CHAPTER 1

GENERAL INTRODUCTION

Part (a)

HAIR HEAVY METALS

Over 99.9% of all animal matter, including the human body, is made of 11 elements, namely, hydrogen, oxygen, nitrogen, sodium, potassium, chlorine, sulphur, magnesium, calcium, phosphorus and carbon with about half of the periodic table represented in the remaining 0.1%. Of this 0.1%, only 10 elements have been recognised as essential and these are: copper, iron, zinc, cobalt, iodine, molybdenum, manganese, selenium, chromium, and fluorine. This group is heterogeneous. Zinc, Fe and Cu are essential parts of many enzymes, whereas Co is found in only one molecule (vitamin B_{12}) and iodine apparently has only one function (thyroxine). The nonessential trace elements include environmentally hazardous metals such as lead, mercury and chromium, and others that may be given as constituents of drugs (eg containing bromine, aluminium and gold).

Hair analysis provides a useful tool for the evaluation of trace mineral status and the detection of exposure to these elements (Shrestha and Schrauzer, 1989). Hair mineral analysis has been widely accepted in general medicine in the U.S. as an "inexpensive screening test" for mineral aberration or imbalances that may indicate the potential for the later development of serious health problems. Furthermore, hair analysis appears a useful guide to help recognise underlying disorders of metabolism that may be associated with violent behaviour (Gordon, 1985).

Scalp hair is not only a reasonably reliable indicator of long-term internal milieu, but may be more easily collected, shipped and prepared for analysis than blood tissue samples. Hair may be used to detect a wide range of trace elements as many trace elements concentrate in hair in appreciable amounts than in other tissues. Zinc, which comprises the largest concentration of the trace metals in hair, is especially well suited for this type of measurement because it is relatively evenly distributed along the hair shaft and insensitive to spurious environmental variations (Gentile, 1981).

Trace elements are accumulated in hair at concentrations that are generally at least ten times higher than those present in blood serum or urine and this may

provide a continuous record of nutritional status and exposure to heavy metals pollutants. For at least 50 years hair has been recognised as potential repository of all the elements that enter the body.

The comprehensive results have been obtained with heavy metal pollutants such as Pb, As, Cd and Hg. Several investigators in Japan, Canada, and the United States have shown that the concentration of these elements in the hair provide an accurate, record of long term exposure and that there is a good correlation between concentration in hair and concentration in internal organs (Maugh, 1978).

Chattopadhyay *et al.* (1977) found that the concentration of Pb in hair was lowest in rural population groups, higher in urban groups, and highest in individuals who live close to lead smelters. These differences are presumed to reflect differing exposure to Pb in automobile exhausts, paint and industrial emissions. Similarly the highest concentration of Hg and Cd was seen in hair from individuals with known exposure to the metals (Maugh, 1978).

The trace metal content of hair has been used by a number of workers to assess the levels of exposure to heavy metal pollution (Petering *et al.*, 1973) as well as to examine the effects of general nutrition, state of health, drug exposure and other factors on the levels of body inorganic body trace elements (Briggs *et al.*, 1972). Unusual concentrations of trace elements in hair may also provide a tool for the diagnosis and therapy of diseases. It has been shown that children with cystic fibrosis have as much as five times the normal concentrations of Na in their hair, but only about 10 % of the normal concentration of tightly bound Ca (Maugh, 1978). Analysis of these two elements in hair may thus be a useful tool both for screening for cystic fibrosis and for assisting in diagnosis of the disease (Maugh, 1978).

Several investigators have shown that marginal Zn deficiencies in the diet can be identified by below normal concentrations of Zn in hair (Maugh, 1978). Previous investigations of animals and man have shown that the tissue content of various trace metals may correlate with the elemental content in the hair. To determine whether hair Zn levels reflect deficiency and sufficiency in man, studies have been made of Zn-deficient male dwarfs, before and after oral therapy with zinc sulphate, and of normal males residing in Cairo, Egypt (Strain *et al.*, 1966). The average Zn content in the hair of the normal Egyptians was 103.3 $\pm$ 4.4 ppm and in the untreated dwarfs 54.1 $\pm$ 5.5 ppm. Hair was also collected periodically from 6 normal males residing in Rochester, New York, throughout an entire year to establish the range of any seasonal variation (Strain *et al.*, 1966).

The quantitative analysis of Pb in hair as an aid in the diagnosis of chronic, mild or subacute Pb poisoning in children was explored by Kopito *et al.* (1967) . In healthy persons the concentration of Pb in scalp hair may be from two to five times greater than in bone, about ten to fifty times higher than in blood and from a hundred to five hundred times greater than that excreted in urine. The diagnosis of Pb poisoning was established on the basis of the clinical picture and laboratory findings, and later confirmed in 16 out of 17 patients through the excretion of large quantities of Pb in urine in response to therapy with chelating agents.

Various factors affecting the trace metal content of human hair were established by Gordus (1973). In a study designed to determine an historical baseline for the intake of trace metals by humans and to provide an evaluation of the present day rate of increase and source of environmental pollution, both present day and historical head hair samples, up to 300 years old, were analysed by neutron activation for more than 30 trace elements. The results were clearly of direct forensic importance. A wide variety of factors such as age, sex, hair structure and colour, geographic location, general diet, and socioeconomic status were considered in evaluating the data.

Hilderbrand and White (1974) evaluated the use of hair as an index of the concentration of certain elements in tissues with regard to the effect of prior cosmetic application and sample washing treatments on the results. The values observed were greatly altered by typical treatments given to human hair. Such changes were not corrected by the commonly used sample wash procedures. Samples of human hair, including pubic hair, may thus not indicate reproducibly and accurately the concentration of some metals (Ca, Mg, Cu and Zn) that would otherwise be present as a general body burden.

Helsby (1976) estimated Hg content in fingernails and body hair. Contamination of dental personnel with Hg was estimated from the analysis of finger nails, toenails and body hair by neutron activation analysis. The results of this study indicated that this technique which is relatively quick and inexpensive can be used to monitor the possible health hazards of Hg to dental personnel.

In a nutritional study, Gershoff *et al.* (1977) estimated trace minerals in human and rat hair. These authors found that both the Zn and Cu content of hair can be varied by altering the diet. In this regard, Underwood *et al.* (1971) monitored nutrition intervention programs in villages of Thailand. Growth, morbidity and haemoglobin values of growing children showed no correlation with hair levels of Zn, Fe, Cu and Mg. Levels of Zn and Fe were higher in boys than in girls.

Klevy (1978) examined hair as a biopsy material. Concentrations of metallic elements in human hair have been related to several demographic variables.

Analysis of hair samples soaked extensively in solutions of salts of metallic elements may not be useful in deciding whether hair exposed *in situ* to air pollution, cosmetics, occupational dusts and fumes has biologically captured the metal or simply adsorbed it to the surface.

Various factors that could well affect trace minerals in hair of human subjects such as age, sex, and contraceptive drugs were studied by Deeming *et al.* (1978). Hair and blood samples were analysed for Zn, Cu, Mg and Fe. Dietary records were kept for the same subjects. In this survey, female subjects had a higher mean hair Zn level than male subjects but serum Zn was not different for the two groups although dietary Zn intake was greater for males. Mean hair Zn increased with contraceptive use while serum Zn decreased, indicating an effect on uptake.

Erten *et al.* (1978) determined hair Zn levels in healthy and malnourished children as a function of age, sex and colour of hair. It was found that the levels of hair Zn increased as a function of age, whereas no statistically significant differences with respect to sex and colour of hair were observed. In a protein-calorie malnourished group of 11 girls and 6 boys between the ages 0 to 3 years, it was found that the hair Zn levels were significantly higher than in a group of healthy subjects of the same age range. As a cautionary note, however, in a protein-calorie malnourished group of seven subjects no correlation was found between hair and serum Zn levels.

Reilly and Harrison (1979) estimated Zn, Cu, Fe and Pb in scalp hair of students and non-students in Oxford. The authors noted a difference in Cu and Zn levels between non-student males and females and also between male and female students. Results for Pb indicated a relatively low level of environmental contamination for all subjects. Given the increase in vehicular traffic since that study, a follow-up survey would be of interest.

Kyle and Ghani (1982) found elevated Hg levels in people from Lake Murray, Western Province, Papua New Guinea and attributed this to the chronic exposure to methyl mercury through fish consumption. In a study of 114 volunteers from the area, the mean methyl mercury concentration in hair was 15.5 mg/kg (range 3.2-50.5). The main source of methyl mercury was barramundi (*Lates calcalifer*) caught in the Lake. For lower levels of fish consumption, the mean methyl mercury levels in hair were 6.4 mg/kg (range 0.62-25.7) and 2.4 mg/kg (range 0.33-9.0).

Reilly and Harrison (1983) determined nutritional implications of levels of Zn, Cu, Fe, and Pb in hair of British and Australian children. Both Fe and Pb were significantly higher in Brisbane than in Oxford. Zinc and Cu did not differ significantly suggesting similar dietary intakes for these two metals but not for Fe.

Zinc and Fe contents were in turn significantly higher in Australian Aboriginals than in Brisbane children. This might be related to genetic factors on one hand (Braefield and Hambridge 1980) or even to malnutrition in Australian Aboriginals on the other hand (Erten *et al.*,1978). Higher Pb contents in Brisbane than in UK children may be due to environmental pollution from leaded petrol in Brisbane at that time.

Sandford *et al.* (1983) determined the concentration of Mg, Ca, Sr, Fe, Zn, Cu and Mn in hair of Nubian mummies and compared levels with modern day samples. These results provided a quantitative method for assessing the nutritional and disease factors contributing to *Cribra orbitalia* (prototic hyperostosis) a frequent pathology in Nubian remains attributed to Fe-deficiency anaemia. A comparison of Fe levels between infants and children with and without *Cribra orbitalia* demonstrated significantly lower Fe levels for the affected group. In addition, concentrations of Mg were also significantly lower for the Nubian subadults with *Cribra orbitalia.*

Suzuki and Hongo (1984) examined elemental contamination of Japanese women's hair which had been cut in the past and preserved. From the intercorrelation of element content and factor analysis by examining the diminution of contents by washing and by comparing the detected levels with the values measured on contemporary Japanese women's hair, the contribution of exogenous contamination to measured hair levels was found to be very strong for Fe, Mn, Cu, Hg, and Pb, moderate for Na and Zn and negligible for Ca, Mg, Sr, K, Hg (organic) and P.

Moon *et al.* (1986) examined 19 trace metals in scalp hair of children and adults in three Indian villages in Alberta, Canada. An increased number of significant metal-metal correlations in hair metal levels for Fort Mackay children suggested a richer source of multiple metal exposure, relative to children in the other two communities.

Jones *et al.* (1987) used hair as the biopsy material to determine a baseline to investigate the levels of certain metals in the area adjacent to the OK Tedi copper-gold mine in Papua New Guinea where mining activity is now in progress. Hair from local people showed a remarkably high Fe content by comparison with previously studied populations. The extreme variations in hair Fe levels were reflected in the differentiated distribution of levels according to location, age and sex. Part of this thesis details those changes as measured against this baseline (hairs collected in 1982 immediately prior to the start of mining activities).

Carvalho *et al.* (1989) determined Cd concentrations in children aged 1-9 years living < 900m from a lead smelter in Brazil. The mean Cd levels in hair (CdH)

were significantly higher in individuals with the following characteristics: female, racial group "dark" or "medium" and children of lead workers. Hair Cd levels increased significantly with an increase in Cd concentration in soil. However, children with the habit of pica (eating sand) had only a slight increase in CdH levels when compared with those without the habit. The marked variations observed in CdH levels suggest the possibility of using these as an epidemiological index in situations of intense chronic environmental pollution.

Shrestha and Schrauzer (1989) compared randomly selected subjects of Darjeeling (India) with those of the residents of San Diego, California (USA). Differences between mean concentrations of Ca, Mg, Cu, Na, and Cd in the two groups were not significant while the concentrations of K, Fe, Mn, and Zn were significantly higher and that of Al significantly lower in hair of the residents of Darjeeling. Concentrations of Pb in four of the Darjeeling hair samples were very high. The mean Mn concentrations in Darjeeling hair was 20 times higher than the mean Mn content in the San Diego hair samples.

Leotsinidis and Kondakis (1990) estimated trace metals in scalp hair of Greek agricultural workers. The concentrations of six metals (Cd, Pb, Cr, Ni, Zn and Cu) were determined. High positive correlations were observed between Pb, Cr and Cu while high negative correlations were observed between Cd and Zn.

Wilhelm *et al.* (1990) measured the concentrations of Cd, Pb and Zn in scalp hair and pubic hair in 41 humans. Scalp hair metal levels were higher than those in pubic hair. The pubic hair Cd, Cu and Pb levels were higher in males than in females whereas both scalp and pubic Zn levels were lower in males than in females.

Wilhelm *et al.* (1991) determined concentrations of Cd, Cu, Pb and Zn in the proximal end of scalp hair and in toenail clippings of children aged 3-7 years living in industrialised areas in Germany. Toenail Cd and Pb levels were much higher than those in hair, while Cu and Zn values were similar in both biological specimens. In toenails, all elements were positively cross-correlated. By contrast, hair Cd and Pb levels were inversely related to Zn.

1.1 Heavy metal toxicity and pollution

Children are at great risk of Pb poisoning because they take in more Pb than adults (they eat more than adults as a percentage of body weight and often suck their fingers after touching walls and windows treated with lead paint). Children also absorb more of the Pb ingested (between 30 and 50%, compared with about 10% in adults). Inside the body, Pb attacks the brain and other organs which, in effect, mistake it for Ca, allowing Pb to attach to essential enzymes and disrupt

vital organ function. But as the body grows, it develops some resistance to the toxicity. However, since Pb never decays, it can accumulate in the body for life and in higher concentration will disrupt other adult organs as well as the brain. Even low blood-lead levels (lower than 10 μg/dL) have been related to a host of problems, including IQ loss, hyperactivity and aggressiveness, reduced attentiveness, slow reaction time, hearing loss, slow growth and problems with balance. Many of these conclusions are still hotly disputed. The lead industry, in particular, argues that scientists still have not proven that small doses of Pb are truly harmful to children (Emerson and Waldman, 1992). Recent medical standards set for a so-called safe limit (10 μg/dL) in plasma (Fett *et al.*, 1992). However, no such limit has been established for hair lead levels, .

Kopito *et al.* (1967) examined Pb in hair of a group of 17 children hospitalised with lead poisoning. The first 6 with high levels of Pb were those with chronic symptoms, and the next 10 those with both acute and chronic symptoms.

Stewart-Pinkham (1989) determined the effect of ambient Cd air pollution on the hair mineral content of children. Hair analyses of 80 children with learning and behavioural problems were assessed. All the children had been exposed for at least two years to air pollution from a refuse-derived fuel incineration plant. All of the patients had increased hair Cd compared with a control group but there was a strong seasonal influence on hair Cd. A neurobehavioral toxic effect associated with enhanced Pb absorption was found in children, who showed evidence of inhibition of pyrimidine-5'-nucleotidase correlated with low hair phosphorus and Zn levels. Thus hair analyses appears to be a useful biological monitor of detecting toxic effects from ambient air Cd levels in subsets of the population at risk for heavy metal toxicity.

It has been known for more than a century that Cd can cause acute poisoning in humans (Friberg, 1949, 1950). Cousins *et al.* (1973) found that the activity in the renal cortex of the Zn metalloenzyme, carbonic anhydrase, was decreased in swine exposed to high concentrations of Cd in the diet. It has also been reported that toxicity of Cd is inhibited in the presence of Zn (Prizek, 1957; Gunn *et al.*, 1961; Powell *et al.*, 1964; Webb, 1972). These observations thus suggest a possible interrelationship between Cd and Zn.

In the past two decades, hair mineral analysis has been used as a medical and behavioral diagnostic tool. There may be a direct relationship between abnormal levels of toxic metals (major and trace elements) in head hair of children and physical, behavioural and learning disabilities (Kracke, 1982; Lester *et al.*, 1982; Marlowe *et al.*, 1983). The existence of brain disorders in individuals have been

attributed to abnormal levels of Pb and Cd by several workers (Thatcher *et al.*, 1982; Needleman *et al.*, 1979; Fishbein *et al.*, 1985).

Corridan (1974) examined the Cu, Pb, As, Hg and Zn content of head hair of 21 school children living near an open-cut metal mine in Ireland. Maximum values for Cu were found to be from 12 ppm to 46.1 ppm which was less than the Cu values of normal subjects in Michigan (USA) ranging from 31 ppm to 130 ppm, although significantly higher than Melanesians in remote underdeveloped areas. In the latter study, by Jones *et al.* (1987), a range of 0.74 to 82.81 ppm Cu was reported. In rural Irish children, a range of 0.4 ppm to 12.2 ppm (mean 3.1 ppm) of Pb was found which was also less than in a study made by Kopito *et al.* (1967) who found a mean value of 24 ppm in 41 Boston (USA) children used as control against children with chronic lead poisoning (mean 282 ppm). A mean level of 20 ppm was found in England in 8 children, non-occupationally exposed to Pb (Kopito *et al.*, 1967).

There are essentially two classes of trace elements. One consists of those elements that are simply toxic (eg. Hg) while the other comprises those that, although toxic in excess, are metabolically essential and these include Cu (Chuttani *et al.*, 1965; Wahal *et al.* 1965). Jamett *et al.* (1991) measured trace elements in the hair of workers of a copper mine and of children living in the vicinity. These researchers found that the mean values for Cu, As, Hg, Cr, Fe and Sb of the children living in the vicinity of the mine were greater than the same parameters in samples of control children living far from the mine.

Jones *et al.* (1987) determined Cu and other elements in the hair of people living in the vicinity of a copper and gold mine in Papua New Guinea prior to the start (1982) of mining operations. The mean level of copper (14.5 ± 6 ppm) was higher than the level (9.3 ± 1.8 ppm) of a control group that lived approximately 1000 km from the mining site.

It was found that, in contrast to that in blood, the amount of Hg in hair is a good index of the degree of Hg contamination of the body (Shimomura *et al.*, 1980). These studies showed that in sexually mature teenagers, males had more Hg than females and adults in general. Moreover, the levels of hair Hg in sexaully mature female teenagers was found to be not proportional to their age. Based on these results the authors suggested that a different endocrine factor was involved in the metabolism of Hg which could participate in a sexual dimorphism of the hair Hg levels. It has also been proposed that Hg can accumulate in the human body not only from sources such as fish but also from other environmental sources, and that hair is a good indicator of accumulated Hg (Airey, 1984).

