Uptake and mobilisation of metals associated with estuarine intertidal sediment by microphytobenthic diatoms

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Declaration of authorship

I, Amani Eva Becker, declare that this thesis has been composed by me and it embodies the results of my own research. Where appropriate I have acknowledged the nature and extent of work carried out in collaboration with others.

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Amani Becker

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Microphytobenthos (MPB), a mixed community of microscopic, photosynthetic organisms, algae and cyanobacteria, inhabiting the top few millimetres of bottom sediment, is a key component of intertidal mudflats. It accounts for a significant proportion of estuarine primary production, forms the base of the food chain and influences sediment distribution and resuspension (through production of extracellular polymeric substances (EPS)). Diatoms dominate the microphytobenthos community in the mid-latitudes of the Northern Hemisphere.

Estuarine sediments, are a sink for metal contaminants derived from fluvial, marine and atmospheric sources. Whilst metal releases to estuaries have declined in recent years due to increased regulation and declining industrial activity, metals previously discharged and which are now locked up in saltmarsh sediments remain a concern. For example, there are indications that saltmarshes are already being eroded, due to climate change related sea level rise, in some locations. This erosion may result in the redistribution of historically contaminated sediment to locations, such as the mudflats, where it is more available to biota, such as the MPB. In addition to causing redistribution, climate change effects, such as increasing temperatures and storminess, may also alter the bioavailability of metals to MPB. Increased concentrations of metals within the MPB could potentially increase their transfer to higher organisms through the food chain with potential impacts for biota.

Whilst planktonic algae have been well studied with respect to metal uptake from the water column, there has been little research involving MPB and uptake of metals from sediment. The extent to which contaminant uptake by microphytobenthic algae occurs and under what conditions is therefore poorly understood.

The research presented uses laboratory, mesocosm and field studies, to gain an understanding of processes governing metal bioavailability and mechanisms for uptake from sediment to the diatoms of the MPB under the complex and variable conditions of intertidal mudflats.

A laboratory study using a single diatom species *Cylindrotheca closterium* found that uptake of cadmium (Cd) varied with sediment properties revealing the importance of sediment particle size and organic matter content in metal bioavailability to diatoms. Additionally, this study showed that the presence of diatoms altered Cd partitioning between sediment, overlying and pore water. Specifically there was an increase in Cd in the overlying and pore water when

diatoms were present, indicating that diatoms mobilise metals from the sediment to the water column potentially increasing metal bioavailability to other biota.

A study was conducted using an intertidal mesocosm to increase the realism of the study system and examine uptake to a natural MPB community. Diatoms were found to have higher concentrations of all the metals analysed (except tin) than other types of algae (filamentous and sheet macroalgae), confirming their importance as a study organism with respect to metal uptake and potential mobilisation through the food chain. Sediment disturbance was shown to increase metal uptake (iron, aluminium, vanadium and lead) from the sediment to algae. This is of concern due to predicted increases in storminess which are likely to increase sediment disturbance, with the likelihood that uptake of metals to diatoms will increase in the future. However, there were also indications of an antagonistic effect of temperature on disturbance, whilst disturbance increased bioavailability and uptake, increasing temperatures reduced uptake of some metals. This highlights the importance of considering the effects of multiple stressors in complex systems.

Field studies showed that concentrations of some metals were related to their position on the mudflat whilst others were related to sampling date, indicating that there may be seasonal controls, such as to the presence of greater diatom biomass in spring and autumn, on metal uptake from the sediment.

The research conducted has increased understanding of metal uptake to microphytobenthic diatoms from sediment and the influence they have in transferring metals from sediment to water, however the research also raises a number of new questions. For example, there appeared to be a link between sediment organic matter content and bioavailability of metals to diatoms, although the relative contribution of the diatoms, other algae, cyanobacteria and EPS to the sediment organic matter warrants further investigation. Furthermore, it has shown that the use of laboratory and mesocosm studies for this type of research can produce similar outcomes to those observed in the field but under more controlled and easily manipulated conditions, although field studies will continue to be vital in improving understanding of metals availability and transfer.

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1. General introduction

1.1. Research context

Intertidal estuarine sediments are known to retain a historical record of contamination from discharges of industrial, agricultural and domestic effluent and waste (Valette-Silver, 1993, Fox et al., 2001, de Souza Machado et al., 2016). The sources of contamination can be derived directly or indirectly from point sources, such as sewage outlets, and diffuse sources within the catchment via riverine inputs, the marine environment, groundwater and from atmospheric deposition. A large proportion of this contamination adsorbs to depositing sediment, and in particular colloids (1nm to 1µm particles (Mayer and Wells, 2011), comprising metallic oxyhydroxides, clays and organic matter (Fitzsimons et al., 2011)). These constituents of the depositing sediment have a net negative surface charge to which metal cations (positively charged) are adsorbed. This temporarily removes contaminants from the aqueous phase, but not the estuarine system as a whole (de Souza Machado et al., 2016).

Whilst it is recognised that estuaries are subject to a wide range of contaminants, e.g. polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorines, metals (including radionuclides) etc., from multiple sources including waste disposal, farming and the chemical, nuclear, manufacturing and extractive industries (Ridgway et al., 2001, Wakefield, 2005, Spencer and O'Shea, 2014), the focus of this research is metal contamination. Metals are naturally ubiquitous in aquatic systems and whilst many (e.g. zinc (Zn), copper (Cu)), are essential micronutrients, but are toxic at high concentrations, others (e.g. lead (Pb)) serve no biological function.

Estuarine systems and their associated coastal wetlands are vulnerable to climate change impacts. The Millennium Ecosystem Assessment (2005) identified pollution and climate change as having had, respectively, very high and moderate impacts on coastal wetlands to date. Additionally, the report warned that impacts of both these drivers are expected to increase rapidly. There is increasing concern in relation to the short and long-term fate of sediment bound metals and the impact these might have on the ecosystems and services that estuarine environments sustain as well as potential impacts on human health (Fairbrother et al., 2007, Burton Jr., 2010, Chapman et al., 2013).

There is also some concern that, due to potential erosion and tidal flooding, as a consequence of climate related sea-level rise, heavily contaminated sediment which is buried in saltmarshes (and therefore currently unavailable to most biota) could be exposed, resuspended and redistributed to areas such as the intertidal mudflats. Once moved, contaminated sediment may become available for ingestion and absorption by organisms that live and feed there (Beldowska et al., 2016).

Other potential climate change related impacts could increase bioavailability of sediment metals. For example, increased storminess (Mölter et al., 2016) could increase sediment resuspension and remobilisation of contaminants to the water column, whilst temperature increases (Philippart et al., 2011) could affect the rate of biological and chemical reactions, both of which have the potential to increase bioavailability. These potential impacts are discussed further in Chapter 2 (Section 2.5).

Sediment contaminants are a source of toxicants for bottom dwelling organisms (Burton Jr., 2010) and, as many commercial species and food chain organisms spend a portion of their life cycle in or on aquatic sediment (Sauriau and Kang, 2000, Laane et al., 2013), this creates an exposure pathway from sediment to higher aquatic organisms, birds, terrestrial wildlife and humans.

As well as direct effects of sediment contaminants on benthic organisms, they may also play a role in contaminant mobilisation. For example, Loach, a type of fish, were found to facilitate release of thallium (Tl) from reservoir sediment (He et al., 2015) due to bioturbation of the sediment, whilst amphipods and bivalves in estuarine sediment facilitated the release of nickel (Ni) and cadmium (Cd) to overlying water. In addition, some recent studies have investigated the mobilisation of arsenic (As) from sediments to water (Kohfahl et al., 2016, Prieto et al., 2016) by algae in freshwater systems. In the first of these studies it was found that inorganic As was transformed to methylated species by algae in ponds, whilst the second found there was transfer of As from sediment to river water in the presence of a diatom dominated biofilm. These studies point to the possibility that algae at the surface of intertidal sediments could be implicated in mobilisation of metals to overlying water. The role of diatoms in contaminant mobilisation is explored further in Chapters 3 (single diatom species controlled laboratory studies) and 4 (controlled mesocosm experiments replicating the community of benthic organisations or microphytobenthos (MPB)).

MPB is a community of microscopic photosynthetic organisms, primarily algae and cyanobacteria, which live on and within the sediment surface. This community forms a biofilm in which individual microorganisms are embedded within a self-produced mucilage of extracellular polymeric substances (EPS). The main component of MPB in the mudflats of the northern hemisphere are the epipelic (mud dwelling) diatoms (Kazemipour et al., 2011), single cell algae with a pennate form which are motile within the sediment. The MPB is a food source to detritus feeders such as invertebrates and molluscs (Kanaya et al., 2008) as well as shore birds (Elner et al., 2005). When MPB becomes suspended in the water column it makes a significant contribution to the food chain in estuarine locations (De Jonge and Van Beusekom, 1992), particularly for zooplankton (David et al., 2016) and filter feeders (Yoshino et al., 2012). It is this role, as the base of the food chain with implications for environmental and human

health, which makes it vital to understand the role of MPB in the cycling and bioaccumulation of contaminants from the estuarine sediments.

Whilst algae are known to have the capacity to take up contaminants, and the phytoplankton and macroalgae have been well studied in this respect (He and Chen, 2014, Suresh Kumar et al., 2015), there has been little research involving the algae component of the MPB (Stronkhorst et al., 1994). The extent to which contaminant uptake by MPB occurs and under what conditions is therefore very poorly understood.

Interest in contamination of sediment and the biological availability of metals has largely focused on invertebrates and molluscs (Araújo et al., 2010). Although it is widely acknowledged that the routes of metal contaminants from sediment to animals include both ingestion and membrane transfer due to proximity (Bryan and Langston, 1992), ingestion of sediment particles has been the main focus (Lee et al., 2015) whilst the contribution of food within the sediment and contamination at the lowest trophic level, benthic algae, appears to have been largely been overlooked.

The need to study contaminant concentrations within epipelic diatoms has been recognised by other workers (Stronkhorst et al., 1994, Absil and van Scheppingen, 1996), but apart from these two studies the focus has been on the biofilm as a whole (Courtney and Clements, 2002, Ancion et al., 2013, McCormick et al., 2014). The mucilage of the biofilm, the EPS has been said to function like a sponge, taking up both nutrients and metals (Bhaskar and Bhosle, 2005). EPS acts as a chelator that binds metals and has been implicated in the sequestering of metals from solution in the water column and into the sediment (Decho, 2000) as well as potentially into the food chain (Ancion et al., 2013). However, the focus of this research will be on contaminant uptake by the epipelic diatoms in the MPB as opposed to the EPS component because the EPS has been comparatively well studied compared with the role of diatoms. Diatoms also form an important link in the food chain and could therefore play an important role in the transfer of contaminants to birds and other wildlife.

Reviews have identified the importance of understanding contaminant behaviour in the more complex real world environment as well as the laboratory setting (Wernberg et al., 2012). It has been recommended that in order to estimate the contribution of microorganisms to metal mobility and successfully interpret and extrapolate results to environmental system scale, studies should investigate multiple aspects of metal accumulation and mobilisation both in the laboratory and the field (Ledin, 2000). In addition, there has been some recent emphasis on investigating the effects of multiple stressors on marine coastal systems (Hewitt et al., 2016). In light of these recommendations and in recognition of the potentially important interactions between climate change and contaminant uptake, e.g. effects of temperature increase on chemical and biological processes and of sediment suspension on contaminant partitioning and

bioavailability (Eggleton and Thomas, 2004) research will be carried out on a range of scales and at increasing levels of complexity.

1.1.1. The ARCoES Project

The research described here is linked to the Engineering and Physical Sciences Research Council (EPSRC) funded Adaptation and Resilience of Coastal Energy Supply (ARCoES) project (ARCoES, 2013) which aims to identify the challenges facing the future security of the UK nuclear energy sector and coastal energy supply as a result of changing patterns of temperature and rainfall, sea-level rise and storms. In particular, it aims to determine threats posed to future energy generation and the distribution network, as well as the surrounding coastline and coastal waters, by flooding, erosion, changing patterns of sedimentation, water temperature and the distribution of flora and fauna in the coastal zone. This is being achieved through modelling of the coast to predict changes for estuaries, beaches, dunes and cliffs in terms of future flooding, erosion, sedimentation, water quality and habitats. There is a particular focus of these models on the North West of England as a test case and this is one of the reasons why the work conducted here was focused on the Ribble estuary and its sediment.

The ARCoES project focuses on the interplay between the two crucially important challenges faced by the UK, the need to reduce greenhouse gas emissions and understand the future impacts of climate change, to improve resilience. As a component of this, there is a need to understand the long-term environmental and health implications of morphological change and remobilisation of contaminated sediments around the UK coastline (Chapter 5). This includes understanding mechanisms for contaminant remobilisation from sediments by biota, such as MPB and is another reason why diatoms were chosen for this study.

1.2. Aims and Hypotheses

This research aims to gain a mechanistic understanding of metal uptake from sediment to the major primary producer of the intertidal mudflat, epipelic diatoms, in the complex estuarine environment and to understand how that might be affected by predicted climate change effects.

In light of the paucity of research into uptake of metals from sediment to microalgae the initial aim is to understand the differences in uptake from either contaminated sediment or contaminated water of a single metal, cadmium (Cd), to a single species of diatom *Cylindrotheca closterium*. This will enable comparison with studies of metal uptake to single species of planktonic and benthic algae from water and the use of previously tested methodology from toxicology research, whilst moving the research forward into a previously understudied area. It is also of interest to understand whether the presence of diatoms, as an

integral component of the sediment, living at the sediment water interface, have any effect on the partitioning of contaminants between system compartments.

In line with the aims of the ARCoES project it is of interest to understand if changes to the existing intertidal mudflat ecosystem due to predicted climate change effects of increasing sea water temperatures and increasing storm frequency and intensity will affect uptake of metals to a microphytobenthic community as opposed to an isolated species. Increased uptake could have implications through the food chain, for the wider environment and human health.

To ensure that findings from controlled studies are applicable in the field the final aim is to understand how metal uptake to MPB varies with height in the tidal frame and seasonally and compare those findings with those from earlier studies.

Working at increasing scales (laboratory microcosm, mesocosm and field) each chapter addresses specific questions. The main questions addressed are:

1. How is uptake to the diatom *C. closterium* affected when Cd is either initially in solution or adsorbed to sediment and is this affected by the organic matter content of the sediment (Chapter 3)?

Hypotheses:

- *Cd concentration in C. closterium is greater when overlying water is the initially contaminated component*
- *Cd concentration in C. closterium is greater when sediment had a lower organic matter content*

2. Does the presence of diatoms (*C. closterium*) affect the partitioning of Cd between system compartments (sediment, overlying water and pore water) (Chapter 3)?

Hypotheses:

- *Sediment Cd concentration is reduced in the presence of C. closterium*
- *Cd concentrations in water increase in the presence of C. closterium*

3. How is metal uptake to a MPB community affected by changes in water temperature and sediment disturbance (Chapter 4)?

Hypotheses:

- *Metal uptake to benthic algae from sediment increases with increasing water temperature*
- *Metal uptake to benthic algae increases with increasing levels of sediment disturbance*

4. Does metal uptake vary at different heights of the tidal frame due to variation in sediment properties (Chapter 5)?

Hypothesis:

- *Contaminant uptake by the diatoms in the MPB varies at different heights in the tidal frame due to variation in sediment organic matter and particle size*

5. Does metal uptake vary at seasonally, between spring and autumn, due to variation in sediment properties (Chapter 5)?

Hypothesis:

- *There is a decrease in metal uptake by the diatoms in the MPB due to an expcted decrease in surface sediment particle size and an increase in sediment organic matter from spring to autumn*

1.3. Thesis structure

The thesis is structured to guide the reader through the questions addressed using increasing scales and levels of realism, from a laboratory study in the controlled environment facility (CEF), through the intertidal mesocosm, to the field. This will facilitate a mechanistic understanding of metal uptake by benthic microalgae in the complex intertidal environment on a continuum from highly controlled to real world (Fig. 1.1.).

The literature review (Chapter 2) gives an overview of previous work in this area and background information on subjects relevant to this study. This information is provided in a single chapter to avoid repetition of information in subsequent chapters. Only two studies (Stronkhorst et al., 1994, Absil and van Scheppingen, 1996) which looked directly at contaminant uptake from the sediment to MPB were found and therefore a range of relevant

study types were identified from which methods were developed, methods specific to each study are discussed within the appropriate chapter.

There are three data chapters. The laboratory experiment based in the CEF (Chapter 3) uses a single diatom species, *C. closterium*, and a natural sediment spiked with a single contaminant, Cd, to investigate the differences in uptake to the algae from water and sediment and the effect that the presence of algae has on contamination within those compartments. This provides a basic understanding of contaminant uptake and partitioning in the system under highly controlled conditions. The mesocosm experiment (Chapter 4) increases realism by introducing a tidal cycle, and naturally varying air temperatures and light levels. In this case a naturally contaminated sediment and its associated algal community was utilised whilst water temperatures and levels of sediment disturbance were manipulated. In Chapter 5 a field study enabled the investigation of a real world setting with its inherent heterogeneity and without any manipulative controls.

The concluding chapter (Chapter 6) summarises the findings of the preceding three chapters and makes recommendations for further investigations based on these conclusions.

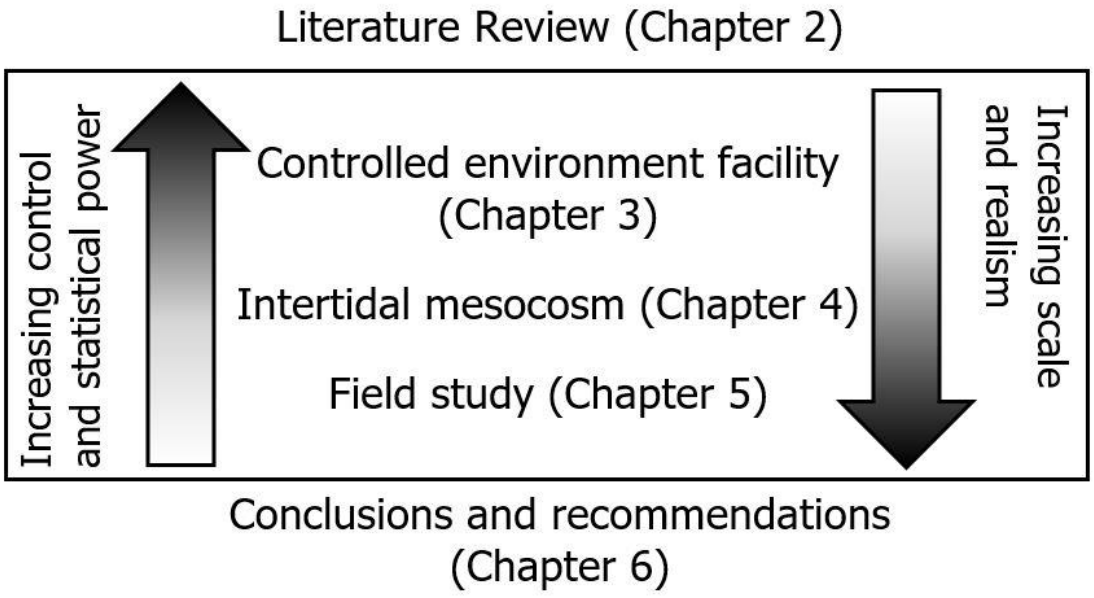


Figure 1.1. Diagram of thesis chapters illustrating trade-off between control and realism at increasing experimental scales

2. Literature Review

2.1. Introduction

The research described in this thesis assesses the role of microphytobenthos (MPB) in metal remobilisation from estuarine intertidal sediment and how that varies due to bioavailability which itself may be potentially be affected by some predicted climate change effects. Chapter 2 therefore discusses several areas: firstly, the importance of estuarine ecosystems, their service provision and propensity to act as a sink for contaminants and specifically metals. It then outlines the composition of the MPB and its role in the intertidal ecosystem, followed by a discussion on the bioavailability of metals in sediment. It then reviews predicted climate change effects that have the potential to influence contamination in estuarine mudflats. Finally it looks at research concerning contaminant uptake by algae, focusing on the few studies regarding uptake to benthic diatoms from sediment in both field and laboratory settings.

2.2. Estuaries

2.2.1. Definition and importance

Estuaries are bodies of water that connect the land and sea. They extend from waters that have fully marine conditions inland to the limit of tidal influence. They are transitional, being neither wholly fresh nor completely saline, with a salinity that varies dependant on the competing influences of fluvial and marine waters from ~1 to >30 (Chapman et al., 2013). Estuaries may be classified in several ways, for example in terms of geomorphic evolution (e.g. fjord or tectonic); by the mixing of salt and fresh water; or by ecosystem (Simenstad and Yanagi, 2011). Chapman and Wang (2001) define three types relative to sediments, these are salt wedge (dominated by river flow); fjord and shallow/partially mixed (both of which are more influenced by tidal currents) and vertically homogenous (in which tides dominate and salinity varies little with depth).

Estuaries are complex and dynamic systems which vary spatially and seasonally due to the influences of the tides, river flow and sediment supply and distribution. Additionally, because of their position at the dual interface of land and sea and fresh and marine waters, estuaries have steep gradients in a range of physical and chemical variables (e.g. salinity, temperature, pH, nutrient availability and particle size). These occur horizontally, from river to sea, laterally, across the width of the estuary (i.e. with height in the tidal frame), and vertically from overlying water to sediment.

The tidal frame is defined as the elevation range between the lowest and highest tides. Tidal frame has been shown to exert controls on benthic algae because microphytobenthos abundance is influenced by sediment particle size and water content (Pratt et al., 2015) which are determined in part by elevation. Species and biomass also vary with changing emersion time and system energy (Jesus et al., 2009, van der Wal et al., 2010). These differences may also exert controls on contaminant uptake due to difference in redox potential of the sediment (Eggleton and Thomas, 2004) and increased time in contact with overlying water (Atkinson et al., 2007). Disturbance of the sediment surface is greater in the higher energy lower level of the tidal frame and sediment resuspension is higher here (Friedrichs, 2012), therefore dissolution of contaminants and potential for uptake by algae may be increased.

Worldwide, estuarine locations are traditionally zones of human habitation due to their provision of important food, climate regulation, pollution control (in terms of retention and removal) and biodiversity resources (Millennium Ecosystem Assessment, 2005).

Estuaries and their associated coastal wetlands provide habitat to a range of organisms for all or part of their lifecycle and are important to marine fish, for their provision of nursery areas, and to migratory birds, as feeding grounds with MPB being a critical dietary component. Whilst there is relatively low biodiversity within estuaries compared to adjacent aquatic environments, the abundance of individual species often increases within estuaries so that they support a higher biomass than either marine or fresh waters (McLusky and Elliott, 2004).

2.2.2. Estuarine sediment as a contaminant sink

As stated in Chapter 1, estuarine pollution is from a wide range of inputs from river and groundwater discharge, atmospheric deposition and activities taking place within the estuaries themselves.

Whilst contamination in estuaries has long been of concern (Matthiessen and Law, 2002) changes in legislation, for example restrictions on the use of lead (Pb) in petrol (Valette-Silver, 1993) and control of discharges to tidal waters, such as the 1974 Control of pollution act in the UK (Matthiessen and Law, 2002), in addition to declines in industrial activity (Plater and Appleby, 2004) have reduced these inputs and improved water quality. However, in countries, such as the UK, which have long histories of industrialisation, metals and other contaminants which were previously discharged to estuarine and coastal areas became associated with the sediment where they remain as a historical record (Valette-Silver, 1993) with the potential for resuspension and redistribution (Turner and Millward, 2002, Cofalla et al., 2012). One concern is that the contaminants buried in generally stable sediments such as those found in salt

marshes might be remobilised through, for example, storm induced erosion events and therefore become more bioavailable than at present.

Estuarine sediments act as a contaminant sink because contaminants attach preferentially to fine particles (colloids, clay particles and organic matter) suspended in the water column due to their high surface area and availability of binding sites (Turner and Millward, 2002). Metals attach to the surface of particles through the process of adsorption, this may be physical or chemical. Clay and organic particles carry negative electrical charges, and are surrounded by cations which undergo cation exchange with metals in the aqueous phase. Chemical (specific) adsorption occurs through the presence of functional groups carried by organic matter which complex metal ions. Estuarine circulation then facilitates the deposition of these fine particles. A turbidity maximum develops at the interface between saline and fresh water which increases sedimentation due to flocculation of aggregated particles which is augmented by salinity (Park and Brown, 1999). There is also an effect of biological aggregation in which small clay particles are ingested by organisms and excreted as part of larger faecal pellets which have a greater settling velocity (Turner and Millward, 2002). Conditions within estuaries such as shelter from large waves and offshore currents also favour settling of sediments from the water column (Friedrichs, 2012).

2.2.3. Mudflats and salt marshes

Much of the fine sediment in estuaries is stored in the salt marshes and mudflats. Salt marshes in particular have been recognised as long-term contaminant stores and for their value as a historical record of past pollution (Valette-Silver, 1993, Fox et al., 1999).

Salt marshes are formed in sheltered locations through the interaction between tidal regime, sea level, sediment supply and vegetation (Allen and Pye, 1992). Salt marsh forms as a succession from the tidal flat environment through colonisation by halophytic pioneer plants (i.e. the grass *Spartina spp.*). The presence of vegetation increase the ability of the marsh to accrete sediment, through stabilisation of the mud surface and trapping sediment in its leaves (Kearney and Fagherazzi, 2016). This allows the salt marsh to gain height, reducing periods of inundation and enabling succession of plant species less tolerant of frequent saltwater inundation. In this way salt marshes becomes a stabilised store of estuarine sediment. Salt marsh is threatened by erosion at the seaward edge (Karimpour et al., 2016) and the ability of salt marshes to keep pace with sea level rise is uncertain (this is discussed further in section 2.5.2).

Tidal flats are low bed slope environments without vegetation which are exposed to the air at low tide (Friedrichs, 2012), they often have salt marsh at the landward boundary. Tidal flats

encompass a range of sediment grain sizes dependant on their position within the estuary with mudflats forming in the central estuary where there is greater mud deposition (Flemming, 2012). There is also a gradation of particle size from the tidal channel with a gradual fining towards the landward edge, i.e. with increasing height in the tidal frame. This is due to the slower deposition of fine material which means it is transported further inland and is generally deposited at slack tide (Wang, 2012).

2.2.4. Summary

Estuaries have been shown to be important ecosystems for both humans and wildlife. They have also been shown to be major pollution sinks, in particular the fine sediments which form salt marshes and mudflats. There is the potential that, due to climate change effects (Section 2.5) sediments currently stored in salt marshes could be resuspended and redistributed to areas of the estuary, such as mudflats, where they may become more readily available to biota, such as MPB.

2.3. The role of microphytobenthos in the intertidal zone

2.3.1. What is microphytobenthos?

The MPB is a community of unicellular, plant-like organisms living in the top few millimetres of marine and freshwater bottom sediment (MacIntyre et al., 1996). It consists of a variety of autotrophic microorganisms that include diatoms, cyanobacteria, euglenoids and dianoflagellates (MacIntyre et al., 1996); diatoms have been shown to dominate in the mid-latitudes of the Northern Hemisphere where this study is focused (Barranguet et al., 1998, Kazemipour et al., 2011) (Fig. 2.1).

Diatoms are single celled algae of the class Bacillariophyceae with an estimated 10^4 to 10^5 species and are present in all aquatic habitats (Smol and Stoermer, 2010). They are made up of both centric and pennate forms that live either individually or as members of a colony, attached to surfaces or in the water column. They have a box like silica frustule made up of two overlapping valves (thecae) held together with girdle bands. Reproduction occurs by cell division, in which the daughter cells maintain one thecae and grow another within it, and sexually through the production of auxospores. Diatoms associated with marine or estuarine sediments can be divided into three life strategies dependant on their relationship with the sediment. Epipelic diatoms are of the pennate form and are motile, moving within the sediment through the excretion of mucilage through a slit called the raphe (Fig. 2.1). Episammic diatoms are predominantly immobile, attaching themselves to sand grains and are therefore subjected

to the transport and mixing of their substratum. The third group are described as meroplanktonic or tychopelagic and exhibit a part planktonic, part benthic lifestyle and include centric and pennate forms. The pennate diatom *Cylindrotheca closterium* exhibits a meroplanktonic lifestyle, spending time as part of the benthos as well as becoming stirred up into the water column where it spends time in the plankton.

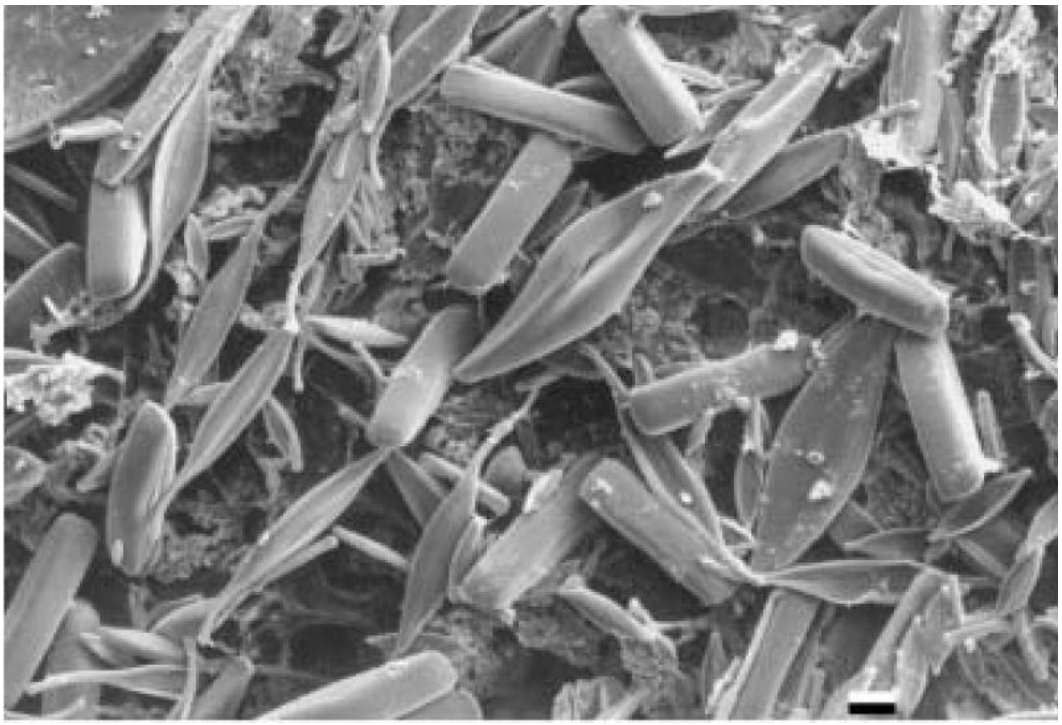


Figure 2.1 Low-temperature scanning electron micrograph of surface sediment with natural diatom assemblage, abundant species include *Gyrosigma fasciola* and *Pleurosigma angulatum*. Scale bar = 10µm (Defew et al. 2002)

Epipelic diatoms dominate in intertidal sediments (MacIntyre et al., 1996). These are mobile within the top few centimetres of the sediment, moving vertically toward the surface and reaching maximal concentration at the surface when the sediment is exposed in the daytime, allowing photosynthesis to take place (Saburova and Polikarpov, 2003). When the surface is inundated by the incoming tide these diatoms retreat beneath the sediment surface, thereby avoiding suspension (Heckman, 1985). This accepted migratory pattern of emergence to the surface during daylight, low tide followed by downwards migration to avoid the incoming tide is somewhat simplistic and there are many variations to this basic pattern dependant on site and species (Consalvey et al., 2004). The main factors controlling epipelic diatom migration appear to be temperature and solar radiation intensity in combination with the tidal cycle (Saburova and Polikarpov, 2003) and cells have been observed to respond to high levels of incident light

or rain showers by migrating below the sediment surface (Paterson, 2001), however, these responses also appear to be species specific (Du et al., 2012).

The depth to which diatoms migrate is controlled by factors such as the depth of light penetration in the sediment and the depth of the anoxic layer (Consalvey et al., 2004) which are in turn affected by sediment properties such as particle size and organic matter content. These variations account for the wide range of reported migration depths from the upper few millimetres (MacIntyre et al., 1996) to maximum depths of up to 80 mm in more coarse sandy sediments (Saburova and Polikarpov, 2003). . Reproduction, by cell division, also takes place at depth (Fig. 2.2). It has been suggested by Saburova and Polikarpov (2003) that this is because as the most favourable nutrient conditions (with nitrogen in its reduced form, NH_{4+}) are found in the deeper sediment.

Diatom migration is achieved through the use of extracellular polymeric substances (EPS). An adhesion/traction model has been proposed (Edgar and Pickett Heaps, 1983) in which EPS is attached to the substrate and connected to free transmember structures which move along the raphe, pulling the diatom forward. Migration therefore requires a suitable substrate for EPS adhesion, with migration speed affected by species and substrate (Hay et al., 1993). Vertical speed of locomotion in natural sediment has been measured as 0.19 μm s^{-1} (Hay et al., 1993), enabling migration of about 0.68 mm in one hour. The migration is utilised within this study for diatom collection in the field and laboratory through a technique devised by Eaton and Moss (1966).

As previously stated epipelic diatoms excrete mucilage EPS composed mainly of sugars (Stal and de Brouwer, 2003) which forms a biofilm at the sediment surface (Miller et al., 1996) and, in addition to aiding diatom movement, provides protection and stabilises the sediment surface (Decho, 2000). De Brouwer & Stal (2002) identified two types of EPS. The first type, described as soluble, was produced continuously and thought to be associated mainly with cell motility, the second, bound, fraction was produced in the light during periods of high growth. This categorisation may be an oversimplification with others describing EPS as being on a range of forms from gel to solution (Decho, 2000).

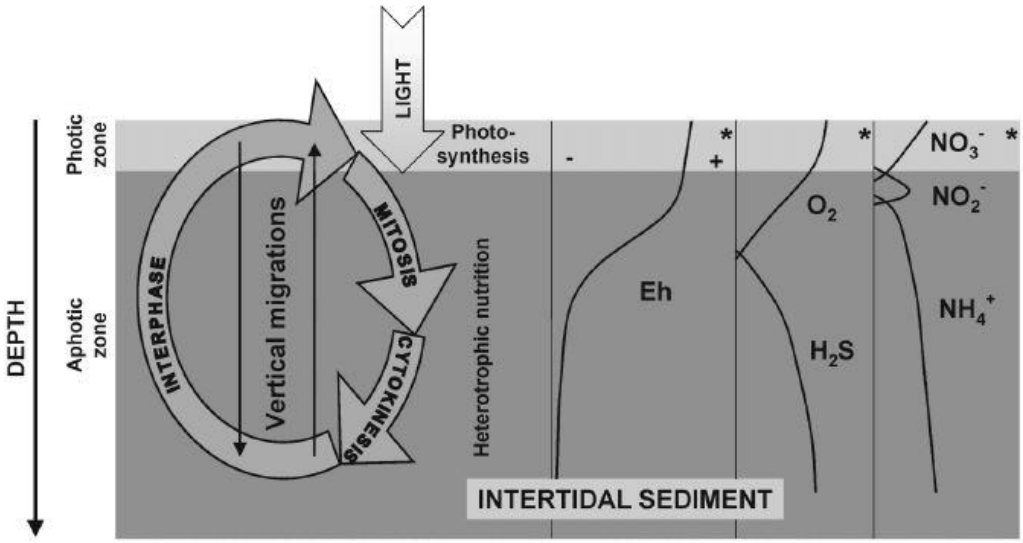


Figure 2.2. Principal scheme of epipelic diatoms activity within intertidal soft sediments (* adapted from Fenchel 1969) (Saburova and Polikarpov, 2003)

2.3.2. Integral component of the sediment

The MPB is an important integral component of sediment (Kromkamp et al., 2006). Through the production of EPS and formation of a biofilm, MPB affect the stability of the sediment surface and influence further deposition of sediment particles (van de Koppel et al., 2001). The MPB biofilm increases sediment stability by decreasing shear stress and increasing cohesive properties (Stal and de Brouwer, 2003). Different species of diatom produce differing quantities of EPS (Underwood, 2010) and therefore may affect sediment stability to varying degrees. Although the biofilm is generally heralded as a sediment stabiliser (Underwood and Paterson, 1993, Ziervogel and Forster, 2006) when this layer is breached it can tear which may result in it peeling off causing rapid erosion and resuspension of the sediment beneath (Miller et al., 1996, Le Hir et al., 2007).

MPB also has an indirect effect on erosion in that, as a food source, it encourages deposit feeders (e.g. *Corophium volutator* and *Hydrobia ulvae*) that bioturbate the sediment (Mouritsen et al., 1998, Orvain et al., 2004). Conversely, in a study of the ragworm, *Hediste diversicolor,* and MPB, (Passarelli et al., 2012) observed enhanced sediment stability and an increase in photosynthetic biomass and EPS indicating more complex relationships.

2.3.3. Part of biogeochemical cycles

The presence of MPB affects nutrient cycling in that it controls the release of certain nutrients to the water column from the sediment (Hochard et al., 2010). For example, Sundbäck and Granéli (1988) found that the release of NH_4 and PO_4 from the sediment was prevented under light levels of ≥10 μEm^{-2} s^{-1} due to increased oxygen production because of photosynthesis and increased nutrient requirements (uptake) by the microbenthic algae. MPB has been shown to mediate nutrient flux between the sediment and water column through the uptake to algae (Cabrita and Brotas, 2000) and by limiting solute diffusion (Miller et al., 1996). The uptake of nutrients by benthic algae has prompted some recent work to develop MPB for use in the phytoremediation of eutrophic sediment (Yamamoto et al., 2008, Kwon et al., 2015).

2.3.4. Primary production and importance as part of the food chain

Benthic diatoms are resuspended into the water column along with the sediment on the incoming tide (Plante et al., 2011) due to a combination of convection currents (caused by the temperature difference between the sediment surface and water), tidal currents and wind action (Baillie and Welsh, 1980). It has been estimated (Koh et al., 2006) that a mean of 33% of benthic algae is resuspended over the course of a flood tide, contributing approximately 66% of chlorophyll a (Chl*a*) to the water column (Koh et al., 2007). Where suspension occurs benthic microalgae form an important part of the estuarine food chain (Baillie and Welsh, 1980, De Jonge and Van Beusekom, 1995, Guarini et al., 1998) particularly as the standing crop of epipelic algae is more abundant and seasonally stable than the phytoplankton (Baillie and Welsh, 1980). MPB has been estimated to supply up to 45% of the organic resources of an estuary (Meleder et al., 2010) with the lighter more nutritious particles being transported greater distances due to hydrodynamic sorting (Miller et al., 1996). Although estimates of the contribution made by MBP to estuary production vary with location and due to the measure used (e.g. Chl*a* or organic resource), there is general agreement that its contribution is significant.

As a food source to deposit feeders the importance of MPB is difficult to disentangle from the detritus of salt marsh and submerged aquatic vegetation, but it is thought that MPB may be preferred as it is nutritionally richer (Miller et al., 1996). MPB is consumed by deposit and suspension feeders (De Jonge and Van Beuselom, 1992, Kanaya et al., 2008, Yoshino et al., 2012). In an assessment of food web dynamics for fish in Portuguese estuaries it was found that MPB made a significant contribution to the diet of the main prey items of economically important fish species (França et al., 2011). Additionally a study in Australia (Melville and Connolly, 2003) also found that microalgae from mudflats made up an important part of the diet of the commercially important sand whiting (*Sillago ciliate*). MPB also represents an

important food source to shorebirds such as the western sandpiper (*Calidris mauri)* and dunlin (*Calidris alpina*) (Elner et al., 2005).

2.3.5. Summary

MPB has been shown to be an extremely important constituent of the intertidal mud flat and wider estuary system. It interacts closely with the sediment having both physical (sediment stabilisation and resuspension) and chemical (nutrient flux) effects. In addition, as the principal primary producer in estuarine systems, it forms a vital component of the benthic and pelagic food web. However, its role in contaminant transfer/bioavailability is not well understood.

2.4. Bioavailability of sediment associated contaminants

Contaminated sediment has been acknowledged as a threat to those organisms that live in or on it, as well as those which feed there (Burton Jr., 2010). However, sediment composition is complex and inherently variable making assessment of bioavailability difficult (Parsons et al., 2007). Speciation of dissolved metals in fresh and marine water is much better understood than that of either estuarine water or sediment (Chapman et al., 2013, de Souza Machado et al., 2016) due to the spatial and temporal heterogeneity in biogeochemical processes caused by cyclical changes in properties such as salinity and pH (Chapman et al., 2013). This may be one reason that so many more studies have been carried out looking at contaminant uptake in planktonic algae than with benthic algae and may explain why studies into benthic algae tend to focus on uptake from overlying water. Conversely, of course, the reason that there is such understanding must be in part because more studies have been carried out.

Metal concentrations and bioavailability within the sediment depend on a range of processes, these include: speciation of metals and their mobilisation to pore and overlying water due to properties of pH, salinity and redox potential (Zhang et al., 2014); controls exerted by major sediment components (e.g. organic carbon (Parsons et al., 2007), particle size (Lee and Cundy, 2001)), Fe- and Mn-oxides (Burton Jr., 2010), acid-volatile sulphides (Ankley et al., 1996)); competition between metals for binding sites in organisms (He and Chen, 2014); and transformation (e.g. methylation) of metals (Bryan and Langston, 1992). In addition, there are effects of resuspension and bioturbation on these processes (Ridgway et al., 2003, Eggleton and Thomas, 2004).

Intertidal sediment is an extremely heterogeneous environment with properties that vary with depth and over the horizontal scale. Properties that vary with depth include irradiance, nutrient availability, pH, O_2 and H_2S concentration (Miller et al., 1996, Saburova and Polikarpov, 2003),

these properties have consequences for both the suitability of habitat for diatom growth and contaminant bioavailability. Irradiance is particularly important for photosynthetic organisms and availability of light (controlled by sediment particle size and mineralogy) influences the depth to which diatoms are found within the sediment (Cartaxana et al., 2011). However, as stated above and illustrated in Fig. 2.2., diatoms do migrate to depths at which conditions would appear to be adverse to their survival (i.e. below the photic zone and where conditions are anoxic) because these locations have greater nutrient availability (Saburova and Polikarpov, 2003). It would be expected that metal contaminants would be less available at these depths, due to the reducing environment.

Pore water is seen as the first bioavailable fraction of sediment contaminants (Chapman et al., 2002) and therefore a major route from sediment to biota. However, it is not the only route of contaminant uptake which is dependent on the life strategy of the biota under investigation and may include overlying water or ingestion of sediment particles. Whilst ingestion is not a factor for algae, diatoms and other algae are exposed to both pore and overlying water and alter their immediate environment through EPS production. This can affect the binding and concentration of metals and consequently their bioavailability (Decho, 2000). For example, the association of silver (Ag) with bacterial EPS appears to increase its accumulation by clams (Bryan and Langston, 1992), and may therefore affect the uptake to the algae itself.

The temporal and spatial changes in the conditions of the estuarine environment with tides and fluxes in fluvial inputs add an extra layer of complexity to the understanding of contaminant bioavailability in these locations. These issues will be addressed through a field study (Chapter 5) in which sampling was executed on a 100m grid to address the spatial variations which might occur due to position in the tidal frame. Sampling was carried out on two dates to enable discussion of temporal changes and address issues of seasonality.

2.5. Climate change

Climate change is predicted to have major impacts on estuaries and coastal seas including warming, sea level rise, increased frequency and/or intensity of storms and a decrease in salinity caused by greater freshwater input due to increased inland rainfall (Hewitt et al., 2016). These changes, alongside secondary impacts caused by human responses to climate change (i.e. changes to sedimentology due to sea defence structures) and other anthropogenic stressors such as increased nutrient inputs and contaminant loads, could have major impacts for shallow coastal ecosystems (Kennish et al., 2014). It has been suggested that, if we are to improve our understanding of the potential consequences of climate change for marine organisms, an increase in research effort is required (Wernberg et al., 2012).

Here the review focuses on the possible changes to temperature, storminess and sea level rise on coastal areas and potential effects to sediment, contaminants and benthic micro algae.

2.5.1. Temperature

Sediment surface temperature is affected by air temperature, solar radiation and sea water temperature with large variations occurring dependant on the season, tide and time of day (Guarini et al., 1997). Sediment temperature also influences the temperature of the water overlying the mudflat due to heat flux which is dependent on the temperature difference. Sediment, which is warmed by the sun, acts as a heat source to the water in the summer and, because it is cooled in the colder air, a sink in the winter (Kim et al., 2010, Rinehimer and Thomson, 2014).

Observed and climate change predicted sea surface temperature increases vary with location and greater increases are anticipated in northern seas, such as the Arctic, Barents and Nordic Seas, (due more pronounced warming at the poles and subsequent decline in sea ice cover which will increase heat absorption) and enclosed seas (due to their contact with warm land areas) (Philippart et al., 2011). In an investigation of changes in sea surface temperatures from 1957 to 2006 it was found that rapid warming occurred from 1982 to 2006 in the Subarctic Gyre and European and East Asian Seas, with a net increase in temperature of 1.35^{o}C in the North Sea and 0.72^{o}C on the Celtic-Biscay Shelf (Belkin, 2009). Predicted increases within the next 100 years range from 1.5 to 5^{o}C for the Celtic-Biscay Shelf (Philippart et al., 2011) and 2.6^{o}C for the Mediterranean (Somot et al., 2008).

Benthic diatoms therefore experience extreme short-term changes in temperature due to large variations between inundation and low tide during which the sediment surface is heated by insolation. The greatest variation in sediment temperatures occur within the top few centimetres (Guarini et al., 1998). Short-term temperature changes impact on photosynthesis which is temperature dependent (Blanchard et al., 1997). The optimum temperature for photosynthesis has been estimated at around 25^{o}C, whilst the maximum temperature at which photosynthesis can occur is about 38^{o}C (Blanchard et al., 1997). Whist benthic diatoms are able to withstand extreme short-term changes in temperature, it is uncertain if they could adapt to long-term temperature changes.

Temperature affects the rate of biological and chemical reactions and it is therefore expected that increasing temperatures will affect metal speciation and solubility and thus bioavailability (Hoffmann et al., 2012). However, there are conflicting reports in the literature concerning the effect of increased temperature on metal uptake from solution by microalgae with increased, decreased and no change all reported (Suresh Kumar et al., 2015). With respect to benthic

algae, warming has been observed to increase nutrient uptake (Alsterberg et al., 2011) and to have an antagonistic effect (reduction in negative effects) of toxicity of copper pyrithione (an antifouling substance) to a benthic community (Alsterberg and Sundbäck, 2013).

2.5.2. Sea level

Global sea levels are predicted to rise due to the transfer of water from the land to the oceans due to the melting of ice sheets and glaciers and expansion of the oceans due to warming. It has been predicted that the likely range of global sea level rise up to 2100 will be in the region of 0.55 to 1.20 m relative to pre-industrial levels (IPCC, 2013). Regionally changes in sea level will be more variable due to climatic variability; ocean currents; gravitational effects; and localised geological processes such as isostatic lift and tectonic plate movement. Additionally anthropogenic processes such as groundwater extraction can cause land subsidence, causing a relative rise in sea level. Sea level extremes, caused by storm surges and wave conditions in coincidence with large astronomical tides, have an increased probability of occurrence under conditions of rising mean sea level, increasing the threat of coastal flooding (Prime et al., 2016), studies of which have been a major focus of the ARCoES project.

There have been a wealth of recent articles discussing sea level rise and erosion of coastal wetlands and whilst it was previously expected that salt marshes would be resilient to sea level rise due to biophysical feedbacks (Simas et al., 2001, Morris et al., 2002) it is now postulated that salt marsh resilience will be lower than previously concluded due to effects such as pond erosion (Mariotti, 2016). Some reports maintain that biophysical feedbacks will enable coastal wetlands to resist sea level rise (Kirwan and Megonigal, 2013) but only if other anthropogenic pressures such as subsidence, eutrophication and reduced sediment availability are avoided, whilst others conclude that erosion will occur due to the combined effects of increased storm intensity, wind fetch, reduced sediment supply and sea level rise (Karimpour et al., 2016). A recent study modelling the impact of sea level rise in South San Francisco Bay predicted increased erosion threatening mud flats and salt marshes (van der Wegen et al., 2016).

Reworking and redistribution of historically contaminated sediment (section 2.2.2) is likely to occur if salt marsh areas are eroded creating a source of metals to estuaries and other coastal locations (Lee and Cundy, 2001, Spencer and O'Shea, 2014). Instances of increased contaminant input due to climate change induced erosion are already being reported (Beldowska et al., 2016). Implications of sea-level rise on the fate of contaminants and their interaction with the ecological dynamics of the estuarine system are poorly understood and require further study (Robins et al., 2016). For this reason it was identified as one of the aims of the ARCoES project.

With regard to the MPB, sea level rise will alter the tidal frame and, because spatial variation in MPB biomass is explained by emersion period and mud content (van der Wal et al., 2010), it is possible there would be reduction in MPB biomass due to loss of habitat.

2.5.3. Storminess

Projections for changes in storm frequency and intensity are extremely uncertain even in the near term (next 20 years) with a great deal of conflicting opinion due to bias in models and poor simulation of features such as ocean circulation and stratospheric dynamics (IPCC, 2013). Although some studies have reported an increase in storm numbers over the last few decades (Feser et al., 2015), others show this to be due to decadal variation and not a long term trend (Lozano et al., 2004). There are predictions for increased storm intensity (defined by higher wind speeds and/or lower atmospheric pressure) over the North Atlantic and Western Europe over the next 100 years (Lozano et al., 2004), but there is a lot of variation dependant on the model used (Feser et al., 2015).

A recent review of 58 studies (Mölter et al., 2016) suggests that there will be variability across the North Atlantic and Europe, with increases in frequency and intensity of storms to the North Atlantic south of 60^{o}N and Central and Western European regions and decreases to Southern Europe and the North Atlantic north of 60^{o}N. For Eastern and Northern Europe the same review indicated an increase in future storminess, but results were less certain than for other regions.

Increased storm frequency and/or intensity could increase rates of coastal erosion, especially in soft sedimentary and low lying areas (Lozano et al., 2004). In estuaries, in addition to contributing to salt marsh erosion due rising sea levels (see above) increased storminess could increase turbidity through disturbance and resuspension of sediment (Roberts, 2012) which could impact upon the MPB.

Disturbance changes chemical properties within the sediment which can cause contaminant mobilisation and have a significant effect on bioavailability (Eggleton and Thomas, 2004). When sediment is disturbed or resuspended into the water column the mixing of anoxic sediment with oxygenated water alters metal speciation and partitioning between the water and sediment increasing the bioavailability to sediment organisms such as the MPB (Atkinson et al., 2007). Disturbance can also increase microbial activity which mobilises metals from sulphide bound complexes (Caille et al., 2003). In a laboratory study of metal release from marine sediment, Atkinson et al. (2007) found that metals released due to physical disturbance of the sediment vary dependant on pH and returned to pre-disturbance concentrations within eight days. Processes of contaminant release from disturbed sediment are complex in that they are compound specific and highly dependent on sediment properties (Eggleton and Thomas, 2004)

and whilst there have been several laboratory studies on contaminant release (Kim et al., 2004, Atkinson et al., 2007) and ecological effects (Chapman, 2007) how this translates to the field remains unclear (Roberts, 2012).

In a recent study on the effect of storms on diatom assemblages (Potapova et al., 2016) it was observed that, post Hurricane Sandy (October 2012), there was an apparent increase in small centric planktonic species over benthic species corresponding with increased contaminant concentrations. It has also been observed that MPB biomass is reduced in a high wind/wave climate and that as a consequence increased storminess is likely to result in a large-scale decrease in MPB (van der Wal et al., 2010). There is the potential for a positive feedback in which reduced MPB biomass and associated reduction in EPS, reduces sediment stability (Defew et al., 2002, Tolhurst et al., 2003) contributing to further disturbance. Biofilm growth is determined by both the intensity and frequency of hydrodynamic disturbance, MPB persists over the neap-spring tidal cycle because there is sufficient time for biofilm development and substrate is stabilised and resuspension reduced (Mariotti and Fagherazzi, 2012), this supports the view that increased storm frequency and/or intensity would reduce MPB at their current locations but it is feasible that they will develop in alternative areas within the estuary especially if this corresponds to movement of fine grained sediment.

2.5.4. Summary

The climate change scenarios described above represent some of the interconnected pressures which may affect contaminant uptake by benthic algae. Some of these (e.g. future patterns of storminess) are still currently uncertain and the potential ecosystem impacts remain poorly understood. Climate change effects are likely to interact with additional related stressors such as increased contaminant and nutrient loads with which they could behave either synergistically or antagonistically (Hewitt et al., 2016). Although there has been much experimentation concerning the ecological effects of climate change over recent years, there is still much to do, particularly in areas investigating combined effects and increasing experimental realism (Wernberg et al., 2012).

Climate change effects on coastal areas and in particular the potential for flooding and erosion due to sea level rise is a major focus of ARCoES project of which this research forms a part. Effects of predicted increases in seawater temperature and storminess on metal uptake to benthic algae will be investigated as part of the mesocosm study (Chapter 4).

2.6. Contaminant uptake by benthic microalgae of the intertidal zone

It has long been established that heavy metals and other contaminants are taken up by algae from the water column making it an important factor influencing the fate and transport of contaminants in natural systems (Schmitt et al., 2001, He and Chen, 2014, Beldowska et al., 2015).

The major pathway for transfer of sediment-associated contaminants to organisms is direct ingestion of sediment during food consumption (Wang et al., 2000) and, in benthic infauna, their close association with the sediment (Ridgway et al., 2001, Adams and Stauber, 2004). As autotrophs benthic microalgae do not receive contaminants by sediment ingestion, but may still receive them through sediment association, possibly taking in contaminants alongside essential nutrients (Adams and Stauber, 2004).

Studies into contaminant uptake by microalgae are carried out for a range of purposes, these include: direct monitoring of contaminants in the environment (Snoeijs and Notter, 1993); understanding of contamination at the lowest trophic level (Stronkhorst et al., 1994); toxicology studies, particularly in the development of bioassays (Adams and Stauber, 2004, Araújo et al., 2010); the use of diatom communities as contaminant indicators (Cunningham et al., 2005); and the study of contaminant partitioning in ecosystems (Ferry et al., 2009). The contaminants studied are most often metals (including some studies of radionuclides (Snoeijs and Notter, 1993) and nanoparticles (Cleveland et al., 2012)), but there has also been some investigation of organic contaminants (e.g. PCBs (Stronkhorst et al., 1994) and compounds such as atrazine, a herbicide (DeLorenzo et al., 1999)). These studies have been carried out using a range of techniques and measure different outputs, dependent on the stated purpose. This review will attempt to synthesis the various methods by examining the objectives and findings of a range of studies with the aim of gaining an overview on the research into contaminant uptake by benthic microalgae, where there are gaps in this research and how the field can be advanced.

Although the main thrust of this review is contaminant uptake by benthic microalgae of the intertidal zone, there will be some diversion to studies involving planktonic algae and algae which are particular to either freshwater or marine locations. This has been necessary due to a paucity of studies focusing on the specified criteria, epipelic diatoms in estuarine locations. The majority of the research into contaminant uptake by primary producers in marine systems has focused on phytoplankton (Knauer and Martin, 1973, Luoma et al., 1998, Heldal et al., 2001, Stewart and Fisher, 2003). Stronkhorst et al. (1994) failed to identify any research into contamination of estuarine benthic diatoms and it would appear, based on a comprehensive review of the literature (using SCOPUS and Science Direct and a range of search terms related to diatoms, estuaries, sediments and contaminants), that there has been very little since that

time. Irving et al. (2009) also noted a lack of research into contaminant uptake by freshwater benthic algae.

2.6.1. Direct monitoring of contaminants in the environment

The uptake of radionuclide contaminants by benthic diatoms and the potential for their use as monitoring organisms was explored by Snoeijs and Notter (1993). In this study diatoms were regarded as intermediate between sediment and water, being more temporary than sediment; their short lifespan allows them to record recent contamination and, unlike water, there is no issue of mixing or dilution as they are reasonably stationary.

The study found there to be a significant relationship between discharges from the nuclear power station at Forsmark, Sweden and concentrations of radionuclides in diatoms. Additionally, the same report (Snoeijs and Notter, 1993) found high caesium concentrations in diatom samples following the Chernobyl accident (April 1986). The epilithic diatoms (those which grow attached to rock substrates) examined in the study were shown to respond quickly to changes in contaminant levels discharged from Forsmark. Uptake varied with distance from the discharge outlet and due to hydrological conditions (when water flow increased, contaminant concentrations in the water were diluted and concentrations in diatoms decreased) and, in the case of caesium fallout from Chernobyl, uptake was observed to increase with increasing water temperature (^{137}Cs uptake was twice as fast where the water temperature was 10^{o}C higher).

It was also observed that up to about 70 % of radionuclides analysed (^{54}Mn, ^{60}Co, ^{65}Zn and ^{110m}Ag) were recycled within the diatom community for a period of about three years (Snoeijs and Notter, 1993). This was thought to be due to the method of reproduction (cell division) and the persistence of the diatom community throughout the year. Surface area/volume ratios were found to be important; with the smaller diatoms adsorbing greater quantities of contaminants (this is also true for planktonic diatom species (Stewart and Fisher, 2003)). Colony size also appeared to have an effect with large blooms having the lowest contaminant concentrations.

The Forsmark study also made a comparison between radionuclide uptake by benthic macroalgae (*Fucus* ssp.) and the diatoms. Diatoms were found to uptake a greater quantity of contaminants, although how much more is uncertain as the macroalgae tend to be covered in epiphytic diatoms which were not removed prior to analysis.

Following this initial study benthic diatoms have been used to monitor radionuclide discharges from all four of Sweden's nuclear power plants (Snoeijs and Weckstrom, 2010).

The study by Snoeijs and Notter (1993) was limited, in relation to this research, in that it was only carried out on epilithic diatoms and was only concerned with uptake from the water

column. Additionally, these studies do not differentiate between contaminants bound externally to the diatom cell surface and intracellular uptake of contaminants.

2.6.2. Understanding of contamination at the lowest trophic level

Production by benthic algae is estimated to be about 10% of the world's marine primary production (Charpy-Roubaud and Sournia, 1990) and MPB is an important food resource in estuarine locations (Kanaya et al., 2008, McCormick et al., 2014, David et al., 2016). In light of these facts, Stronkhorst et al (1994) proposed that understanding the level of contamination in benthic diatoms was important in recognising the effects of contamination on growth rates; species composition; and in understanding the accumulation of toxicants in the food chain. The first and second of these have been taken up through the study of toxicology and the development of bioassays (Araújo et al., 2010) and tools such as the Trophic Diatom Index (TDI, discussed below) (Kelly and Whitton, 1995), with studies into accumulation of contaminants (using benthic microalgae as the starting point) still lacking.

Stronkhorst et al. (1994) recognised the problem of separating microalgae from the sediment and developed a technique by means of their natural migratory rhythm (based on an earlier method (Eaton and Moss, 1966)) to achieve this using lens tissue and nylon gauze. This was the basis for the investigation of uptake of a variety of contaminants (trace metals, PCBs and PAHs) to epipelic diatoms from intertidal sediment in the field. Based on this, the same sampling method forms the basis of the diatom collection in the field and laboratory within this study.

In a study, carried out at the Scheldt estuary, the Netherlands, Stronkhorst et al. (1994) found levels of zinc (Zn), chromium (Cr), cadmium (Cd) and copper (Cu) in benthic diatoms of to be similar to those reported for pelagic diatoms at the same location, and that levels were lower than those in the underlying sediment. It was speculated that this was due either to low bioavailability of the metals present, or to the short lifespan of diatoms (which would prevent them from reaching equilibrium with the pore water).

Absil and van Scheppingen (1996) followed up the study by Stronkhorst et al (1994) at the same location in order to adjust the technique to enable metals analysis to be carried out on diatom samples of just a few milligrams. They found a linear correlation between levels of lead (Pb) and Zn in the sediment and levels in diatoms (as contaminant levels in sediment increased, so did levels in the diatoms), but no relationship was found between Cu and Cd levels in sediment and the diatoms. Bioconcentration factors were found to be higher in sediments with lower metal concentrations which may suggest that the metal uptake is somehow limited. Dissolved metals in the pore water were found to be low due to anoxia which causes the

precipitation of metal sulphides, however it was suggested that oxidation during low tide may result in locally elevated bioavailability of metals (Absil and van Scheppingen, 1996). Concentrations were found to be in the same range as those reported by Stronkhorst et al. (1994).

To the best of my knowledge and confirmed by Stronkhorst (2013, pers. comm., 10th December), there are no further published follow ups to these two studies, however as they were carried out for a Dutch government department (the National Institute for Coastal and Marine Management, part of the Ministry of Transport, Public Works and Water Management) it is feasible that further work may have been undertaken, but has gone unpublished in the peer reviewed literature. An MSc thesis was produced by a student at Hochschule Zittau/Görlitz, in conjunction with University of Stirling, (Scherf, 2002) using similar methods to the above studies to investigate uptake of radionuclides from the sediment to the MPB. Of the three radionuclides investigated, they reported uptake of both caesium-137 and americium-241, but not potassium-40 to the MPB and observed possible effects of sediment organic matter content, particle size and water content on uptake. The influence of these sediment properties on uptake will be investigated further as part of this research.

In contrast to the scarcity of studies examining benthic diatoms, there are several studies that look at contaminant uptake by pelagic diatoms (Heldal et al., 2001, Stewart and Fisher, 2003, Deng et al., 2013). Studies by Stewart and Fisher (2003), on the accumulation of polonium-210, and Deng et al. (2013), which looked at mercury (Hg), distinguished between intracellular and surface bound contaminants, a division which has not, as far as I know, been attempted with epipelic diatoms. This is one of the reasons for the focus on these diatoms within this study.

2.6.3. Toxicology studies for the development of bioassays

There has recently been some research into toxicity testing of sediment using benthic diatoms (Adams and Stauber, 2004, Mauffret et al., 2010), this was summarised in a review by Araújo et al. (2010). Toxicity testing looks at the effect of the contaminants on the organism (in this case benthic diatoms) in terms of growth rates (Adams and Stauber, 2004), mortality etc., but there is seldom any measure of the uptake of contaminants by the study organism. Toxicity testing also tends to focus on the response of a single species, for benthic diatoms this is often *Cylindrotheca closterium* as opposed to investigation using natural assemblages.

Mauffret et al. (2010) investigated the toxicity of Linear Alkylbenzene Sulphonate (a surfactant) to the benthic diatom *C. closterium* in spiked sediment and a water only system. There were differences in toxicity to the diatom in sediment and water; growth rate inhibition was higher when sediment was present. These differences were attributed to the properties of the natural

sediment, particularly grain size, but also to the presence of autochthonous predators and diatoms and sediment nutrient content (Mauffret et al., 2010). Sediment particle size was previously implicated in growth inhibition by Moreno-Garrido et al. (2003). It was found that when the percentage silt in a sand substrate was increased growth was reduced so that optimum growth occurred on a substrate of 90% sand, 10% silt. This is despite the fact that a mud substrate is the natural habitat of the epipelic diatom (MacIntyre et al., 1996) which may point to discrepancies between laboratory studies and toxicology effects in the field.

Although, as previously noted, there are few studies of marine algae which take sediment into account and few toxicology studies that report uptake, a recent study by Atay et al. (2013) attempted both in an investigation of differences in toxicity of Cu and Zn to the marine diatom *Phaeodactylum tricornutum* with and without sediment. It was found that both growth inhibition and bioaccumulation were higher when sediment was present. However, in assessing the bioaccumulation in the presence of sediment there was no separation of the algae from the sediment prior to analysis. It is therefore impossible to ascertain whether bioaccumulation in the algae truly increased in the presence of sediment in this study.

Araújo et al. (2010) suggested that, despite being key in intertidal zones, very few toxicology studies are carried out using benthic diatoms (as opposed to larger benthic organisms) because the microorganisms are difficult to separate from other biota present within the sediment. Some toxicology studies (De Orte et al., 2014) have been carried out using sediment elutriate in order to avoid the problem of separation, but the validity of these methods has been questioned (Chapman et al., 2002). In addition to issues of separation there is the difficulty of carrying out abundance counts in the presence of sediment particles (Adams and Stauber, 2004). These difficulties may also be reasons that so few studies have investigated contaminant uptake by benthic microalgae.

2.6.4. Diatoms as contaminant indicators

MPB communities, of which the diatoms are a large component, are sensitive indicators of environmental change which often results in species shifts, a feature used in environmental monitoring. For example the TDI (Kelly and Whitton, 1995) which uses diatom community composition to monitor eutrophication in rivers and is one of the tools used to determine ecological status as part of the EU Water Framework Directive in the UK (Juettner et al., 2012).Uptake of contaminants is not measured as part of these monitoring procedures.

Studies into the effects of contaminants on benthic diatoms have been carried out in the field looking at the effects of heavy metals on community composition (Cunningham et al., 2003) and the potential for their use as indicators of contamination (Cunningham et al., 2005). These

studies are analogous to the TDI in that they investigate the use of benthic diatoms in assessing contamination of the sediment. Contaminant levels within the sediment are measured and compared with species composition; there is no measurement of contaminant within the diatoms.

2.6.5. The study of contaminant partitioning in ecosystems

There are several recent studies, mainly carried out by the US National Oceanic and Atmospheric Administration (NOAA), which explore the uptake of contaminants to benthic microalgae as part of ecosystem and food web investigations (Ferry et al., 2009, Cleveland et al., 2012). These studies employed the use of a modular estuarine mesocosm (Pennington et al., 2007) adding contaminants to the water element of the system and then measuring uptake by the various components (i.e. sediment, water and various biota).

These studies, using field collected sediment, have been used to study a range of contaminants such as gold (Ferry et al., 2009) and silver (Cleveland et al., 2012) nanoparticles, atrazine (DeLorenzo et al., 1999), a herbicide, and insecticides Endosulfan (Pennington et al., 2004) and Fipronil (Wirth et al., 2004), however they have been little used in the study of metals excluding nanoparticles and water is always the initially contaminated compartment.

2.6.6. Summary

Although it has long been recognised that microalgae take up contaminants, and the potential importance of this for ecosystem and human health has been acknowledged, there has been very little investigation of the potential for the uptake of contaminants from the sediment by the MPB. This is thought to be due in part to the difficulties involved in separating the algae from the sediment. However, by using the separation technique devised by Stronkhorst et al (1994) it should be possible to carry out studies in which the motile fraction of the MPB (primarily epipelic diatoms) can be successfully removed from the sediment. Consequently, using this separation technique in this study will enable the analysis of contaminant uptake to algae and comparison with concentrations in both the sediment and water under both field and laboratory conditions.

2.7. Conclusion

Whilst it is widely agreed that an understanding of contaminant uptake by MPB and the potential for these contaminants to move through the food chain is important in terms of both environmental and human health it has been shown that to date there has been very little work carried out in this area. This is surprising as there is considerable research investigating planktonic algae in this regard and some interest in the use of benthic microalgae in toxicology studies. It seems probable that this omission is due to the complexities of bioavailability of contaminants in the intertidal zone coupled with the difficulties of separating benthic microalgae from the sediment.

A review of the literature has confirmed that there are very few studies into contaminant uptake by epipelic diatoms. The importance of epipelic algae as the major component of the MPB acting to stabilise the sediment surface and forming a vital part of the estuarine food chain has been discussed. This highlights the significance of this research in beginning to better understand the mechanisms for contaminant uptake in a complex environment.

Previous studies have mostly investigate uptake of contaminants from the water column as opposed to the sediment. A comparison of uptake from these compartments will be addressed in the laboratory study discussed in Chapter 3.

Sediment properties such as organic matter content and particle size increase complexity in studies of contaminant uptake from sediment due to their potential effects on contaminant bioavailability. This will be addressed in the laboratory study (Chapter 3) through manipulation of the sediment to achieve two sediment treatments. It is also a factor in the field study (Chapter 5) in which comparisons are made with position in the tidal frame which causes natural variation in particle size due to the decrease in tidal energy with increasing height.

The majority of studies involving contaminant uptake by algae are carried out in either fresh or marine water, not more complex estuarine locations. This is addressed through the use of an intertidal mesocosm (Chapter 4), which introduces a tidal cycle to the sediment, and a field study (Chapter 5).

Climate change will impact estuarine areas in ways which, although uncertain, are likely to cause increased water temperatures and sediment disturbance which could influence bioavailability of metals as well as MPB growth. Research into effects of increasing water temperatures and sediment disturbance will be carried out through the use of an intertidal mesocosm and is reported in Chapter 4.

By utilising and refining a combination of equipment and techniques, such as following ecotoxicology methodology (Araújo et al., 2010) in Chapter 3 and using planktonic gauze for sediment/algae separation in the field (Chapter 5) as recommended by Stronkhorst et al. (1994), it will be possible to address some of these issues and gain fuller comprehension of contaminant uptake by epipelic diatoms.

3. Cadmium partitioning in the presence of the diatom *Cylindrotheca closterium*

3.1. Introduction

It is well known that algae have the capacity to take up contaminants from water (Chapter 2, Section 2.5). Phytoplankton, which has been well studied in this respect, accumulates trace metals to concentrations much higher than the surrounding water (Genter, 1996). The capacity of algae to remove metals from water is so well accepted that there has been considerable recent interest in its use for bioremediation, via the removal of metals from the water column (El-Sheekh et al., 2016, Das et al., 2016, Delrue et al., 2016). There has, however, (as discussed in Chapter 2, Section 2.5.2) been very little research involving benthic algae and, in particular, uptake of metals from sediment. It seems probable that the paucity of research in this area is due to the complexity of the bioavailability of metals in the intertidal zone (Chapman and Wang, 2001) coupled with difficulties in separating MPB from the sediment (Araújo and Moreno-Garrido, 2015).

It is understood from studies of the plankton that, as well as potentially removing metals from the dissolved to particulate fraction (de Souza Machado et al., 2016), decomposing algal cells may release metals to the water column (GESAMP, 1985). In addition, a fraction of the extracellular polymeric substances (EPS) produced by algae dissolves easily in water (Stal and de Brouwer, 2003), potentially releasing any associated metals to the water column. It is hypothesised, therefore, that (as stated in Chapter 1) alongside the potential to take up metals from the sediment, benthic algae may affect partitioning between water and sediment, remobilising metals from the sediment to the water column from where the bioavailability is increased. Algae can also alter their immediate environment, increasing oxygen levels and pH through photosynthesis (Yamamoto et al., 2008) which may cause mobilization of metals from sediment to the water column.

