METAL CONCENTRATIONS IN MUSSELS FROM ST. JOHN'S HARBOUR, NEWFOUNDLAND

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Title Page

METAL CONCENTRATIONS IN MUSSELS FROM ST. JOHN'S HARBOUR, NEWFOUNDLAND

by

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Abstract

St. John's Harbour is an estuarine environment that has been at the centre of much of Newfoundland and Labrador's industrial, commercial and social activity since the settlement of the province's first immigrants. Historical practices such as atmospheric discharges from coal burning and current activities such as high-volume municipal sewage dumping and frequent ocean vessel trafficking has thus lead to contamination of this environment by a range of pollutants associated with such activities. Surprisingly there is little published quantitative information regarding seawater metal abundance and distribution within the harbour. In this study, the concentrations of 12 metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd and Pb) were determined in the soft tissue of indigenous blue mussels that were collected from 6 sites within St. John's Harbour and 5 outer harbour sites, including one control site. The tissue was dissolved in a closed-vessel microwave system and analyzed by ICPMS.

Statistical analysis indicated significant differences ($p < 0.005$) of median concentrations for most metals between inner and outer harbour regions. Zinc and Pb concentrations were an order of magnitude higher at the inner harbour sites, while Cd was an order of magnitude lower at the inner sites. Median inner and outer region values were 149 and 87 $\mu g \cdot g^{-1}$ for Zn, 1.74 and 0.58 $\mu g \cdot g^{-1}$ for Pb, 398 and 1133 $\mu g \cdot g^{-1}$ of Cd, respectively. The magnitude of between-region concentration differences was not as great for the other analytes. Like Cd,

concentrations of As and Se were significantly higher in the outer harbour region, all other metals being significantly higher in the inner region, except Cr and Ni, for which no significant concentration differences between regions was indicated ($p > 0.05$). The 85th percentile Zn concentration from the inner region and that of Se from both regions were higher than 85th percentile mussel concentrations reported by the NOAA National Status and Trends program. The spatial distribution of metals within each region was uniform, excepting one site on the south side of the harbour that showed elevated concentrations for several analytes. No indication of a correlation between the metal concentrations in mussels and proximity to sewage and storm drain outflows was found.

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Abbreviations and Symbols

amu	atomic mass unit
cps	counts per second
DFO	Department of Fisheries and Oceans, Government of Canada
GPS	Global Positioning System
HEPA	High Efficiency Particulate Air
HNO_3	nitric acid
ICPMS	Inductively Coupled Plasma - Mass Spectrometry
ln(X)	natural logarithm of a value X (base e)
OSC	Ocean Sciences Centre, Logy Bay, St. John's, Newfoundland
rps	rotations per second
TFE	tetrafluoroethane
wt.	weight

Chemical Symbols and Names

Symbol	Name	Symbol	Name	Symbol	Name
Ag	Silver	Hg	Mercury	Se	Selenium
As	Arsenic	Mn	Manganese	Sr	Strontium
Cd	Cadmium	Mo	Molybdenum	Sn	Tin
Cr	Chromium	Ni	Nickel	Ti	Titanium
Co	Cobalt	Pb	Lead	V	Vanadium
Cu	Copper	Sb	Antimony	Y	Yttrium
Fe	Iron	Sc	Scandium	Zn	Zinc

Units of Measure

Used in Text	Description
‰	Parts per thousand (salinity)
°C	Degrees Celsius (temperature)
g	Grams (mass)
K	Kelvin (temperature)
$km \cdot hr^{-1}$	Kilometres per hour (distance rate)
m	Metres (distance)
mm	Millimetres (distance)
ml	Millilitre (volume)
$mS \cdot cm^{-1}$	Millisiemens per centimetre (conductivity)
N	Normal (concentration)
Pa	Pascals (pressure)
ppb = $ng \cdot g^{-1}$	Parts per billion = nanograms per gram
ppm = $\mu g \cdot g^{-1}$	Parts per million = micrograms per gram
s	Seconds (time)
torr	Torr (pressure)
W	Watts (power)

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1. INTRODUCTION

St. John's Harbour is an estuarine environment polluted by a variety of sources, in particular past and present untreated municipal and industrial wastes from sewage and storm drain outflows. Studies of surface sediments from the harbour have shown enriched levels of heavy metals such as Ag, Cd, Cu, Hg, Mo, Ni, Pb, Sb, Sn, V and Zn, associated with samples from locations near these outflows (Reid, 1998; Miskell, 2000). Results from the analysis of nearshore sediments from outside the mouth of the harbour showed a negative correlation between heavy metal accumulation and distance from the shoreline of St. John's Bay (Miskell, 2000). Little is known about the extent to which metals are being exchanged between the sediments and the water above. The presence of heavy metals in the water column can lead to contamination of a broad range of organisms in all trophic levels. Many small marine organisms, both flora and fauna, can tolerate the accumulation of high concentrations of metals (Jackson and Jackson, 1996). Animals such as fin fish that would feed on large quantities of these small, contaminated organisms would then accumulate the pollutants within their own bodies. As a pollutant is carried up the food chain its concentration increases within organisms of increasing tropic level. This process is commonly known as bioaccumulation. Thus, it is important to determine metal concentrations in the water column because even low level concentrations of contaminants in small organisms that are at the bottom of the food chain may lead to large concentrations in higher organisms living in the outer marine

environment. Chemical analysis of blue mussels (*Mytilus edulis* and *trossulus*), which, over the course of their lives, accumulate metals from the water in which they feed, has been shown to be an effective method for assessing heavy metal pollution in water bodies (e.g., NOAA, 1993).

The objectives of this study were to determine:

- the concentrations of heavy metals in soft tissue of the indigenous population of blue mussels from St. John's Harbour and the adjacent marine environment;
- whether mussels from within-harbour sites have higher concentrations of heavy metals than mussels sampled from outside the harbour;
- if a relationship exists between the spatial distribution of concentrations of metals in mussels and in sediments previously analyzed from within and outside of the harbour.

The principle scientific hypothesis for this study is that mussels sampled within St. John's Harbour will contain higher concentrations of metals than those sampled from outside the harbour as a result of their proximity to anthropogenic pollution sources.

1.1 Heavy Metal Pollution in Estuarine Environments

Municipal and industrial pollution of harbours and estuarine environments is a problem of global concern. Many large-scale monitoring programs are on going and small-scale individual studies have been done in order to assess metal

contaminant levels in seawater. *Mytilus edulis* is commonly used as a bioindicator in much of this research. The National Status and Trends (NS&T) Mussel Watch, National Oceanic and Atmospheric Administration (NOAA), has been monitoring contamination of shellfish in the coastal United States since 1986 and Gulfwatch was established in 1991 to monitor pollution in the Gulf of Maine.

Lobel et al. (1991) studied *Mytilus edulis* from an unpolluted site in Bellevue, NF, for a variety of elements, including Mn, Cu, Zn, As, Se, Sr, Cd and Pb. The purpose of this study was to examine relationships between soft tissue concentrations and mussel characteristics, such shell size and shape.

Moukrim et al. (2000) studied *Mytilus galloprovincialis* from Agadir Marine Bay, Morocco, in an area polluted with industrial and municipal untreated wastewater. Samples were also collected from an unpolluted control site and both sets of samples were analyzed for Cu, Zn and Cd. According to the authors, these elements were chosen because previous studies in this area indicated that they were the most abundant metals in the wastewater. Concentrations of Cu and Zn were significantly higher at the polluted site, whereas Cd levels were significantly higher at the control site. Similar findings of high Cd concentrations at unpolluted sites were reported for *Mytilus trossulus* from the Kuril Islands, in the Pacific Ocean between Russia and Japan (Kavun et al., 2002). Natural sources were believed to be the cause of the high Cd levels in both studies.

As part of the National Oceanic and Atmospheric Administration (NOAA), National Status and Trends (NS&T) Program in the United States, Turgeon et al. (1989) studied *Mytilus edulis* from eleven sites within Long Island Sound, NY, USA. This study showed a correlation between levels of metals (such as, Cr, Cu, Cd and Pb) in mussels and in sediments. Concentrations of these analytes were higher in the narrow, western end of the sound compared to those samples taken closer to the mouth of the sound in the east. Poor water circulation and high pollution input of wastewater treatment effluent, direct soil runoff, and industrial effluent, in the west-end of the sound, were the reasons given for the observed enrichments.

The Gulfwatch program for the Gulf of Maine, ME, USA, encompasses 56 sites around the perimeter of the gulf, including several from New Brunswick and Nova Scotia, Canada. Chase et al. (2001) described three patterns of metal distribution within the gulf: uniform (Fe, Ni, Cu, Zn and Cd), uniform with isolated hot spots (Cr and Pb), and an increasing gradient from north to south (Ag). Chromium concentrations were high at sites in New Hampshire and Massachusetts, the former where tanning centres thrived for most of the 20th century. Lead concentrations were highest in the most populated areas: Boston Inner Harbour, MA; Portland Harbour, ME and Boothbay Harbour, ME. Lead concentrations were also high in commercial ports of Nova Scotia (Chase et al., 2001). Enrichments were thought to be due to human activities.

1.2 Using Mussels as Bioindicators of Water Quality

The rationale for using the soft tissue of mussels to monitor seawater contamination was first presented by Goldberg in 1975. Since then much literature has been published (Jørgensen, 1990; Widdows & Donkin, 1992; NOAA, 1993; O'Connor, 1994; Sericano 2000) that supports and expands on the advantages of using mussels as bioindicator organisms, many of these advantages are outlined in the following paragraphs.

When selecting a type of shellfish to use as a bioindicator in a water quality study, bivalve molluscs are preferred because these are the only class of shellfish that filter the water as they feed, thus accumulating contaminants that exist in the water, even at trace levels (Environment Canada, 2001). Within the Bivalvia class, the sedentary *Mytilus* genus (commonly called "mussels") is favoured over mobile organisms. As *Mytilus* generally grow as a group in one bed, the concentration of contaminants contained within an individual correlate with the average contaminant concentrations in the surrounding water. These beds or clusters usually contain sufficient numbers of mussels so that they may be resampled if desired. Mussels tolerate fluctuations of salinity and water temperature and can survive in heavily polluted environments, making them exemplar monitors for contaminated estuarine environments.

There are several advantages to using mussels to analyze water quality rather than testing the water directly. The concentration of contaminants in water

samples varies seasonally, daily and even hourly in high flow areas with high input sources, such as sewage and storm drain outfalls. Mussels are very efficient water filters in that they can process up to 300 times their weight in water per hour (Environment Canada, 2001) and they bioconcentrate contaminants by factors of 10^2 to 10^5 making chemical detection more probable (NOAA, 1993). Often concentrations in the soft tissue of just one organism can be detected which enables knowledge to be gained about the within-site variability of samples. In contrast, hundreds of costly analyses of water samples are needed to gain statistical information from just one site and longer monitoring periods are required. Another advantage to analysing an organism rather than the water itself is that the amount of contaminant that is absorbed by the organism is indicative of the bioavailability of the contaminant in the study area.

Mytilus edulis and *M. trossulus* (or "blue mussels") are appropriate for coastal studies in Atlantic Canada because they are commonly found on rocks and wharf pilings in intertidal and shallow subtidal zones (Canadian Department of Fisheries and Oceans, 1996; Innes & Bates, 1999). Therefore, the results from this study may be compared to those in other parts of Newfoundland as well as with those from other Atlantic Provinces.

1.3 Background Information on St. John's Harbour

1.3.1 Geography and Characteristics

St. John's Harbour is located in the northeastern region of the Avalon Peninsula of the island of Newfoundland, Canada (Figure 1.1). It is a protected harbour, as the mouth is not as wide as the inner portion of the harbour. Thus the portion leading to the ocean is named, and will be referred to in this thesis as, the *Narrows*. St. John's Bay is the nearshore area east of the Narrows extending south toward Freshwater Bay (Figure 1.2). There is a shallow shelf at a depth of approximately 10 metres or less that runs along the shoreline from Logy Bay to Cape Spear. This water depth is maintained around the shoreline perimeter of St. John's Harbour. The prevailing water current travels southward along the coastline from north of Logy Bay into the north side of the Narrows, counter clockwise around the harbour, cycles back out through the south side of the Narrows, and then continues southward along the shoreline to Freshwater Bay (Figure 1.3) (Jacques Whitford Environmental Limited, 1995).

The geology of the study area has been mapped and is reported in detail by King (1990). The rocks are primarily late Precambrian and consist of two groups: the Signal Hill Group, with 5 formations, and the St. John's Group, with 3 formations. These groups are mainly composed of sedimentary rock consisting of a variety of shales and sandstones (King, 1990). Figure A.1 in Appendix A provides a more complete description of the area.

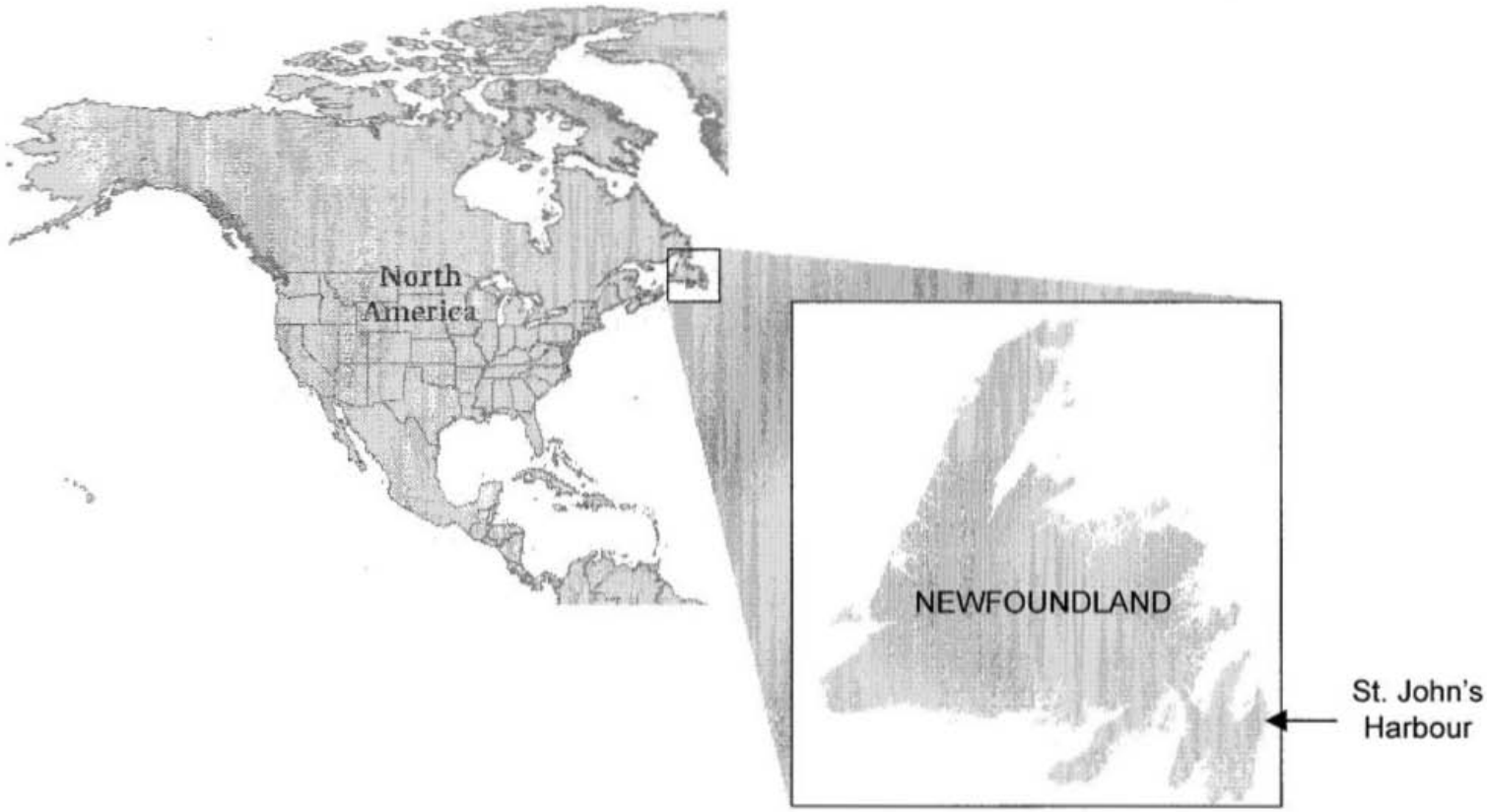

Figure 1.1 Maps of North America and Newfoundland: St. John's Harbour is located on the most easterly part of the island, as indicated by the arrow. (North America map adapted from *www.mapquest.com*; Newfoundland map adapted from Memorial University of Newfoundland, *www.heritage.nf.ca*.)

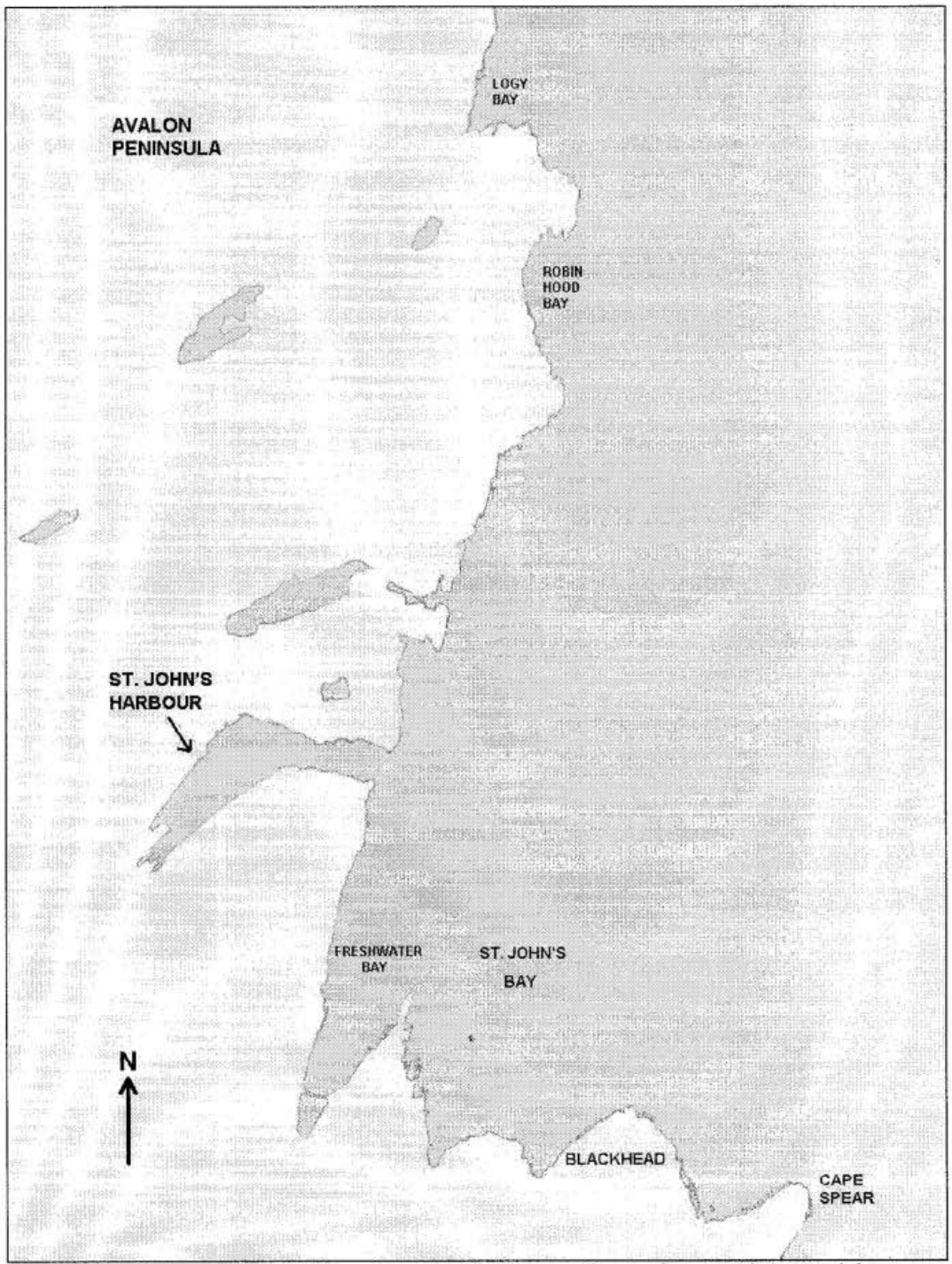

Figure 1.2 Map of Study Area from Logy Bay to Cape Spear (Adapted from LC4846, The Canadian Hydrographic Service, 1999)

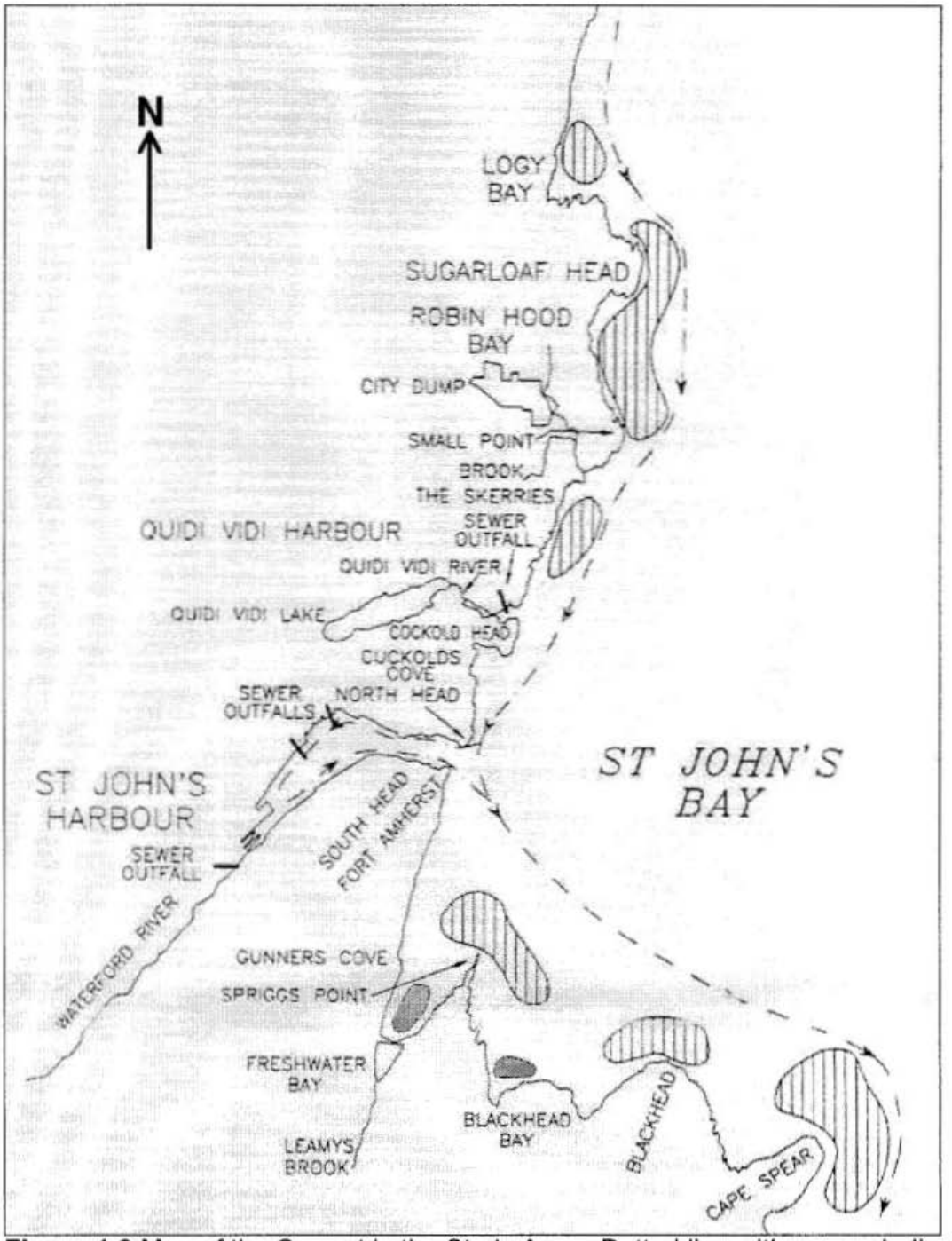

Figure 1.3 Map of the Current in the Study Area: Dotted line with arrows indicate prevailing current, regions shaped with vertical lines indicate stronger current and regions shaded grey indicate weaker current (adapted from JWE, 1995).

1.3.2 Previous Research on St. John's Harbour

Past and present municipal waste from the cities of St. John's and Mount Pearl and part of the town of Paradise is a major anthropogenic pollution source to the waters and surface sediment of St. John's Harbour (NDAL, 1996; Miskell, 2000). These communities have a combined population of over 135 000 people (Statistics Canada Census, 1996) and a developed land area of 120 km^2 (NDAL, 1996). Figure 1.4 gives an overview of this area including drainage basins and waterways.

Studies of surface sediments taken within St. John's Harbour (Reid, 1998; Miskell, 2000) reported enriched concentrations of heavy metals, including Ag, Cd, Cu, Hg, Mo, Ni, Pb, Sb, Sn, V and Zn associated with sample sites near sewer and storm drain discharge points. Miskell also analyzed nearshore sediments from outside the mouth of the harbour. The highest concentrations of the above mentioned metals were located within the harbour just inside the Narrows and directly east of the mouth of the Narrows. This indicates that some of the contaminated sediment within the harbour is escaping to the outer nearshore environment (Miskell, 2000).

Empirical evidence gathered in a study conducted by Jacques Whitford Environment Ltd. (1995) is consistent with Miskell's results. Marine pollution was mapped along the north-eastern Avalon Peninsula coastline based on the results of a survey of St. John's Harbour users, such as local fishers, fisheries

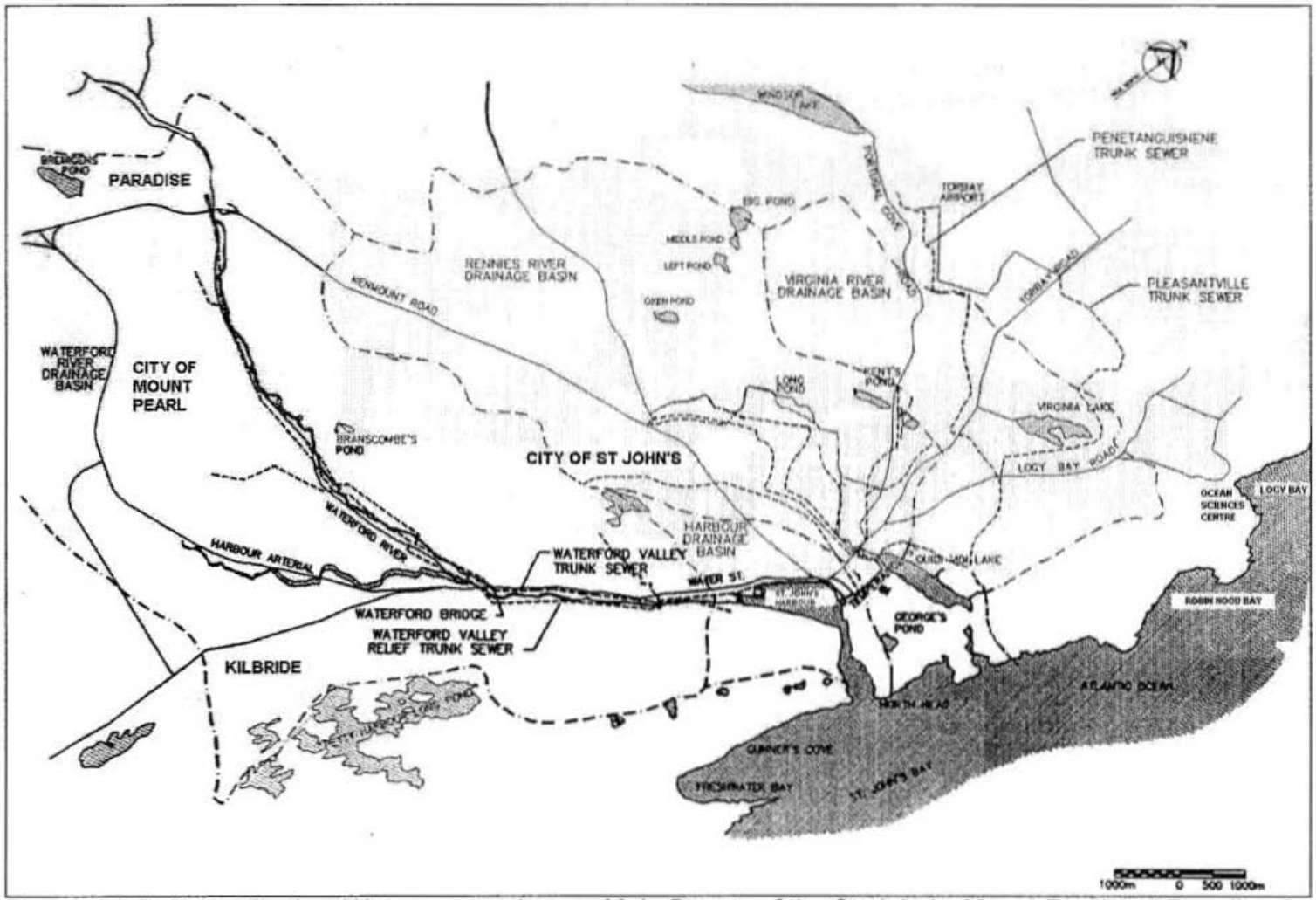

Figure 1.4 Drainage Basins, Waterways and some Main Streets of the St. John's, Mount Pearl and Paradise Area (Adapted from NFDAL, 1996.)

organisations and commercial vessel operators. Among other questions, they were asked to report the type and how much waste material they had seen and when and where they had seen it. The waste material was grouped into categories and then a map reflecting a compilation of all the responses was developed. Survey respondents were also asked to identify the fishing grounds for a variety of commonly fished species in the area. Upon comparing the wastes map and the fishing grounds maps, an obvious overlap exists in the area from St. John's Harbour to Cape Spear. Cod, lobster, salmon, capelin, squid, herring and lumpfish were reportedly fished in this area where oil, sewage, sanitary waste, plastic bags and household trash was also sighted. Pollution plumes were reported in the area outside the Narrows where surface sediments were identified as contaminated by Miskell (2000).

These studies show that suspended, highly visible waste is escaping from the harbour and that nearshore sediment in St. John's Bay is contaminated with the same heavy metal pollutants that exist in the harbour. In both cases, the source of the pollution was suspected to be from sewage and storm drain outfalls.

1.3.3 Sources of Heavy Metals

In addition to sewage, there are a variety of industrial activities that occur around the perimeter of the harbour and may contribute to the concentration of metals in the harbour environment. The harbour's extensive use as a multinational shipping facility causes contaminants from ships' fuel, oil and bilge water to be

added to the harbour waters. Paint from the hulls of ships as well as a defunct paint-manufacturing plant that was located on Water Street, just below Temperance Street may be present and past sources of lead contamination . High concentrations of Cd (6.5 ppm), Zn (950 ppm) and Pb (400 ppm) were observed in St. John's Harbour surface sediments by Reid (1998) approximately half way along the south side of the harbour. Several industrial buildings are located in that area. Located directly above this site, on South Side Hills, are several large commercial oil storage tanks.

Between the early 1800's to the 1950's, coal was a popular fuel for home-heating among the residents of St. John's – most of whom were living near the harbour (Christopher et al., 1993). In a study of sediment cores from lakes in the St. John's area, Christopher et al. (1993), found high concentrations of Fe, Co, Zn, As, Cd and Pb, the result of atmospheric deposition of coal emissions. Selenium is also know to have concentrations as high as 4 ppm in coal ash, as Se accumulates in the organic material from which the coal is eventually formed (Keller, 2000). Thus, these elements entered the harbour directly from the atmosphere and possibly through runoff from soils and drainage from lakes enriched in these metals.

1.4 Background on Mussels

1.4.1 Anatomy

A *Mytilus edulis* shell is somewhat egg-shaped with the anterior end narrower than the posterior (Figure 1.5). The exterior colouring can be various shades of blue or light brown (Brylinsky, 1989). It consists of two valves, hence the class name, Bivalvia, which are joined along the midsection of the dorsal side. Posterior and anterior muscles, called adductors, control the opening and closing of these valves (Figure 1.6). There are ridges along the lateral sides of the valves that form concentric arcs that centre on the anterior-most point, called the umbo (Figure 1.5). The byssus threads emerge from the mid-ventral side of the shell and are used to attach the mussel to a substrate. These are formed from protein-based material that is emitted from the mussel foot as it presses against the substrate. Once the threads have hardened, the foot is recalled back inside the shell (Brylinsky, 1989).

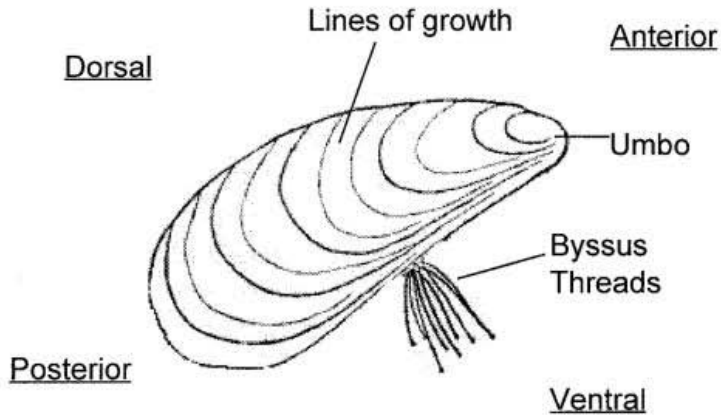

Figure 1.5 The External Anatomy of *Mytilus edulis* (Adapted from Brylinsky, 1989.)

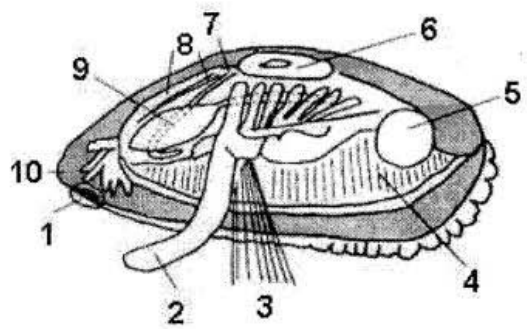

Figure 1.6 Diagram of Mussel Soft Tissue: 1=anterior adductor muscle, 2=foot, 3=byssus threads, 4=gills, 5=posterior adductor muscle, 6=heart, 7=digestive gland, 8=intestine, 9=stomach, 10=hinge (adapted from Yoon, website).

1.4.2 Metabolic Processes

Much research has been done regarding the ways in which metals are introduced to, stored in, and expelled from mussels (Fisher et al., 1996; Gagnon and Fisher, 1997; Boisson et al., 1998; Vercauteren and Blust, 1999; Griscom et al., 2000). A few of the many complex metabolic factors that are necessary to consider in a biomonitoring study are briefly presented below. Metals enter the organism as dissolved and particulate phases. In particulate phase, the metal is adsorbed onto an inorganic (sediment) or organic particle. Once inside the mussel, the metal may preferentially be stored in one or more parts of the soft tissue and/or in the shell. The mussel will release some of the metal back into its environment, either through feces or through chemical replacement reactions in the shell.

1.4.3 Causes of Variability in Sampled Mussels

Annual Reproductive Cycle

Metal concentrations found in mussel tissue vary seasonally. During the reproductive cycle changes in feeding habits and respiration rates may affect the uptake and clearance rates of contaminants. Thus mussels sampled during spawning may not accurately represent levels of contaminants that exist at other times of the year. The USEPA recommends sampling one month before or after the spawning period (USEPA, 1995). Generally, the dry weight of the soft tissue will be lowest in the winter months and highest in the summer, with a decrease

back to winter month levels after spawning (Thompson, 1984). For this reason, it is preferable to collect samples prior to spawning season.

Many natural environmental factors such as water temperature, salinity, food availability and tidal exposure collectively regulate growth and reproduction in the mussel. In areas such as estuaries where these variables differ from year to year, the reproductive cycle of *Mytilus edulis* will also vary. Once the gametes are fully developed within the mussels an increase in water temperature or change in salinity can start the spawning process. These types of environmental triggers ensure optimum conditions for fertilization and development of new organisms (Newell, 1989). A 1965 study by Bayne reported successful fertilization *of M. edulis* at temperatures between 5 - 22°C and salinity values between 15 and 40‰ (Lutz & Kennish, 1992). The spawning season of the blue mussel in Newfoundland is between May and August according to information published by the Canadian Department of Fisheries and Oceans (1996). Thompson (1984) reported late July as the spawning period for subtidal *M. edulis* from Bellevue, Trinity Bay, NF, when the water temperature was approximately 15°C.

In this study, mussels were sampled during the month of June.

Age

Concentrations of metals in mussels are directly related to the length of time the organisms have been exposed to the pollutants. In a study, such as the one

reported in this thesis, where the mussels were naturally growing in the study area, the duration of exposure for each sample is equal to its chronological age. As aging methods such as the one described by Lutz (1976), were too time-consuming to be performed for this study, shell length was used as a measure of age, assuming mussels two centimetres in length to be approximately two years of age (Payne, 1999). This was considered a reasonable assumption since, except for tidal exposure, the natural environmental conditions of the sample sites were similar.

Water Temperature and Salinity

While *Mytilus edulis* survive in subzero temperatures, they actively grow in water temperatures between 3 and 20°C (Seed & Suchanek, 1992). This allows them to live in urban estuarine environments where water temperatures frequently fluctuate due to the elevated temperatures of inflows from rivers, storm and sewer drainpipes and the cooler seawater brought in by the ocean currents. Fluctuations in seawater temperature at the sites sampled in this study are not known, as only one reading was taken (at the time of sampling) per site.

Mytilus can live in waters having a broad range of salinities, 18 to 31‰ being the most successful range for mussel growth. Therefore, the actual salinity of water affects mussel growth less than the rate at which the salinity changes. Sudden changes in salinity often occur in estuarine environments due to large influxes of freshwater. The time it takes the mussel to adjust to such environmental

changes ranges from days to weeks, depending on the presence of other environmental factors (Brylinsky, 1989). However, salinity changes of less than 10 parts per thousand do not affect the activity of the blue mussel (Newell, 1989). The rate of salinity change at the sites sampled in this study is not known.

No studies examining specifically the relationships between water temperature or salinity and metal uptake were found during the literature search for this study. However, growth increases logarithmically between the temperatures of 3 and 20 °C Seed and Suchanek (1992) and growth may be retarded by fluctuations in salinity (Brylinsky, 1989). Therefore, since growth is also related to metal uptake (Widdows and Donkin, 1992), temperature and salinity readings measured in this study were examined statistically.

Genetics

Mytilus edulis is sometimes referred to as a "species complex" that encompassed three species: *M. edulis*, *M. galloprovincialis* and *M. trossulus* (Innes & Bates, 1999). Innes and Bates (1999) reported only small morphological differences among intertidal *M. edulis* and *M. trossulus* from Tray Town, Chance Cove and Bellevue, in eastern Newfoundland. In a 1995 publication, Bates and Innes reported no apparent geographic pattern for the occurrence of each species but noted that samples taken from two unsheltered sites (i.e. sites with high tidal exposure) contained higher numbers of *M. trossulus* than the sheltered sites.

A study of *Mytilus* speciation of subtidal samples in the Bellevue and Chance Cove area by Comesaña et al. (1999) reported no fixed relationship between wave exposure and the ratio of *M. edulis* to *M. trossulus* for their sample sites. They suggested that their results did not support those of Bates and Innes (1995) possibly because differences due to wave exposure may not be as evident in subtidal mussels as they are in the intertidal mussels.

Penney and Hart (1999) examined the distribution of *M. edulis* and *M. trossulus* at forty sites around the entire coastline of the island of Newfoundland. At each site, a mixture of the two species was found with *M. edulis* dominant at the majority of the sites, including a site located on the most eastern part of the Avalon peninsula, south of St. John's Harbour.

Based on the above information it may be assumed that both species of mussels were present in the study area described in this thesis. Further, as all samples were taken from the subtidal zone, it is probable that *M. edulis* were more numerous.

Innes and Bates (1999) favoured the use of genetic markers as a reliable method for species identification of mussels. Determination of these markers (or alleles) requires a portion of soft tissue from each mussel to undergo protein analysis by electrophoresis (Bates & Innes, 1995). The possibility of determining the species of a representative portion of the mussels used in this study was examined, but rejected. One reason for this decision was that it would add a source of

contamination since metal knives would have to be used, as it would be difficult to remove the small portion of tissue without using a tool sharper than a plastic knife. Also, there are conflicting results in the published literature regarding the need for determining this parameter. Results from a study by Lobel et al. (1989) suggest *M. trossulus* and *M. edulis*, in an unpolluted environment, have different capacities for accumulating elements due to differences in growth rates. Seed and Suchanek (1992) stated that while mussel genotype plays a role in growth variation, its effect is most likely insignificant relative to the effects of environmental factors. Given the difficulties with species identification and the inconclusive evidence for a species preference in metal uptake, the ratio of *M. trossulus* and *M. edulis* at each of the sample sites in this study was not determined.

Tidal Exposure

Tidal exposure (or wave action) may affect the distribution of *Mytilus* species living in an intertidal zone but seems to have less effect on subtidal mussels (Comesaña, 1999). Only subtidal mussels were sampled in this study.

Shell shape is also affected by tidal exposure. Mussels living in sheltered areas usually have thin, elongated shells where as, shells experiencing strong wave action are thicker and more rounded (Brylinsky, 1989).

2. MATERIALS AND METHODS

Note that the author of this thesis conducted all field and laboratory work, data calculations and statistical analysis. Two others participated in the sample collection.

2.1 Rationale

The goal of the mussel sampling and chemical analysis program was to determine the intrinsic concentrations of several heavy metals in a statistically significant number of individuals of a common size at a variety of sites inside and outside of St. John's Harbour. A variety of materials and instruments were employed to meet this goal. They are listed in Appendix B.

Chemical analyses were made using inductively coupled plasma mass spectrometry (ICPMS), which can provide precise and accurate data for a large number of metals in a single aliquot of sample. Special procedures were developed to avoid metal contamination of the mussels during sampling and preparation for chemical analysis. These included the use of plastic sampling tools as described below. This is particularly important for ICPMS analysis because the detection limits of the technique are at or below the nanogram per gram level for many heavy metals. Sample digestion in acid for ICPMS analysis was carried out using high pressure, closed vessel microwave heating. In addition to ensuring breakdown of organic compounds, necessary for ICPMS analysis, the large volume digestion vessels of the microwave unit allowed

digestion of the entire soft tissue of an individual mussel. This, eliminated the need to homogenize the dried soft tissue before dissolution and hence there was a reduced opportunity for sample contamination during the homogenizing process (USEPA, 1995).

2.2 Health and Ethics Issues

2.2.1 Human Health Safety Precautions

Under the advisement of an Industrial Hygienist working for the Provincial Government of Newfoundland and Labrador, in the Department of Environment and Labour, the author of this thesis was inoculated for the Hepatitis A virus prior to collecting samples for this research. This was considered to be a reasonable safety precaution since mussels are known to be virus carriers (Shumway, 1992) and most of the test organisms were exposed to sewage containing human waste.

2.2.2 Animal Care Guidelines

No fixed guidelines were available, as the current policy and procedures guidelines of Memorial University of Newfoundland, with respect to animal care and experimentation are limited to vertebrate animals (Animal Care Services, MUN, 2001).

2.3 Sample Collection

2.3.1 Choice of Sample Sites

Figures 2.1(a) and (b) are maps of the study area showing all sample sites. Sample sites located inside the harbour and in the Narrows were chosen based on their proximity to sewage outfalls. The objective was to sample from locations as close to the sewage outfalls as possible while obtaining a distribution of sites that would be representative of the entire harbour perimeter. Outer harbour sites 11, 14, 15 and 16 were chosen to represent the St. John's Bay area because they were easily accessible by land. Steep cliffs and the open coastline made the region between sites 14 and 15 unavailable for sampling from land or boat. Since, the ocean current flows southward along the coastline (JWE, 1995), Logy Bay was chosen to be the control site as it is upstream of the harbour and was previously deemed to be an unpolluted environment (Lobel et al., 1991).

There were no mussels growing on any of the wharf pilings located on the most northwestern part of the harbour. Similarly, mussels were not present in the area surrounding the Prescott Street outfall. It is interesting to note that at site 5, mussels were growing on only one of the pier pilings. This particular piling was no longer being used to support the pier, but stood, with many other old, half-rotted pilings, situated behind the newer ones (Figure 2.2). The mussel growth on this one piling was quite prolific which provides a possible explanation for the absence of mussels on the wharf on the northwestern part of the harbour.

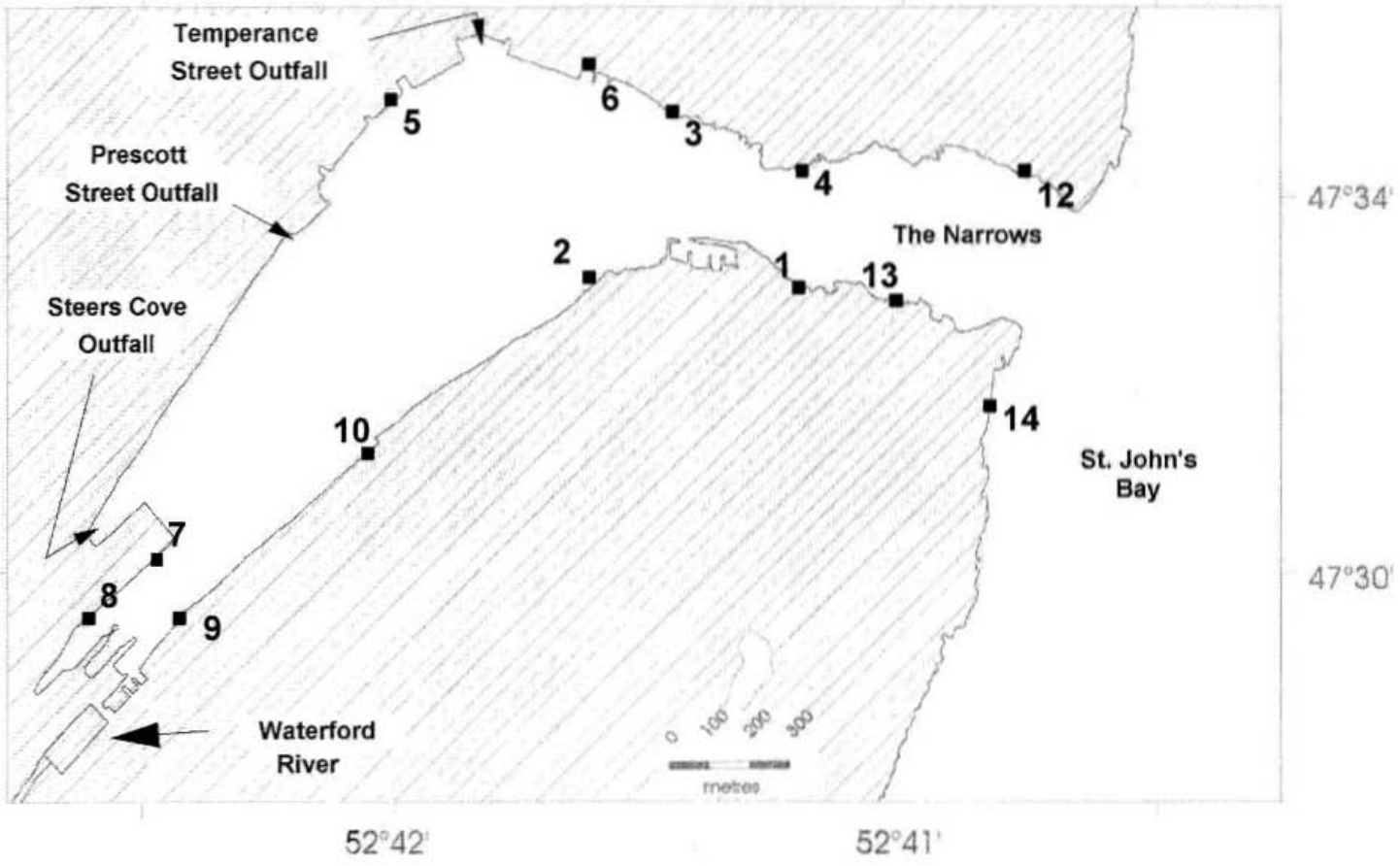

Figure 2.1 (a) St. John's Harbour Mussel Sample Collection Sites: Sample sites outside the range of this map are shown in Figure 2.1 (b). Sewage outfalls are labelled and indicated by arrows.

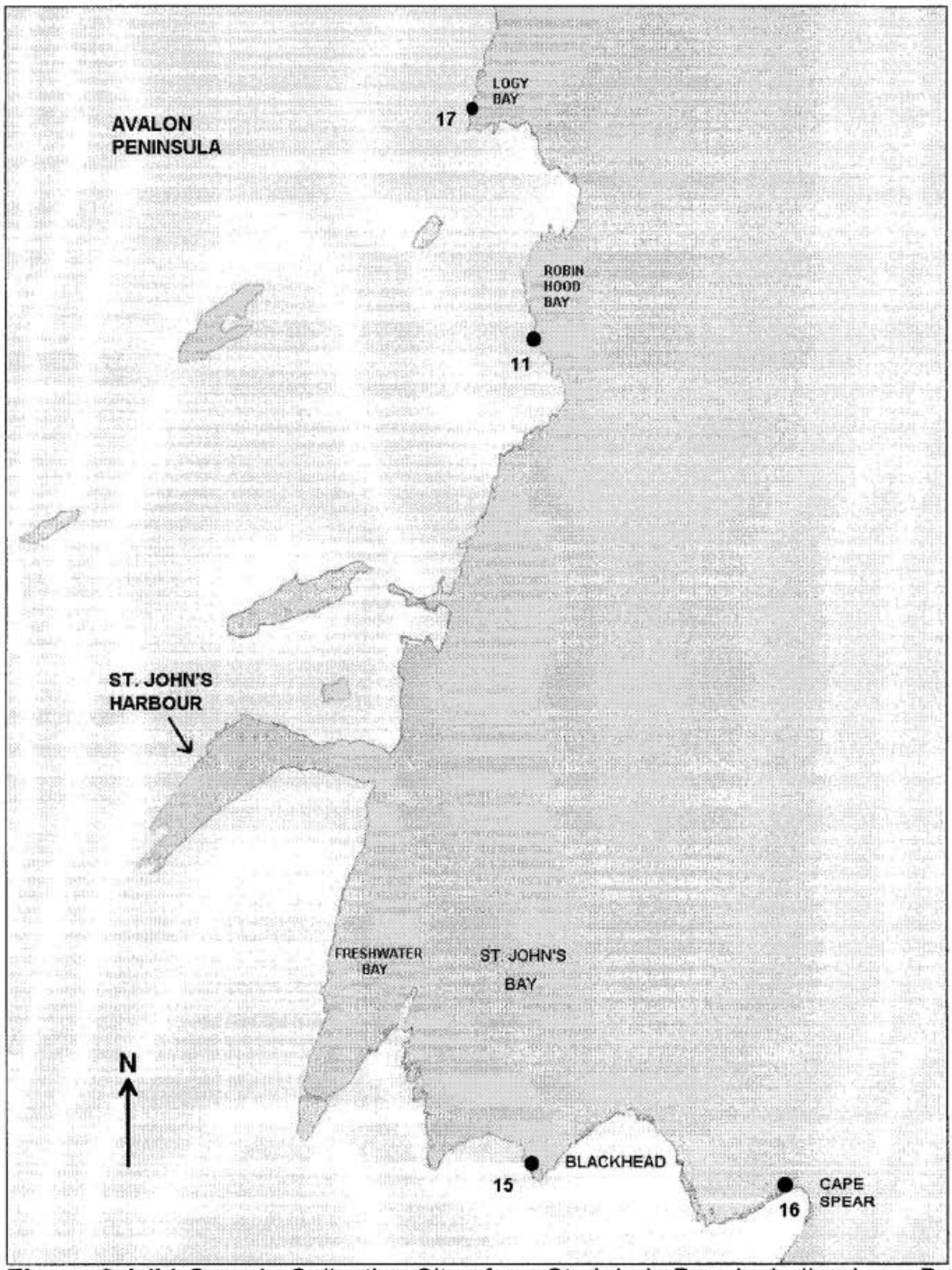

Figure 2.1 (b) Sample Collection Sites from St. John's Bay, including Logy Bay Control Site: (Adapted from LC4846, The Canadian Hydrographic Service, 1999)

Figure 2.2 A Photograph of Sample Site 5: Mussels were sampled from the second piling from the left. Note that mussels are not growing on any other pilings in the area.

That is, it is likely that mussel growth was prohibited due to lack of suitable substrate material rather than due to poor water quality in this area. This inference is supported by the fact that samples were acquired from sites 7, 8 and 9, in an area with similarly poor water quality.

2.3.2 Sampling Procedure

All materials that could have potentially been in physical contact with the mussel tissue during sample collection were soaked in 4N HNO_3 acid for 24 to 48 hours and rinsed with distilled, deionized water prior to use.

Sampling was carried out in June 1999. Approximately 30 to 50 mussels were collected from each of the 17 sample sites. This ensured that there would be a sufficient number of individuals analyzed from each site to test for statistically significant differences between sites (Nicholoson and Fryer, 1996). Individual mussels from each site had shell lengths of 2 centimetres to 5 centimetres. Sites 2 to 10 were sampled from a small open boat and sites 1 and 11 to 17 were sampled from land. Depending on the availability at each site, mussels were sampled from either pier pilings or shoreline rocks, then placed inside plastic Ziploc bags and stored in a cooler, on ice. Separate bags were used for each sample site and each bag contained a seawater-soaked paper towel that served to keep the mussels moist (Hatfield, 1999). Samples that were growing on pilings were pulled from substrates using a plastic rake or by hand; those collected from rocks were sampled by hand. At all sites, gloves were worn when

collecting and storing mussels. Sampling dates and times, and the substrate from which the mussels were sampled are reported in Table 2.1.

The mussels were transported to the Ocean Sciences Centre (OSC), Logy Bay, St. John's, NF, where epifauna, flora and any connecting byssus threads were removed from the exterior of the shells using soft bristled non-metal brushes. The samples were placed in a marine tank in order to undergo a six-day period of depuration, when any sediment particles that may have been present in the soft tissue were flushed out. The flow-through marine tanks at the OSC were chosen because they had been successfully used for this purpose by Lobel et al. (1989). The tanks were of a sufficient volume (one meter cubed), that all samples in this study could be kept in one tank, however segregation of the mussels by sample site was necessary. This was achieved using rectangular, lidded plastic cages (Figure 2.3) into which several holes were bored to allow a free flow of water through the container (Hatfield, 1999). Mussels from each site were placed in the acid-cleaned cages except for those from site 1. At the time when site 1 was sampled, the containers had not yet been prepared, however a container was not needed in this case as only mussels from site 1 were in the tank at that time.

It should be noted that on June 9, it was observed that the bottom of the tank was peppered with fine-grain organic and clastic particles. According to the technician who worked in the tank laboratory, small particles of algae and silt

Table 2.1 Parameters Recorded During Sample Collection: In the "Time" column, (c) and (f) = starting time and finishing time, respectively. "pH" and "Temp" refer to the pH and temperature of the water at the sample site; N.R. = not recorded. Area = Approximate area of sample collection. Sample growing substrate is shown as being either pier pilings (P) or rocks (R). "Date Frozen" is the date the samples were removed from the OCS tank.

Site #	Date Collected	Time	pH	Temp (°C)	Area	Pier (P) Rock (R)	Date Frozen
1	June 03	15:15	N.R.	N.R.	1-2 (m^2)	R	June 09
2	June 09	09:12 (f)	7.75 ± 0.05	5.6	2 pilings	P	June 15
3	June 09	09:21 (c)	7.79 ± 0.02	5.3	2 (m^2)	R	June 15
4	June 09	09:45 (f)	7.90 ± 0.03	6.1	2 (m^2)	R	June 15
5	June 09	10:30 (f)	7.87 ± 0.01	5.6	1 piling	P	June 15
6	June 09	10:45 (f)	8.03 ± 0.02	6.5	2 (m^2)	R	June 15
7	June 09	11:20	7.67 ± 0.01	7.5	1 piling	P	June 15
8	June 09	11.56	7.75 ± 0.03	5.8	1 piling	P	June 15
9	June 09	12:21	7.64 ± 0.03	8.7	1 piling	P	June 15
10	June 09	12:42	7.64 ± 0.03	7.9	1 piling	P	June 15
11	June 11	11:20 (c) 11:35 (f)	8.20 ± 0.03	8.9 ± 0.1	2 (m^2)	R	June 17
12	June 14	13:55 - 14:15	8.04 ± 0.03	9.0 ± 0.1	2 (m^2)	R	June 20
13	June 15	14:40	7.85 ± 0.01	10.1 ± 0.1	0.5 (m^2)	R	June 21
14	June 15	15:20	7.91 ± 0.02	9.6 ± 0.1	0.5 (m^2)	R	June 21
15	June 18	05:30 - 06:00	7.92 ± 0.02	8.4	< 0.5 (m^2)	R	June 24
16	June 18	06:30	7.66 ± 0.03	8.7	1.5 (m^2)	R	June 24
17	June 18	07:45	7.78 ± 0.02	8.7 ± 0.1	<0.5 (m^2)	R	June 24

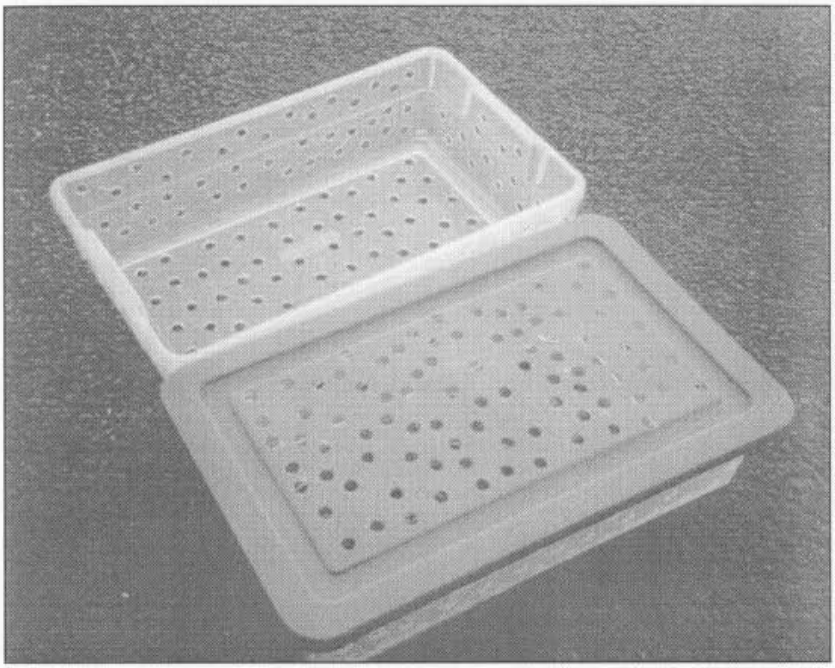

Figure 2.3 A Photograph of Mussel Cages – Plastic containers used to keep samples of differing sites separate during the depuration period. Container dimensions are (32 x 21 x 9) centimetres. Twelve cages were made in total.

often pass through the seawater intake valve filters. Had this been known prior to the commencement of this study, a cleaner environment would have been attained for the depuration process. To prevent any substantial accumulation of particles, the tank was drained, rinsed and refilled June 9, 14, 18 and 21. For each of these rinses, the mussel containers were not exposed to air for more than 3 to 5 minutes.

The temperature and pH of the water in the tank, on June 17, were 8.3 ± 0.2°C and 8.08 ± 0.03, respectively. On June 24, temperature, pH and water conductivity were recorded as 7.6 ± 0.1°C, 8.05 ± 0.2 and 50.4 ± 0.1 $mS \cdot cm^{-1}$, respectively.

After the depuration period, the samples from each site were separately rinsed with seawater and placed into new, acid-cleaned Ziploc bags and stored in a conventional freezer at approximately -5°C. The dates when the samples from each site were removed from the OSC tank are provided in Table 2.1.

2.3.3 Ancillary Measurements

Ancillary measurements that were taken during sample collection were the temperature, pH and depth of the water at which the samples were taken and the area and global positioning coordinates of each sample site. As no portable conductivity meter was available, water samples were returned to the laboratory for conductivity measurements. A detailed list of the instruments used is given in

Appendix B. Weather conditions such as wind speed and direction, and precipitation measurements (Environment Canada, 1999) are listed in Appendix C.

At each sample site, seawater was collected in a plastic pail and the temperature was measured using a calibrated portable instrument with an uncertainty of ± 0.1°C. This method of temperature measurement is similar to that used by NOAA (1993). A pH reading was then measured using a portable pH meter that had been calibrated to an accuracy of ± 0.2 just prior to sample collection. Readings for temperature and pH can be found in Table 2.1.

The water depth at which the mussels were sampled was not measured quantitatively; most were taken between 0.25 metres and 0.50 metres below the low tide mark. The recorded depths in Table 2.2 are estimates reported by the individual who collected them, at the time of sampling. It was difficult to estimate the depth of samples from site 4 because waves were rising and falling against the rock with an amplitude of approximately 1 metre. However, it is believed that the mussels were subtidal because they were underwater at the trough of the wave swells and it was very near the time of low tide. While no depth was recorded for site 5, a rake was used to retrieve mussels that were deeper than arms-length, thus it is believed that they were subtidal. At site 6 there were very few mussels available, those collected were small and below the low tide mark.

Table 2.2 Estimates of Low Tide and Sample Depth: Approximate water height was calculated using the time and water height (ht.) at low tide (DFO, 1999) and the time of collection. The recorded depths are estimates, not measured values. N.R. means measurement was not recorded.

Date	Site	Low Tide Time	Time Collected	Low Tide ht. (m)	Calculated Height (m)	Recorded Depth (m)
June 03	1	15:50	15:15	0.3	0.3	0.10 - 0.15
June 09	2	09:20	09:12	0.3	0.3	0.25
June 09	3	09:20	09:21	0.3	0.3	0.10 - 0.30
June 09	4	09:20	09:45	0.3	0.3	N.R.
June 09	5	09:20	10:30	0.3	0.3 - 0.4	N.R.
June 09	6	09:20	10:45	0.3	0.4	N.R.
June 09	7	09:20	11:20	0.3	0.4	0.50
June 09	8	09:20	11:56	0.3	0.5	0.50
June 09	9	09:20	12:21	0.3	0.6 - 0.7	0.50
June 09	10	09:20	12:42	0.3	0.6 - 0.7	0.50
June 11	11	11:10	11:20 - 11:35	0.2	0.2- 0.3 0.2- 0.3	N.R.
June 14	12	13.50	13:55 - 14:15	0.2	0.2 0.2- 0.3	0.15 - 0.25
June 15	13	14:40	14:40	0.2	0.2	0.30 - 0.40
June 15	14	14:40	15:20	0.2	0.2- 0.3	0.30 - 0.40
June 18	15	05:15	05:30 - 06:00	0.2	0.2 0.2- 0.3	0.20
June 18	16	05:15	06:30	0.2	0.3	0.25 - 0.30
June 18	17	05:15	07:45	0.2	0.5 – 0.6	> 0.50

During low tide in Robinhood Bay (site 11), a large stretch of rocky beach is exposed along with many beds of small intertidal mussels. The samples collected from this site are believed to be subtidal because they were at a depth of about 1 metre, in a bed with mussels much larger than those growing in the intertidal zone. Samples were collected during or very near the time of low tide. Estimates of water level deviations from the height at low tide were calculated by interpolating values of tide levels published by the Canadian Department of Fisheries and Oceans (DFO) (1999) (Table 2.2). These results show that the mussels used in this study were sampled near or below the 0.0 metre water level. An average lower low tide was reported to be 0.3 metres (Canadian DFO, 1999), thus mussels growing at the 0.0 metre water level would have been subtidal most of the time.

The size of each sample site was intended to be kept within an area of a few metres squared, as this would ensure samples from the same site had been exposed to the same environmental conditions and diet (Lobel et al., 1989). As with depth, the area was not measured quantitatively, but estimated by the sampling team. For most sites where samples were collected from rocks the area was between 0.5 and 2 square metres. For those where the mussel substrate was pier pilings, mussels were taken from one piling per site except site 2 where two adjacent pilings were used (Table 2.1).

The geographic location of the sites were recorded on a map and measured using a handheld Global Positioning System (GPS) meter. The meter was not taken in the boat on June 9 but GPS readings of the closest available land point to the sites were later recorded. Locations that were obtained from the map, LC4846, The Canadian Hydrographic Service (1999) and those from the GPS meter are tabulated in Table 2.3.

Ideally the salinity of the water at each sample site would have been measured, but there was no salinity meter available for use in this study so conductivity was measured instead. A sample of seawater was collected from each site, in an acid-cleaned plastic Snap-Seal vessel and stored on ice until transported to a cold storage room (7°C) at Memorial University. The seawater was tested in a laboratory, where each sample was brought to 25°C using a warm-water bath and inverted 30 times prior to taking readings. After inserting the conductivity electrodes into the sample solutions, a one-minute stabilization time was allowed before conductivity and temperature readings were recorded, using the same recently calibrated (±0.5% accuracy) instrument for both measurements. Between samples the electrodes were rinsed three times with distilled, deionized water then patted dry with a Kimwipe tissue. The protective casing that surrounds the electrode is made of plastic, so in order to avoid inducing an electrostatic field, it was patted dry in a dabbing motion rather than wiped in strokes.

Table 2.3 Geographical Locations of Sample Sites: Geographical Position System (GPS) readings for sites 1 and 11 to 17 were taken at time of collection and thus may be more accurate than those read from the map. The longitudinal map reading for sites 15 and 16 are given to the nearest second because the map for these sites had a 1:60 000 scale. The other readings were taken from a map with a 1:5000 scale and are thus given to the nearest 0.1 second.

Site	GPS Readings		Readings from Map	
#	Latitude	Longitude	Latitude	Longitude
1	N 47° 33' 53.8"	W 52° 41' 18.6"	N 47° 33' 54.0"	W 52° 41' 13.8"
2	N 47° 33' 51.2"	W 52° 41' 40.9"	N 47° 33' 50.3"	W 52° 41' 38.3"
3	Not Recorded	Not Recorded	N 47° 34' 06.6"	W 52° 41' 26.9"
4	N 47° 34' 02.2"	W 52° 41' 14.5"	N 47° 34' 02.2"	W 52° 41' 12.0"
5	N 47° 34' 07.4"	W 52° 42' 04.9"	N 47° 34' 07.6"	W 52° 42' 01.2"
6	N 47° 34' 12.4"	W 52° 41' 44.1"	N 47° 34' 10.3"	W 52° 41' 36.8"
7	N 47° 33' 33.9"	W 52° 42' 34.0"	N 47° 33' 31.5"	W 52° 42' 28.6"
8	N 47° 33' 21.6"	W 52° 42' 40.5"	N 47° 33' 25.7"	W 52° 42' 37.8"
9	N 47° 33' 27.2"	W 52° 42' 30.7"	N 47° 33' 25.6"	W 52° 42' 25.5"
10	N 47° 33' 40.1"	W 52° 42' 07.7"	N 47° 33' 39.5"	W 52° 42' 03.2"
11	N 47° 35' 44.8"	W 52° 39' 41.1"	Not Recorded	Not Recorded
12	N 47° 34' 04.0"	W 52° 40' 48.5"	N 47° 34' 01.7"	W 52° 40' 45.0"
13	N 47° 33' 52.0"	W 52° 41' 06.4"	N 47° 33' 51.4"	W 52° 41' 00.4"
14	Not Recorded	Not Recorded	N 47° 33' 43.9"	W 52° 40' 49.8"
15	N 47° 31' 41.4"	W 52° 39' 33.7"	N 47° 31' 39"	W 59° 39' 37"
16	N 47° 31' 31.8"	W 52° 37' 23.3"	N 47° 31' 33"	W 59° 37' 25"
17	N 47° 37' 32.6"	W 52° 39' 53.3"	Not Recorded	Not Recorded

Conversion from conductivity units of millisiemens per centimetre ($mS \cdot cm^{-1}$) to salinity units of parts per thousand is a non-trivial calculation. For this study, the conversion was done using following formula (USEPA, 1997):

$$salinity = A + B(c\text{-}k) + C(c\text{-}k)^2 + D(c\text{-}k)^3 + E(c\text{-}k)^4 + F(c\text{-}k)^5$$

Where c = conductivity ($mS\ cm^{-1}$) at 25°C; k is a constant (32.188) and the coefficient values are:

$A = 20$
$B = 0.69608$
$C = 1.3094 \times 10^{-3}$
$D = -1.1918 \times 10^{-5}$
$E = 1.7392 \times 10^{-7}$
$F = -3.1112 \times 10^{-9}$

Table 2.4 shows the conductivity and temperature measurements and the calculated salinity values.

