

CHAPTER III

EXPERIMENTS



3.1 Materials

3.1.1 Preparation of Standards

Stock solutions of $\text{Cd}(\text{NO}_3)_2$, $\text{Cu}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$ and $\text{Zn}(\text{NO}_3)_2$ in a concentration of 1 mg/cm^3 each, were purchased from BDH. Standard solutions with other concentrations were prepared by diluting appropriate amount of the stock solutions with tri-distilled water.

3.1.2 Sample Collection and Storage

Samples of sea water were collected from the east coast of the Gulf of Thailand at the depth of 0.5 m and 10 m from the surface and also at the bottom from several stations around the province of Chonburi, Rayong and Chanburi (Fig 3.1). The sampling operations were performed on February 20-23, 1979, by the Marine Fishery Division, Department of Fishery. After collection, the samples were filtered through 0.45μ polypropylene millipore filters. Aliquots of 5 dm^3 were stored in polyethylene bottles which were previously cleaned with concentrated analytical grade nitric acid and tri-distilled water. In order to prevent the loss of trace elements by adsorption on the polyethylene container wall and bacteria

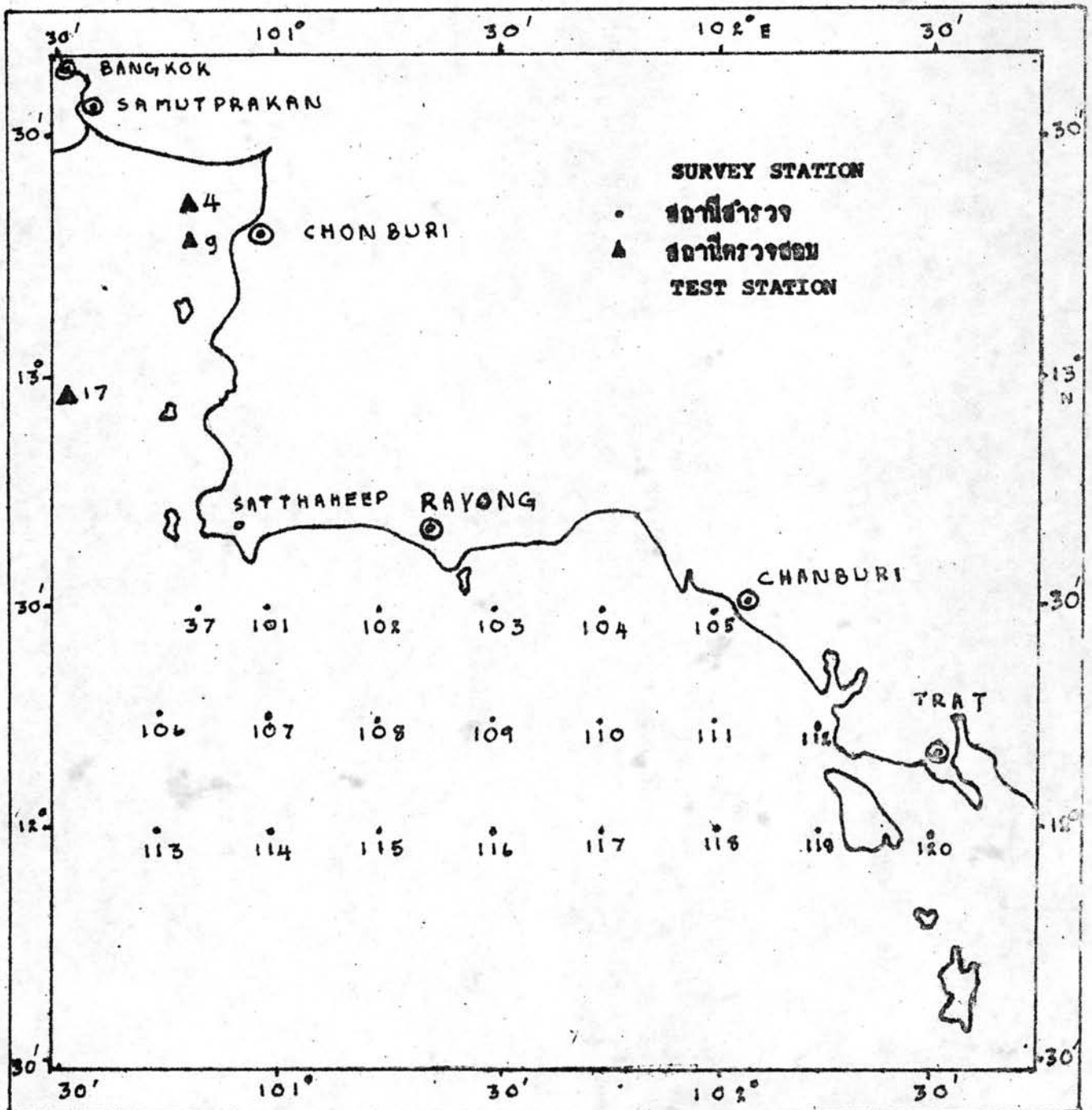


Fig. 3.1 Location for Sampling along the east coast of the Gulf of Thailand 1979.

growth as well as to stabilize the trace elements and to destroy the trace elements which are bound in colloidal particles, concentrated nitric acid was added to make a 1% acid solution.

In order to evaluate the precision of the methods, replicate analyses were carried out for all four elements. A duplication of both methods required at least 16 dm^3 sea water, i.e., 8 dm^3 for each method. In order to obtain sufficient volume of sample, sea water from two neighbouring stations are mixed as recorded in Table 3.1 A total of five samples were thus attained.

3.1.3 Reagents

Buffer solutions of pH 4, 5, 6, 7, 7.6 and 8 were prepared as follows:

An acetate buffer, pH4, was prepared by mixing 150 cm^3 0.1 M sodium acetate with 850 cm^3 0.1 M acetic acid.

An acetate buffer, pH5, was prepared by mixing 640 cm^3 0.1 M sodium acetate with 360 cm^3 0.1 M acetic acid.