Benthic algae represents a significant position at the base of the estuarine food chain, providing an important food source for a wide range of organisms, ranging from infaunal deposit feeders (e.g. polychaetes, molluscs and crustacea) (Kanaya et al., 2008) to filter feeders (e.g. bivalves) when the mud flat is inundated (Yoshino et al., 2012) and shorebirds when it is exposed (McCormick et al., 2014). This makes benthic algae a potential contaminant exposure route for other organisms.

As a first step towards understanding contaminant uptake from sediment by benthic algae, the uptake of cadmium (Cd) to a single diatom species, *Cylindrotheca closterium* ((Ehrenberg) Lewin & Reimann (1964)), has been investigated through the use of a microcosm in a

controlled environment facility (CEF). Changes in Cd partitioning between water and sediment in the presence and absence of diatoms was also studied.

3.1.1. *Cylindrotheca closterium*

C. closterium is a pennate diatom described as tychopelagic (spending the major portion of its lifecycle as part of the benthos, but become part of the plankton due to physical processes) or meroplanktonic (spending part of their lifecycle in the plankton) in recognition of its part planktonic, part benthic existence (Round, 1981). The suspension of *C. closterium* into the water column and its ability to remain part of the plankton is due to its lightly silicified valves. Transportation out of the sediment and into the better lit water column at times of sediment inundation can increase productivity by extending the time during which photosynthesis can occur (Kingston, 2009). *C. closterium has been observed to have a* higher growth rate than other diatom species (Tanaka, 1984, Silva-Aciares and Riquelme, 2008). Of 10 diatom species *C. closterium* had the highest cell division rate (1.60 divisions day^{-1}), almost twice that of the next highest *Cocconeis scutellum* which had a growth rate of 0.86 divisions day^{-1} (Tanaka, 1984). 1.23 divisions day^{-1} were reported by Moreno-Garrido et al. (2003) for *C. closterium* grown on sand in an f2+Si media without EDTA. These facts and that it has long been grown in culture (Kingston, 2009) and used in toxicology studies (Araújo et al., 2010) make *C. closterium* a good candidate for use in laboratory studies of contaminant uptake from sediment.

It should be recognised that whilst algae collected from different locations and at different times may be morphologically similar enough to be identified as the same species, they may demonstrate physiological differences or ecological preferences (Brand et al., 2013). In culture collections different isolates are recognised as distinct strains and assigned a unique identifying number. Optimum culture conditions may differ, even for closely related strains (Brand et al., 2013). Utilising an identified culture strain improves the repeatability of laboratory experiments, in that the algae would be expected to respond in the same way to the same conditions.

Whilst there are advantages to using a species of diatom that can be easily grown in laboratory culture and has been used in previous toxicology studies, the potential issues should also be noted. Laboratory isolates may undergo physiological changes, which may mean they respond differently to their counterparts in the field. Algae cultures grown in laboratory conditions have been observed to have a smaller cell size and changes in pigment composition occur as the algae adjusts to lower light levels by increasing photosynthetic capacity (Defew et al., 2002). Motility has also been observed to decrease with culture age (Kingston, 2009), which could present difficulties in certain applications.

3.1.2. Cadmium (Cd)

Cd is present in the environment from anthropogenic sources as a result of mining, smelting and industrial use (Maret and Moulis, 2013). A recent study of the sediments of the Seine estuary (Hamzeh et al. 2016) found Cd concentrations in the sediment ranging from 1 mg kg^{-1} to 6 mg kg^{-1} with variation occurring seasonally and with distance upstream. The apparent effects threshold (AET) for Cd in marine sediment is 3 mg kg^{-1} (Buchman, 2008) (Chapter 5, Table 5.9). However, in growth inhibition tests with *C. closterium*, Moreno-Garrido et al. (2003) found an EC_{50} value of 79 mg kg^{-1} for Cd, suggesting diatoms have a much higher tolerance than other organisms.

It has been suggested (Moreno-Garrido et al., 2003) that uptake of Cd by microalgae may cause bioaccumulation and biomagnification through the food chain. Additionally, as the excretion of Cd is slow and it could accumulate in the livers and kidneys of birds and mammals (Moreno-Garrido et al., 2003). Cd is also known to be damaging to the kidneys and affects bone metabolism and the cardiovascular system of mammals (Maret and Moulis, 2013).

Cd uptake and toxicity was previously investigated to the freshwater diatom *Navicula pelliculosa* by Irving et al (2009) and the freshwater green algae *Pseudokirchneriella subcapita* by Paquet et al (2015). Uptake and toxicity of Cd to three species of marine microalgae has also previously been investigated (Wang and Wang, 2009). All of these studies involved uptake from water only.

This study investigates two main questions. Firstly, how is Cd uptake to *C. closterium* affected by initially contaminated compartment (water or sediment) and sediment properties? Secondly, what effect does the presence of *C. closterium* have within the system in terms of Cd concentrations in all compartments?

Hypotheses

- *Cd concentration in C. closterium is greater when overlying water, as opposed to sediment, is the initially contaminated compartment*
- *Cd concentration in C. closterium increases with time*
- *Cd concentration in C. closterium is greater when sediment has lower organic matter content*
- *There is both extracellular and intracellular uptake of Cd by C. closterium from both the sediment and the overlying water*

- *Sediment Cd concentration is reduced in the presence of C. closterium*
- *Cd concentration in the overlying water increase in the presence of C. closterium*
- *There is uptake of Cd from sediment to diatoms*

3.2. Methods

3.2.1. Water preparation

Artificial sea water was prepared by adding synthetic sea salt, Tropic Marin® (Wartenberg, Germany) to Milli-Q® water to a salinity of 22. For chemical composition see Atkinson and Bingman (1996). This was used as the base for both the growth media used to culture *C. closterium* prior to the experiment and "light" media used during the experimental phase. All growth (f/2+Si, see Appendix 3-1 for details) and experimental media were autoclaved and allowed to stabilise for a minimum of two days prior to addition of filter sterilised vitamins (vitamins are destroyed by heating) and subsequent use.

During the experimental phase a reduced Ethylenediaminetetraacetic acid (EDTA) f/2+Si media (f/2+Si media without the addition of trace metals stock, see Appendix 3-1 for details), hereafter referred to as light media, was used to avoid chelating effects of EDTA on Cd. Satisfactory growth of control cultures of *C. closterium* during 96 hours in this media was checked prior to the experiment.

Experimental contamination of water

A 10 mg L^{-1} Cd stock solution (anhydrous $CdCl_2$; Sigma Chemical Co., St Louis, MO, USA) was made up in 22 salinity artificial seawater. This was used to spike the water and sediments.

6.75 ml of Cd stock solution was made up to 1L with the addition of light media, resulting in a final contamination level of 11.280 ± 0.083 mg L^{-1}. Water pH was adjusted to pH 8.2±0.2 with the addition of 1M NaOH solution.

3.2.2. Sediment preparation

Surface (top 50mm) intertidal sediment was obtained in September 2014 from Loch Fleet (a tidal basin), Scotland (57° 56'N, 4° 2'W) (Fig. 3.1) using a flat shovel and stored in the dark at 4°C prior to processing. It was assumed that this sediment would be low in pollutants, due to the site location (remote from any industrial activities in an area with a relatively low human population) and its designations as a RAMSAR site (for intertidal mudflats and sandflats); a Site

of Special Scientific Interest (SSSI); a Special Area of Conservation (SAC); and a Special Protection Area (SPA). This was confirmed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis which showed Loch Fleet sediments to have a background Cd concentration of 0.11±0.01 mg kg^{-1}. Additionally, six replicate samples were analysed to check for other potential metal contaminants. Nothing was found of concern, for example the range of concentrations for aluminium (Al), iron (Fe), copper (Cu), zinc (Zn) and silver (Ag) were as follows: Al, 1.15 to 3.42 mg kg^{-1}; Fe, 2.71 to 12.64 mg kg^{-1}; Cu, 0.016 to 0.021 mg kg^{-1}; Zn, 0.012 to 0.030 mg kg^{-1}; and Ag, 3.63 to 7.77 µg kg^{-1}. These were all well below SQUIRT (Screening Quick Reference Table) (Buchman, 2008) effects levels in marine sediment (Chapter 5, Table 5.9). Permission to access the site and remove sediment was obtained in advance through discussion with Scottish Natural Heritage (SNH) who manage the site as a National Nature Reserve (NNR) in partnership with the land owner, Sutherland Estates and the Scottish Wildlife Trust (SWT).

All sediment was sieved to 2mm to remove macrobenthos and homogenised. Half of the sediment (hereafter referred to as unprocessed) received no further treatment and was stored at 4°C in the dark prior to use. The other half (hereafter referred to as processed) was autoclaved (121 °C, 20 minutes) to destroy any remaining meiobenthos and microbiota. It was then washed by mixing distilled water through the sediment for 15 minutes at a ratio of about 10:1 water to sediment. The sediment was then left in the water for 8 hours before draining. This was repeating twice over a 24-hour period following which the sediment was dried for 18 hours at 105 °C. Processed sediment was stored dry and at room temperature prior to rewetting (see below) for use.

Figure 3.1. Loch Fleet, Scotland, 57°56'N, 4°2'W, sediment collection location marked in yellow. Map from Google Maps

Experimental contamination of sediment

A previous study (Moreno-Garrido et al., 2003) showed little growth inhibition of *C. closterium* with Cd concentrations of up to 20 mg kg^{-1}. A sediment contamination level of 10 mg kg^{-1} was therefore selected to ensure growth and survival of the diatoms for the duration of the experiment, whilst still allowing for easy measurement of Cd.

Following recommendations of Simpson et al. (2004) all sediment manipulations were carried out in a glove box filled with oxygen free nitrogen (OFN). Spiked sediments were prepared in 1L plastic bottles and mixed initially using a glass rod (for five minutes) followed by agitation for 12 hours on a mechanical shaker. Sediments were equilibrated at room temperature (18 – 21 °C) in the preparation bottle while stored in an oxygen free atmosphere for a minimum of 40 days (Simpson et al., 2004) to allow equilibration of Cd between sediment and pore water. To create the spiked sediments 6.75 ml of the same Cd stock solution used to contaminate the water was either made up to 375 ml with artificial seawater (salinity of 15) and added to 1 kg dry processed sediment or made up to 63.25 ml with Milli-Q® water and added to 1131.75 g wet unprocessed sediment, to achieve an 8:3 sediment water ratio. This resulted in a final contamination level of 10.31±0.12 mg kg^{-1} for both sediment types, with an interstitial water of 22 salinity.

For uncontaminated sediments 375 ml artificial seawater (15 salinity) was added to 1 kg dry processed sediment and 63.25 ml Milli-Q® water was added to 1131.75 g wet unprocessed sediment. Uncontaminated sediments were treated in the same manner as contaminated sediments with respect to containers, storage and mixing. Uncontaminated sediments had a final contamination level of 0.11 ±0.01 mg kg^{-1} (the background level in Loch Fleet), with an interstitial water of 22 salinity.

Both contaminated and uncontaminated portions of sediment were monitored for pH during equilibration period and at the start of the experiment. Sediment pH was adjusted to pH 8±0.2 with the addition of 1M NaOH solution directly following contamination following the recommendations of Hutchins et al. (2007), and was then monitored and further adjusted over the following three days.

3.2.3. Organisms and culture conditions

A strain of *C. closterium* ((Ehrenberg) Lewin & Reimann (1964)) isolated from Loch Roag, Scotland in 2004 was purchased from CCAP (Culture Collection of Algae and Protozoa). Cells were cultivated in f/2 media (Guillard and Ryther, 1962) enriched with silicon dioxide (SiO_2) (f/2+Si) with a base of artificial sea water (Tropic Marin®) of 22 salinity (see Appendix 3-1 for details). Diatoms were cultivated on a substratum of purified sea sand (Fisher Scientific) in 250 ml and 500 ml glass Erlenmeyer flasks at 15°C. Cultures were illuminated under a 12/12-hour light/dark cycle at an irradiance of 60µmol photons $m^{-2}s^{-1}$ in the controlled environment facility (CEF) at University of Stirling for a minimum of six weeks prior to the experiments being carried out (Fig. 3.2). During the culturing period flasks were swirled gently every second day to ensure mixing of the nutrients. Media was renewed every four days, by adding 30 ml of existing culture to a fresh media. Cultures were checked for continued motility and bacteria every eight days by microscope observation.

Diatom cells were harvested for experimental use during the exponential growth phase (three day old cultures). To harvest cells the cultures were gently shaken by hand until all cells were in suspension. The sand was then allowed to sediment out for approximately 10 seconds and the cell suspension was poured off.

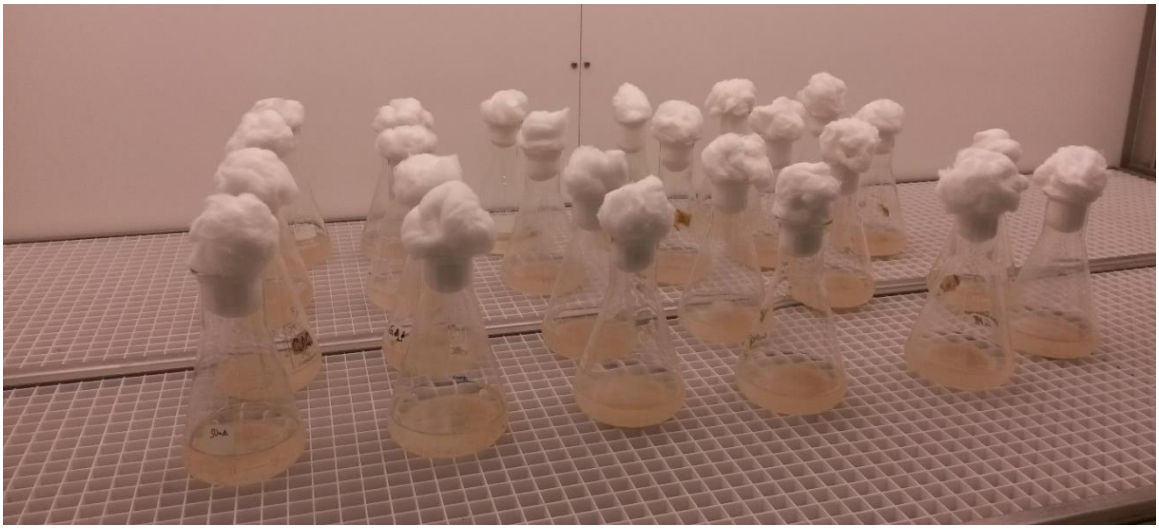


Figure 3.2. Cylindrotheca closterium cultures in controlled growth facility (CEF) at University of Stirling

3.2.4. Experiment setup

Experimental treatments (Fig. 3.3) were run in triplicate, two containing a *C. closterium* culture (enabling measurement of both total and internal contaminant uptake) and one without diatoms.

Experiments were run in 250 ml circular plastic pots. Pots for treatments which included sediment were filled to a depth of 8 mm with either processed or unprocessed sediment, which was either contaminated or uncontaminated. Pots for treatments without diatoms had with 100 ml light media added, treatments with diatoms had 70 ml light media added and were topped up with 30 ml of *C. closterium* culture in growth media at a cell density of 10^6 cells ml^{-1}. Cell density of the cultures was checked prior to use by carrying out counts under a microscope using a haemocytometer.

The experiment ran for a total period of 96 hours (following U.S. Environmental Protection Agency guidelines for toxicity tests (USEPA, 2002)) in the CEF illuminated under a 12/12-hour light/dark cycle at an irradiance of 60 µmol photons m^{-2} s^{-1} at 15 ^oC, with the pots randomly distributed (Fig. 3.4). Sampling was carried out at three, 24 and 96 hours. The experiment was repeated three times for replication purposes. Replication had to be carried out consecutively as each replication generated 72 pots and it was not possible to process three times the number of diatom samples within the required 24-hour period after sampling.

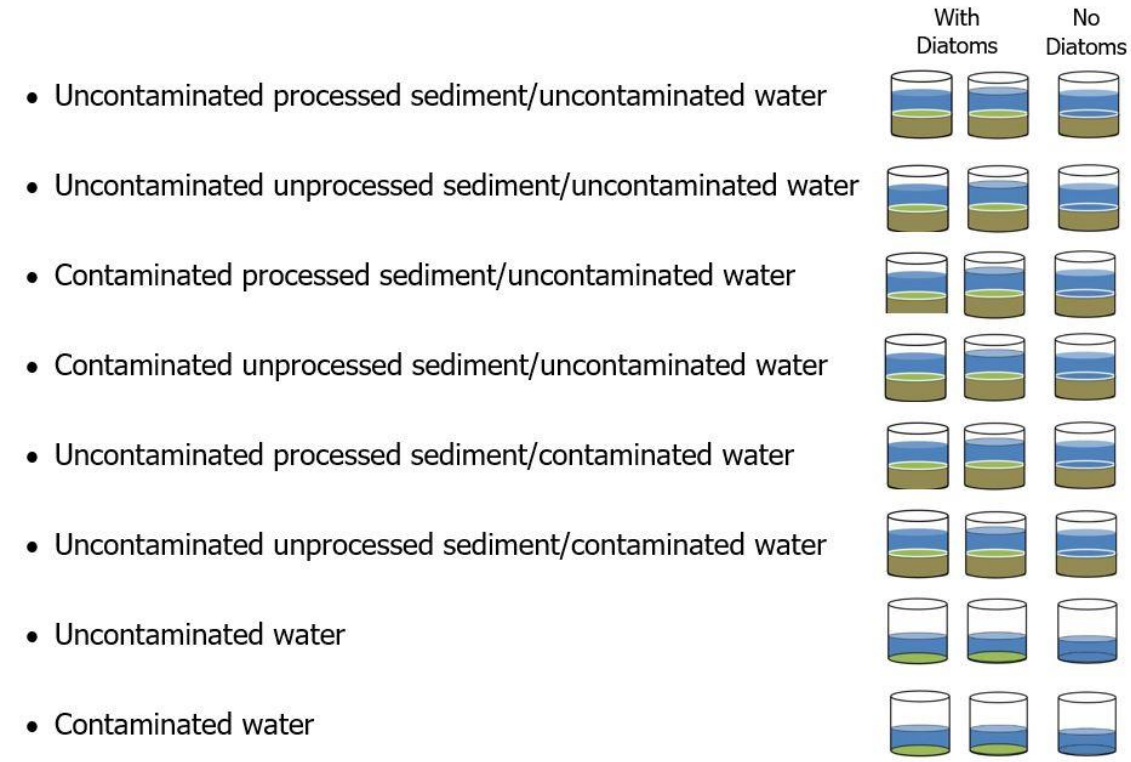


Figure 3.3. Experimental treatments

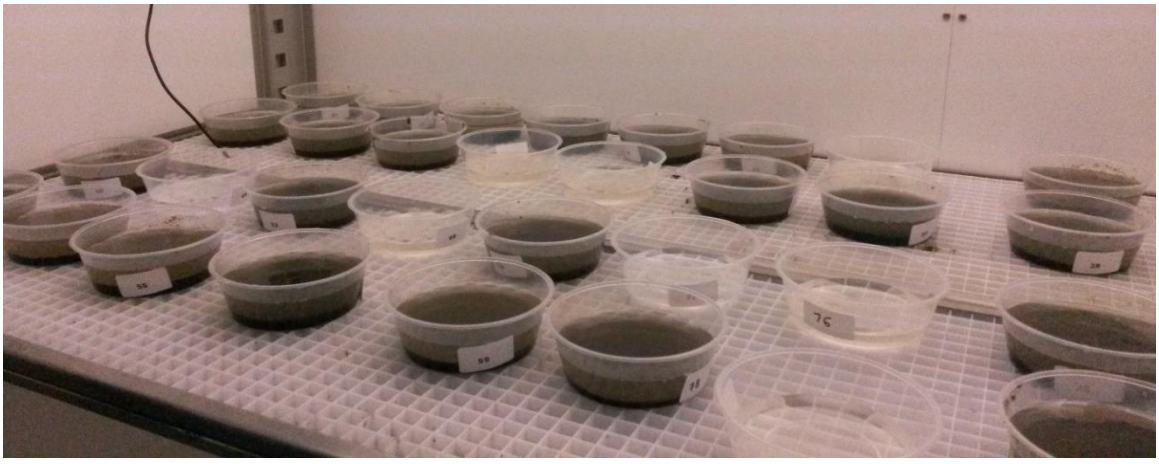


Figure 3.4. Experiment in controlled environment facility (CEF) at University of Stirling

3.2.5. Sampling

At the end of each time period 24 containers were removed from the controlled environment chamber. The components (overlying water, diatoms, pore water and sediment) were then separated out for further processing and analysis.

Overlying water

A vacuum pump was fitted with a pipette tip to enable the overlying water in containers with sediment to be siphoned off to the level of the top of the sediment. Clean tubing was used for each container and the water samples were retained for further analysis. Where no sediment was present water was poured directly into 50 ml centrifuge tubes, which were spun at 180 relative centrifugal force (RCF) for 7 minutes to separate diatoms and water samples. Water samples were decanted to acid washed plastic bottles and stored at 4^{o}C until further processing which was carried out within 24 hours.

Diatoms

Following the removal of overlying water from containers which had both sediment and diatoms present, two circular pieces of lens tissue (Whatman) were placed on top of the sediment. The lower piece completely covered the sediment surface, acting as a barrier to potential sediment contamination of the upper lens tissue layer. The second, slightly smaller circle, acted to trap diatoms which move towards the light and onto the surface of the lens tissue. This method of removing diatoms from the sediment surface is known as the lens tissue method (Eaton and Moss, 1966). All containers were returned to the controlled growth chamber (under the same conditions as previously described) for a further 15 hours to allow the diatoms to migrate to the surface lens tissue.

To ensure migration of the diatoms into the lens tissue to enable their separation from the sediment it was necessary to make sure that there was good contact between the bottom sheet of tissue and the sediment surface and between the two layers of lens tissue. To achieve this each layer was smoothed on to the surface using forceps to push out any air bubbles. It was also necessary that the sediment surface and lens tissue remained damp throughout the process, as one function of diatom migration is avoidance of desiccation. This was achieved by leaving approximately 0.5 mm of overlying water on the sediment surface, sufficient to soak into the lens tissue and remain damp for the required 15-hour period.

Prior to carrying out the laboratory experiment a trial was carried out to compare collection of diatoms using different materials, namely lens tissue and nylon mesh (80 µm pore size,

Normesh, UK). It was found that whilst the nylon mesh was easier to manipulate (particularly when washing diatoms from it), it dried out more quickly and did not make as good a contact with the sediment surface, and therefore a lower mass of algae was collected. For the laboratory scale experiment the lens tissue was selected as the more appropriate material.

Following this 15-hour period (12 hours in the dark followed by three in the light to encourage migration to the sediment surface), the upper lens tissues were removed to 50 ml centrifuge tubes containing 30 ml saline (22) water. Lower lens tissues, which were used to prevent contamination of the diatom sample with sediment, were removed and discarded.

Centrifuge tubes containing the upper lens tissues were agitated by hand for one minute, following which the lens tissue was removed to a plastic weighing boat. The lens tissue was then washed down using a wash bottle filled with saline water to remove diatoms attached to the lens tissue into the centrifuge tube. The lens tissue was then turned over and the process repeated until there was 100 ml of saline water (with diatoms) in two 50 ml centrifuge tubes. The use of weigh boats gave rigidity to the lens tissue during washing. A new weigh boat was used for each sample.

Diatoms were centrifuged at 180 RCF for seven minutes, following which they were left to settle for 10 minutes before the supernatant was removed by pipette until there was 5 ml remaining in each tube, these were then combined to give a single 10 ml diatom sample from each container.

There were a pair of diatom samples for each experiment set up to enable measurement of both internal and total Cd (Fig. 3.3). To achieve a similar mass of algae for internal and total Cd analysis these paired samples were combined and separated into two clean 50 ml centrifuge tubes (Fig. 3.5).

Pore water

Pore water was extracted from sediment by removing all the sediment from each container to two 50 ml centrifuge tubes. These were spun at 3100 RCF for 20 minutes in a refrigerated centrifuge at 4^{o}C. Pore water was removed from the surface by pipetting and the sample from both tubes was combined in an acid washed plastic sample bottle. Pore water samples were stored at 4^{o}C for further processing, which was carried out within 24 hours.

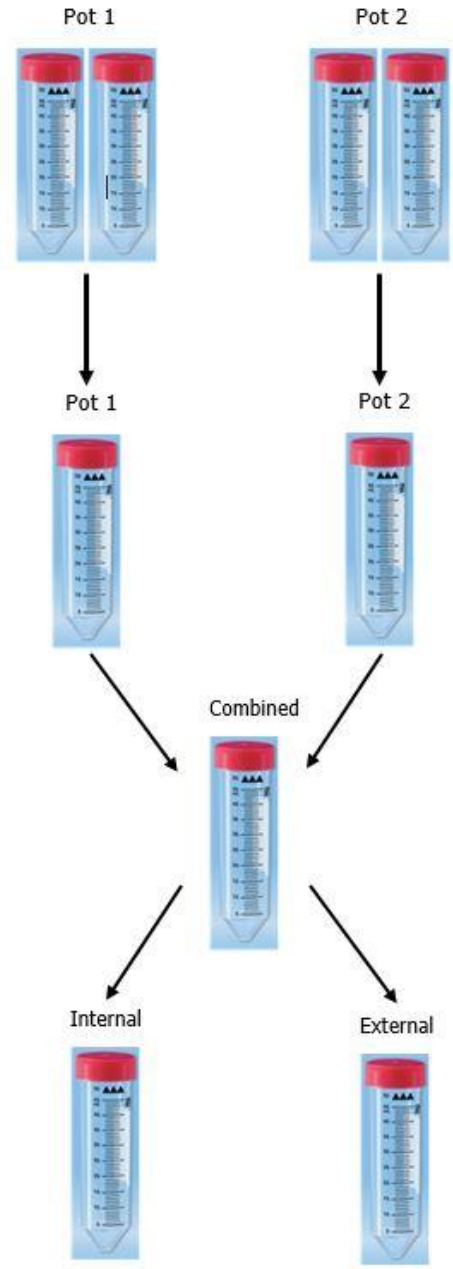


Figure 3.5. Schematic of diatom washing and combination for each paired sample

Sediment

Following the removal of the pore water sediment was dried at 105°C for 24 hours and separated into aliquots for determination of Cd concentration, organic matter content and particle size distribution.

3.2.6. Sample processing

A flow chart of the various steps in sample preparation for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis is shown in Figure. 3.6.

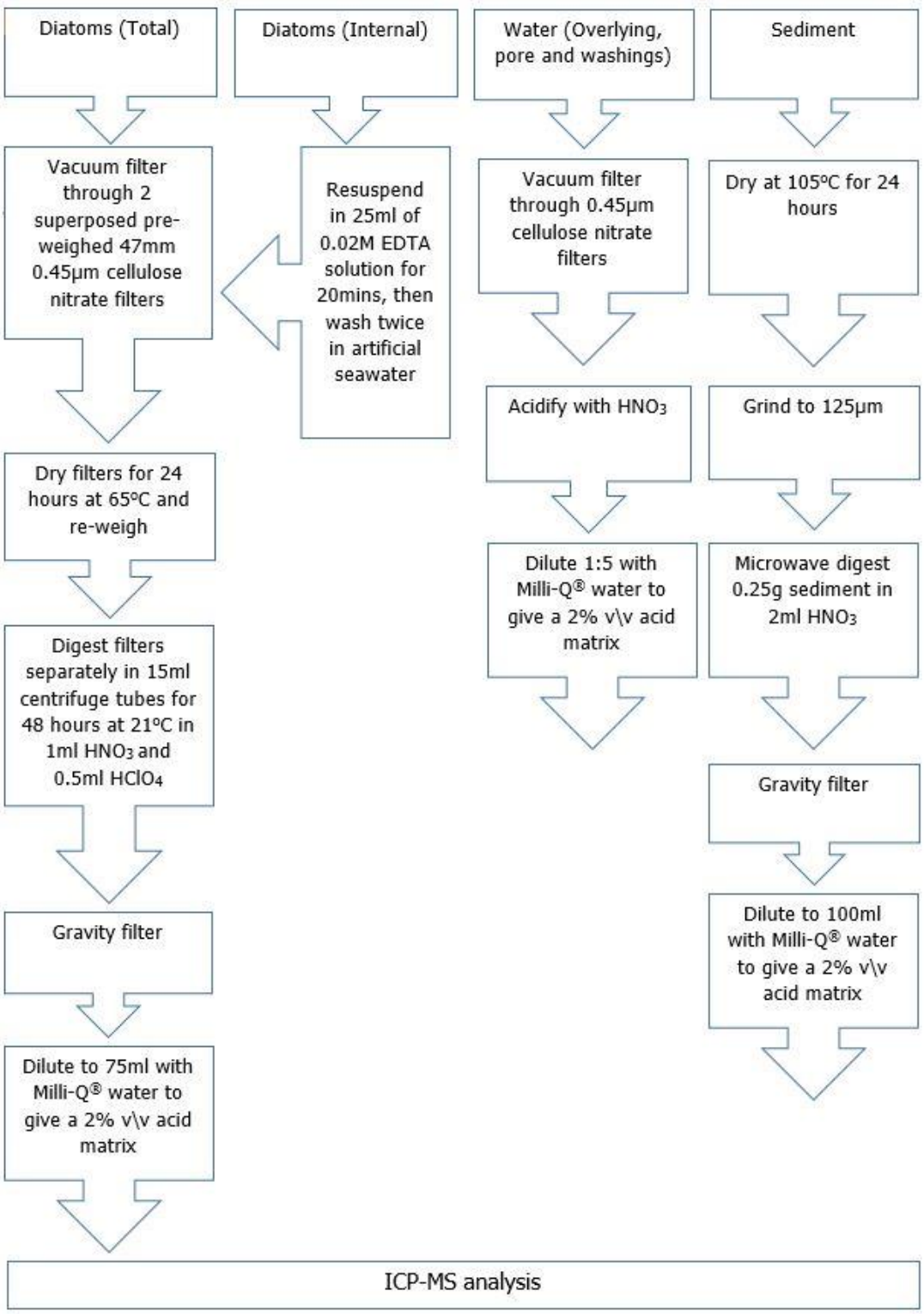


Figure 3.6. Sample processing flow chart

Determination of Cd uptake and adsorption by the diatoms

Determination of Cd uptake (intracellular) and adsorption (external) followed the method set out by Perez-Rama et al (2010).

Total Cd was determined by filtration of the suspended sample through two pre-weighed superposed 47 mm, 0.45 µm cellulose nitrate filters (Whatman™). Filters were dried at 65 ^{o}C for 24 hours and reweighed to determine diatom mass. Filters were then separately digested for 48 hours with 1 ml nitric acid (HNO_3) (70% w/w) and 0.5 ml Perchloric acid ($HClO_4$) (72 % w/w). Resultant samples were diluted to 50 ml, gravity filtered and made up to 75 ml with Milli-Q® water to give a 2 % v/v acid matrix. Cd was measured on both filters using IICP-MS. The lower filter acted as a blank.

Intracellular Cd was measured by resuspending the diatom pellet in 25 ml of 0.02M EDTA solution made up in artificial seawater (of 22 salinity) for 20 minutes. Cells were centrifuged and washed twice in artificial seawater and the resulting suspension was digested as for total Cd. Washing in EDTA removes Cd adsorbed to the surface of the diatoms allowing intracellular Cd to be measured (Pérez-Rama et al., 2010). Supernatant from the EDTA wash was retained for further analysis.

Cd adsorbed to the outside of the diatom cell was calculated by subtracting the intracellular concentration from the total Cd concentration (Eq. 3.1).

Eq. 3.1. External Cd_{Alg} (mg kg^{-1}) = Total Cd_{Alg} (mg kg^{-1}) – Internal Cd_{Alg} (mg kg^{-1})

Determination of Cd concentrations in sediment

Sediment aliquots for Cd concentration analysis were ground to 125µm with a pestle and mortar and 0.25g was weighed into Teflon™ tubes to which 2 ml HNO_3 (70% w/w) was added prior to microwave digestion. Samples were gravity filtered and diluted to 100 ml with Milli-Q® water to give a 2% v/v acid matrix in preparation for analysis by ICP-MS.

Determination of Cd concentrations in water

All water samples (overlying water, pore water and lens tissue and EDTA washings) were treated in the same manner. Samples were filtered through a 47mm 0.45µm cellulose nitrate filters diluted to 1:5 with Milli-Q® water and acidified to 2% v/v with concentrated HNO_3 (70% w/w) in preparation for analysis by ICP-MS.

ICP-MS analysis

Algae, sediment, pore and overlying water samples were analysed for Cd using a XSERIES 2 ICP-MS (Thermo Scientific, Germany) using collision cell technology (CCT) to reduce potential polyatomic interferences. Multi element standards (Merck, Germany) certified by the National Institute of Standards and Technology (NIST), internal standards (scandium (Sc) and rhodium (Rh)) and blanks were included in each run. All samples were run in triplicate and reference samples included in each run to check accuracy and consistency. The instrumental limit of detection (LoD) for Cd is given in Appendix 5-3.

Sediment particle size

Twenty percent of the sediment samples from both sediment types (a total of 32 samples) were analysed for particle size. This number gave a representative random selection from each sediment type and was sufficient for statistical analysis. Sediment samples were homogenised and added to 50 ml plastic sample bottles to a depth of approximately 0.5cm and topped up to 1.5cm with distilled water. 2 ml of dispersant sodium hexametaphosphate, $(NaPO_3)_6$, was added to aid deflocculation and samples were agitated mechanically for 30 minutes.

A Coulter LS 230 laser granulometer was used to determine particle sizes from 2 to 2000µm within each sample. A magnetic stirrer was used to create a vortex ensuring a representative sample was obtained. Five measurements were made of each sample and the mean used. Particle sizes were classified according to the Udden – Wentworth classification scheme (Wentworth, 1922) for grain size and are given as a percentage volume frequency.

Sediment organic content

Twenty percent of the sediment samples randomly selected from both sediment types (a total of 32 samples) were analysed for organic content using the loss on ignition (LOI) method.

Approximately 2g of dry sediment was accurately weighed into pre-weighed crucibles and further dried for two hours at 105^{o}C to ensure a constant dry weight. Samples in crucibles were then reweighed before ignition in a muffle furnace (450^{o}C for 8 hours). Samples were cooled in a desiccator for one hour before reweighing and percentage organic matter was calculated as per equation 3.2.

Eq. 3.2. Organic matter (%) = 100 - (((sediment + crucible after ignition) – crucible (g)/ (sediment + crucible before ignition) – crucible (g)) x 100)

3.2.7. Bioconcentration and concentration factors

The bioconcentration factor (BCF) and concentration factor (CF) were applied to describe the Cd distribution between compartments. BCF was calculated from the total Cd accumulated by the algae as follows:

Eq. 3.3. $BCF_{Sed} = Cd_{Alg}$ (mg kg^{-1}) / Cd_{Sed} (mg kg^{-1})

Eq. 3.4. $BCF_{Wat} = Cd_{Alg}$ (mg kg^{-1}) / Cd_{Wat} (mg L^{-1})

Where C_{Alg} is Cd concentration in *C. closterium*, C_{Sed} is concentration in sediment and C_{Wat} is concentration in overlying water.

CF was calculated from the Cd accumulated by the sediment or water as follows:

Eq. 3.5. $CF_{Sed} = Cd_{Sed}$ (mg kg^{-1}) / Cd_{Wat} (mg L^{-1})

Eq. 3.6. $CF_{Wat} = Cd_{Wat}$ (mg L^{-1}) / Cd_{Sed} (mg kg^{-1})

3.2.8. Statistical analysis

All statistical analysis was carried out using R (3.2.3) in RStudio. The lme4 package (Bates et al., 2015) was used to perform linear mixed effects analysis of the relationships between Cd concentration (in diatoms, sediment and water) and the experiment treatments. As fixed effects contaminated compartment, time, sediment type and diatom presence (without interaction terms) were entered into the model. As a random effect there was an intercept for the effect of experiment run (equation 3.7).

Eq. 3.7. CdPPM ~ Contamination + Sediment + Time + (1|Run)Deviations from homoscedasticity and normality were checked by visual inspection of the residual plots. P-values and Chi-Square values were calculated using likelihood ratio tests of the full model with the effect in question against the model without the effect in question and are reported to two significant figures. A sample model output is provided in Appendix 3-2.

3.3. Results

3.3.1. Sediment properties (pre-experiment)

Sediment properties prior to the start of the experiment are shown in Table 3.1. Prior to contamination of sediments for use, processed sediment was found to be slightly coarser with a lower percentage of particles in the clay (<4μm) to fine silt size (8 - 16μm) classifications and a higher percentage in the medium silt (16 - 32μm) to fine sand (128 - 256μm) classes than unprocessed sediment. Processed sediment also had a lower percentage organic matter content. Cd concentration in the sediments (prior to addition of Cd stock solution) was slightly higher in the unprocessed sediment.

Table 3.1. Properties and cadmium concentration of sediments prior to prior to the experiment and addition of cadmium (values given as mean±standard deviation, n=3)

Sediment	Processed	Unprocessed
Particle size (%)		
Clay (<4μm)	4.40±0.42	5.18±0.19
Very fine silt (4 - 8μm)	3.75±0.45	4.36±0.26
Fine silt (8 - 16μm)	5.93±0.76	6.38±0.10
Medium silt (16 - 32μm)	8.05±1.01	7.86±0.03
Coarse silt (32 - 64μm)	11.10±1.03	9.79±0.31
Very fine sand (64 - 128μm)	9.71±0.76	8.94±0.13
Fine sand (128 - 256μm)	42.01±2.57	40.84±0.91
Medium sand (256 - 512μm)	15.05±1.88	16.67±0.13
Coarse sand (512 - 1024μm)	0.00±0.00	0.00±0.00
Very coarse sand (1024 - 2000μm)	0.00±0.00	0.00±0.00
Water in wet sediment (%)	N/A	22.70±0.07
Organic matter (%)	1.20±0.09	1.54±0.10
Cadmium ($mgkg^{-1}$)	0.10±0.04	0.12±0.03

3.3.2. Sediment organic matter

At the end of the experimental period both sediment types, processed and unprocessed, had very low organic matter content. Mean organic matter content of the processed sediment was 1.04±0.04%, 0.41% lower than the unprocessed sediment at 1.45±0.06% ($p = 2.61*10^{-7}$, Table 3.2, Fig. 3.7). This difference is probably due to the sediment processing, organic matter is likely to have been flushed out of the sediment during the washing phase of the process.

Table 3.2. Analysis of Variance (ANOVA) table of comparison of sediment organic matter at the end of the experiment, n=26, $p=2.61*10^{-7}$

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio
Sediment type	1.092	1	1.092	49.99
Residuals	0.524	24	$S^2=0.0218$	

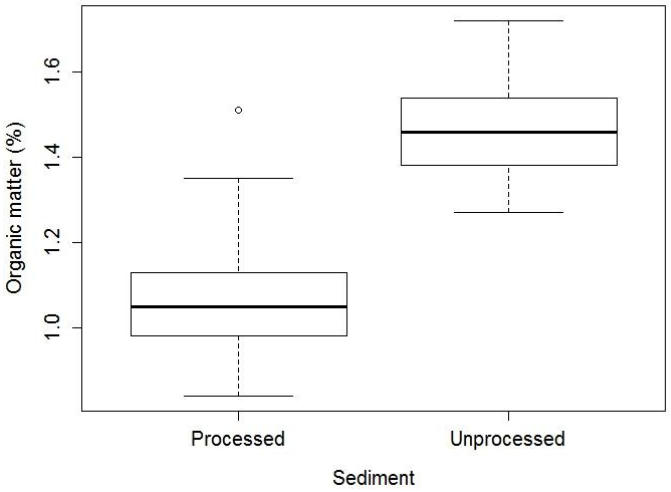


Figure 3.7. Percentage organic matter in processed and unprocessed sediment following experiment, n=32, outliers (◦) calculated as Q1-1.5IQR and Q3+1.5IQR

3.3.3. Sediment particle size

Both sediment types have the highest percentage of particle size in the fine sand bracket (processed sediment 45.46±3.40 % and unprocessed 35.40±6.02 %), neither had particles greater than 512 µm (medium sand). Unprocessed sediment has a greater percentage of particles in the smaller size fractions, clay ($p=7.4*10^{-3}$) and silt ($p=1.1*10^{-4}$), whilst the processed sediment has a higher percentage of particles in the sand size fraction ($p=1.3*10^{-4}$, Fig. 3.8). As with the organic matter, these differences are likely to be due to the sediment processing with smaller particles being lost during the washing and drying of the processed sediment.

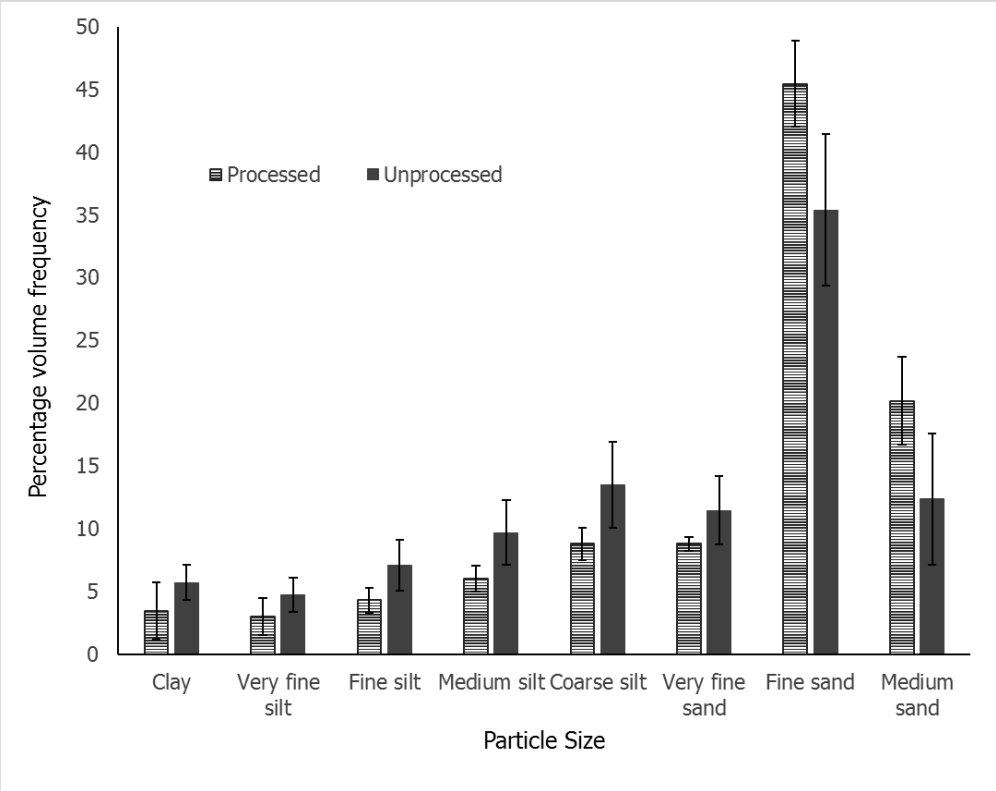


Figure 3.8. Comparison of sediment particle size in processed and unprocessed sediments at the end of the experiment as percentage volume frequency classified according to Udden – Wentworth scale, n=32

3.3.4. Algal growth

Mass of algae retrieved was used as a proxy for algal growth. The only set up in which there was consistent growth of *C. closterium* over the 96-hour experimental period was that in which water was uncontaminated and there was no sediment present (Fig. 3.9 (c)). The average dry weight of algae recovered from this set up increased from 4.46±0.96 mg after 3 hours to 10.62±1.91 mg after 96 hours (χ^2=21.4, p=2.3*10^{-5}, r^2=0.98). In all other configurations the weight of algae recovered either remained the same or decreased over the course of the experiment (Fig. 3.9).

There was an effect of Cd contamination in treatments without sediment, with higher growth when there was no Cd (χ^2=6.4, p=1.2*10^{-2}, r^2=0.61).

It appears growth is restricted in the presence of sediment. The weight of algae collected from uncontaminated, processed sediment decreased from 4.72±2.75 mg after 3 hours to 2.02±0.50 mg after 96 hours (Fig. 3.9 (a)), whilst that collected from uncontaminated, unprocessed sediment decreased from 6.08±2.45 mg after 3 hours to 3.75±0.82 mg after 96 hours (Fig. 3.9 (b)).

There was greater recovery of *C. closterium* from containers with unprocessed (4.48±2.69 mg, Fig. 3.9 (b), (e) and (h)) as opposed to processed sediment (2.68±2.37 mg, Fig. 3.9 (a), (d) and (g)), whether these were contaminated (sediment or water) or uncontaminated (χ^2=7.8, p=5.3*10^{-3}, r^2=0.33). There was no difference in growth in treatments with sediment due to Cd contamination (χ^2=1.6*10^{-1}, p=9.2*10^{-1}).

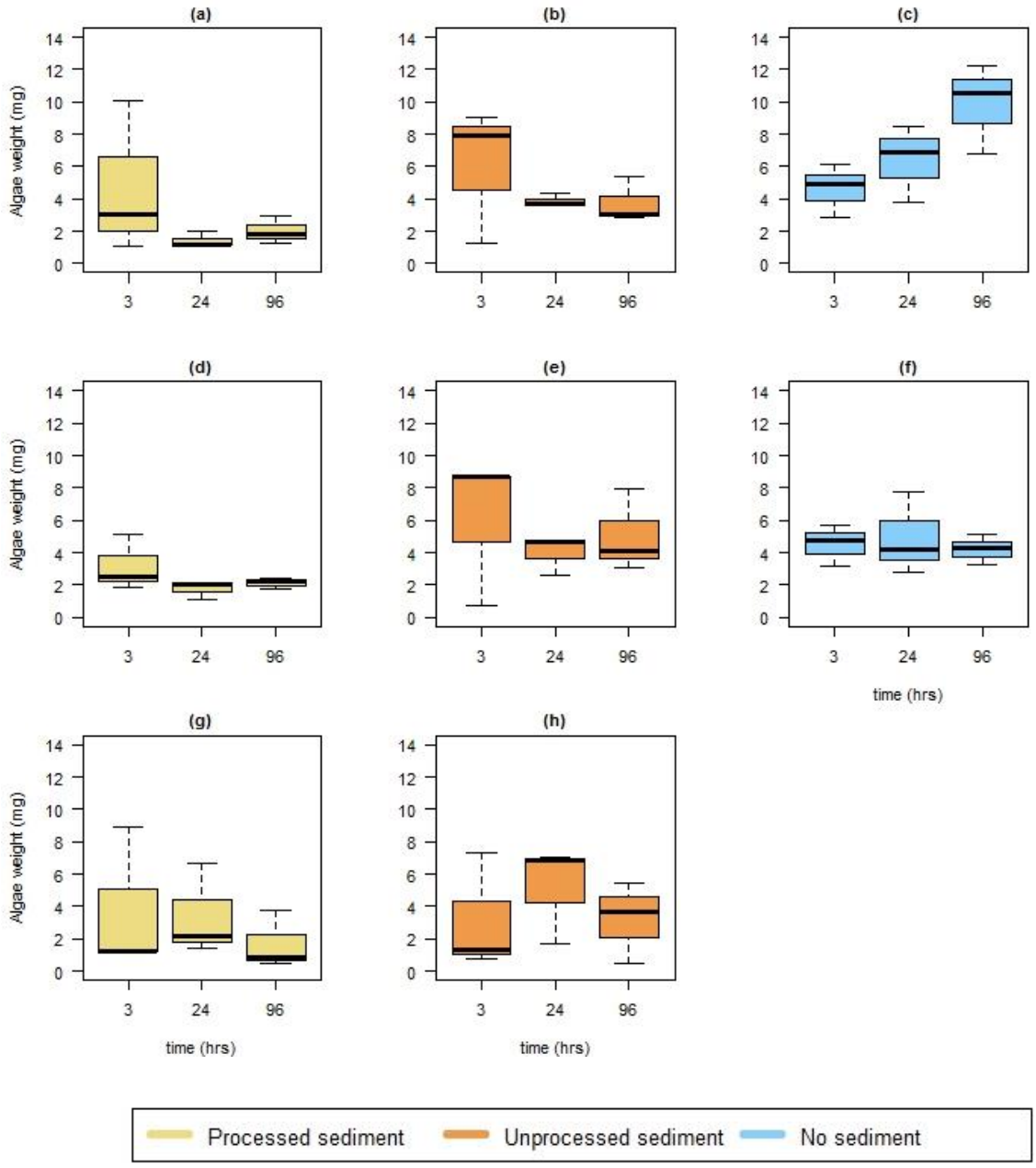


Figure 3.9. Mass of *C. closterium* (mg DW) retrieved at three time points when (a), (b) and (c) there is no contamination, (d), (e) and (f) water is initially contaminated and (g) and (h) sediment is initially contaminated

3.3.5. Uptake of Cd to *C. closterium* from sediment and water

Uptake of Cd to *C. closterium* was greater when water was the initially contaminated compartment (χ^2=73.5, p<2.2*10^{-16}, r^2=0.87) irrespective of whether sediment is processed or unprocessed (Figs. 3.10 and 3.11).

When water is the initially contaminated compartment (Fig. 3.10), there is an indication of a difference in Cd uptake to *C. closterium* between sediment treatments (χ^2=5.9, p=5.2*10^{-2}, r^2=0.32). A comparison of the three treatments (processed, unprocessed and no sediment) using a Tukey's honest significant difference (HSD) test reveals that uptake of Cd to *C. closterium* is greater from water only (no sediment) than when the sediment is processed (p=3.6*10^{-2}), but that there is no difference in uptake between unprocessed sediment and water only (p=0.38) or processed and unprocessed sediment (p=0.48). There is no change in Cd concentration in *C. closterium* over time (χ^2=7.9*10^{-1}, p=6.7*10^{-1}).

When sediment is the contaminated compartment (Fig. 3.11), there is greater uptake of Cd to *C. closterium* from processed than from unprocessed sediment (χ^2=20.7, p=5.5*10^{-6}, r^2=0.86). After 96 hours the mean Cd concentration in *C. closterium* from processed sediment is 201±34 mg kg^{-1}, about four times the concentration in algae from unprocessed sediment, 52±17 mg kg^{-1}. Again there is no change in Cd concentration over time (χ^2=4.6, p=0.10).

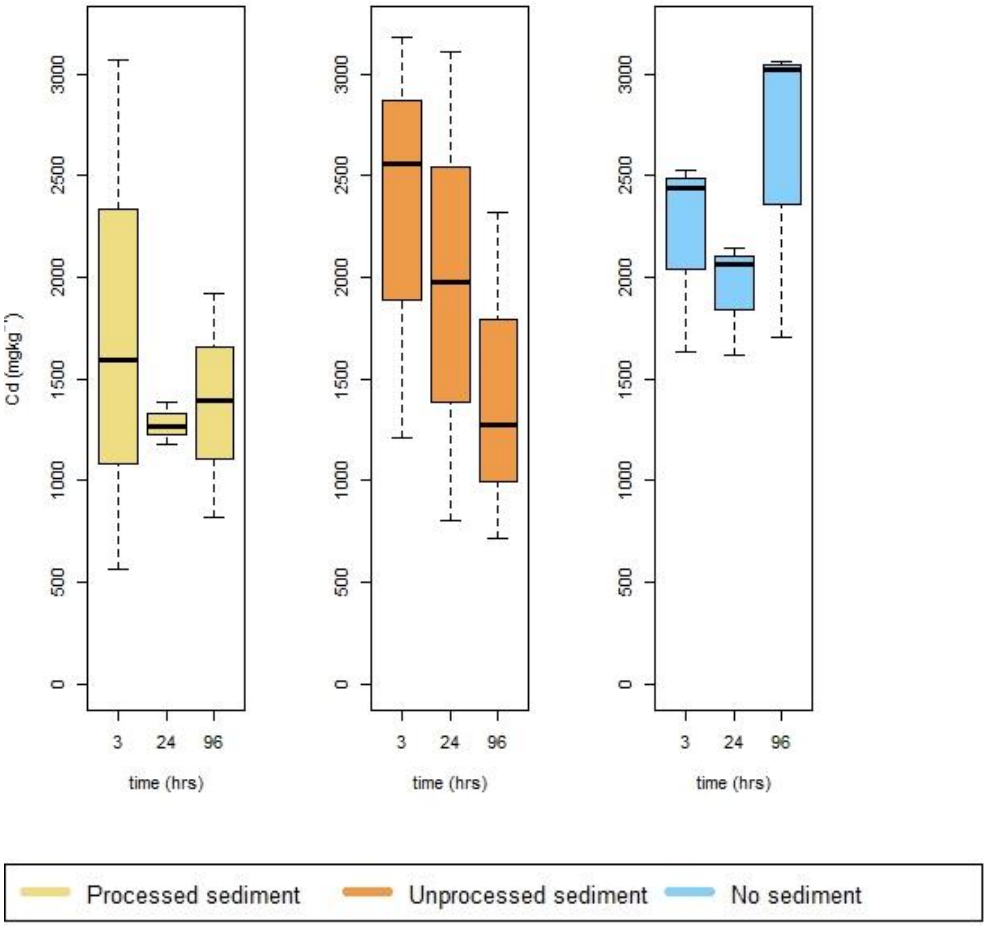


Figure 3.10. Total Cd uptake (internal plus adsorbed, mg kg^{-1} DW) to *C. closterium* when water is the initially contaminated component

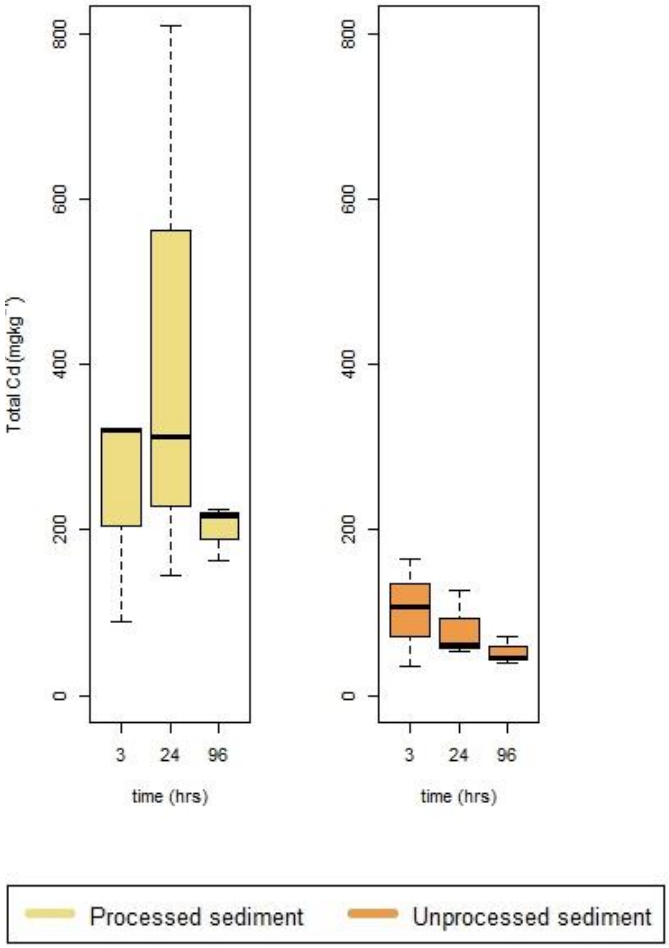


Figure 3.11. Total Cd uptake (internal plus adsorbed, mg kg^{-1} DW) to *C. closterium* when sediment is the initially contaminated component

3.3.6. Bioconcentration factor

The total (both externally adsorbed and internally absorbed) BCF of Cd in *C. closterium* related to both sediment and water is shown in Table 3.3 as the range of BCF values at each time point for each of the treatments related to either sediment (BCF_{Sed}) or water (BCF_{Wat}) Cd concentrations (n=3). If BCF is more than 1 then Cd is bioconcentrated (i.e. higher in the algae than the contaminated compartment).

BCF_{Sed} is higher in processed than unprocessed sediment when sediment is initially contaminated. It is initially higher in unprocessed than processed sediment when water is initially contaminated, but values converge to similar levels by 96 hours. BCF_{Sed} decreases across all treatments over the 96-hour period.

BCF_{Wat} is highest when sediment is initially contaminated (because there is very little Cd in the water). When the water is the initially contaminated compartment it is higher when sediment is present. When there is no sediment present BCF_{Wat} remains fairly constant for the duration of the experiment.

Table 3.3. Bioconcentration factor from contaminated sediment (BCF_{Sed}) and water (BCF_{Wat}) to *C. closterium* given as the range (n=3) to two significant figures

BCF_{Sed} (Cd_{Alg}/Cd_{Sed})			
Time (hrs)	**3**	**24**	**96**
Contaminated processed sediment	8.0 - 37	14 - 71	15 - 25
Contaminated unprocessed sediment	3.0 - 22	5.0 - 13	4.0 - 9.0
Contaminated water, processed sediment	110 - 560	180 - 240	90 - 260
Contaminated water, unprocessed sediment	310 - 860	130 - 700	100 - 260
BCF_{Wat} (Cd_{Alg}/Cd_{Wat})			
Contaminated processed sediment	5700 - 14000	5700 - 15000	520 - 8900
Contaminated unprocessed sediment	4000 - 51000	1400 - 28000	210 - 1700
Contaminated water, processed sediment	130 - 18000	900 - 11000	2200 - 7600
Contaminated water, unprocessed sediment	420 - 2200	390 - 6900	710 - 3600
Contaminated water, no sediment	190 - 300	210 - 240	200 - 380

3.3.7. External and internal Cd in *C. closterium*

Internal contamination is consistently higher than external contamination, i.e. absorption to the diatom cell was greater than external adsorption, for all treatments at all time points (χ^2=19.7, $p=9.0*10^{-6}$, r^2=0.46) apart from at 24 hours where the processed sediment is initially contaminated (Fig. 3.12 (d)).

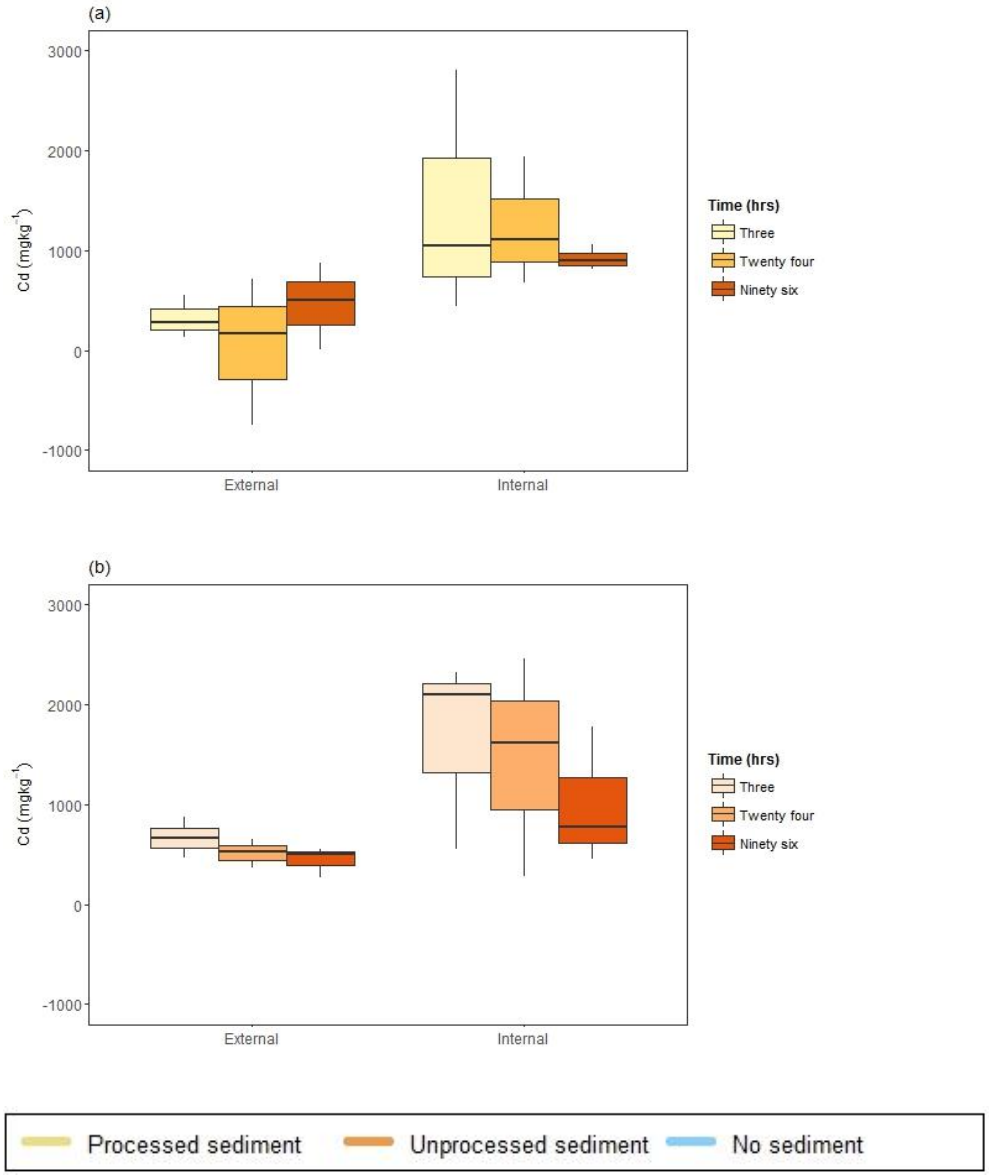


Figure 3.12. (Continued on next page). Cd uptake (external and internal, mg kg^{-1} DW) to *C. closterium* when (a) Sediment is processed and water is initially contaminated (b) Sediment is unprocessed and water is initially contaminated (c) No sediment and water is initially contaminated (d) Sediment is processed and initially contaminated (e) Sediment is unprocessed and initially contaminated. *Note difference in y-axis scale in plots (d) and (e)*

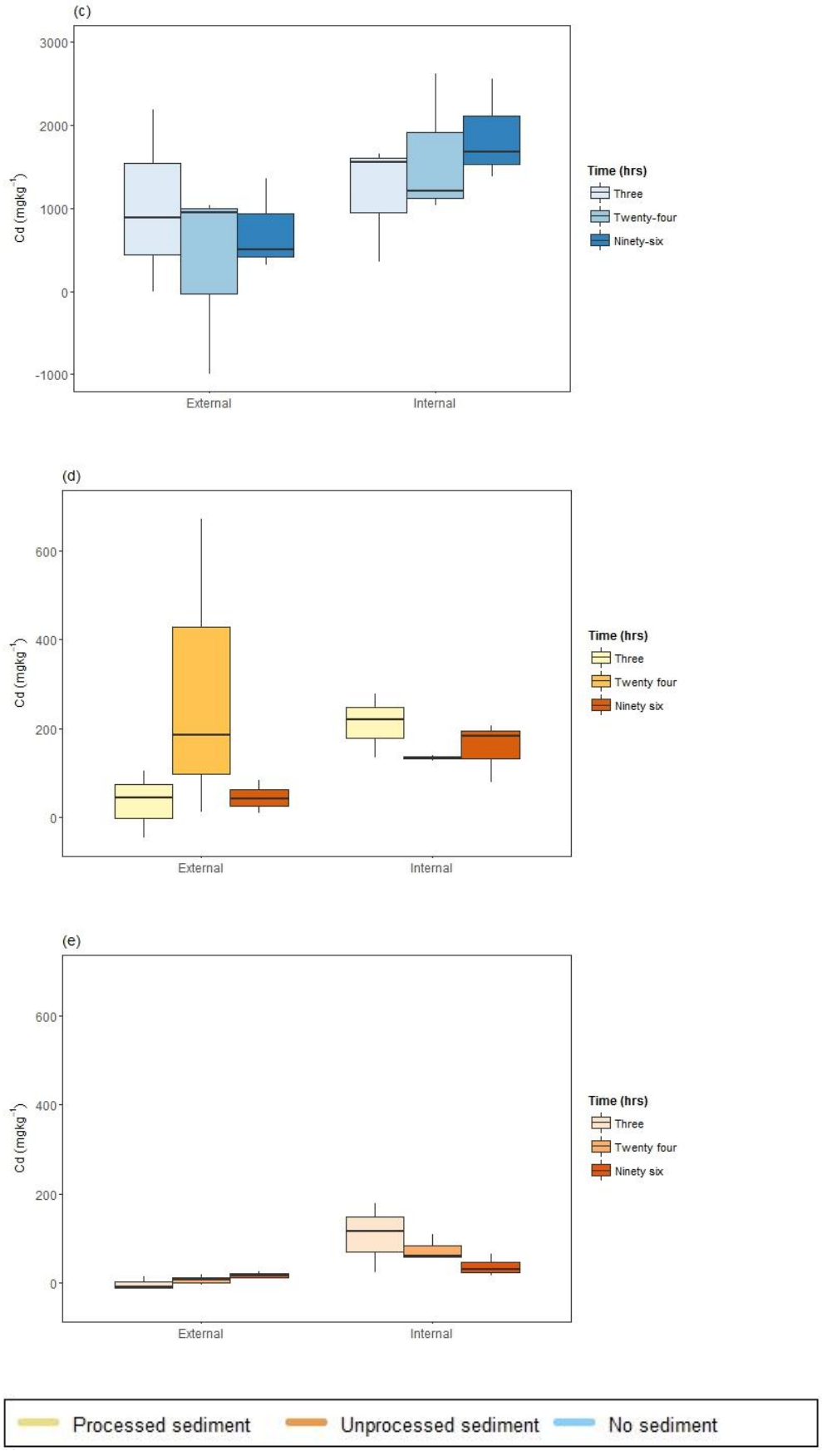


Figure 3.12. (Continued from previous page)

3.3.8. Cd concentrations in sediment (when water initially contaminated)

When water is initially contaminated, Cd concentration in the sediment increases over time (χ^2=30.2,
p=2.8*10^{-7}, r^2=0.59) (Fig. 3.13). There is no difference in Cd uptake to sediment from water due to the different sediment types (χ^2=2.5, p=1.1*10^{-1}). The presence of diatoms slows the uptake of Cd from the overlying water to the sediment for both processed and unprocessed sediment (χ^2=4.2, p=4.1*10^{-2}).

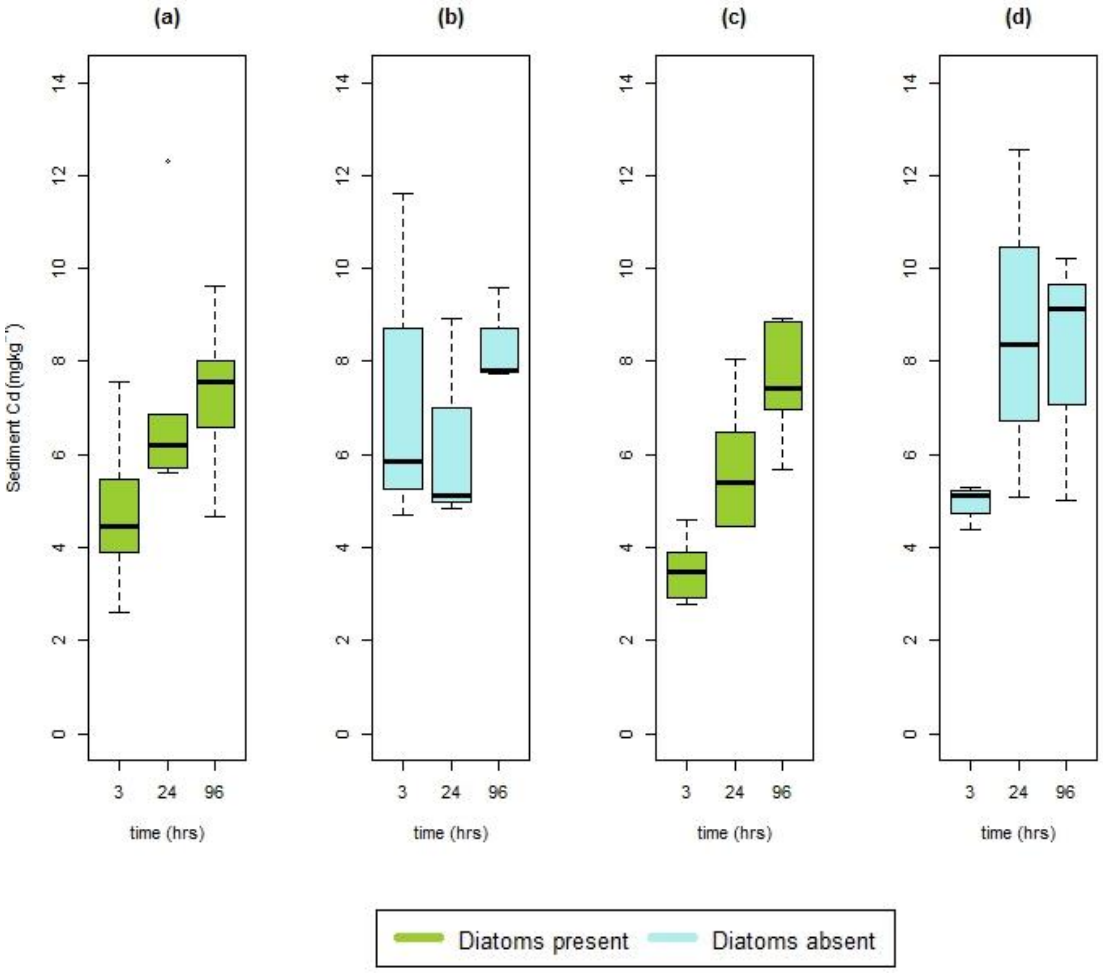


Figure 3.13. Cd concentrations in sediment (mg kg^{-1} DW) when water is the initially contaminated component and (a) and (b) Sediment is processed and (c) and (d) Sediment is unprocessed, outliers (◦) calculated as Q1-1.5IQR and Q3+1.5IQR

3.3.9. Cd concentrations in sediment (when sediment initially contaminated)

When sediment is initially contaminated, Cd concentration in the sediment does not change over time (χ^2=7.0, p=7.3*10^{-2}, r^2=0.52), due to the presence of *C. closterium* (χ^2=1.8, p=1.8*10^{-1}) or due to sediment type (χ^2=3.6, p=5.7*10^{-2}, Fig. 3.14).