Table 2.4 Conductivity and Salinity at 25°C: Conductivity (millisiemens per centimetre, $mS \cdot cm^{-1}$) and Temperature (°C) values were measured with an Orion Conductivity Meter. Salinity (parts per thousand, ‰) was calculated using a conversion formula. Measurements were not taken at site 1.

Site #	Temp (°C) ± 0.1	Conductivity ($mS\ cm^{-1}$) ± 0.1	Salinity (‰)
2	24.9	37.3	23.6
3	25.1	44.4	28.7
4	24.9	47.4	30.9
5	25.1	46.3	30.1
6	24.9	44.9	29.0
7	25.0	42.7	27.4
8	25.0	46.1	29.9
9	25.0	24.2	14.5
10	24.9	30.1	18.6
11	24.9	48.8	31.9
12	25.0	48.3	31.5
13	25.0	47.7	31.1
14	25.1	48.6	31.7
15	24.9	48.5	31.7
16	25.0	36.3	22.9
17	24.9	45.1	29.2

2.4 Sample Preparation

All materials that were in contact with the sample tissue during sample preparation were soaked in 4N HNO_3 acid for 24 to 48 hours and rinsed with distilled, deionized water prior to use. Powder-free, vinyl medical examination gloves were worn at all times in the laboratory.

2.4.1 Tissue Removal

Prior to shucking, the frozen mussels were weighed on a top-loading balance (± 0.01 grams). The length, width and height of the shells, as defined in Figure 2.4, were measured to the nearest 0.05 millimetres using plastic Vernier callipers[†] (Table A.3, Appendix D). While practising shucking techniques on store-bought mussels, it was observed that keeping the samples completely frozen facilitated tissue removal. Therefore, each batch of mussels was kept on dry ice as the individual samples were processed.

A plastic knife was inserted between the two valves at the mid-point on the ventral side, where the byssus threads emerge (Figure 1.5). Using the knife for leverage, the valves were pried open along the ventral side toward the posterior end where the posterior adductor mussel was cut (Figure 1.6). Any byssus threads still connected to the soft tissue inside the shell were then removed. The mussel was positioned with the anterior side pointing downward so that as the

[†] Relationships between shell size and metal concentrations were not analyzed in this study.

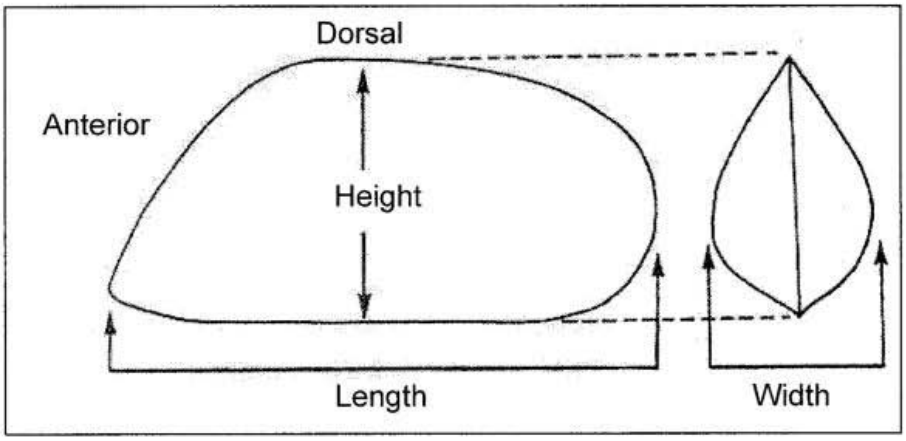

Figure 2.4 Mussel Shell Dimensions – The lateral view of the mussel shell shows the length to be the distance from the anterior-most point to the posterior most point and the height to be where the maximum distance is measured from the dorsal to the ventral sides. The posterior-end view shows the width to be the greatest distance across the lateral axis of the shells. (Adapted from Seed (1968).)

soft tissue was cut from the shell with the plastic knife, and placed directly into a pre-weighed, plastic centrifuge tube. The tube was then capped, the wet weight of the sample was measured using an analytical balance (± 0.0001 grams), and the sample was stored on dry ice in preparation for lyophilization.

The shells from each sample were heated at approximately 70°C for three days (Dabinett, 1999) then stored in appropriately labelled, zip-sealed sample bags. The shells were not analyzed in this study but were kept for possible future work.

2.4.2 Lyophilization

The mussels were lyophilized in 50 millilitre centrifuge tubes. The tubes were sturdy enough to withstand low-temperature freezing and batches of 30 tubes could fit into the chamber simultaneously.

Prior to the start of the lyophilization process, samples were frozen using dry ice and the instrument condenser temperature was lowered to -75 °C. The sample lids were removed as the sample tubes were loaded into the chamber. The chamber was then sealed and the pressure was lowered to approximately 0.1 torr (13 pascals). The samples remained in the chamber for 2.5 to 3 days, as this duration gave the highest percentage of weight loss due to drying when this procedure was tested on store-bought mussels.

When lyophilization was complete the samples were raised to room temperature (approximately 20°C) while the pressure within the chamber was kept at 0.1 torr.

The vaccum was released, as the pressure was allowed to come to ambient atmospheric pressure, and as the tubes were removed from the chamber they were re-capped with their respective lids. The tubes were weighed on the same analytical balance as before and dry weights were calculated. Each tube was individually sealed with Parafilm, then each batch of tubes was sealed inside appropriately labelled Ziploc plastic bags and stored in a cold storage room at approximately 7°C. A total of 243 mussels were lyophilized. Table A.3 in Appendix D lists wet and dry weights of samples.

2.4.3 Creating Composite Samples

Using composite samples of individual mussels from a collection site is advantageous because it reduces the number of prepared samples to be analyzed, thus decreasing the time and costs associated with the research. During sample preparation there are two stages at which tissues from two or more mussels could be combined, each having its advantages and disadvantages. Freeze-dried tissue could be combined prior to digestion, or tissues could be combined during the digestion process.

An advantage to homogenizing dry tissue prior to digestion is that many samples may be included in the composite with only a small number of sub-samples requiring digestion and further analysis. The problem, however, is finding a process that will homogenize the material without introducing metal contamination. Two metal-free methods of homogenizing the mussel tissue were

tested on store-bought, lyophilized samples for potential use in this study. The first attempt at homogenization involved grinding the tissue using a pestel and mortar that were constructed from 100 per cent Teflon. This method was unsuccessful because the pressure of the pestel did not blend the tissue into a powder, but merely flattened it against the mortar. In the second method, a grinding mill was used with 3/4" by 2" polystyrene grinding vials and 1/8" methacrylate grinding balls. While this method did produce a powder, it did not appear to be homogenous as it contained fibrous white spindles and small white chunks of tissue throughout. These chunks were thought to be the adductor muscles of the mussels. Both methods tested were considered to be ineffective ways of homogenizing mussel tissue, thus composite samples were not created at this stage in this study.

Creating composite samples at the digestion stage of sample preparation was, to some extent, viable in this study. One limitation of homogenizing mussel tissue during digestion is the size of the digestion vessel. Oxidation of organic material causes gas formation that increases the pressure inside the vessel. Therefore, only a limited amount of organic material can be safely added without exceeding the maximum pressure threshold of the vessels. Only the smaller mussels used in this study were suitable for forming composites using a process that is described further in section 2.4.4.

2.4.4 Microwave Digestion

Samples

Due to time restrictions imposed by a Master of Science program and availability of funding, it was necessary to limit the number of sample sites analyzed chemically in this study. After re-examination of the sample site locations, it was decided that sites 1, 6, 8, 9, 12 and 15 could be omitted while maintaining a good representation of the study area.

Approximately five mussels from each of the remaining sites were initially digested, analysed for metal content and statistically analysed. Based on the preliminary results from this data set, it was decided that a sample size of 10, from each site, would be sufficient to achieve statistically significant results. The microwave system that was used has a six-vessel capacity, therefore for each digestion cycle, five vessels contained sample material and acid; and the sixth was used as a blank, containing just acid. Only nitric acid (HNO_3) was used, as it is preferred for use in ICPMS analysis due to the few interferences caused by the acid, itself. Also, HNO_3 is a strong oxidizing agent (Yokogawa Analytical Systems Inc., 1997). Battelle Ocean Sciences used a similar microwave digestion method in which only nitric acid was used to digest 0.3 grams of freeze-dried oyster and mussel tissue (NOAA, 1993).

Most mussels were prepared as individual digests, however some of the smaller mussels were digested as composite samples, which contained two mussels

each. For the individual digests approximately 0.2 – 0.3 grams of the lyophilized material was weighed directly into a 120 millilitre Teflon microwave vessel and 4-5 millilitres of 8N distilled nitric acid (HNO_3) was added. Vessels that were used as blanks contained 5 millilitres of 8N distilled nitric acid. These samples were digested by either a temperature or power-regulated microwave program where the temperature was brought to 150°C for a programmed length of time. Table 2.5 provides details of the microwave programs.

A temperature-dependant digestion program that was designed using store-bought mussels was initially used to digest the study samples. However, before all the samples were digested, the microwave thermocouple became inoperable, thus a power-dependent program was designed based on power versus time graphs generated from samples digested using the temperature-dependant program. The power-dependant program was used to digest the remaining 25% of the samples. The program used to digest each particular sample is identified in Table A.3, Appendix D.

After the vessels had cooled to room temperature, they were then opened inside a fumehood and the solutions were swirled until all the orange-brown gas that had formed during the digestion had evolved. The clear, pale-yellow solutions that remained in the vessels were poured into Snap-Seal containers and made up to 60 grams with distilled, deionized water. This volume was based on dilution procedures used by the ICPMS facility at Memorial University.

Individual samples and two of the composite samples that weighed more than 0.3 grams were digested in two stages. Approximately half of the material to be digested was weighed into the microwave vessel and 4 millilitres of distilled 8N nitric acid was added. All two-stage digestions were power-regulated using similar programs as those described for the one-stage digestions (Table 2.6). After the vessels had cooled and the gas had evolved from each, the remaining dried mussel tissue was weighed into the vessels. One millilitre of distilled 8N nitric acid was added to each vessel and a second digestion program was run. Samples were diluted to 60 grams in the same manner as those that followed the one step digestion process.

Two-stage digestions were necessary for the larger samples because gas production increases with increased weight of dried organic material. The vessels used in this study were designed with an automatic venting safety feature if a maximum internal pressure were to be reached. It was decided that it would be better to develop a two-stage procedure rather than risk the venting of some vessels that could have resulted in sample loss and incomplete digestion.

Reference Materials

Two mussel tissue certified reference materials (CRMs): BCR 278R and GBW 08571; as well as two mussel tissue Standard Reference Materials (SRMs): NIST 2976 and 2977; were used in this study (Table A.4, Appendix E). These were digested by both one and two-stage methods and using both the temperature

Table 2.5 Temperature Regulated Microwave Digestion Programs: Each digestion was completed using two steps. The first raised the temperature inside the vessels to 150°C and the second step was to maintain this temperature for the given amount of time. Values in bold-type highlight the differences among the five programs. Time is given in seconds (s), temperature in degrees Celsius (°C) and the maximum power (max P) in watts (W).

	T1			T2			T3			T4			T5		
	time (s)	temp (°C)	max P (W)	time (s)	temp (°C)	max P (W)	Time (s)	temp (°C)	max P (W)	time (s)	temp (°C)	max P (W)	time (s)	temp (°C)	max P (W)
step 1	300	150	750	300	150	**800**	300	150	800	300	150	800	300	150	800
step 2	300	150	700	300	150	**750**	**600**	150	750	**720**	150	750	**300**	150	900

Table 2.6 Power Regulated Microwave Digestion Programs: Each digestion was completed using 10 steps. These programs were designed based on power versus time graphs generated from samples run under the temperature-dependant programs. Time is given in seconds (s) and the maximum power (max P) in watts (W).

	P1		P2		P3	
	time (s)	max P (W)	time (s)	max P (W)	time (s)	max P (W)
step 1	60	600	60	600	60	600
step 2	60	160	60	160	60	160
step 3	60	550	60	500	60	**550**
step 4	30	150	30	100	30	**150**
step 5	30	450	30	400	30	**450**
step 6	60	600	60	600	60	600
step 7	30	500	30	500	30	500
step 8	30	800	30	800	30	800
step 9	30	500	30	500	30	500
step 10	510	300	**600**	300	600	300

and power-regulated microwave programs, except for NIST 2977, which was digested using only a power-regulated program. Reference material digests were similarly diluted to 60 grams using distilled, deionized water and stored in plastic Snap-Seal vials.

2.5 Sample Analysis

2.5.1 Choice of Analytes

The list of analytes (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd and Pb) was comprised of those elements that were reported in available mussel tissue reference materials (Table A.4, Appendix E). NIST 2976 reference material was used as the external calibration standard in this study (section 2.5.5), thus elements with published values for NIST 2976 and at least one of either BCR 278R or GBW 08571 were included in the list of analytes. NIST 2977 was not available for purchase when the analytes were selected.

2.5.2 Internal Standard Solution

It is well known that ICPMS instrument matrix effects may be strongly mass-dependent, with atomic mass units less than 80 affected most and severe drift occurring when an analytical run extends over long periods of time (Eggins et al., 1997). Drift is often also mass dependent with low mass analytes exhibiting high drift. To correct for drift and matrix effects, an internal standard solution comprised of known concentrations of elements that are not among those to be determined in the samples may be mixed, inline, with all samples, reference materials and blanks. Elements that are chosen for an internal standard should not create spectral interference with analyte peaks (thus monoisotopic elements are preferred) and should cover the mass range of the analytes. In addition, concentrations should far exceed intrinsic levels of each element found in the

samples to ensure high counts and thus low relative standard deviations, while remaining within the pulse-counting range of the detector.

For this study, an internal standard multi-element solution was designed using certified single-element solutions of Sc, Y, Ag, Tl and Bi. The solutions were drawn from their respective storage bottles using a new, acid-cleaned, 5 millimetre plastic syringe for each. These were sequentially dispensed into the same 1 litre, acid-cleaned Nalgene bottle and weighed using an analytical balance. Weights and concentrations are given in Table A.5, Appendix E.

2.5.3 Dilution of Samples and Reference Materials

Vials containing either sample or reference material solutions were only opened within a fumehood.

The Snap-Seal vials containing the solutions were inverted 30 times, to ensure the homogeneity of the solutions, before aliquots were poured directly into 10 millilitre test tubes. The required weight of each aliquot was calculated based on a dilution factor of 5000 (see Appendix E for calculations). The weight of most aliquots ranged from 0.2 to 0.5 grams and 0.2N distilled nitric acid was added until the final weight of the diluted solution was 10 grams. The tubes were then capped and placed in a test tube rack.

The dilution factor of 5000 ensured that the analytes in the solution were adequately concentrated to produce high count rates without creating large

amounts of precipitate on the cones of the ICPMS. Such precipitate can block the orifices of the cones, which leads to a decrease in instrument sensitivity during an analytical experiment. The dilution factor was determined experimentally by testing a range of aliquot weights from solutions of store-bought mussels. The first target aliquot weight was 2.0 grams, resulting in an average dilution factor of 1200 and a decrease in instrument sensitivity was observed toward the end of the run. Target aliquot weights of 1.0 gram (average dilution factor of 2300) and 0.5 grams (dilution factor of 4100) also showed a loss in sensitivity throughout the run. The dilution factor was set to 5000 which ensured relatively stable sensitivity for the duration of the run, analyte concentrations well above detection limit and sample aliquots large enough to be poured directly from the Snap-Seal vials, into the test tubes.

2.5.4 ICPMS Analysis

An HP4500*plus* quadrupole ICPMS at Memorial University of Newfoundland was used in this study.

ICPMS Tuning

The instrument parameters were tuned using an optimizing solution containing a concentration of 10 parts per billion for each of ^{7}Li, ^{59}Co, ^{89}Y, ^{103}Rh, ^{133}Cs, ^{169}Tm, ^{209}Bi and ^{238}U.

Sample Introduction to Ion Detection

The combination of a laminar flow clean hood and a HEPA filter has been shown to reduce airborne contaminants in laboratory air by three orders of magnitude for Fe, Cu and Pb and one order of magnitude for Cd (USEPA, 2000). Thus the sample introduction region of the ICPMS was fitted a HEPA filter clean hood system. The prepared tubes were inverted 30 times, then the caps were removed within the clean hood and the solutions were sequentially introduced into the ICPMS using an automatic sampler.

The order in which the test tubes were analyzed was one flush (0.5N HNO_3), one blank (0.2N HNO_3), six unknowns, one NIST 2976 (primary calibration standard), one GBW 08571 (secondary calibration standard), one flush, one blank. This sequence repeated for four or five cycles, with the unknowns of the last cycle consisting of one sample solution and five solutions of reference materials. After the first cycle, duplicate tubes that contained solutions of unknown samples that had been tested previously within the run, and that had similar dilution factors, were analysed throughout the run.

Both the analyte solution and the internal standard solution were drawn through 0.5 millimetre (inner diameter, ID), TFE Teflon tubing to the peristaltic pump assembly. The analyte solution and internal standard were then pumped through the two roller pump assembly in 1.02 millimetre (ID) and 0.19 millimetre (ID), three-collar tygon tubing, respectively, at a rate of 0.50 rotations per second.

The peristaltic pump assembly ensures a stable flow of solution during sample introduction. The solutions flowed into separate 0.5 millimetre (ID) TFE Teflon tubing and were subsequently mixed in a hexagonal-shaped, Teflon, three-way connector. The same sized TFE Teflon tubing was used to transfer the mixed solution from the three-way connector to a concentric nebulizer, where it was converted to a fine spray and was sprayed into the spray chamber. The uptake time from sample tube to spray chamber was 60 seconds, at 0.50 rps followed by a stabilization time of 20 seconds, at 0.10 rps.

Only fine droplets of aerosol pass from the spray chamber to the torch where contained analytes became ionized in approximately 8000 K argon plasma. The sampler and skimmer aluminum cones further sampled the central part of the ion stream. The ions were then focused into the quadrupole mass analyzer using an Omega ion optic lens. From there the analyte ions were converted to electron pulses and subsequently counted by digital circuitry.

Data Acquisition Method

Data was collected in peak-hopping mode (one data point per peak) using HP supplied data acquisition software. An integration time was set for each atomic mass unit (amu) (Table 2.7) based on the expected analyte sensitivity at each mass. Quadupole settle time was 1-2 milliseconds, except between ^{111}Cd and ^{205}Tl amu (2-3 milliseconds). Three within-tube repetitions were analyzed, for a total acquisition time of 596 seconds per tube. The detector automatically

Table 2.7 Integration Times of Determined Elements: Integration time per atomic mass units (amu) = the time in seconds that the ICPMS spends acquiring data for that amu. Given are the amu, chemical symbols and integration times per mass unit, in seconds. An asterisk after the chemical symbol indicates that the element was part of the internal standard solution.

amu	Element	Integration time (s)	amu	Element	Integration time (s)
45	**Sc***	5	**88**	**Sr**	5
53	**Cr**	15	**89**	**Y***	5
55	**Mn**	5	**109**	**Ag***	5
57	**Fe**	5	**111**	**Cd**	15
59	**Co**	10	**203**	**Tl***	10
60	**Ni**	10	**205**	**Tl***	5
65	**Cu**	5	**206**	**Pb**	20
66	**Zn**	5	**207**	**Pb**	20
75	**As**	10	**208**	**Pb**	15
77	**Se**	20	**209**	**Bi***	5

switched from pulse-counting to analogue mode if count rates exceeded a pre-set limit (approximately 6 million counts).

2.5.5 Data Reduction Algorithm

Using the ICPMS software, the counts for each atomic mass unit, for each test tube were saved in separate files as the data were collected. These counts were then transferred to a spreadsheet-based algorithm that was written specifically for this study by the author of this thesis. In this section, the calculations within the algorithm are described and an example of each calculation is given in Appendix F.

Three replicate samples were taken within each tube - that is, the sample probe did not leave the tube in between replicates. Measuring replicates in this way aids in identifying and eliminating any anomalous readings (spikes in count rates) throughout the run. Therefore three sets of counts were recorded for each amu. These counts were then divided by their respective integration time per amu, the unit of these quotients is counts per second (cps). It was the mean cps of the three "intra-tube" replicates that was the base value of the rest of the data calculations and will be referred to as "mean cps" (Equation 1, Appendix F)

Drift/Matrix Correction

Precipitation of the analyte solution matrix in the interface or lens region of the instrument can progressively decrease the ability of the instrument to detect ions of certain atomic mass units. As well, matrix-rich solutions, particularly organic-

rich solutions, exhibit variable analyte responses due to space-charge effects in the plasma (Beauchemin et al., 1987). Drift/matrix factors must be used to correct for such changes in sensitivity that occur throughout each run and between solutions containing different amounts of dissolved matrix. External standardization was attempted using mixed solutions containing known concentrations of single element solutions of the analytes to be determined. However, these organic-free, standard solutions did not yield acceptable results for mussel tissue reference materials treated as unknowns and analyzed many times over several months. Thus, to correct for drift/matrix effects and to calibrate count rate data, replicate tubes of a mussel tissue reference material, NIST 2976, were included in each cycle of tubes throughout each analysis run. NIST 2976 was used for both calculating drift correction and external standardization, using its certified and recommended concentration values. The tube containing this solution was analyzed after the six unknowns in the cycle sequence that was described in section 2.5.4. Mussel, reference material, GBW 08571, whose metal concentratios are somewhat less well-known than NIST 2976, was used as a secondary calibration standard.

Drift correction factors were calculated for the elements that were present in the internal standard solution, for each test tube. The mean cps of all blank, sample and reference material solutions were divided by the mean cps of the drift correction reference material (NIST 2976) (Equation 2, Appendix F). Factors

were then calculated for the unknown analytes by interpolating between the factors for the internal standard analytes (Equation 3, Appendix F).

Once the factors were determined, a drift/matrix correction was applied to each analyte, including those in the internal standard, by dividing the mean cps of the blank, sample or reference material, by its respective drift/matrix factor (Equation 4, Appendix F).

Blank Subtraction

The blanks that were used throughout the run record the count rates in the analyte solutions that can be attributed to 0.2N nitric acid and laboratory contaminants. The drift-corrected cps of a blank that marked the beginning of a new set of unknowns were subtracted from the drift-corrected cps of each of the samples and/or reference materials in that set (Equation 5, Appendix F). Blank subtraction was not performed for analytes in the internal standard solution.

Sensitivity

The sensitivity of each analyte (count rate divided by concentration) was calculated using the same reference material (NIST 2976) that was used for drift/matrix correction. For each cycle of tubes a separate sensitivity was calculated. The published concentration of each analyte in NIST 2976 was divided by the dilution factor associated with each tube. The mean cps was then divided by that quotient to obtain a value for sensitivity in cps per unit of concentration (Equation 6, Appendix F).

Detection Limit

The detection limit for each analyte was calculated by dividing the standard deviation of the mean cps of all the blanks in the run by the mean sensitivity calculated for that particular analyte, then multiplying the quotient by 3 (Equation 7, Appendix F).

Concentration

The blank-subtracted mean cps for an analyte from a given tube was multiplied by the dilution factor for that tube and divided by the sensitivity calculated for the standard in the cycle in which the tube was analyzed (i.e. not the mean sensitivity) (Equation 8, Appendix F).

2.5.6 Spectroscopic Interference

To minimize the effect of spectroscopic interferences caused by acids used to dissolve the samples, only HNO_3 was used in this study. As the elements that make up this acid (H, N and O) are already present in the plasma, HNO_3 does not introduce additional chemical species to the analysis (Evans and Giglio, 1993). However, chloride ions may form during the digestion of organic materials, even when not introduced by using HCl for dissolution. Therefore, results were examined for possible interferences caused by polyatomic ions $^{40}Ar^{35}Cl^+$ and $^{40}Ar^{37}Cl^+$ on ^{75}As and ^{77}Se, respectively. All analyses run on certified reference materials produced concentrations that were within the uncertainty limits for As and Se. Based on the precision and accuracy of these

measurements, it was assumed that most of the chloride generated from the digestion of the organic matrix was vented in gas phase from the microwave vessels before sample dilution.

2.5.7 Statistical Methods

The software application that was used for all statistical analyses included in this thesis was MINITAB, version 12.

For those mussels digested in pairs (Section 2.4.4), only one mean concentration is reported. However, entering one data point for two mussels would misrepresent the real sample size of some collection sites. Therefore, for the purpose of the statistical analysis, the mean concentration of each composite sample was entered twice.

Mussels

Initially, exploratory data analysis techniques were used to examine the concentration data, to detect any trends or relationships and to identify possible outliers that may have been present. Boxplots, correlation comparisons, scatter plots, principal component analysis, and cluster analysis were performed; and descriptive statistics, such as measures of location, measures of spread and error measurements, were examined. Methods of confirmatory data analysis were conducted to further examine and verify the observations made from the exploratory analyses. Mann-Whitney, a nested analysis of variance (ANOVA) model was formulated via a general linear model, one-way ANOVA on Ranks with Tukey multiple comparison tests, and bootstrapping techniques were used.

Firstly, it was necessary to determine the distribution (or shape) of the data. *Normal* (or *Gaussian*) distributions were preferable, as such distributions are

required when applying parametric tests in statistical analyses. While many nonparametric tests exist, there are none that parallel parametric methods for multiple comparisons. Boxplots of the concentrations and the natural logarithm (log) of the concentrations were plotted versus sample site for each element. The log-transformed data were more similar to a Normal distribution, that is, there were fewer outliers and the heights of the boxes (the interquartile range) were more similar across the sample sites. Thus the data were assumed to be lognormal and all subsequent calculations were performed on the log-transformed concentrations. Note that the validity of this assumption was further tested throughout the statistical analysis. Boxplots were the first technique used to examine the data because they graphically represent many descriptive statistics for each site that could then be compared to the other sites.

Using the median value of the log-transformed concentrations of each analyte for all sites, a correlation matrix was generated using Pearson's correlation coefficient (r) to examine relationships between pairs of elements. Scatter plots of each pair were then plotted to determine whether relationships that were identified by the correlation statistics were valid and/or if trends existed among the sample sites.

A principal component analysis (PCA) was used to determine if the data were grouped by sample site. The median of the log-transformed concentrations of each analyte per site was used to make the correlation matrix on which the PCA

was based. PCA is a statistical technique used to transform a set of correlated variables into an uncorrelated set of factors. These factors are linear combinations of the original variables that account for the total variance of the original data. Maximum variance is explained using a minimum number of factors.

To confirm the findings of the principal component analysis, in this study, a hierarchical cluster analysis of observations was performed. The main function of a cluster analysis is to group data into categories (clusters) without any a priori knowledge of the said clusters. Initially, each observation is recognized as being distinctly different and hence, individually classified as a category. Then algorithms are used to successively join increasing disparate observations, thus increasing the amount of observations per cluster, while decreasing the number of clusters. The result of the final algorithm is one cluster containing all observations. The joining of clusters is based on the similarity of or distance between the clusters. A linkage criterion is chosen to determine when clusters are similar enough, or when the distance is small enough for them to be joined (StatSoft, 2001). In this study, the Euclidean distance measure was used on standardized, log-transformed data from each site and a single linkage method was employed. That is, considering two clusters (A and B), these were joined when the distance between an observation in cluster A and an observation in cluster B was less than the Euclidean distance between the two clusters

(StatSoft, 2001). Final grouping (or final partitioning) was based on three clusters.

To examine the observations of the exploratory data analysis, confirmatory tests were employed, starting with a Mann-Whitney analysis. The Mann-Whitney test is nonparametric thus data are not required to have a certain distribution curve, to be homoscedastic (i.e. equal variance among sites within a region) or to fit to any other parameters. This test is used to determine if there is a statistically significant difference between the median values of two data sets. In this study this technique was used to compare the log-transformed concentrations of the inner and outer harbour regions. A separate analysis was done for each analyte and all data were included – i.e. the concentrations within each site were not averaged prior to the test application. P-values of less than 0.05 were considered statistically significant.

When conducting statistical analyses it is usually a good idea to use more than one technique to test the data. Therefore following the nonparametric test, a parametric, nested analysis of variance (ANOVA) was designed. This was used to test for statistically significant differences between the means of the log-transformed concentrations from the inner and outer regions, while at the same time, testing for differences among the sites within each region. Since the factors did not have the same number of values, the general linear model method of ANOVA was employed. Boxplots of the log-transformed concentrations versus

the regions were plotted because the spread of the data, the means, the medians and any possible outliers for the three regions can easily be visually compared using side-by-side boxplots. The residuals for each nested ANOVA were tested for Normality, and plotted against the fitted values and against sample site order. Such usage of regression diagnostics was necessary to see if the assumptions of ANOVA were met. The Normality test checks the distribution of the residuals, while the plots of the fitted values and of observation order allow for easy visual inspection of trends and possible outliers.

ANOVA is a parametric test that can only be used effectively when the data for each level are Normally distributed and are approximately homoscedastic (Bower, 2000). Therefore, to guard against a loss of statistical power in the case that the assumptions of the nested ANOVA were not valid, further testing was done to check for differences among the sites within each region.

A rank transform approximation to the Kruskal-Wallis test called *ANOVA on the ranks* was used, for each region, to test for significant differences between the mean rank of each site to the overall mean rank of all sites within the given region. Performing the parametric one-way ANOVA test on the ranks of the data is preferable to using the nonparametric Kruskal-Wallis test because ANOVA on the ranks allows the application of parametric Tukey multiple comparison tests, which identify the statistical significance of the differences between each pair of sites within a region. Levene's test for homogeneity of variance was used as well

as the diagnostic tests listed for nested ANOVA to verify the ANOVA assumptions. Levene's test was chosen to determine the homogeneity of variance of the rank-transformed data among the sites within each region because it is based on the sample median and does not require a Normal distribution.

Two final nonparametric techniques were used to check the within region results obtained from the parametric ANOVA on the ranks and Tukey tests. To parallel the ANOVA on the ranks test the bootstrapping technique was used; to check the multiple comparison Tukey test a Mann-Whitney test was conducted on several of the site-pairs were used. Macros were written to perform bootstrapping on the sites within the regions. In brief, using Cr from the inner region as an example, the following algorithm was used in the macro. A data set containing all Cr values (n=67) from all inner harbour sites was sampled 67 times, with replacement, then this new data set was broken into subsets that reflected the sample sizes of each site within the inner harbour region. Next, for all pair combinations of the sites in the inner region, median differences were calculated and squared. All of the squared differences were then added together – this value was called the sum of the squared differences (SSD). This marked the end of a do-loop that was repeated 1000 times. The number of times, out of 1000, that the computer generated SSDs that were greater than or equal to the observed SSD was then counted and divided by 1000 to give the p-value. Note that the p-value represents the probability of getting a SSD greater than or equal

to the observed value, in 1000 tries. The observed SSD was calculated not using the macro, but using the median values from the actual data.

Water

For each sample site, the pH, temperature and salinity of a sample of seawater were measured. Each parameter was then statistically analyzed using the parametric one-way ANOVA to test for differences between the means of inner and outer regions. Boxplots were graphed and the residuals were tested for Normality, and plotted against fitted values and observation order. Since there were few data points available (one per site), the nonparametric Mann-Whitney test was run on the pH, temperature and salinity values as well.

3 RESULTS

3.1 Concentrations

As described in section 2.5.5 of this thesis, the standard reference material NIST 2976 was used as an external standard, against which the concentrations of all samples were determined.

3.1.1 Reference Materials

Lists of the trace elements analyzed in the mussel tissue reference materials used in this study and their expected concentrations can be found in Table A.4, Appendix E.

Two different bottles of the reference material GBW 08571 were analyzed during this study. Therefore, to differentiate between the two bottles henceforth in this thesis, the first bottle will be called GBW(1) and the second bottle, GBW(2).

Accuracy and Precision

For the concentrations of the analytes in the reference materials, an acceptable level of accuracy was determined using the criteria defined by NOAA, 1993. Measurements were considered to be accurate if (a) measured values are within 30% of the uncertainty limits from the respective Certificate of Analysis documents and (b) analyte concentrations are at least one order of magnitude higher than their detection limits.

Tables 3.1, 3.2 and 3.3 give the central tendency values, measures of spread, precision and accuracy for the reference materials.[‡] Figure 3.1 shows the accuracy of the median values of the measured concentrations relative to the certified values. The median measured concentrations of all the analytes, for which BCR 278R is certified, were within 30% of the given uncertainty range with the exception of Cr, which was 38% lower than the lower uncertainty limit. The median concentrations of Ni, Cu, As and Cd were within the given uncertainty range for both GBW(1) and GBW(2) and all other analytes were within 30% of the uncertainty range, except Cr which was 39% lower than the lower limit for GBW(1). For NIST 2977, the median concentrations of Co, As, Se, and Pb were within the given uncertainty range, the other elements were within 30% of the uncertainty range, except Cr which was 61% lower than the lower limit. All elements for all reference materials were greater than one order of magnitude higher than their detection limits.

3.1.2 Samples

Table 3.4 gives a summary of the metal concentrations found in the mussels. All metal concentrations for each sample are listed in Appendix G. The detection limit and sensitivity of each analyte for each day that the samples were run on the ICPMS are reported in Appendix H.

[‡] Values given for NIST 2976 were acquired by running the reference material as an unknown against itself.

Table 3.1 Experimental Concentrations for Reference Material GBW 08571: Medians, means and their associated uncertainty values are given in $ng \cdot g^{-1}$. amu = atomic mass unit; IQR = interquartile range; %RIQR = percent relative interquartile range; Accuracy Ratio = certified value divided by the median measured value. Number of observations = 14 (GBW1) & 7 (GBW2)

amu	Analyte	Median	IQR	% RIQR	Mean	SD	% RSD	Accuracy Ratio
		GBW (1)						
53	**Cr**	310	44	14	307	37	12	1.84
55	**Mn**	7507	393	5	7517	465	6	1.36
57	**Fe**	174975	15301	9	174413	13886	8	1.26
59	**Co**	822	43	5	824	58	7	1.14
60	**Ni**	977	520	53	1012	300	30	1.05
65	**Cu**	7063	294	4	7098	558	8	1.09
66	**Zn**	127893	6110	5	127249	8852	7	1.08
75	**As**	6187	432	7	6215	425	7	0.99
77	**Se**	3084	914	30	3517	1254	36	1.18
88	**Sr**	15400	1071	7	15605	1098	7	0.83
111	**Cd**	4082	150	4	4062	290	7	1.10
206	**Pb**	1440	86	6	1441	110	8	1.36
207	**Pb**	1427	83	6	1426	111	8	1.37
208	**Pb**	1424	75	5	1421	105	7	1.38
		GBW (2)						
53	**Cr**	347	77	22	356	60	17	1.64
55	**Mn**	7473	4162	56	9332	3709	40	1.36
57	**Fe**	175066	92867	53	225151	92023	41	1.26
59	**Co**	816	483	59	1040	443	43	1.15
60	**Ni**	1003	564	56	2371	3604	152	1.03
65	**Cu**	7155	2756	39	8669	3046	35	1.08
66	**Zn**	127135	75838	60	157807	65271	41	1.09
75	**As**	6177	3537	57	7660	3058	40	0.99
77	**Se**	3456	2244	65	5249	4560	87	1.06
88	**Sr**	15285	9088	59	18905	7477	40	0.84
111	**Cd**	4103	2451	60	5075	2125	42	1.10
206	**Pb**	1430	860	60	1799	774	43	1.37
207	**Pb**	1429	832	58	1770	744	42	1.37
208	**Pb**	1428	799	56	1745	709	41	1.37

Table 3.2 Experimental Concentrations for Reference Material BCR 278R: Medians, means and their associated uncertainty values are given in $ng \cdot g^{-1}$. amu = atomic mass unit; IQR = interquartile range; %RIQR = percent relative interquartile range; Accuracy Ratio = certified value divided by the median measured value. NA = Ratio not available due to no published certified value. Number of observations = 10

		BCR 278R						
amu	Analyte	Median	IQR	% RIQR	Mean	SD	% RSD	Accuracy Ratio
53	**Cr**	443	96	22	455	76	17	1.76
55	**Mn**	6477	564	9	6487	817	13	1.19
57	**Fe**	107738	16443	15	111046	15374	14	NA
59	**Co**	321	34	10	324	38	12	NA
60	**Ni**	963	656	68	1297	861	66	NA
65	**Cu**	8577	818	10	8701	1127	13	1.10
66	**Zn**	74035	8637	12	74996	9778	13	1.12
75	**As**	5503	337	6	5583	641	11	1.10
77	**Se**	1364	503	37	1534	509	33	1.35
88	**Sr**	18211	1233	7	18548	2198	12	NA
111	**Cd**	297	21	7	312	49	16	1.17
206	**Pb**	1877	172	9	1945	328	17	1.07
207	**Pb**	1853	171	9	1914	327	17	1.08
208	**Pb**	1841	170	9	1893	330	17	1.09

Table 3.3 Experimental Concentrations for Reference Materials NIST 2976 and NIST 2977: Medians, means and their associated uncertainty values are given in $ng \cdot g^{-1}$. amu = atomic mass unit; IQR = interquartile range; %RIQR = percent relative interquartile range; Accuracy Ratio = certified value divided by the median measured value. Values given for NIST 2976 were acquired by running the reference material as an unknown against itself. Number of observations = 7 (2976) & 4 (2977)

		NIST 2976						
amu	Analyte	Median	IQR	% RIQR	Mean	SD	% RSD	Accuracy Ratio
53	Cr	417	76	18	417	53	13	1.20
55	Mn	31471	986	3	31339	906	3	1.05
57	Fe	165872	11054	7	164398	8779	5	1.03
59	Co	582	40	7	574	40	7	1.05
60	Ni	778	196	25	2120	3491	165	1.20
65	Cu	3757	276	7	5908	6269	106	1.07
66	Zn	128981	8005	6	127044	8267	7	1.06
75	As	12700	686	5	12491	471	4	1.05
77	Se	1675	387	23	1864	566	30	1.07
88	Sr	88856	3828	4	88499	3438	4	1.05
111	Cd	799	24	3	794	25	3	1.03
206	Pb	1206	200	17	1308	256	20	0.99
207	Pb	1199	199	17	1305	256	20	0.99
208	Pb	1201	194	16	1303	260	20	0.99
		NIST 2977						
53	Cr	1327	131	10	1306	112	9	2.95
55	Mn	20612	1816	9	20549	1104	5	1.16
57	Fe	244100	14971	6	246608	15919	6	1.12
59	Co	421	28	7	421	18	4	1.14
60	Ni	5501	251	5	5467	221	4	1.10
65	Cu	8595	408	5	8583	658	8	1.10
66	Zn	127013	7377	6	126784	4566	4	1.06
75	As	8267	508	6	8257	327	4	1.07
77	Se	1845	965	52	1898	598	32	0.96
88	Sr	87391	2736	3	87821	2783	3	0.79
111	Cd	153	11	7	152	8	6	1.17
206	Pb	2341	153	7	2385	152	6	0.97
207	Pb	2367	164	7	2411	146	6	0.96
208	Pb	2345	158	7	2387	151	6	0.97

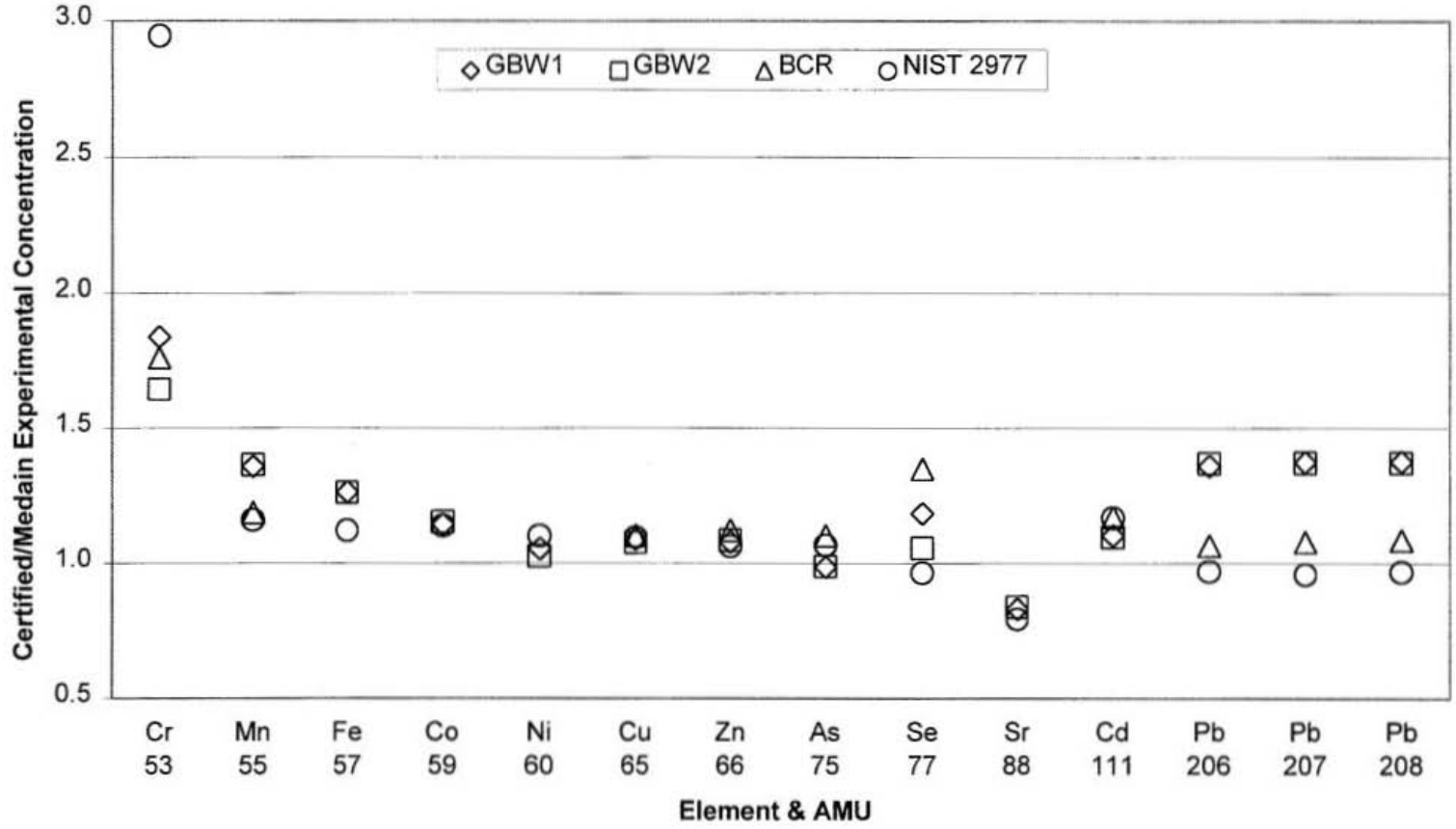

Figure 3.1 Accuracy of Reference Material Concentrations: Graph shows how well the median experimental concentration values agree with the certified expected values in the form of a ratio of certified/experimental values. A ratio value of unity indicates that the certified and experimental values were the same; greater than and less than unity indicates that the experimental value was less than and greater than the certified value, respectively.

Table 3.4 Summary of Experimental Mussel Concentrations: The median ($\mu g \cdot g^{-1}$), interquartile range (IQR) and percent relative interquartile range (%IQR) are given for each element per sample site.

Site	Statistic	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
2	Median	0.94	4.67	164	0.30	1.31	8.05	186	7.52	2.63	74	0.75	2.30	2.29	2.26
	IQR	0.15	1.91	49	0.075	0.21	1.94	115	1.8	0.53	21	0.36	2.0	2.0	2.0
	%IQR	16	41	30	25	16	24	62	24	20	29	48	87	87	87
3	Median	0.77	4.96	114	0.22	1.80	7.19	127	6.34	2.17	60	0.36	1.88	1.87	1.86
	IQR	0.19	0.43	24	0.031	0.47	1.09	49	0.62	0.43	10	0.10	0.57	0.56	0.56
	%IQR	24	9	21	14	26	15	39	10	20	17	27	30	30	30
4	Median	0.82	6.57	111	0.23	1.68	6.75	123	6.40	2.80	56	0.67	1.28	1.28	1.29
	IQR	0.24	3.47	27	0.046	0.74	2.18	46	1.5	0.57	7	0.16	0.41	0.42	0.41
	%IQR	30	53	24	20	44	32	37	24	20	13	24	32	33	32
5	Median	0.78	5.66	124	0.23	1.16	8.16	155	5.77	2.47	68	0.43	1.89	1.88	1.87
	IQR	0.19	2.12	29	0.026	0.34	1.21	52	0.43	0.59	22	0.19	1.87	1.83	1.80
	%IQR	25	38	23	11	30	15	33	7	24	32	44	99	97	97
7	Median	0.78	4.39	122	0.24	1.15	7.47	150	5.10	2.62	63	0.31	1.61	1.60	1.60
	IQR	0.18	2.61	41	0.051	0.29	2.10	76	0.80	0.57	11	0.073	0.50	0.49	0.50
	%IQR	23	59	34	21	25	28	50	16	22	17	23	31	31	31
10	Median	0.66	4.65	114	0.22	1.19	6.77	148	5.15	2.83	60	0.36	1.45	1.45	1.43
	IQR	0.34	2.75	43	0.031	0.92	1.37	36	0.41	0.55	17	0.14	0.61	0.62	0.60
	%IQR	52	59	38	14	77	20	25	8	19	29	39	42	43	42
11	Median	0.77	4.43	98	0.18	1.31	5.11	78	7.34	4.57	56	1.13	0.22	0.22	0.22
	IQR	0.27	1.96	22	0.028	0.41	2.31	27	2.3	1.4	11	0.19	0.065	0.058	0.059
	%IQR	35	44	23	16	32	45	35	31	30	20	17	30	27	27

Table 3.4 Continued

Site	Statistic	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
13	**Median**	0.55	3.95	101	0.22	1.09	5.62	87	6.82	3.05	54	0.44	1.26	1.26	1.26
	IQR	0.22	2.36	51	0.081	0.17	1.50	128	2.8	1.5	30	0.37	0.34	0.34	0.34
	%IQR	39	60	50	37	16	27	147	41	51	56	86	27	27	27
14	**Median**	0.86	3.56	88	0.18	1.40	4.88	81	5.59	4.49	53	1.43	0.58	0.58	0.59
	IQR	0.18	1.11	14	0.025	0.38	2.30	13	1.6	1.1	11	0.47	0.19	0.18	0.19
	%IQR	21	31	16	14	27	47	16	29	24	20	33	33	31	32
16	**Median**	0.61	2.43	104	0.19	1.04	5.39	92	6.42	3.33	53	1.10	0.66	0.66	0.66
	IQR	0.11	2.94	24	0.046	0.41	3.35	22	1.7	1.1	10	0.27	0.34	0.35	0.35
	%IQR	17	121	23	25	39	62	24	27	33	18	25	52	53	53
17	**Median**	0.60	5.14	73	0.20	0.77	5.84	90	7.36	4.18	55	2.14	0.44	0.44	0.43
	IQR	0.18	3.20	26	0.044	0.50	1.86	29	2.0	1.2	8	1.6	0.029	0.031	0.032
	%IQR	29	62	36	21	65	32	32	27	29	15	73	7	7	7

3.2 Statistics

3.2.1 Mussels

For the statistical data analysis, lead concentrations determined from only the 206 isotope were used, as there was little difference in values derived from the 206, 207 and 208 isotopes.

Exploratory Data Analysis

Descriptive statistics of the original and log-transformed data are given for each analyte in Appendix I.

Boxplots

The boxplots of the log-transformed concentrations versus sample site are included in Appendix J. Upon examining these, it appeared that at least two of the five outer harbour sites had medians lower than the inner sites for Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr and Pb. However, the medians of As, Se and Cd were seemingly higher at the outer harbour sites.

Dependent on the analyte, the two sites within the Narrows (sites 4 and 13) were closer in concentration values to either the inner harbour sites or the outer harbour sites. In the boxplots of Co, As, Se, Cd and Pb the medians of sites 4 and 13 were similar to the inner harbour sites, for Sr they were more like the outer harbour sites. For Cr, Mn, Ni, Cu and Zn the median for site 4 appeared to

be similar to the inner sites and the median for site 13 appeared to be similar to the outer sites.

There were possible outliers identified in each of the plots, which indicates that the log-transformed data may not be Normally distributed. Within each plot, the box lengths, which represent interquartile range, were similar for most sites, however site 13 had a notably larger range for most analytes.

Correlation Matrix

The correlation matrix of the log-transformed concentrations (Table 3.5) showed statistically significant positive or negative correlation coefficients for many of the elements, with p-values less than or equal to 0.05. All pair-wise combinations of Fe, Co, Cu, Zn, Sr and Pb produced high positive Pearson correlation constants (r-values). Selenium had a large negative coefficient value when paired with each of these metals and a high positive coefficient when correlated with Cd. Significant negative r-values resulted when Cd was paired with Fe, Cu, Zn and Pb. The only significant r-value for each of Cr and Ni was produced when they were paired together, forming a positive relationship. There were no statistically significant correlation coefficients for any element paired with Mn or As.

Scatter Plots

The scatter plots of the paired elements showed the data points, in many cases, to be roughly grouped in regions of inside harbour sites, outside harbour sites and, to a lesser extent, sites in the Narrows. In some cases when the correlation

Table 3.5 Correlation Matrix of Median Log-Transformed Concentrations for All Sites: The top number in each cell is Pearson's correlation constant, the bottom number is the p-value. Correlation constants that are in bold-type have p-values < 0.05 and are considered to be statistically significant.

	ln(Cr)	ln(Mn)	ln(Fe)	ln(Co)	ln(Ni)	ln(Cu)	ln(Zn)	ln(As)	ln(Se)	ln(Sr)	ln(Cd)
ln(Mn)	0.349										
	0.292										
ln(Fe)	0.542	0.170									
	0.085	0.618									
ln(Co)	0.352	0.453	**0.789**								
	0.288	0.161	0.004								
ln(Ni)	**0.639**	0.281	0.429	0.113							
	0.034	0.403	0.188	0.741							
ln(Cu)	0.392	0.571	**0.761**	**0.867**	0.204						
	0.233	0.067	0.007	0.001	0.548						
ln(Zn)	0.454	0.443	**0.835**	**0.877**	0.224	**0.956**					
	0.161	0.172	0.001	0.000	0.507	0.000					
ln(As)	-0.074	0.042	-0.116	0.022	-0.149	-0.207	-0.299				
	0.828	0.901	0.734	0.948	0.663	0.541	0.371				
ln(Se)	-0.175	-0.343	**-0.731**	**-0.721**	-0.388	**-0.860**	**-0.809**	0.292			
	0.606	0.302	0.011	0.012	0.239	0.001	0.003	0.384			
ln(Sr)	0.561	0.404	**0.830**	**0.847**	0.120	**0.871**	**0.889**	-0.045	**-0.609**		
	0.073	0.218	0.002	0.001	0.725	0.000	0.000	0.896	0.047		
ln(Cd)	-0.056	-0.262	**-0.624**	-0.504	-0.384	**-0.649**	**-0.627**	0.539	**0.837**	-0.440	
	0.870	0.437	0.040	0.114	0.244	0.031	0.039	0.087	0.001	0.175	
ln(Pb)	0.242	0.316	**0.747**	**0.828**	0.321	**0.841**	**0.846**	-0.356	**-0.925**	**0.666**	**-0.767**
	0.473	0.344	0.008	0.002	0.336	0.001	0.001	0.283	0.000	0.025	0.006

coefficient and p-values indicated the presence of a linear relationship between the data points, while plotted along a straight line, were also obviously grouped by site. Two examples of this are the ln(Zn) versus ln(Pb) and the ln(Se) versus ln(Pb) plots (Figure 3.2 (a),(b)). In other cases where the p-value was much greater than 0.05, such as with ln(Cr) versus ln(Cu) (Figure 3.2 (c)), there appeared to be two separate linear relationships, one positive and one negative. This illustrates the need to examine the data using both a correlation matrix and scatter plots.

Principal Component Analysis

Figure 3.3 (a) is a score plot of the second versus the first component resulting from the PCA that showed distinct grouping of inner and outer sites, with the exception of site 2 which did not plot near the other sites. Site 4 was closer to the inner sites than the outer sites and site 13 was close to site 16 and to the outer sites in general. This supports observations from the boxplots that sites 4 and 13 behaved more like the inner and outer sites, respectively. The third versus first component score plot (Figure 3.3 (b)) showed similar relationships as those in the second versus first component plot. However, in the third versus first component plot, site 2 was grouped with the inner sites and site 17 seemed to stray from the outer sites.

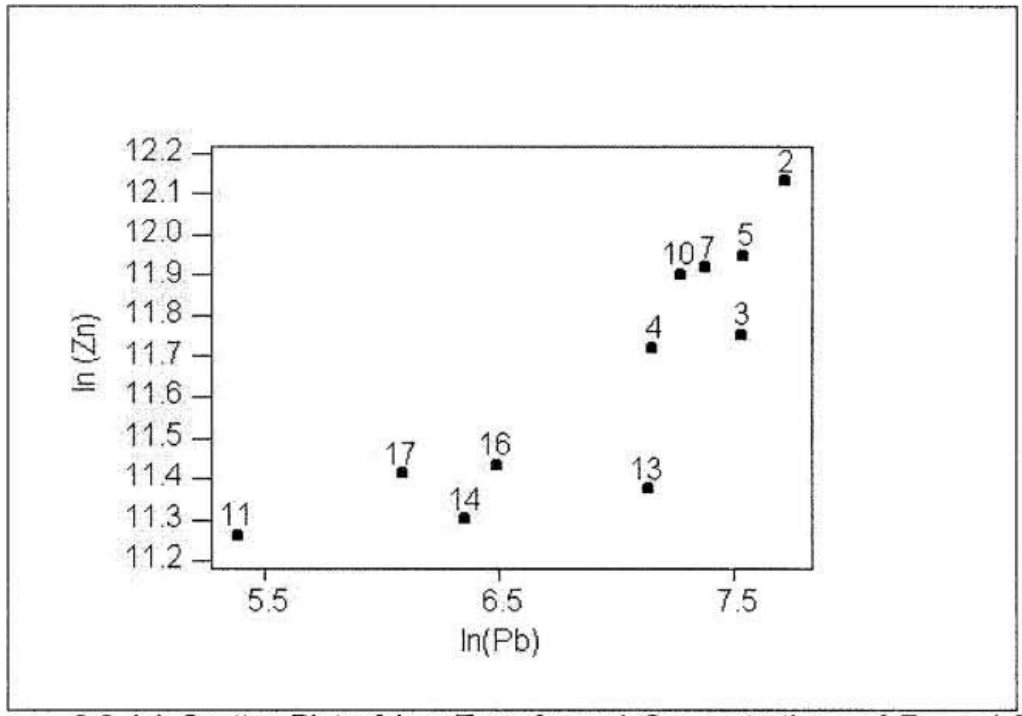

Figure 3.2 (a) Scatter Plot of Log-Transformed Concentrations of Zn and Pb: The Pearson's correlation coefficient of this pair is 0.846, with a p-value of 0.001. Outer harbour sites (11, 13, 14, 16 and 17) appear to have linear, almost horizontal slope. Inner harbour sites (2, 3, 4, 5, 7 and 10) appear to be linear, with a much larger positive slope than the outer sites. Concentration units before log-transform were ($ng \cdot g^{-1}$).

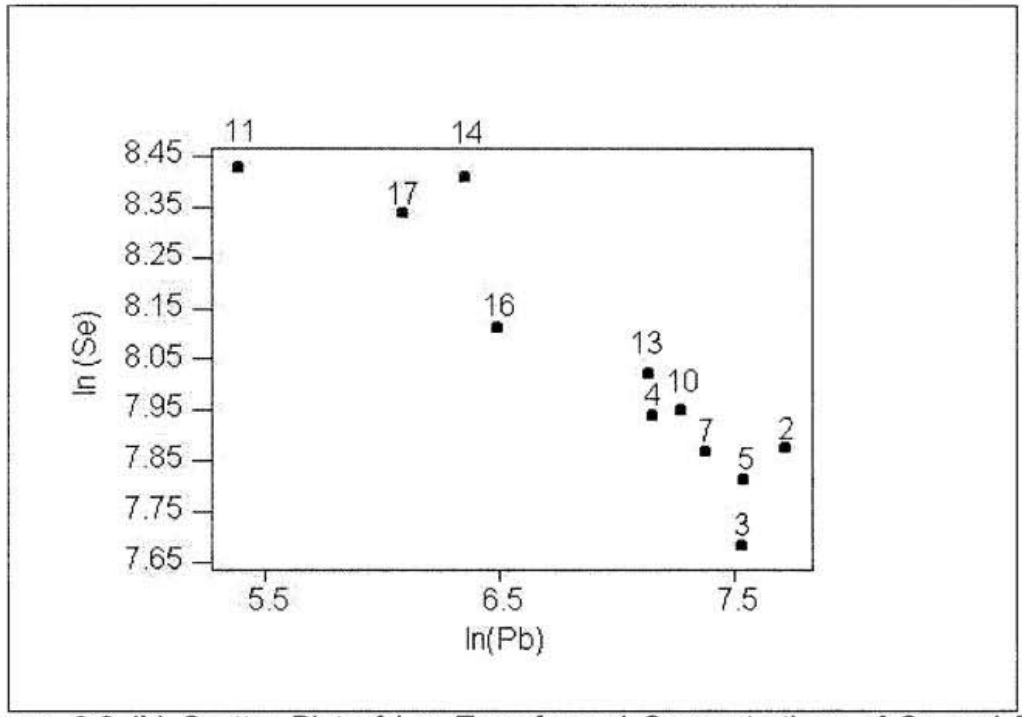

Figure 3.2 (b) Scatter Plot of Log-Transformed Concentrations of Se and Pb: The Pearson's correlation coefficient of this pair is -0.925, with a p-value of 0.000. While this indicates that a negative correlation existed between selenium and lead, it also shows that the data is roughly grouped into inner and outer harbour regions. (Site numbers greater than or equal to 11 are outer harbour, less than 11 are inner harbour sites.) Concentration units before log-transform were ($ng \cdot g^{-1}$).

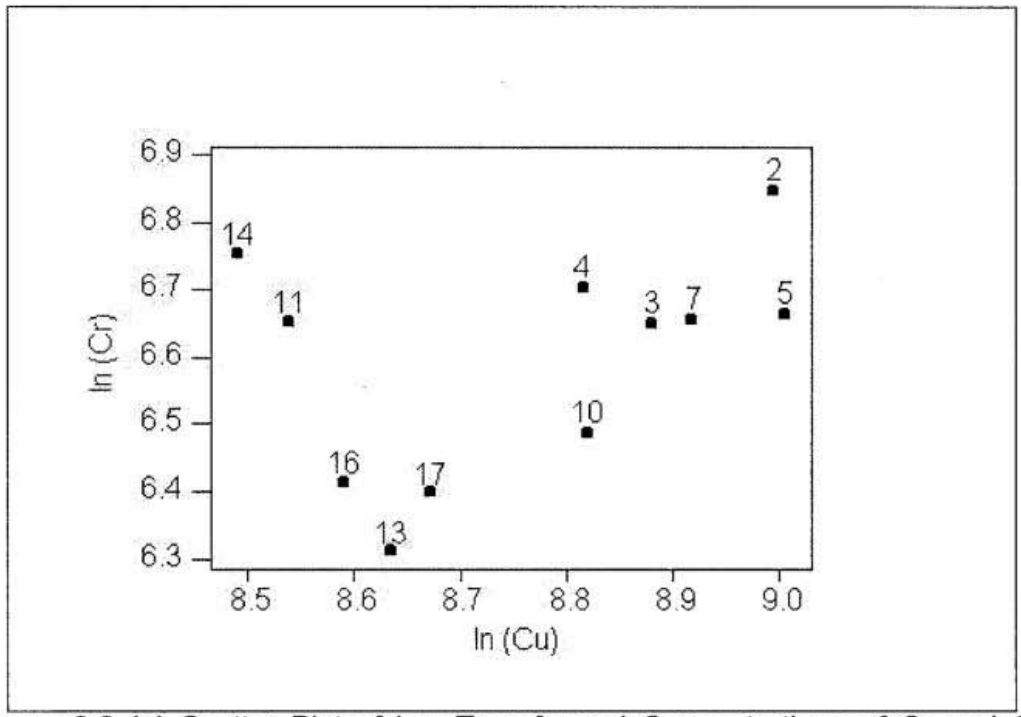

Figure 3.2 (c) Scatter Plot of Log-Transformed Concentrations of Cr and Cu: The Pearson's correlation coefficient of this pair was 0.392, with a p-value of 0.233. In this plot, it appears that the inner harbour sites (2, 3, 4, 5, 7 and 10) may have a positive linear relationship, while the outer sites (11, 13, 14, 16 and 17) appear to be negatively correlated. Concentration units before log-transform were ($ng \cdot g^{-1}$).

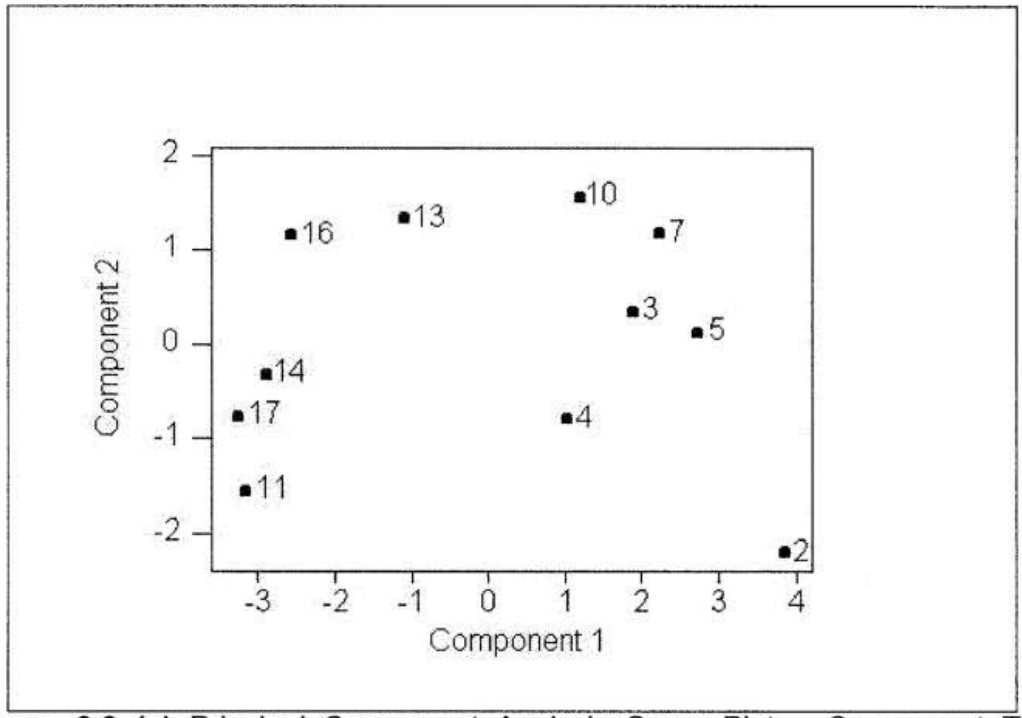

Figure 3.3 (a) Principal Component Analysis Score Plot – Component Two versus Component One: Two groups present, outer sites 11, 13, 14, 16 and 17 and inner sites 3, 4, 5, 7 and 10. Site 2 does not appear to be in either group.

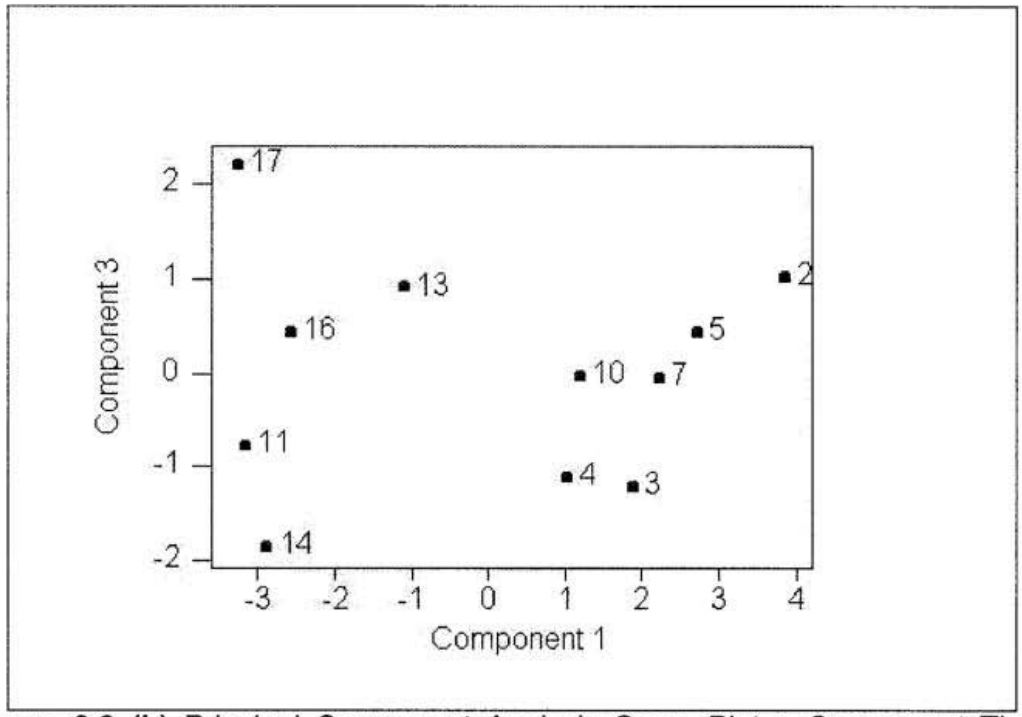

Figure 3.3 (b) Principal Component Analysis Score Plot – Component Three versus Component One: Same inner and outer site grouping as seen in Figure 3.3a. Site 2 appears to be grouped with the inner sites in this plot.

The majority of the variance in the original log-transformed data is accounted for by the first component (58.4%), the second and third components are responsible for much less variance, 13.0% and 11.6%, respectively. Of the components beyond the third, each account for less than 10% of the variance thus these will not be examined. The coefficients for the first three components are listed in Table 3.6.

Interpretation of the results of a PCA is subjective but general assumptions can be made. Within a component, variables that have coefficients whose values are not close to zero are well represented by that specific component. Within the first principal component Fe, Co, Cu, Zn, Sr and Pb all have positive values ranging from 0.325 to 0.362. The negative coefficients of Se, -0.336 and Cd, -0.282, may be seen as contrasting values to the positive coefficients. These findings are similar to the relationships seen in the correlation matrix. That is, pairwise combinations of the elements with positive coefficient values were highly positively correlated. Likewise, Se and Cd were positively correlated with each other, but negatively correlated with Fe, Cu, Zn and Pb. Selenium was also negatively correlated with Co and Sr.