Kyle and Ghani (1982) measured levels of Hg in the hair of people of Lake Murray, Papua New Guinea, who were chronically exposed to methylmercury through fish consumption. The study found the mean methylmercury concentration in the hair (15.5 mg/kg) was higher than the Hg levels in the hair of a nearby control group (6.4 mg/kg). The main source of these high Hg levels was found to be barramundi (*Lates calcarifer*).

Interrelationships of blood and hair Hg concentrations were estimated by Phelps *et al.* (1980) in a North American population exposed to methylmercury. Mercury levels in newly formed hair were found to reflect those in blood while the absolute concentration in native hair was found to be 300 times that in blood. There was a linear relationship between organic and inorganic Hg levels in both hair and blood samples. Furthermore, the concentration of organic and inorganic Hg in hair remained constant and this makes hair a good indicator of body Hg load.

Arsenic in hair has been used as an index for monitoring the exposure of workers to As, because when As is absorbed into the human body, even if the amount is small, a portion of this is deposited in the skin, hair and nails where it binds to the keratin matrix (Yamamura and Yamauchi, 1980). The concentration of As metabolites in hair, blood and urine was determined by Yamamura and Yamauchi (1980) in workers exposed to arsenic trioxide. It was found that the total As concentration in the hair of the workers was very high and inorganic arsenate accounted for the major portion.

1.2 Variations due to age, sex and race

Most trace elements, excluding Zn, are present in higher concentrations in hair of women than in men (Creason *et al.*, 1975). Males, however, have significantly higher ($p < 0.001$) levels of Pb, Cd and Cr in all age groups with the exception of Cd at ages 81-100. Hair levels of Ca, Mg, Cu, Zn and Ni were significantly higher in females for all ages with the notable exception of Mg at ages 11-18 and Cu at ages 35-46. The sex-related differences in Ca, Mg and Cu disappeared in extremely elderly populations (ages 81-100). Hair Zn levels decline with advancing age in both sexes. After age forty, there is a dramatic and significant increase in hair Ni in both sexes (Gordon, 1985). Wide fluctuations occur around birth, puberty, pregnancy and menopause. In both baby girls and boys, the hair Cu concentration increases sharply during the first three months of life and declines between three and six months (Gibson *et al.*, 1980; McDonald *et al.*, 1982). Zn concentration declines after birth in normal male infants who are not breast-fed,

but this is not true in the case of female infants and breast-fed male infants (Petering *et al.*, 1971; Deeming *et al.*, 1978).

Variations with age are also seen in hair Cr and Cd (Hambidge 1974; Gross *et al.*,1976). The concentrations of Zn are higher in black than in blond hair. Black hair contains more Cd but less Pb than brown hair. Red hair contains more Zn, Cd and Ni but less than brown or black hair. One study detected no significant difference between grey and pigmented hair from the same head (except for a higher Pb concentration in the latter), though hairs from different regions of the scalp vary (Holzbecher and Ryan 1982; Grandjean 1983) and the trace element composition of scalp hair differs from that of pubic and axillary hair (Thatcher and Lester 1983). Such variations may be partly racial, but these are difficult to separate from environmental influences. The mean concentrations of Pb, Mn, Sr, Fe, Cu and Ni are significantly higher in Europeans than in Orientals and concentrations of Pb, As, Cr and Mn are higher still in African blacks. The mean concentration of Pb has been reported to be 5-18 times higher in African blacks than in whites (Hilderbrand and White 1974).

1.3 Diagnostic and forensic value of heavy metal analysis

Heavy metal analysis of human hair is an experimental technique that has considerable clinical promise. The metabolism of metallic elements has been implicated in several diseases of high prevalence (Klevay, 1978). Hypercholesterolaemia and aortic and cardiac injury in animals may be the result of copper deficiency (Klevay, 1978). These findings may be of importance in the aetiology of ischaemic heart disease in humans. Congenital abnormalities and difficulties during labour are associated with low serum Zn values early in pregnancy (Klevay, 1978). Hypertension in animals may be due to long-term exposure to small amounts of Cd (Klevay, 1978). Further research and development of hair analysis into a standard clinical method, may be quite useful in the evaluation of the process basic to such diseases.

To determine whether hair Zn levels reflect Zn deficiency and sufficiency in humans as found in swine, Strain *et al.* (1966) analysed hair from zinc-deficient male Egyptian dwarfs, before and after oral therapy with zinc sulphate, and of normal males residing in Cairo. The average Zn content in the hair of the controls was 103.3 $\pm$ 4.4 ppm and in the untreated dwarfs 54.1 $\pm$ 5.5 ppm. Oral zinc sulphate therapy produced an average hair Zn level in the dwarfs of 121.1 $\pm$ 4.8 ppm and clinical alleviation of the Zn deficiency syndrome. The use of hair as an indicator of the body burden of an element shows great promise as a tool for both research and forensic medicine (Bagehi and Ganguly, 1941; Perkons and Jervis,

1962). The highly publicised forensic use of hair analysis was reported in 1961 by Forshufvud and co-workers when they found that Napoleon Bonaparte's hair had a very high As content suggesting that he probably died of As poisoning (Forshufvud *et al.*, 1961).

1.4 Possible relationship between hair protein and heavy metal accumulation

The proteins of hair are present in the main histological components that make up a hair, namely the cortex, the outer covering of cuticle and, in most hairs, a central core or medulla. The study of hair proteins in solution requires very harsh conditions in order to make them dissolve. The first requirement is the cleavage of disulphide bonds and this can be achieved by oxidation, reduction or sulphitolysis. The extractibility is considerably lower for human and animal hair, than for wool but the exact cause of this is not yet known (Gillespie, 1983). The medulla of medullated hair can not be solubilised by any of the procedures that dissolve the proteins of the cortex and cuticle. The main cause of this may be extensive cross-linking by isopeptide bonds and proteolysis can only remove the medulla protein from the medulla structure.

Since the physicochemical matrix of hair is so dependent on the microfibrillar protein structure it is reasonable to expect that, to some extent, the selectivity and rate of heavy metal accumulation in the hair could be affected by relatively minor changes in hair proteins. In an early study, Montagna (1962) suggested that the granular layer of hair as revealed by staining, showed that it was heavily mineralised. Further work using chelating agents confirmed that Ca and Mg are present in the granular layer (Jarrett and Spearman, 1964). The activity of the various enzyme systems involved in keratinisation can be increased by Mg. Magnesium can function as a catalyst in the exothermic reaction for the production of disulphide bonds from cysteine which is a key reaction in the production of a stable keratin molecule (Jarrett and Spearman, 1964). It was estimated by Purser (1979) that wool growth can be affected by a supply of many minerals and it was found that the trace elements Cu and Zn were directly required for wool growth. Deficiency of Cu causes different diseases including Menkes' kinky hair syndrome. In Menkes' syndrome the changes in hair, skin and pigmentation are greatly enhanced (Danks, 1972). The profound changes in free sulphydryl groups in hair keratin which are consistent with Cu deficiency, cause the production of kinky hair in Menkes' syndrome (Danks, 1972). As the amine oxidases which modify the lysyl residues depend on Cu, deficiency of Cu prevents the formation of lysine derived cross-linkages between elastin. Similarly the

altered physical and chemical properties of wool associated with copper deficiency may be explained by the excess of free sulphydryl groups, as the formation of disulphide bonds between the polypeptide chains of keratin fibres is copper-dependent (Danks, 1972). A deficiency of Cu reduces the amount of wool grown as well as the tensile strength of the fibers so produced while Zn deficiency inhibits wool growth and can cause cessation of fibre growth (Underwood, 1977; Reis and Downes, 1980). Another function of Zn is its role in cystine metabolism which is very important for wool growth (Hsu, 1976). A deficiency of Zn caused rats and mice to lose hair (Hsu, 1976). McClain *et al.* (1973) determined the fundamental role of Zn in the process of cross-linking of collagen. It was observed that β-collagen components from Zn-deficient animals were increased in comparison to those from zinc-supplemented animals.

The aims of this section of the present work were to develop appropriate methods for electrophoretic analysis of human and animal hair proteins as well as feather proteins and to examine differences between and among different species for comparative purposes. The longer term aim involves researching relationships between hair heavy metal accumulation and hair proteins both within and between species although the precise elucidation of such relationships is beyond the scope of this thesis.

Part (b)

HAIR PROTEINS

Keratins are a group of proteins found in abundance in hair and nails. The presence of numerous disulphide bonds between polypeptide chains creates difficulty in solubilizing by normal protein solvents, and they are normally extracted by reduction followed by carboxymethylation (Marshall, 1980).

Downes *et al.* (1963) determined the separate synthesis of fibrillar and matrix proteins in the formation of keratin. From electron microscopic evidence, these workers suggested that relatively sulphur-poor microfibrils are synthesised in the lower regions of the follicle and as cells containing them move through the keratinous zone entirely different proteins, sulphur-rich and non-fibrous proteins are synthesised there *de novo*. These proteins form a cementing matrix for fibrils which are covalently linked by disulphide bonds. An alternative pathway is that the matrix proteins may be synthesised directly on the filaments, establishing an actual peptide linkage with existing polypeptides.

The proteins of fully hardened keratins can be solubilised by either oxidation (yielding keratoses) or reduction (yielding kerateines). Following these treatments, proteins can be fractionated into two main groups. One group is poor in sulphur and of high molecular mass and consists of α-keratose and kerateine A. The other is rich in sulphur and of low molecular mass and consists of δ-keratose and kerateine B. These two different protein types are usually termed low-sulphur proteins and high-sulphur proteins respectively, and are derived from fibrillar microfibril and matrix components of the original keratin.

Gillespie and Inglis (1965) concluded that electrophoretic analysis of high-sulphur proteins shows potential as a means for identifying α-keratins. These researchers proposed that all keratins containing high-sulphur proteins are similar to high-sulphur proteins of sheep (Merino) wool. All of these proteins contained large amounts of S-carboxymethylated cysteine (SCMC, cysteine in the native protein), proline and serine. These latter two amino acids (proline and serine) are present in relatively constant amounts and would suggest that all α-keratins contain high-sulphur proteins as structural elements.

The protein fractions of high-sulphur types examined so far are electrophoretically heterogenous. Despite considerable differences between nail, horn, quill and baleen on the one hand and wool and hair on the other, it is quite surprising that amino acid analyses of these keratins are very similar to each other (Gillespie and Inglis, 1965). On the other hand, different proportions of high-

sulphur proteins in the keratins may be of significance. For example, the horny keratins contain a lower percentage of high-sulphur proteins than hair. Low-sulphur proteins, which generally do not differ from keratin to keratin, are regarded as constituent proteins of filaments.

Gillespie *et al.* (1968) examined a S-carboxymethylated high-sulphur protein component S-carboxymethyl kerateine (SCMK-B_2) prepared from five different wool samples and also from bovine hair. These components showed similarities in amino acid composition and also gave very similar peptide maps after tryptic and chromatographic digestion. It was proposed that a sequence of at least 21 amino acids was common to all the SCMK-B_2 preparations. These workers further suggested that this sequence may be a common feature of a protein present in all wool types examined so far, and also present in proteins of bovine hair with perhaps minor variations in the N-terminal sequence.

Proteins of normal hair and cysteine-deficient hair from mentally retarded siblings were determined by Pollitt and Stonier (1971). It was evaluated that normal human hair gives a major high-sulphur protein of higher molecular weight and S-carboxymethyl cysteine than any isolated protein from normal wool. The proteins from cysteine-deficient hair could also be divided into high-sulphur and low-sulphur proteins. It was also determined that there was a lower proportion of high-sulphur proteins in cysteine-deficient hair than in normal hair. Furthermore, the high-sulphur proteins from cysteine-deficient hair had an abnormal amino acid composition. It was proposed that there were changes in high and very high-sulphur proteins in these mentally retarded siblings compared to the corresponding proteins in normal hair.

Darskus (1972) studied the heterogeneity of the high-sulphur fraction of reduced and S-carboxymethylated proteins from wool by starch gel electrophoresis in aqueous urea-acetic acid at pH 2.4. This study revealed the presence of many components that differed in both composition and molecular weight from the soluble derivatives of high-sulphur proteins. When electrophoresis was carried out at 3^{o}C instead of 20^{o}C, the only change was an approximate 50 % decrease in the mobility of each band, thus indicating that protein degradation during electrophoresis did not contribute to the observed heterogeneity.

In a study made by Day (1972) interspecific variations in hair proteins of twenty-two species of mammal and feather proteins of one bird species were examined. Highly purified fractions were compared by amino acid composition analysis, fingerprinting and partial sequencing. Differences were found not only between species but also within species. These inter and intraspecific differences

between species but also within species. These inter and intraspecific differences may be genetically determined (Gillespie, 1965), although non-genetic factors, such as diet, are known to influence the chemical composition of keratins (Reis and Schinkel, 1964). The results of these studies (Day, 1972) were found to be in close agreement with the general finding of other workers that the keratins do exhibit differences between species. The protein patterns resulting from electrophoresis of keratin extracts from twenty-three species (twenty-two species of mammals and one bird species) were used as characters to classify the species and many of the species were found to be closely related. This classification was not significantly different from one based on morphological characteristics (Day, 1972).

Baden *et al.* (1973) carried out a comparative study of physicochemical properties of human keratinised tissues, especially hair and nail. The keratinised tissues exhibited considerable heterogeneity in composition but all of these contained structural proteins as major constituents. In mammals, these structural proteins are a mixture of α-fibrous and globular proteins in varying proportions. The electrophoretic pattern of hair and nail proteins appeared to be identical as confirmed by 10 % SDS-PAGE. On the other hand, amino acid analysis of the fractionated nail and hair proteins revealed different composition patterns. This indicates that there is a striking similarity in physical and chemical properties of hair and nail, although there are some clear differences but both showed a similar modulus of elasticity by sonic velocity as well as by mechanical methods. Hair and nail are also quite similar in their capacity to absorb water at varying relative humidities. The differences observed between total sulphur and half cysteine content of hair and nail could be explained in part by variation in the amount of matrix component. The relative amount of protein precipitated at pH 4.5 with 0.5 M KCl was greater for hair than nail. These results indicate the extent of diversity observed in keratinised tissues.

Hrdy and Baden (1973) investigated biochemical variations of hair keratins in human and non-human primates. Human hair from various racial groups was analysed by amino acid analysis, polyacrylamide gel electrophoresis, X-ray diffraction studies and stress-strain analysis. indicated A marked similarity in human SCMK fractions among the races was determined by amino acid analysis of SCMK-A and SCMK-B. No large differences were found between races and the results were comparable to the normal amino acid composition of hair as reported for Europeans by Crewther *et al.* (1966). On the other hand, facial and body hair also showed the same pattern, however, non-human primate samples were polymorphic with each family examined exhibiting a different pattern.

Marshall *et al.* (1977) have made a study of high-sulphur proteins isolated from keratins to determine the value of using their electrophoretic pattern as an aid in classification of closely related animals. The high-sulphur proteins of 35 different species, covering nine orders of the class Mammalia, were examined after SDS-PAGE at pH 2.6. From these result, it appeared that, although animals of the same species generally have identical electrophoretic patterns, animals from different species within a genus or even from different genera do not invariably have dissimilar patterns. Hair analysis may well be of use as a supplementary tool in animal classification due to the ease of collection of hair samples in comparison with tissue samples, and relative simplicity of the experimental technique, coupled with the high level of specificity observed in certain cases.

Matrix proteins of human hair were used as a tool for identification of individuals by Lee *et al.* (1978). These researchers investigated the principal structural proteins (keratins) of hair located in the cortex and divided them into two major groups: the fibrous proteins which constitute the microfibrillar moiety and the matrix proteins which consist of an amorphous matrix in which microfibrils are embedded. The study of the electrophoretic protein patterns from over 300 individuals showed individual differences in the number and amounts of these protein components. It was concluded that the electrophoretic variants may prove of greater value as markers in forensic medicine by comparison with the limited value of studying such properties as colour, size, shape and surface characteristics in hair identification.

Marshall (1980) observed genetic variations in both low and high-sulphur protein fractions from human nail. It was suggested that all keratins contained the same molecular architecture with filaments composed of a group of proteins termed low-sulphur proteins, embedded in a non-filamentous matrix. The matrix consisted of two classes of proteins, one rich in sulphur (high-sulphur proteins) and the other rich in glycine and tyrosine (high-tyrosine proteins). Moreover, the relative proportions of individual protein components and also the relative amounts of protein associated with keratins were not the same. This was a result of the different functional requirements of the keratins. Although human hair and nail consisted of similar proteins, hair had a higher proportion of high-sulphur proteins, especially very high-sulphur rich components.

Budowle and Acton (1981) developed a photochemical stain for detection of variable electrophoretic patterns of hair proteins. Five different isoelectric focussing patterns were detected from a limited number of individuals. Polychromatic differences of proteins with similar isoelectric points were also examined. Due to the fact that the bulb of a hair strand is vascularized, it is

possible that differences shown for hair proteins may be related to plasma proteins known to be polymorphic.

Marshall and Gillespie (1982) designed a relatively sensitive (3 mg sample) two-dimensional polyacrylamide gel electrophoretic procedure which provided a high degree of discrimination between proteins of human hair. Hair samples from seven individuals spanning three generations of one family were examined. Eight areas of variability were found in electrophoretic patterns which corresponded to differences in one or more proteins. The advantage of this procedure was that it showed potential for forensic analysis of small samples, possibly even part of a single human hair fibre. The authors suggested that significant variations observed between the two-dimensional sodium dodecyl sulfate gel electrophoresis (2D-SDS-PAGE) patterns of different individuals arise mainly from genetic and environmental factors, air and sunlight weathering as well as cosmetic treatments.

In a later publication, the same authors (Marshall *et al.*, 1985) described an improved 2D-SDS-PAGE procedure which resolved many more protein spots. It was suggested that the two-dimensional technique can discriminate between different species and, in some cases, even discriminate between certain individuals within a species. It was concluded that the examination of a large number of samples was required to construct a suitable representative library of human hair proteins.

Gerhard (1987) described a fast and simple procedure for routine typing of human head hair. Hair samples from 445 different individuals were examined and eight characteristic polypeptide patterns (phenotypes) were found. These were termed K_1 to K_8. Further findings showed that K_1, K_2 and K_3 occur frequently, especially K_1 which was found in approximately 79 % of the individuals under study. The molecular mass of these polypeptides ranged from 45 to 60 kDa. This study proposed that electrophoretic keratin typing was a promising tool for hair analysis in genetic and forensic investigations.

