FINAL REPORT

ICA PROJECT NO. 223

THE BIOLOGICAL IMPORTANCE OF COPPER

A Literature Review

June, 1992

ICA PROJECT 223

Preface

In 1973 the International Copper Research Association Incorporated initiated a grant to review the literature dealing with the biological importance of copper in marine and estuarine environments. This was followed by a second review in 1978. It was then apparent that a very large number of publications concerning copper in the marine environment were appearing each year 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. It was apparent from the literature that was reviewed that most of the concepts on the importance and the effects of copper could be applied to all environments. An understanding of the environmental chemistry of copper could be applied to medicine as well as agriculture, the marine environment as well as soils. The reviews thus pointed out the broad application of concepts about the biological importance of copper.

The present review includes literature for the period 1989-1990 although a number of earlier works are included and, where appropriate, a few appearing early in 1991 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, kindly provided the metals section of the Marine Pollution Research Titles as a source of European as well as North American References.

The 1991 review was written using 3,198 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 has been updated to be comparable 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 1991 references, now contains 26,364 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. 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 recent literature identifies various aspects of the biological importance of copper. This includes the requirements for copper exhibited by organisms as well as the various functions that copper has in organisms. It also includes the value of copper to man and its use to control pest organisms. Recent work on the detrimental effects of excess copper are covered. Evidence continues to accumulate that it is the chemistry of copper in various environments that dictates biological availability. There is also consideration of evidence that the tolerance of the organism modulates the biological effect of excess copper. Some examples of information provided by the 3,198 references used in the 1991 review include:

Metallic copper has been used by man for thousands of years (Hauptmann et al., 1989) for tools, artwork and jewelry. Kallfass and Hoerz (1989), for example, discuss some of their metallographic work on a Bronze Age tin bronze bracelet found in Southern Germany.

Copper is widely used in agriculture as a nutrient supplement and is also used in agricultural practices ranging from the preparation of Sea Buckthorn fruits for harvesting (Demenko and Korzinnikov, 1990) to the handling of food materials.

Copper is essential for living organisms and, as such, is considered a nutrient. It is not surprising that conditions of copper deficiency do occur and that supplementation may be necessary for optimum growth of plants and animals. Prohaska (1990a) reviews some of the biochemical functions of copper and the changes that can occur as a result of deficiency.

Copper is found in a number of enzymes and is involved in connective tissue and structural protein formation (Curley, 1987; Tinker et al., 1988). Copper status may directly or indirectly affect brain activity, nerve physiology and hormonal regulation (e.g. Dake and Amemiya, 1991; Hall et al., 1990; Penland et al., 1989).

Stabel and Spears (1989) discuss the effect of copper on immune function and disease resistance, in part a result of the role that copper plays in combating infection. Klevay (1989; 1990b) notes the relationship between copper deficiency and ischemic heart disease, the leading cause of death in the United States. It is not surprising that along with the increased knowledge about the importance of copper to health there is an associated increase in the use of copper in medicine. As an example, Badawi (1990) and Sorenson et al. (1989) discuss the possible role of copper in the control of cancer.

Since high levels of copper can be detrimental to organisms, there is widespread use of the metal for control of pests. The use of copper to reduce algal fouling and corrosion is one example (Callow and Edyvean, 1990). Peacock et al. (1989) describe a copper-based gelcoat which they consider to be environmentally suitable and a cost-effective method for many marine structures. In a patent document, Sakamoto (1989) discuss metal-containing antifouling coatings for fish nets.

The sophisticated nature of the factors affecting metal uptake by aquatic organisms are discussed by Simkiss and Taylor (1989). In the introduction to "Copper Bioavailability and Metabolism", Kies (1989a) points out that there are numerous dietary factors that can affect availability of copper in food. In an excellent histopathological study of copper in the human liver, Elmes et al. (1988a) comment that the molecular state of copper appears to determine its hepatotoxicity.

Whether as a nutrient or as a biological deterrent, the chemistry of copper limits biological availability, limits the amount of the metal that will be taken up by the organism and limits the amount which will enter into the metabolic machinery of the organism. Cromwell et al. (1989), for example, found that copper sulfate (CUS04) provided a much better growth stimulant for swine than copper oxide (CuO). (Incidentally, the 1973 restriction on the level of copper in animal feeds has been withdrawn by the U.S. Department of Health and Human Services (Food and Drug Administration) (Hoeting, 1990).)

The chemistry of the receiving environment has been shown to affect the biological impact of a synthetic effluent designed to simulate electroplating industry wastes (Le Du et al., 1990). Mineralization of the water appeared to be even more important than organic carbon content in the freshwater environment used.

Various techniques have been developed to minimize the input of anthropogenic metals including copper to receiving waters. Mesuere and Fish (1989) present evidence that small dentention ponds can be a useful way of retaining particulate copper in stormwater runoff from parking lot areas. Leak (1990) discusses a technique designed for metal recovery and removal in radiator repair shops. The technique enables the operator to recycle up to 80 percent of the hot caustic (boil-out) solutions used in the trade. Tsai and Nixon (1990) discuss a technical assistance program for small metal-plating operations to assist them in overcoming the increasingly stringent waste disposal regulations.

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I - THE BIOLOGICAL IMPORTANCE OF COPPER

I.1 COPPER AS AN ESSENTIAL METAL

Introduction

Copper is essential for life as well as being widely used in industry and in the arts. It is also a metal that can be alloyed, changing its chemical and structural nature (e.g. Barke, 1990). Technological developments in the use of copper date back through antiquity as indicated by archaeological work on copper (Hauptmann, 1989; Hauptmann et al., 1989) and bronzes (e.g. Kallfass and Hoerz, 1989; Schneider, 1989) from the late Egyptian period (745-330 B.C.) and Bronze Age (approx. 1000 B.C.). Moesta and Schlick (1989) discuss the technological capabilities of a Bronze Age furnace. Bronze has long been used in coins (Mao, 1989). Blue and green copper pigments have been found in ancient Egyptian wall paintings which Schiegl et al. (1990) indicate form a key to the accurate chronology of bronze technology in ancient Egypt. Archeological materials containing copper have been examined by determine technological capabilities of ancient man (e.g. Carlson, 1989). Northover and Salter (1990) discuss the use of copper, bronze and brass by Celtic craftsman who worked in Britain around the time of the Roman conquest (43 A.D.). Evidence of use of native copper by North American native peoples continues to accumulate (e.g. Wayman, 1989). Copper is also useful as a tool in certain analytical techniques (e.g. Suzaki et al., 1989) as well as in medicine.

Copper has a chemical ability to bind with many organics such as enzymes (e.g. Chu and Shaw, 1991). "The ease of complex formation, coupled with the high stability of many of the resultant complexes, gives copper the potential to interact strongly with organic compounds in living tissues" (Nickless et al., 1989a). This is one of the reasons why copper is an essential metal and also a reason for it being useful as a cidal agent when used in excessive amounts. "Copper is a heavy metal, causing deficiency symptoms in all organisms when concentrations are too low, and toxic effects when concentrations are too high" (Sloof et al., 1989). This concept is apparent in a number of publications, including articles in "Excess and Deficiency of trace Elements in Relation to Human and Animal Health in Arctic and Subarctic Regions" edited by Låg (1990) and released by the Norwegian Academy of Science and Letters. The requirements for copper and the ability to tolerate excess copper vary widely (e.g. Zhirmunsky and Tarasov, 1990). As a result there has been a great deal of work on the nature of the metabolic processes that require copper (e.g. Hurley et al., 1988) as well as the effects of excess copper.

A number of reviews address the importance of copper to organisms. These include general reviews of essential trace elements (e.g. Aggett, 1990; Noda, 1983; Shenkin, 1988, 1989, 1990). A brief but excellent discussion of the physiological role of copper is provided by Wachnik (1988) while Sharda (1988) provides a review of copper uptake and discussion of copper-containing agents within the body. Two collections of individual articles on biochemical as well as physiological aspects of trace elements including copper are found in the work edited by Caroli et al. (1989 and Hurley et al. (1988). Gibson (1989) provides an assessment of trace element status in humans and Favier (1988) reviews the value of essential trace elements in clinical biology. Because of its negative effect on growth and normal physiological function, the possibility of copper deficiency is often evaluated in various groups of humans (e.g. Smith et al., 1988) as well as animals (e.g. Ivanov et al., 1989a; Pott et al., 1989a). Gooneratne et al. (1989) and MacPherson (1989) review the effects of deficiency in ruminants and Prohaska (1990a) discusses biochemical changes occurring as a result of copper deficiency. A number of articles in Lei (1990a) review various aspects of the interaction between copper and lipid metabolism (Brannon, 1990; Carr and Lei, 1990b; Cunnane, 1990; Hassel and Lei, 1990; Lei, 1990b,c; Lei and Carr, 1990; Medeiros, 1990) and related topics (Allen, 1990; Allen and Mathias, 1990; Klevay, 1990b). Steinhauser (1990) provides a brief review of the agricultural significance of copper for soils, crops and animals.

A number of factors affect the biological availability of copper, whether from the environment or from food, and the metabolism of the metal (e.g. Kies, 1989a). Interactions between trace elements (e.g. Momcilovic, 1988; Mann et al., 1989; Pond et al., 1990) form an important topic in any discussion of trace metal balance, copper deficiencies and effects of excess metal. Organics that transport copper within an organism can affect its availability (e.g. metallothionein - Abdel-Mageed and Oehme, 1990a). The importance of certain, key copper-containing organics such as copper-zinc superoxide dismutase to normal metabolic function requires an adequate supply of copper. This enzyme, in particular, has been examined because of its metabolic importance as well as its genetic and evolutionary history (de Jesus et al., 1989; Getzoff et al., 1989; see also the discussion of "Life in Oxygen ..." in Gutteridge, 1990). The interaction of zinc and copper is suggested by Askari et al. (1990b) who report decreased urinary loss of zinc as well copper under conditions of copper deficiency.

The use of copper by man is diversifying as a result of technological changes. A number of applications were discussed at "Copper '90" held in Sweden, at Västerås, in 1990 (The Institute of Metals, 1990). In the "Minerals Yearbook, 1988 - Copper - Jolly and Edelstein (1988) discuss the nature of the copper industry and discussions of legislation directly or indirectly affecting the production and uses of copper. In a document issued by the National Institute of Public Health and Environmental Protection in The Netherlands, Janus et al. (1989) discuss some of the environmental concerns related to the use of copper, primarily in agriculture.

Introductory Comments on the Role of Copper in Organisms

Copper participates with organics in a number of biochemical processes whether in plants (e.g. Saur, 1990c), animals or humans (Sharda, 1988; Wachnik, 1988). As a result, copper deficiency can be associated with biochemical changes, something that has stimulated a great deal of biochemical and nutritional research (Prohaska, 1990a).

Certain aspects of connective tissue and structural protein formation and maintenance have been linked to an adequate supply of copper (Curley, 1987; Tinker et al., 1988). This is true for both invertebrates (e.g. Webb et al., 1989) and vertebrates (e.g. Sato et al., 1990). Copper is important in the maintenance of the cardiovascular system in humans (e.g. Kinsman et al., 1990) and has been suggested to play an important role in the maintenance of immune function and disease resistance (e.g. Soderberg et al., 1989; Stabel and Spears, 1989). Changes in concentration of copper have been associated with ischemia (Chen et al., 1990). As well, changes in the enzyme Cu, Zn-superoxide dismutase have been found with open-heart surgery (Hamano et al., 1990). In plants, tolerances of corn to drought and photooxidative herbicide tolerances have been associated with elevated levels of the enzyme (as well as glutathione reductase; Malan et al., 1990). In fact, the information accumulating about CuZn superoxide dismutase suggests the evolution of a unique organometallic compound (e.g. Getzoff et al., 1989) and an important relationship of this enzyme to the evolution of certain primitive groups of plants (de Jesus et al., 1989; Lesser, 1989). Stability of organic compounds has been attributed to the copperin the organic complex under certain conditions (e.g. Bobbio and Guedes, 1990). Copper, either directly or indirectly, plays a role in the hormonal regulation of the body (e.g. Mitsuma et al., 1989) and a role in the functions of nucleic acids (e.g. Palaniandavar, 1989).

Since copper may occur at very low levels in the oceanic environment, the possibility of metal deficiency has been suggested (e.g. Rainbow and Abdennour, 1989). However, the availability of copper to an organism is controlled more by the chemical species of copper than the total copper concentration. Uptake and utilization, from the environment or from food, is also affected by interacting agents, such as other trace metals (Kies, 1989a). This implies that it is the balance as well as the availability of trace metals that is important (e.g. Fungwe et al., 1990). With this as background, deficiencies in dietary copper intake by humans and laboratory animals have been related to a number of physiological conditions including inflammation (DiSilvestro, 1990), osteoporosis (Heaney, 1988), respiratory problems (Schwartz and Weiss, 1990; see also Badawi et al., 1990) and various cardiovascular-related problems (e.g. Peterson and Lee, 1990).

Changes occur in the dietary intake of copper as a result of either changing food habits (e.g. Grupe and Schutkowski, 1989) or food availability. Malnutrition is considered to be the most prevalent serious disease in the world (e.g. Golden and Golden, 1988). These authors review trace metal conditions in the three major forms of overt malnutrition, pointing out the importance of metalloids and trace metals, including copper. Evaluations of copper requirements are frequent and will be discussed in greater detail in a later section. The values range from less than 1 mgÀday (e.g. Turnlund et al., 1990) to 2-3 mgÀday (e.g. Bogden et al., 1990). Milne et al. (1990b) report that with healthy adult men, intakes below 0.9 mgÀday can result in signs of copper depletion. However, there is controversy surrounding the amount of copper that is required to maintain normal metabolism. Some of this may be a result of the numerous other chemical and physiological factors that affect both metal uptake and utilization, giving different results under different conditions or with a physiologically different group of individuals. Copper nutrition during pregnancy is of concern (e.g. Mameesh et al., 1990) and Moser-Veillon et al. (1990) comment (abstract of talk) that "Copper intake needs to be increased during lactation to compensate for Cu secreted into breast milk". Hambidge (1989) discusses trace element requirement sin premature infants, commenting on the need for a better understanding of copper flux in the body. The author points out aht (page 397) "It is recommended that the formula-fed premature infant receive 100 to 120 µg Cu/kg/day, even though the infant fed human milk manages on a much lower intake". Trace element nutrition in children is of continuing importance because of the functions of metals in growth and development (e.g. Curtis, 1990; Lönnerdal, 1988; Milner, 1990). Copper deficiency can occur from a combination of disease and low metal availability in food (Phillip et al., 1990). Since there are biochemical and physiological changes with increasing age, in animals as well as humans, there can also be changes in copper requirements and the ability of the body to metabolize copper as well as other trace metals (e.g. Bogden et al., 1990; Massie et al., 1990; Struck and Hillesheim, 1990).

Research on copper requirements and uptake by plants continues to be of importance to determine favourable conditions for growth (e.g. McFarlane, 1989b; Sánchez et al., 1989), to develop appropriate fertilizers (e.g. Arshad et al., 1989; Dowbenko et al., 1990; Komarov et al., 1987) and to make use of plants for the treatment of polluted waters (e.g. Sridhar, 1988) and metal-rich land. Testing for copper deficiency is an important aspect of this work since low soil copper levels have been correlated with yield loss and disease problems in certain plants (Evans, 1988). Copper deficiency has also been found in plants as a result of chemical properties of the soil rather than soil copper levels (e.g. Robson and Snowball, 1990). Copper deficiencies in wild and domestic ruminants can also be related to deficiencies in plants used as food materials (e.g. Froslie, 1990; McFarlane et al., 1990; Pott et al., 1989a-c; Wilson, 1989; Yu et al., 1988). In these cases, copper supplementation can minimize the effects of deficiency (e.g. Wilson, 1989). However, success of supplementation can be affected by the nature of the supplement and the interaction of factors affecting metal availability and the physiological state of the animal (e.g. Norton et al., 1990). Olkowski et al. (1990), for example, note an interaction of dietary sulphur, copper and molybdenum that can affect the immune function in sheep.

Introductory comments on the effects of copper deficiency

The number of effects is large. Individually, they will be discussed at appropriate places throughout the review. They are best known in cultivated crops, domestic animals (reviewed in Gooneratne et al., 1989 and MacPherson, 1989) and humans. In plants, copper deficiency can affect photosynthesis (Casimiro et al., 1990; Droppa and Horváth, 1990; Henriques, 1989) and cause irregular plant growth (e.g. Olson et al., 1990).

In laboratory animals and humans, dietary copper deficiency has been associated with reduced immune function (DiSilvestro and Carlson, 1990; Lukasewycz and Prohaska, 1990; Prohaska and Lukasewycz, 1989; Windhauser, 1988; see also Kishore et al., 1990a) and anaemia (Akers et al., 1990; Boyne, 1990; Prohaska, 1990b,c). Changes in blood chemistry and blood components have been reported with copper deficiencies (Babu and Failla, 1990; Bala et al., 1990a-c; Failla et al, 1990b; Johnson and Dufault, 1989, 1990a,b; Lynch and Klevay, 1990; Ralston et al., 1989) as they have with lymphoid cells (Kramer et al., 1990a,b). Halas and Klevay (1989) provide some evidence that copper deficiency can elevate blood pressure.

The relationship between ischemic heart disease and copper deficiency is reviewed by Klevay (1989). Impaired tissue formation and maintenance has been linked to copper deficiency, particularly with the cardiovascular system (Farquharson et al., 1989; McCormick et al., 1989b; Saari and Johnson, 1990; Tinker et al., 1990). Variation in copper intake may cause significant changes in the superoxide dismutase levels in certain cardiac tissue (Askari et al., 1990). Copper deficiency in hens has been linked to abnormal development of eggs, in part because of impaired protein formation of the egg shell membrane. Because of its role in certain enzyme systems, the antioxidant status of an organism can be affected by copper deficiency (e.g. Hammermueller, 1990; Hathcock et al., 1990; Lynch and Strain, 1989, 1990; Rosenbaum et al., 1990; Saari et al., 1990a). Hypercholesterolemia is found in copper-deficient rats (e.g. Johnson, 1990), possibly a result of defects in cholesterol metabolism after its release from the liver (Koo et al, 1990) although other factors may be responsible (e.g. Al-Othman et al., 1990; Carr, 1989; Hassel et al., 1990; Yount et al., 1990a), including increased hepatic cholesterol synthesis (Carr and Lei, 1990; Yount et al., 1990b).

Brain activity, nerve physiology and hormonal regulation may, directly or indirectly, be affected by copper status (e.g. Dake and Amemiya, 1991; Hall et al., 1990; Penland et al., 1989). Prohaska and DeLuca (1988, page 109) point out that "Copper is required for development and homeostasis of the nervous system but there exists much controversy as to specific changes that occur when copper is limiting". Deficiency produces a change in the distribution and metabolism of norepinephrine and dopamine, possibly a result of reduced hormone synthesis due to a limiting copper-containing enzyme, dopamine-β-monooxygenase activity as well as elevated turnover (Gross and Prohaska, 1990; Prohaska et al., 1990; see also Prohaska and deLuca, 1988 and Sun and O'Dell, 1990). Changes in the levels of both hormones in the heart are suggested to precede and may contribute to the development of cardiac hypertrophy (Seidel et al., 1990). Saari et al. (1990b) present evidence that although copper deficiency can lead to hypercholesterolemia and cholesterol feeding causes a reduction in copper status, there is no interrelationship in terms of inflammation and thrombosis. Copper deficiency has been asociated with deficits in renal function (Noordewier and Saari, 1990) and toxic effects of some drugs on renal microvillar enzyme activity (Reeves et al., 1990).

Copper deficiency can be exacerbated by dietary fructose in rodents (e.g. Burns et al., 1990b; Fields and Lewis, 1990c; Fields et al., 1990) but not in pigs and probably not in humans (Schoenemann et al., 1990a,b). With rodents, Cunnane et al. (1990) report that fructose, as a dietary carbohydrate, can affect tissue fatty acid and lipid composition in the copper-deficient rat. They comment (abstract) that "Changes in the long chain fatty acids in heart phospholipids may be related to the higher mortality commonly observed in rats fed a copper-deficient diet containing fructose". Tissue enzyme activity and exocrine function in the pancreas can also be affected in copper-deficient rats (Dubick and Majumdar, 1988; Mylroie et al., 1988), especially rats fed a high-fructose, low-copper diet (Lewis and Fields, 1989). Tissue sorbitol concentrations are reported to be affected by the high-fructose, low-copper dietary combination, presumably a result of the effect of copper deficiency on the metabolism of glucose, fructose and sorbitol (Lewis et al., 1990a). There is an interaction of amino acids and copper nutriture, copper deprivation affecting the plasma amino acid profile in rats and sulfur amino acid nutriture affecting the nature and severity of symptoms from copper deficiency (Shuler et al., 1990). Copper may also play an important role in amino acid metabolism in the brain (Farms et al., 1991). Interaction of copper levels with the concentration of at least one group of peptides (plasma atrial natriuretic peptides) can also exist and may be important in cardiac function although the effect is most apparent in male rats (Bhathena et al., 1988). Sex differences in the expression of copper deficiency have also been reported by Lynch (1988) and Fields et al. (1988). Reversible impairment of testicular function has been reported as an effect of experimentally induced copper deficiency in rams (Van Niekerk and Van Niekerk, 1989c,d).

I.2 USES OF COPPER

As a result of its chemical and physical properties, copper is widely used in industry, medicine, agriculture and the arts. This section of the review will use recent literature to discuss some of the uses which man makes of copper.

Copper as a nutrient supplement for plants

When concentrations of biologically available copper are low, supplementation can stimulate plant growth and improve plant quality (e.g. Grigoryan et al., 1990; Hazra et al., 1987; Wu and Wu, 1990). This continues to be demonstrated for seed germination as well as growth at all stages (e.g.

Aleshin et al., 1989; Duka et al., 1990). Supplementation can, for example, improve biomass production in pine seedlings (Saur, 1991) as well as improve ruminant forage quality (Goodwin-Jones, 1990; Sherrell, 1989). Copper fertilization has also been used when concentrations of competing elements (e.g. molybdenum, phosphorus) are excessively high, producing a detrimental ratio of the element with copper (Saur, 1989b; Stark and Redente, 1990b).

Interactions with other nutrients, such as nitrogen and phosphorous, as well as certain trace metals is an important consideration in any supplementation since tissue copper concentrations can be affected (e.g. Jurkowska et al., 1987) or can affect (Venkateswarlu and Misra, 1987) tissue nutrient and trace metal concentrations (e.g. Seliga, 1990). The effect of copper supplementation can often be seen in beneficial changes in the general nature of the organism (e.g. Singh, 1983) or the concentrations of various organics in the tissues (e.g. amino acids; Szakal and Tolgyesi, 1990). However, supplementation under deficient conditions does not always "cure" physiological problems in plants even though it improves some of the symptoms of the problems (e.g. Hopmans, 1990). This may be due to interactions between the physiological properties of the plant and the chemical and physical properties of the environment, which can affect the results of supplementation (e.g. Schum et al., 1988). These also make it difficult to use either tissue or soil copper levels to indicate the copper status of the plant (McFarlane, 1989a).

A variety of copper-containing fertilizers have been developed, in granular (Ciparis et al., 1990; Glabisz and Grzmil, 1989; Meerovskaya et al., 1989) and liquid form (Bezuevskii et al., 1990; Brazauskiene et al., 1990), to be used as a seed dressing (Kudashkin, 1989) as a soil enrichment, and as a mixture to control bacterial and fungal infections as well as increase plant growth (Feng, 1989a). Requirements for supplemental copper are dictated by the concentration of soil copper present, its biological availability, the nature of the plant and the nature of the fertilizer (e.g. Jasiewicz, 1990). Dowbenko et al. (1990) reviews copper fertilizer requirements on peat soils, Xu and Dong (1989) the copper status of permanently waterloged soils, and Mathur and Levesque (1989) discuss soil tests for copper, iron, manganese and zinc in histosols. Concern has been expressed about long-term fertilization effects on soil copper levels although Schwab et al. (1990), however, report little effect on organic-extractable copper in a pasture soil that had continuous fertilization for 40 years. Much of the applied copper is bound by soil organic material and rendered unavailable to plants (e.g. Gorlach, 1989). Application techniques do need to be considered in terms of the nature of the plant (e.g. Sanderson and Gupta, 1990).

Copper in fermentation

Since copper is an essential metal, provision of appropriate amounts of the metal is important in fermentation. Changes in the amounts of copper can affect the output of a fermentation process. Jernejc et al. (1990), for example, comment on the interaction of metal (copper and manganese) supplementation and lipids in citric acid excretion of *Aspergillus niger*. Kaneko et al. (1990) report that one lactic acid bacterium (*Lactococcus lactis lactis* 3022) produced more biomass when grown aerobically with hemin and Cu^{2+} and, as well, converted most of the glucose substrate to diacetyl and acetoin.

Copper as a nutrient supplement in animals

Supplementation is used either when feed copper levels are inadequate or there is a need to control microorganisms and parasites. In 1990 the U.S. Department of Health and Human Services withdrew a proposed restriction on the level of copper in animal feed materials (Hoeting, 1990). This proposed restriction (15 ppm copper) was withdrawn because of the need for copper supplementation and the lack of substantial evidence against its use. Continued improvement of feed additives is indicated by patent documents (e.g. Dixon, 1989).

Copper deficiency can produce a number of physiological problems in both laboratory (e.g. Yokoi et al., 1989, 1990a,b) and domestic animals. Parker et al. (1989) review the types and effects of feed additives for swine, pointing out that copper is a required nutrient and possesses antibacterial properties. Feed conversion is improved by some copper supplementation although pork fat softening can occur at high levels (Astrup and Matre, 1987). When growth and physiological properties are compared, copper supplementation effects differ between germ-free and conventionally-reared pigs (Shurson et al., 1990). Although normally used at concentrations of 6-11 ppm, high dietary copper (125-250 ppm) is often used to promote growth of pigs (Cromwell et al., 1983; Shurson et al., 1988; Ward et al., 1989a). Copper sulfate is the most frequently used chemical in supplementation; Stansbury et al. (1990) note that inorganic and organic complexed compounds were not more efficacious than coper sulfate for nursery or growing pigs. Cromwell et al. (1989) note that copper in the oxide form is largely unavailable to weanling pigs and is ineffective as a growth promoter. Use of high zinc and adequate copper (12 ppm) has been reported to produce a decrease in HDL cholesterol in plasma of young pigs (Klevay and Pond, 1990a). Irie (1990) notes no apparent change in the fat characteristics of pigs supplemented up to 200 ppm copper, with a kapok meal. Copper treatment of rapeseed meal has been reported to improve its value as a food source for pigs (Schöne et al., 1990).

Supplementation of sheep can reduce some gut parasite infestations (Bang et al., 1990b) and has been shown to improve nutrient retention, in gestating ewes grazing Montana winter range (Harris et al., 1989). The form of supplementation used includes copper oxide wire particles (Bang et al., 1990a) and soluble-glass boluses which prolong the supplementation period although with the potential for bolus loss by the animal (Driver et al., 1988c; Matsui et al., 1989; Millar et al., 1989a). Uptake of copper from copper oxide wire particles from the gut can also be affected by nematode infection which increase gut pH (Bang et al., 1990a).

Mineral deficiencies are not uncommon in both natural and cultivated pastures (e.g. Tokarnia et al., 1988). Regius Möcsényi et al. (1990b) used tissue concentrations in certain parts of the organism to indicate mineral status and deficiency/sufficiency levels of dietary metals. When deficiency is diagnosed, ruminants can be supplemented with copper to maintain adequate tissue levels and minimize the risk of several morphological and physiological problems caused copper deficiency (e.g. Booth et al., 1989; Bridges and Moffit, 1990; Dill et al., 1989). Since molybdenum can reduce copper uptake by ruminants, copper supplementation is especially important with high molybdenum diets (Wittenberg et al., 1990; Wod et al., 1989). As with sheep, supplements can be oral, wire particles (Dunbar et al., 1988; Ruksan et al., 1988) or glass boluses (Driver et al., 1988b), or injected.

Copper in medicine

Medicines containing copper are widely used for both animals and humans (see review in Collery et al., 1990).

Inflammation - copper salicylate and copper phenylbutazone are topically applied as anti-inflammatory agents in animals (Auer et al., 1990a). Sorenson et al. (1989) state (abstract) that copper diisopropylsalicylate has "... antiinflammatory, antiulcer, anaticonvulsant, anticancer, anticarcinogenic, antimutagenic, and radiation recovery activities and it prevents reperfusion injury". Other recent work on the nature and anti-inflammatory activity of copper complexes includes that of Auer et al. (1990b), Brumas and Berthon (1990), Dendrinou-Samara et al. (190), Ferrari et al. (1989), Frechilla et al. (1990a,b), Hijleh (1989), Jackson and Kelly (1989, 1990), Kishore (1990a), McGahan (1990), Moiny et al. (1990), Parashar et al. (1990), Roch-Arveiller et al. (1990a,b), Szabová et al. (1990), Thunus and Dauphin (1990), Vaille et al. (1990). Peptide copper complexes have been developed for healing wounds in horses (Pickart, 1989).

Cardiovascular problems - as a transition metal, copper is implicated in enhancing and assisting in the deleterious effects induced by free radicals in cardiac injury (e.g. Appelbaum et al., 1990). As a result, it is the organometallic agent that is frequently found to be beneficial. Examples of recent studies on or description of copper-containing agents found to be beneficial in the range of cardiovascular problems includes that of Appelbaum et al. (1990), Hori (1989), Imaizumi et al. (1990). A copper-containing agent, copper(II) pyruvaldehyde bis(N^4-methylthiosemicarbazone) or [Cu]PTSM, has shown potential as a flow tracer in positron emission tomography (PET). Work on this agent includes Bergmann et al. (1990), Barnhart and Green (1990), Fujibayashi et al. (1989, 1990), John and Green (1990), Mathias et al. (1990b), Shelton et al. (1989)

Abnormal growth (tumours, cancer) - since copper can be used as a growth-control agent, it has been used in a number of medications that control abnormal growth in animals and humans (see review by Badawi, 1990). Recent work includes Apelgot and Guillé, 1989a,b, 1990b; De Pauw-Gillet et al., 1990; Guillé and Apelgot, 1989, 1990; Monti et al. (1990), Nayak et al., 1990; Nistor et al. (1990), Oikawa et al. (1990), Ozawa et al., 1990; Ozawa and Hanaki, 1991; Rabinovitz and Fisher (1989), Tarasiuk et al. (1990), Ujjani et al. (1990). This also includes the use of copper in postirradiation treatment (Jagetia and Ganapathi, 1990; Soderberg et al., 1990) and as a label for tumour targeting (e.g. Moi et al., 1990; Morphy et al., 1990; Roberts et al., 1989).

Other uses for copper in medicine include use in filters for diagnostic x-ray equipment. Thorsen et al. (1990) compared copper and niobium filters and conclude (abstract) that a "... copper filter is better than a niobium filter in achieving a considerable dose reduction to the patient, and is far less expensive". Copper vapour laser treatment of vascular malformations (e.g. port-wine stains) is well known. Mordon et al. (1990) provide a review of its use by members of the French Society of Medical Lasers. Recent work includes Nemeth and Reyes (1990a,b), Pickering et al. (1990), Reyes and Nemeth (1990). Copper chelate sepharose has been used to concentrate staphylococcal enterotoxin from food extract (Dickie and Akhtar, 1989). The authors comment (abstract) that "The method should prove useful as a partial purification and concentration step in the analysis of foods incriminated in food poisoning outbreaks".

Copper in dentistry

"Compared to conventional amalgams, high copper amalgams offer improved corrosion and mechanical properties that lead to clinical superiority" (abstract in Marshall et al., 1990). As a result, there is continuing use of copper use in dental amalgams. In a study of 767 dental restorations using high-copper alloy amalgams, Smales et al. (1990) found that all restorations remained clinically satisfactory; large restorations on occlusal surfaces of molar teeth showed the greatest deterioration. Osborne and Norman (1990) discuss the results of a 13-year clinical assessment of 10 amalgam alloys, 9 copper and one traditional amalgams. They state the alloys that had the lowest loss rates and the best mechanical properties over that time period. Copper will also inhibit growth of supragingival plaque bacteria and thus reduce bacterial acid production (Eisenberg et al., 1989).

The use of copper in contraceptives

A variety of intrauterine devices (IUD's) contain copper as an active element. "In clinical trials it has been clearly shown that copper not only increased contraceptive efficacy, but also decreased the expulsion rate and the occurrence of complications, such as hemorrhage" (page 752 in Hernandez-Perez et al., 1989). Continued development and widespread use of IUD's has led to improvements that reduce the risk of uterine perforations and other detrimental side effects (Edelman and van Os, 1990b). Hirvonen and Idänpään-Heikkilä (1990) carried out an exhaustive study of cardiovascular death among women under 40 years of age that used low-estrogen oral contraceptives and intrauterine devices in Finland. Based on their 95% confidence limits, there does not appear to be a detrimental effect of intrauterine devices on cardiovascular death figures. A number of recently-published studies provide information on the effectiveness and problems of copper-containing IUD's. These include Edelman and van Os (1990a; Multiload 250, Multiload 375), Puraviappan et al. (1989; Multiload 250 IUD), Rowe et al. (1990; TCu380A, TCu220C, Multiload 250), Sivin et al. (1990a; Gyne T 380) and Sivin et al. (1990b; Copper T 380Ag).

Copper in chemistry

Many of the chemical reactions important to organisms and humans involve metals. With copper, the ability to interact with organics makes it a useful tool to test chemical reactions as well as chemical properties of organics and organometallic agents. Organocopper reagents are used in substitution reactions (e.g. Bolitt et al., 1989). They frequently serve as catalysts (e.g. Brik, 1990; Desimoni et al., 1990; Keegstra et al., 1990; Marjit and Sharma, 1989; Nozaki, 1990). Certain copper complexes are used to mediate reactions (e.g. Cervello et al., 1990; Nair and Sells, 1990). Since copper is biologically active, the synthesis of appropriate organocopper complexes is an important step in the development of pharmaceutical agents as well as pesticides (e.g. Dilanyan et al., 1989). This is followed by an evaluation of the structure and biological activity of promising compounds (e.g. Nagar, 1989).

Copper can catalyze oxidation processes and thus be important in intentionally-controlled chemical reactions as well as in food spoilage. Sessa et al. (1990) report that L-Ascorbic acid plus cupric sulfate inactivates soybean Kunitz trypsin inhibitor. Copper inhibition of hydrogenase has been reported (Fernandez et al., 1989). Oyama et al. (1988) mention the use of copper or zinc as a catalyst in a survey on synthesis technologies of methanol as an alternative fuel.

Copper is used in the analysis of a number of organic materials. It has recently been discussed for the determination of sugar concentration (Liu and Liu, 1988), the activity of a soil microbe enzyme (Capalash et al., 1990), and estimation of the degree of polymerization of plant polysaccharidase-generated oligosacharides (Doner and Irwin, 1990). Cupric acetate and cupric sulfate are used to stain neutral lipids, fatty acids and phospholipids for quantification (Rustenbeck and Lenzen, 1989). Potentiometric determination of amino acids can be accomplished with copper sulfate and a copper electrode (Lati et al., 1989). Hara et al. (1990) describe the use of a 10-phenanthroline-hydrogen peroxide-copper(II) chemiluminescence system to measure proteins. Proteins can also be measured by densitometric measurement after copper iodide staining (Root and reisler, 1990a,b). Nakajima et al. (1990) describe the use of copper in the determination of zink pyrithione in antidandruff preparations. Ligand-exchange chromatography of nucleosides, nucleic acid bases and amines has been accomplished with a copper-containing agent (Ersöz et al., 1989). Separation and concentration of proteins is possible with the use of copper-stained sodium dodecyl sulfate polyacrylamide gels (Vanfleteren and Peeters, 1990). Copper-chelex is described as a selective adsorbent for certain derivatives of phenolic substances (Ohta et al., 1990). In association with certain gels, copper can be used to immobilize protein (Moriya et al., 1989).

Copper as a pesticide

Since high concentrations of biologically available copper are toxic, the metal can be used in the control of noxious plants and animals as well as a plant and food preservative (e.g. Zemanova, 1988). In a patent document, Nielsen (1990) describes a moss killer which used "atomized elemental zinc and atomized elemental copper". Lüderitz et al. (1989a) discuss the use of copper in the control of algae, commenting on the time for application and the way to estimate uptake by the algae. A good deal of effort has been spent in developing pesticides to control pests of economically important plants. These include agents to combat plant viruses (e.g. Davarski et al., 1989) as well as bacteria (e.g. Feng, 1989b; Ling, 1989), algae (Lim and Hew, 1990) and fungi (e.g. Feliciano et al., 1989; Ptaszkowska et al., 1989; Vargas de Alvarez et al., 1990). Galoux and Bernes (1990) discuss 11 different commercial wettable antifungal formulations containing copper. The discussion includes quality control which is required for the official approval of pesticides in Belgium. Application efficiency is important in the commercial use of pesticides; Nichols et al. (1990) discuss the use of cupric ion (abstract)"... as a tracer to measure the spray application efficiency of agricultural chemicals on plants". Persistence as well as shelf life of copper-containing pesticides is important, with continuing evidence of the benefit of metal complexes (Prasad et al., 1990). Since some disease organisms or, in animals disease conditions (e.g. Sunila and Farley, 1989), can tolerate high concentrations of copper, the concentration needed for effective action becomes important. Side effects of pesticides are also of concern, both in terms of effect on the plant and effect on non-target species. In a discussion of the effects of pesticides on a type of bean plant, Schnelle and Hensley (1990) report no long-term effect from 20 different pesticides, including copper-containing agents.

Recent literature includes the description and/or evaluation of copper-containing fungicides for the treatment of diseases in a wide range of plants (e.g. Anonymous, 1989). These include oilseed plants like rape (Ansari et al., 1990; Saha and Singh, 1985) and groundnut (Murugesan and Mahadevan, 1988). They include various grains (Piening et al., 1989; Tolstikov et al., 1989), rice (Narashimhan et al., 1990) and legumes such as the peanut (Osborne and Taylor, 1989), guar (Mathur and Shekhawat, 1988), herbs (Thomas et al., 1989) and soybean (Prathuangwong and Choochoa, 1989, 1990). Root crops discussed in recent fungicide literature include the onion (Ahmed and Goyal, 1988), potato (Bhattacharyya et al., 1987), carrot (Muniz and da Ponte, 1988) and parsnip (Cerkauskas and McGarvey, 1988). Other crops include the cucurbit, a gourd, (Li and Liu, 1990), tomato (Gorska-Poczopko et al., 1987), squash (Azevedo and Silva, 1988), beans (Sharma, 1988), the cocoa (De Figueiredo, 1988; Rudgard et al., 1990; Singh, 1989a), fruit trees (e.g. McMillan, 1990), tobacco (Liang, 1990) and coffee (Quijano-Rico, 1987). Quijano-Rico (1987) provides a good review of the biology and effects of coffee rust as well as its control. Control of fungal diseases on trees, with copper-containing agents is discussed for eucalypts (Ruiz et al., 1987), nut trees (e.g. Banihashemi, 1990; Riggert et al., 1989), date palm (Khatri and Shekhawat, 1989) poplars (Khan et al., 1990b) and conifers (e.g. Franich, 1988; Presnell and Nicholas, 1990). It has long been used with grape pests (e.g. Ventura et al., 1988), changes the species composition of certain organism groups in vineyards (Marchesini and Gambaro, 1989) but is reported to be effective without detrimental effects to parasitoids of insect pests (Mani and Krishnamoorthy, 1989). Other uses of fungicides are given in Santos and Resende (1988), Servontain and Knox-Davies (1990)

A variety of copper-containing agents have been used for animal pests. Nematode parasites of plants have been treated with copper (Rovesti et al., 1988; Tanda, 1988) although with only limited success. Copper tape has been used as a slug repellant, for plant protection (Suzuki et al., 1990a). Use of copper sulfate is widespread, ranging from control of pests (e.g. Khajuria and Bali, 1988; Schwartz and Capatos, 1990) to control of diseases arising from the use of Thiram, a fungicide (Wu et al., 1990b). Copper sulfate has been used as a slug toxin (Young and Wilkins, 1989) as well as evaluated as a means of inhibiting earthworm castings in turf (Baldwin and Bennett, 1990). It has also been effectively used in the control of a parasitic mite found with commercial bee hives (Guiraud et al., 1989). The authors note (summary) that "Copper residues in honey never significantly increased from natural levels, ...". Copper sulfate is used in the control of subterranean termites (Roomi et al., 1990), has been found to be partially effective for the control of leeches on marine turtles (Choy et al., 1989) and is effective against swimmers' itch which is caused by an infective stage of nonhuman flukes (Yescott, 1989). Copper is one of the agents that was considered for use against a maize rootworm in South Africa (see references in Drinkwater, 1989).

Pesticides include disinfectants, agents used to reduce the impact of or potential for infection (e.g. Sondossi et al., 1990). They include agents like copper sulphate when it is used to control outbreaks of liver flukes (Bielecki, 1987). They also include copper complexes used as antiviral agents (Ivanov et al., 1989; Tomas et al., 1989) or against organisms like *Escherischia coli* (Vairamani et al., 1989) and malaria-producing protozoans (Meshnick et al., 1990). The relationship between copper in antiviral agents and virus growth is complex and not well understood, appearing to be related to cell receptor and viral metabolism (e.g. Calvert and Simon, 1990). Kovacic et al. (1989b) report evidence that drugs used against parasitic worms are effective either because they cause oxidative stress or disturb electron transport within the worm. Control of parasitic worms can involve control of their intermediate hosts, usually molluscs. As a result, the release rate of molluscicides becomes an important factor (Helaly et al., 1990).

Electrolytically generated ionic copper and silver has been used in the disinfection of water systems, against bacteria and protozoans, under reduced levels of free chlorine (Cassells et al., 1990; Yahya et al., 1990; see also Yahya and Straub, 1990). Landeen et al. (1989) comment that systems used in disinfection of *Legionella pneumophila* showed increased inactivation rates with added copper and silver (400:40 µgÀL Cu:Ag). Comparable results are reported by LeChevallier et al. (1990). Copper in water pipes and in drinking water has been shown to reduce microbial growth (e.g. Schoenen and Schlömer, 1989; Versteegh et al., 1989). Nuttall (1990), in a discussion of controlling bacterial growth including *Legionella*, comments (abstract) "... that copper is probably the only common plumbing material which has the ability to suppress bacterial growth". However, bacterial colonization of copper pipes has been reported (Tuschewitzki, 1990) and Schulze-Röbbecke et al. (1990) found no influence of copper pipes on *Legionella* in a hospital hot water system. The effect of copper as an antibacterial agent in water supply systems is affected by the chemistry of the water (e.g. pH) as well as water temperature.

Copper, corrosion and biodeterioration

Exposure of copper and its alloys in aquatic environments can cause corrosion, a factor which is important in ship building and ship maintenance (Lenard et al., 1989). However, the release of enough copper from either a metal source or from a metal-containing compound can inhibit biofouling in both aquatic and terrestrial environments. As a result, copper-containing compounds have been routinely used for the preservation of wood against fungal decay (e.g. Newbill and Morrell, 1990). Pendleton (1989) notes the beneficial effect against marine borer attack of copper-containing sheathing and coatings on marine pilings at Pearl Harbor. Williams and Knox-Holmes (1989) and Williams et al. (1989) discuss marine biofouling solutions for closed seawater systems, noting that a "copper/chlorine" system was the most efficient at reducing fouling both inside steel pipes and on titanium heat exchange surfaces. Callow (1990; see also Callow and Edyvean, 1990) provides an excellent review of ship fouling, commenting on the problems with tributyl tin and copper. She points out that new compounds are being developed, such as those containing dithiocarbamates which act synergistically with copper (e.g. Oishi et al., 1989). Peacock et al. (1989) describe a copper-based gelcoat for use on fiberglass vessels, a coating that contains 70 wt.-% copper particles.

New coatings continue to be developed against biofouling in terrestrial and marine environments (e.g. Göttsche and Marx, 1989; Maynard, 1990; McIntyre and Pasek, 1990; Moewius et al., 1989; Price and Brady, 1989). Absorption of copper and arsenic by *Eucalyptus* has been of concern although Khan (1989) reports that arsenic did not affect the movement of copper. Recent discussions of preservative treatment include ammoniacal copper arsenate on aspen waferboard (Gertjejansen et al., 1989), copper naphthenate with pressure application (De Groot et al., 1988), and copper-chrome-arsenate (CCA) on wilt-diseased coconut palm (Gnanaharan and Dhamodaran, 1989). Soltis and Winandy (1989) note negligible effect on strength, of CCA treatment of southern pine lumber although it did with loblolly pine (Winandy, 1989). Doi (1989) reports that CCA treatment of wooden sills was not fully effective in suppressing surficial fungal growth under the fungus cellar conditions of the test. In an evaluation of CCA treatment, Green et al. (1989) note that treatment prior to soil burial of wood reduces moisture uptake and, as a result, leaching of the preservative. Concern for release of wood preservatives into the environment has sponsored work on alternatives, including natural metabolites (e.g. Smith et al., 1989).

Other biologically important uses of copper

Although the uses of copper in heavy industry are outside the scope of this review, uses in other areas such as historical uses in the arts are included. Copper has had a long and noble history in both medicine and the arts; it is difficult not to include this in a review of the biological importance of copper.

There is a longstanding historical use of copper in jewelry and a technical ability to produce alloys with the metal. This continues to be indicated by information from archaeological findings such as the tin bronze Bronze Age bracelet (ca. 1000 B.C.) described by Kallfass and Hoerz (1989). Schiegl et al. (1990) provide an interesting discussion of apparent technology-related changes in the preparation of copper pigments used in ancient Egyptian wall paintings. The authors relate this to the chronology of bronze technology in ancient Egypt. Copper pigments from other paintings have yielded considerable information on the technological ability of man at various times through history (e.g. Naumova et al., 1990). Technological changes in the alloying of copper are also indicated by the decorative metallurgy of the Celts who used both coating and surface treatment techniques in the first century A.D. (Northover and Salter, 1990). Modern analytical techniques, such as neutron activation analysis, are now being used to elucidate the nature of the material and the technology used with copper in the past. Wayman (1989), for example, used neutron activation analysis to examine copper artifacts from the Inuit people of the

Canadian Arctic. He comments (page 70) that "This work has shown how INAA data, in combination with microstructural analysis, can help archaeologists to answer questions related to the origin of copper artifacts within one culture in which both native copper and smelted copper were used to produce aesthetically similar artifacts".

The combination of modern technology and the tractability of copper continue to produce items for today's needs. Surface coatings of copper on silicon (Walker et al., 1990) and carbon fiber reinforcement of copper matrix composites (Yulin and Liu, 1990) are two examples of the types of things that can be done with today's technology. The uses of these "high-tech" items range from the field of electronics to the removal of undesirable materials from fluids (e.g. Voecks and Sharma, 1990). Other, less "high tech" uses are commonly found. Tripathy et al. (1990) discuss the use of totally copper-jacketed bullets to reduce airborne lead in a covered firing range. Gourdin (1990) discusses the characteristics of copper shaped-charge liner materials. Copper-coated plant containers have been used to control root growth and improve transplant success in a variety of plant types (Struve and Rhodus, 1990). Strips of metal have been used as an animal feed preservative (Halley et al., 1990). In patent documents, Ichise and Kuroda (1989) and Seto and Yokoyama describe copper-containing adsorbents for preservation of fresh vegetables and fruits. Both remove ethylene and acetaldehyde and reduce their effect on spoilage. Copper has also been used in an antimicrobial film for packaging food (Mita et al., 1989). Copper chloride has been used to prevent ethylcarbamate formation in fruit brandies and spirits (Christoph, 1989). Hayakawa (1989) describes the use of copper sulfate in the drying of cut flowers. Copper chromite catalysts have been used in the hydrogenation of fatty acids and fatty acid esters to fat alcohols (Schneider et al., 1987).

Hashizume et al. (1990) describe a new copper alloy for lead frames used in electronics. Bugga et al. (1989), in a patent application, briefly describe a copper chloride cathode for a secondary battery. Physical and chemical properties of certain copper oxide superconductors are discussed by Golub et al. (1990) and Kusuhara et al. (1990). Solar power cells containing copper are discussed by Gillette et al. (1990; see also Kapur et al., 1989, Mooney and Hermann, 1990 and Ullal et al., 1990). Sulfur dioxide can be removed from flue gases by supported copper and iron absorbents (e.g. Melson, 1988). Filters made from charcoal with small quantities of copper, chromium and silver ("Whetlerite") can remove poisonous agents such as cyanogen chloride from air (Krishnan and Birenzvige, 1989).

