Chapter II

MATERIALS AND METHODS

The Chao-Praya River and the Area of Study

The Chao-Praya River originated from four major tributaries, namely the Ping, the Wang, the Yom and the Nan Rivers. After coming into concurrence at their lower reaches, they form the great Chao-Praya River flowing through the central plain for about 365 km towards the Gulf of Thailand. This vast area (about 2.5 million acres) along both banks is open as paddy-cultivated land. The whole system of the Chao-Praya River flows down broadening as it does so, and enters the shallow merky waters in the northern part of the Inner Gulf of Thailand.

The Gulf of Thailand is the shallow part of the continental shelf of the South China Sea, and is situated between the Thai-Malayan and the Indochina penisulae. The coast of the vast central plain is to the North. The gulf is composed of two parts, the outer and the inner gulf. The inner part with the square shape lying on the northernmost head of the gulf has an approximate area of about 10,000 sq.km and an average depth of about 15 m. However, generally speaking, the mountains in the North, the flat central plain, and the Gulf of Thailand in the South all influence the hydrographic characteristics of the lower section of the Chao-Praya River.

The region is mainly influenced by the monsoons. The southwest monsoon, or what is known as the summer monsoon, is roughly from May to September, bringing appreciable rainfall throughout the region. This period

is known as the rainy season. The monsoon does not always bring continuous rainfall since a period of drought usually exists in June or July which is due to local atmospheric disturbances. From November to February, the northeast monsoon season is controlled by the northeast wind. It is known as the cool season because it is a relatively dry and cool period of the year as the northeast wind blows from the anticyclonic centre in Siberia. The region along the east coast of the Thai-Malayan penisula receives rainfall during this period of the year. The influences of the cyclonic disturbances can sometimes either cause the region to flood, or produce a normal or drought conditions. The arrival of substantial rainfall in Thailand, is then, the occurrence of the southwest monsoon through anti-cyclonic disturbances. The water discharges of the Chao-Praya River also depend upon the amount of rainfall, which is usually high from September through December and shows a drop from March to June. It has been estimated that the total annual rainfall of all major rivers in the country would be in the order of 220,000 million m^3 of which a million m^3 flows through the Chao-Praya River annually (Kambhu, 1961).

Ideal characteristics of tides in the Gulf of Thailand are represented by a combination of semi-diurnal and diurnal types, showing the greatest tidal range during the dry season. The tidal influences along with saline intrusion can be detected as far as Nonthburi, a province 62 km from the river mouth. This phenomena is drawing attention from authori ies seeking solutions to the problems of how to prevent vegetation, orchards and farms from being destroyed by the salty water intrusion, especially during the summer months when the down-fresh water is at a minimum.

Regarding Thailand's geologic condition, the top soil consists of deteriorated material which is mainly derived from sandstone. Down to a depth of about 1 to 5 meters a layer of impervious slippery blue clay dominates. The near-surface layers are bleached through by the hot tropical sun during the daytime and finally become lateristic. This type of clay, like that in the central plain, makes the land suitable for cultivation.

Upon meandering through various kinds of soil on the way to the mouth, the Chao-Praya River carries particles of various sizes which are principally of clay and silt size. The sedimentary particles carried along with the river are mostly in suspension, whereas sand grains and cobbles are left on the river bed. The river bottom in the Bangkok area is chiefly of silt and clay. These particles are transported in suspension with quantity of sand on the river bed. They travel along the lower part of the river down to the mouth and may be deposited all along the water course when the conditions are favourable. The silting activities in the river are affected by the upland supply of silt and clay particles and occur almost exclusively during the rainy season. Other factors also govern the depositing processes.

