Cadmium in the Coastal Marine Environment: Pathways for Cadmium in Oysters and using the Cadmium: Phosphorus Ratio as an Indicator of Biogeochemical Processes

by

Priya Anka Lekhi

B.Sc., University of British Columbia, 2002

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Chemistry)

UNIVERSITY OF BRITISH COLUMBIA

September 2006

Abstract

Pacific oysters collected on the coasts of British Columbia (BC) have shown Cd levels of around 2 $\mu g g^{-1}$ and higher (wet weight) which has resulted in the loss of some international markets. During 2004-2005, two farm sites on Vancouver Island were monitored for various parameters including the Cd concentration in oysters, dissolved and particulate Cd in seawater, temperature and salinity. One part of this study investigated the source and transfer of Cd to oysters by focusing on the role of dissolved and particulate Cd in seawater. Results show that dissolved Cd is the main source of Cd to oysters and a seasonal trend was observed for Cd in oysters, where levels were lowest during periods of warmer temperatures. Factors such as input of water masses and sediment diagenesis were found to directly affect dissolved Cd and thereby, influence the Cd levels in oysters. Particulate matter was not a significant source of Cd to oysters and actually has a negative effect. This effect is likely due to the uptake of dissolved Cd by phytoplankton and to the uptake of phytoplankton by oysters, which effectively increases the tissue mass and dilutes the Cd content in oysters.

The other half of this study looked at the marine environment of these two locations using the Cd:P ratio as an indicator of many biogeochemical processes. Dissolved Cd and P, as well as particulate Cd, P and Ti were analyzed biweekly at one depth and throughout the water column three times during the year. In addition, a surface marine sediment sample was also analyzed. Cd:P is consistent in the deep open ocean, due to respiration processes but this consistency breaks down in the coastal waters. These two sites demonstrate that the coastal Cd-P ratio can be affected by phytoplankton species composition, phytoplankton abundance, sedimentary input, physical mixing of water masses with varying Cd-P ratios, and sediment diagenesis. Biological activity, highly affected by species composition and abundance, is generally the main factor affecting the Cd-P ratio, but other factors can also lead to variations of the ratio on smaller time and spatial scales.

Table of Contents

List of Tables

List of Figures

Acknowledgements

There are so many people that helped me throughout this project. I would like to thank my supervisor, Kristin Orians for her insight, patience, and guidance. I would also like to thank Maite Maldonado for all of the time and effort she put into helping me improve this thesis. Thank you to David Cassis- I will never forget all those hours we spent sampling and writing together. I learned a lot from you and thanks for putting up with my music during those long drives! Steve Calvert, Maureen Soon and Bert Mueller were all so helpful and instrumental to the completion of my studies. A special thanks to Maureen for being resourceful and kind. I would like to thank Nadene Ebell and Leah Walberg for collecting the samples and Chris Pearce for compiling the oyster data. To the Orians research group- in general, thanks for all the support and good chats. Specifically, I want to thank Sabrina for training me and for always being willing to answer my questions. I want to thank Cheryl for all of her guidance and tutoring and I want to thank Alyssa for all her great suggestions and for being so resourceful. Outside of science, I want to thank the extended team at TAG for making UBC more enjoyable and more brightening up many of my working days. Finally, I can not forget all my friends and family: Your constant support, patience and humor make this crazy life relaxing and delicious! Everyday I feel blessed for being able to share the trials and triumphs of life with such loving and beautiful people. I love you all.

Dedication

To my parents,

Your inner strength and hard work inspires the success I have in my life.

Co-Authorship Statement

This thesis was part of a larger project where biological and chemical oceanographers from UBC worked in collaboration, along with the Department of Fisheries and Oceans (DFO) and the Shellfish Growers Association (BCSGA). I worked closely with David Cassis, PhD Candidate, department of Oceanography. We co-authored the Technical Report for this project (cited in Chapters 2 and 3). Chapter 2 encompasses my part of this larger project. My contribution included the sample preparation and analysis of dissolved Cd, particulate Cd, Ti and P as well as preparation and analysis of the major and minor element in the marine sediment samples. The interpretations of the data and a version of the introduction of this chapter was co-authored with D. Cassis. Chris Pearce (DFO) compiled the oyster data. Chapter 3 is my own interpretations but the biomass data was analyzed by D. Cassis and the section in methods, 3.2.5 "Quantitative Phytoplankton" was written by D. Cassis.

1 Chapter 1: Introduction

1.1 *Research Project Description and Main Goals*

Oysters grown along the Pacific coast have shown relatively high concentrations of cadmium (Cd) at around 2 μgg^{-1} (wet weight). In late 1999 and early 2000, successive batches of oysters were rejected by the Hong Kong Food and Environmental Hygiene Department because the Cd concentrations in the product exceeded the 2 μgg^{-1} wet weight standard set by this market. In other markets, the limits vary between 1 μgg^{-1} (European Union) to 3.7 μgg^{-1} (United States Food and Drug Administration recommended guideline). Health Canada has no concentration limits for Cd in oysters but does have consumption guidelines for Canadian consumers. With the current concentrations of cadmium, BC oyster farmers are limited mainly to local and US consumption. The loss of markets is a fairly recent problem but the issue with Cd in oysters dates further back. Environment Canada and Department of Fisheries and Oceans show data on wild oysters collected in northern extent of the Strait of Georgia, where Cd residues were approaching 2 μgg^{-1}, 27 years ago (Kruzyski, 2002).

The project's main goals were to investigate the seasonal trend of Cd in cultured oysters and to study the pathways of Cd to oysters. Two farm locations, on the east and west coasts of Vancouver Island, were monitored for one year for Cd in oysters and for several environmental parameters including dissolved and particulate Cd. This research initiative was a cooperative effort where biological and chemical oceanographers from UBC worked in collaboration, along with members of the British Columbia Shellfish Growers Association (BCSGA), and the Department of Fisheries and Oceans (DFO).

1.2 *Background*

1.2.1 Sources of Cd in Seawater

Cadmium, like most elements, exists in the earth's crust and in the marine environment. The crustal abundance of Cd is around 0.15 μgg^{-1} whereas in surface seawater, Cd averages 0.11 ngg^{-1} (Lide, 2006).

Although Cd is naturally present in seawater, there is also input from anthropogenic sources. Anthropogenic uses of Cd include electroplating and coating of metals and alloys for protection against corrosion, pigments in paints and in phosphate fertilizers. Smaller amounts of cadmium compounds are used as stabilizers in plastics, as catalysts in pesticides and in the production of nickel-cadmium batteries. In the crust, Cd is most commonly found as a sulfide deposit associated with the zinc ore, sphalerite (ZnS), and is released into the environment during the processing of zinc. As a result of these uses, Cd can be released and subsequently enter the marine environment.

1.2.2 Vertical Distribution in the Open-Ocean

There are three major categories of distribution profiles for trace metals in the oceans: conservative, nutrient-type and scavenged. Conservative metals are non-reactive and have profiles that are governed by physical processes which affect all ions, such as dilution or concentration by evaporation. Scavenged metals are reactive and typically have maxima at the surface and/or bottom water, at the point of input (atmospheric, riverine or marine sediments). A minimum in the distribution is observed at a distance from the source due to removal via adsorption onto sinking particles. A nutrient-type distribution shows surface water depletion and an increase with depth to a maximum coincident with that of nutrients such as phosphate and nitrate, followed by relatively high concentrations that decrease slightly with depth (Fig 1-1). Nutrients are removed from the surface waters by phytoplankton and are subsequently regenerated at depth through the process of respiration as the biological matter sinks. Inter-ocean fractionation is observed for all nutrient types. Deep water formed in the North Atlantic travels south, around Antarctica, and ends up in the North Pacific Ocean, making the deep water of the North Pacific the "oldest" deep water. This old water has accumulated nutrients from sinking particles for ~1000 years so the concentration at depth in the North Pacific is 2-5 times greater than the concentration of the fairly "young" deep water of the North Atlantic (Bruland and Franks 1983). Cadmium was initially thought to be a non-essential element but exhibits a nutrient type distribution and correlates with phosphate and nitrate (Fig. 1-1).

1.2.3 Upwelling

Deeper, colder, nutrient and Cd-rich waters can be transported to the surface through the process of upwelling. The local coasts of Vancouver Island are recognized as upwelling regions which is mainly wind-driven (Thomson *et al.*, 1989). Winds push away surface water which causes deeper water to rise and replenish the surface water. Upwelling can also be caused by deeper water forcing its way upward by tidal currents that are deflected upward by underwater ridges or other topographic features on the channel bottom. This type of upwelling allows water to be raised from deeper depths than wind-driven upwelling. Because upwelling brings in nutrient rich water up to an area which is nutrient-depleted, upwelling regions tend to be highly productive.

1.2.4 Biochemical cycling of Cd

Cd is present in dissolved and particulate forms in seawater. In the dissolved form, it is mainly present as chloro-complexes. Dissolved cadmium includes simple hydrated metal ions, metal ion complexes with inorganic anions and metal ion complexes with organic ligands. Particulate sources include cadmium sorbed onto suspended mineral and organic particles and cadmium present in phytoplankton and mineral particles. Although dissolved cadmium has a nutrient-type profile in the open ocean, it was not initially known to have any biological role. It has now been shown that cadmium can indeed have a nutrient role by substituting for zinc in the metalloenzyme, carbonic anhydrase, in the marine diatom, *Thalassiorsira weissflogii* (TW) (Price and Morel, 1990). Carbonic anhydrase is a metalloenzyme which functions to hydrolyze HCO_3^- to CO_2. During photosynthesis, the enzyme Rubisco coverts inorganic carbon, in the form of CO_2, to organic carbon. However, in the oceans, inorganic carbon primarily exists as HCO_3^-. Carbonic anhydrase (CA) is used to speed up the conversion of HCO_3^- to CO_2 . Zinc is a cofactor of CA and since carbon acquisition is essential to cell growth, the amount of zinc may limit the rate of primary production and CO_2 fixation. Subsequent studies have demonstrated that Cd can accelerate the growth of phytoplankton species other than TW in zinc-limiting conditions (Lee and Morel, 1995, Cullen *et al.*, 1999, Lane and Morel 2000). Moreover, it has been shown that Cd can be directly used in a Cd-specific form of

the enzyme under zinc limiting conditions (Lane and Morel, 2000; Cullen *et al.*, 1999). Given the role of cadmium in the hydrolysis of HCO_3^- to CO_2, it lends itself to the notion that the levels of CO_2 play a role in the uptake of cadmium. Studies have demonstrated that at low pCO_2 (100 μgg^{-1}) the uptake of cadmium is two to three times higher than at greater levels of CO_2 due to the increase activity of carbonic anhydrase in such conditions (Cullen *et al.*, 1999). The beneficial role of Cd has only been observed over a narrow range of free Cd (Cd^{2+}) for studied species. Outside this range, Cd could have no effect or it could be toxic (Lee and Morel, 1995).

Cadmium's toxicity at high concentrations is either due to the destructive replacement of Zn by Cd in enzymes or by the ineffective replacement of Mn by Cd. Due to non-specific binding, similar sized metals will compete for binding sites. These sites can either be for the uptake of a certain metal along the cell membrane, for internal transport, or for a metabolic role within the cell. In marine diatoms, at low levels of Zn, Cd is taken up by a single low-Zn inducible system but with high levels of zinc, Cd uptake is by the Mn transport system (Sunda and Huntsman, 1998b). Mn is an essential nutrient as it serves as a cofactor in certain enzymes. Thus, cells have transporting sites intended for Mn along their membranes, but because Cd is chemically similar, in terms of its ionic radius and coordination geometry (Sunda and Huntsman, 1998a), it will bind to those sites and get transported into the cells. It is thought that free cadmium ions are mainly available for phytoplankton uptake. Up to 70% of total cadmium in surface waters up to 175m in the Central North Pacific ocean is complexed with organic ligands (Bruland, 1992) which generally do not dissociate rapidly enough to free Cd^{2+} for uptake by phytoplankton (Sunda and Huntsman, 1998a). A bulk of the remaining Cd will be held in chloro-complexes, governed by salinity (as Cl^- varies conservatively with salinity). Chlor-complexes are labile so Cd in this form can be taken up by phytoplankton (Sunda and Huntsman, 1998). The resistance to Cd toxicity varies greatly among phytoplankton species and it is suggested that most species produce proteins rich in sulfur and nitrogen, such as phytochelatin, in response to Cd stress (Payne and Price, 1999; Lee and Morel, 1995). The congruence between the dissolved chemistry of the ocean and the composition of phytoplankton was described by Alfred Redfield in 1934. He found that the average elemental composition of N:P in phytoplankton, under non-limiting conditions was 16:1, which matched the average dissolved N:P ratio in the oceans. The present-day Redfield ratio refers to

the stoichiometric ratio of C:N:P of 106:16:1. Whether it is life that is dictating what is present in its environment or the environment dictating what is present in life, the Redfield ratio has been fundamental in our understanding of the biochemistry of the oceans. Although the ratio is an average, it serves as a reference that can be indicative of biochemical processes. In accordance to the importance of trace metals in controlling primary production, this ratio has been extended to include trace metals. Experimental analysis on specified representative phytoplankton species have demonstrated an average elemental stoichiometry of C:N:P:Cd to be 124:16:1:0.00021 (Ho *et al.*, 2003). Thus, a ratio of Cd-P in particulate matter similar to 0.00021:1, suggests that particulate matter is of biogenic origin.

1.2.5 The Global Cd:P relationship

It has been demonstrated that dissolved Cd is very closely correlated with dissolved phosphate (Boyle *et al.*, 1976 and Bruland *et al.*, 1978). This relationship suggests that cadmium is taken up by phytoplankton at the surface and is re-mineralized at depth upon oxidation of sinking organic particles. . The strong correlation between Cd and PO_4^{3-} lead to investigations of a global oceanic Cd-P relationship. Plots of dissolved Cd versus PO_4^{3-} show good linearity for different data sets collected in various regions with the regressions of each region being similar to one another. Combining all data of the deep ocean (>1000m) onto one plot, shows two clusters with separate linear regressions of Cd versus phosphate (fig. 1-2). The break in the slope of the line is commonly referred to as the "kink" in the relationship. Presently, it is considered that that the global Cd-P relationship is best described by two separate Cd-P regressions; one for phosphate concentrations lower than 1.3μM which describes North Atlantic data and one for phosphate concentration higher than 1.3μM (Boyle, 1988), which describes the Indian-Southern-and Pacific Ocean data. The higher nutrient data corresponds to older water masses. This strong relationship has been used with records of the Cd/Ca ratios in $CaCO_3$ shell deposits to reconstruct past Cd distributions and via, the Cd-P ratio, P distributions (Boyle, 1988). A criticism of this technique is that is assumes that the Cd-P ratio has remained constant through time and from region to region (de Baar *et al.*, 1994).

The mystery behind the breakdown of the Cd-P ratio at the surface waters and the kink of the relationship has been widely studied. The dissolved Cd-P ratio changes widely in the surface layers of the ocean (<1000m); the ratio rapidly increases from the surface to the thermocline, reaching higher values in deep waters (de Baar *et al.*, 1994). The deviation of the ratio in surface waters has been credited to the role of phytoplankton in the uptake of Cd and phosphate. The dissolved ratios in the surface waters are lower (de Baar *et al.*, 1994) which suggests the preferential take up of Cd over PO_4^{3-} by phytoplankton followed by the sinking and remineralization of relatively high Cd-P particulate matter (Saager and de Baar, 1993). As mentioned, it has been demonstrated that the uptake of cadmium by phytoplankton is related to levels of Zn and Mn (Sunda and Huntsman, 1998) and ρCO_2 (Cullen *et al.*, 1999). The interactions of these metals with phytoplankton can affect the Cd-P ratio. For example, under conditions of low Zn and Mn, and relatively higher Cd, phytoplankton take up Cd by their Mn transport system (Sunda and Huntsman, 2000) and under conditions of low ρCO_2, the cellular concentrations of cadmium increases (Cullen *et al.*, 1999). This has strong implications for the use of Cd-P as a paleoceanographic tracer as with levels of ρCO_2 changing throughout time, the levels of Cd-P will by affected. Moreover, in conditions of Fe-limitation, the cellular Cd content increases with decreasing Fe availability (Sunda and Huntsman, 2000, Cullen *et al.*, 2003). The preferred uptake of dissolved Cd in iron-limiting conditions reduces the dissolved Cd-.P ratio.

The kink of the global Cd-P relationship occurs when PO_4= 1.3 μM. It has been proposed that the kink is due to the input of Cd-depleted Subantarctic waters into the intermediate waters of the global ocean, (Frew and Hunter, 1992) and the formation of Antarctic bottom water which has a major component of near surface waters which presumably has a high Cd-P ratio (Frew, 1995). The role of phytoplankton in explaining this kink has also been investigated. Specifically, the mysterious kink has been link to an older and now resolved oceanographic mystery: the High Nutrient Low Chlorphyll (HNLC) regions. Normally, phytoplankton growth is limited by levels of the dissolved macronutrients (nitrate, phosphate and silicate) but regions of the subarctic Pacific, the equatorial Pacific, and the Southern Ocean are characterized by high nutrient concentrations and yet low phytoplankton growth and thus, low levels of chlorophyll (Fig 1-3). In 1990, John Martin first proposed the "Iron Hypothesis" to explain this phenomenon in which he suggests the limited phytoplankton growth is due to lack of iron in these regions. These regions are all far

from any large deserts, so dust (and therefore iron) deposition is very limited. Evidence has shown that the ratio of Cd-P in the surface waters of HNLC regions is lower than in a nearby highly productive Fe-replete region (Cullen, 2006). This suggests that the kink may be due hyperaccumulation of Cd by Fe-limited phytoplankton in high latitude HNLC regions. In iron sufficient areas, phytoplankton take up less Cd at the surface and have a relatively lower Cd-P ratio. Once they sink and are remineralized, the same low Cd-P ratio is released in the deep water. In iron-limited areas, phytoplankton have a larger intracelluar Cd-P ratio which translates to a smaller dissolved Cd-P ratio in the surface waters. Once the remains of the phytoplankton, carrying the same high intracellular Cd-P ratio, sink and remineralize, the dissolved Cd-P ratio in the deep waters increases (Cullen, 2006).

The variation in the global Cd-P relationship regionally and over time and the proposed reasons for these variations has strong implications for the use of this relationship in paleoceanography. For example, during the last glacial maximum, when levels of atmospheric CO_2 were lower than present-day, the dissolved Cd-P ratio would be lower than the modern ratio. However, the Cd-P relationship with its variations is also a useful reference to understanding biochemical interactions.

1.2.6 Geochemical Cycling of Cd

Marine sediments serve as a reservoir or as a sink for metals in overlaying water. There are different pathways for cadmium to the sediments depending on its form. Cadmium that is taken up by phytoplankton is non-reversibly bound to either the soft tissue or hard parts of the organism; the metal is only released upon remineralization of the host (Boyle *et al.*, 1976). Thus, dead phytoplankton sinks to the ocean floor as organic detritus or fecal material and is deposited. Cadmium that is adsorbed onto the surface of organic or inorganic particles also settles to the sediments. If organic matter containing cadmium is remineralized, cadmium is released in the dissolved form. Dissolved cadmium can also be removed from seawater to the sediment through precipitation due to changes in redox conditions at the bottom waters, near the sediment-water interface. As organic matter sinks from the surface, it begins to become oxidized:

$$CH_2O + O_2 \rightleftharpoons CO_2 + H_2O \quad (1)$$

When O_2 runs out in the deeper water, or in sediment pore waters, alternate oxidants are used. First nitrate is reduced (2), followed by Mn and Fe reduction (3 and 4) and lastly in the water column there is reduction of sulfate (5):

$$CH_2O + NO_3 \rightleftharpoons CO_2 + N_2 + NH_3 + H_2O \quad (2)$$

$$CH_2O + MnO_2 \rightleftharpoons CO_2 + Mn^{2+} + H_2O \quad (3)$$

$$CH_2O + FeOOH \rightleftharpoons CO_2 + Fe^{2+} + H_2 \quad (4)$$

$$CH_2O + SO_4^{2-} \rightleftharpoons HCO_3^- + HS^- \quad (5)$$

Organic matter remaining in the sediments after these oxidants are used up may undergo fermentation. The preservation of organic matter in the sediments depends on the balance between supply and removal rates. Under the condition of a large supply of organic matter to the bottom waters, either due to high primary productivity in the overlaying water column, or to shallow water depths, which decreases the chance for the organic matter to become oxidized as it sinks, the waters near the sediment interface can become anoxic. This will lead to the formation of HS^-, which preferentially precipitates Cd under reducing conditions, as CdS, to the sediments. Along the coast of BC, in many areas, Cd levels in the sediments are enriched (Pederson *et al.*, 1989). This enrichment has been attributed, not to anthropogenic input but to the coupling of high organic matter deposition and sulfate reduction which enables dissolved Cd from overlaying seawater to diffuse into organic-rich sediments and becomes fixed in the solid phase as CdS. Depletions of Cd in water columns have been measured in anoxic basins such as Saanich inlet (Jacobs *et al.*, 1985). Thus, a combination of shallow depths and highly productive area resulted in an enrichment of Cd in the sediments and a low deep water dissolved Cd-P ratio. The sediments are not necessarily a permanent sink,

as Cd can undergo natural post-depositional remineralization through oxidation (if bottom water O_2 is present).

1.3 *Cadmium in Oysters*

Many studies have focused on metals in oysters and the following is a review of some of this research. The species cultivated in the Pacific Northwest and used in this project is the Pacific Oyster, *Crassostrea gigas* (Thunberg). Some studies center on the American oyster, *Crassostrea virginica* but the two species are closely related. As well, studies on other bivalve mollusks, such as species of mussels are included where appropriate. Mussels are known to bioaccumulate Cd and have been used as indicators of marine Cd contamination (Geret *et al.*, 2002) and of Cd in upwelling regions (Lares and Orians, 1997).

1.3.1 Accumulation

Oysters are filter feeders, filtering up to five to eight litres of water per hour. Cilia on the gills of oysters generate water current through the gills. Because of these extraordinary filtration rates, oysters are able to bioaccummulate metals up to concentrations four or five orders of magnitude higher than what is present in seawater ($\mu g g^{-1}$ in oysters, $0.1 ng g^{-1}$ in dissolved form and $0.01 ng g^{-1}$ in particles). Cadmium is not known to have a cellular function in oysters so it is assumed that cadmium uptake is accidental. Cadmium can be taken up in two ways. One is by the direct uptake and sequestration of dissolved Cd by the gills and the other is the uptake of ingested particulate material through the mouth to the stomach (Roesijadi and Robinson, 1994). Once in the gill, dissolved Cd can be re-distributed to the other internal organs on its way to being eliminated in the kidneys or accumulated in association with metallothioneins (Roesijadi and Robinson, 1994). Metallothioneins are a family of low-molecular mass, metal binding proteins. They are present in the digestive gland and in gills and their roles are to sequester metals and to transport metals to internal organs. The production of metallothioneins has been found to be a response to exposure to high

levels of metals and has been associated with increased tolerance to metal concentrations (Roesijadi and Klerks, 1989; Geret *et al.*, 2002).

Suspended particles and phytoplankton are trapped in the mucus of the gills, sorted, and transported to the mouth, where they will be eaten, digested and transported to internal organs. The metal contained in these particles can then follow one of three processes: storage as inorganic inclusions, storage as metallothioneins, or elimination via the renal and digestive pathways (Roesijadi and Robinson, 1994). Oysters are capable of preferentially ingesting organic material and rejecting inorganic particles (Newell and Jordan, 1983). Sorting of particles can be done by three mechanisms: pre-ingestion by cilia on the gills where excess food and large plankton are eliminated in pseudofeces, by the labial palps (flaps that sort particles before reaching the mouth) and by post-ingestive selection in the gut (Shumway, 1996). Selected particles then enter the digestive system. These two pathways are summarized in Fig 1-4.

1.3.2 Elimination of metals

Cadmium is eliminated by the kidneys and in feces. From a commercial stand point, depuration of metals could be a viable tool to alleviate the problem of Cd in oysters, if the elimination rate was economically reasonable. In early studies, depuration experiments were unsuccessful (Greig and Wenzloff, 1978; Zaroogian, 1979). Oysters were experimentally contaminated then allowed to depurate for 48 or 56 weeks, respectively in laboratory aquaria filled with natural seawater. In the latter study, there was a decrease in the Cd content per oyster but this was accompanied by a decrease in the weight of the oysters, which led to steady Cd concentrations. In contrast, Van Dolah *et al.* (1987) has shown clear Cd depuration after 140 days. A field study which translocated oysters from a metal-rich estuary to a clean site had observed a rapid decrease in Cd and after four months-levels decreased from 2.8 μgg^{-1} to 1 μgg^{-1} (Geffard *et al.*, 2002). The half-life of cadmium in soft tissues from these studies has been calculated to be 150 days (Van Dolah *et al.*, 1987) and 137days (Geffard *et al.*, 2002), which may not be workable for commercial use. Cd elimination was also shown to be related to gonad development and spawning (Frazier, 1975). Cd levels would increase

in oysters in months leading up to spawning and then released with the release of gonads. Thus, Cd concentrations in oysters may vary seasonally.

1.3.3 Factors Affecting Cd Cycling in Oysters

There are many environmental factors that influence both the accumulation and depuration of cadmium in oysters. Speciation of trace metals has been known to significantly affect phytoplankton growth and influence phytoplankton species composition (Bruland *et al.*, 1991). Generally, the bioavailable form of a metal to phytoplankton is considered to be its free ion (M^{2+}) (Sunda and Huntsman, 2000). The speciation of cadmium in the dissolved form has a direct effect on accumulation of Cd in oysters as Cd bound to organic ligands is rendered biologically unavailable to oysters (Hung, 1982). It has also considered that the toxic form of Cd to aquatic organisms is free Cd (Cd^{2+}) (Sunda, 1979). In a study where oysters were subjected to salinity ranging from 10 to 30 PSU (Phelps *et al.*, 1985), a negative relationship was found between salinity and Cd in oysters. The explanation for these results was that as salinity increases in seawater, more Cl^- binds to Cd and there is less free Cd but unlike organic ligands, chloride complexes are labile so the conclusion that Cd-chloride complexes are biologically unavailable to oysters is unexpected. Salinity can affect Cd in oysters in other ways; An increase in salinity increases pumping rate (Shumway, 1996), which is the rate water is transferred over the gills. A higher pumping rate can correspond to the higher levels of uptake of dissolved cadmium, which is opposite to the lab results found by Phelps *et al.*, (1985) where a non-typical range of salinities was used. Salinity also signifies water masses; deeper, colder, nutrient-rich waters have high salinity in many regions.

Temperature also affects Cd accumulation; growth is also more rapid in warmer temperatures (Shumway, 1996). Growth, in terms of tissue mass, of oysters is inversely related to metal concentrations as an increase in tissue mass dilutes the pool of metals (Zaroogian, 1979). At higher temperatures, oysters have been observed to eliminate Cd at a faster rate (Van Dolah *et al.*, 1987). As well, temperature, like salinity can also signify water masses, with colder water often carry higher dissolved nutrients and Cd.

The quality of particulate food can influence metal cycling in oysters. An abundance of quality food, defined as having a high proportion of organic particles, has been demonstrated to decrease particle handling time on the labial palps (Milke and Ward, 2003). The handling time can influence the amount of energy available for growth and reproduction, which, as discussed earlier, are two processes that affect metal concentrations in oysters.

1.4 *Research Objectives*

The principle objectives of this research are to elucidate the role of dissolved and particulate cadmium in the surrounding seawater on high cadmium concentrations in BC oysters, to study the complex biogeochemical cycling particular to coastal environments and to investigate the Cd-P relationship both in the dissolved and particulate in coastal environments. This research initiative was supported by the Aquaculture Collaborative Research and Development Program (ACRDP), British Columbia Shellfish Growers Association (BCSGA), and the Department of Fisheries and Oceans (DFO).

The thesis is organized as follows: Chapter one presents an introduction to the research and chapters two and three are written as manuscripts. Chapter two details the role of environmental factors on the pathway of Cd to oysters, while chapter three details the biogeochemical interactions at these two coastal sites in terms of the Cd-P relationship. Both chapters include some repetition. The appendices contain detailed information on the methods used in this study.

1.5 *Figures*

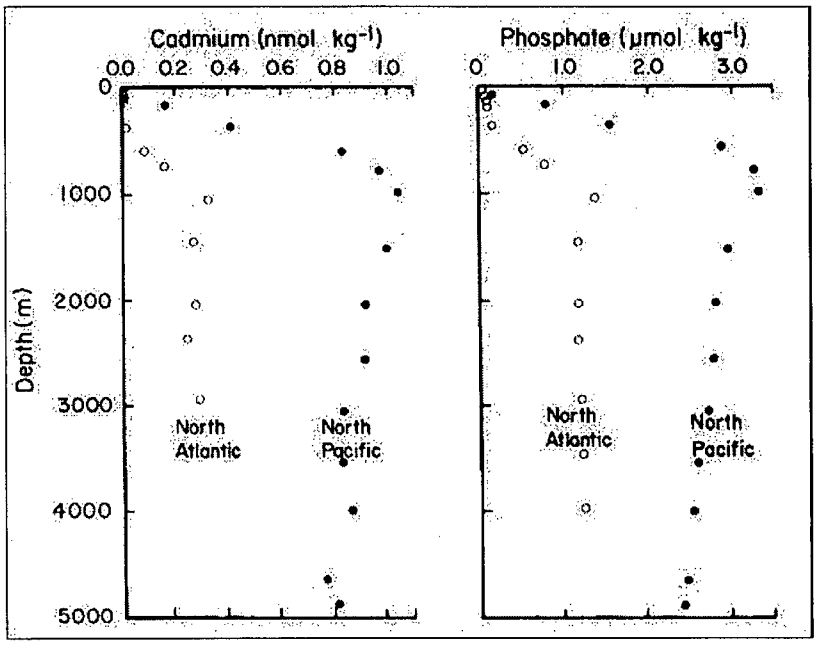

Figure 1-1: Vertical distributions of Cd and PO_4^{3-} in the open oceans. (Bruland and Franks, 1983)

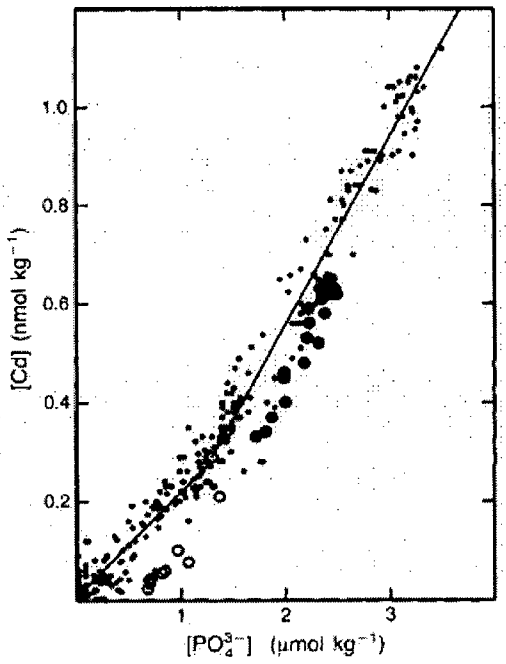

Figure 1-2: [Cd] against $[PO_4^{3-}]$ for open ocean samples >1000m deep. Taken from Frew and Hunter, (1992)

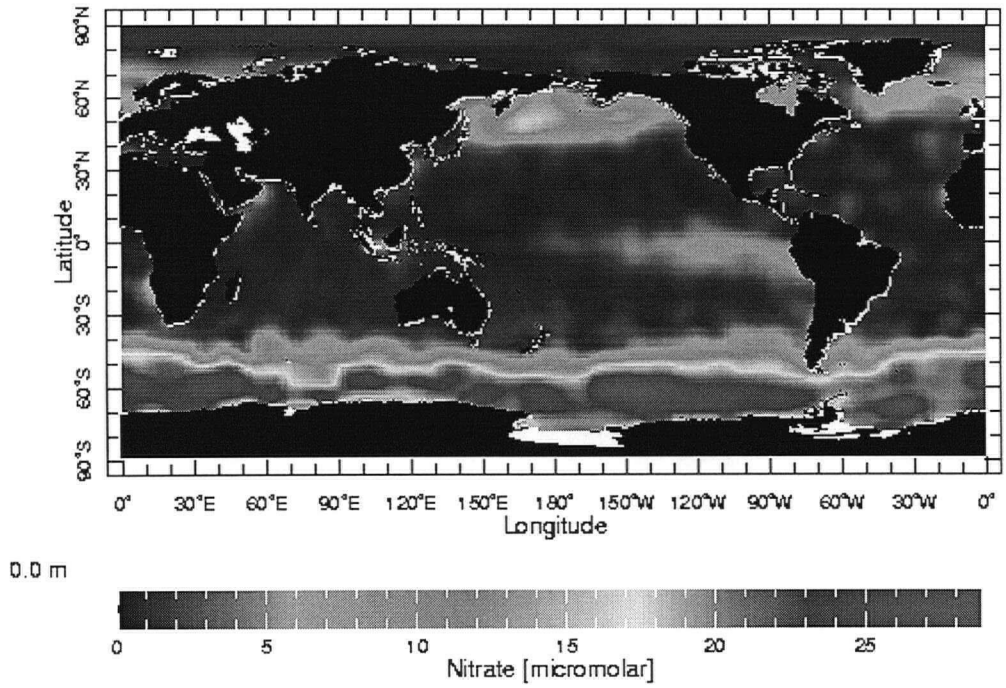

Figure 1-3: Map of annual average nitrate concentrations in the surface waters of the oceans. This image shows the high levels of nitrate in the subarctic Pacific, the equatorial Pacific and the Southern Ocean. Data from the Levitus World Ocean Atlas 1994 (www.atmosphere.mpg.de/enid/1vv.html).

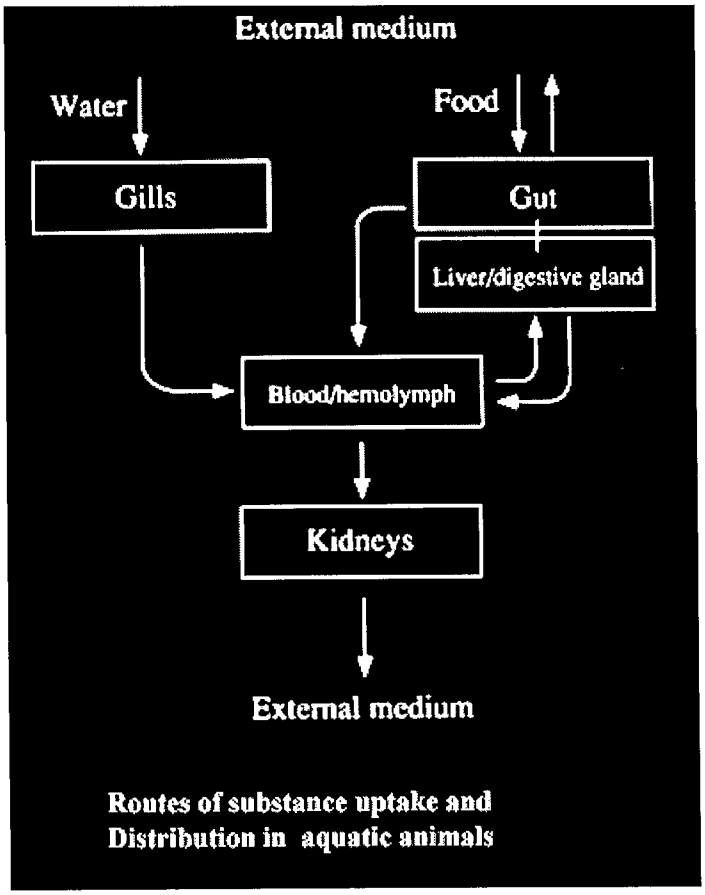

Figure 1-4: Uptake pathways of metals in aquatic animals. Taken from Roesijadi and Robinson (1994).

1.6 *References*

Boisson, F., Goudard, F., Durand, J.P., Barbot, C., Pieri, P., Amiard, J.C. and. Fowler S.W. 2003. Comparative radiotracer study of cadmium uptake, storage, detoxification and depuration in the oyster *Crassostrea gigas*: potential adaptative mechanisms. Marine Ecology Progress series. 254: 177-186.

Boyle, E.A., Sclater, F. and Edmond, J.M., 1976. On the marine geochemistry of cadmium. Nature, 263: 42-44.

Boyle, E.A, 1988. Cadmium: Chemical tracer of deepwater paleoceanography. Paleoceanography 3: 471-489.

Brooks, R.R., and Rumsby, M.G., 1965. The biochemistry of trace metal uptake by some New Zealand bivalves 10: 521-527.

Bruland, K.W. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. Limnology and Oceanography 37: 1008-1017.

Bruland, K.W., Donat, J.R., Hutchins, D.A., 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. Limmnology and Oceanography 36: 1555-1577.

Bruland, K.W. and Franks R.P., 1983. Mn, Ni, Cu, Zn and Cd in the western North Atlantic, In: Trace Metals in Sea Water (Wong, C.S. *et al.*, Ed), NATO Conference Series, Series IV, Marine Sciences, volume 9, Plenum Press, NY, pp 395-414.

Bruland, K.W., 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. Earth Planetary Science Letters 47: 176-198.

Bruland, K.W., Knauer, G.A., Martin, J.H., 1978. Cadmium in northeast Pacific waters. Limnology and Oceanography 23: 618-625.

Chong, M. 2003. A comparison of techniques for quantification of particulate trace metals. Honours thesis presented to UBC. 40p.

Cullen, J.T. 2006. On the nonlinear relationship between dissolved cadmium and phosphate in the modern global ocean: Could chronic iron limitation of phytoplankton growth cause a link?. Limnology and Oceanography, 51: 1369-1380.