Garner and Zinkle (1990) and Garner et al. (1990) review the irradiation experiments on copper alloys, for potential use of copper and copper alloys for high heat flux applications in fusion power devices. In an International Copper Association and Copper Development Association-sponsored study, Van Konynenburg et al. (1990) discuss "background studies in support of a feasibility assessment on the use of copper-base materials for nuclear waste packages in a repository in tuff". CDA materials have also been evaluated for containers by Bullen and Gdowski (1988) and Gdowski and Bullen (1988). Other work done on copper-based materials, for the Nevada Nuclear Waste Storage Investigations Project (Yucca Mountain Project), include evaluation of copper as a container material (Kass, 1989) and copper-Zircaloy interactions (Smith, 1990). Copper has also been considered as a container material for nuclear waste at Hanford, Washington (Hoover et al., 1987) and for the Swedish nuclear waste management program (Ekbom and Bogegaard, 1989).

I.3 COPPER AND ORGANISMS

The physiological requirements of an organism dictate the need for a certain amount of copper (e.g. Wachnik, 1988) whether in food or directly, from the environment. This means that copper will be found in the organism. As an indication of metal uptake and concentration above background values, Slin'ko (1988) notes that the temporal variability of particulate copper and zinc is correlated with changes in biomass in estuarine areas. As a result of metal uptake, tissue copper concentrations have been used as an indication of metal deficiency (Wachnik, 1988), adequacy or excess (e.g. Kisgeci, 1989). This is often with the assumption that metal within the organism is being used or is available for use. However, Rainbow et al. (1990) discuss the need for an understanding of the accumulation strategy adopted by an organism before concluding whether the tissue concentration is high, low or of little significance. In the introduction to "Copper Bioavailability and Metabolism", Kies (1989a) comments that "Dietary, environmental and genetic causes may result in symptoms of copper deficiency or copper toxicity ...". These causes along with metal speciation form an important parts of the review of literature on tissue copper concentrations.

I.3.1 TISSUE COPPER CONCENTRATIONS UNDER NORMAL CONDITIONS

Microorganisms and plants

Tissue metal concentrations vary widely in both microorganisms and plants. Mantere-Alhonen and Vuorinen (1989) report concentrations ranging from 44.4-102.0 mg/kg (dry weight) in some lactic acid bacteria and bifidobacteria while Satake et al. (1990) found concentrations as high as 17,900 mg/kg (reported as µg/g) in copper mosses growing under a copper roof of the Tsukuba shrine in Japan. In fact, the distribution of plants capable of growing in high levels of copper tends to be restricted to soils with high metal concentrations (Garcia and Clark, 1989). Tissue copper concentrations frequently differ within the plant (e.g. Kotzé and de Villiers, 1989; Markert, 1990; Mozafar, 1990; Wasserman, 1989). Okada et al. (1990) report that in the stems of an oak (*Quercus mongolica*), maximum copper concentrations were at the boundary between heartwood and sapwood. In a stand of loblolly pine (*Pinus taeda*), Jordan et al. (1990) found an inverse relationship between radial growth and the copper in xylem tissue. (Radial growth expressed as basal area increment.) In an evaluation of rootstock effect in 'Redhaven' peach, Brown and Cummins (1989) report that (abstract) "Differences among rootstocks were most evident in the foliar levels of Ca, Mg, Fe, Mn, B, and Cu".

Tissue concentrations can change as a result of the nature of the growth medium or growth conditions, whether natural or artificial (e.g. Bræke and Håland, 1990; Dubik et al., 1990; Geyer et al., 1990; Kawai et al., 1990; Locascio and Rhue, 1990; Rao and Indusekhar, 1989; Setälä and Nuorteva, 1989; Vetter, 1989b; Wang et al., 1989). This can be due to the concentration and availability of copper in the medium, seasonal variations and, in artificial media, root restriction due to the size of containers (Dubik et al., 1990). However, availability and uptake are also affected by interaction with other trace metals as well as by the concentration and type of major nutrients (e.g. Jasiewicz, 1989; Sauer, 1990a). Soil moisture level can affect soil mineral levels although the relationship does not hold for mineral uptake, at least for alfalfa and sainfoin in a fine loam (Kidambi et al., 1990). Soil pH is important in controlling bioavailability and uptake, tissue metal levels generally decreasing with increasing pH (e.g. Jasiewicz, 1989). Tissue copper concentrations can also change seasonally (e.g. Buwalda and Meekings, 1990; Guillard and Allinson, 1989; Rönnberg et al., 1990) as a result of physiological changes in the organism and changes in metal bioavailability (Helmisaari, 1990). Demands for copper and other nutrients change during the growth of a plant, with resultant changes in uptake and potential changes in tissue concentrations. Kotzé and de Villiers (1989) found copper accumulation mainly in the first 27 weeks after bud break in kiwifruit vines. Laszlo (1990), working with soybean, reports that copper content in the seed coat initially increased, then dropped, corresponding with an increase in the developing embryo. Kunelius and Sanderson (1990) found similar copper concentrations in forage rape, stubble turnip and forage radish 46, 75 and 101 days after seeding.

Levine et al. (1989) found higher copper concentrations in soils, foxtail, and earthworms from sludge-treated plots than from fertilizer and control plots. However, concentrations in bluegrass and brome, although higher in sludge-treated than fertilizer-treated plots, were not higher than plants from control plots. They suggest a soil-plant barrier to uptake of copper as well as a reduced bioavailability of copper in sludge. Higher tissue metal levels have been associated with the use of pesticides (Sovljanski, 1989b). However Robson and Snowball (1990) found that the herbicide chlorsulfuron induced symptoms of both copper and zinc deficiencies within wheat shoots, by reducing metal uptake. Treatment of foods and medicines after harvesting may affect tissue metal levels. Rincón et al. (1990) found a loss of copper in peas as a result of the canning process. Irradiation of foods and natural products used for medicinal purposes is more frequently being done; with ginseng (*Panax ginseng*), Kwon et al. (1990a) found no demonstrable change in tissue copper levels with γ irradiation.

Animals

Metal concentrations in animal tissues and skeletal parts have been used to monitor conditions today and in the past. Linn et al. (1990) use copper:calcium ratios in seventeenth-century Galapagos coral to estimate copper concentrations in surface water in the seventeenth century. Their estimates infer surface water concentrations similar to those of today (0.7-1.4 nmol/kg). However, in a discussion of heavy metal levels in marine invertebrates, Rainbow (1990, page 74) comments that "The concentrations of heavy metals in the bodies (and tissues) of marine invertebrates, ... , depend on the accumulation strategy adopted by each species for each metal". As with plants, it is the nature of the individual as well as the availability of the metal that is important. Thus organisms such as those occurring in metal-rich hydrothermal vent sites may have higher tissue copper values (Cosson-Mannevy et al., 1989) than those in metal-poor areas (e.g. Rainbow and Abdennour, 1989). Reviews of literature concerning tissue copper levels and factors affecting them are included in the book "Heavy Metals in the Marine Environment" edited by Furness and Rainbow (1990). These include discussions of levels in marine invertebrates (Rainbow, 1990) and vertebrates (Thompson, 1990). Normal as well as abnormal factors affect metal availability in nature (e.g. Feeder et al., 1990) while a range of physiological processes control metal levels within the organism (e.g. Eriksen et al., 1990a). Kroupa et al. (1990) points out that with the peppered moth *Biston betularia,* the concentrations of metal (Fe, Zn, Mn, Pb, Cu) were not significantly different between melanic and typical forms.

Regulation of metal uptake and accumulation is discussed later in this review. Obviously, however, it is important in considering tissue metal concentrations. Ozoh (1990b) found copper accumulation independent of water concentration in a marine annelid suggesting that tissue metal levels are not directly related to water intake. Regulation of metals in polychaete worms (annelids) is also indicated by the work of Athalye and Gokhale (1991). Control of tissue copper levels is more apparent in some organism groups than in others. With crustaceans, Powell and White (1990) found no apparent regulation in two species of barnacles and suggest that, as a result, barnacles may be suitable biomonitors of copper. Al-Thaqafi and White (1991) present evidence that, in the barnacle *Elminius modestus*, copper and zinc accumulation and loss can occur rapidly. In the Antarctic krill *Euphausia superba*, Yamamoto et al. (1990b) suggest that changes in the amounts of copper-binding proteins are a result of physiological condition. They speculate that the changes may be the cause for variations of tissue copper that they found between sexes and life history stages of the species. However, internal changes in total, soluble and organically-complexed (e.g. haemocyanin) copper may be related to copper availability as suggested for the mesopelagic decapod crustacean *Systellaspis debilis* (Rainbow and Abdennour, 1989). Changes in tissue copper concentrations may also occur on a seasonal basis (Rainbow and Moore, 1990) as a result either of changes in physiological condition (Arumugam, 1989; Finerty et al., 1990) or metal availability (Alliot and Frenet-Piron, 1990; Heliövaara and Väisänen, 1990b; Roth-Holzapfel and Funke, 1990). Differences in metal concentration have also been noted between populations of a species (e.g Krantzberg and Stokes, 1990) as well as ages and sizes, even within members of the same life history stage (e.g. Krantzberg,1989b). Hemminga et al. (1989), however, did not find obvious time-related fluctuations with copper in larvae of a stem-boring beetle that lives in a species of salt-marsh plant.

In molluscs, Chu et al. (1990a) report seasonal variations in copper for a mussel (*Perna viridis*) but not a rock oyster (*Saccostrea cucullata*) in Tolo Harbour, Hong Kong. Greville and Morgan (1990) found that although there are differences in tissue metal levels between species of terrestrial slugs, there is a high level of variability within a species. They suggest that this is high enough to limit the use of slugs as biomonitors. However, when general trends are of interest and adequate geochemical and biological information exists, the use of molluscs can be of benefit in monitoring studies (e.g. Luoma et al., 1990). A number of recent references discuss metal concentrations in mussels, in part a continuation of the "Mussel Watch" program started in the 1980's (e.g. Ward, 1990b). Borchardt et al. (1989) provide evidence that, in *Mytilus edulis*, metal concentration is related to body condition and shell length. They were able to "normalize" the metal levels to a "standard sized and conditioned mussel" with the use of multiple regression techniques. Coimbra and Carraca (1990) discuss metal levels in regard to life history stages in *M. edulis* and Chou and Uthe (1991) comment that, unlike oysters, starvation did not result in significant tissue copper loss.

Tissue metal levels in fish are frequently used as an indication of environmental conditions (e.g. Gokhale and Patil, 1989; Ismail and Samin, 1988; Morozov and Petukhov, 1986; Pujin et al., 1990; Saiki and Palawski, 1990; Singh et al., 1991) or to compare conditions in different environments (e.g. Vaz Carreiro and Moura, 1989). However, in an examination of heavy metals in a Louisiana water system, Ramelow et al. (1990) present evidence that (abstract) "Differences in elemental concentrations (are) related to organism mobility and not to site-specific variations in metal loadings". McLean et al. (1991) report low copper values in a species of fish (*Cheilodactylus fuscus*) collected near primary outfalls near Sydney, Australia. Protasowicki (1989) found that essential elements such as copper are accumulated primarily in organs (abstract) "... which play an important role in the functioning of (the organism)". Physiological state and growth affects tissue metal levels in fish as it does in other organisms (e.g. Miramand et al., 1991; Poston and Ketola, 1989; Takahashi, 1990a,b). Tissue copper concentrations in other vertebrates (birds - Warren et al., 1990; Shetty et al., 1990. Mammals - Ma and Broekhuizen, 1989; Marcovecchio et al., 1990) are also used in an attempt to monitor conditions or the nature of the physiological condition of the organism.

Tissue copper concentrations in domesticated animals are used to indicate potential deficiency conditions as well as to measure changes in requirements with growth and as a result of organism type (e.g. Kaur and Chopra, 1990; Krusic et al., 1990; Sreekumar and Nirmalan, 1989b; van Niekerk and van Niekerk, 1989e; van Niekerk et al., 1990b). van Niekerk et al. (1990) measured plasma copper, and other elements, in ewes and lambs of 3 different breeds of sheep, for 120 days after birth. They report differences during the period as well as between the breeds. In a study of trace mineral concentrations of goat milk, Park and Chukwu (1989) found differences between breeds as well as changes during the first 5 months of lactation. Seasonal changes in tissue copper concentrations have also been evaluated, to indicate stress or changes in food sufficency (e.g. Nazki and Rattan, 1990). Distribution of copper within domesticated animals also provides an indication of physiological condition as well as changes associated with reproduction or growth. Copper concentrations in cervical mucus of buffalo change during the oestrous cycle (Eltohamy et al., 1990). Copper deficiency has been associated with reversible impairment of testicular function in rams (van Niekerk and van Kiekerk, 1989c). Hill et al. (1988) and Leighton et al. (1990) examined metal (Cu, Zn, Mn, Fe) accumulation during foetal growth in deer. Liver copper concentrations were high and (Leighton et al., 1990) rate of accumulation appeared to be elevated during the last month of gestation. Indirect analysis of copper, through its enzyme activity, has been used to indicate metal status as well as physiological condition, in animals ranging from cattle to mink (Työppönen, 1988). (The use of ceruloplasmin has been shown to be valid as an indicator only of plasma copper concentrations.)

Tissue and food mineral levels are frequently compared to determine food adequacy as well as the need for and effect of trace metal supplementation (Anke et al., 1989; Boila and wittenberg, 1990; Harris et al., 1989; Ivan et al., 1990; Macneil et al., 1990; Musalia et al., 1989; Régius-Mocsényi, 1990; Tokosová, 1989). Régius-Mocsényi et al. (1990), for example, report an estimated 24% copper deficiency for ruminants feeding on fodder crops in Hungary. This type of comparison has been applied to higher levels in the human food chain, Holm et al. (1988) evaluated the contents of elements in animals used as human food. No final conclusions were drawn and they note (abstract) that "The comprehensive data pool of 15 elements requires some cheap method of evaluation if clear information is to be passed on to the consumer ...".

Laboratory animals provide a means of examining the metabolism of copper and the causal factors for particular tissue copper levels (e.g. Amarowicz et al., 1989b). That is, providing the consideration for differences in response between animal types and between breeds of one type (e.g. Verheesen and Nederbragt, 1988). As with other animals, and also with plants, there are also changes that occur with age (Dowdy et al., 1990; Ischiropoulos et al., 1990; Palm et al., 1988, 1990; Struck and Hillesheim, 1990; but also see Kurobe et al., 1990) and physiological condition (e.g. sex - Fields et al., 1988; Mehta and Eikum, 1989a; Uchino et al., 1990). Symonds and Charmley (1990) discuss a surgical technique for the long-term collection of bile in the pig and the measurement of biliary excretion of copper and zinc. Their results indicate an output during 24 hours of approximately 5.8% of the daily intake of copper. Hepatic copper concentrations in dogs have been studied to better understand cell copper associations and the effect of disease on these associations (Thornburg et al., 1990). The location of copper within a tissue is important in an understanding of the metabolism of the metal as well as the tissue (e.g. Hirayama, 1990; Hirayama and Amemiya, 1990). Radiocopper use in dogs has recently been described, as a mechanism to provide a short-term tracer of blood flow (Barnhart et al., 1990). In rats, neutrophils (a type of white blood cell) are used as an indicator of copper status, the activity decreasing rapidly by suboptimal copper intake (Failla et al., 1990b). Metal-metal interactions occur within organisms that affect metal metabolism and tissue copper concentrations. Gordon and Ellersieck (1988) note the importance of this in laboratory studies and propose a dietary range of iron, zinc and copper to reduce the impact in studies with rats. Stimulants such as nicotine (Morrill et al., 1990) and commercial explosives (Jiang et al., 1991) can also affect trace element levels in tissues as well as the metabolism of the organism.

Humans

Tissue levels of copper, and other trace metals, can provide information about the nature of humans, and the environment, both in the present and past (e.g. Pavlov et al., 1989; Qin et al., 1990). Concentrations in dental enamel from skeletons of prehistoric natives in South Dakota (U.S.A.) have been used to infer dietary differences and movement of populations (Schneider and Blakeslee, 1990). Based on trace element analysis of 2nd millennium B.C. human bones, Grupe and Schutkowski (1989) inferred a dietary shift in humans living in the Oman peninsula. Hisanaga et al. (1989) used differences in the concentrations of trace metals in ancient (3,000 to 120 years before present) Japanese bones to infer lifetime and postmortem environmental conditions. Fortunately, the analytical techniques now available are capable of measuring very low levels of copper and other metals and providing some information on the chemistry of the metal in the tissue (e.g. Dever and Bresee, 1990).

Copper concentrations in human tissues, given in recent references, are provided in table 5. These include values used for a variety of topics ranging from a listing of tissue levels to the examination of differences between normal individuals and those prone to problems such as dental caries (Grasso et al., 1989). The need for tissue reference values is pointed out by Woittiez and Iyengar (1988). Turnlund and Keyes (1990a) followed the loss of copper injected in the plasma to estimate copper turnover. The regional distribution of trace elements in the brain has been examined to provide background information (Andrási et al., 1988; Duflou et al., 1989) against which disease effects can be compared (e.g. Duflou et al., 1988). Nishimuta (1990) comments on the importance of assessing metal flux as well as tissue-specific concentrations in normal and abnormal conditions.

Gibson (1989) reviews procedures for and problems in assessing chromium, copper, selenium and zinc status in humans. From an analysis of total body burdens and tissue concentrations of copper and lead, Saltzman et al. (1990) suggest that (abstract) "... the use of blood samples as a convenient clinical measure of body burdens for these metals may be of limited value". With the intent of examining easily-obtained human tissues for trace metal status, Anke et al. (1988c) used domestic animals to relate tissue concentrations to trace metal status. Copper status was best reflected by tissue copper concentrations in the cerebrum, at least in goats. In humans, copper status is often judged on the basis of blood copper levels (e.g. Milne et al., 1988, 1990a). Copper requirements and flux through the body have been estimated from intake and loss, the latter in urinary, salivary and fecal concentrations (e.g. Turnlund et al., 1990). The effects of exercise on trace metal requirements continues to be evaluated. Although controversial, there is evidence that although physical training may cause an increase in blood copper levels it does not produce adverse effects on copper nutriture (Becchi et al., 1988, 1989; Lukaski, 1989;

Marrella et al., 1990b; Singh et al., 1990a; Vlcek et al., 1989; but see Anderson et al., 1988; Resina et al., 1990 and Singh et al., 1988).

Changes occur in some tissue metal concentrations during and after gestation. Aggett (1988) reviews the metabolism of trace elements in pregnancy and lactation. He reports the calculation that a pregnant woman requires 75 µg of elemental copper daily to meet the requirements of pregnancy. Moser-Veillon et al. (1990) state that copper intake needs to be increased during lactation to compensate for copper secreted into breast milk. As a result of poor nutritional status with pregnant adolescent teenagers from urban, poor environments, the long-term nutritional status of the young mothers may be compromised (Scholl et al., 1990a,b). Fu et al. (1990) present evidence that levels of serum copper begin to rise during the sixth to eighth week of gestation. Lockitch et al. (1990) note that serum copper concentrations more than doubled between 12 weeks and term and returned to control levels by the sixth week after parturition. The increase in serum copper during gestation in humans is in contrast to the decrease noted during gestation in rats (Suzuki et al., 1989a).

The non-protein-bound copper fraction of the mother may be involved in supplying the fetus with copper (Noubah and Al-Awquati, 1990). M'bofung and Subbarau (1990) note that copper content of the placenta of male babies is higher than that of femal babies. From this and other evidence they suggest that the concentration of certain trace elements, in particular zinc and copper, may have an influence on the outcome of pregnancy. Kapu et al. (1989) comment on the negative correlation of maternal serum copper with neonate serum levels, suggesting that this (page 22) "... may indicate that beyond physiological level of copper the neonate has devices to reduce placental transfer of maternal copper as this metal increases in the mother". Zinc and copper balances in preterm infants are estimated by Sievers et al. (1990) although the variability of the data suggests the need to better understand the processes of copper retention. Hambidge (1989) reviews some of the trace element requirements in premature infants, commenting on the use of blood superoxide dismutase activity as an indicator of copper status. Copper uptake and copper retention in the neonate are also of concern in terms of the type of formula used (Stack et al., 1990). As an individual ages there may be changes in metal requirements and tissue metal concentrations (e.g. Bales et al., 1990; Fuller et al., 1990; Laitinen, 1990; Nishimuta et al., 1989; Vivoli et al., 1987). Copper status and copper deficiency can be affected by the use of medications such as estrogen or drugs such as alcohol (Fields and Lewis, 1990b; Fischer et al., 1990b; Mehta and Eikum, 1989b) or environmental contaminants (e.g. Ward, 1989). Mertz (1989) reviews the role of trace elements in the aging process. Copper intake and status in the elderly are of concern because of nutritional status as well as physiological condition (e.g. Allegrini et al., 1988; Panemangalore and Lee, 1990; Umoren, 1989). As a result, there is ongoing work on the effect of nutrient supplementation on trace-element status of humans as well as laboratory animals used in the study of human problems (e.g. Matsuda et al., 1989). Ewers et al. (1990), however, report that tissue copper in the human kidney cortex did not increase with age.

I.3.2 ORGANISM COPPER LEVELS UNDER ABNORMAL CONDITIONS

A wide range of agents can affect the tissue concentration of copper and copper-containing organics. Hormones are one group of agents (e.g. Droliya et al., 1989); DiSilvestro (1988b) reviews their on copper metalloprotein levels, commenting on the limited information known about the topic and the importance of a greater understanding. Drugs and dietary agents can affect copper status (e.g. Brown and Strain, 1990). The consequences of copper deficiency have been reported to be affected by dietary carbohydrates although the findings of different workers are contradictory (Schoenemann et al., 1990b). Metal-metal interactions may also be important, copper deficiency has been associated with decreased excretion of both copper and zinc (Askari et al., 1990b). Copper levels in certain tissues can be affected by iron deficiency (Shukla et al., 1989, 1990), at least in rats. The discussion of recent literature on copper levels under abnormal conditions must thus be considered in terms of dietary and physiological conditions as well as environmental factors affecting both metal bioavailability and the nature of the organism.

Anthropogenic metal effects on copper levels have been reported. Alder leaf copper concentrations are reportedly high in the zone of emission of a copper smelter in Poland (Mejnartowicz, 1986). Amelioration of high soil copper effects can be accomplished by control of pH as well as metal-binding by organic components of composted materials (e.g. Traulsen, 1987). Anthropogenic metal has also been linked to elevated copper levels in oysters (Han and Hung, 1990), terrestrial isopods (Hopkin, 1990), insects (Butovskii, 1989), fish (Sulaiman et al., 1991) and laboratory animals (e.g. Skreblin et al., 1988). Spierenburg et al. (1988) report that copper levels in cattle tend to be lower in the neighbourhood of zinc refineries in a region of the Netherlands. Low levels of lead are reported to cause a decrease in the copper concentrations of the cerebellum and forebrain of guinea pig dams but an increase in the fetuses (Sierra et al., 1989). The treatment of lead-exposed children with the chelating agent EDTA (ethylenediaminetetraacetic acid) can cause a loss of copper with the need for some supplementation (Chisolm and Thomas, 1989). Metal exposure in the workplace can lead to departures from normal tissue copper concentrations (e.g. Bentur et al., 1988; Bogdanovic et al., 1989) although this is dependent on the nature of the exposure.

Malformation in plants may or may not be associated with changes in tissue copper concentration (e.g. Mozafar, 1990). An understanding of the processes involved in disease transmission can be of value. In a patent document, Thomas et al. (1990) describe overproduction of the copper-containing enzyme superoxide dismutase by stress-tolerant, copper-resistant tobacco plants. These plants were obtained by fusing the *Escherichia coli* superoxide dismutase gene to a vector, the cauliflower mosaic virus, which was then used to "infect" tobacco cells. The tobacco plants were then regenerated from these "transformants". Superoxide dismutase performs a protective function by scavenging superoxide radicals, a function which may alter aging and disease processes associated with free radical reactions. Working with the fruit fly *Drosophila melanogaster*, Seto et al. (1990) note that extra superoxide dismutase did not markedly affect oxygen metabolism or longevity.

Anemia in mink has been associated with abnormal trace metal levels, including copper (Työppönen and Lindberg, 1988). Gastrointestinal nematode parasites have been reported to interfere with copper uptake and metabolism in sheep (Bang et al., 1990a) and bovine respiratory virs in cattle (Orr et al., 1990). In sheep, low tissue copper levels are associated with growth retardation and susceptibility to infection (Suttle et al., 1988b). However, sheep are susceptible to chronic copper poisoning, with elevated plasma copper concentrations (Stahr et al., 1989; Zervas et al., 1990). Added dietary or intravenously-administered molybdenum is one way of treating excess copper (Fungwe et al., 1989), initially causing a release of copper from the liver with a subsequent drop in plasma copper (Howell and Kumaratilake, 1990; van Ryssen and Barrowman, 1988). High molybdenum or sulfate in the diet can, however, produce low copper levels, and copper deficiency (van Niekerk and van Niekerk, 1989a) both in the adult and offspring (van Niekerk and van Niekerk, 1989b). Low serum copper levels have been reported for Simmenthal cows with reproductive disorders (Hahn et al., 1989).

In humans and laboratory animals, tissue trace metal concentrations are useful in studies of nutrition and various diseases (Hassan et al., 1990; Massie and Sternick, 1989; Saltzman et al., 1990; Yokoi et al., 1990a). Changes in concentration also occur as a result of environmental conditions (e.g. Corhay et al., 1988), prolonged emotional stress (Gribauskas et al., 1988) and aging (Allegrini et al., 1988), the latter often in combination with abnormal tissue conditions (e.g. Garland, 1990). Alterations in both copper metabolism and requirements can change as a result of injuries (e.g. Bhattacharya et al., 1989; Lan et al., 1989). Indicators of copper status have been examined in an attempt to provide a simple expression of trace metal needs and metabolism. These include blood (e.g. Wu et al., 1989) and various blood components (e.g. Failla et al., 1990a; Hamrin et al., 1988) as well as cerebrospinal fluid (e.g. Belliveau et al., 1990) and hair (Donma et al., 1990; Mzhel'skaya et al., 1989a; Takizawa et al., 1989; Zhang et al., 1988). Ward et al. (1989b) report (abstract) "... that elevated serum copper concentration is an accurate and minimally invasive index of lung injury in irradiated and monocrotaline-treated rats". This was part of a study to determine if thracic radiotherapy for lung cancer would produce a change in serum copper that would indicate clinical outcome of treatment (Molteni et al., 1989).

Medicinal and non-medicinal agents have been examined for their effect on tissue metal concentrations. Smoking has been associated with increased serum copper levels (e.g. Jolly et al., 1990; McMaster et al., 1988b). Salgó (1988), working with pregnant women, reports elevated serum copper levels in those that smoked, particularly those that were heavy smokers. Infants of smoking mothers had higher serum copper ($p<0.05$) than infants from non-smoking mothers (Ahlsten et al., 1989). Serum levels of copper were elevated in wine-using chronic alcoholic patients, a condition which the author attributes to copper in the wine (Tavares do Carmo, 1988). In rats given a high dose of ethanol, liver copper levels (and zinc and selenium) decreased although iron increased, all likely contributing to an ethanol-induced oxidative stress (Rouach et al., 1990). The use of the drug disulfiram in the treatment of alcoholics causes a redistribution of zinc and copper (Grandjean et al., 1990), particularly of copper in the brain (Singh et al., 1989a). Copper has been reported to be significantly increased in the plasma ($p<0.01$) and erythrocytes ($p<0.001$) of heroin addicts (Ruiz Martinez et al., 1990) and decreased in the urine and plasma of cocaine-treated individuals (Smith and Gibbons, 1990). Certain environmental pollutants have also been found to increase tissue copper levels (e.g. in the kidney and liver by 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDD; Elsenhans et al., 1990). Hair copper levels were lower in women exposed to carbon disulfide in the workplace (Wang and Bao, 1988). Evaluations of medicinal agents include their effect on tissue metal concentrations. Paternain et al. (1990) report that the oral administration of the metal complexing agent DMSA (2,3-dimercaptosuccinic acid) to pregnant rats caused a significant reduction in hepatic copper and an increase in intestinal copper; whole-fetus copper levels varied. Etrinate (a retinoid) is reported to increase plasma copper levels (Krari et al., 1989). The use of a surfactant (LF-57) to promote dissolution of urinary calculi appears to solubilize the incorporated copper (Ivanova et al., 1989). The use of a gold drug (auranofin) in the treatment of an arthritic patient was associated with an increase in urine copper levels (Elder et al., 1990). In ovariectomized female laboratory rats, maintenance of normal serum copper levels requires hormone (estradiol) supplementation (Brenner and Koo, 1990). Based on the work of Arowojolu et al. (1989), the use of multiload intra-uterine contraceptive devices does not appear to affect mean serum copper levels. Infertility in males has been associated with changes in seminal plasma metal concentrations (Wang and Liu, 1989) although details are not provided in the symposium abstract. (Ishikawa et al., 1989, did not find seasonal changes in copper in semen from infertile males.)

Inherited abnormalities are frequently associated with abnormal tissue metal concentrations. A red blood cell enzyme deficiency (glucose-6-phosphate dehydrogenase or G6PD) is one of the major public health problems in many parts of the world (Karunanithy et al., 1990). The authors note that (abstract) "Both the serum magnesium and copper content were found to be significantly lower in the G6PD-deficient subjects compared to that in the non-deficient control". Carl et al. (1990) note that genetically epilepsy-prone rats had significantly lower liver copper levels than controls. To determine if epilepsy-induced tissue trace element concentrations could be a result of the seizures, Carl et al. (1989b) induced seizures in rats with kainic acid. Copper was lower in the kidney of treated rats although the reduction was not significant. Groner et al. (1990), using an animal model of Down's syndrome, found elevation of Cu- Zn-superoxide dismutase activity that appeared to interfere with neurotransmission, a feature which they feel could contribute to the neurobiological abnormalities of the syndrome.

Cowan et al. (1989) found elevated serum and urine copper levels in morbidly obese patients. They suggest that this may be a result of metabolic insufficiency and hepatobiliary impairment. Although Stuart et al. (1990) report unusually low serum copper values in anorexia nervosa patients, Mira et al. (1989) found no differences in plasma copper concentrations between normal women and those with eating disorders. Fecal loss of copper is reported as a result of diarrhea (Ruz and Solomons, 1990). Diet, physiological status and tissue copper concentation have been correlated in work on diabetic rats (Oster et al., 1990). Lewis et al. (1989) found reduced pancreatic copper concentrations in a corpulent rat model of non-insulin-dependent diabetes. In a second rat model of diabetes (streptozotocin induced), low serum copper concentrations could be improved by somatotocin (Tu et al., 1988). In comparison with control rats, diabetic dams had higher concentrations of hepatic and kidney manganese, copper, zinc and metallothionein while their fetuses had lower concentrations than the controls (Uriu-Hare, 1988). Juntti-Berggren et al. (1990) report evidence that changes in the elemental composition of the pancreatic B cells in diabetic mice may not be related to the onset of diabetes. Cu,Zn-superoxide dismutase activity appears to be affected in long-term diabetics and serum levels may reflect prolonged disturbance resulting from a relative or absolute decline of insulin (Inagaki et al., 1989). The glycated form of the enzyme found in erythrocytes is increased in diabetic patients with ocular complications.

Abnormal metabolism of copper is often associated with irregularities in the storage and mobilization of the metal within the body (e.g. Chen et al., 1989). Supplementation may be advisable during periods of physiological stress, whether in laboratory animals (e.g. Yokoi et al., 1988) or humans (e.g. Mansell et al., 1989). Elmes et al. (1988a) state that, in the liver, "The molecular state of copper appears to determine both its hepatotoxity (sic) and our ability to demonstrate it histochemically and we suggest that 'invisible' copper is non-toxic CuMT" (metallothionein-bound copper). They later infer that the copper will become biologically active if the metallothionein detoxifying mechanism breaks down or its capabilities are exceeded. Mulder et al. (1990) notes (summary) "... that human MT is a unique metalloprotein with age and disease-dependent characteristics". Lower-than-normal serum copper concentrations have been reported for patients with viral hepatitis (Zhang, 1989). Aidid (1990) reports elevated concentrations of copper in the fingernails of a biliary artresia patient. Metal concentrations in human cystic bile can be affected by disease, including calculi (Sato, 1989). Elevated liver copper concentrations are reported by Andersson et al. (1990b) in dogs with chronic hepatitis and liver cirrhosis. In muscle cells cultured from a one-year-old patient with Menkes' disease, van den Berg et al. (1990c) note that copper uptake was elevated. Data available from both human cases and animal models of the disease suggest that there is a generalized defect in copper metabolism (e.g. Dirik et al., 1989; Garnica et al., 1988). Work by a number of individuals and groups suggests that the basic defect "... affects cellular copper transport or the intracellular delivery of copper to cellular transport systems" (abstract - Castillo et al., 1990). Yamamoto et al. (1990) used a copper chelating agent (D-penicillamine) with suckling mice to produce what they term "... a useful animal model not only for Menke's kinky hair disease but also for mitochondrial encephalomyopathy". Copper and zinc metabolism has been examined in patients with Wilson's disease, another inherited disease of copper metabolism in which hepatic copper levels can be excessively high (e.g. Lee et al., 1990c; Mzhel'skaya et al., 1989b). Animal models of this disease include the pig (e.g. Srai et al., 1988) and the guinea pig (e.g. Bingle et al., 1990b). Although infrequent, the deposition of excess copper in Wilson's disease can be associated with other physiological problems (O'Donnell and Myers, 1990).

Tissue metal concentrations have been measured in individuals with kidney and urinary tract problems ranging from urinary tract stones (Durak et al., 1990) to renal failure. In a study of children with phenylketonuria, Reilly et al. (1990) found higher than normal dietary intakes of copper. Stec et al. (1990) report that, in children with nephrotic syndrome, there was above normal urinary loss of copper which may be the cause of the low copper levels found in patients with the disease. Asayama et al. (1990) note that both manganese and Cu,Zn-superoxide dismutase were elevated in lymphocytes and monocytes (but not erythrocytes) of children and adolescents with renal failure adequate to require dialysis.

Elevated plasma copper levels occur in individuals with chronic or acute rheumatic diseases (Marrella et al., 1990a) and diseases such as lupus and scleroderma (Oana et al., 1989). Concentrations can often be correlated with biochemical indices of inflammation (Peretz et al., 1988). Inflammation increases plasma levels of ceruloplasmin, a copper-containing protein, although levels of Cu,Zn-superoxide dismutase may decrease in the liver (DiSilvestro, 1989; DiSilvestro and Marten, 1990). This is of interest because both have an antioxidant function while the distribution and activity level differs under the stress of inflammation. Although plasma copper levels may be affected by infection, Shanbhogue and Paterson (1990) report no significant change after surgery and removal of the septic focus. Extensive copper loss can occur in multiple trauma patients with the need to consider supplementation (Clark and Drewnowski, 1990c). Overt copper deficiency has been observed in HIV-infected men (Cabrejos et al., 1990).

Ward et al. (1988) report wide variations in copper concentrations in cerebrospinal fluid from patients with neurological diseases. Below normal values were found with multiple sclerosis and motor neurone disease. Degeneration of the nervous system can be accompanied by changes in copper levels in hair although the nature of the change appears to be unique to parts of the nervous system (e.g. Oishi et al., 1990). In anenocephalic (brain incompletely developed) and spina bifida fetuses, kidney copper concentrations are reported to be elevated as are zinc and iron levels in several tissues (Fehily et al., 1988).

Copper plays several roles in the cardiovascular system and has been implicated directly or indirectly in cardiovascular problems (e.g. McMaster et al., 1988b; Sui et al., 1989; Zhang et al., 1989b). Laitinen et al. (1989) found that serum copper concentrations related negatively to HDL-cholesterol levels in Finnish girls and boys. McMaster et al. (1988b) did not find this in an all-age population in Northern Ireland nor did Oncu et al. (1988) . Borella et al. (1987) report higher copper excretion in urine of hypertensives than normotensives although this was not found in a second study (Bergomi et al., 1989). Bergomi et al. (1989) did find a positive relationship between blood pressure levels and serum copper values. Myocardial infarction is associated with high levels of copper and low levels of zinc (Acartürk et al., 1988; Meissner et al., 1990), at least during the first 24-48 hours; Oncu et al. (1988) report a return to normal levels after two weeks. Cardiac surgery has been associated with decreased serum copper concentrations (Fraser et al., 1989; Zhao et al.,1989) which Fraser et al. (1989) suggest could be due to hemodilution. They (Fraser et al., 1989) also found serum copper levels increasing gradually during the first seven days after the operation, in both cardiac surgery and cholecystectomy surgery patients. Marniemi et al. (1988) note elevated serum copper and copper/zinc ratio in coronary by-pass patients. Cu,Zn-superoxide dismutase appears to be released from erythrocytes during cardiopulmonary bypass operations with a subsequent decrease which is suggested to be due to use of the enzyme against free radicals generated during the surgery (Hamano et al., 1990).

Abnormal growth of cells is often associated with changes in tissue copper levels (e.g. Casaril et al., 1989; Cheng and Mao, 1989; Zaichik et al., 1989) as is medical treatment of the problem. Serum copper concentrations in women increase with gynecological tumours in the order 'normal < benign < malignant' (Çetìnkaya et al., 1988). The authors suggest that the serum Cu/Zn ratio could be used as an indicator of malignant gynecological tumours, as does Gal et al. (1989) for ovarian cancer. Boman and Selin (1988) note that cisplatinum treatment of patients with testicular tumour caused an increase in skeletal muscle copper, zinc and selenium levels. Serum copper concentrations are elevated in patients with gastric cancer (Shim, 1988; Wang et al., 1988). Copper concentrations are reportedly higher in cirrhotic human liver cells (Laursen et al., 1990) and Takikawa (1990) found elevated serum copper levels in patients with liver cirrhosis and hepatocellular carcinoma. Beguin et al. (1990) comment (abstract) that "In 50 patients with chronic lymphocytic leukemia, it was observed that (1) serum Cu and Cu/Zn ratio were useful indices of the disease activity, ...". Superoxide dismutase activity is reported to decrease in mice during the development of lymphatic leukemia (Madej et al., 1988), presumably a result of the response of the enzyme against free radicals produced by the disease. However, Sawaki and Isono (1985) found that superoxide dismutase activity increases in patients with various cancers.

I.3.3 COPPER AND THE RESPONSE OF THE ORGANISM

The response of an organism to copper can be of a physiological, morphological or behavioural nature. It can be direct or indirect, a reaction to one or more factors affected by the metal (e.g. Abdel-Mageed and Oehme, 1990b; Kaneko et al., 1990; Yagodin et al., 1988 (reference not seen)). However, in all cases, the "response" is not to all chemical species of copper, only to those that are biologically available, able to be taken up by the organism and incorporated into its metabolic machinery. A variety of studies have appeared in the recent literature, dealing with the response or the nature of the metal species causing the response, from outside or within the organism. Some of these are considered in reviews by Langston (1990), Janus et al. (1989), Vernet (1989a) and Reish et al. (1990). More and more information is becoming available on metal fluxes into the organism (e.g. Simkiss and Taylor, 1989) and on the adaptations of organisms to metals, including copper, a situation which needs to be addressed when evaluating anthropogenic impact (Cairns and Niederlehner, 1989). In addition to literature on the response of the organism there are also a number of publications that consider the fate of anthropogenic metals once introduced into environments (e.g. Chapta and Boyer, 1990). This section of the review will consider literature on the response of the organism although with reference to that on metal bioavailability.

Microorganisms and plants

There are numerous metal-microorganism interactions that have been examined (e.g. Poole and Gadd, 1989). Copper is known for its ability to inactivate bacteria (e.g. Belay and Daniels, 1990; Lebedev et al., 1989, 1990a,b), a response beneficial in water supply systems (Landeen et al., 1989; Yahya et al., 1989; Yahya and Straub, 1990). Inactivation of other microorganisms is also valuable in the control of pest organisms (e.g. Lüderitz et al., 1989b). However, bacteria and other microorganisms, as well as certain organics, can mediate corrosion of copper and copper-nickel piping systems (e.g. Geesey and Bremer, 1990; Gianotto et al., 1989; Wagner et al., 1987). Copper can reduce the resistance of food-poisoning microorganism spores to heat although this can be done more efficiently by iron (Kihm et al., 1990). Metals can affect soil microorganisms (Murugesan and Mahadevan, 1989) with detrimental effects on microbially-mediated soil processes (Bååth, 1989; Gabteni and Gallali, 1988; Klinkenberg et al., 1988; Moon, 1990; Schuller, 1989; but see also discussion in Mandal and Parsons, 1989) as well as processes in other media (Domka et al., 1990; Jackson, 1989; Kagalou et al., 1989), including laboratory media (e.g. Larrondo and Calvo, 1990). Reduction in activity can be from a variety of processes that affect the viability of microorganism cells. Copper can induce loss of potassium from bacterial (*Pseudomonas syringae*) cells (Cabral, 1989) as well as ribose-containing molecules (Cabral, 1990). Lebedev (1989b) notes copper-induced plasmolysis of *Escherichia coli* cells accompanied by a significant loss of potassium.

Fernandez et al. (1989) note copper inhibition of hydrogenase activity in a bacterium (*Desulfovibrio gigas*). Abbas and Edwards (1990) affected production of an organic (actinorhodin) by *Streptomyces coelicolor.* Copper sulfate supplementation was associated with a decrease in nitrate reduction by mixed rumen microorganisms (Takahashi et al., 1989). In contrast, Wadud et al. (1987) report increased activity in cultures of methanogenic bacteria with the addition of metal salts (Zn, Mn, Co, Cu, Ni, Mo). (Since the culture medium was buffalo dung, this may be a result of complexing agent-induced metal deficiency.) Agents capable of complexing biologically available copper can reduce detrimental effect (e.g. Klinkenberg et al., 1990). This has been demonstrated with natural and synthetic organics (e.g. Gupta, 1990; Nakamura, 1990). When levels of available copper become too low an apparent deficiency occurs, affecting processes as varied as germination (Yumnam and Reddy, 1989) and enzyme activity.

Copper is widely used as a fungicide, especially with economically important plants (Banihashemi, 1990; Girond et al., 1989). It has been shown to be effective against grape fungi without harming a parasitoid that feeds on the grape mealybug insect pest (Mani and Krishnamoorthy, 1989). However, tolerance to excess copper is often found in fungi (e.g. Hashem, 1989), particularly by the spores (e.g. Lokesha and Someshekar, 1989). This allows colony formation even after exposure to elevated concentrations (Feliciano et al., 1989). Poitou and Olivier (1989) report that mycelial growth can be decreased by excess copper as can the uptake of other cations (K, Mg, Ca). With *Achlya americana*, Al-Rekabi et al. (1990) found that copper can delay the appearance of spore-producing bodies although the number of these bodies is higher when they do appear. This is not always the case. In coniferous forests with high soil metal levels in northern Sweden, Rühling and Söderström (1990) found reduced spore-producing bodies in a group of fungi. Part of the effect of deficient and excess copper, on fungal growth, may involve Cu, Zn-superoxide dismutase and its effect on the superoxide radical anion, O_2^- (Greco et al., 1990). (Malan et al. (1990), for example, found that stress-tolerant maize inbreds had high levels of Cu, Zn-superoxide dismutase and glutathione reductase.) Copper supplementation can cause changes in metabolite production (e.g. Gora and Clijsters, 1989; Gaál et al., 1988). With fungi, this is a factor useful in fermentation (Jernejc et al., 1990). The effects of fungi on plants is varied, including the potential to change metal uptake from soil (Heggo et al., 1990).

Copper is of real or potential value as an algicide (e.g. Steele et al., 1989). Lüderitz et al. (1989a) review the physiological and ecological aspects of the application of copper sulfate as an algicide in aquatic systems. In another paper, Lüderitz and Nicklisch (1989) comment that blue-green algae have lower EC_{100} values than diatoms, a feature that is beneficial since algicide treatment in fresh water is frequently against blue-greens, with the need to retain other species. However, it is difficult if not impossible to make broad generalizations about the effect of copper. This is partly due to the effect of environmental parameters on metal concentrations and bioavailability (e.g. Arzul and Gentien, 1990; Nakamura, 1990). It is also due to the species-specific differences that are found in the response and tolerance of phytoplankton to copper (e.g. Lasheen et al., 1990; Metaxas and Lewis, 1991a; Ning et al., 1990b; Tadros et al., 1990). Primary production can be reduced by excess metals in the water (including copper; Jindal and Verma, 1988) and in the sediments (Nalewajko et al., 1989). This is due to a reduction in photosynthesis (e.g. Takamura et al., 1988), a result of inhibition of photosystem I electron transport (Droppa and Horváth, 1990) and photosystem II activity by Cu^{++} (Renganathan and Bose, 1990) as well as chlorophyll degradation (Robinson and Choi, 1989). Twiss et al. (1989) discuss an assay technique for determining the effect of copper on photosynthesis in the chlorophyte *Scenedesmus acutus.* However Bastien and Côté (1989) report the possibility of a tolerance mechanism after long-term exposure of *Scenedesmus quadricauda* to copper. This could affect the applicability of any assay technique. With the diatom *Nitzschia palea*, sub-lethal levels of added copper are reported to affect growth rate and the ratio of carbohydrates, proteins and lipids (Sathya and Balakrishnan, 1988). Coppellotti (1989) reports that with the flagellate *Euglena gracilis*, excess copper caused an increase in free cysteine but did not affect the concentration of total acid-soluble thiols, including glutathione.

The response of terrestrial plants to copper is a result of exposure to the metal either in the soil or as an aerosol. Copper deficiency is not uncommon and can lead to yield loss and disease problems in economically important crops (e.g. Evans, 1988). In the sugar beet, deficient leaves had reduced chlorophyll content (Henriques, 1989). Chloroplast ultrastructure (wheat) can be affected by deficiency (Casimiro et al., 1990). Stem deformity in a pine (*Pinus radiata*) has been associated with soil copper deficiency (Downes and Turvey, 1990; Hopmans, 1990). Growth in plants can be optimized by addition of copper, whether for nutrition or the control of diseases (e.g. McFarlane, 1989a; Murugesan and Mahadevah, 1988). The copper nutrition status of stock plants can also be important, to the success of cultivars (Schum et al., 1988) and seed viability. Seliga (1990) notes that copper increases total nitrogen content and nitrogenase activity in root nodules of yellow lupine inoculated with a nitrogen-fixing microorganism. Copper-containing fertilizers can improve plant vigor (e.g. Kuczynska, 1989; Zietecka, 1989). Copper is used in the final maturation of sea buckthorn fruits, in conjunction with surface-active agents copper ions initiate abscission of the fruit, improving the potential for mechanical harvesting (Demenko and Korzinnikov, 1990). However, work with copper-containing fertilizers continues to demonstrate the effect of soil organic matter (and other factors) in binding copper and reducing its bioavailability (e.g. Gorlach, 1989). Fertilizer application techniques are important, to deliver the supplement in a suitable chemical form and in a suitable manner. (A similar statement can be made about application of pesticides (Franz, 1989; Fulton, 1989; Lye et al., 1990; Rudgard et al., 1990).) Sanderson and Gupta (1990) suggest that soil applications of copper or zinc are preferable to foliar sprays with Russet Burbank potatoes grown on Prince Edward Island. They present evidence that, even with evidence of copper deficiency, foliar sprays can cause a phytotoxic response.

Reduced metal bioavailability can produce a condition of metal deficiency or it can reduce the impact of excess metal. Balsberg Påhlsson (1989) comments on the difficulty of stating what is a toxic concentration because (abstract) "... the degree of toxicity is influenced by biological availability of the metals (Zn, Cu, Cd, Pb) and interactions with other metals in the soil, nutritional status, age and mycorrhizal infection of the plant". Reduced growth or biomass is reported as one effect of excess copper when it occurs in high concentrations, by itself (Benson and Kelly, 1990; Eleftheriou and Karataglis, 1989; Lee et al., 1990b; Moon et al., 1990; Pernestål and Li, 1990; Rhoads et al., 1989; Rauta et al., 1989; Yang et al., 1990), when other factors may be limiting (e.g. Arshad et al., 1989; Chao and Wang, 1990; Kuduk, 1988; Nuorteva, 1988) or when copper occurs in a pesticide (Cheng, 1988; Milhaud et al., 1989). Copper inactivation of proteins (e.g. Sessa et al., 1990) is one of the ways of reducing growth. Barcelo and Poschenrieder (1990) review the effect of heavy metals, including copper, on plant water relationships. Copper-induced damage is reported to affect the permeability of roots of higher plants (De Vos, 1991; De Vos et al., 1989) which would affect plant water relationships as well as uptake of nutrients and ions. Yang and Skogley (1990), for example, noted the potential effect of copper on potassium uptake. Elevated levels of soil copper have been associated with reduced iron uptake, apparently a result of metal-metal competition (Nickless et al., 1989a). However, Nickless et al. (1988b) present evidence that copper uptake by *Lolium perenne* seedlings was not affected by chromium and arsenic. Copper has been reported to inhibit pollen germination and pollen tube growth in *Amaryllis vittata* (Bhandal and Bala, 1989). Wastewater containing copper has been reported to inhibit plant growth and germination (e.g. Gukasyan et al., 1989). Metal-containing sewage sludge, when applied to agricultural lands, can have an adverse effect on microbial processes, including mycorrhizal infection that could be of benefit, especially with seedling growth (Koomen et al., 1990).