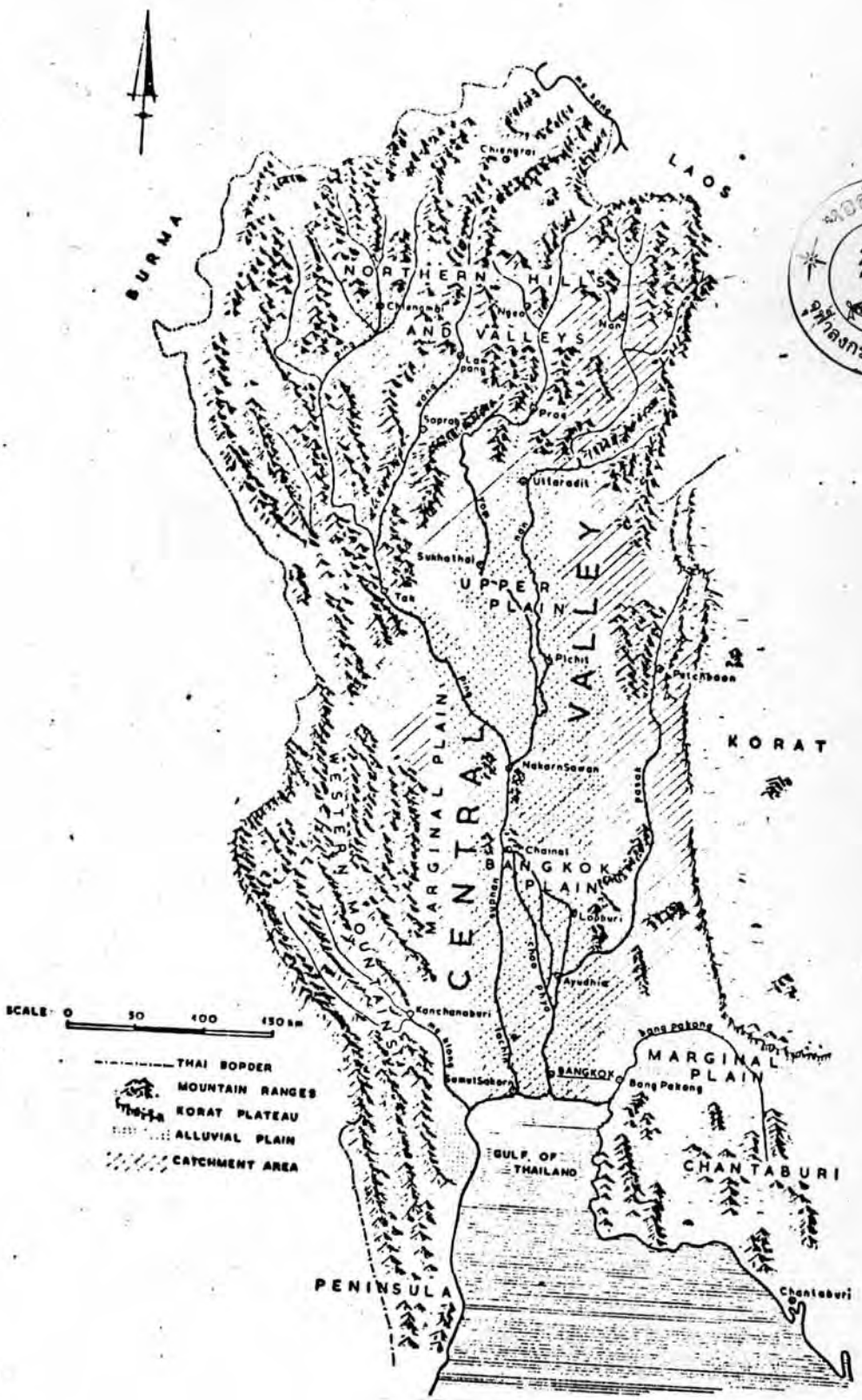

Figure 2: Physiographic Provinces of the Chao-Praya River System (Kambhu, 1961)

Materials and Methods

Many attempts have been made to determine quantitatively the concentration of trace amounts of lead and mercury in the aquatic environment. The methods of determination differ considerably due in part to the forms of the two metals involve in the processes. The conventional methods for lead include:

a) the spectrographic analysis of residues after the evaporation of the samples in acidified condition,

b) the colorimetric analysis of the products after concentrating the chemically produced complexes using a solvent extraction of the resulting lead-dithizone complex (which produces stable red colouration of the complex from the interaction of lead and dithizone - Diethyl dithiocarbamate,

c) the concentration of solvent extraction with APDC (Ammonium pyrolidine dithiocarbamate) in the presence of MIBK - Methyl - isobutyl-ketone (Parker, 1972, Gardner, 1974). The subsequent analysis by the use of the atomic absorption spectrophotometric method is becoming more popular for rapid and precise analysis.

d) Methods of analysis using more complicated instruments such as NAA or Neutron Activation Analysis, these are used where the instruments are available.

e) Isotope dilution, the known amount of isotopic lead is spiked into the sample and after a period of aging and acidification the amount is withdrawn from the solution and finally determined isotopically.

f) The direct determination of lead by the use of the atomic absorption spectrophotometer after complete digestion with suitable acid mixture. This method is reported to be one of the most rapid and reliable measures (Ward et al., 1975, Welch et al., 1975, Mierau, 1975).

