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TECHNIQUES FOR MONITORING HUMAN EXPOSURE TO AIRBORNE TRACE METALS

A thesis submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy awarded by the
Council for National Academic Awards.

by

DAVID R TENNANT

THE POLYTECHNIC OF NORTH LONDON

November 1983

ACKNOWLEDGMENTS

I would like to express my thanks to my supervisors, Dr. Ron Rees and Mr. Chris Huskett for their advice and guidance.

At the Polytechnic of North London I would like to thank Mr. Frank Nobee and Ms. Kiortyahann for technical advice and assistance. At the Health and Safety Executive Dr. David Gompertz, Mr Eric Pryde and Mr. Norman Smith have given me every help and encouragement. I would also like to thank Dr. Trevor Ogden for reading part of the manusript and my other collegues for giving up their hair so willingly!

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Declaration:

While registered as a candidate for this degree the author has not been registered for another award of the CNAA or of a University during the research programme.

David R Tennant

PART I

A Passive Sampler for Trace Metals in Airborne Respirable Particles

1.1.1 Trace elements in ambient air

Considerable concern has been expressed about the levels of trace elements in urban atmospheres over recent years. While these are a natural component of any aerosol there is abundant evidence that levels are increasing, particularly of the more toxic trace metals.

The composition of the aerosol varies worldwide but the principal contrast is between urban and rural aerosols (Table 1.1.1).

The composition of trace elements in an urban atmosphere will be the sum of the local background level plus any contribution from industrial processes, power generation, transport, incineration and other element releasing processes, plus re-entrained material deposited previously.

1.1.2 The absorption of airborne trace metals

Of primary importance when studying the levels of trace elements in urban air is the particle size distribution of these elements. If there is to be any value in

TABLE 1.1.1 Total concentrations of some elements in air at remote and urban locations. (1.2.3)

Element	Background	Urban	
	ng kg^{-1}	ng kg^{-1}	ng m^{-3}
Al	0.67 - 43	370	
As	0.006 - 0.19	6.4 - 15	
Br	1.1 - 6.7	1.1 - 320	
Ca	0.4 - 200	-	
Cd	ND - 0.4	2.8	ND - 130
Cl	5.9 - 2100	4600	
Co	0.040	1.4 - 4.5	ND - 30
Cr	<0.03 - 0.29	6.1 - 14	6 - 65
Cu	0.51 - 0.72	19 - 57	110 - 846
Fe	29 - 40	680 - 940	610 - 2590
Mn	1.2 - 1.8	25 - 31	23 - 85
Na	2.7 - 1200	1960	
Ni	< 2	13 -66	12 - 120
Pb	3.6 - 11	340 - 500	630 - 2210
Sb	0.16 - 0.24	4.0 - 7.3	
V	0.24 - 1.3	17 - 21	39 - 240
Zn	0.027 - 8.1	260 - 310	150 -830

measuring element concentrations in urban air it must be in the context of assessing the data for toxic risk. Toxic risk is inextricably bound to the ability of the respiratory system to retain or absorb any toxic compound present (4).

The human respiratory system does not exhibit 100% retention characteristics for inhaled particles over the whole size spectrum (4,5). The most important factors determining the pattern of deposition within the respiratory tract are (5):

1. The dimensions of the respiratory system.

2. Respiratory patterns, especially tidal volume, the rate of breathing and nose or mouth breathing.

3. Secondary responses such as diseased states or allergic conditions.

The minimum retention is normally about 0.5 μm where about 25% of the inhaled aerosol is retained (5,6,7). Above 0.1 μm, deposition in the nasopharyngeal regions increases with increasing particle size. Particles with an aerodynamic diameter greater than 20 um are almost exclusively retained in the nose (6). Below 1.0 μm

pulmonary deposition increases with decreasing particle size (5). For particles below 0.1 um, the efficiency of deposition approaches in value the fraction of the total tidal volume which reaches the pulmonary air spaces of the deep lung.

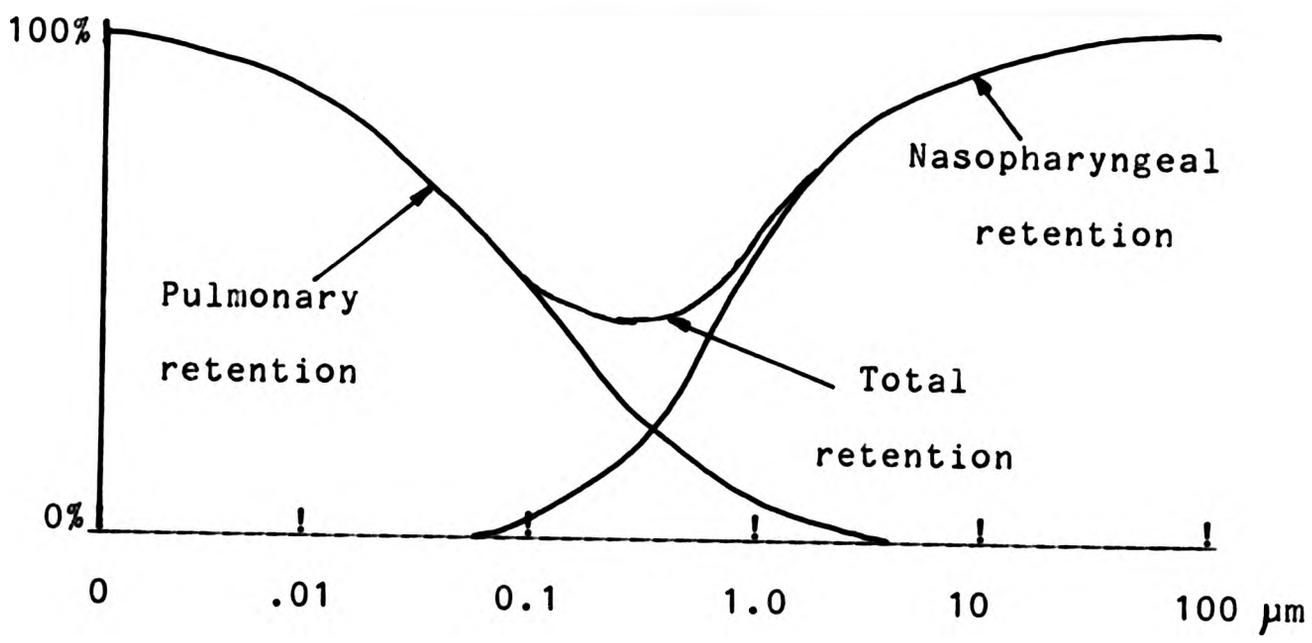

Figure 1.1.1 Idealised particle retention curve for different regions of the lung.

The upper respiratory tract serves mainly as a protection to the rest of the system. Particles which are collected here are either swallowed or expelled with nasal clearance. Particles which are deposited in the conducting airways are gradually raised by the mucociliary escalator to be swallowed. Trace elements are generally poorly absorbed in the gastro-intestinal tract. Only about 5-15% of inorganic lead associated with particles is extracted (4). The only particles of pathological interest are those which penetrate to, and

are deposited in, the pulmonary air spaces (6). Particles may remain in the alveoli until they dissolve or are phagocytised. Material collected by phagocytes is introduced to the blood stream via the lymphatic channels. Mouth breathing can introduce more of the larger particles to the alveoli because the protection of the nasopharyngeal region has been avoided (8). The rate of breathing also affects the retention efficiency of the deep lung (6).

Clearly, as particle diameter decreases so the hazard associated with airborne toxic trace elements increases. The Medical Research Council has defined "respirable dust" mass measurement criteria which reflect the relative hazard from different particle sizes (Fig 1.1.2). The dynamics of lung particle retention have been confirmed by more recent studies (10).

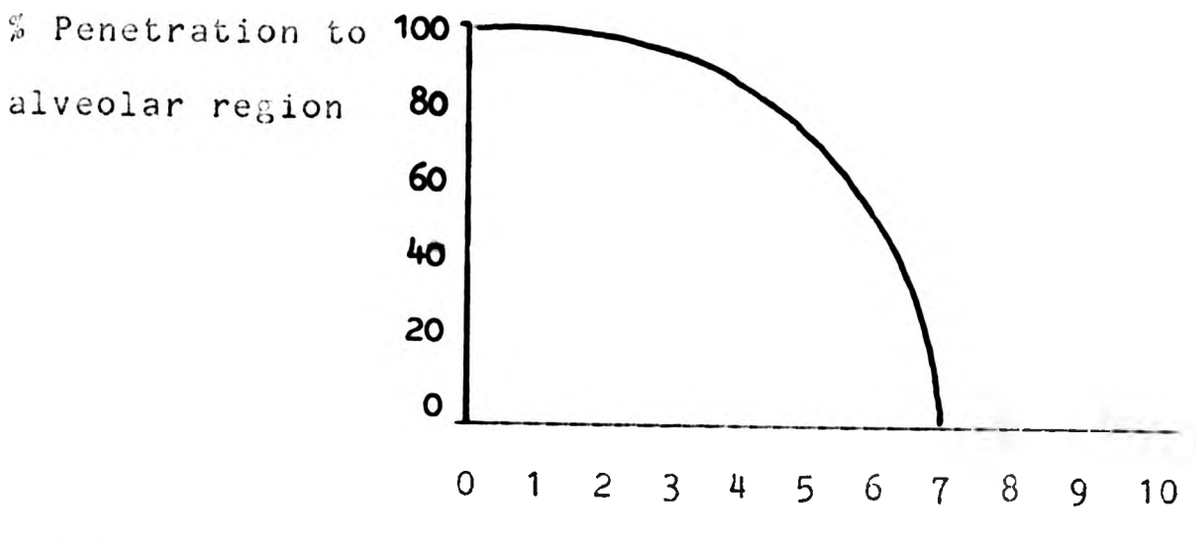

Figure 1.1.2 Medical Research Council "Respirable Dust" Curve.

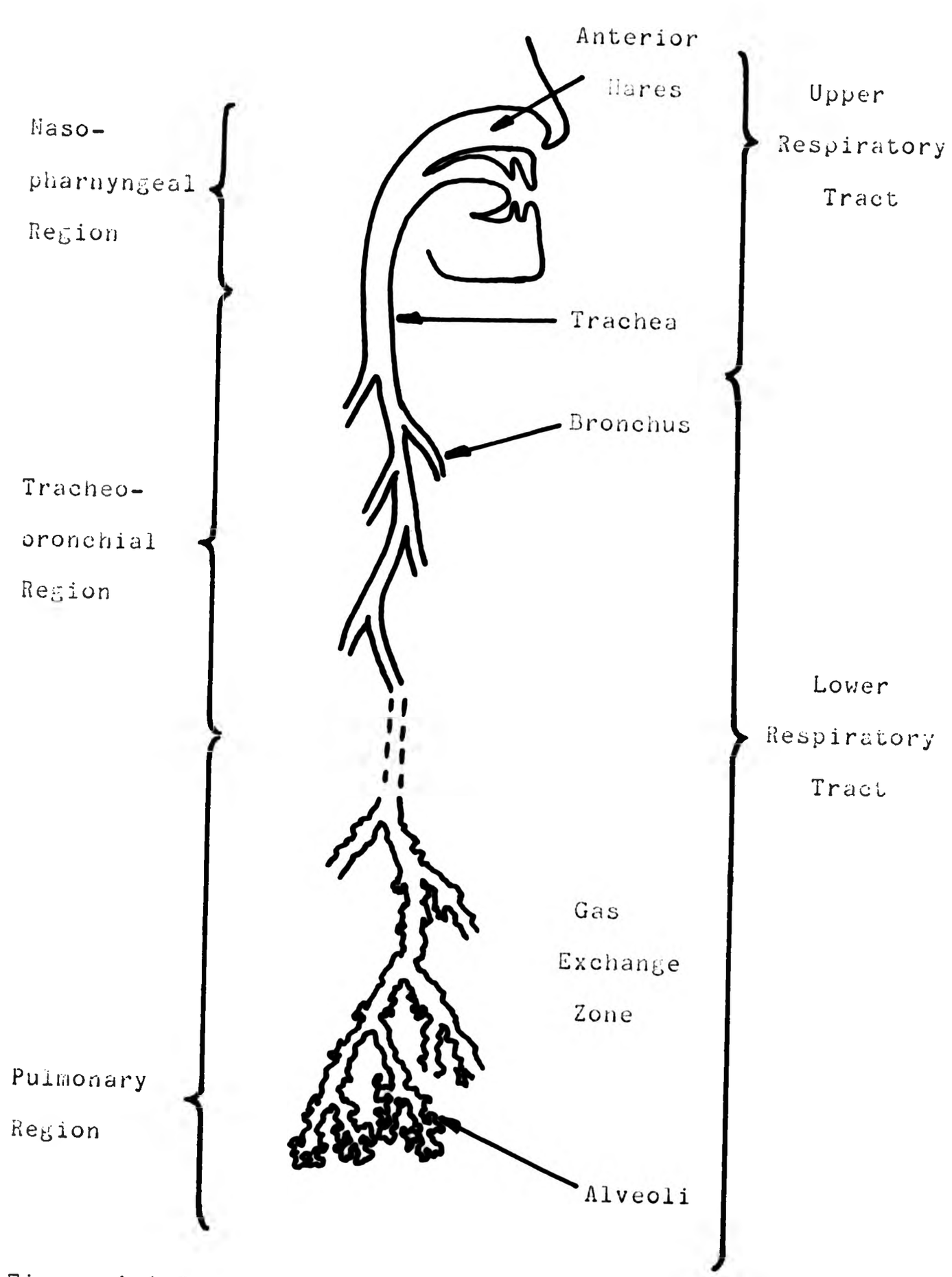

Figure 1.1.3 Diagrammatic representation of the human respiratory tract (8).

1.1.3 Size distribution of Airborne Trace metals

Airborne material can cover a size spectrum varying from Aitken nuclei at about 0.002 μm to wind blown dusts up to 100 μm in diameter (8,11). The size distribution at any location will depend upon the sources of the aerosol, its age and the degree of turbulence resuspending previously settled larger particles (Fig 1.1.4).

The finer particles are generated mainly by combustion processes through the condensation of gases and vapours. These particles coagulate and act as condensation nuclei for other gases and vapours so that the size distribution shifts to a larger size range as the aerosol ages. The primary aerosol is relatively short-lived but the secondary aerosol becomes more stable as the processes of coagulation and condensation become less favourable. The particle size range above 1 μm tends to be generated by mechanical processes such as erosion. Its stability will depend upon local turbulence, wind-speed and precipitation.

In general, particles greater than 9 μm settle very rapidly, particles sized between 1-9 μm may remain airborne for short periods but it is only the sub-micron fraction that has a significant residence time (12).

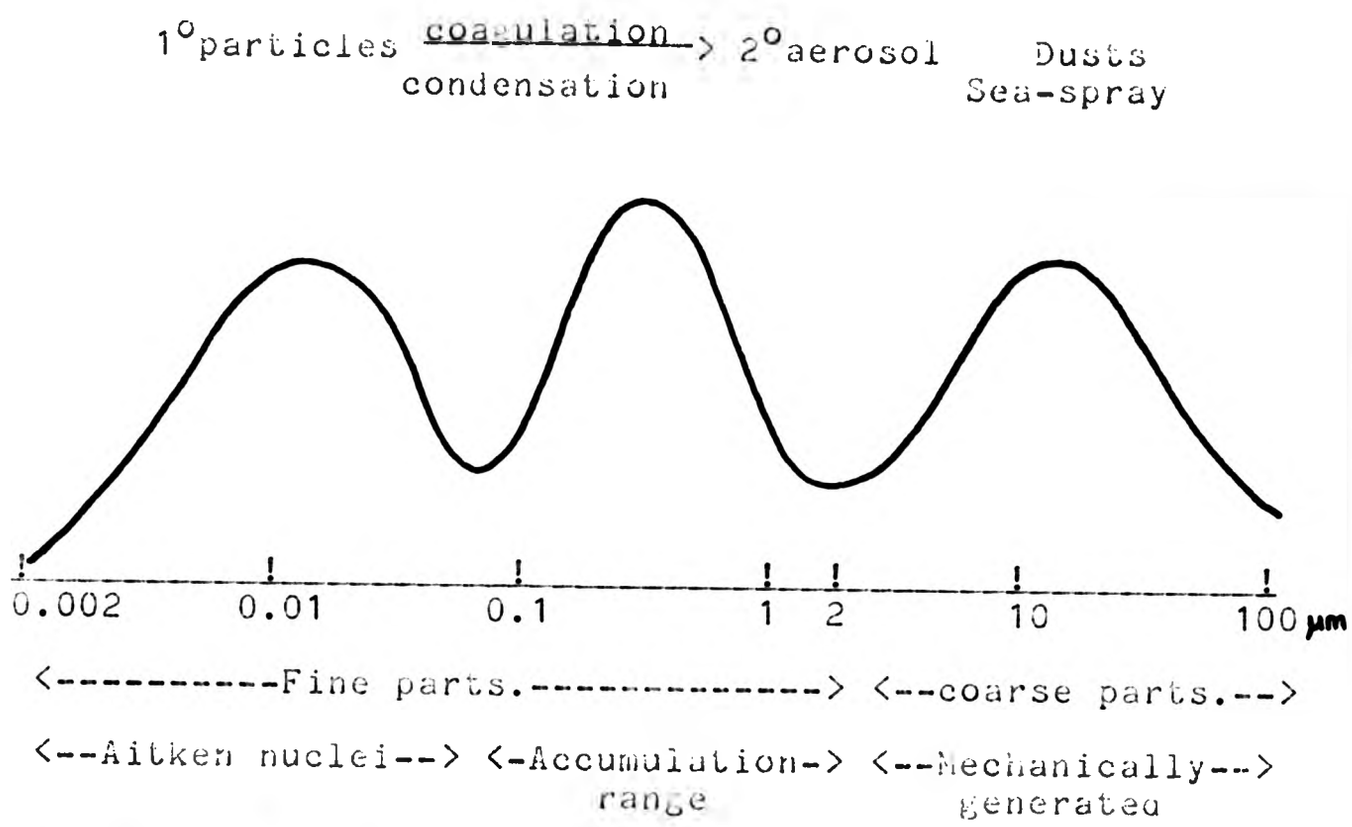

Figure 1.1.4 Multi-modality of the ambient aerosol (8).

In a study of the size distribution of aerosols in California, Hidy et al (11) found that trace elements from anthropogenic sources were concentrated in the sub-micron fraction of the aerosol. Components such as toxic trace metals, carbon-compounds, bromides and oxides of sulphur and nitrogen had a maximum mass concentration at around 0.5 µm, corresponding to the secondary aerosol. The major components, silicon, aluminium, iron, sodium and chlorine, had a maximum mass concentration at about 10 µm, reflecting their natural origin.

The size distribution of trace metals in urban air tends to reflect their principal origin (Table 1.2).

Table 1.2 The size distribution of some trace metals in USA urban air (13).

	Total Suspended Particulates ($\mu g\ m^{-3}$)	% Less than or equal to 1μm
Fe	0.5 - 1.5	10 - 37
Pb	1.0 - 3.5	53 - 79
Zn	0.1 - 1.7	10 - 65
Cu	0.1 - 0.6	16 - 60
Ni	0.04 - 0.11	28 - 52
Mn	0.01 - 0.31	13 - 38
V	0.02 - 0.15	41 - 79

Because of its size distribution and known toxic effects, lead probably poses the greatest inhalation hazard in urban air. In Cincinnati 70% of airborne lead was associated with particles less than 1 μm in diameter and the mass median equivalent diameter (MMED) of lead particles in USA urban air ranged from 0.08 to 0.64 μm (14,15). The range of sizes reflects the source and age of the aerosol. Paciga et al (16) found that near a roadside the lead aerosol was predominantly

associated with sub-micrometer particles whereas in the vicinity of lead smelters the lead was biased towards particles greater than 3.3 μm. They suggest that roadside lead poses a greater health hazard than that emitted from smelters.

Natusch and Wallace (4,17) have investigated the mechanisms of particle formation in combustion processes. In the cooling flue gases the more volatile metals including Pb, Cd, Se, As and Ni are adsorbed on to refractory particles. Smaller particles have a higher surface area to mass ratio and the average concentration per unit mass, (C_x) of a volatizable species, x, should depend upon the particle diameter, Dp according to the form

$$C_x \propto C_o + \frac{C_a}{Dp}$$

C_o = Average intrinsic concentration of x in refractory particle.

C_a = Average concentration added by absorption.

The volatile trace metals are thus preferentially concentrated on the smaller particles in the combustion products. The final distribution will depend upon the particle sizes available. In coal fired power plants the metals are associated with fly-ash particles (18,9). A significant amount of sub-micron material is generated by the 'bursting' and fragmentation of larger fly-ash

particles. Although the incineration of domestic wastes can contribute significantly to the levels of toxic trace metals in the urban atmosphere (20), the low temperature of combustion will tend to generate a larger aerosol (17).

Although the ramifications of the addition of lead to petrol are still the subject of debate (21,22,23) there is little doubt that this is the major source of lead in urban air (24,25,26). Lead is added to petrol as tetramethyl or tetraethyl lead as an anti-knock agent and reacts with the lead scavengers 1,2-dichloroethane and 1,2-dibromethane in the combustion chamber (25). The lead is emitted as lead halide compounds in the form of very dense sub-micron particles. These particles remain suspended in the aerosol and are highly mobile. The principal loss mechanism is believed to be diffusion to upper atmospheric levels and 90% of the lead is in a form suitable for long-range dispersion (26).

Organic lead may be present in the urban atmosphere in trace amounts (28,29) (Table 1.1.3). Since organic lead is regarded as being considerably more toxic than inorganic lead simultaneous monitoring may be justified. However, since all organic lead is eventually broken

down to inorganic lead it would appear that inorganic lead is a greater cause for concern except in the immediate vicinity of filling stations (30).

Table 1.3 Organic lead in air (28,29)

Location	Total Pb (ngm^{-3})	% Organic Pb
Rural	92 - 112	ND
Residential	146 - 317	2.5 - 1.6
Urban	1267 - 3870	12.4 - 20.8
Pb smelter	92 - 1445	ND - 5.1
Petrol station	789 - 892	23.9 - 24.3

Robinson et al (31) suggest that lead may be present in the atmosphere in an inorganic 'molecular' form. Surface imperfections of crystals may not be subject to the theoretical dynamics of volatilisation when subjected to physical pounding and the heat of the road surface. Thus an inorganic molecular vapour may be formed. The authors have detected molecular forms of PbO, $PbCl_2$ and $PbBr_2$ which could not be collected on conventional filters.

It is apparent that the physical form of airborne toxic trace metals is of great significance. The environmental effect of trace metals in small particles, or

concentrated at particle surfaces, may be much higher than might be expected because of their enhanced availability to aqueous leaching in biological systems (32,33).

1.1.4 Factors Affecting Levels of Trace Elements in Urban Air

At a given urban location considerable fluctuations in the levels of trace metals may be encountered (34). In the USA and the UK levels are usually higher in the winter (1,13,34). This is due to a combination of effects including lower mixing heights and the use of more heating and transport fuels in these months. In Glasgow, periods of fog and calm weather were found to be associated with higher levels, while rain was found to clear the atmosphere (35). Robinson *et al* detected higher values of 'molecular' lead on warm, still days (31), and in general the concentration of lead has been found to be highest on dry, still days, washed out on wet days and dispersed on windy days (34).

Lawther *et al* (37) report that the ratio [Pb] street/[Pb] roof is lower for lead than for NO, CO and hydrocarbons. This may be because the other components are unstable and might be lost from the aerosol or it

might be that Lawther's technique failed to detect the primary lead aerosol at street level. Huskett (38) studied the concentration of lead in air in tower blocks in Islington, London. he detected no significant increase or decrease with height although an earlier study using moss bags had implied an increase with height. It seems possible that techniques which do not account for the whole particle size distribution may introduce bias into the results. It may also be the case that the techniques used in these studies were windspeed dependant.

Air concentrations of lead have been found to vary linearly with traffic volume and follow a quadratic decay function with respect to distance from the roadway (39). Normal 'downtown' levels of 2-3 $\mu g\ m^{-3}$ were found to be elevated up to 40 $\mu g\ m^{-3}$ during rush hour. The most important factor affecting local time-averaged values was found to be wind direction.

There is some dissent over the relationships between indoor and outdoor levels. Andersen (40) found a close correlation between indoor and outdoor levels of particulates and SO_2 but indoor levels were 30% to 50% lower. Nosandreas (41) considered that large particles

were excluded from the indoor atmosphere but that lead, which is associated with smaller particles, may maintain indoor concentrations similar to those found outside.

1.1.5 Techniques for Monitoring Trace Metals in Air

A range of techniques is available for monitoring trace metals in air varying in objectives and principals and degree of sophistication. The most common techniques rely on drawing air at a constant rate through a filter. If an air sample is drawn slowly through a filter (of small area) this is referred to as 'low-volume' sampling. If the sample is drawn rapidly through a filter (of large area) this is referred to as high-volume sampling which has been americanised to 'Hi-Vol' sampling. The 'Hi-Vol' filter is made from glass-fibre and its dimensions are 5" x 7". It is mounted on a device somewhat resembling a vacuum cleaner. Its principal applications are in 'spot' sampling or in intermittent sampling at the same time each day. Since they do not smooth out short-term fluctuations they have no application in this study.

Low volume sampling is employed to provide a measure of total suspended particulates (TSP) over time periods extending from days to weeks. The choice of filter

material is very important. Some filters do not collect certain particle sizes efficiently, other are subject to blocking and others may introduce analytical difficulties (42,43,44). Cellulose fibre (paper) filters are often the least efficient in the sub-micron size range (45). The retention characteristic of filters is often expressed in terms of the % penetration or retention of a 0.3 μm mass median diameter aerosol. Mass median diameter is defined as that diameter above or below which 50% of the mass of the aeorosol lies. It is a convenient parameter with which define an aerosol but gives no information about the distribution of particle sizes. Since the particle diameter of some hazardous components may be less than 0.3 μm it is desirable to have some notion of filter efficiencies for very small particles. Spurny et al (46) have investigated the retention of 0.3 μm and 0.03 μm particles by various types of filter (Table 1.1.4).

It can be assumed that Millipore AA and HA filters will have retentions greater than 97% across the whole size spectrum as long as relatively low face velocities are maintained (47).

Table 1.1.4 Retention of small particles by various filter types

Filter	Material	Pore size μm	$E_{(0,03)}$	$E_{(0.3)}$
Membrane				
Nuclepore	Polycarbonate	0.50	97.8	99.3
Nuclepore	"	1.00	86.8	52.2
HA-Millipore	Cellulose acetate	0.46	99.9	98.8
AA-Millipore	"	0.80	99.9	97.1
SS-Millipore	"	3.00	99.9	91.2
Fibre				
Gelman-AGF	Glass-fibre	-	99.9	99.0
PF-41-Whatman	Paper	-	53.9	22.4

Face velocity: 5 cm sec^{-1}.

$E_{(0.03)}$ and $E_{(0.3)}$ are mean collection efficiencies for particle diameters 0.03 and 0.3 um.

When a membrane filter is run for long periods 'plugging' of the pores may occur. This will affect the resistance across the filter and in turn the flow rate will fall. Smith and Suprenant (45) have investigated changes in pressure differential with filtration time and some of their findings are summarised in Table 1.1.5.

Table 1.1.5 Change of pressure drop across air sampling media with operating time

Filter	Pressure drop (ins of water) Initial	120 hrs	480 hrs	600 hrs
Whatman No 41	0.35	2.15	4.3	-
Millipore AA	2.3	2.6	3.2	3.4
Millipore HA	5.4	5.7	8.1	8.6
Hurlbut Glass Paper	0.95	1.1	1.25	(8.4)*
AEC All-Glass	0.7	0.7	0.85	(5.6)*

Approximate dust load: 63 $\mu g\ m^{-3}$

Flow rate: 5 ft min^{-1} (= 2.54 cm sec^{-1}),
*28 ft min^{-1} (= 14.2 cm sec^{-1})

The overall performance of millipore filters is generally better than paper filters but not as good as glass-fibre filters. However, membrane filters have other properties which make them superior in many applications. The membrane filter may be totally digested for chemical analysis making extraction techniques unnecessary. Membranes may be rendered transparent by the addition of a fluid of similar

refractive index, so that they can be examined by optical microscopy (47). They may also be collapsed to form a clear solid film which is a suitable mounting medium for both optical and electron microscopy (48). When the filter itself is used as the electron microscopy substrate, only minimal disturbance of the collected particles occurs. This is of particular importance when collecting particles to study size distributions.

The measurement of dust fall into glass funnels and bottles can provide information about the cycling of elements but is of little value in assessing airborne concentrations. The rate of fall-out will depend upon the size distribution of the aerosol and to a large extent on the prevailing meteorological conditions (49). In urban areas a similar approach has been to analyse dust collected inside homes. This has proved of value in assessing the toxic metal hazard to urban dwellers (50).

Leaves and twigs have been found to be reliable indicators of deposition to vegetation (51,52), and indigenous mosses have been shown to possess a unique ability to absorb cations from the air (53), making them useful indicators of local airborne pollution (54). Goodman *et al* (55) and Little and Wiffen (26) have

described the use of moss-bags as monitors of fall-out of trace metals to vegetation. The moss-bag comprises about 10 g of acid-rinsed sphagnum moss collected from some remote area, packed loosely into a ladies 'bun-net' and suspended from a 1 m bamboo cane. A refinement of the technique is a two-dimensional 'wallet' which allows some assessment of the direction of the source (53). This technique has been employed in a number of successful studies of trace metal transport and distribution (53,56,57). The moss-bag has several features which commend it; it is cheap; simple to operate; requires no power supply; it is easy to analyse chemically and generates data which is simple to interpret. The moss-bag is an excellent model for the mass transfer of airborne material to vegetation. However, it is highly unsuitable as a monitor for trace metals in the urban environment, where inhalation hazard is the principal concern. For particles greater than 10 um the moss bag is very efficient, but it is extremely inefficient in the critical 0.05-0.20 μm size range (26,58). Its collection characteristics are also highly dependent upon wind speed (58).

Some of the techniques used in occupational hygiene monitoring attempt to discriminate between the "respirable" and "non-respirable" components of the

aerosol.The American Conference of Governmental Industrial Hygienists has defined criteria for the assessment of the respirable component of an aerosol (Fig 1.1.5). The Health Effects Research Laboratory of the USA Environmental Protection Agency recommends that a standard for inhaled particles should extend to 15 μm to allow for the worst case situation (mouth breathing) (59). However, this is now to be reduced to 10 μm (59A). The recommended minimum cut-off brings the criteria broadly into line with the earlier AGGIH standards. The 'respirable' component may be defined as those particles sufficiently small to be aspirated into the human alveoli (60). The considerable inter-individual differences have been discussed above so it is clear that any definition must be highly idealised. However, any method which does not take this distinction into account will provide a crude and perhaps misleading indication of inhalation hazard. This is best illustrated by observing that one 10 μm particle has the same mass as one million 0.1 μm particles (and possibly the same trace metal content too). Only a few resuspended particles of road dust are needed in order to achieve the same lead content as a more representative sample of smaller, respirable particles (61). The ratio of inhalable particle concentration to total suspended particles is reported to range

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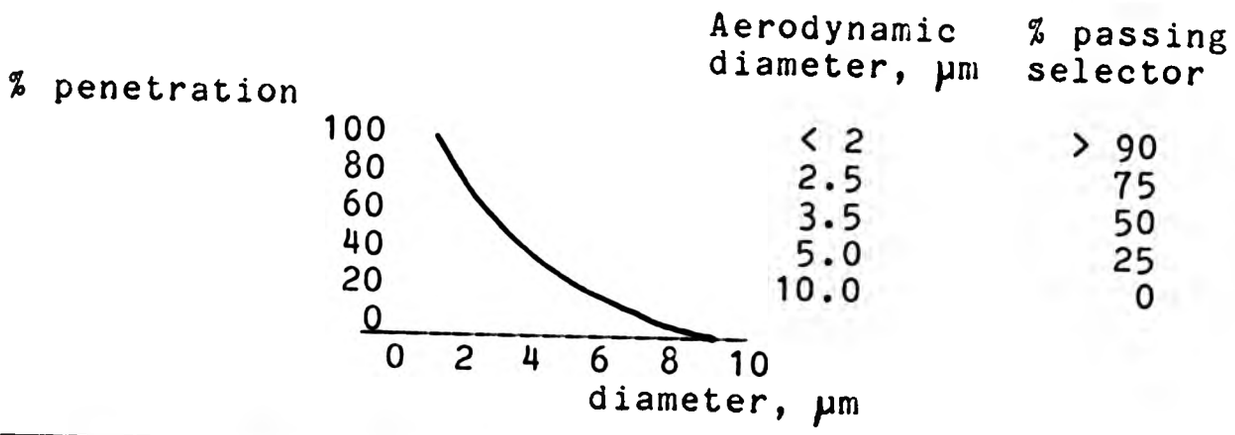
from 0.5 to 0.7 (62).

A number of dichotomous samples are available on the market and typical of these are the Cassella 'Hexlet' and 'Personal Dust Sampler'. Both of these are designed to conform to the MRC retention curve and separate the aerosol by removing large particles in an elutriator and collecting the 'respirable' component on a filter. The division is somewhat arbitrary but it is a considerable improvement on Total Suspended Particulates sampling when an assessment of inhalation hazard is required.



Aerodynamic diameter, μm	% passing selector
< 2	> 90
2.5	75
3.5	50
5.0	25
10.0	0

Figure 1.1.5 The ACGIH 'Respirable' Mass measurement Criteria (3)

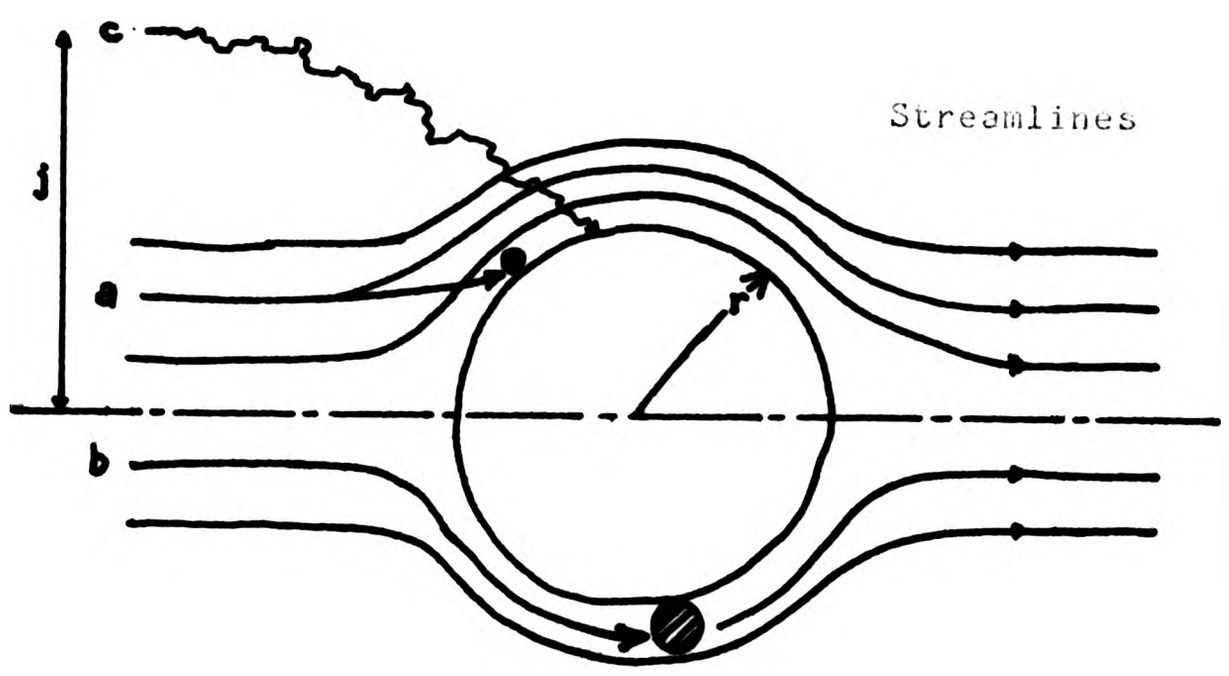
Cascade impactors are designed to provide a serial separation of the particles according to their aerodynamic properties. Particles may be collected on up

to 8 stages where each stage is defined by its cut-off mass median equivalent diameter (MMED). Equivalent diameter is the diameter of a unit density sphere which has the same inertial characteristics as a given particle. A detailed discussion of the use of the Anderson 2000 impactor appears in Section 1.2.1.

1.1.7 Small Particle Dynamics

The moss-bag is a passive sampler for dry deposition to vegetation. The aim of this work is to apply this type of technique, with all its benefits to the problem of sampling for respirable hazard. It is necessary to consider some of the physical mechanisms involved in the collection of particles from gaseous media. Most of the theory has been developed around industrial filtration and dust control equipment, although there has been some recent work in the field of passive dosimetering for vapours and gases (63,64).

Six mechanisms have been identified in the capture of particles; inertial impaction, interception, gravitation, diffusion, electrostatic attraction and temperature gradient motion (Figure 1.1.6).



a: Impaction

b: Interception

c: Diffusion

r: radius of collector

j: displacement

Figure 1.1.6 Trajectories of particles subject to various collection mechanisms. (After George and Poehlein (63)).

Inertial impaction occurs when particles deviate from the streamlines of the carrying gas as it flows around or over the collector. It is most significant for larger (heavier) particles at high gas velocities.

It is the dominant mechanism in the filtration of industrial gases and in 'Hi-Vol' sampling techniques and collection of particles by the moss-bag. It is also responsible for the retention of larger particles in the nasopharyngeal regions of the respiratory system. The inspired air passes through the nasal hairs and over the internal convolutions of the air passages at high speed. The efficiency of collection is related to particle mass and gass velocity since collection is dependent on particle inertia where

particle inertia = mu m = mass

u = velocity

Particle shape and density will also be a factor, which is why theoretical criteria are always expressed in 'equivalent diameter' for unit density spheres.

Interception is only important for large particles which follow the streamlines and make contact with the collector surface. In most cases the effect can be considered to be negligible. Gravitation is only significant for large particles at low gas velocities. It is the mechanism involved in dust fall techniques.

Diffusion only affects those particles small enough to be subject to Brownian motion (random motion of the particle caused by the buffeting by gas molecules). The rate of collection is related to the rate at which particles can cross a layer of laminar flow adjacent to the collector surface (see Fig 1.6). Loffler (65) states that the collection efficiency is inversely related to the Peclet number, Pe, where

(1) $Pe = \frac{Dc\ Vo}{d}$

Dc = Diameter of cylindrical collector
Vo = Gas velocity
d = diffusion coefficient

(2) $d = \frac{K\ T\ C}{3\ u\ Dp}$

K = Boltzman's constant
T = Absolute temperature
C = Cunningham correction factor (0.50 - 0.75)
U = dynamic viscocity of gas
Dp = diameter of the particle

For a given system at a fixed temperature, collection efficiency can be seen to be inversely proportional to the diameter of the collector (Dc), the diameter of the particle (Dp) and the velocity of the gas flow (V).

Diffusion collection is the principal mechanism involved in the retention of very small particles in the lung. The lung is a highly branched system and the passage diameter is reduced at each bifurcation. As the diameter

of the alveolar passages approaches the root mean square (RMS) displacement of the particles due to Brownian motion, the probability of a given particle reaching the walls approaches 1/2 (5). The number of particles retained is also related to the rate and depth of breathing. If breathing is slower, then the time that the particles spend in the narrow passages is increased, and the probability of a particle reaching the wall is increased. Deep breathing may admit more particle-containing air to the alveoli and allow a more complete change of air.

Electrostatic attraction between two oppositely charged bodies, or the induced force where only one body is charged, can enhance collection efficiencies. The collection efficiency may be greater than 100% where the collection efficiency, n, for a fibre, is defined as the ratio of the number of particles collected by a unit length of fibre a, in unit time, to the total number which flow toward the projected surface of the fibre in unit time.

(3) $$n = \frac{a}{D_c V_o n_o}$$

D_c = fibre diameter
V_o = Upstream gas velocity
n_o = upstream particle concentration
a = number of particles collected per unit length per unit time

The force between two point charges can be calculated by Coulomb's law. In most cases the collector is uncharged and capture then relies on image forces between the charged particle and the collector. This force depends on the charge on the particle and on the dielectric constant of the collector material. Dielectric constant is defined as the ratio of the capacitance of a capacitor with the space between the plates being filled with the dielectric material, to the capacitance of the same capacitor in vacuo, and is given the symbol, ϵ. The dielectric constant is related to the behaviour of bonding electrons. When the material is subjected to an electrostatic field some electrons are able to modify their orbits so that there is a net, though slight, transfer of negative charge towards the positive pole of the field. The surfaces of the material then carry a charge. If there are polar bonds present in the structure these may align with the field and increase the charging effect (66). If a charged particle approaches a dielectric, an induced charge, of opposite sign to that of the particle, will form on the surface of the dielectric and the two bodies will be attracted towards each other. Some typical dielectric constants are given in Table 1.1.6. The unexpected dielectric of polyethylene is due mainly to the formation of extraneous carbonyl group dipoles during the

manufacturing process. The dielectric properties of terylene (polyethylene terephthalate, ICI) and nylon 6,6 (polyhexamethylene adipamide, ICI) vary with humidity and temperature (69). The dielectric of nylon is much higher when it is wet but terylene is unaffected.

Table 1.1.6 Dielectric Constants for some Common Materials (67,68)

Glass	5-10
Mica	6
Ebonite	2.8
Polyethylene	2.28 + 2.01 (p - 0.92) p = density
Terylene	3-3.2
Nylon 6,6	3.8-7
Nylon 6,6 (wet)	20.0
Water	81
Alcohol	26

When electrostatic forces are active particles may be collected even if they originate at a distance j, off-set from the streamline which is greater than the radius of the collecting fibre (r) (See Fig 1.1.6). Thus the collection efficiency may be increased to significantly greater than 1 (as defined in equation 3). Electrostatic forces are most effective when particle inertia is low. Thus they tend to enhance diffusive collection but be ineffective when inertia is high(Fig 1.1.7).

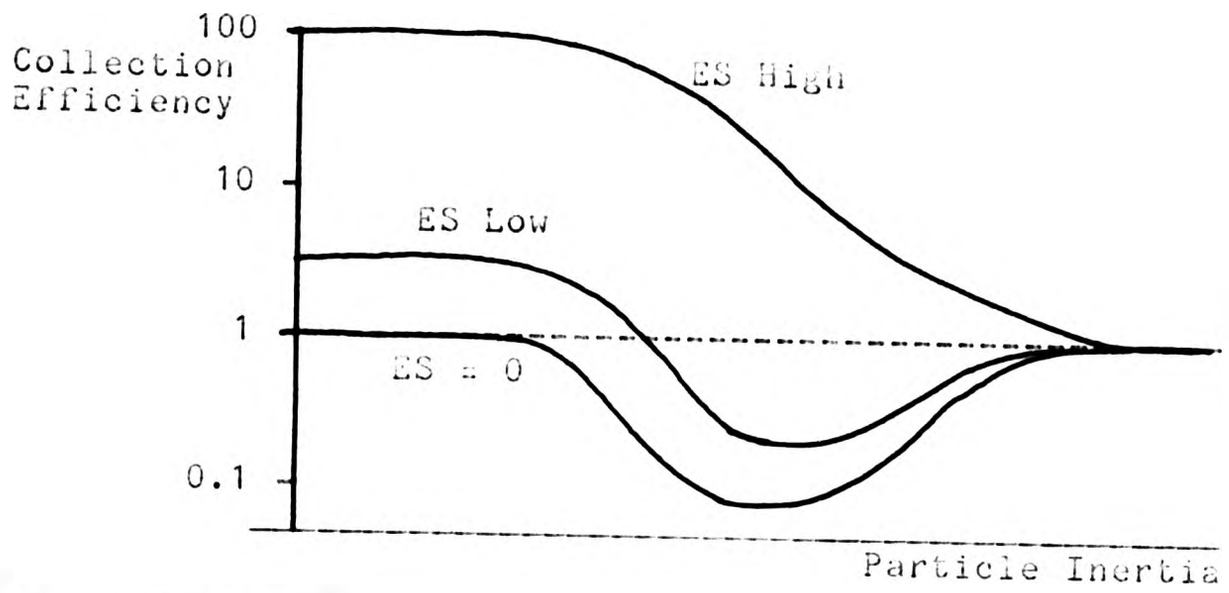


Figure 1.1.7 Idealised effects of electrostatic forces on collection efficiency as particle inertia changes (adapted from 63 and 70).

ES = Electrostatic effect

Many anthropogenic sources emit particles which carry predominantly positive or negative charges and electrostatic charge is a universal feature of equilibriated aerosols (70). As a rule 40% to 50% of particles in the sub-micron size range carry electrical charges (71). In addition, fine particles from combustion sources frequently carry strong uni-polar charges. Even elementary charges (Q = ±1) on particles may have marked effects. Only 16% of uncharged methylene blue particles were retained on a wool felt filter whereas 99% of elementary charged particles were retained (65). The effect of particle charging is

reported to be insignificant in lung deposition unless the charge is in excess of ±10 (71). This is probably because diffusional deposition of very small particles is almost 100% anyway and the charge aids the collection of some of the more marginal, larger particles.

Temperature gradients between surfaces can cause convective cells to be established. This may facilitate a transfer of air particles from the air body to the surfaces by thermophoresis. No application of this principal in this work is foreseen.

Wind tunnel studies of deposition to moss bags and grass trays have demonstrated a 'U' shaped collection efficiency vs particle size curve which is broadly similar to the lung retention curve (56,72). Thus the mechanisms of diffusion and inertial impaction are active in these systems. The collection efficiency by inertial impaction and the minimum size of particle affected by the mechanism is dependent upon wind-speed.

1.1.7 Design parameters for a passive sampler

A passive sampler for respirable trace elements in urban atmospheres should have all of the positive attributes of the moss-bag. It should be cheap, simple to operate, require no power supply, be easy to analyse chemically and it should generate data which it is easy to interpret. In addition, it should be biased to collect only those particles which can reach the deep lung and to exlude all particles outside this size range. It has been shown that in the lung and the moss-bag, particles in the respirable size range are collected predominantly by a diffusive transfer mechanism. It is plain to see that a passive sampler for respirable particles should operate on this principal too.

Diffusion across a boundary layer to a collecting medium is a mechanism which is seeing increasing use in passive sampling devices for gaseous species in air (64,73). Inorganic gases, including NH_3,CO_2, Cl_2, NO_2 and CO and organic gases and vapours including CCl_4, toluene and benzene have been monitored in this way. Usually the gas is adsorbed on to a solid such as activated charcoal, from which it can be later desorbed for analysis. The rate of mass transfer across the fixed

path is defined by Fick's 1st. law:

(4).

$$w = - DA \frac{dc}{dx}$$

w = mass transfer rate
D = diffusional coefficient
A = cross-sectional area of the diffusion path
$\frac{dc}{dx}$ = concentration gradient across diffusion path

It can be shown that the mass transferred in unit time is proportional to the concentration of the pollutant in the air (64). The technique is reported to have a precision of better than 5% which is as good as most active systems. Sources of error include variations in the geometry of the collector, variations in factors which may affect the diffusion coefficient (temperature and pressure) and saturation of the sorbent.

