# OPTIMIZATION OF ANALYTICAL PROCEDURES AND DETERMINATION OF LEVELS OF MERCURY, CADMIUM AND LEAD IN FISH, WATER, SEDIMENTS, SOIL AND PLANTS FROM NAIVASHA AREA, KENYA

BY



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A Thesis submitted in partial fulfilment for the Degree of MASTER OF SCIENCE in the University of Nairobi



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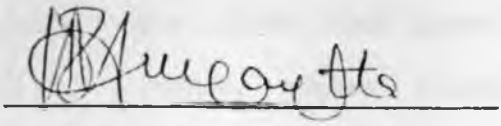
## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.



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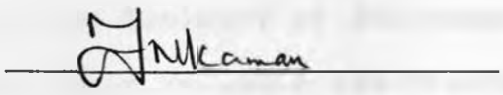

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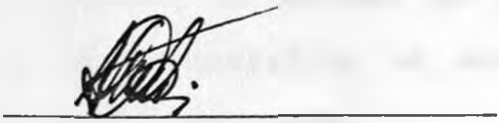

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## DEDICATION

This thesis is dedicated to my dad, AYUB GITITA and mom, ESTHER W. GITITA, who have tiressly worked hard for my sake.

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| | |
|---|---|
| FAAS | Flame Atomic Absorption Spectrophotometry |
| CVAAS | Cold Vapour Atomic Absorption Spectrophotometry |
| ED-XRF | Energy- Dispersive X-ray Fluorescence analysis |
| ppm | parts per million (mg/kg or mg/l) |
| ppb | parts per billion (ng/g or ng/ml) |
| TEL | Tetra-ethyl lead |
| TML | Tetra-methyl lead |
| VGA | Cold Vapour Generation Accessory |
| HDPE | High density Poly ethylene |
| NaDDTC | Sodium diethyl dithio carbamate |
| APDTC | Ammonium pyrrolidine dithio carbamate |
| EPA | Environmental Protection Agency |
| MCA | Multi-channel analyzer |
| x | The Horizontal axis variable of the curve |
| y | The vertical axis variable of the curve |
| $R^2$ | The square of correlation coefficient |
| --- | Value not determined |
| E.N.A. | VGA-76 equipment not available |
| D.L. | Detection limit |
| N.A. | Data not available |
| N.D. | Value not detected |
| N | Total number of samples of one kind |

| | |
|---|---|
| n | Number of sub-samples, each of which was analysed in triplicate |
| S.D. | Standard deviation of the mean |
| CV | Coefficient of variation |
| NVF | Naivasha fish |
| KABF | Kaburu Dam fish |
| TIL | Tilapia fish |
| BB | Black bass |
| LNW | Lake Naivasha water |
| RMW | River Malewa water |
| BH-NW | Bore-hole Naivasha water |
| OW | Olkaria Well |
| LNSD | Lake Naivasha sediment |
| RMSD | River Malewa sediment |
| HSL | Highway soil |
| AgrSL | Agricultural soil |
| OW-SL | Olkaria well-soil |
| VSL | Virgin soil |
| (H)TPl | Highway Terrestrial plant |
| overnight | Period of about 12 hours |

## ABSTRACT

The analysis of heavy metals in digested samples depends on parameters such as pH, temperature, time and digestion procedures. These parameters were investigated in order to obtain optimum experimental conditions for the determination of mercury, cadmium and lead in fish, unfiltered water, sediment, soil and plant samples from Naivasha area, Kenya. The aim was to determine the level of pollution, regarding these heavy elements in the area. The techniques used were Flame and Cold Vapour Atomic Absorption Spectrophotometry (FAAS and CVAAS), and Energy-Dispersive X-ray Fluorescence analysis (ED-XRF).

The AAS absorbance and XRF intensity for mercury, cadmium and lead were found to be pH dependent. Maximum values were found in the lower pH region, <1.5, with AAS and 1.6-2.7 with ED-XRF for mercury and lead. The detection limit of mercury with CVAAS was found to be lower than with XRF. Inter-comparison of mercury and lead, in a cross-section of the digested samples, between AAS and XRF techniques showed excellent correlation coefficients of 0.9982 and 0.9999 respectively.

In this project, it was found that by using a 3:1:1 ($HNO_3$:$HClO_4$:$H_2SO_4$) acid mixture (vol/vol), higher mercury sensitivity was obtained than with a

3:1 ($HNO_3$:$HClO_4$) acid mixture, using Cold Vapour AAS as the analytical technique. The former digestion method was tested on two International Atomic Energy Agency standard reference materials, MA-M-2/TM and MA-A-2(TM), which gave high mercury recoveries of 77.7% and 100% respectively.

Comparison between stannous chloride and sodium borohydride as reducing agents in CVAAS technique was also carried out. Stannous chloride exhibited higher sensitivity, stability and lower limit of detection than sodium borohydride. Prolonged oxidation time (at least overnight) of the digested samples gave high correlation coefficient (0.977) and regression coefficient (0.991), between the two reducing agents. The detection limits of mercury with $SnCl_2$ and $NaBH_4$ as reducing agents were found to be 0.243 and 2.303 ng/ml respectively. It was found that mercury concentration reading was negatively correlated with the digestion temperature. A digestion temperature of 50 $\pm$ 3$^{o}$C was found to be preferable.

For the determination of cadmium and lead with flame AAS, a digestion mixture of 3:1 nitric and perchloric acids was used. Different models of AAS equipment, located in different labs, were compared and the detection limit and sensitivity values found to be close to those given in the literature.

The sample concentration levels for the three heavy elements investigated (mercury, cadmium and lead) were found to range, respectively, as follows: fish: 0.052 - 1.521 mg/kg, 0.008 - 0.562 mg/kg, 0.514 - 5.111 mg/kg (wet weight); water and condensed steam: 0.019 - 0.143 mg/l, 0.045 - 0.200 mg/l, 0.970 - 5.525 mg/l; sediments: 0.209 - 0.269 mg/kg, 0.530 - 2.129 mg/kg, 13.710 - 28.084 mg/kg (dry weight); soils: 0.104 - 0.256 mg/kg, 0.479 - 2.668 mg/kg, 12.133 - 37.497 mg/kg (dry weight) and plants: 0.097 - 0.153 mg/kg, 0.305 - 2.961 mg/kg, 4.465 - 27.926 mg/kg (dry weight).

The concentration of the three heavy elements in fish and sediments compare well with the recommended and literature values. There was little indication of pollution with respect to these elements. However, the levels of the three elements in the water samples indicated probable pollution. On the other hand, soils and plants had elevated cadmium levels, whereas mercury and lead were within the expected range.

# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

The most severe problems of non-occupational environmental poisoning by heavy metals are caused by lead, mercury and, to a certain extent, cadmium. Moreover, a potential risk of environmental poisoning due to human activities still exists. This research concentrates on these three elements, which have been the focus of considerable research, environmental assessment and management [1].

### 1.1 ENVIRONMENTAL ASPECT OF NON-ESSENTIAL TRACE ELEMENT POLLUTANTS

Metals are an integral component of the environment and of living matter. Some of them play basic roles in living organisms, and hence the term "essential element" is applied. Some exhibit beneficial biological influence when present in certain concentrations, though the mechanisms are often obscure. Others have no evident positive biological influence and these are known as non-essential elements. However, toxic elements may

be encountered with any metal whenever its dose exceeds a certain "critical" level [2].

Mercury, cadmium and lead are non-essential elements, whose toxicity is of major environmental concern. They are jointly grouped under "major pollutants". Some 200,000 tons of lead are deposited on the earth annually due to the use of tetra-ethyl lead and tetra-methyl lead in fuels [3]. Alkyl mercury compounds are still present in many lakes at toxic concentrations [3]. However, with the recognition of the hazard, the risk of further alkyl mercury poisoning is decreasing. Cadmium poisoning may increase as the world consumption of the metal increases [3].

### 1.1.1 MERCURY

Mercury and its compounds are used in agriculture and industry. They are used in fungicides and in a broad spectrum of industries including agriculture, paint, pharmaceuticals, dental procedures, cosmetics, skin lightening creams, plastics, tanning, pulp and paper processing. Mercury is also used in the electrolytic production of chlorine and caustic soda; in mercury cells, fluorescent lamps, thermometers, barometers and polarographs.

Mercury fungicides have been used in treatment of seeds for more than half a century and their safety record is good, except alkyl mercury whose agricultural use has been recently discontinued in several countries because of its accumulation in terrestrial food chains [4].

Mercury is one of the most studied toxic elements in our environment. Investigation of mercury traces back as far as 1928 [5]. Today, there is considerable interest in health risk associated with the use of a particular mercury compound balanced against nature and its benefit, imparted directly or indirectly by the use of a specific formulation [6,7]. Though naturally occuring components contain a certain level of mercury (inorganic or organic), increasing use of mercury enriched formulations may cause deviations from threshold levels depending on the character of the sample of interest. This makes it desirable to have a reliable picture of current levels of mercury and make probable predictions for the future. Mercury levels in sediments, fish, water, blood and cosmetics have been explored extensively [8-15].

The increasing concern with mercury stems from repeated outbreaks of epidemics of methyl mercury poisoning [16,17]. This has occured due to

consumption of cereals treated with mercury or to the consumption of animals which have consumed such cereals. Methyl mercury toxicity has been reported by Lu [16]. In this incident, mercury released from a nearby industrial plant to the waters of Minamata Bay in Japan was converted to methyl mercury by micro-organisms. The fish in the bay absorbed this methyl mercury upto very high concentrations. Fish constitutes a large part of the Japanese diet in the area and as a result, many people died after eating the fish. The outbreak of methyl-mercury poisoning in Iraq was caused by the consumption of home made bread prepared from wheat treated with a methyl mercury fungicide [16,17]. Bakir [17] reported the average cncentration of mercury in wheat as approximately 8 mg/kg. In another incident in Ghana, a total of 17 out of 65 persons died in Keta Government Hospital following ingestion of stolen maize which had been treated with Merkuran, a product containing 2% ethyl mercuric chloride. The maize was reported to contain 15-20 mg/kg of mercury [16].

Three types of mercury poisoning can be distinguished: mercury vapour, inorganic mercury and alkyl mercury. Soluble inorganic mercury salts are highly toxic. Mercury (II) binds to thiol groups, thus almost all proteins can bind mercury to some

extent and are potential targets for mercury poisoning. Methyl mercury reacts with thiol groups to give very stable complexes. Quite stable complexes are also formed with nitrogen donors [3]. Methyl mercury compounds are absorbed via the skin, the respiratory tract and there is complete absorption from the digestive tract [16]. The more severe forms of mercury poisoning include brain damage, central nervous system disorders and birth defects. Less severe symptoms of mercury toxicity include insomnia, dizziness, fatigue, drowsiness, weakness, depression, tremors, and sometimes, in still more severe cases, death [7, 18]. Metallic mercury vapour and organic mercury (methyl- and phenyl-) are the major health risks [6,19,20]. Absorption of mercury compounds is also well documented [21-25].

#### 1.1.1.1 DISTRIBUTION OF MERCURY IN THE ENVIRONMENT

The average total concentration of mercury in rocks, soils, freshwater and marine sediments is within the range of 0.03-0.05 mg/kg [1]. The overall range is much wider; in parent rock, it is 0.004-0.7 mg/kg, most values being < 0.1 mg/kg. Much higher levels may be found in marine sediments as a result of submarine volcanism [1,26].

(a) Soils

The concentrations of mercury in soil are variable but low. The range is about 10 to 300 ng/g; however, the levels can exceed 500 mg/kg in mineralized areas. A representative continental mean concentration is 70 ng/g [27,28]. Background levels of mercury in soils are not easy to estimate due to widespread mercury pollution. Nevertheless, data for various soils on a world-wide basis show that mean concentrations of mercury in surface soils do not exceed 0.400 mg/kg [29]. The highest mean levels of mercury were reported for various soils of Canada (0.400 mg/kg) and for paddy soils of Japan (0.350 mg/kg) and Vietnam (0.300 mg/kg) [29]. Similarly, in organic and clay soils of the United States, the highest average concentrations were found to be 0.280 mg/kg in some soils (histosols) and 0.130 mg/kg in loamy soils [29].

(b) Sediments

The concentration of mercury in marine sediments is of the order of 0.05 to 1.2 ppm (ppm = mg/kg) in the open ocean and <1 ppm in the coastal regions. A representative level in sediments of both freshwater and marine areas is 0.330 ppm [27,28]. According to Kamau et al. [8], a preliminary survey of the total

mercury content in environmental samples from different regions of Kenya indicated that the range of mercury in sediments was 0.03-0.1 mg/kg.

(c) Atmosphere

Concentrations of mercury in air are of the order of a few $ngm^{-3}$ in remote areas and up to 50 $ngm^{-3}$ in urbanized areas. The range of levels in rural areas has been given as 3-9 $ngm^{-3}$ over non-mineralized areas and 7-53 $ngm^{-3}$ over mineralized areas [27]. Values of 0.005-0.06 $ngm^{-3}$ from Norway and 0.025 $ngm^{-3}$ in the Jung Fraujoch mountains probably represent background levels [1]. Natural degassing of the earth's crust and oceans result in the annual release of some 25-30,000 tons of mercury into the atmosphere [1]. It is expected that elemental mercury vapour is the major form of mercury in air [1]. At a semi-rural site in England, the mercury concentration in air averaged 1.4 $ngm^{-3}$ over the years 1957-74 [27]. Other estimates of mercury concentrations in air include: 7 $ngm^{-3}$ in urban areas (range 0.5-50), 4 $ngm^{-3}$ in rural remote continental areas (range 1-10), 1.5 $ngm^{-3}$ over continental shelf areas, and 0.7 $ngm^{-3}$ over oceanic and polar regions [27,28].

(d) Water

Mercury concentrations in "unpolluted" fresh waters are in the range 0.01-10 ng/ml, with the majority of values below 0.1 ng/ml [1]. Concentrations of total mercury in the open ocean average 0.05 ng/ml and it has been suggested that the natural background may be as low as 0.01 ng/ml [1]. However, wide variations (0.07-1.1 ng/ml) in deep-sea mercury levels have been reported, possibly due to submarine volcanism [1,26]. Mercury is strongly bound to particulate matter in freshwaters and probably mostly in dissolved form in the ocean. Typical mercury concentrations are 20-60 ng/l in fresh water and 10-30 ng/l in the ocean [27,28]. Uptake by biota of methyl mercury in water is quite efficient, so that even low concentrations in water can lead to high concentrations in fish [27].

Drinking water generally contains low mercury concentration and makes only a small contribution to exposure in the general population. According to WHO standards for drinking water regarding metallic contents [30], the maximum permissible level of mercury is in the range of 0.001-0.005 mg/l. Preliminary work has shown that the concentration of total mercury in representative Kenyan waters was in the range of 0.12-0.18 ng/ml (ppb) [8].

(e) Plants, vegetables and meat

The levels of mercury in plants and vegetables range from 0.001 to 0.3 ppm, most levels being < 0.01 ppm. Total mercury levels in meat are in the range of 0.001-0.05 ppm [1].

(f) Fish

Fish are known to accumulate mercury to high concentrations in the form of methyl mercury, and even in non-contaminated fresh waters, concentrations in fish muscle are of the range 0.03-0.2 mg/kg (wet weight basis). Marine fish from non-contaminated areas usually have mercury levels of 0.1-0.2 ppm (wet weight basis) with the exception of large predatory species, e.g. sword fish, tuna and halibut, in which values between 0.2 and 1.5 mg/kg are often found [1,10]. According to Kamau et al. [8], the range of mercury in representative Kenyan fish was 0.004-0.03 mg/kg (ppm). The maximum recommended mercury level for fish is 0.5 mg/kg [31].

### 1.1.2 CADMIUM

Cadmium accompanies zinc to the extent of about 0.5 % in many of its ores, and is obtained as a by-product of its manufacture. Roughly 5000 tons of cadmium are used annually, mainly in plating, pigments, alkaline batteries and metallurgy [3].

Cadmium has no known biological function. It is toxic to all organisms. Plants have no metabolic requirement for cadmium. The element can readily disrupt the normal functioning of plant enzymes because of its affinity for binding to a number of sites especially those containing the sulphydryl grouping [32]. Also due to its physical-chemical similarity to zinc, an essential divalent ion, it can interfere with many key metabolic processes by replacing zinc, especially at the active sites of enzymes. The isolation from kidney and liver of a cadmium-containing metallo-protein, metallo-thionein, suggests that the protein is involved in detoxification process [3]. Cadmium causes a reduction in iron content of land plants; reduced growth and complete failure.

On aquatic plants, it causes inhibition of frond development in duckweed, and chlorosis in Najas guadalupensis [32].

In mammals, some of the prominent effects associated with cadmium are: pulmonary and testicular lesions; renal dysfunction, poor bone mineralization, anemia, liver and kidney damage, retarded growth, disturbed carbohydrate metabolism and inhibition of drug-metabolizing enzymes. From studies on rats, it has been shown by Matsubara-Khan and Machida [33] that cadmium is cumulative and its concentration increases with age.

Acute cadmium poisoning (10 mg) can cause serious symptoms, which lead to nausea, salivation, vomiting, diarrhoea and abdominal pain. The toxicity of cadmium taken by mouth is partly offset by the vomiting it frequently induces [3].

Chronic cadmium poisoning due to long-term, low-level dosage, is primarily an industrial hazard. A severe outbreak of chronic cadmium poisoning occured along the Jintsu river of north-west Japan, and was known as "Itai-itai" disease. The region is near an abandoned non-ferrous metal mine and several hundred people died from cadmium poisoning [3].

Experiments in animals have shown that toxic cadmium effects may be prevented by administration of other metals during or prior to cadmium exposure [32]. For example, cadmium induced anemia can be prevented by excess iron while cadmium induced

testicular necrosis can be prevented by the administration of zinc, cobalt or selenium. Also the nutritional status influences the metabolism and toxicity of cadmium. Absorption of cadmium from the gastro-intestinal tract is increased if there is a deficiency in calcium and iron.

In nature, cadmium occurs in two oxidation states: the metallic state and the $Cd^{2+}$ state. The metallic state is rare and only occurs in some certain samples of native zinc. Potential sources of pollution include: smelting of zinc, lead and copper, burning of plastics, and dissolution from galvanized iron objects [34]. The use of phosphate rock fertilizers containing cadmium (1-100 mg/kg) and of sludge from combined industrial and domestic sewage treatment works (concentrations up to 30 mg/kg) are major sources of soil contamination by cadmium [1]. Weathering of rocks and erosion contribute less cadmium to the aquatic environment than the activities of man.

The excretion of cadmium is usually low. In humans, the body burden is 10-60 mg of cadmium. The concentration in urine may be around 0.5-2.0 ng/ml [1]. Urinary excretion increases with renal damage.

### 1.1.2.1 DISTRIBUTION OF CADMIUM IN THE ENVIRONMENT

#### (a) Soils

Cadmium is present in the earth's crust at an average concentration of 0.2 ppm (mg/kg) [35]. The natural concentration in soil normally ranges from 0.01 to 0.7 ppm. Cadmium is bound in clay and basic soil but is more mobile in sandy and acidic soils. Cadmium concentrations in soil are generally less than 1 ppm in non-polluted areas. Average values of 0.2 to 0.4 ppm in uncontaminated soil have been suggested with 0.9 ppm in the organic fractions [35]. The average concentration in soil from 91 samples from farming areas in the U.S.A. was 0.57 ppm [35]. Cadmium concentrations in contaminated soil may reach 800 ppm [35]. According to Kabata-Pendias and Pendias [29], the average content of cadmium in soils lie between 0.07 and 1.1 ppm. According to these authors, the background cadmium levels in soils apparently should not exceed 0.5 ppm, and all higher values reflect the anthropogenic impact on the cadmium status in top soils.

Cycling of cadmium and lead has largely been focused on pathways from point sources, mostly industrial. Local patterns of decreasing Cadmium and

lead in soils and plants with distance from heavily travelled roads (North America) has been reported by Lagerwerff [36].

(b) Sediments

According to Alala [37], the range of cadmium concentration in sediments from seven of the Kenyan lakes was 1-3 ppm. Similar work from Winam Gulf of Lake Victoria has been published by Wandiga and Onyari [38], who reported that the range of cadmium concentration was 0.0-1.0 mg/kg (ppm). It is also reported that in contaminated waters, cadmium levels in sediments can reach values in excess of 100 ppm [1].

(c) Atmosphere

Atmospheric levels of cadmium in rural areas of industrialized countries range from 0.1 to 20 $ngm^{-3}$. The annual average air concentrations of cadmium in highly urbanized areas is in the range of 2-90 $ngm^{-3}$ with most values around 10-20 $ngm^{-3}$ [1]. The concentration of cadmium in air was determined for a semi-rural site in southern England during 1957-74 as 3 $ngm^{-3}$ although the uncertainty was fairly high [35].

(d) Water

In fresh waters, not known to be contaminated, most cadmium levels are below 1 ng/ml (ppb) [1]. The concentration of cadmium in Mediterranean Sea water usually ranges from 0.02 to 1.9 ppb [1]. The open ocean concentration of cadmium is of the order of 0.1 ng/ml. In contaminated waters, dissolved cadmium levels are mainly dependent upon pH [1]. Concentrations up to 10 ppb are found in mining areas. High concentration of cadmium can be found on suspended particulates as high as 700 ppb -especially at neutral and alkaline pH [1].

The WHO recommended maximum permissible level of cadmium in drinking water is 0.01 mg/l (ppm) [30]. According to Alala [37], the range of cadmium concentration in water samples from seven Kenyan lakes was 0.002-0.116 ppm.

(e) Plants

Background levels in plant leaves have been estimated to range from 0.05 to 0.2 ppm [35]. According to other literature [29], the grand mean concentrations for all cereal grains range from 0.013 to 0.22 ppm, grasses range from 0.07 to 0.27 ppm, and legumes range from 0.08 to 0.28 ppm (all on dry weight basis). Application of inorganic sludge

fertilizer, adding up to 10 ppm to Cd in surface soil increased the Cd concentrations in grain and potatoes ten to fifteen fold over background levels [35]. In general, the fruit and seeds of plants contain less cadmium than the leaves [35].

Plants differ in their ability to take up cadmium from the soil. The uptake is pronounced in some grasses, wheat, lettuce and also tobacco. In rice grown in highly contaminated soils, cadmium levels exceed the natural level in rice by a factor of 10-15, reaching 0.5-1 ppm [1]. The concentration of cadmium in tobacco may be relatively high (1-2 ppm) and smokers can possibly absorb as much cadmium from smoke as from food. Intake is 100-200 ng per cigarette with high absorption (about 50 %) [35].

(f) Fish

No maximum allowable concentration has been recommended for cadmium in food [39]. In Britain, no official limits have been estimated but in Hong Kong, cadmium is completely banned in sea food [40]. Some current legislative limits for cadmium in sea food are 2.0 mg/kg (wet weight) in Australia and the United States of America; 1.0 mg/kg (wet weight) in New Zealand [40,41]. A study by Wandiga and Onyari

[38] has shown that the concentration of cadmium in the muscle tissues of fish from the Winam Gulf of Lake Victoria was in the range of 0.04-0.12 mg/kg (wet weight), which were far below the legislative limits cited above. It was also shown that the concentration of the element in the marine fish species were slightly higher than the lake fish. The concentration values obtained in muscle for marine species (from the Indian Ocean) were in the range 0.04-0.38 mg/kg for cadmium (wet weight).

### 1.1.3 LEAD

Consumption of lead throughout the industrialized world has more than doubled during the last 30 years [3]. Between 1968 and 1977, the world production of refined lead increased from 3.55 to 4.27 million tons [42]. North America today produces approximately one million tons of lead annually, or about 10 lbs per inhabitant. The battery industry is one of the largest single users of lead, but leaded petrol accounts for more than 20 % of the total lead consumed per year. Tetra-ethyl lead (TEL), tetra-methyl lead (TML) and mixed lead alkyls are used as antiknock additives to improve the combustion characteristics of gasoline [3]. However, today, with

the introduction of catalytic converters in automobiles, use of unleaded gasoline is becoming popular.

Diet is the major source of lead in man. Lead poisoning most frequently results from the absorption of lead through the gastro-intestinal tract rather than the respiratory tract. Chronic lead poisoning was a major cause of illness throughout the period of the Roman Empire [3]. The principal source of contamination has been the use of lead compounds in the manufacture, storage, transportation and cooking of some foodstuffs, as well as the use of lead-based agricultural insecticides [3].

The toxicity of lead is based on the fact that it is a potent enzyme inhibitor because it binds sulphydryl (SH) groups [43]. It also inhibits the synthesis of heme and utilization of iron in the body [43]. The pathological effects of lead are observed in three organ systems: the nervous system, kidney and hematopoietic system. Other effects which may occur are endocrine and reproductive abnormalities [43]. Children with overt lead poisoning have central nervous system symptoms ranging from ataxia to stupor, coma to convulsions [43]. Adults may have encephalopathy from lead intoxification. Anemia is an early manifestation of acute or chronic lead

intoxification. The anemia that occurs in lead poisoning results from shortened erythrocyte life span and impairment of heme synthesis [43].

Most naturally occuring bacteria as well as plants in field conditions can tolerate relatively high environmental levels of lead without overt toxicity. In aquatic organisms, the effect of lead seems more pronounced in higher forms than in lower ones. In fish, lethal effects occur only at very high concentrations. Long term destructive effects are found in more sensitive fish species such as rainbow trout, brook trout and oysters at levels close to 0.1 mg/l [1].

### 1.1.3.1 DISTRIBUTION OF LEAD IN THE ENVIRONMENT

Lead is a world wide pollutant of the atmosphere, concentrated in urban areas from the combustion of tetra ethyl lead in gasoline. Local pollutants are from mines and lead based paint pigments.

Lead is relatively abundant in the environment. It is a natural constituent of air, water and the biosphere. Human beings ingest a certain amount in food, water and air [34]. The dietary intake of lead by man is in the range of 0.1-0.5 mg of lead a day [1].

(a) Soils

Whereas the average content of lead in rocks and soils is about 20 ppm, in certain uncontaminated areas, the levels can be higher by one order of magnitude. The range of "uncontaminated" soil concentration given thereof is 2-200 ppm [1]. Some literature [35] give the average content of lead in the earth's crust as 13 ppm, with a range of 1-500 ppm. The lowest values are in those of sedimentary or alluvial origin, while the highest concentrations occur in the upper horizon of the soil [35]. The levels of lead in agricultural soils are typically in the range 20 to 80 ppm, with mean values of around 40 to 50 ppm [35]. The values generally reflect underlying mineralization. Agricultural soils from 226 farms in England and Wales have previously been analysed [35]. Four samples per farm were taken from a depth of 15 cm. There had been no application of sewage sludge or other potentially contaminating materials. The median lead concentration was 42 ppm [35].

According to a report on the distribution of lead in an uncontaminated surface soil, compiled by Khan [44], the baseline levels in a number of different geographical areas are in the region of 48-160 ppm in England, 20-80 ppm in Scotland, 6-155

ppm in the U.S.A., 21-108 ppm in Canada and 20-114 ppm in some tropical soils from Cameroun. The lead content of some agricultural soils of England and Wales was found to vary quite widely, the mean being 57 ppm [44].

According to Kabata-Pendias and Pendias [29], the terrestrial abundance of lead indicates a tendency for lead to concentrate in the acid series of magmatic rocks and argillaceous sediments in which the common lead concentrations range from 10 to 40 ppm, while in ultra-mafic rocks and calcareous sediments, its range is from 0.1 to 10 ppm. According to these authors, values for the natural lead occurence in top horizons of different soils from various countries show that amounts range from 3 to 189 ppm, while mean values for soil types range from 10 to 67 ppm with an average of 32 ppm. High lead levels (above 100 ppm) have been reported only for Denmark, Japan, Great Britain and Ireland and most probably reflect the impact of pollution [29]. Davis [45] stated that an upper limit for the lead content of a normal soil could be established as 70 ppm.

(b) Sediments

The concentration of lead in sediments in the open ocean has been given as 150 mg/kg while that

near the shore as 40 mg/kg [1]. The Mediterranean Sea sediments have lead concentrations ranging from 9 to 300 ppm [46]. According to Alala [37], the overall range of lead concentration in sediments from seven Kenyan Lakes was 6.7-210 ppm and the one for Lake Naivasha was 16.3 and 16.67 ppm for two sites of the lake. According to Wandiga and Onyari [38], the range of lead concentration in sediments from the Winam Gulf of Lake Victoria was 6.0-69.4 mg/kg.

(d) Water

Surface water, as well as drinking water lead levels are usually below 10 ng/ml (ppb) [1]. However, much higher levels can be found in certain soft water areas and in cases where lead pipes are still in use. Lead levels in ground waters are in the range of 1-500 ppb, while in hot springs, they can sometimes exceed 1 ppm [1].

Limited data on the lead content of contaminated surface waters and sea waters do not point to values at great variance with those reported for non-contaminated waters. Sea water in the Mediterranean has concentrations ranging up to 7.2 ppb [1]. Lead concentration in deep ocean water is about 0.01-0.02 ng/ml and 0.3 ng/ml in surface ocean water [35]. Dissolved lead in rivers in unpolluted

areas is < 0.1 ng/ml [35]. Usually reported values of 1-10 ppb are probably inaccurate due to use of insensitive analytical methods. The concentration in rain-water is 10-30 ppb but may be 0.1-0.5 mg/l in areas of heavy traffic [35]. The World Health Organization- recommended limits for lead in municipal water supplies are 0.1 mg/l (ppm) [3,30]. Alala's work [37] showed that levels of lead in water from seven Kenyan lakes studied were in the range of 2-600 ppb, of which the Lake Naivasha water indicated a range of 5-7 ppb in two samples.

(e) Plants

Increased lead levels in the soil are reflected by increased lead concentrations in plants, including vegetables [1]. Lead enters plants by root uptake from soil or by direct deposition from air [35]. Plant uptake of lead depends upon the plant species and the soil conditions. Uptake is greater under conditions of low pH, low Cation Exchange Capacity (C.E.C.), low organic matter and low phosphate levels. It has been reported that approximate concentration of lead in leaves and twigs of woody plants is 2.5 ppm, grasses is 1ppm, and vegetables and cereals is in the range of 0.1-1 ppm [1]. Background levels of lead in grass in Denmark are

reported to be about 2 ppm on a dry weight basis [35].

Natural lead in plants growing in uncontaminated and unmineralized areas appears to be quite constant, ranging from 0.1 to 10 ppm (dry weight) and averaging 2 ppm [29]. The full range could also be < 1.2 to 15 ppm, when all values are included [29].

(f) Fish

Among the aquatic biota of the Mediterranean, lowest concentrations of lead were found in fish (1.5-1.8 ppm) and the highest in mussels- up to 480 ppm, dry weight [46]. According to Wandiga and Onyari [38], the overall mean concentration of lead in Lake Victoria fish was in the range of 0.39-1.08 mg/kg (wet weight), while for the fish from the Indian Ocean, it was in the range of 1.22-6.48 mg/kg (wet weight). The authors found that, in general, the metal content in marine and lake fish were too low in muscle tissues to pose any danger to fish eaters.

The World Health Organization has recommended a maximum limit of 5.0 mg/kg in food [39,40]. Other countries have set the following legislative maximum limits of lead in food products [41,47]: Australia, New Zealand and Britain, 2.0 mg/kg; Poland, 5.0 mg/kg; France, 2.5 mg/kg; Thailand, South Africa and

Namibia, 5.0 mg/kg; Hong Kong, 6.0 mg/kg; Kenya, 10.0 mg/kg for marine and fresh water animal products, although a value of 0.5 mg/kg for food products (not specified) is also enforced [38].

(g) Lead in Humans

Lead is transported in blood and distributed primarily to bone. About 90 % of the total body burden is found in bone. The concentrations in tissues (men and women over 16) were reported to be 9-34 mg/kg (wet weight) in bone, 1 mg/kg in liver, 0.8 mg/kg in kidney cortex and 0.02 to 0.8 mg/kg in brain cortex [35]. Lead in blood of individuals with only normal background exposure is 100 to 200 ng/ml (ppb) on average. There is no age difference but slightly higher levels in males. There is also a clear difference in levels from urban to rural areas. Higher values are found in populations living near highways and lead smelters [35].

## 1.2 RESEARCH OBJECTIVES

Not much detailed work on levels of mercury has been reported in Kenya, though mercury and other trace elements have been determined in cosmetics and sediments obtained from Lake Victoria and the Indian Ocean [48-50]. According to generalized map showing

the mercuriferous belt of the earth [51], Kenya does not appear to fall within the mercury zones, because little or no data has been published [8].

According to Kamau et al. [8], a preliminary survey of mercury content in environmental samples, mainly water, sediments, fish and commercial products and blood samples has shown that Naivasha area had more mercury than other areas in Kenya. The overall objective of this research project was to confirm this finding. The slightly higher levels of mercury in Naivasha than other areas was partly attributed to volcanic origin and probable agro-chemical usage. The present work was aimed at analysing various samples from Naivasha region with respect to mercury, cadmium and lead. Moreover the research in Naivasha area was also to provide a first detailed study on mercury content in the area. This was compared with values from other areas in the literature. The results obtained from this study were also compared to the preliminary work which has been done in the same area [8].

The objectives of the research project can therefore be summarized as follows:

(a) To carry out a preliminary investigation and inter-comparison of some experimental conditions

and parameters for analysis of mercury, cadmium and lead using Cold Vapour and flame Atomic Absorption Spectrophotometry (CVAAS & FAAS); mercury and lead using Energy-Dispersive X-Ray Fluorescence analysis (ED-XRF).

(b) To review experimental conditions for mercury, cadmium and lead analysis with respect to digestion procedures, temperature and pH; optimize these conditions in order to develop best procedures for the present work.

(c) To determine the level of pollution by heavy metals, especially mercury in Naivasha area.

(d) To investigate possible influence of the heavy metal content (particularly mercury) by the agricultural, volcanic and other activities in the area.

(e) To obtain up-to-date results for levels of mercury, cadmium and lead and compare them with previous data obtained in the same area and other parts of the world.

(f) To give suitable recommendations with regard to heavy metal (mercury, cadmium and lead) pollution in the area.

# CHAPTER II

## THEORY OF THE ANALYTICAL TECHNIQUES

The major analytical technique used in the present project was Flame and Cold-Vapour Atomic Absorption Spectrophotometry (FAAS & CVAAS). This is therefore discussed below in detail as well as in literature [52,53]. Energy Dispersive X-Ray Fluorescence Analysis (ED-XRF) was also used for comparison of certain parameters with AAS and to support the principal analytical method (AAS) by analysing some representative digested samples. As such, the latter method (XRF) is briefly discussed, by outlining its basic principles, but the details can be obtained in the literature cited.

### 2.1 ATOMIC ABSORPTION SPECTROMETRY

Atomic Absorption Spectrometry is an instrumental analytical technique used in the determination of elements. The technique is simple, rapid and applicable to a large number of metals in

various types of samples, e.g. geothermal, river, lake and waste waters. The technique is also applicable in widely varying fields such as clinical chemistry, ceramics, petroleum chemistry, metallurgy, mineralogy, biochemistry, soil analysis, water supplies and industrial effluents.

### 2.1.1 BASIC PRINCIPLES

Atomic Absorption Spectrometry is based upon the absorption of radiation by free atoms [54]. The basic reaction underlying AAS may be stated as follows:

$$R + h\nu \longleftrightarrow R^* \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where:

R is the ground state atom;

$R^*$ is the excited state atom;

h is the Planck's constant and $\nu$ is the frequency.

An atom is said to be in the ground state when its electrons are in their lowest energy levels. When energy is transferred to such atoms, by means of thermal or electrical excitation, a number of different excitation states result throughout the population. The ground state atom, therefore absorbs

energy to yield the excited state, which in turn emits radiation following de-excitation process. Absorption or emission of light is therefore associated with the process of transition of atoms from one steady state to the other.

For the steady states m and n with energies $E_m$ and $E_n$ respectively, when $E_n > E_m$, then (m to n) transition results in the absorption of light and (n to m) transition results in emission of light with frequency $\nu_{mn}$, given by:

$$\nu_{mn} = \frac{(E_n - E_m)}{h} \qquad \text{.....(2)}$$

According to Einstein's quantum theory of radiation [37], there may be three types of transitions between levels m and n:-

(a) Emission (n to m) transitions from the excited state to a lower energy state taking place spontaneously.

(b) Absorption transitions (m to n) from a lower to a higher energy state taking place in response to the action of external radiation with a frequency, $\nu_{mn}$. Subscript mn implies frequency of transition is from level m to n.

(c) Emission (n to m) transitions from an excited state to a lower energy state, stimulated by external radiation of the same frequency, $\nu_{mn}$.

The (n to m) emission transitions thus include two types of transitions: (i) spontaneous transition taking place without any external source and (ii) transitions stimulated by external radiation.

The (m to n) absorption transitions are always stimulated by external radiation. This phenomenon forms the integral part of Atomic Absorption Spectrometry.

The proportion of excited to ground state atoms in a population at a given temperature can be considered with the aid of the Boltzmann relation [52,53]:

$$(N_n/N_m) = (G_n/G_m) \exp(-(E_n - E_m)/kT) \quad .....(3)$$

where N is the number of atoms in a state n or m; G is the statistical weight of a particular state, k is the Boltzmann constant and T is the kelvin temperature.

Since $E_n - E_m = \frac{hc}{\text{wavelength}}$, it follows

that for a fixed T, $N_n/N_m$ increases with wavelength. This latter ratio is very small over the temperature ranges of typical flames [55] (3-4000 $^{o}C$), and has a value of around $10^{-10}$ - $10^{-4}$; sodium at 3,000 K has a $N_n/N_m$ value of $6 * 10^{-4}$. This means there is very low proportion of atoms in the first excited state, n compared to that in the ground state, m. Absorption by atoms takes place within very narrow spectral regions of the order of hundredths of angstroms. In the laboratory, only those transitions involving the ground state are observed, yielding simple spectra. Absorption involving the ground state are therefore known as resonance lines. This means that there is little possibility of coincidence of resonance lines, and therefore very little spectral interference [56], thus accounting for one of the main advantages of AAS.

