water (Schock and Neff, 1988). Reiber (1989) investigated some electrochemical kinetic parameters on copper plumbing surfaces and notes the effects of corrosion inhibitors, aging of the corrosion scale, dissolved oxygen, pH and residual chlorine. He found that, contrary to some reports, orthophosphates significantly reduced corrosion rates on copper surfaces. Uptake of copper by foods and drugs occurs from containers and cooking utensils (e.g. Gajek et al., 1987; Sugita et al., 1988). It can also occur from raw materials used in preparation of the products (Boulos and Smolinski, 1988; Osborne and Laal-Khoshab, 1989) and techniques such as grinding (Panduwawala et al., 1988). Corrosion of copper from copper-containing implements occurs as a result of organic acids and such things as food colouring agents (Talati and Patel, 1988, 1989). O'Neil and Tanner (1989) point out that milk is an effective carrier of copper from brass utensils to infants. In a review of copper in brewing technology, Cejka et al. (1989) report that copper contamination of beer can occur with fluid transport in copper pipes, especially under poor maintenance procedures. In the production of pot still whisky, an undesirable organic (ethyl carbamate or urethane) can be formed in the presence of copper (Riffkin et al., 1989b). Copper can also be a problem in wines and demetallization procedures are frequently used to reduce the metal-associated turbidity and poor taste (Chobanova and Nachkav, 1989). Reduced value and flavour can occur in foods as a result of copper-accelerated oxidation processes, particularly foods with lipids (Decker and Hultin, 1988, 1989; Jeno et al., 1988; Prasad and Bhat, 1987; Salih et al., 1989).

I.3.13 ORGANISMS AS INDICATORS OF COPPER BIOAVAILABILITY AND EFFECT

It seems only common sense to use organisms as indicators in attempts to measure the biological availability of copper. As well, "Biological monitoring procedures in the laboratory and in the field are the most effective and cost-efficient tools for water quality monitoring and control" (abstract, Westlake and Ralston, 1988). Similarly, biological monitoring is both appropriate and effective in evaluating conditions of copper deficiency. Animal models can also be used to examine metal availability under physiological stress. Cornelius (1988), for example, comments that (page 1315) "Animals with hepatic disorders are a unique research resource of spontaneous 'experiments of nature' ...". Concentration or activity of proteins such as enzymes and metallothionein are widely used for assaying either metal availability or organism well being (e.g. Coffey, 1988; Ecker et al., 1989). Engel (1988), however, notes the effect of factors other than those tested, on metallothionein concentration. Although in a somewhat abstract sense, the use of organisms to recover metals can be considered in this discussion; metal removal efficiencies are determined and loading capacities estimated (e.g. Gadd, 1988).

A variety of aquatic bioassay programs are discussed in "Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals", edited by Cairns and Pratt (1988). Höllwarth (1988b) reviews the possibilities and limits of bioindicators. Several other recent papers and recently received manuscript reports consider the general uses of bioassay organisms (e.g. Birge et al., 1987; Pereira et al., 1987; Tatem and Portzer, 1985; Wang, 1986). Newman and McIntosh (1989) discuss the appropriateness of *Aufwuchs* to monitor trace element bioaccumulation in aquatic biota. *Aufwuchs* is material that accumulates on submerged surfaces, usually consists of both biotic and abiotic components, often is inadequately defined and even when defined, may not be a reliable estimator of metal bioavailability and potential for trophic transfer of metal. Several estuarine organisms have been examined as indicators of environmental quality in the Elbe, Weser and Ems river estuary (Zauke et al., 1987). Under natural conditions, communities of invertebrates have been used to indicate impact of metals in various environmental situations (e.g. Duzzin et al., 1988). This is because community components can be affected by excess metal as well as other environmental conditions, affecting species composition as well as abundance. Since the species in the community can vary in metal tolerance, it is important to understand the nature of the community members (Warwick, 1988b). It is also important to understand environmental conditions appropriate to application of the bioassay results (e.g. Clements et al., 1989b; Schimmel et al., 1989). Under somewhat artificial conditions, the "Standardized Aquatic Microcosm" (SAM) is a multispecies assay used to evaluate effects of excess metal on freshwater aquatic organisms (Ladis et al., 1988b). Analogous systems have been developed for terrestrial environments (e.g. Weidemann et al., 1987). Programs have also been developed for measuring the concentration of aerosol-transported metal (Ross and Bengtsson, 1988). Zhirmunsky and Khristoforova (1986) discuss bioassay techniques for assessment of contaminant levels in the marine environment.

There is an increasing awareness of the importance of bacterial interactions with metals (Beveridge and Doyle, 1989). Their response is affected by properties of the environment (e.g. Wilke, 1988) which, with rapid growth rates, makes bacteria useful in bioassay programs. As well, metal-binding properties of microbial films allow some bacteria to grow on metal surfaces and produce metabolites useful in examining metal-organism interactions (e.g. Murgel et al., 1989). Certain species of bacteria are also capable of high levels of copper retention and rapid growth under high levels of metal bioavailability (Solanellas and Bordons, 1988). These organisms have been used in prospecting, as indicators of metal (Shrivastava and Alexander, 1988; von Holy et al., 1988), and in microbial leaching of low grade ores (Acevedo and Gentina, 1989; Khalid and Malik, 1988).

Recent literature includes reports on the use of microbial assay techniques for detrimental effects of metals in soils (Gulyás and Kovács, 1988), water and of various chemicals (Liu et al., 1989a). Pratt and Cairns (1988) found adverse effects on bacterial colonization by a cadmium and copper mix of <1 μg Cd:18 μg Cu/L even though low levels of copper enhanced the species numbers. Bioluminescent bacteria are being widely used in estimating detrimental effects of excess biologically available copper as well as other metals (Ankley et al., 1989; Bülow and Klein, 1987; Reteuna et al., 1989; Ulitzur and Barak, 1988; Zholdakov et al., 1989).

Algae have been used to estimate the effects as well as availability of copper (Twiss et al., 1989a). They have also been used as general environmental monitors of metal effects and metal levels in natural waters (e.g. Anderson and Hunt, 1988; Khristoforova, 1985; Langston, 1988; Say et al., 1986). Momper and Redmann (1987) discuss the suitability of several macroalgae as "active monitors of heavy metal contamination ..." (synopsis). In a rather interesting set of observations, Zolotukhina et al. (1988) report that changes in the oxidative activity of macroalgae culture medium can be used as an indication of ionic activity in natural environments. With microalgae, the effect of metals on photosynthesis can be easily measured with fluorescence techniques (Samson and Popovic, 1988). Changes in the apparent rate of sediment deposition of scales from chrysophytes (golden-brown algae) have been used as evidence of paleolimnological changes in lakes (Dixit et al., 1989). Walsh et al. (1988) discuss the advantages of the marine diatom *Minutocellus polymorphus* as a bioassay organism. Swain et al. (1986) discuss the use of enclosures to assess the impact of copper sulfate treatments on phytoplankton in lakes. They note that the major effect of treatment is a change in the secondary succession. However, there are interactions of metals with other parameters on organisms, causing changes in metal effects. Hall et al. (1989b) found that algae were more sensitive to excess copper in batch cultures under phosphorous limitation than under nitrogen limitation. They comment (abstract) that "This suggests a need for careful consideration of the parameters used to measure toxicity and of the nutrient limiting the final yield of batch cultures in metal toxicity studies". Stauber and Florence (1989) also report an effect of the culture medium on copper toxicity, to the marine diatom *Nitzschia closterium* and the freshwater green alga *Chlorella pyrenoidosa.*

Lichens and mosses are widely used to sample aerosol metals. Lloyd et al. (1988) includes these, along with soils and synthetic material as low cost, low technology samplers of particular value because numerous samples can be obtained at relatively low cost. Recent publications and manuscript reports dealing with the use of these organisms include Herzig and Urech (1988), Herzig et al. (1989), Macher (1987), Mouvet et al. (1988), Puckett (1988). Beck and Ramelow (1990) made use of the metal accumulating ability of lichens to successfully monitor dissolved metals in natural waters.

Plants are useful for assaying aerosol metal and soil metal bioavailability. Rebele and Werner (1987) noted generally elevated concentrations of metals (Cd, Pb, Zn, Cu) in five common species of plants in industrial areas in Berlin. Unlauff-Zimmerman and Kreimes (1987) used what they term a "passive monitoring" forest system to evaluate long term changes in air-borne pollutants. Their system included animals as well as two species of forest trees. Landolt et al. (1989) report that metal concentrations in and on Norway spruce needles could be useful in evaluating aerosol metal input; Krivan and Schäfer (1989) used metal levels in surface deposits on spruce needles in a similar manner. In the latter, metal concentrations in the atmosphere could be correlated with metal concentrations in surface deposits. Crop plants have been used as indicators, both of aerosol metal and soil metal availability. Gajewski et al. (1987), for example, report that near a Polish copper smelting works, (summary) the "Content of Cu and Zn in bean and buckkwheat ranged from several to 1440 ppm and to 300 ppm for Cu and Zn, respectively". Seed germination has also been used as a bioassay mechanism, including early growth of seedlings (e.g. Baghdady, 1988). Mahmood et al. (1987) used activity of the copper metallo-enzyme ascorbate oxidase as an indicator of plant copper status in peanut plants.

Selection of plants for sites such as mine tailings is not an assay, *per se*, although growth of these plants provides an indication of the biological impact of a site (e.g. LeFèbvre and Demoulin, 1989; McLaughlin and Crowder, 1988; Mitchell et al., 1988; Taylor et al., 1989). Macnair (1989) discusses a new species of plant endemic to copper mines in California, which appears to have evolved in response to the selective pressure of mineralized soils. Something of a "bioassay in reverse" is the determination of copper levels adequate for controlling particular pest plants (Rocchio, 1988). Another type of "assay" is determining the ability of a plant species to remove metals, usually from water (Jain et al., 1989).

A range of invertebrate animals have been used as bioassay organisms to determine the effect of copper. Recent literature includes hydroid coelenterates (Santiago-Fandino, 1989; Ph.D. thesis), planarian flatworms (Rauscher, 1988; Ph.D. thesis) and the free-living soil nematode *Caenorhabditis elegans* (Williams and Dusenberv, 1987). In the latter case, the organism is well known for its use in cytological and genetic work. Polychaete worms have been used in wetland (Simmers, 1984) and estuarine (Grant et al., 1989; Langston, 1988) studies of metal-containing sediments. Grant et al. (1989) used the occurrence of metal-tolerant specimens of the polychaete *Nereis diversicolor* as an indication of metal impact. Another polychaete (*Dinophilus gyrociliatus*) has been suggested as a candidate for pore-water toxicity tests (Carr et al., 1989). Earthworms and related oligochaetes have been used to predict the impact of metal-containing sediments (i.e., dredged material; Rhett et al., 1988) and soils (Celardin and Landry, 1988; Morgan and Morgan, 1988). Based on its sensitivity to metals (including copper), sea urchin sperm has been described as a quick and useful means of biomonitoring metals in marine waters (Dinnel et al., 1989). A freshwater mollusc and crustacean plankton are discussed by Balogh (1988a) as monitors of anthropogenic metal effects in Lake Balaton (Hungary). Abaychi and Mustafa (1988) found that the freshwater clam *Corbicula fluminea* would accumulate metals in relation to concentration in the water. However, they also found an effect of size and season on metal uptake. Transplanted organisms are often used to measure metal uptake in both fresh and salt water environments. Hinch and Green (1989), however, note genetic differences in the freshwater clam *Elliptio complanata* that may affect metal uptake (Cu, Zn, Mn, Cd) by soft tissues. They comment (abstract) that "The use of freshwater clams as transplant biomonitors must be reassessed since there is a strong source component to growth and metal uptake". Oyster larvae have been used as indicators of metal effect. Phelps and Warner (1990), for example, report the LD_{50} of the pediveliger larva of *Crassostrea gigas* to be 313 mg/L, at least with a solution used to enrich the sediment. Phelps (1989) also found that burrowing speed of the clam *Mya arenaria* could be reduced by sediment copper levels of 51.4 mg/g sediment. The use of some animals to eliminate unwanted anthropogenic metals (e.g. Kurihara et al., 1987) is not a "bioassay" mechanism although it is a use of the organism to deal with unwanted metal.

Mussels such as *Mytilus edulis* and *Perna viridis* are widely used to indicate metal availability and impact in the marine environment (e.g. Langston, 1988; Roesijadi, 1987, 1988; Wesley and Raj, 1983) although problems with their use continue to be uncovered. The concentration of copper in the shell has been used for monitoring purposes (e.g. Lindstrom et al., 1988, 1989). However, Fischer (1986) notes that the copper:soft body weight is more meaningful than the copper:shell weight. Lobel et al. (1989) note a variability in tissue metal concentration in *Mytilus edulis* and a method for evaluating this variability. They comment (abstract) "... that some elements (e.g. the alkali earth elements and B, Mg and Cu) had extremely low residual variability while other elements ... showed unusually high degrees of residual variability". With cadmium, not copper, Fischer (1989) notes that relating body burdens of metal to shell weight overcomes some of the nutritional dependence of tissue metal concentrations. Using previously-published data, Amiard-Triquet (1987) found that changes in oyster and mussel tissue metal concentrations (including copper) are effected by physiological factors. Chan (1989), working with the green mussel *Perna viridis* noted temporal fluctuations and spatial variability in the Hong Kong area although no consistent seasonal pattern was found. In part as a result of variability in tissue metal concentration, other traits have been examined in *Mytilus edulis* as well as other bivalves. Kramer et al. (1989), for example, used the valve movement response of both freshwater (*Dreissena polymorpha*) and marine (*Mytilus edulis*) mussels as an effective monitoring tool. Chemical properties of the organism have been used to indicate stress (e.g. Zaroogian et al., 1988). Changes in concentrations of metal-binding proteins have been difficult to evaluate in *Mytilus edulis* although Roesijadi and Morris (1988) may now have a workable assay technique.

A number of arthropods are used as bioassay organisms. In recent literature, Belanger et al. (1989) discuss the maintenance and use of the freshwater crustacean *Ceriodaphnia dubia* in toxicity studies. They note that resistance to copper can be affected by diet and maintenance techniques and that LC_{50} values increase with increasing water hardness. Nimmo et al. (1990) used the species to detect nonpoint sources of metals from mine drainage. Powell and White (1989) examined the effect of copper and cadmium on the feeding activity (cirral beat) of two sessile barnacles. Activity was reduced by 80 ppb Cu. Rainbow et al. (1989) used tissue metal concentrations of several amphipod crustaceans as an indication of copper bioavailability. They recommended that one species (*Orchestia gammarellus*) be used as a biomonitor for copper and zinc in British coastal waters. Martin et al. (1989b) evaluated a mysid crustacean for routine complex effluent toxicity testing. LC_{50} and no effect levels for copper were 27 and <11 µg/L. Hatakeyama and Sugaya (1989) successfully used a freshwater shrimp as a bioassay for herbicides. In an evaluation of several different types of invertebrates, Alliot and Frenet-Piron (1988) chose a shrimp (*Palaemonetes serratus*) as the best indicator of the biological impact of anthropogenic trace metal (Cu, Pb, Zn). A number of aquatic insects, and insect larvae, have recently been discussed as bioassay organisms (Khangarot and Ray, 1989b; Lynch and Popp, 1988; Popp et al., 1989). Work also includes the effects of metals on food webs. Clements et al. (1989a), for example, noted an effect of copper on net-spinning caddisflies with an increase in predation on one of the two species present. They comment that species interactions are important and can not be predicted from single species bioassays. Novak (1985) notes greater accumulation of copper by males than females in the carabid beetle *Harpalus rufipes* and suggests that males could be a good bioindicator of copper bioavailability. Wittassek (1987b) examined the effects of copper in vineyard soils on faunal elements; spiders were noted as good biological indicators.

The metal-complexing agent metallothionein and its analogues have been used to monitor the health of organisms as well as the health of the environment, with plants and animals. Engel (1988) examines some of the problems of doing this. Using three organisms - the oyster *Crassostrea virginica*, the blue crab *Callinectes sapidus* and the beaked whale *Mesoplodon europaeus* - Engel notes how natural environmental and physiological factors can affect the mobilization and partitioning of metals and the variability in metallothionein levels. In oysters, the reproductive cycle was important, in blue crabs, growth and molt cycle, in the whale the type of food and habitat.

Fish are widely used in water quality studies because they are commercially valuable, can be readily grown for use in the laboratory, and a few species have been routinely used, providing background information on toxicity (e.g. Hughes, et al., 1989). In natural environments, fish kills have been used as an indication of poor water quality. Legorburu and Canton (1989) linked some fish kills in Basque rivers to effluents from an aluminium anodizing factory, cyanide and copper wastes. Hutchinson and Sprague (1989) measured the acute lethality of several metals to the American flagfish (*Jordanella floridae*). They report copper LC_{50} values of 1.4 µg/L at pH 5.3 but note that the values are pH dependent. Avoidance of high metal concentrations by the fathead minnow (*Pimephales promelas*) has been used as a means of assessment (Hartwell et al., 1988) although the authors note that fish acclimated to elevated concentrations do not respond as readily as fish under normal conditions. The same authors (Hartwell et al., 1989) examined avoidance and toxicity in the golden shiner (*Notemigonus crysoleucas*). They note that simple toxicity tests do not provide as realistic an evaluation of toxicity as a combination of toxicity and behavioural tests. Miyashita and Egami (1988) report differences in susceptability to heavy metals and pesticides, between inbred strains of the Medaka (*Oryzias latipes*). This indicates the importance of calibrations of fish from different breeding stock. Toxicity tests can also be replaced or supplemented with physiological and biochemical properties. Overnell and McIntosh (1988) report that cytosolic liver copper concentration or heat stable liver copper concentration can be used as an indication of excess dietary copper in the dab (*Limanda limanda*). Metabolic changes produced by metals, in preparations of isolated animal mitochondria have been found to affect the ration of reduced to oxidized nicotinamide adenine dinucleotide (Blondin et al., 1988). The authors suggest that this provides a suitable bioassay for predicting acute toxicity in fish.

Metal concentrations in field-collected feathers have been used as an indicator of metal concentration and effect (Cosson et al., 1988; Hahn et al., 1989). So also have tissue metal levels from field-collected rodents (Fendick et al., 1989). Laboratory rodents have been widely used as assay organisms for both metal effects and physiological abnormalities. In these cases, tissue metal concentrations (Molteni et al., 1988; Ward et al, 1989), morphological/anatomical irregularities (e.g. Rahiman et al., 1988) or physiological/biochemical changes (e.g. Bises et al., 1988; Herbert et al., 1990; Takahashi and Shimizu, 1988) are used as diagnostic factors. Many of these same factors are used in humans (e.g. Shinar et al., 1989; Van den Berg et al., 1989).

I.3.14 TOXICITY

When considering any toxic effects of copper it is essential to remember that copper is found naturally, is indispensable for life, and that any potential harm "... is linked to a great number of factors such as ... geochemical behaviour and the physiology of the target species considered" (page 267 in Alzieu, 1988). Some of the considerations needed are provided in volume 1 of the "Handbook on the Toxicology of Metals" edited by Friberg et al. (1986). One of the chapters (Nordberg et al., 1986), for example discusses the factors that may influence the dose-response relationships between metals and organisms. Good discussions of the importance of metal speciation and toxicity are provided in Flemming and Trevors (1989), Hirose (1990) and Morrison et al. (1989). With major groups of organisms, Wong and Chau (1988) examined the toxicity of metal mixtures to phytoplankton and the National Technical Information Service (1989g) cites references to laboratory and field studies on the effects of metals to freshwater fish. Howell and Gooneratne (1987) discuss the metabolism of copper in a review of the pathology of copper toxicity in animals. They also discuss the prevention and treatment of copper poisoning in sheep, noting the importance of metal-metal interactions. Hernberg (1986) reviews "General aspects of the prevention of metal poisoning", commenting on some of the economic and technical problems associated with the topic.

Metal toxicity is considered not only in terms of organism mortality but also in terms of organism physiology and species composition (Weinstein and Birk, 1989). Efforts to provide a basis for assessment include the U.S. National Animal Poison Information Network Database which can be used for ecological risk assessment (Beasley and Schaeffer, 1989). Bioassays are widely used to evaluate the toxic effects of metals ranging from single substances (e.g. Pereira et al., 1987) to complex effluents (Schimmel et al., 1989) and effluent-containing media (e.g. Vives-Rego et al., 1988). Results of these assays are used to evaluate organism effect, species composition, identification of component effect in a complex mixture (e.g. Schimmel et al., 1989; Silver and Misra, 1988), effectiveness of treatment processes (Hill and Kocornik, 1986; Lee and Jones, 1988) and derivation of permissible metal levels (e.g. Abbasi and Soni, 1986). In making these assessments there are problems both of analysis and interpretation. Lee and Jones (1988), for example, comment on this in a discussion of toxic wastewater effluent treatment. They state (page 659) that "The net result of the current problems in the development of technically valid criteria for heavy metals and approaches for their implementation into appropriate water quality standards is that the design engineer and the wastewater treatment plant operator do not have available to them defensible numeric standards for heavy metals that can be used to determine the degree of treatment necessary to protect aquatic life in the receiving waters". A number of approaches have been taken in an attempt to evaluate the biological impact of metals in aquatic and terrestrial environments (e.g. Landis et al., 1988b). The U.S. Environmental Protection Agency (1988d) provides excerpts (for copper) from the individual state water quality standards establishing pollutant-specific criteria for interstate surface waters.

A major portion of the toxicity literature deals with effects on specific groups of organisms or specific organisms. Examples of this are given below, other examples are given elsewhere in this review, including the discussion of bioassay organisms.

1. Microorganisms (including fungi but not algae) - Bhattacherjee et al., 1988; Bossier and Verstraeta, 1989; Bülow and Klein, 1987; Casioda, 1988; Chao and Lin, 1989; Colpaert and Assche, 1987; Daniel and Nilsson, 1988; de Carvalho and Milanez, 1988; Dias et al., 1987; Diels et al., 1989; Francis and Petersen, 1989; Garrett and Schwartz, 1988; Kohen and Chevion, 1988; Kramer et al., 1987; Kuznetsova et al., 1989; Landeen et al., 1989a; Macreadie et al., 1989; Malvick and Bender, 1988; Menkissoglu and Lindow, 1988; Menkissoglu et al., 1988; Moorman et al., 1989; Nieto et al., 1989a,b; Platen et al., 1987; Roberts et al., 1990; Rouch et al., 1989b; Sato et al., 1988a; Shuttleworth and Unz, 1988; Sundin et al., 1989; Tomlin and Forster, 1988.