Of all of the processes currently employed, the most recommended methods of analysis are Neutron Activation Analysis, analysis using atomic absorption spectrophotometer and the isotope dilution technique. The discrepancy of the results from these various methods is said to be caused mainly by the contamination of lead during collection and laboratory operation, so in order to minimize the undesired contamination great care has to be taken during the operation.

For the determination of total mercury in the various samples, the methods of determination also vary widely. The colorimetric method of mercury-dithizone complex results from the reaction of mercury and dithizone, but the procedure is reported to be less satisfactory due to the relative lack of sensitivity. Neutron Activation Analysis is suitable for the rapid determination of a large number of samples but the method is limited by the availability of the instrument. The Neutron Activation Analysis is also enhanced by the enrichment and chemical separation of the sample determined. In modern analyses of mercury, the most recommended method is to use the flameless atomic absorption spectrophotometer or cold vapour atomic absorption spectrophotometer. The method is recommended as being rapid and reliable for the determination of trace amounts of total mercury content in the samples.

Sampling and Storing Techniques for the Samples

Two periods of sampling were assigned to the area of study. The The study area was divided into 9 stations. The same sampling techniques were exercised at stations I, II and III in the inner gulf through station IX in the river.

1. Water sample. The surface-water samples were sampled and stored in 2½-litre polyethylene bottles where a pH of about 2 was kept throughout the time of storation by putting 2 ml of HNO_3 per 1 litre of the water sample.

2. Biological sample. Fish, prawns and molluscs were collected and at a certain station seagulls were also collected. All the biological samples were then forzen.

3. Sediment. A core sampler was employed to collect the sediment cores by using the PVC core linings. Two cores were also forzen.

The sampling procedures were repeated at each station to collect the three types of samples. The water and sediment samples from stations I, II and III were collected on January 28, 1976 during a cruise of the research vessel "Fishery I". Collections were also made on May 18, 1976. The cores, water and biological samples from station IV to IX inclusive were collected during the cruise of the "Marine Science II". Water and biological samples from stations IV to IX inclusive were again collected during May 17-19, 1976 using the same techniques and the same sites. A diagram of the sampling sites is given in Figure 3. The samples of sediment cores, the water and biological samples were consequently collected in January and May.

Stations:

I	Lat 13° 10′ 00″ N., Long 100° 30′ 00″ E.
II	Lat 13° 25′ 00″ N., Long 100° 35′ 00″ E.
III	Lat 13° 29′ 00″ N., Long 100° 36′ 00″ E.
IV	Below South Bangkok Power Plant
V	Above Prapadaeng District
VI	Above Summit Oil Refinery
VII	Bangkok Bridge
VIII	Inlet of Padungkrungkasaem Canal
IX	Above Rama VI Bridge

At all stations, the sampling were made at low tide except at station IV+V at high tide.