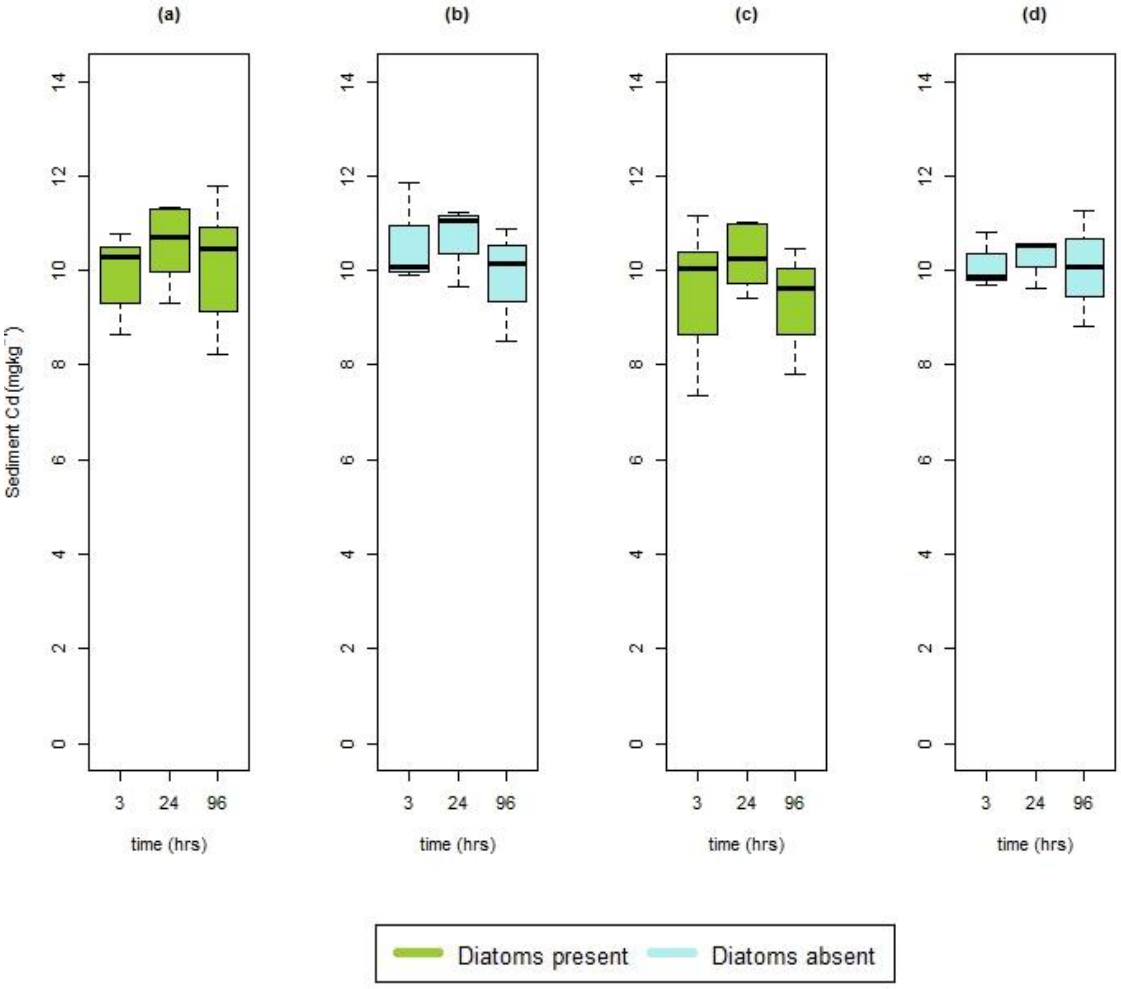


Figure 3.14. Cd concentrations in sediment (mg kg^{-1} DW) when sediment is the initially contaminated component and (a) and (b) Sediment is processed and (c) and (d) Sediment is unprocessed

3.3.10. Cd concentrations in water (when water initially contaminated)

When water is the initially contaminated component the Cd concentration in water decreases over time (χ^2=30.6, p=2.3*10^{-7}, r^2=0.77). For both sediment types, this decrease happens more quickly where *C. closterium* is present than when it is not (χ^2=6.7, p=9.7*10^{-3}). Cd concentrations in the water are higher when the sediment is unprocessed (χ^2=17.5, p=2.9*10^{-5}), this is particularly noticeable at three hours (Fig. 3.15), especially where there are no algae present (Fig. 3.15 (d)).

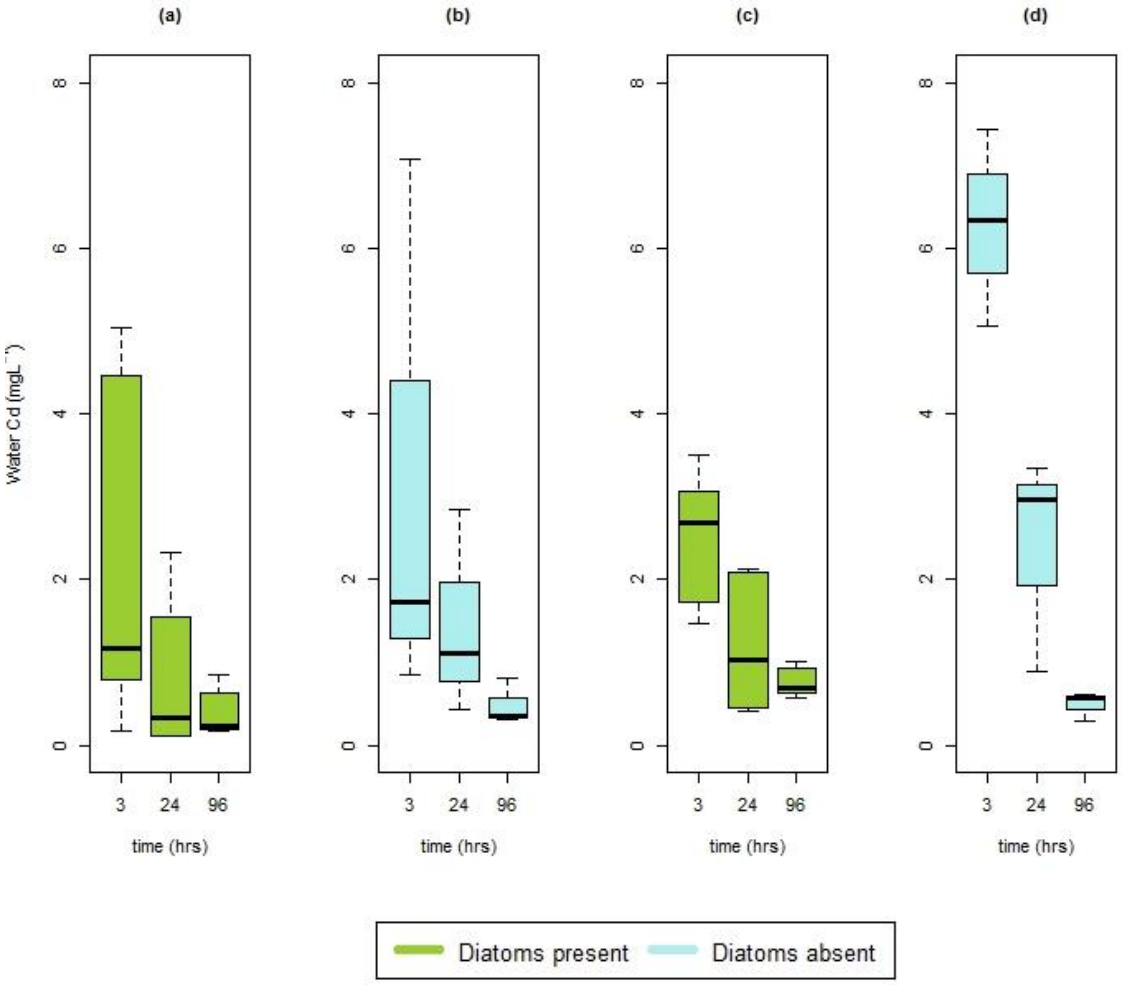


Figure 3.15. Cd concentrations in water (mg L^{-1}) when water is the initially contaminated component and (a) and (b) Sediment is processed and (c) and (d) Sediment is unprocessed

3.3.11. Cd concentrations in water (when sediment initially contaminated)

When the sediment is the initially contaminated compartment, Cd concentrations in the water increase over time (χ^2=50.7, p=9.6*10^{-12}, r^2=0.77, Fig. 3.16). Cd concentrations in water are greater in the presence of *C. closterium* (χ^2=5.9, p=1.5*10^{-2}), however there is no difference in contamination due to the different sediment treatments (χ^2=1.8, p=1.8*10^{-1}).

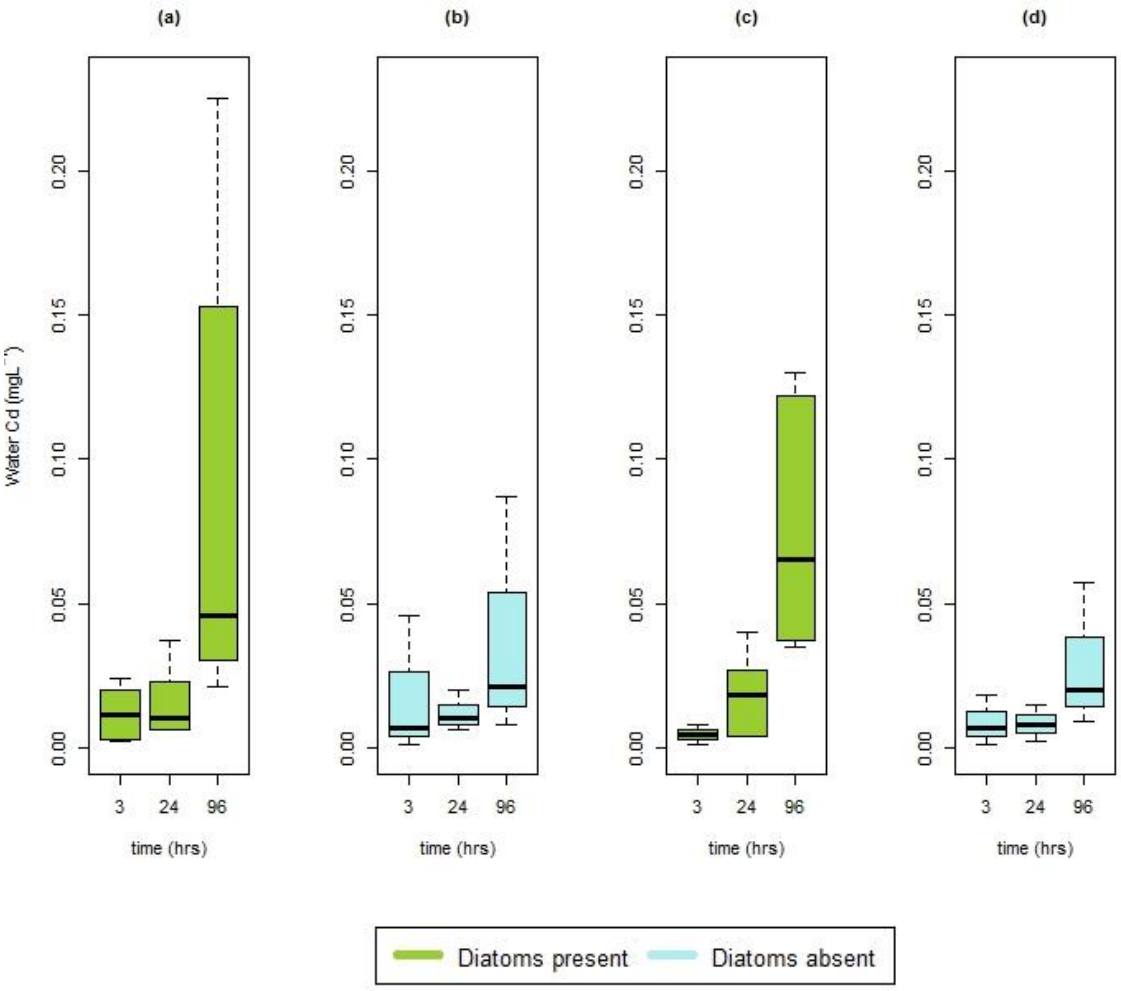


Figure 3.16. Cd concentrations in water (mg L^{-1}) when sediment is the initially contaminated component and (a) and (b) Sediment is processed and (c) and (d) Sediment is unprocessed

3.3.12. Concentration factor

CF_{Sed} and CF_{Wat} related to sediment and water contamination are shown in Table 3.4 as the range of CF values at each time point for all the treatments which included both sediment and water. If CF is more than 1 then Cd is concentrated in that compartment.

CF_{Sed} is higher than CF_{Wat} across all treatments at all time points. When sediment is initially contaminated CF_{Sed} is higher in treatments without *C. closterium* and where water is initially contaminated CF_{Sed} is higher when *C. closterium* is present.

There is no concentration of Cd from the sediment to the water (i.e. CF_{Wat} is always <1) except at three hours when the water is initially contaminated. This shows that there is quick equilibration of Cd between water and sediment, however, this is mediated by *C. closterium* (Section 3.3.10, Fig. 3.16).

Table 3.4. Concentration factor from contaminated sediment and water to sediment (CF_{Sed}) and water (CF_{Wat})

CF_{Sed} (Cd_{Sed}/Cd_{Wat})			
Time (hrs)	**3**	**24**	**96**
Contaminated unprocessed sediment with *C. closterium*	1700 - 5300	240 - 2600	76 - 230
Contaminated processed sediment with *C. closterium*	450 - 4800	280 - 1800	49 - 400
Contaminated unprocessed sediment without *C. closterium*	550 - 8500	690 - 5200	200 - 1000
Contaminated processed sediment without *C. closterium*	260 - 13000	550 - 1500	120 - 1100
Contaminated water, unprocessed sediment with *C. closterium*	7.9E-01 - 2.5	3.0 - 11	7.0 - 14
Contaminated water, processed sediment with *C. closterium*	7.7E-01 - 32	2.4 - 100	9.5 - 45
Contaminated water, unprocessed sediment without *C. closterium*	7.1E-01 - 8.7E-01	2.8 - 5.7	15 - 18
Contaminated water, processed sediment without *C. closterium*	6.6E-01 - 13	1.7 - 20	10 - 27
CF_{Wat} (Cd_{Wat}/Cd_{Sed})			
Contaminated unprocessed sediment with *C. closterium*	2.7E-04 - 7.1E-04	3.8E-04 - 4.1E-03	4.3E-03 - 1.3E-02
Contaminated processed sediment with *C. closterium*	1.7E-04 - 6.9E-04	5.4E-04 - 3.6E-03	2.5E-03 - 2.0E-02
Contaminated unprocessed sediment without *C. closterium*	1.2E-04 - 1.8E-03	1.9E-04 - 1.5E-03	9.7E-04 - 5.1E-03
Contaminated processed sediment without *C. closterium*	8.0E-05 - 3.9E03	6.7E-04 - 1.8E-03	8.9E-04 - 8.5E-03
Contaminated water, unprocessed sediment with *C. closterium*	4.0E-01 - 1.3	9.4E-02 - 3.4E-01	7.2E-02 - 1.4E-01
Contaminated water, processed sediment with *C. closterium*	3.1E-02 - 1.3	1.0E-03 - 4.1E-01	2.2E-02 - 1.1E-01
Contaminated water, unprocessed sediment without *C. closterium*	1.2 - 1.4	1.7E-01 - 3.5E-01	5.7E-02 - 6.7E-02
Contaminated water, processed sediment without *C. closterium*	7.4E-02 - 1.5	4.9E-02 - 5.9E-01	3.6E-02 - 1.0E-01

3.3.13. Cd concentrations in pore water

It was not possible to extract pore water from all experimental treatments due to the small (if any) quantities collected and the properties of the sediments (processed and unprocessed) which affected their ability to hold water. From a potential total of 162 pore water samples 102 were successfully extracted. Of these 67 were from contaminated treatments (34 where sediment was initially contaminated and 33 where water was initially contaminated). Fewer samples were extracted from processed (n=14) than unprocessed (n=53) sediment and fewer were from samples without diatoms (n=26) than with diatoms (n=41). Across the time periods there were 22 samples extracted at three hours, 22 at 24 hours and 23 at 96 hours.

The issues with extraction have implications in terms of replication. Whilst graphical indications are that there may be a difference in pore water concentrations due to sediment type (Fig. 3.17), the fact that there is only one replicate for processed sediment with *C. closterium* when water is initially contaminated (Fig. 3.17 (c)) makes meaningful comparison difficult. With this in mind there was no statistical test made between Cd concentrations in pore water for different sediment types.

There was higher Cd concentration in pore water when the overlying water was the initially contaminated compartment ($\chi^2=20.1$, $p=7.2*10^{-6}$, $r^2=0.59$) and higher concentration, regardless of initially contaminated compartment, when *C. closterium* was present ($\chi^2=32$, $p=1.9*10^{-8}$, Fig. 3.18). There is some indication that contamination of pore water may increase over time ($\chi^2=5.9$, $p=5.3*10^{-2}$).

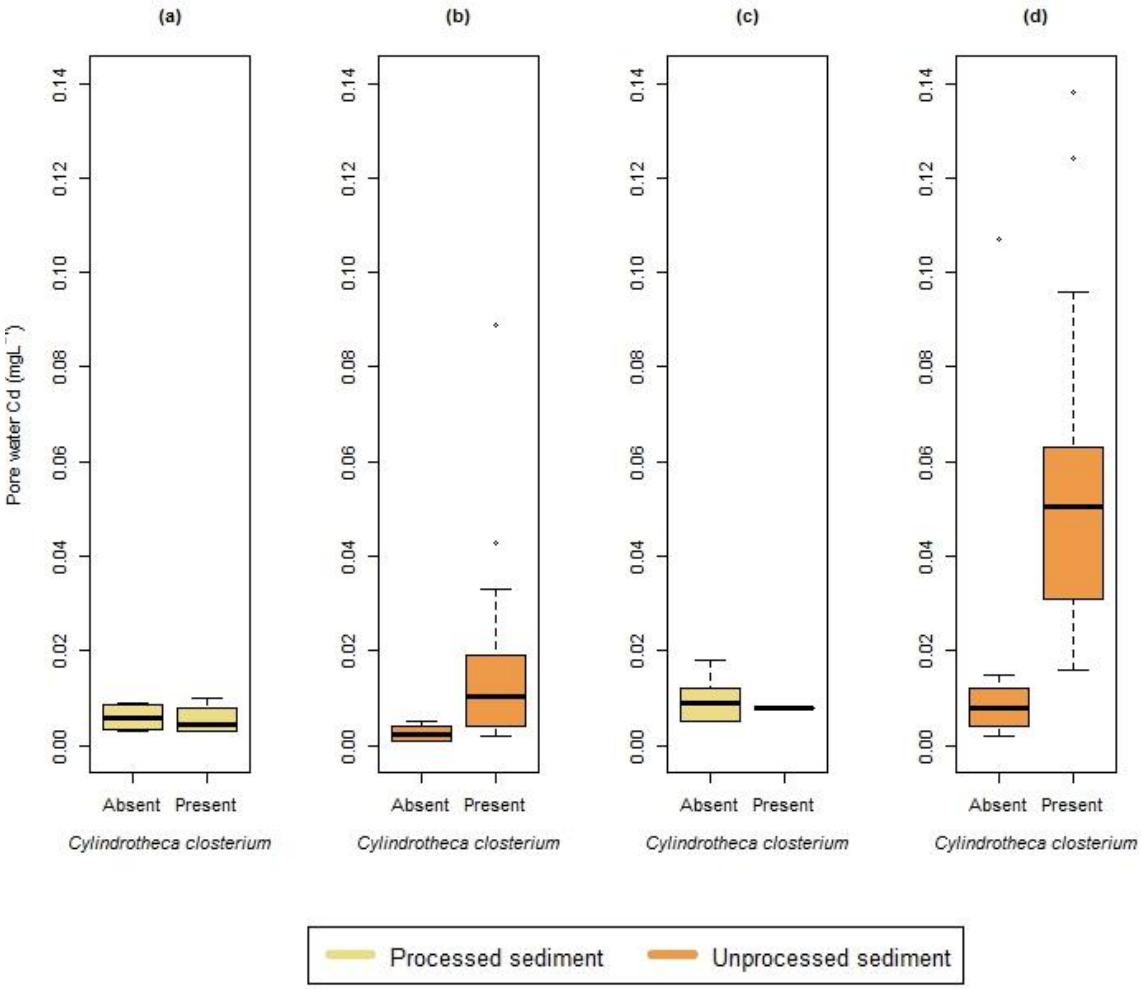


Figure 3.17. Cd concentrations in pore water (mg L^{-1}) when (a) and (b) Sediment is initially contaminated and (c) and (d) Water is initially contaminated, outliers (◦) calculated as Q1-1.5IQR and Q3+1.5IQR

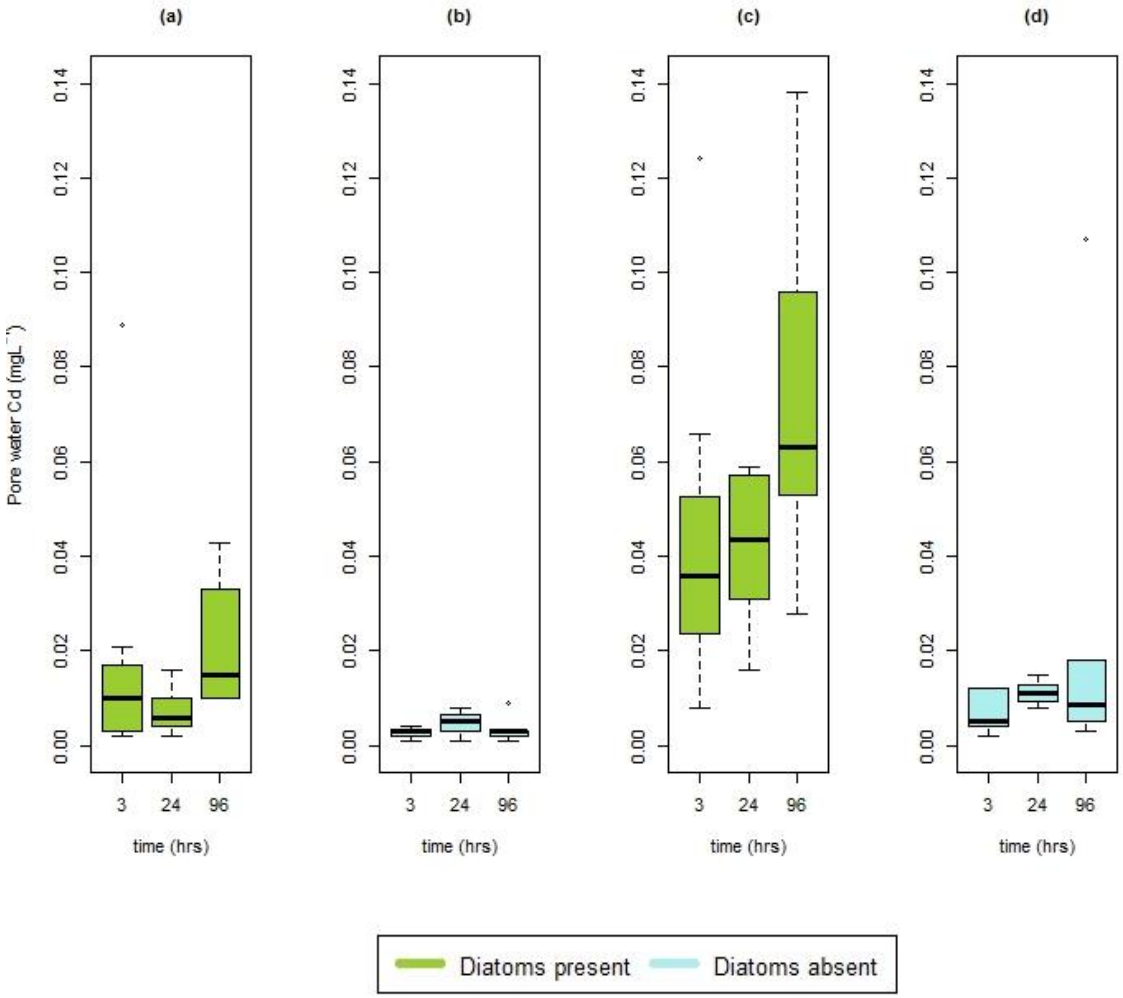


Figure 3.18. Cd concentrations in pore water (mg L^{-1}) when (a) and (b) Sediment is initially contaminated and (c) and (d) Water is initially contaminated, outliers (◦) calculated as Q1-1.5IQR and Q3+1.5IQR

3.4. Discussion

As stated in the introduction the aims of the laboratory study were twofold, firstly to examine how uptake of Cd by the diatom *C. closterium* (Cd_{Alg}) is affected by the various treatments and secondly to look for any effects of *C. closterium* on the Cd concentrations of the water (Cd_{Wat}) and sediment (Cd_{Sed}).

3.4.1. Algal growth

The mass of *C. closterium* retrieved from each treatment may be used as an indicator of microalgae response to the presence/absence of sediment, differences in sediment properties and presence/absence of Cd contamination.

C. closterium growth was shown to be greatest in uncontaminated treatments without sediment present. In these containers there was growth throughout the 96 hours, this was the only treatment in which such continuous growth was observed. When water treatments were contaminated (no sediment) growth at three hours was similar to that observed in uncontaminated treatments (4.61±1.71 mg and 4.52±2.54 mg respectively). However, whilst the algae biomass increased in uncontaminated water without sediment, no further growth was observed in Cd contaminated treatments without sediment; algae weights remaining constant throughout the experiment period. This result suggests that there was some toxic effect of Cd at concentrations of 10 mg L^{-1} on growth of *C. closterium*, this is in contrast to effects found by other researchers in toxicology. For example, Moreno-Garrido et al. (2003), using a similar light media (f/2+Si without trace metals stock) as this study, found little growth inhibition below 20 mg kg^{-1} Cd and EC_{50} for growth inhibition of *C. closterium* at Cd concentrations of 79 mg kg^{-1} (almost eight times the levels used in this experiment).

There also appears to be a distinct effect of the presence of sediment on *C. closterium* growth. None of the treatments containing sediment show any increase in the weight of algae recovered over the 96 hours of the experiment. At three hours mean algal weight in uncontaminated treatments with processed sediment (4.72±4.31 mg) was similar to that of treatments without sediment (4.61±1.71 mg), but there was no increase in weight in treatments with processed sediment thereafter. In uncontaminated treatments with unprocessed sediment mean weight after three hours (6.08±3.83 mg) was greater than that observed in water only (4.61±1.71 mg), again however there was no increase in the weight of algae recovered from the unprocessed sediment treatment over the 96-hour period.

As there were no other potentially toxic levels of contamination found in the sediment (Section 3.2.2), the effect of sediment on algal weight may be due to two of factors. It may be that growth of *C. closterium* is reduced in the presence of sediment or perhaps that their motility has been reduced affecting the diatoms ability to migrate into the lens tissue. In toxicity tests using artificial sediment, Moreno-Garrido et al. (2007) reported a negative effect on *C. closterium* when the percentage of silt sized (<0.63 µm) particles in sediment was greater than 15% (with the remainder of the sediment consisting of sand particles). This effect on growth was thought to be due to a "shadowing" effect in which the algae becomes buried under finer sediment particles which settle out of the water column more slowly following disturbance than the larger algae cells, therefore burying them. However, it was also reported (Moreno-Garrido

et al., 2003) that there was no effect of particle size if the cultures were not disturbed during the course of the experiment. In the current study the *C. closterium* cells were the final element added to each treatment (after sediment and water) and the pots were not disturbed or shaken for the duration of the experiment, it therefore seems unlikely that this was the cause of reduced weight.

In a growth inhibition test of the surfactant Linear Alkylbenzene Sulphonate, Mauffret et al. (2010) found a lower ErC_{50} (EC_{50} in terms of reduced growth rate) in treatments containing a natural sediment with a high percentage of fine particles (34.1±6.0% clay) than in those without sediment. Mauffret et al. (2010) suggested two additional possible reasons for an effect of sediment on algal growth. They postulate that firstly diatoms might be grazed by autochthonous predators which had not been completely removed from the sediment and secondly that diatoms may have been adversely affected by nutrients released from the natural sediment. In the current study algal weights recovered from the processed sediment, which was autoclaved, washed and dried prior to use, were lower than those recovered from the unprocessed sediment, which was sieved to 2mm only. This would suggest that there is no effect of autochthons predators, which would not have survived in the processed sediment. An adverse effect of nutrients also seems unlikely as increased release of nutrients would be expected to stimulate growth and potentially mask toxic effects (Adams and Stauber, 2004).

Another possible reason for a reduction in the weight of algae retrieved from the sediment is an effect on diatom motility. Algae were retrieved from treatments containing sediment by use of their natural motility, therefore impairment of this ability would result in lower weights being recovered. Kingston (2009) found that following inoculation of *C. closterium* culture (in the exponential growth phase) to fresh f/2 media motility declined after two days in flasks that were continuously stirred and after three days in flasks that were shaken once daily. After six days he found a reduction in both treatments to 39.5% of the initial motility. It is conceivable that, although the treatments in this experiment received no agitation (to prevent the possible smothering effects suggested by Moreno-Garrido et al. (2007)), reduction in motility caused retrieval of lower weights of algae from treatments containing sediment than those without (treatments with water only did not rely on the motile function of algae for retrieval). It is possible that there was growth in these treatments, but, due to reduced motility, that this was not observable from the weights recovered.

In addition to differences between pots with and without sediment there was a difference in the weight of algae retrieved from treatments containing processed and unprocessed sediment types with lower weights of algae retrieved from treatments with processed sediment. It is possible that the unprocessed sediment had higher nutrient content than processed sediment, although nutrient concentrations were not measured in this experiment, it is conceivable these were flushed from the processed sediment during its handling (specifically washing) prior to the

experiment. It is also feasible that, as unprocessed sediment was not autoclaved it may contain autochthons algae (which has survived cold storage in the dark and the spiking process) and which is contributing to the total weight of algae. This may be evidenced by the higher weight of algae in unprocessed sediment at three hours in uncontaminated and water contaminated treatments (Fig. 3.9 (b) and (e)).

3.4.2. Cadmium uptake to *C. closterium* from sediment and water

The total concentration of Cd in *C. closterium* (Cd_{Alg}) was shown to be greater by an order of magnitude in treatments where water was the initially contaminated component (Figs. 3.10 and 3.11). This is in agreement with the findings of the limited number studies in both marine (Stronkhorst et al., 1994, Absil and van Scheppingen, 1996) and freshwater environments (Laube et al., 1979), which include contaminated sediment. No previous studies comparing uptake of metals from water and sediment directly could be found.

In laboratory experiments with freshwater algae, Laube et al. (1979) found that after 72 hours, in systems containing both water and sediment, when water was initially contaminated Anabaena and *Ankistrodesmus braunii* had Cd concentrations of ~9800μgg^{-1} dry weight and ~5500μgg^{-1} dry weight respectively. When sediment was initially contaminated uptake was much lower at ~20μgg^{-1} dry weight for Anabaena and ~8.5μgg^{-1} dry weight for *A. braunii*. Unfortunately, as previously stated, there are very few studies of uptake of contaminants to algae from sediment and therefore very little to compare the results observed here against.

Where sediment was the initially contaminated component, there is a difference in Cd_{Alg} between sediment treatments. There was greater uptake of Cd to *C. closterium* from processed than from unprocessed sediment. This may be due to two factors; firstly unprocessed sediment has a higher organic matter content than processed sediment (Table 3.1 and Fig. 3.7), secondly a greater percentage of particles in unprocessed sediment were in the smaller size classes compared to the processed sediment (Fig. 3.8). Metals will preferentially attach to organic matter and to smaller sediment particles. It is therefore suggested that the stronger attachment of Cd to the unprocessed sediment results in lower contamination of *C. closterium*. Indeed Eimers et al. (2002) found that increasing organic matter content of sediment increased Cd partitioning to sediment, reduced Cd in the overlying water and reduced Cd accumulation in the benthic detritivore (*Asellus racovitzai*, Isopoda). In the current study, there was an indication ($p=5.7*10^{-2}$) that there may be a corresponding difference in sediment contamination due to sediment type (Fig. 3.14). The small size of this difference is probably due to the comparative volume of sediment and algae compartments.

In treatments in which water was the initially contaminated component Cd_{Alg} was greater when there was no sediment present than when the sediment is processed. There is no difference between treatments with unprocessed sediment and those without sediment. This was surprising as treatments with processed sediment might be expected to act in a more similar way to those treatments without sediment due to the lower organic matter and larger particle size. Figure 3.4 showed Cd_{Alg} from treatments with processed sediment were lower after three hours than in other treatments (~1500 mg kg^{-1}) and that there was no change in concentration over time whereas in treatments with unprocessed sediment Cd_{Alg} after three hours was ~2500 mg kg^{-1} (similar to that in water alone) decreasing over time to ~1300 mg kg^{-1} at 96 hours (a level similar to that of the processed sediment).

There was no change in contamination of algae over time when either sediment or water was initially contaminated, although results for unprocessed sediment (Figs. 3.10 (b) and 3.11 (b)) suggest that, was the experiment to be run for a longer time, a reduction in Cd concentration over time may occur. This is in contrast to a study by Irving et al. (2009) which found a linear increase in Cd uptake (per unit mass) to mats of the freshwater diatom *Navicula pelliculosa* from water contaminated to 7 μgL^{-1}. A result which may be due to the short time period (five hours) of the Irving et al. study. Metal concentrations in planktonic algae have been seen to go through two phases of uptake an initial rapid passive uptake phase and then slower active uptake period (Ledin, 2000). In the Irving et al. (2009) study Cd concentrations in diatoms were fairly low ~25 μgg^{-1} after five-hour exposure from an initial concentration in the water of 7μgL^{-1}, approximately two orders of magnitude less than in the current study after three hours (albeit from a much higher initial concentration of 11 mg L^{-1}) (Fig. 3.10 (c)). A recent mesocosm study (Bere and Tundisi, 2012) found uptake to a natural periphyton community from water contaminated to 10 μgL^{-1} of <500 μgg^{-1} after two weeks' exposure, this is still considerably lower than the Cd concentrations observed in the current study.

3.4.3. Bioconcentration factor (BCF)

BCF from both sediment and water is greater than 1 across all treatments, bioaccumulation of Cd to *C. closterium* was occurring whether the initial contamination was in the water or the sediment. Where there is sediment present BCF_{Sed} and BCF_{Wat} decreases to some extent across all treatments, possibly due to equilibration occurring between the sediment and water compartments. Although the sediment was stored for 40 days following contamination to allow equilibration between sediment and pore water (Section 3.2.2), there was no equilibration between sediment and overlying water prior to the start of the experiment, Cd was therefore moving between those compartments during through the course of the experiment.

There are very few studies which report transfer of metals from sediment to algae. A pilot study carried out in the Scheldt estuary by Stronkhorst et al. (1994) found a concentration factor (conc. in algae/conc. in sediment) of 0.01 to 0.30 for Cd in diatoms and therefore no bioconcentration. However, a follow up study by Absil and van Scheppingen (1996) in the Westerschelde estuary found concentration factors ranging from 0.01 to 16.3 with greater concentration factors occurring at sites with lower Cd concentrations in the sediment ($<1.5 \mu g g^{-1}$ Cd). The second study shows agreement with the results reported here, a BCF range of 3.0 to 71 across all sampling points where sediment was initially contaminated. It should be noted that spiked sediments used in this study were allowed to equilibrate for a minimum of 40 days in an oxygen free environment (Simpson et al., 2004) to ensure that Cd was bound to the sediment and pore water concentrations were low (Figs. 3.17 and 3.18). Reported BCF values may differ from those observed in the current study because the above cited studies (Stronkhorst et al., 1994, Absil and van Scheppingen, 1996) were both conducted in the field where Cd uptake is likely to be affected by environmental conditions very different from those of a laboratory study. In particular, there is no water exchange during the laboratory experiment and the volume of overlying water relative to the volume of sediment is much smaller than in the field. The lack of a tidal cycle in the laboratory means that it is possible for equilibrium between compartments to be reached, whilst in the field there is constant cycle between reductive and oxidative conditions. Resuspension of sediment, due to the tide and waves, will also cause remobilization of metals. In addition, in estuarine locations, salinity is variable due to the varying influence of fresh and marine water which in turn affects partitioning (Chapman et al., 2013) and bioavailability (Atkinson et al., 2007) of metals and therefore BCF (Mountouris et al., 2002). The differences between laboratory and field studies will be further explored in Chapter 5.

In the natural environment Cd uptake to algae from water is reported to have a BCF of 1000 (Fowler, 2002). A study in Daya Bay, South China, (Qiu, 2015) reported BCF values for Cd in water of 4440 in phytoplankton and 5870 in the macroalgae *Ulva fasciata*. These values are an order of magnitude greater than those observed in the current study (190 to 380, Table 3.3). This difference may be due to the algal species studied, the fact that samples were taken from higher salinity water or, as with uptake from sediment, the variation in conditions between the laboratory and field.

There have been some recent laboratory studies for uptake of metals (in this case copper and zinc) to freshwater (Atay & Ozkoc, 2010) and marine (Atay et al., 2013) algae in which the water is contaminated and sediment is either present or absent. Unfortunately, these studies fail to separate the algae from the sediment and give a combined BCF (conc. in algae+sediment/conc. in water) which is always higher in treatments where sediment is

present. This is probably because a combined sediment and algae value is reported rather than because contaminant uptake to algae is greater in the presence of these sediments.

There have been some recent field studies that compare Cd concentration in organisms, other than algae, to the concentration in the sediment. Qiu (2015) reported BCF values of 2.24 for the shrimp *Penaeus merguiensis*, 1.95 for the crab *Portunus pubescens,* 14.8 for the shellfish *Veremolpa scabra* and 0.93 for the planktivorous fish *Stromateoides argenteus* from sediment containing 0.28±0.10μg^{-1} Cd at Daya Bay, South China, showing bioconcentration (all values >1) in organisms other than the fish. A study in the San Pedrito Lagoon, Mexico, (Mendoza-Carranza et al., 2016) reported BCFs for trophic groups Crustaceans 0.08, planktivorous fish 0.18 and detritivorous fish 0.41 from a sediment concentration of 3.99±1.67 mg kg^{-1}, showing no bioconcentration (all values <1) in these organisms from sediment. BCF values reported in these studies are lower for all organisms (apart from the shellfish) than those observed for *C. closterium* in this study. These two studies also give widely differing results from each other with higher BCFs from the site with a lower Cd concentration. These differences are not particularly surprising and will be due to different responses from species and other environmental factors, BCF is a simple ratio and other workers have developed more complex models (Luoma and Rainbow, 2005) which may better predict contaminant uptake. However, these models require information on loss rates of Cd from diatoms which are currently poorly understood (Wu et al., 2016).

3.4.4. External v internal cadmium concentration for *C. closterium*

The range of values for Cd_{Alg} was often quite variable, probably due to the small amounts of algae extracted. Unfortunately, this has resulted in some negative values being obtained for external contamination at three and 24 hours as these were calculated by extracting internal Cd_{Alg} from total Cd_{Alg}. That said, internal Cd_{Alg} is higher than external Cd_{Alg} for all but one treatment, processed contaminated sediment at 24 hours (Fig. 3.12 (d)).

This differs from other studies of Cd uptake by microalgae. In a study of the planktonic marine microalga *Tetraselmis suecica* (Pérez-Rama et al., 2010) found that after 72 hours there were higher intercellular than extracellular Cd concentrations at initial water concentrations of ≤6 mg L^{-1} Cd. At higher initial concentrations (≥15 mg L^{-1}) external concentrations were higher, this was thought to be due to the toxic effect of Cd on the algae. At 24 hours the same study found that all external concentrations were higher than internal, suggesting that active uptake and incorporation to the interior of the cell is a slower process than external adsorption. A study of Cd uptake from water to a natural freshwater biofilm (Morin et al., 2008) found that, although internal Cd increased over the duration of the study (six weeks), it was always less than 50% of the total accumulation and therefore less than externally attached Cd. In a study

of Cd uptake capacity from water by the marine diatom *Phaeodactylum tricornutum* (Torres et al., 2014) found that at low concentrations (up to 10 mg L^{-1}) about 90% of Cd was internal to the cell although this decreased over time and with increasing Cd concentrations. These results (Torres et al., 2014) were in agreement with an earlier study (Torres et al., 1998) in which, at concentrations of ≤10 mg L^{-1}, intracellular Cd was greater than extracellular throughout the 96-hour experiment, in line with the results of the current study. Differences in these results may indicate a difference due to algal type (*T. suecica* is a green algae) and salinity as well as differences due to Cd concentration. The study carried out with the same algal type (diatoms), in seawater (Torres et al., 1998, Torres et al., 2014), found results similar to those presented here.

3.4.5. Cadmium in sediment

When water is the initially contaminated compartment

In addition to examining Cd uptake to *C. closterium* under different contamination conditions, effects of the presence of algae on the Cd concentrations in the other experiment compartments i.e. sediment (Cd_{sed}) and water (Cd_{wat}) were also studied.

It has been observed (Fowler, 2002) that small plankton species may be important in the redistribution of metals in marine environments. Algae, particularly planktonic species, may be useful in reducing contamination in water and it is due to this property there are currently numerous studies investigating the phytoremediation potential of various algae species in both the freshwater and marine environments (Delrue et al., 2016, El-Sheekh et al., 2016). There have also been some studies investigating the potential for phytoremediation of eutrophic sediments by benthic algae (Yamamoto et al., 2008, Kwon et al., 2015), in which, as well as removing excess nutrients, the algae are thought to enhance the activity of aerobic bacteria through oxygen production.

In this study it was found that, when water was the initially contaminated compartment Cd_{sed} increased over time. This was entirely expected, muddy sediments in estuaries have long been known to act as a sink for many pollutants (Fairbrother et al., 2007). However, in treatments where *C. closterium* was present, Cd_{sed} at three hours was lower than in treatments without algae for both the processed and unprocessed sediment (Fig. 3.13). This would appear to indicate that algae take up contaminants from the water more readily and over shorter timescales than the sediment does. This was seen in a study of metal sorption onto sediment components (Calmano et al., 1988) where Cd had a stronger affinity for cells of the green algae *Scenedesmus quadricauda* than the mineral sediment constituents (bentonite, aluminium oxide, goethite and quartz).

Cd_{sed} was seen to increase in treatments with contaminated water over the period of the experiment so that at 96 hours Cd_{sed} in treatments with *C. closterium* were close to levels seen in treatments without algae. It is recommended that experiments are run for longer periods in order to understand if concentrations in sediment with algae do eventually equal concentrations in sediment without algae.

An important property of diatoms is the production of EPS which is acknowledged for its ability to bind metals (Bhaskar and Bhosle, 2005). A previous study (Schlekat et al., 1998) show that EPS produced by bacteria increase the binding of Cd to sediment. This is counter to the effect seen here, which may be due to the short time of the current experiment or the different properties of diatom and bacterial EPS (Gutierrez et al., 2012).

When sediment is the initially contaminated compartment

When sediment was initially contaminated there was no difference in contamination levels due to the presence of *C. closterium* or over time (Fig. 3.14). This may be due to comparative volumes of sediment and algae, with a much greater volume of sediment providing many more binding sites, so although we see uptake of Cd from the sediment by algae (Fig. 3.11 and Table 3.3) this does not alter levels in the sediment.

There were indications that there may be a difference in sediment contamination during the course of the experiment due to the slight difference in sediment properties ($p=5.7*10^{-2}$) as it appeared there were slightly lower levels of Cd in unprocessed sediment (Fig. 3.14). This is unexpected because the Cd was not attaching to the algae (Cd_{Alg} is lower in *C. closterium* from unprocessed sediment (Fig. 3.11) nor did there appear to be a greater release to the water from unprocessed sediment (Fig. 3.16).

3.4.6. Cadmium in water

When water is the initially contaminated compartment

Following on from the effects seen in sediment contamination when water was the initially contaminated component, Cd levels in the overlying water of those treatments were examined (Fig. 3.15). It was found that (as expected) Cd_{Wat} decreased over time as Cd became increasingly associated with the sediment. However, it was also observed that Cd_{wat} decreased more quickly when *C. closterium* was present. In both processed and unprocessed sediment, the Cd_{Wat} was lower at three hours in treatments with *C. closterium* than those without. This effect was particularly evident in the unprocessed sediment treatments where the Cd_{Wat} with *C. closterium* present was approximately half that of the treatments without diatoms. Following

the initial difference between treatments at three hours, contamination in the water reduced to a similar value across all treatments by 96 hours. It appears therefore that the diatoms were initially removing the Cd from the water more quickly than the sediment alone, but that over time the effect of either sediment or sediment plus diatoms was similar.

It was initially supposed that the observed accelerated decrease in Cd_{Wat} in the presence of diatoms was due to their associated EPS which might cause Cd to bind to the sediment more effectively than in treatments without algae (Schlekat et al., 1998). However, as described above (Section 3.4.5), uptake to sediment was slowed by the presence of algae (Fig. 3.13). It must therefore be concluded that, in the presence of diatoms, Cd was moving from the water in a two stage process initially to the diatoms and then to the sediment. It may be that although Cd has stronger affinity for algal cells than mineral components of the sediment (Calmano et al., 1988), over time Cd becomes associated with increasing volumes of EPS and thereby part of the sediment.

There was also a difference in Cd_{Wat} due to sediment type (Cd_{Wat} was lower when the sediment was processed) (Fig. 3.15), although no corresponding difference in uptake to sediment was observed (Fig. 3.13) and uptake to *C. closterium* from contaminated water with processed sediment was lower than other treatments (Fig. 3.10).

It was counter to expectation that Cd_{Wat} in processed sediment was reduced more quickly than in treatments with unprocessed sediment. The unprocessed sediment contains greater organic matter (Table 3.1 and Fig. 3.7) and had smaller particle size (Fig. 3.8) (with greater surface area) than the processed sediment, there was also a greater quantity of *C. closterium* recovered from unprocessed sediment (Fig. 3.9 (b), (e) and (h)). All of these factors suggest that Cd would be removed more quickly from the water in treatments containing unprocessed sediment. This aspect would warrant further investigation as it cannot be explained with the information currently available.

When sediment is the initially contaminated compartment

It was observed that where the sediment was the initially contaminated component, although levels were very low, Cd_{Wat} increased over time (Fig. 3.16). It was further noted that Cd_{Wat} was greater in the presence of *C. closterium* than when there was no algae present which suggests that the diatoms were somehow facilitating the release of Cd into the overlying water.

This release may be due to two mechanisms, firstly the alteration of redox conditions at the sediment water interface due to the presence of algae and secondly the excretion by diatoms of EPS.

Release of metals from sediment occurs due to disturbance (resuspension and mixing, neither of which took place here) and by changes in the properties of the overlying water (pH, dissolved oxygen (DO) concentration and salinity) (Simpson et al., 2004). The presence of benthic algae alters properties at the sediment water interface by increasing oxygen through photosynthesis (Yamamoto et al., 2008). Indeed there have been recent studies (Yamamoto et al., 2008, Kwon et al., 2015) to investigate the potential for bioremediation of eutrophic sediments using MPB. In a field study in Hiroshima Bay, Japan, Yamamoto et al. (2008) observed an increase in redox potential and a decrease in acid-volatile sulphides in sediment in locations seeded with MPB. Conversely, a study in France (Amouroux et al., 2003), using benthic chambers on the sediment surface, found a positive flux of Cd from the sediment to the water column with increasing respiration, i.e. when the available oxygen was reduced. These properties at the sediment interface are important in contaminant flux and these changes may account for releases to the overlying water observed here, although, as these properties were not measured it is not possible to comment further.

EPS is a catch all term which encompasses a wide variety of large microbially-secreted molecules, comprised primarily of polysaccharides, with a wide range of physical states (from a thick gel to dissolved organic carbon (DOC) in solution), chemical properties and biological roles (e.g. protecting cells from desiccation and suspension) (Decho, 2000). Secretion of EPS by diatoms is closely related to their motility (Smith and Underwood, 1998). Production of EPS takes place in response to changing conditions of light, nutrient availability and physiological condition in response to which its chemical and physical composition vary (De Brouwer and Stal, 2002). In a study of *C. closterium* EPS De Brouwer and Stal (2002) distinguished two fractions, soluble (present in the culture supernatant) and bound (extracted using 30^{o}C water). It is proposed, as has been previously suggested (Wakefield, 2005), that this soluble fraction may contribute contaminants to the overlying water. It should also be noted that adsorption of Cd to inorganic particles is reduced in the presence of dissolved organic matter (Simpson, 1981), so if EPS has increased DOM this may result in a higher concentration of Cd in the water column.

3.4.7. Concentration factor

There was movement of Cd between the sediment and water compartments through the duration of the experiment. When sediment was initially contaminated CF_{Sed} quickly decreased through the course of the experiment and when water was initially contaminated CF_{Sed} increased. The opposite was observed for CF_{Wat}, which increased over time when sediment was the initially contaminated compartment and decreased when water was initially contaminated (Table 3.4). This was due to the process of equilibration of Cd between the two compartments;

had the experiment run for a longer period these figures were expected to stabilise as equilibrium was reached.

Equilibration between the two compartments of sediment and water appeared to be mediated to some extend by the presence of algae, which was in line with observations described above (Figs. 3.13, 3.15 and 3.16 and sections 3.4.5 and 3.4.6).

3.4.8. Cadmium in pore water

Due to the difficulty extracting pore water there were fewer replicates (particularly for treatments with processed sediments) available for analysis, it is therefore difficult to make comparisons between Cd_{Pore}, Cd_{Wat}, Cd_{Sed} and Cd_{Alg}. The higher concentrations in pore water when overlying water was the initially contaminated compartment were probably because the sediment and water concentrations did not have time to equilibrate over the course of the experiment. Whilst sediment and pore water concentrations were stored for 40 days to allow equilibration to occur as recommended by Simpson et al. (2004) (Section 3.2.2), when contaminated water was added to uncontaminated sediment at the start of the experiment equilibration occurred only during the experiment (Table 3.4, Sections 3.3.12 and 3.4.7).

Cd_{Pore} was extremely low across all treatments, even lower than Cd_{Wat} when sediment was initially contaminated, but the effect of the presence of *C. closterium* was clearly that pore water concentrations were higher than in treatments without (Fig. 3.18). Reasons for this are probably related to availability of oxygen as described for water concentrations (above) but this would require further investigation.

In addition, there was an apparent increase of Cd_{Pore} over time, particularly where diatoms are present (Fig. 3.18), it would be of interest to continue this experiment over a longer time period to discover how this develops.

3.5. Conclusion

It has been established that diatoms take up a significant quantity of Cd from the sediment as well as the water column and that this differs with sediment properties. It has also been shown that the presence of diatoms alters Cd concentrations in overlying and pore water. Further investigation is required to establish the controls on contaminant uptake from sediment and to better understand the differences that occur due to other sediment properties. Additionally there is more work to be carried out in understanding why the presence of diatoms increases Cd in the dissolved phase. However, this study has shown that, using these methods, under

controlled laboratory conditions, it is possible to carry out studies of metal uptake from sediment using benthic diatoms and paves the way for these further questions to be answered.

With so few previous studies in this area there is great scope for further work with other metals and contaminants of concern as well as other algae species. Furthermore studies could take into consideration variations in contaminant concentration, salinities and temperatures as have been carried out in studies of contaminant uptake from the water column (Pérez-Rama et al., 2010, Xu and Morel, 2013). It would also be of interest to increase the scale of the study in both time and space to extrapolate results to the estuarine system. Recent reviews have highlighted the need to understand metal behaviour in complex real world environments, as well as the laboratory (Wernberg et al., 2012) and ensure that results observed in controlled settings tally with field observations (Belzunce-Segarra et al., 2015).

Chapters 4 and 5 will begin to address these concerns. In Chapter 4, the range of metals will be increased and effects of temperature and algal type will be investigated in a more complex mesocosm system. In Chapter 5 a field study addresses issues of seasonality and variation in uptake across the tidal frame.

4. Effects of water temperature and sediment disturbance on contaminant uptake from sediment to different types of algae

4.1. Introduction

Whilst chemical analysis of sediment provides a quantitative measure of the degree of contamination, assessing bioavailability and food chain transfer is less straightforward (Burton Jr., 2010). This is especially true in the sediments of the intertidal mudflat that represent a complex system subject to constant change due to tides, seasons and weather events. These factors can all affect metal bioavailability through variations in salinity (Chapman et al., 2013), redox conditions (Eggleton and Thomas, 2004) and resuspension of sediment (Atkinson et al., 2007). All of which could potentially impact contaminant uptake by microphytobenthos (MPB). Additionally, changing seasonal conditions of light availability and temperature will affect the growth of MPB (Barranguet et al., 1998) which may also influence contaminant uptake (Suresh Kumar et al., 2015).

The MPB experiences a wide range of temperatures with seasonal variation and due to the immersion/emersion cycle. For example, in the summer, the temperature can change over the course of a few hours from ~10 ^{o}C (when covered by the tide) to 30 ^{o}C or more (when exposed) (Alsterberg et al., 2011). Temperature has an effect on photosynthesis which has been found to be reduced at temperatures exceeding 25 ^{o}C and to cease at temperatures over ~35 ^{o}C (Morris, 2005). The same study also found there was very little photosynthesis in the biofilm at temperatures of 5 ^{o}C and below.

Over the next 100 years seawater temperatures in the Celtic-Biscay shelf are predicted to rise by between 1.5 ^{o}C and 5 ^{o}C (Philippart et al., 2011). This sea area covers the location of the Adaptation and Resilience of Costal Energy Supply (ARCoES) project test case area of the North West English coast and the Ribble estuary field site discussed in Chapter 5. Studies on the effect of temperature on uptake of metals by microalgae from water are not consistent and various reports have shown increased, decreased and no effect of temperature on metal uptake by microalgae (Monteiro et al., 2012). There may also be variation with algal species and type of metal (Suresh Kumar et al., 2015).

In Chapter 3 it was observed that a single metal, cadmium (Cd), was taken up from both contaminated water and sediment by the diatom *Cylindrotheca closterium*. However, this simplified system had stable temperature and light and did not experience any tide. Additionally, there was only a single species of algae represented, which is very different from the mixed biofilm of a natural intertidal mudflat.

There is a recognised need to ensure that effects observed under laboratory conditions are maintained in more complex environments (Belzunce-Segarra et al., 2015). Additionally, in a review of climate change experiments Wernberg et al. (2012) identified the need for increased effort in the areas of, amongst others, combined effects of climate change and non-climate change stressors; responses of primary producers; reduced pseudo-replication; and increased realism. These needs can be met with the use of a mesocosm. These issues are assessed in this chapter through the use of a mesocosm study in which an algal community is grown on sediment in large tanks, under natural light, with controls on water temperature and levels of sediment disturbance.

4.1.1. The use of mesocosms in marine benthic research

Mesocosm use has increased in popularity in recent years as it enables the study of processes at close quarters, with replication and some level of control, but under more realistic environmental conditions than laboratory or microcosm studies can achieve.

What is a Mesocosm?

The term mesocosm was first used in the book *"Marine Mesocosms"*, edited by Grice and Reeve (1982), to describe experimental ecosystems larger than the laboratory scale, bench top microcosm but excluding very large enclosures which might constitute a sub-unit of the natural environment. Mesocosm size was later defined as between $1m^3$ and $1000m^3$ by Lalli (1990), in order to distinguish them from microcosms ($<1m^3$). These distinctions are somewhat arbitrary and are not strictly adhered to (many studies describing much smaller systems as mesocosms) but offer a rough guide.

Mesocosms are experimental enclosures that attempt to replicate the conditions of the ecosystem being studied so that the processes occurring in the natural system also occur within the mesocosm. They offer the ability to control or manipulate the system in order that the natural systems and the impact of any potential changes to it (e.g. due to climate change effects) can be better predicted. A mesocosm may be established by creating a copy of the system of interest (Lauth et al., 1996, Pennington et al., 2007) or by isolating part of the system *in situ* in order to study the processes (de Wilde, 1990).

The Marine Ecosystem Research Laboratory (MERL) in Rode Island, USA, (an example of a recreated system) was established in 1976 to simulate the estuarine environment of Narragansett Bay (de Wilde, 1990). It consists of fourteen outdoor cylindrical enclosures of 1.8m diameter and 5.5m depth (Doering et al., 1989). These have been used to run a range of

long-term (lasting more than one year) and shorter experiments observing ecosystem response to pollutants, stratification, salinity gradients and the behaviour of trace metals and hydrocarbons (Wade and Quinn, 1980, Doering et al., 1989).

Large, in situ experiments were carried out in the 1980s using the Bremerhaven Cassions, large floating structures which were lowered onto and enclosed areas of mudflat in the Wadden Sea (Fig. 4.1) as described by Farke et al. (1984). These were operated as flow through systems with pollutant studies required the addition of contaminants to the inflowing water on every rising tide (Farke et al., 1984).

A smaller in situ experiment was recently completed in the Mar. Menor lagoon, Spain, in which 3.25 cm^2 biofilm coated glass slides were deployed for 20 days and subject to periodic treatments of dissolved nutrients and metals (lead (Pb) and zinc (Zn)) to investigate interactive effects (Belando et al., 2017). The study found there was a change in community composition and increase in metal uptake due to increased nutrient or metal loads or when treatments were applied together.

Figure 4.1. The Bremerhaven Cassions in intertidal area, German Bight (Farke et al., 1984)

An advantage of recreating the system, over the in situ method, is that it allows for better controlled replication and for a range of experiments to be carried out by simply resetting the experiment and starting afresh, it also avoids problems of introducing potential contaminants to natural systems. The ex situ mesocosm offers the ability to work with the benthos in a close to

natural environment without the inherent access difficulties of working on the intertidal flat and it allows close monitoring and frequent measurement of the system properties. That said, in situ mesocosms do have the advantage that, although they are nominally isolated, they most closely replicate the environmental conditions and processes of the natural system.

Early mesocosms developed for the study of estuarine systems were generally without replication, so whilst some idea could be gained of how a system operated, there could be no statistical analysis of results. Pennington et al. (2007) identified this lack of replication as a significant failing in mesocosm experiments and developed the intertidal replication mesocosm which, in part due to its flexibility, has been utilised for numerous experiments carried out by the National Oceanic and Atmospheric Administration (NOAA). These include several studies into the effects of pollutants in the intertidal system (DeLorenzo et al., 1999, Wirth et al., 2004). The system consists of a series of pairs of tanks, one housing sediment and biota and the second acting as a reservoir for the tidal water (Fig. 4.2). Water is pumped up from the bottom tank to simulate the rising tide and by having a pair of tanks in each set up, as opposed to a single supply, true replication is achieved. Pennington et al. (2004) described the modular estuarine mesocosm system as an opportunity to "answer questions that are basically unanswerable in laboratory toxicity tests with statistical power that is lacking in field tests".

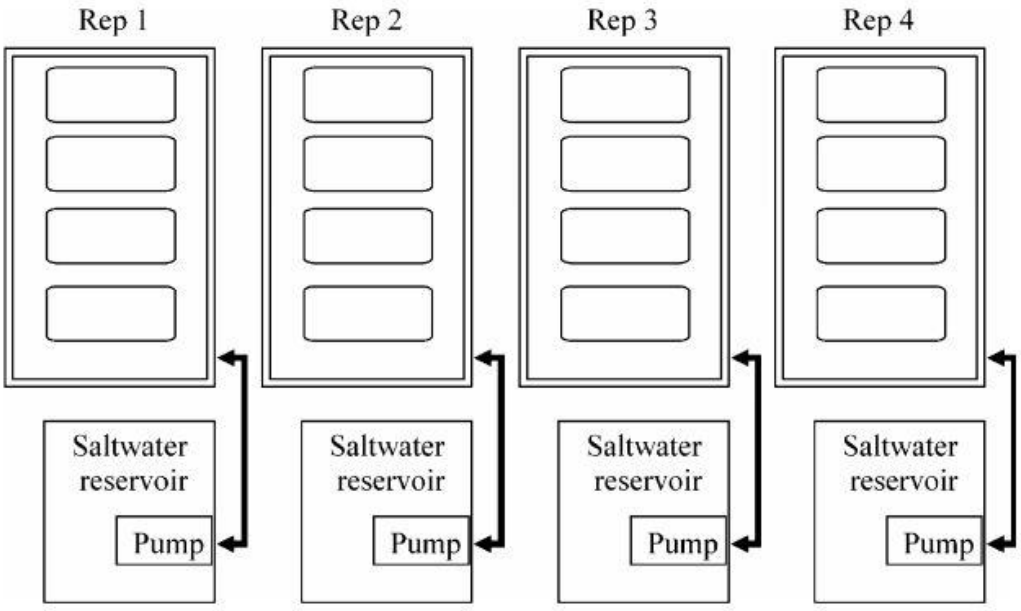


Figure 4.2. Diagrammatic illustration of the replicate mesocosm designed by Pennington et al. (2007) and used by Balthis et al. (2010)

Balthis et al. (2010) used the mesocosm design by Pennington et al. (2007) to investigate a subtidal as opposed to intertidal habitat installing sediment without saltmarsh vegetation. Spiked sediment slurries were added to field collected sediment (in contrast to previous studies which added contaminant to water). This particular study did not measure uptake of the contaminant, but instead took community measures such as diversity and infaunal density of benthic macrofauna.

Ferry et al. (2009) carried out a study into uptake of gold nanoparticles using a similar mesocosm design, but, in this instance, it was intertidal and included saltmarsh vegetation. It was shown that the nanoparticles partitioned between biofilms, sediments, plants, animals and sea water and that multi species biofilms had the second highest concentration per mass of the nanoparticles after the filter feeding clam *Mercenaria mercenaria*. These studies highlight the potential for the use of these types of mesocosm in studying both community effects and pollutant partitioning within the estuarine ecosystem.

Mesocosm studies have demonstrated the potential for studying natural MPB assemblages in the laboratory. Defew et al. (2002) showed that there was no significant difference between the species richness and photophysiological response of diatom assemblages on collection and after 14 days in a laboratory mesocosm making them a useful experimental model. Orvain et al. (2003) confirmed, through the use of scanning electron microscopy, that natural diatom migration patterns were maintained in a tidal mesocosm. Additionally, the MERL experiments, which were carried out over longer timescales, have shown that transplanted benthic communities survive well in tanks (de Wilde, 1990). These studies reveal the suitability of natural algal benthic communities for use in these types of research projects.

The use of mesocosms provides the opportunity to control and manipulate a close to natural system, whilst providing the opportunity to take multiple measurements and carry out replication. Previous studies have demonstrated the opportunity to examine effects of contaminants and climate change in a natural benthic community whilst maintaining control over some of the variables. For the current study this enables measurement of metal uptake from sediment to a natural MPB community under a tidal cycle, whilst maintaining control of water temperatures and levels of sediment disturbance.

The main question addressed by the mesocosm study here is whether contaminant uptake by MBP will be affected by predicted increases in water temperature due to climate change? In addition variation in uptake between algae types and the effect of disturbance of the sediment surface on contaminant uptake will be examined.

Hypotheses

- *Metal uptake by algae from sediment increases with increasing water temperature*
- *Epipelic diatoms take up a greater concentration of metals than macroalgae (filamentous and sheet)*
- *Disturbance increases metal concentration in overlying water*
- *Disturbance increases metal concentration in algae*
- *Metal concentrations in the water decrease with increasing algal biomass*

4.2. Methods

4.2.1. Mesocosm construction

The mesocosm design followed that of Lauth et al. (1996) and incorporated the modifications of Pennington et al. (2004). The design here consists of nine pairs of tanks (Fig. 4.3) maintained, on a wooden platform, in a polytunnel facility at University of Stirling (Fig. 4.4). In the present study the tanks were run to model an estuarine intertidal sediment habitat.

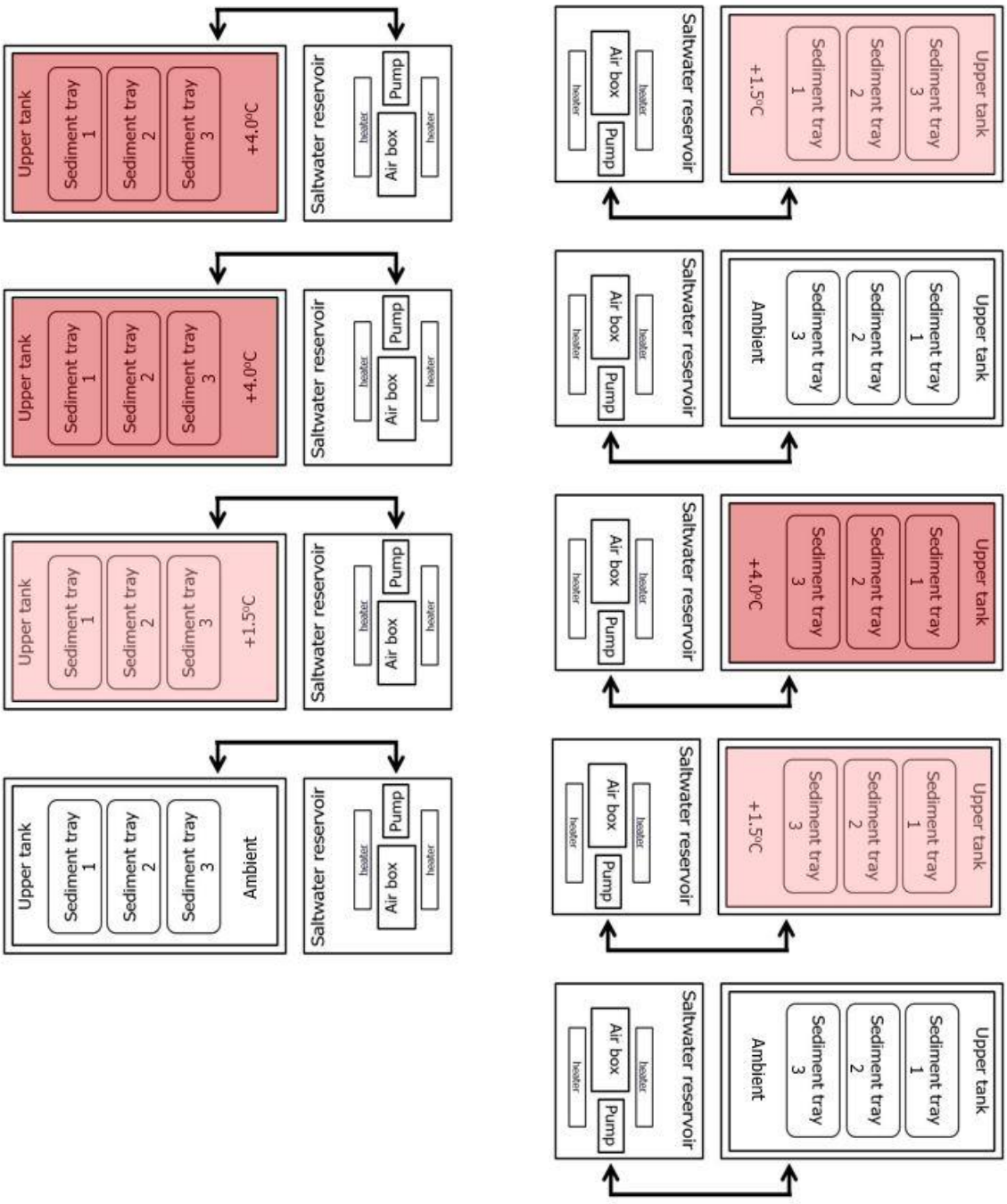


Figure 4.3. Schematic of nine replicate mesocosm units

Polytunnel

The mesocosm was situated within a polytunnel in the gardens of University of Stirling. Prior to the construction of the mesocosm platform, the polytunnel was refurbished with new plastic sheeting and netting. A clear polythene (Lumisol Clear AF, Visqueen®, Stevenston, UK) was selected as the most transparent film available with less than 30% light diffusion and more than 97% light transmission. This was chosen to allow outdoor light levels to be as closely matched as possible. The sides of the polytunnel to a height of 1m were fitted with shade netting. This reduced the possibility of warming on the lower water tanks and helped improve airflow and reduce air temperatures within the polytunnel, particularly during the summer months. In addition the polytunnel was fitted with two air circulation fans which were programmed to operate if the air temperatures rose above 18 °C so that the air temperature in the polytunnel did not rise to unrealistic levels.

Platform

A wooden platform 3.45 m by 2.75 m (Fig. 4.5) was constructed on a levelled and paved area in the polytunnel. The platform comprised of a lower shelf ~20 cm above the floor for the lower water tanks and two upper shelves for the upper tidal sediment tanks which were separated by a central walkway and accessed by wooden steps. Two further shelves were fitted underneath the walkway to hold the temperature controllers and other electrical equipment.

Tanks

There were nine pairs of tanks, each consisting of an upper (135L) and lower (250L) tank. The lower tank acted as a storage sump for the tidal water and contained the water pump and heaters (Fig. 4.6). The upper tank contained sediment trays representing the mud flat (Fig. 4.8).

The upper and lower tanks of each pair were connected by two lengths of reinforced plastic tubing (25mm diameter) through holes in the upper tank (Fig. 4.7). A timer operated aquarium water pump (Compact 1000, EHEIM GmbH, Deizisau, Germany) was fitted to the lower of these tubes which, when switched on, pumped water to the upper tank to simulate the rising tide. The second tube attached to the top of the upper tank and acted as an overflow, controlling the maximum tidal height. Non-toxic aquarium sealant was used on all connections.

The lower tanks were insulated around four sides and on the base using roofing insulation to prevent sudden/extreme fluxes in water temperature. The upper tanks were insulated on three sides only so that access to the water connections was maintained (Fig. 4.7).