The diffusion of fine particles to vegetation is related to surface roughness or 'hairiness' (72). The rate of diffusion is also related to the diameter of a collecting fibre and the material from which the fibre is made. Wind speed has a marked effect on the collection of larger sized particles and may cause turbulant diffusion of smaller particles. So if the collection is to be by Browinian diffusion to a fibrous medium, then a number of design considerations must be considered:-

1. The collecting medium must have a minimal fibre diameter and a high packing density to ensure maximum collection of particles.

2. The collecting medium must be protected from direct wind in order to prevent impaction of the larger, non-respirable particles.

3. Ambient air must be able to exchange freely with air surrounding the collecting medium.

4. The dimensions and geometry of the sampling device should be standardised in order to improve precision.

1.1.8 Prototype design for the passive sampler

In order to satisfy the design considerations it was proprosed that the sampler should comprise some piece of fine material held on a frame of fixed dimensions, supported inside a cover to reduce the wind-speed. A net cone would be a suitable device for the cover because it would (i) collect larger particles by impaction while allowing smaller particles through, (ii) deflect in the wind to protect the surface of the collector, (iii) allow air to circulate freely around the collector and (iv) protect it from the rain (Fig. 1.1.8).

The design is based upon a conical cover of mesh material within which is suspended the sampling medium. The mesh that was selected was green coloured P.V.C. greenhouse shading and it was supported on a frame made from plastic coated wire. Some simple experiments with an air line and pieces of paper showed that it could effectively reduce windspeed.

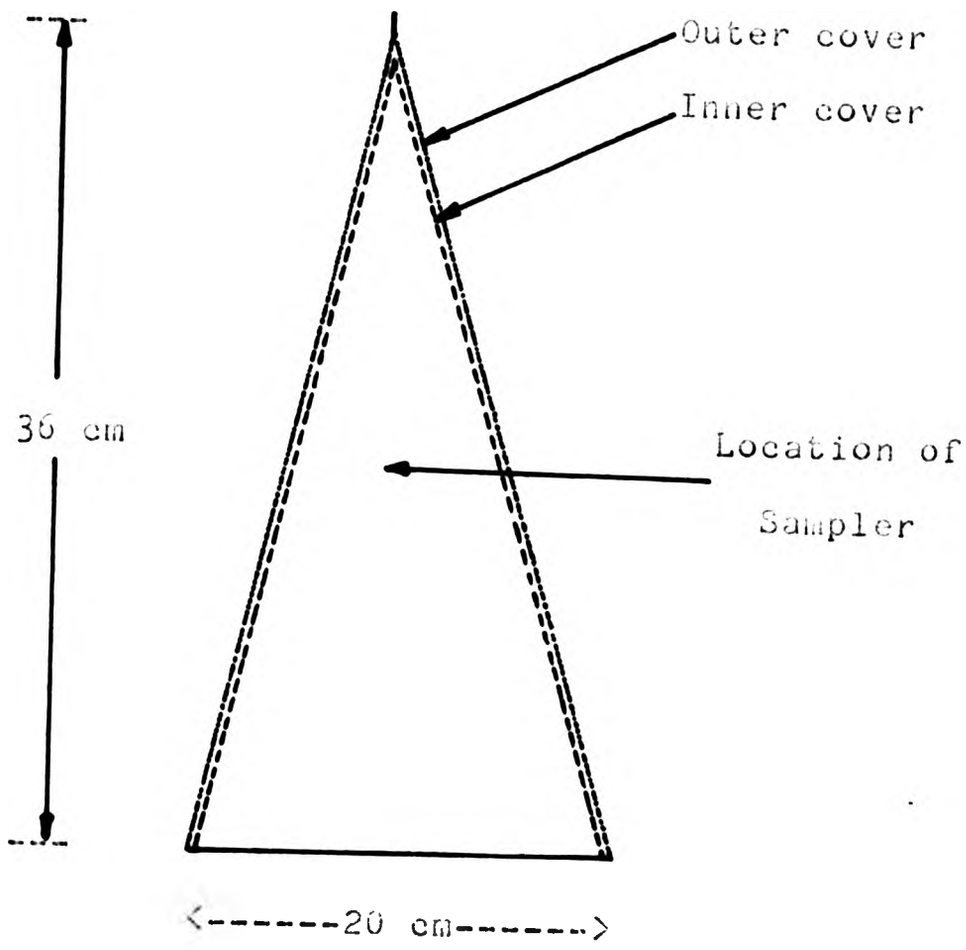


Figure 1.1.8 Design of Cover.

1.1.9 Plan of work

Although the design is well removed from the geometry and structure of the human lung, it was hoped that particle collection would occur by the same principles and that the device would collect a representative sample of respirable particles for analysis for toxic trace metals.

It was proposed that a number of these covers should be constructed, and a variety of sampling materials suspended inside them. The prototype samplers would then be exposed alongside a conventional air sampling apparatus at a suitable site. The aerosol particle size distribution at the sampling site would be assessed using a cyclone, and compared with the particle size distribution of particles collected by the passive samplers as assessed by microscopy. The accumulation of trace metals by the conventional sampling technique and the prototype samplers could also be compared.

The selection of sampling media was based upon availability, within the criteria of minimal fibre diameter and standardisation. The idea of using rubber filaments for aerosol sampling as described by Watson

(81) is particularly interesting. The rubber filaments are formed by drawing out threads of Dunlop rubber solution. The filaments can be less than 1 μm in diameter and are thus very delicate.

The final choice of media for testing was:

Glass wool

Angora wool yarn

Nylon fibre yarn

Terylene fibre yarn

Rubber solution filaments

Lens cleaning tissue

All the samples were mounted on a balsa wood frame (7 cm x 7 cm). The glass wool was held by pulling it out across the frame and glueing it in place. The yarns were wrapped round the outside of the frame a fixed number of turns. The rubber solution was drawn out across the frame and stuck on the it, and the lens tissue was glue to one face of the frame.

1.2 Characterisation of the Urban Aerosol

1:2:1 Introduction

A number of samplers are available which can provide data as the particle size distribution of the aerosol. Some devices rely on light scattering, filtration, or microscopic sizing, but these do not take into account density and other properties which are responsible for the dynamics of such particles in air and in the lung (74). Devices which apply impaction as the collection mechanism take these dynamic properties into account. As a consequence they do not catagorize the aerosol according to its true particle diameter but according to its equivalent diameter as defined as "the diameter of a sphere of unit density having the same terminal setting velocity as the sampled particle" which means that they have identical aerodynamic characteristics (75). Inertial impaction devices all have a sudden deviation to the air flow. They can be divided into two categories; those with many small jets and those with one large jet. Both types may be used to provide a sample which can be used to estimate the component of the aerosol, although they do not operate on identical principals. Typical of the small jet type is the Andersen 2000 Inc. cascade impactor (74). This device

divides the aerosol into a series of defined particle size components. It is up to the scientist to interpret the data obtained. The small jet size means that relatively precise cut-offs between stages can be obtained. In the large jet type samplers such as the Casella personal sampler, the air path is designed so that the spread of collection efficiency, caused by the ability of particles to deviate from the axis of air flow, approaches the collection efficiency profile of the human lung. The Casella device has been designed to conform to the "Johannesburg Curve" (fig. 1.2.1) (76).

The Andersen 2000 Inc. Cascade impactor consists of a series of perforated aluminium discs interposed with glass plates. Air is drawn through the device at a constant rate (1 cu.ft. min^{-1}) so that it is forced to change direction sharply at the glass plates (fig. 1.2.2) (77). Particles of a given aerodynamic size will have sufficient inertia to deviate from the streamlines and impinge on the glass plate. At each stage the perforations are smaller and the distance between the aluminium and glass plates reduced so that smaller particles impinge on the glass plate (Table 1.2.1). At the final stage a Millipore AA (0.8 μm pore size) filter collects all the particles less than 0.4 μm equivalent diameter (fig. 1.2.3).

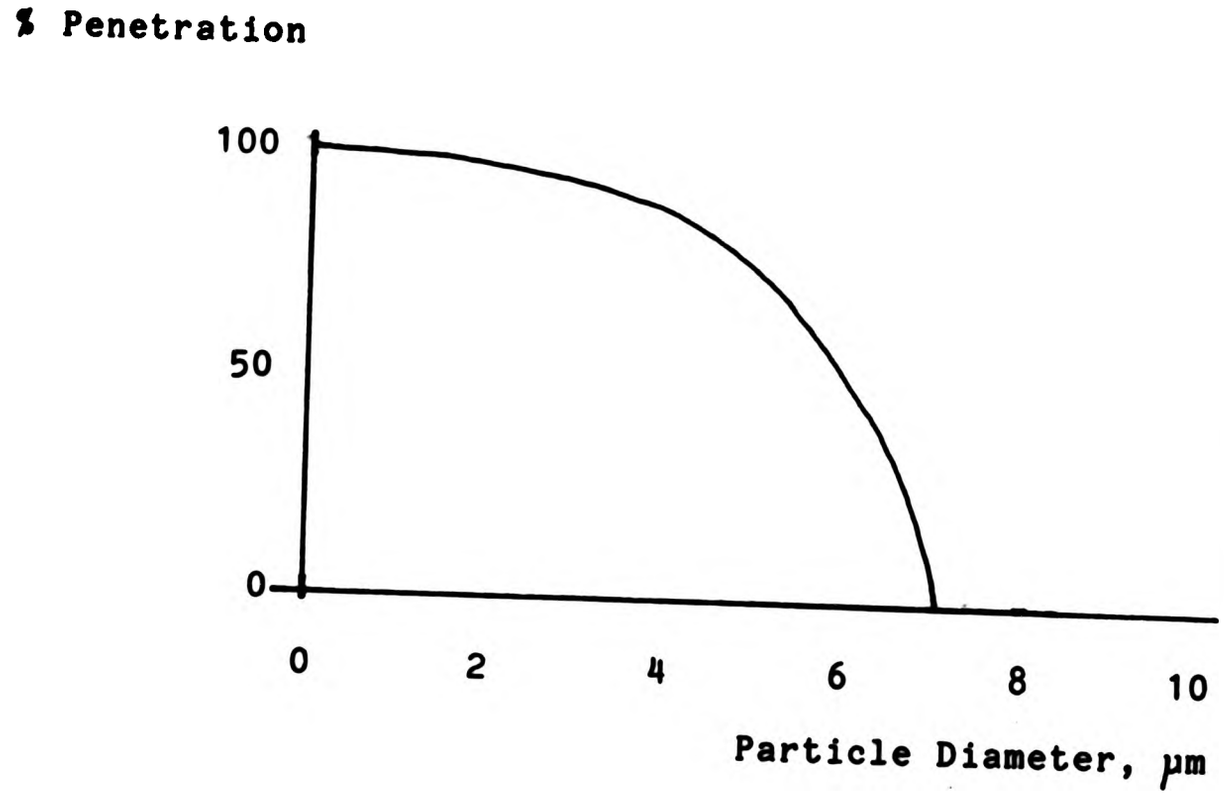


Figure 1.2.1 The "Johannesburg Conference" Curve.

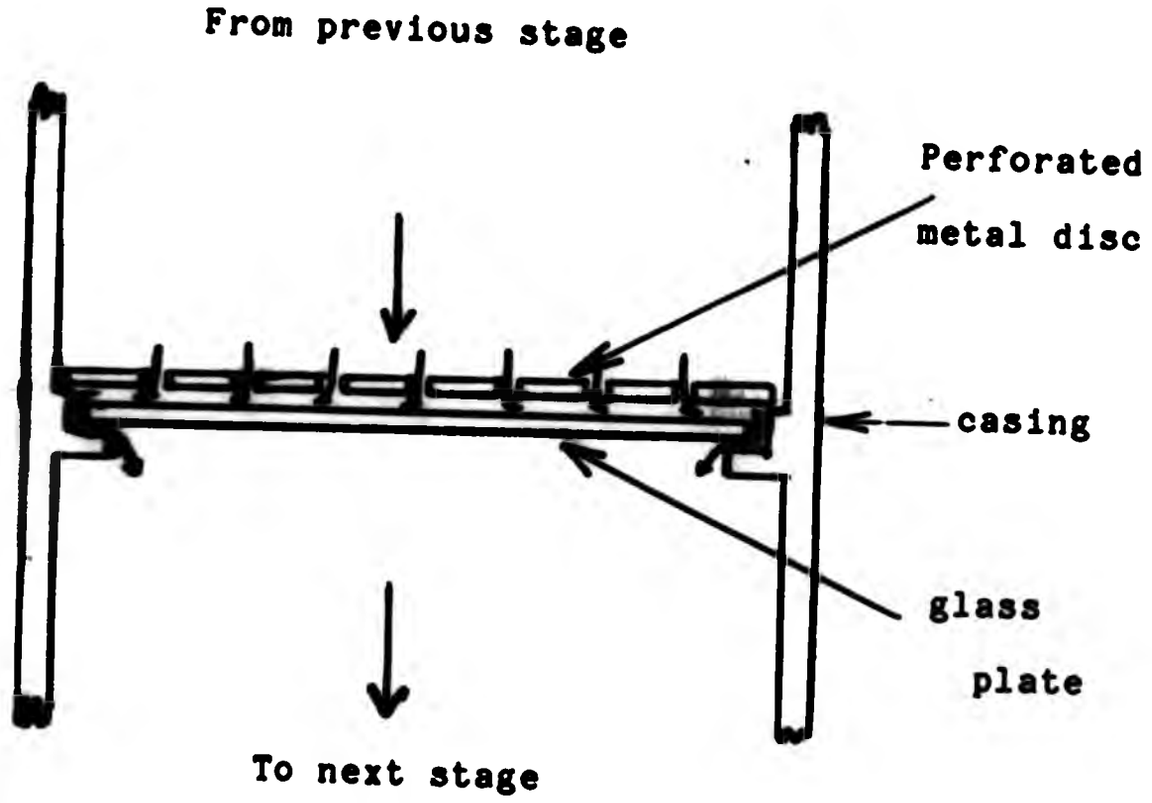


Figure1.2.2 Detail of particle collection

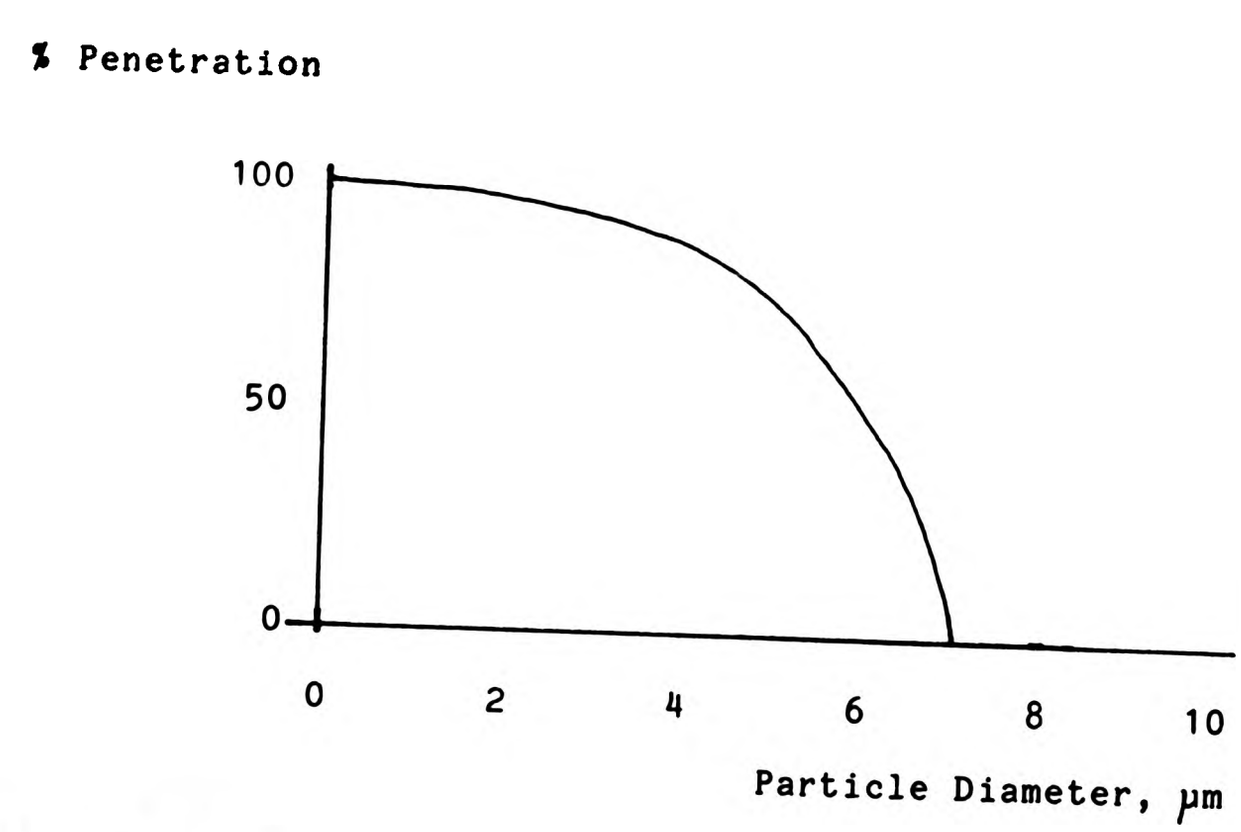


Figure 1.2.1 The "Johannesburg Conference" Curve.

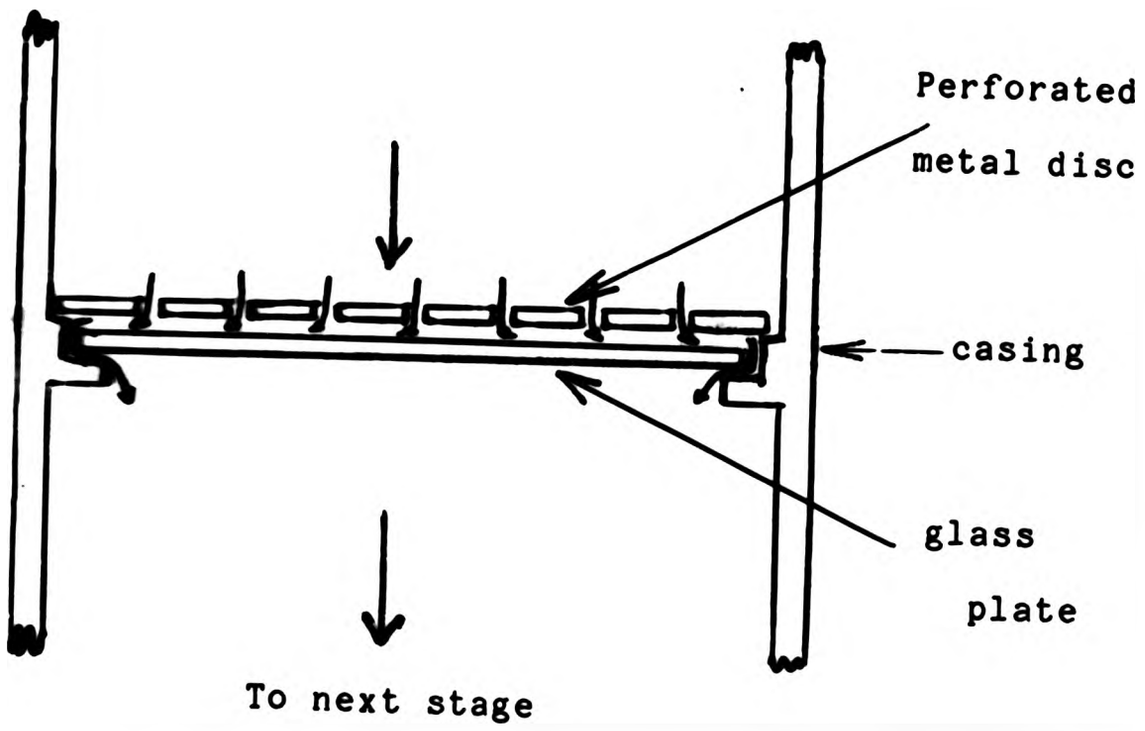


Figure1.2.2 Detail of particle collection

Table 1.2.1

Collection Parameters of Andersen Impactor

Stage	Effective Cut-off Diameter (ECD) μm
0	11
1	7
2	4.7
3	3.3
4	2.1
5	1.1
6	0.6
7	0.4
Filter	<0.4

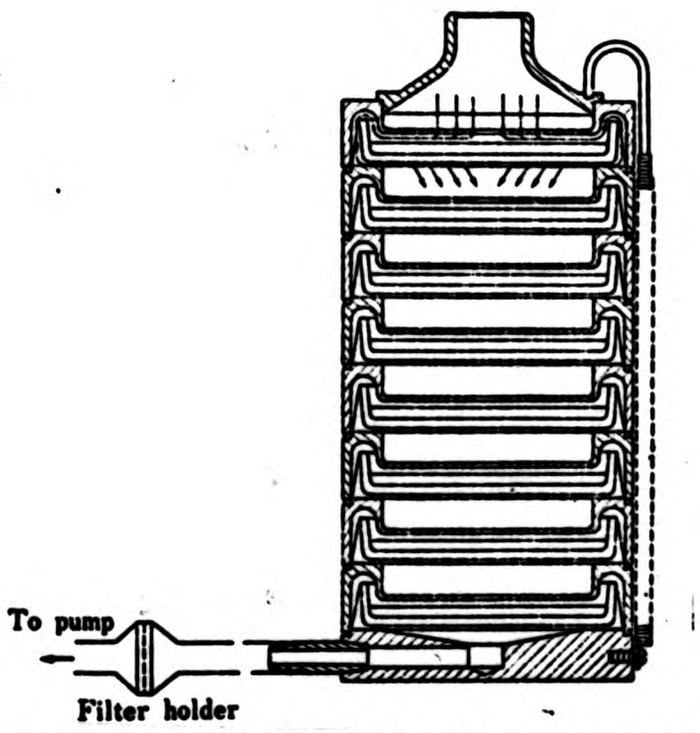


Figure 1.2.3 Cross-sectional view of an Andersen sampler (78).

The impactor should, in theory, provide unambiguous results. However, in practice, there are a number of important shortcomings (2):

Bounce-off: Particles bounce off the glass plate and proceed to a later stage.

Re-entrainent: Particles are re-entrained by the air-stream.

Wall-losses: Particles diffused and sometimes impact on to the impactor walls and other surfaces instead of the glass plates.

Cross-sensitivity: Collection efficiency does not change from 0 to 100% at a fixed particle size, so particles of a given size may be distributed on to two or more plates.

Discreetness: A limited number of stages introduces problems when interpreting the discreet mass data in terms of a continuous distribution of mass as a function of size.

Each stage will therefore have its own collection characteristics so the notion of 'effective cut-off diameter' is introduced. The ECD is defined as the diameter for which the collection efficiency for a given stage is 50% (75). Its use assumes that a given stage collects all particles greater than the ECD and none less than it.

1.2.2 Method

Details of the operating technique for the Andersen Impactor appear in setion 1.7.2.

The impactor was run for periods of 8, 24 and 48 hours during May and June 1979. The percentage results were averaged from four runs to obtain a weighted average.

1.2.3 Results

The weighted average percentage of the total, for each stage (Table 1.2.2) is normally used to obtain an estimate of the Mass Median Equivalent Diameter (MMED) of that aerosol. A cumulative size distribution curve is prepared by plotting log ECD for each stage against the per cent mass accumulated greater or equal to this diameter on a normal probability scale (fig. 1.2.4). A long-normal size distribution is assumed and a line of best fit is calculated and drawn. The MMED corresponds to the 50 percentile of the cumulative mass curve.

These data indicate a mass median equivalent diameter of 1.3 μm for particles in the air at the North London site.

Table 1.1.2 Per cent mass collected on each stage

Stage	% Mass	Cumulative %
F	27.1	27.1
7	13.6	40.7
6	9.7	50.4
5	6.8	57.2
4	5.8	63.0
3	6.3	69.3
2	4.0	73.3
1	10.3	83.6
0	15.8	99.4

1.2.4 Discussion

The use of the mass median equivalent diameter for characterisation of the ambient aerosol has been criticised for its implied assumption of a mono-modal log-normal size-mass distribution (79,80). In an industrial situation particles will probably originate from one principal source and the size-mass distribution

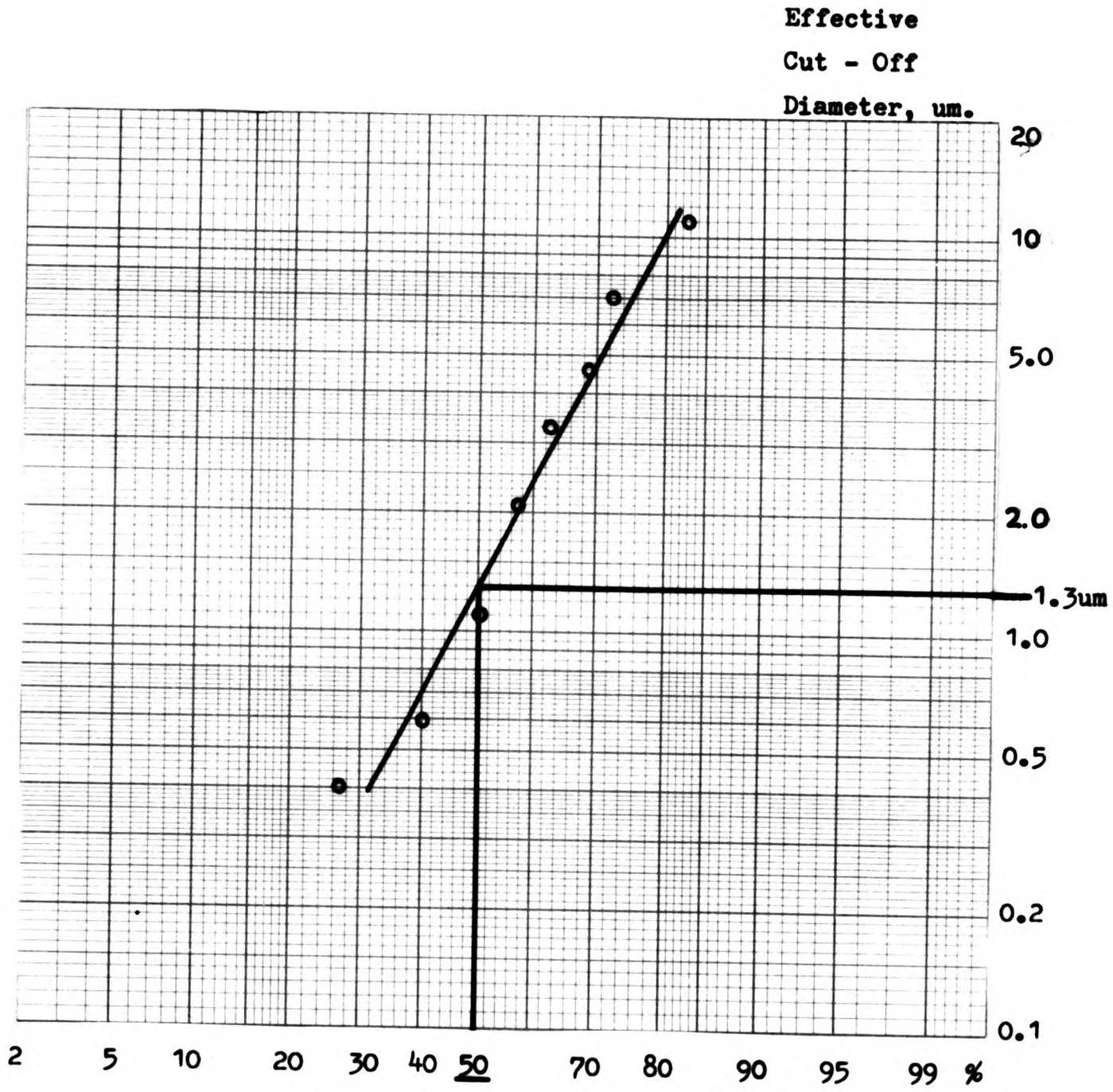


Figure 1.2.4 Total aerosol cumulative weight distribution.

may be expected to be log-normal. The urban aerosol, however, may orginate from a variety of different sources and a single log-normal distribution should not be assumed. Indeed, a number of aerosols have been found to be multi-model (13,78,80). If a control measure is introduced which is based on the assumption of log-normality it may be ineffective (79).

An assessment of the size-mass distribution can be obtained by plotting the mass collected on each stage divided by the size interval against the log of the mid point of each stage (fig. 1.2.5). The empirical data deviate from the best-fit log-normal curve most at the upper and lower limits. The elevated point at 9 μm following a minimum at 6.3 μm may indicate a small secondary distribution. Interpretation of the final point should be guarded because an arbitrary upper limit of 20 μm is assumed.

The log-normal line of best fit appears to under-estimate the mass of particles in the 0.4 - 0.5 μm size range. The cascade impactor is reported to be poor at characterising the sub-micron portion of the aerosol (82). However, if the technique is under-estimating this part of the aerosol, then it might also under-estimate the respirable hazard. It appears that a major part of the mass of the aerosol at the

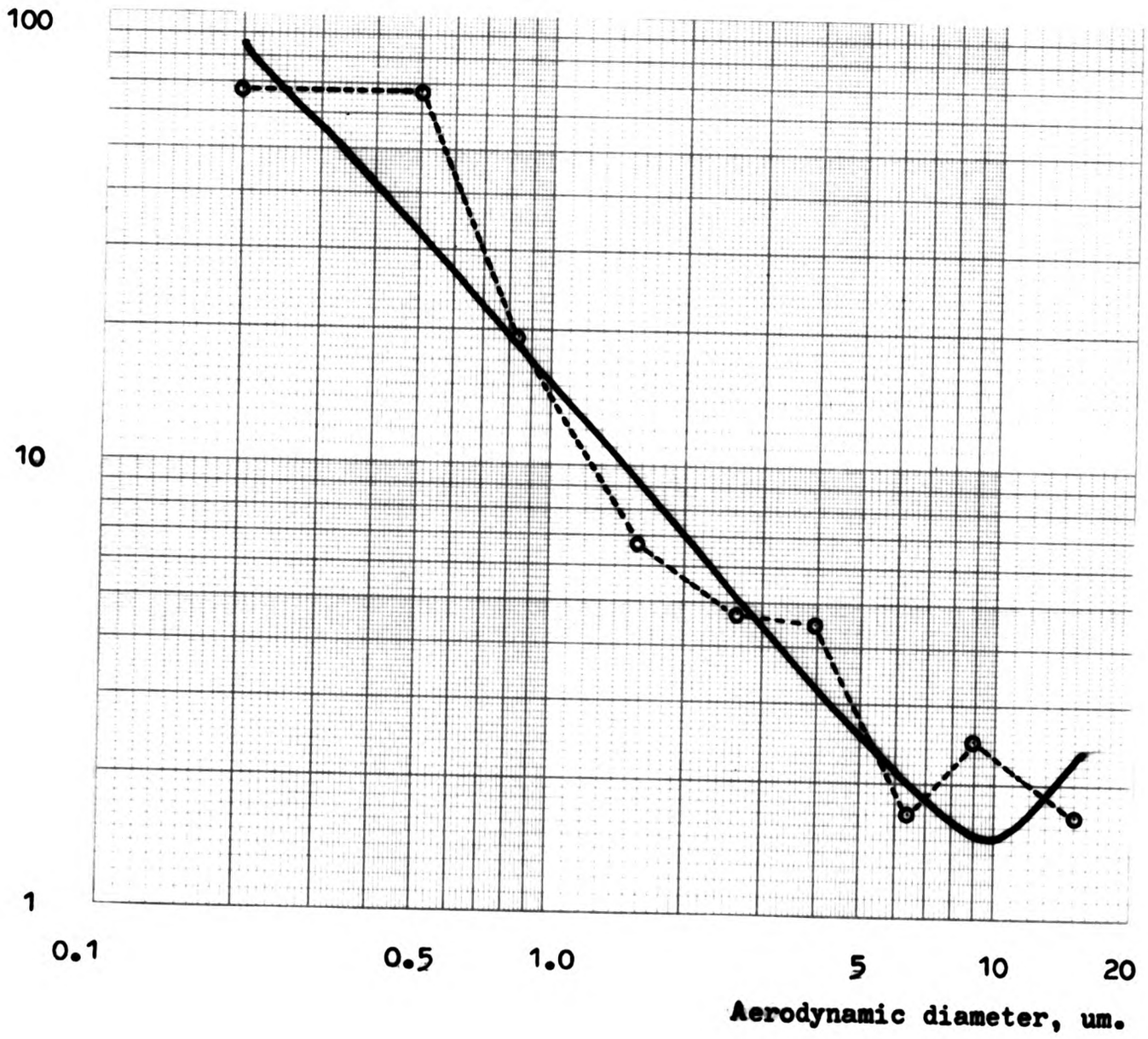


Figure 1.2.5 Particulate mass frequency distribution.

North London site falls within the respirable range. The most likely source of particles at this size (adjacent to the A1 road) is motor vehicles. The small secondary distribution may reflect re-suspended particles. The relatively high mass found in the 0.4 - 0.6 μm size interval indicates that the passive sampler should be designed to be efficient over this size interval if it is to provide a realistic indication of inhalation risk.

1.3 Estimation of Particle Size Collection Characteristics by Optical and Electron Microscopy

1:3:1 Introduction

Small samples of exposed and unexposed sampler media from the north London site were examined by optical and scanning electron microscopy. The nature of the collecting fibres and of the collected particles was studied. During the course of the experiment the design of the sampler cover was modified. The early cover was a cone of plastic mesh (ca. 0.4 cm. mesh). On later covers an additional covering of fine net (c.a. 0.05 cm. mesh) was employed to prevent large particle impaction.

1.3.2 Optical Microscopy

Table 1.3.1 Relative Fibre Dimensions

Material	Fibre Dimensions
Terylene Yarn	19 - 22 µm round
Cotton Wool	15 x 6 µm eliptical
Lens Tissue	7 x 9 µm eliptical
Glass Wool	7 x 12 µm round
Angora Wool Yarn	15-35 µm round, "ropey"
Brushed Synthetic Yarn	25-30 µm round," curly"
"Evo-stick" filaments	5-100 µm round, "knobbly"

Table 1.3.2 Particle size ranges visible at various magnifications (mean diameter or range) µm.

Material	Magnification			
	x50	x100	x400	x1000
Angora Wool	5-10	11-22	12	1.2
Nylon Fibre	5-10	11	6	2.5
Glass Wool	5-10	6-17	3	1.6
Terylene Fibre	-	-	5-17	2.5
Glue Filaments	5-20	11	5	0.5
Lens Tissue	10-100	10-20	3	2.1

The membrane filter from the final stage of the Anderson Cascade Impactor appeared as a mottled grey and black mass at X100 and X400 magnifications. At X1000

particles can be seen clustered around the mouths of the pores. Many of the particles appeared as agglomerated masses and these masses had diameters in the size range 0.5 to 1.5 μm. The fundamental particle was difficult to see but appeared to have a diameter of about 0.2 μm.

Materials exposed in the early design of cover collected particles in two fairly distinct size ranges. The larger class had range of about 5 - 100 μm and the smaller class less than about 3 μm. The larger particles were not counted at higher magnification because of the limited field of view. The smaller class were not seen at lower magnifications. As magnification increased, more small particles could be seen and the minimum diameter that could be resolved fell. Therefore the minimum diameter cannot be defined unless magnification can be increased.

It was found to be impossible to give an accurate estimate of the particle size distribution using cumulative frequency techniques. This is because the number of particles visible is very small; the visible size distribution is dependent upon the magnification used, and the optical microscope becomes unreliable for particles less than 0.5 μm (84).

1.3.3 Electron Microscopy

Small samples of exposed materials were mounted and coated by Ms Kiortyahan of the Polytechnic of North London Electron Microscope Unit. They were examined using a Philips Scanning Electron Microscope. The most interesting frames were recorded as photomicrograms and a selection of these is reproduced in plates I to V.

Unexposed fibres showed no discerible particles at all. Exposed samples showed two classes of particle. Agglomerated particles appeared to be made up of smaller units, and had diameters of the order of 1-2 μm. The primary particles which comprised the larger agglomerated particles and appeared independently, had diameters less than 0.5 μm. These primary particles appeared to have a minimum diameter at about 0.14 μm although this may reflect the lower limit of resolution of the photomicrograms. The lower limit of resolution will depend on the instrument and conditions employed.

1.3.7 Discussion of Microscopy Results

The information obtained in this study is of an essentially qualitative nature. No sucess was achieved in estimating the particle size distribution by microscopy, but some indication of the character of the aerosol sampled was obtained. Samplers with the early

hoods appeared to collect particles from two size distributions with approximate arithmetic mean diameters of 15 μm and 3 μm with a minimum at about 6 μm. In the later hoods (with an additional fine mesh outer cover) the larger particles seemed to be totally excluded and the smaller particles were less abundant above 2 μm. Electron microscopy showed that these particles extended down to, and probably below 0.14 μm.

These observations would indicate an aerosol with at least two modes; one centred at around 15 μm and a second at about 1 μm with a minimum at about 6 μm. Little and Wiffen (26,85) studied the aerosol size distribution at a site adjacent to the M4 Motorway. They identified three classes of particles (Table 1.3.3).

The particles seen in this study would appear to correspond to Little and Wiffen's categories 2 and 3, with some larger agglomerated particles outside Little and Wiffen's range. These probably reflect the urban setting of the passive samplers, where larger re-suspended particles are more like to occur. Little and Wiffen were able to observe primary particles as small as 0.1 μm. In this study 0.14 μm was about the smallest size that could be discerned.

Table 1.3.3 Particle size distribution of Motorway aerosol (85)

Category	Size (μm)	Appearance	Supposed Origin
1	0.1	Opaque, discrete rounded	Primary vehicle exhaust particles
2	0.1-1.0	Diaphanous chain aggregates	Mostly diesel smoke
3	0.5-5.0	Dense, flaky or amorphous	Carbonaceous particles from exhaust, re-suspended road dust

The early design of cover allowed the impaction of particles up to about 100 um in diameter. This was clearly undesirable so the cover was modified with an outer layer of finer mesh in order to exclude these larger particles. Examination of the later design showed that the larger size range had been effectively excluded.

It is not possible to assess the precise collection charateristics of a particle sampling device from studies such as these. For an accurate evaluation an aerosol of known dimensions would need to be generated and the sampler exposed under controlled conditions,

such as in a wind tunnel. However, we can see that with the modified cover the device excludes larger particles in the non-respirable range while still collecting some smaller respirable particles. A knowledge of the dynamics of particle collection indicates that diffusion is the most likely mechanism of collection if wind-speed has been adequately controlled, and allows the tentative prediction that the sampler has collection characteristics which approximate to those of the human respiratory system.

1:4 Analytical Techniques for the Determination of Trace Metals in Airborne Particulate Samples

1:4:1 Introduction

Preparation and analysis proceeds through three stages: Ashing of organic material to reduce the bulk of the sample and free trace metals, digestion under acidic and oxidizing conditions to solublise metals, and the determination of the concentration of metals in the solution by electrochemical, spectroscopic or other means.

The technique recommended for extracting heavy metals from glass-fibre filter materials is low temperature ashing followed by Soxhlet extraction (165). In low

temperature ashing an oxygen plasma is formed by passing oxygen through a high frequency electro-magnetic field. Oxygen radicals so formed react with the sample at a temperature of 50^{o}-250^{o}C. This method prevents the significant loss of metals Pb, Zn, Cd, Hg, Th, Se and As which may occur when glass fibre filters are ashed at 550^{o}C in a muffle furance (165). It has been suggested that the losses of metals during muffle furnace ashing are due to the formation of insoluble silicates rather than volatilisation (166). The losses were insignificant for Pb, Zn and Cd added to paper filters. Ashing may be unnecessary for glass fibre filters (165). Collected metals can be extracted into concentrated HCL on a low heat (168).

For organic matrices, including cellulose acetate membrane filters, ashing at 500^{o}-550^{o}C should not result in a loss of metals so long as the temperature is raised gradually from cold (169). An acid mixture ($HNO_3/HClO_4$) has also been used successfully for the determination of metals in membrane filters (39).

Burnham (170) found that while atomic absorption spectrophotometry (AAS) was relatively free of chemical and spectral interferences, centrifuging of solutions was necessary in order to reduce matrix effects from glass-fibre filters. Beyer (168), however, determined

metal concentrations in glass-fibre filter extracts by AAS and found no significant interferences for Cd, Cr, Co, Cu, Pb, Mn, Ni or Zn. The important difference is that Burnham dry ashed while Beyer used wet ashing. The interferences encountered by Burham may have been caused by silica from the filter in suspension (165).

It was considered desirable that an analytical technique should be employed which could be applied to all the materials in order to provide a reliable basis for the comparison of results. Wet ashing followed by AAS was thought to be the most promising technique. There are some doubts about interferences from glass-fibre filters but these can be avoided by the simple expedient of using only cellulose-acetate membrane filters.

Some unexposed membrane filters and some unexposed samples of materials suitable as passive sampler collection media were analysed in order to determine blank values and detection limits.

1:4:2 Method

Samples were digested in a nitric/perchloric acid mixture and analysed by atomic absorbtion spectrophotometry. Full details of the technique appear in section 1.7.1.

1:4:3 Results

Detection limits were determined on six reagent blank samples. The detection limit was calculated as twice the standard deviation of the six blank readings (Table 1.4.1).

Six unexposed membrane filters and six samples each of silk fabric, angora wool, glass wool and lens cleaning tissue were analysed by the method to obtain blank values (Table 1.4.2).

Table 1.4.1 Instrumental Detection Limits ($\mu g\ ml^{-1}$)

Element	Pb	Cu	Cd	Zn
Detection Limit,	0.04	0.30	0.02	0.10

Table 1.4.2 Blank Concentrations ($\mu g\ g^{-1}$)

	Pb	Cu	Cd	Zn
Membrane filter	N/D	N/D	N/D	N/D
Silk	1.5	N/D	N/D	11.1
Angora Wool	N/D	N/D	N/D	39.5
Glass Wool	10.3	11.6	44.8	52.7
Lens Tissue	N/D	N/D	N/D	N/D

N/D = Below detection Limit

1:4:3 Discussion

The technique has been found to be practicable in all cases except glass wool. Glass wool resists acid attack and has to be removed and rinsed before analysis. Glass wool was also found to have very high blank concentrations and so was excluded as a potential collecting medium. The membrane filters were found to have low blank concentrations as were lens cleaning tissues. Angora wool and silk had small blank values but it was considered that these levels would be insignificant if the materials were effective collectors of airborne particles.

These preliminary studies gave reagent blank signals which were indistinguishable from deionised water signals. However, during later work high reagent blanks were occasionally encountered. To investigate the possible source of contamination a small sample of laboratory dust was collected and analysed. It was found to contain 451 $\mu g\ g^{-1}$ Cu, 1944, $\mu g\ g^{-1}$ Pb and 70 $\mu g\ g^{-1}$ Cd. Even a small amount of laboratory dust finding its way into a sample would result in a considerable enhancement of the signal. Unfortunately, the probability that a sample will become contaminated is far greater than the probability that a reagent blank will be contaminated, because of the extra handling

involved. Steps were taken to reduce the risk of contamination and the incidence of high blanks was reduced

1:5 Preliminary Survey

1:5:1 Introduction

A location was selected within the Polytechnic of North London site where the passive samplers could be exposed undisturbed and where there was a power supply. The chosen site was on a low roof and was surrounded by higher buildings. This was probably not ideal but the number of available sites was limited. The location was about 100 m to the north-east (and thus down-wind) of the busy Holloway Road. Holloway Road is part of the A1 linking the Midlands and North of the UK with the City of London.

1.5.2 Results

Membrane filters have been reported to be prone to clogging. For the first one-month exposure period the gas meter was read frequently to observe the effect of particle collection on flow rate (Fig. 1.5.1).

The flow was linear at 109.7 cu.ft. per day (= 0.70 $lmin^{-1}$). The flow rate was found to be consistent at about 110 cu.ft. per day throughout the trials.

The weight of the metals Pb, Cd, Cu and Zn collected on the membrane filters was divided by the volume of air sampled to obtain monthly estimates of the average concentration of each metal in the air (Table 1.5.1)

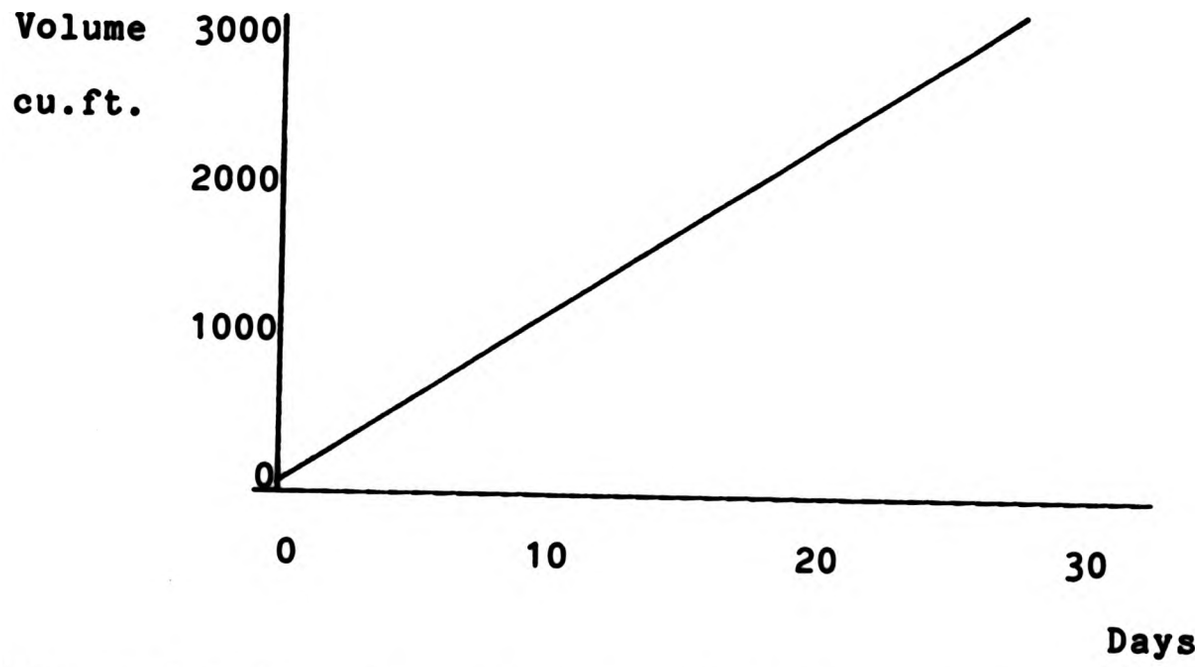


Figure 1.5.1 Low volume sampling flow rate.

The weight of the metals Pb, Cd and Cu collected on the sampling media was divided by the number of days exposure to obtain a value for the mean monthly deposition (Table 1.5.2).

