



T1900156660

**HAIR HEAVY METAL AND PROTEIN ANALYSIS
IN OCEANIA**

Effects of mining and lifestyle changes

by

Sitwat Lubna Khawar

M.Sc. (Pakistan), M.Phil (Pakistan)

A thesis submitted for the degree of Doctor of Philosophy at the
University of New England.

Armidale, Australia.

March, 1995.

Dedicated to Iqbal and Hasan

Declaration

The whole of the experimental work presented in this thesis was carried out by myself in the Department of Biochemistry/Microbiology. The conclusions are my own, reached after numerous critical discussions with Dr. Graham Jones and Dr. Ken Watson. The substance of this thesis has not been submitted for any other degree or qualification, and any help received in the preparation of this thesis and all sources used or literature cited have been acknowledged.

SITWAT LUBNA KHAWAR

Acknowledgments

In the name of God The Most Merciful and Compassionate and His Last Prophet Mohammad (Peace be upon him). I would like to express my sincere gratitude to my dignified supervisors, Dr. Graham Jones and Associate Professor Ken Watson for their learned advice, support and encouragement given through out the course of my study. Special thanks to Evan Thompson for his generous help with the statistics. I would like to thank all my lab colleagues and well wishers for their constant help.

I am thankful to the staff of the Media Resource Unit for their assistance in producing the photographs of the gels.

I am thankful to my parents for their prayers. In particular I would like to thank my husband for his constant support, sacrifice and patience through out my studies. Special thanks to my four year old son Hasan who has suffered a lot during my lab work.

Special thanks to Prof. John Lourie and his wife Lucinda for collecting hair samples from Ok Tedi. I am also thankful to Dr. Tukatau Taufa for collecting hair samples from the Central Highlands and Dr. Bart Curie for collecting hair samples from Australian Aboriginals at Groote Eylandt.

I would like to thank Les Dale and Owen Farrell of CSIRO (Lucas Heights, Sydney) for helpful advice and performing analysis of hair heavy metals.

I am also thankful to technical and secretarial staff at UNE for valuable support and assistance.

SITWAT LUBNA KHAWAR

Abbreviations

1D	One-dimensional
2D	Two-dimensional
ANSTO	Australian Nuclear Science and Technology Organisation
APS	Ammonium persulfate
As ₂ O ₃	Arsenic trioxide
ATP	Adenosine triphosphate
CdH	Cadmium in hair
CSR	Commonwealth Sugar Refineries
dl	Decilitre
DMAA	Dimethyl arsinic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
HC	High cysteine
HPLC	High performance liquid chromatography
IAA	Iodoacetic acid
ICPMS	Inductively coupled argon mass spectrometry
IEF	Isoelectric focussing
IFs	Intermediate filaments
KCl	Potassium chloride
LC	Low cysteine
M _r	Relative molecular mass
NaOH	Sodium hydroxide
ppb	Part per billion
ppm	Part per million
SCMC	S-carboxymethylcysteine
SCMK	S-carboxymethylkerateine

SCMKs	S-carboxymethylkerateines
SEM	Standard error of mean
SDS	Sodium dodecyl sulfate
SDS-	Sodium dodecyl sulfate polyacrylamide gel
PAGE	electrophoresis
TEMED	N, N, N', N, -tetramethylenediamine
Tris	Tris (hydroxymethyl) diaminomethane
TTD	Trichothiodystrophy
VHC	Very high cysteine
VLC	Very low cysteine

Abstract

Hair has advantages over other tissues as a biological sampling milieu since it can be easily and atraumatically collected, stored and analysed. Hair preserves a mid to long range record of exposure to many metals (some, like lead, toxic in high levels) that can be studied and analysed. In this thesis, a group of Melanesian people living close to a copper and gold mine (Ok Tedi) in Papua New Guinea and another group from the same racial background living far from the mine site have been examined to obtain evidence for enhanced exposure to metals that may have occurred as a consequence of mining operations. For comparative purposes, hair samples from Australian Europeans (predominantly Anglo-Celtic origin) living in a non-industrial rural environment (Armidale, NSW) and Australian Aboriginals living close to a manganese mine (Groote Eylandt) were also analysed. Results showed that each group displayed a characteristic pattern of metal distribution in hair. Distressingly high levels of copper, lead and mercury analysed in the Aboriginal group may be related to environmental pollution and/or maladaptive lifestyle practices among that group while the levels shown by test sites (Ok Tedi) were found to be largely well below the previously published values concerning Westernised urban populations. It was concluded that the elevated heavy metal levels among the Melanesian Ok Tedi population by comparison with the Melanesian control group may be correlated less with the mining activity and more with a changed life style and diet. A longitudinal analysis of key heavy metals (Fe, Zn, Cu, Pb, Cd and Hg) from 1983 (premining) to 1990 (6 years post mining) showed generally that the Ok Tedi group changed from a profile strongly resembling the Melanesian controls to one much closer to the Australian European control group in Armidale. Given the extensive rapid Westernisation and current, almost total, dependence of the Ok Tedi population on processed food provided by the mining consortium, this transition is perhaps not too surprising.