Sediment trays

Each upper tank contained three rectangular plastic trays (36 x 29.5 cm, 14.5 cm high, created from 9 L washing up bowls, Fig.4.8). These were drilled with 25mm diameter drainage holes (one on each side and one on the base) which were fitted with fibreglass mesh to prevent sediment escaping (Fig. 4.10). There were additionally four holes around the top of each tray to ensure that water drained from the sediment surface when the tide receded. Trays were placed on 2cm high stanchions (made from rings of drainage pipe) to ensure free drainage (Fig.4.8).

Water supply

The lower water tanks were connected to two large (1000 L) water supply tanks, containing distilled water, via a series of plastic tubes and taps (Fig. 4.4). This enabled the lower tanks to be topped up with distilled water by operation of a hand pump as necessary throughout the duration of the experiment.

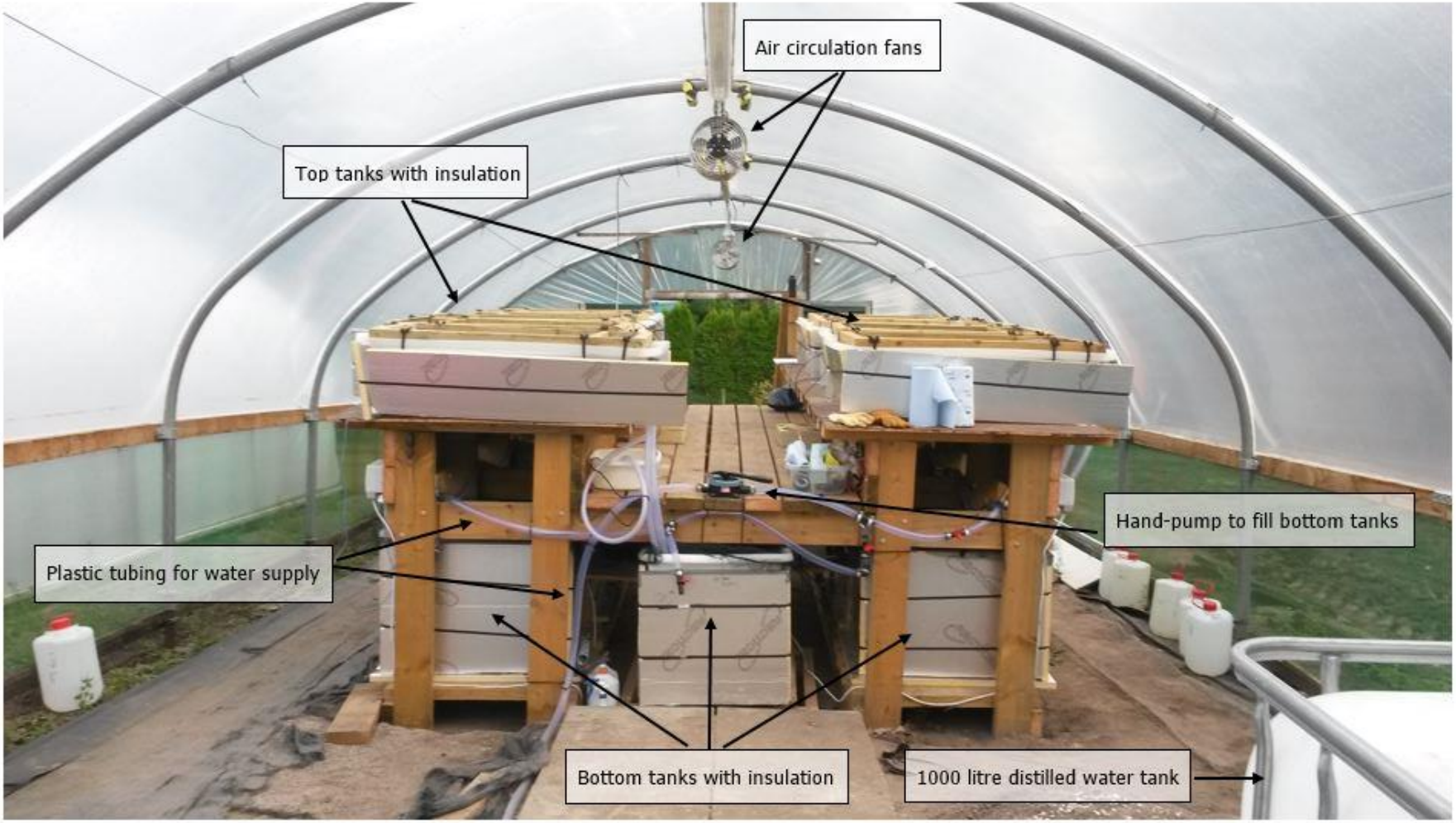


Figure 4.4. Complete mesocosm showing insulated tanks and water supply

Figure 4.5. Wooden platform in polytunnel

Figure 4.6. Inside of lower tank showing water pump, heaters and air box

Figure 4.7. Upper and lower tanks showing tubing connections and insulation

Figure 4.8. Sediment trays in upper tank showing stanchions and drainage holes

4.2.2. Water and sediment

Water

Lower tanks were filled with 150L artificial sea water (prepared by adding synthetic sea salt, Tropic Marin® (Wartenberg, Germany) to distilled water to a salinity of 22). Salinity was maintained by topping up the tanks with distilled water to a marked level on a weekly basis in the warmer months and biweekly in colder weather when evaporation was less. Salinity was checked with the use of a refractometer on a weekly basis and adjusted through addition of distilled water if necessary.

To maintain the pH and oxygenation of the water each lower tank contained a plastic box, with holes drilled around the base and lid, filled with crushed oyster shells through which air was continuously pumped by means of a Hailea V60 air pump (Guangdong, China) (Fig. 4.6).

In order to simulate a tidal cycle, water was pumped from the lower sump tank to the upper sediment tank twice daily. Sediment was inundated for a total of eight hours per day with two low tides from 04:00 to 08:00 and 16:00 to 20:00. Low tides were set (using analogue timers) to occur at the same time each day, there was no lag.

Sediment preparation

Sediment trays were filled to a depth of 6.5 cm with a mix of pre-washed silver sand and 0.5 cm gravel, topped with a layer of fiberglass mesh (to prevent migration of the gravel) followed with a 2 cm layer of pre-washed silver sand. Sand and gravel were washed firstly in tap water, to remove any fine particles, and then three times in distilled water, prior to use.

Intertidal sediment was collected in 10L buckets from approximately the top 50mm of the mudflats at Lytham St. Anne's, Lancashire, UK (53°43'58"N, 2°57'37"W). Following collection, the sediment was sieved to 2 mm (to remove mieofauna), homogenised and stored at 4 °C in the dark. The sediment was thoroughly mixed prior to use firstly during the sieving process when the contents of two 10L buckets were sieved together. Secondly, sediment was homogenised by mixing the contents of all buckets together in an 84L plastic box a third at a time (Fig. 4.9). Finally (having been returned to the buckets for transport to the polytunnel) sediment was mixed in an 84L plastic box prior to adding to the sediment trays. The intertidal sediment was added on top of the sand layer and made flush with the top of the tray on the 15th September 2015, immediately prior to the start of the experiment (Fig. 4.10).

Sediment was sampled immediately prior to use and analysed for organic matter content, particle size and metals (sampling point M00). No additional organisms were added to the experiment, only the natural algae community contained within the collected sediment was utilised.

Figure 4.9. Mixing sediment following sieving

Figure 4.10. Sediment tray with layers of sand and gravel, sand and sediment

4.2.3. Experiment setup

The experiment was initially run to investigate the effects of increased water temperature on metal uptake to algae. This phase ran from 15th September 2015 to 28th March 2016, with a break from 14th November to 2nd December 2015 due to a failure of the heating system. Following the first phase, a sediment disturbance element (described below) was introduced alongside the temperature manipulation, which ran for four weeks from the 8th April to 3rd May 2016.

Temperature control

The nine pairs of tanks were set up in groups of three to achieve three true replicates. For each group of three, the tank water was held at either ambient temperature or heated to +1.5 °C above and +4 °C above ambient temperature. The distribution of temperature treatments on the mesocosm platform is shown in Figure 4.3. Ideally tanks would have been randomly sited, but this was not possible due to the temperature control wiring which dictated treatment position to some extent. It was noted that different sides of the platform experienced slightly different temperatures due to the position relative to the sun. This effect was more apparent at the start of the experiment, when the sun was higher and daylight hours were longer, than in the winter (Fig. 4.12). The effect of variation in conditions between tanks due to position was accounted for in statistical analysis by making the category "Tank" a random variable.

Regulation of the water temperature was achieved using controllers, one per tank group, built using Arduino™ boards (Fig. 4.11) which were wired to receive input from a thermistor in each of the lower tanks. The controller recorded the water temperatures in each tank every five seconds and calculated the difference in water temperature between the tanks in each group. The 200W aquarium heaters (V^{2}Therm, TMC, Chorleywood, UK) in the lower tanks were switched on and off by the controller to maintain the temperature differences between the tanks. The programming code for the controllers is available in Appendix 4-1.

The heating system failed on the 14th of November 2015 due to an internal shut off within the aquarium heaters which activated when the water temperature dropped below 7 °C. To resolve this the heaters were dismantled and the internal control system removed. The heaters were then resealed and reconnected and the experiment was restarted on 2nd December 2015.

The air temperature within the polytunnel was recorded hourly along with the water temperature and sediment temperature in one sediment tray of each tank. Outside air temperatures for the period of the experiment were obtained from a weather station at Grangemouth Refinery (56°0'32"N, 3°41'6"W, approximately 10 miles from the polytunnel) from the Met Office.

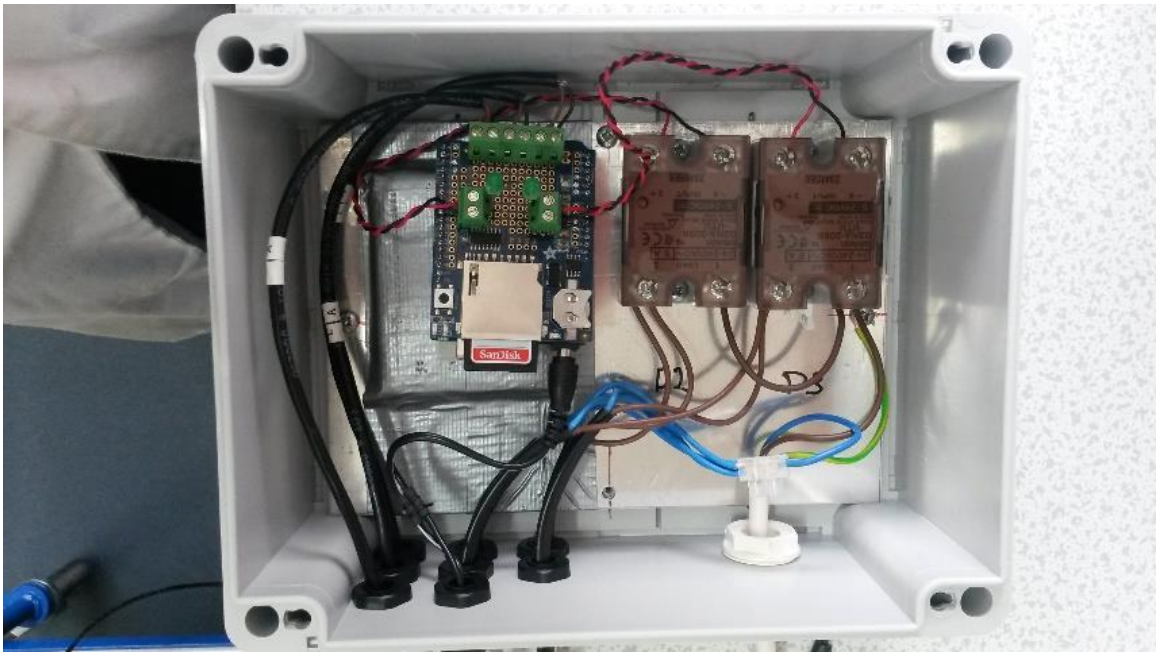


Figure 4.11. Temperature controller in waterproof box showing Arduino board and heater switches

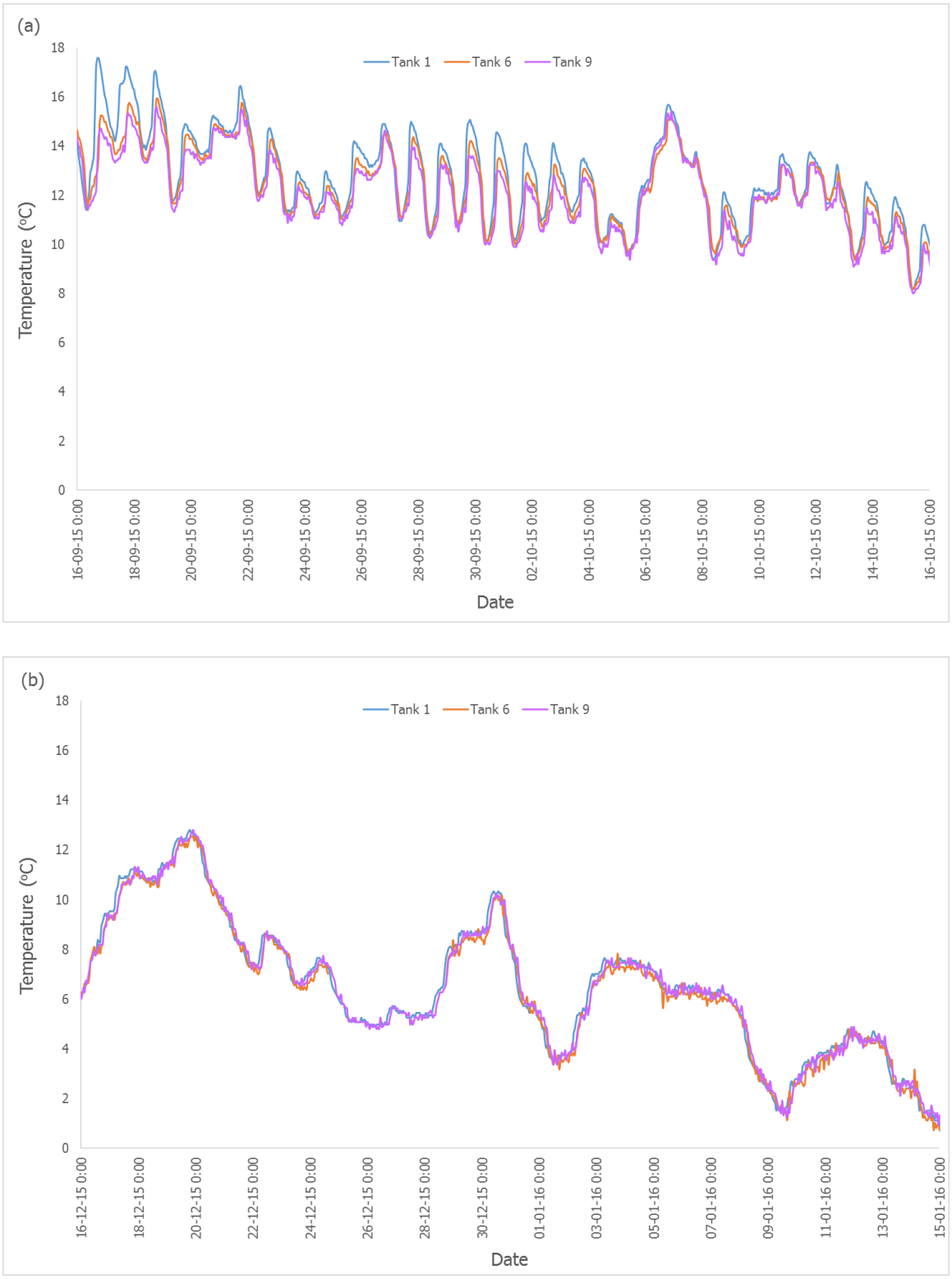


Figure 4.12. Water temperature in ambient treatment tanks in September 2015/October 2015 and December 2015/January 2016.

Disturbance of sediment surface

From the 8th of April the sediment surface in each tray was disturbed using a wide toothcomb dragged across the sediment surface to a depth of 5mm (Fig. 4.13). This was carried out to simulate the increasing levels of disturbance which might be experienced due to increasing storm frequency, a potential effect of climate change (Chapter 2, Section 2.4.3). There were three levels of disturbance, once, twice and three times per week, in sediment trays one, two and three of each tank respectively (Fig. 4.3). The method of disturbance chosen was similar to those employed in other studies (Cowie et al., 2000, Whomersley et al., 2010, Kenworthy et al., 2016), in all of which a rake was used to disturb intertidal sediments at varying frequencies. The temperature treatments were maintained throughout this period.

Replication was affected by the disturbance treatments because to collect enough diatoms for metals analysis, samples must be combined across tanks of the same temperature. For example, in order to obtain a sample of diatoms disturbed twice a week at ambient temperature, samples from tray two of the three tanks at ambient temperature were combined. During the disturbance period of the experiment there was, therefore, replication of temperature treatments and of disturbance levels, but no replication of the combined effect of temperature and disturbance.

Figure 4.13. Disturbance of sediment surface (with temperature probe inserted in sediment surface of right-hand tray)

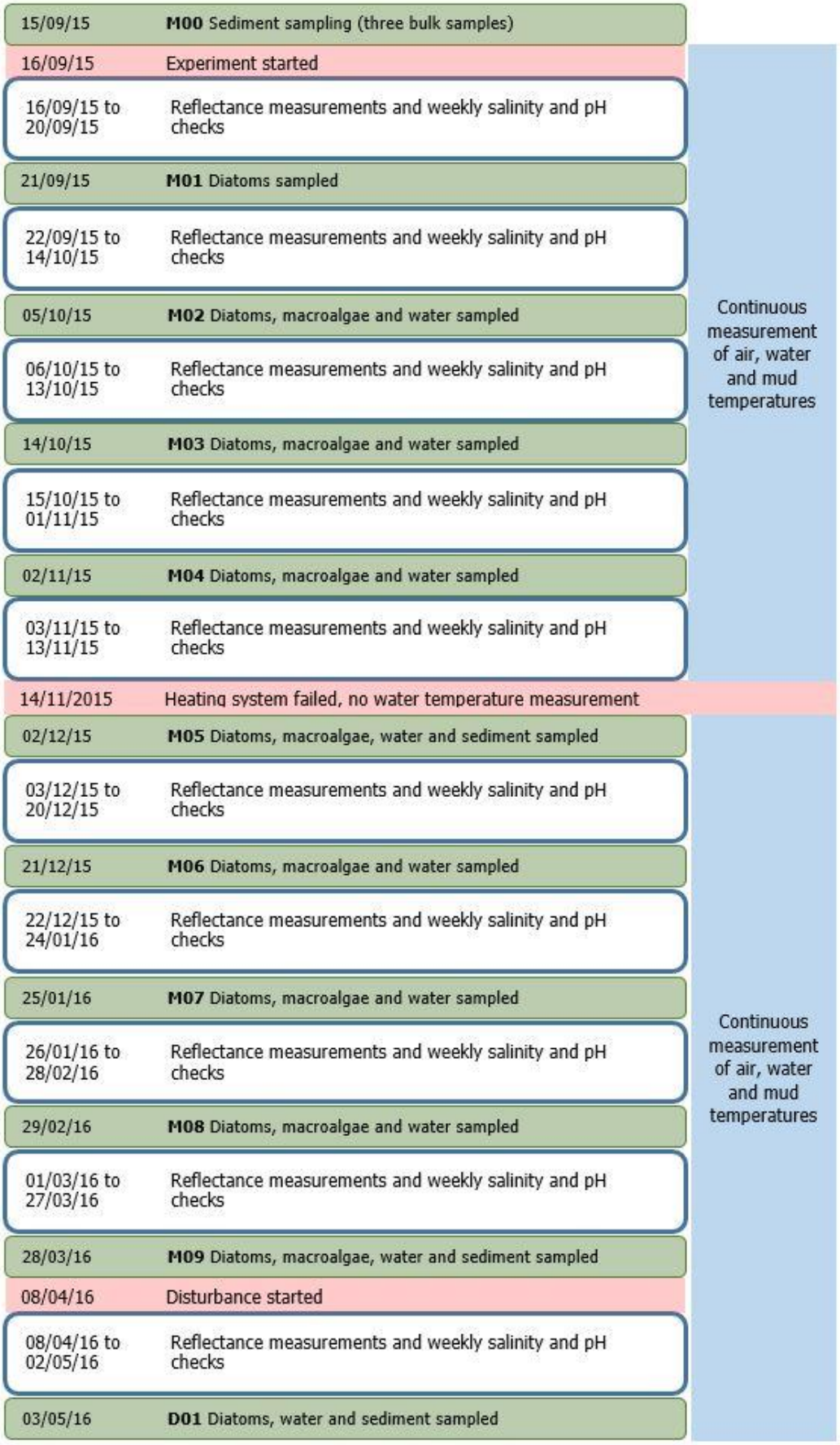


Figure 4.14 Sampling regime

4.2.4. Reflectance measurements (algal growth)

Growth of algae through the course of the experiment was monitored using Normalised Difference Vegetation Index (NDVI) as a proxy for biomass. NDVI is a very commonly used remote sensing technique used to assess vegetation cover and as a measure of its health and vitality. It operates on the premise that healthy vegetation, through the presence of chlorophyll and active photosynthesis absorbs red light in the visible spectrum whilst cell structure causes high reflectance in the near infrared (NIR) area of the electromagnetic spectrum. This results in reflectance spectra with a distinct trough at about 675nm (Fig. 4.15). Although there are a wide range of vegetation indexes developed to study different vegetation types (Meleder et al., 2010), NDVI has been shown to be a robust proxy for Chlorophyll *a* (chl *a*) in muddy sediment (Kromkamp et al., 2006).

In order to fully quantify biomass as chl *a* mgm^{-2} using NDVI it would be necessary to set up a transformation function using destructive sampling techniques which were not suitable for this study. So in this experiment NDVI has been used as a proxy to measure relative biomass levels through the course of the experiment.

Reflectance measurements were taken with the ASD FieldSpec® UV/NIR throughout the course of the experiment, initially daily and then with larger gaps, switching to weekly as the rate of change slowed. Measurements were made of the central tray (tray 2) in each tank throughout the course of the temperature only phase of the experiment, to avoid shading effects of the tank on trays 1 and 3, although as the natural light levels changed the tray was lifted out and illuminated with photographic lights (see Fig 4.16). At the final temperature only sampling point M09 (Fig. 4.14), measurements were taken from all trays. During the disturbance phase reflectance measurements were obtained from all trays on a weekly basis. When measurements were taken from all trays these were lifted out of the tanks and lit.

Reflectance measurement

The bare fibre optic was held vertically at a distance of 30 cm above the centre of the sediment surface so that a circular area of diameter 13 cm was within view of the instrument. For each tank the reflected upwelling radiance was measured from a white reference panel (Spectralon™) (Lu_r) followed by a reflectance measurement of the sediment surface (Lu_s). Spectral reflectance (R) is equal to Lu_s/Lu_r. Spectra were visually checked for any obvious errors simultaneous to measurement and if necessary measurements were repeated.

NDVI was calculated as follows:

Eq. 4.1. $(R_{750} - R_{673}) / (R_{750} + R_{673}) = NDVI$

where R_{750} is the reflectance ratio at 750nm in the NIR and R_{673} is the red reflectance ratio at 673nm (Rouse et al., 1973).

Initially measurements were carried out using the available daylight, however as the year progressed and light intensity diminished, it was necessary to carry out reflectance measurements with the use of photographic lights (Video Light 8 300W, Kaiser Fototechnik GmbH, Germany) (Fig.4.16). The change from natural light to photographic lights was made on the 12th November 2015, after sampling point M04. A comparison of reflectance measurements with and without the use of lights showed a linear relationship in which the use of lights gives a slightly higher NDVI value than without (Fig. 4.17).

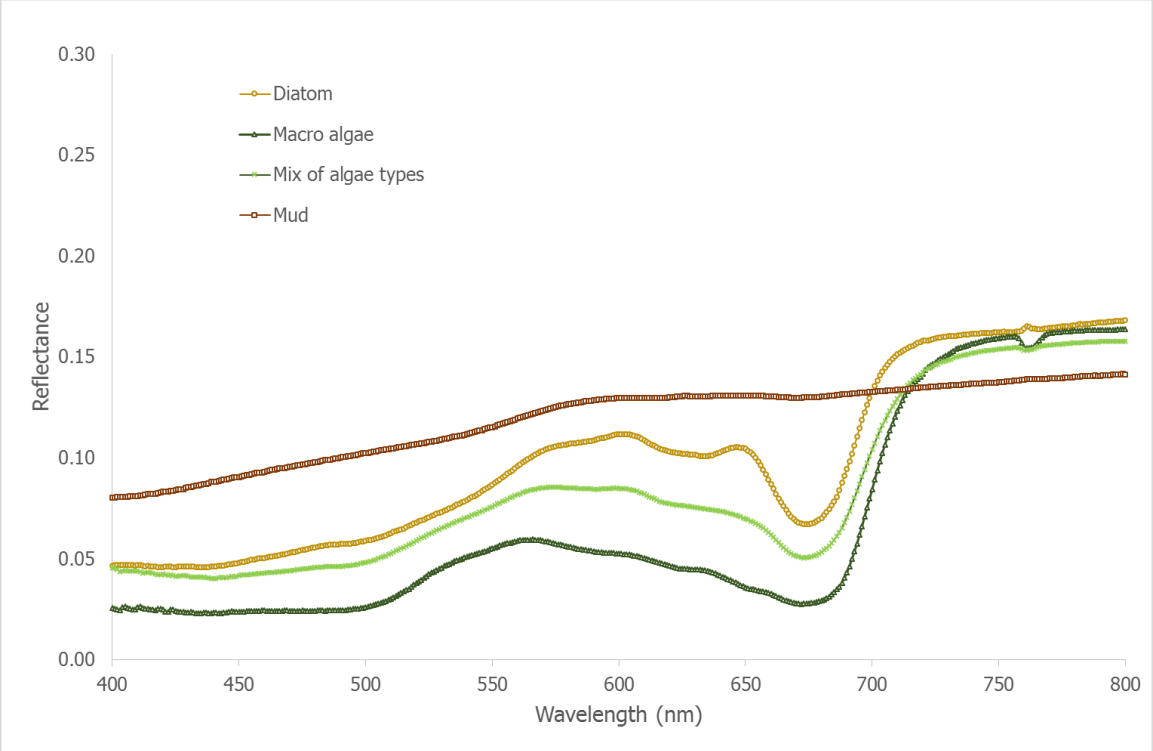


Figure 4.15. Reflectance spectra showing characteristic spectra for sediment dominated by different algae types measured in this project

Figure 4.16. Lighting setup for reflectance measurements used to calculate NDVI and monitor biomass

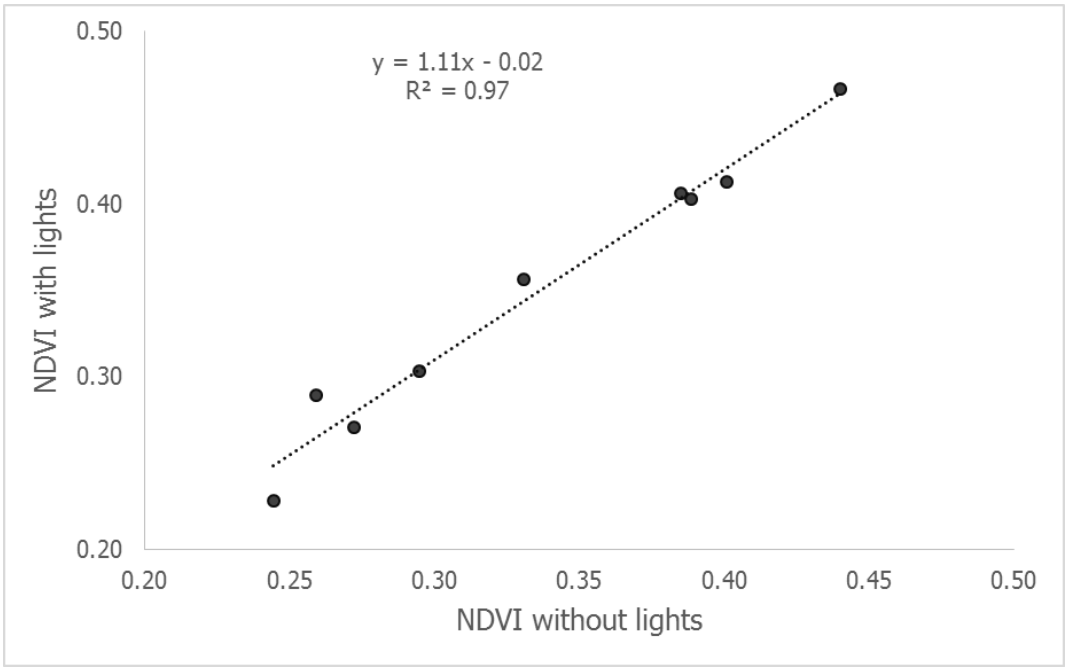


Figure 4.17. Relationship of sediment surface NDVI measured with and without the use of photographic lights

4.2.5. Temperature

Unfortunately the sediment temperature monitors (Squirrel, Grant Instruments, Cambridge, UK) failed on several occasions and although data appeared to have been recorded, these could not be downloaded. Sediment temperature data were therefore available for all tanks from 15/09/15 to 28/10/15 and for tanks five to nine from 28/10/15 to 23/12/15 and 28/02/16 to 4/05/16. There were no sediment temperature data for 24/12/15 to 27/02/16.

Due to the failure of the water heaters, there were no water temperature data available from 24/11/15 to 2/12/16 when the controllers were removed to repair the system.

4.2.6. pH

Water pH was checked throughout the course of the experiment on a weekly basis (at the same time as salinity checks) using an HI-98127 pocket pH tester (Hanna Instruments Ltd, UK). Water pH remained stable at pH 8 to 8.5 and was similar between all tanks.

4.2.7. Sampling and analysis

Overlying water

Overlying water was sampled at each sampling point (except M00 and M01, Fig. 4.14) whilst the top tank was flooded (i.e. prior to the tide going out) (Fig 4.18). A 125 ml water sample was collected from each tank in acid washed plastic sample bottles and immediately returned to the laboratory. In the laboratory samples were filtered through a 47 mm 0.45 µm cellulose nitrate filter (Whatman™) diluted to 1:5 with Milli-Q® water and acidified to 2% v/v with concentrated (70% w/w) nitric acid (HNO_3) in preparation for analysis by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

Figure 4.18. Flooded top tank

Diatoms

Collection of diatoms was carried out from 8.30am on each sampling day, as soon as the simulated tide had receded. Diatoms were sampled using a variation of the previously described lens tissue method (Chapter 3, Section 3.2.5). In the mesocosm sheets of lens tissue 200mm by 150mm were placed on the sediment surface of each sediment tray. This was topped with two sheets of lens tissue 150mm by 100mm. The bottom sheet of lens tissue was used to separate the upper pieces from the sediment. The upper two pieces were used to collect the algae sample. Two upper layers of lens tissue were used in an attempt to collect the maximum amount of algae with the rationale that by providing a deeper surface layer (i.e. two pieces thick) more algae could collect there and that if the light intensity was too great or the surface became too dry the algae would remain in the middle layer as opposed to the surface of the sediment and not move through the lens tissue. This method was devised as it had previously been observed (when trialling the method in the mesocosm with two layers of lens tissue) that whilst a large quantity of algae was migrating into the lower sheet of lens tissue, there was little evidence of algae in the upper sheet.

Four hours later, at 12.30 pm, the top two lens tissue layers were collected into a 50 ml centrifuge tube containing 20 ml artificial salt water and returned to the laboratory. Once in the laboratory, each sheet of lens tissue was opened out flat on the base of a plastic tray (new for each tank and acid washed prior to use). The lens tissues were then washed with 150 ml salt water dispensed from a wash bottle to remove attached diatoms to three 50 ml centrifuge

tubes. Diatoms were centrifuged at 180 rotational centrifugal force (RCF) for seven minutes and left to settle for 10 minutes before supernatant was removed by pipette. The remaining samples in each tube were combined to give one 30 ml sample per tray. These samples were further centrifuged, supernatant removed and combined to give a single diatom sample for each tank (Fig. 4.19).

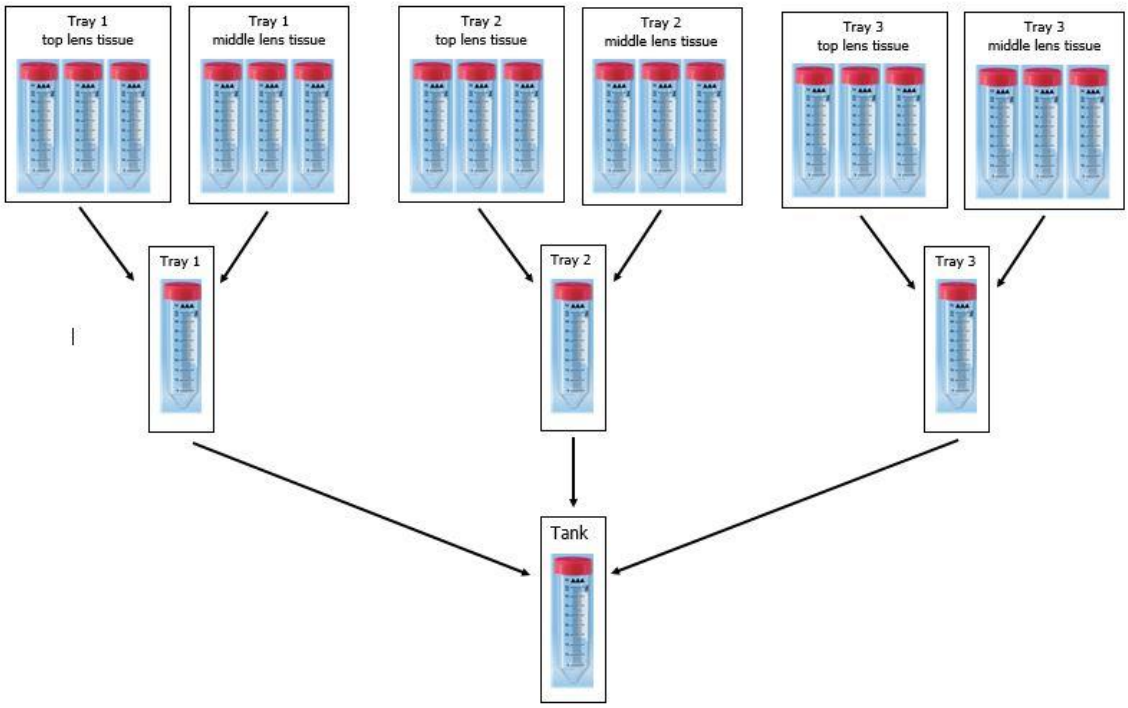


Figure 4.19. Schematic of diatom washing and combination for each tank

Filamentous algae

Following collection of lens tissues, filamentous macroalgae was sampled from the sediment surface (from the tanks in which they were present, Table 4.1) using tweezers and stored in 50 ml centrifuge tubes with 20 ml salt water for return to the laboratory.

Filamentous algae were washed in salt water dispensed from a wash bottle to remove any attached sediment or diatoms. Washed algae samples were checked under a microscope to ensure that this was effective, and samples were found to be clean of both sediment and diatoms.

The suspended diatom and filamentous algae samples were prepared for analysis as described in Chapter 3 (Section 3.2.6). Following vacuum filtration through two superimposed pre-weighed 47 mm, 0.45 µm cellulose nitrate filters (Whatman™), filters were dried at 65 ^{o}C for 24

hours and reweighed in advance of being separately digested in 15 ml centrifuge tubes for 48 hours with 1 ml HNO_3 and 0.5 ml perchloric acid ($HClO_4$) (72% w/w). The resultant samples were filtered and diluted to 75 ml with MilliQ® water to give a 2% v/v acid matrix for analysis by ICP-MS. The lower filter acts as a blank and metal concentrations (mg kg^{-1}) were determined as follows:

Eq. 4.2. weight of algae (g) = (weight of upper filter – initial upper filter weight (g)) - (weight of lower filter – initial lower filter weight (g))

Eq. 4.3. metal concentration in algae (mg kg^{-1}) = (sample volume (L) x (metal concentration in upper filter (ppb) - metal concentration in lower (blank) filter (ppb)) / weight of algae (g)

Sheet macroalgae

Sheet macroalgae was sampled in the same way as filamentous algae. On return to the laboratory sheet macroalgae was washed to remove any sediment or attached diatoms and any holdfasts were cut off as these had sediment attached. The macroalgae was then washed in MilliQ™ water to remove any salt and dried in foil trays at 65 ^oC for 24 hours. Once dry the macroalgae was lightly crushed with an agate pestle and mortar before being weighed into 15 ml centrifuge tubes. It was then digested in the same manner as other algae samples, but with additional blanks as there were no filters, and analysed by ICP-MS. Concentrations were calculated as per equation 4.2, above, using the average concentration from the blanks in place of the lower filter.

Due to variations in availability, it was not possible to collect filamentous and sheet macroalgae from all tanks at every sampling point. Table 4.1 gives details of which algal types were collected from which tanks at the various sample points.

Table 4.1. Algae collected at each sampling point

Sampling point		Algal group collected from tanks		
Name	**Date**	**Diatoms**	**Filamentous**	**Sheet macroalgae**
M01	21-09-15	All	None	None
M02	05-10-15	All	1, 4, 5, 8	1, 2, 3, 5, 6, 8
M03	14-10-15	All	4, 9	1, 2, 3, 4, 5, 6, 7, 8
M04	02-11-15	All	1, 2, 4, 5, 7, 8, 9	All
M05	02-12-15	All	1, 2, 4, 5, 7, 8, 9	All
M06	21-12-15	All	All	All
M07	25-01-16	All	All	All
M08	29-02-16	All	All	All
M09	28-03-16	All	1, 2, 3, 4, 5, 8 ,9	All
D01	03-05-16	All	None	None

Sediment

Sediment was sampled four times in the course of the experiment in a variety of ways dependant on the situation (Fig. 4.14 and Table 4.2). Prior to the start of the experiment three large (~ 500 g dry weight (DW)) subsamples of the sieved, homogenised sediment were taken, this is henceforth referred to as sample point M00.

Following the breakdown of the heating system and subsequent repair a sample of sediment was taken as a surface scrape from a ~5 cm^2 area of corner of tray 3 in each tank, to confirm sediment metal concentrations at the restart of the study. The sample was small (approximately 3 g DW) as the experiment was ongoing and disturbance of the surface or removal a large amount of material from the system would have been detrimental. This was sampling point M05.

At the end of the temperature only phase of the experiment (sampling point M09) small cores (1.6 cm diameter) of the top 2.5 cm of sediment were taken using 50 ml syringes with their ends cut off. These cores were immediately flash frozen in liquid nitrogen and stored at -40 °C. Prior to analysis these sediment cores were sectioned into upper (0 to 5 mm) and lower (5 to 20 mm) samples.

Finally, at the end of the disturbance phase (sampling point D01) samples were taken to reflect the different states of the sediment, disturbed upper layer and compacted lower layer. The disturbed sediment layer (approximately 5mm) was scraped off into plastic sample bag followed by removal of the lower compact layer (5 to 20mm) to a second sample bag (Fig. 4.20).

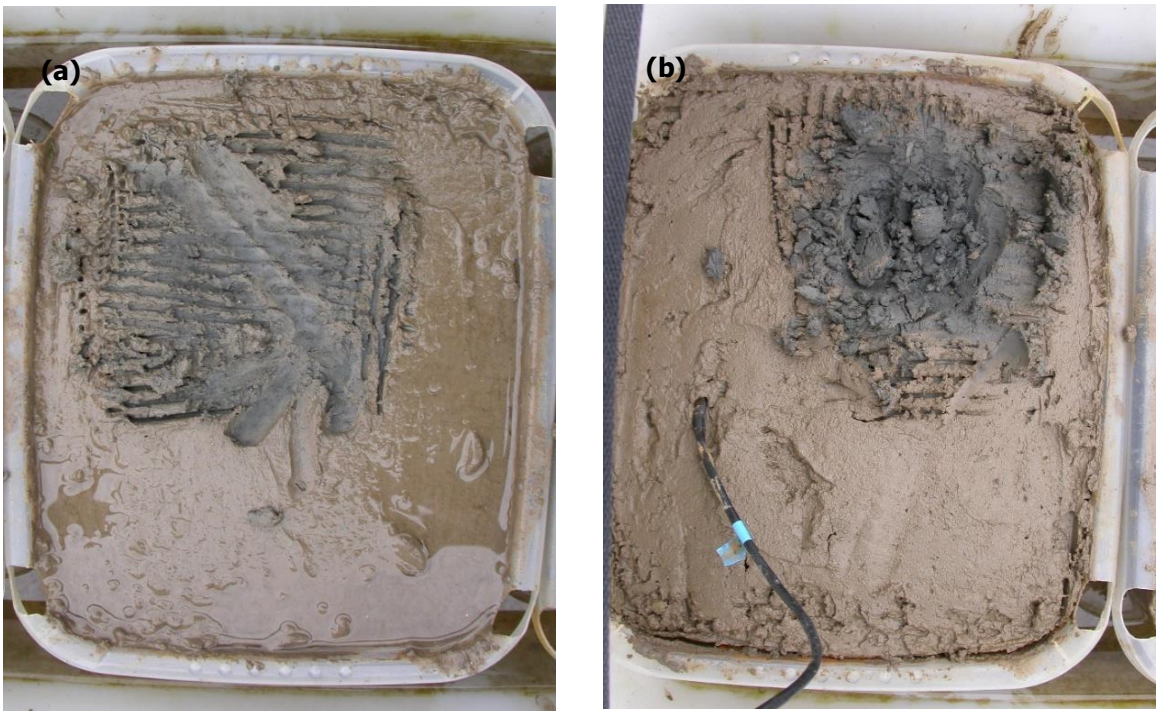


Figure 4.20. Sampling layers of the disturbed sediment at sampling point D01 showing collection of (a) the surface disturbed layer and (b) the compacted lower layer (with temperature probe inserted in sediment surface of right-hand tray)

The sediment analysis carried out at each sampling point is shown in Table 4.2. Water content was obtained by weighing the sediment in a pre-weighed foil container, before drying for 24 hours at 105 ^{o}C and reweighing the dry sample. Percentage water content was calculated as per equation 4.4, below.

Eq. 4.4. 100 - (((sediment + foil after drying) – foil (g)) /
((sediment + foil before drying) – foil (g)) x 100) = water content (%)

Percentage organic matter was measured through loss on ignition (LOI). Approximately 2 g of dry sediment was accurately weighed (weight recorded) into pre-weighed ceramic crucibles. Samples were heated in a furnace at 450 ^{o}C for 8 hours, then removed to a desiccator to cool before being reweighed. Percentage organic matter was calculated as per equation 4.5, below.

Eq. 4.5. 100 - (((sediment + crucible after ignition) – crucible (g)) / ((sediment + crucible before ignition) – crucible (g)) x 100) = organic matter (%)

Sediment particle size was determined with the use of the Coulter LS 230 laser granulometer for all three M00 samples and a third of M09 and D01 samples (39 samples in all) as described in Chapter 3 (section 3.2.6). Particle sizes were classified according to the Udden – Wentworth classification scheme for grain size (Wentworth, 1922) and are given as a percentage volume frequency.

Subsamples of sediment were prepared for metals analysis by grinding to 125 µm in a pestle and mortar (particle size was checked by passing samples through a sieve). A subsample of approximately 0.25 g was accurately weighed (weight recorded) into Teflon™ tubes to which 2 ml HNO_3 was added followed by digestion using a MARS Xpress (CEM Corporation) microwave digestion system using a standard programme (US EPA 3051_16, CEM Corporation (2009)). Samples were then filtered and diluted to 100 ml with Milli-Q® water to give a 2% v/v acid matrix in preparation for analysis by ICP-MS.

Table 4.2. Sediment sample description and analysis carried out

Sampling point		Sample type	Analysis			
Name	Date		Water content	Organic matter	Particle size	Metals
M00	15-09-15	Three bulk samples	No	Yes	Yes	Yes
M05	02-12-15	Surface scrape from tray 3 in each tank	No	No	No	Yes
M09	28-03-16	Syringe cores from all trays	Yes	Yes	Yes	Yes
D01	03-05-16	Scrape of surface (0-5mm) and compact (5-20mm) layers from all trays	Yes	Yes	Yes	Yes

4.2.8. ICP-MS analysis

Algae, sediment and overlying water samples were analysed for 14 elements (aluminium (Al), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), Zn, arsenic (As), silver (Ag), Cd, tin (Sn) and Pb) identified by Fairbrother et al. (2007) in their Framework for Metals Risk Assessment for the US EPA.

Analysis was carried out using an XSERIES 2 ICP-MS (Thermo Scientific, Germany) using collision cell technology (CCT) to reduce potential polyatomic interferences. Multi element standards (Merck, Germany) certified by the National Institute of Standards and Technology

(NIST), internal standards (scandium (Sc) and rhodium (Rh)) and blanks were included in each run. Accuracy was assessed by comparing reference sample determinations (CRM-142R: Community Bureau of Reference) with recorded values and all samples were run in triplicate. The instrumental limit of detection (LoD) for all analytes is given in Appendix 5-3.

4.2.9. Statistical analysis

All statistical analysis was carried out using R (version 3.2.3) in RStudio. The lme4 package (Bates et al., 2015) was used to perform linear mixed effects analysis of all examined relationships. To account for the effect of variation in temperature and light conditions due to tank position the random effect for all analysis was an intercept for tank. Fixed effects for all investigated response variables are shown in Table 4.3, with an example model for metal concentration in sediment shown in equation 4.6. Deviations from homoscedasticity and normality were checked by visual inspection of the residual plots. Chi-square (X^2) and p-values were calculated using likelihood ratio tests of the full model with the effect in question against the model without the effect in question and are reported to two significant figures. A sample model output is provided in Appendix 4-2.

Eq. 4.6. $Mn \sim Level + Organic + Temperature + Disturbance + (1|Tank)$

Table 4.3. Fixed effects used in linear mixed effects models

Response variable	Fixed effects					
	Sampling point	Temperature treatment	Disturbance level	Sediment depth	Organic matter	Algae type
Sediment properties						
Organic matter	X	X	X	X		
Water content	X	X	X	X		
Grain size	X	X	X	X		
Metal concentrations						
Sediment		X	X	X	X	
Water	X	X				X
All algae	X					
Diatoms	X	X	X			
Biomass		X				

4.3. Results

4.3.1. Temperatures

Air temperatures within the polytunnel during the period of the experiment ranged from a low of -3.0 °C in the early morning of two days in January and February 2016, to a high of 26.0 °C on the afternoon of 16th September 2015. Daily minimum temperatures in the polytunnel were similar to those recorded outside at Grangemouth Refinery (the closest available Met office weather station, approximately 10 miles away). Whilst the maximum air temperatures were similar from the end of October 2015 to the end of February 2016 outside of these dates maximum air temperatures inside the polytunnel were higher (by up to 8 °C) than those recorded outdoors (Fig. 4.21). The range of air temperatures in the week prior to each sampling point varied from a difference of 20.5 °C in the autumn and spring to difference of 11.5 °C in the winter (Fig. 4.23 and Table 4.4).

The control system worked as expected and maintained water temperatures at ambient plus 1.5 °C and plus 4.0 °C throughout the experiment (Fig. 4.22), except for a period of 18 days from 14th November 2015 (when internal controls in the heating units overrode the system, see section 4.2.2) until 2nd December 2015, when the situation was resolved and the system repaired and restarted. Water temperatures followed air temperatures and were lowest in January prior to sampling point M07 and highest in September prior to sampling point M01 in ambient and +4.0 °C treatments respectively (Table 4.4). Minimum water temperatures in the ambient treatment were almost always higher than minimum air temperatures, although they were lower for a few days in December 2015 (Fig. 4.23 (b)).

Sediment temperatures were lowest in December 2015 with a minimum of 0.08 °C recorded on 13th December 2015 and highest in September 2015 with a maximum of 25.6 °C on 18th September 2015 in ambient and +4.0 °C tanks respectively. Sediment temperatures were influenced by both air and water temperatures, with air temperatures exerting the greatest influence when the sediment surface was exposed and water temperatures having greater influence during inundation periods (Fig. 4.23 and Appendix 4-3). The seasonal sediment temperature patterns are clearly visible in Fig. 4.23. The greater range of air temperatures during the warmer months of autumn and spring (September to October and March to May, corresponded to sampling points M01, M02, M03, M09 and D01) and caused a greater range of sediment temperatures with highs and lows controlled by air temperature (Fig. 4.23 (a)). In the colder winter months (November to February, sampling points M04 to M08) the range of sediment temperatures was more strongly influenced by water temperature. This was particularly evident in the plus 4.0 °C treatment (Fig. 4.23 (b)). Due an equipment failure (see section 4.2.5), sediment temperatures were not recorded prior to sampling points M07 and M08.

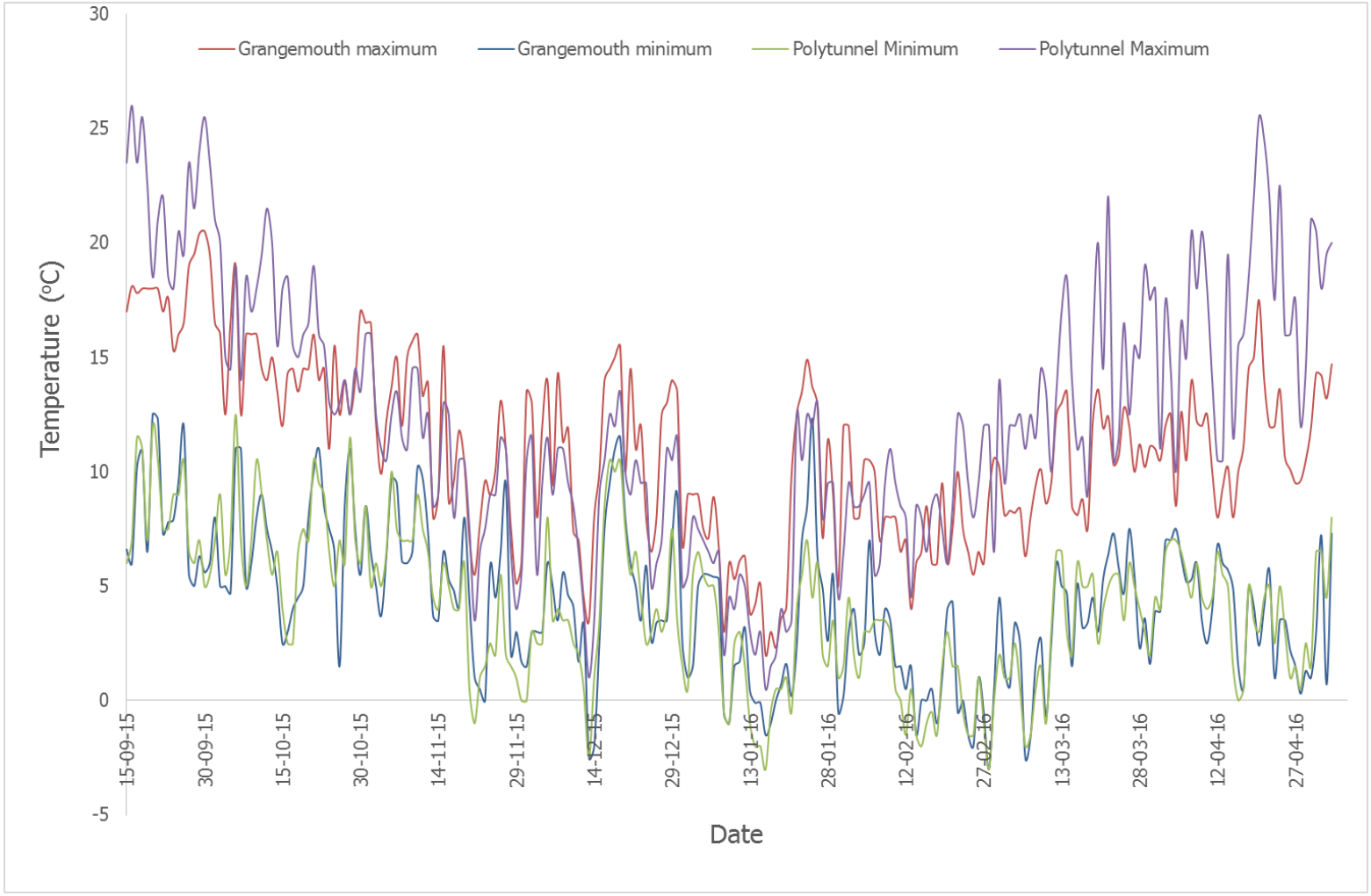


Figure 4.21. Minimum and maximum air temperatures in the polytunnel and at Grangemouth Refinery through the duration of the experiment

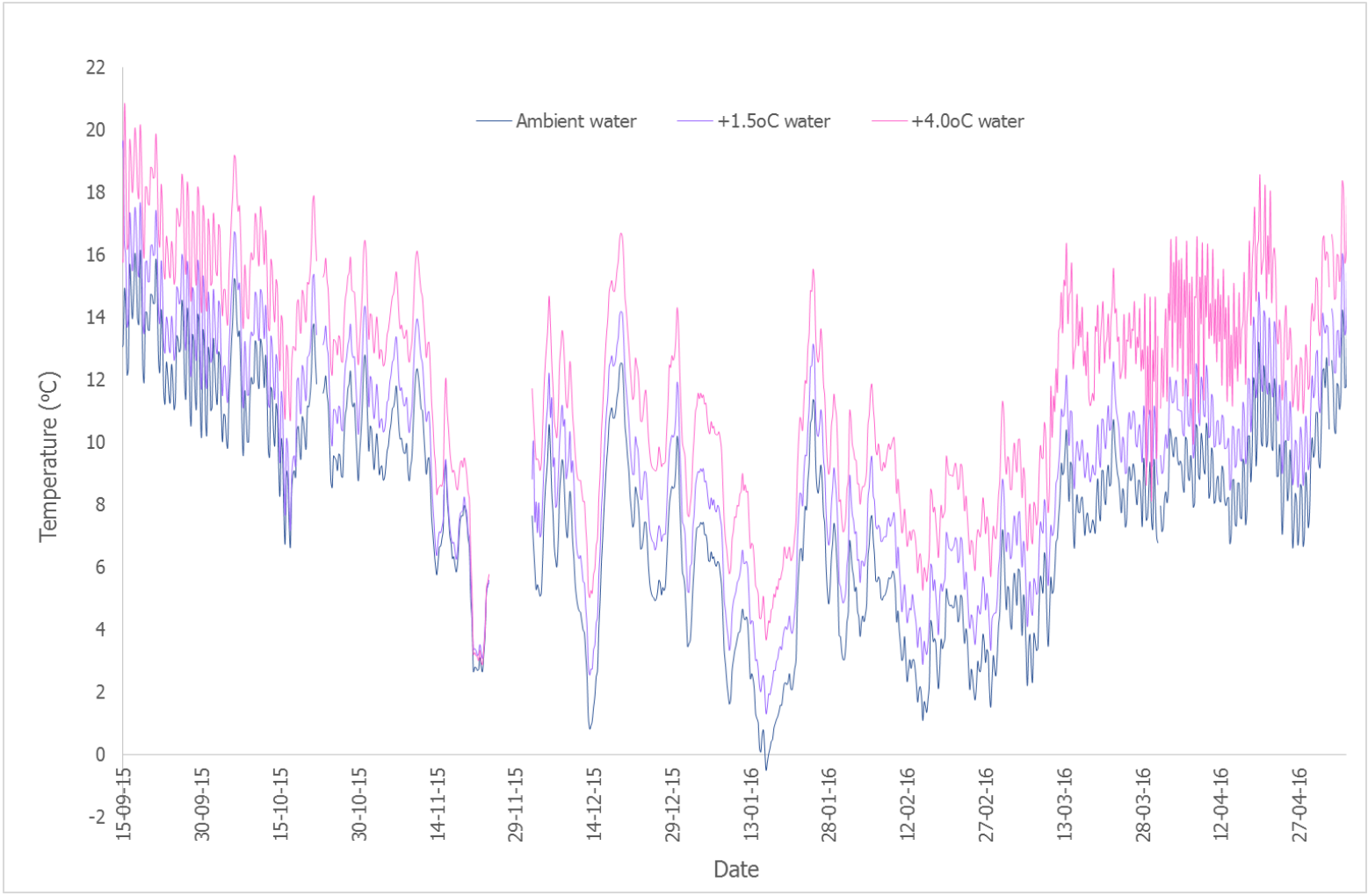
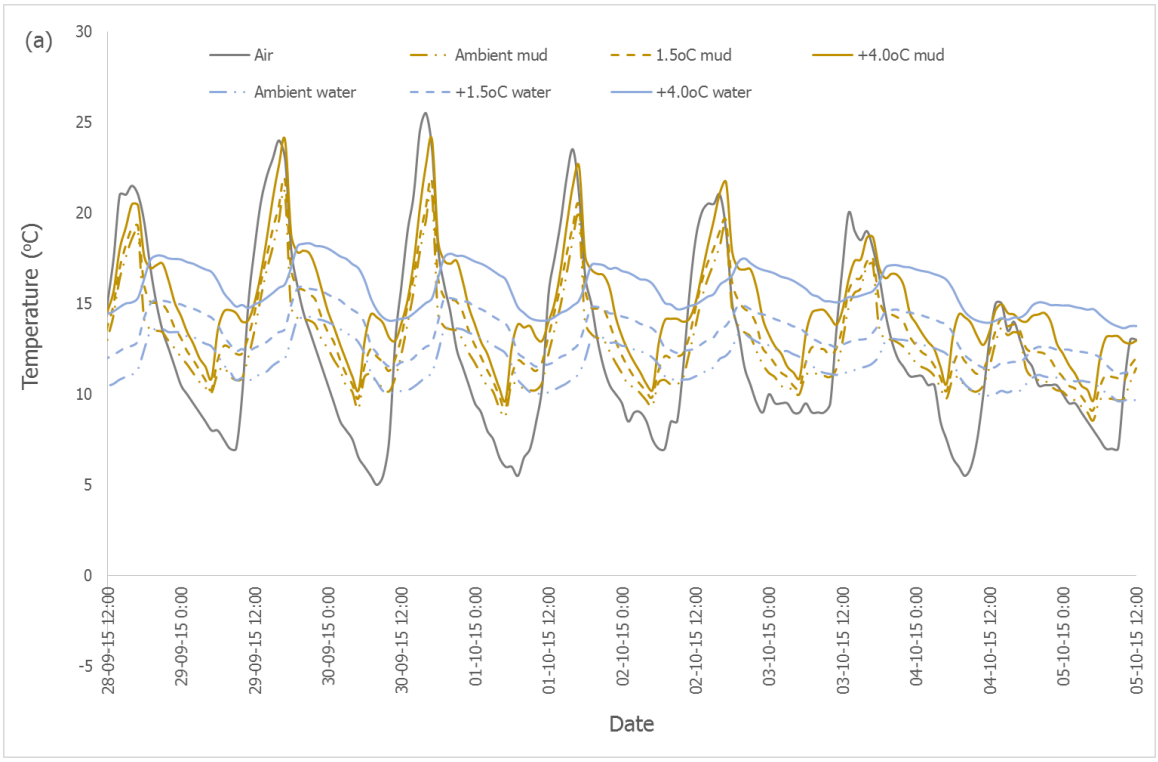


Figure 4.22. Water temperatures in ambient, +1.5 °C and +4.0 °C tanks through the duration of the experiment



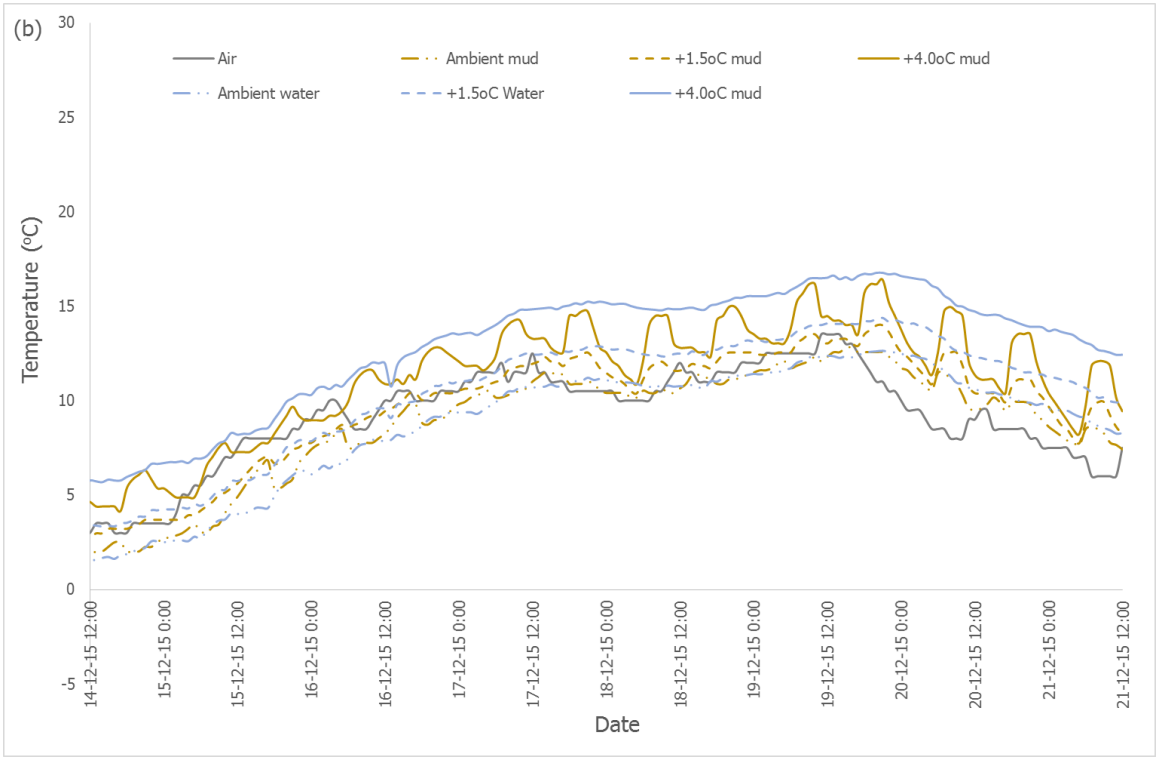


Figure 4.23. Temperatures in air, sediment and water in the week prior to (a) sampling point M02 on 05/10/2015 and (b) sampling point M06 on 21/12/2015

Table 4.4. Minimum and maximum temperatures in air, water and sediment under three temperature treatments in the week prior to each sampling point. Sediment temperatures are missing at sampling points M07 and M08 and water temperatures missing at sampling point M05 due to equipment failure.

Sampling point	Air		Water						Mud						Sampled			
			Ambient		plus 1.5°C		plus 4.0°C		Ambient		plus 1.5°C		plus 4.0°C					
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	D	W	M	S
M01	6.0	26.0	11.49	16.13	13.01	20.92	15.41	21.64	10.46	22.08	10.48	23.68	11.52	25.60				
M02	5.0	25.5	9.63	14.29	11.14	15.93	13.65	18.33	8.56	21.12	9.12	21.84	9.68	24.00				
M03	5.0	21.5	9.34	13.56	10.84	15.10	13.36	17.58	7.36	18.00	7.60	18.32	8.08	19.52				
M04	5.0	16.0	8.71	12.87	10.20	14.37	12.43	16.54	7.52	14.60	7.84	15.08	8.24	16.16				
M05	0.0	11.5	-	-	-	-	-	-	0.92	10.28	1.88	10.64	0.80	10.16				
M06	2.0	13.5	1.33	12.66	3.04	14.37	5.41	16.77	1.28	12.80	2.60	14.00	4.16	16.40				
M07	-0.5	12.5	0.86	11.38	2.70	13.15	5.14	15.56	-	-	-	-	-	-				
M08	-3.0	12.5	1.10	5.54	2.73	7.23	5.14	9.57	-	-	-	-	-	-				
M09	3.5	22.0	6.84	11.14	8.43	12.85	10.04	15.94	4.64	17.36	5.36	17.96	6.56	18.32				
D01	0.5	21.0	6.65	12.98	8.33	14.63	10.70	16.97	3.44	16.76	3.68	17.60	4.16	17.36				

Key to samples taken	
D	Diatoms
W	Overlying water
M	Macro algae (filamentous and sheet)
S	Sediment

4.3.2. Sediment

Organic matter

The percentage organic matter in the sediment increased following disturbance (D01) in both the upper (0 to 5mm) and lower (5 to 20mm) sediment layers by 0.32% (χ^2=11.7, p=6.3*10^{-4}, r^2=0.13) compared to temperature treatment only (M09) (Fig. 4.24).

There was a difference in percentage organic matter between the upper and lower sediment layers in both the temperature only (M09) and disturbance (D01) phases of the experiment. At sampling point M09 there was 0.50% more organic matter in the upper sediment layer (χ^2=12.6, p=3.8*10^{-4}, r^2=0.21), with no difference due to temperature treatment (χ^2=5.8*10^{-2}, p=0.97) (Fig. 4.25). At sampling point D01 there was 0.51% more organic matter in the upper sediment layer (χ^2=34.7, p=3.9*10^{-9}, r^2=0.63). There was no difference due to the level of disturbance (χ^2=1.5, p=0.48) and, as with M09, there was no difference in organic matter content due to temperature (χ^2=1.2, p=0.56) (Fig. 4.26).