In the second component, As, which was represented the most poorly in the first component, has the highest absolute coefficient with a value of –0.535, followed closely by Cr (-0.494) and Cd (-0.428). Nickel is represented the best by the third component, with a coefficient of –0.708. Manganese was not represented

Table 3.6 Pricipal Component Analysis Coefficients for First Three Components

	Coefficients		
Variable	**Component 1**	**Component 2**	**Component 3**
ln(Cr)	0.185	-0.494	-0.435
ln(Mn)	0.184	-0.293	-0.016
ln(Fe)	0.332	-0.085	-0.041
ln(Co)	0.333	-0.130	0.296
ln(Ni)	0.151	-0.146	-0.708
ln(Cu)	0.360	-0.026	0.163
ln(Zn)	0.362	-0.013	0.115
ln(As)	-0.105	-0.535	0.342
ln(Se)	-0.336	-0.237	-0.005
ln(Sr)	0.325	-0.233	0.202
ln(Cd)	-0.282	-0.428	0.146
ln(Pb)	0.344	0.210	0.044

well by either of these components, having its highest absolute coefficient value in the second component (-0.293).

Cluster Analysis

The final partitioning of the cluster analysis was based on three clusters because, while the PCA clearly showed two groups, it was thought that a third group might be formed from those sites located in the Narrows. As shown in the dendrogram (Figure 3.4), the results of the cluster analysis support those of the principal component analysis. The outer harbour sites 11, 13, 14, 16 and 17 were within one cluster. The inner harbour sites 3, 4, 5, 7 and 10 were within another cluster; site 2 was unique in a third cluster. Table 3.7 lists the amalgamation steps and associated similarities and distances. According to these results, site 2 was not grouped within either the inner or outer harbour regions. However, as only methods of exploratory statistical analysis had been used thus far, and since site 2 was geographically located inside the harbour, it was decided that site 2 would be included with the sites of inner harbour region for the confirmatory statistical analyses.

Confirmatory Data Analysis

Mann-Whitney Test – Comparing Inner and Outer Regions

The Mann-Whitney test indicated for all analytes, except for nickel, that there was a statistically significant difference between the median concentrations of the mussel tissue from inner harbour sites to those of the outer harbour sites.

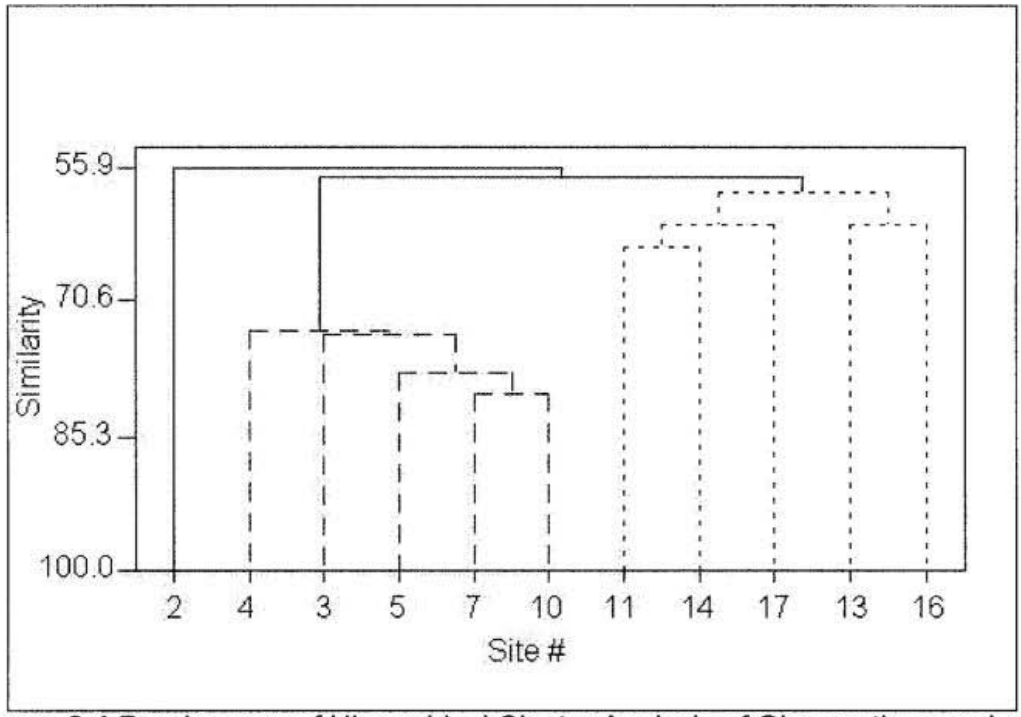

Figure 3.4 Dendrogram of Hierarchical Cluster Analysis of Observations on Log-Transformed Medians: The differing line styles illustrate the three clusters.

Table 3.7 Amalgamation Steps of Cluster Analysis: Each step represents the joining of two clusters, starting with a total of eleven clusters (one for each site). Distance and similarity levels are calculated values indicating how much the two clusters that were joined are alike. The number of the "New cluster" is the lower of the two clusters joined.

Step	# of Clusters	Similarity level	Distance level	Clusters joined		New cluster
1	10	80.80	1.481	5	6	5
2	9	78.36	1.668	4	5	4
3	8	74.15	1.993	2	4	2
4	7	73.77	2.022	2	3	2
5	6	64.75	2.718	7	9	7
6	5	62.27	2.909	8	10	8
7	4	62.24	2.911	7	11	7
8	3	58.71	3.183	7	8	7
9	2	56.99	3.316	2	7	2
10	1	55.90	3.400	1	2	1

The p-values were less than 0.003 for all but Cr, which was very close to showing no significant distance with a p-value of 0.0491, and Ni (0.174). After viewing these results, it was decided that another set of Mann-Whitney tests should be done, this time excluding site 2 data from all analyte sets. This was done to verify that the observed differences were not due to disparate concentrations seen at site 2 in the PCA and cluster analyses. There was little difference in the p-values for this secondary set of tests, except for that of Cr ($p = 0.225$), where no significant difference was identified. This change in significance with the omission of site 2 is logical noting that the highest Cr median concentration of all the sites was at site 2 (Table 3.4). P-values for both sets of Mann-Whitney tests are found in Table 3.8.

Nested ANOVA – General Linear Model

The results of the nested ANOVA showed no statistically significant difference between the means of the inner and outer harbour regions for the log-transformed Cr, Ni and As data (p-values were 0.145, 0.168 and 0.287, respectively). For all other analytes a significant difference was detected between the regions, with p-values less than 0.02. This test also detected significant differences in the mean log-transformed concentrations among the sites within the regions for all analytes, with p-values less than 0.02, except Mn, Cu and Sr (p-values were 0.391, 0.673 and 0.058, respectively). All p-values are shown in Table 3.9. In cases where a difference among the sites within a region was detected, the nested ANOVA method does not provide information regarding

Table 3.8 P-values of Mann-Whitney Tests Comparing Inner and Outer Regions: For both sets of tests, the outer region sample size was 56. The respective sample sizes for the inner region were 67 and 57 for the analyses including and excluding site 2. P-values were adjusted for ties.

Analyte	Including site 2	Excluding site 2
ln(Cr)	0.0491	0.2245
ln(Mn)	0.0002	0.0003
ln(Fe)	0.0000	0.0000
ln(Co)	0.0000	0.0000
ln(Ni)	0.1735	0.2191
ln(Cu)	0.0000	0.0000
ln(Zn)	0.0000	0.0000
ln(As)	0.0025	0.0002
ln(Se)	0.0000	0.0000
ln(Sr)	0.0000	0.0002
ln(Cd)	0.0000	0.0000
ln(Pb)	0.0000	0.0000

which of the two, or if both regions, contained sites that were statistically different.

The regression diagnostics indicated that the assumptions made for the nested ANOVA test were violated. The side-by-side boxplots of the two regions showed many outliers and half the analytes failed the formal test for Normality (p-values less than 0.05) (Table 3.9) of the residuals of the log-transformed data. For most analytes, the residuals versus fitted value and residuals versus observation order plots indicated the presence of some possible outliers however, most data appeared to be approximately homoscedastic. Cobalt plots are given as an example in Figures 3.5 (a) and 3.5 (b). However, from the residuals versus fitted value plot for Zn (Figure 3.6) it appeared that the variance increased with concentration which indicates that the ANOVA assumptions may not have been valid.

ANOVA on the Ranks – Within Region Testing

For the inner harbour region, the one-way ANOVA test on the ranks showed no statistically significant differences between the overall mean rank of all inner sites and the mean ranks of Cr, Mn, Ni and Cu. Significant differences were detected for all other analytes. P-values are shown in Table 3.10. Boxplots showed few outliers and the box heights (interquartile ranges) were similar across the sites for most analytes. Similarly, the residuals versus fitted value plots showed uniform variance for most analytes, however some showed one site with less variance than the others. The ranked Fe residuals versus fitted value plot is

Table 3.9 Nested ANOVA Results: P-values are given for the inner versus outer harbour comparison (In/Out) and for the comparison of sites within the regions (Site(In/Out)). The number of sites per region and the total number of samples per region are given for the In/Out factor. The number of samples per site are given for the Site(In/Out) factor, where "S2=10" means 10 samples from site 2, etc. The Pearson correlation constant [r] and the p-value for the Normal Probability Plots of the nested ANOVA residuals are listed under the "Normality Test" heading.

Factor	**Levels**	**Sample sizes**
In/Out	2 Regions	In = 6 sites (67 mussels); Out = 5 sites (56 mussels)
Site(In/Out)	11 Sites	S2=10; S3=10; S4=11; S5=11; S7=13; S10=12; S11=17; S13=7; S14=12; S16=10; S17=10

		In/Out	**Site(In/Out)**	**Normality Test**	
	Analyte	**p-value**	**p-value**	**r**	**p-value**
	ln(Cr)	0.145	0.005	0.9907	0.0882
	ln(Mn)	0.002	0.391	0.9938	> 0.1000
	ln(Fe)	0.002	0.004	0.9935	> 0.1000
	ln(Co)	0.001	0.008	0.9879	0.0370
	ln(Ni)	0.168	0.003	0.9705	< 0.0100
	ln(Cu)	0.000	0.673	0.9878	0.0365
	ln(Zn)	0.003	0.016	0.9928	> 0.1000
	ln(As)	0.287	0.000	0.9896	0.0581
	ln(Se)	0.008	0.000	0.9854	0.0141
	ln(Sr)	0.015	0.058	0.9768	< 0.0100
	ln(Cd)	0.002	0.000	0.9959	> 0.1000
	ln(Pb)	0.004	0.000	0.9754	< 0.0100

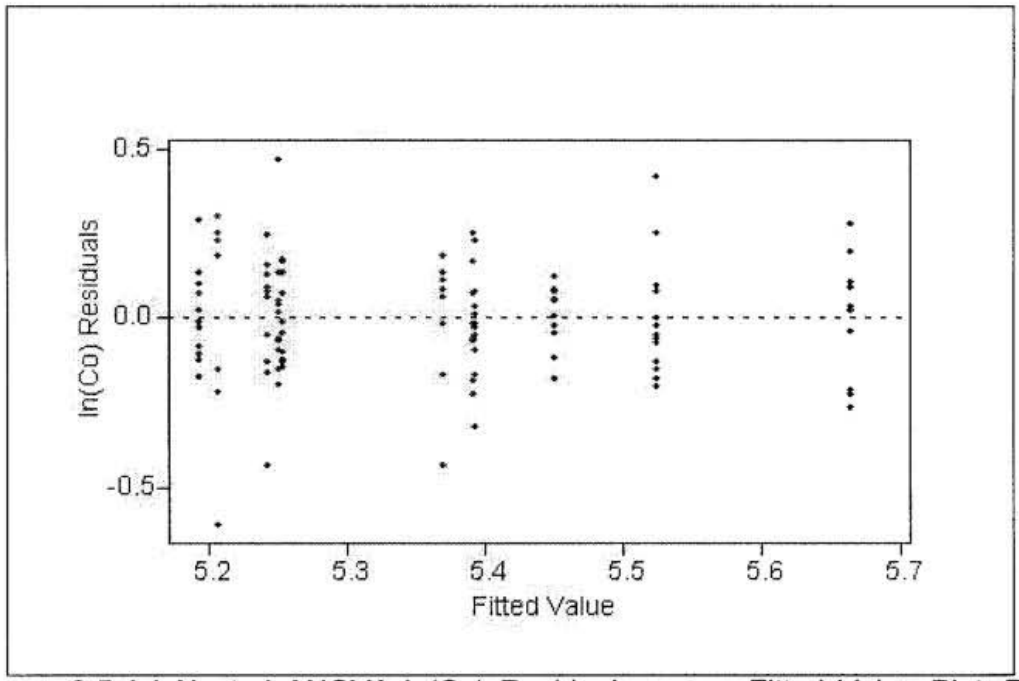

Figure 3.5 (a) Nested ANOVA ln(Co) Residuals versus Fitted Value Plot: The vertical spacing of points is approximately uniform among the data sets. Possible outliers have y-values near ± 0.5.

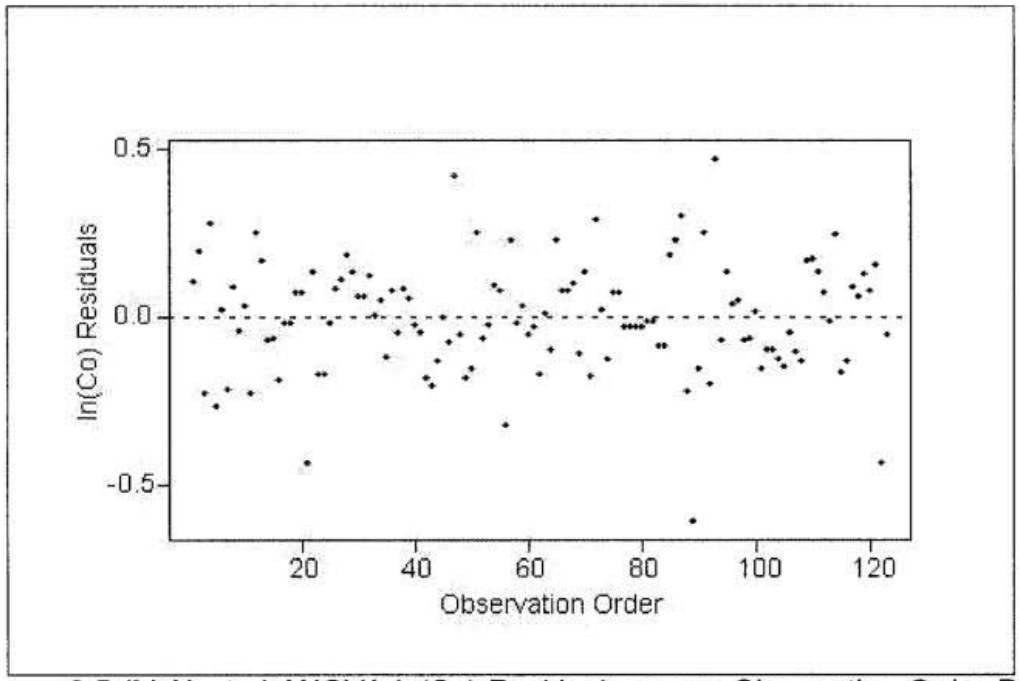

Figure 3.5 (b) Nested ANOVA ln(Co) Residuals versus Observation Order Plot: The random distribution of points above and below the x-axis indicate that the ANOVA assumptions may have been valid for this analyte, however presence of outlying points supports a violation of these assumptions.

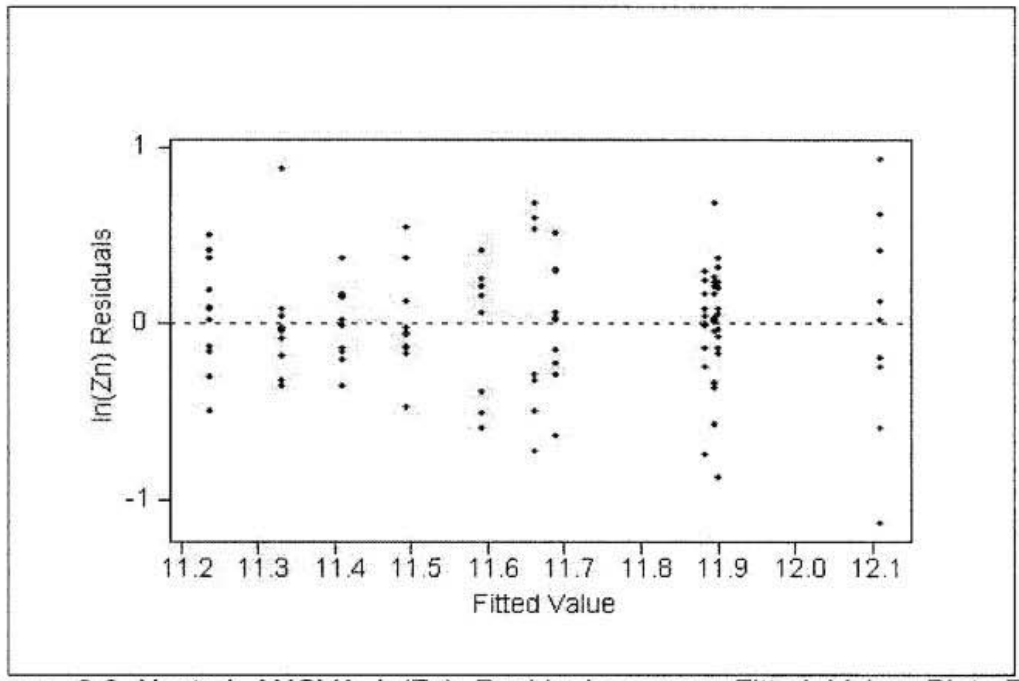

Figure 3.6 Nested ANOVA ln(Zn) Residuals versus Fitted Value Plot: The increased vertical spread of the points as the values increase along the x-axis indicates that variance may be increasing with concentration and thus the ANOVA assumptions may have been violated.

Table 3.10 Results of ANOVA on the Ranks and Bootstrap Within Region Analyses: Sample sizes per site are: site 2 = 10, site 3 = 10, site 4 = 11, site 5 = 11, site 7 = 13, site 10 = 12, site 11 = 17, site 13 = 7, site 14 = 12, site 16 = 10, site 17 = 10.

	Inner Sites		Outer Sites	
Analyte	**ANOVA**	**Bootstrap**	**ANOVA**	**Bootstrap**
Cr	0.215	0.204	0.000	0.008
Mn	0.863	0.325	**0.209**	**0.023**
Fe	0.014	0.023	0.083	0.019
Co	0.007	0.002	0.743	0.059
Ni	0.111	0.139	0.000	0.023
Cu	0.175	0.363	0.893	0.843
Zn	**0.038**	**0.137**	0.116	0.421
As	0.000	0.001	**0.012**	**0.169**
Se	**0.007**	**0.079**	**0.001**	**0.016**
Sr	**0.001**	**0.124**	0.985	0.973
Cd	0.000	0.000	0.000	0.000
Pb	**0.001**	**0.061**	0.000	0.000

shown as an example in Figure 3.7, other analytes with similar plots were Cr, Co and Se. Residuals versus order plots were randomly scattered for all analytes. The p-values of the Normality tests were greater than 0.1 for all analytes except Fe (0.05), Co (0.06) and Pb (0.05).

For the outer harbour region, the one-way ANOVA test on the ranks showed no statistically significant differences between the overall mean rank of all outer sites to the mean ranks of Mn, Fe, Co, Cu, Zn and Sr. Significant differences were detected for each of Cr, Ni, As, Se, Cd and Pb. P-values are shown in Table 3.10. Boxplots of the outer region were similar to those of the inner region, that is, few outliers and similar box heights. Residuals versus fitted value plots indicated homoscedasticity among the sites. Residuals versus order plots showed randomly distributed points with few outliers. All analytes passed the Normality test with p-values greater than 0.1, except Sr (p=0.08). Boxplots, residuals versus fitted value and residuals versus order plots, for all analytes, from the ANOVA on the ranks of the inner and outer regions are in Appendix K.

The results from Levene's test for homogeneity of variance support the diagnostic plots in finding no differences in variance of the ranked data for any analyte across the sites within each region. P-values for each analyte were greater than 0.1 (Table 3.11). This, and the fact that all analytes passed the Normality test indicates that the ANOVA assumptions were valid for the ranked data.

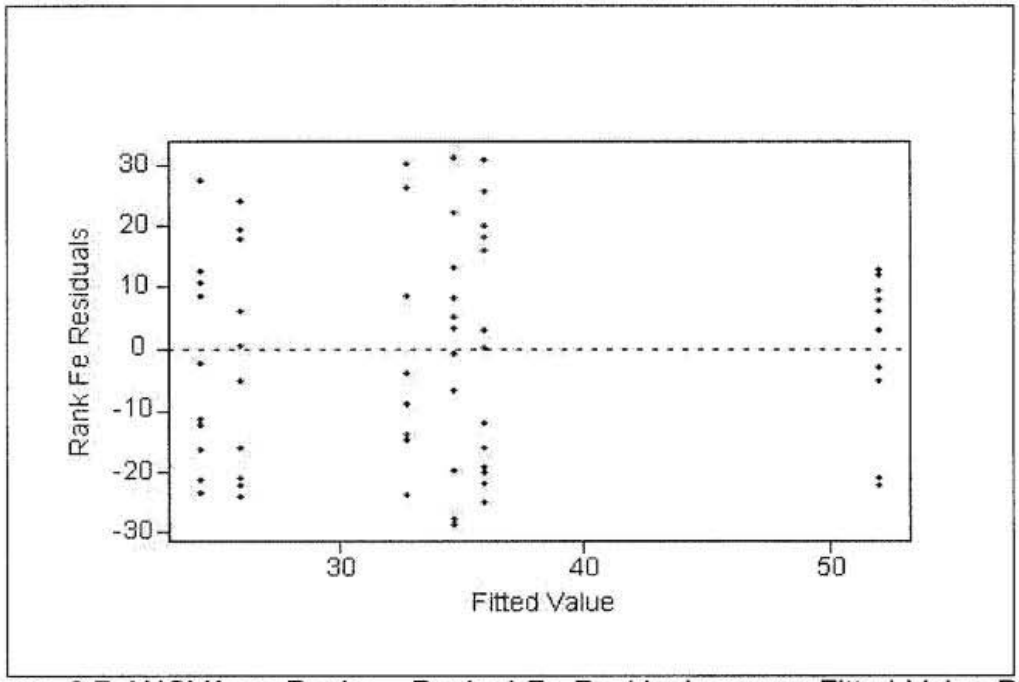

Figure 3.7 ANOVA on Ranks – Ranked Fe Residuals versus Fitted Value Plot: Inner region comparison of Fe showing one site with less variance than the others.

Table 3.11 P-values of Levene Test for Homogeneity of Variance on Rank-Transformed Data: Both inner harbour and outer harbour regions are shown. Sample sizes are 67 and 56 mussels for inner and outer, respectively.

Analyte	Inner	Outer
Cr	0.770	0.866
Mn	0.157	0.217
Fe	0.600	0.412
Co	0.946	0.285
Ni	0.595	0.866
Cu	0.663	0.940
Zn	0.776	0.488
As	0.507	0.222
Se	0.442	0.726
Sr	0.493	0.176
Cd	0.728	0.105
Pb	0.300	0.398

Bootstrapping versus ANOVA on Ranks - Within Region Testing

For the inner region, the bootstrapping technique supported the ANOVA on the ranks results for most analytes. The only discrepancies were in the cases of Zn, Se, Sr and Pb, where ANOVA indicated a significant difference, and bootstrapping did not. For the outer region, the differences were similar to those of the inner region for As, but for Mn and Fe, bootstrapping detected a significant difference, where ANOVA did not. P-values for both methods are listed in Table 3.10. One of the reasons for these discrepancies is that bootstrapping analyzes differences in medians, while ANOVA looks at differences in means. Therefore if data is skewed, the differences between the medians will not equal the differences between the means, which may lead to the identification of significant differences by one method that are not identified by the other. Another possible reason for the differing results is that ANOVA's test statistic calculations, like those of the t-test, involve division by the sum of the total squared deviations. In cases where the data range is large and/or where outliers are present, this denominator can be large, causing the quotient (i.e. test statistic) to be small, and hence giving a large p-value. Therefore, due to ANOVA's sensitivity to outliers, their presence may cause this method to erroneously indicate no significant difference, when in fact one may exist.

Tukey versus Mann-Whitney – Within Region Comparisons

In all cases where the Tukey test indicated significant differences between site-pairs, so did the Mann-Whitney test. In some cases the Mann-Whitney test

detected significant differences, when the Tukey test did not. For example, in the inner region both tests indicated that there was a significant difference between ranked Fe concentrations from sites 2 and 4, and that there was no difference between sites 2 and 7. The Mann-Whitney test also detected a difference between sites 2 and 3, but the Tukey test did not. The primary reason for the discrepancy between the two methods is the difference in their acceptable error values. The Mann-Whitney test was performed with an acceptable error (alpha) value of 0.05. Tukey, however, operates on a *family error value* of 0.05, where the individual error value may be much lower. This was the case in this analysis; the individual error values for the inner and outer regions were 0.005 and 0.007. Lower alpha values allow less chance of finding a significant difference, regardless of which method is used. Table 3.12 shows the analytes that were significantly different (alpha 0.05) in median rank value for each site-pair within both the inner and outer regions.

Differences among Sites within the Inner Region

For Fe and Co all significant differences between median rank values occurred when sites were paired with site 2. Zinc and Se concentrations were significantly different at most of the sites paired with site 3. Most sites paired either with site 2 or site 4 showed significantly different median values for Sr. Significant differences of Pb medians were found for several pairs of sites.

Table 3.12 Results of Mann-Whitney Tests on Sample Sites Within Regions: Each cell represents the outcome of a Mann-Whitney test (alpha 0.05) between a pair of sample sites, which are identified by the numbers on the row and column headers. The analytes listed are for cases where significant differences were found between the median rank values of the two sites. The underlined analytes were significantly higher at the site in the column header. Those that are not underlined were higher at the site in the row header.

Inner Region

Site	2	3	4	5	7
3	Fe Co Zn Se Sr Cd				
4	Fe Co Sr Pb	Se Cd Pb			
5	Fe Co As	Zn As Se Cd	Sr Pb		
7	As Sr Cd	Zn As Se	As Sr Cd Pb	Cd	
10	Fe Co As Sr Cd Pb	Zn As Se	As Cd	As Cd Pb	

Outer Region

Site	11	13	14	16
13	Cr Ni Se Cd Pb			
14	As Cd Pb	Cr Ni Se Cd Pb		
16	Cr Mn Ni Zn As Se Pb	Cd Pb	Cr Ni Zn Se Cd	
17	Cr Fe Ni Cd Pb	Se Cd Pb	Cr Ni Cd Pb	Fe Cd Pb

Differences among Sites within the Outer Region

For Cr and Ni rank values, sites 11 and 14 were significantly different than all other outer region sites, but were not significantly different than each other. Only the site 11 versus 16 site-pair showed significant differences in Mn rank values. Sites 11 and 16 indicated significant differences in Fe rank values when paired with site 17. For each of Fe, Zn and As only two site-pairs were found to be significantly different. Most sites paired with sites 13 and 16 were found to have significantly different values for Se. Cadmium and Pb median values were significantly different in all but one site-pair, site 11 versus 16 and site 14 versus 16, respectively.

Statistical Summary

Lead

Median lead concentrations in mussels were an order of magnitude higher inside the harbour and in the Narrows (including site 13) (1.3 – 2.3 ppm) than median values observed outside the harbour (0.21 – 0.66 ppm). Levels were highest along the east end of the harbour at sites 2, 3 and 5, lower near the west end at sites 7 and 10 (Table 3.4). The median concentration at the Logy Bay control site (site 17) (0.44 ppm) was less than those at outer sites south of the Narrows (0.58 & 0.66 ppm). Samples from locations in the Narrows had lower median concentrations than those from inside the harbour. Spatial distribution for this analyte is illustrated well in Figure A.17, Appendix L.

Arsenic

The Mann-Whitney statistical analysis indicated that there was a significant difference between the median As concentrations of the inner and outer harbour, with higher As levels in the outer region. However, inspection of median concentrations at each site (Table 3.4 and Figure A-15, Appendix L) shows the inner sites that are closest to the Narrows to be similar in As concentration to some outer sites. Arsenic concentrations in samples from sites 3 and 4 are comparable to those of site 16. The highest median As concentrations were found in samples from site 2 (7.52 ppm), the Logy Bay control site (site 17) (7.36 ppm) and at site 11 (7.35 ppm), located in Robinhood Bay.

Cadmium

Median Cd concentrations at all the outer harbour sites, except site 13, were an order of magnitude greater than Cd concentrations at the inner sites. Mussels from the control site (site 17) contained the highest Cd concentrations overall.

Selenium

Median Se concentrations are significantly higher in the outer harbour region (3.05 – 4.57 ppm) than in the inner harbour region (2.17 – 2.84 ppm). The Se median concentration at the Logy Bay control site (site 17) was 4.18 ppm.

Cobalt, Copper and Zinc

The median concentrations of Co, Cu and Zn in mussels were all significantly higher within the inner harbour region than at sites in the outer region. At the

Logy Bay control site (site 17), median concentrations of these metals (Co=2 ppm, Cu=58 ppm, Zn=90 ppm) were not significantly different than the other outer harbour sites. The only significant differences among the inner region sites were with site 2 and site 3. The median Co concentration at site 2 (3.0 ppm) was significantly higher than the other inner region sites, except site 7 (2.4 ppm). The median Zn concentration at site 3 (127 ppm) was significantly lower than the other inner region sites, except site 4 (123 ppm). In the outer region, the median Zn concentration at site 16 (92.3 ppm) was significantly higher than those at sites 11 (77.8 ppm) and 14 (80.8 ppm). Zinc concentrations were an order of magnitude higher in the inner region (123 – 186 ppm) than in the outer region (77.7- 92.3 ppm).

Manganese and Iron

Median Mn and Fe concentrations in mussels were significantly higher in the inner harbour region compared to the outer region. The distribution of the median site concentrations was near uniform within both regions, for both analytes. The median Mn value for site 16 was low compared to the other sites but the interquartile range indicates that some mussels from that site contained Mn levels comparable to the other outer region sites (Figure A.17, Appendix L). The lowest median Fe concentration (72.5 ppm) over all sites was at the Logy Bay control site (site 17). The Mn median concentration at this site (5.1 ppm) was higher than the other outer harbour sites, which ranged from 2.4 to 4.4 ppm.

Strontium

Median Sr concentrations in mussels were significantly higher at sites within the inner harbour region. The highest values were from site 2 (74.1 ppm) but the spatial distribution was fairly uniform within the inner (56.1 – 74.1 ppm) and outer (53.1 – 56.0 ppm) regions (Figure A.18 Appendix L and Table 3.4). The median concentrations at the Logy Bay control site (site 17) and at the Robinhood Bay site (site 11) were the highest of the outer harbour range, 55.3 ppm and 56.0 ppm, respectively.

Chromium and Nickel

The nested ANOVA and Mann-Whitney statistical tests did not find concentrations of Cr and Ni significantly different between the inner and outer harbour regions. Overall, higher concentrations, for both analytes were measured inside the harbour and levels at the control site (17) and at Cape Spear (site 16) were among the lowest.

In summary, the statistical results indicate that the sample sites, for all analytes, with the exception of chromium and nickel, can be grouped into two major categories, inner harbour sites and outer harbour sites. Geographically this division is located between sites 4 and 13, which are located in the Narrows (Figure 3.8).

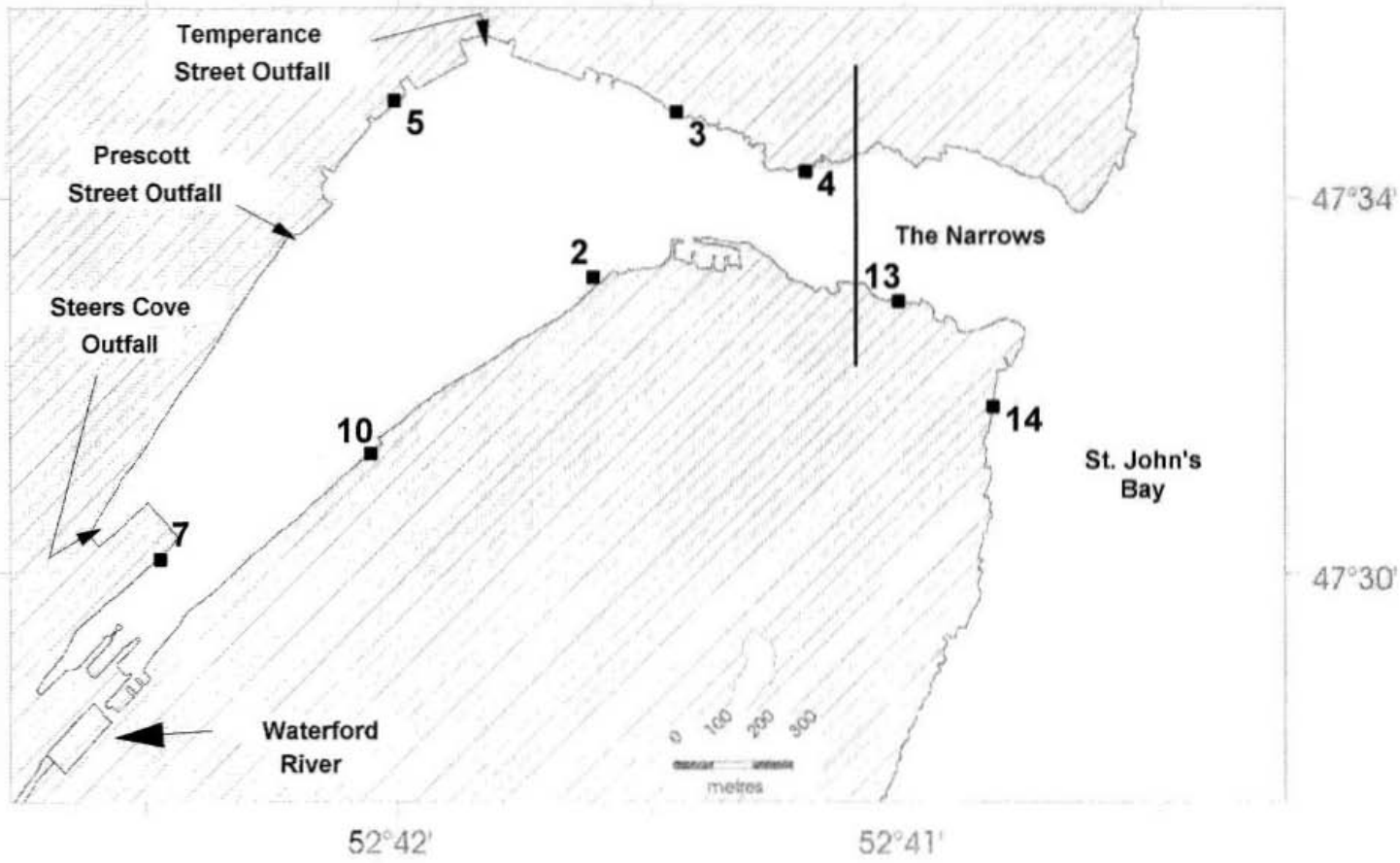

Figure 3.8 Map Of Sample Sites Showing Division Line Between Inner and Outer Regions: Left of the vertical line is the inner region and to the right of the vertical line is the outer region. Sites shown are those from which mussels were analysed - sites 11, 16 and 17 are not shown.

3.2.2 Water

The results of the one-way ANOVA indicated no significant difference between the mean measurements in the inner region to those of the outer region for pH and salinity. However a difference was detected between the temperature means of the two areas, which was also seen in the temperature boxplots. No outliers were seen in the boxplots for the three analyses (Figures 3.9 (a), (b) and (c)) and the variances (box heights) of the two regions appeared to be similar. The residuals of the pH, temperature and salinity tests all passed the Normality tests with p-values greater than 0.1000 for pH and temperature, and 0.067 for salinity. With the small sample sizes, it was difficult to determine if there were any patterns or trends in the residuals versus observation order and the residuals versus fitted value plots. The results of the Mann-Whitney tests supported those of the one-way ANOVA, finding significant differences between the temperature medians ($p = 0.008$), but not for pH ($p = 0.411$) or salinity ($p = 0.171$).

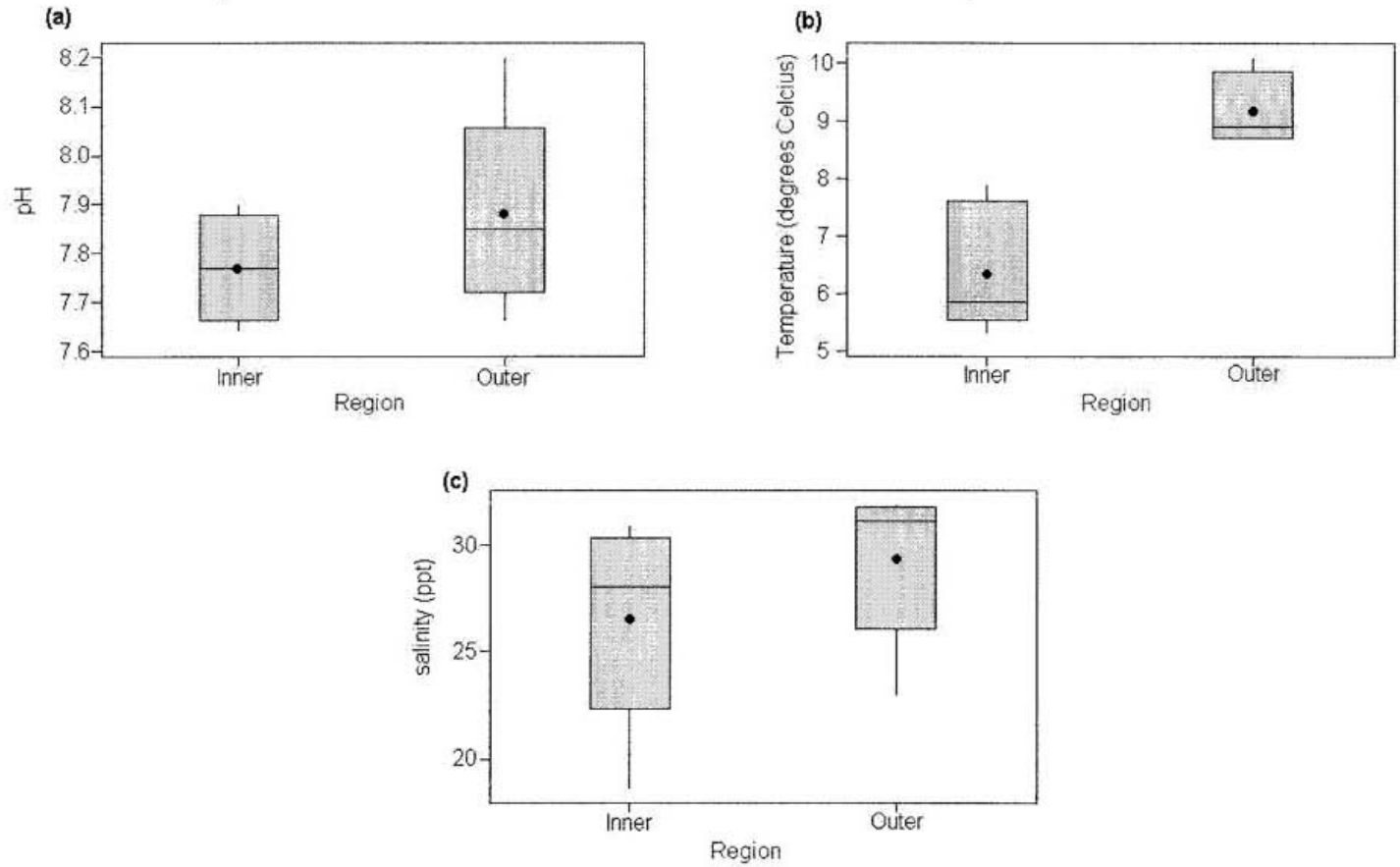

Figure 3.9 Side-by-Side Boxplots of Seawater pH, Temperature and Salinity Values: Figures (a), (b) and (c) show pH, temperature (°C) and salinity (‰), respectively. The horizontal line within the boxes indicates the median value, the filled circle indicates the mean.

4. DISCUSSION

4.1 Metal Concentrations in Mussels

The metal concentrations of the mussel tissue determined in this study were accumulated through the organisms' filter-feeding process. Whether the metal entered the organism in dissolved phase or while adsorbed to a particle will affect the extent to which the mussel retains the metal in its tissue. These metabolic processes will be discussed in the more detail for Cd, Zn and Pb, which showed the most difference in concentration between the inner and outer harbour regions. The metabolism of Se will also be discussed, as this metal showed high concentrations both inside and outside of the harbour compared to other mussel studies. First, however, some of the geochemical factors that affect the sediment/water column exchange of these metals will mentioned.

4.1.1 Geochemical Factors

There are three factors that determine the absolute concentration of a metal ion in sediment: (1) the metal abundance of the parent material, (2) deposition from external sources (e.g. atmosphere, water tributaries), (3) the binding ability of the sediment to keep the metal from transferring into the above water column (Rate et al., 2000).

A description of the geology of the study area, which relates to the first factor, and its possible pollutant sources, which relates to the second factor, are outlined in section 1.3. There are many complex mechanisms involved in the third factor

and these are difficult to evaluate in this work due to the limited information available from previous studies of sediments in this area. However, two factors affecting the exchange of metal ions between marine sediments and the water column can be discussed: the effect of the reduction potential at the water sediment interface and the presence and abundance of organic material in the sediment.

Whether an environment is oxic (i.e. oxidizing) or anoxic (i.e. reducing) can greatly affect the behaviour of metals that exist in more than one oxidation state. An example of such a metal that is a good indicator of the effects of reduction potential in sediment environments is iron. In seawater iron is present mostly in the Fe^{3+} (ferric) oxidation state which in oxic sediments will form ferric oxides (Marshall and Fairbridge, 1999). These red coloured oxides often coat other solids present , such as quartz (Marshall and Fairbridge, 1999) and act as bonding sites for metal ions such as Pb^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, and Sr^{2+} (Rose et al., 1979) dissolved in water.

From King's (1990) report, the land surrounding the study area is broken into 5 rock formations: Renews Head (St. John's Group) and Gibbet Hill, Quidi Vidi, Cuckhold and Blackhead (Signal Hill Group) (Figure A.1, Appendix A). Red sandstone is reported as a major component of the formations that cross the east end of the harbour (Quidi Vidi) , the Narrows (Cuckhold) and the outer harbour sites (Cuckhold & Blackhead). Chemical analysis of the Quidi Vidi formation

showed the percentage of oxidized iron (Fe_2O_3) (4.75 %) to be ten times that of the percentage of reduced iron (FeO) (0.46%) present. In comparison, green sandstone from the Gibbett Hill formation (crossing the central part of the harbour) showed approximately the same overall iron content (~5%), but the oxidized iron to reduced iron ratio was 2.54 to 2.82 (King, 1990).

The partitioning of metals in oxic sediments is more complex than in anoxic sediments (Griscom et al., 2000). An oxic environment can be transformed to an anoxic environment when organic matter is introduced due to oxygen depletion as bacteria break down the organic matter. In this situation, ferric oxides would be reduced to ferrous (Fe^{2+}) ions which could precipitate as a hydroxide ($Fe(OH)_2$) or, in the presence of sulfide ions, precipitate out as sulfides (Marshall & Fairbridge, 1999). Precipitating sulfides could strip chalcophile (i.e. "sulfur-loving") metals such as Pb, Cu, Zn, Ni and Cd from the water column. It can be inferred that due to the organic inputs from sewage effluent to St. John's Harbour, a reduced sediment environment may exist in the inner harbour region. However, no published dissolved oxygen data was found to support this inference.

Cadmium

According to Marshall and Fairbridge (1999), cadmium is enriched in shale and depleted in sandstone. As seen in Figure A.1, Appendix A, sandstone is the predominant rock material of the study area, with a small amount of black shale

in the Renews Head formation. Based on this qualitative knowledge of the geology of the area, it seems unlikely that the natural rock abundance of this element affected the concentrations found in the mussels in this study. A more probable source of cadmium pollution to the sediments is from deposition of coal emissions produced by the residents of St. John's during the 1800's to the mid 1900's, as seen in a study of St. John's lake sediments (Christopher et al., 1993). Although these emissions would have affected the entire study area, it seems likely that, due to the close proximity of the residents to the harbour, more ash would have been deposited in the harbour than outside the Narrows. Other possible sources of Cd pollution are from municipal sewage and storm drain outflows, oil from ships that frequent the harbour and waste from industries that surround the harbour. However, with the exception of site 13 in the Narrows, median Cd concentrations in mussels of all outer region sites are an order of magnitude greater than those of the inner region sites – the control site, at Logy Bay, having the highest overall Cd concentration. In contrast, sediments inside the harbour have been found to have Cd concentrations of approximately 2 ppm near the mussel sample sites, while sediments outside the harbour contained less than 0.1 ppm (Reid, 1998; Miskell, 2000). Considering these points, it can be inferred that differences between cadmium concentrations inside and outside the harbour may be influenced by geochemical differences in the sediments which lead to differences in the bioavailability of Cd.

In oxic environments, Cd will bind to hydrous iron oxides, but in seawater, Cd ions complex with chlorine ions forming soluble $CdCl^+$ and $CdCl_2$. In anoxic environments and in the presence of sulfide ions, Cd will form very stable, solid CdS (Marshall & Fairbridge, 1999). Reid (1998) and Miskel (2000), reported sediment sulfur values 5000-15000 ppm higher inside the harbour compared to values in the Narrows and near Fort Amherst (site 14). Therefore, one explanation for the low Cd concentrations inside the harbour is that the metal was not available for uptake due to the probable anoxic environment (as inferred above).

Other published articles have reported elevated Cd concentrations in mussels at unpolluted sites, as seen in this study. Lobel et al. (1991) observed Cd concentrations in mussels from an unpolluted site in Bellevue, NF that were similar to those from the outer region in this study (Table 4.1). In a study of Agadir Marine Bay, Morocco, Moukrim et al. (2000), reported significantly higher ($p < 0.001$) Cd levels in mussel species sampled from a unpolluted control site (4 ppm, dry wt.) than those from a site near untreated municipal waste outflows (0.8 ppm). Mussels sampled from "pristine" locations in the Kuril Islands, Russia, were also high in Cd concentrations, ranging from 3.5 to 34.0 ppm, dry wt. (Kavun et al., 2002). The authors of both of these articles suggested that the high Cd levels in these areas might have been due to natural, rather than anthropogenic sources. A natural source of Cd, proposed by Martin et al. (1976) was deep, off-shore, Cd-rich water transported to shore via upwelling processes.

Table 4.1 Study Results Compared to Metal Concentrations from an Unpolluted Site in Bellevue, Newfoundland: Bellevue statistics are based on 69 samples collected at one site (Lobel et al., 1991). Sample sizes for Inner and Outer harbour data are 67 and 56, respectively. All concentrations given in micrograms per gram, dry weight.

	Mean			Minimum			Maximum		
Analyte	**Bellevue**	**Inner**	**Outer**	**Bellevue**	**Inner**	**Outer**	**Bellevue**	**Inner**	**Outer**
Mn	5.91	5.52	4.22	2.58	2.98	1.60	11.17	13.2	8.50
Cu	6.62	7.36	5.76	4.40	3.69	3.13	9.65	10.0	12.58
Zn	79.8	151	94	48.0	58.7	46.2	197.1	465	230
As	12.9	6.00	6.84	8.5	3.13	3.29	20.2	8.83	11.6
Se	5.45	2.63	3.89	3.44	1.41	1.33	7.78	4.35	5.95
Sr	29.3	67	56	14.7	32.0	28.7	145.6	110	153
Cd	1.59	0.455	1.46	0.86	0.152	0.239	3.94	0.947	4.44
Pb	1.09	1.99	0.597	0.47	0.626	0.170	2.66	13.9	2.45

This model attempted to explain high Cd levels that were found in phytoplankton in California, USA, coastal waters. O'Connor (2002), also speculated that high levels of Cd in north California NOAA, National Status &Trends mussels may be due to this upwelling process. A similar mechanism could be operating outside St. John's Harbour and should be investigated in future studies.

Zinc

Geochemically, zinc is similar to cadmium (Marshall & Fairbridge, 1999), therefore if Cd is bioavailable in a given environment, Zn will most likely be bioavailable too, and vice versa. However, using radiolabeled oxic sediment and mussels and clams, Griscom et al., (2000) showed that while 1.5 times more Cd was extracted from the sediment than Zn, the assimilation efficiency[‡] of Zn was twice that of Cd. Vercauteren & Blust (1999) reported that for the same exposure concentration, Zn uptake in the soft tissue of *M. edulis* is ten times faster than that of Cd. They also determined that Zn at concentrations 100 times higher than Cd will inhibit Cd uptake. While Cd concentrations 10 times higher than Zn will inhibit the uptake of Zn. While the exposure concentrations of these metals are not known for the study presented in this thesis, concentrations of Zn in the mussel tissue from the inner harbour sites were three orders of magnitude greater than the corresponding Cd concentrations. For the outer harbour sites, Zn concentrations were two orders of magnitude higher than Cd concentrations.

[‡] Assimilation Efficiency represents the bioavaillability of a metal and is specific to a given organism and set of conditions (Griscom, et al., 2000).

Concentrations of Zn and Cd found in surface sediment, near mussel site 10, are 950 and 6.5 ppm, respectively (Reid, 1998). Therefore it is possible that the ratio of exposure concentration of Zn to Cd was great enough to inhibit uptake of cadmium by the mussels in both the inner and outer harbour regions. The affect of an exposure ratio greater than 100:1 on Cd uptake was not reported by Vercauteren & Blust (1999).

Lead

Lead is a ubiquitous metal that commonly accumulates with Zn, Cu, Cd and Fe in ore deposits (Mashall & Fairbridge, 1999). It has many entry sources to a harbour water environment, including natural weathering, municipal and industrial outflows, and atmospheric deposition. However, in oxic environments the concentration of Pb found in the water column is lower than might be expected because it readily absorbs onto Fe and Mn oxides and insoluble organic particles. Pb may also form complexes with chloride ions, hydroxide ions and soluble organic mater. In anoxic environments Pb is reduced to the Pb^{2+} ion, which in the presence of S, precipitates out as lead sulfide (Rose et al., 1979).

Sediments near the mussels sites that had the highest concentrations of lead (sites 2, 3 and 5) have been measured to be between 300 and 350 ppm (Reid, 1998; Miskell 2000). Sediment concentrations in the Narrows and outside the harbour were between 50 and 100 ppm. Therefore, a correlation may exist between the Pb concentration in the sediment and that found in the mussels.

Storelli and Marcotrigiano (2001) reported similar findings in a study where the highest Pb concentrations in fish were found in areas with the highest Pb levels in sediments. Published research suggests that dissolved phase Pb accumulates more readily in organisms. Borgmann and Norwood (1999) studied Pb bioavailability in amphipods (*Hyalella azteca*) from sediment and dissolved phases. The authors speculated that lead in Pb-spiked sediments becomes available for uptake by organisms through dissolution into seawater; particle-bound Pb was not considered bioavailable to these amphipods. Boisson et al. (1998) noted the same preference for dissolved Pb in Pb bioaccumulation in mussels.

Selenium

Selenium is not associated with silicate minerals, having low abundances in sandstone and quartz, but can be found in sulfur deposits with As and Cu (Rose et al., 1979). Several oxidation states of Se are possible in aqueous environments, such as the reduced ion, selenide (Se^{2-}), elemental (Se^{0}) and oxidized selenoanions selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Schlekat et al., 2000). A study on the bioavailability of particle-bound Se to clams collected in the Carquinez Strait of San Francisco Bay found Se^{0} and adsorbed selenoanions to be unavailable for uptake by the clams (Schlekat et al., 2000), citing Se ingested with radiolabeled diatoms to be the most bioavailable pathway. As noted for Cd, a possible source of Se to the study area is through coal emissions (Keller, 2000) from St. John's residents in the 1800 and 1900s.

No Se concentrations in sediments from the study area were available for comparing to mussel concentrations.

Copper

Copper minerals are associated with Pb and Zn and have a similar natural abundance in sandstone as Pb (Rose et al., 1979). Also, like Pb, Cu complexes with organic matter and Fe and Mn oxides and weaker complexes are formed with chloride ions (Marshall & Fairbridge, 1999). In conditions where the pH is greater than 5, Cu^{2+} will react with water to form copper hydrides ($CuOH^{+}$ and $Cu(OH)_2$) (Rose et al., 1979).

Copper concentrations in mussels from inner harbour sites were significantly higher than outer sites. Similar results have been found in other studies. Moukrim et al. (2000), reported significantly higher Cu and Zn concentrations ($p < 0.05$) at the polluted site (5.4 ppm and 198 ppm) in their mussel study compared to the control site (4.6 ppm and 195 ppm).

In a study of *Mytilus edulis* in British Colombia, Canada, by Grout and Levings (2001), high concentrations of Cu in mussels were associated with sites where the water was very turbid and concentrations of chlorophyll *a* were low. While the water at the west end of St. John's Harbour was completely opaque, Cu concentrations from site 7 were about the same as those from site 5 and 2, where the water was clear. Grout and Levings used chlorophyll *a* as a measure

of phytoplankton abundance. These measurements were not taken for this study.

4.1.2 Biological Factors

Mussel depuration was determined to be necessary for this study to ensure observed tissue concentrations were not partly due to the within-gut release of sediment-bound metals that had been adsorbed onto the surface of ingested sediment particles. Some studies that have analyzed mussel tissue for metal concentration did not depurate samples prior to analysis (e.g. NOAA, (O'Connor, 1998)) or the depuration period was shorter than that used in this study (Lobel et al., 1991). Thus it should be considered whether the depuration period of this study may have been too long, causing metal concentrations to be lower, and less representative of their environments. Laboratory studies where mussels are placed in clean seawater after having been contaminated generally follow a two-stage release rate for metals (Boisson et al., 1998). A two-stage release model for Pb, as described by Boisson et al. (1998), revealed that the first stage involves a rapid release (half-life of 1.4 days) of Pb that had not been accumulated into the tissue. The second stage was much longer (2.5 months) and represents accumulated Pb released from soft tissue. Gagnon and Fisher (1997) reported similar two-stage depuration processes for Co and Cd. Thus it is probable that the six-day duration of depuration used in study was not too long and that the observed concentrations accurately represent the amount of metal accumulated into the tissue. It should be noted, however, that the differences in

depuration times or absence of this process may present a confounding factor when comparing data to other published observations.

The general health of mussels affect their capacity to accumulate metals, thus it is important to know if the organisms could have been affected by the body burden of metals they were carrying. O'Connor (2002) stated that the lowest concentrations of Ni, Cu, Zn, Cd and Pb that are known to affect the survival, growth and reproduction of mussels are an order of magnitude higher than the highest NOAA Mussel Watch observations. Since the concentrations of these elements in this study are lower than the maximum NOAA measurements, their health was probably not affected by their metal burdens. However, no data on any mollusks are available for body burdens of As and Se, in this regard (O'Connor, 2002). Also, mussel health may be affected by other poorly documented factors for St. John's Harbour such as bacterial contamination (Bower, 1992), polycyclic aromatic hydrocarbons and polychlorinated biphenyls (O'Connor, 2002) levels associated with raw sewage dumping.

4.1.3 Physical Factors

Since the metals in the soft tissue of the mussels had accumulated over the period of their lifetime, the one reading of salinity and pH taken at each site do not represent the variability of these parameters that occurred seasonally and with daily tidal flushing, over this time frame.

4.2 Comparisons Between Mussel and Sediment Metal Concentrations

In this section, comparisons between the metal concentrations determined in the mussels from this study are compared to metals found in St. John's Harbour and area surface sediments by Reid (1998) and Miskell (2000).

High levels of As (35 to 45 ppm) were reported in sediment samples taken near sites 7 and 10 (where the lowest mussel concentration were found), and lower concentrations (15 to 25 ppm) (Reid, 1998) were found near sites 3 and 4 (where ~ average mussel concentrations were determined). Concentrations in sediments from outside the Narrows (Miskell, 2000) are generally lower than those from the inner harbour sites reported by Reid (1998) – the opposite trend seen in mussel concentrations. Thus, there is no evidence to support a positive relationship between As concentrations in mussels and sediments. According to Valette-Silver et al. (1999), finding a positive correlation between the metal concentrations of bivalves and spatially-associated sediments is rare.

Inner harbour sediment samples in close proximity to mussels sites were uniform in Cr concentration (70-80 ppm) (Reid, 1998). There was one hot spot in Reid's study, between mussel sites 2 and 10 on the south side of the harbour. While the median mussel concentration was highest at site 2, the interquartile range of site 10 indicates the presence of some concentrations comparable to those of site 2. These values are listed in Table 3.4 and comparisons are illustrated in

Figure A.16 in Appendix L. Outer harbour sediment concentrations measured by Miskell (2000), do not show any trend and are similar to those found inside the harbour.

Both Reid (1998) and Miskell (2000), reported uniformly distributed Ni concentrations in sediments within the harbour, with the exception of sediment from a site near Temperance Street outfall where the Ni concentration (43 ppm) was twice that of the other sites. Concentrations were relatively low (5 ppm) in the Narrows and continued to be low along the coastline of Freshwater Bay. Nickel concentrations in mussels measured in this study were approximately uniform across all sites with the highest concentrations at sites 3 and 4, and the lowest at the control site.

Similar results were found in a study of the Gulf of Maine, where Cr and Ni concentrations in mussels were mainly uniform, albeit with a few high Cr concentrations in mussels at sites where high Cr concentrations were reported in sediment samples (Chase et al., 2001).

There is no apparent relationship between the mussel concentrations of Cu and Zn, and the concentrations of these elements in the spatially-associated sediments of the Reid (1998) and Miskell (2000) reports. There is an indication

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* Comparisons for some metals have already been made previously in the discussion and thus will not be repeated in this section.

in the sediment data that cobalt concentrations are slightly higher inside the harbour, but this remains to be demonstrated by further work.

Manganese and Fe sediment concentrations (Reid, 1998; Miskell, 2000) appear to be approximately uniform from the inner harbour, into St. John's Bay. Thus no relationship between mussel and sediment data is apparent.

Relatively high sediment Sr concentrations (190-203 ppm) were measured near sites 2 and 5 (Reid, 1998; Miskell, 2000) – sites from which the highest Sr concentrations in mussels were collected. However, Miskell (2000) measured higher concentrations (322-490 ppm) at locations in the Narrows, an area where the Sr content of mussels was relatively low. Thus, a clear correlation between sediment and mussel Sr concentrations is not apparent, based on these observations.

4.3 Possible Anthropogenic and Natural Sources

Anthropogenic Sources

A study of lake sediment in St. John's (Christopher et al., 1994) provides good evidence that coal burning has been a source of Fe, Co, Zn, As, Cd and Pb contamination of St. John's Harbour. The metals reach the harbour sediments and water through both direct atmospheric deposition of coal emissions and via storm drain runoff from contaminated lakes and soils. In the harbour they are potentially available for ingestion by the filter-feeding mussels. Other anthropogenic sources of contamination include runoff from soils contaminated

from vehicle emissions, ship fuels and bilge water. Seepage from older oil storage tanks located on South Side Hills may also be a source of pollution, as mentioned in section 1.3.3. It is the understanding of the author that some of the older storage tanks have been removed within recent years.. Raw sewage effluent is an obvious contaminant of the harbour and would be expected to contain elevated levels of the metals studied here.

In their bivalve and sediment study of several locations along the southeastern coast of the United States, Valette-Silver et al. (1999) suggested agricultural use of arsenic-containing pesticides on soils and As-enriched, marine phosphate deposits as anthropogenic and natural As sources, respectively. However, neither the use of these types of pesticides in the St. John's area, nor the phosphate content of sediments in St. John's Bay, were examined in this study.

No clear correlation between increased concentrations in mussels and distance from outfalls were seen for any of the elements in the study presented in this thesis. This suggests that harbour waters are well mixed over the time-scale of mussel growth (~2 years). However, the complex environmental interactions associated with using mussels as bioindicators, mentioned previously in this chapter, complicate such a correlation assessment. In a contaminated system with multiple pollution sources, it is difficult to link the loading of a particular metal to a particular source.

Natural Sources

The most probable natural source of all analytes is the shale and sandstone bedrock (King, 1990) that predominates in the St. John's area. Also, cobalt occurs naturally in soil and seawater and is ubiquitous in the environment (ATSDR, 1992). In conjunction with the high mixing capacity of the harbour waters, a widespread bedrock metal source could explain the uniformity of the spatial distributions of most analytes within the inner region. In a similar mussel study of Long Island Sound, NY, USA, Turgeon et al. (1989) stated one reason for increased concentrations of Cr, Cu, Cd and Pb in the west-end versus the eastern end of the sound, was decreased mixing capacity in the west-end. The significant difference between the median temperature of the inner and outer St. John's Harbour sites was due to differences in water depth of the sample sites rather than poor water circulation and mixing. The five outer sites were shoreline environments where mussels grew on rocks, in warm shallow water relative to the colder deeper water environments of most of the inner harbour mussels, which were attached to pier pilings.

In NOAA Mussel Watch studies, greater than 80% of the high levels of Ni, As, Se and Cd were found at rural locations – indicating that that these high concentrations may be caused by natural occurrences (O'Connor, 2002). Further, O'Connor (2002) stated that a natural source is more probable when high concentrations are found in a region of sample locations rather than for an individual site. This may also be the case in St. John's Harbour.

4.4 Comparisons with Other Mussel Study Data

Cadmium concentrations found in this study were lower than those reported by Gulfwatch and the NOAA Mussel Watch Program (Table 4.2). The mean Cd mussel concentration from Bellevue (Lobel et al., 1991) is about 0.1 ppm higher than the mean Cd concentration from the outer harbour region. However, the maximum value for the outer harbour region is 0.5 ppm greater than that of the Bellevue study (Table 4.1).

Compared to the NOAA Mussel Watch data (Table 4.2), Zn concentrations from the inner harbour region are higher at the 15^{th} and 50^{th} percentiles. The median Zn inner harbour concentration is also higher than the median Gulfwatch level. Copper concentrations from this study are lower than both NOAA and Gulfwatch values. Copper concentrations from Bellevue were similar to those from the inner harbour region, while Zn levels were more like those observed in the outer region (Table 4.1). No published concentrations of Co in mussels were found for comparison with the St. John's Harbour data.

The 15^{th} percentile median Pb concentration of the inner region samples is higher than that of the NOAA data, but for the 50^{th} and 85^{th} percentiles, both harbour regions are less than the values reported by NOAA and Gulfwatch (Table 4.2). The mean Pb concentration of the inner harbour region is almost twice the value found in Bellevue (Table 4.1).

Table 4.2 Study Results Compared to Published Metal Concentrations of Gulfwatch and NOAA National Status & Trends Mussel Watch Programs: Gulfwatch data (Chase et al., 2001) represents *M. edulis*, sampled 1991-1997. NOAA data represents *M. edulis* and *M. californianus* sampled 1986-1998 (http://ccmaserver.nos.noaa.gov), except for Cr, 1986-1995. Values in bold type indicate a St. John's Harbour value is greater than the corresponding NOAA value. An asterisk indicates that a St. John's Harbour value is greater than the corresponding Gulfwatch value. All concentrations are given in micrograms per grams dry weight.

	15th Percentile				50th Percentile				85th Percentile			
	Gulfwatch	**NOAA**	**Inner**	**Outer**	**Gulfwatch**	**NOAA**	**Inner**	**Outer**	**Gulfwatch**	**NOAA**	**Inner**	**Outer**
Cr	N.A.	0.97	0.60	0.54	1.68	1.70	0.784	0.729	2.69	2.90	1.08	0.93
Mn	N.A.	8.623	3.775	2.606	N.A.	17.85	4.98	4.24	N.A.	40.7	7.446	5.898
Fe	N.A.	230	99	69	389	410	120	92	570	890	177	113
Ni	N.A.	0.99	0.820	0.738	1.43	1.74	1.275	1.231	2.11	3.2	1.958	1.638
Cu	N.A.	7.4	5.831	4.101	6.87	9.7	7.47*	5.22	8.63	12.7	8.776*	7.027
Zn	N.A.	81.7	**101**	64	96	120	**144***	84	131	178	**193***	114
As	N.A.	6.6	4.975	5.100	N.A.	9.5	5.77	6.81	N.A.	14.6	7.273	8.233
Se	N.A.	1.636	**2.169**	**2.916**	N.A.	2.39	**2.60**	**3.80**	N.A.	3.482	3.104	**5.044**
Cd	N.A.	0.947	0.278	0.894	1.57	1.73	0.407	1.224	2.20	3.5	0.692	1.815
Pb	N.A.	0.869	**1.127**	0.218	2.62	2.2	1.613	0.486	5.23	6.1	2.696	1.046

The 15th and 50th percentiles of Se for both regions are greater than the respective percentiles of the NOAA Mussel Watch Program. As well, the 85th percentile of the outer harbour region is higher than that of NOAA (Table 4.2). Selenium concentrations from Bellevue (Lobel et al., 1991) were higher than either harbour region (Table 4.1).

The range of median As concentrations over all sites is 7.52 ppm to 5.10 ppm, which are less than the national median reported by NOAA for *Mytilus* spp. for the coastal United States (Table 4.2). Mean As concentrations reported in mussels collected from an unpolluted site in Bellevue (Lobel et al., 1991) were twice as high as those observed in this study (Table 4.1).

Concentrations of Cr and Ni measured in this study were less than those reported by the NOAA Mussel Watch or Gulfwatch programs (Table 4.2).

Manganese and Fe concentrations from both regions were low compared to NOAA Mussel Watch data. NOAA median Mn concentrations were approximately four times those of the two regions, while median Fe values were between 3 and 4.5 times lower than either NOAA or Gulfwatch data (Table 4.2). Manganese values from the inner harbour region were comparable to concentrations reported in the Bellevue study (Table 4.1).

No Sr data was available from the NOAA Mussel Watch or from the Gulf of Maine mussel study. The Bellevue mean Sr concentrations is approximately 25 ppm lower than the outer region median values observed in this study and 30-45 ppm lower than the inner region concentrations (Table 4.1).

4.5 Public Health Regulation of Shellfish Contaminants

While the purpose of this study was not to determine if St. John's Harbour mussels are fit for human consumption, the following information has been included as a point of interest. Note that this information deals only with metal loadings and does not address concerns of bacterial or other forms of mussel contamination.

The United States Food and Drug Administration (FDA) has established safety guidelines for concentrations of five metals in shellfish: As, Cd, Cr, Ni and Pb (USFDA, 1993). In this study, the median concentrations of these metals, for all sites, were less than the FDA limits (Appendix M). However, Pb concentrations greater than that recommended for children between the ages of two and five years, at the 90^{th} percentile consumption rate, were found in three mussels from site 5. The concentrations of samples 0508, 0509 and 0516 were 4.1, 5.5 and 14 ppm, respectively. A concentration of 14 ppm exceeds the guidelines for the same group of children consuming at the mean rate and for pregnant women that fall under the mean and 90^{th} percentile consumption rates. No explanation for the anomalous concentration of sample 0516 is speculated since the sample size

is relatively small, n=11. This sample was collected and prepared in the same way as the other mussels in this study and concentrations of the other analytes appear to be similar to those found in the rest of the mussels from site 5. Therefore, it is unlikely that the high lead concentration in sample 0516 is due to post-collection contamination.

4.6 Future Work

Several lines of investigation seem warranted:

Analysis of the metal content of the mussel shells collected in this study, using either solution ICPMS or laser-ablation ICPMS, for comparison with the soft tissue results. Hard shell analyses may present a temporal record of metal contamination not accessible through soft tissue work.