2. Plants -
    - A. Algae - Albertano and Pinto, 1986; Anderson and Hunt, 1988; Bastien and Cote, 1989; Bozeman et al., 1989; Drbal et al., 1985; Eloranta et al., 1988; Gupta, 1985, 1989a; Hall et al., 1989a,b; Kowaleska, 1988 (review); Lyngby and Brix, 1987; Macnair, 1989; Mikryakova, 1987 (hornwort); Novikov et al., 1989; Roberts et al., 1990; Samson and Popovic, 1988; Sengar and Sharma, 1987; Twiss et al., 1989b,c; Walsh et al., 1988; Watanabe et al., 1988
    - B. Commercial crop plants - Baghdady, 1988; Fabian and Dezsi-Devay, 1987; Hyuuga, 1987; Krauze and Bobrzecka, 1987; McCarter, 1989; Muramoto, 1989; Tobita et al., 1988
    - C. Miscellaneous - Gapochka et al., 1988; Nishizono et al., 1989a; Rocchio, 1988; St-Cyr and Crowder, 1989; Waddell and Kraus, 1990

3. Animals -
    - A. Flatworms - Rauscher, 1988
    - B. Nematode worms - Vranken et al., 1986, 1988
    - C. Polychaetes, earthworms and leeches - Grant et al., 1989; Hateley et al., 1989; Ozoh, 1986; Tatem and Portzer, 1985; Yang and Zhang, 1989
    - D. Molluscs -
        - 1. Clams and mussels - Chan, 1988a; Kramer et al., 1989; Miller, 1988a; Prabhudeva and Menon, 1986
        - 2. Snails and slugs - Greville and Morgan, 1989b; Rao and Jayasree, 1987
    - G. Crustaceans -
        - 1. *Daphnia* and other cladocerans - Belanger et al., 1989; Bodar et al., 1989; Khangarot and Ray, 1989a; Pokethitiyook et al., 1987; Vardia et al., 1988
        - 2. Ostracods - Vardia et al., 1988
        - 3. Copepods - Sullivan and Ritacco, 1988; Sunda et al., 1990
        - 4. Amphipods, isopods and mysids - de Nicola Giudici and Guarino, 1989; de Nicola Giudici and Migliore, 1988; Martin et al., 1989b; Migliore and de Nicola Giudici, 1988; Tatem and Portzer, 1985
        - 5. Shrimps and crabs - Hatakeyama and Sugaya, 1989; Knowlton, 1988a,b; Uma Devi and Rao, 1989
    - H. Insects - Khangarot and Ray, 1989b; Krantzberg and Stokes, 1989; Marchal-Segault, 1989; Nectoux and Bounias, 1988
    - I. Fish -
        - 1. Freshwater - Blondin et al., 1988; Elgaard and Guillot, 1988; Hartwell et al., 1988, 1989; Hughes and Nemcsok, 1988; Hutchinson and Sprague, 1989; Jana and Sahana, 1989; Legorburu and Canton, 1989; Munkittrick and Dixon, 1989; Misra et al., 1989
        - 2. Saltwater - Steele, 1989; Torres et al., 1987
    - J. Chickens - McCormick and Fleet, 1988
    - K. Sheep and goats - Goh and White, 1988; Martin et al., 1988; Smith, 1989; Wu, 1988
    - L. Cattle and horses - Auer et al., 1989; Jenkins and Hidiroglou, 1989; Koizumi et al., 1989
    - M. Laboratory mammals - Glushchenko et al., 1989; Mason and Edwards, 1989a,b; Smith et al., 1989b; Solecki, 1989

4. Cells - Freedman and Peisach, 1989b; Fuentealba, 1988; Fuentealba et al., 1989a; Iyengar and Mendiratta, 1988; Maroti and Bognar, 1988; Morazzoni et al., 1988

Resistance to elevated levels of copper is reported for most major groups of organisms. It is common in bacteria, especially fecal coliforms (e.g. Bhattacherjee et al., 1988; Dias et al., 1987) and fungi (e.g. Daniel and Nilsson, 1988; Kuznetosva et al., 1989; Moorman et al., 1989). It normally occurs in response to repeated exposure to anthropogenic copper (e.g. Colpaert and van Assche, 1987) and can involve a plasmid-based resistance (Diels et al., 1989; Malvick and Bender, 1988; Sundin et al., 1989; Thriene et al., 1988) as well as polysaccharides and other organics (Chao and Lin, 1989;

Rouch et al., 1989b). Tolerance of copper is often associated with tolerance of antibiotics (Bossier and Verstraeta, 1989). When considering "tolerance" with microorganisms, however, it is essential to remember that the physicochemical characteristics of the environment affect metal bioavailability and hence toxicity (Collins and Stotzky, 1989). Soil organic matter, particularly humic substances, can moderate the detrimental effects of copper (e.g. Mathur, 1983). Since tolerance differs from species to species as well as within a species, the microorganism community can shift in response to excess copper and variable copper tolerance (e.g. Casioda, 1988). Metal tolerance can be beneficial to the organism in a number of ways. It can allow an organism to exist under adverse conditions and grow chemolithoautotrophically on sulfide ores (Huber and Stetter, 1989). In this way it can also be beneficial to man when used to recover metals from tailings piles. However, copper- and nickel-tolerant marine sulphate reducing bacteria can play a role in the corrosion of copper-nickel alloys in marine environments (Chamberlain et al., 1988b). In metal-contaminated soils, interactions of plants with metal-tolerant fungi can reduce metal uptake by the plant (Angle et al., 1989; Evans and Sylvester, 1988).

In plants, copper uptake and, to some extent, toxicity can vary between and within a species (e.g. Tobita et al., 1988). General water or soil conditions are important (e.g. Belanger et al., 1987; Ortega et al., 1988). In addition, uptake and toxicity can be affected by nutrient (phosphate, nitrogen) concentrations (Fabian and Dezsi-Devay, 1987; Gupta, 1985, 1989a; Hall et al., 1989a,b; Twiss et al., 1989b,c). In an evaluation of long-term effect of continued Bordeaux mixture application to grapevines, Hyuuga (1987) comments that countermeasures for excess soil copper include the application of organic matter, phosphate or lime (to raise soil pH). Copper uptake can be affected by the physical and chemical nature of the plant root. In a species found in metalliferous habitats, copper ions have been reported to have an affinity for the cell wall rather than passing across the cell wall into the cytoplasm (Nishizono et al., 1989a). Control of metal concentrations within the organism is apparent in a number of plants (e.g. Alberts et al., 1990) although this is limited to certain ranges of metal concentrations. Toxicity can also be a result of the time of exposure, long exposure can cause phenotypic adaptation with subsequently higher survival (e.g. Gapochka et al., 1988).

In a discussion of the effects of metals on eelgrass (*Zostera marina*), Lyngby and Brix (1987) comment (page 444) that "The metal concentrations which reduced the growth of eelgrass in this investigation are in general much higher than those observed in natural and polluted waters. Significant phytotoxicity on eelgrass due to metal contamination therefore probably does not occur." They continue, however, to point out that eelgrass does enter detrital food chains, complete with contained metals, and may constitute a pathway for metal transport to higher trophic levels. As with microorganisms, plants that are tolerant to one set of conditions are often tolerant to excess metals, including copper (e.g. Albertano and Pinto, 1986; St-Cyr and Crowder, 1989). These are the species that are often found inhabiting areas of mineralization, including mine tailings (e.g. Macnair, 1987, 1989).

Copper tolerance in animals can be associated either with survival or changes in behaviour (e.g. Elgaard and Guillot, 1988; Hartwell et al., 1988, 1989; Steele, 1989). Tolerance, as indicated by survival, often changes with stages in the life history. Younger animals are usually less tolerant than adults whether it is with polychaete worms (Ozoh, 1986), some crustaceans (de Nicola Giudici and Migliore, 1988; Martin et al., 1989b; Sullivan and Ritacco, 1988) and some fish (e.g. Munkittrick and Dixon, 1989). However, in the crustacean *Daphnia magna*, the egg and embryo exhibit greater tolernace than the juvenile and adult stages (Bodar et al., 1989). Seasonal changes in tissue metal concentrations (e.g. Greville and Morgan, 1989b) may be associated with changes in the physiology of an organism, an indication that there may also be changes in metal tolerance. As with other types of organisms, however, it must be remembered that the effect of excess metal on an organism is controlled by environmental factors acting either on the biological availability of the metal or the physiological nature of the organism. The toxicity of copper to estuarine molluscs can, for example, be affected by salinity (Miller, 1988a). Diet and water hardness have been reported to affect the toxicity of copper to a freshwater crustacean (Belanger et al., 1989) as have pH (Hughes and Nemcsok, 1988; Hutchinson and Sprague, 1989) and the physicochemical properties of the metal (Khangarot and Ray, 1989a). Tolerance to copper can be inherited (Grant et al., 1989; Hateley et al., 1989) although selective pressure acting on the genome can produce a more resistant organism (Klerks and Levinton, 1989). Evidence is available suggesting that chronic exposure to elevated metal bioavailability may result in an increase in tolerance (Krantzberg and Stokes, 1989). However, this may be a result of the production of metal complexing agents (e.g. metallothionein; Misra et al., 1989) rather than selection of a more tolerant genotype.

The route of administration of copper can affect the impact of the metal. Toxic effects with chicks have been reported when copper was given intraperitoneally but not when it was given intravenously (McCormick and Fleet, 1988). In sheep, dietary copper can be toxic if the copper:molybdenum ratio is greater than 10:1 (Martin et al., 1988), a factor which must be considered not only for prepared food materials but also for natural foods, affected by metal concentrations in the soil (Smith, 1989). Dietary copper levels in milk replacer for calves is also of concern, high levels of copper can be toxic without adequate levels of zinc and molybdenum (Jenkins and Hidiroglou, 1989).

At the cellular level, Viarengo (1989) discusses the effect of heavy metals to be detrimentl, or be regulated. The ability of copper to react with organics within a cell is a key to the reason why copper is essential and can also be detrimental, interacting with the wrong organic. It is one of the reasons why copper-containing drugs are effective agents for controlling certain types of irregular cell growth. At the same time, however, these agents can be toxic to the wrong cell (e.g. Morazzoni et al., 1988). Ringenberg et al. (1988) reviews the possible hematologic effects of excess metal, listing the effects of copper as (page 1134, table 3) "Coombs-negative hemolytic anemia, acute abdominal pain, nausea, vomiting, CNS depression, and acute renal tubular necrosis". In a discussion of cellular mechanisms of toxicity and tolerance in the copper-loaded rat, Fuentealba (1988) and Fuentealba et al. (1989a) point out that lysosomal binding of copper is protective whereas copper entering the nucleus causes irreversible damage. One of the mechanisms of tolerance is metallothionein. Discussed earlier, Freedman and Peisach (1989b) propose that the level of resistance to copper toxicity is mediated by the concentration of copper-metallothionein.

Several copper-containing agents have been identified as potentially toxic chemials. These include copper sulfate (e.g. Lamont and Duflou, 1988; Miksch and Schürmann, 1988), copper napthenate (e.g. Angerhofere and Metker, 1988), Bordeaux mixture (e.g. Rahiman et al., 1988), yttrium barium copper oxide (London, 1988) and brass dust (Landis et al., 1988a) as well as a range of copper-containing drugs. Copper-containing antifouling compounds are routinely considered as toxic agents (Fingerman, 1988) although they are far superior to tin-containing compounds (e.g. Henderson, 1988) and are rapidly replacing them in many instances. As discussed in an earlier section, de la Court (1988) provides the leaching rate necessary to prevent fouling by certain groups of organisms.

In evaluating the toxic effects of any copper-containing compound, consideration must include a number of hydrographic, chemical and physiological properties govern the success of an antifouling agent (Lindner, 1988) (or other cidal agent, O'Sullivan et al., 1989). Tolerance is certainly important (e.g. Watanabe et al., 1988) but so also is the medium. Whether natural or artificial, the physical and chemical properties of a soil or aquatic medium can buffer the metal supply (Grün and Guenter, 1988; Laass, 1987; Hino and Takahashi, 1989a,b; Mackey and Higgins, 1988; Staubeb and Florence, 1989). Naturally-occurring agents such as humic acids have the ability to complex copper and reduce the impact of excess metal (e.g. Graneli et al., 1989). As an example, Flemming and Trevors (1988a) demonstrated the effects of sediment properties in reducing the effect of high levels of copper on sediment respiration. Chemicals produced by the organism can also buffer excess copper. These include a number of organics that either sorb or complex metals such as copper (Kozarac et al., 1989; Mench et al., 1988; Nishizono et al., 1988, 1989b; Ogiwara and Kodaira, 1989; Schultz and Hutchinson, 1988; Strange, 1988; Sunda and Gessner, 1989; Zhou and Wangersky, 1989c). Specific agents discussed in recent literature include melanin (Gadd and de Rome, 1988), pectin substances (Katseva et al., 1988b), siderophores (Kosakowska et al., 1988) and various peptides and proteins such as phytochelatins and metallothioneins (e.g. Cureton-Brown and Rauser, 1989; Drasch et al., 1989; Gordon, 1989a; Mehra et al., 1988; Misra, 1989; Rauser, 1989; Salt et al., 1989; Sato et al., 1989; Schreiber et al., 1990; Tomsett and Thurman, 1988) or agents that are analogous to these last two (e.g. Verkleij et al., 1989). These are often agents that are produced either in response to the presence of excess metal or whose concentration varies seasonally, or in response to organism growth (Morelli et al., 1989; Ogiwara and Kodaira, 1989; Vieira and Nascimento, 1989; Wangersky et al., 1989;

Williams et al., 1989; Zhou et al., 1989). The injury to animals, by excess copper, can also be moderated by dietary factors such as the vitamin E status in copper-overloaded rats (Sokol et al., 1988).

The uneven distribution of metal availability in a soil, lake or saltwater body is a result of the uneven distribution of metal and the factors affecting its availabity. As noted by Provini et al. (1989, page 220), "Different lakes have different sensitivities to different toxic substances". Hirose (1988) presents a multimetal complexation model which is reported to assist in determining the relationship between the total and free ion concentrations of the various metals. Moffett et al. (1990) discuss the distribution and potential sources and sinks of copper "chelators" in the Sargasso Sea. In the North Pacific, Coale and Bruland (1990) report seasonal variations in the distribution of strong copper-complexing ligands which follow seasonal variations in the depth of the mixed layer, at least at one station. It is interesting to note that they could not relate the change to shifts in primary productivity measured in the general area. In contrast, Mackey and Szymczak (1988) noted that an increase in copper complexing capacity in an Australian estuary could be related to a phytoplankton bloom. Lakes, estuaries and coastal marine areas are important because they can receive metal-containing materials from runoff and aerosols. Changes in conditions within these areas can affect metal concentrations (e.g. Forstner et al., 1989; Riedel and Sanders, 1988) which may cause changes in metal bioavailability in both water and sediments (e.g. Luoma, 1989).

II - COPPER AND MAN

II.1 USES OF COPPER

Copper is widely used. Whether for implements, medicine or agriculture, the metal provides a wealth of benefits in today's World as well as the opportunity for increased benefits in the World of tomorrow. To provide an indication of the value and importance of copper to man, and of some of the problems related to its use, some of the recent references and patents are given below, in table 1.

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

Agriculture - fertilizers, yeast and plant nutrients - Blue, 1988; Chernyi and Strel'tsov, 1988; Cheng, 1987; Gangwar et al., 1988; Gupta, 1989b; Javadi, 1988; Jokinen and Tahtinen, 1987a; Jurkowska and Rogoz, 1988; Komarov et al., 1988; Kuduk, 1988; Lasztity, 1988a; Laszkiewicz et al., 1987a,b; Lubis et al., 1988; Lungu and Toma, 1988; Luyindula, 1988; Mabbett, 1987; Mamaeva et al., 1989; Mamedkhanov, 1989; Mathur et al., 1989; Morris et al., 1987; Oliveira et al., 1989; Ovchinnikova et al., 1988; Pal et al., 1987; Parkhomenko et al., 1987; Ruszkowska et al., 1986; Singh and Misra, 1986; Sviklas, 1988; Szakal and Tolgyesi, 1989; Treiman, 1988

Agriculture - livestock - Cameron et al., 1989; Chase, 1987; Gonzalez et al., 1988b; Gooneratne and Christensen, 1989; Hamada et al., 1988b; Hennig et al., 1988; Hidiroglou and Proulx, 1988; Heitman et al., 1989; Jensen et al., 1989; Johri et al., 1986; Langlands et al., 1989; McPhee and Cawley, 1988; Menten, 1988; Menten et al., 1987; Omarkozhaev, 1988; Pimentel et al., 1989; Polasek et al., 1987; Ruda et al., 1988; Ruszbach et al., 1988; Shurson et al., 1987a,b,c.

Agriculture - miscellaneous - Arnold and Struve, 1989a,b; Diaz et al., 1987; Hayakawa, 1988; Matsumura and Honda, 1987; Mauer, 1987; Moens et al., 1986; Nakajima and Sumi, 1988; Wenny and Woollen, 1989; Yokota, 1989.

Biofouling and Corrosion - Alzieu et al., 1987; Blunn and Gareth-Jones, 1988*; Chamberlain and Garner, 1988a; Chugoku Marine Paints Ltd., 1983; Clayton, 1987; de la Court, 1988; Diprose et al., 1989; Dowd, 1988; Evans, 1988; Ford et al., 1988; Furukawa Electric Co. Ltd., 1984a,b; Gaffoglio, 1987; Giudice et al., 1984a; Henderson, 1988; Jolley et al., 1989; Jones et al., 1986*; Kamimoto et al., 1989; Maeda et al., 1989; Manfredi et al., 1987; Mellouki et al., 1989; Moraru et al., 1983; Nippon Steel Corp., 1984; Ohsugi et al., 1989; Pyne, 1987; Rascio et al., 1988; Tadros, 1989; Woods et al., 1987.

Dentistry - Drake and Waerhaug, 1989; Mante et al., 1989; Moore et al., 1989b; Parker, 1989.

Industry - food and water - Al-Obaidi et al., 1987; Cejka et al., 1989; Hajdu and Jenei Kiraly, 1988.

Industry and Environment - Flytzani-Stephanopoulos et al., 1987; Govindaraj et al., 1987; Hagenmaier et al., 1987; Kyotani et al., 1989; Melson, 1988; Shah and Leshock, 1988; Stelman, 1988; Stelman et al., 1987; Yahata et al., 1988.

Medicine - contraceptives - Apelo et al., 1989; Batar, 1988; Baveja et al., 1989; Bratt et al., 1988; Champion et al., 1988; Holland and White, 1988; Skandhan, 1988; Wildemeersch et al., 1988.

Medicine - nutrient supplementation - Fujita et al., 1989; Kinzel, 1989.

Medicine - pharmacology - Badawi et al., 1987; Bressan et al., 1989; Chang et al., 1989; Crispens and Sorenson, 1988, 1989; Denko, 1989; Egner et al., 1988; Emerit et al., 1989; Garuiti et al., 1988; Ghandour et al., 1989; Hernandez et al., 1987; Kasemeier et al., 1987; Kovacic et al., 1988; Loven, 1987; Morphy et al., 1989; Okuyama et al., 1987; Pickart, 1987, 1988b,c; Salari et al., 1987; Soderberg et al., 1987a,b; Solaimen, 1988; Sorenson, 1987a, 1988d, 1989a,b; Sorenson et al., 1989a,b; Takamura et al., 1989b; Torregrosa et al., 1987; Van der Goot et al., 1987.

Medicine - miscellaneous - Cohenford et al., 1989; Matsuda et al., 1989;

Pest Control - Abbaiah and Reddy, 1989; Abdel-Rahman et al., 1988; Aggarwal and Mehrotra, 1987, 1988; Albuquerque et al., 1987; Ando et al., 1988a,b; Anderson and Dechoretz, 1988; Arki et al., 1985; Baicu, 1987; Cardeilhac and Whitaker, 1988; Cerkauskas and McGarvey, 1987; Chauhan and Singh, 1988; Cheng, 1989; Choudary et al., 1989; Cook and Culshaw, 1989; Csutak et al., 1988; Darvas et al., 1987; Das Gupta et al., 1988; De and Sengupta, 1988; De Pauw-Gillet et al., 1987; de Zwart et al., 1989; Dillard, 1988; Du Plessis, 1987; Duso, 1988; Feng, 1988; Forster, 1988; Forster and Olson, 1988; Gadoury and Pearson, 1988; Garrett and Schwartz, 1988; Garza Lopez, 1988; Ge and Xu, 1989; Goetzschel et al., 1988; Gorska-Poczopko et al., 1986; Grzybowska, 1986; Gupta and Bhardwaj, 1988; Gupta and Khare, 1988; Gupta and Srivastava, 1988; Haag et al., 1988; Haidar and Nath, 1987; Hesse et al., 1988; Johri et al., 1986; Jollands and Jollands, 1989; Jollands et al., 1989; Joseph et al., 1987; Jurkowska and Wojciechowicz, 1988; Katircioglu and Gurcan, 1987; Kleemann and Claus, 1989; Kawale et al., 1989; Landeen et al., 1989b; Lepp and Dickinson, 1987; Lin et al., 1989b; Malavolta, 1988; Mandoki, 1988; Mantecon, 1989; Maringoni, 1988, 1990; McGuire, 1988; Mehta et al., 1989b; Miki and Ueda, 1988, 1989; Mollin et al., 1989; Moorman et al., 1989; Muniz and da Ponte, 1989; Murugesan and Mahadevan, 1987, 1988; Nakajima, 1988; Nazif, 1988; Nectoux et al., 1988; Onsando, 1988; Orozco Santos, 1987; Padule and Shinde, 1989; Pal and Chatterjee, 1989; Patel et al., 1988; Paul and Cairns, 1988; Pereira et al., 1988; Pinto de Torres and Carreño I, 1988; Pradhan et al., 1988; Raman and Cook, 1988; Ramanathan and Sivapalan, 1986; Rawal and Ullasa, 1988a,b; Redl and Purkhauser, 1988; Reed, 1988; Rondelaud, 1988b; Sarhan and Jalal, 1989; Schuster et al., 1989; Sfintitchi, 1988; Shenoi and Abdul Wajid, 1988; Shiroshita et al., 1989; Shukla et al., 1987; H. Singh and Saha, 1989; H. Singh and Singh, 1988; R. Singh, 1988; R. Singh and Dwivedi, 1987; Skopenko et al., 1989; Song et al., 1987; Stachewicz et al., 1987; Sunami, 1988; Swain et al., 1986; Teviotdale et al., 1989a,b,c; Thind and Bedi, 1988; Thurman and Gerba, 1988; Ullasa and Amin, 1988; Utikar and Shinde, 1987; Van Bruggen et al., 1988; Westerdahl and Getsinger, 1988; Wisniewski, 1988; Wittmann and Fickert, 1988a,b; Wu et al., 1985; Xu and Guo, 1988; Zuivertz et al., 1988.