Animals

As with all organisms, copper is essential for invertebrate animals, participating in the function of certain enzymes (e.g. Webb et al., 1989). Since elevated levels can be detrimental, copper is an agent widely used to control invertebrate pests (e.g. Bounias et al., 1990; Butovskii and Roslavtseva, 1989; Kovacic et al., 1989b; Yescott, 1989). However, there is a continuing interest in the effect of pesticides and heavy metals on invertebrate animals (Blynn et al., 1989; Jepson, 1989), including the duration of application of the agents (Helaly et al., 1990). Like plants, invertebrates respond to changes in metal bioavailability. As a result, some species have been used as indicator organisms (e.g. Ahsanullah and Williams, 1991; Barr et al., 1990; Nipper et al., 1989; Ozoh, 1990a; Salánki and V.-Balogh, 1989; Williams and Dusenbery, 1990a). Moore and Winner (1989) note, however that as a result of organism variability, single-species toxicity tests should not be used to indicate the response of an entire community (see also Lithner, 1989). The physiological state of an organism changes with stress whether from copper *per se* or from the sum total of anthropogenic effects (e.g. Luoma et al., 1990; Savari et al., 1991a). The change is due to the availability of the anthropogenic agent(s), whether in food (Amiard-Triquet et al., 1989) or the environment, as well as the nature of the organism. Differences in response to added copper have, for example, been noted between females, males and juveniles of the isopod crustacean *Idothea baltica* (Giudici and Guarino, 1989). The nature of the effect of copper on organisms is important in evaluating the impact of excess available metal. Yevich (1990) discusses comparative histopathological effects of metals on marine organisms and Kadhim (1990) reviews literature on the genetic effects of mutagens and carcinogens to marine mussels. George (1990) reviews the biochemical and cytotoxic effects of heavy metals to marine animals.

Excess copper can affect physiological processes within the organism (e.g. Kovacic et al., 1989b) as well as ecologically-important processes such as feeding activity. Salánki and V.-Balogh (1989) report that added copper (10 and 100 μg/L) reduced the duration of filtration of a freshwater mussel (*Anodonta cygnea*). Filtration rate by the green mussel *Perna viridis* was reduced significantly by 25 μg/L copper with the same amount of mercury (Krishnakumar et al., 1990). Copper sulfate was one of the metals which Butovskii and Roslavtseva (1989) found acted as an antifeedant to gypsy moth caterpillars. A mixture of hydrocarbons and copper are reported to produce changes in the structure of the digestive epithelium of the mussel *Mytilus edulis* (Lowe and Clarke, 1989) as are a mixture of mercury and copper (Krishnakumar et al., 1990). At high copper concentrations (>75 ppb) reduced fertilization success of a polychaete is reported (Ozoh and Jones, 1990a) and (50-200+ ppb) embryonic development is affected and survival after hatching is decreased in the cuttlefish *Sepia officinalis* (Paulij et al., 1990). (Bournias-Vardiabasis et al. (1990) report changes in fruit-fly embryonic cells after exposure to metal ions, including copper.) Gamete maturation in the giant sea scallop (*Placopecten magellanicus*) can also be affected by excess copper (Gould et al., 1990). Decreased gas (oxygen) exchange occurs when two species of fiddler crabs are exposed to excess copper (Devi and Rao, 1989). Similar results were obtained when two freshwater snails were exposed to a copper-containing herbicide (Cutrine-Plus; Christian and Tesfamichael, 1990). Gainey and Kenyon (1990) note decreased cardiac activity in *Mytilus edulis* when exposed to excess copper; 0.43 ppm produced a 50% reduction in heart rate. In mussels, excess copper has been found to affect the structure of the gill cells (Viarengo et al., 1990)and, with prolonged exposure, to cause the formation of renal cysts (Sunila, 1989). In oysters, copper is reported to affect immunity, at least partly by reducing the viability of hemocytes (Cheng, 1989, 1990). Comparable results with hemocyte viability have been obtained with other bivalves (Miller and Feng, 1987; Suresh and Mohandas, 1990b) and the shore crab *Carcinus maenas* (Truscott and White, 1990). Boitel and Truchot (1990), working with the shore crab, found a copper-related acidosis problem in the hemolymph which was more severe in half-strength than full-strength seawater. Using bivalves, Suresh and Mohandas (1990a) demonstrated that copper can destabilize blood-cell membranes causing release of acid phosphatase into the hemolymph. Results from Viarengo (1990) support this finding. Rózsa and Salánki (1990) propose that, in molluscs, heavy metals (Hg, Cd, Cu, Pb, Zn) regulate physiological and behavioural events by modulating ion channels in neuronal membranes.

Copper is found in vertebrate animals in a large array of materials ranging from the livers of fish to the teeth of alligators (Sato et al., 1990). As with invertebrate animals, the source is either food or the environment. It is also important to keep in mind that whether the total concentration of the metal is high or low, it is the chemistry of the metal that affects its bioavailability. Natural agents such as humic substances and anthropogenic agents such as wastewater can reduce metal bioavailability and thus metal effect (e.g. Young et al., 1990). This is one of the reasons why in natural environments, high concentrations of copper, and other heavy metals, can often not be identified as a toxic factor (e.g. Hall et al., 1987). Hall et al. (1989) comments (abstract) that "Mortality of striped bass in the Susquehanna River (Chesapeake Bay) may have been partly caused by copper and lead concentrations; however, other nonidentified factors were likely more important". In a study of haematological disorders found in fishes from polluted waters in two Indian estuaries, Rao et al. (1990) suggest that synergistic effects of toxicants is more important than the effects of each toxicant separately. In an examination of acute toxicity of formalin and copper sulfate to striped bass fingerlings, Reardon and Harrell (1990) note (abstract) "... a significant variation in tolerance (to copper sulfate) levels due to changes in salinity". This is a result of tolerance to low salinity as well as elevated copper concentrations. Smith et al. (1990) discuss the impact of effluent from the OK Tedi copper mine on the fisheries resource in a river in New Guinea. They found no evidence of acute toxicity attributable to copper and no evidence for copper accumulation from particulates. Although they state that there should not be any toxic effects from particulate-associated copper in the river, they fail to address the effect of increased sediment load on food organisms for fish in the river.

Avoidance of a food odor (alanine) by two species of fish has been noted as a result of sublethal concentrations of copper (Steele et al., 1987). The authors point out the importance of sublethal effects on the ecology of the organism. Heavy metals (Cu, Zn, Cd, Pb) have been reported to induce histopathological changes in carp taste buds (Jiang, 1989). (Dietary copper deficiency has not been found to alter taste sensitivity in the adult male rat (Brosvic and Hecht, 1989).) Skeletal deformities of European smelt (*Osmerus eperlanus*) in the estuary of the Elbe River have been compared with tissue and environmental concentrations of metals, including copper (Pohl, 1990). In laboratory situations it is easier to identify causative factors although often difficult to make application of results to natural conditions. Hogan (1990) reports a reproducible cardiac response of the Japanese rice fish (*Oryzias latipes*) to excess copper. Work with liver histological structure in fry of the milkfish (*Chanos chanos*) has shown that histology (abstract) "... is capable of revealing sensitively and selectively even subtle effects of environmentally induced changes in fish" (Segner and Braunbeck, 1990). Other body systems such as blood chemistry may not be as responsive to added copper (e.g. Dawson, 1990) although cupric chloride has been demonstrated to aggregate the red blood cells of the goosefish (*Lophius americanus*; Bourke and Borgese, 1990). An age-related progressive condition of abnormal hepatic copper storage in white perch (*Morone americana*) has been related to a cytoprotective response similar to the condition found in mammals (Bunton and Frazier, 1990). Singh and Reddy (1990) found that copper sulfate intoxication by Indian catfish (*Heteropneustes fossilis*) could be indicated by hematological and biochemical parameters as well as hepato-somatic indices. In an examination of the effects of pesticides on carp, Asztalos et al. (1990) note tissue damage from copper sulfate and liver cell death with a combination of copper sulfate and methidathion. Copper sulfate-induced alterations in fibroblasts from rainbow trout cell cultures have been reported by Mayer et al. (1988) and suppression of antibody-producing cells is reported in rainbow trout spleen sections exposed to copper (Anderson et al., 1989).

Adequate copper is important in domesticated animals. In birds, for example, deficiency has been linked to reduced melanin deposition (Grau et al., 1989) and reduced weight (Choi and Paik, 1989). Inadequate levels of copper in naturally-occurring feed materials are not uncommonly reported (e.g. Tokarnia et al., 1988). In a review of trace element status in northern regions of Norway, Froslie (1990) points out that trace element deficiencies are more common in domestic than in wild ruminant animals. This is a result of the lack of physical barriers in wild animals, allowing them to move to different areas. Booth et al. (1989) report that 10g of oxidized copper wire particles provided adequate liver copper stores for up to five months in farmed red deer grazed on a marginally copper-deficient property. A balance of trace metals is imperative for proper development. Bridges and Moffitt (1990) point to the importance of dietary zinc on copper metabolism of weanling foals. Zinc can inhibit copper uptake with the potential for improper cartilage formation and maintenance in ruminants (Bridges and Moffitt, 1990) and pigs although the background of the animals is important (e.g. Pond et al., 1990). Copper supplementation is advisable for a number of reasons, including the control of parasite burdens (e.g. Bang et al., 1990b). There is widespread use of dietary copper to improve growth and to assist in the control of microbial and parasite infestation in pigs (e.g. Shurson et al., 1990; Stansbury et al., 1990). Some consideration of level of supplementation is important because of the potential for detrimental effects with high levels of copper-containing agents (e.g. Choi and Paik, 1989; Kerr, 1990; Ward et al., 1989a; Wilczek et al., 1989).

Laboratory animals provide a means of evaluating the effects of copper deficiency or excess in relation to factors that are indicative of conditions found in either domestic animals or humans (e.g. Bladé and Arola, 1989). They have, for example, been used to determine the effect of copper deficiency on brain activity (Penland et al., 1989) and to test the anti-inflammatory activity of copper complexes (e.g. Auer et al., 1990a; Frechilla et al., 1990a,b). Popovich and Golikov (1988) note effects of direct injection of pesticides (including copper-containing pesticides) on the functional status and energy metabolism of the rat heart. Nutritional factors enter into the effects of copper deficiency, especially with dietary carbohydrates (Cunnane et al., 1990; Fields and Lewis, 1990c; Ghafghazi et al., 1990). Some relationship has been suggested between copper and cholesterol, in the regulation of inflammation and thrombosis although Saari et al. (1990b) report evidence that the two act separately. Copper deficiency has been linked to changes in the ratio of saturated to unsaturated fatty acids (Rosenbaum et al., 1990). This is suggested to be linked to the effect of copper on the oxidant status of the organism. Evidence is accumulating that copper-deficient animals are prone to oxidative damage, through reduced levels of the copper-containing antioxidants superoxide dismutase, ceruloplasmin, catalase and cytochrome oxidase (e.g. Carville, 1988; Saari et al., 1990a). Since inflammation elevates activity levels of ceruloplasmin, marginal copper status could limit the potential antiinflammatory actions of this agent (DiSilvestro, 1990). However, the effect of copper deficiency is not always obvious as shown by the inconsistent relationship between paw edema and liver Cu,Zn-superoxide dismutase activity and plasma ceruloplasmin activity in the copper-deficient rat (Kishore et al., 1990a).

Copper deficiency has been associated with changes in amino acid levels in rat auditory structures, on the basis of which the authors suggest that copper may have a very specific role in amino acid metabolism in the brain (Farms et al., 1991). The authors (Farms et al., 1990) did not observe a copper-deficiency effect on enzyme activities in rat auditory structures. However, copper deficiency has been associated with detrimental effects as a result of reduced enzyme levels (Reeves et al., 1990) implying the importance of copper to enzymes throughout most of the body. Mitsuma et al. (1989) report that injection of copper-binding peptide into rats caused a significant increase in thyrotropin-releasing hormone while plasma thyrotropin levels decreased. Hall et al. (1990) report reduced thyroxine levels in copper-deficient rats and suggest that a low copper diet may influence thermoregulation. Copper deficiency as well as excess can affect connective tissue composition (e.g. Agarwal et al., 1990; Farquharson et al., 1989; Jagetia and Ganapathi, 1990; Kaji et al., 1990; Massie et al., 1990) although the overall effect of this on the mechanical capabilities of the organism can be difficult to discern (e.g. Crist and Askari, 1990). Klevay (1990) notes decreased vitamin D metabolites in plasma of rats deficient in copper and suggests that low dietary copper in the U.S. may contribute to the high prevalence of osteoporosis.

Cytological effects of copper deficiency are numerous and can produce a change in blood cell composition (e.g. Johnson and Dufault, 1990a) as well as a shift in the ratios of various cell types in haemopoietic tissues (e.g. Bala et al., 1990b,c; Kramer et al., 1990a). Kramer et al. (1990b) suggest that copper deficiency is associated with enhanced activity or number of suppressor macrophages in the spleen. As an indication of the complexity of the copper-cell relationship in metabolism, Calvert and Simon (1990) report that the treatment of interferon-producing mouse L cells with two structurally unrelated chelators of copper ions completely stopped the ability of interferon to inhibit mengovirus growth. Copper-containing drugs are frequently used to treat conditions involving abnormal cell growth (e.g. De Pauw-Gillet et al., 1990; Nayak et al., 1990; Oikawa et al., 1990). The relationship between copper and the abnormal growth of cells and tissues can be affected by the nature of the organism as well as the nature of the cells, something that must be considered in the treatment of abnormal growth (e.g. Apelgot and Guillé, 1989a,b, 1990b; Guillé and Apelgot, 1989, 1990). Brem et al. (1990), for example, report that anticopper treatment inhibited the invasive spread of gliosarcoma cells in the rat brain. They suggest that this is a result of the biological role of copper in the neoplastic spread of brain tumour cells.

The cytological effects of copper deficiency can be related to the immune response of an organism. Windhauser (1988, Ph.D. thesis) notes that the response is affected by severe dietary copper deficiency although not always by marginal deficiency. Bala et al. (1990a) comment that, for newborn Lewis rats, (page 285) "... development of the immune system in the newborn may be particularly vulnerable to suboptimal Cu nutriture". There may also be a time-related effect of copper deficiency, noted by Prohaska and Lukasewycz (1989) in an evaluation of copper supplementation during development of weanling, copper-deficient Swiss albino mice. With older Swiss albino mice, copper deficiency appears to also affect the immune system (Lukasewycz and Prohaska, 1990) suggesting that, throughout life, copper nutriture is important.

Copper chloride can induce taste responses in the gerbil (Somenarain and Jakinvich, 1990), a result of excess copper. However, copper deficiency does not appear to alter taste sensitivity, at least in the adult male rat (Brosvic and Hecht, 1989). With the ferret, Rudd et al. (1990) note an emetic action by copper sulfate, a result of gastric irritation possibly facilitated by afferent vagal and sympathetic innervation. Gastric ulcer formation in the rat has been associated with reduced gastric mucosal copper-zinc superoxide dismutase activity (Ogino et al., 1990). Penland et al. (1989) examined brain electrophysiology in adult rats, noting evidence of increased laterality of brain activity with copper deficiency. In a study of the optic nerve in copper deficient rats, Dake and Amemiya (1991) note evidence of demyelination or dysmyelination.

Mice and rats have been used to evaluate the effects of excess copper. Bogoslovskaya et al. (1989) report that highly-dispersed copper powder was more bioavailable than copper sulfate when injected into rats. Szabová et al. (1990) determined mouse LD_{50} doses of a copper-containing drug used in rheumatoid arthritis. They did not, however, find evidence of significant pathomorphological changes in organs of the test animals. Exposure to metal salts via intraperitoneal injection, has been associated with a reduction in oxygen consumption and deep body temperature (i.e., hypometabolism and hypothermia; Gordon et al., 1990a). The authors note that above a 3.3 mg/kg threshold dose of cupric chloride, there was a linear relationship between dose and effect. With chick embryos, high concentrations of copper (10+ µg/egg), as copper sulfate, has been reported to be both toxic and teratogenic (Gilani and Alibhai, 1990). Inhalation of powdered copper is reported to have histological effects (e.g. copper chloride - Ermachenko and Ermachenko, 1987; copper-containing superconductor material - London et al., 1990). Copper has been used to induce diaphragm contractures in mice, apparently a result of increased entry of calcium through the outer membrane of muscle cells (Lin-Shiau et al., 1989). Excesses of several metals (Cd, Cu, Hg) have been reported to inhibit intestinal absorption of some sugars and amino acids by binding to proteins of cells involved in the uptake (Rodriguez-Yoldi et al., 1989). Excess copper in laboratory animals and humans may be associated with myocardial (Hyacinthe et al., 1990), kidney (e.g. Kone et al., 1990; Madej and Radzanowska, 1988) and hepatocellular damage (Sokol et al., 1990), the latter as a result of oxidant injury to liver cell mitochondria. A good deal of work continues to be done on the compartmentalization and flux of copper in the body, particularly with the liver and bile (e.g. Aykac et al., 1989). The amount of copper associated with important copper-binding agents (metallothionein, superoxide dismutase), has been used as an indication of the physiological status of the organism (e.g. Chung et al., 1988; Evering et al., 1990). Excess copper has been related to changes in cell viability (e.g. Brown et al., 1988/89; Mason and Grinstein, 1990; Ng and Liu, 1990; Sun and O'Dell, 1990), including hemolysis (Caffrey et al., 1990). Yu and Zhang (1989) present evidence that copper can inhibit lactate dehydrogenase activity in rat spermatozoa, a factor which may cause the loss of sperm motilitiy.

The major application of work on trace elements and laboratory animals is the understanding and treatment of human malfunctions. The use of copper in rheumatoid arthritis (e.g. Pullar et al., 1989) and its effect on inflammation is an example of this. Secondary applications include domestic animals and the effects of byproducts of industry and society. Caroli (1989) includes many of these applications under inorganic biochemistry, the discipline which he states "... embraces toxicological, clinical, nutritional and ecotoxicological aspects of element interactions with living organisms". This includes both the use of copper in medicines and the explanation of effects of excess copper (e.g. Bogdanovic et al., 1989; Chahboun et al., 1990; Dash, 1989; Nakatani and Kobayashi, 1990; Terada et al., 1990). The application to immune function and other functions, includes what Ohsawa (1990) terms the environmental status of heavy metals.

The amount of copper required for normal human metabolism varies and is affected by the chemical nature of the copper ingested as well as the physiological state of the human (e.g. Allegri et al., 1990; Heaney, 1988). Environmental factors and habits of the individual can influence the concentration of copper in humans as well as other animals (e.g. Stransky, 1990; Wolf et al., 1989). The role that copper plays in diseases such as osteoporosis is largely unknown (Baslé et al., 1990, 1990; Heaney, 1988) which makes prediction of requirements difficult, especially for those in poor health. This may not be a major factor since there is some evidence that within normal ranges, copper absorption is not affected by the amount of dietary copper (Milne et al., 1990b). The authors point out that sex differences need to be considered when determining the effect of copper depletion on various indices of copper status.

Copper is often linked with oxidation processes in materials ranging from foods (e.g. Kanner and Shapira, 1989) to the bleaching of melanin pigments (Korytowski and Sarna, 1990). Toxic effects of metal ions have, for example, been linked to their ability to catalyze peroxidation of lipids (e.g. Gelvan and Saltman, 1990; Knight and Voorhees, 1990). Kawakishi et al. (1990) found increased oxidation of a protein with copper added to the reaction mixture. Breakage of the nucleic acid DNA molecule is enhanced by copper (e.g. Inoue et al., 1990; Rahman et al., 1989a) and copper complexes (e.g. Piccinini et al., 1990). Bhattacharyya et al. (1989) report that copper, as copper sulfate, increased the radiosensitivity of thymine, with ^{60}Co γ-ray irradiation. The ability of copper to interact with oxidation processes has been of value in designing metal complexes that act as radiosensitizers for ionising radiation-induced damage to cells. Conversely, the effect of treatment with radiation and/or drugs (e.g. Slavik et al., 1989) can affect tissue copper concentrations and can have toxic side effects (e.g. Monti et al., 1990). Soderberg et al. (1990) found that pre- and postirradiation treatment with a copper-containing drug (Cu-DIPS) increased radiation recovery, postirradiation treatment enhancing recovery of blood cell production in bone marrow and spleen.

Domestic and industrial uses of copper have caused concern about human health effects. Copper from copper tubing is reported to increase levels in drinking water and thus affect water quality (e.g. López de Sá, 1989). Nordberg (1990) notes that acidic water may dissolve copper from piping systems but that neutralization can reduce the effect. He notes (page 892) that "In Sweden, a limit for copper in acid water has been set at 3,000 mg/L. Neutralization is recommended when this limit is exceeded". Acid water-related elevated copper levels in drinking water have been reported to cause Indian Childhood Cirrhosis (Eife et al., 1989; Madsen et al., 1990) although this may be a result of hypersensitivity to excess copper (e.g. Nordberg, 1990). The effects of continued exposure to aerosol copper in and near industrial sites have been examined for effects on the respiratory tract (reviewed by Nemery, 1990) as well as other systems. In a study of the effect of copper-containing dusts, Belyaev and Shmeleva (1989) found evidence of cytotoxic products of lipid peroxidation. However, since exposure is frequently to more than one metal it is often difficult to isolate the effects of any excess copper (e.g. Sunde and Haldorsen, 1990). Bogdanovic et al. (1989) report no major hepatotoxic effects although some decrease in enzyme activity, from chronic exposure to copper and aluminum in a cable factory in Yugoslavia.

I.3.4 COPPER IN DENTAL AMALGAMS

The use of copper in dental amalgams continues to be evaluated (e.g. Goehlich and Marek, 1990; Icenhower et al., 1990; Marhsall et al., 1990; McKinney et al., 1990; Osborne and Norman, 1990). Deterioration of any alloy is of concern and, with copper as well as other alloys, this tends to occur more frequently with the large restorations (e.g. Smales et al., 1990). Osborne and Gale (1990) comment that the width of the restoration is a critical factor with tooth position and alloy differences being of lesser importance. However, Nelson and Mahler (1990) present evidence that at least one low copper alloy exhibits a delayed expansion sufficient that it might later induce root fracture of the tooth.

One of the concerns about copper alloy amalgams is the release of ionic metal into solution in saliva or the oral cavity in general (e.g. Johansson et al., 1989; Örtendahl et al., 1989; Zivko-Babic et al., 1989). Kaga et al. (1990), report that (abstract) "... any cellular or tissue reaction to amalgams is mainly a response to dissolved Cu and Zn ions" while Bumgardner et al. (1990a,b) found reduced proliferation in cultured lymphocytes/monocytes and T-cells when exposed to copper alloys. However, the nature of the cell culture medium be considered in toxicity tests (Hao et al., 1990). Soileau and Lucas (1990) report that (abstract) "... although significant Cu levels were measured in the gingival tissues adjacent to the crowns, Cu elevations were not detected in the ... blood, liver, spleen, kidney, or lymph nodes". Handling of the amalgam is important. Polishing of the as-cast condition of copper-containing alloys may increase the dissolution of copper from the alloy (Wataha et al., 1990) while burnishing has been reported to reduce microleakage more than that found with polishing (Icenhower et al., 1990). Loss of copper is certainly a factor to consider, but not only in terms of tissue metal concentrations - copper will inhibit plaque bacteria and bacterial pathogens when used as an oral rinse (Eisenberg et al., 1989; Lee et al., 1990e) or when released from copper amalgams. However, sensitivity to constituents of dental alloys may be a factor, Namikoshi et al. (1990) note the potential for an allergic reaction to copper in a low percentage of a small number of individuals.

I.3.5 COPPER IN CONTRACEPTIVE DEVICES

In a very brief but excellent discussion of long-term use of copper intrauterine devices (IUD), Newton and Tacchi (1990) point out that the IUD is now probably the second most commonly used reversible method of birth control. Copper-bearing IUDs became available in the 1970's and gave lower pregnancy rates than inert devices, the copper enhancing the contraceptive effect of the device, in part through its action on the endometrium (e.g. Hernandez-Perez et al., 1989; Liu et al., 1988) and on fertilization (e.g. Cordonnier et al., 1989). A variety of copper-bearing devices have been used and continue to be tested and improved for effectiveness and safety (e.g. Puraviappan et al., 1989; Rowe et al., 1990; Sivin et al., 1990a,b; Srisupandit, 1988; Wildemeersch et al., 1988). Edelman et al. (1990) discuss the duration of use, frequency of replacement, of IUDs suggesting that as a result of copper loss and surface deposits, removal and replacement should occur at intervals of about five years.

Safety of the wearer is of concern, Edelman and van Os (1990b) pointing out that (abstract), at least with two units "... (Multiload Cu375 and TCu380), uterine perforations are rare, there is no evidence of an increased risk of infertility, and there probably is no incresed risk of ectopic pregnancy after IUD removal". They continue with the statement that "Any increased risk of pelvic inflammatory diseasemay be limited to the initial months of IUD use". The incidence of intrauterine IUD breakage increases with duration of use (e.g. Edelman and van Os (1990a). Goldstuck et al. (1990) provide information on the forces required to break copper-bearing devices, commenting that the decreased fracture forces associated with used MLCu 250/375 devices may explain the reports of intrauterine breakage with Multiload devices. Surface deposits on IUDs are primarily oxides of copper although, with time, they include calcium carbonate and, to a small extent, magnesium substituted for the calcium (Martin et al., 1988). Randic et al. (1990) discuss the nature of nucleated cells adherent to copper-bearing IUDs, noting a difference in cells between devices removed from successful users and those with accidental pregnancy. Their conclusion is a question, does the cell difference indicate anything related to the antifertility nature of the IUD? Allergic reactions have been noted, one case to nickel in a copper IUD (Grinsted, 1990). Inflammation is a factor with some IUD users (Fahmy et al., 1990). As well, changes in the endometrium, bleeding and increased menstrual flow have been associated with the use of IUDs (e.g. Chi et al., 1990; Milsom et al., 1990; Wang et al., 1990a; Zhu et al., 1989a,b) although this is reduced by a relatively new IUD device, the Cu-Fix (Pizarro et al., 1989; Wildemeersch et al., 1988). Cases of uterine perforation by an IUD, although rare, are reported as are cases where both the uterus and rectum are perforated (Sepúlveda, 1990). Hirvonen and Idänpään-Heikkilä (1990) review cardiovascular death among women under 40 years of age using low-estrogen oral contraceptives and IUD in Finland from 1975 to 1984. No solid evidence of effect from copper-bearing IUDs is presented. In a study of the development of human embryos in the presence of a copper IUD, Barash et al. (1990) comment that from their small sample number (abstract) "... the copper-releasing intrauterine device has no deleterious effects on fetal development".

I.3.6 AGENTS TO CONTROL FOULING AND DETERIORATION OF WOOD AND METAL

The use of copper in antifouling agents and in wood preservatives is based on the ability of high concentrations of the metal to inhibit or stop organism growth. This has sponsored concern about the biological effects of metal released from these agents and an evaluation of this by a number of workers (e.g. U.S. National Technical Information Service, 1989e). There is also appropriate concern about the nature of treatment, McCoy (1987), for example, commenting that "Effective control of biofouling generally requires adequate diagnosis of the problem followed by application of an appropriate treatment technique". Callow (1990) and Callow and Edyvean (1990) provide excellent reviews of ship fouling (and corrosion in Callow and Edyvean, 1990) problems and solutions, including a discussion of the chemical nature of detrimental effects of coper on algal metabolism. New types of antifouling agents continue to be developed, agents that will prolong the action of the antifoulant and in doing so will reduce the rate of metal release into the environment (e.g. Peacock et al., 1989). There is also a continuing attempt to relate environmental factors to the nature of the antifouling agent (e.g. Edyvean and Silk, 1989) as well as its biological effect (e.g. Augler et al., 1989.

Biological fouling of water supply systems is fa continuing problem (Mittelman and Geesey, 1987). Fouling follows a definite order, with microorganism and then biofilm formation occurring followed by macroorganisms. Techniques are being developed for disinfecting water supply systems efficiently and with minimal environmental effect (e.g LeChevallier et al., 1990; Williams and Knox-Holmes, 1989; Williams et al., 1989). Fouling is frequently associated with corrosion of piping. Corrosion of copper can be induced by organisms, primarily bacteria, algae and fungi (e.g. Wagner et al., 1987) and byproducts of their metabolism (e.g. Gianotto et al., 1989; Jolley et al., 1988, 1989). Gaylarde (1989) provides a review of the microbial corrosion of metals, discussing effect and the mechanisms involved as well as techniques to minimize the effect. However, there is an interaction of the physical, chemical and biological factors causing corrosion which can make identification of primary causal mechanisms difficult (e.g. Wood and Fry, 1989). A number of techniques have been used to examine and model corrosion by biopolymers (Geesey and Bremer, 1990; Gianotto et al., 1989; Jang et al., 1990b; Jolley et al., 1988, 1989).

Biodeterioration of wood has long been a problem in both terrestrial and aquatic environments. Copper, in association with an organic (e.g. De Groot et al., 1988; Göttsche and Marx, 1989) or in association with chrome and arsenic (CCA), has long been successfully used to reduce the rate of biodeterioration (e.g. Doi, 1989; Gnanaharan and Dhamodaran, 1989; Newbill and Morrell, 1990; Pendleton, 1989). Application treatments and success of treatment can vary from site to site as well as between different types of wood and different wood uses. King et al. (1989) present results which suggest that (page 190) the "... knowledge of CCA permanence and performance in wood is not as precise as is required for accurate prediction of performance, ..." and suggest that more testing is needed. Smith et al. (1990) used direct-scan X-raying techniques to assess preservative (e.g. CCA) retention and distribution in wood. Agents are being tested as new controls against biodeterioration, in part as a result of the rapid leaching rates of some copper-containing agents as well as concern about environmental effects (e.g. Galarneau et al., 1990; Roomi et al., 1990). Concern has also been expressed about the health of individuals working with chemical preservatives. Gilbert et al. (1990), however, report no adverse health effects or increased incidence of mortality in wood-treating workers in Hawaii, when compared with nonexposed individuals.

I.3.7 PHYSIOLOGICAL EFFECTS OF DEFICIENT AND EXCESS BIOLOGICALLY AVAILABLE COPPER

"The response of an organism to deficient as well as excess biologically available copper involves metabolic processes. These processes are biochemically driven and physiologically expressed on the health and well being of the organism" (page 29, Final Report, I.C.A. Project 223, 1990). Wachnik (1988) reviews the physiological role of copper and the problems of copper nutritional deficiency. The author discusses the importance of the metal in eliminating metabolically detrimental agents from the body, in the synthesis of various organics and in the processes that provide energy for metabolism. Persson (1989) presents some general information on some of the mechanisms involved in metal ion-related diseases. Participating in the maintenance of immune function is a very important role for copper in domestic animals (e.g. Suttle et al., 1988b), laboratory animals (e.g. Soderberg et al., 1989) and humans (Stabel and Spears, 1989). These benefits are achieved only with an adequate supply of copper indicating the importance of nutritional adequacy and metal availability in the environment. It is also important to recognize that a variety of drugs can affect the copper status (e.g. Paternain et al., 1990) and complicate the availability and metabolism of copper. Excess biologically available copper can be detrimental, a situation which has sponsored a great deal of research on aquatic (e.g. National Technical Information Service, 1990c) as well as terrestrial plants and animals. This section of the review examines the physiological importance of copper using references on the effects of both copper deficiency and excess.

Muscle and connective tissue

Copper deficiency as well as the interaction between copper and metabolites of physiological imbalance have been linked with improper tissue formation and maintenance (e.g. Chace et al., 1990, 1991a,b). Elastin in major blood vessels can have reduced cross-linking as a result of copper deficiency although copper repletion can correct the effect, at least in certain cases (Tinker et al., 1988; 1990). Some of the proteins associated with heart tissue in copper-deficient rats have been reported to have a consistent decrease or diminished level of a polypeptide (McCormick et al., 1989b).

Cardiovascular problems

In a discussion of ischemic heart disease and copper deficiency Klevay (1989) comments (page 203) that "More than 50 similarities between animals deficient in copper and people with ishemic heart disease have been identified. ... More aspects of ... ischemic heart disease can be explained by considering this illness to be a problem of copper deficiency than by considering any of several other explanations that have been offered." Klevay (1990b) also provides an excellent review of copper and ischemic heart disease in a series of reviews edited by Lei (1990a). These include Allen (1990), Allen and Mathias (1990), Brannon (1990), Carr and Lei (1990b), Cunnane (1990), Hassel and Lei (1990), Lei (1990b,c), Lei and Carr (1990) and Medeiros (1990). Since ischemic heart disease is heart disease associated with poor blood circulation, it involves a large number of factors associated with the circulatory system as well as the heart. The effects of severe disease conditions, such as acute myocardial infarction, are often diagnosed by copper levels as well as levels of zinc and certain hormones (Meissner et al., 1990).

Copper status has been associated with the condition of the lining of the arteries which, when in poor condition are a main contributor to ischemic heart disease (e.g. Allen, 1990; Kinsman et al., 1990). Carter et al. (1990), however, present evidence that copper deficiency may not produce the same results with all animals. Much of the work related to the association of copper deficiency and ischemic heart disease concerns lipids and lipid metabolism (e.g. Schuschke et al., 1990; Yount et al., 1990a). Cunnane et al. (1988) note that marginal copper depletion can cause significant changes in coronary perfusion pressure and force of contraction. Dietary copper deficiency has been associated with changes in heart weight (Saari and Johnson, 1990) and size (Seidel et al., 1990) as well as increases in cardiac sodium and decreases in potassium (Halas and Klevay, 1989). Cardiovascular abnormalities have been found in the pig when fructose is consumed with a low copper diet (Scholfield et al., 1990). Lynch and Strain (1989) report reduced cardiac antioxidant enzyme activities in lactose- and sucrose-fed rats.

Although physiological problems with the heart and cardiovascular system are usually associated with copper deficiency, excess copper can have cardiovascular effects. The nature of membranes can change with copper deficiency (e.g. Hassel et al., 1990), transport across the pericardial membrane can be influenced by metal salts, including $CuCl_2$ (Arif et al., 1990) and changes can occur in the nervous system (e.g. Salánki and Hiripi, 1990; Sugawara and Chen, 1990). Evidence from Zhang et al. (1990d) indicates that $CuCl_2$ can affect the nerve-muscle cell relationship in the heart. Rhee and Dunlap (1990a,b) note changes in blood pressure of anaesthetized rabbits after injection of large amounts of copper (<3 to 10 mg/kg) with the higher dose causing shock. Hyacinthe et al. (1990) report that copper and ascorbate are toxic to the myocardium in isolated, perfused rat heart preparations. This is presumably a result of exacerbation of the injurious effects of free radicals, a feature that can be ameliorated by certain metal complexing agents such as neocuproine (Appelbaum et al., 1990). Drugs used for chemotherapy may have cardiotoxic effects that can be modulated by the copper status of the organism. With adriamycin, Fischer and Johnson (1990) found evidence that copper deficiency will increase oxidative damage by the drug. This was felt to be associated with the reduced Cu,Zn superoxide dismutase activity associated with copper deficiency. Production of oxygen free radicals during ischemia (Chen et al., 1990) has led to the use of recombinant Cu,Zn Superoxide dismutase as a treatment (Hori et al., 1989; Imaizumi et al., 1990). Villani et al. (1990) report that copper only marginally if at all, affects the cardiotoxicity produced by doxorubicin. Hasinoff (1990) notes that both Fe^{3+}-adriamycin and Cu^{2+}-adriamycin react directly with a cardioprotective agent (ICRF-187) which results in the displacement of the metal ion from its complex with adriamycin.

Enzymes

The activity of many copper-containing organics requires copper, deficiency of the metal results in a reduction in activity with resultant disability in the metabolism of the organism (e.g. Singh et al., 1990b). In contrast, an excess of copper can lead to inactivation of organics as a result of the excess metal either replacing a normally-occurring cation or binding at a site on the organic and producing an abnormal compound. Van Assche and Clijsters (1990b) discuss the effects of metals on enzyme activity in plants, pointing out that there are (abstract) "Two mechanisms of enzyme inhibition (that) predominate: (1) binding of the metal to sulphydryl groups, involved in the catalytic action or structural integrity of enzymes, and (2) deficiency of an essential metal in metalloproteins or metal-protein complexes, eventually combined with substitution of the toxic metal for the deficient element". Excess copper can inhibit enzyme activity whether in fungi (e.g. Ali and Abdel-Moneim, 1989) or shrimp (e.g. Chu and Shaw, 1989) or a variety of tissues (e.g. Cadelis and Vijayalakshmi, 1989; Jenkins and Kramer, 1989; Kang et al., 1988; Moreno et al., 1989) as well as purified enzymes (e.g. Nagamori et al., 1990). The inhibition may, however, not be by just the metal but rather by an organic complex containing the metal. And this may be beneficial instead of detrimental. Badawi et al. (1990), for example, discuss the possibility of copper complexes inhibiting a lipoxygenase. On the basis of its activity they suggest that copper (or zinc) may have an important metabolic role in the normal function of the human respiratory system. Enzyme action and copper effect, is also pH dependent (e.g. Chu and Shaw, 1991; Wigfield and Goltz, 1990b). Another consideration is that the effects of metal deficiency or metal excess, on enzyme activity, can be affected by other factors. Lewis and Fields (1989), for example, report reduced activity of amylase and lipase with nutritional copper deficiency; however, activity was lower in rats consuming fructose than in those consuming starch. Interactive effects of a number of metals and metalloids, with copper have been suggested (e.g. Boyne and Arthur, 1990; Emerick and Kayongo-Male, 1990b,c). Chemical characteristics of enzymes and the role of copper in their activity continue to be of importance (e.g. Gabriel et al., 1990; Nagamori et al., 1990; Oblender and Carpentieri, 1990a

Excess metal in soils has been linked to reduced phosphatase activity (Doelman and Haanstra, 1989), and reductase activity (Fu and Tabatabai, 1989). Similar results have been obtained with excess metal in aquatic systems (e.g. Accomando et al., 1990). Phosphatase activity in plants can also be inhibited by copper or by a phytic acid complex of copper (inositol hexaphosphate-Cu^{+2}; Martin and Evans, 1989, 1990). The reduced soil reductase activity is of interest because, of the two distinct types of nitrite reductases known, one contains a copper center (Coyne et al., 1989), is active in denitrifying bacteria (e.g. Coyne et al., 1990) and indicates the requirement for a suitable source of the metal. An additional expression of the importance of copper to reductases is its ability to enhance ribonucleotide reductase activity in human lymphocytes (Oblender and Carpentieri, 1990b) and its requirement for appropriate reductase activity in cholesterol biosynthesis (Yount et al., 1990b).

Cell copper concentration has been linked to enhanced ascorbate oxidase activity in plants (Sekiya et al., 1990). Oxidase and peroxidase activity in plants and animals can be inhibited by deficient or excess biologically available copper (e.g. Donlan et al., 1989; Fischer et al., 1989; Lobarzewski et al., 1990). The role of copper can thus be linked to the regulation of enzyme activity in a broad range of metabolically important processes in blood cells (e.g. Farant and Wigfield, 1990; Rossi et al., 1990; Scuderi, 1990). Similar responses are found in the liver (e.g. Cicero et al., 1990; Laidley et al., 1988) and pancreas (e.g. Dubick and Majumdar, 1988). Kwon et al. (1990b) report inactivation of a proteinase inhibitor by copper (as $CuSO_4$) in the presence of hydrogen peroxide. The protein in question is an agent that protects connective tissue from degradation by the enzyme elastase. The interaction between copper-proteinase inhibitor-elastase is important for maintenance of certain connectives and an imbalance has been associated with rheumatoid arthritis (Kwon et al., 1990b). (Similar copper relationships have been found with lipoxygenases (Aihara et al., 1990a).) At low concentrations ionic copper has been found to stimulate the activity of purified poly (ADP-ribose) polymerase but activity is inhibited at elevated levels of ionic copper (Zaalishvili et al., 1990). The interaction of copper, the polymerase and two polyamines may be associated with the normal metabolism of brain cell nuclei. The effect of copper deficiency is not, however, always apparent, some enzymes do not form useful indicators of copper deficiency (Mameesh et al., 1989).

Cellular antioxidant protection is provided through the enzyme Cu, Zn-superoxide dismutase (SOD) which catalytically removes superoxide radicals (Gutteridge, 1990). As a result, SOD activity is often indicative of physiological condition (e.g. Asayama et al., 1990; Malan et al., 1990). Cu, Zn-superoxide dismutase has a long and noble, although poorly known history in the evolution of life on earth (e.g. Lesser, 1989). Copper deficiency has been linked to reduced SOD activity in plants and animals (Askari et al., 1991; Wu and Wu, 1990). Intriguingly, red blood cell SOD activity levels can be used to assess existing human copper status because copper repletion will increase SOD activity in both young and old red blood cells (DiSilvestro, 1988a). Askari et al. (1990a) found that variation in copper intake may cause changes in the SOD levels of rat cardiac myocytes. Panda et al. (1990b), however, report that erythrocyte SOD activity did not correlate with other parameters of copper nutriture. Similar results have been obtained by Tran et al. (1989). SOD activity has also been shown to be resistant to changes in copper availability in rabbit liver but not in whole blood or plasma (Ferrell et al., 1990). Elevated intracellular SOD activity has been related to biological damage (e.g. Harisch et al., 1989; Inagaki et al., 1989), possibly a result of the generation of free hydroxyl radicals (Yim et al., 1990a). In diabetics, abnormal changes in erythrocyte SOD have been related to cataracts and retinopathy (Kinoshita et al., 1990) and elevated serum activities to nephropathy (Inagaki et al., 1989). The association of the genetic makeup of individuals with Down syndrome with SOD activity suggests that, in these individuals, there is an overexpression of the gene for Cu, Zn-superoxide dismutase (Groner et al., 1990; see also Mote et al., 1990). (Seto et al. (1990) report that overexpression of SOD does not affect life span in the fruit fly.)

The stability of the Cu, Zn-superoxide dismutase molecule is reported to be due to the presence of zinc (Kajihara et al., 1990). Taylor et al. (1990) presents evidence that zinc deficiency reduces the ability of the organism to increase SOD activity when needed. Other work on the nature of SOD includes Bertini et al. (1990), Greco et al. (1990), Harris and Dameron (1988), Makita et al. (1989b,c, 1990), Sanchez-Moreno et al. (1989), Steinkühler et al. (1990), Valentine and Nick (1990), Yano (1990). Because of its broad application in therapy and diagnosis, techniques have been developed for large scale purification of human erythrocyte Cu, Zn-superoxide dismutase (Jungbauer et al., 1989).

Inflammation

Superoxide dismutase activity as an antioxidant is important in cell protection and, as a result, can be affected by inflammation reactions (e.g. DiSilvestro and Marten, 1990). Copper deficiency is often linked to enhanced damage caused by oxygen toxicity (e.g. Akers et al., 1990; Hathcock et al., 1990) and to increased inflammation (Kishore, 1990b). Excess copper can also be linked to oxidative damage although it can be difficult to separate the damage occurring as a result of excess ionic iron from that produced by excess copper (Gelvan and Saltman, 1990). Effects of inflammation are varied, causing an increase in plasma levels of the copper-containing protein ceruloplasmin (e.g. DiSilvestro, 1989). These effects can be reduced by treatment with copper in combination with several other drugs (e.g. aspirin - McGahan, 1990; D-penicillamine - McGahan and Grimes, 1990, Mita and Matsunaga, 1990; indomethacinate - Roch-Arveiller et al., 1990a). The effect of D-penicillamine and other, suitable metal-complexing agents is difficult to identify (e.g. Auer et al., 1990b) although they may either buffer the metal supply to the tissues or the organometallic complex may act as the anti-inflammatory agent (e.g. Brumas and Berthon, 1990; Ferrari et al., 1989; Parashar et al., 1990).

Atypical growth

The balance between trace metal demands and growth can be affected by a range of factors (e.g. Tejwani and Hanissian, 1990). Some of these are associated with perturbations causing or resulting from the irregular production of metabolites or irregular growth of cells. Copper is one of the trace metals that can, for example, affect the biosynthesis of melanin (Jara et al., 1990). One of the many causes of ulcer formation has been linked to reduced activity of Cu,Zn-superoxide dismutase, at least in a laboratory animal that has been treated with a strong metal chelating agent (Ogino et al., 1990). Deficiency has also been linked to reductions in a broad range of other cell activities (e.g. Babu and Failla, 1990). Although many problems of irregular growth can be overcome with copper-containing agents, excess copper can cause atypical production of some proteins in pregnant mice (Mizejewski et al., 1990).

Even though nutritional copper deficiency has not been directly linked to all irregular cell growth (e.g. Kishore et al., 1990a), it can affect metabolism and as such, may have an indirect effect. Treatment of irregular growth, such as tumours, can also have an effect on tissue copper concentrations and the requirements for nutritional copper (e.g. Boman and Selin, 1988) although the association with SOD activity is controversial (e.g. Fischer et al., 1990a; Oka et al., 1990; Sawaki and Isono, 1985). Kovacic et al. (1986) suggest that anti-cancer drugs generally function by charge transfer resulting in formation of toxic oxy radicals which destroy the neoplasm. The direct and indirect associations of copper with cell growth have sponsored evaluation of the experimental role of copper in cancer treatment. Badawi (1990) comments (page 51) that "... the future use of copper chelates with SOD(superoxide dismutase)-like activity to treat neoplastic diseases without killing transformed cells has some exciting possibilities". An indication of the value of copper is given in the four papers by Apelgot and Guillé (1989a,b) and Guillé and Apelgot (1989, 1990) on the treatment of mice bearing a krebs ascitic tumour by means of a protocol based on radioactive copper (^{64}Cu). Interest in trace metal status has also arisen as a result of the concern that trace metal deficiency can affect the activity of antitumour drugs like bleomycin (Lyman et al., 1989).

Human diseases of inherited copper imbalance

A number of diseases involve copper imbalances (e.g. Barrow, 1988). These are Diseases involving trace metal imbalances can frequently characterized by abnormal liver metal concentrations (e.g. Aoyama et al., 1990; McMaster et al., 1988a; Sato et al., 1989; Tanner, 1990). In contrast, in diseases such as Down Syndrome, there may be an overexpression of copper-containing enzymes (Krmpotic-Nemanic et al., 1989; Minc Golomb et al., 1990) or metal-transporting organics. In several inherited diseases involving copper there is increasing evidence that the change from prenatal to neonatal metabolism includes a lack of ability to adequately mobilize and metabolize the metal (Bingle et al., 1991). This can lead to accumulation of large amounts of copper in the liver and detrimental effects on liver cells as well as the rest of the body (e.g. Schilsky et al., 1989c). Work on these diseases in humans is often difficult because of the relatively low incidence of the diseases as well as the requirements for animal models to test concepts and treatments (e.g. Jori et al., 1990).

"Menkes' kinky hair syndrome is a lethal x-linked neurodegenerative disorder of copper metabolism, with low serum copper concentrations, tissue-specific copper sequestration, and decreased activities of cuproenzymes in a number of cell types" (Castillo et al., 1990; see also Garnica et al., 1988, Goto et al., 1989). High concentrations of copper are accumulated in fibroblasts in both humans and the macular and mottled or blotchy mouse animal models used most frequently for work on this syndrome (Palida et al., 1988; Tanaka et al., 1990; Waldrop and Ettinger, 1990b). However, Kodama et al. (1989) report evidence of copper deficiency in mitochondria of cultured skin fibroblasts from patients with Menkes' Syndrome. van den Berg et al. (1990c) report copper uptake in cultured muscle cells to be similar to that found in fibroblasts from Menkes' patients. Although elevated levels of metallothionein are reported for Menkes' disease (Waldrop and Ettinger, 1990b), low levels of one or more other copper-binding proteins have been noted in the brindled mouse (Palida, 1989; Palida et al., 1988).

Recent case reports of Menkes' disease include Dirik et al. (1989), Ichihashi et al. (1990) and Lo et al. (1989). Tonnesen et al. (1989a,b, 1991) discuss the diagnosis and incidence of Menkes' disease, commenting (Tonnesen et al., 1991) that for five European countries the incidence is 1 patient per 298,000 live-born babies. Copper treatment of humans and the brindled mouse can reduce the apparent effects of the disease (e.g. Kasama and Tanaka, 1989; Tanaka et al., 1989; Westman and Morrow, 1989) although, at least in the mouse, delays in treatment are reported to produce irreversible changes (Fujii et al., 1990).