Table 1.5.1 Mean monthly concentrations of metals in air

Month	Pb	Cd	Cu	Zn
	$\mu g.m^{-3}$	$ng.m^{-3}$	$ng.m^{-3}$	$\mu g.m^{-3}$
Jan.	1.24	8.8	58.2	0.26
Feb.	1.26	2.4	62.8	0.24
Mar.	0.56	2.6	39.4	0.16
Apl.	0.64	1.6	32.8	0.28
May.	0.74	7.8	65.4	0.54
Jun.	0.44	4.4	59.2	0.58
Jul.	0.22	4.4.	13.8	0.20
Aug.	1.68	7.8	33.6	0.10
Sept.	0.46	2.0	27.4	0.12
Oct.	0.22	1.8	16.2	0.06
Nov.	0.42	8.8	19.8	0.08

1.5.3 Discussion

The results of the trial run are presented in graphical form in figures 1.5.2, 1.5.3, and 1.5.4. It can be seen that there are no obvious relationships between the measured concentrations of the metals in air and the amounts accumulated by any of the sampling media. It was considered that lead was the most suitable metal for investigating correlations between the different sampling methods because the metal is present at relatively high concentrations and is believed to be associated with small particles.

Table 1.5.2 Mean monthly deposition of trace metals to various sampling media

/over.

Month	Angora Wool			Silk			Lens Tissue			Cotton Gauze		
	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu
Jan.	0.11	N.D	-	X	X	X	X	X	X			
Feb.	0.20	N.D	-	0.18	0.18	.0 6	0.19	1.60	0.01			
Mar.	0.09	N.D	-	0.19	3.80	.03	0.19	1.42	0.02			
Apl.	0.12	N.D	-	X	X	X	0.16	1.83	0.01	0.08	1.00	.02
May.	0.10	N.D	-	0.19	1.79	.20	0.20	1.25	0.02	X	X	X
Jun.	X		-	0.09	0.70	.16	0.12	0.33	0.01	X	X	X
Jul.	0.10	N.D	-	0.04	0.22	.14	0.11	0.33	0.07	0.05	N.D	N.D
Aug.	0.16	N.D	-	0.15	N.D	.05	0.09	0.48	0.02	0.17	1.28	0.01
Sept.	0.12	N.D	-	0.25	0.97	.10	0.12	1.22	0.02	0.09	N.D.	0.04
Oct.	X	N.D	-	0.55	6.00	.17	0.10	1.10	0.02	0.72	0.10	0.05
Nov.	0.17	N.D	-	0.21	N.D	.02	X	X	X	0.16	1.42	0.02

Pb : $\mu g.g^{-1}$ Cd and Cu : $ng.g^{-1}$

Pearson's 'r' Test (Appendix A) was used to calculate correlation coefficients between the various sampling methods (Table 1.5.3). For this size sample the 'r' value would have to be greater than 0.7 in order to be statistically significant at the $p < 0.025$ level. All of the data was plotted graphically using the Trivector Microcomputer System 'Regression Graphics' programme (Appendix B).

Table 1.5.3 Correlation coefficients for amount of lead collected by each sampling method

	Cotton Gauze	lens Tissue	Silk	Angora
Air concentration	.22	.10	.22	.43
Angora Wool	.98	.06	.01	-
Silk fabric	.93	.03	-	-
Lens cleaning tissue	.41	-	-	-

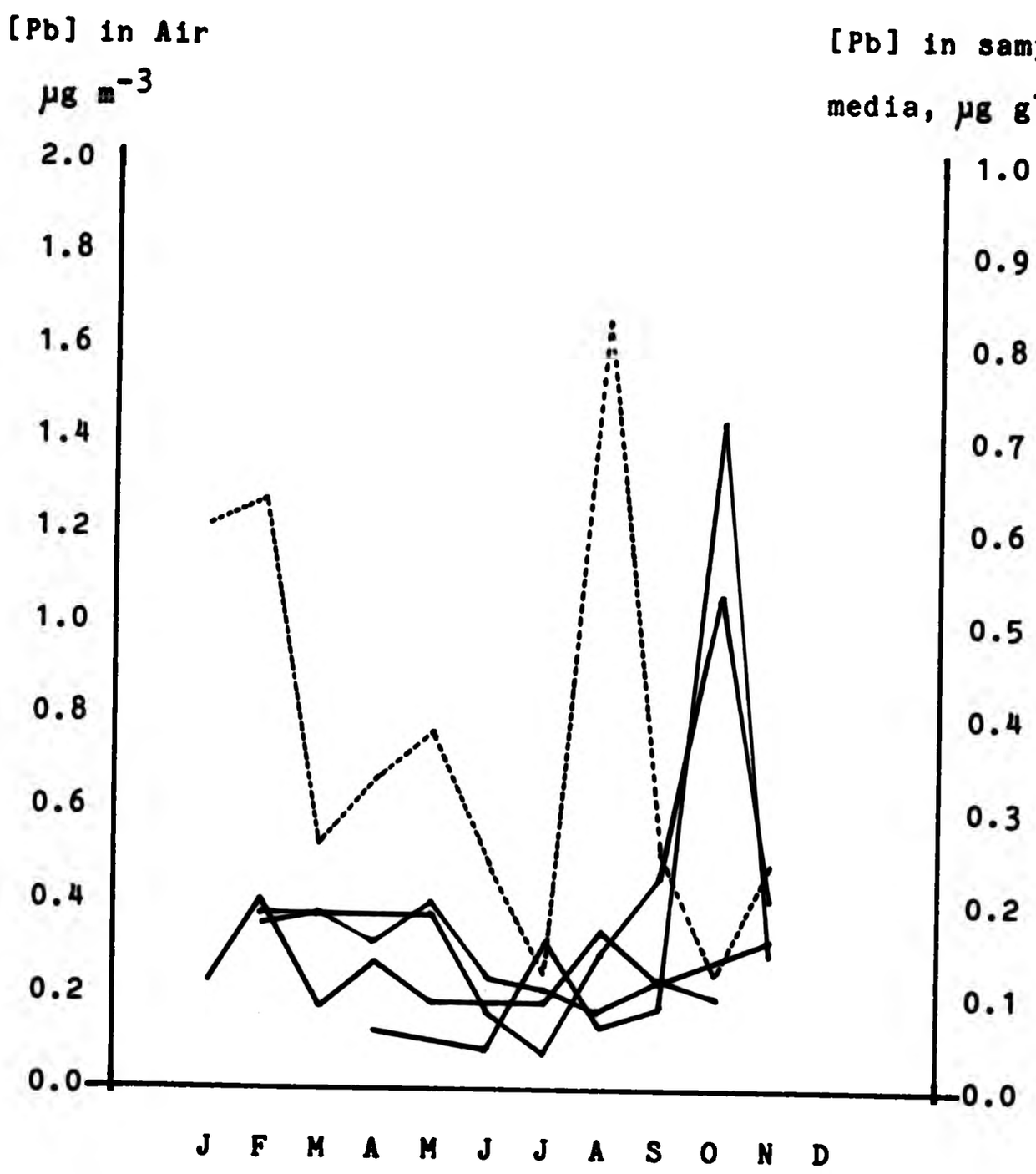


Figure 1.5.2 Monthly distribution of lead collected by sampling methods.

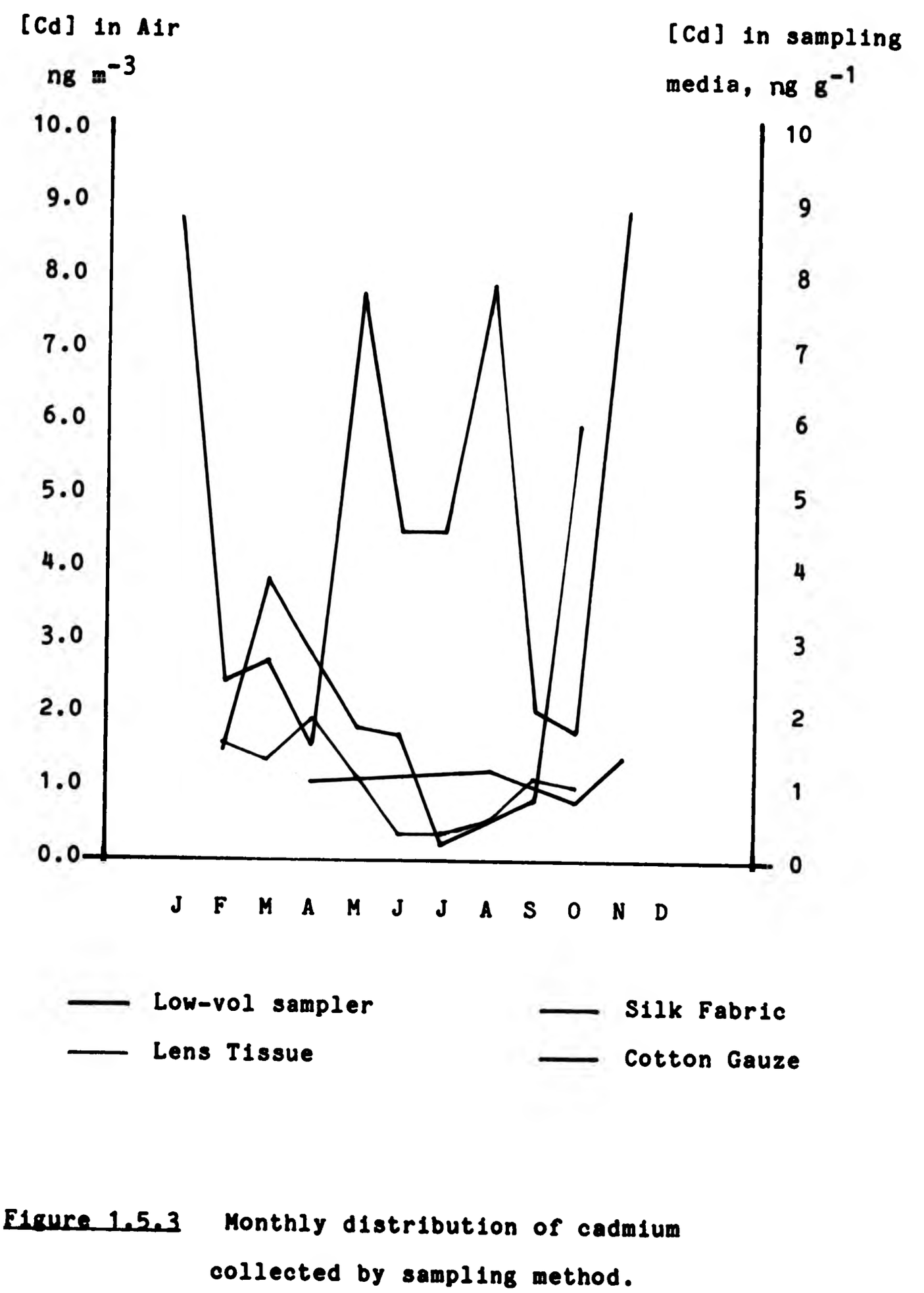


Figure 1.5.3 Monthly distribution of cadmium collected by sampling method.

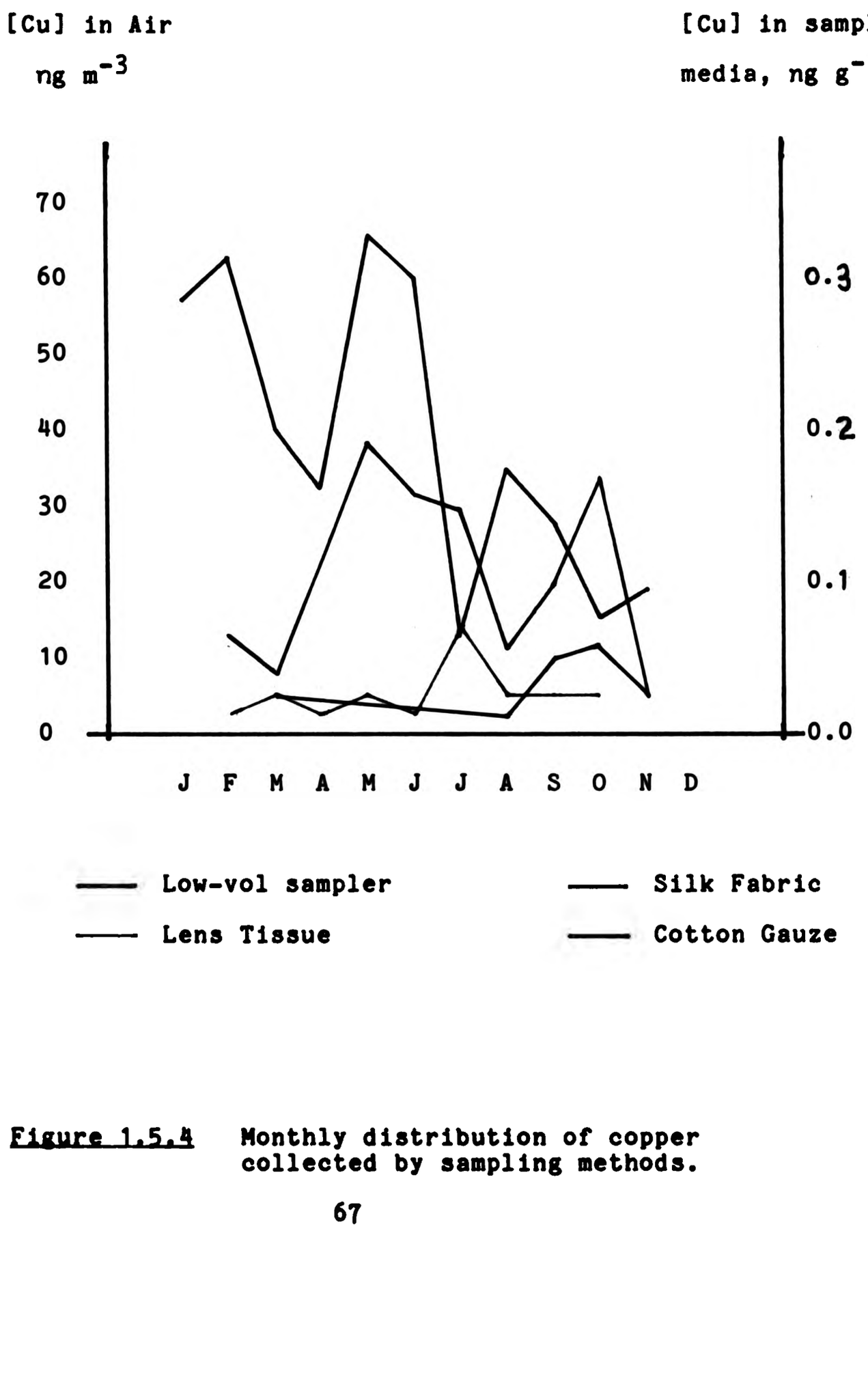


Figure 1.5.4 Monthly distribution of copper collected by sampling methods.

It was noticed that on several of the scatter diagrams one point in particular was isolated from the other data points. The air concentration value for August and the silk passive sampler value for October were found to be causing this effect. Strictly speaking a data point should never be excluded as an outliner unless there is some specific reason for editing that particular point. This practice is never justified purely on the grounds that it improves the statistical result! In this case each point is the highest value found using the two sampling methods and contamination of the sample is suspected. However, no statistical inferences will be drawn from the re-calculated correlation coefficients (Table 1.5.4). These recalculated values exclude the air concentration for August, and the silk and cotton gauze data points for October. An examination of the scatter diagrams for the statistically significant correlations between cotton gauze and angora and silk samples cast some doubts on the correlations (Figs. 1.5.5, 1.5.6). The high 'r' value generated by the gauze and silk data is caused by the correlation calculations giving undue weight to the coincidence of suspect outliers in the data. The calculation of the correlation coeffiecient assumes that the data is normally distributed. In this example it is clearly not the case.

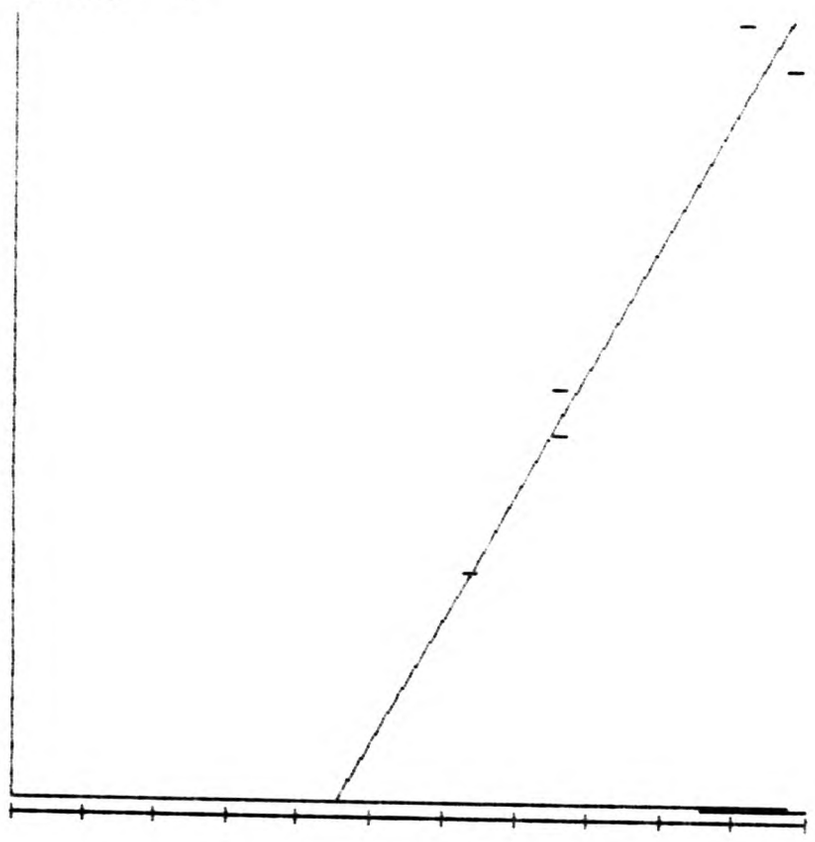
LINEAR REGRESSION

SAMPLE : ANGORA / GAUZE n = 5

Y = -.12 + 1.73 X

COEF. OF CORRELATION = .980

Full scale = .17



Full Scale= .17

Figure 1.5.5 Regression equation between Angora Wool and Cotton Gauze passive samplers.

LINEAR REGRESSION

SAMPLE : SILK / GAUZE n = 5

Y = .08 + 1.34 X

COEF. OF CORRELATION = .929

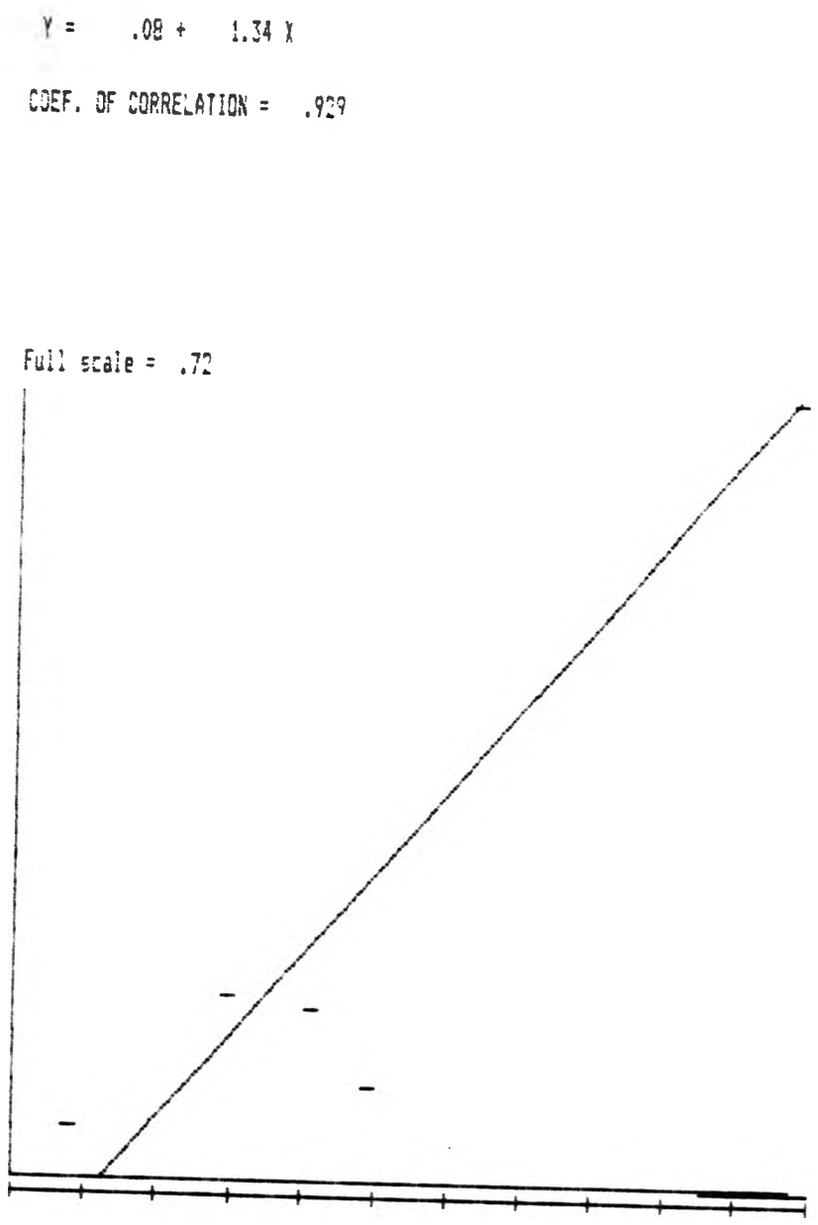


Figure 1.5.6 Scatter diagram of Cotton Gauze/Silk Fabric regression.

When these are removed the 'r' value adopts a more modest proportion (Table 1.5.4). The correlation between cotton gauze and silk is more interesting. The data conforms closely to the regression line but intercepts about half-way along the 'angora' axis indicating a high endogenous concentration. The correlation is significiant at the $p < 0.001$ confidence level but since there are only five data points may be considered to be suspect.

Table 1.5.4 Re-calculated correlation coefficients for lead collected by different sampling methods

	Cotton Gauze	Len Tissue	Silk	Angora Wool
Air concentration	.23	.78	.38	.38
Angora Wool	*	*	.01	
Silk fabric	.46	.57		
Lens Cleaning Tissue	.53			

* Unaffected

The re-calculated correlation between lead in air and the mass of lead accumulated by the lens tissue sampler although less dramatic, is more encouraging. The

correlation is significant ($p < 0.025$) but the intercept lies well up the y (lens tissue) - axis (Fig 1.5.7). This correlation can be improved further and the y-intercept reduced if the February data point is removed. This 'data adjustment' also improves the air concentration/silk fabric correlation to 0.81 and this may justify the action to some extent.

The poor correlations found between mean monthly lead concentrations in air, as measured by a conventional filtration method, and the amounts accumulated by any of the sampling media under test may be attributable to five possible causes:

1. Differences between the particle size bias of different sampling techniques may result in differences in metal accumulation.

2. The design of the passive samples may cause their collection efficiencies to be subject to external variables such as wind speed, relative humidity, wind direction or rainfall.

3. The response of the passive sampler may be time-dependent so that it is more 'active' at the beginning of the sampling period.

LINEAR REGRESSION

SAMPLE : AIR / TIZZUE ♦ n = 8

Y = .09 + .10 X

COEF. OF CORRELATION = .784

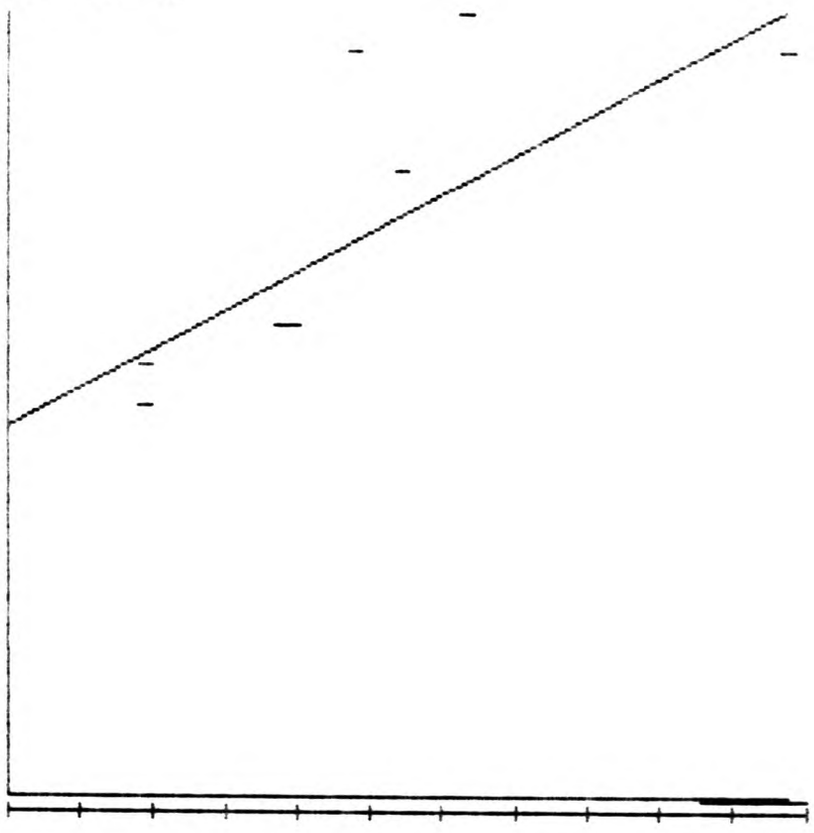


Figure 1.5.7 Correlation and regression between lead in air and that accumulated by Lens Tissue passive samplers.

4. Many of the analyses of the passive sampling media were carried out near the detection limit of the flame atomic absorption technique. The level of analytical error is much higher at low concentrations.

5. High or inconsistent blank values in the sampling media and contamination during sample exposure, retrieval or analysis may introduce an extra element of variability.

Replicate analyses of unexposed materials have shown the analysis to be reproducible and blank values to be low. It is possible that the North London site, which is close to a main road but behind buildings, has an unusual or highly variable lead particle size distribution. The buildings may trap the larger secondary particles which may be re-suspended by the strong wind eddies around the tower block. This might cause large variations in the concentration of aggregated secondary lead particles which the membrane filter would collect. The passive samplers, which have been designed to collect only respirable particles would not accumulate lead under these circumstances.

High values which occur infrequently and stand out against the bulk of the data are indicative of contamination. It is not reliable to exclude such data from the analysis of results although this may be done in order to investigate suspected trends. It is clearly important to ensure that this contamination does not occur in the future. Since the design of the cover must also be critical to the sampling behaviour of the device some attention must also be paid to standardising and improving this in the future.

This trial has not been as successful as the author would have wished. Any trends that exist are clouded by undefinable variations from other sources. The conclusions that can be drawn are:-

1. Exposed concentrations are significantly greater than unexposed, so it can be assumed that the passive sampler collects metals from the atmosphere .

2. When the pumped membrane filter collects more lead, the lens tissue and silk fabric passive sampling media also tend to accumulate more lead. So it can be assumed that the rate of accumulation is related to airborne concentrations.

Of course, perfect correlation and linear regression between lead in air, as measured by a pump filter, and the lead accumulated by the passive sampler is not to be expected, or even wished for. The membrane filter collects 'Total Suspended Particulates' whereas the passive sampler is intended to collect respirable particles only. The maximum degree of correlation that could be achieved is only to be guessed at.

The lens cleaning tissue is the most promising choice for further work. This is because it is manufactured 'clean' and thus has a low blank value, it is readily available in a standard form (Whatman 551 Lens Cleaning Tissues) and has shown some promise in this preliminary trial.

1.6 Temporal Response of Passive Samplers

1.6.1 Introduction

The relatively poor performance of the passive samples when compared to conventional air sampling techniques indicated that the accumulation of metals by the samplers should be studied in greater detail.

Unfortunately it was not possible to study the performance of the sampling devices in the controlled conditions of a wind tunnel with monodisperse aerosols. Instead it was decided to compare the accumulation of lead over different exposure times in the ambient aerosol. It was necessary to devise a scheme of exposures so that although the samplers were exposed for different time periods, the total exposure would be the same so that a direct comparison could be made. This experiment was performed twice using a different cover design on each occasion. The first cover used (Cover B) was a modification of the original conical cover with an extra layer of finer mesh. The second cover (type C) was a new design based on a wire frame with a fine nylon mesh cover. This design allowed a larger ample area, equal to one complete lens cleaning tissue (10 cm x 15 cm), and a more complete sheltering of the sampling medium from direct wind (Fig 1.6.1).

1.6.2 Method

Passive samplers were exposed according to a scheme which ensured that the total exposure time and conditions would be identical irrespective of the exposure period selected (Fig 1.6.2).

Lens cleaning tissue was the only sampling medium used in these trials since it had been shown to be the most promising.

Samplers were digested and analysed by the methods described in the techniques section.

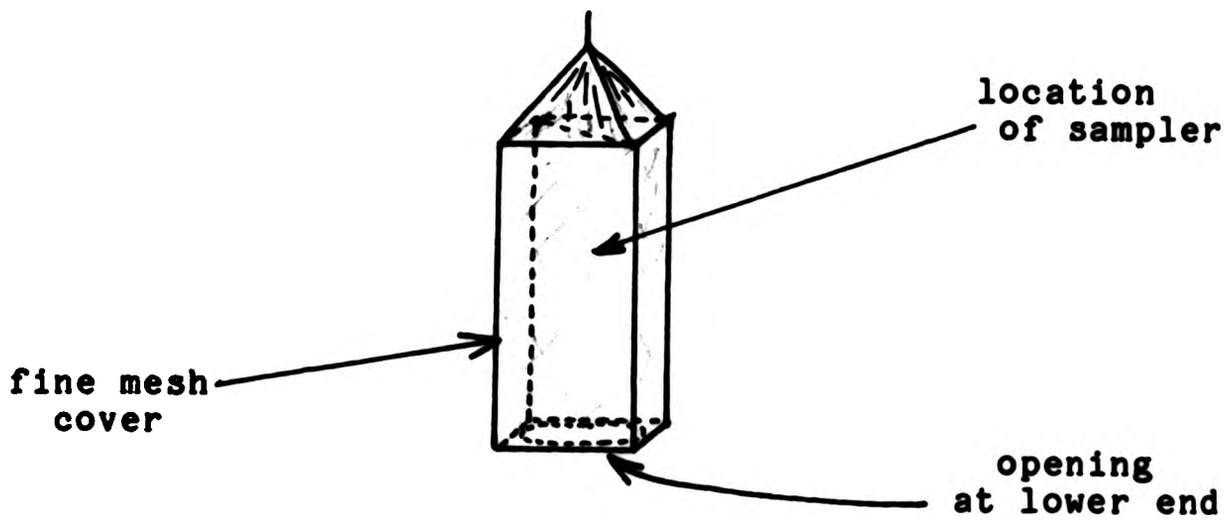


Figure 1.6.1. Cover type 'C'.

Sampling Period	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1 Week	<--->	<--->	<--->	<--->	<--->	<--->	<--->	<--->
2 Weeks	<--------->	<--------->	<--------->	<--------->				
4 Weeks	<------------------->	<------------------->						
8 Weeks	<------------------------------------->							

Figure 1.6.2 Passive sampler exposure scheme

1.6.3 Results

Cover Type B :

Exposure period:	1	2	4	8	wks.
Number of samplers:	16	8	4	2	
Mean conc of Pb:	0.57	1.13	2.60	3.70	ppm
RSD:	84	48	15	17%	

Cover Type C :

Exposure period:	1	2	4	8	wks.
Number of samplers:	16	8	4	2	
Mean conc of Pb:	3.20	3.90	5.48	7.59	ppm
RSD:	93	82	47	30%	

These results are better illustrated graphically (Figs 1.6.3 and 1.6.4).

1.6.4 Discussion

The results indicate that the amont of lead accumulated by the passive samplers is related to the duration of exposure. The results achieved with Cover Type B are more promising than those achieved with Cover Type C. With Cover Type B the rate of accumulation appears to be linear up to about 4 weeks and falls off slightly

thereafter. The rate of accumulation is about 0.65 μg/week. The estimated precision does not fall within acceptable limits until the fourth week. This is probably because analytical errors, small random contaminations and analyte losses contribute a relatively large component at the shorter exposure times.

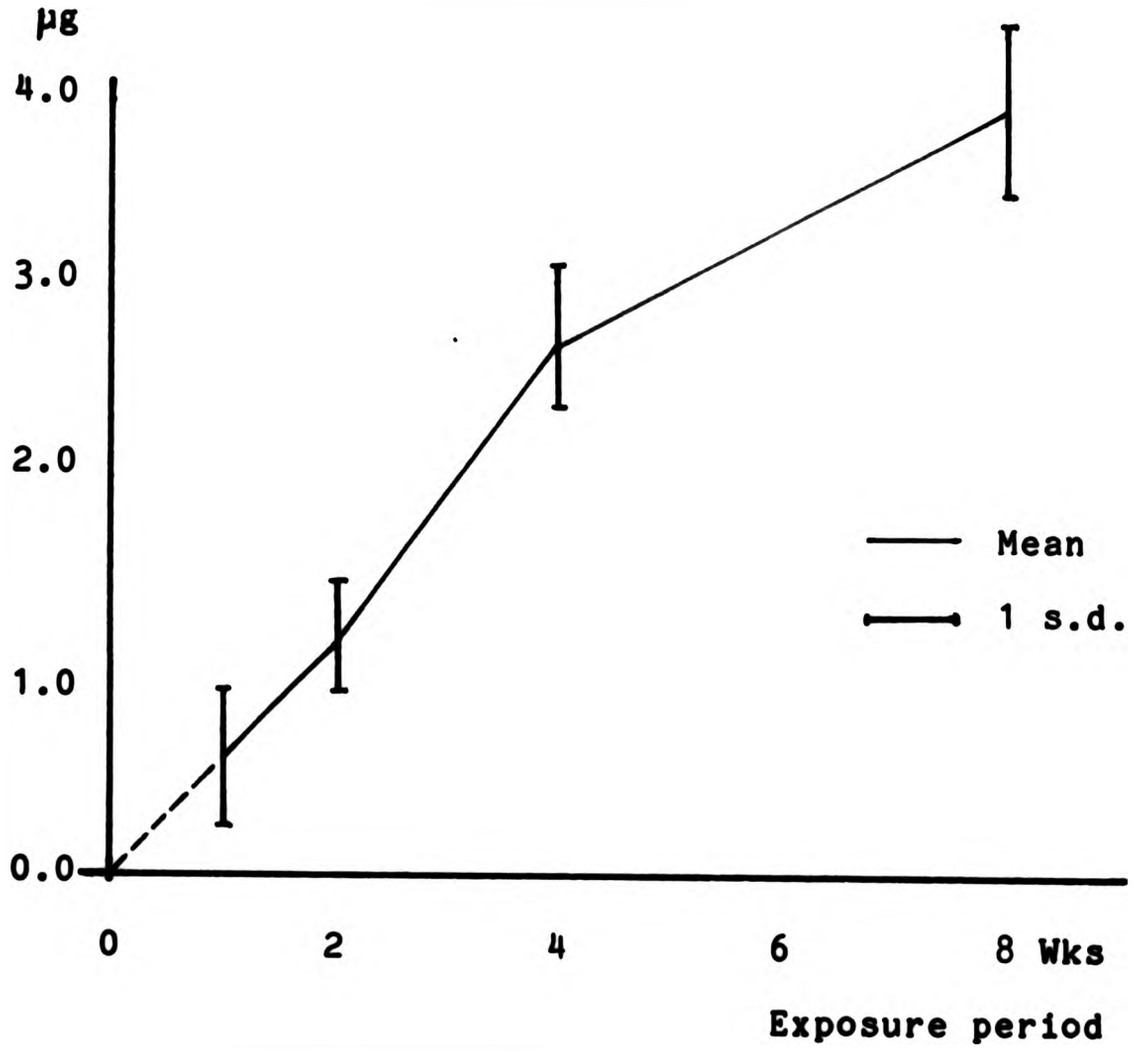


Figure 1.6.3 Accumulation of lead by type 'B' cover.

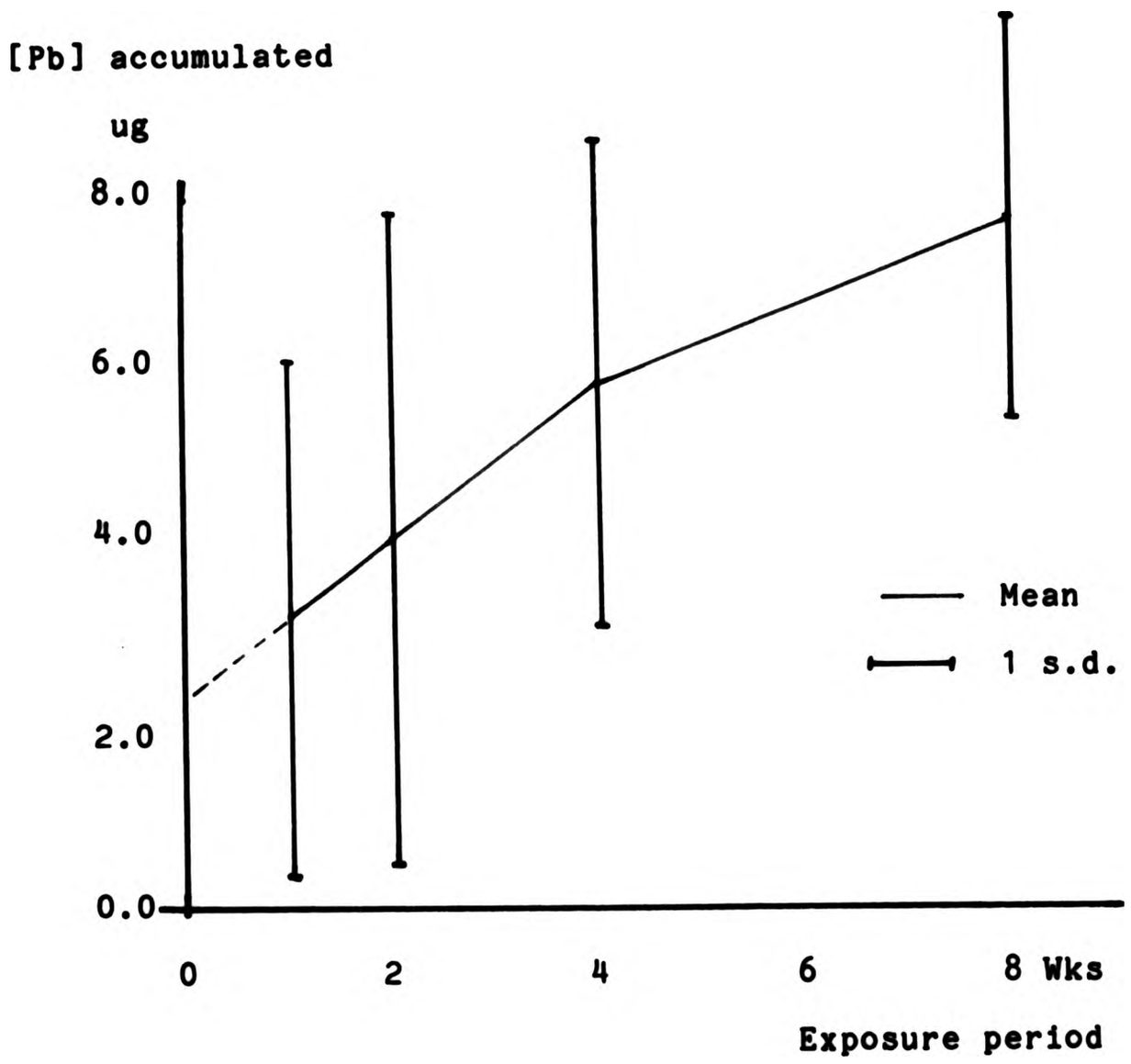


Figure 1.6.4 Accumulation of lead by type 'C' cover.

It is reassuring to observe that if the slope joining the first two means is extrapolated back, it almost coincides with the origin. It was felt that the low level of precision might be attributable, to some extent, to variations in the parameters between individual covers. As a consequence it was decided to

develop a further cover modificaiton. Cover Type C has the advantages of a larger sampling area (10 cm x 15 cm compared with 9 cm x 9 cm) and an outer covering which is drawn down so that only a small aperture, about 7 cm squared is available for air to exchange freely. It was hoped that the almost doubling of the sampling area would give a better analyte: contaminant ratio and that the smaller aperture would prevent larger wind-borne particles from impacting on the surface of the sampler. The results achieved with Cover Type C however, were disappointing. The rate of accumulation had only increased very slightly to about 0.75 µg/week and the precision was very much poorer after every exposure period. It can also be seen that if the slope joining the first two means is extrapolated backwards it crosses the y-axis at about 2.4 µg. The immediate indication is that there is a significant degree of contamination being introduced. A likely source of contamination is the cover itself. The 'C' type covers had already been used for a series of trials for the electron microscope study and were already discoloured by accumulated air-borne material. The design of the cover was such that it had to be pushed back over the frame by hand when the sampler was changed. This operation provided ample opportunity for contamination by falling dust and from the hands.

There is no doubt that the passive samplers are collecting lead from the air. The low level of precision obtained probably reflects the effects of unmeasured variables such as meteorological factors and slight variations in hood dimensions, in addition to contamination. The results achieved with the 'B' type hood after four weeks show just how good the device can be under optimal conditions, whereas the results achieved with the 'C' type hood demonstrate the effects of contamination.

1.7 Concluding Summary on Passive Sampler

The concentrations of trace metals in the urban atmosphere sampled in this study were slightly lower than those found in previous similar studies (Table 1.7.1).

Lead in urban air is reported to be concentrated in the sub-micron fraction of the aerosol (16). In this study the mass median equivalent diameter (MMED) of the aerosol was found to be 1.3μm, and 46% of this North London aerosol was less than 1μm.

Table 1.7.1 Concentrations of trace metals in ambient urban air.

	Concentration found in this study	Concentration found in previous studies (3,13,28,29)	
Pb	0.72	0.63 - 3.50	$\mu g\ m^{-3}$
Cd	4.80	n.d - 130	$ng\ m^{-3}$
Cu	40	100 - 846	$ng\ m^{-3}$
Zn	0.24	0.1 - 1.7	$\mu g\ m^{-3}$

This indicates that the aerosol was relatively "young" with a significant primary component. It seems highly likely that there would be a high respirable to non-respirable ratio for lead in such an aerosol.

The passive sampler was designed so that it would be biased to sample the respirable part of the aerosol. The aim was to develop a device that would provide an index of "risk" to the urban dweller from respirable toxic trace metals. It was felt at the design stage that the geometry of the protective cover would be critical to the performance of the sampler. Microscopic examination of exposed sampling media revealed that the first hood design allowed particles greater than 10μm to be accumulated by the sampling medium. When a fine mesh

outer layer was added to the cover the upper limit of collection was reduced to about 2μm. Particles were visible down to about 0.14μm, indicating that the sampler was effectively collecting in the respirable range.

The collection of lead by the passive samplers did not correlate well with that collected by a conventional "low volume" sampling system. A perfect correlation was not expected because of differences in sampling bias between the two techniques. The lens cleaning tissue sampling medium was found to perform best overall.

The accumulation of lead by the lens tissue passive sampler was studied over several time periods. The sampler was found to perform best after an exposure of four weeks.

The passive sampler shows considerable promise as a cheap, simple and reliable monitor for respirable trace metals in air. The effects of wind speed, temperature and relative humidity, and the sampling bias of the device are difficult to study under ambient conditions. Such variables could only be reliably controlled under wind-tunnel or exposure chamber conditions with

mono-disperse aerosols. Such studies would have to be undertaken before complete confidence could be placed on the reliability of results obtained with the passive sampler.

The theories of small particle dynamics have been studied and the design for a size-selective passive sampler for trace metals in airborne respirable particles developed. Trials showed that the device collected particles over the desired size spectrum in sufficient quantities for analysis. Further studies will be required for complete validation.

PAGINATION ERROR

Plate I Lens cleaning tissue X300.

Plate I Lens cleaning tissue X300.

