A SIMPLE NON-DESTRUCTIVE TECHNIQUE FOR THE ANALYSIS OF MERCURY IN CREAMS

Maina Charles

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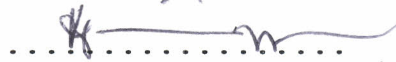
DEDICATION

Dedicated to the sacred memory of my departed mother,
Mary Njeri, R.I.P.
1954-1994

DECLARATION

This thesis is my own composition and apart from the acknowledged assistance is a record of my own research. The material has not been presented before the University of Nairobi or any other establishment for an academic award.



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This thesis has been submitted with our approval as university supervisors

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ABSTRACT

The sale of cosmetic creams is unrestricted in Kenya. Accusations have been levelled on these products due to their having dysfunctious effects on consumers as a result of addition of non conventional skin lightening ingredients like mercury .

Mercury occurs widely in the biosphere and has long been known as a toxic element presenting hazards associated with ingestion and inhalation. No vital function for the element in living organisms has yet been found. The toxic properties of mercury have evoked increasing concern lately due to the extent of its use in industries, agriculture and in pharmaceutical preparations like cosmetic creams. Mercury containing creams are left on the skin and therefore the possibility of exposure to mercury exists. This exposure leads to poisoning manifestations which can be neurological and nephrotic.

Previous workers in this subject have used an analytical method which involved heating specimens to high temperatures during acid digestion. Considering the volatility of mercury, this method may not have been most reliable hence in this study an essentially non-destructive testing technique has been utilised to determine the levels of mercury in imported cosmetic creams sold in Kenya. Samples analysed for each brand were collected in two groups six months apart. The aim of this kind sampling was to study possible variations in mercury concentration in each brand within the six months period.

The analysis showed the following mercury concentration ranges (µg/g) within brands: Madonna(green) 20867-33508; Madonna(red)14330-22167; Pimplex 3742-9949; Shirley(original)

5444-32270; Bestlady 8837-16187; Topsine 1182-1969; Fennel 3743-5444; Shirley(new), Dermovate, Topshirley had no mercury within the lower limit of detection of the Energy Dispersive X-ray Fluorescence (EDXRF) detector. The following variations between the two sample groups were also observed: Madonna(green) 7.3%; Madonna(red) 30%; Pimplex 121%; Shirley(original) 40%; Bestlady 7.4%; Topsine 3.7%; Fennel 14%; Shirley(new),Dermovate, Topshirley 0%. Most of the brands analysed (70%) had mercury levels above the Kenya Bureau of Standards recommendation of 0% and were also above levels reported in previous studies. 30% of the brands showed no mercury concentration within the lower limit of detection of the Energy Dispersive X-ray Fluorescence (EDXRF) detector system used. thus it was observed that most imported cosmetic cream brands contain high levels of mercury and therefore the Kenya Bureau of Standards should find ways of preventing the entry of these products into the country.

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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

Cosmetic creams are commonly used by women as medication for clearing blemishes, blackheads, wrinkles, pimples and for enhancing their facial appearance.

Basically cosmetic creams constitute an emulsion and an emulsifier. The composition can range from liquid through semi-liquid to gel type according to the type of emulsion and quantity of thickening agent which may be present. The composition may also include anti-oxidants such as vitamin E or vitamin C . Other conventional ingredients such as preservatives, perfumes and alcohol may be present. In Table 1,typical compositions of water-in-oil and oil-in-water emulsion creams are presented.

In addition, Some cosmetic creams contain skin bleaching agents like mercury(WHO,1991). Mercury containing creams have for a long time been used by the dark-skinned people to obtain a lighter skin tone, probably due to inhibition of skin pigment formation (Barr *et al*,1972). Although the use of these mercury containing creams produces the desired effects, it has been observed that users develop clinical disorders similar to those of mercury poisoning (Findlay *et al*,1980).

People suffering from mercury poisoning exhibit chest pain, dyspnoea, coughing,haemoptysis, impairment of pulmonary functions and there is evidence of intestinal pneumonitis (Mcfarland and Reigel ,1978). Acute mercurial pneumonitis was reported among four men after they were exposed to mercury

vapour while attempting gold ore purification using a gold-mercury amalgam and sulphuric acid(Levin *et al*,1988).

Table 1. Typical compositions of cosmetic creams

Water-in-oil emulsion

Ingredients	Concentration(%w/w)
distearyldimethyl ammonium chloride	0.50
light mineral oil	17.30
beeswax	3.00
liquid lanolin	5.00
borax	0.50
magnesium sulphate	0.20
demineralised water	73.30
perfume	0.20
Total	100.00

Oil-in-water emulsion

non-ionic emulsifying water	2.00
acetyl alcohol	8.00
light mineral water	10.00
beeswax	0.60
demineralised water	79.06
mixed esters of p-hydroxy benzoic acid	0.14
perfume	0.20
Total	100.00

(Beechams group of companies patent (1978), UK)

Since by the very nature of application cosmetic creams are left on the skin, if they contain mercury then the possibility of exposure to the element via the skin and through inhalation

exists. There is no emperical data showing the relative importance of the different exposure routes but evidence indicates that total exposure to mercury through the skin as a result of application of cosmetic creams is substantial.

Barr *et al*(1973) reported that in a group of sixty African women who used cosmetic creams containing 5-10% ammoniated mercury had a mean urinary level of 109μg/l. Out of this group, a subgroup of 26 of these women suffering from nephrotic syndrome had a mean urinary level of 150μg/l.In a similar study by Marzulli and Brown(1972) it was reported that a group of six women who had used cosmetic creams containing 1-3% ammoniated mercury for two years had urinary levels of 28-600μg/l.

Evidence through the study of Lauwerys *et al*(1987) shows that mercury can be transfered from a mother to a baby. In their study they reported the case of a woman who had given birth and who had used during pregnancy and lactation a soap and cream containing 1% of mercury. The urinary mercury contents of the mother was 784μg/l four months after birth at a time when she was still using the soap and cream. Although no mercury containing soap or cream was used on the baby's skin and the lactation period lasted only one month, the baby's blood at the age of three months contained 19μg/l and the urine 274μg/l.Despite the ban imposed on the manufacture, distribution and selling of mercury containing creams in North America, Europe,there are still some companies that manufacture them for export.

In Kenya such products have continued to be imported and their sale is so unrestricted that they can even be bought from hawkers. accusations have been levelled at some cosmetic cream

brands on account of their having dysfunctious effects on the consumers (Jacinta and Marion 1995, Barr *et al*,1972). These effects have ranged from minor skin irritations to major internal organ complications like kidney damage. These symptoms closely relate to those of mercury poisoning and could be due to addition of mercury in some cosmetic creams. Other than the study by Wandiga and Jumba(1982) , there has been no other documented studies done locally on mercury containing creams. In the study by wandiga and Jumba, it was reported that samples analysed showed levels of mercury ranging from zero to 4.9μg/g.

In a preliminary study of this work (Maina *et al*,1994), it was determined that while most locally manufactured cosmetic cream brands had no mercury, most imported brands had appreciable levels of mercury with one sample recording 30000μg/g mercury.

The study by Wandiga and Jumba (1982) utilised an analytical method (Cold Vapour Atomic Absorption Spectroscopy) that involved heating the specimen to high temperatures. Considering the volatility of mercury, this method may not have been most reliable. In this study, a non-destructive testing method namely Energy Dispersive X-ray Fluorescence (EDXRF) has been utilised.

1.1 Objectives of the study project.

The purpose of this project was to:

- Develop a simple and effective non-destructive sample preparation technique which can be used to determine the elemental contents in liquid and semi-solid samples.

- Develop a system calibration method which removes the need to calibrate the analytical system with standards matching the composition of the unknown samples.

- use the developed non-destructive analytical technique to determine the mercury levels in imported cosmetic creams sold in Kenya.

CHAPTER 2

LITERATURE REVIEW AND THEORETICAL PRINCIPLES

Mercury occurs widely in the biosphere and has long been known as a toxic element presenting occupational hazards associated with both ingestion and inhalation(Clarkson,1987). No vital function in living organisms has yet been found for the element. The toxic properties of mercury have evoked increasing concern lately due to the extent of its use in industry and agriculture (WHO,1976) and the recognition that alkyl derivatives of the element are more toxic than most other chemical forms(WHO,1991).

2.1. Physical and chemical properties of mercury

Elemental mercury has a relative atomic mass of 200.59g. In its elemental form, mercury is a heavy silvery,oduorless and volatile liquid at room temperature (WHO,1980). At 20°c, the specific gravity of mercury is 13.456 and the vapour pressure is 0.0012mmHg(Cotton and Wilkinson, 1972). Thus a saturated atmosphere at 20°C would contain approximately 15mg/m^3. This concentration is 300 times greater than the recommended health -based occupational exposure limit of 0.05mg/m^3 (WHO, 1980).

Mercurials differ greatly in their solubility depending on whether the mercurial is organic or inorganic and whether the solvent is polar or non-polar. In addition it also depends on the chemical state of mercury. Solubility values in water are: 2μg/l for elemental mercury(30°C) ; 2mg/l for mercurous chloride (25°C) and 69g/l for mercuric chloride (20°C)(Linke,1958; CRC, 1972). The solubility of methylmercury

chloride in water is higher than that of mercurous chloride by about three orders of magnitude, and is related to the high solubility of methylmercury cation in water (Clarkson *et al*,1988a). Certain species of mercury are soluble in non-polar solvents. These include elemental mercury and halide compounds of alkyl mercurials (Clarkson *et al*,1988b)

In its vapour form mercury is more soluble in plasma, whole blood and haemoglobin than in distilled water (Hursh,1985).

2.1.1. Sources of mercury.

Mercury and methylmercury are naturally occurring substances to which all living organisms have been exposed to varying degrees depending on natural, biological, chemical and physical processes(Clarkson,1987). Modern technological developments involving the use of mercury compounds are responsible for the discharge of large and variable amounts of the element into the environment.

The main industrial source is the Chloralkali industry where brine is electrolysed in mercury cells in which the cathode is a flowing sheet of liquid mercury(Murozumi 1967). Other major sources of mercury are to be found in the manufacture of electrical apparatus(Goteli,1989), dental and pharmaceutical preparations(Barr *et al*,1973).

2.1.2. Mercury intake routes

Inhalation of mercury vapour is the most common route of uptake of elemental mercury. Approximately 80% of inhaled mercury vapour is retained. The retention occurs almost entirely in the

alveoli where it is almost 100%. The retained amount is the same whether inhalation takes place through the nose or the mouth (WHO,1976; Hursh *et al*,1976).

Although liquid metallic mercury is poorly absorbed, once ingested, it is converted to the mercuric form which is better absorbed into the bloodstream. It has been observed that humans who accidentally ingested several grams of metallic mercury showed increased blood levels of mercury (WHO, 1976). The absorption in humans of inorganic mercury from foods was estimated by WHO (1976) to be ≈ 7% on average and by Elinder *et al* (1988) to be less than 10%. The data was mainly obtained from tracer studies on human volunteers who received single oral doses of protein bound inorganic mercuric compounds. Absorption in young children may be considerably greater. Kostial *et al* (1978,1983) observed an average absorption in new-born rats of 38% mercury, six days after an oral dose of $HgCl_2$. The absorption in older animals was only about 1%.

Studies on experimental animals reveal that inorganic salts of mercury ($HgCl_2$) may be absorbed in significant amounts through the skin. Skog and Wahlberg (1974) reported that 5% of mercury in a 2% water solution of $HgCl_2$ was absorbed through the skin of guinea pigs over a five hour period. From this data it is infered that mercury can also be absorbed through the human skin. Recently, studies on human volunteers (Hursh *et al*,1989) indicate that uptake of metallic mercury vapour via the skin, is about 1% of uptake by inhalation. It is to be expected that the use of cosmetic creams containing mercury salts causes substantial absorption and accumulation of mercury into the body. However

there is no information on how much mercury is absorbed through the skin as a result of this usage(Barr *et al* 1973)

2.1.3. Distribution of mercury in human tissues and fluids

From studies on animals and humans (WHO,1976; Khayat andDecker,1983a,1984), it has been shown that mercury has an affinity for ectodermal and endodermal epithelial cells and glands. It accumulates in, for instance, the thyroid, pituitary, brain, kidney, liver, pancreas, testes, ovaries and prostate. Within the organs, the distribution is not uniform. This explains why the biological half-life may differ not only between organs but also within an organ.

Mercury has been previously detected in all the tissues of human accident victims with no known exposure to mercury(Howie and Smith,1967). The mean concentrations cluster between 0.5 and 2.5 ppm Hg (dry weight). The highest mercury levels were present in the skin, nails and hair. Among the internal organs,Clarkson(1973) pointed out that the kidneys generally carry the highest mercury concentration followed by the liver, spleen, intestinal walls, heart, skeletal muscles and lungs. For example, Joselow and Goldwater (1967) reported a mean of 2.7μg mercury, wet weight, for kidney compared with means ranging from 0.05-0.3μg/g of mercury for all other tissues including the liver and lungs. Goldwater (1964)reported that 74% of a normal population drown from sixteen countries had blood mercury concentrations less than 5μg/l and 98% had less than 50μg/l.

Significant amounts of mercury are also found in the brain. Takahata (1970) in their study of two autopsy cases with chronic

mercury poisoning reported levels in the human brain several times higher than those in the liver and other organs except the kidney.It should be noted that organ distribution of mercury can be dramatically affected by moderate intake of alcohol . Alcohol reduces levels in the lungs and increases levels in the liver severalfold(Magos *et al*,1972)

2.1.4. Metabolic transformation and rate of elimination of mercury from the human body.

Metabolic transformation of metallic mercury results into its conversion to the divalent ionic form. This oxidation reaction has been shown to take place rapidly in the red blood cells (Clarkson, 1961). Studies indicate that it probably takes place in most other tissues (Kudsk, 1973). The process is enzyme mediated and the catalase complex is the most likely site of biochemical oxidation(Magos *et al*, 1974). Despite the rapid oxidation in the red cells some elemental mercury may remain dissolved in the blood long enough for it to be carried to the blood-brain barrier and to the placenta. Its lipid solubility and diffusibility allow rapid transit across these barriers. The oxidation of mercury in brain and foetal tissues convert it to the ionic form which is less likely to cross the blood-brain and placental barriers. Thus oxidation in these tissues serves as a trap to hold the mercury and leads to its accumulation in this tissues.(WHO,1976).