A citrate buffer, pH6, was prepared by mixing 404 cm^3 0.1 M sodium hydroxide with 596 cm^3 0.1 M sodium citrate (21.014 g citric acid + 200 cm^3 1M sodium hydroxide dilute to 1 dm^3).

A phosphate buffer, pH7, was prepared by mixing 612 cm^3 1/15 M disodium hydrogen phosphate with 388 cm^3 1/15 M potassium dihydrogen phosphate.

Table 3.1 Preparation of Sea Water Samples .

Date of Sample Collection	Sample No.	Station	Depth at Collection point (meter from surface)	Total Depth of the Station (meter)	Volume of Aliquot Collected (dm ³)
21/2/79	1	17	0.5	18	5.0
	1	17	10.0	18	5.0
	1	17	17.0	18	5.0
22/2/79	1	37	0.5	26	5.0
	1	37	10.0	26	5.0
	1	37	25.0	26	5.0
22/2/79	2	106	0.5	23	5.0
	2	106	10.0	23	5.0
	2	106	22.0	23	5.0
22/2/79	2	113	0.5	35	5.0
	2	113	10.0	35	5.0
	2	113	34.0	35	5.0
22/2/79	3	107	0.5	20	5.0
	3	107	10.0	20	5.0
	3	107	19.0	20	5.0
22/2/79	3	101	0.5	15	5.0
	3	101	14.0	15	5.0
22/2/79	4	108	0.5	25	5.0
	4	108	10.0	25	5.0
	4	108	24.0	25	5.0
22/2/79	4	102	0.5	18	5.0
	4	102	10.0	18	5.0
	4	102	17.0	18	5.0
23/2/79	5	115	0.5	40	5.0
	5	115	10.0	40	5.0
	5	115	39.0	40	5.0
23/2/79	5	109	0.5	25	5.0
	5	109	10.0	25	5.0
	5	109	24.0	25	5.0

A phosphate buffer, pH7.6, was prepared by mixing 885 cm³ 1/15 M disodium hydrogen phosphate with 115 cm³ 1/15 M potassium dihydrogen phosphate.

A tetraborate buffer, pH8, was prepared by mixing 558.5 cm³ 0.1 M sodium hydroxide with 441.5 cm³ 0.05 M sodium tetraborate.

A 2% (W/V) aqueous sodium diethyl-dithiocarbamate was prepared daily and filtered before use.

A 1% (W/V) aqueous ammonium pyrrolidine-dithiocarbamate was prepared daily and filtered before use.

The reagents used are summarized in Table 3.2 together with the production sources.

3.1.4 Preparation of Chelex Columns

The glass column, 8 mm in diameter and 30 cm in length was closed at one end with a piece of sintered glass over a teflon stopcock. The column was then filled with tri-distilled water. An aqueous slurry of chelex-100, Na-form, with a particle size ranging between 50-100 mesh was poured into the column to a height of 10 cm. The upper end of the column was stoppered with another piece of sintered glass.