Technology - science and medicine - Al-Mashikhi and Nakai, 1988; Arai et al., 1988; Barnhart et al., 1989; Chauhan et al., 1989; Das Gupta et al., 1989; Foucault and Rosset, 1987; Green and John, 1989; Harder, 1988; Jones and Bradshaw, 1989; Krotyuk, 1987; Mathias et al., 1989; Matyushichev et al., 1987; McPherson et al., 1989; Moriya et al., 1989b; Root and Reisler, 1989; Saracoglu and Eryilmaz, 1987; Stover, 1989; Velasco, 1989; von Muenchhausen and Sulkowski, 1988; Walker et al., 1989; Yu et al., 1989b.

Wood Preservation - Barnes, 1987; Briscoe, 1987; Collett, 1988; Eaton et al., 1989a; Hale and Eaton, 1988; Hein and Alkaitis, 1989; Johnson et al., 1988a; Leightley, 1987; Maksimenko et al., 1988; Metzner and Seepe, 1988; Ostmeyer et al., 1989; Pugel, 1987; Raghu-kumar et al., 1988; Schnippenkoetter et al., 1988; Shaler et al., 1988; Sharma et al., 1988b; Suzuki and Higaki, 1988.

Miscellaneous - Carter and Razi, 1989; Ivarsson and Oesterberg, 1988; Jacob et al., 1988; Keller, 1979; McClanahan and Bradley, 1988.

* Work supported by International Copper Association.

II.2 ANTHROPOGENIC COPPER - NATURE AND EFFECTS

Copper can be introduced into the environment either naturally or through man's activities. The latter occurs through processes associated with the extraction and recovery of the metal as well as in metal-forming and metal use. As a result, copper can be transported in the atmosphere (e.g. Andren, 1988; J-GESAMP, 1989; Murphy, 1988; Ontario Ministry of the Environment, 1984; Varady, 1988), can be elevated in terrestrial environments (e.g. Smith, 1990), in receiving waters and in sediments and organisms in these waters (e.g. Regner, 1988; Wallace, 1987). There is also the possibility of changes occurring in the biology of an area from the sum of all industrial effects, usually not just those associated with copper. Discussions of biological effects are reported in a variety of publications including proceedings of agencies and societies (e.g. Canadian Association on Water Pollution Research and Control, 1988; Dixon et al., 1988; Steubing, 1987), symposia and reviews of specific regions (e.g. Bender and Huggett, 1987; Jacobs et al., 1987) or specific problems (e.g. Albaiges, 1989; Hood et al., 1989; Lester et al., 1986) and reports by and for government-supported agencies (e.g. Bavarian State Institute for Water Research. In a Ph.D. thesis, Bent (1984) for example, reports a change in planktonic bacteria in association with metal-refinery effluent discharge to the Humber River estuary (U.K.). Changes in bacterial community structure in rivers have been associated with changes in heavy metal concentration and pH (e.g. Dean-Ross and Mills, 1989). Effects do, however, tend to be species-specific even with bacteria (e.g. Ibrahim, 1989). The determination of metal speciation is critical to the estimation of metal impact, Bent (1984) as an example reporting increased concentration of ionic metal or "simple" inorganic complexes in the vicinity of the metal refinery.

Reviews of impacts and effects of metals, including copper, are numerous and varied in their intent. High levels of copper and lead in soils have been used as markers of ancient site occupance in Greece (Davies et al., 1989) and evidence of contamination with modern man (Bridges, 1989; Laub, 1988; Mogollon et al., 1988). Discharge of metal-containing materials into aquatic environments has also been used as an indication of anthropogenic effect (e.g. Qasim et al., 1988). Friberg et al. (1986) discuss some of the general aspects of metal toxicology in volume I of the "Handbook on the Toxicology of Metals" (Elsevier). This series is a comprehensive review that considers the effects of metallic elements and their compounds on biological systems. Evaluations of effects of metals, for government agencies, are common. Trotter (1987) discusses effects in a water quality document prepared for the Ontario (Canada) Ministry of the Environment, H.J. Singleton (1987) prepared a "Water Quality Criteria for Copper" for the British Columbia (Canada) Ministry of Environment and Parks. The U.S. Environmental Protection Agency (1988d) provides a "Water Quality Standards Criteria Summaries: A compilation of state/federal criteria" on copper. In a study for the Australian government, McMahon (1989) examines the impact of marinas on water quality, including a discussion of the accumulation of metals (Pb, Cu, Zn, Hg). Other discussions of effects of metals on water quality are found in Anderson (1986), Gerlach (1988a,b), Gucinski et al. (1988), Hart and Lake (1987), Helz and Huggett (1987), Kremling et al. (1987), Kuntz (1988a), Lockerbie, 1987b,c, Meller, 1988, Muir and Sudar (1987), Newman and Aragg (1988), New York City (1986), Phillips (1989), Pollman and Danek (1988), Poulton et al. (1986), Prarie Provinces Water Board (Canada; 1981), Schmidtke (1988), Strachan (1988), Sylvestre et al. (1987), Sheehan and Lamb (1987), Wachs (1988) and Wolfe and O'Connor (1988). Discussions of metals in sediments are found in Irion and Müller (1987), Krumgalz (1989), Krumgalz et al. (1989), Kuntz (1988b), National Oceanic and Atmospheric Administration (1988), Rossmann (1988), Provini et al. (1989) and Xia et al. (1988).

Hamilton (1988) discusses the pattern of relative abundances of chemical elements in both living (biotic) and non-living (abiotic) systems. Flemming and Trevors (1989) provide a review of the chemistry, biological availability and biological effects of copper to microorganisms in the environment. Bouquegneau and Joiris (1988) discuss the fate of metals as well as organochlorines in marine organisms while Sicko-Goad and Stoermer (1988) review the general effects of anthropogenic materials on phytoplankton, primarily in freshwater. General effects of pollutants are discussed by Bender and Huggett (1987) for Chesapeake Bay shellfish and by Favretto and Favretto (1988) for the mussell *Mytilus galloprovincialis*). Krantzberg and Stokes (1989) discuss metal bioaccumulation in a group of insect larvae (chironomids) from acidic lakes in Canada while Suzuki et al. (1988b) discuss metal bioaccumulation by mayfly larvae in a Japanese river. With fish, contaminant concentrations are disscussed by Buijse (1987) for yellow perch and spottail shiner in the St. Lawrence River, Johnson (1989c) discusses metal concentrations in fish scales in a Canadian lake (Lake Opeongo) although no tabular values are provided for copper. Fish have been used as assay organisms, to determine water quality as well as permissible metal levels (e.g. Abbasi and Soni, 1986). Alzieu (1988) points out the difficulty in evaluating the impact of anthropogenic metal input on the North Sea ecosystem and Hunn (1988) points out the difficulty in determining the significance of contaminant residues in fishes. Zhirmunsky and Khristoforova (1986) discuss some of the techniques available to assess contamination levels in the marine environment and Rump and Krist (1988) provide a "Laboratory Manual for the Examination of Water, Waste Water and Soil". Lapin and Krasyukov (1988; in Russian) provide a discussion of the analysis of the organic forms of heavy metals for natural water monitoring purposes.

With terrestrial plants, effects of aerosol input of anthropogenic materials, including metals are of concern (e.g. Fritze, 1988). Concern has also been expressed for possible effects of copper used as pesticides. Examples of this include a discussion of effects on grapevines (Hyuuga, 1987) and pine seedlings (Huber et al., 1989). Hopkin (1989) provides a good review of metals in terrestrial invertebrates, discussing both beneficial and detrimental effects as well as sources, uptake and some aspects of the metabolism of metals. Soczo et al. (1988) review soil treatment techniques for removing organics and heavy metals from soils in the Netherlands.

In these reviews it is pleasing to see an increasing consideration of the importance of metal speciation and environmental conditions in relating metal concentration to biological effect. In a Ph.D. thesis, Bent (1984) reports a change in planktonic bacteria in association with metal-refinery effluent discharge to the Humber River estuary (U.K.), changes which he relates to changes in ionic metal. Changes in bacterial community structure in rivers have been associated with changes in heavy metal concentration and pH (e.g. Dean-Ross and Mills, 1989). Singleton (1987) comments (page 11) that "In setting water quality objectives for waterbodies where the copper concentration exceeds the criteria as a result of existing discharges, the form of copper stated in the objectives needs to be defined in advance. In view of the dependence of copper toxicity on the complexing capacity of a waterbody, an assessment ... would have to be performed on a site-specific basis to determine if the biota are being harmed." As an addition to this, monitoring programs need to be carefully considered to accomplish their intent. Balls (1989b) points out some of the problems and pitfalls in trend monitoring of dissolved trace metals in coastal sea water.

In the evaluation of a general effect of contaminants (e.g. Sanders, 1987), a bioindicator can be used as a monitor (e.g. Macher, 1987). However, the effects of high levels of anthropogenic metal need to be evaluated not in terms of general effects on an organism but, more importantly, in terms of effects on specific organ systems (Nordberg, 1988).

Mining, smelting and metal-working

Extraction and smelting of copper for the benefit of mankind has been occurring for thousands of years (Bamberger et al., 1988; Presslinger, 1988). However, the byproducts of these processes can be released (e.g. Curzydlo, 1988; Haque, 1987; Justyn and Ruzicka, 1984; Leita et al., 1989) and can be of concern if they provide substances detrimental to the environment or to human health. As a result, a number of agencies and individuals have examined the nature and volume of byproduct materials that are released from copper mining, smelting and metal working. Progress and summary reports of these often appear in government publications (e.g. Bennett and Knapp, 1989; Environment Canada, 1988; Godin et al., 1985; Jaques, 1988). Varady (1988) discusses the effects of copper smelting in the U.S.-Mexico border region. He points out that although expensive and unprofitable, reduction in various types of aerosol materials from smelting is important and is playing a role in binational issues between the U.S. and Mexico. Airborne particulate exposure in dust-generating operations like ore crushing can be sufficiently severe to require respiratory protection equipment within the area of the operation (e.g. Romo-Kröger et al., 1989). Emissions of copper oxide aerosol and SO_2 from copper smelters may result in sulfur oxides on the surface of particles that could affect smelter workers or residents living near the smelter (Chen et al., 1989b).

Release of metals from mine wastes is dependent on the nature of the waste materials, the supply of water for leaching and the pH of the medium (e.g. Schafer and Smith, 1989; Singh and Subramanian, 1988). Hakansson et al. (1989b) discusses long-range spreading of metals from a mine waste deposit in Sweden, noting a non-conservative behaviour of the metals due to sedimentation processes. Brook and Moore (1988) discuss bed sediments from a stream affected by mining and smelting operations. They note that copper is derived mainly from the "oxidizable" phase of the sediment samples. Effects of smelting operations have been related to changes in microorganisms in both soil (Fritze et al., 1989) and water (e.g. Anagnostidis and Roussomoustakaki, 1988; Mann et al., 1989a; see also the book "Metal Ions and Bacteria" by Beveridge and Doyle, 1989). However, some microorganisms are extremely useful for microbial leaching of copper from low grade ores (e.g. Acevedo and Gentina, 1989; Bosecker, 1987; Chaudhury et al., 1988; Golab and Orlowska, 1988a; Khalid and Malik, 1988; Olson and Kelly, 1986; Orlowska et al., 1986). Golab and Orlowska (1988b) report that the efficiency of microorganism leaching can be associated with the chemistry of the medium, as affected by the leaching organisms. Evidence of mining and smelting effects have been recorded for estuarine sediments and organisms (e.g. Frantzen et al., 1989; Henriques and Fernandes, 1988), ombrotrophic bogs which are affected by aerosol materials (e.g. Fjeldstad et al., 1988), plants (Borgegard and Rydin, 1989; Fernandes and Henriques, 1989; Zbierska et al., 1987), slugs (Greville and Morgan, 1989a), coral reefs (Brown, 1987), honey bees (Bromenshenk et al., 1988), small mammals (Hunter et al., 1989), and marine mammals (Wagemann, 1989). Effects of accidental discharges of copper-containing agents range from fish kills from copper-containing wastes (Legorburu and Canton, 1989) to the impact of an oil and copper spill from the sinking of a freighter (Hyland et al., 1989). High levels of soil metals and byproducts of smelting have been used to recommend against the growth of sugar beets near a copper smelter (Szerszen and Laskowski, 1987). However, many of the problems are those produced as a result of antiquated mining and smelting facilities and techniques. As stated by Kelly (1988; Preface) "... with forethought, planning and, it must be admitted, capital expenditure on the part of the industrialist, nature can exist quite happily alongside an industrial plant". The application of this pleasant statement exists in such documents as the "Generic *in situ* Copper Mine Design Manual" of Davidson et al. (1988).

Industry

The uses of copper in industry are of general benefit to man. However, loss of metal in aerosols, effluents and leachates from byproducts does introduce copper into the environment. Losses of metal are reported for both ancient and modern man (Davies et al., 1989) although the nature of the losses has changed, aerosol input increasing after the industrial revolution (e.g. Tendel and Wolf, 1988). Estimates of input amounts are difficult to make accurately in most cases and are often subject to great variability both within and between industries. Integration over extended time periods, say in sediments, can provide an overall indication of input (e.g. Wallace, 1987) although sources of the copper are often difficult to identify. Krumgalz (1988b) suggests that (abstract) "..., under certain conditions, the intercorrelations between various heavy metal concentrations can be successfully used to identify the sources of anthropogenic heavy metals in an area under consideration". These correlations can be used as "fingerprints" for determining the source(s) of metal or metals.

Elevated soil copper levels have been associated with industrial activities (Shahin et al., 1988). Some of these levels are high enough to be of sufficient concern to require remedial response (e.g. U.S. Environmental Protection Agency, 1988b,c). Leachate from metal-containing wastes such as fly ash can transport copper away from the source although the details of transport are difficult to identify (e.g. Theis et al., 1989). In general, soil copper levels tend to decrease away from the metal source (e.g. Stirban et al., 1987) although the change will be affected by the nature of the metal release (aerosol or effluent) and the nature of the soil. In effluents, copper concentration and biological effect is controlled by the nature of the effluent and the speciation of the metal (Comber et al., 1988; Tenorio et al., 1988). Accumulations of anthropogenic metals in both freshwater and saltwater environments have been associated with industrial and mine drainage as well as ocean disposal of sludges

(Quevauviller et al., 1989; Wallace et al., 1988; Zhang et al., 1989). In Hamilton Harbour (Canada), which has been impacted by industrial and municipal pollutant loadings over a long time period, copper concentrations in the water have been noted to exceed the Provincial Water Quality Objectives (5 µg/L) in 26% of the samples in the adjacent western Lake Ontario (Poulton et al., 1986). In the sediments, copper concentrations decreased away from the Burlington Ship Canal with only a minor influence evident for copper offshore although Lum (In Poulton et al., 1988) notes an increase in comparison to nearshore mean values. The patterns of accumulation are not simple and are dependent on water circulation. Yoshimura et al. (1988), for example, note higher metal concentrations in sediments from certain small, enclosed Japanese bays surrounded by factories than in sediments from more open waters in highly industrialized areas. Sediment accumulations reflect the nature of the effluent material, particle size and diagenetic processes that have occurred subsequent to the release of the metal (e.g. Benoliel et al., 1988; Calmano et al., 1988; Griffin et al., 1989).

The impact of atmospheric metal deposition on the primary productivity of a Newfoundland lake was sufficient to cause the authors (Rybak et al., 1989, abstract) to comment that "It seems that primary production in oligotrophic, acidifying lakes in Newfoundland is controlled more by nutrient loading and the possible toxicological effects of heavy metals, than directly by water acidity". Metal uptake by terrestrial plants occurs near an industrial source of metal (Bednarova and Kubisova, 1985; Bulinski et al., 1987). However, the accumulation of copper by plants near a chemical plant in Prerov, Czechoslovakia (Bednarova and Kubisova, 1985; page 8, translation) "... does not, ... , change too much even at higher levels of soil copper ...". Soil levels of copper can be elevated by the use of fungicides in agricultural areas, particularly vineyards (e.g. Stritar and Pavlovic, 1988). Macek and Repe (1987) comment that (synopsis) "In general the contamination of grapes was low, especially the contamination with copper residues". As with terrestrial plants, metal uptake may also occur in aquatic plants and animals in response to increases in anthropogenic metal (e.g. Rozema et al., 1988; Vale and Cortesao, 1988). However, tissue metal concentrations will be a result of metal chemistry as well as metal concentration.

Sewage, sludges and wastewater

A major source of anthropogenic copper and other metals is that found in sewage, sludges and wastewater (Nimmo et al., 1989; Wallace, 1987). Some of these agents can be used as a source of fertilizer (e.g. Bauduin et al., 1987; Dumitru et al., 1987; Gallardo-Lara and Nogales, 1987; Genevini et al., 1987; Giggey et al., 1989; Lubis et al., 1988; Paris et al., 1987; Wolski et al., 1986) under appropriate conditions (Drzymala and Mocek, 1987; Imagawa et al., 1987; Rutherford et al., 1987). However, copper concentrations in soils, sediments, water and crops have been reported to be elevated with release or agricultural use of sludges, wastewaters and human and porcine sewage (Adamu et al., 1989; Aboulroos et al., 1989; Barbera, 1987; Beaudoing et al., 1985; Bishop and DeWaters, 1988; Cabrera et al., 1989; Christie and Beattie, 1989; Couillard and Grenier, 1989; Drbal and Vebr, 1987; Keller, 1988; Meeus-Verdinne et al., 1988; Petruzzelli et al., 1989a; Reddy et al., 1989b; Sauerbeck and Styperek, 1987; Wachs, 1987; Zhang et al., 1988; see also Quigley et al., 1984). Metals in sludge may reduce its value as a fertilizer (Duquet and Vedy, 1989). This is because the metals in sludge-amended soils can reduce plant growth (e.g. Giller et al., 1988). However, Gettier et al. (1988), in an ICA-supported study, report that application of up to 638 mg/kg of wet Cu-enriched pig manure did not cause an environmental hazard. Stark et al. (1986) point out the importance of understanding the distribution of metals following application of sewage sludge. Oosthoek and Vam (1987) provide an excellent discussion of the use and problems of using composted wastes as fertilizers. They comment that the source of the waste and the extent of the composting dictate the metal concentration and influence of compost on soil quality. Lee and Jones (1988) provide an assessment of the degree of treatment required for toxic wastewater effluents, pointing out the difficulties of relating total metal content to biological impact. They comment (page 672) that "The US EPA has recommended an approach that is designed to begin to address the evaluation and testing issues pertinent to this type of effluent evaluation approach." They support the use of a metal concentration guideline but advocate amplification with site-specific hazard assessment studies to consider the ability of the environment to affect metal toxicity. The U.S. National Technical Information Service provides citations for references on sewage and industrial waste treatment appearing between January 1977 and July 1989

(NTIS, 1989d) and on wastewater treatment (June 1970-November 1987; NTIS, 1989e; December 1987-July 1989; NTIS, 1989f).

Fluctuations in trace metals routinely occur in sewage and sludges (e.g. Melcer et al., 1988; Monteith, 1987; Nevissi et al., 1988). Much of the variability is due to changes in the nature of the material although at least some is a result of analytical techniques; techniques for the determination of metal concentrations in various waste materials need to be considered in terms of waste composition, extraction efficiency and accuracy of measurement (e.g. Alimonti et al., 1988). Techniques have also been proposed for detection of excess metals originating from the use of sludge as a fertilizing agent (e.g. Gutenkunst, 1988).

Several major studies have recently examined the effect of sewage, sludge and waste input into receiving waters. Dethlefsen (1988), for example, discusses the input of anthropogenic materials into the German Bight of the North Sea. Moore and Davies (1987) review the monitoring results of disposal of sewage sludge from the region of Edinburgh (U.K.) into nearby marine waters. Voutsinou-Taliadouri et al. (1989) discuss sediment metal concentrations in areas receiving sewage from Athens (Greece). In an evaluation of water quality in New York harbour, the New York City Department of Environmental Protection (1986) noted high copper concentrations at all stations with values ranging between 19-150 µg/L. They note a harborwide (page 11) "... significant long-term decreasing trend in Cu ... most evident in the Hudson, and East Rivers". Stanford and Young (1988) note that the largest source of chemical pollutants to the New York Bight Apex in the 1970's and 80's was the barge dumping of dredge material rather than sewage and sludges. In an evaluation of wastewater discharges by the five largest municipal wastewater discharges, to Southern California marine waters between 1977-1981, Nishikawa-Kinomura et al. (1988) note a range of total copper emission between 336-417 tons per year.

Metals in sludge can inhibit sludge treatment (e.g. Hickey et al., 1989; Tomlin and Forster, 1988). Shuttleworth and Unz (1988) examined the effect of copper, zinc, and nickel on the growth of filamentous bacteria in a growth medium. They report that, of the metals tested, copper was the most inhibitory to the test bacteria. Vives-Rego et al. (1988), working with the effects of metals on seawater bacteria electron transport system activity, report an EC_{50} value of 0.8 mg/L for copper, second only to the value for mercury. Sakai et al. (1989a) note adsorption of copper in wastewater by activated sludge with little toxic effect on oxygen consumption, at least at pH values less than 4. Metal tolerance does occur in bacteria and has been reported for coliform bacteria (Bhattacherjee et al., 1988). As a result, metal-tolerant bacteria are often used in sludge treatment (e.g. Kasan and Baecker, 1989a).