The conversion of organic to inorganic mercury may increase or decrease the total rate of excretion of mercury from the body. Where the organic group of the organomercurial is large,

conversion to the inorganic form increases the solubility while where the organic group is small (eg CH_3 -) convertion to the inorganic form decreases the solubility because this kind of organomercurial is more soluble in physiological fluids than the inorganic ionic mercury. If an organomercurial is more soluble in the physiological fuids of the body and therefore more rapidly excreted than inorganic mercury their biotransformation will decrease the overall excretion rate. This has been demonstrated in the case of diuretic chloro merodrin where the molecule is almost completely excreted within 24 hours but inorganic mercury remains in the body for much longer period. (Clarkson *et al*, 1973). In the case of methyl mercury, biotransformation may play a role in determining the rate of excretion of total mercury from the body (Swensson and Ulfvarson, 1968,). Inorganic mercury accounts for about 50% of the total mercury in the faeces, the principal pathway of excretion following single or chronic doses of methyl mercury compounds.

2.1.5. Biological half-life of mercury.

The body accumulates a metal when uptake exceeds elimination. At a certain stage a steady state may be reached when uptake and elimination are equal. A common way to express the elimination is in terms of biological half-life(WHO,1991). The biological half-life would be the time taken for the amount of mercury in the body to fall to one-half. This concept is meaningful only if the elimination can be approximated to a single exponential first order function. This will be true if the distribution and turn over of a metal in different tissues of the body are faster than

the elimination from the body as a whole. If elimination from one organ is slow compared with that from other organs, then the calculation of biological half-life for the whole body may be completely misleading from the toxicological point of view (WHO 1973; Nordberg, 1976)

Studies on experimental animals (Miettinen, 1973) indicate that the elimination of mercury after exposure does not follow a single first order exponential function path and thus the accumulation and elimination of mercury from the body is much more complex. As such, the pattern of elimination of mercury when administered to human beings is dose and time dependent (Piotrowski *et al*, 1969) and the half-life can only be determined on organ basis as illustrated in table 2.

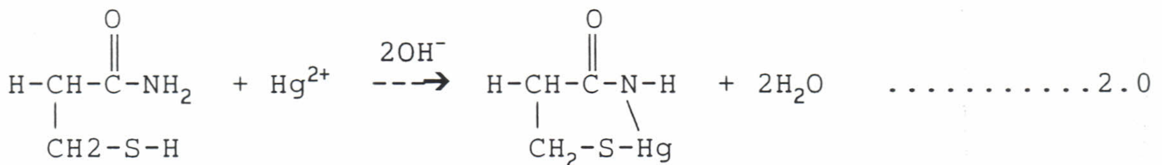
Table 2. Accumulation of mercury and biological half-life

		clearance in half-life(days)		
No of subjects	mercury intake (μg/kg/day)	body	blood	hair
5	tracer	70	---	---
15	tracer	76	50	---
5	up to 5	---	---	33-120
5	up to 5	---	58-164	---
16	up to 50	---	65	---
48	up to 50	---	---	---

(source: WHO,1991)

2.1.6 Mercury poisoning.

The mechanism of mercury poisoning is not well understood but Kark (1979) proposes that a possible mechanism is the interference with enzyme functions by bonding to sulfhydryl groups. In his study he reviewed the available evidence regarding the inhibitory effect of mercury ions on different enzyme systems and reported that mercury concentrations at which enzyme inhibition appears are consistent with concentrations at which toxic effects are observed. Ligands capable of bonding with the mercuric ions eg sulfhydryl groups are associated with proteins. Peptides containing these groups react with mercury in the basic medium of the blood and give rise to peptide-mercury complexes thus stoping polymerisation of the peptide molecules in protein synthesis(Kark,1979).



Possible mechanism of mercury bonding with sulfhydryl peptide

Hg2+ ions also react with DNA and RNA and may change the tertiary structure of the molecules(Groenwedel and Davidson,1966) Inhibition of protein synthesis as shown in equation 2.1 has been obseved in cell systems at mercury concentrations of $2x10^{-5}$ mol/l (Nakada et al,1980). A large dose of soluble mercury salts taken orally act as a corrosive poison(WHO,1991) When mercury gains entrance to the circulatory system it is rapidly taken by the

tissues. It is to be expected that mercury will be deposited in excretion organs which will therefore be prone to damage. The fatal dose of corrosive sublimate is about 200mg to 300mg. 400mg is the smallest quantity known to have caused death of a boy aged fourteen in three weeks (Thomas *et al*, 1979).

Chronic poisoning may be as a result of injurious medical administration or continuous absorption for those working with the metal or its salts or long-term application to the skin(Barr *et al*, 1972). The signs and symptoms of chronic poisoning include excessive salivation, loosening of teeth with inflamed gums, neurological conditions such as tremors and mental symptoms e.g. erethism(Clarkson,1987). Observation of more than one thousand individuals exposed to mercury vapour indicated that classical signs and symptoms of mercury poisoning may be expected to appear after chronic exposure to air concentrations of mercury above $0.1mg/m^3$ (Ladd , 1966). Soluble mercuric compounds eg dimethyl and diethyl mercury which are used in medicinal preparations are extremely poisonous and by far the most common cause of acute poisoning(WHO,1991).

Pathological findings demonstrate that methyl and ethyl mercurial are primarily neurotoxic (Hunter *et al*,1940; Hunter and Russel, 1954). Takeuchi (1975) in his study on the health effects of alkyl mercury in Japan, reported on changes in the diameter of the peripheral nerves in patients suffering from heavy exposure to methyl mercury. A joint FAO/WHO expert meeting (1972) established (basing on its metabolism and elimination) tolerable weekly intake of mercury of 0.3 mg of total mercury per person of which no more than 0.2 mg should be present as methyl mercury.

Dermatitis has been reported after skin contact with phenyl mercury (Goldwater, 1973). Symptoms have ocassionaly been reported to relate to amalgam fillings (Frykholm,1957; Thomson and russel,1970;Duxbury *et al*,1982). In most cases the main symptoms were facial dermatitis, sometimes with erythematous and urticarial rashes. In the 1940s, "Pink disease " (Acrodynia) was reported in children below five years of age as a reult of the use of mercurous chloride in teething powder and ointments. affected children became irritable and had difficulty in sleeping. Profuse sweating, photophobia and generalised rash followed. The extremities became cold , painful, red and swollen and the skin desquamated. Neither the occurance of the disease nor its severity was dose related. After the withdrawal of teething powder preparations by the main United Kingdom manufacturers in 1953, there was a dramatic decline in the occurance of pink disease. Calomel is not the only mercurial that can cause pink disease. Mercury dispersed from Fluorescent bulbs(Tunnessen *et al*,1987) and the use of nappies treated with phenylmercury(Gotelli et al,1985) have also been responsible for pink disease.

In the Iraqi outbreak (Bakir et al,1973) seed grain treated with methylmercury fungicidewas used to prepare homemade bread in rural communities through out the country. consumption probably began in October-November 1971 and the first cases of severe poisoning were admited to hospital in December 1971. over 400 deaths attributed to methylmercury merc recorded. both sexes and all ages were affected. More recently studies of canadian indian population groups exposed seasonally over a long period

of time to methylmercury through fish consumption recorded levels of exposurein blood and hair lower than those reported in the Minamata and Niigata outbreaksin Japan. The highest blood level of mercury recorded in Canada was 600µg/l (Wheatly,1979).

Administration of penicillamine is said to pprevent the process of mercury poisoning(Jumba,1997) personal communication ,1997).

2.1.7 Analysis of mercury.

The most commonly used analytical methods for the quantification of mercury compounds are Cold Vapour Atomic Absorption(CVAA)(Farant *et al*, 1981; Wandiga and Jumba, 1982), Neutron activation (WHO,1976), Gas chromatography (Horvat *et al*,1988), and X-ray fluorescence (Jaklevic *et al*, 1978)

Energy Dispersive X-ray Fluorescence Analysis (E.D.X.R.F.)is one of X-ray fluorescence methods available for elemental determination in different materials. It is the method chosen for this work because, coupled to the Fundamental Parameters Technique (F.P.T.) (Sparks *et al*, 1975), it has the advantage of being multielemental and removing the need to use standards matching the composition of the unknown samples (which is the general practice with other methods).

2.2. X-RAY FLUORESCENCE ANALYSIS

There are four stages in the scheme of X-ray emission analysis technique, namely:

- The excitation of an atom achieved by bombardment of the inner shell electrons with high energy photons. This results into an unstable atom.

- De-excitation of the unstable atom i.e. an electron from the outer shells shifts into the inner shell where a vacancy was created. This process is accompanied by the emission of characteristic x-rays.

- The detection of a characteristic emission line of the element in question by means of an energy dispersive spectrometer

- The conversion of the emission line intensity to elemental concentration by use of a suitable relationships.

All elements will give one or more sets (series) of characteristic emission and the number of these lines measured by the spectrometer will depend upon the resolution of the spectrometer. Most commercial spectrometers are able to detect the majority of K and L lines plus a few of the M series of the high atomic number elements(Jenkins,1981).

Most spectrometers lose sensitivity at low energy and their ability to detect the low atomic number elements generally falls off sharply around $Z=12$ because low Z elements produce X-rays that are not energetic enough to penetrate the detector material and produce distinct peaks above the background.This fact combined with the low penetration of low energy X-rays makes the method largely unsuitable for measurement of elements with $Z<9$. Sensitivities with these spectrometers reach a few μg/g range and the method is equally applicable for high or trace concentrations levels. Accuracies of the order of a few tenths

of a percent are achievable with analysis times of the order of minutes for scanning spectrometers and even less for multi-channel instruments (Jenkins *et al*, 1981)

The X-ray spectrometry method is essentially non-destructive and measurable signals are obtained from as little as one milligram of specimen. Optimum specimen sizes range from 0.1g to 5g of solid material and the method is equally applicable to liquids as well as gas analysis. A wide range of calibration standards combined with the development of 'Fundamental' type algorithms relating X-ray emission intensity and elemental composition allows the use of the X-ray method in the analysis of a wide variety of samples(Jenkins,1981).

2.2.1. Quantitative analysis using Fundamental Parameters Technique (F.P.T.)

For quantitative analysis of a given sample, it is necessary to measure the intensities of characteristic lines and to relate them to the concentration of elements present. In most cases the intensity of the emitted line is dependent upon the influence of other elements making up the specimen. The latter are called matrix effects. Since the penetration of characteristic X-ray lines is small (typically 1 to 1000 microns) it is vital that the specimen be homogeneous over the depth of the specimen contributing to the measured signal(Giauge et al,1979). Accurate results can only be obtained when the source, spectrometer and counting equipment are themselves stable and free from systematic errors. The relationship between elemental concentration and the observed fluorescence X-ray intensity I_i is given by equation 2.1

(also see fig 1)(Sparks,1975),whose derivation is based on the following assumptions:

- A monochromatic radiation source is used to excite the characteristic X-rays from the sample.
- The sample is homogeneous i.e. the density is well defined and constant throughout the sample.
- A fixed geometry of the sample, source and detector orientation is maintained.(see fig 1)

$$I_i = G_o K_i \rho_i d \frac{1-\exp(-a\rho d)}{a\rho d} \qquad 2.1$$

where:

ρ_i/ρ = the concentration of element i in the specimen

ρ_i = is the density of element i.

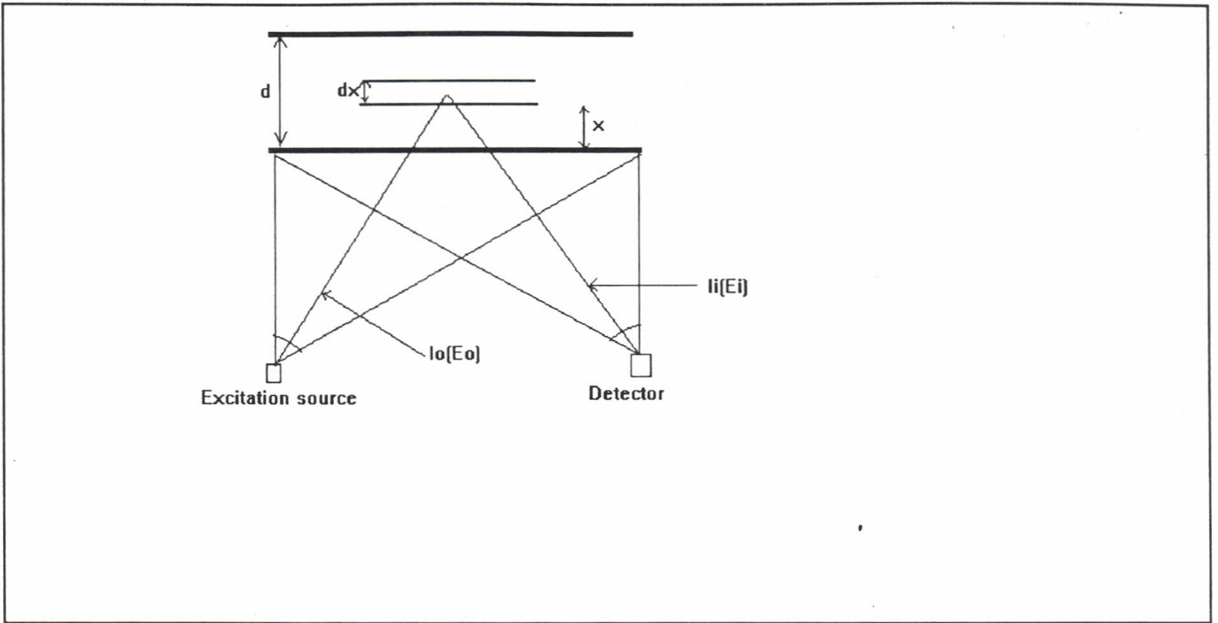


Fig 1 Sample geometry for analysis of intermediate thick samples with E.D.X.R.F. using Fundamental Parameters(Jenkins,1981).

I_i = intensity of characteristic x-rays from element i in sample

$G_o = I_o\ \Omega_1\ \Omega_2\ \mathrm{cosec}\phi_1$ (c/s) is the geometric constant.

Ω_1 = solid angle of sample as seen from radiation source

Ω_2 = solid angle of sampleas seen from detector

ϕ_1 = angle of incidence of primary radiation

ϕ_2 = effective angle of emergence of characteristic radiation from the sample.