A follow-up study of mussels and sediments from site 2, with further investigation into present and past industrial activity of the immediate surroundings.

Isotope ratio analysis comparing Pb isotope ratios from mussel, sediment and sewage samples from various locations within St. John's Harbour. This would provide information on possible sources of Pb and its bioavailability from the sediments.

Sediment studies to identify mineral fractions associated with particular metal loadings within St. John's Harbour. Further investigation into possible natural sources of As, Se and Cd in the study area.

4.7 Conclusions

The soft tissue of indigenous blue mussels, collected from 11 sites in and around St. John's Harbour, were analyzed for concentrations of 12 elements, using microwave digestion and ICPMS. From the results of statistical analyses, the study area was divided into inner and outer regions, with the dividing line located approximately halfway through the Narrows, between sites 4 and 13. The results of this study support the scientific hypothesis that mussels sampled within St. John's Harbour will contain higher concentrations of metals than those sampled from outside the harbour, for seven of the 12 analytes. All analytes, except Cr and Ni, were found significantly different based on median concentrations of the sites within each region. Zinc and Pb concentrations were an order of magnitude higher at inner region sites, while Cd levels were equally elevated at outer region sites. The differences between median site concentrations were not as great for the other analytes. Arsenic, Se and Cd concentrations were significantly higher in the outer region, the other metals that showed significant differences were higher in the inner region. According to NOAA National Status & Trends program criteria the 85th percentile concentrations for Zn from inner region sites and Se from both inner and outer regions are high. The moderate to low levels of metals (excepting Zn and Se) in the mussels is somewhat surprising given the high level of dumping of untreated sewage in St. John's Harbour. This suggests that if the sewage carries significant quantities of these metals, they are largely sequestered in the bottom sediment and in a form that makes them not generally

bioavailable. There was an overall uniform spatial distribution of metals within each region, with the exception of many high values associated with site 2. Mussels from site 2, in the inner region, contained the highest overall median concentrations of all sites for Cr, Fe, Co, Zn, As, Sr and Pb. The notable peak in heavy metal concentration at this site was unexpected as there were no sewage outfalls in the area and past sediment studies did not indicate elevated metal concentrations at sites sampled closest to this area. There was little or no evidence to support a positive correlation between analyte concentrations and the proximity of mussel sites to previously sampled sediment sites and sewage outfalls, respectively. It is probable that the uniformity of the metal concentrations within the inner region is due to the high mixing capacity of the harbour water.

LITERATURE REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1992). "Toxicological Profile for Cobalt." Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://atsdr1.atsdr.cdc.gov/

Animal Care Services, Memorial University of Newfoundland (2001), http://www.mun.ca/acs/

Bates, J.A. & Innes, D.J. (1995) " Genetic Variation Among Populations of *Mytilus* spp. in Eastern Newfoundland", Marine Biology, Vol. 124, pp. 417-424.

Beauchemin, D., McLaren, J.W. & Berman, S.S. (1987) "Study of the Effects of Concomitant Elements in Inductively Coupled Plasma Mass Spectrometry", Spectrochimica Acta, Vol. 42B (3), pp. 467-490.

Boisson, F., Cotret, O. & Fowler, S.W. (1998), "Bioaccumulation and Retention of Lead in the Mussel *Mytilus galloprovincialis* Following Uptake from Seawater", The Science of the Total Environment, Vol. 222, pp. 55-61.

Borgmann, U. & Norwood, W.P. (1999) "Assessing theToxicity of Lead in Sediments to *Hyalella azteca*: the Significance of Bioaccumulation and Dissolved Metal", Canadian Journal of Fisheries and Aquatic Sciences, Vol. 56, pp. 1494-1503.

Bower, K.M. (2000) "Analysis of Variance (ANOVA) Using MINITAB", Scientific Computing and Instrumentation Online, www.scimag.com (February) pp. 64-65.

Bower, S.M. (1992) "Diseases and Parasites of Mussels" in Gosling E. (Editor) The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 543-563.

Brylinsky, M. (1989) Biology of the Blue Mussel (*Mytilus edulis*): A Manual Prepared for Participants of the Water Quality Monitoring Programme for Mussel Growers,Publication No. 9 of the Acadia Centre for Estuarine Research, Wolfville, Nova Scotia, 45 pp.

Canadian Department of Fisheries and Oceans (DFO) (1996) "Blue Mussel", series, Coastal Zone Species Profile, No. 7, Fisheries and Oceans Canada, Ottawa, Ontario, 2 pp.

Canadian Department of Fisheries and Oceans (DFO) (1999) "Canadian Tide and Current Tables, Volume 1 Atlantic Coast and Bay of Fundy", Canadian Hydrographic Service, Fisheries and Oceans Canada, Ottawa, Ontario, 96 pp.

Canadian Hydrographic Service (1999) "Newfoundland, Southeast Coast, Motion Bay to Cape St. Francis" (Map) LC4846, Minister of Fisheries and Oceans, Canada.

Chase, M.E., Jones, S.H., Hennigar, P., Sowles, J., Harding, G.C.H., Freeman, K., Wells, P.G., Krahforst, C., Coombs, K., Crawford, R., Pederson, J. and Taylor, D. (2001) "Gulfwatch: Monitoring Spatial and Temporal Patterns of Trace Metal and Organic Contaminants in the Gulf of Maine (1991-1997) with the Blue Mussel, *Mytilus edulis* L", Marine Pollution Bulletin, Vol. 42 (6), pp. 491-505.

Christopher, T.K., Davenport, P.H. & Burden, E.T. (1993) "The Effect of Urban and Industrial Development on the Geochemistry of the Watersheds in the St. John's Area: Preliminary Results", Current Research, Newfoundland Department of Mines and Energy, Geological Survey Branch, Report 93-1, pp. 419-433.

Christopher, T.K., Davenport, P.H. & Burden, E.T. (1994) "The Pollution History of a City from the Sediment Record of Urban Lakes", Trace Substances, Environment and Health, 27, University of Missouri-Columbia. Columbia, MO, United States, pp. 145-152.

Comensaña, A.S., Toro, J.E., Innes, D.J. & Thompson, R.J. (1999) "A Molecular Approach to the Ecology of a Mussel (*Mytilus edulis – M. trossulus*) Hybrid Zone on the East Coast of Newfoundland, Canada", Marine Biology, Vol. 133, pp. 213-221.

Cossa, D. (1988) "Cadmium in *Mytilus* spp.: Worldwide Survey and Relationship Btween Seawater and Mussel Content", Marine Environmental Research, Vol. 26, pp. 265-84.

Dabinett, P. (1999) Personal communication. Faculty member of the Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Eggins, S.M., Woodhead, J.D., Kinsley, L.P.J., Mortimer, G.E., Sylvester, P., McCulloch, M.T., Hergt, J.M. & Handler, M.R. (1997) "A Simple Method for the Precise Determination of ≥ 40 Trace Elements in Geological Samples by ICPMS using Enriched Isotope Internal Standardisation", Chemical Geology, Vol. 134, pp.311-326.

Environment Canada (1999) "Monthly Meteorological Summary for St. John's, NF for June 1999", Environment Canada Atlantic Region, Atmospheric Environmental Branch.

Environment Canada (2001) "Shellfish and Water Quality", online fact sheet last updated April 26, 2001. http://www.atl.ec.gc.ca/epb/factsheets/sfish_wq.html

Evans, E.H. & Giglio, J.J (1993) "Interferences in Inductively Coupled Plasma Mass Spectrometry: A Review", Journal of Analytical Atomic Spectrometry, Vol. 8, pp.1-18.

Fisher, N.S., Teyssié, J.-L., Fowler, S.W. & Wang, W.-X. (1996) "Accumulation and Retention of Metals in Mussels from Food and Water: A Comparison under Field and Laboratory Conditions", Environmental Science and Technology, Vol. 30, pp. 3232-3242.

Gagnon, C. & Fisher, N.S. (1997) "The Bioavailability of sediment-bound Cd, Co, and Ag to the Mussel *Mytilus edulis*", Canadian Journal of Aquatic Science, Vol. 54, pp. 147-156.

Griscom, S.B., Fisher, N.S. & Luoma, S.N. (2000) "Geochemical Influences on Assimilation of Sediment-Bound Metals in Clams and Mussels", Environmental Science and Technology, Vol. 34, pp. 91-99.

Goldberg, E.D. (1975) "The Mussel Watch – A First Step in Global Marine Monitoring", Marine Pollution Bulletin Vol. 6 (7) page 111.

Grout, J.A. & Levings, C.D. (2001) "Effects of Acid Mine Drainage from an Abandoned Copper Mine, Britannia Mines, Howe Sound, British Colombia, Canada, on Transplanted Blue Mussels (*Mytilus edulis*)", Marine Environmental Research, Vol. 51, pp. 265-288.

Hatfield, E. (1999) Personal communication. Employee of the Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Innes, D.J. & Bates, J.A. (1999) "Morphological Variation of *Mytilus edulis* and *Mytilus trossulus* in Eastern Newfoundland", Marine Biology, Vol. 133, pp. 691-699.

Jackson, A.R.W. & Jackson, J.M. (1996) Environmental Science: The Natural Environment and Human Impact, Longman Group Ltd., Harlow, 370 pp.

Jacques Whitford Environmental Limited (1995) "Fisheries Inventory Study", JWE Project No. 748, St. John's, Newfoundland, 33 pp. plus appendices.

Jørgensen, C.B. (1990) Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology, Olsen & Olsen, Fredensborg, Denmark, 140 pp.

Kavun, V.Y., Shulkin, V.M. & Khristoforova, N.K. (2002) " Metal Accumulation in Mussels of the Kuril Islands, North-West Pacific Ocean", Marine Environmental Research v.51 pp.219-226.

Keller, E.A. (2000) Environmental Geology, 8th Edition, Prentice Hall, Inc., New Jersey, USA, 562 pp.

King, A.F.(1990) Geology of the St. John's area Government of Newfoundland and Labrador, Department of Mines and Energy, Geological Survey Branch, Report 90-02, GS# 001N/0560, 102 pp, enclosures (map, figure).

Lobel, P.B., Belkhode, S.P., Jackson, S.E. & Longerich H.P. (1989) "A Universal Method for Quantifying and Comparing the Residual Variability of Element Concentrations in Biological Tissues using 25 Elements in the Mussel *Mytilus edulis* as a Model", Marine Biology Vol. 102, pp. 513-518.

Lobel, P.B., Longerich, H.P., Jackson, S.E. and Belkhode, S.P. (1991) "A Major Factor Contributing to the High Degree of Unexplained Variability of Some Elements Concentrations in Biological Tissue: 27 Elements in 5 Organs of the Mussel *Mytilus* as a Model", Archives of Environmental Contamination and Toxicology Vol. 21, pp. 118-125.

Lutz, R.A. (1976) "Annual Growth Patterns in the Inner Shell Layer of *Mytilus edulis* L."Journal of the Marine Biological Association of the United Kingdom, Vol. 56, pp. 723-31.

Lutz R.A. & Kennish M.J. (1992) "Ecology of Larval and Early Postlarval Mussels", in Gosling E. (Editor) The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 53-85.

Marshall, C.P. & Fairbridge, R.W. (editors) (1999) Encyclopedia of Geochemistry, Kluwer Academic Publishers, Dordrecht, The Netherlands, 712 pp.

Martin JH, Bruland KW & Broenkow WW (1976) "Cadmium Transport in the California Current", in Windom HL & Duce RA (editors) Marine Pollutant Transfer, Lexington Books, Lexington, pp. 159-184.

Miskell, M. (2000) "Anthropogenic Pollution in St. John's Harbour and the Degree of Contamination of the Nearshore Zone East of the Narrows: Heavy Metals in the Surface Samples." Honours Dissertation, Memorial University of Newfoundland, St. John's, NF, 86 pp.

Moukrim, A., Kaaya, A., Najimi, S., Roméo, M., Gnassia-Barelli, M. & Barnonne, J.F. (2000) " Assessment of the Trace Metal Levels in Two Species of Mussels from the Agadir Marine Bay, South of Morocco", Bulletin of Environmental Contamination and Toxicology v. 65 pp. 478-485.

Newell, R.I.E. (1989) "Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (North and Mid-Atlantic): Blue Mussel" Biological Report 82 (11.102), TR EL-82-4, Fish and Wildlife Service, US Department of the Interior, 25 pp.

Newfoundland Design Associates Ltd. (NFDAL) (1996) "Assessment of Sewage Treatment Processes and Development of a Strategy for Implementation of a Sewage Treatment and Disposal System for the St. John's Harbour Drainage Area", not published.

Nicholson, M.D. & Fryer, R.J. (1996) Contaminants in Marine Organisms: Pooling Strategies for Monitoring Mean Concentrations. ICES Techniques in Marine Environmental Sciences No. 18, March 1996

NOAA (1993) National Status and Trends Program for Marine Environmental Quality, "Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992, Vol. 1 Overview and Summary of Methods", Lauenstein G.G. & Cantillo A.Y. (Editors) NOAA Technical Memorandum NOS ORCA 71, Silver Spring, MD, 117 pp.

O'Connor, T.P. (1994) "The NOAA National Status and Trends Mussel Watch Program: National Monitoring of Chemical Contamination in the Coastal United States" in Cothern C.R. & Ross P. (Editors) Environmental Statistics, Assessment and Forcasting, Lewis Pub., Boca Raton, FL, pp. 331-349.

O'Connor, T.P. (1998) "Mussel Watch Results from 1986 to 1996", Marine Pollution Bulletin, Vol.37 (1-2), pp. 14-19.

O'Connor, T.P. (2002) "National Distribution of Chemical Concentrations in Mussels and Oysters in the USA", Marine Environmental Research, Vol. 53, pp. 117-143.

Payne, J.F. (1999) Personal communication. Scientific Investigator, Environmental Sciences Section, Newfoundland Region, Fisheries and Oceans Canada, Government of Canada.

Penney, R.W. and Hart, M.J. (1999) "Distribution, Genetic Structure, and Morphometry of *Mytilus edulis* and *M. trossulus* within a Mixed Species Zone", Journal of Shellfish Research, Vol. 18 (2), pp. 267-274.

Rate, A.W., Robertson, A.E. & Borg, A.T. (2000) "Distribution of Heavy Metals in Near-Shore Sediments of the Swan River Estuary, Western Australia", Water, Air and Soil Pollution, Vol. 124, pp. 155-168.

Reid, P. (1998) "Marine Pollution in St. John's Harbour: Heavy Metal Contamination in Surficial Sediments" Honours Dissertation, Memorial University of Newfoundland, St. John's, NF, 56 pp.

Rose, A.W., Hawkes, H.E. & Webb, J.S. (1979) Geochemistry in Mineral Exploration, Second Edition, Academic Press Inc. (London) Ltd., England 657 pp.

Seed, R. (1968) "Factors Influencing Shell Shape in the Mussel *Mytilus edulis*", Journal of the Marine Biological Association of the United Kindom, Vol. 48, pp. 561-584.

Seed, R. & Suchanek, T.H. (1992) "Population and Community Ecology of *Mytilus*", in Gosling E. (Editor) The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 87-169.

Sericano, J.L. (2000) "The Mussel Watch Approach and its Applicability to Global Chemical Contamination Monitoring Programmes", Int. J. Environment and Pollution, Vol. 13 (1-6), pp. 340-350.

Shumway, S.E. (1992) "Mussels and Public Health", in Gosling E. (Editor) The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 511-542.

Statistics Canada (1996) "Statistical Profile of Canadian Communities", Statistics Canada's Web Site, http://ceps.statcan.ca/english/profil/PlaceSearchForm1.cfm

StatSoft, Inc. (2001) Electronic Statistics Textbook, Tulsa, OK, USA, http://www.statsoft.com/textbook/stathome.html.

Storelli, M.M. & Marcotrigiano, G.O. (2001) "Heavy Metal Monitoring in Fish, Bivalve Molluscs, Water, and Sediments from Varano Lagoon, Italy", Bulletin of Environmental Contamination and Toxicology, Vol. 66, pp. 365-370.

Thompson, R.J. (1984) "The Reproductive Cycle and Physiological Ecology of the Mussel *Mytilus edulis* in a Subarctic, Non-Estuarine Environment", Marine Biology, Vol. 79, pp. 277-288.

Turgeon, D.D., Gottholm, B.W., McMahon, K.D. & Robertson, A. (1989) "National Status and Trends Program for Marine Environmental Quality: Toxic Contaminants in Long Island Sound", NOAA/NOS/OMA/OAD, Rockville, Maryland, USA.

U.S. Environmental Protection Agency (USEPA) (1995) Guidance for Assessing Chemical Contamination Data For Use in Fish Advisories, Volume 1: Fish Sampling and Analysis Second Edition, EPA Publication No. EPA 823-R-95-007, Office of Science and Technology, Office of Water, USEPA, Washington, DC.

U.S. Environmental Protection Agency (USEPA) (1997) "Conductivity Meters", The Volunteer Monitor: The National Newsletter of Volunteer Water Quality Monitoring, Vol. 9, (1), http://www.epa.gov/volunteer/spring97/

U.S. Food and Drug Administration (USFDA) (1993) "Food and Cosmetic Guidance Documents", http://www.cfsan.fda.gov/~dms/guidance.html

Valette-Silver, N.J., Riedel, G.F., Crecelius, E.A., Windom, H., Smith, R.G. & Dolvin, S.S. (1999) "Elevated Arsenic Concentrations in Bivalves from the Southeast Coasts of the USA", Marine Environmental Research, Vol. 48, pp. 311-333.

Vercauteren, K. & Blust, R. (1999) "Uptake of Cadmium and Zinc by the Mussel *Mytilus edulis* and Inhibition by Calcium Channel and Metabolic Blockers", Marine Biology, Vol. 135, pp. 615-626.

Widdows, J. & Donkin, P. (1992) "Mussels and Environmental Contaminants: Bioaccumulation and Physiological Aspects", in Gosling E. (Editor) The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture, Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 383-424.

Yokogawa Analytical Systems Inc. (1997) "HP 4500 Application Handbook", Revision 1.2.

Yoon, Jeong-Yeol, "Research of Jeong-Yeol Yoon – New Bioadhesive, MEFP-1", http://www.bol.ucla.edu/~wamozart/acad2.htm, 1998-2001.

APPENDIX A

Geological Map of the Study Area

Legend for Figure A.1: (cited from King (1990))
St. John's Group
(8) Renews Head Formation: thin, lenticular bedded, dark-grey sandstone and minor shale.
Signal Hill Group
(9) Gibbett Hill Formation: thick bedded, greenish-grey sandstone, siltstone and tuff; minor red sandstone and siltstone and greenish-grey conglomerate
(10) Quidi Vidi Formation: red and green sandstone, siltstone and mudstone; minor pebble conglomerate and intraformational breccia
(11) Cuckold Formation: red conglomerate and sandstone.
- (11a) Cabot Tower Member: medium- to coarse-grained red sandstone and interbedded red pebble conglomerate;
- (11b) Cape Spear Member: red pebble to cobble conglomerate and coarse-grained red sandstone; contains exotic clasts;
- (11c) Skerries Bight Member: pebble conglomerate, passing into coarse grained red sandstone at top

(12) Blackhead Formation:
- (12a) Petty Harbour Member: red sandstone and granule conglomerate;
- (12b) Maddox Cove Member: red mudstone and sandstone; minor tuff;
- (12c) Spriggs Point Member: red sandstone and mudstone (lower part); greenish-grey, red and white sandstone and mudstone (upper part);
- (12d) Deadman's Brook Member: green mudstone and sandstone;
- (12e) Cliff Point Member: red and green sandstone, siltstone and mudstone

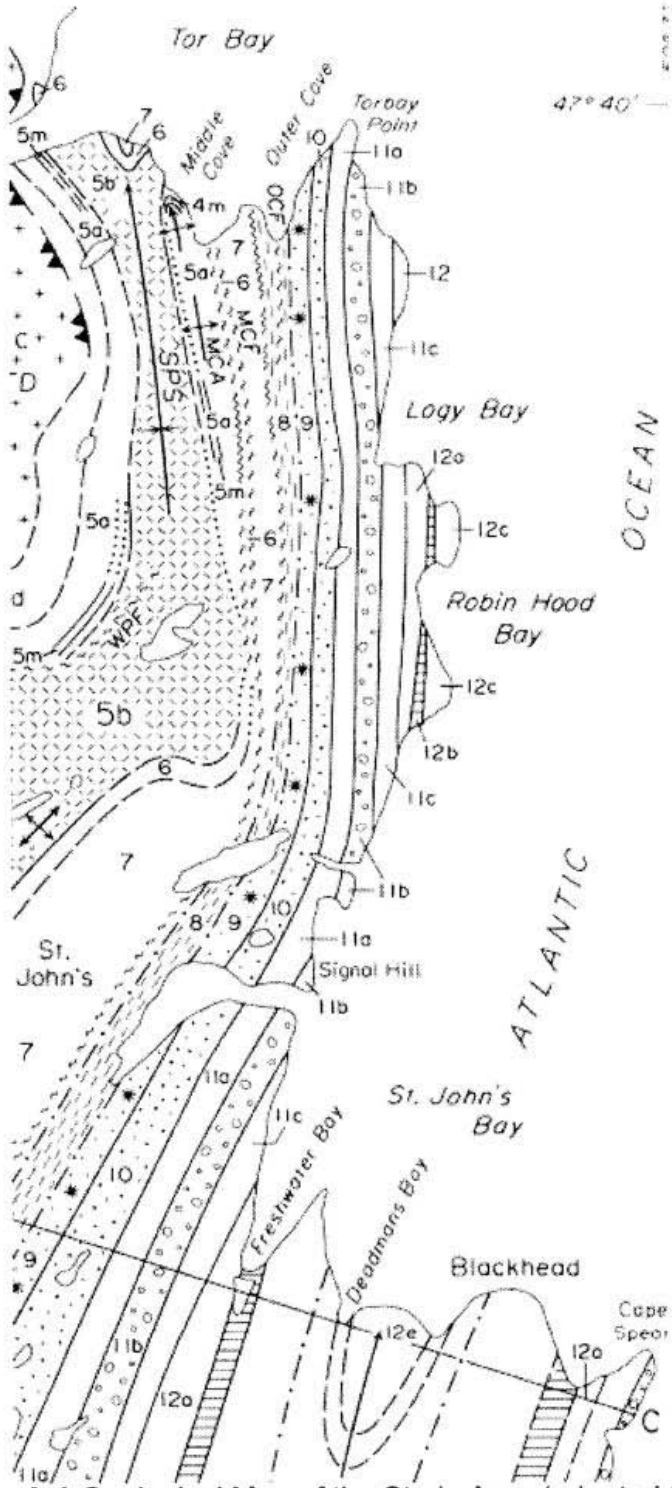

Figure A.1 Geological Map of the Study Area (adapted from King (1990))

APPENDIX B

Materials and Instrument Information

Materials

bags – plastic, able to be closed tightly, Ziploc brand

brushes – nylon bristles

containers – plastic, 120 ml, Corning Snap-Seal Sample Container
– plastic, 1000 ml, screw-top bottle, Nalgene
– plastic, rectangular containers, Rubbermaid (mussel "cages")
– polystyrene, 12 ml capacity, grinding vials, SPEX CertiPrep

gloves – vinyl, non-powdered surgical

grinding balls – methacrylate (plexiglas), 0.32 cm diameter, SPEX CertiPrep

knives – plastic serrated

rake – plastic

syringe – sterile, 5ml, Henke-Sass, Wolf GMBH brand

tubes – plastic, centrifuge tubes, 50ml, VWR (freeze-drying preparation)
– plastic, test tubes, 11.5 ml, Sarstedt (ICPMS analysis)

weighing boats – plastic, Wiley & Sons brand

wipers – low-lint, disposable, Kimwipe
– paper towels

wrapping film – thermoplastic, flexible and self-adhesive, Parafilm M

Solutions and Acids

Buffer Solutions – VWR Cat. No. 34170-130, pH 7.00 ± 0.01 @ 25°C
– VWR Cat. No. 34170-127, pH 4.00 ± 0.01 @ 25°C
(used to calibrate pH meter)

HNO_3 – Nitric Acid, reagent grade, Fisher Scientific (distilled in-house)

Water – distilled and deionized (Deionization process involves a four column Nanopure purification system.)

Instruments

- Portable digital temperature probe used for testing seawater in the field.
  VWR - TRACEABLE® to NIST Calibration
  Cal. Pt.: 10/20/97, Due: 10/20/99, Control Company ISO 9001 Certified
- Portable pH meter used for testing seawater in the field.
  Corning® pH-30 Sensor, Resolution: 0.01, Accuracy: ± 0.2
- GARMIN® GPS 12

S/N: 36136650, Software 4.00, Copyright 1996 – 1998
Garmin Corperation, Olathe, KS, USA

- ORION® Conductivity/Temperature Meter – model 122

	Conductivity	Temperature
Ranges:	0.00 μS/cm to 199.9 mS/cm	-5 to 50°C
Accuracy:	±0.5% of measured value	±0.2°C ± 1 digit (0 to 50°C)

  Temperature Compensation: Automatic, related to 25°C (fixed)
  Temperature Coefficient: 2.1% per °C (fixed)
  Conductivity Cell: 012210
  Cell Constant: 0.609/cm ± 1.5%

- Balances – Sartorius B610 Top-loading (2 decimal places, maximum = 600g)
  – Sartorius R200D Analytical (6-digit capacity, maximum = 200g)
- Calipers – plastic, Vernier style
- Oven – Sybron Thermolyne Oven (drying mussel shells)
- Freeze-Dryer – Lab Con Freeze Dry-5
- Mixer-Mill – SPEX Certiprep
- Milestone Microwave, Ethos Plus system
  – six, 120ml Teflon, vessel carrousel
  – Ni/Cr, Teflon-coated temperature thermocouple.
- Inductively Coupled Plasma Mass Spectrometer – HP4500*plus*

APPENDIX C

Weather Conditions on Sample Collection Days

Table A.1 Weather Conditions on Sample Collection Dates: Information in this table was taken from the Monthly Meteorological Summary for St. John's, NF for June, 1999; Environment Canada Atlantic Region, Atmospheric Environmental Branch. Measurements apply to the following position: Lat. 47° 37 N, Long. 52° 44 W; Altitude: 132 meters. N.E. means no entry or no occurance.

Date Collected	Site #	Temp range (°C)		Rel. Humidity (%)		Rainfall (mm)	Winds		Bright Sunshine (hours)
		Max	Min	Max	Min		Ave speed (km h^{-1})	Prevailing direction	
June 03	1	15.2	4.9	100	87	0.5	11.6	NE	0.4
June 09	2	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	3	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	4	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	5	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	6	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	7	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	8	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	9	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 09	10	12.5	-2.1	94	46	trace	13.5	WNW	11.7
June 11	11	24.2	9.0	73	38	N.E.	28.2	WSW	8.8
June 14	12	26.5	13.9	95	48	N.E.	16.7	W	10.4
June 15	13	23.4	14.5	99	71	N.E.	30.0	WSW	7.8
June 15	14	23.4	14.5	99	71	N.E.	30.0	WSW	7.8
June 18	15	16.3	7.3	100	85	22.1	12.9	E	1.3
June 18	16	16.3	7.3	100	85	22.1	12.9	E	1.3
June 18	17	16.3	7.3	100	85	22.1	12.9	E	1.3

Table A.2 Wind Conditions at Sample Collection Times: Hourly wind speeds (kilometres per hour, km h^{-1}) and directions near times when mussels samples were collected.

Date/	Hourly winds (km h^{-1})										
Time	05:30	06:30	07:30	08:30	09:30	10:30	11:30	12:30	13:30	14:30	15:30
June 03										11 ESE	7 ESE
June 09				19 WNW	15 W	26 WNW	19 NW	17 NNW	15 NE		
June 11						31 WSW	31 WSW	28 WSW			
June 14									7 SE	13 SE	
June 15										37 WSW	37 WSW
June 18	15 W	19 NNW	15 N	13 NNE							

APPENDIX D

Shell Dimensions, Weights & Digestion Programs of Mussel Samples

Table A.3 Measurements of Processed Mussels: Given, for each prepared mussel, are the shell dimensions of length, width and height in millimeters (mm); the total weight of tissue and shell, the wet weight (wet wt.) and dry weight (calc'd dry wt.) of tissue in grams (g); and the percent (%) weight lost after freeze-drying (wt. lost). The microwave digestion program (digestn prog) for those samples that were digested is also given. T1, T2, T3, T4 & T5 represent temperature programs, P1, P2 & P3 represent power programs (Tables 2.5 & 2.6). N.A. indicates that the sample was neither digested nor further used in this study.

	shell dimensions			weights (g)				
sample code	length (± 0.05)	height (± 0.05)	width (± 0.05)	total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.	wt. lost (%)	digestn prog
0101060100	18.20	7.70	6.40	1.84	0.7662	0.0835	89.10	N.A.
0102060100	23.65	9.30	7.95	2.98	1.2813	0.1442	88.75	N.A.
0103060100	22.45	9.40	8.35	3.34	1.5240	0.1794	88.23	N.A.
0104060100	18.55	6.90	6.75	2.19	0.8158	0.1147	85.94	N.A.
0105060100	17.00	7.05	5.75	1.68	0.6901	0.0857	87.58	N.A.
0106060100	17.60	7.55	5.40	1.54	0.7443	0.0894	87.99	N.A.
0107060100	15.65	7.00	5.85	1.40	0.5922	0.0917	84.52	N.A.
0108060100	23.20	9.75	10.00	3.32	1.2138	0.1624	86.62	N.A.
0109060600	35.30	13.20	14.35	8.35	4.4372	0.2752	93.80	N.A.
0110060600	24.55	10.50	8.00	3.09	1.7617	0.1285	92.71	N.A.
0111060600	28.65	12.60	11.45	5.44	3.0494	0.2483	91.86	N.A.
0112060600	20.45	8.70	6.70	2.39	1.0607	0.0836	92.12	N.A.
0113060600	20.90	9.75	5.95	2.17	1.1478	0.0821	92.85	N.A.
0114060600	24.70	11.45	7.35	2.99	1.6493	0.2115	87.18	N.A.
0115060600	32.55	15.85	9.60	6.27	3.2132	0.3511	89.07	N.A.
0116060600	24.90	11.45	8.55	3.90	1.8306	0.1296	92.92	N.A.
0201052400	34.90	8.65	9.85	5.60	3.5162	0.4186	88.10	N.A.
0202052400	40.05	12.10	14.30	9.79	6.1717	0.5278	91.45	N.A.
0203052400	35.60	16.40	9.55	5.65	3.6278	0.3385	90.67	P2
0204052400	32.60	11.80	10.25	5.40	3.2602	0.2181	93.31	T1
0205052400	36.40	16.75	15.55	10.13	6.3279	0.6936	89.04	N.A.
0206052400	35.00	14.95	10.10	6.34	3.8286	0.3964	89.65	P2
0207052400	32.85	16.25	10.15	4.69	2.6256	0.2695	89.74	T1
0208052400	37.60	15.95	13.55	9.11	5.7367	0.5951	89.63	N.A.
0209081800	27.05	10.60	9.65	2.55	0.8606	0.1058	87.71	T2
0210081800	28.65	13.70	9.40	4.70	2.4178	0.2676	88.93	T2
0211081800	26.60	11.15	6.75	2.86	1.6874	0.1919	88.63	T2
0212081800	26.65	12.50	8.60	3.72	2.1491	0.1616	92.48	T2
0301021800	29.05	12.40	9.65	4.32	2.3361	0.3111	86.68	N.A.
0302021800	28.95	12.90	7.55	3.62	2.1928	0.2595	88.17	T2
0303021800	22.30	9.05	5.80	2.05	1.0911	0.1221	88.81	T2
0304021800	22.00	9.05	6.80	2.68	1.3108	0.1705	86.99	T2
0305021800	21.15	8.90	5.45	1.90	1.0468	0.1071	89.77	T2

sample code	shell dimensions length (± 0.05)	height (± 0.05)	width (± 0.05)	weights (g) total (± 0.01)	wet wt. ($\pm 10^{-5}$)	calc'd dry wt.	wt. lost (%)	digestn prog
0306021800	33.10	13.40	12.55	7.09	3.7061	0.3357	90.94	N.A.
0307021800	22.20	10.45	6.60	2.27	1.2712	0.1543	87.86	N.A.
0308021800	20.25	8.80	5.40	1.86	0.8509	0.0541	93.64	N.A.
0309061500	34.35	16.20	11.10	6.77	3.9257	0.4893	87.54	N.A.
0310061500	27.80	11.85	9.50	4.50	2.5738	0.2882	88.80	N.A.
0311061500	24.45	11.25	5.90	2.66	1.4960	0.1752	88.29	T2
0312061500	20.80	8.95	4.90	1.64	1.0696	0.1169	89.07	T2
0313061500	30.60	15.60	10.25	5.40	3.0119	0.2402	92.02	T2
0314061500	21.10	18.55	7.20	2.34	1.1839	0.1583	86.63	T2
0315061500	21.85	9.15	5.50	2.07	1.1813	0.1399	88.16	T2
0316061500	25.05	9.90	7.70	3.08	1.6978	0.2117	87.53	T2
0401021500	40.80	11.00	13.75	9.07	4.8540	0.4658	90.40	N.A.
0402021500	25.80	12.45	10.10	3.70	1.5222	0.2041	86.59	T2
0403021500	28.55	13.05	8.50	4.00	1.7368	0.2227	87.18	T1
0404021500	23.20	9.30	7.40	2.23	0.7099	0.1130	84.08	T2
0405021500	20.85	10.15	6.50	1.75	0.5574	0.0656	88.23	N.A.
0406021500	20.80	9.75	6.95	2.04	0.6865	0.0854	87.56	T2
0407060900	24.40	10.25	7.55	2.92	1.4649	0.1922	86.88	T2
0408060900	23.95	10.35	7.20	2.61	1.4300	0.1583	88.93	T3
0409060900	25.85	9.90	8.20	3.30	1.6681	0.2033	87.81	T2
0410060900	27.60	12.75	8.50	3.66	2.1255	0.2576	87.88	T1
0411060900	25.20	10.60	6.50	2.83	1.3744	0.1554	88.69	P2
0412060900	28.65	12.05	8.70	3.90	2.0515	0.2503	87.80	T1
0413060900	22.10	9.85	5.65	2.11	1.0527	0.0784	92.55	N.A.
0414060900	28.80	10.50	8.20	3.03	1.6348	0.2017	87.66	T2
0504052400	29.00	12.20	8.95	3.55	2.0795	0.2549	87.74	T1
0505052400	31.60	15.85	11.30	6.02	2.9772	0.3273	89.01	P1
0506052400	41.30	19.90	15.60	13.18	6.7295	0.6643	90.13	N.A.
0507052400	39.00	15.30	13.25	6.25	3.5777	0.4543	87.30	P2
0508052400	33.45	11.45	9.30	3.95	1.8456	0.2205	88.05	T1
0509052400	35.90	15.50	11.25	6.73	4.0720	0.2659	93.47	T1
0510052400	40.00	18.20	12.40	8.50	5.5123	0.5896	89.30	N.A.
0511052400	32.80	14.25	8.40	4.94	3.0541	0.2765	90.95	T3
0512081800	30.20	13.30	8.70	4.24	2.5469	0.3175	87.53	P1
0513081800	24.00	10.85	6.25	1.97	1.0847	0.1206	88.88	T2
0514081800	27.30	11.45	7.10	3.16	1.8550	0.2154	88.39	T2
0515081800	28.65	14.50	7.90	4.13	2.5827	0.3725	85.58	P2
0516081800	29.35	13.50	10.35	4.55	2.8589	0.2153	92.47	T2
0601060100	21.35	10.95	11.45	1.80	1.0046	0.0874	91.30	N.A.
0602060100	27.75	12.95	10.35	5.37	2.5637	0.3055	88.08	N.A.
0603060100	30.60	13.00	10.20	3.64	1.6957	0.1543	90.90	N.A.

sample code	shell dimensions			weights (g)			wt. lost (%)	digestn prog
	length (± 0.05)	height (± 0.05)	width (± 0.05)	total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.		
0604060100	24.50	10.50	9.45	2.59	1.3502	0.1427	89.43	N.A.
0605060100	21.90	10.95	7.20	2.25	1.1404	0.1282	88.76	N.A.
0606060100	11.95	7.90	4.50	1.45	0.7786	0.0770	90.11	N.A.
0607060100	30.90	12.85	9.30	4.22	2.6343	0.2077	92.12	N.A.
0701021500	37.00	15.35	11.25	8.20	4.6781	0.4179	91.07	N.A.
0702021500	43.25	19.20	13.30	10.04	6.4217	0.7265	88.69	N.A.
0703021500	40.45	17.35	17.55	10.29	6.2644	0.7665	87.76	N.A.
0704021500	33.65	14.40	11.40	5.09	2.7120	0.3619	86.66	P2
0705021500	33.50	8.20	12.35	5.47	3.0556	0.3842	87.43	P2
0706021500	34.05	15.30	13.70	6.01	3.3022	0.4227	87.20	N.A.
0707021800	36.60	15.10	11.35	6.62	4.3050	0.3840	91.08	N.A.
0708021800	14.80	11.75	8.05	3.07	1.8485	0.2133	88.46	T1
0709021800	18.80	9.25	5.45	1.63	0.9914	0.1061	89.30	N.A.
0710021800	43.10	17.55	15.20	10.91	7.0144	0.8529	87.84	N.A.
0711030200	37.60	15.30	14.60	9.38	5.7654	0.7035	87.80	N.A.
0712030200	47.15	19.70	18.65	15.28	8.3361	0.9758	88.29	N.A.
0713030200	38.55	17.45	17.70	10.90	6.8498	0.7648	88.83	N.A.
0714052400	39.20	19.90	11.95	10.25	6.3580	0.7002	88.99	N.A.
0715052400	30.45	13.00	11.85	5.00	3.3069	0.2639	92.02	T1
0716052400	48.70	18.60	17.40	12.62	7.7280	0.8491	89.01	N.A.
0717060100	36.35	16.25	13.65	8.05	5.0842	0.5440	89.30	N.A.
0718060100	34.80	15.95	12.10	7.40	4.3411	3.4391	20.78	N.A.
0719060100	39.75	11.20	17.05	10.46	6.6188	0.7130	89.23	N.A.
0720060600	33.15	15.25	13.25	6.84	4.0283	0.4405	89.06	N.A.
0721060600	35.20	15.65	13.70	6.58	4.0543	0.5220	87.12	N.A.
0722060600	32.95	13.65	12.65	6.26	4.0699	0.3408	91.63	P2
0723060900	29.65	13.45	10.80	4.30	2.6463	0.3041	88.51	P1
0724060900	25.25	12.20	11.50	4.15	2.8787	0.2846	90.11	T4
0725060900	23.70	12.10	8.95	3.17	1.9540	0.2166	88.92	T1
0726061500	25.85	12.40	8.05	3.23	2.1811	0.2073	90.50	T2
0727061500	30.00	13.10	10.40	4.49	2.8578	0.2839	90.07	T4
0728061500	33.95	10.95	13.85	7.64	4.7930	0.4489	90.63	N.A.
0729081800	25.80	13.30	10.55	3.02	1.6179	0.2043	87.37	T2
0730081800	28.45	11.15	8.95	3.91	2.2427	0.2859	87.25	T4
0731081800	29.40	13.30	8.70	4.84	2.4406	0.3302	86.47	P1
0801021800	29.85	14.40	12.55	5.14	3.2966	0.3458	89.51	N.A.
0802021800	33.10	15.95	12.60	6.37	3.7854	0.4222	88.85	N.A.
0803021800	33.25	15.90	11.00	5.33	3.0154	0.2837	90.59	N.A.
0804021800	28.60	14.40	10.00	4.70	2.7979	0.3130	88.81	N.A.
0805021800	21.00	10.65	6.15	1.94	1.0470	0.1185	88.68	N.A.
0806021800	26.85	12.55	7.90	3.07	1.9422	0.1951	89.95	N.A.

sample code	shell dimensions length (± 0.05)	height (± 0.05)	width (± 0.05)	weights (g) total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.	wt. lost (%)	digestn prog
0807021800	21.95	10.60	6.55	2.16	1.2281	0.1446	88.23	N.A.
0808021800	26.90	11.75	9.90	3.78	2.3969	0.2596	89.17	N.A.
0809060900	31.55	15.20	9.95	4.72	2.8862	0.3555	87.68	N.A.
0810060900	33.60	16.20	14.90	6.65	3.8173	0.4851	87.29	N.A.
0811060900	31.35	14.20	11.60	5.43	3.4476	0.3884	88.73	N.A.
0812060900	21.00	10.45	7.30	2.17	1.2825	0.1362	89.38	N.A.
0813060900	23.55	9.55	9.25	2.87	1.7223	0.2032	88.20	N.A.
0814060900	29.05	13.20	9.35	3.97	2.3802	0.2390	89.96	N.A.
0815060900	32.55	13.80	12.65	6.72	3.8705	0.3248	91.61	N.A.
0816060900	27.65	13.60	10.40	3.82	2.3459	0.3022	87.12	N.A.
0901030200	36.75	14.00	13.30	7.25	4.9797	0.4812	90.34	N.A.
0902030200	25.35	12.80	7.45	3.19	1.9082	0.2094	89.03	N.A.
0903030200	45.55	19.50	12.85	11.79	6.6341	0.7319	88.97	N.A.
0904030200	31.00	12.20	9.65	4.78	2.9359	0.2075	92.93	N.A.
0905030200	30.10	12.55	8.95	4.20	2.6468	0.2455	90.72	N.A.
0906030200	23.70	11.75	9.30	3.03	1.8442	0.1946	89.45	N.A.
0907030200	33.70	13.65	11.00	5.27	3.2309	0.2859	91.15	N.A.
0908030200	38.50	16.30	10.35	6.74	4.2992	0.4741	88.97	N.A.
0909030200	32.55	16.65	10.90	5.93	3.5118	0.4280	87.81	N.A.
1001021500	37.30	15.75	11.00	7.23	4.4778	0.6475	85.54	N.A.
1002021500	44.85	19.25	17.35	12.69	8.0849	0.9147	88.69	N.A.
1003021500	28.65	13.70	11.10	4.43	2.4445	0.2779	88.63	T2
1004021500	28.40	15.10	9.00	3.85	2.0939	0.2803	86.61	T4
1005021500	29.15	11.80	9.90	3.23	1.5393	0.1758	88.58	T2
1006021500	27.10	12.30	9.05	2.96	1.3151	0.1898	85.57	T1
1007021500	24.65	10.35	6.85	2.10	1.0259	0.1844	82.03	T1
1008060900	23.50	10.60	5.75	2.19	1.3605	0.1097	91.94	T2
1009060900	33.00	13.75	11.90	6.26	4.0107	0.3679	90.83	N.A.
1010060900	27.75	12.80	8.70	3.79	2.3303	0.2305	90.11	T1
1011060900	31.25	13.80	10.00	5.45	3.1795	0.4051	87.26	N.A.
1012060900	34.45	15.85	12.15	7.27	4.6087	0.4827	89.53	N.A.
1013060900	27.90	13.20	9.40	4.45	2.6304	0.3265	87.59	P2
1014060900	28.90	14.15	9.20	4.53	2.6821	0.3240	87.92	P1
1015060900	37.70	16.05	13.95	9.02	5.8143	0.5387	90.73	N.A.
1016081800	25.15	13.25	10.45	4.17	2.4480	0.3336	86.37	P2
1017081800	29.90	15.15	10.95	5.24	3.1987	0.2814	91.20	T4
1018081800	23.20	10.55	6.85	1.95	0.9226	0.1270	86.23	T2
1101021800	30.50	13.20	11.15	4.66	1.9592	0.3031	84.53	N.A.
1102021800	24.70	11.15	7.90	2.24	1.1698	0.1693	85.53	T1
1103021800	22.00	9.65	8.10	2.17	1.1799	0.1688	85.69	T1
1104021800	19.95	7.80	6.05	1.48	0.7055	0.1127	84.03	T1

sample code	shell dimensions length (± 0.05)	height (± 0.05)	width (± 0.05)	weights (g) total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.	wt. lost (%)	digestn prog
1105021800	20.40	9.70	6.10	1.61	0.6728	0.1037	84.59	T2
1106021800	20.00	8.05	6.65	1.81	1.0231	0.1090	89.35	T2
1107021800	19.00	7.50	5.70	1.55	0.8591	0.1063	87.63	T2
1108021800	15.35	7.20	4.05	0.98	0.5546	0.0661	88.08	N.A.
1109021800	16.15	6.50	4.35	0.99	0.4559	0.0679	85.11	N.A.
1110061500	19.30	8.90	6.05	1.46	0.6196	0.1184	80.89	N.A.
1111061500	21.25	8.60	6.75	2.11	1.1841	0.1521	87.15	P2
1112061500	20.85	8.35	5.15	1.60	0.9001	0.1108	87.69	N.A.
1113061500	22.85	9.75	8.20	2.45	1.2827	0.1150	91.03	N.A.
1114061500	25.45	10.50	6.75	2.46	15.410	0.1805	98.83	P2
1115061500	27.70	10.70	12.75	3.86	1.7111	0.2888	83.12	N.A.
1116061500	20.75	9.15	7.10	1.96	1.0178	0.1542	84.85	P2
1117061500	23.60	10.15	6.45	2.30	1.3174	0.1604	87.82	P2
1118081800	25.75	11.50	8.65	2.74	1.2348	0.2092	83.06	T2
1119081800	29.10	12.55	9.85	3.38	1.3419	0.2448	81.76	T2
1120081800	29.70	13.25	11.20	5.06	1.9697	0.2940	85.07	N.A.
1121081800	26.85	13.05	9.60	3.80	1.7499	0.1719	90.18	T2
1122081800	25.05	10.60	9.90	2.83	1.2265	0.2306	81.20	T2
1201060100	23.95	9.80	7.75	2.39	1.4057	0.1622	88.46	N.A.
1202060100	26.65	10.85	8.35	2.93	1.4862	0.1539	89.64	N.A.
1203060100	20.75	9.70	6.75	1.98	1.0007	0.0966	90.35	N.A.
1204060100	22.60	9.75	7.00	2.18	1.2412	0.1534	87.64	N.A.
1205060100	24.30	8.75	6.30	2.07	1.2589	0.1383	89.01	N.A.
1206060100	21.50	9.45	6.70	2.21	1.2116	0.1912	84.22	N.A.
1207060100	23.20	9.55	7.40	2.29	1.2536	0.1615	87.12	N.A.
1208060100	20.45	8.25	5.80	1.77	0.8802	0.1276	85.50	N.A.
1301030200	32.10	14.60	10.20	6.23	3.3525	0.3154	90.59	P1
1302030200	30.60	12.45	9.50	5.15	2.5225	0.3403	86.51	P2
1303030200	39.10	15.70	15.00	9.14	4.9513	0.3103	93.73	P1
1304030200	28.45	10.90	8.35	4.36	2.1589	0.2575	88.07	T2
1305030200	24.50	10.85	6.95	2.69	1.5859	0.2243	85.86	T2
1306030200	25.90	11.20	6.80	2.96	1.5071	0.2098	86.08	T2
1307030200	32.00	8.10	10.15	5.53	2.9392	0.3347	88.61	P2
1401030200	28.05	11.90	9.50	3.74	2.2549	0.2950	86.92	P2
1402030200	30.25	13.70	10.95	4.94	2.9839	0.3074	89.70	N.A.
1403030200	29.20	13.30	11.15	3.69	2.1340	0.2284	89.30	T1
1404030200	26.40	12.55	8.50	3.27	2.0861	0.2450	88.26	T1
1405030200	25.30	11.30	6.95	2.52	1.5723	0.1666	89.40	T3
1406030200	26.70	11.45	7.60	2.60	1.4834	0.2086	85.94	T2
1407030200	21.10	9.15	6.30	1.45	0.8286	0.1173	85.84	T2
1408061500	26.00	11.70	9.05	2.78	1.6324	0.1975	87.90	T2

sample code	shell dimensions length (± 0.05)	height (± 0.05)	width (± 0.05)	weights (g) total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.	wt. lost (%)	digestn prog
1409061500	28.85	11.95	9.75	4.05	2.4831	0.3068	87.64	P2
1410061500	27.35	10.90	9.05	2.85	1.6297	0.2183	86.60	T2
1411061500	22.10	9.05	6.25	1.93	1.0650	0.1183	88.89	T2
1412061500	29.75	13.10	8.50	3.71	1.9865	0.2226	88.79	T1
1413061500	33.95	11.50	12.60	5.95	3.4965	0.4757	86.39	N.A.
1414061500	33.10	13.00	12.70	5.39	3.3000	0.3819	88.43	N.A.
1415061500	25.95	10.30	7.65	2.76	1.7648	0.2102	88.09	T2
1501060600	21.85	8.40	8.30	1.87	1.1447	0.1177	89.72	N.A.
1502060600	20.60	8.20	6.95	1.77	1.0453	0.1202	88.50	N.A.
1503060600	15.25	5.45	4.20	0.60	0.2789	0.0460	83.51	N.A.
1504060600	16.45	6.40	4.55	0.95	0.5757	0.0688	88.05	N.A.
1505060600	24.50	9.80	7.35	1.88	1.0084	0.1444	85.68	N.A.
1506060600	21.40	7.00	6.35	1.37	0.7198	0.0999	86.12	N.A.
1507060600	20.85	8.20	5.85	1.65	0.9585	0.1268	86.77	N.A.
1508060600	17.90	7.05	5.25	1.12	0.5873	0.0794	86.48	N.A.
1509081800	24.30	11.45	11.00	1.89	0.9928	0.1630	83.58	N.A.
1510081800	24.75	10.40	9.85	3.28	1.9108	0.2259	88.18	N.A.
1511081800	24.40	9.95	7.40	2.35	1.2793	0.2026	84.16	N.A.
1601021500	40.25	16.75	14.90	10.14	4.2793	0.7451	82.59	N.A.
1602021500	31.00	12.60	10.10	4.66	2.4666	0.3420	86.13	N.A.
1603021500	33.70	14.15	11.35	6.06	3.1017	0.4381	85.88	N.A.
1604021500	27.40	11.85	10.20	4.43	2.0958	0.3058	85.41	P1
1605021500	24.75	9.55	9.05	2.95	1.3069	0.1910	85.39	T2
1606060600	29.00	10.90	9.50	3.78	2.2406	0.2491	88.88	T1
1607060600	19.55	7.70	5.65	1.51	0.7850	0.0703	91.04	N.A.
1608060600	28.10	13.45	9.85	3.99	2.1820	0.2770	87.31	T2
1609060600	31.00	13.40	9.45	3.71	1.9336	0.2472	87.22	T1
1610060600	28.05	9.90	10.65	4.17	2.0883	0.2630	87.41	T3
1611060600	44.55	11.65	17.55	14.92	6.5646	0.8782	86.62	N.A.
1612060600	18.80	8.40	5.75	1.45	0.8879	0.1059	88.07	N.A.
1613060600	22.35	12.95	8.20	2.62	1.3190	0.2076	84.26	T1
1614081800	27.70	11.75	9.40	3.66	1.6582	0.2503	84.91	T2
1615081800	28.40	11.75	9.75	3.54	1.9031	0.2198	88.45	T2
1616081800	25.50	9.90	8.75	3.48	1.4286	0.1868	86.92	T2
1701052400	28.15	11.30	9.00	3.55	2.1076	0.2489	88.19	T1
1702052400	27.55	12.65	9.95	4.14	2.5128	0.2591	89.69	T1
1703052400	31.50	12.60	12.35	4.63	2.6557	0.3718	86.00	N.A.
1704052400	26.75	12.65	8.70	3.55	2.0437	0.2766	86.47	T3
1705052400	26.05	11.95	8.45	3.45	2.1952	0.2594	88.18	T1
1706052400	23.40	10.00	7.70	2.60	1.6928	0.2121	87.47	T2
1707052400	21.30	9.90	7.80	2.49	1.2114	0.1601	86.78	T2

sample code	shell dimensions			weights (g)			wt. lost (%)	digestn prog
	length (± 0.05)	height (± 0.05)	width (± 0.05)	total (± 0.01)	wet wt. (± 10^{-5})	calc'd dry wt.		
1708052400	23.70	8.85	7.75	2.53	1.4895	0.1689	88.66	T2
1709052400	23.75	9.85	9.35	3.34	1.8272	0.2376	87.00	N.A.
1710081800	29.75	12.40	10.90	4.07	2.1700	0.2944	86.43	P1
1711081800	27.05	11.30	8.85	3.30	1.6138	0.2356	85.40	T2
1712081800	31.60	13.95	11.05	3.53	1.7425	0.2975	82.93	P1

APPENDIX E

Reference Material Analytes and their Expected Concentrations,

Composition of Internal Standard Solution
&
Dilution Factor Calculations

Table A.4 Reference Material Analytes and their Expected Concentrations: Concentrations are given in $ng \cdot g^{-1}$. All values are certified for GBW, no information regarding the derivation of uncertainty values was provided. All given values are certified for BCR, the uncertainty is the half-width of the 95% confidence interval of the given certified value. NIST uncertainty values are calculated by multiplying the standard error of the mean of the available values, by the Student's t-value for a 95% confidence interval with the degrees of freedom corresponding to each element. NR = no value referenced for the corresponding element in that standard.

Analyte	GBW 08571	BCR 278R	NIST 2976	NIST 2977
Cr	570 ± 80	780 ± 60	500 ± 160[b]	3910 ± 470[b]
Mn	10200 ± 1800	7690 ± 230	33000 ± 2000[b,c]	23930 ± 290[a]
Fe	221000 ± 14000	NR	171000 ± 4900[a]	274000 ± 18000[b]
Co	940 ± 60	NR	610 ± 20[b,d]	480 ± 130[b]
Ni	1030 ± 130	NR	930 ± 120[b]	6060 ± 240[a]
Cu	7700 ± 900	9450 ± 130	4020 ± 330[a]	9420 ± 520[a]
Zn	138000 ± 9000	83100 ± 1700	137000 ± 1300[a]	135000 ± 5000[b]
As	6100 ± 1100	6070 ± 130	13300 ± 1800[a]	8830 ± 910[b]
Se	3650 ± 170	1840 ± 100	1800 ± 150[a]	1780 ± 160[b]
Sr	12800 ± 1100	NR	93000 ± 2000[b,c]	69300 ± 4200[a]
Cd	4500 ± 500	348 ± 7	820 ± 160[a]	179 ± 3[a]
Pb	1960 ± 90	2000 ± 40	1190 ± 180[a]	2270 ± 130[a]

[a] Certified NIST values.
[b] Reference NIST values.
[c] Reference concentrations of NIST 2976 where material homogeneity and method bias were not considered in the uncertainty value.
[d] Reference concentration of NIST 2976 where material homogeneity and method bias were considered in the uncertainty value.

Table A.5 Weight and Concentration of Each Analyte in the Internal Standard Solution: Suppliers of the certified ICP standard solutions are [a]SCP Science or [b]Aldrich Chemical Company. The concentration provided by the supplier (orig conc) and the concentration in the internal standard solution (aliquot conc) are given in parts per billion ($ng \cdot g^{-1}$). The aliquot weights are given in grams (g).

analyte	lot #	orig conc	aliquot wt. (g)	aliquot conc
Sc[a]	SC0158880	10250	20.32	208
Y[a]	SC0164907	10190	5.05	51.4
Ag[a]	SC9285822	10150	5.01	50.8
Tl[b]	17903TR	10500	7.72	81.0
Bi[a]	SC9285824	10670	0.53	5.7

Dilution Factor Calculations

Let initial wt_1 = dry wt. of mussel sample
final wt_1 = total wt. of mussel solution (i.e. mussel sample dissolved in acid and diluted with water)

initial wt_2 = wt. of aliquot taken from mussel solution
final wt_2 = wt. of aliquot diluted with 0.2N nitric acid

DF = dilution factor

$$DF = \frac{final\ wt_1}{initial\ wt_1} \times \frac{final\ wt_2}{initial\ wt_2}$$

The aliquot weights required to produce dilution factors of 5000 were determined by assuming at target *final* wt_2 value of 10 grams and by rearranging the above formula, to give:

$$initial\ wt_2 = \frac{final\ wt_1}{initial\ wt_1} \times \frac{10\ g}{5000}$$

Example: Using data of sample 1708.

$$initial\ wt_2 = \frac{61.8716}{0.1692} \times \frac{10\ g}{5000} = 0.7313\ g$$

APPENDIX F

Data Reduction Calculations & Examples

The mussel sample used throughout the example calculations was sample 1704, analyzed May 3, 2001.

$$Mean\ cps = \frac{mean\ of\ replicate\ counts}{integration\ time} \qquad \text{(Equation 1)}$$

Example 1: ^{53}Cr

$$Mean\ cps_{Cr} = \frac{\left(\frac{26245 + 27404 + 28710}{3}\right) counts}{15\ s} = 1830\ cps$$

$$Drift\ Corr'n\ Factor = \frac{mean\ cps\ of\ sample}{mean\ cps\ of\ NIST\ 2976_{cycle}} \qquad \text{(Equation 2)}$$

Example 2: ^{45}Sc; 2nd tube cycle

$$Drift\ Corr'n\ Factor_{Sc} = \frac{304609\ cps}{318830\ cps} = 0.955$$

$$Interpolated\ Factor = \left(\frac{amu_{unk} - amu_{LIS}}{amu_{UIS} - amu_{LIS}}\right) \times \left(factor_{UIS} - factor_{LIS}\right) + factor_{LIS} \qquad \text{(Equation 3)}$$

Where, unk = unknown analyte, LIS = Lower Internal Standard, UIS = Upper Internal Standard, amu = atomic mass unit, and factor = the drift /matrix correction factor (calculated by Equation 2).

Example 3: Unknown analyte = ^{53}Cr, lower and upper internal standards are ^{45}Sc and ^{89}Y, respectively.

$$Interpolated\ Corr'n\ Factor_{Cr} = \left(\frac{53 - 45}{89 - 45}\right) \times (0.959 - 0.955) + 0.955 = 0.956$$

$$Drift\ Corrected\ Mean\ cps = \frac{Mean\ cps}{Correction\ Factor} \qquad \text{(Equation 4)}$$

Example 4: ^{53}Cr

$$Drift\ Corrected\ Mean\ cps_{Cr} = \frac{1830\ cps}{0.956} = 1915\ cps$$

$$\underset{Mean\ cps}{Blank\ Sub'd} = Drift\ Corr'd\ Mean\ cps - Drift\ Corr'd\ Mean\ cps_{blank} \qquad \text{(Equation 5)}$$

Example 5: ^{53}Cr, blank from 2nd tube cycle

$$\underset{Mean\ cps_{Cr}}{Blank\ Sub'd} = 1915\ cps - 813\ cps = 1102\ cps$$

$$Sensitivity = \frac{Mean\ cps\ of\ NIST\ 2976_{cycle}}{\left(\frac{given\ NIST\ 2976\ concentration}{dilution\ factor_{tube}}\right)} \qquad \text{(Equation 6)}$$

Where the mean cps in the numerator has been drift-corrected and blank-subtracted.

Example 6: ^{53}Cr; 2nd tube cycle

$$Sensitivity_{Cr} = \frac{969\ cps}{\left(\frac{500\ ppb}{5175}\right)} = 10032\ cps \cdot ppb^{-1}$$

$$Detection\ Limit = \left(\frac{s\tan dard\ deviation\ of\ blanks}{Mean\ Sensitivity}\right) \times 3 \qquad \text{(Equation 7)}$$

Example 7: ^{53}Cr

$$Detection\ Limit_{Cr} = \left(\frac{20\ cps}{9859\ cps \cdot ppb^{-1}}\right) \times 3 = 0.0061\ ppb$$

$$Concentration = \left(\frac{Mean\ cps}{Sensitivity_{cycle}}\right) \times dilution\ factor_{tube} \qquad \text{(Equation 8)}$$

Where the mean cps in the numerator has been drift-corrected and blank-subtracted.

Example 8: ^{53}Cr, 2nd tube cycle

$$Concentration_{Cr} = \left(\frac{1102\ cps}{10032\ cps \cdot ppb^{-1}}\right) \times 5748 = 631\ ppb$$

APPENDIX G

Analyte Concentrations for Mussel Samples

For Tables A.6 (a) - (k):
Subscripts after dates indicate in which of the four May 03 sample sets the sample was run - "A" indicating the first, "B" the second, etc. An asterisk after the sample number indicates that the sample was prepared using a two-step digestion procedure. Rows containing two samples represent composites. The median, mean, interquartile range (IQR), standard deviation (st dev), percent relative IQR (%IQR) and percent relative standard deviation (%RSD), for each analyte, are given below the concentrations. Data from composite samples were entered twice in calculating the statistics (Section 2.5.5).