Both metal chemistry and environmental properties affect metal speciation and metal disposition in sewage sludge or sludge-treated fields (e.g. Baron et al., 1989; Gibbs and Angelidis, 1989; Greiner and Deacon, 1983; Lake et al., 1989; Legret et al., 1988; MacNicol and Beckett, 1989). As a result, treatment of sludge (and compost) may be considered before activated sludge treatment (Licsko, 1988; van Roosmalen et al., 1988) or before use as a fertilizer, in both cases to reduce metal concentration (e.g. Campanella et al., 1988; Cheung, 1989; Krauss et al., 1987; Ried, 1988; Sheppard and Mayoh, 1986; Tsai and Cheung, 1988). (As noted in Bishop (1988), Francis et al. (1988), Kasan and Baecker (1989b), Peters (1988), Wu et al. (1988b) and Zirschky et al. (1989), this also can apply to other wastes and wastewaters.) Particle size is important, metals including copper are frequently associated with the finest particle size in sewage sludge (Campbell et al., 1987; Petruzzelli et al., 1989; Schulze and Gunkel, 1988). McGrath and Lane (1989) used a two-dimensional dispersion model to help determine the reasons for apparent losses of metals in long-term field experiments with sewage sludge. Competitive interactions for metals, between soil organic matter and sludge organic matter also affect soil metal chemistry and plant availability as well as uptake (Adams and Kissel, 1989; Strnad et al., 1988). The same is true for metals in other media, such as fly ash (Menicagli et al., 1988). Soil characteristics have also been important in the design and use of a bentonite/soil liner for containing heavy metal sludges (Olsta, 1989). Soil pH is considered to be of paramount importance in controlling soil metal availability (e.g. Rothe et al., 1988a,b). Hall et al. (1988a), however, comment that soil pH is not the only factor in relating soil copper to copper levels in ryegrass grown on sludge-treated soils.

Dredged materials

Dredged materials are essentially aquatic sediments exposed to reworking and oxidation. Sources of metals in dredged materials include metals in river sediments, in materials from manganese nodule processing (Bigham, 1989) and in marine sediments (Wainwright et al., 1988). They often incorporate industrial and municipal wastes whether in harbour sediments (Senten, 1989) or elsewhere. They include not only metals but also organics which can affect metal speciation (Förstner, 1989). Many of the problems associated with dredging and ocean dumping are considered in volume 3 of "Oceanic Processes in Marine Pollution" edited by Champ and Park (1989). Bramley and Rimmer (1988) evaluate the uses of dredged materials on land and conclude that dredged materials may be of value in many reclamation projects.

Hall (1989) examined the effects of dredging and reclamation in Trinidad and noted increases in sea-water levels of Cu,Pb, and Ni subsequent to dredging. Henry et al. (1989), working with the sediments of Table Bay Harbour (South Africa), noted high copper levels in some but not all areas of boat repairs which they attribute to vessel repairs. In an examination of dredged polluted sediments, Boothman (1987) associated the copper, cadmium and chromium with the oxidizable fraction. Palermo et al. (1988a), Palermo and Thackston (1988b) and Thackston and Palermo (1988) discuss tests for dredge material effluent quality.

Anthropogenic copper from the production and use of fossil fuels

Fossil fuels contain copper which can be released to the environment either during combustion or in residual material. Sahu (1987), Wilson (1988), Wahlström and Pohjola (1987) and Koelling et al. (1988), for example, comment on the availability of metals from coal ash. However coal ash stabilized with cement has been proposed as an alternative settlement substratum for oyster larvae. They report no significant difference in tissue copper concentrations between oysters grown on control and ash-based substrate.

Atmospheric deposition of copper-containing materials may increase soil metal levels and uptake by plants (Mulchi et al., 1990). Sato et al. (1988b) notes, however, that there is negligible effect of emissions from a 25-year old coal-fired power plant on soil metal levels in the plant vicinity. The change in pH produced by atmospheric deposition of sulfides from fossil fuel burning does have an effect, not only on the pH of lakes but also on their chemistry (e.g. Kelso et al., 1987), including the biological availability of copper and other metals. In an examination of 5 lakes near Sudbury, Ontario, Dillon et al. (1988b) report that the acidic lakes were conservative with respect to Cu and Ni while the circum-neutral lakes were effective sinks for the 2 metals.

Concern is continually expressed about changes in sediment metal concentrations in the vicinity of offshore oil-well drilling platforms. Barium levels may be elevated in the barium-rich mud used with copper present incidentally. Boothe and Presley (1989), working in the northwestern Gulf of Mexico found that copper showed no consistent trend around the six wells studied, with respect to the barite drilling mud that was being used. Similar results have been found in the Beaufort Sea (Boehm et al., 1986). Neff et al. (1989) comment (abstract) that "Our results support the hypothesis that much of the metals apparently accumulated from barite-contaminated sediments in tissues of marine animals was actually in the gut or gills as unassimilated barite particles. We conclude that metals associated with drilling mud barite are virtually nonbioavailable to marine organisms that might come in contact with discharged drilling fluid solids". Barchard and Mahon (1986) note (abstract) "... that long-term effects of heavy metals from drilling fluids would be limited, especially if formulations that contained relatively pure barite are used".

The use of fossil fuels in cars, trucks, aircraft and boats releases both particulate and soluble metals in aerosols and fluids. Some of this material becomes atmospheric fallout from vehicle exhaust (Suzuki and Hirai, 1987), some is mobilized from road dusts and some is released from oils leaking onto roadways (and being released from road materials). Major servicing areas for vehicles may provide important sources of anthropogenic metals. Roadway catchments for fuel-based metals have been developed and evaluated for their ability to retain heavy metals (e.g. Morrison et al., 1988) although results indicate only limited retention ability.

Although not a "fossil fuel" source of copper, the use of copper-containing antifouling agents on vessels provides a source of anthropogenic metal for release into the environment (Robbe, 1988). Elevated levels of copper have, for example, been reported near shipyards (e.g. Marcus and Mathews, 1987). Concern has been expressed about these agents, as indicated by the report to the House of Lords (1989) on anti-fouling paints. Actually, with the reduced use of tin in antifouling agents, copper is being more widely used. As an indication of this, evidence given in the House of Lords (1989) report states (page 1) that the Nature Conservancy Council (NCC) "... is not aware that copper-chrome-arsenic based compounds have caused environmental problems in the UK". In a comparison of oyster shell thickness and condition index measurements, Marcus et al. (1989) found no evidence that heavy metals were a major pollutant around recreational marinas in coastal South Carolina.

Copper deposits

Prospecting for copper-containing ores in todays World includes the use of organisms. Metal-tolerant microorganisms and macroorganisms often concentrate the metal. As such, their presence and their metal concentrations can be used as indicators of potentially useful mineralizations. von Holy et al. (1988) used bacterial isolates (*Thiobacillus* types) in an investigation of the importance and feasibility of microbiological prospecting for sulphide ore deposits. They comment that the technique (abstract) "... could therefore be developed into a valuable *in situ* tool for the field geologist who does not have immediate access to high-resolution geochemical analysis". Shrivastava and Alexander (1988), however, report failure of geomicrobiological techniques to identify two base metal deposits in India. Perhaps the use of geomicrobiological techniques should be considered in the same manner as the use of geochemical techniques where anomaly recognition for multi-element effects is important (Stanley and Sinclair, 1987). Shyam (1986) discusses botanical "prospecting" for copper in an Indian copper mineralization area. A number of techniques are used for recovery of copper from areas of mineralization (e.g. Thayer et al., 1987); the use of microorganisms is mentioned in the following subsection.

Mehrtens (1986) reviews the geological history and geochemical processes associated with the Coed-Y-Brenin porphyry copper deposit in North Wales. Browning and Beske-Diehl (1987) used paleomagnetic dating to age the native copper deposits in the Portage Lake volcanics of Michigan. Reports of metalliferous marine sediments include those of the Atlantis-II-Deep in the Red Sea (Guney et al., 1988). Several papers discuss the source, transport and precipitation of metals in stratiform copper deposits (e.g. Haynes and Bloom, 1987a,b). Association of copper-rich deposits with evaporites and oil-field brines led Sverjensky (1987) to suggest that evaporating shallow water marine basins form potential areas of mineralizations. Copper-containing offshore deposits, particularly of manganese nodules, are not only difficult to mine but require special equipment and provide special problems. Bigham (1989) discusses the oceanic disposal of wastes from manganese nodule processing. The manganese nodule "problem" has played an important role in the United Nations Law of the Sea conferences, a long-term series of international discussions which will ultimately provide a management policy for the use of metals in manganese nodules (e.g. Mahmoudi, 1987).

Recovery - Metal and Environment

Reduction of anthropogenic metal release is dependent either on the recovery from metal-containing materials or isolation of the material. This can range from simply enclosing the emissions of an industrial system such as a furnace (e.g. Hugk and Landau, 1987) to government regulation of the release of materials (e.g. Dogterom, 1986). Higgins and Desher (1989) review requirements for reducing the impacts of byproducts of metal finishing and processing operations. Hoffmann (1987) evaluates potentially harmful materials in effluents and Dobosz and Sebastian (1988) characterize sludges produced by the metallurgical industry and discuss their potential environmental effects. Vachon et al. (1987) review the treatment of acid mine water and the problems of sludge formed by metal precipitation after neutralization. Metal removal from sewage sludges can be achieved, at least partially, by acid treatment (Ried, 1988) as well as overland flow (Zirschky et al., 1989) although the latter is not recommended without testing for soil suitability. Harper (1988), in an excellent discussion of the "practicability of reducing heavy metal inputs", points out the problems of reducing metal input (to the North Sea from the U.K.) from what he calls a managerial rather than technical viewpoint.

Higgins (1989) discusses various techniques for removing waste byproducts in his book on "Hazardous Waste Minimization Handbook". Johannes et al. (1989) provide useful guidelines for electroplating/metal finishing wastewater treatment although the discussion concerns chromium rather than copper. Various techniques have been advocated for treating waste solutions, including homogeneous oxidation catalysis (Brooks, 1989), coprecipitation (Pardus and Regan, 1989), ultrafiltration (Dunn et al., 1989 although not a paper concerned with copper), complexatiion-ultrafiltration (Chaufer and Deratani, 1988), ion-exchange (de Haan et al., 1989), crystallization (Schöller et al., 1988), air-stripping (Pearson and Bowers, 1988) and the use of iron hydroxides (Edwards et al., 1989). Microbial and plant accumulation of metals has been demonstrated (e.g. Dorneanu et al., 1988; Francis et al., 1988; Gadd, 1988; Jain et al., 1989; Kasan and Baecker, 1989a; LeFebvre and Demoulin, 1989; Mann and Fyfe, 1988; Mann et al., 1989a,b; Mikryakova, 1987; Solanellas and Bordons, 1988; Taylor et al., 1989) not only to reduce environmental concentrations but also to recover copper from mine spoils and areas of low mineralization. Volesky (1988) discusses the use of microbial cells for biosorption (not uptake). (see also Brierley et al., 1989, Darnall and Gardea-Torresdey, 1989 and Glombitza and Iske, 1987 and the use of biofilms discusssed by Lion et al., 1987.) Bioprecipitated tin and iron oxides and oxyhydroxides are been demonstrated to scavenge metals (Cu, Pb, Zn, Ni, Cd, Th) from acidic mine effluents (Mann and Fyfe, 1989). Fly ash is advocated for sorption of copper and cadmium in soils (Petruzzelli et al., 1988). Montmorillonite has been reported to be better than kaolinite (both clay minerals) for sorption of cations in aquatic environments (Raymahashay, 1987). Silver (1988) describes artificial wetland systems for decreasing pH and metals from effluents emanating from disturbed land systems. Flue gas cleaning is advocated as a means of reducing metal emissions to a low level (Carlsson, 1988), a mechanism of filtration also useful for dust reduction (Hugk and Landau, 1987). Plant uptake of metals from aquatic systems has been widely used as a mechanism to reduce metal concentrations, especially in water with low pH (e.g. Ahlf et al., 1989; Lehtonen, 1989) where metal availability is high. Various types of bioreactors have been evaluated as a mechanism to remove metals from municipal sludge (Tyagi et al., 1989) as have mud snails for submerged paddy soil treated with composted sewage sludge (Kurihara et al., 1987). Metal removal from soils is somewhat more difficult although extraction techniques have been developed (e.g. Assink and rulkens, 1988; Tuin et al., 1988).

In a 1986 publication the U.S. Environmental Protection Agency provides some of the technologies and costs for the treatment and disposal of waste byproducts (including copper) from water treatments for the removal of inorganic and radioactive contaminants in drinking water. In a discussion of solid-solution interfaces, the "Trace Inorganic Substances Research Committee" (1988) provides some chemical and physical information on details of the partitioning of inorganic contaminants between solution and particulate phases. Effective treatment, metal removal, also occurs in natural systems such as certain types of wetlands (Harper et al., 1986). Besides metal removal from drinking water and ground water, effort has also been made to maintain appropriate copper levels in wine (Wucherpfennig et al., 1989a,b), primarily to improve the taste and ageing quality of the wine.

Copper-containing byproducts of industry that have been used for improving plant growth include steel furnace slag, used as a liming material for peat poor in mineral content (Myhr, 1987). The author notes, however, that the change in pH may produce a soil copper deficiency. Copper-containing agents have been used to enrich fertilizers (e.g. Avshister et al., 1988; Sviklas, 1988; Szakal et al., 1988; Wolski and Glinski, 1986)

III. COPPER SPECIATION AND ITS BIOLOGICAL IMPORTANCE

III.1 COPPER SPECIATION

The properties of copper have long been of interest to man. Even today, some of the basic properties such as colour (Guerrero et al., 1989; Kauffman and Jorgensen, 1989) or behaviour in solutions (Mazurkiewicz et al., 1988) sponsor interest and study. Reactions with other chemicals, such as sulfur (Reda, 1988), become important in determining the chemical uses of copper as well as some reactions in the environment. The understanding of metal speciation is important not only because it provides an indication of geochemical conditions in the environment (e.g. Zonta et al., 1988) but also because it indicates the biological availability of both natural and anthropogenic copper. Lapin and Krasyukov (1988), for example, discuss the importance of humic substances in the aqueous phases of copper. With increasing technical ability, man is now able to examine some of the chemical relationships between copper and organisms (e.g. Mazzucotelli et al., 1988), relationships that provide an indication of the physiological condition of the organism, or the biological impact of metal excess or deficiency.

Consideration of the properties of copper includes those of machined and finished products. Reiber (1989) investigated the electrochemical kinetic parameters on copper plumbing surfaces. Some of these properties better allow selection of copper based alloys for particular uses, as for example in polluted seawater (Manfredi et al., 1987). The control of corrosion requires an understanding not only of the factors that affect the metal but also the response of the metal to those factors (Cheng et al., 1989; De Sanchez and Schiffrin, 1988). This is important not only to improving the durability of the copper-containing item but also reducing the input of anthropogenic metal. Schock and Neff (1988) note that for lead, copper and zinc in drinking water, corrosion of brass valves and fittings (abstract) "... (is) a significant cause of metal concentrations that exceeded maximum contaminant levels". In a patent document, Hoover (1989) Joley et al. (1989) describe the measurement of biocorrosion of copper by food agents and bacteria. In a discussion on the chemical fate of brass dust in waters of varying hardness, Muse (1988) points out that it may cause a detrimental effect, either by dissociation into soluble copper and zinc or ingestion by organisms.

Copper speciation in soils is dependent on soil physical-chemical properties. In a brief review (Chinese), Kong (1987) discusses the chemistry of copper in soils. A number of papers present information on copper in soils of various regions (e.g. Agaev, 1988; Singh et al., 1988). Use of supplementary copper for plant growth is also discussed in terms of soil properties (e.g. Gembarzewski et al., 1989) and potential detrimental effects (e.g. Krauze and Bobrzecka, 1987). Sequential extraction techniques have been used to examine metal binding and metal bioavailability in soils (e.g. Duees, 1987; Gembarzewski et al., 1987). This also provides an indication of the metal retention by soils, a topic reviewed by Evans (1989) in terms of anthropogenic metal, metal bioavailability and metal toxicity. Tests of metal retention and migration in soils (Kumari et al., 1988; Li, 1988; Van Bladel et al., 1988) provide an indication of residence time and some of the chemical factors that affect residence time. Shuman (1988) comments that organic matter can affect not only the distribution of metals (Mn, Cu, Fe, Zn) in soils but also metal bioavailability although less effect was noted for copper than manganese and iron. With humic soils, copper lability can be affected (Berggren, 1989), an important factor in bioavailability. Kuznetsov et al. (1988) discusses copper speciation in reclaimed peat-bog soils and Shotyk (1988) provides a good review of metal (including copper) chemistry in peats and peatland waters.

Discussions of copper speciation in aquatic environments are found in a broad range of publications. A number of papers appear in "Heavy Metals in the Hydrological Cycle" edited by Astruc and Lester (1988). The majority of these papers appear elsewhere in the present review because they consider biological uptake and impact rather than metal speciation. Physical and geochemical characteristics of copper are discussed in examinations of certain freshwater systems. Li (1987), for example, discusses model-calculated chemical species in the Yellow River (China) while Zhang et al. (1989) used the concentration of heavy metals (Cu, Cd, Pb, Zn) in the Xiangjiang River (China) to examine the input of anthropogenic metals from industrial centers. This includes the changes that can occur in freshwater systems as a result of water passing over a major waterfall

(Johnson et al., 1989a) and flood events (Hart et al., 1988). Ferris et al. (1989) note that copper is one of the metals bound by microbial biofilms, a mechanism for removing filterable metal onto an organic surface. Betti and Papoff (1988), in a discussion of trace element evaluation of an aqueous ecosystem, comment on the problems of measurement and analysis and the difficulties of determining metal species. Effects of sewage and wastewater introduction include effects on metal speciation as well as metal concentration. Schulze and Gunkel (1988) discuss the distribution and transformation of heavy metals in the activated sludge process in municipal sewage treatment.

Reviews of metal chemistry in seawater include the short paper by Kuwamoto (1984) and a number of reviews of specific reactions (e.g. Soli and Byrne, 1989). Nimmo et al. (1989) examined copper speciation in Liverpool Bay (U.K.), noting that almost all copper (98-99%) occurred as relatively (compared to nickel) unstable organic complexes. They also noted a difference between months, the mean log a_{CuL} values higher in September than in May. This is not surprising since Zhou et al. (1989) note short term changes in copper-complexing ability of water in a phytoplankton bloom. Morelli et al. (1989) report growth phase-related changes in the copper-complexing ability of exudates produced by the chain-forming diatom *Skeletonema costatum*. Concentrations may also change as a result of atmospheric events (e.g. rainfall, Khokiattiwong and Limpsaichol, 1986), if for no other reason than dilution. van den Berg et al. (1987) provide evidence that only strongly complexed copper is kept in solution. Hirose (1988) developed a multimetal complexation model to evaluate the relationship between total and free ion concentrations. Later, using a model, Hirose (1990) suggests that copper, when present as an organic complex, is associated with particulate organic matter. The author comments (abstract) that "Metals, whose free ion is buffered by organic and/or inorganic ligands, may be used as indicators of the presence of particulate organic matter in the marine environment". This supports a conclusion by Balls (1989a) that (abstract) in estuarine and coastal waters "... particle-water interactions are important and that studies which examine only one phase are unlikely to further our knowledge of trace metal behaviour in such environments". Ion interactions have been shown to be important in the oxidation of Cu (I) (Millero, 1989). Sharma and Millero (1989) discuss the oxidation of Cu (I) with hydrogen peroxide in natural waters, commenting on the need for reliable rate equations for the oxidation, to better understand the role of hydrogen peroxide in surface ocean water. Hering and Morel (1989) report that the rate of formation of metal complexes with strong ligands can be slow and suggest that, as a result, the concentration of strong complexing agents in seawater may be underestimated when using metal addition techniques. Metal concentrations in seawater and sediments are presented in tabular form elsewhere. Major works include the study of Kremling and Pohl (1989) on spatial and seasonal variability of dissolved cadmium, copper and nickel in north-east Atlantic surface waters.

Windom et al. (1989b) presents trace metal concentrations in estuarine and coastal marine sediments of the southeastern United States. Bao et al. (1988) present geochemical characteristics of elements in sediments of the North Pacific, the results of China's first oceanographic survey in the North Pacific. They note differences in metal concentration between sediment types, a result of source and diagenetic processes. Selective partitioning of metals to various size-fraction particles is noted by Krumgalz (1989) in contaminated sediments. Mobilization and transfer can also occur if physico-chemical conditions change (Calmano et al., 1988). Copper is particularly noted for sorption on freshly precipitated iron hydroxide and on algal cell walls.

III.2 BIOLOGICAL IMPORTANCE OF SPECIATION

General discussions of biological availability frequently consider either metal deficiency with humans and economically important organisms or metal toxicity. In these and other cases it is really a discussion of what species of metal can be acquired, mobilized, and used by the organism whether it is a wheat stalk or a human. Zumkley and Spieker (1988) comment that our knowledge of metal bioavailability is insufficient and that, with humans as other organisms, there are age-specific abnormalities in trace element metabolism. Morrison et al. (1989), in an article entitled "Metal speciation and toxicity" comment that (abstract) "For example, copper ions are very toxic while copper bound to natural organic matter is innocuous". What they should have said is that copper ions are biologically available, that excess ionic copper may be toxic. An understanding of the interactions of copper with other metals as well as organics is also critical to an understanding of copper bioavailability. Changes in the forms of dietary molybdenum ingested by ruminants will, for example, reduce copper absorption (Gawthorne, 1987).

