

NWRI CONTRIBUTION - 90-101

**MULTIVARIATE OBSERVATIONS OF THE  
DISTRIBUTION OF POLYCHLORINATED  
BIPHENYLS IN ENVIRONMENTAL  
COMPARTMENTS OF TWO HARBOURS**

by

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## **MANAGEMENT PERSPECTIVE**

Soft-independent modelling of class analogy, (SIMCA) using a principal components analysis technique has been applied for the assessment of concentration patterns of PCBs in different compartments of the aquatic system, namely water, sediment and biota. The technique is useful for the evaluation of the sources of PCBs and pollutants in general, at different locations, particularly in areas of concern in the Great Lakes Basin. The results of this investigation suggest that the PCB content of the samples analyzed could be described as being derived from an Aroclor or Aroclors-mixtures.

**Dr. John Lawrence**

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## **EXECUTIVE SUMMARY**

Multivariate observations of the distribution of PCBs in water, sediment and selected biota samples using the principal components SIMCA program was applied to analytical data obtained from samples collected in Wheatley Harbour, Lake Erie and Hamilton Harbour, Lake Ontario. The results show that the concentration patterns of individual homologues were similar in sediments and biota samples. In the water samples, a greater concentration of the lower chlorinated biphenyls were present.

## RESUME POUR LA DIRECTION

Des observations multivariées de la distribution des PCB dans un choix d'échantillons d'eau, de sédiments et du biote, recueillies à l'aide du programme aux composantes principales SIMCA, ont été appliquées aux données d'analyse provenant d'échantillons prélevés dans le port de Wheatley sur le lac Erié et dans le port de Hamilton sur le lac Ontario. Les résultats indiquent que les concentrations étaient semblables dans des échantillons homologues de sédiments et de biote. Dans les échantillons d'eau, la concentration des biphenyles chlorés de masse moléculaire inférieure était plus élevée.

## PERSPECTIVE: GESTION

La modélisation SIMCA (Soft-Independent Modelling of Class Analogy) qui s'appuie sur l'analyse des composantes principales a été appliquée à l'évaluation des concentrations de PCB dans différents compartiments du système aquatique, notamment l'eau, les sédiments et le biote. La technique sert à évaluer les sources de PCB et de polluants en général, en différents endroits, particulièrement dans les régions d'intérêt du bassin des Grands Lacs. Les résultats de cette étude indiquent que la teneur en PCB des échantillons analysés pourrait être décrite comme étant dérivée d'un Aroclor ou de mélanges d'Aroclors.

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## **ABSTRACT**

The objectives of this study were to identify, classify and categorize polychlorinated biphenyl (PCB) residues in samples collected from the Hamilton and Wheatley Harbour environmental compartments. The study is built around the use of a principal components analysis method, namely the soft independent modelling of class analogy (SIMCA) technique. This multivariate method is widely used for evaluating differences and observing similarities among multiple objects. The results obtained from this work confirm that the gas chromatographic data sets obtained with the samples provide a good approximation of the pattern derived from a determination of the composition of commercial Aroclors in water, sediment and biota samples. The data analysis technique provides insight into the origin of PCB contamination in environmental samples and indicates pathways for the environmental degradation or bioaccumulation of PCBs. This investigation contributes some evidence that multivariate reduction techniques are suitable for the investigation of complex data sets in environmental studies.

## INTRODUCTION

Polychlorinated biphenyls (PCBs) have been identified and quantified in water, sediment and fish in the Great Lakes (1,2). Concentrations of widely distributed PCB-residues in Lake Huron, Georgian Bay and North Channel sediments are considerably lower than those found in Lake Ontario and Erie (3,4).

A major problem associated with the analysis and toxicology of PCBs is the multiplicity of possible congeners. This situation leads to complex chromatographic traces when PCB contaminated samples are analyzed with open tubular column gas chromatography. The composition of PCB mixtures in commercial products such as Aroclors has been determined and certain batches can be used as secondary analytical standards. The composition of PCBs found in environmental samples is often different from that of commercial Aroclors (5) and this can be attributed to biological processes and degradation.

Most of the quantitative data for PCBs have been reported as total loading values or total PCBs. The interpretation of these data is very difficult and it has no real meaning for environmental chemists. High resolution gas chromatography provides much more information, because the chromatograms allow the quantitation of almost all PCB congeners. Each mixture of an Aroclor is characterized by a somewhat different distribution of congeners and homologues leading to a characteristic fingerprint chromatographic trace. The quantification of individual congeners is possible but it is of very little practical use to toxicologists. From a toxicology standpoint, the concerns are limited to no more than twelve specific isomers known to influence the activity of the Aryl Hydrocarbon Hydroxylase (AHH) enzyme system.

Degradation and weathering of PCBs in sediments, water, fish and biota is a natural process. The kinetics of the decomposition varies with the chlorine content and the position of the chlorine atom on the biphenyl moiety. In the case of PCBs mixture, the quantitation of individual congeners and their grouping into homologue series can be taken as the best approach to determine their composition. The resulting data are more robust than those obtained from

the quantitation of individual congeners. The individual congener peaks are prone to systematic errors due to unpredicted interferences, especially in complex environmental samples of different origin (6). In the study described herein, an attempt was made to obtain graphic evidence of differences in the pattern of PCB contamination between an area greatly impacted by industrial pollution and one mildly polluted. Contamination in the mildly polluted area was suspected to be arising from agricultural activities and only a background PCB contamination was expected. Two different harbours were selected for this study, the Hamilton Harbour representative of heavy industrial activities due to two large steel plants located nearby, and the Wheatley Harbour, representative of a rural agricultural area characterized by a busy fishing industry. Both harbours provide habitats for many species of fish and biota. To our knowledge, Wheatley Harbour is neither used for the disposal of municipal wastes nor for the dumping of hazardous and industrial wastes.

Our objectives for the work described below were to investigate the distribution of specific homologues of PCBs in biota and to compare the patterns with those in water and sediment. To this end, we used SIMCA (Soft Independent Modelling of Class Analogy) technique based on principal components analysis of the data. Chemometric intercomparison employs reference data sets which simulate analytical measurements for the purpose of assessing the quality of data for structurally different chlorinated biphenyls. The method is used to draw conclusions about biodegradation from data obtained with the training sets representing time zero kinetics. Such chemometric intercomparisons offer the opportunity to initially include all data that are detected in a sample, with some to be discarded later as outliers or false positives.