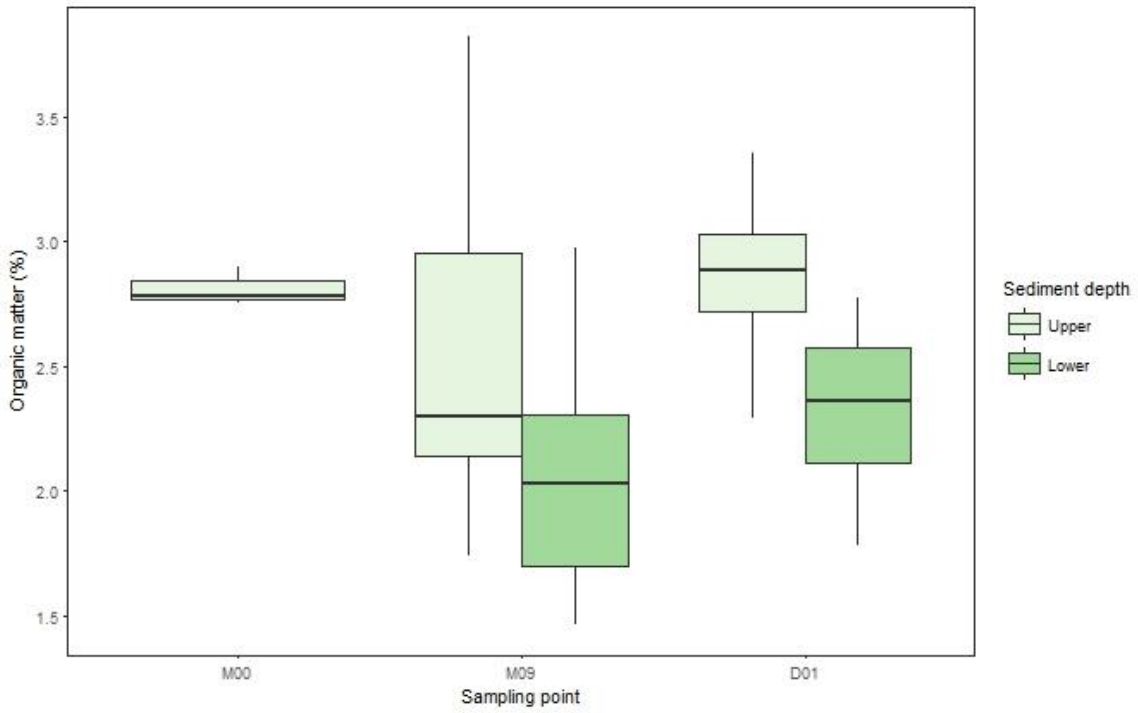


Figure 4.24. Percentage organic matter content of sediment prior to experiment start (M00, n=3) and in upper (0 to 5mm) and lower (5 to 20mm) depths at end of temperature (M09) and disturbance (D01) experiment periods, n=27

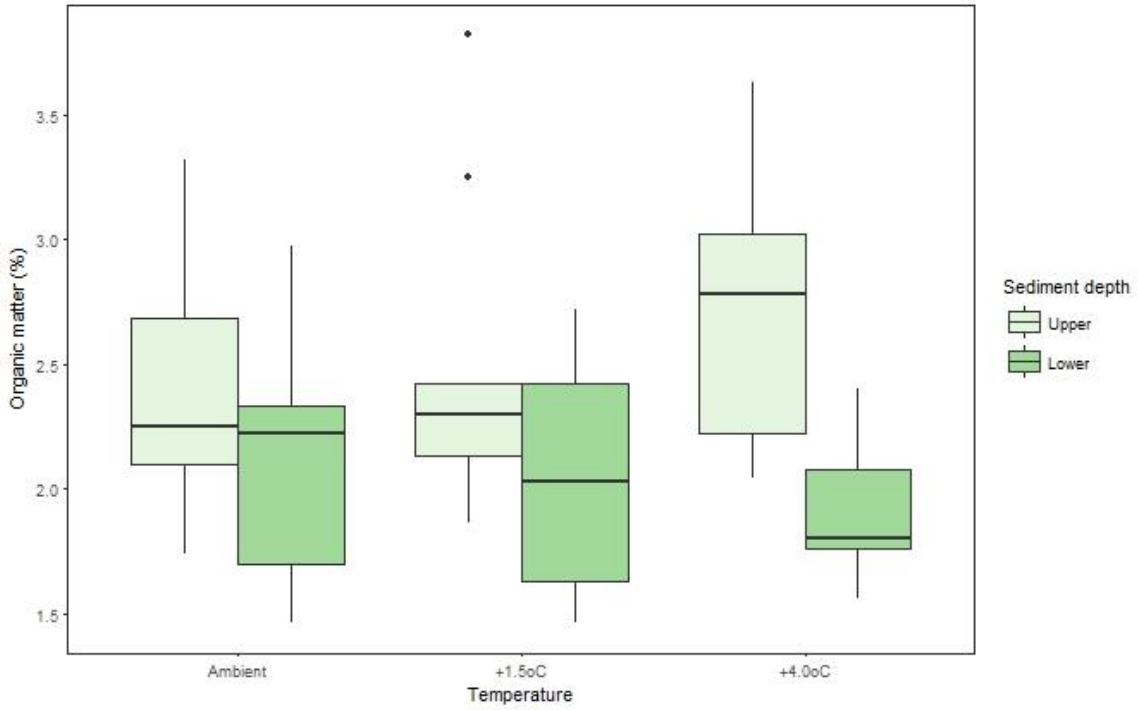


Figure 4.25. Percentage organic matter content of sediment in upper (0 to 5mm) and lower (5 to 20mm) depths at three temperature treatments at end of temperature (M09) experiment period, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

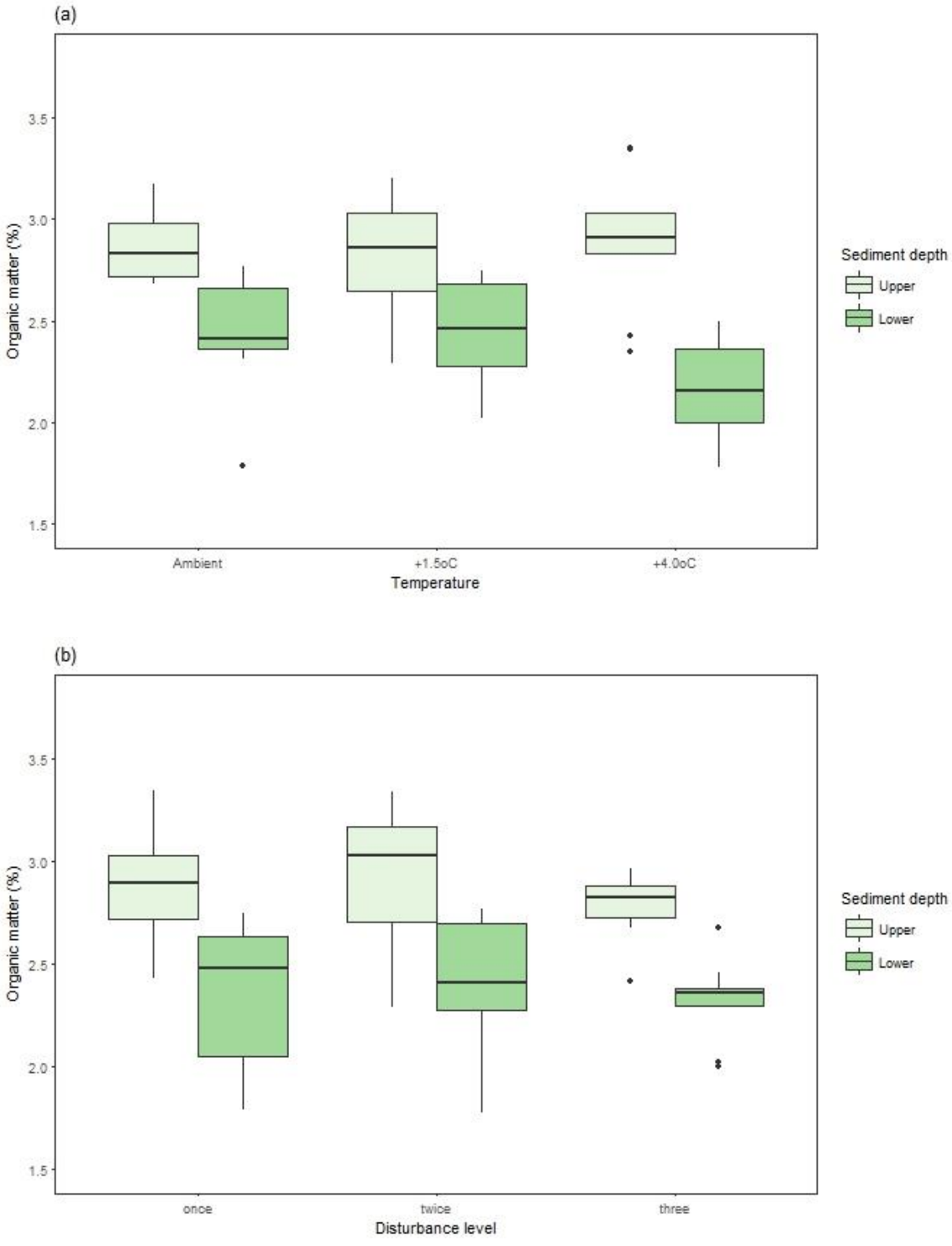


Figure 4.26. Percentage organic matter content of sediment in upper (0 to 5mm) and lower (5 to 20mm) depths at (a) three temperature treatments and (b) three levels of disturbance at end of disturbance (D01) experiment period, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Particle size

There was an increase in the percentage of clay sized particles between sampling points M09, the end of the temperature only phase of the experiment, and D01, following disturbance (χ^2=29.2, p=6.6*10^{-8}, r^2=0.71, Fig. 4.27). Furthermore, there was a difference in the percentage of clay sized particles with sediment depth following the disturbance phase, D01 (χ^2=4.4, p=3.5*10^{-2}), with 2.6% more clay in the upper sediment layer.

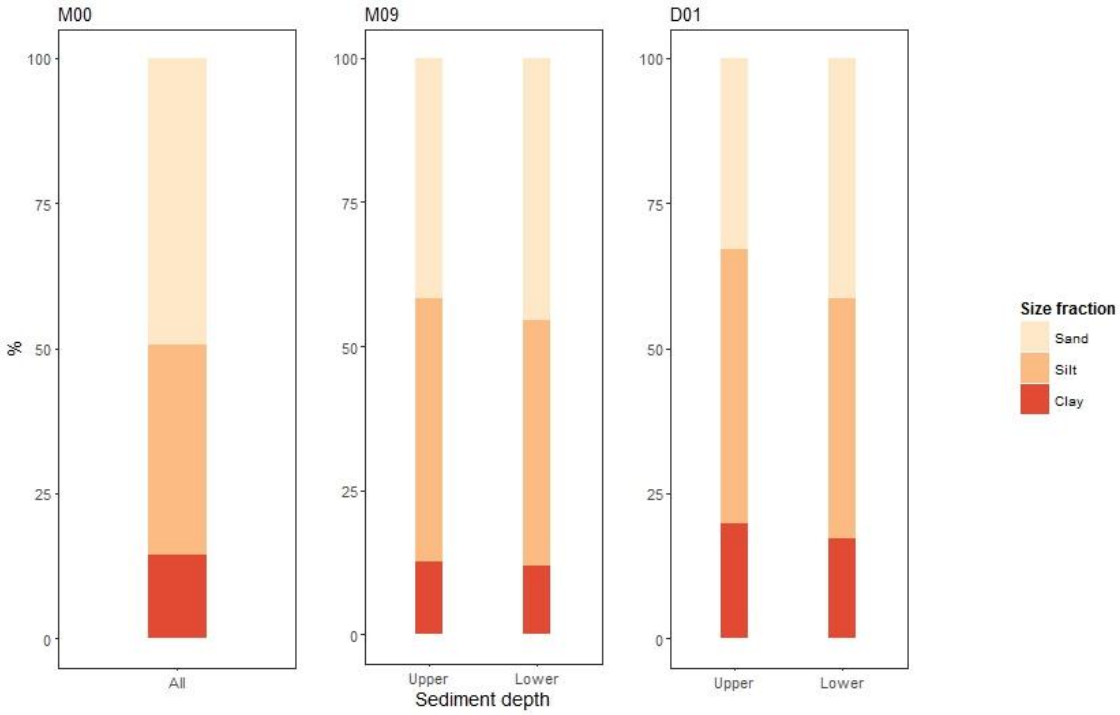


Figure 4.27. Percentage of particle size fractions at sediment depths in the starting sediment (M00), at the end of the temperature only phase (M09) and at the end of the temperature plus disturbance phase (D01) of the experiment

Water content

Water content of the sediment was altered due to disturbance (D01), increasing in the upper (0 to 5mm) sediment layer (when compared to temperature treatment only (M09)) by 3.63% (X^2=34.4, p= $4.5*10^{-9}$, r^2=0.65). Conversely the water content of the lower (5 to 20mm) sediment layer decreased during the disturbance phase (D01) (compared to the temperature only experiment (M09)) by 4.58% (X^2=90.6, p<$2.2*10^{-16}$, r^2=0.89), (Fig. 4.28)

Whilst there was no difference in the upper and lower sediment layers in the temperature only phase of the experiment (M09, χ^2=0.43, p=0.51, r^2=0.65), there was a difference in the upper and lower depths during the disturbance experiment (D01, χ^2=86.2, p<$2.2*10^{-16}$, r^2=0.88). However, there was no difference in percentage water content at sampling point D01 due to either temperature (χ^2=0.70, p=$7.1*10^{-1}$) or disturbance treatment (χ^2=4.9, p=$8.6*10^{-2}$, Fig. 4.29).

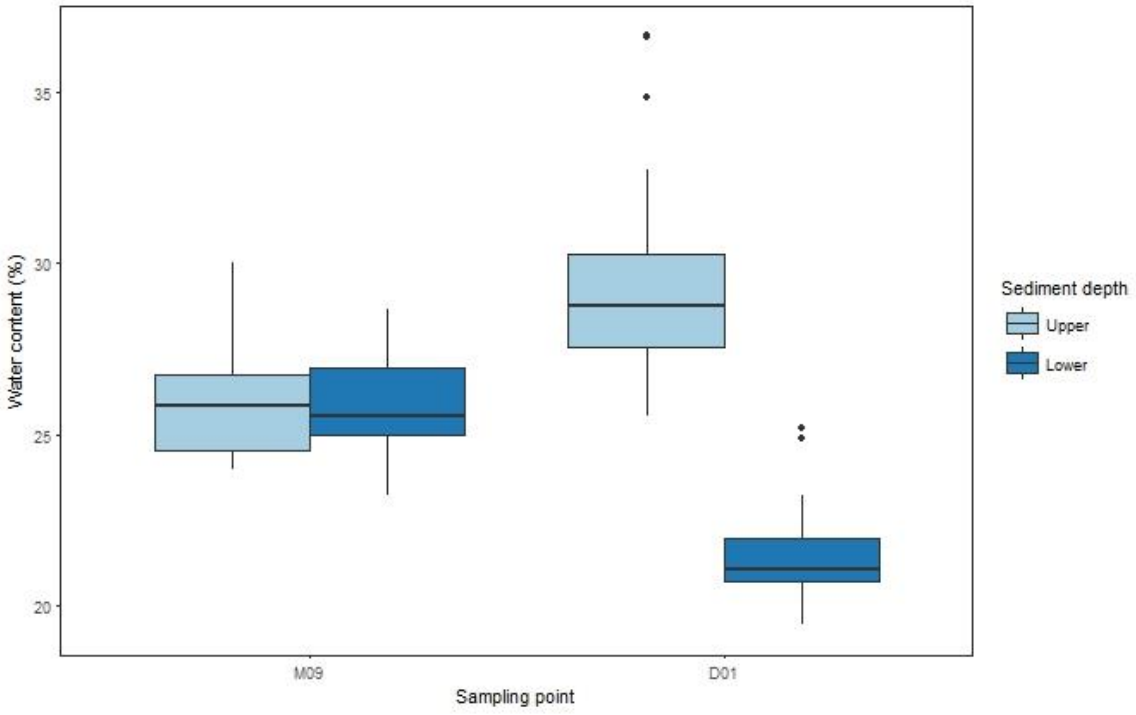


Figure 4.28. Water content of sediment in upper (0 to 5mm) and lower (5 to 20mm) depths at end of temperature (M09) and disturbance (D01) experiment periods, n=27, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

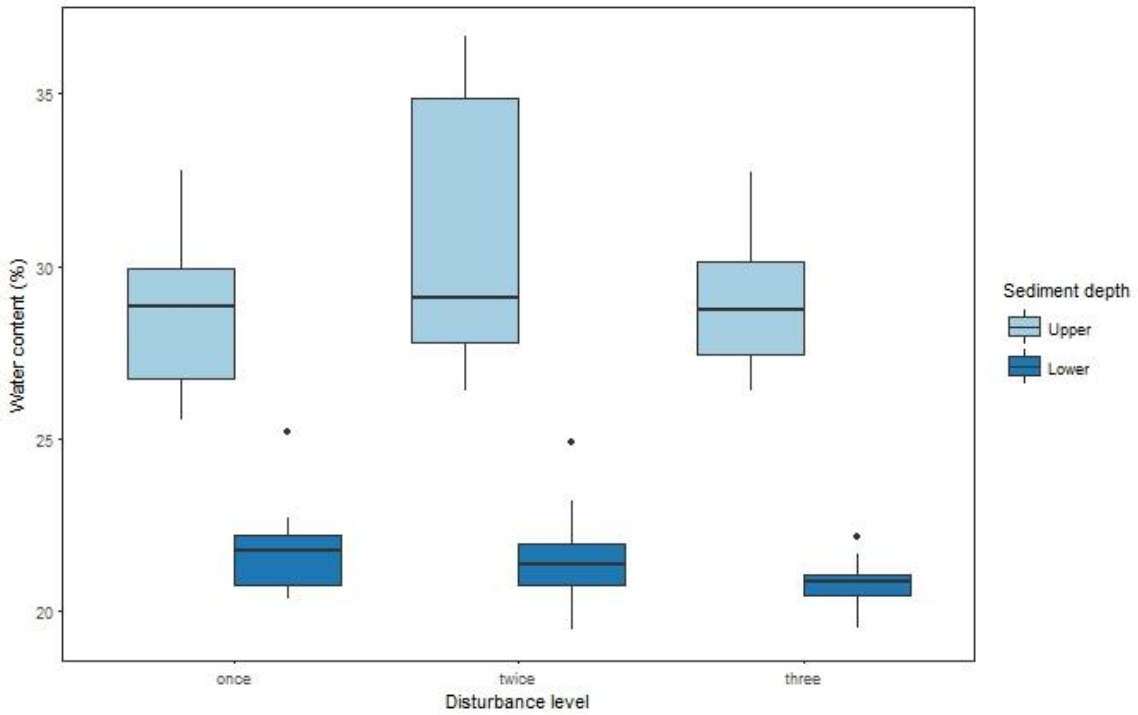


Figure 4.29. Water content of sediment in upper (0 to 5mm) and lower (5 to 20mm) depths at three levels of disturbance at end of disturbance (D01) experiment period, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Metals concentration

Concentrations of all analytes in sediment originally deployed in the mesocosm (M00) are given in Table 4.5.

Table 4.5. Initial sediment concentrations of analytes in mg kg^{-1} DW unless otherwise stated (mean ± standard deviation (SD), n=3)

Analyte	Initial Sediment Concentration
Aluminium (%)	0.30 ± 0.02
Vanadium	8.5 ± 0.65
Chromium	10 ± 0.85
Manganese	580 ± 40
Iron (%)	0.99 ± 0.07
Cobalt	3.7 ± 0.27
Nickel	8.1 ± 0.69
Copper	8.3 ± 0.51
Zinc	57 ± 2.8
Arsenic	5.2 ± 0.38
Silver	0.15 ± 0.08
Cadmium	0.15 ± 0.05
Tin	0.80 ± 0.05
Lead	19 ± 1.2

A range of metals (Al, V, Cr, Mn, Fe, Co, Ni, Zn, As and Sn) had very similar patterns of concentration. Zn, Mn and Sn are shown as examples of this pattern (Figs. 4.30 to 4.34), boxplots for the remaining metals in this group are given in Appendix 4-4. The most notable feature of this concentration pattern was the wide range of concentrations for +1.5 ^{o}C and +4.0 ^{o}C temperature treatments and the higher concentration for the ambient temperature treatment at sample point M05. Apart from Al and Sn (non-essential, basic metals) and As (a non-essential, semimetal) these are all essential, transition metals. Essential metals are to some extent required for growth and therefore some biological uptake would be expected, in contrast non-essential metals have no biological function.

Although Mn, Fe and Sn have concentration patterns similar to the others, the initial concentrations, M00, were higher than at sampling points M09 and D01 (Figs. 4.31 and 4.32 and Appendix 4-4).

Concentrations of Cu in the sediment have some similarity with other transition metals, but the range of concentrations at sampling points M09 and D01 was much wider (Fig. 4.33). There was also an increase in concentration from the starting sediment, M00, to the end of the experiment, D01.

The pattern of Pb concentration in the sediment differed from other metals (Fig. 4.34). Like Cu, Pb concentrations in sediment increased from the starting sediment, M00, to the end of the experiment D01.

Eight of the metals analysed (Mn, Fe, Co, Ni, Zn, As, Sn and Pb) showed an increase in the sediment concentration from sampling point M09, the end of the temperature only phase, to D01, the end of the disturbance phase of the experiment.

Sediment concentrations of Ag and Cd (non-essential transition metals) were very different from other metals and each other (Appendix 4-4) with some high outlier values at sampling point D01. Concentrations of both these metals were very low.

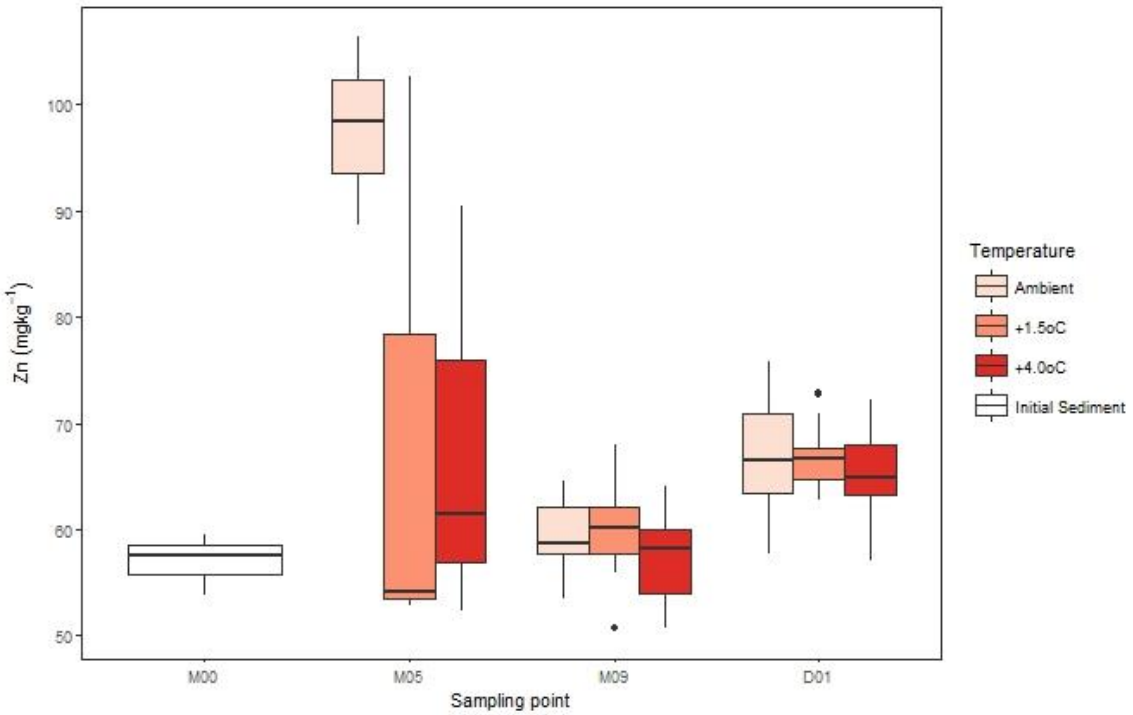


Figure 4.30. Sediment concentrations (mg kg^{-1} DW) of zinc in the initial sediment, M00, and at three sampling points (M00 n=3, M05 n=3, M09 and D01 n=18), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

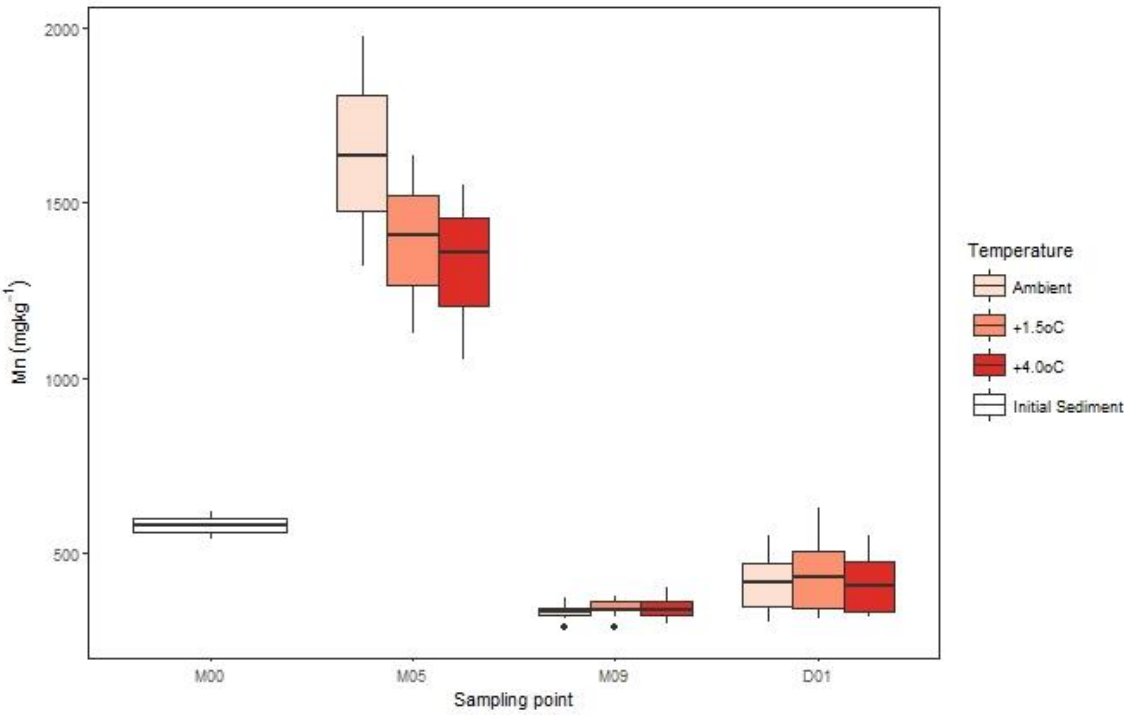


Figure 4.31. Sediment concentrations (mg kg^{-1} DW) of manganese in the initial sediment, M00, and at three sampling points (M00 n=3, M05 n=3, M09 and D01 n=18), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

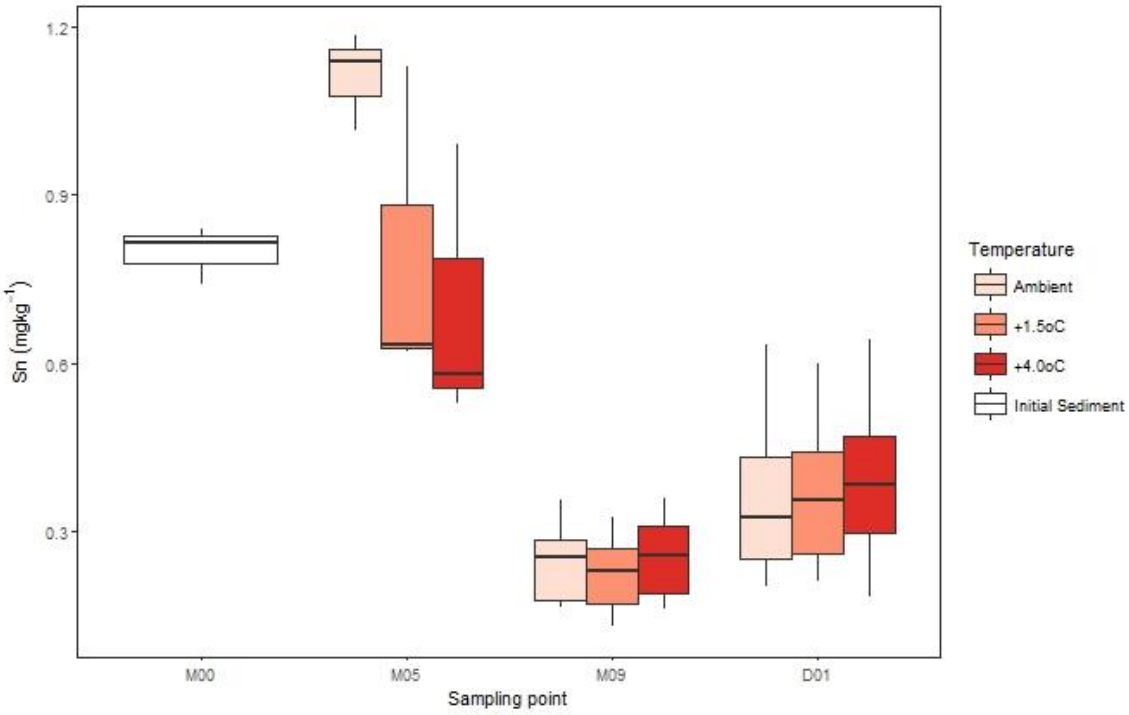


Figure 4.32. Sediment concentrations (mg kg^{-1} DW) of tin in the initial sediment, M00, and at three sampling points (M00 n=3, M05 n=3, M09 and D01 n=18)

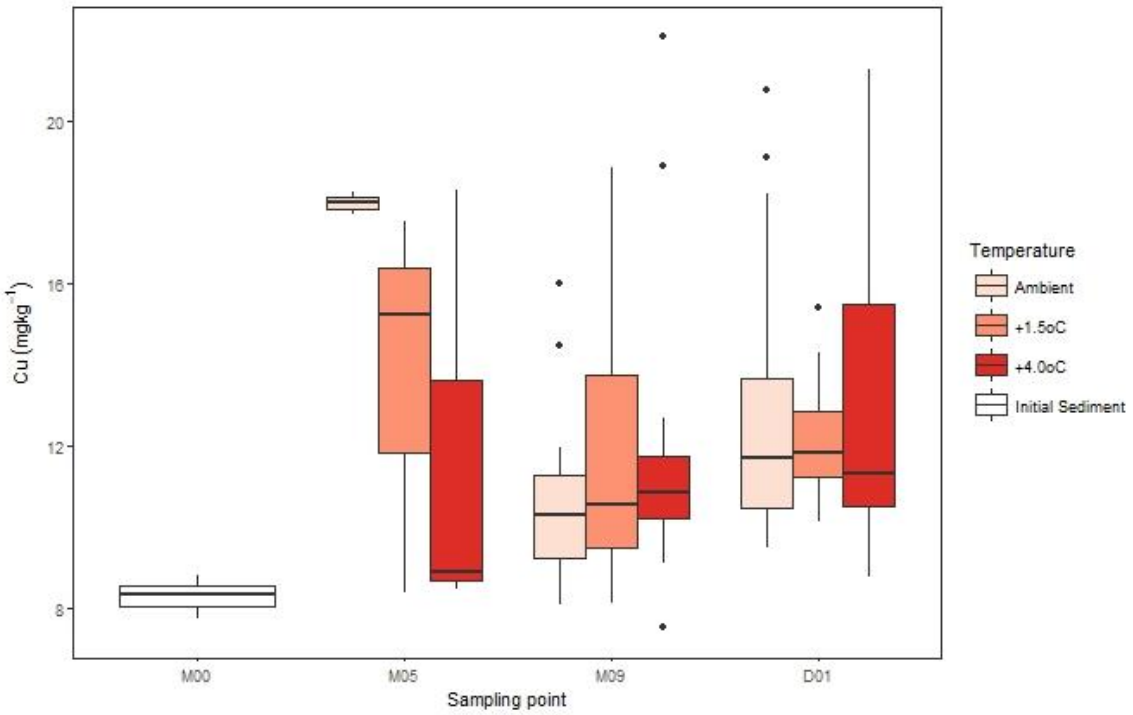


Figure 4.33. Sediment concentrations (mg kg^{-1} DW) of copper in the initial sediment, M00, and at three sampling points (M00 n=3, M05 n=3, M09 and D01 n=18), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

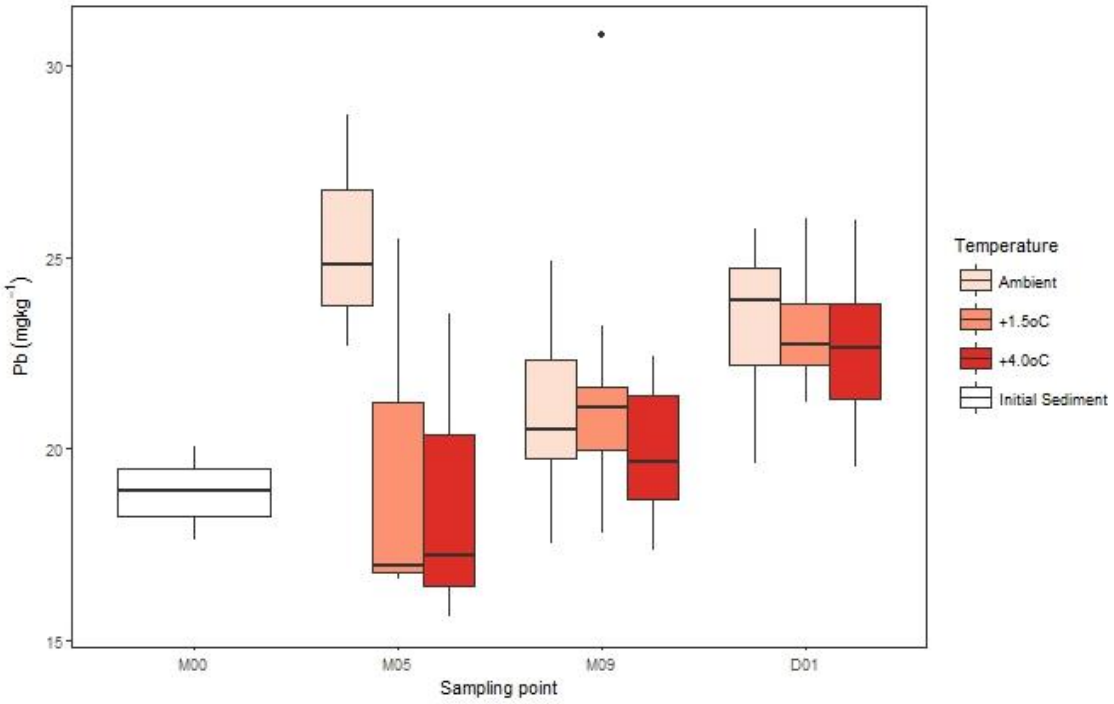


Figure 4.34. Sediment concentrations (mg kg^{-1} DW) of lead in the initial sediment, M00, and at three sampling points (M00 n=3, M05 n=3, M09 and D01 n=18), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

There was a difference in concentration between the top and bottom (disturbed and compacted) layers of sediment at sample point D01 for Mn, Fe, As and Pb (Figs. 4.35 to 4.38). The shallower, disturbed sediment had a higher concentration of Mn, Fe and As than the deeper compacted sediment. Conversely, Pb concentrations were lower in the upper sediment.

Sediment concentration of Mn was 143 mg kg^{-1} higher in the disturbed upper layer of sediment (χ^2=104.4, p<2.2*10^{-16}, r^2=0.91, Fig. 4.35). There was an effect of organic matter which, for each percentage increase, resulted in Mn concentration increases of 37.2 mg kg^{-1} (χ^2=5.0, p=2.5*10^{-2}). There was an additional effect of disturbance on Mn concentrations with a decrease of ~22.5 mg kg^{-1} with increased disturbance (χ^2=7.0, p=3.0*10^{-2}). There was no change in sediment Mn concentrations due to temperature treatment (χ^2=3.7, p=1.6*10^{-1}).

At sampling point D01 Fe had a higher concentration in the upper disturbed sediment (χ^2=45.6, p=1.5*10^{-11}, r^2=0.69, Fig. 4.36). Concentration was also affected by organic matter content (χ^2=8.9, p=2.9*10^{-3}), increasing by 630 mg kg^{-1} for every percentage increase in organic matter. There was no difference in sediment Fe concentrations due to either temperature treatment (χ^2=1.6, p=4.5*10^{-1}) or disturbance level (χ^2=2.5, p=2.9*10^{-1}).

Arsenic was 1.82 mg kg^{-1} higher in the upper sediment compared to the deeper, undisturbed sediment (χ^2=124.2, p<2.2*10^{-16}, r^2=0.95, Fig. 4.37) at sampling point D01. There was also an increase of 0.63 mg kg^{-1} As with increasing percentage organic matter (χ^2=16.5, p=5.0*10^{-5}). Neither disturbance level (χ^2=2.4, p=3.1*10^{-1}) or temperature (χ^2=2.3*10^{-1}, p=8.9*10-^{1}) had any effect on sediment As concentrations.

At sampling point D01 Pb had a higher concentration in the deeper compacted sediment than the disturbed upper sediment layer (χ^2=5.8, p=1.6*10^{-2}, r^2=0.51, Fig. 4.38). There was a difference due to organic matter, with Pb increasing with increasing organic matter (χ^2=5.6, p=1.8*10^{-2}), but no difference due to either temperature treatment (χ^2=0.46, p=7.9*10^{-1}) or disturbance level (χ^2=0.56, p=7.6*10^{-1}).

At sampling point M09 only Mn (χ^2=31.4, p=2.1*10^{-8}, r^2=0.57, Fig. 4.39) and As (χ^2=11.9, p=5.5*10^{-4}, r^2=0.50, Fig. 4.40) showed a difference in concentration with depth. The differences were less than those observed following sediment disturbance (sampling point D01) decreasing by 31 mg kg^{-1} and 0.27 mg kg^{-1} in the lower sediment layer for Mn and As respectively.

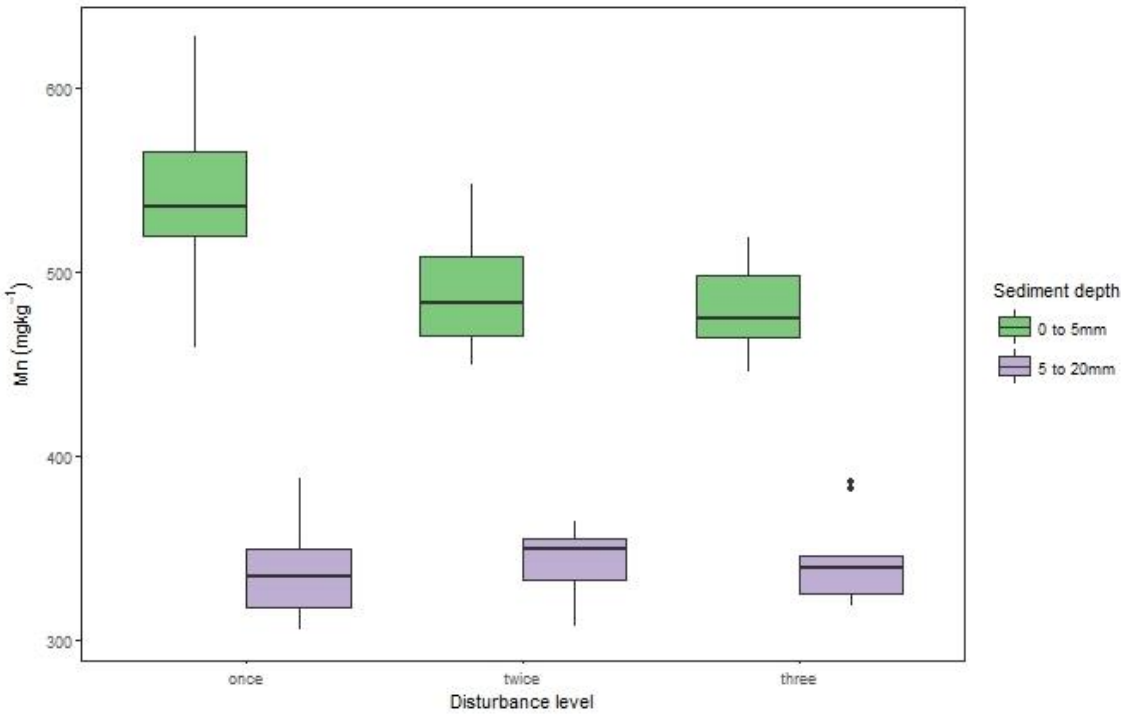


Figure 4.35. Sediment concentrations (mg kg^{-1} DW) of manganese in the disturbed sediment, D01, at three disturbance levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

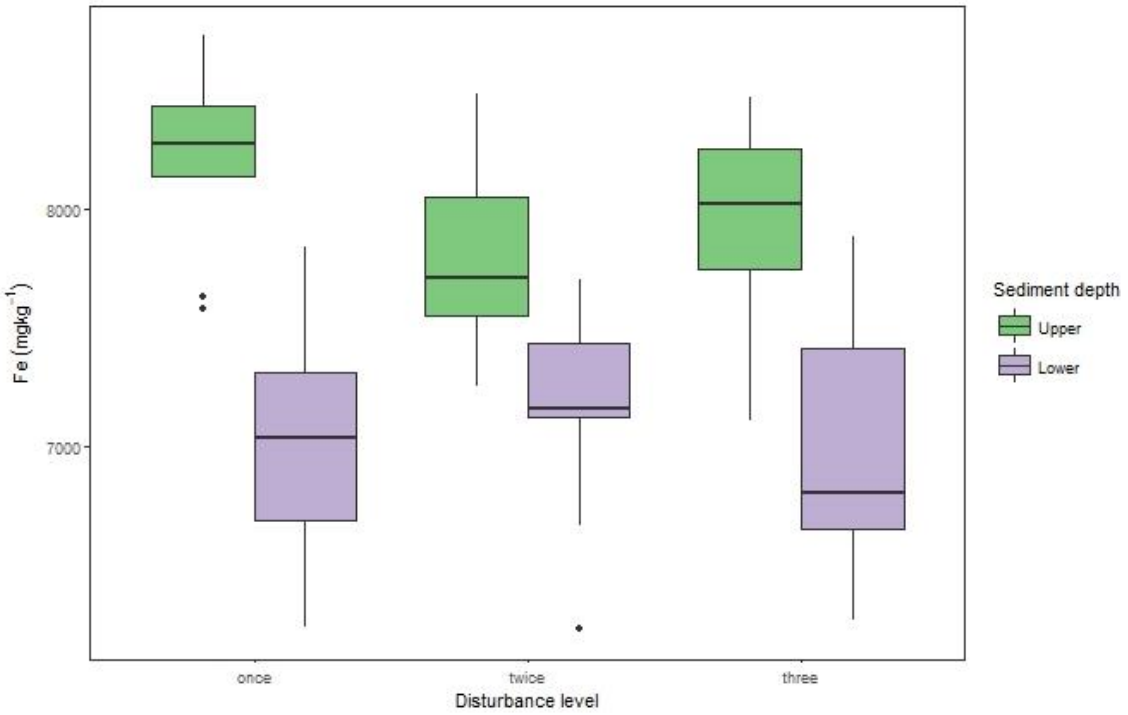


Figure 4.36. Sediment concentrations (mg kg^{-1} DW) of iron in the disturbed sediment, D01, at three disturbance levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9 , outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

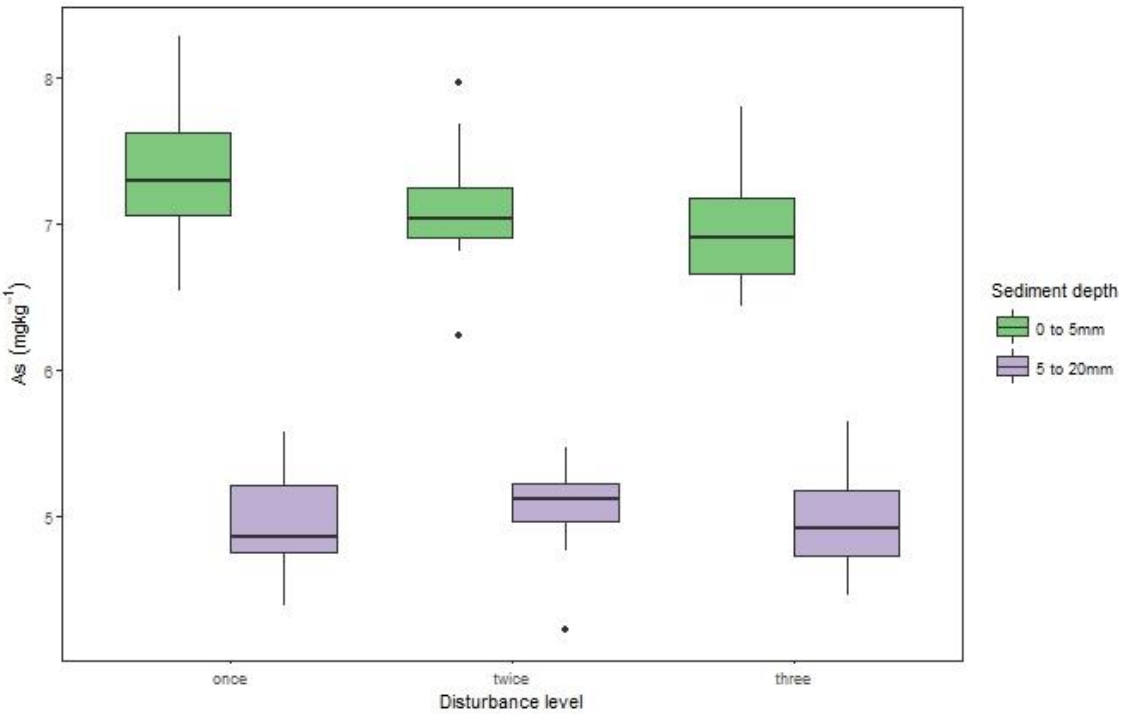


Figure 4.37. Sediment concentrations (mg kg^{-1} DW) of arsenic in the disturbed sediment, D01, at three disturbance levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

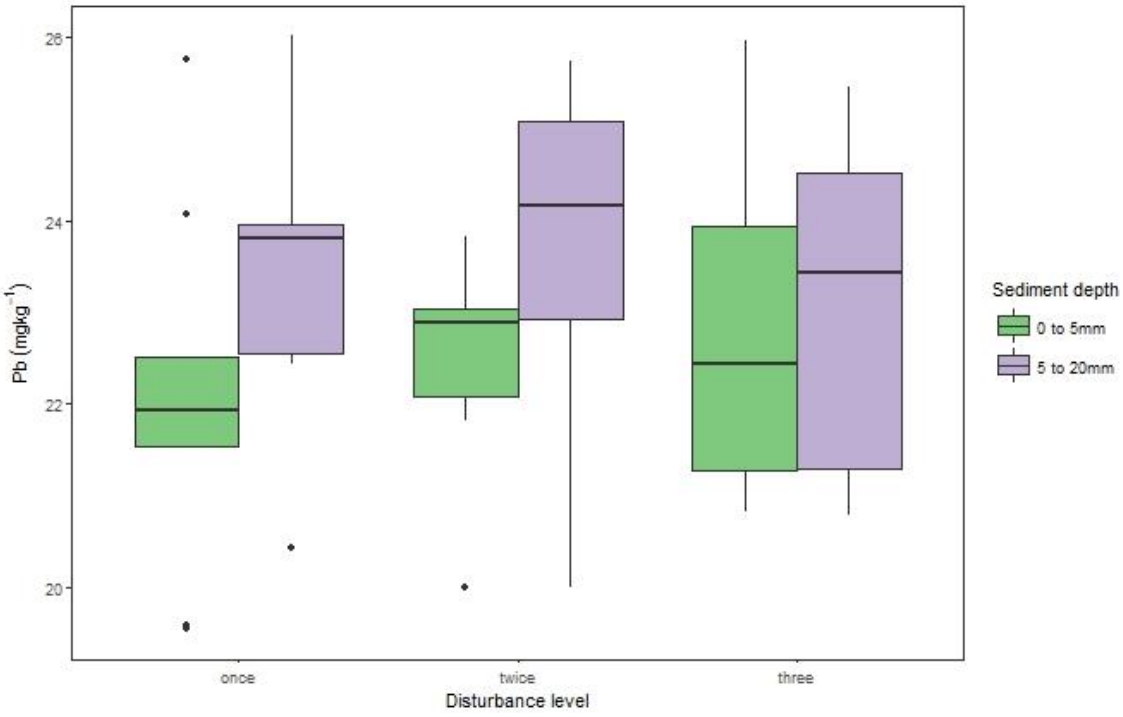


Figure 4.38. Sediment concentrations (mg kg^{-1} DW) of lead in the disturbed sediment, D01, at three disturbance levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

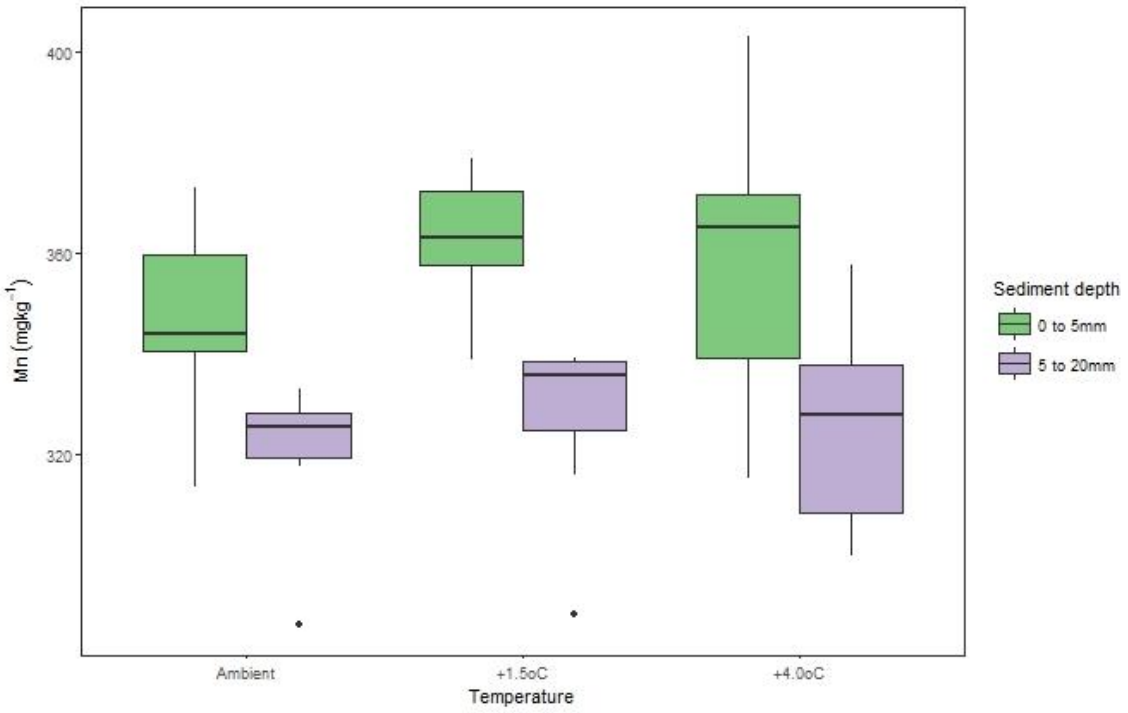


Figure 4.39. Sediment concentrations (mg kg^{-1} DW) of manganese at M09, for three temperature levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

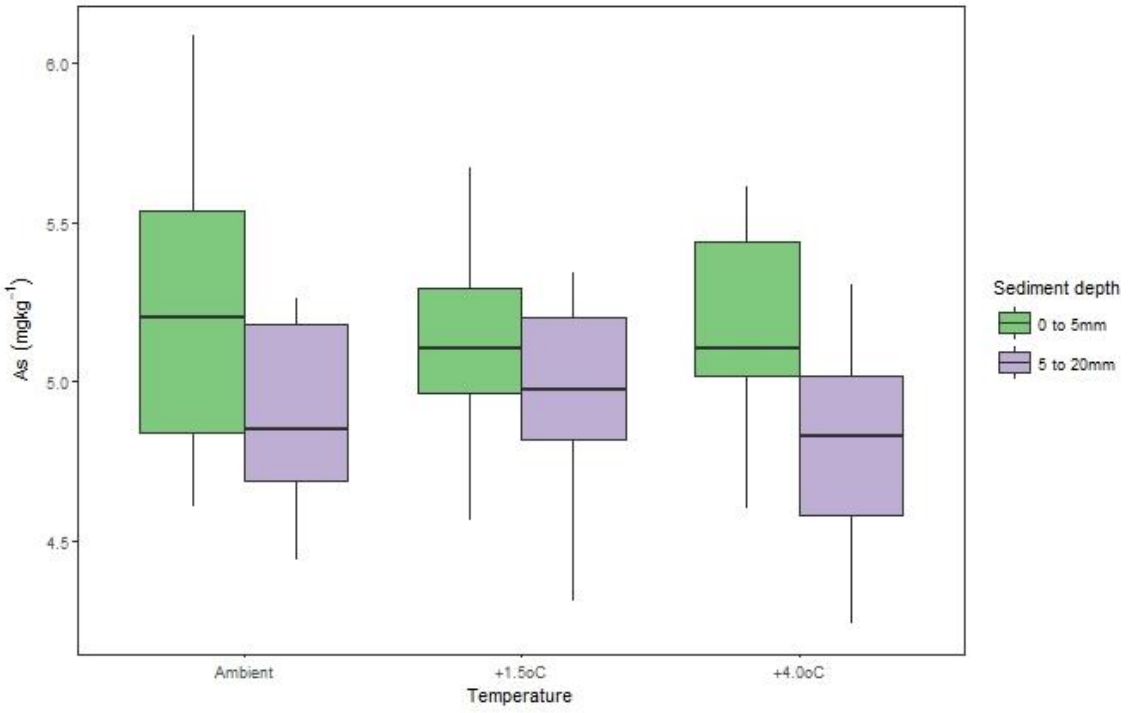


Figure 4.40. Sediment concentrations (mg kg^{-1} DW) of arsenic at M09, for three temperature levels and two sediment depths, 0 to 5mm (disturbed) and 5 to 20mm (compacted) layer, n=9

4.3.3. Overlying water

Unlike sediment, there were few correlations between concentrations of metals in the overlying water. Correlations were found for Co-Mn (0.78) and Fe-Cr (0.75), however Fe and Cr have over 50% of their data below the LoD.

Many of the metals analysed were below the LoD at the majority of sampling points, these were Cr, Fe, Ag, Cd, Sn and Pb. Metals which had between 50 and 75% of data above the LoD were Al, Ni, Cu and Zn the data for these are presented in Appendix 4-5. There were no obvious patterns to these data (which appear mainly static with some outlier values) with the exception of Al, which appears to follow a similar curve to that of biomass (Fig. 4.45 and Appendix 4-5).

V, As and Co had detectable values for all samples, whilst Mn was below the LoD in just one sample, graphs of the concentrations of these metals at each sample point are shown below (Figs. 4.41 to 4.44).

There was a marked increase in V in overlying water from sample point M02 to M03. Concentrations then decreased to a low at sampling point M09, they then increased slightly again following the disturbance phase, D01 (Fig. 4.41). Concentrations of V in overlying water were shown to change between sampling points (χ^2=240.1, p<2.2*10^{-16}, r^2=0.96) with higher concentrations in tanks at +4.0 ^{o}C (χ^2=7.6, p=2.3*10^{-2}).

Apart from the increased concentration at sampling point M03, concentrations of Co in the overlying water increased from M02 to M06 and then decreased to D01 (Fig. 4.42). This pattern appeared to follow that of biomass (Fig. 4.45). Concentrations of Co in overlying water vary with sampling point (χ^2=47.7, p=1.1*10^{-7}, r^2=0.60) and were lower in tanks with water temperatures at +4.0 ^{o}C (χ^2=8.0, p=1.8*10^{-2}).

Concentrations of Mn in overlying water had a similar pattern to Co (Figs. 4.42 and 4.43). Concentrations varied at sampling points from M02 to M07 (χ^2=120.62, p<2.2*10^{-16}, r^2=0.80), increasing to a peak at M06, but were very low and apparently static from M08 to D01. There were differences due to temperature (χ^2=7.0, p=3.0*10^{-2}), but these do not appear consistent (Fig. 4.43).

There was no apparent trend in concentrations of As in overlying water (Fig. 4.44) although they were higher from M04 to M07 (χ^2=108.1, p=<2.2*10^{-16}, r^2=0.86), there was no spike in concentration at M03 as was observed with other metals. There was no variation in As concentration in overlying water due to temperature (χ^2=1.2, p=5.4*10^{-1}).

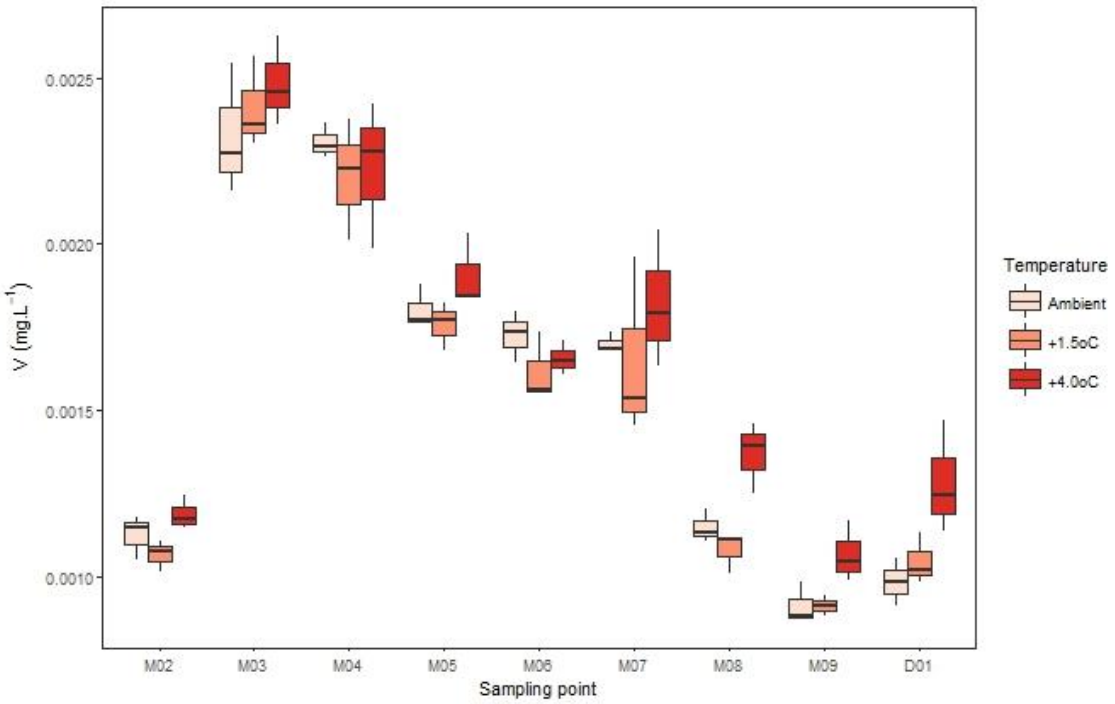


Figure 4.41. Concentrations of vanadium (mg L^{-1}) in the overlying water at nine sampling points (n=3)

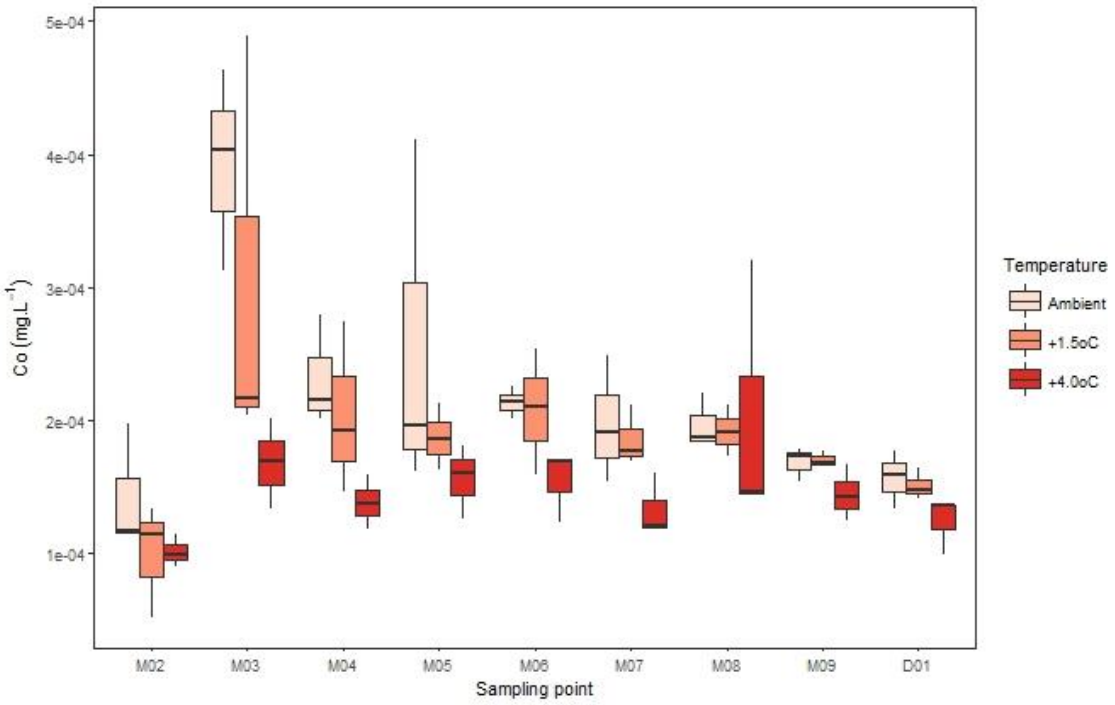


Figure 4.42. Concentrations of cobalt (mg L^{-1}) in the overlying water at nine sampling points (n=3)

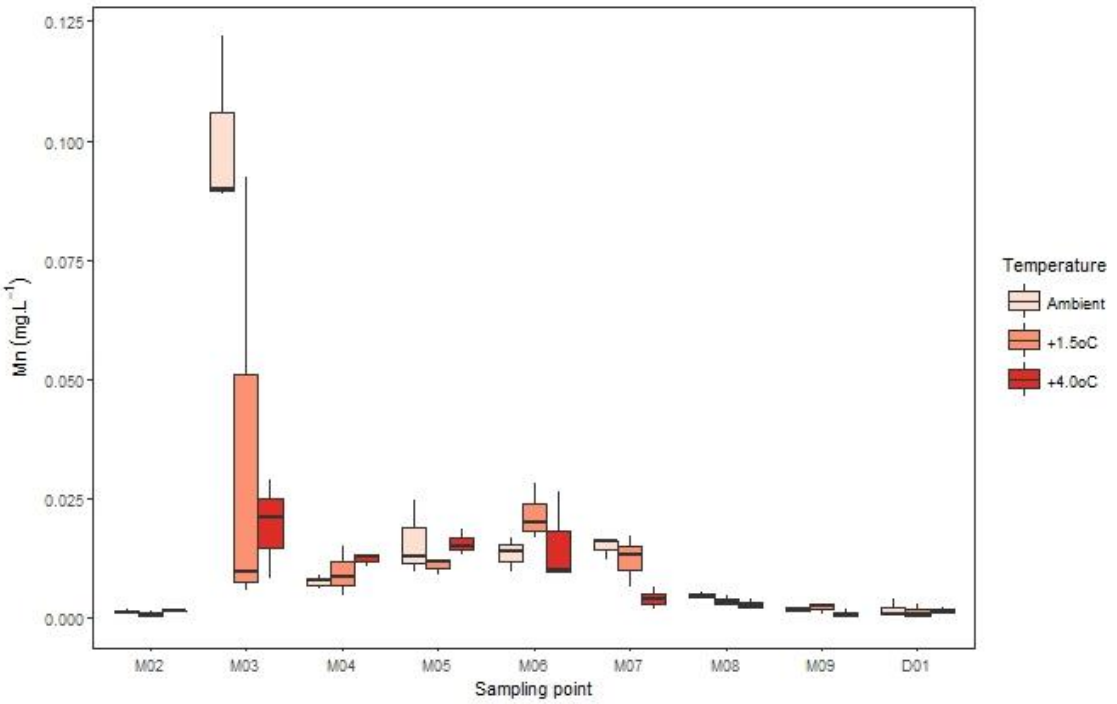


Figure 4.43. Concentrations of manganese (mg L^{-1}) in the overlying water at nine sampling points (n=3)

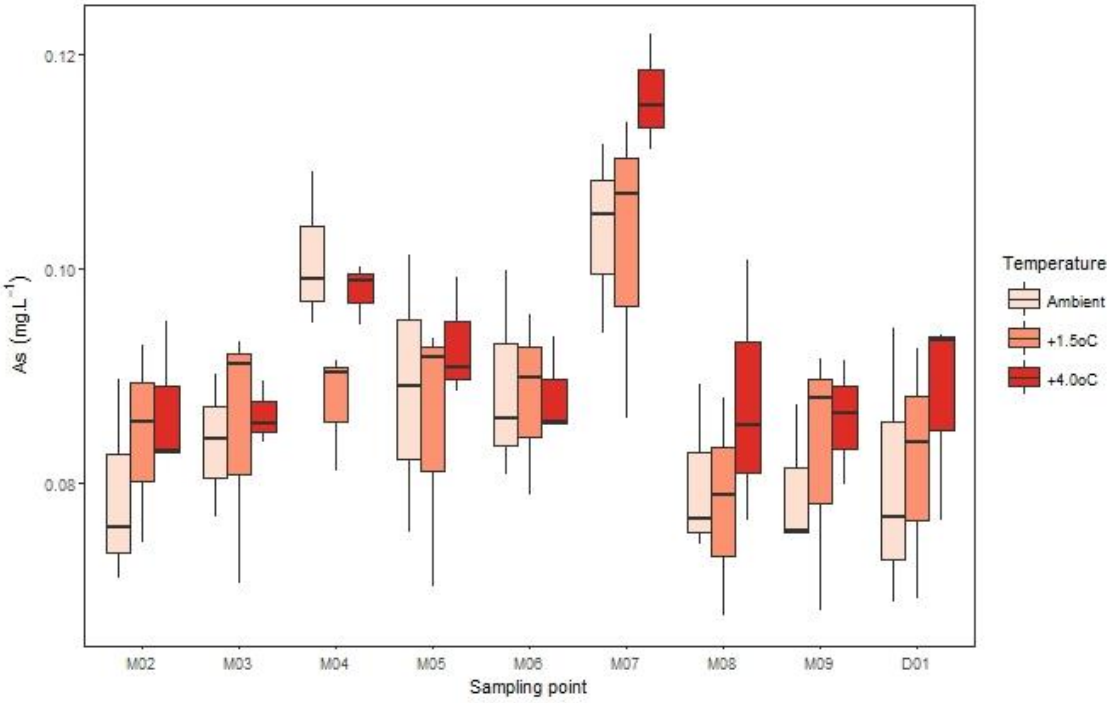


Figure 4.44. Concentrations of arsenic (mg L^{-1}) in the overlying water at nine sampling points (n=3)

4.3.4. Algal biomass

Biomass on the sediment surface was measured using NDVI as a proxy. Following an initial increase in average biomass across all tanks (Fig. 4.45) from an average NDVI of 0 at the beginning of the experiment to about 0.40 by day 14, there was a sharp decline to around 0.25 on day 22 after which it quickly increased again until it reached an average of about 0.45 at day 50. From then on average biomass was fairly stable until around day 115 when biomass started to decline to an NDVI of about 0.35 around day 175 where it levelled out again. Following the end of the temperature only experiment period on day 200 the biomass dropped sharply to an NDVI of about 0.15 at the start of the disturbance experiment on day 205. Biomass continued to decline throughout the disturbance period of the experiment (Fig. 4.45).

The changing biomass up to the end of the temperature only period of the experiment can be seen in Figure 4.46., and the effect of disturbance in Figure 4.47.

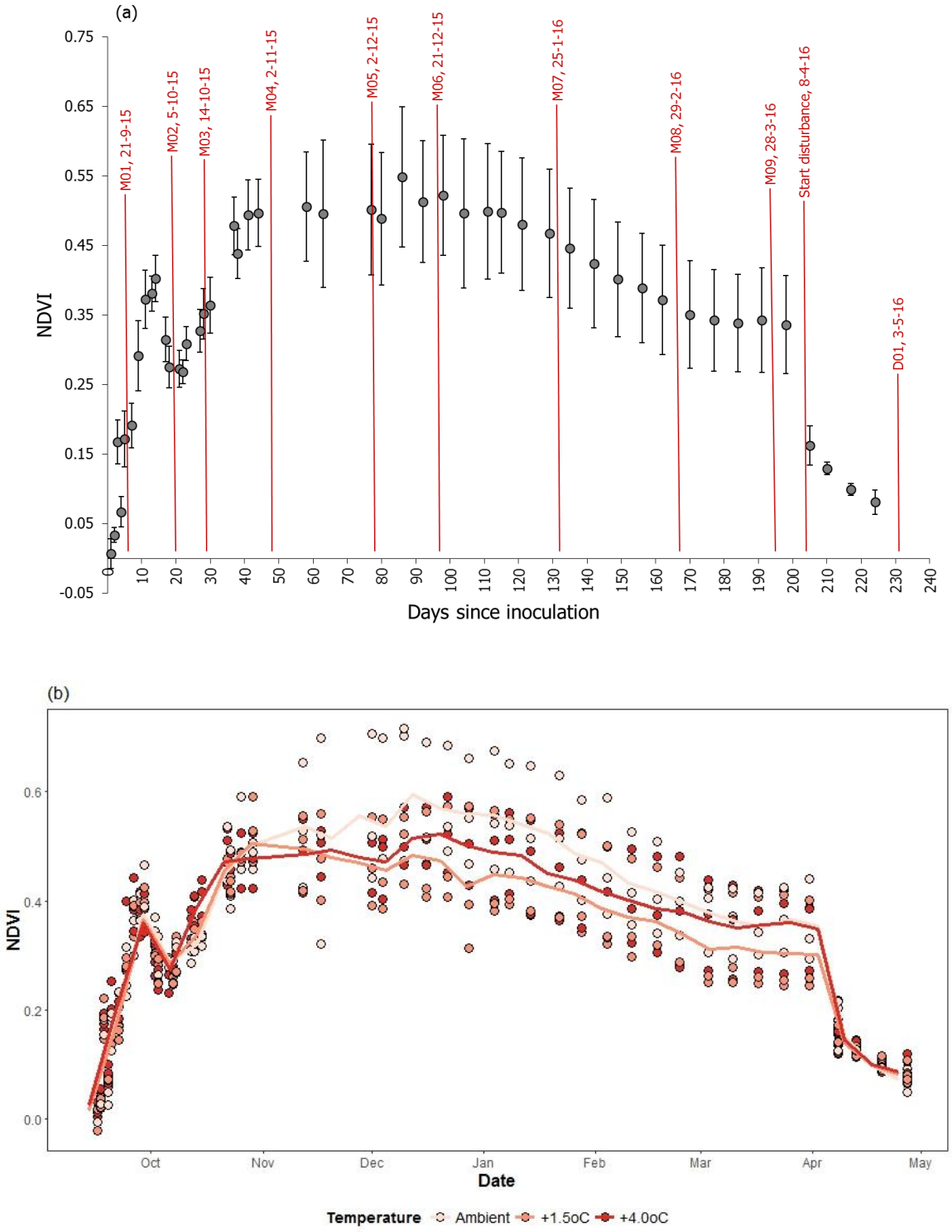


Figure 4.45. Average algal biomass (using NDVI as a proxy) throughout the duration of the study as (a) an average across all tanks with sampling points and start of disturbance experiment indicated and (b) for individual tanks with temperature treatment shown.

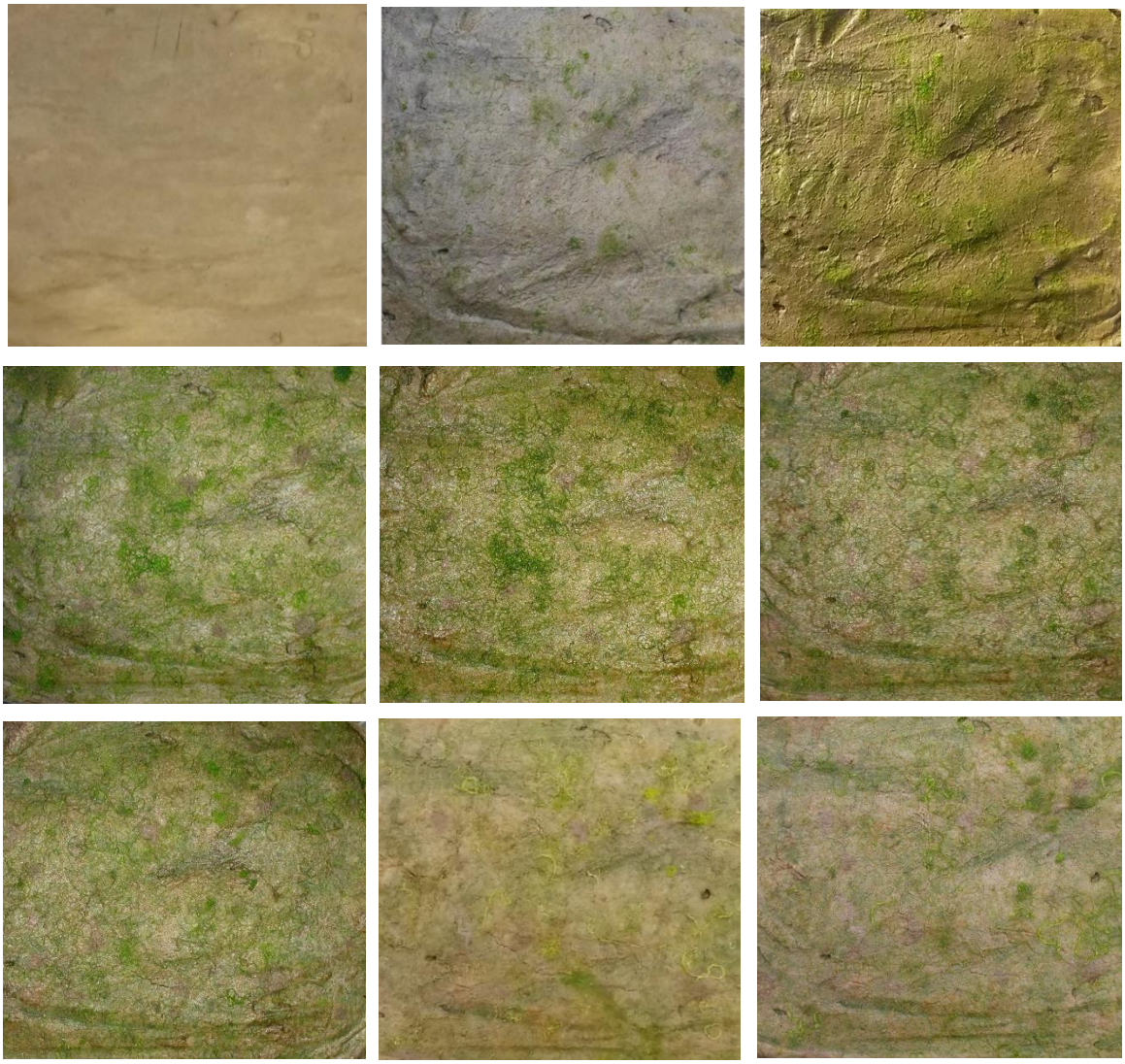


Figure 4.46. Algal growth in Tank 4 on days 13, 27, 37, 58, 77, 98, 135, 170 and 198 of temperature experiment

Figure 4.47. Growth in Tank 4 during disturbance phase of experiment, day 217

There was a difference in growth due to temperature treatment (χ^2=24.4, p=4.9*10^{-6}, r^2=0.86), with the highest growth occurring in the ambient temperature tanks and the lowest in the plus 1.5 ^oC treatment, although this varied through the course of the experiment (Fig. 4.45 (b)).