The availability of copper to plants is important and estimates of availability are often taken when metal deficiencies or excesses are anticipated (e.g. Cox, 1987; Gembarzewski, 1987; Gupta et al., 1988b; Haque, 1987; Luchese and Bohnen, 1987; San Valentin et al., 1986; Willaert and Verloo, 1988). This is also true when knowledge is needed about the effect of copper application as a nutrient (Das and Mandal, 1988; Jasiewicz and Gorlach, 1987; Sedberry et al., 1986), as anthropogenic copper (Gallardo-Lara and Nogales, 1987), or when used as a cidal agent (Menkissoglu and Lindow, 1988). The degree of copper accumulation in plant roots is a good indicator of copper supply to plants (McLaughlin and Crowder, 1988; Ruszkowska and Lyszcz, 1988). In metal-enriched soils, root metal concentrations can be excessive (Haque, 1987). Geochemical interactions play important roles (e.g. Cadahía et al., 1988; Godfrin et al., 1989; Joshi et al., 1988; Luoma, 1989; McCall and Smith, 1988; Szakmany, 1986; Weil and Holah, 1989). Since ionic copper forms an available source of metal in water (e.g. Menkissoglu et al., 1988; Sunda et al., 1987) and soils, pH and Eh often correlate with copper availability rather than total metal (Aboulroos et al., 1989; Clark and Gourley, 1987; Dean-Ross and Mills, 1989; Dutta et al., 1989; O'Sullivan et al., 1989) or uptake by plants (Sillanpää, 1987). In media such as sewage and wastewater, factors such as ammonia can also be important (Sato et al., 1988a). Availability is also related to organic content because of the metal complexing properties of natural as well as anthropogenic organic material (e.g. Apte et al., 1989; Bezborodov, 1989; Coale and Bruland, 1990; Domergue and Védy, 1989; Hirose, 1990; Kosakowska et al., 1988; Marquenie and Bowmer, 1988; Mathur, 1983; Moffett et al., 1990; Nakashima, 1988; Riedel and Sanders, 1988; Zhou and Wangersky, 1989c). As such, the measurement of complexing capacity forms a source of data (Mackey and Szymczak, 1988; Qian et al., 1986; Zhou and Wangersky, 1989b) which can provide information important in understanding metal bioavailabity (e.g. Wangersky et al., 1989; Zhou and Wangersky, 1989a). The presence of humic substances can reduce the availability of metals, including copper (Piccolo, 1989). (Aplincourt et al. (1988) discuss some of the chemical relationships between copper and humus-like organics.) In contrast, Treeby et al. (1989) demonstrated mobilization of soil Fe, Zn, Cu and Mn by root exudates from iron-deficient barley. Metal-metal interactions can affect copper availability. Karamanos et al. (1989) note that high Mn:Cu ratios led to copper deficiency in Canola, a condition that could be corrected either by adding copper or increasing the concentration of sulfur. An increase in sulfur can cause an increase in extractable soil copper (Modaihsh et al., 1989).

Delhaize et al. (1987) provide a good review of copper in plants, its relation to soils and availability to animals. Since humans, along with most economically important animals obtain the major portion of their copper from foods, availability must consider the chemistry of the food (e.g. Kumar et al., 1987; Talati and Patel, 1988) as well as that of the animal (e.g. Smith, 1989). Tissue copper levels have been used as indicators of metal bioavailability (e.g. Ledoux, 1987) although this is more an indication of uptake and metabolism that simply bioavailability. Copper supplementation is often used to improve the copper status of animals, a situation which must also be evaluated in terms of metal availability and the physiology of uptake (e.g. Edmiston and Bull, 1988).

III.3 COPPER-CONTAINING ORGANOMETALLICS

The field of organometallic chemistry has grown rapidly in the recent past, as indicated in the textbook by Powell (1988) entitled "Principles of Organometallic Chemistry". The biological importance of copper complexes is discussed in a series of papers from a symposium on the subject, edited by Sorenson (1987). Information on specific groups of organics, including information on copper, is included in the Proceedings of the Second Forum on Peptides edited by Aubry et al. (1989), the volume on metalloproteins edited by Otsuka and Yamanaka (1988), and specific chapters in these and other major works, chapters which include metalloproteins (Hanson and Wilson, 1989), copper proteins (Nakamura, 1988), hemocyanin (Makino, 1988), plastocyanin (Katoh. 1988) and superoxide dismutase (Asada, 1988). The importance of copper-containing organometallic agents in controlling metal availability in natural waters continues to be examined (e.g. Bezborodov, 1989) and techniques developed to isolate and identify major groups of agents (e.g. Apte et al., 1989). The biological roles and activities of copper-containing organics in humans and other organisms are not always easy to identify although considerable effort is being made to do this (e.g. Ferris et al., 1989; Kovacic et al., 1989; Tanaka, 1988; Tyeklar and Karlin, 1989) as well as to better understand the chemical structure and properties of the compounds (e.g. Bernardo et al., 1989; Hasnain, 1987; Lukasiewicz and Wozniak, 1989; Smiley and Triantafyllakis, 1988; Verma et al., 1989).

Major groups of copper-containing organometallic compounds and environmentally important organic copper-complexing agents are given below. Selected references are given here with others used elsewhere in the review.

Humic substances

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

Review: Shotyk, 1988.

Occurrences: Benes and Pabianova, 1987; Fischer et al., 1986.

Chemistry: Alberts et al., 1989; Berggren, 1989; Dolidze, 1988; Gregor et al., 1989; Hering and Morel, 1990; Hiraide et al., 1988; Lovgren and Sjoberg, 1989; Senesi, 1986; Ugrekhelidze and Dolidze, 1987; Verweij et al., 1989; Zhuang et al., 1988.

Biological importance: Graneli et al., 1989; Mathur, 1983.

Amino acids

Organic acids containing the amino group NH_2. They are the building blocks of proteins. Albourine et al., 1989; Albrigo and Taylor, 1989; Anderegg and Ripperger, 1989; Bottari et al., 1989; Carrell et al., 1988; Chen et al., 1989a; Ehrenberg et al., 1989; Henry and Delpuech, 1989; Johnson et al., 1989b; Lomozik and Wojciechowska, 1989; M'Boungou et al., 1989; Prasad et al., 1989; Sarkar et al., 1988a; Satyanarayana and Reddy, 1989a,b; Scrimin et al., 1988; Shanthi et al., 1988; Suzuki et al., 1989; Todd and Arnold, 1989; Zhou et al., 1988b.

Nucleic acids and copper-containing agents affecting these acids

Nucleic acids are organic acids responsible for the genetic makeup of an organism or the transmission of genetic information to the operation of the cell. Grisvard et al., 1989; Hudson and Mascharak, 1989; John et al., 1989; Mellano and Cooksey, 1988; Onori et al., 1987a; Pawlowski et al., 1989; Reed and Douglas, 1989; Sagripanti and Kraemer, 1989; Shahabuddin et al., 1989; Shirahata et al., 1989; Stockert, 1989; Tamilarasan et al., 1989; Veal and Rill, 1988, 1989; Wang and van Ness, 1989; Yamamoto and Kawanishi, 1989.

Copper-containing drugs

A number of copper-containing "drugs" are listed elsewhere in this section since the articles discuss the chemistry of the compounds rather than their action as drugs. Articles listed in this sub-section discuss the compunds as drugs as well as dealing with their chemistry. Abdel-Gawad et al., 1987; Ali et al., 1988b; Antholine et al., 1987, 1989; Berners-Price and Sadler, 1988a,b; Bressan et al., 1989; Casy and Taylor, 1989; Cook and Ternai, 1989; Elliott, 1987; Emerit et al., 1989; Ghandour et al., 1989; Hasinoff, 1989; Hay et al., 1989; Hoey, 1987; Iizuka and Maeda, 1988; Johansson, 1989; Kaden et al., 1989; Knapp et al., 1989b; Mercer-Smith et al., 1988; Mezyk and Armstrong, 1989; Mohanan and Aravindakshan, 1989; Mollin et al., 1989; Morazzoni et al., 1988; Morphy et al., 1989; Nishikawa et al., 1989; Parashar et al., 1987; Patel et al., 1989; Patil et al., 1989; Shetty and dMelethil, 1987; Sorenson, 1989b; Steiner et al., 1988; Tsipis et al., 1988; Ueno et al., 1988; Underhill et al., 1989; Vaselinovic and Kapetanovic, 1989.

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". These may fail to function correctly under copper deficiency.

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

Reviews - Czapski and Goldstein, 1988

Activity under normal conditions - Dameron and Harris, 1987; Emerit et al., 1989; Ischiropoulos et al., 1989; Kajihara et al., 1988a; Konstantinova and Russanov, 1988; Ohkuma et al., 1987

Activity and function under abnormal or deficient conditions - Coffey, 1988; DiSilvestro and dMarten, 1989; Hendricks-Munoz et al., 1989; Jewett et al., 1989; Kinoshita et al., 1988; Lee et al., 1988; Mylroie et al., 1987; Palozza et al., 1988; Paoletti and Mocali, 1988; Saito et al., 1989; Sakagishi and Hayashi, 1988; Steinkuhler et al., 1988; Taniguchi et al., 1988, 1989.

Biochemistry, chemistry - Banci et al., 1989a,b; Becker, 1987; Bertini et al., 1988a; Calabrese et al., 1989b; Desideri et al., 1987, 1988, 1989a; DiSilvestro, 1989; Frigerio et al., 1989; Fukui et al., 1988; Hallewell et al., 1989; Hasnain et al., 1986; Kanematsu and Asada, 1989a,b; Nadziekjo et al., 1989; Roe et al., 1988; Schinina et al., 1986; Tomita et al., 1988; Viezzoli and Wang, 1988; Willingham and Sorenson, 1987

Genetics - Gralla et al., 1988; Hass et al., 1989; Kajihara et al., 1988b; Phillips et al., 1989; Scioli and Zilinskas, 1988; Seto et al., 1989

SOD-like copper complexes - Foye and Ghosh, 1988b; Luo et al., 1987, 1988; Parashar et al., 1987; Shen et al., 1987

Hydroxylases: Blumberg et al., 1989a,b; Kawaguchi et al., 1988; Scott and Eidsness, 1988, Scott et al., 1988.

Nucleases: Graham, 1988; Murakawa et al., 1989; Sigman and Chen, 1989; Sigman and Spassky, 1989; Sigman et al., 1988a,b; Thederahn et al., 1989.

Oxidases: Beinert, 1988; Bertini et al., 1988b; Brenner and Klinman, 1989; Brenner, 1988; Chan, 1988b; Copeland et al., 1988; Dooley, 1988; Dyer et al., 1989; Esaka et al., 1988; Fry and Peschek, 1988; Gasparian and Nalbandyan, 1987; George et al., 1987; Gotoh et al., 1989; Greenaway and O'Gara, 1987; Hasinoff et al., 1989; Klinman et al., 1988; Kroneck et al., 1989; Kobayashi et al., 1989b; Lewinsohn, 1988; Lewinsohn et al., 1989; Li et al., 1988, 1989; Lindsay et al., 1987; Markossian et al., 1989; McCormick et al., 1989; Messerschmidt et al., 1989; Mondovi et al., 1988; Morgan et al., 1989; Morpurgo et al., 1988; Muller et al., 1989; Numata et al., 1989; Ohkawa et al., 1989; Ralston and Milne, 1989; Sakurai et al., 1989; Salerno et al., 1989; Tur and Lerch, 1989; Wrigglesworth et al., 1988; Yamada et al., 1987; Yewey and Caughey, 1988.

Reductases: Jin et al., 1989; Jones et al., 1988; Kroneck et al., 1988; Lee et al., 1989; Riester et al., 1989; Scott et al., 1989; Serafini et al., 1989; Viebrock and Zumft, 1988; Yount et al., 1989.

Miscellaneous enzymes: Angleton and Van Wart, 1988a,b; Ginalska et al., 1989; Green, 1989; Homer and Pierce, 1989; Laschi and Rossi, 1989; Morton, 1989; Tu et al., 1989.

Blue Copper Proteins: Adman et al., 1989; Casella et al., 1989; Desideri et al., 1989b; Farver and Pecht, 1989; Karlsson et al., 1989a,b; Hanna, 1988; Hanna et al., 1988; Huber, 1989;

Haemocyanins and haemocyanin-like compounds: Balaji et al., 1989; Begley et al., 1989; Depledge and Bjerregaard, 1989; Fenton, 1989; Lang, 1988; Latour, 1988; Linzen, 1989; Mazur and Gondko, 1988; Pate et al., 1989; Piguet et al., 1989; Tan et al., 1989; Volbeda and Hol, 1989a,b; Volbeda et al., 1989; Vuillaume et al., 1989; Westmoreland et al., 1989.

Miscellaneous organics: Antholine et al., 1989; Bailey et al., 1988; Baron et al., 1989; Baumy and Brule, 1988; Bellosta and Czernecki, 1989; Bernardo et al., 1989; Block et al., 1988; Burch et al., 1988; Calabrese et al., 1989a; Casassas et al., 1989; Cazorla et al., 1988; Chikvaidze, 1988; Chin and Jubian, 1989; Costes et al., 1989; Cousins and Barber, 1987; Cowan, 1988; DeZwart et al., 1987; Durell et al., 1988, 1989; Eaton et al., 1989b; Fuchs et al., 1987; Fujiwara and Tasumi, 1988; Gan, 1988; Gao et al., 1989; Goode et al., 1988; Gargano et al., 1989; Harrington, 1989; Harrington et al., 1987; Harris, 1987; Jeffery, 1989; Kachurin et al., 1989; Kaivarainen and Rozhkov, 1987; Karlin et al., 1988; Karpinski, 1989; Katseva et al., 1988; Kiss et al., 1989; Kowalewska and Hoffmann; Kohzuma et al., 1989; Kratsmar-Smogrovic et al., 1988; Kuriata et al., 1988; Lau et al., 1989; Laubry et al., 1988; Lehn and Rigault, 1988; Linderman and Lonikar, 1988; Liu and Pang, 1989; Malhotra and Sharma, 1988; Manoharan et al., 1989; Martin and Evans, 1988, 1989; Massoudipour et al., 1989; Matsumoto et al., 1989; McLean and Hagaman, 1989; Menger and Tsuno, 1989; Micera et al., 1989; Mikaelyan and Nalbandyan, 1988; Mikaelyan et al., 1988; Moriya et al., 1989a; Mostafa et al., 1987; Onori et al., 1987b; Orbán, 1988; Penner-Hahn et al., 1989; Pennings et al., 1988; Permyakov et al., 1988; Pickart and Lovejoy, 1987; Prabhakar et al., 1989; Ranga et al., 1986; Rao et al., 1988b; Reddy, 1988; Reddy et al., 1989a; Reed and Madhu, 1987; Revankar and Mahale, 1989; Rogic and Naumski, 1989; Rush et al., 1988; Sakharov and Skibida, 1988; Salehi et al., 1988; Sandhu and Sharma, 1989; Shinar et al., 1989; Singh and Srivastava, 1989; Sorrell, 1989; Steele et al., 1988; Stepien et al., 1989; Suzuki et al., 1989; Tabak et al., 1989; Tamaru et al., 1989; Temizer and Sarisoy, 1987; Tripathi et al., 1988; Tschudin et al., 1989; Vajtner and Suskovic, 1988; Varnagy et al., 1988; Yanada et al., 1989; Yang and Babitch, 1988; Yip and Hutchens, 1988.

Hormones: Barnea and Bhasker, 1989; Gibson and Glembotski, 1987; Jellinck and Newcombe, 1988; Jungclas et al., 1989; Medici et al., 1989.

Metallothionein-like organics

Metallothionein and metallothionein-like organics transport copper within the organism. Since they bind copper they can assist the organism either in elimination of excess metal or storage of metal for subsequent use.

General, including reviews: Engel, 1988; Engel and Brouwer, 1989; Hamer and Winge, 1989 (proceedings of a colloquium on metal ion homeostasis).

Occurrences: Drasch et al., 1989; Eriksen et al., 1988; Freedman et al., 1989b; Gekeler et al. (phytochelatins); Goncalves and Conceicao, 1989 (metal-binding in an alga); Grill, 1989 (phytochelatins); Melkonyan and Nalbandyan, 1989; Mehra et al., 1988; Melkonyan et al., 1989; Nishizono et al., 1988 (copper-thiolate complex in a fern); Roesijadi and Morris, 1988; Vieira and Nascimento, 1988 (copper complexation in a chlorophyceaean).

Chemistry and/or function: Beltramini et al., 1989; Brouwer and Brouwer-Hoexum, 1989; Brouwer et al., 1989; Eannetta and Steffens, 1989 (phytochelatins); Ebadi et al., 1987; Grider and Cousins, 1989; Grill et al., 1988 (phytochelatins); Huibregtse et al., 1989; Kleczkowski and Barej, 1989; Malikayil et al., 1989; Richards, 1989d; Roesijadi et al., 1989; Stillman et al., 1989; Tanaka et al., 1990b; Thurman et al., 1989 (phytochelatins); Viarengo et al., 1989

Detoxification: Czaja et al., 1988; Freedman et al., 1988; Misra, 1989; Verkleij et al., 1989 (metal-binding compounds in *Silene*).

Genetics: Aladjem et al., 1988; Bergman and Timblin, 1989; Erraiss et al., 1989; Farr and Hunt, 1989; Furst and Hamer, 1989; Furst et al., 1988a,b; Jacobs et al., 1989; Macreadie et al., 1989; Maiti et al., 1989; Munger et al., 1989; Romeyer et al., 1988; Rouch et al., 1989a,b; Skroch et al., 1990; Sugimoto et al., 1988; Timblin, 1988.

Miscellaneous -

Rubidium sphaeroides - copper has been used to study exchange reactions with Rb sphaeroides. Calvo et al., 1989.

Zeolites - copper has been used as a probe to study the coordination sites of these hydrated silicates of aluminum. Anderson and Kevan, 1988; Packet and Schoonheydt, 1988.

Fenton-like reactions - the reaction between H_2O_2 and a transition metal in association with a ligand. Sutton, 1989; Sutton and Winterbourn, 1989.

III.4 ADSORBING AGENTS

Adsorption is used to describe metal binding to particle surfaces. Adsorption is also used to note the binding (**but not uptake**) of metal by some biological surfaces. Since particulates form an important fraction of aquatic and terrestrial environments, it is appropriate to have a discussion of the topic using the relatively few papers that have recently appeared on the adsorption of copper. It is most important to note that even with the obvius importance of particle adsorption, little is really known about the factors that affect the uptake and exchange of metals by particles (Jenne and Zachara (1987). And this needs remedying because the chemistry of a metal adsorbed to a particle (including colloids) is different than when it is in the dissolved state. It is also important to relate metal-particle to metal-solution chemistry because metals can be adsorbed either as an ion or as a complex (e.g. Goldberg et al., 1988). Clegg and Sarmiento (1989) use a chemical model to address some of the problems associated with the scavenging of metal ions by marine particulate matter.

The interaction of clay mineral particles and trace metals has been examined not only to examine chemical interactions but also to relate this to changes in chemical parameters such as pH and organic matter (Zhang and Liu, 1988; Zheng et al., 1987). Xia et al. (1987) make application of this information to adsorption of Pb, Cd and Cu to fine sediments in the Yangtze River estuary, pointing out that the adsorption can be described by the Freundlich equation. Gallacher and Pulford (1989) note that, with sorption of copper by soil from solution, the Freundlich lines were all curved but could be resolved into two straight lines. In contrast, copper adsorption by any soil could not be described by the Langmuir equation. Clay minerals such as montmorillonite have been proposed as a mechanism to adsorb excess metal from solution (Raymahashay, 1987). The addition of organics, such as humic acids, to clay minerals has been demonstrated to increase the adsorption of copper (Galicia and Schindler, 1989). This may demonstrate what is happening in natural environments where a combination of clay minerals and organics would be expected. However, interactions with both natural and anthropogenic organics (e.g. fertilizers, pesticides) can affect the copper adsorption capabilities of trace metals such as copper (Raman and Rao, 1986; Slosiarikova et al., 1986; Zima and Pis, 1987). Altmann and Leckie (1987) discuss results with a composite-isotherm model that examines adsorption and complexation characteristics of trace metals with humic substances and with hydrous oxides. They note the problems of attempting to use simple models to explain sorption by structurally complex agents such as humic matter or bacterial surfaces.

Biological material can act as a site of adsorption as well as a mechanism that modifies metal adsorption by clay minerals. In an evaluation of metal transfer from polluted sediments, Calmano et al. (1988) noted measurable enrichment of copper on algal cell walls as well as on freshly precipitated iron hydroxide. (Aluminum hydroxides are also noted to adsorb copper and other metals from solution (Pavlova and Sigg, 1988).) Chitosan, derived from a natural polysaccharide, has been proposed as a mechanism to adsorb excess metal from solution (McKay et al., 1989). In contrast to the effect of biological material on adsorption, clay minerals have been used as metal buffers with livestock. Schwarz and Werner (1987), for example, note lower liver copper levels in some mini-goats after long-term bentonite application.

Several recent studies address the specifics of "adsorption" of copper to biological surfaces, either to examine the physical-chemical nature of the relationship or the biological importance of processes associated with metal-organism interactions. Möhl et al. (1988) used magnetic resonance techniques to examine copper(II) ions adsorbed on deactivated bacteria. Binding capabilities of cell walls from cultured cells are described by Venkateswerlu and Stotzky (1989). Gadd and de Rome (1988) report that the addition of fungal melanin to a copper-containing culture of a fungus reduced the detrimental effects of excess copper. Rao and Venkobachar (1990) describe the copper adsorption characteristics of byproducts of biological and industrial processes. Allan and Jarrell (1989) evaluated copper adsorption to corn and soybean root cell walls using a surface complexation model originally designed for use with metal oxide surfaces. They note a difference between organic and inorganic surfaces which they can attribute to the characteristics of the surfaces. Wilhelm et al. (1988) examined uptake of several metals (including copper) by human scalp hair as well as the elution of the metals. They noted a binding capacity of Al > Cd > Cu > Pb > Zn and an elution capacity ranging between 0.1-11.8% for copper under the experimental conditions used.

IV - METAL-METAL INTERACTIONS IN ORGANISMS

Copper competes with several other metals for sites on organic ligands as well as for sorption sites on both inorganic and organic surfaces. The interactions with other metals are thus important not only for uptake but also for the performance of normal metabolic functions. In an excellent review of copper interactions, Gawthorne (1987) comments that "... it would not be an exaggeration to say that interactions with other trace elements are a part of the normal state of copper nutrition". Gawthorne (1987) then goes on to talk about the interactions of copper with molybdenum, sulfur, zinc, iron, cadmium, selenium, lead, nickel, ascorbate, drugs and hormones. This list of metals, metalloids and organics provides an indication of the numerous things with which copper interacts and which can affect the actions of copper in organisms. To provide an indication of the wide range of interaction effects, they can change the chemistry of compounds (e.g. Lukasiewicz and Wozniak, 1989), the varous reactions associated with photosynthesis (e.g. Jana and Bhattacharjee, 1988) and the tolerance of certain organisms to cadmium, through the interaction of copper with metallothioneins (Erraiss et al., 1989).