## **MATERIALS AND METHODS**

Sediment, water and biota samples were collected from Hamilton and Wheatley Harbours at sampling sites shown in Figures 1 and 2.

## **Sediment Sampling and Analysis**

Sediment samples were collected with an Ekman dredge from a boat. Surface sediment (0-3 cm layer) was sub-sampled into prewashed glass jars for the determination of PCBs. The jars were covered with Al-foil and frozen within 48 hours.

Sediment samples (5 grams dry sample equivalent) were extracted using ultrasonic extraction with 1:1 n-hexane-acetone mixture (5). The extract was partitioned with water and then back extracted with benzene. The combined organic extracts were dried with anhydrous  $\text{Na}_2\text{SO}_4$ , reduced in volume, cleaned on a gel permeation chromatograph (ABC Autoprep-1002A) and silica gel. All solvents employed for the extraction were pesticide grade purity. The water used in the process was filtered via a Millipore filter (pore size 2 mm).

## **Water Sampling and Analysis**

Water samples were collected from the middle of the water column with a van Dorn bottle at sampling stations in each harbour. Each water sample was stored in a solvent rinsed bottle.

Duplicate 2 litre aliquots of water were collected and were extracted using 250, 100, and 100 mL volumes of methylene chloride for the serial extraction of PCBs and all base/neutral contaminants at pH 11. In order to determine the recovery of the analyte of interest through the extraction method, all water samples were spiked with 86.2 ng of decachlorobiphenyl resulting into a decachlorobiphenyl concentration of 43.1 pg/mL. The methylene chloride extract was collected in a 500 mL Erlenmeyer flask. The combined extract was poured through a drying column containing a 10 cm layer of anhydrous  $\text{Na}_2\text{SO}_4$  and the eluate was collected in a 500 mL round-bottom flask. Ten mL of iso-octane was added as a keeper and the sample was evaporated on a Buchi rotary evaporator to 10 mL. The extraction procedure was 90 to 100 percent efficient.



To check on the purity of used solvents, the entire extraction and clean-up procedure was repeated using appropriate amounts of solvents to obtain solvent blanks.

### **Biota Samples and Analysis**

Specimens such as *Physa integra* (gastropoda), *Zonitoides arboreus* (gastropoda), *Stagnicola polustris* (gastropoda), fish (*Eupo-motis gibbosus*), and Isopods were collected from the nearshore and offshore area between Station A and B in Wheatley Harbour. Isopods and *Physa integra* were collected from the area between Stations 2 and 4 in Hamilton Harbour.

Approximately 5 grams of oligochaete worms (wet weight) was collected from the offshore zone at each sampling station in both harbours. After washing them with distilled water, the oligochaetes were allowed to ingest clean sediments for 48 hours to displace the original sediment from the harbour and to void these sediments in clean water prior to analysis. All biota samples were collected into a n-hexane pre-washed jars and Al-foil and stored frozen for further analysis. No attempt was made to separate soft parts from shells of collected snails.

The known amount of biota and worms were analyzed by decomposing them in concentrated hydrochloric acid (80 mL) at ambient temperature overnight. Eighty mL of organics free water (hexane washed) was added to the sample. The mixture was transferred to a separatory funnel and extracted with three volumes of 20 mL each of n-hexane for PCB analysis. The hexane extract was concentrated to 5 mL volume and processed through the cleanup procedure as described above.

If sulfur was present in a sample (in sediment), it was removed by vortex-stirring of the final extract with a drop of mercury.

## Gas Chromatography

Open tubular columns (OTCs) were used for all gas chromatographic analyses. The chromatograms represented in Figure 3 were obtained on a 30 m x 0.25 mm I.D. SE-52 WCOT column coated with 0.2 mm film thickness. A Varian Vista 6000 gas chromatograph fitted with a cold on-column injector was used. The average linear velocity for the carrier gas (hydrogen) was 45 cm/s. After holding the initial temperature of the oven at 75 C for one minute, it was programmed to 120 C at 40 C/min, then to 240 C at 1 C/minute. The injector temperature was programmed from 75C to 280 C at 100 C/min. Nitrogen was used as the detector make-up gas at a flow rate of 20 mL/min. To reduce the detector dead volume, the WCOT column exit was installed right up at the detector source. The PCB peaks were identified as in previous work (6) and quantified as described elsewhere (7).

## Multivariate Analysis of PCBs

The basic idea of principal components analysis is to describe the dispersion of an array of n-points in p-dimensional space by introducing a new set of orthogonal linear coordinates so that the sample variances of the given points with respect to these derived coordinates are in decreasing order of magnitude. Thus, the first principal component is such that the projections of the given points onto it have maximum variance among all possible linear coordinates. The second principal component has maximum variance subject to being orthogonal to the first one.

SIMCA multivariate data analysis is one of several linear cluster analysis techniques. It employs the variance-covariance matrix to determine principal component positions (8). These methods should reduce dimensionality in multivariate data sets so that the presence of systematic variation can be investigated. The method of principal components has been discussed in detail in several texts (9,10,11,12).

In this study, we were looking for similarity among samples represented by data tables. The data tables discussed here have the matrix format consisting of the PCB homologue compositions of Aroclors 1248, 1254 1260 and 1262 as determined by analysis with high resolution gas chromatography (HRGC).