For dilute solutions the relation between the intensity of incident and transmitted monochromatic light for an absorbing species is given by the following equation [53,57]:

$$(I/I_o) = 10^{-kcl} \quad \text{.....(4)}$$

where:

I = intensity of the transmitted light beam.

$I_o$ = intensity of the incident light beam.

k = a constant, the extinction coefficient or absorptivity, dependent upon type of solvent, temperature and wavelength of light.

c = concentration of absorbing species.

l = depth of solution traversed by the light (path length).

This relationship is referred to as the Lambert-Beer or Bouguer- Beer Law, and following recent procedures for standardization [57], is written:

$$A = -\log (I/I_o) = kcl \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where A is the absorbance, the negative logarithm of the transmittance and k, c and l have the usual meanings given above.

The above relationship (equation 5), commonly called Beer's Law, forms the basis of quantitative Atomic Absorption Spectrophotometric determination. It relates the absorbance of a solution directly to the concentration of an absorbing species.

## 2.1.2 INSTRUMENTATION AND TECHNIQUES

The schematic diagram of the essential components of an atomic absorption spectrophotometer is shown in Figure 1.

The most important components are the spectral source, which emits the spectrum of the element of interest e.g. a hollow cathode lamp; an atom cell, in the present work, a flame or Cold Vapour Generation Accessory kit, in which mercury vapour is formed by reduction with $SnCl_2$ or $NaBH_4$; a monochromator for spectral dispersion of the source radiation and an exit slit for selection of the wavelength of the analyte resonance line; a detector, normally a photo multiplier tube, to permit measurement of the radiation intensity at the resonance line and an amplifier and display system for recording of the absorption values.

The instruments used in this project were:-

(i) (a) Varian Techtron AA-10 Atomic Absorption Spectrophotometer, equipped with

(b) Varian Model 76 Vapour Generation Accessory VGA-76.

(ii) Perkin Elmer Atomic Absorption Spectrophotometer, model 2380.

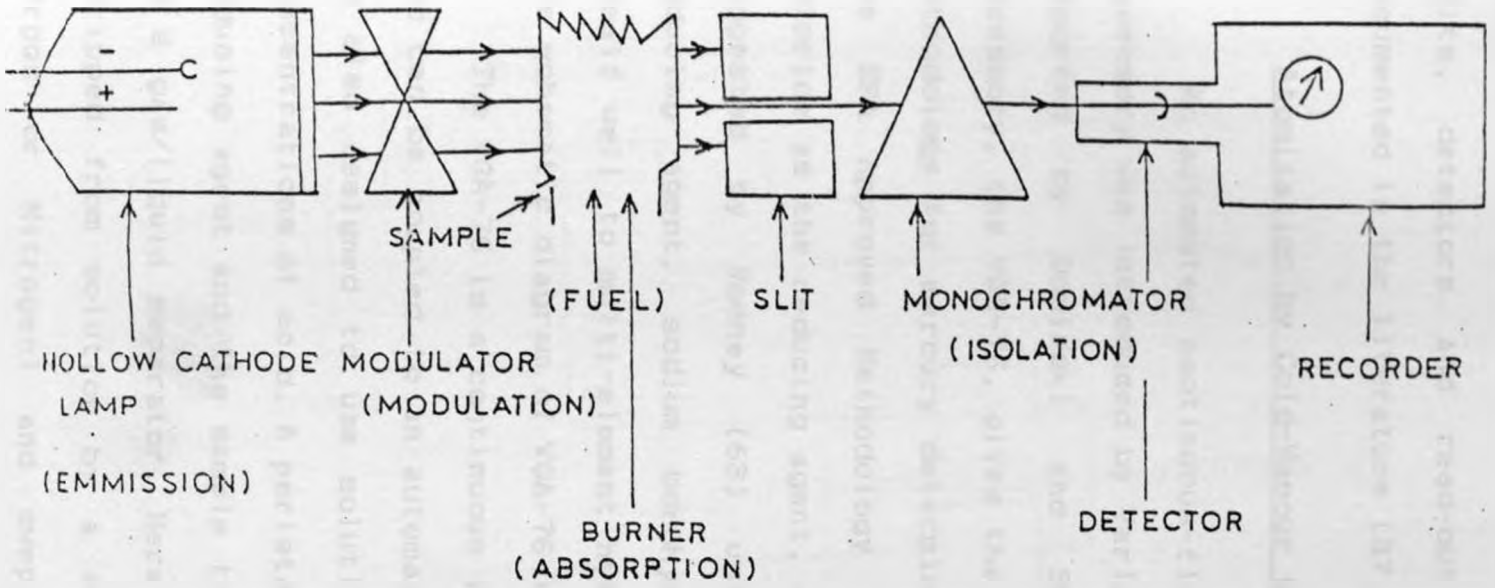





Figure 1: A schematic representation of the essential components of an Atomic Absorption Spectrophotometer (58)

The functions, principles and techniques of spectral sources, flame atomization, monochromators, slits, detectors and read-out systems are well documented in the literature [37,52-60].

Atomization by Cold-Vapour Generation (VGA-76)

An automated continuous-flow vapour generation accessory was introduced by Varian in March 1984, as reported by Dominski and Shrader [61]. This accessory, the VGA-76, gives the analyst a choice of methodology for mercury determinations. One may use the EPA Approved Methodology [62] with stannous chloride as the reducing agent, or another technique suggested by Rooney [63] using an alternative reducing agent, sodium borohydride, which lends itself well to multi-element hydride analysis [61]. The schematic diagram of VGA-76 is shown in Figure 2.

The VGA-76 is a continuous flow vapour generator and can be coupled to an automatic sampler [64]. It is also designed to use solutions containing high concentrations of acid. A peristaltic pump pushes the reducing agent and the sample through a mixing coil to a gas/liquid separator. Here, mercury vapour is stripped from solution by a stream of inert gas (Argon or Nitrogen) and swept into a (quartz) flow-thru cell positioned in the AAS burner

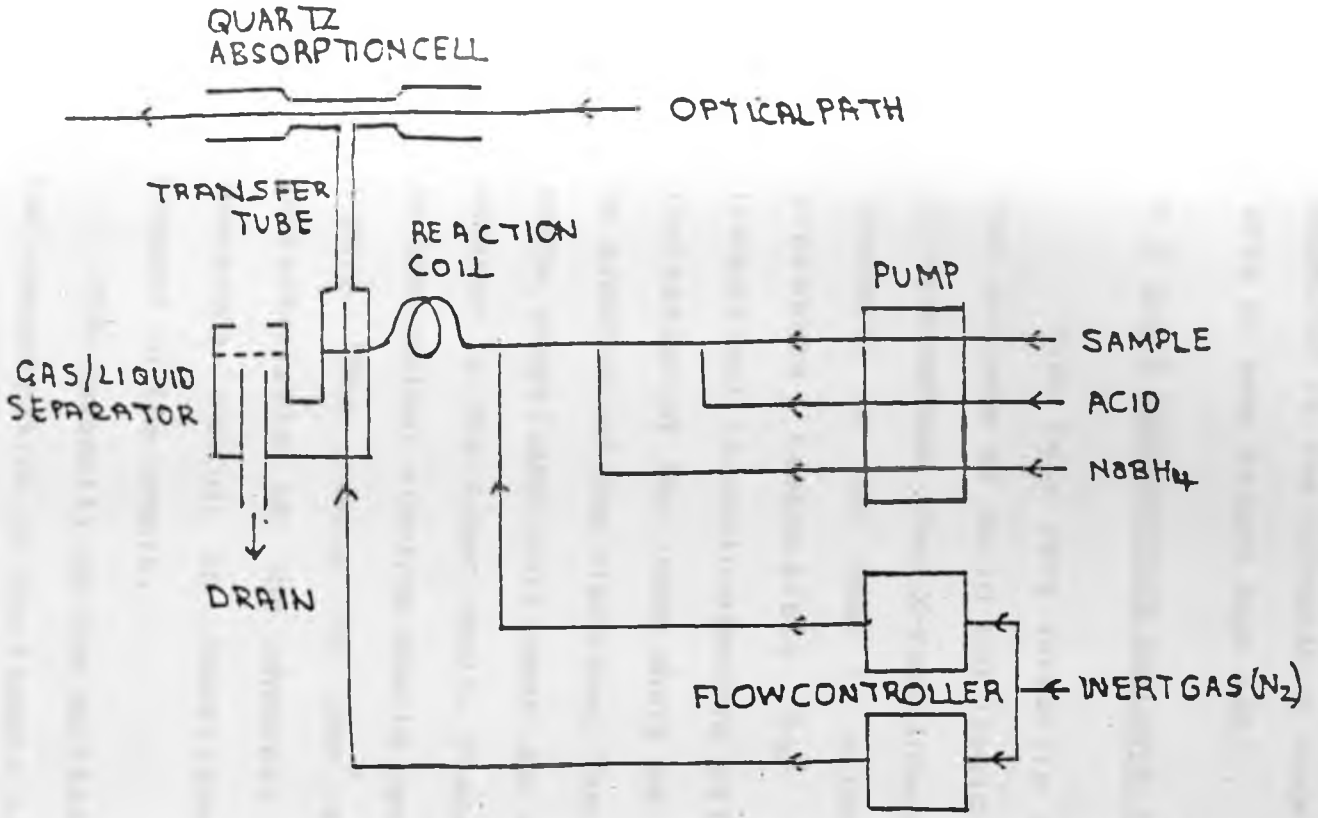



Figure 2: A schematic representation of the Cold vapour Generator kit (VGA-76) (61)

compartment. This system produces a continuous analytical signal, not a transient peak as with earlier vapour generation systems. Absorbances are measured in the integration mode instead of the peak area or peak height mode [65].

## 2.2 X-RAY FLUORESCENCE ANALYSIS (XRFA)

This is a very versatile analytical technique for analysis of solid and liquid samples [37,66-72]. In this method, when X-rays interact with matter, the intensity of the beam is attenuated. One of the processes responsible for this attenuation (reduction) is photo-electric effect where, there is ionization of the inner shell of the atom, resulting in ejection of the electrons. The atom in the excited state stabilizes only when an electron fills the vacancy in the inner shell. Transition of electrons in the orbital electron shells results in emission of X-rays. The energy of the emitted X-rays is characteristic of the chemical element. This is therefore useful in identifying the element(s) present in the sample.

The intensity of the emitted ray is related to the concentration of the element in the sample by the following equation [69,71]:

$$I_i = G_o K_i C_i A_{corr} \quad \text{.....(6)}$$

where:

$I_i$ = Intensity of element i in counts $sec^{-1}$

$G_o$ = Geometrical constant in counts $sec^{-1}$

$K_i$ = Relative excitation and detection efficiency of element i in $cm^2 g^{-1}$

$C_i$ = Concentration of element i in $gcm^{-2}$

$A_{corr}$ = Absorption correction factor

For fairly "thin" samples, equation (6) becomes:

$$I_i = G_o K_i C_i \quad \text{.....(7)}$$

In the present work, $^{109}Cd$ Radioisotope (25mCi) was used as the source of excitation while the detector was Si(Li). All the representative samples analysed by XRF were digested and treated as liquid samples. According to Kinyua [66] and Luke [70], it is necessary to correct the measured X-ray intensities for absorption energies below 6 kev. When the filter load and deposit per unit area are small, a sample can be regarded as "thin" for X-rays exceeding 6 kev. In such a case, linear relationship between measured intensities and mass per unit area of respective elements are obtained and matrix effects may be neglected. With regard to this fact therefore, equation (7) was assumed in all the

present analysis for mercury and lead. It was generally not possible to analyse for cadmium using $^{109}Cd$ source. More details of this technique may be obtained in the literature cited above.

# CHAPTER III

## EXPERIMENTAL TECHNIQUES

### 3.1 SAMPLING AND SAMPLE PRESERVATION

The geographical area of study showing the sampling sites in Naivasha region is shown and depicted in Figure 3. These included some parts of Lake Naivasha and River Malewa, which feeds the former; Naivasha Town, surrounding residential and agricultural areas; Olkaria Geothermal area; two bore holes and three sites along the Great North Road i.e. along new Nairobi-Nakuru Highway. These are shown by numbers on the map. The specific names and location of the sampling sites are given under the appropriate sections under "Results and Discussion".

The geology of the Naivasha area has been discussed by Thomson and Dodson [73]. The greater part of the area lies within the floor of the Rift Valley. The rocks of the area fall into two main groups: lacustrine deposits; lavas and pyroclastics. The lavas range from under-saturated basic rocks (tephrites) to acid rocks (rhyolites and obsidians) with numerous gradations in between. The pyroclastics, some consolidated and others

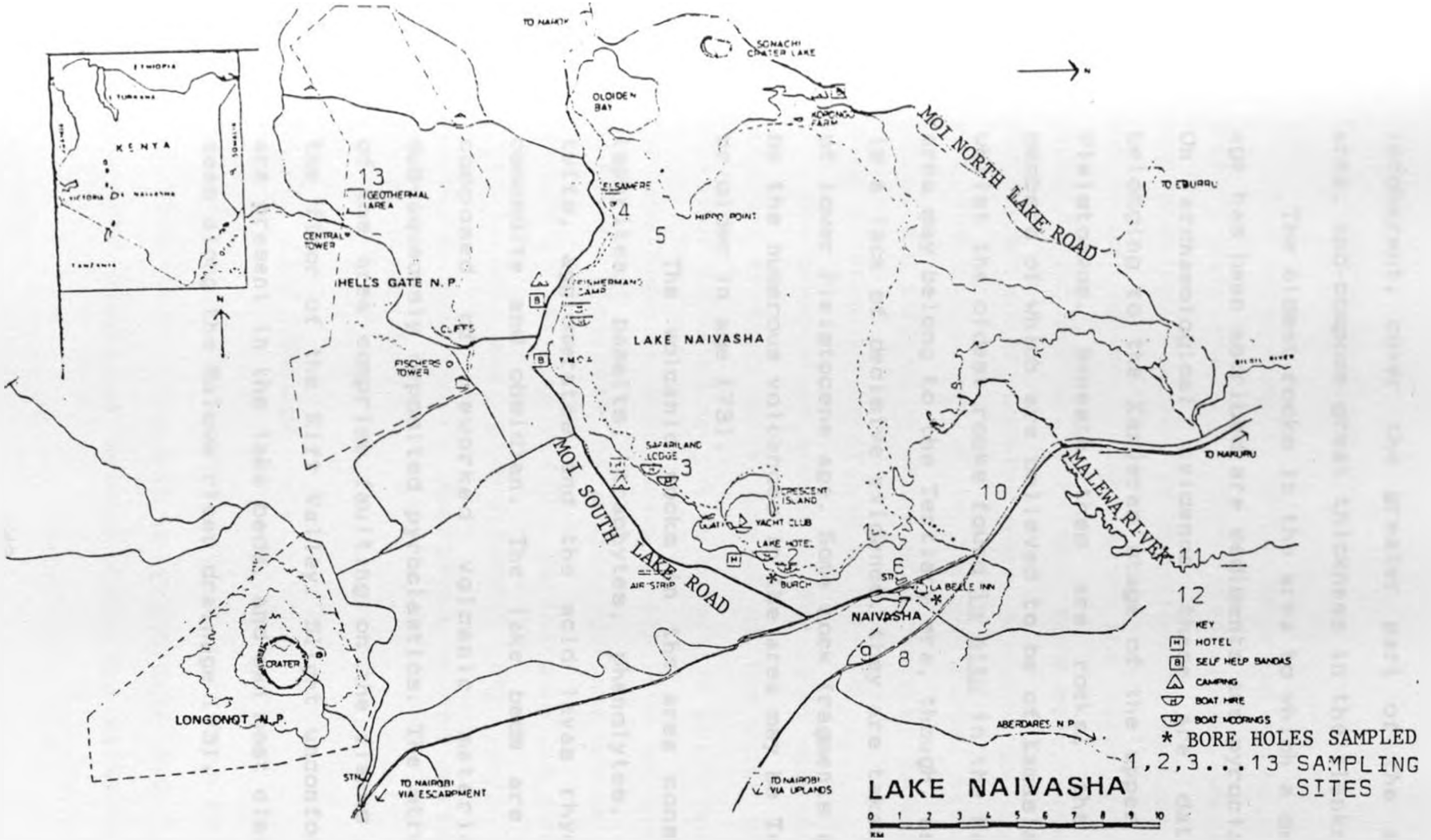



Figure 3: The map of Lake Naivasha and its environs showing the sampling sites, labelled 1 to 13 (74)

incoherent, cover the greater part of the surface area, and compose great thickness in the flanks.

The oldest rocks in the area to which a definite age has been ascribed are sediments and pyroclastics. On archaeological evidence these are dated as belonging to the Kanjeran stage of the upper middle Pleistocene. Beneath them are rocks, the upper members of which are believed to be of Kamasian age, whilst the oldest rocks found *in situ* in the Naivasha area may belong to the Tertiary era, though, as there is a lack of decisive evidence, they are taken to be of lower Pleistocene age. Some rock fragments ejected by the numerous volcanoes in the area may be Tertiary or older in age [73].

The volcanic rocks in the area consist of tephrites, basalts, trachytes, phenolytes, ashes, tuffs, agglomerates and the acid lavas rhyolites, comendite and obsidian. The lake beds are mainly composed of reworked volcanic material or sub-aqueously deposited pyroclastics. The structures of the area comprise faulting on the flanks and in the floor of the Rift Valley. Slight unconformities are present in the lake beds, and can most clearly be seen along the Malewa river drainage [73].

### 3.1.1 WATER SAMPLING

The water samples (N=18) were collected in high density poly ethylene (HDPE) containers, which had been previously washed as described in section 3.2.2 and rinsed several times with the water from the point of collection. The lake, bore hole, river and geothermal water samples were transferred directly into the containers while the geothermal steam was cooled and sampled by means of a "Weirber Separator", using a technique described by Bor [58].

The temperature and pH of the water samples were recorded at every site. The pH of the water samples was later adjusted to $\leq 2$ using nitric acid (Analar). The addition of acid greatly inhibits or prevents metabolic processes of micro-organisms which cause changes in the sample [75]. Furthermore, it prevents flocculation and precipitation of, say, metal compounds and reduces adsorption on the surface of the container [37].

### 3.1.2 FISH SAMPLING

The two common species of fish in Lake Naivasha i.e. Blackbass (*Micropterus salmoides*) and *Tilapia* sp. were bought at Nairobi City and Naivasha fish

markets. Careful interrogation of the fish mongers was done, to ascertain that the fish had come from Lake Naivasha. For comparison purposes, four tilapia fish from Kaburu dam were also bought at Gikomba market, Nairobi.

The fish samples (N=25) were transported in polythene paper bags and preserved in the deep freezer. Small sub-samples of the muscle tissues from the dorso-lateral part ventrally to the dorsal fin were removed using stainless steel blades and forceps, transferred into small plastic (HDPE) bottles and stored in the deep freezer for approximately up to four months until the time of analysis.

### 3.1.3 SEDIMENT SAMPLING

Due to lack of sophisticated equipment for deep-water sampling, Lake Naivasha sediment sampling was restricted to the shores, to a depth of about 50 cm below the water surface. However, due to the shallowness of River Malewa, off-shore sediment sampling was done along the river course, to a depth of about 40 cm below the water surface. Sediments were collected using a stainless steel scoop, which was washed at intervals with the water in the sediment environment. The samples (N=7) were

transferred into polythene and cellulose bags and dried in the open air for about four days, before analysis.

Most of the lake and river sediments were collected at the sites where the water samples were collected.

### 3.1.4 SOIL SAMPLING

This was done at various sites at Naivasha Town, along the Great North Road i.e. Nairobi-Nakuru road, in agricultural farms near Lake Naivasha and Naivasha Veterinary Research Station and in the vicinity of three geothermal wells at Olkaria. The specific areas sampled are given in the section under "Results and Discussion".

The samples (N=11) were dug to a depth of about 15 cm below the soil surface. They were transferred into clean polythene and cellulose paper bags and later dried at room temperature in the laboratory for about four days, before analysis.

### 3.1.5 PLANT SAMPLING

The plant samples (N=17) were of two major types. These were: Aquatic plants from Lake Naivasha, which included *Salvinia molesta*. *Cyperus papyrus* and terrestrial plants including Leleshwa (*Tarchonanthus*

camphoratus), pepper tree (Schinus molle), sodom apple (Solanum incanum), kikuyu grass (Pennisetum clandestinum), napier grass (Pennisetum purpureum) and Nicotiana glauca from Naivasha Town, along the Nairobi-Nakuru Highway, in agricultural farms near Lake Naivasha and Naivasha Veterinary Research Station and in the vicinity of four geothermal wells at Olkaria. Most of the terrestrial plants were sampled at the same sites where the soil samples were collected. The above species were chosen because they were the most common and available in the varying geographical regions of the area studied.

The free-floating aquatic fern, Salvinia molesta was collected wholly, while for the other plants, part of the aerial portion including the twigs and leaves were cut. The samples were transferred into polythene and cellulose paper bags and dried in the open air in the laboratory for about four days, before analysis.

## 3.2 CHEMICALS AND APPARATUS REQUIRED

### 3.2.1 REAGENTS

The chemicals used were of analytical grade. These included: Nitric acid, $HNO_3$, 70 % (from Berk

Spencer Acids Ltd.); Sulphuric acid, $H_2SO_4$, 96 % (Codex Farmacopea); Perchloric acid, $HClO_4$, 70 % (Riedel-de Haen); Hydrochloric acid, HCl, 35.4 % (Gainland Chemical Company); Cadmium chloride, $CdCl_2$ $2.5H_2O$, Lead (II) nitrate, $Pb(NO_3)_2$, Mercury (II) chloride, $HgCl_2$, Stannous chloride, $SnCl_2.2H_2O$ and Ammonia solution, $NH_3$ (were from BDH Chemicals Ltd.); Sodium borohydride, $NaBH_4$ (from Aldrich Chemical Co. Ltd.); Sodium hydroxide, NaOH and Hydroxylamine hydrochloride, $NH_2OH.HCl$ (were from May and Baker Ltd.); Potassium permanganate, $KMnO_4$ (SD's Lab. Chemical Industry) and Sodium diethyl-dithio carbamate (NaDDTC), $(C_2H_5)_2N.CS.SNa$ $2.5H_2O$ (Sigma Chemical Company).

### 3.2.2 WASHING OF APPARATUS

All labware (digestion beakers, pipettes, burettes, volumetric flasks, etc.) and sample containers (high density poly ethylene) were rinsed with tap water, then soaked for at least 24 hours in washing detergent, thoroughly rinsed with running tap water and acid washed with 10 % (vol/vol) nitric acid, for at least 24 hours. The washing procedures were completed by rinsing the labware when required for immediate use, with deionised or double distilled

water and dried at 80-100°C, where necessary or at 50-70°C for the plastic containers.

### 3.2.3 PREPARATION OF REAGENTS

#### 3.2.3.1 STOCK STANDARD SOLUTIONS

The following stock solutions containing 1000 mg/l (ppm) of mercury, cadmium and lead respectively were made and finally stored in one litre poly propylene bottles. Serial dilutions were made from these in order to obtain lower concentrations when required.

(a) 1000 ppm $Hg^{2+}$ Stock Solution

0.6802 gm of $HgCl_2$ (Assay 99.5 %) was dissolved in about 250 ml of deionized water. To enhance the solubility, the resulting solution was diluted with 0.5 M $H_2SO_4$ to 500 ml in a 0.5 litre volumetric flask.

(b) 1000 ppm $Cd^{2+}$ Stock Solution

1.0261 gm of $CdCl_2$ $2.5H_2O$ (Assay 99%) was dissolved in about 200 ml of deionized water and diluted to 500 ml in a half-litre volumetric flask.

(c) 1000 ppm $Pb^{2+}$ Stock Solution

0.8033 gm of $Pb(NO_3)_2$ (Assay 99.5 %) was dissolved in about 100 ml of deionised water and diluted to 500ml in a half-litre volumetric flask.

3.2.3.2 OTHER REAGENTS

(a) Potassium Permanganate, $KMnO_4$ Solution, 6 % wt./vol.

24 gm of $KMnO_4$ was dissolved in about 200 ml of deionized water and warmed in order to dissolve. The solution was then allowed to cool and diluted to 400 ml with deionised water. This gave a 6 % wt./vol KMnO4 solution. The solution was kept away from direct light and prepared the same day it was required.

(b) Hydroxylamine hydrochloride solution, $NH_2OH.HCl$, 20 % wt./vol

50 gm of $NH_2OH.HCl$ was dissolved in 150 ml of deionised water. The dissolution process was endothermic and so the solution had to be warmed to room temperature and diluted to 250 ml with deionised water.

(c) Stannous chloride, 25 % $SnCl_2.2H_2O$ wt./vol in 20 % HCl vol/vol

Deionised water was added to 52.632 gm of 95% (BDH) $SnCl_2.2H_2O$ in a 200 ml volumetric flask. The solution was diluted to the mark with deionised water. This formed a cloudy milky suspension, A. Into another 200 ml volumetric flask was trasferred 113 ml of 35.4% vol/vol HCl. This was diluted to the mark with deionised water and mixed with suspension A in a half-litre flask or beaker and covered with a watch glass. The mixture was boiled while stirring until a clear colourless solution resulted. This was cooled and transferred into a plastic bottle, ready for use.

(d) Sodium borohydride, 0.3 % $NaBH_4$ wt./vol, 0.5 % NaOH (wt./vol)

0.6 gm of $NaBH_4$ was dissolved in a 200 ml flask with deionised water. To this was added 0.5% NaOH, made by dissolving 1.0 gm of NaOH pellets in 200 ml of solution with deionised water. After use, this solution had to be kept in the fridge, or else it had to be prepared afresh the same day it was required.

(e) Sodium diethyl dithiocarbamate, 2 % NaDDTC (wt./vol)

5 gm of NaDDTC was dissolved in about 100 ml of double-distilled water in a 250 ml volumetric flask and diluted to the mark with double-distilled water.

## 3.3 ANALYTICAL PROCEDURES- ATOMIC ABSORPTION SPECTROPHOTOMETRY

### 3.3.1 PRELIMINARY INVESTIGATION OF CERTAIN EXPERIMENTAL PARAMETERS

In order to compare the experimental conditions with those given in the literature, a preliminary investigation to compare some parameters such as solution pH, delay time, digestion temperature, detection limit and digestion matrix was attempted. This was done in order to attain optimum conditions which give the highest elemental recoveries and sensitivities. For this to be achieved, the recommended optimum instrumental conditions for Atomic Absorption Spectrophotometry, given in section 3.3.6 were employed.

### 3.3.1.1 SOLUTION pH

By means of serial dilutions of the stock solutions prepared (Section 3.2.3.1), 500 ml solution of a mixed standard containing 0.1 mg/l of mercury, 1 mg/l of cadmium and 2 mg/l of lead was made. 50 ml of this solution was separately transferred into nine beakers and the pH varied from 0.9 to 8.1, using nitric acid and ammonia solution. The resulting solutions were transferred into clean, high density polyethylene bottles, ready for absorbance measurement with Varian AA-10 Atomic Absorption Spectrophotometer (Cold-vapour and Flame) for mercury, cadmium and lead as per the manufacturer's recommendations.

### 3.3.1.2 DELAY TIME

In AAS determination, this is the time in seconds which elapses between the probe reaching the fully down position and the start of the actual measurement. For manual sampling, it is the time which elapses between "READ" and the start of the actual measurement. This delay allows the flame to stabilize before the actual reading is taken. For most applications, the flame will be stable after about 5 to 10 seconds from the start of aspiration.

For vapour generation analysis with the VGA-76, the delay period required is generally about 40 seconds; for mercury, it is about 45 seconds [64,76]. Alternatively, delay time can be defined as the total time which elapses during the complete reduction of mercuric ions by stannous chloride or sodium borohydride to elemental mercury.

Determination of optimum delay time was only done for mercury, using a stop clock. The absorbance of mercury for a standard solution containing 0.03 ppm Hg was read at different times, using Varian Spectr AA 10 attached to VGA-76 and $SnCl_2$ as reducing agent. Between two successive readings, the system was rinsed with deionised water.

### 3.3.1.3 DIGESTION TEMPERATURE

The wet-digestion procedure of various substances in their native form is much dependent on the temperature. At very low temperature, digestion may not be complete due to incomplete destruction of the organic and other interfering matter present. On the other hand, if the temperature is too high, there is a possibility of losing some of the volatile elements such as mercury in the sample. Therefore, the temperature that will give maximum AAS absorbance and hence highest elemental recovery is a compromise

between these two extremes. In this work, the temperature was kept at about 50°C for mercury and 130-140°C for cadmium and lead.

3.3.1.3.1 MERCURY

Both analytical methods for mercury determination using the EPA Approved methodology with stannous chloride as the reducing agent [62] and sodium borohydride [63] were used. A representative fish muscle sample, F1, which had earlier shown presence of an appreciable level of mercury was digested at different temperatures using the procedure described in section 3.3.3.1. In the present work, temperature was varied from 47 to 108°C. Temperatures close to the room temperature were avoided as these would require longer digestion time [65, 77]. In order to investigate the effect of covering the reaction vessel on the mercury reading, one beaker containing the sample was covered, while the other one containing the same sample was left open and both digested at the same temperature. After the outlined digestion procedure was complete, the contents were transferred into high density polyethylene bottles ready for mercury analysis using Cold Vapour Atomic Absorption Spectrophotometry, as per manufacturer's recommendations.

### 3.3.1.3.2 CADMIUM AND LEAD

Using the same digestion medium that was used for mercury determination, it was found that lead concentration values could not be determined accurately in such medium. It was therefore not possible to obtain a concentration-temperature curve for lead. According to some literature [36,59,78], determination of both cadmium and lead can be done at the same digestion temperature. Furthermore, it is known that cadmium is more volatile than lead [59], which implies that lead can be determined at slightly higher temperatures without loss of the element by evaporation.

The same representative fish sample, F1, was therefore digested at different temperatures, ranging from 50 to 130 $^{o}$C as described in section 3.3.3.2 and analysed for cadmium using flame AAS in the usual recommended way.

### 3.3.1.4 COMPARISON BETWEEN SODIUM BOROHYDRIDE AND STANNOUS CHLORIDE AS REDUCING AGENTS FOR MERCURY DETERMINATION WITH CVAAS

As explained in section 3.3.1.3.1, the representative fish muscle sample, F1, was digested at different temperatures on the same day, giving a

number of sub-samples, which would be expected to give different mercury concentration readings when analysed with Cold vapour AAS. These sub-samples were analysed for mercury, successively with sodium borohydride and stannous chloride and the results with the two reducing agents compared.

In order to compare the effects of prolonged oxidation period on mercury concentration readings with the two reducing agents above, a set of different analytical samples and standard reference materials were oxidized by $KMnO_4$ overnight at room temperature after the initial digestion with acid. As in the previous case, this set of samples was analysed for mercury, successively with the two reducing agents ($NaBH_4$ or $SnCl_2$) for inter-comparison.

Comparison between $NaBH_4$ and $SnCl_2$ for reproducibility and stability of single-element and mixed triple-element standard solutions was also done. The standards were made as described in sections 3.3.2.1 and 3.3.2.2. They were preserved and analysed for mercury with the two reducing agents over a period of about ten weeks. This is described more fully in the next section.

### 3.3.2 CALIBRATION STANDARDS

The standards for calibration graphs were made by serial dilutions of stock standard solutions made as previously described. The same acid matrix used for digestion of the samples was incorporated in these standards. This would compensate for any effect of the digestion acid mixture on the sample. It also lowers the pH of the final solution to the optimum range that gives maximum element recovery as found in section 4.1.1.2.

#### 3.3.2.1 SINGLE-ELEMENT CALIBRATION STANDARDS

These were made in line with the specifications giving the linear range for a particular element with the AAS instrument, usually given in the accompanying manual. They were made separately for the three elements as given in the following sub-sections.

##### 3.3.2.1.1 MERCURY

Using the proper sizes of volumetric flasks, pipettes and burettes, the 1000 mg/l Hg stock solution was serially diluted in order to give the following concentrations: 0.01, 0.02, 0.03, 0.04, 0.05, 0.07 and 0.09 mg/l. Each standard solution contained 7 ml of 3:1:1 ($HNO_3$, $HClO_4$, $H_2SO_4$)

(vol/vol) acid mixture for every 100 ml. This was the digestion acid mixture used for the determination of mercury in the samples. The standards were stored in high density polyethylene (HDPE) bottles.

3.3.2.1.2 CADMIUM

The cadmium standards were made using the same procedure described for mercury. The final cadmium concentrations of the standards were: 0.05, 0.10, 0.25, 0.50, 1.00, 1.50, 2.00 and 2.50 mg/l. Each of these standards contained 8 ml of 3:1 ($HNO_3$ and $HClO_4$) (vol/vol) acid mixture for every 100 ml, which was used for digestion in cadmium and lead determination. The standards were stored in HDPE bottles.

3.3.2.1.3 LEAD

The technique for making lead calibration standards was similar to that for mercury and cadmium. 8 ml of the 3:1 acid mixture was added for every 100 ml, diluted to the mark with deionised water and the standard solutions containing 0.1, 0.2, 0.25, 0.5, 0.6, 1, 2, 2.5, 5 and 10 mg/l of lead were stored in HDPE bottles.

### 3.3.2.2 MIXED TRIPLE-ELEMENT STANDARDS

These were made from combinations of standards within the calibration range of the three elements as listed down in the preceding sub-sections. The following mixed standards containing mercury, cadmium and lead were made (Table 1), each containing 7 ml of acid mixture for every 100 ml solution and stored in HDPE bottles. The standard mixing was based on random combinations.

TABLE 1. CONCENTRATION OF MERCURY, CADMIUM AND LEAD IN THE MIXED STANDARDS

| Mixed Standard Code | Concentration in mg/l of Mercury | Cadmium | Lead |
|---|---|---|---|
| MSTD 1 | 0.01 | 0.10 | 0.25 |
| MSTD 2 | 0.01 | 0.50 | 0.50 |
| MSTD 3 | 0.02 | 0.50 | 0.50 |
| MSTD 4 | 0.02 | 1.00 | 0.50 |
| NSTD 5 | 0.05 | 1.00 | 1.00 |
| MSTD 6 | 0.05 | 1.00 | 2.50 |
| MSTD 7 | 0.07 | 2.00 | 2.50 |
| MSTD 8 | 0.07 | 2.00 | 5.0 |
| MSTD 9 | 0.09 | 2.50 | 2.5 |
| MSTD 10 | 0.09 | 2.50 | 0.25 |

### 3.3.2.3 COMPARISON OF ABSORBANCES FOR STANDARDS MADE FROM DIFFERENT CONCENTRATIONS OF MERCURY STOCK STANDARDS

One stock solution contained 1000 ppm of mercury while another intermediate standard containing 50 ppm of the element was made by serial dilution of the former. Both were kept for at least 2.5 months and from these, the single-element calibration standards for mercury were separately made, treated and stored as described in section 3.3.2.1. The mercury absorbances of the two sets of standards were compared using Cold vapour AAS, with $NaBH_4$ as the reducing agent.

### 3.3.2.4 EFFECT OF ACID MIXTURE IN CALIBRATION STANDARDS ON MERCURY ABSORBANCE

To investigate the role the acid mixture (7ml for every 100 ml of standard solution) plays on the stability and absorbance sensitivity, a set of calibration standards were made from the 50 ppm mercury intermediate standard as described in section 3.3.2.3. The only deviation from the previous method was the omission of the acid mixture from the standards. The mercury absorbances of the two sets of

standards (with and without acid mixture) were compared.

To investigate the effect of changing the relative amount of the acid mixture in the calibration standards on the absorbance, two sets of single-element mercury standards were made, according to the method described previously, except that one set contained a uniform volume of 7 ml of acid mixture for every 100 ml of solution, while the other set contained different volumes of acid mixture, in the range 0.007-0.035 ml, proportional to the mercury standard concentration. The mercury absorbances and instrumental sensitivity of the two sets were compared.

### 3.3.2.5 DETECTION LIMIT, INSTRUMENTAL SENSITIVITY AND STANDARD SOLUTION STABILITY USING DIFFERENT AAS MODELS AND EXPERIMENTAL CONDITIONS

For the determination of standard solution stability, the calibration stadards were analysed from time to time and the absorbance readings determined using a particular AAS model and experimental conditions. Detection limits were determined graphically and statistically while

instrumental sensitivity was done by graphical methods, using the calibration standards.

For mercury, the above parameters were determined by use of sodium borohydride and stannous chloride as reducing agents, using Varian Spectr AA 10 AAS model attached to VGA-76 Cold Vapour generation kit. For cadmium, a VARIAN SPECTR AA 10-flame AAS model at a wavelength of 228.8 nm with the recommended optimum conditions at one lab (A) and the same model and conditions at another lab (B) was employed. The parameters investigated were also compared with those obtained at another lab (C) using flame PERKIN ELMER 2380 AAS model at 228.8 nm and the recommended optimum operating conditions. Finally, lead calibration standards for lead were analysed at 217 and 283.3 nm and the values obtained at different labs (A, B and C) compared, using VARIAN SPECTR AA-10 and PERKIN ELMER 2380 AAS models and the optimum instrumental conditions recommended.

### 3.3.2.6 REPRODUCIBILITY AND STABILITY OF MIXED STANDARD SOLUTIONS

The mixed triple-element standards were also preserved in high density polyethylene bottles and analysed from time to time over a period of about 10 weeks in order to determine reproducibity, stability

and inter-elemental effects. As before, for mercury determination, the two reducing agents ($NaBH_4$ and $SnCl_2$) were compared.