ρ = specimen density.

d = specimen thickness.

$K'_i = \sigma_i\ \omega_i\ f_i\ (1-1/J_k)_i\ \epsilon_{(Ei)}$ (cm^2/g) is the relative excitation detection efficiency.

$a = \sum\mu_{(Eo)}\csc\phi_1 + \sum\mu_{(Ei)}\csc\phi_2$ - Total absorption for the

primary and fluorescence radiation in the sample.

$\mu_{(Eo)}$ = total specimen mass attenuation coefficient at primary radiation

$\mu_{(Ei)}$ = total mass attenuation coefficient for the fluoresced energy E_i.

ω_i = fluorescence yield of element i.

$\sigma_{i(Eo)}$ = total photoelectric mass absorption coefficient for element i at energy .

$\epsilon_{(Ei)}$ = efficiency of detector at the fluoresced energy Ei

f_i = Fractional radiative rate.

J_k = absorption jump of the K edge of the photoelectric absorption.

2.2.2. Particle size, heterogeneity and surface texture effects

Derivation of the basic excitation equation 2.1 assumes homogeneous and flat specimen. This means that the relatively thin surface layer that emits the measured x-ray spectral line intensity contains all elements homogeneously distributed in the specimen in their true concentration ratios. A specimen is said to be homogeneous or heterogeneous according to whether all its particles have the same chemical composition or the material is a mixture of particles having two or more compositions.

A solid is said to have uniform or nonuniform particle size according to whether all particles have the same size or the material is a mixture of particles of different sizes.

Emitted line intensity may be affected by the particle size and particle size distribution in a solid - even if the

composition is homogeneous since a large particle will absorb the incident radiation more than a small particle. Emitted line intensity may be affected by heterogeneity even if the particle size is uniform since different chemicals in the dispersed differentlyin the specimen will absorb the primary and secondary radiations differently.

These effects arise when the specimen is nonuniform with respect to composition or particle size over distances about the same as the pathlength of the analyte line X-rays.In addition the emitted line intensity may also be affected by the surface texture of a solid even if both the composition and particle size are uniform. The emitted intensity is affected by the orientation of the grind or polish marks with respect to the directions of the primary and secondary radiation beams.

Clearly if the composition, heterogeneity, particle size , particle size distribution and/or surface texture vary among samples and standards, the measured analyte line intensities are likely to be difficult to correlate with each other and with the analyte concentration. The severity of the effects increase as heterogeneity and nonuniformity of the analyte increases and energy of the analyte decreases.

Particle size effects become less severe as the specimen thickness decreases below infinite thickness. Surface texture may have one or more of the following three major effects;

(a) The pathlength of the primary and analyte line radiation with the specimen may vary from point to point.

(b) Because the measured analyte line x-rays leave the surface at ≈ 90° to the incident primary radiation there is

the possibility of shielding and shadowing effects.

(c) Extremely coarse surface topography may actually influence the effective distance between the radiation source and specimen.

These effects become progressively more severe as; the energy of the analyte line decreases; the mass absorption coefficient of the specimen and more important, analyte line x-rays increase and the energy of the radiation source decreases.

2.2.3. Correction of matrix effects

Generally a sample cannot be completely transparent (absolutely thin) and as such one prepares intermediate thick samples. To correct for matrix effects ,the emission-transmission method is used(Giauge *et al*,1979) The specimen is mounted on a substrate of a pure target element (see Fig 2) and calculations for absorption effects performed using the undersaid relationships and a suitable quantitative analysis software like the Quantitative Analysis of Environmental Samples software (QAES, Kump,1993). According to Beer's, $I=I_o e^{-\mu d}$ where I is the transmitted intensity, I_o is the incident intensity, μ (=$a\rho$, see equation 2.1) is the attenuation coefficient and d is the sample thickness. Thus $\mu d=In(I_o/I)$. According to Giauge *et al*(1979), I_o represents the intensity of the pure target element(I_T), I represents the difference between the intensity of the pure target and the sample (I_{TS}) and the intensity of the sample alone (I_S). Thus $\mu d = In(I_T/(I_{TS}-T_S))$

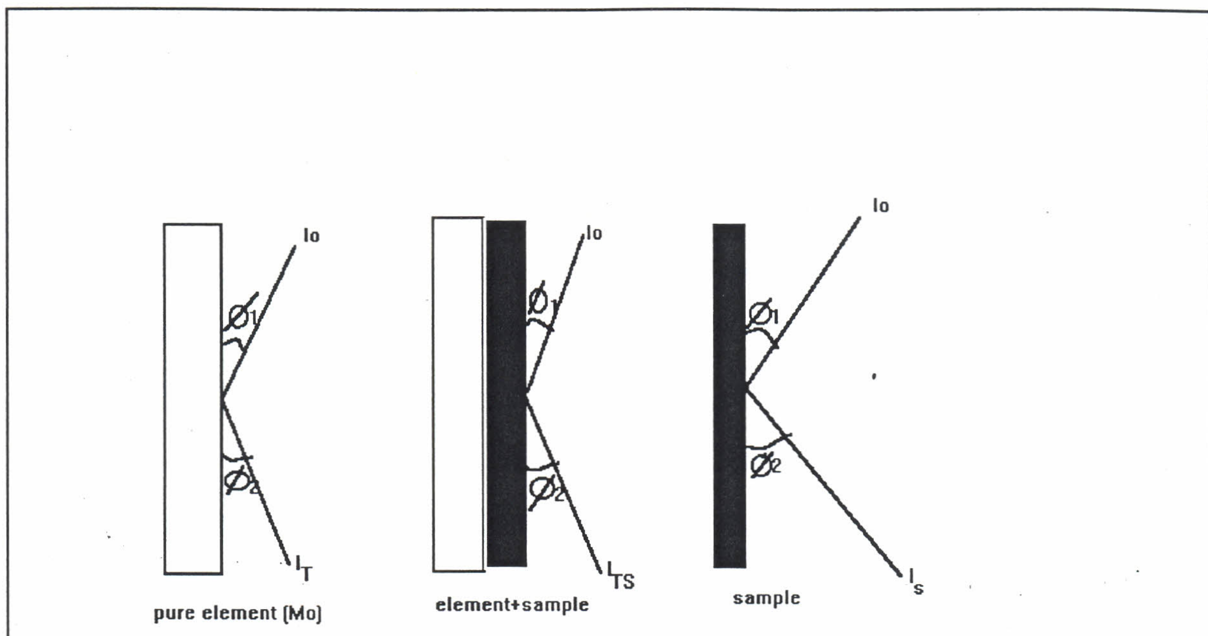


Fig.2 The emission-transmission method for matrix absorption corrections(Giauge *et al*,1979)

2.2.4. Spectrum analysis

Least-square fitting of photopeaks

Spectrum analysis encompasses the technique of extracting from a recorded spectrum the most accurate information possible about characteristic line energies and intensities. The recorded spectrum is considered to be a histogram with the x-axis representing energy intervals (channel numbers) and the y-axis representing the number of counts recorded in the time t in each interval or channel number. Provided peak shapes are accurately depicted, least-square fitting provides the most accurate measurement of the component peak intensities for overlapping peaks.

Spectrum analysis can accomplished by use of a suitable software like the Analysis of X-ray spectrum by Iterative Least-

square fitting (AXIL) which utilises the least-square fitting procedure for spectrum analysis(Van Espen *et al* 1986). The leastsquare fitting procedure assumes that the intensities can be approximated by a gaussian shape i.e. for a region of spectrum containing the background and n overlapping peaks the function could be described by the equation

$$y(x) = f_o(x) + \Sigma y_i(x) \quad ....2.2$$

where $f_o(x)$ describes the background while $y_i(x)$ describes one of the peaks(Jenkins,1981).

$$y_i(x) = (A_i/\sigma_i\sqrt{2\pi}) \exp(-(x_i-x_p)^2/2\sigma_i^2) \quad ...2.3$$

where A_i= the area of the peak

σ_i= width of the peak

x_i= energy of the i_{th} element

x_p= the peak centroid

The background function is a quadratic function and takes the form :

$$f_o(x) = c_o + c_1x + c_2x^2 \quad ......2.4$$

Thus the fitted spectrum takes the form

$$y(x) = \Sigma c_i\ y_i(x) .... \ 2.5$$

where the y_i are the number of counts in channel i and c_i are the parameters to be determined by optimizing the fit i.e.

$$\chi^2 = \Sigma(y_i-y(x_i))^2/y_i \ .... \ 2.6$$

is to be minimised. y_i is the measured data, $y(x_i)$is the calculated data in the ith channelfrom equation 2.3.

2.2.5. Quantitative X-ray Analysis of intermediate thick samples using emission-transmission method

The EDXRF analysis of intermediate thick samples using the emission-transmission method (Fig 2) can be applied to a variety of samples. However, this method needs to perform many calculations due to finding absorption correction factors. This is often the most labour-consuming stage of the analysis. Thus the development of a computer software (Holynska *et al*, 1993) was necessary to reduce the time needed for an analysis.

The algorithm of the program (fig 3), is based on the formula for intensity of the x-ray line of the ith element (equations 2.1,2.7) emitted from an intermediate thick sample.

$$I_i = G_o\ I_o\ \epsilon_{(Ei)}\ K_i'_{(Eo)}\ C_i\ Ms\ F_{abs} \quad \ldots\ldots\ldots\ldots 2.7$$

where; G_o is the geometrical constant, I_o is the intensity of the primary radiation ,$\epsilon_{(Ei)}$ is the detection efficiency for the radiation of energy of E_i ,$K_i'_{(Eo)} = \sigma^i_{(Eo)}\ \{1-1/J^i\}\ \omega^i\ f^i$ is the relative detection efficiency and $\sigma_{i(Eo)}$ is the photoelectric mass absorption coefficient for the radiation of energy E_o

J_i is the jump of mass absorption coefficient at the absorption edge, ω_i is the fluorescence yield, f_i is the fractional radiative rate, C_i is the weight fraction of the ith element , M_s is the mass per unit area of the sample. Equation 2.7 reduces to

$$I_i = S_i C_i F_{abs} \quad \ldots\ldots 2.8$$

where $S_i = G_o\ I_o\ \epsilon_{(Ei)}\ K_i'_{(Eo)}$ is the Sensitivity of the detector

system for element i in the given matrix , C_i is the concentration of element i in the sample in μg/g and F_{abs} is the absorption correction factor. This Fundamental Parameters Technique has the advantage of removing the need to calibrate the EDXRF system with standards marching the composition of the unknown samples and replaces it by a process of determining absorption within the sample itself i.e. the Emission-Trans mission method in which essentially three measurements of characteristic x-rays for:sample, sample with target and target a lone are performed. Peak areas are calculated by Analysis of X-ray by Iterative Least square fitting (AXIL) program (Van Espen *et al*, 1986) and stored in files while quantitative analysis was by QAES software (Kump,1993). The library data from the AXIL files contain atomic mass values, photoelectric mass absorption coefficients, fluorescence yields, energies and fractional radiative rates for for x-ray lines, energies of absorption edges. The jumps of mass absorption coefficients are calculated with the use of formulas as given by Poehn *et al* (1985).All other data are entered through the operator interface.

In calculating the absorption correction factor (F_{abs}) the software uses the equation below:

$$F_{abs}=\frac{1-\exp[-\ln(\frac{I^{T}}{I^{TS}-I^{S}})]}{\ln(\frac{I^{T}}{I^{TS}-I^{S}})} \quad .....2.9$$

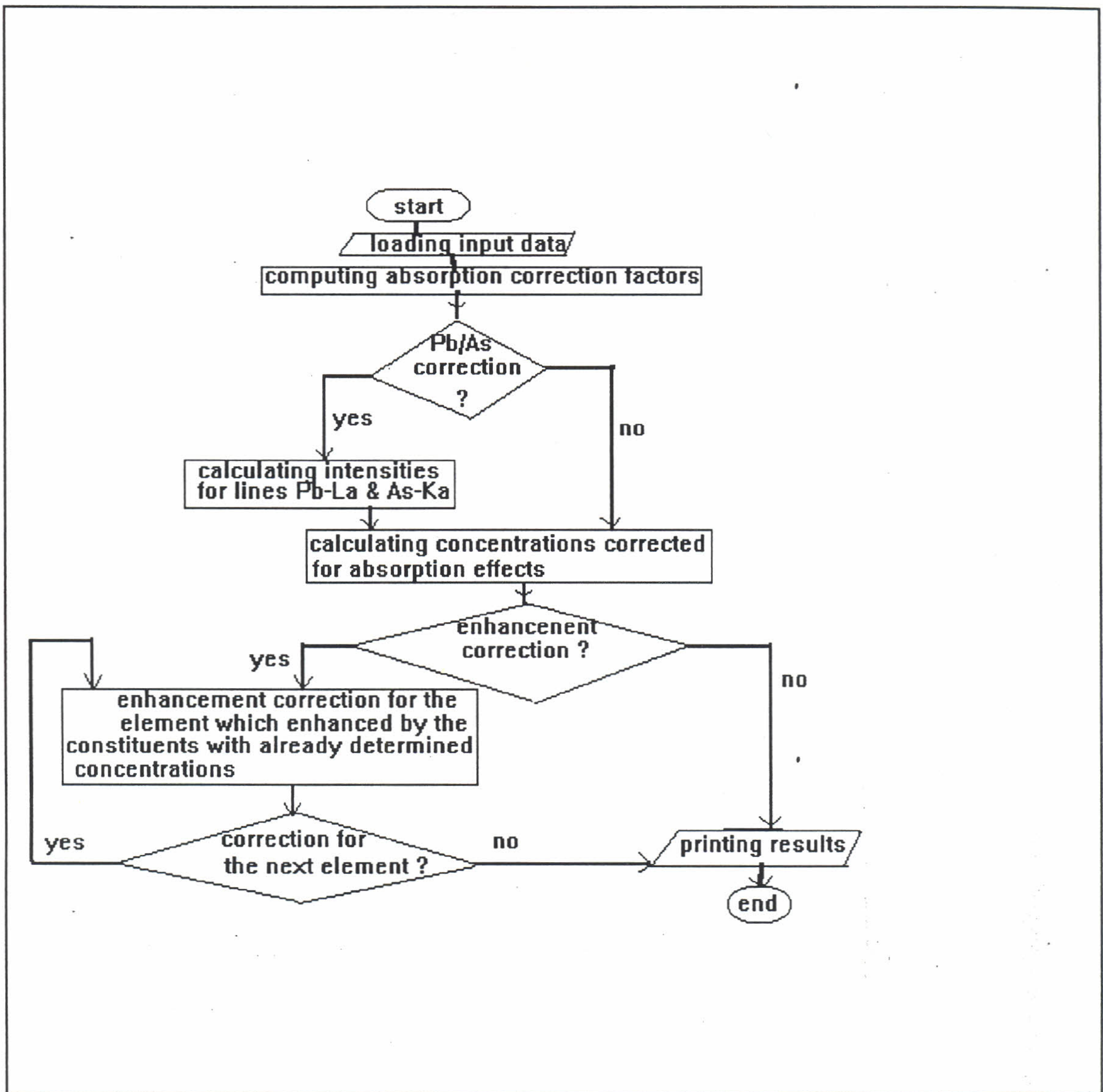


Fig 3 The flowchart of the program.(Holynska and Wegzynek,1993)

where;

I^S = intensity of the measured characteristic x-ray line for sample

I^{TS} =intensity of the measured characteristic x-ray line for

sample with target.