Cullen, J.T., Sherrell, R.M., 2005. Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages. Limnology and Oceanography 50: 1193-1204.

Cullen, J.T., Chase, Z., Coale, K.H., Fitzwater, S., Sherrell, R.M., 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. Limnology and Oceanography 48: 1079-1087.

Cullen, J.T., Lane, T.W., Morel, F.M.M, Sherrell, R.M., 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature 402: 165-167.

Cullen, J.T., Lane, T.W., Morel, F.M.M., Sherrell, R.M. 1990. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature, 402: 65-167..

de Baar, H.J.W., Saager, P.M., Nolting, R.F. and van der Meer, J., 1994. Cadmium versus phosphate in the world ocean. Marine Science, 46: 261-281.

Engel, D.W., Sunda, W.G. and Fowler, B.A. 1981. Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters. *In* F.J. Vernberg, A Calabrese, F.P. Thur-berg, and W.B. Vernberg (Eds.). Biological Monitoring of Marine Pollutants. Academic Press, New York. 127-144.

Engel, D.W. (1999). Accumulation and cytosolic partitioning of metals in the American oyster *Crassostrea virginica*. Marine Environ. Res. 47: 89–102

Ettajani, H., Berthet, B., Amiard, J.C. and Chevolot, L. 2001. Determination of cadmium partitioning in microalgae and oysters: contribution to the assessment of trophic transfer. Archives of Environmental Contamination and Toxicology. 40: 209-221.

Fisher, N.S., Teyssie, 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 30: 3232-3242.

Frazier, J.M. and George, S.G. 1983. Bioaccumulation of cadmium in marine organisms. Environ. Health Perspect. 28: 75-79.

Frazier, J.M, 1975. The dynamics of metals in the American Oyster, *Crassostrea virginica*. I. Seasonal Effects. Chesapeake Science 16: 162-171.

Frew, R.D. 1995. Antarctic bottom water formation and the global cadmium to phosphorus relationship. Geophysical Research Letters 22: 2348-2352.

Frew, R.D., Hunter, K.A., 1992. Influence of Southern ocean waters on the cadmium-phosphate properties of the global ocean. Nature 360: 144-146.

Geffard A., Amiard, J.C. and Amiard-Trinquet, C.. 2002. Kinetics of metal elimination in oysters from a contaminated estuary. Comparative Biochemistry and Physiology Part C. 131: 281-293.

Geret, F., Jouan, A., Turpin, V., Bebianno, M.J., Cosson, R.P., 2002. Influence of metal exposure on metallothioneins synthesis and lipid peroxidation in two bivalve mollusks: the oyster (*Crassostrea gigas*) and the mussel (*Mytilus edulis*). Aquatic Living Resources 15: 61-66.

Greig, R.A. and Wenzloff, D.R. 1978. Metal accumulation and depuration by the American oyster, *Crassostrea virginica*. Bull.Environ. Contam. Toxicol. 20: 499-504.

Hardy J.T., Sullivan, M.F., Crecelius, E.A., Apts, C.W. 1984. Transfer of cadmium in a phytoplankton-oyster-mouse food chain. Archives of Environmental Contamination and Toxicology. 13: 419-425.

Ho, T-Y., Quigg, A., Finkel, Z., Milligan, A., Wyman, K., Falkowski, P., Morel, F.M.M. 2003. The Elemental composition of some marine phytoplankton. Journal of Phycology 39: 1145-1159.

Hung, Y-W., 1982. Effects of temperature and chelating agents on cadmium uptake in the American oyster. Bulletin of Environmental Contamination and Toxicology 28: 546-551.

Jabobs, L., Emerson, S., Skei, J., 1985. Partitioning and transport of metals across the O_2/H_2S interface in a permanently anoxic basin: Framvaren Fjord, Norway, Geochimica et Cosmochimica Acta 49: 1433-1444.

Kruzynski, G.M., Addison, R.F., Macdonald, R.W., 2002. Proceedings of a Workshop on Possible Pathways of Cadmium into the Pacific Oyster (Crassostrea gigas) as Cultured on the Coast of British

Columbia, Institute of Ocean Sciences, March 6-7, 2001. Canadian Technical Report of Fisheries and Aquatic Sciences 2405.

Kruzynski, G. 2004 Cadmium in oysters and scallops: the BC experience. Toxicology Letters 148: 159-169

Kusuki Yutaka, (1977). Retention of small particles by the gills of the Japanese oyster. Bulletin of the Japanese Society of Scientific Fisheries 43, 1391-1396.

Lane, T.W., and Morel, F.M.M. 2000. A biological function for cadmium in marine diatoms. Proceedings of the National Academy of Sciences. 97(9): 4627-4631.

Lares, M.L., and Orians, K.J. 1997. Natural Cd and Pb variations in *Mytilus californianus* during the upwelling season. Sci. Total Environ. 197: 177-195.

Lee, J.G., and Morel, F.M.M., 1995. Replacement of zinc by cadmium in marine phytoplankton. Marine Ecology Progress Series 127: 305-309.

Lide, D.R., (ed) 2006. Abundance of elements in the Earth's Crust and in the Sea, in CRC Handbook of Chemistry and Physics, Internet Version 2006, Taylor and Francis, Boca Raton, FL, 2006.

Martin, J.H. 1990. Glacial-interglacial CO_2 change: The Iron Hypothesis. Paleoceanography 5: 1-13.

McNee, Jay J. 1997. The Post-Depositional Cycling of Cd, Cu, Mo and Zn in Several Hydrographically Distinct B.C. Fjords. Ph.D. Thesis presented to the University of British Columbia.

Milke, L.M. and Ward, J.E. 2003. Influence of pre-digestive particle processing in bivalves II: Residence time in the pallial cavity and handling time on the labial palps. Journal of Experimental Marine Biology and Ecology. 293: 151-172.

Newell, R.I.E., Jordan, S.J. 1983. Preferential ingestion of organic material by the American Oyster *Crassostrea virginica.* Marine Ecology Progress Series, 13: 47-537.

Payne, C.D. and Price, N.M. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. Journal of Phycology. 35: 293-302.

Pederson, T.F., Waters, R.D. and Macdonald, R.W., 1989. On the natural enrichment of cadmium and molybdenum in the sediments of Ucluelet Inlet, British Columbia. The Science of the Total Environment, 79: 125-139.

Phelps, H.L., Wright D.A. and J.A Mihursky. 1985. Factors affecting trace metal accumulation by estuarine oysters *Crassotrea virginica.* Marine Ecology Progress Series, 22:187-197.

Price, N.M., Morel, F.M.M. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. Nature, 344: 658-660.

Redfield, A.C., 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *In* Daniel, R.J. [Eds] James Johnstone Memorial Volume. Liverpool University Press. pp 176-192.

Reinfelder, J.R., Wang, W.X., Luoma, S.N., Fisher, N.S. 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Marine Biology, 129: 443-452.

Ren, J.S., Ross, A.H., Schiel, D.R. 2000. Functional descriptions of feeding and energetics of the Pacific oyster Crassostrea gigas in New Zealand. Marine Ecology Progress Series, 208: 119-130.

Roesjadi, G. and Klerks, P. 1989. A kinetic analysis of cadmium-binding to metallothionein and other intracellular ligands in oyster gills. Journal of Experimental Zoology, 251:1-12.

Roesjadi, G. and Robinson, W.E. 1994. Metal Regulation in aquatic animals: mechanisms of uptake, accumulation, and release. In D.C. Malins and G. Ostrander (Eds.). Molecular Mechanisms in Aquatic Toxicology. Lewis Publishers, Boca Raton. 387-420.

Roesijadi, G. 1996. Environmental factors: response to metals. *In* The Eastern Oyster *Crassostrea virginica.* V.S.Kennedy, R.I.E. Newell, and A.F. Eble (Eds.). Maryland Sea Grant, College Park, Maryland. 515-537.

Saager, P.M., and de Baar, H.J.W., 1993. Limitations to the quantitative application of Cd as a paleooceanographic tracer, based on results of a multi-box model (MENU) and statistical considerations. Global Planetary Changes 8: 69-92.

Shumway, S. 1996. Natural environmental factors. *In* The Eastern Oyster *Crassostrea virginica.* V.S.Kennedy, R.I.E. Newell, and A.F. Eble (Eds.). Maryland Sea Grant, College Park, Maryland. 467-503

Sunda, W.G., Huntsman, S.A., 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. Limmnology and Oceanography 45: 1501-1516.

Sunda, W.G., Huntsman, S.A., 1998. Processes regulating cellular metal accumulation and physiological effects: Phytoplankton as model systems. The Science of the Total Environment 219: 165-181.

Sunda, W.G., Huntsman, S.A., 1998. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn and Mn in seawater. Environmental Science and Technology 32: 2961-2968.

Sunda, W.G., Engel, D.W., and Thuotte, R.M. 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp, Palaemonetes pugio.: Importance of free Cd ion. Environmental Science and Technology, 12: 409-413.

Taylor, S.R. and McLennan, S.M., 1985. The Continental Crust: its Composition and Evolution. Blackwell Scientific, Oxford, 312 pp.

Thompson, R.E. 1981. Oceanography of the British Columbia Coast. Canadian special publication of fisheries and aquatic sciences, 56. 291p.

Van Dolah, F.M., Siewiki, T.C., Collins, G.W., and Logan, J.S. 1987. Effects of environmental parameters on the elimination of cadmium by eastern oysters, *Crassostrea virginica.* Archives in Environmental Contamination and Toxicology, 16:733-743.

Yang, L., (1993) Dissolved Trace Metals in the Western North Pacific. MSc Thesis presented to UBC.

Zaroogian, G. 1979. Studies on the depuration of cadmium and copper by the American oyster *Crassostrea virginica.* Bulletin of Environmental Contamination and Toxicology, 23:117-122.

2 Chapter 2: The Role of Dissolved and Particulate Cadmium in the Accumulation of Cadmium in BC Cultured Oysters[1]

Priyanka Lekhi, D. Cassis, C. Pearce, M.T. Maldonado, and K.J. Orians

2.1 *Introduction*

For the last 7 years, oysters grown on the Pacific coast have shown relatively high concentrations of cadmium (Cd) at around 2 μgg^{-1} wet weight. In late 1999 and early 2000, successive batches of oysters were rejected by the Hong Kong Food and Environmental Hygiene Department because the Cd concentrations in the product exceeded the 2 μgg^{-1} wet weight standard set by this market. In other markets, the allowable Cd limits vary between 1 μgg^{-1} (European Union) to 3.7 μgg^{-1} (United States Food and Drug Administration recommended guideline). Health Canada has no concentration limits for Cd in oysters but does have consumption guidelines for Canadian consumers. With the current Cd concentrations in oysters, BC shellfish farmers are limited mainly to local and US markets. The loss of markets is a fairly recent problem but the issue with of relatively high Cd in oysters dates further back. For example, 27 years ago, Environment Canada and Department of Fisheries and Oceans show data on wild oysters collected in the northern Strait of Georgia, with Cd residues approaching 2 μgg^{-1} (Kruzyski, 2001). The geographic distribution of oysters with high levels of Cd within BC does not seem to follow a pattern. Possible point sources of Cd have been identified, such as fresh water inputs, mines or mineral deposits, leaching of farm equipment, fertilizers, logging activities, and municipal waste water disposal (Kruzynski, 2004). However, there is no clear correlation between the location of these Cd sources and and high Cd content in oysters.

The relatively high concentrations of dissolved Cd found in seawater in the North Pacific could be partially responsible for higher concentrations of Cd in oysters on the Pacific coast compared to the Atlantic coast (Kruzynski, 2004). Cd has a long residence time in seawater (appr. 50,000 years). Due to the deep ocean

[1] A version of this chapter will be submitted for publication to Marine Ecology Progress Series.

circulation, deep water formed in the North Atlantic travels south, around Antarctica and ends up in the North Pacific Ocean. This water has accumulated Cd from sinking particles for ~1000 years, so the Cd concentration at depth in the North Pacific is 2 to 5 times greater (values ranging from 0.08-0.1 ngg^{-1} in water deeper than 1000m) than the concentration of the fairly "young" deep water of the North Atlantic (0.02-0.03 ngg^{-1} in water deeper than 1000m) (Bruland and Franks, 1983). This difference is also reflected in typical surface water Cd levels. Dissolved Cd values at ~7m deep on a transect between the English Channel and the open Northeast Altantic ocean had values up to 0.009 ppb (Kremling and Pohl, 1989), which is 8-13 times smaller than values reported in surface waters on the east and west coasts of Vancouver Island (Paulson *et al.*, 1991; Lares and Orians, 1997).

Cadmium can be toxic to phytoplankton at higher levels because it can ineffectively replace essential metals in enzymes or inactivate enzymes. Although Cd was not initially known to have any biological role in the marine environment, its distribution profile in the open ocean follows that of a nutrient such as phosphate. It has now been shown that Cd can indeed have a nutrient role by substituting for zinc in the metalloenzyme carbonic anhydrase (Price and Morel, 1990). Oysters are able to bioaccumulate metals from seawater to highly elevated concentrations due to their extraordinary filtration rates. Cadmium is not known to be a nutrient for oysters, and thus, is considered to be taken up accidently. However, there are many factors that may influence the bioaccumulation of Cd in oysters, such as salinity, temperature, chemical and physical speciation of the metal and the growth/ physiology of the oysters (Roesjadi, 1996).

Oysters are able to absorb Cd in its dissolved form, as well as selectively take in Cd contained in particulate matter (Roesjadi and Robinson, 1994). Dissolved Cd includes simple hydrated metal ions, Cd ions complexed to inorganic anions and to organic ligands. Particulate sources include Cd sorbed onto suspended mineral and organic particles and Cd present in mineral particles and in phytoplankton. All of these species vary with respect to their biological availability to aquatic organisms (Bendell-Young and Arifin, 2004; Sunda, 1979). There has been controversy over whether Cd in the dissolved form or Cd associated with particles, is the primary source of cadmium to the oysters. The most likely source of Cd to the oysters has been considered to be their food: phytoplankton and other filtered particles (Reinfelder *et*

al., 1997; Kruzynski *et al.*, 2002). Oysters preferentially ingest organic rich materials and reject inorganic particles (Newell and Jordan, 1983). Moreover, retention efficiencies of oysters have been reported to be optimal with particles sized 3-6μm (Kusuki, 1977) or 3-12μm (Pauley *et al.*, 1988).

The aim of the present work was to study the natural seasonal variability of Cd in oysters and to investigate the role of dissolved and particulate Cd in the accumulation of Cd in oysters over an annual cycle. These variables were sampled from two farm locations in Vancouver Island in 2004-2005. Other environmental and local factors, such as oyster growth, temperature and salinity were also studied for their direct and indirect effects on Cd in oysters. Particulate Cd was partitioned into fractions of 0.4-3μm, 3-20μm and >20μm to determine if a certain size range is correlated to Cd in oysters. In addition to these variables, particulate titanium (Ti) and phosphorus (P) were also analyzed in order to characterize the particulate matter as mainly organic or inorganic. Titanium is the ninth most abundant element in the Earth's crust; it is a ubiquitous constituent of rocks, soils and sediments and has been used as a conservative tracer for marine sediment analysis (Skrabal, 1995; Murray and Leinen, 1996). The main source of particulate Ti would be from chemical weathering of rocks and from resuspended marine sediments. Phosphorus serves as a nutrient for phytoplankton in the dissolved phase. Particulate P can be associated with organic material as well as with terrigenous materials (Keefe, 1994). By comparing the particulate Cd:P ratio in samples to the extended Redfield stoichiometric formula for the average cellular content of phytoplankton (Ho *et al.*, 2003), it can be judged whether particulate Cd is associated with phytoplankton. A surface sediment sample was also sampled at each location to study any influence of sedimentary input or of diagenesis at the surface sediments on the dissolved or particulate Cd of the overlaying water column.

2.2 *Materials and methods*

2.2.1 Sampling

At two locations on Vancouver Island (Figure 1), samples of seawater and oysters were taken at 5m depth biweekly during the summer/ spring months and monthly during the winter. The site in Deep Bay is located

on the east coast of Vancouver Island (Baynes Sound, Strait of Georgia) and site in Lemmens Inlet is located on the west coast of Vancouver Island (Clayoquot Sound). Temperature and salinity data were collected on site using a ClineFinder® digital temperature probe and a manual refractometer. Seawater was collected using a peristaltic pump equipped with Tygon® polyethylene tubing and in-line filter holders. Filtered seawater for dissolved Cd analysis was collected in two 500mL acid-washed HDPE bottles and frozen until transport to UBC. Upon arrival, samples were acidified for storage until they were analyzed (within two months of collection). Initially, seawater was filtered through trace metal clean polycarbonate filters, pore sized 0.4μm, 3.0μm, 10μm, and 20μm. Due to excessive clogging, in December 2004, the three smaller sized polycarbonate filters were replaced with cellulose acetate filters sized 0.4μm, 3.0μm. Once collected, these filters were frozen until analysis. In addition, a surface marine sediment sample was collected in February 2005 for one location at each site.

2.2.2 Oyster analysis

The oysters sampled at the Deep Bay were grown out for one year before sampling and were cultured using tray culture method. The oysters from the Lemmens Inlet were grown out for two years and cultured using the string culture method. Each oyster sample contained 5 individual market-sized oysters that were collected by farm personnel and transported to Norwest Laboratories in Greater Vancouver for analysis using Canadian Food Inspection Agency (CFIA) Cd Testing Protocols and CFIA Cd Laboratory Testing Guidelines. Each oyster was processed individually. During the biweekly sampling, oysters were analyzed whole, while three times during the sampling year, oysters were partitioned into two body components, gut (includes the digestive gland, stomach and esophagus) and tissue other than the gut to determine the Cd distribution in the animals. Oysters were measured (shell length), weighed, shucked and the remaining soft tissue was weighed (wet and dry weight) and analyzed for moisture and Cd concentration.

2.2.3 Particulate analysis

Particulate matter suspended in seawater was collected by filtering 2-4L of seawater into size fractions of 0.4-3μm, 3.0-20μm and greater than 20μm. Polycarbonate filters sized 3μm and 10μm collected at the beginning of the project were combined to form one sample. All filters were microwave digested with HNO_3, HCl, and HF in a 4:4:1 ratio. Prior to digestion, polycarbonate filters were first dissolved in concentrated NH_4OH for 24 hours. After evaporation of the concentrated acids, 50% HNO_3 was added and samples were heated for further digestion. Digests were diluted to 2N HNO_3 before analysis on the GFAAS for Cd using standard additions. In addition, particulate titanium (Ti) and phosphorus (P) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using indium (In) as the internal standard. Procedural blanks were less than 10% of measured concentrations for Cd and Ti and less than 3% of measured concentrations for P. The precision of the filter blanks varied up to 4% for Cd and Ti and 6% for P.

2.2.4 Sediment analysis

Surface sediment samples were collected at one location at each site using a Petit Ponar® Grab sampler, into two acid-washed specimen containers and frozen until analysis. Samples were freeze-dried in an Edwards 4k Modulyo Freeze dryer for one week. Once dry, samples were ground in a Hertzog HSM-100 tungsten-carbide mill. Inorganic carbon was determined colorimetrically on CM5014 and 5011 CO_2 Coulometers. Total carbon was measured by a Carlo-Erba CNS analyzer Model NA 1500 gas chromatograph (Verardo *et al.*, 1990). The measurement of major and minor elements was done by a Phillips PW 1400 x-ray fluorescence spectrometer. Sample preparation of the major elements (Na, K, Ti, Si, P, Mn, Al, Ca, Fe, and Mg) involved preparation of fused glass discs following the method in the Katanax® K1 manual. For the analysis of minor elements (Mn, Mo, I, Br, Cu, Zn, Zr, Sr, Cr, Ba, Rb, Ni, V, Y and Pb), samples were run as pressed-powder pellets (McNee 1997).

The Cd content in sediment samples was determined following the same digestion and analysis procedure used for the filters. The accuracy and precision of the procedure was determined by measurements of coastal sediment standard reference materials (PACS-2 and MESS-2, Table 2-1), from the National Research Council of Canada. The recovery for both reference materials was 120%. Although high, these results may be due to the small sub-sample of reference material used for the procedure, containing mostly large particles, which would be comparatively enriched in Cd, P and Ti.

2.2.5 Dissolved cadmium analysis

All seawater samples were processed in a HEPA filtered, positive pressure air supply trace metal clean laboratory. One 500mL bottle was divided into two replicates and adjusted to pH 6 before being pre-concentrated using 2mL of a Chelex®-100 ion exchange resin at a pumping rate of 0.8mL/min (Yang, 1993). Samples were eluted with 2N HNO_3 and analyzed on a Varian Spectra-300/400 GFAAS using Zeeman background correction and standard addition. The accuracy and precision of the seawater analysis were determined by measurements of standard reference materials for coastal ocean seawater (CASS-4) from the National Research Council of Canada and from measuring replicates of deep seawater collected from "Line P" stations (stations extending from Juan de Fuca Strait, to Ocean Station Papa at 50°N 145°W, in the Pacific Ocean) spiked or unspiked with Cd. The efficiency of extraction for CASS-4 was found to be 100-105%; the variation of replicate unspiked analysis was less than 3%. The precision of all replicate samples were within 5% or were re-analyzed.

2.3 *Results*

2.3.1 Deep Bay

2.3.1.1 Seasonal variability of Cd in oysters

The oysters in Deep Bay had Cd levels ranging from 1.2 to 3.6 μgg^{-1}, with standard deviations ranging from 0.08 to 0.8 μgg^{-1} wet weight (Fig. 2-2, Table 2-2). The data suggests a seasonal trend with the highest oyster Cd concentrations observed during the end of winter and early spring, when temperature was lower, and lower concentrations during summer and fall. The inverse correlation between temperature and Cd in oysters is significant ($r = -0.6$, $P < 0.01$). Cadmium concentrations in oysters were predominately above the Hong Kong allowable limit of 2 μgg^{-1} during the sampling year after November 2004.

Cadmium concentrations in the gut and in other oyster tissues were determined for oysters three times during the sampling year (Fig. 2-3 and Table 2-3). There was a substantial difference between the Cd concentration in the gut and in other tissues, as Cd was highly concentrated in the gut (8-12 μgg^{-1}) compared to that in other tissues (1.2-2.0 μgg^{-1}).

2.3.1.2 Oyster Growth

The average shell length of the oysters increased over time, starting from an initial shell length of 78mm, reaching up to 116mm by the end of the sampling year (Fig. 2-4). From the increase in shell length, it is apparent that they were actively growing during the year. The dry meat weight of the oysters followed a seasonal trend and generally increased over the year (Fig. 2-4, Table 2-2). The dry weight of the oysters ranged from 2-8g during the sampling year. From August to October, there was a rapid increase in oyster meat weight and this corresponded to a general plateau in concentration of Cd in oysters (Figures 2-2 and 2-4). From November to February, there was a plateau in meat weight and this corresponded to an increase

in Cd concentration in oysters. From February to the end of the sampling year, there was an increase in oyster meat weight and this corresponded to a general decrease in Cd concentration.

2.3.1.3 Dissolved cadmium

The concentrations of the dissolved Cd in seawater, and Cd in oysters followed a similar trend through the sampling year (Fig. 2-5). Cd in the dissolved form was greatest in the winter months and lowest in the summer and spring months. The range observed was from 0.45 to 0.81 nM (Fig. 2-5, Table 2-4). Among all the variables, dissolved Cd showed the strongest correlation with Cd in oysters and this relationship is highly significant ($r = 0.7$, $P < 0.001$).

2.3.1.4 Particulate Cd, Ti and P

Total particulate Cd was generally lowest in the winter months and greatest during the fall and summer (Figures 2-6, 2-7, Table 2-5). Values of total particulate Cd ranged from 0.008 to 0.05 nM. Generally, most of the particulate Cd was present in the 3-20μm size fraction throughout the sampling year. The correlations between fractions of particulate Cd and this metal in oysters were negative but only the >20μm fraction reached significance level ($r = -0.02$, $P > 0.05$; $r = -0.3$, $P > 0.05$; $r = -0.6$, $P < 0.01$ for the 0.4-3μm, 3-20μm and >20μm fractions, respectively) (Fig. 2-6, Tables 2-2, 2-6). Total particulate Cd was also not significantly correlated with Cd in oysters.

Values for total particulate phosphorus (P) ranged from 0.042 to 0.269 μM and varied widely throughout the sampling year. (Fig. 2-7, Table 2-5). Particulate P concentrations were normally higher in the 3-20μm size fraction throughout the year (Table 2-6). Total particulate Ti ranged from 0.0099 to 0.058 μM, with an exceptional event in November 2004 where levels exceeded 0.15 μM (Fig. 2-7, Table 2-5). Aside from this event, particulate Ti presented a small variability within its range of concentrations, not displaying any marked seasonal trend. Particulate Ti was very high in the 3-20μm fraction and very low in the smallest

fraction (Table 2-6). There was no significant correlation with the total particulate P and Ti and Cd in oysters ($P > 0.05$) (Fig. 2-7).

Samples of suspended particulate matter showed a fairly consistent ratio of Cd:P between 0.04 and 0.18 (nM:μM), increasing in the winter to around 0.3 (Fig. 2-8 and Table 2-5). The Cd:Ti ratio varied significantly and without a seasonal pattern over the sampling year (Fig. 2-8, Table 2-5), indicating that Cd does not follow titanium.

2.3.1.5 Sediment sample

A grab sample of the top layer of the marine sediment was collected at the Deep Bay, at approximately 25m bottom depth. This sample contained 0.76 $\mu g g^{-1}$ of particulate Cd and 1.2% of organic carbon (Table 2-7). Materials which contain more than 0.6 $\mu g g^{-1}$ Cd are prohibited from marine disposal under the Canadian Ocean Dumping Control Act (Pedersen *et al.*, 1989) and most coastal marine sediments worldwide fall below this level. Thus, the sediments in Deep Bay are enriched in Cd.

The Cd:Ti ratio in the sediments was 0.08 mmol kg^{-1}: mol kg^{-1} (Table 2-7) and was low compared to that found in the suspended particulate matter (0.08-2.57 nM:μM, Fig. 2-8). The sedimentary Ti value at Deep Bay (4029$\mu g g^{-1}$) was higher than the crustal Ti value of 3000 $\mu g g^{-1}$. The sedimentary Cd:Ti ratio is also higher than the crustal Cd:Ti ratio of 0.014 mmol kg^{-1}: mol kg^{-1} (Taylor and McLennan, 1985), which points towards a Cd enrichment. The ratio of Cd:P in the sediments was 0.62 mmol kg^{-1}: mol kg^{-1} and was higher than the ratio of Cd:P found in suspended particulate matter (0.04 to 0.31 nM:μM) throughout the year (Fig. 2-8 and Table 2-5) and higher than the crustal Cd:P ratio (0.14 mmolkg^{-1}: molkg^{-1}).

2.3.2 Lemmens Inlet

2.3.2.1 Cadmium in oysters

At this site the concentrations of Cd found in the oysters ranged from 1.4 to 2.5 μgg^{-1} and remained below the 2 μgg^{-1} level most of the year (Fig. 2-9, Table 2-8). Standard deviation ranged from 0.061 to 0.61 μgg^{-1} wet weight. There was less variation in Cd levels in oysters at this site, making it difficult to draw correlations with other variables. The Cd concentrations in oysters showed a general decrease after April, 2005. During late winter and early spring, where temperature was the lowest, the Cd levels in oysters was greatest. The inverse correlation between temperature and Cd in oysters is significant ($r = -0.4$, $P < 0.05$) but not as strong or as significant as the relationship found in Deep Bay ($r = -0.6$, $P < 0.01$). Also, as seen on the Deep Bay, there was notably greater Cd concentrations in the gut (7.43 to 11.23 μgg^{-1} wet weight) compared to other tissues (0.94 to 1.65 μgg^{-1} wet weight) (Fig. 2-10 and Table 2-3).

2.3.2.2 Oyster Growth

The average shell length of the oysters in Lemmens Inlet did not increase over the course of the sampling year and varied from 124mm to 159mm (Fig. 2-11, Table 2-8). As indicated by the large error bars, the meat dry weight at this site observed no statistically significant change for most of the year, with the exception of the period between February and April, when the dry weight was lower. This decrease in tissue weight corresponds with a slight increase in Cd concentration.

2.3.2.3 Dissolved cadmium

The dissolved Cd levels ranged from 0.22 to 0.46 nM. The correlation between dissolved Cd and Cd in oysters does not reach the significant level ($r = 0.2$, $P > 0.05$) but this may be due to the low variation of Cd in oysters and in the dissolved phase (Fig. 2-12 and Table 2-9). The dissolved Cd is lower at this site compared to the Deep Bay and correspondingly, the Cd in oysters is also lower at this site.

2.3.2.4 Particulate Cd, Ti and P

The levels of total particulate Cd at Lemmens Inlet (0.010-0.061 nM) were very similar to those determined in Deep Bay (0.008-0.05nM) (Figures 2-13, 2-14, Table 2-10). There was no obvious seasonal trend, as observed in Deep Bay, and most of the particulate Cd was present in the 3-20μm fraction (Fig. 2-13, Table 2-11). Size fractionated particulate Cd and Cd in oysters was weakly inversely correlated but only the 3-20μm fraction reached the significance level ($r = -0.2$, $P > 0.05$; $r = -0.5$, $P < 0.02$; $r = -0.4$, $P > 0.05$ for 0.4-3μm, 3-20μm and >20μm, respectively) (Fig. 2-13). Total particulate Cd was also not significantly correlated with Cd in oysters.

Total particulate phosphorus ranged from 0.023 to 0.505 nM (Table 2-10, Fig. 2-14), and was mainly present in the 3-20μm fraction with very little in the smallest fraction (Table 2-11). Total titanium ranged from 0.013 to 0.267 nM with high variability throughout the sampling year (Table 2-10, Fig. 2-14). Particulate Ti is mainly present in the 3-20μm fraction, followed by the smallest fraction. Only in two cases was the particulate Ti was highest in the >20μm fraction (Table 2-11). Both particulate P and particulate Ti were not significantly correlated with Cd in oysters ($P > 0.05$).

The Cd:P changed throughout the sampling year, only remaining constant between 0.14 and 0.19 (nM:μM) during the winter (Fig. 2-15, Table 2-10). Although the Ti varies through the year (Fig. 2-14), the ratio of Cd:Ti is fairly constant around 0.20 nM:μM (Fig. 2-15, Table 2-10). During two sampling events, the Cd:Ti ratios in the suspended particulate was higher than the norm (0.63 and 4.36 nM:μM) (Table 2-10).

2.3.2.5 Sediment samples

Lemmens Inlet is approximately 15m deep. A grab sample of the top layer of the sediment showed an enrichment of Cd and organic carbon, with values of 2.24 $\mu g g^{-1}$ and 3.1%, respectively (Table 2-7). The level of sedimentary Cd is four times higher than the limit of 0.06 $\mu g g^{-1}$ set for marine disposal under the Canadian Ocean Dumping Control Act (Pedersen *et al.*, 1989). The Cd: Ti ratio in the sediments was found

to be 0.27 (mmol kg^{-1}: mol kg^{-1}) (Table 2-7) at this site, which is greater than the crustal Cd:Ti ratio of 0.014 (mmol kg^{-1}: mol kg^{-1}) (Taylor and McLennan, 1985). Most of the year, the ratio of Cd:Ti in suspended particulate (Table 2-10) was very close to what is in the sediments (ranging from 0.08 to 0.3 nM:μM, excluding August, 2004). Similarly, the Cd:P ratio in the Lemmens Inlet sediment sample was determined to be 0.21 (mmol kg^{-1}: mol kg^{-1}) (Table 2-7), in close agreement with the Cd:P ratio in the suspended particulate matter (0.14-0.19 nM:μM)

2.4 *Discussion*

Several laboratory studies have suggested suspended particulate matter as the primary source of Cd for bivalves (Bendell-Young and Arifin, 2004; Reinfelder *et al.*, 1997). Others have predicted that the accumulation of metals in aquatic organisms from both the dissolved and particulate form (Wang, 2002; Hardy *et al.*, 1984). In this study most of the Cd was found to be concentrated in the gut tissues of oysters, which may also point towards food as the source of Cd. However, after compiling the data from both sites, the results indicate that dissolved Cd was the main source of Cd to oysters as it had the strongest positive correlation of all the variables (Fig. 2-16). This correlation was highly significant ($P < 0.001$). Particulate cadmium is not a source of Cd to oysters and was actually negatively correlated. In addition to the direct impact of dissolved Cd on Cd in oysters, there were also many variables, including physical mixing of water masses, oyster growth, temperature, salinity and sediment diagenesis, which either directly, or indirectly affected Cd in oysters. The variables were very different for each site as summarized in Table 2-12, which lead to different annual cycles of Cd concentrations in oysters.

2.4.1 Dissolved Cd and Cd uptake by Oysters

Although the correlation between dissolved Cd and Cd in oysters was significant in Deep Bay and in the compiled dataset, this correlation did not reach significance in Lemmens Inlet. However, there was little variability in both parameters at this site (Fig. 2-15). Moreover, an important difference between the two sites was that both dissolved Cd and Cd in oysters were both lower in Lemmens Inlet (Table 2-12) This

observation, combined with the significant correlation in the compiled data set (Fig. 2-17) emphasizes the significance of dissolved Cd to Cd in oysters. The variability of Cd concentrations in oysters was affected by variability in dissolved Cd present in the surrounding seawater. In Deep Bay, local oceanographic inputs may have affected dissolved Cd while at Lemmens Inlet, fluxes into anoxic sediments affected dissolved Cd.

Deep Bay is a protected estuary, approximately 25m deep, whose water is mainly from the Strait of Georgia. During the spring and summer months the central Strait of Georgia is highly influenced by the Fraser river and other fresh water inputs. In the winter, the water of the Strait of Georgia is influenced by the Juan de Fuca Strait (Thomson, 1981). The salinity of the seawater close to the surface at Deep Bay ranged from 23 to 29 PSU (Table 2-4). This is low compared to oceanic salinities of 35 PSU, indicating an important fresh water influence throughout the year. Fresh water could also contribute to the dissolved Cd levels, as it could bring in Cd from terrestrial and anthropogenic sources. For example, a surface sample taken from Fraser River at Hope, BC was determined to have an enriched Cd concentration of 13.3 nM in 1991 (Holms, 1997). However, another surface fresh water sample taken from Toba Inlet, on the coast of BC (100 miles north of Vancouver), was determined to have Cd concentration of only 0.20 nM (Lekhi, unpublished data). If fresh water was a source of enriched dissolved Cd, intrusions of dissolved Cd would be seen with lower salinity would correspond to higher dissolved Cd (Fig. 2-5). However, salinity is positively correlated with dissolved Cd at Deep Bay ($r = 0.6$. $P < 0.05$), indicating that oceanic water is a more significant source of dissolved Cd compared to fresh water. Cadmium levels in the Juan de Fuca Strait has been reported to range between 0.71-1.1 nM in 1980 (Paulson *et al.*, 1991), which are similar to the values of dissolved Cd measured in the winter in Deep Bay in 2004. In November 2004, the salinity at Deep Bay slightly increased to 29PSU, which could suggest an influence from the Juan de Fuca Strait (whose salinity has been reported to range from 31.5-32.4PSU at 5m -Rensel *et al.*, 2002). The influence of the Juan de Fuca Strait in the winter and from the Fraser River throughout the year, could contribute to variations in the dissolved Cd at Deep Bay. The correlation between dissolved Cd and Cd in oysters was strongest at this site, so variations in dissolved Cd was often accompanied by variability in Cd in oysters (Fig. 2-5).

Lemmens Inlet is a protected bay on the west side of Vancouver Island and is only approximately 15m deep. The waters of this site had higher salinities than the site at the Deep Bay. Nearshore waters are dominated by the open ocean California Current, which carries cooler, saline water, while during the winter the slightly weaker Davidson Current carries warmer water from the south along the coast into this region (Thomson, 1981). Cadmium is enriched in surface California current water at a level of 0.16nmol kg^{-1} (van Geen and Luoma, 1993) and can get as high as 0.92nmol kg^{-1} in upwelling locations (Merrin, 2002). However, the dissolved Cd concentrations for this particular site were lower than surrounding areas, such as Amphridite Point, on the west coast of Vancouver Island, where levels averaged around 0.62 nM (Lares and Orians, 1997). These lower concentrations can be explained by the sediment dynamics. The redox chemistry could be a factor in reducing the Cd in the dissolved phase through the transfer of Cd from the dissolved to the solid phase. The sampling site was shallow and the sediments contained high levels of organic carbon, reaching 3.1%, which may give rise to anoxic conditions at the sediments. Under anoxia, sulphur is reduced and can form an insoluble phase with Cd, which settles to the sediments. There is an enrichment of Cd in these sediments with a level of 2.24 μgg^{-1} (Table 2-7) which is typical of nearby areas such as Ucluelet inlet where surface sediment Cd levels range from 1.7 to 3.1 μgg^{-1} and organic C range from 3.5 to 7.4 % (Pederson *et al.*, 1989). The levels of manganese (Mn) found in the sediments support the notion that the conditions are anoxic at the sediments. Mn was found to be at 410 μgg^{-1}, which is considered within "background" levels. However, under oxic conditions, Mn is present as MnO_2, an insoluble brown solid that settles to the sediments, increasing the levels of sediment Mn considerably. Thus, the absence of excess Mn suggests that Mn is present in its reduced form: Mn^{2+}, which is soluble (Pederson *et al.*, 1989). Depletions of Cd in water columns have been measured in anoxic basins such as Saanich Inlet (Jacobs *et al.*, 1985). Thus, a combination of shallow depths and highly productive area resulted in an enrichment of Cd in the sediments and low dissolved Cd in the overlaying water column. This depletion in dissolved Cd at the Lemmens Inlet was accompanied by relatively lower levels of Cd in oysters (Table 2-12).