In a discussion of the gene makeup of an individual with Wilson's disease La Farrer et al. (1989) comment that the disease (page 997) "... is an inborn error of copper metabolism associated with decreased biliary copper excretion resulting in copper deposition in and subsequent damage to various tissues, primarily the liver, brain and kidneys". Since copper excretion is accomplished by at least two pathways (Houwen et al., 1990), dealing with Wilson's disease can be difficult. The effect of the disease is expressed in a variety of ways, including the normal high liver copper concentrations (e.g. Mzhel'skaya et al., 1989b) and a change in the nature of ceruloplasmin, a copper-containing antioxidant in human blood (Saenko et al., 1990). When Bedlington terriers exhibit copper toxicosis they exhibit symptoms of Wilson's disease (Nederbragt et al., 1988a) and provide a useful model for studies of the disease and its treatment. Copper-containing bodies in the liver of eider ducks have also been compared with conditions in Wilson's disease (Norheim and Borch-Iohnsen, 1990b).

Recent discussions of case histories include Davies et al. (1989), Dening and Berrios (1989) and Enomoto et al. (1989). Dening and Berrios (1989) discuss variability in expression of the disease. Some of the variability can make diagnosis difficult, especially in the early stages (e.g. Crumley, 1990) although urinary copper excretion is a diagnostic feature. Identification of copper-sulfur complexes in the liver is also considered important in the diagnosis and management of patients with Wilson's disease (Hishida et al., 1989). Although often considered to have a fatal outcome, the use of a decopperizing agent (D-penicillamine or triethylene tetramine hydrochloride) or zinc supplementation, as well as proper short- and long-term care does allow survival (Bachmann et al., 1989; Chehter et al., 1989; Dubois et al., 1990; Hartard et al., 1990; Marrella et al., 1989). McArdle et al. (1990) suggest that D-penicillamine acts by removing copper from some intermediary ligand, making it available to increase the levels of metallothionein. The use of zinc is to reduce copper uptake across the gut, either by metal-metal competition or the zinc induction of metallothionein with resultant chelation of copper (e.g. Brewer and Yuzbasiyan-Gurkan, 1989; Brewer et al., 1989, 1990; Lee et al., 1989; see also Dastych, 1990b). Work with zinc in both normal and Wilson's disease patients suggests that zinc metabolism is normal in both cases even though copper metabolism is perturbed in those with Wilson's disease (Lee et al., 1990c).

Miscelleneous

Copper deficiency and excess has been associated with changes in blood cells and platelets (e.g. Caffrey et al., 1990; Johnson and Dufault, 1989) as well as the concentration of certain blood plasma components (e.g. Bhathena et al., 1988; Shuler et al., 1990; Wegger, 1988). It has also bee associated with changes in hormones (Kozlowski et al., 1990a; Prohaska et al., 1990; Sovago et al., 1990). Hassan et al. (1990) report the association of copper with certain amino acids in patients with thyrometabolic diseases. A number of divalent metals, including copper, have been examined for their effects on the kidney and kidney elements (e.g. Templeton and Chaitu, 1990) and copper deficiency has been related to changes in renal function (Noordewier and Saari, 1990). In the liver, calcium is important but its excretion can be affected by a range of metals, including copper (Yamaguchi and Uematsu, 1990).

I.3.8. THE INTERACTION OF COPPER WITH ORGANICS

The evolution of life on Earth appears to have involved minerals of prebiotic nature combined with photochemical reactions (e.g. Kuma et al., 1989). Copper appears to have played an important role in the evolution of life because of its ability to react with both inorganics and organics (e.g. Boichenko, 1987 - in 1990 ICA review). As a result of its biological importance and application to the requirements and needs of mankind, there has been continued interest in the coordination chemistry of copper and other metals with biological molecules (Aplincourt et al., 1990; Kawakishi and Uchida, 1990; Kawakishi et al., 1990; Kozlowski et al., 1990c; Mignani et al., 1990; Suzuki and Karasawa, 1990; Taniguchi et al., 1990). Copper-mediated chemical reactions with organics are of major interest not only in the field of chemistry (e.g. Capdevielle et al., 1990; Hara et al., 1990; Maichle et al., 1990; Paatero et al., 1990; Ryden and Hunt, 1990; Temerk et al., 1990; Todd et al., 1990; Weber, 1990) but also in medicine and nutrition (see for example Elkins, 1989; Hutchens and Yip, 1990a,b; Kovacic et al., 1989, 1990c; Lovstad, 1990; Reinaud et al., 1990). It becomes apparent that, witbh organics, the applications of chemistry to knowledge about copper and copper knowledge to chemistry are of importance to industry, agriculture and medicine as well as to academia (e.g. Botros and Vijayalakshmi, 1989; Dehnad and Förstner, 1989; Scrimin et al., 1989). It also becomes apparent that the uptake and utilization of copper by plants can be affected by organics such as herbicides (Robson and Snoball, 1990) or even plant hormones (Gurevich and Yakushkina, 1989). In a study of heliotrope alkaloids and copper, Howell et al. (1988) obtained evidence that the combination of heliotrope and excess copper can be more damaging than either alone. Synergy and antagonism is not uncommon with organics and copper or other metals and copper and can affect copper-organic interactions in organisms (e.g. Bienvenu and Kergonou, 1990).

Organisms produce organometallic compounds for a variety of purposes. Some of these are used for immediate metabolic requirements (e.g. enzymes) while others (e.g. antibiotics, metal-transporting agents, pheromones; Demain, 1989) are used for a variety of other functions. Some of the byproducts of bacterial metabolism serve as metal-binding polymers and can accumulate on copper pipes or on copper-bottom paint and sheathing (Geesey, 1987; Geesey and Jang, 1990). Exposure to elevated levels of metals can often initiate or increase the production of these agents. Cochrane et al. (1991) notes, for example, that exposure of a rotifer (*Brachionus plicatilis*) to sublethal doses of $CuSO_4$ caused a 4-5-fold increase in a protein (58,000 Da). Byproducts of metabolism can affect metal speciation (e.g. Cai et al., 1989) and be involved in the control of metal bioavailability either directly, as in siderophores, or indirectly, as a result of degradation of organic matter. These agents are found in both aquatic (e.g. Chen et al., 1988a) and terrestrial environments. Differences in the organic composition of foods can also affect metal bioavailability (e.g. Brätter et al., 1988).

Copper and organics in transport systems

Copper reacts with peptides and proteins associated with transport or transport systems in both plants (e.g. Zolotukhina et al., 1989) and animals (e.g. Matsushita et al., 1989; Morgan et al., 1989). In a number of invertebrate animals it is an integral part of the blood respiratory pigment haemocyanin. Along with the iron-containing respiratory pigment haemoglobin, it is found in many although not all crustaceans (Waite and Walker, 1988). The nature and activity of haemocyanin continues to be of interest as shown by the recent work of Ma et al. (1990) on horseshoe crabs. Work by Arumugam (1989) suggests that in addition to a respiratory function, haemocyanin as well as some other copper-binding proteins of crabs may regulate copper concentrations in the haemolymph during periods of stress. Rainbow and Abdennour (189) found that changes in total body copper concentrations were related to changes in haemocyanin that occurred during growth in a mesopelagic crustacean.

In vertebrates and humans, copper in serum or plasma is mainly bound to the proteins caeruloplasmin, albumin and transcuprein (Lyon and Fell, 1990). Suzuki et al. (1990f) report that cupric ions injected intravenously into rats were preferentially bound to albumin. Caeruloplasmin appears to mediate the transfer of copper from the plasma into the cell (Harris and Percival, 1989), possibly through receptors whose frequency may be tissue-specific (Forrester et al., 1990). The activity of ceruloplasmin is of interest because of its oxygen-transporting capability (e.g. Winyard et al., 1989). Activity can be be affected by a number of factors, possibly including excessive dietary iron (Pellett et al., 1990).

Ascorbic acid can affect the concentration of serum mineral elements, including copper (e.g. Tsao et al., 1990). Total body copper levels can, for example, be reduced by ascorbic acid supplementation of copper-adequate rats (Van den Berg et al., 1990b). Stack et al. (1990), however, note little effect on copper absorption of ascorbic acid supplementation with low-birth-weight infants. The relationship between copper and ascorbic acid is complex, the organic being able to oxidize proteins, especially in the presence of excess copper; the metal being able to catalyze oxidation of ascorbate at low concentrations but the organic serving as an antioxidant at high concentrations (Varma et al., 1990). The rate of oxidation can, however, be reduced by certain organic compounds (e.g. Smith and Gore, 1990). Yu et al. (1990) note an ascorbate-enhanced copper toxicity on bovine corneal endothelial cells.

Nucleic acids

Copper is able to react with nucleic acids (e.g. Blagoi et al., 1991; Chikvaidze and Gvritishvili, 1990). Some of the reactions are considered in the review of "Metal-DNA Chemistry" edited by Tullius (1989) and in papers appearing in "Water and Ions in Biomolecular Systems", the results of a UNESCO conference (Vasilescu et al., 1990). Others are considered in the variety of papers on coordination chemistry, function and roles of nucleic acids (e.g. Bütje and Nakamoto, 1990; Prütz et al., 1990; Tajmir-Riahi, 1990). Copper can, for example, modify the effect of ionizing radiation on components of nucleic acids (Bhattacharyya et al., 1989; Frenkel and Tofigh, 1989; Portugal, 1989). Ozawa et al. (1988) suggest that copper(II)-peptide or -nucleotide complexes can yield OH radicals which can competitively react with deoxyribose and peptides or nucleotides. Tachon (1989) notes that the presence of copper causes an increase in single-strand breakage of DNA by hydrogen peroxide (see also Kobayashi et al., 1990). The amount of breakage with copper and hydrogen peroxide can be modulated by histidine (Tachon, 1990). Breakage can also be effected by organics in combination with copper (Inoue et al., 1990; Kawanishi and Yamamoto, 1991; Kawanishi et al., 1989a; Lickl et al., 1989; Rahman et al., 1989a; Rao and Pandya, 1989; Yamamoto and Kawanishi, 1991a,b). The effects or actions of copper can frequently but not always (e.g. Piccinini et al., 1990) be offset by reactions with copper-chelating agents (e.g. Coloso et al., 1990) or other complexing agents, including carbohydrates (Nagy et al., 1990; Wapnir and Balkman, 1990a). In addition, the effects of copper, whether with nucleic acids or metabolism, can be influenced by organics which change uptake and utilization of the metal (e.g. Lewis et al., 1990a,b; Oberleas and Chan, 1990; Strain and Lynch, 1990; Wood and Stoll, 1990).

Metallothionein-like organics

Metal buffering systems occur in organisms, to play important roles in metal homeostasis and often in metal transport (e.g. Iwai, 1988). The nature of the buffering system for copper varies amongst major groups of organisms but still serves the same functions whether it is metallothionein itself (e.g. Abdel-Mageed and Oehme, 1990a) or some of the peptides found in plants (e.g. Steffens, 1990). Several reviews of the nature and role of these organics have appeared in the recent literature (general - Reddy and Prasad, 1990; plants - Grill et al., 1990; Rauser, 1990; Steffens, 1990; animals - Bremner and Beattie, 1990).

In recent literature, evidence for metal buffering capability, in one form or another, has been reported for bacteria (e.g. Diels and Mergeay, 1989; Harwood-Sears and Gordon, 1990), fungi and lichens (Nishiyama et al., 1990; Purvis et al., 1990), plants (e.g. Nishizono et al., 1988; Palma et al., 1990; van't Riet et al., 1987; Wadey, 1988) and animals (e.g. Acey et al., 1989; Baer and Thomas, 1991; Cosson, 1989; Engel and Brouwer, 1989; Hogstrand et al., 1990; McCormick and Lin, 1988; Nott and Nicolaidou, 1989; Petering et al., 1988). Literature dealing with the nature and production of phytochelatins includes Matsumoto et al. (1990), Mendum et al. (1990). Characterization of metallothionein in animals and humans continues to be an important topic (e.g. Mulder et al., 1990), a topic which must be related to the nature and function of other coper-binding proteins (e.g. Schilsky et al., 1989b).

Hogstrand et al. (1990) note that metallothionein and hepatic levels of copper and zinc in perch reflected the water concentration of copper and zinc near a brassworks in Sweden. Dutton and Majewski (1988) report a correlation of hepatic metallothionein and hepatic metal burdens in fish near a metal smelter in Manitoba (Canada). The relationship between metals and metallothionein is also reported for two subtropical fish species (Hogstrand and Haux, 1990) although results with a third species suggests that high concentrations of hepatic metallothionein, zinc and copper can occur in fish from an uncontaminated location. Copper tolerance has been linked to metallothionein levels (e.g. Elmes et al., 1988b). Suzuki et al. (1989b) provide evidence suggesting that, in rats at least, the uptake of copper by the liver depends on the induction of metallothionein synthesis. Stillman et al. (1989) developed a mechanism for measuring copper-metallothionein levels in tissues and suggest that it will be useful in evaluating patients with impaired copper metabolism. This will provide some alternatives to present situations since problems do exist in evaluating the status of genetically-related copper imbalance (e.g. Elmes et al., 1988a; Evering et al., 1990; Waldrop and Ettinger, 1990b). Changes in metallothionein levels also occur during development which can affect the trace-metal status of organisms (Mercer et al., 1988; Paynter et al., 1990; Suzuki et al., 1990d) and may mimic some of the problems found in inherited disorders of copper metabolism.

Although the expression of metallothionein-effect is ultimately controlled by the genetic makeup of the organism (Zafarullah et al., 1989, 1990), the relationship between metallothionein and metals, within the organism, is affected by the "chemistry" of the organism (e.g. Xia et al., 1990). This includes the effects of hormones (DiSilvestro, 1988b), the effects of pH (Nederbragt et al., 1988a; Xia et al., 1990), and the effects of other metals (e.g. Lee et al., 1989; Suzuki et al., 1990c). In principle, whether it is with the ameliorative effects of copper on the side effects of tumour drugs (e.g. Reeves, 1990) or excess metals, the role of metallothionein is moderated by the chemistry of the organism in which it operates.

Copper in lipids

Lipids are found throughout the body and in human and animal food materials. There is a wide variety of lipids which play a number of roles in normal as well as abnormal metabolism. Copper is found in association with lipids ranging from lipid material in the hair (Attar et al., 1990) to body fat. In growing pigs, copper nutriture can affect the serum concentration of certain fat-soluble oily phenolic compounds (alpha tocopherols) derived from food materials (Dove and Ewen, 1990). Copper deficiency in laboratory rats has also been associated with alterations in plasma lipoprotein composition (Al-Othman et al., 1990). Cholesterol is a group of steroid alcohols derived from lipids and, with some forms of cholesterol, associated with atherosclerosis. Copper deficiency is reported to stimulate cholesterol synthesis from high-density lipoproteins, in the liver (Carr, 1989; Carr and Lei, 1990; Koo et al., 1990). The uptake of lipoproteins during copper deficiency, and subsequent conversion to cholesterol is of obvious concern and has formed the basis for a number of recent studies (e.g. Hassel et al., 1990; Zhang and Lei, 1990). So also has the interaction of copper and fats and fat components (e.g. Carter et al., 1990; Cunnane, 1989; Knight and Voorhees, 1990b; Lenz et al., 1990; Ohta et al., 1989; Parthasarathy et al., 1990; Silverman et al., 1990a). As usual, the story is often not simple and straight forward. Peterson et al. (1990), for example, present evidence of an interactive effect of sucrose and copper deficiency in elevating serum cholesterol.

Organic copper complexing agents in the environment

Copper complexes with organics account for an appreciable amount of the total dissolved copper and can affect metal bioavailability (e.g. Hansen et al., 1990; Hiraide et al., 1989). These organics include both natural and anthropogenic materials and can range from vitamins (Abo El Maali et al., 1989) to degradation products of plants. Depending on the definition of "dissolved", they may also include colloidal organic matter (e.g. Sigleo and Means, 1990). Lapin and Yedigarova (1990) review the interaction of exudates (exometabolites) of aquatic organisms with heavy metal ions. They provide a list of the major groups of organics found in algal exudates, a list that includes carbohydrates, organic acids, lipids, polypeptides, protein-like substances, free amino acids and phenols. In a study of copper-complexing exudates from the the marine diatom *Chaetoceros muelleri* (a member of the phytoplankton), Ning et al. (1990a) note that the greatest production of exudates occurs after the major growth phase, when the cells are limited by environmental parameters. Robinson and Brown (1991) measured significant amounts (10-30 X 10^{-8} M) of copper complexation in material released by another phytoplankton organism, the dinoflagellate *Gymnodinium sanguineum*, during an extended bloom. Similar results have been obtained in large culture chambers (mesocosm) by Slauenwhite and Wangersky (1991). However, the importance of these exudates in natural environments is open to debate. Slauenwhite et al. (1991), note little change in the concentration of complexed copper during a spring phytoplankton bloom in Bedford Basin (Halifax, Canada). In a Ph.D. thesis, Hering (1988) notes obvious metal complexation in the laboratory but difficulty in demonstrating this in the field. The amount of exometabolites produced and released can be appreciable, with algal-polysaccharides enough to be considered for the control of excess anthropogenic metal under certain conditions (Jang et al., 1990a,b; Kaplan et al., 1988). Biocorrosion of copper is also noted for alginic acid polysaccharides (Jolley et al., 1989). This information suggests that even though the importance of the exudates is open to debate, the binding of metals does occur in nature.

In soils, metal concentrations and bioavailability are both affected by organic material, particularly organic degradation products (e.g. Il'in, 1988; Senesi and Sposito, 1989; Senesi et al., 1989a). Changes in metals and metal bioavailability are apparent as a result of changes in pH (e.g. Senesi & Sposito, 1989; Singh, 1989b), metal concentrations as well as availability and the possible effect of plant metabolites acting as metal-complexing agents (Linehan et al., 1989). Feedback mechanisms exist where, for example, changes in copper and copper bioavailability affect the concentration and composition of metal-complexing agents in the plant (e.g. Slusarczyk amd Ruszkowska. 1986) which affect the concentration of bioavailable metal either within or external to the organism. Water soluble organic substances such as fulvic acids are important copper complexing agents (e.g. Strnad, 1987). Since they are water soluble they can, for example, act as transport materials for complexed metals (Elpat'ievskiy and Lutsenko, 1990), especially with soil acidification (Berggren et al., 1990). However, Fischer (1987) notes no significant influence of increasing soil pH on the stability of copper complexes.

Detrimental effects of excess copper can be ameliorated by the reduction of metal availability with either synthetic or natural complexing agents (Ademoroti, 1990; Gupta, 1990; Lüderitz et al., 1989c; Mackey and O'Sullivan, 1990; Manahan, 1989; Maslennikov, 1989; Shanmukhappa and Neelakantan, 1990; Versteegh et al., 1989). However, some of the metal-complexing agents are themselves detrimental (e.g. Gershon et al., 1989). Eutrophication may also modify the bioavailability of copper as a result of the increased organic loading (Tracey, 1990). McCarthy (1989) reviews the effect of humic substances on the availability and toxicity of organic and inorganic contaminants in aquatic environments. Although Perdue (1989) and McCarthy (1989) discuss the general effects of humics on metal speciation, both point out that the complex interactions with various environmental components makes generalizations difficult although metal complexation will affect metal bioavailability. Other recent papers discussing characteristics of the complexation of copper with humic-like substances include Giesy and Alberts (1989), Buffle et al. (1990), Li et al. (1988a), Nor and Cheng (1989) and Susetyo et al.

(1990). The interactions of copper and complexing agents in the laboratory and in the field have been studied with a variety of techniques (Ephraim and Marinsky, 1990; Lund et al., 1990; Midorikawa et al., 1990; Ryan et al., 1990; Sun et al., 1990b; van Den Berg et al., 1990a; Ventry et al., 1990; Zhang et al., 1990a).

The use of information on trace metal-organic interactions in agriculture includes evaluation of the effect of fertilizers on tissue copper levels in crops (e.g. Warman, 1990). Information of this general type can also be of importance in evaluating the effect of toxic agents such as paraquat (Sion et al., 1989) as well as naturally-occurring toxins (e.g. Davis et al., 1990; Nunn et al., 1989). The application of information on metal-organic interactions can also be made to the properties of sewage and sewage sludges (Senesi et al., 1989b) and the effects they may have on reclaiming copper mine spoils (Sabey et al., 1990) or the effect on plant tissue metal levels when used as a fertilizing agent. Coal carbonization wastewater has been demonstrated to be an effective complexing agent for copper (Pandey and Kumar, 1990). A final use is that of lignin, from pulp and paper waste, for metal removal of metal in industrial effluents (Varma et al., 1989). However, use of these agents must be dictated by environmental conditions which affect both metal complexation and bioavailability (e.g. Manahan, 1989). The influence of major electrolytes and pH on flocculation of trace metals and humic acid in seawater (Li et al., 1988a) can, for example, be either detrimental or benefical to the use of humic acids in effluent treatment. Varma et al. (1990) note that the optimum pH for maximum metal removal by lignin, from industrial effluent, is 5.5 for copper and 6.5 for cadmium.

I.3.9. THE EFFECTS OF COPPER ON GROWTH

The growth of all living organisms requires copper and yet excess copper can limit growth. However, limitation depends on the biological availability of the copper that is present as well as the physiological nature of the organism. Organisms vary in their requirements for metals and in their tolerance to excess available metals. As an example, the sensitivity to excess metal can play an important role in the soil microbial community (Domsch, 1989). However, variation in the requirements for and tolerance of copper can be found not only between different types of organisms but also between different phases in the growth of a single organism. There is often sufficient variability in test data to make generalizations difficult, about the effects of excess copper. This is a topic that has been addressed by Conquest and Taub (1989) for bioassay data from tests using the "Standardized Aquatic Microcosm".

Several trace metals (B, Cu, Zn) have been reported to improve development and germination of a blue-green alga (Yumnam and Reddy, 1989). In contrast, excess copper can reduce the growth of algae (e.g. Nyholm, 1990; Zhang et al., 1990b) as well as terretrial plants (e.g. De Vos, 1991). This makes copper a widely-used agent in controlling the growth of fouling algae (e.g. French and Evans, 1988). Synergistic effects have been noted between copper and other metals, that cause reduction in growth (e.g. Lasheen et al., 1990). French and Evans (1988) report a synergistic effect between zinc and copper that they suggest could be used to produce more effective antifouling coatings.

Uptake of metals by plants is associated with metal bioavailability (e.g. Bell et al., 1991). Transport and placement within the organism is dependent on the physiology of the organism. In an examination of mineral contents of soybean seed coats and embryos, Laszlo (1990) found an increase in seed coat Cu and Mn followed by a decrease with an increase in embryo content. He suggests that this, and results with other metals, is due to ion-specific events. The similarity between Cu and Mn is interesting because these two metals appear to compete for uptake sites. Results with Cu and Fe (Nickless et al., 1989a) suggest the possibility of these two metals competing for the same uptake site. An understanding of the metal requirements of plants is essential to optimal plant development (e.g. McFarlane, 1989b). It is also important to understand the properties of the soil, or other growth medium, since they dictate many of the factors controlling bioavailability (e.g. Sánchez et al., 1989). Combinations of these factors often enter into the evaluation of effects of trace element-containing fertilizers (e.g. Saur, 1989a, 1990b). Consideration of these factors has also been made in designing copper-containing equipment such as growth containers (Struve and Rhodus, 1990).

In invertebrates, growth and reproduction can be influenced by excess bioavailable copper. Beneficial use of this has been made in a number of parasites and parasite carriers or vectors. Marva et al. (1989), for example, note that copper interfers with the growth of a malaria-causing protozoan (*Plasmodium falciparum*). They also note a synergistic effect of ascorbate and copper, a result of the pro-oxidant nature of the ascorbate. Although detrimental to the growth of benthic organisms (e.g. Piraino and de Nicola, 1990), elevated concentrations of copper can be used to reduce fouling by the same organisms. Knowledge of metal availability and growth response of the organism can be of use in evaluating the effects of anthropogenic metal either by itself (e.g. van Gestel et al., 1989) or in combination with other agents (e.g. Comber et al., 1989; Misitano and Schiewe, 1990; Widdows et al., 1990). Mani and Thontadarya (1988), for example, report no effect of commonly-used fungicides (including copper oxychloride and Bordeaux mixture) on the developmental stages of an insect used to control the grape mealybug.

Copper is frequently stored in the developing invertebrate, for use in various enzyme systems and in the copper-containing blood pigment haemocyanin, when it is present (e.g. Paulij et al., 1990; Yamamoto et al., 1990b). In contrast, other invertebrates maintain relatively constant body levels throughout development (e.g. Timmermans and Walker, 1989). Embryonic development can be affected at elevated copper concentrations. Paulij et al. (1990) note that cuttlefish (*Sepia officinalis*) embryos hatched earlier and had lower survival potential at high copper concentrations (50-200 ppb Cu^{2+}), when compared with control embryos. With the brine shrimp, embryonic development appears to be normal at 63 ppb Cu but is arrested at 6.3 ppm Cu (Acey et al., 1990). Comparable effects of copper have been noted for fish. Metal-induced (including copper) retardation or cessation of embryonic development has, for example, been reported by van Coillie et al. (1989) and Wu et al. (1990c) and in. Developmental changes in the nucleic acids responsible for metallothionein production have been attributed to metal reglation in the rainbow trout (*Onchorynchus mykiss*) by Olsson et al., 1990. Changes in tissue copper concentrations have also been found with growth in cultured yellowtail although the nature of the changes tend to be tissue-specific (Takahashi, 1990a).

The accumulation of an adequate supply of copper by domestic animals is important to their well being, particularly during embryonic and postembryonic development. Uptake, however, can be affected by interactions with molybdenum and sulfur (Grace et al., 1988). During both foetal and early neonatal life sheep, and other mammals like the deer, have elevated concentrations of copper in their liver (Leighton et al., 1990; Mercer et al., 1988). Metallothionein and zinc levels are also elevated in the foetal liver of sheep and the copper is associated with the metallothionein. Paynter et al. (1990) conclude that the major function of the metallothionein (abstract) "... in the foetal liver is protection of the liver against the potentially toxic accumulation of zinc". (This is also suggested for fetal rat small intestinal mucosal cells, by Lim and Gordon, 1990.) An imbalance of copper, and the factors affecting copper uptake and utilization, can cause ill health in domestic animals. This is particularly true in young animals, a situation which has sponsored a good deal of research on mineral composition and metal balances and imbalances in food and tissues of brood stock (e.g. Van Kiekerk and Van Niekerk, 1989c,d) and young animals (e.g. Purdy et al., 1990; Shetty et al., 1990; Stansbury et al., 1990; Suttle et al., 1988a). Much of this work is justified on the basis of use of animal tissues and products as food sources for humans (e.g. Park and Chukwu, 1989).

Age-related changes in tissue copper concentrations have been reported in laboratory animals (Palm et al., 1988, 1990; Uchino et al., 1990). This is expressed not only as changes in total copper but also changes in the activity of certain copper-containing enzymes, such as Cu, Zn-superoxide dismutase (Mariucci et al., 1990). Since changes do occur, it implies required changes in copper nutriture. An understanding of the effects of copper deficiency during early development of laboratory animals has thus been an important area of research (e.g. Prohaska, 1990b,c) as has an understanding of the effects of nutrients, alcohol and drugs on copper utilization (e.g. Baek, 1988; Fields et al., 1990). Metal transport from mother to fetus plays a role in this (e.g. Lee et al., 1990d), as suggested by most groups working in the field. Information from these studies has been used to indicate potential effects of copper deficiency in prenatal and neonatal development in humans.

Changes in copper levels have also been reported in humans during pregnancy (Fu et al., 1990) and, in fact, throughout life. As with laboratory animals, these can often be seen as changes in copper-containing enzymes or copper-transport, copper-buffering agents (Fuller et al., 1990). Copper values are reported to be lower in neonate (umbilical cord blood) than maternal serum (Kapu et al., 1989; Okonofua et al., 1990). Mbofung and Subbarau (1990) report a relationship between placental copper levels and birth weight, suggesting that (abstract) "... the concentration of ... zinc and copper in the human placenta may have an influence on the outcome of pregnancy". A great deal of importance is placed on trace element nutriture, especially in early life (Curtis, 1990; Dörner et al., 1989; Kakker and Kapoor, 1989; Lönnerdal, 1988; Milner, 1990) and in the elderly (Mertz, 1989).

I.3.10 COPPER AND ORGANISM BEHAVIOUR

The response of an organism towards a chemical condition is a result of the physiological/biochemical effect of the chemical. However, the actual response can be a change in either the physiological/biochemical nature of the organism or its behaviour. A change in colour of lichens as a result of copper complexation (e.g. Purvis et al., 1990) is an example of a physiological/biochemical change. In contrast, a sudden change in the swimming response of a fish exposed to copper is an example of a behavioural response although it may also be physiological, a result of nerve-muscle stimulii. With fish, excess copper has been reported to produce changes in respiration, movement, conditioned reflex behaviour and feeding/foraging response (Alkahem, 1989; Sandhenirich and Atchison, 1989; Simonaviciene, 1989a,b; Steele et al., 1987, 1990). Avoidance response to copper has also been reported (Svetsyavichyus, 1989). Increased swimming activity was noted with green turtles when treated with copper sulfate to eliminate leech parasites (Choy et al., 1989).

Changes in behaviour have been used as an assay to determine water quality. Evans and Samosir (1990) noted that excess copper inhibited the to food-containing agar blocks by a marine ciliate, in a manner comparable with a growth bioassay using the same organism. Based on work with copper, Simonaviciene (1988) advocates the response to food by goldfish as a mechanism to evaluate the quality of the aquatic environment. Using rainbow trout exposed to chromium and copper compounds, Anestis and Neufeld (1988) present evidence that avoidance-preference responses can be used to screen detrimental agents and establish water quality standards. Welch et al. (1989) used tilapia (an economically important fish) exposed to copper at various temperatures to evaluate the sensitivity of temperature preference tests. The suggest that (abstract) "... based on the results of this study, temperature preference tests can be used for establishing minimum acceptable toxicant levels".

I.3.11 COPPER AND THE STRUCTURE OF ORGANISM COMMUNITIES

The term community, when applied to organisms, means an assemblage of populations living in a prescribed area or physical habitat. The species composition or structure of a community can be affected by deficient or excess amounts of biologically available copper as well as by the interactions which occur between the organisms within a community. Although the nature of the community must be examined if reactions to deficiencies or excesses of metal are concerned, many organisms have some ability to "adapt" to changing conditions (e.g. Domsch, 1989). Even with this, however, detrimental effects of anthropogenic metal (including copper) have been reported for communities in soils (Domsch, 1989), freshwater wetlands (Crowder et al., 1988), lakes (Yan and Miller, 1984) and saltwater (e.g. Di Geronimo, 1990; Sandulli and de Nicola-Giudici, 1990; Steimle et al., 1990b; Warwick et al.). A variety of papers discuss natural communities (e.g. Qin et al., 1989) as well as those affected by anthropogenic metal, including the effects of sewage sludge (e.g. Glockemann and Larink, 1989) and urban runoff (e.g. Mesuere and Fish, 1989).

The use of insecticides and copper-containing fungicides intentionally changes the community structure in an affected area (e.g. Marchesini and Gambaro, 1989). Metalliferous soils such as mine-spoil heaps contain unique communities (e.g. Garcia and Clark, 1989) made up of species tolerant to high metal bioavailability. Changes in metal availability are often followed by changes in species composition whether on land or in aquatic systems. Deniseger et al. (1990) evaluated the effects of decreasing metal conentrations in a lake after changes were made in tailings disposal from a copper-lead-zinc mine. They comment on the appearance of a phytoplankton community first of a very few species then gradually changing to greater species diversity, in both phytoplankton and zooplankton. Changes in metal availability have also been related to organic matter, changes that may affect the time of appearance of phytoplankton red tide blooms (Arzul and Gentien, 1990).

The effect of copper on a community can differentially affect predator and prey (e.g. Doucet and Maly, 1990) or host and parasite (e.g. Marva et al., 1989) or host and symbiont (Kreuzer and Kirchgessner, 1990a). In the latter case, this may have an indirect effect on the host if the symbiont participates in food digestion. As well, a community of organisms contains members which can affect metal bioavailability by their exudates. This can produce an indirect interaction between species, reducing the detrimental effects of excess copper (Metaxas and Lewis, 1991b). The early stages of microfouling of copper-nickel alloys or copper-containing antifouling paint may also do this, the tolerant initial stages such as certain bacteria (e.g. Gomez de Saravia et al., 1989) reducing the availability of copper to subsequent colonization stages. In metal-containing soils, certain fungi may reduce the availability of copper, providing an apparent "tolerance" for other organisms (e.g. Griffioen and Ernst. 1989). "Tolerance" may also be a laboratory-related term, growth of large laboratory populations under metal-supplemented laboratory conditions may have little ecological significance for sparse populations in natural environments (Duxbury and McIntyre, 1989). Moore and Winner (1989) provide evidence from which they conclude that it is difficult to predict community responses in the field from single-species tests in the laboratory. Even community-level toxicity tests in the laboratory may have only limited, if any, application to the field as a result of field conditions (Clements et al., 1990) and the adaptation and resistance of ecosystems to stress (Cairns and Niederlehner, 1989). Misra et al. (1989b) found that, with comtaminant concentrations in two species of fish (Atlantic cod and European flounder), there was a difference in response to changes in contaminant levels with time between the species as well as within each species. Lifshits and Korsak (1989), however, suggest on the basis of work done with with copper, that (abstract) "The adaptative potentials of hydrobiont communities in various zones of the oceans can be compared in experiments involving exposure to negative factors".

I.3.12 COPPER IN FOOD AND FOOD CHAINS

As an essential trace element, copper is obtained either from the medium in which the organism lives or the food which it ingests, or from both. Nutritional regulation of an organism thus becomes important in terms of trace elements (e.g. Chandra, 1989) and their availability. Reviews of trace element sources, requirements and metabolism include those of Shenkin (1988, 1989, 1990) while Aggett (1990) includes a discussion of copper in his review of "Malnutrition and Trace Element Metabolism". Factors associated with the uptake and metabolism of copper are discussed by Johnson (1989b) and include metal-metal interactions (e.g. Abdulla and Chmielnicka, 1990; O'Dell, 1989). Kies (1989a) was the editor of a volume entitled "Copper Bioavailability and Metabolism" which includes a number of articles on the requirements for copper. This section deals with copper in foods or as a nutrient in soils. The transport of copper after uptake becomes an important factor in the metabolism of copper (e.g. Harris and Percival, 1989) although it is more fully discussed in the section on metal uptake. References dealing with the roles of copper, for example in lipid metabolism (Lei, 1990a), are also discussed separtely, in appropriate sections of this review.

At least part of the chemical makeup of an organism can be directly or indirectly associated with trace metal effects. The composition of saccharides in oat plants has, for example, been related to the supply of copper as well as the time of metal supplementation (Slusarczyk and Ruszkowska, 1986). Feeding responses of certain fish have been reported to be affected by excess copper (Steele et al., 1987) as have taste responses in gerbils (Somenarain and Jakinovich, 1990). However metal availability is important, a factor which must be considered in evaluating the effect of anthropogenic materials (e.g. Gries and Garbe, 1989; van Haren et al., 1990). In filter-feeding invertebrates, the chemical nature of the different food particles can vary, thereby changing not only total metal intake but also metal availability (Amiard-Triquet et al., 1989). Since many filter-feeding organisms also take up "dissolved" copper in the water (e.g. Amiard et al., 1989), the chemical nature of this fraction is also important (Han and Hung, 1990). Eutrophication can thus be important through modification of metal availability as well as metal concentration (e.g. Tracey, 1990). Uptake of copper from food is dependent on the nature of the digestive tract and digestive processes, subsequent accumulation occurs as a result of metabolic processes associated with transport, use and storage of the metal (Nott and Nicolaidou, 1989). Storage and loss of metal is, however, dependent on the nature of the organism as well as the concentration of metal available for uptake. Decreases in metal levels can occur in invertebrates such as barnacles if the organism is transferred from a contaminated to uncontaminated site (Powell and White, 1990). Exposure of terrestrial isopods to elevated levels of copper is followed by isolation of the metal in insoluble intracellular granules in the hepatopancreas (Hopkin, 1990). However, the presence of nickel in the diet is reported to reduce uptake and storage of copper in the digestive gland (hepatopancreas) (Alikhan and Storch, 1990). Storage of excess metal can also occur in the larval exuvium ("skin") of insects and lost when moulting occurs (Heliövaara and Väisänen, 1990a).

The use of copper in food of domestic animals is widespread and the measurement of copper levels in commercial and natural foods is common (e.g. Estrada Ruiz et al., 1988). Appropriate levels of copper are often associated with improved growth and reduced parasite burden. However, excess copper can be detrimental, altering enzyme activities (Jenkins and Kramer, 1989) and affecting survival under certain conditions. Copper deficiency in ruminants can be a severe problem, arising as a result of deficiencies in feedstuffs (e.g. McFarlane et al., 1990; Régius-Mocsényi et al., 1990a). As a result, copper is one of the elements evaluated both in feeds and animals (e.g. Millar et al., 1986). This includes the copper status of unique materials such as kraft pulp mill fiber waste which has been proposed as a feedstuff for beef cattle (Bilawchuk et al., 1989). Recent studies on the copper status of ruminants includes work on cattle (Kumagai et al., 1990a; Régius-Mocsényi, 1990), horses (Régius-Mocsényi, 1990), sheep (Régius-Mocsényi, 1990) and goats (Musalia et al, 1989). Much of this work considers the potential value or effects of trace metal supplementation. Work dealing more specifically with supplementation includes Norton et al. (1990) on Merino ewes and lambs, Dill et al. (1989) on steers and Ott and Asquith (1989) on growth and skeletal development of yearling horses. Supplementation can be accomplished in a variety of ways, including the use of oral oxidised copper wire (Booth et al., 1989; Wilson, 1989; Wood et al., 1989), soluble-glass boluses (Matsui et al., 1989; Millar et al., 1989a) and injectable copper (Dill et al., 1989). Oxidized wire has also been used to reduce parasite burdens in lambs (Bang et al., 1990b).

The effect of various feeds on forage intake (Harris et al., 1989) and trace metal status is also important (e.g. Steinbrecher et al., 1990). There is concern about the effect of anthropogenic metal on soil and fodders as well as its uptake by domestic animals (e.g. Kerr, 1990; Vrzgula et al., 1986). Metal bioavailability in feeds thus becomes an important factor (Ibrahim et al., 1990). This includes the interaction of copper with factors independent of metal speciation but which can still affect uptake and utilization. These are factors such as metals and metalloids (Madej and Radzanowska, 1988) as well as growth promoters (Tokosova, 1989). Howell et al. (1988) report that the ingestion of heliotrope (containing toxic pyrrolizidine alkaloids) by sheep can influence tissue concentrations of copper. Cattle on high molybdenum foods benefit with copper supplementation (Boila and Wittenberg, 1990; Wittenberg et al., 1990). This is as a result of the competition between the two metals. This can be at least partially corrected by copper fertilization of soils with high levels of molybdenum (e.g. Stark and Redente, 1990b).

Parker et al. (1989) discuss the various feed additives used with swine, commenting on the use of copper sulfate to provide the copper needed by the organism as well as to serve as an antibacterial agent. Ivan (1989) and Shurson et al. (1990) discuss the relationship between dietary copper levels and microbiological status. High levels of copper, chelated and as $CuSO_4$, have been reported to benefit feed utilization, growth and body composition in adult and young pigs (Astrup and Matre, 1987; Cromwell et al., 1983; Dove and Ewan, 1990; Irie, 1990; Shurson et al., 1988; Stansbury et al., 1990). Cromwell et al. (1989), however, suggest that copper in CuO is largely unavailable and inefective as a growth promotant for weanling pigs. There is some concern about potential interactions of various food types with copper uptake and metabolism (e.g. Schöne et al., 1990) as well as the metal-metal interaction that occurs with zinc. The latter is an apparent interference with copper metabolism even when dietary copper is not marginal (Klevay and Pond, 1990b). The use of high levels of copper increases liver copper concentrations (e.g. Ward et al., 1989a). High levels have also been suggested to increase the copper content of soils and sediments in areas where swine are raised (Arzul et al., 1990), through release of copper-containing fecal material. The potential effects of excess copper have been evaluated by various government agencies. In 1973, for example, the U.S. Food and Drug Administration proposed limitation of the use of copper in poultry and swine feeds. This was withdrawn in 1990 with the comment "... that the available data and information do not require restricting supplemental levels of copper salts in swine and poultry feeds to 15 ppm" (page 10468 in Hoeting, 1990). The further consideration was that the use of copper in animal feeds for nutritional purposes can be regulated under an existing regulation (FDA 21 CFR 582.80).

The mineral composition of food materials for animals and humans is an important consideration whether it is the composition of pigeon milk (Shetty et al., 1990) or use of nutrient supplements (e.g. Qin Pei, 1991). Kies (1989b) discusses food sources and biological availability of dietary copper for humans while Lurie et al. (1989) use previously-published data to evaluate the copper content of foods. Other work discussing metal concentrations in human foods includes Augier et al., 1989, Ellen et al., 1990a, Holm et al. (1988), van Dorst et al. (1989)and Watanabe et al. (1988) to name a few. Copper values for various food materials are presented in tabular form later in this review. The foods examined in the recent literature are widespread, including both solids and liquids (e.g. Natesan and Ranganathan, 1990). Other work includes the effects of various materials on metal availability (e.g. Vinson et al., 1989; Yasui and Tanaka, 1989) as well as effects from alcohol (Frimpong and Louis-Charles, 1989).

As a result of the concern for adequate nutritional status for young humans, a good deal of work has been devoted to the concentration and availability of metals in human and cow's milk. Coni et al. (1990b), for example, provides reference values for essential and toxic elements in human milk. The mean value for copper is given as 0.43 mg/kg human milk based on data from four population groups each with 9 subjects. (Minimum and maximum concentrations were 0.08 and 1.00 mg/kg.) These values compare with a mean value of 0.39 mg/kg reported by Cumming and Fardy (1988) from an Australian study. Goat milk is widely used as a food material, with a mean concentration of 1.41 mg/kg (ppm) copper reported by Park and Chukwu (1989). Values appear to range widely, Chunk et al. (1989), for example, reporting less than 0.1 mg/kg (ppm) for raw milk samples in the vicinity of Seoul, Korea. Metal-fortified (Zn, Fe, Cu) milk is reported to be a mechanism for improving human nutritional status (Momcilovic et al., 1988). However, milk-based diets are suggested to induce copper deficiency under certain conditions, possibly due to casein and soluble milk proteins which reduce metal availability (Lynch et al., 1990). With young pigs fed a dried skim milk-based diet, Schoenemann et al. (1990a) indicated that the consequences of copper deficiency are not differentially influenced by the carbohydrate fraction.

Food handling and storage can be affected by excess copper. It can also affect trace metal concentrations in the food. Copper is reported to augment the nonenzymic browning of foods such as grapefruit juice (Kanner and Shapira, 1989; Rendleman and Inglett, 1990). An increase in the concentration of several metals, including copper, has been noted over a two year period, in canned juices (Arvanitoyannis, 1990a), canned vegetables (Arvanitoyannis, 1990c), and canned meat (Arvanitoyannis, 1990b). Decreases in copper-complexed lactoferrin have been reported in buffalo milk as a result of heating (Maheshwari and Bhati, 1990). Scholtissek and Barth (1989), however, report that heat treatment of milk does not affect copper bioavailability. Control of metal levels in food production has been of importance in the production of cheese (e.g. Fabricius, 1989) and spirits (e.g. Christoph, 1989; Girond et al., 1989). High levels of metals have also been reported to inhibit aerobic digestion of waste cheese whey (Cimino and Caristi, 1990). Fermentation of cassava to produce a traditional Nigerian food (Fufu) is reported to cause a reduction in copper levels (Oyewole and Odunfa (1989).

Concentrations of metal can be elevated in food materials as well as drinking water (e.g. Roggi et al., 1990) although any biological effect will be determined by metal speciation as well as metal concentration. Recent references dealing with heavy metals in drinking water are listed by the National Technical Information Service (1989f,g). Schintu et al. (1989) report the possibility that reservoir sediments serve as a potential source of heavy metals in drinking water. Madsen et al. (1990) discuss the potential for high copper content in drinking water and some of the risks which they believe are involved. Several techniques have been recently discussed to remove metals from food materials (e.g. Ivanov et al., 1990; Keurentjes et al., 1990). However, copper can be useful in the food industry. In an evaluation of the use of natural pigments to replace synthetic pigments used in foods Bobbio and Guedes (1990) found that copper chlorophyll was considerably more stable than magnesium chlorophyll. An emulsifier (phosphatidic acid) in food technology is reported to act as a carrier for biologically important metal ions such as copper (Rauch et al., 1989). Copper can also serve as a preservative for fresh produce (Ichise and Kuroda, 1989) as well as other natural products.

Understanding the interaction of copper with nutrients is important not only for an understanding of nutrient uptake and metabolism but also of copper metabolism in laboratory animals and humans (e.g. Askari et al., 1990b). Copper is reported to inhibit the absorption of D-galactose and L-phenylalanine, possibly because the metal binds to proteins forming part of the uptake mechanism (Rodríguez-Yoldi et al., 1989). However, the absorption and tissue distribution of copper can apparently be influenced by the dietary level and source of proteins (Brzozowska et al., 1989; Link et al., 1990) and possibly certain fatty acids (e.g. Fang, 1989) as can the ingestion of tin (Hight et al., 1990). Copper uptake and utilization can be reduced by dietary fiber (e.g. Amarowicz et al., 1989b; Kies and Umoren, 1989; Kim and Vanderstoep, 1989; Kincaid et al., 1990; Stachowiak and Gawecki, 1989; see also Anastasia et al., 1990). It has been reported that dietary macronutrient composition can affect copper deficiency, something that is refuted by the work of MacDonald et al. (1990). However, macronutrient composition may increase kidney copper levels in diabetic rats (Oster et al., 1990). Kies and Harms (1989) found a positive effect of calcium on copper utilization in humans, possibly because of its neutralizing effect on the high levels of ascorbic acid present in the diet. Ascorbic acid can interact with minerals in the digestive tract (Dabrowski and Köck, 1989) and, with fructose and sucrose, can interfere with copper utilization and exacerbate copper deficiency (Johnson, 1989a, 1990; Johnson and Murphy, 1988; Van den Berg et al., 1990b). Stack et al. (1990), however, report that absorption of copper was not altered by ascorbic acid in low birth-weight infants fed a cows' milk formula.

Copper can complex with carbohydrates (e.g. Angyal, 1990) and a number of recent papers address the possible effects of carbohydrates on copper uptake, flux, excretion and deficiency. Kreuzer and Kirchgessner (1990a) found only small effects of differences in dietary carbohydrate composition on trace element excretion in wethers. The type of dietary carbohydrate appears to play a major role in the expression and severity of copper deficiency (e.g. Lynch and Strain). Fructose exacerbates the effects of copper deficiency (Burns et al., 1990b; Cunnane et al., 1990; Fields and Lewis, 1990c; Koh, 1990; Lewis et al., 1990b; Scholfield et al., 1990). Exception to this is noted by Schoenemann et al. (1990b) for pigs although it is not supported by Scholfield et al. (1990) who also worked with pigs. Fields et al. (1989) report that during pregnancy and lactation, fructose consumption can affect the copper status of the offspring if there is already a tendency towards copper deficiency. There is also some sexual difference in the effects of fructose on the expression of copper deficiency, male rats being more prone than female to the effects of deficiency (Fields et al., 1988). In a Ph.D. thesis Lynch (1988) addressed the interaction of sex and dietary factors on copper deficiency in the rat. He suggests that there is a complex interaction of sex, age and diet. Vitamin status is important from a cause and effect status. Vitamin E has been used to moderate copper deficiency although Silverman et al. (1990a,b) note that it does not protect laboratory rats against the severity of copper deficiency induced by fructose consumption. Klevay (1990a) reports decreased vitamin D metabolites in the plasma of rats deficient in copper. Dulin et al. (1990), however, found no affect of copper status on vitamin A metabolism. Alcohol consumption has been suggested to aggravate copper deficiency under certain conditions (Fields and Lewi, 1990a,b). In contrast, beer increases the longevity of rats fed a diet deficient in copper (Klevay, 1988). Some effects of copper deficiency can be mediated by beer (Klevay and Moore, 1990) and garlic oil (Lure et al., 1990). This mixture, it would appear, should cure almost any ill!