Copper-iron interactions

Iron and copper interact from the organism level down to the molecular level. It can enhance fouling of 90/10 copper-nickel alloys (Chamberlain and Garner, 1988a). Iron utilization can be affected by ascorbic acid in rats, moreso in copper deficient than copper replete rats, moreso in copper deficient females than males (Johnson, 1989a; Johnson and Murphy, 1988). Utilization of dietary iron may also be affected by copper levels in other organisms (e.g. Lepper et al., 1989). This is not to imply that interactions always occur, they don't (e.g. Hunter et al., 1989) although the competition between the two metals is always a factor. In cultures of rat small intestine cells, iron and copper are inhibitory above certain metal levels and the effect of any metal-metal interaction is additive. Iron can displace copper in ovotransferrins, a group of iron-binding proteins found in egg white (Xian-Xuan et al., 1989). In contrast, copper can interact with iron in a heme-like environment causing a series of spectral changes (Burch et al., 1988).

Copper-manganese interactions

The uptake of soil copper by plants can be affected by the concentration of soil manganese, uptake decreasing with increasing manganese (Crawford et al., 1989; Jasiewicz and Gorlach, 1987). Mn-induced copper deficiency can, however, be reduced by increased sulfur (Karamanos et al., 1989). Borchmann and Zajonic (1988b) note that a higher tolerance of soil copper deficiency in certain plants was accompanied with greater sensitivity to soil manganese deficiency (and vice versa). Working with a green alga, Noro (1985) noted that manganese uptake from a growth medium could be inhibited by the addition of zinc and copper. At least part of the Mn/Cu interaction is associated with the interaction of the metals on superoxide dismutase activity (e.g. Chang and Kosman, 1989). Fernandes and Manso (1988) note that in human erythrocytes, (abstract) "Manganese decreases the formation of methemoglobin and partially inhibits lipid peroxidation induced by copper ...".

Copper-molybdenum interactions

Nordberg et al. (1986) discuss some of the interactions between copper and molybdenum in ruminants. Molybdenum, frequently with sulfur as thiomolybdate, is capable of reducing tissue copper levels in humans as well as a number of economically important animals (Chase, 1987; Golfman and Boila, 1989; Gooneratne et al., 1989a,c,d; Ke and Symonds, 1989; Kincaid, 1988; Kincaid and White, 1988; Lamand, 1989; McArdle et al., 1989b; Read et al., 1989; Sas, 1987, 1989; Symonds and Ke, 1989; Wang et al., 1988b; White et al., 1989a; Wittenberg and Boila, 1988; Yang and Yang, 1989). Falke and Anke (1987) note, however, that molybdenum supplementation to goats produced an increase in blood serum and organ copper levels as well as an increase in fecal copper loss (which should not have occurred with tissue copper increases). Certain soils and forage crops growing on them have elevated levels of molybdenum (e.g. Regiusne Mocsenyi, 1988) and can produce a copper deficiency if used as feed materials. Thiomolybdate has been advocated as a treatment for animals and humans with excess copper (e.g. Gooneratne et al., 1989b,e; Kumaratilake and Howell, 1989b;

Vrzgula et al., 1988). Gooneratne et al. (1989a), however, warn about the potential for copper deficiency because of excess binding of copper by thiomolybdate.

Copper-zinc interactions

The addition of zinc to some types of soils can modify the copper nutrition of plants (Gupta and Gupta, 1987; Sarkunan et al., 1989; Taban and Turan, 1987). A similar statement can be made in aquatic environments. Wong and Chau (1988) noted that of a group of 10 metals, the Zn-Cu combination was the most toxic to freshwater algae and phytoplankton. However, a reduction in the zinc alleviated the toxicity. These results suggested (abstract) "... that water quality objectives should not be based on toxicity data with individual metals". Nordberg et al. (1986) review some important copper-zinc interactions in a discussion of the factors influencing the effects and dose-response relationships of metals. Korbashi et al. (1989) reports that zinc acts in a protective fashion with the microorganism *Escherichia coli*, against copper-mediated paraquat-induced damage. A protective effect has been noted in other cells, possibly as a result of zinc-mediated induction of metallothionein production (Schilsky et al., 1988). Gangwar et al. (1989) found that the activities of some enzymes in rice were better related to the Zn:Cu ration than were metal concentrations in leaves.

With a nematode worm species, a synergistic zinc-copper relationship has been suggested for the response to elevated levels of metals (e.g. Vranken et al., 1988). Supplementation of high levels of zinc to channel catfish is not believed to impair the copper status of the fish (Gatlin et al., 1989). Dietary supplementation of zinc has been reported to have an effect on zinc:copper levels in other animals (e.g. Bonomi et al., 1988; Kabat, 1988), including humans (Castillo-Duran et al., 1988; Liu et al., 1988a; Sievers et al., 1988; Turnlund et al., 1988; Turnlund and Keyes, 1990; Yadrick et al., 1989). Dietary zinc:copper ratios may be more critical when diets are low in either or both zinc and copper (Frimpong and Magee, 1989). Correlations between zinc and copper concentrations in the horse kidney are reported by Koizumi et al. (1989). Zinc may interact with copper at the sites of absorption and utilization; excess zinc may be accumulated more rapidly than excess copper (suggested from case report of Ogden et al., 1988). Ingestion of excess zinc is reported to decrease tissue copper levels (from Stahl et al., 1989). However, Stahl et al. (1988) found that ingestion of excess zinc by hens did not have (abstract) "... consistent effects on the iron or copper content of eggs ...". Zinc deficiency in diabetic rats has been associated with a greater likelihood of excess tissue copper (Deebaj et al., 1989). Abnormal metabolism of zinc and copper is reported with physiological problems, including atherosclerosis (e.g. Chang et al., 1988), liver cirrhosis (e.g. Liu et al., 1988a), certain types of cancer (e.g. Wasowicz et al., 1989). Not surprisingly, interactions between zinc, copper and physiologically important organics have been demonstrated (e.g. Rao, 1988; Yang et al., 1988). Zinc supplementation is an effective treatment for the high tissue copper levels in animals and humans with Wilson's disease (Brewer et al., 1990; Milanino et al., 1989b; Rossaro et al., 1987), an autosomal recessive disorder.

Copper-cadmium interactions

Cadmium has been reported to inhibit copper activation of an enzyme (nitrous oxide reductase) in a microorganism, at least under conditions of copper deficiency (Minagawa and Zumft, 1988). In a study of factors aggravating cadmium health effects in old rabbits, Nomiyama et al. (1990) noted aggravation of effects by excessive dietary zinc or copper, a condition not found in younger animals. Copper (as well as Se, Cd and Zn) levels in the kidney cortex were "signficantly lower" in autopsy tissues from cadmium exposed humans than in nonexposed subjects (Kido et al., 1988).

Interactions of copper with other metals, metalloids and nutrients

Cobalt - In Sprague-Dawley rats, cobalt produced a substantial increase in urinary excretion of copper, over a 72-h period after a single dose of cobalt (250 μmol/kg body weight; Rosenberg and Kappas, 1989a; see also Rosenberg and Kappas, 1989b).

Mercury - Working with the uptake of mercury from aqueous solution by duckweed, Mo et al. (1989) note that (abstract) "The presence of copper ion suppressed the mercury uptake significantly".

Lead - Sone (1989) notes that, in male Wistar rats, lead appears to have some effect on body copper distribution but the mechanism is unknown. Flora et al. (1989) note that copper supplementation caused a reduction in lead uptake and lead-induced alterations in rats.

Nickel - Copper-nickel interactions in soil are reported to affect metal concentrations in rice (Sarkunan et al., 1989). Vranken et al. (1988) report that copper and nickel act synergistically in terms of detrimental effect to a nematode worm species. The uptake and regulation of nickel may be affected by copper tolerance in crayfish (Alikhan and Zia, 1989).

Tin - Rader et al. (1990) report that (abstract) "Sn significantly depletes Cu in rats fed +Cu and -Cu diets. The effects may occur through decreased Cu absorption, increased fecal Cu losses, or effects of Sn on Cu functions". (Also reported in Rader et al., 1989.)

Boron - Boron may interact with plant uptake of a number of elements from the soil, including copper, (Mozafar, 1989). In a review on "The Making of an Essential Element" McBride (1989) comments that boron may be involved in the metabolism of copper in humans.

Barium - van Veggel et al. (1989) report that barium can affect the organization of copper-containing organic complexes.

Magnesium - Working with the bacteria *Escherichia coli*, Lebedev et al. (1989) conclude that excess copper increased the uptake of magnesium simply by non-specific influx of cations into the cell.

Platinum - In rats, Reeves et al. (1990) report that some of the toxic effects of cis-diamminedichloroplatinum (II) were enhanced by low copper status.

Selenium - Working with the interactive effects of selenium and copper on the flatworm *Dugesia dorotocephala*, Rauscher (1988) found that selenium initially appeared to antagonize the effects of copper toxicity but subsequently seemed to have synergistic effects. In sheep, White et al. (1989b) found that long-term ingestion of moderate levels of copper influenced the metabolism of selenium, possibly a result of excess copper rather than metal-metal interaction. With dairy cows, supplemental copper did not influence absorption of dietary selenium (Koenig et al., 1989). Selenium supplementation in humans has been associated with a slight but not significant tendency to decreasing urine and blood values of zinc, copper, iron and lead and in plasma copper levels of women taking oral contraceptives (Nève and Vertongen, 1988).

Calcium - Calcium and copper may be antagonistic, in terms of uptake by an aquatic plant (*Hydrilla*; Anderson and Dechoretz, 1986) and by brown trout fry (Sayer et al., 1989). In dairy calves, pancreas copper levels were reduced with certain calcium-supplemented diets (Alfaro et al., 1988).

Potassium - Factors that affect the availability of soil potassium, for plant uptake, are reported to be more strongly influenced by copper than by cadmium (Yang and Skogley, 1989).

Phosphorous - Gembarzewski and Korzeniowska (1989) report that, for spring wheat, the optimal application of phosphorous is dependent on soil copper content, phosphorous requirements increasing with increasing soil copper levels. However, the effect of pH on copper speciation can change the relationship between copper and phosphorous (e.g. Wallace and Cha, 1989). Other factors also appear to both directly and indirectly affect this relationship (El-Shourbagy et al., 1989; Grün and Scholz, 1988; Javadi, 1988; Modaihsh et al., 1989). These factors appear to be important in forages since phosphorous fertilization procedures have been associated with poor growth and a reduction in cattle liver copper (Wadsworth et al., 1988).

Nitrogen - Copper uptake and tissue concentration in plants may be affected by the nitrogen status of the soil and by the protein status of the plant (e.g. Kadar and Shalaby, 1985).

V. UPTAKE AND ACCUMULATION OF COPPER BY ORGANISMS

That copper is essential for organisms is accepted and continues to be demonstrated (e.g. Adams et al., 1989). However, to be of use to an organism, copper must first be acquired either from an environmental source or from food. After uptake, the metal must be transported to the site where it is needed or to a storage site for later use. This section uses recent literature in a discussion of some of the aspects of the uptake and transport of copper by organisms. Summary information on the subject is available in Beveridge and Doyle (1989), Camakaris (1987), Girchev and Tzachev (1988), Hopkin (1989) and Stamp (1988). Sunda et al. (1987) use their own work to point out that the free ion concentrations indicate metal bioavailability in aquatic systems. Morrison et al. (1989) comment (abstract) that "In polluted waters the toxicity of heavy metals to aquatic plants and animals depends on their physicochemical form (i.e., speciation). For example, copper ions are very toxic while copper bound to natural organic matter is innocuous." The importance of metal speciation is apparent, but for bioavailability, the concentration of the biologically available state and the nature of the organism will dictate whether there is enough excess metal to be detrimental. As Howell and Gooneratne (1987) point out (page 54) "Low concentrations of copper in the diet are necessary for survival, but high concentrations may be toxic". A variety of agents both in the environment and in the food affect metal availability (Baumy and Brule, 1988; Raghib and Blincoe, 1989), agents that range from amino acids to clay mineral particles to capsular polymers in bacteria (Geesey and Jang, 1989).

In a discussion of copper accumulation by bacteria in sea water, Stupakova et al. (1989) point out (abstract) that "The copper accumulation factor K ... is higher by 1-3 orders of magnitude than the analogous quantities for other marine organisms". Sorption of copper by bacteria is a major uptake mechanism in aquatic and terrestrial environments (Cotter and Trevors, 1988; Geesey and Jang, 1989; Mullen et al., 1989). Cotter and Trevors (1988), however, found no conclusive evidence that Cu^{2+} was accumulated in *Escherichia coli* cells by an active transport mechanism. Zoroddu et al. (1989) used electron paramagnetic resonance with a thermotolerant bacteria and found evidence suggesting the presence of strong ligand "fields". They suggest that copper could be taken up either by active or passive transport. Rouch et al. (1989a) used mutant types of *E. coli* to model copper metabolism. Accumulation of metals is high in certain bacteria (e.g. Golab and Orlowska, 1988a), Solanellas and Bordons (1988) report copper retention up to 3.8% of cell dry weight with copper-resistant bacteria isolated from activated sludges. As such, they may be able to act as metal-transporting agents in water (Knowles, 1987). Schreiber et al. (1990) discuss the production of an extracellular copper-binding compound by a heterotrophic marine bacterium (*Vibrio alginolyticus*), produced in response to elevated levels of added copper. They comment that this suggests a potential role of bacterial metabolites to modulate copper ion activity in seawater. In an examination of the effects of long-term pig slurry application, Christie and Beattie (1989) did not find phytotoxic concentrations of copper and did not find inhibition of nitrifying microbial populations in the soil. Mann and Fyfe (1989) note that acidophilic microorganisms play an important role in the metal budget of mine-tailings waste. By efficiently sequestering aqueous metals and promoting nucleation of oxide minerals they retard metal dispersion into the natural environment.

Plant acquisition of copper from soils is dictated by the nature of the soil, the chemistry of the metal and the nature of the plant. Sillanpää (1987) provides a discussion of the micronutrient status of soils in different parts of the World and Youssef (1988) reviews the various factors that influence the mineral profiles of tropical grasses in the Caribbean, Latin America and Africa. The assessment of soil copper bioavailability is accomplished primarily by metal extractability or plant growth and metal concentrations (e.g. Gembarzewski, 1987; Gembarzewski et al., 1987). As Cox (1987) points out, however, (page 103) "Relatively little research has been conducted to interpret soil tests for Cu, especially field calibration (of soil tests)."

In an evaluation of the micronutrient nutrition of a pine species, Saur (1989) notes that quartz soils characteristically have low levels of copper. Using HCl and EDTA (ethylenediaminetetraacetic acid) extraction techniques, Jokinen and Tahtinen (1987b) noted closer correlation between extractability and oat growth in peat soils than coarse mineral soils. Gettier et al. (1989) failed to find a relationship between soil copper additions and corn leaf copper concentrations even though soil metal extraction (DTPA) indicated increased copper concentrations present. In "heavy soil not contaminated with heavy metals", Gorlach and Gambus (1988) found elevated root copper concentrations in Italian ryegrass. Borgegard and Rydin (1989) used birch biomass and root penetration to estimate the upward movement of copper from copper tailings, through a soil covering. They comment that leaf concentrations of copper were related to above and below ground biomass more than between soil and leaf metal concentrations. Soil pH is an important factor, affecting metal speciation and metal bioavailability in natural and anthropogenic metal-containing soils (e.g. Angle et al., 1989). However, in sewage sludge-treated soils, Hall et al. (1988a) comment (abstract) that "Cu levels in grass exhibited little change with pH ...". (Tissue copper concentrations may (e.g. Reddy et al., 1989b) or may not (e.g. Keller, 1988; Paris et al., 1987) be affected in plants grown on refuse or sludge-treated soil.) Jokinen and Tahtinen (1987a) examined the effect of pH on the efficiency of copper sulfate as a nutrient for oats and comparing effect with grain yield. An increase in soil pH caused a drop in grain yield and copper uptake, copper fertilization was highest without increasing pH (liming) as well as in soils with the greatest liming. The former is presumably due to an increase in ionic metal and bioavailability, the latter is unknown. (Possibly from agents in the lime itself.) Soil liming has been shown to have different effects on copper uptake and plant yield, depending in part on the nature of the plant (e.g. Gambus, 1987). Salinity effects on soil copper availability are of interest in areas where irrigation waters have elevated salt contents. Borchmann and Zajonc (1988a) found little effect of salinity on the dynamics of soil copper under the conditions of their experiments. Metal-metal interactions can have an effect (e.g. Wallace and Abou-Zamzam, 1989). As an example, Jasiewicz and Gorlach (1987) report that plant uptake of copper introduced as copper sulfate, decreased with increasing soil manganese oxide concentration. Other agents that have been shown or suggested to affect soil copper bioavailability include lithogen (Grupe and Kuntze, 1988) and sulfur, the latter through copper speciation and its effect on the metal-metal interaction of copper and molybdenum (e.g. Karamanos et al., 1989).

There is an interaction of copper and nitrogen with some plants, at least in certain soils. Kadar and Shalaby (1985) note that with a calcareous chernozem and barley, an excess of nitrogen as NO_3, increases plant uptake of certain cations, including copper. Lasztity (1988b,c) reports comparable findings with calcareous chernozem and a calcareous sandy soil, with winter wheat, rye and triticale although with NPK fertilization; a seasonal change in copper uptake is also reported. The rate of copper accumulation in triticale was found to be highest prior to milky ripening (Lasztity, 1988b; Lasztity and Biczok, 1988). (As shown by Clark et al. (1989), Reboredo et al. (1988) and others, seasonality in accumulation is not uncommon.) In a silty loam soil, Ikram-Ul-Haq et al. (1987) note that with *Coriandrum sativum,* (abstract) "the quantitative uptake of copper, ..., decreased in the order of nitrophos >dia-ammonium hydrogen phosphate >Urea >ammonium sulphate". With solanaceous fruit vegetables (tomato, eggplant and sweet pepper), Zhong and Kato (1988) report highest dry weight and fruit yield with a 7:3 ratio of nitrate nitrogen and ammonium nitrogen, at least in sand culture under glasshouse conditions. Higher concentrations of ammonium nitrogen severely inhibited growth and fruit development as well as reducing the concentration of cations, including copper (see also Zhong et al., 1988). Wallace (1989) reports that for monocots, ammonium increased uptake of copper. Large doses of PK (phosphorus and potassium) fertilizer on chalky soils have been associated with reduced copper levels in triticale (Lasztity, 1989).

The interaction of nitrogen and copper in certain soils implies that the uniqueness of a soil is important when considering soil copper bioavailabity. This has formed at least part of the reasoning behind work on various soil types and soils in various regions (e.g. Aboulroos et al., 1989; Malaiskaite, 1987; Rahmatullah et al., 1988; Rozema et al., 1988; Weil and Holah, 1989; Willaert and Verloo, 1988). A major reason for the uniqueness of a soil is the concentration of metal-binding agents such as humic substances. A result of the decomposition of plants, and possibly animals, humics have been shown to play important roles in controlling soil metal bioavailabity (e.g. Piccolo, 1989). Organic matter from anthropogenic sources, with or without humics, can also affect availability both in terrestrial soils and aquatic sediments (e.g. Barbera, 1987; Marquenie and Bowmer, 1988). As a result of the various factors, copper deficiencies may occur through both total copper and bioavailability. In these cases, supplementation is important for plant growth (e.g. Melendez et al., 1986).

As in the terrestrial environment, the uniqueness of aquatic environments includes the control of metal bioavailability, including copper. The presence of metal complexing agents, the presence of particles, and the occurrence of metal-metal competition, to name a few factors, all affect metal uptake. The source of the water and its history dictate the source and the concentration of metal. Nakashima (1988), for example, presents evidence suggesting that deep sea water is deficient in organic complexing agents which would complex metals. Complexing capacity of water has been an important factor in evaluating the biological availability of natural and anthropogenic copper (Mackey and Szymczak, 1988; Uchiyama et al., 1988).

The nature of the plant affects metal uptake as a result of chemical processes and physiological state (e.g. Baker and Brooks, 1989; Clark and Gourley, 1987; Gomes et al., 1988a; Mahan et al., 1989). Immobilized blue-green algae (*Nostoc calcicola*) cells have, for example, been shown to take up more copper than free cells (Singh et al., 1989c). Response to environmental factors is often unique to the species of organism. In a study of sorption by an alga, Greene and Darnall (1988) report a temperature dependence, higher sorption at elevated temperatures. Copper uptake by blue-green algae is reportedly higher with increasing temperature (Sharma and Azeez, 1988), at least to a point. Demon et al. (1989), however, noted little effect of temperature on uptake by an alga and a fungus. As a result of species uniqueness, some species of plants form particularly important storage and release agents in natural environments (e.g. Alberts et al., 1987; Kraus, 1988a). Others store metals in certain parts of the plant and can be used as bioindicators (e.g. Hogan and Morrison, 1988; Killingbeck and Costigan, 1988).

Actual uptake is considered to include initial association with the cell followed by uptake across the cell membrane and then transport within the plant (or cell). Sorption of copper to root cell walls may occur (Allan and Jarrell, 1989) although this may be affected by sorbed materials such as plaque-forming iron (Otte et al., 1987). Ross and Parkin (1989) note that with the fungus *Candida utilis*, copper uptake was biphasic, (abstract) "... initial binding being followed by energy-dependent transport into the cells". Recent work on copper uptake by microorganisms and plants includes Gadd and de Rome (1988), Geesey et al. (1988), Nishizono et a. (1989a), Venkateswerlu and Stotzky (1989). The ability of some microorganisms to release metal-binding agents is of academic interest (e.g. Guckert and Jean-Louis Morel, 1988; Mench et al., 1988; Morel et al., 1987; Treeby et al., 1989) but has been of value in leaching of oxides (Golab and Orlowska, 1988b). Metal tolerance has been related to the ability of tissues, particularly roots, to release one or more organic agents that rapidly bind excess metal (e.g. Cureton-Brown and Rauser, 1989). Many of these agents are normally produced for the uptake and transport of metals within the plant (e.g. Grill et al., 1988). Effects of both natural and synthetic complexing agents have been related to copper uptake in an attempt to understand not only the uptake process but also the effect of organic ligands on uptake (e.g. Gaisser et al., 1987; Iwasaki and Takahashi, 1989) and transport within the plant (Albrigo and Taylor, 1989).