### 3.3.3 DETERMINATION OF MERCURY, CADMIUM AND LEAD IN ALL THE ENVIRONMENTAL SAMPLES

Different methods for determination of mercury, cadmium and lead have been described, as given in the literature cited in the text. The original intention in this project was to attempt to use one of these methods to determine all the three elements simultaneously. A number of digestion media and experimental conditions were therefore varied in order to achieve suitable and reliable results. The following sub-sections describe the analytical procedures that were employed for the determination of the three elements.

#### 3.3.3.1 DETERMINATION OF TOTAL MERCURY

According to Welz [56], the most successful procedure for the determination of mercury traces, was proposed by Puluektov and co-workers in 1964. As reported by the same author [56], the method was later thoroughly investigated by Hatch and Ott. The procedure is used most frequently nowadays, and was employed in the present work. The different digestion

media used for the determination of this element can be obtained from various researchers [8,50,65,77,79-82].

Initially, in the following experimental procedure, the digestion temperature of 70°C, which is used by some researchers [8,50,79] was employed in this project. However, other literature [83] recommend a temperature range of 50-60°C. It was therefore found necessary to carry out a preliminary investigation of mercury sensitivity dependence on digestion temperature. A representative fish sample, F1, which had indicated presence of mercury was used. The optimum conditions within the experimental range were then applied for all the environmental samples to be analysed.

The ranges of mass or volume of sample digested for the determination of mercury were as follows: 0.5-2 gm of fish (wet weight) was used, while 2 ml of water and 0.5-1 gm of sediments, soil and plants (dry weight) were digested. To a carefully determined mass or volume of the sample contained in a 250 ml conical flask or beaker was added 7 ml of a 3:1:1 mixture (vol/vol) of concentrated nitric, sulphuric and perchloric acids. For sediments and soils, the mixture was cooled in an ice bath and 2 ml of 12 M HCl acid added, as recommended by Stewart and Bettany

[83], but this was not necessary for the other types of samples.

The flasks and contents were placed on a water bath for about 1-1.5 hours, initially at 70$^{o}$C. This temperature was later reviewed in the following procedure. The representative fish sample, F1 was digested at different temperatures in the range 47-108 $^{o}$C. The mercury sensitivity dependence on the digestion temperature will be discussed in section 4.1.3 under "Results and Discussion". In order to avoid volatilization of mercury, it was found necessary to cover the digestion vessels with watch glasses.

The digestion vessels were then removed from the water bath and cooled in an ice bath and 50 ml of 6 % wt./vol $KMnO_4$ solution was added slowly with constant shaking until effervescence was complete. One set of samples was left in this oxidation medium at least overnight before the second digestion step.

Further digestion was allowed to continue for 2-2.5 hours in the regulated water bath at the "optimum" temperature determined as above. The digests were then removed from the water bath and cooled to room temperature. 15 ml of 20 % wt./vol hydroxyl amine hydrochloride solution was added slowly, while shaking the vessel, to avoid frothing

and possible loss of the flask contents. The digestion process yielded a clear and almost colourless, homogeneous solution, with some purple or pale-brown silica particles at the bottom. The final digest was either decanted or filtered through a previously washed Whatman No.1 filter paper into a 100 ml volumetric flask. The residue was then rinsed several times with deionised water and added to the mark with some gentle stirring.

Blanks of 2 ml of deionised water were treated under the same conditions. Blank digestion went along with every group of samples digested so as to ensure removal of carry-over interference or contamination.

The uptake rate of standards and samples was in the range 6-8 ml/min, as determined by the inner diameter of the peristaltic pump tubing (0.081"). The two remaining pump channels (Figure 2) each have an inner diameter of 0.030" and produced an uptake rate of approximately 0.8-1 ml/min. The solutions pumped through these two channels also contribute to total sample acidity and the effect of the acid content of these solutions was examined by Dominski and Shrader [61].

One channel contained the reducing agent, stannous chloride at a fixed HCl concentration of 20%. The second was used to increase the acid

concentration if necessary. Best results were obtained if this channel pumped distilled water. Any HCl in this channel degraded the sensitivity. Therefore, the final HCl concentration of solutions in the system after mixing was within the optimum range of 5 to 7.5 % (about 60% HCl) [61]. In this project, the inert gas used was dry and pure nitrogen.

Mercury absorbance was read at 253.7 nm in triplicate. For calibration, standards containing 0.01, 0.02, 0.03, 0.04 and 0.05 mg/l, made and preserved as described in section 3.3.2.1.1 were used. A calibration graph was then plotted with the aid of a Computer interfaced with the AAS. Higher concentrations were avoided as it was difficult to optimize the instrumental parameters. Using the calibration graph, the final read-out for the unknown sample digests was then read in concentration units.

### 3.3.3.2 DETERMINATION OF TOTAL CADMIUM AND LEAD

In order to save time, it was decided to use the same acid mixture for wet digestion of all types of samples. Different acid mixtures used in the literature were reviewed so as to employ a method that yields high elemental recovery, with high accuracy and precision. A survey of the available

literature showed that most researchers and authors recommend use of a mixture of nitric and perchloric acids in varying ratios [37,72,84-90], which was used in this project.

The ranges of mass or volume of sample digested for the determination of cadmium and lead were as follows: 0.7-2 gm, of fish (wet weight), 1-2 ml of water and 0.4-1 gm of sediments, soil and plants (dry weight). To a carefully determined mass or volume of the sample contained in a 250 ml beaker or conical flask was added 8 ml of a 3:1 vol/vol mixture of concentrated nitric and perchloric acids. The digestion vessels were covered with watch glasses and transferred to a hot plate, whose temperature was controlled to about 80-140$^{o}$C. The mixture was then heated to about 130-140$^{o}$C until all the brown fumes of $NO_2$ were completely expelled. Further digestion was allowed to continue until dense white fumes appeared. Further heating was done for about 20 minutes and the solution allowed to cool at room temperature. The sides of the beaker or conical flask and watch glass cover were then washed down with 15-20 ml of warm deionised water. Depending on the solution, the digest was either decanted or filtered through a Whatman No.1 filter paper, initially into a 100 ml volumetric flask. However, using five fish

samples, it was found that the concentrations of cadmium and lead in this diluted volume were below the detection limits. When these solutions were pre-concentrated by evaporation factors in the range of 2.5 to 3.3, the concentrations of the two elements were well above the detection limits. For the determination of cadmium and lead, a final diluted volume of 50 ml was therefore used throughout the project. The filter paper contents were washed and the solutions diluted to the mark with deionised water together with thorough shaking. The final solution had its pH adjusted to below 2, using nitric acid and stored in HDPE bottles.

Analysis was then done using flame AAS and the optimum operating conditions as per the manufacturer's recommendations. The standards made in sections 3.3.2.1.2 and 3.3.2.1.3 were used for calibration in the same manner as those for mercury determination (section 3.3.3.1).

### 3.3.4 EVALUATION OF ANALYTICAL PROCEDURE: DIGESTION AND ANALYSIS OF CERTIFIED REFERENCE MATERIALS

In order to assess the reliability and accuracy of the digestion and analytical procedures, it was necessary to digest and analyse standard reference materials, containing the elements of interest. These were subjected to the same procedure as those for the samples and the concentration of the three elements obtained compared to the literature values. The percentage recovery of the elements determined the reliability of the method. For this work, three biological standard reference materials from the International Atomic Energy Agency (IAEA) and one geological reference material were used.

#### 3.3.4.1 MUSSEL TISSUE (MA-M-2/TM)

This is an IAEA reference material and was dried to constant weight at 85$^{o}$C before digestion. The reported concentrations of the three heavy metal elements were as follows: 0.95 $\pm$ 0.11 mg/kg of mercury, 1.32 $\pm$ 0.22 mg/kg of cadmium and 1.92 $\pm$ 0.58 mg/kg of lead (non-certified) [91].

### 3.3.4.2 FISH FLESH HOMOGENATE (MA-A-2 (TM))

This is also an IAEA reference material and was also dried to constant weight at 85°C before digestion. The reported concentrations of the three heavy metal elements were as follows: 0.47 ± 0.02 mg/kg of mercury, 0.066 ± 0.004 mg/kg of cadmium and 0.58 ± 0.07 mg/kg of lead [91].

### 3.3.4.3 HORSE KIDNEY (H8)

This was also an IAEA reference material, which had been dried to constant weight at 85°C before digestion. The provisional heavy metal element concentrations are: 0.91 ± 0.08 mg/kg of mercury and 189 ± 5 mg/kg of cadmium. Its concentration for lead was not reported [91].

### 3.3.4.4 ZINC-TIN-COPPER-LEAD ORE, MP-1

This was a certified reference geological material from Department of Energy, Mines and Resources Branch, Ottawa, Canada. The reported provisional concentration of lead in the ore was 1.93 ± 0.03 % [92].

### 3.3.5 EVALUATION AND IMPORTANCE OF DIGESTION: COMPARISON OF DIGESTED AND UNDIGESTED WATER SAMPLES

In order to assess the importance of digesting the samples for the determination of total elemental concentrations, a set of 15 water samples was selected from the whole batch from the area of study. These included:- the lake, bore-hole, domestic, geothermal and river water or condensed steam. They were analysed for mercury, cadmium and lead. Ten of these samples were later digested and their levels of mercury, cadmium and lead compared to those obtained before digesting the samples.

### 3.3.6 OPTIMUM OPERATING CONDITIONS

Tables 2 and 3 show the optimum operating conditions of the spectrophotometers used in the present work.

TABLE 2. RECOMMENDED OPTIMUM OPERATING CONDITIONS FOR FLAME AND COLD-VAPOUR ATOMIC ABSORPTION ANALYSIS OF MERCURY, CADMIUM AND LEAD, USING VARIAN SPECTR AA-10 AAS [61,93]

| OPERATING PARAMETERS | MERCURY (Cold-Vapour) | CADMIUM | LEAD |
|---|---|---|---|
| Wavelength (nm) | 253.7 | 228.8 | 217.0 |
| Slit width (nm) | 0.5 | 0.5 | 1.0 |
| Lamp Current (mA) | 4 | 3 | 5 |
| Flame | ___ | A-A | A-A |
| Flame stoichiometry | ___ | Oxidizing | Oxidizing |
| Sensitivity | 0.30 ppb | 0.02 ppm | 0.17 ppm |
| Optimum working range (mg/l) | 0.1 | 0.5-2 | 5-20 |
| Detection limit | 0.2ppb | 0.0323ppm | 0.020ppm |

A-A = Air-Acetylene, ppm = mg/l, ppb = ng/ml

TABLE 3. RECOMMENDED OPTIMUM OPERATING CONDITIONS FOR FLAME ATOMIC ABSORPTION ANALYSIS OF CADMIUM AND LEAD, USING PERKIN ELMER, MODEL 2380, AAS [94,95]

| OPERATING PARAMETERS | CADMIUM | LEAD | |
|---|---|---|---|
| Lamp current intensity (mA) | 4 | 10 | 10 |
| Spectral band pass (nm) | 0.7 | 0.7 | 0.7 |
| Wavelength (nm) | 228.8 | 217.0 | 283.3 |
| Oxidant (air) flow rate ($cm^3/min$) | 50 | 50 | 50 |
| Acetylene flow-rate ($cm^3/min$) | 15 | 15 | 15 |
| Sensitivity (mg/l) | 0.022 | 0.15 | 0.49 |
| Linear working range (mg/l) | 2 | 20 | 20 |

## 3.4 ANALYTICAL PROCEDURES- X-RAY FLUORESCENCE ANALYSIS

As shown earlier, not much work has been done with this analytical technique. However, in this project, some preliminary work was done in order to determine some optimum parameters for the determination of mercury and lead in liquid or digested samples using $^{109}Cd$ source and Si(Li) detector.

### 3.4.1 DETERMINATION OF OPTIMUM pH, COMPLEXING TIME AND DETECTION LIMITS FOR MERCURY AND LEAD

Since the three elements (mercury, cadmium and lead) were to be determined in the project, it was necessary to determine the optimum pH using different matrices involving the three elements. Standard solutions containing these elements were made and the effect of changing pH on the metal recovery by complexing and irradiating with $^{109}Cd$ source was studied.

In order to accomplish this, 50 ml of a solution containing 10 ppm (i.e. 10 mg/l) of mercury and mixed solutions containing 0.1 ppm mercury, 1 ppm cadmium and 2 ppm lead were transferred into each of a series

of beakers and the pH adjusted in the range 1.0-7.2 by using analar nitric acid and ammonia solutions. To each of these was added 10 ml of 2 % sodium diethyl dithio carbamate (analar NaDDTC) and allowed to complex for about 25-30 minutes. After filtering over 45 micron millipore filter papers, the samples were dried at room temperature for at least one day and excited with Cadmium-109 source. The detector used was Si(Li) with 25 micron Be window entrance. The spectra were collected on the Multi-channel analyzer (MCA), then transferred and analysed for mercury and lead on the Digital Professional 350 micro-computer, with Quantitative XRFA software from IAEA [96]. Later on, IMC Data Systems Computer with Canberra S100 was used for data analysis with the same software.

Likewise, for the determination of optimum complexing time for mercury and lead, the same volume of the mixed standard solution was transferred into a series of beakers and the pH of all the solutions adjusted to around 2. The complexing time was varied from 2 to 60 minutes with the other analytical procedures remaining the same as previously described.

In order to determine the lowest level of the element deposited on the millipore filter that could be detected using XRF technique, different volumes

(in the range 0-100 ml) of the mixed standard solution were diluted to 100 ml with double distilled water. The corresponding concentrations were: 0.02 to 0.1 mg/l for mercury and 0.4 to 2 mg/l for lead. Complexing, filtration, drying and analysis for mercury and lead of the deposit was done as previously described.

### 3.4.2 INTER-COMPARISON BETWEEN AAS AND XRF RESULTS

In order to confirm or assess the reliability of the AAS data obtained, it was found necessary to analyse a few representative samples, which had been digested as described in section 3.3.3. Since $^{109}Cd$ was used as the source of excitation and due to other peak interferences, it was not possible to analyse for cadmium using XRF, as already mentioned in section 2.2. The volume of the digested and complexed solution depended on its availability after AAS analysis.

About 3.9-36 ml of the diluted sample digests were transferred into clean beakers, which had been washed as described in section 3.2.2. It was ensured that their pH was in the optimum range obtained (about 2), according to the results given in section 4.1.2.1. Complexing for about 15 minutes, filtration and drying of the deposit on the nucle-pore filters

were done as in the preceding sections. Analysis of the sample for mercury and lead was done with IMC Data Systems Computer, installed with Canberra S100 and Quantitative XRFA software from IAEA [96].

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 OPTIMIZATION AND EVALUATION OF THE ANALYTICAL PROCEDURES

### 4.1.1 CALIBRATION STANDARD SOLUTIONS- ATOMIC ABSORPTION SPECTROPHOTOMETRY

#### 4.1.1.1 OPTIMUM DELAY TIME FOR MERCURY ANALYSIS

The determination of optimum delay time was done as described in section 3.3.1.2. In AAS determination, this is the time in seconds which elapses between pressing the key "READ" and the start of the actual measurement [76]. Alternatively, it can be visualized as the total time which elapses during the complete reduction of mercuric ions by stannous chloride or sodium borohydride to elemental mercury. The different mercury absorbances at different time intervals using a 0.03 mg/l mercury standard and $SnCl_2$ as reducing agent are recorded in Table 4 and the trend displayed in Figure 4. The results show that a delay time of 50-70 seconds or more gave maximum absorbance. This time limit seems to agree

with the values recommended for the VGA76 mercury determination using Cold Vapour Atomic Absorption Spectrophotometry as given in the literature [64,65,76]. This is as expected because the analysis and instrumental operation was done according to the manufacturer's recommendations and therefore ensured optimum delay time for stable mercury absorbance readings.

TABLE 4. MERCURY ABSORBANCE READINGS OF A 0.03 mg/l STANDARD SOLUTION AT DIFFERENT DELAY TIMES[a].

| Delay time (sec) | Mercury Absorbance(units) |
|---|---|
| 0 | 0.007 |
| 3 | 0.009 |
| 13 | 0.011 |
| 23 | 0.212 |
| 28 | 0.309 |
| 33 | 0.398 |
| 38 | 0.444 |
| 43 | 0.461 |
| 48 | 0.469 |
| 53 | 0.469 |
| 58 | 0.470 |

TABLE 4 (contd.)

| Delay time (sec) | Mercury Absorbance(units) |
|---|---|
| 63 | 0.471 |
| 73 | 0.471 |
| 83 | 0.471 |
| 93 | 0.471 |

[a] Varian Spectr AA10 AAS attached to Cold Vapour Generator VGA 76

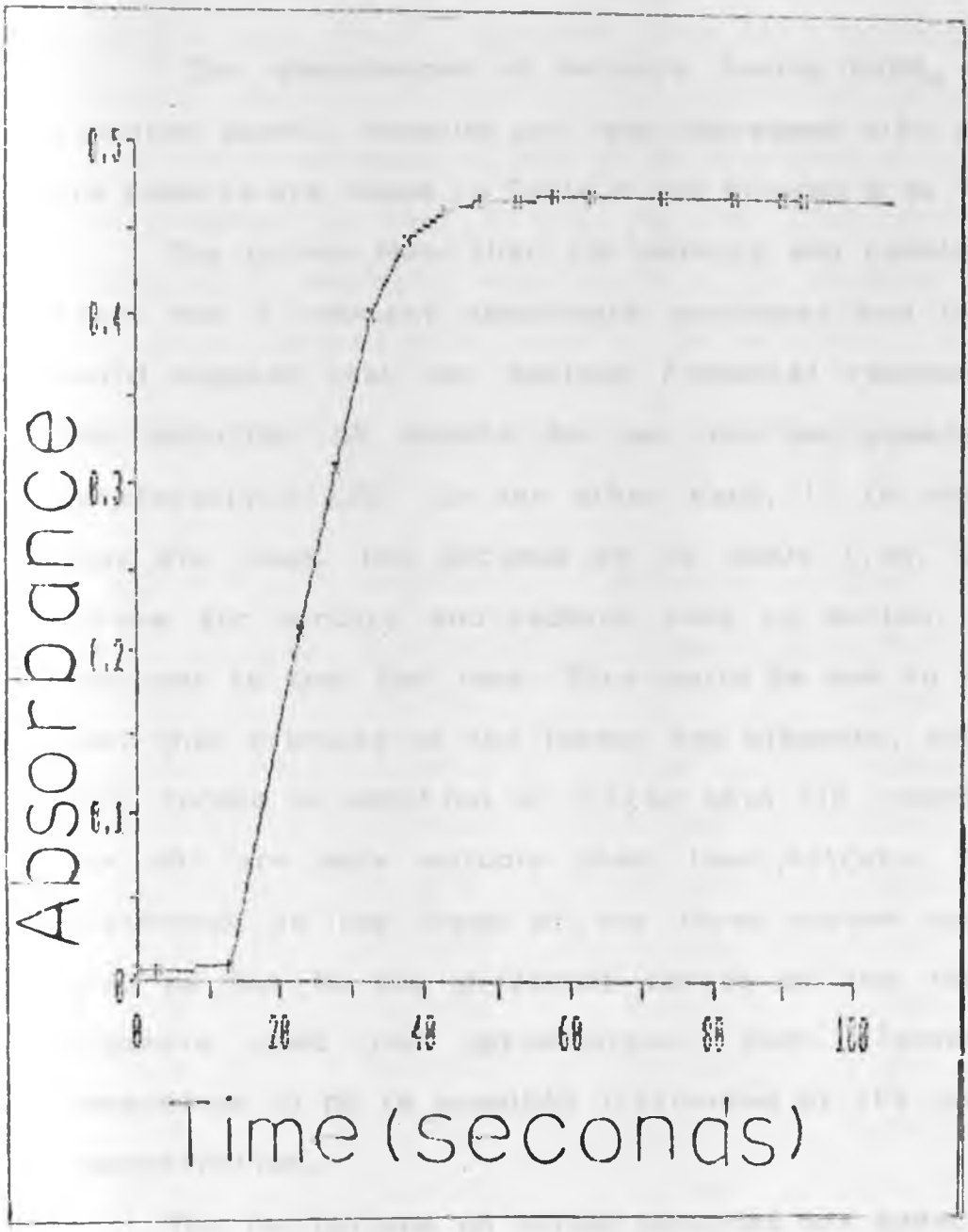



Figure 4: Graph of mercury absorbance vs. delay time in seconds in Cold Vapour AAS determination

#### 4.1.1.2 OPTIMUM pH FOR ELEMENT ANALYSIS

The absorbances of mercury (using $NaBH_4$ as reducing agent), cadmium and lead decreased with pH. The results are shown in Table 5 and Figures 5 to 7.

The curves show that for mercury and cadmium, there was a constant absorbance decrement and this would suggest that for maximum elemental recovery, the solution pH should be as low as possible (preferably < 1.5). On the other hand, it is shown that for lead, the optimum pH is about 1.43. The curves for mercury and cadmium have no maxima, in contrast to that for lead. This could be due to the fact that nitrates of the former two elements, which were formed on addition of nitric acid (in lowering the pH) are more soluble than lead nitrate. The difference in the trend of the three curves could also be due to the different levels of the three elements used for optimization. Each element's dependence on pH is possibly influenced by its ionic concentration.

The low optimum pH values obtained are expected because at high pH values, the elements will be precipitated as, say hydroxides. It is known from the general rules of solubility that the hydroxides of mercury, cadmium and lead are insoluble [97]. These

would therefore decrease the concentration of ions in solution and hence suppress the AAS absorbance signals. Another possible explanation is that at high pH, there is possible flocculation and adsorption of the elements on the surface of the container or formation of secondary equilibria in the presence of $OH^-$, which removes metal ions in the solution.

TABLE 5. MERCURY, CADMIUM AND LEAD ABSORBANCE READINGS[b]

| pH | MERCURY (0.1 mg/l) | CADMIUM (1 mg/l) | LEAD (2 mg/l) |
|---|---|---|---|
| 0.9 | 0.0788 | 0.129 | 0.0353 |
| 1.5 | 0.073 | 0.119 | 0.0343 |
| 2.0 | 0.0608 | 0.1183 | 0.035 |
| 3.0 | 0.0540 | 0.1160 | 0.0337 |
| 4.3 | 0.0559 | 0.1163 | 0.033 |
| 4.8 | 0.0475 | 0.110 | 0.029 |
| 5.8 | 0.0422 | 0.1123 | 0.029 |
| 7.0 | 0.0343 | 0.104 | 0.0197 |
| 8.1 | 0.0292 | 0.0963 | 0.0177 |

[b] using Varian Spectr model AA10 AAS

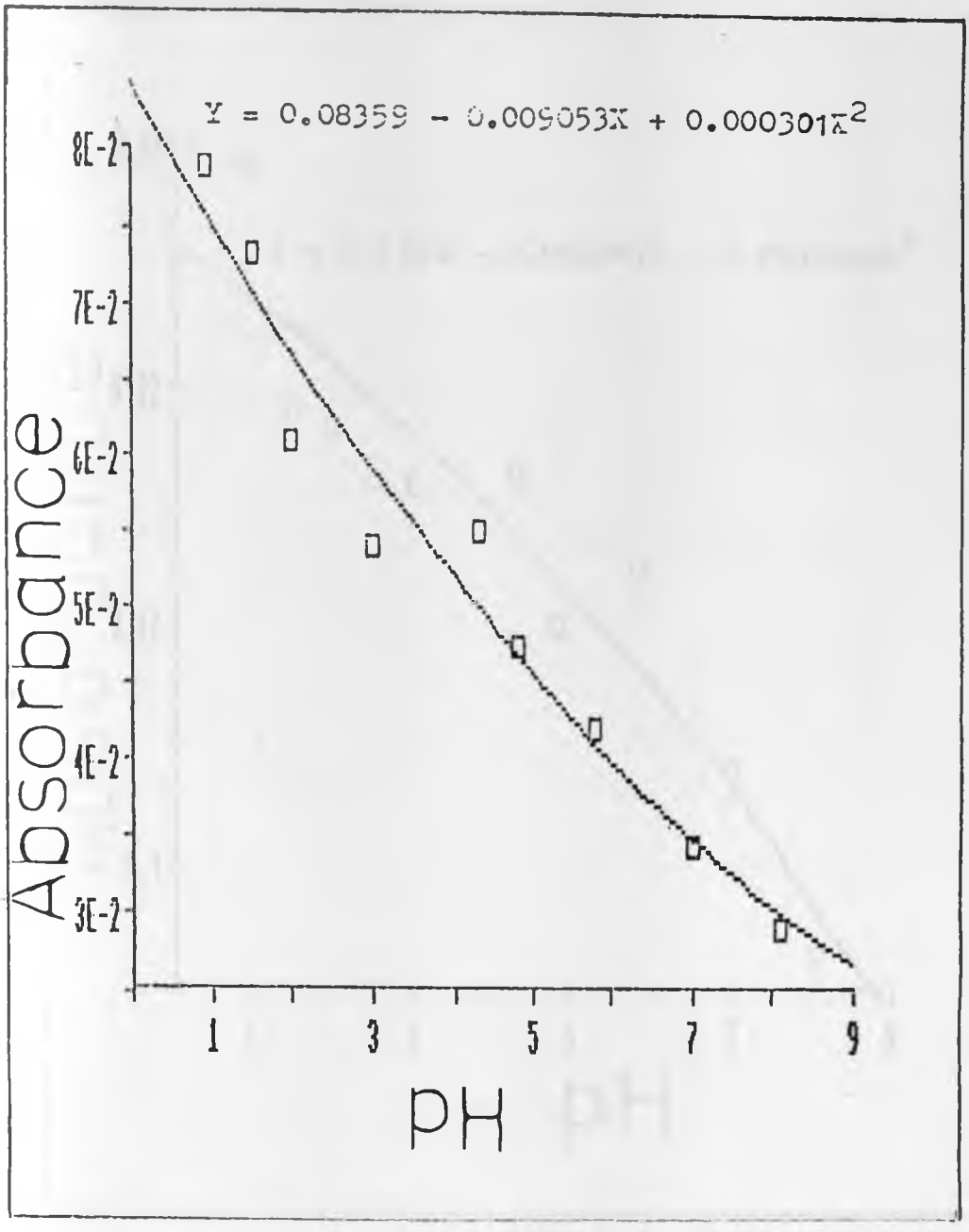



Figure 5: Graph of mercury AAS Absorbance vs. pH

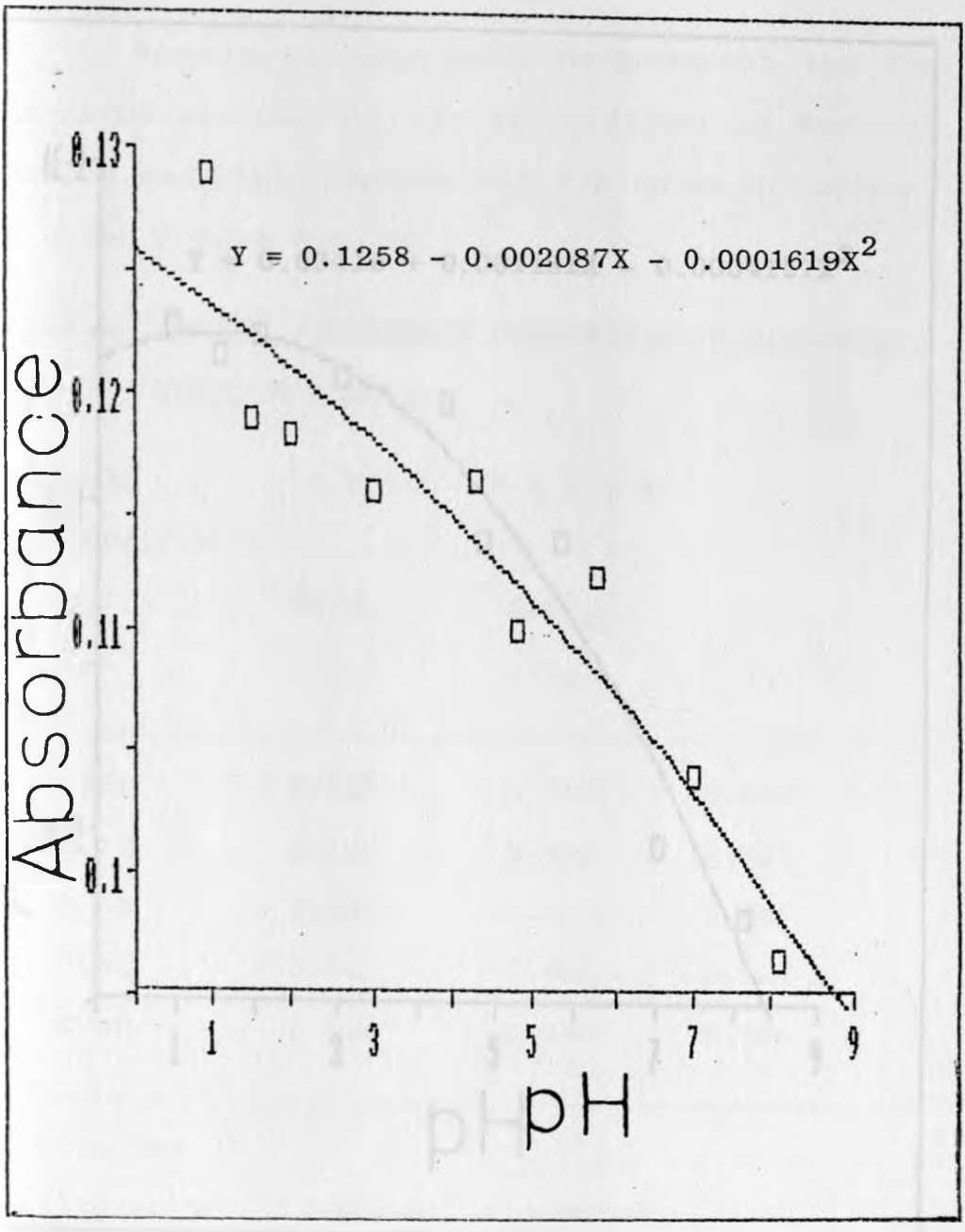



Figure 6: Graph of cadmium AAS Absorbance vs. pH

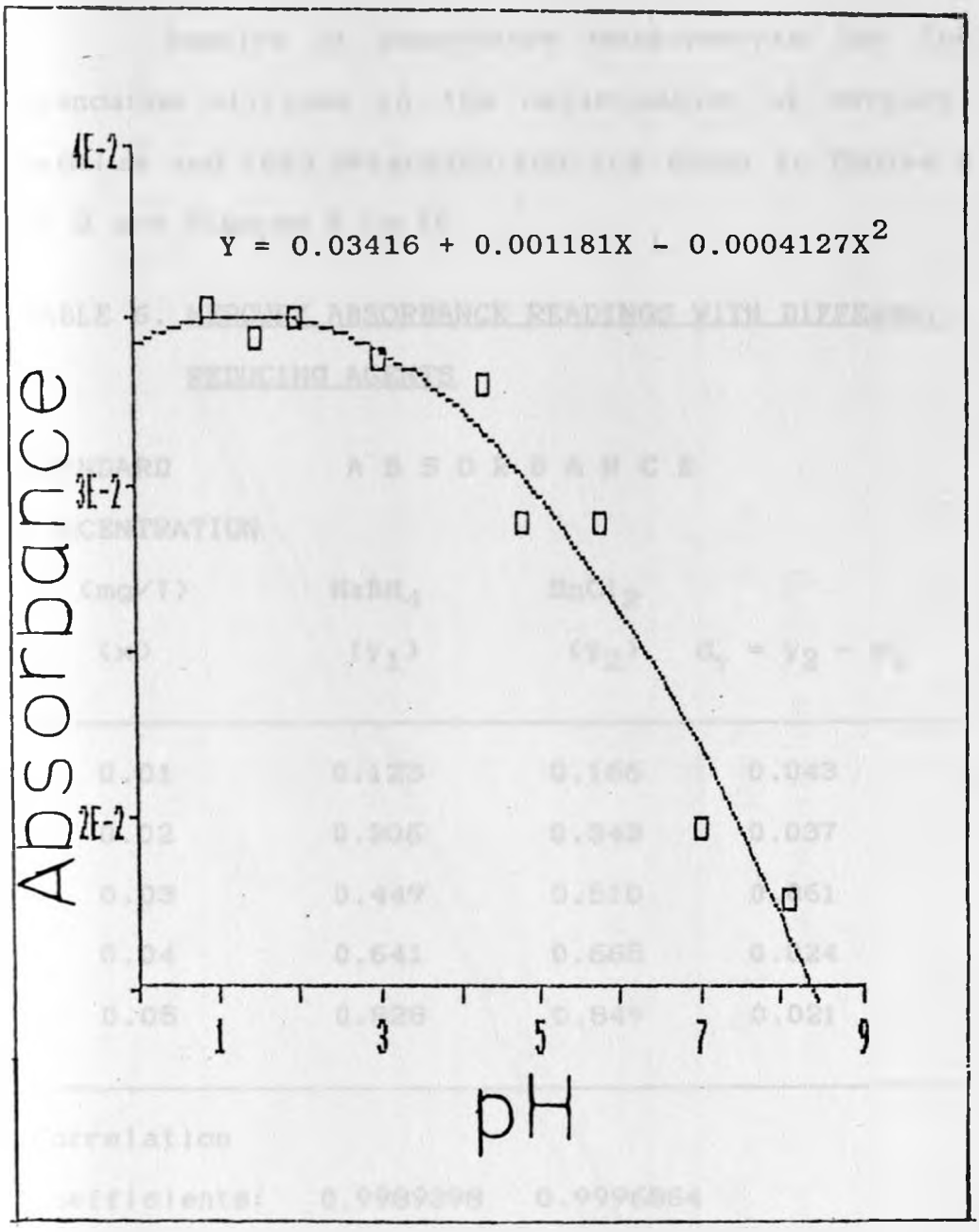



Figure 7: Graph of lead AAS Absorbance vs. pH

### 4.1.1.3 STANDARD CALIBRATION

Results of absorbance measurements for the standards utilized in the optimization of mercury, cadmium and lead determination are shown in Tables 6 to 8 and Figures 8 to 10.

TABLE 6. MERCURY ABSORBANCE READINGS WITH DIFFERENT REDUCING AGENTS

| STANDARD CONCENTRATION (mg/l) (x) | A B S O R B A N C E $NaBH_4$ ($y_1$) | $SnCl_2$ ($y_2$) | $d_y = y_2 - y_1$ |
|---|---|---|---|
| 0.01 | 0.123 | 0.166 | 0.043 |
| 0.02 | 0.306 | 0.343 | 0.037 |
| 0.03 | 0.449 | 0.510 | 0.061 |
| 0.04 | 0.641 | 0.665 | 0.024 |
| 0.05 | 0.828 | 0.849 | 0.021 |

Correlation coefficients: 0.9989398 0.9996864

Regression Equations: $y_1 = 17.45x - 0.0541$

$y_2 = 16.88x + 0.0002$

Mean difference, $d_y = 0.0372 \pm 0.0161$

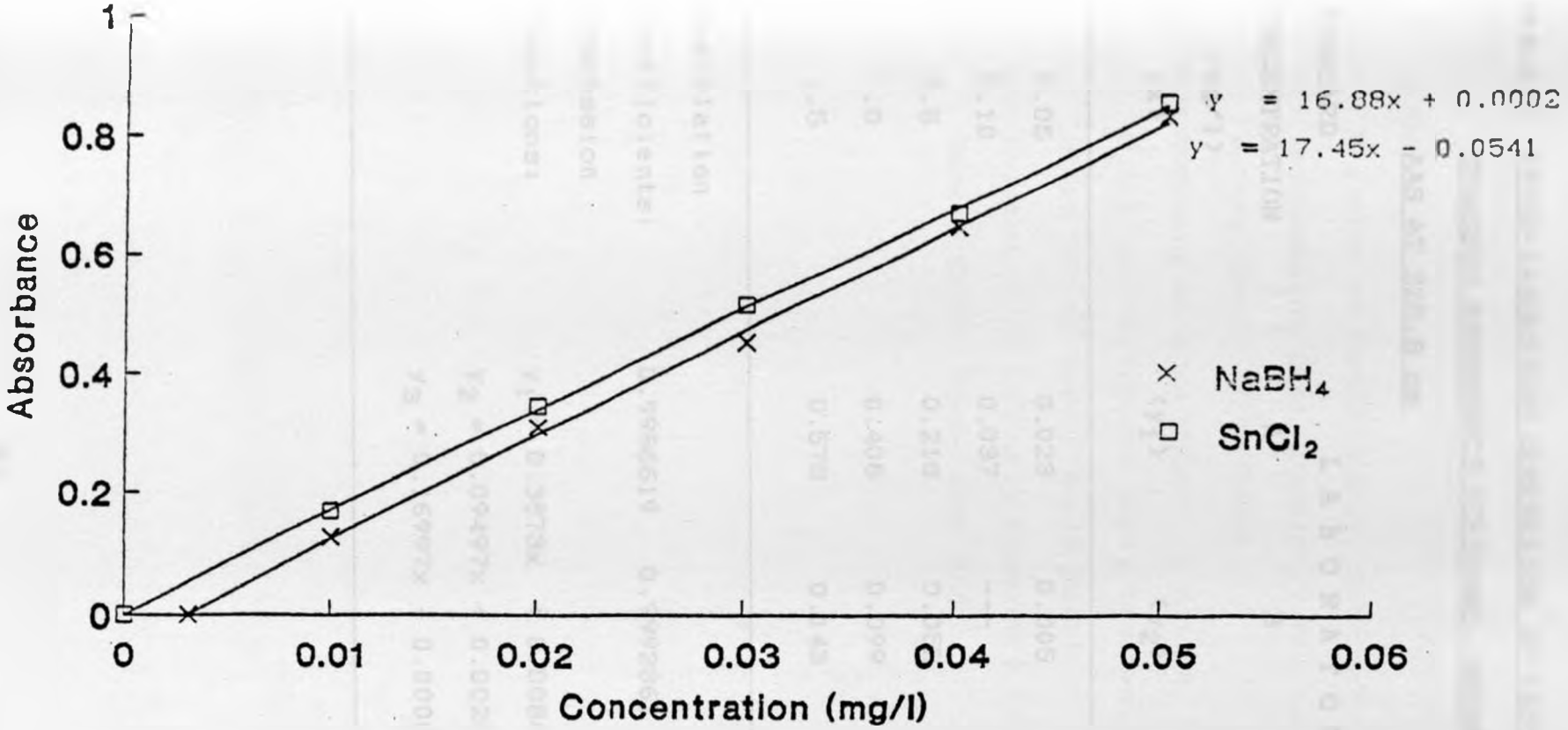



Figure 8. Calibration Graph for mercury,using CV-AAS with $NaBH_4$ and $SnCl_2$ as reducing agents

TABLE 7. INTER-LABORATORY COMPARISON OF CADMIUM STANDARD ABSORBANCE READINGS, USING FLAME AAS AT 228.8 nm

| STANDARD CONCENTRATION (mg/l) (x) | LABORATORY A ($y_1$) | B ($y_2$) | C ($y_3$) |
|---|---|---|---|
| 0.05 | 0.023 | 0.005 | 0.0095 |
| 0.10 | 0.037 | --- | 0.015 |
| 0.5 | 0.218 | 0.052 | 0.088 |
| 1.0 | 0.406 | 0.099 | 0.172 |
| 1.5 | 0.578 | 0.143 | 0.254 |
| Correlation coefficients: | 0.9986619 | 0.9992861 | 0.9998053 |
| Regression Equations: | $y_1 = 0.3873x + 0.0084$ | | |
| | $y_2 = 0.09497x + 0.0023$ | | |
| | $y_3 = 0.16997x + 0.0006$ | | |

TABLE 7 (contd.)