Table A.6 (a) Analyte Concentrations (ppm) for Samples from Site 2

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
May 03$_{(C)}$	0201	0.730	8.106	141	0.321	0.839	8.055	340	7.524	3.688	66.8	0.867	3.475	3.443	3.380
May 03$_{(D)}$	0202*	0.934	4.711	193	0.351	1.372	8.046	206	6.466	2.963	88.5	0.764	3.169	3.148	3.105
May 03$_{(C)}$	0203*	1.036	4.637	139	0.230	1.313	6.226	59	7.525	3.071	72.7	0.383	1.274	1.274	1.267
Feb. 20	0204	0.893	5.789	196	0.383	1.402	9.424	465	8.833	2.489	109.8	0.788	3.485	3.431	3.400
May 03$_{(C)}$	0206	0.696	3.839	117	0.222	0.751	6.146	143	8.131	3.438	70.3	0.546	1.613	1.621	1.605
Feb. 20	0207*	1.047	6.246	181	0.296	2.124	8.304	149	8.305	2.498	74.8	0.423	1.869	1.839	1.830
May 03$_{(A)}$	0209	1.202	4.215	179	0.233	4.440	9.952	186	5.333	2.566	93.4	0.821	2.726	2.732	2.698
Feb. 20	0210	0.902	4.254	149	0.316	1.179	7.732	101	7.927	2.322	73.4	0.947	3.433	3.414	3.381
Feb. 20	0211	0.950	4.215	116	0.278	1.207	6.885	278	6.239	2.603	64.8	0.417	1.134	1.125	1.122
Feb. 21	0212*	1.558	6.318	192	0.299	1.311	9.280	186	5.822	2.660	100.2	0.737	1.231	1.234	1.227
	median	0.942	4.67	164	0.298	1.312	8.05	186	7.52	2.632	74	0.751	2.30	2.29	2.26
	mean	0.995	5.23	160	0.293	1.594	8.00	211	7.21	2.830	81	0.669	2.34	2.33	2.30
	IQR	0.15	1.9	49	0.075	0.21	1.9	115	1.8	0.53	21	0.36	2.0	2.0	2.0
	st dev	0.25	1.3	31	0.053	1.1	1.3	120	1.2	0.45	15	0.21	1.0	1.0	0.98
	%IQR	16	41	30	25	16	24	62	24	20	29	48	87	87	87
	% RSD	25	26	20	18	67	16	57	16	16	19	31	43	43	43

Table A.6 (b) Analyte Concentrations (ppm) for Samples from Site 3

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 23	0302	0.485	4.897	96	0.175	7.145	5.465	60	4.769	1.532	50.8	0.231	1.312	1.304	1.285
Feb. 23	0304	0.980	5.028	180	0.284	1.986	7.227	73	8.413	2.569	60.5	0.472	1.737	1.723	1.708
Feb. 23	0311	0.939	4.550	193	0.260	1.649	7.083	140	7.780	2.139	65.5	0.407	2.516	2.502	2.455
Feb. 23	0313*	0.655	5.426	115	0.205	9.162	5.507	65	6.826	1.825	83.2	0.403	1.745	1.744	1.727
May 03$_{(A)}$	0314*	0.668	9.256	107	0.206	1.160	7.154	116	6.044	2.174	46.9	0.379	1.553	1.552	1.530
Feb. 23	0316	0.471	3.967	105	0.183	0.658	5.842	164	5.423	1.631	38.5	0.152	1.141	1.149	1.126
May 03$_{(A)}$	C0303 C0312	0.772	4.782	112	0.216	1.506	7.241	134	6.269	2.169	58.5	0.300	2.220	2.203	2.189
May 03$_{(A)}$	C0305 C0315	0.847	5.216	132	0.236	1.942	8.231	127	6.414	2.382	62.7	0.357	2.008	1.992	1.986
	median	0.772	4.963	114	0.216	1.796	7.19	127	6.341	2	60	0.357	1.876	1.868	1.856
	mean	0.744	5.312	128	0.227	2.866	6.92	114	6.462	2	59	0.336	1.846	1.836	1.818
	IQR	0.19	0.43	24	0.031	0.47	1.1	49	0.62	0	10	0.097	0.57	0.56	0.56
	st dev	0.17	1.4	33	0.033	2.9	1.0	35	1.0	0	12	0.093	0.43	0.42	0.42
	%IQR	24	9	21	14	26	15	39	10	20	17	27	30	30	30
	% RSD	23	27	25	15	100	15	31	16	16	20	28	23	23	23

Table A.6 (c) Analyte Concentrations (ppm) for Samples from Site 4

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 23	0402	0.391	3.173	58	0.140	0.737	4.162	102	4.113	1.406	32.0	0.315	0.629	0.629	0.621
Feb. 20	0403	0.734	7.495	121	0.246	2.379	7.997	123	7.245	2.459	62.2	0.670	1.340	1.328	1.319
May $03_{(A)}$	0407	0.816	6.569	92	0.182	1.955	8.830	95	7.118	2.902	46.1	0.535	0.920	0.924	0.921
May $03_{(B)}$	0408	1.052	3.909	111	0.182	2.953	6.410	63	6.398	2.805	59.9	0.580	1.345	1.346	1.348
Feb. 23	0409	0.730	3.701	100	0.212	1.052	5.042	161	5.315	2.225	53.3	0.675	1.488	1.506	1.467
Feb. 20	0410	0.456	5.982	84	0.233	1.016	8.024	162	8.636	2.593	54.7	0.430	0.742	0.739	0.734
May $03_{(C)}$	0411	0.847	7.139	119	0.240	1.387	8.268	199	6.272	4.352	59.9	0.707	1.660	1.652	1.639
Feb. 20	0412*	0.684	4.048	100	0.258	1.820	5.261	89	6.887	2.141	54.0	0.691	1.561	1.560	1.550
Feb. 23	0414	0.849	8.623	124	0.246	1.235	8.016	122	8.067	2.846	56.1	0.514	1.088	1.091	1.079
May $03_{(B)}$	C0404 C0406*	1.088	7.446	144	0.228	1.683	6.750	127	5.640	2.915	82.4	0.688	1.280	1.280	1.287
	median	0.816	6.57	111	0.228	1.683	6.75	123	6.40	2.805	56.1	0.670	1.280	1.280	1.287
	mean	0.794	5.96	109	0.218	1.627	6.86	125	6.48	2.687	58.5	0.590	1.212	1.212	1.205
	IQR	0.24	3.5	27	0.046	0.74	2.2	46	1.5	0.57	7.4	0.16	0.41	0.42	0.41
	st dev	0.23	1.9	26	0.036	0.65	1.5	38	1.3	0.72	14	0.13	0.33	0.33	0.33
	%IQR	30	53	24	20	44	32	37	24	20	13	24	32	33	32
	% RSD	29	32	24	17	40	22	31	20	27	25	22	27	27	27

Table A.6 (d) Analyte Concentrations (ppm) for Samples from Site 5

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 20	0504*	0.363	7.087	88	0.264	0.740	8.297	62	5.815	2.342	62	0.432	1.668	1.648	1.642
May 03$_{(C)}$	0505	0.866	4.731	140	0.234	1.199	7.975	128	5.903	2.469	64	0.787	2.722	2.681	2.678
May 03$_{(D)}$	0507	0.759	8.518	133	0.245	1.163	9.527	216	6.070	3.301	58	0.492	2.544	2.521	2.504
Feb. 20	0508	0.375	4.709	131	0.207	0.892	9.058	187	5.767	2.231	85	0.704	4.198	4.122	4.057
Feb. 20	0509	0.784	3.625	177	0.251	5.714	8.156	137	7.100	2.344	103	0.910	5.533	5.533	5.456
May 03$_{(B)}$	0511	0.813	4.985	114	0.222	1.000	7.286	124	5.641	2.625	83	0.405	1.840	1.839	1.855
May 03$_{(C)}$	0512	0.788	6.812	124	0.253	1.201	9.512	202	5.716	3.158	60	0.431	1.328	1.317	1.311
May 03$_{(B)}$	0513	1.327	5.660	202	0.246	1.816	6.412	161	5.248	2.714	71	0.494	1.890	1.877	1.868
Feb. 23	0514	0.698	6.372	120	0.228	1.381	8.029	143	5.961	2.440	68	0.411	1.449	1.447	1.409
May 03$_{(C)}$	0515	0.595	6.877	91	0.223	1.006	8.621	155	5.181	3.701	46	0.327	1.506	1.501	1.486
Feb. 23	0516	1.116	3.206	102	0.195	0.818	4.768	181	5.368	1.900	84	0.286	13.87	14.12	13.78
	Median	0.784	5.66	124	0.234	1.163	8.16	155	5.767	2.469	68	0.432	1.89	1.88	1.87
	mean	0.771	5.69	129	0.234	1.539	7.97	154	5.797	2.657	71	0.516	3.50	3.51	3.46
	IQR	0.19	2.1	29	0.026	0.34	1.2	52	0.43	0.59	22	0.19	1.9	1.8	1.8
	st dev	0.28	1.6	35	0.021	1.4	1.4	43	0.52	0.53	16	0.20	3.7	3.8	3.7
	%IQR	25	38	23	11	30	15	33	7	24	32	44	99	97	97
	% RSD	37	28	27	9	92	18	28	9	20	23	38	105	107	106

Table A.6 (e) Analyte Concentrations (ppm) for Samples from Site 7

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
May $03_{(D)}$	0704	0.684	4.388	155	0.205	0.939	5.729	104	5.101	2.466	61	0.265	1.693	1.693	1.677
May $03_{(D)}$	0705	0.600	3.936	101	0.221	0.809	6.209	102	4.565	2.615	65	0.312	3.084	3.078	3.063
Feb. 20	0708*	0.606	4.240	99	0.251	1.105	8.640	181	5.665	2.522	63	0.370	1.986	1.974	1.978
Feb. 23	0715	0.586	3.415	130	0.233	1.146	8.189	83	5.939	2.620	85	0.260	1.046	1.044	1.039
May $03_{(C)}$	0722	0.801	6.325	192	0.382	1.250	8.775	290	6.346	3.805	104	0.550	3.285	3.264	3.238
May $03_{(B)}$	0723	0.777	8.336	122	0.239	1.233	7.473	185	4.890	3.217	62	0.265	1.609	1.604	1.597
May $03_{(B)}$	0724	0.784	3.715	112	0.210	1.275	5.013	174	4.796	2.240	63	0.317	1.679	1.682	1.669
Feb. 23	0725	0.644	3.587	103	0.216	0.452	6.748	149	4.981	2.298	63	0.338	1.464	1.468	1.464
Feb. 23	0726	1.196	6.010	233	0.324	1.709	10.02	192	6.561	2.876	89	0.426	1.933	1.932	1.933
May $03_{(B)}$	0727	0.757	5.926	102	0.237	1.048	7.114	150	4.922	3.264	65	0.328	1.527	1.526	1.518
Feb. 23	0729	0.856	3.615	144	0.246	0.988	6.595	105	4.923	2.262	73	0.305	1.433	1.439	1.436
May $03_{(B)}$	0730*	1.193	7.859	145	0.276	1.803	8.988	141	5.718	3.039	58	0.279	1.245	1.242	1.223
May $03_{(C)}$	0731	0.823	8.549	108	0.272	1.382	8.699	151	5.446	3.041	54	0.207	1.223	1.213	1.208
	Median	0.777	4.39	122	0.239	1.146	7.47	150	5.101	2.620	63	0.312	1.609	1.604	1.597
	mean	0.793	5.38	135	0.255	1.165	7.55	154	5.373	2.790	70	0.325	1.785	1.782	1.773
	IQR	0.18	2.6	41	0.051	0.29	2.1	76	0.80	0.57	11	0.07	0.50	0.49	0.50
	st dev	0.20	1.9	40	0.050	0.35	1.5	54	0.63	0.47	15	0.087	0.68	0.67	0.67
	%IQR	23	59	34	21	25	28	50	16	22	17	23	31	31	31
	% RSD	25	36	30	20	30	19	35	12	17	21	27	38	38	38

Table A.6 (f) Analyte Concentrations (ppm) for Samples from Site 10

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 23	1003	0.427	2.979	67	0.160	0.820	3.693	172	3.129	1.417	38	0.233	1.024	1.012	1.002
May $03_{(B)}$	1004	1.081	4.767	138	0.276	1.548	6.542	196	5.188	2.811	59	0.373	1.086	1.080	1.081
Feb. 23	1005	0.808	3.729	114	0.217	1.917	5.894	113	4.693	2.618	76	0.220	1.353	1.350	1.318
Feb. 20	1006	0.652	5.557	118	0.228	1.670	8.781	69	5.023	2.254	62	0.456	0.728	0.730	0.719
Feb. 20	1007	0.538	6.583	86	0.209	0.685	8.503	159	5.096	2.663	43	0.221	0.752	0.755	0.744
Feb. 20	1010	0.662	3.855	88	0.214	1.113	6.523	196	5.282	2.145	70	0.391	1.926	1.925	1.900
May $03_{(C)}$	1013*	0.626	4.527	114	0.186	0.729	6.743	126	5.935	3.066	57	0.287	1.844	1.855	1.830
May $03_{(C)}$	1014	0.652	3.780	110	0.223	0.854	5.476	145	4.710	2.858	54	0.269	1.123	1.124	1.121
May $03_{(C)}$	1016	0.617	3.929	97	0.200	0.804	6.802	151	5.572	2.883	56	0.394	2.528	2.532	2.512
May $03_{(B)}$	1017	0.927	13.19	143	0.277	1.276	8.667	186	5.657	3.165	86	0.354	1.547	1.547	1.540
May $03_{(A)}$	C1008 C1018	1.100	6.589	139	0.238	2.140	7.474	144	5.150	3.098	73	0.418	1.621	1.625	1.605
	Median	0.657	4.65	114	0.220	1.194	6.77	148	5.150	2.835	60	0.364	1.450	1.448	1.429
	mean	0.766	5.51	113	0.222	1.308	6.88	150	5.049	2.673	62	0.336	1.429	1.430	1.415
	IQR	0.34	2.7	43	0.031	0.92	1.4	36	0.41	0.55	17	0.14	0.61	0.62	0.60
	st dev	0.23	2.7	24	0.033	0.56	1.5	37	0.70	0.51	14	0.085	0.53	0.53	0.52
	%IQR	52	59	38	14	77	20	25	8	19	29	39	42	43	42
	% RSD	30	50	22	15	43	21	24	14	19	22	25	37	37	37

Table A.6 (g) Analyte Concentrations (ppm) for Samples from Site 11

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 21	1102	1.011	5.008	160	0.200	1.850	7.037	110	8.854	5.549	48	1.239	0.327	0.321	0.322
Feb. 21	1103	1.165	3.279	127	0.162	1.999	4.152	83	7.198	3.971	49	1.141	0.251	0.245	0.248
Feb. 21	1104	1.005	6.352	100	0.206	3.691	6.878	92	11.61	5.885	46	0.990	0.221	0.217	0.220
Feb. 21	1118*	0.721	3.090	98	0.151	1.227	4.436	65	5.674	2.896	45	1.230	0.263	0.263	0.266
Feb. 21	1119	0.771	6.882	122	0.241	1.891	7.803	115	8.209	4.172	41	1.689	0.614	0.605	0.618
May $03_{(A)}$	1121	0.934	4.161	87	0.184	1.457	6.002	126	7.111	5.132	96	1.165	0.530	0.530	0.530
Feb. 21	1122	0.469	5.311	63	0.159	0.989	6.674	92	9.237	3.197	64	1.219	0.186	0.186	0.189
May $03_{(A)}$	C1105 C1110	0.637	5.911	93	0.194	1.270	6.463	66	10.38	4.568	46	1.133	0.218	0.215	0.218
May $03_{(A)}$	C1106 C1112	0.809	5.603	84	0.175	1.308	5.110	56	7.345	3.768	57	0.891	0.217	0.215	0.214
May $03_{(A)}$	C1107 C1113*	0.775	3.640	79	0.175	1.235	4.101	78	7.558	3.498	78	1.364	0.167	0.170	0.171
May $03_{(C)}$	C1111 C1114	1.026	3.234	107	0.178	1.648	3.514	46	6.127	4.778	58	1.041	0.183	0.186	0.186
May $03_{(C)}$	C1116 C1117	0.737	4.430	105	0.166	1.192	4.961	84	6.596	5.281	56	1.085	0.234	0.235	0.237
	Median	0.775	4.43	98	0.175	1.308	5.11	78	7.34	4.568	56	1.133	0.219	0.215	0.219
	mean	0.826	4.69	100	0.181	1.554	5.37	79	7.88	4.388	58	1.159	0.261	0.259	0.262
	IQR	0.27	2.0	22	0.028	0.41	2.3	27	2.3	1.4	11	0.19	0.065	0.058	0.059
	st dev	0.18	1.2	22	0.021	0.62	1.3	23	1.7	0.88	15	0.19	0.12	0.12	0.12
	%IQR	35	44	23	16	32	45	35	31	30	20	17	30	27	27
	% RSD	22	26	22	12	40	25	29	21	20	25	17	48	47	47

Table A.6 (h) Analyte Concentrations (ppm) for Samples from Site 13

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
May $03_{(C)}$	1301*	0.681	3.032	101	0.220	1.162	5.622	84	7.534	3.100	71	1.140	1.428	1.430	1.423
May $03_{(C)}$	1302	0.530	8.497	104	0.230	1.091	12.58	199	8.240	3.910	54	0.624	1.258	1.263	1.256
May $03_{(B)}$	1303*	0.886	3.928	168	0.246	1.272	5.778	212	7.506	3.048	153	0.880	2.458	2.450	2.433
Feb. 23	1304	0.551	3.950	82	0.147	0.904	4.749	87	4.838	1.898	42	0.402	1.438	1.422	1.412
Feb. 23	1305	0.376	1.738	44	0.099	0.568	3.442	57	4.277	1.329	29	0.239	0.975	0.968	0.958
Feb. 23	1306	0.436	5.917	44	0.156	1.066	4.854	70	4.599	1.771	32	0.352	1.077	1.075	1.064
May $03_{(C)}$	1307*	0.716	5.766	124	0.236	1.148	6.827	230	6.820	3.664	65	0.436	1.104	1.106	1.097
	Median	0.551	3.95	101	0.220	1.091	5.62	87	6.82	3.05	54	0.436	1.258	1.263	1.256
	mean	0.596	4.69	95	0.191	1.030	6.26	134	6.26	2.67	64	0.582	1.391	1.388	1.378
	IQR	0.22	2.4	51	0.081	0.17	1.5	128	2.8	1.5	30	0.37	0.34	0.34	0.34
	st dev	0.18	2.2	44	0.056	0.23	3.0	76	1.6	1.0	42	0.32	0.50	0.50	0.50
	%IQR	39	60	50	37	16	27	147	41	51	56	86	27	27	27
	% RSD	30	47	46	29	23	47	56	26	38	66	55	36	36	36

Table A.6 (i) Analyte Concentrations (ppm) for Samples from Site 14

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
May $03_{(C)}$	1401	0.445	5.110	50	0.157	0.474	6.592	76	6.470	5.947	44.7	1.015	0.419	0.421	0.425
Feb. 21	1403	0.705	4.344	111	0.306	2.637	6.998	91	6.961	4.413	69.7	2.372	1.221	1.231	1.231
Feb. 21	1404*	0.946	3.244	105	0.179	1.368	4.263	61	4.774	3.223	58.5	1.463	0.499	0.499	0.500
May $03_{(B)}$	1405	0.721	3.618	85	0.218	2.673	12.38	201	5.780	5.650	62.1	2.397	0.679	0.666	0.674
Feb. 21	1406	0.902	2.646	93	0.199	1.435	4.396	87	5.080	3.299	51.1	1.452	0.598	0.601	0.609
May $03_{(A)}$	1408*	1.051	3.498	119	0.201	1.959	4.473	80	5.268	4.666	60.8	4.107	0.685	0.688	0.685
May $03_{(D)}$	1409	0.815	5.378	99	0.179	1.412	6.866	87	6.852	4.565	49.0	1.004	0.646	0.650	0.649
Feb. 21	1410	0.777	3.113	86	0.180	1.146	4.517	58	5.159	2.975	46.1	1.609	0.772	0.756	0.768
Feb. 21	1412	0.751	2.888	85	0.194	0.986	3.970	80	5.393	3.582	69.6	1.398	0.450	0.455	0.450
May $03_{(A)}$	1415	0.950	3.365	88	0.164	1.607	3.793	70	5.193	3.828	50.8	1.309	0.452	0.453	0.454
May $03_{(A)}$	C1407 C1111	0.918	4.316	88	0.174	1.398	5.233	81	6.795	4.583	53.4	1.332	0.559	0.563	0.563
	Median	0.859	3.558	88	0.179	1.405	4.88	81	5.586	4.489	53.4	1.425	0.579	0.582	0.586
	mean	0.825	3.820	91	0.194	1.541	5.73	88	5.877	4.276	55.8	1.733	0.628	0.629	0.631
	IQR	0.18	1.1	14	0.025	0.38	2.3	13	1.6	1.1	11	0.47	0.19	0.18	0.19
	st dev	0.16	0.87	17	0.039	0.63	2.4	37	0.83	0.93	8.4	0.87	0.22	0.22	0.22
	%IQR	21	31	16	14	27	47	16	29	24	20	33	33	31	32
	% RSD	19	23	19	20	41	42	42	14	22	15	50	34	34	34

Table A.6 (j) Analyte Concentrations (ppm) for Samples from Site 16

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
May $03_{(B)}$	1604	0.592	2.084	108	0.169	1.274	5.571	83	4.919	2.641	44.7	0.820	1.204	1.198	1.199
Feb. 21	1605*	0.655	1.888	83	0.165	0.978	4.690	111	5.036	2.500	48.7	0.902	0.617	0.611	0.615
Feb. 21	1606	0.481	4.587	92	0.183	0.639	9.339	143	8.332	3.411	66.0	1.168	0.657	0.654	0.657
May $03_{(A)}$	1608	0.565	1.904	80	0.173	1.078	3.429	61	5.390	2.498	48.4	1.267	0.471	0.473	0.472
Feb. 21	1609	0.632	2.691	116	0.168	0.729	5.200	86	5.821	2.735	56.3	1.030	0.375	0.370	0.373
May $03_{(B)}$	1610	0.629	2.164	102	0.226	1.041	4.602	85	6.301	3.249	52.2	1.681	0.660	0.660	0.668
Feb. 21	1613	0.512	6.556	87	0.228	0.764	9.755	170	7.034	3.681	39.9	0.979	1.234	1.225	1.224
May $03_{(B)}$	1614	0.534	4.988	114	0.219	1.294	7.720	92	7.280	4.421	54.1	1.177	0.781	0.775	0.786
Feb. 21	1615	0.758	5.298	127	0.206	1.468	8.057	93	9.863	3.778	60.7	0.967	0.504	0.500	0.502
May $03_{(A)}$	1616	0.739	1.812	105	0.189	1.038	4.485	96	6.547	4.282	58.7	1.519	0.907	0.914	0.913
	Median	0.611	2.43	104	0.186	1.040	5.39	92	6.42	3.330	53.1	1.099	0.658	0.657	0.663
	mean	0.610	3.40	101	0.192	1.030	6.28	102	6.65	3.320	53.0	1.151	0.741	0.738	0.741
	IQR	0.11	2.9	24	0.046	0.41	3.3	22	1.7	1.1	10	0.27	0.34	0.35	0.35
	st dev	0.092	1.8	15	0.025	0.27	2.2	32	1.6	0.72	7.9	0.27	0.29	0.29	0.29
	%IQR	17	121	23	25	39	62	24	27	33	18	25	52	53	53
	% RSD	15	52	15	13	26	36	31	23	22	15	24	40	40	39

Table A.6 (k) Analyte Concentrations (ppm) for Samples from Site 17

Date Analysed	sample	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	^{206}Pb	^{207}Pb	^{208}Pb
Feb. 21	1701	0.577	6.107	79	0.243	0.820	6.637	107	7.207	4.273	57.3	4.316	0.442	0.441	0.435
Feb. 21	1702	0.555	2.593	66	0.161	0.577	4.475	78	6.536	3.127	61.2	1.577	0.431	0.428	0.428
May $03_{(B)}$	1704*	0.595	2.139	63	0.166	1.063	4.045	89	4.982	3.366	49.2	1.436	0.389	0.386	0.391
Feb. 21	1705	0.537	5.105	60	0.207	0.635	6.827	77	7.924	4.449	58.8	1.728	0.464	0.460	0.459
Feb. 23	1706	0.749	2.756	93	0.202	0.714	5.175	105	5.799	3.406	55.7	2.775	0.450	0.443	0.453
May $03_{(A)}$	1707	0.696	6.842	89	0.215	1.159	6.031	106	7.509	4.089	53.5	4.441	0.534	0.533	0.535
May $03_{(A)}$	1708*	0.893	5.862	89	0.205	1.414	5.647	73	8.852	4.640	59.6	2.430	0.440	0.439	0.434
May $03_{(B)}$	1710	0.890	5.738	105	0.221	1.400	7.803	131	7.997	5.517	54.9	1.639	0.432	0.423	0.426
Feb. 23	1711	0.607	1.602	54	0.123	0.650	3.134	63	3.290	1.827	33.5	1.844	0.235	0.236	0.236
May $03_{(B)}$	1712	0.430	5.167	63	0.180	0.627	6.123	92	8.039	5.423	46.6	3.359	0.534	0.518	0.527
	Median	0.601	5.14	73	0.203	0.767	5.84	90	7.36	4.18	55.3	2.14	0.441	0.440	0.435
	mean	0.653	4.39	76	0.192	0.906	5.59	92	6.81	4.01	53.0	2.55	0.435	0.431	0.432
	IQR	0.18	3.2	26	0.044	0.50	1.9	29	2.0	1.2	8.2	1.6	0.029	0.031	0.032
	st dev	0.15	1.9	17	0.035	0.33	1.4	20	1.7	1.1	8.2	1.1	0.083	0.081	0.082
	%IQR	29	62	36	21	65	32	32	27	29	15	73	7	7	7
	% RSD	23	43	23	18	36	25	22	25	28	16	44	19	19	19

APPENDIX H

Detection Limits and Sensitivities of ICPMS Analytical Runs

Table A.7 Detection Limits of ICPMS Analytical Runs: Detection limit concentrations, in parts per billion (ppb), when the samples presented in this study were analysed. The analysis run started on May third was divided into four parts for data reduction. The subscript (A) denotes the first set of samples and reference standards, (B) the second, (C) the third and (D) the fourth.

	Analysis Date						
	Feb. 20	Feb. 21	Feb. 23	May 03 (A)	May 03 (B)	May 03 (C)	May 03 (D)
	Concentrations (ppb)						
^{53}Cr	0.0135	0.0405	0.0066	0.0099	0.0061	0.0173	0.0078
^{55}Mn	0.0041	0.0089	0.0018	0.0072	0.0053	0.0074	0.0049
^{57}Fe	4.3740	6.9718	2.9170	1.9853	1.5195	3.2044	1.4055
^{59}Co	0.0008	0.0026	0.0007	0.0026	0.0008	0.0018	0.0024
^{60}Ni	0.0078	0.0522	0.0262	0.0398	0.0329	0.0065	0.0489
^{65}Cu	0.0264	0.1047	0.0120	0.1059	0.1240	0.0259	0.0444
^{66}Zn	0.2412	0.8252	0.1222	0.3801	0.1753	0.3191	0.3110
^{75}As	0.0033	0.0070	0.0029	0.0067	0.0058	0.0112	0.0023
^{77}Se	0.0550	0.1369	0.0128	0.0945	0.1362	0.2381	0.1232
^{88}Sr	0.0037	0.0191	0.0044	0.0048	0.0059	0.0031	0.0045
^{111}Cd	0.0007	0.0041	0.0010	0.0041	0.0038	0.0075	0.0044
^{206}Pb	0.0158	0.0152	0.0048	0.0048	0.0120	0.0045	0.0034
^{207}Pb	0.0149	0.0147	0.0044	0.0054	0.0110	0.0039	0.0035
^{208}Pb	0.0150	0.0146	0.0046	0.0047	0.0113	0.0037	0.0032

Table A.8 Mean Sensitivity of ICPMS Analytical Runs: Sensitivity, in counts per second (cps) per parts per billion (ppb), when the samples presented in this study were analysed. The analysis run started on May third was divided into four parts for data reduction. The subscript (A) denotes the first set of samples and reference standards, (B) the second, (C) the third and (D) the fourth.

	Analysis Date						
	Feb. 20	Feb. 21	Feb. 23	May 03 $_{(A)}$	May 03 $_{(B)}$	May 03 $_{(C)}$	May 03$_{(D)}$
	Mean Sensitivity (cps ppb^{-1})						
^{53}Cr	2636	2095	1949	11298	9859	9712	8431
^{55}Mn	12084	10571	9830	44479	45464	43977	44587
^{57}Fe	237	209	205	874	894	839	850
^{59}Co	9164	8097	8147	33453	34507	32955	33501
^{60}Ni	770	2123	1771	12862	7634	7684	6530
^{65}Cu	2554	2187	2526	8771	9042	8592	8858
^{66}Zn	1784	1451	1761	5379	5428	5324	5443
^{75}As	1634	1374	1947	4355	4439	4366	4487
^{77}Se	189	153	200	330	278	235	250
^{88}Sr	17809	17282	24940	36901	37532	37671	39581
^{111}Cd	2937	2702	4344	5868	5906	5955	6397
^{206}Pb	15013	13228	11246	15312	14488	15179	16408
^{207}Pb	12673	11074	9485	13625	12949	13506	14619
^{208}Pb	29989	25993	22640	34313	32513	34124	36824

APPENDIX I

Descriptive Statistics of Original and Log-Transformed Data

Clarification of column headers:

Analyte = chemical analyzed

Site = sample site number

N = number of samples analyzed

Mean = arithmetic mean (sum of data values divided by N)

StDev = standard deviation of sample (N-1 divisor)

Variance = variance (StDev squared)

Q1 = first quartile (25^{th} percentile)

Median = median (50^{th} percentile)

Q3 = third quartile (75^{th} percentile)

IQR = interquartile range (Q3 minus Q1)

Min = minimum value

Max = maximum value

Skew = skewness value (level of distribution asymmetry)

Kurtosis = kurtosis value (amount distribution differs from the Normal (or Gaussian) distribution)

Table A.9 Descriptive Statistics of Original Data ($\mu g \cdot g^{-1}$)

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
Cr	2	10	995	247	6.10E+04	852	942	1086	234	696	1558	1.30	2.45
	3	10	744	174	3.02E+04	613	772	870	258	471	980	-0.42	-0.77
	4	11	794	233	5.42E+04	684	816	1052	368	391	1088	-0.36	-0.49
	5	11	771	283	7.99E+04	595	784	866	271	363	1327	0.42	0.51
	7	13	793	200	3.98E+04	625	777	840	215	586	1196	1.27	1.04
	10	12	766	233	5.41E+04	619	657	1043	423	427	1100	0.43	-1.20
	11	17	826	178	3.17E+04	729	775	1008	279	469	1165	0.08	-0.24
	13	7	597	176	3.10E+04	436	551	716	280	376	886	0.48	-0.39
	14	12	825	160	2.56E+04	729	859	939	211	445	1051	-1.08	1.83
	16	10	610	92	8.42E+03	529	611	676	148	481	758	0.35	-0.74
	17	10	653	152	2.32E+04	551	601	784	234	430	893	0.54	-0.55
Mn	2	10	5233	1349	1.82E+06	4215	4674	6264	2049	3839	8106	1.14	0.79
	3	10	5312	1445	2.09E+06	4724	4963	5269	545	3967	9256	2.67	7.97
	4	11	5957	1907	3.64E+06	3909	6569	7446	3537	3173	8623	-0.31	-1.60
	5	11	5689	1609	2.59E+06	4709	5660	6877	2168	3206	8518	0.07	-0.62
	7	13	5377	1912	3.65E+06	3665	4388	7092	3427	3415	8549	0.64	-1.18
	10	12	5507	2728	7.44E+06	3799	4647	6588	2789	2979	13194	2.26	6.13
	11	17	4689	1228	1.51E+06	3460	4430	5757	2298	3090	6882	0.19	-1.30
	13	7	4690	2227	4.96E+06	3032	3950	5917	2885	1738	8497	0.57	0.25
	14	12	3820	868	7.53E+05	3146	3558	4337	1191	2646	5378	0.54	-0.72
	16	10	3397	1773	3.14E+06	1900	2428	5066	3166	1812	6556	0.72	-1.18
	17	10	4391	1909	3.64E+06	2480	5136	5923	3444	1602	6842	-0.34	-1.76
Fe	2	10	160389	31492	9.92E+08	133718	163972	192267	58548	116150	196307	-0.27	-1.69
	3	10	128462	32688	1.07E+09	106603	113623	144333	37730	95506	192781	1.32	0.62
	4	11	108719	25709	6.61E+08	91611	110934	123970	32359	58055	144102	-0.41	0.15
	5	11	129332	34564	1.19E+09	101746	124234	140369	38623	88439	202257	1.03	0.83
	7	13	134518	40249	1.62E+09	102908	122323	149967	47060	99192	233447	1.50	1.99
	10	12	112759	24401	5.95E+08	90529	113539	138658	48128	67341	142850	-0.39	-0.78

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	11	17	99671	22195	4.93E+08	84484	97648	106894	22410	63202	159695	1.13	2.36
	13	7	95230	44159	1.95E+09	43892	101304	123536	79644	43786	168310	0.38	-0.10
	14	12	91380	17212	2.96E+08	85489	87839	103490	18000	49926	119211	-0.86	2.68
	16	10	101460	15494	2.40E+08	85927	103660	114313	28387	80170	126827	0.07	-1.09
	17	10	76159	17158	2.94E+08	62382	72522	90152	27770	53648	104979	0.35	-1.28
Co	2	10	293	53	2.85E+03	232	298	329	96	222	383	0.13	-0.79
	3	10	222	33	1.11E+03	200	216	242	43	175	284	0.50	-0.04
	4	11	218	36	1.28E+03	182	228	246	64	140	258	-1.16	0.76
	5	11	233	21	4.36E+02	222	234	251	29	195	264	-0.45	-0.45
	7	13	255	50	2.50E+03	219	239	274	56	205	382	1.64	2.69
	10	12	222	34	1.12E+03	202	220	238	36	160	277	0.08	0.26
	11	17	181	21	4.55E+02	166	175	194	28	151	241	1.36	2.80
	13	7	191	56	3.17E+03	147	220	236	89	99	246	-0.71	-1.12
	14	12	194	39	1.54E+03	174	180	201	27	157	306	2.41	6.78
	16	10	193	25	6.29E+02	169	186	221	52	165	228	0.40	-1.72
	17	10	192	35	1.22E+03	165	204	217	52	123	243	-0.69	0.35
Ni	2	10	1594	1066	1.14E+06	1094	1312	1583	489	751	4440	2.52	6.91
	3	10	2866	2856	8.16E+06	1420	1796	3276	1856	658	9162	1.81	2.06
	4	11	1627	647	4.18E+05	1052	1683	1955	903	737	2953	0.72	0.37
	5	11	1539	1416	2.01E+06	892	1163	1381	489	740	5714	3.06	9.73
	7	13	1165	355	1.26E+05	964	1146	1329	365	452	1803	0.02	0.73
	10	12	1308	556	3.10E+05	808	1195	1855	1047	685	2140	0.42	-1.49
	11	17	1554	621	3.85E+05	1231	1308	1749	518	989	3691	2.80	9.29
	13	7	1030	232	5.40E+04	904	1091	1162	258	568	1272	-1.53	2.56
	14	12	1541	630	3.97E+05	1202	1405	1871	670	474	2673	0.60	0.51
	16	10	1030	267	7.12E+04	755	1040	1279	524	639	1468	0.08	-0.81
	17	10	906	327	1.07E+05	633	767	1219	586	577	1414	0.68	-1.27
Cu	2	10	8005	1311	1.72E+06	6720	8051	9316	2596	6146	9952	-0.07	-1.05
	3	10	6922	1005	1.01E+06	5758	7191	7489	1730	5465	8231	-0.35	-0.97

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	4	11	6865	1524	2.32E+06	5261	6750	8024	2763	4162	8830	-0.52	-0.93
	5	11	7967	1404	1.97E+06	7286	8156	9058	1772	4768	9527	-1.23	1.69
	7	13	7553	1469	2.16E+06	6402	7473	8737	2335	5013	10021	-0.11	-0.84
	10	12	6881	1462	2.14E+06	6051	6773	8246	2195	3693	8781	-0.66	0.74
	11	17	5369	1332	1.77E+06	4127	5110	6569	2442	3514	7803	0.24	-1.18
	13	7	6264	2975	8.85E+06	4749	5622	6827	2078	3442	12579	1.98	4.51
	14	12	5727	2379	5.66E+06	4296	4875	6798	2501	3793	12384	2.25	5.84
	16	10	6285	2237	5.00E+06	4573	5386	8378	3805	3429	9755	0.49	-1.38
	17	10	5590	1408	1.98E+06	4368	5839	6685	2317	3134	7803	-0.29	-0.38
Zn	2	10	211319	120404	1.45E+10	132328	186203	293324	160996	58675	464779	1.05	1.01
	3	10	114001	35337	1.25E+09	71393	127073	135251	63857	60057	163899	-0.56	-0.99
	4	11	124513	38267	1.46E+09	94554	123117	160675	66121	63346	198724	0.43	0.16
	5	11	154187	43027	1.85E+09	127811	154739	187031	59220	61793	215835	-0.67	0.96
	7	13	154487	54006	2.92E+09	104717	150080	183095	78378	83154	290291	1.16	2.39
	10	12	149945	36563	1.34E+09	130556	147831	182808	52252	68634	195891	-0.78	0.96
	11	17	78941	23146	5.36E+08	60355	77722	91687	31332	46232	125941	0.49	-0.33
	13	7	134275	75604	5.72E+09	70494	87235	212022	141528	56587	230037	0.36	-2.50
	14	12	87861	37018	1.37E+09	71282	80782	87241	15960	58323	200897	2.99	9.79
	16	10	102027	31967	1.02E+09	84429	92301	119354	34925	61356	170102	1.27	1.37
	17	10	92090	20341	4.14E+08	75890	90446	106211	30320	63454	131320	0.52	-0.04
As	2	10	7211	1171	1.37E+06	6135	7525	8175	2040	5333	8833	-0.33	-1.24
	3	10	6462	1048	1.10E+06	5889	6342	7065	1176	4769	8413	0.45	0.54
	4	11	6485	1294	1.67E+06	5640	6398	7245	1605	4113	8636	-0.08	-0.06
	5	11	5797	520	2.71E+05	5368	5767	5961	593	5181	7100	1.52	3.73
	7	13	5373	629	3.95E+05	4906	5101	5829	923	4565	6561	0.69	-0.65
	10	12	5049	704	4.96E+05	4788	5150	5500	711	3129	5935	-1.90	5.23
	11	17	7877	1686	2.84E+06	6596	7345	9046	2450	5674	11607	0.89	0.01
	13	7	6259	1639	2.69E+06	4599	6820	7534	2935	4277	8240	-0.17	-2.32
	14	12	5877	832	6.92E+05	5168	5587	6795	1628	4774	6961	0.21	-1.93

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	16	10	6652	1553	2.41E+06	5302	6424	7543	2242	4919	9863	0.97	0.66
	17	10	6814	1693	2.87E+06	5595	7358	8008	2413	3290	8852	-1.06	0.70
Se	2	10	2830	449	2.02E+05	2496	2632	3163	667	2322	3688	0.97	-0.14
	3	10	2097	336	1.13E+05	1777	2169	2382	606	1532	2569	-0.55	-0.60
	4	11	2687	719	5.17E+05	2225	2805	2915	690	1406	4352	0.75	3.04
	5	11	2657	529	2.79E+05	2342	2469	3158	816	1900	3701	0.78	0.08
	7	13	2790	468	2.19E+05	2382	2620	3129	747	2240	3805	0.74	0.09
	10	12	2673	513	2.63E+05	2345	2835	3090	745	1417	3165	-1.50	2.24
	11	17	4388	878	7.70E+05	3633	4568	5207	1574	2896	5885	-0.01	-1.05
	13	7	2674	1005	1.01E+06	1771	3048	3664	1893	1329	3910	-0.15	-1.90
	14	12	4276	932	8.69E+05	3370	4489	4645	1276	2975	5947	0.36	-0.55
	16	10	3320	718	5.16E+05	2606	3330	3904	1298	2498	4421	0.28	-1.36
	17	10	4012	1118	1.25E+06	3306	4181	4836	1530	1827	5517	-0.50	0.26
Sr	2	10	81490	15450	2.39E+08	69434	74130	95127	25693	64832	109819	0.78	-0.74
	3	10	58771	11970	1.43E+08	49828	59501	63382	13555	38522	83180	0.36	1.50
	4	11	58453	14452	2.09E+08	53333	56108	62166	8833	31951	82432	0.28	0.79
	5	11	71313	16115	2.60E+08	60407	68066	83591	23184	45582	103333	0.50	0.23
	7	13	69681	14539	2.11E+08	61303	63398	78835	17532	54159	104413	1.46	1.50
	10	12	62178	13883	1.93E+08	54513	60209	72991	18478	37963	85727	-0.14	-0.43
	11	17	57552	14509	2.10E+08	46355	55977	61025	14670	40664	95946	1.41	1.89
	13	7	63644	42246	1.78E+09	32115	54426	70733	38618	28716	152569	1.92	4.16
	14	12	55762	8396	7.05E+07	49419	53406	61750	12332	44729	69674	0.54	-0.77
	16	10	52970	7863	6.18E+07	47467	53146	59194	11727	39862	66032	-0.02	-0.40
	17	10	53019	8230	6.77E+07	48511	55292	58992	10480	33511	61192	-1.65	3.02
Cd	2	10	669	208	4.32E+04	422	751	833	411	383	947	-0.32	-1.64
	3	10	336	93	8.69E+03	283	357	404	121	152	472	-0.71	0.45
	4	11	590	129	1.67E+04	514	670	688	174	315	707	-1.13	0.45
	5	11	516	198	3.91E+04	405	432	704	299	286	910	1.02	0.04
	7	13	325	87	7.59E+03	265	312	354	89	207	550	1.51	3.06

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	10	12	336	85	7.28E+03	242	364	412	170	220	456	-0.24	-1.61
	11	17	1159	193	3.71E+04	1041	1133	1235	194	891	1689	1.19	2.59
	13	7	582	323	1.04E+05	352	436	880	528	239	1140	0.96	-0.13
	14	12	1733	869	7.56E+05	1315	1425	2181	867	1004	4107	2.14	5.02
	16	10	1151	275	7.56E+04	951	1099	1330	379	820	1681	0.91	0.07
	17	10	2555	1136	1.29E+06	1624	2137	3598	1975	1436	4441	0.82	-0.85
^{206}Pb	2	10	2341	1011	1.02E+06	1263	2298	3444	2180	1134	3485	0.01	-2.14
	3	10	1846	431	1.86E+05	1493	1877	2220	727	1141	2516	-0.20	-0.68
	4	11	1212	332	1.10E+05	920	1280	1488	568	629	1660	-0.57	-0.65
	5	11	3505	3679	1.35E+07	1506	1890	4198	2692	1328	13872	2.65	7.50
	7	13	1785	677	4.58E+05	1339	1609	1960	621	1046	3285	1.50	1.61
	10	12	1429	526	2.77E+05	1040	1450	1788	749	728	2528	0.56	0.19
	11	17	261	124	1.55E+04	185	218	257	73	167	614	2.23	4.40
	13	7	1391	502	2.52E+05	1077	1258	1438	362	975	2458	2.00	4.44
	14	12	628	216	4.65E+04	464	579	684	220	419	1221	2.05	5.29
	16	10	741	294	8.65E+04	496	659	981	486	375	1234	0.76	-0.47
	17	10	435	84	6.99E+03	421	441	482	61	235	534	-1.46	3.71
^{207}Pb	2	10	2326	998	9.97E+05	1264	2286	3418	2154	1125	3443	0.00	-2.15
	3	10	1836	424	1.80E+05	1490	1868	2203	713	1149	2502	-0.18	-0.67
	4	11	1212	332	1.10E+05	924	1280	1506	582	629	1652	-0.58	-0.66
	5	11	3509	3751	1.41E+07	1501	1877	4122	2621	1317	14117	2.68	7.66
	7	13	1781	672	4.52E+05	1341	1604	1953	613	1044	3264	1.49	1.58
	10	12	1430	529	2.79E+05	1029	1449	1798	769	730	2532	0.56	0.15
	11	17	259	122	1.50E+04	186	215	254	68	170	605	2.25	4.41
	13	7	1388	500	2.50E+05	1075	1263	1430	356	968	2450	2.00	4.46
	14	12	629	216	4.68E+04	466	582	683	217	421	1231	2.14	5.74
	16	10	738	293	8.59E+04	493	657	985	492	370	1225	0.74	-0.53
	17	10	431	81	6.58E+03	414	440	475	61	236	533	-1.48	3.77
^{208}Pb	2	10	2302	981	9.62E+05	1257	2264	3380	2123	1122	3400	0.00	-2.15

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	3	10	1818	422	1.78E+05	1469	1857	2189	720	1126	2455	-0.26	-0.76
	4	11	1205	329	1.08E+05	921	1287	1467	546	621	1639	-0.62	-0.61
	5	11	3459	3653	1.33E+07	1486	1868	4057	2571	1311	13778	2.67	7.60
	7	13	1773	669	4.47E+05	1330	1597	1956	626	1039	3238	1.48	1.52
	10	12	1415	523	2.74E+05	1022	1429	1774	752	719	2512	0.57	0.22
	11	17	261	124	1.55E+04	188	218	257	70	171	618	2.27	4.58
	13	7	1378	498	2.48E+05	1064	1256	1423	359	958	2433	1.98	4.41
	14	12	631	217	4.70E+04	466	586	682	217	425	1231	2.10	5.53
	16	10	741	292	8.53E+04	495	663	985	490	373	1224	0.71	-0.55
	17	10	432	82	6.77E+03	417	435	476	59	236	535	-1.42	3.66

Table A.10 Descriptive Statistics of Log-transformed Data

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
ln(Cr)	2	10	6.877	0.232	0.054	6.744	6.848	6.988	0.244	6.545	7.351	0.62	1.00
	3	10	6.585	0.254	0.064	6.409	6.650	6.768	0.359	6.156	6.888	-0.77	-0.44
	4	11	6.631	0.333	0.111	6.528	6.704	6.958	0.430	5.970	6.992	-0.96	0.38
	5	11	6.581	0.395	0.156	6.388	6.664	6.764	0.376	5.895	7.190	-0.58	0.20
	7	13	6.649	0.232	0.054	6.437	6.656	6.733	0.296	6.373	7.087	0.85	0.24
	10	12	6.599	0.305	0.093	6.429	6.488	6.947	0.518	6.056	7.003	0.00	-0.82
	11	17	6.694	0.226	0.051	6.592	6.653	6.916	0.325	6.150	7.061	-0.54	0.72
	13	7	6.353	0.297	0.088	6.077	6.312	6.573	0.496	5.928	6.786	-0.02	-0.80
	14	12	6.694	0.225	0.051	6.591	6.754	6.845	0.253	6.098	6.958	-1.76	4.13
	16	10	6.403	0.150	0.022	6.270	6.414	6.514	0.244	6.176	6.631	0.10	-0.84
	17	10	6.458	0.231	0.053	6.312	6.399	6.662	0.351	6.063	6.795	0.11	-0.38
ln(Mn)	2	10	8.536	0.240	0.058	8.346	8.450	8.743	0.397	8.253	9.000	0.78	-0.35
	3	10	8.552	0.222	0.049	8.461	8.510	8.569	0.109	8.286	9.133	2.20	6.35
	4	11	8.639	0.354	0.125	8.271	8.790	8.915	0.644	8.062	9.062	-0.55	-1.50
	5	11	8.607	0.299	0.089	8.457	8.641	8.836	0.379	8.073	9.050	-0.45	-0.51

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	7	13	8.534	0.344	0.118	8.207	8.387	8.861	0.654	8.136	9.054	0.39	-1.55
	10	12	8.531	0.399	0.159	8.243	8.444	8.793	0.550	7.999	9.488	1.18	1.95
	11	17	8.420	0.267	0.071	8.148	8.396	8.658	0.511	8.036	8.837	-0.07	-1.47
	13	7	8.347	0.517	0.268	8.017	8.281	8.686	0.669	7.461	9.047	-0.54	0.40
	14	12	8.225	0.224	0.050	8.054	8.177	8.375	0.321	7.881	8.590	0.21	-0.95
	16	10	8.013	0.505	0.255	7.550	7.789	8.530	0.980	7.502	8.788	0.45	-1.84
	17	10	8.280	0.519	0.269	7.812	8.544	8.686	0.874	7.379	8.831	-0.67	-1.24
ln(Fe)	2	10	11.967	0.205	0.042	11.800	12.004	12.167	0.367	11.663	12.187	-0.44	-1.44
	3	10	11.738	0.232	0.054	11.577	11.641	11.871	0.294	11.467	12.169	1.07	0.13
	4	11	11.568	0.263	0.069	11.425	11.617	11.728	0.303	10.969	11.878	-1.09	1.66
	5	11	11.740	0.253	0.064	11.530	11.730	11.852	0.322	11.390	12.217	0.49	-0.03
	7	13	11.774	0.267	0.071	11.542	11.714	11.918	0.376	11.505	12.361	1.05	0.44
	10	12	11.610	0.233	0.054	11.413	11.640	11.840	0.427	11.118	11.870	-0.79	0.09
	11	17	11.488	0.213	0.045	11.344	11.489	11.580	0.236	11.054	11.981	0.33	1.09
	13	7	11.361	0.509	0.259	10.689	11.526	11.724	1.035	10.687	12.034	-0.43	-0.99
	14	12	11.404	0.216	0.047	11.356	11.384	11.547	0.191	10.818	11.689	-1.77	5.16
	16	10	11.517	0.154	0.024	11.361	11.549	11.647	0.286	11.292	11.751	-0.14	-1.23
	17	10	11.218	0.225	0.050	11.041	11.188	11.409	0.368	10.890	11.562	0.11	-1.45
ln(Co)	2	10	5.665	0.184	0.034	5.450	5.695	5.793	0.343	5.405	5.949	-0.14	-1.04
	3	10	5.392	0.148	0.022	5.296	5.375	5.489	0.193	5.167	5.647	0.17	-0.33
	4	11	5.369	0.184	0.034	5.203	5.429	5.506	0.303	4.940	5.555	-1.45	1.82
	5	11	5.450	0.091	0.008	5.404	5.454	5.527	0.123	5.274	5.576	-0.59	-0.24
	7	13	5.525	0.178	0.032	5.39	5.48	5.61	0.23	5.33	5.95	1.27	1.45
	10	12	5.393	0.154	0.024	5.31	5.39	5.47	0.16	5.08	5.62	-0.38	0.67
	11	17	5.193	0.111	0.012	5.11	5.17	5.27	0.16	5.02	5.48	0.99	1.70
	13	7	5.206	0.337	0.114	4.99	5.39	5.46	0.47	4.60	5.51	-1.05	0.16
	14	12	5.251	0.175	0.031	5.16	5.19	5.30	0.14	5.05	5.72	1.95	4.85
	16	10	5.252	0.129	0.017	5.13	5.22	5.40	0.27	5.11	5.43	0.33	-1.77
	17	10	5.242	0.198	0.039	5.10	5.31	5.38	0.28	4.81	5.49	-1.13	1.38

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
ln(Ni)	2	10	7.241	0.496	0.246	6.99	7.18	7.35	0.36	6.62	8.40	1.42	3.01
	3	10	7.632	0.793	0.629	7.25	7.49	7.91	0.66	6.49	9.12	0.97	0.71
	4	11	7.322	0.404	0.163	6.96	7.43	7.58	0.62	6.60	7.99	-0.14	-0.31
	5	11	7.141	0.560	0.313	6.79	7.06	7.23	0.44	6.61	8.65	2.22	5.83
	7	13	7.010	0.350	0.122	6.87	7.04	7.19	0.32	6.11	7.50	-1.25	3.00
	10	12	7.091	0.432	0.187	6.69	7.08	7.52	0.83	6.53	7.67	0.10	-1.74
	11	17	7.297	0.306	0.093	7.12	7.18	7.47	0.35	6.90	8.21	1.79	4.27
	13	7	6.910	0.271	0.074	6.81	7.00	7.06	0.25	6.34	7.15	-1.92	3.92
	14	12	7.255	0.456	0.208	7.088	7.248	7.531	0.442	6.161	7.891	-0.96	2.44
	16	10	6.906	0.270	0.073	6.627	6.947	7.154	0.527	6.460	7.292	-0.35	-0.84
	17	10	6.753	0.347	0.121	6.451	6.640	7.103	0.652	6.358	7.254	0.45	-1.63
ln(Cu)	2	10	8.976	0.168	0.028	8.812	8.994	9.140	0.328	8.724	9.206	-0.30	-1.00
	3	10	8.833	0.150	0.023	8.658	8.881	8.920	0.262	8.606	9.016	-0.54	-0.99
	4	11	8.809	0.242	0.058	8.568	8.817	8.990	0.422	8.334	9.086	-0.83	-0.31
	5	11	8.966	0.201	0.040	8.894	9.006	9.111	0.217	8.470	9.162	-1.69	3.24
	7	13	8.911	0.202	0.041	8.764	8.919	9.076	0.312	8.520	9.212	-0.45	-0.56
	10	12	8.812	0.240	0.058	8.707	8.821	9.016	0.309	8.214	9.080	-1.37	2.76
	11	17	8.559	0.252	0.063	8.325	8.539	8.790	0.465	8.165	8.962	-0.07	-1.22
	13	7	8.666	0.402	0.162	8.466	8.634	8.829	0.363	8.144	9.440	1.12	2.38
	14	12	8.594	0.336	0.113	8.366	8.490	8.824	0.459	8.241	9.424	1.45	2.36
	16	10	8.689	0.355	0.126	8.428	8.591	9.031	0.604	8.140	9.185	0.13	-1.32
	17	10	8.597	0.275	0.076	8.381	8.672	8.807	0.427	8.050	8.962	-0.82	0.28
ln(Zn)	2	10	12.112	0.595	0.354	11.783	12.135	12.586	0.803	10.980	13.049	-0.35	0.39
	3	10	11.592	0.356	0.127	11.175	11.753	11.815	0.640	11.003	12.007	-0.86	-0.95
	4	11	11.688	0.319	0.102	11.457	11.721	11.987	0.530	11.056	12.200	-0.38	0.37
	5	11	11.900	0.341	0.117	11.758	11.949	12.139	0.381	11.032	12.282	-1.70	4.02
	7	13	11.895	0.336	0.113	11.559	11.919	12.118	0.559	11.328	12.579	0.16	0.14
	10	12	11.884	0.289	0.084	11.778	11.904	12.116	0.338	11.137	12.185	-1.62	3.54
	11	17	11.236	0.296	0.088	11.006	11.261	11.427	0.421	10.741	11.744	-0.09	-0.62

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	13	7	11.662	0.588	0.345	11.163	11.376	12.264	1.101	10.944	12.346	0.16	-2.35
	14	12	11.330	0.310	0.096	11.174	11.300	11.377	0.203	10.974	12.211	2.19	6.66
	16	10	11.493	0.290	0.084	11.344	11.433	11.684	0.340	11.024	12.044	0.62	0.68
	17	10	11.409	0.219	0.048	11.237	11.413	11.574	0.337	11.058	11.785	0.08	-0.54
ln(As)	2	10	8.871	0.169	0.028	8.722	8.926	9.009	0.287	8.582	9.086	-0.52	-1.06
	3	10	8.762	0.162	0.026	8.680	8.755	8.861	0.181	8.470	9.038	-0.01	0.56
	4	11	8.758	0.209	0.044	8.638	8.764	8.888	0.250	8.322	9.064	-0.62	0.63
	5	11	8.662	0.086	0.007	8.588	8.660	8.693	0.105	8.553	8.868	1.23	2.85
	7	13	8.583	0.114	0.013	8.498	8.537	8.670	0.172	8.426	8.789	0.55	-0.86
	10	12	8.516	0.163	0.026	8.473	8.547	8.613	0.139	8.048	8.689	-2.39	7.08
	11	17	8.952	0.204	0.042	8.794	8.902	9.110	0.316	8.644	9.359	0.55	-0.47
	13	7	8.711	0.274	0.075	8.434	8.828	8.927	0.493	8.361	9.017	-0.29	-2.35
	14	12	8.670	0.141	0.020	8.550	8.628	8.824	0.274	8.471	8.848	0.13	-1.89
	16	10	8.780	0.223	0.050	8.575	8.768	8.927	0.352	8.501	9.196	0.54	-0.27
	17	10	8.792	0.298	0.089	8.627	8.904	8.988	0.361	8.099	9.088	-1.60	2.53
ln(Se)	2	10	7.937	0.152	0.023	7.822	7.876	8.058	0.236	7.750	8.213	0.78	-0.51
	3	10	7.636	0.169	0.029	7.481	7.682	7.776	0.295	7.334	7.851	-0.79	-0.40
	4	11	7.863	0.276	0.076	7.707	7.939	7.977	0.270	7.249	8.378	-0.58	2.67
	5	11	7.868	0.193	0.037	7.759	7.812	8.058	0.299	7.550	8.216	0.39	-0.15
	7	13	7.921	0.163	0.027	7.775	7.871	8.048	0.273	7.714	8.244	0.44	-0.57
	10	12	7.870	0.229	0.053	7.758	7.950	8.036	0.277	7.257	8.060	-1.98	4.35
	11	17	8.367	0.206	0.043	8.197	8.427	8.558	0.361	7.971	8.680	-0.31	-0.87
	13	7	7.823	0.413	0.171	7.480	8.022	8.206	0.726	7.192	8.271	-0.49	-1.50
	14	12	8.339	0.219	0.048	8.122	8.409	8.444	0.322	7.998	8.691	-0.01	-0.87
	16	10	8.087	0.217	0.047	7.865	8.111	8.268	0.403	7.823	8.394	0.07	-1.58
	17	10	8.255	0.325	0.105	8.104	8.338	8.481	0.378	7.510	8.616	-1.33	2.38
ln(Sr)	2	10	11.293	0.182	0.033	11.148	11.214	11.463	0.314	11.080	11.607	0.60	-1.10
	3	10	10.962	0.208	0.043	10.816	10.994	11.057	0.241	10.559	11.329	-0.37	1.16
	4	11	10.947	0.260	0.067	10.884	10.935	11.038	0.154	10.372	11.320	-0.66	1.87

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	5	11	11.152	0.226	0.051	11.009	11.128	11.334	0.325	10.727	11.546	-0.07	0.16
	7	13	11.134	0.190	0.036	11.024	11.057	11.272	0.249	10.900	11.556	1.18	0.66
	10	12	11.013	0.237	0.056	10.906	11.006	11.198	0.292	10.544	11.359	-0.63	0.03
	11	17	10.934	0.229	0.053	10.744	10.933	11.018	0.274	10.613	11.472	0.92	0.52
	13	7	10.911	0.564	0.318	10.377	10.905	11.167	0.790	10.265	11.935	0.85	0.89
	14	12	10.919	0.148	0.022	10.808	10.886	11.031	0.223	10.708	11.152	0.33	-0.93
	16	10	10.867	0.151	0.023	10.767	10.881	10.989	0.221	10.593	11.098	-0.33	-0.24
	17	10	10.866	0.178	0.032	10.790	10.920	10.985	0.196	10.420	11.022	-2.00	4.50
ln(Cd)	2	10	6.457	0.341	0.116	6.043	6.621	6.724	0.681	5.948	6.853	-0.54	-1.60
	3	10	5.774	0.331	0.110	5.639	5.879	6.002	0.363	5.021	6.158	-1.43	2.20
	4	11	6.354	0.256	0.065	6.242	6.507	6.534	0.292	5.752	6.561	-1.53	2.02
	5	11	6.185	0.359	0.129	6.003	6.068	6.557	0.554	5.656	6.813	0.49	-0.50
	7	13	5.754	0.246	0.061	5.580	5.743	5.869	0.290	5.331	6.310	0.72	1.35
	10	12	5.786	0.270	0.073	5.488	5.896	6.022	0.534	5.395	6.124	-0.43	-1.56
	11	17	7.043	0.158	0.025	6.948	7.032	7.119	0.171	6.793	7.432	0.62	1.23
	13	7	6.239	0.544	0.296	5.865	6.078	6.780	0.915	5.478	7.039	0.24	-0.84
	14	12	7.373	0.401	0.161	7.182	7.262	7.675	0.493	6.912	8.320	1.30	1.72
	16	10	7.024	0.228	0.052	6.857	7.000	7.190	0.333	6.709	7.427	0.54	-0.47
	17	10	7.762	0.424	0.179	7.392	7.658	8.182	0.790	7.270	8.399	0.47	-1.42
ln(^{206}Pb)	2	10	7.664	0.470	0.220	7.141	7.722	8.144	1.003	7.034	8.156	-0.21	-2.00
	3	10	7.494	0.248	0.062	7.306	7.535	7.705	0.399	7.040	7.830	-0.62	-0.33
	4	11	7.060	0.312	0.097	6.824	7.155	7.305	0.481	6.444	7.415	-0.99	0.01
	5	11	7.865	0.715	0.512	7.317	7.544	8.342	1.025	7.192	9.538	1.46	1.91
	7	13	7.432	0.334	0.111	7.198	7.384	7.581	0.383	6.953	8.097	0.90	0.54
	10	12	7.201	0.379	0.144	6.947	7.277	7.488	0.541	6.591	7.835	-0.21	-0.60
	11	17	5.489	0.365	0.133	5.219	5.387	5.550	0.331	5.119	6.420	1.67	2.46
	13	7	7.193	0.307	0.095	6.982	7.137	7.271	0.289	6.882	7.807	1.50	2.69
	14	12	6.400	0.293	0.086	6.138	6.360	6.528	0.390	6.038	7.108	1.18	2.14
	16	10	6.539	0.390	0.152	6.206	6.490	6.881	0.675	5.928	7.118	0.16	-0.72

Analyte	Site	N	Mean	StDev	Variance	Q1	Median	Q3	IQR	Min	Max	Skew	Kurtosis
	17	10	6.054	0.230	0.053	6.040	6.088	6.174	0.135	5.461	6.280	-2.13	5.79
ln(^{207}Pb)	2	10	7.659	0.467	0.218	7.142	7.715	8.137	0.995	7.025	8.144	-0.21	-2.00
	3	10	7.490	0.245	0.060	7.304	7.531	7.698	0.395	7.046	7.825	-0.60	-0.37
	4	11	7.060	0.312	0.097	6.829	7.155	7.317	0.488	6.444	7.410	-1.00	0.03
	5	11	7.860	0.720	0.519	7.314	7.537	8.324	1.010	7.183	9.555	1.49	2.04
	7	13	7.430	0.333	0.111	7.199	7.380	7.577	0.379	6.951	8.091	0.89	0.53
	10	12	7.201	0.380	0.145	6.936	7.276	7.493	0.557	6.593	7.837	-0.19	-0.65
	11	17	5.486	0.360	0.129	5.226	5.372	5.536	0.310	5.137	6.405	1.75	2.62
	13	7	7.191	0.307	0.094	6.980	7.141	7.266	0.286	6.875	7.804	1.49	2.71
	14	12	6.401	0.290	0.084	6.143	6.366	6.525	0.382	6.044	7.115	1.26	2.49
	16	10	6.535	0.392	0.153	6.200	6.489	6.886	0.686	5.914	7.111	0.13	-0.72
	17	10	6.045	0.225	0.051	6.025	6.087	6.160	0.135	5.463	6.279	-2.14	5.83
ln(^{208}Pb)	2	10	7.650	0.463	0.214	7.136	7.706	8.125	0.989	7.023	8.131	-0.21	-2.00
	3	10	7.479	0.248	0.062	7.290	7.524	7.691	0.402	7.026	7.806	-0.65	-0.37
	4	11	7.054	0.313	0.098	6.825	7.160	7.291	0.466	6.431	7.402	-1.03	0.09
	5	11	7.851	0.716	0.512	7.304	7.532	8.308	1.004	7.179	9.531	1.48	2.00
	7	13	7.425	0.334	0.111	7.189	7.376	7.579	0.390	6.946	8.083	0.87	0.48
	10	12	7.190	0.381	0.145	6.929	7.262	7.479	0.550	6.577	7.829	-0.20	-0.59
	11	17	5.494	0.360	0.130	5.235	5.387	5.549	0.314	5.144	6.426	1.75	2.68
	13	7	7.183	0.308	0.095	6.970	7.136	7.261	0.291	6.865	7.797	1.47	2.64
	14	12	6.404	0.291	0.085	6.143	6.373	6.526	0.383	6.051	7.115	1.22	2.30
	16	10	6.539	0.390	0.152	6.204	6.496	6.884	0.681	5.920	7.110	0.11	-0.74
	17	10	6.049	0.226	0.051	6.032	6.075	6.163	0.132	5.465	6.282	-2.10	5.74

APPENDIX J

Boxplots - Log-Transformed Concentration versus Sample Site

For Figure A.2 (a) – (l):
Outliers are identified by an asterisk and labeled by the sample number, where the first two digits and the last two digits represent the site number and sample number, respectively. Solid circles show mean values and the horizontal line within a box indicates a median value. The box length represents the interquartile range and vertical lines beyond the box ends indicate that there is at least one data point outside the interquartile range, but within the 95% confidence interval.