I^T =intensity of the pure(M_o) target a lone

At first the program calculates the expression $In\{I^T/(I^{TS} - I^S)\}$ for the x-ray line selected by the operator. Next, fitting the function against the energy is performed taking into account expected absorption edges. The fitting is linear except for energies greater than the absorption edge. In that case, parabolic fitting is carried out.

2.3. Sources of error in EDXRF quantitative Analysis

The basic equation (2.1) used in quantitative analysis by the Fundamental Parameters Technique is an oversimplification of the true processes of radiation interaction with matter that results in the characteristic X-ray intensities of the sample elements.

Effects due to absorption are only considered while it is true that enhancement effects, which depend on the sample composition can cause additional uncertainties in the determination of concentrations of elements of interest. These are ignored for practical purposes. Sources of error in this respect were considered as from:

1: Sample	**2: Equipment**
absorption	**systematic**
enhancemewnt	short-term drifts
heterogeneity	long-term drifts
mixing	sample repositioning
weighing	reseting operation conditions
contamination	crystal deterioration

2.3.1. Measurement of x-ray intensity.

2.3.1.1. The net counting error.

It is important that a sufficient number of counts be taken of each analytical line, and if necessary on the associated background, to ensure that the counting error is minimised . It is also important that no systematic errors be introduced in the measurement of the intensity, such as those that may accrue due to counting loss (mainly those due to deadtime in the detection and counting circuitry)

In energy dispersive spectrometers where peak-to-background ratios are relatively low, background subtraction is necessary. Although the background level may remain constant during short periods of time, over a period of several hours significant fluctuations may be observed(Jenkins,1981).

2.3.2. Estimation of background

In those cases where the background is significant and therefore has to be measured, problems may occur in actual background measurement. The true background is measured at the analyte energy when the analyte concentration is zero. Such a measurement is impracticable since the background itself is dependent upon the matrix composition.

In energy dispersive spectrometers, a computer software applied least-squares fitting technique is usually employed.

2.3.3. Problems in Quantitative Analysis.

Quantitative analysis by x-ray emission involves a series of steps, each of which must be carefully controlled if accurate data are to be obtained. Although accuracies of the order of a few tenths of one percent are possible and indeed being obtained by many hundreds of x-ray laboratories all over the world, a thorough understanding of specimen heterogeneity and absorption phenomena is necessary.

There are three major stages in quantitative determination: the preparation of the specimen from the sample taken for analysis the excitation of a suitable emission line for each element to be analysed and the measurement of its intensity and the conversion of the measured intensity into elemental concentration. The relationship between the measured x-ray intensity and elemental concentration based on equation 2.1 may also be put in the form:

$$C = K.I.M.S \qquad \ldots\ldots 2.10$$

where M represents the inter-element effects and S, the specimen heterogeneity. This expression shows that four factors may affect the accuracy in the measurement of C, namely;

(a). The factor K: (sensitivity) This is a factor which depends upon the design of the spectrometer and the conditions under which the spectrometer is operated. For a given instrument is constant only where all measurements are performed under constant conditions. It is almost impossible to calculate K accurately. Thus in practice its value is

determined by calibration.(b) The Intensity I: This is the net intensity of the measured energy peak above the background. For error levels above the limits set by the mechanical precision or electrical stability of the instrument, counting statistics alone will determine the precision in the measurement of I and hence in the precision in the estimation of C. (c) Inter-element effects M: This may include primary and secondary absorption effects. Since these effects all lead to systematic errors in the measurement of the true intensity, the accuracy obtained in the measurement of C will be directly dependent upon how well the quantitative method employed corrects for or minimises inter-element effects. (d) Specimen heterogeneity S: This will depend mainly upon the penetration depth of the measured x-rays relative to the average particle size of the specimen. Where both penetration depth and particle size are of the same order it will also depend upon the elemental distribution within a particle. At the present state of art, the only reliable way of reducing or eliminating problems arising from specimen inhomogeneity lies in controlling the particle size influence by suitable specimen preparation

CHAPTER 3.

EXPERIMENTAL PROCEDURES

3.1 Sampling and Sample Preparation

3.1.1. Sampling

100 samples representing ten brands of imported cosmetic creams were bought from various shops in Nairobi. Sampling was done such that the first five samples of each brand were bought during the month of March,1995 while the last five samples were bought during the month of Setember , 1995 in an attempt to randomise the sampling and' acquire samples manufactured under different batch numbers . The September samples had higher batch numbers than the March samples and the objective of this kind of sample collection was to study possible variations in levels of mercury between the two groups of samples. All samples were acquired and preserved in their normal commercial package to prevent atmospheric exposure that would enhance deterioration and contamination.

3.1.2. Sample preparation

For each sample the specimen for analysis was prepared by warming the sample to melt (≈ 40° to 50°C) on a water bath. The molten sample was then poured on a 2.5 cm diameter mylar-backed aluminium ring so as to acquire the configuration of a uniform sample pellet when cooled. Apart from the slight warming no other physical or chemical treatment was applied to the sample. The samples were then irradiated with ^{109}Cd excitation source and their respective spectra acquired over a time such that the error in the area of the L1 peak of mercury

had good statistics.

3.1.3. Sensitivity measurements

To determine the sensitivity of the detector system for mercury,an oil standard certified to contain 300μg/g of Pb, Hg, Bi,and u was used to determine the number of counts per second per microgram which is sensitivity of the detector for each of the L-lines element. The values obtained were plotted against the atomic number of the elements to give the sensitivity curve.

3.1.4. Accuracy of measurements

To determine the accuracy of the detector system for mercury samples of known amount of mercury ranging from 1000μg/g to 50μg/g were irradiated and the experimental mercury measurements in them compared to the certified values.

3.1.5. Precision of measurements

In order to determine the reproducibility of the detector system five specimen of the standard solution containing 1000μg/g were made by pippetting about 5 cm^3 of this solution. the specimen were irradiated and the experimental measurements compared to the certified value.

3.1.6. Sample irradiation

Samples were irradiated ^{109}Cd excitation source and their respective spectra acquired over a time such that the error in the $L_{\alpha 1}$ peak of mercury had good statistics. the spectra were developed using an S100 PC based multichannel analyser.

3.1.7. Absorption Correction.

To correct for matrix absorption effects, first, the intensity of mercury in the sample was measured, then the intensity of a pure molybdenum target mounted behind the sample was measured and finally the intensity of the pure target element alone was measured. The emission-transmission method (equation 2.9) used to determine the absorption correction factor.

3.1.8. Data deconvolution.

Spectral data deconvolution was done using the AXIL software while the quantitative analysis was achieved by use of the Quantitative Analysis of Environmental Samples (QAES) software.

3.1.9 Detection limit

The lower limit of detection of the EDXRF spectrometer was determined as described from the equation 3.1 using the intensity of the $L_{\alpha 1}$ peak for mercury.

$$DL = \frac{3}{M}\sqrt{\frac{C_b}{T_b}} \quad \ldots\ldots. 3.1$$

Where C_b is the number of background counts, T_b is the background time and M is the sensitivity of the detector in c/s/μg.

3.2 Instrumentation

The instrumentation used in this study is shown in fig 4.

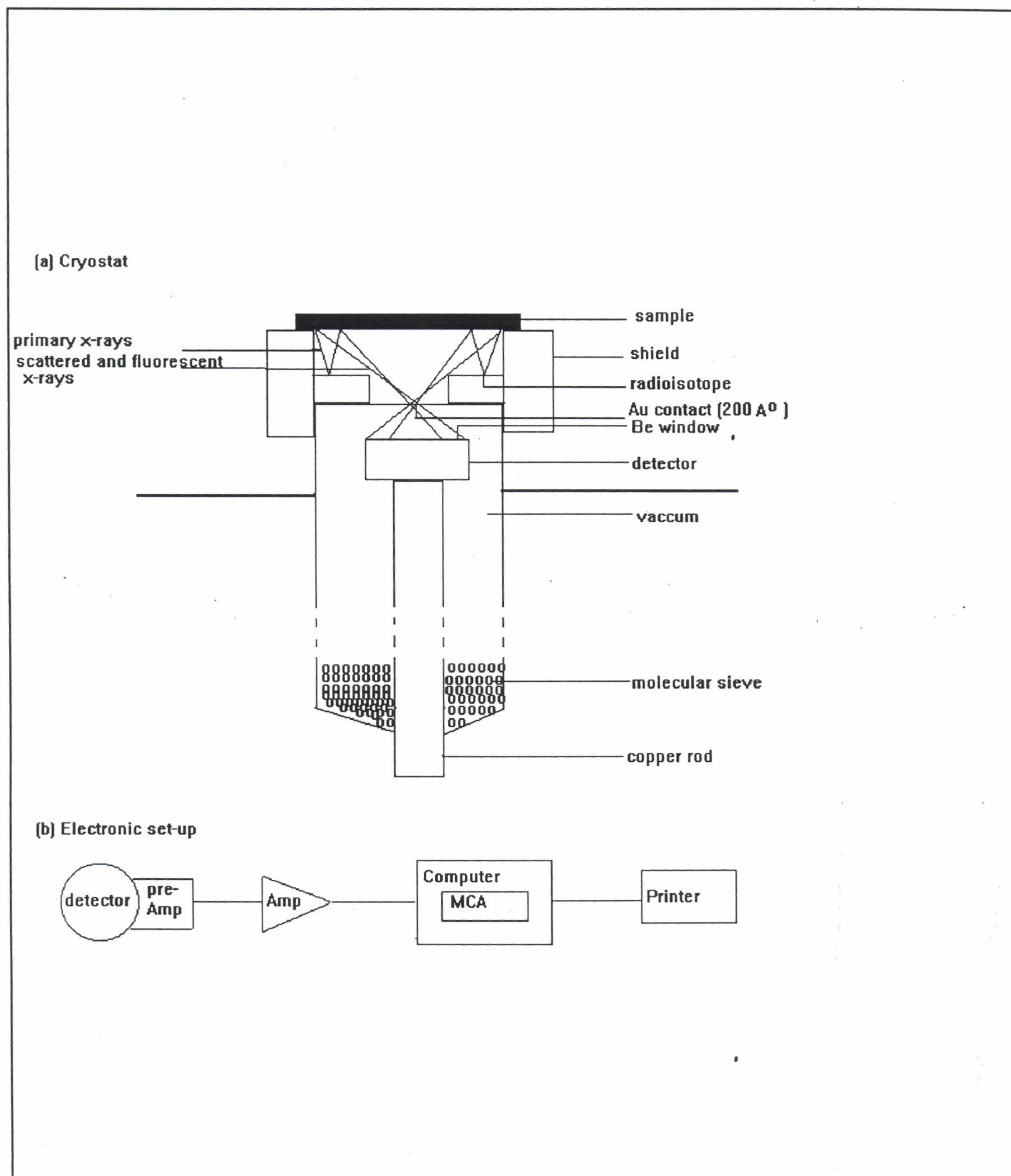


Fig 4 Schematic diagram of EDXRF system.
(Kinyua, 1982)

and the parameters of the detector used were:

Type: $28mm^2$ x 5mm thick   EG&G Ortec

air path: 1.6cm

detector sample distance: 2.1cm

detector sensitive radius:0.3cm

detector sensitive depth: 0.5cm

collimator radius: 0.47cm

effective sample radius: 1.0475cm

beryllium window thickness:25 microns

dead layer: 0.63 microns   gold layer contact: 200Å

incidence angle: 53.3°   takeoff angle: 72.2°

source activity: 20mCi (1st May 1994)

detector resolution: 180eV at Mn K_α line (5.9 keV)

energy of primary radiation: 22.1 keV (Ag k_α line)

3.3 AAS analysis of cosmetic cream

An exercise to compare the EDXRF measurements with AAS measurements was done. The following method for AAS sample preparation was employed:-

0.1g of the cream sample was weighed into a $150cm^3$ conical flask. Two to five drops of water were added and $5cm^3$ of analytical grade sulphuric acid introduced from a pipette. The contents were then placed in a temperature regulated waterbath at 70°C for an hour. The highly coloured digests were cooled and $50cm^3$ of analytical grade permanganate solution added and further digestion continued for two hours in the waterbath. $15cm_3$ of $NH_2OH.HCL$ solution were added to reduce the excess $KMnO_4$. The resulting mercuric ions solution was then reacted with $SnCl_2.H_2O$

and the mercury vapour produced therefrom pumped to the AAS detector and its absorbance read at 253.7nm. The amount of mercury in the sample was determined using the relationship

$$A = E\ c\ d \ldots\ldots 3.3$$

where E is the molar absorptivity of the AAS ($mol^{-1}cm^{-1}$), c is the concentration of mercury in the sample(mol), d is the length of the AAS cell and A is the recorded absorbance.

CHAPTER 4

RESULTS AND DISCUSSION.