The accumulation of dissolved Cd in oysters is a two-step process. Dissolved metals are taken up directly by shellfish and other aquatic organisms through their exposed body surfaces, such as the gills. Once in the gill, Cd can be re-distributed to the other internal organs on its way to being eliminated in the kidneys and liver, or accumulated in association with metallothioneins in the gills or in the digestive gland, which is part of the gut (Roesijadi and Robinson, 1994). Oysters are not considered to have specific uptake mechanisms for Cd, thus Cd is believed to be internalized through existing uptake pathways for essential metals (Roesijadi and Robinson, 1994). Therefore, the concentrated levels of Cd in the gut of the oyster can be attributed partially to the small size of the gut and to the transfer of dissolved Cd from the gills to the digestive gland where Cd is accumulated by metallothioneins.

2.4.2 Oyster growth, temperature, salinity and Cd uptake by oysters

Seasonally, Cd in oysters is lowest during periods of high temperature and greatest in periods of low temperature. The variability of Cd during the sampling year may be reflecting changes in tissue mass of the oysters as reported by Zaroogian (1979), in addition to reflecting the uptake or release of metals. The oysters at the Deep Bay were grown out for one year before sampling began in 2004. Based on the shell length and dry meat weight data, these oysters were actively growing throughout the sampling year (Fig. 2-4). An increase in meat weight may lead to stabilization of the Cd concentration in the oyster whereas during a plateau or decrease in meat weight, the Cd concentration increases. This was seen in the results of this site (section 2.3.1.2). At Lemmens Inlet, the oysters were grown out for two years before sampling began in 2004. Based on the shell length and dry meat weight, these oysters were not in their active growth stage (Fig. 2-11). The differences in the variability in the Cd in oyster data between both sites may be reflective of the oyster growth stage. As seen in table 2-12, the Cd concentrations in oysters varied by 2.4 μgg^{-1} over the sampling year, whereas, in Lemmens Inlet, the Cd concentrations in oysters only varied by 1.1 μgg^{-1}. This difference in variablility can be attributed to the dissolved Cd levels but the dissolved Cd only varied by 0.36 and 0.24 nM throughout the sampling year in Deep Bay and Lemmens Inlet, respectively. Thus, the variation in Cd in oysters is also due to other factors. In Deep Bay, where oysters are actively growing and changing their tissue mass, the levels of Cd were quite variable. In Lemmens

Inlet, where the oysters were not actively growing, the levels of Cd were not so variable. The correlation between Cd in oysters and tissue mass was significant for the compiled dataset (Fig. 2-16) The difference in growth rate is likely due to the different ages of the oysters and the different culture conditions at the two sites.

As seen for both sites, temperature has a strong and significant negative effect on Cd levels in oysters. During periods of low temperature, Cd levels in oysters were generally higher. This relationship is stronger and highly significant in the Deep Bay (Fig. 2-2; $r = -0.6$, $P < 0.01$) and less strong in Lemmens Inlet (Fig. 2-9; $r = -0.4$, $P < 0.05$), likely due to the small seasonal variability in the data. Temperature can indirectly affect Cd in oysters by directly affecting dissolved Cd levels. For example, during upwelling events, colder, deeper water is brought to the surface that is rich in dissolved Cd and in colder seasons, phytoplankton are less abundance which leads to minimal uptake of dissolved Cd levels, leading to higher dissolved Cd and a greater accumulation of Cd in oysters. The correlation between temperature and dissolved Cd was only significant in Deep Bay where there was an obvious seasonal trend. Temperature can also directly affect oysters; during summer, the oysters can have clearance and oxygen consumption rates of about double of what they experience during winter, which would lead to the oyster retaining less Cd (Van Dolah *et al.*, 1987, Ren *et.al*, 2000). Growth is more rapid in warmer temperatures (Shumway, 1996), increasing tissue mass, and diluting the pool of metals, decreasing Cd concentration.

Salinity can also affect oyster physiological responses (Shumway, 1996). In this study, there is such a small range in salinity that in the compiled dataset, the correlation between salinity and Cd in oysters does not reach significance. The relationship between dissolved Cd and salinity is weakly positive and significant for Deep Bay only, likely signifying a seasonal effect where colder, saline waters are enriched in dissolved Cd leading to higher levels of Cd in oysters.

2.4.3 Particulate Cd

Another important difference between both sites was the characterization of particulate Cd. During periods of high phytoplankton abundance, the Cd:P ratio in Deep Bay (with a mean value of 0.14 and 0.12 nM:μM during Aug. to Nov. and Mar. to Aug, respectively) (Fig. 2-8, Table 2-5) was similar to the extended Redfield stoichiometric formula for the average cellular content of phytoplankton, of 0.21 (mmol:mol) (Ho *et al.*, 2003). This suggests that that particulate Cd is mainly associated with phytoplankton. Although the average of 0.14 and 0.12 found in Deep Bay is lower than the extended Redfield ratio, the Cd-P ratio varies from species to species. For example, a small celled estuarine diatom, *Thalassiosira eccentrica* had a Cd-P ratio of 0.15 mmol:mol, whereas, a benthic dinoflagellate, *Amphidinium carterae* has a Cd-P ratio of 0.73 mmol:mol (Ho *et al.*, 2003). The phytoplankton species composition in Deep Bay during the sampling year mainly consisted of diatoms, some of which were physiologically similar to *Thalassiosira eccentrica* (Cassis *et al.*, 2006). A ratio of 0.14 and 0.12 (nM:μM) may just reflect the species composition at Deep Bay. Moreover, the extended Redfield ratio is determined for the intracellular composition of phytoplankton but phosphorus can bind to phytoplankton extracellularly (Sanudo-Wilhemy *et al.*, 2004), which would cause the particulate Cd-P ratio determined in this study to decrease. The variable Cd:Ti ratio in suspended particulate matter throughout the year and its lack of similarity to the Cd:Ti ratio in the sediments (Tables 2.5, 2.7 and Section 2.3.1.4), suggests that terrigenous materials either from rain, runoff or the sediments was not a dominant source of particulate Cd in the water column. Thus, the particulate Cd in Deep Bay was mainly organic. In Lemmens Inlet, the Cd:P ratio varied throughout the year and only came close to the extended Redfield ratio of 0.21 (mmol:mol) (Ho *et al.*, 2003) during the winter, suggesting that particulate Cd at this site is not mainly associated with phytoplankton. Titanium varied greatly throughout the year, but the Cd:Ti ratio remained relatively constant around 0.2 (nM:μM) and was very close to the ratio found in the sediments (Table 2-7, 2-10). The variation of Ti indicates the input of terrigenous materials during rain/runoff events and because the ratio of Cd:Ti is fairly constant, input of terrigenous materials is likely bringing in inorganic particulate Cd. Accumulation of Cd by the oysters from this particulate matter would be expected to be low, as most of the silt and other inorganic particules are rejected into pseudo-feces (Roesijadi, 1996). In Deep Bay, one may expect the particulate Cd to be a

source for the oysters because it is mainly associated with phytoplankton. However, particulate Cd was not found to be a major source of Cd to oysters, regardless of its characterization.

Several studies have indicated suspended particulate matter as a source of Cd for oysters under laboratory conditions (Bendell-young and Arifin, 2004; Reinfelder *et al.*, 1997; Hardy *et al.*, 1984)) but in this study, particulate matter was not found to be a vector of Cd. In Deep Bay, the particulate Cd is mainly organic and oysters do take up organic rich particles, so what happens to the Cd contained in these particles once they are ingested by oysters? Metals taken up by aquatic organisms can either by accumulated in a tightly bound or easily mobilized form (Roejadi and Robinson, 1994). If metals are easily mobilized they will depurate faster but metals that are tightly bound, such as those bound by metallothioneins, will depurate at a much slower rate. Cd taken up by phytoplankton will be ingested and then only after solubilization in the gut, is it taken up by oysters. Our data suggests that while dissolved Cd is taken up directly and will be sequestered and bioaccumulated, Cd associated with phytoplankton taken up by oysters will not be tightly bound and will depurate quicker.

Total particulate Cd from the combined data set displayed a negative correation with Cd in oysters ($r = -0.5$, $P < 0.01$). Particulate matter that is associated with phytoplankton is likely effecting Cd in oysters by directly affecting dissolved Cd. In Deep Bay, where the particulate Cd is mainly associated with phytoplankton, there was a seasonal trend in dissolved Cd. Levels were lowest in the spring and summer months, corresponding to typical periods of phytoplankton abundance, and highest in the winter months, corresponding to the typical period of minimal phytoplankton abundance. Phytoplankton take up dissolved Cd and this effectively removes the form of Cd that oysters bioaccumulate. In Deep Bay, there was a significant inverse correlation between particulate Cd and dissolved Cd ($r = -0.5$, $P < 0.05$), signifying the important role of phytoplankton on dissolved Cd. In Lemmens inlet, where the particulate Cd is mainly inorganic, there was no seasonal trend in dissolved Cd and there is no significant correlation between particulate Cd and dissolved Cd. Since Cd is a non-reactive metal (Bruland *et al.*, 1994), a relationship between inorganic particulate Cd and dissolved Cd would not be expected. However, a significant inverse correlation was still observed for the combined dataset (Fig. 2-16). The second manner in which Cd

associated with phytoplankton can negatively affect Cd in oysters is by adding to the tissue mass of the oysters after ingestion. In the combined data set, there was a significant correlation between particulate Cd and tissue mass (Fig. 2-16).

2.5 *Conclusions*

This project is one of the first to look at the variables effecting the accumulation of Cd in oysters in the field. How all the variables fit in this study, along with the significant correlations from the combined data set is depicted in the figure 2-16. The main source of Cd to oysters was found to be dissolved Cd. Highly concentrated levels of Cd were found in the gut of the oyster, which is partially due to the small size of the gut and mainly due to the transfer of dissolved Cd from the gills to the digestive gland where Cd is accumulated by metallothioneins (Roesjadi and Robinson, 1994). Significant influences on dissolved Cd included sediment diagenesis, which can lead to the drawdown of dissolved Cd, as was seen in Lemmens Inlet, and the effect of water mixing and primary production, as was seen in Deep Bay. Particulate Cd was not a source of Cd to oysters, indicating that particulate Cd is likely accumulated by oysters in an easily mobilized form, allowing Cd from particles to depurate quicker. Instead, particulate Cd was found to be negatively correlated with Cd in oysters. Organic particulate Cd has a direct negative affect on dissolved Cd and a positive affect on oyster tissue growth. Oyster tissue growth directly affects Cd in oysters with increases in tissue mass leading to lower Cd concentrations. Inorganic particulate Cd does not play a role in Cd in oysters as oysters preferentially reject inorganic particles and since Cd is a non-reactive metal, inorganic particulate Cd does not affect dissolved Cd. Temperature mainly had a seasonal effect on dissolved Cd, where periods of low temperature resulted in higher dissolved Cd, which also corresponded to higher levels of Cd in oysters. Both temperature and salinity signified mixing of different water masses which changes the dissolved Cd and indirectly affects Cd in oysters.

The motivation for this study was to determine the pathway of Cd to oysters and ultimately help BC shellfish farmers work around the Cd levels in their products. A pathway has been determined but, unfortunately, it is one that is the least controllable. However, the lowest Cd levels in oysters were seen

when dissolved Cd was low and temperature and particulate matter was high. Choosing sites with low dissolved Cd and harvesting oysters during the summer and spring months, when the oysters are actively growing and there is a bloom of phytoplankton, is the best option for shellfish farmers.

2.6 *Figures*

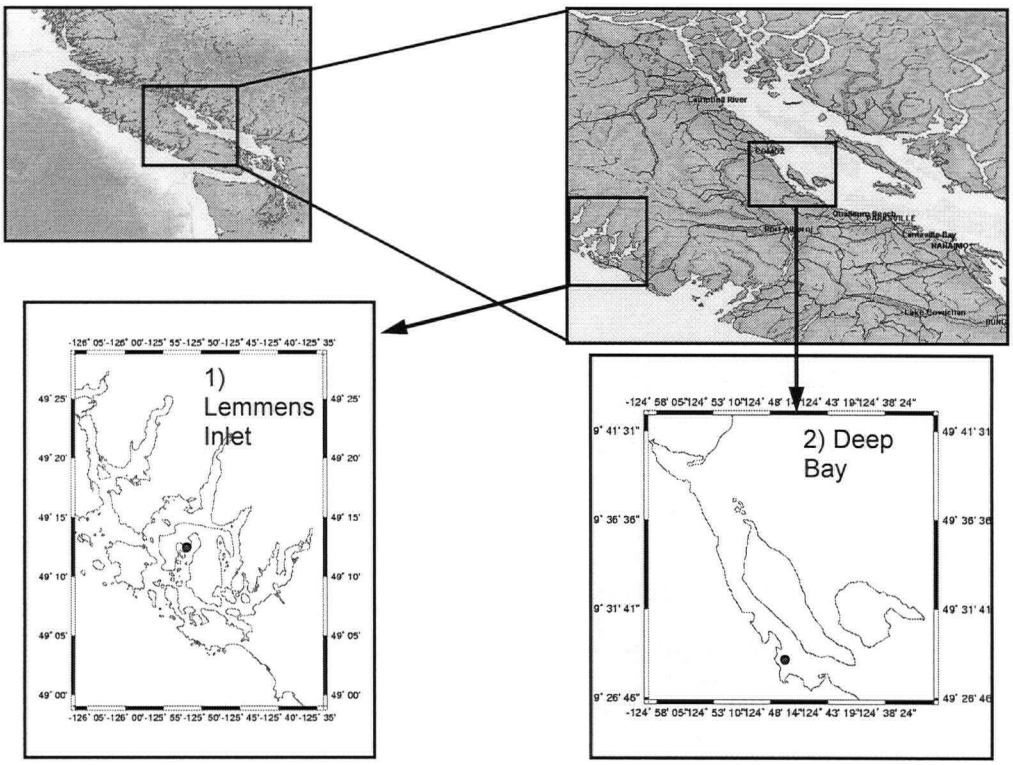

Figure 2-1: Location of the sampling stations (Deep Bay and Lemmens Inlet)

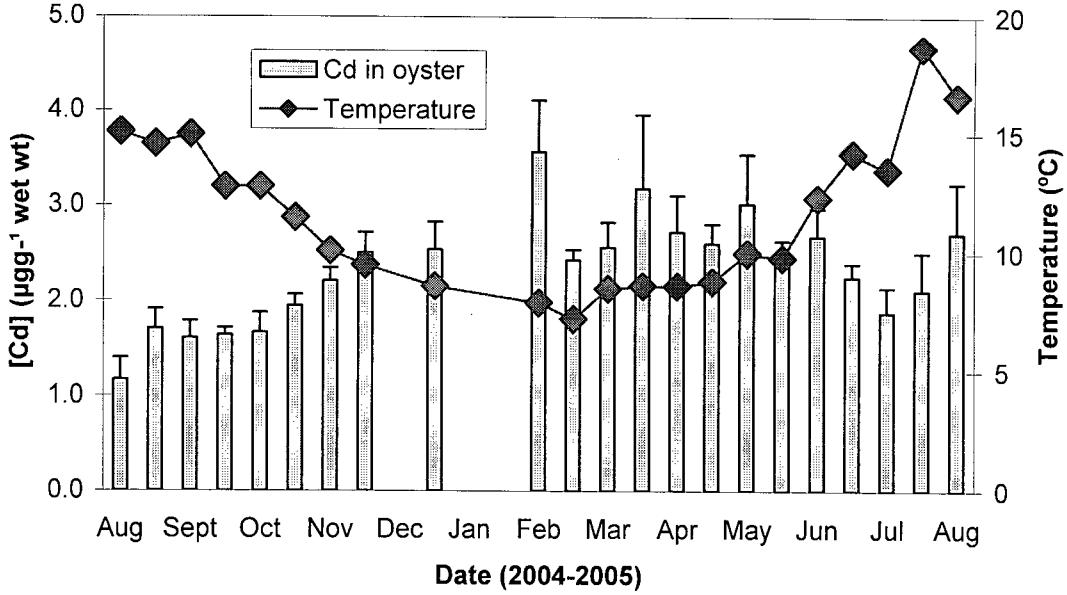

Figure 2-2: Deep Bay: Seasonal variability of Cd in oysters overlayed with temperature data. Inverse correlation between temperature and Cd in oysters is significant ($r = -0.6$, $P < 0.01$).

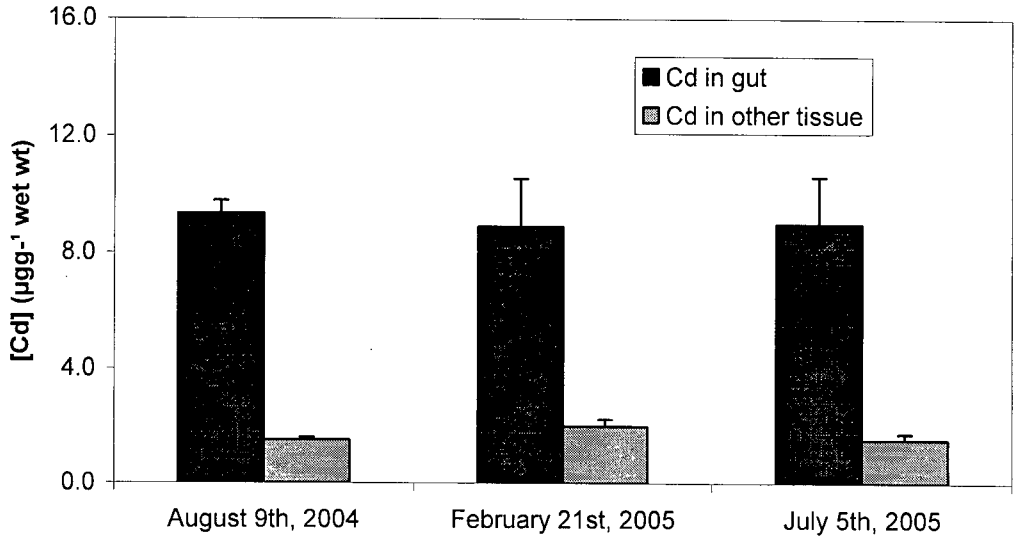

Figure 2-3: Deep Bay: Concentration of Cd in the gut and in tissues other than the gut of oysters sampled at 5m during the three vertical sampling events.

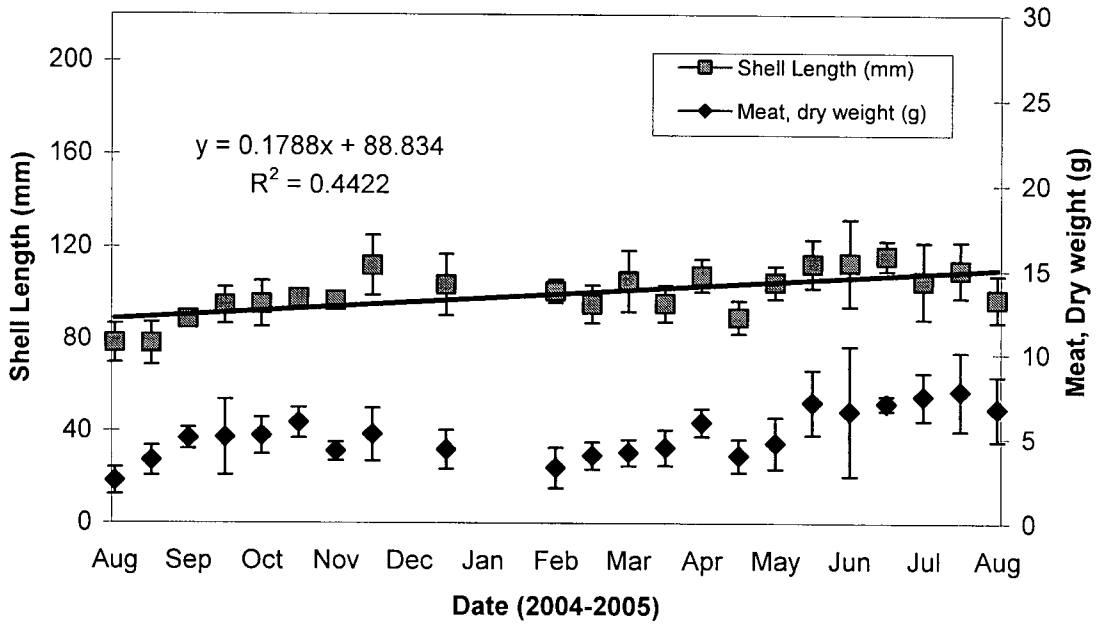

Figure 2-4: Deep Bay: Oyster growth over time: Changes in shell length and dry meat weight over time.

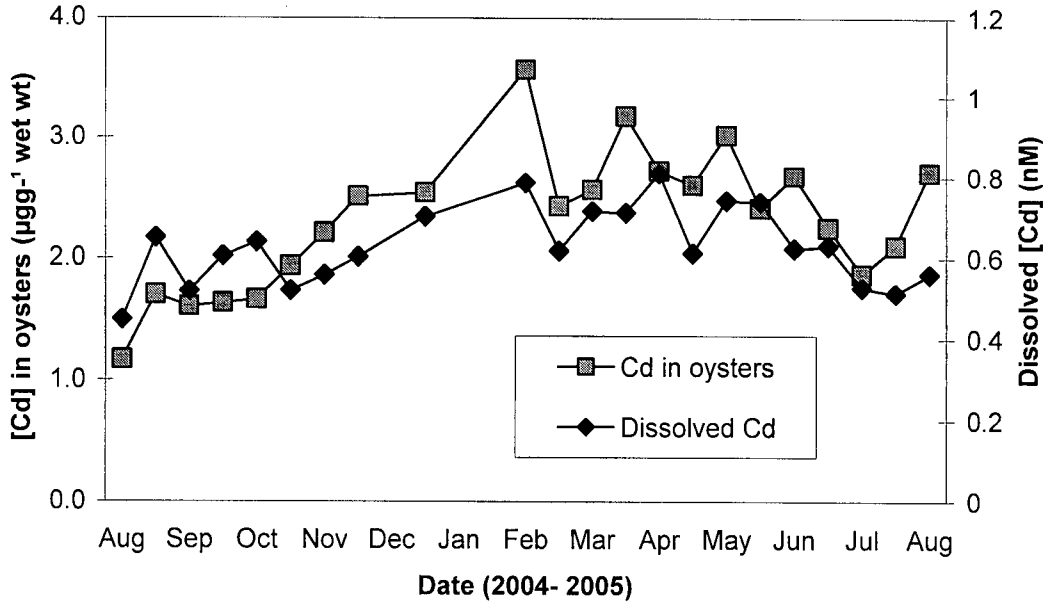

Figure 2-5: Deep Bay: Comparison of dissolved Cd and Cd in oysters. Dissolved Cd is significantly correlated with Cd in oysters ($r = 0.7$, $P < 0.001$). Error bars for Cd in oyster data have been removed for clarity. Error bars for dissolved Cd data are too small to be visible.

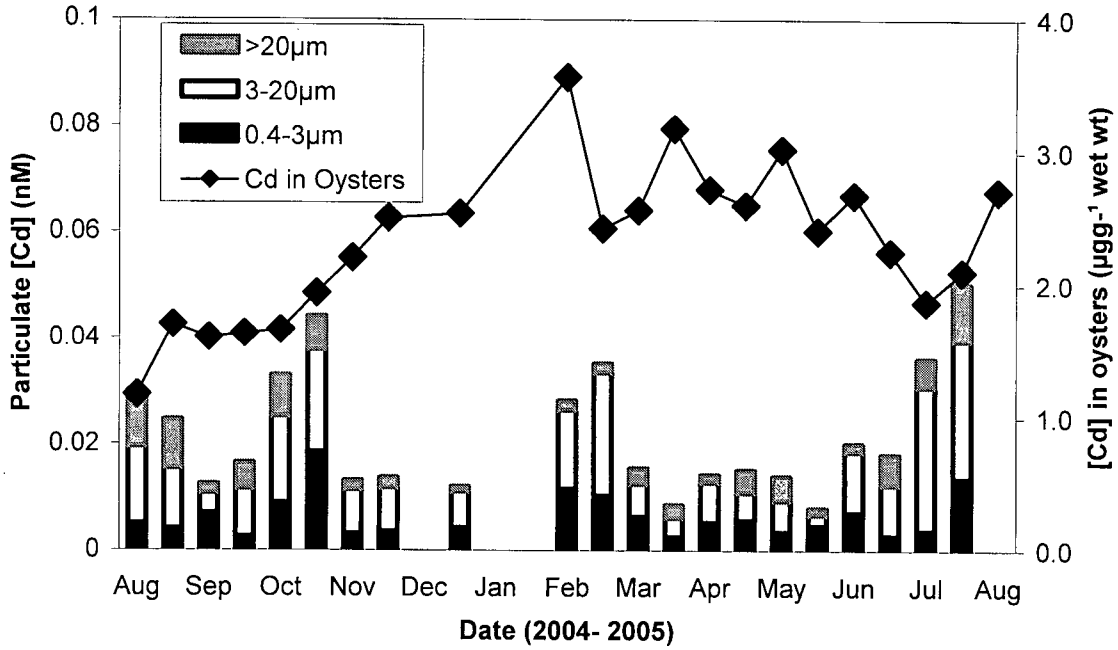

Figure 2-6: Deep Bay: Comparison of fractionated particulate Cd and Cd in oysters. Correlations between the smaller fractions of particulate Cd and Cd in oysters were weakly negative and insignificant ($r = -0.02$ and $r = -0.3$, $P > 0.05$ for 0.4-3µm and 3-20µm, respectively). For the >20µm fraction, the inverse correlation was significant ($r = -0.6$ and $P < 0.01$). Error bars for Cd in oyster data have been removed for clarity.

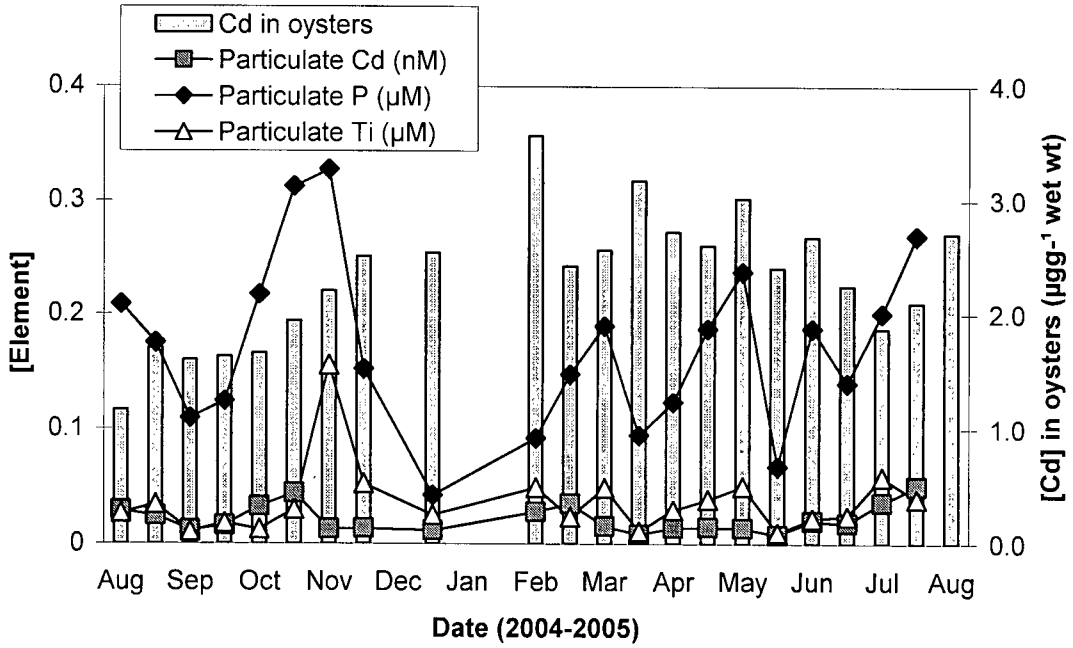

Figure 2-7: Deep Bay: Comparison of total particulate Cd (nM), phosphorus (µM), titanium (µM) and Cd in oysters ($\mu g g^{-1}$). Total particulate Cd, P, and Ti are not significantly correlated with oysters as $P > 0.05$. Error bars for Cd in oyster data have been removed for clarity.

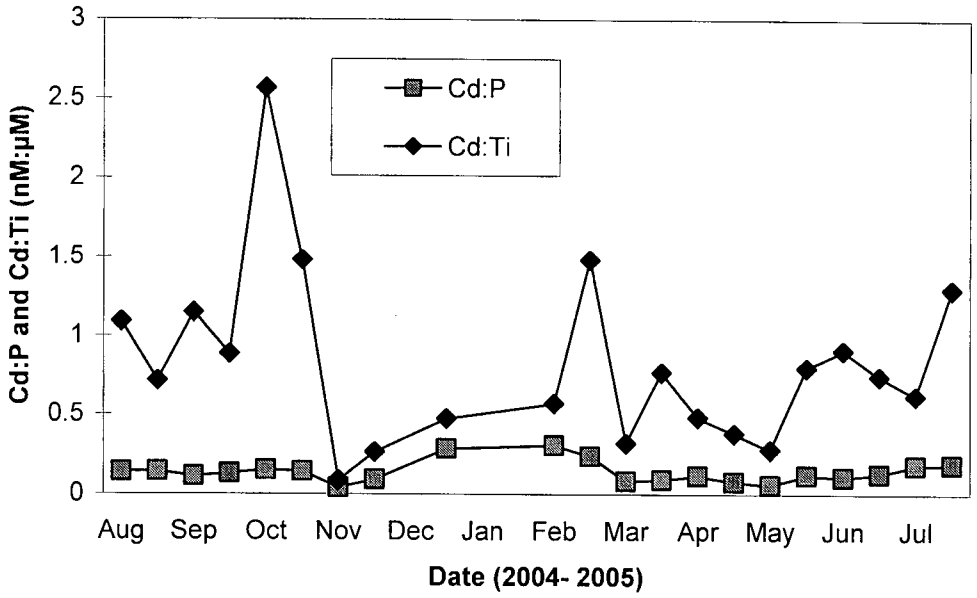

Figure 2-8: Deep Bay: Ratios of Cd:P and Cd:Ti in particulate samples.

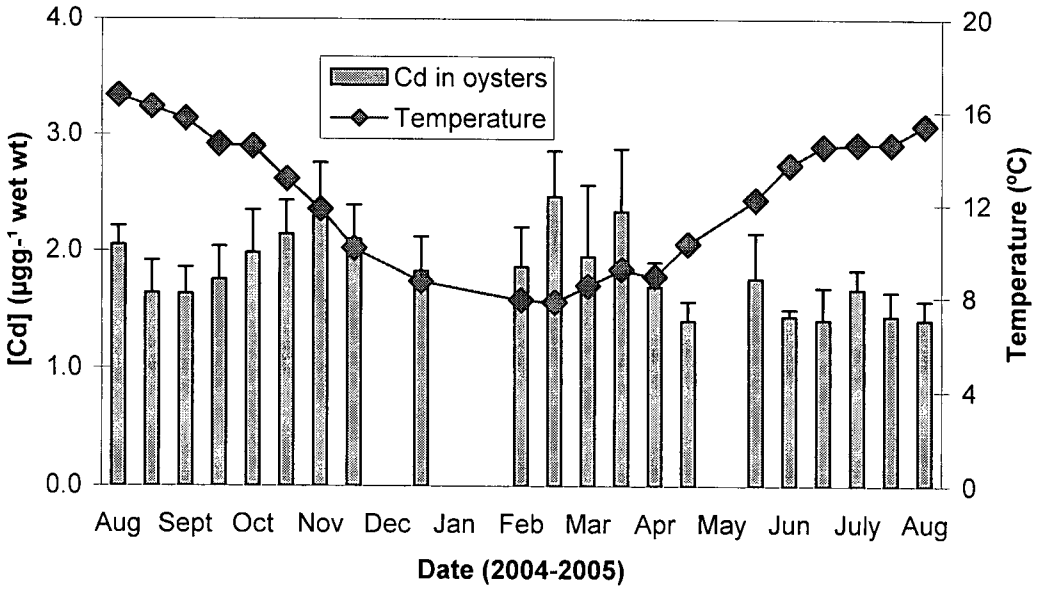

Figure 2-9: Lemmens Inlet: Seasonal Variability of Cd in oysters overlayed with temperature. Temperature and Cd in oysters have an inverse correlation ($r = -0.4$ and $P < 0.05$).

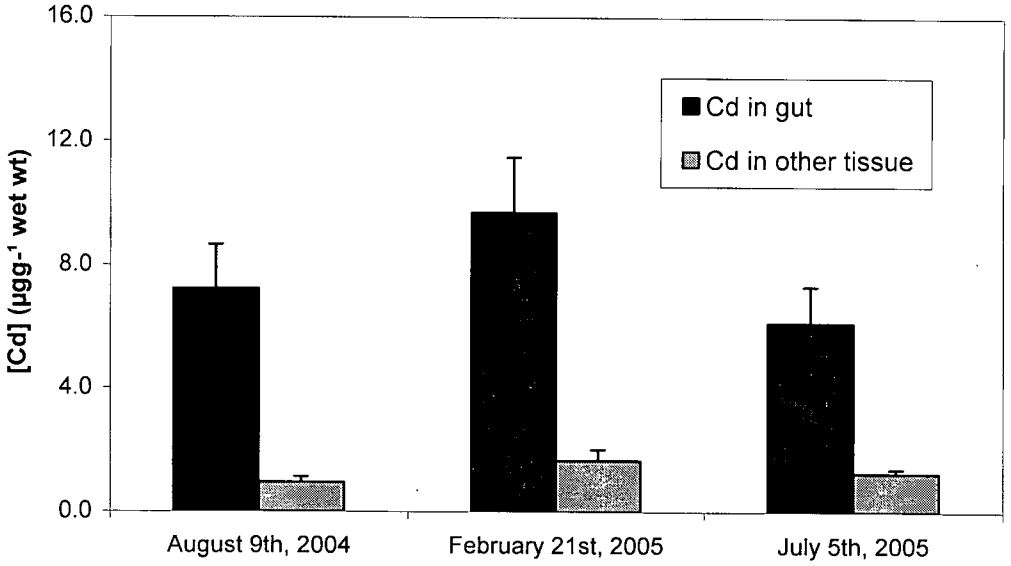

Figure 2-10: Lemmens Inlet: Cadmium concentrations in the gut and in tissues other than the gut of oysters.

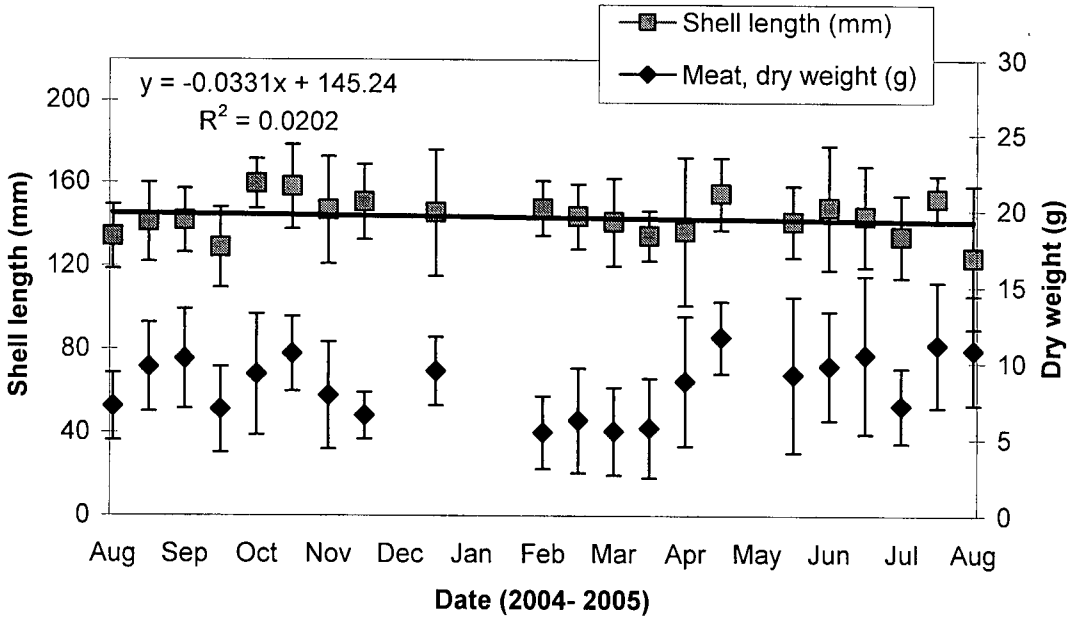

Figure 2-11: Lemmens Inlet: Oyster growth over time; changes in shell length and meat, dry weight.