Quantitative sample data from analysis of training sets (Table 1) and individual samples were stored on the hard disk of an EXCEL-OME 386 microcomputer in linear arrays from the data base. The concentration data obtained from each analysis were expressed as fractional parts and were normalized to a sum of 100. The normalized data were analyzed by calculating principal components sample scores, and visualized as Theta 1 vs. Theta 2 plots. Variable loading data of homologues were expressed in Beta 1 vs. Beta 2 plots by using the program CPRINT from SIMCA-3XQ software package (Principal Components, Columbia, MO 65201). Principal components analysis (PCA) illustrates the data matrix  $X$  (9) properly transformed and scaled down on the hyperplane defined by the previously calculated loading matrix  $P$ , expressed as  $F$ -dimensional hyperplane  $x$   $p$  dimensional space. The resulting coordinates of the objects on this hyperplane, the score matrix  $T$ , are interpreted visually, allowing an evaluation of the similarities between objects based on their closeness in the factor space. In the multivariate analysis of PCBs, these are represented as data vectors, where the sum of concentration of individual congeners containing the same number of chlorine atoms is one variable, identified as an homologue. The homologue group identification is essential when translating chromatographic fingerprints to the  $X$  data vector. It is essential that a given group of peaks always appears as the same  $x$ -variable. As part of a separate study in this area, Stalling et al(12) concluded that PCB residues are adequately described only by an isomer-specific quantitation and the summation of variables into the homologue series grouping and that the approach may lead to erroneous classification results when compared to the results derived from the modelling of isomer data. On the basis of our analytical experience, we regard this assumption as not valid because homologue groupings are based on the isomer specific quantitation. Contrary to the conclusions of Stalling et al. we consider that the data sets obtained with this method to be more rugged when they are grouped together rather than when individual isomers matrices are

used. Individual peaks can be influenced by residues contributing to the signal of many variables, as even open tubular column chromatography does not separate all components of various pesticides and contaminants often present as mixtures in environmental samples. This error is somewhat masked when individual congeners are grouped into a particular homologue series.

## RESULTS AND DISCUSSION

The sample results from data sets were analyzed in four classes representing Aroclors (n=4), water samples (n=2), sediment samples (n=6) and biota (n=3 in Hamilton Harbour and n=4 in Wheatley Harbour). Results from these two harbours are treated separately but all classes and training set data are depicted together for each harbour to illustrate sample score plots in two or three dimensional space. Principal components analysis of the total data set and the Aroclor class provided models containing two principal components. Since class assignment is based on the distribution of PCB homologues in the samples, the data for each sample were normalized to a mean of zero. This step makes all the samples equivalent in terms of total PCB concentration.

In the classification analysis, a two-dimensional graphic display of the training sets and unknowns was obtained by plotting a principal components plot for each harbour separately. The results obtained with samples from Wheatley Harbour and Hamilton Harbour are shown in Figure 4 and Figure 5, respectively. Both figures show that all samples in both harbours are similar to Aroclor 1254 because they cluster around the point A 54 representing Aroclor 1254.

Even more illustrative principal components plots are shown in the three dimensional graphs (Figures 6 and 7) depicting each harbour separately. As shown in the Hamilton Harbour plot, there are significant differences between water samples taken at Station 1 and 2 (◆). It is evident that this dissimilarity is very significant when comparing the water samples to the sediments (■) and biota

(▲) samples. These samples are clustered in the close vicinity to Aroclor 1254. The inset plot (Fig.4 and Fig.5) over the 3-D graph illustrates a top view of the X-Y projection. The sediment samples are characterized by a pronounced shift towards higher chlorinated PCBs (Aroclor 1260) as illustrated by the data from Stations 3 and 6, respectively. Sediment samples collected at these two stations contained the highest concentration of PCBs among the Hamilton Harbour sediment samples.

The sediment samples from Wheatley Harbour are also closely grouped around Aroclor 1254, with the exception of Station B (■2) and Station E (■6) which are shifted towards Aroclor 1262.

As shown in Table 4, levels of PCBs in sediment samples from the Hamilton Harbour area were up to ten times greater than those from the Wheatley Harbour. Distribution patterns of PCB homologues in sediments from all locations at the Wheatley Harbour were generally similar. The same observation can be applied to the Hamilton Harbour, except for Station 3 where a high level of nonachlorobiphenyl congeners were detected. However, there were significant differences in patterns of individual homologues between the harbours.

Distribution of homologues and concentration of total PCBs in biota collected in both harbours are shown in Table 5. In Wheatley Harbour, accumulation of PCBs were detected in all biota samples. *Physa integra* and *Stagnicola polustris* accumulated similar quantities of PCBs but *Zonitoides arboreus* accumulated less. In Hamilton Harbour, oligochaete worms accumulated greater concentrations of PCBs than *Physa* and Isopods.

Generally, the concentration patterns of PCB homologues in biota samples were more similar to the patterns observed with sediment samples than with water samples in both harbours. Concentrations of penta and hexachloro biphenyls were significantly greater than the other homologues in both sediments and oligochaetes from Wheatley Harbour. However, the concentration of total PCBs in oligochaetes from Wheatley Harbour was higher than those from Hamilton Harbour. Similar PCB patterns were found in oligochaetes collected from both harbours.

Similar concentration patterns of homologues were observed in all other types of biota obtained from Wheatley Harbour. In the case of oligochaete samples collected in Hamilton Harbour, the concentration pattern of homologues differed from that observed in the other biota samples collected in Wheatley Harbour. These results may reflect the differences in the size of each harbour, which affects the homogeneity of sediments and water. Wheatley Harbour represents a much smaller ecosystem with water depth about 3 m, and frequent mixing by an inflowing stream and occasional back-flow from Lake Erie. Consequently, this system is more homogeneous than Hamilton Harbour which has a much larger surface area and water depth of about 9 meters. The components of higher chlorinated products such as penta, hexa and hepta chlorinated biphenyls were found in greater concentrations in most of the sampled biota in both harbours.

As stated before, the principal components modelling of multivariate data such as those observed in PCB mixtures is more easily interpreted from a graphical representation of sample similarity. The use of graphical programs such as AXIS and ROTECA lead to improved graphical representations that enhance the analysis of the multivariate data. The AXIS program processes the data through translation, rotation and scaling operations. The AXIS program improves the quality of the original 3-D illustration capacity of SIMCA on the screen. However, the graphical 3-D illustration on the screen is not effectively reproduced when printed with a plotter. This deficiency comes from the inadequacies of the BASIC programming language for the description and presentation of calculated data sets. A typical presentation of the 3-D plot obtained with AXIS for both harbours is shown in Figures 6 and 7, respectively. When produced on a monitor screen, the representations provide an even better description of the samples in a 3-D graphical format, and an opportunity to rotate the graph to obtain the most informative view.