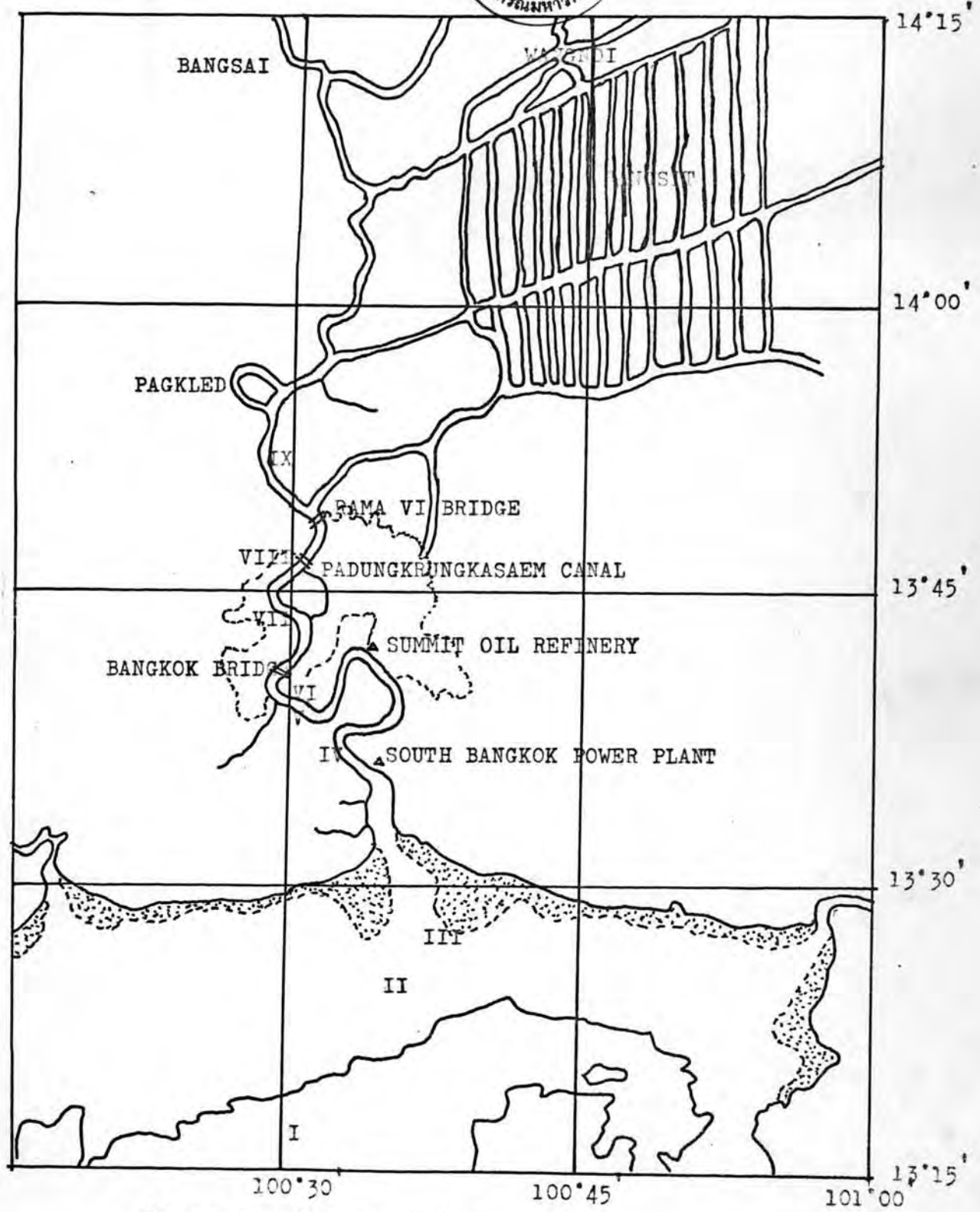

Figure 3: Sampling Sites

Pretreatment of the Samples

All the preserved samples were pretreated before being analysed. Each sediment core was treated as follows:-

- running water was run over the frozen PVC core lining to melt the outside of the core
- the sediment core was extruded from the core lining by forcing a piston through the bottom of the core onto a plastic plate
- the extruded core was sectioned into slices of 1-cm thick. The desired layer and portion of each slice is illustrated in Figure 4

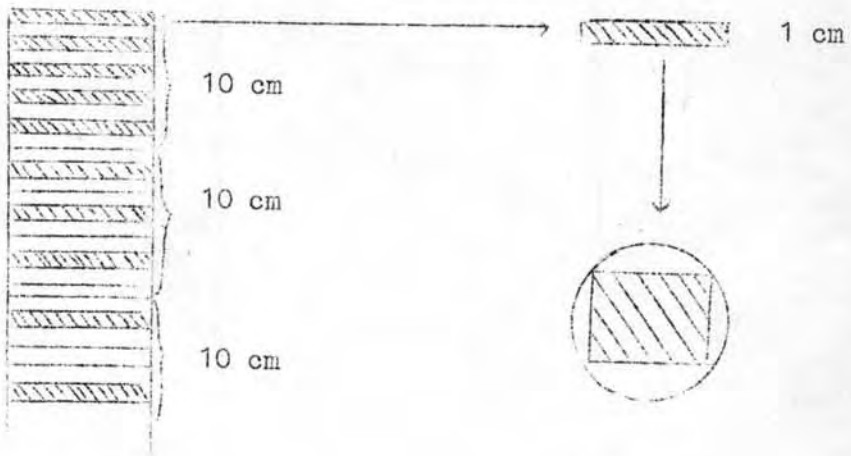

Figure 4. Diagram for Core Operation

The water samples were filtered to separate the particulate species from the dissolved species.