It was further noted that hair samples expressing the same keratin or phenotype often yielded banding patterns which were different in their total intensities, i.e. contents of total protein, even if the same quantity of hair material was used for extraction. This was true not only with hair from different individuals but also with hair originating from different head sites from one individual. These types of differences may reflect differences in accessibility of the hair fibers to solubilising agents depending on the structure of the hair cuticle and cortex.

Marshall (1987) analysed hair proteins by SDS-PAGE using a simplified method without S-carboxymethylation, protein fractionation and lyophilisation. Hair samples from human, rat, guinea pig, rabbit, gerbil, cow and sheep were

Marshall (1987) analysed hair proteins by SDS-PAGE using a simplified method without S-carboxymethylation, protein fractionation and lyophilisation. Hair samples from human, rat, guinea pig, rabbit, gerbil, cow and sheep were analysed, compared and molecular mass determined. These molecular sizes were found to be consistent with those obtained using physical methods. It was suspected that the relative lack of prominent lower molecular mass bands in human hair reflects poor extractability i.e. protein recovery was unusually low about 30 % in this case, while samples with recovery > 60 % gave more intensive but diffuse bands. Despite these difficulties, in all cases the characteristic human protein pattern was unaffected. It was suggested that high resolution patterns distinguished the hair proteins of various species and it was proposed that this method had the potential for application in forensic science and the textile industry.

Shimoshima *et al.* (1988) determined the influence of protein malnutrition on amino acid composition, trace metals and tensile strength of rat hairs. It was determined that Mg, Zn and Fe contents in hair increased in protein-deficient rats as compared with controls. However, it should be noted that many investigators have reported variable results for metals in hair protein of malnourished children and there appears to be no common agreement among the data. For example, it was pointed out by Suzuki (1985) that changes in trace metals in hair should be cautiously regarded as an indicator of nutritional status.

Nagai *et al.* (1991) examined the affect of hair dyes and bleach on hair protein patterns by isoelectric focussing (IEF). In this study, it was determined that metallic dyeing, oxidative dyeing and bleaching induce changes in IEF protein patterns and in intensity of hair protein bands. These results further indicated that hair treated with a permanent hair dye and hair which has been bleached can be differentiated from untreated hair by its characteristic IEF protein pattern. Secondly, the metallic dyed hair as well as acidic oxidative dyed hair could be distinguished from hair that had been treated with other dyes. These results may thus prove useful for forensic cases requiring hair identification.

1.5 Different classes

Proteins of hair can be accordingly classified as follows:

a) High sulphur proteins.
b) Very high sulphur proteins.
c) Low sulphur proteins.
d) Tyrosine containing proteins.

1.6 Occurrence

1.6.1 High-sulphur proteins (matrix proteins)

High sulphur proteins together with the high glycine/tyrosine proteins, could be referred to as matrix or filament-associated proteins. There are few other examples of such proteins in epidermal cells (Bernstein, 1983). The high-sulphur or cysteine-rich proteins are a heterogenous group in hair and are found in different amounts in various keratins (Gillespie and Frenkel, 1974). Around 20 mol % S-carboxymethylcysteine (SCMC) present in wool and the S-carboxymethylkerateines-B (SCMK-B) fractions of the hair of some animals can be as high as 30 mol % (Gillespie, 1983). The exact number of high-sulphur proteins is not known. The fractionation by chromatography on DEAE-cellulose or gel electrophoresis of each fraction yielded at least 35 separate proteins and a similar number were distinguishable after 2D-SDS-PAGE of SCMK-B (Darskus, 1972).

Sulphur rich proteins are the main constituents of all hard α-keratins examined so far but they vary in amounts from 7 to 10 % for some horns and up to 40 to 50 % in some hair (Gillespie, 1991). The cystine content of these proteins ranges differently for different keratins, eg. it may be 17 % for the components of rhinoceros horn and up to 35 % for certain components of mouse hair. They constitute one of the richest sources of cystine found in nature. These proteins are also different from other proteins because of their extreme heterogeneity in both mass and charge.

1.6.2 Very high-sulphur proteins

The discussion of the high-sulphur protein group remains incomplete without specifying the ultra-high sulphur proteins of wool which were first described as a result of cystine-enrichment of experimental sheep (Reis, 1979). It was later estimated that the ultra-high sulphur proteins locate themselves on a diagonal in SDS-PAGE at pH 8.9 and approximately one third of the amino acid residues in these proteins are half-cystine residues. Not much is known about them and it is presumed that they are encoded by a separate set of genes. Their increased content of S-carboxymethyl groups may result in greater molecular mass than for the high-sulphur proteins and anomalous electrophoretic mobility in the presence of SDS. In mouse and human hair an equivalent group of proteins appears to be present but, as for the wool proteins, no single species has been isolated and sequenced (Gillespie *et al.*, 1980; Gillespie *et al.*, 1982).

1.6.3 Low-sulphur proteins

These are mainly the proteins of intermediate filaments (IFs). IFs comprise of mostly, if not exclusively, multiple subunit proteins that are partly helical and that are referred to as low-sulphur proteins (Fraser *et al.*, 1972; Jones, 1976).

The presence of α-helix separating the low-sulphur proteins from other keratins, reaches a level of about 53 % for Merino and 55 % for Lincoln sheep. Crewther *et al.* (1966) estimated the average helix content for the proteins of 12 species and found that it was 45 % with a range from 37 to 51% which may indicate the presence of non-helical protein impurities rather than inherent differences in the α-helical content of different low-sulphur proteins.

1.6.4 High-tyrosine proteins

These are small proteins containing a relatively large proportion of glycine and aromatic amino acids, particularly tyrosine, are present in variable amount in mammalian keratins. Although these have been generally referred to as high-tyrosine proteins, some authors (eg Crewther, 1976) prefer to call them high-glycine-tyrosine proteins. The amounts of these proteins range differently for different keratins eg. almost zero in some keratins like human hair, up to 30-40 % in others such as echidna quill (Gillespie, 1972a). The high-tyrosine proteins are the most easily solubilised of the wool proteins and there are two major families of high-tyrosine proteins: type 1 is poor in cystine but rich in phenylalanine while the opposite is true for type 2 components.

1.7 Synthesis

Keratin synthesis starts in the cortex and cuticle. Firstly, the trichohyalin granules appear in the medulla and inner root sheath, and secondly, a fibrillar material of unknown composition is detectable in the outer root sheath. The synthesis of keratin in the cortex and cuticle occurs at the same time, increasing in rate as the cells migrate up the follicle until the cells are replete and become extensively cross-linked and die (Orwin, 1979).

It was indicated by electron microscopy of metal-stained thin sections (Rogers, 1959 a,b) that a different proportion and arrangement of microfibrils is the characteristic of each type of cortical cell. The orthocortical cell consist of 60 % microfibrils arranged in a whorl pattern with little amounts of intermicrofibrillar matrix, while the lower microfibrillar content (30 %) of the paracortical cell is subsequently associated with greater amounts of matrix (Whiteley and Kaplin 1977; Kaplin and Whiteley 1978; Orwin 1979). This variation in microfibrillar arrangement results in different proportions of microfibril and matrix. It was suggested by other workers (Chapman, 1976) that the microfibrils (low-sulphur

proteins) and the matrix (high-sulphur and high-glycine/tyrosine proteins) are synthesised concurrently in the orthocortex. In the paracortex the microfibrillar proteins are synthesised prior to the synthesis of matrix proteins. At first the synthesis of matrix protein is slow and then it increases sharply until there is dual synthesis of both. The existence of a separate mechanism of synthesis in the mesocortex is not known.

The fibre cuticle varies in thickness from the single layers of wool to six layers in human hair. Keratin synthesis in the cuticle cell is initiated by the progressive appearance of cystine-rich granules. These are deposited initially around the cell periphery and later along the inner root sheath side of the cell (Happey and Johnson, 1962; Roth and Helwig, 1964; Wood and Orwin, 1980). During the process of keratinization, the granules first fuse and are then transformed into lamellar networks (Woods and Orwin, 1980). There is no further information about the fate of these granules. In the fibre the keratin proteins are all exposed to variation via genetic, dietary and physiological factors (Frenkel *et al.,*. 1974; Reis, 1979; Gillespie *et al.,* 1982).

1.8 Methods of separation

1.8.1 Electrophoretic methods

For each of three types (high-sulphur, low-sulphur and high-tyrosine-glycine) of constituent proteins, high resolution separations using both 1D-and 2D-PAGE have already been developed. Two dimensional electrophoresis of the high-sulphur proteins represents an improvement on 1D-gels because the unseparated protein which hinders much of the banding in 1D-gels (Marshall *et al.,* 1977) may be separated from other components by 2D-gels (Gillespie and Marshall, 1981; Marshall, 1981). However, it should be noted that the three classes of proteins are generally present in variable proportions and that protein stains (eg. Coomassie and silver stain) are taken up by certain high sulphur components less than by other keratin proteins. This problem was overcome by Marshall (1981) by differential staining and labelling the proteins with ^{14}C-iodoacetic acid and bands visualised by fluorography coupled with computer based scanning and analysis (Marshall *et al.,* 1985). Hair proteins have also been separated by isoelectric focussing (Budowle and Acton, 1981) as well as the sensitive technique of capillary isotachophoresis (Mikaye and Seta, 1987).

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Although chromatographic methods have been widely practiced for the preparation and characterisation of keratin proteins but only the high performance liquid chromatographic (HPLC) procedures can accomplish a resolution comparable to 2D-gels. Thus HPLC proves to be a promising technique for the quantitative resolution of high-sulphur protein mixtures (Said *et al.,* 1986).

Human hair low-sulphur proteins can be separated from contaminating high-sulphur proteins by dissolving in 6M guanidine hydrochloride and 2 volumes of acetone, the remaining low-sulphur protein preparation has a much lower content of SCMC. Furthermore, low-sulphur proteins of human hair contain considerably more S-carboxymethylcysteine (SCMC) than the low-sulphur protein of Lincoln wool (Gillespie and Marshall, 1981). Reverse phase HPLC has also been used to purify hair proteins (Said *et al.,* 1987) but zinc precipitation has advantages in removing high-sulphur protein contaminants.

Two major families of proteins, known as components 7 and 8 respectively and a minor family component 5, each comprising of a number of individual chains can be separated by two-dimensional separations involving a combination of SDS-PAGE and IEF after fractionation from an extract of the SCM-kerateines (Gillespie, 1991).

High-sulphur proteins can be isolated in the form of α-keratoses after oxidation (Alexander and Earland, 1950) or as SCMK-B after reduction followed by alkylation with iodoacetate (Gillespie, 1959). Most workers use SCMK-B in their studies because firstly, specific reaction are involved in the preparation and secondly, separation is much better after electrophoresis or chromatography.

The high-tyrosine proteins can be easily dissolved. Good yields can be obtained by extraction of wool with mercaptoethanol alone at pH 9.5, but these proteins can be usually separated by zinc precipitation of a total extract using the fractionation scheme of Gillespie, 1991:

Human hair cystine-rich proteins can be separated into 27 fractions by reverse-phase high-performance liquid chromatography. Size-exclusion high performance liquid chromatography can be employed for molecular mass analysis of these keratin proteins (Said *et al.*, 1986).

1.10 Clinical and forensic applications

The relative amounts of the constituent proteins can be determined by the origin of the species and the type of keratins. Moreover, mutations can cause quite drastic changes in the protein composition of wool and hair. A mutation named as trichothiodystrophy (TTD) results in the growth of hair with a much lower cystine content than normal in human hair (Pollit and Stoner, 1971; Gold and Kachra, 1974; Baden, 1976; Price, 1979; Price *et al.*, 1980) which may be the result of a disturbance of high-sulphur protein synthesis. Relative proportions of the constituent proteins of wool and human hair are sensitive to some extent to dietary manipulation.

The origin of unknown samples may be identified by the electrophoretic patterns. The advantage of the 2D-procedure is that it gives many more protein spots in the electrophoretic pattern than does the 1D-method (Marshall *et al.*, 1984). It appears from previous discussions that the 2D-technique can discriminate between different species and in some cases even discriminate between certain individuals within a species because this procedure is relatively sensitive and is capable of producing a pattern from a small part of a single hair fibre (Marshall *et al.*, 1985).

Lee *et al.*(1978) described an electrophoretic system which can readily display the various matrix components of hair keratins. Studies of these electrophoretic patterns from over 300 individuals have shown individual differences in the number and amounts of these protein components. They suggest that the electrophoretic variants in fibrous components could be used as markers in forensic medicine. The technique is reproducible and sufficiently practical to permit relatively simple screening of a large number of samples.

1.11 Polymorphism

Identical groups of proteins have been determined from the hard keratins belonging to different animals, although the relative proportions may differ. These observations have been reported for horn, hoof, wool, echidna, quill, claw and human hair and nail, the latter pair also contains some tissue-specific IF polypeptides (Marshall and Gillespie, 1977; Marshall, 1983). It has been

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The first requirement can be easily fulfilled as keratin proteins from different individuals of the one species generally produce similar patterns with minor differences in the proportion of individual protein components. However, it is quite difficult to meet the second requirement and even the greater resolving power of 2D-electrophoresis may not produce satisfactory results (Darskus and Gillepie, 1971; Simonsen, 1976; Marshall *et al.*,1977).

Polymorphism in keratin was first reported with the observation of an additional band in electrophoretic patterns of the high-sulphur proteins of some samples of Merino wool (Darskus and Gillespie, 1971).

Polymorphism has been demonstrated for both the low-sulphur and high-sulphur proteins of human hair and nail and shown to be inherited in an autosomal

way (Baden *et al.*, 1975; Baden, 1976; Marshall, 1985). In a study made by Marshall (1980) of more than 100 samples of Caucasian nail, 30% contained an extra high-sulphur electrophoretic band, although a third of these variants also contained an additional low-sulphur protein component. The latter was a new type of polypeptide. Lee *et al.* (1978) and Marshall (1985) found sufficient variation in human hair high-sulphur components among a Caucasian population to suggest their use for forensic and taxonomic purposes.

CHAPTER 2

Comparison of scalp hair heavy metals at control and test (Ok Tedi) sites in Papua New Guinea

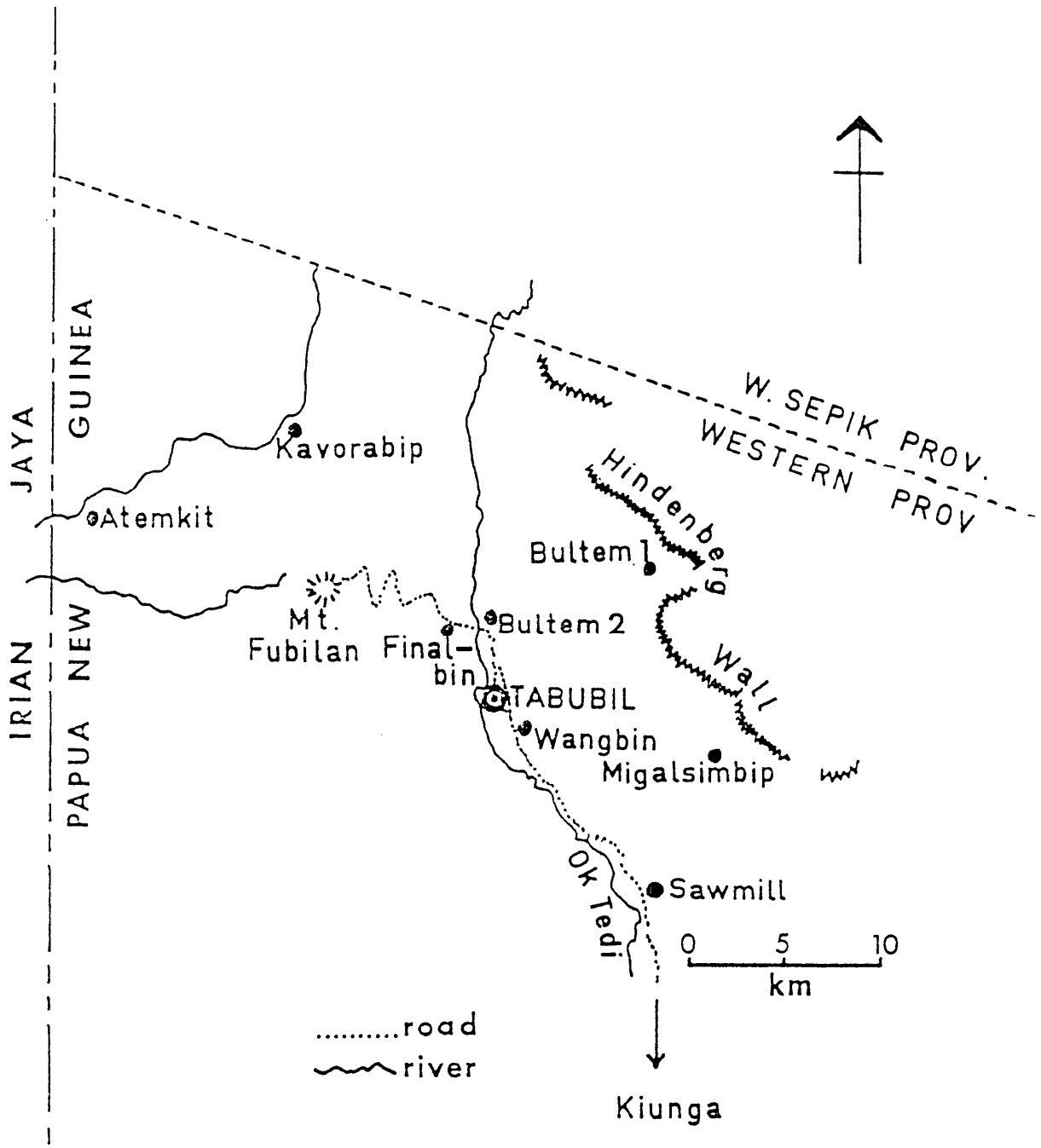
2.1 INTRODUCTION

Papua New Guinea consists of the eastern portion of the island of New Guinea and about 600 smaller islands, with a total land area of about 463,000 square kilometres. Its topography is mainly rugged, with steep mountains constituting the backbone of the country with a northern and southern plain. About 70% of the land area is forested, although this is rapidly diminishing predominantly by courtesy of Malaysian and Indonesian logging interests. The country is culturally diverse. Its three million inhabitants include native speakers of over one third of the world's active languages (O'Fairchellaigh, 1984).