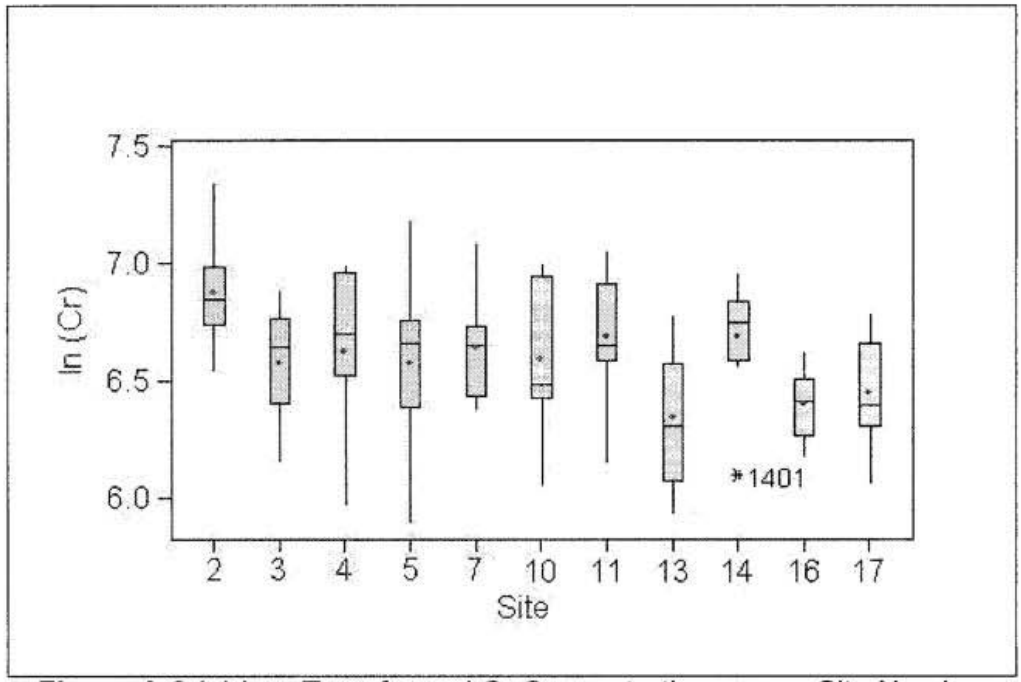

Figure A.2 (a) Log-Transformed Cr Concentration versus Site Number

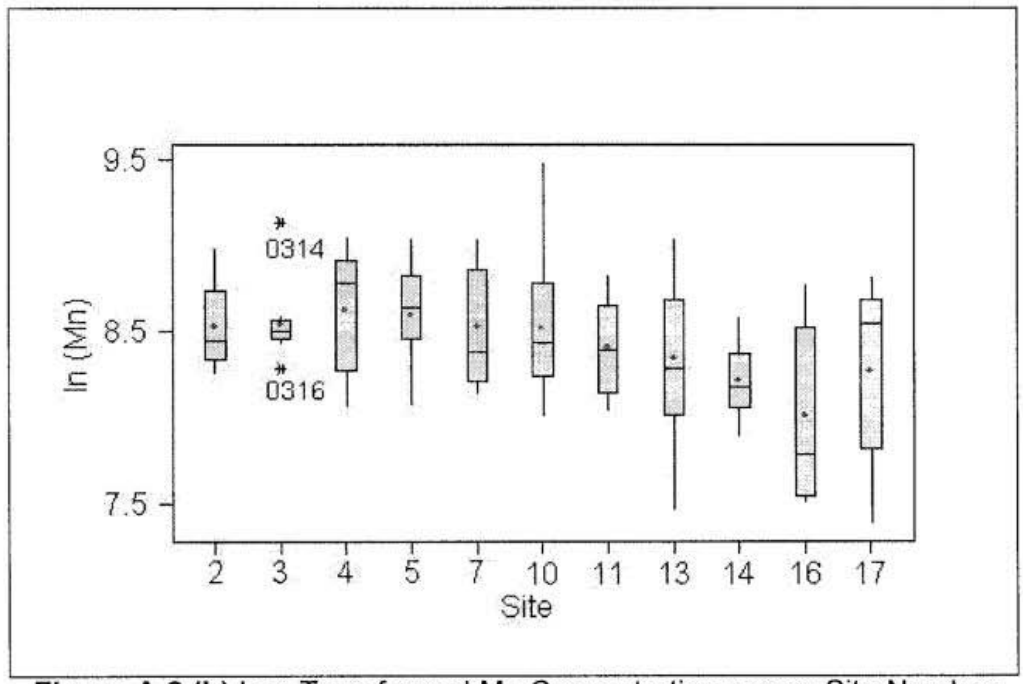

Figure A.2 (b) Log-Transformed Mn Concentration versus Site Number

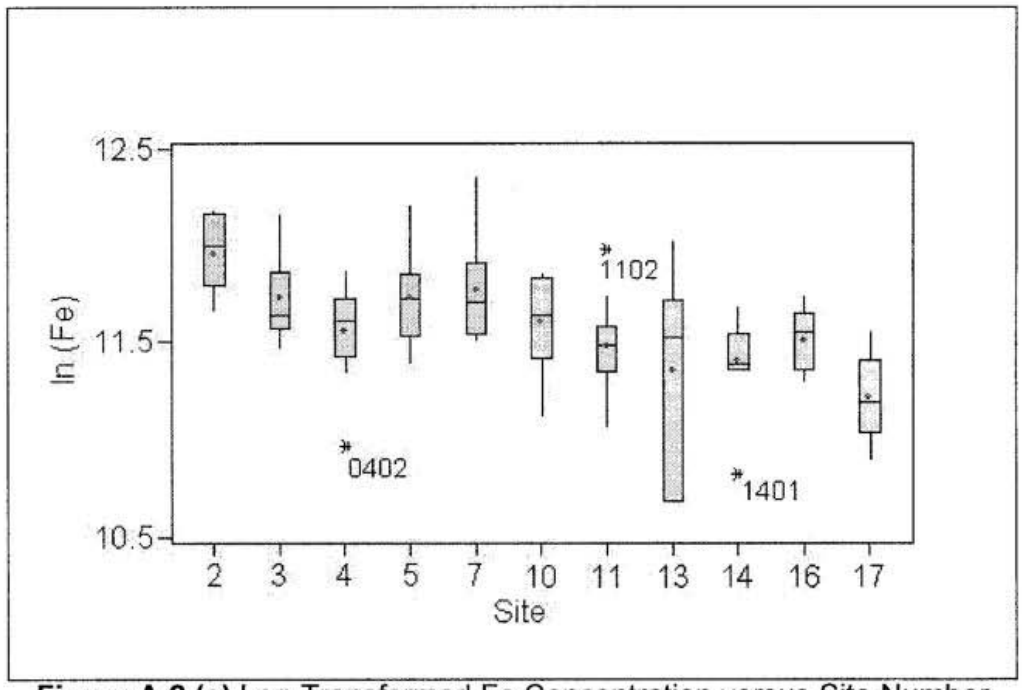

Figure A.2 (c) Log-Transformed Fe Concentration versus Site Number

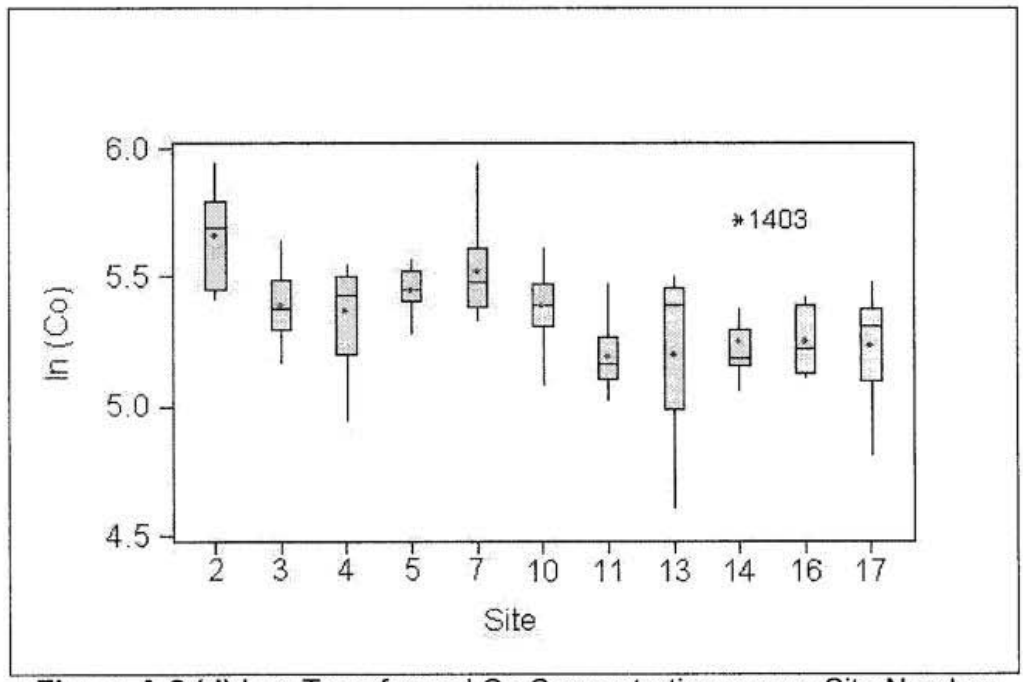

Figure A.2 (d) Log-Transformed Co Concentration versus Site Number

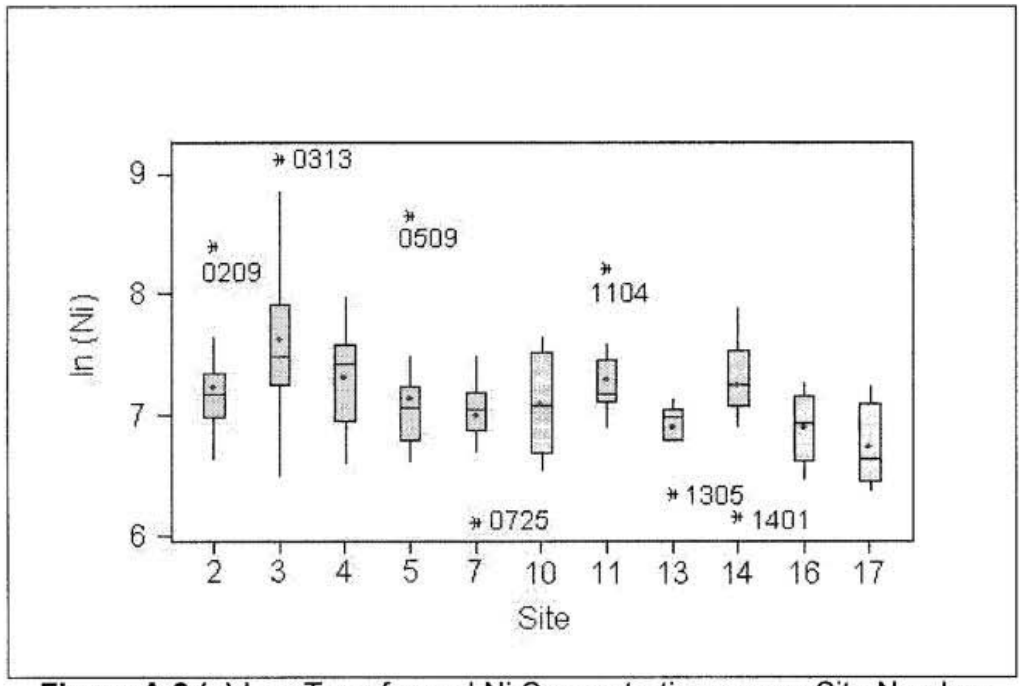

Figure A.2 (e) Log-Transformed Ni Concentration versus Site Number

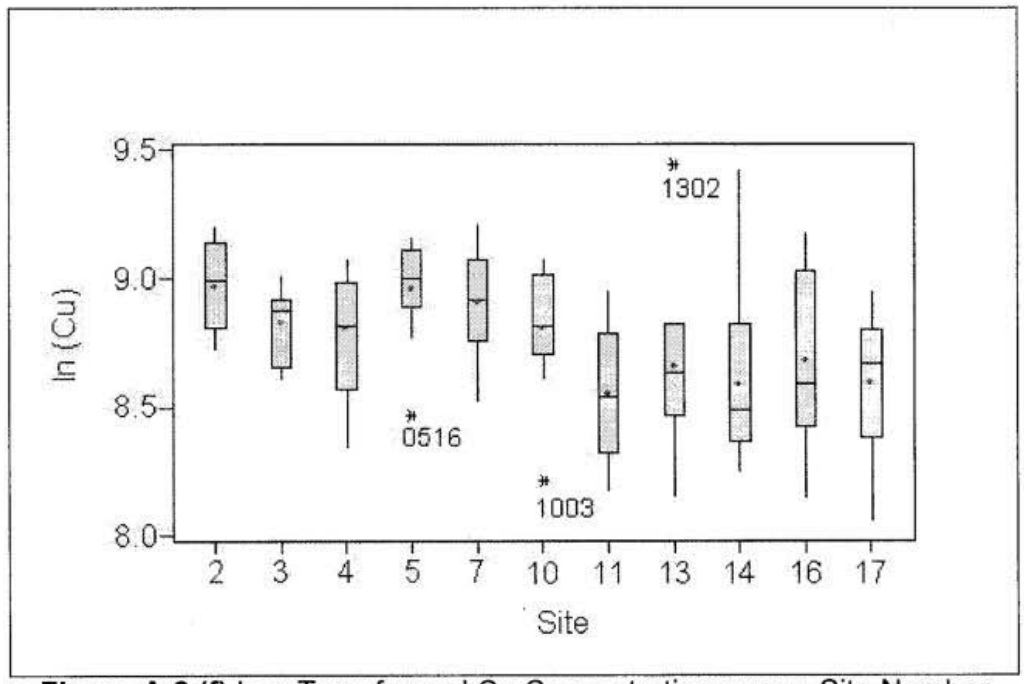

Figure A.2 (f) Log-Transformed Cu Concentration versus Site Number

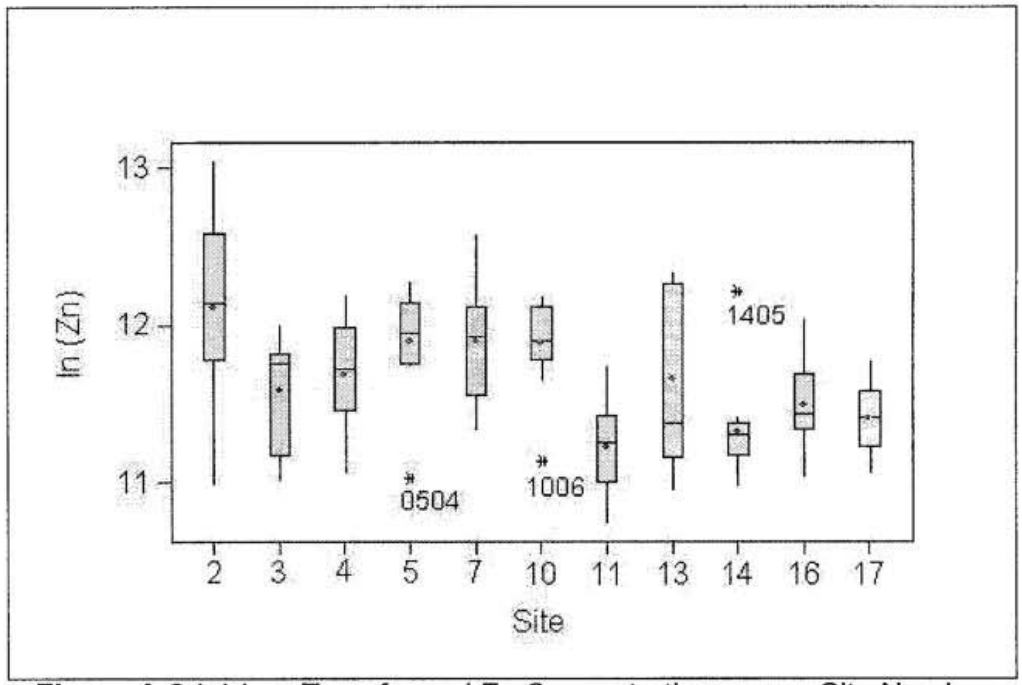

Figure A.2 (g) Log-Transformed Zn Concentration versus Site Number

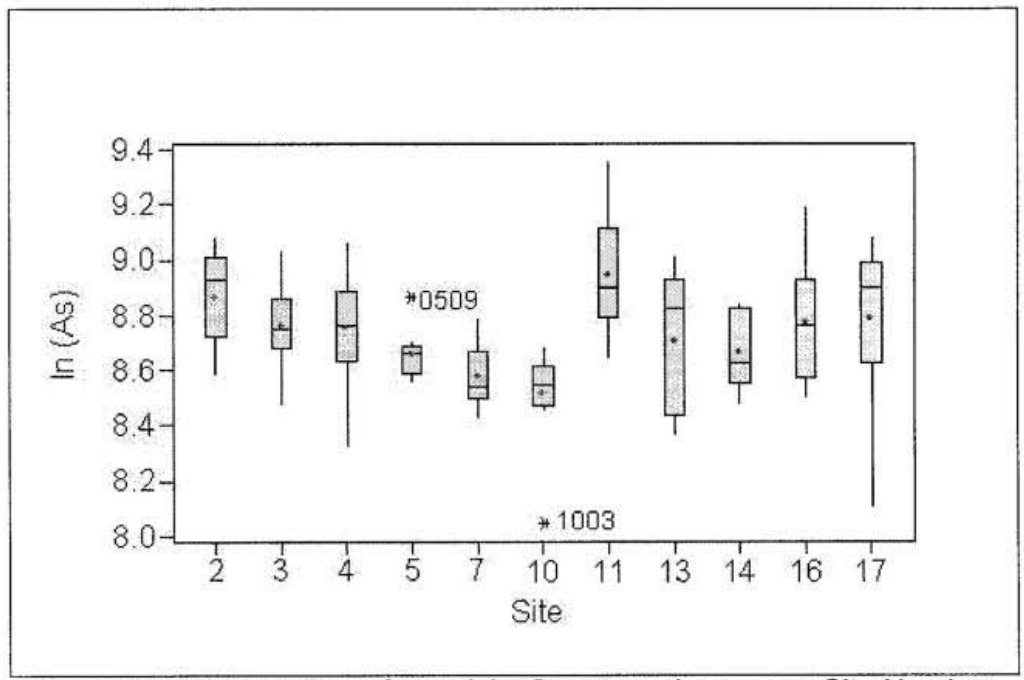

Figure A.2 (h) Log-Transformed As Concentration versus Site Number

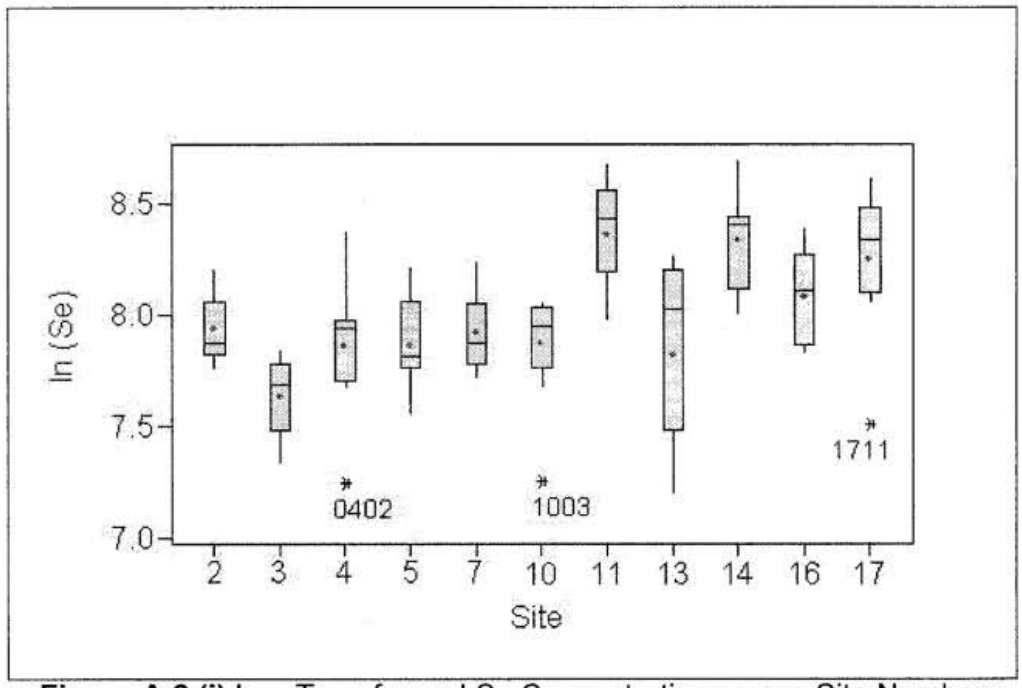

Figure A.2 (i) Log-Transformed Se Concentration versus Site Number

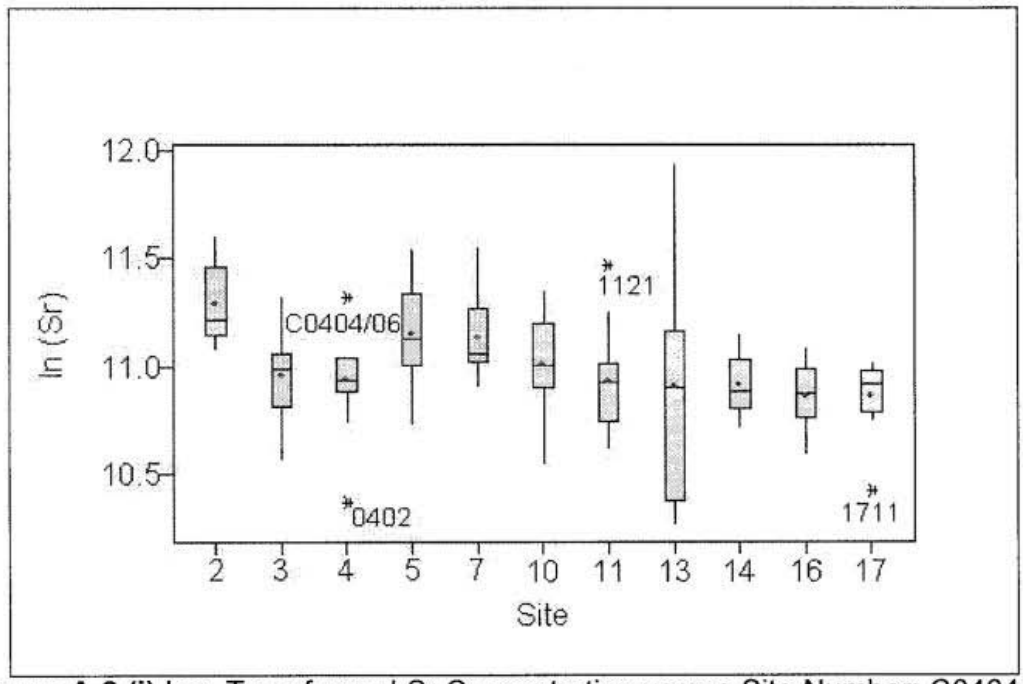

Figure A.2 (j) Log-Transformed Sr Concentration versus Site Number: C0404/06 represents a composite solution of samples 4 and 6 from site 4.

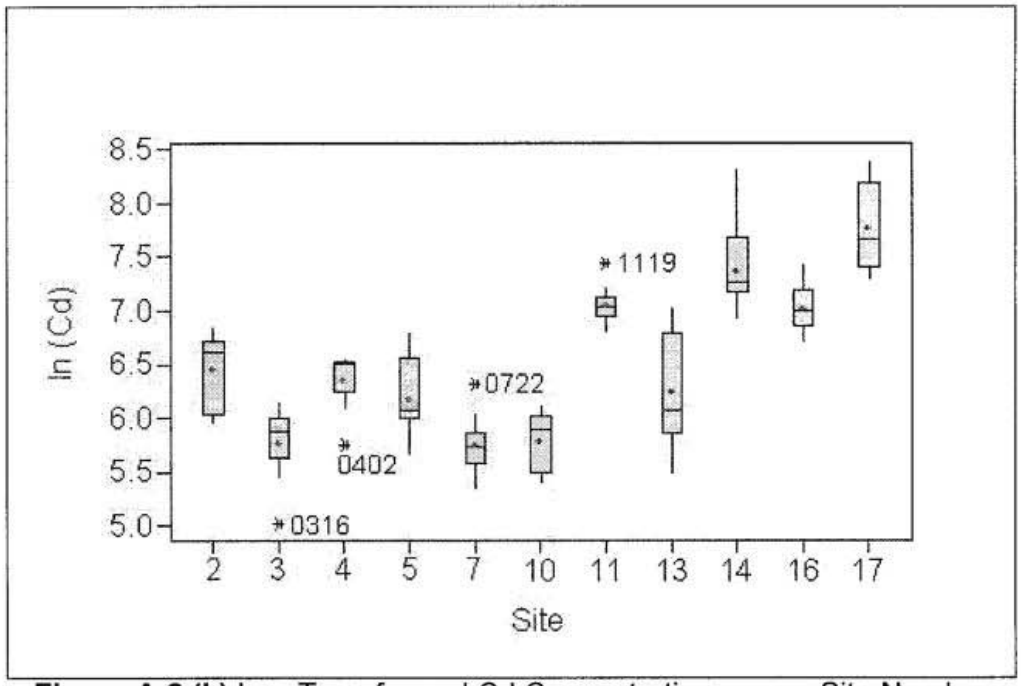

Figure A.2 (k) Log-Transformed Cd Concentration versus Site Number

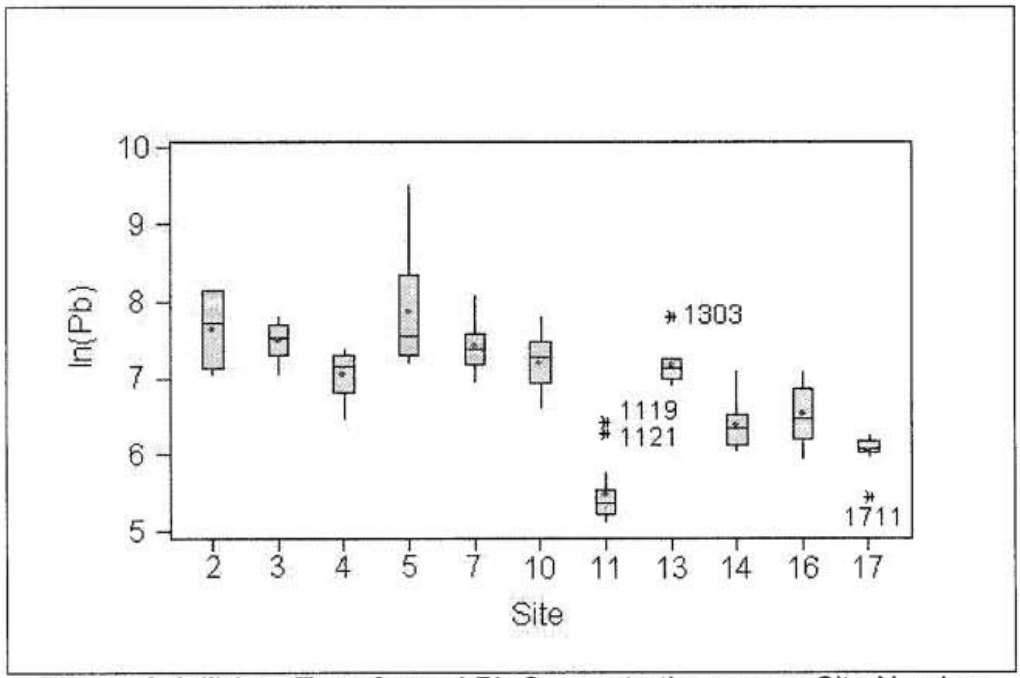

Figure A.2 (l) Log-Transformed Pb Concentration versus Site Number

APPENDIX K

Boxplots, Residuals versus Fitted Value & Residuals versus Order Plots, for All Analytes, from the ANOVA on the Ranks of the Inner & Outer Regions

Boxplots:
Outliers are identified by an asterisk and labelled by the sample number, where the first two digits and the last two digits represent the site number and sample number, respectively. Solid circles show mean values and the horizontal line within a box indicates a median value. The box length represents the interquartile range and vertical lines beyond the box ends indicate that there is at least one data point outside the interquartile range, but within the 95% confidence interval.

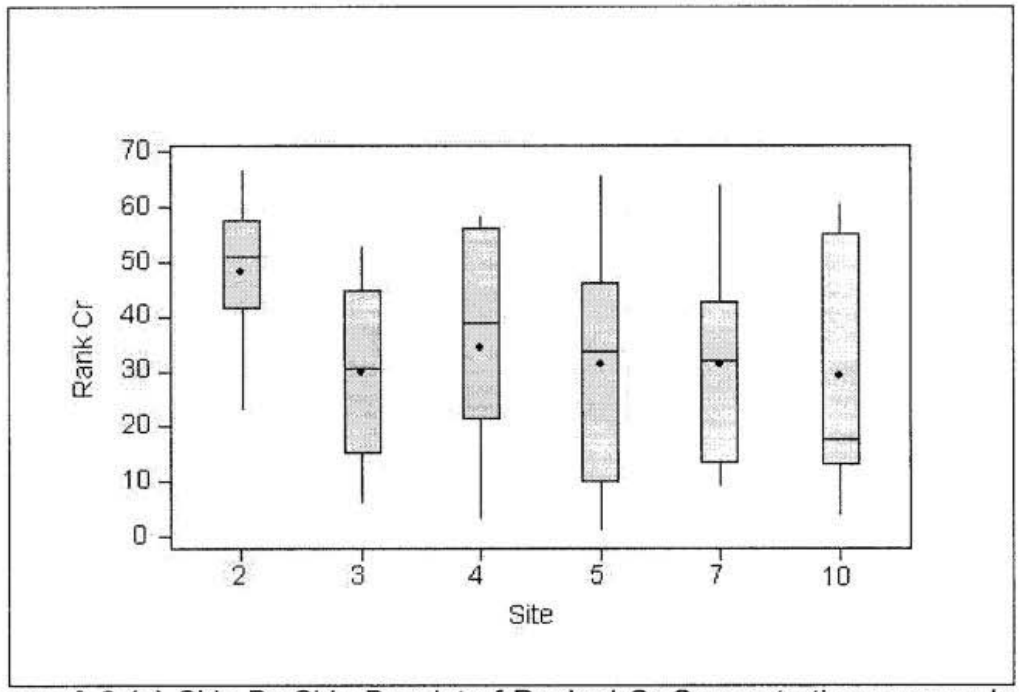

Figure A.3 (a) Side-By-Side Boxplot of Ranked Cr Concentrations versus Inner Region Site Numbers

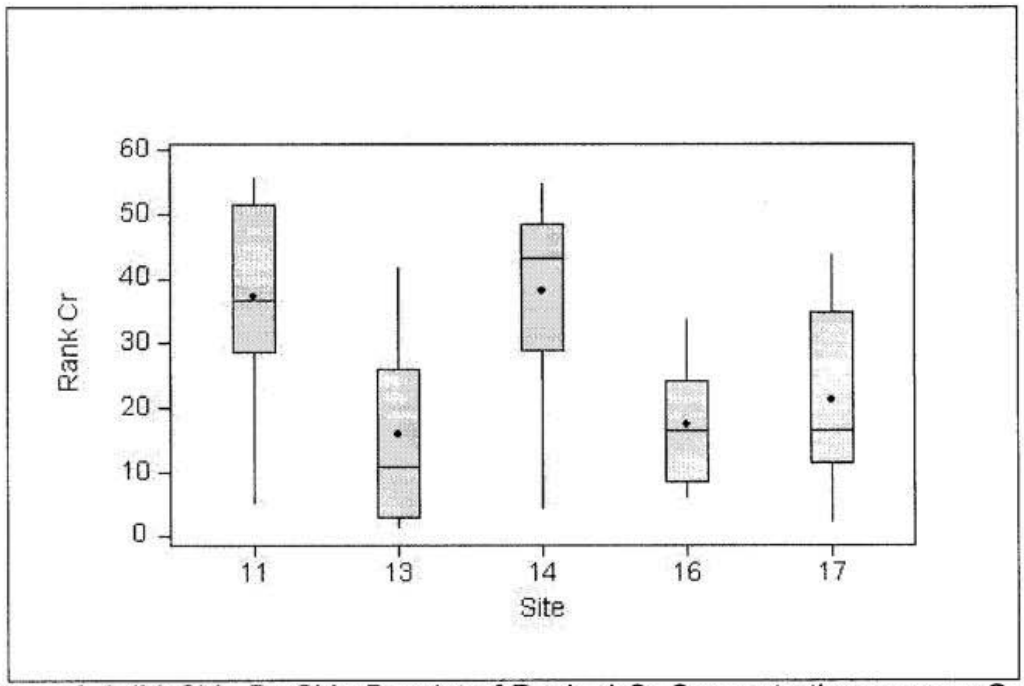

Figure A.3 (b) Side-By-Side Boxplot of Ranked Cr Concentrations versus Outer Region Site Numbers

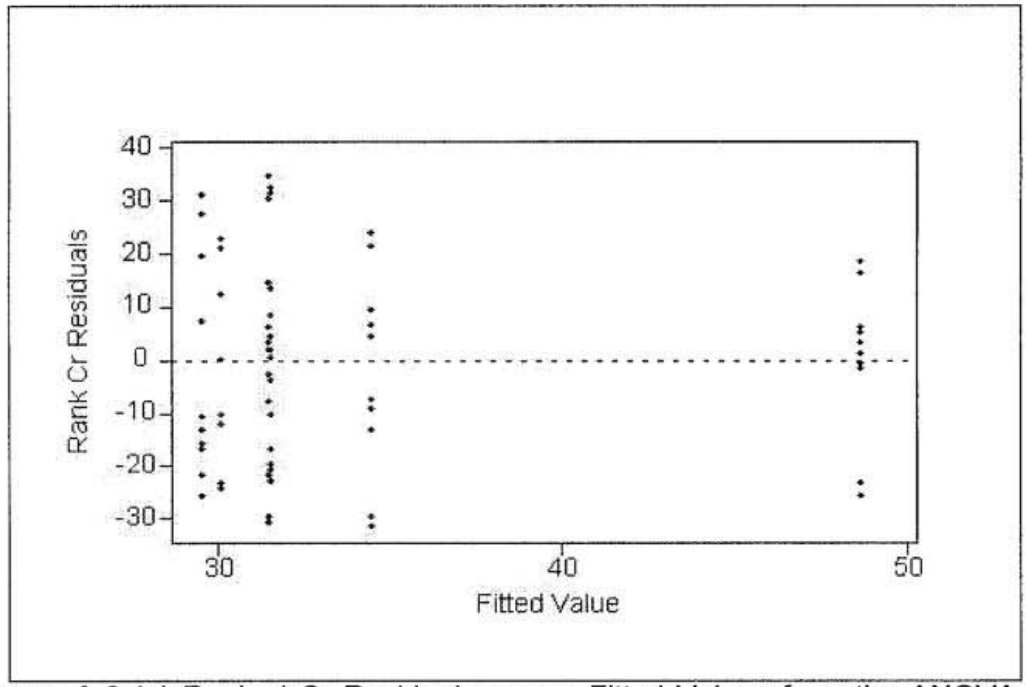

Figure A.3 (c) Ranked Cr Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

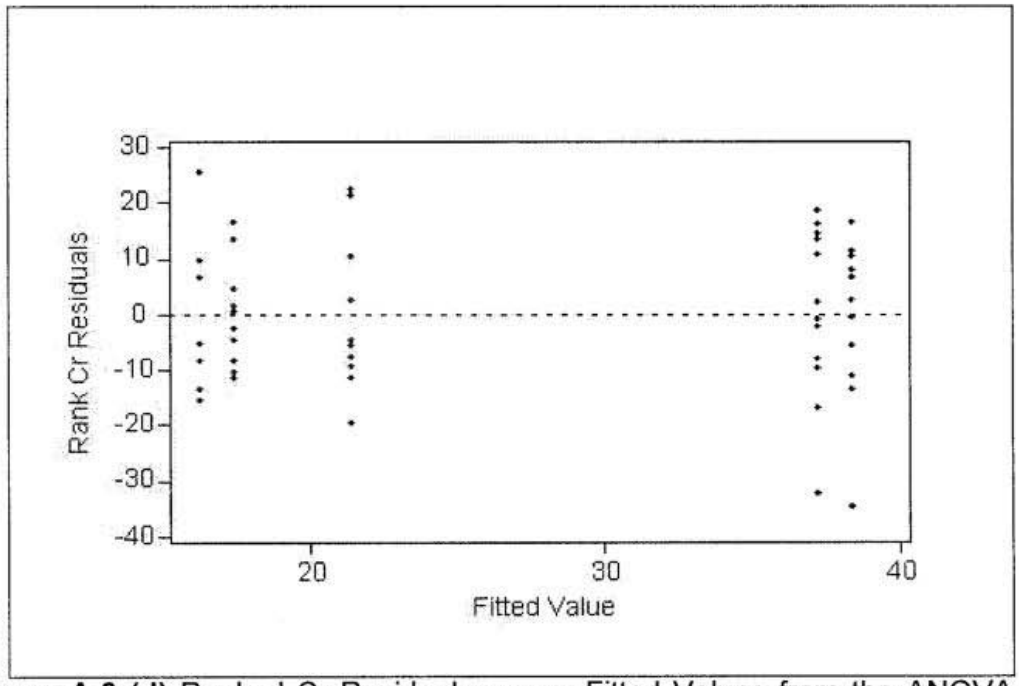

Figure A.3 (d) Ranked Cr Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

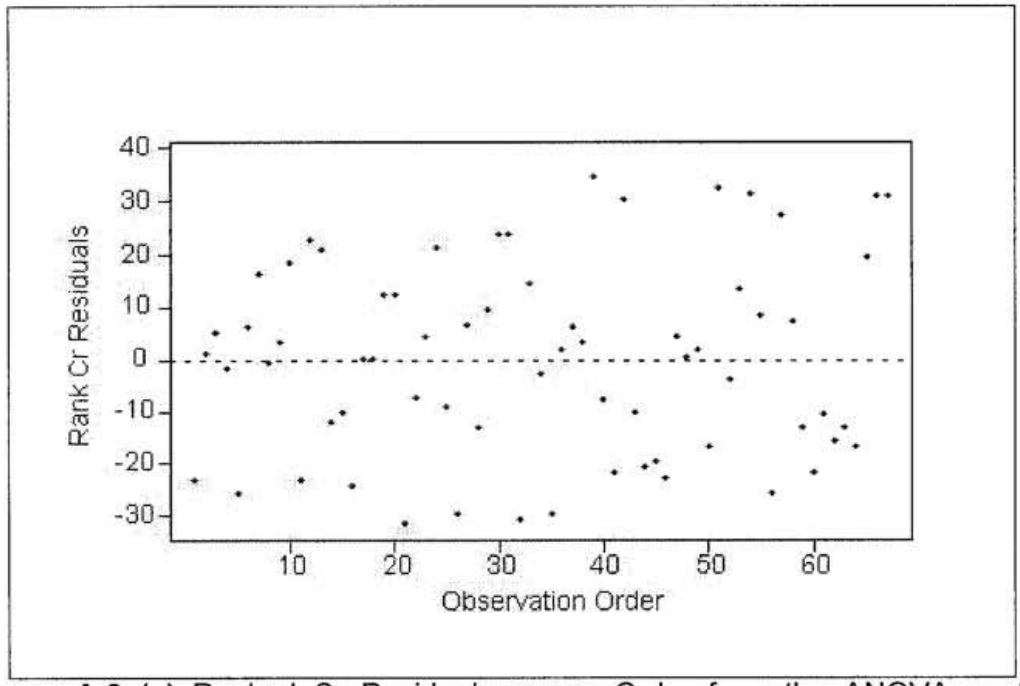

Figure A.3 (e) Ranked Cr Residuals versus Order from the ANOVA on the Ranks of the Inner Region

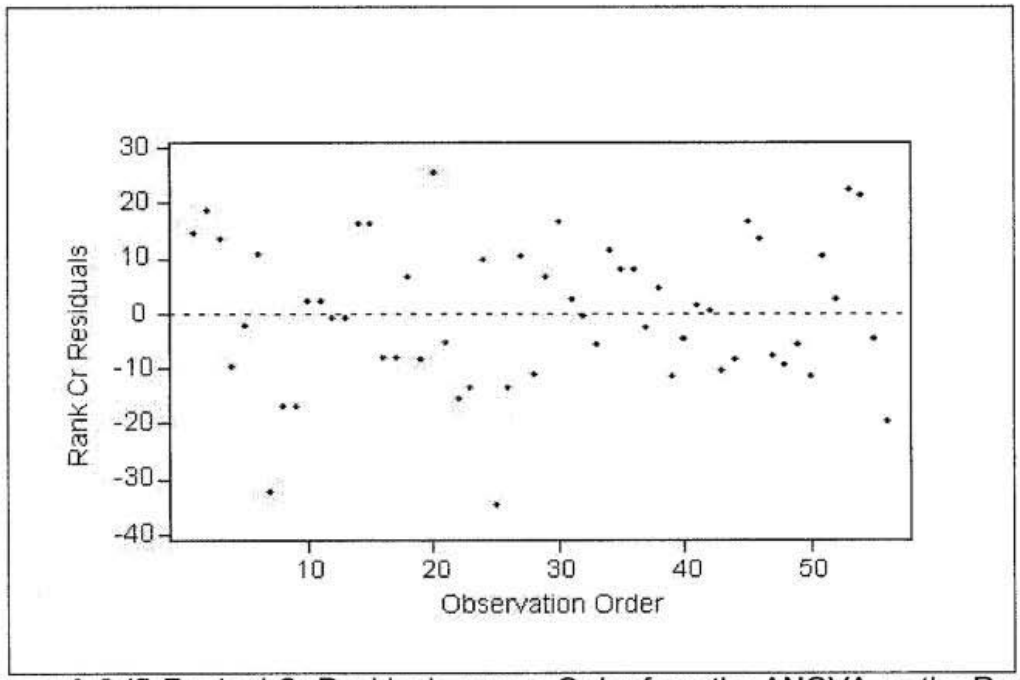

Figure A.3 (f) Ranked Cr Residuals versus Order from the ANOVA on the Ranks of the Outer Region

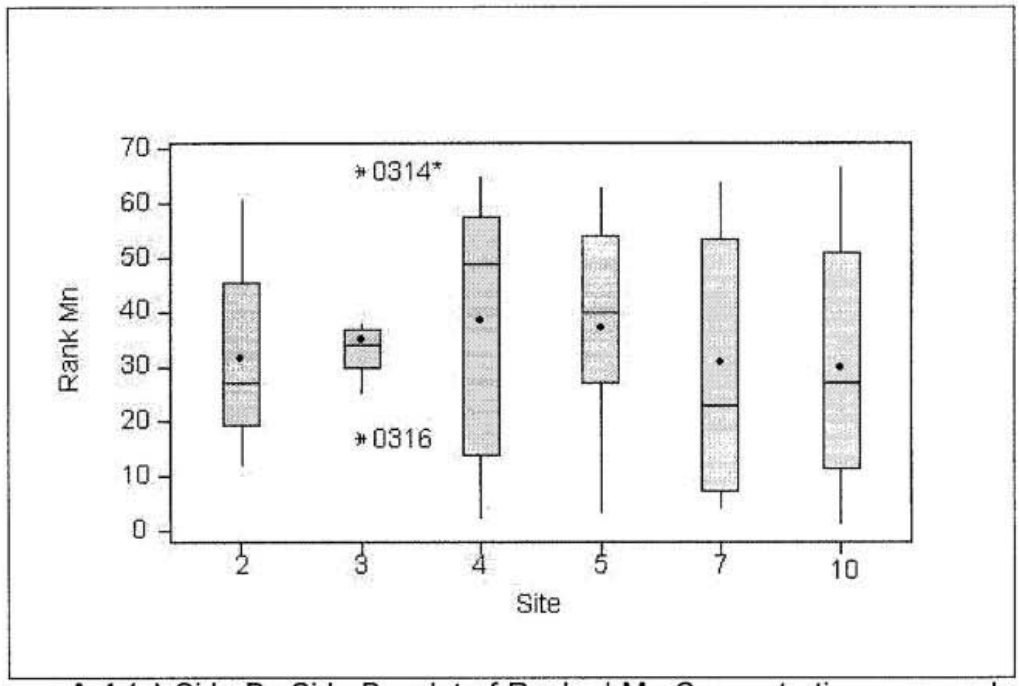

Figure A.4 (a) Side-By-Side Boxplot of Ranked Mn Concentrations versus Inner Region Site Numbers

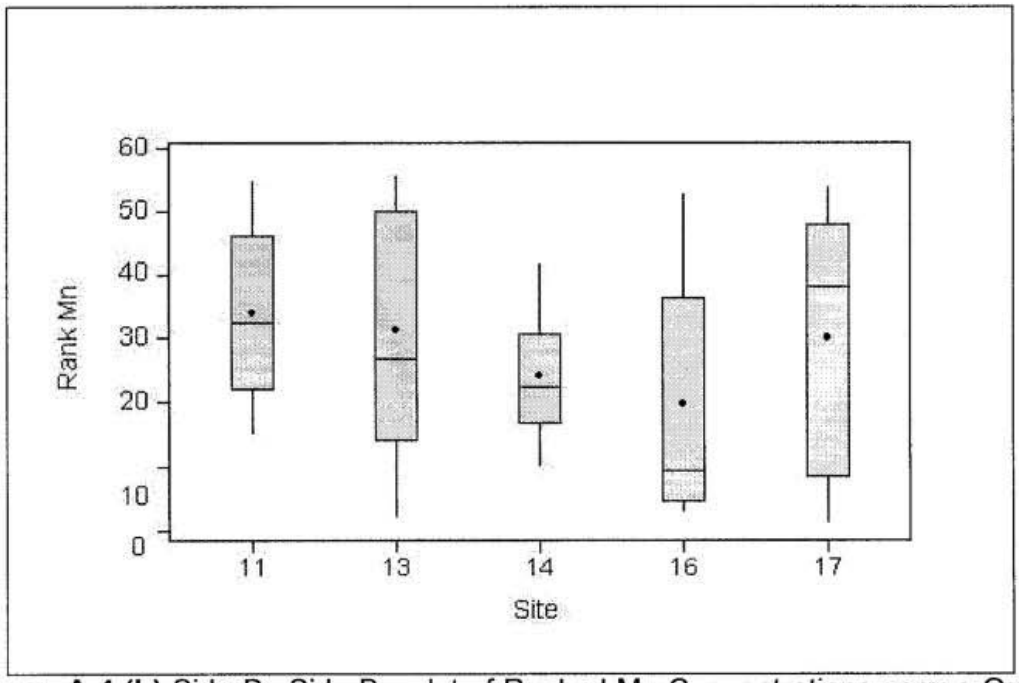

Figure A.4 (b) Side-By-Side Boxplot of Ranked Mn Concentrations versus Outer Region Site Numbers

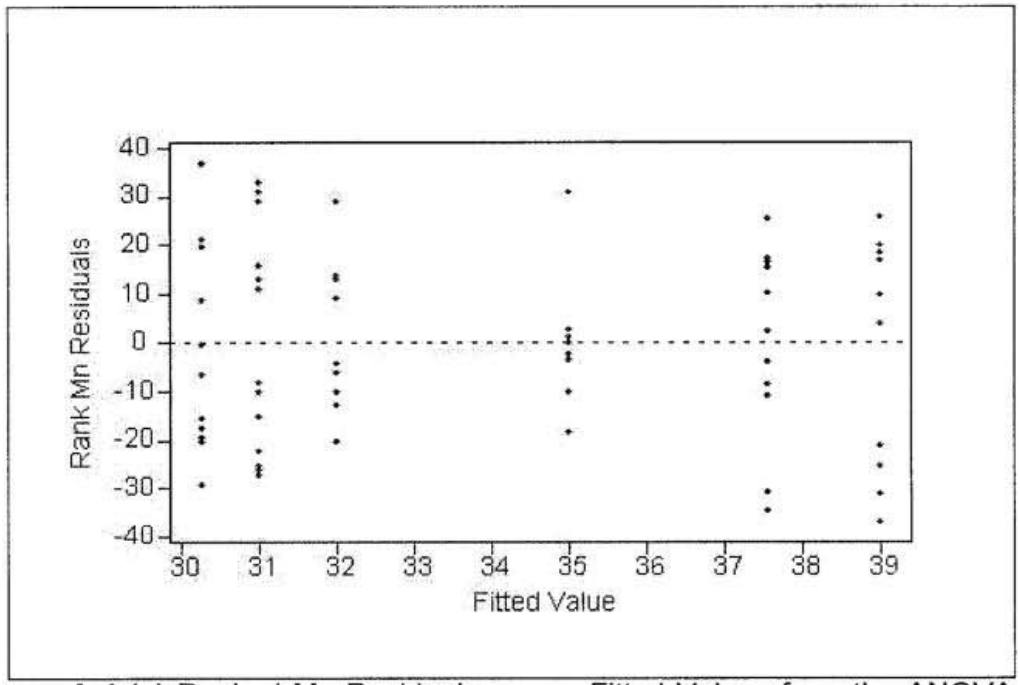

Figure A.4 (c) Ranked Mn Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

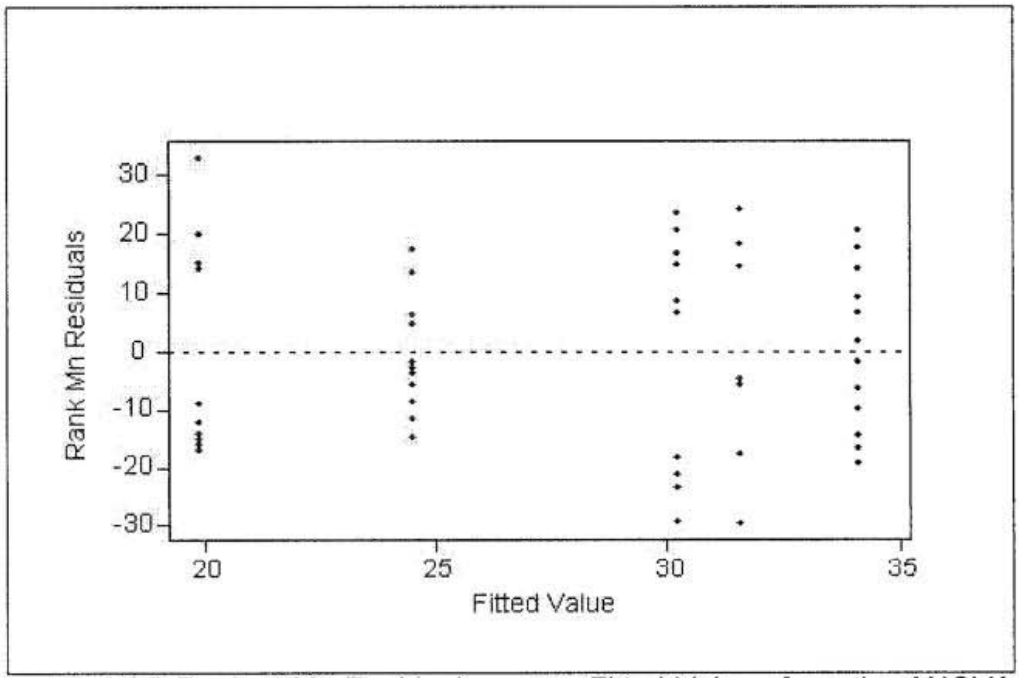

Figure A.4 (d) Ranked Mn Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

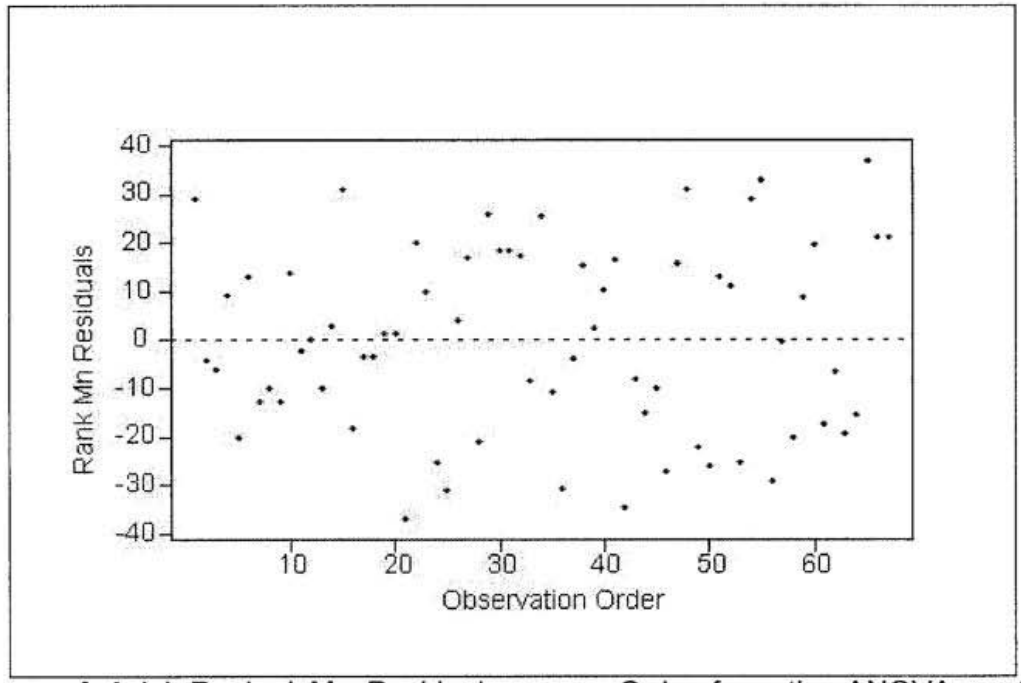

Figure A.4 (e) Ranked Mn Residuals versus Order from the ANOVA on the Ranks of the Inner Region

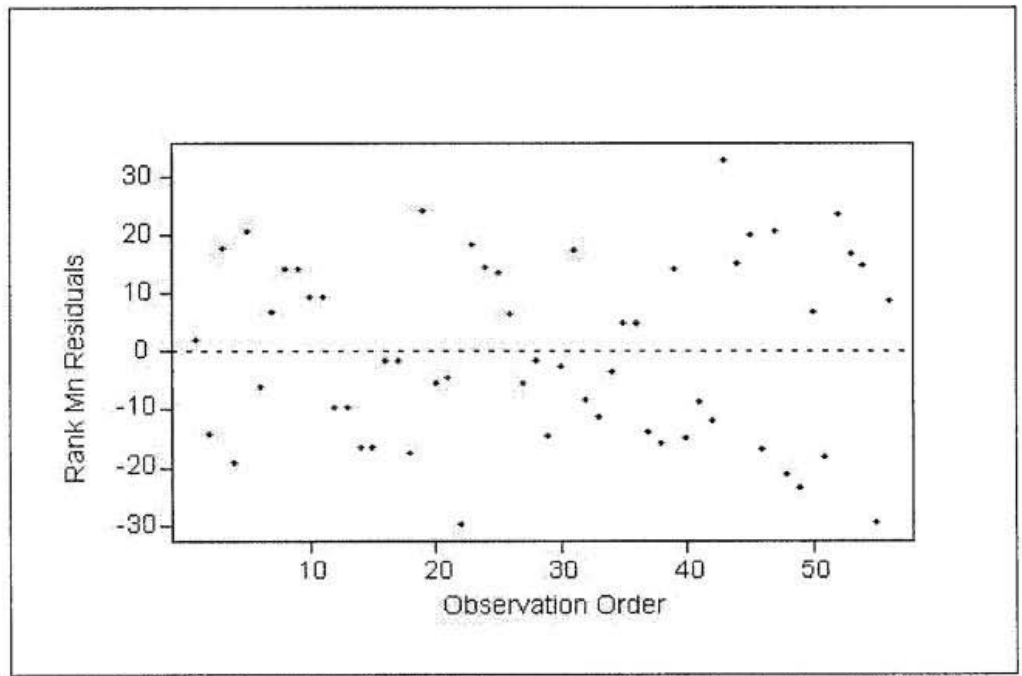

Figure A.4 (f) Ranked Mn Residuals versus Order from the ANOVA on the Ranks of the Outer Region

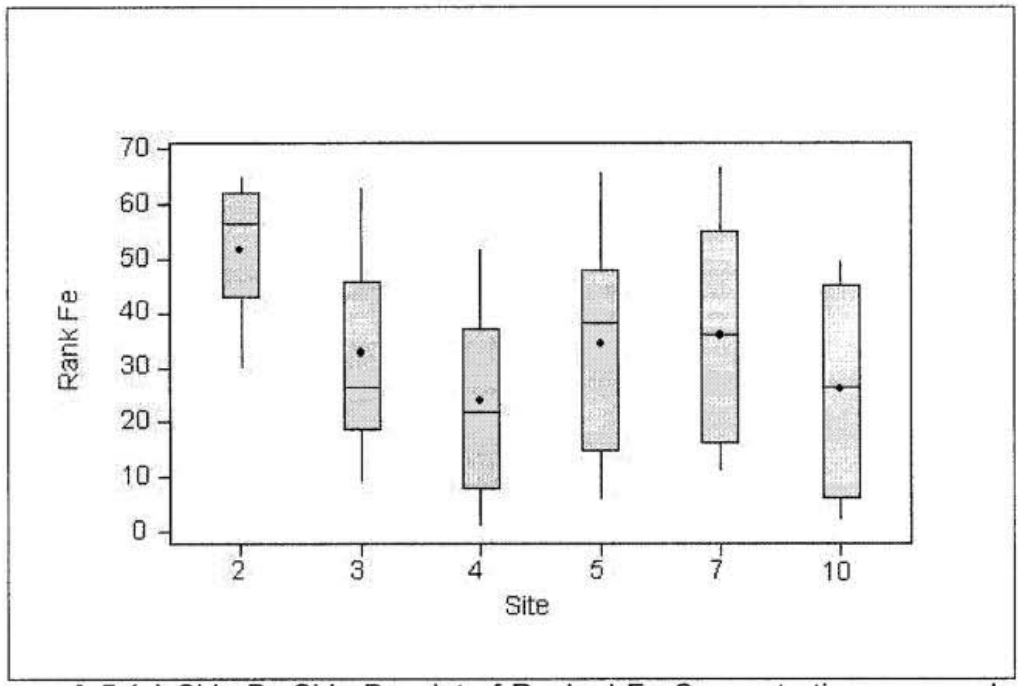

Figure A.5 (a) Side-By-Side Boxplot of Ranked Fe Concentrations versus Inner Region Site Numbers

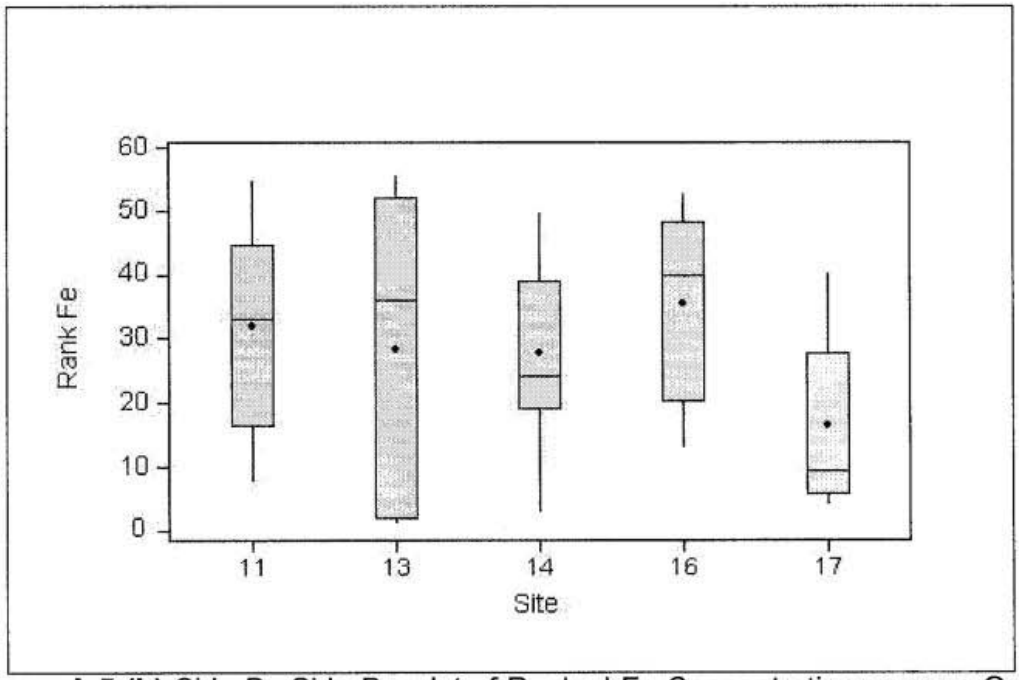

Figure A.5 (b) Side-By-Side Boxplot of Ranked Fe Concentrations versus Outer Region Site Numbers

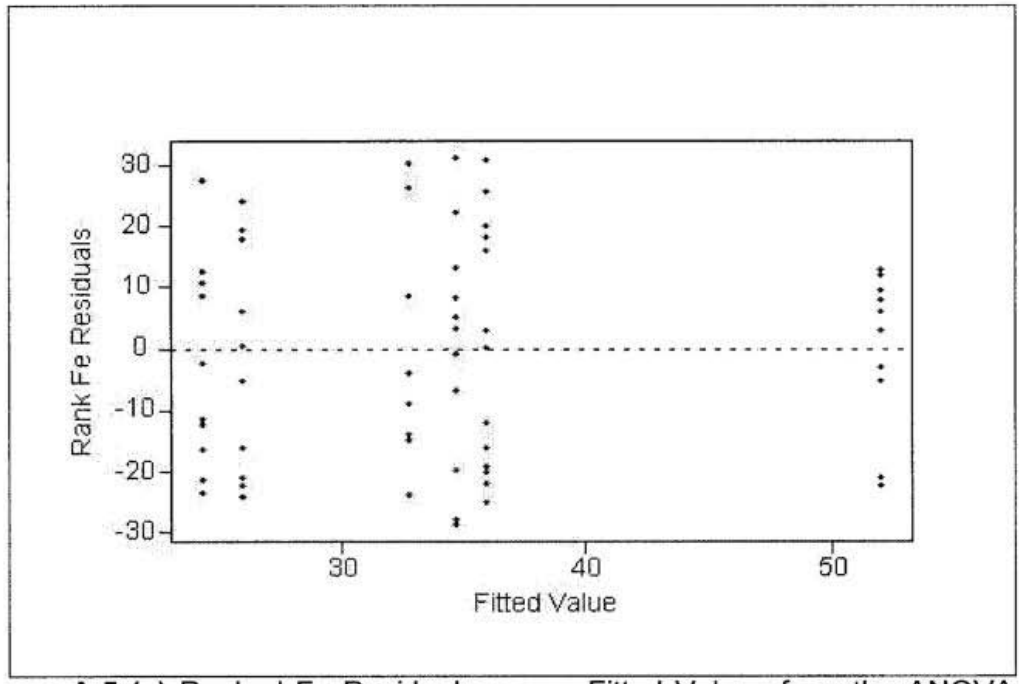

Figure A.5 (c) Ranked Fe Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

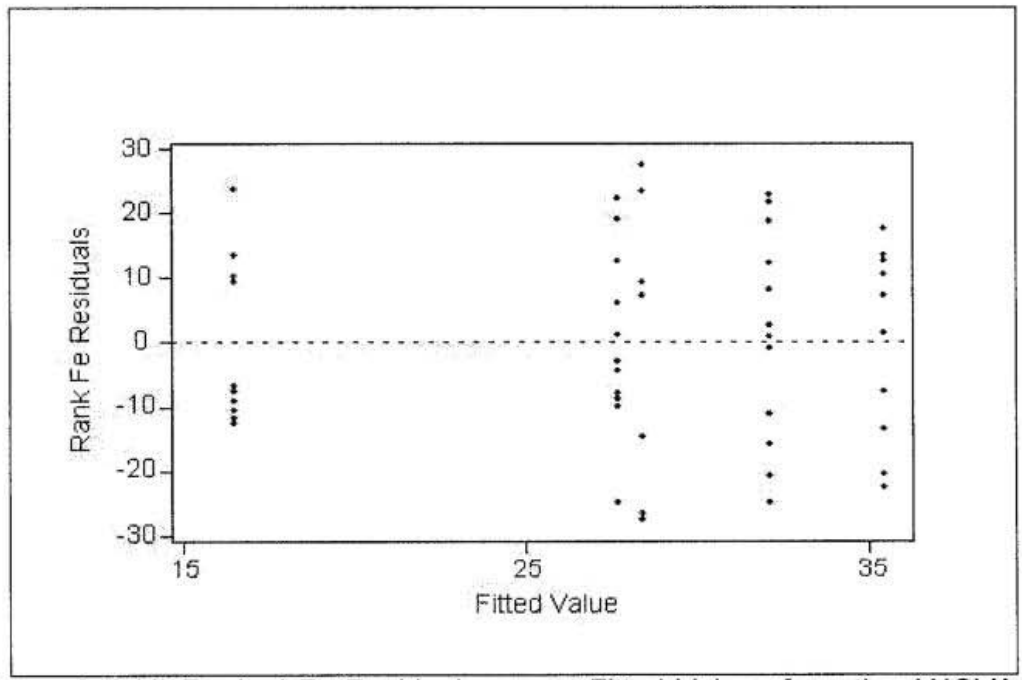

Figure A.5 (d) Ranked Fe Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

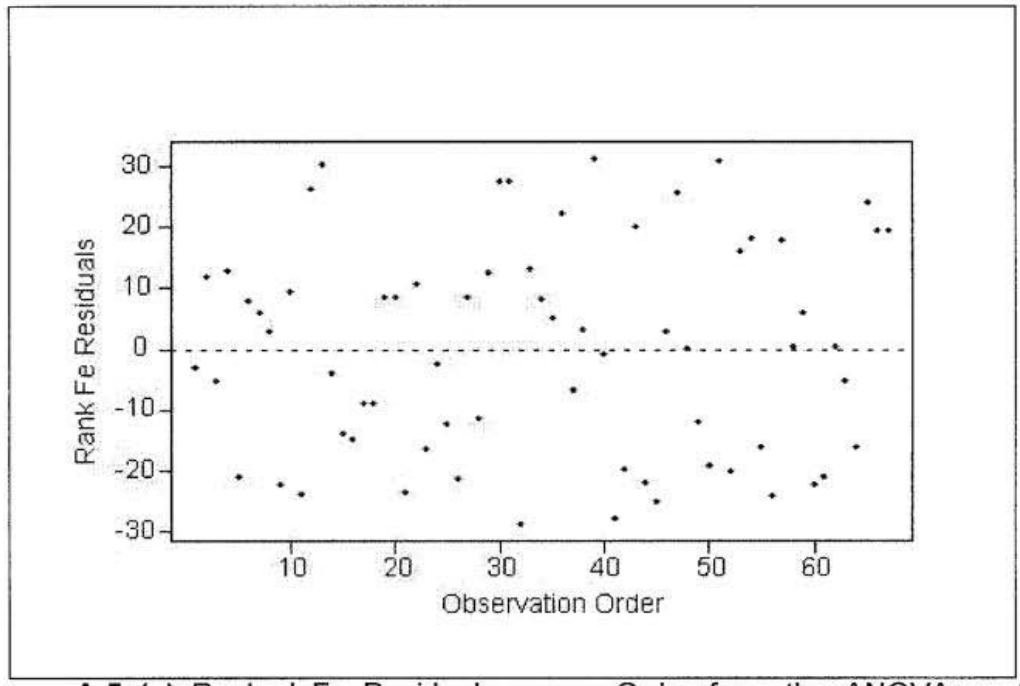

Figure A.5 (e) Ranked Fe Residuals versus Order from the ANOVA on the Ranks of the Inner Region

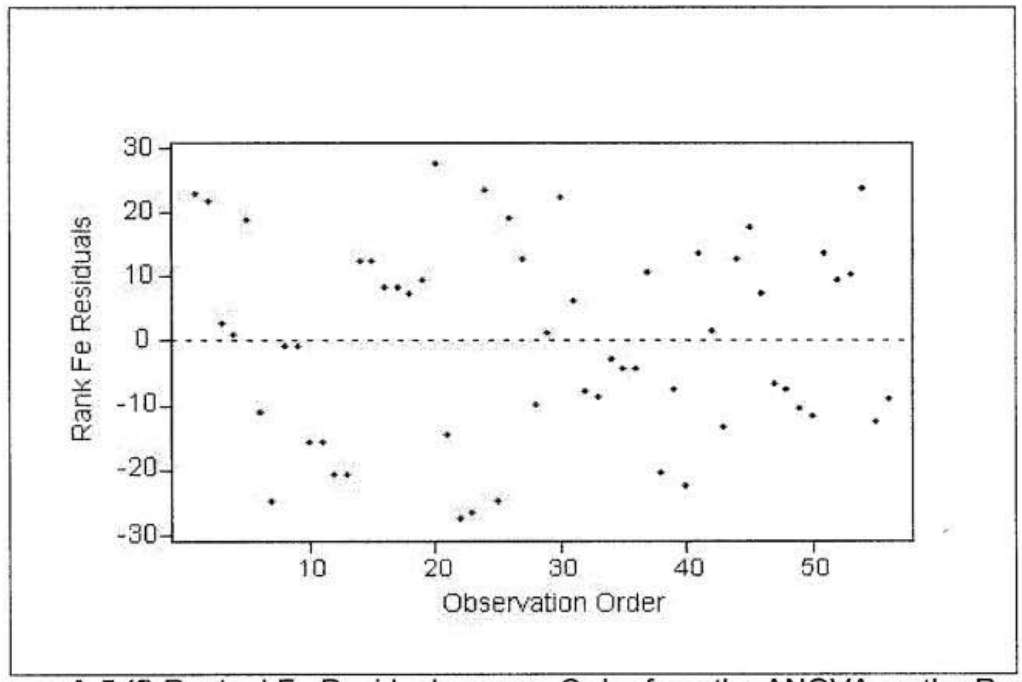

Figure A.5 (f) Ranked Fe Residuals versus Order from the ANOVA on the Ranks of the Outer Region

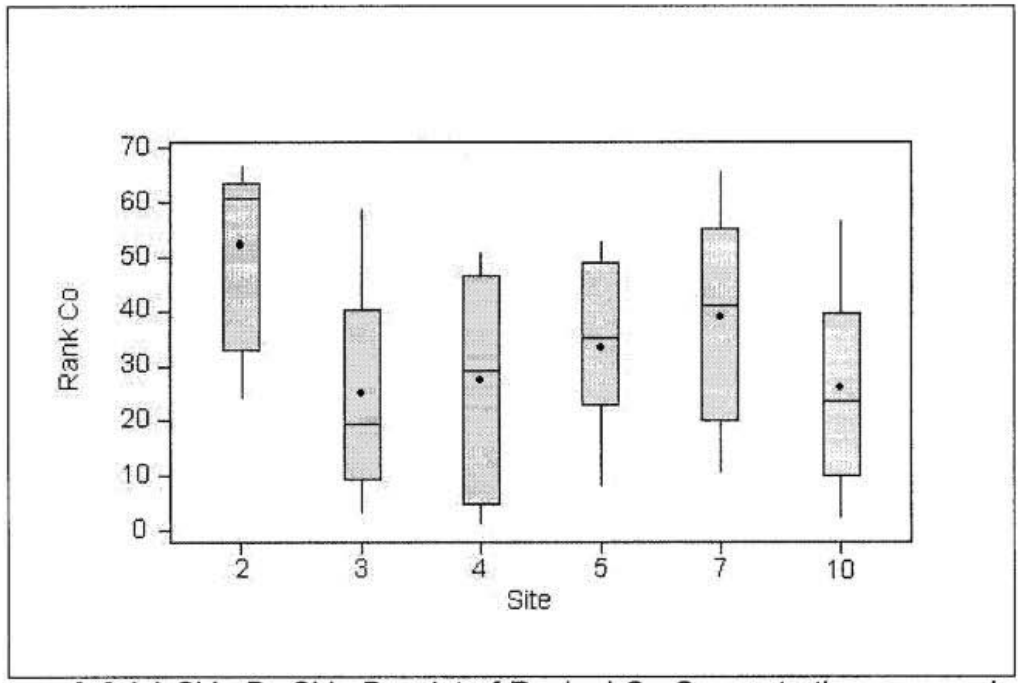

Figure A.6 (a) Side-By-Side Boxplot of Ranked Co Concentrations versus Inner Region Site Numbers

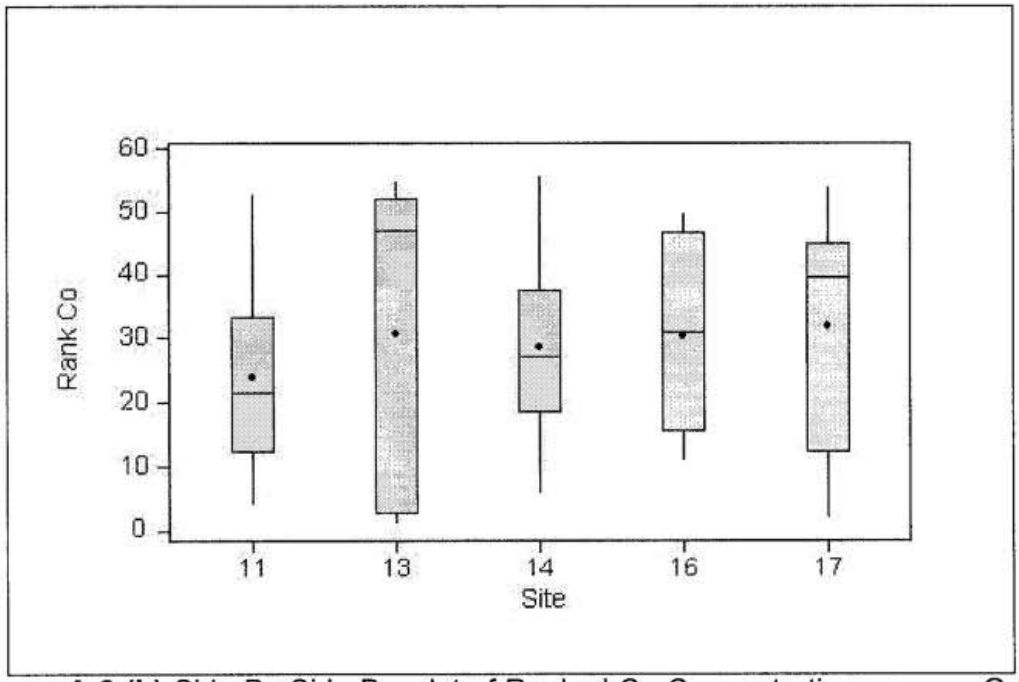

Figure A.6 (b) Side-By-Side Boxplot of Ranked Co Concentrations versus Outer Region Site Numbers