## CONCLUSIONS

The pattern of PCBs in water, sediment and biota samples show that homologues of PCBs are not evenly distributed, as based on the solubility of these homologues in water. The patterns suggest that no similarity exist with any training set sample. However, sediment samples show that all profiles cluster around Aroclor 1254, which is the sole source of contamination in both harbours. The data obtained from biota samples also cluster around the Aroclor 1254 showing that all biota species examined came into contact with the local source of contamination. These observations support the importance of carefully evaluating the homologue distribution in any attempt to assess profiles of PCB contamination. These profiles can be easily evaluated every year in order to detect trends. Such information can contribute to the improvement of the modelling techniques and consequently our knowledge about the distribution of PCBs in our ecosystem would be expanded.

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**Table 1****Composition of Individual Homologue Groups in Selected Aroclors**

<b>AROCLOR</b>	<b>1248</b>	<b>1254</b>	<b>1260</b>	<b>1262</b>
Monochloro-	0.0	0.0	0.0	0.0
dichloro-	1.0	0.0	0.3	0.4
trichloro-	28.3	0.3	0.6	1.0
tetrachloro-	54.6	15.0	0.7	0.7
pentachloro-	11.4	44.1	8.0	2.4
hexachloro-	2.6	35.1	42.0	25.5
heptachloro-	1.4	4.6	35.6	43.5
octachloro-	0.6	0.8	11.8	24.3
nonachloro-	0.1	0.1	0.9	2.2
decachlorobiphenyl	ND	ND	0.1	0.5

**Table 2. Residues of PCBs in Water at Two Harbours (ng/L)**

PCB-homologues	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-	Total
<b>HAMILTON HARBOUR :</b>							
Station 1	39.1	68.8	69.7	24.4	10.3	2.4	214.7
Station 2	26.3	33.3	18.8	5.0	1.6	1.0	86.0
<b>WHEATLEY HARBOUR :</b>							
Station 1	36.2	148.5	91.0	13.0	2.3	0.1	291.1
Station 2	13.9	35.9	27.3	7.4	1.8	0.1	86.4

**Table 3. Distribution of PCBs in Sediments (mg/kg)**

Homologue	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-	Nona-	Total
<b>HAMILTON HARBOUR</b>								
Station 1	97.4	134.9	324.6	284.5	30.9	70.9	5.3	948.5
Station 2	41.0	72.4	174.3	140.3	146.2	31.4	2.3	608.0
Station 3	733.0	1065.0	3321.0	3289.0	4044.0	985.0	748.0	14185.0
Station 4	235.7	451.0	777.5	451.3	462.7	112.0	8.8	2499.0
Station 5	205.9	441.6	724.8	443.6	350.5	75.8	6.1	2248.0
Station 6	148.4	267.4	825.6	744.2	865.1	206.3	15.8	3073.0
<b>WHEATLEY HARBOUR</b>								
Station A	28.8	111.2	474.0	358.0	175.8	26.6	2.4	1176.8
Station B	5.6	20.0	67.8	51.0	16.8	2.0	2.8	166.0
Station C	14.8	64.6	252.2	140.8	91.6	14.6	1.4	580.0
Station D	5.8	30.8	103.2	53.0	31.2	5.6	0.4	230.0
Station E	5.6	34.0	155.4	110.0	40.2	5.8	0.1	351.1
Station F	21.4	54.2	189.8	132.2	59.6	7.7	0.5	465.0

Table 4

## Distribution of PCBs in Biota (mg/kg wet weight)

Homologue	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-	Total
<b>HAMILTON HARBOUR</b>							
Isopoda	16.7	32.2	37.9	17.5	5.3	ND	109.6
Physa integra	5.5	36.5	71.2	32.0	12.0	1.1	158.3
Oligochaetes	32.6	39.5	54.5	45.3	30.5	4.8	207.2
<b>WHEATLEY HARBOUR</b>							
Fish (Eupomotis							
gibbosus)	7.0	10.3	26.5	21.6	3.9	ND	69.3
Zonitoidus	5.1	17.3	64.8	46.3	16.3	1.6	151.4
arborens							
Physa integra	5.6	14.3	27.9	21.2	3.1	ND	72.1
Oligochaetes	22.4	40.2	102.8	72.2	22.6	2.5	262.7
Lymnea stagnal.	1.2	7.4	22.7	24.5	2.9	ND	58.7

## CAPTIONS

- Figure 1.** Sampling Stations in Wheatley Harbour.
- Figure 2.** Sampling Stations in Hamilton Harbour.
- Figure 3.** Aroclor Training Sets.  
Identification of the number of chlorine atoms is shown at the top of each peak.
- Figure 4.** Principal Components Plot of the Data for the Aroclors, ★  
Water (◆), Sediment (■) and Biota (●) Samples from the Wheatley Harbour showing that all Samples are Similar to Aroclor 1254.  
Aroclor assignment : Aroclor 1248 = A48; Aroclor 1254 = A54;  
Aroclor 1260 = A60; Aroclor 1262 = A62.
- Figure 5.** Principal Components Plot of the Data for the Aroclors,  
Water (◆), Sediment (■), and Biota (●) Samples from the Hamilton Harbour showing that Samples are Similar to Aroclor 1254.
- Table 2.** Residues of PCBs in Water at Two Harbours (ng/L).
- Table 3.** Distribution of PCBs in Sediments (mg/kg).
- Table 4.** Distribution of PCBs in Biota (mg/kg wet weight).
- Figure 6.** 3-D Illustration of the Principal Components for the Wheatley Harbour Data Shown in Fig.4 using AXIS Program.
- Figure 7.** 3-D Illustration of the Principal Components for the Hamilton Harbour Data Shown in Fig.5 using AXIS Program.