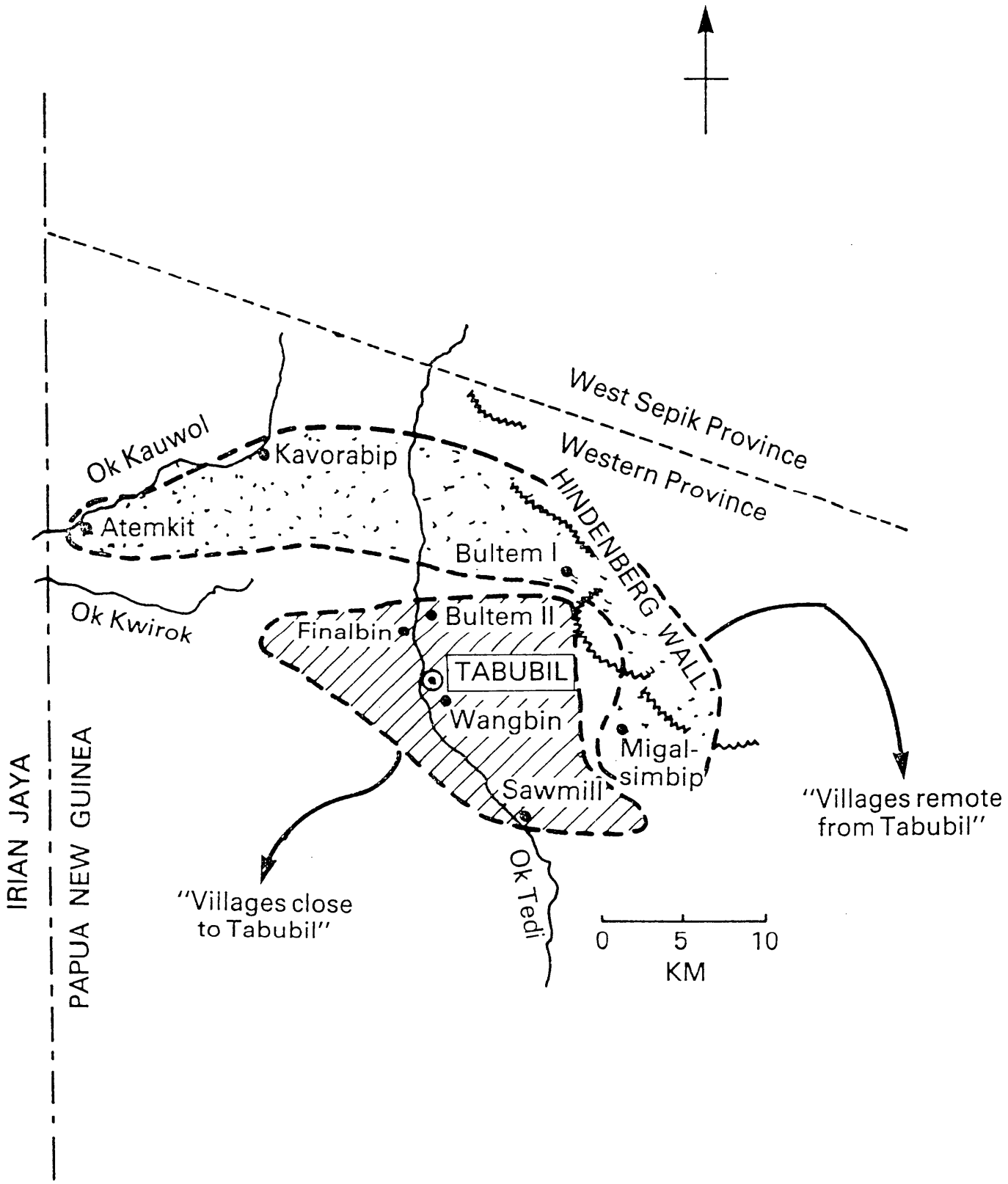
The copper and gold deposits of the Ok Tedi are in the Star Mountains. The Ok Tedi region of the Star Mountains is an area of several thousand square kilometres in the North-Western corner of Western Province of Papua New Guinea. The copper-gold mine is on Mt. Fubilan (elevation 2053 m) which is connected by 5 km of road to the mill at Folomian. A further 17 km away is the village of Tabubil which is the largest community with a population of 4000. Average rainfall at Mt. Fubilan is 10,000 mm and at Tabubil 8,000 mm per annum, and this region is covered by dense tropical forest (Rush and Seegers, 1990).

In the late 1960s a significant deposit of copper and gold was discovered. The mining operation was planned in three phases. The first phase was started in 1984 and this involved mining and processing of gold ore. The second phase commenced in 1987 and was concerned with the mining and treatment of both gold and copper ore. The third and final stage which started in 1988 included the mining of copper ore (Rush and Seegers, 1990).

It is known that mining operations can change the levels and distribution of different heavy metals of the environment in the vicinity of the mine. This pollution not only affects the nearby atmosphere but can be widespread and often has deleterious effects on the health of people living close to the mine (Jones *et al.*, 1987). In this study, scalp hair has been used as the biopsy material since hair samples are easy to collect, store and transport and have an established history of use as an indicator of mid-to long-term body heavy metal burden.



Detailed map of Ok Tedi region of Papua New Guinea showing mine site (Mt. Fubilan), mine settlement (Tabubil) and local villages involved in the study.



Grouping of villages involved in the study.

A medical survey was performed between December 1982 and May 1983 to get the baseline (pre-mining) information about the health and nutritional status of the local population living in the vicinity of the mine. Jones *et al.* (1987) determined the 1982 hair heavy metal levels of this population and these levels were used as a baseline in the present study. A group of people in the Mt. Obree area living a predominantly traditional life style (and living about 700 km from the mine site) were selected as a control grouping in an attempt to throw light on the complex network of social, dietary and environmental interactions which necessarily affect heavy metal burden. For the present survey, hair was collected from all the local villages in the vicinity of the mine site and, in addition, hair was also collected from eleven villages in the Mt. Obree area of the Central Highlands, North-West of the capital, Port Moresby, and remote from the mine site. Hair was collected in late 1990, about six years into the projected 30 years life of the mine. At this stage significant amounts of gold were still being won, although the production of copper was becoming of increasing importance.

Metals such as Cd, Pb, Hg and Cu in hair are known to be toxic at certain levels and their levels in the environment can be increased due to mining operations (Warren, 1973). The present study was designed to monitor the levels of 14 metals (Mg, Al, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Hg and Pb) in the hair samples of people living in the vicinity of the mine and to compare these levels to a population of eleven villages living remote from the mining site. In this way, two populations; one retaining traditional life styles and the other fully exposed to a "Westernised" way of life (and particularly the physical and cultural detritus resulting from mining activities) could be compared.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Common chemicals were purchased from Merck/BDH Biochemicals (Melbourne, Victoria) or Sigma Chemical Company (Sydney, NSW) and were either AnalaR or Univar grade. Triton X-100 was purchased from Sigma, CSR (Commonwealth Sugar Refineries, Sydney) supplied absolute alcohol and Merck/BDH provided acetone. Ultra pure nitric acid was purchased from Merck. Polycarbonate tubes with plain caps were supplied by Disposable Products (Adelaide, SA). The microwave used for digesting hair samples was model PMO-600 from Palsonic.

All solutions were made with glass-distilled rainwater which was purified through a Milli-Q (Millipore, Sydney) water filtration system.

2.2.2 Origin and preparation of samples

The subjects of this study belonged mostly to the Wopkaimin population group, with a small minority of the people consisting of those who had been living in the Ok Tedi region for a long time, and these were the Ningerum people. Hair samples were also collected from a control group consisting of eleven villages in the Mt. Obree area of the Central Highlands of Papua New Guinea, remote from the mining site.

Hair samples were cut, using clean stainless steel scissors, from at least four parts of the head (front, back, crown and side). An attempt was made to cut the hair as close to the scalp as possible to avoid the surface accumulation of trace and heavy metals. Approximately one gram of hair was collected from each individual. After cutting, the hair sample was placed in a dry plastic bag and labelled before it was transferred to the laboratory. The hair samples were not washed before sampling.

In the laboratory, hair samples were washed thoroughly with Triton X-100 (1%), a non-ionic surfactant (Harrison *et al.,* 1969), rinsed at least three times with double-distilled water, washed with acetone and again rinsed with double-distilled water, and finally oven dried at 80^{o}C for 15 hours.

2.2.3 Digestion of hair samples

Fifty milligrams of washed, dried hair samples were microwave digested in polycarbonate tubes with plain caps, in 5 ml of ultra pure constant boiling point nitric acid. Digests were made up to a fixed volume (25 ml) with Milli-Q water and a suitable aliquot was withdrawn for elemental analysis by inductively coupled plasma argon mass spectrometry (ICPMS) (Horlick *et al.,* 1987) at the CSIRO, Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights Laboratories, Sydney using the following standard and sample solutions.

2.2.3.1 Standard solution

A standard solution was made by mixing 0.5 ml of HNO_3, 0.5 ml of indium solution (10 ppm solution was prepared by dilution of a 1000 ppm In solution) and 0.5 ml of 10 ppm of a multi-element solution and made up to volume (30 ml) with Milli-Q water.

The multielement standard solution consisted of 500 µg/L Fe, Al; 100 µg/L Mg, Cr, Ni, Mn, Co, Cu, Zn, As, Se, Mo, Cd, Pb; 20 µg/L Hg, all in 10 % v/v HNO_3.

2.2.3.2 Sample solution

0.1 ml of 10 ppm indium solution was added to the sample solution (50 mg of digested hair in 5 ml of HNO_3) and made up to volume (30 ml). Sample solutions were introduced into the spectrometer at a flow rate of 0.7 ml/min. The sensitivity of the instrument was 3 to 4 million cps/ppm In, with a background of < 10 cps (@ mass 220).

2.2.3.3 Calculations

Semi-quantitative analysis: A response curve (second order fit, mass vs instrument response) was used to initially calculate the concentrations of all samples. A response curve was generated each day by running a blank and a 100 µg/L each of Be, Mg, Co, Y, In, Bi and U standard. The multielement standard (see 2.2.3.1) was then used to quantify the elements of interest.

The concentration of heavy metal obtained from the instrument was in parts per billion (ppb). This was converted to parts per million (ppm) taking into account the appropriate dilution factor.

2.2.3.4 Statistical analysis

Statistical analyses were performed using an Osborne 386-SX computer and the Minitab statistical program.

a) Means and standard errors of mean were calculated for each of the villages for 14 trace and heavy elements.

b) Histograms with significance levels, for each of the metals were drawn for pooled controls and test groups.

c) The Student t-test was applied to the data between pooled controls and test villages for each of the 14 elements.

2.3 RESULTS

Tables 2.1 and 2.2 show the mean concentrations, with standard errors of the mean (SEM) for each of 14 elements i.e Mg, Al, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Hg and Pb in the eleven control villages and six test villages. Those results in the test (Ok Tedi) villages which show significant difference from the global (pooled) control (Mt. Obree) data for any given metal are indicated.

Figures 2.1 to 2.8 show the same data plotted as histograms for control and test sites for each of the above 14 elements. As the mean concentrations of Se and Hg for the test sites are ten times higher than the concentrations of control sites, separate histograms for control and test sites have to be drawn as it was difficult to fit these comparatively high and comparatively low values on the same scale.

Magnesium levels at 4 out of 6 of the test sites were similar to control sites. However, test villages 2 and 4 were markedly high in Mg.

With the exception of test village 2, levels of Al for the test villages were slightly lower than the values for control villages.

Chromium levels for test villages were considerably higher than controls.

The mean concentrations of Fe for test and control villages were similar except for test village 2 which showed a mean Fe concentration of 765.4 $\pm$ 52.6 mg/kg which is remarkably high by comparison with any former study of any other population group.

The mean values for Mn of most of the test sites were lower than the control sites with the exception of test villages 2 and 4 which showed a mean concentration of for Mn of 101.61 $\pm$ 9.15 and 97.8 $\pm$ 11.0 mg/kg respectively.

The levels of Co for control as well as test sites showed a highly heterogenous trend of high and low values for that element. No consistent and comprehensive difference between tests and controls was evident.

Considerable variation in Ni levels existed in the control populations, so although the test populations tended to show higher levels, a consistent and comprehensive trend was not evident.

The mean concentrations of Cu for the test villages were higher than the control sites, with the exception of control village 3 which showed a concentration of 19.62 $\pm$ 8.20 mg/kg, comparable to that for the levels shown by test sites.

On the other hand, mean Zn values of test villages were significantly, consistently and comprehensively higher than the values shown by control sites.

The mean values of As, Se, Cd, Hg and Pb for test villages were again significantly, consistently and comprehensively higher by comparison with the mean concentrations for control villages. Indeed, for Hg and Se, different scales

were required for presentation of results since test sites showed approximately ten times higher levels of these elements than the control sites.

MELANESIAN VILLAGES

CONTROL	TEST
(Mt. Obree area, PNG)	(Ok Tedi area, PNG)
1. TABU	12. FINALBIN
2. BEDIKA	14. ATEMKIT
3. BOEA	15. BULTEM
4. ADORA	16. KAVORABIP
5. BORO	17. MAGALSIMBIP
6. SOMORI	19. WANGBIN
7. ABORA	
8. MIMAI	
9. LARONU	
10. ABOANA	
11. DOOBI	

CODES FOR LEVELS OF SIGNIFICANCE

@ 0.05%

* 0.01%

0.001%

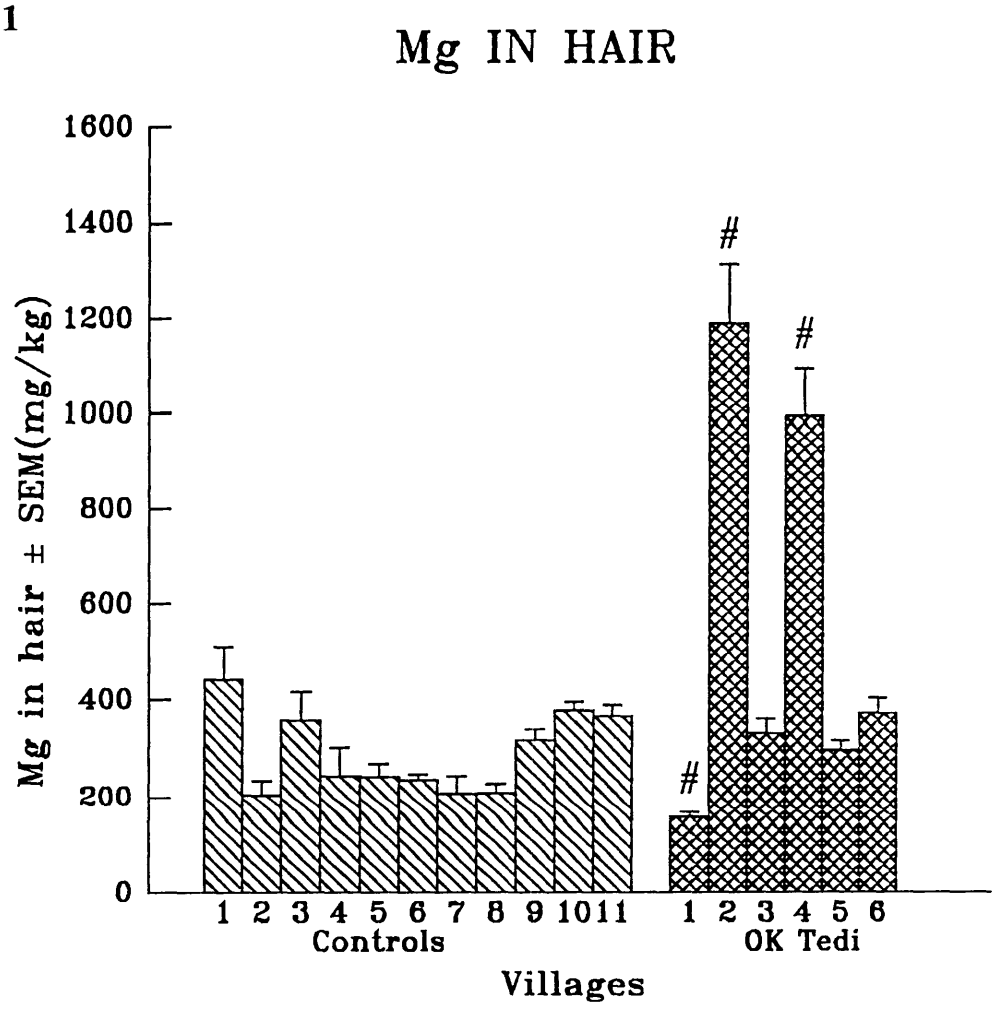
TABLE 2.1 MEAN TRACE METAL LEVELS (mg/kg) IN HAIR FROM CONTROL AND OK TEDI SITES

CONTROL VILLAGES	Mg	Al	Cr	Fe	Mn	Co	Ni
1	442.1 ± 68.3	299.9 ± 46.2	0.9796 ± 0.0911	215.3 ± 35.7	52.49 ± 6.39	0.1149 ± 0.022	1.951 ± 0.595
2	205.5 ± 28.6	197.4 ± 27.3	0.994 ± 0.197	186.2 ± 23.4	44.02 ± 8.11	0.0746 ± 0.146	1.145 ± 0.154
3	356.0 ± 61.0	288.9 ± 29.5	0.779 ± 0.106	240.1 ± 27.1	66.3 ± 11.4	0.0984 ± 0.034	0.7191 ± 0.05
4	244.4 ± 58.3	297.2 ± 43.0	0.8968 ± 0.0577	298.5 ± 57.7	37.63 ± 6.34	0.0683 ± 0.017	0.980 ± 0.251
5	242.3 ± 27.1	208.0 ± 26.2	1.222 ± 0.432	215.5 ± 31.6	50.60 ± 6.66	0.0861 ± 0.027	0.673 ± 0.122
6	235.8 ± 13.0	211.2 ± 14.5	0.8692 ± 0.0613	260.9 ± 15.5	22.82 ± 1.15	0.1774 ± 0.013	0.674± 0.035
7	207.1 ± 37.3	263.5 ± 45.9	0.5967 ± 0.0844	253.1 ± 46.7	16.64 ± 1.57	0.1503 ± 0.017	0.815 ± 0.153
8	208.9 ± 19.0	61.71 ± 6.76	0.884 ± 0.359	144.5 ± 22.3	14.21 ± 1.62	0.1061 ± 0.023	0.630 ± 0.110
9	315.8 ± 21.7	111.4 ± 14.9	0.604 ± 0.200	162.4 ± 13.0	18.47 ± 1.63	0.1518 ± 0.019	0.523 ± 0.121
10	377.8 ± 17.6	147.6 ± 12.5	0.8537 ± 0.0634	281.1 ± 14.5	24.73 ± 1.60	0.2302 ± 0.016	2.330 ± 0.312
11	364.4 ± 23.7	189.4 ± 16.3	1.4959 ± 0.0518	333.5 ± 15.3	25.90 ± 2.07	0.3526 ± 0.026	2.343 ± 0.200
TEST VILLAGES							
1	156.7 ± 9.73#	27.33 ± 2.00#	1.4864 ± 0.0932#	273.14 ± 8.01#	18.17 ± 1.34#	0.0940 ± 0.009#	1.806 ± 0.059#
2	1189 ± 124#	644.0 ± 73.9#	3.272 ± 0.150#	765.4 ± 52.6#	101.61 ± 9.15#	0.2781 ± 0.033@	2.071 ± 0.150#
3	329.7 ± 29.4	84.63 ± 6.80#	1.5036 ± 0.0693#	222.82 ± 7.06	20.10 ± 1.85#	0.0833 ± 0.006#	1.760 ± 0.101*
4	991 ± 104#	258.9 ± 48.1	1.8112 ± 0.0912#	295.8 ± 39.6	97.8 ± 11.0#	0.03208 ± 0.03*	1.489 ± 0.104
5	295.8 ± 19.4	209.9 ± 25.6	2.778 ± 0.275#	249.2 ± 15.6	18.58 ± 1.28#	0.1369 ± 0.009#	2.003 ± 0.153#
6	370.6 ± 32.5	116.0 ± 27.4@	2.851 ± 0.153#	272.9 ± 15.7	12.18 ± 1.47#	0.3175 ± 0.036*	2.007 ± 0.185*