Growth at each temperature treatment was initially similar with lines of mean biomass overlapping. This changed on day 20, following sampling point M02, when growth at different temperature treatments altered such that the highest biomass was observed in the hottest temperature treatment (+4.0 ^oC) and lowest biomass was in the coldest treatment (ambient) (Fig. 4.45 (b)). There was another change at sampling point M04 at the start of November so that by sampling point M05 at the start of December highest biomass was in the ambient treatment and the lowest in the plus 1.5 ^oC treatment, a condition which persisted until the end of the temperature only phase of the experiment.

For a period of approximately three months from November to February one of the ambient temperature treatment tanks (Tank 6) had a notably higher biomass than other tanks. This was due to an abundance of macroalgae (*Enteromorpha sp.*), which covered the sediment surface (Appendix 4-9).

The average mass of diatoms retrieved by the lens tissue method for each temperature treatment at each sampling date is shown below (Fig. 4.48). The greatest mass was collected on the first sampling date, M01, with wide variation between the tanks indicated by the large error bars. Mass of microalgae collected remained fairly stable thereafter with no apparent pattern between temperature treatments. Disturbance, sample point D01, increased the mass of microalgae collected by this method (χ^2=5.5, p=2.0*10^{-2}, r^2=0.52).

There was no apparent pattern to the mass of algae collected by the lens tissue method following different levels of sediment surface disturbance. However, a greater mass of microalgae was collected from tanks at ambient temperature than those in the +4.0 ^oC treatments at all levels of disturbance (Fig. 4.49).

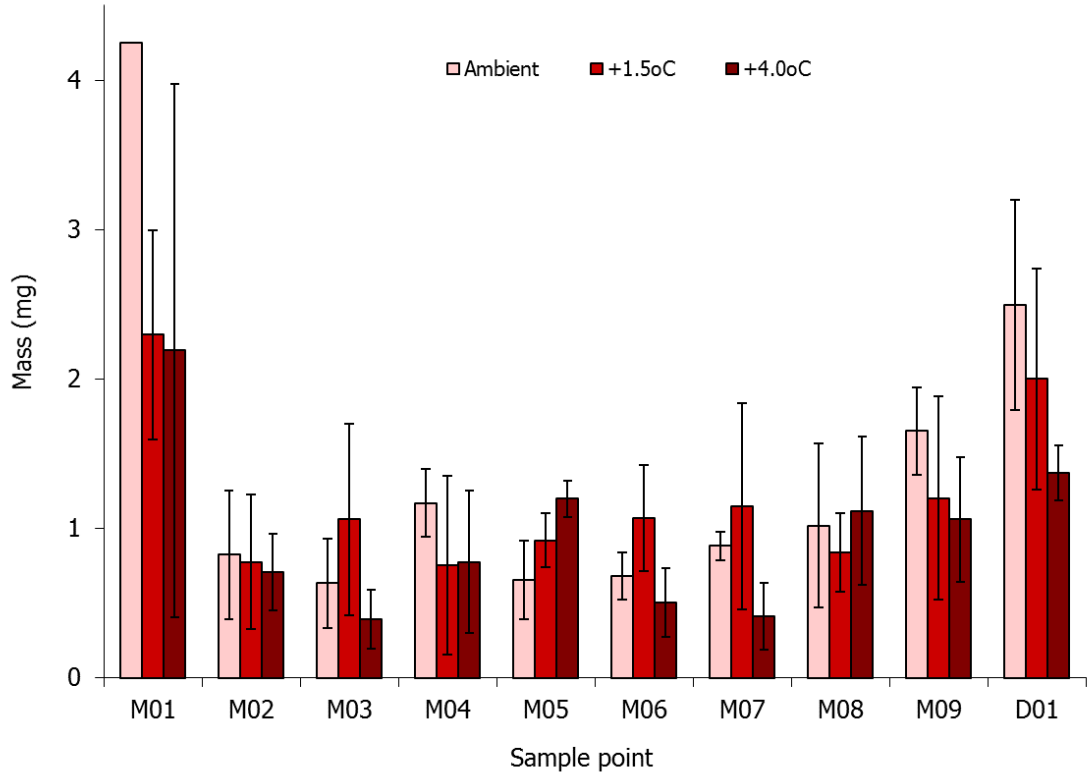


Figure 4.48. Average (n=3) mass of diatoms retrieved from each temperature band at all sample points. Error bar for ambient temperature is not shown as it is too large (4.25±4.83 mg)

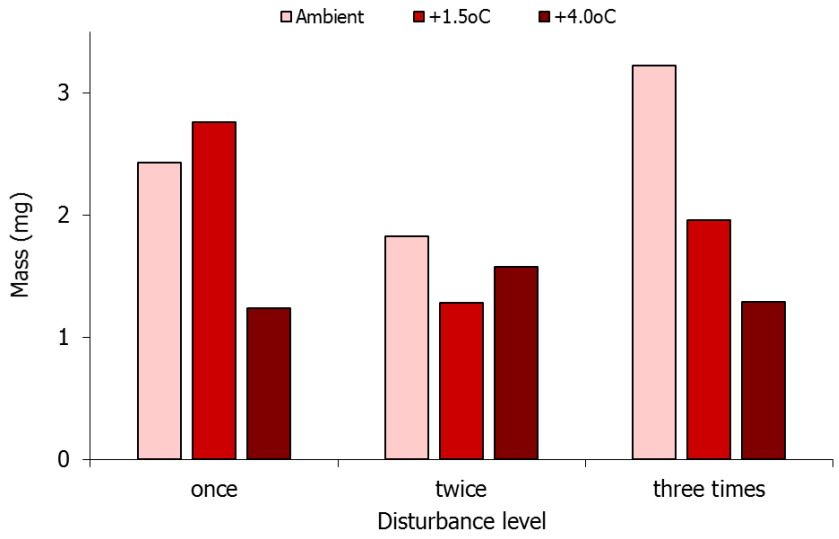


Figure 4.49. Mass of diatoms retrieved from each temperature treatment at each level of disturbance for sample point D01. Samples from each disturbance level were a combined across 3 tanks of the same temperature (n=1)

4.3.5. Metal concentration in algae

The sheet macroalgae present in all tanks was identified as *Enteromorpha sp.* (Fig. 4.50). Microscope examination revealed two species of filamentous macroalgae present in the tanks, but species were not identified (Fig. 4.51). Motile algae collected by lens tissue are collectively referred to as diatoms, the dominant algae type present in these samples (Fig. 4.52).

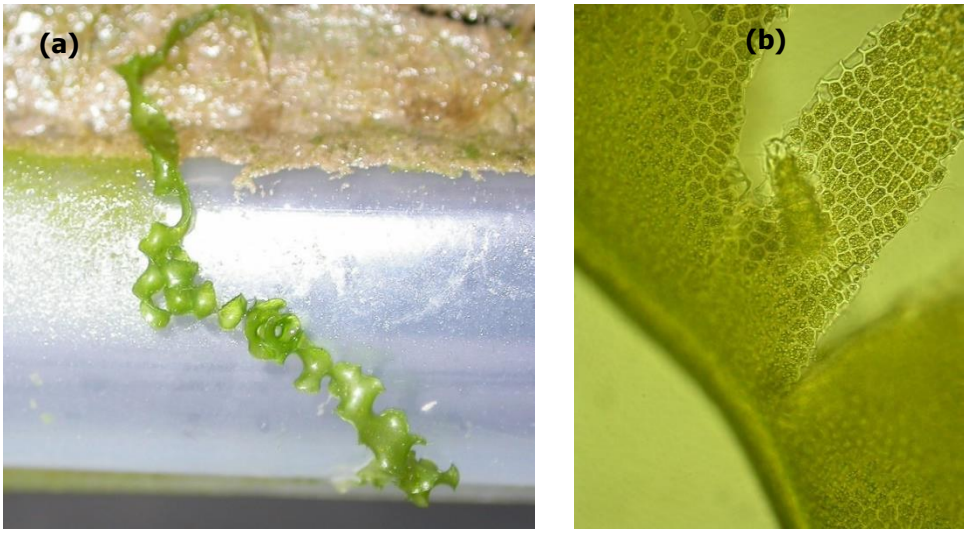


Figure 4.50. *Enteromorpha sp*. (a) in sediment tray and (b) under microscope at x400 magnification

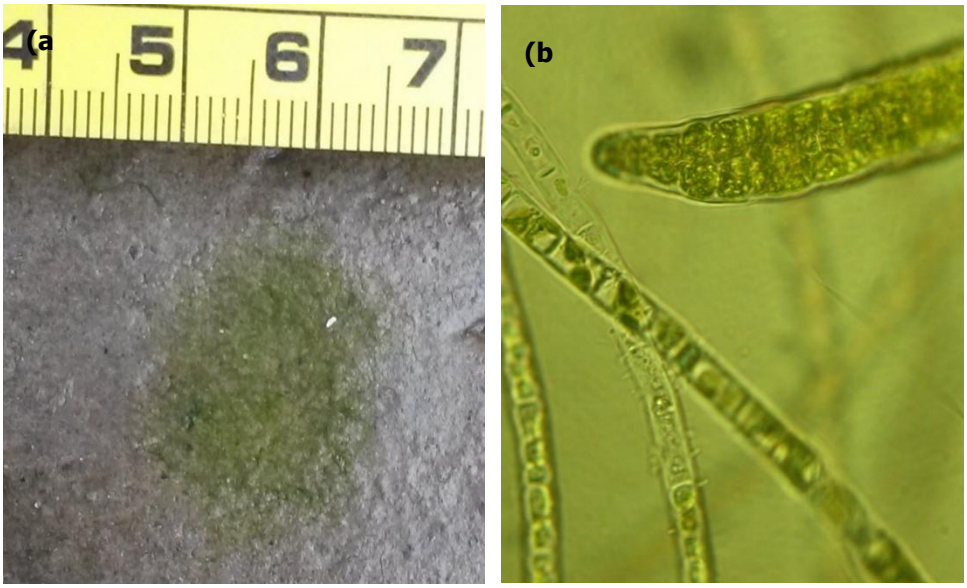


Figure 4.51. Filamentous algae (a) on sediment surface and (b) under microscope at x630 magnification

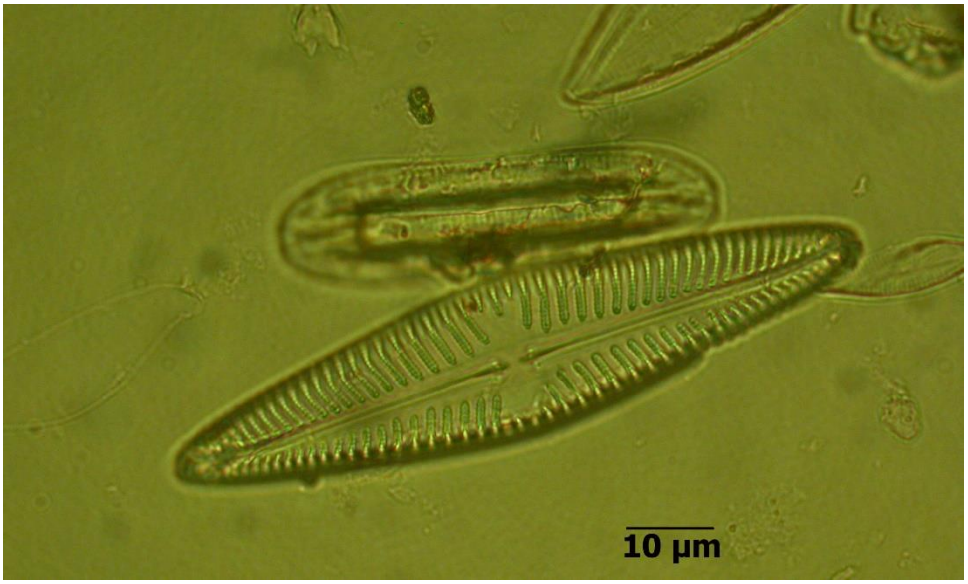


Figure 4.52. Diatom, *Navicula sp*.

Uptake by different algae types

For the majority of analytes investigated diatoms had higher concentrations of metals than either filamentous or sheet macroalgae. Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn and Pb all showed a similar pattern across sampling points (Figs. 4.53 and 4.54 and Appendix 4-6). For these metals, concentrations across algal groups were similar at sample point M02, but by sampling point M04, there were higher concentrations of these metals in diatoms than other algal types. Concentrations of both Mn (χ^2=185.4, p<2.2*10^{-16}, r^2=0.70) and Cu (χ^2=112.4, p<2.2*10^{-16}, r^2=0.65) were higher in diatoms than in filamentous or sheet macroalgae (Fig. 4.53 and 4.54). Concentrations of As across algal groups differed slightly from the above metals in that, although diatoms always had the highest As concentration, concentrations in filamentous algae were similar at sampling point M09 (Fig. 4.55). Cd and Ag concentrations were also greater in diatoms than other algae (Appendix 4-6).

Only one metal, Sn, showed a very different pattern of concentration across the algal types (Fig. 4.56). Concentrations of Sn in sheet macroalgae were initially below the LoD, but increased over the course of the experiment until, at sampling points M08 and M09, sheet macroalgae had concentrations of Sn greater than either filamentous macroalgae or diatoms (χ^2=52.2, p=4.6*10^{-12}, r^2=0.82, Fig. 4.56).

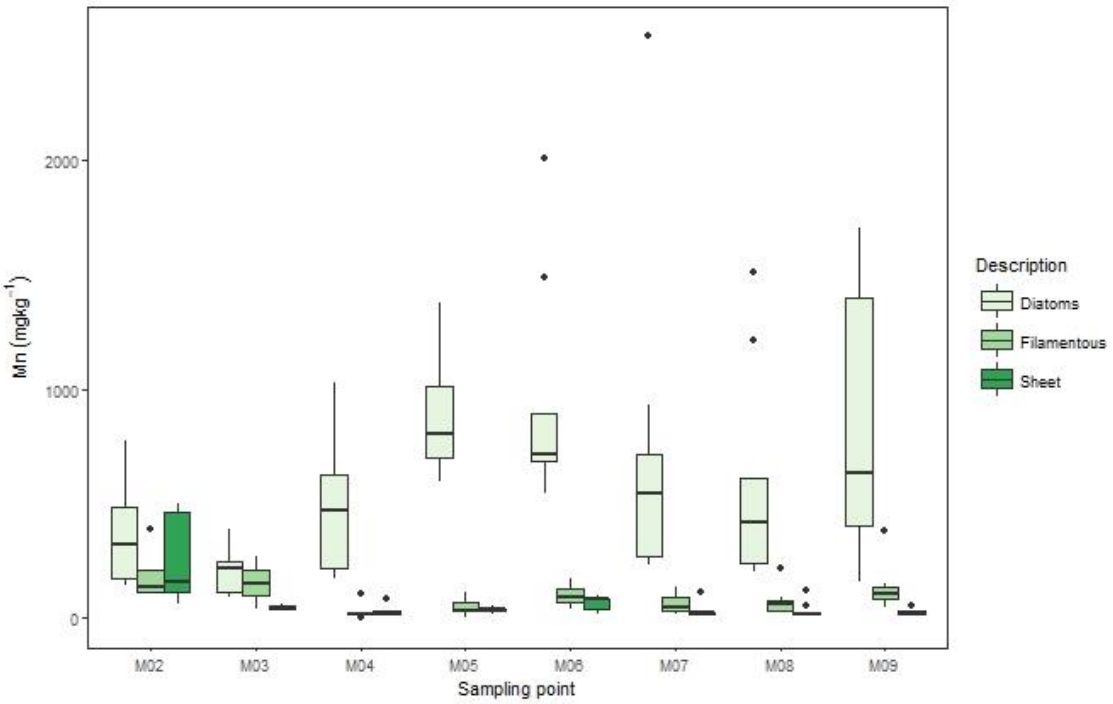


Figure 4.53. Concentrations of manganese (mg kg^{-1} DW) in each algal group at sampling points M02 to M09, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

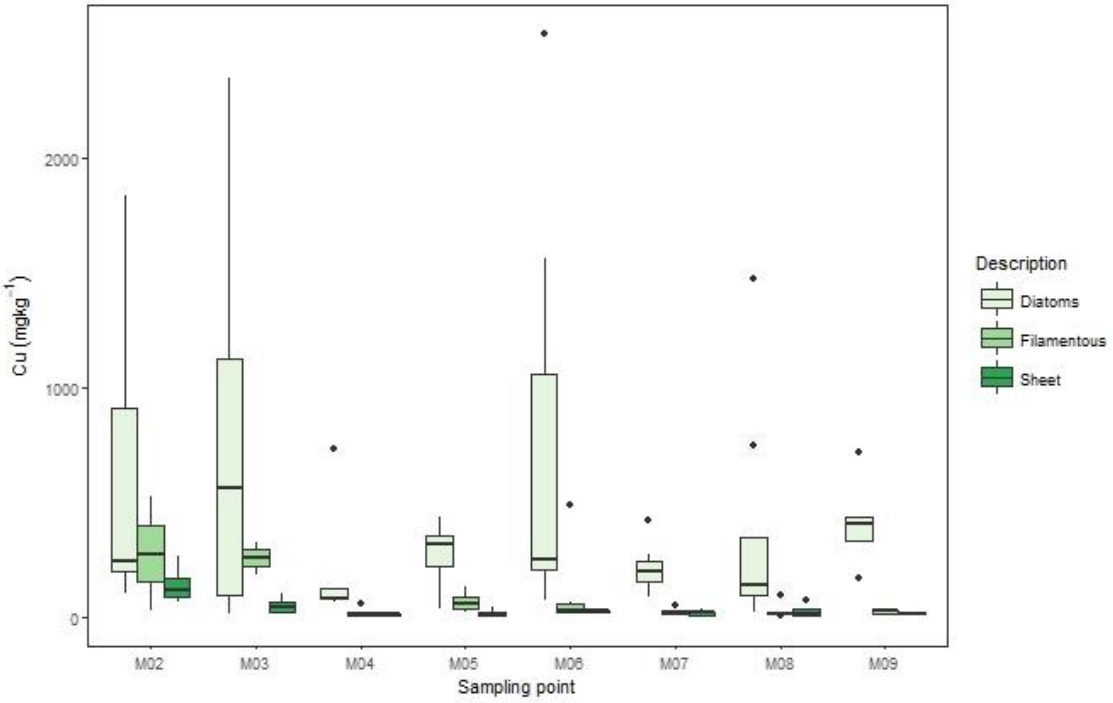


Figure 4.54. Concentrations of copper (mg kg^{-1} DW) in each algal group at sampling points M02 to M09, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

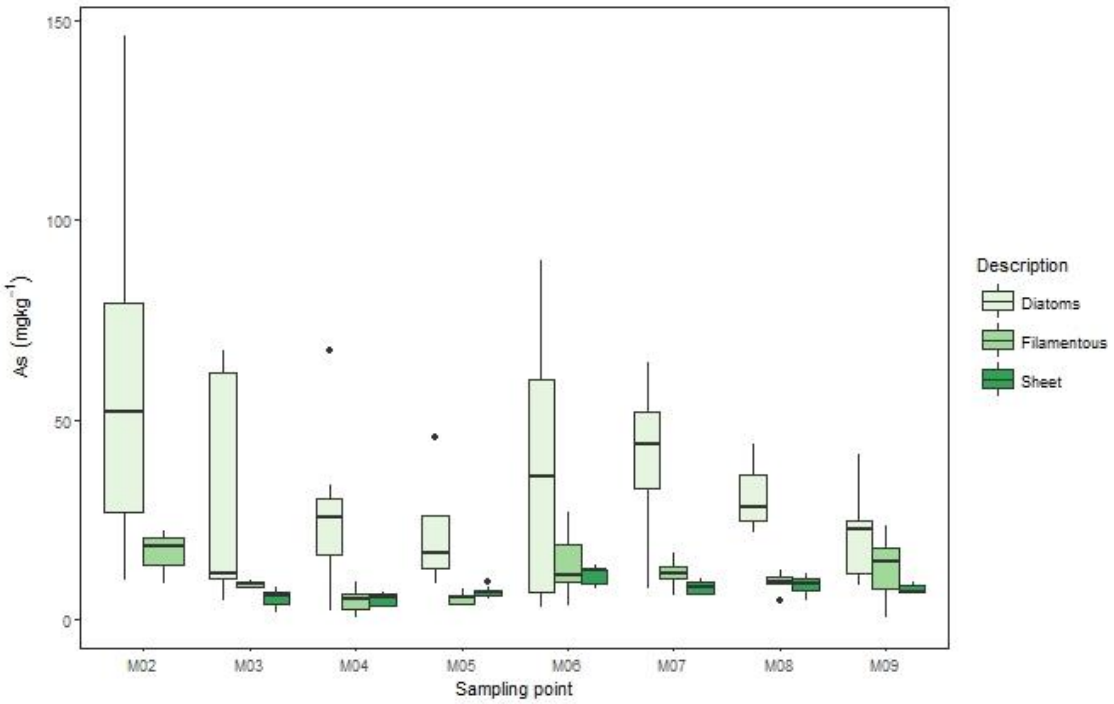


Figure 4.55. Concentrations of arsenic (mg kg^{-1} DW) in each algal group at sampling points M02 to M09, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

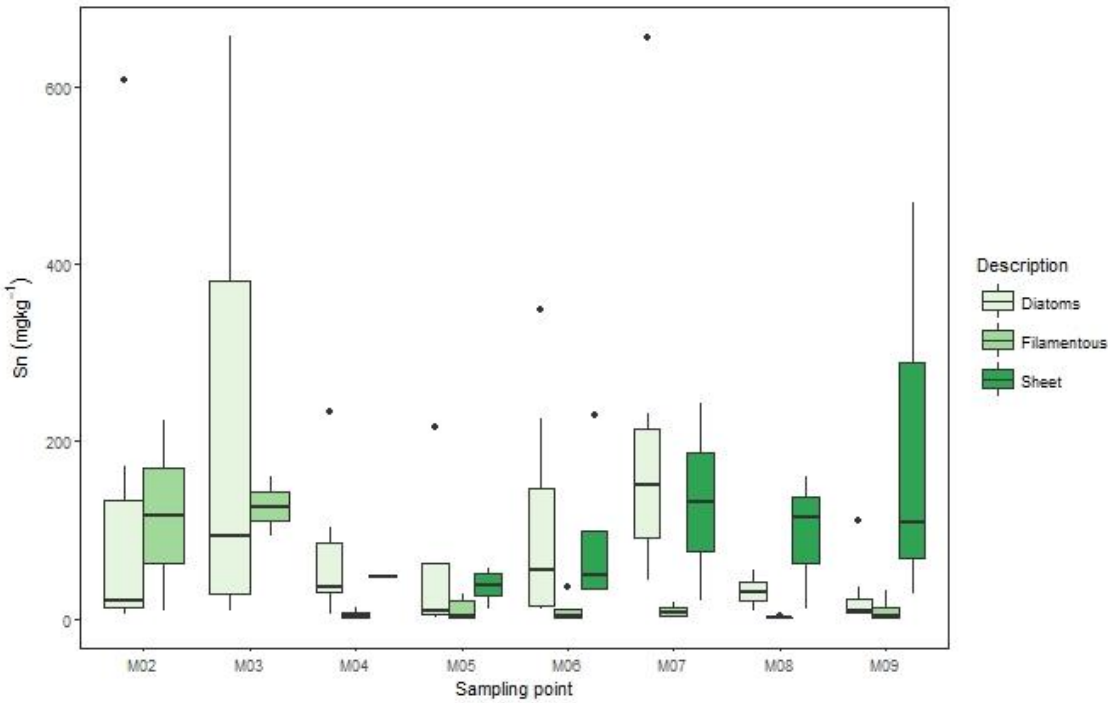


Figure 4.56. Concentrations of tin (mg kg^{-1} DW) in each algal group at sampling points M02 to M09, n=9, outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Internal and external uptake to diatoms

The concentration of metals internal and external to the diatom cells varied dependant on the metal. None of the metals had a significant difference in concentration internal and external to the diatom cells (Fig. 4.57).

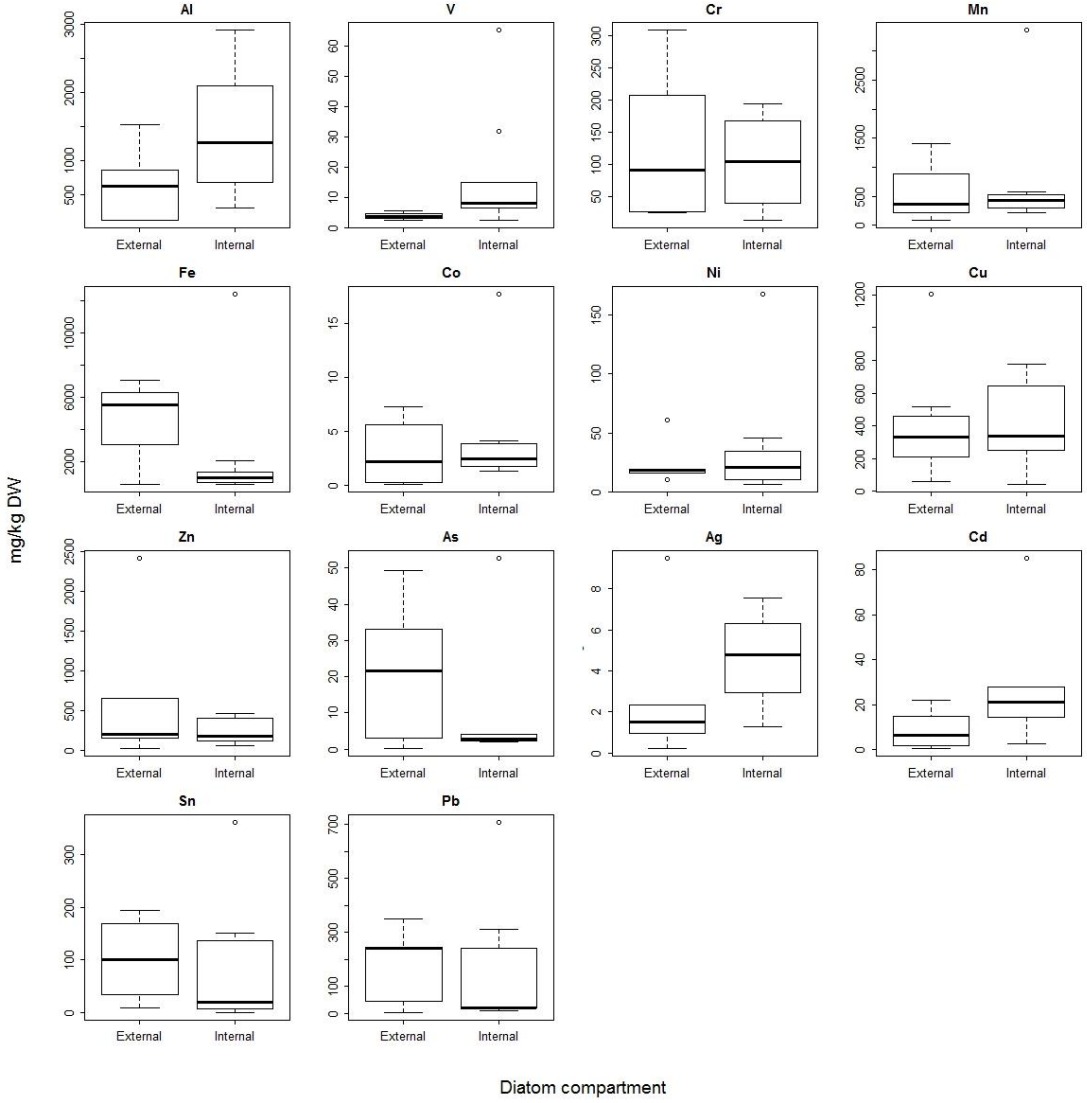


Figure 4.57. External and internal concentrations of analytes in diatoms (mg kg^{-1} DW) from sampling point M01, n=9, outliers (◦) calculated as Q1-1.5IQR and Q3+1.5IQR

Effect of temperature on metal uptake to diatoms

There was no clear effect of temperature treatment on uptake by diatoms for any of the analytes (Figs. 4.58 to 4.60 and Appendix 4-7).

To account for the effect of seasonal temperature changes on contaminant uptake a comparison was made between metal concentration and average sediment temperature in the four days prior to sampling (Figs. 4.61 to 4.63 and Appendix 4-8). Sampling points M07 and M08 were excluded as equipment failure meant there were no sediment temperature data available over this period. This also failed to reveal any relationship, although the highest concentrations all occur at higher temperatures.

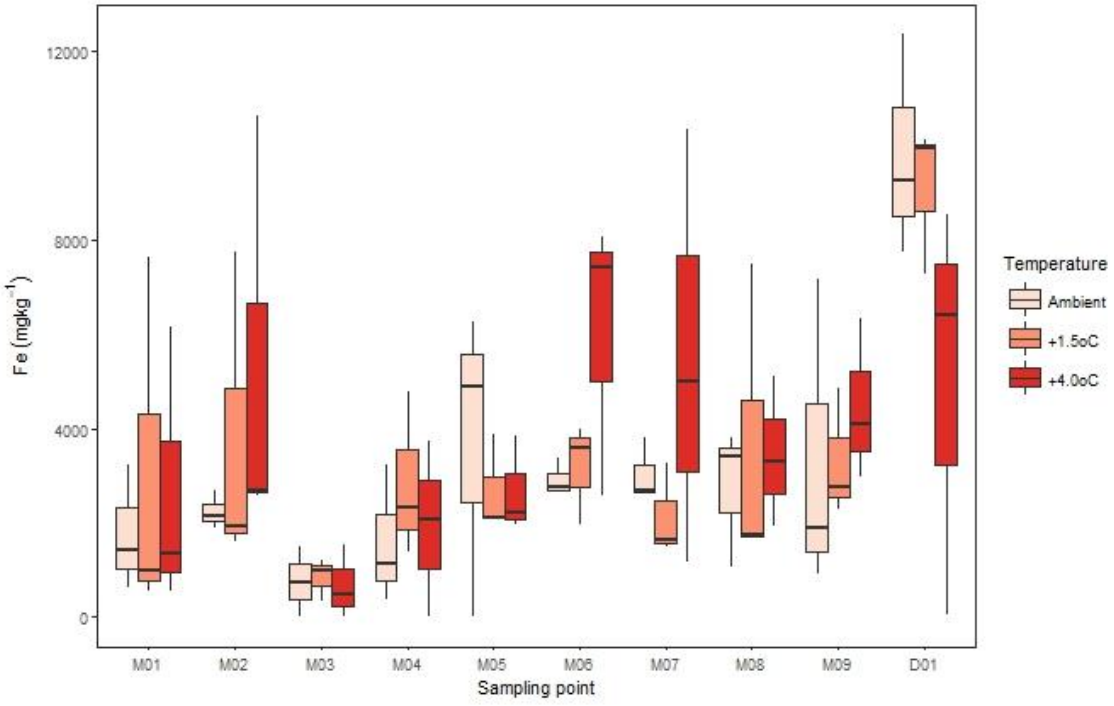


Figure 4.58. Concentration of iron in diatoms (mg kg^{-1} DW) at all sampling points

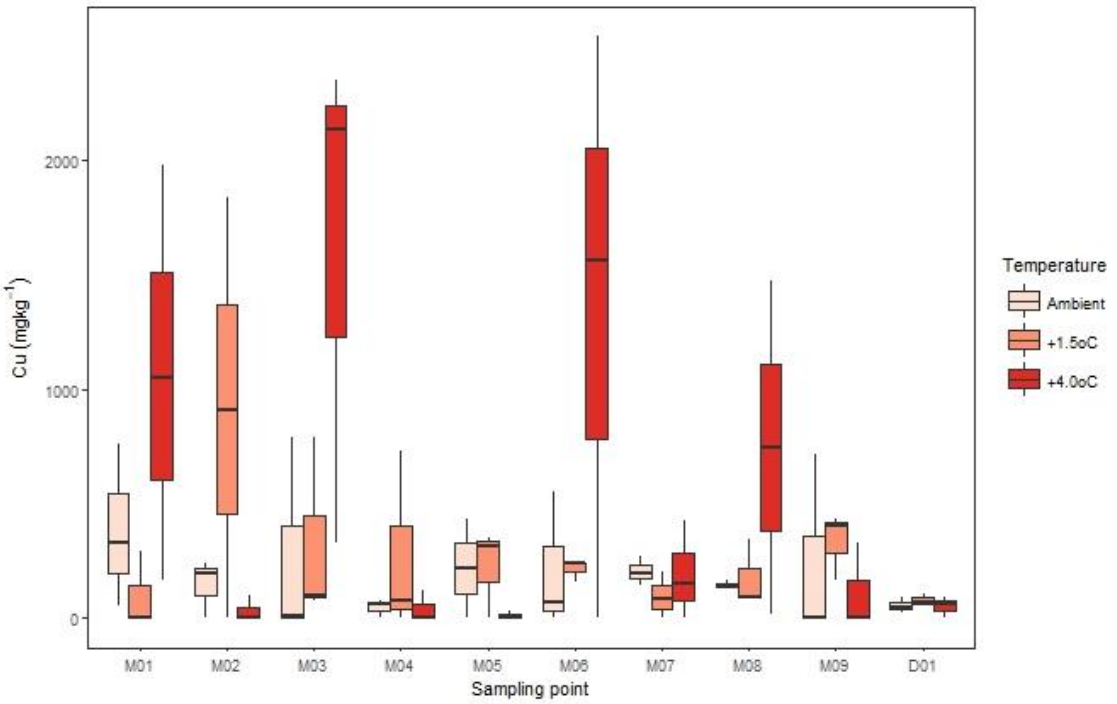


Figure 4.59. Concentration of copper in diatoms (mg kg^{-1} DW) at all sampling points

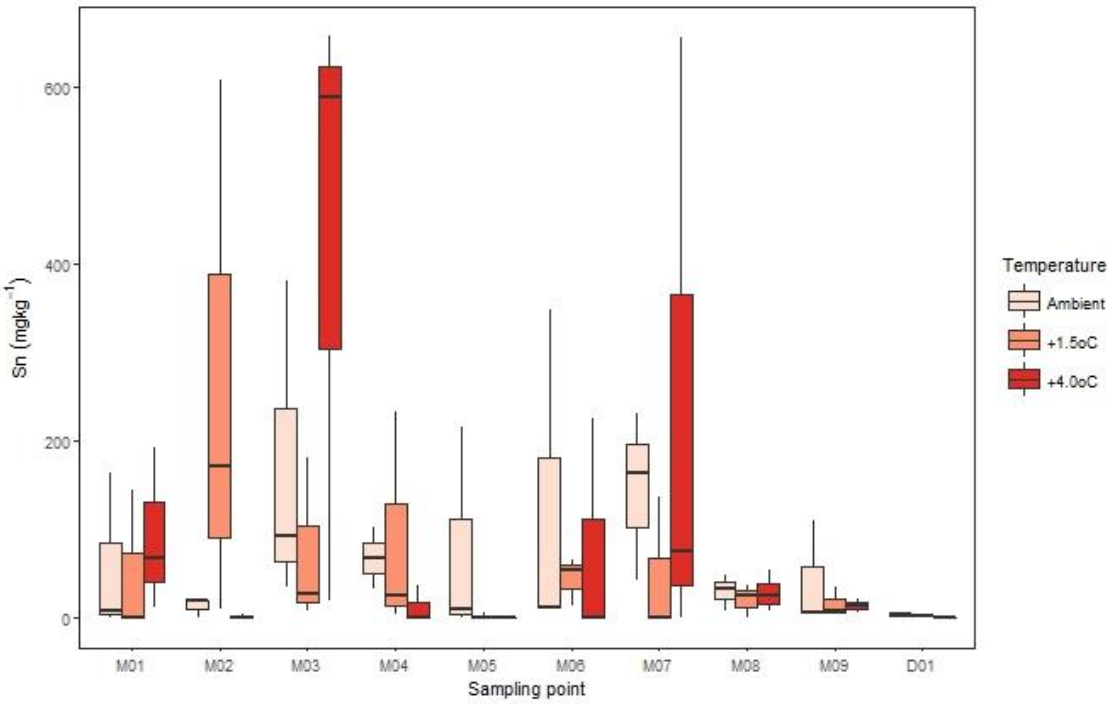


Figure 4.60. Concentration of tin in diatoms (mg kg^{-1} DW) at all sampling points

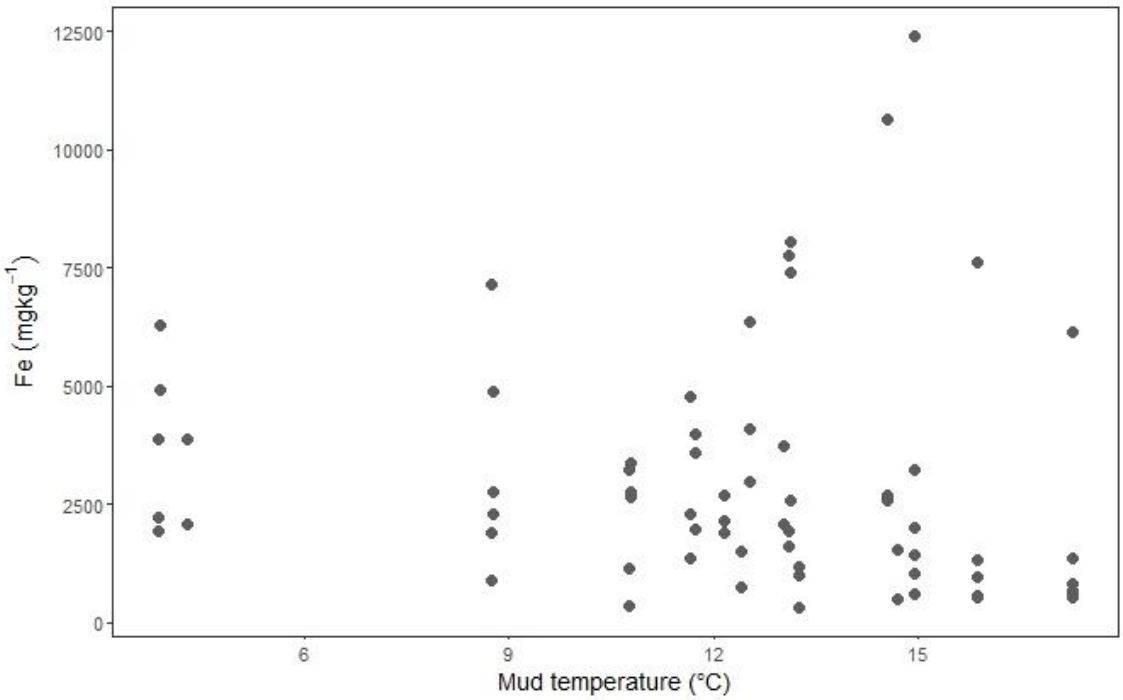


Figure 4.61. Concentration of iron in diatoms (mg kg^{-1} DW) at all sampling points M01 to M06 and M09 against average sediment temperature

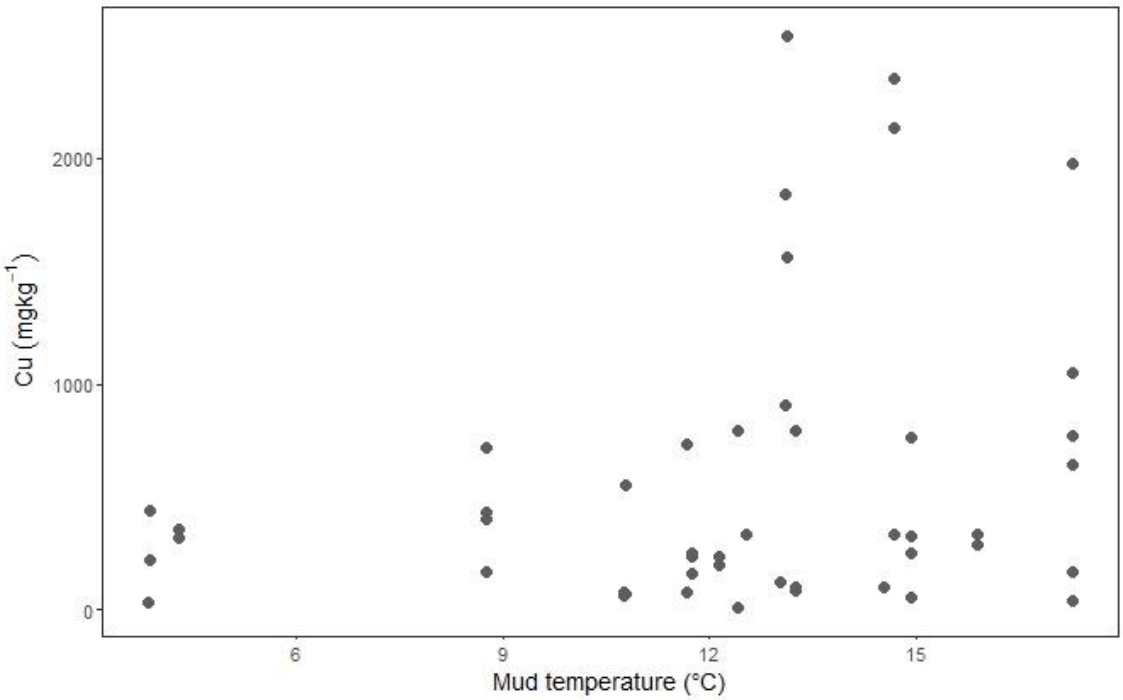


Figure 4.62. Concentration of copper in diatoms (mg kg^{-1} DW) at all sampling points M01 to M06 and M09 against average sediment temperature

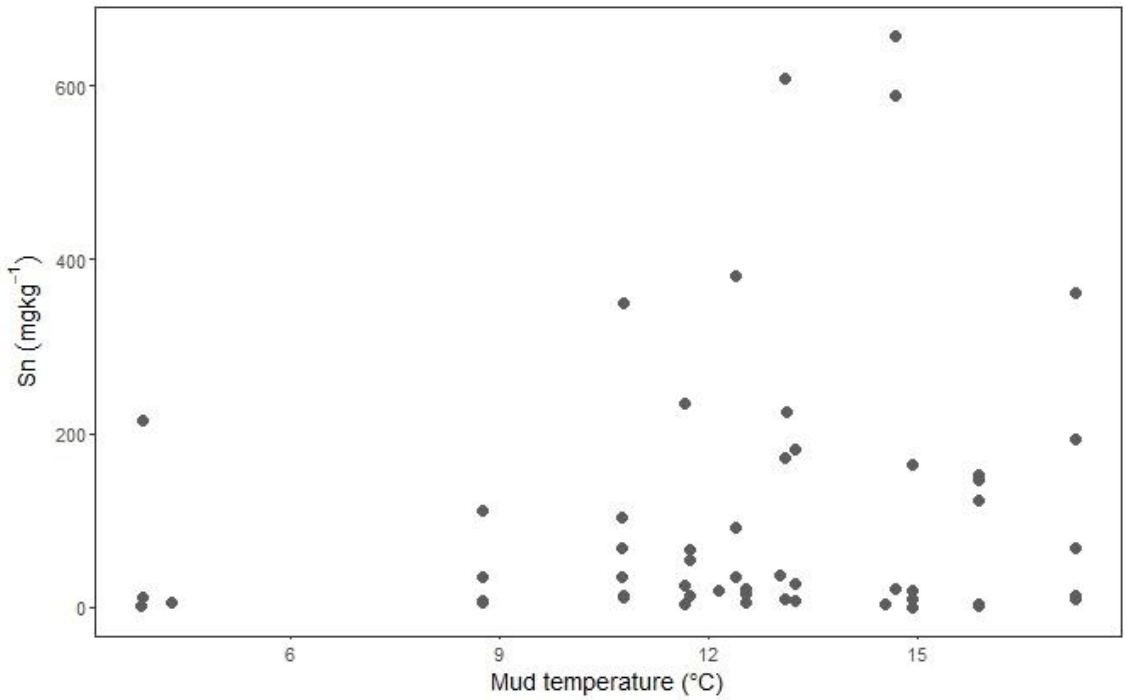


Figure 4.63. Concentration of tin in diatoms (mg kg^{-1} DW) at all sampling points M01 to M06 and M09 against average sediment temperature

Effect of disturbance on metal uptake by diatoms

There was some effect of disturbance on metal concentration in diatoms. For Fe the concentration in diatoms was greatest when the sediment was disturbed (χ^2=28.0, p=1.2*10^{-7}, r^2=0.31, Fig. 4.58). For other metals the concentration was greater after disturbance (sampling point D01) than at the end of the temperature only phase of the experiment (sampling point M09). Examples of this were Al (χ^2=11.3, p=7.8*10^{-4}, r^2=0.54), V (χ^2=10.7, p=1.1*10^{-3}, r^2=0.48) and Pb (χ^2=4.5, p=3.4*10^{-2}, r^2=0.77) (see figures in Appendix 4-7). In contrast to other metals, Cu showed a decline in concentration in diatoms following disturbance of the sediment (χ^2=17.1, p=3.5*10^{-5}, r^2=0.73, Fig. 4.59). Cu was also the only metal for which the level of disturbance had a linear effect on diatom concentration, i.e. concentration in diatoms decreased with increasing level of disturbance (χ^2=10.4, p=5.5*10^{-3}, r^2=0.83, Fig. 4.64).

A second effect of disturbance of the sediment surface was a decline in diatom uptake with increasing temperature. This appeared to occur for several metals (Al, V, Mn, Fe, Co, Zn, As, Sn and Pb) (Fig. 4.65), however the effect was only statistically significant for Mn (χ^2=6.9, p=3.1*10^{-2}, r^2=0.54), Zn (χ^2=6.4, p=4.0*10^{-2}, r^2=0.51), As (χ^2=11.9, p=2.6*10^{-3}, r^2=0.88) and Sn (χ^2=7.3, p=2.5*10^{-2}, r^2=0.66).

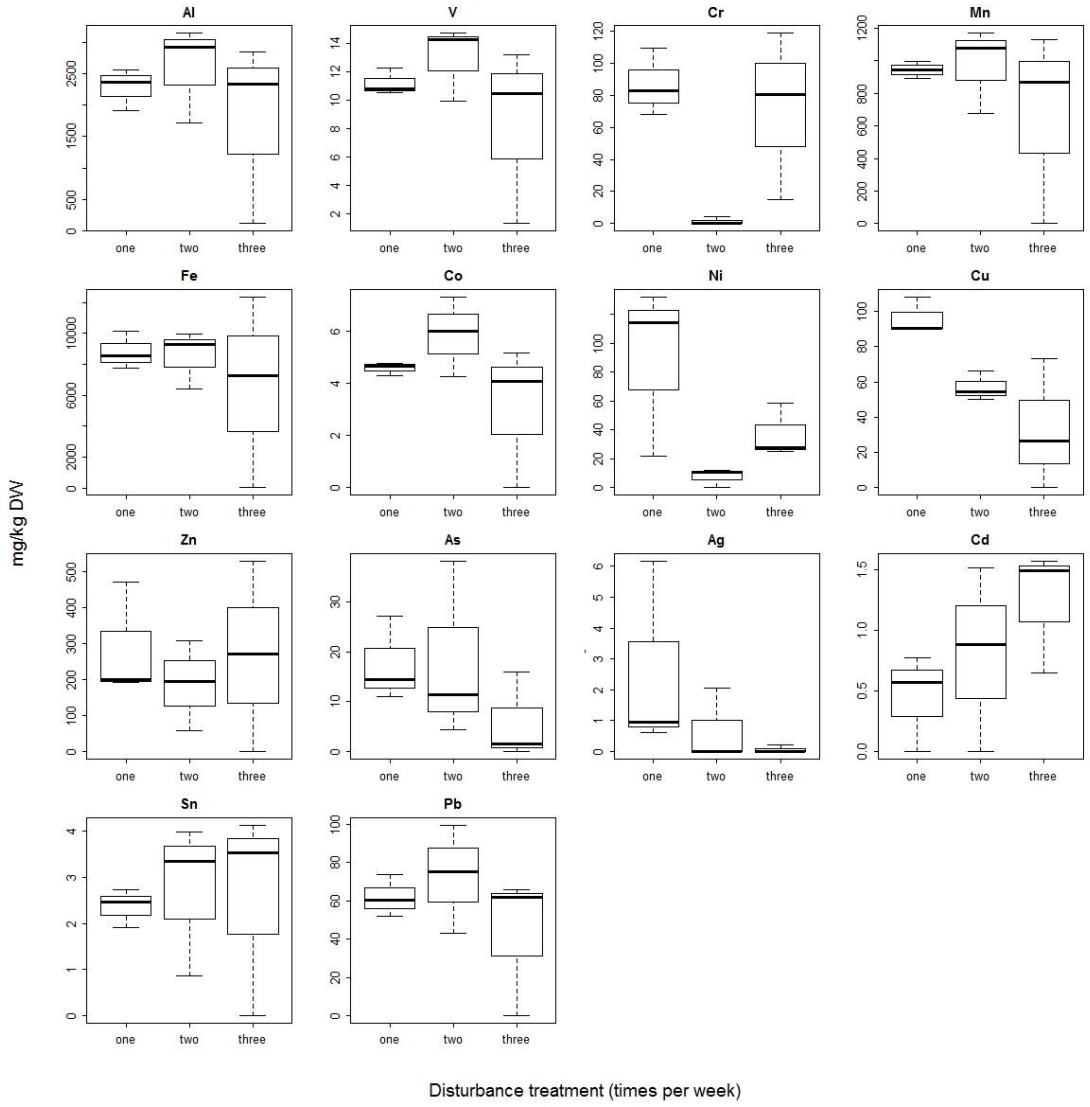


Figure 4.64. Concentration of metals in diatoms (mg kg^{-1} DW) at sampling point D01, following disturbance of sediment surface, at each disturbance level, n=3

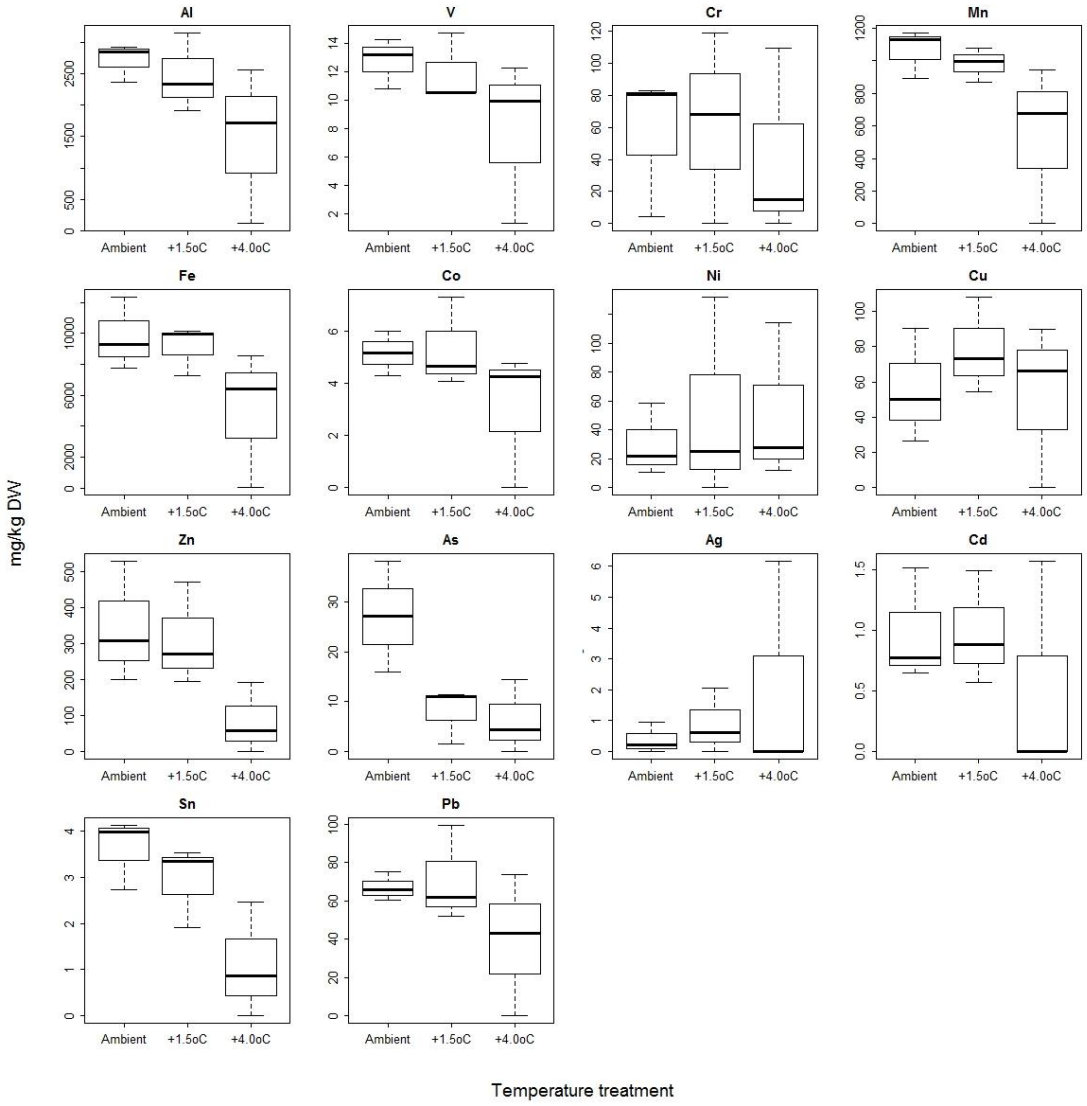


Figure 4.65. Concentration of metals in diatoms (mg kg^{-1} DW) at sampling point D01, following disturbance of sediment surface, at each temperature treatment, n=3

4.4. Discussion

The main questions addressed in this study were whether uptake of metals to benthic microalgae was affected by (i) changes in temperature or (ii) sediment disturbance, and if there were differences in metal uptake from sediment by the different types of algae present.

4.4.1. Sediment

Organic matter

The percentage organic matter increased in both the upper (0 to 5mm) and lower (5 to 20mm) sediment depths between sample point M09, taken at the end of the temperature treatment experiment phase, and D01, following the temperature plus disturbance treatments, by 0.32%. The difference between percentage organic matter in the upper and lower sediment was similar at both sampling points, M09 and D01 (0.50 and 0.51% respectively). It seems likely that the increase in percentage organic matter is simply due to the disturbance which incorporated the filamentous and sheet macroalgae from the surface into the sediment. There was no difference due to temperature treatment or disturbance level which corresponded to the lack of difference in biomass between temperature treatments prior to the disturbance phase of the experiment.

Particle size

A difference was observed between the percentage of clay sized particles at the end of the temperature only (sampling point M09) and disturbance (sampling point D01) phases of the experiment, with an increase in clay sized particles following sediment disturbance. Furthermore, there was a difference in clay sized particles between the upper (0 to 5mm) and lower (5 to 20mm) sediment layers at sampling point D01, with a higher percentage of clay particles in the upper sediment. This is thought to be due to the settling out of the sediment particles following disturbance of the sediment surface. Larger particles would be expected to settle first and therefore at a greater depth, whilst the smaller particles would settle last at the top of the sediment (Wang, 2012).

Water content

Sediment water content was altered due to sediment disturbance, increasing in the upper (0 to 5mm) layer and decreasing in the lower (5 to 20mm) layer at sampling point D01 compared to M09. This led to a difference in water content between the upper and lower layers of sediment

at D01, which was not apparent at M09. The average water content of all the sediment (both layers) did not change between the sampling points, but the distribution of water did change. The change in water content was not related to organic matter as percentage organic matter increased equally at both depths between these sampling points.

The reason for the difference in water content between the upper and lower sediment layers at sampling point D01 is thought to be the change in the percentage of clay sized particles present in these layers following disturbance of the sediment surface (Section 4.3.2 and above). Smaller clay particles have a higher porosity and therefore hold more water than larger silt and sand particles (Avnimelech et al., 2001).

Metal concentration in sediment

All the essential, transition metals analysed, except Cu, showed a similar pattern of concentration in the sediment throughout the course of the experiment. In addition, the basic metals, Al and Sn, and the semimetal As also followed a similar pattern to the transition metals.

There was a higher concentration of all analytes in the sediment at sampling point M05. This is believed to be due to the nature of the sample taken (a small scrape of the top few millimetres) which was necessary to preserve the integrity of the sediment surface for the remainder of the temperature only period of the experiment. This scrape probably contained a higher percentage of organic matter than other sediment samples (although this could not be verified in the laboratory due to its small volume). However, sediment sampled at points M09 and D01 showed 0.5% more organic matter in the upper 0 to 5mm than the deeper 5 to 20mm layer (Section 4.3.2). It is therefore possible that the M05 scrape, taken from a depth of less than 5mm, had an even higher organic matter content. It is known that metals generally have an affinity for organic matter (Burton Jr., 2010) and metal concentrations were often greater in the upper (0 to 5mm) layer of sediment samples from sampling points M09 and D01 (Figs. 4.35 to 4.37). The similarity between different metals at this (M05) and other sampling points suggests that nothing unusual occurred within the sediment at this sampling point, with the variation due simply to the nature of the sample taken. In addition, these samples were taken just as biomass peaked, increasing the likelihood that high concentrations are due to a high percentage organic matter.

There was a decrease in sediment concentrations of Fe, Mn and Sn from the starting sediment, M00, to sampling point M09 at the end of the temperature only experiment phase. This is thought to be due to uptake by the algae which had a greater biomass at M09. Whilst it could be argued that the sediment sample contains the biomass fraction, and therefore a difference in sediment concentration could not be due to algae uptake, the sample will have contained

proportionally more sediment than algae (due to the depth of the sediment sample). Additionally, any macroalgae from the surface of sediment samples was removed, it is therefore possible that the observed decrease in metal concentration was due to uptake by algae.

For several metals (Mn, Fe, Co, Ni, Zn, As, Sn and Pb) there was an increase in sediment concentration from sampling points M09 to D01. It is believed that this is due to the sediment disturbance which removed sheet and filamentous macroalgae from the sediment surface into the top layer of sediment where it broke down causing a decrease in biomass in all tanks (Fig. 4.45). This would have led to a release of metals from the algae and a corresponding increase in concentration of metals in the sediment.

A difference in sediment concentrations of Mn and As with depth was observed at sampling point M09 and D01. Concentrations of both these analytes decreased from the upper (0 to 5mm) to lower (5 to 20mm) sediment level at both sampling points. This difference was more pronounced in the D01 samples where a corresponding increase with organic matter was also observed. At sampling point D01, there was also a higher concentration of Fe within the upper sediment compared to the deeper, more compacted layer. This difference may be due to the input and decomposition of organic matter (as described above), which could have influenced concentrations in the upper 0 to 5mm more than the deeper sediment layer as the disturbance was carried out only to a depth of 5mm. It may also be due to the higher percentage of clay particles at the shallower depth following sediment disturbance (Fig. 4.27). Metals have a greater affinity for smaller sediment particles due to their greater surface area (Donazzolo et al., 1984).

In contrast to other metals which showed variation in concentration with sediment depth at sampling point D01, Pb had a lower concentration in the upper sediment layer compared to the deeper more compacted sediment. This is unexpected due to the higher levels of organic matter in the upper layer which were also shown to affect Pb concentrations, although to a lesser extent than sediment depth.

4.4.2. Analyte concentrations in water

As and Cu had concentrations in overlying water greater than those described as producing acute effects in marine water (Chapter 5, Table 5.11) (Buchman, 2008).

For some analytes (Mn, Co, As, Al and Ni (Figs. 4.41 to 4.43 and Appendix 4-5) the pattern of concentration in water appeared to follow that of algal biomass (Fig. 4.45). There were higher concentrations of these analytes in the water at sampling points M04 to M07, when biomass was greatest. This is contrary to the findings of a recent study of metal transfer from sediment to river water (Prieto et al., 2016), which found that water concentrations of As and Mn were

reduced in the presence of a diatom dominated biofilm. However, it is in agreement with findings in Chapter 3 (sections 3.3.11 and 3.4.6) in which it was shown that Cd concentrations in overlying water increased in the presence of the diatom *Cylindrotheca closterium*. This was thought to be potentially due to two mechanisms, the alteration of redox conditions at the sediment surface due to the presence of algae, or excretion of metals in the extracellular polymeric substances (EPS), some fraction of which is likely to be soluble in water.

For three analytes (V, Mn and Co) there was a spike in overlying water concentration at sampling point M03 (Figs. 4.41 to 4.43). This was particularly evident in the ambient temperature treatment for Mn and Co, but for V it occurred across all temperature treatments at this sampling point. For Mn and Co this spike was no longer evident by sampling point M04, but for V the spike at M03 represented a peak from which concentrations then declined for the remainder of the temperature only period of the experiment (i.e. to sampling point M09). It has been suggested that biological uptake is responsible for the removal of V from surface water in estuaries (Santos-Echeandia et al., 2009). However, the decline observed here is not in line with either increasing biomass or increased concentration in algae (Appendices 4-5 and 4-6). This may be because concentrations in water were very small and therefore any changes are unlikely to be observed in other sample compartments. With respect to Mn and Co, there was a decline in concentrations of these metals in all algal types from sampling point M02 to M03, from means of 290 mg kg^{-1} and 1.5 mg kg^{-1} to 110 mg kg^{-1} and 0.5 mg kg^{-1} respectively (Fig 4.53 and Appendices 4-5 and 4-6). This may be responsible for a release of these metals to the water column.

V was the only metal for which there was an effect of water temperature on water concentrations. V showed an increase in concentration at the +4.0 ^{o}C treatment at most sampling points. This differs from a study of benthic flux of trace metals in the Venice Lagoon (Turetta et al., 2005), which found reduced concentrations of V in water at increasing temperatures.

4.4.3. Algal biomass

Biomass was found to differ with temperature treatment, with the highest growth occurring in the ambient temperature treatment tanks, followed by the plus 4.0 ^{o}C and 1.5 ^{o}C treatments. These differences occurred from the end of November 2015 until the end of the temperature only phase of the experiment (Fig. 4.45(b)). Tank 6, which was set to ambient temperature, appeared to have had a higher biomass than other tanks from mid-November 2015 to February 2016. Reasons for this are unknown although it was observed that the biomass in tank 6 consisted largely of macroalgae compared with the other tanks and this is suspected to have influence the biomass readings (Appendix 4-9).

There was a discrepancy between the mass of microalgae recovered via the lens tissue method and the NDVI measured. The largest mass of diatoms was recovered at sampling point M01, although NDVI was low, thereafter the mass collected decreased (Fig. 4.48) although biomass (as measured by the NDVI) increased (Fig 4.45). The reduction in mass of diatoms collected is thought to be due to several reasons; a reduction in diatom mass; an increase in filamentous and sheet macroalgae; a reduction in diatom migration; and difficulty in keeping lens tissue fully in contact with the sediment surface. Diatoms were initially the dominant form of algae accounting for the majority of NDVI-recorded biomass. This can be seen in the initial growth (Fig. 4.45 (a)) where biomass increased from day zero to day 14, followed by a decrease to day 22 after which it started to increase again. This dip in biomass represents the point at which the algal mass was visually observed to switch from dominance of microalgae to forms more easily visible to the naked eye, i.e. macroalgae (Fig. 4.46). At sampling point M01, when the largest mass of diatoms was collected via the lens tissue method, NDVI-biomass was increasing and was wholly made up of microalgae, i.e. not visible to the naked eye. By sampling point M02 NDVI-biomass had declined and both filamentous and sheet macroalgae were present, this likely accounts for the low mass of diatoms collected via the lens tissue method.

Later in the experimental period, although patches of biofilm were visible on the sediment surface (Appendix 4-9), these did not appear to migrate (i.e. there was no change observed as the tide encroached). A reduction in migration was possibly due to a lack of sediment disturbance. The lack of sediment disturbance was confirmed by the water clarity during the immersion period (Fig. 4.18) and the lack of movement in the sediment surface over time (Fig. 4.46). This was due to the slow incoming tide which filled from the bottom of the tank as opposed to the effect of normal incoming tides as seen in estuaries. Although diatom migration patterns are related to tides and light levels (Consalvey et al., 2004) lack of disturbance of the sediment surface may have reduced this function. Such a corresponding reduction in diatom migration would reduce the effectiveness of the lens tissue method.

The possibility that migration was reduced due to lack of sediment disturbance is upheld by the fact that although biomass was very low at sample point D01 (Fig. 4.45), the mass of algae retrieved by lens tissue is greater than at all other sampling points except M01 and M09 (Fig. 4.48). The reduction in total biomass during the disturbance period of the experiment could also be due to the destruction of the sheet and filamentous macroalgae, which were observed to decline throughout the disturbance period and were no longer apparent by the sampling point D01 (Figs. 4.13 and 4.47).

The final possible reason for a reduction in diatom mass collected from sampling points M02 to M09 is the physical effect of the presence of macroalgae. Macroalgae at the sediment surface made it difficult to make complete contact between the sediment surface and the lens tissue.

This will have reduced the area of lens tissue into which diatoms could migrate, potentially contributing to a reduction in diatom mass collected.

The decline in biomass observed throughout the disturbance period of the experiment (Fig. 4.45) is probably due to the frequency of disturbance. It has been predicted via modelling (Mariotti and Fagherazzi, 2012) that frequent disturbance of the sediment surface reduces biomass as there is insufficient time for recovery. Hence the period of lower disturbance during the neap period of the spring-neap tidal cycle allows recovery and stabilisation of the sediment surface, so the MPB can better withstand the higher energy of a spring tide. Whilst frequent high levels of disturbance due to diurnal tides do not allow a long enough period of recovery and biomass declines. A similar conclusion was reached in a study which observed MPB biomass at the sediment surface by remote sensing (van der Wal et al., 2010) which concluded that increased disturbance due to increased storminess would result in a decrease in MPB biomass.

4.4.4. Metal uptake by algae

Uptake by different algae types

Three groups of algae were defined in the experiment: migratory diatoms (Fig. 4.52); filamentous macroalgae (Fig. 4.51); and sheet macroalgae (Fig. 4.50, identified as *Enteromorpha sp.*).

Diatoms were found to have a greater concentration of most metals compared with macroalgae types (either as filamentous or sheet). The only metal for which diatoms had a lower concentration was Sn, which was more concentrated in the sheet algae than either the diatoms or filamentous algae (Fig. 4.56) at the end of the temperature only period of the experiment (M09).

Greater uptake of metals by diatoms was probably due to their small size which means they have a large surface area to mass ratio and therefore adsorb more metal than an equivalent mass of larger algae. The size and volume of microalgae has previously been reported to affect both uptake (Khoshmanesh et al., 1997) and sensitivity (Levy et al., 2008) to metals. This is thought to be due to the size-dependence of their biochemical composition, metabolism, growth and loss processes (Suresh Kumar et al., 2015).

Higher uptake of metals by diatoms may also be due to their closer association with the sediment. The microalgae sampled had the capacity for migration within the sediment and it has been reported that depth affects nutrient uptake to algae, due to the changes in speciation of nutrients with depth in the sediment (Saburova and Polikarpov, 2003), it is therefore

proposed that the same may be true for metals. In this experiment it was seen that diatoms had a higher concentration of most metals than macroalgae.

Accumulation of metals is related to species biogeochemical composition (Malea and Kevrekidis, 2014). For example, amongst the macroalgae, brown algae (Phaeophyta) have a higher capacity for metal uptake than either green (Chlorophyta) or red (Rhodophyta) algae (He and Chen, 2014), which is related to alginate content (Zeraatkar et al., 2016). It has also been reported that algae of the same genus, but different species, vary in their capacity to take up metals from water (Suresh Kumar et al., 2015). A recent study of macroalgae carried out in Greece found interspecific variation in trace element concentrations and that filamentous forms often had higher metal concentrations than sheet algae (Malea and Kevrekidis, 2014). In this study, concentrations in the filamentous and sheet forms appear to be similar for most metals (Figs. 4.53 to 4.55 and Appendix 4-6). However, concentrations of Cr were higher in filamentous than sheet algae at most sampling points (Appendix 4-6) and Sn concentrations in sheet algae were higher than in filamentous algae from sampling point M04 onwards (Fig. 4.56).

In addition to the above, it was observed that concentrations of most metals remained fairly stable in diatoms throughout the course of the temperature only phase of the experiment, whilst concentrations in filamentous and sheet algae often started with concentrations comparable to diatoms but which then declined through the course of the experimental period (Figs. 4.53 to 4.55 and Appendix 4-6).

Internal and external uptake to diatoms

Although uptake of contaminants by both live and dead algae is possible, this study was only concerned with uptake by live algae, which were still capable of migration through the lens tissue. Live microalgae take up metals by passive removal in which metals are externally accumulated, a reversible process, and active intracellular accumulation, which is slower and usually irreversible (Suresh Kumar et al., 2015). Externally bound metals on the diatoms may be transferred to EPS, which has been observed to increase in production in the presence of some metals (Pistocchi et al., 2000). In order to better understand the location of the metals studied internal and external concentrations were quantified. This could only be carried out for the first collection of algae at sampling point M01 due to the lower weights recovered at later sampling points (Fig. 4.48). It was not possible therefore to determine if internal and external concentrations changed with time and the differences in temperature and disturbance.