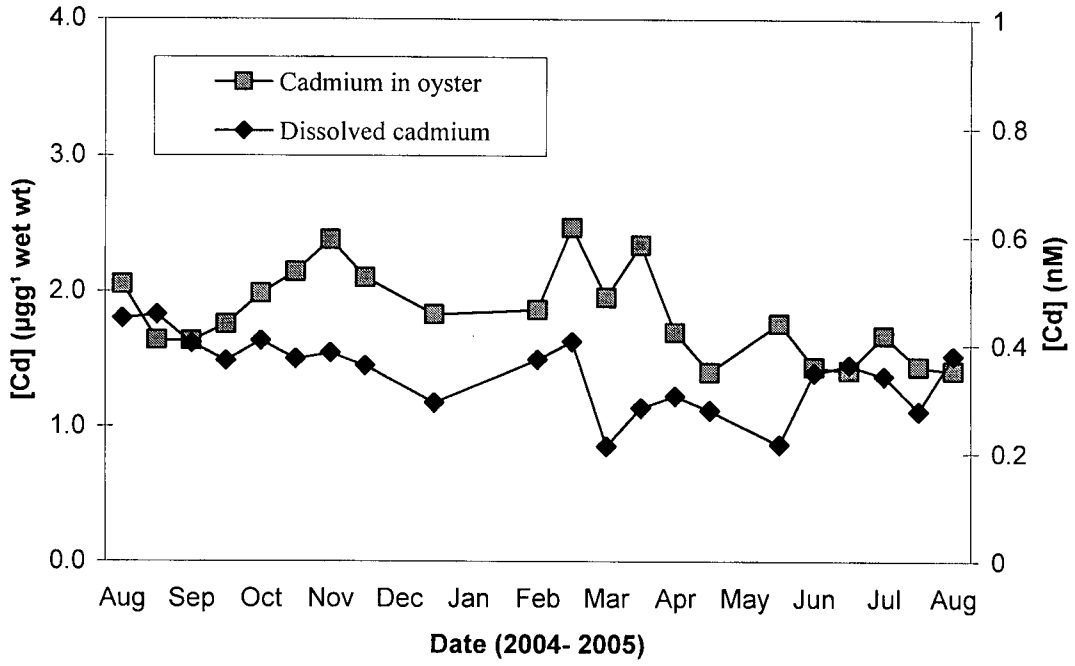

Figure 2-12: Lemmens Inlet: Comparison of dissolved Cd and Cd in oysters. Dissolved Cd is correlated with Cd in oysters (r = 0.2) but it is not significant (P> 0.05). Error bars for Cd in oyster data have been removed for clarity. Error bars for dissolved Cd data are too small to be visible.

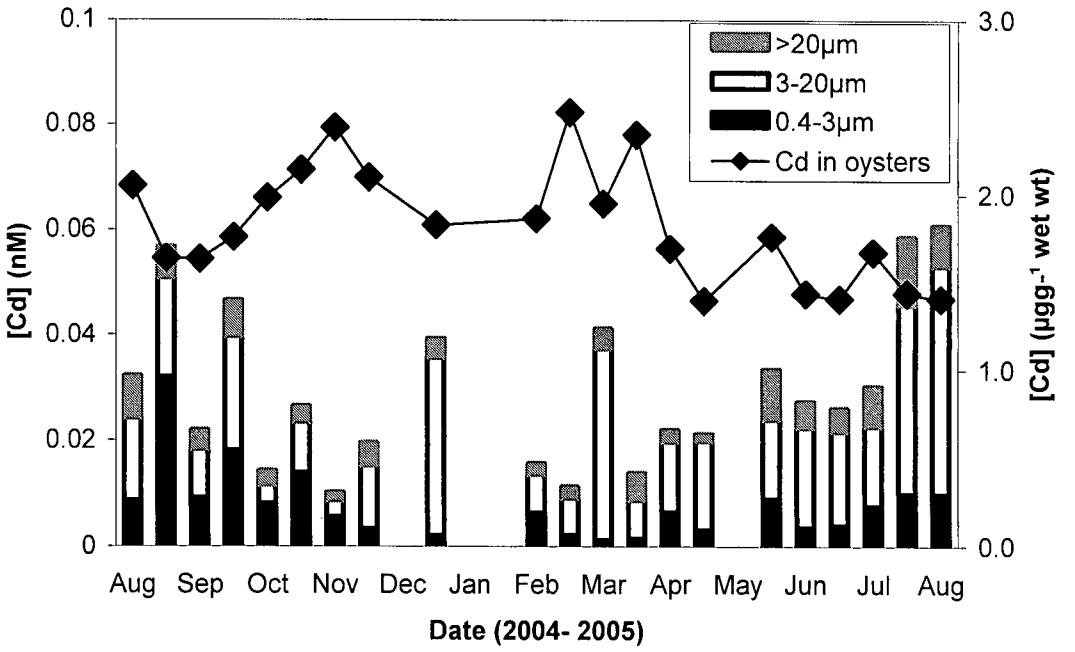

Figure 2-13: Lemmens Inlet: Comparison of fractionated particulate Cd and Cd in oysters. Particulate Cd and Cd in oysters are inversely correlated (r= -0.1 to -0.5) but only the 3-20µm fraction is significantly correlated (P< 0.05). Error bars for Cd in oyster data have been removed for clarity.

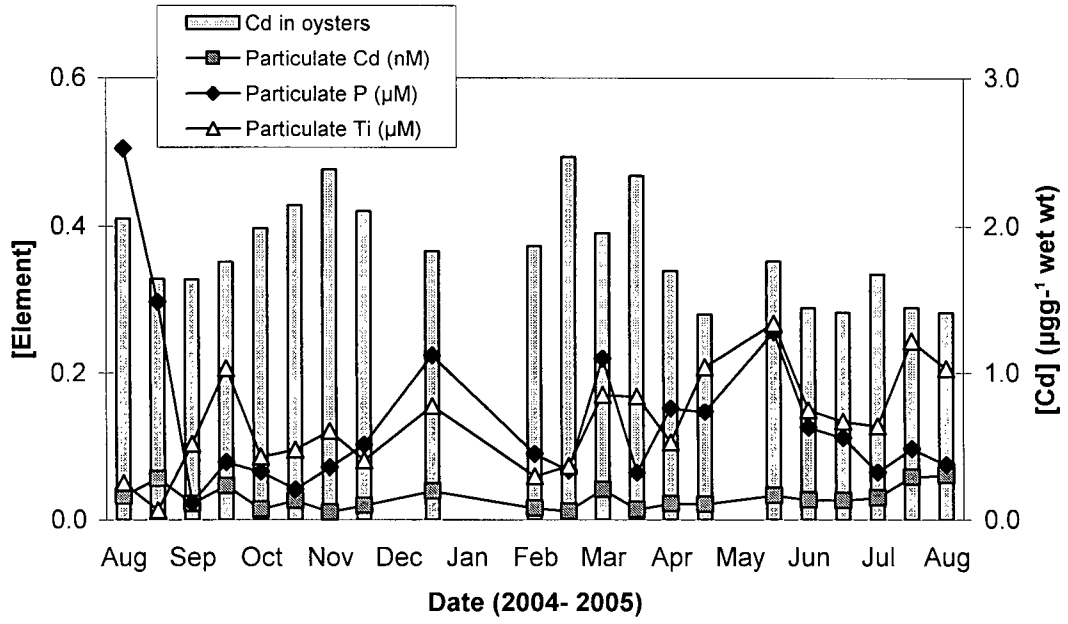

Figure 2-14: Lemmens Inlet: Total particulate Cd (nM), phosphorus (µM) and titanium (µM) and Cd in oysters (µgg^{-1}). Particulate Cd is significantly inversely correlated with Cd in oysters (r = -0.5, P<0.02) Particulate P and Ti are not significantly correlated. Error bars for Cd in oyster data have been removed for clarity.

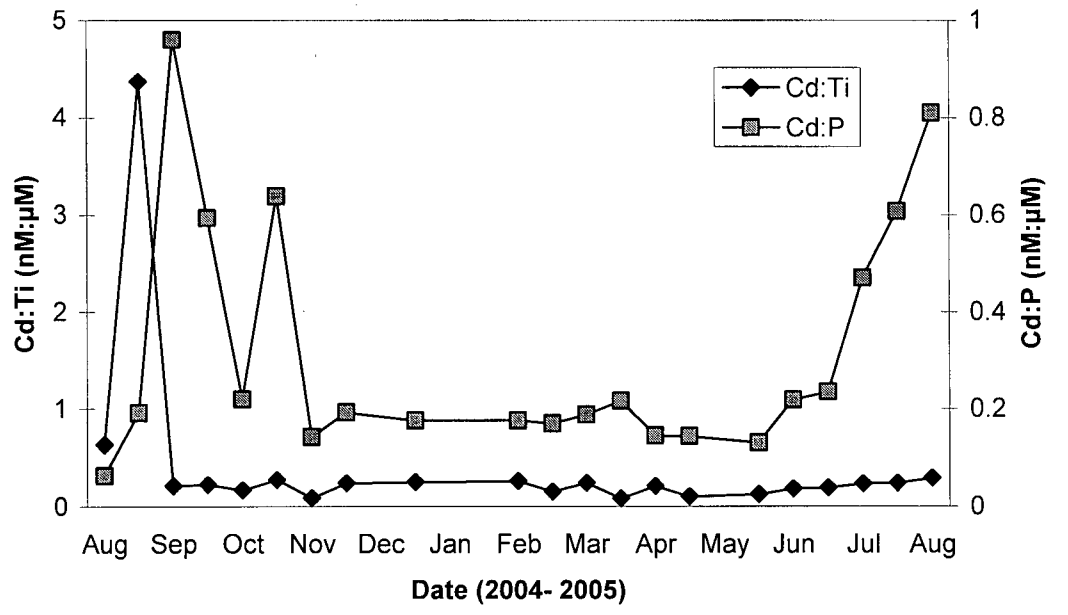

Figure 2-15: Lemmens Inlet: Ratios of Cd:Ti and Cd:P (nM:µM) and Cd in oysters (µgg^{-1}).

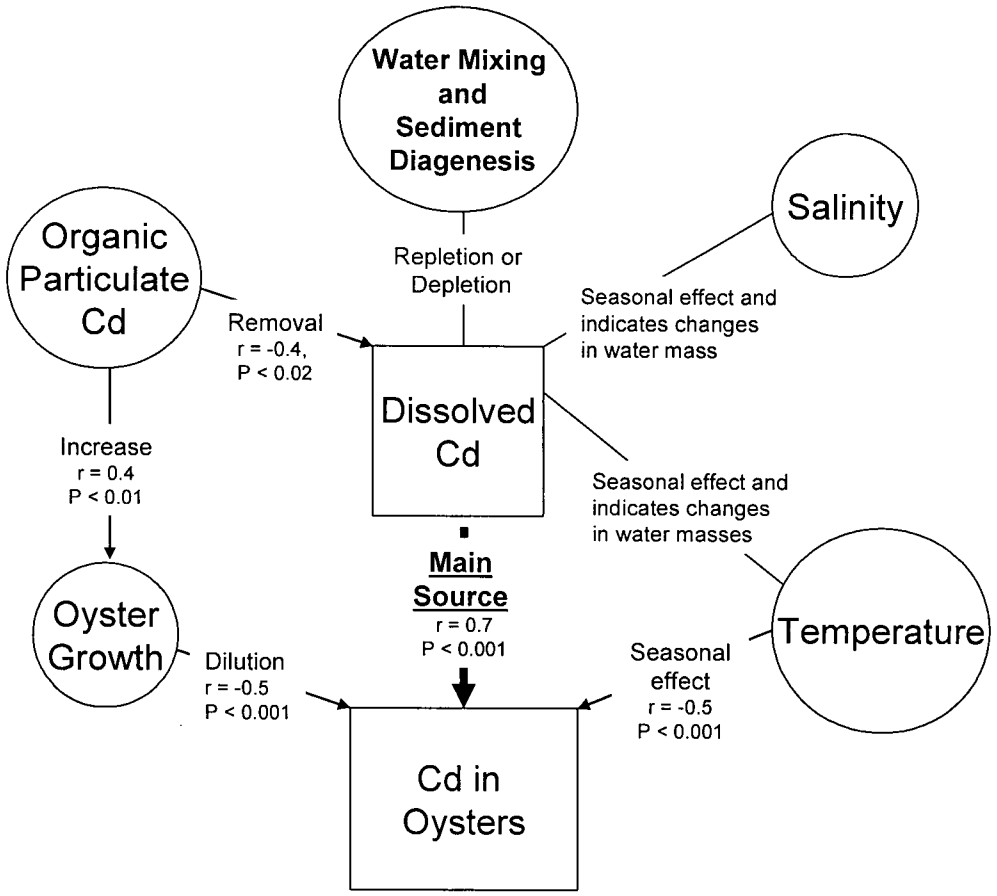

Figure 2-16: Summary of the factors affecting Cd accumulation in oysters. Statistics given are for the combined data set and only for significant correlations. Arrows represent significant correlations, as indicated. Lines indicate variable affects, as specifed. The statistics given for organic particulate Cd is actually for total particulate Cd.

2.7 *Tables*

Table 2-1: Accuracy and precision of Cd, P and Ti concentrations in the analysis of suspended particulate and sediment samples using Coastal Sediment Reference Materials.

Standard	Certified [Cd] (ugg^{-1})	Found [Cd] (ugg-1) (n= 3)	Certified [P] (%)	Found [P] (%) (n=3)	Certified [Ti] (%)	Found [Ti] (%) (n=3)
PACS-2	2.11 ± 0.15	2.53 ± 0.08	0.096 ± 0.004	0.115 ± 0.001	0.443 ± 0.032	0.480 ± 0.004
MESS-2	0.24 ± 0.01	0.303 ± 0.002	0.12 ± 0.01	0.127± 0.008	0.437[a]	0.534 ± 0.010

[a]Not a certified value. Value from one set of XRF results. (pers. Comms., Willie, S., National Research Council of Canada)

Table 2-2: Deep Bay: Biweekly oyster Cd concentration, meat dry and wet weight, and shell length collected at 2-5m.

Date	[Cd] (μgg^{-1} wet weight)	Std. dev.	Meat, dry weight (g)	Std. dev.	Meat, wet weight (g)	Std. dev.	Shell length (mm)	Std. dev.
09-Aug-04	1.17	0.23	2.47	0.81	9.58	2.34	78	9
24-Aug-04	1.70	0.20	3.69	0.89	14.98	3.73	78	9
07-Sep-04	1.60	0.18	5.01	0.63	20.64	1.91	89	3
20-Sep-04	1.63	0.08	5.05	2..24	20.92	7.53	95	8
05-Oct-04	1.66	0.21	5.16	1.09	20.58	4.54	95	10
19-Oct-04	1.94	0.12	5.93	0..90	22.52	3.24	98	4
02-Nov-04	2.21	0.14	4.23	0..55	16.64	1.44	97	4
30-Nov-04	2.51	0.21	5.22	1.56	20.78	5.16	112	13
28-Dec-04	2.54	0.29	4.33	1.16	18.16	5.54	104	13
07-Feb-05	3.57	0.54	3.25	1.21	14.11	4.37	101	5
20-Feb-05	2.43	0.11	3.98	0.80	16.62	3.59	95	8
07-Mar-05	2.56	0.26	4.15	0.78	16.60	2.11	105	13
21-Mar-05	3.18	0.78	4.45	1.06	18.32	2.56	96	8
04-Apr-05	2.72	0.38	5.94	0.82	22.74	3.22	108	7
17-Apr-05	2.60	0.21	3.96	0..98	15.70	3.15	90	7
01-May-05	3.02	0.52	4.71	1..53	17.82	4.60	105	7
20-May-05	2.41	0.22	7.13	1..91	24.54	5.73	113	10
06-Jun-05	2.68	0.29	6.60	3.85	25.78	13.05	113	19
21-Jun-05	2.25	0.14	7.07	0.42	30.38	2.50	116	6
06-Jul-05	1.87	0.26	7.47	1.42	31.32	6.14	105	17
17-Jul-05	2.10	0.41	7.78	2..32	30.04	7.04	110	12
04-Aug-05	2.71	0.53	6.74	1..91	24.82	5.71	98	10

Table 2-3: Oyster Cd concentration in the gut and in other tissues for Deep Bay and Lemmens Inlet.

	[Cd] gut ($\mu g g^{-1}$ wet weight)	Std. dev.	[Cd] other tissue ($\mu g g^{-1}$ wet weight)	Std. dev.
August 9th, 2004				
Deep Bay	9.33	1.96	1.52	0.08
Lemmens Inlet	7.23	1.42	0.94	0.18
February 21st, 2005				
Deep Bay	8.88	2.69	1.97	0.25
Lemmens Inlet	9.69	1.79	1.65	0.36
July 5th, 2005				
Deep Bay	8.97	1.74	1.53	0.21
Lemmens Inlet	6.10	1.21	1.25	0.13

Table 2-4: Deep Bay: Biweekly temperature, Salinity, dissolved Cd collected at 6m.

Date	Salinity (PSU)	Temp (°C)	[Cd] (nM)
09-Aug-04	23.5	15.1	0.45
24-Aug-04	27	14.6	0.65
07-Sep-04	27.1	15	0.52
21-Sep-04	27.1	12.8	0.61
05-Oct-04	26	12.8	0.64
19-Oct-04	24	11.5	0.52
02-Nov-04	29	10.1	0.56
30-Nov-04	29	9.5	0.60
28-Dec-04	27	8.6	0.70
07-Feb-05	27	7.9	0.79
20-Feb-05	27	7.2	0.62
07-Mar-05	28	8.5	0.72
21-Mar-05	28.5	8.6	0.71
04-Apr-05	28	8.6	0.81
17-Apr-05	27.1	8.8	0.61
01-May-05	27	10	0.74
20-May-05	28.5	9.8	0.74
05-Jun-05	27	12.3	0.62
20-Jun-05	26	14.2	0.63
06-Jul-05	27	13.5	0.53
17-Jul-05	24	18.7	0.51
05-Aug-05	26	16.6	0.56

Table 2-5: Deep Bay: Biweekly total particulate Cd (nM), P (μM), Ti (μM) collected at 6m and ratios of particulate Cd:P (nM:μM) and Cd:Ti (nM:μM).

Date	Total Cd (nM)	Total P (μM)	Total Ti (μM)	Cd:P (nM: μM)	Cd: Ti (nM: μM)
09-Aug-04	0.029	0.21	0.027	0.14	1.09
24-Aug-04	0.025	0.18	0.034	0.14	0.71
07-Sep-04	0.012	0.11	0.011	0.11	1.17
21-Sep-04	0.016	0.12	0.018	0.13	0.90
05-Oct-04	0.033	0.22	0.013	0.15	2.57
19-Oct-04	0.044	0.31	0.030	0.14	1.48
02-Nov-04	0.013	0.33	0.16	0.040	0.084
30-Nov-04	0.014	0.15	0.052	0.090	0.26
28-Dec-04	0.012	0.042	0.025	0.28	0.47
07-Feb-05	0.028	0.092	0.049	0.31	0.57
20-Feb-05	0.035	0.15	0.024	0.24	1.48
07-Mar-05	0.016	0.19	0.049	0.082	0.32
21-Mar-05	0.0085	0.094	0.011	0.090	0.77
04-Apr-05	0.014	0.12	0.030	0.11	0.48
17-Apr-05	0.015	0.19	0.039	0.080	0.39
01-May-05	0.014	0.24	0.050	0.059	0.28
20-May-05	0.0079	0.067	0.0099	0.12	0.80
05-Jun-05	0.020	0.19	0.022	0.11	0.91
20-Jun-05	0.018	0.14	0.024	0.13	0.74
06-Jul-05	0.036	0.20	0.058	0.18	0.62
17-Jul-05	0.050	0.27	0.039	0.18	1.29
05-Aug-05	-	-	-	-	-

Table 2-6: Deep Bay: Biweekly fractionated particulate Cd, P, Ti collected at 6m.

Date	Particulate Cd (nM)			Particulate P (μM)			Particulate Ti (μM)		
	0.4-3μm	3.0-20μm	>20μm	0.4-3μm	3.0-20μm	>20μm	0.4-3μm	3.0-20μm	>20μm
09-Aug-04	0.0049	0.0144	0.0097	0.0290	0.1044	0.0754	0.0018	0.0182	0.0067
24-Aug-04	0.0040	0.0113	0.0092	0.0339	0.1282	0.0128	0.0012	0.0307	0.0024
07-Sep-04	0.0070	0.0037	0.0017	0.0536	0.0433	0.0125	0.0070	0.0018	0.0018
21-Sep-04	0.0026	0.0089	0.0050	0.0162	0.0541	0.0542	0.0006	0.0099	0.0077
05-Oct-04	0.0088	0.0163	0.0077	0.0853	0.0829	0.0493	0.0018	0.0087	0.0023
19-Oct-04	0.0185	0.0192	0.0064	0.0810	0.1880	0.0434	0.0012	0.0216	0.0070
02-Nov-04	0.0031	0.0082	0.0017	0.0268	0.2886	0.0117	0.0012	0.1501	0.0045
30-Nov-04	0.0035	0.0081	0.0020	0.0161	0.1327	0.0035	0.0025	0.0486	0.0012
28-Dec-04	0.0042	0.0067	0.0010	0.0128	0.0267	0.0028	0.0023	0.0229	0.0000
07-Feb-05	0.0115	0.0148	0.0017	0.0236	0.0643	0.0039	0.0039	0.0435	0.0020
20-Feb-05	0.0102	0.0231	0.0018	0.0388	0.0886	0.0204	0.0022	0.0185	0.0031
07-Mar-05	0.0063	0.0061	0.0031	0.0348	0.1341	0.0206	0.0037	0.0400	0.0051
21-Mar-05	0.0026	0.0035	0.0024	0.0342	0.0240	0.0361	0.0021	0.0043	0.0047
04-Apr-05	0.0053	0.0074	0.0015	0.0571	0.0576	0.0088	0.0057	0.0217	0.0022
17-Apr-05	0.0056	0.0051	0.0043	0.0800	0.0893	0.0179	0.0069	0.0265	0.0056
01-May-05	0.0035	0.0058	0.0046	0.0709	0.1011	0.0654	0.0025	0.0333	0.0142
20-May-05	0.0046	0.0021	0.0012	0.0441	0.0151	0.0077	0.0033	0.0039	0.0028
05-Jun-05	0.0070	0.0115	0.0016	0.0959	0.0805	0.0109	0.0052	0.0140	0.0029
20-Jun-05	0.0027	0.0095	0.0058	0.0291	0.0706	0.0405	0.0049	0.0129	0.0065
06-Jul-05	0.0038	0.0269	0.0054	0.0420	0.1175	0.0411	0.0029	0.0404	0.0151
17-Jul-05	0.0135	0.0260	0.0109	0.1412	0.1030	0.0247	0.0138	0.0208	0.0044
05-Aug-05	0.0753	-	0.0129	0.109	-	0.267	0.0238	-	0.021

Table 2-7: Metal analysis in sediment samples at both sites. For ratios, units converted to mmol kg^{-1} for Cd and mol kg^{-1} for other elements.

[Element] (ugg^{-1})	Deep Bay	Lemmens Inlet
P	661	955
Ti	4029	3526
Mn	534	410
Org C	12000	31000
Cd	0.76	2.24

Ratios (mmol kg^{-1} : mol kg^{-1})	Deep Bay	Lemmens Inlet
Cd:P	0.62	1.31
Cd:Ti	0.080	0.27
P:Ti (mol kg^{-1}:mol kg^{-1})	0.13	0.21

Table 2-8: Lemmens Inlet: Biweekly oyster Cd concentration, meat dry and wet weight, and shell length collected at 2-5m.

Date	[Cd] ($\mu g g^{-1}$ wet weight)	Std. dev.	Meat, dry weight (g)	Std. dev.	Meat, wet weight (g)	Std. dev.	Shell length (mm)	Std. dev.
10-Aug-04	2.05	0.16	7.13	2.22	25.95	8.39	134	15
24-Aug-04	1.64	0.28	9.73	2.91	32.85	13.25	141	19
07-Sep-04	1.63	0.22	10.26	3.26	41.30	14.04	142	15
21-Sep-04	1.76	0.28	6.94	2.82	22.10	10.04	123	19
05-Oct-04	1.98	0.37	9.25	3.96	47.80	13.87	159	12
19-Oct-04	2.14	0.29	10.59	2.45	49.10	9.27	158	20
02-Nov-04	2.38	0.38	7.86	3.52	28.70	16.25	147	26
30-Nov-04	2.10	0.30	6.54	1.53	34.35	5.60	151	18
28-Dec-04	1.83	0.29	9.46	2.23	46.50	9.33	146	30
09-Feb-05	1.86	0.34	5.42	2.39	24.55	9.61	148	13
21-Feb-05	2.47	0.39	6.22	3.43	21.10	10.62	144	16
09-Mar-05	1.95	0.61	5.51	2.86	22.42	14.88	141	21
22-Mar-05	2.34	0.54	5.73	3.25	29.90	13.69	134	12
06-Apr-05	1.69	0.21	8.79	4.26	25.30	17.86	137	36
19-Apr-05	1.40	0.17	11.67	2.36	54.05	6.90	155	17
25-May-05	1.76	0.39	9.23	5.12	5.54	5.12	141	17
08-Jun-05	1.44	0.06	9.81	3.56	8.89	3.56	148	30
22-Jun-05	1.41	0.28	10.52	5.18	11.75	5.18	144	24
05-Jul-05	1.67	0.17	7.19	2.46	4.83	2.46	134	20
20-Jul-05	1.44	0.21	11.20	4.11	13.20	4.11	152	11
28-Jul-05	1.41	0.17	10.84	3.59	14.50	3.59	124	34

Table 2-9: Lemmens Inlet: Biweekly temperature, Salinity, dissolved Cd collected at 6m.

Date	Salinity (PSU)	Temp (°C)	[Cd] (nM)
10-Aug-04	28.5	16.7	0.45
24-Aug-04	30.0	16.2	0.46
07-Sep-04	30.0	15.7	0.40
21-Sep-04	29.0	14.6	0.37
05-Oct-04	29.0	14.5	0.41
19-Oct-04	31.0	13.1	0.37
02-Nov-04	25.0	11.8	0.39
30-Nov-04	25.0	10.1	0.36
28-Dec-04	25.0	8.7	0.29
09-Feb-05	25.0	7.9	0.37
21-Feb-05	24.5	7.8	0.41
09-Mar-05	26.0	8.5	0.21
22-Mar-05	27.0	9.2	0.28
05-Apr-05	25.0	8.9	0.30
22-Apr-05	24.0	10.3	0.28
25-May-05	25.0	12.2	0.22
08-Jun-05	27.0	13.7	0.35
22-Jun-05	30.0	14.5	0.36
05-Jul-05	26.0	14.6	0.34
20-Jul-05	28.0	14.6	0.28
28-Jul-05	28.0	15.4	0.38

Table 2-10: Lemmens Inlet: Biweekly total particulate Cd (nM), P (uM), Ti (uM) collected at 6m and ratios of particulate Cd:P (nM:uM), and Cd:Ti (nM:uM).

Date	Total Cd (nM)	Total P (μM)	Total Ti (μM)	Cd:P (nM: μM)	Cd: Ti (nM: μM)
Aug-04	0.032	0.51	0.051	0.07	0.63
24-Aug	0.057	0.30	0.013	0.19	4.36
07-Sep	0.022	0.023	0.10	0.96	0.21
21-Sep	0.047	0.079	0.21	0.59	0.23
05-Oct	0.014	0.065	0.086	0.22	0.17
19-Oct	0.027	0.042	0.095	0.64	0.28
02-Nov	0.010	0.072	0.12	0.14	0.08
30-Nov	0.020	0.10	0.081	0.19	0.24
28-Dec	0.039	0.22	0.15	0.18	0.25
09-Feb	0.016	0.090	0.060	0.18	0.26
20-Feb	0.011	0.067	0.074	0.17	0.16
09-Mar	0.041	0.22	0.17	0.19	0.24
22-Mar	0.014	0.064	0.17	0.22	0.08
06-Apr	0.022	0.15	0.11	0.15	0.21
22-Apr	0.021	0.15	0.21	0.14	0.10
25-May	0.033	0.26	0.27	0.13	0.13
08-Jun	0.027	0.13	0.15	0.22	0.18
22-Jun	0.026	0.11	0.13	0.23	0.20
05-Jul	0.030	0.065	0.13	0.47	0.24
20-Jul	0.059	0.097	0.24	0.61	0.24
28-Jul	0.061	0.075	021	0.81	0.30

Table 2-11: Lemmens Inlet: Biweekly fractionated particulate Cd, P, Ti collected at 6m.

Date	Particulate Cd (nM)			Particulate P (μM)			Particulate Ti (μM)		
	0.4-3μm	3.0-20μm	>20μm	0.4-3μm	3.0-20μm	>20μm	0.4-3μm	3.0-20μm	>20μm
Aug-04	0.009	0.015	0.008	0.188	0.284	0.033	0.013	0.033	0.004
24-Aug	0.032	0.019	0.006	0.165	0.111	0.020	0.005	0.007	0.001
07-Sep	0.009	0.009	0.004	0.005	0.016	0.001	0.057	0.036	0.010
21-Sep	0.018	0.022	0.007	0.024	0.049	0.005	0.116	0.079	0.011
05-Oct	0.008	0.004	0.003	0.046	0.012	0.007	0.062	0.014	0.010
19-Oct	0.014	0.010	0.003	0.006	0.032	0.003	0.056	0.032	0.008
02-Nov	0.006	0.003	0.001	0.048	0.022	0.002	0.096	0.020	0.004
30-Nov	0.003	0.012	0.004	0.004	0.079	0.019	0.023	0.049	0.010
28-Dec	0.002	0.033	0.004	0.002	0.206	0.015	0.007	0.139	0.008
09-Feb	0.006	0.007	0.002	0.004	0.076	0.010	0.010	0.045	0.004
20-Feb	0.002	0.007	0.002	0.002	0.057	0.008	0.016	0.045	0.012
09-Mar	0.001	0.036	0.004	0.002	0.191	0.027	0.011	0.146	0.013
22-Mar	0.002	0.007	0.005	0.001	0.035	0.028	0.018	0.072	0.078
06-Apr	0.007	0.013	0.002	0.006	0.133	0.012	0.023	0.076	0.006
22-Apr	0.003	0.017	0.001	0.003	0.117	0.027	0.034	0.156	0.018
25-May	0.009	0.015	0.009	0.025	0.104	0.126	0.051	0.091	0.124
08-Jun	0.004	0.019	0.005	0.004	0.099	0.023	0.027	0.102	0.020
22-Jun	0.004	0.018	0.004	0.003	0.088	0.020	0.031	0.091	0.013
05-Jul	0.008	0.015	0.008	0.009	0.034	0.022	0.055	0.048	0.024
20-Jul	0.010	0.036	0.013	0.004	0.061	0.032	0.043	0.122	0.077
28-Jul	0.010	0.043	0.008	0.003	0.061	0.012	0.037	0.145	0.024

Table 2-12: Summary of pertinent results at 5m deep, given in ranges for each site.

Parameter	Deep Bay		Lemmens Inlet	
	Range	Median	Range	Median
Cd in oysters (µgg-1 wet wt)	1.2-3.6	2.32	1.4-2.5	1.83
Shell length (mm)	78-116	100	124-159	143
Dissolved Cd (nM)	0.45-0.81	0.63	0.22-0.46	0.35
Particulate Cd (nM)	0.008-0.05	0.022	0.010-0.061	0.03
Particulate P (µM)	0.042-0.269	0.18	0.023-0.51	0.14
Particulate Ti (µM)	0.0099-0.058	0.037	0.013-0.27	0.13
Cd:P (nM:µM)	0.08-0.18	0.12-0.14 (except winter); suggest part. Cd is mainly organic	0.14-0.19	Variable
Cd:Ti (nM:µM)	0.084-1.48 (with one value at 2.57)	Variable	0.08-0.30 (with two outliers)	0.20 (except Aug/04) consistent; suggests part. Cd is mainly inorganic
Salinity (PSU)	23.5-28.5	26	24-31	27

2.8 *References*

Bendell-young, L., Arifin, Z., 2004. Application of a kinetic model to demonstrate how selective feeding could alter the amount of cadmium accumulated by the blue mussel (*Mytilus trossolus*). Journal of Experimental Marine Biology and Ecology 298: 21-33.

Boyle, E.A., Sclater, F. and Edmond, J.M., 1976. On the marine geochemistry of cadmium. Nature, 263: 42-44.

Brooks, R.R., and Rumsby, M.G., 1965. The biochemistry of trace metal uptake by some New Zealand bivalves 10: 521-527.

Bruland, K.W., Orians, K.J., Cowen, J.P., 1994. Reactive trace metals in the stratified central North Pacific. Geochimica et Cosmochimica Acta 58: 3171-3182.

Bruland, K.W. and Franks R.P., 1983. Mn, Ni, Cu, Zn and Cd in the western North Atlantic, In: Trace Metals in Sea Water (Wong, C.S. *et al.*, Ed), NATO Conference Series, Series IV, Marine Sciences, volume 9, Plenum Press, NY, pp 395-414.

Cassis, D., Lekhi, A., Pearce, C., Ebell, N., Walberg, W., Maldonado, M.T., Orians, K.O., 2006. Pathways of Cadmium in Cultured Pacific Oysters and the Effects of Environmental Parameters. Can. Tech. Rep. Fish. Aquat. Sci. In press.

Chong, M. 2003. A comparison of techniques for quantification of particulate trace metals. Honours thesis presented to UBC. 40p.

Cullen, J.T., Lane, T.W., Morel, F.M.M., Sherrell, R.M. 1990. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature, 402: 65-167.

de Baar, H.J.W., Saager, P.M., Nolting, R.F. and van der Meer, J., 1994. Cadmium versus phosphate in the world ocean. Marine Science, 46: 261-281.

Engel, D.W., Sunda, W.G. and Fowler, B.A. 1981. Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters. *In* F.J. Vernberg, A Calabrese, F.P. Thur-berg, and W.B. Vernberg (Eds.). Biological Monitoring of Marine Pollutants. Academic Press, New York. 127-144.

Engel, D.W. (1999). Accumulation and cytosolic partitioning of metals in the American oyster *Crassostrea virginica*. Marine Environ. Res. 47: 89–102

Ettajani, H., Berthet, B., Amiard, J.C. and Chevolot, L. 2001. Determination of cadmium partitioning in microalgae and oysters: contribution to the assessment of trophic transfer. Archives of Environmental Contamination and Toxicology. 40: 209-221.

Fisher, N.S., Teyssie, 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 30: 3232-3242.

Frazier, J.M. and George, S.G. 1983. Bioaccumulation of cadmium in marine organisms. Environ. Health Perspect. 28: 75-79.

Greig, R.A. and Wenzloff, D.R. 1978. Metal accumulation and depuration by the American oyster, *Crassostrea virginica*. Bull.Environ. Contam. Toxicol. 20: 499-504.

Hardy J.T., Sullivan, M.F., Crecelius, E.A., Apts, C.W. 1984. Transfer of cadmium in a phytoplankton-oyster-mouse food chain. Archives of Environmental Contamination and Toxicology. 13: 419-425.

Holms, G.B., 1997. State of water quality of Fraser River at Hope, 1979-1995. Canada-British Columbia Water Quality Monitoring Agreement. British Columbia, Ministry of the Environment, Land and Parks. ISBN 0-7726-3002-X. 297

Ho, T-Y., Quigg, A., Finkel, Z., Milligan, A., Wyman, K., Falkowski, P., Morel, F.M.M. 2003. The Elemental composition of some marine phytoplankton. Journal of Phycology 39: 1145-1159.

Jabobs, L., Emerson, S., Skei, J., 1985. Partitioning and transport of metals across the O_2/H_2S interface in a permanently anoxic basin: Framvaren Fjord, Norway, Geochimica et Cosmochimica Acta 49: 1433-1444.

Keefe, C.W., 1994. The contribution of inorganic compounds to the particulate carbon, nitrogen, and phosphorus in suspended matter and surface sediments of Chesapeake Bay. Estuaries 17: 122-130.

Kremling, K. and Pohl C., 1989. Studies on the special and seasonal variabilities of dissolved cadmium, copper and nickel in north-east Atlantic surface waters. *Mar. Chem.*, 27, 43–60.

Kruzynski, G.M., Addison, R.F., Macdonald, R.W., 2002. Proceedings of a Workshop on Possible Pathways of Cadmium into the Pacific Oyster (Crassostrea gigas) as Cultured on the Coast of British Columbia, Institute of Ocean Sciences, March 6-7, 2001. Canadian Technical Report of Fisheries and Aquatic Sciences 2405.

Kruzynski, G. 2004 Cadmium in oysters and scallops: the BC experience. Toxicology Letters 148: 159-169

Kusuki Yutaka, (1977). Retention of small particles by the gills of the Japanese oyster. Bulletin of the Japanese Society of Scientific Fisheries 43, 1391-1396.

Lane, T.W., and Morel, F.M.M. 2000. A biological function for cadmium in marine diatoms. Proceedings of the National Academy of Sciences. 97(9): 4627-4631.

Lares, M.L., and Orians, K.J. 1997. Natural Cd and Pb variations in *Mytilus californianus* during the upwelling season. Sci. Total Environ. 197: 177-195.

McNee, Jay J. 1997. The Post-Depositional Cycling of Cd, Cu, Mo and Zn in Several Hydrographically Distinct B.C. Fjords. Ph.D. Thesis presented to the University of British Columbia.

Merrin, C. 2002. Sources of Mn, Al, Cd, and Cu to Coastal Waters of the California Current System. PhD thesis presented to UBC.

Milke, L.M. and Ward, J.E. 2003. Influence of pre-digestive particle processing in bivalves II: Residence time in the pallial cavity and handling time on the labial palps. Journal of Experimental Marine Biology and Ecology. 293: 151-172.

Murray, R. W., Leinen, M., 1996. Scavenged excess aluminum and its relationship to bulk titanium in biogenic sediment from the central equatorial Pacific Ocean. Geochimica et Cosmochimica Acta, 60: 3869-3878.

Newell, R.I.E., Jordan, S.J. 1983. Preferential ingestion of organic material by the American Oyster *Crassostrea virginica*. Marine Ecology Progress Series, 13: 47-537.

Pauley, G.B., Van Der Raay, B., Troutt, D., 1988. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)-Pacific oyster. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.85). U.S. Army Corps of Engineers, TR EL-82.4. 28pp.