The application of information on nutritional copper has been recently applied to both metal requirements and physiological condition. This includes copper uptake from various food materials under normal conditions (e.g. Shiraishi et al., 1989; Tamura et al., 1989a) and the copper requirements of laboratory animals and humans with respect to physiological stress (e.g. Reilly et al., 1990; Schwartz and Weiss, 1990). Information on copper nutrition has also been applied to work on total parenteral nutrition (e.g. Mansell et al., 1989; Yokoi et al., 1988, 1989) although this should also include evaluation of both biochemical and physiological factors (e.g. McCormick et al., 1989a). Higher serum copper levels have been associated with lower serum total cholesterol (Umoren, 1989). Copper deficiency has been reported in an infant with respiratory distress (giardiasis) fed with copper-poor cow's milk (Phillip et al., 1990).

Nutritional copper levels, requirements and uptake have been examined for laboratory animals and humans (Kallman, 1989; Turnlund, 1989; Turnlund et al., 1990; see review by Gibson, 1989 for humans). Other references concern these situations in men and women (Barberá et al., 1989; Ellen et al., 1990b; Iyengar, 1988b; Milne et al., 1988, 1990b), postpartum and never pregnant women (Moser-Veillon et al., 1990), infants (Allegrini et al., 1987; Curtis, 1990; Hambidge, 1989; Johnson and Canfield, 1989), children (Allegrini et al., 1989; Lönnerdal, 1988, 1989; Molner, 1990), athletes (Anderson et al., 1988) and adults and the elderly (Allegrini et al., 1987, 1988; August et al., 1989; Bogden et al., 1990). Mameesh et al. (1990) examine zinc and copper nutrition in terms of pregnancy outcome. Hambidge and Krebs (1989) discuss the upper limits of zinc, copper and manganes in infant formulas (200 µg Cu/100 kcal). Låg (1990) is the editor of a series of articles on excess and deficiency of trace elements in relation to human and animal health in arctic and subarctic regions. Grupe and Schutkowski (1989) used element concentrations in skeletal, mollusc and soil samples to evaluate dietary changes in prehistoric humans on the Oman peninsula. They relate elevated skeletal copper concentrations to the use of copper-rich molluscs as a major food source. In doing so they assume food-chain transfer without moderation by the consumer, something that is not widely accepted for copper (e.g. Campbell et al., 1988).

Food-chain transfer of metals continues to be controversial with continuing evaluation of effects at certain types of industrial sites (e.g. Vermeer and Castilla, 1989). The evidence accumulating about copper indicates that an increase of metal (biomagnification) does not occur at higher trophic levels. Rather, that there is metabolic control of copper concentrations by organisms at each trophic level (e.g. Timmermans et al., 1989). Although not with copper, Nott and Nicolaidou (1990a,b) provide evidence of the transfer of

metal detoxification along marine food chains. Mtimuni et al. (1990) could not find a significant relationship between mineral (including copper) concentrations in soils, plants and animal tissues in a study at Malawi. Lin and Li (1988) report elevated fecal copper concentrations in a small planktonic crustacean when fed copper-enriched algae, suggesting either selectivity in uptake or excretion of copper from the food. Kalfakakou and Kallistratos (1987) do report biomagnification of copper in the trophic chain of a lake in Greece. In an intertidal food web Timmermans et al. (1989) do note higher copper concentrations in deposit feeders than in filter feeders, higher copper levels in organisms that use hemocyanin as a blood pigment, and a negative concentration/body-size relationship in some invertebrates. Evidence of copper regulation is reported for a variety of organisms (e.g. Norheim and Borch-Iohnsen, 1990b).

I.3.13 ORGANISMS AS INDICATORS OF COPPER BIOAVAILABILITY

There is widespread use of organisms to monitor the bioavailability of copper and continued work on metal chemistry pertinent to the measurement of bioavailability. Monitoring is accomplished by evaluating tissue metal concentrations as well as organism growth and metabolism. It includes the use of metal-complexing agents such as phytochelatins (e.g. Hilpert et al., 1989). Recent reviews include Khristoforova (1989) who includes methods to assess the status of large algae and invertebrate animals impacted by heavy metals. Brinckman and Olson (1990) provide an overview of chemical techniques to monitor organometals of biological origin. Results from a U.S. national status program, on trends of chemical contamination in coastal and estuarine U.S. systems are discussed by O'Connor et al. (1989). McCain et al. (1989) discuss the results of the Pacific Coast portion of a U.S. National Benthic Surveillance Project which included evaluation of metal impact. Schmitt and Brumbaugh (1990) discuss results from the freshwater fish portion of the U.S. National Contaminant Biomonitoring Program for the period 1976-1984. For copper, they comment (page 739) that "None of the indicator statistics (...) for copper changed from 1980-81 to 1984, after a slight decline from 1978-79 to 1980-81 (...)". Studies of areas include Nuorteva (1990) on Finnish forest biocoenoses and Barr et al. (1990) on the Humber estuary (Britain). Gunkel (1989a) and Malins (1989) discuss the use of biomonitoring and Genjatulin (1990) and Lifshits and Korsak (1989) the use of mathematical models to evaluate contamination of aquatic systems. Johansen et al. (1991) review the biomonitoring program used at a lead-zinc mine in Greenland. Crowder et al. (1989) discuss the usefulness of several species as bioassays of metal contamination in sediments of the Bay of Quinte, Lake Ontario, Canada. Data analysis includes the use of principal component analysis and partial least squares regression (Piepponen and Lindström, 1989) as well as multivariate ecotoxicological mapping of organism-environment associations (e.g. Vogt, 1990).

A wide range of test methods is available for biomonitoring work (Poels et al., 1982). Cairns and Niederlehner (1989) discuss the capacity of ecosystems to adapt to anthropogenic stress, commenting on the use of microsystems for studies of environmental stress. The use of microcosms for bioassay work is discussed in Lasserre (1990), Meador (1988) and Niederlehner et al. (1990). Work on other types of systems includes Pilson (1990), Li et al. (1990a). Li et al. (1990a) note that in a marine enclosure, half-removal times for added copper is 16-29 days, a feature which must be considered in evaluating the effects of added copper. Some of the problems in ecosystem experiments are presented in Guanguo (1990). McNaught (1989) discusses the use of ingestion, reproduction and respiration in zooplankton as a functional bioassay for PCBs and heavy metals. Long et al. (1990) provide an evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. They found differences between tests and between organisms. Sediment composition and chronology has been used as a tool for environmental impact investigations (Battiston et al., 1989). In a Ph.D. thesis, Gutekunst (1988) reviews sewer slime analysis as a method of spotting heavy metal-bearing waste water discharge. An overview of automated biomonitoring is provided by Diamond et al. (1988), including discussion of organism sensitivity and the need for multiple species. The multispecies approach is described by Misra et al. (1989b) for fish in the general region of the North Sea.

As with all monitoring programs, techniques are of primary importance. Test accuracy is important, a factor that can be affected by the nature of the collections (e.g. Krumgalz et al., 1989). Intralaboratory precision of saltwater short-term chronic toxicity tests is discussed by Morrison et al. (1989). The development and use of reference materials for both chemical and biological assay work is essential for interlaboratory comparisons of metal concentrations and speciation (e.g. Daniel and Theiller, 1990; Holm, 1990; Miller-Ihli, 1989; Tamba et al., 1989).

A variety of organism types have been used in toxicity tests and monitoring programs to evaluate metal bioavailability. Microorganisms used include *Escherichia coli* (Jardim et al., 1990), *Aeromonas hydrophila* (Flemming and Trevors, 1989) and *Klebsiella pneumoniae* (Podbielski et al., 1989). Yeast respiratory activity has been used as a rapid test of metal stress (Haubenstricker et al., 1990b). Mosses continue to be widely used as indicators of aerosol metal effects (Grodzinska et al., 1990; Markert and Weckert, 1989; Pilegaard and Rasmussen, 1989; Ross, 1990a; Steinnes et al., 1987) as do lichens (Bartók, 1988; Blium and Tiutiunnik, 1989; Garty, 1989; Gorshkov, 1989; Herzig et al., 1990) and bryophytes (Ward and Sampson, 1989). Mosses have also been used for monitoring metal concentrations and bioavailability in freshwater (Mouvet et al., 1987).

Algae are widely used as biomonitors, in part because of their widespread occurrence but also because unicellular algae are relatively easy to grow in the laboratory. Wong (1989) discusses the use of algal assays to interpret toxicity guidelines for natural waters. The term "Cu equivalent" is used rather than EC_{50} values in an attempt to improve the comparisons of metal effect. Yu (1990) discusses chemical species of copper in culture solutions of freshwater algae, an important topic when the algae are used for bioassay purposes. Variation in response of algae is also important, providing an indication of organism sensitivity (e.g. Tadros et al., 1990). Bioassay organisms discussed in recent literature include diatoms (Tadros et al., 1990) and common green algae such as *Chlorella* (Wren and McCarroll, 1990), *Scenedesmus* (Nyholm, 1990; Twiss et al., 1989) and *Ulva* (Ho, 1990a,b). Phillips (1990) discusses the use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters, commenting that for the organism to be of value, tissue metal concentrations should reflect environmental bioavailability. The brown seaweed *Fucus vesiculosus* has been used as an indicator of copper bioavailability in fish farms (Rönnberg et al., 1990). Kondratev et al. (1990) discuss the use of aerial spectrophotometry to measure plant biomass in the Black Sea area, which they attempt to relate to heavy metal effect. Kapkov and Trishina (1990) provide tissue metal concentrations of commercially-harvested macroalgae from the White Sea (values in table on plant tissue metal levels).

A number of aquatic vascular plants have been used as biomonitoring agents (e.g. Gavrilenko and Zolotukhina, 1989; Gonzalez et al., 1989; Gunkel, 1989b; Havas and Hutchinson, 1987; Jenner and Janssen-Mommen, 1989; Taraldsen and Norberg-King, 1990). Salabarria Fernandez et al. (1989) used tissue metal concentrations of seaweeds, seagrasses and a mangrove species, along with several animal species, to indicate metal concentrations in a Cuban estuary. Terrestrial plants have also been used as biomonitors. This includes indicators of metal uptake in commercial food plants (e.g. Cheung et al., 1989) and evaluation of metal availability in mine and smelter soils (e.g. Van Assche and Clijsters, 1990a). Evaluation of aerosol input of metals and acids has been attempted with tree tissue metal levels (e.g. Ilgen and Nebe, 1989) as well as soil chemistry at the base of trees (Venanzoni and Werner, 1988).

Invertebrate animals have been widely used for metal bioassay purposes. V.-Balogh et al. (1989), for example, used a series of invertebrates to quantify heavy metal uptake in Lake Balaton (Russia). They have also been used to monitor metal and organic concentrations in the Dutch Wadden Sea (Essink, 1989) although the publication does not include copper. Invertebrates have also been used to evaluate metal availability from dredged material (e.g. Parrish et al., 1989). Rainbow et al. (1990), however, point to the need for a better understanding of metal uptake and what tissue metal concentrations really mean. Winner et al. (1990), for example, note greater sensitivities of both phytoplankton and zooplankton to copper in the spring than in the summer or fall. Repeatability and reproducibility of data from organism in aquatic microcosms is also of concern (Conquest and Taub, 1989).

Invertebrate organisms evaluated for or used in recent bioassay and toxicity work include ciliate protozoans (Evans and Samosir, 1990), nematodes (Williams and Dusenbery, 1990a,b), rotifers (Couillard et al., 1989), polychaetous anellids (Carr et al., 1986; Comber et al., 1989; Ozoh, 1990a) and earthworms (van Gestel et al., 1989). Bivalve molluscs are widely used, including freshwater (Belanger et al., 1990; Doherty, 1990; Salánki et al., 1989) and saltwater (Savari et al., 1991a; Zorba et al., 1988) clams. Phelps (1990) discusses the use of an estuarine sediment burrowing bioassay for shipboard use, with the soft-shell clam *Mya arenaria*. Mussels continue to be used as a means of evaluating metal concentrations and bioavailability on a global basis (e.g. Boehm et al., 1988; Borchardt et al., 1989a,b; Chan et al., 1986; Chernova et al., 1988; Chou and Uthe, 1990; Johnson, 1988; Kadhim, 1990; Khristoforova and Kavun, 1988; Lauenstein et al., 1990). van Haren et al. (1990) provides a model approach to predict accumulations in terms of bioavailability. They do, however, point out that (abstract) "... additional laboratory experiments should be done for a better understanding of why there is poor agreement between the few field observations and the simulations". Lobel et al. (1990) point out that there are genetic differences between populations of *Mytilus* that have not been adequately considered in monitoring programs. Truchet et al. (1990) evaluate the storage of metals in two species of gastropod molluscs (*Littorina littorea* and *Scrobicularia plana*). Another gastropod, the pond snail *Lymnaea stagnalis* is used in monitoring heavy metals in fresh water (Rózsa et al., 1988) while slugs (also gastropods) are used in terrestrial environments (e.g. Greville and Morgan, 1990).

A number of crustaceans have recently been used in biomonitoring studies in both aquatic (e.g. Ahsanullah and Williams, 1991; De Nicola et al., 1990; Moore and Winner, 1989; Nipper et al., 1989; Weeks and Moore, 1991) and terrestrial environments (e.g. Morgan et al., 1990). Of these, barnacles are frequently used (Chan et al., 1986; Powell and White, 1990). Weeks and Moore (1991) provide evidence that moulting does not seriously affect copper concentrations in four species of amphipod crustaceans. This is also suggested for chironomid insects by Timmermans and Walker (1989). Dhavale (1990) does point out that metal effects are subject to a number of environmental variables, at least with the crab *Scylla serrata*. Recent publications dealing directly or indirectly with the use of insects as monitors include Benton and Guttman (1990), Kroupa et al. (1990), Timmermans and Walker (1989) and Veleminsky et al. (1990).

The use of vertebrates for monitoring purposes frequently involves the use of physiological processes as well as tissue metal levels (e.g. Munkittrick et al., 1990). With fish this includes metal levels in otoliths ("ear stones") (Protasowicki and Kosior, 1988) and blood (Banerjee and Homechaudhuri, 1990). Hogan (1990) briefly discusses the use of cardiac contractions of fish embryos to estimate the effect of copper. Two studies that provide applicable concepts, although do not use copper are Weis et al. (1990), using the rate of fin regeneration to test sublethal effects of wastes and Dethlefsen (1989) which discuses the effects of contaminants on fish reproduction. Adams et al. (1990) comment that a monitoring program should involve a suite of slected stress responses at several levels of biological organization. Metallothionein levels in fish have been used as an indication of metal effects (e.g. Dutton et al., 1988; Hogstrand and Haux, 1990). Behavioural responses have also been found to be of general use in monitoring programs (Diamond et al., 1990) as well as with particular compounds such as copper (e.g. Anestis and Neufeld, 1988). Welch et al. (1989) report that temperature preference tests are a sensitive indicator of chronic effects of excess copper in a cultured fish (tilapia). Elezaj et al. (1989) examined metal availability near a lead-zinc smelting facility using tissue metal levels in a natural population of land turtles. Tissue metal concentrations in birds have also been used to indicate the availability of metals in industrial aerosol emissions (Nyholm, 1987, 1989) and industry in general (e.g. Walsh, 1990).

Domestic and laboratory animals as well as cells or cell cultures (e.g. Failla et al., 1990a; Klein et al., 1990a; Weglarz et al., 1990) are used in biomonitoring. This is to allow evaluation of metal availability in food sources (e.g. Rengers, 1988), metal flux through systems (e.g. Ting et al., 1990), or potential effects of metal on humans. The activities of copper-containing enzymes are useful indicators of metal availability and various enzyme assay systems have been developed to allow measurement (e.g. Aitsu et al., 1989; Nakano et al., 1990; Porstmann et al., 1990). Cu, Zn-superoxide dismutase is one enzyme that has been used (DiSilvestro, 1988; Kurobe et al., 1990a,b). Ceruloplasmin has also been recently used, by Työppönen (1988) as an indicator of copper availability in mink, as have other enzymes (e.g. Mameesh et al., 1989). Metallothionein activity is considered an indication of metal exposure in laboratory as well as field animals (e.g. Stillman et al., 1989). Chemical activity of isolated cells or cell components has been used as an indication of copper status (Failla et al., 1990a,b; Haubenstricker et al., 1990a; Knobeloch et al., 1990). Laboratory animals can also be called "monitors" when they are used to indicate the effects of various chemicals on copper availability or metabolism (e.g. Yamamoto et al., 1990a). Serum copper concentrations can also be used to "monitor" physiological condition in an organism (e.g. Ward et al., 1989b). In some cases, the use of copper isotopes is beneficial in tracing copper flux in the body (Barnhart et al., 1990; Mathias et al., 1990a).

The use of reference materials is highly recommended when tissue metal concentrations are used to estimate metal availability or the effect of physiological condition on tissue metal concentrations. Tissue reference materials provide a means of comparing the results of analysis with those done on the same tissue in other laboratories. This is true whether the tissue is from a field or laboratory animal (e.g. Kucera et al., 1990) or from humans (e.g. Iyengar, 1988a; Senofonte et al., 1989; Vanhoe et al., 1989).

I.3.12 TOXICITY

The noun "toxicity" is defined as the quality, relative degree, or specific degree of being toxic or poisonous. As such, it is a term often used in documents discussing detrimental as well as beneficial effects of copper (e.g. Janus et al., 1989). Unfortunately, there is often the misunderstanding that a metal is detrimental until proven beneficial (i.e., guilty until proven innocent). Copper is an essential metal, required for life; too much copper can be detrimental if it is in a biologically available chemical form. For this latter reason, discussions of toxicity for an essential metal must include reminders that it is the amount of metal that is biologically available not just the total amount of metal that is present.

Recent reviews of toxicity that include discussions pertinent to copper include Langston (1990) on marine ecosystems, Somerville and Walker (1990) on pesticide effects on terrestrial wildlife, George (1990) on biochemical and cytological assessments of metal toxicity in marine animals, and England et al. (1989) on the ordering of metal-ion toxicities in different species with extrapolation to man. Recent work of a generalized nature, that includes copper toxicity, has been done on the pelagic region (e.g. Lifshits and Korsak, 1989), sediments (e.g. Crowdere et al., 1989), dredged materials (e.g. Parrish et al., 1989), and soils (e.g. Tu and Qing, 1989). These and other works include a variety of toxicity and bioassay tests, including a number of microbial tests (e.g. Domsch, 1989), respiration and carbon dioxide production (e.g. Haubenstricker et al., 1990a,b; Jardim et al., 1990), other physiological as well as biochemical tests (e.g. Knobeloch et al., 1990; Oleskiewicz and Sharma, 1990), a variety of growth and life cycle inhibition tests (e.g. Carr et al., 1986 Lee et al., 1990b; Nyholm, 1990). Methods of study of aquatic animals and application of results are reviewed by Abel (1989). Other discussions of bioassay protocol and use are found in Genjatulin (1990). Morrison et al. (1989) provide data from an intralaboratory study of precision of saltwater short-term chronic toxicity tests using copper sulfate as one agent. Clements et al. (1990) found a greater impact of copper exposure under laboratory conditions than in the field. They suggest that this may have resulted from the inability of certain organisms to acclimate to laboratory conditions and advocate field mesocosms for experimental work. Strain-dependent differences in copper accumulation and excretion have been reported in rats (Verheesen and Nederbragt, 1988).

Factors that affect toxicity are numerous and are the same factors that affect metal uptake and tissue metal concentrations. Some authors have related metal accumulation to concentration of total metal (e.g. Drbal and Veber, 1989) although, fortunately, more and more work uses the concept of metal and environment chemistry (e.g. Krantzberg and Stokes, 1987; Lithner, 1989; Slauenwhite et al., 1991; Young et al., 1990) and bioavailability. Bengtsson and Tranvik (1989) reviewed literature on metal effects on forest soil invertebrates and suggest that copper concentrations less than 100 mg per kilogram soil is a maximum allowable metal concentration that will cause no adverse effects. Environmental parameters that have been suggested to affect availability include mineralization of the water in aquatic environments (Le Du et al., 1990), pH (Lüderitz and Nicklisch, 1989a; Lüderitz and Scholz, 1989; Meador, 1988; Versteegh et al., 1989), salinity and temperature (Dhavale, 1990; Ozoh and jones, 1990b), sorbing agents such as clay minerals (Nagy-Bozsoky et al., 1989; Walker et al., 1989), metal complexing capacity (e.g. Chen et al., 1988a; Lüderitz et al., 1989c; Nakamura, 1990; Slauenwhite and Wangersky, 1991; Sun et al., 1990b; Winner et al., 1990) and physiological status of the organism. In terms of metal complexing agents, considerable work has been done on the nature and importance of humic substances in aquatic (e.g. Manahan, 1989; McCarthy, 1989; Shanmukhappa and Neelakantan, 1990) and terrestrial (Senesi et al., 1989b) environments. In laboratory work, environmental conditions also include culture methods and composition of the various media used (e.g. Okamura et al., 1989). What these factors imply is that measuring "toxicity" must include some expression of metal bioavailability. Campbell and Tessier (1989) discuss availability in sediments and include some of the thornier issues relating to expression of metal effect on organisms in terms of metal chemistry. Metal availability must also be incorporated into evaluations of effluents and the dumping or using metal-containing sludges (e.g. Gambrell and Patrick, 1989). Finally, the interaction of copper with other agents such as other metals or alkaloids (e.g. Howell et al., 1988) should be considered as they can affect toxicity.

A major portion of the toxicity literature concerns effects on specific groups of organisms or on specific organisms. Most of these references are used elsewhere in the review, where discussions of effects are found:

1. Microorganisms - Reviews - Bååth, 1989; Gadd and White, 1989; Poole and Gadd, 1989.

    Bacteria - Bååth, 1989, Bowman et al., 1990; Cherdyntseva, 1988; Flemming and Trevors, 1989; Harwood-Sears and Gordon, 1990; Jeanthon and Prieur, 1990a; Nakatsu et al., 1987; Poole and Gadd, 1989; Stormer and Falkinham, 1989; Walker et al., 1989.

    Fungi and yeasts - Cimino and Caristi, 1990; Franich, 1988; Gadd and White, 1989; Gershon et al., 1989; Hashem, 1989.

    Cyanobacteria (blue-green algae) - Lüderitz and Nicklisch, 1989a; Lüderitz and Scholz, 1989; Lüderitz et al., 1989b,c.

2. Plants - Reviews - Balsberg Påhlsson, 1989; Tyler et al., 1989.

    Mosses, lichens and ferns - Shaw, 1990; Tyler, 1989.

    Algae - Bastien and Côté, 1989; French and Evans, 1988; Jindal and Verma, 1989; Lüderitz and Nicklisch, 1989b; Metaxas and Lewis, 1991a,b; Morrison et al., 1989; Nakamura, 1990; Ning et al., 1990b; Nyholm, 1990; Rao and Latheef, 1989; Shanmukhappa and Neelakantan, 1990; Sun et al., 1990a; Swartzman et al., 1990. Takamura et al., 1988; Twiss et al., 1989; Wong, 1989; Wong et al., 1989; Wren and McCarroll, 1990; Zhang et al., 1990b.

    Vascular plants - Balsberg Påhlsson, 1989; Cheung et al., 1989; De Vos, 1991; Lee et al., 1990b; Rhoads et al., 1989; Tu and Qing, 1989; Tyler et al., 1989; Wadey, 1988.

3. Animals - Reviews - Bengtsson and Tranvik, 1989; Clements et al., 1990; Tyler et al., 1989.

    Protozoans - Bonnemain and Dive, 1990; Cassells et al., 1990; Lifshits and Korsak, 1989.

    Coelenterates - Piraino and De Nicola, 1990.

    Rotifers - Couillard et al., 1989.

    Nematode worms - Williams and Dusenbery, 1990a,b.

    Annelids -

        Polychaete worms - Fernandez and Jones, 1990; Jenner and Bowmer, 1990; Ozoh and Jones, 1990b; Parrish et al., 1989.

        Oligochaetes (earthworms) - Gal and Bouche, 1988.

    Molluscs -

        Oysters - Parrish et al., 1989.

        Mussels - Accomando et al., 1990; Gainey and Kenyon, 1990; Hawkins et al., 1990; Johnson, 1988.

        Clams - Belanger et al., 1990; Jenner and Bowmer, 1990; Sunila and Farley, 1989.

        Snails and slugs - Nott and Nicolaidou, 1990b; Vareille-Morel and Debord, 1990; Young and Wilkins, 1989.

    Crustaceans -

        Cladocerans (e.g. *Daphnia*) - Belanger and Cherry, 1990; Lithner, 1989; Moore and Winner, 1989.

        Anostracans (e.g. *Artemia*) - Rao and Latheef, 1989.

        Ostracods -Khangarot, 1987.

        Mysids - Morrison et al., 1989; Parrish et al., 1989.

        Isopods and amphipods - Giudici and Guarino, 1989.

        Shrimp - Parrish et al., 1989; Tanaka, 1988.

        Crabs - Boitel and Truchot, 1990; Burton and Fisher, 1990; Dhavale, 1990; Hilmy et al., 1985.

    Insects - Benton and Guttman, 1990; Darlington and Gower, 1990; van de Guchte and van Urk, 1989.

    Echinoderms - Morrison et al., 1989.

4. Fish - Banerjee and Homechaudhuri, 1990; Bucke et al., 1990; Burton and Fisher, 1990; Denizeau and Marion, 1990; Ebele et al., 1990; Hamilton and Buhl, 1990; Khangarot, 1988; Morrison et al., 1989; Reardon and Harrell, 1990; van Coillie et al., 1989; Wakabayashi and Mizorogi, 1989; Wu et al., 1990c; Young et al., 1990. Also see references listed in National Technical Information, 1990c,e

5. Domestic animals - Howell et al., 1988; Yu et al., 1990; Zervas et al., 1990.

6. Laboratory animals other than rats and mice - Abbasi and Soni, 1989; Gilani and Alibhai, 1990; Rhee and Dunlap, 1990b.

7. Rats and mice - Fuentealba et al., 1989; Gordon et al., 1990a; Nakatani and Kobayashi, 1990; Verheesen and Nederbragt, 1988.

8. Cell cultures - Ng and Liu, 1990; Okamoto et al., 1989.

9. Humans - evidence obtained from accidental (Nakatani and Kobayashi, 1990) and suicidal (Dash, 1989) cases

A number of organisms are able to exist and even thrive in environments containing high levels of total copper (e.g. von Frenckell and Hutchinson, 1989; Winge et al., 1990). In a discussion of one of these situations, Diels and Mergeay (1989) discuss chemical as well as physiological properties which may enable this to occur. They include an enzyme-driven efflux mechanism and metal crystallization in the medium. Ernst et al. (1990) discuss some of the possible reasons for metal resistance in the plant *Silene vulgaris*, including changes in the potential for membrane uptake of metal. Bryson et al. (1990) discuss the possibility that, at least with one coliform bacterium, chemical changes in the nature of the copper are produced by the organism. Exposure to copper-enriched soils, as well as water, can also lead to acclimation with resultant increase in tolerance (e.g. Huysman et al., 1988). With copper resistance in the coliform *E. coli*, excess copper is reported to modify the uptake, storage and efflux of the metal (Brown et al., 1990b). In a second publication, the authors (Lee et al., 1990a) discuss this in terms of the ability of the organism to regulate copper transport and metabolism.

Fungicide resistance has been frequently reported for fungi isolated from plants exposed to fungicides (e.g. Moorman and Lease, 1989). Part of this may be due to tolerance differences between parts of the fungi (e.g. Schmidt and Moreth-Kebernik, 1989). Part may be due to genetic mutation (e.g. Phelan et al., 1990). (Fungi growing in a symbiotic relationship with *Agrostis capillaris* may assist in the uptake of metal (Griffioen and Ernst, 1989).) In plants, storage of accumulated metal is important in metal-rich environments (e.g. Sieghardt, 1990). Population levels of an organism may also be important, acting as a "diluent" for existing available copper (e.g. Duxbury and McIntyre, 1989).

Evaluation of copper-resistance indicates the importance of the genetic structure of the organism (Cooksey, 1990; Dittaponqpitch, 1989). Bender et al. (1990) characterize pHX10A, a copper resistance plasmid in a bacterium found on tomatoes. Other copper-resistant bacteria have been isolated from wild and domesticated plants (e.g. Cooksey et al., 1990b). Cooksey and Azad (1989) and Cha and Cooksey (1989) attempt to relate copper resistant capability of a bacterial spot disease organism to genetic structure. Silver et al. (1989) provide an analysis of the DNA sequence of bacterial plasmids that have the genetic ability to confer metal resistance to copper and a number of other metals and metaloids.

Gowrinathan and Rao (1990) note reduction in detrimental effects of excess copper in a planktonic diatom, in association with either sorption to the outer surface of the cell or production of a metal-complexing agent within the cell. Secondary metabolites may act as metal-binding agents (Hershberger et al., 1989) and reduce the concentration of biologically available metal and detrimental effect from excess metal (e.g. Lapin and Yedigarova, 1990). Copper-binding compounds have been isolated from plants exposed to high levels of copper (van't Riet et al., 1987). Copper complexation has been reported to increase, as a function of cell density, during an extended bloom of the dinoflagellate *Gymnodinium sanguineum* (Robinson and Brown, 1991). Geesey and Jang (1990) discuss extracellular polymers produced by microorganisms, which they suggest act as effective modulators of metal-ion concentration at the cell surface. Kaplan et al. (1988) discuss the relationship between the chemical structure of algal polysaccharides and their metal-complexing properties. Steffens (1990) reviews the nature and structure of heavy metal-binding peptides of plants. One major group of these are phytochelatins, peptides produced for normal metal metabolism as well as in response to elevated levels of metal. The nature and production of these peptides can, however, be limited by the availability of glutathione (Mendum et al., 1990). In an evaluation of binding of copper and cadmium by proteins from aquatic algae, Zolotukhina et al. (1989) point out the ability of the metals to form nonspecific complexes with the proteins. In another paper (Zolotukhina and Gavrilenko, 1990) they point out that (abstract) the "Accumulation of Cu and Cd in proteins on the whole is nonspecific and inversely proportional to protein molecular weight". They also found that manganese in proteins reduces the ability to bind copper suggesting a mechanism for the Mn-Cu antagonism found by Kazumi et al. (1987 - discussed in an earlier I.C.A. review). Nishizono et al. (1988) have isolated a copper-binding protein from the root cytoplasm of a metal-tolerant plant (*Salix bakko*) growing near a mine in Japan. Tukendorf (1989) isolated and characterized copper-binding proteins from spinach chloroplasts grown under high copper concentrations. (The molecular weight was estimated at 2,000 daltons.) Palma et al. (1990) isolated and characterized low-molecular-weight copper-binding proteins from a plant grown with supplemental copper. Similar work has also been done with animals (e.g. Cochrane et al., 1991). Many of these metabolites are discussed in a review of "Heavy metal-binding proteins/peptides ..." by Reddy and Prasad (1990), a review that does not include metallothioneins.

Metal resistance is found in animals and bacteria living in regions of mineralization, such as hydrothermal vents (Jeanthon and Prieur, 1990). Adaptation to elevated copper levels in animals as well as other organisms, can occur for a variety of reasons. Optimum conditions of other variables (e.g. salinity and temperature) will increase the ability of an organism to tolerate some increase in metal (e.g. Ozoh and Jones, 1990a). Metal-metal interactions can reduce metal uptake or incorporation into the body (e.g. Schilsky et al., 1989c). Sequestration of excess metal is an important adaptation, one which occurs in a number of aquatic (Phillips and Rainbow, 1990) as well as terrestrial animals. Isolation of the metal within the body does occur in a number of invertebrate groups, in association with phosphate granules (Simkiss and Taylor, 1990). Several gastropod molluscs are known to be able to compartmentalize excess metal within mineralized granules as phosphates and within lysosomes in association with sulfur (Nott and Nicolaidou, 1989). Similar findings are recorded for an insect (Darlington and Gower, 1990). Other invertebrates appear able to reduce the uptake of excess metal (e.g. Denneman, 1990). These findings as well as a number of other observations, indicate that there is a reduction in copper in food chains (Nott and Nicolaidou, 1990a,c).

One of the more important metal-complexing agents in animals is metallothionein. As a metal-transporting protein it is normally found in the body. In rainbow trout, at least, variations may occur as a result of changing metal requirements during growth (Olsson et al., 1990). However, exposure to elevated metal concentrations will often cause an increase in the protein (e.g. Elmes et al., 1988b; Fuentealba and Haywood, 1988) indicating an ability to reduce the concentration of biologically available metal. Bremner and Beattie (1990) review metallothioneins, including their functions as well as nature and synthesis/degradation. Hogstrand et al. (1990) report that liver tissue levels of copper, zinc and metallothionein reflected environmental levels of the metals in perch collected in the region of a brassworks in Sweden. Because of the relationships between metal levels and metallothionein levels, metallothionein levels have been used as an indicator of heavy-metal exposure in a variety of animals (e.g. Cosson, 1989 - birds; Hogstrand and Haux, 1990 - fish). Relationships between pH, trace elements (Cd, Zn, Cu) and metallothioneins have been examined (Xia et al., 1990) with the intent of evaluating the effect of pH on metal binding. Zafarullah et al. (1989) discusses heavy-metal-ion-induced metallothionein gene expression in salmonid tissues, using $CuCl_2$ as one of three metals to induce expression in rainbow trout. The same authors (Zafarullah et al., 1990) discuss the metal regulation of metallothionein production in rainbow trout, providing evidence that it is the metals rather than gene-regulated protein synthesis that is important.

II - COPPER AND MAN

II.1 USES OF COPPER

Copper is used for a variety of biological purposes as well as in industry (e.g. Institute of Metals "Copper '90" list of presentations). These include uses in medicine, agriculture as well as a wide variety of uses in pest control. There are numerous recent references concerning the uses and importance of copper, as well as problems arising from the use of the metal. These are cited throughout the entire review. To give an indication of the value and the importance of copper to man, some of the recent references and patents are given below, in table 1.

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

Agriculture - fertilizers, yeast and plant nutrients - Aleshin et al., 1989; Bezuevskii et al., 1990; Brazauskiene et al., 1990; Ciparis et al., 1990; Dowbenko et al., 1990; Duka et al., 1990; Feng et al., 1989a; Glabisz and Grzmil, 1989; Gorlach, 1989; Grigoryan et al., 1990; Hazra et al., 1987; Hopmans, 1990; Jasiewicz, 1990; Jurkowska et al., 1987; Komarov et al., 1987; Koomen et al., 1990; Krut'ko et al., 1989; Kudashkin, 1989; Meerovskaya et al., 1989; Murugesan and Mahadevan, 1988; Piening et al., 1989; Schum et al., 1988; Sherrell, 1989; Singh, 1983; Szakál and Tölgyesi, 1990; Venkateswarlu and Misra, 1987; Zeng et al., 1989.

Agriculture - livestock and laboratory animals - Abdel-Monem and Anderson, 1990; Badawi et al., 1990; Bogoslovskaya et al., 1989; Bridges and Moffitt, 1990; Cromwell et al., 1983; Dixon, 1989; Driver et al., 1988b,c; Duerckheimer et al., 1989; Dunbar et al., 1988; Goodwin-Jones, 1990; Hoeting, 1990; Hyuusa, 1983; Irie, 1990; Klevay and Pond, 1990a; Matsui et al., 1989; Millar et al., 1989a; Pentek et al., 1989; Ruksan et al., 1988; Shenkin, 1988; Sherrell, 1989; Shuler et al., 1990; Wilson, 1989; Wu et al., 1990b; Yokoi et al., 1989; Young, 1990.

Agriculture and forestry - fungicides, microbicides and antiphytoviral agents - Ahmed and Goyal, 1988; Anonymous, 1989; Ansari et al., 1990; Azevedo and Silva, 1988; Banihashemi, 1990; Bhattacharyya et al., 1987; Cerkauskas and McGarvey, 1988; Coziahr, 1989; Csutak et al., 1989; Davarski et al., 1989; De Figueiredo, 1988; Feliciano et al., 1989; Feng, 1989b; Franich, 1988; Galoux and Bernes, 1990; Gorska-Poczopko et al., 1987; Guiraud et al., 1989; Halley et al., 1990; Hugener and Roth, 1990; Hugk et al., 1990; Ichimura et al., 1989; Khan et al., 1990b; Khatri and Shekhawat, 1989; Li, 1988a,b; Li and Liu, 1990; Liang, 1990; Lim et al., 1990; Ling, 1989; Mathur and Shekhawat, 1988; Matsuno and Nishina, 1984; Maynard, 1990; McMillan, 1990; Milhaud et al., 1989; Muirhead et al., 1989; Muniz and Da Ponte, 1988; Murugesan and Mahadevan, 1989; Narashimhan et al., 1990; Nasu et al., 1990; Okutsume and Watanabe, 1989; Osborne and Taylor, 1989; Peter et al., 1990; Prasad et al., 1990; Prathuangwong and Choochoa, 1989, 1990; Presnell and Nicholas, 1990; Prohaszka, 1989; Ptaszkowska et al., 1989; Quijano-Rico, 1987; Riggert et al., 1989; Riha et al., 1990; Rossmoore, 1990; Rovesti et al., 1988; Rudgard et al., 1990; Ruiz et al., 1987; Saha and Singh, 1985; Santos and Resende, 1988; Schnelle and Hensley, 1990; Serfontein and Knox-Davies, 1990; Sharma, 1988; Singh, 1989a; Sinha, 1988; Suzuki et al., 1990a; Szego et al., 1989; Tanda, 1988; Thomas et al., 1989; Tolstikov et al., 1989; Tomas et al., 1989; Vargas de Alvarez et al., 1990; Ventura et al., 1988; Wu, 1989; Yamada et al., 1989; Zhang et al., 1989a.

Agriculture - miscellaneous - Auer et al., 1990a; Hayakawa, 1989; Ichise and Kuroda, 1989; Khajuria and Bali, 1988; Schwartz and Capatos, 1990; Seto and Yokoyama, 1990; Someya, 1990; Struve and Rhodus, 1990.

Biofouling - Callow and Edyvean, 1990 (an excellent discussion of algal fouling and corrosion); Lenard et al., 1989; Oishi et al., 1989; Ozeki and Hattori, 1989; Peacock et al., 1989; Pendleton, 1989; Price and Brady, 1989; Sakamoto, 1989 (patent for metallic coatings for fish nets); Williams and Knox-Holmes, 1989; Williams et al., 1989.

Dentistry - Eisenberg et al., 1989; Osborne and Norman, 1990;

Industry - food and water quality - Cassells et al., 1990; Dickie and Akhtar, 1989; Landeen et al., 1989; Mita et al., 1989; Nuttall, 1990; Schoenen and Schlömer, 1989; Schulze-Röbbecke et al., 1990; Tripathi et al., 1990; Tuschewitzki, 1990.

Copper in industry - Bolitt et al., 1989; Brik, 1990; Bugga et al., 1989; Bullen et al., 1988; Capalash et al., 1990; Cervello et al., 1990; Desimoni et al., 1990; Doner and Irwin,1990; Ekbom and Bogegaard, 1989; Ersöz et al., 1989; Garner and Zinkle, 1990; Garner et al., 1990; Gdowski and Bullen, 1988; Gillette et al., 1990; Golub et al., 1990; Gourdin, 1990; Hara et al., 1990; Hoover et al., 1987; Jernejc et al., 1990; Kaneko et al., 1990; Kapur et al., 1989; Kass, 1989; Keegstra et al., 1990; Krishnan and Birenzvige, 1989; Kusuhara et al., 1990; Lati et al., 1989; Liu and Liu, 1988; Marjit and Sharma, 1989; Melson, 1988; Mooney and Hermann, 1990; Moriya et al., 1989; Nair and Sells, 1990; Nakajima et al., 1990; Ohta et al., 1990; Oyama et al., 1988; Root and Reisler, 1990a,b; Rustenbeck and Lenzen, 1989; Schneider et al., 1987; Smith, 1990; Sondossi et al., 1990; Thunus and Dauphin, 1990; Ullal et al., 1990; Vanfleteren and Peeters, 1990; Van Konynenburg et al., 1990*; Voecks and Sharma, 1990; Walker et al., 1990; Yaha et al., 1990; Yulin and Liu, 1990.

Medicine - contraceptives - Edelman and van Os, 1990a,b; Hernandez-Perez et al., 1989; Hirvonen and Idänpään-Heikkilä, 1990; Puraviappan et al., 1989; Rowe et al., 1990; Sivin et al., 1990a,b; Smales et al., 1990; Srisupandit, 1988; Wildemeersch et al., 1988.

Medicine - nutrient supplementation - Shenkin, 1988, 1990;

Medicine - pharmacology - (see articles in Collery et al., 1990) - Apelgot and Guille, 1990b; Appelbaum et al., 1990; Auer and Seawright, 1990b; Badawi, 1990; Barnhart and Green, 1990; Bergmann et al., 1990; Brumas and Berthon, 1990; Damerau and Wischnewsky, 1989; Dilanyan et al., 1989; Ferrari et al., 1989; Frechilla et al., 1990a; Fujibayashi et al., 1989, 1990; Hori, 1989; Jackson and Kelly, 1989, 1990; Jagetia and Ganapathi, 1990; John and Green, 1990; Kishore, 1990a; Mathias et al., 1990b; McGahan, 1990; Meshnick et al., 1990; Moi et al., 1990; Moiny et al., 1990; Monti et al., 1990; Mordon et al., 1990; Morphy et al., 1990; Nagar, 1989; Nayak et al., 1990; Nistor et al., 1990; Oikawa et al., 1990; Parashar et al., 1990; Pickart, 1989; Rabinovitz and Fisher, 1989; Roberts et al., 1989; Roch-Arveiller et al., 1990a,b; Shelton et al., 1989; Shing and Folkman, 1990; Soderberg et al., 1990; Sorenson et al., 1989; Szabová et al., 1990; Tarasiuk et al., 1990; Thorsen et al., 1990; Vaille et al., 1990; Yoshino, 1989.

Medicine - miscellaneous - Bienvenu and Kergonou, 1990; Nemeth and Reyes, 1990a,b; Pickering et al., 1990; Reyes and Nemeth, 1990.

Pest Control - Baldwin and Bennett, 1990; Bielecki, 1987; Cassells et al., 1990; Choy et al., 1989; Ichimura et al., 1989; Ivanov et al., 1989b; Kamiya and Ueda, 1990; Kovacic et al., 1989b; Landeen et al., 1989; Murugesan and Mahadevan, 1988; Nagar, 1989; Nielsen, 1990; Ono et al., 1989; Roomi et al., 1990; Sakuma et al., 1990; Schoenen and Schlömer, 1989; Sunila and Farley, 1989; Tuschewitzki, 1990; Vairamani et al., 1989; Yescott, 1989; Young and Wilkins, 1989; Zemanová, 1988.

Wood preservation - Brayman, 1989; De Groot et al., 1988; Doi, 1989; Gertjejansen et al., 1989; Gnanaharan and Dhamodaran, 1989; Göttsche and Marx, 1989; Göttsche et al., 1989; Green et al., 1989; Khan, 1989; King et al., 1989; Kitada, 1989; McIntyre and Pasek, 1990; Moewius et al., 1989; Ruddick et al., 1990; Soitis and Winandy, 1989; West, 1990; Winandy, 1989.

* ICA-supported research -

II.2 ANTHROPOGENIC COPPER - NATURE AND EFFECTS

Environmental copper is derived from both natural and anthropogenic sources. High concentrations can naturally occur in water near hydrothermal vents (Lein et al., 1989; Zhirmunsky and Tarasov, 1990) and in hot springs (Veldeman et al., 1991) or associated with manganese nodules (Earney, 1990). They may also occur in sediments, as a result of diagenetic processes or estuarine circulation (e.g. Pertsov et al., 1990). Organisms living in these areas are either tolerant to excess copper (e.g. Lein et al., 1989; Zhirmunsky and Tarasov, 1990) or the copper is not in a biologically available state. Anthropogenic copper can occur from a variety of effluent discharges (e.g. Arzul and Maguer, 1990) as well as the use of copper for antifouling (e.g. Lenard et al., 1989; Wagner et al., 1987), biological control of noxious organisms (e.g. Lüderitz et al., 1989a) or even in medicine (e.g. Ujjani et al., 1990). In aquatic environments, metal concentration is a result of the amount of metal released as well as the dynamics of both the metal chemistry and circulation (e.g. Rönnberg et al., 1990). Reservoir sediments have been shown to be a source of metals (including copper) for drinking water (Schintu et al., 1989) as have groundwater in some industrialized areas (e.g. Zahn, 1988). Even water distribution systems are reported to be sources of copper for drinking water (Eife et al., 1989); O'Day (1989) comments on the importance of evaluating corrosion in water distribution systems. Industrial input, as aerosol copper, has been demonstrated with lichen bioassay techniques (e.g. Walther et al., 1990).

The increasing information on metal speciation has led to greater interest in and better evaluation of the chemistry of natural and anthropogenic copper in the environment (e.g. de Kruif et al., 1988; Furness and Rainbow, 1990; Janus et al., 1989; Jenkins, 1982; Jepson, 1989; Langston, 1990; Lindberg and Hutchinson, 1987a,b; National Swedish Environment Protection Board, 1989; Nriagu, 1989; Sors and Sabbioni, 1987; Vernet, 1989a; Wollast, 1982). This is a result of improving technology, more accurate sample collection, wider use of reference standards (e.g. Schauenburg et al., 1990; Tamba et al., 1989) and being able to address increasingly more difficult questions. As a result, details and questions about water and air quality can be better addressed (e.g. Madelain, 1990; Michaelis, 1990). Formerly nieve questions such as "What constitutes ecosystem health" (Rapport, 1989) can be addressed in terms of metal bioavailability. Information on chemistry is also of value in evaluating the roles of metals in industrial operations such as anaerobic digestion (e.g. Oleszkiewicz and Sharma, 1990) or the problems and treatments of pitting and corrosion of copper and copper alloys (Bremer and Geesey, 1990; Gaylarde, 1989; National Technical Information Service, 1990b; Pizzini et al., 1990; Yunker, 1990).

There is increasing concern about environmental quality (e.g. Aston, 1990; Dayton, 1991) and the means of environmental protection (e.g. articles in Orio, 1988). Kennedy and Gaugush (1988), for example, discuss water quality in reservoirs used by the U.S. Army. Shofstahl and Hardy (1989) discuss a technique for detection of priority pollutant metals. Lampe and Mitchell (1990) provide the results of the U.S. Environmental Protection Agency's Semiannual U.S. Acid Rain Audit Program (NPAP). Sloof et al. (1989) report that, at least for the Netherlands, the current copper load in drinking water poses no health hazard in terms of toxicity. They also note (abstract) that "On the other hand, there are groups of people with an insufficient intake of copper". There have been several recent reviews and evaluations of metal-containing environments and metal levels in organisms in these environments. They include regions or sites in the Black Sea (Mihnea et al., 1991), the Mediterranean Sea (Ganoulis, 1991), West and Central Africa (Makaya et al., 1988), India (Chandra, 1988), northern Europe (e.g. Beddig and Sündermann, 1988; Korzeniewski and Neugebauer, 1991; Kubiznakova et al., 1989; Lithner et al., 1990; Maenhout, 1989; Makarova and Moiseenko, 1990; Ottley and Harrison, 1991; Portman, 1989; Steinnes et al., 1989b; Verta et al., 1989) and the United Kingdom (Barr et al., 1990; Franklin, 1990; Grant and Middleton, 1990; Slinn, 1990). They also include sites and regions in the United States (Anderson and Proctor, 1990; Bender, 1989; Brosnan et al., 1987; Crecelius et al., 1989a; Decker et al., 1989; MacDonald, 1989; McCain et al., 1989; O'Connor et al., 1989), Canada (Kay, 1989; Reid et al., 1987; Samis et al., 1990; Wainwright and Humphrey, 1988), South America (Trucco et al., 1990), New Zealand (Glasby et al., 1990) and Japan (Hoshika et al., 1991). The Stockholm Environmental Institute (1990) reviews policy considerations for the environment of the Baltic Sea region. Although there is no specific comment on copper, this review provides an indication of changing nature of outlook about major environmental problems. There is continued reevaluation of environmental quality criteria (e.g. Kempic, 1988; Long, 1989) as well as attempts to model the transport and fate of anthropogenic materials, including metals (e.g. Chapra and Boyer, 1989, 1990; Klomp, 1990). This includes work towards reduction of metal in aerosols (e.g. Brna et al., 1989; Fournier et al., 1990). Recovery of severely-affected sites continues to be attempted, as for example in the U.S. Superfund Projects (e.g. Ikalainen and Allen, 1989).