For some time now the levels of different metals in hair have been thought to partly depend on the matrix proteins. This has led to the idea that the matrix proteins of human hair belonging to different racial groups may absorb metals differentially. A central idea of this thesis is to explore possible relationships between the presence of metallic elements and the nature and disposition of matrix proteins in the different groupings studied. Relatively minor changes in hair proteins may affect the rate of heavy metal accumulation in hair and therefore impact significantly on group to group comparisons. The long term aim of the work of which this thesis is a part is to search for a possible relationship between hair heavy metal accumulation and hair proteins within and between species.

Hair samples from several hundred different individuals representing several human racial groupings (Australian European, Australian Aboriginals, Melanesians, Africans and Chinese) were analysed for keratins (major hair proteins) by one and two-dimensional polyacrylamide gel electrophoresis. The use of ^{14}C -iodoacetic acid to label S-carboxymethylatedkerateines allowed the assignment on autoradiographs of cysteine containing proteins. Polymorphic variation of hair proteins in the mid-range low cysteine kerateine zones was confirmed. In addition to these variations, further variations in high cysteine zones (molecular masses, 45-30 kDa) were also noted. Furthermore, homologous proteins from mammalian (both placental and marsupial) species were compared with respect to their molecular masses and cysteine content using laser densitometry of gels. The relative cysteine content of Coomassie stained gels (as an estimate of proteins) were compared with autoradiographs (as an estimate of cysteine). These results may be quite useful forensically as an adjunct to fibre analysis since they yield more information per analysis than previous procedures using more laborious two-dimensional electrophoretic separation. Finally, a simple and rapid two-dimensional electrophoretic method for peptide mapping of human and animal hair and feather proteins has been developed. The protease digestion pattern was reproducible and characteristic of each sample.

Publications arising from this thesis

1. Khawar, S. L., Watson, K. and Jones, G. L. (1995) **High resolution one-dimensional electrophoretic separation and partial characterisation of human head hair proteins.** Electrophoresis, 16: 110-115.
2. Khawar, S. L., Watson, K. and Jones, G. L. **Changes in the hair heavy metal levels in populations near the Ok Tedi mine site, Papua New Guinea.** Poster presented at International Environmental Biometrics Conference Sydney, Australia. 14-15 December, 1992.
3. Khawar, S. L., Watson, K., Taufan, T., Lourie, J. A. and Jones, G. L. **Improved separation and partial characterisation of S-carboxymethylated human hair proteins.** Poster presented at 38th Annual Conference of Australian Society for Biochemistry and Molecular Biology, Gold Coast, Australia. 26-29 September, 1994.
4. Khawar, S. L., Watson, K. and Jones, G. L. **An electrophoretic comparison of hair and feather proteins from various species of mammals and birds.** Poster presented at 38th Annual Conference of Australian Society for Biochemistry and Molecular Biology, Gold Coast, Australia. 26-29 September, 1994.