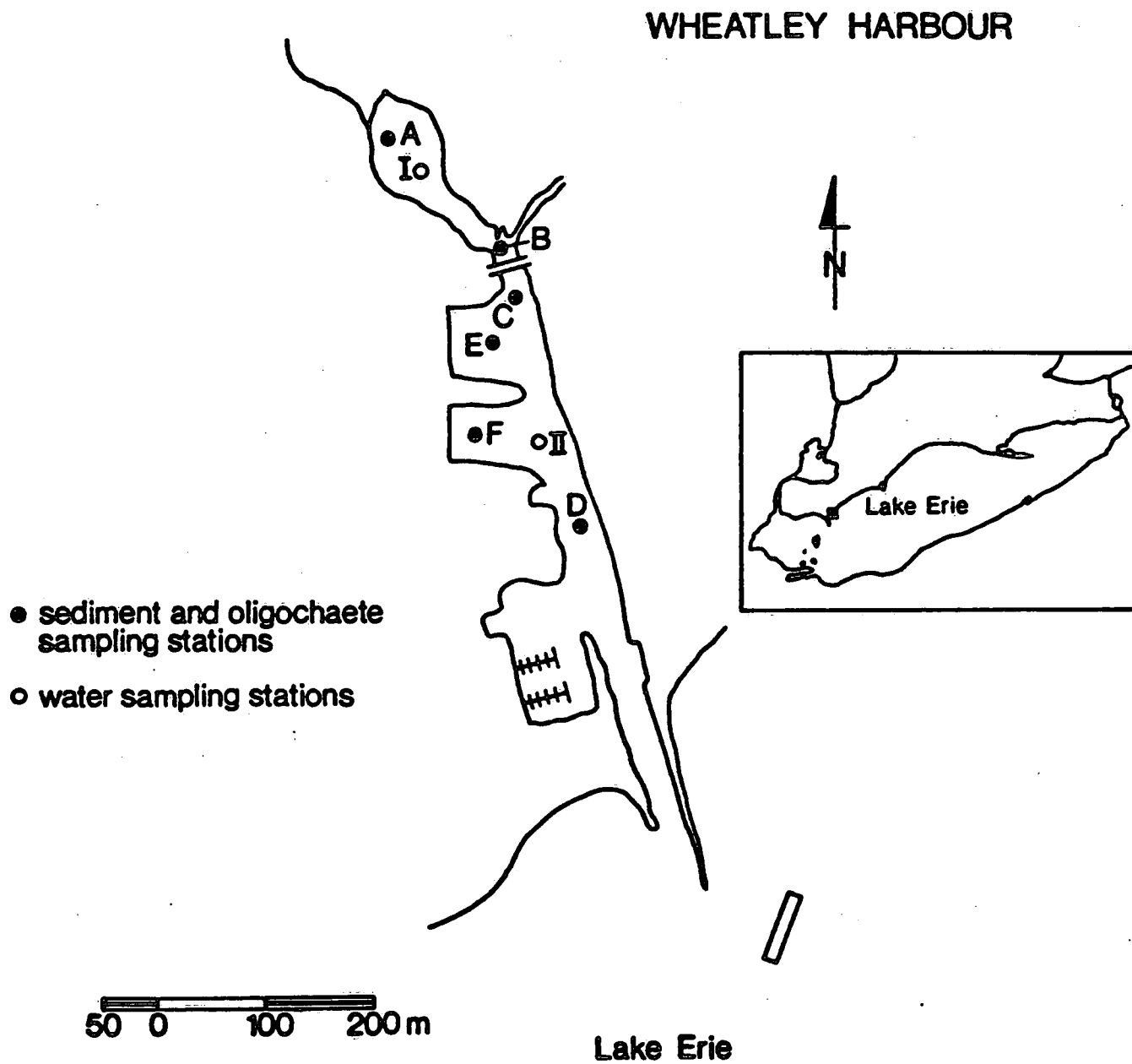


Figure 1. Sampling stations in Wheatley Harbour.