4.1. Sensitivity of the EDXRF system for mercury.

The measurement of sensitivity of the detector system for Hg, Pb, Bi and U produced the results shown in table 3

Table 3 Sensitivity measurements

Element	Atomic Number	Sensitivity
Hg	80	0.0129
Pb	82	0.0166
Bi	83	0.0227
U	92	0.0237

A plot of sensitivity (S) against atomic number (Z) generated as described in section 3.1.3 produced an S-shaped curve (Fig 5) which showed sensitivity to increase as Z increases. However the sensitivity reaches a plateau between Z=83 and 92 probably due to high absorption of the characteristic X-rays of these elements by the lower z elements. This was the shape of curve to be expected(Jenkins, 1981)

4.2 Accuracy of measurements

The following results (table 4) were obtained for the analysis of known concentration mercury solutions. Using the student's t-test for small samples (n<30), comparison of the experimental measurements with the certified values showed no significant difference . This showed that the analytical technique and the sample preparation method adopted in this study were valid and that the sensitivity determined to calibrate the

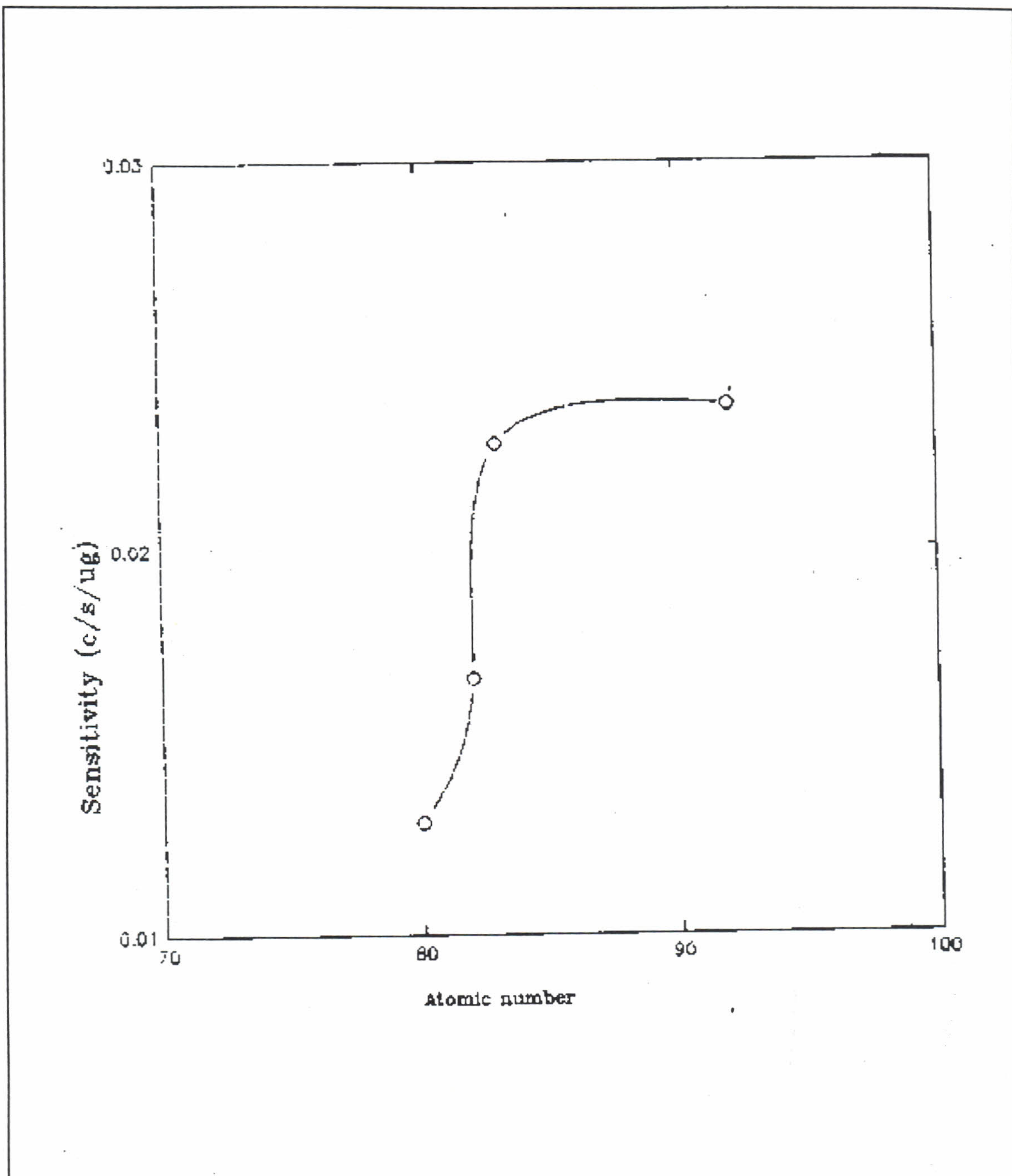


Fig. 5 Elemental sensitivity of the EDXRF system

detector system was accurate.

Table 4 Results of analysis of mercury standards

Certified concentration (µg/g)	Exprimental concentration (µg/g) n=3
1000	1000.2±1.4
500	496.7±5.2
100	99.6±0.8
50	49.2±0.6

4.3 Results of precision of measurements.

The 5 specimen made for precision measurements produced the results in table 5. Comparison of this experimental measurement with the certified value showed no significant difference implying that the sample preparation method had good reproducibility and that a measurement made from a sample prepared using this method can be taken as precise.

Table 5 Precision of measurements

Specimen number	Certified conc. (µg/g)	Experimental conc. (µg/g)
1	1000	1002.3±1.8
2	1000	1000.5±0.8
3	1000	998.9±1.2
4	1000	1001.2±0.7
5	1000	1000.8±0.3

4.4 Detection limit

A detection limit of 3.3 µg/g was determined for the technique used. The detection limit determined in this study was rather high due to the effect of the low atomic number elements found

in the emulsion, emulsifier and other conventional additives found in cosmetic creams which due to their low energy x-rays and scattering tend to raise the backgound intensity (C_b/T_b) thereby raising the detection limit.

4.5 mercury concentrations in cosmetic creams

Table 6 mean mercury concentrations in cosmetic creams

Brand	Mean Hg conc for March samples(μg/g)	mean Hg conc for Sept.samples (μg/g)
Madonna(green)	31033±2017	28756±1947
Madonna(Red)	16079±1034	20909±1356
Pimplex	4350±287	9644±630
Shirley(Original)	13649±886	10697±687
Bestlady	14232±994	12367±803
Topsine	1552±103	1619±109
Fennel	4191±264	4787±300
Topshirley	BLD	BLD
Dermovate	BLD	BLD
Shirley(New)	BLD	BLD

BLD = Below Limit of Detection

Madonna(Green) cream: This brand of cosmetic cream labelled " MEDICATED CREAM" and " FOR EXPORT ONLY" was found to have the highest mean mercury concentration ≈ 2.9±0.2% for the ten samples analysed. Samples collected in March 1995 had a mean mercury concentration 3.1±0.2% while samples collected in September 1995 had a mean of 2.87±0.19%, implying a varioation of 7.3% in mercury concentrations between the two sample groups (Fig 6).

Madonna (red) cream: This cosmetic cream brand labelled " MEDICATED CREAM" and "FOR EXPORT ONLY" was found to have a mean mercury concentration of ≈ 1.8±0.1% for the ten samples analysed. Samples collected in march 1995 had a mean mercury concentration of 1.6±1% while samples collected in September had a mean of 2.1±1.4% implying a variation of 30% in mercury concentration between the two sample groups(Fig 7)

Pimplex cream: This cosmetic cream brand labelled " MEDICATED CREAM" was found to have a mean mercury concentration of 0.7±0.05% for the ten samples analysed. Samples collected in March 1995 had a mean mercury concentration of 0.4±0.03% while samples collected in September 1995 had a mean mercury concentration of 1.0±0.06%, implying a variation of 121% between the two sample groups(Fig 8).