3.1.5 Regeneration of Chelex Resin

The ion exchange resin, chelex-100 was stirred at room temperature with excess 1M nitric acid. The resin was washed thoroughly twice with tri-distilled water, then stirred with

Table 3.2 Reagents and the Production Sources.

Reagent (A.R. grade)	Company
Ammonium pyrrolidine-dithiocarbamate 98% min	BDH
Chelex-100 50-100 mesh Na-form	Bio Rad Laboratories
Chromosorb W-DMCS 60-80 mesh	PACKARD INSTRUMENT
Chromosorb W-DMCS 80-100 mesh	PACKARD INSTRUMENT
Chromosorb W-DMCS 100-120 mesh	PACKARD INSTRUMENT
Citric acid	RIEDEL DE HAEN AG SEELZE
Disodium hydrogen phosphate	MERCK
Potassium dihydrogen phosphate	MERCK
Quartz wool	RIEDEL DE HAEN AG SEELZE
Sodium acetate	MERCK
Sodium diethyl-dithiocarbamate	J.T. BAKER
Sodium tetraborate	RIEDEL DE HAEN AG SEELZE
Absolute ethanol	MERCK
Acetic acid (glacial)	RIEDEL DE HAEN AG SEELZE
Chloroform	BDH
Hydrochloric acid	RIEDEL DE HAEN AG SEELZE
Nitric acid	RIEDEL DE HAEN AG SEELZE
Phosphoric acid	RIEDEL DE HAEN AG SEELZE

excess 1M excess sodium hydroxide. The resin was finally washed thrice with tri-distilled water.

3.1.6 Preparation of Chromosorb Columns

The glass column, 8 mm in diameter and 30 cm in length was closed at one end with quartz wool. The column was then filled with chromosorb W-DMCS with a particle ranging between 100-120 mesh, to a height of 2 cm. The upper end of the column was stoppered with quartz wool. Absolute ethanol was allowed to pass through the column to displace air in the void volume. The ethanol was then displaced by tri-distilled water.

3.1.7 Regeneration of Chromosorb

In regeneration, the chromosorb W-DMCS was washed thrice with absolute ethanol and then rinsed with tri-distilled water.

3.2 Apparatus

3.2.1 Atomic Absorption Spectrophotometer.

A Varian-Techtron model AA6 atomic absorption spectrophotometer was used in the present study. Hollow cathode lamps were used as radiation sources. The optimum conditions were set according to the manufacturer's recommendation, which are summarized in Table 3.3. The results are read by a digital readout which displays only temporary. A picture of the Varian-Techtron model AA6 atomic absorption spectrophotometer (AAS) is reproduced in Fig. 3.2.