TABLE 2.2 MEAN TRACE METAL LEVELS (mg/kg) IN HAIR CONTROL AND OK TEDI SITES

CONTROL VILLAGES	Cu	Zn	As	Se	Cd	Hg	Pb
1	7.728 ± 0.372	91.3 ± 14.8	0.1655 ± 0.0365	0.365 ± 0.058	0.257 ± 0.068	0.310 ± 0.380	2.74 ± 0.780
2	11.01 ± 1.03	107.8 ± 14.7	0.2705 ± 0.0344	0.730 ± 0.066	0.266 ± 0.031	0.806 ± 0.155	10.03 ± 2.59
3	19.62 ± 8.20	111.2 ± 10.8	0.1826 ± 0.0485	0.598 ± 0.128	0.192 ± 0.032	0.401 ± 0.163	6.36 ± 2.53
4	9.44 ± 1.55	70.8 ± 12.1	0.2686 ± 0.0631	0.712 ± 0.132	0.220 ± 0.095	0.390 ± 0.046	5.29 ± 1.43
5	10.62 ± 0.81	120.35 ± 9.33	0.2802 ± 0.0504	0.888 ± 0.160	0.193 ± 0.024	0.666 ± 0.089	7.80 ± 2.11
6	9.215 ± 0.307	99.13 ± 3.96	0.2047 ± 0.0239	0.802 ± 0.035	0.274 ± 0.040	0.443 ± 0.096	3.45 ± 0.49
7	9.935 ± 0.815	111.5 ± 11.6	0.1953 ± 0.0302	0.691 ± 0.159	0.337 ± 0.072	0.802 ± 0.301	3.86 ± 0.764
8	8.99 ± 0.779	147.6 ± 14.8	0.1205 ± 0.0286	0.443 ± 0.109	0.124 ± 0.019	0.241 ± 0.053	2.86 ± 0.590
9	9.614 ± 0.869	109.16 ± 7.28	0.2362 ± 0.0251	0.514 ± 0.047	0.309 ± 0.076	0.363 ± 0.054	2.84 ± 0.421
10	9.347 ± 0.439	130.0 ± 16.1	0.5589 ± 0.0447	0.746 ± 0.069	0.180 ± 0.025	0.284 ± 0.017	2.69 ± 0.478
11	11.70 ± 1.05	208.3 ± 13.8	0.7229 ± 0.0537	1.031 ± 0.059	0.293 ± 0.025	0.656 ± 0.051	6.29 ± 0.755
TEST VILLAGES							
1	10.99 ± 0.460	158.15 ± 6.38#	0.777 ± 0.0369#	1.899 ± 0.104#	0.641 ± 0.113#	0.984 ± 0.049#	7.63 ± 0.544#
2	16.44 ± 1.04#	292.3 ± 12.7#	0.7259 ± 0.067#	5.63 ± 0.336#	0.667 ± 0.057#	7.98 ± 0.456#	5.51 ± 0.582
3	13.50 ± 0.727#	302.9 ± 12.4#	0.6215 ± 0.0505#	0.951 ± 0.077	0.714 ± 0.051#	5.32 ± 0.33#	16.11 ± 4.23*
4	10.34 ± 0.799	261.7 ± 18.2#	0.2145 ± 0.0438#	4.19 ± 0.211#	0.954 ± 0.158#	13.48 ± 6.41@	5.01 ± 0.609
5	14.93 ± 0.955#	314.0 ± 14.1#	0.7187 ± 0.0658#	4.92 ± 0.180#	1.681 ± 0.271#	8.49 ± 0.67#	13.99 ± 0.99#
6	22.65 ± 1.76#	431.4 ± 21.0#	0.6851 ± 0.086#	6.278 ± 0.320#	1.420 ± 0.173#	7.26 ± 0.56#	20.23 ± 2.50#

Figure 2.1


Mg IN HAIR
Mg in hair ± SEM(mg/kg)
1600
1400
1200
1000
800
600
400
200
0
#
#
#
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

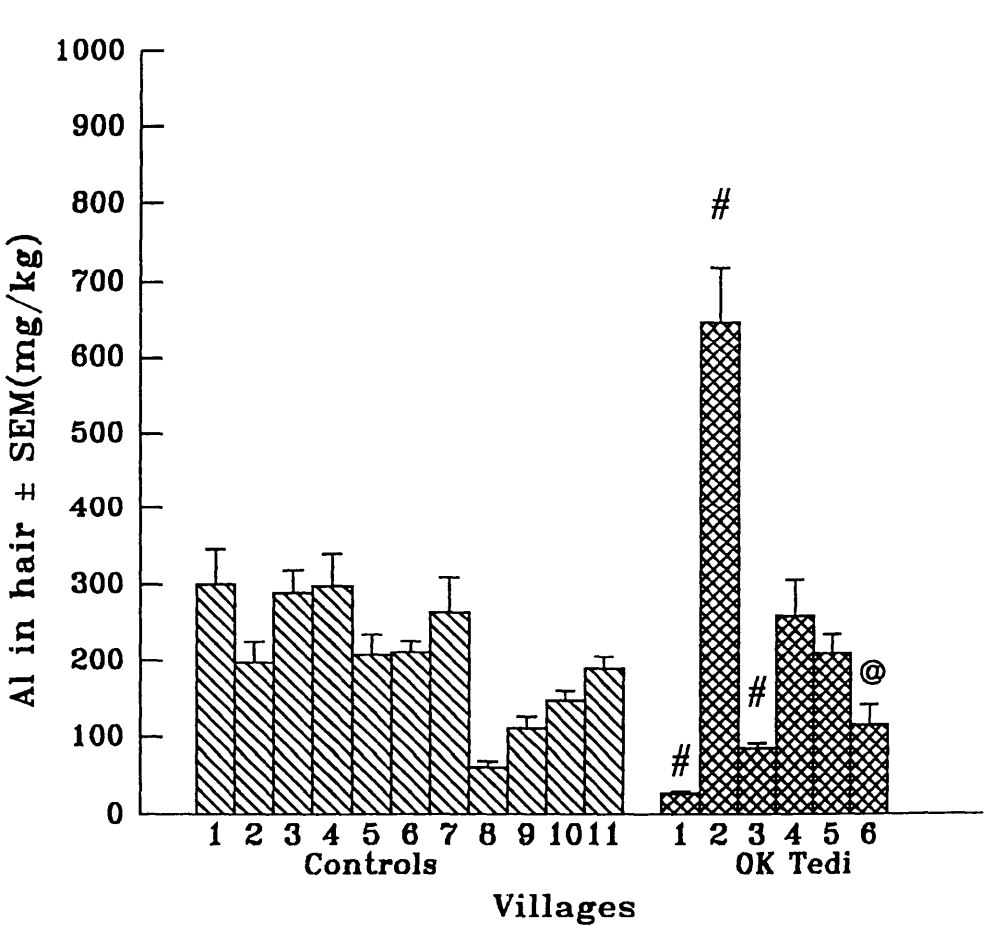
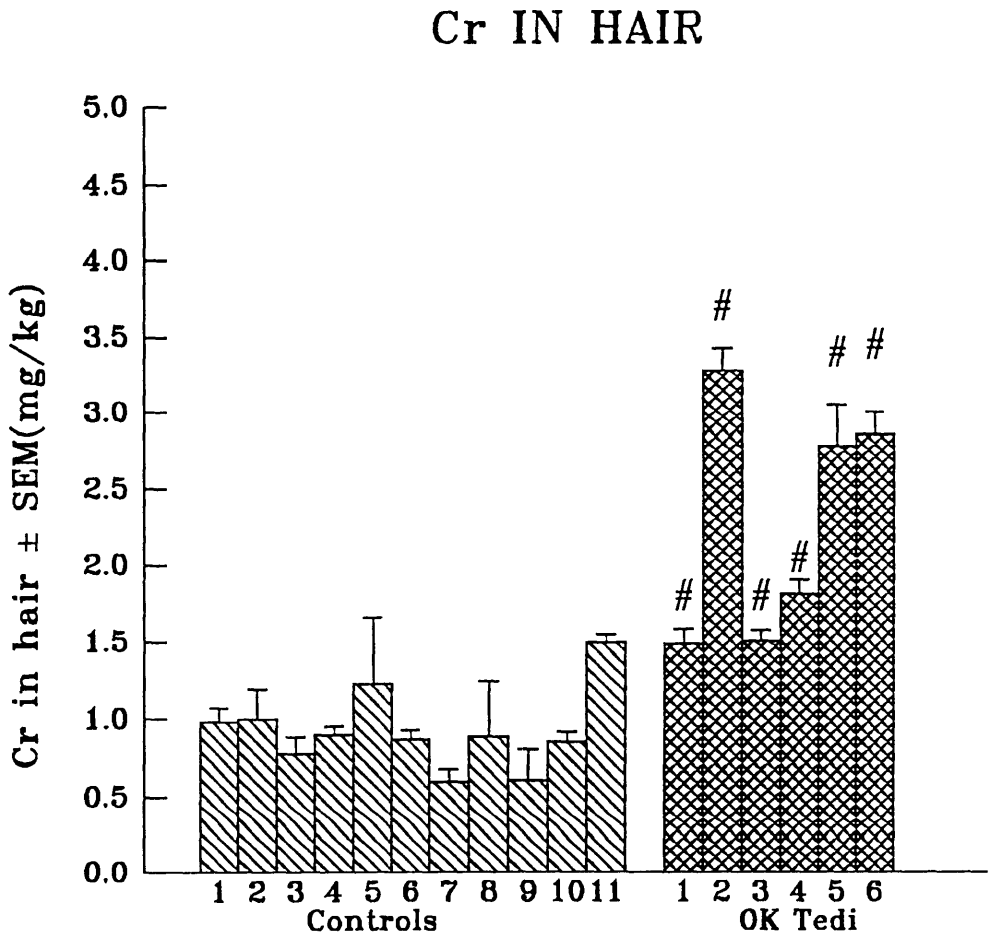

Al IN HAIR
Al in hair ± SEM(mg/kg)
1000
900
800
700
600
500
400
300
200
100
0
#
#
#
@
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

Figure 2.2


Cr IN HAIR
Cr in hair ± SEM(mg/kg)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
#

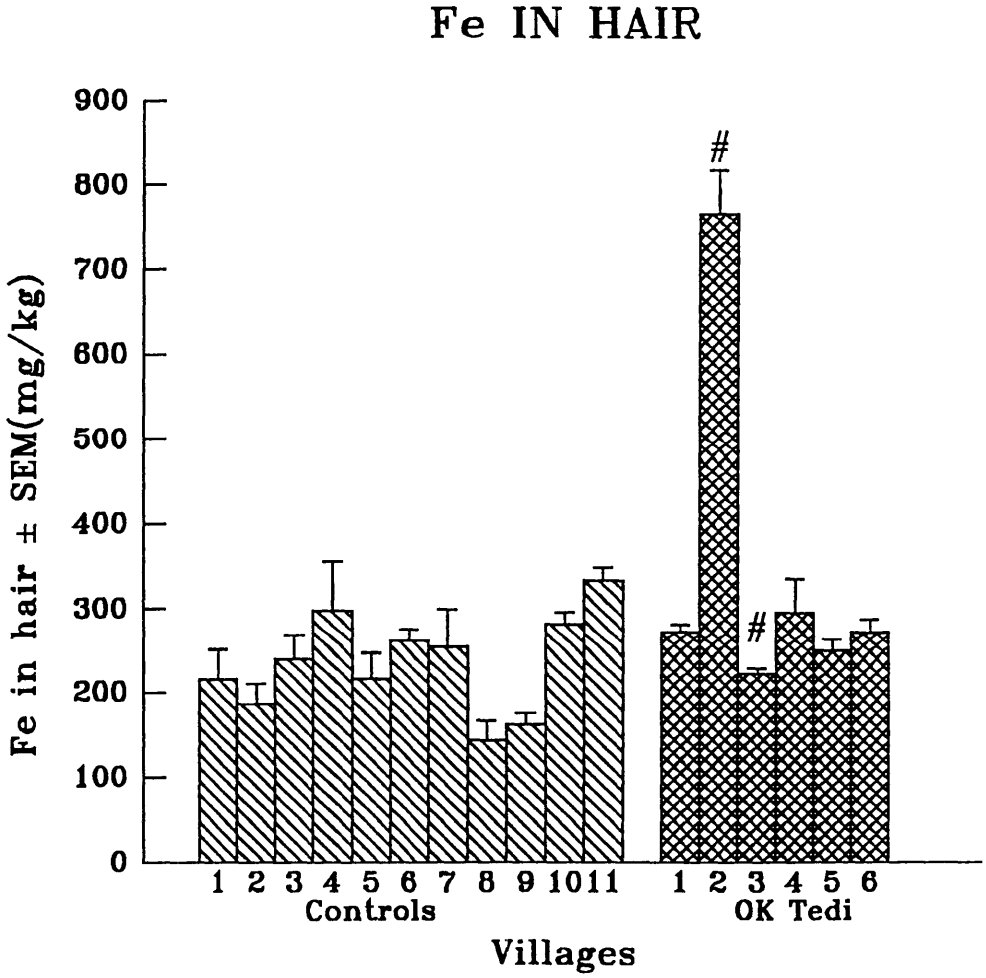
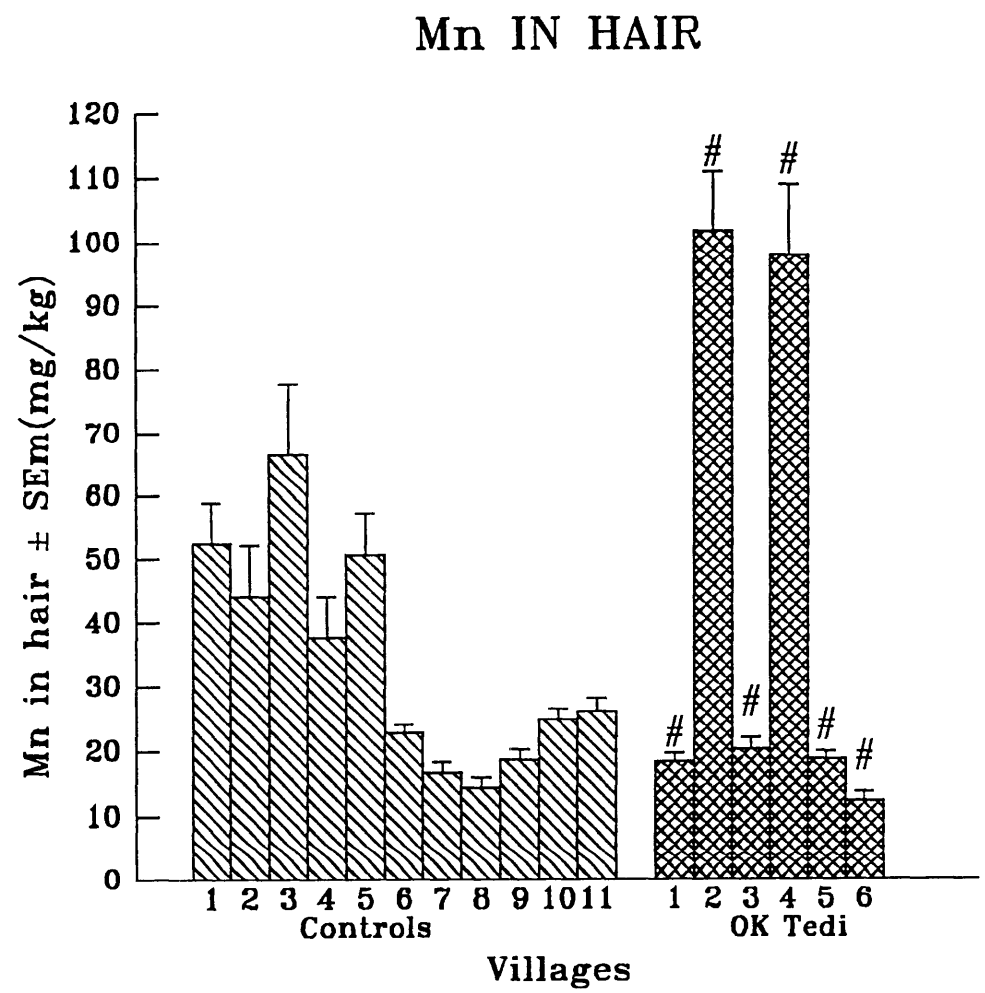

Fe IN HAIR
Fe in hair ± SEM(mg/kg)
900
800
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages
#

Figure 2.3


Mn IN HAIR
Mn in hair ± SEm(mg/kg)
120
110
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