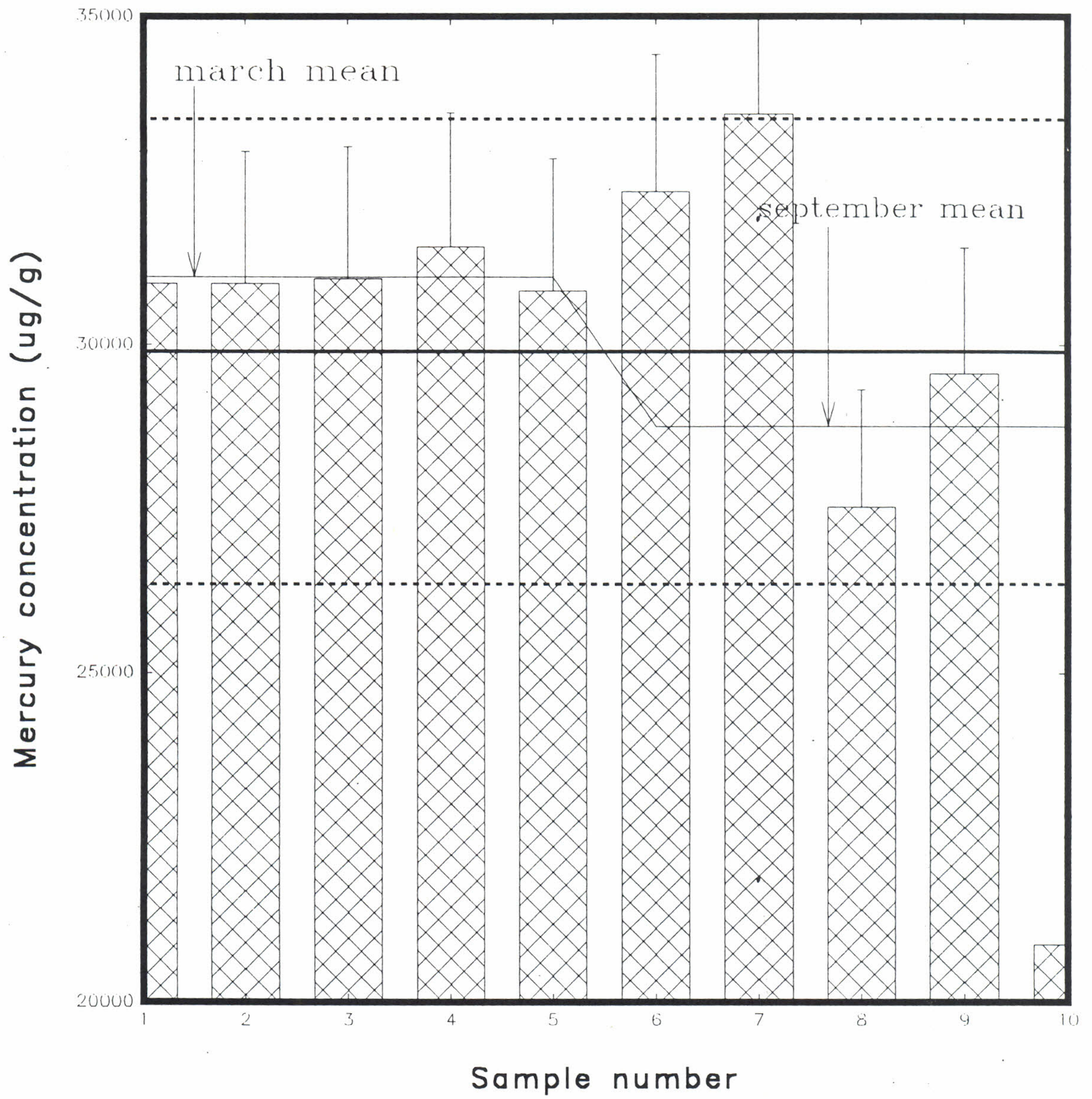


Fig 6 Concentration levels in Madonna(green) cream

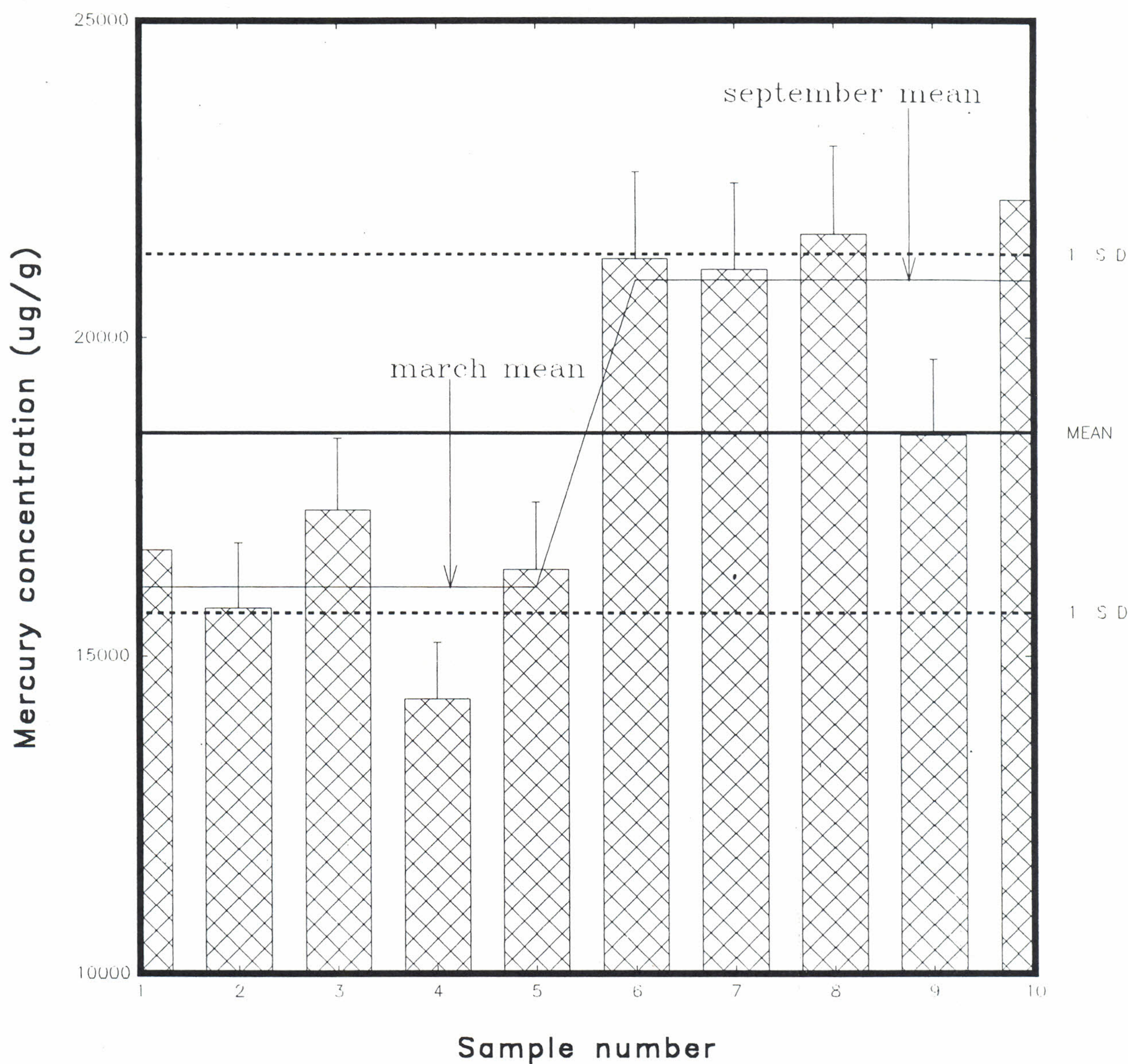


Fig 7 Concentration levels in Madonna(red) cream

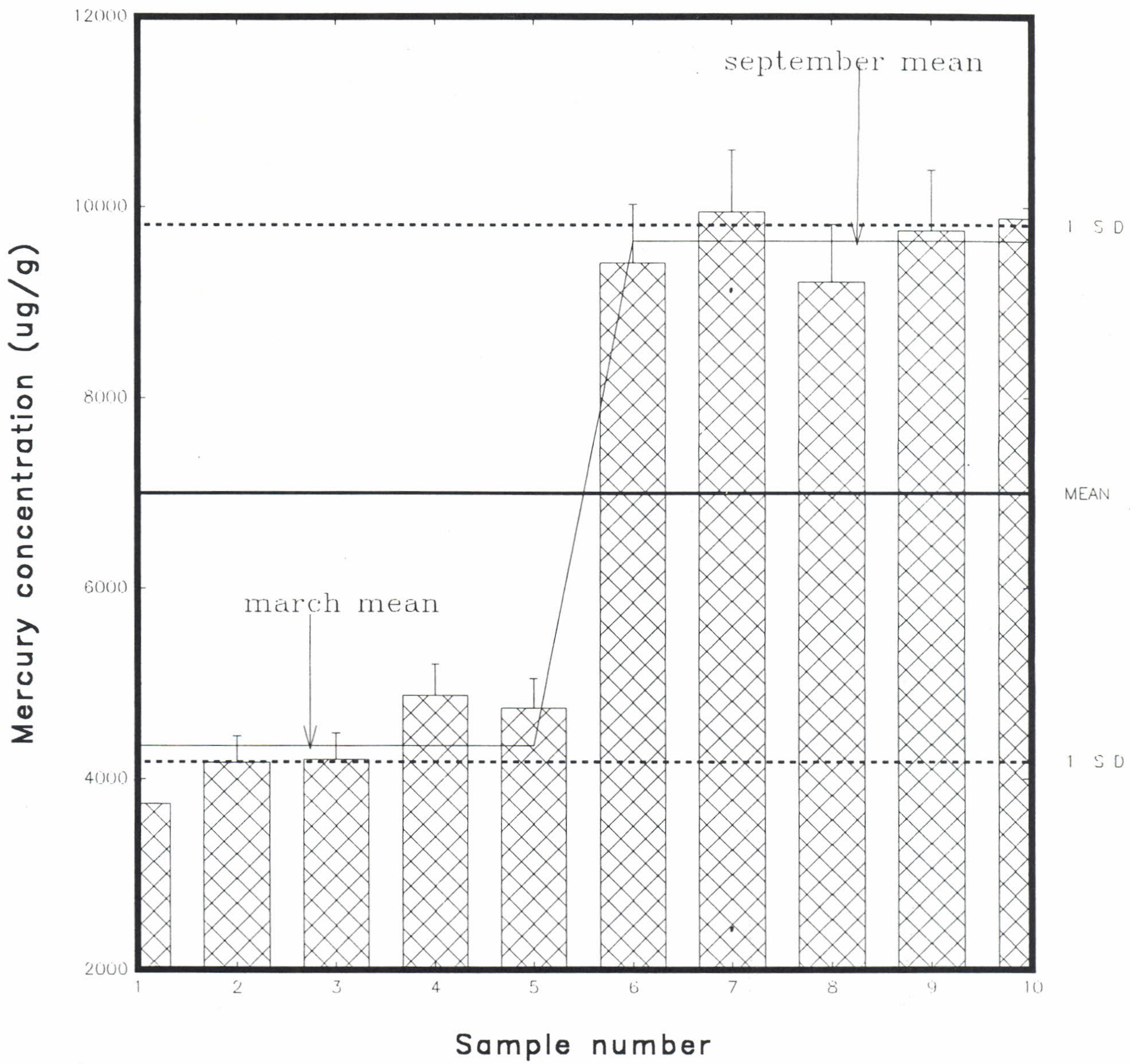


Fig 8 Concentration levels in Pimplex cream

Shirley (original) cream: This cosmetic cream brand labelled "MEDICATED CREAM" and " FOR EXPORT ONLY" showed a mean mercury concentration of 1.5±0.08% for the ten samples analysed. Samples collected in March 1995 had a mean mercury concentration of 1.8±0.09% while samples collected in September 1995 had a mean mercury concentration of 1.1±0.07%, implying a variation of 40% between the two sample groups(Fig 9).

Bestlady cream: This cosmetic cream labelled " MEDICATED CREAM" has a mean mercury concentration of 1.47±0.1% for the ten samples analysed. Sapmles collected in march 1995 had a mean mercury concentration *of 1.42±0.1% while samples collected in September* 1995 had a mean mercury concentration of 1.53±0.1%, implying a variation of 7.4% in mercury concentrations between the two sample groups (Fig 10)

Topsine cream: This cosmetic cream brand labelled " MEDICATED CREAM " was found to have a mean mercury concentration of 0.16±0.01 for the ten samples analysed. Samples collected in March 1995 had a mean mercury concentration of 0.15±0.01% while samples collected in September 1995 showed a mean mercury concentration of 0.16±0.01% implying a variation of 3.7% between the two sample groups(Fig 11).