Model AA-6 Atomic Absorption Spectrophotometer

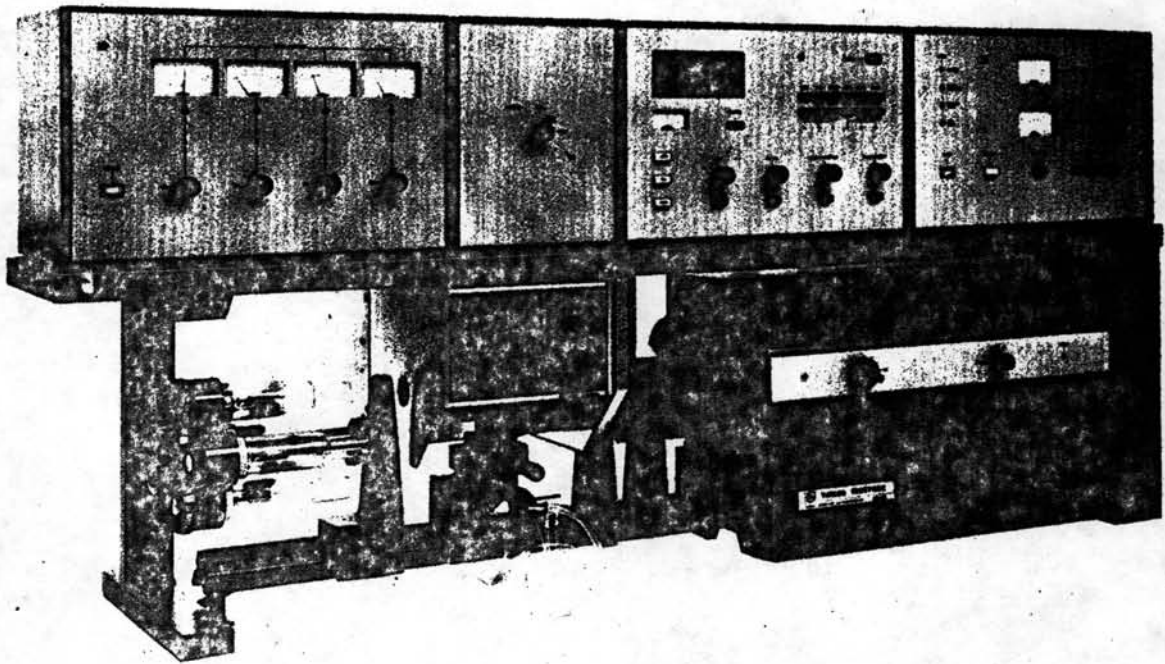


Fig. 3.2 Varian-Tehtren model AA-6.

Table 3.3 Operation conditions for the analysis of Cd, Cu, Pb and Zn by the Varian-Techtron AA-6 atomic absorption spectrophotometer.

Element	Light source	Lamp current mA	Wave length nm	Slit width μm
Cd	Cd-lamp	3	228.8	0.5
Cu	Cu-lamp	3	324.75	0.5
Pb	Pb-lamp	5	283.3	0.5
Zn	Zn-lamp	5	213.86	0.5

3.2.2 pH-meter

A Beckman Zeromatic II was used for the measurement of pH.

3.2.3 Suction-pump

A Welch Duo-Seal pump was used for drying the solid support after the adsorption of the metal 1-pyrrolidine-dithiocarbamate.

3.3 Preparation of Stripped Sea Water

Buffer solution of pH7.6 was allowed to flow through a column of chelex-100, 20 cm in height until the pH of the effluent remained the same as the feed. A volume of 20 dm³ of sea water, buffered at pH7.6 by the addition of 12M sodium

hydroxide, was passed through the column with a flow rate of $1.0 \text{ cm}^3/\text{min}$. Trace elements were concentrated on the chelex-100 column and thus separated from the major components (8). The absorbance and concentration of the four elements in the stripped sea water at pH 7.6 are given in Table 3.4. As high absorbance was measured in the stripped ~~sea water~~ **for Zn and Cd**, the stripped sea water was restripped for a second and a third time. Constant absorbance was obtained everytime (See Table 3.4). This indicates that the absorbance was not caused by incomplete adsorption of the first strip. Similar experiments were performed on Cd and Zn at other pH. The results in Table 3.5 show that a constant absorbance was measured for Zn at every pH-value and for Cd when the pH is higher than 7. As Zn is widely distributed and the contents in tri-distilled water from trial experiments was found to be between 4-7 $\mu\text{g}/\text{dm}^3$, the absorbance of Zn in the stripped sea water originated undoubtedly from the small concentration of Zn in the reagents used. As for Cd there might exist some complex species (when the pH is higher than 7) which absorbs light at the wavelength of Cd. Thus in every determination including the spiking experiments, the samples after the preconcentration procedure were restripped and the absorbance was considered as blank.

Table 3.4 Absorbance and concentration of Cd, Cu, Pb and Zn in stripped sea water at pH7.6.

	No. of Experiment	Cu		Cd		Pb		Zn	
		Absorbance	Concentration (ppb)	Absorbance	Concentration (ppb)	Absorbance	Concentration (ppb)	Absorbance	Concentration (ppb)
First strip	1	un detect.	un detect.	0.009	0.618	un detect.	un detect.	.078	7.15
	2	"	"	0.009	0.618	"	"	.080	7.28
	3	"	"	0.009	0.618	"	"	.081	7.34
	4	"	"	0.009	0.618	"	"	.082	7.42
	5	"	"	0.009	0.618	"	"	.082	7.42
	6	"	"	0.009	0.618	"	"	.083	7.51
Second strip	1	un detect.	un detect.	0.009	0.618	un detect.	un detect.	.078	7.15
	2	"	"	0.008	0.549	"	"	.080	7.28
	3	"	"	0.009	0.618	"	"	.080	7.28
	4	"	"	0.008	0.549	"	"	.080	7.28
	5	"	"	0.009	0.618	"	"	.082	7.42
	6	"	"	0.009	0.618	"	"	.082	7.42
Third strip	1	un detect.	un detect.	0.009	0.618	un detect.	un detect.	0.080	7.28
	2	"	"	0.009	0.618	"	"	0.080	7.28
	3	"	"	0.008	0.549	"	"	0.083	7.51
	4	"	"	0.008	0.549	"	"	0.082	7.42
	5	"	"	0.009	0.618	"	"	0.082	7.42
	6	"	"	0.009	0.618	"	"	0.082	7.42

Table 3.5 Absorbance and concentration of Cd and Zn in stripped sea water at various pH.