The biological samples were first measured and weighed. Only the pectoral muscle in fish and the chest muscle in birds were disected. For molluscs and prawns, the whole body was wrapped with aluminium foil and disected in entirety.

Determination of the Total Lead Concentration

1. Instruments used

1.1 Varian Techtron Atomic Absorption Spectrophotometer Model AA-4 and Model AA-5.

2. Reagents used

2.1 Acid mixture; 2 volumes of redistilled nitric acid, HNO_3, was mixed with 1 volume of concentrated perchloric acid, $HClO_4$.

2.2 Stock solution of lead; 1.8308 gm of lead acetate $(CH_3COO)_2 Pb \cdot 3H_2O$ was dissolved in 1.00 ml of concentrated acetic acid, CH_3COOH. The content was then diluted to 100 ml, giving a concentration of 1.00 mgPb/ml

2.3 Working solution of lead standard; 0.1 ml of the standard solution was diluted to 100 ml with acid mixture to give a concentration of 1.00 ugPb/ml The standard solution wi the concentration of 0.1, 0.5, 1.0, 5.0 and 10.0 ppm was used.

2.4 Buffer solution; concentrated hydrochloric acid, HCl, and ammonium solution, NH_4OH, were used as buffer adjuster-solution.

2.5 APDC solution; 1.00 gm of APDC was dissolved in the tridistilled water and the content transfered into a separating funnel. 10.00 ml of MIBK was added and the solution was shaken vigorously for 3 minutes. The two phases were carried out separately and the lower layer was drained through a filter paper. A new solution was prepared daily.

2.6 MIBK solution; redistilled MIBK was used in the extraction procedure.

3. Sample Size

3.1 Sediment sample of about 1.0-2.0 gm wet weight.

3.2 Water sample separated into particulate and dissolved varieties. Particulate species on the milliporefilter, both whole and dissolved, required $\frac{1}{2}$ litre of filtrate.

3.3 Biological sample of about 1.0-5.0 gm wet weight.

4. Digestion of the Samples

4.1 The biological and sediment samples were wet ashed. The sample was weighed in a 250-ml Erlenmeyer flask and the acid mixture (about 20.0 ml) was added.

4.2 The samples were heated in flasks on a hot plate to $95\pm2^{\circ}C$ for hours, or until a dense white fume ceased to appear, indicating that the reaction was complete.

4.3 The samples were cooled to room teperature and the sediment samples were centrifuged at 2,000 rpm. for about 10 minutes. The clear supernatant was then transfered to a 100-ml polyethylene bottles. The digested samples were also treated in the same way.

4.4 The aliquot of $\frac{1}{2}$ litre of the water samples was digested in the same procedure.

5. Extraction Procedure

5.1 The pH of the digested samples was adjusted to around 2 by adding the adjuster-solution.

5.2 An APDC solution of about 5.0 ml was added to the samples and the pH of the solution was raised to around 4.8-5.2 by adding the same adjuster-solution.

5.3 About 50 ml of the resulting solution was transfered into a 250-ml separating funnel.

5.4 The solution in the funnel was throughly mixed and shake vigorously with 10 ml of MIBK solution for 3 minutes.

5.5 The two layers were separated and the lower layer was drained off. The upper dark layer was stored in a stoppered glass tube. If the upper layer showed the presence of colloidal or suspended particle, the solution was filtered or centrifuged at 2,000 rpm. for 5 minutes.

5.6 The chelated samples were analysed for lead content instrumentally from the stored extracted samples. It was possible to determine the lead content within 5 hours.

6. Direct Measurement of Lead.

Only the water samples were determined by using the chelation technique using the Varian Techtron AAS Model AA-5. The digested biological and sediment samples were determined in their aqueous forms by using the Varian Techtron AAS Model AA-4.

The reagent blanks were duplicated during each operation.