Understanding metal geochemistry in natural and anthropogenically-affected environments better allows identification of metal sources, fate and biological impact (e.g. Abu-Hilal and Badran, 1990; Anderson et al., 1989b; Brügmann and Lange, 1989; Christensen and Tjell, 1989; Flügge et al., 1988; Irion and Müller, 1990; Landsberger et al., 1990; Nichols et al., 1988; Rang and Schouten, 1989; Skei and Naes, 1989; Sugano and Sato, 1982; Zeman and Slaymaker 1988; United Nations Environment Programme, International Atomic Energy Agency, 1990). The transport pathways of metals in food webs is important for an understanding of metal impact and retention (e.g. Crowder et al., 1988; Mackie and Yan, 1987; Small, 1989; U.S. National Oceanic and Atmospheric Administration, 1989). Metal concentrations in the environment and organisms have been used to locate anthropogenic sources of metal and to evaluate the need for and problems with decontamination (e.g. Kilpatrick-Howard et al., 1988; Piepponen and Lindström, 1989; Schmoyer et al., 1988a,b; U.S. Environmental Protection Agency, 1988a-c). Although total metal concentrations are used for safety and health administration (e.g. Tripathi et al., 1990), metal bioavailability is controlled by environmental conditions as well as metal concentrations. Conditions in natural water systems can control the biological impact of copper (e.g. Lithner, 1989). Acidic water has been suggested to dissolve copper from piping systems with detrimental effects to humans (Nordberg, 1990). Other work infers a risk of adverse health effects from the oral intake of heavy metals, with the need to test for the concentrations of these metals (Danielsson and Huazhang, 1989) in foods. There is also the need to examine their biological availability in aquatic environments, including sediments (Campbell and Tessier, 1989). The ability of the organism to combat the heavy metal is important (e.g. Almar and Dierickx, 1990) and needs to be incorporated into any major program on the availability and impacts of metals.

Recent literature on the biological effects of anthropogenic metals, including copper, to aquatic animals is discussed in Abdel (1989) and on saltwater organisms by Reish et al. (1990). Fluxes of copper, and other metals, to forests, and the effect on forest systems is reviewed by Borg and Johansson (1989), Nuorteva (1990) and Tyler (1989a). Rüppell (1988) reports the results of a working group analysis of heavy metal load in selected organism groups in the Oker valley (Germany). Misra et al. (19989b) discusses the monitoring of contaminant levels using a multispecies approach (actually 2 species of fish) on the Belgian coast between 1978-1985. Community structure effects of "anthropogenic disturbance" in Hamilton Harbour, Bermuda are discussed in Warwick et al. (1990). With plants, recent literature includes discussions of the impact of metals on aquatic macrophytes (e.g. Strzelecki et al., 1990) and trees (Garrec, 1989; Ilgen and Nebe, 1989; Jordan et al., 1990; Ward and Homer, 1989). Scott (1990) records increased concentrations of copper and other metals in coral skeletons in Hong Kong. A number of studies have examined copper and other metals in bivalve molluscs, including oysters (Hiraoka, 1991; Murphy, 1990; O'Connor and Lauenstein, 1990; Wilson et al., 1990), freshwater clams (Pynnönen, 1990), the common cockle (Savari et al., 1991a) and a mussel (*Arca zebra*; Leavitt et al., 1990; Widdows et al., 1990). Effect of anthropogenic metals on fish is discussed in several publications (e.g. Bucke et al., 1990; Hall et al., 1989; Pohl, 1990), on the frog *Rana tigrina* (Abbasi and Soni, 1989) and on mammals such as the harbour seal by Skaare et al. (1990).

Mining, Smelting and Metal-Working

The long use of copper by man has been a result of the tractable nature of the metal. The development of technology to extract and work copper can be traced back through the bronze age (e.g. Moesta and Schlick, 1989). The development of technology continues today, with improvement, for example, in leach-mining capability (U.S. Department of the Interior, 1990), in the ability to evaluate metal behaviour (e.g. Veblen and Ilton, 1989) or the nature and potential of alloys (e.g. Kim, 1990). A discussion of recent changes in the U.S. copper industry is provided by the U.S. Office of Technology Assessment (1990). Extraction and working of copper as well as other metals can have an environmental effect, a result of byproducts of the operations. These are often considered in general evaluations of the environment (e.g. Rapport, 1989) as well as particular situations (e.g. Hodson, 1988; U.S. Environmental Protection Agency, 1989b). They also enter into the preparation of effluent limitations. The U.S. Environmental Protection Agency (1989a), for example, provides effluent limitations guidelines and standards for primary copper smelting, primary electrolytic copper refining, secondary copper and metallurgical acid plants. Titles relating to acid mine drainage are given by the U.S. National Technical Information Service (1989a,b) for the period January 1977-December 1989. The biological effect of copper-containing effluents is related to the chemistry of the effluent as well as the receiving medium and the nature of the organism (Alloway, 1990; Lewis, 1990).

The real or potential environmental effect of mining and smelting copper-containing ores has been examined for a number of mine sites. This includes the final Environmental Impact Statement for the Exxon Coal and Minerals Co. zinc-copper mine in Wisconsin (Wisconsin Department of Natural Resources, 1986). Aldwell (1990) discusses examples of mining in Ireland and its impact on the environment, including some details on how environmental problems are handled. The influence of copper mining and smelting activities on metal levels as well as impact are discussed in a number of recent references (e.g. Frenzel et al., 1990; Bailey, 1988; Karlsson et al., 1989; Kwong and Nordstrom, 1989; Salomons and Eagle, 1990; Vale and Cortesao, 1989). There is evidence of release of metals from leachate or tailings although the chemistry of the environment is obviously important in the nature of the release whether it is from mining (e.g. Håkansson et al., 1989) or industry (e.g. Baudo et al., 1989; Camusso et al., 1989a; Mosello et al., 1989). Sandén and Carlsson (1990) used simulated concentrations and mass transport of zinc, copper and cadmium to model metal transport through a lake, from tailing deposits of an abandoned copper mine. They point out the potential interaction of hydrology and biology in affecting metal concentrations and transport.

Concern has been expressed about the biological impact of mining and industry. These concerns relate to the physical effects of the operation on natural conditions (e.g. Silva and Rosa, 1989) as well as to the byproducts of mining, smelting and metal working (e.g. Arzhanova and Elpat'evsky, 1988; Belyaev and Shmeleva, 1989; Fuge et al., 1989; Vermeer and Castilla, 1989). Although there are numerous bacteria and plants that are resistant to high levels of copper (e.g. Garcia and Clark, 1989; Gomez de Saravia et al., 1989; Nakatsu et al., 1987; Obbard and Jones, 1989), high concentrations of copper can be detrimental to growth of many plants if the metal is in a biologically available state. These high concentrations can be found in areas of mineralization (Eleftheriou and Karataglis, 1989) as well as areas affected by mining or industry (e.g. Gukasyan et al., 1989; Kuduk, 1988). One effect of high levels of biologically available copper is a reduction in species number and thus species diversity (Hatakeyama et al., 1988). It is important, however, to relate metal concentration to conditions in the receiving environment. Smith et al. (1990), for example, used both chemical and bioassay evidence to infer no toxic effects from overburden and partially treated ore residues introduced into a river from a mine in New Guinea. In contrast, Deniseger et al. (1990) found reduced species diversity of phytoplankton in a lake receiving effluent or leachate from a mining operation in British Columbia. Metal uptake from mine drainage streams has been noted for certain insect larvae (Gower and Darlington, 1990) and the toad *Bufo justasper* (Lee and Stuebing, 1990). With regard to the metal uptake noted by Deniseger et al. (1990) for fish in the lake receiving effluent, the three salmonid species examined behaved differently. One of the reasons for the difference in response to excess metal, in both vertebrates and invertebrates, is the difference in their ability to buffer excess metal (e.g. Brough and White, 1990; Deniseger et al., 1990). There may also be an ability to reduce uptake of metals as indicated for cadmium, zinc and lead in a carnivorous beetle (Denneman, 1990).

The environmental impact of smelters and metal-working industries is has been discussed in a number of recent publications, including a book entitled "Environmental Impacts of Smelters" edited by Nriagu (1984). Belyaev discusses some of the effects of the industry in his paper on the "Experimental-hygienic evaluation of the combined effect of the leading adverse factors of the production of blister copper" (in Russian; document not examined for I.C.A. review). Documentation of metal release from smelting and metallurgical plants is given in a number of references (e.g. de Lima and Patchineelan, 1989; Dumontet et al., 1990; Lundberg, 1987; Roszyk and Szerszen, 1988a,b; Sarritzu et al., 1989). Tsai and Nixon (1990) provide an assessment of waste management options for metal plating operations, particularly for small industrial operations. The publication also describes technical assistance and training programs given by Environment Canada. The U.S. Environmental Protection Agency (1990a) provides a document on the characterization and treatment of wastes from metal-finishing operations. Analytical determination of plating fluid components is discussed by Sopok (1990) in a U.S. Army Armament Research document.

The biological and chemical effects of smelter emissions are discussed in Yan and Miller (1984). The influence of copper smelter emissions on tissue metal levels is discussed for alder leaves (Mejnartowicz, 1986). Sutherland and Martin (1990) document a reduction in growth of a conifer (*Pseudotsuga menziesii*) as a result of what they term "air pollution" from copper smelting. Air pollution has also been suggested to be responsible for changes in lichen cover on pine trunks (Gorshkov, 1989). In soybeans, heavy metal uptake from metal-rich soils can be affected by mycorrhizal fungi (Heggo et al., 1990) suggesting a potential secondary factor in the effects of smelter emissions on tissue metal concentrations. Franzin (1984) found biological acclimation and adaptation in response to changes in atmospheric emissions and liquid effluents from base metal smelters at Flin Flon, Manitoba Canada. Regulation of metal levels does occur in many animals (e.g. Rantataro et al., 1989) although fish metal concentrations of copper and several other metals are reported to be highest in lakes having highest anthropogenic metal (Harrison and Klaverkamp, 1990). Nicolaidou and Nott (1990) note species differences in uptake of metals (including copper) by marine gastropods near a ferro-nickel smelting plant on the coast of Greece. Increases in metabolism have been reported for small mammals in a zone exposed to effluents of a copper-smelting plant (from translation of Kovalchuk and Mikshevich, 1988). Elevated copper levels have been reported in sheep near a copper smelter in eastern Europe (Vrzgula et al., 1986) and elevated liver copper as well as elevated mortality have been found for ewes after feeding copper oxide from what is termed "copper works" (Vrzgula et al., 1989). Spierenburg et al. (1988) report decreased copper levels in cattle in the neighbourhood of zinc refineries in the Netherlands.

Wang (1990) reports inhibition of millet seed germination after exposure to effluent samples from an acid bath from a metal-engraving industry. Metals in aerosols from smelters may have a detrimental effect on plant growth in forests (e.g. Vutov and Penev, 1987) and have been associated with increases in activity of two enzymes in alder leaves (Mejnartowicz et al., 1986). Le Du et al. (1990) found an effect of the receiving water on the toxicity of an electroplating industry effluent. They report (abstract) that "It appears that the mineralization of the water, more than the total organic carbon (TOC), is an important parameter for the expression of toxicity". Other factors include the interactions of the effluent components, Dive et al. (1989) state in a discussion of electroplating industry wastes (abstract) that "... chemical analysis of the effluents gives an unrealistic evaluation of the risks of such wastes, which is dependent upon multiple interactions occurring between components. As the residence times of these metallic components in water are different, interactions will be modified after discharge". High concentrations of metals in soils have been a factor in categorizing sites for listing on the U.S. National Priorities List (e.g. the Kerr-McGee site and Aladdin Plating Site inclusion on the U.S. Agency for Toxic Substances and Disease Registry, 1988a, 1989b). Concern has been expressed for worker exposure to copper and other metals in fumes and gases in industrial operations (e.g. van der Wal, 1990). Copper is one of several metals reported to be able to induce asthma and alveolitis in workers in metal recycling plants (Halloy et al., 1990). Exposure to selenium has been reported to be of potential damage to workers in copper refineries (Holness et al., 1989).

Recovery of metals and energy from industrial processes is of long-term environmental benefit as well as a possible financial benefit. Susko and Scheiner (1990) present an infrared spectroscopic study of flocculants obtained through a process developed by the U.S. Bureau of Mines for recovering metals from mining and mineral-processing waste streams. Patterson et al (1987) discuss the morphological aspects of copper hydroxide precipitation. Recovering heat from copper vapours is used at a brewery company to reduce energy outlay for heating boiler water (U.S. Department of Energy, 1988). Evaluation of metals in mining wastes can be used to indicate the availability of metals (e.g. Clevenger, 1990). Removal of metals from electroplating wastewaters (e.g. Süss and Russ, 1988) and from tailings pond overflow (Zaidi, 1980) is becoming of increasing importance both for the recovery of the metal and the reduction in input of metal to the environment.

Waste materials including sewage, sludges and wastewater

The impact of municipal and industrial waste materials is of continuing health concern for public health agencies (e.g. U.S. Agency for Toxic Substances and Disease Registry, 1987, 1988e, 1989d,h). Many of these materials contain copper and are deposited in waste material sites as bulk materials (e.g. Rousseaux et al., 1989) or sludges (e.g. Tijero et al., 1990). Techniques continue to be developed to analyze the nature of and to deal with waste products and sludges, to reduce bulk deposition and leaching of metals and organics from waste products (Barth et al., 1989; Teraoka and Nakashima, 1990; Tien and Huang, 1989). Reduction of metal levels is also used to bring trace metal concentrations down to levels acceptable for human and agriculture use (e.g. Regnier et al., 1982; Tran, 1989).

The U.S. National Technical Information Service (1989h) provides references concerning nutrient removal, heavy metal recovery and case studies of wetlands used for wastewater treatment. Use of sewage sludge can be beneficial and, when used properly, has been shown to increase agricultural and forest productivity without environmental problems (Cavallaro and Villarrubia, 1989; Davis, 1989; Gillies et al., 1989; Sabey et al., 1990). Likewise, proper wastewater use can provide nutrients without producing adverse metal concentrations in irrigated plants (Meshref et al., 1989). However, tissue and soil metal concentrations are affected by the nature of the wastewater or sludge as well as the nature of the soil or plant (Beckett, 1989; Berthet et al., 1989; Petruzzeli et al., 1989; Werner et al., 1989; Védy and Greter-Domergue, 1989). Greter-Domergue and Vedy (1989) note that the high affinity of copper for the disolved organic substances (in sludge-composted soils) diminishes its bioavailability. Even with this, the use of wastewater or sludges as fertilizers can increase soil and plant metal concentrations (Grigoryan, 1990) over long time periods (Berrow and Burridge, 1990; Burridge and Berrow, 1984; Dowdy et al., 1989). That is without the essential knowledge about the concentration of copper and other metals in sludges (e.g. Akhter, 1990) and the biological effects of the metals (e.g. Chaney, 1989). Gupta (1987), for example, points out the need to know the degree of metal saturation in soils as well as the metal-binding capacity of the soils.

Solid wastes such as municipal sludges, may beneficially alter the physical properties of the soil (Chang et al., 1989). However, long-term application of manure, fertilizers and sewage sludge can lead to an increase in metal concentrations and possibly bioavailability (Lal and Mathur, 1989) although this is not always true (see Levine et al., 1989). The addition of copper to pig feed can increase the tolerance of the organisms as well as the transport of metals away from the sources (e.g. Huysman et al., 1988). Crop uptake of metals from sludges has been demonstrated in fungi (Zabowski et al., 1990) as well as other plants. However, the level of copper uptake appears to depend on the nature of the sludge, the soil, and the plant (e.g. Kiemnec et al., 1990). Whatever factors are important, the effects can be direct (e.g. increases in tissue copper or shifts in organism enzyme activities; Schuller, 1989) or indirect as for example, the influence on soil nutrient availability (e.g. Yang and Skogley, 1990). Clover nitrogen fixation has, for example, been shown to be affected by long-term contamination of metal (Giller et al., 1989; Koomen et al., 1990). Crop uptake in sludges can also be translated to effect on higher levels in the food chain. Glockemann and Larink (1989) demonstrate dietary intake by livestock and humans (Gambrell and Patrick, 1989) with a resultant negative effect on faunal diversity in a pond. Köck et al. (1989b) note accumulation of copper by small mammals at a waste disposal site. Metal levels in soils can also be ameliorated by the nature of the soil. Alberici et al. (189), for example, note trace metal increases in soil, vegetation and voles from mine land treated with sewage sludge.

Incineration of waste materials releases metals as aerosols. Characterization of some municipal waste ash samples is provided by the U.S. Environmental Protection Agency (1990b) and Mumma et al. (1990) provide the results of a national survey of elements in municipal incinerator ashes. The nature of the aerosol as well as the ash (e.g. particle size) is a function of the waste material and the conditions of incineration. Rachwalsky (1989), for example, advocates the use of oxygen instead of air for high-temperature refuse incineration. Important aspects of municipal solid waste combustion are reviewed in Rood, 1988 and the 1988 World Health Organization manuscript on emissions of heavy metal and PAH compounds

Moriyama et al. (1989) review the concentration of metals in domestic wastewater. In wastewaters as well as refuse, sewage and sludges, the lability of copper and other metals is of importance because it indicates the potential for release of metals, for example to soils or the water table (e.g. Elliott et al., 1990; Hernando et al., 1989; Naohara et al., 1990; Schoer and Förstner, 1987). Conversely, the reaction of sludge metals with soil fractions (e.g. humic substances) provides an indication of the ability of natural substances to complex metals and reduce their bioavailability (e.g. Senesi et al., 1989b). The lability of metals in wastewater and sludges is also an indication of biological impact and the ability for reduction of metal concentrations (e.g. Goldstone et al., 1990; Lo and Chen, 1990; Melcer and Bridle, 1985). The same can be said for copper and other metals in waste products, such as peat residue and grape debris (Garcia et al., 1990). References dealing with treatment of wastewater and industrial streams are given by the U.S. National Technical Information Service (1989i,j, 1990d). Techniques for metal removal from waste waters also include biological uptake mechanisms (Ilangovan et al., 1990; Rai and Chandra, 1989). Contamination of fish can occur in areas affected by sewage (e.g. Joseph, 1989); uptake by fish grown in wastewater is discussed by Hadjmohammadi and Ghaziaskar (1989).

Degradation of digested sewage sludge in marine environments has been modelled (Nedwell and Lawson, 1990) with the suggestion that metals would be immobilized by the sediment. Metal-containing wastes may also be transported away from discharge sites in current-swept aquatic systems (e.g. Haertling, 1989). This will, however, be dependent on the current velocity as well as the volume and nature of the discharged material. Sites of major discharges are frequently sites of accumulation of contaminated sediments (e.g. Anderson et al., 1989b). Geochemical processes occurring shortly after introduction can affect the lability of copper. Paulson et al. (1991) obtained experimental evidence of initial flocculation with subsequent remobilization of copper in marine organic material and sewage. They suggest that the remobilization is responsible for the increased levels of dissolved copper in the bottom waters of Puget Sound (Washington). Differences in treatment of sludges may also be responsible for differences in the distribution of oxidizable and reducible metals which occurs after ocean dumping of sludges (Gibbs and Angelidis, 1989). Part of this is a result of the nature of the particle, including metal concentration and sinking rate; slower-settling microflocs are reported to contain most of their metals in the reducible phase and are transport greater distances than fast-settling flocs (Angelidis and Gibbs, 1991).

The biological impact of discharged sewage and sludges will be affected by factors discussed in the previous paragraph. Steimle et al. (1990b) and others report the presence of increasingly tolerant species, and reduced species diversity, with increasing sediment contamination by sewage and sludge. Various physiological disturbances have also been noted in fishes from waters receiving sewage and wastewater (e.g. Rao et al., 1990). There can also be an increase in tissue concentrations of some anthropogenic material near outfalls or dumping grounds although this is not a consistent finding with copper (e.g. McLean et al., 1991; Roy-Burman et al., 1989). Recovery of areas after cessation of sludge discharge (e.g. Thompson and Dorsey, 1989) suggests that the rate of biological and physical change is controlled by the amount of accumulated anthropogenic material as well as the nature of the surrounding environment, especially current exchange in marine regions. Recovery can also be achieved by pretreatment of discharged materials (Young et al., 1990).

Anthropogenic copper from industry and agriculture

Introduction of copper into soils and the atmosphere occurs as a result of industry, including the agricultural industry. Although uses of fertilizers are reported to increase soil copper concentrations, this is not always the case. Schwab et al. (1990) note that after 40 years of continuous fertilization with N and P, chelate-extractable copper levels were unaffected. However, drainage from agricultural lands is reported to contain concentrations of metals high enough that they may be detrimental to fish in receiving waters (e.g. Hamilton and Buhl, 1990). Leaching of copper from copper-based preservatives (Bergholm (1985), from wastes (Sakai, 1985) or from accidental release (Ward, 1989a) can also increase metal concentrations in soils or receiving waters.

Aerosol metal from industrial sites is a major source of copper to soils and aquatic sites (e.g. Makarova and Moiseenko, 1990; Ndiokwere and Ezihe, 1990; Pacyna and Münch, 1989; Steinnes, 1990). Aerosol copper can also originate from indoor sources of dust (e.g. Raza et al., 1990). Soils and plants in areas receiving aerosol input from industrial and minerological areas may also have elevated metal levels, including copper (e.g. Braginskiy and Myrlian, 1990; Ding, 1989; Kortesharju, 1989; Lin et al., 1989). High levels of metals may also be associated with physiological imbalance of plants, needle loss of conifers, for example (Mankovska et al., 1989). Effects on animals are also apparent (e.g. Onwumere and Oladimeji, 1990) although affected by the nature of the animal species and their food relationships. Heliövaara and Väisänen (1989; 1990b), for example, note that tissue concentrations of copper in sawfly larvae feeding on pine were lower than their food. They also note a decrease in metal concentrations with increasing distance from a copper smelter in southwest Finland. Uptake is, however, affected by metal bioavailability and a number of other factors such as metal-metal interaction. Grazing animals, such as sheep, also accumulate metals from available metals in food although uptake is controlled by metal-metal interactions as well as metal speciation (e.g. Bires, 1989) and the physiological state of the animal (e.g. Bires et al., 1990).

Introduction of anthropogenic copper into aquatic environments from industry and agricultural sources is often detectable from metal concentrations in water, sediments and organisms (e.g. Fabris et al., 1986; Landsberger et al., 1990; Moiseenko and Kudratsjeva, 1990). Excess metal can affect species composition and metabolic functions of microbial flora (Kagalou et al., 1989) as well as other organisms if the metal (e.g. Hatakeyama et al., 1988) is in a biologically available form. Determining biological availability in areas of high metal concentrations often includes measurement of cupric ion activity (e.g. Camusso et al., 1989b).

Health assessments and remedial action are required by government agencies when release of anthropogenic metal is considered to be excessive. Recent examples of this, that are pertinent to copper, are provided by the U.S. Agency for Toxic Substances and Disease Registry (1988b,c,d; 1989a,c,e,f,g; 1990a,c).

Copper from transportation and power sources

Release of copper and a number of other metals from roadways provides a source of anthropogenic metal (e.g. Harrop et al., 1990). Ward (1989b) points out that eleven elements (V, Cr, Mn, Co, Ni, Cu, Zn, Br, Cd, Ce, Sm) have elevated levels with increasing mean traffic densities on British motorway environments. Copper release is associated with the wear of tires and brake pads as well as possible combustion of lubricating oils (Ward, 1990a); maximum concentrations of released metal are found in surface soils, from dust-born particulates as well as surface runoff. In a study of roadside dusts and soils in Cincinnati, Ohio, Tong (1990) reports average copper levels as high as 1882.5 µg/g in dust samples from a curb. Abdullah and Latiff (1988) report soil copper levels ranging from 1.4-7.1 ppm (mg/g) near five suburban roads at Koala Lumpur. Soils near a railway in Japan are reported to contain elevated copper levels (128-150 ppm) with levels decreasing away from the railway (Suzuki et al., 1987). Aerosol metal levels have also been shown to be elevated within vehicles, with a tendency for copper-enriched fine-grained particles to be higher in cars with a filtered ventilation system (Valtink and Liegmahl, 1989). Higher levels of copper have also been associated with fine-grained particles in drainage from urban areas, including roadways (Xanthopoulos and Hahn, 1990). As a result, urban catchment areas and detention ponds can be enriched with copper as well as other metals, especially lead (Revitt et al., 1990; Yousef et al., 1990). Mesuere and Fish (1989) note that copper was the dominant metal in a dentention pond system in a parking lot near Portland, Oregon. They state (abstract) that "Copper was found to be deposited in the pond sediments in a small but highly concentrated plume (up to 130 mg kg^{-1}), extending axially from the runoff inlet pipe". They also note that dentention ponds can be a useful management practice for runoff from parking lot areas. Stormwater runoff from storm drains is a possible mechanism for input of roadway anthropogenic copper into receiving waters (Schmoyer et al., 1988b). Uptake of roadway metals by plants and animals does occur near roadways. Butovskii (1989), for example notes copper levels of 11.7-37.9 µg/g in insects from agricultural ecosystems located near roads. Ivonin and Shumakova (1990) advocate the use of forest belts as a means of reducing roadway metal transport from roads into adjacent agricultural areas.

Coal-burning is and has been a major source of aerosol metals, including copper (Kubiznakova et al., 1989; Zhou et al., 1990). There is also a biological impact (e.g. Garrec, 1989) as indicated by metal concentrations in soils and plant tissues (Long and Davis, 1989; Mulchi et al., 1990). Ash from coal may, however, be beneficial as a soil addition, to modify physical properties of soils (Chang et al., 1989). However, ash as well as other byproducts of coal burning often contain elevated levels of metals although the chemistry of the medium infers that not all of the metal will be biologically available (e.g. Nerin et al., 1990; Pandey and Kumar, 1990). In a study of coal-ash leachates, van der Sloot and Wijkstra (1990) demonstrated an effect of abosrbents and complexants on reducing metal release from stabilized waste products. Plant uptake of metals grown on flyash dumps revealed differential uptake by two plants although both plants showed a similar trend of accumulation - root < stem < leaf > seeds (Kumawat and Dubey, 1989). Jenner and Janssen-Mommen (1989) suggested use of the duckweed *Lemna minor* for monitoring pulverized fuel ash leachates. They note an order of metal toxicity of Cd > Cu > Zn > As(Arsenite) > Se(Selenite) > Ge > B > Mo. In a mesocosm examination of pulverized fuel ash leachate, Jenner and Bowmer (1990) note that metal accumulation differed widely in different invertebrate animals. Retorted oil shale disposal piles also form a source of leachate metal although plant uptake is again species dependent (Stark and Redente, 1990a). Metal contamination has been noted in soils under pylons used to hold power transmission lines although copper levels do not increase (Herms and Peterson, 1990) even though copper is often used in power transmission cables. Reduction of metal concentrations in contaminated materials can be done although often at considerable expense. Lin et al. (1987) and Premuzic and Lin (1989) describe the successful treatment of brine sludges from geothermal power plants with strains of acidophilic bacteria to reduce metal levels and toxicity.

Widespread use of fossil fuels is associated with acid rain and its effect on freshwater and soil pH (e.g. Steinberg and Högel, 1990). Soil pH is affected by the nature of the soil as well as atmospheric fallout; Bergkvist (1987) notes, in Sweden, soil solution pH is lowest in spruce forests and highest in soils in forest regeneration areas. Kreutzer et al. (1989) found increased concentrations of ionic copper after acid irrigation of a Norway spruce stand (*Picea abies*). However, sensitivities to acid precipitation and metal bioavailability are species specific (Majumdar et al., 1989). Levels of copper are often found to be elevated in lakes near a source of copper and affected by acid rain (e.g. Yan and Miller, 1984). In a study of acidification effect on metal fluxes in Swedish forest lakes, Borg et al. (1989) found maximal metal concentration when water discharge was highest and pH lowest. However, they report that Fe, Al, Cu and Pb were largely deposited in the sediments of lakes and pH had a relatively small influence on metal sedimentation. In a controlled acidification study of a Wisconsin lake, Mach and Brezonic (1989) found no increases in dissolved copper after acidification. Yan et al. (1989) found that metal concentrations (including copper) in the zooplankton of 38 Canadian Shield lakes varied with lake location, acidity and community composition; seasonal succession of zooplankton species was important in the temporal variability in tissue metal concentrations. As indicated by freshwater clams, it is often difficult to link tissue metal concentrations in animals with acid stress (e.g. Pynnöen, 1990). This appears to be true even though growth patterns in at least one species, the Asiatic clam *Corbicula fluminea*, are reported to be a good indication of anthropogenic copper levels (Belanger et al., 1990).

Copper from dredged sediments

Sediment dredged from channels and harbours contains copper and other metals. The amount of the metal is controlled by the nature of the sedimentary material and the proximity of a natural or anthropogenic source of the metal. Metal can be released both during dredging and disposal of the dredged material although the amount released and its biological availability is quite variable. Ludwig and Sherrard (1989) discuss a sediment elutriate test designed to predict the upper limits of metal release during dredging operations. Dredged sediments can also sorb metals suggesting an equilibrium (sorbed minus desorbed) that could actually reduce metal concentrations in affected areas (e.g. Liao and Chen, 1989).

Although toxicity of dredged material is often not toxic (e.g. Parrish et al., 1989), some biological effects have been reported. Flatfish (dab, *Limand limanda*) maintained on contaminated harbour sludge are reported to have a lower antibody titre than those on reference sediments (Bucke et al., 1990). Beyer et al. report that copper concentrations in biota correlated with those from soil in confined disposal facilities for dredged material.

Reduction in metal concentrations or impact

The reduction in metal concentrations in mining and industry continues to be of importance (e.g. Gabler and Jones, 1988; Zaidi, 1989). Langer (1990) presents a technique for increasing the efficiency of reclaiming plastic and copper from cable scrap. Lützke and Klee (1987) measured heavy metal emissions from an industrial waste incineration plant in Germany and suggest measure for reduction of metal emissions. Lützke and Sobottka (1987a,b) provide a similar measurement and suggestions for a stationary fluidized-bed combustion system and (1987c) for a brown-coal-fueled power plant boiler. Lützke et al. (1987) provide similar discussions for several industries. Waterland et al. (1990) report an average collection efficiency of 43 percent for an ionizing wet scrubber in a rotary kiln incinerator. Fleischhauer and Korte (1990) discuss a "... reliable and cost-effective method for establishing cleanup criteria for heavy metals (including copper) at hazardous waste sites". Thompson and Dorsey (1989) review the changes that have occurred in metal concentrations and biological composition of a sludge discharge location after cessation of sludge input. Removal of metals from municipal wastes and sludges is examined by Hofstede et al. (1989), Ilangovan et al. (1990), Lo and Chen (1990) and Tran (1989). Hassett and McClimans (1989) report on the use of municipal solid waste incinerator bottom ash as a construction material. They comment that metals such as copper appear to be immobilized when the ash is incorporated into masonry products. Reduction in metals from sludge waters is discussed in Goldstone et al. (1990) and Melcer and Bridle (1985). Isolation of metals (including copper) from ground and river water is discussed by Lieser et al. (1989). They used activated carbon to isolate metals in order to identify the chemical form. Removal of metals from aqueous systems, including industrial waste streams is discussed in several recent references (e.g. Fletcher and Akgerman, 1989; Jones and Stewart, 1990; Regnier et al., 1982; Shengjun and Holcombe, 1990; Tijero et al., 1990; Yongxiang et al., 1990). Techniques for recovering copper from wastewaters are provided by Jang et al., (1990a), Kilbane et al. (1990), Ma et al. (1989) and Süss and Russ (1988). Pahlman and Khalafalla (1988) discuss the use of lignochemicals from the paper industry and humic acids from peat and coal, to remove heavy metals from process waste streams. Lignin use for heavy metal removal is also advocated by Verma et al. (1990). Gomez-Lahoz et al. (1989) discuss some of the dynamics of removing copper and cadmium from industrial wastewaters using sodium borohydride. Recovery of copper from plating operations is examined by Tsai and Nixon (1990), as a means of reducing metal input into the environment. Leak (1990) describes a simple and apparently inexpensive system for reducing metal concentrations in hazardous waste streams from radiator repair shops.

Leaching of metals from ores and from materials such as coal often includes the use of organisms. Tuttle et al. (1989), for example, review the chemical and microbiological factors influencing the leaching of trace metals from coal. The use of microorganisms to recover metal from a variety of agents is discussed in the U.S. Department of Energy publication (1990a) entitled "Harnessing Microalgae". Biological recovery is discussed for geothermal residual sludges, by Presmuzic and Lin (1989). Although the information in these publications is important, one needs to know the ability of the organism to take up metal as well as the impact of excess metal on organisms (e.g. Monniot, 1990). The use of organisms to recover copper and other metals from natural systems such as sediments requires an understanding of metal bioavailability and techniques to measure bioavailability (e.g. Campbell and Tessier, 1989). Darnall and Hosea (1990) discuss innovative technologies for metal biorecovery. These include the use of artificial wetlands (Wildeman et al., 1990) as well as organisms, either under free or controlled conditions (e.g. Venkateswerlu and Stotzky, 1989). Interactions between metals and microorganisms (Kuhn and Pfister, 1989; Poole and Gadd, 1989) offers one group of mechanisms for biorecovery. The use of bacteria is used to leach metals from ores (e.g. Serebryanaya and Vishevnik (1989) or metal mine soils (Obbard and Jones, 1989). Members of one group of bacteria (*Thiobacillus*) are widely used, to reduce the impact of metals in various media, including geothermal brine residues (Lin et al., 1987) and leaching of copper-containing ores (e.g. Huber and Stetter, 1990). Sugio et al. (1990) demonstrate the reduction of cupric ions with elemental sulfur by *Thiobacillus ferrooxidans* and Belyi et al. (1989) review the mechanisms underlying the ability of this species to isolate metals. Metal-organism interactions are discussed for the metal-containing yeast *Saccharomyces cerevisiae* by Straube (1990). Metal recovery is noted for algae (e.g. Greene and Bedell, 1990; Lopez et al., 1990; Maeda and Sakaguchi, 1990) and cupric ion adsorption is reported for fungi (Gadd, 1990; Wainwright and Grayston, 1989), including the yeast *Saccharomyces cerevisiae* (Huang et al., 1990). Huang et al. (1989a,b) describes the removal of copper and several other metals from groundwater with several species of fungi. Aquatic mosses have been reported to be effective not only in the monitoring of metal concentrations but also in water treatment (Prasad et al., 1989). Bog conditions supporting moss plants have been found to reduce the impact of acid mine water discharged from coal operations (Falbo and Weaks, 1990). Various vascular plants have also been used in the recovery of copper from natural or industrial situations (e.g. Dieberg et al., 1987; Okieimen and Okundaye, 1989; Rai and Chandra, 1989; Sen and Mondal, 1990; Sridhar, 1988). One over-riding factor is the cost of operation of biological systems to recover metals, a factor which needs to be examined very carefully in terms of cost of operation and overall benefit, as discussed in McNulty and Thompson (1990).

Use of a clay mineral (bentonite) is reported to assist in the recovery of copper, iron and zinc from wine (Soulis et al., 1989b) and pectic acid is reported to be an effective chemical for recovery of the same metals, plus nickel, in vegetable oils (Ivanov et al., 1990). Keurentjes et al. (1990) describe a membrane extraction procedure for removing metals from edible oil. Leaching of metals from thermally-decontaminated soils is discussed by Mooren and van der Heide (1989).

III - COPPER SPECIATION AND ITS BIOLOGICAL IMPORTANCE

III.1 METAL SPECIATION

Copper occurs in a wide range of chemical forms and states - particulate, colloidal, dissolved, organic, inorganic. As a result, knowledge of copper speciation is essential to an understanding of the biological as well as economic importance of copper. In essence, the:

chemistry of the metal is involved in geochemical processes which dictate the mineral form.

chemistry of the metal is considered in any metal-finishing process.

chemistry of the metal is involved in geochemical processes which control metal bioavailability. This is the ability of an organism to take up the metal and use it in metabolic processes.

chemistry of the metal is involved in the use of copper in medicine and agriculture.

A number of excellent discussions of copper have appeared in recent literature including those in a text on marine geochemistry (Chester, 1990) and discussions in papers from the proceedings of an international workshop on Trace Element Analytical Chemistry in Medicine and Biology (edited by Brätter and Schramel, 1988). Other papers include Fischer et al. (1990c) and, in an older paper, Wollast (1982). Papers on the properties of copper include Hering (1988), Ribot et al. (1989) and Sugar and Musgrove (1990); copper is used in teaching student about chemical techniques as well as chemical properties of metals (e.g. Tanaka and Koga, 1990). Interactions of metals with chemicals in natural environments continue to be explored to understand natural processes and rates of reaction (e.g. Hering, 1988; Hering and Morel, 1990; Vazquez et al., 1989a). This is also the case for the interactions of metals with organics, in order to facilitate analytical processes (e.g. Suh and Arnold, 1990) as well as an understanding of biochemical processes in organisms. Papers on the chemistry of mining wastes include Clevenger (1990) and are discussed in another section of the review.

Discussions of biological availability appear in the Manual on Aquatic Ecotoxicology edited by de Kruijf et al. (1988). Measurements of availability frequently are associated with metal complexation (Sun et al., 1990b; Zhang et al., 1990a). Evaluations of metal complexation are important, especially in terms of biological processes and products (e.g. Harris and Ramelow, 1990; Slauenwhite and Wangersky, 1991). The relationship between geological properties and trace element availability considers metal properties (e.g. Anke et al., 1988b) as well as metal-metal interactions (e.g. St-Cyr and Crowder, 1990). Measurement of environmentally important organometallic substances of biological origin is reviewed by Brinckman and Olson (1990). In a paper on the regulation of heavy metal concentrations in natural aquatic systems, Schindler (1989) discusses copper in terms of "simple steady state models". He points out that the steady state model together with a surface complexation model can be used to provide an indication of the factors that control the fate of trace metals in natural aquatic systems. Humic substances can, for example, affect metal speciation (e.g. Ephraim and Marinsky, 1990). This is a factor that has been considered in terms of water-treatment processes (Perdue, 1989). Removal of copper from aqueous solutions is discussed in several papers, including Varma et al. (1989) who used lignin as a removal agent.

pH and organic ligands play important roles in metal speciation as well as transport and deposition of metals in aquatic environments (e.g. Lazerte et al., 1989). Copper-organic interactions have been examined to identify the nature of the organic complexing agents as well as their effect on metal bioavailability. Mackey and O'Sullivan (1990), for example, report that organic and copper-organic complexes in a seawater-filled bag become more polar as a phytoplankton bloom progressed. Hung et al. (1990) comment that nonpolar copper-organic complexes indicate anthropogenic agents. Strongly binding organic complexes have been isolated from natural systems (e.g. Hansen et al., 1990). Physical and chemical changes are important to metal speciation in aquatic environments (e.g. Stiller and Sigg, 1990) and are normally considered in chemical speciation models (Kramer and van de Meent, 1988). In Lake Orta, a freshwater lake in Italy, liming has been advocated as a means of changing the pH and decreasing the concentration of dissolved copper that originally came from industrial use of the lake water (Mosello et al., 1989).

Copper concentrations in aquatic sediments are indicative of transport of metal through the water as well as the nature of the sediments and the chemical processes occurring on and within the sediments (e.g. Martincic et al., 1990a). As such, sediment metals are frequently classified into general categories that include "adsorptive and exchangeable", "bound to carbonates", "bound to reducible phases", "bound to organic matter and sulphides" and "residual metals". The distribution of copper within these categories has been used as an indication of anthropogenic metal as well as metal from natural sources (Pardo et al., 1990). Work on metal accumulation and speciation in sediments has also appeared in Ajayi and Mombeshora (1989), Belokon (1989), Elsokkary and Müller, 1989; Fernex et al. (1989), McKee et al., 1989b, Rauret et al. (1989), Sager et al. (1990) and Steinberg and Högel (1990). Exchange between the water and sediments is in both directions, sedimentation into the sediments, remobilization from the sediments back into the water (e.g. Allen et al., 1990; McKee et al., 1989a,b; Rae, 1989; see also Brand, 1989). Paulson et al. (1991) note high levels of release of sewage-derived copper during sedimentation and from benthic sediments within 10 km of an outfall in Puget Sound. Effects of hydrothermal systems are to produce high concentrations of sulfide metal species (e.g. Veldeman et al., 1991).

Speciation of metals, including copper, in estuaries is of importance because estuaries form the major entry point of terrestrial metals into marine environments. Yeats (1988) discusses processes affecting trace metal fluxes through the St. Lawrence estuary, commenting (page 6) that "dissolved copper ... generally appears to behave conservatively". In a later paper (Yeats, 1990) he points out that the St. Lawrence estuary provides a natural laboratory for studying processes that affect the reactivity and transport of trace chemicals. Other recent studies of copper and other metals in particular estuaries include Allen et al. (1990), Cossa (1990), Elbaz-Poulichet et al. (1991), Lapin et al. (1990), Piron et al. (1990), Rae (1989), Rajan et al. (1989), Shi et al. (1988) and Shibu et al. (1990). Trace metal-nutrient relationships in estuaries are discussed by Windom et al. (1991) who note that copper values covary closely with silica. The increasing interest in colloidal associations of metals is apparent in the review of Sigleo and Means (1990) who note a definite tendency for association of copper with organics. There definitely is an association of copper with organics in estuarine sediments (e.g. Allen et al., 1990; Piron et al., 1990; Rajan et al., 1989; Shi et al., 1988)

With soils, recent work on copper and other metals includes the use of chemical models to estimate metal sorption (e.g. Christiansen, 1989) and speciation. Soil properties are key factors in evaluating the requirements for copper in fertilizers (e.g. Jasiewicz, 1990). In soils affected by salts there can be a reduction in plant uptake of copper even though an adequate metal supply is provided (Arshad and Quraishi, 1990). Metal contamination potential was a key topic at a 1988 meeting of an "Experts' meeting of the Dechema working-group" in Oberursel Germany. Soil factors that were considered to have the greatest influence on metal speciation and retention included pH, Eh (redox potential) and concentrations of clays, iron and manganese oxides, and humic substances. In a Ph.D. thesis, Stam (1989) discusses acidic precipitation and notes its effects on soil chemistry and bioavailability of aluminum, manganese and copper. Metal associations and soil composition have been evaluated (e.g. Jiang et al., 1990b; Rabenhorst and Fanning, 1989), especially in terms of metal sorption/desorption (e.g. Gaszczyk, 1989, 1990a,b) and metal bioavailability (e.g. Ruszkowska et al., 1989c; Singh et al., 1989b). Extraction of soil copper by water is influenced by the pH and dissolved organic carbon concentration in the water (Duffy et al., 1989). Groundwater can thus form a source and a transport mechanism for copper. Wells et al. (1989) comment on the importance of mobile organic particulate matter in groundwater, on the transport of transition metals in aquifers. Bioavailability of transported copper is, however, dependent on the nature of the organic (e.g. Greter-Domergue and Vedy, 1989). Concentrations of copper and other metals in soils near mines, smelters and metal-finishing plants continue to be examined (e.g. Sarritzu et al., 1989).

Atmospheric and runoff transport of natural and anthropogenic copper is of continuing interest, to obtain estimates of metal fluxes as well as potential impact from industrial activity. Fly ash from thermal power plants is, for example, a source of copper as a silicate, oxide, sulfide and metal (Nerín et al., 1990). Bergkvist et al. (1989) review fluxes of Cu, Zn, Pb, Cd, Cr and Ni in temperate forest ecosystems, noting that the mobility of copper is strongly dependent on the solubility of organic matter.

In an evaluation of metal fluxes to Swedish forest lakes, Borg and Johansson (1989) note highest runoff transport of several metals (Zn, Cd, Cu, Pb, Hg) in acidified areas. Kreutzer et al. (1989) note that acid irrigation has caused measurable changes in the chemistry of the soil of a mature Norway spruce stand. They comment that (abstract) "Liming ... led to a significant increase of dissolved organic C, which is associated with mobilization of metals such as Pb, Cu and Al in organic complexation". The extractability and mobility of heavy metals, including copper, in organic wastes such as aerobic sewage sludge and peat residue can be reduced by composting (García et al., 1990).

III.2 COPPER BIOAVAILABILITY

A number of factors affect the biological availability of copper. Many of these have been or will be discussed in other sections of this review. This short section serves as a review of the organic agents that can affect bioavailability and the nature of their effects. This is important not only to better understand the availability of copper in the environment but also the availability of copper in food materials. This is exemplified by two sentences in the introduction to a review of the roles of copper and the problems of copper deficiency (Wachnik, 1988; page 755):

> "The course of development of diagnostic methods has made it possible to study the role of copper in the etiopathology of some diseases. The problem of dietary copper deficiency is drawing the attention of many scientists nowadays."

Another review that stresses the importance of metal bioavailability is that of Kies (1989a). There is some evidence that research is tending towards an integration of biochemical and physiological work to understand the entire organism rather than just its parts (e.g. Momcilovic, 1988). In a Ph.D. thesis, Rengers (1988) evaluates a model for determining copper bioavailability in food. Metal bioavailability is more and more being considered in evaluating the impact of copper in the environment. Bergseth (1990) discusses the problem of identifying a tolerance limit for metals in soils above which plants take up excess metal. Problems of metal speciation and bioavailabiity are now considered by many individuals (see Manahan, 1989 for example, or the papers in de Kruijf et al., 1988).

Lack of relationship between soil copper concentrations and plant uptake or tissue metal levels can often be attributed to metal availability (e.g. Bell et al., 1991; Jiang et al., 1989; Mtimuni et al., 1990). The geological origin and geochemical nature of the site as well as environmental conditions and fertilization thus become important (Anke eg al., 1988b; Mathur and Lévesque, 1989; Ruszkowska et al., 1989c). In a discussion of copper bioavailability in terrestrial environments, Alloway (1990) notes relatively low bioavailability as a result of strong adsorption by organic and mineral colloids. It is also commented that although this is advantageous in contaminated soils because it reduces phytotoxicity, it increases the deficiency problems in soils of marginal status. Humic substances, or their precursors, are important metal complexing agents in soils (e.g. Senesi et al., 1989a) and are being examined by a variety of techniques (e.g. Ephraim and Marinsky, 1990; Singh, 1989b). Soil pH can affect both metal sorption and complexation, thus changing availability as well as metal concentration; uptake by plants is thus dependent on the interactions occurring with a number of factors (e.g. Demir et al., 1990; Folkeson et al., 1990). The influence of metal supplementation (e.g. fertilizers) on availability is dependent on the chemical conditions of the soil as well as the chemical nature of the supplement (Bednarek, 1989; Gambrell and Patrick, 1989; Gorlach, 1989; Jasiewicz, 1990; Kuczynski and Felinski, 1989; Pezzarossa et al., 1990). Verma et al. (1989) notes a relationship between the cation-exchange capacity of rice roots and the uptake of several metals, including copper. Agricultural practices such as intensive forage cropping and flooding can also affect the speciation of copper in soils (Gallardo-Lara and Torres-Martín, 1990; Misra et al., 1989a; see also Sánchez et al., 1989). In aquatic sediments, metal concentrations are used to indicate anthropogenic input (e.g. Johansson, 1989; Szefer, 1990b). However sediment extraction techniques are important and continue to be evaluated to relate metal concentrations to bioavailability (e.g. Campbell and Tessier, 1989). Physical and chemical properties of copper and other metals play major roles in bioavailability (e.g. Gunn et al., 1989; Krantzberg, 1989a). Depledge and Rainbow (1990) review metal uptake and metal flux in marine invertebrate animals They comment on the importance of speciation in uptake and metal-binding agents such as metallothionein in regulation of tissue metal levels.

Naturally-occurring organics continue to be examined in aquatic environments, to better understand the speciation of metals such as copper and to evaluate metal bioavailability under conditions of both deficiency and excess (Gardner and Gunn, 1989; Hansen et al., 1990; Hering and Morel, 1990; Lapin and Yedigarova, 1990; Lüderitz et al., 1989c; Mackey and O'Sullivan, 1990; McCarthy, 1989; Robinson and Brown, 1991; Shanmukhappa and Neelakantan, 1990; Slauenwhite et al., 1991; Strnad, 1987; Tracey, 1990; Zhang et al., 1990b). Techniques used to determine copper complexing capacity include polarography (e.g. Zhang et al., 1990a) and bioassay (e.g. Sun et al., 1990b). Warning about techniques used to measure the effects of copper on biological systems have also appeared in the recent literature (e.g. Renganathan and Bose). Mathematical models have also been used to estimate metal speciation in the environment and metal affinity for proteins in organisms (Suh and Arnold, 1990).