Plate II Glass Fibre 5,250X

Plate II Glass Fibre 5,250X

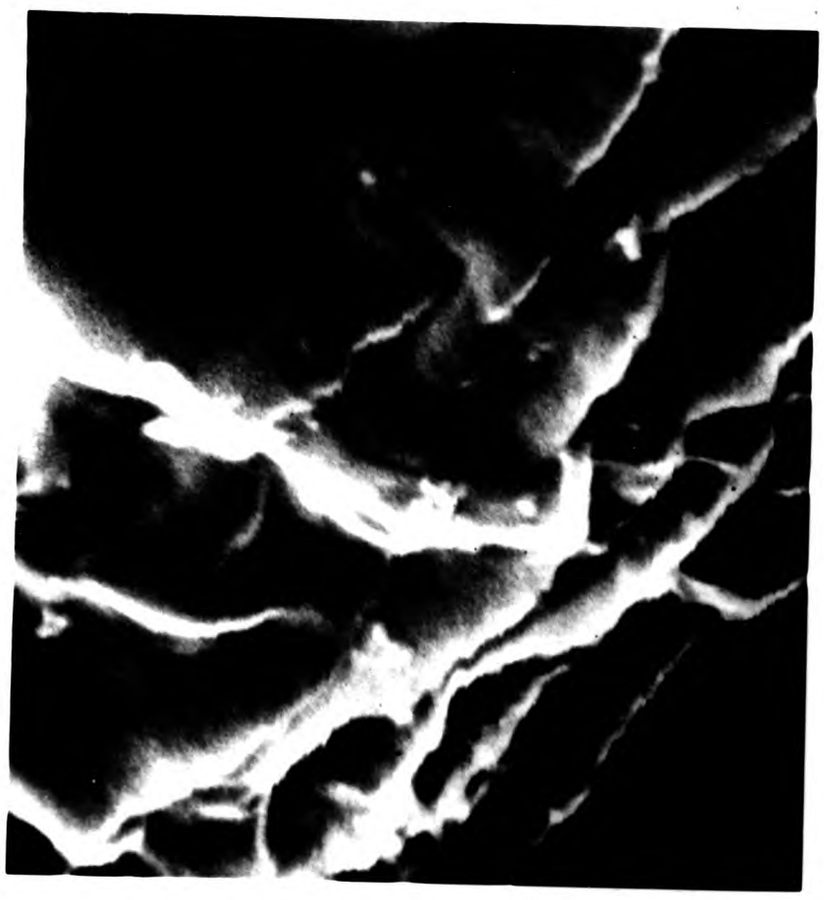


PLATE III Lens cleaning tissue 23,500X

PLATE III Lens cleaning tissue 23,500X

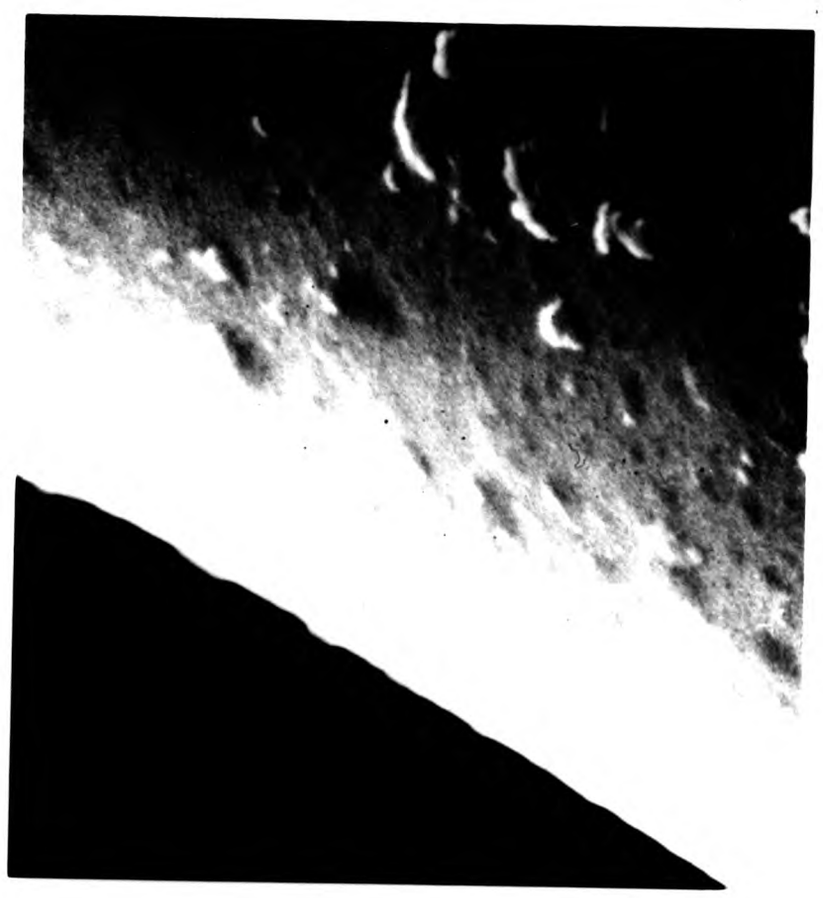


Plate VII Glass wool 22,500X

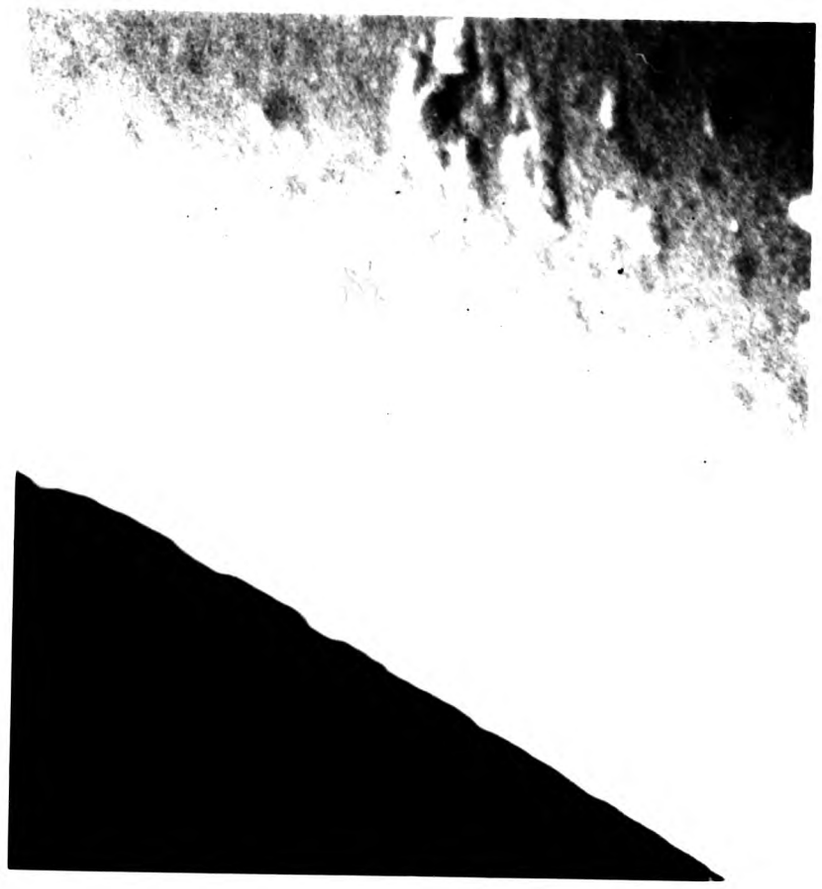


Plate VII Glass wool 22,500X

PLATE V Type 'C' passive samplers

PLATE V Type 'C' passive samplers

Plate VII Varian AA6 Graphite tube Atomiser.

Plate VII Varian AA6 Graphite tube Atomiser.

Plate VI Perkin-Elmer AA4000/HGA400 system.

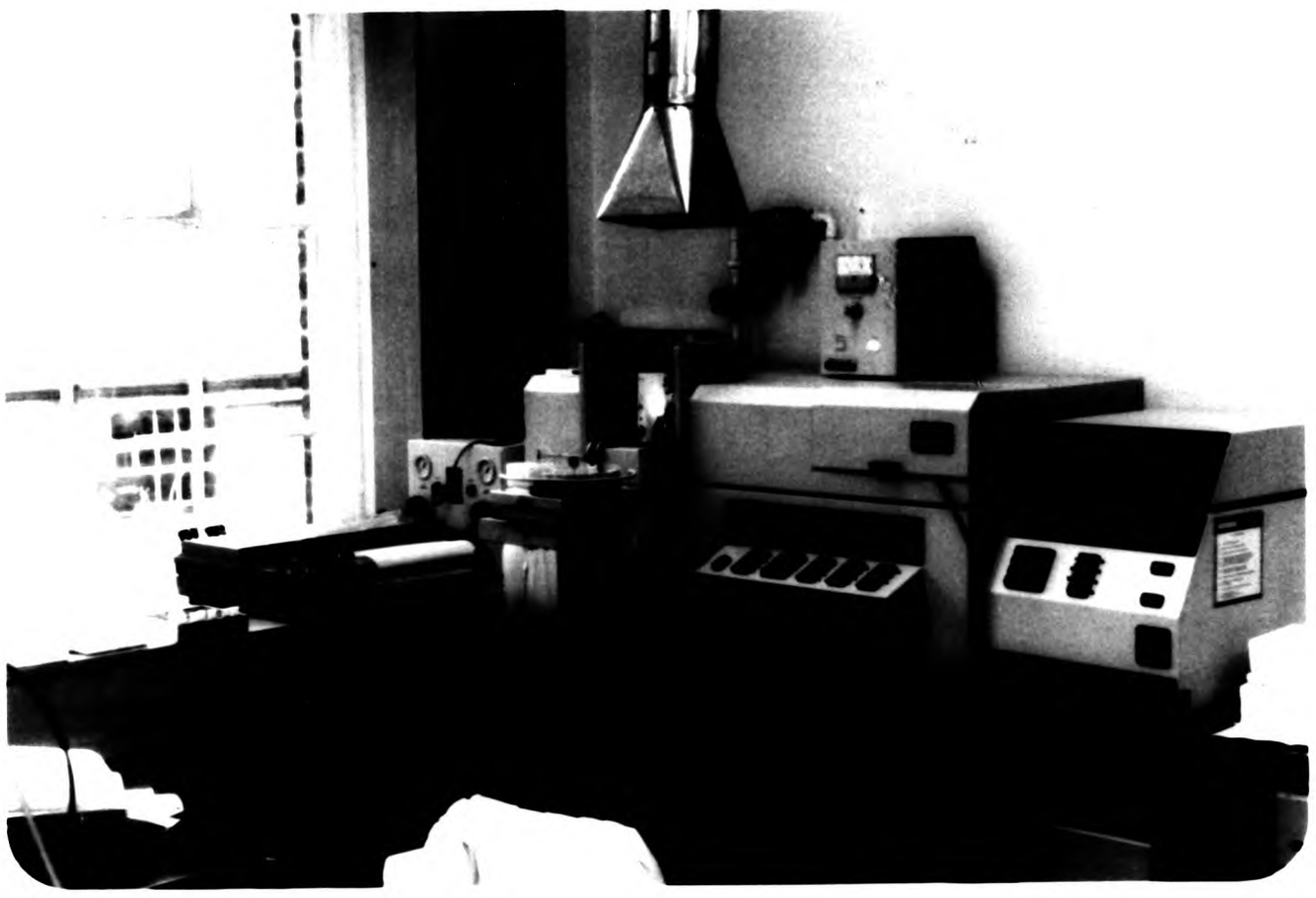


Plate VI Perkin-Elmer AA4000/HGA400 system.

PART II

The Use of Hair as a Biological Monitor for Exposure to, and Uptake of Environmental Trace Metals.

2.1.1. Introduction

Hair has many attractions as a biological monitor. it is easily and painlessly collected in sufficient quantities for analysis; it can be stored for indefinite periods, it contains levels of some trace elements significantly higher than many other tissues, and it is easily analysed (86). To be a reliable biological monitor a tissue should reflect acute and chronic exposure to the toxin and should be as homogenous as possible (87). Blood and urine are the media most commonly used as biological monitors. These fluids reflect many of the criteria for a biological monitor, but their value as biological monitors will depend on the dynamics of the toxin within the biological system (fig. 2.1.1) Blood levels would not be a good monitor for toxins with a short half-time in blood, and urine is subject to considerable variation in excretory patterns (88).

Hair will tend to reflect the deposition of metals to hard tissues and both hair and deciduous teeth have been used to evaluate uptake of lead (89,90). Deciduous teeth are relatively easy to collect, but the highest concentration of lead in milk teeth is in the root and this is reabsorbed before the tooth is shed (90).

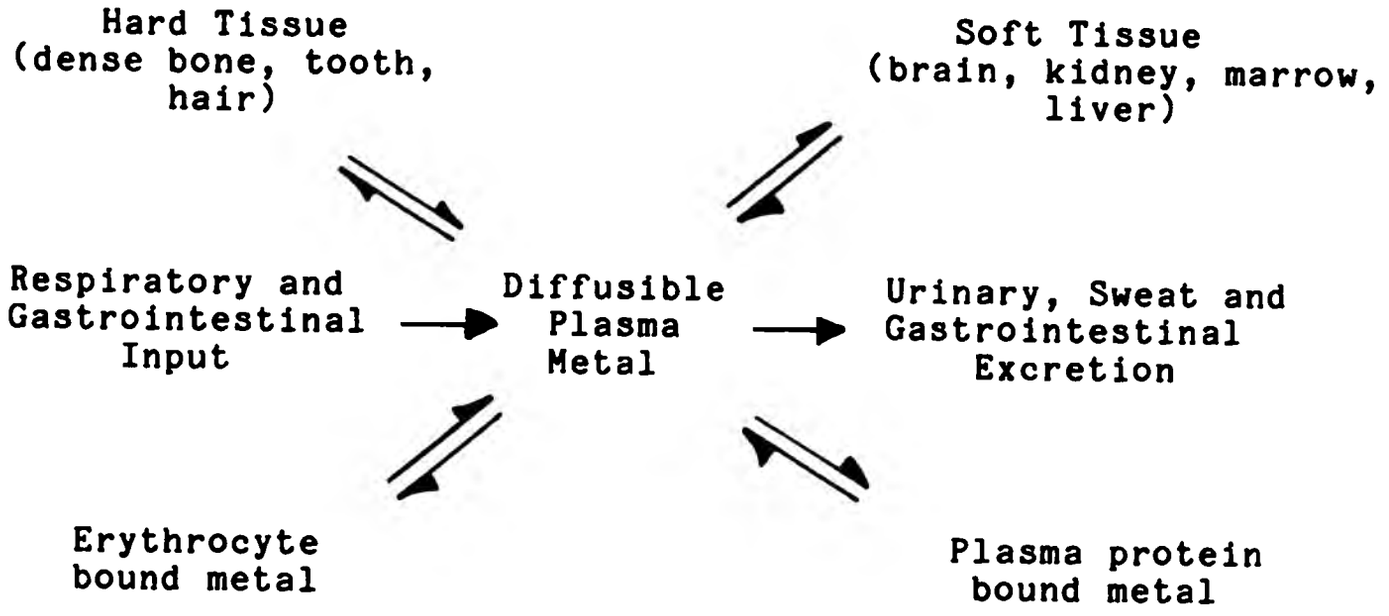


Figure 2.1.1 Dynamics of trace metals in biological systems (88).

Dentine also accumulates high lead levels and this tissue is usually selected for tooth analysis. Whole blood is the preferred medium for occupational monitoring of lead exposure but this is not practicable for monitoring large populations at ambient levels. Blood and urine also, are only temporary repositaries for the metals while in hair the metals may become irreversibly bound to the hair matrix (91). The value of hair as a biological monitor is becoming increasingly well established. Indeed, a woman has been found guilty of arsenic poisoning and sentenced to death on the forensic evidence of elevated arsenic levels in the hair and nails of the deceased (92). However, in the more subtle circumstances of non-occupational exposure the

interpretation of hair data is beset with difficulties; the concentrations of some elements may vary considerably from site to site on the head (93); some components may be lost during shampooing or other treatments, and others may be accumulated by contamination from the environment.

Although measurements made in any modern laboratory can generally be assumed to be accurate, comparisons of data obtained from different laboratories can show large variations in absolute values (94). Differences may reflect differences between the sample populations and, especially in the particular case of hair, to variations in sampling, pre-treatment and analytical techniques (Table 2.1.1).

The purpose of this part of the thesis is to identify the problems associated with the use of human hair as a monitor for trace metals, and to investigate a technique through which these might be overcome.

2.1.2 The Physiology of Human Hair

This subject has been rigorously reviewed by Hopps (86). The growth of individual hairs is cyclic. Each hair undergoes a long growing phase (anagen) and a short resting phase (telogen). The ratio of time spent in

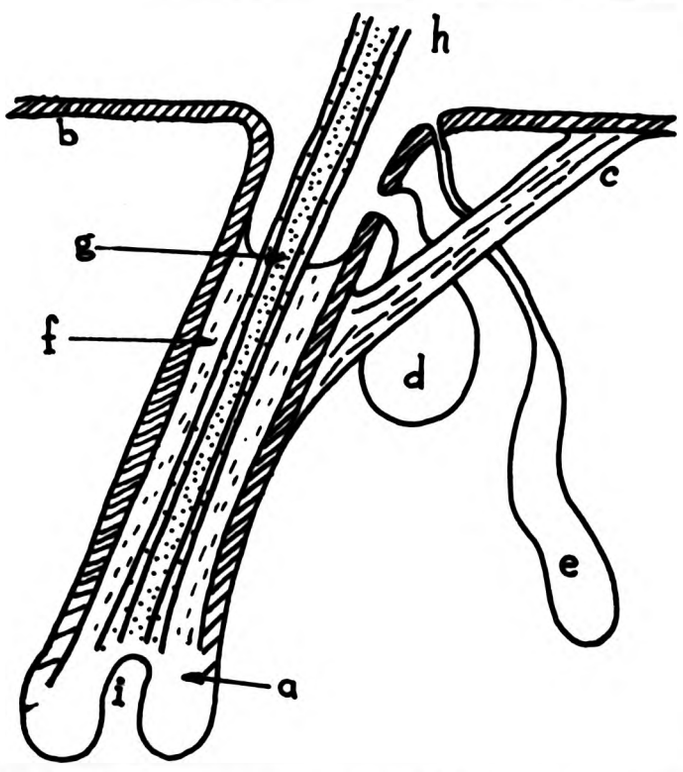
the two phases varies considerably according to the region of the body.

Table 2.1.1 'Normal' (unexposed) concentrations of some trace metals in hair (range of geometric means reported, μg/g)(91,93,94).

Ag	0.27
Al	10
As	0.085-0.14
Au	0.01
Cd	0.35-1.18
Cr	0.58-0.81
Cu	5.7-49.8
Fe	15.5-40.6
Hg	0.44-25.0
Mn	0.40-2.15
Ni	0.18-3.6
Pb	3.60-27.6
V	0.03-0.26
Zn	159-210

On the scalp growth continues for years and the anagen phase is on average nine times as long as the telogen phase. Hairs held in the follicle during the telogen phase are easily dislodged by combing so most hair is in the anagen phase. There is no co-ordinated growth/rest periodicity in humans such as causes seasonal moulting in many species. In any region of the human skin there is a mosaic pattern of follicles in all stages of the growth cycle. The growth rate of anaphase hair is approximately constant and continuous. The rate of growth has been estimated to be 7.1 to 12.0 mm per month

for adults and 10.1 to 13.5 mm per month for children (97) or 0.4 mm. per day (98). The rate varies considerably between individuals and 1 cm. per month may be used as an approximation.



The follicle is a minute organ. The matrix (a) extends through the epidermis (b) and is connected to the dermal tissue. The smooth muscle, arrectores pilorum (c), raises the hair causing 'goose-pimples.' There is a sebaceous gland (d), and in the axillary, pubic and perineal areas, an apocrine gland. Sweat glands are also closely associated with the follicle in the skin (e).

f : inner sheath
g : medulla
h : hair shaft
i : papilla

Figure 2.1.2 The Hair follicle

The follicle comprises a cluster of matrix cells within which the hair develops for a period of several days. During this time it is exposed to circulating blood and lymph and to extracellular fluids. As the hair proceeds along the inner sheath the outer layers harden, 'locking-in' the metabolic products accumulated during the hair formation (fig. 2.1.2).

Keratinisation occurs toward the distal end of the follicle. Keratin comprises a group of proteins containing a high proportion of disulphide bonds which make it remarkably resistant to enzymic digestion and by all but the strongest chemicals. A typical keratin molecule comprises a two or three stranded cable of highly oriented polypeptide chains of low sulphur content wound into a helix with secondary folds and distortions, associated with an unoriented matrix of high sulphur content. The most important bond stabilising the secondary and tertiary structure of the keratin molecule is the disulphide linkage. This linkage is formed by two adjacent cysteine residues contained in adjacent polypeptide chains and it is this linking which bestows on the molecule its remarkable physical and chemical stability.

The cysteine residue is also of significant importance because metals a have considerable affinity with the sulphhydryl groups combining with them to form mercaptides or metallo-proteins. About 14% of the hair matrix comprises cysteine residues and electron micrcoscopy has shown that these residues tend to be concentrated in the outermost sections of the hair; the eco-cuticle. This high sulphur content is probably responsible for the incorporation of metals from body fluids during the formation of the hair and may aid the

entry, inclusion and diffusion of metals into the hair after it has emerged from the scalp.

2.1.3 Endogenous Deposition of Trace Metals

As each hair develops in the follicle it is in intimate contact with the circulating body fluids and metals in these fluids will readily combine with sulphhydryl groups in the keratin. Thus hair will tend to reflect the relative concentrations of trace metals in the body fluids at the time that it is formed.

Trace metals may also be incorporated into the hair via the endogenous route from the follicular and scalp secretions. These are frequently considered as exogenous sources even though they originate from the body fluids. Sebum from the sebaceous glands comprised water, free and combined fatty acids, waxes and unsaponifiable matter including squalene, cholesterol, hydrocarbons and alcohols. The role of sebum in the incorporation of metals into hair is unclear. It may protect the hair from adsorption of exogenous metals (99) or it might provide a physical or chemical means of incorporation of these metals into the hair (100). Eccrine sweat varies widely in composition according to individual and enrironmental conditions. Water, urea, and sodium and potassium are the principal constituents, although amino, lactic and pyruvic acids are also

present. Eccrine sweat is a quantitatively important source of trace metals that may become incorporated into the hair after its formation (Table 2.1.2). Studies have shown that arsenic appears in sweat soon after it is administered leading to rapid contamination of the extruded portion of individual hairs (101). However, in cases of acute poisoning, no significant levels were found other than in root sections of anphase hairs. This suggests that follicular component is much greater than the contribution from sweat.

Table 2.1.2 Trace Metals in Eccrine Sweat ($\mu g/100\ cm^3$)

	Hopps (86)	Kuno (100)
Cu	6	6
Fe	2-45	24-200
Mg	4-6	200-1190
Mn	3-7	6
Zn	93 ± 26	-

The principal source of endogenous metals in hair is the diet. Inhaled particles will contribute to some extent but this is unlikely to be significant except in the cases of some minor trace metals which are common air pollutants. The zinc content of human hair has been shown to have a direct relationship to the availability of zinc in the diet and to its metabolism (102).

However, protein-deficient children have been found to have depressed zinc in hair while the content of manganese is elevated (103). The age of the individual may also affect the concentrations of elements depositing in hair. From birth to the early twenties there is a gradual decrease in the concentration of Cl, I, Al, K, V, Mn, Co, As and Br while the concentrations of the elements Mg, Ca, Cu, and Zn gradually increase (104). After this initial increase, Cu and Zn slowly decline until 80 years (102). Sex may also affect the concentrations of trace metals in hair (Table 2.1.3).

Table 2.1.3 Trace metals in hair of males and females, µg/g (105)

	Cu	Cd	Mn	Hg	Ni	Pb	Zn
M :	10.7-41.6	9.1-9.3	<1-3.9	0.2-12	1.0-7.2	11-50	75-194
F :	11.4-61.4	0.2-3.3	<1-2.2	0.2-30	0.7-7.5	7.6-59	65-308

The iron content is lower in the hair of women and this may reflect menstruation (106). Hair concentrations of certain trace metals have also been found to be lower in certain diseased conditions (106, 107, 108).
Differences in trace metal contents may also occur in different racial groups, hair colour, hair treatments and medical treatments (106,109).

The arsenic content of locks of hair taken from the Emperor Napoleon before and just after his death have revealed very high levels. Chronologies of the time indicate that Napoleon may have suffered from the symptoms of arsenic poisoning (110). Napoleon was not necessarily poisoned by an adversary; it has been found that in damp conditions arsenic may be released into the atmosphere by the effects of fungus on wallpaper pigments. The situaiton may have been made worse by the ministrations of his physicians.

The mechanisms of incorporation of trace metals into the hair are not well understood. Cross-sectional trace metal profiles of root bulbs and emerged hairs were studied by proton-induced X-ray emission (PIXE) analysis (111,112). The elements Fe, Cu, Zn, S, K and Ca were detected in root bulbs but As, Se and Pb were not. The elements As, Se and Pb were detected at the surface of the hair after its formation. The authors conclude that Cu and Zn are accumulated from blood during the first stage of hair formation, and that Pb, As and Se are introduced from sebum or sweat after the hair has emerged.

The endogenous deposition of trace metals into hair is poorly understood and appears to be subject to a wide degree of variability. Madeiros (112) considers that

hair should only be used on a population basis when monitoring the essential trace metals. Hair may have more utility on an individual basis when monitoring the toxic trace metals.

2.1.4 Exogenous Accumulation of Trace Metals

The hair is exposed to trace metals in water, cosmetic preparations and airborne particles. Several workers have reported increases in the concentrations of certain non-essential trace metals with increasing distance from the scalp (99, 100, 101, 114, 115). This indicates immediately that hair is absorbing these metals from the environment, since the further each hair has grown from the scalp, the longer it has been exposed. The essential trace metals including Fe, Cu and Zn showed no increase with length, indicating their endogenous origin (97, 116). It must, however, be pointed out that this gradient has not always been seen, even among lead workers (117).

Hair is hygroscopic and has a porosity of about 20% (w/w) (118). Absorption of fluids is very rapid; 75% may be absorbed in only four minutes. Many soluble substances may be absorbed along with water, sweat, cosmetics or other fluids which may come into contact with the hair. A sample of hair, submerged in a

sweat-like solution containing 13 μg/ml of lead contained more lead than the solution after four days (119). Waste hair has been proposed as an adsorbent for the removal of metal contaminants from hide processing wastes (119).

A 75 mg sample of hair was found to take up 471 μg, 481 μg, 493 μg and 457 μg of Cr^{3+}, Cu^{2+}, Cd^{2+} and Hg^{2+} respectively from solutions containing 500 ug of the metals. The concentrations of Cu and Zn have been found to be higher after using dyes and certain shampoos (120).

Airborne contaminants may also be a significant source of trace metals to the hair. An uncontaminated sample of hair accumulated significant amounts of arsenic from the air when exposed for four weeks in a gold mine (121).

Exogenous metals may become incorporated into hair so that they are chemically indistinguishable from those of endogenous origin. The mechanisms of adsorption and absorption are not well understood but it may be assumed that the sulphydryl groups of the cysteine residues are involved.

2.1.5 The Use of Human Hair as a Biological Monitor

Where an essential element is present as a trace component in a biological tissue, homestatic mechanisms ensure that the probability that the conentration of the element in a particular tissue sample will be above, or below, the population mean, will be about equal. The distribution can be assumed to be normal and this has been found to be the case for the distribution of the essential metals in hair (122,123). When an element is non-essential and occurs as a contaminant the probability that the concentration in a particular tissue will be higher than the arithmetic mean, will be much lower than the probability that the concentration will be below the arithmetic mean, at or near the background level. The frequency distributions of the toxic metals have been found to be skewed (115,123). For this reason a log-normal distribution is frequently assumed for the toxic metals in hair, and the geometric mean is thus often employed.

A number of studies have investigated the relationships between the levels of trace metals in hair and other biological tissues and fluids (101,122,124,125,126,). Blood levels of metals will tend to return to normal after exposure as the metals are cleared to the tissues.

The deposition into hair will tend to reflect tissue clearance and as a result may be a better index of dose. However, hair may also accumulate metals directly from the environment and this inevitably leads to some ambiguity when interpreting the results. These factors mean that a linear correlation between blood and hair levels of trace metals is unlikely to occur.

Blood lead is reported to be normally distributed in the population with the median coinciding with the mean at about 30 µg/100 ml (122). Chattopadhay et al (124) found a reasonable correlation between blood lead and the natural log of hair lead concentrations. The relationship was only significant for individuals who were in a steady state with respect to lead intake and excretion. In cases of massive or irregular doses the correlation did not hold. When studying 1 cm. root segments only, Grandjean et al (101) found strong correlations between hair lead, blood lead and urinary lead. They also found a strong correlation between hair lead and urinary ALA (δ-amino levulinic acid) which is an indicator of interference with haem synthesis. They found considerable variation from hair to hair so several 1 cm root segments were analysed to obtained an average. A blood lead level of 60 µg/100 ml corresponded to a hair lead of 70 µg/g (or 3 µg/cm). Other workers have found weaker relationships between

blood and hair lead (125, 126).

The hair metal pool only represents 0.5 to 1.0% of the total body pool of lead and gives a poor indication of the amount of lead accumulated in the bones (127). Teeth can give an indication of the total accumulated dose but provide no information on the current status in the way that blood does (128). Hair may turn out to be a compromise between the two, providing an indication of the rate of recent deposition to hard tissues.

It is particularly important to identify cadmium exposed individuals before kidney damage takes place (129). Blood cadmium has not been found to be a reliable indicator of body burden. In autopsy studies a significant correlation was achieved between hair cadmium and kidney cadmium (130). While hair was found to give a better correlation with liver and kidney cadmium than blood or urinary cadmium, it was still unable to provide a quantitative estimate of body burden (131). It also failed to identify those with impaired kidney function as indicated by elevated urinary β_2 microglobulins. It seems likely that with cadmium as with lead, hair has the potential to indicate the recent rate of deposition to the target tissues.

Hair mercury levels were found to be related to blood mercury levels but were 300 times higher (132). In a

population consuming fish which had been contaminated with methyl-mercury, fluctuations in the levels of mercury in the hair were observed. These were shown to coincide with seasonal availability of the fish. Phelps (132) found that blood mercury followed a constant ratio to the concentration of methyl-mercury in the target tissue (the brain) and was directly proportional to the daily intake of those consuming contaminated fish. Hair mercury may provide a permanent record of levels of methyl-mercury in the brain (fig. 2.1.3).

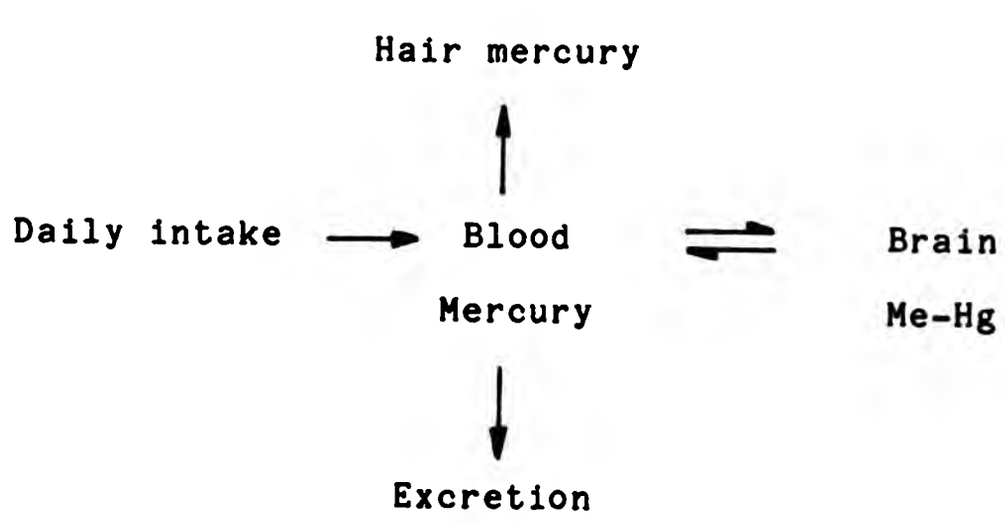


Figure 2.1.3 Deposition of mercury to hair.

Using this relationship between blood and hair mercury levels Al-Shaharistan et al (133) used hair mercury levels to estimate the biological half-life of methyl-mercury in the brain after exposure had ceased.

The example of hair mercury indicates the potential value of hair as a biological indicator. However, it must be stressed that in this case the intake was entirely via the diet. With other metals and in other circumstances there may be a considerable exogenous component.

2.1.6 Exposure Studies with Hair

Hair samples have been found to be useful in numerous studies of environmental and occupational expsoures to trace metals. A number of studies have indicated that hair levels of the toxic trace metals tend to follow environmental exposure gradients while the essential trace metals do not (134, 135,136). Lead invariably followed exposure gradients, while copper, zinc and iron invariably did not. The metals manganese, arsenic, cadmium and aluminium were less well defined; sometimes following the exposure trend and at other times not (124, 136, 137, 139, 140). The particular combination of metals following exposure gradients will presumably reflect the sources in a particular locality.

In occupational circumstances it is not surprising to find that those handling toxic metals accumulate higher levels in their hair. Fergusson et al (141) found a mean lead concentration of 363 µg/g in the hair of 16 battery factory employees, compared with 10.4 µg/g in

203 controls. It is quite surprising to find that the lead in the hair of the families of the employees was raised to 67 µg/g. Cadmium battery workers can have hair cadmium levels up to 140 times the normal range (0.13-0.54 µg/g) (142, 143). Mercury workers, and also dentists, frequently have elevated levels of mercury in their hair (144, 145).

Where individuals are not occupationally exposed to toxic metals, differences might be expected to be more subtle. Populations living in the vicinity of metal refineries have been found to have hair metal levels above those of a normal urban population (Table 2.1.4) (124, 135, 146, 147).

Table 2.1.4: Trace Metals in Hair of Three Populations ($\mu g\ g^{-1}$) (148)

	Rural	Urban	Urban, Nr. Pb refinery.
As	.45-1.7	.32-2.8	.55-5.2
Cd	.25-2.7	.26-3.7	.40-8.8
Hg	.28-3.5	.24-5.4	.20-6.1
Pb	.50-30	.50-40	5.0-355
Se	1.3-24	1.2-38	1.5-50
Zn	40-450	40-485	45-205

The concentration of lead is raised as expected, but the cadmium level is also raised slightly because cadmium is frequently associated with lead in ores and may be released during the refining process.

Hair lead levels have been found to vary with soil lead levels (125, 149) and type of plumbing (Table 2.1.5) (150). If these differences in hair lead are entirely attributable to lead in pipes, then some of these high values may reflect endogenous accumulation.

Table 2.1.5: Lead in Hair according to Environment and Plumbing ($\mu g\ g^{-1}$) (150)

Location	Number	Range	Geometric Mean
East Kilbride	81	1.4-69.7	10.2
Glasgow (No Pb)	104	2.1-334	28.9
(Part Pb)	54	9.7-350	51.5
(All Pb)	35	10.0-267	57.7

Cadmium only shows minor differences between urban and rural populations (see Table 2.1.4 and reference 135). Whanger (151) found differences between different occupational groups but found even greater differences between smokers and non-smokers. Smokers had a mean

hair cadmium of 1.66 ug/g whereas non-smokers had a mean hair cadmium of only 0.90 ug/g. Ely et al (152) could detect no difference between smoking and non-smoking households.

Many studies have been made of the levels of toxic and essential metals in hair. Some have even gone on to discuss the behavioural and toxicological significance of the data (152, 153, 154). Cheraskin and Ringsdorf (155) suggest that hair lead levels of 20 ppm and above may be indicative of lead intoxication. Unfortunately, few authors acknowledge the difference between endogenous and exogenous metals, and none has provided a reliable technique to discriminate between the two.

2.1.7: Discriminating between Endogenous and Exogenous Components

Katz (156) has observed considerable differences in values obtained by different workers at different locations. Not only do values for exposed populations vary widely but 'background', 'unexposed' or 'normal' values do also. This apparent lack of agreement can be attributed to four possible sources:

(a) Sampling bias. It has been shown that hair taken from different regions of the scalp, different distancesfrom the scalp, different age groups, etc.

may have differing concentrations of trace metals.

(b) Pre-analysis treatments. Different washing processes may introduce bias.

(c) Analytical bias. Identical materials analysed by apparently similar techniques can produce remarkably different results. This is especially the case at low concentrations which approach instrumental detection limits.

(d) There may be real differences reflecting different exposures to the metals.

In 1975 Kopito and Surachman (157) observed that "Since there are no commonly accepted 'standard procedures' for sampling, treating and analysing hair, comparison with absolute values obtained by other workers is presently difficult, if not impossible." This situation remains unchanged.

Shapcott (158) states that "obviously it is impossible, from analysis of untreated hair, to state categorically which proportion is "true" metal content and which is present as a contaminant." Most workers who have recognised this problem have put their faith in washing procedures as a means of removing 'external contamination' (Table 2.1.6).

Table 2.1.6: Some Wash Procedures used to Pre-treat Hair

Wash Procedure	Reference
2 hr Soxhlet extraction in diethylether	159
1% EDTA, 10 min boiling water, hot water rinse acetone	117
Acetone, ether, acetone	102
Soak and wash in water, soak and wash in methanol	105
Non-ionic detergent, purified diethylether	109
1% mild detergent, warm deionized water, 1% HNO_3	143
Detergent, deionised water, alcohol, EDTA	123
Double distilled water, absolute ethanol, diethylether	135,148
1% DECON-75, deionised water 0.1 M Na EDTA	160
Hot ether, detergent, EDTA	161

In addition to these procedures, soap and shampoo solutions, carbon tetrachloride, benzene, hexane, hydrochloric acid, and sodium hydroxide have all been used to pretreat hair (94). Salmeda et al (162) compared four washing techniques after an initial rinse in hexane:-

1. Organic solvent - Acetone
2. Complex forming agent - 1% NA_2 EDTA
3. Ionic detergent - 1% Na lauryl sulphate
4. Non-ionic detergent - 1% Triton X-100

They found that the duration of the wash was the most important factor and recommended that washing should be continued until the concentration of a particular element could be reduced no more by further washing. Similar studies have indicated that hot water, organic solvents and detergent washes remove similar amounts of elements but that complex forming agents may remove a lot more (114, 161, 163). Deionised water and organic solvents are intended to remove deposits held by fatty residues on the surface of the hair, and have been found to remove only minor amounts of trace metals (87,135,148). Chattopadhyay et al (135) believe that this indicates that there has been only minimal external deposition of metals to the hair pool. They are, however, overlooking the complex physico-chemical associations that can exist between metals and the matrix (Table 2.1.7).

Table 2.1.7: <u>Physico-Chemical Associations between Trace Metals and Hair</u>

Exogenous	1. Metals in particles adhered to surface.	Increasing ease of removal ↑
	2. Metal ions adsorbed onto hair surface	
Endogenous	3. Metal ions held by weak chemical bonds (eg H-bonds) within the hair matrix.	
	4. Metals firmly bound and incorporated into hair structure.	

Metals in particles which are adhered to the hair surface are easily removed by rinsing, but the metal ions which have migrated, and have become adsorbed onto or within the hair matrix may not be so readily removed. No physical or chemical distinction can be made between such metals of exogenous origin, and those which are endogenous to the hair.

Thus any washing procedure, however reproducible, can only make an arbitrary division between the metals in the hair. Grandjean (101) concludes that "Endogenous lead which has diffused into the hair is impossible to remove without disturbing the original lead content of the hair," and this, of course, applies to any other metal. In the opinion of Chittleborough (94), "Washing the hair is to be avoided because such treatments remove, to a lesser or greater degree, the unique and pristine character of that sample and its relationship to its original owner." However, he later admits that "a knowledge of the separate contributions of both the endogenic and exogenic sources may prove to be quite valuable to the environmental scientist." Clearly, it is necessary to be able to distinguish between the sources of trace metals in hair before a valid interpretation of hair data can be made.

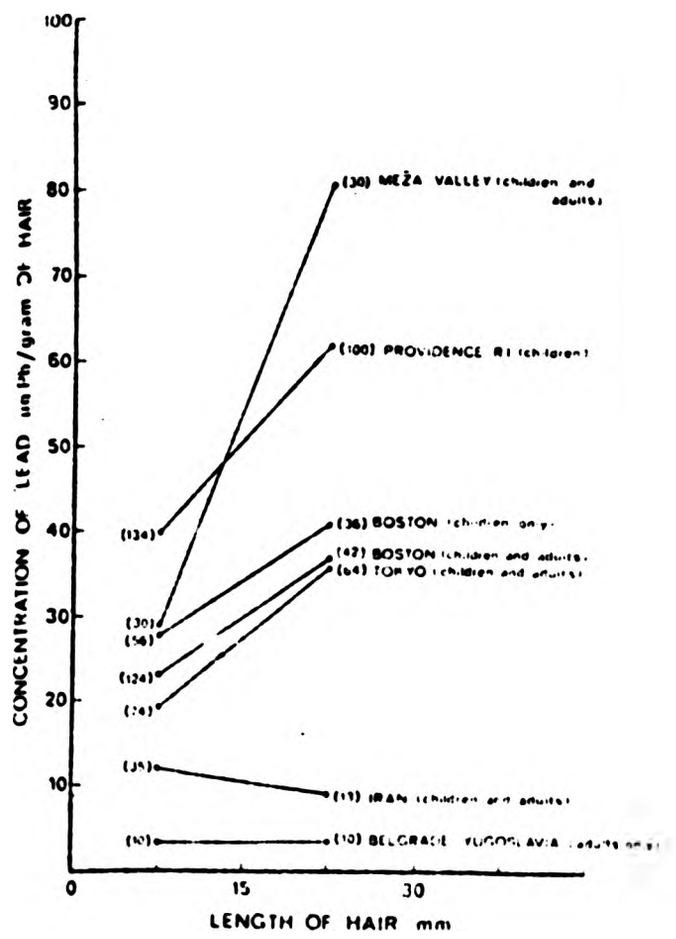


Figure 2.1.8: Concentration of lead in two adjacent hair 0-10 mm and 15-30 mm distant from scalp. Value given for mid-point of segment; number of observations in parentheses. (157).

2.1.8 Hypothetical Model for Hair as a Biological Monitor

Hair may preserve a record of trace metal concentrations in the blood stream. It does not provide an index of total body burden in the same way that a deciduous tooth might for lead, and neither does it give an estimate of current exposure as would a blood level figure. Instead, it would provide an index of blood levels over a period

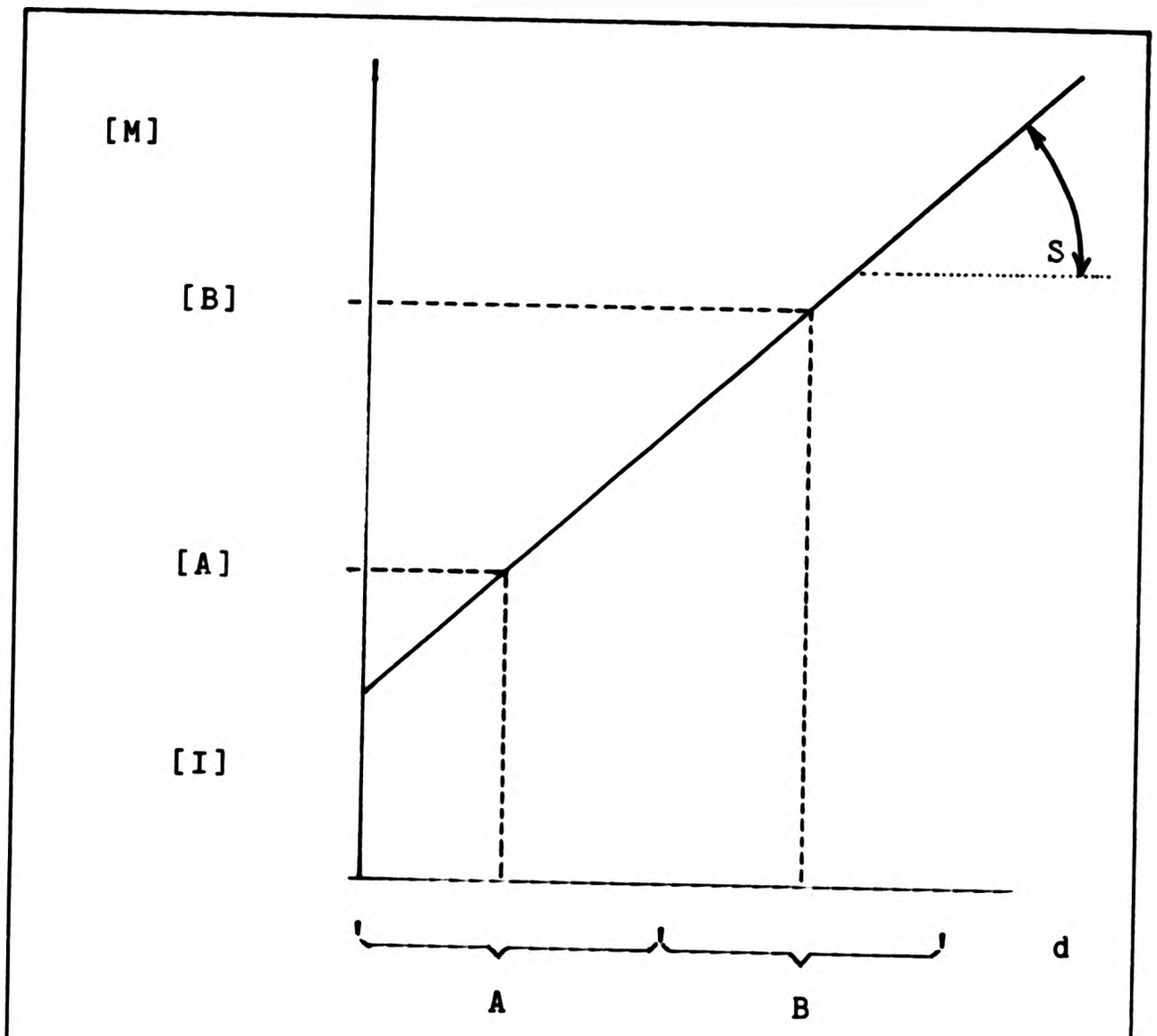
of a few weeks, depending on the hair length sampled. If a long strand were sampled and segmented, then a long-term record of changing tissue levels might be obtained.

Once the hair has emerged from the follicle though, it is subject to the environment in which the individual lives. The air, water and cosmetics which come into contact with the hair may contain trace metals and cause them to accumulate in it. So hair could be a useful indicator of the levels of trace metals in the individuals' environment. However, normal hair washing might cause metals to be leached from the hair and alter the record.

If, for a given individual, exposure to all sources of trace metals is relatively constant and the individual's metabolic state is stable, then we can assume that the flux of trace metals to the individual's hair via both endogenous and exogenous routes will be constant and continuous. It could then be expected that the concentration of a given metal in a hair would be at a level reflecting the endogenous contribution at the root, and increase along the hair at a rate reflecting the degree of environmental exposure.

It is proposed that hair can provide an index of both tissue level and environmental exposure. If a hair sample is cut from as near to the scalp as possible, divided into two equidistant portions and analysed separately, then the value for the rate of increase with length so obtained (the slope) may be used as an index of environmental exposure, and the concentration of metal at the root, obtained by extrapolation (the intercept), can be used as an index of tissue levels (Figure 2.1.9). The model assumes that the rate of accumulation will be linear in a steady state system. Of course this will depend upon other factors including personal hair hygiene, contributions from sweat and sebum, the effects of cosmetic treatments on the hair and physical and chemical changes which may occur as a result of ageing.

The object of this part of the work is to test the methodology outlined above to see if hair can be useful as a dual index, and to investigate some of the other factors which may influence trace metal levels in hair.



d = Distance from scalp
[M] = Concentration of metal
A = Segment A
B = Segment B
a = Mean length of segment a
b = Mean length of segment b
[A] = Concentration of metal in A
[B] = Concentration of metal in B

$$\text{Slope} = S = \frac{[B] - [A]}{b - a}$$

Intercept = I = [M] when d = 0
= [B] - Sb

2.2 Trace Metal Analysis of Hair

2.2.1 Introduction

Prior to analysis by conventional atomic absorption techniques the hair must be ashed to reduce the bulk of organic material and digested under acidic and oxidising conditions to solublise the metals. Much of the early parts of this work were performed by flame atomic absorption spectrophotometry (aas) using the Pye SP9. Later analyses were carried out by atomic absorption spectrophotometry with electrothermal atomisation using the Perkin-Elmer AA 4000 spectrophotometer, equipped with an HGA 500 graphite tube atomiser.

2.2.2 Analytical Techniques

All hair samples were analysed by atomic absorption spectrophotometry. Details of the analytical proceedure appear in section 2.7.1.

2.2.3 Accuracy and Precision

As this work progressed, and the author's appreciation of the practice and pitfalls of analytical chemistry developed, more checks on the reliability of the

analyses were introduced.

The accuracy of the flame aas was confirmed by comparing a spiked digest calibration curve with an aqueous calibration curve (Figure 2.2.1). That the two calibration curves are approximately parallel indicated that there were no matrix interferences within the flame. The 217.0 nm lead absorption line is believed to be relatively free from spectral interferences. The mean concentration of lead was 14.4 ppm with a relative standard deviation of 25%. The mean concentration of copper was 13.6 ppm with a relative standard deviation of 7.5%. Cadmium analysis was also studied at this stage but the relative standard deviation of 275% obtained indicated that there was little point in proceeding with cadmium analyses.

AAS with electrothermal atomization is more prone to interferences than flame aas. This is because:

1. The analyte and matrix are concentrated within the light beam. This means that although better sensitivity is achieved it is at the expense of having much more non-specific 'background' absorption.