# HAMILTON HARBOUR

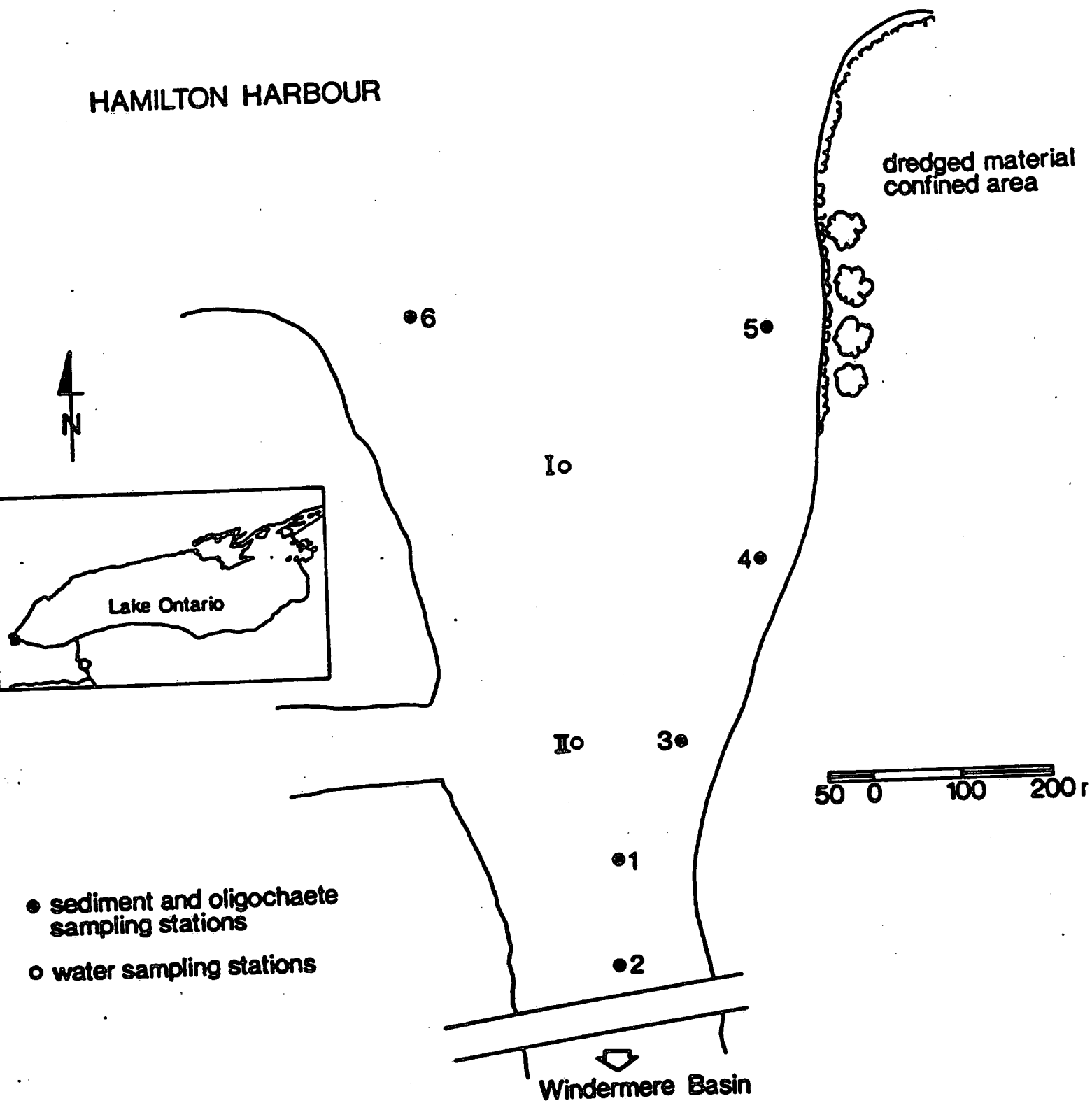
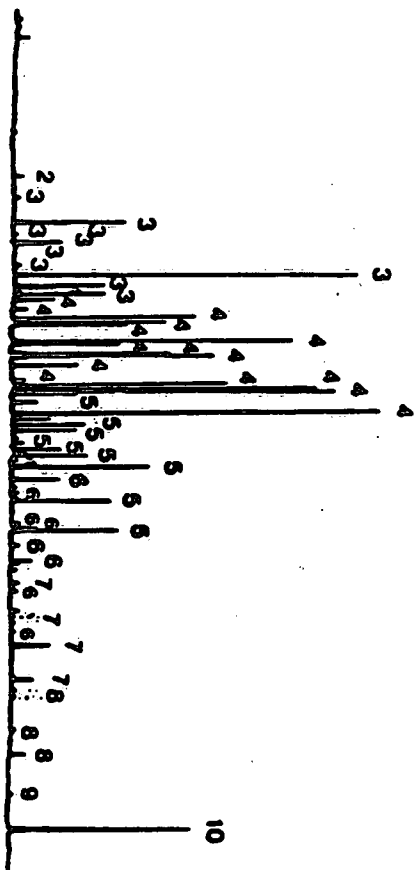
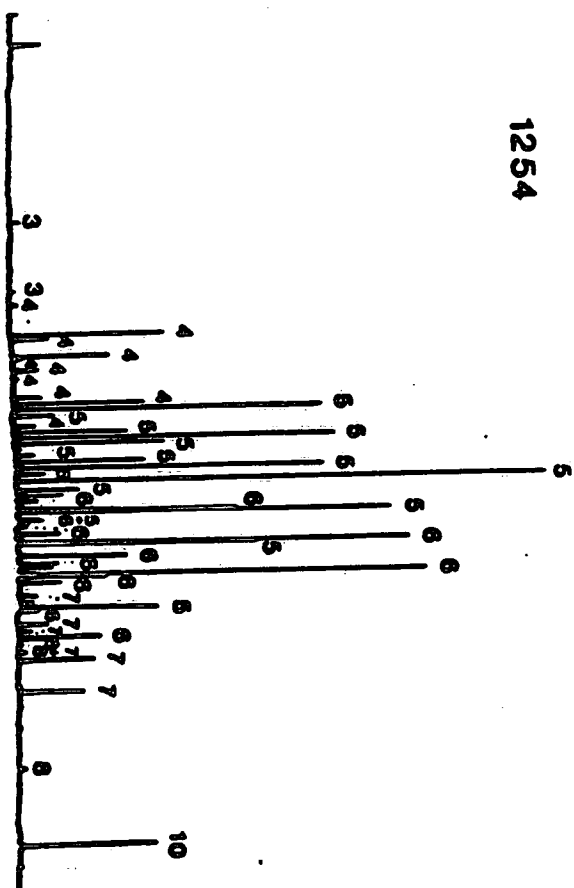


Figure 2. Sampling stations in Hamilton Harbour.