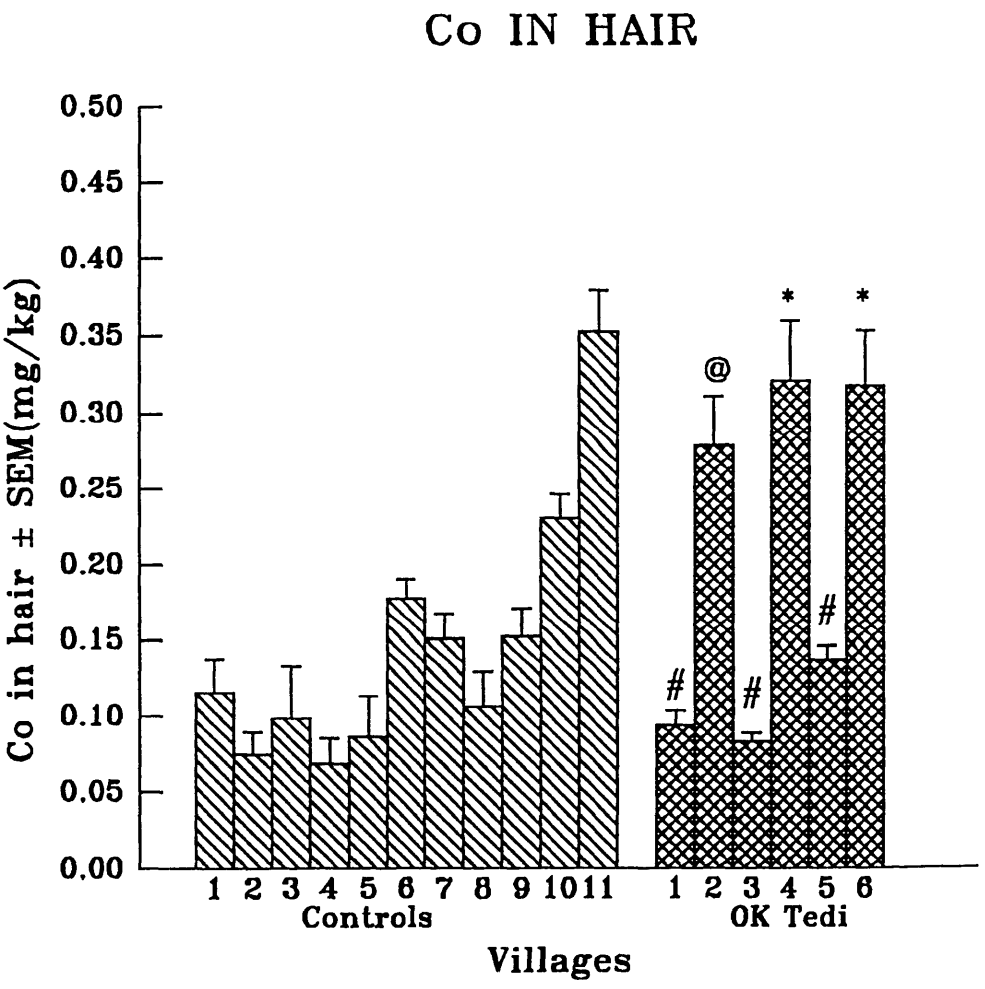
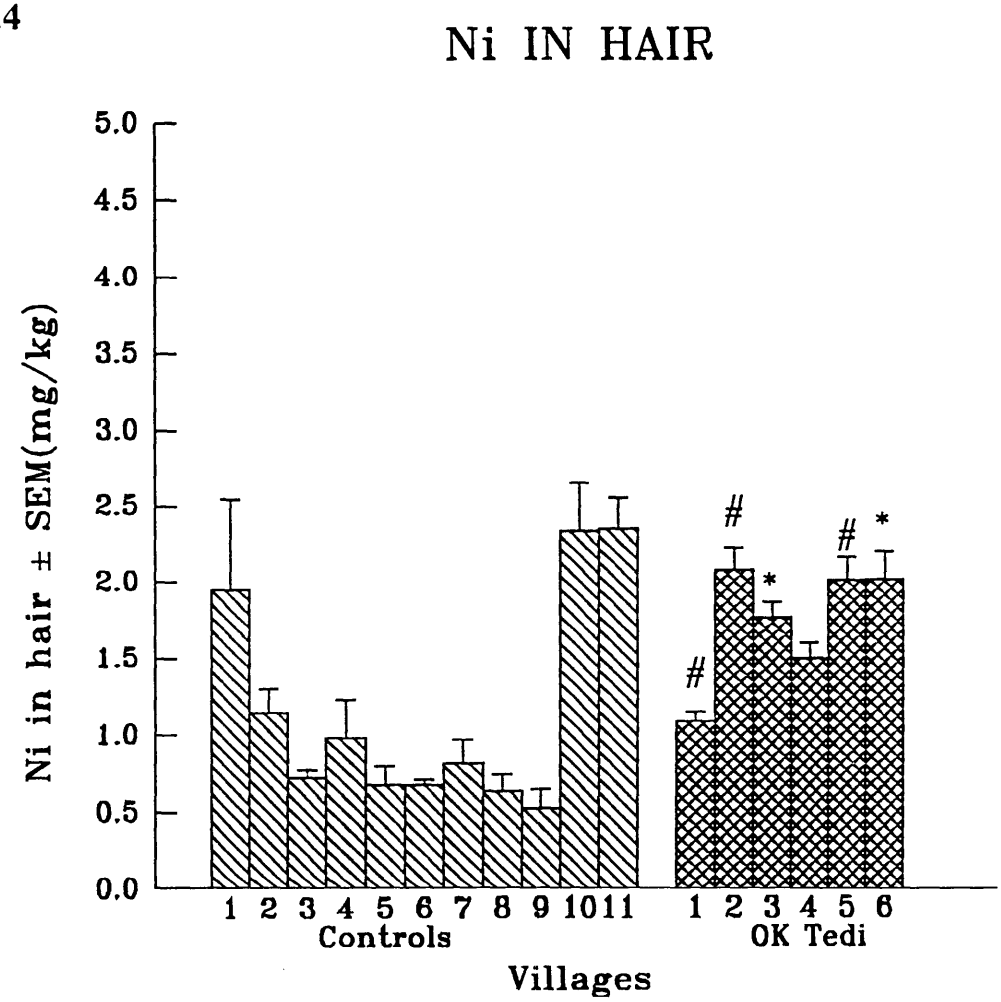

Co IN HAIR
Co in hair ± SEM(mg/kg)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

Figure 2.4


Ni IN HAIR
Ni in hair ± SEM(mg/kg)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

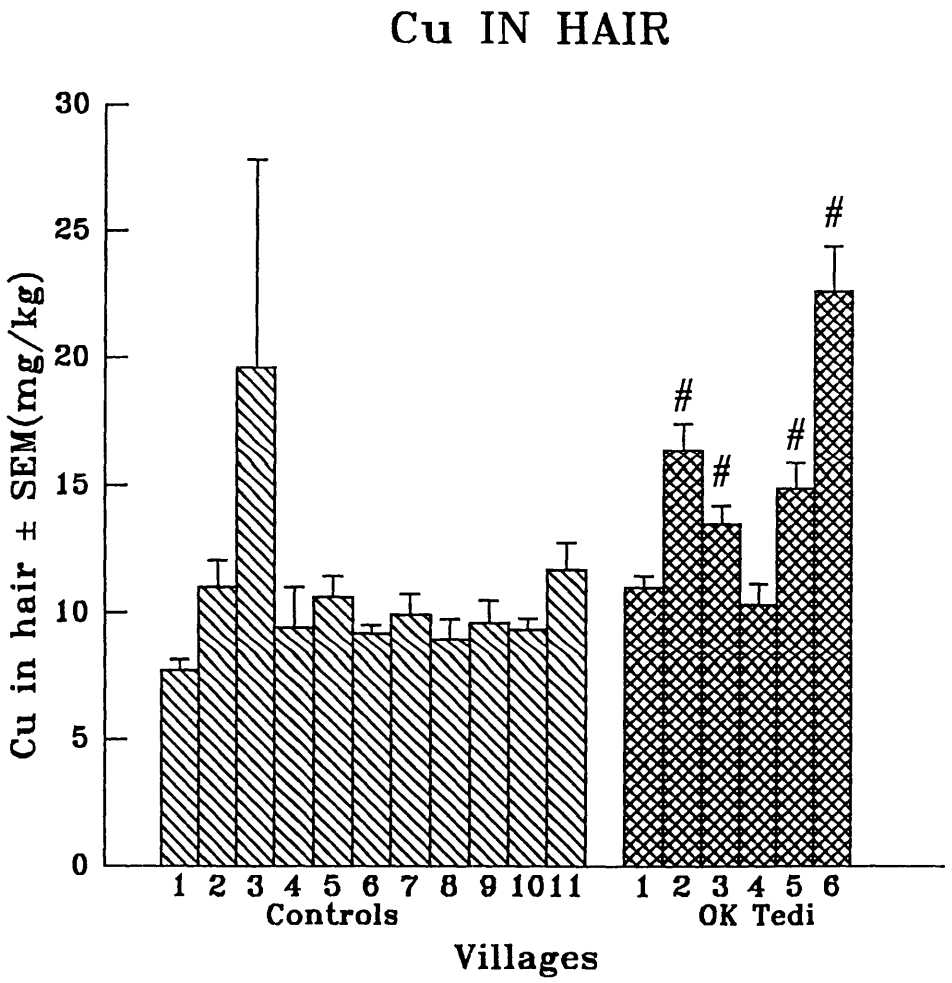
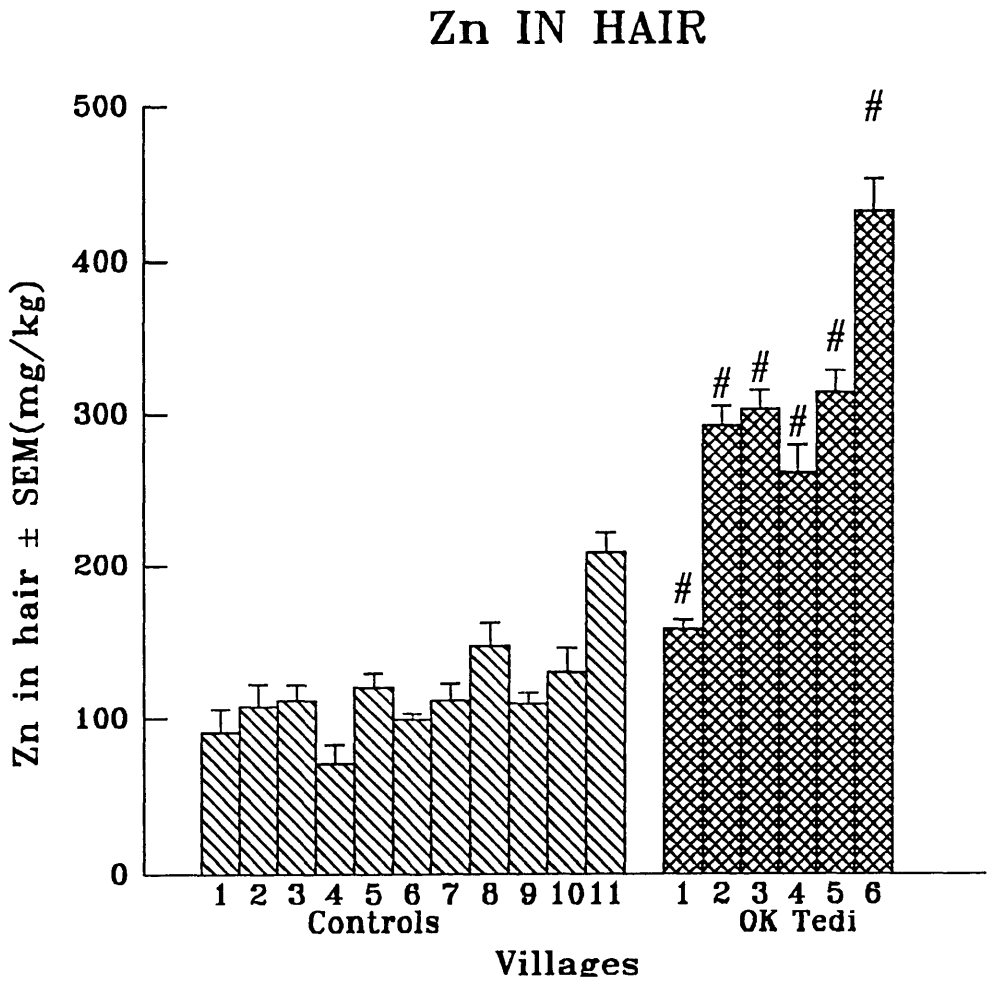

Cu IN HAIR
Cu in hair ± SEM(mg/kg)
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

Figure 2.5


Zn IN HAIR
Zn in hair ± SEM(mg/kg)
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

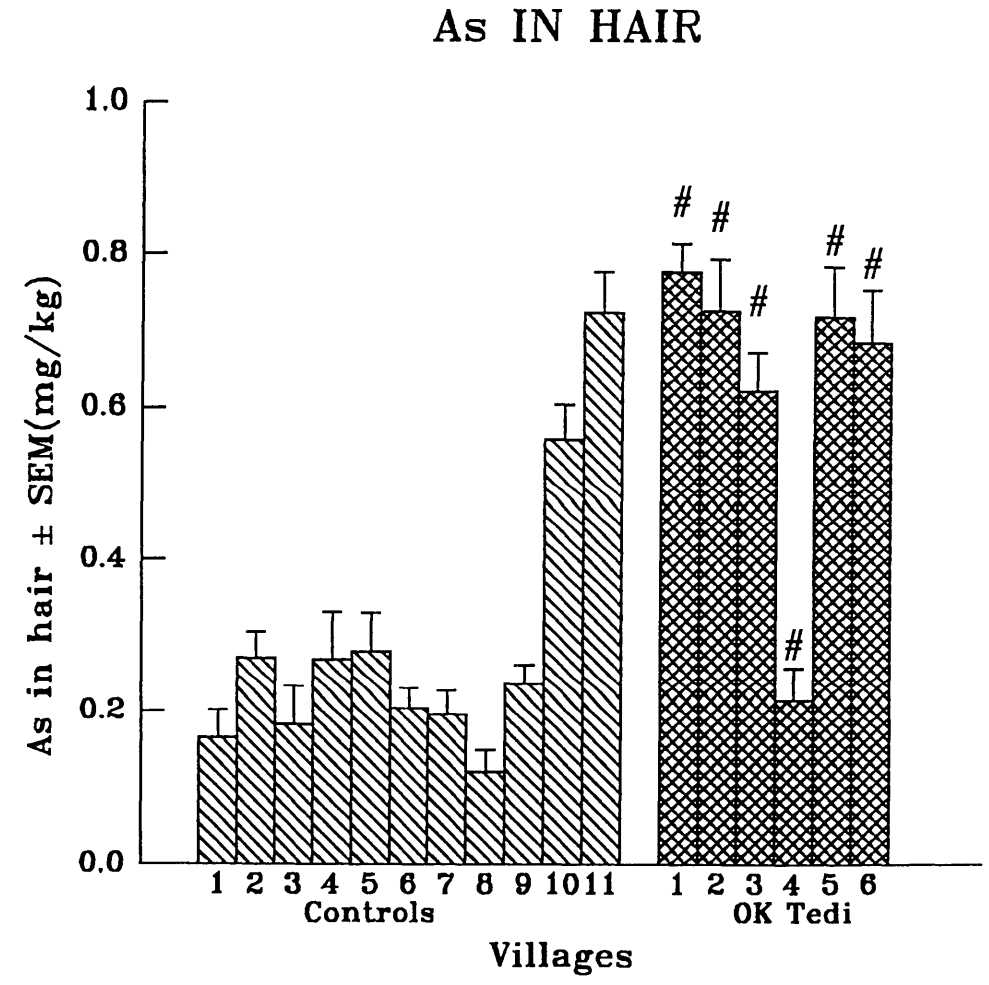
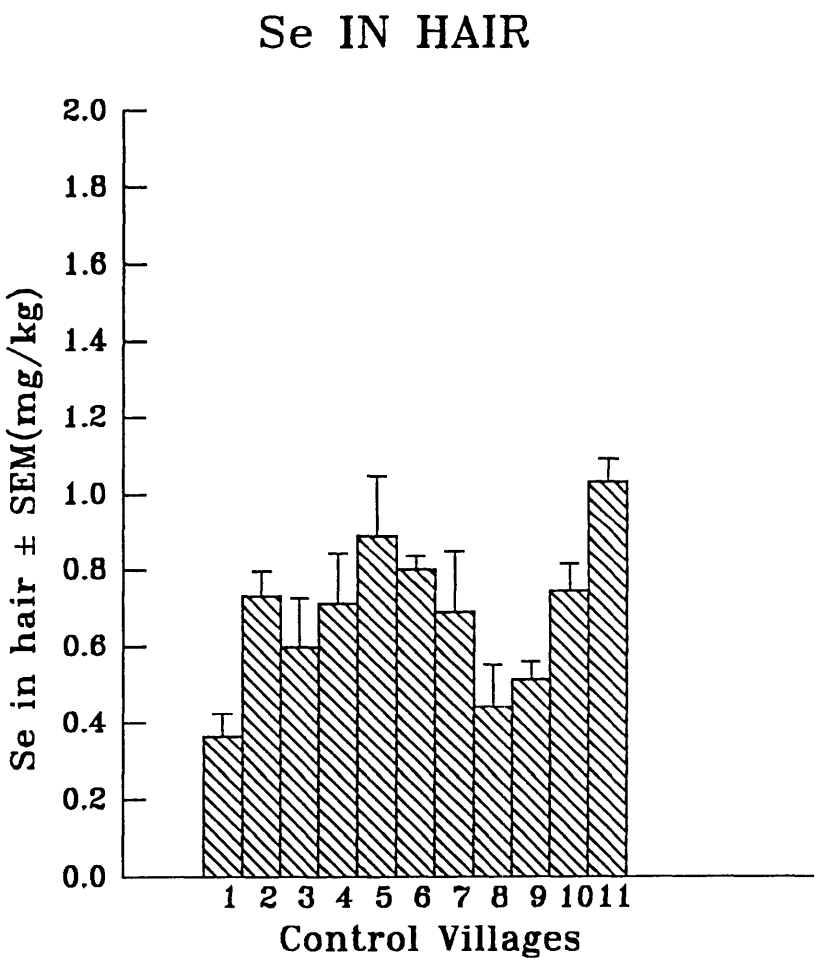

As IN HAIR
As in hair ± SEM(mg/kg)
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

Figure 2.6


Se IN HAIR
Se in hair ± SEM(mg/kg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11
Control Villages