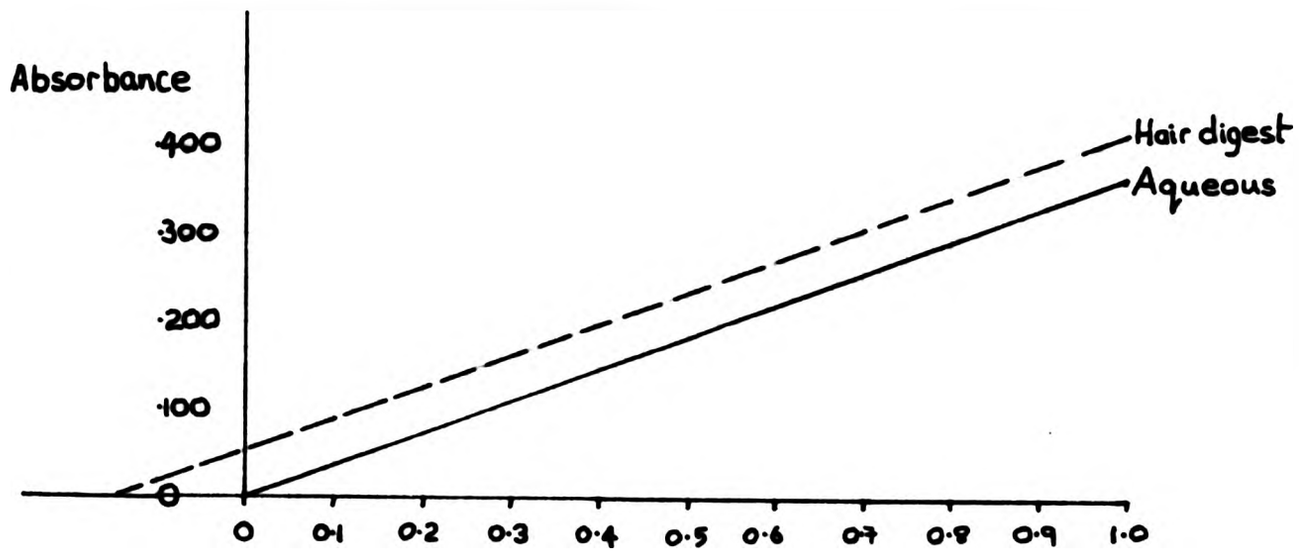


Figure 2.2.1: Aqueous and spiked hair digest calibration curves for flame aas

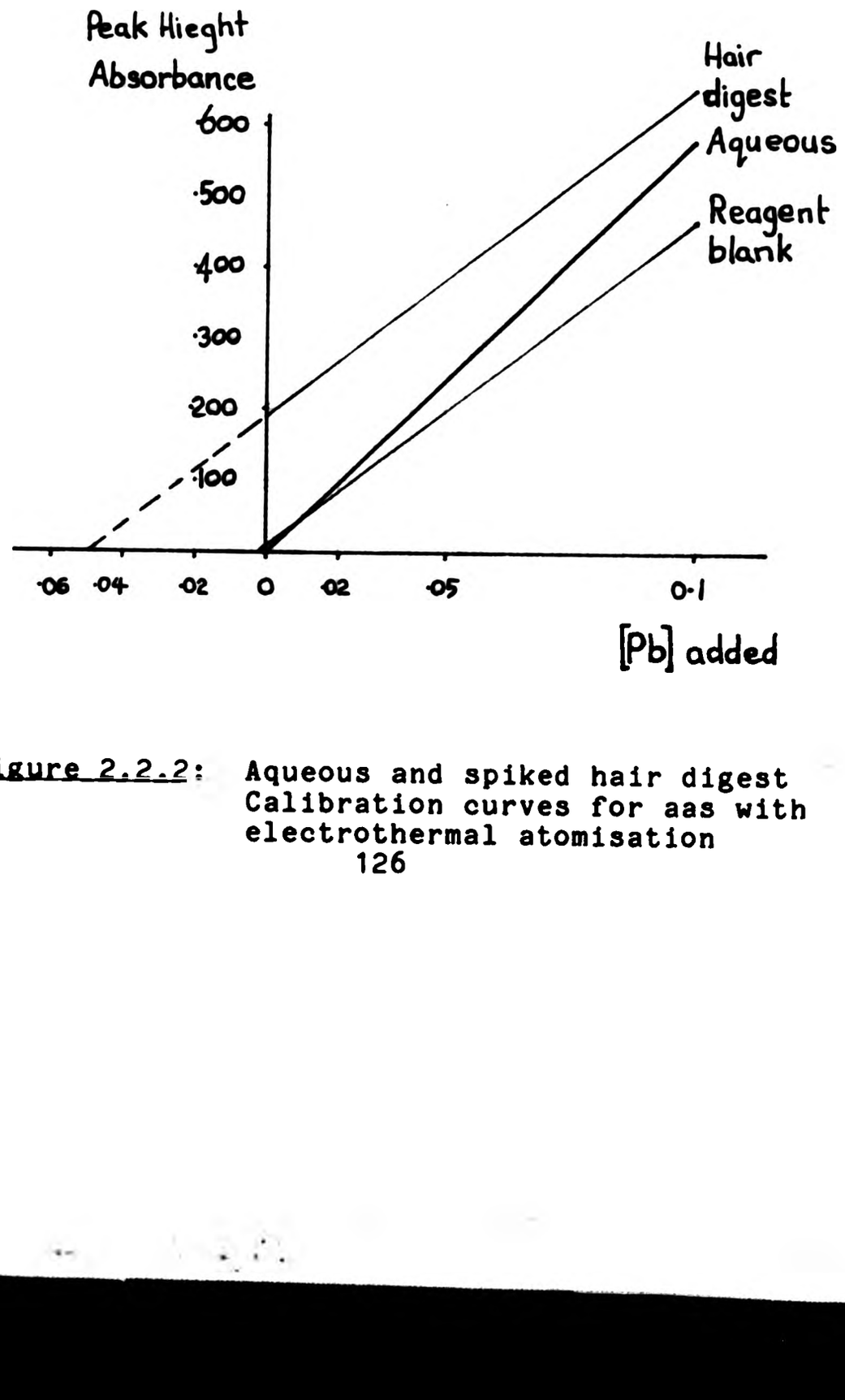


Figure 2.2.2: Aqueous and spiked hair digest Calibration curves for aas with electrothermal atomisation

2. The tube will not be evenly heated so the analyte may be atomised, and then condense, or react with other elements present in the matrix at cooler parts of the tube, to be re-atomized later.

3. The analyte may react with the surface of the tube, or the bulk of the matrix, thus inhibiting or enhancing atomisation. This effect may be reduced or increased in the presence of other matrix elements.

A number of hair digest solutions and a reagent blank were analysed by standard additions and compared with an aqueous calibration curve (fig 2.2.2). The spiked hair digest solutions were found to be parallel to each other but not to the aqueous calibration curve. This showed that calibration with aqueous standards was not satisfactory. It was noted that the spiked hair digest calibration curves were parallel to the spiked reagent blank calibration. It is possible that the interference which causes a slight reduction in sensitivity for lead is due to the presence of a relatively high acid concentration. Since the acid concentration is common to all hair digest and reagent blank solutions, and in this study the calibration curves were found to be parallel, it was considered that matched matrix calibration would

be most suitable for these analyses. The matched matrix standard were prepared by taking a small aliquot (ca 0.5 ml) from each sample to be analysed and combining them. The composite sample was then spiked with aqueous lead standards and analysed as described above. The concentration of lead in the calibration composite was used as one quality control index for the analyses. A sample of the composite was re-diluted and included at the beginning of each analytical run to check that the sensitivity did not vary. A record of the values obtained gives an indication of the precision of the analysis.

Five composite samples analysed on the same day but in different runs gave a mean concentration of 0.039 ppm with a standard deviation of .0045 ppm (RSD = 1.15%). This illustrates the excellent level of precision that can be achieved with the best modern instrumentation. A further quality control check was made by carrying over the calibration composite from a previous analytical session. This quality control check was placed at the end of each analytical run and was used in the same way as the first quality control check. In addition it would indicate any day to day changes, such as errors in calibrations, analyte loss, or contamination. This material was only diluted once per session so that it

could be preserved for as many sessions as possible. The level of precision achieved for one sample analysed on four separate occasions was 3.2% at a concentration of 0.068 ppm.

The overall performance of the technique was assessed in a recovery study. Early recovery studies were disappointing because of difficulty in obtaining true homogeneity of the sample. However, when a cryogenic mill became available the exercise was repeated. The cryogenic mill cools the sample in a bath of liquid nitrogen and pounds it with a reciprocating metal weight. The hair shatters at the cold temperature and a well-mixed powder is obtained.

The results achieved in therecovery study were quite satisfactory (Table 2.2.1). The concentration of lead in the hair sample was 14.4 ppm.

Careful selection of conditions and continued quality control has ensured that both flame and flameless atomic absorption spectrophotometry are reliable techniques for the analysis of lead in hair.

Table 2.2.1 Recovery Study Results

Addition	lead conc^{n} in solution	Coefficient of variation	Recovery
0	0.072	7.7%	-
1 ug Pb	0.169	4.4%	97.0%
2 ug Pb	0.265	5.1%	96.5%

2.3 Pilot Survey

2:3:1 Protocol

A limited pilot survey was mounted with the objects of testing the methodology, and studying the relationships between the concentration of metals in hair, and hair length.

Samples of sub-occipital hair were collected from sixth-form students from urban and rural schools in London and Hertfordshire. All the students also completed a short questionnaire about their hair and hair washing habits (Appendix C). Samples were cut from as close to the scalp as possible and labelled carefully at the proximal end with a piece of adhesive tape.

They were stored in sealed polythene bags prior to analysis by flame atomic absorption spectrophotometry.

2:3:2 Results

A full table of analytical results and questionnaire responses appears in Appendix D. The data obtained from individuals are considered in two populations; one "urban" from London suburbs and Letchworth, and a "rural" population who attended Hadham Hall School in Hertfordshire. The distribution of the first two 3 cm segment concentrations of lead and copper are illustrated graphically in figures 2.3.1 and 2.3.2.

The first observation that can be made is that none of the distributions conform to a normal distribution. This means that the use of the mean as a measure of central tendency and standard deviation as a measure of spread are inappropriate. The distribution of the data can also be presented as a cumulative frequency distribution and this can be used to provide a more suitable measure of central tendency, the median (50th. percentile), and interquartile range, which is the difference between the cut-off point for the lower 25% of scores and the cut-off point for the upper 25% of scores (fig. 2.3.3. and 2.3.4.).

They were stored in sealed polythene bags prior to analysis by flame atomic absorption spectrophotometry.

2:3:2 Results

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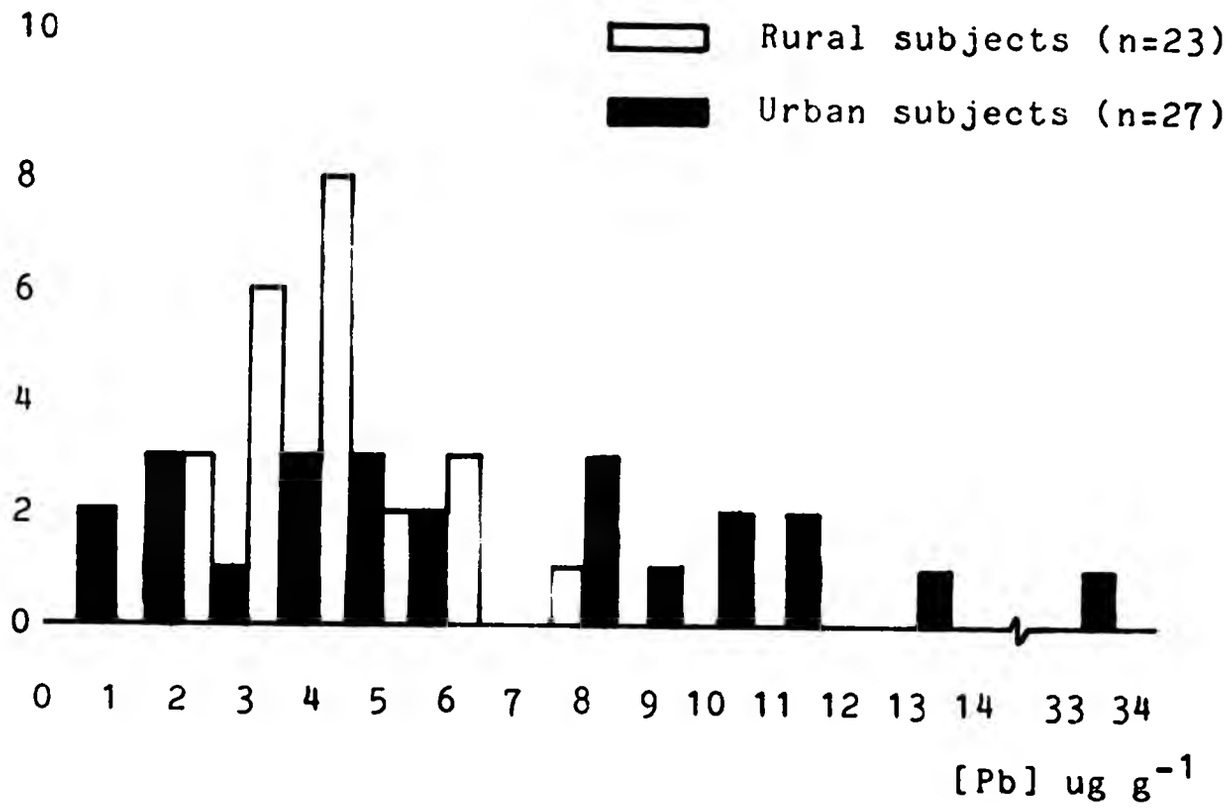


Figure 2.3.1 Histogram of lead concentrations in first two 3cm hair segments.

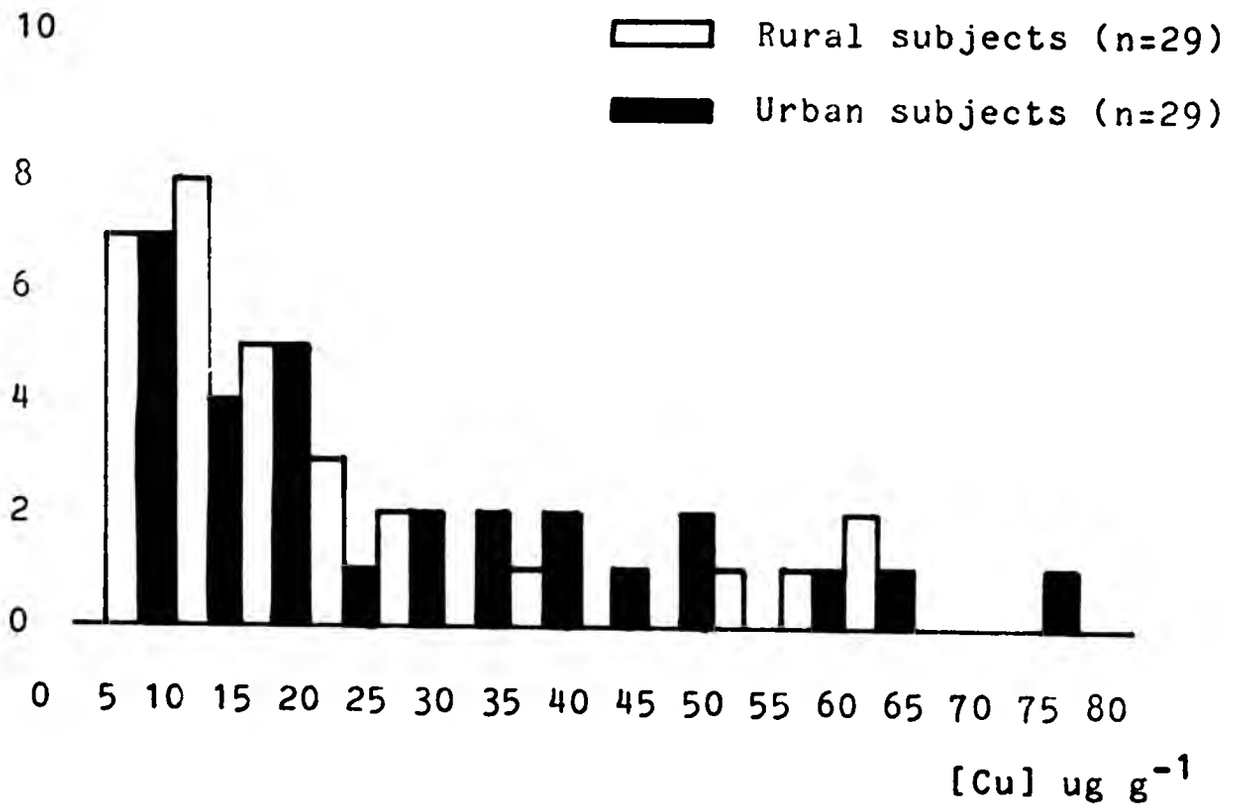


Figure 2.3.2 Histogram of copper concentrations in first two 3cm hair segments

The characteristics of the groups of data are presented in Tables 2.3.1. and 2.3.2. The data may also be divided in the first (proximal) and second (distal) 3 cm. segments and the data split in this way are presented in Tables 2.3.3. and 2.3.4.

Table 2.3.1 Distribution Characteristics of Lead in Hair ($\mu g\ g^{-1}$)

	Median	Interquartile Range
Rural	3.3	1.5
Urban	5.3	6.4

Table 2.3.2 Distribution Characteristics of Copper in Hair ($ug\ \mu^{-1}$)

	Median	Interquartile Range
Rural	15	9
Urban	23	40

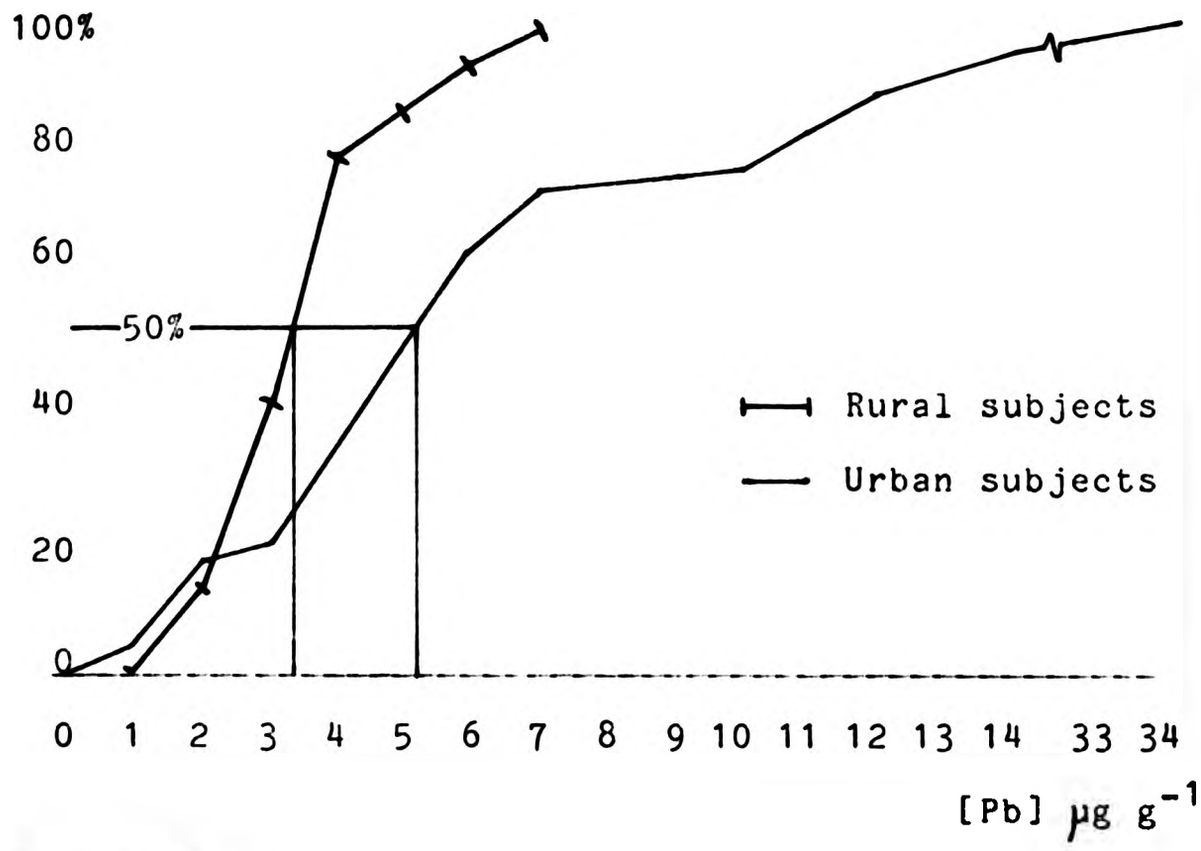


Figure 2.3.3 Cumulative frequency curve for lead in hair

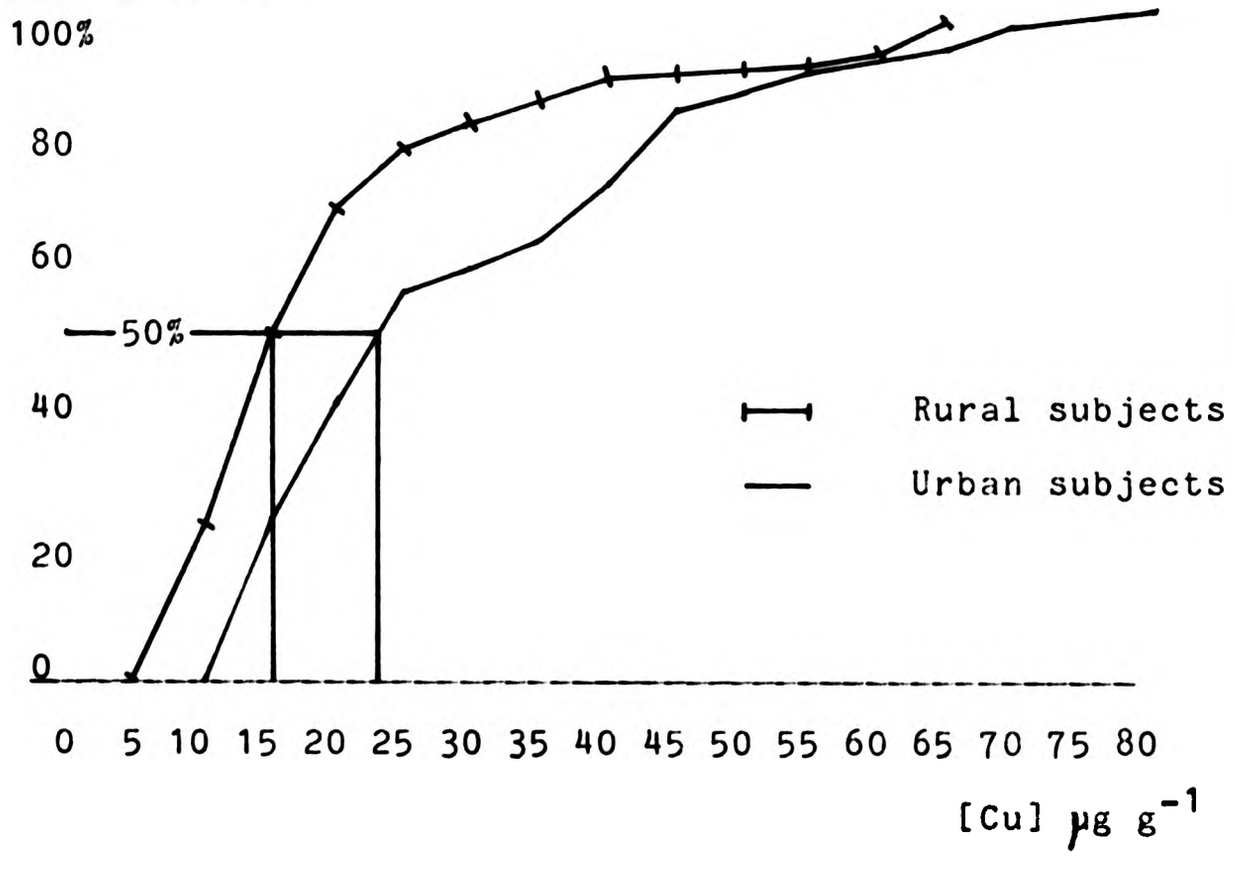


Figure 2.3.4 Cumulative frequency curve for copper in hair

Table 2.3.3. Proximal and Distal Lead Concentrations ($\mu g\ g^{-1}$).

	Section	Median	Interquartile Range
Rural	Proximal	3.2	1.2
	Distal	3.3	1.7
Urban	Proximal	4.8	4.2
	Distal	6.4	6.0

Table 2.3.4 Proximal and Distal Copper Concentrations ($\mu g\ g^{-1}$).

Copper	Section	Median	Interquartile Range
Rural	Proximal	15	7
	Distal	15	14
Urban	Proximal	23	17
	Distal	28	23

2.3.3 Discussion

The results can be interpreted more easily when presented graphically (figs. 3.2.5. and 3.2.6.). However, caution should be exercised when making interpretations because of the limited number of observations and the relatively large degree of variation within each group. The difference between proximal and distal values was investigated within each group using Wilcoxons matched pair test (Appendix E). The only significant difference was for 'urban' lead values ($p = 0.025$). The difference between 'urban' copper proximal and distal values was not found to be statistically significant although the median values certainly indicate this trend. Man-Whitney's test was used to compare data from various origins (Appendix E). All the 'urban' lead values were significantly greater than the 'rural' lead values ($p = 0.05$), and when the distal values were taken alone, an even more signficant difference was observed ($p = 0.01$). When all the copper proximal and distal data were put together the concentration in 'urban' hair was significantly greater than in 'rural' hair (0.025). This difference was not significant when proximal and distal values were considered separately.

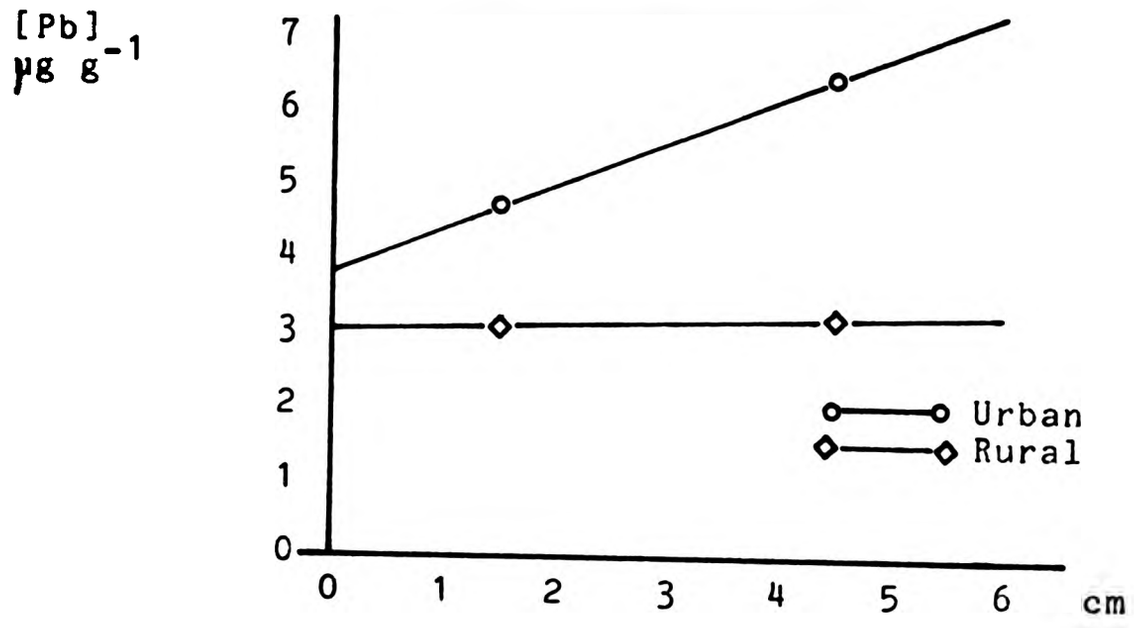


Figure 2.3.5 Change in Lead Concentration With Hair Length.

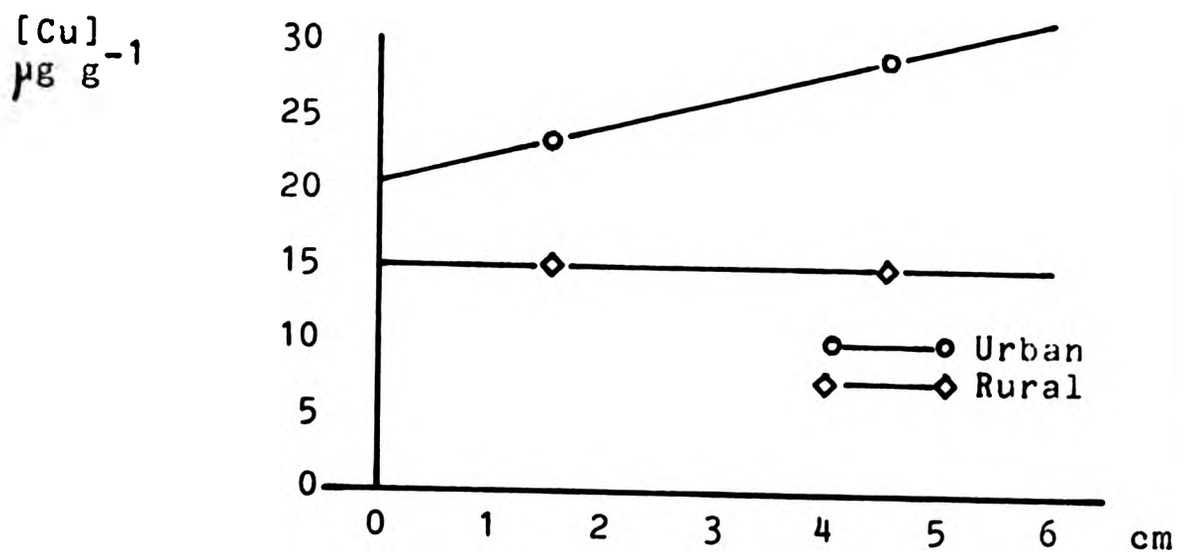


Figure 2.3.6 Change in Copper concentration with hair length.

Since the 'urban' distal lead values were significantly greater than the proximal values it was decided to calculate slopes and intercept values for these data (Table 2.3.5). These data were investigated using Mann-Whitney' s test and the slopes of the 'urban' data were found to be significantly greater than the slopes of 'rural' data ($p = 0.01$). There was no significant difference between the intercept values.

It can be concluded that the background concentration of lead in hair, as shown by the rural populations, is in the range 1.0 - 5.5 $\mu g\ g^{-1}$ (90% limits) and that concentrations may extend beyond 13 $\mu g\ g^{-1}$ (95% cut-off) in an urban population. The wide range of copper concentrations found in the hair of a normal population makes discrimination between 'exposed' and 'normal' difficult. Although there is some evidence that urban residents do show higher concentrations, the data has been summed to obtain a normal range of 0 - 65 $\mu g\ g^{-1}$ (90% limits) .

The significantly higher gradients of lead concentration with length (slopes) found in the urban residents may indicate greater exposure to environmental lead and suggests the potential merit of this value as an index of exposure. It is interesting to note that two negative slope values appear in the urban data and that

Table 2.3.5. Slope and Intercept Values for Hair Lead Data

Urban:

Rural:

almost half of the rural subjects show a negative slope. This indicates that the relationship between exposure and concentration gradient is not as simple as has so far been implied and that there are other factors at work.

Lead intercept values did not show a significant difference between groups. This may mean that either each group had a similar endogenous contribution to lead in hair or that the intercept value is not a reliable indicator of the endogenous component. The extrapolation to calculate the intercept point assumes a linear relationship between hair length and concentration. While hair has been shown to accumulate lead it seems unlikely that it will do so in a truly linear fashion.

This preliminary work has shown that there is some value in studying the way in which the concentration of lead changes with hair length, and that the relationship may be used to make observations about the environment in which populations live.

2.4 Factors affecting the uptake of lead by hair

2.4.1 Protocol

This part of the work was initially planned as a national survey of trace metals in hair. It was hoped that by studying hair lead profiles of groups of adolescents from various environments, some conclusions could be drawn about environmental factors which affect the uptake of lead by hair. Introductory letters, questionnaire forms, polythene sample bags and instructions were sent to twenty schools with sixth forms, throughout the United Kingdom. Only six schools responded to the request and of these only 33 samples were labelled at the proximal end and were large enough to be useable. Only 11% of the sampling kits sent out were returned with a useful sample! For examination of the effects of washing, etc on hair lead these data have been combined with the data from the preliminary survey (Section 2.3) in order to provide a better sample size ($n = 57$). In these cases the data base will be refered to as "combined data".

2.4.2 Results

A detailed Table of results appear at Appendix F. The results are summarised in Table 2.4.1.

Table 2.4.1 Results of National Survey of Hair Lead

Source	n	Median	Interquartile range
Bristol	7	8.0	13.7
Isle of Wight	8	4.4	4.4
Manchester	8	10.6	8.6
Swansea	7	5.4	6.0
Tobermory	2	10.0	2.4
Combined Data	57	5.6	7.3

$\mu g\ g^{-1}$

The combined data are presented graphically in figures 2.4.1 and 2.4.2. The median of the combined data is 5.6 $\mu g\ g^{-1}$ and the interquartile range 7.3 $\mu g\ g^{-1}$. The form of the distribution has a log-normal appearance but the cumulative frequency distribution does not give a straight line when plotted on log-probability graph paper (fig.2.4.3). Statistical tests which assume a fixed statitical distribution would be inappropriate for these data so non-parametric methods will be used. Slope an intercept values were calculated from the proximal and distal concentrations (Table 2.4.2).

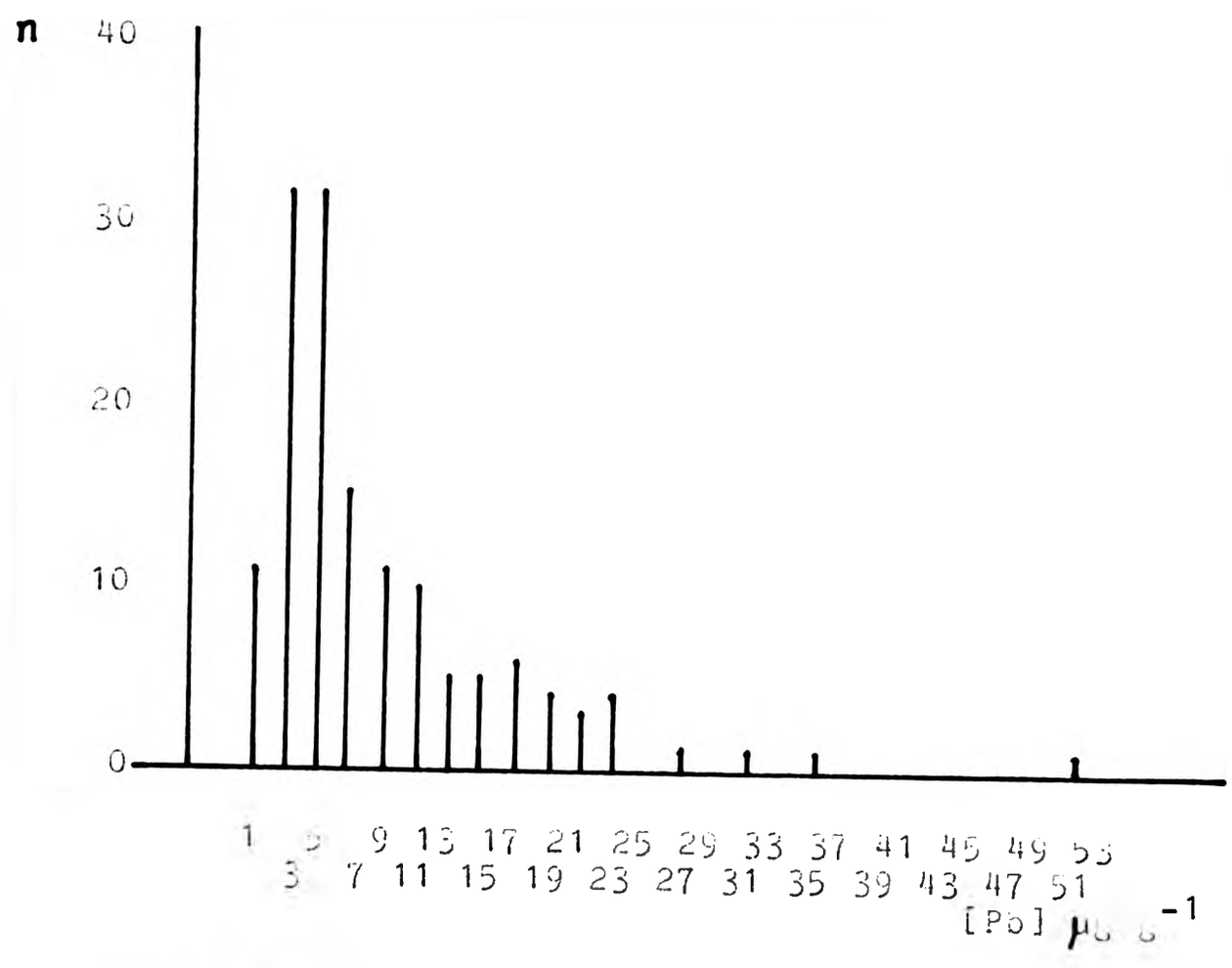


Figure 2.4.1: Concentration distribution of lead in hair.

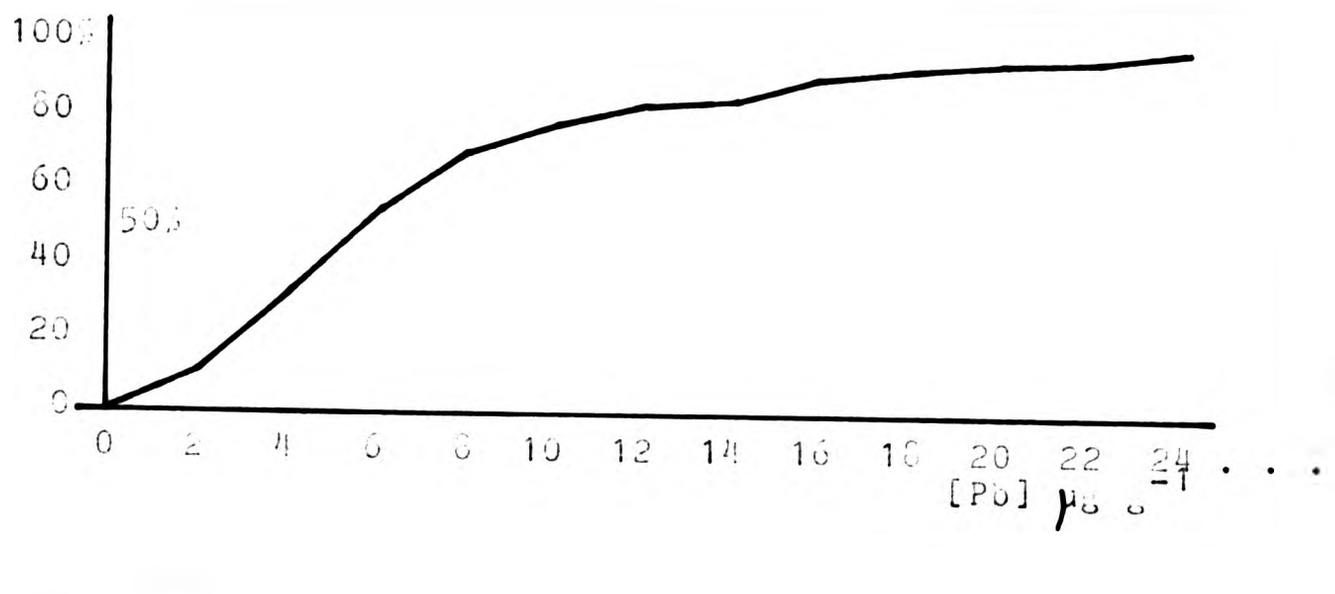


Figure 2.4.2: Cumulative frequency distribution for combined hair lead data.

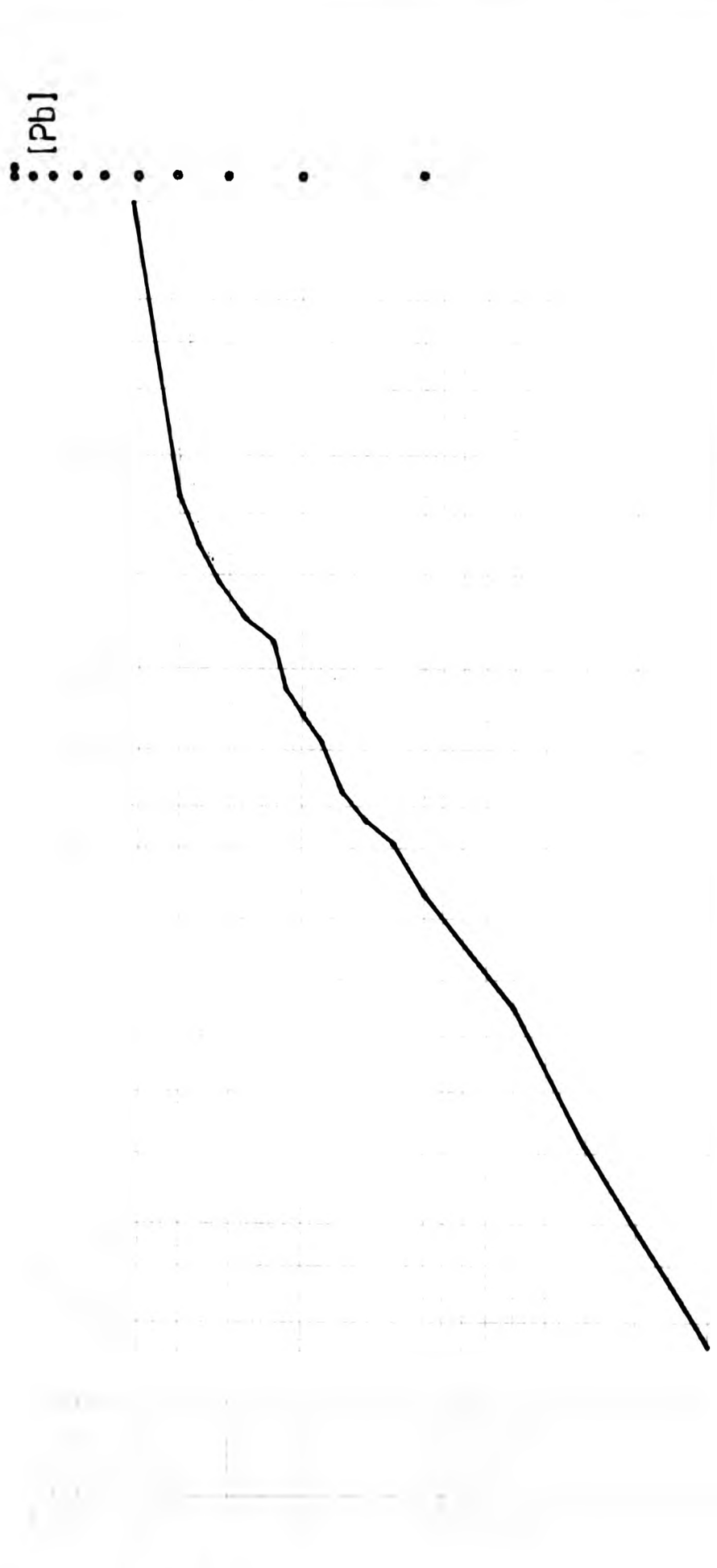


Figure 2.4.3: Log-probability curve of cumulative hair lead data.

Table 2.4.2 Hair Lead Values for each residential Location

BRISTOL

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
B101	3.7	4.5	.67	1.9
B087	3.7	45.1	4.47	-15.4
B105	18.1	4.5	-.01	5.16
B110	12.7	23.4	1.57	-7.2
B111	4.3	22.3	2.03	1.11
B112	3.5	21.2	4.47	.1
B113	3.1	21.4	.83	1.1

DAVIS

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
D102	9.2	9.2	-.13	9.2
D103	2.1	5.3	.61	3.3
D104	4.1	7.3	.61	1.5
D105	3.1	7.3	1.47	-2.8
D106	3.5	8.1	1.47	-2.4
D107	6.1	7.1	.15	.4
D108	7.3	4.8	-1.87	7.1
D109	8.1	17.3	1.21	-1.4
D110	3.5	7.1	.30	3.1

TORRANCE

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
T102	10.8	11.4	.27	11.2
T103	9.8	8.7	-.30	10.7

BASKET

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
B201	5.7	1.7	.53	5.4
B202	4.1	5.1	1.87	3.4
B203	1.3	1.3	.33	1.1
B204	3.5	8.1	2.13	2.7
B205	3.1	10.1	3.13	5.1
B206	4.9	11.7	3.53	7.1
B207	5.1	12.1	3.53	7.1

MANCHESTER

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
M101	3.5	5.5	.13	3.7
M102	3.9	7.1	1.83	1.1
M103	11.1	17.3	1.71	2.1
M201	11.5	21.5	2.17	13.1
M202	11.1	13.7	1.71	3.2
M203	13.1	23.7	3.17	2.5
M208	3.3	9.1	1.03	1.5
M209	11.1	15.7	1.71	3.2
M211	12.5	7.3	-.77	9.3

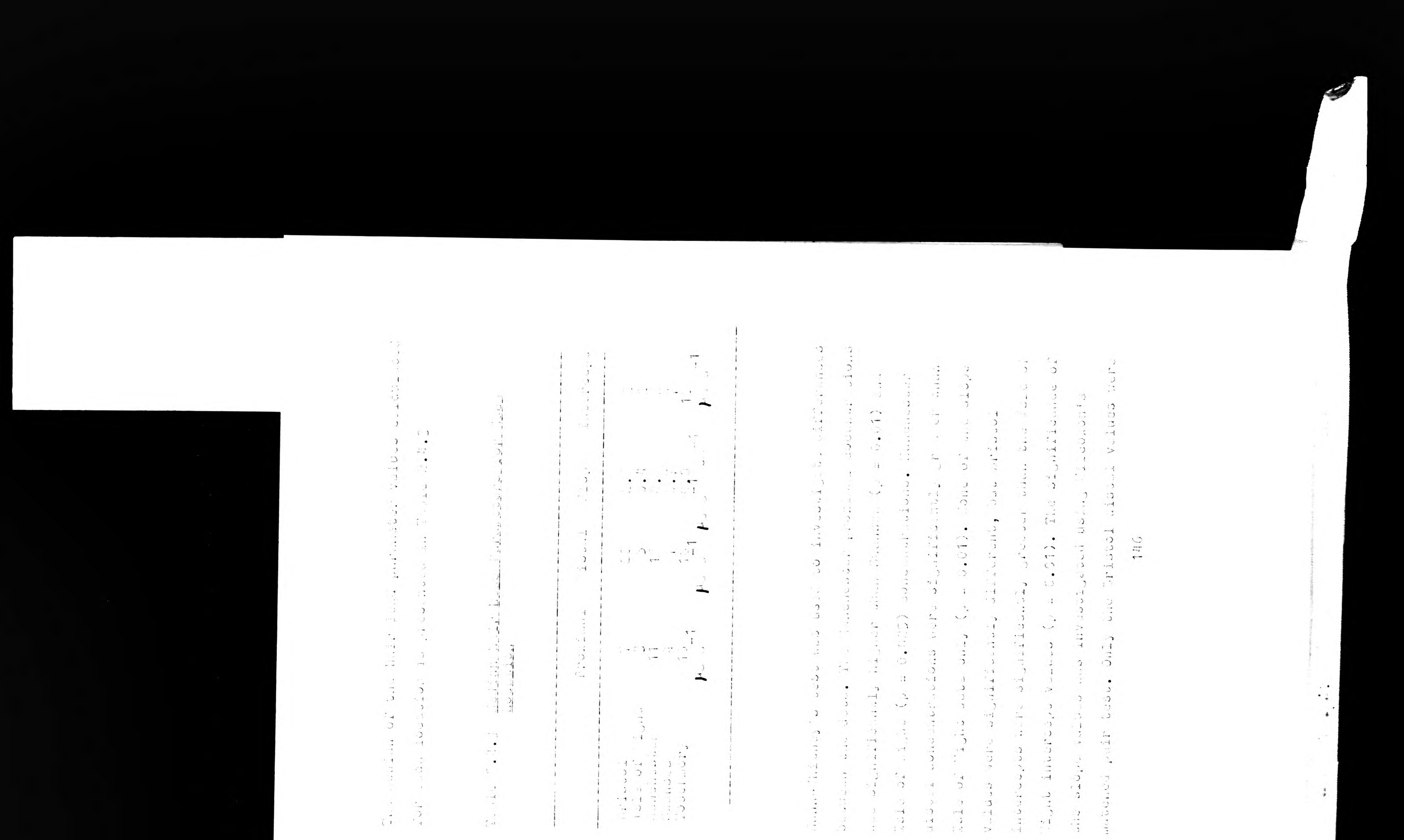


Table 2.4.4 Hair lead parameters for various environments (medians)

	Proximal	Distal	(P + D average)	Slope	Intercept
Urban	11.0	16.0	(14.0)	0.8	8.0
Inter-mediate	5.0	7.0	(6.0)	0.7	3.0
Rural	4.5	5.5	(5.5)	0.4	2.0
	$\mu g\ g^{-1}$			$\mu g\ g^{-1} cm^{-1}$	$\mu g\ g^{-1}$

Mann-Whitney's test was used to look for significant differences between each group and the rest of the data. There were no significant differences at this level. When the urban and rural data were considered without the intermediate data the mean concentration data was significantly different ($p = 0.005$). Both proximal ($p = 0.01$) and distal data ($p = 0.025$) when taken separately gave significant results. The intercept value of urban subjects was significantly greater than rural subject ($p = 0.05$), but slope differences were not significant.