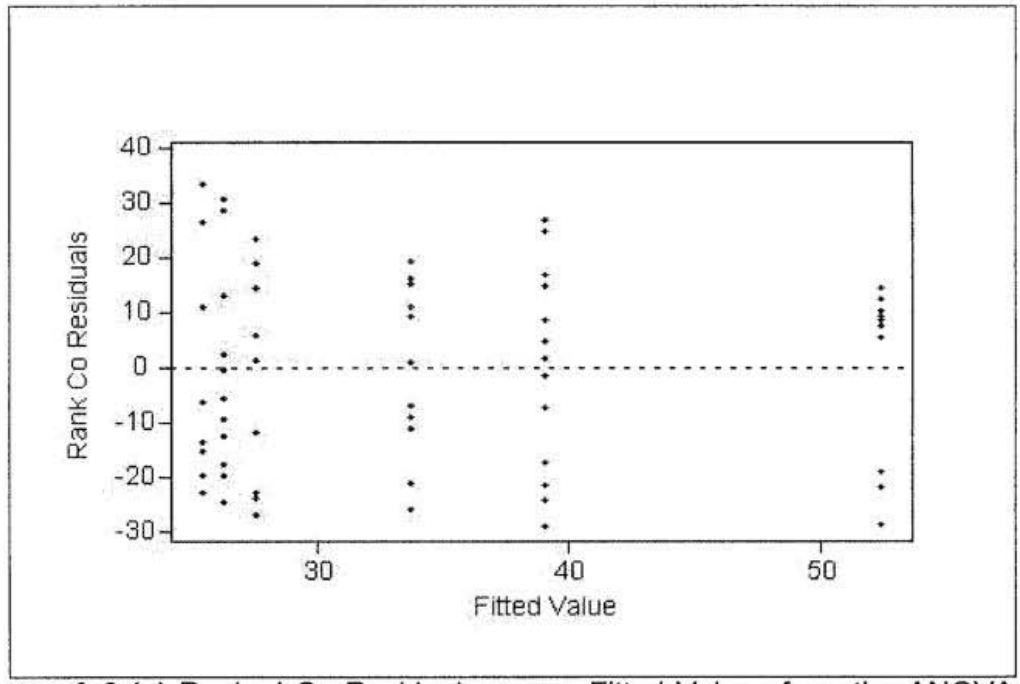

Figure A.6 (c) Ranked Co Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

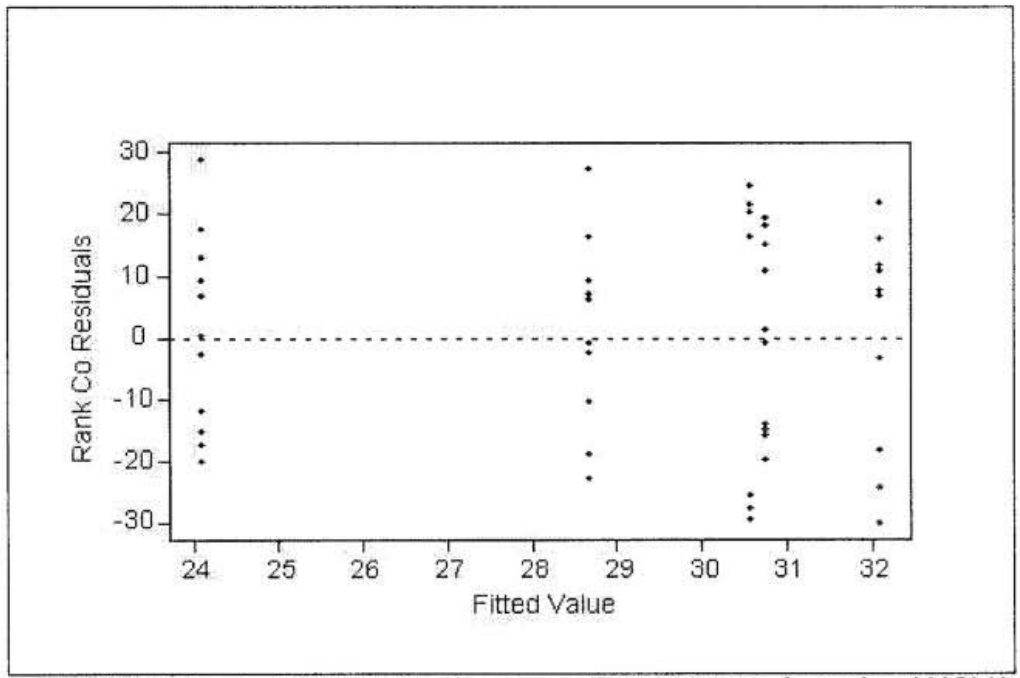

Figure A.6 (d) Ranked Co Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

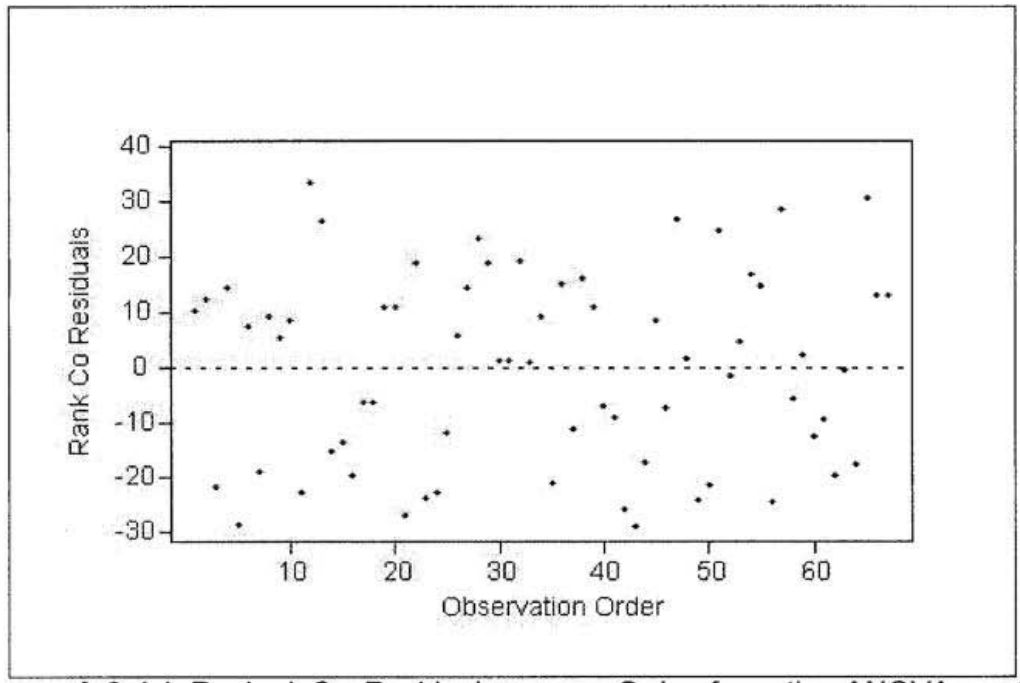

Figure A.6 (e) Ranked Co Residuals versus Order from the ANOVA on the Ranks of the Inner Region

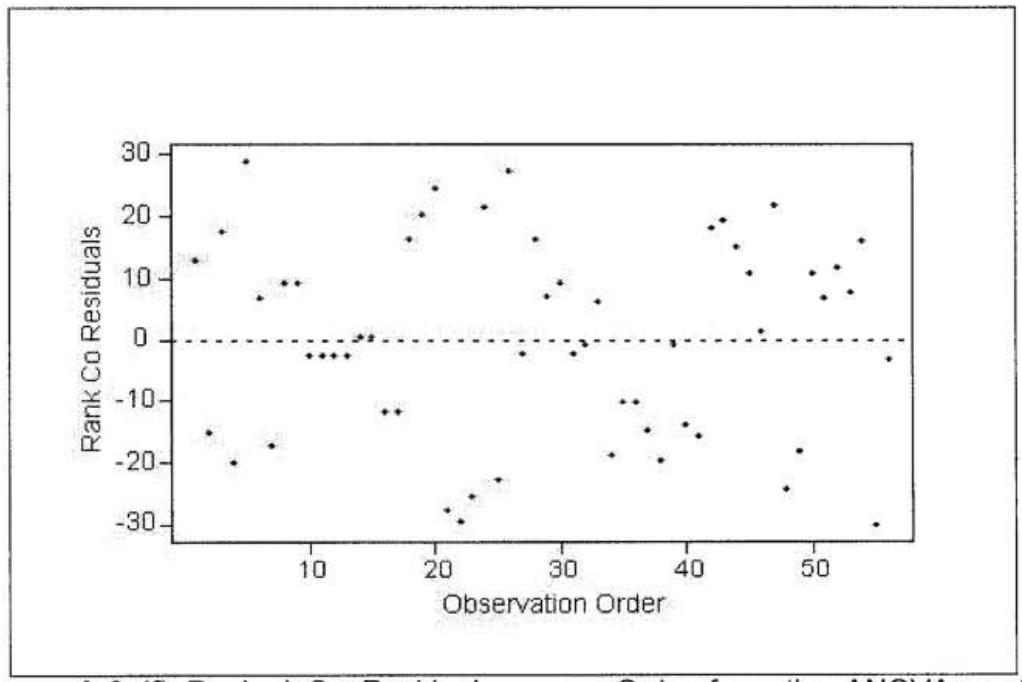

Figure A.6 (f) Ranked Co Residuals versus Order from the ANOVA on the Ranks of the Outer Region

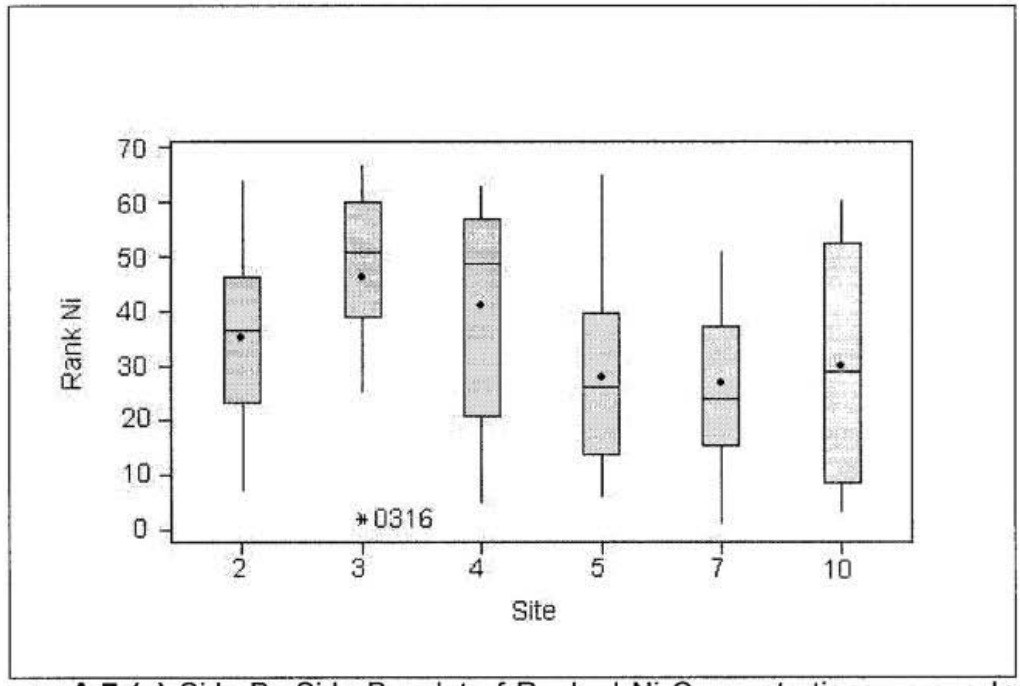

Figure A.7 (a) Side-By-Side Boxplot of Ranked Ni Concentrations versus Inner Region Site Numbers

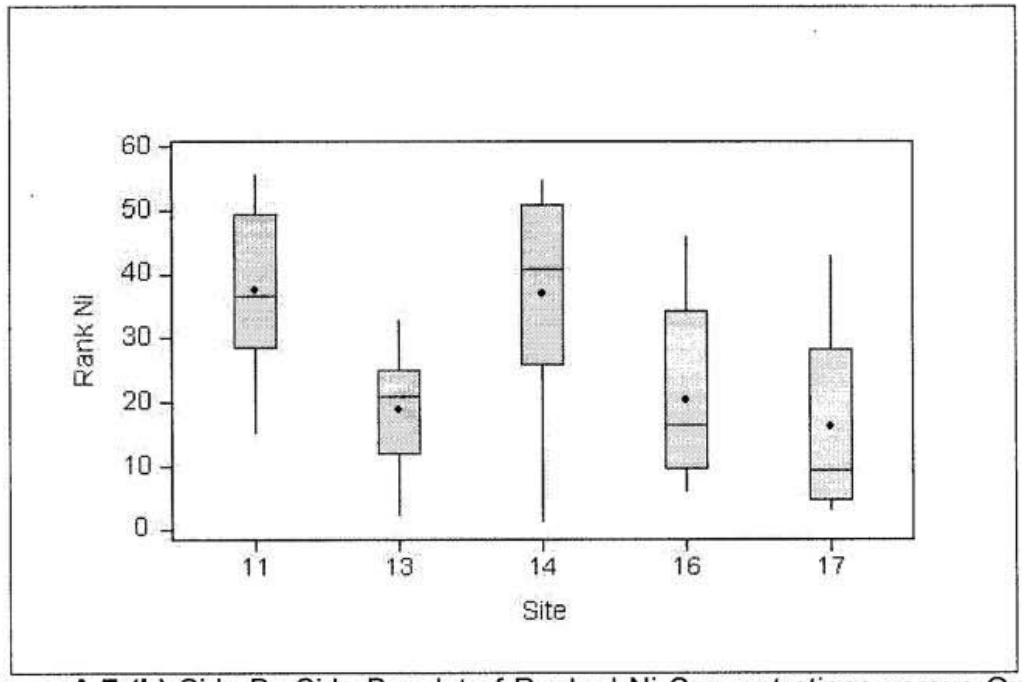

Figure A.7 (b) Side-By-Side Boxplot of Ranked Ni Concentrations versus Outer Region Site Numbers

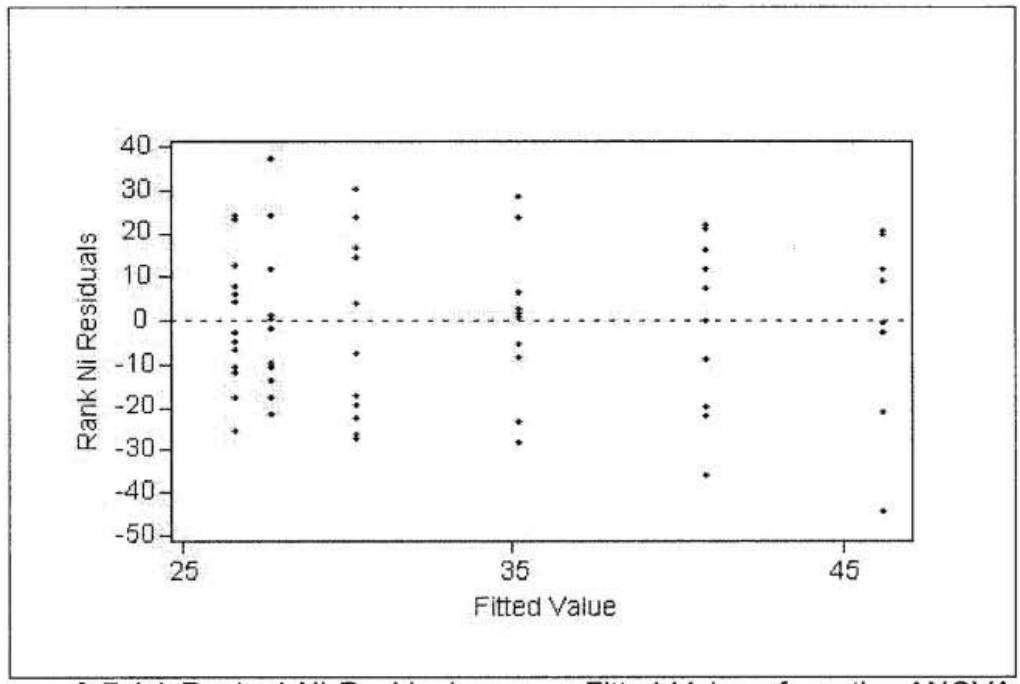

Figure A.7 (c) Ranked Ni Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

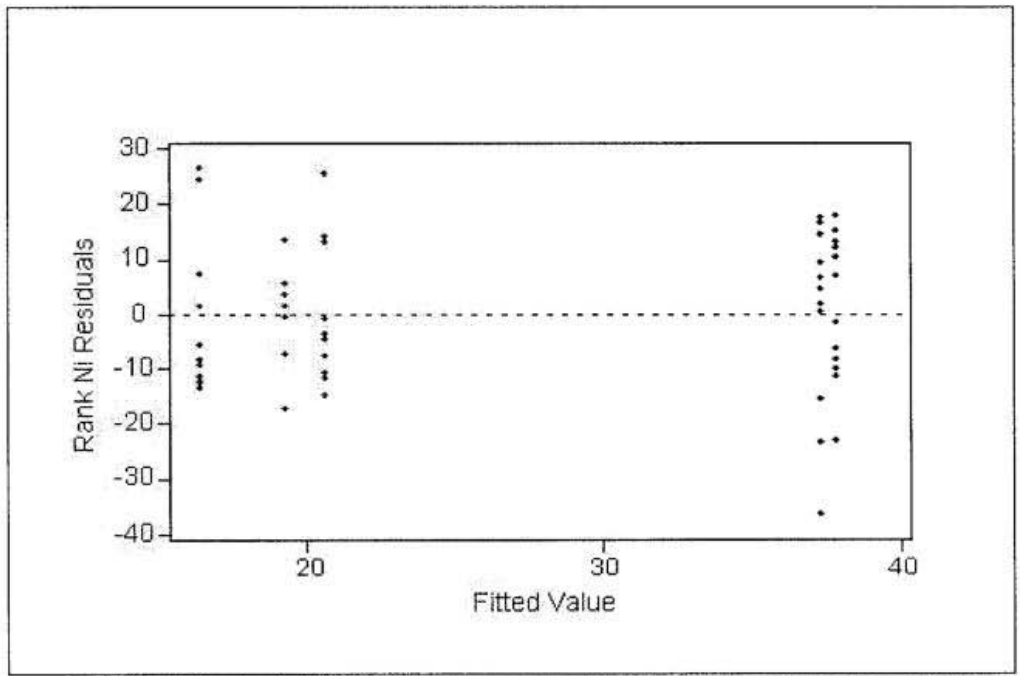

Figure A.7 (d) Ranked Ni Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

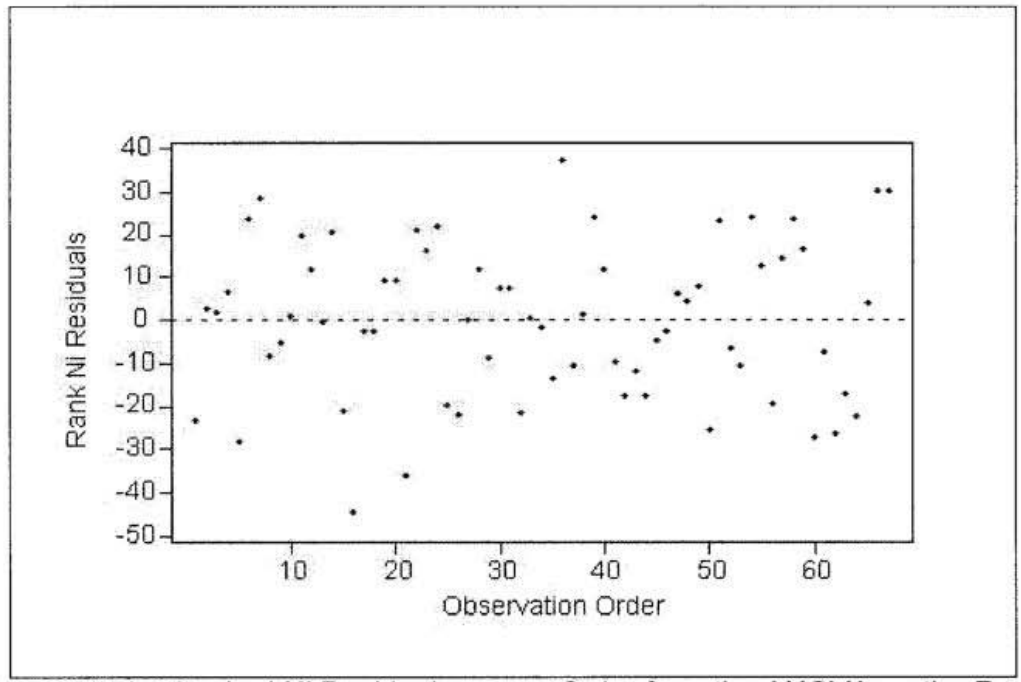

Figure A.7 (e) Ranked Ni Residuals versus Order from the ANOVA on the Ranks of the Inner Region

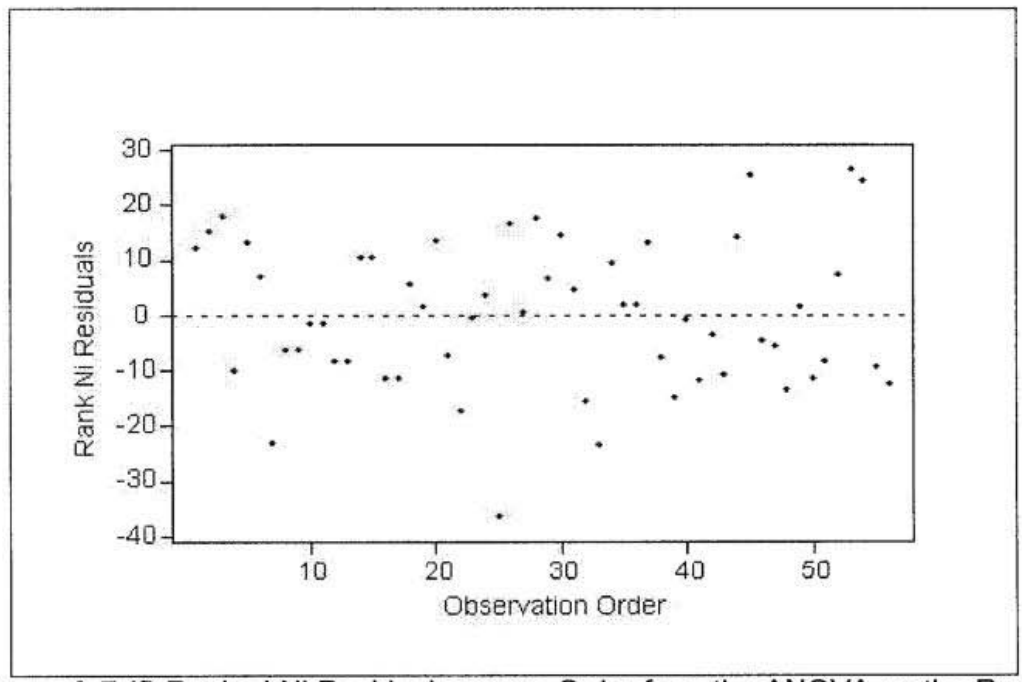

Figure A.7 (f) Ranked Ni Residuals versus Order from the ANOVA on the Ranks of the Outer Region

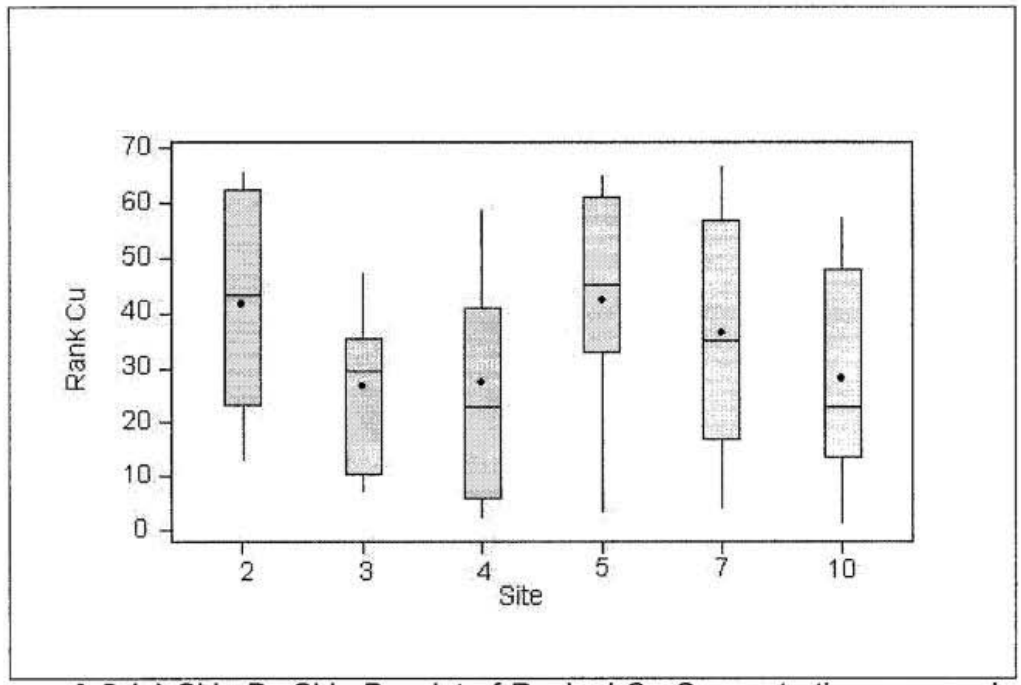

Figure A.8 (a) Side-By-Side Boxplot of Ranked Cu Concentrations versus Inner Region Site Numbers

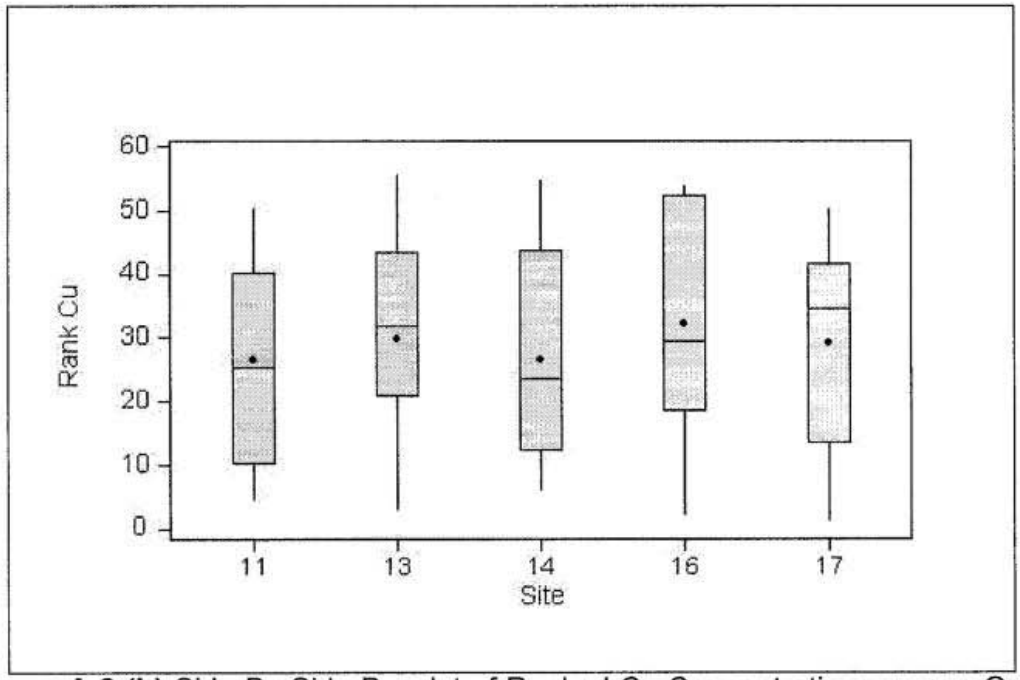

Figure A.8 (b) Side-By-Side Boxplot of Ranked Cu Concentrations versus Outer Region Site Numbers

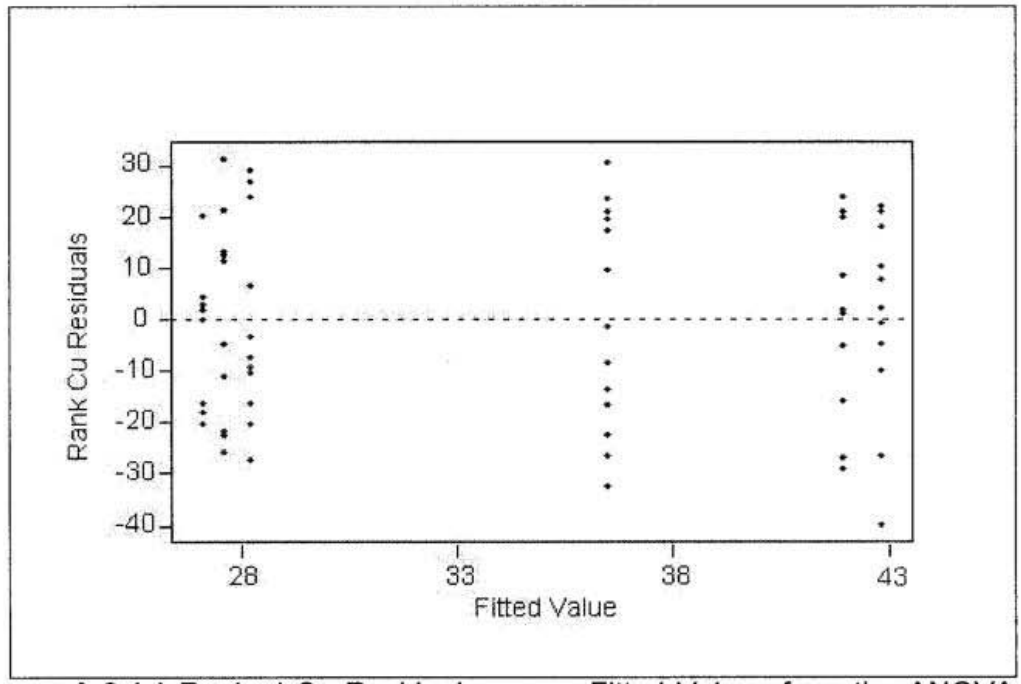

Figure A.8 (c) Ranked Cu Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

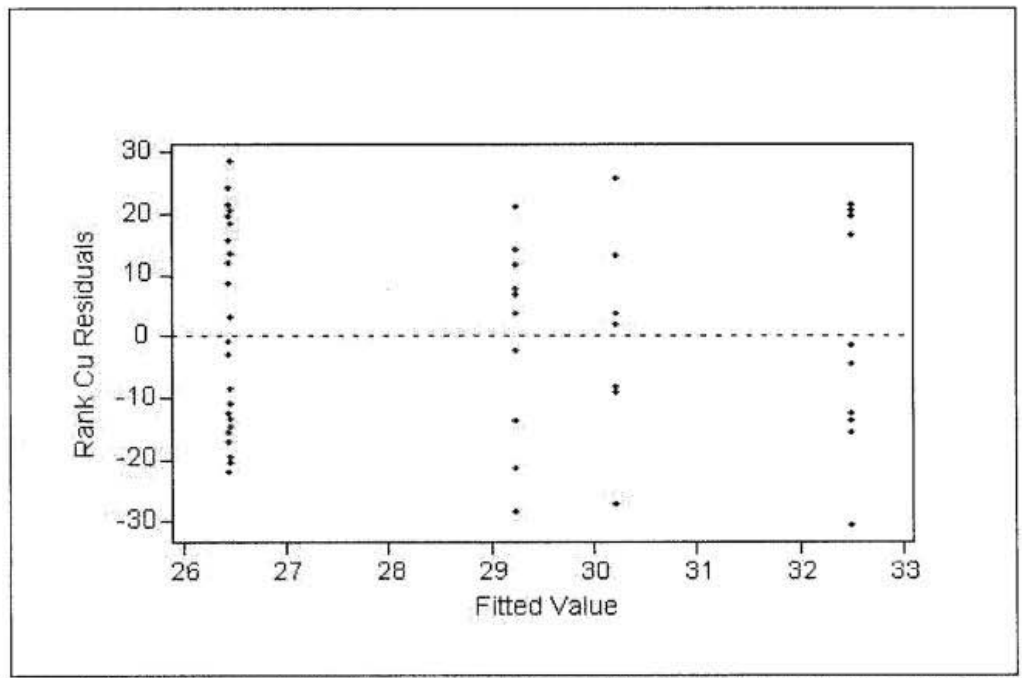

Figure A.8 (d) Ranked Cu Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

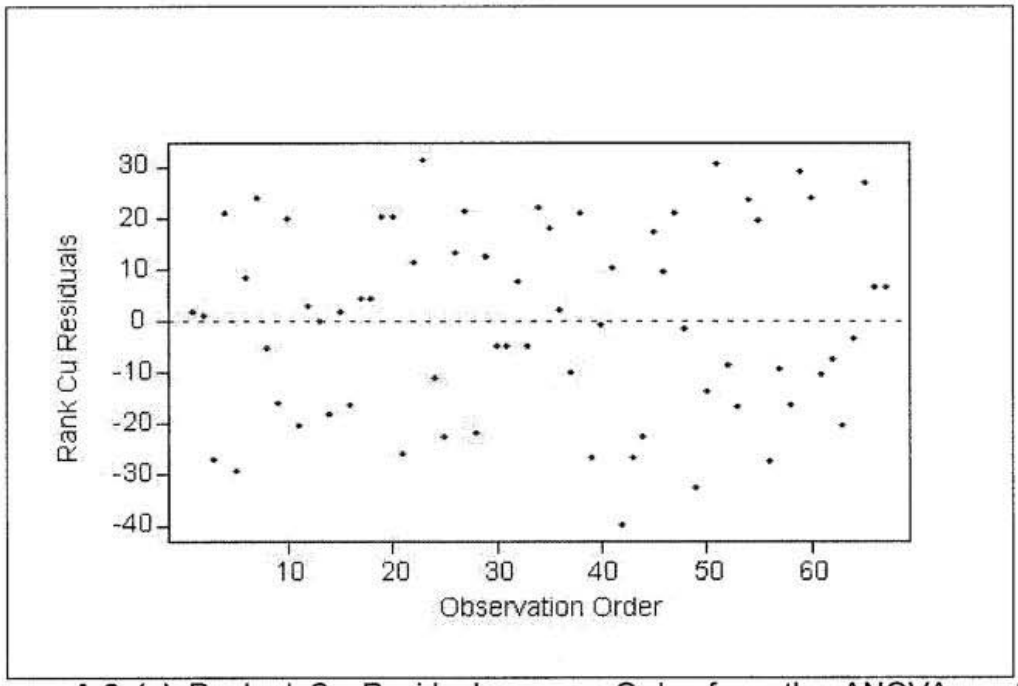

Figure A.8 (e) Ranked Cu Residuals versus Order from the ANOVA on the Ranks of the Inner Region

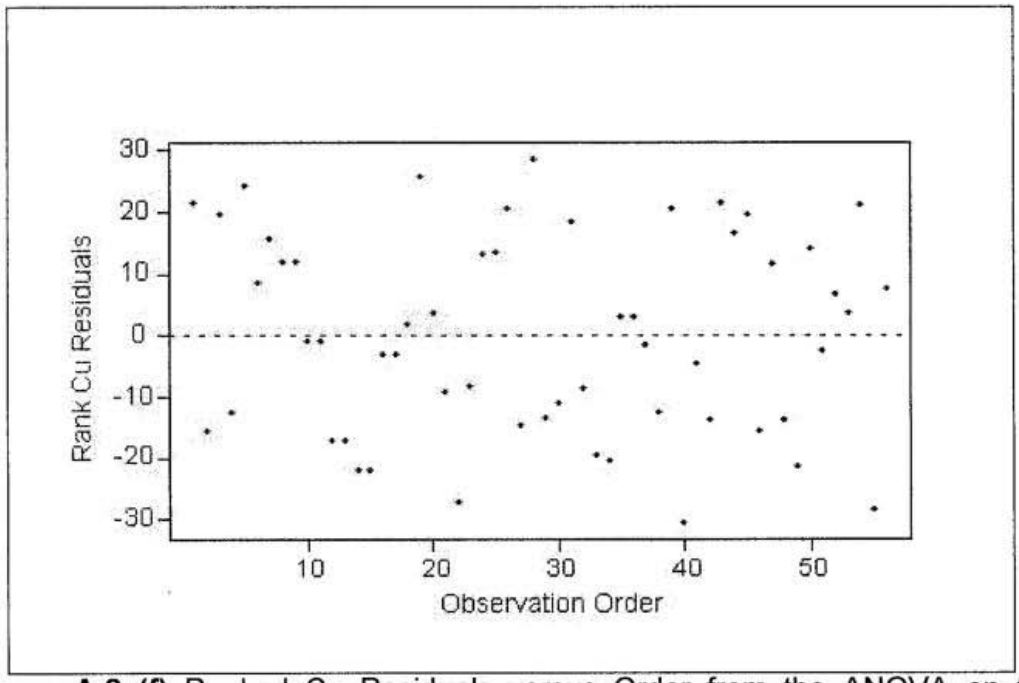

Figure A.8 (f) Ranked Cu Residuals versus Order from the ANOVA on the Ranks of the Outer Region

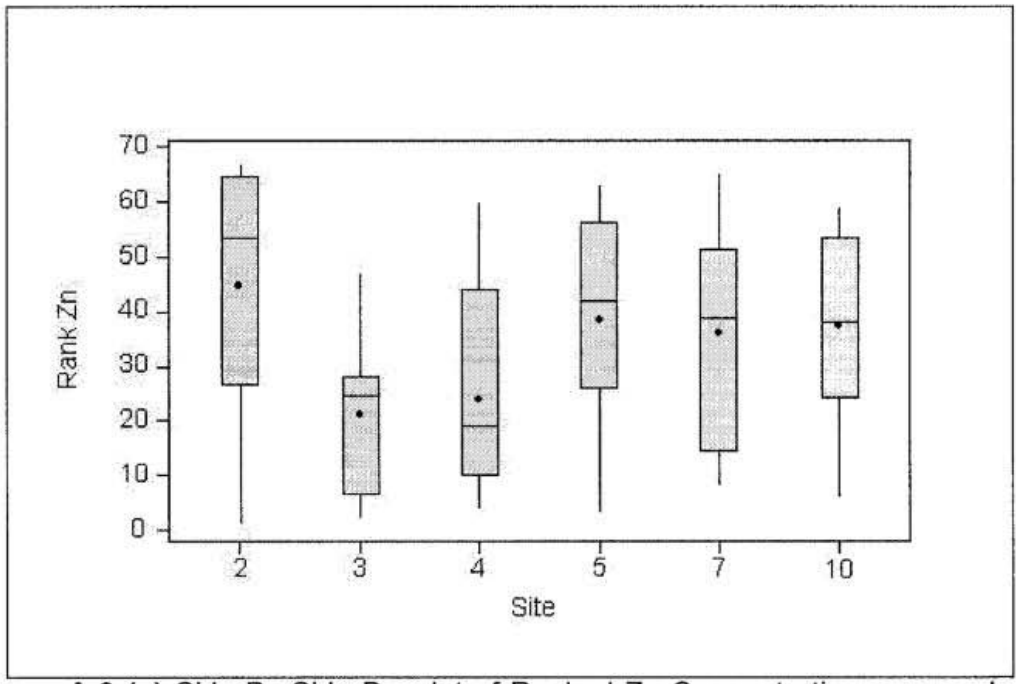

Figure A.9 (a) Side-By-Side Boxplot of Ranked Zn Concentrations versus Inner Region Site Numbers

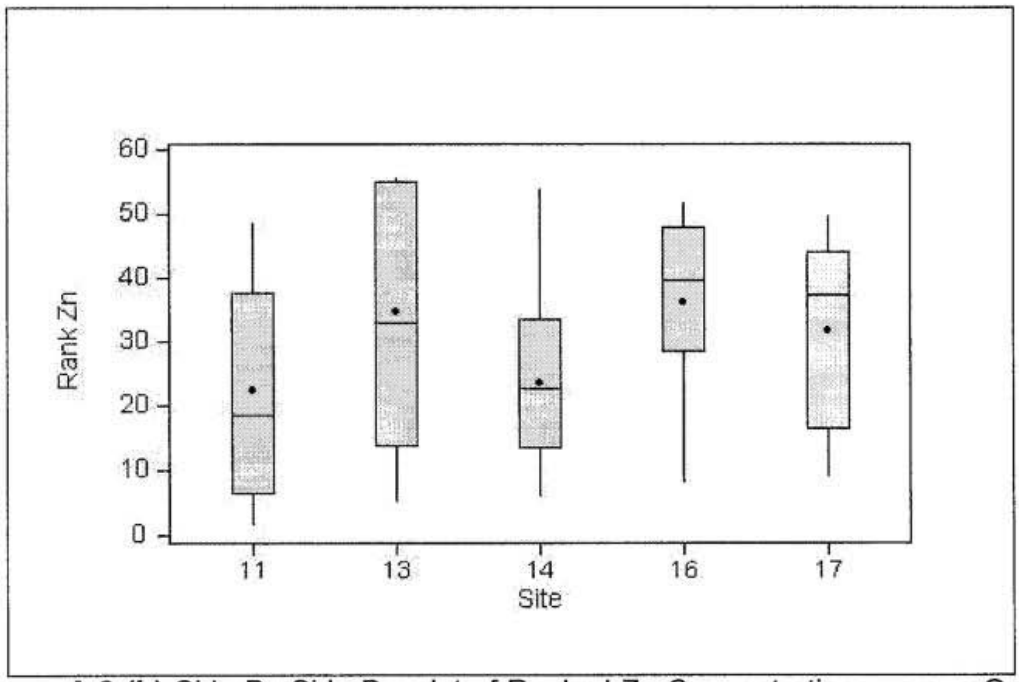

Figure A.9 (b) Side-By-Side Boxplot of Ranked Zn Concentrations versus Outer Region Site Numbers

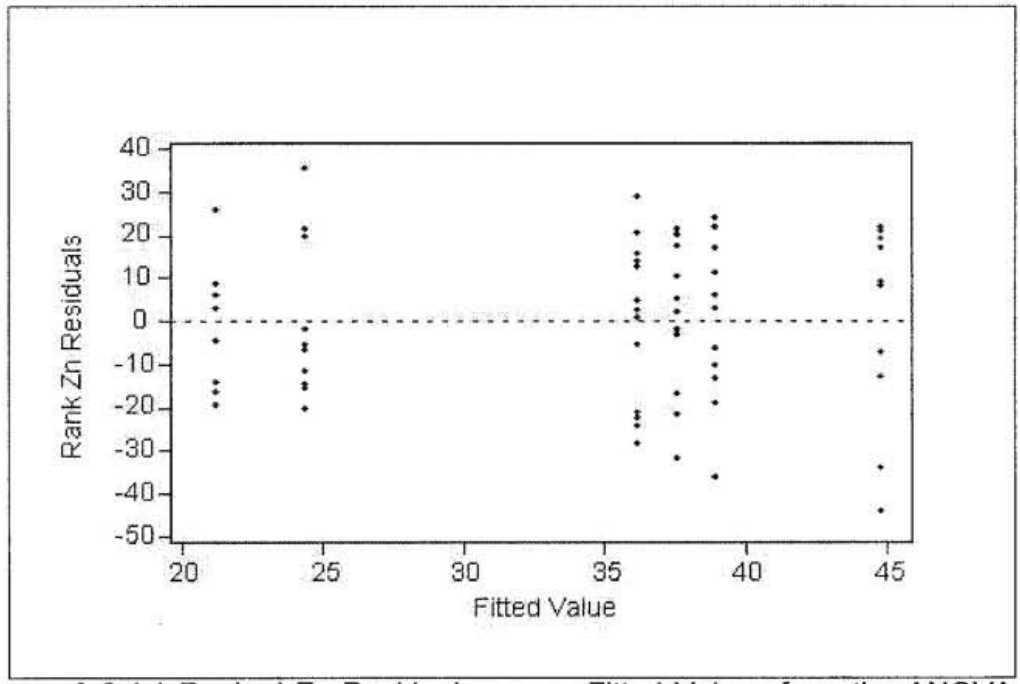

Figure A.9 (c) Ranked Zn Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

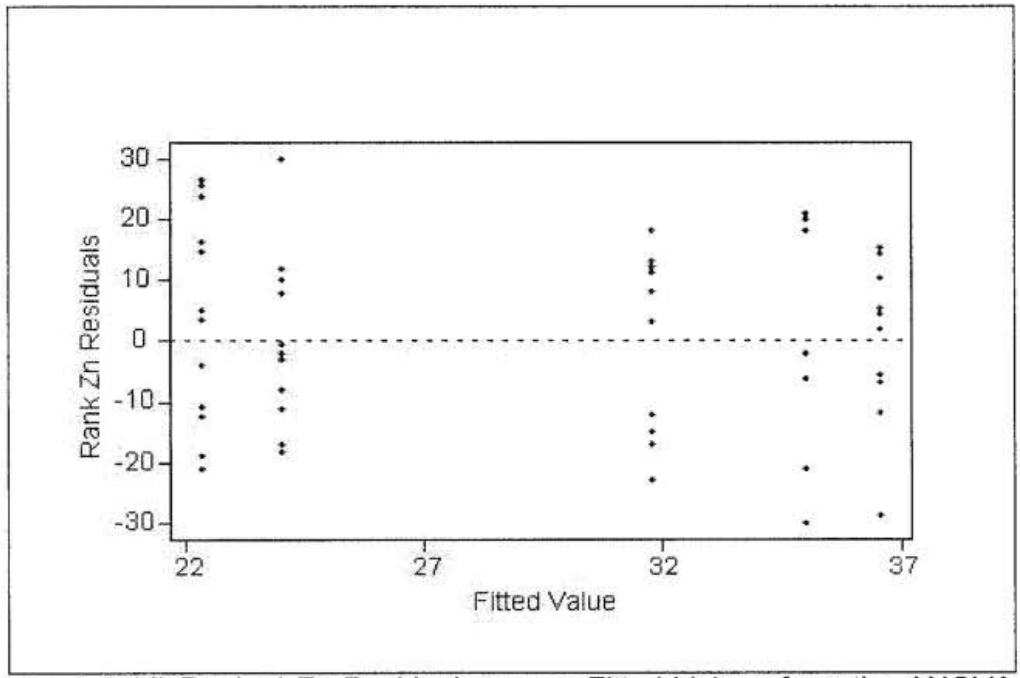

Figure A.9 (d) Ranked Zn Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

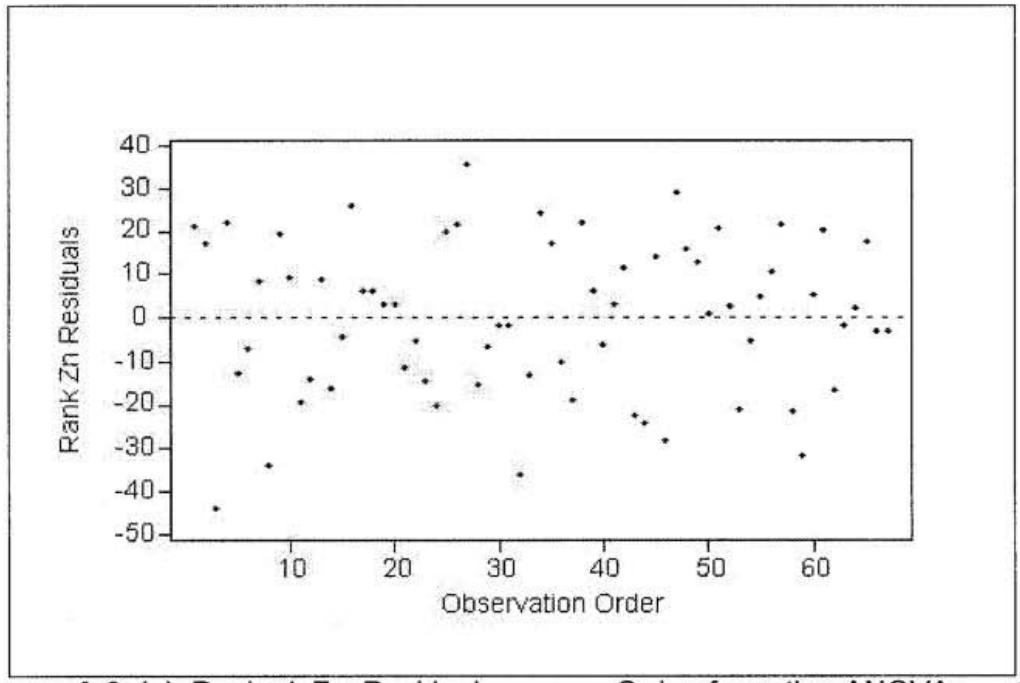

Figure A.9 (e) Ranked Zn Residuals versus Order from the ANOVA on the Ranks of the Inner Region

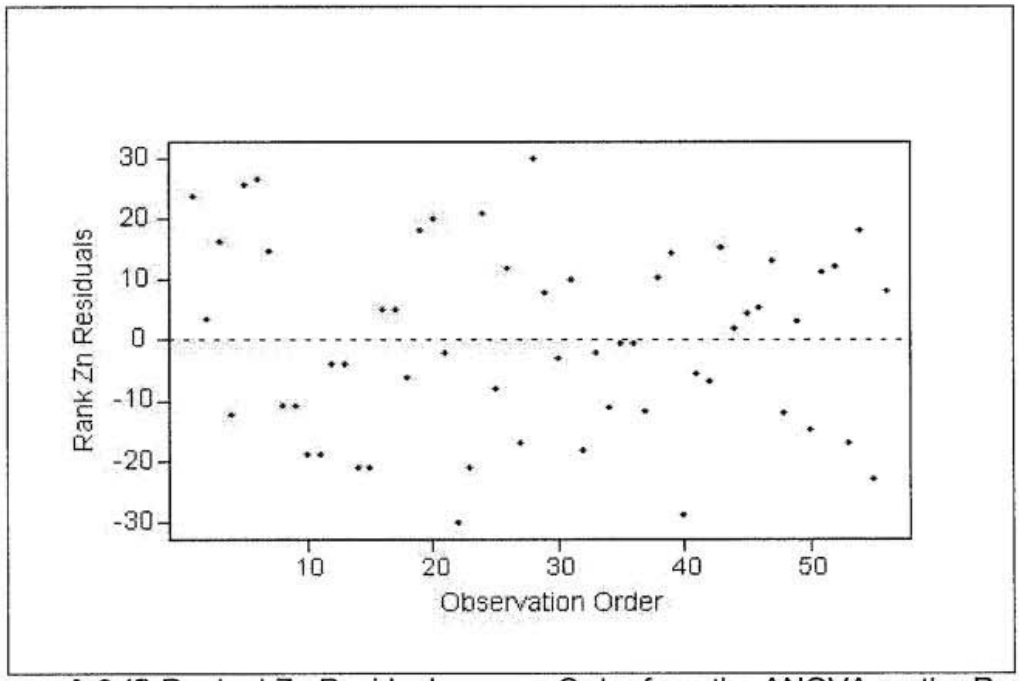

Figure A.9 (f) Ranked Zn Residuals versus Order from the ANOVA on the Ranks of the Outer Region

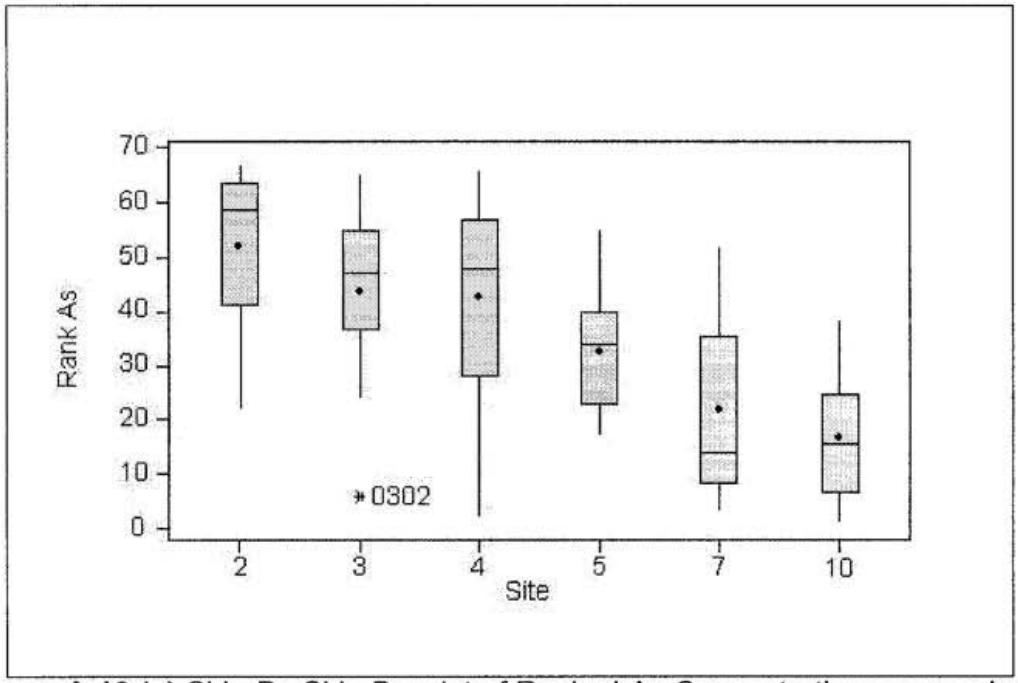

Figure A.10 (a) Side-By-Side Boxplot of Ranked As Concentrations versus Inner Region Site Numbers

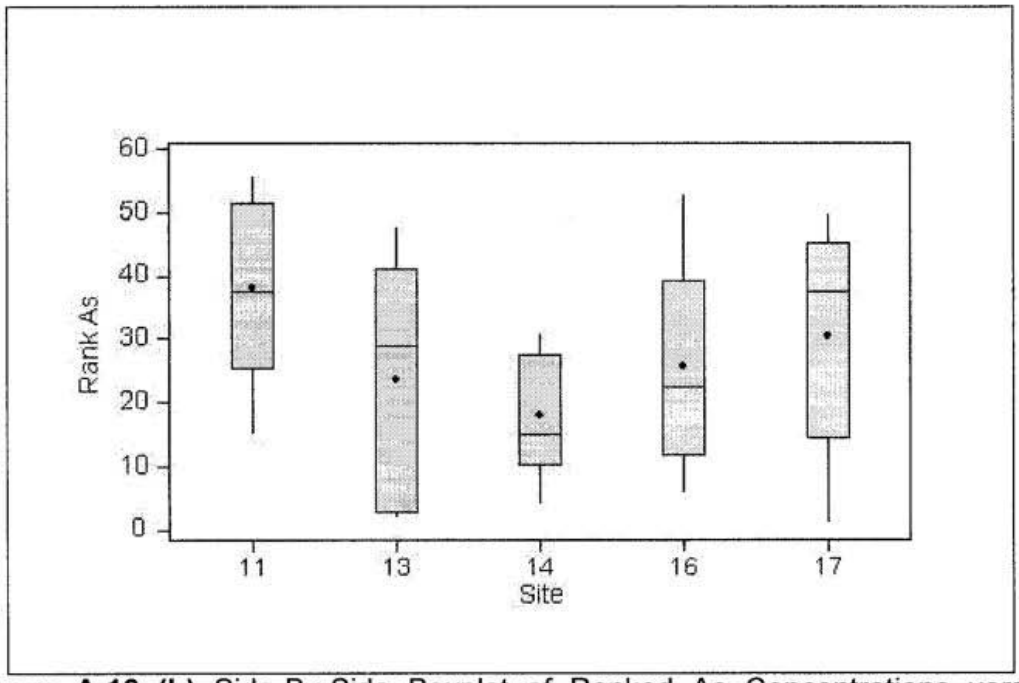

Figure A.10 (b) Side-By-Side Boxplot of Ranked As Concentrations versus Outer Region Site Numbers

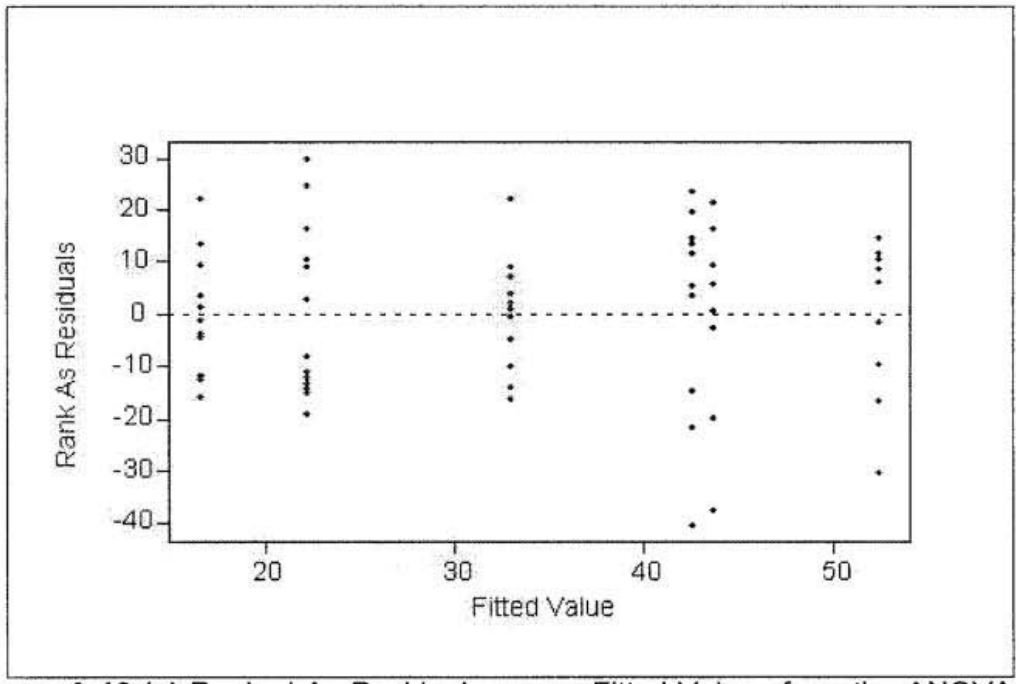

Figure A.10 (c) Ranked As Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

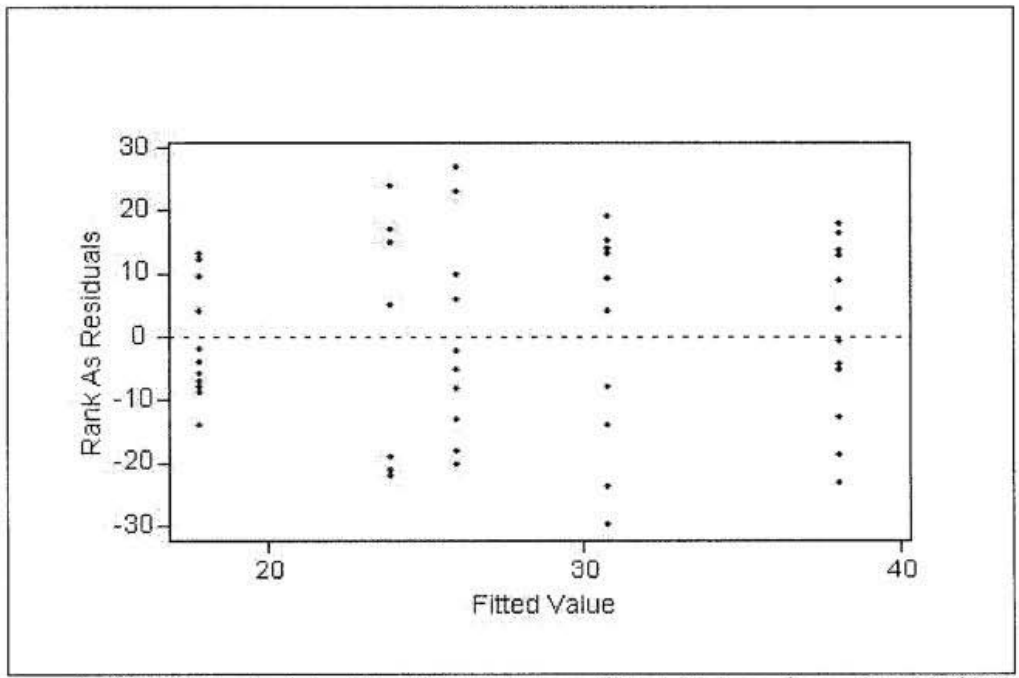

Figure A.10 (d) Ranked As Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

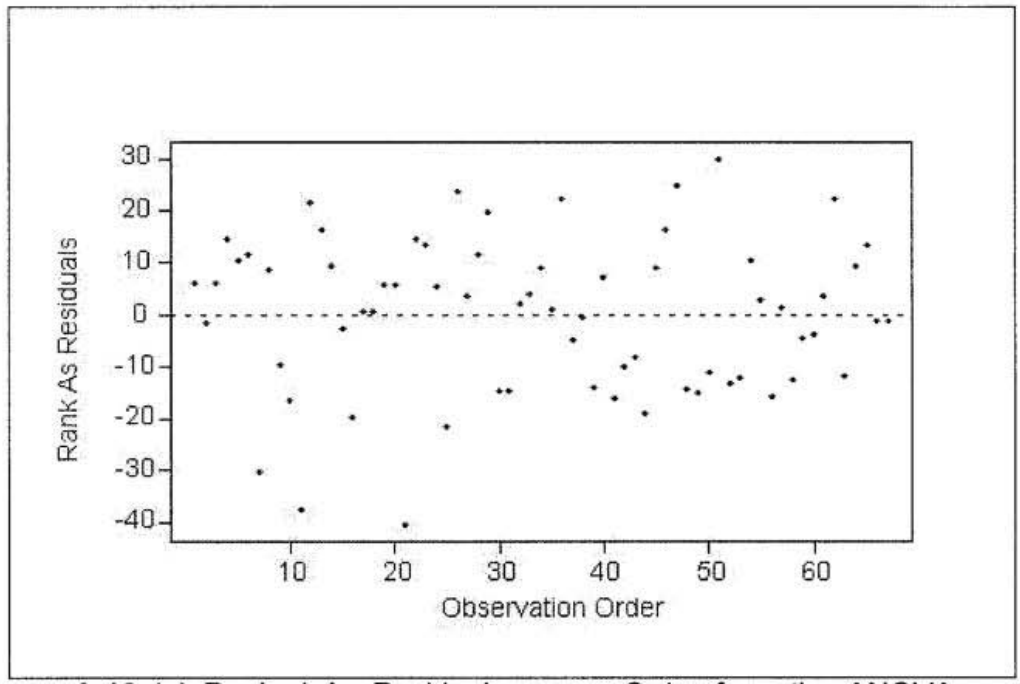

Figure A.10 (e) Ranked As Residuals versus Order from the ANOVA on the Ranks of the Inner Region

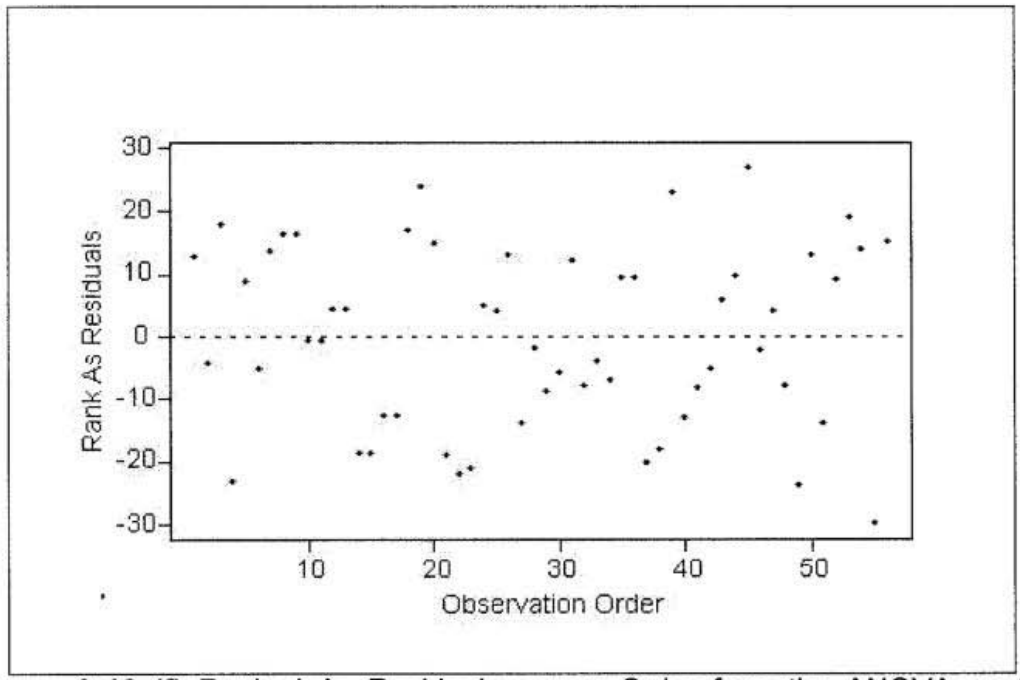

Figure A.10 (f) Ranked As Residuals versus Order from the ANOVA on the Ranks of the Outer Region

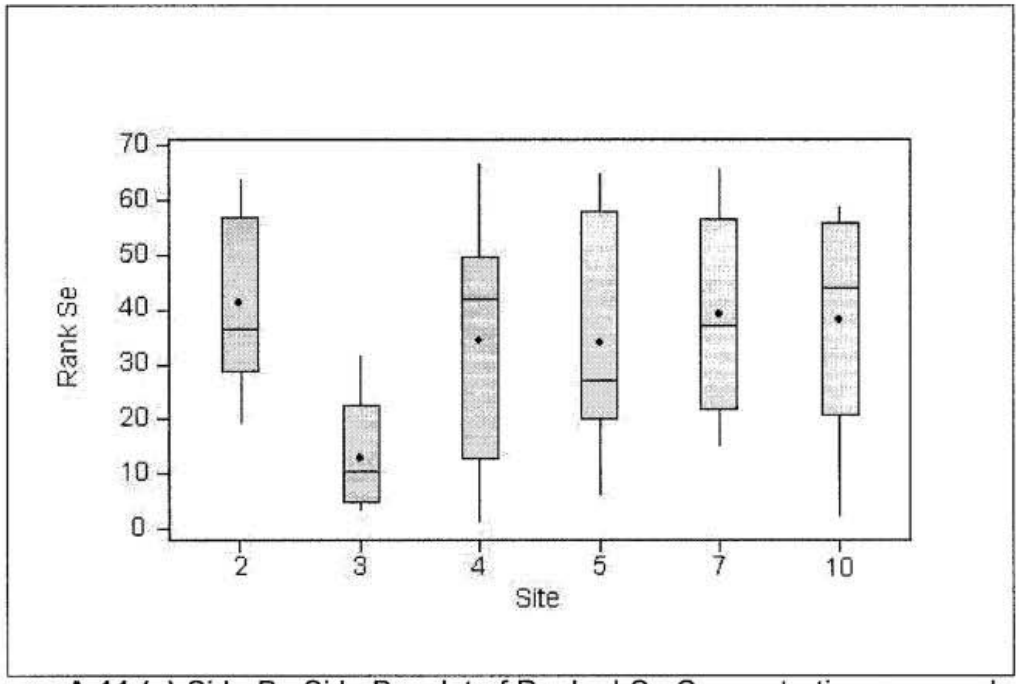

Figure A.11 (a) Side-By-Side Boxplot of Ranked Se Concentrations versus Inner Region Site Numbers

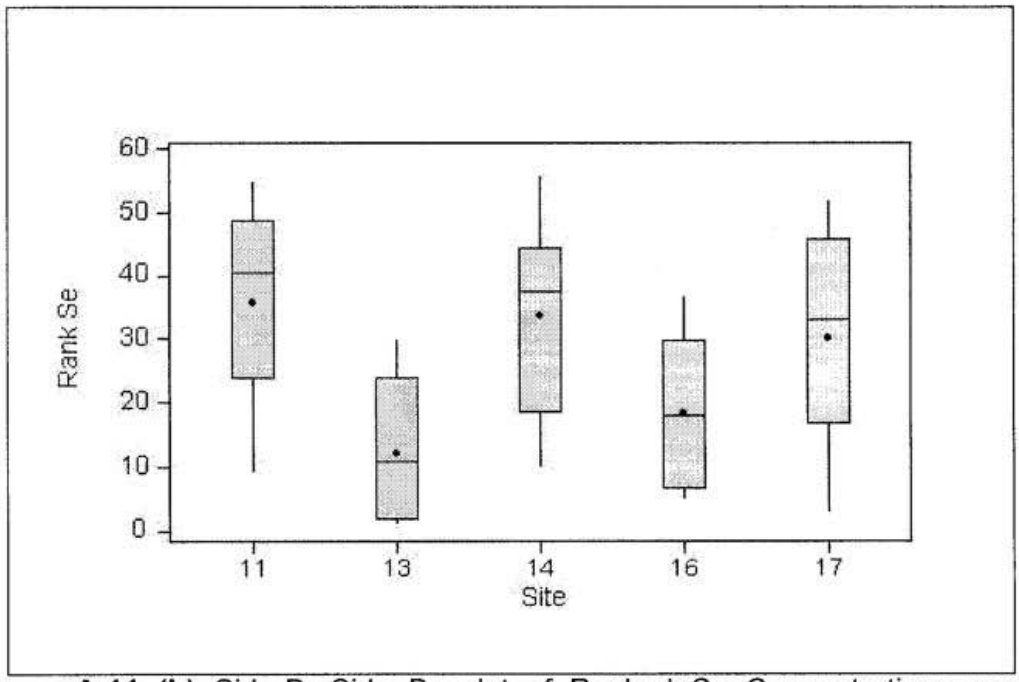

Figure A.11 (b) Side-By-Side Boxplot of Ranked Se Concentrations versus Outer Region Site Numbers