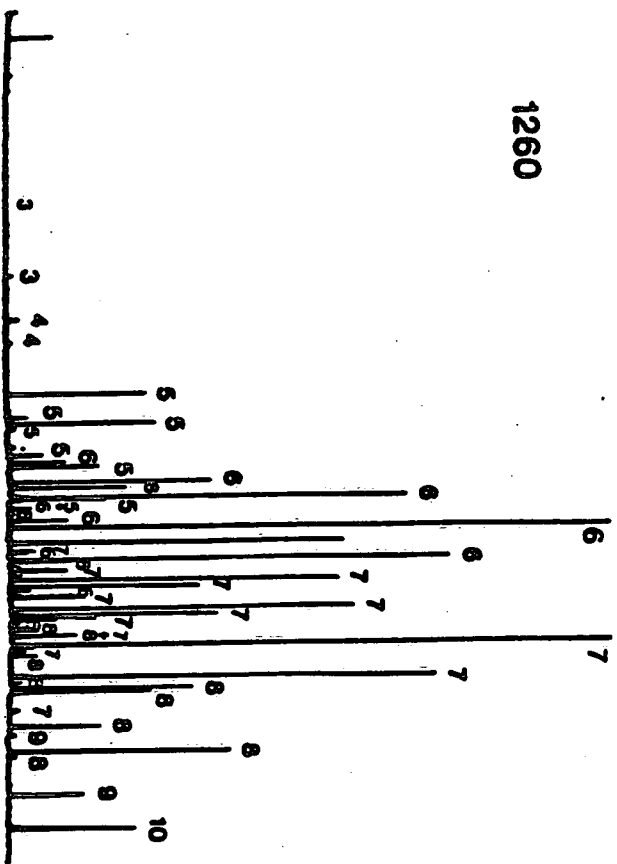
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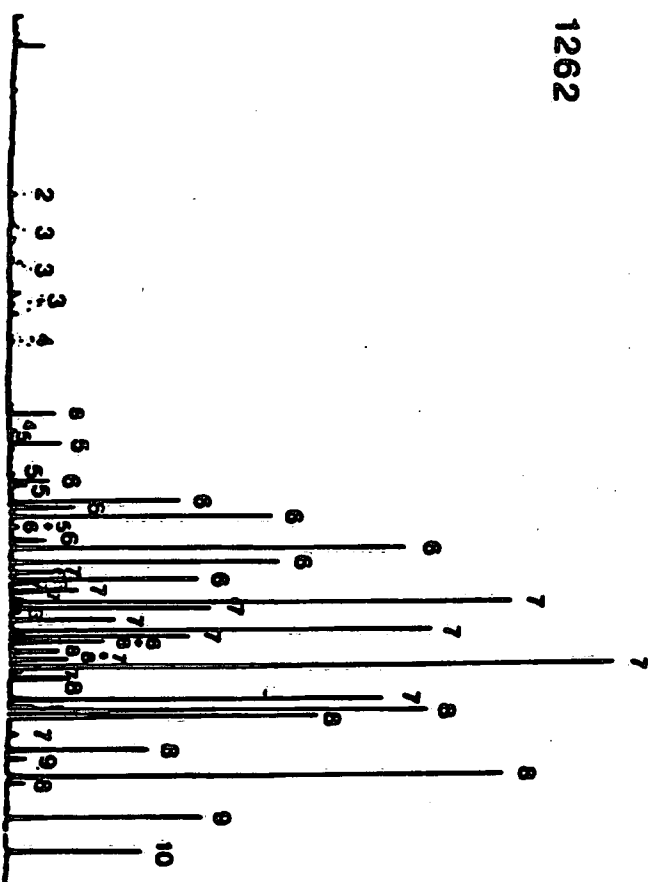
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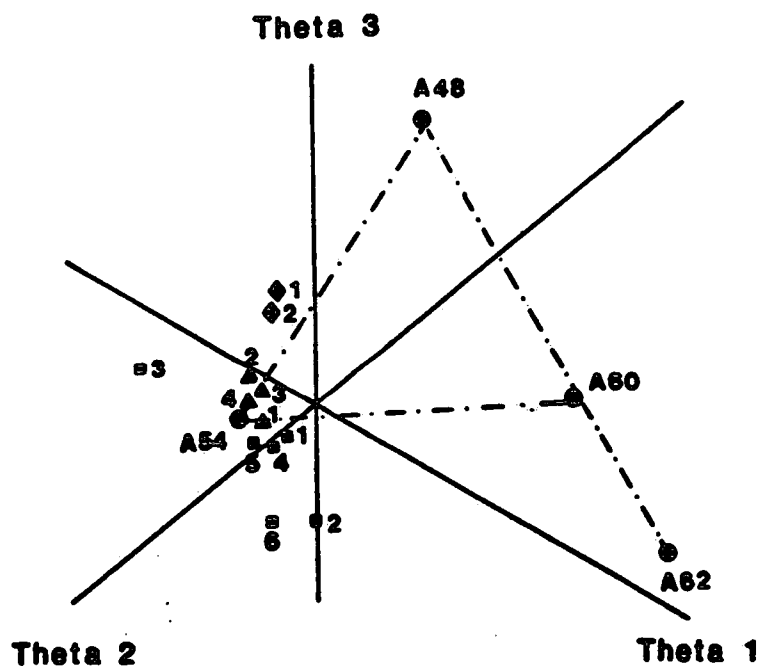
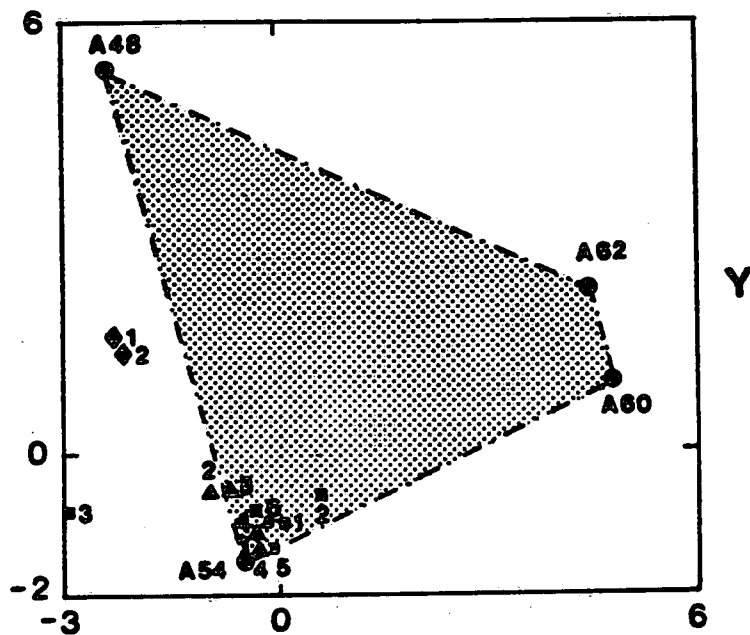
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1262