Paulson, A.J., H.C. Curl, Jr., R.A. Feely, T. Geiselman, K. A. Krogslund, and S. Hanson (1991) Trace metal and ancillary data in the open waters of Puget Sound: 1981-1985. NOAA Data Report. ERL PMEL-31 Pacific Marine Environmental Laboratory, Seattle, Washington, 56pp. + appendices.

Payne, C.D. and Price, N.M. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. Journal of Phycology. 35: 293-302.

Pederson, T.F., Waters, R.D. and Macdonald, R.W., 1989. On the natural enrichment of cadmium and molybdenum in the sediments of Ucluelet Inlet, British Columbia. The Science of the Total Environment, 79: 125-139.

Phelps, H.L., Wright D.A. and J.A Mihursky. 1985. Factors affecting trace metal accumulation by estuarine oysters *Crassotrea virginica*. Marine Ecology Progress Series, 22:187-197.

Price, N.M., Morel, F.M.M. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. Nature, 344: 658-660.

Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W-X., 1998. Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach. The Science of the Total Environment 219: 117-135.

Reinfelder, J.R., Wang, W.X., Luoma, S.N., Fisher, N.S. 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Marine Biology, 129: 443-452.

Ren, J.S., Ross, A.H., Schiel, D.R. 2000. Functional descriptions of feeding and energetics of the Pacific oyster Crassostrea gigas in New Zealand. Marine Ecology Progress Series, 208: 119-130.

Rensel, J.E., Forster, J.R.M, 2002. Strait of Juan de Fuca, offshore finfish mariculture: Literature review and preliminary field study results. *prepared for* National Oceanic and Atmospheric Administration. pp 1-92.

Roesjadi, G. and Klerks, P. 1989. A kinetic analysis of cadmium-binding to metallothionein and other intracellular ligands in oyster gills. Journal of Experimental Zoology, 251:1-12.

Roesjadi, G. and Robinson, W.E. 1994. Metal Regulation in aquatic animals: mechanisms of uptake, accumulation, and release. In D.C. Malins and G. Ostrander (Eds.). Molecular Mechanisms in Aquatic Toxicology. Lewis Publishers, Boca Raton. 387-420.

Roesijadi, G. 1996. Environmental factors: response to metals. *In* The Eastern Oyster *Crassostrea virginica*. V.S.Kennedy, R.I.E. Newell, and A.F. Eble (Eds.). Maryland Sea Grant, College Park, Maryland. 515-537.

Sanudo-Wilhelmy, S.A., Tovar-Sanchez, A., Fu, F-X., Capone, D.G., Carpenter, E.J., Hutchins, D.A., 2004. The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. Nature 432: 897-901.

Shumway, S. 1996. Natural environmental factors. *In* The Eastern Oyster *Crassostrea virginica*. V.S.Kennedy, R.I.E. Newell, and A.F. Eble (Eds.). Maryland Sea Grant, College Park, Maryland. 467-503

Skrabal, S.A., 1995. Distributions of dissolved titanium in Chesapeake Bay and the Amazon River Estuary. Geochimica et Cosmochimica Acta, 59: 2449-2458.

Sunda, W.G., Engel, D.W., and Thuotte, R.M. 1979. Effect of chemical speciation on toxicity of cadmium to grass shrimp, Palaemonetes pugio.: Importance of free Cd ion. Environmental Science and Technology, 12: 409-413.

Taylor, S.R. and McLennan, S.M., 1985. The Continental Crust: its Composition and Evolution. Blackwell Scientific, Oxford, 312 pp.
Thompson, R.E. 1981. Oceanography of the British Columbia Coast. Canadian special publication of fisheries and aquatic sciences, 56. 291p.

Van Dolah, F.M., Siewiki, T.C., Collins, G.W., and Logan, J.S. 1987. Effects of environmental parameters on the elimination of cadmium by eastern oysters, *Crassostrea virginica.* Archives in Environmental Contamination and Toxicology, 16:733-743.

van Geen, A., Luoma, S.N., 1993. Trace metals (Cd, Cu, Ni, and Zn) and nutrients in coastal waters adjacent to San Francisco Bay, California. Estuaries 16: 559-566.

Verardo, D.J., Froelich, P.N., and McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. Deep-Sea Res. Part A, 37:157-165.

Wang, W-X., 2002. Cd and Se aqueous uptake and exposure of green mussels *Perna viridis*: influences of seston quantity. Marine Ecology Progress Series 226: 211-221.

Ward J.E., Levinton, J.S., and Shumway, S.E. 2003. Influence of pre-digestive particle processing in bivalves I: Transport velocities in the ctenidium. Journal of Experimental Marine Biology and Ecology, 293: 129-149.

Yang, L., (1993) Dissolved Trace Metals in the Western North Pacific. MSc Thesis presented to UBC.

Zaroogian, G. 1979. Studies on the depuration of cadmium and copper by the American oyster *Crassostrea virginica.* Bulletin of Environmental Contamination and Toxicology, 23:117-122.

3 Chapter 3: Coastal Cd-P Ratios: The Effects of Biogeochemical Cycling[2]

Priyanka Lekhi, D. Cassis, M.T. Maldonado and K.J. Orians

3.1 *Introduction*

In 1934, Alfred Redfield proposed that marine plankton have a consistent elemental N:P ratio of 16:1, which is similar to the ratio of dissolved NO_3^- and PO_4^{3-} in seawater. His hypothesis suggested that marine biological activity determines the chemical composition of the oceans. This theory, combined with the present day Redfield stoichiometric ratio of C:N:P of 106:16:1, has been a fundamental reference to our understanding of the biogeochemistry of the oceans. It has been extended to explain the distributions of some trace metals, such as cadmium. The profile of dissolved Cd is closely related to the profile of dissolved phosphate (Boyle *et al.*, 1976, Bruland *et al.*, 1978 and de Baar *et al.*, 1994) and so is classified as nutrient- like. The relationship suggests that cadmium, like phosphorus, is taken up by phytoplankton at the surface and is re-mineralized at depth after the sinking of organic particles. Price and Morel (1990) have suggested that a reason for this nutrient-type behavior is the substitution of Cd in oceanic phytoplankton for the essential micronutrient zinc in the enzyme, carbonic anhydrase. There is also evidence of a Cd-specific form of the enzyme (Cullen *et al.*, 1999, Lane and Morel, 2000). The strong correlation between dissolved Cd and dissolved PO_4^{3-} has lead to investigations of a global, oceanic Cd-P relationship. Global data sets of Cd and PO_4^{3-} show that they are linearly related, with the regressions of different regions being similar to one another. However, at the surface layers of the oceans (<1000m), the Cd-P relationship changes widely; the ratio rapidly increases from the surface to the thermocline, reaching higher values in deep waters (de Baar *et al.*, 1994). Combining all data of the deep ocean (>1000m) onto one plot, shows two clusters with separate linear regressions of dissolved Cd versus dissolved phosphate (Fig. 3-1). The break in the slope of the line is commonly referred to as the "kink" in the relationship. Presently, it is considered that the global

[2] A version of this chapter will be submitted for publication in Marine Chemistry.

dissolved Cd-P relationship is best described by two separate Cd-P regressions; one for phosphate concentrations lower than 1.3μM (North Atlantic data) where the Cd-P ratio is about $0.18x10^{-3}$, and one for phosphate concentrations higher than 1.3μM (Boyle, 1988), (Indian-Southern and Pacific Ocean data) where the Cd-P ratio is $0.33\text{-}0.35x10^{-3}$ (deBaar *et al.*, 1994).

The close correlation between dissolved Cd and PO_4^{3-} in the deep ocean has been linked to the remineralization of organic particles over large time and depth scales (de Baar *et al.*, 1994, Cullen, 2006). Assuming that the ocean is in a steady-state, phytoplankton could be expected to take up Cd and P in a similar ratio than the dissolved Cd-P ratio in the deep water, if the dissolved Cd-P ratio is due to the remineralization of organic particles. The Cd-P ratio in the extended Redfield stoichiometric formula for the average cellular content of phytoplankton is 0.21 mmol:mol (Ho *et al.*, 2003). This average is based on few representative species with the ratio varying from species to species and with the composition of the growth media. For example, Cd-P ratios are greater for oceanic phytoplankton species than coastal species (Ho *et al.*, 2003), indicative of the substitution of Cd for Zn in species that live in low Zn regions of the ocean (Sunda and Huntsman, 2000). Laboratory experiments have shown that in coastal diatoms, cellular [Cd] are correlated with free $[Cd^{2+}]$ in the medium but cellular [Cd] are inversely correlated with free Zn and Mn concentrations (Sunda and Huntsman, 1998 and 2000). The Cd-P ratio of phytoplankton also increases when they are iron-limited. This is due to the hyperaccumulation of Cd (Sunda and Huntsman, 2000, Cullen, 2003), and has been linked to low dissolved Cd-P ratios in high latitude surface High Nutrient Low Chlorophyll (HNLC) regions (Cullen, 2006). Considering these variations, it is surprising that the average cellular Cd-P ratio is similar to the deep ocean dissolved Cd-P ratios, emphasizing the important influence of primary production on oceanic chemical composition.

The deviation of the dissolved Cd-P ratio in surface waters and the kink in the global Cd-P relationship have been linked to the uptake of Cd and PO_4^{3-} by phytoplankton (de Baar *et al.*, 1994, Cullen, 2006). In coastal regions, the Cd-P ratio is also not consistent. Coastal regions are characterized by high primary production supported by large amounts of nutrients supplied by rivers, coastal upwelling and eddy diffusion. Usually, coastal waters are not very stratified as the waters are shallow and vertical mixing

returns regenerated nutrients to the euphotic zone. The dissolved deep oceanic Cd-P ratio has been used in paleoceanography where records of the Cd/Ca ratios in $CaCO_3$ shell deposits have been used to reconstruct past Cd distributions and to extrapolate P distributions (Boyle, 1988). Moreover, the Cd-P ratios have been used as a reference to further understand the biogeochemistry of the oceans, much like the original Redfield ratio with a similar principle linking the biology of the oceans to its chemical composition. Although the oceanographic environment is very different for coastal waters compared to the open ocean, the Cd-P ratio may still serve as a tool to understanding biological or physio-chemical processes.

In this study, we explore the coastal Cd-P ratios in the dissolved and particulate forms at two sites on Vancouver Island, BC (Fig. 3-2). To address the challenge of characterizing particulate matter in the field as organic or inorganic, particulate samples were also analyzed for titanium (Ti). Titanium is the ninth most abundant element in the Earth's crust; it is a ubiquitous constituent of rocks, soils and sediments and has been used as a conservative tracer for marine sediment analysis (Skrabal, 1995; Murray and Leinen, 1996). The main source of particulate Ti would be from the chemical weathering of rocks and from resuspended marine sediments. In addition, a surface sediment was sampled from each site and analyzed to study any influence of sedimentary input or of diagenesis at the sediment surface on the Cd-P ratios of the overlaying water column. The project was initially designed to investigate the trophic transfer of Cd to oysters (Cassis *et al.*, 2006). Thus, the chosen sites were the locations of two oyster farms. This part of the study compares the Cd-P ratios over time and depth in the dissolved and particulate phases of two locations in order to investigate the effects of biogeochemical cycling on the Cd-P ratio in coastal waters.

3.2 *Materials and Methods*

3.2.1 Sampling

At two locations on Vancouver Island (Fig. 3-2), samples of seawater were taken at 5m depth biweekly during the summer/ spring months and monthly during the winter. The east coast site is located in Deep Bay (Baynes Sound, Strait of Georgia) and the west coast site is in Lemmens Inlet (Clayoquot Sound).

Temperature and salinity data were collected on site using a ClineFinder® digital temperature probe and a manual refractometer. Seawater was collected using a peristaltic pump equipped with Tygon® polyethylene tubing and in-line filter holders. Unfiltered seawater was collected for quantitative phytoplankton and dissolved PO_4^{3-}analysis. Filtered seawater for dissolved Cd analysis was collected in two 500mL acid-washed HDPE bottles and frozen until they were transported to UBC. Upon arrival, samples were acidified for storage until analysis (within two months of collection). Initially, seawater was filtered into fractions (Cassis *et al.*, 2006) using polycarbonate and cellulose acetate filters, but for the purpose of this study, only the combined totals are reported for particulate samples. In addition to the biweekly sampling, three vertical profiles were sampled during the year. With a bottom depth of approximately 25m, seawater samples were collected at 1m, 5m, 12m and 20m at Deep Bay and at Lemmens Inlet, which has a bottom depth of 15m, seawater was sampled at 1m, 6m, 9m, and 14m. A surface marine sediment sample was collected in February 2005 at one location for each site.

3.2.2 Dissolved cadmium analysis

All seawater analyses were made in a HEPA filtered, positive pressure air supply trace metal clean laboratory. One 500mL bottle was divided into two replicates and adjusted to pH 6 before being pre-concentrated on a Chelex®-100 ion exchange resin at a flow rate of $0.8mLmin^{-1}$ (Yang, 1993). Samples were eluted with HNO_3 (2N, Seastar® grade) and analyzed on a Varian Spectra-300/400 GFAAS using standard addition and Zeeman background correction. The accuracy and precision of the seawater analysis were determined by measurements of standard reference materials for coastal ocean seawater (CASS-4) from the National Research Council of Canada, and from measuring replicates of deep seawater collected from "Line P" stations (stations extending from Juan de Fuca Strait, to Ocean Station Papa at 50°N 145°W, in the Pacific Ocean) spiked or unspiked with Cd. The efficiency of extraction for CASS-4 was 100-105%; the variation of replicate unspiked analyses was less than 3%. The precision of all replicate samples were within 5% or were re-analyzed.

3.2.3 Dissolved Phosphate

Frozen unfiltered seawater samples transported from the sampling sites, were thawed and analyzed for phosphate using a Bran Luebbe Autoanalyzer 3 continuous flow autoanalyzer. A standard curve was prepared and run following the specifications of the instrument prior to the analysis of the samples.

3.2.4 Particulate analysis

All filters were microwave digested with Seastar® grade HNO_3, HCl, and HF in a 4:4:1 ratio. Prior to digestion, polycarbonate filters were first dissolved in concentrated NH_4OH for 24 hours. After evaporation of the concentrated acids, 50% HNO_3 was added and samples were heated for further digestion. Digests were diluted to 2N HNO_3 before analysis on the GFAAS for Cd using standard addition. Particulate titanium (Ti) and phosphorus (P) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using indium (In) as the internal standard. Procedural blanks were less than 10% of measured concentrations for Cd and Ti, and less than 3% of measured concentrations for Ti and P. The precision of the filter blanks varied up to 4% for Cd and Ti and up to 6% for P.

3.2.5 Quantitative phytoplankton

Water mounts were analyzed quantitatively for microphytoplankton and small zooplankton, by the Utermöhl method with modifications described in Hasle (1978). Gently homogenized water samples were placed in 10, 25 or 50mL settling chambers, for 24 hours. The detailed composition of these samples was obtained by means of observation, identification, and enumeration of the settled particles in a known volume, under a Zeiss Axiovert 10 inverted microscope.

Carbon biomass per cell estimates were based on determinations of biovolume of local microalgae (Haigh, *et al.,* 1992). These biovolume measurements were used to obtain carbon estimates using Strathman's equation (1967) for diatoms, and Montagnes *et al.* (2001) for other groups. The different algal groups and

species have marked differences in size and carbon content, with most of the large diatoms cells being filled with vacuoles (Mullin *et al.*, 1966). This method allows a better estimate of the actual contribution of each species to the total of the phytoplanktonic community, rather than their numeric abundance alone.

3.2.6 Sediment analysis

Surface sediment samples were collected at one location at each site using a Petit Ponar® Grab sampler, into two acid-washed specimen containers and frozen until analysis. Samples were freeze-dried in an Edwards 4k Modulyo Freeze dryer for one week. Once dry, samples were ground in a Hertzog HSM-100 tungsten-carbide mill. Inorganic carbon was determined colorimetrically on CM5014 and 5011 CO_2 Coulometers. Total carbon was measured by a Carlo-Erba CNS analyzer Model NA 1500 gas chromatograph (Verardo *et al.*, 1990). The measurement of major and minor elements was done by a Phillips PW 1400 x-ray fluorescence spectrometer. Sample preparation of the major elements (Na, K, Ti, Si, P, Mn, Al, Ca, Fe, and Mg) involved preparation of fused glass discs following the method in the Katanax® K1 manual. For the analysis of minor elements (Mn, Mo, I, Br, Cu, Zn, Zr, Sr, Cr, Ba, Rb, Ni, V, Y and Pb), samples were run as pressed-powder pellets (McNee, 1997).

Cd, P and Ti content in sediment samples was determined following the same digestion and analysis procedure used for the filters for each element. The accuracy and precision of the procedure was determined by measurements of coastal sediment standard reference materials (PACS-2 and MESS-2, Table 3-1), from the National Research Council of Canada. The recoveries were 120-125%, 104-120% and 110-121% for Cd, P and Ti, respectively. Although high, these results may be due to the small sub-sample of reference material used for the procedure, containing mostly large particles, which would be comparatively enriched in Cd, P and Ti

3.3 *Results*

3.3.1 Deep Bay

3.3.1.1 Characterization of Particulate Cd and P

Particulate Cd displayed no significant seasonal trend and values ranged from 0.0079 to 0.050 nM (Fig. 3-3 and Table 3-2). Values for particulate phosphorus (P) varied from 0.042 to 0.33 nM (Fig. 3-3 and Table 3-2). Particulate Ti ranged from 0.0099 to 0.058 nM throughout the year, with an exceptional event in November 2004 where levels exceeded 0.15 nM (Fig. 3-3 and Table 3-2). Aside from this event, particulate Ti presented little variability and did not display any marked seasonal trend.

Samples of suspended particulate matter showed a fairly consistent ratio of Cd:P between 0.08 and 0.18 (nM:μM) for much of the sampling year, and increased in the winter to 0.3 (Fig. 3-4 and Table 3-3). The overall Cd:P ratio over the year averaged at 0.14 nM:μM. Excluding the winter, this Cd:P ratio (with a mean value of 0.14 and 0.12 nM:μM from Aug. to Nov. and from Mar. to Aug., respectively) was similar to the extended Redfield stoichiometric formula for the average cellular content of phytoplankton, of 0.21 (mmol:mol) (Ho *et al.*, 2003). The Cd:Ti ratio varied significantly over the sampling year (Fig. 3-4 and Table 3-3), indicating that Cd does not follow titanium. The P:Ti ratio varied from 1.86 to 17 (μM:μM) throughout the year at 5m and was inconsistent throughout the sampling year, indicating that P does not correlate with Ti (Fig. 3-4 and Table 3-3).

3.3.1.2 Dissolved Cd-P

The dissolved Cd-P ratio varied from 0.25 to 0.82 (nM:μM) throughout the year and was only constant in the winter months from November to April where it averaged 0.27 (Fig. 3-5 and Table 3-3). The ratio was highest in the summer and lowest in the winter.

3.3.1.3 Annual trends of P and Cd in the dissolved and particulate phase

Both the dissolved P and Cd display marked seasonal trends with values greatest in the winter and lowest in the summer and spring months while particulate P and Cd displayed a weaker seasonal trend. In general, the annual cycles of particulate and dissolved P are mirror images of each other (Fig. 3-6). However, this inverse relationship does not reach the significance level ($P > 0.5$). The annual cycles of particulate Cd and dissolved Cd are generally opposite, and this inverse relationship is statistically significant but with a weak correlation ($r = -0.5$, $P < 0.05$).

3.3.1.4 Vertical Profiles of Cd-P ratios

In the dissolved, the Cd-P ratio remains constant throughout the water column, except during the August 2004 sampling event (Fig. 3-8) when there was a depletion of P in the upper water column (Fig. 3-9). During this event, the water column is well-stratified (Fig. 3-10) and there was a bloom of diatoms. The biomass in the upper layer was between 400-600μgCL^{-1} (Fig. 3-11 and Table 3-4), corresponding to high dissolved Cd:P. Also in August 2004, the particulate Cd-P remains constant at 0.12-0.14 from 1m to 12m (the particulate Cd data at 20m was lost in the preparation). In February 2005, the water column was well mixed as indicated by the salinity, temperature and dissolved Cd and P data (Fig. 3-10 and Table 3-4). Thus, the dissolved Cd-P ratio is constant throughout the water column during this event. In February the biomass was very low (Fig. 3-11) so the particulate Cd-P is likely dominated by inorganic particles. The particulate Cd-P ratio at this event increases with depth, as it gets closer to the sediments. In July, 2005, the dissolved and particulate ratios remain consistent throughout the water column, except at 5m, where the particulate Cd-P ratio increases. There is an unusually high Ti signal at 5m at this time. This indicates the input of terrigenous materials, containing higher cadmium, causing the ratio to increase (Table 3-4).

3.3.1.5 Sediment Sample

A grab sample of the top layer of the marine sediment was collected at Deep Bay, at approximately 25m deep. This sample contained 0.76 $\mu g g^{-1}$ of particulate Cd and 1.2% of organic carbon (Table 3-5). Materials which contain more than 0.6 $\mu g g^{-1}$ Cd are prohibited from marine disposal under the Canadian Ocean Dumping Control Act (Pedersen *et al.*, 1989) and most coastal marine sediments worldwide fall below this level, indicating an enrichment of Cd in this site.

The P:Ti ratio in the sediment sample was 0.13 mol kg^{-1}: mol kg^{-1}, which was significantly below the P:Ti ratio in the suspended particulate matter (with values of 1.7-17 μM:μM) (Table 3-5 and 3-3). The Cd:Ti ratio at this site was 0.08 mmol kg^{-1}: mol kg^{-1} in the sediments and was mainly lower than what was present in the suspended particulate matter, where the ratios ranged from 0.08 to 2.57 nM:μM (Table 3-5 and 3-3). This indicates that both P and Cd have an important organic component and are re-mineralized in the deeper water column. Comparing the Cd:Ti value to the crustal Cd:Ti ratio of 0.014 mmol kg^{-1}: mol kg^{-1} (Taylor and McLennan, 1985), points towards a Cd enrichment in the sediments. The ratio of Cd:P in the sediments was determined to be 0.62 mmol kg^{-1}: mol kg^{-1} and was higher than the ratio of Cd:P in suspended particulate matter (0.04 to 0.31 nM:μM) throughout the year (Table 3-5 and 3-3), which also suggests the importantance of organic component in suspended particulate P.

3.3.2 Lemmens Inlet

3.3.2.1 Characterization of particulate Cd and P

The levels of particulate Cd (0.010-0.061 nM) were very similar to those determined in Deep Bay (Fig. 3-12 and Table 3-6). During the sampling year, there was no obvious seasonal trend and the Cd remained fairly constant throughout the year. Particulate phosphorus levels ranged from 0.023 to 0.51 nM (Fig. 3-12 and Table 3-6), and titanium ranged from 0.013 to 0.27 nM with high variability in both P and Ti throughout the sampling year (Fig. 3-12 and Table 3-6).

Although the Ti varies through the year, the ratio of Cd:Ti is fairly constant at 0.2 nM:μM with the exception of one sampling event in August 2004, where the Ti concentration is very low (Fig. 3-13 and Table 3-7). The particulate P:Ti ratio varied from 0.22 to 1.50 μM:μM, with two events where the ratio reached 10 and 22. There is a larger range in the P:Ti ratio than in the Cd:Ti, ratio but it is still fairly constant (Fig. 3-13 and Table 3-7). In contrast, the Cd:P ratio changed throughout the sampling year, only remaining constant between 0.14 and 0.19 during the winter (Fig. 3-13).

3.3.2.2 Dissolved Cd-P

The Cd-P ratio in the dissolved phase in Lemmens Inlet was fairly constant throughout the year, ranging from 0.23 to 0.44 (nM:μM) with one sampling event in August 2004, where the Cd-P ratio was 1.92 (Fig. 3-14 and Table 3-7). The P at this sampling event was considerably lower, presumably as a result of a dinoflagellate bloom (Table 3-6) where the biomass reached 8300μgCL^{-1}.

3.3.2.3 Annual trends of P and Cd in dissolved and particulate phases

Dissolved P and Cd show different trends than what was seen in Deep Bay. Levels are highest in late summer and fall of 2004 and lowest in the spring and early summer of 2005. The particulate Cd and P do not display a seasonal trend. Both the annual cyles of particulate and dissolved P and particulate and dissolved Cd over the year are, in general, mirror images (Figures 3-15, 16). The inverse relationship between particulate and dissolved P is significant ($r = 0.7$, $P < 0.01$) but the correlation between particulate and dissolved Cd is not significant.

3.3.2.4 Vertical profiles of Cd-P

In August 2004, during a bloom of dinoflagellates, with the biomass exceeding 8000ugC/L, the dissolved Cd-P ratio reached a maximum at 6m and varied at other depths (Figures 3-17, 18 and Table 3-8). This bloom corresponded with a drawdown of dissolved P (Fig. 3-19) and as a result, the dissolved Cd-P was

very high at 6m. There was also a minimum in the dissolved Cd-P profile at 14m during this event due to an enrichment of P. This was accompanied by an increase in salinity, indicating the influx of oceanic water (Table 3-8). During both the February and July sampling events, the dissolved Cd-P ratio is constant at 0.3 nM:μM. In both August and February, the particulate vertical Cd-P ratios vary from 0.06-0.10 nM:μM in the upper layer and slightly increase as depth increases, where Ti also shows a maximum (Fig. 3-18 and Table 3-8). In July 2005, the particulate Cd-P profile is quite different from the other vertical profiles and also during this time, there is a small bloom of diatoms throughout the water column (Fig. 3-17 and Table 3-8). The ratio is higher and the increases at 9 and 14m, due to an enrichment of particulate Cd (Figures 3-18, 3-19).

3.3.2.5 Sediment Samples

At ~ 15m, in Lemmens Inlet, the sediment surface showed an enrichment of Cd and organics, with values of 2.24 $\mu g g^{-1}$ and 3.1%, respectively (Table 3-5). The Cd: Ti ratio in the sediments was found to be 0.27 mmol kg^{-1}: mol kg^{-1} (Table 3-5) at this site, which is greater than the crustal Cd:Ti ratio of 0.014 mmol kg^{-1}: mol kg^{-1} (Taylor and McLennan, 1985). Most of the year, the ratio of Cd:Ti in suspended particulate (0.2 nM:μM) (Table 3-7) was very close to sediment values. The Cd:P ratio in the Lemmens Inlet sediment sample was determined to be 1.31 mmol kg^{-1}: mol kg^{-1} and the suspended particulate Cd:P ratio was always well below that (0.07 to 0.81 nM:μM). The P:Ti ratio in the sediments was 0.21 μM:μM, which is comparable to the ratio found in suspended particulate matter, at specific times of the year (Tables 3-5,3-7).

3.4 *Discussion*

The Cd-P ratio in the deep oceans is governed by internal cycling of these two elements, which is controlled by respiration and ocean circulation processes. In the Northeast Pacific waters, off the coast of California, vertical distributions of particulate Cd and P mirror that of dissolved Cd and P. This suggests that the trophic transfer of one phase to the other is controlling the internal cycling of Cd and P (Knauer and Martin, 1981). Partitioning between phases for elements in the ocean can be either due to the production

and remineralization of organic tissue or to the adsoption of dissolved elements onto particles. Cadmium is not known to be a particle- reactive metal (Bruland *et al.*, 1994) so its transfer between phases will be mainly due to biological activity. Phosphate strongly adsorbs onto iron oxides, which are present in oxic waters (Bjerrum and Canfield, 2002). Dissolved phosphate has also been found to adsorb on the surface of phytoplankton in surface waters (Sanudo-Wilhemy *et al.*, 2004; Fu *et al.*, 2005). The transfer of P between phases can be attributed to direct uptake or remineralization by phytoplankton or by adsorption onto phytoplankton or iron oxides. Both Lemmens Inlet and Deep Bay displayed biweekly trends between both dissolved and particulate P and dissolved and particulate Cd that were inversely correlated. The general inverse relationship between the phases signifies the important role of biology on Cd and P in both coastal sites on a larger time scale. However, these inverse relationships were weak and only significant between particulate and dissolved Cd at Deep Bay and between particulate and dissolved P at Lemmens Inlet. Furthermore, the Cd-P ratios in the two phases were not inversely correlated at either site. This suggests the influence of other factors besides trophic transfer, on the Cd-P ratio on a smaller time scale. It is also important to note that the particulate Cd and P are an order of magnitude smaller than the dissolved Cd and P, respectively, so changes in the particulate phase at these sites may not translate to a detectable change in the dissolved phase. The study of these two sites highlights the processes which affect this ratio, including biological processes, physical processes (such as riverine input) and geochemical processes (such as sediment diagenesis). The contribution of the processes to the Cd-P ratio varies with site, season and depth.

3.4.1 Deep Bay

3.4.1.1 Biweekly Cd-P ratios

Deep Bay is a protected estuary located on the southwestern part of Baynes Sound, on the west coast of the Strait of Georgia (Fig. 3-2). At this site, the particulate Cd and P was determined to be mainly associated with organic material. Particulate P can be associated with organic material as well as with terrigenous materials (Keefe, 1994) but it is commonly considered that most of the P in this phase is either present in or adsorbed on phytoplankton. The ratio of C:P found in sinking particulate material in the oceans has a ratio

of 106–117:1, suggesting a Redfield relationship where most of the P in this form is associated with marine organic matter (Redfield *et al.*, 1963; Benitez-Nelson, 2000). However, a study in the estuary, Chesapeake Bay, demonstrated that 34-77% of the total particulate P is organic, with higher percentages associated with higher phytoplankton abundance (Keefe, 1994). These results suggest that a significant amount of particulate P could be inorganic. In order to source the particulate P and Cd, both elements were normalized to Ti. In Deep Bay, the ratio of P:Ti in suspended particulate matter is not constant and is not comparable to the ratio in the sediments (Fig. 3-4, Table 3.3 and 3.5). Since particulate P is generally associated with phytoplankton and the ratios of P:Ti in Deep Bay do not show to the contrary, the particulate P at this site is considered to be mainly associated with organic material. The particulate Cd-P ratio remained constant during the periods of phytoplankton abundance (Figures 3-4, 3-11) and was similar to the extended Redfield ratio of 0.21(mmol:mol), suggesting that Cd is also predominantly organically bound (Fig. 3-4). Although 0.12-0.14 nM:μM (the mean range during periods of phytoplankton abundance) is lower than the extended Redfield ratio, the Cd-P ratio varies with phytoplankton species. For example, a small celled estuarine diatom, *Thalassiosira eccentrica,* has a Cd-P ratio of 0.15 (mmol:mol), whereas a benthic dinoflagellate, *Amphidinium carterae,* has a Cd-P ratio of 0.73 (mmol:mol) (Ho *et al.*, 2003). The phytoplankton species composition in Deep Bay during the sampling year mainly consisted of diatoms, which were similar physiologically to *Thalassiosira eccentrica,* (Cassis *et al.*, 2006). A ratio of 0.14 and 0.12 (nM:μM) may therefore, reflect the species composition at Deep Bay. Moreover, the extended Redfield ratio is determined for the intracellular composition of phytoplankton but phosphorus can bind to phytoplankton extracellularly (Sanudo-Wilhemy *et al.*, 2004), which would cause the particulate Cd-P ratio determined in this study to decrease. In the winter when phytoplankton abundance is minimal, the particulate Cd-P increases to 0.3 due to low particulate P. During this time, less dissolved P is taken up or adsorbed by phytoplankton (Fig. 3-6). Thus, throughout the year, the Cd-P ratio in particles is governed by primary production.

The particulate Cd-P ratio is controlled by primary production and is constant through most of the year but the dissolved Cd-P ratio in Deep Bay is not consistent throughout the year (Fig. 3-5). From August to November, 2004, and from April to August, 2005, the dissolved Cd-P ratio at this site increases,

corresponding to periods where the dissolved P is lowest due to primary production (Table 3-3). Dissolved Cd also exhibits a seasonal trend and is highest in the winter and lowest in the spring and summer, but dissolved P changes to a greater extent (Figures 3-6 and 3-7). During the periods of low dissolved Cd and P, the ratio randomly varies which can be caused by the influence of water masses with different Cd-P ratios. The hydrographic characteristics of Baynes Sound indicate that during the summer there is a strong southbound flow driven by wind (Thomson, 1981). The water in this flow is mainly from the Strait of Georgia, highly influenced by the fresh water input of the Fraser and other rivers. Total Cd levels in the Fraser river at Hope, BC, have been reported to range from 1.2-13.3 nM between 1991 and 1995 (Holms, 1997). During the same time period and at the same location, total phosphorus levels ranged from 0.81-19.4 μM (Holms, 1997). The Cd-P ratio at the highest and lowest values of Cd were 0.75 and 1.4 (nM:μM), respectively. The Fraser river contributes about 80% of the freshwater runoff into the Strait. Its riverine discharge starts to increase in March, reaches a maximum in June, gradually decreases in July, August, and September. The discharge then remains near minimum levels throughout the rest of the year (McFarlane et al., 1998). The variability in the dissolved Cd and P concentrations and in the Cd-P ratio, combined with the range in the Fraser River input, could plausibly translate to higher and variable Cd-P ratios during the summer and spring at Deep Bay (Fig. 3.5). Edmond *et al* (1981), Boyle *et al* (1982) and Sharpe (1982) also reported Cd and P concentrations in estuaries. They found high Cd-P ratios of 0.3-0.7 nmol:μmol, which was attributed to riverine input (Kudo, 1995).

In the winter, there is less variability in the data and the dissolved Cd-P ratio at Deep Bay approaches the North Pacific ratio of 0.33×10^{-3} (deBaar, *et al.*, 1994). An explanation for this is that the wind blows in a northernly direction in the winter, bringing with it high salinity and low temperature waters (Fig. 3-6, Table 3-4), indicative of oceanic input. Furthermore, the relationship between dissolved Cd-P ratios and salinity from both sites indicate that at higher salinities, the Cd-P ratio is closer to the North Pacific ratio (Fig. 3-21). The water in Deep Bay is highly influenced during the winter by the Juan de Fuca Strait (Thomson, 1981). The Juan de Fuca Strait is characterized with a salinity range of 31-33 PSU (Rensel and Forester, 2002) and can be an important channel of oceanic input to the Strait of Georgia (Thomson, 1981). Thus,

during periods of oceanic input, the Cd-P is fairly constant, but at other times during the year, the dissolved Cd-P ratio is variable, and mainly controlled by riverine input.

3.4.1.2 Vertical Cd-P ratios

During the August 2004 sampling event, the water is well stratified (Fig. 3-9) and there is a difference between the dissolved Cd-P ratio in the upper and lower layer (Fig. 3-8). Throughout the water column, the dissolved Cd concentrations are lower relative to other times of the year (Fig. 3-10 and Table 3.2 and 3.4), which indicates the uptake of Cd. However, the dissolved Cd-P ratio is high in the upper layer due to a larger drawdown of dissolved phosphate relative to dissolved Cd (Fig. 3-10). During this event, there was a bloom of diatoms (Fig. 3-11), which are known to have low intracellular Cd-P ratios (0.15 mmol:mol in species *Thalassiosira eccentrica* ; Ho *et al.*, 2003). Moreover, P adsorbs onto the surface of phytoplankton so during a bloom, there could be a significant transfer of dissolved P to the particulate phase. During this bloom in August 2004, diatoms take up both dissolved Cd and P with dissolved P also adsorbing onto the surface of phytoplankton. This leaves behind a high dissolved Cd-P ratio in the upper water column. In the deeper water column (>10m), organic matter exported from the surface with a low extra-and intra-cellular Cd-P ratio, is oxidized. As a result, there is a low dissolved Cd-P ratio in the deeper waters. Organic particles carrying the same Cd-P ratio (0.12-0.14 nM:μM) are prevalent down to 12m (No Cd value at 20m), as the biomass of the bloom extends to 20m (Table 3-4).

The February 2005 sampling event is characterized by very low phytoplankton abundance (Fig. 3-11). The water column is well mixed, as indicated by the temperature and salinity data (Table 3-4). This leads to a constant dissolved Cd-P ratio throughout the water column at around 0.3 nM:μM, which is indicative of oceanic input in the winter. The particulate Cd-P ratio increases with depth (Fig. 3-8) and is mainly due to inorganic particles since there is hardly any biomass during this event. The Cd:Ti ratio in suspended particles had little variability throughout the water column, only ranging from 0.9-1.4 nM:μM (Table 3-4). This ratio is similar to the Cd:Ti ratio in the sediments (0.08 mmol kg^{-1}: mol kg^{-1}), indicating that during this event, the particulate Cd is mainly associated with inorganic particles. At depths closer to the

sediments, there is the input of terrigenous materials from the sediments, bringing in particles with larger Cd-P ratio (Cd-P ratio in the sediments is 0.62 mmol kg^{-1}:mol kg^{-1}- Table 3-5) and increasing the Cd-P ratio.

During the July 2005 sampling event, the water was well-mixed and the dissolved Cd-P ratio was constant at around 0.3 nM:μM. The particulate Cd-P ratio was also consistent throughout the water column, except at 6m, where there is an enrichment of particulate Cd. This enrichment corresponds to an increase in particulate Ti in the upper water column (Table 3-4), indicating the input of terrigenous particles. Thus, the enrichment of Cd may be due to the input of inorganic particles containing a higher Cd-P ratio.