Submitted for publication

5. Khawar, S. L., Watson, K. and Jones, G. L. **A comparative electrophoretic analysis of mammalian hair and avian feather proteins.**
6. Khawar, S. L., Watson, K. and Jones, G. L. **Peptide mapping of S-carboxymethylated hair and feather proteins using two-dimensional electrophoresis.**

List of Tables

Tables -----	Page
2.1 Mean trace metal levels in hair from control and Ok Tedi sites--	33
2.2 Mean trace metal levels in hair from control and Ok Tedi sites--	34
3.1 Mean trace metal levels in hair from each of nineteen groupings (control, Ok Tedi, Australian Aboriginals and Australian Europeans)-----	62
3.2 Mean trace metal levels in hair from each of nineteen groupings (control, Ok Tedi, Australian Aboriginals and Australian Europeans)-----	63
3.3 Pooled levels of heavy metals among different groupings -----	64
3.4 Correlation among different elements for control villages -----	65
3.5 Correlation among different elements for test villages -----	66
3.6 Correlation among different elements for Australian Aboriginals	67
3.7 Correlation among different elements for Australian Europeans	68
3.8 Correlation among different elements for global pool -----	69
4.1 Changes in hair heavy metals in the village of Atemkit -----	92
4.2 Changes in hair heavy metals in the village of Bultem -----	94
4.3 Changes in hair heavy metals in the village of Finalbin -----	96
4.4 Changes in hair heavy metals in the village of Kavorabip-----	98
4.5 Changes in hair heavy metals in the village of Migalsimbip ----	100
4.6 Changes in hair heavy metals in the village of Wangbin -----	102
4.7 Changes in hair heavy metals levels for pooled Ok Tedi samples	104
6.1 Major SCMK zones identified by laser densitometry of SDS-PAGE after Coomassie (protein) stain and fluorography (cysteine content)	130
6.2 Distribution of SCMK polymorphism in the high cysteine (30-40 kDa) region -----	131
6.3 Distribution of SCMK polymorphism in the low cysteine (45-60 kDa) region -----	132

6.2	Distribution of SCMK polymorphism in the high cysteine (30-40 kDa) region-----	131
6.3	Distribution of SCMK polymorphism in the low cysteine (45-60 kDa) region -----	132

List of Figures

Figure-----	Page
2.1 Mg and Al in hair -----	35
2.2 Cr and Fe in hair -----	36
2.3 Mn and Co in hair-----	37
2.4 Ni and Cu in hair-----	38
2.5 Zn and As in hair-----	39
2.6 Se in hair-----	40
2.7 Hg in hair -----	41
2.8 Cd and Pb in hair-----	42
3.1 Mg and Al in hair -----	70
3.2 Cr and Fe in hair -----	71
3.3 Mn and Co in hair-----	72
3.4 Ni and Cu in hair-----	73
3.5 Zn and As in hair-----	74
3.6 Se in hair-----	75
3.7 Hg and Pb in hair -----	76
4.1 Longitudinal changes in Atemkit-----	93
4.2 Longitudinal changes in Bultem-----	95
4.3 Longitudinal changes in Finalbin -----	97
4.4 Longitudinal changes in Kavorabip -----	99
4.5 Longitudinal changes in Migalsimbip-----	101
4.6 Longitudinal changes in Wangbin-----	103
4.7 Longitudinal changes for Ok Tedi pool -----	105
6.1 SDS-PAGE separation of human hair proteins after S-carboxymethylation and staining with Coomassie blue -----	121
6.2 SDS-PAGE of the same hair samples as in Fig. 6.1 and staining with silver nitrate -----	122
6.3 Fluorograph of proteins of human head hair following	

S-carboxymethylation with ¹⁴ C-IAA-----	123
6.4 Laser densitometer traces of protein gel stained with Coomassie blue and following fluorography from human (Chinese) -----	124
6.5(a) Protein profile of different individuals following separation by SDS-PAGE and Coomassie blue staining -----	125
6.5(b) Protein profile of different individuals following separation by SDS-PAGE and silver nitrate staining -----	126
6.5(c) Schematic representation of putative electromorphs of lanes 2, 9 and 12 from Fig. 6.5(b)-----	127
6.6(a) Coomassie stained protein gel showing phenotypic variation in the low cysteine region -----	128
6.6(b) Fluorograph of S-carboxymethylated proteins showing phenotypic variation in the low cysteine region ----	129
6.7(a) Two-dimensional polyacrylamide gel electrophoretic pattern of proteins from human (African) hair -----	133
6.7(b) Two-dimensional polyacrylamide gel electrophoretic pattern of proteins from human (European) hair-----	134
6.7(c) Two-dimensional polyacrylamide gel electrophoretic pattern of proteins from human (European) hair -----	135
7.1(a) SDS-PAGE of human and animal hair proteins after S-carboxymethylation-----	145
7.1(b) SDS-PAGE of human and animal hair proteins after S-carboxymethylation-----	146
7.1(c) SDS-PAGE of animal hair proteins after S-carboxymethylation-----	147
7.1(d) SDS-PAGE of human and animal hair and avian feather proteins after S-carboxymethylation -----	148
7.2 SDS-PAGE of same hair samples as in Fig. 1d -----	149
7.3(a) Fluorograph of proteins of human and animal hair following	