--- = Not determined

Lab A = Ministry of Public Works, Materials Testing and Research Dept; Chemistry Section, using Varian Spectr AA-10 model

Lab B = Ministry of Environment & Natural Resources, Mines and Geological Dept; Geochemical Lab, using Varian Spectr AA-10 model

Lab C = University of Nairobi, Chemistry Lab, using Perkin Elmer, model 2380 AAS

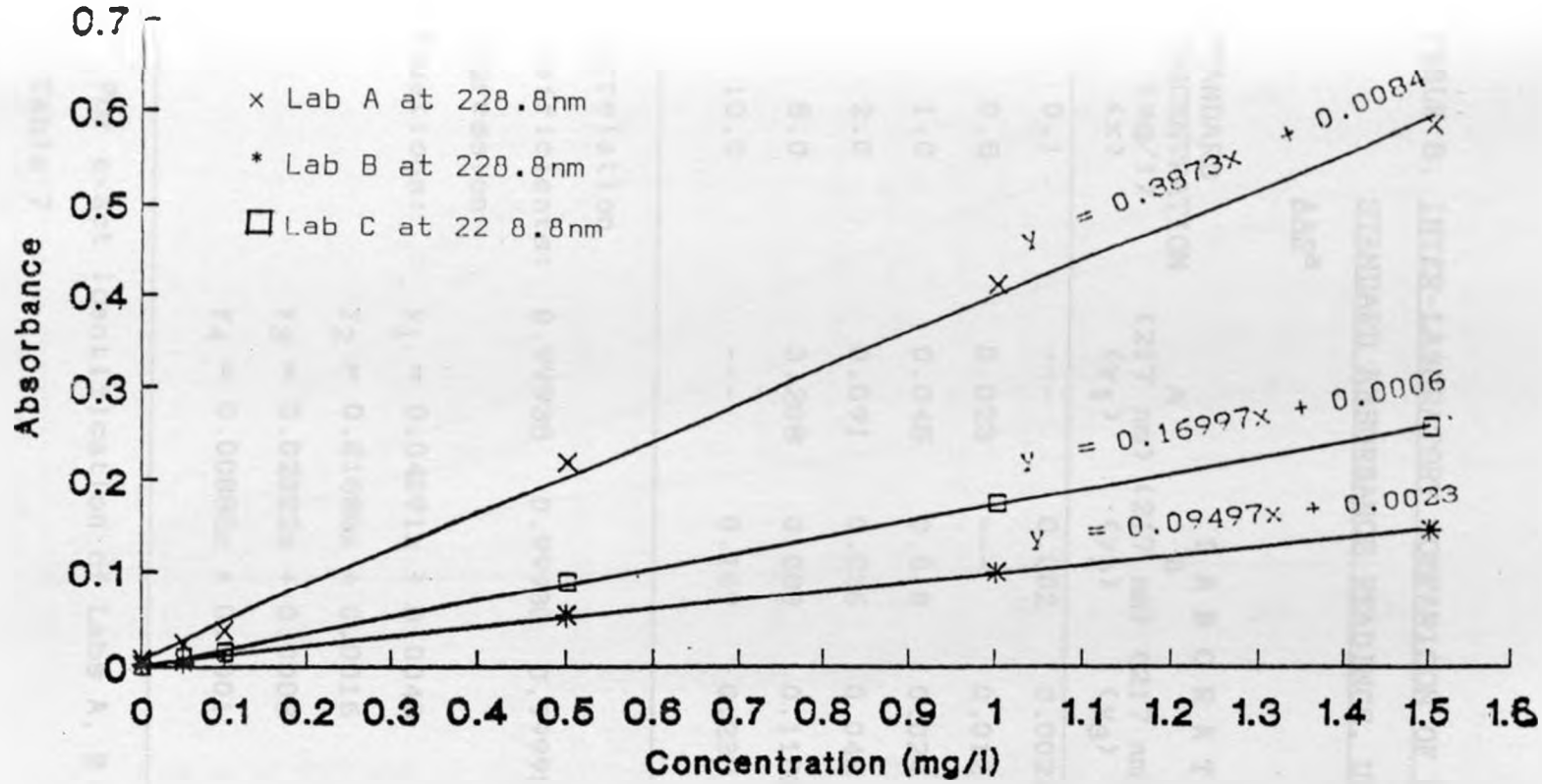



Figure 9. Calibration Graph for cadmium using flame AAS with different models and at different labs

TABLE 8. INTER-LABORATORY COMPARISON OF LEAD STANDARD ABSORBANCE READINGS, USING FLAME AAS[a]

| STANDARD CONCENTRATION (mg/l) (x) | LABORATORY A (217 nm) ($y_1$) | B (217 nm) ($y_2$) | C (217 nm) ($y_3$) | C (283.3 nm) ($y_4$) |
|---|---|---|---|---|
| 0.1 | --- | 0.002 | 0.0024 | 0.001 |
| 0.5 | 0.023 | --- | 0.012 | 0.00475 |
| 1.0 | 0.045 | 0.018 | 0.024 | 0.00867 |
| 2.0 | 0.091 | 0.036 | 0.048 | 0.0180 |
| 5.0 | 0.208 | 0.088 | 0.1182 | 0.0440 |
| 10.0 | --- | 0.169 | 0.233 | 0.0887 |
| Correlation coefficients: | 0.99935 | 0.99980 | 0.99995 | 0.99995 |

Regression Equations:

$y_1 = 0.04091x + 0.0048$

$y_2 = 0.01686x + 0.0016$

$y_3 = 0.02322x + 0.0008$

$y_4 = 0.00885x + 0.0001$

[a] For exact identification of Labs A, B and C, see Table 7

--- Not determined

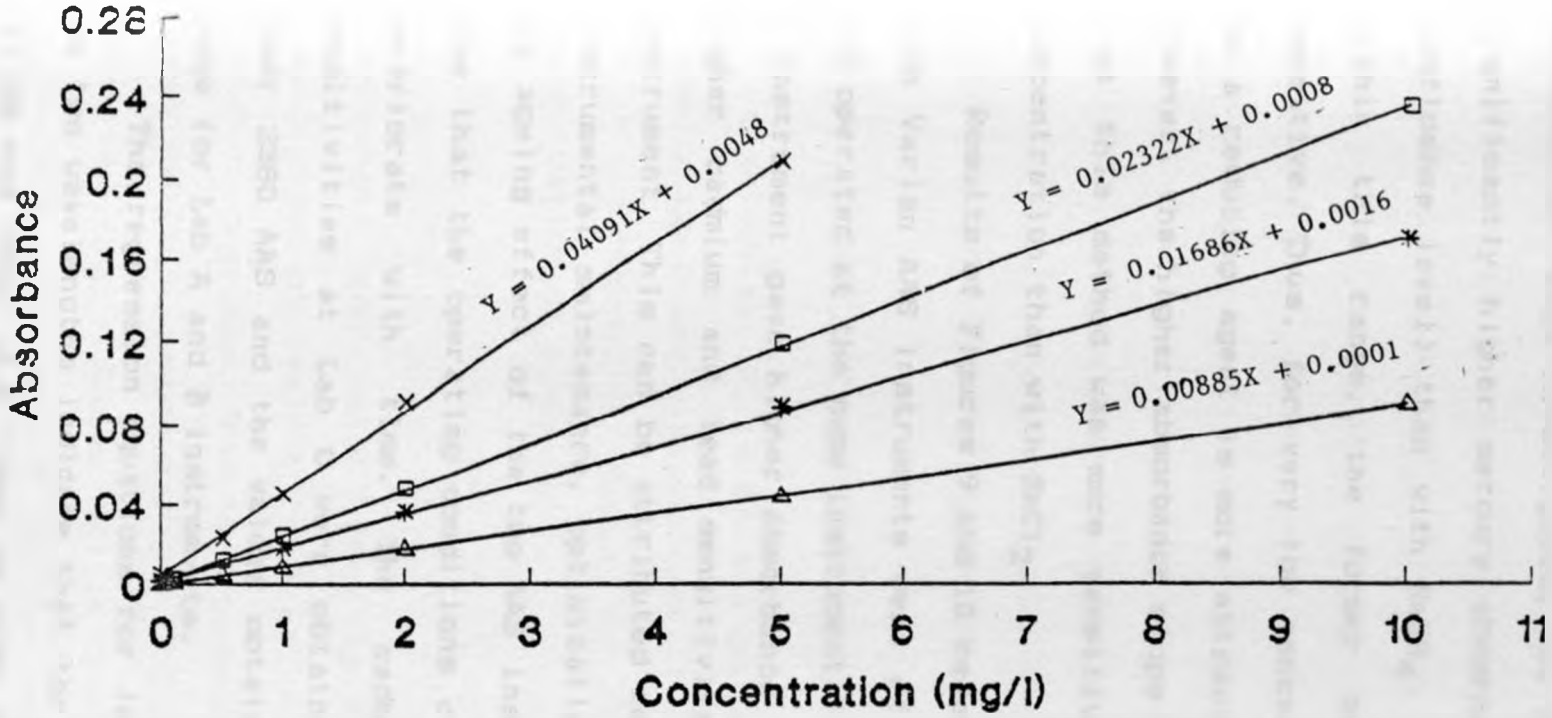



Figure 10. Calibration Graph for lead using flame AAS with different models and at different labs

Table 6 and Figure 8 show that within the calibration range investigated, the $SnCl_2$ method gave significantly higher mercury absorbances (at a 99% confidence level) than with $NaBH_4$. This shows that within this range, the former method was more sensitive. Thus, for very low concentrations, $SnCl_2$ as a reducing agent is more attractive than $NaBH_4$. However, the higher absorbance slope with $NaBH_4$ shows that this method was more sensitive to change in concentration than with $SnCl_2$.

Results of Figures 9 and 10 reveal that although both Varian AAS instruments were of the same model and operated at the same instrumental conditions, Lab A instrument gave higher absorbance values and also higher cadmium and lead sensitivities than Lab B instrument. This can be attributed to differences in instrumental maintenance, optimization and probably the ageing effect of the two AAS instruments. It is true that the operating conditions of an instrument deteriorate with time. The cadmium and lead sensitivities at Lab C were obtained with Perkin Elmer 2380 AAS and the values obtained lie between those for Lab A and B instruments.

The regression equations for lead at Lab C at the two wavelengths indicate that the sensitivity at 217 nm was about 2.6 times as high as at 283.3 nm,

which is very close to the values given in the literature [95]. Most of the analytical sample data was obtained at 217 nm with Varian Spectr AA-10 and Perkin Elmer, model 2380 AAS.

The calibration data showed excellent correlation of absorbance values with sample concentration for different or similar instruments. There is excellent linearity and obedience to Beer's Law [53,57]. The calibration range covered almost all the analytical sample concentrations and there was hardly any need of diluting the samples.

#### 4.1.1.4 SENSITIVITY AND DETECTION LIMIT

Sensitivity, S, is defined as the ratio of the change in the instrument response, I (output signal), with a corresponding change in the stimulus, C (concentration of analyte):

$$S = \frac{dI}{dC} \qquad [53]$$

Sensitivity may also be expressed as the concentration of analyte required to cause a given instrument response. This is also referred to as the characteristic concentration [61]. In Atomic Absorption Spectroscopy, it is expressed as the concentration in mg/l of analyte that produces an

absorbance of 0.0043(4) absorbance unit i.e. 1.0 % absorption [53,78].

As the concentration of the analyte approaches zero, the signal disappears into the noise, and the detection limit is reached. A quantitative definition of the detection limit is that concentration of analyte which produces an output signal twice the root mean square of the back-ground noise (a signal-to-noise ratio of 2). Detection limits are generally defined at a 95% confidence level [53], by the following formula [61,78]:

$$\text{Detection Limit} = \frac{2SD * \text{Standard concentration}}{\text{Mean absorbance of standard}}$$

where SD = Standard deviation of absorbance readings.

Using the above definitions, the sensitivity and detection limits of mercury, cadmium and lead with different AAS equipments in different labs or in different reaction media were calculated and the values shown in Table 9.

TABLE 9. INTER-LABORATORY COMPARISON OF SENSITIVITY AND DETECTION LIMITS FOR DIFFERENT AAS MODELS

(a) SENSITIVITY (mg/l)

| ELEMENT | LabA | LabB | LabC |
|---|---|---|---|
| MERCURY at 253.7 nm | | | |
| (i) $NaBH_4$ method | 0.0034 | E.N.A | E.N.A |
| (ii) $SnCl_2$ method | 0.0003 | E.N.A | E.N.A |
| CADMIUM at 228.8nm | 0.0056 | 0.0217 | 0.0223 |
| LEAD | | | |
| (i) at 217 nm | 0.0197 | 0.1676 | 0.1537 |
| (ii) at 283.3 nm | --- | --- | 0.4866 |

TABLE 9 (contd.)

(b) DETECTION LIMIT (mg/l)

| | Lab A | | Lab B | | Lab C |
|---|---|---|---|---|---|
| ELEMENT | Stat. | Graph. | Stat. | Graph. | Stat. |
| MERCURY | | | | | |
| at 253.7 nm | | | | | |
| (i) $NaBH_4$ | 0.0023 | 0.0031 | E.N.A | E.N.A | E.N.A |
| method | ±0.000012 (n = 11) | ±0.000019 (n = 3) | | | |
| (ii) $SnCl_2$ | 0.0002 | 0.0002 | E.N.A | E.N.A | E.N.A |
| method | ±0.000002 (n = 13) | ±0.000000 (n = 3) | | | |
| CADMIUM | | | | | |
| at 228.8nm | 0.0154 | --- | 0.0323 | 0.0366 | 0.0069 |
| LEAD | | | | | |
| (i) at 217 nm | 0.0198 | --- | --- | 0.0139 | 0.0532 |
| (ii) at 283.3 nm | --- | --- | --- | --- | 0.0858 |

TABLE 9 (contd.)

N.B. Labs A, B, C represent the same labs and AAS models as listed under Table 7

Stat. = Statistically calculated value

Graph. = Graphically obtained value

E.N.A = VGA-76 equipment not available

--- = Not determined or not practicable

---

Results in Table 9 show that for mercury analysis, there was significant difference between the statistically and graphically obtained values for detection limits at all tabulated significant levels (say 0.05) with the $NaBH_4$ reduction method, but none (at all the levels), when the $SnCl_2$ method was used. It also shows that the latter method was 11.3 times as sensitive, with a detection limit of 11.5 times that of $NaBH_4$. This could be due to the fact that the $SnCl_2$ reducing method involves a metal-metal reaction:

$$Hg^{2+} + Sn^{2+} \xrightarrow{HCl} Hg^{0} + Sn^{4+} \quad \text{.....(8)}$$

with mercury and tin having similar electronic energies, while the $NaBH_4$ one involves a metal-metalloid reaction:

$$Hg^{2+} + 2BH_4^- \xrightarrow{HCl} Hg^0 + B_2H_6 + H_2 \quad \ldots\ldots\ldots (9)$$

The presence of $B_2H_6$ and $H_2$ also seem to reduce the mercury sensitivity, possibly by partially shifting the equilibrium of Equation (9) to the left.

Table 9 also shows that for the analysis of cadmium at 228.8 nm, the sensitivity of the AAS at Lab A was about 3.9 times that of Lab B. This was also previously shown in Table 7 as discussed in section 4.1.1.3. The former laboratory had a better detection limit, with a magnitude of 2.1 times. The sensitivities at Labs B and C were nearly the same. The same trend is observed for the determination of lead at 217 nm.

The values of sensitivity and detection limit obtained for the determination of cadmium and lead at Lab C, are very close to those obtained in the literature [38,78,94,95]. At 217 nm, the sensitivity and detection limit for lead were 3.2 and 1.6 times better than at 283.3 nm respectively.

### 4.1.1.5 DAY TO DAY REPRODUCIBILITY AND STABILITY OF SINGLE- ELEMENT CALIBRATION STANDARDS

#### 4.1.1.5.1 MERCURY

The results of mercury absorbance and the regression parameters, obtained by analysing the single-element mercury standards on different days and in different manners of preparation, using sodium borohydride and stannous chloride as reducing agents have been summarized in Table 10 and calibration curves shown in Figure 11. In most cases, the linear correlation of the curves was fairly high, as reflected by the high correlation coefficients. This shows fair obedience to Beer's Law.

TABLE 10 (a) RESULTS FOR MERCURY ABSORBANCE READINGS OF SINGLE-ELEMENT STANDARDS ON DIFFERENT DAYS[a]

| Set No. | Storage time (days) | Reducing agent | ABSORBANCE READINGS FOR: Mercury concentration (mg/l) 0.01 | 0.02 | 0.03 | 0.04 | 0.05 |
|---|---|---|---|---|---|---|---|
| 1. | 0 | | 0.121 | 0.276 | 0.413 | 0.546 | 0.652 |
| 2. | 10 | $NaBH_4$ | 0.123 | 0.306 | 0.449 | 0.641 | 0.828 |
| 3. | 35 | | 0.100 | 0.216 | 0.302 | 0.404 | 0.547 |
| 4. | 23* | | 0.171 | 0.350 | 0.491 | 0.630 | 0.825 |
| 5. | 44 | $SnCl_2$ | 0.166 | 0.343 | 0.510 | 0.665 | 0.849 |
| 6. | 48 | | 0.141 | 0.313 | 0.448 | 0.606 | 0.786 |
| 7. | 69 | | 0.152 | 0.344 | 0.468 | 0.628 | 0.820 |
| 8.[b] | 0 | | 0.099 | 0.231 | 0.398 | 0.543 | 0.661 |
| 9.[c] | 4 | $NaBH_4$ | 0.062 | 0.136 | 0.225 | 0.344 | 0.444 |
| 10.[d] | 4 | | 0.003 | 0.014 | 0.029 | 0.118 | 0.176 |

TABLE 10 (b). RESULTS FOR REGRESSION ANALYSIS PARAMETERS FOR MERCURY CALIBRATION CURVES ON DIFFERENT DAYS[a]

| Set No. | Storage time (days) | Reducing agent | Regression equation | Correlation coefficient |
|---|---|---|---|---|
| 1. | 0 | $NaBH_4$ | y=13.32x+0.002 | 0.9978348 |
| 2. | 10 | | y=17.45x-0.0541 | 0.9989398 |
| 3. | 35 | | y=10.82x-0.0108 | 0.9963455 |
| 4. | 23* | $SnCl_2$ | y=15.88x+0.017 | 0.9981122 |
| 5. | 44 | | y=16.88x+0.0002 | 0.9996864 |
| 6. | 48 | | y=15.83x-0.0161 | 0.9989841 |
| 7. | 69 | | y=16.20x-0.0036 | 0.9976096 |
| 8.[b] | 0 | $NaBH_4$ | y=14.36x-0.0444 | 0.9985445 |
| 9.[c] | 4 | | y= 9.72x-0.0494 | 0.9965547 |
| 10.[d] | 4 | | y= 4.50x-0.067 | 0.9418844 |

TABLE 10 (contd.)

[a] Standards were made from 1000 mg/l stock solution and contained 7 ml of 3:1:1 (vol/vol) ($HNO_3$,$HClO_4$,$H_2SO_4$) acid mixture per 100 ml solution, unless indicated otherwise

[b] Standards containing 0.007-0.035 ml acid mixture volume in increasing order

[c] Standards made from 50 mg/l stock solution

[d] Standards made from 50 mg/l stock solution and contained no acid mixture

* values for days 0-22 not obtained with $SnCl_2$

x = mercury concentration (mg/l), ranging from 0.01 to 0.05 mg/l

y = Absorbance, with Varian Spectr AA10 AAS attached to Cold Vapour VGA-76 kit

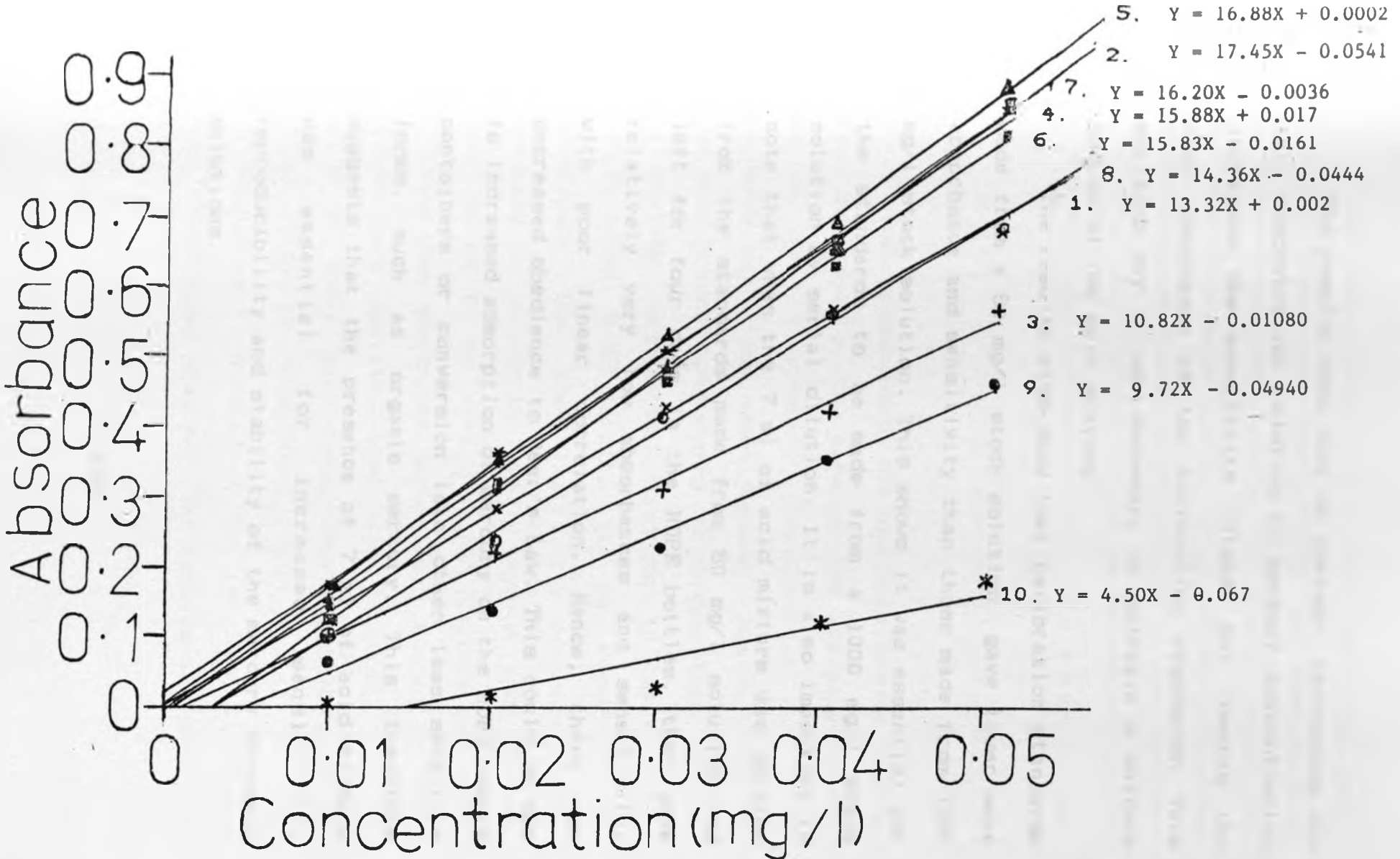



Figure 11. [redacted]ury calib[redacted]ion curves

The results show that on average, increasing the acid concentration relative to mercury concentration increased the sensitivity (slope) but lowered the mean absorbance of the calibration standards. This explains why it was necessary to maintain a uniform volume of the acid mixture.

The results also show that calibration standards made from a 50 mg/l stock solution gave lower mean absorbance and sensitivity than those made from 1000 mg/l stock solution. This shows it was essential for the standards to be made from a 1000 mg/l stock solution by serial dilution. It is also important to note that when the 7 ml of acid mixture was omitted from the standards made from 50 mg/l solution and left for four days in the HDPE bottles, they gave relatively very low absorbances and sensitivity, with poor linear correlation. Hence, there was decreased obedience to Beer's Law. This could be due to increased adsorption of mercury on the HDPE sample containers or conversion into other less sensitive forms, such as organic mercury. This therefore suggests that the presence of 7 ml of acid mixture was essential for increased sensitivity, reproducibility and stability of the mercury standard solutions.

The data also clearly shows that the $SnCl_2$ reducing method gave more stable and reproducible standard absorbances than the $NaBH_4$, with the possible explanation that $SnCl_2$ method involves metal-metal reaction, while the $NaBH_4$ one involves metal-metalloid reaction and hence reduced efficiency in transfer of electrons, especially after a long time.

4.1.1.5.2 CADMIUM

Most researchers and analysts prefer making fresh standards on the same day of analysis of the samples due to possible deterioration of the standards with time [58]. However, due to lack of time and proper consistency in preparation of the calibration standards, leading to carry-over interferences and errors, it is essential to investigate the possibility of preparing the standards in a certain matrix medium and storing them, so as to be used for AAS Calibration when required. The standards, which were stored in HDPE bottles were analysed from time to time, over a period of about six weeks. Two digestion matrix media: I(3:1:1 $HNO_3$,$HClO_4$,$H_2SO_4$) and II(3:1 $HNO_3$,$HClO_4$) were compared in the two Labs A and B, using Varian model AA10 AAS. The results are shown in

Table 11 and the calibration curves obtained on different days for the two media are shown on Figure 12. These show that for a period of about one month (Regression equations 1-4), the absorbance sensitivity did not vary appreciably.

The data shows that presence of sulphuric acid does not seem to reduce the solubility and hence the sensitivity of the absorbance signal for cadmium. This could be attributed to the fact that the nitrate and sulphate of cadmium are both soluble in water [97].

TABLE 11 (a). ABSORBANCE READINGS FOR CADMIUM STANDARDS AT 228.8 nm ON DIFFERENT DAYS

| Set No. | LAB | Matrix medium | Storage time (days) | ABSORBANCE READING FOR: cadmium concentration (mg/l) 0.05 | 0.10 | 0.50 | 1.00 | 1.50 |
|---|---|---|---|---|---|---|---|---|
| 1. | | I | 9 | --- | 0.035 | 0.182 | 0.355 | --- |
| 2. | A | I | 23 | --- | 0.037 | 0.198 | 0.377 | --- |
| 3. | | I | 31 | --- | 0.037 | 0.189 | 0.367 | --- |
| 4. | | II | 3 | 0.023 | 0.037 | 0.218 | 0.406 | 0.578 |
| 5. | B | I | 35 | --- | 0.005 | 0.027 | 0.055 | --- |
| 6. | | II | 44 | 0.005 | --- | 0.052 | 0.099 | 0.143 |

--- = Not determined

TABLE 11 (b). REGRESSION ANALYSIS PARAMETERS OBTAINED FOR THE CALIBRATION CURVES OF CADMIUM ON DIFFERENT DAYS, AT 228.8 nm[a]

| Set No. | LAB | Storage time (days) | Matrix medium | Regression equation | Correlation coefficient |
|---|---|---|---|---|---|
| 1. | | 9 | I | y=0.35516x+0.00125 | 0.9998523 |
| 2. | A | 23 | I | y=0.37697x+0.00295 | 0.9994387 |
| 3. | | 31 | I | y=0.36623x+0.00234 | 0.9998269 |
| 4. | | 3 | II | y=0.38730x+0.00840 | 0.9986619 |
| 5. | B | 35 | I | y=0.05557x-0.00064 | 0.9999870 |
| 6. | | 44 | II | y=0.09497x+0.00234 | 0.9992861 |

[a] For sets of standard solutions containing 0.05-1.5 mg/l of cadmium

x = cadmium concentration (mg/l)

y = Absorbance, with Flame Varian Spectr AA10 AAS

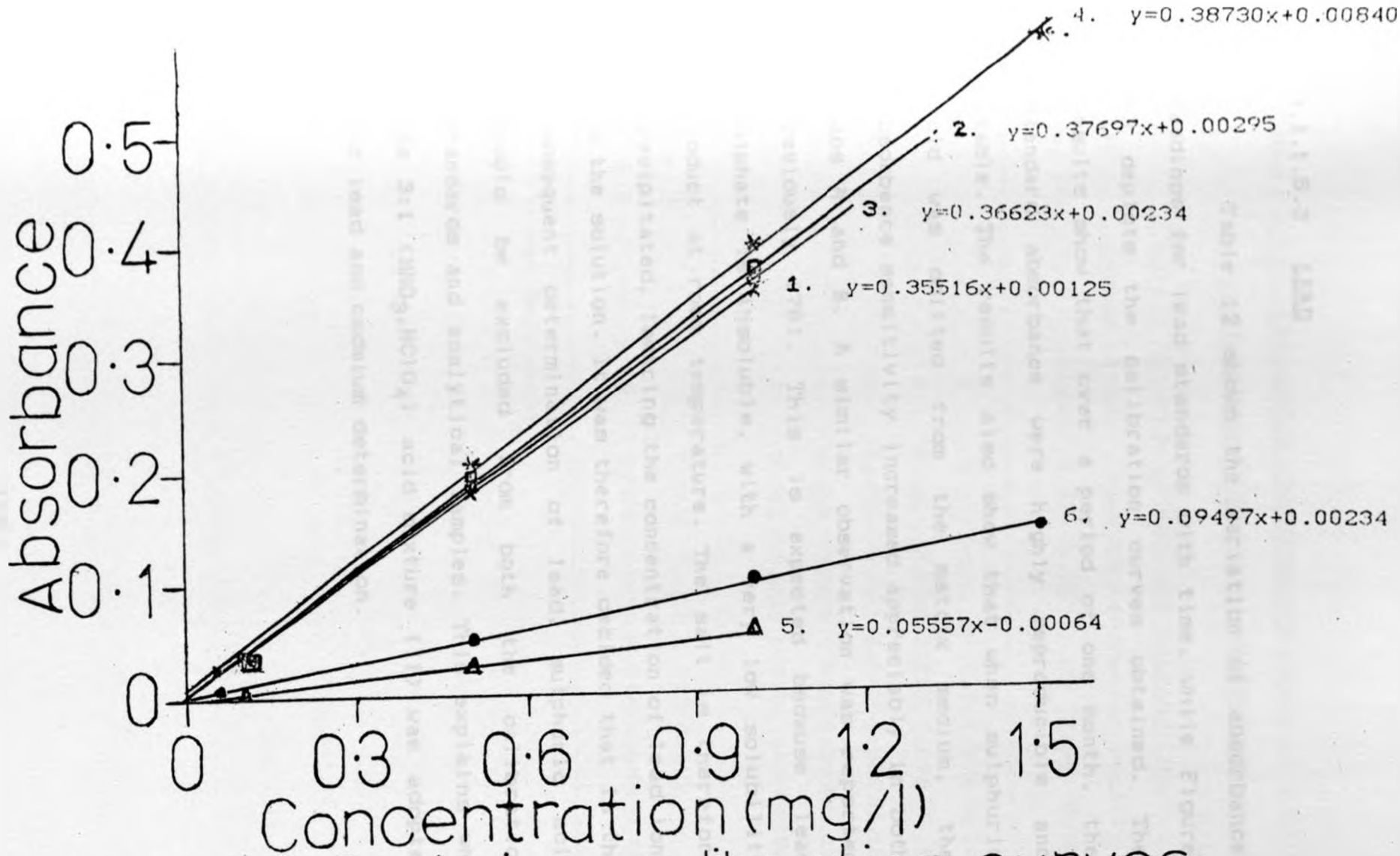



Figure 12·Cadmium calibration curves

#### 4.1.1.5.3 LEAD

Table 12 shows the variation of absorbance readings for lead standards with time, while Figure 13 depicts the calibration curves obtained. The results show that over a period of one month, the standard absorbance were highly reproducible and stable. The results also show that when sulphuric acid was omitted from the matrix medium, the absorbance sensitivity increased appreciably in both Labs A and B. A similar observation was reported previously [78]. This is expected because lead sulphate is insoluble, with a very low solubility product at room temperature. The salt is therefore precipitated, lowering the concentration of lead ions in the solution. It was therefore decided that in the subsequent determination of lead, sulphuric acid should be excluded from both the calibration standards and analytical samples. This explains why the 3:1 ($HNO_3$,$HClO_4$) acid mixture (II) was adopted for lead and cadmium determination.