No statistically significant difference was found between internal and external concentrations of any of the metals analysed here. In Chapter 3 (Section 3.3.7, Fig. 3.12) higher internal uptake

of Cd were observed in the laboratory experiment and this has been reported in studies of uptake from water by others (Pérez-Rama et al., 2010, Torres et al., 2014), however, concentrations of Cd in the sediment were much lower here (0.15±0.05 mg kg^{-1} compared to 10.31±0.12 mg kg^{-1} in the spiked laboratory sediment). A recent laboratory study into uptake of As from freshwater sediment to a diatom dominated biofilm also found a uniform distribution between the outside and inside of cells (Prieto et al., 2016).

Effect of temperature on metal uptake to diatoms

No consistent effect of temperature treatment on uptake of any of the analytes to diatoms was observed in this study (Figs. 4.48 to 4.63 and Appendices 4-6 and 4-7).

Previous studies (all of which were concerned with uptake of metals from water) have reported a range of effects of temperature on metal uptake by microalgae and the results are not consistent. For example, Rangsayatorn et al (2002) found no effect of temperatures (ranging from 20 to 40 °C) on Cd uptake by the cyanobacteria *Spirulina platensis*. Whilst others report an effect of either increased (Snoeijs and Notter, 1993, Sari and Tuzen, 2009) or decreased (Gupta and Rastogi, 2008, Tuzen et al., 2009) uptake with increasing temperature. It is acknowledged that temperature effect is likely to vary with both the metal (Suresh Kumar et al., 2015) and algal species (Zeraatkar et al., 2016), which may account for such disparity in results but this makes it difficult to say what the longer term consequences of increased temperatures due to climate change might lead to.

The lack of variation of metal uptake with temperature observed here may be due to other factors within the mesocosm such as biomass, organic content of the sediment, concentrations in sediment or water, which due to competing effects may have masked any clear effect of temperature. The temperature range investigated here was very small (0 to 4 °C) in line with predicted temperature increases for the Celtic-Biscay Shelf in the next 100 years (Philippart et al., 2011). Additionally, the temperature variation exerted by air temperature through the course of the experiment (section 4.3.1), although realistic for intertidal algae, complicates the interpretation of results. This is because the sediment surface and associated algae were subject to temperature controlled by both the overlying water and the air temperatures which meant that, particularly during warmer months, temperatures did not vary significantly between tanks whilst the tide was out (Fig. 4.23).

Effect of disturbance on metal uptake by diatoms

An increase of metal uptake to diatoms following disturbance of the sediment surface was observed for Fe, Al, V and Pb. However, there was no difference in uptake due to the different levels of disturbance (once, twice or three times per week) for these metals (Fig. 4.64). Sediment disturbance is known to alter its chemical properties, potentially causing mobilisation of metals and affecting bioavailability (Eggleton and Thomas, 2004). For example, disturbance will increase oxygenation and the depth of the oxic sediment layer (Atkinson et al., 2007). It is well accepted that disturbance causes the release of metals from sediment to the water column (Eggleton and Thomas, 2004, Atkinson et al., 2007). Release of metals to water makes them more available for uptake by biota and as has been shown for Cd (Chapter 3, Figs. 3.10 and 3.11) uptake of metals from contaminated water is greater than from contaminated sediment.

A second potential cause of increased metal availability, in this study, following disturbance are metals released from decaying macroalgae. A sharp decline in biomass was observed at the start of the disturbance period of the experiment (Fig. 4.45) and by sampling point D01 there were no macroalgae present within the sediment trays (Fig 4.47). It is possible that metals were released from macroalgae to pore water and were readily taken up by diatoms before becoming attached to sediment particles. Again, this is similar to the process seen in the laboratory experiment (Chapter 3) in which, when the water was initially contaminated, Cd was taken up more readily by the diatoms and the presence of diatoms appeared to slow uptake by the sediment (Sections 3.3.5 and 3.3.8).

It is also possible that decomposition of macroalgae, responsible for releasing metals back to the system, may also be implicated in reducing uptake of metals (Mn, As and Sn) at warmer temperatures during the disturbance phase of the experiment (Fig. 4.65). Decay is increased at warmer temperatures and as decay requires oxygen, this would decrease oxygen levels in the sediment. Dissolved oxygen is also decreased at warmer water temperatures (Buentello et al., 2000). Furthermore, in experiments examining the effect of disturbance and dissolved oxygen (DO) on metal release to water Atkinson et al. (2007) found that at lower DO levels metals remained in solution for longer. These factors may have combined to create the pattern of decreased uptake observed here.

Finally, although a clear dependence of solubility on temperature has not been established for many metals, it is known to affect Fe (Hoffmann et al., 2012) and represents another potential explanation for the decrease in uptake of some metals with increasing temperature following sediment disturbance.

In contrast to other metals, Cu showed a decline in uptake to diatoms due to disturbance and a decrease with increasing levels of disturbance. A decrease in dissolved Cu at increasing disturbance levels was previously reported (Xu et al., 2015) and could explain the decrease in uptake to diatoms seen in this study.

4.5. Conclusion

It has been shown that, whilst there was no consistent variation in metal uptake to diatoms with increasing water temperature, there was a change in uptake of some of the metals investigated due to sediment disturbance. Additionally, findings indicated that temperature may have some effect on uptake to diatoms when considered in combination with sediment disturbance. It would be of interest to study the effects of disturbance over a longer period both separately from temperature and in combination to understand if there are either synergistic or antagonistic effects of multiple stressors. It would also be of interest to introduce other factors such as changes in pH, nutrient availability and salinity to investigate their effects on contaminant uptake by MPB.

A difference in uptake by the various algal groups in the mesocosm was observed with diatoms taking up a greater concentration of all metals, except Sn, than either sheet or filamentous macroalgae. Whilst reasons for this are currently unknown, it is speculated that it may be due to their size and closer association with the sediment. This study has shown that, as an important food source to biota such as invertebrates, shellfish and birds, epipelic diatoms and other sediment associated microalgae would warrant increased scrutiny in terms of uptake of metals and other potential contaminants.

Whilst the use of the mesocosm was successful in that it proved the potential to maintain a microphytobenthic community for a long period under semi-controlled conditions, it is important that this be compared with real world conditions to show that results obtained are analogous to those in the field. In Chapter 5 a field study facilitates these comparisons and investigates how spatial variations across the mudflat and seasonality affect metal uptake to benthic microalgae.

5. Metal concentrations in benthic algae at Lytham St. Annes

5.1. Introduction

Benthic micro algae are of primary importance in the estuarine food chain. They are directly consumed by deposit feeders such as the mud snail, *Hydrobia ulvae* (Haubois et al., 2005) and shorebirds (McCormick et al., 2014). When diatoms become suspended into the water column they are consumed by suspension feeders such as the common cockle, *Cerastoderma edule* (Sauriau and Kang, 2000), the pacific oyster and *Crassostrea gigas* (Riera and Richard, 1996) both of which are harvested for human consumption. The potential for diatoms to take up metal contaminants is therefore of concern to both environmental and human health.

Contamination of estuaries is an historical (Fox et al., 1999) and current issue (Spencer and O'Shea, 2014) of ongoing concern (Du Laing et al., 2009, McCormick et al., 2014, Beldowska et al., 2016). A recent review paper (de Souza Machado et al., 2016) describes the importance of metal uptake to phytoplankton in the estuarine system and points out that whilst on the one hand they may remove contaminants from the water column and return them to sediment through uptake, death and sedimentation, they have an equally important role as part of the estuarine food chain in transfer of metal upwards through uptake followed by predation (contaminants are taken up by other organisms (e.g. mussels) more readily in the particulate than aqueous phase. These observations are also relevant to benthic microalgae which also take up metals in the estuary and are an important constituent of the food chain.

Whilst it has been shown that diatoms (*Cylindrotheca closterium*) take up cadmium from the sediment as well as overlying water in a laboratory microcosm (Chapter 3) and that a range of metals are taken up from the sediment by the diatom community in an intertidal mesocosm (Chapter 4), there is a requirement to show that this also occurs in the more heterogeneous field setting. Furthermore, while laboratory bioassays are often used to assess the risks of contaminated sediment to organisms (Adams and Stauber, 2004, Moreno-Garrido et al., 2007, Araujo et al., 2010), the conditions to which these organisms are subject in the field are more dynamic and heterogeneous than can be replicated under laboratory conditions (Belzunce-Segarra et al., 2015). This is due, for example, to exchanges of large volumes of water (Mann et al., 2010) and the presence of a range of other organisms, which can through processes such as bioturbation affect contaminant bioavailability (Atkinson et al., 2007).

To date, whilst there have been multiple studies of toxicity of contaminated sediment to benthic algae (Araújo and Moreno-Garrido, 2015) there have been very few studies measuring metal uptake from sediment to diatoms (Prieto et al., 2016) and only two field studies were identified (Stronkhorst et al., 1994, Absil and van Scheppingen, 1996). The need to examine this issue

further, in particular with regard to the food chain has recently been acknowledged (McCormick et al., 2014).

Previous studies have identified the difficulty of separating diatoms from the sediment in quantities sufficient to carry out chemical analysis (Stronkhorst et al., 1994). These issues are compounded by the nature of working in intertidal areas, which have restricted working times due to the tides and present difficulties in transporting equipment and keeping samples clean due to the muddy nature of these locations. It has previously been shown that benthic microalgae continue to migrate through the surface sediment following removal to the laboratory (Defew et al., 2002) and it is postulated that this quality could be utilised to solve the issues of collecting algae samples in the field.

5.1.1. Field site

The Ribble estuary is located on the North West coast of England. It is a funnel shaped, partially mixed estuary lying in a roughly East-West orientation. It is approximately 30km in length, from Lytham to the tidal limit at the M6 motorway east of Preston and about 15km wide at the mouth (from Lytham St. Anne's to Southport). It is macrotidal, experiencing semi-diurnal tides with a range of up to 10m. It has two tributaries downstream of the tidal limit, the Rivers Darwen and the Douglas, both of which enter the estuary channel from the south. The channel was extensively engineered at the turn of the 20th Century with training walls completed in 1913 to improve navigation to Preston Docks. This has resulted in a straight narrow channel, prone to sediment infilling. Dredging of the estuary ceased in the early 1980s, this has resulted in extensive silting of the channel since. The port at Preston was formally closed by the Preston Dock Closure Act in October 1981.

Irish Sea environmental contaminants consist of radionuclides, non-radioactive metals and organics primarily produced by the heavy industry and electrical power generation facilities of the north west of England (Ridgway et al., 2003). The estuary average signature for the Ribble reported by Ridgway et al. (2001) shows contamination in Mg, Ca, Fe, V Cr, As, Rb and Sr. These are the metals with which the sediment is enriched in comparison to the catchment signature. There is a winnowing out of metal carrying sediments by strong tidal and fluvial currents in the inner estuary, these are redeposited further out to sea. The Environment Agency of England, in investigations related to implementation of the EU Water Framework Directive, indicated that the Ribble Estuary has excessive levels of tributyltin and a range of insecticides in addition to a high load of the metals Cr, Fe, Cu, Zn, Cd and Pb (Hagger et al., 2012).

The extensive sand- and mudflats, which are exposed at low tide provide an important food resource to overwintering birds, making the potential uptake of metals to them a pertinent issue at this location.

5.1.2. Aims

The aim of this study was to compare concentrations of inorganic analytes in the sediment of the mudflat with concentrations in the benthic algae and to look at whether contamination and uptake varies with the tidal frame or seasonally in either the sediment or algae. This will allow trends seen in the laboratory and mesocosm studies to be tested, to understand if observations made in more controlled environments are also applicable to the real world. The tidal frame is defined in Chapter 2 (section 2.2.1) as the elevation range between the lowest and highest tides.

Hypotheses

- *Sediment contamination varies with height in the tidal frame*
- *Concentrations of metals in diatoms are related to concentrations in sediment and position in the tidal frame*
- *There is a seasonal variation in metal concentration in epipelic diatoms*

5.2. Method

The study measured metal contaminants in sediment, surface and pore water and motile algae (internal and external). Additionally sediment particle size, organic matter content and mass of algae were investigated.

Fieldwork was carried out twice, in September 2014 and April 2015, to allow for the examination of metal behaviour at different seasons and to look for possible changes due to differences in temperature and other weather conditions preceding the sampling date. These dates were chosen because Montani et al. (2003) showed that diatom numbers peaked in late summer and early spring.

5.2.1. Field sampling

Sample locations

Sampling was carried out at low tide on an area of mudflat close to the jetty at the RNLI lifeboat station, Lytham St. Anne's (Fig.5.1.). Samples were taken at 10 locations on a 100m by 100m grid (Fig.5.2) on 25th September 2014 and 23rd April 2015. With respect to the tidal frame locations 1, 4, 7 and 10 are defined as "high", 2, 5 and 8 are defined as "mid" and 3, 6 and 9 are "low".

The above dates were selected to coincide with the spring tide to ensure that the mudflats were at maximum possible extent to assist with sampling and maintain consistency. In September 2014 there was a low tide at Blackpool of 1.2m and a high tide of 8.7m, sampling was started shortly after low tide (06:49) at 7am. In April 2015 low water at Blackpool was 1.2 m at 09:26 (sampling started slightly prior to this at 9am) and there was a high tide of 8.6m.

Weather conditions were good on both occasions with clear skies and low winds throughout the sampling period.

Figure 5.1. Field site at Lytham St. Anne's on the Ribble estuary, 53°43'58"N, 2°57'37"W. Map from Google Maps

Figure 5.2. Layout of sample grid (including tidal frame heights) at Lytham St. Anne's. Map from Google Maps

Diatom sampling

Algae were sampled from the sediment surface using a modification of the lens tissue method developed by Stronkhorst et al. (1994) from the method of Eaton and Moss (1966). This method utilises the natural motility of epipelic diatoms, which migrate to the sediment surface at low tide during daylight, to separate them from the sediment. Two sheets of lens tissue (Whatman), 200 by 300 mm, were placed on the sediment surface. This was then topped with a sheet of gauze (160 x 210 mm nylon mesh, pore size 80 µm, Normesh Ltd., UK), which was lightly pressed on to the surface using forceps to remove air bubbles and allow the interstitial water to soak through the three layers, effectively sticking them together and to the sediment surface. This method was further refined through the use of a shallow plastic tray (38 x 24 x 7cm) from which the base was cut away. This was pressed onto the sediment around, but not touching the lens tissue/gauze layers, acting as a wind break. This both prevented the lens tissue and gauze from being blown away (it was observed by Stronkhorst et al. (1994) that even low wind velocity caused problems in deploying the tissue) and stopped any sediment being blown onto the top of the gauze whilst not overshadowing the collection area (Fig. 5.3).

The sampling arrangement was left on the sediment surface for one hour, following which the upper gauze layer was removed with forceps to a pre-labelled 250ml HDPE sample bottle containing 50ml of artificial seawater (Tropic Marin® at salinity of 22).

In order to collect enough algae to measure metals both internal and external to the cells a pair of lens tissue/gauze samples were collected at each location (Fig. 5.3).

The nylon gauze was selected over lens tissue as the top layer for field diatom sampling over lens tissue because of the size of the collection area. Although lens tissue was observed to make better contact with the sediment surface, such a large sheet of lens tissue is difficult to handle in both the field (during placement and collection) and the laboratory (during the washing process to remove diatoms) due to its flimsy structure. The nylon gauze is a stiffer material which is easier to collect from the sediment surface and to wash in the laboratory. Although it was previously observed that lens tissue had a tendency to dry more quickly than lens tissue (section 3.2.5), this was of less concern during field sampling as the sheets were left in place for a much shorter time (one hour, due to tidal constraints) than in either the mesocosm or laboratory sampling described in chapters 3 and 4 (four and 15 hours respectively).

Figure 5.3. Diatom sampling set up

Sediment sampling

Sediment samples were collected adjacent to the algae samples at each of the 10 sampling locations (Fig. 5.4). This was carried out after the diatom samples were collected in order to avoid contamination of the algae due to splashing. Sediment was sampled in two ways, firstly by sediment scrape and secondly using a mini core.

Sediment scrapes were taken using a flat trowel to remove the top 5 mm of sediment from a 0.5 m^2 area, to collect a 1250 cm^3 sample (approximately 1kg wet weight). This was scooped into a sealable polythene sample bag which was sealed, placed inside a second sample bag and stored in the dark in a cool box for return to the laboratory within 24 hours.

0-20 mm and 20-40 mm samples were collected using a mini corer (pre-cut sections of plastic pipe, 100 mm long with a 60 mm diameter, giving a 285 cm^3 sample). The mini corer was longer than the desired sample depths to ensure the integrity of the sediment and associated pore water was maintained. The mini corers were pushed into the sediment using a small piece of plywood and a rubber mallet until flush with the surface. A trowel was then used to dig around the outside of the tubing until it could be easily extracted. Tubes containing sediment were wrapped in a double layer of clingfilm and then placed in sample bags, upright in a cool box to preserve interstitial water.

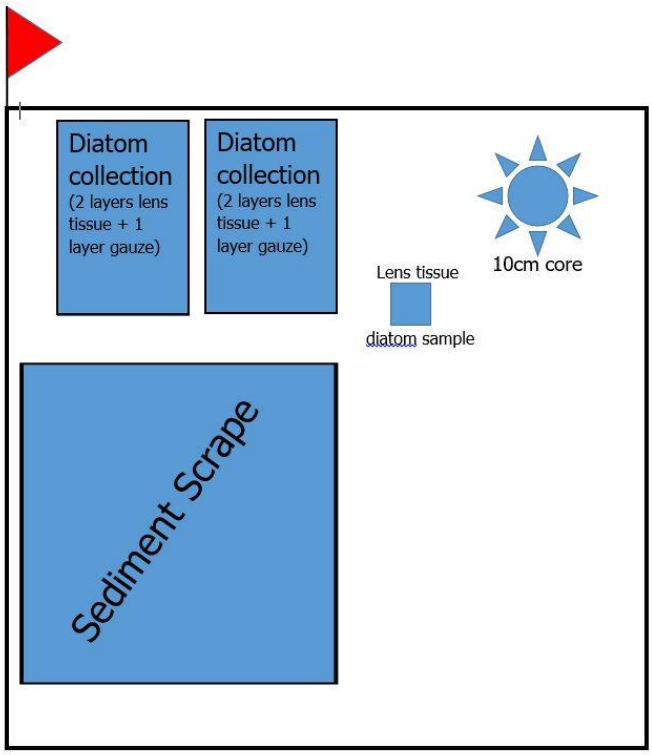


Figure 5.4. Layout of sample collection at each sampling point

Overlying water

Overlying water was sampled from the jetty as the tide came in following collection of sediment and diatom samples. Three 1L samples were taken using HDPE bottles held below the water surface. All field samples were stored in the dark in a cool box for return to the laboratory within 24 hours.

5.2.2. Laboratory sampling

On return to the laboratory a second separate sample of diatoms was taken for comparison with the sample collected in the field and to test whether (i) it was possible to increase the amount of algae collected, and (ii) it is possible to avoid field sampling for diatoms with its inherent problems with potential sediment contamination.

A subsample of the sediment scrape was spread (with the use of a flat edged trowel) onto a shallow (5 mm) plastic tray (345 x 265 mm). This was then covered with two layers of lens tissue and a layer of gauze (two sheets side-by-side so that the tray was completely covered), giving the same sample area as in the field (Fig. 5.4). Trays were left, close to a window but out of direct sunlight, overnight until the time of the next daylight low tide. Diatoms have been shown to continue migrating through the sediment, in the absence of tides for several days following collection (Serôdio et al., 1997, Defew et al., 2002, Saburova and Polikarpov, 2003), which could enable effective sampling in the laboratory.

This was observed to be a very successful collection method in that the collected gauze was blackened with diatoms, whilst there was little evidence of diatoms on the lower lens tissue and the sediment surface was pale underneath.

The gauze was then removed to a pre-labelled 250 ml HDPE sample bottle containing 50 ml of artificial seawater (salinity of 22) for processing in the same manner as field collected samples.

Figure 5.5. Diatom collection in the laboratory

5.2.3. Laboratory processing

Diatoms

Sample bottles containing gauze sheets were agitated by hand for one minute. The gauze was then removed to a plastic tray where it was washed down with 250 ml artificial sea water (salinity 22) dispensed from a wash bottle to remove the attached diatoms. The collected water, combined with that from the sample bottle was split between six 50 ml centrifuge tubes. These six sub-samples were centrifuged at 180 RCF for seven minutes and then left to settle for 10 minutes before the supernatant was removed and the subsamples recombined to a single 50ml centrifuge tube.

The two gauze samples from each sample point (Fig. 5.2) were combined and homogenised by manual agitation and then split (by pipetting) into two clean 50 ml centrifuge tubes. These paired samples were then further processed, see below, for analysis of metal uptake to diatoms (total and intracellular, from which the adsorbed fraction was calculated).

The process was repeated for the diatoms collected by laboratory method.

Diatom Subsample 1 – total metal concentration

Total contaminants were determined by vacuum filtration of the subsample through two superimposed, pre-washed and pre-weighed, 47 mm, 0.45 µm cellulose nitrate filters (Whatman). The filters were then dried at 65 ^{o}C and re-weighed to determine the weight of the algae sample (Eq. 5.1).

Eq. 5.1. weight of algae (g) = (weight of upper filter – initial upper filter weight (g)) - (weight of lower filter – initial lower filter weight (g))

Filters were then removed to 15 ml centrifuge tubes and 0.5 ml 72 % perchloric acid ($HClO_4$) followed by 1 ml nitric acid (HNO_3) was added. Samples were left to digest for 48 hours at room temperature. Resultant samples were made up to 75 ml with deionised water to give a 2 % v/v acid matrix. These samples were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with the lower filter acting as a blank. Subtracting contaminants in the lower filter from those in the upper enables the elimination of dissolved contaminants which are present in both filters.

Diatom Subsample 2 – intracellular metal concentration

Samples for measurement of intracellular metal concentrations were processed by re-suspending the diatom pellet in 25 ml of 0.02 M Ethylenediaminetetraacetic acid (EDTA) solution made up in artificial seawater (at salinity of 22) for 20 minutes. Cells were then centrifuged and washed a further two times in artificial seawater. The resulting suspension was then filtered, digested and analysed as for total metals concentration as above.

Washing the algae cells in EDTA removes metals adsorbed to the surface of the diatoms thereby enabling measurement of internal metals only.

The fraction of metals adsorbed to the outside of the diatom cells was calculated by subtracting the intracellular metal concentration from the total metal concentration (Eq. 5.2, as described for Cadmium (Cd) in Chapter 3, Section 3.2.6).

Eq. 5.2. External M_{Alg} (kg mg^{-1}) = Total M_{Alg} (kg mg^{-1}) – Internal M_{Alg} (kg mg^{-1})

where M_{Alg} is algae metal concentration normalised to the dry weight (DW) of algae.

Pore water

Analysis of pore water and sediment from the mini cores was carried out at two depths, 0 to 20 mm and 20 to 40 mm. To achieve this sediment was pushed out of the core from the base and sliced into two 20 mm sections. The sediment was put into 50 ml centrifuge tubes (two per core slice) and interstitial water was separated from the sediment by centrifuging at 3100 RFC for 10 minutes in a refrigerated (4 ^{o}C) centrifuge. Pore water was removed to HDPE sample bottles and centrifuging was repeated to maximise pore water removal. The retrieved pore water sample was immediately vacuum filtered through 0.45µm cellulose nitrate filters, diluted 1:5 with Milli-Q® water and acidified to 2 % v/v with HNO_3.

Sediment

Following pore water removal sediment samples were dried for 48 hours at 105^{o}C, sieved to 2mm and split into aliquots for measurement of organic matter by loss on ignition (LOI), particle size and metal concentration analysis using ICP-MS.

Sediment organic matter

The percentage organic matter content of sediment was measured through LOI in the 0 to 20 mm and 20 to 40 mm sections. Approximately 2 g of dry sediment of dry sediment was accurately weighed into pre-weighed ceramic crucibles. Samples were heated in a furnace at 450^{o}C for eight hours, then removed to a desiccator to cool before re-weighing. Percentage organic matter is calculated as per equation 5.3, below.

Eq. 5.3. Organic matter (%) = 100 - (((sediment + crucible after ignition) – crucible (g)/ (sediment + crucible before ignition) – crucible (g)) x 100)

Sediment particle size

Particle sizes within each sample (0 to 20 mm and 20 to 40 mm) from 2 to 2000 µm were determined with the use of a Coulter LS 230 laser granulometer. Samples were prepared by filling a 50 ml HDPE sample bottle with sediment to a depth of approximately 5mm followed by the addition of distilled water to a total depth of about 15 mm. To aid deflocculation, 2 ml of dispersant sodium hexametaphosphate (Calgon) was added and samples were agitated mechanically for 30 minutes. Samples were then placed on a magnetic stirrer for 5 minutes to create a vortex, ensure it was well mixed and that a representative sub-sample was obtained.

Five measurements were made of each sub-sample and the mean used. Particle sizes were classified according to the Udden – Wentworth classification scheme for grain size (Wentworth, 1922) and are given as a percentage volume frequency.

Overlying water

Overlying water samples were vacuum filtered through a 47 mm, 0.45 µm cellulose nitrate filter. A 20 ml aliquot of each sample was acidified with 2 ml HNO_3 and made up to 100 ml with Milli-Q® water to give a 1:5 dillution in a 2% v/v acid matrix for metals analysis by ICP-MS.

ICP-MS analysis

Algae, sediment, pore and overlying water samples were analysed for a total of 14 elements (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Ag, Cd, Sn and Pb). The metals and metalloids analysed were 14 of the 23 identified by Fairbrother et al. (2007) in their Framework for Metals Risk Assessment for the US EPA and the same as those analysed in the mesocosm experiment (Chapter 4).

Analysis was carried out using the XSERIES 2 ICP-MS (Thermo Scientific, Germany) using collision cell technology (CCT) to reduce potential polyatomic interferences. Multi element standards (Merck, Germany) certified by the National Institute of Standards and Technology (NIST), internal standards (scandium (Sc) and rhodium (Rh)) and blanks were included in each run. Accuracy was assessed by comparing reference sample determinations (CRM-142R: Community Bureau of Reference) with recorded values and all samples were run in triplicate. The instrumental limit of detection (LoD) for all analytes is given in Appendix 5-3.

Some elements in were present in concentrations too low to measure and were therefore given zero values. This issue occurred with algae, pore water and overlying water samples, particularly those collected in April 2015. In algae this was possibly due to the low sample weights available for analysis. Details of percentage of samples treated in this way for each compartment on both sampling dates are given in Appendix 5-1.

5.2.4. Bioconcentration factor

The bioconcentration (BCF) factor was applied to enable the comparison of uptake of the various analytes from the sediment to algae. BCF was calculated from total analyte concentration in algae and sediment as follows:

Eq. 5.4. $BCF = M_{Alg}\ (kg\ mg^{-1}) / M_{Sed}\ (kg\ mg^{-1})$

where M_{Alg} is the analyte concentration in algae and M_{Sed} is the concentration of the same analyte in the sediment.

5.2.5. Normalisation

Minimisation of differences due to sediment bulk characteristics may be achieved procedurally i.e. by sieving to reduce grain-size characteristics, or through normalisation. Normalisation reduces the variability between samples that arises from sediment properties such as grain size and organic matter. Geochemical normalisation is used to compensate for mineralogical as well as granulometric variability in the sediment. There are several acknowledged techniques for normalisation which include the use of simple metal normaliser ratios, "double" normalisation which involves the comparison of ratios observed at the study site with ratios present in the upper crust and regression based normalisation (Matys Grygar and Popelka, 2016). A decision was made to normalise the analysed elements within the sediment to percentage organic matter.

Sediment metals were normalised to percentage organic matter using the following formula:

Eq. 5.5. $M_{NOR} = M\ (mg\ kg^{-1}) / O\ (\%)$

where M_{NOR} is the normalised concentration, O is the percentage organic matter content of the sediment and M is the measured concentration in the sediment (mg kg^{-1} DW).

5.2.6. Statistical analysis

All statistical analysis was carried out using R (3.2.3) in RStudio. The lme4 package (Bates et al., 2015) was used to perform linear mixed effects analysis of the relationships between metal concentration in diatoms and the concentrations in sediment and pore water. As fixed effects concentration in sediment or pore water, tidal frame position (which is a proxy for sediment particle size and organic matter content) and date (without interaction terms) were entered into the model. As a random effect there was an intercept for the effect of date (equation 5.6).

Eq. 5.6. $As \sim T.Pore.As + Date + tidal.frame + (1|Date)$

Differences in diatom mass collected in the laboratory and field were also examined using the lme4 package. In this case the model fixed effects were collection method, date and tidal frame position (without interaction terms) and again there was a random intercept for the effect of date (equation 5.7).

Eq. 5.7. Mass.mg. ~ Collection + Date + tidal.frame + (1|Date)

To examine seasonal variation in pH the linear fixed effect model was fitted with month as the fixed and transect as a random intercept (equation 5.8).

Eq. 5.8. ph ~ Month + (1|Transect)

Finally, the variation of sediment percentage silt and organic matter content within tidal frame was investigated. In this case the fixed effects were tidal frame position, sediment depth and date with a random intercept for the effect of date (equation 5.9).

Eq. 5.9. Organic ~ tidal.frame + Depth + Date + (1|Date)

Deviations from homoscedasticity and normality were checked by visual inspection of residual plots. Outliers were identified using Cook's distance and points with a value of >1 were classified as being influential. P-values and Chi-Square values were calculated using likelihood ratio tests of the full model with the effect in question against the model without the effect in question and are reported to two significant figures. A sample model output is provided in Appendix 5-3.

Diatom samples with concentrations below the LoD were set to zero for statistical analysis details of the percentage of samples treated in this way for each analyte on each collection date are given in Appendix 5-2.

5.3. Results

5.3.1. Sediment

Particle size

The percentage of silt sized particles varied with level in the tidal frame (χ^2=6.5, p=3.8*10^{-2}, r2=0.22) and with depth (χ^2=6.6, p=3.7*10^{-2}), but not with date (χ^2=1.7, p=1.9*10^{-1}, Fig. 5.6).

The percentage of clay sized particles varied with level in the tidal frame (χ^2=11.3, p=3.5*10^{-3}, r2=0.26), but not with depth (χ^2=3.7, p=0.16) or date (χ^2=3.3, p=6.9*10^{-2}, Fig. 5.7).

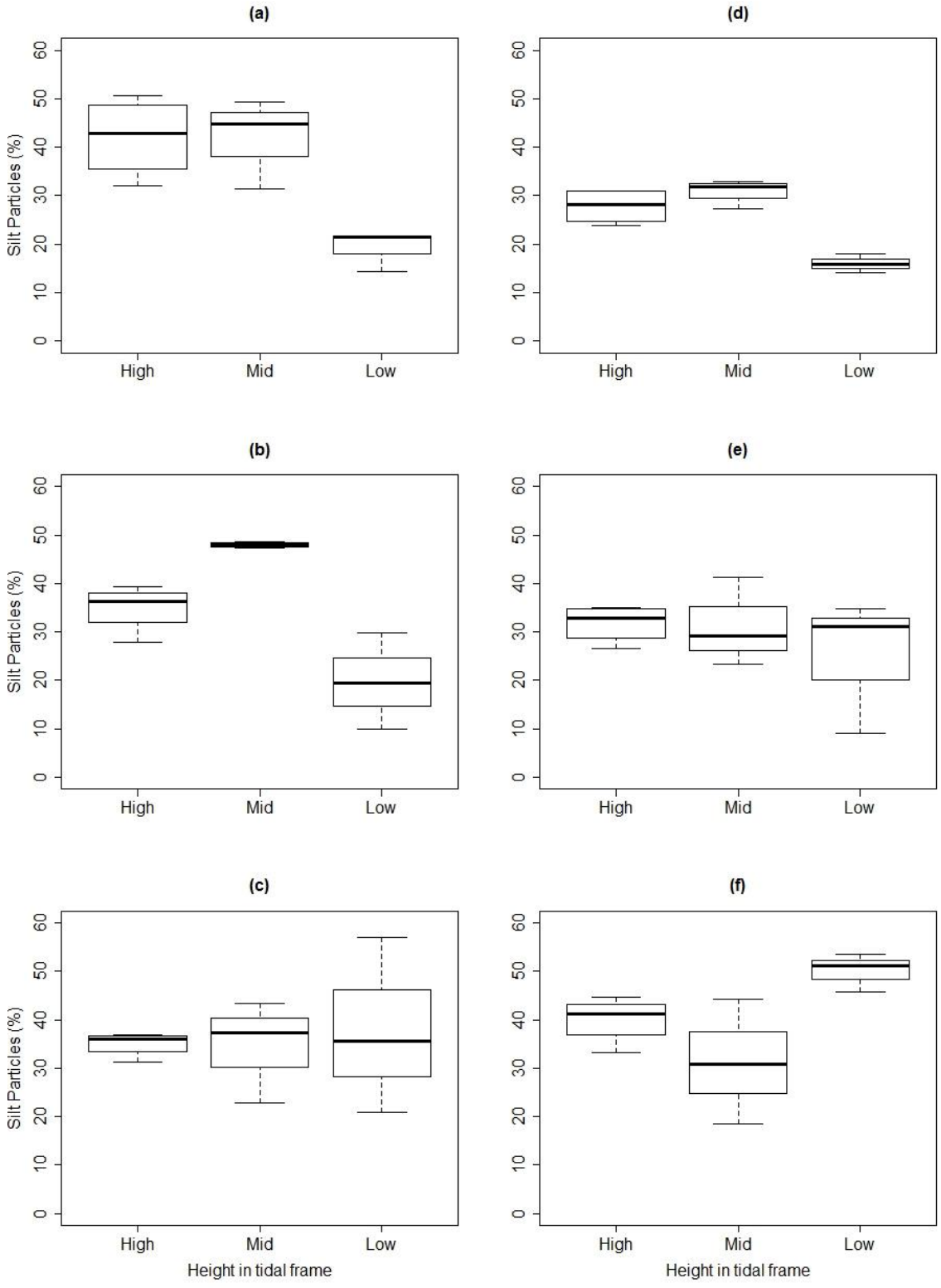


Figure 5.6. Percentage of silt sized (4 - 63µm) particles at three heights in the tidal frame in September 2014 at (a) sediment surface (b) 0 to 20mm deep and (c) 20 to 40mm deep and April 2015 at (d) sediment surface (e) 0 to 20mm deep and (f) 20 to 40mm deep

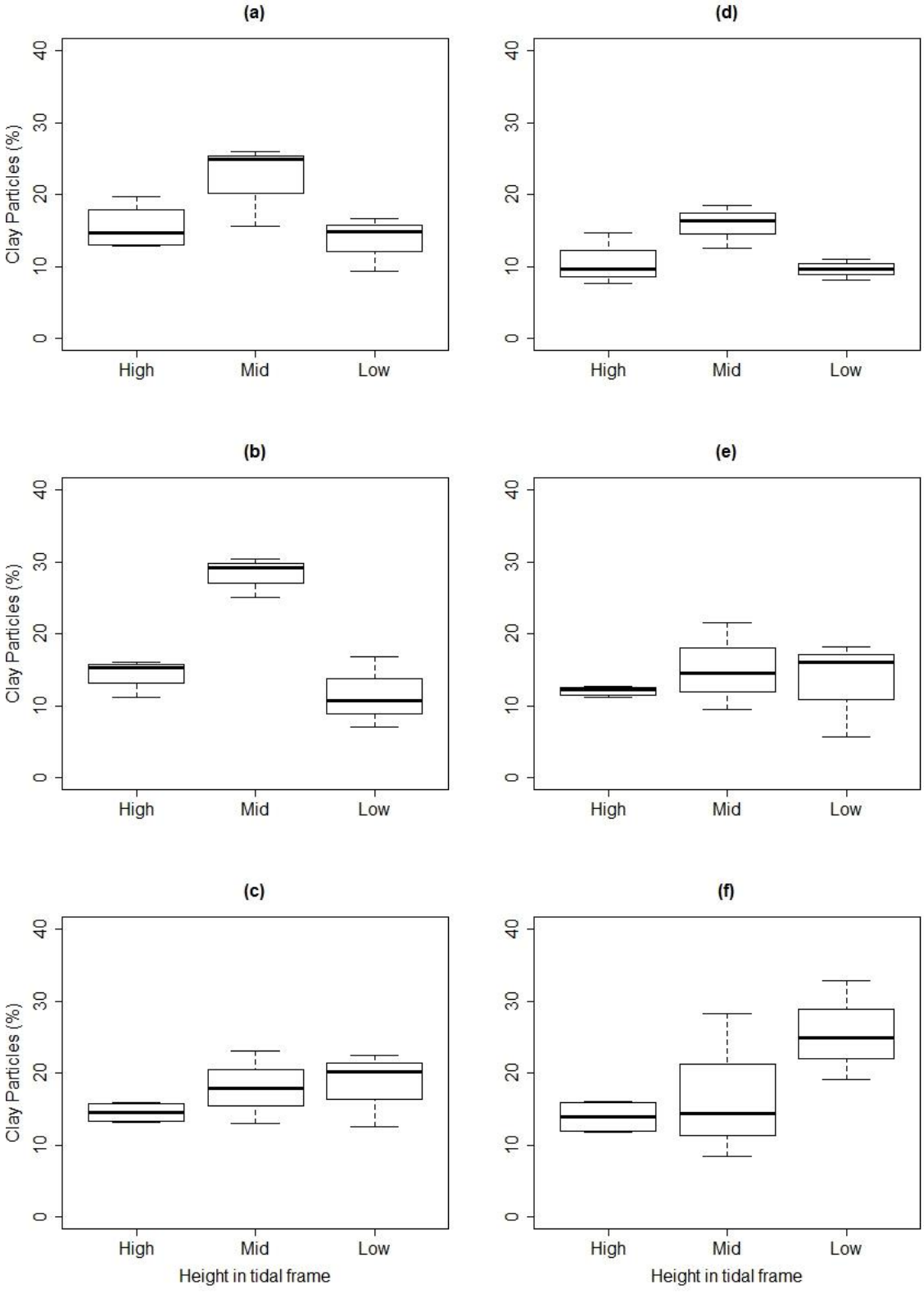


Figure 5.7. Percentage of clay sized (<4μm) particles at three heights in the tidal frame in September 2014 at (a) sediment surface (b) 0 to 20mm deep and (c) 20 to 40mm deep and April 2015 at (d) sediment surface (e) 0 to 20mm deep and (f) 20 to 40mm deep

Organic matter

The percentage of sediment organic matter changed with date (χ^2=5.8, p=1.6*10^{-2}, r^2=0.30), with greater quantities in September, and with position in the tidal frame (χ^2=8.5, p=1.4*10^{-2}), but not with depth (χ^2=2.3, p=3.1*10^{-1}) (Fig. 5.8). There was a correlation between percentage organic matter and percentage silt content in the sediment (Table 5.2).

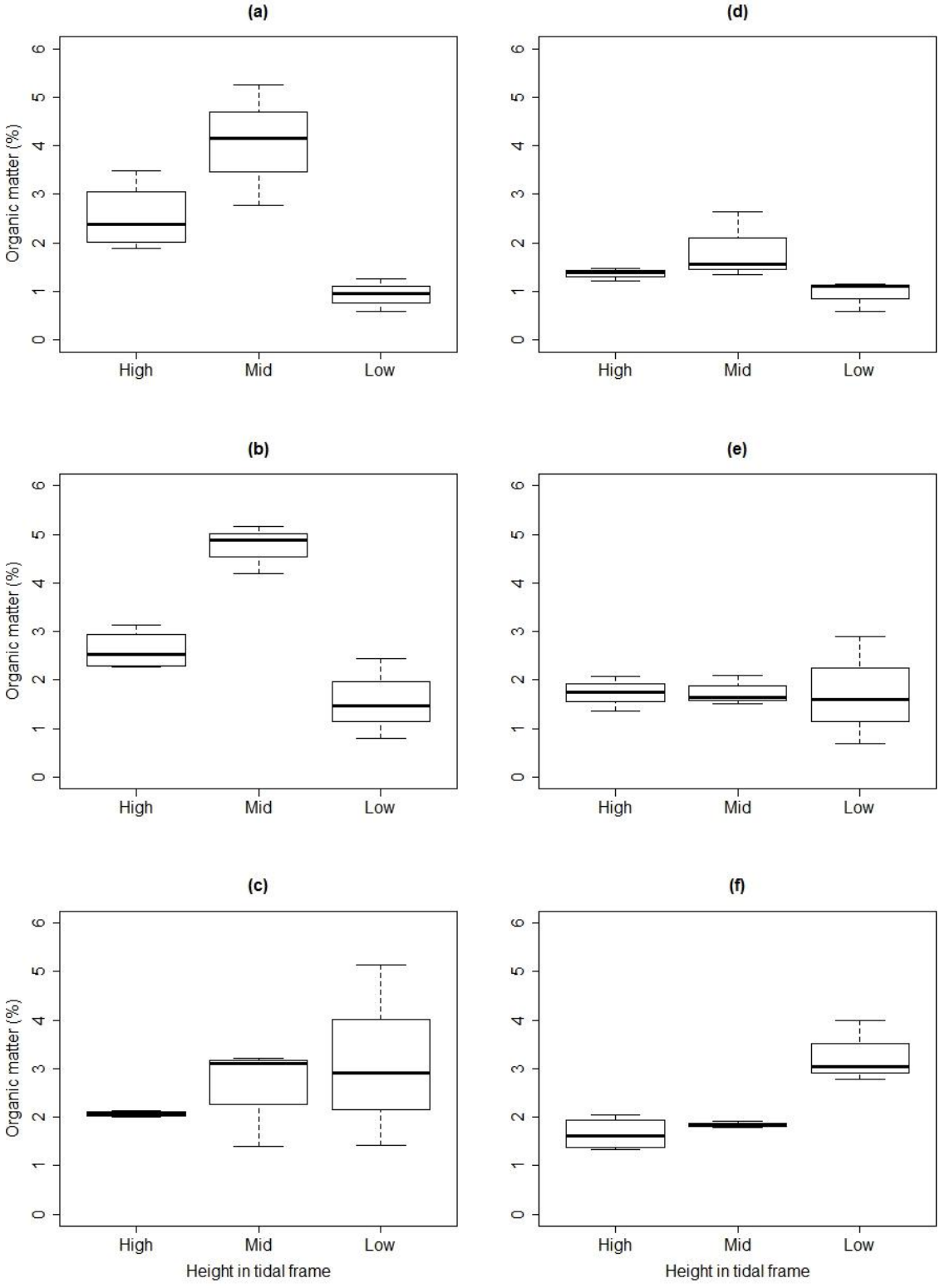


Figure 5.8. Percentage organic matter at three heights in the tidal frame in September 2014 at (a) sediment surface (b) 0 to 20mm deep and (c) 20 to 40mm deep and April 2015 at (d) sediment surface (e) 0 to 20mm deep and (f) 20 to 40mm deep

Sediment pH

Seasonal sediment pH data were recorded as part of another project working at the same location (Sneddon, 2016, pers. comm., 19th December). Data were collected on four occasions during 2014 along three transects on the mudflat at Lytham St. Anne's. The "East" transect runs from the high to low tidal frame through sampling locations 1, 2 and 3.

Sediment pH varied seasonally (χ^2=93.6, p<2.2*10^{-16}, r^2=0.51) across all transects (Fig. 5.9). On the East transect, in September, pH varied between ~7.5 and 7.8 and in March and May it varied between ~8.1 and 8.5 (Fig. 5.10).

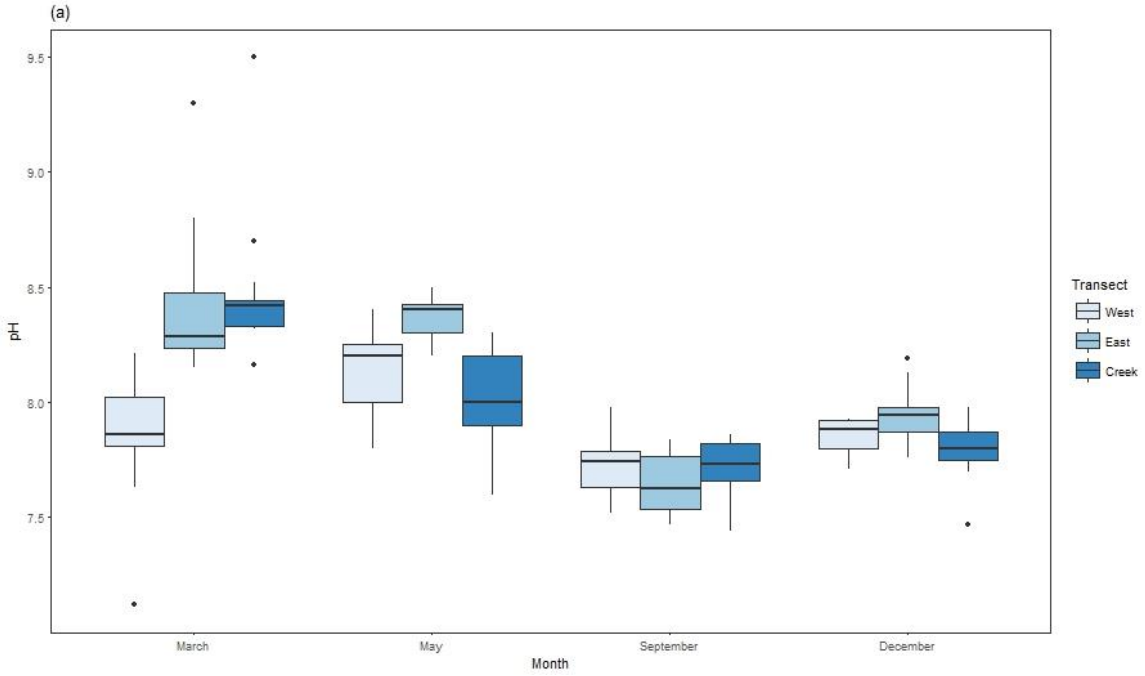


Figure 5.9. Seasonal variation in sediment pH at each transect (West n=11, East n=13 and Creek n=12), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

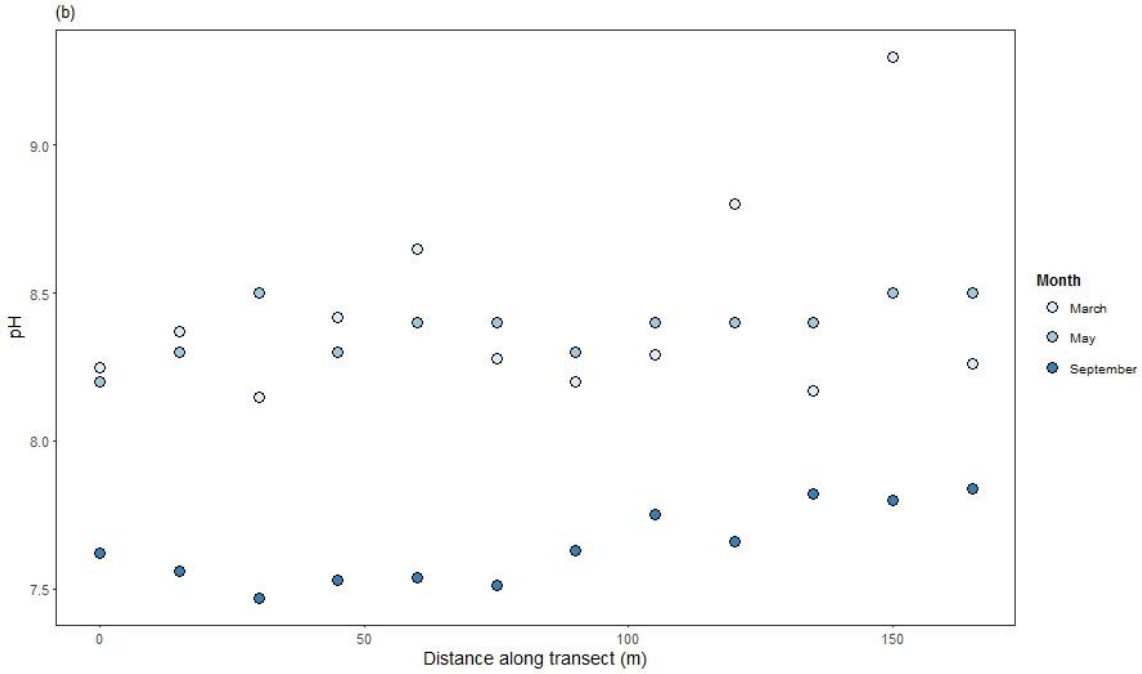


Figure 5.10. Seasonal variation in sediment pH with distance from the start (top of tidal frame) of the East transect

Metal concentration

Total metal sediment data for the top (0 to 20mm) of sediment cores in September 2014 and April 2015 are presented in Table 5.1. These data indicated greater spatial variability in concentrations of analytes in sediment in September than in April. In September 2014 concentrations were highest in the middle of the tidal range and lowest in the sediment low in the tidal frame, closest to the main channel (see Fig. 5.2). Metal concentrations in sediment collected in the high tidal frame in September 2014 were very similar to the initial sediment concentration in the in the mesocosm, due to the bulk of that sediment being collected at that location on the same date. One notable difference was in the Cu concentration, which in the mesocosm sediment was closer to concentrations observed in April 2015 (Tables 4.4 and 5.1).

There was a strong relationship between the percentage of organic matter and metal concentrations (excluding Cd) in the sediment. This was particularly evident for Al, V, Cr, Fe, Co, Ni, Zn and Pb (Fig. 5.12 and Table 5.2). There were also correlations between the concentrations of several metals in the sediment. Notably, those identified above as being correlated with percentage organic matter also showed a strong correlation with each other.

Metal concentrations in sediment were measured at three depths (surface scrapes, 0 to 20mm and 20 to 40mm). Patterns of concentration were similar for all metals (Fig 5.11 and Appendix 5-4). Metal concentrations were similar across all three depths although some analytes had a higher concentration in deeper sediment, particularly in samples from April 2015.

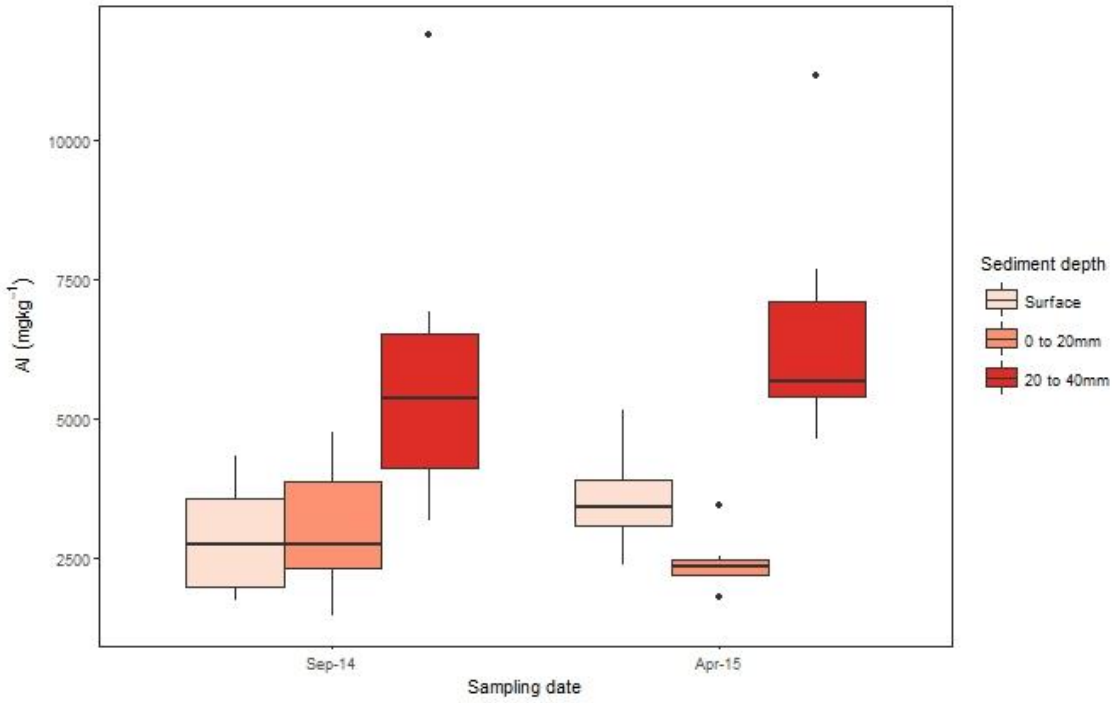


Figure 5.11. Concentration of aluminium in sediment (mg kg^{-1} DW) at three depths, samples for all tidal frame heights combined (n=10), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Table 5.1. Mean ± SD sediment analyte concentration (mg kg^{-1} DW unless specified) in the top 20mm for September 2014 and April 2015 at different heights in the tidal frame (High n=4, Mid n=3 and Low n=3).

Analyte	September 2014			April 2015		
	High	Mid	Low	High	Mid	Low
Aluminium (%)	0.28 ± 0.03	0.44 ± 0.03	0.20 ± 0.04	0.23 ± 0.01	0.24 ± 0.01	0.26 ± 0.08
Vanadium	8.3 ± 0.52	11 ± 0.85	6.8 ± 0.57	7.4 ± 0.36	7.9 ± 0.36	8.3 ± 1.6
Chromium	10 ± 1.1	4.7 ± 0.50	1.6 ± 0.83	1.7 ± 0.29	1.8 ± 0.31	1.7 ± 1.1
Manganese	620 ± 67	740 ± 100	370 ± 56	370 ± 49	410 ± 22	410 ± 75
Iron (%)	0.95 ± 0.07	1.3 ± 0.1	0.72 ± 0.08	0.81 ± 0.03	0.89 ± 0.05	0.93 ± 0.23
Cobalt	3.5 ± 0.25	5.2 ± 0.39	2.5 ± 0.45	3.0 ± 0.08	3.2 ± 0.15	3.3 ± 0.83
Nickel	7.9 ± 0.97	12 ± 0.92	5.4 ± 1.0	6.5 ± 0.37	6.8 ± 0.50	7.0 ± 2.2
Copper	12 ± 0.49	18 ± 2.2	11 ± 2.1	8.6 ± 2.3	8.4 ± 1.0	9.1 ± 3.1
Zinc	57 ± 4.6	84 ± 6.7	40 ± 12	50 ± 2.7	53 ± 9.7	52 ± 18
Arsenic	5.2 ± 0.31	6.8 ± 0.88	5.0 ± 0.82	4.1 ± 0.30	5.1 ± 0.51	6.2 ± 2.1
Silver	0.10 ± 0.02	0.15 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.13 ± 0.09	0.10 ± 0.05
Cadmium	0.25 ± 0.22	0.28 ± 0.16	0.12 ± 0.14	0.35 ± 0.29	0.19 ± 0.07	0.24 ± 0.11
Tin	0.73 ± 0.11	1.5 ± 0.23	0.62 ± 0.19	0.62 ± 0.13	0.56 ± 0.06	0.58 ± 0.21
Lead	19 ± 1.7	33 ± 3.8	12 ± 4.8	17 ± 1.7	18 ± 2.9	19 ± 7.2

Table 5.2. Correlation matrix of analytes and sediment properties in top 20mm of sediment

	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Ag	Cd	Sn	Pb	Organic
V	0.982	1.000													
Cr	0.991	0.975	1.000												
Mn	0.853	0.817	0.821	1.000											
Fe	0.990	0.992	0.985	0.831	1.000										
Co	0.993	0.984	0.991	0.842	0.992	1.000									
Ni	0.992	0.978	0.992	0.854	0.986	0.992	1.000								
Cu	0.826	0.791	0.823	0.784	0.811	0.798	0.833	1.000							
Zn	0.956	0.929	0.974	0.776	0.962	0.966	0.962	0.784	1.000						
As	0.732	0.757	0.744	0.516	0.782	0.721	0.710	0.710	0.760	1.000					
Ag	0.613	0.619	0.674	0.464	0.661	0.636	0.640	0.529	0.736	0.612	1.000				
Cd	0.158	0.097	0.166	0.263	0.164	0.197	0.145	0.161	0.239	0.132	0.138	1.000			
Sn	0.861	0.836	0.862	0.786	0.839	0.851	0.871	0.921	0.812	0.615	0.509	0.127	1.000		
Pb	0.970	0.966	0.987	0.764	0.975	0.981	0.972	0.793	0.976	0.762	0.695	0.214	0.841	1.000	
Organic	0.962	0.920	0.959	0.847	0.932	0.945	0.959	0.866	0.917	0.679	0.567	0.156	0.864	0.923	1.000
Silt	0.824	0.760	0.849	0.735	0.805	0.836	0.842	0.603	0.883	0.462	0.691	0.325	0.674	0.832	0.808

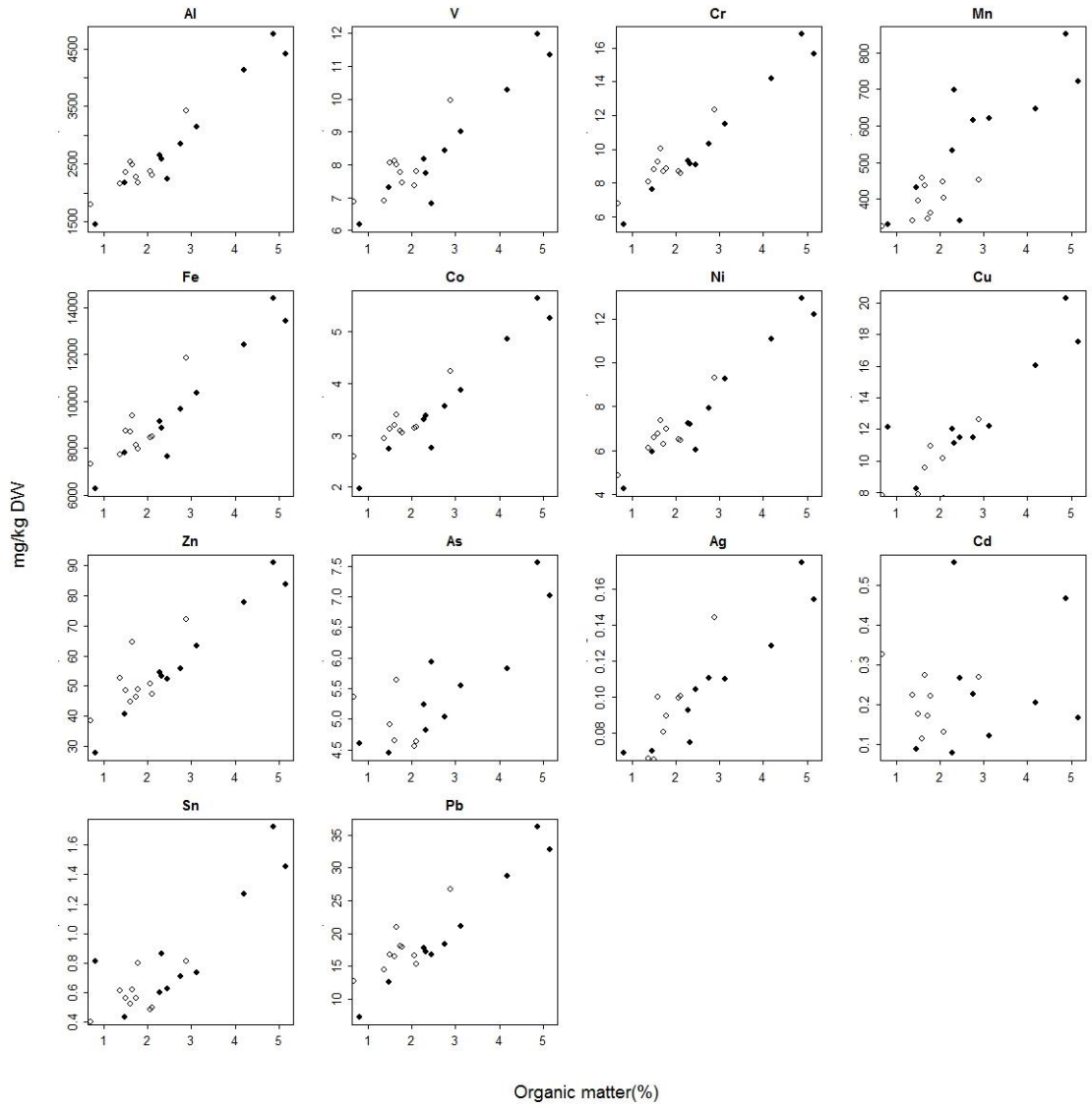


Figure 5.12. Relationship between organic matter content and analyte concentrations (mg kg^{-1} DW) in top 20mm of sediment core on September 2014 (black points) and April 2015 (white points)

5.3.2. Pore water

Pore water was analysed for all sites except site 3 where the volume retrieved was too small for analysis (probably due to larger sediment particle size at this location, there were are larger percentage of sand size particles in the low tidal frame, Figs. 5.7 and 5.8). The majority of analytes were at least two orders of magnitude lower in pore water than in sediment.

Table 5.3. Concentration (µg L^{-1}) of analytes in sediment pore water from the top 20mm of sediment in September 2014 and April 2015 at different heights in the tidal frame (High n=4, Mid n=3 and Low n=2). Where no value is given more than 10% of samples had concentrations below the detection limit (see Appendix 5-2).

Analyte	September 2014			April 2015		
	High	Mid	Low	High	Mid	Low
Aluminium	15 ± 7.8	31 ± 7.8	33 ± 25	-	230 ± 160	74 ± 6.2
Vanadium	5.7 ± 5.3	8.5 ± 9.6	12 ± 8.7	6.0 ± 0.88	7.4 ± 2.8	2.7 ± 0.14
Chromium	0.95 ± 0.34	0.97 ± 0.25	0.95 ± 0.35	3.7 ± 2.3	4.8 ± 4.9	2.0 ± 2.3
Manganese	6000 ± 5900	20000 ± 5700	5900 ± 2400	2500 ± 5100	1900 ± 2500	1600 ± 2200
Iron	19 ± 14	30 ± 6.4	14 ± 5.1	18 ± 10	550 ± 760	45 ± 3.0
Cobalt	2.1 ± 1.0	4.0 ± 0.64	1.4 ± 0.14	1.0 ± 1.2	1.8 ± 0.21	1.1 ± 0.21
Nickel	3.2 ± 1.8	3.2 ± 2.3	2.4 ± 0.35	6.2 ± 1.6	5.4 ± 1.6	3.2 ± 0.14
Copper	18 ± 12	14 ± 9.6	8.3 ± 4.5	-	-	-
Zinc	27 ± 9.9	35 ± 20	18 ± 0.57	100 ± 60	57 ± 12	24 ± 5.8
Arsenic	230 ± 10	230 ± 26	220 ± 14	220 ± 29	250 ± 9.2	240 ± 39
Silver	0.38 ± 0.41	0.33 ± 0.11	0.40 ± 0.00	0.48 ± 0.10	16 ± 23	0.35 ± 0.35
Cadmium	-	-	0.50 ± 0.42	7.7 ± 9.2	1.1 ± 1.2	0.50 ± 0.42
Tin	-	-	-	-	-	-
Lead	-	-	-	17 ± 28	2.6 ± 1.8	0.50 ± 0.56

5.3.3. Overlying water

Analytes in overlying water were present in similar or lower concentrations than in pore water (Table 5.4)

Table 5.4. Analyte concentration (μg L^{-1}) in overlying water on two dates (n=3). Where no value is given more than 33% samples had concentrations below detection limit (see Appendix 5-2).

Analyte	September 2014	April 2015
Aluminium	21.9±21.6	17.3±20.0
Vanadium	11.8 ± 0.86	2.6 ± 0.23
Chromium	0.86 ± 0.06	1.3 ± 0.15
Manganese	-	71 ± 3.3
Iron	7.8 ± 12	6.7 ± 2.7
Cobalt	0.10 ± 0.00	0.37 ± 0.06
Nickel	0.97±1.1	2.0 ± 0.38
Copper	4.7 ± 1.1	-
Zinc	25 ± 4.5	4.0 ± 0.68
Arsenic	210 ± 23	190 ± 5.4
Silver	0.16 ± 0.06	-
Cadmium	-	-
Tin	-	-
Lead	-	26 ± 0.20

5.3.4. Benthic algae

Biomass

The only difference between the quantities of algae collected in the field in September 2014 and April 2015 was in the lower part of the tidal frame (χ^2=7.2, p=7.3*10^{-3}, r^2=0.35) where a lower mass was collected during the September 2014 sampling campaign (Fig. 5.13 and Table 5.5).

In April 2015 the mass of algae collected in the field was similar across all heights in the tidal frame (Fig. 5.13). In September, for field collected samples, there was a difference in the mass of algae collected from the middle section of the tidal frame which was greater than that from the lower section (χ^2=5.5, p=1.7*10^{-2}, r^2=0.44, Fig. 5.13).

There was no difference in the mass of algae collected by the laboratory or field methods in April 2015 (χ^2=3.7*10^{-1}, p=5.4*10^{-1}, r^2=0.31, Fig. 5.15 and Table 5.5). A greater mass of algae

was collected through the laboratory method in September 2014 (χ^2=11.3, p=7.9*10^{-4}, r^2=0.72), most notably for algae in the middle of the tidal frame although there are differences at all heights in the tidal frame (Fig. 5.14). The difference in mass with tidal frame height is visible in Fig. 5.5 where the three trays with sediment from the middle of the tidal frame have a darker surface indicating a greater migration of diatoms.