These results and those of the preliminary survey indicate that hair lead may effectively discriminate

between groups living in urban and rural environments. If the 'normal' range established in the preliminary study are accepted (1.0 - 5.5) then all of the Manchester subjects had elevated levels. In the rural group, 3 out of 8 of the Isle of Wight subjects showed elevated levels but both of the Tobermory subjects had high levels (median = 10 $\mu g\ g^{-1}$).

Clearly the environment is an important factor affecting hair lead levels, but not the only one.

2.4.4 Effect of Water Quality on Hair Lead Levels

Water hardness is a factor which has been implicated in some lead poisoning cases and has been shown to be associated with hair lead levels (150). Heavy metals are more soluble in low pH waters collected in granitic catchment areas. Metal containing minerals are associated with igneous mother-rocks and these may be leached out by the soft water, giving a high background level. The antiquated plumbing of many older properties frequently includes lengths of lead piping. If soft water is allowed to stand in such systems, the lead may become dissolved and be present in drinking water. This is not such a problem in hard water areas where heavy metals have a lower solubility in the water.

Science teachers were asked to estimate the hardness of water in the catchment area of their school. The Isle of Wight has hard water, Tobermory, Manchester and Swansea have soft water, and Bristol has an intermediate supply. The results are presented in Table 2.4.5.

Table 2.4.5 Lead Parameters for Different Water Types

	Proximal	Distal	(P + D Average)	Slope	Intercept
Hard	3	5	(4)	0.4	1.0
Inter-mediate	7	20	(14)	2.8	2.0
Soft	8	11	(7)	0.6	6.5
			μg g-1	μg g-1cm-1	μg g-1

The only significant differences that occurred were between the soft and hard groups for proximal, distal, proximal and distal average and intercept values. It is interesting to note that the expected trend is only observed in proximal values and in the extrapolated intercept values. It may be that the effects of water type are only significant when the hair has just

emerged. As the hair grows other factors may become more important. One would need to have much more data from diverse locations to confirm this hypothesis.

2.4.5 Sexual Differences in Hair Lead Levels

The combined data was divided according to the sex of the participants and the hair lead parameters calculated (Table 2.4.6).

Table 2.4.6 Hair Lead parameters for Males and Females

	Proximal	Distal	(P + D average)	Slope	Intercept
Male	6	9	(8)	0.6	4
Female	4	5	(5) $\mu g\ g^{-1}$	0.4 $\mu g\ g^{-1} cm^{-1}$	3 $\mu g\ g^{-1}$

Although the females showed a generally lower level of lead in their hair, this difference was not found to be significant.

2.4.6 The Effects of Personal Hair-washing Habits on Hair Lead Levels

Each participant in the study completed a questionnaire (Appendix C), which included two questions about hair washing habits:

1. How often do you wash your hair?
2. How Long is it since you last washed your hair?

The differences between hair washing frequency groups is presented in Table 2.4.7.

Table 2.4.7 Differences in hair lead parameters for different wash frequency groups

	n	proximal	Distal	Slope	Intercept
Daily	4	6	8	0.1	4
Every other day	32	4	6	0.3	4
Twice weekly	17	9	16	1.8	6
			μg g-1	μg g-1cm-1	μg g-1

The 'daily' group was too small for there to be any significant differences. The distal concentrations and slope values of the 'twice weekly' group were

significantly greater than the 'every other day' group ($p = 0.01$ and 0.05), but the proximal concentrations were not significantly different. This is particularly important because it suggsts that hair washing affects the rate of accumulation rather than the initial concentration of lead in hair at the proximal end. It seems that the frequency of personal hair washing is an important consideration when considering the accumulation of lead by hair. The time elapsed since the hair was last washed seems to be a less important factor (table 2.4.8). There are no significant differences between any of the data.

Table 2.4.8 Differences in hair lead parameters with time elapsed since last wash

Days elapsed	n	Proximal	Distal	Slope	Intercept
0	7	6	9	0.6	4
1	17	4	6	0.2	3
2	11	4	5	0.5	4
3	8	4	7	0.4	3
4	1	4	6	0.6	3
5	1	7	20	4.5	3
			μg g-1	μg g-1cm-1	μg g-1

It appears that the cumulative effect of the frequency

hair washing is more important than the effect of the most recent wash.

It was felt that hair washing could be one of the most important factors affecting lead levels in hair, so a minor study was mounted. Samples of hair were obtained from eight volunteers before and after their normal hair wash. The concentration of lead in the first two 3 cm segments (proximal and distal) was determined and the slope and intercept values calculated (Table 2.4.9. a full table of results appears in Appendix F).

Table 2.4.9 Hair lead parameters before and after normal washing

	Proximal	Distal	Slope	Intercept
Before washing	5.2	5.7	0	5.2
After washing	3.5 $\mu g\ g^{-1}$	3.9 $\mu g\ g^{-1}$	0.2 $\mu g\ g^{-1}\ cm^{-1}$	3.4 $\mu g\ g^{-1}$

The values for the concentration of lead were significantly higher before washing ($p = 0.01$). Only the slope was not significantly affected. It is particularly important to notice that the intercept value was significantly reduced ($p = 0.025$). This indicates that

washing is able to remove lead from throughout the length of the hair and is not only effective on the distal segments where much of the accumulated lead is believed to be associated with 'surface contamination'. This finding casts doubt on the value of the slope and intercept as indicators of exposure and uptake, especially on an individual basis.

2.4.7 The Effect of Hair Colour on the Level Lead in Hair

Dividing the combined data according to hair colour did not produce any evidence of any relationship between hair colour and hair lead (Table 2.4.10).

Table 2.4.10: Effect of Hair Colour on Hair Lead Parameters

Colour	n	Proximal	Distal	Slope	Intercept
		$\mu g\ g^{-1}$		$\mu g\ g^{-1} cm^{-1}$	$\mu g\ g^{-1}$
Blonde	4	5	6	1.0	4
Light Brown	16	6	9	0.8	3
	14	4	6	0.9	3
Dark Brown	14	4	6	0.4	4
Black	2	4	6	0.4	4

2.4.8 Other factors which may affect the level of lead in hair

All participants in the study were asked if they had red hair (possibly associated with iron), if they used a dandruff control shampoo (some contain zinc pyrithione) and if they smoked at all. The number of positive replies was too small to be statistically useful.

Diet, season and other factors may also significantly affect the levels of trace metals in hair. Such variables would have to be taken into account on an individual basis if a more detailed study of the influences on lead levels in hair were to be undertaken.

2.4.9 Discussion

The combined data of lead in hair shows a highly skewed distribution which has a median at 5.6 $\mu g\ g^{-1}$ and an interquartile range of 7.3 $\mu g\ g^{-1}$. Nearly 50% of the subjects studied had hair lead levels above the background range established in the preliminary survey (1.0-5.5 $\mu g\ g^{-1}$).

Significant inter-regional differences in lead levels were found even though subject numbers were small, and

urbanisation of the environment was found to be an important factor. Soft water was also found to be associated with higher lead levels. Only two specimens were obtained from Tobermory but both had a relatively high lead concentration. It is suggested that this might be related to the soft water supply and that the small slope values reflect the rural environment. The Isle of Wight is also a predominantly rural area and produced a slope value similar to that of Tobermory. The lower lead concentrations found may reflect the hard local water supply. Manchester has soft water and a high level of urbanisation and here the slope was high and the lead concentrations was high also. The Bristol data are interesting because of the very high slope value and relatively low proximal lead concentration. The Head of Science stated that although the catchment was predominantly suburban, it included the intersections of the M4, M5 and M32 motorways and some aerospace industry installations. It is also about 5 miles to the west of the lead smelter complex at Avonmouth. These factors probably contribute to a relatively high exposure level, and the low proximal concentration could reflect a water supply which may be harder than the Head of Science suspects, if it originates in the Mendips.

There were found to be significant relationships between hair washing habits and lead levels. While this would call into question the validity of interpreting hair lead data on an individual basis, it need not detract from the data on a group basis, as long as hair washing habits are fairly uniform.

The presence of negative slope and intercept values has cast doubt upon the usefulness of these parameters. It was noted that negative slope values occurred more often when the author was not present at the time of sampling. It is suspected that in these cases the proximal end had not been correctly labelled. However, since it has been found that normal hair washing can significantly reduce the levels of lead found in the hair, it may be that some of these negative values could be genuine. Negative intercept values are quite obviously erroneous and in the statistical tests have been adjusted to zero. While intercept values have been found to follow expected trends throughout this work, no attempt has been made to interpret their significance.

2.5 A Local Survey of Lead in Children's Hair

2.5.1 Introduction

Children are at a higher risk from airborne lead than are adults (169). They have a much higher rate of pulmonary ventilation and smaller blood volume than adults. They are also at greater risk of exposure to environmental lead because of childhood habits like pica and putting dirty fingers in mouths. So for a given level of exposure young children might be expected to show elevated blood lead values and toxic effects more frequently (169).

2.5.2 Protocol

Hair samples were obtained from 2-4 year old children attending pre-school playgroups in West London. The area was specifically chosen because of the proximity of the Cromwell Road/M4 extension which is one of the busiest arterial routes into London. Parents were asked to give the address of each child.

Hair samples were sectioned into the first two 3 cm distal portions and analysed according to the procedure described in Section 3.5.

2:5:3 Results

The results of the analyses and the calculated slope and intercept values are presented in Table 2.5.1. The median value and interquartile range for each parameter is presented with Table 2.5.2.

Table 2:5:1 Hair Lead parameters for Chiswick children

Sample	Proximal	Distal	Slope	Intercept
CC03	10.0	15.0	1.67	7.5
CC04	8.0			
CC07	39.0	42.0	1.00	37.5
CC07	12.0	22.0	3.33	7.0
CC09	10.0	24.0	4.67	3.0
CC10	29.0	45.0	5.33	21.0
CC11	19.0	20.0	.33	18.5
CC12	29.0	38.0	3.00	24.5
CC13	13.0	11.0	-.67	14.0
CC14	30.0			
CC15	14.0	15.0	.33	13.5
CC16	13.0	17.0	1.33	11.0
CC18	13.0	17.0	1.33	11.0
CC19	7.0	12.0	1.67	4.5
CC20	17.0	12.0	-1.67	19.5
CC22	14.0	15.0	.33	13.5
CC23	33.0			
CC25	9.0	11.0	.67	8.0
CC26	13.0	15.0	.67	12.0
CC27	22.0	23.0	.33	21.5
	ppm	ppm	ppm/cm	ppm

Table 2.5.2 Hair lead parameters for Chiswick children

	n	Median	Interquartile Range	
Proximal sections	20	14.5	8.5	$\mu g\ g^{-1}$
Distal sections	17	17.5	10.0	$\mu g\ g^{-1}$
(All sections)	37	(15.5)	(10.0)	$\mu g\ g^{-1}$
Slopes	17	1.1	1.8	$\mu g\ g^{-1}\ cm^{-1}$
Intercepts	17	12.5	11.0	$\mu g\ g^{-1}$

The location of each child's place of residence was plotted on a map of the area together with downwind vectors to the nearest main road, and major through routes (figure 2.5.1).

2:5:4 Discussion

All of the children in this survey had hair lead levels higher than the upper limits of the background range established in the preliminary survey (5.5 $\mu g\ g^{-1}$). The median concentration of lead in the hair of the children in this study was more than twice that found in the 'urban' adolescents in the preliminary survey, and was greater than any of the median concentrations of lead in the hair of adolescents in the National survey. The data are not directly comparable, but they suggest that the Chiswick children are exposed to higher levels of environmental lead than are the adolescents.

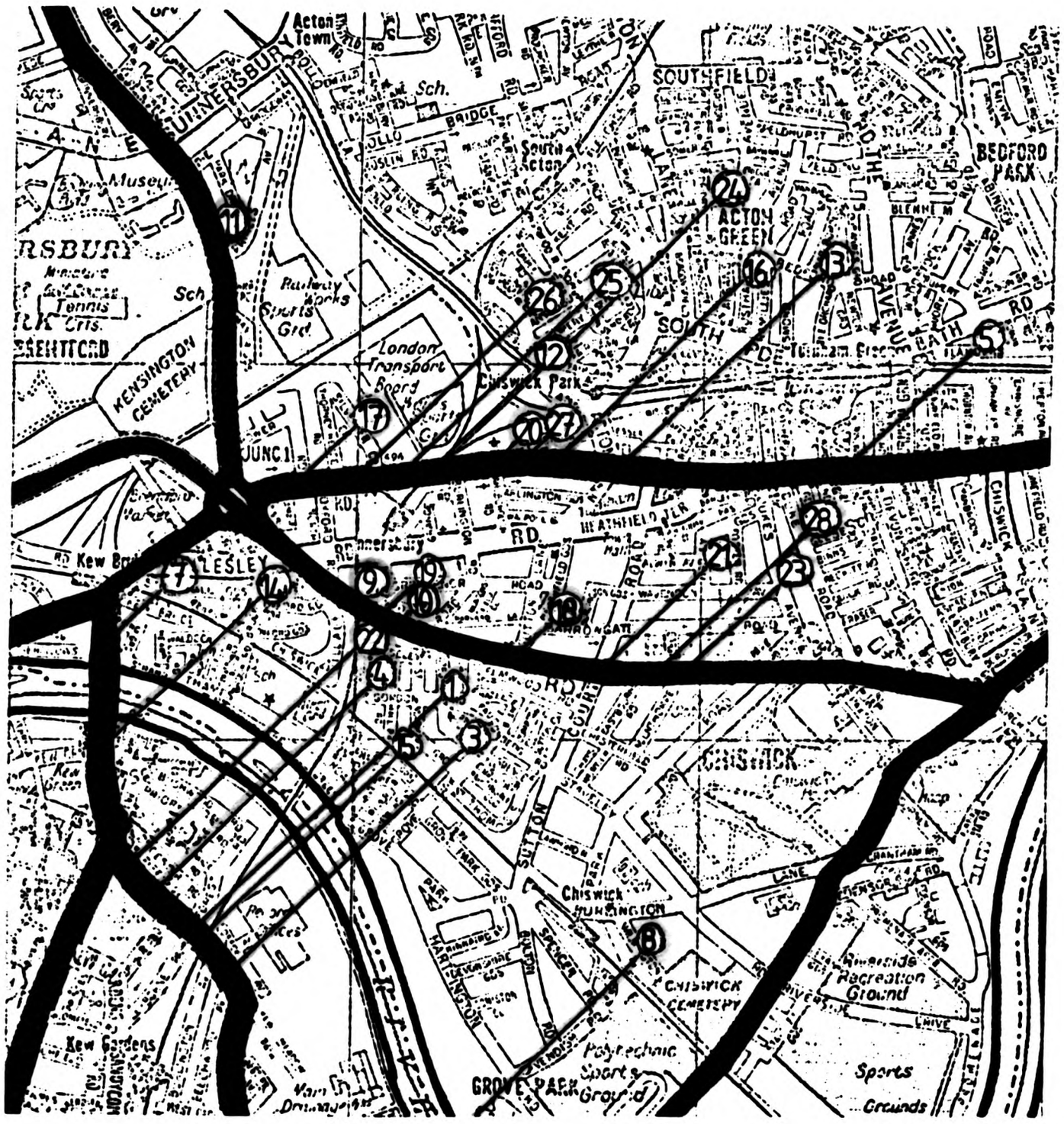


Figure 2.5.1 Map of sampling area.

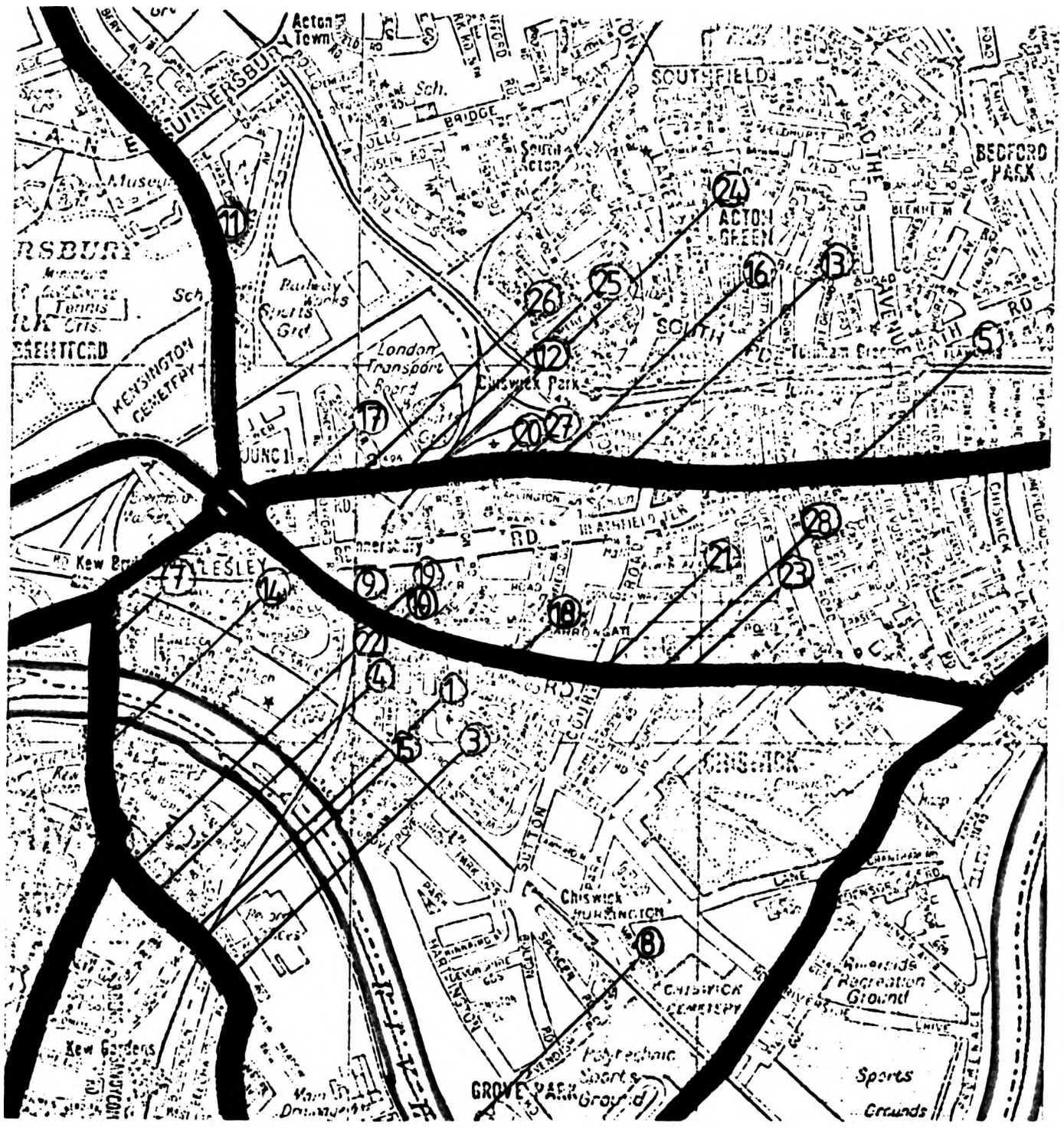
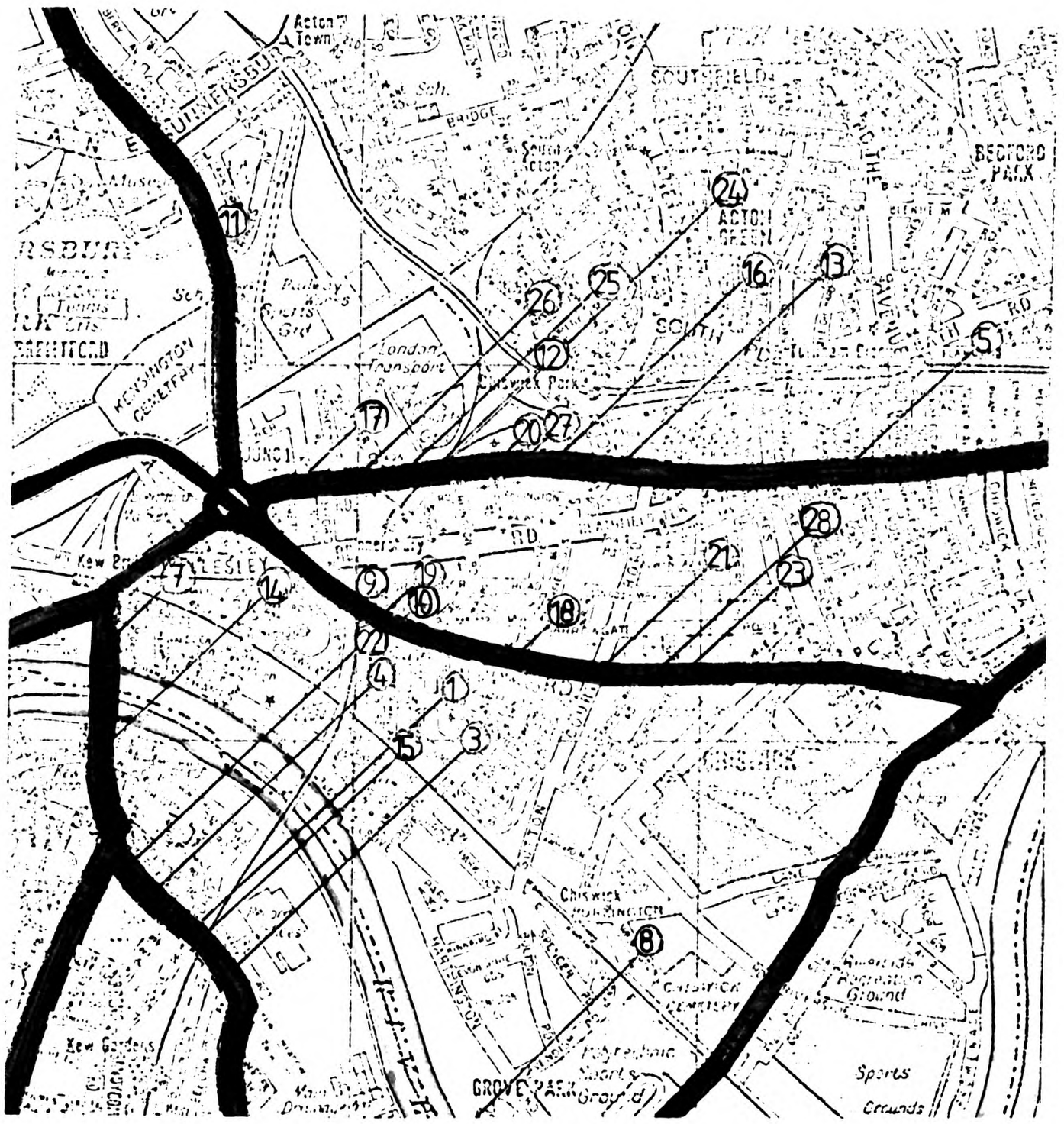


Figure 2.5.1 Map of sampling area.



○ Suggested location to visit from major tourist route

Figure 1.3.1 Map of Study Area

The children came from predominantly middle-class backgrounds (subjective assessment) and there is no significant industry in the area. The most likely sources of environmental lead are the busy main roads that run through the area. The concentration of lead in the hair of each subject was compared with the downwind distance to the nearest main road using the regression graphics programme (Appendix B).

Only when proximal and distal data were taken together was a significant correlation observed (figure 2.5.2) (p=0.025). While the regression equation showed the expected negative change in lead concentration with increasing distance from the reoad, the scatter-diagram shows that the data fell into three fairly distinct groups:

1. Short distance, intermediate lead.
2. Intermediate distance, high lead
3. High distance, low lead.

It was felt that these groupings were caused by errors in estimating the distances from the road. The analysis was repeated using the distance to the nearest main road irrespective of direction (fig. 2.5.3.) The correlation was again significant at a low level (p = 0.025) and the grouping seen previously had been dispersed.

LINEAR REGRESSION

SAMPLE : CHISWICK CHILDREN: DISTANCE / PROXIMAL + DISTAL

Y = 23.35 + -.16 X

COEF. OF CORRELATION = .333 N= 37

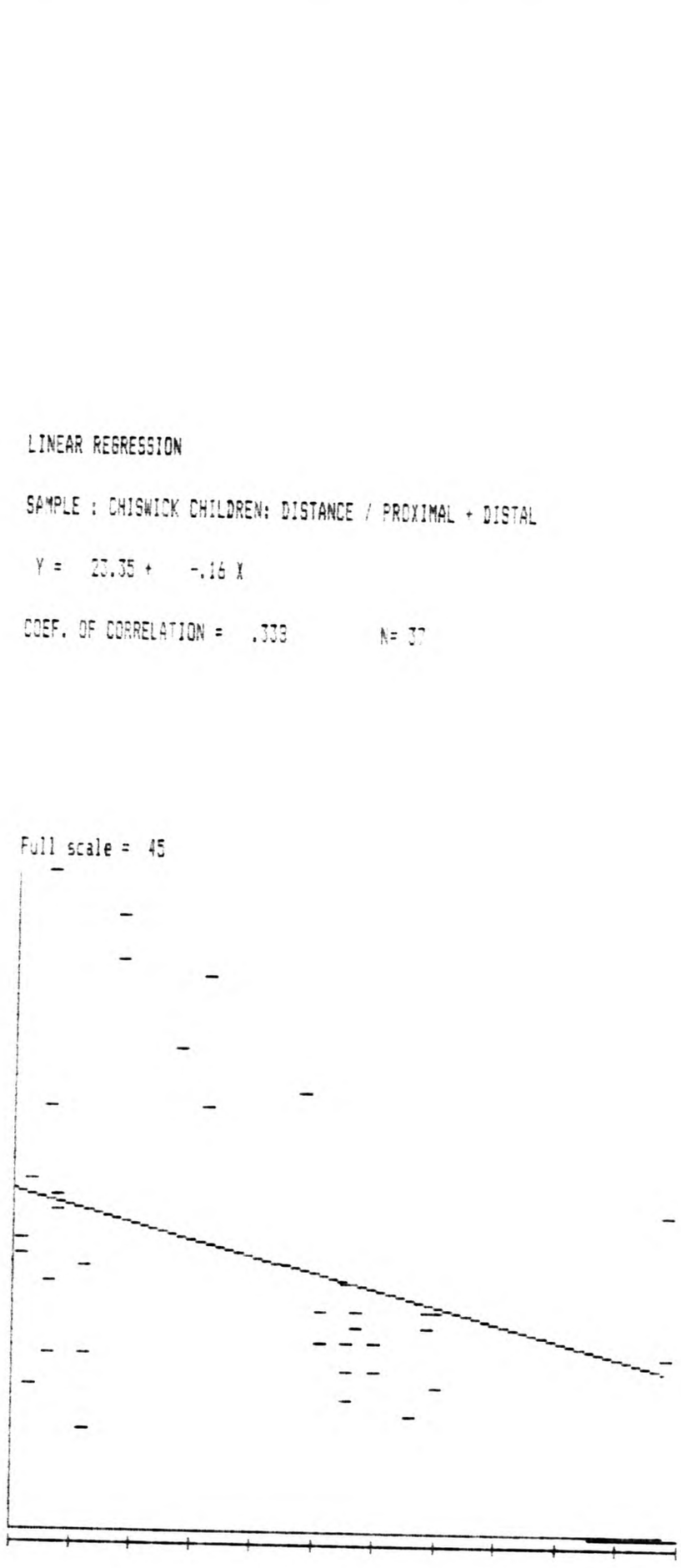


Figure 2.5.2 Relationship between hair lead in children and distance downwind to nearest major road.

LINEAR REGRESSION

SAMPLE : CHISWICK CHILDREN DISTANCE / HAIR LEAD

Y = 22.81 + -.33 X

COEF. OF CORRELATION = .322

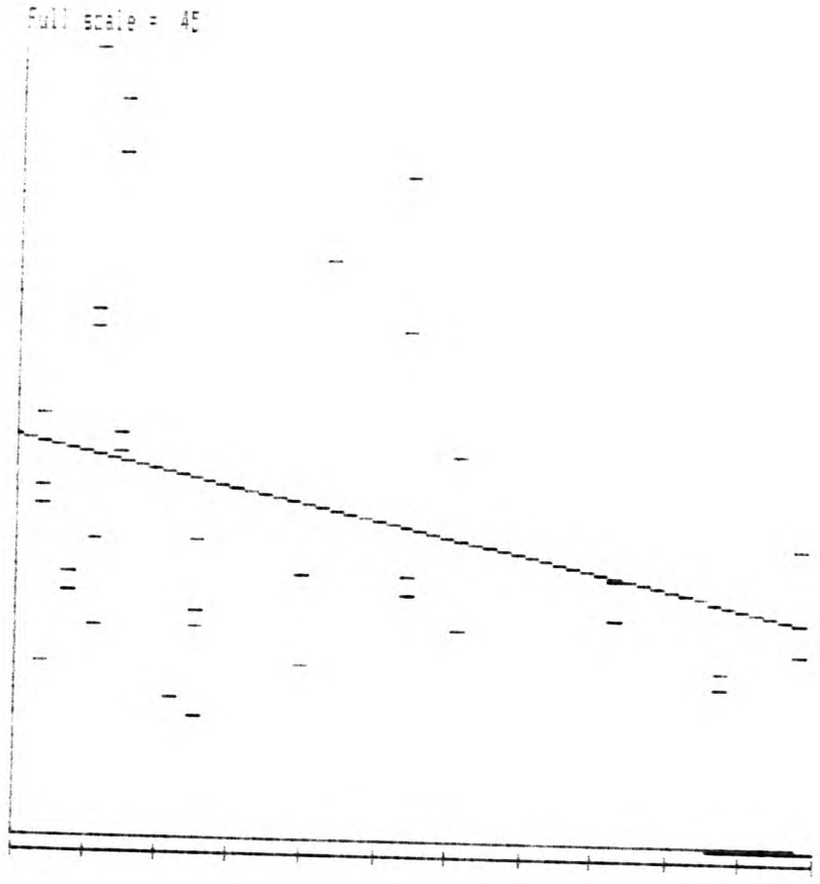


Figure 2.5.3 Relationship between hair lead in children and distance to nearest main road.

Considering the high degree of scatter in the data it would be inappropriate to allocate much confidence in the regression equation. However, it is interesting to note that a similar equation was generated by each analysis: [Pb] in hair = -0.33(distance from road)+23.

Analysis of slope and intercept data with respect to distance from the road did not provide any significant correlations. However, the regression equations generated by the data were negative as expected.

This study suggests that infants living in urban areas have a higher risk of exposure to environmental lead than other groups. Major roads are significant sources of lead in the hair of these children.

2.6 The Direct Determination of Lead in Hair

2.6.1 Introduction

Several authors have observed an increase in the concentrations of some trace metals from the roots to the tips of hairs (99, 157, 165). The early parts of this work have been based on an underlying assumption that the concentration of lead in hair increases from root to tip in a near linear way, that reflects the type

and degree of exposure. It was considered highly desirable that a more detailed investigation of this relationship should be made.

The modern graphite furnace atomic absorption system is sufficiently sensitive to detect the levels of lead found in single hairs (159). Alder and his co-workers have made extensive studies of the technical aspects of single strand hair analysis for toxic metals (159, 166, 167, 168). They found that solid sampling gave fast and reliable results, but required painstaking attention. They also identified a serious calibration problem; the analysis is destructive of the sample, so standard additions cannot be used. Silk and animal hairs, artificial fibres, protein materials and ion-exchange resins have all been investigated as possible calibration matrices but with only limited success. In earlier studies in this thesis, using wet chemical ashing followed by graphite furnace aas, it was found that the presence of acid in the digest solution caused the most significant matrix effect. In solid sampling no acid is required and it is hoped that matrix effects will therefore be minimal. An absolute determination of the concentration in hair (although desirable) is not necessary so long as reliable relative values are obtained.

Access to two lead working firms was obtained with the kind assistance of Dr M A Samuel of the Employment Medical Advisory Service. Single strands of hair were plucked from the back of the head of each subject, and at the same time bulk hair samples were obtained (for wet ashing and analysis). Samples were placed in sealed polythene bags and a staple was inserted at the proximal end. Dr Samuel took a blood sample from each subject (5 ml potassium EDTA). Single strands of hair were also obtained from colleagues at the Health and Safety Executive, Occupational Medicine Laboratories for use as a control (unexposed) group.

2.6.2 Analytical techniques

Individual hairs were cut into 9mm segments and analysed directly using the method described in section 3.7. Whenever it was possible more than one hair from the same head was analysed and the results combined.

2.6.3 Solid sampling results

All the results of segmented analysis of single hairs was processed using the "Regression Graphics" programme (Appendix B), written specifically for the purpose, to be run on the Trivector Microcomputer system at the HSE

Occupational Medicine Laboratories. This programme calculates a linear regression equation from the data and calculates Pearsons' correlation coefficient (R). The statistical significance of the correlation coefficient must be found from tables. The programme then plots all the data points and joins them up (or their arithmetic averages if more than one value is entered). The line of the calculated linear regression equation is then plotted over the top. In the segment analysis diagrams the atomic absorbance signal is generated on the y-axis against hair length on the x-axis. Space does not permit all the diagrams created in this way to be shown, but a selection is presented below (Figs 2.6.1 to 2.6.4). Note that in all these diagrams the x-axis has been set to a maximum of 6 cm but that the y-axis varies from 0.5 to 3.0 absorbance units. A 2mm segment length was used for some of the samples which generated very high signals, if a extra hair was available. All the segment analysis results, converted from absorbance units to nanograms and corrected for different segment lengths are presented, along with correlation coefficients and levels of significance in Table 2.6.1. Median values for slopes and intercepts were calculated using statistically significant data only (Table 2.6.2).

LINEAR REGRESSION

SAMPLE : HSE 05

Y = 15.46 + 14.42 X

COEF. OF CORRELATION = .767

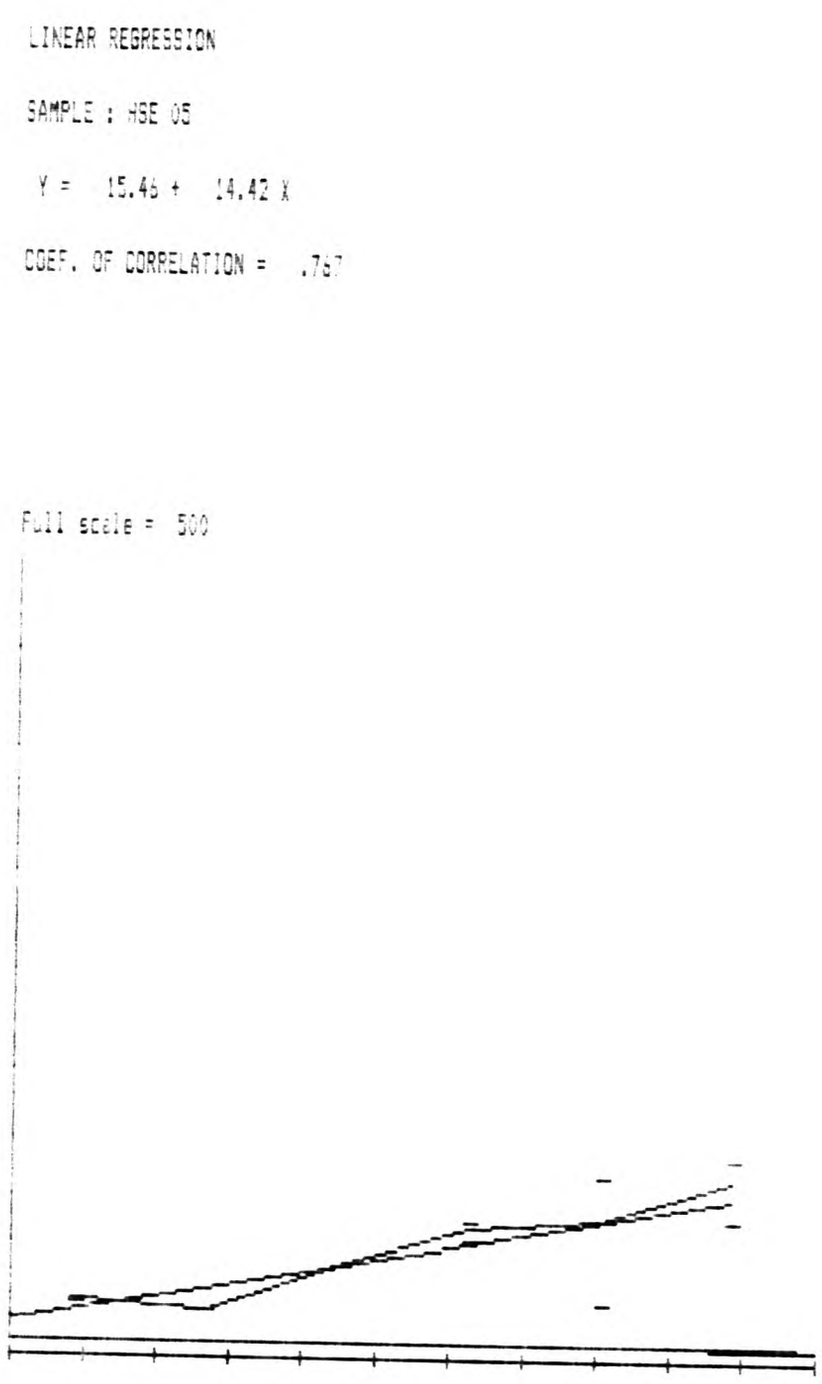


Figure 2.6.1 Hair lead segment diagram for a normal subject.

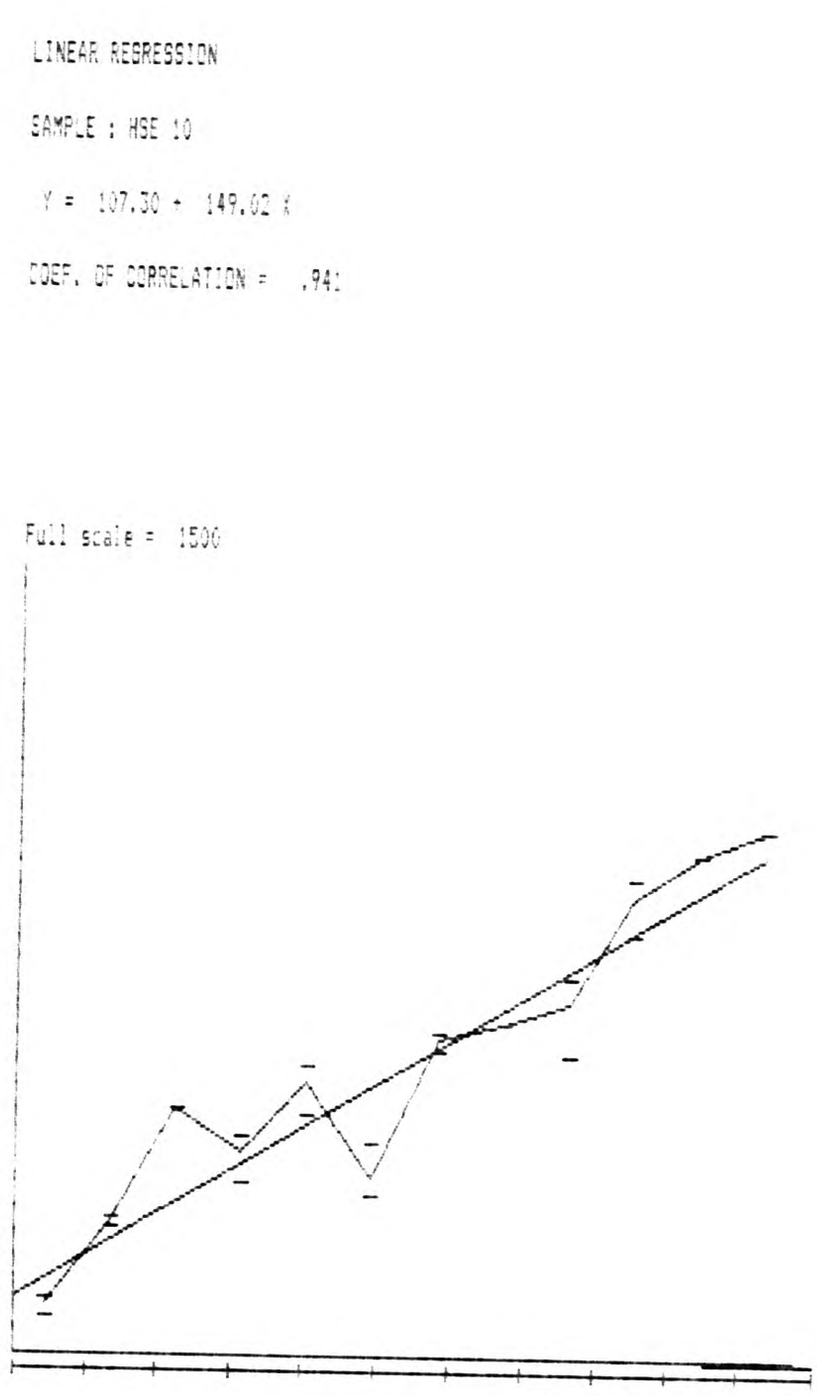


Figure 2.6.2 Hair lead segment for normal subject,

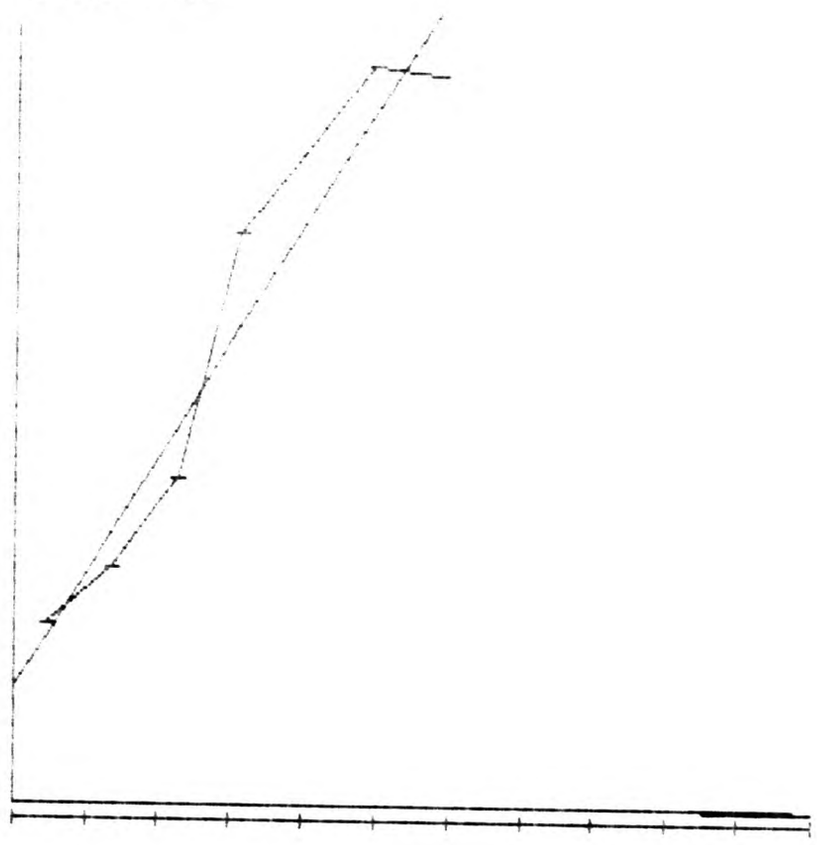
LINEAR REGRESSION

SAMPLE : G/K 03

Y = 371.17 + 658.40 X

COEF. OF CORRELATION = .969

Full scale = 2500



Full Scale= 6

Figure 2.6.3 Hair lead segment diagram for occupationally exposed subject.