TABLE 12 (a). ABSORBANCE READINGS FOR LEAD STANDARDS AT 217.0 nm ON DIFFERENT DAYS

| Set No. | LAB | Matrix medium | Storage time (days) | ABSORBANCE READING FOR: lead concentration (mg/l) 0.25 | 0.50 | 1.00 | 2.50 | 5.00 |
|---|---|---|---|---|---|---|---|---|
| 1. | | I | 3 | --- | --- | 0.050 | 0.094 | 0.178 |
| 2. | | I | 6 | 0.012 | 0.025 | 0.047 | 0.095 | 0.194 |
| 3. | A | I | 23 | 0.009 | 0.021 | 0.043 | 0.089 | 0.173 |
| 4. | | I | 31 | 0.010 | 0.021 | 0.042 | 0.092 | 0.179 |
| 5. | | II | 3 | 0.012 | 0.023 | 0.045 | --- | 0.208 |
| 6. | B | I | 35 | 0.003 | 0.005 | 0.010 | 0.022 | --- |
| 7. | | II | 40 | 0.006 | --- | 0.018 | 0.045 | 0.088 |

--- = Not determined

TABLE 12 (b). REGRESSION ANALYSIS PARAMETERS OBTAINED FOR THE CALIBRATION CURVES OF LEAD ON DIFFERENT DAYS, AT 217.0 nm.[a]

| Set No. | LAB | Storage time (days) | Matrix medium | Regression equation | Correlation coefficient |
|---|---|---|---|---|---|
| 1. | | 3 | I | y=0.03216x+0.01620 | 0.9993822 |
| 2. | | 6 | I | y=0.03754x+0.00516 | 0.9990026 |
| 3. | A | 23 | I | y=0.03388x+0.00432 | 0.9989309 |
| 4. | | 31 | I | y=0.03518x+0.00371 | 0.9995683 |
| 5. | | 3 | II | y=0.04091x+0.00481 | 0.9993488 |
| 6. | B | 35 | I | y=0.00845x+0.00102 | 0.9991496 |
| 7. | | 40 | II | y=0.01686x+0.00157 | 0.9998000 |

[a] For sets of standard solutions containing 0.5-10 mg/l of lead

x = lead concentration (mg/l)

y = Absorbance, with Flame Varian Spectr AA10 AAS

Matrices I and II represent the same matrices as those with corresponding numbers in Table 11

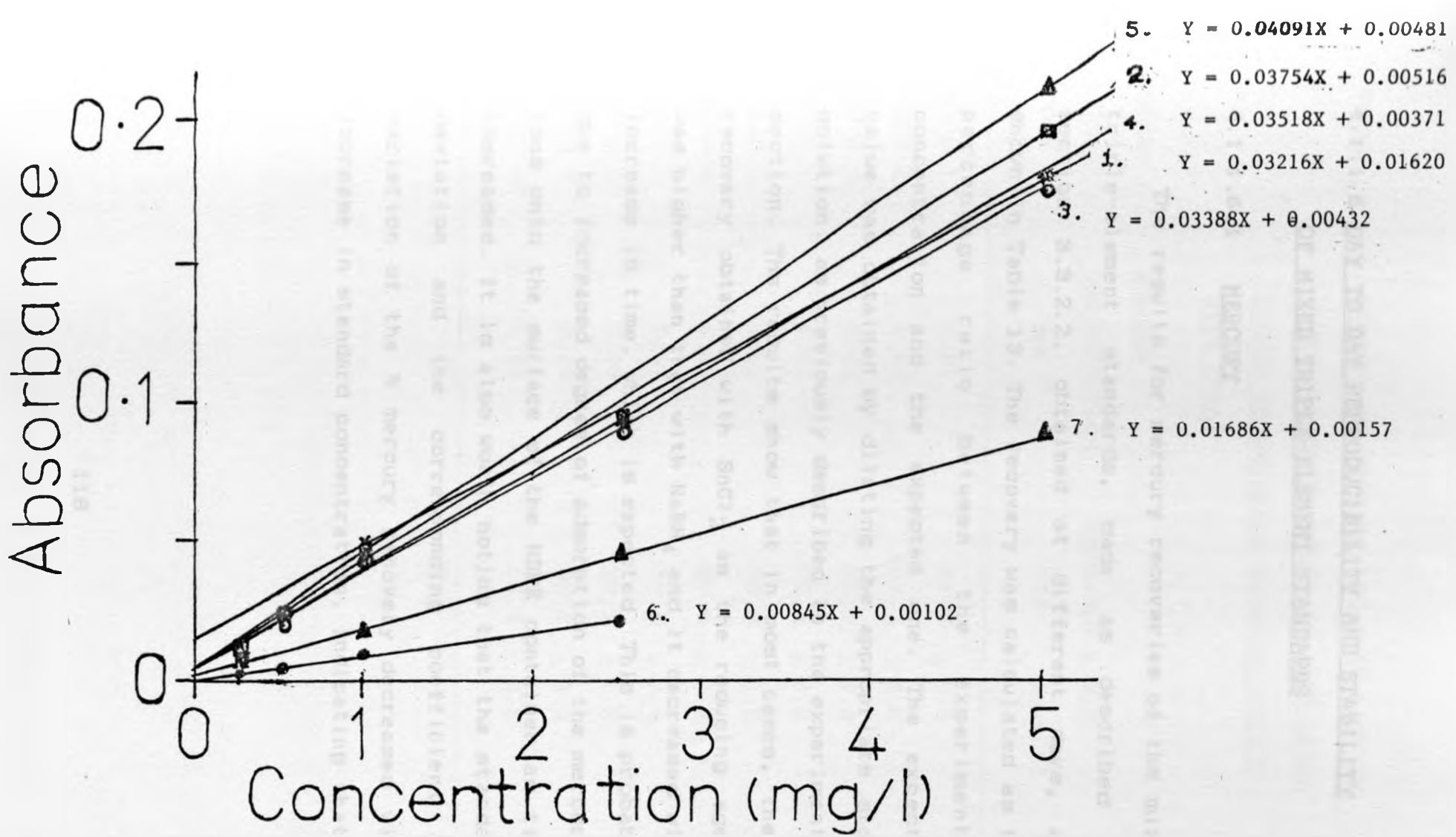



Figure 13. Lead calibration curves

### 4.1.1.6 DAY TO DAY REPRODUCIBILITY AND STABILITY OF MIXED TRIPLE-ELEMENT STANDARDS

#### 4.1.1.6.1 MERCURY

The results for mercury recoveries of the mixed triple-element standards, made as described in section 3.3.2.2, obtained at different days, are shown in Table 13. The recovery was calculated as the percentage ratio between the experimental concentration and the expected one. The expected value was obtained by diluting the appropriate stock solution, as previously described in the experimental section. The results show that in most cases, the % recovery obtained with $SnCl_2$ as the reducing agent was higher than that with $NaBH_4$ and it decreased with increase in time, which is expected. This is probably due to increased degree of adsorption of the mercuric ions onto the surface of the HDPE container as time increases. It is also worth noting that the standard deviation and the corresponding coefficient of variation of the % mercury recovery decreased with increase in standard concentration, indicating that

TABLE 13 (a). MERCURY RECOVERIES AND STABILITY OF MIXED STANDARDS AFTER 10 DAYS USING SODIUM BOROHYDRIDE REDUCING METHOD WITH CVAAS

| MIXED STANDARD CODE | MERCURY CONCENTRATION (mg/l) | RECOVERY ± S.D. (%) (n = 3) |
|---|---|---|
| MSTD 1 | 0.01 | 61.8 ± 0.4 |
| MSTD 2 | 0.01 | 101.1 ± 0.6 |
| MSTD 3 | 0.02 | 85.9 ± 0.3 |
| MSTD 4 | 0.02 | 95.4 ± 0.2 |
| MSTD 5 | 0.05 | 88.2 ± 0.2 |
| MSTD 6 | 0.05 | 89.6 ± 0.2 |
| MSTD 7 | 0.07 | 89.3 ± 0.4 |
| MSTD 8 | 0.07 | 88.1 ± 0.2 |
| MSTD 9 | 0.09 | 85.3 ± 0.3 |
| MSTD 10 | 0.09 | 85.5 ± 0.2 |

TABLE 13 (b) MERCURY RECOVERIES OF MIXED STANDARDS AT DIFFERENT DAYS USING STANNOUS CHLORIDE REDUCING METHOD WITH CVAAS

| MIXED STANDARD CODE | CONCENTRATION (mg/l) | RECOVERY (%) AT DAY: 13 | 48 | 69 | Mean ± SD[a] (%) (n = 3) | CV (%)[b] |
|---|---|---|---|---|---|---|
| MSTD 1 | 0.01 | 76.0 | --- | --- | | |
| MSTD 2 | 0.01 | 99.8 | 73.4 | 54.0 | 75.7 ± 23.0 | 30.4 |
| MSTD 3 | 0.02 | 85.5 | --- | --- | | |
| MSTD 4 | 0.02 | 96.2 | 84.4 | 76.1 | 85.6 ± 10.1 | 11.8 |
| MSTD 5 | 0.05 | 92.2 | --- | --- | | |
| MSTD 6 | 0.05 | 91.6 | 85.6 | 83.5 | 86.9 ± 4.2 | 4.8 |
| MSTD 7 | 0.07 | 90.9 | --- | --- | | |
| MSTD 8 | 0.07 | 92.0 | 89.1 | 86.1 | 89.1 ± 3.0 | 3.3 |
| MSTD 9 | 0.09 | 87.7 | --- | --- | | |
| MSTD 10 | 0.09 | 87.3 | 84.2 | 79.0 | 83.5 ± 4.2 | 5.0 |

For the concentration of the three elements in the mixed standards, see Table 1

n = number of sample replicates

--- = Not determined

[a] SD = Standard Deviation of % recovery

[b] CV (%) = Coefficient of variation of the mean recovery (stability measure) = $\frac{SD * 100\%}{\text{mean recovery}}$

the more concentrated solutions were relatively more stable. This could be due to the relatively high rate of adsorption on the HDPE containers and analytical errors in pipetting the solutions and AAS signal measurement at low concentrations. The results show that the presence of the other two elements of interest i.e. cadmium and lead does not seem to affect the mercury concentration significantly and that the presence of the 7 ml acid mixture was essential for solution stability.

The low recovery at low concentration may also be attributed to detector response. At low concentrations close to the detection limit, the detector is not able to distinguish the real signal from the random noise and therefore may yield erroneous results. On the other hand, the low recovery at high concentrations may be due to factors that lead to deviation from Beer's Law at high concentration, such as non-atomic absorption by molecular species.

4.1.1.6.2 CADMIUM

Due to insufficient recovery data for cadmium with time, no generalized conclusions can be made. However, a decrease in recovery with ageing of the solution, similar to the other elements was observed.

Though not complete, the data available showed that the cadmium recoveries were high and therefore the presence of the other two elements (mercury and lead) did not seem to affect the recovery of cadmium in its analysis.

#### 4.1.1.6.3 LEAD

The lead recovery data for mixed triple-element standards is shown in Table 14. Within the period investigated (3-49 days), the results show that lead recoveries were high and therefore the presence of the other two elements (mercury and cadmium) did not affect the recovery of lead in its analysis. Recoveries greater than 100% were probably due to analytical and statistical errors. It should also be noted that, as stated earlier, lead is relatively abundant in the environment, especially in urban areas, due to the combustion of tetra ethyl lead in gasoline [34]. It is therefore possible that lead was present in the laboratory environment, thus registering higher levels than those originally dissolved in the standard solutions.

TABLE 14 (a) LEAD RECOVERIES FOR MIXED STANDARDS AT DIFFERENT DAYS USING FLAME AAS

| MIXED STANDARD CODE | LEAD CONCENTRATION (mg/l) | LEAD RECOVERY (%) AT DAY: (± S.D., n=3) 3 | 6 | 49 |
|---|---|---|---|---|
| MSTD 1 | 0.25 | 63.6±0.8 | 100±10.8 | --- |
| MSTD 2 | 0.5 | 85.2±1.1 | 84±1.8 | --- |
| MSTD 3 | 0.5 | 103.4±0.4 | 112±19.0 | --- |
| MSTD 4 | 0.5 | 109 ±0.8 | 84±3.1 | --- |
| MSTD 5 | 1.0 | 116.6±0.1 | 90±1.5 | --- |
| MSTD 6 | 2.5 | 105.4±0.3 | 95±0.7 | 89.3±0.6 |
| MSTD 7 | 2.5 | 100.5±0.1 | 99±0.8 | --- |
| MSTD 8 | 5.0 | 89.3±0.1 | 93±0.5 | 77.7±0.1 |
| MSTD 9 | 2.5 | 102.8±0.4 | 96±0.9 | --- |

n = number of sample replicates

--- = Not determined

For the concentration of the three elements in the mixed standards, see Table 1

TABLE 14 (b). STATISTICAL ANALYSIS OF LEAD RECOVERIES FOR MIXED STANDARDS AT DIFFERENT DAYS

| Mixed Std. code | Actual Pb conc. (mg/l) | Storage time (days) | Mean ± SD[a] recovery (%) | CV (%)[b] |
|---|---|---|---|---|
| MSTD 1 | 0.25 | 3-6 | 81.8 ± 25.7 | 31.5 |
| MSTD 2 | 0.50 | 3-6 | 84.6 ± 0.8 | 0.9 |
| MSTD 3 | 0.50 | 3-6 | 107.7 ± 6.1 | 5.6 |
| MSTD 4 | 0.50 | 3-6 | 96.5 ± 17.7 | 18.3 |
| MSTD 5 | 1.00 | 3-6 | 103.3 ± 18.8 | 18.2 |
| MSTD 6 | 2.50 | 3-49 | 96.5 ± 8.2 | 8.5 |
| MSTD 7 | 2.50 | 3-6 | 99.5 ± 1.3 | 1.3 |
| MSTD 8 | 5.0 | 3-49 | 86.7 ± 8.0 | 9.2 |
| MSTD 9 | 2.5 | 3-6 | 99.2 ± 5.1 | 5.2 |

TABLE 14 (contd.)

[a] SD = Standard Deviation of mean % recovery

[b] CV (%) = Coefficient of variation of the mean recovery (stability measure) = $\frac{SD * 100\%}{\text{mean recovery}}$

For the concentration of the three elements in the mixed standards, see Table 1

### 4.1.2 CALIBRATION STANDARD SOLUTIONS- X-RAY FLUORESCENCE ANALYSIS (XRFA)

#### 4.1.2.1 OPTIMUM pH FOR ELEMENT ANALYSIS

In the determination of metals in liquid samples using ED-XRF, the pH of the sample solution has to be taken into account. This is because at very low pH, the solution is very stable and extraction with a complexing agent is not highly efficient. On the other hand, at very high pH, the heavy metals are precipitated (e.g. as insoluble hydroxides) before extraction can be done. The optimum pH depends on the type of complexing agent and the element being analysed. For example, it was found [66] that using ammonium-1- pyrrolidine-dithio-carbamate (APDC), a pH value of 4.0 gave the highest recovery for copper. It is generally reported that the optimum pH for most heavy metals when using sodium diethyl dithio carbamate (NaDDTC) is in the range 5-6 [100]. Because of this varying range of pH dependence, it was found necessary to first investigate the most appropriate pH for the present work, particularly the optimum pH for mercury and lead, when NaDDTC was used as the chelating agent. Table 15 shows the recovery of mercury and lead in different standard combinations

at different pH. Figures 14 to 17 show the curves after fitting the data.

TABLE 15. XRF % RECOVERY OF MERCURY AND LEAD AT DIFFERENT pH

| | SINGLE ELEMENT STANDARDS[a] | MIXED STANDARDS | | | |
|---|---|---|---|---|---|
| | | Double-element standards[b] | | Triple-element standards[c] | |
| Solution pH | %MERCURY | %MERCURY | %LEAD | %MERCURY | %LEAD |
| 1.0 | --- | --- | --- | 52.1 | 83.1 |
| 1.5 | --- | 35 | 84 | --- | --- |
| 1.6 | 92.8 | --- | --- | --- | --- |
| 2.0 | --- | 55 | 86 | --- | --- |
| 2.5 | --- | --- | --- | 41.4 | 82.5 |
| 3.0 | 87.2 | 11 | 73.5 | --- | --- |
| 3.4 | --- | --- | --- | 27.0 | --- |
| 4.0 | --- | 7.5 | 50 | --- | 81.3 |
| 4.05 | 91.3 | --- | --- | --- | --- |
| 5.2 | 91.4 | 16 | 35 | --- | --- |
| 5.3 | --- | --- | --- | 49.1 | 81.6 |
| 6.2 | --- | 23 | 40.3 | --- | --- |
| 6.3 | --- | --- | --- | 41.0 | --- |
| 7.0 | --- | 12 | 39.8 | 4.3 | 63.5 |
| 7.2 | 84.8 | --- | --- | --- | --- |

a Standards containing 10 ppm mercury
b Standards containing 0.1 ppm mercury, 2 ppm lead
c Standards containing 0.1 ppm mercury, 1 ppm cadmium, 2 ppm lead
--- indicates that recovery was not determined at these pH's, but only at the specific values shown

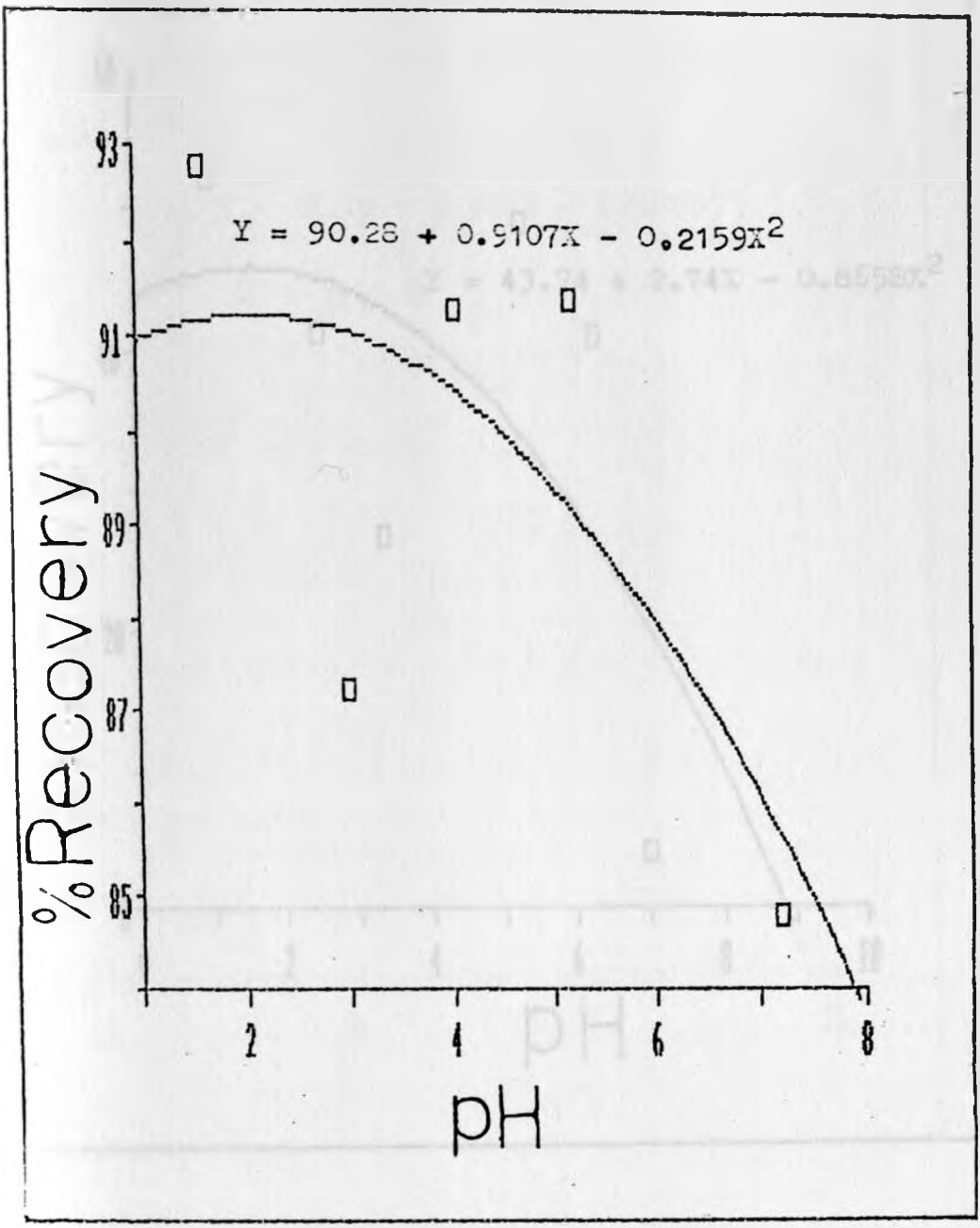



Figure 14: XRF Mercury recovery at various pH

(10 ppm Hg standard)

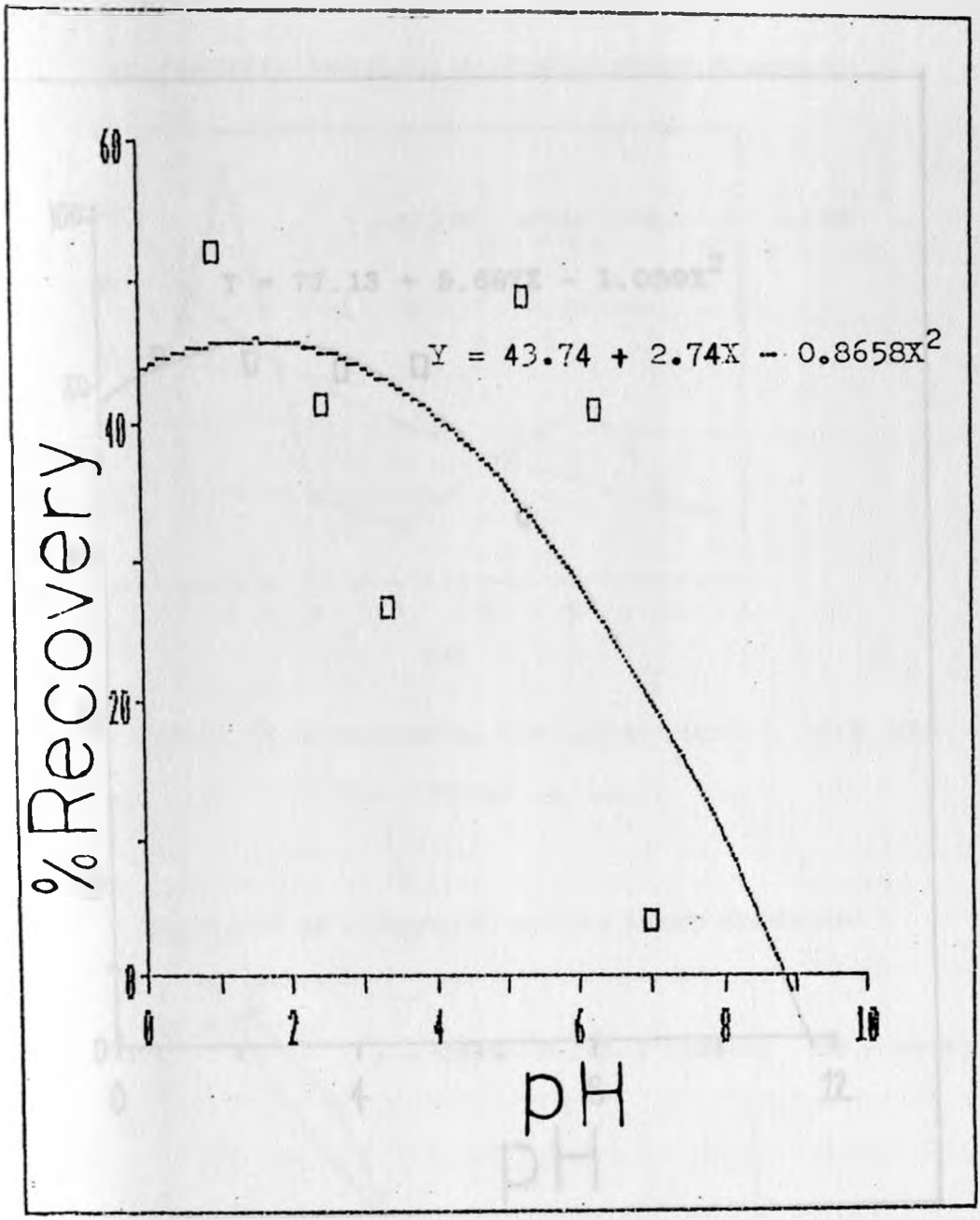



Figure 15: XRF Mercury recovery at various pH
(Triple-element standard)

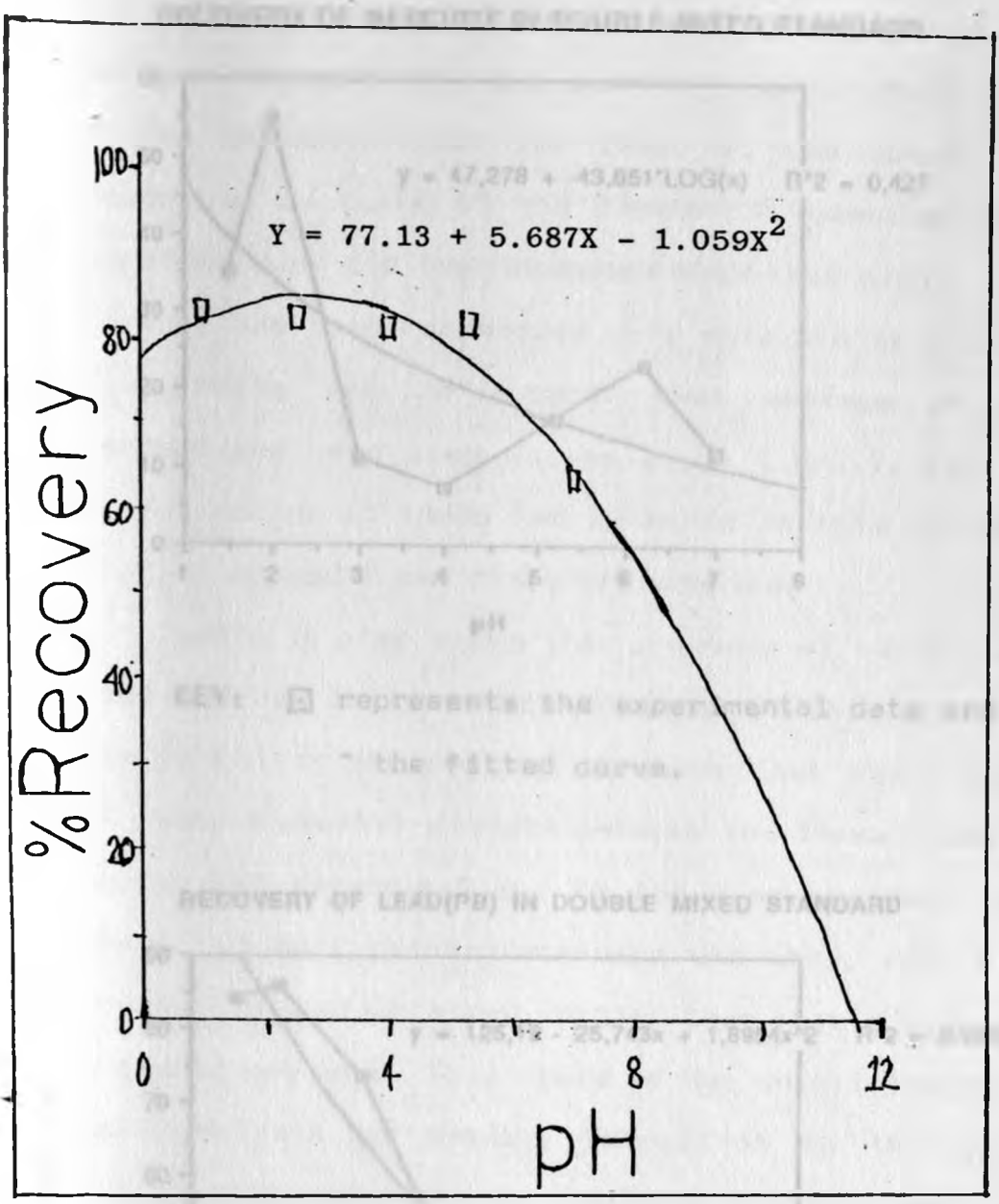
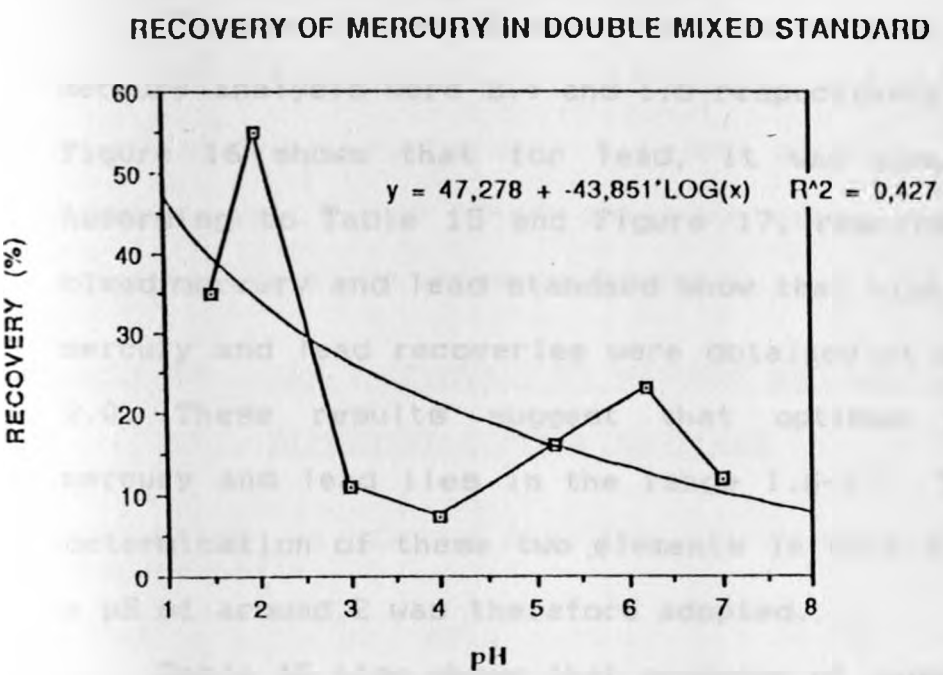



Figure 16: XRF Lead recovery at various pH
(Triple-element standard)




KEY: ⊡ represents the experimental data and

~ the fitted curve.

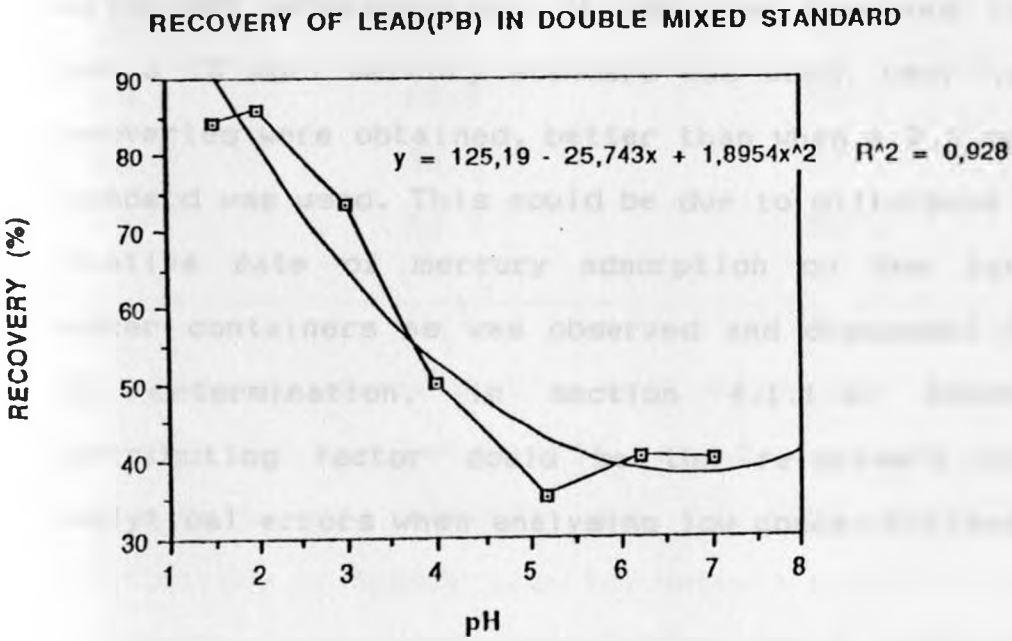



Figure 17: XRF curves for mercury and lead recovery at various pH

Figures 14 and 15 show that the optimum pH for mercury analysis were 2.1 and 1.6 respectively, while Figure 16 shows that for lead, it was about 2.7. According to Table 15 and Figure 17, results for a mixed mercury and lead standard show that high mercury and lead recoveries were obtained at a pH of 2.0. These results suggest that optimum pH for mercury and lead lies in the range 1.6-2.7. For the determination of these two elements in this project, a pH of around 2 was therefore adopted.

Table 15 also shows that presence of cadmium and lead lowered the recovery of mercury. This could be due to matrix effects and it shows that there could be inter-elemental effects between the three elements during XRF determination. It was also observed that when a 10 mg/l mercury standard was used, very high recoveries were obtained, better than when a 0.1 mg/l standard was used. This could be due to difference in relative rate of mercury adsorption on the pyrex beaker containers as was observed and discussed for AAS determination, in section 4.1.1.6. Another contributing factor could be the relatively high analytical errors when analysing low concentrations.

### 4.1.2.2 OPTIMUM COMPLEXING TIME

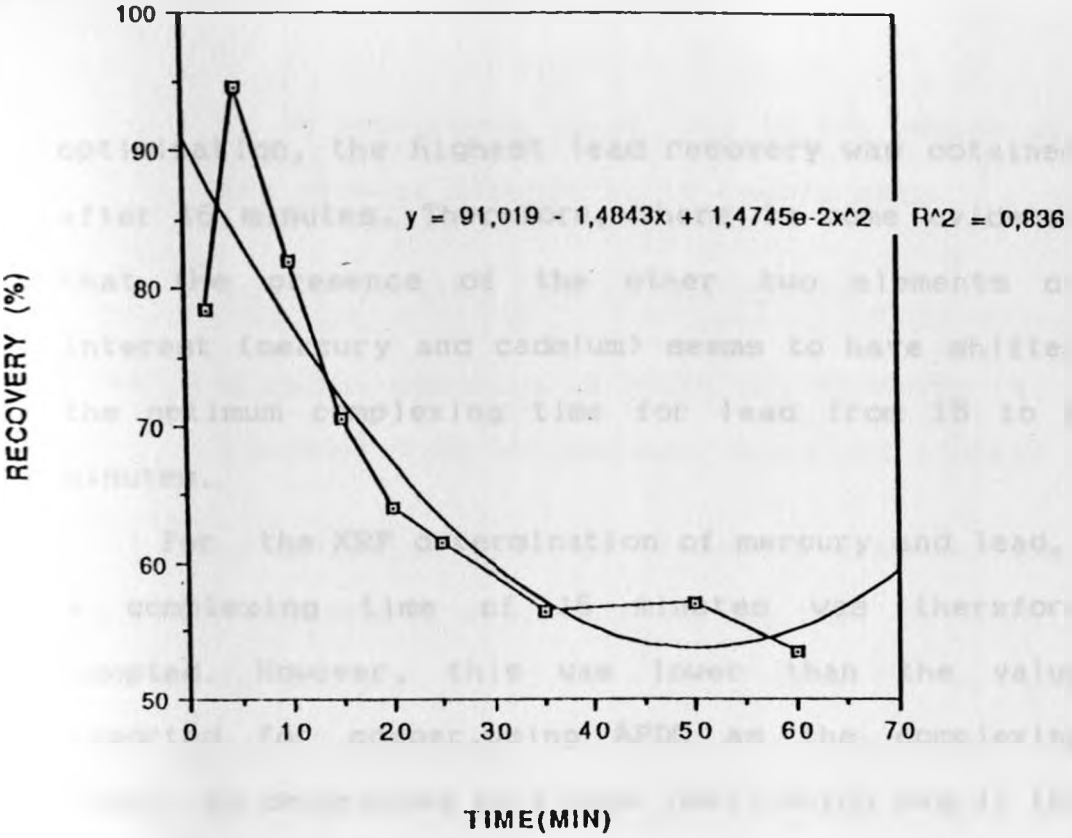
The extraction results of mercury and lead in a mixed standard containing 0.1 ppm mercury, 1 ppm cadmium and 2 ppm lead obtained after different reaction times are shown in Table 16.

TABLE 16. XRF % RECOVERY OF MERCURY AND LEAD AT DIFFERENT COMPLEXING TIMES

| COMPLEXING TIME (mins) | % MERCURY RECOVERY | % LEAD RECOVERY |
|---|---|---|
| 2 | 42.1 | 78.5 |
| 5 | 46.5 | 94.5 |
| 10 | 60.5 | 82 |
| 15 | 70 | 70.5 |
| 20 | 60.7 | 64 |
| 25 | 59.0 | 61.5 |
| 35 | 58.9 | 56.5 |
| 50 | 58.1 | 57 |
| 60 | 54.7 | 53.5 |

The data shows that in most cases, the mercury recovery was far below 100%, relative to that of lead. This was possibly due to high chelating inefficiency of NaDDTC with low mercury concentration (0.1 ppm). It is likely that other lighter ions were

preferentially chelated and so most of the mercury ions were filtered off in the solution. The results also show that the highest recoveries for mercury and lead were obtained after complexing times of 15 and 5 minutes respectively. These results are shown graphically in Figure 18. From these results, the optimum complexing times for these two elements seem to be slightly different. It should be noted, however that the standard used was a triple-element mixed standard. According to Munyithya [101], when a single-element standard containing lead, was used for




KEY: ⊡ represents the experimental data and

~ the fitted curve.

**XRF COMPLEXING TIME FOR MERCURY**

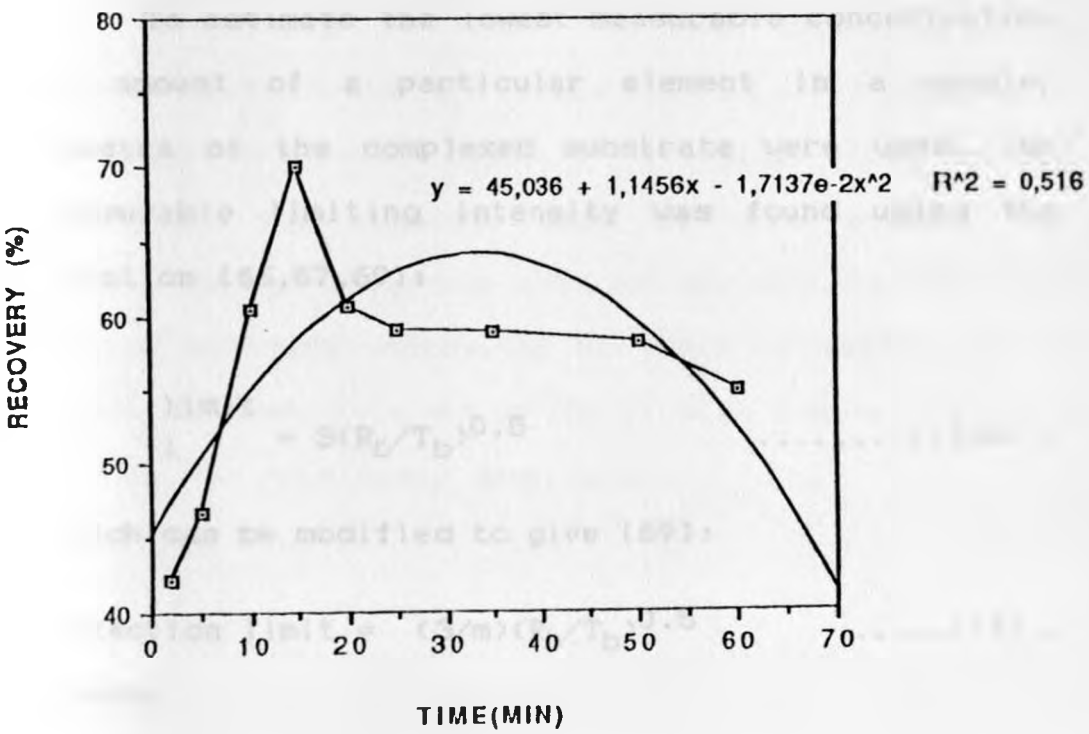



Figure 18: XRF complexing time.

optimization, the highest lead recovery was obtained after 15 minutes. Therefore, there is some evidence that the presence of the other two elements of interest (mercury and cadmium) seems to have shifted the optimum complexing time for lead from 15 to 5 minutes.

For the XRF determination of mercury and lead, a complexing time of 15 minutes was therefore adopted. However, this was lower than the value reported for copper,using APDC as the complexing agent, as determined by Kinyua [66], which was in the range of 30-35 minutes, with single-element standard.

#### 4.1.2.3 DETERMINATION OF DETECTION LIMITS

To estimate the lowest measurable concentration or amount of a particular element in a sample, spectra of the complexed substrate were used. The measurable limiting intensity was found using the relation [66,67,69]:

$$I_i^{limit} = 3(R_b/T_b)^{0.5} \quad \text{.....(10)},$$

which can be modified to give [69]:

$$\text{Detection limit} = (3/m)(R_b/T_b)^{0.5} \quad \text{.....(11)},$$

where:

$R_b$ is background count rate in the region of the peak of element i, in counts/sec.

$T_b$ is counting time on background.

m is the gradient of graph of intensity vs. concentration or absolute amount of element i.

The detection limit of element i with XRF could also be defined in a similar way to that for AAS, as given in section 4.1.1.4, with intensity replacing the absorbance signal. In order to determine the detection limits of mercury and lead with XRF, both graphical and formula methods have been used. The elements were extracted at a pH of about 2, with NaDDTC.