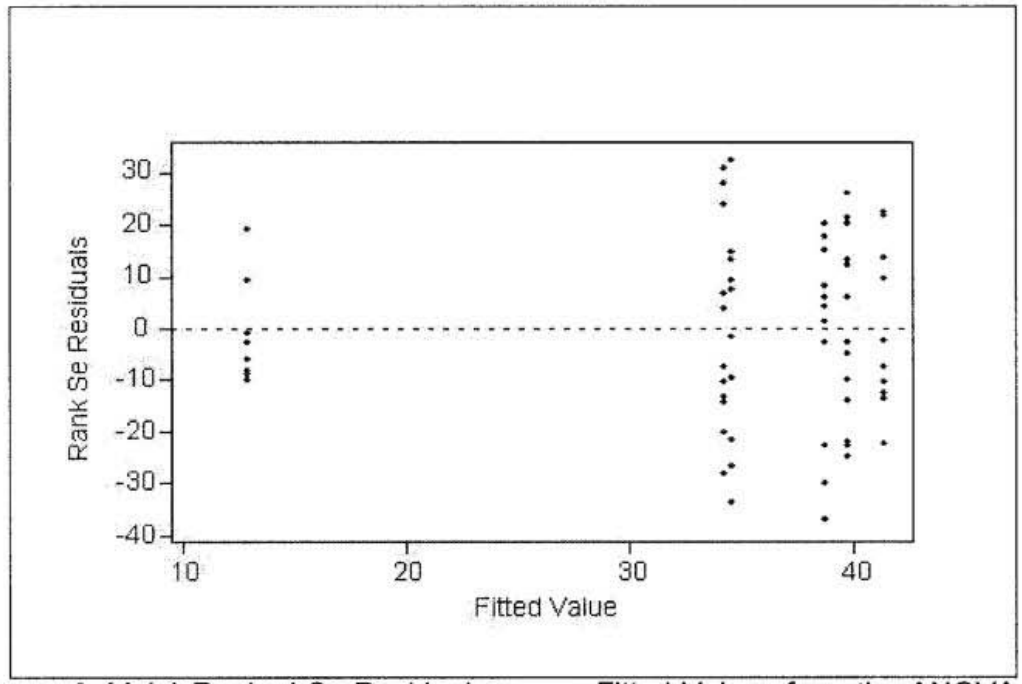

Figure A.11 (c) Ranked Se Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

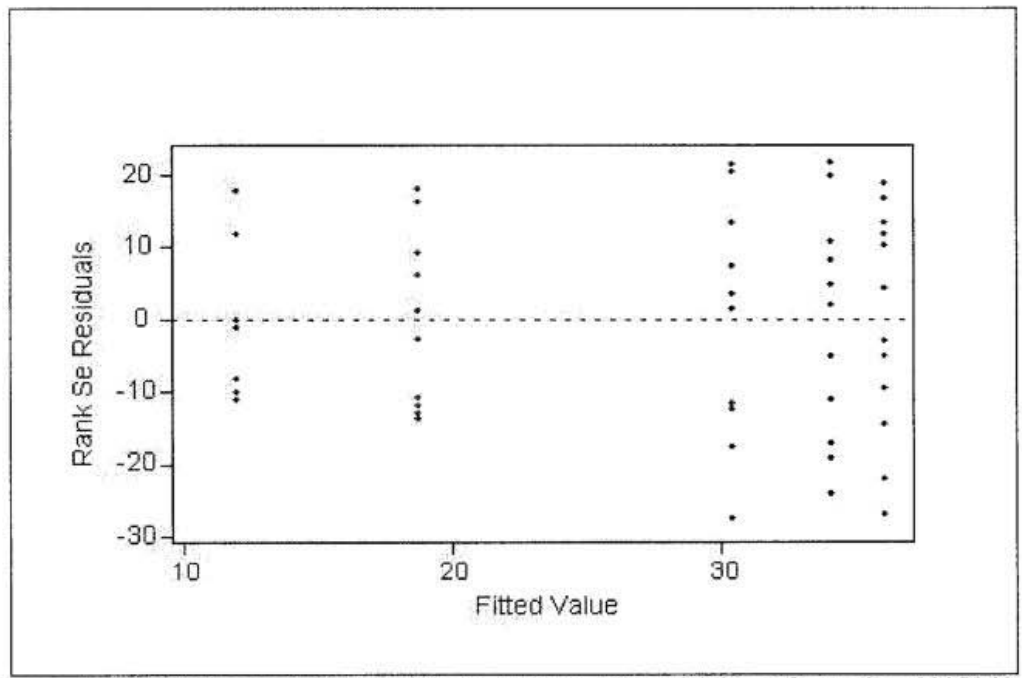

Figure A.11 (d) Ranked Se Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

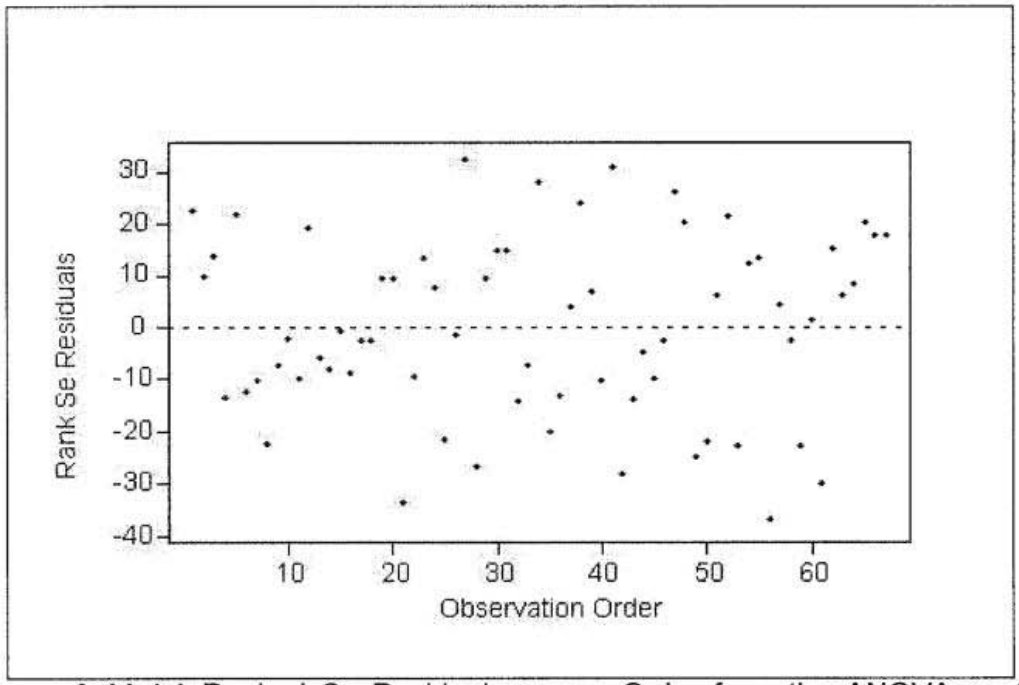

Figure A.11 (e) Ranked Se Residuals versus Order from the ANOVA on the Ranks of the Inner Region

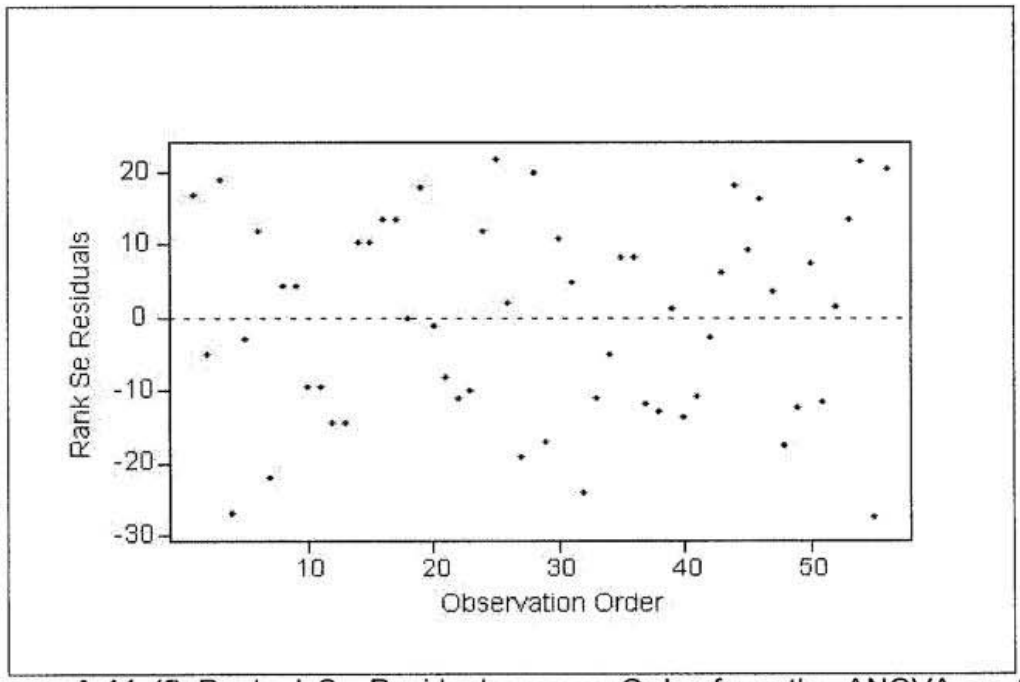

Figure A.11 (f) Ranked Se Residuals versus Order from the ANOVA on the Ranks of the Outer Region

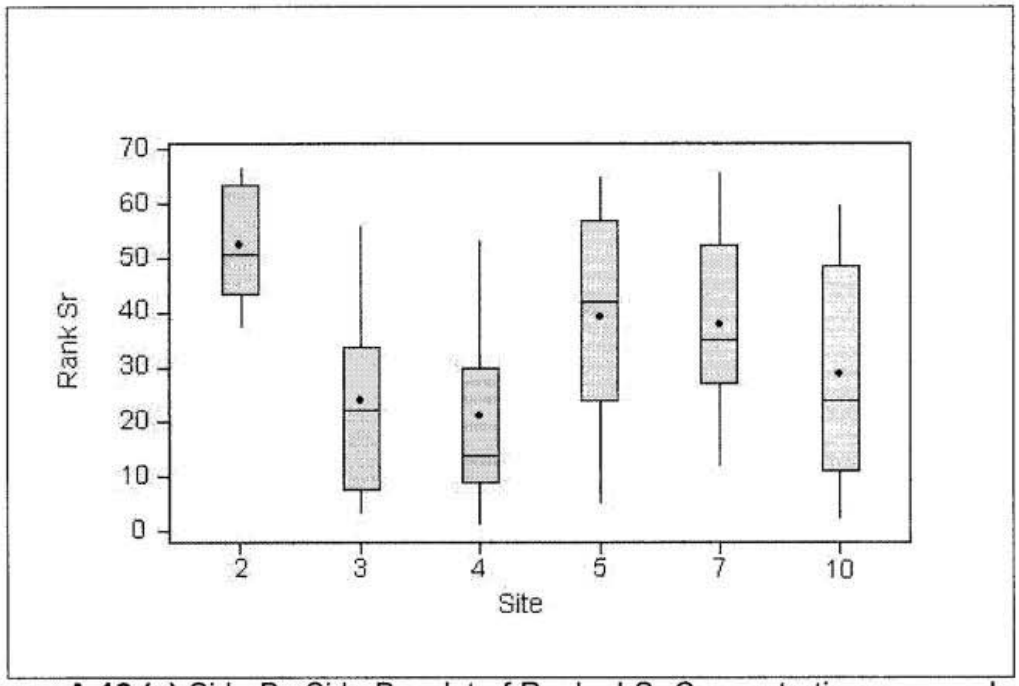

Figure A.12 (a) Side-By-Side Boxplot of Ranked Sr Concentrations versus Inner Region Site Numbers

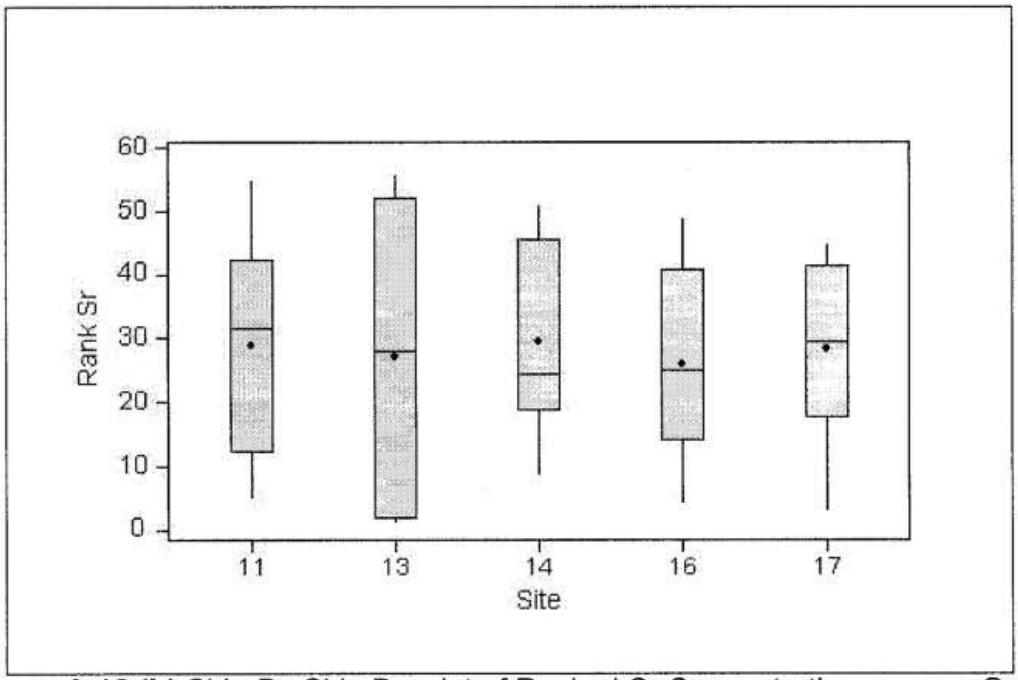

Figure A.12 (b) Side-By-Side Boxplot of Ranked Sr Concentrations versus Outer Region Site Numbers

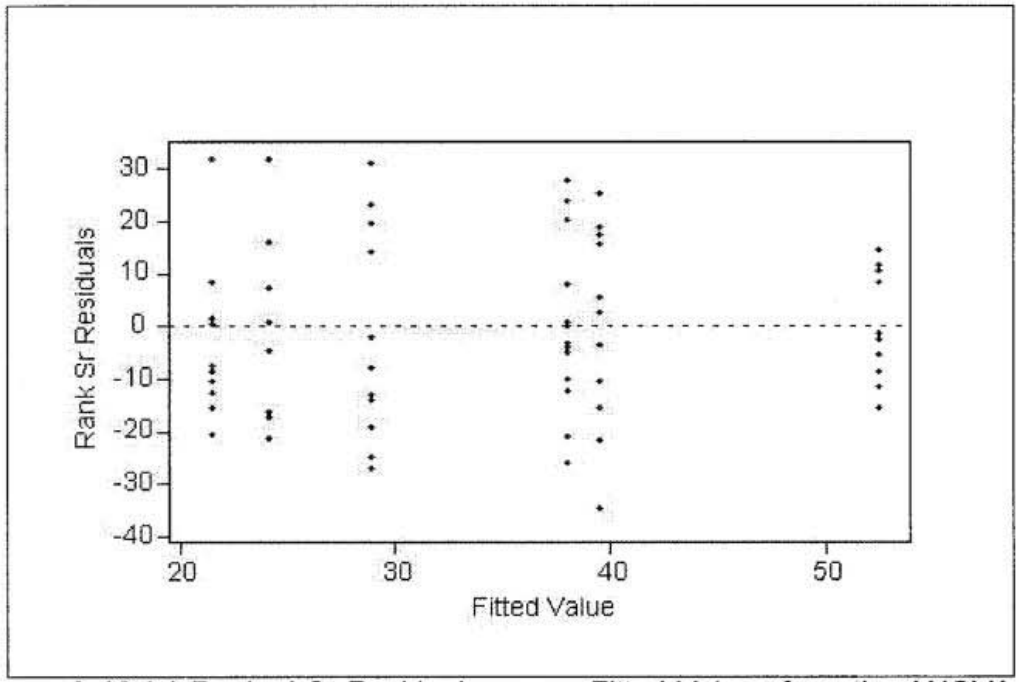

Figure A.12 (c) Ranked Sr Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

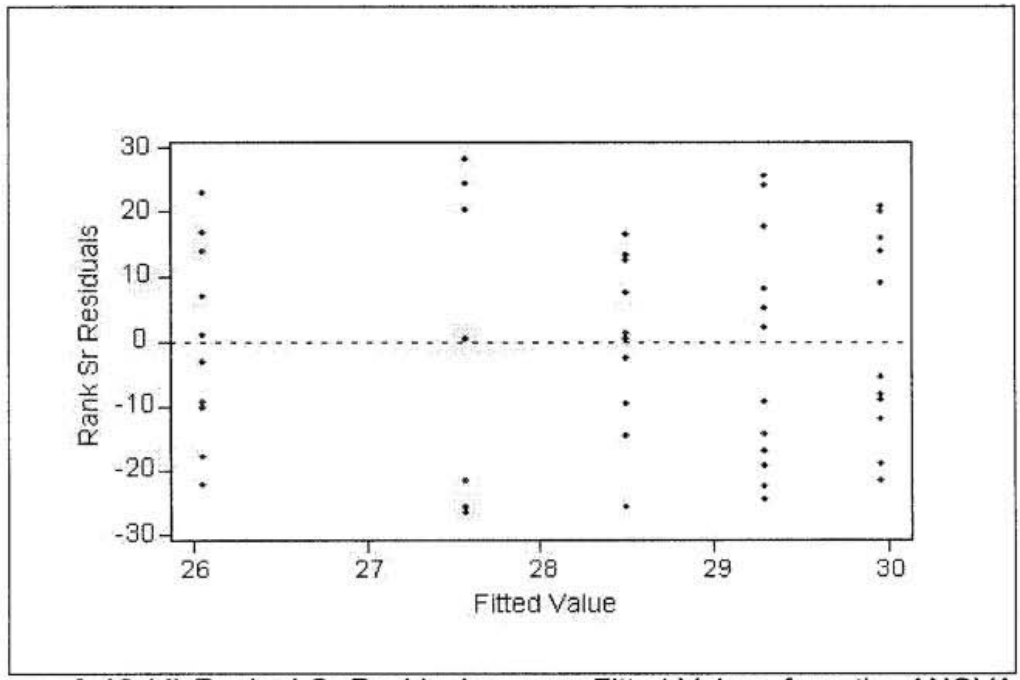

Figure A.12 (d) Ranked Sr Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

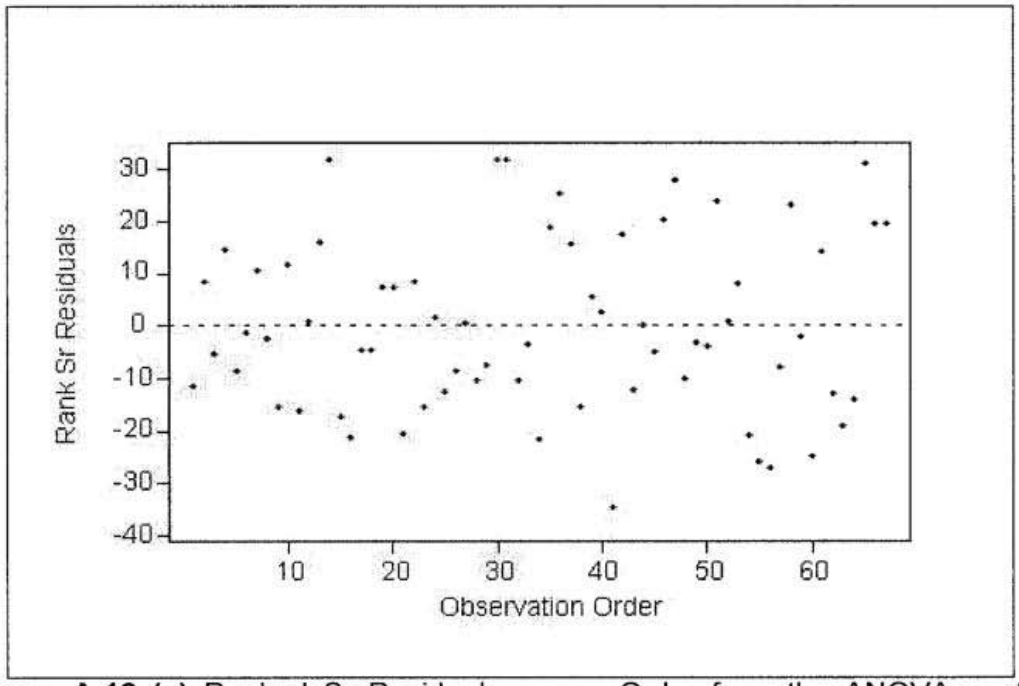

Figure A.12 (e) Ranked Sr Residuals versus Order from the ANOVA on the Ranks of the Inner Region

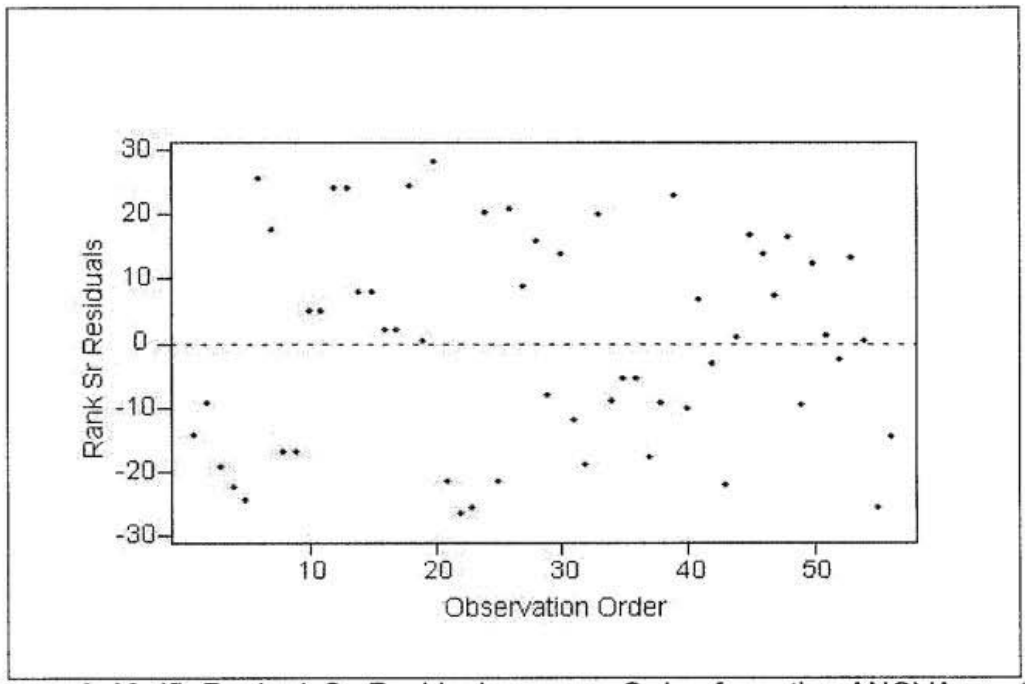

Figure A.12 (f) Ranked Sr Residuals versus Order from the ANOVA on the Ranks of the Outer Region

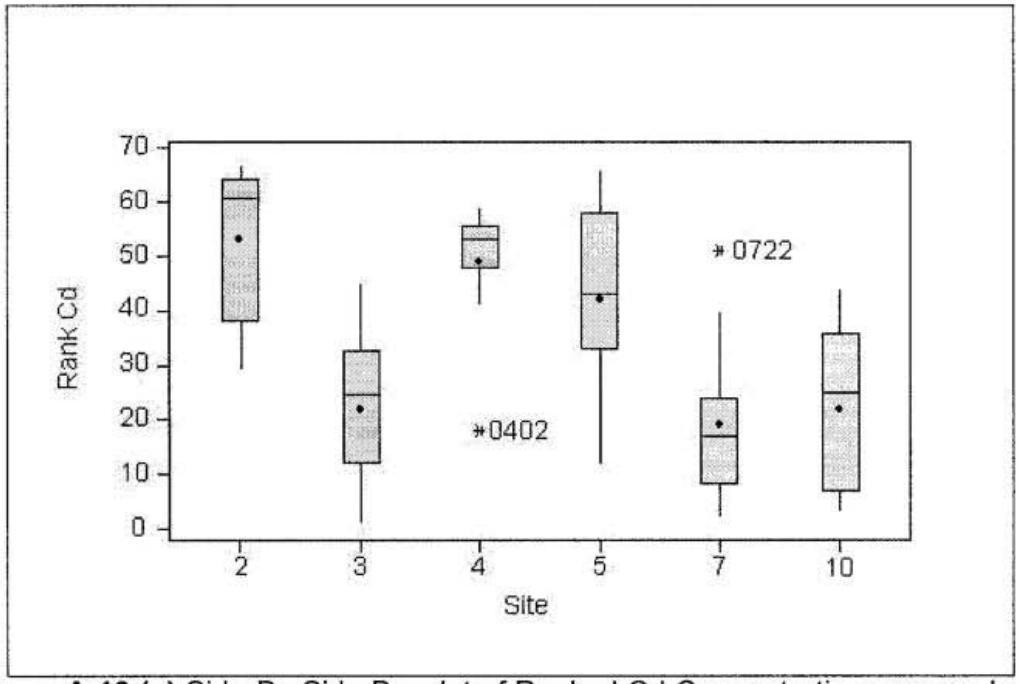

Figure A.13 (a) Side-By-Side Boxplot of Ranked Cd Concentrations versus Inner Region Site Numbers

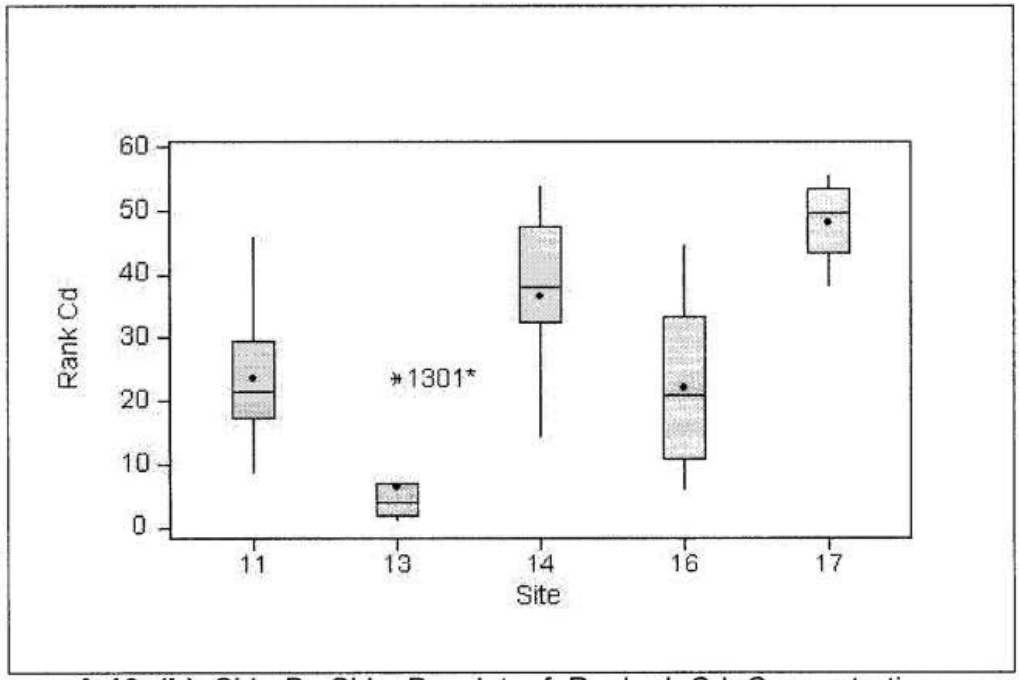

Figure A.13 (b) Side-By-Side Boxplot of Ranked Cd Concentrations versus Outer Region Site Numbers

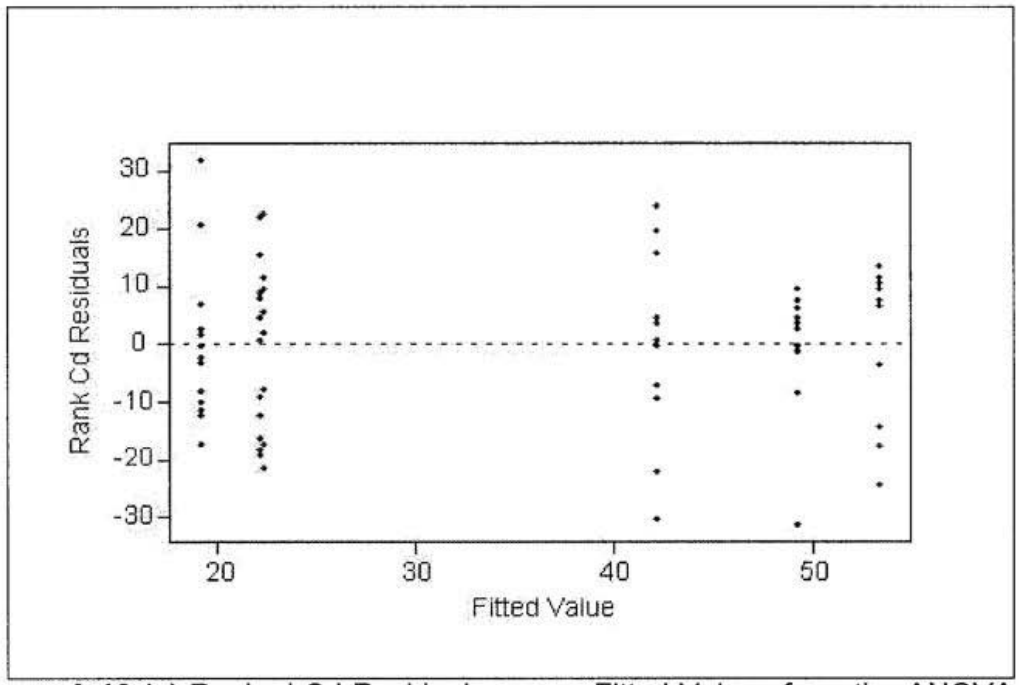

Figure A.13 (c) Ranked Cd Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

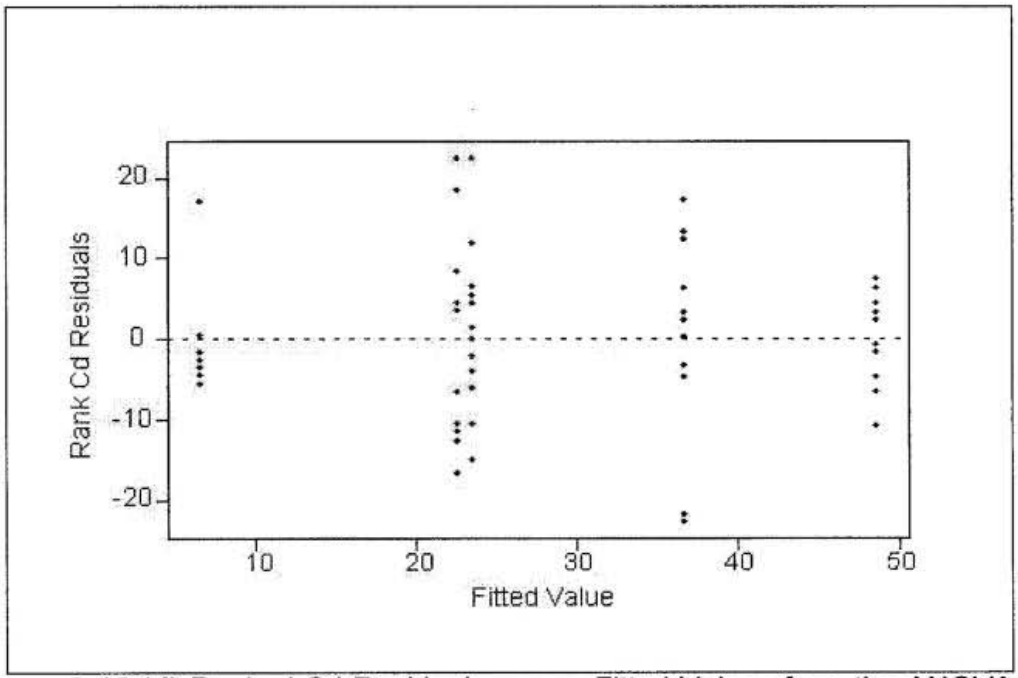

Figure A.13 (d) Ranked Cd Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

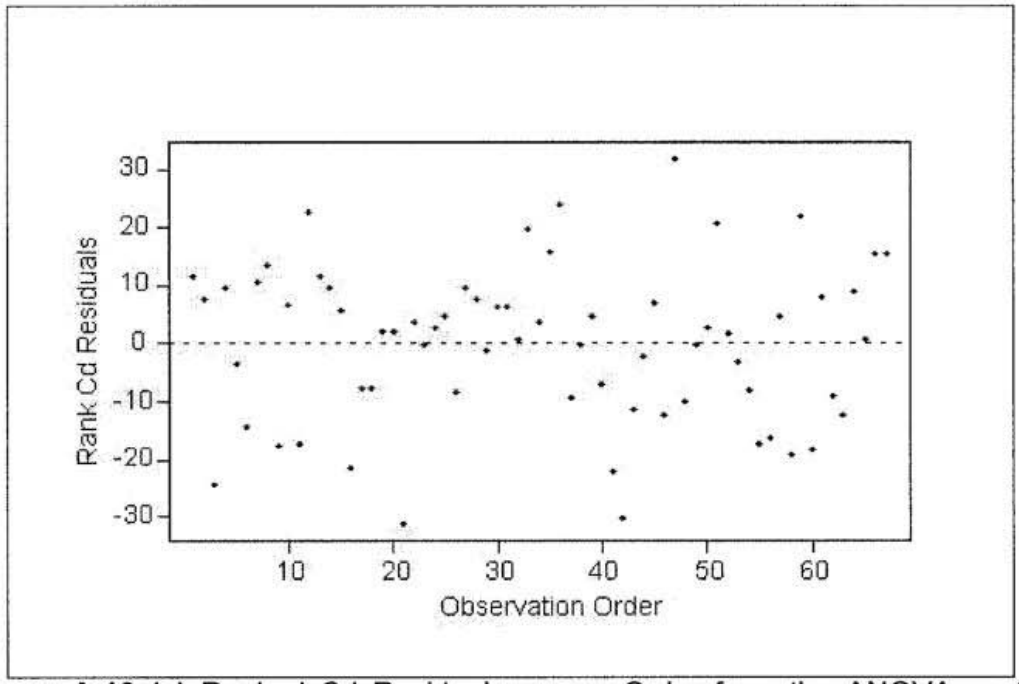

Figure A.13 (e) Ranked Cd Residuals versus Order from the ANOVA on the Ranks of the Inner Region

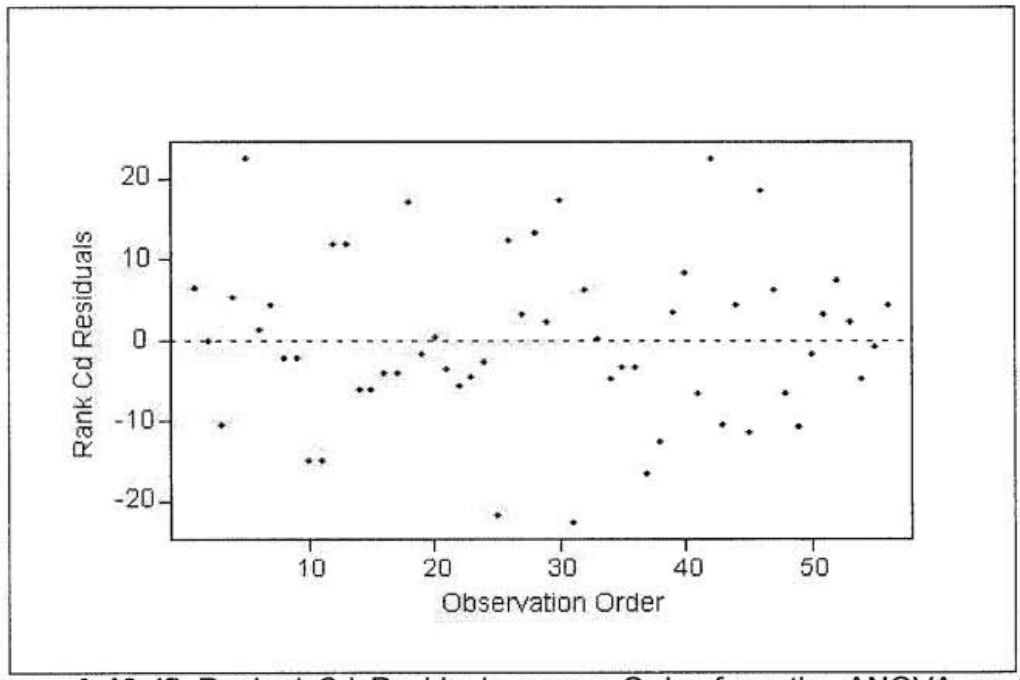

Figure A.13 (f) Ranked Cd Residuals versus Order from the ANOVA on the Ranks of the Outer Region

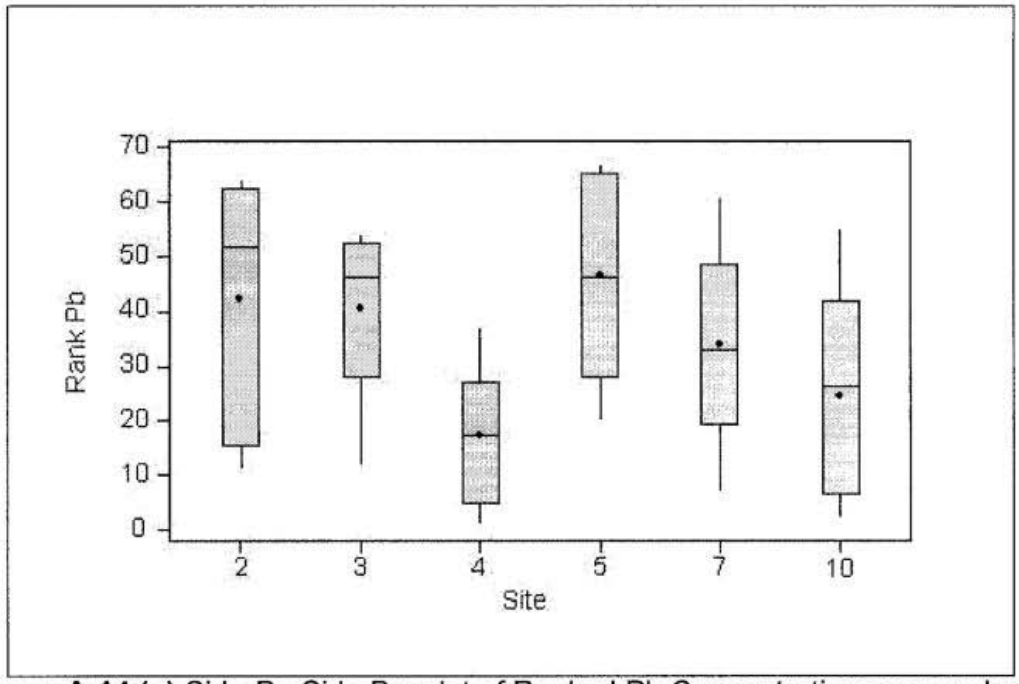

Figure A.14 (a) Side-By-Side Boxplot of Ranked Pb Concentrations versus Inner Region Site Numbers

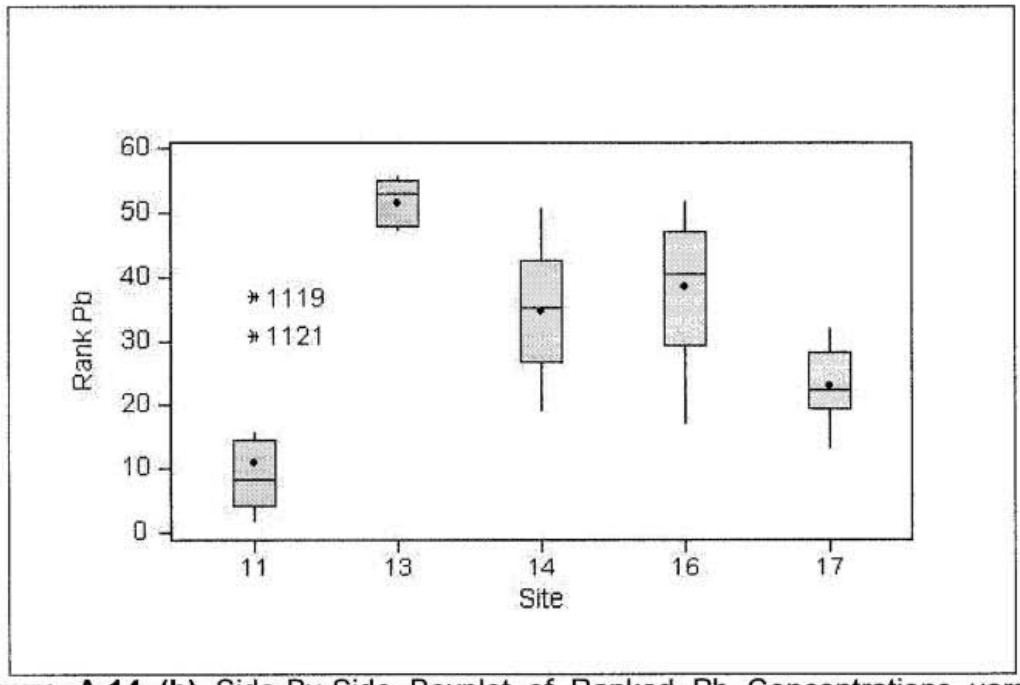

Figure A.14 (b) Side-By-Side Boxplot of Ranked Pb Concentrations versus Outer Region Site Numbers

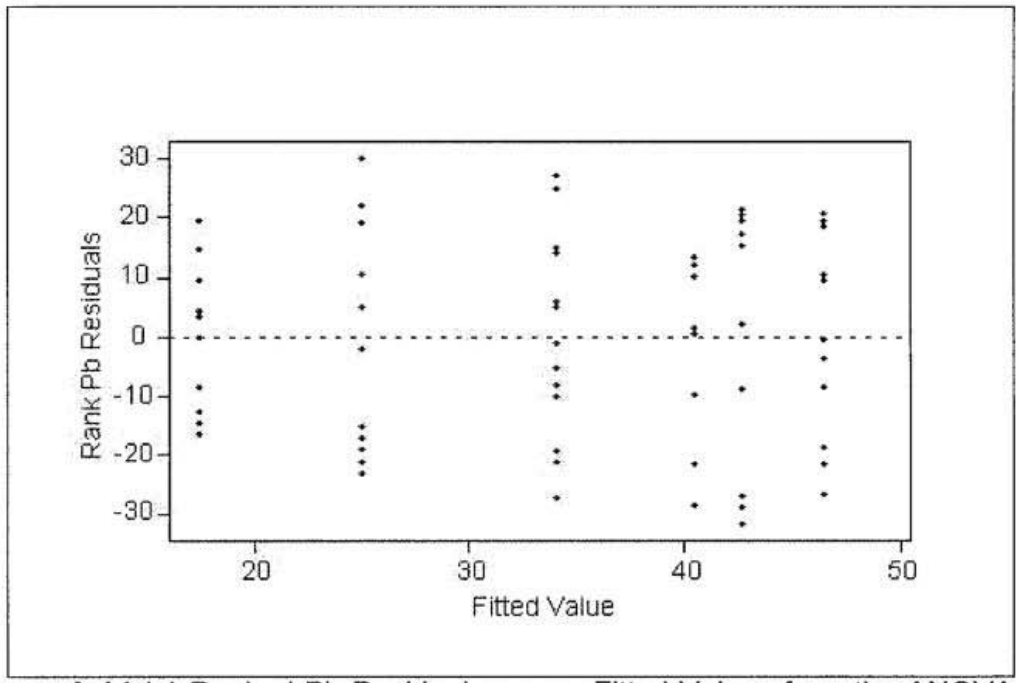

Figure A.14 (c) Ranked Pb Residuals versus Fitted Values from the ANOVA on the Ranks of the Inner Region

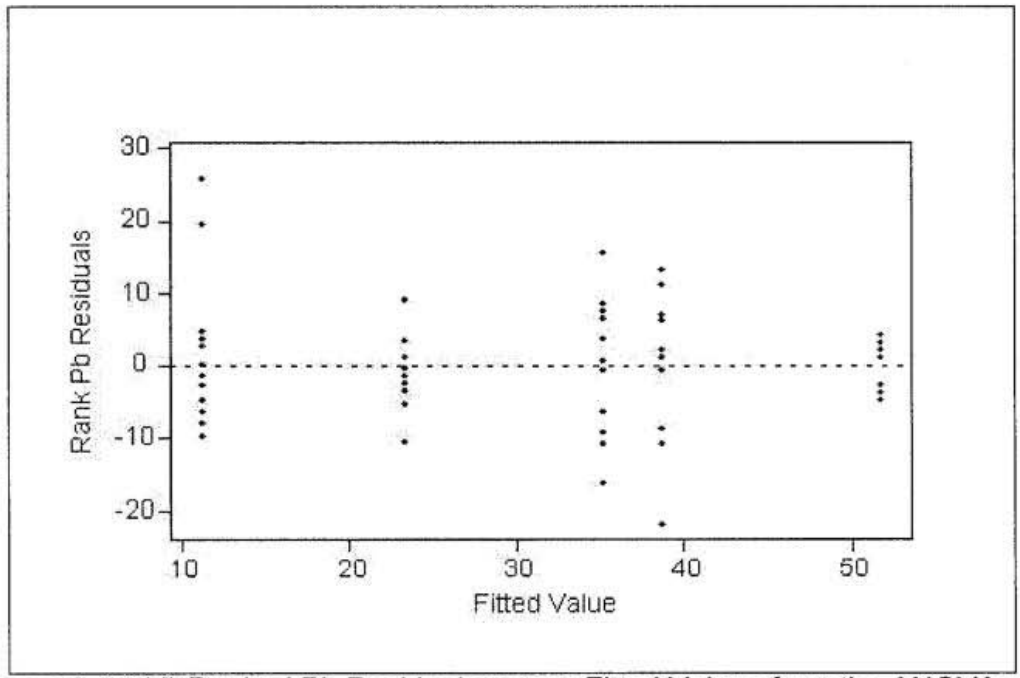

Figure A.14 (d) Ranked Pb Residuals versus Fitted Values from the ANOVA on the Ranks of the Outer Region

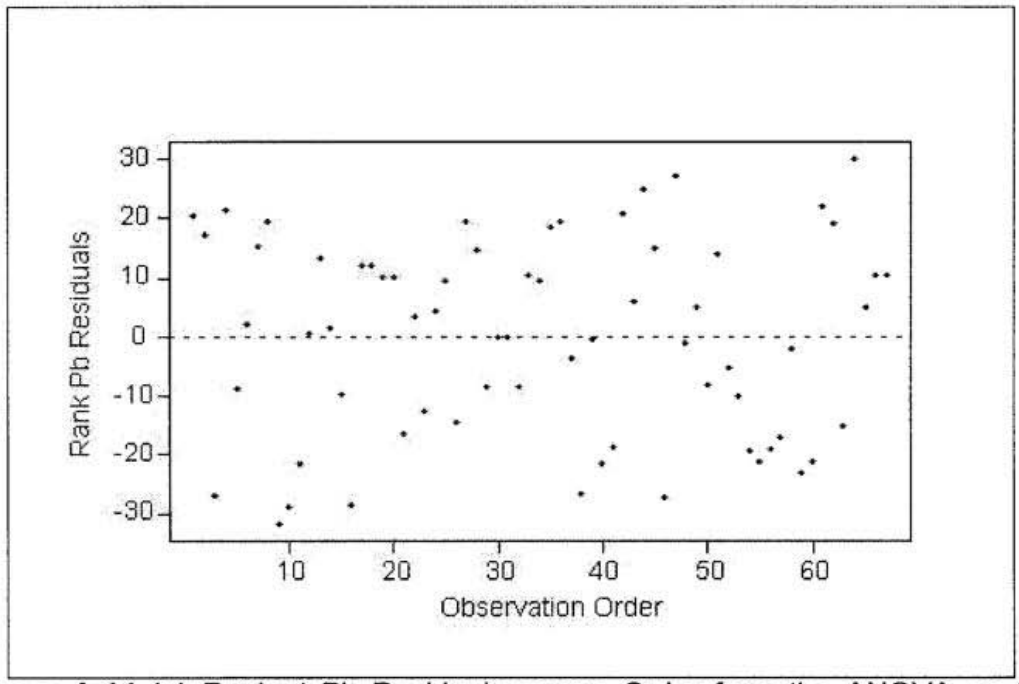

Figure A.14 (e) Ranked Pb Residuals versus Order from the ANOVA on the Ranks of the Inner Region

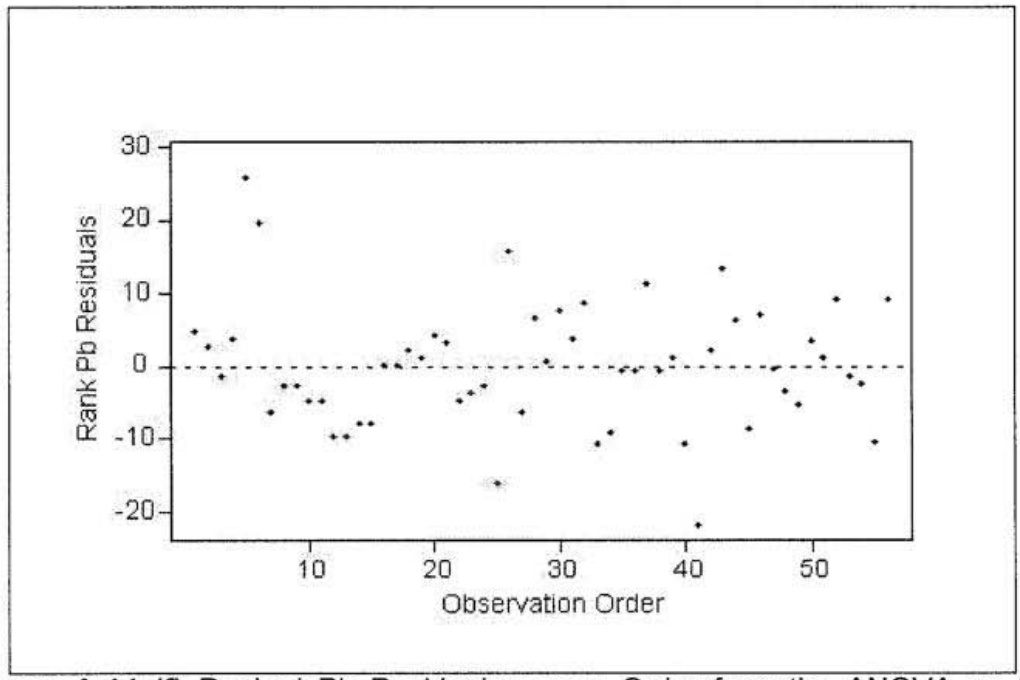

Figure A.14 (f) Ranked Pb Residuals versus Order from the ANOVA on the Ranks of the Outer Region

APPENDIX L

Charts Illustrating Changes in Analyte Concentrations from West to East

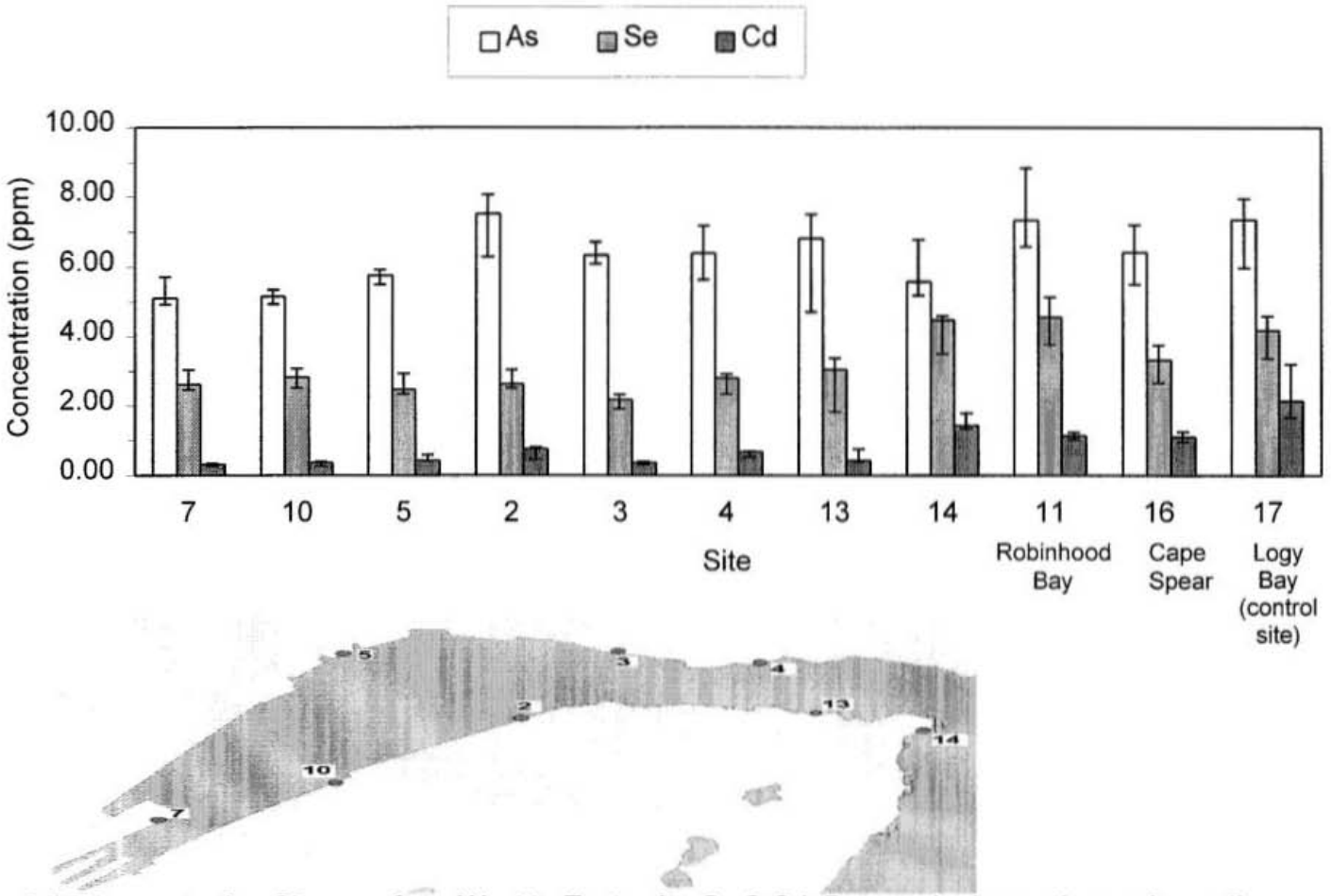

Figure A.15 Concentration Changes from West to East – As, Se & Cd: Bar graph shows change in median concentrations (μg·g-1) from inner to outer harbour regions. Error bars represent IQR. Geographic locations of sample sites are identified on the elongated map of St. John's Harbour below the bar graph.

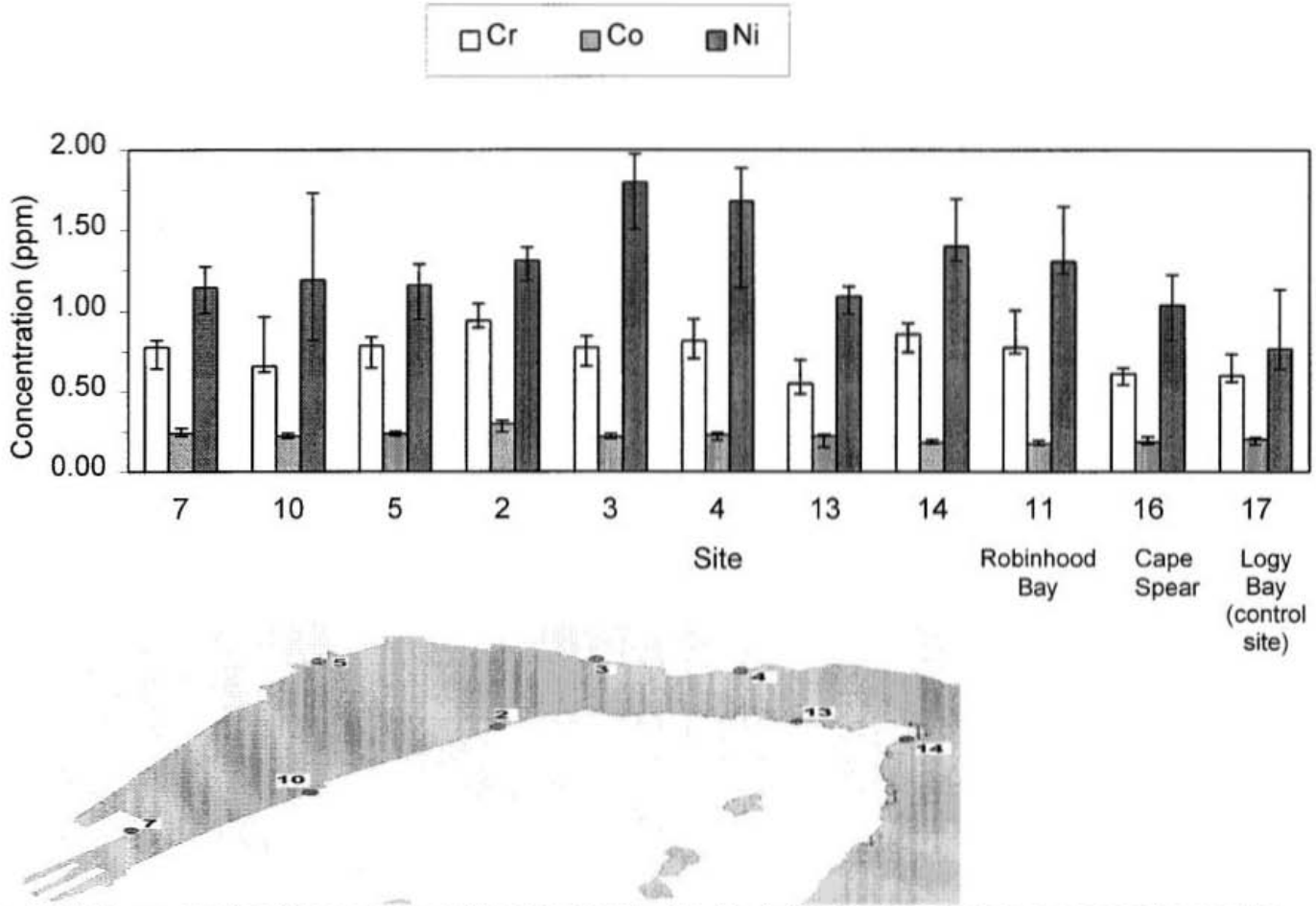

Figure A.16 Concentration Changes from West to East – Cr, Co & Ni: Bar graph shows change in median concentrations (μg·g-1) from inner to outer harbour regions. Error bars represent IQR. Geographic locations of sample sites are identified on the elongated map of St. John's Harbour below the bar graph.

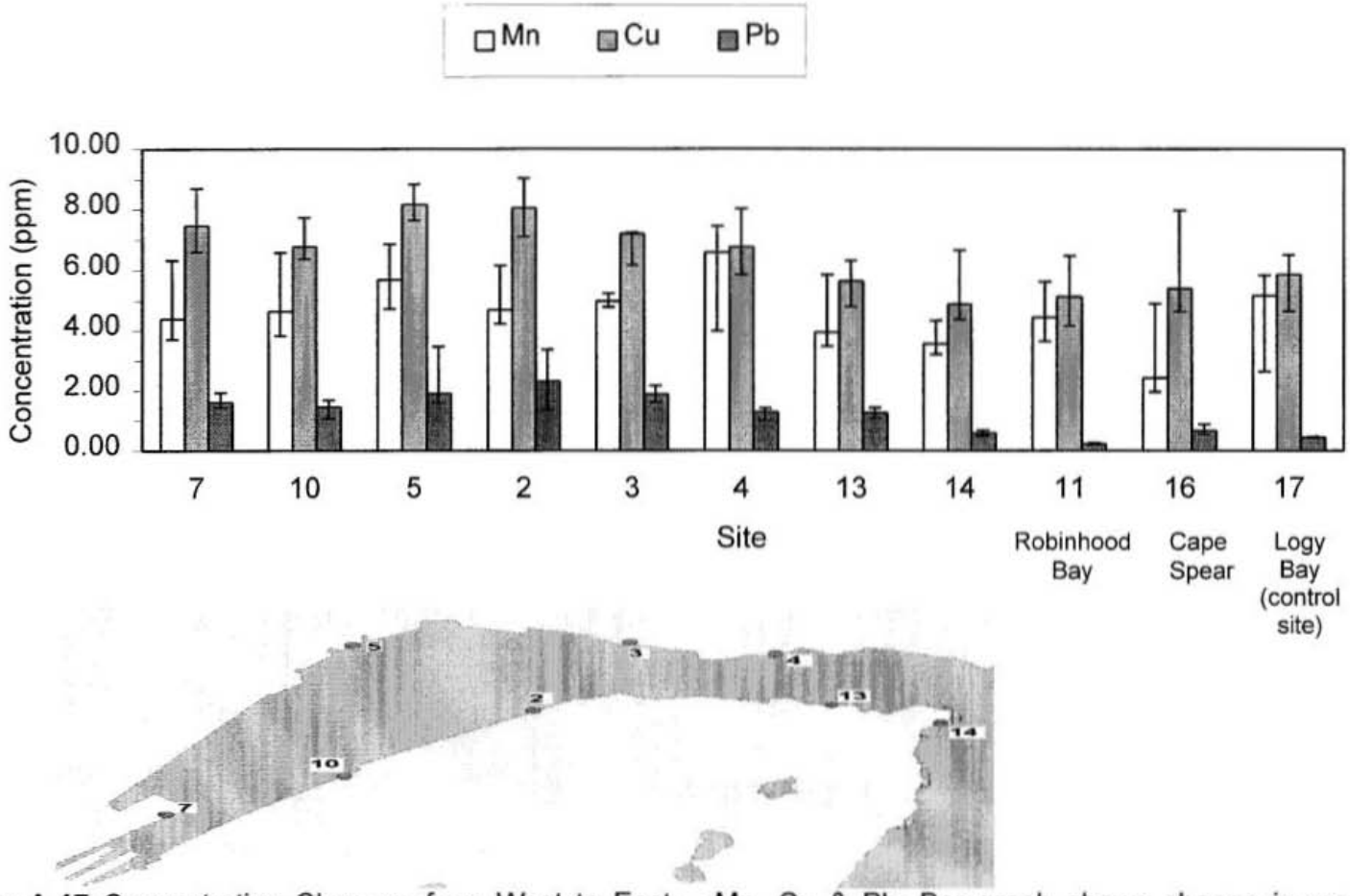

Figure A.17 Concentration Changes from West to East – Mn, Cu & Pb: Bar graph shows change in median concentrations (μg·g-1) from inner to outer harbour regions. Error bars represent IQR. Geographic locations of sample sites are identified on the elongated map of St. John's Harbour below the bar graph.

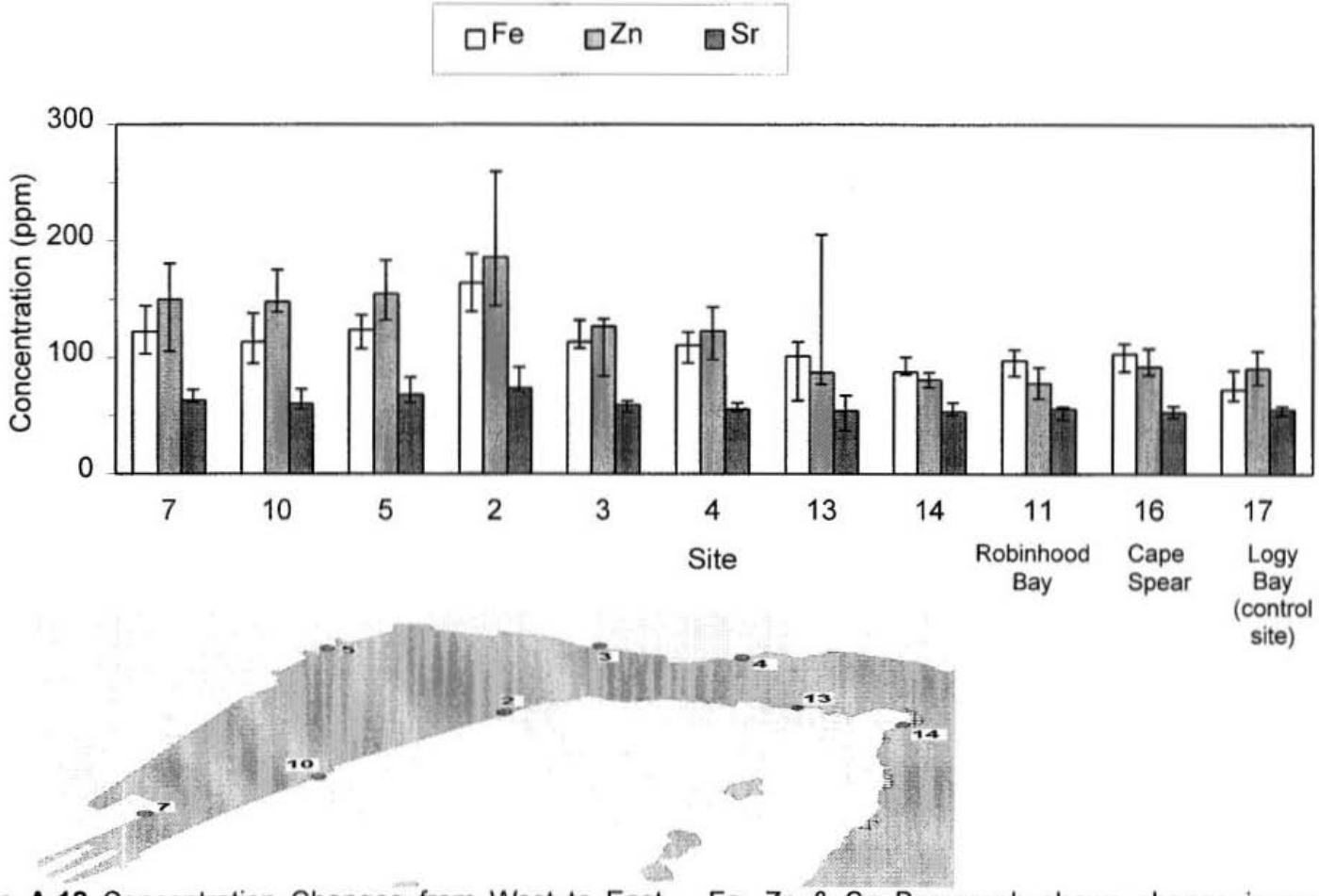

Figure A.18 Concentration Changes from West to East – Fe, Zn & Sr: Bar graph shows change in median concentrations (μg·g-1) from inner to outer harbour regions. Error bars represent IQR. Geographic locations of sample sites are identified on the elongated map of St. John's Harbour below the bar graph.

APPENDIX M

FDA Guidelines for Shellfish Consumption Compared to Observed Concentrations

Table A.11 FDA Guidelines for Shellfish Consumption Compared to Observed Concentrations: The guidelines (USFDA, 1993) are for individuals who consume shellfish at the mean rate and at the 90th percentile rate. Median concentrations observed for the inner and outer regions are shown. All values are expressed as $\mu g \cdot g^{-1}$, dry weight.

Element	Age Group	FDA Mean	FDA 90th percentile	Inner Region	Outer Region
Cr	2+ yrs (all ages)	100	65	0.784	0.729
	18-44 yrs (male/female)	85	55		
Ni	Adults	600	400	1.275	1.231
As	2+ yrs (all ages)	650	430	5.77	6.81
	2-5 yrs (male/female)	550	275		
	18-44 yrs (male/female)	550	360		
Cd	2+ yrs (all ages)	30	20	0.407	1.224
	18-44 yrs (male/female)	25	15		
Pb	Children 2-5 yrs	7.5	4.0	1.613	0.486
	Pregnant Women	10.5	7.0		
	Adults 18-44 yrs	31.5	21.0		