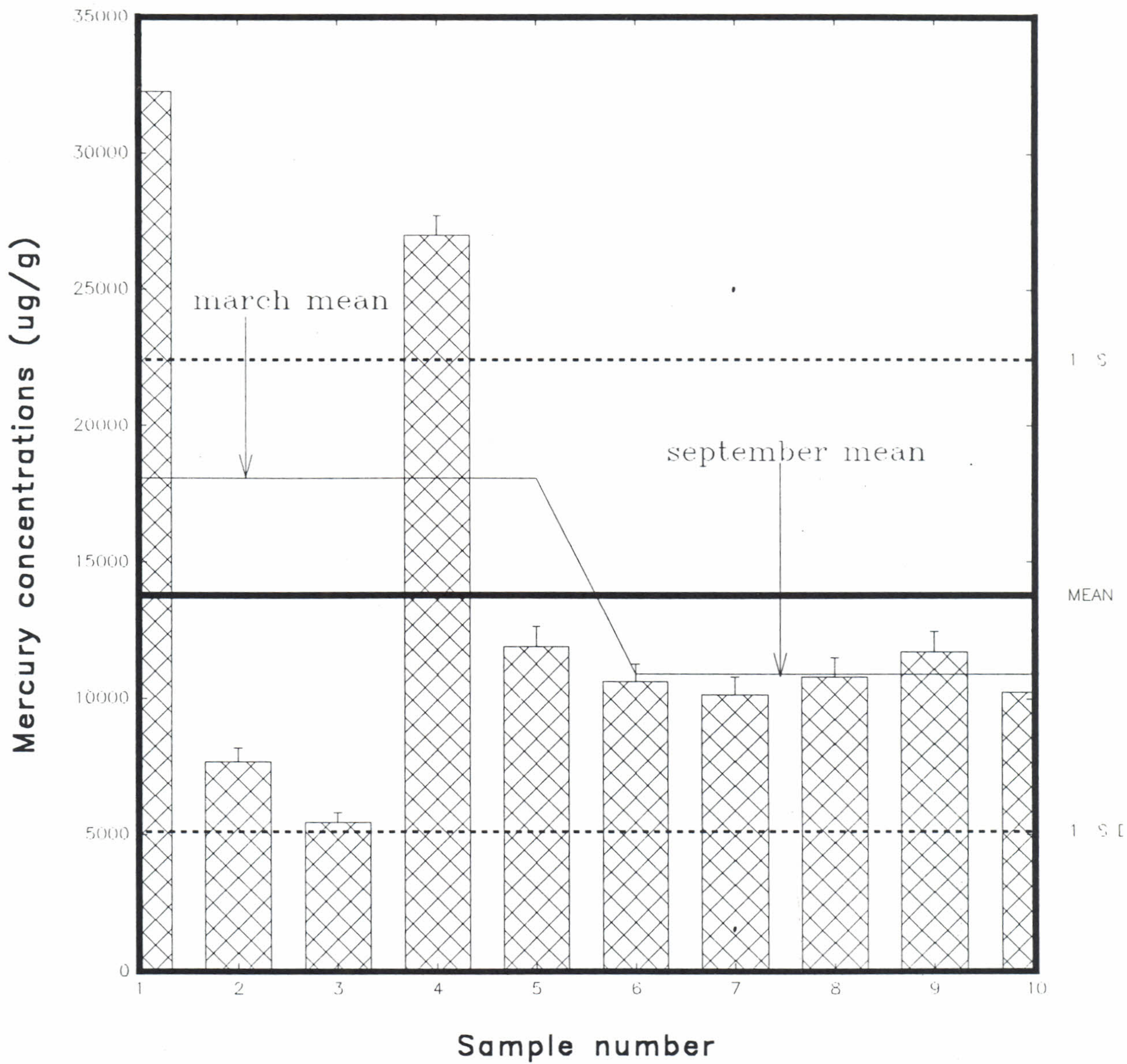


Fig 9 Concentration levels in Shirley(original) cream

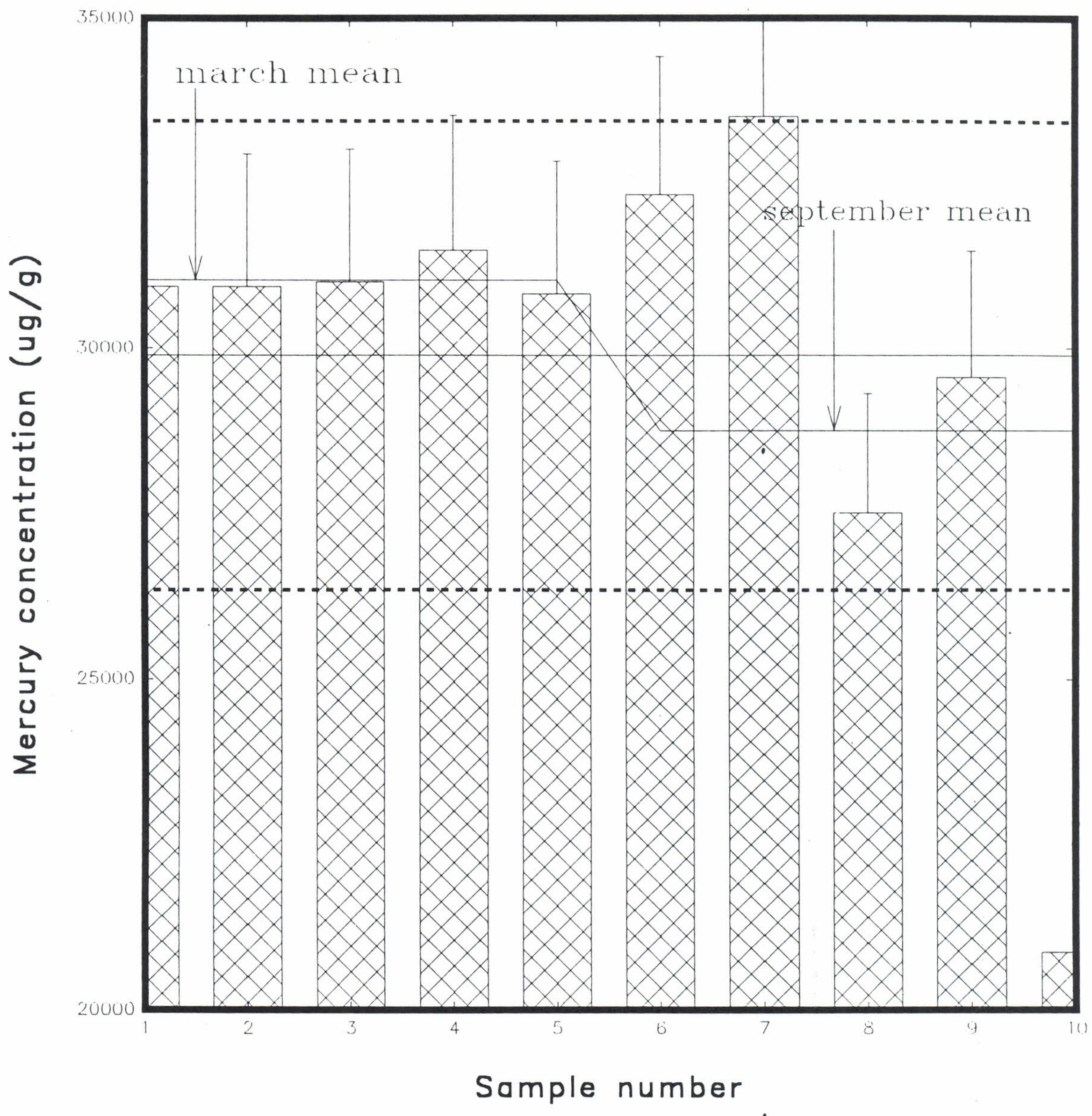


Fig 10 concentration levels in Bestlady cream

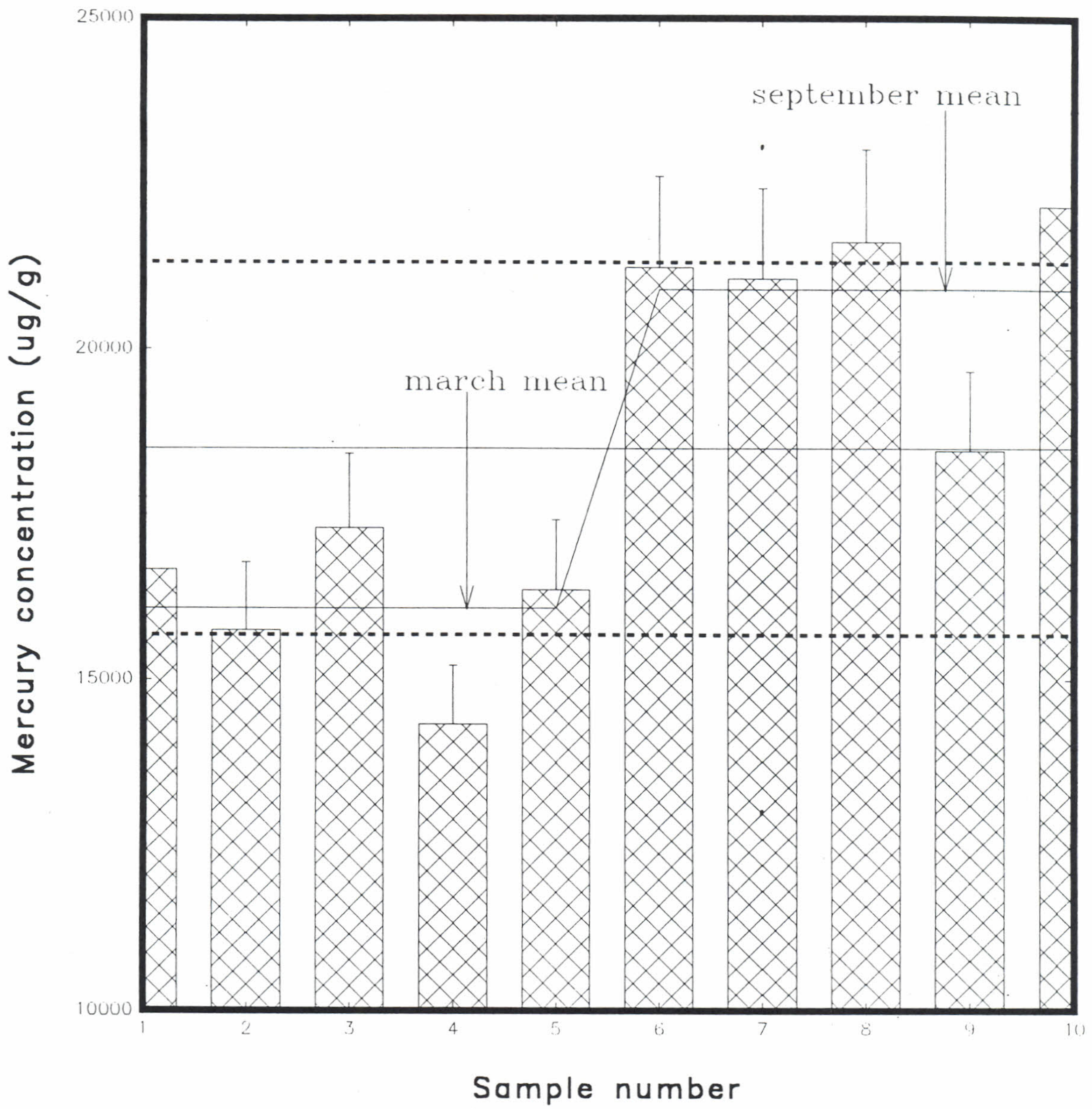


Fig 11 Concentration levels in Topsine cream

Fennel cream: This cosmetic brandhad a mean mercury concentration of 0.43±0.03% for the ten samples analysed. samples Collected in March 1995 had a mean mercury concentration of 0.41±0.03% while samples collected in September 1995 had a mean mercury concentration of 0.48±0.03%, implying a variation of 14% between the two sample groups(Fig 12).

Shirley(new),Dermovate,Topshirley creams: This cosmetic cream brands were found to have no mercury within the lower limit of detection of the EDXRF system. Other elements found in the creams analysed were Ca, Ti, Fe, Co, Cu, and Zn. However their concentrations were within alowable limits.

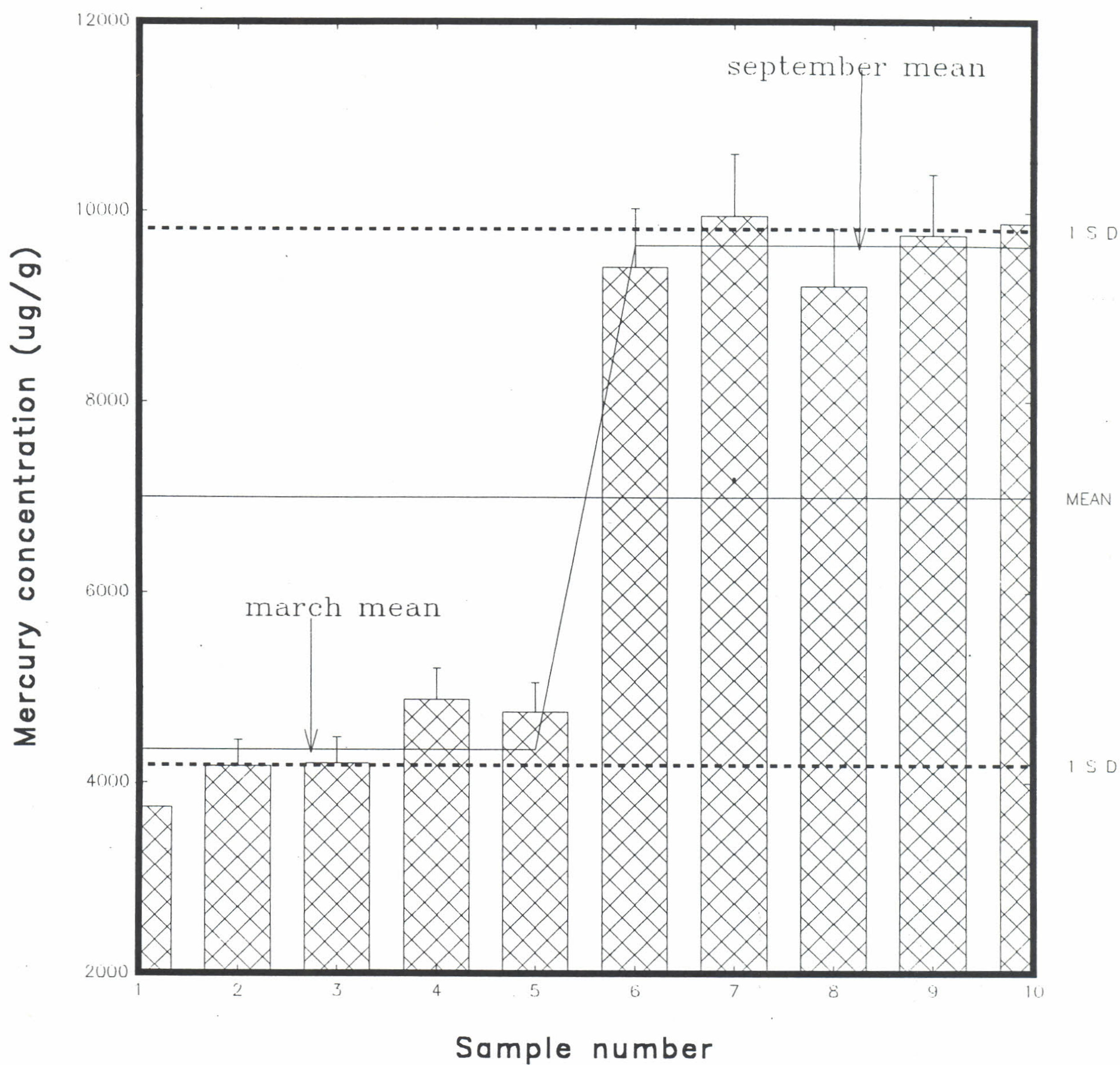


Fig 12 Concentration levels in Fennel cream

4.5.1. Variations in mercury concentrations

The aim of this exercise was to statistically analyse the difference in levels of mercury between samples collected in March and september,1995. The method of Least Significant Difference (LSD) between means of the two sample groups was calculated to determine whether the means were significantly different at the 95% confidence interval. μ_m=March mean, μ_s=september mean, SD?= whether the two means are significantly different.

Table 7 Variations in mercury concentrations.

Brand	μ_m(µg/g)	μ_s(µg/g)	LSD	SD?
Madonna(g)	31033±2016	28756±1946	87.4	yes
Madonna(r)	16079±1034	20908±1356	35.2	yes
Pimplex	4349±287	9644±630	21.8	yes
Shirley(o)	13684±886	10697±687	28.6	yes
Bestlady	14231±994	12366±802	30.1	yes
Topsine	1551±103	1618±109	10.5	yes
Fennel	4191±264	4787±300	17.1	yes
Shirley(n)	BLD	BLD	0.0	no
Dermovate,	BLD	BLD	0.0	no
Topshirley	BLD	BLD	0.0	no

Table 8 Comparison between EDXRF and AAS measurements

Sample number	Hg level(µg/g)EDXRF	Hglevel(µg/g)AAS
Bestlady (B19)	30186±1051	29888
Madonna(green)(Mg4	31479±2046	30563
Shirley(orig)(SO4)	26980±724	25695
Pimplex(P7)	9949±451	9651
topshirley(TS1)	BLD	BLD

No significant difference was found between the AAS and EDXRF measurements at the 95% confidence interval.

4.5.2. Discussion.

The results of the cosmetic creams obtained in this study showed mercury concentrations to range from the lower limit of detection to about 30000μg/g. When these results were compared to those reported by Wandiga and Jumba (1982) it was observed that in the one and a half decade period between the studies, mercury has increasingly become a prominent ingredient in cosmetic creams despite the ban imposed on the manufacture, distribution ad selling of mercury containing creams. Moreover in this study, the level of mercury within samples of the same brand was found to vary considerably. Significant variation was also observed among the mean concentration levels of different brands.

This variation among samples of the same brand and among mean concentration levels of different brandsimply that the manufacturers of these mercury containing brands do not adhere to any quality control procedures and that a consumer has no assurance on the amount of mercury to be found in the next purchase of the same brand she has been using previously or if she decides to to change brands, she has no surity that the new brand will not be of worse health risk than the the previous brand.

According to WHO(1991), cosmetic creams are usually applied and left on the skin and if they contain mercury then the risk 0f exposure by absorption through the skin and through inhalation

exists. With regard to the high levels of mercury found in some of the cosmetic creams analysed in this study, it can be inferred that these products are undoubtedly a major health risk. Skog and Wahlberg (1974) reported that 5% of mercury in a 2% $HgCl_2$ solution was absorbed through the skin of guinea pigs over a five hours period. If the results of this study can be applied to human beings, then the amount of mercury absorbed through the human skin after application of cosmetic creams containing such high levels of mercury as was found in this study is certainly lethal. Furthermore, Hursh *et al* (1989) reported that uptake of mercury via the skin is about 1% of uptake by inhalation. thus, with respect to the WHO (1991) study and its report that uptake via inhalation also exists when a mercury containing cream is applied to the skin, it can therefore be expected that application to the skin of cosmetic creams containing such high levels of mercury as was determined in this study not only subjects the consumer to considerable absorption through the skin but also high uptake through inhalation.

Clarkson(1987) reported that dimethyl and diethyl mercury compounds are used in medicinal preparations such as cosmetic creams due to their high solubility in physiological fluids like blood. Also WHO(1991) reported that mercuric compounds are extensively poisonous and by far the most common cause of of acute poisoning. With regard to this two studies, and the fact that cosmetic creams are made from emulsions of light organic matter, it can be inferred that cosmetic creams containing such high levels of mercury as was found in this study will be highly absorbed into the body and subsequently couse acute poisoning if

the the mercurial present has a light organic moiety (eg methyl or ethyl). Also , Swensson and Ulfvarson (1987) reported that tissue oxidation of organomercurials transforms them to the mercuric ion form. This ion is less soluble in physiological fluids than methyl mercury and therefore biotransformation of methylmercury to the mercuric ion form reduces solubility of methylmercury and subsequently reduces the excretion rate of mercury resulting in its accumulation in tissues. Considering the high levels of mercury found in the cosmetic creams analysed in this study, if the mercury present in the cosmetic cream is a low alkyl mercurial such as methyl and ethyl mercury, then biotransformation would lead to accumulation of mercury in organs such as the kidney. As such in Kenya increasing rates of occurance of internal and external organ complications presenting symptoms similar to mercury poisoning could actually be due to mercury poisoning as a result of use of cosmetic creams containing high levels of of mercury as was found in this study.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The results of this study showed mercury levels in cosmetic creams to be much higher than those reported in the study by Wandiga and Jumba in 1982. Thus cosmetic cream products especially imported brands should be analysed on a regular basis using state-of-the-art technology especially in this era of market liberalisation. Those found to be infringing the imposed ban should be destroyed and impounded and legal action taken against their local importers and manufacturers internationally.

Further studies especially on the absorption of mercury through the human skin should be done so as to give a comprehensive picture of the effects of using mercury containing creams as cosmetic creams. The results of this study indicate a need for the Kenya Bureau of Standards to sample all the brands of cosmetic creams in the Kenyan market and have them analysed with a view to imposing stricter regulations on the importation, sales and marketing of this class of products. All the brands found to contain mercury should be banned from the market and all imported brands should be screened at the point of entry. Since cosmetic creams are sold as over-the-counter prescriptions, consumers should be careful especially with imported creams and where doubts arise the consumer should present the product to a quality control laboratory.

REFERENCES

BAKIR,F., DAMLUJI,S.F., AMIN-ZAKI,L., MURTADHA,M. (1973) Methylmercury poisoning in Iraq,Science,18:230

BARR,R.D., WOODGER,G.A., REES,P.H., KUNGU,A. (1972) Nephroptic syndrom in adult africans in Nairobi. Br.Med.Jr. 131.

BARR, R.D. WOODGER,G.A. (1973) Levels of mercury in urine correlated to cosmetic creams. Br. Med.J.