The sedimentary Cd-P ratio was found to be 0.62 (mmol kg^{-1}: mol kg^{-1}), which is higher than the suspended particulate matter present in the overlaying water column (0.06-0.31 nM:μM) and the crustal Cd-P ratio of 0.02 (mmol kg^{-1}: mol kg^{-1}) (Lide, 2006). This high Cd-P ratio is due to a combination of high Cd and low P. The Cd was found to be 0.76 μgg-1, which is considerably higher than the crustal value of 0.15 μgg-1 (Lide, 2006). The crustal P is 0.1% (Lide, 2006), and this site had P levels slightly lower with a value of 0.0661%. At this site, the particulate P is mainly associated with organics, and as such, the low P in the sediments may be due to high remineralization rate of organic particulate P in the water column. The enrichment of Cd may be due to suboxic conditions at the surface sediments, which leads to the precipitation of dissolved Cd to CdS. Suboxic conditions at surface marine sediments occur under highly productive and shallow areas. Details of this process are described for Lemmens Inlet, where the Cd-P ratio in the overlaying column is considerably more affected by the process than at Deep Bay (section 3.4.2.2).

3.4.2 Lemmens Inlet

3.4.2.1 Biweekly Cd-P ratios

The most significant difference between the site in Lemmans Inlet and the site in Deep Bay is that the particulate Cd at Lemmans Inlet is mainly inorganic. Although the Ti varied greatly throughout the year,

the Cd:Ti ratio remained relatively constant at 0.2 nM:μM and was close to the ratio found in the sediments (0.27mmol kg^{-1}:mol kg^{-1}) (Table 3.6 and 3.8). The P:Ti ratio was also somewhat constant throughout the year (0.22-1.29 μM:μM- excluding August 2004) and comparable to the sedimentary P:Ti (0.21 mmol kg^{-1}:mol kg^{-1}) (Table 3.6 and 3.8). Phosphorus in the sediments is higher than in Deep Bay, indicating an important input of inorganic suspended particulate P in Lemmens inlet. The Cd:P ratio varied throughout the year (0.07-0.96 nM:μM) and only came close to the Redfield ratio (0.21 mmol:mol) during the winter, when phytoplankton abundance is minimal (Figures 3-13, 3-17).The variation of particulate Cd:P during the spring and summer months could be due P-limiting conditions, which would lead to varying phytoplankton intracellular Cd-P ratios. However, in Lemmens Inlet conditions were not P-limiting (Cassis *et al.*, 2006). Particulate Cd-P ratio in the surface of northeast Pacific waters, off the coast of California was determined to be 0.33 pmol:nmol and attributed to phytoplankton matter (Knauer and Martin, 1981) but the Cd-P ratio at this site was mainly below this value (0.07-0.96 nM:μM). This suggests that particulate Cd at this site is not mainly associated with phytoplankton and the variations in particulate Cd-P are due to non-biological factors. The variation of Ti indicates the input of terrigenous materials during rain/ runoff events. Because the ratio of Cd:Ti is fairly constant, input of terrigenous materials is likely bringing in inorganic particulate Cd and, to a lesser extent, inorganic P.

In contrast to the particulate Cd-P ratio, the dissolved Cd-P ratio is consistant throughout the year. Phytoplankton take up Cd and P in the dissolved form in a constant ratio but the particulate matter is highly influenced by inorganic material which alters the particulate Cd-P ratio. The Cd-P ratio in the dissolved phase has a mean value of 0.30nM:μM from late August 2004 to late July 2005 (Fig. 3-14). This ratio is significantly lower than the Cd-P ratio in a nearby coastal area, Amphritrite Point, on the west coast of Vancouver Island, where levels ranged from 0.44-3.0 nM:μM during an upwelling season (Lares and Orians, 1997). The waters of Lemmens Inlet had higher salinities relative to Deep Bay. The dissolved Cd-P ratio in Lemmens Inlet is very similar to the oceanic dissolved Cd-P ratio in the deep Pacific that has a value of 0.33 nM:μM. Nearshore waters are dominated by the open ocean California Current, which carries cooler water. During the winter, the slightly weaker Davidson Current carries warmer water from the south along the coast into this region (Thomson, 1981). Phosphate and cadmium are enriched in surface

California current water with levels of 0.6μmol kg-1 and 0.16nmol kg-1 respectively and has a Cd-P ratio of 0.27 (van Geen and Luoma, 1993). In the surface waters off the coast of Washington, the Cd-P ratio has been reported to be 0.33-0.35 (Jones and Murray, 1984). Thus, the dissolved Cd-P ratio is influenced by oceanic waters with a similar Cd-P ratio in addition to primary production.

3.4.2.2 Vertical Cd-P ratios

In August 2004, the water column was well stratified (Fig. 3-20) and there was a massive bloom of dinoflagellates as the biomass exceeded 8000ugCL^{-1} at 1m and 6m depths (Fig. 3-17, Table 3-8). The particulate Cd-P ratio was around 0.06 nM:μM at 1m and 6m. Dinoflagellates generally have a larger intracellular Cd-P ratio than diatoms, reaching up to 0.73 mmol:mol for some species (Ho et al., 2003) but this is not reflected in the upper water particulate Cd-P ratio. Usually dissolved P is above 1μM throughout the year and the water column (Tables 3-6, 3-8) but during this sampling event, the phosphate is lower in the water column down to 9m, with a minimum at 6m. With such a large abundance of biomass, it is likely that dissolved phosphate is being adsorbed onto the surface of the phytoplankton (Sanudo-Wilhelmy *et al.*, 2004). This leads to a lower particulate Cd-P ratio and a high dissolved Cd-P ratio, which is most significant at 6m. Salinity and temperature varied throughout the water column as well, so the variation in dissolved Cd-P ratio may be governed by both biological activity and intrusions of oceanic water masses brought in by the California current during the summer. The particulate ratio at 14m is high where there is an unusually high level of Ti (Table 3-8). This suggests that particulate matter from the sediments, which contains a higher Cd-P ratio (1.31 mmol:kg^{-1}:mol kg^{-1}- Table 3-5) is contributing to the particulate Cd-P ratio in the water column.

In February 2005, the water column was well mixed and the dissolved Cd-P ratio is constant at around 0.3 nM:μM (Fig. 3-20, 3-18). This is likely due to the influence of water brought in during the winter by the Davidson current, which has a dissolved Cd-P ratio of 0.33-0.35 nmol:μmol off the coast of Washington (Jones and Murray, 1984). Particulate Cd-P ratio ranges from 0.07 to 0.18, increasing with depth (Table 3-8). The biomass is minimal at this time of year (Fig. 3-17, Table 3-8) so the particulate Cd-P ratio is likely

dominated by inorganic particles. This is supported by the Cd:Ti ratio. In suspended particles, the Cd:Ti ratio had little variability at depths below 1m (0.23-0.28 nM:μM) and close to the sediment Cd:Ti ratio (0.27 mmol kg^{-1}: mol kg^{-1}) (Tables 3-5, 3-8). Terrigenious suspended material coming from the sediments which contain a higher Cd-P ratio effects the ratio in the water column.

The dissolved Cd-P ratio during the July 2005 sampling event is constant throughout the water column at around 0.3 nM:μM. The particulate Cd-P ratio is generally higher throughout the water column relative to other sampling events, varying from 0.16 to 0.27 nM:μM and increasing in the deeper water column (Fig. 3-18). Again, in deeper waters that are close to the sediments, there is the input of Ti and consequently, input of inorganic material with a higher Cd-P ratio (Table 3-8). During this sampling event, there was a small bloom of diatoms, and in particular, the species *Chaetocervos curvisetus* (Cassis *et al.*, 2006). This species is one of the major species contributing to the bulk of the limited biomass in the iron depleted Antarctic Circumpolar Current (Smetacek *et al.*, 2003). In iron-limiting conditions, species tend to have higher Cd-P ratios (Sunda and Huntsman, 2000; Cullen et al., 2003). Therefore, it is possible that the particulate Cd-P ratio was higher at this time because the prevalent species takes up higher amount of Cd. The results of a study which placed iron limited species in iron replete conditions showed a decrease of Cd-P in particulate matter (which included the group *Chaetoceros*) from 0.62 to 0.36 (mmol:mol) (Cullen *et al.*, 2003). A Cd-P ratio of 0.36 (mmol:mol) is still a high ratio compared to other diatoms (Ho *et al.*, 2003). Although the conditions at Lemmens Inlet were likely not iron-limiting, the abundant species, *Chaetocervos curvisetus* may have a relatively higher intracellular Cd-P ratio compared to the other diatoms that were prevalent throughout the year.

In the sediments, the Cd-P ratio is high at 1.31 mmol kg^{-1}: mol kg^{-1} (Table 3-5). This ratio is higher than the Cd-P ratio in the overlaying seawater (0.07-0.96 nM:μM) and is also double of what was found in Deep Bay. The high ratio at this site is due to an enrichment of Cd. Crustal P is roughly 0.105% (Lide, 2006) and at this site P was found to be 0.0955%. However, Cd at this site was 2.26 $\mu g g^{-1}$, which, compared to the crustal abundance of 0.1 $\mu g g^{-1}$, is highly enriched. Conversely, both the dissolved Cd and P concentrations were lower in Lemmens Inlet relative to Deep Bay. Close to the sediments, the dissolved Cd-P ratio at

Lemmens Inlet was slightly lower due to a slight depletion in dissolved cadmium. Both the depletion in dissolved Cd and the enrichment of Cd in the sediments is related to sediment dynamics. The redox chemistry could be a factor in lowering the Cd in the dissolved phase through the transfer of Cd from the dissolved to the solid phase. The sampling site was shallow and the sediments contained high levels of organic carbon, reaching 3.1%, which gives rise to anoxic conditions at the sediments. Under anoxia, sulphur is reduced and can form an insoluble phase with Cd, which can settle to the sediments. The enrichment of Cd in these sediments is typical of nearby areas, such as surface sediment samples from Ucluelet Inlet which had Cd levels of 1.7 to 3.1 μgg^{-1} and organic C that ranges from 3.5 to 7.4 % (Pederson *et al.*, 1989). As seen in all three vertical profiles, the sediments can serve as a source of particulate Cd in the form CdS. The levels of manganese (Mn) found in the sediments support the notion that the sediments are anoxic. Mn concentrations were 410 μgg^{-1}, which is considered within "background" levels (Pederson *et al.*, 1989). However, under oxic conditions, Mn is present as insoluable MnO_2, that settles to the sediments, increasing the levels of sediment Mn considerably. Thus, the absence of excess Mn suggests that Mn is present in its reduced form, Mn^{2+}, which is soluble (Pederson *et al.*, 1989). Depletion of Cd in the water column has been observed in anoxic basins such as Saanich Inlet (Jacobs *et al.*, 1985). Thus, a combination of shallow depths with high productivity resulted in an enrichment of Cd in the sediments and a high sedimentary Cd-P ratio. Van Geen *et al.* (1995) demonstrated that Cd accumulation is enhanced in suboxic sediments in the California continental margin. This suggests that oceanic Cd, underlying productive continental margins, is affected by the reducing conditions at the sediments, which in turn, affects the Cd-P ratio.

3.5 *Conclusions*

The investigation of the Cd-P ratio at these two sites not only lead to the characterization of each site, but also identified the processes which affect the coastal Cd-P ratio. Particulate matter in Deep Bay is mainly governed by the production of organic matter and its waters are highly influenced by varying input from the Fraser River and the Juan de Fuca Strait. In contrast, there is a strong presence of inorganic particles with

input of terrigenous materials from the sediments in Lemmens Inlet. The waters of this site were governed by oceanic input with sediment diagenesis leading to the drawdown of dissolved Cd.

These two sites demonstrate that the coastal Cd-P ratio can be affected by phytoplankton species composition and phytoplankton abundance, physical mixing of water masses with varying Cd-P ratios, terrigenous input and sediment diagenesis. Different phytoplankton species have different intracellular Cd-P ratios and we saw the affect on the dissolved Cd-P in August 2004 in Deep Bay. A bloom of diatoms with presumably low intracellular Cd-P ratios left a high dissolved Cd-P in the upper water column. Physical mixing of water masses with different Cd-P ratios lead to an inconsistent dissolved Cd-P ratio during the spring and summer in Deep Bay, where there is varying input from the Fraser river throughout the year. The effect of terrigenous materials from the sediments was most commonly seen in Lemmens Inlet during the vertical sampling events. Anoxic conditions at the surface marine sediments lead to the drawdown of dissolved Cd in Lemmens Inlet which translated to lower dissolved Cd-P ratios at 14m. Moreover, the conversion of dissolved Cd to CdS precipitates at the bottom sediments also served as a source of particulate Cd in Lemmens Inlet as consistently at 14m, the particulate Cd-P ratio was higher. All these processes affect the ratio and the dominating variable changes over time and depth, highly influenced by seasons.

Although there were many factors that affected the chemical composition of the marine environment at these two sites, biological activity was generally the main factor. Its influence was variable, which was reflected in the Cd-P ratios in both phases. Physio-chemical processes, also affected the marine composition and in turn, affected the Cd-P ratios. Similar to the Redfield ratio, the Cd-P ratio can serve as a tool to further understand the biogeochemical cycling and to explain the distributions of these elements in a coastal area.

3.6 *Figures*

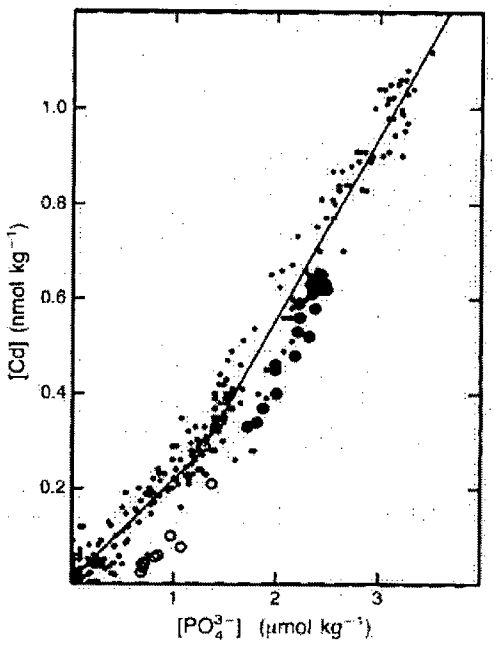

Figure 3-1: [Cd] against [PO_4^{3-}] for open ocean samples >1000m deep. Taken from Frew and Hunter (1992).

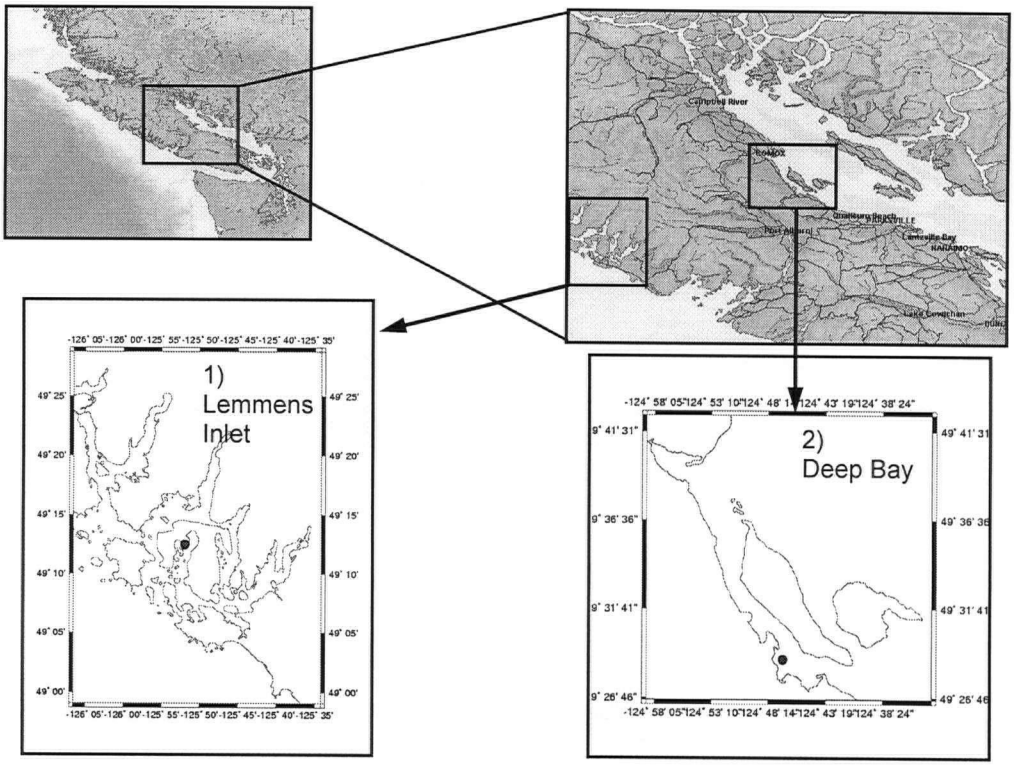

Figure 3-2: Location of sampling stations in Deep Bay (1) and Lemmens Inlet (2) coasts of Vancouver Island.

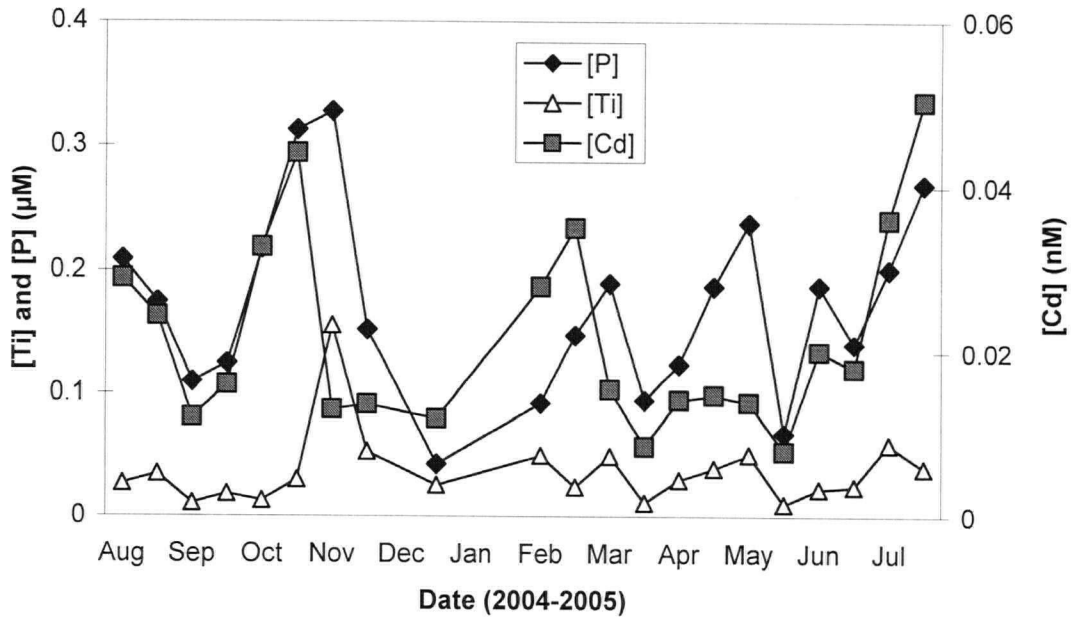

Figure 3-3: Deep Bay: Biweekly sampling of particulate Cd, P and Ti sampled at 5m. Ti and P (µM) are plotted on the primary axis, while Cd (nM) is plotted on the secondary axis.

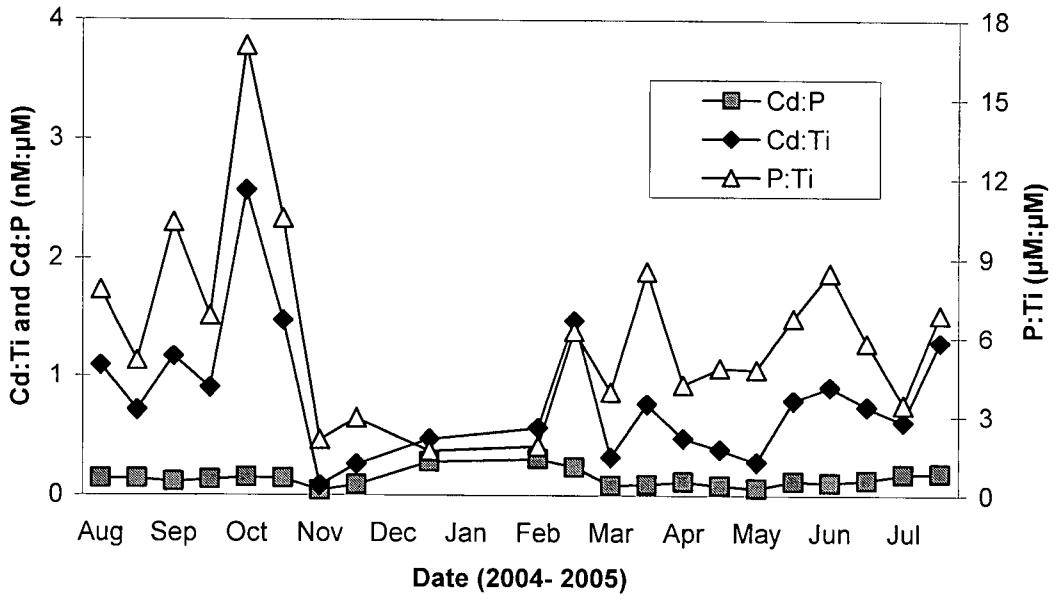

Figure 3-4: Deep Bay: Biweekly particulate ratios of Cd:P, Cd:Ti and P:Ti sampled from 5m. The ratios of Cd:P and Cd:Ti (nM:µM) are plotted against the primary axis, while P:Ti (µM:µM) is plotted against the secondary axis.

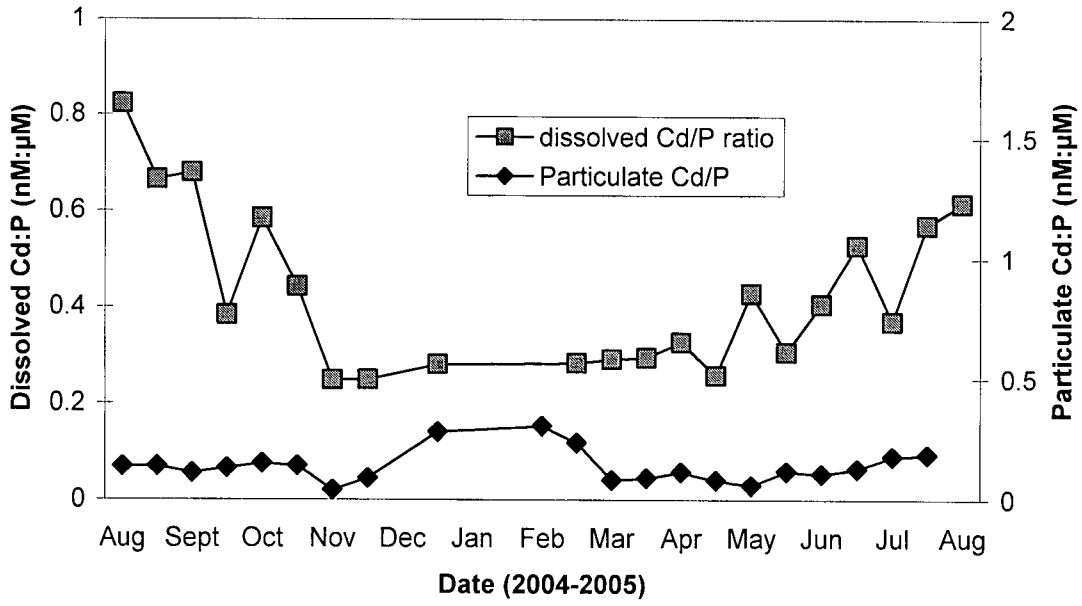

Figure 3-5: Deep Bay: Biweekly Dissolved and Particulate Cd-P (nM:µM) ratios sampled at 5m.

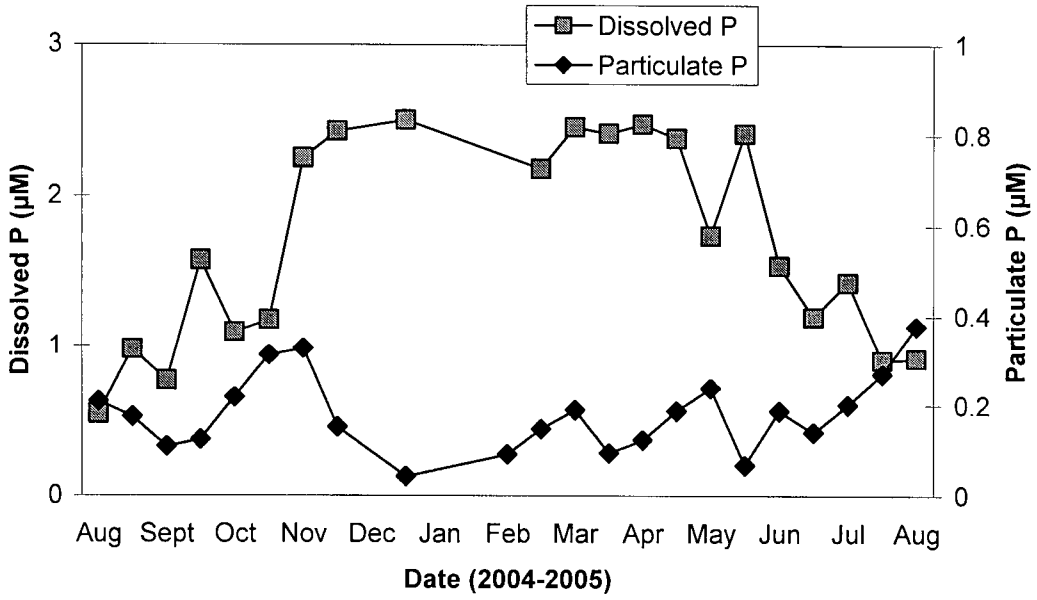

Figure 3-6: Deep Bay: Biweekly dissolved and particulate P (µM) samped at 5m.

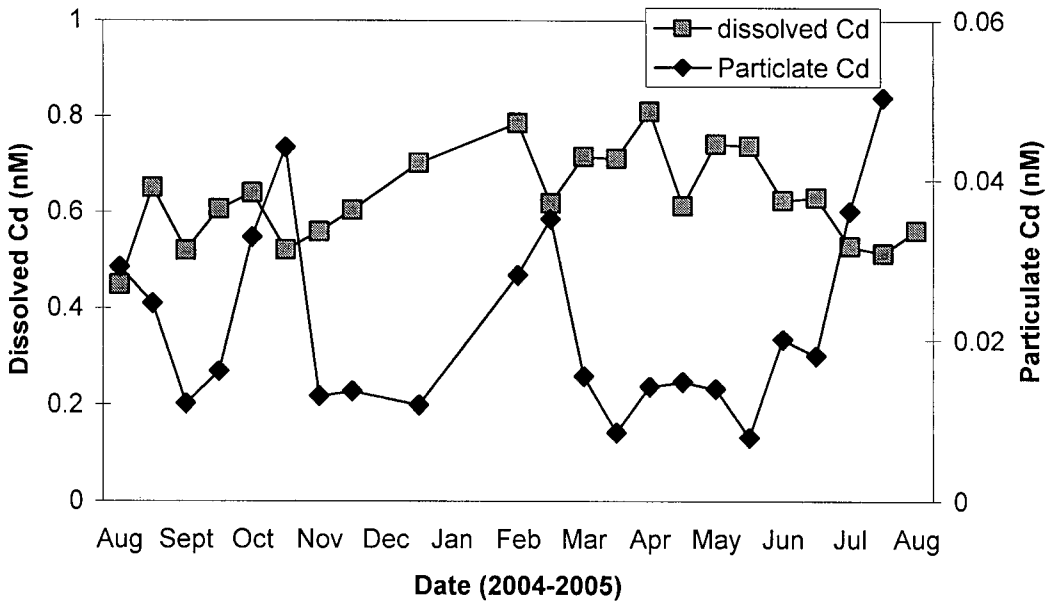

Figure 3-7: Deep Bay: Biweekly dissolved and particulate Cd (nM) sampled at 5m.

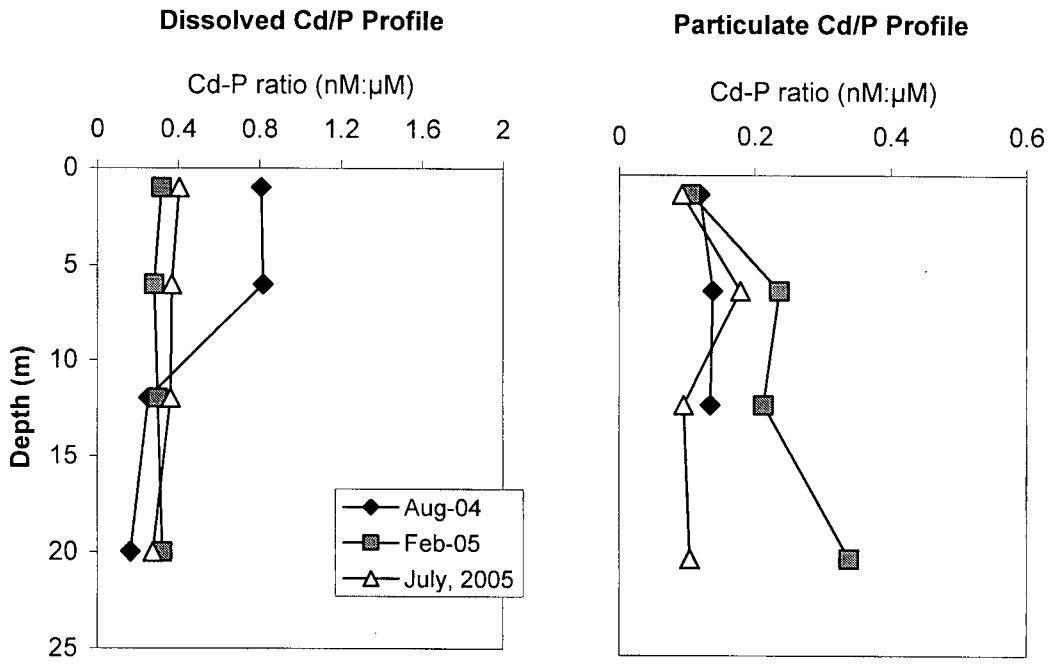

Figure 3-8: Deep Bay: Vertical profiles of Cd-P ratios (nM:µM) in dissolved and particulate.

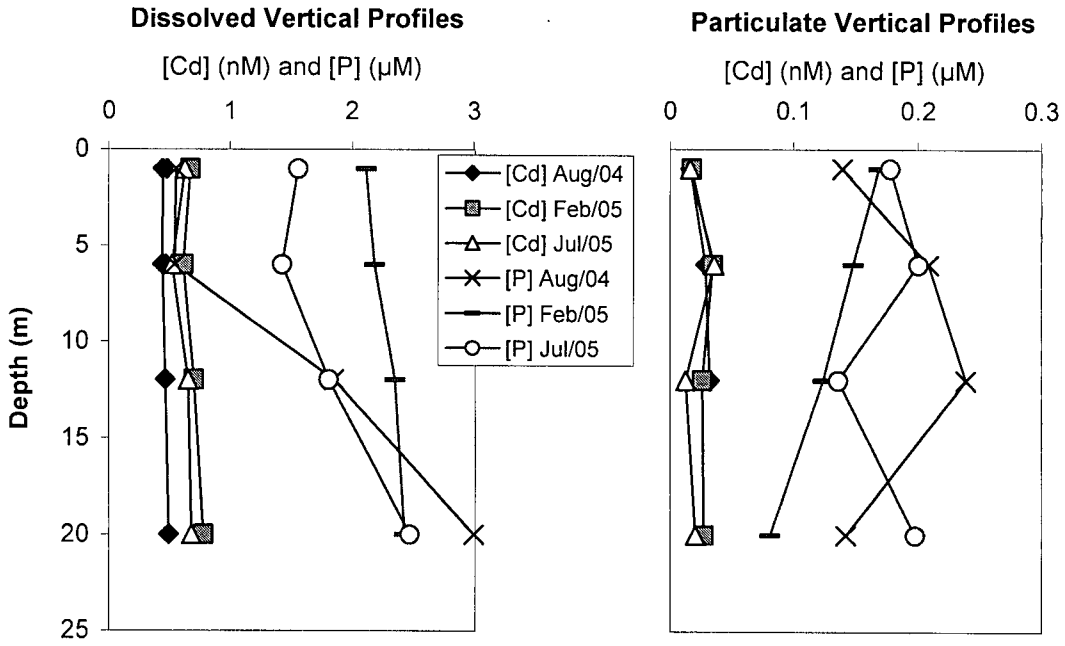

Figure 3-9: Deep Bay: Cd (nM) and P (µM) vertical profiles in the dissolved and particulate form.

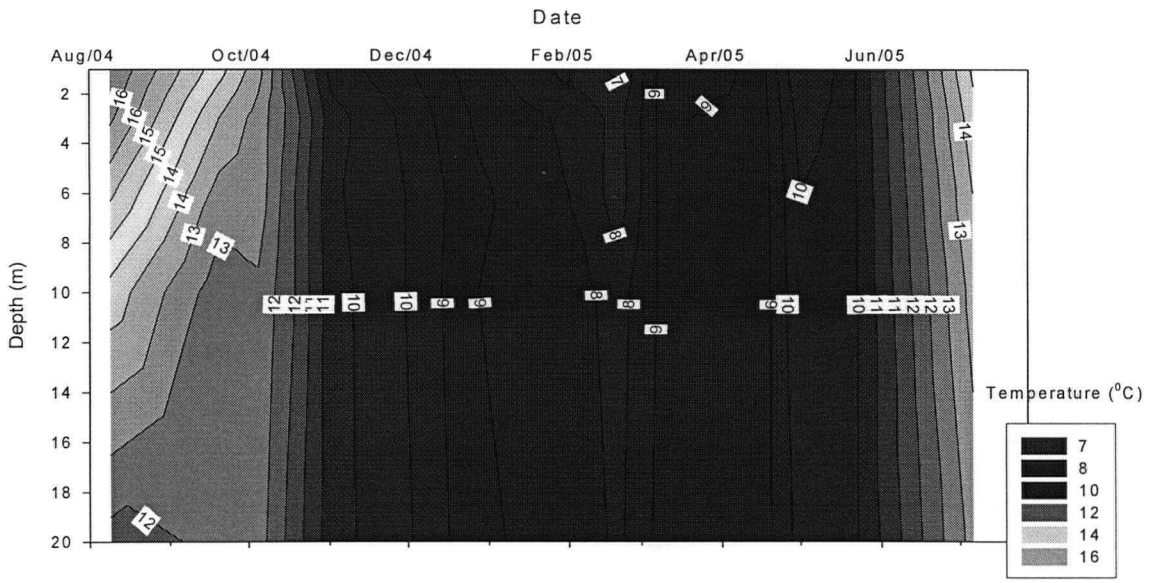

Figure 3-10: Deep Bay: Biweekly temperature profile for 2004 and 2005. Taken from Cassis *et al.*, 2006.

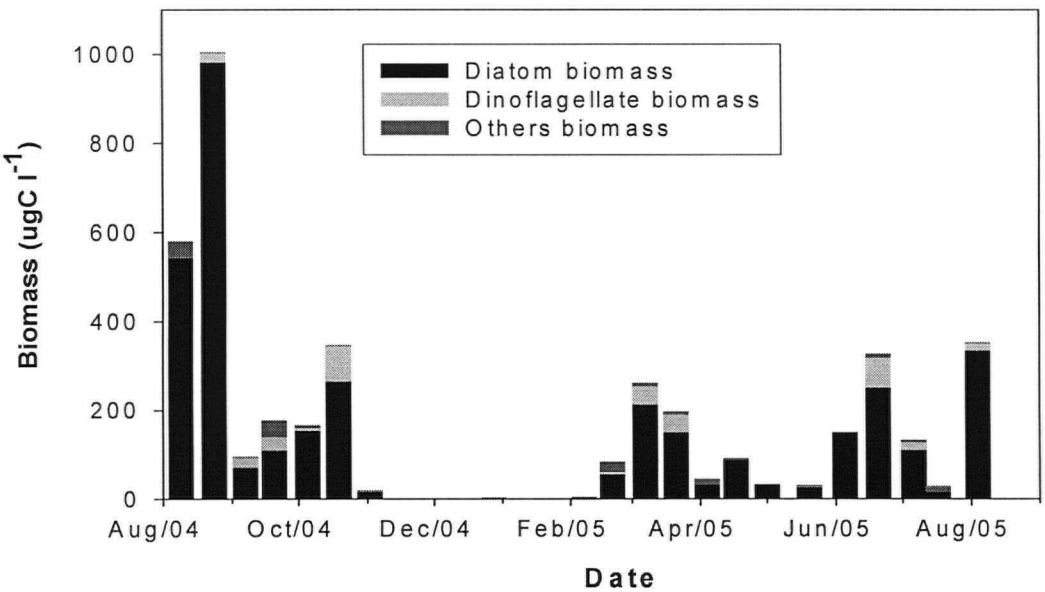

Figure 3-11: Deep Bay: Phytoplankton biomass (μgC L^{-1}) at 5m deep. Taken from Cassis *et al.*, 2006.