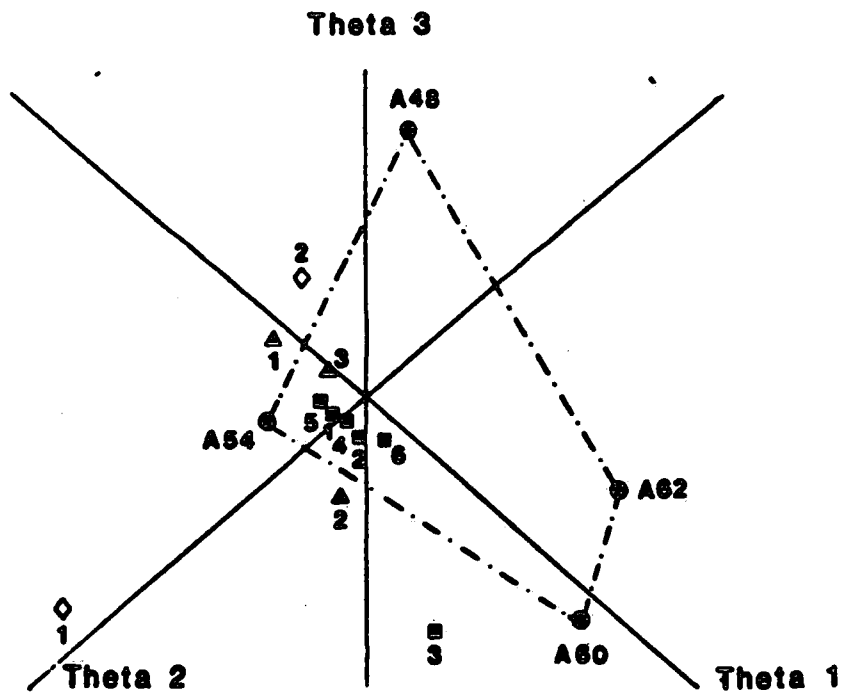
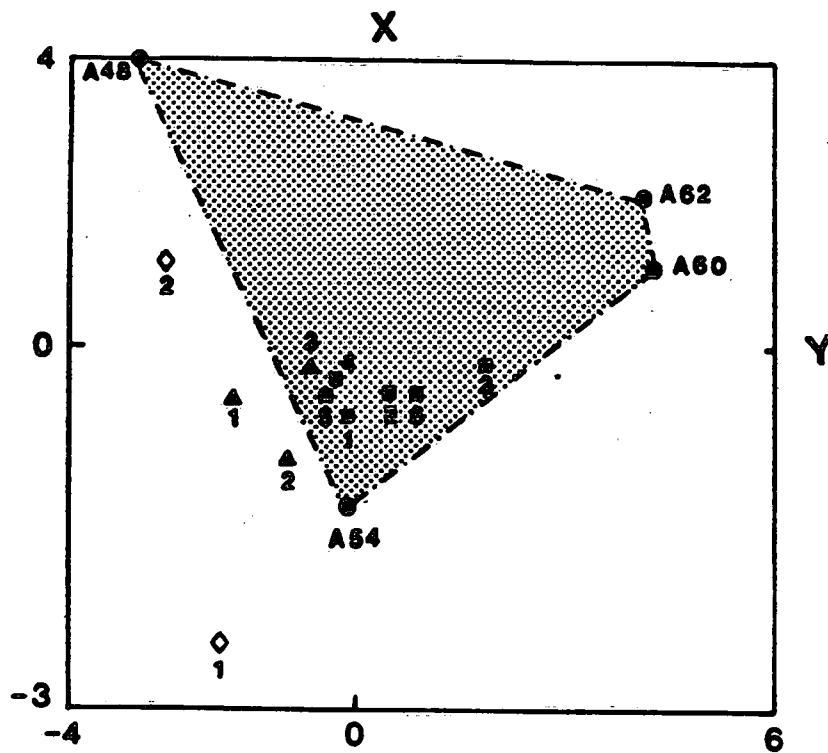


# WHEATLEY HARBOUR

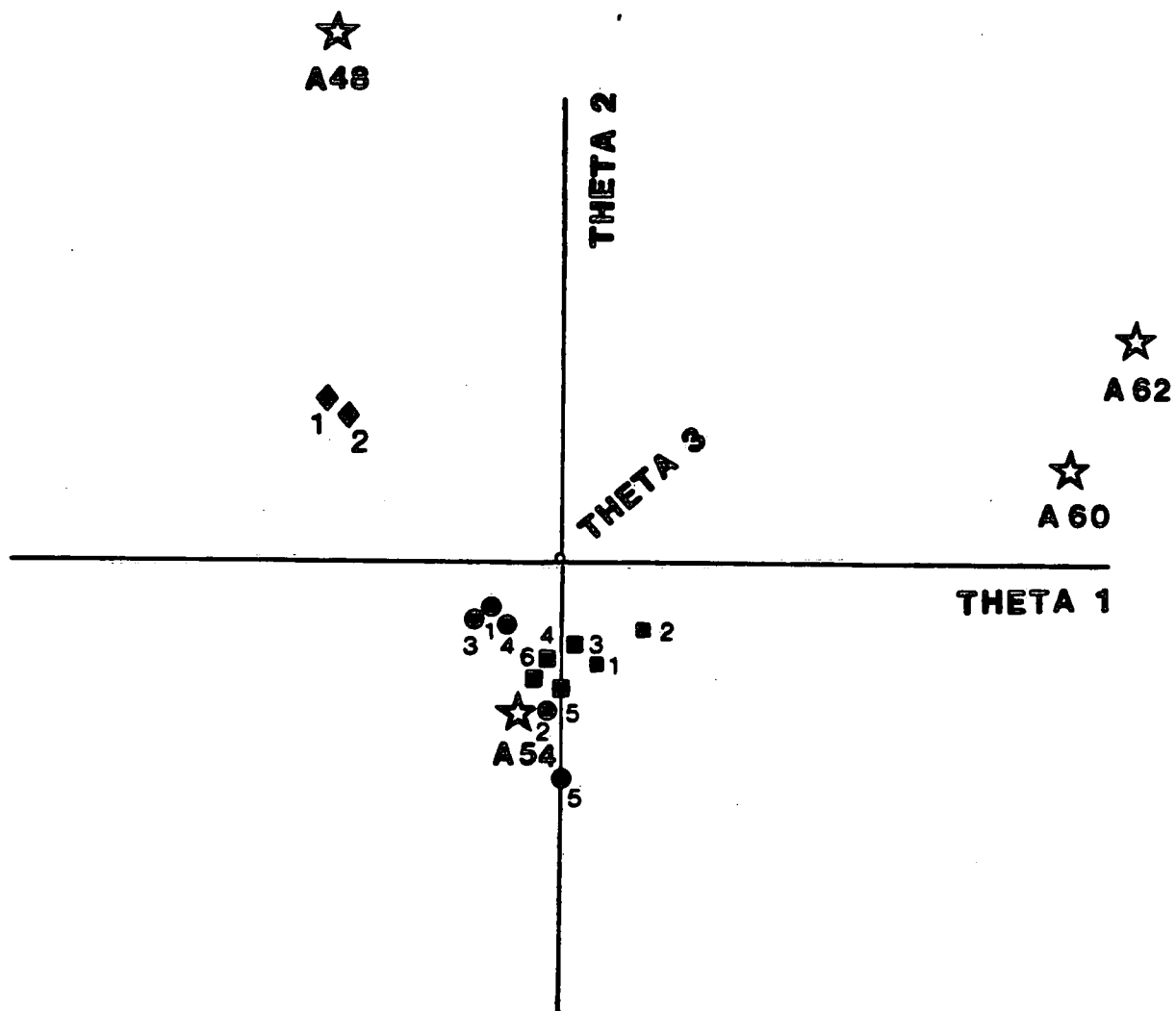




# HAMILTON HARBOUR



# Wheatley Harbour



# Hamilton Harbour