No. of Experiment	pH5				pH6				pH7				pH7.6				pH8.0			
	Zn		Cd		Zn		Cd		Zn		Cd		Zn		Cd		Zn		Cd	
	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ⁿ (ppb)	Absorbance	Conc ² (ppb)	Absorbance	Conc ⁿ (ppb)
1	.076	7.02	-	-	.078	7.15	-	-	.080	7.28	.008	0.549	.080	7.28	.009	0.618	.080	7.28	.009	0.618
2	.078	7.15	-	-	.078	7.15	-	-	.080	7.28	.008	0.549	.082	7.42	.009	0.618	.080	7.28	.008	0.549
3	.076	7.02	-	-	.076	7.02	-	-	.082	7.42	.008	0.549	.082	7.42	.009	0.618	.080	7.28	.008	0.549
4	.076	7.02	-	-	.078	7.15	-	-	.082	7.42	.008	0.549	.083	7.51	.009	0.618	.082	7.42	.008	0.549
5	.076	7.02	-	-	.078	7.15	-	-	.080	7.28	.008	0.549	.082	7.42	.009	0.618	.078	7.15	.008	0.549
6	.076	7.02	-	-	.080	7.28	-	-	.078	7.15	.008	0.549	.080	7.28	.009	0.618	.082	7.42	.008	0.549

- = un detectable

3.4 Preconcentration by Chelex-100

3.4.1 Method

Buffer solution of the appropriate pH value was allowed to flow through the column of chelex-100 until the pH of the effluent remained the same as the feed. A volume of 4 dm³ of sea water sample was passed through the column with a flow rate of 1.0 cm³/min. The column was then washed with 200 cm³ of tri-distilled water with a flow rate 5.0 cm³/min. Cadmium, copper, lead and zinc were eluted with 2M HNO₃. A total of four fractions, 10 cm³ each, were collected. The content of the elements in the fractions was determined. The pH of this stripped sea water was readjusted to pH7.6 and was then allowed to pass through another column of chelex-100 with a flow rate of 1.0 cm³/min. The column was washed with 200 cm³ of tri-distilled water with a flow rate of 5.0 cm³/min. The column was then washed with 4 portions of 2M HNO₃, 10 cm³ each, called blank.

3.4.2 Effect of pH on the Recovery Yield of Cd, Cu, Pb and Zn through Chelex-100

Standard solutions of Cd, Cu, Pb and Zn in a concentration of 2, 4, 10 and 30 µg respectively were spiked into 1 dm³ of stripped sea water. The pH of the solution was varied between 5-8 by the addition of 12M sodium hydroxide. The solution was poured into a chelex-100 column which was previously buffered to the required pH. The flow rate was

kept constant at $1 \text{ cm}^3/\text{min}$. The column was then washed with 200 cm^3 tri-distilled water. Cd, Cu, Pb and Zn were eluted with 40 cm^3 , 2M HNO_3 at a flow rate of $1 \text{ cm}^3/\text{min}$. The eluate was collected in four fractions. The absorbance of the blank in each fraction was corrected.

3.4.3 Effect of Flow Rates on the Recovery of Cd, Cu, Pb and Zn from Sea Water by Chelex-100

The amount of Cd, Cu, Pb and Zn in 1 dm^3 sea water were determined in the same manner as described in Section 3.4.1. The pH of the solution was fixed at pH7.6. The flow rate was varied between $1.0\text{-}4.0 \text{ cm}^3/\text{min}$.

3.4.4 Effect of Nitric Acid Concentration on the Stripping of Cd, Cu, Pb and Zn from Chelex-100

The optimum molarity of nitric acid as eluting agent was investigated. Cd, Cu, Pb and Zn were first adsorbed on the chelex column at pH7.6. Nitric acid with a concentration varying from 1 to 4 molar was allowed to flow through the column. A total of four fractions, 10 cm^3 each, were collected. The content of the elements in each fraction was determined.