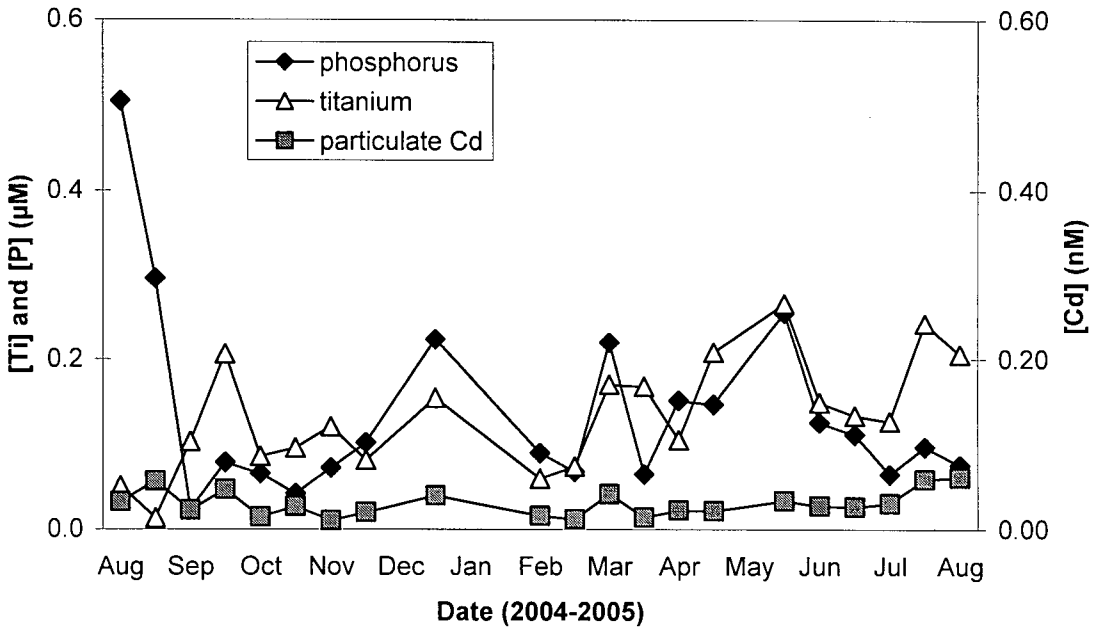

Figure 3-12: Lemmens Inlet: Biweekly particulate Cd (nM), P (µM), and Ti (µM) sampled at 5m. Ti and P are plotted against the primary axis and Cd is plotted against the secondary axis.

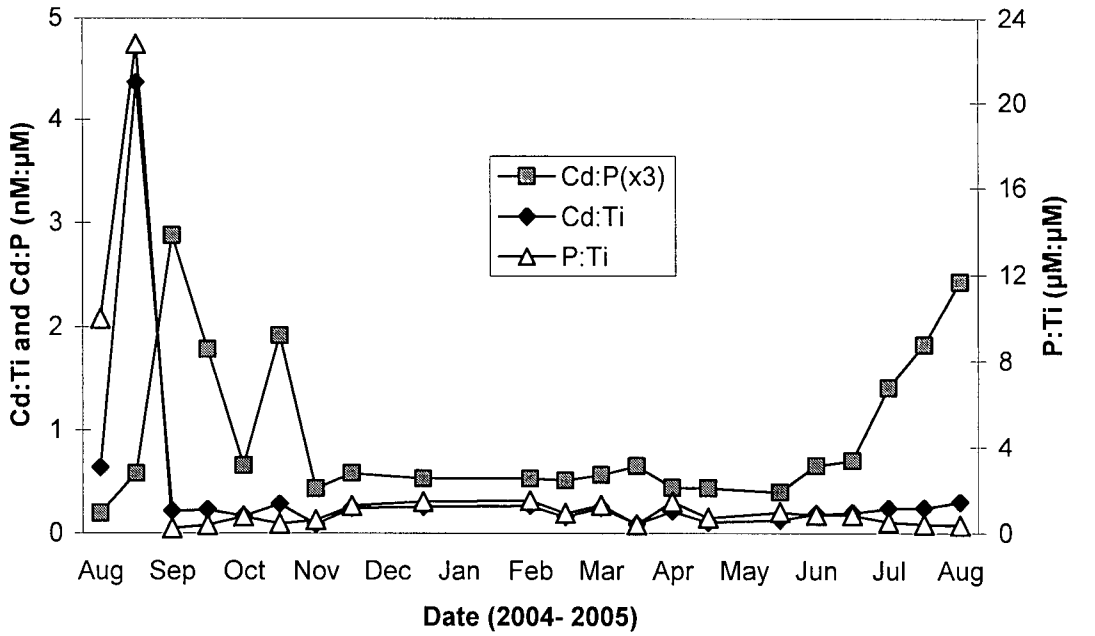

Figure 3-13: Lemmens Inlet: Biweekly ratios of particulate Cd:Ti, Cd:P (nM:µM) and P:Ti (µM:µM) sampled at 5m. Cd:P ratio is multiplied by 3 to clearly illustrate its structure. Cd:Ti and Cd:P is plotted against the primary axis and P:Ti is plotted against the secondary axis.

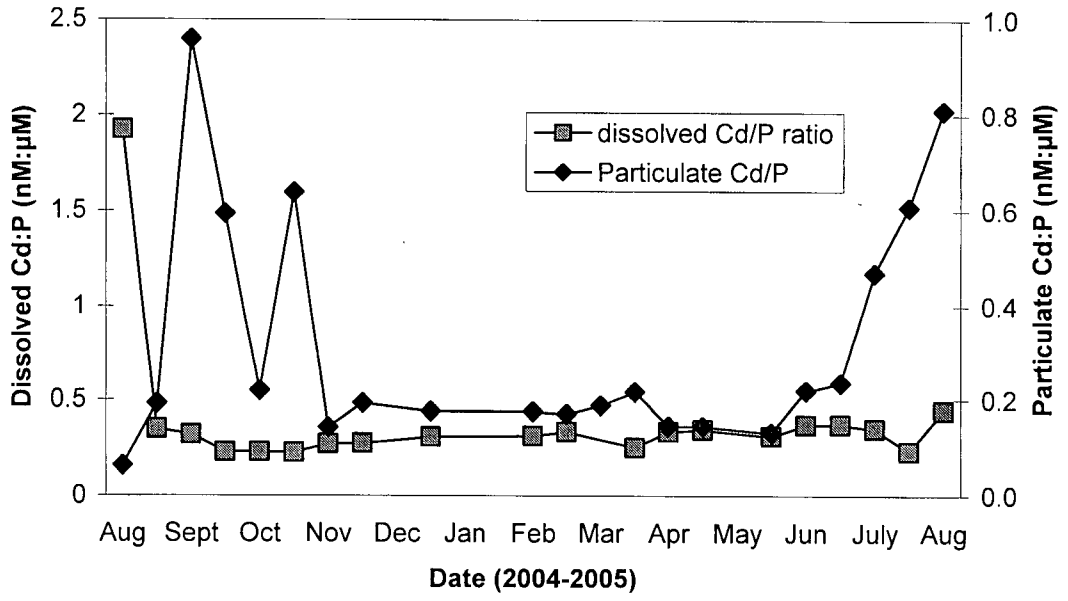

Figure 3-14: Lemmens Inlet: Biweekly dissolved and paritculate Cd-P ratios (nM:µM) sampled at 5m in 2004 and 2005.

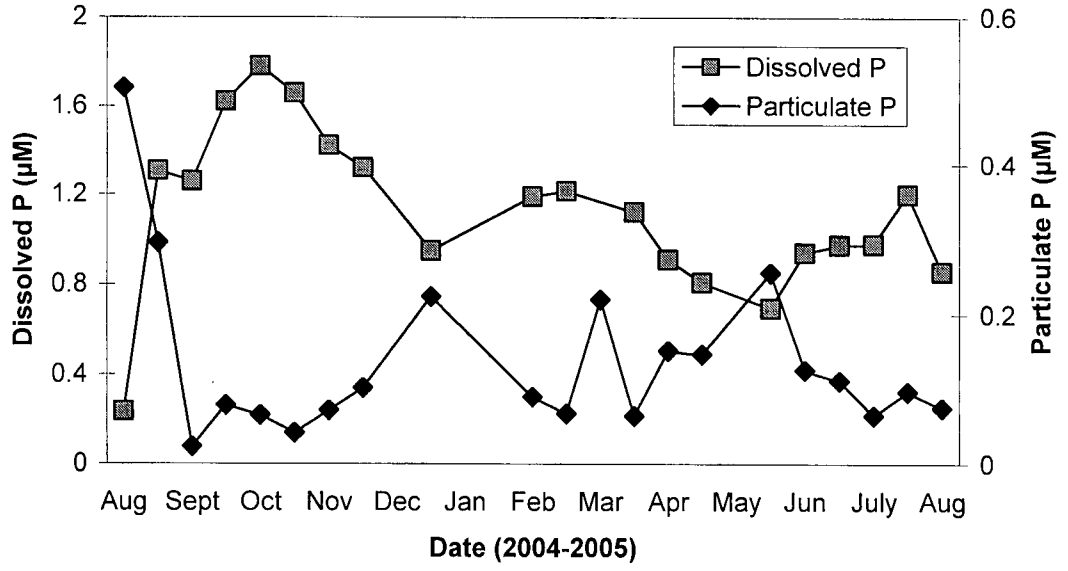

Figure 3-15: Lemmens Inlet: Biweekly sampling of dissolved and particulate P (µM) sampled at 5m in 2004 and 2005.

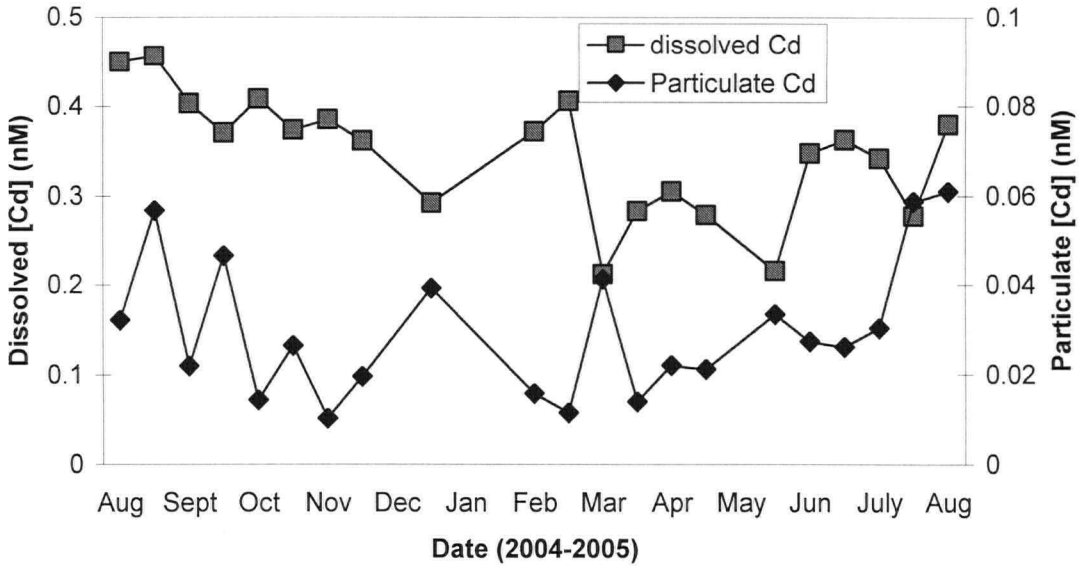

Figure 3-16: Lemmens Inlet: Biweekly sampling of dissolved and particulate Cd (nM) sampled at 5m in 2004 and 2005.

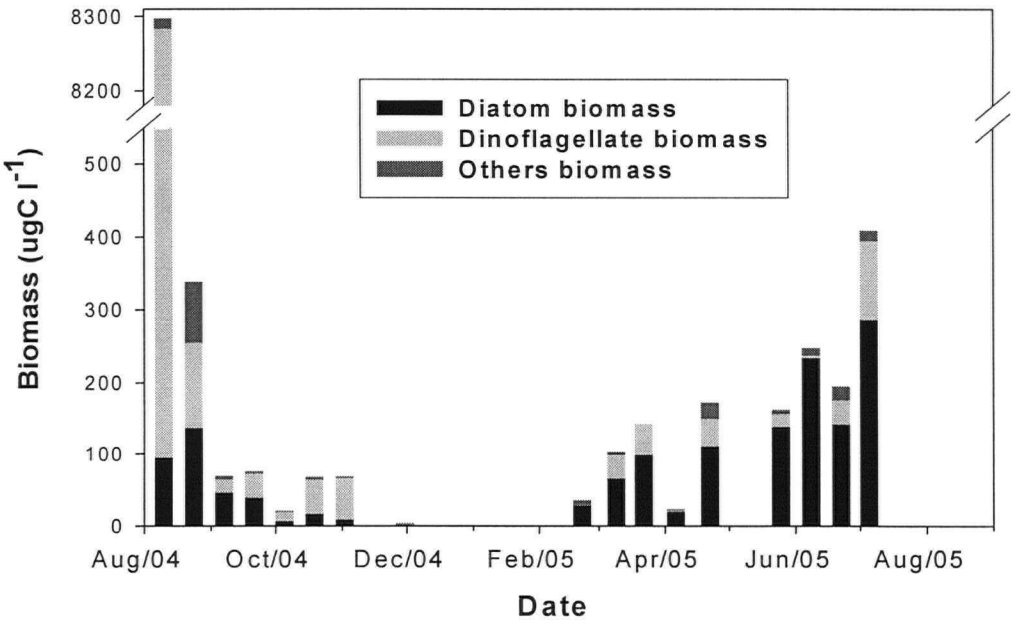

Figure 3-17: Lemmens Inlet: Biweekly biomass sampled at 5m in 2004 and 2005 (taken from Cassis et al., 2006).

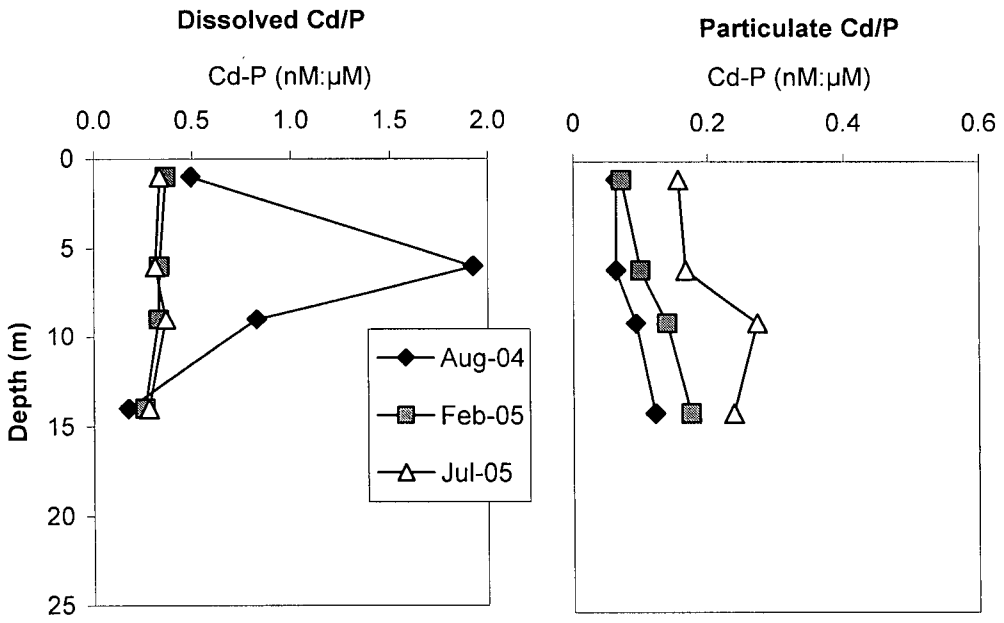

Figure 3-18: Lemmens Inlet: Vertical profiles of Cd-P ratios (nM:µM) in the dissolved and particulate.

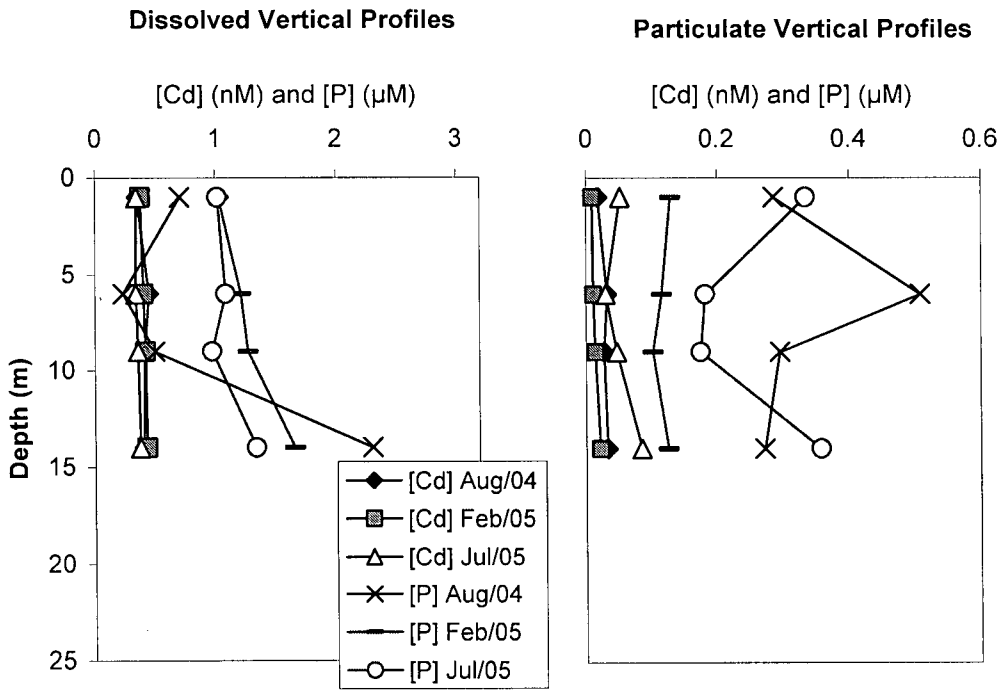

Figure 3-19: Lemmens Inlet: Cd (nM) and P (µM) vertical profiles in the dissolved and particulate form.

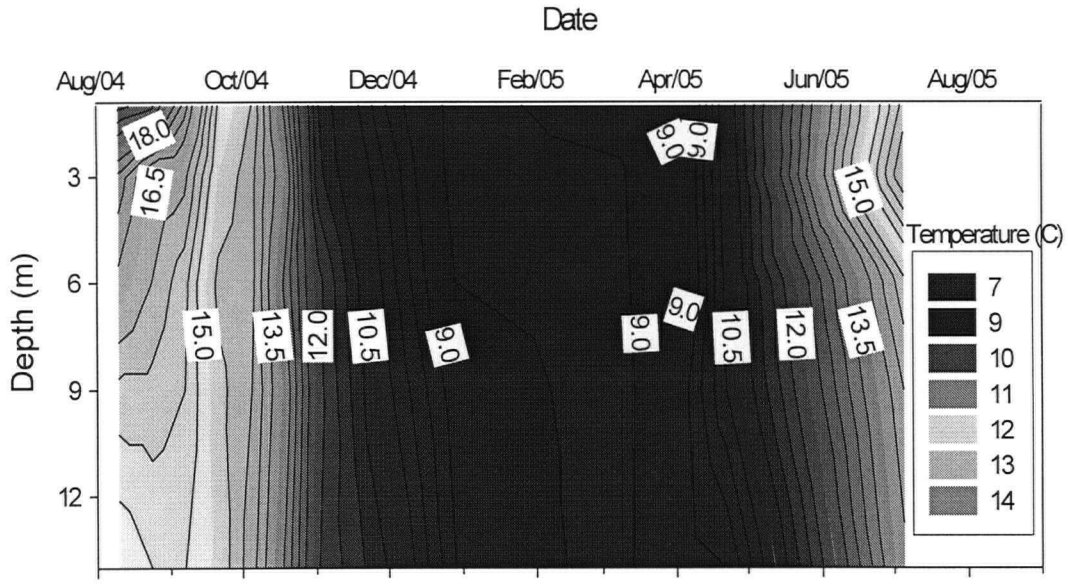

Figure 3-20: Lemmens Inlet: Biweekly Temperature profile in 2004 and 2005. Taken from Cassis et al., 2006.

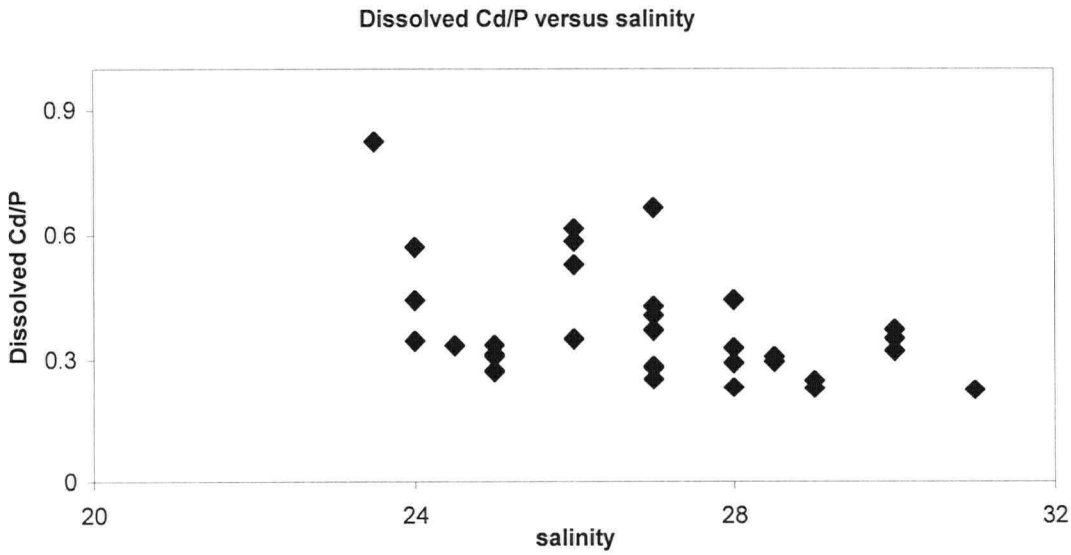

Figure 3-21: Dissolved Cd-P ratios (nM:μM) from both sites versus salinity.

3.7 *Tables*

Table 3-1: Accuracy and precision of Cd, P and Ti concentrations in the analysis of suspended particulate and sediment samples using Coastal Sediment Reference Materials.

Standard	Certified [Cd] (ugg^{-1})	Found [Cd] (ugg-1) (n= 3)	Certified [P] (%)	Found [P] (%) (n=3)	Certified [Ti] (%)	Found [Ti] (%) (n=3)
PACS-2	2.11 ± 0.15	2.53 ± 0.08	0.096 ± 0.004	0.115 ± 0.001	0.443 ± 0.032	0.480 ± 0.004
MESS-2	0.24 ± 0.01	0.303 ± 0.002	0.12 ± 0.01	0.127± 0.008	0.437[a]	0.534 ± 0.010

[a]Not a certified value. Value from one set of XRF results. (pers. Comms., Willie, S., National Research Council of Canada)

Table 3-2: Deep Bay: Biweekly Data sampled at 5m

Date	Salinity (PSU)	Temp (°C)	Dissolved [Cd] (nM)	Dissolved [P] (µM)	Total part. Cd (nM)	Total part. P (µM)	Total part. Ti (µM)	Total Biomass (µgC L^{-1})
09-Aug-04	23.5	15.1	0.45	0.54	0.029	0.21	0.027	579.7
24-Aug-04	27	14.6	0.65	0.98	0.025	0.18	0.034	1003.8
07-Sep-04	27.1	15	0.52	0.76	0.012	0.11	0.011	94.5
21-Sep-04	27.1	12.8	0.61	1.58	0.016	0.12	0.018	176.7
05-Oct-04	26	12.8	0.64	1.10	0.033	0.22	0.013	165.7
19-Oct-04	24	11.5	0.52	1.18	0.044	0.31	0.030	346.0
02-Nov-04	29	10.1	0.56	2.25	0.013	0.33	0.16	18.4
30-Nov-04	29	9.5	0.60	2.43	0.014	0.15	0.052	1.2
28-Dec-04	27	8.6	0.70	2.50	0.012	0.042	0.025	1.6
07-Feb-05	27	7.9	0.79	-	0.028	0.092	0.049	3.2
20-Feb-05	27	7.2	0.62	2.18	0.035	0.15	0.024	85.0
07-Mar-05	28	8.5	0.72	2.45	0.016	0.19	0.049	261.5
21-Mar-05	28.5	8.6	0.71	2.41	0.0085	0.094	0.011	203.9
04-Apr-05	28	8.6	0.81	2.48	0.014	0.12	0.030	44.9
17-Apr-05	27.1	8.8	0.61	2.38	0.015	0.19	0.039	94.6
01-May-05	27	10	0.74	1.74	0.014	0.24	0.050	65.6
20-May-05	28.5	9.8	0.74	2.41	0.0079	0.067	0.0099	30.3
05-Jun-05	27	12.3	0.62	1.54	0.020	0.19	0.022	152.6
20-Jun-05	26	14.2	0.63	1.19	0.018	0.14	0.024	338.9
06-Jul-05	27	13.5	0.53	1.43	0.036	0.20	0.058	132.9
17-Jul-05	24	18.7	0.51	0.90	0.050	0.27	0.039	28.5
05-Aug-05	26	16.6	0.56	0.91	-	-	-	362.1

Table 3-3: Deep Bay: Biweekly ratios of dissolved and particulate Cd:P, particulate Cd:Ti and particulate P:Ti

Date	Dissolved Cd:P (nM:μM)	Particulate Cd:P (nM:μM)	Particulate Cd:Ti (nM:μM)	Particulate P:Ti (μM:μM)
09-Aug-04	0.82	0.14	1.09	7.81
24-Aug-04	0.67	0.14	0.71	5.09
07-Sep-04	0.68	0.11	1.17	10.35
21-Sep-04	0.38	0.13	0.90	6.84
05-Oct-04	0.58	0.15	2.57	17.01
19-Oct-04	0.44	0.14	1.48	10.48
02-Nov-04	0.25	0.040	0.084	2.10
30-Nov-04	0.25	0.090	0.26	2.91
28-Dec-04	0.28	0.28	0.47	1.67
07-Feb-05	-	0.31	0.57	1.86
20-Feb-05	0.28	0.24	1.48	6.23
07-Mar-05	0.29	0.082	0.32	3.89
21-Mar-05	0.29	0.090	0.77	8.51
04-Apr-05	0.33	0.11	0.48	4.17
17-Apr-05	0.26	0.080	0.39	4.80
01-May-05	0.43	0.059	0.28	4.74
20-May-05	0.31	0.12	0.80	6.74
05-Jun-05	0.41	0.11	0.91	8.46
20-Jun-05	0.53	0.13	0.74	5.78
06-Jul-05	0.37	0.18	0.62	3.43
17-Jul-05	0.57	0.18	1.29	6.89
05-Aug-05	0.61	-	-	-

Table 3-4: Deep Bay: Vertical Sampling Data.

Depth	Temp (°C)	Salinity (PSU)	Cd (nM)	P (μM)	Particulate Cd (nM)	Particulate P (μM)	Particulate Ti (μM)	Biomass (μgC L^{-1})	Dissolved Cd:P (nM:μM)	Particulate Cd:P (nM:μM)
August 9th, 2004										
1m	16.5	25	0.45	0.55	0.017	0.14	0.028	480.11	0.81	0.12
6m	15.1	23.5	0.45	0.54	0.029	0.21	0.027	579.69	0.82	0.14
12m	13.8	27	0.47	1.85	0.032	0.24	0.030	267.87	0.25	0.14
20m	11.8	27	0.49	3.01	-	-	-	225.25	0.16	-
February 20th, 2005										
1m	6.9	27	0.67	2.11	0.018	0.17	0.027	93.75	0.32	0.11
6m	7.2	27	0.61	2.18	0.035	0.15	0.024	85.04	0.28	0.24
12m	7.8	27	0.70	2.35	0.026	0.12	0.025	73.13	0.30	0.21
20m	7.9	27	0.78	2.43	0.027	0.081	0.031	12.58	0.32	0.34
July 6th, 2005										
1m	14.1	27.5	0.63	1.56	0.017	0.18	0.065	138.12	0.40	0.094
6m	13.5	27.5	0.52	1.43	0.036	0.20	0.058	132.88	0.37	0.18
12m	13.2	27	0.65	1.81	0.013	0.14	0.032	141.68	0.36	0.096
20m	12.7	27	0.68	2.47	0.021	0.20	0.11	67.87	0.28	0.10

Table 3-5: Sediment Data and Ratios for both sites. Ratios given are in ugg^{-1}: ugg^{-1}

[Element] (ugg^{-1})	Deep Bay	Lemmens Inlet
P	661	955
Ti	4029	3526
Mn	534	410
Org C	12000	31000
Cd	0.76	2.24

Ratios (mmol kg^{-1} : mol kg^{-1})	Deep Bay	Lemmens Inlet
Cd:P	0.62	1.31
Cd:Ti	0.080	0.27
P:Ti (mol kg^{-1}:mol kg^{-1})	0.13	0.21

Table 3-6: Lemmens Inlet: Biweekly Data sampled at 5m.

Date	Salinity (PSU)	Temp (°C)	Dissolved [Cd] (nM)	Dissolved [P] (µM)	Particulate Cd (nM)	Particulate P (µM)	Particulate Ti (µM)	Total Biomass (µgC L^{-1})
10-Aug-04	28.5	16.7	0.45	0.23	0.032	0.51	0.051	8297.3
24-Aug-04	30.0	16.2	0.46	1.31	0.057	0.30	0.013	337.9
07-Sep-04	30.0	15.7	0.40	1.26	0.022	0.023	0.10	69.2
21-Sep-04	29.0	14.6	0.37	1.62	0.047	0.079	0.21	75.8
05-Oct-04	29.0	14.5	0.41	1.78	0.014	0.065	0.086	20.1
19-Oct-04	31.0	13.1	0.37	1.66	0.027	0.042	0.095	67.8
02-Nov-04	25.0	11.8	0.39	1.42	0.010	0.072	0.12	68.0
30-Nov-04	25.0	10.1	0.36	1.32	0.020	0.10	0.081	3.5
28-Dec-04	25.0	8.7	0.29	0.95	0.039	0.22	0.15	0.0
09-Feb-05	25.0	7.9	0.37	1.19	0.016	0.090	0.060	-
21-Feb-05	24.5	7.8	0.41	1.22	0.011	0.067	0.074	35.5
09-Mar-05	26.0	8.5	0.21	-	0.041	0.22	0.17	101.9
22-Mar-05	27.0	9.2	0.28	1.12	0.014	0.064	0.17	141.1
05-Apr-05	25.0	8.9	0.30	0.91	0.022	0.15	0.11	22.8
22-Apr-05	24.0	10.3	0.28	0.81	0.021	0.15	0.21	173.0
25-May-05	25.0	12.2	0.22	0.69	0.033	0.26	0.27	162.8
08-Jun-05	27.0	13.7	0.35	0.94	0.027	0.13	0.15	247.3
22-Jun-05	30.0	14.5	0.36	0.98	0.026	0.11	0.13	194.6
05-Jul-05	26.0	14.6	0.34	0.98	0.030	0.065	0.13	408.4
20-Jul-05	28.0	14.6	0.28	1.20	0.059	0.097	0.24	-
28-Jul-05	28.0	15.4	0.38	0.86	0.061	0.075	0.21	-

Table 3-7: Lemmens Inlet: Biweekly dissoved and particulate Cd:P, particulate Cd:Ti and particulate P:Ti

Date	Dissolved Cd:P (nM:μM)	Particulate Cd:P (nM:μM)	Particulate Cd: Ti (nM:μM)	Particulate P:Ti (μM:μM)
Aug-04	1.92	0.07	0.63	9.97
24-Aug	0.35	0.19	4.36	22.8
07-Sep	0.32	0.96	0.21	0.22
21-Sep	0.23	0.59	0.23	0.38
05-Oct	0.23	0.22	0.17	0.76
19-Oct	0.23	0.64	0.28	0.44
02-Nov	0.27	0.14	0.08	0.60
30-Nov	0.27	0.19	0.24	1.25
28-Dec	0.31	0.18	0.25	1.45
09-Feb	0.31	0.18	0.26	1.50
20-Feb	0.33	0.17	0.16	0.91
09-Mar	-	0.19	0.24	1.29
22-Mar	0.25	0.22	0.08	0.38
06-Apr	0.33	0.15	0.21	1.44
22-Apr	0.34	0.14	0.10	0.71
25-May	0.31	0.13	0.13	0.96
08-Jun	0.37	0.22	0.18	0.84
22-Jun	0.37	0.23	0.20	0.83
05-Jul	0.35	0.47	0.24	0.51
20-Jul	0.23	0.61	0.24	0.40
28-Jul	0.44	0.81	0.30	0.37

Table 3-8: Lemmens Inlet: Vertical sampling data

Depth	Temp (°C)	Salinity (PSU)	Cd (nM)	P (μM)	Particulate Cd (nM)	Particulate P (uM)	Particulate Ti (uM)	Biomass (μgC L^{-1})	Dissolved Cd:P (nM:μM)	Particulate Cd:P (nM:μM)
August 10th, 2004										
1m	19	30	0.35	0.71	0.018	0.28	0.012	8194.43	0.50	0.063
6m	16.7	28.5	0.45	0.23	0.032	0.51	0.051	8297.32	1.93	0.064
9m	15.8	29	0.42	0.50	0.028	0.30	0.025	230.88	0.83	0.094
14m	15	30	0.41	2.31	0.033	0.27	0.082	50.96	0.18	0.12
February 21th, 2005										
1m	7.5	26	0.38	1.03	0.0092	0.13	0.017	104.76	0.37	0.072
6m	7.8	24.5	0.41	1.22	0.011	0.11	0.048	35.46	0.33	0.10
9m	7.9	25	0.42	1.27	0.014	0.10	0.052	-	0.33	0.14
14m	7.9	27	0.43	1.66	0.022	0.13	0.080	4.59	0.26	0.18
July 5th, 2005										
1m	16	26	0.34	1.02	0.052	0.33	0.085	269.00	0.34	0.16
6m	14.6	26	0.34	0.98	0.030	0.18	0.042	408.42	0.31	0.17
9m	14.1	27	0.36	1.09	0.048	0.17	0.063	292.55	0.37	0.27
14m	13.7	27	0.38	1.34	0.086	0.36	0.21	155.15	0.28	0.24

3.8 *References*

Abe, k. Variation in the cadmium concentration related to phosphate in the surface layer of the Equatorial Pacific. Journal of Oceanography 61: 783-788.

Benitez-Nelson, C.R., 2000. The biogeochemical cycling of phosphorus in marine systems. Earth-Science Reviews 51: 109-135.

Bjerrum, C.J., Canfield, D.E., 2002. Ocean productivity before about 1.9 Gyr ago limited by phosphorus adsorption onto iron oxides. Nature 417: 159-162.

Boyle, E.A., Sclater, F. and Edmond, J.M., 1976. On the marine geochemistry of cadmium. Nature, 263: 42-44.

Boyle, E.A., Huested, S.S., Grant, B., 1982. The chemical mass balance of the Amazone Plume II. Copper, nickel and cadmium. Deep-sea Research 11A: 1355-1364.

Boyle, E.A, 1988. Cadmium: Chemical tracer of deepwater paleoceanography. Paleoceanography 3: 471-489.

Bruland, K.W., Orians, K.J., Cowen, J.P., 1994. Reactive trace metals in the stratified central North Pacific. Geochimica et Cosmochimica Acta 58: 3171-3182.

Bruland, K.W. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. Limnology and Oceanography 37: 1008-1017.

Bruland, K.W., Donat, J.R., Hutchins, D.A., 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. Limmnology and Oceanography 36: 1555-1577.

Bruland, K.W. and Franks R.P., 1983. Mn, Ni, Cu, Zn and Cd in the western North Atlantic, In: Trace Metals in Sea Water (Wong, C.S. *et al.*, Ed), NATO Conference Series, Series IV, Marine Sciences, volume 9, Plenum Press, NY, pp 395-414.

Bruland, K.W., 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. Earth Planetary Science Letters 47: 176-198.

Bruland, K.W., Knauer, G.A., Martin, J.H., 1978. Cadmium in northeast Pacific waters. Limnology and Oceanography 23: 618-625.

Cassis, D., Lekhi, A., Pearce, C., Ebell, N., Walberg, L., Maldonado, M.T., Orians, K.J., 2006. Pathways of Cadmium in cultured Pacific oysters and the effects of environmental parameters. Canadian Technical Report of Fisheries and Aquatic Sciences pp 1-95. *In press.*

Cullen, J.T. 2006. On the nonlinear relationship between dissolved cadmium and phosphate in the modern global ocean: Could chronic iron limitation of phytoplankton growth cause a link?. Limnology and Oceanography, 51: 1369-1380.

Cullen, J.T., Sherrell, R.M., 2005. Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages. Limnology and Oceanography 50: 1193-1204.

Cullen, J.T., Chase, Z., Coale, K.H., Fitzwater, S., Sherrell, R.M., 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. Limnology and Oceanography 48: 1079-1087.

Cullen, J.T., Lane, T.W., Morel, F.M.M, Sherrell, R.M., 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature 402: 165-167.

Cullen, J.T., Lane, T.W., Morel, F.M.M., Sherrell, R.M. 1990. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature, 402: 65-167.

de Baar, H.J.W., Saager, P.M., Nolting, R.F. and van der Meer, J., 1994. Cadmium versus phosphate in the world ocean. Marine Science, 46: 261-281.

Edmond, J.M., Boyle, E.A., Grant, B., Stallard, R.F., 1981. The chemical mass balance in the Amazon plume, I. The nutrients. Deep-sea Research 28A: 1339-1374.

Elderfield, H., Rickaby, R.E.M, 2000. Oceanic Cd/P ratio and nutrient utilization in the glacial Southern Ocean. Nature 405: 305-310.

Frew, R.D. 1995. Antarctic bottom water formation and the global cadmium to phosphorus relationship. Geophysical Research Letters 22: 2348-2352.

Frew, R.D., Hunter, K.A., 1992. Influence of Southern ocean waters on the cadmium-phosphate properties of the global ocean. Nature 360: 144-146.

Fu, F-X., Zhang, Y., Leblanc, K., Sanudo-Wilhelmy, S., Hutchins, D., The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces. Limnology and Oceanography 50: 1459-1472.