S-carboxymethylation with ^{14}C -IAA and separation by SDS-PAGE -----	150
7.3(b) Flouorograph of proteins of human and animal hair following S-carboxymethylation with ^{14}C -IAA and separation by SDS-PAGE -----	151
7.4(a) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from baboon hair -----	152
7.4(b) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from sheep hair -----	153
7.4(c) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from goat hair-----	154
7.4(d) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from rabbit -----	155
7.4(e) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from rat-----	156
7.4(f) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from cat -----	157
7.4(g) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from dog-----	158
7.4(h) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from kangaroo -----	159
7.4(i) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from possum -----	160
7.4(j) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from mountain pygmy possum -----	161
7.4(k) Laser densitometer traces of SDS-PAGE gels of S-carboxymethylated hair proteins from marsupial shrew ----	162
7.4(l) Laser densitometer traces of SDS-PAGE gels of	

S-carboxymethylated feather proteins from chicken -----	163
7.4(m) Laser densitometer traces of SDS-PAGE gels of	
S-carboxymethylated feather proteins from duck -----	164
7.5(a) Two-dimensional polyacrylamide gel electrophoretic pattern of	
hair proteins from sheep -----	165
7.5(b) Two-dimensional polyacrylamide gel electrophoretic pattern of	
hair proteins from dog -----	166
7.5(c) Two-dimensional polyacrylamide gel electrophoretic pattern of	
hair proteins from cat -----	167
7.5(d) Two-dimensional polyacrylamide gel electrophoretic pattern of	
hair proteins from human (Australian Aboriginal) -----	168
8.1(a) Tryptic cleavage pattern of human hair proteins	
(Australian European of Anglo-Celtic origin)-----	176
8.1(b) Fluorograph of the same sample (Australian European of	
Anglo-Celtic origin) as represented in Fig. 8.1(a) -----	177
8.1(c) Fluorograph of human hair proteins (Australian Aboriginal)-	178
8.2(a) Tryptic cleavage pattern of hair proteins from goat (Angora)	
hair proteins-----	179
8.2(b) Fluorograph of the same sample (goat) as represented in	
Fig. 8.2(a)-----	180
8.3(a) Tryptic cleavage pattern of feather proteins from chicken ---	181
8.3(b) Fluorograph of the same sample (chicken) as represented in	
Fig. 8.3(a)-----	182
8.4(a) Fluorograph of hair proteins from rat-----	183
8.4(b) Fluorograph of hair proteins from possum -----	184
8.4(c) Fluorograph of hair proteins from mountain pygmy possum-	185
8.4(d) Fluorograph of feather proteins from duck -----	186

TABLE OF CONTENTS

Declaration	<i>i</i>
Acknowledgements	<i>ii</i>
Abbreviations	<i>iii</i>
Abstract	<i>v</i>
Publications arising from this thesis	<i>vii</i>
List of Tables	<i>viii</i>
List of Figures	<i>x</i>
Table of Contents	<i>xiv</i>
CHAPTER 1: INTRODUCTION	1
Hair heavy metals	1
1.1 Heavy metal toxicity and pollution	7
1.2 Variations due to age, sex and race	9
1.3 Diagnostic and forensic value of heavy metal analysis	10
1.4 Hair proteins	11
Hair Proteins	13
1.5 Different classes	18
1.6 Occurrence	19
1.6.1 High sulphur proteins (matrix proteins)	19
1.6.2 Very high sulphur proteins	19
1.6.3 Low sulphur proteins	20
1.6.4 High tyrosine proteins	20
1.7 Synthesis	20
1.8 Methods of separation	21
1.8.1 Electrophoretic methods	21
1.8.2 Chromatographic methods	22
1.9 Fractionation of SCMK	23