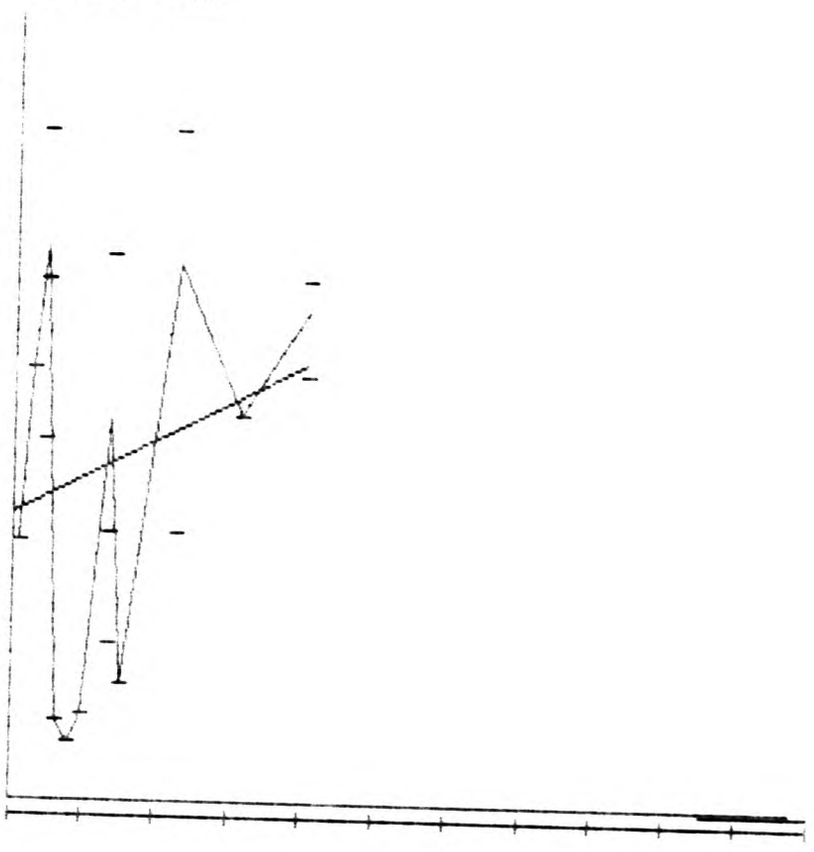
LINEAR REGRESSION

SAMPLE : G/K 05

Y = ******* + 257.24 X

COEF. OF CORRELATION = .232

Full scale = 3000



Full Scale= 6

Figure 2.6.4 Hair lead segment diagram for occupationally exposed subject (2 mm hair segments).

Table 2.6.1 Results of Hair Solid Sampling Analysis

Sample		n	Slope	Intercept	'R'	Sig.%
HSE	02	9	0.06	0.04	.74	1.0
	03	16	0.02	0.20	.23	-
	04	17	0.13	0.10	.86	0.1
	05	9	0.02	0.03	.77	1.0
	07	9	-0.27	1.85	.56	-
	10	19	0.28	0.20	.94	0.1
	12	12	0.07	0.07	.94	0.1
	13	17	0.19	0.43	.80	0.1
	15	15	0.02	0.10	.59	1.0
	16	12	0.08	0.36	.78	0.1
	17	11	0.15	0.22	.77	1.0
	18	10	0.05	0.03	.75	1.0
	19	12	0.17	0.72	.88	0.1
	20	17	0.15	0.10	.93	0.1
	21	17	0.03	0.13	.50	2.5
G/G	02	5	-0.31	3.33	.31	-
	03	20	0.56	0.68	.94	0.1
	04	14	0.54	1.61	.84	0.1
	06	11	-0.34	2.80	.39	
	08	8	0.30	0.28	.97	0.1
	11	6	0.49	2.66	.67	-
	14	5	0.03	2.26	.26	-
	15	5	0.31	0.30	.99	0.1
	51	5	0.97	4.67	.50	-
	52	20	0.89	1.70	.83	0.1
	53	16	0.32	0.31	.96	0.1
	54	5	0.86	1.35	.94	1.0
	55	6	0.11	0.07	.55	-
			$ng\ cm^{-2}$	$ng\ cm^{-1}$		

Table 2.6.1 cont'd.

G/K	01	9	0.44	0.46	.93	0.1
	02	9	0.41	1.59	.79	1.0
	03	5	0.47	1.76	.39	-
	04	5	0.58	2.21	.62	-
	05	17	0.86	3.33	.23	-
	06	5	2.86	3.33	.50	-
	07	5	- 0.50	3.33	.58	-
	08	6	1.22	0.69	.97	0.1
	09	5	0.51	2.74	.48	-
	10	22	0.32	0.38	.87	0.1
	11	9	- 0.18	1.17	.60	5.0
	12	5	0.97	2.68	.84	5.0
	13	9	0.29	0.63	.70	2.5
	14	21	0.13	0.09	.78	0.1
			ng cm^{-2}	ng cm^{-1}		

Table 2.6.2 Median slope and intercept values for each group (interquartile range in parenthesis).

	Slope, $ngcm^{-2}$	Intercept $ngcm^{-1}$
Normal	0.09 (0.11)	0.10 (0.23)
Exposed	0.45 (0.31)	0.73 (1.42)
p	0.01	0.01

These results indicate that both slope and intercept values can effectively discriminate between occupationally exposed and normal individuals when a statistically significant correlation between hair length and lead concentration is obtained.

About 62% of the segment analysis results show a significant positive correlation between lead concentration and hair length. In most cases where a correlation was not found the lead concentration was very high. This might reflect differences in the pattern of uptake at high environmental levels or may be due to analytical errors. It is not possible to dilute a solid hair sample to bring it into the calibration range so analysis at a less sensitive line was attempted. Sample GG 11 was analysed at 217.0 nm and at 283.3 nm (Figures 2.6.5 and 2.6.6). The less sensitive line gave a much more satisfactory result for this sample. It was not possible to employ the 283.3 nm line throughout because the majority of the samples had already been analysed (destructively) at 217.0 nm.

The slope and intercept values obtained for each individual were compared using the "regression graphics" computer programme. The scatter diagram showed that the data were highly skewed towards lower values so a log-normal transformation was performed on each set of data. A high level of correlation was obtained from the log-transformed data ($p=0.001$) indicating a high level of association between the variables (Fig. 2.6.7). Slope values are almost certainly associated with levels of exposure to environmental lead.

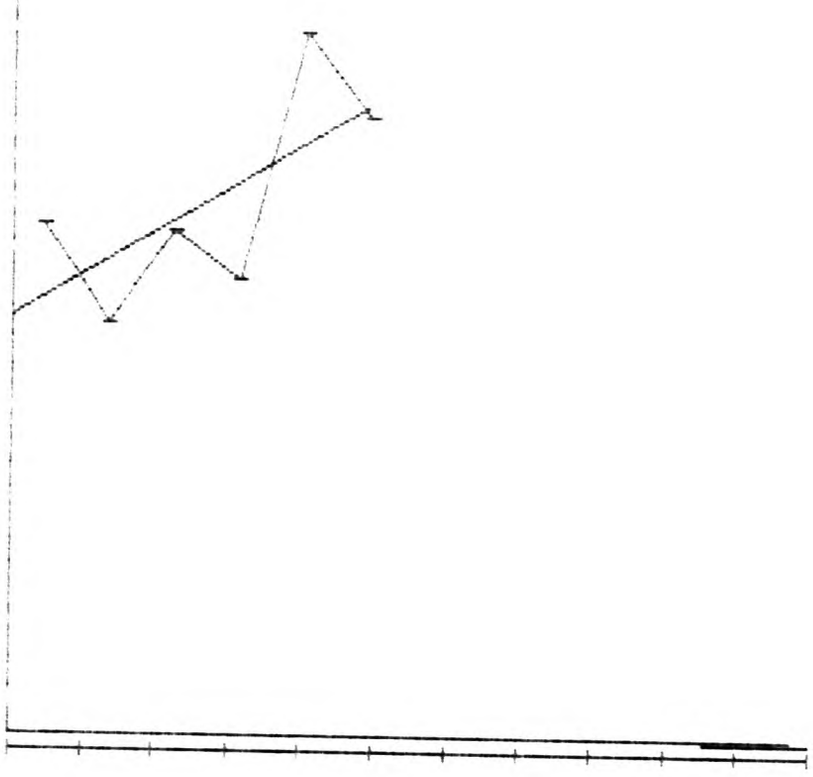
LINEAR REGRESSION

SAMPLE : S/G 11

Y = 796.84 + 147.89 X

COEF. OF CORRELATION = .671

Full scale = 1500



Full Scale= 6

Figure 2.6.5 Sample GG 11 Analysed at the 217.0 nm lead line

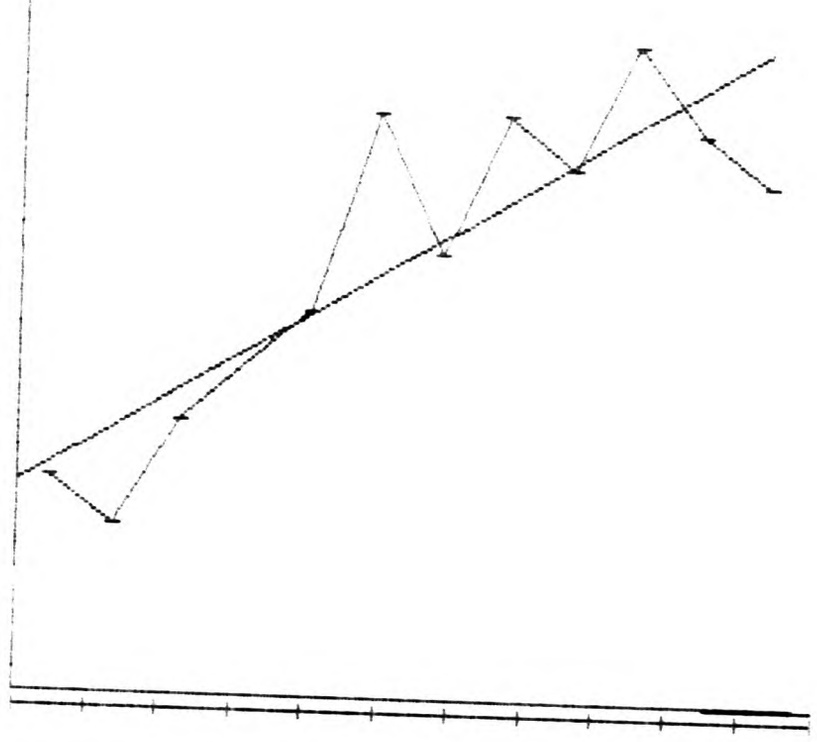
LINEAR REGRESSION

SAMPLE : G/G 11 - - - - - - - - **283.3 nm**

Y = 267.96 + 97.53 X

COEF. OF CORRELATION = .359

Full scale = 1000



Full Scale= 6

Figure 2.6.6 Sample GG 11 analysed at the 283.3 nm lead line.

LINEAR REGRESSION

SAMPLE : SLOPE / INTERCEPT - ALL SEGMENTS n=25

Y = .89 + .87 X

COEF. OF CORRELATION = .916

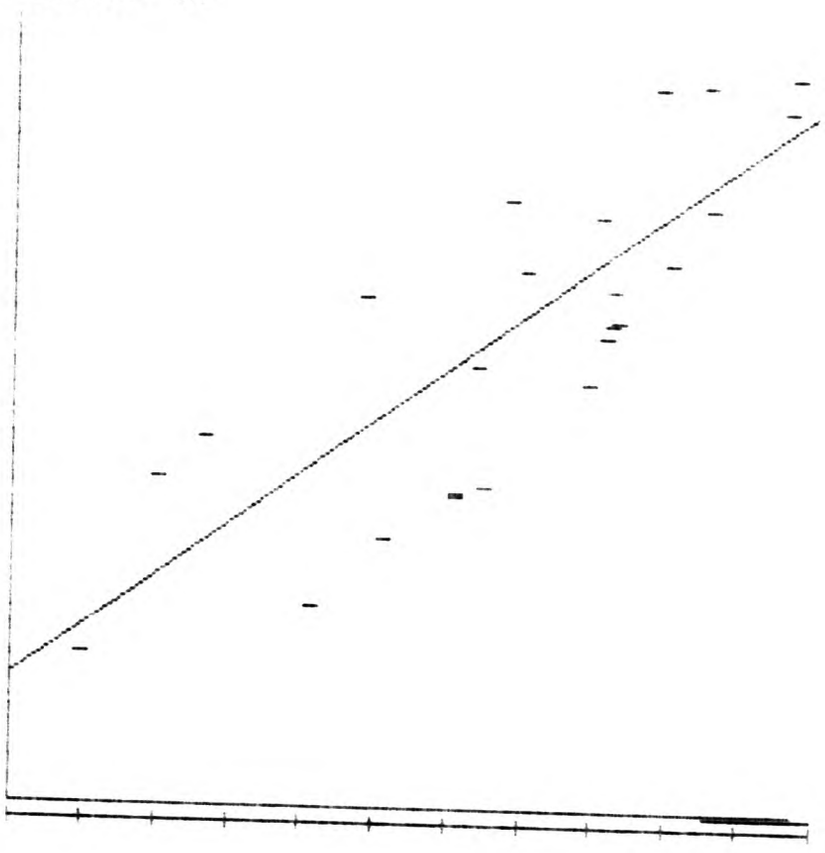


figure 2.6.7 Relationship between hair slope values (y-axis) and intercept values (x-axis)

If the hypothesis that the extrapolated intercept values reflect the endogenous component is true, then these results indicate that high levels of endogenous hair lead are associated with high exposure levels and that intercept values may be useful indicators of uptake.

The regression equation indicates that an intercept value of about 0.02 ng cm^{-1} would be associated with a slope value of zero. This figure may be taken to be the background endogenous concentration when there is no environmental exposure, and will reflect that component contributed by the diet.

2.6.4 Results of analysis after wet ashing

Parameters obtained for the analysis of lead workers' hair by wet chemical digestion are presented in Table 2.6.3. The data are summarised in Table 2.6.4. For some subjects only sufficient hair was available for proximal concentration to be estimated.

Table 2.6.3 Hair Lead Parameters, Exposed Group.

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
S011	121.0	183.0	22.67	105.0
S302	144.0	237.0	47.67	71.0
S303	5.0	61.0	3.00	46.0
S304	23.0	59.0	1.00	12.0
S015	550.0			
S306	22.0	20.0	-1.00	23.0
S307	3.0	7.0	-1.00	8.0
S308	12.0	1.0	-4.00	23.0
S09	28.0	50.0	7.00	17.0
S311	36.0	77.0	17.00	91.0
S012	5.0	4.0	-1.00	5.0
S313	27.0	25.0	1.00	8.0
S06.4	18.0	21.0	1.00	16.0
S315	20.0			
S016	12.0	11.0	-1.00	13.0
S317	20.0	24.0	4.00	12.0
S018	17.0	14.0	-3.00	18.0
S05.	22.0			
S052	106.0			
S053	54.0			
S054	35.0			
S055	21.0			

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
S001	28.0	12.0	-4.00	34.0
S002	240.0	166.0	-26.00	280.0
S003	55.0	77.0	11.00	33.0
S004	24.0	24.	-3.00	39.0
S005	449.	592.0	17.00	177.0
S006	230.0			
S007	113.	124.0	1.00	9.0
S008	71.			
S009	16.0			
S010	18.0			
S011	14.0	14.0	0.	14.0
S012	84.0	51.0	-11.00	82.0
S013	12.0			
MEANS :	115.7	96.7	-6.44	125.0
	SDF	SDB	SDB/SF	SDF

Table 2.6.4 Median values of hair lead parameters (Exposed group)

	Median	Interquartile Range
Proximal concentration	26	66
Distal concentration	34	53
Slope	0.4	22
Intercept	19	67

The high number of negative slope values occurring in the data is extremely disappointing. The level of association between proximal and distal values was investigated using the regression graphics programme. The data was found to be highly skewed towards smaller values so natural logs were used (Figure 2.6.8). The high level of association found indicates that the analytical technique is probably reliable. It is felt that the high incidence of negative values may be due to a failure to correctly identify the proximal end. No correlation between slope and intercept data was found when all the data was considered. However, when slope values less than 1 were excluded a signficiant degree of correlation was observed for the natural log data ($p=0.025$) (Figure 2.6.9). This shows that if any relationship between slope and intercept values does exist then it may be being obscured by some weakness in either the technique or the theory.

2.6.5 Results of blood lead analyses

The individual results of the blood lead analyses was presented in Table 2.6.5. The median concentration is 31 µg/100 ml and the interquartile range 28 µg/100 ml. The distribution did not appear to be skewed towards high or low values and so was assumed to approximate to the normal distribution. Blood lead concentrations in a

normal population are usually less than 20 µg/100 ml and 25% of these values fall into that range. Only two subjects showed dangerously high blood lead levels (GC 5, GG 52) and one (GG 52) showed signs of interference with haemoglobin synthesis, as indicated by a slightly raised free erythrocyte protoporphyrin level. The data represent a typical occupationally exposed group.

2.6.6 Hairlead/blood lead inter-relationships

Too few of the significant segment analyses and wet chemical analyses coincided to make comparisons of slope and intercept data possible. A significant correlation was observed when the natural logs of significant segment intercepts were compared with the natural logs of wet ashed proximal concentrations ($p=0.025$). However, the scatter diagram shows a disappointingly high level of spread (Figure 2.6.10).

The data were used to assess the degree of association between blood lead and hair lead levels as estimated by the two techniques. All the hair lead data was observed to be skewed toward lower values so natural logarithms of the concentrations have been used throughout. Blood lead values appear to approximate to a normal distribution so they have not been subjected to a log-transformation.

Table 2.6.5 Lead Exposed Workers' Blood Lead Concentrations

Sample		Blood Lead Concentration	Sample		Blood Lead Concentration
GG	1	37	GK	1	42
	2	30		2	48
	3	34		3	43
	4	38		4	34
	5	68		5	40
	6	31		6	29
	7	8		7	30
	8	15		8	33
	10	39		9	36
	11	17		10	23
	12	8		11	17
	13	19		12	22
	14	24		13	14
	15	24		14	11
	16	12			
	17	20			
	18	17			
	51	20			
	52	69			
	53	34			
	54	41			
	55	25			

µg/100 ml

In the scatter-diagrams the hair data has been multiplied by a factor of 100. This is because the graphics part of the programme cannot cope with negative values created by the log-transformation of values less than one.

A significant correlation was achieved between the lead intercept concentration values achieved by segment analysis and blood lead concentrations ($p=0.05$). Examination of the scatter diagram reveals a somewhat

weak association and the regression equation may not be valid (Figure 2.6.11). No correlation was observed between segment analysis slope values and blood lead concentrations.

When blood lead concentrations were compared with proximal values by wet ashing (hair data not log-transformed) a very high degree of correlation was observed ($p=0.001$). When the scatter diagram was examined the hair lead data were found to be skewed towards lower values, as expected (Figure 2.6.12). When the natural logs of the hair lead data were considered an even higher/degree of correlation was observed (Figure 2.6.13). The "least squares best fit" regression equation for the blood lead/hair lead association found was:

$$\ln [Pb]_{hair} = 0.06 [Pb]_{blood} + 2$$

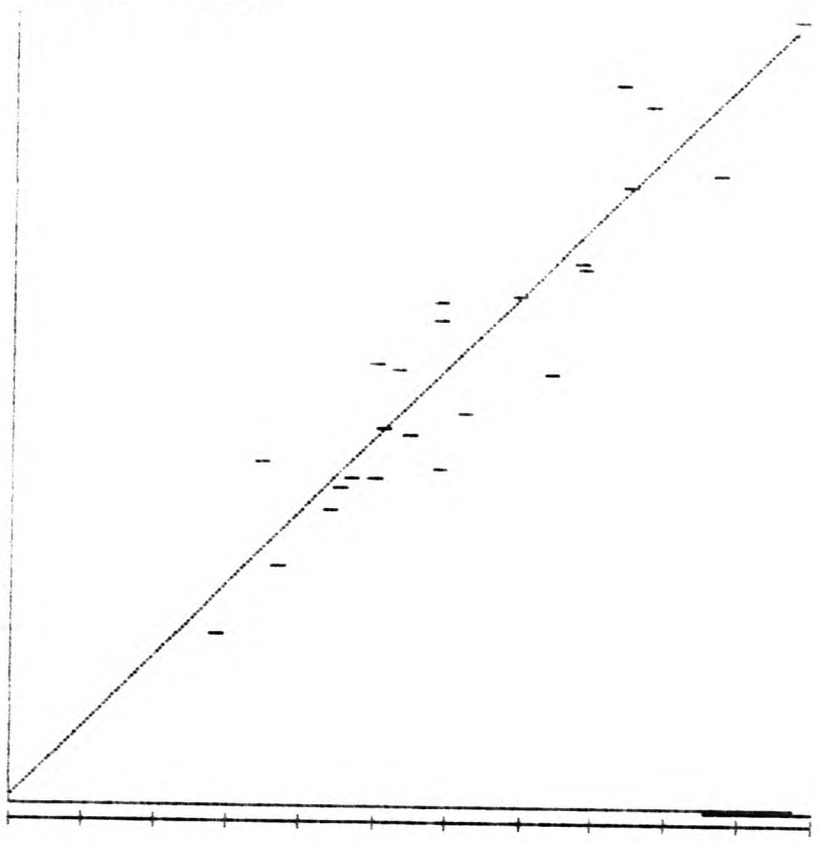
LINEAR REGRESSION

SAMPLE : WET PROXIMAL VS. WET DISTAL (TO SHOW DEGREE OF ASSOCIATION ONLY)

Y = .06 + 1.02 X

COEF. OF CORRELATION = .929 N= 24

Full scale = 6.3875063



Full Scale= 6.10702289

Figure 2.6.8 Association between proximal and distal concentration (natural Logarithims)

LINEAR REGRESSION

SAMPLE : WET SLOPE VS.WET INTERCEPT [VALUES >1 ONLY]

Y = .98 + .93 X

COEF. OF CORRELATION = .706 N= 9

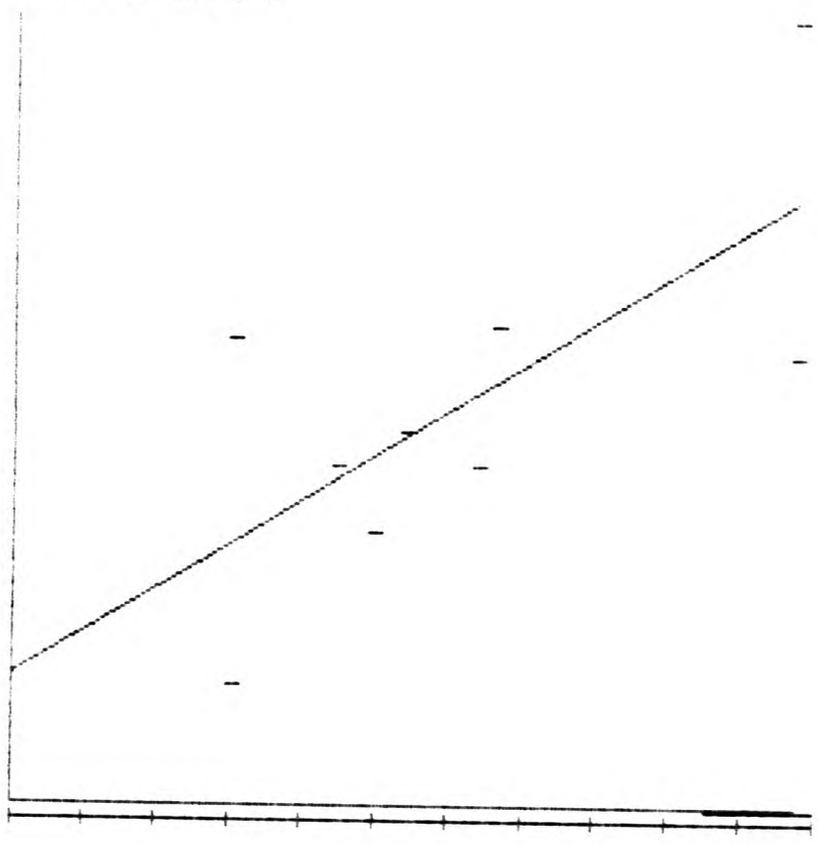


Figure 2.6.9 Correlation between slope and intercept values obtained by wet ashing and analysis.

LINEAR REGRESSION

SAMPLE : SEGMENT INTERCEPTS (x10) / WET PROXIMALS

Y = 2.09 + .90 X

COEF. OF CORRELATION = .545 N= 14

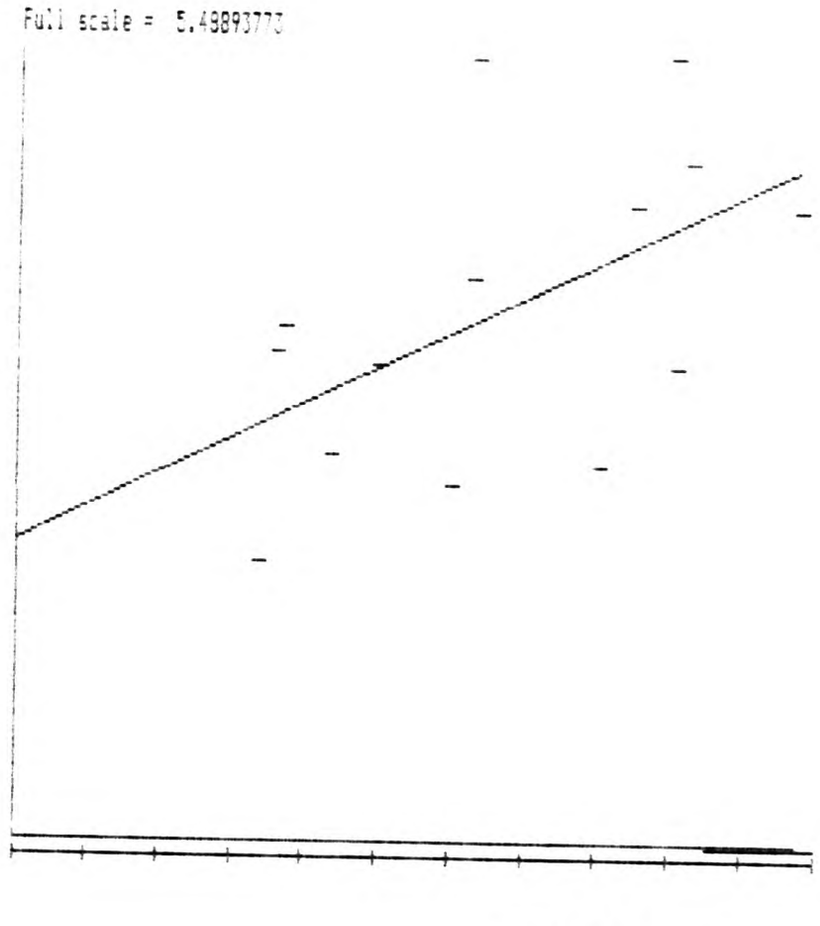


Figure 2.6.10 Correlation between significant segment intercepts and wet ashed proximal values.

LINEAR REGRESSION

SAMPLE : BLOOD LEAD / SEGMENT INTERCEPTS

Y = 3.07 + .03 X

COEF. OF CORRELATION = .547 N= 14

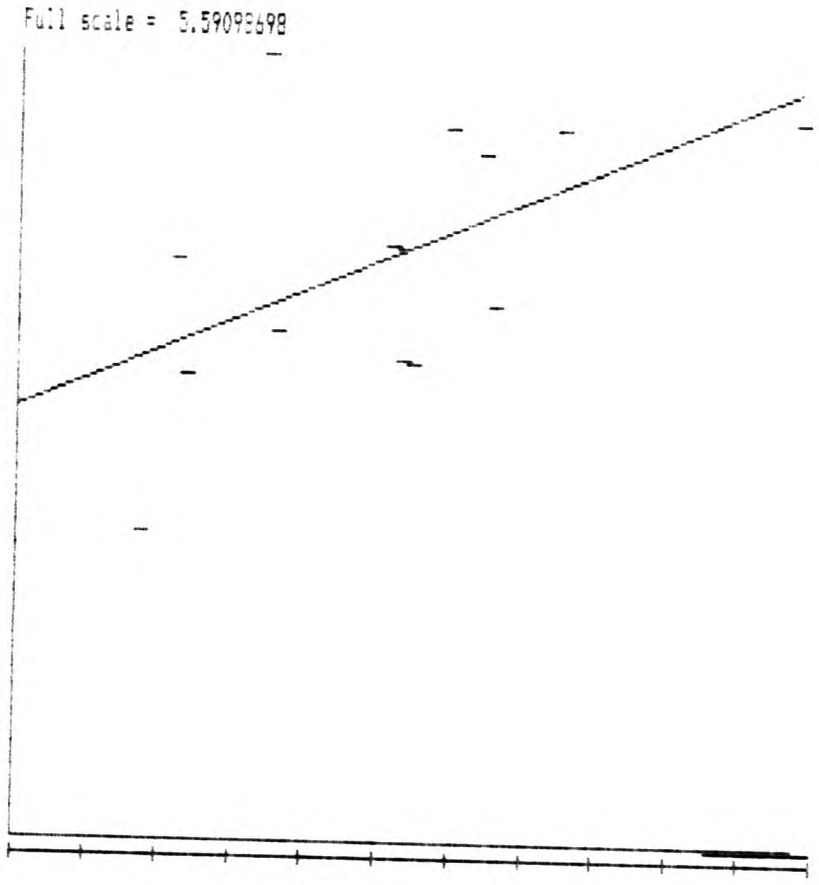


Figure 2.6.11 Correlation between log of segment intercepts (x-axis) and blood concentrations (y axis)

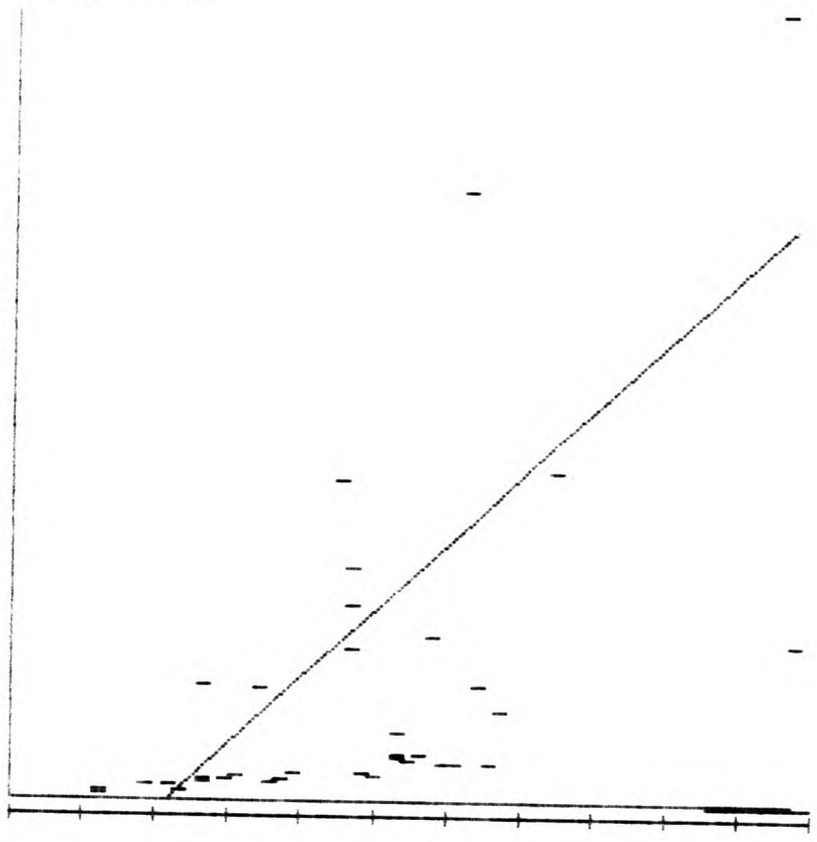
LINEAR REGRESSION

SAMPLE : BLOOD LEAD / WET PROXIMALS

Y = 70.65 + 5.13 X

COEF. OF CORRELATION = .582 N= 35

Full scale = 580



Full Scale= 69

Figure 2.6.12 Blood lead (x-axis), proximal hair lead (y-axis) correlation

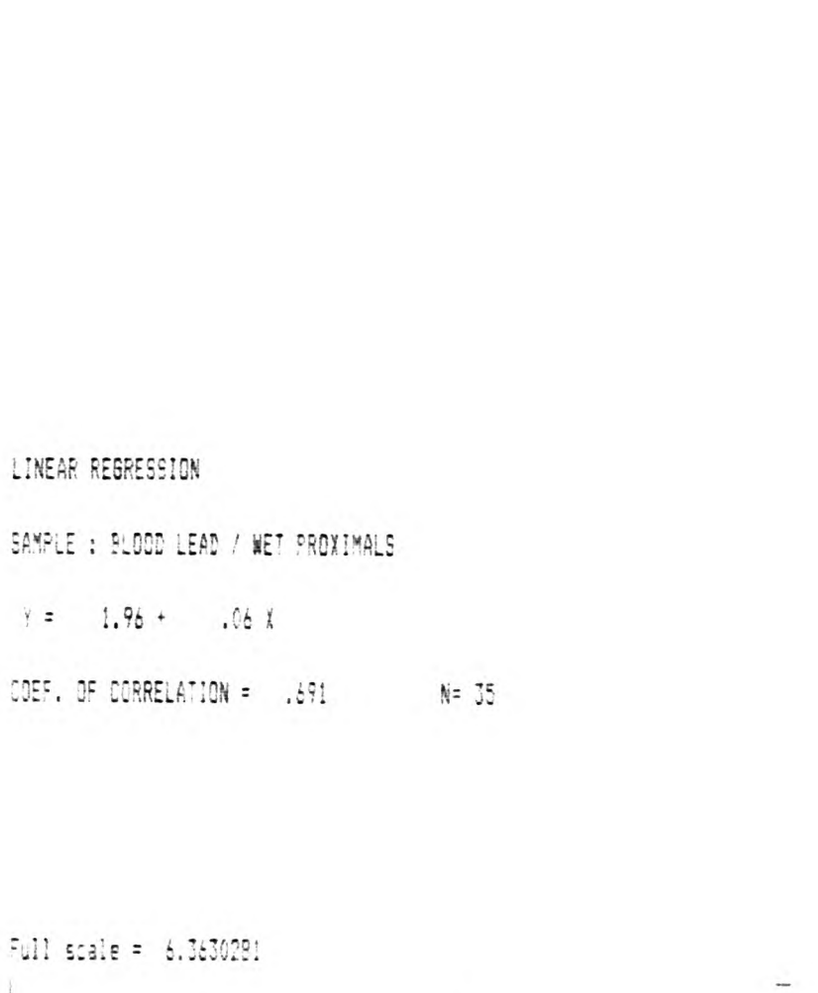
LINEAR REGRESSION

SAMPLE : BLOOD LEAD / WET PROXIMALS

Y = 1.96 + .06 X

COEF. OF CORRELATION = .691 N= 35

Full scale = 6.3630281



Full Scale= 69

Figure 2.6.13 Blood lead (x-axis) vs. ln Hair lead (proximal sections) (y-axis) correlation

LINEAR REGRESSION

SAMPLE : WET INTERCEPT VS. BLOOD LEAD

Y = 2.42 + 6.82 X

COEF. OF CORRELATION = .636 N= 23

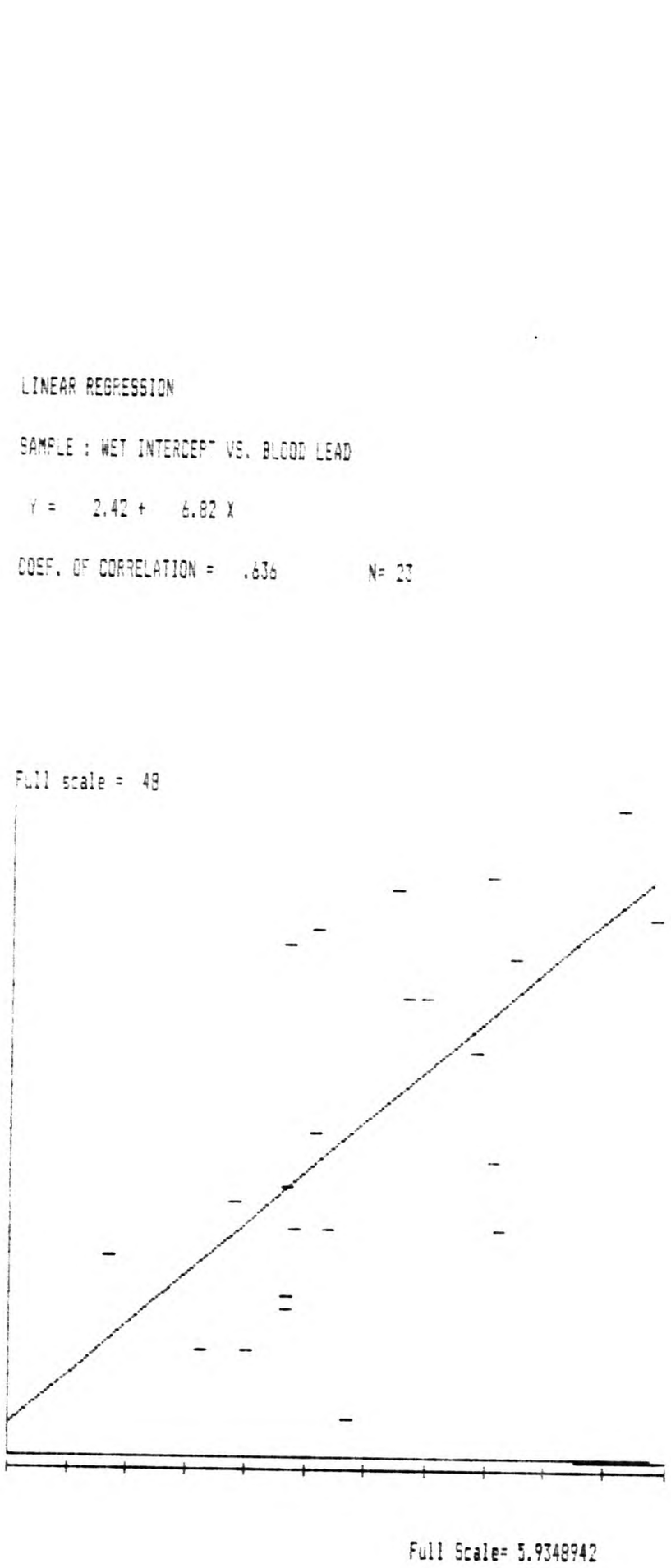


Figure 2.6.14 Correlation between blood lead values and log of intercept values obtained by wet ashing

This equation would generate the following hair lead values for the range of blood lead values found in this study:

Blood Lead	Hair Lead
0	7
10	14
20	25
30	45
40	82
50	148
60	270
70	493
µg/100 ml	µg g^{-1}

A significant correlation was observed between blood lead values and intercept values achieved by wet ashing ($p=0.001$) (fig 2.6.14). However, the degree of scatter was much greater than when proximal sections were considered.

2.6.7 Discussion

Solid sampling analysis was found to be satisfactory so long as the strictest standards of cleanliness were

adhered to. Analysis at the 217.0 nm lead absorption line caused sensitivity problems when very high lead levels were encountered so the use of the 283.3 nm line is recommended.

The expected increased in lead concentration with hair length was observed in two-thirds of the samples. Both slope and extropolated intercept values were high in the occupationally exposed group. The cut-off between the exposed and non-exposed groups occurred at about 0.3 ng cm^{-2} (slopes) and 1.0 ng cm^{-1} (intercepts). The results of this study were very slightly lower than those reported in two earlier studies (101, 126). This may be because the analytical procedure used in this study did not determine the higher concentrations successfully. The natural logs of slope and intercept values were found to have a high order of correlation suggesting a common origin: lead exposure.

The level of correlation between the results of analysis of hair lead by the two techniques was disappointingly poor. It is felt that this lack of agreement may be due to imprecision in the solid sampling technique which relies upon the analysis of only two or three individual hairs.

Blood lead values were only weakly associated with hair lead slope and intercept values as determined by the solid sampling technique. However, the concentration of lead in proximal sections of hair, as determined by the wet ashing technique showed a strong correlation with blood lead levels. The regression equation:

$$\ln[Pb]_{hair} = 0.06[Pb]_{blood}^{+2}$$

was very similar to that calculated from Chattopadhyay's data (124):

$$\ln[Pb]_{hair} = 0.05[Pb]_{blood}^{+2}$$

It should be noted that Chattopadhyay et al quote a different equation in their paper:

$$\log [Pb]_{hair} = 0.8[Pb]_{blood} + 0.025$$

but this is not supported by their data and is thought to be in error.

A hair lead concentration of 82 $\mu g\ g^{-1}$ was found to be associated with a blood lead concentration of 40 μg/100 ml. It is suggested that 80 $\mu g\ g^{-1}$ lead in hair may be taken as a minimum level associated with raised occupational risk. This figure should not be applied to other non-occupational groups and in particular to children, who at any given exposure level may be at significantly greater risk than adults (169).

1.7 Concluding summary of the use of human head hair as a biological monitor.

Trace metals may become incorporated into hair during its' formation, be absorbed from sweat and sebum or be accumulated from the environment after the hairs' emergence. Trace metals may vary with hair length and with position on the scalp. Random hair samples are therefore unlikely to be good monitors of exposure or uptake. The object of this work has been to investigate the potential value of hair as a biological monitor.

It was proposed that the concentration of metals at the roots of the hair would be related to endogenous deposition, and that the rate of increase with distance from the scalp would be related to exogenous accumulation.

In a preliminary study of two groups of urban and rural dwelling adolescents the rate of increase in lead concentrations (slope) was able to discriminate between the different exposure ratings. Extrapolated root concentrations (intercepts) were not significantly different. The rural group was used to estimate a

background range, which may be defined as "the concentration of lead in the proximal 3cm of sub-occipital hair collected from a group of adolescents with minimal exposure". The background range was 1 - 6 $\mu g\ g^{-1}$, and this could be extended up to about 10 $\mu g\ g^{-1}$ in order to include 'normal' urban adolescents.

Urban and rural copper concentrations did not differ significantly and a normal range of 8 - 65 $\mu g\ g^{-1}$ was found. This is slightly higher than the range of values reported by other authors (6 - 50 $\mu g\ g^{-1}$) (91,93,94).

In a more extensive study of adolescents throughout the United Kingdom, hair lead levels were found to be related to the degree of urbanisation and water hardness. Sex and hair colour appeared to make no difference. Personal hair washing habits were found to be an important factor; washing tending to remove metals from the hair. However it was felt that this would not be significant as long as the data were considered only on a group basis.

Children aged 2 - 4 yrs living in an urban area were found to have hair lead levels that were higher than any of the adolescent groups. There was a weak inverse relationship between the individual hair lead levels and

the distance from a major road, to the child's home. Leaded petrol is thus implicated as a possible source. These infants may be at much higher risk than the other groups studied because of the combined effects of increased exposure and the greater susceptibility of small children to toxic effects.

Single hairs complete with roots were collected from a normal and an occupationally exposed group. The hairs were analysed longditudinally and a significant positive correlation between hair length and lead concentration was found in 62% of the subjects. Both the slope and intercept values could discriminate between the exposed and normal populations.

Bulk hair samples (about 1g of sub-occipital hair cut close to the root) and blood samples were also taken from the exposed group. The proximal 3cm hair lead concentrations correlated well with the blood lead values and the regression equation was calculated:

$$\ln[Pb]_{hair} = 0.06[Pb]_{blood} + 2$$

The single hair analysis results did not correlate well with the bulk hair or blood lead results. It was felt

that this was because the single hairs were subject to too much biological and analytical variability.

The most useful parameter found throughout this study has been the concentration of lead in the proximal 3cm of sub-occipital hair. The calculated slope and intercept values were sometimes useful supplements, and were particularly interesting in the single hair analyses. However, negative values often occured and these were a clear indication of some weakness in either the theory or technique.

It is not clear whether the proximal concentration reflects endogenous deposition or exogenous accumulation, although it seems most likely that it comprises a component of each. However, since it has been shown to be related to blood lead concentrations, degree of urbanisation, water hardness and occupational exposure it clearly shows great promise as a screening tool for assessing potential risk. A concentration of 80 $\mu g\ g^{-1}$ was estimated as being the minimum level of risk for an occupationally exposed adult population. This figure cannot be applied to the other groups.

Normal hair lead values reported in the literature, for rural and urban populations, fall into the range 0.5 to 40 $\mu g\ g^{-1}$. The proximal concentration ranges and median for each group studied here are presented in table 2.7.1.

Table 2.7.1. Proximal hair lead concentration for each study group ($\mu g.g^{-1}$).

	Range	Median
U.K. Adolescents	1 - 20	6
Urban -"-	1 - 34	5
Rural -"-	2 - 8	3
Urban Infants	7 - 39	15
Occupationally exposed adults	8 - 850	26

Many papers have been published on this subject and many authors have commented on the need for a standardised approach. All too few have followed that advice and nearly every paper introduces some new variation. In that respect this work is no exeption. It does, however,

provide a solution to the problem which is simple, straightforward and quick:

1. Take sub-occipital hair only.
2. Cut as close as possible to the scalp.
3. Don't wash.
4. Analyse only the proximal 3cm portion.

Further work is required to investgate more fully the mechanisms of metal incorporation, accumulation and loss. Then, with a standardised technique, data can be collected which will be direcly comparable, and a better understanding of trace metal toxicology aquired.

Hair is not strictly a 'biological monitor' because although it is a biological product, it can accumulate environmental, as well as systemic metals. It should perhaps be called a 'natural personal monitor' instead.

PART III

Experimental Details

3.1 Equipment:

Pye SP9 atomic absorption spectrophotometer system

Pye SP9 Video electrothermal atomisation furnace

Varian AA5/AA6 atomic absorption spectrophotometer

Varian CRA 90 electrothermal atomisation funace

Varian 9176 chart recorder

Perkin Elmer 4000 atomic absorptionspectrophotometer

Perkin Elmer HGA 400 electrothermal atomisation furnace

Perkin Elmer AS 40 sample dispenser

Perkin Elmer 56 chart recorder

Trivector 'Trilab III' microcomputer system

Hamilton Microlab 'M' diluter/dispenser

3.2 Reagents:

Nitric Acid	-	BDH 'Analar' grade s.g.= 1.42
Perchloric Acid	-	BDH 'Analar' grade
Pb, Cd, Cu & Zn Standard solutions	-	BDH 'Spectrosol' 1000ppm
Triton X-100	-	Fisons Scintillation grade

3.3 Chemical Analysis of Air Sampling Materials

Samples of collecting material or membrane filters were weighed and placed in clean glass beakers (50 cm^3). Concentrated nitric acid (10 cm^3) was added and the beakers covered with clean watch-glasses. The beakers were glaced on a hotplate in a fume cupboard and allowed to boil gently. When the volume was reduced to about one half the beakers were removed from the hotplate and allowed to cool. Concentrated perchloric acid (2 cm^3) was then added and the beakers were placed back on the hotplate. The temperature of the hotplate was raised until white fumes of perchloric acid began to be evolved and heating was continued until about 2 cm^3 remained . The solutions were allowed to cool down and the watch-glasses were rinsed into the beaker using dilute nitric acid (5%). The beakers were then rinsed twice into volumetric flasks (10 cm^3 membrane filters, 5 cm^3 other samples) with dilute nitric acid (5%) and made up to volume. Sample solutions were stored in polythene bottles (25 cm^3).