Table 17 shows the XRF intensities and background counts for different absolute masses of mercury and lead, using a collection time of 500 seconds. The absolute mass of each element was obtained by measuring the appropriate volume of a mixed standard containing 0.1 mg/l of mercury and 2 mg/l of lead, diluted to 100 ml with double-distilled water, as previously described.

TABLE 17. XRF DATA USED TO CALCULATE DETECTION LIMITS OF MERCURY AND LEAD

| ELEMENT | MASS OF ELEMENT (micrograms) | INTENSITY (cts/sec) | BACKGROUND (counts) | DETECTION LIMIT (micrograms)[a] Formula | Graph |
|---|---|---|---|---|---|
| MERCURY | 2 | 0.12 | 618 | | |
| | 4 | 0.29 | 840 | | |
| | 5 | 0.48 | 928 | 1.558 | 1.033 |
| | 6 | 0.60 | 954 | ± 0.174 | |
| | 10 | 1.02 | 1158 | | |
| LEAD | 40 | 4.28 | 1198 | | |
| | 80 | 7.52 | 1351 | | |
| | 100 | 9.2 | 1673 | 2.118 | 12.701 |
| | 120 | 8.73 | 1397 | ± 0.147 | |
| | 200 | 21.6 | 1639 | | |

[a] Values obtained by formula and graphical methods respectively

In order to obtain the graphical detection limits for mercury and lead, as given in Table 17, graphs of intensity against the absolute masses of each element on the millipore filters were plotted. These are shown in Figures 19 and 20. The detection limit was determined as the mass of the element when its intensity approached zero, as given by the regression equation i.e. the mass (or concentration) intercept in the intensity-mass (or concentration) graph. Table 17 shows that the detection limit for mercury obtained by the formula method was about 1.5 times that obtained by the graphical method, while for lead, the graphical value was about 6 times that obtained by the formula method. These variations could be attributed to analytical and statistical errors in reading the intensity, the uncertainty in the calibration limits of the masses of each element and possibly, contamination. As in AAS, one would expect the linearity to be lost beyond a certain value, where the linear correlation will be poorer. However, the values obtained by the formula method seem to be more reliable than the graphical ones, because on average, the former were closer to the values given in the literature, such as 1 ppb in 500 ml solution [66].

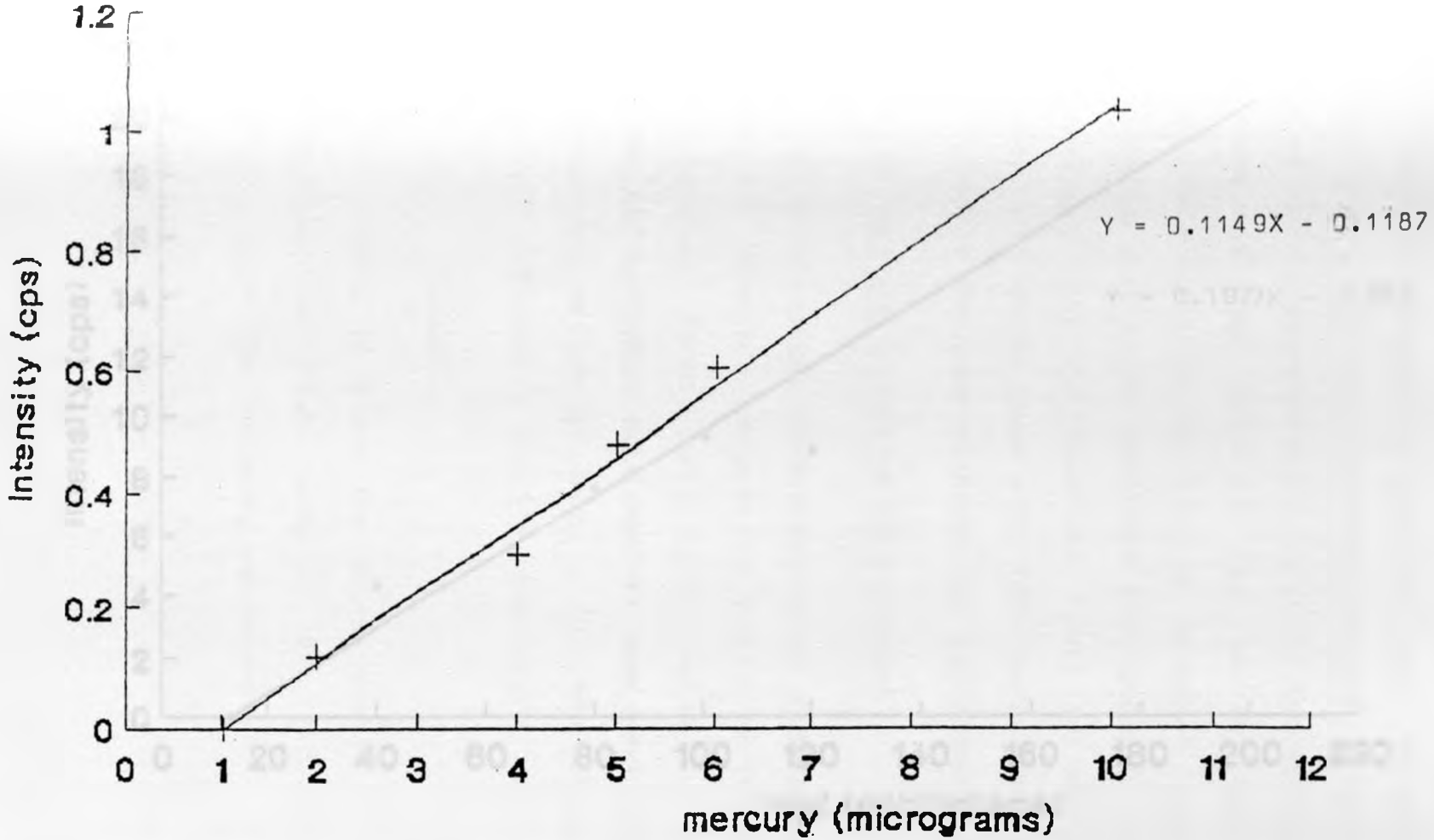



Figure 19. XRF mercury intensity vs. absolute amount of mercury on millipore filter

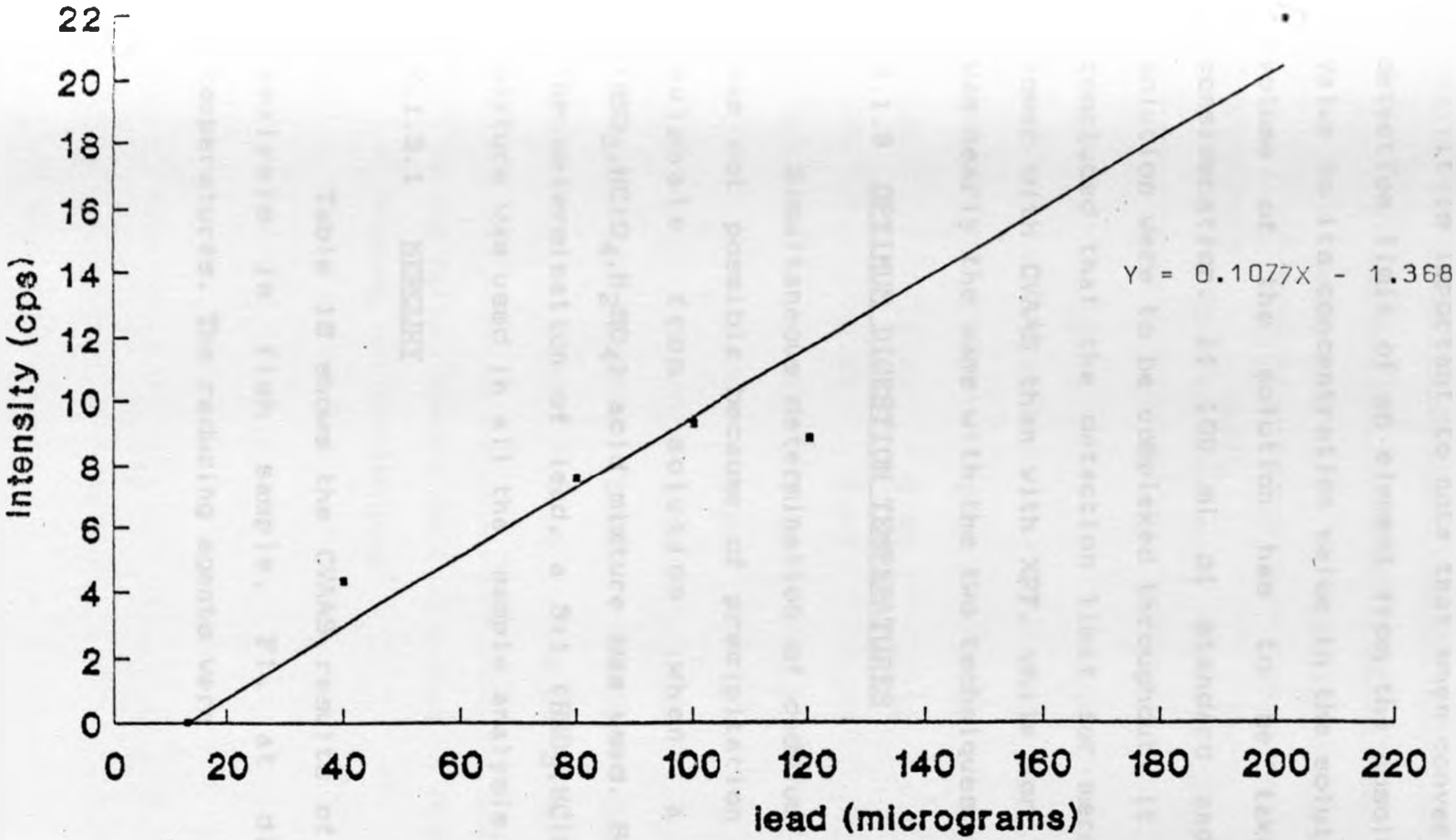



Figure 20. XRF lead intensity vs. absolute amount of lead on millipore filter

It is important to note that when converting the detection limit of an element from the absolute mass value to its concentration value in the solution, the volume of the solution has to be taken into consideration. If 100 ml of standard and sample solution were to be complexed throughout, it could be concluded that the detection limit for mercury was lower with CVAAS than with XRF, while for lead, it was nearly the same with the two techniques.

### 4.1.3 OPTIMUM DIGESTION TEMPERATURES

Simultaneous determination of cadmium and lead was not possible because of precipitation of lead sulphate from solution when a 3:1:1 ($HNO_3$,$HClO_4$,$H_2SO_4$) acid mixture was used. Hence for the determination of lead, a 3:1 ($HNO_3$,$HClO_4$) acid mixture was used in all the sample analysis.

#### 4.1.3.1 MERCURY

Table 18 shows the CVAAS results of mercury analysis in fish sample, F1, at different temperatures. The reducing agents were

TABLE 18. RELATIVE MERCURY CONCENTRATION READINGS (ng/ml) AT DIFFERENT TEMPERATURES WITH $NaBH_4$ AND $SnCl_2$ AS REDUCING AGENTS[a]

| TEMPERATURE (°C) | $NaBH_4$ | | $SnCl_2$ | |
|---|---|---|---|---|
| | Covered | Uncovered | Covered | Uncovered |
| 47 | 4.13 | --- | --- | --- |
| 50 | 2.64 | --- | 2.49 | --- |
| 55 | 1.92 | --- | 2.03 | --- |
| 60 | 1.67 | 2.66 | 1.59 | 0.88 |
| 65 | 1.41 | 0.83 | 1.72 | 0.67 |
| 70 | 0.83 | 0.03 | 1.49 | 0.36 |
| 75 | 1.49 | 1.30 | 1.22 | 1.21 |
| 80 | 0.74 | 0.00 | 1.13 | 0.38 |
| 85 | 0.01 | 0.19 | 0.57 | 0.56 |
| 91 | 2.30 | 0.50 | 1.04 | 0.08 |
| 108 | 1.02 | 0.16 | 0.55 | 0.00 |

[a] using Varian Spectr AA10 attached to VGA-76

--- = reading not determined

sodium borohydride and stannous chloride, while the reaction vessel was both covered and uncovered. The redox mixture, potassium permanganate and hydroxyl-amine hydrochloride were sequentially added as previously described.

For both the $NaBH_4$ and $SnCl_2$ reducing methods, it can be observed in Table 18 that on average, there was decrease of mercury concentration reading with increasing digestion temperature. This was due to loss of mercury at higher temperatures. This was confirmed by the fact that, in most cases, the values obtained when the digestion vessel was covered were generally higher than those when the vessel was left open, as shown in Table 18.

The trends of the mercury concentration readings (sensitivity) with change in digestion temperature for the data given in Table 18 is shown in Figures 21 to 25. The negative correlation of mercury reading with digestion temperature indicates that although some researchers use a temperature of 70°C [8,50], lower temperatures gave higher recoveries and so within the investigated temperature range, a temperature range of 50 $\pm$ 3°C seemed to give the best results. Therefore, a temperature of around 50°C was used in the digestion of all the samples.

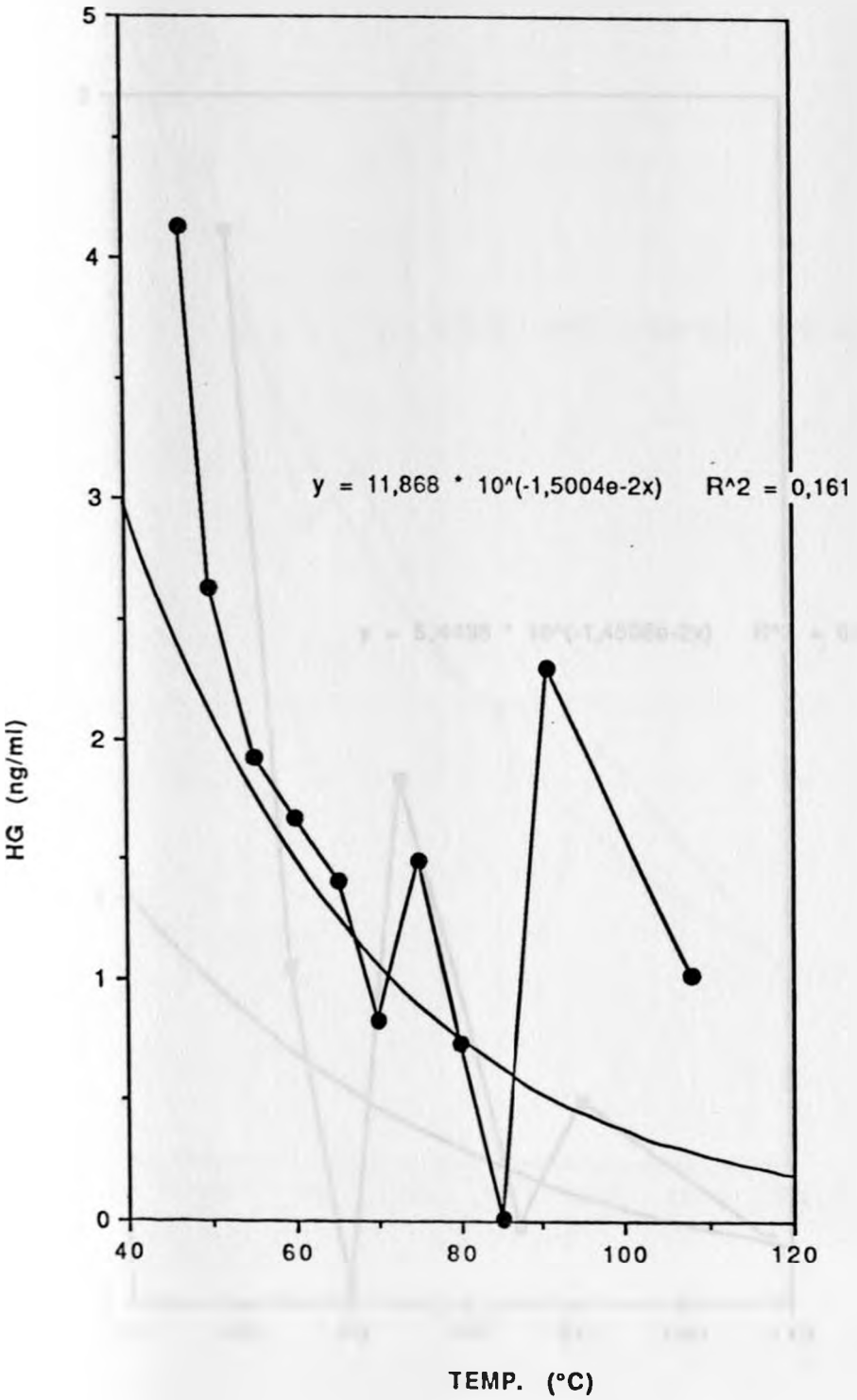



FIGURE 21. MERCURY SENSITIVITY WITH NaBH4 (COVERED)

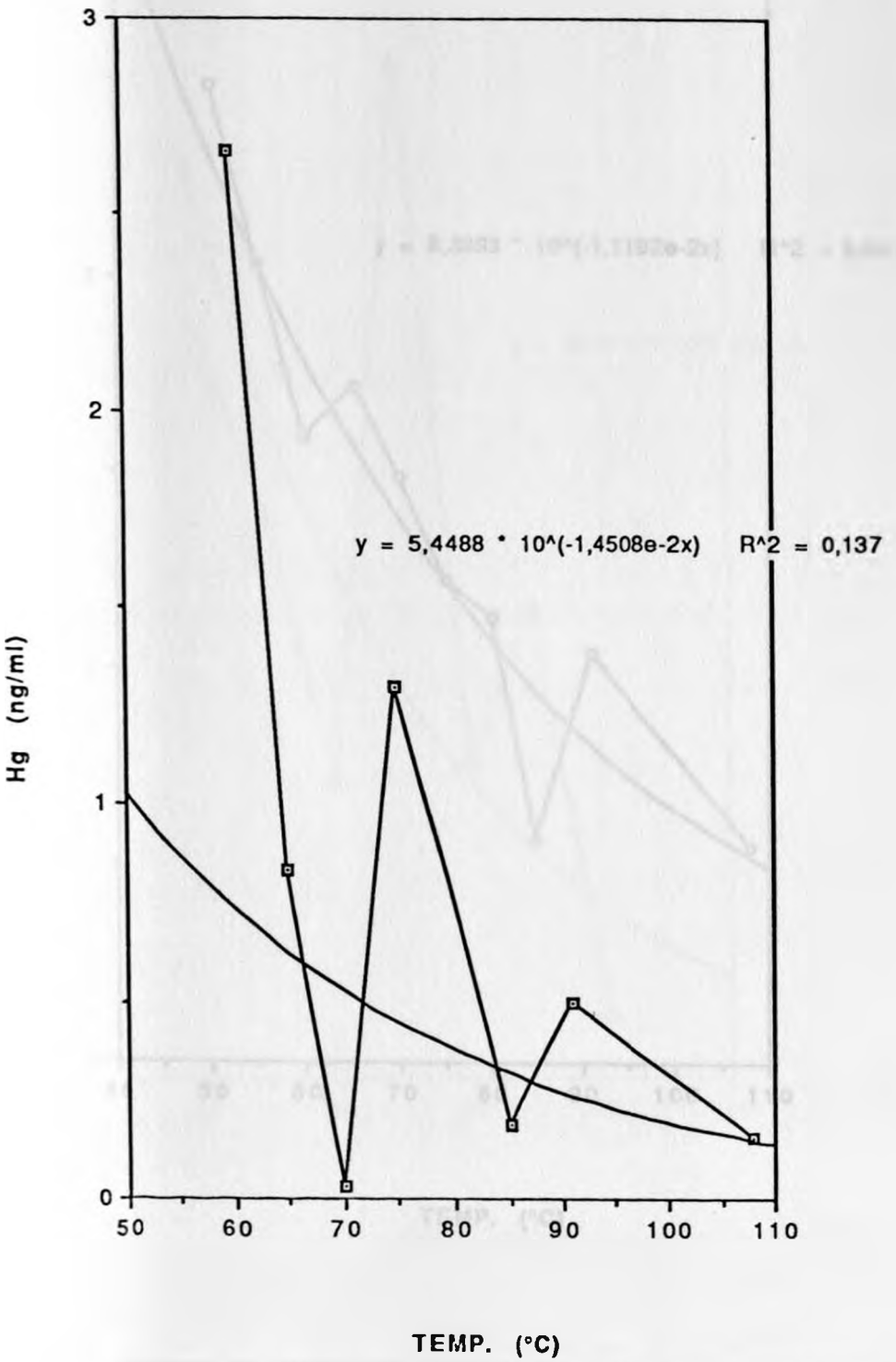



FIGURE 22. MERCURY SENSITIVITY WITH NaBH4 (UNCOVERED)

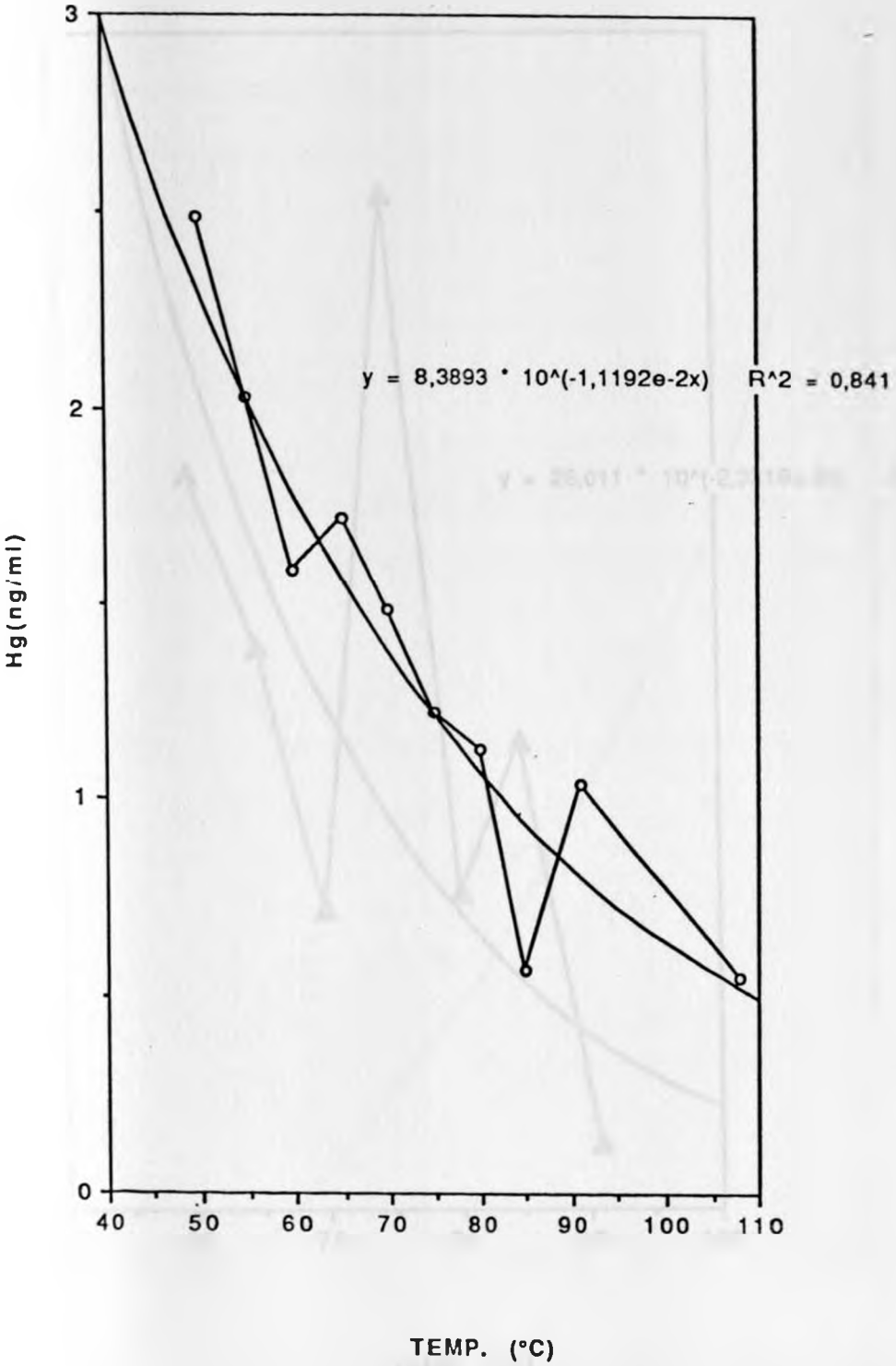



FIGURE 23. MERCURY SENSITIVITY WITH SnCl2(COVERED)

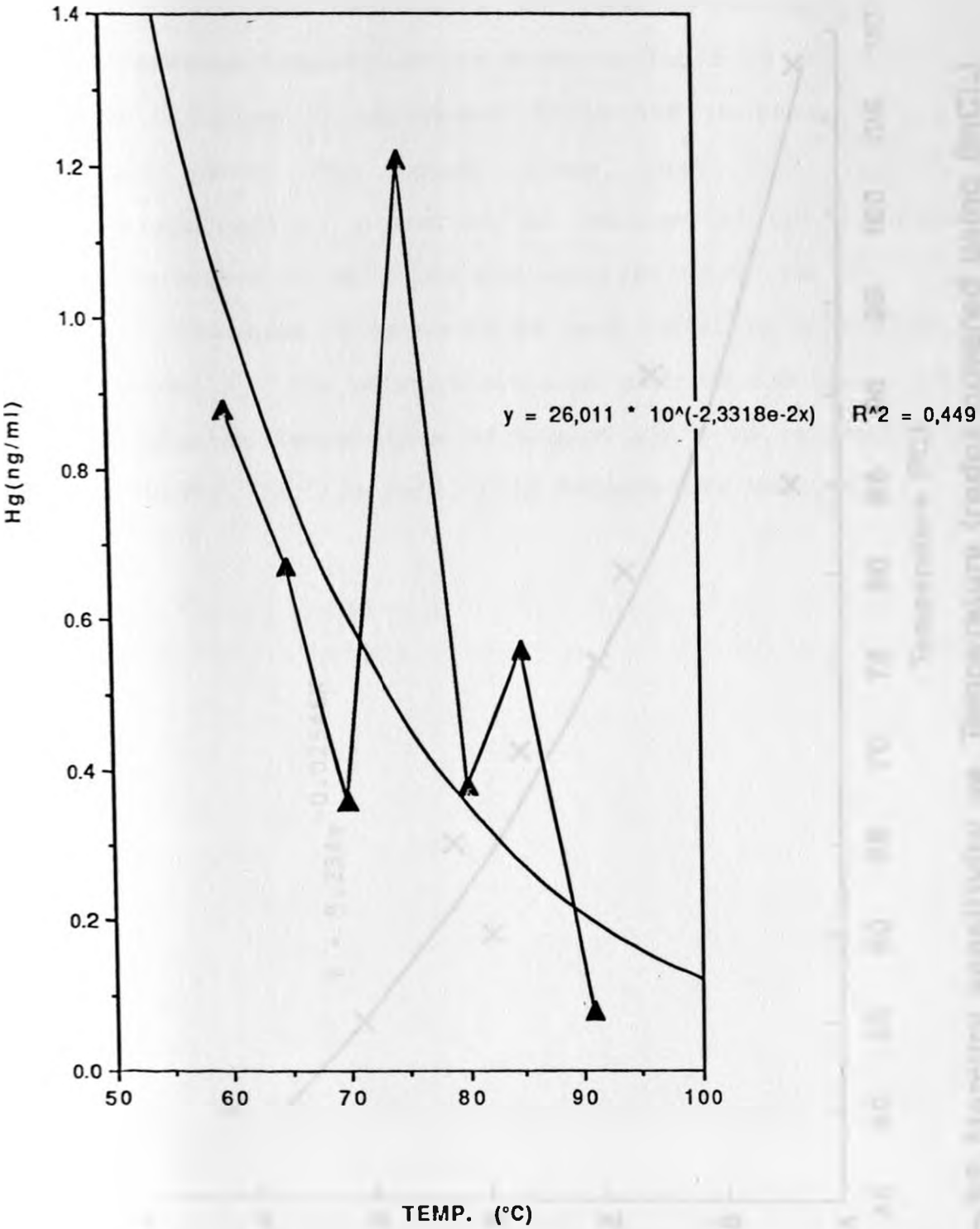



FIGURE 24. MERCURY SENSITIVITY WITH SnCl2(UNCOVERED)

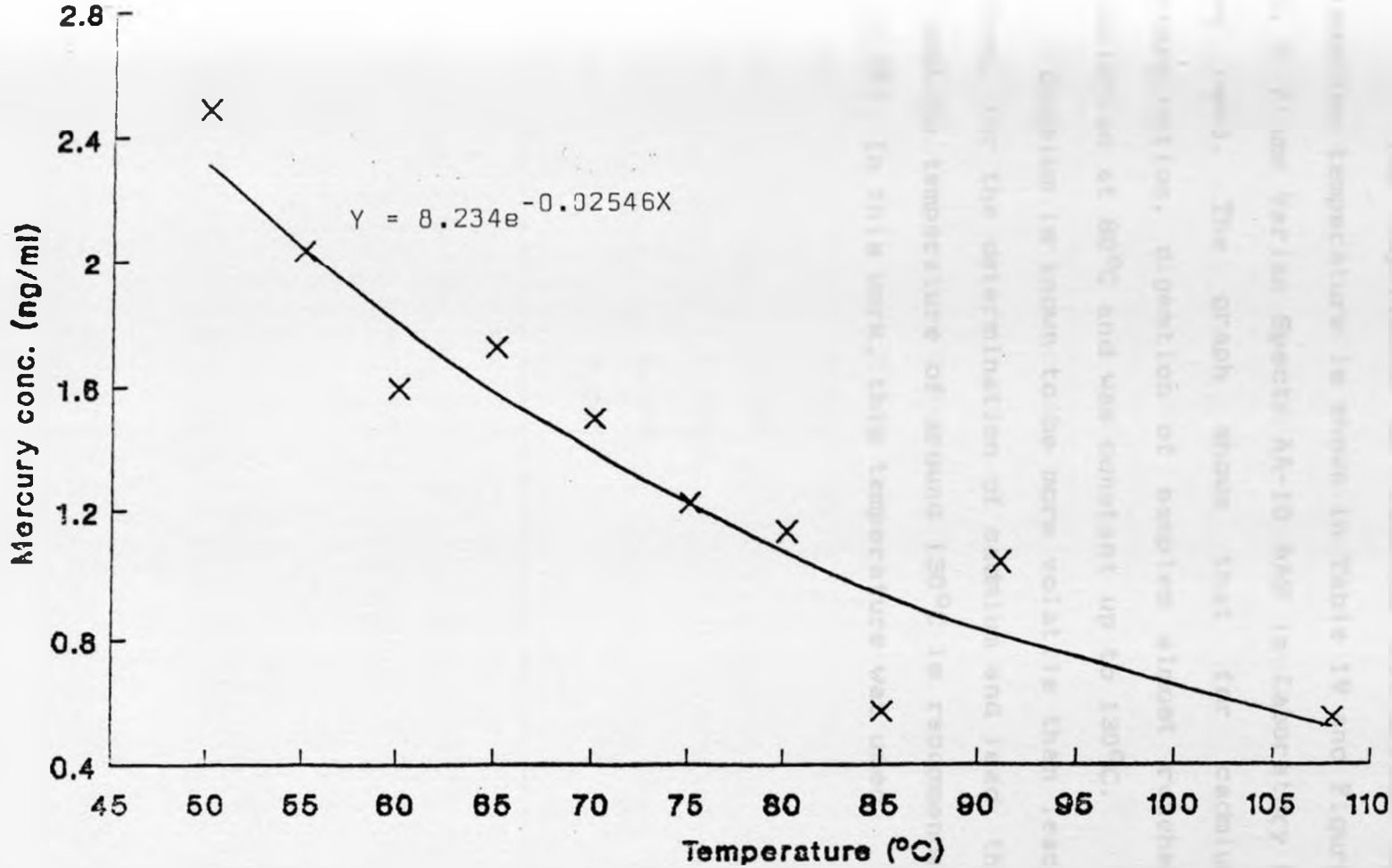



Figure 25. Mercury sensitivity vs. Temperature (redox covered using $SnCl_2$)

#### 4.1.3.2 CADMIUM

The dependence of cadmium extraction on digestion temperature is shown in Table 19 and Figure 26. A flame Varian Spectr AA-10 AAS in Laboratory A was used. The graph shows that for cadmium determination, digestion of samples almost reached completion at 80$^{o}$C and was constant up to 130$^{o}$C.

Cadmium is known to be more volatile than lead. Hence, for the determination of cadmium and lead, the digestion temperature of around 130$^{o}$C is recommended [38,78]. In this work, this temperature was used.

TABLE 19. DEPENDENCE OF CADMIUM CONCENTRATION SENSITIVITY ON TEMPERATURE

| Temperature (°C) | Relative concentration instrumental response (mg/l) |
|---|---|
| 50 | 0.000 |
| 55 | 0.002 |
| 60 | 0.009 |
| 65 | 0.015 |
| 70.5 | 0.044 |
| 75 | 0.049 |
| 80 | 0.060 |
| 85 | 0.060 |
| 91 | 0.060 |
| 108 | 0.060 |
| 130 | 0.060 |

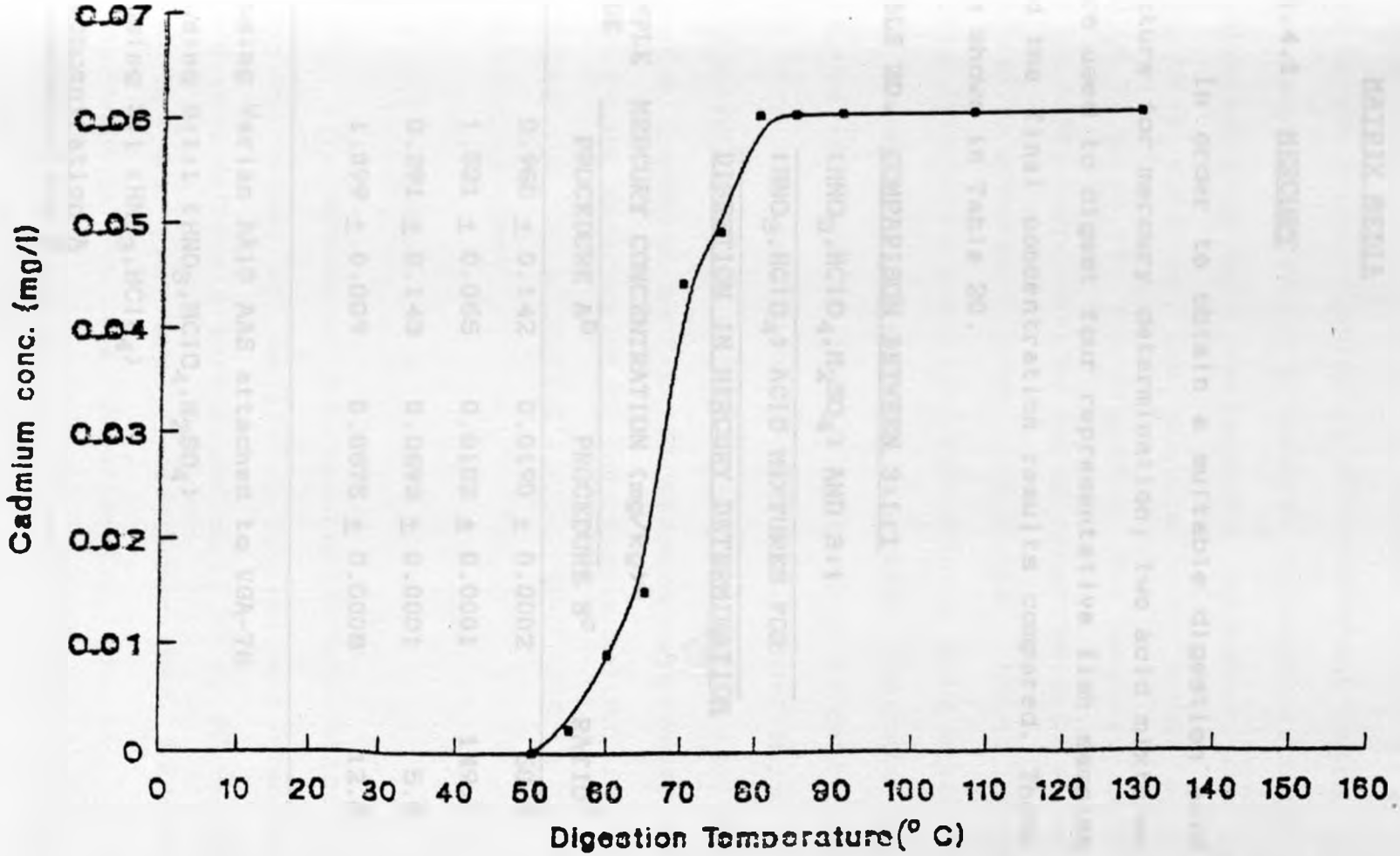



**Figure 26.** Cadmium Concentration Sensitivity at various Temperatures

### 4.1.4 RESULTS FOR COMPARISON OF DIFFERENT DIGESTION MATRIX MEDIA

#### 4.1.4.1 MERCURY

In order to obtain a suitable digestion acid mixture for mercury determination, two acid mixtures were used to digest four representative fish samples and the final concentration results compared. These are shown in Table 20.