CEC, WORKING GROUP OF EXPERTS (1974) Non-organic M i c r o p o l l u t a n t s of the environment, Methods of Analysis, Report. Luxemberg, Commission of the European Communities,VF/1966/74e

CLARKSON,T.W. (1961) Studies on the equilibration of mercury vapour with blood, Rochester, University of Rochester atomic energy project, U.R., 582 P.299

CLARKSON,T.W.& ROTHSTEIN,A. (1964) Effects of amino acids on brain uptake of methyl mercury. Health Phys., 10:239

CLARKSON,T.W.& VOSTAL,J. (1973) Mercurials, mercury ion and sodium transport, In: Lant,A.F.& Wilson G.M., ed. Mordern diuretic therapy in the treatment of cardiovascular diseases, Armsterdam,Excerpta medica, p.229

CLARKSON,T.W.(1973) Mercury in western environment. pp 332-336

CLARKSON T.W.,(1987) ed. mercury, mercurials and mercaptans, Springfield, C.C. Thomas, p.233

CLARKSON,T.W.,FRIBERG,L.,HURSH,J.B.,NYLANDER,M. (1988a) The prediction of intake of mercury vapour from amalgams. ed. Biological monitoring of metals, New York, London,Plenum

Press,pp247-264

CLARKSON,T.W.,HURSH,J.B., SAGER,P.R., SYVERSSEN,T.L.M.(1988b) Mercury. biological monitering metals. NY,London, plenum press. pp199-246

COTTON,F.A.& WILKINSON,L. (1972) Advanced inorganic chemistry-a comprehensive text,New York, Wiley Inter-Science Publications. Third edition.

CRC (1972) Handbook of chemistry & physics, 32nd ed. CRC Press

DUXBURY,A.J.,EAD, R.D., McMURROUGH,S.,WATTS, D.C., (1982) Allergy to mercury in dental amalgams. Br. Dent.J., 152: 47-48

ELINDER,G.G., GERHARDSON,L., OBERDOESTER,G.(1988) Biological monitoring of metals. ed. biological monitoring of metals, New York, London, Plenum press. pp 1-71

ENGLESON,G.& HERNER,T.(1952) Acta. pediatr.scand.,41:289

FARANT,J.B.,BRISSETTE,D., MONCION,L., BIGRAS,L., CHARTRAND,A. (1981)- Improved cold vapour atomic absorption tech. for the micro-determination of total and inorganic mercury in biological samples. J.anal.tixicol. 5: 47-51

FINDLAY ET AL(1980) chronic hydroquinone poisoning by skin lightening creams. Med. J. 57: 187

FRYKHOLM,K.O. (1957) Mercury from dental amalgam. Its toxic and allergic effects and some comments on occupational hygiene. Acta. Odont. Scand.,22:1-108

GIAUGE,R.D., GARRET,R.B. ,GODA,L.Y. (1979) Determination of trace elements in light matrices by x-ray fluorescence spectrometry with incoherent scattered radiation as an internal standard,Analyt.Chem.51:511

GOLDWATER,L.J. (1973) Aryl and alkoxyalloyl mercurials. In: Miller,M.W.& Clarkson,T.W., ed. mercury, mercurials and mercaptans, Springfield, C.C. Thomas, p.56

GOLDWATER,L.J. (1964) Mercury, a history of quicksilver. R. Inst. Public health Hyg. J. 27: 279-397

GOTELLI,C.A.,ASTOLFI,E.,COX, E.,CLARKSON,T.W.(1985) Early biochemical effects of an organic mercury fungicide on infants. Science, 227:638-640

HOLYNSKA,B., WEGZYNEK,D.(1993) software for Fundamental Parametres analysis of intermediate thick samples.appl.Radiat. isot. vol 45: 401-404

HORVAT ET AL (1988) Trace elements in human placenta, blood and hair.

HOWIE,R.A., SMITH,H. (1967) J.forensic Sci. soc. 7: 90

HUNTER,D. (1969) Diseases of occupations, London, Little, Brown and Co., p.306

HUNTER,D.,RUSSEL,D.S.(1954) Focal celebral and celebellar attrophy in human subjects due to organic mercury compounds. J.Neurol. 17:235-241

HURSH,J.D.,CLARKSON,T.W.,CHERIAN,M.G.(1976) Clearance of mercury vapour inhaled by human subjects arch.environ.health . 31: 302.

HURSH,J.D. (1985)Particle coefficients of Hg^{203} J.appl.toxicol. 5: 327-332

HURSH,J.B.,CLARKSON,T.W.,MILES,E.,GOLDSMITH,M.A.(1989)Percutan eous absorption of mercury vapour by man. Arch. Environ. Health 44(2): 120-127

INTERNATIONAL ATOMIC ENERGY AGENCY (1972) Mercury contamination in man and his environment, Vienna, IAEA TRS No. 137

INTERNATIONAL LABOUR ORGANISATION (1970) Permissible levels of toxic substances in the working environment, Geneva, ILO, Occupational Safety and Health series 20

JAKLEVIC,J.M., FRENCH,W.R.,CLARKSON,T.W., GREENWOOD,M.R.(1978) X-ray fluorescence analysis applied to small samples in: Barret,C.S., Lyden,D.E., ed.,Advances in X-ray analysis. New York,London. Plenum press Vol.121 pp 171-185

JACINTA N.W., MARION N, personal communication,1995

JAN KRUGER (1973) Instrumentation in applied nuclear chemistry, Plenum Press, New York/London.

JENKINS,R. (1975) Gas flow propotional counters in X-ray spectroscopy. phillips scientific reports.F56

JENKINS,R. *et al* (1981) Quantitative x-ray spectrometry, Marcel Decker Inc. pp. 161-203

JOINT FAO/WHO MEETING ON FOOD ADDITIVES (1972) Evaluation of mercury, cadmium, lead and the food additives amaranth, diethylpyrocarbamate and octyl gallate, WHO food additives series, No.4, FAO nutrition meeting series report No. 51a

JOSELOW,M.M.& GOLDWATER,L.J. (1967) Faetal distribution of mercury following introduction of methylmercury into porcine maternal circulation. Arch. environ. Health,15:155

JUMBA,1997 personal communication ,1997).

KARK,P.(1979)clinical and neurochemical aspects of inorganic mercury intoxication. in : Handbook of clinical neurology, Armsterdam, oxford,Elsevier Science publishers, vol36, p 147-197

KENYA BUREAU OF STANDARDS (1968) Specifications for skin lightening creams. KS03-597, 1986

KHAYAT,A.,DENCKER,L.,(1983a) Whole body and liver distribution

of inhaled mercury vapour in the mouse: Influence of ethanol and aminotriazole pretreatment. J. Appl. Toxicol. 3: 66-74

KHAYAT,A.,DENCKER,L.(1984)Organ and celular distribution of inhaled metallic mercury in the rat and marmoset monkey: Influence of ethyl alcohol pretreatment. Acta Pharmacol. Toxicol. 55:145-152

KOSTIAL,K., KELLO,D.,JUGO,S.,RABAR,I.,(1978)Influence of age on metal metabolism and toxicity. Environ. Health.. Perspect, 25:81

KOSTIAL,K.,SIMONOVIC,I.,RABAR,I.,BLANUSA,M.,(1983) Age and intestinal retention of mercury and cadmium in rats. Environ. Res. 31:111-115

KUDSK,F.N. (1973) Biological oxidation of elemental mercury, In:Miller,M.W. & Clarkson,T.W., ed. mercury, mercurials and C. Thomas, p.355

KUMP,P.(1993) Quantitative Analysis of Environmental Samples (QAES) Ljubljana,Slovenia.

LADD,A.C. (1966) J. Occup. Med., 8:127

LAVI,N.M. (1984) A practical approach to Quantitative Analysis in x-ray Fluorescence Analysis. MSc Thesis, University of Nairobi.

LAUWERYS ET AL(1987) Prenatal and early postnatal intoxication by inorganic mercury resulting from maternal use of mercury containing soaps and creams. Toxicol. 6: 253-256

LENIHAN,J.M. & SMITH,H. (1967) Nuclear activation technics in life science. IAEA, Vienna.

LEVIN,M.,JACOBS,J.,POLOS,P.G.,(1988) Acute mercury poisoning and mercury pneumonitis from gold ore purification. Chest. 94:554-556

LINKE,W.F.(1958) Inorganic & metal-organic compounds. NY, Van

Nostrand Reinhold Co. Inc.

LINQVIST,O.JERNELOV,A.JOHANSON,K. (1984) Mercury in the Swedish environment. rep. no.1816.

MAC COMMITTEE (1969) Reduction of mercuric ions in inhalation nof mercury in mice .Arch. environ. Health, 19:891

MCFARLAND,R.B.,RIEGEL,H. (1978) Chronic mercury poisoning from single brief exposure. J. Occup. Med. 20(8):532-534

MAINA,C.,MAINA,D.M. KINYUA, A.M. (1994) Energy Dispersive X-ray Fluorescence Analysis of Cosmetic creams sold in Kenya. A paper presented at the 4th Aga Khan Conerence, Nairobi,1994

MAGOS,L.& BUTLER,W.H. (1972) Food, Cosmet. Toxicol., 10:513

MAGOS,L.,WEBB,M.,BUTLER,W.H.(1974) The effect of cadmium pretreatmenton the nephrotic action and kidney uptake of mercury in male and female rats. Br. J. Exp. Pathol. 55: 589-594

MARZULLI AND BROWN (1972) Potential harzards of applied mercury. J. soc. cosmet. 23:875-886

MIETTINEN,J.K. (1973) Absorption and elimination of dietary mercury (Hg2+) and methyl mercury in man. In: Miller M.W.&

MUROZUMI,M.(1967) Electrochemistry. Technol. 5: 236

NAKADA,S.,NOMOTO,A.(1980) Effects of methylmercury and inorganic mercury on protein synthesis mammalian cells. Ecotoxicol. environ. saf. 4:184-190

NIIGATA REPORT (1967) Report on the cases of mercury poisoning in Niigata, Tokyo, Ministry of Health and Welfare.

NIOSH (1973) Criteria for a recommended standard. Occupational exposure to inorganic mercury. Washington Public Health Service, U.S. Department of Health, Education and Welfare.

NORDBERG,G.F., ed.(1976) Effects and dose-response relationships

of toxic metals, Proceedings from an international meeting organised by the sub-committee on the toxicology of Metals of the Permanent Commission and International Associations on occupational Health, Tokyo, 18-23 November. Amsterdam, Elsevier.

PIOTROWSKI *et al* (1969) Health effects of methyl mercury. MARC report.

PIOTROWSKI,J.,TROJANOWSKI,B.,MOGILNICKA,E.M. (1975) Excretion kinetics and variability in urinary mercury in workers exposed to mercury vapour. Int . Arch . Arbeits . Med. (in press)

POENH,C., WERNISCH,J., HANKE,W. (1985) Least-squares fits of fundamental parameters. X-ray spectrometry, 14:120

RESEARCH COMMITTEE ON MINAMATA DISEASE (1975) Pathological, Clinical and epidemiologicalresearch about Minamata Disease, 10 years after, Kumamoto, Kumamoto University.

SKOG,E. & WAHLBERG,J.E. (1974) J.Invest. Derm.,43:187

SPARKS,C.J. (1976) Advanced x-ray analysis., 19:19

SPARKS,Jr.,C.J. (1975) Quantitative x-ray Fluorescence using Fundamental Parameters. Advances in x-ray analysis, 19, **Gould ,R.W.** *et al*, ed. p.20, Plenum Press, New York.

STORM,E. & ISRAEL,H.I. (1970) Nuclear data tables.

SWENSON,A. & ULFVARSON,U. (1968) Methylmercury in fish. a toxicological epidemiology and evaluation of risks. Poult.sci., 48:1567

TAKAHATA,N. (1970) Polio Psychiat. Neurol. Jpn., 24:59

TAKEUCHI T.,(1975) Minamata disease. chronic occurance from pathological viewpoint. Jap. Env. Agency. p 28-62

TASK GROUP ON METAL ACCUMULATION(1973)Environ . Physiol . Biochem

. , 3: 65TEJNING,S. (1970) Mercurycontentsin blood plasma of non-fisheaters. Department of occupational medicine, University hospital, Lund, Report No. 700406

THOMAS,D.J.,SMITH,J.C.(1979)Partial characterisation of low molecular eight methylmercury complex in rat 'cerebrum. Toxicol. Appl. Pharmacol. 47:457-556

THOMSON,J.,RUSSEL,J.A., (1970)Dermatitis due to mercury following amalgam dental restorations. Br.J. Dermatol., 82:292-297

TUNNESSEN,W.W.,MCMAHON,K.J.,BASER,m. (1987) Acrodynia: exposure to mercury from fluorescent light bulbs. Pediatrics, 79(5):786-789

VAN ESPEN,P., JANSENS,K., NOBELS,J (1986)PC Software for the analysis of complex x-ray spectra. Chemo. Lab. 1:109

WANDIGA,S.O. & JUMBA,I.O. (1982) Determination of mercury levels in body beauty soaps and creams sold in Kenya and Norway, Dept. of Chem. Univ. of Nairobi.

WEGRZYNEK,D., HOLINSKA,B. (1993) Simoultaneous analysis of trace concentration of Lead and Arsenic by energy dispersive x-ray fluorescence spectrometry. Appl. Radiat. Isot. 8:1101

WEGRZYNEK,D., HOLINSKA,B.,PTASINSKI,J. (1993) The Fundamental Parameters method for energy dispersive x-ray fluorescence analysis of intermediate thick samples with the use of monochromatic excitation. x-ray spectrometry. 22:80

WHEATLY,B(1979) Methylmercury in Canada: Exposure of indian and Inuit residents to methylmercury in Canadian environment, Ottawa Department of National health and welfare, Medical services branch, p200

WORLD HEALTH ORGANISATION (1971) International standards for

drinking water, 3rd ed., Geneva.

WORLD HEALTH ORGANISATION (1972) WHO techrep ser. 505

WORLD HEALTH ORGANISATION (1972) WHO Tech Rep. Series 505.

WORLD HEALTH ORGANISATION (1973) Environmental health cryteria 1. Mercury. Geneva. WHO. p132

WORLD HEALTH ORGANISATION (1976) Environmental health cryteria 1 ; Mercury, Geneva, WHO, 132pp.

WORLD HEALTH ORGANISATION (1980) Health risk evaluation for methylmercury- an interim review- EHE/EHC/ 80.22 WHO, Geneva.

WORLD HEALTH ORGANISATION (1980) Recommended health-based limits in occupational exposure to heavy metals; Geneva, WHO, 116pp. WHO-TRS No. 647

WORLD HEALTH ORGANISATION (1991) Environmental health cryteria 118. Inorganic mercury. WHO Geneva,1991