The eluting curves of Cd, Cu, Pb and Zn were investigated. The stripped sea water was spiked with 2, 4, 10 and 30 ug of Cd, Cu, Pb and Zn respectively. The elements were separately adsorbed on a chelex-100 column at pH7.6 and at a flow rate of $1 \text{ cm}^3/\text{min}$. The elements were then eluted with

2M HNO_3 . Fractions of 2 cm^3 were collected and the content of the element in each fraction determined.

3.5 Preconcentration by Reverse Phase Chromatography

3.5.1 Method

Ammonium pyrrolidine-dithiocarbamate (APDC) is used as chelating agent in the extraction of many elements prior to atomic absorption spectrophotometric determination (9,10). It was shown that dithiocarbamates can be directly adsorbed on activated charcoal (11) or a silanized surface (12); the capacity of such surfaces being higher than that of liquid extractants. Adsorption of dithiocarbamate on silanized surface of kieselguhr (chromosorb W-DMCS) was chosen for the present studies. The important characteristics of extraction agents in reverse phase procedures include the low water solubility of the organic phase and the strong adsorptive forces between the organic phase and the surface of the carrier. Since the diffusion of the complexes into the organic layer is relatively rapid, high flow rates are possible (13).

A volume of 120 cm^3 of the pH5 buffer solution and 160 cm^3 1% APDC were added to 4 dm^3 of sea water. The pH of the solution was adjusted to exactly 5 by the addition of 12M sodium hydroxide or 17M acetic acid. The solution was then poured into a chromosorb W-DMCS column at a flow rate of $4.0 \text{ cm}^3/\text{min}$. The column was washed with 3 cm^3 tri-distilled water

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and dried by air suction for five minutes. The elements Cd, Cu, Pb and Zn were eluted with 10 cm³ chloroform. The complexes in chloroform were stripped back with 10 cm³ 6M **nitric acid** and the solution was ready for measurement. The absorbance of the reagent blank were determined by commencing the whole process on the stripped sea water, i.e. to restrip the sample. No detectable absorbance was found in the restripped sea water for the four elements. The restripping process was commended, however, in every determination to assure complete elution of the first strip. Two days are required for the determination of one sample.

3.5.2 Effect of pH on the Recovery Yield of Cd, Cu, Pb and Zn by Reverse Phase Chromatography

The recovery yield of the elements at pH between 4-7 were determined by spiking 2, 4, 10 and 10 µg of Cd, Cu, Pb and Zn respectively into 900 cm³ stripped sea water prepared as described in Section 3.3, 30 cm³ of the appropriate buffer solution and 40 cm³ 1% APDC were added. The final pH of 4-5 was obtained by the addition of either sodium hydroxide or acetic acid. A final pH of 6 was obtained by the addition of either sodium hydroxide or citric acid, pH7 was obtained by the addition of either sodium hydroxide or phosphoric acid. The solution was poured into a column filled with 60-80 mesh chromosorb W-DMCS at a rate of 4.0 cm³/min. The column was washed with 3 cm³ tri-distilled water and dried by air suction

for 5 minutes. The dithiocarbamate complexes adsorbed on the column were eluted with 10 cm^3 chloroform, then stripped back with 10 cm^3 6M HNO_3 . The concentration of the elements was measured by the atomic absorption spectrophotometer. Similar experiments were performed on 100-120 mesh chromosorb W-DMCS.

The effectiveness of sodium diethyl-dithiocarbamate at pH between 4-7 as extracting agent was also investigated. First the recovery yield of the element were determined by spiking 2, 4, 10 and 10 μg of Cd, Cu, Pb and Zn respectively into 900 cm^3 stripped sea water. 30 cm^3 Buffer solution of the appropriate pH and 40 cm^3 1% sodium diethyl-dithiocarbamate were added. The final solution was adjusted to the appropriate pH as described in section 3.5.2. Then the solution was poured into a column filled with 100-120 mesh chromosorb W-DMCS at a rate of $4.0\text{ cm}^3/\text{min}$. The column was washed with 3 cm^3 tri-distilled water. The dithiocarbamate complexes were eluted with 10 cm^3 chloroform, then stripped back with 10 cm^3 6M HNO_3 . The concentration of the elements was determined by AAS.