The relationship between speciation and uptake by or effect on organisms continues to be important in work on organisms (e.g. Chen et al., 1988a; Cosson-Mannevy et al., 1989; Cromwell et al., 1989; Domsch, 1989; Hung et al., 1989; Nakamura, 1990; van Eck et al., 1989; Versteegh et al., 1989; Zanetti et al., 1990) as well as food (e.g. Kies, 1989b; Vinson et al., 1989). This is indicated by the variety of forms of copper supplements for domestic animals (e.g. Cromwell et al., 1989; Wilson, 1989; Zanetti et al., 1990). It is of course, also necessary to consider physiological and biochemical factors associated with the organism (Belanger and Cherry, 1990; Gordon, 1989; Johnson, 1989b). Numerous examples of this occur. Bremer and Geesey (1990 - Cu90 talk) comment, for example, that copper-corroding biofilms (introduction) "... are often comprised of a consortia of physiologically distinct microorganisms bound together by a polymeric matrix". Efficient use of bacteria to leach copper ores requires an understanding of the organism as well as the chemistry of the process (Sugio et al., 1990). The action of copper-containing antitumour agents requires not only an understanding of the chemistry of the compound but also its action in organisms (e.g. Cao Vazquez and Diaz Garcia, 1989; Garcia Minsal et al., 1989).

Metal-metal interactions and carbohydrates such as fructose have been implicated in the control of copper uptake and status within the organism (Angyal, 1990; Fields et al., 1989; Johnson, 1989a; Kies and Harms, 1989). The action of molybdenum and sulfur in particular, can affect copper status in sheep and some other domestic animals (Olkowski et al., 1990). Fiber and phytate are also important agents that affect copper uptake and utilization (Kim and Vanderstoep, 1989). Copper transport within the organism involves organic complexation (e.g. Merceer-Smith et al., 1989); interactions of copper with some of these and some other organics within the organism have been associated with abnormal copper metabolism (e.g. Yamamoto et al., 1990a). Goode et al. (1989) provide a good review in their discussion of the mechanisms of copper transport and delivery in mammals.

Although the concentrations of environmental copper can be affected by the activities of man, the biological availability of any added metal is dependent on metal speciation. For this reason it is important to understand metal speciation and metal bioavailability in anthropogenic metal as well as the changes that occur once the metal is added to the environment (e.g. Häni, 1990; Santiago et al., 1989; Stam, 1989; van de Guchte and van Urk, 1989; Verloo et al., 1987). This is true whether the metal is in agricultural byproducts (e.g. Petruzzelli, 1989), human sewage (Chaney, 1989) or industrial effluents (Lewis, 1990). Du et al. (1990) suggest a method for evaluating the interaction of electroplating wastes with receiving waters, to estimate biological impact. Acidic precipitation can alter metal speciation in soils and fresh waters, introducing a complicating variable to the understanding of speciation and bioavailability (Belanger and Cherry, 1990; Kreutzer et al., 1989; Nordberg, 1990; Stam, 1989). Changes in both biological and environmental parameters can occur and affect metal speciation, bioavailability and thus uptake and metabolism (e.g. Chu et al., 1990a). Techniques for metal depuration are suggested for commercially important shellfish harvested from areas receiving anthropogenic metal (Hiraoka, 1991).

III.3 ORGANIC COMPLEXING AGENTS

Natural and synthetic organic ligands play an important role in influencing the speciation of copper and affecting the interaction between the organism and the metal (Cochrane et al., 1991; Gardner and Gunn, 1989; Hansen et al., 1990; Ram and Raman, 1988; Reddy and Prasad, 1990; Zolotukhina et al., 1989). Hering (1988) points out that although copper complexation is normal in natural waters, the chemistry and metal associations of naturally-occurring complexing agents is not well understood. This even with the increasing amount of information on the chemistry of natural and synthetic organic ligands that can bind with copper (e.g. Aplincourt et al., 1990; Apte et al., 1990a; Bermond and Malenfant, 1990; Buffle et al., 1990; Jolley et al., 1988; Matsushita et al., 1989; Nozaki, 1990; Panda et al., 1990a; Ram and Raman, 1988; Reddy and Prasad, 1990; Roe and Valentine, 1990; Scrimin et al., 1989; van den Berg et al., 1990a; Werner and Erker, 1989; Tullius, 1989). This also includes a better understanding of metal speciation in foods and environmental media (e.g. Brätter et al., 1988). The metal-organic relationship can, for example, be affected by metal-metal interactions with a change in biological effect (e.g. Bienvenu and Kergonou, 1990). Replacement of cadmium with copper has been reported for some proteins although each metal may be bound separately (Suzuki et al., 1989c). Redox potentials can change with changes in the nature of a copper-containing compound, with subsequent shift in activity towards agents like hydrogen peroxide (Ozawa et al., 1988). Metal ion binding sites in proteins may be associated with hydrophobicity based on the occurrences of hydrophobic atomic groups (Yamashita et al., 1990). Metals in ceramics have been examined to understand the reactions to and associations with inorganic as well as organic materials in the ceramics (e.g. Gray, 1990).

Humic substances

A diverse group of organics derived from the breakdown of biological material, primarily plant material. Many humic substances are capable of complexing copper and probably play important roles in natural environments as well as those affected by man.

Occurrences: Hiraide et al., 1989; Senesi and Sposito, 1989 (leaf litter, not humic substances *per se*).

Complexing capacity: Giesy and Alberts, 1989; Hatira et al., 1990.

Chemistry: Cabaniss, 1990; Filella et al., 1990; Fischer, 1987; Gregor et al., 1989; Hatira et al., 1990; Lund et al., 1990; Maslennikov, 1989; Nor and Cheng, 1989; Ram and Raman, 1988; Senesi, 1990; Senesi and Sposito (1989); Strnad, 1987; Susetyo et al., 1990; Weis and Frimmel, 1990.

Copper-containing enzymes

Enzymes are proteins that control the rate at which reactions occur in all organisms. As such they are considered to be extremely important. A number of enzymes contain copper as a nucleus and 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 - de Jesus et al., 1989.

Activity (concentration) - Ischiropoulos et al., 1990; Mariucci et al., 1990; Natoli et al., 1990.

Activity (concentration) under abnormal or deficient conditions - Asayama et al., 1990; Askari et al., 1990a; Inagaki et al., 1989; Makita, 1989a; Oka et al., 1990; Thomas et al., 1990.

Biochemistry, chemistry, genetics - Banci et al., 1989, 1990a-e; Bauer et al., 1990; Bertini et al., 1990; Cannon and Scandalios, 1989; Carri et al., 1990; Chang, 1989; Ciriolo et al., 1990; Eifler et al., 1989; Galeotti et al., 1989; Goldstein et al., 1990; Greco et al., 1990; Hashimoto et al., 1989; Hsu et al., 1990; Kajihara et al., 1990; Kanematsu and Asada, 1990; Karpinski et al., 1990; Makita, 1989b; Ming and Valentine, 1990; Paci et al., 1990a,b; Porstmann et al., 1990; Redford et al., 1990; Roe et al., 1990; Rosato et al., 1990; Sakamoto et al., 1990; Sanchez-Moreno et al., 1989; Shen et al., 1990a; Sines et al., 1990; St. Clair, 1989 (Ph.D. thesis); Steinkühler et al., 1990; Steinman and Ely, 1990; Tabatabal et al., 1990; Vig et al., 1989; Yano, 1990.

Functions - Belzer et al., 1990; Ceballos et al., 1989; Fischer et al., 1990a; Imaizumi et al., 1990; Kelner and Bagnell, 1990; Montesano et al., 1989; Yim et al., 1990a,b.

Miscellaneous - Beck et al., 1990a; Chang and Kosman, 1990; Damerau and Wischnewsky, 1989; Harris and Dameron, 1988; Jornot et al., 1990; Jungbauer et al., 1989; Lesser, 1989; Tsukamoto et al., 1989.

Hydroxylases: Blackburn et al., 1990.

Laccases: Cole et al., 1990a,b; Karhunen et al., 1990; Klemens and McMillin, 1990; Quinton-Tulloch et al., 1989; Tamilarasan and McMillin, 1989.

Nucleases: Bruice et al., 1990; Sigman, 1990; Thederahn et al., 1990; Yoon et al., 1990.

Oxidases: Aihara et al., 1990a; Azzi and Müller, 1990; Casella et al., 1989b; Clark et al., 1990a; Cogoni et al., 1989; Coleman et al., 1989; Collison et al., 1989; Covello and Gray, 1990; Dooley et al., 1990; Gabriel et al., 1990; Gacheru et al., 1990; Hoshi et al., 1989; Kroneck et al., 1990; Leoni et al., 1990; Li, 1990 (Ph.D. thesis); Mather et al., 1990; Oda et al., 1989; Ohkawa et al., 1990; Peiffer et al., 1990; Piantadosi, 1989; Salerno et al., 1990; Savini et al., 1990; Sekiya et al., 1990; Shinmyo, 1989; Singh et al., 1990c; Solomon et al., 1990; Yoshikawa and Caughey, 1990.

Reductases: Ahlers et al., 1990; Coyne et al., 1989, 1990; Oblender and Carpentieri, 1990a,b; Scott et al., 1989; Suzuki et al., 1989d; Yount et al., 1990b.

Miscellaneous enzymes: Ali and Abdel-Moneim, 1989; Andrawis and Kahn, 1990; Baasov and Knowles, 1989; Balla et al., 1990b; Barton and Price, 1990; Brenner and Wu, 1990; Kalyanaraman et al., 1990; Kang et al., 1988; Kelley and Vessey, 1990; Lobarzewski et al., 1990; Loeffler et al., 1989; Martin and Evans, 1989, 1990; Menif and Martell, 1989; Ming et al., 1990; Nagamori et al., 1990; Nilsson, 1989; Shaw and Chu, 1989; Shaw et al., 1989; Suh et al., 1990; Suresh and Mohandas, 1990a; Thomas and Poznansky, 1990; Wigfield and Goltz, 1990a,b; Witsell et al., 1990; Yoon et al., 1990; Zaalishvili et al., 1990.

Ceruloplasmin - a copper-transport agent in the blood

Bingle et al., 1990; Bligh et al., 1990; Cannistraro, 1990; Cogoni et al., 1990; Musci et al., 1990; Ryll'kov et al., 1990; Winyard et al., 1989; Zgirski and Frieden, 1990.

Hemoglobin, hemocyanin and selected heme-containing proteins

Ma et al., 1990; Muhoberac et al., 1990; Salerno et al., 1990; Vanka, 1989 (Ph.D. thesis); Zolla et al., 1990. (See also Waite and Walker, 1988.)

Blue-copper proteins (other than those listed elsewhere)

Brader and Dunn, 1990; Collyer et al., 1990; Corin and Gould, 1990; Dinarieva and Netrusov, 1989; Drews et al., 1990; Gullotti et al., 1990; Holt et al., 1990; Kim and Kim, 1990; Kitajima et al., 1990; Messerschmidt and Huber, 1989; Rydén and Hunt, 1990; Sakurai et al., 1990b; Shepard et al., 1990; Urushiyama and Tobari, 1990; Zaitsev et al., 1989.

Complexing agents of nutritional importance

A number of organics in foods bind copper and can affect metal supply to the organism as well as modulate uptake.

Phytates: Champagne and Fisher, 1990; Champagne et al., 1990; Oberleas and Chan, 1990.

Metallothionein-like organics

Metallothionein and metallothionein-like organics transport copper within the organism. They can also bind excess metal, assisting the organism either in metal elimination or storage in an innocuous state. Elmes et al. (1988a) suggest that metallothionein-bound copper in the human liver is non-toxic.

General, including reviews: Grill et al., 1990; Reddy and Prasad, 1990; Steffens.

Occurrences and condition of occurrences: Andersen et al., 1989; Arumugam, 1989; Cochrane et al., 1991; Cosson, 1989; Elmes et al., 1988a; Mercer et al., 1988; Palida et al., 1990; Petering et al., 1988l Woodall, 1988; Zafarullah et al., 1989; Zolotukhina and Gavrilenko, 1990.

Chemistry, separation and/or function: Klein et al., 1990a,b; McCormick and Lin; Mulder et al., 1990; Nederbragt and de Wit, 1988; Nederbragt et al., 1988a; Nishiyama et al., 1990; Paynter et al., 1990; Richards, 1989; Schilsky et al., 1989a,b; Stillman et al., 1989; Tukendorf, 1989; Xia et al., 1990.

Metal regulation: Elmes et al., 1988a,b; Olsson et al., 1990; Suzuki et al., 1990d.

Genetics: Buchman et al., 1990a,b; Culotta and Hamer, 1990; Culotta et al., 1989; Dixon et al., 1990; Etcheverry, 1990; Hackett et al., 1990; Hamer, 1990; Hamer et al., 1990; Harlow et al., 1989; Zafarullah et al., 1990.

Phytochelatins: Grill et al., 1990; Loeffler et al., 1989; Matsumoto et al., 1990; Mendum et al., 1990; Steffens, 1990.

Copper, nucleotides and nucleic acids

Antonelli et al., 1989; Apelgot and Guillé, 1990a; Blagoi et al., 1988; Bütje and Nakamoto, 1990; Covello and Gray, 1990; Dizdaroglu et al., 1990; Gannett and Toth, 1990; Hutchens et al., 1989; John and Douglas, 1989; Kashige et al., 1990; Kawanishi et al., 1989b; Kenani et al., 1989; Kobayashi et al., 1990; Kornilova et al., 1989, 1990; Lickl et al., 1989; Macreadie, 1990; Nagy et al., 1990; Palaniandavar, 1989; Pan and Coleman, 1990; Portugal, 1989; Prütz et al., 1990; Que and Palacios, 1990; Stern et al., 1990; Tachon, 1989, 1990; Tajmir-Riahi et al., 1990; Tamilarasan and McMillin, 1990; Turpin et al., 1990; Veal, 1989 (Ph.D. thesis).

Miscellaneous copper-containing or copper-activated complexes

Properties: Aalten, 1989 (Ph.D. thesis); Abo El Maali et al., 1989; Ajiboye and Brown; Andersson et al., 1990a; Baker et al., 1990b; Basto and Machado, 1990; Baumgartner et al., 1990; Belokon et al., 1990; Berardi et al., 1990; Bessenbacher et al., 1989; Birdsall and Taylor, 1989; Bocian and Procyk, 1990; Brader et al., 1990; Byrnes et al., 1990; Capdevielle et al., 1990; Carrondo et al., 1990; Casassas and Izquierdo-Ridorsa, 1990; Casella et al., 1989a; Cha and Cooksey, 1989; Chace et al., 1990; Chakraborty and Bhattacharya, 1990; Cheng and Hulce, 1990; Chikvaidze, 1990; Cochran and Schultz, 1990; Cramer et al., 1990; Dallavalle et al., 1989; Daniele et al., 1990; Davis et al., 1990; Delben and Muzzarelli, 1989; Delben et al., 1989; De Rosch and Trogler, 1990; Diez et al., 1990; Dilli et al., 1990; Duerr and Czarnik, 1989; Durell et al., 1990; Eaton et al., 1990; El Maali et al., 1990; El-Saadani et al., 1988; Farkas and Buglyo, 1990; Farkas and Kiss, 1989, 1990; Fife and Pujari, 1990; Frenkel and Tofigh, 1989; Fujita et al., 1990; Gavioli et al., 1990a,b; Gener, 1989; Ghadiri and Choi, 1990; Gojon et al. 1990; Gonzalez and Damodaran, 1990; Gullotti et al., 1989; Habermann and Humlová, 1989; Haendler, 1989; Hanaki et al., 1990; Haynes and Vonwiller, 1990; Heald and Cotton, 1990; Hursthouse et al., 1990; Iverson et al., 1990; Jiang et al., 1990a; John and Green, 1990; John et al., 1989; Kant et al., 1990; Kawakishi and Uchida, 1990; Kawakishi et al., 1990; Khodari et al., 1989; Kiss et al., 1989, 1990; Knapp et al., 1990; Ko and Godin, 1990; Kovacic et al., 1989a; Kozlowski et al., 1990a,b; Lenz et al., 1990; Liang and Sigel, 1989, 1990; Liu et al., 1990a; Lommen and Canters, 1990; Lomozik and Wojciechowska, 1989; Maichle et al., 1990; Manjula et al., 1990; Marchettini et al., 1990; Marjit and Sharma, 1989; Martell and Shanbhag, 1990; Massa et al., 1989; Massoud et al., 1990; McMullen et al., 1989; Menabue and Saladini, 1990; Micera et al., 1990; Morgan et al., 1989; Nagata et al., 1990; Nandi et al., 1990; Narasimhamurthy et al., 1989; Nekroshus et al., 1990; Nishida, 1990; Norman et al., 1990; Nunn et al., 1989; Ohta et al., 1989; Pandeya and Patel, 1990; Purvis et al., 1990; Radomska et al., 1990; Rafter, 1990; Rahman et al., 1989b; Rai et al., 1990; Rao et al., 1989; G. Reddy et al., 1990a; K.H. Reddy, 1990; K.V. Reddy et al., 1990b; Reinaud et al., 1990; Rendleman and Inglett, 1990; Ross and Solomon, 1990; Salmon et al., 1990; Sandhu and Jaswal, 1990; Sanz Alaejos et al., 1990; Sarkar and Bandyopadhyay, 1989a,b; Saxena and Srivastava, 1990; Shemyakina, 1988; Shen et al., 1990b; Shuaib et al., 1990; Simoes Goncalves et al., 1990; Souaya, 1987; Souaya and Hanna, 1988a,b; Souaya et al., 1988; Sovago et al., 1990; Subczynski et al., 1990; Suzuki and Karasawa, 1990; Suzuki et al., 1990f; Szabó-Plánka et al., 1989; Takehira et al., 1989; Taniguchi et al., 1990; Tare et al., 1989; Tauler et al., 1990; Tkach and Kandaskalova, 1988; Uchida and Kawakishi, 1990a-c; Uchida et al., 1990a,b; Ullah and Bhattacharya, 1990; Varma et al., 1990; Wilczok et al., 1989; Wu et al., 1990a; Xiao et al., 1990a; Yang et al., 1989b; Yatsimirskii and Mosin, 1989; Yip et al., 1989; Zainal et al., 1990; Zhang and Lei, 1990.

Copper and organometallic drugs: Balla et al., 1990a; Brumas et al., 1989; Calvert and Simon, 1990; Chary et al., 1990; Dendrinou-Samara et al., 1990; El-Khateeb et al., 1989; El Maali et al., 1989; Fischer et al., 1989; Hijleh, 1989; Hogale et al., 1989; Ibrahim et al., 1989; Jackson and Kelly, 1990; Lyman et al., 1989; Meshnick et al., 1990; Morphy et al., 1990; Nagar, 1989; Pezeshk and Pezeshk, 1990; Tarasiuk et al., 1990; Ujjani et al., 1990; Vaille et al., 1990.

Bioactivities (drugs, radioisotope labels, etc.): Aihara et al., 1990b; Cao Vazquez and Diaz Garcia, 1989; Cole et al., 1989; Dilanyan et al., 1989; Garcia Minsal et al., 1989; Hasinoff, 1990; Kovacic et al., 1990a; McPherson et al., 1990; Mercer-Smith et al., 1989; Muralidharan and Freiser, 1990; Prasad et al., 1990; Roberts et al., 1989.

III.4 ADSORPTION AND ADSORBING AGENTS

The various types of reactions that occur between particles and metals are important to the environmental chemistry of copper. Adsorption and desorption reactions can change the concentration of copper as well as its chemistry and biological availability. Often, however, the interactions occurring on the surface of a particle are complex, involving metal-metal competition as well as the interaction with a range of components of the medium (e.g. Flemming et al., 1990; Park and Huang, 1989). Plavsic and Cosovic (1989), for example, point out the importance of understanding the effect of organic matter when it forms a coat on the particle.

Studies of sorption are applicable to natural environments as well as anthropogenic media. Since adsorption reactions can be used to remove metals from a medium, sorption techniques have been used in determining metal concentrations (e.g. Calvet and Msaky, 1990). Recent literature includes detailed examinations of copper sorption reactions with silica (Conklin and Waterbury, 1990), calcium (Compton and Pritchard, 1990; Papadopoulos and Rowell, 1989), sulfide minerals (Yuhuan et al., 1989) and chitosan (Delben and Muzzarelli, 1989). Discussion of sorption reactions in soils can be found in Duquette and Hendershot (1990), Gaszczyk (1989, 1990a,b) and Schulte (1988). The importance of pH in adsorption of copper in soils is discussed by Godfrin and van Bladel (1990) and Msaky and Calvet (1990). Because of their common occurrence in soils, sorption by iron colloids has been examined (Lamy et al., 1989). Particulate trace metals in fresh waters (e.g. Zhang and Huang, 1988) and reactions of metals with river muds (Salim and Bloh, 1989) are important to an understanding of metal transport as well as metal chemistry. Work continues on adsorption and desorption of copper by dredged sediments in an attempt to understand the effects of dredging and dredge sediment disposal (Liao and Chen, 1989). Bilinski et al. (1990) examined adsorption on inorganic solids in the Krka River, noting a tendency of higher adsorption at the highest of the salinities tested. Adsorption of metals to sludge continues to be examined (e.g. Tien and Huang, 1989) as does the stability of metals in sludge-composted soils. Metal-saturated montmorillonite clays have also been used to remove unwanted organics such as herbicides from soils (Pusino et al., 1989).

In seawater, organics affect copper speciation (e.g. Hansen et al., 1990) and adsorption of copper by organic particles such as chitin is important (Gonzalez-Davila and Millero, 1990). There is an increasing interest in and knowledge of metal sorption by organisms. Recent work discusses adsorption of copper and other metals by bacteria (Mullen et al., 1989), fungi (Muraleedharan and Venkobacher, 1990) and cyanobacteria as well as algae (Crist et al., 1990; Greene and Darnall, 1990; Harris and Ramelow, 1990). Ulberg et al. (1990) provide evidence indicating that the sorptive capacity of living cells considerably exceeds that of inactivated cells and inorganic sorbents. Savvaidis et al. (1990) describe a method for the rapid and semi-quantitative assessment of the extent of metal ion binding to microbial cell surfaces. Gardea-Torresdey et al. (1990, abstract) "... indicate that carboxyl groups on algal cells are responsible for a great pportion of copper(II) and aluminum(III) binding, ...".

Metal adsorption by dietary fiber is discussed in terms of potential effect on metal bioavailability to humans (Stachowiak and Gawecki, 1989). They report that copper was best absorbed on cellulose preparations with pH 5.02 and 8.23". Removal of metals from dilute aqueous solutions by sorption agents is being examined for foods such as wine (Soulis et al., 1989b) and for solutions used in or produced by industry (Jang et al., 1990a). Agents for this latter series of purposes include yeasts (Huang et al., 1990), algae such as *Chlorella* and *Scenedesmus* (Harris and Ramelow, 1990) and plant byproducts such as maize cob meal (Okieimen and Okundaye, 1989). Walker et al. (1989) discuss the interaction of bacterial cell envelopes with clay minerals, in an evaluation of the ability of the composite to immobilize heavy metals from solution.

IV. METAL-METAL INTERACTIONS IN ORGANISMS

Metals can interact to affect the uptake of copper as well as to change the concentrations needed for beneficial as well as detrimental effects (Momcilovic, 1988). References dealing with metal effects (e.g. Boulos and von Smolinski, 1990; Evans and Weingarten, 1990; Tyler et al., 1989) may infer or allude to interactions but not adequately cover their biological importance. Part of this is due to the complexity of many reactions, as Bremner (1988) points out for cadmium-zinc-copper interactions which can affect both humans and grazing animals. He comments that they can involve both 2-way and 3-way interactions and may also be influenced by iron status. Bremner (1988) also provides a brief but good review of copper-iron-molybdenum interactions. A number of other references review various metal-metal interactions. These include:

> Abdulla and Chmielnicka (1990 - effect of toxic metals on trace element metabolism), Gavrilenko and Zolotukhina (1989 - metal interactions and metal uptake by aquatic plants), Jamall and Roque (1990 - cadmium-induced alterations in ocular trace elements), Oleszkiewicz and Sharma (1990 - effect of metals on anaerobic digestion), Serebryanaya and Vishevnik (1989 - trace elment effect on bacterial leaching of manangese), von Frenckell and Hutchinson (1989 - co-tolerance to metals by a grass)

COPPER-IRON INTERACTIONS - in a discussion of mineral interactions relevant to nutrient requirements, O'Dell (1989) comments that excess iron can affect copper uptake and metabolism. Yokoi et al. (1990b) present evidence that the inefficient utilization of iron is involved in copper deficiency. Abdel-Mageed and Oehme (1990c) suggest that the interactions between copper and iron are not adequately understood, that further information is needed to better understand dietary requirements. This is important in the sense that plant and animal tissue metals provide a source of copper for humans. It is also important because the preparation and handling of food materials can affect metal concentrations (e.g. Diaz Romero et al., 1989). Cell uptake of copper and iron may occur at the same site in plants, producing metal-metal competition (Nickless et al., 1989a). Copper can, however, increase the reduction of iron(III) to iron(II) by a uronic acid, in plant uptake of iron (Deiana et al., 1990). In rats, Kattelmann and Gordon (1990) provide evidence that at marginal dietary concentrations, iron utilization is enhanced by copper. Similar evidence is presented (Gordon et al., 1990b) for the increase of iron retention with adequate dietary iron. Zinc can, however, affect the iron-copper relationship (e.g. Greger et al., 1988) in a somewhat complex manner, although metallothionein may be involved in buffering the effect of zinc (Lim and Gordon, 1990).

Tissue metal concentrations have been related to metal-metal interactions. Humphries et al. (1988) report that moderate increases (150 mg/kg) in dietary iron intake can decrease liver copper retention in calves. Copper-iron effect has been demonstrated in rats for iron concentrations in the brain (Shukla et al., 1989), liver, kidney and spleen (Gordon and Ellersieck, 1988; Shukla et al., 1990). However, there is evidence that tissue metal concentrations are affected by physiological condition as well as metal bioavailability (e.g. Lust et al., 1990). Johnson and Murphy (1988) note an effect of high dietary iron and ascorbic acid on copper and iron utlization during copper deficiency. Excess dietary iron has also been associated with a decrease in the activity of a copper-containing enzyme (ceruloplasmin) (Pellett et al., 1990) which is necessary for iron mobilization in the body (Abdel-Mageed and Oehme, 1990b).

COPPER-MOLYBDENUM INTERACTIONS - the interactions of copper and molybdenum often include sulfur; sulfurlike spectra are reported for both metals (Kaufman et al., 1990). Biologically important interactions between the metals have been reported for both plants (e.g. Agarwala et al., 1989) and animals. High dietary molybdenum and sulfate can often produce an apparent copper deficiency in ruminants, by affecting copper excretion and/or metabolism (Fungwe et al., 1989; Gooneratne et al., 1988; Humphries et al., 1988; van Niekerk and van Niekerk, 1989a). As an example, Auza et al. (1989) report that excess molybdenum can cause anemia due to decreased copper as well as liver cell damage, the latter probably from a direct effect of molybdenum. Detrimental effects of high dietary molybdenum and sulfate have also been associated with the reproductive cycle of female sheep and high pre-weaning mortality in lambs (van Niekerk and van Niekerk, 1989b). Although ram testicular function can be affected by copper deficiency, produced by excess molybdenum (van Niekerk and van Niekerk, 1989c), van Ryssen et al. (1990) found no obvious affect of excess molybdenum on ram fertility. Some of the effects appear to be the result of complexes between copper-binding proteins which have become associated with tetrathiomolybdate (Allen and Gawthorne, 1988). As a result of the potential detrimental effects of excess molybdenum, guidelines have been established for molybdenum in irrigation waters (Albasel and Pratt, 1989) and copper fertilization is advocated for molybdenum-enriched soils (Stark and Redente, 1990b). Because of its ability to cause excretion of copper, high levels of dietary molybdenum can be used to reduce the tissue copper levels in hypercuprotic sheep (Howell and Kumaratilake, 1990; van Ryssen and Barrowman, 1988). Kerr (1990) points out the importance of a balance between copper and molybdenum in livestock feeds, to reduce the chances of hypercuprosis.

Although evidence does suggest a molybdenum-copper interaction in other animals, the evidence is not always as conclusive as in ruminants (e.g. Anke et al., 1988a). Meyer et al. (1990) report that increasing dietary molybdenum in the rat causes a dose response decrease in serum copper but no significant change in ceruloplasmin or hematocrit and an increase in bone copper. Chen et al. (1988b), with female rats, found that molybdenum injections had no effect on copper utilization while copper injections improved molybdenum utilization. Fungwe et al. (1990) does suggest that supplemental molybdenum may influence oestrous activity and embryogenesis in rats.

COPPER-ZINC INTERACTIONS - the interactions of these two metals are considered to be "... of nutritional significance ..." (O'Dell, 1989, abstract) when zinc is in excess and copper is at low levels. Abdel-Mageed and Oehme (1990c), however, comment that (abstract) "The effect of zinc supplementation on the bioavailability of copper and iron is a matter of conjecture". Greger et al. (1988) briefly discuss copper as a mediator of zinc/iron interactions. From these three references, and many others, it is apparent that metal-metal interaction does occur but that, at least with zinc and copper, not a great deal is known. High levels of dietary zinc are reported to interfere with copper metabolism (e.g. Klevay and Pond, 1990b) and to reduce tissue copper levels (e.g. Bridges and Moffitt, 1990; Gordon and Ellersieck, 1988; Stahl, 1990). This has an application, the treatment of hypercupremic conditions such as Wilson's disease with zinc to reduce tissue copper levels (Brewer et al., 1989, 1990; Lee et al., 1989, 1990c; Schilsky et al., 1989c). The treatment can be enhanced by the use of the amino acid histidine which enhances the inhibition of copper absorption by zinc (Wapnir and Balkman, 1989). (The chemistry of both metals and their interactions with organics is of great importance in attempting to understand metal-metal interactions (e.g. Kajihara et al., 1990; Taylor et al., 1990; Yeh and Knochel, 1989).) Teraki et al. (1984) however found (translation) elevated liver copper concentrations in pregnant rats and their fetuses. The copper:zinc ratio has been used as a marker of ovarian cancer (Gal et al., 1989). Zinc deficiency has been associated with increases in copper in some, but not all, tissues of some animals (Gupta et al., 1989; Kalinowski and Chavez, 1990). Physiological adaptations, possibly a result of metallothionein action, is also considered to be important in the interaction of copper and zinc (Mercer et al., 1988; Pond et al., 1990; but see Chung et al., 1988). Mehta and Mehta (1988) comment that in the rat, the effect of interaction of zinc and copper on haemoglobin levels was not significant. The effect of citrate may be important in determining the concentration and interaction of zinc and copper in human milk although the evidence is somewhat contradictory (Atkinson and Lonnerdal, 1988).

Whether from environmental levels (e.g. Bires, 1989; Tyler, 1989b) or from food, the interaction of zinc and copper (and other metals) is considered to be important. Both are reported to act synergistically in their inhibitory effects on fouling organisms (French and Evans, 1988). Both can act synergistically and have detrimental effects in effluents (e.g. Cimino and Caristi, 1990; Fernandez and Jones, 1990). But a copper and zinc balance may be affected by arteriosclerosis (Sui et al., 1989) or may be important for cellular immunity in the elderly (Bogden et al., 1990). Panemangalore and Lee (1990) note that zinc intake at even 50% of the recommended daily amount can maintain normal levels of plasma zinc and copper and erythrocyte zinc levels in the elderly. Serum zinc and copper levels are reported to diminish in burn patients (Bhattacharya et al., 1989) although the authors report that aggressive hyperalimentation will grossly elevate their urinary levels.

COPPER-CADMIUM INTERACTIONS - in a discussion of interactions between components of electroplating industry wastes, Dive et al. (1989) point out (abstract) that the "Effects of copper and cadmium are strongly synergistic, but this synergism disappears in the presence of trivalent chromium". They stress the multiple interactions of components when they suggest that chemical analysis of electroplating industry effluents gives an unrealistic evaluation of the risks from the effluent. Lasheen et al. (1990) provide evidence that cadmium, copper and chromium act synergistically on the growth of Nile water algae. In laboratory rats, long-term exposure (3 weeks) to cadmium has been associated with an increase in copper in the kidneys (Chmielnicka et al., 1989). In contrast, Cempel et al. (1988, abstract) report that "A single intravenous administration of cadmium to rats was found to ... decrease copper concentration in the kidney". Ocular copper levels in rats have been reported to increase in rats fed low-selenium diets and treated with cadmium (Jamall and Roque, 1990). Iguchi et al. (1990) present evidence of cadmium interference with copper in a copper-containing enzyme (bone lysyl oxidase). In humans, cadmium-copper interactions (in terms of metal concentrations) are reported to occur in the placenta at "normal" levels of cadmium exposure and over a very short time period (Kuhnert et al., 1989). Some of the differences between various references may be a result of metallothionein effect. Cadmium supplementation over time does induce measurable metallothionein synthesis which can cause an accumulation of other metallothionein-bound metals, including copper (e.g. Brown et al., 1990a; Sayed and Friedberg, 1990; Suzuki et al., 1990c). However, there does not appear to be any overall trend to provide the generalizations needed for management (e.g. Brown et al., 1990a).

INTERACTIONS OF COPPER WITH OTHER METALS, METALLOIDS AND NUTRIENTS - when considering the biological availability and importance of copper, it is difficult to restrict the discussion just to metals. Metals, metalloids, sulfur, and nutrients can interact with copper to affect uptake, tissue metal concentrations and biological effect.

Manganese can compete with copper at uptake sites on the cell. This has been discussed in previous I.C.A. literature reviews and is again suggested by the report that excess manganese is associated with reduction in root copper in cucumber plants (Crawford et al., 1990b). In rats, **tin** can induce copper depletion, possibly as a result of decreased copper uptake and increased fecal loss or disturbed metal metabolism (Hight et al., 1990; Rader, 1991; Reicks and Rader, 1990; Rader et al., 1990). **Nickel** replacement of copper and zinc in copper-zinc superoxide dismutase is discussed by Ming and Valentine (1990). They state that the derived compounds can serve as good structural models for copper-zinc superoxide dismutase. In a study of the copper-containing blood pigment hemocyanin, Serafin and Gondko (1990) report binding of **cobalt**. The sites of binding include those other than the active center of the molecule. Nickel and **lead** can both disturb the metabolism of copper within an organism (Vodichenska and Razboinikova, 1989). In a review of lead and copper interactions, Miller et al. (1990) note that lead ingestion can induce symptoms of copper deficiency. Sierra et al. (1989), working with pregnant guinea pigs, found that dietary lead caused a decrease in copper in the blood, cerebellum and forebrain of the dams but an increase in the fetuses, in a dose-dependent manner. In the treatment of lead poisoning, the use of a metal complexing agent ($CaNa_2EDTA$) may affect tissue levels of copper as well as lead (Chisolm and Thomas, 1989). Working with winter wheat, Jensen and Aoalsteinsson (1989) note that copper (as $CuCl_2$; 10-500 μM)) reduced the active but not the passive uptake of **rubidium**. A reduction in passive and, to some extent, active uptake was noted by Jensen and Gussarsson (1990) for birch roots. Working with a bacterium (*E. coli*), Lebedev et al. (1990b) found evidence that copper cound induce an influx of **magnesium** into the cell. Saur (1990a) notes a potential synergism between copper and **boron** uptake in the maritime pine.

Metallothionein increase in response to **mercury** supplementation may be associated with an increase in bound rather than free copper (Skreblin et al., 1988) with a resultant perturbation of tissue copper. Copper supplementation can affect the pH optimum of certain enzymes (e.g. Farant and Wigfield, 1990) which may affect the metabolism of mercury, as well as other metals in the organism. **Vanadium** at high dietary levels can be toxic to chicks. In a study of the effects of dietary copper on vanadate toxicity, Hill (1990) reports that "The addition of copper to a corn-soybean diet at levels of 200 mg/kg and above lessened the growth-retarding effect of vanadate for chicks". Interactions of copper with **chromate** and **arsenate** have been reported for *Lolium perenne* seedlings (Nickless et al., 1989b). Direct or indirect interactions of copper with precious metals may occur. Roe et al. (1990) provide **silver**-binding constants for some derivatives of copper-, zinc-superoxide dismutase while Rafter (1990) reports that **gold** sodium thiomalate, used in the treatment of rheumatoid arthritis, removed copper(II) bound to bovine serum albumin. **Selenium** and copper can interact in an antagonistic manner, as suggested by the protective nature of selenium on parenchymatous organs when given with excess copper to hens (Madej and "Radzanowska, 1988). Millar et al. (1989b) report that when lambs are given selenium and copper supplements, the copper significantly increases blood selenium levels. This does not occur with selenium-deficient lambs. In a study of copper and **iodine** in pig diets with a particular rapeseed meal (high glucosinolate), Schöne et al. (1990) found that iodine deficiency was associated with a drastic increase in liver copper levels. Treatment of the rapeseed meal with copper(II) increased the blood serum zinc content and level of an important enzyme (alkaline phosphatase).

Emerick and Kayongo-Male (1990a-c) present evidence that, at least in the rat, **silicon** may increase copper utilization. Sulfur, as sulfide, may directly or indirectly affect copper uptake in ruminants. Nederbragt et al. (1988b) suggest, however, that there is little direct influence in the gut of ruminants and that compartmentalization of both copper and sulfur has to be considered; in other words an indirect effect. With plants, the dose and form of **nitrogen** fertilization may be important to growth as well as plant tissue copper levels. Jasiewicz (1989) found that an increase in ammonium nitrate in a fertilizer caused an increase in the copper content in the tops of rape. Kumar et al. (1990), however, reports a mutually antagonistic effect of nitrogen and copper on copper levels in wheat; copper in shoots and roots was lowest with ammonium. (Antil et al., 1988, also reports this relationship.) **Phosphorus** has been implicated in regulating copper transport and accumulation in the blue-green alga *Nostoc calcicola* (Verma et al., 1991). In contrast, Saur (1989b, 1990d) found that the presence of phosphate in soils strongly depresses the copper concentrations in needles of the maritime pine *Pinus pinaster*. In support of a negative effect, Timmer and Teng (1990) and Teng and Timmer (1990) found that excessive phosphorus fertilization may induce copper deficiency in nursery-grown hybrid popular cuttings.

V. UPTAKE AND ACCUMULATION OF COPPER BY ORGANISMS

Uptake of copper, whether by plants, animals or humans, from the environment or from food, is dependent on the metal being in a biologically available chemical form (Kies, 1989a). Since copper is an essential metal, there are often physiological capabilities to regulate metal concentrations once the metal is within the organism (e.g. Thompson, 1990; Wachnik, 1988). Metal bioavailability, metal concentration and metal metabolism are thus all important in examining uptake and the potential effects of deficiency (Janus et al., 1989; Prohaska, 1990a; Wachnik, 1988) as well as excess of metal. Simkiss and Taylor (1989) provide an excellent, general discussion of metal fluxes across the membranes of aquatic organisms. One of the more interesting reviews, albeit with humans in mind, is the discussion of "Metal-protein interactions in transport, accumulation, and excretion of metals" by Sarkar (1989).

The uptake of copper has been examined for a wide range of organisms, including bacteria (e.g. Cooksey et al., 1990a; Sakurai et al., 1990a), plankton (e.g. Revis et al., 1989), fungi and yeasts (Gadd and White, 1989), bryophytes (e.g. Brown and Brown, 1990) and both aquatic and terrestrial plants and animals (e.g. Bohac and Pospisil, 1989; Drbal and Veber, 1989; Rainbow et al., 1990). Some of this is discussed in the review of heavy-metal ecology by Tyler et al. (1989). The U.S. National Technical Information Service provides bibliographies of bioaccumulation references for both freshwater and marine communities (N.T.I.S., 1989c,d). They also provide references for uptake of heavy metals by shellfish and marine plants (N.T.I.S., 1990f), and bioaccumulation by fish (N.T.I.S., 1990a). Applications of uptake information are also available in recent references. Szefer (1991), for example, notes that in the southern Baltic biosphere, plankton are important for translocating certain trace metals (Fe, Pb, Cu) from surficial waters to bottom sediments. Transport and redistribution of trace metals by particles of biological origin is important in aquatic environments and has been discussed, for the marine environment, by Fowler (1989). References of application include some of the references on pollution effects of metals listed in bibliograpies provided by the U.S. National Technical Information Service (N.T.I.S., 1990e,f,g). Uptake of copper by humans is of obvious importance and has been examined by a number of workers (e.g. Johnson, 1989b). Some unique situations have been described as, for example, the uptake of copper across the skin of an individual exposed to an explosion of copper azide (Bentur et al., 1988).

The availability of environmental copper has been examined both for nutritional adequacy of plants and animals and for possible effects of elevated metal concentrations. Metal speciation is obviously important in both aquatic and terrestrial environments (e.g. Lewis, 1990). Bell et al. (1991), for example, note a relationship between free metal activity (Fe, Cu, Zn, Mn) and metal concentrations in barley plants. Alliot and Frenet-Piron (1990) note a relationship between metals in sea-water and metal accumulation in a species of shrimp in south Britany. They report seasonal increases of anthropogenic metal (Cd, Cu, Pb, Zn) in both water and shrimp, high in spring and summer and low in winter, which they attribute to anthropogenic metal. However, they do not consider seasonal physiological changes in the organism which affect tissue metal concentrations (Al-Thaqafi and White, 1991). Anthropogenic metal from atmospheric fallout is suggested as an important source of trace metals in marine environments (e.g. Szefer, 1990b) as well as on land (e.g. Braekke, 1990). Atmospheric input of metals is often associated with a reduction in pH, a condition which increases the chance for the free metal ion to be present and uptake to occur. This can have an impact on the apparent toxicity of copper to plants (e.g. Luderitz and Nicklisch, 1989a) as well as to the concentration of metal in drinking water (Nordberg, 1990).

In aquatic environments, the chemical relationship of copper with both water and sediment can affect the availability of metal for uptake by organisms (e.g. Monniot et al., 1990; Samant et al., 1990; van Eck et al., 1989). This includes metal-metal interactions. Salim and Bloh (1989), for example, note metal-metal competition for uptake sites on river muds, a situation which could affect the concentration and species makeup of metal in the overlying water column. pH of the overlying water is always important in determining the amount of metal reaching the sediment and metal bioavailability (e.g. Krantzberg, 1989a) as well as uptake (e.g. Lüderitz and Scholz, 1989). When evaluating the meaning of tissue metal concentrations of organisms in sediments, uptake must be related to the ability of the organism to eliminate or physiologically isolate excess metal. Organisms from metal-contaminated sediments are frequently shown to have enhanced ability to regulate copper (e.g. Krantzberg and Stokes, 1987). Byproducts of organism metabolism can also affect metal speciation in water and sediments. Geesey (1987) reports that bacteria isolated from metal-laden sediments in a fresh-water system can produce exopolymers which exhibit a high affinity for bacteria, with a stability constant of 7.3 x 10^8 and a maximum binding ability of 37 nmoles copper per mg exopolymer. Algal polysaccharides are also capable of binding copper (Kaplan et al., 1988), thereby affecting metal speciation in the overlying water. Phosphates may play a role in metal uptake by certain phytoplankton although the relationship is not obvious (Watanabe et al., 1989). The presence of anthropogenic agents such as sewage (e.g. Gunn et al., 1989)(e.g. Gunn et al., 1989) or synthetic metal complexing agents (e.g. Triton X-100; Gupta, 1990) also affects metal speciation and uptake.

The uptake of copper from soils is also dependent on metal chemistry as well as the nature of the organism. Elemental concentration in plant tissues is often compared with various soil metal-extracting agents in an attempt to evaluate copper availability (e.g. Demir et al., 1990). Food chain relationships take soil-plant-animal metal transfer into account, using copper levels in forage crops and animal tissues as an indication of uptake and metal availability (e.g. Anke et al., 1988b; Regius-Micsenyi et al., 1990a). In a paper from Cu90, Alloway (1990) reviews some of the factors that affect copper bioavailability in terrestrial environments. Jiang et al. (1989) discusses the relationship between chemical form and bioavailability of copper in some soils from middle-eastern China, pointing out the importance of soil organic components in reducing availability in soils receiving anthropogenic copper. Copper adsorption by soils is also important, a factor which is controlled by the nature of the soil particles (Tu and Qing, 1990) as well as well as other chemical components. Results from the study of soil-plant-animal relationships are not always apparent. Mtimuni et al. (1990), for example, fouond low or nonexistent correlation coefficients among soil, plant and animal tissues at a ranch in Malawi. However, when the nature of the copper is known it is possible to infer some relationships, even to use copper uptake as a tracer (Nichols et al., 1990 - copper was applied as the cupric ion in a study of herbicide efficiency).

Uptake in terrestrial plants is affected by soil pH as well as metal-complexing plant metabolites released into the root zone by the plant (Saur and Gomez, 1989; Sinclair et al., 1990; Youssef and Chino, 1989). Linehan et al. (1989) and Sinclair et al. (1990) note that there are seasonal changes in metal concentrations within the root zone which may result from the plant-released agents. Other factors that affect availability, uptake and tissue metal concentrations include flooding (Misra et al., 1989a) and fertilization (Jasiewicz, 1989; Srikumar and Öckerman, 1990; Warman, 1990). Beckett and van Staden (1990) note that seaweed concentrate had little effect on copper uptake although it did stimulate zinc uptake. Saur (1990a) notes that phosphate fertilization increases copper content of the roots of pine species but (Saur, 1990d) depresses the concentration of copper in the needles. The effect of phosphorus may be indirect, acting on an intermediary agent (e.g. fungi) which affects metal uptake by the plant (Eivazi and Weir, 1989). Vesicular-arbuscular mycorrhizal fungi may also affect plant tissue metal concentrations (Heggo et al., 1990). In organic-rich soils, copper fertilization is often beneficial to uptake, providing the amount of metal required by the plant (e.g. Kuczynska and Felinski, 1989). In other soil types, where a copper deficiency may exist, care in application is needed to reduce the chances for detrimental effects of copper (e.g. Sanderson and Gupta, 1990). Liming is often used to increase soil pH and reduce the availability of copper and copper uptake by plants (Jasiewicz, 1989).

The response of the cell to metals, including copper, is a component of the process of metal uptake. With aquatic organisms (as well as others), Simkiss and Taylor (1989) suggest that both ion pumps and endocytosis may be involved in metal transfer across membranes. In microorganisms (as well as macroorganisms), cell wall constituents have been implicated in metal binding (Green and Darnall, 1990; Premuzic and Lin, 1987). Ulberg et al. (1990) report that, with bacteria and microalgae, sorption of copper is greater by living cells than inactivated cells. In a microorganism (*Cunninghamella blakesleeana*), Venkateswerlu and Stotzky (1989) found greater cell wall binding of cobalt and copper if the cell wall contained elevated levels of phosphate and chitosan. From a number of studies (e.g. McKnight et al., 1990) it becomes apparent that the chemistry of the cell wall affects sorption. Techniques for measuring metal binding by microbial cells include the use of surface-bound cationic dyes which are replaced by metals (Savvaidis et al., 1990). Verma and Singh (1990) note a biphasic uptake of copper in a blue-green alga, rapid binding of the cation to the cell wall followed by metabolism-dependent intracellular uptake. Similar results (but with nickel and chromium, not copper) have been reported for the green alga *Closterium monoliferum* by Chaudhary and Sastry (1988). Singh and Verma (1988) found uptake to follow a pattern that could be related to models with Michaelis-Menten type kinetics, in the blue-green alga *Nostoc calcicola*. They (Verma et al., 1991) also noted a possible role of phosphorus in regulating copper accumulation and transport in the same blue-green alga. Metallothionein has been considered as a mechanism involved in metal uptake although, with copper in a yeast (*Saccharomyces cerevisiae*), Lin and Kosman (1990) found no direct involvement in uptake, as distinct from retention where it plays an important role. Distribution within microorganisms and plants, after uptake, can be studied with metal flux (Shaw et al., 1987) or stable isotope techniques (Yoshioka et al., 1987). The effect of phytates mis important in binding copper and other metals and affecting uptake and distribution within the organism (Oberleas and Chan, 1990).

In rooted plants, relative uptake of different metals may vary from species to species (Godt, 1989). Tissue metal levels may also differ between root and shoot. Cation-exchange capacity is important in uptake and may vary with plant type as well as stage of growth (e.g. Verma et al., 1989). Excess available copper may bind all available copper-uptake sites on the cell (Iwasaki et al., 1990a). From work on ryegrass seedlings, Thornton and Macklon (1989) provide evidence that differing contributions of cell wall adsorption and symplasmic absorption may account for differing effects of external copper concentration on uptake by the same tissue. Root plaque on the common reed *Phragmites australis* is a major source of iron and a minor source of copper for uptake by the plant although availability is affected by several factors, including pH (St-Cyr and Crowder, 1990). St-Cyr and Crowder (1987) also noted that the iron plaque is not an efficent means of reducing uptake of heavy metals. Organics in sediments where the reed lives may also be important, affecting metal bioavailability and, as a result, metal uptake (Suzuki et al., 1989e). The effect of root plaque has also been reported for the roots of the salt marsh plant *Spartina maritima* (Vale et al., 1990). Uptake of copper can also occur across the leaf cuticle although uptake from solution, in a precipitation event, does not appear to be at biologically significant rates (Scherbatskoy and Tyee, 1990).