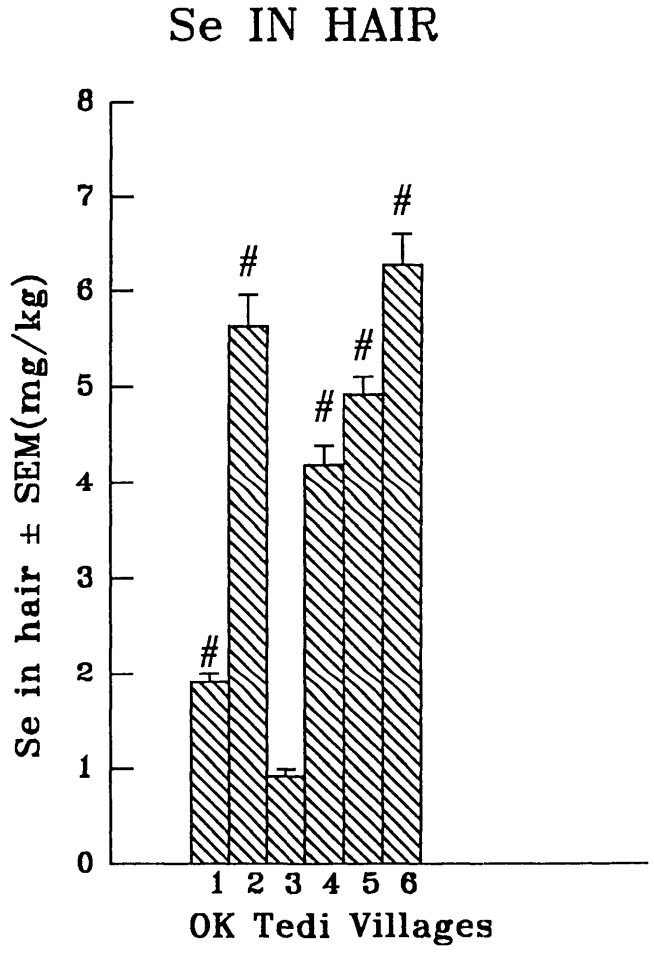
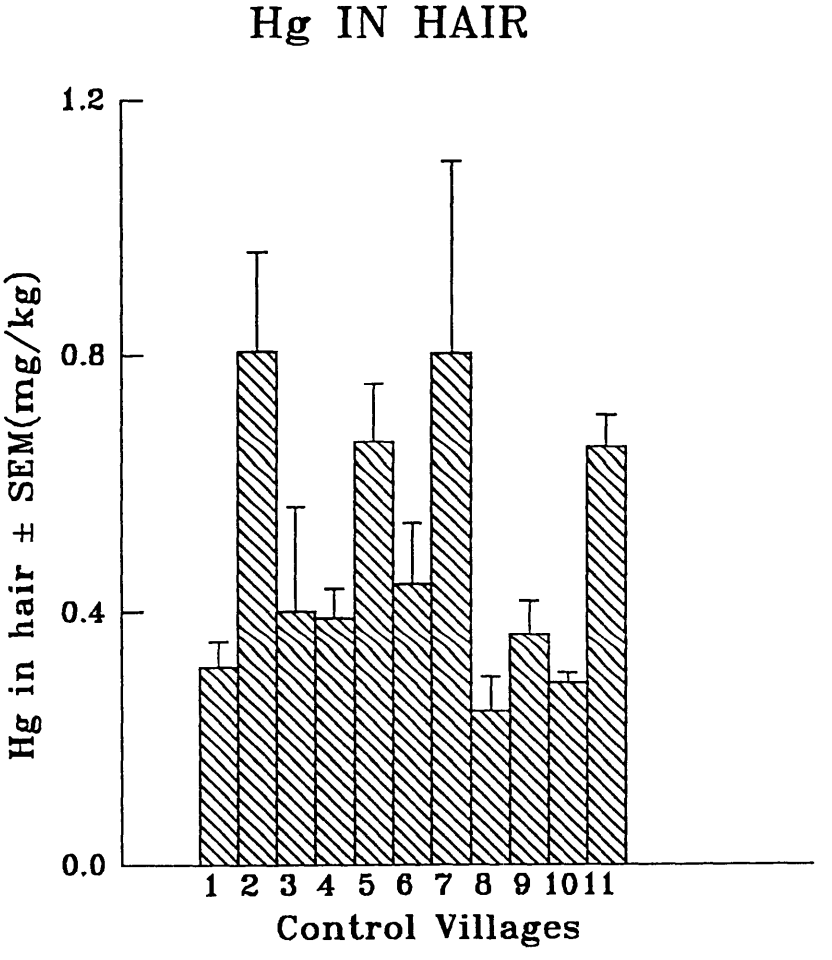

Se IN HAIR
Se in hair ± SEM(mg/kg)
8
7
6
5
4
3
2
1
0
#
#
#
#
#
1 2 3 4 5 6
OK Tedi Villages

Figure 2.7


Hg IN HAIR
1.2
0.8
0.4
0.0
Hg in hair ± SEM(mg/kg)
1 2 3 4 5 6 7 8 9 10 11
Control Villages

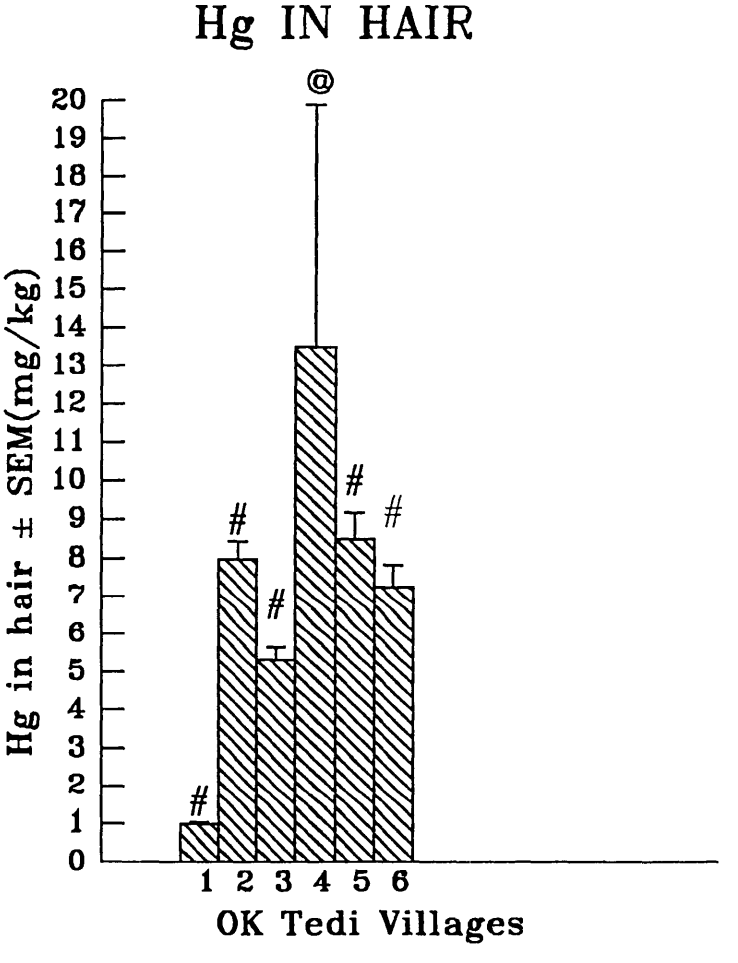
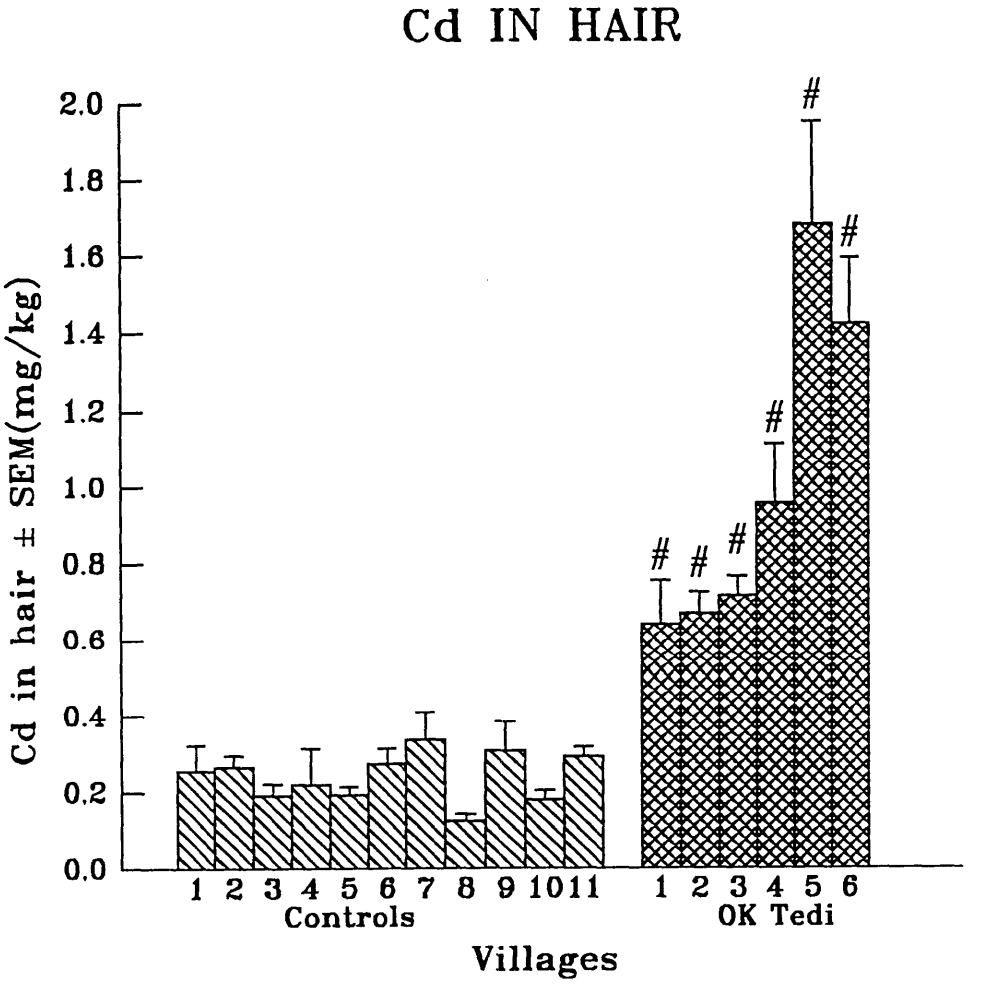
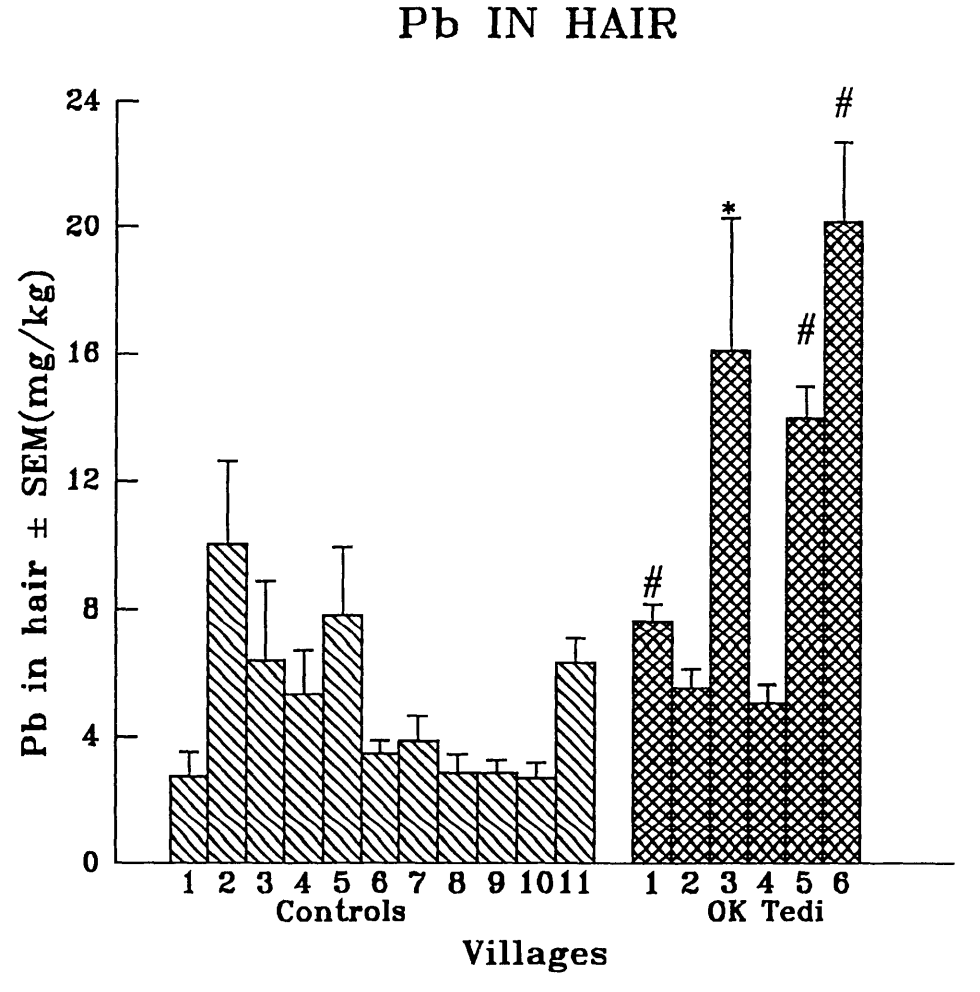

Hg IN HAIR
@
#
#
#
#
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Hg in hair ± SEM(mg/kg)
1 2 3 4 5 6
OK Tedi Villages

Figure 2.8


Cd IN HAIR
Cd in hair ± SEM(mg/kg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages


Pb IN HAIR
Pb in hair ± SEM(mg/kg)
24
20
16
12
8
4
0
1 2 3 4 5 6 7 8 9 10 11
Controls
1 2 3 4 5 6
OK Tedi
Villages

2.4 DISCUSSION

In spite of the fact that some authors have expressed some doubt about the use of hair as a reliable indicator of trace metals, hair has for some time been widely used as a biopsy material for the determination of different heavy and trace elements, as well as to examine the extent of environmental exposure (Briggs *et al.*, 1972; Petering *et al.*, 1973).

It has been noted by a number of workers that the process of extraction and treatment of minerals greatly affects the environment and the surroundings of a mine, including the health of individuals living in the vicinity of the mine (Jamett *et al.*, 1991).

Hair has been used for monitoring environmental and occupational exposure for the determination of heavy metal concentrations because hair serves as a reliable record of exposure to heavy metals as they accumulate in a relatively short time and tend to increase with increasing exposure. It has, for instance, been recommended that the concentration of Cd in hair, as compared to blood and urine samples, is a good biological monitor of the extent of exposure of communities or individuals to this element (Carvalho *et al.*, 1989). Hair analysis has thus been widely accepted as a valuable tool in epidemiological studies (Wilhelm *et al.*, 1991).

In this part of the discussion the mean concentrations of each of the 14 elements will be compared between each individual test village and the global mean of the control villages.

Magnesium

Magnesium is the fourth most abundant cation in the body and is associated with many different biological processes. Its involvement is enough to suggest that this element has some single fundamental role (Aikawa, 1971). It appears that Mg plays an important role in essential cellular processes, eg. in the structure of the DNA double helix as well as of ribosomes and in energy transduction (i.e. Mg ATP) (Aikawa, 1971).

Magnesium is an element that while not directly related to mining activity, is very widespread in mining burden and the general detritus generated by mining activity. In addition, different foodstuffs contain varying concentration of Mg eg. nuts contain high concentrations, while fats show the least amount of Mg.

The mean concentrations of Mg for test villages 2 and 4 were significantly higher than the pooled controls, while the levels of Mg for village 1 were significantly lower than the pooled controls (Fig. 2.1). One possible speculation about the variation of Mg levels between and among the control and test villages is that concentrations of Mg may depend on the type of foodstuff consumed in

each particular village. Clearly, however, there is overall no comprehensive increase in hair Mg in the test villages by comparison with control villages.

Aluminium

The mean values of Al for the test sites were significantly lower than the control sites, with the exception of village 2, which showed a significantly higher level of Al as compared to pooled controls (Fig. 2.1). On the other hand, the overall values of Al for both pooled controls and test sites were higher than previously reported values (Shrestha and Schrauzer, 1989) in a different population.

Shrestha and Schrauzer (1989) determined the concentrations of Al in Darjeeling (India) and San Diego (USA) hair samples, and found that Al concentrations in Darjeeling hair were lower (2.4 $\mu g\ g^{-1}$) than those in the San Diego hair (10.6 $\mu g\ g^{-1}$). These researchers related the low values of Al with the limited use of canned food and beverages in the Darjeeling area and related the high levels in San Diego to the extensive use of beverages contained in Al cans and the use of Al containing drugs. With respect to the present study, the people from the areas of study use different clays for painting their hair, faces and/or bodies. The use of these special clays or ashes might result in anomalously high values of Al through surface adsorption. Aluminium, despite its apparent lack of a biological role, is one of the most abundant elements in the minerals which constitute the Earth's crust and therefore would be expected to be present at a high level in mining-related detritus and consequently sediments entering rivers and streams in the vicinity of the mine.

Chromium

There is some evidence that deficiencies of Zn and Cr occur in particular populations, due to inadequate intake and/or poor availability of these two elements. Hambidge (1974) reviewed Cr nutrition in human. It was evident from these studies that tissue Cr levels decreased with increasing age. It was suggested that deficiency in the United States might occur due to less intake and losses of Cr in processing of foodstuffs such as flour and sugar (Schroeder *et al.,* 1962; Hambidge and Baum, 1972).

The most common function of Cr is its involvement in the glucose tolerance factor. The content of Cr in food may be reduced by refining processes (Czerniejewski *et al.,* 1964; Schroeder, 1968, 1971; Schroeder *et al.,* 1970).

The mean values of Cr for the test (Ok Tedi) sites were significantly higher compared to the values of the pooled controls (Fig. 2.2). Values for the pooled controls on the other hand, were very similar to the mean value of 0.73 mg/kg seen for hair Cr in Greek agricultural workers (Leotsinidis and Kondakis, 1989)

Hambidge *et al.* (1972) observed that there was no significant increase in the mean values of Cr for different sections of hair that had been exposed for many months to the external environment, and ruled out the environment as a cause of contamination or loss of Cr from hair shafts. The uptake of Cr mainly depends on its presence in foodstuffs, with the highest amount of Cr present in spices, with lesser amounts in meats.

It was also noticed by Schroeder *et al.* (1962) that cooking in stainless steel utensils might be responsible for Cr in the diet, Particularly if acidic foodstuffs were prepared. It was noted that Cr, found in high concentrations in stainless steel, can leach out during the cooking process.