3.5.3 Effect of Particle Size of the Solid Support on the Recovery Yield of Cd, Cu, Pb and Zn by Reverse Phase Chromatography

The effect of the particle size of chromosorb W-DMCS were investigated by varying the mesh size between 60-80, 80-100 and 100-120 mesh. The pH of the solution was fixed at

5.0. The conditions of the experiments were similar to those of Section 3.5.2.

3.5.4 Effect of Flow Rates on the Recovery Yield of Cd, Cu, Pb and Zn by Reverse Phase Chromatography

The recovery yield of the elements were determined by spiking 2, 4, 10 and 10 μg of Cd, Cu, Pb and Zn respectively into 900 cm^3 of the stripped sea water, 30 cm^3 of the pH5 buffer solution and 40 cm^3 1% APDC were added. The pH of the final solution was adjusted to pH5 by the addition of either sodium hydroxide or acetic acid. The solution was poured into a column filled with 60-80 mesh chromosorb W-DMCS at flow rates between 2-8 cm^3/min . The column was washed with 3 cm^3 tri-distilled water and dried by air suction for 5 minutes. The dithiocarbamate complexes adsorbed on the column were eluted with 10 cm^3 chloroform with a flow rate of 1.0 cm^3/min , then stripped back with 10 cm^3 6M HNO_3 . The concentration of the elements was measured by AAS.

3.5.5 Effect of Eluting Agents on the Recovery Yield of Cd, Cu, Pb and Zn by Reverse Phase Chromatography

The recovery yields of the elements using chloroform, benzene and toluene as eluting agents were determined. The experiments were performed in the same manner as described in Section 3.5.4. The pH was fixed at pH5.0 and the flow rate at 4.0 cm^3/min .

3.5.6 Effect of Acid Concentration on the Stripping of Cd, Cu, Pb and Zn from Chloroform

2, 4, 10 and 10 μg of Cd, Cu, Pb and Zn were spiked into 50 cm^3 stripped sea water added 5 cm^3 buffer pH5.0, 5 cm^3 chloroform was added. The mixture was shaken for two minutes. The organic layer was washed with 10 cm^3 tri-distilled water. The trace elements in the organic phase were stripped back into the aqueous phase either by 10 cm^3 hydrochloric acid with a concentrate varying between 0.5-10.0 molar or by 10 cm^3 nitric acid at a concentrate varying between 1.0-8.0 molar. The concentration of the elements in the stripped solution was determined by AAS.

3.6 Calibration Curves

Series of pure aqueous standard solutions with concentrations extending between the two limiting points of the concentration scale were prepared from the stock solutions. After the instrument was aligned and optimum conditions were set, the absorbance was calibrated with reference to these standard solutions which concentrations were exactly known. The working curves might change slightly from day to day due to small variations in lamp and flame conditions. The instrument was recalibrated for each batch of samples. Some typical results showing the relation between the absorbance and the concentration of the elements under investigation are shown as examples in Table 3.6 and plotted in Fig. 3.3-3.6.

Table 3.6 Relation between absorbance and concentration.

Element	Concentration (ppm)	Absorbance
Cd	0	0
	0.10	0.014
	0.20	0.028
	0.30	0.042
	0.40	0.057
	0.50	0.070
Cu	0	0
	0.50	0.019
	1.00	0.038
	1.50	0.058
	2.00	0.076
	2.50	0.095
Pb	0	0
	1.00	0.015
	2.00	0.030
	3.00	0.045
	4.00	0.061
	5.00	0.075
Zn	0	0
	0.10	0.022
	0.20	0.044
	0.30	0.066
	0.40	0.089
	0.50	0.110