Determination of the Total Mercury Concentration

1. Instruments used.

1.1 Mercometer Model 20001-(Anti Pollution Technology Corporation, Holland, Michigan, USA.).

2. Reagents used

2.1 Reducing agent; 20 gm of hydroxylamine, $NH_2OH.HCl$, 20 gm of NaCl, 33 gm of $SnCl_2.2H_2O$, 1.0 gm of Hydrazine sulphate and 9.0 ml of H_2SO_4 (Con.) were mixed together in a 1000-ml beaker and the content was diluted to 1000 ml with tridistilled water.

2.2 Acid mixture; 1 volume of concentrated redistilled HNO_3 were 1 volume of concentrated reagent grade H_2SO_4 were mixed together.

2.3 Saturated $K_2S_2O_8$; $K_2S_2O_8$ and tridistilled water were dissolved in the 1000-ml bottle.

2.4 Stock solution of mercury; 1.350 gm of $HgCl_2$ and 0.1 N HCl were dissolved and then diluted to 1000 ml in the 1000-ml volumetric flask. This solution gave a concentration of 1.000 mgHg/ml.

2.5 Standard solution of mercury; 0.1 ml of the stock solution was pipetted into the 100-ml volumetric flask and was diluted to 100 ml giving a concentration of 1.00 μgHg/ml.

2.6 Working standard of mercury; 0.1 ml of standard solution was pipetted into the 100-ml volumetric flask and diluted with tridistilled water to 100 ml. This solution gave a concentration of 1.00 ngHg/ml. Standards of 10, 30, 50, 100 and 150 ngHg were used during the analysis.

3. Sample Size

The biological and sediment samples used in the determination were the same as those in the determination for lead. The dissolved species in the water sample of about 180 ml was used for each sample.

4. Digestion of Samples

4.1 The weighed samples were put into 250-ml Erlenmeyer flask.

4.2 20.0 ml of acid mixture was added into each flask containing the sample.

4.3 The flask were loosely capped with glass stoppers and digested in a water bath to 95±2°C for 20 minutes or until the digest was clear.

4.4 The 10.0 ml of saturated $K_2S_2O_8$ was added to each flask and then 50 ml of tridistilled water (except in the case of the water sample). The solution of all non-aqueous samples was then swirled in an Erlenmeyer flask.

4.5 The content in the flask in a water bath was then allowed to digest at the indicated temperature for a further 2 hours.

4.6 After the digestion was completed, all the flasks containing the digested samples were removed from the water bath and allowed to cool to room temperature.

5. Measurement of the Total Mercury

5.1 The total content of the digested samples were quantitatively transfered to a 500-ml two-neck bottle.

5.2 40 ml of reducing agent was added to, the bottle and the ground glass stopper was immediately put into place. The solution was then swirled gently for 30 seconds.

5.3 The scale reading was read from the scale readouts, and the reagent blanks were duplicated for each analysis.

Statistical Analyses

1. Mean Value ($\bar{X}$). Since the analyses were carried out many times in relation to various samples, then the mean value can be calculated as follows:-

$$\bar{X} = X_1+X_2+X_3+ \ldots\ldots\ldots\ldots X_n/N$$

where $X_1, X_2, X_3 \ldots\ldots\ldots\ldots X_n$

X_n = mean value of each sample

N = total number of samples

2. Standard Deviation (σ). The standard deviation value can be calculated for each mean value of samples from the following relationship:

$$\sigma^2 = \sqrt{\frac{\Sigma(X-\bar{X})^2}{N}}$$

3. Analysis of variance. The analysis of variance was used to determine the difference in the mean values of the varieties of water in each season concerned. The test was from the hypothesis that all mean values were equal, assuming that the variances of each set were equal (Snedecor, 1956, Mendenhall, 1969).

$$H_0 : M_1 = M_2 = M_3$$

mean value of each species in the water sample

$$F = \frac{\text{Mean square of sample means}}{\text{Mean square of individual}}$$

If the calculated value of F was less than the value of F from the Variance Analysis Table at the same degree of freedom, the hypothesis was accepted. There would be no statistical difference in varieties in each season for the water sample. If the calculated value of F was greater than the one from the Variance Analysis Table, the hypothesis was rejected. So, for the varieties in each season there would exist significant difference of the water sample.