Gobeil, C., Silverberg, N., Sundby, B., Cossa, D., 1987. Cadmium diagenesis in Laurentian Trough sediments. Geochimica et Cosmochimica Acta 51: 589-596.

Haigh, R., Taylor, F.J.R. and Sutherland, T.F. 1992. Phytoplankton ecology of Jervis Inlet, a fjord system on the British Columbia coast. I. General features of the nano-and microplankton. Marine Ecology Progress Series 89: 117-134.

Hasle, G.R. 1978. Using the inverted microscope. In: Sournia, A. (Ed). Phytoplankton Manual. UNESCO. Monographs on Oceanographic methodology 6: 337p.

Ho, T-Y., Quigg, A., Finkel, Z., Milligan, A., Wyman, K., Falkowski, P., Morel, F.M.M. 2003. The Elemental composition of some marine phytoplankton. Journal of Phycology 39: 1145-1159.

Holms, G.B., 1997. State of water quality of Fraser River at Hope, 1979-1995. Canada-British Columbia Water Quality Monitoring Agreement. British Columbia, Ministry of the Environment, Land and Parks. ISBN 0-7726-3002-X. 297

Jabobs, L., Emerson, S., Skei, J., 1985. Partitioning and transport of metals across the O_2/H_2S interface in a permanently anoxic basin: Framvaren Fjord, Norway, Geochimica et Cosmochimica Acta 49: 1433-1444.

Jones, C.J., Murray, J.W., 1984. Nickel, cadmium, and copper in the northeast Pacific off the coast of Washington. Limnology and Oceanography 29: 711-720.

Keefe, C.W. 1994. The contribution of inorganic compounds to the particulate carbon, nitrogen, and phosphorus in suspended matter and surface sediments of Chesapeake Bay. Estuaries 17: 122-130.

Knauer, G.A., Martin, J.H., 1981. Phosphorus-cadmium cycling in northeast Pacific waters. Journal of Marine Research 39: 65-76.

Kudo, I., Kokubun, H., Matsunaga, K., 1996. Cadmium in the southwest Pacific Ocean Two factors significantly affecting the Cd-PO_4 relationship in the ocean. Marine Chemistry. 55-67.

Lane, T.W., and Morel, F.M.M. 2000. A biological function for cadmium in marine diatoms. Proceedings of the National Academy of Sciences. 97(9): 4627-4631.

Lares, M.L., and Orians, K.J. 1997. Natural Cd and Pb variations in *Mytilus californianus* during the upwelling season. Sci. Total Environ. 197: 177-195.

Lee, J.G., and Morel, F.M.M., 1995. Replacement of zinc by cadmium in marine phytoplankton. Marine Ecology Progress Series 127: 305-309.

Lide, D.R., (ed) 2006. Abundance of elements in the Earth's Crust and in the Sea, in CRC Handbook of Chemistry and Physics, Internet Version 2006, Taylor and Francis, Boca Raton, FL, 2006.

Locher, B.M, van der Meer, J., deBaar, H.J.W., Saager, P.M., de Jong, J.T.M, 1997. The global Cd/phosphate relationship in deep ocean waters and the need for accuracy. Marine Chemistry 59: 87-93.

Martin, J.H. 1990. Glacial-interglacial CO_2 change: The Iron Hypothesis. Paleoceanography 5: 1-13.

McFarlane, G.A., King, J.R., Beamish, R.J., Kurashov, V., Ivanov, V.V., 1998. The use of the Pacific Atomosphere Classification system as an indicator of future long-term climate impacts on the early life history of salmon from the Fraser River. Dept. of Fisheries and Oceans, Science Branch-Pacific region. (NPAFC Doc. No. 320) 13p.

McNee, Jay J. 1997. The Post-Depositional Cycling of Cd, Cu, Mo and Zn in Several Hydrographically Distinct B.C. Fjords. Ph.D. Thesis presented to the University of British Columbia.

Montagnes, D.J.S. and Franklin, D.J. 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: reconsidering some paradigms. Limnology and Oceanography 46 (8): 2008-2018.

Mullin, M.M., Sloan, P.R. and Eppley, R.W. 1966. Relationship between carbon content, cell volume, and area in phytoplankton. Limnology and Oceanography 11: 307-311.

Murray, R.W., Leinen, M., 1996. Scavenged excess aluminum and its relationship to bulk titanium in biogenic sediment from the central equatorial Pacific Ocean. Geochimica et Cosmochimica Acta. 60: 3869-3878.

Noriki, S., Hamahara, K., Harada, K., Particulate flux and Cd/P ratio of particulate matter in the Pacific ocean. Journal of oceanography 55: 693-703.

Paulson, A.J., H.C. Curl, Jr., R.A. Feely, T. Geiselman, K. A. Krogslund, and S. Hanson (1991) Trace metal and ancillary data in the open waters of Puget Sound: 1981-1985. NOAA Data Report. ERL PMEL-31 Pacific Marine Environmental Laboratory, Seattle, Washington, 56pp. + appendices.

Payne, C.D. and Price, N.M. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. Journal of Phycology. 35: 293-302.

Pederson, T.F., Waters, R.D. and Macdonald, R.W., 1989. On the natural enrichment of cadmium and molybdenum in the sediments of Ucluelet Inlet, British Columbia. The Science of the Total Environment, 79: 125-139.

Price, N.M., Morel, F.M.M. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. Nature 344: 658-660.

Redfield, A.C., 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. *In* Daniel, R.J. [Eds] James Johnstone Memorial Volume. Liverpool University Press. pp 176-192.

Rensel, J.E., Forster, J.R.M, 2002. Strait of Juan de Fuca, offshore finfish mariculture: Literature review and preliminary field study results.*Prepared for* National Oceanic and Atmospheric Administration 92pp.

Saager, P.M., and de Baar, H.J.W., 1993. Limitations to the quantitative application of Cd as a paleooceanographic tracer, based on results of a multi-box model (MENU) and statistical considerations. Global Planetary Changes 8: 69-92.

Sanudo-Wilhelmy, S.A., Tovar-Sanchez, A., Fu, F-X., Capone, D.G., Carpenter, E.J., Hutchins, D.A., 2004. The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. Nature 432: 897-901.

Sharpe, J.H., Culberson, C.H., Church, T.M., 1982. The chemistry of the Delaware estuary. General considerations. Limmnology and Oceanography 27: 1015-1028.

Smetacek, V., Assmy, P., Henjes, J., 2004. The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles. Antarctic Science 16: 541-558.

Sunda, W.G., Huntsman, S.A., 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. Limmnology and Oceanography 45: 1501-1516.

Sunda, W.G., Huntsman, S.A., 1998. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn and Mn in seawater. Environmental Science and Technology 32: 2961-2968.

Sunda, W.G., 1991. Trace metal interactions with marine phytoplankton. Biolgical Oceanography 6:411-42

Strathman, R.R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnology & Oceanography, 12: 411-418.

Taylor, S.R. and McLennan, S.M., 1985. The Continental Crust: its Composition and Evolution. Blackwell Scientific, Oxford, 312 pp.

Thompson, R.E. 1981. Oceanography of the British Columbia Coast. Canadian special publication of fisheries and aquatic sciences, 56. 291p.

Van Geen, A., McCorkle, D., Klinkhammer, G.P., 1995. Sensitivity of the phosphate-cadmium-carbon isotope relation in the ocean to cadmium removal by suboxic sediments. Paleoceanography 10: 159-169.

Van Geen, A., Luoma, S.N., 1993. Trace metals (Cd, Cu, Ni, and Zn) and nutrients in coastal waters adjacent to San Francisco Bay, California. Esturaries 16: 559-566.

Verardo, D.J., Froelich, P.N., and McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. Deep-Sea Res. Part A, 37:157-165.

Yang, L., (1993) Dissolved Trace Metals in the Western North Pacific. MSc Thesis presented to UBC.

4 Conclusions

4.1 *Contributions to the field of Chemical Oceanography*

The biogeochemistry of cadmium in the marine environment has been the subject of much research. Its mysterious nutrient-type profile in the open ocean lead to investigations of a nutrient role for cadmium in phytoplankton and it has been determined that cadmium does have a nutrient role as a cofactor in the metalloenzyme carbonic anhydrase. Cadmium's close correlation to phosphate in the deep ocean has lead to the use of Cd-P ratio in paleoceanography. Recently, the interest in cadmium in the coastal environment has been sparked by the uptake of cadmium by BC cultured oysters and its affect on marketability and potential health concerns. This study on the biogeochemistry of cadmium, as it relates to oysters at two locations on Vancouver Island has many implications. Firstly, this the first field studies to show that the uptake of cadmium by oysters is mainly through the dissolved form. Thus, any biogeochemical processes which affect dissolved cadmium will, in turn, affect Cd levels in oysters. Another important implication is the role of particulate Cd on Cd in oysters. Its main affect was not as a source of Cd to oysters, but rather, as how it affects Cd in the dissolved phase and by increasing the tissue mass of oysters, thereby, diluting Cd concentrations.

The method used in this project to characterize particulate Cd at the two sites was not typical but worked very well. The use of Ti as tracer of terrigenous materials is well-documented but the comparison of particulate Cd:Ti and Cd:P ratios to each other, to the extended Redfield ratio and to sedimentary ratios was a novel technique for this purpose and in this type of study. In the field, it is difficult to analyze for trace metals in organic particulate matter because trace metal clean filters can not be analyzed for carbon. Studies instead rely mainly on culture experiments or use more contamination-prone filters. Analysis of particulate matter for P and Ti can be a useful and relatively easy option to characterize particulate matter for field work.

The Cd-P ratio in the deep ocean has been used to re-create past distributions of phosphate and cadmium. This study used the coastal Cd-P ratio in the dissolved and particulate form as a reference to understand biogeochemical cycling, similar to the manner in which the Redfield ratio is used. The particulate and dissolved Cd-P ratios, combined with biological, physical and particulate Ti data, was able to indicate dominating processes, either specifically in a sampling event, or over a larger scale. Initially, the main objective for this part of the study was to use all the collected data and offer a characterization of these two sites. The inclusion of the Cd-P ratio served as a tracer of different processes. For example, when the particulate Cd-P ratio was consistent and close to the extended Redfield ratio, biological activity seemed dominant. When the particulate Cd-P was high, this may have been indicative of an input of terrigenous particulate Cd or of a specific phytoplankton species that hyperaccumulates Cd. Inconsistency in the dissolved Cd-P ratio over time may have been tracing the influence of varying water masses with different Cd-P ratios. The coastal Cd-P ratio is a valuable tool that can indicate or lead to information about biogeochemical processes and phytoplankton species compositon.

4.2 *Future Research*

In terms of looking at Cd accumulation in oysters, there is much more to uncover. It has been demonstrated that the uptake of cadmium by phytoplankton is modulated by the presence or absence of other trace metals. Perhaps similar relationships can be determined for oysters; is there a nutrient role for cadmium in oysters and if so, how do the interactions of other metals affect this role? One of the most useful questions for BC shellfish growers would be to uncover the toxicity of Cd in oysters to humans; How much of the Cd present in oysters actually transfers to humans upon consumption and does this depend on the presence of other metals, such as Zn, in oysters?

In order to further the use of a Cd-P ratio as a Redfield-type reference for biogeochemical cycling, this tool should be applied to other coastal locations. Such a reference can be fruitfully studied to offer clues that can characterize a site. The environment of the coastal ocean is extraordinarily complex. The proximity to and interaction of anthropogenic activity, land and surface marine sediments with physical, chemical and

biological ocean processes leads to elevations in biogeochemical processes. The distributions of Cd, combined with P and Ti can reflect many of these processes.

A Appendix A: Acid Washing

All plastics (bottles, pipet tips, AA cups, digestion vessels etc) were cleaned in a multiple-stage acid wash to minimize contamination of trace metals. Plastics that were not bottles were placed in plastic containers during the cleaning procedure. First plastics were soaked in Extran® for two days to remove residual organics and then rinsed ten times with distilled, deionized water (DDW). Reagent grade 4N HCl was added and plastics were placed in an oven overnight at 60°C. The acid was removed and plastics were rinsed six times with DDW. The plastics were then soaked with 1N environmental grade HNO_3, heated in the oven at 60°C and left at room temperature for two days. Again, the plastics were drained of the acid and rinsed four times with DDW before being filled with a final solution of 0.1N ultra pure Seastar® HNO_3. Plastics sat in this final solution until two days before use when they were rinsed with DDW four times and dried in a laminar flow hood overnight. The reagents for steps 1-3 were re-used for cleaning plastics but the final solution was newly made for each use.

All filters (polycarbonate and cellulose acetate) were acid washed by soaking them in 0.2N ultra-pure Seastar® HCl for one week, followed by soaking them in DDW until use.

Chelex®-100 resin used for the pre-concentration of seawater was rinsed by gravity elution. Columns were packed with 2mL of Chelex®-100 and rinsed with 40mL of 2.0N Seastar HNO_3 initially and then with 30mL of 2.0N Seastar® HNO_3 after sample was pumped through. The Chelex®-100 was replaced after three samples were pumped. An alternate cleaning procedure for Chelex®, when analyzing more contaminant-prone metals, is more rigorous and involves shrinking and expanding the chelex using NH_4OH and acid (Appendix B and Price *et al.*, 1988). However, the method used is suitable for Cd analysis.

B Appendix B: Seawater and Sediment Sample Preparation

B.1 Seawater Pre-Concentration

All seawater samples were processed in a trace metal clean lab (high efficiency particle air filtered-HEPA, positive pressure air supply). Upon receiving the samples, they were thawed and acidified to pH 2 using ultra-pure 6N HCl (Seastar® Chemicals) and stored. At a later date, samples were pH adjusted to pH 6 using concentrated ultra-pure ammonium hydroxide and buffered with a solution of ammonium acetate (made with ultra pure reagents from Seastar® Chemicals). 500mL samples were split into two replicates of 250mL and then pumped at 0.76-0.84mL/min using a peristaltic pump through 2mL of Chelex®-100 resin.

Chelex®-100 resin is a polystyrene divinyl benzene structured resin with iminodiacetate functional groups which act as chelating groups in binding polyvalent metal ions. The Chelex® resin is supplied in the sodium form and is dissolved and stored in a 0.3M ammonium acetate buffer at pH 6, which converts the resin to the ammonium form. Both ammonium and sodium are weakly held ions which allows the cadmium present in seawater to be readily adsorbed as it is pumped through the resin. Cadmium is eluted from the column by gravity elution with 10mL of 2N HNO_3 (1). The resin is re-generated by flushing with at least two columns of ammonium acetate buffer back to a pH of 6 (2):

$$\text{Resin-Cd} + HNO_3 \longrightarrow \text{Resin-H} + Cd(NO_3)_2 \quad (1)$$

$$\text{Resin-H} + NH_4Ac \longrightarrow \text{Resin-}NH_4 + HAc \quad (2)$$

The quantity of ions exchanged is a function of pH. Exchange is generally very low below pH of 2 and for most metals, optimal recover is achieved after a pH of 5 (Yang, 1993). The structure of Chelex® changes with pH:

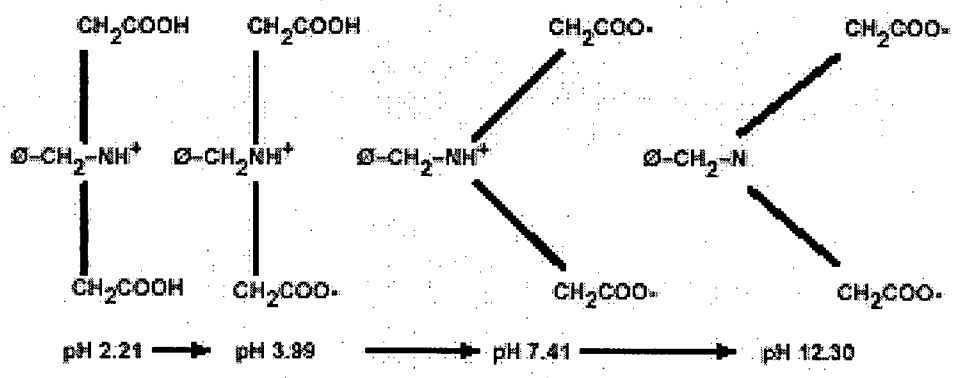

Figure B-1: Change in structure of Chelex resin with increasing pH. Taken from Biorad Chelex®-100 Resin Instructional Manual.

After elution, samples are run on the GFAAS using Zeeman background correction and quantified using standard addition method (Appendix C).

B.2 Suspended Particulate Microwave Digestion

Suspended Particulate matter was collected by filtration of 2-3L water samples through acid-cleaned 47mm diameter polycarbonate filters, pore sized 0.4μm, 3.0μm, 10μm and 20μm. Filters were analyzed in the following fractions: particulate matter sized between 0.4-3um, between 3.0-20um (the 3 and 10um sized polycarbonate filters were combined to form one sample) and greater than 20um. Due to excessive clogging, cellulose acetate filters sized 0.4μm and 3.0μm replaced the three smaller pore-sized polycarbonate filters. Once the filtration of seawater was complete, filters were folded, placed in acid-cleaned culture cups and frozen until analysis where they were placed in pre-weighed 6mL Teflon screw-cap vials (Savillex Inc., Minnetonka, MN). Polycarbonate filters were first dissolved in 3-4mL concentrated NH_3 (aq) for 24 hours before digestion. The vials were placed on a hotplate and ammonia was evaporated to dryness. This process disintegrated the polycarbonate filters. Polycarbonate and cellulose acetate filters were microwave digested with 1mL HNO_3, 1mL HCl, and 250μL HF. The digestion was performed in a closed system CEM Microwave Digestion System-205. In microwave digestion the solid filters and particulate matter are digested in closed vessels by the concentrated acids under a high pressure and

temperature atmosphere. As the acids absorb microwave energy the pressure in the closed digestion vessels increases and this allows the temperature of the acid mixture to further increase. HNO_3 is added to oxidize the organics to CO_2, HF is added to digest the silicates and HCl is added to digest inorganic particles. The digestion program involves ramping up the pressure to 40 PSI and maintaining that pressure for 45min followed by cooling the vessels for 1hour at atmospheric pressure. The program is repeated twice. Once digested, the acid mixture is evaporated on a hotplate. Next, 50% HNO_3 was added to the remaining residue (either a black or clear pellet) and samples were heated at low setting to prevent boiling for further digestion and again the acid was evaporated off. Digests were diluted to 4mL 2N HNO_3 and weighed before analysis by standard addition method on the GFAAS for Cd and by inductively coupled plasma mass spectrometry (ICP-MS) for Ti and P, using Indium (In) as the internal standard (Appendix C).

The use of a closed microwave system over the conventional method of heating acids over a hotplate reduces the time required for digestion, the amount of acid needed and the loss of volatile elements. Moreover, it also reduces blank levels for trace metal analysis by minimizing exposure to laboratory air.

B.3 Sediment

Surface sediment cores (0-2cm) were collected at one location at each site using a Petit Ponar® Grab sampler, provided by Lorax Environmental Services Ltd into two acid-washed speciman containers and frozen until analysis. Samples were freeze dried in an Edwards 4k Modulyo Freeze dryer for one week. Once dry, samples were ground in a Hertzog HSM-100 tungsten-carbide mill and stored in the same specimen containers.

C Appendix C: Analytical Methods

C.1 Dissolved and Suspended Particulate

C.1.1 Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

All Cd analysis was performed using a Varian Spectra-300/400 graphite furnace atomic absorption spectrophotometer equipped with Zeeman background correction and an autosampler. GFAAS measures the absorbance of a vaporized and atomized sample that has been injected into a graphite tube. In this analysis, samples were deposited onto a graphite furnace tube with a L'vov pyrolytic platform rather than using "off the wall" partition tubes. The advantage of using a platform is that the vaporization of the sample is delayed until the graphite tube has reached a stable and high temperature. This allows time for the atmosphere of the furnace to reach a high temperature, subsequently minimizing chemical interferences in the light path. The disadvantage is that the graphite tube wears out quicker because more heat is required to achieve the desired temperature. However, with Cd, since the atomization temperature (1800°C) is not relatively high, platform tube is preferred.

The process of atomic absorption is stepwise. The drying step involves slowly increasing the temperature to remove the solvent from the sample, then samples are ashed at a relatively higher temperature to remove the organic and some inorganic material. After ashing, the temperature is ramped up rapidly to generate free ground state atoms in the light path in milliseconds. The absorption of the ground state atoms of the light beam generated by a hollow cathode lamp is then measured. Instrument parameters were modified from those used by previous users and are given in Table C-1. The drying and ashing temperatures and time were optimized for each sample matrix based on a trial and error. The concentration of the analyte is determined by standard addition technique to account for signal suppression/ enhancement caused by the sample matrix which would not be detected by external calibration. GFAAS is a single element technique. The detection limits are quite low and the method is relatively inexpensive.

Table C-1: GFAAS Operation conditions for dissolved Cd

Description	Conditions		
Instrument Mode	Absorbance		
Calibration Mode	Standard Additions		
Measurement mode	Peak Area		
Lamp Current	4mA		
Slit width	0.5nm		
Wavelength	228.8		
Slit Height	Reduced		
Sample Introduction	Automixing		
Furnace Parameters Step No.	**Temp (C)**	**Total Time (sec)**	**Gas Flow (L/min)**
1	300	8	3
2	300	45	3
3	700	5	3
4	700	3	3
5	700	1	0
6	2000	1	0
7	2000	2	0
8	2000	1	3
9	2300	2	3
10	40	13.3	3

Zeeman background effect

The GFAAS was equipped witb Zeeman background correction to correct for interfering species that may be produced during atomization or in the sample matrix. This type of correction is based on the observation that atomic orbitals which are degenerate, will split into different energy levels under a magnetic field (Fig. C-1). This splitting of the energy levels gives the uniformly spaced multiplet splitting of the spectral lines which is called the Zeeman effect. (when the electron spin is included, there is a greater variety of splitting patterns than that which is shown in Fig. C-1).

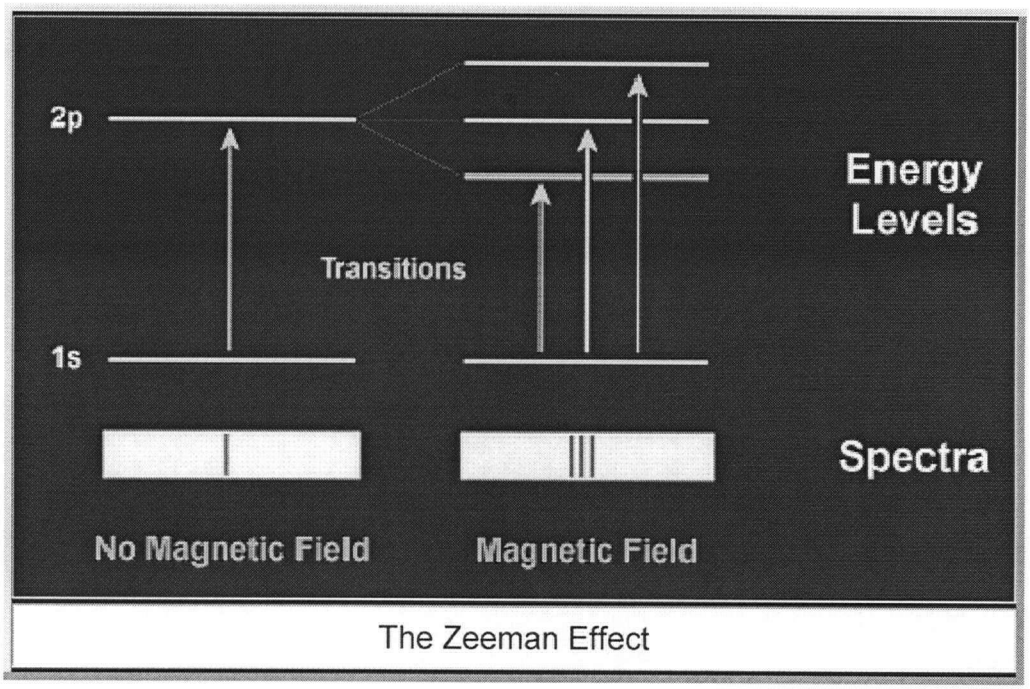

Figure C-1: Illustration of the Zeeman effect. Taken from http://csep10.phys.utk.edu/astr162/lect/light/zeeman-split.html

The components of these split spectral lines include the central or pi component, (whose position remains at the original wavelength) which absorbs the radiation polarized parallel to the direction of applied magnetic field, and sigma components (whose position is shifted from the original wavelength and are to the right and left of the pi component) which absorb radiation polarized perpendicular to the magnetic field. The sum of the component absorbances equals the absorbance of the original line.

In the SpectrAA-300/400, the Zeeman background correction applies a modulated magnetic field with a fixed polarizer to the sample. The fixed polarizer is oriented to reject the component of the hollow cathode lamp emission polarized parallel to the magnetic field. Thus, the pi component is not measured when the magnetic field is on. When the magnetic field is off, there is an analytical atomic signal plus background signal. When the field is on, the analytical signal splits into its pi and sigma components and the pi component is rejected by the fixed polarizer. Thus, when the magnetic field is on, there is only background signal at the analytical wavelength. The absorbances measured from the field off and field on conditions are subtracted to yield the background corrected atomic absorbance.

C.1.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is highly sensitive and suitable for multi-element trace level analyses. Phosphorus (P)-31 and Titanium (Ti)-47 and 49 in the particulate samples were determined by a Thermo Finnigan Element 2 ICP-MS in medium resolution. The relative isotopic abundances were 100% for P-31 and 7% and 49% for Ti-47 and Ti-49, respectively. For this analysis, particulate samples were digested in the same procedure as described by Appendix B-2. The remaining sample digests were diluted ten times and an internal standard, indium (In) was added, making the final solution of 1% HNO_3 and an In concentration of 10ppb. An external calibration method was used, with standards of a blank, 0.1ppb, 1ppb, 10ppb and 100ppb, all made in 1% HNO_3. The standards and samples were blank subtracted after normalizing to the 10ppb ^{115}In internal standard. The use of the internal standard as well as the dilution of the samples helped minimize the matrix effects.

In ICP-MS, the argon plasma serves to convert elements to atomic ions and these ions are then introduced to the mass spectrometer under high vacuum. The Element 2 is equipped with a double focusing mass spectrometer which uses a magnetic sector and an electric sector to separate and focus the ions. The magnetic sector disperses the ions based on both their energy and mass and the electric sector disperses the ions based only on energy and focuses the ions onto the exit slit. In High Resolution ICP-MS, the reverse Nier Johnson Geometry, where the electric sector follows the magnetic sector, is used. The resolution of the instrument can be changed by changing the slit width. These samples were run in medium resolution to resolve interfering polyatomic ions. In medium resolution, the entrance and exit slits of the mass spectrometer is narrowed and the peaks of interfering species are distinguishable from the analyte peak. However, as you decrease the slit width, you decrease sensitivity. Some interfering species for P-31 include $^{15}N^{16}O$ and $Ni\text{-}62^{++}$; for Ti-47, interfering species include $^{46}Ti^{1}H$ and $^{31}P^{16}O$; for Ti-49, the species $^{48}Ti^{1}H$ and $^{48}Ca^{1}H$. ICP-MS is fast, multi-element analysis with low detection limits and a large linear range.

C.2 Sediment Analysis

C.2.1 Major and Minor Elements using X-Ray Fluorescence (XRF)

The measurement of major and minor elements was made by a Phillips PW 2400 XRF instrument. XRF involves the bombardment of a prepared sample by x-rays. Electrons present in the inner most shells of the atoms will be ejected and an excited ion remains. The electrons from higher energy level shells of this excited ion transition down to fill the 'gap' left by the ejection electron. In this transition process, the ions fluoresce, emitting characteristic x-rays and return to their ground state. By determining the exact energy of the fluorescence, the identity of the element is known and by measuring the intensity of the fluorescence, the concentration of the element is known.

Sample preparation for the major elements (Na, K, Ti, Si, P, Mn, Al, Ca, Fe and Mg) involved fusing glass beads using the procedure outlined in the Katanax® K1 Fluxer manual. This process consisted of mixing 0.4g of an oxidized sample (described below) with 6.0g of a 50/50 blend of lithium metaborate/tetraborate. This mix is placed in a platinum/ gold crucible and heated to approximately 1000°C. At this temperature, the lithium borate blend is liquefied and dissolves the sample to form a homogeneous material. Finally, the molten-like material is poured onto a platinum mold and cooled, forming a glass disk. Prior to fusion, samples were oxidized to prevent the formation of metal-crucible alloys at the high temperature through the process of calcination. Calcination involves heating samples in an oven and burning the carbon to CO_2. Lastly, 25% weight/volume LiI is also added (500μL) to the solid mixture before fusion because it is a non-wetting agent and will make the melted product less prone to sticking to platinum ware.

Minor elements analyzed by XRF included Mn, Mo, I, Br, Cu, Zn, Zr, Sr, Cr, Ba, Rb, Ni, V, Y and Pb. Samples were run as pressed-powder pellets backed by boric acid, formed in a stainless steel die under ~ 10 tons hydraulic pressure. The pellets were made with 4g of sediment.

C.2.2 Carbon, Nitrogen and Sulfer

Total carbon, nitrogen and sulfer were measured by gas-chromatography on a Carlo-Erba CNS analyzer Model NA 1500. Dried sediment samples were accurately weighed (5-10mg) on a microbalance into small tin cups. A few mg of V_2O_5 were added to the samples to aid in complete oxidation of the sample to its gaseous components (N_2, CO_2 and SO_2). The sample cups were crimped shut and loaded into a sample carrousel on the instrument. The sample is introduced into a combustion column reactor and the eluted combustion products are carried through by helium gas into a second column, the reduction reactor where nitrogen oxides are reduced to N_2. Before entering the chromatographic column, analytes are swept through a water-absorbing filter. Detection is by thermal conductivity.

C.2.3 Inorganic and Organic Carbon

Inorganic carbon was determined by coulometry on a Coulometrics 5010 coulometer; organic carbon was derived by subtracting inorganic carbon from total-carbon as measured by the Carlo-Erba CNS.

Coulometry measures inorganic carbon by converting inorganic carbon into CO_2 through the addition of 10% HCl to dried sediment. Dried samples (25-100mg) were accurately weighed on a microbalance and placed in a clean glass reaction tube. The reaction tube containing the sample was first flushed with CO_2-free air for two minutes to remove residual atmospheric CO_2, placed in a heating block on the instrument and a few mL of 10%HCl is added. The CO_2 which is liberated from the sample is carried through a blue solution of monoethanolamine that reacts with CO_2 to form hydroxyethlycarbamic acid, which is clear. The change in transmittance of the ethanolamime solution is detected by photodetection monitors, which responds by reducing water at an electrode and generating a current. When the solution returns to its original color, the current stops and therefore, the amount of current generated is directly proportional to the amount of inorganic carbon present in the sample.

D Appendix D: Analytical Figures of Merit

D.1 Recoveries and Precision of Dissolved Cd

The accuracy of this technique was evaluated by the analysis of Cd in trace metal seawater reference standards from the National Research Council of Canada. One reference sample, Nearshore Seawater Reference Material (CASS-4), was split into duplicates and analyzed following the same method as samples (Table D-1).

Table D-1: Recovery of standard reference materials for dissolved Cd analysis

CASS-4	Found Concentration (ng/L)	Certified Concentration (ng/L)
Replicate A	0.025_1	0.024 ± 0.003
Replicate B	0.024_5	

Recovery tests involved spiking a known amount of Cd into seawater samples (from station "P" located at 145°W, 50°N, in the Northeast Pacific) and processing them through the full pre-concentration procedure (Appendix B-1). These spiked samples were run each batch of 12 samples (run in replicate). Recoveries were calculated from the difference between the spiked and unspiked seawater samples (Tables D-2, D-3) and precision was calculated by the using relative standard deviation (RSD) of the unspiked values over the sampling year. The precision within one processing set was smaller, with an RSD of 3% and was calculated from the replicate samples.

Table D-2: Recoveries from spiked seawater for dissolved Cd

Seawater Sample No.	1	2	3	4	5	6	7	8	9
Recovery %	93.3	93.2	95.9	102.3	111.4	108.0	96.4	95.4	109.1
Average %	$100._5 \pm 7._3$								

Table D-3: Cd concentration determined for unspiked samples over the sampling year

Seawater Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13
[Cd] (ppb)	0.0358	0.0353	0.0333	0.0337	0.0338	0.0334	0.0295	0.0301	0.0301	0.0276	0.0316	0.0310	0.287
Average [Cd] (ppb)	$0.031_8 \pm 0.002_7$												
RSD	$8.0\,\%$												

D.2 Accuracy of Suspended Particulate and Sediment analysis

The accuracy of these techniques was assessed by the analysis of trace metal marine sediment reference standards from the National Research Council of Canada. There are no certified reference materials for suspended particulate matter, but the marine sediment samples underwent the same digestion and analytical methods as the suspended particulate. The accuracy and precision of the suspended particulate analysis for Cd, P and Ti and of sediment analysis for Cd using microwave digestion was determined by triplicate measurements of coastal sediment standard reference materials (PACS-2 and MESS-2) (Table D-4).

Table D-4: Accuracy and precision of Cd, P and Ti concentrations in the analysis of suspended particulate and sediment samples using Coastal Sediment Reference Materials

Standard	Certified [Cd] (ppm)	Found [Cd] (ppm) (n= 3)	Certified [P] (%)	Found [P] (%) (n=3)	Certified [Ti] (%)	Found [Ti] (%) (n=3)
PACS-2	2.11 ± 0.15	2.53 ± 0.08	0.096 ± 0.004	0.115 ± 0.001	0.443 ± 0.032	0.480 ± 0.004
MESS-2	0.24 ± 0.01	0.303 ± 0.002	0.12 ± 0.01	0.127± 0.008	0.437[a]	0.534 ± 0.010

[a]Not a certified value. Value from one set of XRF results. (pers. Comm., Willie, S., National Research Council of Canada)

The recoveries were 120-125%, 104-120% and 110-121% for Cd, P and Ti, respectively. Although high, these results may be due to the small sub-sample of reference material used for the procedure, containing mostly large particles, which would be comparatively enriched in Cd, P and Ti. These recovery tests would have been repeated, if time permitted but will be repeated before publication. Because 100% recoveries were expected for the reference material, the data was not corrected based on these high recoveries.

D.3 Limits of Detection

D.3.1 Dissolved Cd

Replicate measurements of the acid blank, 2N HNO_3 were used to estimate the GFAAS instrument detection limit. The instrument detection limit (IDL) is calculated from the sum of the average of the signal of blank samples and three times the standard deviation. Replicate measurements of the procedural blank, in which triplicates of 200mL of DDI water were processed through the same procedure as seawater samples, were used to estimate any contribution of Cd from the resin. The results were very low and equal to blank values, illustrating that Chelex®-100 is a good choice for Cd pre-concentration. The detection limit for seawater was then determined by using three times the standard deviation from the instrument blank and accounting for a concentration factor of approximately 20 (the concentration factor applied to all seawater samples).

Table D-5: Instrument and pre-concentration procedural detection limits for Cd (IDL and PDL)

IDL (n= 82) (ppb)	PDL (ppb) (n=3)
0.002_6	0.001_2

D.3.2 Suspended Particulate Cd, P and Ti

Replicates of acid-cleaned polycarbonate and cellulose acetate filters were digested following the same procedure (Appendix B-2) and analyzed on the GFAAS for Cd and on the ICP-MS for Ti and P (Table D-6). Although these procedural blanks seem high, they are relatively low as compared to the concentrations of the metals that were actually measured.

Table D-6: Filter procedural blanks on GFAAS (for Cd) and ICP-MS (for Ti and P)

Filter Type (n= 3)	Cd (ppb)	% of measured [Cd]	Ti (ppb)	% of measured [Ti]	P (ppb)	% of measured [P]
Polycarbonate 20μm	0.0310 ± 0.0007		0.79 ± 0.03		0.30 ± 0.02	
Cellulose Acetate 0.4μm	0.0280 ± 0.0004	<10%	2.1 ± 0.07	<10%	0.6 ± 0.03	<3%
Cellulose Acetate 3.0μm	0.025 ± 0.001		5.4 ± 0.2		2.7 ± 0.1	

E Appendix E: Complete Sediment Data

Table E-1: Complete results from surface sediment samples taken in February, 2005.

	[Element] ($\mu g g^{-1}$)	
	Deep Bay	**Lemmens Inlet**
Al	67589	60589
Ca	55451	64477
Fe	28248	30076
K	7994	6027
Mg	11968	28625
Na	28644	35836
P	**661**	**955**
Si	279255	264816
Ti	**4029**	**3526**
V	149	116
Cr	87	80
Mn	**534**	**410**
Co	9.9	9.9
Ni	27.3	28.2
Cu	18.9	19.9
Zn	51.5	69.6
Rb	24.0	48.7
Sr	460	362
Y	12.8	11.8
Zr	99	89
Ba	343	286
Pb	10.6	11.3
Nb	11.6	9.6
Org C	**12000**	**31000**
Cd	**0.76**	**2.24**

References for Appendices

McNee, Jay J. 1997. The Post-Depositional Cycling of Cd, Cu, Mo and Zn in Several Hydrographically Distinct B.C. Fjords. Ph.D. Thesis presented to the University of British Columbia.

Price, N.M., Harrison, G.I.,Hering, J.G., Hudson, R.J., Nirel, P.M.V., Palenik, B., Morel, F.M.M. 1988. Preparation and chemistry of artificial algal culture medium aquil. Biological Oceanography 6: 443-461.

Verardo, D.J., Froelich, P.N., and McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. Deep-Sea Res. Part A, 37:157-165.

Yang, L., (1993) Dissolved Trace Metals in the Western North Pacific. MSc Thesis presented to UBC.