Fig. 3.3-3.6 Calibration curves of Cd, Pb, Cu and Zn

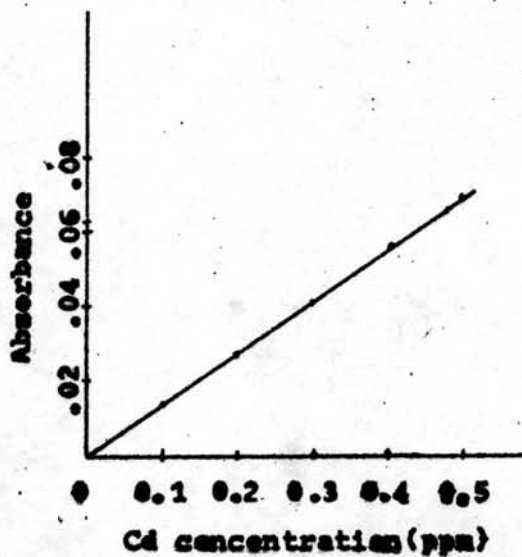


Fig. 3.3 : Cd

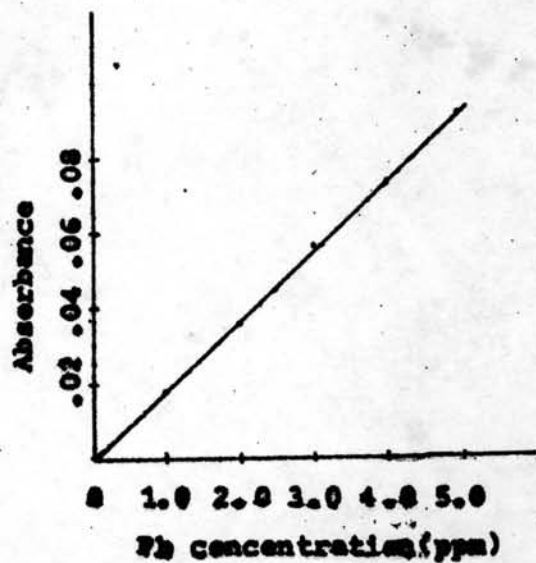


Fig. 3.4 : Pb

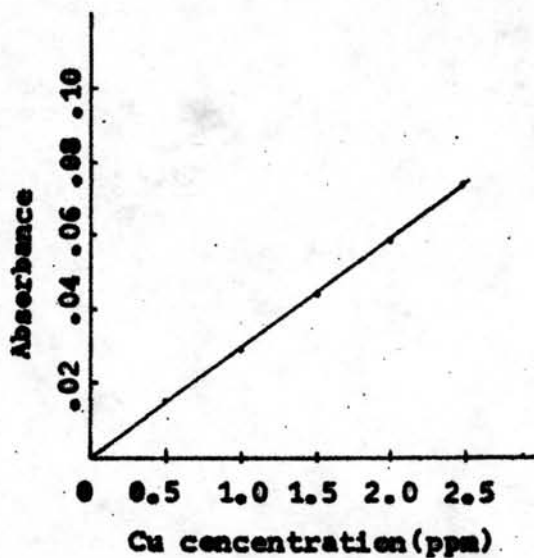


Fig. 3.5 : Cu

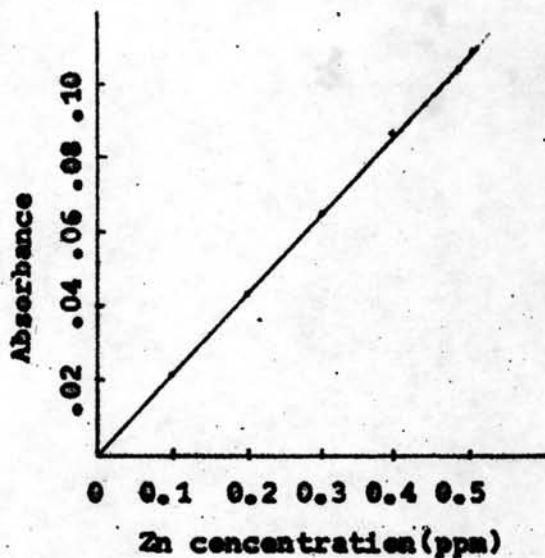


Fig. 3.6 : Zn

3.7 Determination of Detection Limits

Standard solutions containing $0.04 \mu\text{g}/\text{cm}^3$ of Zn and Cd, $0.4 \mu\text{g}/\text{cm}^3$ of Cu and $0.80 \mu\text{g}/\text{cm}^3$ of Pb were prepared.

The operation conditions of the AAS for the analysis of these elements were set according to Table 3.1. Tri-distilled water was aspirated through the burner at least two minutes for cleaning the spray chamber. Each standard solution was aspirated ten times, with aspiration of tri-distilled water as blank before and after each injection. The absorbance of the standards were calculated by subtracting the mean of the adjacent blank, then the mean \bar{X} and the standard deviation, S.D, of the absorbance were calculated. The detection limit was calculated from equation 2.16. The results of the measurements and a calculation example are given in Appendix I, where as the results of the detection limits are given in Table 3.7.

Table 3.7 Detection limits of the spectrophotometer
of the elements Cd, Cu, Pb and Zn .

Element	Detection Limit (ppm)
Cd	0.006
Cu	0.036
Pb	0.105
Zn	0.009