Under certain conditions, the tissue metal concentrations of certain microorganisms and plants can be used as an indication of biologically available copper. As such, these organisms can be used to monitor background environmental levels of available copper (Gavrilenko and Zolotukhina, 1989; Prasad et al., 1989). Plants that accumulate metal can also be used as a means of reducing metal levels in effluents (Gadd, 1990; Nor, 1990; Sridhar, 1988; Straube, 1990) and soils (Mullen et al., 1989). The process of uptake in lichens is reviewed by Tyler (1989b) who also comments on the tolerance of some members of this group. A number of lichens and mosses are tolerant to copper and, in fact, there are "copper mosses" which occur in copper-rich environments (Satake et al., 1990). Copper deficiency occurs in a number of areas and plants (e.g. Olson et al., 1990), affecting the availability and uptake of soil metal.

Variations in plant tissue metal concentrations have been associated with differences in uptake and accumulation. Seasonal variations in tissue metal concentrations have been noted in a range of plants (e.g. Buwalda and Meekings, 1990; Kotzé and de Villiers, 1989; Malea and Haritonidis, 1990). Algal resistance to metal accumulation can vary with organism species (Maeda and Sakaguchi, 1990) as well as over time. High copper accumulation can be induced in the yeast *Saccharomyces cerevisiae* by menadione, a drug (2-methyl-1,4-naphtoquinone) (Funk and Schneider, 1989).

Metal uptake by animals can occur either from the environment or from food, or from both (e.g. Rao and Latheef, 1989). Like plants, tissue metal concentrations in animals have been used to indicate environmental conditions as well as physiological status (e.g. Alikhan, 1989; Ozoh, 1990b; Powell and White, 1990; Spicarova, 1989). However, although linear relationships between exposure time and uptake have been demonstrated in some animals (e.g. Zia and Alikhan, 1989), physiological processes affecting metal retention may limit the usefulness of animals as indicators (e.g. Amiard et al., 1989; Nott and Nicolaidou, 1989). Decreases in metal concentrations or bioavailability are suggested to occur along food chains with a number of metals (e.g. Zn, Mn - Nott and Nicolaidou, 1990a,c; Cu - Campbell et al., 1988). Rengers (1988) was only moderately successful when he used three indicators of copper uptake in the rat to evaluate the bioavailability of nutritional copper. Cheggour et al. (1990) note that both abiotic parameters and physiological processes play important roles in the seasonal changes in tissue metal concentrations of a polychaete worm and a bivalve mollusc in a Moroccan estuary. In a review of heavy metal levels in marine invertebrates Furness (1990) comments (page 74) that "The concentrations of heavy metals in the bodies (and tissues) of marine invertebrates, ..., depend on the accumulation strategy adopted by each species for each metal". The time spent in an organism, the so-called "biological half-life", will also vary from metal to metal and organism to organism (e.g. Marmolejo-Rivas and Páez-Osuna, 1990). There is also more evidence of physiological control of the uptake and accumulation of essential metals (nutrient metals) such as copper than for the non-essential metals such as cadmium (e.g. Ahsanullah and Williams, 1991). Storage of copper within an organism may be affected by elevated levels of another metal, the presence of nickel in the diet of a terrestrial isopod ("pill-bug") can, for example, adversely affect the uptake and hepatopancreatic storage of copper (Alikhan and Storch, 1990).

In evaluating the adequacy of dietary copper it is important to consider both copper absorption and endogeonous losses (Turnlund, 1989). Turnlund et al. (1990) provide evidence that, in young men confined to a metabolic research unit for 90 days, <0.8 mg/day is adequate to maintain copper status. Whether in domestic animals or in humans, the availability of copper in the diet is important. Copper in certain forms (e.g. Cu0) has been reported to be largely unavailable as a growth promotant, at least for weanling pigs (Cromwell et al., 1989). In contrast, copper sulfate and copper chloride provide much more available sources (e.g. Ivan et al., 1990). (Copper used to supplement dietary copper (e.g. Driver et al., 1988a) also needs to be in a form suitable for supplementation.) It thus appears that although food sources of dietary copper may be important (e.g. Kies, 1989b), the chemistry of those sources must also be very seriously considered (e.g. Fang, 1989; Vinson et al., 1989). The nature of the food source as well as the nature of the organism using that food source become important in determining the concentration as well as availability of copper (e.g. Brzozowska et al., 1989; Emanuele and Staples, 1990; Ivan, 1989; Kreuzer and Kirchgessner, 1990a; Ramelow et al., 1990; Trinidad et al., 1990). (Marits and Iscan, 1990, suggest that trace metals (Mg, Mn, Cu, Zn) in the humerus and femur of prehistoric man can be used for paleonutritional studies.) In addition, although uptake is important, utilization within the organism after uptake is critical to the well-being of the animal. Uptake and utilization can vary between species, strains of a single species (e.g. Verheesen and Nederbragt, 1988) and even within the life history. Utilization of copper can be affected by a variety of agents including food additives (e.g. Rauch et al., 1989). Ascorbic acid is reported to affect uptake and utilization of copper in rainbow trout (Dabrowski and Köck, 1989) and rats (Van den Berg et al., 1990b). Fructose has been shown to alter some indices of copper status in rats and humans fed diets low in copper (Johnson, 1989a; Koh, 1990). Fields et al. (1989) found when rats consumed a diet containing fructose during pregnancy it affected carbohydrate metabolism in the placenta and fetus. This may have a deleterious effect on the prenatal development of copper deficient offspring. Wapnir and Balkman (1990a) present evidence that the effect of fructose is not directly on the uptake of copper from the rat small intestine. Excesses of some amino acids are reported to interfere with copper absorption in rats (Wapnir and Balkman, 1990b). Lykken et al. (1990), however, comment from a study of ^{67}Cu absorption and retention in men consuming sulfur amino acids, that sulfur amino acid manipulations did not significantly affect copper absorption or biological half-life. Dietary fiber is reported to inhibit the uptake of dietary copper (Kies and Umoren, 1989) although the evidence is not conclusive (see Kincaid et al., 1990; Wood and Stoll, 1990). Stachowiak and Gawecki (1989) do present evidence that, *in vitro*, copper is sorbed by cellulose and, to some extent on apple pomace. They (Gawecki and Stachowiak, 1989) found release of copper from fibers with human gastric juice. The uptake of copper can also be affected by metals and metaloids, magnesium and selenium can increase apparent fecal losses of copper (Kies and Harms, 1989). In contrast, calcium increases copper utilization, possibly from neutralizing the effect of ascorbic acid on inhibiting uptake (Kies and Harms, 1989). Iron, zinc and copper may also interact, affecting the uptake and utilization of copper (August et al., 1989; Gordon, 1989).

Uptake and metabolism of copper can change with age. This has been examined for individual components of organisms as well as organisms of various ages. With human red blood cells of various ages, Chu et al. (1990b) found no measurable alteration in copper levels. In a study of the influence of aging on copper metabolism in rats, Dowdy et al. (1990) found that aging may influence copper utilization by reducing apparent absorption and by impairing endogenous copper turnover. A great deal of work has been done on the relationship between metal levels during pregnancy and uptake by foetuses and newborns of various ages. Some of this has been with domestic animals. Hill et al. (1988), working with the Chinese water deer, report changes in foetus copper concentration during development; they also found seasonal changes of foetuses conceived at different times of the year. Grace et al. (1988) note an increase in swayback, a disorder caused by copper deficiency, in lambs born to ewes given high levels of molybdenum, a metal which competes with copper for uptake. In work on copper transport from mother to foetus at the end of gestation in rats, Lee et al. (1990d) concluded that ceruloplasmin is probably the main source of copper from the mother. The copper, after entering the placenta, may be transferred to transcuprein and albumin in the fetal circulation then taken to the liver for storage and incorporation into fetal ceruloplasmin. Possibly in a comparable manner, foetal deer accumulate high concentrations of copper in the liver during the last month of gestation (Leighton et al., 1990). In young infants and preterm infants, uptake of copper is better from breast-milk than from unsupplemented cow's milk formula (Dörner et al. (1989). The authors conclude that breast-milk copper is more available and recommend that cow's milk formula be fortified with copper up to a level of at least 600 μg/L. In a discussion of trace elements in infant formulas, Lönnerdal (1989) recommends an upper limit of 1.2 mg/L for copper.

Organisms exposed to high levels of metal may be affected if the metal is in a biologically available form. Far too often, however, it is the concentration of metal rather than its availability which is evaluated. As a result, evaluation of metal effect can infer bioavailability and impact of anthropogenic copper (e.g. van de Guchte and van Urk, 1989). When an organism is exposed to excess biological available metal it needs to have mechanisms to tolerate the metal and to store or eliminate excess metal that is taken up. As an example of this, Jeanthon and Prieur (1990a) found copper resistance in 88% of the bacteria isolated from a hydrothermal vent polychaete worm living under metal-rich conditions. In general, organisms living near hydrothermal vents are metal-tolerant (e.g. Cosson-Mannevy et al., 1989). Microorganisms used in bioleaching of ores or tailings piles are not only tolerant but capable of metabolizing the metal-containing material (e.g. Huber and Stetter, 1990; McNulty and Thompson, 1990; Wainwright and Grayston, 1989). Plant uptake of excess elements (As, B, Cu, F, Mo, Se) in retorted oil shale disposal piles is considered a potential hazard for ruminants grazing in the region (Stark and Redente, 1990a). In urban and industrial environments, uptake from metal-enriched rain and snow is increased by a reduction in pH (Gjengedal and Steinnes, 1990), a result of increasing metal bioavailability. As a result of both increased aerosol metal and often increased metal availability, tissue metal concentrations can be elevated in plants and animals within a fallout zone of aerosol metal from industry. This has been demonstrated for animals in both laboratory (Vrzgula et al., 1989) and natural environments (Göttingen Univ., 1984).

In aquatic environments, uptake of excess copper can occur from metal-rich sediments (e.g. Eriksen et al., 1990a,b; Gries and Garbe, 1989; Huiskes and Nieuwenhuize, 1990; Roy-Burman et al., 1989) as well as from the water (e.g. Sen and Mondal, 1990). This makes treatment of waste effluents possible as well as causing concern for naturally-occurring plants and animals. Truchet et al. (1990) report excess copper in molluscs taken from estuaries receiving anthropogenic metal in Great Britain. The nature of the material entering estuaries or other water systems includes a variety of metal-containing anthropogenic agents. Some of these, such as fuel ash, contain copper which can be acquired by organisms (Jenner and Bowmer, 1990). Analogous situations occur in terrestrial environments, with organisms from contaminated urban sites exhibiting elevated tissue copper levels (e.g. Berger and Dallinger, 1989). Tolerance of plants to high levels of metals can be due to a mechanism or mechanisms within the plant or to a symbiotic relationship with certain fungi (Griffioen and Ernst, 1989). A number of microorganisms and algae release metabolites which reduce the availability and uptake of excess metal (e.g. Gowrinathan and Rao, 1990; Green and Bedell, 1990; Huang et al., 1990). Phytochelatins and metallothionein and its analogues are produced by a wide range of organisms, to bind excess metal within the organism (Elmes et al., 1988a; Engel and Brouwer, 1989; Hogstrand et al., 1990; McCormick et al., 1989a; Olsson et al., 1990; Petering et al., 1988; Rauser, 1990; Suzuki et al., 1990b,c,d,e; Zolotukhina and Gavrilenko, 1990). Whether aquatic or terrestrial, organisms in metal-rich environments are frequently able to isolate excess metal in apparently unavailable form(s) within their tissues (e.g. Brough and White, 1990; Darlington and Gower, 1990; Hopkin, 1990; Nott and Nicolaidou, 1990b; Phillips and Rainbow, 1990; Reddy and Prasad, 1990; Simkiss and Taylor, 1990). This is not always the case although organisms with reduced ability to do this still tend to concentrate excess copper in the same tissues. Thus Moiseenko and Kudratsjeva (1990) report elevated liver copper concentrations in freshwater fishes in metal-enriched effluent-receiving waters on the Kola Peninsula in Russia. Badgers foraging in the metal-rich forelands of the river Meuse (Netherlands) have elevated levels of copper in the kidney (Ma and Broekhuizen, 1989).

The uptake, flux and metabolism of copper involves a number of processes. Initially, a supply of metal is required, whether from the environment, from food, or even from the organism itself (e.g. Zivko-Babic et al., 1989). Secondly, some mechanism(s) to allow uptake into the organism or the cell (e.g. Alda and Garay, 1990; Barnea and Katz, 1990; Barnea et al., 1990; Katz and Barnea, 1990; Lebedev, 1989a; Liubimov et al., 1988; McArdle et al., 1988, 1989; Nederbragt, 1988). Thirdly, transport to various sites within the cell or to various tissues in multicellular organisms (e.g. Barnhart et al., 1989; Brown et al., 1990b; Waldrop et al., 1990; Waldrop and Ettinger, 1990a) and finally, localization within the tissues. Hirayama (1990), for example, notes copper in rat ocular tissue and suggests that it plays roles as components of some metalloenzymes. However, amounts of a copper-containing enzyme like superoxide dismutase, may not be linked to the copper status (Tran et al., 1989). Copper may appear early in the development of a tissue (e.g. Sauer et al., 1989) and may play a role in its formation. Another factor is that the organism may store copper for use during periods of low copper availability (e.g. Arumugam, 1989). In reality, however, very little is known about copper turnover in organisms although the use of stable isotope techniques offers hope for improvement in the future (Levenson and Janghorbani, 1990; Ting et al., 1990).

The metabolism of copper, once within the organism, deals with copper-binding proteins which act as transport agents. Within microorganisms, cellular requirements for copper are normally small but still require carefully regulated transport systems to deliver copper to specific enzymes (Camakaris et al., 1990; Rouch et al., 1988). Within plants, especially higher plants, glutathione may function as an organic carrier for copper transport (Albrigo and Taylor, 1990). A number of organics can act as transport agents in animals, including synthetic agents of a porphyrin nature (Mercer-Smith et al., 1989) as well as ceruloplasmin. Ceruloplasmin is a blue glycoprotein with a molecular weight of about 130,000 Daltons and containing some 6 atoms of Cu (Van den Hamer, 1988; see also Bligh et al., 1990). The nature of its role in copper transport is not completely understood although the nature of the copper binding sites has been recently examined (Zgirski and Frieden, 1990). As well, two other transport compounds are involved - albumin and transcuprein (Goode et al., 1989). In a ceruloplasmin-based delivery system, copper-containing ceruloplasmin is suggested to bind to the membranes of specific cells resulting in a transfer of bound copper into the cell cytosol (Harris and Percival, 1989; Montaser et al., 1990). The suggestion by Davidson and Harris (1990) and Percival and Harris (1990) is that the plasma membrane has the capacity to remove copper from ceruloplasmin with the plasma membrane accumulating the copper without accumulating the ceruloplasmin protein. The levels of ceruloplasmin are reduced in the foetus and the neonate although levels of copper are elevated (Bingle et al., 1990a). Other copper-transporting agents include the tripeptide glycyl-L-histidyl-L-lysine (Iwai, 1988, 1990) as well as histidine (e.g. Waldrop et al., 1988). Histidine residues localized on cell surfaces may form an important mechanism for copper uptake (e.g. Botros and Vijayalakshmi, 1989). Linder et al. (1988) provide the structure and function of transcuprein, an effective agent for the transport of copper by mammalian blood plasma. Almar and Dierickx (1990) suggest that the binding of heavy metals (Hg, Cu, Cd) with glutathione transferase in humans may have a protective function.

One of the more frequent organism responses to excess is an increase in production of one or more metal-binding agents, to reduce the size of the active copper pool within the organism (e.g. Kosman et al., 1990). Reduction in the pool includes changes in copper speciation (e.g. Bryson et al., 1990). Depledge and Rainbow (1990) discuss regulation and accumulation of trace metals in marine invertebrates, including agents responsible for transport and storage. Details of the uptake and metabolism of copper within particular cell types are found in a number of references. These include a range of subjects, like the publication of Richards and Steele (1988) on zinc, copper and iron metabolism by turkey embryo hepatocytes, and Aggett et al. (1989) on the effect of picolinic acid on metal translocation across lipid bilayers. Metal loss can occur in fecal material or in urine (e.g. Ishihara et al., 1990). In mammals, the measurement of metal excretion in bile has proven useful in evaluating metal flux and requirements. Symonds and Charmley (1990) describe a technique for long-term colleciton of bile in the pig as well as the measurement of biliary excretion of copper and zinc. They note a 24-hour output of approximately 5.8 percent of the daily intake of copper. In cattle, excess copper or a combination of dietary molybdenum and sulfur enhances biliary excretion of copper in cattle, with higher rates of excretion in Simmental than in Angus cattle (Gooneratne et al., 1988). Dijkstra et al. (1991) note a marked circadian rhythm in bile copper excretion in rats. Excretion via bile can, however, be increased by glutathione (Houwen et al., 1990; Nederbragt, 1988; see also Nederbragt, 1989). Use of drugs to treat certain physiological disorders (e.g. chronic bronchitis) may have some effect on copper loss (e.g. Hjortso et al., 1990).

Irregularities in uptake and metabolism of copper may occur with physiological irregularities. Bunto and Frazier (1990), for example, describe an age-related, progressive condition of abnormal hepatic copper storage in white perch. Acute, dehydrating diarrhea has been associated with an increased fecal loss of copper (Ruz and Solomons, 1990). Arthritis can induce changes in tissue copper concentrations which may or may not be reversed with aspirin treatment, depending on the tissue (Kishore, 1990a). Several diseases are associated with possible disorders of copper transport and resultant accumulation of excess copper in the liver (e.g. Barrow, 1988). These include Wilson's disease and Indian Childhood Cirrhosis. Lack of adequate bile flow, or cholestasis is associated with atypical copper uptake by liver cells (e.g. Ochs et al., 1988). In individuals with Menke's disease or in mouse models of the disease, irregular production of metal complexing agents associated with copper transport may be associated with irregular copper uptake by liver cells (Palida et al., 1988; Tanaka et al., 1990). Abnormal uptake of copper has also been reported for muscle cells of Menke's disease patients (van den Berg et al., 1990). In Wilson's disease, high liver copper, low plasma copper and caeruloplasmin levels are characteristic. These are also found in neonatal mammals including man, but they change to the adult mode of copper metabolism shortly after birth (Srai et al., 1988). Treatment of the disease includes the use of metal complexing agents and excess zinc, which competes with copper (e.g. Dörner et al., 1988).

VI. CHANGES OCCURRING IN COPPER AFTER INTRODUCTION INTO NATURAL ENVIRONMENTS

Copper entering a new environment is exposed to conditions that may cause a change in metal speciation and biological availability. This occurs with the introduction of aerosol metal into soils, riverine metals into salt water, industrial aerosols into the atmosphere. The biological importance of this pertains to the availability of copper for uptake by plants, the satisfaction of metal deficiency conditions. Conversely, it applies to the introduction of anthropogenic metal and whether it will have a detrimental impact. Reviews pertinent to this discussion include "Marine Geochemistry" by Chester (1990), the publications in Vernet (1989a,b) and the publication entitled "Trace Metals in Lakes" edited by Nriagu (1989). Information from national and international programs such as the Mussel Watch program (e.g. Lauenstein et al., 1990) provides evidence of a general nature on metal uptake and its relationship to environmental conditions. Of concern is the methodology of work on metal analysis as well as the application of this work to an understanding of metal speciation and flux (e.g. Voelkening, 1989; Wollast, 1982). Sources of metals as well as metal transport are of obvious importance both in terms of natural (e.g. Arimoto et al., 1989; Wagner, 1989a) and anthropogenic copper (e.g. Beckett, 1989; Beddig et al., 1988). Knowledge about speciation and flux can also be used in examining the fate of anthropogenic metals (e.g. Chapra and Boyer, 1989; Horowitz et al., 1989; Pentcheva, 1988) and the management of anthropogenic metal sources such as groundwater (e.g. Schiffer, 1989) or wastewater and sewage (Chaney, 1989), or even the potential impact of copper-containing fertilizers (e.g. Hyuusa, 1983). This information can also be used in examining metal uptake and flux through plants and animals (e.g. Alikhan, 1989).

The biological importance of changes that occur in copper after introduction into natural environments, as well as affected environments, are discussed in the articles in Tyler (1989a), for forest ecosystems. Skei and Næs (1989) discuss experimental work on polluted sediments, commenting on the poor understanding of events that occur in nature. Transport of metals by sedimentation formed an important topic in a Mediterranean workshop (International Atomic Energy Agency, 1990). Even such effects as the sulfide discolouration of copper oxide-containing antifouling paint (Edyvean and Silk, 1989) are indicators of the complex metal-environment interactions that naturally occur. The associations of copper with ligands (e.g. Apte et al., 1990a; Buffle et al., 1990; Hering, 1988; Perdue, 1989) is of increasing importance in understanding metal speciation and biological availability in both aquatic and terrestrial environments as well as the use of copper in industry and the medical and agricultural communities. The same applies with sorption of copper to particles (e.g. Flemming et al., 1990).

Copper in Estuarine Environments

Estuaries are considered as regions where rivers enter the sea (infrequently where they enter lakes). They are areas where flow rate decreases and major ion concentration increases. Both of these changes are associated with changes in dissolved and particulate chemistry of metals, including copper (e.g. Fischer et al., 1990e; Krom et al., 1990). Luoma (1990) discusses some of the processes affecting metal concentrations in estuarine and coastal marine sediments. In a laboratory study, Salomons and Brill (1990) simulated changing estuarine conditions and modelled settling particles, salt wedge effect and sorption-desorption phenomena causing the estuarine release of copper in the Fly river which drains the region of the OK Tedi Mine site in New Guinea. Slin'ko (1988) reports temporal variability of particulate copper in estuarine areas which was correlated with changes in biomass. Flocculation in estuaries involves humic substances as well as metals (e.g. Li et al., 1988a), and can affect metal composition and bioavailability. Phytoplankton (e.g. Slauenwhite and Wangersky, 1991) as well as macrophytes such as seagrasses, marshgrasses and mangroves form a source as well as a sink for copper in estuaries (e.g. Silva et al., 1990; Wasserman, 1989). The same may also be said for metal uptake by animals (e.g. Cheggour et al., 1990). However, many benthic animals affect sediment metal speciation as a result of burrowing (bioturbation). Anthropogenic effects are apparent in a number of areas, such as the input into the North Sea of dredged material from Rotterdam harbour (Klomp, 1990). Arzul et al. found a relationship between estuarine sediment copper levels and intensive pig farming in an area on the Brittany coast. Degradation of sewage, whether from pigs or humans can affect sediment metal concentration and speciation (e.g. Nedwell and Lawson, 1990; Paulson et al., 1991). Metal segregation has also been reported during the sedimentation of sludge after ocean dumping (Angelidis and Gibbs, 1991).

Sigleo and Means (1990) review organic and inorganic components in estuarine colloids, commenting (page 131) that "The chemical composition of colloidal organic matter is a major factor influencing the interaction of colloids with respect to other materials in the system". He (1987) reviews the aggregation of suspended fine-grained matter, including copper-conatining material, in estuaries. Koliadima and Karaiskakis (1990) in a discussion of metals and suspended particulate material, report an inverse relationship between concentration of metals in particles and the size of the particles. The rate of sedimentation of copper-containing particles in estuaries is also affected by particle size as well as river flow (e.g. Hungspreugs et al., 1990). Relationships between salinity and particulate copper concentrations have also been reported (e.g. Bilinski et al., 1991). With an estuary this can suggest an effect of increasing major ion concentration on extraction/sorption by/from particulates. It may also be associated with differences in the sources of the salt water (e.g. Harper, 1991). Windom et al. (1991) present evidence from three estuaries that copper covaries with silica, an indication of weathering of silicate minerals within the estuarine environment. They suggest that copper and silica are not fractionated during weathering and transport from the continents to the oceans.

Recent literature includes discussions of copper flux and speciation in a number of important estuaries. Concentrations of copper provided in some of these publications are listed in table 2. Other publications contain previously published data. Dorten et al. (1991), for example, uses published data to estimate fluxes of dissolved copper into the Mediterranean Sea. They estimate riverine input as 2,850-10,000 x 10^3 moles/year in comparison with input through the Strait of Gibraltar (145,000 x 10^3 moles/year), input from the Black Sea (8,000-16,800 x 10^3 moles/year) and atmospheric input (80,000-170,000 x 10^3 moles/year).

Estuaries that are discussed in the currently-reviewed literature include -

estuaries of rivers entering the Mediterranean (Dorten et al., 1991), Adige R. (Boldrin et al., 1989; Guerzoni, 1990), Krka R. (Bilinski et al., 1991; Elbaz-Poulichet et al., 1991), Nile R. (Abdel-Moati, 1990), Po R. (Ferrari and Farrario, 1987; Guerzoni, 1990; Perin et al., 1989), Venice Lagoon (Albani et al., 1989).

Volga R. (Lapin et al., 1990).

Gironde R. (Jouanneau and Weber, 1989), Loire R. (Piron et al., 1990), Sado R. (Vale and Cortesao, 1989), Tagus R. (Vale, 1990).

Clyde R. (Balls, 1990), Humber R. (Grant and Middleton, 1990), Mersey R. (Head et al., 1990), Severn R. (Allen et al., 1990; Apte et al., 1990b; Harper, 1991; Rae, 1989).

Elbe R. (Fanger et al., 1990), Scheldt R. (Zwolsman and van Eck, 1990), Vistula R. (Szefer, 1990a), Weser R. (Jathe and Schirmer, 1989).

Estuaries in Florida (Schropp et al., 1990), Savannah R. (Windom et al., 1991), Delaware R. (Church et al., 1988), Narragansett Bay (Bender, 1989), New York Harbour (Brosnan et al., 1987), New Brunswick harbours (Ramos-Pérez et al., 1989; Samant et al., 1990), Medway R. (Windom et al., 1991), St. Lawrence R. (Cossa, 1990; D'Anglejean and Ramesh, 1990; Yeats, 1988, 1990).

Fraser R. (Swain and Walton, 1990b), Boundary Bay (Canada) (Swain and Walton, 1990a), Elliott Bay (Washington) (Schmoyer et al., 1988a), Puget Sound (Crecelius et al., 1989b), San Francisco Bay (MacDonald, 1989), Mexican coastal lagoons (Hansen et al., 1990).

Frade R. (Brazil) (Drude de Lacerda et al., 1989b), estuaries in southern Rio de Janeiro (Pestana et al., 1989)

Avon-Heathcote R. (New Zealand) (Rodrigo, 1989), Fly R. (New Guinea) (Salomons and Eagle, 1990).

Visakhapatnam harbour (India) (Subrahmanyam and Kumari, 1990), Cochin estuary (Nair et al., 1990, 1991), Periyar and Muvattupuzha (Shibu et al., 1990), Thane creek (Athalye and Gokhale, 1989), Vellar R. (Rajan et al., 1989).

Bang Pakong and Mae Klong Rivers (Thailand) (Hungspreugs et al., 1990), Maekong R. (Thailand) (Windom et al., 1991), San-in district (Japan) (Shinkawa, 1981), Jiulong estuary and Xiamen Bay (China) (Hong and Lin, 1990), Huangpu and Suzhou rivers (China) (Oai et al., 1989), Yangtse R. (Perin et al., 1989), Yellow R. (Xue et al., 1988), Geum R. (Korea) (Byrd et al., 1990).

Copper in Freshwater Environments

Copper concentrations have been determined in freshwaters, sediments, interstitial and groundwaters. Coale and Flegal (1989) also estimated residence times for a number of metals in the epilimnion of Lake Erie (950 days) and Lake Ontario (660 days). Copper associated with suspended particulates is often measured since this can represent a major portion of the total metal concentration (e.g. Zhang and Huang, 1988; Zhang et al., 1990c). Bodo (1989), for example, suggests that during peak flows into Toronto Harbour and then Lake Ontario, the suspended particulate copper can be 80-90% of the total copper. Sedimentation of the suspended fraction will occur over time, reducing the total metal concentration in the water column but introducing the metal into the benthic sediments. Johannson (1989), working in central and northern Sweden, provides evidence of anthropogenic input to lakes, of copper and other metals. Mining is reported as an important source of anthropogenic copper in certain freshwaters (e.g. Axtmann and Luoma, 1987). However, relatively high levels can also occur in freshwater as a result of the geology of the region; Mogollón et al. (1990) provide evidence which they say indicates higher metal concentrations leach from metavolcanic and ultramafic rocks than metasedimentary rocks. Ledin et al. (1989) note that concentrations of heavy metals in groundwaters from igneous crystalline bedrock in Sweden are related to concentration levels in the bedrock as well as the pH of the water.

The affinities of copper for fine-grained particles and certain organics affect not only metal speciation but also vertical and horizontal transport (e.g. Lazerte et al., 1989; Nriagu and Wong, 1989; Shafer and Armstrong, 1990) and bioavailability. Particles of a biogenic nature can also transport copper from the water to the sediments which can impart a seasonal change in sedimentation rate (e.g. Camusso et al., 1989a). Deposition of particulate copper occurs as river-borne particles enter lakes and sediment out of the water as a result of the reduced rate of water flow. As a result, lakes can serve as a sink for natural as well as particulate copper of anthropogenic origin (e.g. Rada et al., 1990). Variability in flow within a river can also produce differences in metal transport over both space and time (Kappenberg et al., 1990). Although this can reduce total metal concentrations in the water (Pentcheva and Damyanova, 1989), metal is transported to the sediments. For this reason, suspended solid metal concentrations are frequently used to indicte the metal available for transport to the benthic sediments. Mayer and Manning (1990), for example, calculate an annual loading of 958 kg Cu to the sediments of Lake Ontario, from suspended solid measurements in Hamilton Harbour. Undisturbed sediments have also been used to provide a history of metal input over time (e.g. Leenaers, 1989; Rang and Schouten, 1989; Verta et al., 1989) although changes in concentration can occur as a result of leaching and diagenetic processes (e.g. Brügmann and Lange, 1989). Leachates have been associated with increased groundwater levels in a former mining area (Karlsson et al., 1989), soils treated with sewage effluents (Magaritz et al., 1990) and solar evaporation basins (Hall, 1989). With an understanding of the operational geochemical processes, however, the effects of new or proposed mine operations can be minimized (e.g. Smith et al., 1990). Since domestic and industrial wastewaters (Moriyama et al., 1989) and sludges (e.g. Védy and Greter-Domergue, 1989) may also contain metals, including copper, an understanding of both the wastewater treatment and receiving water is advisable to minimize the release of excess metal in available form. Use of sewage sludge as a fertilizer also requires this (e.g. Dowdy et al., 1987) as does aerosol input, especially in association with changes in pH (e.g. Borg et al., 1989; Mach and Brezonik, 1989). These factors are important in the evaluation of metal uptake by organisms in regions receiving anthropogenic copper (e.g. Köck et al., 1989a) as well as in natural areas with metal-rich sediments or water, whether fresh water or salt water (e.g. Lein et al., 1989). In areas either receiving or going to receive anthropogenic metal this implies the need for both initial evaluation of potential effect (e.g. Swain and Walton, 1990b) and, when necessary, adequate biomonitoring (e.g. Gunkel, 1989a). Work from these areas needs to be evaluated in terms of variability in metal concentration, fractionation (particulate:"dissolved") and bioavailability, then compared with control areas to determine actual or potential impact (e.g. Zeman and Slaymaker, 1988). With an area expected to receive anthropogenic copper, it may be possible to establish relationships or associations which allow anticipation or modelling of metal transport (Aldrin et al., 1989; Müller et al., 1990; Sandén and Carlsson, 1990) and biological availability to effect better management. In using models, however, the reader is warned that widespread use without adequate monitoring programs can be very misleading. Marcus (1989) points out, for example, that only under proper circumstances can dilution mixing models be used to estimate suspended sediment metal concentrations in unmonitored stream channels of a creek in Colorado. Sandén (1988) discusses the dynamics of metal (Cd, Cu, Zn) transport in an old mining area in Sweden. Use of a model for the simulation of water flow allowed comparison of transport dynamics in different areas and suggested that transport was greater downstream than close to the source of the metal. Vasilikiotis et al. (1990) examined metal (Cu et al.) transport in a Greek river in terms of mobile and refractory phases of the metals with the sediments. Water and sediment chemistry continues to be shown to be of primary importance in estimating trace metal mobilization and transport in freshwater systems (e.g. Brooks and Moore, 1989; Drude de Lacerda et al., 1990; Elsokkary and Müller, 1989; Gunkel and Sztraka, 1986).

A number of general models have been proposed for the distribution of trace metals (e.g. Gu et al., 1988). Whether in the water or associated with sediments, however, variability in physical and chemical properties (e.g. Sager et al., 1990; Stiller and Sigg, 1990; van der Weijden and Middelburg, 1989) can make general models difficult to apply universally. Schindler (1989) discusses the behaviour of trace metals (Zn, Cu, Pb, Cd) in terms of simple steady state models and some of the problems associated with their use. Fine details of geochemical partitioning are important in evaluating metal source as well as remobilization from the sediments (e.g. Brand, 1989; McKee et al., 1989a,b), details that can be difficult to resolve with general models. As an example, albeit in a unique situation, Lyons et al. (1987) note a 100 fold copper enrichment in the sulfidic zone below a mat of blue-green algae in hypersaline lake sediments. Heavy metal transfer and retention are important, especially at dumpsites for dusts and sludges from the metalprocessing industry. Wagner (1989b) comments on transfer and retention in clay, for dumpsite considerations, noting that retardation values determined by a diffusion technique seem to be more realistic for predictive modelling. Complexation properties of organics in freshwater, groundwater, interstital water and sorbed to surfaces also need to be considered in terms of transport, retention and fate of released metals (e.g. Dehnad and Förstner, 1989). Hiraide et al. (1989) and Lund et al. (1990) note some of the properties for humic substances, and Lund et al. (1990) discuss some of the complexities of interpreting titration curves used for estimating complexation. Since humic substances can reduce the biological availability of trace metals and some organics (McCarthy, 1989), they have been suggested for the treatment of hazardous-waste chemical species (Manahan, 1989). An understanding of their capabilities is important since they may have secondary effects on biota as a result of altering the transport and fate of both metals and organics (McCarthy, 1989). Varma et al. (1989) used lignin to remove copper from solution and suspension. They note that it is affected by solution pH as well as the amount of lignin used, with a pH optimum for removal of between 4.4 and 5.0.

Copper in Marine Environments

Coastal input accounts for most of the copper entering marine environments; much of this is from river input (e.g. Fabiano et al., 1990; Korzeniewski and Neugebauer (1991). The introduction of anthropogenic metal continues to be of concern and to sponsor work on environmental effects (e.g. Mihnea et al., 1991) as well as metal transport and speciation. This is because of the nature and effect of changes that occur in the speciation of copper after introduction into marine environments. Burton and

Statham (1990) provide a somewhat brief review of the chemistry of metals in seawater and Ruiz Pino et al. (1990) discuss trace metal cycles in the Mediterranean Sea using a box model (copper is not one of the metals discussed). The effect of hydrogaphic conditions on the distribution and speciation of copper is important, Riso et al. (1990) noting differences in concentration between well-mixed and stratified zones in western Brittany coastal waters. As expected, copper concentration varied much less in a well-mixed zone than across a zone of stratification in a stratified water column. They attribute some of the effect of stratification to complexation and/or adsorption phenomena. In an examination of particulate trace metals in the western Bay of Bengal, Satyanarayana et al. (1990b) note that particulate copper profiles showed a continuous decrease from the surface to the bottom. They also found a correlation between particulate copper and particulate organic carbon suggesting biological uptake near the surface and transport to the sediments. Matsumura et al. (1989) report dissolved copper levels increasing with depth in the Shikoku Basin on the east coast of Japan. Values were low near the surface (0.5-2.0 nmol/L) and high near the bottom (4.2-4.7 nmol/L). The difference between particulate and dissolved concentrations can be a result of scavenging of dissolved copper by inorganic particles or uptake by organisms; the nature and variation in both types of particles will be controlled by regional as well as seasonal factors. As an example of an "extreme" condition, Zhirmunsky and Tarasov (1990) describe a hydrothermal vent-type situation and ecological community in a flooded volcano in the Kurile Islands. Concentrations of metals are extremely high in the vent water (Cu = 25 µg/L) and both metal chemistry and biological communities are driven by the nature of the system. Copper in extreme conditions such as these can have an effect on water chemistry. As a catalyst for example, it may under certain conditions, influence the rate of oxidation of H_2S in seawater (Vazquez et al., 1989a).

Changes in copper speciation can occur as a result of organics in salt water. Hansen et al. (1990) suggest that naturally-occurring organic ligands may be responsible for the biological availability of copper in three Mexican coastal lagoons that they examined. In a study of the Tamar estuary (U.K.), van den Berg et al. (1990a) found very low cupric ion activities as a result of organic ligands. Mackey and O'Sullivan (1990) found relatively constant metal-complexing capacity in a plankton-containing enclosure but note a change in the nature of the copper complexes as a phytoplankton bloom developed. Organic and metal-organic compounds became more polar as the bloom progressed.

The flux of copper in particles from terrestrial environments to marine environments or from the overlying water to the sediments is important not only because it transfers metal from one biological environment to another but also because it is from one chemical environment to another. As a result, attention continues to be focused on the scavenging of metals and cycling of particles (e.g. Clegg and Whitfield, 1990). Transport of particulate metal is primarily in inshore waters, associated with the vertical flux of copper (e.g. Heussner et al., 1988) as well as localized flux produced by particular topographic and/or hydrographic features (e.g. Bothner, 1989; Zhan et al., 1989). Guerzoni (1990) examined the transport of trace metals by sedimentation in the Adriatic Sea, commenting that prodeltas of rivers appear to be important sites for pollutant trapping. Lithner et al. (1990) and Szefer (1990a) comment on the importance of anthropogenic metals, including copper, in the flux of metals to the Baltic Sea. In an evaluation of the flux of metals in the North Sea, in the clay fraction (< µm), Irion and Müller, (1990) note the importance of river input as well as water circulation. Work on copper associated with suspended particles includes that of Baker et al. (1990a) who report evidence of suspended particulate copper and cadmium from a copper and gold mine. Variability in the vertical flux of metals into the deep-ocean sediments has been noted and, at least in one case (Jickells et al., 1990), suggested to be a result of variable input of major river systems. The authors, however, note the contribution of biogenic material and association with particulate organic carbon, a feature that appears to be widespread in both offshore and nearshore marine environments (Fowler, 1989; Lin and Li, 1988; Szefer, 1991). The effect of biogenic material is widespread in sediments, affecting redox conditions as well as the concentration of interstitial copper as well as other metals (Shaw et al., 1990).

Deep sea sediments are usually fine-grained, a result of aerosol input, the distance from riverine sediment sources and the relatively slow sedimentation rate of fine-grained materials. Elevated concentrations of copper can be associated with some fine-grained material, producing somewhat higher concentrations of copper in surficial deep-sea sediments (Brügmann and Lange, 1989; Fernex et al., 1989). However, other factors can also affect sediment metal concentrations. Klamer et al. (1990) report an average of 21.8 µg/g copper in muds from the southern North Sea and suggest that very little (0.03-0.3%) of the total metal input to the North Sea reaches this area. In contrast, in the Seto Inland Sea in Japan, Hoshika et al. (1991) states that 70% of the natural and anthropogenic copper input is in the benthic sediments. Once within the bottom sediments, chemical conditions affect the distribution between solid (sediment) and liquid (insterstital waters) phases (Kosov and Demidova, 1987). In an excellent piece of work, Kosov and Demidova (1987) conclude that metal concentrations in Atlantic interstitial waters are several times larger than those in the Pacific. They believe that this may be due to higher sedimentation rates in the Atlantic, causing lower diffusion losses to the bottom water. They also discuss the large-scale geochemical zones where similar processes affect copper chemistry. Both large-scale and small-scale processes affect the distribution and speciation of copper in sediments, small-scale processes often producing variability and complexity in the metal chemistry of a benthic system (e.g. Pentcheva and Damyanova, 1988). This can produce small-scale changes in the biological availability and bioaccumulation of sediment-associated copper (e.g. Pertsov et al., 1990; Szefer, 1990b).

Copper in Terrestrial Environments, particularly Soils

Copper is present in soils in varying amounts and species, as a result of the geological background of the soil as well as the input of aerosol copper and the nature of organisms using the metal (e.g. Sillero et al., 1988). Bergkvist (1989) reviews the fluxes of several metals in temperatre forest ecosystems (Cu, Pb, Zn, Cd, Ni, Cr) with particular concern for metal deposition, fate and speciation. Other reviews include discussions of global cycles of heavy metals and their biological cycling on oceanic islands (Dobrovol'skii, 1988) and critical metal concentrations for forest soil invertebrates (Bengtsson and Tranvik, 1989). Grigal and Ohmann (1989) examined spatial patterns of elemental concentrations in soils of forest stands in the north central U.S.A.; they found little change in copper, in contrast to some other elements. Although fertilizers may contain elevated concentrations of metals (e.g. Petruzzelli, 1989), Christensen and Tjell (1989) report that neither soil types nor long term fertilization practices influenced soil copper concentrations in the soils that they examined. (But see Ruszkowska et al., 1989c, for contrast.) In contrast, elevated concentrations of anthropogenic copper have been reported from snow and some soils as a result of urban and industrial activity (e.g. Dumontet et al., 1990; Ecker et al., 1990; Ivonin and Shumakova, 1990; Leharne et al., 1990; Rang and Schouten, 1989; Schönhard and von Laar, 1990). Evidence of changes in aerosol metal is shown in Antarctic ice copper concentrations, from both natural and anthropogenic sources, since the last glacial maximum (Boutron, 1987). Queirolo and Valenta (1989) found no significant difference in tissue copper concentrations between oak growth rings from a pristine region and a region receiving atmospheric pollution. The fact that copper is an essential metal may explain the ability of many organisms to control both uptake and tissue metal concentration (e.g. Nuorteva, 1990).

The mobility, and biological availability of soil copper is controlled by physical and chemical conditions in the soil as well as the nature of the metal-containing source material (e.g. Braginskiy and Myrlian, 1990; Godt, 1989). This also means that various agricultural practices can have an impact on copper concentrations as well as availability (Gallardo-Lara and Torres-Martin, 1990). There is an increase in solubility of copper in acid soils (Folkeson et al., 1990; Kreutzer et al., 1989; Palko and Yli-Halla, 1990). The problems arising from acid mine drainage include the low pH and supply of metal in an area of mineralization (e.g. Kwong and Nordstrom, 1989). However, liming to raise the pH can be associated with an increase of dissolved organc carbon which causes increased mobilization of metals such as Pb, Cu and Al through organic complexation (Kreutzer et al., 1989). Organics in the soil, especially in litter and humus are able to complex and transport soluble metal as precipitation percolates through the soils (Berggren, 1990; Klamberg et al., 1989; Markert and Thornton, 1990; Radogna, 1989; Senesi et al., 1989a). This may reduce the concentration of surficial heavy metal (Elpat'ievskiy and Lutsenko, 1990) but it can also cause its introduction into groundwater. However, metal complexing agents in soils and compost will also reduce metal bioavailability (e.g. Hernando et al., 1989). The combined effect of organic and inorganic components of the soil modifies the role of each in metal mobility and bioavailability (e.g. Arshad and Quraishi, 1990; Singh, 1989b). In contrast to the effect of organics on the transport of copper, Kumari et al. (1988) talk about the effect of copper and cadmium on the mobility of soil amino acids. They comment that the mobility of amino acids was reduced in copper-ammended soils, possibly in repsonse to sorption as well as complexation processes. Obviously, percolation of copper-containing fluids through the soil is dependent on the physical nature of the soil, percolation through sand is faster than through clay. The interaction of chemical and physical features has been examined to determine transport, mobility and availability of copper (e.g. Ruszkowska et al., 1989a,b; Struck and Ostapczuk, 1990). Suitable bioassay organisms (e.g. Sheppard, 1991) and/or extraction techniques are required to evaluate metal availability in soils.

Aerosol Copper

Chester and Murphy (1990) comment that (page 47) "There is a 'veil' of aerosol-associated trace metals present in the atmosphere over the whole of the World Ocean". They could have said 'over the whole world' since there is introduction of metal into and deposition of metal out of the atmosphere over the entire world. Schroeder (1987) provides evidence suggesting that the atmosphere plays an important role in the environmental impact of aerosol metals. These two references thus suggest that not only is the atmosphere a source and a sink for copper and other metals but that it also plays a role in metal chemistry, at least prior to deposition and, in some cases, afterwords. Historical evidence of natural and anthropogenic aerosol copper can be found in recent snow and ancient ice samples (e.g. Boutron, 1987; Ecker et al., 1990; Wolff, 1990).

One source of aerosol metal is waste incineration (e.g. Rachwalsky, 1989), another is the use of fossil fuels to produce power (e.g. Pacyna and Münch, 1989). Others include high-temperature processes in steel and iron manufacturing, cement production, a variety of other industries (e.g. Vaychis et al., 1989) and, perhaps the most widespread, gasoline combustion. Measuring the concentration of aerosol copper is difficult because of the often low concentration of metals (e.g. Brinckman and Olson, 1990). Sampling routines as well as frequency of sample collection become imnportant items in producing realistic evaluations of metal sources, chemistry and biological availability (Ross, 1990b). The use of lichens as a collecting mechanism offers not only a way of "trapping" aerosol metal but also provides an indication of metal uptake (Walther et al., 1990). Associations of metals with different types of aerosol particles becomes important in dictating distance of transport (e.g. Cornille et al., 1989) as well as chemical associations of the metal.

Völkening and Heumann (1990) found a range of <0.02-20 ng/m^3 copper in the near-surface aerosol over the Atlantic Ocean from 60° south to 54° north. In a discussion of atmospheric input of copper into the North Sea, Ottley and Harrison (1991) report estimates between 380-14,100 tons/year from the literature. The important thing in an evaluation, for example for areas like the North Sea, is to evaluate not just the source but also the prevailing winds (Chester and Bradshaw, 1991). The location of the receiving environment is also important, natural rather than anthropogenic sources of metal are, for example, important in northern Norway (Steinnes, 1990). In contrast, soils in southern Norway receive major inputs of aerosol copper of anthropogenic origin (Steinnes et al., 1989a,b). Johansson (1989) found low concentrations of sediment copper and reports a generally low anthropogenic input, presumably through both aerosol and riverine metal, in lakes in northern Sweden. Lake sediments have been used to chronicle atmospheric inputs in Canada; Wong et al. (1984) reporting sedimentation rates of 0.7-9.4 gùm^{2}ùyr^{-1} in ten remote lakes in Ontario. Past atmospheric deposition of metals has also been chronicled in a peat core from a bog in northern Indiana. Cole (1990c) estimate a presettlement (1339-1656 A.D.) copper accumulation rate of 0.266 mgùm^{-2}ùyr^{-1} and a postsettlement peak accumulation rate of 20.83 mgùm^{-2}ùyr^{-1} (1970-73). Purghart et al. (1990) note decreased concentrations of particulate aerosol copper with increasing elevation in rural Switzerland. They did, however, find variations which could be associated with atmospheric conditions. In the atmosphere of Madrid, Santamaria et al. (1990) found a bimodal distribution of particle size with metal levels tending to be higher in the smaller particles. In airborne particles sampled in the southern Appalachian mountains, Reisinger (1990) identified both anthropogenic (automobile, etc.) and natural (crustal) metal-containing material. Natural (crustal) copper is apparent in rural areas (Morales et al., 1990) but tends to be swamped by copper in anthropogenic (automobile, etc.) aerosols in urban environments like the Los Angeles Basin of California, Cincinnati Ohio and Chicago Illinois (Noll et al., 1990) or Beijing China (Zhou et al., 1990). Aerosols of marine origin form an important source of metal to oceanic areas (e.g. Arimoto et al., 1989) or island areas like New Zealand (Arimoto et al., 1990). Separating natural from anthropogenic sources of aerosol metals, including copper, can be difficult in areas like the Mediterranean although Migon and Caccia (1990) provide a probability density function for each metal which they suggest allows separation.

A number of chemical changes can occur in a copper-containing aerosol particle after impact. These changes can affect the biological availability of the metal. In aquatic as well as terrestrial environments, increased availability is associated with low pH (Sigg and Zobrist, 1989), a factor which can also be affected by aerosol input (e.g. Braekke, 1990). Since atmospheric deposition of copper can occur on the plants, transport to the soil can take place after uptake, through stemflow processes (Regina and Gallardo, 1989). The ability of at least some plants to transmit copper to the soil as well as to accumulate required copper from the soil implies a regulatory capability. This is supported by work such as Queirolo and Valenta (1989) who found no significant difference in the average concentrations of copper in oak tree rings from polluted and unpolluted regions.