Recent literature discusses copper uptake in a number of different types of animals. Viarengo (1989) provides an excellent review of some mechanisms involved in the regulation and toxicity of heavy metals in marine invertebrate organisms. Harland and Nganro (1990) found that the sea anemone *Anemonia viridis* did not take up copper in proportion to external concentrations. They suggested that regulation was achieved by mucus (possible complexation with mucopolysaccharides) and the expulsion of symbiotic algae when present. Tissue metal concentrations in an aquatic oligochaete worm are reported to be related to the concentration of copper in the adsorbed/exchangeable fraction of the sediment in which the organism lived (Broberg and McMasters, 1988). Uptake and depuration of copper in the clam *Arca subcrenata* is reported to be rather slow (Cui et al., 1987) and, in the euryhaline ribbed mussel *Guekensia demissa*, affected by extremes in salinity (Miller, 1988a). Mussels continue to be one of the more frequently examined groups of animals, in part because of their abundance and ease of use (e.g. Chan, 1988a), part because of their economic importance, and part because of their supposed suitability as bioindicators (e.g. Lakshmanan and Nambisan, 1989). Accumulation of copper in other bivalves has also been examined in part because of the reputed usefulness of the organism as indicators (Cain et al., 1987). Some bivalves have unique methods of metal acquisition. *Pinna bicolor*, for example, has large quantities of metal-sequestering nephroliths produced by the giant kidneys (Reid and Brand, 1989). Kidney granules containing copper are reported in a number of bivalves although not always associated with copper (e.g. Sullivan et al., 1988). Khomik and Karchenko (1989) examined uptake as part of their evaluation of bivalves, particularly the shells of freshwater bivalves, as mechanisms to remove metals from solution in a waterway (Dnieper-Donbass Canal, U.S.S.R.). Berger and Dallinger (1989) examined uptake by a terrestrial snail, commenting that copper assimilation efficiency exceeded 95% and that 49% of the ingested copper was found in foot and mantle tissues. With zooplankton in Xiamen Harbour, Lin and Li (1988) found little metal regulation, that tissue copper concentrations were proportional to copper levels in seawater over the range of concentrations found in the harbour. In a comparative study, Rainbow and White (1989) found little metal regulation in an amphipod crustacean (*Echinogammarus pirloti*) and a barnacle (*Elminius modestus*) but evidence of metal regulation in the shrimp *Palaemon elegans*. Anil and Wagh (1987) discuss copper concentrations in the barnacle *Balanus amphitrite*, reporting elevated tissue metal concentrations but using tissue dry weight:water concentration which makes comparisons more difficult than if tissue wet weight had been used. Dallinger and Prosi (1988) and Prosi and Dallinger (1988) describe membrane-limited vesicles (lysosomes) rich in several metals (Cu, Zn, Pb, Cd) in a terrestrial isopod. Lake pH and sediment redox conditions affect metal availability to benthic and near-benthic organisms (Young and Harvey, 1988). Uniqueness of the organism also is important; metal concentrations will be higher in certain body tissues of multicellular organisms (e.g. Khan et al., 1989). Concentrations in tissues are a result of the metabolism and transport systems of organisms as well as the physiological state of the organism (e.g. Depledge, 1989; Villegas-Navarro and Villarreal-Trevino, 1989; Zia and Alikhan, 1989). In fish, tissue concentrations have been correlated to exposure concentrations (e.g. Daramola and Oladimeji, 1989) as well as the ability to regulate uptake and storage. Copper concentrations are reportedly higher in ovaries than testes of the Ayu, a salmonoid (*Plecoglossus altivelis*; Ikuta, 1988). Nakagawa and Ishio (1989) note that uptake of several metals, including copper, by eggs of a fish (*Oryzias latipes*) was in part a result of metal being bound by the egg membrane. Accumulations of copper by fish have been recorded over time with preserved material (Johnson, 1989c) although the failed to show that fish tissue concentrations over time reflect temporal trends in metal loadings entering lakes. Other work (Dai and Zhang, 1988) suggests no distinct evidence of accumulation of metals from dietary sources. The U.S. National Technical Information Service (1989a) provides a reference list on bioaccumulation of heavy metals by fish for the period January 1977-May 1989.

In a study of soil invertebrates in copper fungicide-treated vineyard soils, Wittassek (1987a) comments that (synopsis) "In general physiological requirement and ecological factors have more influence on copper accumulation than food chain". Tissue accumulation of copper by chicks has been suggested as an indication of metal bioavailability (Ledoux, 1987), something that the author did not find for sheep. This is in part due to the nature of metal metabolism in the two types of organisms (e.g. Kumaratilake and Howell, 1989a). Metal binding by organics such as amino acids, plasma proteins and metallothionein have been shown to be major factors in the fate of copper after uptake (e.g. Freedman et al., 1989a). In turkeys, for example, Richards (1989a) reports evidence that copper administered to hens is transferred to eggs after being bound by plasma proteins (see also Richards, 1989b; Richards and Steele, 1987).

The availability of plant copper to animals is important. The nature of the diet dictates the amount and chemical nature (and availability; Calhoun et al., 1987; Kume et al., 1983). Copper as chloride, acetate or sulfate, added to silage can be accumulated by sheep to levels of the haemolytic stage of copper poisoning (Charmley and Ivan, 1989). However, silage treatment is important. O'Kiely et al. (1989a) report that liver copper levels in cattle were reduced when the animals were fed silage preserved with sulphuric acid but not when the silage was preserved with formic acid (see also O'Kiely et al., 1989b). The authors (O'Kiely et al. 1989c) also found that copper supplementation counteracted the effect of the sulphuric acid preservative. Copper supplementation to animals is recognized as a means of overcoming a dietary deficiency (e.g. Solaiman and Maloney, 1989), especially with pregnant animals to reduce the chance for deficiency in offspring (e.g. Naylor et al., 1989). However, ligands such as salicylate can cause a reduction in maternal serum copper and an increase in fetal liver copper, at least in rats (Gunther and Vormann, 1989) Various forms of copper have been used as supplements (e.g. Langlands et al., 1989). Not only is the form of copper important, so also is the route of administration (Koh et al., 1989; McCormick and Fleet, 1988). Recent work also includes a continued examination of copper transport and the fate of copper after uptake (e.g. Baerga et al., 1989; Goode and Linder, 1989; Johnson and Lee, 1988; Müller, 1988) as well as metal tolerance (e.g. Fuentealba, 1988). Metal-metal interactions can, for example, affect uptake, metabolism and transport (Jenkins, 1989; Ke and Symonds, 1989; Koenig et al., 1989; Mason et al., 1989a; Moshtaghi-Nia et al., 1989; Rosenberg and Kappas, 1989b).

As with plants, the nature of the animal dictates many of the parameters around copper uptake and transport. Prabhakara Rao et al. (1988), for example, note that uptake is higher at elevated temperatures in a mollusc (*Cerithidea cingulata*), at least within the range of temperatures that they tested. This is not surprising, metabolic rate in poikilotherms tends to vary with temperature, within tolerance limits. Jenkins (1987) discusses metal metabolism in marine invertebrates and reviews some earlier work in which copper accumulation and subcellular distribution in developing crab larvae were regulated at cupric-ion activities equivalent to or lower than that of the estuary from which the organisms were collected. In an evaluation of copper deficiency in the rat, Nielsen (1988) report differences between male and female rats in their response to copper deficiency. He suggests that this is due to a difference in sulfur amino acid metabolism. Hartter et al. (1988) report sex-related differences in the number of copper-carrier sites in the hypothalamic and hippocampal tissue of the rat; gonadal steroids appear to modulate the process of copper uptake by the rat brain.

With humans, copper uptake and metabolism are important because of the roles played by the metal during normal metabolism. As a result, several studies have examined copper intake and loss in humans and laboratory animals (e.g. Rawson and Medeiros, 1989; Solomons, 1989; Turnlund, 1989a,b) as well as requirements and toxicities (Gregor, 1989). Although uptake may occur across the skin in humans (Bentur et al., 1988) or uniquely, from particulate copper (e.g. Greenberg, 1988), it is usually from food, in the intestinal tract. Gregor (1987) provides a general discussion of food supplementation as well as metal bioavailability. (Salmenperä et al. (1989) point out that concentrations of plasma copper and ceruloplasmin in healthy full-term infants are resistant to dietary supplementation.) Dietary fiber and various organic ligands have been examined for the potential to affect copper uptake and utilization (e.g. Failla and Seidel, 1988; Fekete et al., 1988; Gordon, 1989b; Jacobs and Domek, 1989; Johnson, 1988b, 1989b,d; Johnson et al., 1988c; Kelsay et al., 1988; Kies, 1989b; Kies et al., 1989; Kim et al., 1988; McArdle et al., 1988; Mesnier et al., 1989; Reiser and Hallfrisch, 1987; Schlemmer, 1989; Wapnir, 1989). Metabolism and transport of copper within an organism may be affected by agents such as ethanol (Yamaoka, 1989) and abnormal physiological conditions (Iqbal et al., 1989; Suttle, 1988). Changes in plasma copper transport protein amd changes in copper concentration have been associated with inflammation (e.g. McGahan et al., 1989b). Serfass et al. (1988) note that abnormal mineral metabolism is a consequence of obesity in a strain of male Zucker rats. Hormonal regulation of trace element metabolism is important (e.g. LeBlondel and Allain, 1989). In diabetic rats, Uriu-Hare et al. (1988) found increased concentrations of liver and kidney copper bound by metallothionein. In contrast, Raz and Havivi (1988) report no apparent change in kidney copper in diabetic rats although they did find increased liver, femur and blood cell copper levels. Changes in trace metal metabolism have been noted with kidney malfunctions (Lindeman, 1989). Yamatani and Okada (1988) note a change in urinary excretion of copper in laboratory-induced rats with nephrotic syndrome. Children on continuous ambulatory peritoneal dialysis have been reported to lose significant amounts of copper (Tamura et al., 1989). Girchev and Natochin (1987) provide evidence (abstract) suggesting that "... renal nerves are involved in the regulation of trace elements, reabsorption and that copper and zinc from the tubular lumen may affect the luminal cell membrane and control the transport of substances from tubular fluid into the blood". Waldrop (1988), in a Ph.D. thesis, and Palida et al. (1989) discusses the effect of Menkes disease on copper uptake, noting that the genetic defects may regulate the amount of copper taken up by fibroblasts. In Wilson's disease, reduced copper transport plays a role in the expression of the elevated liver copper concentrations characteristic of the disease (e.g. Bingle et al., 1988; Evans et al., 1989). Biliary excretion of copper is the main route of copper excretion (Dijkstra et al., 1989; Gross et al., 1989; Houwen et al., 1989) and copper granules tend to accumulate in the liver when biliary excretion of copper is impaired (Miyamura et al., 1988). Hatta et al., 1985 provide a review (in Japanese) on the role of the liver in copper and zinc metabolism.

The transport of copper is an extremely important topic (Harris, 1990) and involves ligands such as ceruloplasmin (Harris, 1987, Harris and Percival, 1989)). However, the mechanisms involved in the transport of copper are not well understood whether it is at the level of the cell or organism. As a result, a number of papers address the chemistry of the processes (e.g. Barnea et al., 1988, 1989; Brouwer and Brouwer-Hoexum, 1989; Crispens et al., 1989; Freedman and Peisach, 1989a; Katz and Barnea, 1988; Lau et al., 1989; Melkonyan et al., 1989; Percival and Harris, 1989; Sakuma et al., 1988) while others consider the chemistry of the organism (e.g. Fuller et al., 1990; Mas and Sarkar, 1988). In the abstract of a symposium paper, Robinson et al. (1988) examine metal transport in two molluscs, commenting on the species-specific importance of plasma and hemocytes in the transport of Cr, Cu, Ni and Zn. Copper resorption by cells such as hepatocytes has been examined because of the important physiological role played by the metal (e.g. Heck et al., 1988).

VI. TRANSPORT OF COPPER AND GEOCHEMICAL CHANGES OCCURING AFTER INTRODUCTION INTO NATURAL ENVIRONMENTS

The transport of copper from source to receiving environment can occur in several ways. Of these, aerosol and riverine transport are the most common. A number of geochemical changes can and usually do occur after introduction of the metal into the receiving environment. Both the transport and the changes are important to an understanding of the amount and nature of copper in the environment as well as the potential for biologically available copper with naturally- and anthropogenically-introduced metal. Sorption by inorganic and organic particles can, for example, affect the concentrations of ionic metal (Jenne and Zachara, 1987). Organisms become important in this discussion since they acquire copper and, in doing so, effect a change on its chemistry (e.g. Knowles, 1987) both while they are living and through decomposition products after death. Humic substances, for example, are a class of biogenic organic compounds important in the carbon cycle (Frimmel and Christman, 1987) but also important in the geochemistry of metals in aquatic and terrestrial environments (e.g. Suffet and MacCarthy, 1989).

Human activities can affect global and regional cycles of trace metals (Astruc and Lester, 1988; Nriagu, 1989) through the release of metal-containing biological (e.g. Wu, 1988b) and industrial materials. Public awareness of the real or hypothesized impact of these activities is increasing and being expressed as reviews of environmental quality (e.g. Lockerbie, 1987d; Saskatchewan government document dated May 1983), international meetings on environmental problems (e.g. 1990 report from the Joint Group of Experts on the Scientific Aspects of Marine Pollution), and the development of methods for investigating the potential for environmental impact (e.g. Battiston et al., 1989) and recovering metals (e.g. Ozimek, 1988). Harper (1988) discusses some of the problems associated with the reduction of riverine metal input into the North Sea, a discussion which points out some of the financial issues and identifies problems of interpretation of metal concentrations and metal impact. Bouquegneaud and Joiris (1988) extend this type of discussion in an evaluation of biological indicators and monitoring. Even with the problems associated with the reduction of anthropogenic metal input and the problems of monitoring, there is an increasing effort for industry and nature "... to live happily side by side; ..." (Kelly, 1988). But this requires a working knowledge of the processes of both nature and industry, especially the effects of one upon the other.

Aerosol Copper

"It is now recognized that the atmosphere is a significant pathway for the transport of many natural and pollutant materials from the continents to the ocean ..." (introductary statements from 1989 report of Joint Group of Experts on The Scientific Aspects of Marine Pollution). Martin et al. (1989a) point out (abstract) that in the Mediterranean region, "... the atmospheric flux of Cu, Pb and Cd exceeds river input by one or two orders of magnitude". "Among the most important natural sources of metals in the atmosphere are: surface waters, soils and vegetation, volcanic activity and forest fires" (Beijer and Jernelöv, 1986, page 68). The major anthropogenic sources are fossil fuels, smelting and industrial emissions, primarily near industrialized areas (e.g. Alebic-Juretic and Klasinc, 1988; Tripathi et al., 1989). Nriagu (1989) provides an estimated 28 (2.3-54) x 10^9 g yr^{-1} copper from natural sources and 35 (20-51) x 10^9 g yr^{-1} from anthropogenic sources (the latter from Nriagu and Pacyna, 1988). The emissions from natural and anthropogenic sources varies from region to region and country to country. In Canada, for example, "Total emissions of copper were 1689 tonnes in 1982 with primary copper/nickel production accounting for 76% of the total" (summary, Jaques, 1987). Considerable variation in emissions occurs with different types of metal recovery and with different metals recovered (e.g. Bennett and Knapp, 1989).

Both wet and dry deposition occurs with aerosol metals. Barrie and Schemenauer (1989) discuss macroscale and microscale processes of wet removal as well as some of the problems of collection, analysis and interpretation. They also point out that it is difficult and time consuming to determine the speciation and soluble/insoluble fraction of metals in wet deposition, a factor which explains the lack of adequate information about aerosol trace metal speciation. The potential for sample contamination must also be considered in any sample-collection program (e.g. Ross and Bengtsson, 1988). Particle size becomes important not only in sample collection but in estimation of source and transport distance; copper is found predominantly on small particles (Mueller and Broecker, 1987).

In a presentation on rainwater contribution to the dissolved chemistry of storm runoff, Ng (1986) gives a mean ratio of 2.3 for rainwater copper:stormwater copper based on the concentration of copper in both. This infers a relatively important wet deposition of atmospheric copper by rainfall. Sakai et al. (1988) suggest that trace metal concentrations in snow can be used as a reliable guide to the concentration of aerosol trace metals. Average metal concentrations in snow near Sapporo, Japan ranged from 3.1-37.6 µg/L, increasing towards the center of the city. Transfer of aerosol metal occurs at the water:air interface with bodies of water ranging from ponds to the Great Lakes. Transfers can be in appreciable amounts near industrialized locations. Andren (1988), for example, provides atmospheric loading values of 110-950 tons per year for copper to lake Michigan (See also Murphy, 1988). The effect of acid precipitation, on aerosol trace metal input, is of constant concern (e.g. Ontario Ministry of the Environment (1984) but is not well understood. Association of metals with sulphates may be pronounced, in industrialized areas, at least with lead and possibly other metals (Gentilizza et al., 1988; Reda, 1988). The biological impact of - a, the metals and - b, the acid is another story or series of stories. Rybak et al. (1989) comment that with oligotrophic lakes in Newfoundland at least, the heavy metals and nutrients may be more important than the acidity.

Dry deposition of aerosol metals is also important and can provide an appreciable input of metals from industrialized regions. Gomes et al. (1988b), for example, estimate an input of 41 kg copper per day from the Fos/mer industrial complex to the northwestern Mediterranean. Modelling input, whether wet or dry, provides an estimate of the importance of aerosol metal transport from particular regions to particularly sensitive areas, such as the North Sea and Baltic Sea (Petersen et al., 1989). Maring and Duce (1989) comment that (abstract) "Atmospheric deposition supplies roughly the same quantity of soluble copper to tropical open North Pacific surface waters as does upwelling to eastern North Pacific surface waters. Atmospheric copper deposition, which appears to be primarily of natural origin, may be the most important input of copper to the surface waters of the central gyre of the North Pacific".

Atmospheric input, whether wet or dry, also provides sources of metal for organisms, whether metal tolerant (e.g. Satake et al., 1988) or intolerant. Schultz et al. (1987) report a 10-15% canopy uptake of the cadmium and copper from atmospheric input to a beech and spruce stand in the Solling area (central Germany). Acidification has been suggested to increase uptake and effect of aerosol-deposited metal by soil and organisms (Schaefer, 1987)

Copper in Freshwater Environments

Water quality objectives are established to provide guidelines for the release or potential release of anthropogenic materials (e.g. Godin et al., 1985; Lockerbie, 1987c,d), including copper. Very often, however, they do not consider the geochemical characteristics of metals in rivers (e.g. Zhang et al., 1989) or other receiving waters. And yet, there is increasing information on the relationship between metal levels in aquatic systems and changes in water quality that can be indicative of metal levels (e.g. Jeffries, 1988) or of biological effect (Hart et al., 1988; Johnson et al., 1989a). Since geochemical properties of freshwater systems will affect the biological availability of copper, and other metals, it would be beneficial if any "excess" metal (e.g. Marsalek and Ng, 1987; Skacel, 1988) could be considered in terms of biological availability rather than just "total metal", a condition which now routinely exists. Continued work on metal bioavailability and multi-element geochemical data (Stanley and Sinclair, 1987) will provide insights into the problem.

Anthropogenic copper can form an important portion of riverine copper in areas of high population density. In a discussion of the environmental geology of the Ganga River Basin in India, Subramanian et al. (1987) note that anthropogenic copper from Delhi forms an estimated 62% of the total copper in the sediments of one of the rivers, the Yamuna. Pollman and Danek (1988) report median values of copper ranging from 27-33 µg/L for urban runoff from 22 cities in the U.S., derived mainly from automotive activity and corrosion of metallic surfaces and fittings. Recent increases in sediment or water copper concentrations have also been noted in other bodies of fresh water and associated with man's activities (e.g. Dorney and Kreutzberger, 1986; Kuntz, 1988b; McCrea et al., 1984; Provini et al., 1989). Chemical changes occurring in copper (and other metals) and metal-containing agents after introduction into fresh water will affect the chemistry of the metal and can dictate its biological availability (Domagalski, 1988) and biological effect (Dixit et al., 1989; Gibson et al., 1987; Reader et al., 1989; Sayer et al., 1989; Young and Harvey, 1988). Depositional processes are dependent upon the physical and chemical nature of the fresh water (e.g. Nakashima, 1987) as is the exchange between sediments, pore waters and water column (e.g. Sakata, 1987). Changes can occur in the chemistry of a body of fresh water, or its sediments, as a result of natural as well as anthropogenic factors, changes that can affect both metal sedimentation and metal exchange (e.g. Alhonen, 1987; Brook and Moore, 1988; Dai, 1988; Johnson and Nicholls, 1988; Lebeuf and Tessier, 1985; Li, 1987). These changes also occur with the transport of metal-containing materials from one body of water to another (e.g. Sloterdijk et al., 1987) or the infiltration of river water into a groundwater aquifer (e.g. Goerlich et al., 1988; von Gunten et al., 1989; Subramaniam and Hoggard, 1988). The presence of humic substances in a receiving water can, for example, produce major changes in metal chemistry and the biological availability of copper (Hiraide et al., 1988; see also the review of peats and peatland waters by Shotyk, 1988).

The introduction of anthropogenic copper into fresh waters can cause an increase in filterable copper. The amount and duration of this increase is affected by a number of factors. With brass dust, decreasing water hardness has been associated with increasing dissociation of copper from the brass (Muse, 1988). With mine waste deposited in shallow water (pH 6.8-7.4), leaching and subsequent sedimentation appear to be associated with the increase and subsequent decrease of copper proceeding away from the dump site (Hakansson et al., 1989b). Microbial biofilms and microorganisms have been found to accumulate copper from the water in both acidic and neutral pH environments (Ferris et al., 1989; Mann and Fyfe, 1988) as do macroorganisms (Duzzin et al., 1988). Seasonal changes in biomass can thus have an impact on metal uptake as well as metal speciation.

Copper in Estuarine Environments

Riverine transport of copper to marine waters involves transport through an estuary, a region of rapidly-increasing major ion concentration and decreasing river flow rate with resultant sedimentation, at least of coarser particles. These estuarine processes affect the chemistry of trace metals, the distribution between the particulate and filterable fractions, and the total concentration in the water column and in the sediments. Some of the factors involved in this are discussed in recent references that deal with specific receiving waters (e.g. Kersten, 1988), input from major river systems (e.g. Salomons, 1988, 1989) as well as a few that deal with input from relatively small freshwater systems that include anthropogenic copper (Paulson et al., 1989a; Wallace, 1987). Bewers and Yeats (1989) discuss ways of estimating trace metal tansport through estuaries, including some of the geochemical processes operating to affect metal transport. Since copper is non-conservative, involving a loss of metal within an estuarine system, consideration of metals in wetlands becomes important in evaluating metal budgets and biological availability to wetland organisms (Kraus, 1988b). Mobilization of metals after changes in physico-chemical conditions, is discussed by Calmano et al. (1988) with regard to possible metal uptake by algal cells and iron hydroxides.