Iron

Iron is essential for the human body as it is an important part of the oxygen carrying pigment haemoglobin. Blood losses associated with parasitic diseases like hookworm and schistosomiasis are known to cause Fe deficiency as well as losses of other trace elements (Waslien *et al.,* 1972).

Mean values of Fe for both control and test villages were very high, with an exceptionally high value (765.4 ± 52.6) in test village 2 (Fig. 2.2). The widespread use of ochre clay daubs as hair and body adornments may be the cause of the extremely high Fe levels in both a pre-mining OK Tedi group (1982) and the Mt. Obree controls by comparison with Western populations (Jones *et al.,* 1987). Mean values for Fe in a range of other populations in the developed world vary between 20-60 μg/kg (Reilly and Harrison, 1979; Shresta and Schrauzer, 1989).

It has been previously reported that the water of the major rivers in the Ok Tedi region are particularly high in particulate and dissolved Fe (Environmental Monitoring Programme OTML, 1983). In summary, the extensive use of ochre clay daubs as facial hair and body adornments by the villagers on different occasions may have resulted in the absorption of Fe externally which has been shown to be very difficult to remove, even by thorough laboratory procedures.

Manganese

Manganese is not only essential for growth but also for reproduction and skeletal development in all species of animals including humans. Normal diets for most species with the exception of chicken contain sufficient Mn to prevent its deficiency (Kemmerer *et al.,* 1931; Orent and McCollum, 1931). A number of Mn metalloenzymes are known including pyruvate carboxylase and superoxide dismutase.

The concentrations of Mn in the hair of test and pooled controls showed a highly variable trend (Fig. 2.3). It was first believed by Bose and Chakravorty (1964) that high concentrations of Mn in the hair of Indians were associated with

its black colour. Following that observation, Cotzias *et al.* (1964) reported that dark hair contained more Mn than light coloured hair. Shrestha and Schrauzer (1989) also determined the Mn values of individuals of Chinese origin and found less Mn in these hair samples than the levels found in Indian hair. On the other hand, Schroeder *et al.* (1966) found high Mn levels in some brands of tea and according to Shrestha and Schrauzer (1989) the high Mn levels in hair was linked with consumption of Darjeeling tea in their study. Therefore, it may be reasonable to associate high Mn levels in hair to the consumption of tea. The extraordinary mean levels of Mn in test villages 2 and 4 may be associated with differential consumption of tea or other beverages/foodstuffs/drugs etc unique to these villages. The levels in the other test and control villages are close to reported means ranging from 8.1 to 40 mg/g for different populations in the developed world (Dissanayake, 1984; Shrestha and Schrauzer, 1989).

Cobalt

Cobalt is an important part of vitamin B_{12}. Underwood (1971) determined, after several years of research, that Co deficiency in ruminants is actually a vitamin B_{12} deficiency that is caused by the inability of the rumen microorganisms, due to insufficient dietary Co, to synthesise vitamin B_{12}.

Mean levels of Co also showed a mixed trend of low and high values among and between test and controls with no consistent definitive difference between tests and pooled controls (Fig. 2.3). This variable trend of Co is somewhat difficult to explain. Test villages 2, 4 and 6 show relatively high levels whereas all other villages are close to the mean of 0.10 mg/kg previously observed in another rural (South American) population (Jamett *et al.*, 1991).

Nickel

Nickel has been known to be essential for both animals and humans since the 1920s. Nickel has a role in metabolism and in the structure of membranes as well as in hormonal control (LaBella *et al.*, 1973). Nickel can easily form a complex with phytic acid and phytate and some vegetables could therefore decrease the availibility of dietary nickel for intestinal absorption (Vohra *et al.*, 1965).

Although three control villages showed relatively high values for nickel, the mean concentrations of Ni for most of the test villages were significantly higher than those of the pooled controls (Fig.2.4). This higher overall concentration of Ni may be positively correlated to extensive use of tinned food, the containers of which partly consist of Ni. Loetsindis and Kondakis (1990) observed what they described as high concentrations of Ni in scalp hair of Greek agricultural workers. These mean levels of 1.02 mg/kg are slightly higher than the levels of most of the control villages in this survey. The higher values of the test (Ok Tedi) villages in

the present survey approach those mean values of 2.64 mg/kg noted for indigenous people exposed to smokestack pollution in Alberta, Canada (Moon *et al.*, 1986).

Copper

The first evidence that Cu plays an important role in the physiology of the vertebrates was reported by Hart *et al.* (1928), who noticed that it was required for the prevention of anaemia. It was shown by Cohen and Elvehjem (1934) that Cu is essential for the elaboration of haeme A, which is a component of cytochrome activity. In short, Cu is an essential part of several important metalloenzymes, which play many biochemical and physiological roles in animals and humans. There are three important roles of Cu related to human health. Firstly, it is involved in haemopoiesis, secondly the maintenance of vascular and skeletal integrity through a role in collagen assembly and thirdly in the structure and function of the central nervous system (Prasad, 1976). Nonetheless, the required daily intake of Cu is rather low and excess intake is highly toxic.

Mean values of hair Cu were significantly higher for most test sites compared to the controls (Fig. 2.4). The trace mineral content of hair, particularly the concentrations of Zn and Cu have been reported to be a good reflection of estimating nutritional status (Strain *et al.*, 1966; Klevay, 1970; Petering *et al.*, 1971). The fact that hair could adsorb significant amount of Cu from the environment as a result of mining has previously been confirmed by several workers (Bate and Dyer, 1965; Bate, 1966; Schroeder and Nason, 1969; Steinners, 1975). The increase in the levels of Cu of the tests site might, therefore, be related to mining activity. However, these levels though are not considered to be abnormal by comparison with previous reports.

In a study made by Jamett *et al.*(1991) eleven trace elements, including Cu, were determined in the hair of workers of a Cu mine and of children living in the immediate vicinity. These researchers found a mean concentration of 33.08 ppm in the hair samples of mine workers and mean levels of 30.8 ppm in the hair samples of children living in the vicinity of the mine whereas children living in a relatively undisturbed rural environment far from the Cu mine showed an average of 17.5 ppm, close to the levels shown by the test villages in the present study.

Reilly and Harrison (1979) found a mean level of Cu (22.9 $\pm$ 1.3 ppm) in non-student males and a level of 34.6 $\pm$ 3.3 ppm in non-student females in Oxford. The main idea was to determine whether or not the Oxford subjects were exposed to environmental pollution. In the same study they observed mean concentrations of 25.3 $\pm$ 1.6 ppm and 33.9 $\pm$ 3.9 ppm in male and female students respectively. It is concluded from these reviews that the results of Cu for the Ok Tedi population

are in good agreement to those reported by other workers for sites not affected by any obvious exogenous Cu pollution and that the values for the control population (Mt. Obree) should be considered low by comparison.

Zinc

The importance of Zn in human nutrition was first recognised by Prasad *et al.* (1963a) and Halsted *et al.* (1972) when these workers reported that zinc deficiency was asssociated with the syndromes of retardation of growth and sexual maturation. Zinc is a part of different metalloenzyme including carbonic anhydrase and superoxide dismutase while the effect of zinc on wound healing has been previously reported by Pories *et al.* (1967).

Several workers have reported that large variation in nutritional intake of available Zn in animals is usually reflected in the content of Zn in hair (Petering *et al.*, 1971). It appears, therefore, that Zn content of human hair may be positively correlated to that of the Zn content of the diet and to Zn metabolism. Zinc has been recognised for a long time as an essential nutrient (Todd *et al.*, 1934; Keilin and Mann, 1939; Underwood, 1971), and the possibility of subclinical deficiencies of Zn has been recognised in humans since the 1970s (Pories *et al.*, 1974).

The overall mean concentrations of Zn for the present study showed significantly higher levels for the Ok Tedi population compared with that of the controls (Fig. 2.5). As confirmed from previous reports of various workers, hair Zn is a good indicator of nutritional status. The people of Ok Tedi consume more tinned food in their diet due to their trend towards Westernisation and this may well be responsible for the high (as compared to control site) but normal (as compared with other populations in the developed world) levels of Zn in hair of the Ok Tedi population. Zinc which is present in hair in relatively large concentrations is well suited for hair analysis as it is relatively evenly distributed along the hair shaft and not sensitive to environmental variations (Obrusnik *et al.*, 1972; Eads and Lambdin, 1973; Valkovic, 1977).

Briggs *et al.* (1972) found a clear-cut connection between Zn levels in hair and general nutrition status in hospitalised Negro children. Previous studies by Prasad (1966) on Zn deficiency-related dwarfism in Iran, showed a correlation between the levels of Zn in hair and its availability in the diet.

Furthermore, a study by Walravens and Hambidge (1978) which determined the levels of Zn in children from a low-income population in Denver, USA found values to be 87 $\pm$ 5.9 mg/kg. These authors concluded that the above group of children were nutritionally deficient in Zn. In this context, then, the levels of Zn in the present control (Mt. Obree) groups should be considered low. Mean values for Zn in populations in the developed world range between 200-400 mg/kg

(Reilly and Harrison, 1979). The levels of Zn in the Ok Tedi (test) villages, therefore, lie well within the"normal" range.

Arsenic

The mean levels of arsenic in hair of 5 out of 6 test villages were substantially higher than the pooled controls (Fig. 2.5). Nevertheless, the higher concentrations of As in these test villages were still well under previously published "normal" ranges.

In a study by Jamett *et al.* (1991), hair samples of 28 mine workers and 32 six-year old children living near a copper mine were investigated for eleven trace elements including As. Jamett *et al.* (1991) suggested that certain elements such as As, which is produced in the form of As_2O_3 during the treatment of minerals, may reach a concentration that could be harmful to human health.These investigators reported the mean value of As for the mine workers to be 15.14 ppm while the children showed a mean concentration of 19.25 ppm. At the same time, a group of people living a rural lifestyle 250 km away from the mine showed a mean concentration of 0.55 ppm. The latter value was higher than the mean values of As shown by the pooled controls of this present study but very similar to the global mean of the Ok Tedi villages.

Yamamura and Yamauchi (1980) examined As metabolites in hair, blood and urine in workers exposed to arsenic trioxide. Arsenic was analysed in hair for various chemical species, and it was found that As^{5+} was present in high amounts, followed by As^{3+} and traces of dimethyl arsinic acid (DMAA). The study concluded that As^{3+} increased in workers exposed to high concentrations of As dust, compared to those who were exposed to low concentrations of dust, while levels of DMAA made no difference.

Selenium

Selenium, with vitamin E and sulphur amino acids has long been known to serve as an antioxidant. Selenium is an essential component of intracellular and extracellular glutathione peroxidases. Deficiencies of Se, vitamin E and/or glutathione often cause increased sensitivity to oxidant stress (Witting, 1980).

Selenium hair concentration of test sites were approximately 10 times higher than that of pooled controls (Fig. 2.6). According to Olson *et al.* (1970) and Burk (1973a), the Se content of food is related to protein content as Se is concentrated in the protein fraction. Foods that are relatively low in protein such as fruits, contain less amounts of Se (Morris and Levander, 1970). On the other hand, Higgs *et al.* (1972) noticed that cooking caused no major losses of Se from most foods. However, there was a loss in Se of approximately 23% when cereals were dry heated. In addition, a few vegetables showed more Se than this amount.

Higgs *et al.* (1972) noticed that cooking caused no major losses of Se from most foods. However, there was a loss in Se of approximately 23% when cereals were dry heated. In addition, a few vegetables showed more Se than this amount. Selenium is also a significant component of antidandruff shampoos as well as some topically applied vermin treatments. The use of antidandruff shampoos and, in particular, other topically applied vermin treatments may be one of the reasons for the massively elevated levels of Se in the hair of individuals from the test villages. Be this as it may, the levels of the control villages are very close to previously published mean values of 0.53 mg/kg (Chuang and Emery, 1978), so the elevated levels in the test villages do require some explanation.

Mercury

Mercury can accumulate in the human body not only from the consumption of contaminated fish but also from other environmental sources both natural and anthropogenic, and hair Hg concentration may be a good indicator of such accumulated Hg (Airey, 1984).

The mean concentrations of Hg in hair of the Ok Tedi test villages were more than ten times that of the pooled controls (Fig. 2.7). The main source of this Hg is thought to be due to consumption of barramundi (*Lates calcarifer*) which has been shown to accumulate Hg naturally from the food chain. Kyle and Ghani (1982) determined mercury levels in people from Lake Murray, Western Province, Papua New Guinea, because they were thought to be chronically exposed to methylmercury through fish consumption. The investigation was initiated from a report that barramundi contained elevated levels of mercury and these people used this fish as an important source of protein (Reynolds and Price, 1979). These investigators found that methylmercury levels in hair of these individuals were significantly higher than those from areas such as Rumginae and Suki, where consumption of fish was low. A mean concentration of 18.0 mg/kg was found which is higher even than the most elevated levels (13.48 mg/kg) of Hg in village 4 of Ok Tedi.

A study made by Marsh and co-workers (1974), estimated a total Hg level of 17 mg/kg in hair of a group of 88 Korean fishermen based at American Samoa. These fishermen lived almost entirely on tuna while they were at sea. Similarly, Turner *et al.* (1980) observed a mean blood Hg level of 0.02 mg/kg in a Peruvian population which was identified to be chronically exposed to long-term consumption of ocean fish. As there is a good correlation between Hg levels in blood and hair with levels in hair at about 300 times that in blood (Kershaw *et al.*, 1980), these levels correspond to a total Hg content in hair of about 25 mg/kg.

However, there may still be a risk of developing subclinical effects of Hg poisoning below 50 mg/kg. Although the present levels of hair Hg of the Ok Tedi populations were significantly higher than those of the Central Highlands (Mt. Obree) population (i.e pooled controls), these levels would not be considered dangerously high. It is worth noting that, as well as the barramundi, the Ok Tedi populations also consume large amounts of tinned fish which they now buy from the trade stores.

Cadmium

Cadmium is known to be a non-essential element and thus its presence in the body should be minimised. Cadmium can compete with some of the essential divalent elements for ligands. It has been observed that the toxicity of Cd is inhibited if sufficient Zn is present in the cell (Gunn *et al.*, 1961; Powell *et al.*, 1964; Webb, 1972; Prizek, 1983).

Leotisindis and Kondakis (1990) determined trace metals in scalp hair of Greek agricultural workers and reported a negative correlation coefficient between Cd and Zn. The metabolic competition between Cd and Zn is well established and this metabolic process in the body influences trace metal concentrations in hair (Hopps, 1977). Shrestha and Schrauzer (1989) determined the levels of Cd of individuals in Darjeeling (India) and San Diego (USA) and found moderately elevated Cd concentrations in three of the four high lead Darjeeling subjects (mean 1.7 $\mu g\ g^{-1}$). These levels were comparable to the high levels (1.68 ± 0.271 ppm) of Cd for one of the test villages of the present Ok Tedi study. Shrestha and Schrauzer (1989) further observed that the concentrations of Cd in all 27 samples of Darjeeling hair were directly correlated with those of Pb and they suggested a common source of exposure that might cause elevation in the levels of Pb and Cd. On the other hand, these workers failed to find a statistically significant correlation between levels of Pb and Cd in hair. In contrast, Petering *et al.* (1973) found a high correlation between Cd and Pb in hair of the residents of Cincinnati, Ohio.

Carvalho *et al.* (1989) estimated Cd in hair of children living near a Pb smelter in Brazil, and found that concentrations of Cd in hair varied inconsistently according to the nutritional status of the children. In addition, the mean Cd hair (CdH) of individuals with Fe deficiency was lower than that of individuals with adequate Fe. Animal experiments have also shown that intestinal Fe absorption is inhibited by Cd concentrations (Hamilton and Valberg *et al.*, 1974; Ragan, 1977). Furthermore, it was found that the mean CdH concentration was significantly higher in children whose fathers worked in the Pb smelter compared with those whose fathers had other types of work (Carvalho *et al.*, 1989)

The effect of ambient Cd air pollution on the hair mineral content of children was observed by Stewart-Pinkham (1989). According to this study, the most important source of indoor air Cd was exposure to passive smoke. Similarly, additional toxic exposures in cigarette smoke could increase the toxicity of Cd in the passive smokers. The study concluded that the variation in CdH with season and variability in groups with toxic exposure make CdH an unreliable marker of Cd air pollution exposure or toxicity. In summary, the values for CdH in the Mt. Obree controls in the present study (Fig. 2.8) were similar to those previously reported for populations in rural Germany (0.35-0.5 mg/kg, Willhelm *et al.*, 1991). The Ok Tedi populations showed a clear increase in Cd although the levels did not reach those of individuals living in an area affected by mining-related pollution (5.0 mg/kg, Carvalho *et al.*, 1989).

Lead

Mean values of Pb, like most of the other heavy metals, were significantly higher in the people of the Ok Tedi region compared with that of control groups. High levels of Pb in children has previously been correlated with the eating of mud or sand (pica habit) while a possible source of Pb in adults may be to the use of bronze cookware and repairing of leaks with Pb solder (Shrestha and Schrauzer, 1989).

Although the levels of Pb of the OK Tedi population were comparably higher to those of pooled controls (Fig. 2.8), these levels were significantly lower than those of a previous study made by Jones *et al.* (1987) for the same population (23.79 μg/g), and about the same level as the mean values for most populations in the developed world (Reilly and Harrison, 1979). The mean levels of Pb in the control villages are about the same as Pb in hair of children in rural Germany (6 mg/kg) (Willhelm *et al.*, 1991). According to Creason *et al.* (1975), urban living and cigarette smoking are the environmental factors that have contributed increases in the Pb levels in human hair. In addition, the residential proximity to industries (Chattopadhyay *et al.*, 1977) and the Pb ingestion habit of petrol sniffing (Underwood, 1971; Hemphill, 1972) may also contribute toward increased body Pb levels.

As there is no standard for hair Pb levels, a comparison of human blood and hair Pb levels suggests that a blood Pb level of 30 μg dL^{-1} would roughly correspond to a hair Pb level of 24 ppm (mg/kg), assuming Pb intake and excretion are in a steady state (Chattopadhyay *et al.*, 1977).

It is concluded from this heavy and trace element study of the Ok Tedi (test sites) and Central Highlands (control sites) that although there is a consistent increase in the levels of several metals (Cr, Zn, Ni, Co, Cd, Hg and Pb), these

elevated levels are still within previously published "normal" ranges. The increase of hair Zn in the Ok Tedi population should be seen as a positive change reflecting improved nutrition. Again, Cu levels have also increased but only to levels consistent with adequate nutrition.