Standard solutions were prepared daily in dilute nitric acid (5%) in a range of concentrations and stored in polyethylene bottles (25 cm^3) (Table 3.1)

Table 3.1 Standard Solution Concentrations (ug ml^{-1})

		Pb	Cd	Cu	Zn
Instrument Adjustment:		10	1.0	10	10
Calibration Standards:	1	0.2	0.1	1.0	0.5
	2	0.5	0.5	5.0	1.0
	3	1.0	1.0	10.0	2.0

All samples and standards were analysed using a Pye SP9 atomic absorption spectrophotometer with automatic background correction, using an acetylene/air flame, under the following conditions (Table 3.2). Sample and standard solutions were determined twice using a 5 second instrumental integration period.

Calibration curves were constructed for each metal daily. Some examples appear at Figure 1.4.1. Slight differences in sensitivity were observed from day to day. These were probably due to slight changes in gas pressures, burner alignment, lamp current and general setting-up.

Table 3.2 Instrument Operating Conditions

Line		Fuel flow rate ($L.min^{-1}$)	Burner Height (cm)	Lamp Current (mA)
	(nm)			
Pb	217.0	18	6	6.0
Cd	228.8	17	4	3.7
Cu	324.7	15	5	7.5
Zn	213.9	17	5	8.0

Slit width: 0.5 nm

Integration time: 5 sec.

Air flow rate: 34 $L.min^{-1}$

3.4 Operation of the Andersen Cascade Impactor

The plates of the impactor were thoroughly cleaned in dilute nitric acid (5%), rinsed twice in deionised water and dried in a drying cabinet. The membrane filter was acclimatized in a desiccator for 4 hours. Plates and filter were accurately weighed on a five-figure balance. The plates and filter were loaded into the impactor and it was taken to its operating location. The flow rate was checked by placing a gas meter in series after the filter.

After running, the filter was again acclimatized for 4 hours. The plates and filter were re-weighed on the five-figure balance, the differences being taken as the mass collected at each stage.

3.5 Optical Microscopy Techniques

Samples of fibres were mounted on glass microscope slides (8 x 2 cm) and held in place with glass cover slips (1 x 1 cm) secured with mounting adhesive. The slides were examined using a Vickers binocular microscope at X50, X100, X400 and X1000 magnifications. The microscope was calibrated using a stage micrometer in order to estimate fibre diameters. The relative cross sectional areas of particles were estimated using the British Standard Graticule (fig.3.2), which is also known as the 'Fairs Graticule'. The graticule is designed so that the areas of the circles double progressively. Each particle is assigned to a class size defined by two adjacent circles, which represent the size limits of that class. The method is reported to be reliable in the range 150 um to 0.38 um (83).

A Millipore membrane filter which had been used to collect an aerosol sample by low volume sampling at the north London site, was cleared using an acetone/acetic acid mixture (10:90). When dry, this produced a clear, hard, transparent sheet within which the collected particles were permanently fixed.

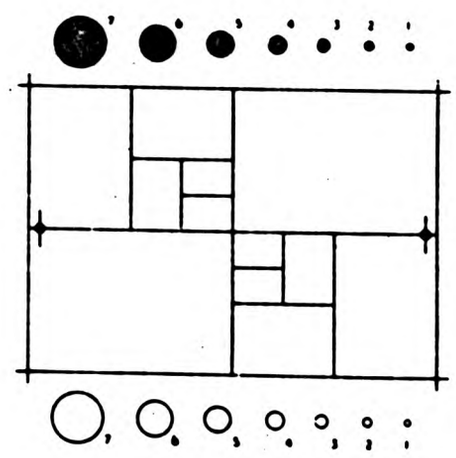
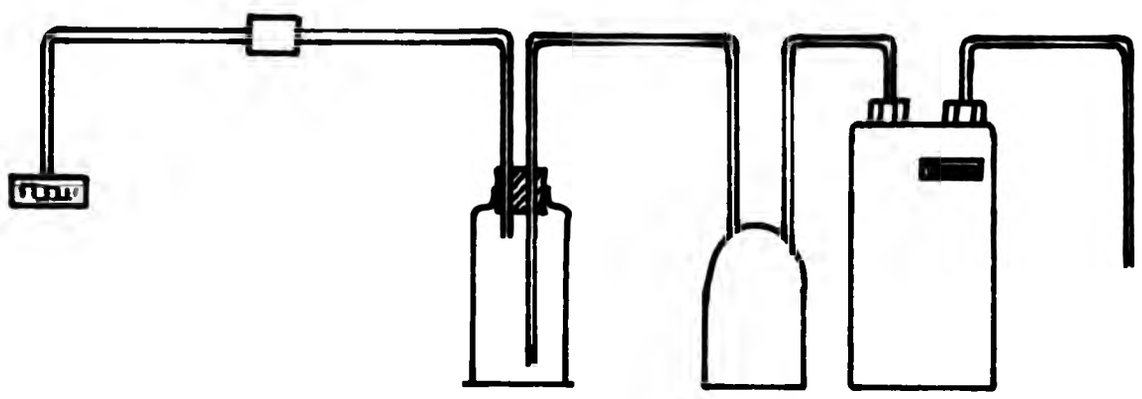


Figure 3.2 British Standard Graticule, (BS 3260) (03)

3:4 Low Volume Sampling Technique

A conventional low-volume sampling train was set up which comprised a filter holder with a 47 mm diameter Millipore H.A. filter (0.46 um pore size), a limiting orifice (2.0 l min^{-1}), an expansion vessel to regulate the flow, a pump (Charles Austin Capex 1k II) and a gas meter (Fig. 3.3).

Samples were exposed for periods of one calendar month and the sampling train run concurrently to obtain a sample of the total aerosol. Exposed samplers and membrane filters were stored in plastic petri dishes kept in an air-tight cabinet until they were analysed.



Inlet Limiting Expansion Pump Flow Exhaust
Filter orifice vessel valve

Figure 3.3 Low-Volume Sampling Train.

3.5 Analysis of Hair by AAS

For analysis by flame aas hair samples were weighed to the nearest 0.5 mg and placed in cleaned glass beakers (75 cm^3). Nitric acid (72%) was added (10 cm^3) and a watch glass placed over the top. The beakers were placed on a hot-plate and the temperature was raised slowly to

about 150°C. When the solutions had been reduced to about one half of the original volume they were removed from the hotplate and allowed to cool. Perchloric acid (70%) was added to each beaker (2 cm^3) and the solutions were replaced on the hot-plate. As the volume of solution approached about 2 cm^3 dense white fumes of perchloric acid were evolved and the beakers were removed from the hot-plate and allowed to cool.

When cooled to room temperature the lower surface of the watch-glass was rinsed into the beaker using dilute nitric acid (10%). The beakers were then rinsed twice into a volumetric flask (5 cm^3) and the volume made up with dilute nitric acid (5%). All solution were stored in closed polythene bottles (25 cm^3) prior to analysis.

Where solutions were to be analysed by aas with electrothermal atomisation it was felt that the potentially hazardous perchloric acid oxidation stage could be excluded because the digest solution would be ashed within the graphite furnace. Sample preparation was essentially the same as for flame aas except that there was no perchloric acid addition and the samples were made up to a final volume of 10 cm^3.

All the glassware and the plastic bottles were soaked in dilute nitric (ca 5%) between use and rinsed twice with deionised water and dried in a warm air cabinet before use.

For flame aas standard solutions were prepared daily in dilute nitric acid (5%) in a range of concentrations and stored in polyethylene bottles (25 cm^3) (Table 3.3).

Table 3.3 Standard Solution Concentrations

		Pb	Cu	
For Instrument adjustment:		10.0	10.0	
Calibration standards:	1	0.2	1.0	
	2	0.5	5.0	
	3	1.0	10.0	ppm

All samples and standards were analysed using the Pye SP9 atomic absorption spectrophotometer with automatic background correction using an air/acetylene flame. Operation conditions were adjusted and optimised daily (Table 3.4).

Table 3.4 Optimal Instrumental Conditions

Metal	Pb	Cu	
Absorption Line	217.0	324.7	nm
Fuel flow rate	18	15	$l.min^{-1}$
Burner height	6	5	cm
Lamp current	6.0	7.5	mA
Band pass	0.5	0.5	nm
Integration time	5.0	5.0	sec
Oxidant flow rate	34.0	34.0	$l.min^{-1}$

Calibration curves were constructed daily (Fig 3.1). Some variation in sensitivity was observed from day to day which was probably due to slight changes in gas pressures, burner alignment, lamp current and general setting-up. Two reagent blank samples were included for every ten hair samples. The instrumental detection limit was calculated as twice the standard deviation of the means of the reagent blank samples. Detection limits were 0.05 ppm for lead and 0.50 ppm for copper.

Hair samples collected later in the study were analysed by aas with electrothermal atomisation using a Perkin Elmer 4000/HGA 400 system (Plate VI). Instrumental conditions were optimised using a 20 ul injection of 1.0 ppm Pb solution. Ashing and atomisation curves were constructed by varying the ash and atomisation temperatures (Fig 3.4). The following operating parameters were used throughout (Table 3.5).

Table 3.5 Graphite Furnace aas Operating Parameters

	Dry	Ash	Atomise	Clean	
Temperature	110	500	1600	2400	^{o}C
Ramp time	10	10	0	1	sec
Hold time	30	20	5	4	sec

Absorption Line:	283.3 nm
Slit width:	0.2 nm
Purge Gas:	Argon, 300 ml min^{-1}
Gas flow on atomise:	0 ml min^{-1}
Injection volume:	20 ul
Deuterium background correction	

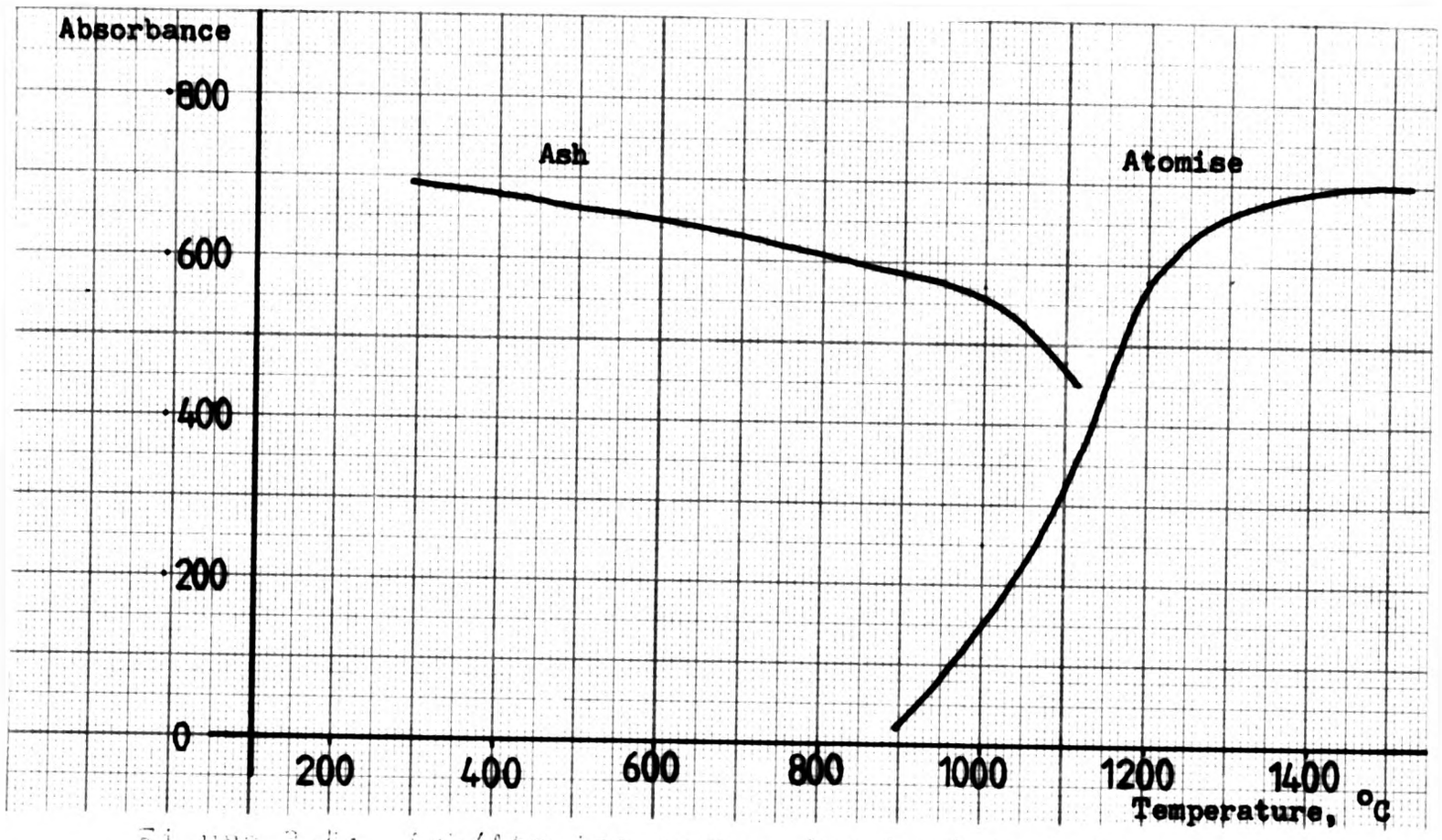


Figure 3.4: Ash/Atomise curves for lead in hair matrix.

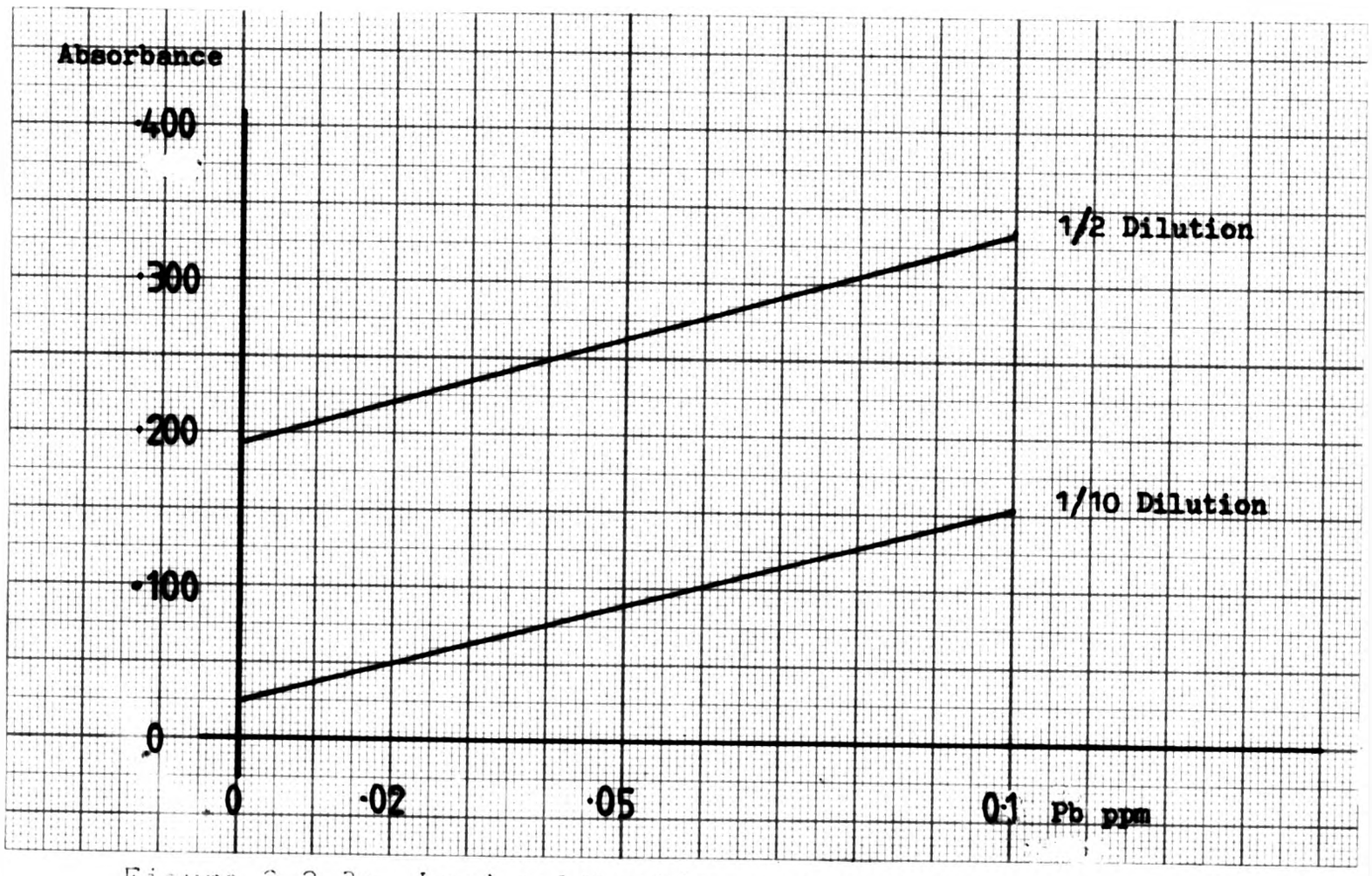


Figure 2.2.3: Lead calibration curve constructed using spiked hair digest solution.

Calibration standards of 0,0.2, 0.5 and 1.0 ppm Pb in dilute acid (5%) were prepared daily. Calibration was achieved by standard additions to a composite hair digest sample. A Hamilton Microlab 'M' diluter/ dispenser was employed using programme 1 (Table 3.6) to give a final dilution of 1+9 for the aqueous standards (giving final concentrations of 0, 0.02, 0.05 and 0.1 ppm) plus a dilution of 1+1 for the hair digest composite in a nitric acid (1.0%)/Triton X-100 (0.1%) diluent. The Triton X-100 surfactant is added to aid sample spreading in the tube. Hair digests are diluted 1+1 with nitric acid/Triton-X using Hamilton Microlab 'M' programme 2 (Table 3.6). Any samples which gave a peak height greater than the top standard were diluted 1+9 with nitric acid/Triton-X (1.0%/0.1%) using diluter programme 3 against standards in 1+9 diluted composite prepared using diluter programme 4 (Table 3.6). Calibration curves for 1+1 and 1+9 diluted hair digest composite were constructed daily (Figure 3.5). Data are collected from the Perkin Elmer 4000/HGA 500 system automatically by a Trivector Systems microcomputer system. Calibrations were performed and presented graphically on the microcomputer before hair digest samples were analysed. Hair digest solution concentration calculations were performed using the microcomputer system.

Table 3.6 Hamilton Diluter Programmes

PROGRAMME 1 STANDARD ADDITIONS (1+1)

STEP	ACTION	VOLUME	SWITCH
1	ASPIRATE DILUENT	400 ul	
2	ASPIRATE STANDARD	100 ul	*
3	ASPIRATE SAMPLE	500 ul	*
4	DISPENSE	1000 ul	*
5	ASPIRATE RINSE	500 ul	
6	DIPENSE RINSE	500 ul	

PROGRAMME 2 DILUTIONS (1+1)

STEP	ACTION	VOLUME	SWITCH
1	ASPIRATE DILUENT	500 ul	
2	ASPIRATE SAMPLE	500 ul	*
3	DISPENSE	1000 ul	*
4	ASPIRATE RINSE	500 ul	
5	DIPENSE RINSE	500 ul	

PROGRAMME 3 STANDARD ADDITIONS (1+9)

STEP	ACTION	VOLUME	SWITCH
1	ASPIRATE DILUENT	800 ul	
2	ASPIRATE STANDARD	100 ul	*
3	ASPIRATE SAMPLE	100 ul	*
4	DISPENSE	1000 ul	*
5	ASPIRATE RINSE	500 ul	
6	DIPENSE RINSE	500 ul	

PROGRAMME 4 DILUTIONS (1+9)			
STEP	ACTION	VOLUME	SWITCH
1	ASPIRATE DILUENT	900 ul	
2	ASPIRATE SAMPLE	100 ul	*
3	DISPENSE	1000 ul	*
4	ASPIRATE RINSE	500 ul	
5	DIPENSE RINSE	500 ul	

3.8 Recovery Study Preparation

Aliquots (0.05 g) of hair which had been homogeneised in a cryogenic mill were accurately weighed into 9 prewashed beakers. To these were added:-

Beaker No	Addition
1, 2, 3	2 ml water
4, 5, 6	1 ml water + 1 ml 1 ppm Pb
7, 8, 9	2 ml 1 ppm Pb

Each beaker was then treated according to the technique outlined above.

3.9 Solid Sampling Technique

Hair strands were placed on a clean sheet of graph paper (1 mm markings) and the proximal (root) end identified. A section (9 mm, corresponding to the length of the graphite furnace) was cut using a cleaned scalpel blade and the hair segment was carefully transferred to the graphite furnace (Plate VII) using a pair of cleaned plastic forceps. Varian AA5 and AA6 spectrophotometers with GTA 90 graphite furnace atomisers were used for the analyses (Table 3.7).

Table 3.7 Furnace conditions for segmental analysis of single hairs

	Dry	Ash	Atomise
Temperature, ^{o}C	200	500	1600
Hold, sec	20	40	1.5
Ramp, $^{o}C\ sec^{-1}$	-	-	600

Line: 217.0 nm
Bandpass: 0.2 nm
Lamp current: 5mA

D_2 background correction

A drying temperature of 200°C was used because it prevented the "curling-up" of the sample that occurred if the temperature was raised too rapidly to 300°C. A 20 second drying time was necessary to accommodate a delay in the data collection system. A 40 second ash time was required to ensure that all smoke had dispersed before atomisation.

Hair filaments were analysed throughout their length as a maximum of twelve segments. When more than one hair was available (and the root end could be identified) up to three from the same head were analysed. In some of the early analyses, occasional high peaks arose within otherwise discrete data. This was controlled by adopting every means to ensure absolute cleanliness, including wearing latex gloves throughout the analyses.

Calibration was performed using aqueous standards. An attempt was made to use small segments of silk thread as a matched matrix calibration material. However, the amount of silk required to match the background absorbance signal generated by the hair was so small that it was impossible to achieve a consistent blank value. Results achieved by electrothermal AAS without

matrix matched calibration should always be interpreted with caution. In this case aqueous calibration was the only course available.

A number of the lead workers' hair gave very high absorbance values so calibration had to be achieved to as high a level as possible. Standards of 0, 0.5, 1.0 and 2.0 ng were used and calibration was achieved up to 1.5 absorbance units

Bulk hair samples obtained from lead workers were divided into 3 cm sections and analysed according to the technique described in Section 2.2.

Blood samples were analysed according to the Health and Safety Executive Occupational Medicine Laboratories standard method for blood lead analysis. Briefly, samples are diluted 1+10 with Nitric acid (1%) Triton X-100 (0.1%) and injected into the furnace. Calibration is by matched matrix standards. The laboratory adopts stringent quality control procedures and performs consistently well in the National External Quality Assurance Scheme.

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Appendix A Pearsons' 'r' test for correlation

Pearson's 'r' describes the degree of linear correlation between two variables. From a sample of N pairs of scores (X, Y) and we wieh to decide whether in general, we can make a useful prediction of the value of Y when we Know the corresponding X score.

We compute r:

$$r = \frac{N\Sigma XY - X\Sigma Y}{\sqrt{\{N\Sigma X^2 - (\Sigma X^2)\ \ (N\Sigma Y^2 - (\Sigma Y)^2)\}}}$$

Where "r" is a measure of correlation whose value lies between -1 and +1. Values of r near to 0 indicate a low level of correlation. Positive values of r indicate a positive correlation and negative values a negative correlation. The statistical significance of r may be found from tables.

Appendix B "Regression Graphics" programme

```
PROG
   50 T$=STRING$(42,
   55 C$=R$(7)
  100 PR
  110 REM VERSION 1.3
  200 P
  300 P
  400 "This program uses least squares method to obtain Y = A + BX for"
  500 "paired data. The data points and regression line may be plotted"
  6?  "then there is an extra option for Segwer Analysis."
  7?0 P
  720 P                                                    DAT 13/02/87"
  730 P
  750 "EXAMPLE IDENTITY"
  760 INPUT A$
  800 "HOW MANY PAIRS OF READINGS?"
  850 INPUT N
  900 LET I=0
 1000 DIM X(N),Y(N),W(N),X(1000
 1110 DIM S(N)
 1120 FOR I=1 TO N
 1200 "ENTER PAIR ";I;"X,Y;"
 1300 INPUT X(I),Y(I
 1400 NEXT I
 1420 "DOC YOU WANT AUTO-SCALING?","Y/N"
 1440 INPUT  B4$
 1450 IF ASC(B4$)=89 THEN 2000
 1500 REMROUTINE TO ENTER MAX VALUES
 1530 "YOUR DATA:"
 1700 P,  Y","  Y
 1800 FOR I=1TON
 1900 "P=IR";I,X(I),Y(I)
 2000 NEXT I
 2100 "WHAT IS MAX. VALUE OF X?"
 2150 INPUT V
 2200 " WHAT IS MAX. VALUE OF Y?"
 2300 INPUT W
 2500 REM REGRESSION ROUTINE
 2600 S1=0
 2700 S2=0
 2800 S3=0
 2900 S4=0
 3000 S5=0
 3100 FOR I =1 TO N
 3200 LET S1=S1+X(I)
 3300 LET S2=S2+Y(I)
 3400 LET S3=S3+X(I)^2
 3500 LET S4=S4+Y(I)^2
 3600 LET S5=S5+X(I)*Y(I)
 3700 NEXT I
 3800 LET M =(N*S5-S2*S1)/(N*S3-S1^2)
 3900 LET C=(S2-M*S1)/N
 4000 LET R=(M*(S5-S1*S2/N))/(S4-S2^2/N)
```

```
4130 ?"SAMPLE : ";N$
4200 ?"Y=";C(1);"+";M(1);"X"
4300 ?
4400 ?"CORRELATION COEFF.=";SGR(R)
4500 ?"N=";N
4600 ?
4700 ?
4800 ?"ENTER  * TO CONTINUE"
4850 INPUT A$
4900 A$=SEG$(A$,1,1)
4950 IF A$<> "X" THEN 5000
5000 REM CURSOR ROUTINE
5010 LET F$=C$+P$(27)+CHR$(42
5120 ? F$
5030 X=5, Y=1
5040 GOSUB 5500
5 50 ?A$
5120 ?
5130 ?"PRINT HARD COPY"
5140 ? "GRAPHICS"
5150 ?"SEGMENTS ANALYSIS"
5180 ? CHR$(20)
5190 GOSUB 5500
5200 GOSUB 5700
5260 ? CHR$(27)+CHR$(42)
5270 GOTO 10100
5280 REM  SUB FOR CURSOR ADDRESSING
5300 X1=Y+32
5310 Y1=X+32
5320 LET A$=CHR$(27
5330 RETURN
5690 REM OUTPUT
5700 X=5,Y=7
5705 GOSUB 5500
5710 ? A$;
5720 ON F GOTO 5730,5750,5770
5730 REM HARD COPY HAS BEEN CHOSEN
5740 GOSUB 6070
5745 GOTO 5260
5750 REM GRAPHICS HAS BEEN CHOSEN
5760 GOTO6200
5770 REM SEGMENTS ANAL HAS BEEN CHOSEN
5780 GOTO 6510
5790 REM SUB TO MOVE CURSOR  .
5800 B=1
5810 ? D$
5820 INPUT CHARACTER D$
5830 IF ASC(D$)=10 THEN 5870
5850 IF ASC(D$)=13 THEN 6050
5860 GOTO 5820
5870 B=B+1
5880 GOTO 5900
5890 B=B-1
5895 ? CHR$(11)
5900 IF B>3 THEN 5930
5910 IF B <1 THEN5960
5920 GOTO5810
5930 ? D-5$ 11);CHR$(11);CHR$(11)
5940 B=1
5950 GOTO 5820
5960 ? CHR$(10);CHR$(10);CHR$(10)
5970 B=3
5980 GOTO5820
6050 B=B
6060 RETURN
6070 REM PRINT HARD COPY ROUTINE
6080 OPEN OUT £, HARD,PRINTER,TF,"    "
6081 ?£ HARD,
6082 ?£ HARD,
6083 ?£ HARD,"LINEAR REGRESSION"
6084 ?£ HARD,
6085 ?£ HARD,"SAMPLE : ";N$
6090 ?£ HARD,
6091 ?£ HARD," Y = ";C(3.2);" +  ";M(3.2);" X"
6092 ?£ HARD,
6130 ?£ HARD,"COEF. OF CORRELATION =";SGR(R)12.3);"          N=";N
6132 ?£HARD,
6140 CLOSE£ HARD
6150 RETURN
6200 REM GRAPHICS ROUTINE
6210 GOSUB 6760
6220 REM DRAW SCATTER GRAM
6230 FOR I=1 TO N
6240 LET X=X(I)
6250 LET Y=Y(I)
6260 LET X=X*500/W
6270 LET Y = Y*255/H
6275 LET X=X+5
6280 USR3+,1,0,X,Y
6285 IF C(I)=0THEN6340
6300 LETX=X-10
6310 USR3+,2,0,X,Y
6320 NEXT I
6330 REM DRAW REGRESSION LINE
6331 LET D=0
6332 FOR I=0 TO N
6333 IF X(I) > D THEN 6335
6334 GOTO 6336
6335 LET D=X(I)
6336 NEXT I
6340 LET X=0,Y=C
6342 IF Y=0 THEN 6350
6344 IF Y<0 THEN 10000
6345 LET Y=Y*255/H
6350 USR3+,0,0,X,Y
6360 LET X=D
6370 LET Y=(M*D)+C
6375 LET X=X*500/W
6380 LET Y=Y*255/H
6390 USR3+,2,0,X,Y
6440 ?"DO YOU WANT HARD COPY?","Y/N"
6450 INPUT CHARACTER Q3$
6460 IF ASC(Q3$)=89 THEN6480
6465 USR3+,5,1
```

```
6470 SCRDE2$=
6480 REM HARD COPY
6485 GOSUB 6070
6486 OPEN OUT 2, HARD.PRINTER.TF," "
6487 ?2 HARD.
6488 ?"HARD,"Full scale = ";W
6490 USR3+,14.169
6495 USR3+,6,1
6498 ?"HARD.
6497 ?"HARD,
6498 ?"HARD,"                                   Full Scale=";W
6499 CLOSE HARD
6500 GOTO 5210
6510 REM GEGS ANAL
6520 GOSUB 6760
6530 FOR I=1 TO N
6531 FOR J=1 TO N
6532 IF X(I) = X(J) THEN 6540
6533 NEXT J
6534 LET N(I) = 1
6535 LET P(I) = Y(I
6536 FOR K = 1 TO N
6537 IF (X(
6538 LET N(I) = N(I)+1
6539 LET P(I) = P(I) + Y(K)
6540 NEXT K
6541 LET G(I) = X(I)
6542 LET M(I) = P(I)/N(I)
6543 NEXT I
6550 REM Draw segments
6551 LET T = 0
6552 FOR I = 0 TO N
6553 LET X1 = G(I)
6554 IF X1 = 1 THEN 6676
6555 FOR J =0 TO N
6558 LET X2 = G(J)
6560 IF X1 = 9999 THEN 6665
6562 IF X2 = 0 THEN 6665
6564 IF X2 < X1 THEN 6679
6566 NEXT J
6567 LET T = T+1
6568 LET X = X1*500/W
6569 IF X1=9999 THEN 6678
6570 LET Y = M(I)*255/W
6572 IF T=1 THEN 6870
6574 USR2+,2,0,X,Y
6576 LET G(I) = 9999
6578 NEXT I
6580 FOR K = 0 TO N
6582 IF G(K) < 9999 THEN 6652
6584 NEXT K
6750 GOTO  6230
6760 REM RDRAW AXES SUBROUTINE
6765 ? CHR$(27)&CHR$(42)
6770 USR3+,13
6780 USR3+,6,1
6790    USR3+,0,0,0
6800 USR3+,2,0,255
6810 USR3+,0,0,1
6820 USR3+,2,0,500,0
6830 RETURN
6840 REM Zero plot subroutine
6850 LET X=X(I
6860 GOTO 6810
6870 REM Set segment plot point
6880 USR3+,6,0,X,"
6890 GOTO 6650
7100 REM Auto-scaling routine
7110 LET V=0
7200 FOR I=0 TO N
7300 IF X(I)>V THEN 7500
7400 GOTO 7600
7500 LET V=X(I)
7600 NEXT I
7700 LET W=0
7800 FOR I=1 TO N
7900 IF Y(I)>W THEN 8100
8000 GOTO 8200
8100 LET W=Y(I)
8200 NEXT I
8300 GOTO 2500
10000 LET V=0
10010 LET D=1-D
10020 LET X=D/M
10030 LET X=X*511/9
10040 GOTO 6350
10100 ?"DO YOU WANT X DATA LOGGED? ";
10101 ?
10110 INPUT CHARACTER Q8$
10120 IF ASC(Q8$)=78 THEN 10137
10125 FOR I = 1 TO N
10130 LET X(I)=LOG(X(I))
10135 NEXT I
10137 ?"DO YOU WANT Y DATA LOGGED? ";
10138 ?
10140 INPUT CHARACTER Q7$
10150 IF ASC(Q7$)=78 THEN 10160
10154 FOR I = 1 TO N
10156 LET Y(I)=LOG(Y(I))
10158 NEXT I
10160 ?"CONTINUE? ";
10161 ?
10170 INPUT CHARACTER Q9$
10180 IF ASC(Q9$)=89 THEN 7000
10185 ?CHR$(7)
10190 END
```

Appendix C
Questionnaire

Polytechnic of North London

Department of Chemistry

Trace Metals Analysis of Human Hair Research Project

1. Your Age: _____ 2. Sex: _____

3a. Name of street in which you live: _____

3b. How long have you lived there? _____

4. How often do you wash your hair?

Daily	☐
Every 2nd day	☐
Twice a week	☐
Once a week	☐
Less than once a week	☐

(tick one box)

5. How long since you last washed your hair?

Today	☐	4 days ago	☐
Yesterday	☐	5 days ago	☐
2 days ago	☐	6 days ago	☐
3 days ago	☐	more	☐

6. What colour is your hair?

Blonde	☐
Light brown	☐
Dark brown	☐
Black	☐

7. Is it ginger?

Very	☐
Slightly	☐
No	☐

8. Is your hair dyed? Yes/No.

9. Do you use a dandruff control shampoo?

Yes/No.

10. Do you smoke?

10–20 a day	☐
5–10 a day	☐
0–5 a day	☐
None	☐

Appendix D

Results Table

Sample No.	Metal Concentration, ppm.		Questionnaire Response						
	Cu	Pb	Sex	4	5	6	7	9	10
London									
4A a	46.8	1.0	F	2	1	4	0	0	0
b	43.6	2.9							
4B a	67.0	2.2							
b	52.9	5.0							
4C a	70.3	5.8							
b	56.8	1.0							
Mean	56.2	3.0							
5A	11.2	1.8							
5B	12.3	3.0							
5C	15.6	4.3							
Mean	13.0	3.0							
7	13.2	8.6	M	3.5	1	2	0	1	0
13A	20.6	5.0	F	2	1	2	0	0	0
13B	29.9	6.9							
Mean	25.3	6.0							
15	12.8	8.3	M	2	3	0	0	0	0
24	14.7	1.4	M	2	1	2	0	0	0
Letchworth									
1A	24.0	4.4	F	2	2	3	0	0	0
1B	30.5	5.9							
1C	29.7	7.7							
Mean	28.1	6.0							
5A	18.4	4.5	M	2	1	3	0	0	0
5B	18.7	10.8							
Mean	18.6	7.7							
7A	19.3	10.7	F	2	2	3	0	0	0
7B	61.6	11.8							
7C	-	37.5							
Mean	40.5	20.0							
9A	21.3	6.1	M	2	1	3	0	1	0
9B	76.0	9.8							
Mean	48.7	8.0							

Sample No.	Metal Concentration Cu	Pb	Sex	Questionairre Response 4	5	6	7	9	10
10A	18.8	5.5	M	2	1	4	0	1	0
10B	14.8	9.1							
Mean	16.8	7.3							
11	38.8	2.2	M	2	2	3	0	0	0
12A	35.1	13.5	M	2	1	1	0	1	0
12B	63.9	33.7							
Mean	49.5	23.6							
13A	52.5	3.6	M	2	2	2	0	0	0
13B	23.4	5.8							
Mean	38.0	4.7							
16A	41.0	31.5	F	2	2	1	0	0	0
16B	-	-							
16C	-	16.4							
Mean	41.0	23.5							
20A	11.7	1.1	F	3.5	2	3	0	0	0
20B	19.8	0.9							
Mean	15.8	1.0							
32A	30.0	11.5							
32B	24.1	5.1							
32C	20.0	2.1							
Mean	24.7	6.3							
Hadham Hall									
14A	10.4	7.9	M	2	2	3	0	0	0
14B	8.2	5.6							
Mean	9.3	6.8							
15A	15.2	3.2	F	2	3	2	0	0	0
15B	18.3	2.2							
15C	16.6	15.2							
Mean	16.7	8.6							
18A	16.5	3.9	M	2	3	3	0	1	0
18B	16.2	1.7							
Mean	16.4	2.8							
19A	10.6	3.7	M	2	2	3	0	1	0
19B	8.9	4.5							
Mean	9.8	4.1							

Sample No.	Metal Concentration, ppm.		Questionnaire Response						
	Cu	Pb	Sex	4	5	6	7	9	10
22A	19.6	4.8	F	3.5	1	3	0	1	0
22B	26.2	5.6							
22C	26.7	3.6							
Mean	24.1	4.6							
24	10.3	2.6	M	2	2	2	0	0	0
29A	51.5	2.3	M	3.5	1	3	0	1	0
29B	60.8	3.1							
29C	63.9	6.4							
Mean	58.7	3.9							
30A	19.4	2.2	M	2	3	2	0	1	0
30B	36.1	3.6							
Mean	27.8	2.9							
31A	11.6	2.9	M	2	2	2	0	0	0
31B	7.8	2.0							
Mean	9.7	2.5							
35A	10.6	N/D	F	1	4	1	0	0	1
35B	11.9	N/D							
35C	12.5	N/D							
Mean	11.7	-							
34A	14.4	2.9	F	2	2	2	0	0	0
34B	14.7	2.9							
34C	14.8	5.0							
Mean	14.6	3.6							
21A	22.9	3.2	M	2	1	2	0	0	0
21B	21.2	3.7							
Mean	22.1	3.5							
26	9.3	5.2							
37A	9.7	3.4	M	1	2	3	0	0	0
37B	9.9	2.4							
Mean	9.8	2.9							

Sample No.	Lead Concentration Proximal	Distal	Sex	Questionnaire Response 4	5	6	7	9	10
Bristol									
093	3-	5	F	3.5	2	2	0	0	0
097	6	50	F	2	0	3	0	1	0
099	16	5	F	3.5	0	3	0	1	0
100	13	23	F	3.5	1	3	1	0	0
101	14	23	M	3.5	2	2	0	1	0
102	7	20	M	1	5	2	0	1	0
103	2	3	F	2	3	1	0	0	0
Isle of Wight									
118	9	9	F	2	3	2	0	0	0
120	2	4	F	3.5	2	2	1	0	0
121	1	6	F	2	1	1	0	0	0
122	1	5	F	2	2	3	0	0	0
129	2	4	F	2	3	2	0	0	0
130	8	5	F	2	1	1	0	0	0
131	5	18	F	3.5	2	2	0	0	0
132	4	4	F	2	1	2	1	0	0
Manchester									
196	7	7	M	2	2	4	0	1	0
197	5	7	M	2	2	2	0	1	0
199	20	17	M	3.5	0	3	0	1	1
200	12	9	M	1.	3	2	0	1	0
202	11	17	M	3.5	3	4	0	1	0
203	19	16	M	3.5	1	4	0	1	0
208	6	9	M	2	0	4	0	1	0
209	11	17	M	3.5	0	2	0	1	0
210	13	18	M	3.5	0	2	0	1	0
Swansea									
289	5	7	M	3.5	0	3	0	1	0
290	4	6	M	1	4	2	0	1	0
291	1	2	F	2	3	2	0	0	1
292	4	3	F	2	0	2	0	0	1
293	9	15	M	3.5	1	1	0	0	0
296	3	11	F	3.5	1	3	0	0	0
299	6	27	M	3.5	0	2	0	0	0

Tobermory

160	11	11	F	2	2	3	0	1	0
162	10	9	F	2	1	3	0	0	0

Appendix E Mann - Whitney test for paired data.

PROGRAMME LIST:

```
 100 "CHR$(27)&CHR$(42)
 110 "*************************************************************************"
 115 "                        "MANN-WHITNEY TEST"
 116 "
 117 "
 118 "A test for difference between two sets of non-parametric, but rankable
 119 "data"
 120 "
 121 "The test computed a value 'R' which must be equal to or smaller than that"
 122 "in the table (see DT) in order to be significant. A significant result"
 123 "indicates a definite difference between the two sets of data when the"
 124 "following conditions are met:"
 125 "     i) The samples were drawn independantly from each other."
 126 "    ii) The scores in each sample were drawn at random."
 127 "   iii) The scores are at least rankable."
 128 "
 129 "A 1-tail test may be used if a correct prediction of the relative"
 130 "magnitudes of the two groups has been made"
 131 "
 132 "Let group with the fewest scores be 'X' data."
 133 "                                                              DRT August '83"
 134 "*************************************************************************"
 299 "
 300 "HOW MANY 'X' DATA";
 301 INPUT N1
 302 "
 305 LET A=1
 315 DIM X(N1)
 317 "
 330 FOR I=1 TO N1
 340 "VALUE ";I;" ";
 350 INPUT X(I)
 355 LET A = -A
 360 NEXT I
 361 "
 362 "
 365 "HOW MANY 'Y' DATA";
 370 INPUT N2
 375 LET B=1
 390 DIM Y(N2)
 410 FOR I=1 TO N2
 420 "VALUE ";I;" ";
 430 INPUT Y(I)
 435 LET B=-B
 455 NEXT I
```

```
470 GOSUB 1000
480 GOSUB 1000
440 REM RANK DATA
450 C=0
470 X=0
480 Y=0
500 FOR I= 1 TO N1
510 IF X(I)=9999 THEN 710
520 FOR J= 1 TO N1
530 IF X(I)=X(J) THEN 710
540 NEXT J
550 FOR K=1 TO N2
555 IF X(I)=Y(K) THEN 800
560 IF Y(I)=Y(K) THEN 620
570 NEXT K
580 LET C=C+1
590 LET X=X+1
600 LET X(I)=9999
610 GOTO 710
620 FOR K=1 TO N2
630 IF Y(K)=9999 THEN710
640 FOR L=1 TO N2
650 IF Y(K)=Y(L) THEN 70
660 NEXT L
670 LET C=C+1
680 LET Y(K)=9999
690 GOTO 710
700 NEXT K
710 NEXT I
711 REM   NEARLY FINISHED
712 FOR I=1 TO N1
714 IF X(I)=9999 THEN 500
716 NEXT I
718 LET T=X(1)*(N1+N2+1)
720 LET R=T-W
726 IF X<0 THEN 750
740 LET R=W
750 ?CHR$(27)&CHR$(42)
751 ?CHR$(27)&CHR$(42)
752 ?"*****************************************************************"
755 ?
760 ?"                    MANN-WHITNEY RESULTS"
761 ?
762 ?
763 ?
764 ?"MEDIAN OF 'X' DATA IS ";M1;"       MEDIAN OF 'Y' DATA IS ";M2
765 ?"                    (N1=";N1;")                    (N2=";N2;")"
766 ?
767 ?
768 ?
770 ?"
771 ?"                    THE VALUE OF 'R' IS ";R
                          \/\/\/\/\/\/\/\/\/\/\/"
772 ?
774 ?
775 ?
776 ?"
777 ?                                SEE D.T. FOR SIGNIFICANCE TABLE"
778 ?"*****************************************************************"
```

```
 950 END
 960 REM AVERAGING EQUAL VALUES
 970 LET C=C+1
 980 LET D=C+1
 990 LET D=C+1
1000 LET D=C+1
1010 LET D=(C+C)/2
1020 LET X=X+D
1030 LET M(I)=9999
1040 LET M(N)=9999
1050 GOTO 710
1060 REM MEDIAN ROUTINE
1070 LET C=0
1080 DIM M(N2),P(N2)
1090 FOR G = 1 TO N1
1100 LET M(G) = X(G)
1110 NEXT G
1120 FOR I= 1TO N1
1130 IFM(I)=9999 THEN1180
1140 FOR J = 1 TO N1
1150 IF M(J) < M(J) THEN 1180
1160 NEXT J
1170 LET C=C+1
1180 LET P(C)=M(I)
1190 LET M(I)=9999
1200 NEXT I
1210 ?"     THINKING . . ."
1220 IF C<N1 THEN 1050
1230 IF A=1 THEN 1240
1240 LETS=(N1+1)/2
1250 LET M1=P(S)
1260 RETURN
1270 LETS1=N1/2
1280 LETS2=(N1+2)/2
1290 LET S1=P(S1)
1300 LET S2=P(S2)
1310 LET M1=(S1+S2)/2
1320 GOTO1230
1330 REM MEDIAN ROUTINE "Y"
1340 LET C=0
1350 FOR G=1 TO N2
1360 LETM(G) = Y(G)
1370 NEXT G
1380 FOR I=1 TO N2
1390 IF M(I) = 9999 THEN 1480
1400 FOR J = 1TO N2
1410 IF M(I) < M(J) THEN 1480
1420 NEXT J
1430 LET C=C+1
1440 LETP(C) = M(I)
1450 LETM(I) =9999
1460 NEXT I
1470 ?" STILL THINKING!"
1480 IF C < N2 THEN 1350
1490 IF B=1 THEN 1540
1500 LET B=(N2+1)/2
1510 LET M2 = P(B)
1520 RETURN
1530 LET S1=N2/2
1540 LET S2=(N2+2)/2
1550 LET S1=P(S1)
1560 LET S2=P(S2)
1570 LET MC=(S1+S2)/2
1580 GOTO 1530
```

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Appendix F Results of hair washing study.

B - Before

A - After

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
DT B	6.9	10.0	1.0	5.4
DT A	5.9	7.1	.4	5.3
PV B	6.5	6.1	-.2	6.8
PV A	2.9	3.1	.1	2.8

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
HM B	5.4	5.5	.0	5.3
HM A	3.0	2.9	-.0	3.0
DB B	5.0	6.5	.5	4.3
DB A	4.9	4.3	-.2	5.2
	ppm	ppm	ppm/cm	ppm

SAMPLE	PROXIMAL	DISTAL	SLOPE	INTERCEPT
JC B	4.8	4.3	-.2	5.0
JC A	3.3	4.3	.3	2.8
JP B	3.3	4.3	.3	2.8
JP A	3.6	3.4	-.1	3.7
JC B	4.8	4.3	-.2	5.0
JC A	1.8	2.7	.3	1.3
CE B	6.0	5.9	-.0	6.0
CE A	4.8	6.9	.7	3.8
	ppm	ppm	ppm/cm	ppm

D554965

END