Riedel and Sanders (1988) discuss the factors affecting the bioavailability of toxic trace elements to estuarine organisms. In this, they provide a reasonable review of the processes associated with estuarine circulation, commenting on some of the processes involved in the changes that occur in metal speciation within an estuary. Alberts et al. (1989) examined the nature of humic substances and their reaction with copper in a salt-marsh estuary in the southeastern U.S.. They point out that the solubilities varied with salinity which suggests the importance of the salt (or major ion) gradient to trace metal speciation within an estuary. Other organics are also important (e.g. Dehnad and Förstner, 1988) both in the evaluation of copper speciation and in an evaluation of metal bioavailability. There is also a direct association of filterable copper with fine-grained sediments (e.g. Krumgalz, 1988a; Xia et al., 1987), including colloids. In fact, the nature of the sediment, as determined from cores, can be used to piece together a suggestion of metal speciation, possibly through time (Battiston et al., 1989) although metal diagenesis may not allow this to be accurately done.

Various extraction techniques have been used to determine the mobility of sediment-bound metals (e.g. Boothman, 1987; Dehnad and Förstner, 1988). El Sayed (1988) provides evidence of variations in trace metal speciation (including copper) induced by chemical and physical changes within the estuary at any one time and over time. Short-term changes in organometallic complexes have been ascribed to rainfall (Khokiattiwong and Limpsaichol, 1986). The implication of this and other studies (Fischer et al., 1986; Noureddin et al., 1988b; Qian et al., 1986) is that the biological availability of copper in an estuary could change over short distances and short periods of time. Changes can also be produced by anthropogenic effects in coastal and estuarine environments, from marinas (McMahon, 1989) as well as sewage.

Riverine transport of copper and other metals, through estuaries, to marine environments is discussed for a number of areas in the recent literature. (Values from these references are included in table 2 when appropriate.) These include Indonesia (Everaarts, 1989; Nolting et al., 1989a) and the Mediterranean Sea (Martin et al., 1989a; van Geen et al., 1988), the marine deltaic area of the Po river in the North Adriatic Sea (Ferrari and Ferrario, 1989), and the Gulf of Gaeta in the Tyrrhenian Sea (Ferretti et al., 1989). Ferretti et al. (1989) note the importance of the two largest rivers in the area but report highest copper concentrations in coarse particles (rather than fine-grained) at depths of 50-100 m, particularly in a region south of a promontory. (The latter suggests the possibility of long-shore transport of copper-containing coarse-grained particles.) Avoine (1986) presents values for copper (and other metals) entering the English Channel through the estuary of the Seine River (France). Quevauviller et al. (1989) examined the distribution of metals in the estuary of the Sado River (Portugal). They report an increase in zinc and copper in the middle estuary due to mining activities and in the lower estuary due to input from urban and industrial sources. Chaussepied et al. (1989) provide a broad-based review of the coastal region north of Calais (France), including metal flux. Transport and chemistry of copper are included, to greater or lesser extent, in many of the references on the North Sea and Baltic Sea (Dethlefsen, 1988; Dogterom, 1986/87; Flügge and Jappelt, 1987; Golimowski et al., 1990; Irmer et al., 1988; Kramer and Duinker, 1988; Kremling et al., 1987; Söderlund and Areskoug, 1989; Steffen, 1987; van den Berg et al., 1987). Nimmo et al. (1989) note the importance of the River Mersey to the copper in Liverpool Bay (Irish Sea).

Barcellos et al. (1988) note that of the estimated 840 grams of copper transported daily in the Arroio Pavuna River (Brazil), 83% is of anthropogenic origin, from the region of Rio de Janeiro. Estimates of metal transport, metal chemistry and water quality are also found in a number of references dealing with rivers and coastal regions in North America (Curl et al., 1987; Griffin et al., 1989; Kuwabara et al., 1989; Paulson et al., 1989b; Stanford and Young, 1988; Sylvestre et al., 1987; Wallace et al., 1988; Windom et al., 1989a,b; Yeats, 1987a, 1988). Lan and Qiao (1986) provide estimates of natural and anthropogenic copper fluxes through the Changjiang estuary (East China Sea), Huang et al. (1987b) and Sun et al. (1988) discuss metal speciation in the Huanghe estuary (Bohai/Yellow Sea) and Matsumoto (1988) discusses residence times of trace metals and nutrients in Tokyo Bay water.

Recent discussions of trace metal concentrations and chemistry also include some rather unique situations and regions, like the distribution of metals in the saltworks of Mesolongi, Greece (Varnavas and Lekkas, 1988). Zonta et al. (1988) provide a brief discussion of metal speciation in the sediments of Venice Lagoon. They suggest that the exposure of the sediments to air, drying, and resubmergence play an important role in the geochemistry of copper. Förstner et al. (1989) examine metal transfer between sedimentary phases and note the dominant role of organic material in binding copper in estuarine sediments. This provides continuing evidence that organics are a major factor in controlling copper bioavailability in aquatic (as well as terrestrial) environments. Langston (1988) used metal levels in a number of organisms to estimate metal bioavailability in the Mersey Estuary (U.K.). There is some question about the use of organisms to determine "metal bioavailability". This is based on the ability of some organisms to control tissue metal levels as well as the source of the metal. Does the metal come from the sediments, the interstitial water or the overlying water column? Perhaps the latter for the salt marsh cordgrass *Spartina alterniflora*, as suggested by the lack of correlation between interstitial water concentrations and tissue concentrations of Cu, Mn and Sn (Alberts et al., 1987). This can have some bearing on metal transport through an estuary, Kraus (1988a) notes an ability of the species to transport an estimated 548.2 g of copper per hectare per year through accumulation and loss in leaf tissue. However, the non-conservative nature of copper in estuaries implies not only the loss of metal within the estuary but also the "filtering" effect on transport of natural and anthropogenic metal from terrestrial regions into the oceans (e.g. Xiao, 1988).

Copper in Saltwater Environments

The differentiation between "estuarine" and "saltwater" environments is subjective and implies overlap in the use of references. Saltwater environments receive copper from river runoff and from atmospheric sources. The relative amounts tend to be controlled by distance from the coast as well as from a runoff source. They are also affected by biological, hydrographic and geochemical processes as well as any nearby anthropogenic copper (e.g. Bothner et al., 1987; Riso et al., 1988; Wolfe and O'Connor, 1988). In an examination of the spatial and seasonal variability of filterable cadmium, copper and nickel in northeast Atlantic surface waters, Kremling and Pohl (1989) note the importance of west European rivers as metal sources. Yeats (1987a) notes (page 8) that in general "... the concentration of copper in coastal waters with salinities between 30 and 35§ will decrease with salinity and will vary between 0.2 and 0.5 µg/L at 30§ and 0.1 and 0.2 µg/L at 35§. Generalizations can be useful although unique situations are frequently reported. van Geen et al. (1988), for example, report entrainment of trace-metal-enriched Atlantic shelf water (10 nM Cu) in the inflow to the Mediterranean Sea.

Trace metal anomalies occur in hydrothermal vent areas (e.g. Gamo et al., 1988) and in closed marine basins (e.g. Haynes and Bloom, 1987), both of which have produced economically important mineralizations. Anomalies also occur in the factors that control metal speciation and the biological availability of copper, discontinuities that occur in both space and time. Mackey and Higgins (1988) point out (abstract) that "The strong copper-complexing capacity of seawater varies over three orders of magnitude". Moffett et al. (1990) note (introduction) that "Copper speciation in seawater is now recognized to be dominated by complexation with naturally occurring organic ligands". They discuss the distribution as well as sources and sinks of copper complexing agents in the Sargasso Sea. Hirose (1988) presents a multimetal complexation model which considers total and free ion concentration. Sun et al. (1988) report "apparent" copper complexing capacities in the Huanghe River estuary (China). A number of authors note the relationship between complexing capacity and biological productivity (e.g. Mackey and Higgins, 1988; Moffett et al., 1990; Wangersky et al., 1989; Zhou et al., 1989) and the seasonal variation which is presumably a result of this (e.g. Mackey and Szymczak, 1988). Even with this variation, Coale and Bruland (1990) note that at least in the North Pacific, the result is to hold the surface water copper(II) ion activities relatively constant.

In addition to metal complexation, sorption by marine particulate matter affects the concentration of filterable and biologically available copper in seawater (Betti and Papoff, 1988; Clegg and Sarmiento, 1989). Inorganic and organic surfaces are often able to sorb metal-containing filterable and particulate materials from seawater or interstitial water in sediments (e.g. Goldberg et al., 1988). The combination of sorption and complexation, combined with the variability that occurs in these processes over both space and time, can produce variability in total and filterable metal concentration as well as metal bioavailability (Krumgalz et al., 1989).

Metal concentration as well as speciation can be affected by anthropogenic materials (e.g. Hall, 1989; Henry et al., 1989; Irion and Müller, 1987). Hoshika and Shiozawa (1987) calculated total inputs of copper into the Seto Inland Sea to be in the order of 870 tons per year with approximately half of that being anthropogenic material. Gibbs and Angelidis (1989) report major differences in the distribution of oxidizable and reducible metals as a result of sludge digestion before ocean dumping. These differences affected the nature and settling rate of the flocs and would appear to affect metal transport away from the dump site. Marine bacteria can affect the deposition of copper, perhaps more effectively than any other group of organisms (Stupakova et al., 1989). In the water column, however, planktonic organisms play important parts in extracting filterable copper from the water and turning it into particulate copper within the organism (Savenko, 1989). Lin and Li (1988) note that, in Xiamen

Harbour (China), the copper increase in zooplankton was proportional to copper levels in seawater as well as the period of exposure of the organisms to the metal-containing medium.

Copper in Soil Environments

Both the concentration and chemical properties of copper in soil are most frequently addressed for agricultural purposes. Thus the "microelement distribution" or "status" of copper, and other metals, in soils becomes important (e.g. Benes and Pabianova, 1987; Kwon and Eun, 1987; Singh et al., 1988; Wu et al., 1988a). This is especially true for copper availability to plants under natural conditions (Van Bladel et al., 1988; Weil and Holah, 1989) and when anthropogenic metal has accumulated in soils (e.g. Ruppert and Schmidt, 1987). Since copper bioavailability is affected by organic complexing agents, relationships between metals and organics is often considered (e.g. Boluda Hernandez, 1988). When humic substances are considered, this relationship can be very meaningful, many humic substances can complex copper and reduce metal bioavailability to crops (Berggren, 1989) or reduce bioavailability of excess anthropogenic metal (e.g. Fjeldstad et al., 1988). Natural metal-complexing agents, like humic substances, offer a mechanism to reduce the impact of excess metal in an area where a lengthy time period would be needed to accomplish the same effect (e.g. Okazaki and Saito, 1989). But the need is to understand the nature of the soil, the nature of the plant and the chemistry of copper which affects metal availability (e.g. Cox, 1987).

A knowledge of the geochemistry of copper can also be useful in identifying areas of mineralization (Mehrtens, 1986; Sverjensky, 1987). The ability of organisms to tolerate high levels of copper can also be used, microbial leaching of copper ores is a recognized technique, used to recover copper from low-grade ores (Bosecker, 1987). Bosecker (1987) points out that about 5% of the annual World production of copper is obtained by bacterial leaching. However, the geochemistry of metals (including copper) is also useful in treating environmental problems such as detention of metal from roadside catchbasins (Morrison et al., 1988) or predicting the fate of soil properties on metal retention and speciation (e.g. Li, 1988).

In a review of the use of municipal refuse compost, Gallardo-Lara and Nogales (1987) point out that excess metal can cause a problem with seed germination and plant growth. Movement of metal away from sewage- or compost-enriched soil is known to occur (McGrath and Lane, 1989), movement through relatively impervious materials such as clay has been demonstrated although to a comparatively small extent with copper when compared with zinc or iron (Quigley et al., 1984). The question that is of importance is what does the chemistry of the metal and of the soil mean to the accumulation of copper by plants? Hogan and morrison (1988) found little evidence that acid deposition alters the uptake and accumulation of metals, including copper, in the sugar maple and yellow birch. In contrast, regular agricultural procedures such as irrigation can have an effect on tissue metal levels in certain plants (Malaiskaite, 1987b). The long-term use of inorganic copper fungicides has been associated with increased tissue accumulation of copper in coffee plants (Dickinson et al., 1988; Lepp and Dickinson, 1987) and soil copper in vineyards (but not accumulation in grapes; Macek and Repe, 1987). Soil copper concentrations can increase with copper fungicide use (e.g. Stritar and Pavlovic, 1988) although the effect on soil microorganisms and plants is affected by sorption and complexation as well as the physiological nature of the organism.

VII - COPPER CONCENTRATIONS - ENVIRONMENT

A large number of copper determinations are made annually in both aquatic and terrestrial environments. Some of these are in major programs to monitor water quality (e.g. Landesant Wasser und Abfall, 1987), others in programs to examine specific regions (Hödrejärv and Ott, 1989) or specific events. A number of recent developments have been made in the collection and analysis of metals in natural and anthropogenic systems (Hoffmann, 1988; Jones et al., 1987; Sedberry et al., 1988). However, the distribution of metals is often irregular, occurring in higher than "normal" concentrations under certain natural (e.g. Villa, 1988) and anthropogenic conditions and lower than "normal" concentrations under other conditions. A good deal of work has been done on sampling and storage of samples (Calabrese et al., 1988; Schwedhelm et al., 1988; Yeats, 1987b), especially with regard to contamination problems (McCrea, 1981). Rheinallt et al. (1989) comment on the sources of variation associated with the sampling of marine sediments for metals; Wopereis et al. (1988) discuss spatial variability of metals in one type of soil. Methods of laboratory handling of samples and methods of analysis are continually examined because of the importance of accurately determining trace metal concentrations (Edgell and Wilbers, 1989; Martin-Goldberg, 1987; Usami et al., 1989). The effects of natural agents are important in the analysis of the types or species of copper (e.g. Catoggio and Porta, 1989; Cleven and Del Castilho, 1988). There is also a continuation of intercalibration tests to determine interlaboratory variability and errors in trace metal determination of known samples (Cheam et al., 1988; Loring, 1987; Schramel, 1989).

Recent literature discussing analytical techniques considers, for the most part, details, problems and improvements of previously-used techniques. Colourimetric techniques for the measurement of copper are discussed by Ceriotti and Rota (1988), Fontana and Olsina (1989), Jenik (1987) and Kasahara et al. (1989). Rau and Chen (1987) discuss the use of a copper ion selective electrode under the influence of silver. Both liquid chromatography and gas chromatography methods have been used for the quantitation of four military fungicides (including copper 8-Quinolinolate; Akkara et al., 1988). Drake et al. (1985) used neutron activation and inductively-coupled plasma-atomic emission spectroscopy to characterize elements in the "soluble fraction of urban snow". They suggest that elemental analysis of urban snow can act as a suitable environmental monitor. The use of various atomic absorption spectrometry techniques, frequently after novel preconcentration techniques, is discussed in Caballero et al. (1987), Geckeler et al. (1989), Liu and Ingle (1989a,b), Ohzeki et al. (1990), Shkinev et al. (1989) and Schöneborn (1987). The U.S. Department of Energy (1987) describes a procedure for the determination of copper in water samples taken to determine if effluents meet national pollutant discharge compliance requirements. Ostapczuk et al. (1988) review the potential of electrochemical methods for metal determination in reference materials. Discussions of voltammetric techniques are found in Bond et al. (1988), Boussemart et al. (1989), Khandekar et al. (1988), Quentel and Madec (1990), van den Berg (1988) and Wu (1986). Mass spectrometry has been used in measuring copper as well as other metals; recent discussions that include copper are Beauchemin and Bermin (1989), Götz and Heumann (1988), Plantz et al. (1989) and Vermeiren et al. (1990). Freimann and Schmidt (1989) describe the use of reflection X-ray fluorescence to analyze metals, including copper, at the ng-µg/kg level in North Sea water. X-ray fluorescence use is also described by Jenkins et al. (1989) and Morales and Zepeda (1988). Various other techniques that have been discussed for copper analysis include energy dispersive X-ray and electron microprobe use for soils (Weber and Kowalinski, 1987) and a novel on-line photoelectron emission for detection of heavy metal aerosols (Niessner et al., 1989). In the measurement of copper complexation, Morrison and Florence (1989) report that anodic stripping voltammetry is not suitable in freshwaters although differential pulse polarography did provide estimates comparable with bioassay results. Various types of ion-exchange resins have been used to isolate labile (readily exchanged) metal from aqueous sediment suspensions (Beveridge et al., 1989). Nourredin et al. (1988a) discuss the use of Chelex-100 for measuring copper complexing capacity of estuarine waters. Cabaniss and Shuman (1988) discuss some of the problems of fluorescence quenching for the measurement of copper-fulvic acid binding.

Values for copper in various environmental samples are presented in table 2. These are values from recent references and should be considered in terms of the conditions for sample analysis presented by the authors.

VIII - COPPER CONCENTRATIONS - ORGANISMS

Many of the problems and techniques for collection and analysis of metal levels in environmental samples apply to organisms. Crompton (1988), in an excellent review of the analysis of organometallic compounds in the environment, includes in "environment" water, air, plants, crops, fish, crustacea and biological materials. Tissue metal levels are important in evaluating nutrient deficiency and excess as well as in the evaluation of man's effect on organism metal levels. And this applies for samples ranging from agriculturally-important plants such as clover (Peaslee and Taylor, 1989) to human tissues (e.g. Braithwaite et al., 1987; Preu et al., 1987). Dubreuil (1988) points out that for organisms used as aquatic bio-monitors, "... there is a great demand for reliable analytical techniques for trace metals in biological samples". As a result of the use of metals in agriculture and the food industry, Voigt and Pohl (1989) comment on the need for adequate and accurate measurement of metals in food and food materials. Skurikhin provides (1989a) a review of USSR standards of determinations of heavy metals and arsenic in food products and (1989b) limits of determination of various methods. In the latter he discusses a standard that can be determined in a test solution and will provide a maximum permitted level and the optimum quantity of a food sample to ensure an adequate amount for analysis.

Metal variability and metal contamination are constant problems in biological as well as environmental samples. Faa et al. (1990), for example, discuss variability of copper levels in biopsy tissue from a cirrhotic liver, pointing out the importance of replicate samples even if with biopsy needle samples through the same puncture site. Dol et al. (1988) discuss the sources of external metal contamination in hair and Lakomaa et al. (1988) comment on avoidance of contamination of serum samples. Reference materials provide a means of checking analytical techniques. As Okamoto (1990) comments (abstract) "The incorporation of appropriate reference materials in the scheme of analysis is the most convenient, cost-effective mechanism by which to assess and maintain analytical data quality". Reference samples have been prepared for a wide range of materials, from food to individual animals and single-cell proteins (Berman and Sturgeon, 1988; Diver et al., 1988; Griepink, 1988; Iyengar, 1990; Jorhem and Slorach, 1988; Kumpulainen et al., 1988; Vercoutere and Cornelis, 1988) and used for routine analysis as well as for interlaboratory comparisons (e.g. Rapin and Blanc, 1988).

There has been a good deal of work on the best method or methods of treatment of biological samples in preparation for analysis. A good deal of the concern is towards the right treatment for a particular type of organism or for a particular type of analysis. Lindstrom et al. (1988) discuss a selective fractionation procedure for isolating different parts of the mollusc *Mytilus edulis*. Isolating specific portions of body systems is important, as pointed out by Garvey et al. (1988). Djarmati and Stankovic (1987) used a wet digestion procedure and passing through a Chelex 100 resin to isolate metals in urine. Methods of tissue digestion are discussed by Agemian et al. (1980), Christie et al. (1989), Matusiewicz et al. (1989) and Nieuwenhuize et al. (1989); sequential extraction procedures by Jordao and Nickless (1989). Casassas et al. (1988; human serum) and Shinde (1988; sodium salicylate-containing solution) discuss techniques for removing copper from salicylic acid. Saleh et al. (1988) evaluated ashing techniques for the determination of iron and copper in palm oil.

Analytical techniques for measuring levels of tissue metals in prepared samples are varied. Because of the ability of copper to react with organics and form coloured compounds (e.g. Atamna et al., 1989; Ichinoki and Yamazaki, 1989), colourimetric techniques are widely used (Abe et al., 1989; Ceriotti et al., 1988; Gnusowski and Zygmunt, 1985; Kuban et al., 1989; Tang et al., 1989; Yuan, 1984) including histochemical staining of tissues with elevated copper levels (e.g. Bunton, 1990). Atomic absorption spectrophotometric techniques are also used widely for materials ranging from salt (Baklanov et al., 1988) to tissues (Buehler et al., 1989; Burguera et al., 1988a; Evenson, 1988; Favier and Ruffieux, 1988; Miller-Ihli, 1988; Molina et al., 1988; Nielepkowicz, 1988; Puri et al., 1989; Sakae et al., 1989; Smirnova et al., 1987; Sun, 1989; Ulberth and Blineder, 1989; Wagley et al., 1989; Weber, 1988; Ybanez et al., 1989). Discussions of the use of electrochemical techniques include Daniele et al. (1989), Romanov et al. (1988) and Sakai et al. (1989b). Using neutron activation, Dybczynski et al. (1989) developed a procedure for measuring what they term "small" amounts of copper in biological materials. (Limit of detection stated to be 15 µg/kg under the conditions of the study.) Other discussions of neutron activation use for copper (and other metals) include Hirai (1990), Mukhamedov and Tillaeva (1987), Schelhorn and Geisler (1988), Suzuki and Hirai (1988) and Zhuang et al. (1989b). Other techniques that are described in recent literature include Inductively Coupled Plasma Mass Spectrometry (Friel et al., 1990; He, 1989; Uchida et al., 1988; Vanhoe et al., 1989), Energy Dispersive X-ray Fluorescence (Sargentini-Maier et al., 1987), Optical Emission Spectrography (Marits and Iscan, 1989), Proton Induced X-ray Emission (PIXE) and Proton Induced Gamma-ray Emission (PIGE) (Lapatto, 1990), Gas Chromatography (Kobayashi et al., 1989a).

The following tables present metal levels in plant, animal and human tissues as well as in human and some animal foods. 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 region to another. In using the tables it should be kept in mind that the methods of collection, preparation and analysis should be provided in the original publication. These must be considered in evaluating the accuracy and usefulness of the metal levels.