TABLE 20. COMPARISON BETWEEN 3:1:1 ($HNO_3$,$HClO_4$,$H_2SO_4$) AND 3:1 ($HNO_3$,$HClO_4$) ACID MIXTURES FOR DIGESTION IN MERCURY DETERMINATION

| SAMPLE CODE | MERCURY CONCENTRATION (mg/kg)[a] | | |
|---|---|---|---|
| | PROCEDURE A[b] | PROCEDURE B[c] | RATIO[d] |
| F1 | 0.960 ± 0.142 | 0.0190 ± 0.0002 | 50.5 |
| F2 | 1.521 ± 0.065 | 0.0102 ± 0.0001 | 149.1 |
| F3 | 0.391 ± 0.143 | 0.0693 ± 0.0001 | 5.6 |
| F4 | 1.099 ± 0.009 | 0.0875 ± 0.0008 | 12.6 |

[a] using Varian AA10 AAS attached to VGA-76

[b] using 3:1:1 ($HNO_3$,$HClO_4$,$H_2SO_4$)

[c] using 3:1 ($HNO_3$,$HClO_4$)

[d] $\frac{\text{concentration A}}{\text{concentration B}}$

The results show that procedure A gave far much higher mercury concentrations than procedure B, with the former method displaying an enhancement factor in the range 5.6-149.1. A general literature survey indicated that most researchers recommend use of sulphuric acid or its mixture with other acids [8,50,77,80-83,99] for digesting the samples. Landi et al. [99] found that by using sulphuric acid alone, better accuracy was obtained than when nitric/sulphuric and nitric/perchloric acid mixtures were used. The literature therefore supports the results in Table 20 and it is clearly evident that for the determination of mercury, especially in biological samples, sulphuric acid had to be included in the digestion matrix. It plays a very important role in the destruction of the organic matter, mainly because it is a strong ionic acid and a good dehydrating agent.

#### 4.1.4.2 CADMIUM

To determine the cadmium concentration in samples, a set of analytical samples and standard reference materials were digested at 70 or 85 $^{o}C$ and treated as previously described for mercury determination in the experimental section. This utilized a reduction/oxidation medium ($KMnO_4$ followed

by $NH_2OH.HCl$). The same set of samples were digested at around 130-150 $^oC$ without the redox mixture and treated for cadmium and lead determination. Table 21 shows the cadmium concentration results obtained with the two procedures.

TABLE 21. RESULTS FOR CADMIUM CONCENTRATIONS IN PRESENCE AND ABSENCE OF REDOX MIXTURE. AT 228.8 nm

| SAMPLE CODE | EXPECTED VALUE (mg/kg) | WITH REDOX MIXTURE (mg/kg) (x) | WITHOUT REDOX MIXTURE (mg/kg) (y) |
|---|---|---|---|
| F1 | | 0.588±0.060 | 0.381±0.068 |
| F2 | | 0.612±0.006 | 0.126±0.018 |
| F3 | | 0.777±0.083 | 0.295±0.005 |
| F4 | | 1.862±0.061 | 0.562±0.005 |
| F5 | | 0.881±0.276 | 0.496±0.035 |
| MA-A-2(TM) | 0.066 ± 0.004 | N.D.[a] | 0.073±0.006 |
| H8 | 189 ± 4.5 | 148.586±3.460 | 141.805±0.425 |
| MA-M-2/TM | 1.32 ± 0.22 | 1.245±0.022 | 1.342±0.043 |

TABLE 21 (Contd.)

Regression equation: y = 0.956646x - 0.3463244

Correlation cefficient = 0.9999647

[a] Not detected (below limit of detection)

The linear correlation obtained was high. The above results show that for nearly all the samples, the values obtained when the redox mixture was used were higher than those obtained when it was absent. This could be due to the fact that the former were digested at the same temperature as that used for mercury determination which, according to Figure 26, showed that loss of cadmium by volatilization had not yet been attained. On the other hand, for the latter sample results (without redox mixture), there was a possibility of cadmium getting lost by volatilization since higher digestion temperatures of up to 150 $^{o}$C were sometimes reached. However, both digestion procedures were reliable because there was no major difference between the values obtained for the IAEA standard reference materials, whose results will be discussed later, in section 4.1.6. It can therefore be concluded that the presence of the redox medium, especially the manganese ion does not seem to affect

the AAS absorbance for cadmium (at 228.8 nm) significantly.

#### 4.1.4.3 LEAD

The comparison results for lead were obtained in the same way as those for cadmium. Table 22 gives the lead concentration data with and without the redox mixture ($KMnO_4$ and $NH_2OH.HCl$).

TABLE 22. RESULTS FOR LEAD CONCENTRATIONS IN PRESENCE AND ABSENCE OF REDOX MIXTURE, AT 217 nm

| SAMPLE CODE | EXPECTED VALUE (mg/kg) | WITH REDOX MIXTURE (mg/kg) (x) | WITHOUT REDOX MIXTURE (mg/kg) (y) | RATIO x/y |
|---|---|---|---|---|
| F1 | | 17.027± 3.086 | 2.091±0.068 | 8.1 |
| F2 | | 14.348± 1.220 | 2.545±0.709 | 5.6 |
| F3 | | 12.824± 5.612 | 2.788±0.318 | 4.6 |
| F4 | | 21.831± 1.397 | 5.111±0.557 | 4.3 |
| F5 | | 25.277± 1.036 | 3.861±0.319 | 6.5 |
| MA-A-2(TM) | 0.58±0.07 | 93.733±24.741 | 0.594±0.064 | 157.8 |
| H8 | N.A.[a] | 62.243± 3.597 | N.D.[b] | 20.7 |
| MA-M-2/TM | 1.92±0.58[c] | 18.677± 1.868 | 3.393±0.240 | 5.5 |

[a] Data not available
[b] Not detected (below limit of detection i.e. < 3mg/kg) with Varian Spectr AA-10 AAS
[c] Non-certified value for IAEA reference material

From Table 22, the lead level values for the redox treated samples were higher than those obtained without the redox mixture, with the former showing a relative enhancement factor of at least four times. By comparing these two values with the mean values given for the IAEA standard reference materials, it is observed that the latter set of values (without redox mixture) were within the expected range, while the former were too high to be reliable. It can therefore be pointed that presence of the redox mixture consisting of $KMnO_4$ and $NH_2OH.HCl$ in the digestion matrix could not allow for the accurate determination of lead with flame AAS and so it had to be omitted in subsequent sample analysis for this element.

The interferences observed in AAS determination of elements is reported in a wide range of Analytical Chemistry text books, journals and manuals. For example, it is reported that large excesses of other elements may interfere with the signal and that 10,000 mg/l of iron enhances the lead signal [95]. In the present work, the $KMnO_4$ used (6% wt./vol.) contained 7,424 mg/l of potassium and 10,424 mg/l of manganese ions. Therefore, excesses of potassium and manganese enhanced the lead signal by a factor in the

range 4-8, if the upper two extreme ratios are neglected.

It is also reported [52,53] that there are often more resonance emission lines from a flame than can be accounted for on the basis of temperature alone, particularly when an organic solvent is present. It has been shown that this enhancement of emission in part is the result of fluorescence brought about by Ultra-violet radiation produced in the flame itself. The enhancement may also be related to a transfer of electrons from stable molecular orbitals of a compound (an oxide for example, such as manganese oxides) to an excited atomic orbital, when the compound is pyrolyzed. The excited electron then drops to its ground state with emission. This can be considered a case of chemiluminescence [52,53]. In this project, oxides or hydroxides of manganese might have formed, giving molecular band spectrum instead of a line spectrum, thus constituting spectral line interference.

### 4.1.5 COMPARISON BETWEEN SODIUM BOROHYDRIDE AND STANNOUS CHLORIDE AS REDUCING AGENTS IN COLD VAPOUR ATOMIC ABSORPTION SPECTROPHOTOMETRY

In order to compare the mercury concentration data between the two reducing agents, sodium

borohydride and stannous chloride in Cold Vapour Atomic Absorption Spectrophotometry, three sets of samples were used. The first set (A) consisted mainly of standard reference materials, which had been digested and left overnight in the oxidizing medium of $KMnO_4$ before digestion was continued the following day. The reagent blank was treated in the same way and the reading subtracted from the samples. The second set (B) was composed of other (digested) liquid samples, whose readings were to be obtained directly without blank correction, while the third set (C) consisted of the representative experimental fish sample, F1, digested in different ways under different conditions such as with no redox mixture, covered; with redox mixture, covered and uncovered and at different temperatures. The digestion and oxidation of the sub-samples was completed the same day (a major deviation from set A). In the third set, the concentration readings were corrected for the reagent blanks. Table 23 (a) displays the actual concentration readings for the three sets, with the two reducing agents, while Table 23 (b) shows the regression analysis of the data.

TABLE 23 (a) COMPARISON OF MERCURY CONCENTRATION READINGS FOR DIFFERENT SAMPLE SOLUTIONS WITH SODIUM BOROHYDRIDE AND STANNOUS CHLORIDE AS REDUCING AGENTS USING CVAAS (Concentration in ng/ml)

| SET A[a] | | | SET B[a] | | |
|---|---|---|---|---|---|
| Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ | Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ |
| 1. | 19.395 | 19.98 | 1. | 2.690 | 2.507 |
| 2. | 17.14 | 18.61 | 2. | 4.39 | 3.545 |
| 3. | 15.88 | 16.98 | 3. | 4.54 | 3.777 |
| 4. | 17.72 | 16.84 | 4. | 3.06 | 3.255 |
| 5. | 16.27 | 12.80 | 5. | 3.00 | 3.60 |
| 6. | 1.37 | 1.14 | 6. | 1.89 | 3.09 |
| 7. | 17.89 | 16.57 | 7. | 1.845 | 3.78 |
| 8. | 14.23 | 12.61 | 8. | 0.54 | 2.91 |
| 9. | 1.70 | 1.58 | 9. | 1.46 | 3.80 |
| 10. | 17.86 | 17.65 | 10. | 0.647 | 3.255 |
| | | | 11. | 0.645 | 2.77 |
| | | | 12. | 1.465 | 4.77 |

TABLE 23 (a) (contd.)

SET B[a]

| Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ |
|---|---|---|
| 13. | N.D. | 2.34 |
| 14. | N.D. | 1.66 |
| 15. | N.D. | 1.57 |
| 16. | N.D. | 2.04 |
| 17. | N.D. | 2.27 |
| 18. | N.D. | N.D. |

SET C[a]

| Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ | Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ |
|---|---|---|---|---|---|
| 1. | 16.52 | 3.64 | 8. | 0.02 | N.D. |
| 2. | 0.66 | 0.29 | 9. | 0.19 | N.D. |
| 3. | 0.40 | 0.26 | 10. | 2.39 | 1.65 |
| 4. | 0.92 | 0.12 | 11. | 1.67 | 1.19 |
| 5. | 0.49 | 0.15 | 12. | 1.42 | 0.75 |
| 6. | 0.44 | 0.10 | 13. | 1.16 | 0.88 |
| 7. | 0.39 | N.D. | 14. | 0.58 | 0.65 |

TABLE 23 (a) (contd.)

SET C[a]

| Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ | Sample No. | WITH $NaBH_4$ | WITH $SnCl_2$ |
|---|---|---|---|---|---|
| 15. | 1.24 | 0.38 | 22. | N.D. | N.D. |
| 16. | 0.49 | 0.29 | 23. | 1.05 | 0.37 |
| 17. | N.D. | N.D. | 24. | N.D. | N.D. |
| 18. | 2.05 | 0.20 | 25. | N.D. | N.D. |
| 19. | 0.77 | N.D. | 26. | 0.25 | N.D. |
| 20. | 2.41 | 0.04 | 27. | N.D. | N.D. |
| 21. | 0.58 | N.D. | | | |

[a] For identification of sets A, B and C, see foot-note of Table 23 (b)

N.D. Not detected

TABLE 23 (b). REGRESSION ANALYSIS PARAMETERS FOR DETERMINATION OF MERCURY USING $NaBH_4$ AND $SnCl_2$ AS REDUCING AGENTS

| SAMPLE SET | REGRESSION EQUATION | CORRELATION COEFFICIENT | SAMPLE SIZE (N) |
|---|---|---|---|
| A | y = 0.991292x-0.348066 | 0.977074 | 10 |
| B | y = 0.500188x+2.241504 | 0.803530 | 18 |
| C | y = 0.239997x-0.054633 | 0.857065 | 27 |

A - Digested samples left in $KMnO_4$ medium overnight; blank corrected

B - Digested samples with direct readings; no blank correction

C - Representative fish sample, F1, digested at different temperatures (range 47-108$^{o}$C) on the same day; with blank correction

x = mercury reading (ng/ml) with $NaBH_4$

y = mercury reading (ng/ml) with $SnCl_2$

Table 23 shows that the first set of samples (A) gave high positive correlation and regression coefficients (0.977 and 0.991 respectively) almost close to unity, as compared with the second and third sets of samples, when digestion and oxidation by $KMnO_4$ was completed on the same day. This partly explains the high deviation between the mercury results obtained with the two reducing agents reported in some literature [50,65]. According to Wandiga and Jumba [50], the range of mercury content in body beauty soaps and creams sold in Kenya and Norway was 222-4920 ng/g when stannous chloride was used as the reducing agent, whereas with sodium borohydride, it was 0.95-1121.86 ng/g. According to Evans et al. [65], the recoveries of mercury in the analysis of a lyophilized dogfish muscle tissue was found to be negatively correlated with the quantity of fish flour digested when sodium borohydride was used but such an effect was not apparent when stannous chloride was used. In this project, it has been established that one of the factors contributing to this difference between the two reducing agents could have been insufficient oxidation time. It appears that when oxidation was done the same day, poor correlation was obtained, with the results favouring the stannous chloride reducing method. It

is therefore recommended that for total mercury determination, it is advisable to react the sample with $KMnO_4$ at least overnight at room temperature for complete oxidation, as recommended by Stewart and Bettany [83]. When samples were thus treated, it did not matter which of the two reducing agents was used as long as digested reagent blanks were prepared the same way and subtracted from all the sample readings. Another factor contributing to the improved correlation between the two reducing agents, could be the difference in the digestion procedures. It can therefore be asserted that the digestion procedure described in the experimental section is suitable for the determination of mercury with the two reducing agents.

The regression equation in set B also shows that for the directly read solutions, the reading obtained with $SnCl_2$ is higher than with $NaBH_4$ up to a concentration of about 4.48 ng/ml, but the relation reverses for higher concentrations. The $SnCl_2$ intercept (2.24 ng/ml) could represent the relative detection limit with $SnCl_2$ over that with $NaBH_4$. Regression analysis data also shows that correlation in set C results was higher but the regression equation drifted considerably from that of set B. Set C results, therefore, relatively favoured the

stannous chloride reduction method. This could be due to the fact that in set C, the same fish sample, F1, at different experimental conditions was used. On the other hand, set B results, which involved different samples, digested and analysed in the same way at roughly the same experimental conditions, gave higher slope between the two reducing agents.

### 4.1.6 ACCURACY AND RELIABILITY OF CONCENTRATION DATA- RESULTS FOR STANDARD REFERENCE MATERIALS

In order to assess the accuracy, reliability and precision of the acquired data, standard reference materials were subjected to the same digestion procedure and their mean concentrations compared with those given in the literature. The results obtained are given in Table 24. There is some good comparison of the analytical results to those expected values of the standard reference materials. However, there are a few cases where the elemental recovery was less than 80 %. For mercury determination, the experimental values given in Table 24 were obtained at a digestion temperature of 70$^{o}$C. As was found earlier (section 4.1.3.1), a temperature of 50 $\pm$ 3$^{o}$C gave higher mercury readings than at 70$^{o}$C, due to volatilization at the higher temperatures. This

explains why mercury determination had to be done at the lower temperatures. For cadmium and lead, the few low recoveries and the extremely high value of 176.7% could be due to incomplete digestion as well as analytical, statistical and sampling errors. However, such extremely high concentrations were not anticipated in the unknown analytical samples.

TABLE 24. ELEMENTAL RECOVERY OF STANDARD REFERENCE MATERIALS (Values in mg/kg dry weight)

| REFERENCE | MERCURY | | CADMIUM | | LEAD | |
|---|---|---|---|---|---|---|
| MATERIAL | Mean | SD. | Mean | SD. | Mean | SD. |
| | (n = 4) | | (n = 2) | | (n = 2) | |
| MA-A-2(TM) | | | | | | |
| Certified value | 0.47 | ±0.02 | 0.066 | ±0.004 | 0.58 | ±0.07 |
| Experimental value | 0.47 | ±0.14 | 0.073 | ±0.006 | 0.59 | ±0.06 |
| Mean recovery (%) | 100 | | 110.6 | | 102.4 | |
| H8 (Horse kidney) | | | | | | |
| Certified value | 0.91 | ±0.08[a] | 189 | ± 4.5[a] | N.A. | |
| Experimental value | --- | | 141.81 | ± 0.43 | N.D.[b] | |
| Mean recovery (%) | | | 75.0 | | | |

TABLE 24 (contd.)

| REFERENCE MATERIAL | MERCURY Mean | SD. (n = 4) | CADMIUM Mean | SD. (n = 2) | LEAD Mean | SD. (n = 2) |
|---|---|---|---|---|---|---|
| MA-M-2/TM | | | | | | |
| Certified value | 0.95 | ±0.11 | 1.32 | ± 0.22 | 1.92 | ±0.58[a] |
| Experimental value | 0.74 | ±0.05 | 1.34 | ± 0.04 | 3.39 | ±0.24 |
| Mean recovery (%) | 77.7 | | 101.7 | | 176.7 | |
| MP-1 (Geological ore) | | | | | | |
| Certified value | N.A. | | 700[a] | | 19300 | ± 300 |
| Experimental value | --- | | 510 | ± 10 | 17130 | ± 120 |
| Mean recovery (%) | | | 72.9 | | 88.8 | |

[a] Non-certified or provisional value

[b] Value not detected (below limit of detection)

n = number of sample replicates analysed

N.A. Data not available

--- Value not experimentally determined

### 4.1.7 EVALUATION AND IMPORTANCE OF DIGESTION OF WATER SAMPLES

Heavy metals may be present in water in various forms such as inorganic ions, organic and elemental. The organic form poses interference in AAS determination. It was therefore essential to determine whether the organic forms were present in the water in significant levels. This was done by comparing the levels of mercury, cadmium and lead in a selected batch of water samples from the area of study before and after digestion. Table 25 shows the concentration data obtained.

#### 4.1.7.1 MERCURY

The results in Table 25 show that only three of the water samples analysed (before digestion) gave detectable mercury concentrations. The rest of the samples had levels of mercury below the limit of detection (about 0.0023 mg/l, which was determined as previously described). This shows that most of the mercury, where present, in most of the water samples was in the organic form. This indicated that there was need to digest the water samples. Of these samples, the few digested showed improvement by

TABLE 25. AAS DATA OF WATER SAMPLES BEFORE AND AFTER DIGESTION (Values in mg/l)[a]

| SAMPLE | MERCURY | | CADMIUM | | LEAD | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| LNW2 | N.D. | 0.0188 | N.D. | 0.1250 | 0.032 | 1.9069 |
| BHNW3 | N.D. | --- | N.D. | 0.0450 | 0.062 | 1.3263 |
| DNW4 | N.D. | --- | N.D. | --- | 0.062 | --- |
| BHNW5 | N.D. | --- | N.D. | 0.1375 | 0.052 | 1.4138 |
| DNW6 | 0.0036 | --- | N.D. | --- | 0.052 | --- |
| OW26W[b] | 0.0034 | 0.0228 | N.D. | 0.1500 | 0.052 | 1.0002 |
| OW26St[c] | N.D. | --- | N.D. | N.D. | 0.062 | 1.2263 |
| OW715St[c] | N.D. | --- | N.D. | N.D. | 0.052 | 1.0583 |
| OW715W[b] | 0.0135 | 0.029 | N.D. | 0.0500 | 0.092 | 0.9916 |
| RMW1 | N.D. | --- | N.D. | --- | 0.032 | --- |
| RMW2 | N.D. | --- | N.D. | 0.0825 | 0.072 | 1.3416 |
| RMW3 | N.D. | --- | N.D. | --- | 0.072 | --- |
| RMW4 | N.D. | --- | N.D. | 0.0750 | 0.072 | 1.1166 |
| RMW5 | N.D. | --- | N.D. | --- | 0.042 | --- |
| RMW6 | N.D. | --- | N.D. | 0.0875 | 0.052 | 1.1593 |
| Laboratory tap water | N.D. | --- | N.D. | 0.1125 | 0.024 | 1.0763 |

TABLE 25 (contd.)

a using Varian Spectr AA 10 AAS and VGA-76

b water sample

c condensed steam sample

LNW = Lake Naivasha water, BHNW = Bore-hole Naivasha water, DNW = Domestic Naivasha water

OW = Olkaria Well

RMW = River Malewa water

N.D.= Not detected (lower than limit of detection)

--- = Not determined

(enhancement) factors in the range 2.2-8.2. Among the few samples analysed, the highest concentration in the undigested Olkaria geothermal well water (OW 715W) of 0.0135 mg/l, and hence the least mercury digestion enhancement factor of 2.2, suggests that there was an appreciable amount of inorganic mercury in the cooled geothermal water from Olkaria. This seems to comply with Bor's view that metals frequently occur in the ionized forms as hydrated cations in geothermal fluids [58].

4.1.7.2 CADMIUM

All the undigested water samples showed that their cadmium concentrations were below the lowest limit of detection (0.0323 mg/l, previously determined (see Table 9)). After digesting and analysing 11 of these samples, 9 of them gave reasonably detectable concentration values in the range of 0.045-0.15 mg/l. Hence, digesting the water samples enhanced the cadmium concentration by factors in the range of 1.4-4.6. Also, this shows that most of the cadmium present in the samples was in the organic form and hence the need to digest the water samples.

#### 4.1.7.3 LEAD

All the undigested water samples gave concentration readings of lead above the limit of detection, in the range of 0.032-0.092 mg/l, indicating presence of some inorganic (ionic) forms of lead in the water. When 11 of these samples were digested, all showed increased concentrations by factors in the range of 10.8-59.6. As in the case of mercury, the least digestion enhancement factor of 10.8 was observed for OW715 water sample, thus supporting the previously asserted view of Bor's [58].

According to Munyithya [101], manganese was not detected in condensed steam sample of OW 715. This therefore ensures that the lead enhancement in the digested samples was not due to chemiluminescence, as previously discussed.

### 4.1.8 OPTIMUM DIGESTION SAMPLE QUANTITIES

Determination of elements, when in very low concentration, requires large quantities of the sample to be digested. This would require large volumes of acid and other appropriate reagents as well as longer digestion time, with a possible risk of elemental contamination. It was therefore, the aim

of this project to use the minimum volume of acids and other reagents for digestion of the environmental samples. In most cases, a fixed volume of the digestion acid mixture of about 7 ml was maintained for this purpose. The values obtained are shown in Table 26. The results show that in most cases, using a reasonable volume of digestion acids (about 7 ml) and reagents, as given in the experimental section, the least quantity of the sample possible (about 1gm or 2ml) had to be digested for highest elemental recovery. This is because when large quantities of the sample are used, incomplete digestion can occur, giving low recoveries. There were only a few exceptional cases, where the elemental concentrations were positively correlated with the quantity of sample digested.

Table 26 also shows that when 5-10 ml of water sample was used, the concentration of cadmium was below the limit of detection. This is because when such large volumes of sample were used, complete digestion may not have been achieved and incomplete destruction of organic matter may have posed interference to the cadmium signal.

TABLE 26. THE CONCENTRATION DATA FOR MERCURY, CADMIUM AND LEAD IN DIFFERENT QUANTITIES OF SAMPLES DIGESTED (ppm)[a]

| SAMPLE TYPE | VOLUME OR MASS | MERCURY Mean ± SD (n = 2) | CADMIUM Mean ± SD (n = 2) | LEAD Mean ± SD (n = 2) |
|---|---|---|---|---|
| OLKARIA STEAM | | | | |
| OW714St (a) | 2ml | 0.0780±0.0007 | 0.200±0.016 | 4.458±0.545 |
| (b) | 5ml | 0.1296±0.0007 | N.D. | 1.845±0.035 |
| (c) | 10ml | 0.1639±0.0004 | N.D. | 0.945±0.018 |
| OLKARIA WATER | | | | |
| OW714W (a) | 2ml | 0.0287±0.0020 | 0.050±0.011 | 5.525±0.150 |
| (b) | 5ml | 0.0138±0.0003 | N.D. | 2.550±0.064 |
| (C) | 10ml | 0.0042±0.0004 | N.D. | 1.400±0.046 |

TABLE 26 (contd.)

| SAMPLE TYPE | VOLUME OR MASS | MERCURY Mean ± SD (n = 2) | CADMIUM Mean ± SD (n = 2) | LEAD Mean ± SD (n = 2) |
|---|---|---|---|---|
| LAKE NAIVASHA WATER | | | | |
| LNW15W (a) | 2ml | 0.0215±0.0014 | N.D. | 3.788±0.088 |
| (b) | 5ml | 0.0107±0.0006 | N.D. | 3.250±0.160 |
| (c) | 10ml | 0.0030±0.0000 | N.D. | 1.865±0.035 |
| OLKARIA PLANT | | | | |
| OW714P[b] (a) | 1gm | 0.1275±0.0261 | 0.248±0.027 | 9.638±0.770 |
| (b) | 2gm | 0.1565±0.0301 | 0.374±0.008 | 8.884±0.978 |
| (c) | 5gm | 0.0950±0.0421 | 0.390±0.008 | 4.457±0.042 |
| OLKARIA SOIL | | | | |
| OW714S1 (a) | 0.5gm | 0.1922±0.0028 | 0.496±0.032 | 25.90±0.351 |
| (b) | 1gm | 0.0617±0.0234 | 0.346±0.020 | 15.32±0.070 |
| (c) | 2gm | 0.0085±0.0007 | 0.462±0.194 | 15.33±0.330 |

TABLE 26 (contd.)

[a] ppm = mg/l for liquids, or mg/kg for solid samples;
about 7 ml of digestion acid mixture was used
S.D. = Standard deviation of the mean
[b] Plant analysed was Tarchonanthus camphoratus
N.D. = Not detected (below limit of detection)
n = number of sample replicates analysed

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From the results just discussed, it was decided that for the sample analysis, with AAS technique, the maximum quantities used should be as follows: 2 ml of water and condensed steam, 1 gm of plant (dry weight) and 1 gm of soil (dry weight). When these quantities were to be used, a maximum volume of about 7 ml of acid or acid mixture had to be used for digestion. However, it should be noted, especially in ED-XRF that 2 ml of the water sample and about 7 ml of the acid could give very high errors due to contamination, mode of analysis and statistical variation. In practice, large volumes of the water sample are normally chelated with a suitable agent, when ED-XRF technique is employed. However, in such cases, some researchers analyse undigested samples [37,66].

## 4.2 ELEMENTAL LEVELS IN SAMPLES FROM NAIVASHA

The samples analysed included fish, water, sediments, soil and plants. The analysis was mainly by AAS.

### 4.2.1 FISH SAMPLES

The concentrations of mercury, cadmium and lead in individual fish samples analysed are shown in Table 27. These included fish samples bought at Naivasha (NVF1-NVF17), Naivasha samples bought in Nairobi (NVF18-NVF21) and those from Kaburu Dam, bought at Gikomba Market, Nairobi (KABF1-KABF4). These latter samples were for comparison purposes only. The results for each of the three elements are discussed below.

TABLE 27. LEVELS OF MERCURY, CADMIUM AND LEAD IN NAIVASHA AND KABURU FISH SAMPLES

in mg/kg (wet weight)

| FISH SAMPLE CODE | MERCURY Mean[a] ± SD[b] (n = 4) | CADMIUM Mean ± SD (n = 2) | LEAD Mean ± SD (n = 2) |
|---|---|---|---|
| NVF1TIL1 | --- | 0.235 ±0.059 | 0.819 ± 0.290 |
| NVF2TIL2 | --- | 0.225 ±0.087 | 0.742 ± 0.199 |
| NVF3TIL3 | 0.052 ± 0.001 | 0.188 ±0.037 | 2.867 ± 0.177 |
| NVF4TIL4 | --- | 0.132 ±0.012 | 2.052 ± 0.711 |
| NVF5TIL5 | --- | 0.099 ±0.030 | 1.574 ± 0.525 |
| NVF6TIL6 | --- | 0.085 ±0.001 | 1.224 ± 0.353 |
| NVF7TIL7 | 0.092 ± 0.021 | 0.279 ±0.163 | 1.768 ± 0.812 |
| NVF8TIL8 | --- | 0.119 ±0.008 | 1.171 ± 0.146 |
| NVF9TIL9 | --- | 0.048 ±0.014 | 1.998 ± 1.160 |
| NVF10TIL10 | --- | 0.008 ±0.003 | 0.514 ± 0.245 |
| NVF11BB1 | --- | 0.089 ±0.047 | 1.079 ± 0.045 |
| NVF12BB2 | 0.058 ± 0.011 | 0.113 ±0.005 | 1.786 ± 0.250 |
| NVF13BB3 | --- | 0.040 ±0.012 | 1.549 ± 0.547 |
| NVF14BB4 | --- | 0.077 ±0.037 | 1.146 ± 0.015 |
| NVF15BB5 | --- | 0.274 ±0.079 | 0.953 ± 0.374 |
| NVF16BB6 | --- | 0.087 ±0.024 | 2.404 ± 0.896 |
| NVF17BB7 | 0.104 ± 0.016 | 0.165 ±0.084 | 2.011 ± 0.654 |

TABLE 27 (contd.)

| FISH SAMPLE CODE | MERCURY Mean[a] ± SD[b] (n = 4) | CADMIUM Mean ± SD (n = 2) | LEAD Mean ± SD (n = 2) |
|---|---|---|---|
| NVF18TIL11 | 0.391 ± 0.143 | 0.295 ±0.010 | 2.788 ± 0.318 |
| NVF19TIL12 | 1.099 ± 0.009 | 0.562 ±0.005 | 5.111 ± 0.557 |
| NVF20BB8 | 0.960 ± 0.142 | 0.381 ±0.068 | 2.091 ± 0.068 |
| NVF21BB9 | 1.521 ± 0.065 | 0.126 ±0.018 | 2.545 ± 0.709 |
| KABF1TIL1 | 0.391 ± 0.018 | 0.496 ±0.035 | 3.861 ± 0.319 |
| KABF2TIL2 | 0.506 ± 0.001 | --- | --- |
| KABF3TIL3 | 0.399 ± 0.080 | --- | --- |
| KABF4TIL4 | 0.255 ± 0.011 | --- | --- |

[a] Mean of four sample replicates

[b] Standard deviation of the replicates

n = number of sample replicates

NVF = Naivasha fish, KABF = Kaburu Dam fish, TIL = Tilapia, BB = Black bass

--- = Not determined

#### 4.2.1.1 MERCURY

Mercury levels in the fish species bought in Nairobi City Market were 10-15 times (higher than) those of the corresponding fish species bought at the Naivasha Market. It is likely that high mercury levels in fish bought in Nairobi were due to contamination, during storage and handling. Another observation is that the mean concentration of mercury in Blackbass (*Micropterus salmoides*) was higher than in the *Tilapia zilli* bought from the same market. This is probably because Tilapia feeds on the algae, fungi, lichen or plankton. Tilapia is in turn eaten by Blackbass. The mean mercury concentration in the Tilapia fish from Kaburu Dam was between the levels obtained in the Tilapia fish from Naivasha and those bought in Nairobi.

The overall results show that 5/8 of the Naivasha fish analysed had mercury concentrations below the toxic level of 0.5 mg/kg [31], while 3/8 were above this limit. However, the upper limit, which is due to the carnivorous species, Blackbass, compares well with the values for large predatory species, e.g. swordfish, tuna and halibut of between 0.2 and 1.5 mg/kg [10]. However, the slight elevation in some mercury values could be due to

bio-methylation of mercury from the aquatic environment or accumulation as a result of age, weight, sex, feeding behaviour of the fish, physical and chemical properties of the lake (alkalinity or pH) or its water-shed.

The results of the present work show that the levels of mercury in Naivasha fish were at least 10 times higher than those obtained in fish from different regions of Kenya, as reported by Kamau et al. [8]. This could probably have been due to difference in digestion procedures or sampling. In the earlier work, a temperature of 70 $^{o}$C was employed, while in the present work, 50 $^{o}$C was used, which was found to be more sensitive than 70 $^{o}$C.

#### 4.2.1.2 CADMIUM

Levels of cadmium in the fish species bought at City Market, Nairobi, were higher than those of the corresponding species bought at the Naivasha Market by a factor of about 2-3 times. The explanation is the same as that given under mercury, i.e. possible contamination in storage and transportation of the fish bought in Nairobi as well as hauling seasonal variation. When the level of the lake is low, one would expect higher concentration of cadmium in the water. This would be reflected by higher

concentration levels of cadmium in the fish. However, if the level of the lake is high, as a result of drainage and erosion, the concentration of cadmium in the water would depend on its concentration in the surrounding soils and farm inputs. This would in turn be reflected in the concentration levels of cadmium in the fish.

Unlike for mercury, the mean cadmium concentration in Blackbass was lower than in the Tilapia. It would therefore be logical to rule out the feeding behaviour of the fish as a possible consequence of cadmium accumulation in the fish. This implies that the possible factors determining the level of cadmium could be age, weight, sex, physical and chemical properties of the lake or its water-shed (alkalinity or pH).

The overall results show that the cadmium concentration values in all the fish were far below the legislative limits of 1.0 and 2.0 mg/kg [40,41]. However, results show that about 52.4 % of the samples gave higher cadmium levels than fish samples from Lake Victoria (0.04-0.12) mg/kg as reported by Wandiga and Onyari [38]. This shows significantly higher pollution of the Lake Naivasha fish. This could be due to handling and storage, or the high cadmium levels in the surrounding soils, as discussed

later. The cadmium in the soil is drained into the lake by rain or blown off into the lake during the windy seasons. It is then transferred to the fish through the food chain and some microbiological processes.

4.2.1.3 LEAD

The results in Table 27 show that the concentrations of lead in the fish species bought in the City Market, Nairobi, were higher than those of the corresponding species bought at the Naivasha Market by a factor of 1.3-2.2 times. This could be due to handling, storage and hauling variations. In addition, the higher traffic volume in Nairobi might cause the higher lead levels. Murakaru [102] has recently shown that exhaust fumes do pollute Nairobi air with lead.

Unlike for mercury, but similar to cadmium, the mean lead concentration in Blackbass was lower than in Tilapia. This is probably due to difference in age, weight, sex, physical and chemical properties of the lake or its water-shed (alkalinity or pH).

The overall results show that most of the fish analysed (95.2 %) registered lead levels lower than the maximum limit of 5.0 mg/kg in food as recommended by the World Health Organization [39,40].

Furthermore, all the values were below the legislative maximum limit in food products set in Kenya [47]. There was therefore no induced health hazard in eating the fish. However, most of the results (76.2 %) were higher than the values obtained for fish from Lake Victoria, 0.39-1.08 mg/kg, as reported by Wandiga and Onyari [38]. This suggests that the Naivasha fish were relatively more polluted than those from Lake Victoria. One of the possible reasons is that Lake Naivasha is very close to the Great North Road, where the traffic density is relatively high. This could also be due to differences in sampling, sampling period and the volume of the lake. Other possible sources of lead could be the fuel used in the motor-boat engines and the volcanic origin of the underlying rock. It should also be noted that the difference in the digestion and analytical methods employed on the two occasions could have yielded different concentration results.

### 4.2.2 WATER SAMPLE ANALYSIS

The results for the analysis of mercury, cadmium and lead in water samples from various sites in Naivasha are shown in Tables 28 to 32. The samples

TABLE 28. LEVELS OF MERCURY, CADMIUM AND LEAD IN THE WATER SAMPLES FROM LAKE NAIVASHA (mg/l)

| SAMPLE AND SITE/LOCATION | Dist.[a] | pH | MERCURY Mean±SD (n =4 ) | CADMIUM Mean±SD (n = 2) | LEAD Mean±SD (n = 2) |
|---|---|---|---|---|---|
| LNW 1 (Eastern shore, north-west of Railway Station) | 1 | 8 | --- | 0.1250 ± 0.0173 | 1.1110 ± 0.3338 |
| LNW 2 (400 m North of Lake Hotel) | 4 | 8 | 0.0188 ± 0.0026 | 0.1250 ± 0.0708 | 1.9069 ± 0.4179 |
| LNW 4 (Safariland Hotel) | 8.7 | 7.0 | --- | 0.1125 ± 0.0086 | 0.9698 ± 0.2290 |
| LNW 5 (Elsamere shore) | 15.7 | 7.9 | --- | 0.1125 ± 0.0197 | 1.8179 ± 0.0960 |
| LNW 15 (Off Elsamere shore, toward Hippo point) | 17.2 | 8.4 | 0.0215 ± 0.0014 | N.D. (<D.L.) | 3.5188 ± 0.3801 |

[a]Distance from sewage treatment plant in Kilometres
LNW = Lake Naivasha water, --- = Value not determined
N.D. = Value not detected, S.D. = Standard deviation
D.L. = Detection limit

TABLE 29. LEVELS OF CADMIUM AND LEAD IN THE WATER SAMPLES FROM RIVER MALEWA (mg/l)

| SAMPLE AND SITE/LOCATION | pH | CADMIUM Mean±SD (n = 2) | LEAD Mean±SD (n = 2) |
|---|---|---|---|
| (a) RMW 2 (near Veterinary Farm Research Station) | 8.5 | 0.0825 ±0.0126 | 1.3416 ±0.5334 |
| (b) RMW 4 (10 m downstream of (a) | 7.8 | 0.0750 ±0.0097 | 1.1166 ±0.4099 |
| (c) RMW 6 (10 m downstream of (b) | 8.1 | 0.0875 ±0.0129 | 1.1593 ± 0.1752 |

RMW = River Malewa water

n = number of sample replicates

TABLE 30. LEVELS OF CADMIUM AND LEAD IN THE WATER SAMPLES FROM BORE-HOLES, NAIVASHA (mg/l)

| SAMPLE AND SITE/LOCATION | pH | CADMIUM Mean±SD (n = 3) | LEAD Mean±SD (n = 3) |
|---|---|---|---|
| BH-NW 3 (Lucita farm) | 8 | 0.0450 ±0.0053 | 1.3263 ±0.3518 |
| BH-NW 5 (Naivasha Boarding Primary School) | 8 | 0.1375 ±0.0327 | 1.4138 ±0.4755 |

BH-NW = Bore-hole Naivasha water

n = number of sample replicates

TABLE 31. LEVELS OF MERCURY, CADMIUM AND LEAD IN THE WATER SAMPLES FROM OLKARIA GEOTHERMAL WELLS (mg/l)

| SAMPLE AND SITE/LOCATION | pH | MERCURY Mean±SD. (n = 4) | CADMIUM Mean±SD. (n = 2) | LEAD Mean±SD. (n = 3) |
|---|---|---|---|---|
| OW 22 | 9.6 | 0.0235 ±0.0007 | 0.1978 ±0.0323 | 2.3024 ± 1.3114 |
| OW 26 | 8.7 | 0.0228 ±0.002 | 0.1500 ±0.0233 | 1.0002 ± 0.2265 |
| OW 714 | 9.3 | 0.0287 ±0.002 | 0.0500 ±0.011 | 5.5250 ± 0.1503 |
| OW 715 | 9.4 | 0.0298 ±0.002 | 0.0500 ±0.0098 | 0.9916 ± 0.2593 |

OW = Olkaria Well

n = number of sample replicates

TABLE 32. LEVELS OF MERCURY, CADMIUM AND LEAD IN THE CONDENSED STEAM SAMPLES FROM OLKARIA GEOTHERMAL WELLS (mg/l)

| SAMPLE AND SITE/LOCATION | pH | MERCURY Mean±SD. (n = 4) | CADMIUM Mean±SD. (n = 2) | LEAD Mean±SD. (n = 2) |
|---|---|---|---|---|
| OW 22 | 4.0 | 0.1433 ± 0.0015 | 0.1892 ±0.0379 | 1.9308 ± 0.4444 |
| OW 26 | 4.1 | --- | N.D. (<D.L.) | 1.2263 ± 0.2104 |
| OW 714 | 4.0 | 0.1238 ± 0.043 | 0.200 ± 0.016 | 4.4583 ± 0.5445 |
| OW 715 | 3.9 | --- | N.D. (<D.L.) | 1.0583 ± 0.5583 |

OW = Olkaria Well

n = number of sample replicates

N.D. = Not detected

D.L. = Detection limit

--- = Not determined

were from Lake Naivasha, River Malewa, bore-holes and Olkaria geothermal wells. Four replicates for mercury and two for cadmium and lead were analysed for each sample, each determined in triplicate.