1.10 Clinical and forensic applications -----	23
1.11 Polymorphism -----	24
CHAPTER 2: Comparison of scalp hair heavy metals at control and test	
(Ok Tedi) sites in Papua New Guinea -----	26
2.1 Introduction -----	26
2.2 Materials and Methods -----	28
2.2.1 Materials -----	28
2.2.2 Origin and preparation of samples -----	28
2.2.3 Digestion of hair samples -----	28
2.2.3.1 Standard solution -----	29
2.2.3.2 Sample solution -----	29
2.2.3.3 Calculations -----	29
2.2.3.4 Statistical analysis -----	29
2.3 Results -----	30
2.4 Discussion -----	43
CHAPTER 3: A comparison of scalp hair heavy metals among different	
populations in Australia and Papua New Guinea -----	54
3.1 Introduction -----	54
3.2 Materials and Methods -----	56
3.3 Results -----	57
3.4 Discussion -----	77
CHAPTER 4: Longitudinal changes in hair heavy metals at test	
(Ok Tedi) sites -----	86
4.1 Introduction -----	86
4.2 Materials and Methods -----	88
4.2.1 Heavy metal analysis -----	88
4.2.2 Protocol A -----	88
4.3 Results -----	89
4.4 Discussion -----	106

CHAPTER 5: Materials and Methods	110
Hair protein analysis	
5.1 Materials	110
5.1 Origin of human hair samples	110
5.3 Origin of animal hair and avian feather samples	111
5.4 Preparation of samples	111
5.5 Digestion buffer	111
5.6 Quantitation of microgram quantities of protein	112
5.6.1 Preparation of protein reagent	112
5.6.2 Protein assay	112
5.7 Method for the incorporation of radioactivity into proteins of hair	112
5.8 S-carboxymethylation with iodoacetic acid	112
5.9 One-dimensional 12.5 % sodium dodecyl sulfate (SDS) gel ---	113
5.9.1 Stock gel solution	113
5.9.2 Lower Tris buffer	113
5.9.3 Upper Tris buffer (for stacking gel)	113
5.9.4 Reservoir buffer (for upper and lower reservoirs)	113
5.9.5 Running buffer	113
5.9.6 Sample buffer	113
5.10 Composition of 12.5 % one-dimensional SDS gel	113
5.11 Sample preparation for gels	114
5.11.1 0.1 % Bromophenol blue	114
5.11.2 0.05 % Coomassie blue	114
5.12 One-dimensional (1D) SDS-PAGE	114
5.13 Staining	114
5.14 Destaining	114
5.15 Scanning laser densitometry	114
5.16 Two-dimensional (2D) electrophoresis	115

5.17	Two-dimensional SDS-PAGE with trypsin-----	115
CHAPTER 6: Electrophoresis of S-carboxymethylated human hair proteins-		
partial characterisation and indication of polymorphism 116		
6.1	Introduction -----	116
6.2	Results -----	118
6.2.1	SDS-PAGE separation of human hair proteins-----	118
6.2.2.	Autoradiography of ¹⁴ C-S-carboxymethylated human hair proteins -----	118
6.2.3.	Laser densitometry -----	118
6.2.4	2D electrophoresis of human hair proteins -----	120
6.3	Discussion -----	136
CHAPTER 7: A comparative electrophoretic analysis of mammalian hair		
and avian feather proteins ----- 140		
7.1	Introduction -----	140
7.2	Results -----	142
7.2.1	The electrophoresis of S-carboxymethylated kerateines from different animals -----	142
7.2.2	Autoradiography of ¹⁴ C-S-carboxymethylated animal and feather proteins -----	142
7.2.3	Laser densitometry -----	143
7.2.4	Two-dimensional electrophoresis of mammalian hair and avian feather proteins -----	144
7.3	Discussion -----	169
CHAPTER 8: Peptide mapping of S-carboxymethylated hair and feather		
proteins using 2D-dimensional electrophoresis ----- 173		
8.1	Introduction -----	173
8.2	Results -----	174
8.2.1	Comparison of tryptic cleavage pattern between Coomassie blue stained gel and autoradiograph -----	174
8.2.2	Radioactive peptide pattern as an indication of species relatedness -----	174

8.3 Discussion-----	187
GENERAL DISCUSSION -----	190
CONCLUSIONS AND INDICATIONS FOR FUTURE WORK -----	194
REFERENCES -----	196
APPENDIX -----	222