#### 4.2.2.1 MERCURY

Due to pressure of time, it was not possible to determine the levels of mercury in River Malewa and the bore-hole samples. However, this is being done separately for publication. The acquired data shows that the concentration of mercury increased in the following order: Lake Naivasha water, Olkaria geothermal water and condensed steam. This indicates that the recent volcanic activities in the area could be a possible source of mercury present in the rocks, soils and in this environment. The condensed geothermal steam registered higher mercury than the cooled geothermal water. This could be due to the escape of mercury into the atmosphere due to its volatile nature. The pH of the condensed steam was far below that of the cooled water. Furthermore, there was an obnoxious smell from the vapours spewed by the geothermal wells, similar to that of rotten eggs. This was due to hydrogen sulphide, resulting in condensed steam which was acidic (pH = 4.0).

This vapour could therefore have carried with itself some mercury or its sulphides. After condensation, the chemical constituents in the steam were concentrated and hence resulted in high mercury levels.

The overall results show that the mercury concentration in the samples were above the maximum permissible level of 0.001-0.005 mg/l in drinking water as recommended by World Health Organization [30]. The values are also far much higher than those obtained for seven Kenyan lakes, including Lake Naivasha by Alala [37]. According to Alala, the concentration range for water samples was <0.0002 to 0.001 mg/l and mercury was not detected around Fisherman's Camp (see Figure 3). The values are also higher than those obtained in the preliminary study of the same area [8]. The present work suggests that there was significant mercury pollution in the aquatic environment of Naivasha area. However, it should be noted that the water samples analysed in this project were not filtered and they probably included suspended particulate matter in the water. The particulate matter may have contributed to the elevation of levels of mercury in some water samples. Other possible reasons for this deviation from literature values could be the difference in the

digestion procedures and the analytical techniques employed. However, mercury pollution in the aquatic environment of the area studied can not be totally ruled out. Volcanic origin may also be playing a significant role in this.

#### 4.2.2.2 CADMIUM

The cadmium levels increased in the following order: River Malewa water (0.075 to 0.088 mg/l), Bore-hole water (0.045 to 0.138 mg/l), Lake Naivasha water (<0.045 to 0.125 mg/l), Olkaria geothermal water (0.05 to 0.198 mg/l) and condensed steam (<0.045 to 0.2 mg/l). The sampling sites for the lake, river and bore-hole water were very close to each other. The relatively high cadmium content in the lake water could possibly be attributed to the sewage treatment works of Naivasha Town Council, which are very close to the lake, near the sampling site for LNW 1 (marked "1" in Figure 3). This is supported by the fact that there seems to be a negative correlation between cadmium concentration and the distance from the sewage treatment plant (see Table 28). After treatment of the sewage, the effluent is discharged into the lake, at the nearest point. The surface area of the main part of the lake is about 130 $Km^2$ [74] and so it would take a long

time for the entire lake to be homogeneously mixed up. It also implies that sampling sites which are far from the treatment works may not have indicated detectable cadmium concentrations. This may explain why Alala could not detect any dissolved cadmium concentration around Fisherman's Camp of the lake [37], which is fairly far from the effluent discharge point. The discharge point was near sampling site 1 (as marked in Figure 3). However, cadmium levels in the lake water obtained in the present work was very close to the values reported for seven lakes by Alala [37].

The geothermal fluid samples had higher cadmium than the other samples, probably due to geological factors such as leaching from rocks and volcanicity. Though the same wells were not sampled, the values obtained in this project were slightly high but close to those reported by Bor [58]. The differences are probably due to the analytical procedure, among other factors. While Bor used chelation-solvent extraction technique, acid digestion was used in this project.

The overall results show that 83.3% of all the water samples analysed had cadmium concentrations above the maximum permissible level of 0.01 mg/l in drinking water as recommended by World Health Organization [30]. In general, the cadmium pollution in this area could be due to the geology of the surrounding area, sewage treatment works and possibly, use of phosphate rock fertilizers (which might have been used in the surrounding agricultural farms).

4.2.2.3 LEAD

The lead levels in the water samples increased as follows: River Malewa water (1.117 to 1.342 mg/l), Bore-hole (1.326 to 1.414 mg/l), Lake Naivasha (0.970 to 3.519 mg/l), condensed Olkaria geothermal steam (1.058 to 4.458 mg/l) and geothermal water (0.992 to 5.525 mg/l). The high lead content in Lake Naivasha water relative to that in River Malewa and bore-hole waters could be contributed by the sewage treatment works. Other contributing factors are the motor-boats and motor-vehicles, which use leaded fuels.

The geothermal fluid samples had higher lead than the other samples, probably due to leaching from rocks and geothermal activity in the area. The values obtained were also far much higher than those

obtained for different wells in the same area as reported previously by Bor [58]. Unlike for mercury and cadmium, the cooled geothermal water had higher mean lead content than the condensed steam. This is because lead is not as volatile as the other two elements and so it is not appreciably carried by the vapours rising into the atmosphere.

The overall results show that the water samples exhibited higher lead levels - greater than the maximum permissible level (0.1 mg/l) in drinking water [30]. These values are also higher than those for the seven lakes, especially the Fisherman's Camp in Lake Naivasha, as reported by Alala [37]. The values are also higher than those for surface water (0.001-0.01 mg/l) and ground waters (0.001-0.5 mg/l) as previously reported [1]. However, hot springs may sometimes register concentrations higher than 1 mg/l [1]. Most of the samples in the present work showed this.

### 4.2.3 SEDIMENT SAMPLES

The results for the analysis of mercury, cadmium and lead in sediments are shown in Table 33. The sediments sampled were from Lake Naivasha and River Malewa and were collected up to 50 and 40 cm below the water surface, respectively.

TABLE 33. ANALYTICAL RESULTS FOR MERCURY, CADMIUM AND LEAD IN SEDIMENTS (in mg/kg dry weight)

| SAMPLE DESCRIPTION AND LOCALITY | Dist[a] | MERCURY Mean ± S.D. (n = 4) | CADMIUM Mean ± S.D. (n = 2) | LEAD Mean ± S.D. (n = 2) |
|---|---|---|---|---|
| LNSD 1 (Eastern shore, west of Naivasha Railway Station) | 1 | 0.267 ± 0.092 | 1.485 ± 0.259 | 28.084 ± 4.050 |
| LNSD 2 (400 m North of Lake Hotel) | 4 | --- | 1.275 ± 0.178 | 16.631 ± 3.685 |
| LNSD 3 (Safariland Hotel jetty, 45 cm below water surface) | 8.7 | 0.209 ± 0.027 | 0.950 ± 0.085 | 18.483 ± 3.404 |

TABLE 33 (contd.)

| SAMPLE DESCRIPTION AND LOCALITY | Dist[a] | MERCURY Mean ± S.D. (n = 4) | CADMIUM Mean ± S.D. (n = 2) | LEAD Mean ± S.D. (n = 2) |
|---|---|---|---|---|
| LNSD 4 (Elsamere shore, 50 cm below water surface) | 15.7 | 0.269 ± 0.058 | 0.530 ± 0.194 | 13.710 ± 2.727 |
| RMSD 2 (30 cm below water surface) | | --- | 2.129 ± 0.558 | 26.764 ± 1.797 |
| RMSD 4 (20 cm below water surface) | | --- | 1.418 ± 0.641 | 20.292 ± 0.814 |
| RMSD 6 (40 cm below water surface) | | --- | 1.426 ± 0.160 | 21.757 ± 1.167 |

TABLE 33 (contd.)

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[a] Distance from sewage treatment plant (in kilometres)

LNSD = Lake Naivasha sediment, corresponding to LNW sample

RMSD = River Malewa sediment, corresponding to RMW sample

n = number of sample replicates

--- = Not determined

#### 4.2.3.1 MERCURY

Only three lake sediments were analysed for mercury. The values were within the overall range of mercury levels of 0.004-0.7 mg/kg, but higher than 0.1 mg/kg, which is the upper limit exhibited in most values, as reported in the literature [1,26]. Volcanicity may be playing an important role in the elevation of some mercury levels.

The mercury levels indicate more than two-fold increase over those obtained by Alala for Fisherman's Camp, Lake Naivasha, but within the overall range reported for the seven lakes [37]. They are also higher than those reported in a preliminary survey of the same area [8]. This could be due to differences in the analytical procedures and sampling. However, increase in mercury levels over the period 1981-1991 could have been possible due to the rapid rate of urbanization of Naivasha Town and increased agricultural activities in the area.

#### 4.2.3.2 CADMIUM

The cadmium levels in Lake Naivasha sediments (0.530 to 1.485 mg/kg) were lower than those for River Malewa's (1.418 to 2.129 mg/kg). This could be due to the geological influences. Lake Naivasha

sediments were mainly composed of clay, while the river sediments included some sedimentary fragments as well, due to erosion or denudation into the river from the parent bed rock material. This implies that the differences in the geology of the area is one of the factors contributing to variable cadmium levels in the two aquatic environments. The pH was of the same range (7-9) and may not have contributed a significant role in the difference.

A notable observation in the results of the lake sediments is the existence of negative correlation between cadmium concentration and the distance from the sewage treatment plant, as shown in Table 33. The distance from the treatment works increased from the location of the sample LNSD 1 to LNSD 4. This clearly supports the view previously asserted, that the variation of cadmium content at various points of the lake was due to the proximity to the treatment plant.

The overall results for cadmium concentration in the sediments show that the values obtained in this work were very close to those previously reported for Winam Gulf in Lake Victoria (0.0-1.0 mg/kg) [38] and for the seven Kenyan lakes (<0.008-3 mg/kg) by Alala [37].

#### 4.2.3.3 LEAD

The lead levels in Lake Naivasha sediment samples (13.710 to 28.084 mg/kg) were lower, on average, than in River Malewa samples (20.292 to 26.764 mg/kg). The difference is mainly due to the chemical composition of the parent rock constituting the sediment material. The high levels of lead in the lake sediments could have been as a result of the effluent discharged from the sewage treatment works.

The overall results indicate that all the sediments analysed exhibited lead concentrations, which were within the lower limits of the ranges given for Winam Gulf of Lake Victoria (6.0-69.4 mg/kg) by Wandiga and Onyari [38] and results from seven Kenyan lakes (6.7-210 mg/kg) by Alala [37]. The values are also lower than the values reported for "uncontaminated" sediments, near the shore (40 mg/kg) [1]. This therefore suggests no major lead increase over the period 1981-1991.

### 4.2.4 SOIL SAMPLES

The results for the analysis of soil samples from different regions of Naivasha area are presented in Table 34. However, due to pressure of time, mercury analysis was not exhaustive. The results show that the concentrations of the three elements in soils were close to the corresponding values in the sediments. This is expected because lake sediment sampling was restricted to the shores. Over the recent past, the periphery of Lake Naivasha has been receding at a tremendous rate. This is expected to continue, though the reason for this has not yet been fully understood. As this process continues, part of the surface that formerly constituted the sedimentary floor of the lake, henceforth becomes dry ground and hence a source of dry soil particles. However, since the soils were from different regions, including some where such sediment-soil interconversion was not known to exist, the close agreement between the two sets of data could be due to geological similarity.

TABLE 34. ANALYTICAL RESULTS FOR MERCURY, CADMIUM AND LEAD IN SOIL SAMPLES FROM NAIVASHA AREA (in mg/kg dry weight)

| SAMPLE AND SITE LOCATION | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ± S.D. (n = 2) |
|---|---|---|---|
| HSL 4 (Naivasha town, near Railway Station) | 0.104 ± 0.007 | 2.281 ± 0.602 | 34.249 ± 4.575 |
| AgrSL 5 (near Lucita farm, 400 m N of Lake Hotel) | 0.203 ± 0.065 | 2.501 ± 0.780 | 18.279 ± 1.599 |
| AgrSL 2 (Flower-irrigated farm, near Panafood) | --- | 1.673 ± 0.461 | 28.982 ± 0.895 |
| HSL 7 (near Prison, 2 m E of Nakuru Road) | --- | 2.155 ± 0.159 | 30.534 ± 3.226 |

TABLE 34 (contd.)

| SAMPLE AND SITE LOCATION | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ± S.D. (n = 2) |
|---|---|---|---|
| HSL 11 (300 m E of Kinangop Road, 3 km S of Naivasha town centre) | --- | 1.565 ± 0.862 | 29.427 ± 4.199 |
| AgrSL 12 (Vet.Farm Res. Stn.) | --- | 1.277 ± 0.303 | 25.798 ± 3.718 |
| VSL 1 (Safariland jetty, 100 m from lake shore, 30 cm deep) | --- | 0.640 ± 0.078 | 13.020 ± 1.860 |
| OW26 SL (50 m from well, 30 cm deep) | --- | 0.969 ± 0.076 | 26.923 ± 0.707 |
| OW714 SL (20 cm deep) | 0.192 ± 0.003 | 0.479 ± 0.024 | 25.900 ± 0.351 |

TABLE 34 (contd.)

| SAMPLE AND SITE LOCATION | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ± S.D. (n = 2) |
|---|---|---|---|
| OW716 SL (dry, at surface, 50 m from well) | 0.256 ± 0.081 | 2.668 ± 0.808 | 12.133 ± 2.890 |
| OW716 SL (dry mud, at the well) | --- | 0.924 ± 0.183 | 37.497 ± 2.678 |

HSL = Highway soil, AgrSL = Agricultural soil,
OW-SL = Olkaria well-soil, VSL = Virgin soil
--- = Not determined

#### 4.2.4.1 MERCURY

For the four samples analysed, the lowest mercury concentration was registered by highway soil, while the highest was by Olkaria geothermal well sample, near OW716. The agricultural sample exhibited intermediate value between this range. Though the sample size was not large enough, the high mercury content present in some soil samples could be as a result of the geological influence in the area, as opposed to the agricultural activities. In a recent study, the presence of mercury has been shown by Munyithya [101] to exist in the geothermal well samples. This is further strengthened by the fact that in most countries, mercury compounds have been banned for use in agriculture [4] and so one does not expect much contribution from this. The low level of mercury in highway soil is expected because the use of mercury and its compounds as additives in gasoline and petrol, or in motor-vehicle manufacture is minimal, if not absent.

The samples analysed indicated that on a world-wide basis, the values were below the upper limit of 0.4 mg/kg in surface soils [29]. These values are also within the expected ranges of 0.01-0.3 mg/kg and 0.004-0.7 mg/kg as previously

reported [1,27]. It can tentatively therefore be said that there was no analytical evidence to confirm probable mercury pollution in the area studied. Any mercury present could be attributed to the background level, as a result of the recent volcanicity in the rift valley area [8].

#### 4.2.4.2 CADMIUM

The cadmium content in the soils increased in the following order: Virgin soil (0.562 to 0.718 mg/kg), Olkaria geothermal soils (0.479 to 2.668 mg/kg), Agricultural soils (1.277 to 2.501 mg/kg) and Highway soils (1.565 to 2.281 mg/kg). This shows that the high vehicle density along the Great North Road highway and agricultural activities employing use of agro-chemicals could both be major contributing factors towards elevated cadmium content in the soils. The geothermal activities at Olkaria also do contribute, to some extent, towards higher cadmium in the surroundings. This is as a result of the condensing vapours being spewed out by the geothermal wells. The condensed vapour was found to contain some cadmium.

The overall results show that the cadmium levels in the soils analysed was above the normal range of natural soils, 0.01 to 0.7 mg/kg [35]. It is likely

that this elevation of cadmium was as a result of use of phosphate rock fertilizers and sludge from both industrial and domestic sewage treatment works [1]. This is supported by the fact that Naivasha area has been subjected to modern extensive methods of farming for many years since the beginning of this century and a wide application of agro-chemicals has been practised. Another possible source of cadmium could be the burning, wear and tear of the motor-vehicle tyres [34]. This is because Naivasha lies along the busy Great North Road with a lot of worn out fragments alongside the road. The existing small scale light industries could also be a contributing factor to the high cadmium levels.

#### 4.2.4.3 LEAD

The lead content in the soils increased as follows: Virgin soil (11.16 to 14.88 mg/kg), Agricultural soils (18.279 to 28.982 mg/kg), Olkaria geothermal soils (12.133 to 37.497 mg/kg) and Highway soils (29.427 to 34.249 mg/kg). This shows that agricultural, geothermal and transportation activities all do contribute to the high lead content in the soil. Among these activities, agriculture seems to contribute the least. The large relative standard deviation of lead levels at the vicinity of

geothermal wells was due to the high variation in the environmental pollution from the geothermal wells. This is controlled by factors such as distance from the geothermal well, wind speed and direction. This is supported by the fact that the highest lead content (37.50 mg/kg) was registered for the soil just at the surface of well OW716. High lead content in the highway soils was probably as a result of the high traffic density along the Great North Road. Tetra-ethyl lead (TEL) is still used as an antiknock additive in some gasoline and motor-vehicle fuels in Kenya.

The overall results indicate that the levels of lead in soils from Naivasha area were in the lower limit of the ranges given for uncontaminated surface soil in various geographical regions of the world [44]. The values were also within 10-40 mg/kg, which is the range given for the acid series of magmatic rocks and argillaceous sediments [29].

### 4.2.5 LEVELS OF MERCURY, CADMIUM AND LEAD IN PLANT SAMPLES

The results for the analysis of the above elements in various aquatic and terrestrial plants are shown in Table 35.

#### 4.2.5.1 MERCURY

Only four plant samples of the following species: Savinia molesta, Tarchonanthus camphoratus and Nicotiana glauca were analysed for mercury. The levels ranged from 0.099 ± 0.003 mg/kg for Nicotiana glauca to 0.153 ± 0.034 mg/kg for Savinia molesta. Salvinia is a floating aquatic fern. Although it is environmentally troublesome to aquatic eco-systems, it can serve as a useful sink of heavy elements, especially mercury.

The results show that the levels of mercury in the four plant samples analysed were within the range 0.001-0.3 mg/kg for plants and vegetables as given in the literature [1].

TABLE 35. CONCENTRATIONS OF MERCURY, CADMIUM AND LEAD IN NAIVASHA PLANT SAMPLES in mg/kg (dry weight)[a]

| SAMPLE CODE, SPECIES & SITE | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ±S.D. (n = 2) |
|---|---|---|---|
| ALN Pl 2 (*Salvinia molesta*) 400 m N of Lake Hotel | --- | 1.130 ± 0.309 | 10.833 ± 1.795 |
| ALN Pl Elsa 13 (*Salvinia molesta*) Off Elsamere, near Hippo point | 0.153 ± 0.034 | 0.907 ± 0.391 | 10.340 ± 3.435 |
| ALN Pl Elsa (Pap) (*Cyperus papyrus*) 100 m E of Elsamere | --- | 0.731 ± 0.066 | 7.691 ± 0.910 |
| TPl OW714 (Lel) (*Tarchonanthus camphoratus*) | 0.142 ± 0.021 | 0.337 ± 0.078 | 9.261 ± 0.533 |

TABLE 35 (contd.)

| SAMPLE CODE, SPECIES & SITE | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ±S.D. (n = 2) |
|---|---|---|---|
| TP1 OW24 (*Schinus molle*) | --- | 0.358 ± 0.089 | 5.801 ± 1.080 |
| TP1 OW26 P1 1 (*Schinus molle*) | --- | 0.305 ± 0.006 | 5.426 ± 2.404 |
| TP1 5- I (ii) (*Schinus molle*) Agricultural, 1 km W of Railway Station | --- | 0.805 ± 0.295 | 8.677 ± 0.945 |
| TP1 1(a) (*Schinus molle*) Vet. Farm Res. Stn. | --- | 0.867 ± 0.464 | 9.846 ± 1.632 |
| (H)TP1 11 (*Schinus molle*) near Prison, 2 m E of Nakuru Road | --- | 0.861 ± 0.088 | 6.658 ± 1.877 |

TABLE 35 (contd.)

| SAMPLE CODE, SPECIES & SITE | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ±S.D. (n = 2) |
|---|---|---|---|
| TP1 OW26 P1 2 (*Solanum incanum*) | --- | 0.583 ± 0.157 | 8.468 ± 1.203 |
| TP1 9 (*Solanum incanum*) Kabati Estate, 2 km S of Naivasha town centre, 500 m E of road | --- | 1.110 ± 0.097 | 10.492 ± 3.665 |
| TP1 1(c) (*Solanum incanum*) Vet Farm Res. Stn. | --- | 0.979 ± 0.236 | 11.271 ± 1.021 |
| TP1 2 (*Solanum incanum*) Vet Farm Res. Stn. | --- | 0.857 ± 0.118 | 11.560 ± 1.318 |

TABLE 35 (contd.)

| SAMPLE CODE, SPECIES & SITE | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ±S.D. (n = 2) |
|---|---|---|---|
| TP1 3 (*Pennisetum clandestinum*) 1.5 km W of Railway Station, near shore | --- | 0.755 ± 0.451 | 5.941 ± 0.501 |
| TP1 6 (*Pennisetum purpureum*) Agricultural, 1 km W of Railway Station | --- | 0.580 ± 0.231 | 4.465 ± 0.197 |
| TP1 OW2 P1 (*Nicotiana glauca*) | 0.101 ± 0.001 | 2.164 ± 0.338 | 11.275 ± 5.000 |

TABLE 35 (contd.)

| SAMPLE CODE, SPECIES & SITE | MERCURY Mean ±S.D. (n = 4) | CADMIUM Mean ±S.D. (n = 2) | LEAD Mean ±S.D. (n = 2) |
|---|---|---|---|
| (H)TP1 3 (*Nicotiana glauca*) at bridge, near Vet. Farm Res. Stn. | 0.097 ± 0.024 | 2.961 ± 0.246 | 27.926 ± 4.131 |

[a] Leaves of the plants were analysed

A = Aquatic, LN = Lake Naivasha, Pl = Plant,

T = Terrestrial, OW = Olkaria well, H = Highway

Vet. Farm Res. Stn.= Veterinary Farm Research Station

S.D. = Standard deviation of the mean

n = number of sample replicates

--- Not determined

### 4.2.5.2 CADMIUM

Among the plant species analysed, Nicotiana glauca had the highest cadmium concentration (2.164 to 2.961 mg/kg (dry weight)). Tobacco is one of the plants which exhibit pronounced cadmium uptake from the soil [1]. It is also reported that the concentration of cadmium in tobacco may be relatively high (1-2 mg/kg) [35]. Since tobacco (Nicotiana tobacum) and Nicotiana glauca belong to the same genus and may have similar chemical characteristics, the high cadmium content registered by the latter species was close to that expected for tobacco.

For the different species analysed, it is apparent that the samples from Olkaria geothermal project had the least cadmium content. The main sources of cadmium are possibly the light industries present in the town, burning and tear of tyres [34] along the Great North Road, the use of phosphate rock fertilizers containing cadmium and sludge from the sewage treament works [1].

The overall results show that the cadmium levels in all the plant samples analysed were higher than the range (0.013-0.28 mg/kg (dry weight)) for plants grown in uncontaminated soils [29]. This could probably be due to the high cadmium content in the

soil, coupled with possibly high cadmium uptake rates for the corresponding plant species. The plants analysed in the present work were feed plants for both domestic and wild animals or generally wild plant species in the sampling sites. Most of the values given in literature pertain to food plants. Kabata-Pendias and Pendias [29] have pointed out that the threshold concentrations in feed plants may be a bit higher than those established for food plants and may differ for each kind of animal.

#### 4.2.5.3 LEAD

Among the plant species analysed, Nicotiana glauca had the highest lead concentration (11.275 to 27.926 mg/kg), while napier grass (Pennisetum purpureum) exhibited the lowest (4.268 to 4.662 mg/kg). The trend for lead concentration in different environments is similar to that already described for cadmium. Industrial and agricultural activities, plus heavy traffic density do contribute more lead in plants than the geothermal activity at Olkaria. It is also certain that the high lead content along the highway was as a result of tetra-ethyl lead additive in motor fuels and high uptake of lead from the soil by some plant species.

The overall results show that 5.9% of the plant samples registered higher lead concentrations than the entire range (<1.2 to 15 mg/kg) for uncontaminated plants [29]. This shows that most of the plant samples had concentration values within the expected range and so there was no significant lead pollution in plants from the area studied. This possibly arises from the fact that the lead content in the soil environment was within the normal range, as previously reported in the text.

## 4.3 COMPARISON OF AAS AND XRF RESULTS

The inter-comparison between AAS and XRF for heavy metal determination has been previously reviewed. According to Alala [37], AAS was found to be a better analytical technique than XRF when analysing liquid samples of very low concentrations (ppb range), while XRF superseded AAS in the analysis of solids or generally highly concentrated samples. Maina [72] compared the XRF and AAS results for the determination of copper, iron and zinc in sewage sludge analysis. He observed that the results for AAS were all about 10% lower than those for XRF, with correlation coefficients in the range of 0.79-0.92.

In the present work, an inter-comparison analysis of both AAS and XRF was done. Elemental sensitivity was increased for both analytical techniques by digestion of representative samples. This was done as described previously in the experimental section. The samples included fish, water, sediments, soil and plants. The inter-comparison data for both AAS and XRF for various digested samples is shown in Tables 36 and 37.

TABLE 36. INTER-COMPARISON RESULTS FOR MERCURY BY AAS AND XRF ANALYSIS

| SAMPLE CODE | SAMPLE TYPE | MERCURY CONNCENTRATION (ppm)[a] | |
|---|---|---|---|
| | | AAS (x) (n = 2) | XRF (y) (n = 2) |
| F3 | Tilapia fish | 0.5433 | 0.4534 |
| F4 | Tilapia fish | 1.0987 | 1.4684 |
| BT/1 | Biclogical tissue | 2.6588 | 3.4050 |
| BT/2 | Biological tissue | 3.3457 | 4.3753 |
| BT/3 | Biological tissue | 2.8517 | 3.9340 |
| FV | Tilapia fish | 0.397 | 0.5512 |
| LNW 2 | Water (near Lake Hotel | 0.021 | 0.02229 |
| LNP 1 (Elsa13) | Plant (*Salvinia molesta*) | 0.124 | 0.10392 |
| AgrSL 5 (Lucita farm) | Agricultural soil | 0.259 | 0.37293 |
| OW716S1 | Olkaria soil | 0.186 | 0.15146 |
| NVF7TIL7 | Tilapia fish | 0.069 | 0.04107 |
| NVF12BB2 | Blackbass fish | 0.0675 | 0.06573 |

Correlation coefficient 0.9982

Regression equation $y = 1.33876x - 0.051163$

[a] ppm = mg/kg for solids or mg/l for liquids

n = number of sample replicates

TABLE 37. INTER-COMPARISON RESULTS FOR LEAD BY AAS AND XRF ANALYSIS

| SAMPLE CODE | SAMPLE TYPE | LEAD CONNCENTRATION (ppm)[a] AAS (x) (n = 2) | XRF (y) (n = 2) |
|---|---|---|---|
| F2 | Blackbass fish | 2.545 | 2.45456 |
| F3 | Tilapia fish | 2.788 | 2.51140 |
| OW715 W | Olkaria water | 0.092 | 0.114 |
| OW22 St (i) | Olkaria steam | 1.6165 | 1.277922 |
| OW22 St (ii) | Olkaria steam | 2.245 | 3.06481 |
| LNP1 (Elsa 13) | Plant (*Salvinia molesta*) | 6.96716 | 5.22526 |
| SL4 (town) (i) | Naivasha soil | 35.55211 | 33.46052 |
| SL4 (town)(ii) | Naivasha soil | 31.59325 | 30.84812 |
| AgrSL 5 | Agricultural soil | 16.254 | 12.87959 |
| LNSD 1 | Sediment (Lake Naivasha) | 26.11291 | 26.52580 |
| LNSD 4 | Sediment (Lake Naivasha) | 10.66639 | 9.93739 |
| NVF7TIL7 | Tilapia fish | 1.40333 | 1.38696 |
| NVF12BB2 | Black bass | 0.88624 | 0.56171 |
| NVF17BB7 (i) | Black bass | 1.99069 | 1.79355 |
| NVF17BB7 (ii) | Black bass | 1.53600 | 1.02803 |
| MP1 | Geological ore | 1.511% (78.3%) | 1.514% (78.4%) |
| Correlation coefficient | | | 0.99999996 |
| Regression equation | | y = 1.001804x - 0.628688 | |

[a] ppm = mg/kg for solids or mg/l for liquids
n = number of sample replicates

### 4.3.1 MERCURY

There seems to be very high correlation between AAS and XRF data for this element. The regression equation also shows that for mercury concentrations below 0.151 ppm, the XRF values were lower than for AAS, but the relation reversed for higher concentrations. This suggests that for low mercury concetrations, AAS is more sensitive. This is probably because the detection limit of mercury with Cold Vapour AAS is lower than with XRF. However, there was good agreement between the two techniques as reflected by the high correlation coefficient, close to 1. When most of the water, soil and plant samples whose mercury data is reported in the present work were analysed by XRF after either liquid chelation or pelletation without digestion, mercury was not detected [101]. Hence, by special digestion techniques of the samples, there was improvement in sensitivity of mercury for both analytical techniques.

### 4.3.2 LEAD

There was excellent correlation between the AAS and XRF data for this element. The regression equation also shows that the AAS data was higher than that for XRF, with the relation reversing for higher concentrations. Within the sample concentration range investigated, it can be deduced that AAS gave better results. However, the regression and correlation coefficients were both closer to 1 than those obtained for mercury, showing better agreement between the two analytical techniques in the determination of lead.

# CHAPTER V

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 CONCLUSIONS

In the present work, review of analytical procedures and analysis of mercury, cadmium and lead in Naivasha area have revealed the following:

(a) AAS absorbance signal for mercury, cadmium, lead and X-Ray Fluorescence intensity signal for mercury and lead were found to be dependent on pH. In AAS, maximum values were obtained in the lower pH region, of less than 1.5, probably due to precipitation at higher pH values, reducing the concentration of ions in solution. On the other hand in XRF, maximum element recoveries were in the pH region around 2.0.

(b) Cold Vapour AAS (CVAAS) absorbance signal for mercury depended on the type of reducing agent used. Within the calibration range, $SnCl_2$ exhibited higher absorbance values, lower detection limit and higher stability than $NaBH_4$. When a set of digested samples were left overnight in $KMnO_4$ oxidizing medium, higher correlation and regression coefficients were

obtained for the two reducing agents than with other sets when the digestion and oxidation were done the same day. This shows "nearly complete" destruction of organic matter to release all mercury.

(c) The sensitivity of different AAS equipments for the determination of cadmium and lead depended on the analytical instrument model and the lab site where the analysis was done. Determination of lead at 217 nm was more sensitive than at 283.3 nm, by a factor of about 2.6 times, with the lower wavelength giving a lower detection limit.

(d) When the same volume of acid mixture used for digestion of samples was incorporated in the calibration standards, the stability for the three elements was high. The same set of calibration standards could be used for a period of up to seven weeks without significant variation in the element recoveries. However, the stability of the standard solutions increased with increasing element concentration, due to decreasing relative rate of adsorption of ions onto the containers. The presence of the three elements (mercury, cadmium and lead) did not appear to interfere with the absorbance signal of

a particular element and hence with the concentration readings of the elements.

(e) For the determination of mercury with CVAAS, the digestion acid mixture containing 3:1:1 ($HNO_3$,$HClO_4$,$H_2SO_4$) (vol/vol) gave sensitivity enhancement factors in the range 5.6-149.1 over 3:1 ($HNO_3$,$HClO_4$) in the analysis of fish samples. This shows that the presence of sulphuric acid in the digestion matrix was essential for mercury determination.

(f) Presence of the reduction/oxidation reagents, $KMnO_4$ and $NH_2OH.HCl$ did not affect the concentration results for cadmium significantly. However, this mixture interfered with the lead signal, by displaying an enhancement factor in the range 4.3-157.8, probably due to $K^+$ ion effect and chemiluminescence caused by the presence of manganese.

(g) Due to the volatile nature of mercury, it tends to get lost at higher temperatures. It was therefore found that mercury concentration reading was higher at 50 $\pm$ 3$^oC$ than at 70$^oC$ (which is used by some researchers) and so the

lower temperature range had to be used in the CVAAS extraction of mercury for the present work.

(h) The trend of the concentration sensitivity vs. temperature curve for cadmium was different from that of mercury. This was because cadmium is less volatile and its extraction could therefore be done at 80-130$^{o}$C without its loss from solution.

(i) The proposed analytical procedures for the extraction of mercury, cadmium and lead were fairly reliable. This is because they were tested on standard reference materials and high element recoveries in the range of 77.7-110.6% were obtained, relative to the mean certified values.

(j) Mercury, cadmium and lead were present in the water samples, mainly in the organic forms. This was shown by the large differences between the results obtained for undigested samples and those obtained after digestion. Consequently, in order to determine total elemental concentrations, all the samples had to be digested.

(k) The high correlation and regression coefficients (close to 1) between the AAS and XRF data for mercury and lead indicate excellent agreement

between the two techniques when all the samples were digested.

(l) The concentration of mercury in most of the fish from Naivasha area was below the maximum recommended level (0.5 mg/kg). However, the slight elevation in some fish could be due to bio-methylation of mercury in the aquatic environment. The levels of cadmium and lead were lower than the maximum limits stipulated by the World Health Organization (WHO), suggesting no probable pollution of these elements in fish.

(m) The levels of mercury, cadmium and lead in most water samples from Naivasha area appeared to be higher than the maximum permissible levels in drinking water as recommended by WHO. This was attributed to the geology of the surrounding area and the proximity to the sewage treatment works. The sewage effluent might have contained high levels of these heavy elements, thus increasing their content in the aquatic systems.

(n) The concentration of mercury, cadmium and lead in the sediments compared well with the recommended and literature values.

(o) In the soil samples from Naivasha, the levels of mercury and lead were well within the normal range for uncontaminated surface soils in various geographical areas of the world. On the other hand, the cadmium content in most of the soils was above the normal range for the natural soils as given in the literature, indicating probable pollution. Possible sources of pollution in this area include use of phosphate rock fertilizers and sludge from both industrial and domestic treatment works, wearing out or burning of motor vehicle tyres along the highway and operation of small scale mercantile industries around the town.

(p) The level of cadmium in the plant samples analysed was higher than the range for plants grown in uncontaminated soils as given in the literature. This was reflected by the high cadmium content in the soils, though atmospheric cadmium pollution could not be ruled out. Mercury and lead levels in most of the plants were within the expected ranges.

## 5.2 RECOMMENDATIONS

According to the results and conclusions indicated above, the following have been recommended for further research:

(a) For effective determination of heavy elements by AAS, it is important to ensure that suitable conditions such as pH, digestion medium, time and temperature are employed in order to obtain accurate and reliable data.

(b) Thorough research should be carried out in order to obtain the optimum pH and complexing time for the determination of most of the commonly investigated heavy metal elements with X-ray Fluorescence Analysis.

(c) While using XRF for the determination of heavy metal elements in different type of samples (especially biological tissues and liquids), it is advisable to digest them in order to obtain detectable amounts comparable to those obtained with AAS.

(d) Research on the determination of heavy metals in Naivasha and other areas should be carried out more comprehensively in order to obtain a clear picture of the heavy metal pollution in the environment. Special attention should be given to the atmospheric environment, sewage sludge and the different types of agrochemicals (pesticides, herbicides and fertilizers) used in Naivasha area, in order to trace the source of the high cadmium content in the soils, plants and the high mercury, cadmium and lead levels in unfiltered water.

(e) The emission of lead along the Great North Road should be monitored in order to determine the extent of its pollution in soils and plants with respect to traffic density.

(f) More research should be carried out in order to determine the role played by the volcanic origin of soils and geothermal projects in enhancing heavy element concentrations in their respective vicinities.

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