Table 5.5. Mean mass (dry weight) of benthic algae (mg) collected by two collection methods, field and laboratory, at two dates

Date	Collection method	Height in tidal frame			
		All	High	Mid	Low
September 2014	Field	8.60 ± 4.14	9.18 ± 3.75	11.51 ± 4.63	4.92 ± 0.95
	Lab	75.98 ± 123.34	15.61 ± 2.01	224.91 ± 144.42	7.53 ± 2.26
April 2015	Field	10.01 ± 1.85	10.17 ± 1.94	10.97 ± 2.41	8.83 ± 0.66
	Lab	10.46 ± 2.02	10.35 ± 1.45	12.06 ± 2.65	9.02 ± 1.05

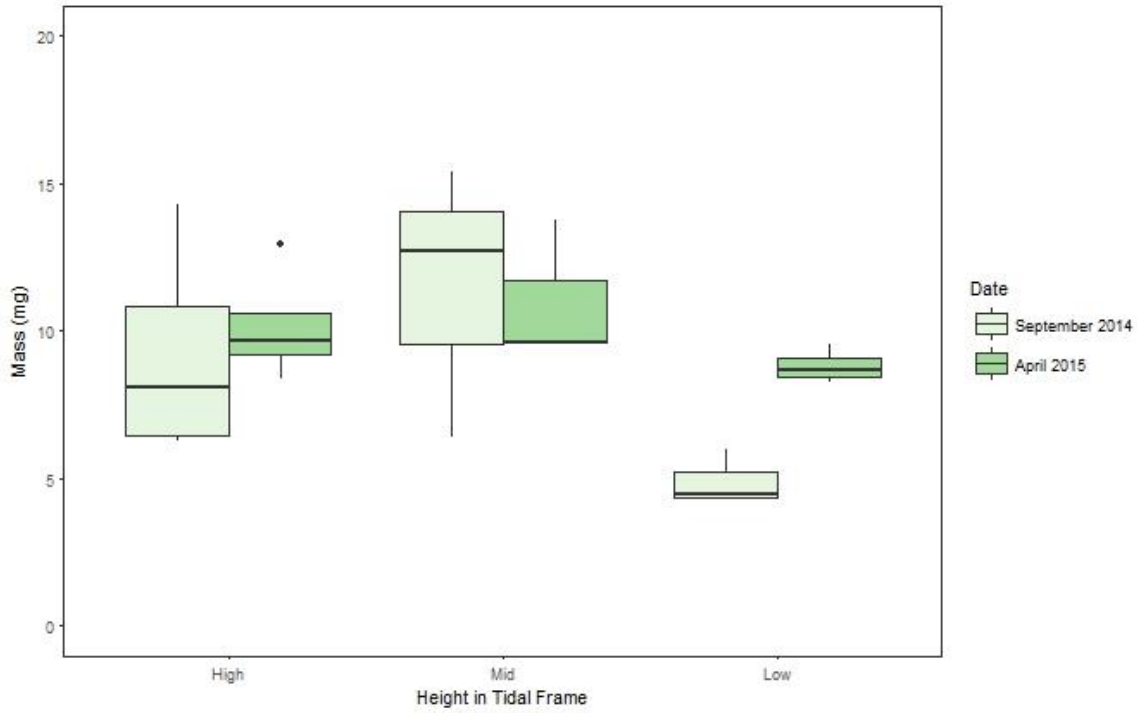


Figure 5.13. Comparison of mass of diatoms (mg) collected in the field on 25th September 2014 and 23rd April 2015 (High n=4, Mid n=3 and Low n=3), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

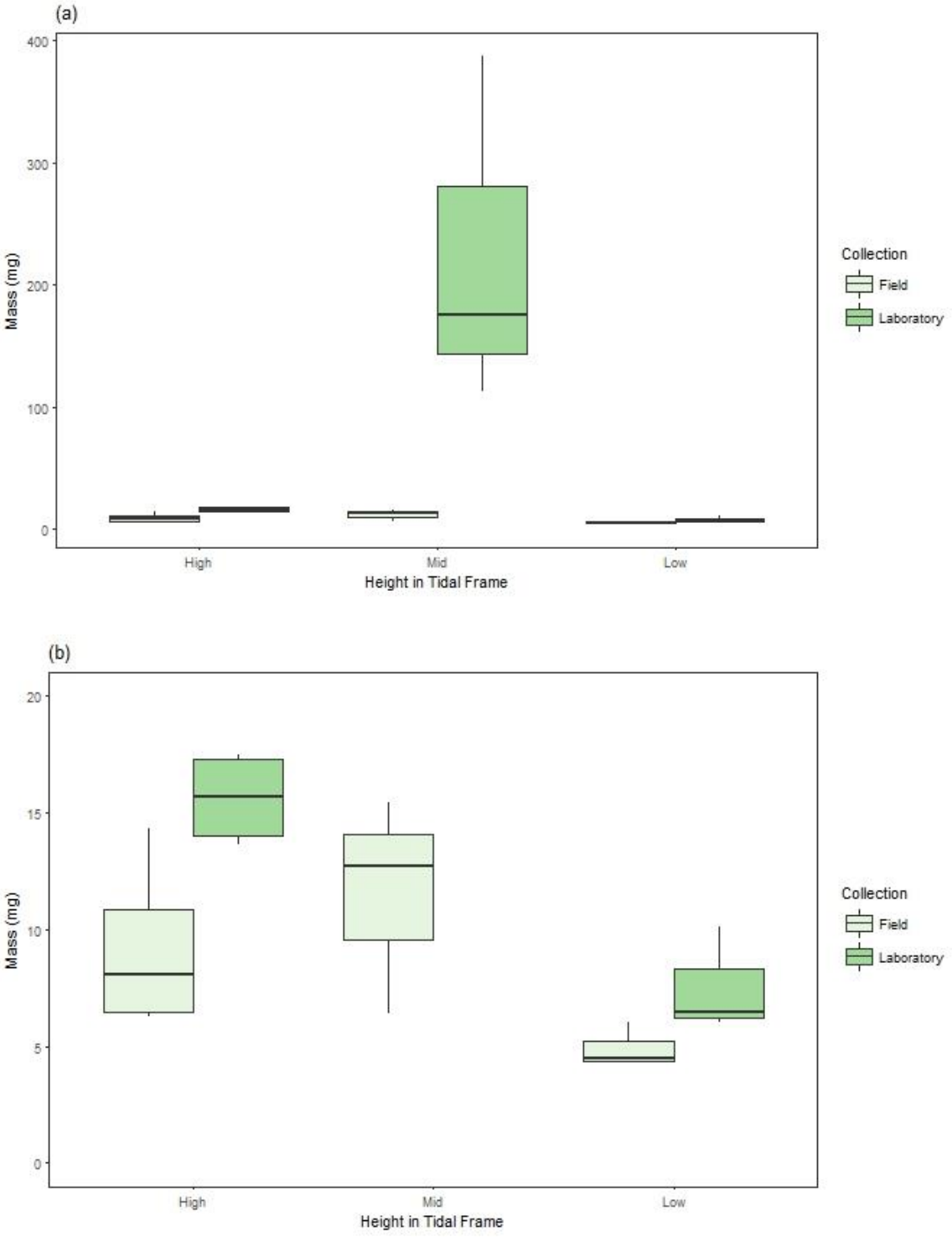


Figure 5.14. (a) Comparison of mass of diatoms (mg) collected in the field and laboratory in September 2014 and (b) with the middle tidal frame, laboratory collection sample removed (High n=4, Mid n=3 and Low n=3)

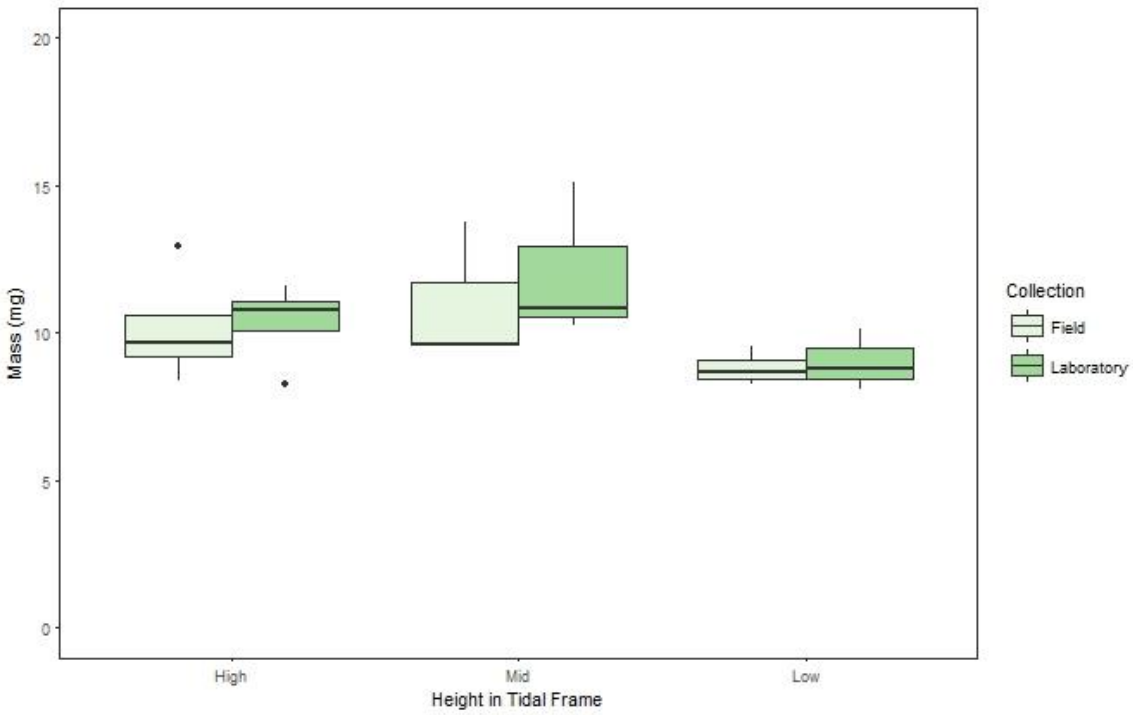


Figure 5.15. Comparison of mass of diatoms (mg) collected in the field and laboratory in April 2015 (High n=4, Mid n=3 and Low n=3), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Difference in analyte concentrations from field and lab separation of diatoms

Concentrations of some metals (Al, V, Fe and Sn) were lower in diatoms which were separated from the sediment in the laboratory as opposed to the field (Figs. 5.16 to 5.19).

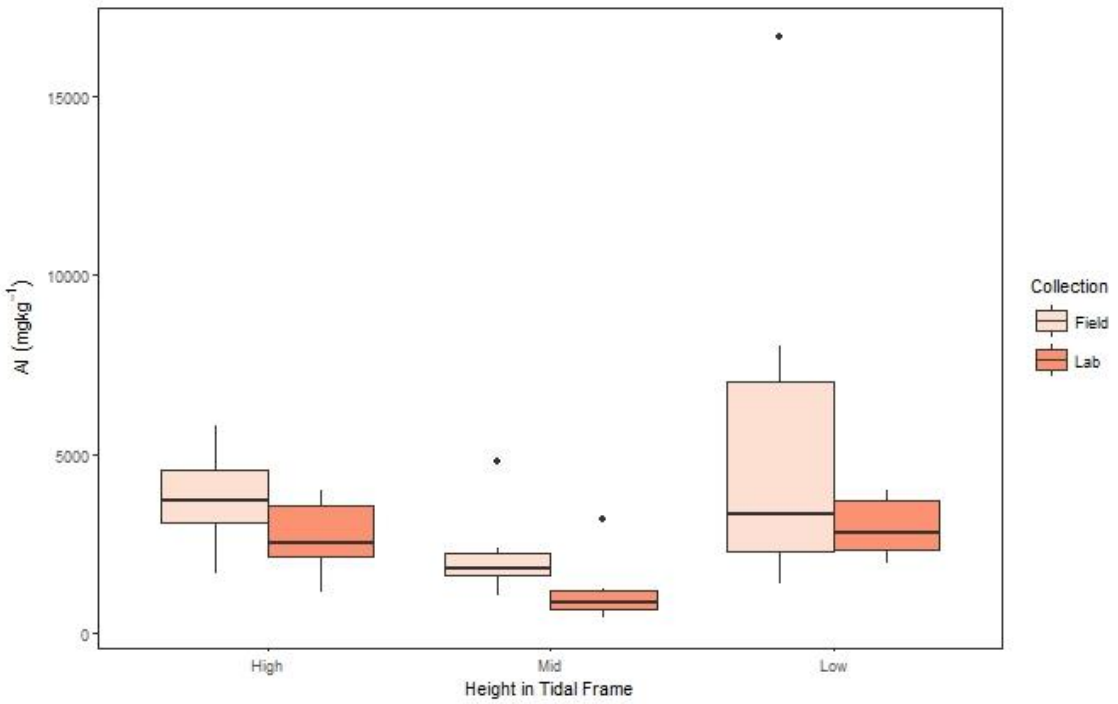


Figure 5.16. Difference in diatom aluminium concentrations (mg kg^{-1} DW) at different levels of the tidal frame when separated from the sediment in the field or laboratory, $p=7.4*10^{-3}$, (High n=8, Mid n=6 and Low n=6), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

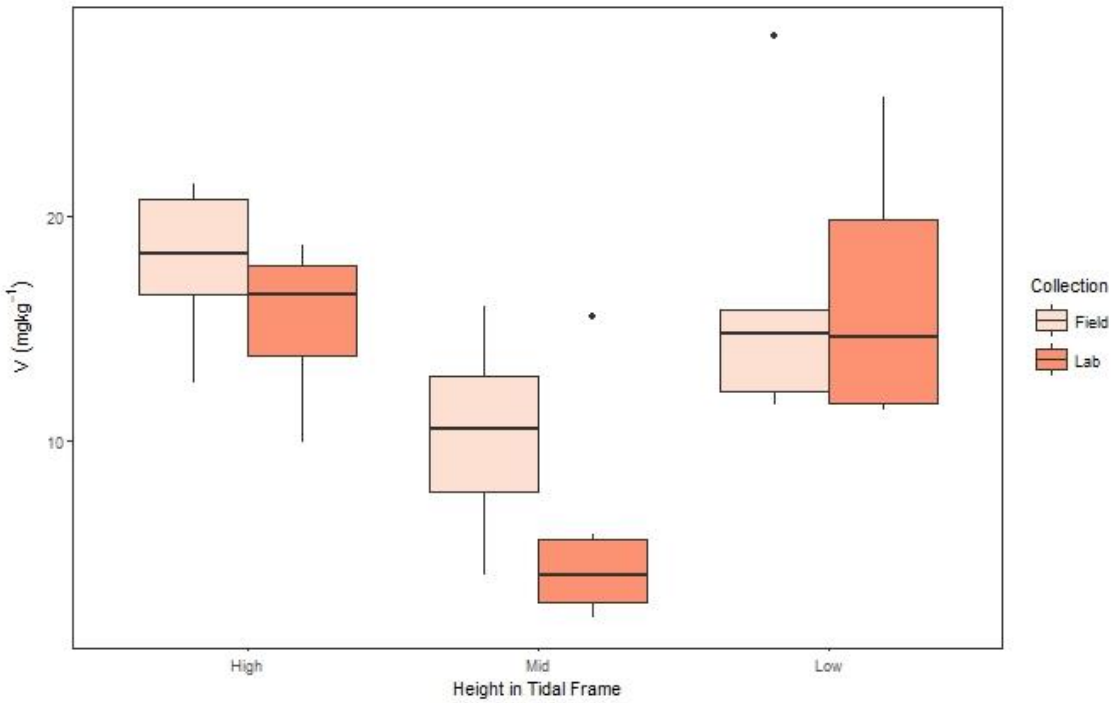


Figure 5.17. Difference in diatom vanadium concentrations (mg kg^{-1} DW) at different levels of the tidal frame when separated from the sediment in the field or laboratory, $p=2.2*10^{-2}$, (High n=8, Mid n=6 and Low n=6), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

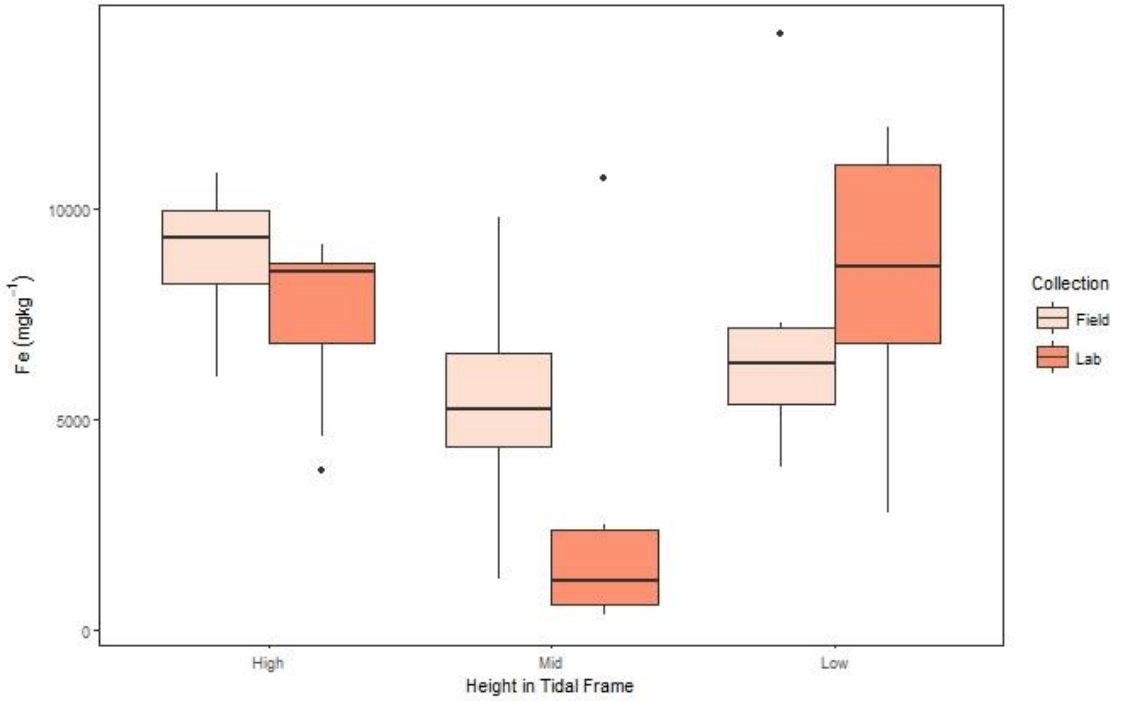


Figure 5.18. Difference in diatom iron concentrations (mg kg^{-1} DW) at different levels of the tidal frame when separated from the sediment in the field or laboratory, p=3.7*10^{-2}, (High n=8, Mid n=6 and Low n=6), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

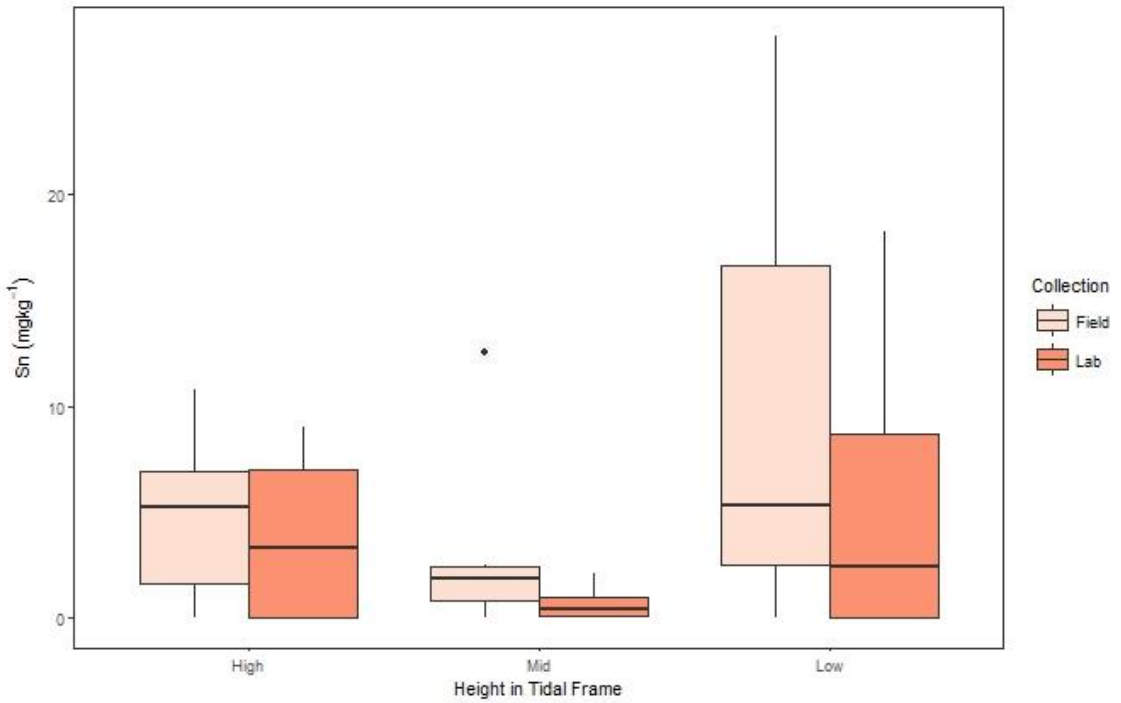


Figure 5.19. Difference in diatom tin concentrations (mg kg^{-1} DW) at different levels of the tidal frame when separated from the sediment in the field or laboratory, p=2.3*10^{-2}, (High n=8, Mid n=6 and Low n=6), outliers (•) calculated as Q1-1.5IQR and Q3+1.5IQR

Analyte concentrations in field separated diatoms

The BCF in diatoms related to sediment is shown in Table 5.6 as the BCF of mean concentrations of each analyte in sediment and algae on both dates. Where the BCF is greater than 1 the analyte is bioconcentrated (i.e. higher in the algae than the sediment).

There was bioconcentration in algae of all metals except Fe. Cd had the highest bioconcentration in diatoms, followed by Cu, Ni and Sn. BCF decreased as follows Cd>Ag>Cu>Sn>Ni>Zn>Pb>Cr>Mn>Al>V>As>Co>Fe in September 2014 samples and Cd>Cu>Ni>Cr>Sn>As>Zn>Pb>V>Ag>Co>Mn>Al>Fe in April 2015 samples. The analyte which shows the biggest change (by an order of magnitude) in bioconcentration was Ag going from a BCF of 23 in September 2014 to 1.8 in April 2015.

Table 5.6. Bioconcentration factors from concentration in sediment to algae given as the mean across all sites (n=10)

Analyte	September 2014			April 2015		
	Sediment (0 to 20mm)	Total Algae	BCF	Sediment (0 to 20mm)	Total Algae	BCF
Al	3000 ± 1100	5400 ± 4500	1.8	2400 ± 420	2500 ± 930	1.0
V	8.7 ± 1.9	15 ± 6.9	1.7	7.8 ± 0.87	15 ± 4.0	1.9
Cr	11 ± 3.6	31 ± 61	2.9	9.0 ± 1.4	57 ± 56	6.4
Mn	580 ± 170	1100 ± 530	2.0	400 ± 50	430 ± 160	1.1
Fe	10000 ± 2700	7700 ± 3500	0.77	8700 ± 1300	7100 ± 2500	0.82
Co	3.7 ± 1.2	4.9 ± 2.3	1.3	3.2 ± 0.42	3.6 ± 1.7	1.1
Ni	8.4 ± 2.9	82 ± 95	9.7	6.7 ± 1.1	124 ± 286	18
Cu	13 ± 3.6	165 ± 120	12	8.7 ± 2.1	220 ± 530	26
Zn	60 ± 20	380 ± 320	6.3	51 ± 10	182 ± 140	3.5
As	5.6 ± 1.0	7.8 ± 8.0	1.4	5.0 ± 1.4	19 ± 22	3.8
Ag	0.11 ± 0.04	2.5 ± 2.3	23	0.10 ± 0.05	0.19 ± 0.26	1.8
Cd	0.22 ± 0.17	12 ± 13	53	0.27 ± 0.19	10 ± 13	37
Sn	0.92 ± 0.42	9.2 ± 8.5	10	0.59 ± 0.13	2.6 ± 3.6	4.4
Pb	21 ± 9.1	87 ± 44	4.2	18 ± 3.9	52 ± 23	2.9

Comparisons of analyte concentrations in total and internal algae, sediment and pore water across the tidal frame on both dates are shown in Figures 5.20 to 5.30. Relationships between total diatom analyte concentrations and sediment analyte concentrations normalised to percentage organic matter are shown in Figure 5.31.

Concentrations of metals Al, V, Mn, Fe, Co and Pb showed similar patterns for all compartments except pore water, particularly for the total (absorbed and adsorbed) concentration in diatom samples (Figs. 5.20 to 5.25). These metals also showed very similar distribution of concentration in the sediment and were shown to be highly correlated with each other (Table 5.2). Sn and Cu showed a similar pattern of concentration in the algae and sediment in September 2014, but whilst the sediment concentrations showed a similar pattern of concentration in April 2015 samples, the algae concentrations did not (Figs. 5.27 and 5.28).

There was one extreme outlier at site 7 in April 2015 with a Cu concentration in the algae of (1700 ± 9.3 kg mg^{-1}), more than four times that of any other sample (Fig. 5.27 (e)), which was excluded from the statistical analysis presented in Table 5.7. With the outlier excluded sampling date was shown to have a significant ($p=2.4*10^{-2}$) influence on Cu concentration in algae with higher concentrations occurring in September 2014. With the outlier included there was no effect of date on Cu concentrations in algae due to date ($\chi^2=1.6*10^{-1}$, $p=9.3*10^{-1}$).

Prior to normalisation of the diatom metal concentrations some analytes (Mn, Cu, Sn and Ag) were affected by date and others were affected by sediment concentrations (V and Fe). Others were influenced by both sediment concentration and date (Al, Co, Zn, Pb) (Table 5.7). Where date had an effect, concentrations of analytes in diatoms were increased in September 2014. The influence of increased sediment metal concentrations was to decrease concentrations in diatoms. There was no effect of tidal frame position on analyte concentration in diatoms.

Following normalisation of sediment metals to organic matter, concentrations of Al, Mn, Co, Zn, Sn, Pb and Ag in diatoms were all shown to vary with date having a higher concentration in September 2014. Concentrations of V, Fe, Co, Pb and Ag all vary with position in the tidal frame (Table 5.8). Following normalisation of sediment analytes to percentage organic matter, there was no longer any influence of sediment analyte concentrations on diatom concentration. This suggests that uptake was influenced by the organic matter content of the sediment.

Only two analytes, Ni and As, showed any relationship between total diatom and pore water concentrations (Figs. 5.32 and 5.33). Ni in diatoms increased with increasing pore water concentrations ($\chi^2=4.4$, $p=3.7*10^{-2}$, $r^2=0.32$), however, examination of the data revealed that this was due to an outlier (Fig. 5.32) and when this point was removed from the model the relationship was no longer significant ($\chi^2=1.0*10^{-1}$, $p=7.5*10^{-1}$, $r^2=0.41$). There was a decrease in As concentration in diatoms with increasing pore water concentrations ($\chi^2=5.7$, $p=1.7*10^{-2}$, $r^2=0.47$).

Table 5.7. Statistical results from lmer models examining influences on analyte concentrations in algae prior to normalisation of sediment analyte concentrations. Significant values ($p<0.05$) in bold

Analyte	Sediment Concentration		Date		Model r^2
	χ^2	p-value	χ^2	p-value	
Aluminium	**5.9**	**1.5E-02**	**5.3**	**2.1E-02**	0.48
Vanadium	**12.8**	**3.4E-04**	2.1	1.5E-01	0.61
Manganese	1.7	1.9E-01	**7.6**	**5.9E-03**	0.66
Iron	**8.5**	**3.6E-03**	3.0	8.7E-02	0.55
Cobalt	**11.0**	**9.0E-04**	**0.7**	**1.7E-02**	0.67
Copper	0.2	6.4E-01	**5.1**	**2.4E-02**	0.37
Zinc	**5.1**	**2.4E-02**	**5.5**	**1.9E-02**	0.44
Silver	1.9	1.7E-01	**5.6**	**1.8E-02**	0.46
Tin	4.8	6.1E-01	**4.8**	**2.8E-02**	0.38
Lead	**8.7**	**3.2E-03**	**6.1**	**1.3E-02**	0.62

Table 5.8. Statistical results from lmer models examining influences on analyte concentrations in algae following normalisation of sediment analyte concentrations to percentage organic matter. Significant values ($p<0.05$) in bold

Analyte	Tidal frame		Date		Model r^2
	χ^2	p-value	χ^2	p-value	
Aluminium	2.6	2.8E-01	**4.9**	**2.6E-02**	0.37
Vanadium	**10.5**	**5.3E-03**	0.6	4.5E-01	0.49
Manganese	5.6	6.1E-02	**14.7**	**1.0E-02**	0.63
Iron	**7.8**	**2.1E-02**	0.9	3.3E-01	0.38
Cobalt	**6.7**	**3.6E-02**	**5.1**	**2.4E-02**	0.46
Copper	1.7	6.9E-01	**5.1**	**2.4E-02**	0.37
Zinc	3.0	2.2E-01	**5.3**	**2.1E-02**	0.41
Silver	**6.6**	**3.8E-02**	**5.6**	**1.8E-02**	0.75
Tin	3.5	1.8E-01	**4.8**	**2.8E-02**	0.38
Lead	**9.7**	**7.8E-03**	**4.3**	**3.8E-02**	0.53

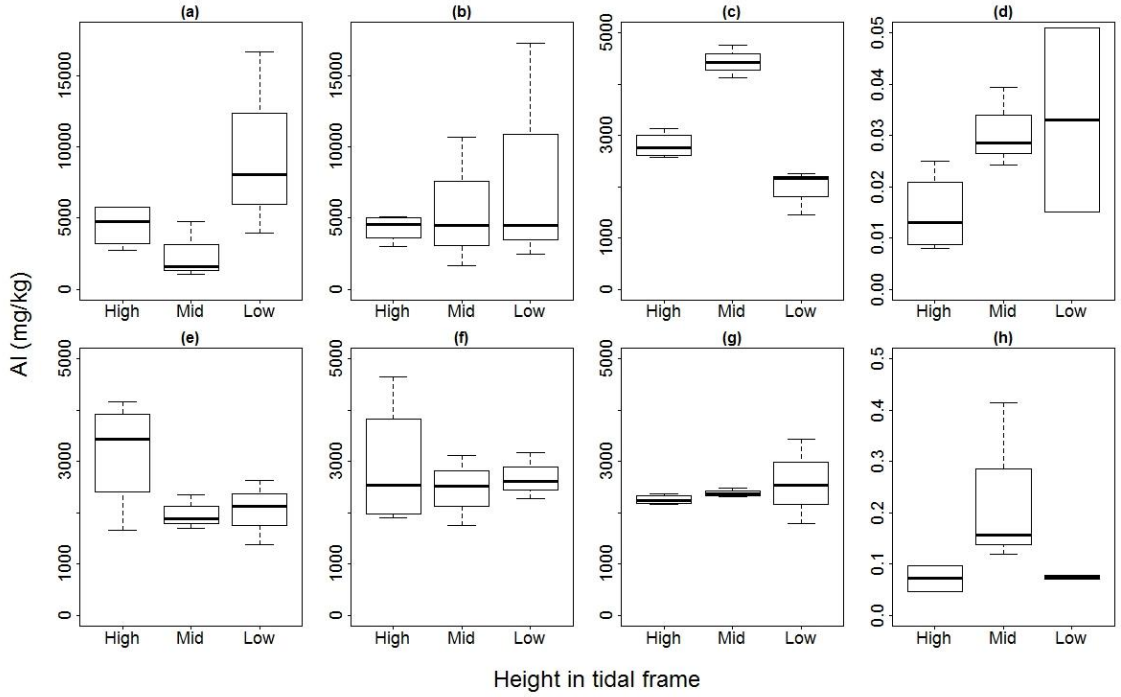


Figure 5.20. Aluminium concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

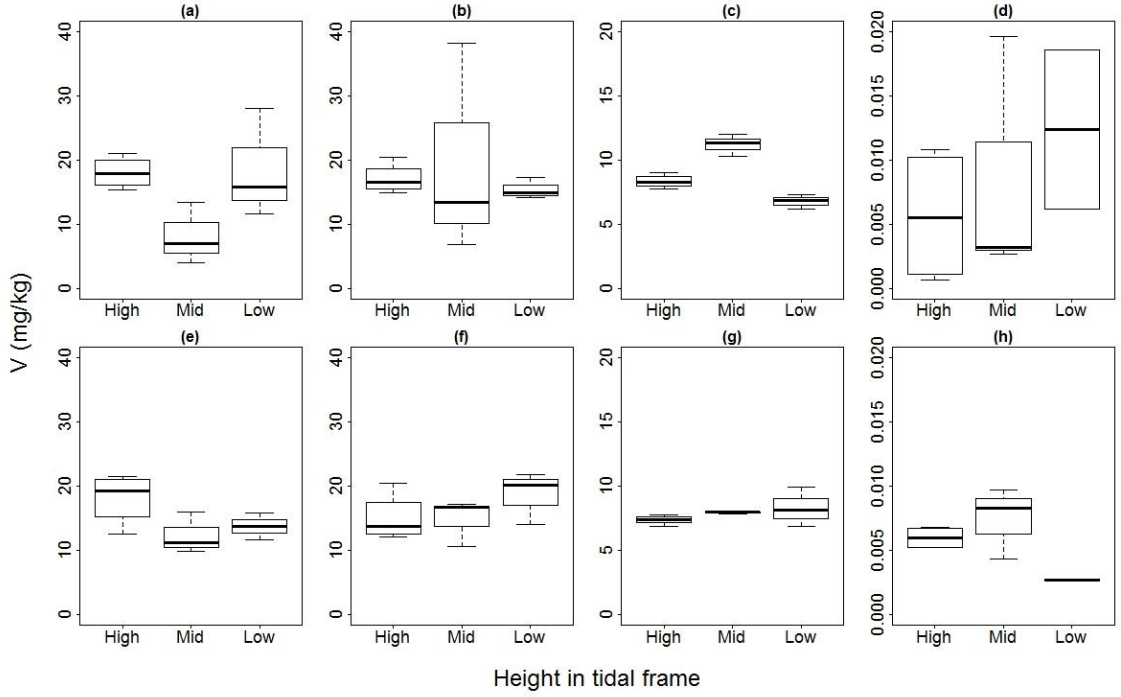


Figure 5.21. Vanadium concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

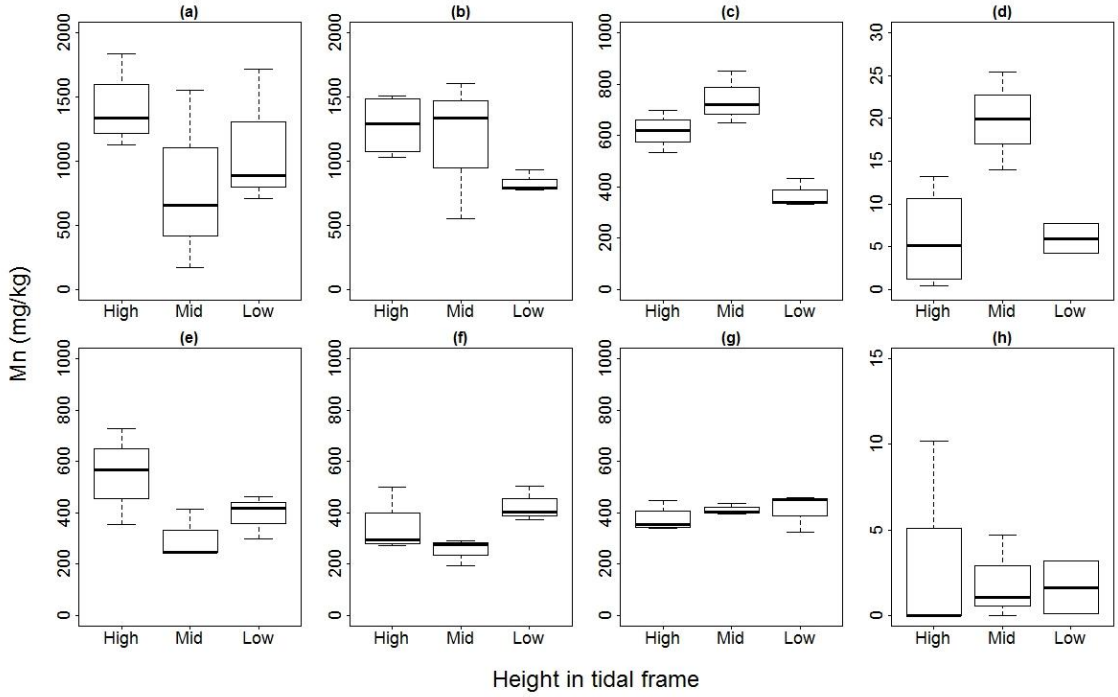


Figure 5.22. Manganese concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

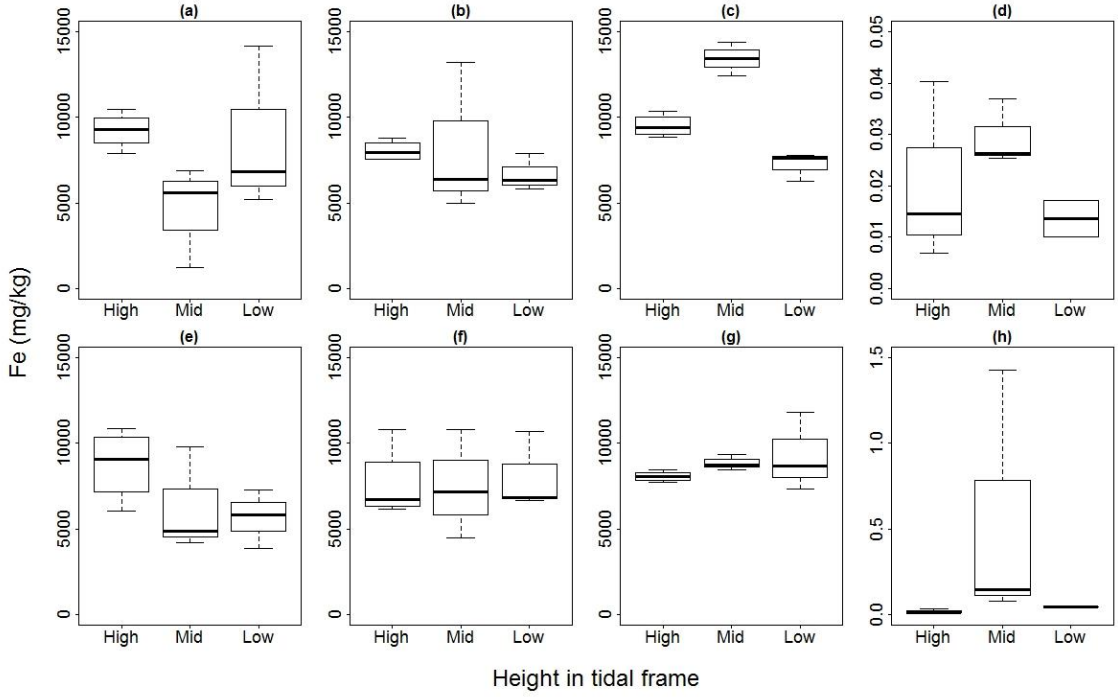


Figure 5.23. Iron concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

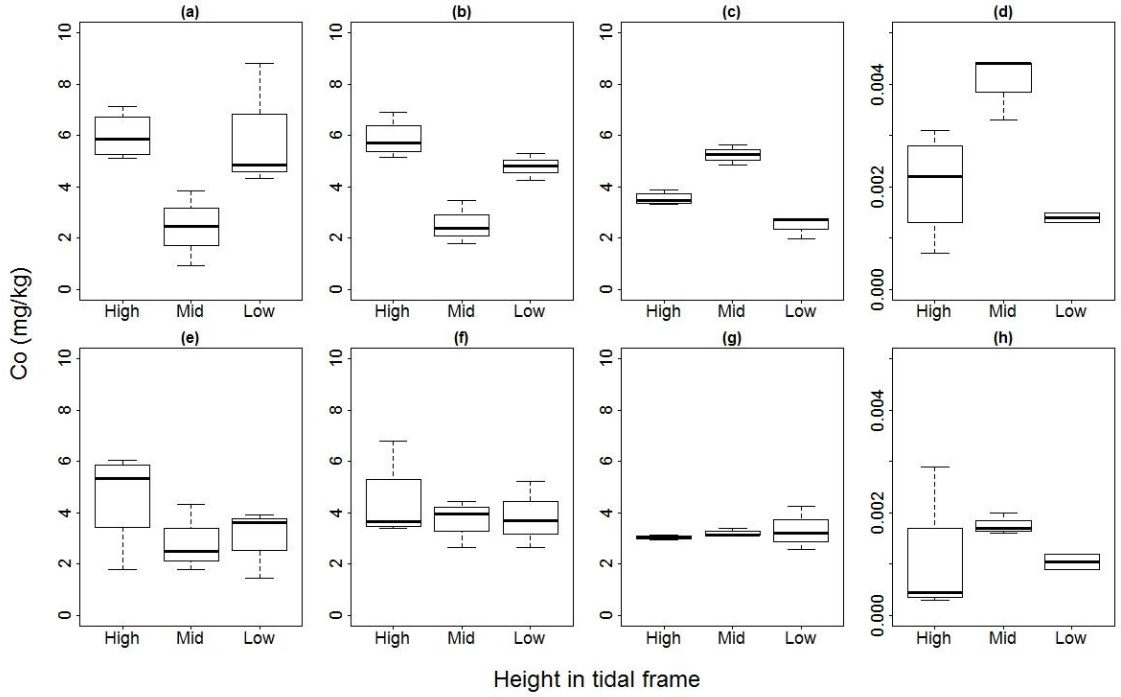


Figure 5.24. Cobalt concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

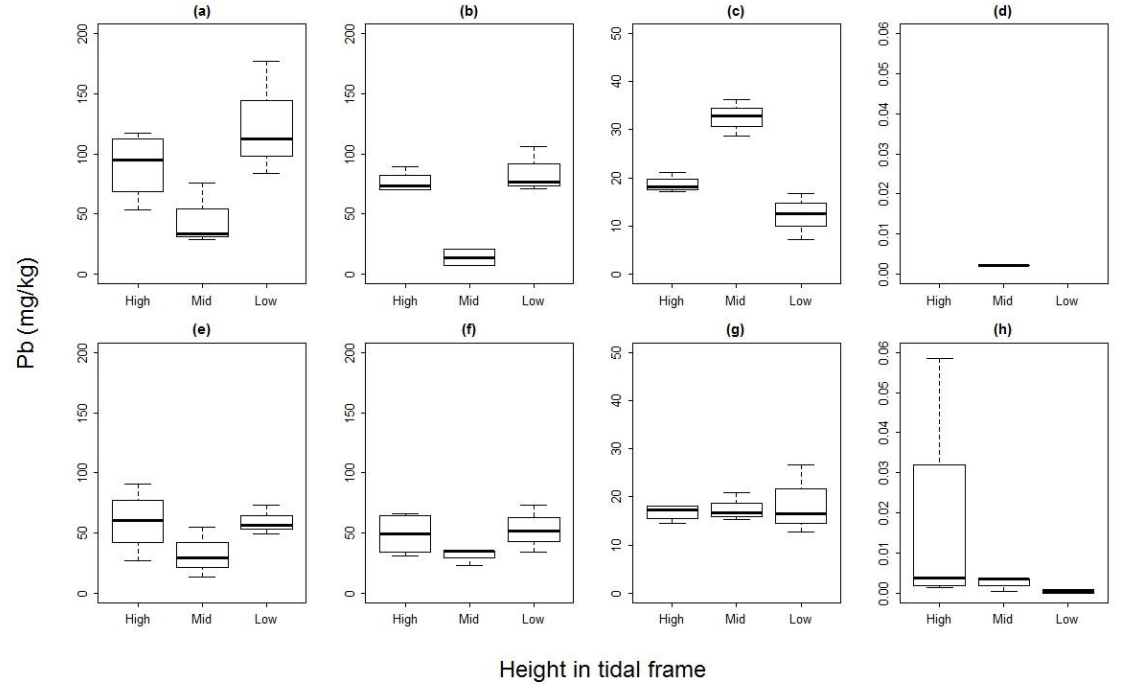


Figure 5.25. Lead concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

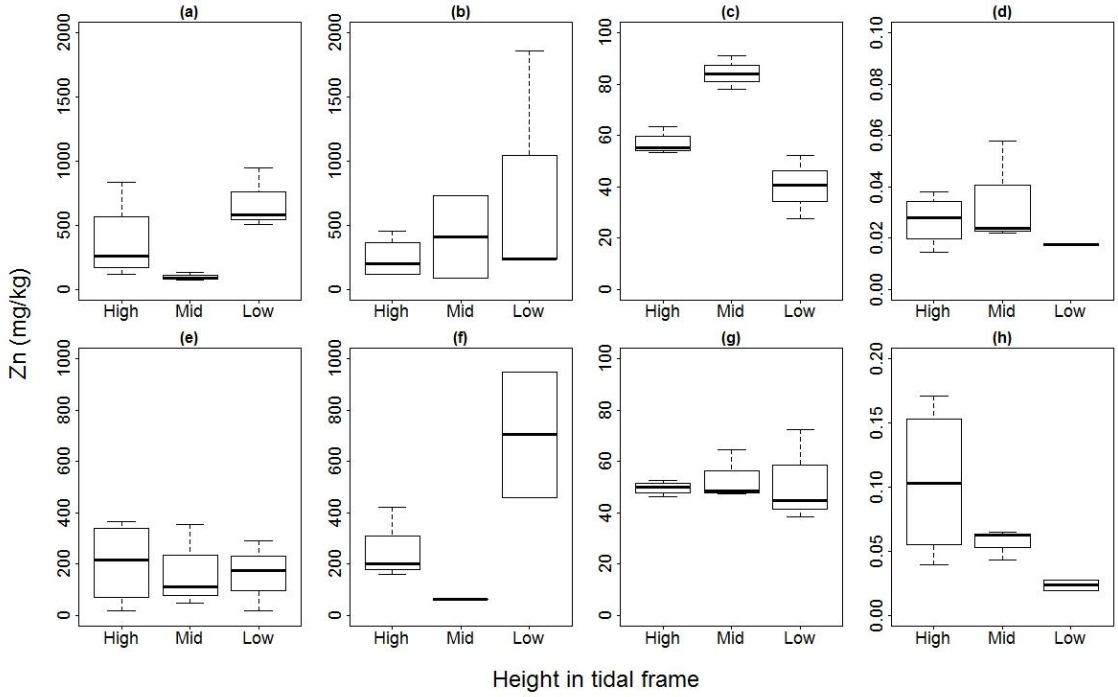


Figure 5.26. Zinc concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

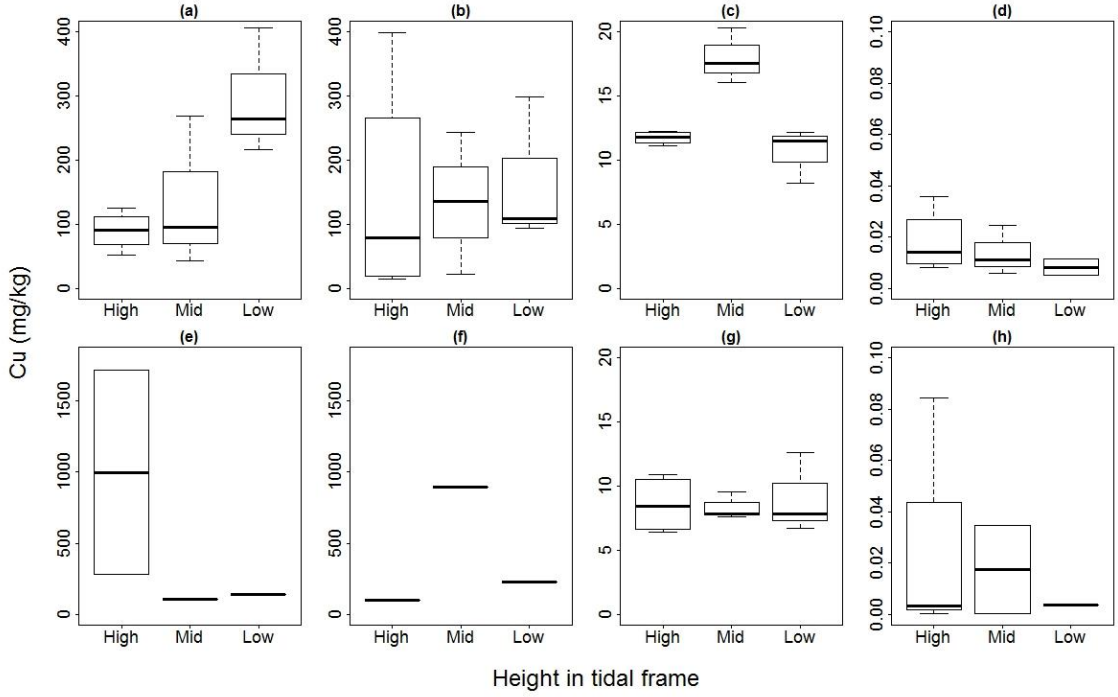


Figure 5.27. Copper concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

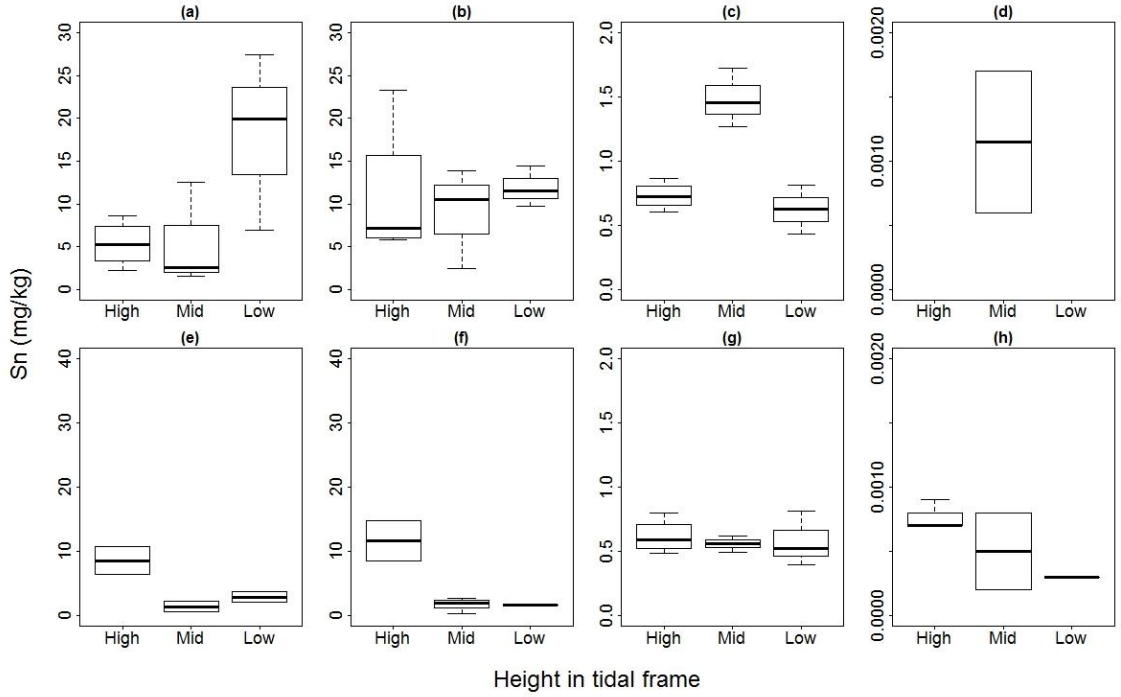


Figure 5.28. Tin concentration in (mg kg^{-1}) (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

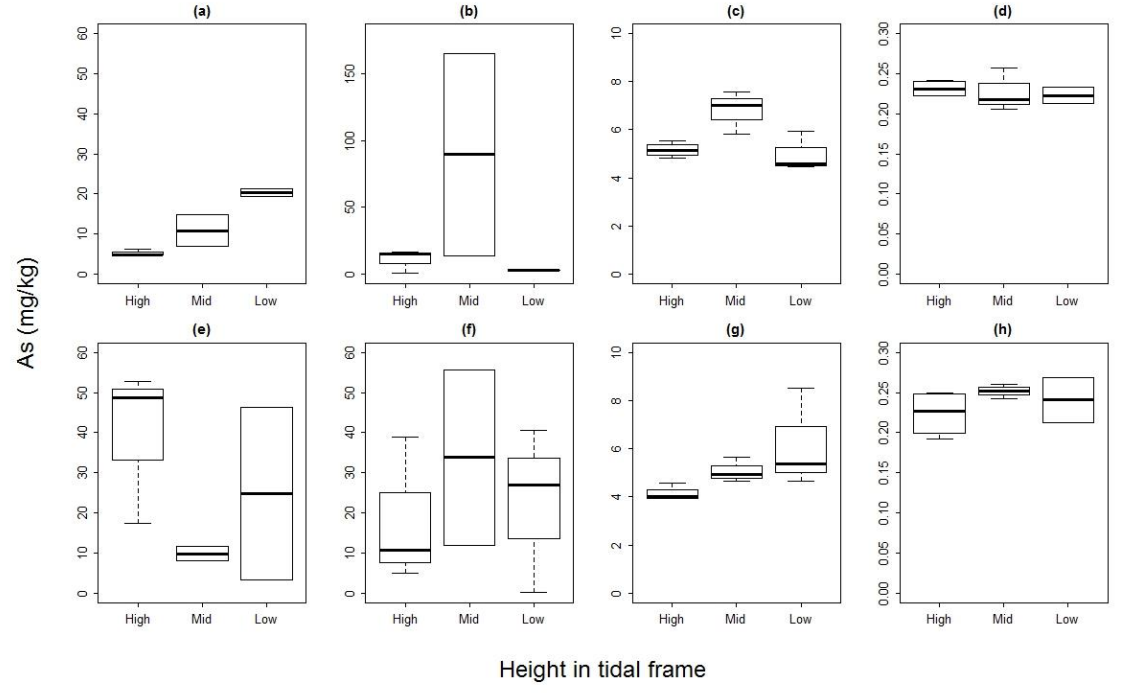


Figure 5.29. Arsenic concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

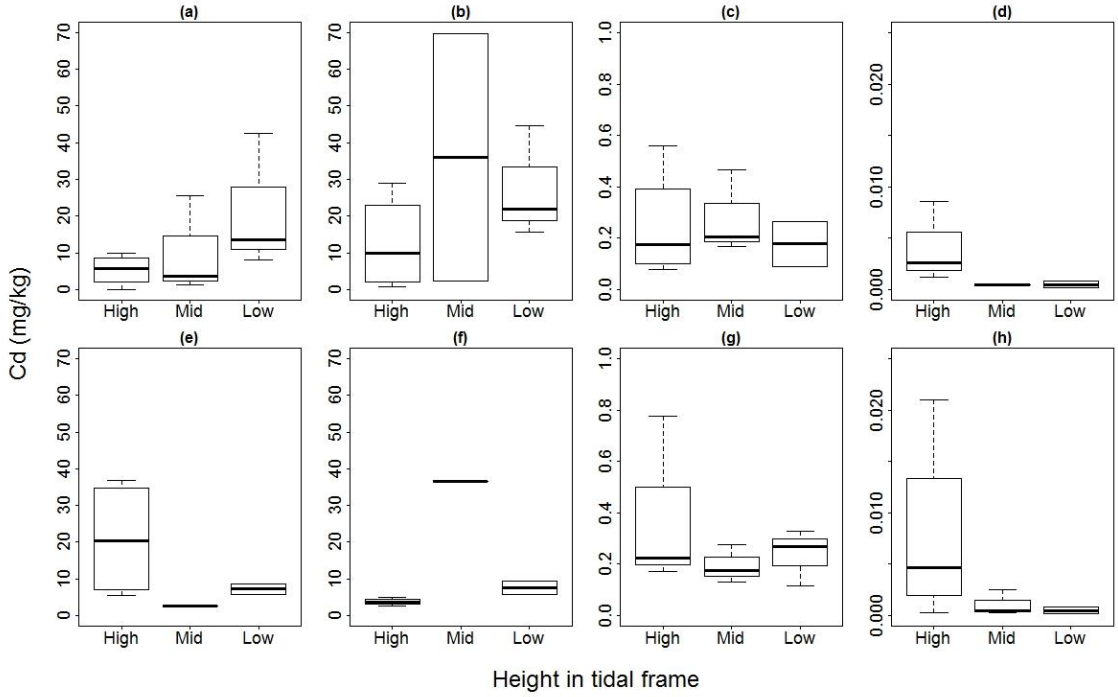


Figure 5.30. Cadmium concentration (mg kg^{-1}) in (a) total algae (b) internal algae (c) sediment (0 to 20mm) (d) pore water (0 to 20mm) in September 2014 and in (e) total algae (f) internal algae (g) sediment (0 to 20mm) (h) pore water (0 to 20mm) in April 2015

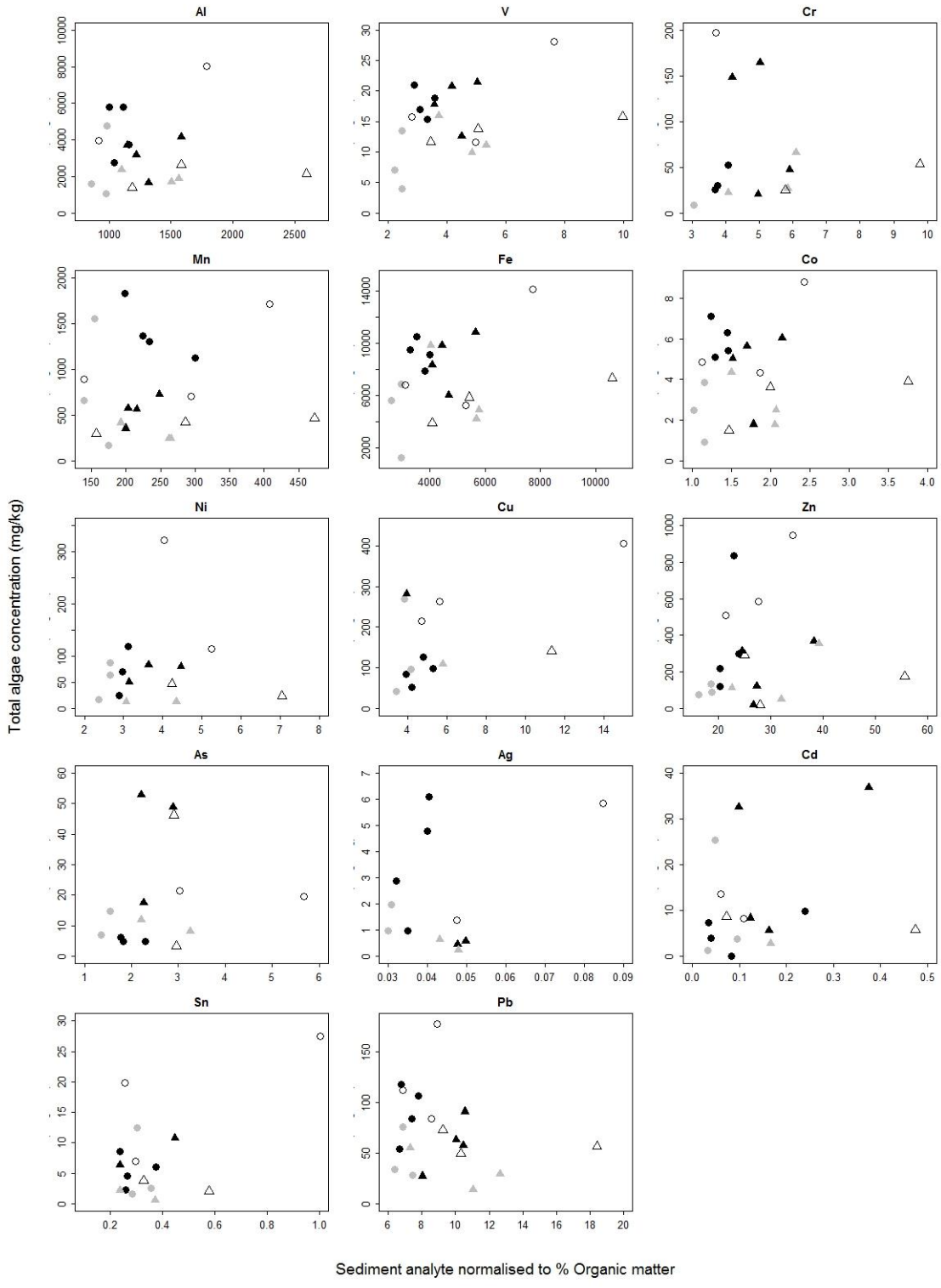


Figure 5.31. Relationships between total algae analyte concentrations (mg kg^{-1} DW) and sediment analytes normalised to percentage organic matter in September 2014 (circles) and April 2015 (triangles) in the high (black), middle (grey) and low (white) tidal frame

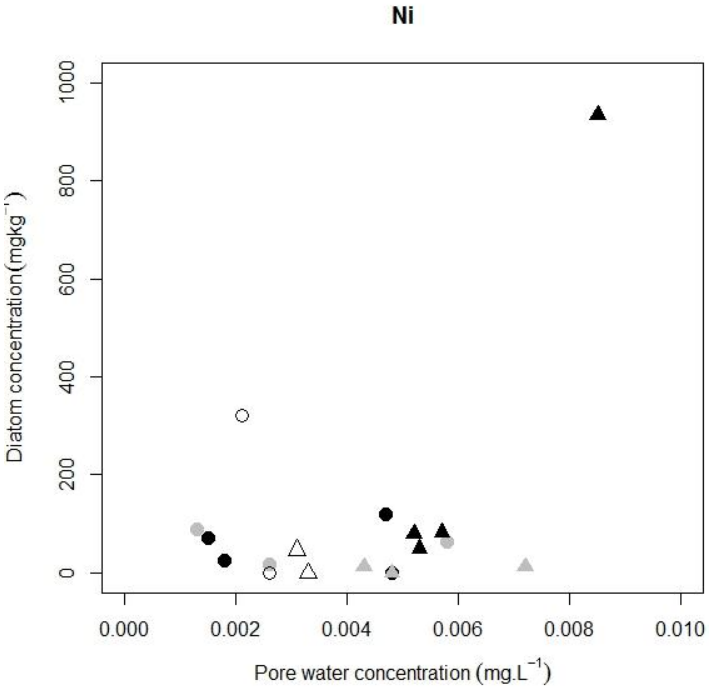


Figure 5.32. Relationship between total diatom (mg kg^{-1} DW) and pore water (mg L^{-1}) concentration of nickel in September 2014 (circles) and April 2015 (triangles) in the high (black), middle (grey) and low (white) tidal frame

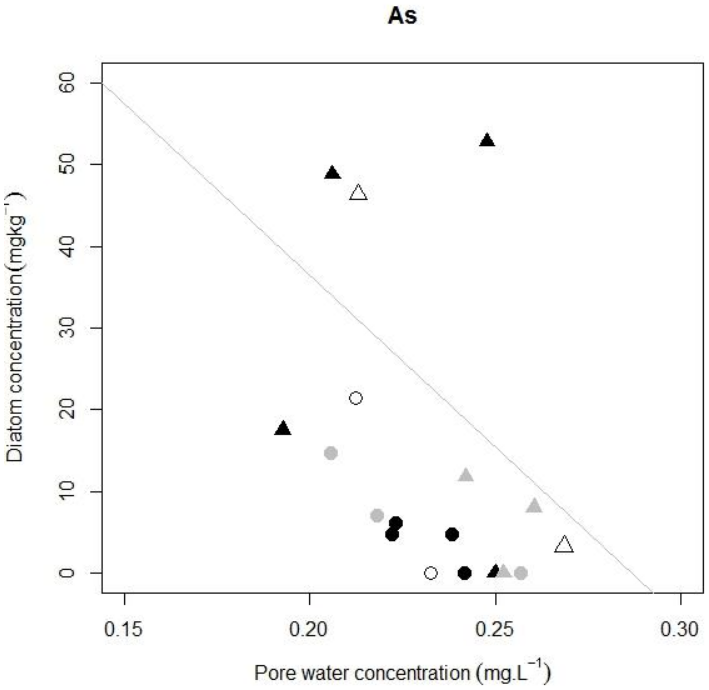


Figure 5.33. Relationship between total diatom (mg kg^{-1} DW) and pore water (mg L^{-1}) concentration of arsenic in September 2014 (circles) and April 2015 (triangles) in the high (black), middle (grey) and low (white) tidal frame

5.4. Discussion

5.4.1. Sediment

Particle size

The greater sediment particle size in the lower part of the tidal frame (Figs. 5.6 and 5.7) indicates that there is higher hydrodynamic energy at this location. This was the expected pattern due to a change in speed of the rising tidal current which would initially be quite fast, gradually slowing as it reaches high tide. This means that larger particle sizes drop out of the water column at the lower part of the tidal frame whilst the smaller particle sizes remain in suspension for longer and are carried further up the mudflat. This is the pattern seen in most tidal flat systems (Friedrichs, 2012). The fact that there is no significant difference in particle size between the two dates suggests that the site is fairly sheltered and did not experience a significant increase in energy (due to increased storminess) over the winter months (this was confirmed by Met Office (2016) reports for the period) which might have imported larger sediment particles to the mudflat. Although it should be noted that whilst sediment responses to storm events can be rapid, they may also be short-lived (Friedrichs, 2012).

There was a correlation between the percentage of silt (4 – 63 μm) particles and the percentage organic matter (Table 5.2) which may be due to a combination of current velocity and sediment cohesion associated with smaller particle sizes. Sediment cohesion reduces sediment resuspension and, therefore, loss of organic matter to the water column (Sakamaki and Nishimura, 2007).

Organic matter

A difference in organic matter as a percentage of sediment was observed between the two sampling campaigns with a higher percentage in September 2014 than April 2015 (Fig. 5.8). There was also a difference with height in the tidal frame which is particularly evident in the September samples when the highest percentage organic matter was in the middle of the tidal frame, followed by the high (most inland) level and with the lowest percentage in the low tidal frame, closest to the estuary channel. A similar pattern was observed in the surface sediment collected in April 2015 (Fig. 5.8 (d)) although it was not as pronounced. There was some correlation between percentage organic matter and silt particles (Table 5.2).

There are two possible reasons for the higher percentage organic matter seen in September 2014, firstly there may be a seasonal increase in inputs from the surrounding estuary and secondly it may be due to the quantity of microscopic benthic algae present within the sediment. Inputs of organic matter from the surrounding estuary might be increased due to an

increase in planktonic algae or input from terrestrial vegetation (i.e. in the form of leaf litter). Studies have shown that benthic algae are generally a minor component of sediment organic matter (Cook et al., 2004), with transported organic material making a bigger contribution (Sakamaki and Nishimura, 2007). Although the contribution of MPB to sediment organic matter has been shown to vary seasonally (Cook et al., 2004), there was no consistent pattern to the variation between sampling sites.

A comparison of organic matter concentration in the top 20mm of sediment in September 2014 and April 2015 (Figs. 5.8 (b) and (e)) with the mass of diatoms collected in the field on those two dates (Fig. 5.13) revealed that percentage organic matter and algal mass had a similar distribution pattern. However, the only difference between mass of diatoms collected in the field on the two dates was in the low tidal frame where the mass of diatoms retrieved in September was lower than in April. Yet, when the laboratory collection of diatoms is considered, there was a greater mass of algae collected in the high and middle tidal frame in September than in April (Figs. 5.14 and 5.15 and Table 5.5) in a pattern that closely followed that of organic matter distribution. It is suggested from these data that, at the current study site, on these dates, benthic microalgae were making a significant contribution to the sediment organic matter content.

Metal concentration

The greatest concentrations of all analytes except Cd occurred in sediment samples from September 2014 (Table 5.1). On this date the majority of metals had their highest concentrations the middle of the tidal frame with the exception of Cr which had its highest concentration in the high tidal frame and Cd which had similar concentrations in the middle and high tidal frame. Higher concentrations in the middle and high tidal frame were probably due to the smaller sediment particle size and higher organic matter contents which most of the metals, with the notable exception of Cd, were highly correlated with (Table 5.2). Strong correlations between sediment metals and organic matter in the sediments of the Ribble estuary were observed in a previous study (Ridgway et al., 2001) with strong positive correlations for Ag, As, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn and Zn and a negative correlation for Cd.

In contrast to the current study, McCormick et al. (2014) found no increase in the total sediment contamination when comparing sediment with an obvious biofilm to adjacent sediment without, which may suggest that there was some other cause on this occasion (e.g. particle size). A possible reason within the current study for higher metal concentrations in September 2014 is that at the time of collection an algal bloom was observed. This was both visually apparent during field sampling across the middle of the tidal frame as a blackening of the sediment surface and evident in the higher mass of algae retrieved by the laboratory

collection method on this date (Table 5.5 and Figs. 5.5 and 5.14), although it was not apparent from the mass of algae collected in the field (see section 5.4.4 for further discussion of variation of diatom mass with collection method). It is uncertain whether the increase in analyte concentrations was due to the algae or the higher organic matter content of the sediment (which may in turn have been due to the larger quantity of benthic algae). Percentages of silt and clay sized particles within the sediment did not change with date (Fig 5.6 and 5.7).

Comparing sediment metal concentrations (Table 5.1) to the SQUIRT (screening quick reference table) (Table 5.9) (Buchman, 2008) showed Mn concentrations to be above the apparent effects threshold (AET), the concentration where biological indicator effects are always observed, at all tidal frame heights on both sampling dates. Sn was above the threshold effects level (TEL), the maximum concentration where no effects are observed, at all tidal frame heights on both sampling dates. Additionally, Cu, As and Pb were all close to or above the TEL in the sediment in the middle of the tidal frame in September 2014. It has been suggested that the application of sediment quality values such as those in the SQUIRT tables may over or underestimate the risk to biota due to the geochemical variability of sediments across different estuaries (Spencer and MacLeod, 2002). However, these tables are useful as a guide and may indicate where further monitoring and investigation is warranted. At the Ribble field site metals that have sediment concentrations at or over the TEL are all also shown to be bioconcentrated in diatoms (Table 5.6). This would indicate that these metals are bioavailable to algae and may therefore be available to other biota both from the sediment and through the estuarine food chain.

Whilst the effects levels in SQUIRTs were devised using organisms other than diatoms a preliminary set of sediment quality guidelines (SQGs) for Cu, Hg, Pb and Zn have been produced using the diatom *Cylindrotheca closterium* and sediments from 17 sites in the south of Spain (Araújo et al., 2010) (Table 5.10). Whilst the level at which there are no observable biological effects (equivalent to TEL) is higher for diatoms, the level at which biological effects are observed (equivalent to AET) is lower. Whilst the sediment analysed in this study was below the level at which there are no observable biological effects for the limited metals available for comparison, the diatom used *C. closterium* (a meroplanktonic species) is thought to be more tolerant to contaminants than truly benthic diatoms as in locations with polluted sediments it is often the only diatom observed (Cibic, 2016, pers. comm., 6th September).

An earlier study of the river Ribble and its estuary carried out by the British Geological Survey (BGS) (Ridgway et al., 2001) compared model catchment signatures, created using background geology, to actual catchment signatures in an attempt to distinguish anthropogenic from natural sources of metals entering the Irish Sea. It found the lower Ribble to be contaminated with Zn, Pb and Sn from anthropogenic sources when compared to the background signature. Cu and As

were noted as being high in the sediment due to naturally high background levels at this location.

Sample "Ribble 3" in the BGS report (Ridgway et al., 2001) is described as being taken 70m from the end of the RNLI jetty, which gives it a location roughly equivalent to samples taken in the low tidal frame in this study. Comparison of the sediment concentration of metals in this study and those measured in the BGS report (Table 5.11) revealed several differences. Even taking in to account variations observed between seasons in this study, sediment concentrations of V and Cr were much lower than those reported by Ridgeway et al. (2001), whilst Cu and Zn concentrations increased. Concentrations of Ni, As, Sn and Pb were broadly similar in the earlier BGS study and this one. A possible reason for these differences is that it is highly probable that levels of contamination in the shifting sediments of the intertidal mudflat vary over time (samples in this study were taken approximately 15 years after those in the BGS report) due to dilution, regulatory improvements and changing contamination sources. Other possible reasons for differences are the sampling and the laboratory analysis methodologies. In the BGS report (Ridgway et al., 2001) samples were described as being collected by walking out onto the mudflats at low tide and taking a sample of whole sediment (i.e. not sieved to 2mm) totalling more than 2kg made up of material collected from several places at each site. This differs from the short (40mm) core method employed here gives no indication of sampling depth which may impact on metal concentrations. There was a slight variation in metal concentration with sampling depth in the current study in which some analytes have a higher concentration at 20 to 40mm (Fig. 5.11 and Appendix 5-4). Furthermore, analysis in this study was carried out using ICP-MS, whilst XRF (x-ray fluorescence spectrometry) analysis was used in the earlier study.

A slightly more recent study of sediment contamination in the intertidal mudflats of the Ribble (Mawby et al., 2004), which analysed metal content by ICP-OES, found levels of Cu and Zn similar to those reported here. Although lower than those reported by Ridgeway et al. (2001), Mawby et al. (2004) found Cr concentrations more than double those measured in this study. Pb, Zn and As concentrations were similar across all three studies. This comparison with earlier reports, in addition to highlighting the difficulties of inter-comparison across analytical methodologies, gives some insight into the variability across time and space within the estuary and highlights the need for continued monitoring of sediment contamination.

Table 5.9. Screening Quick Reference Table for metals in marine sediment (Buchman, 2008). All concentrations in mg kg^{-1} unless specified.

Analyte	Threshold Effects Level (TEL)	Effect Range Low (ERL)	Probable Effects Level (PEL)	Effects Range Median (EMR)	Apparent Effects Threshold (AET)
Aluminium (%)	-	-	-	-	1.8 (Neanthes)
Vanadium	-	-	-	-	57.0 (Neanthes)
Chromium	52.3	81	160	370	62.0 (Neanthes)
Manganese	-	-	-	-	260 (Neanthes)
Iron (%)	-	-	-	-	22 (Neanthes)
Cobalt	-	-	-	-	10.0 (Neanthes)
Nickel	15.9	20.9	42.8	51.6	110 (Echinoderm larvae)
Copper	18.7	34	108	270	390 (Microtox & Oyster Larvae)
Zinc	124	150	271	410	410 (Infaunal community)
Arsenic	7.24	8.2	41.6	70	35.0 (Bivalve)
Silver	0.73	1	1.77	3.7	3.10 (Bivalve)
Cadmium	0.68	1.2	4.21	9.6	3.00 (Neanthes)
Tin	0.05	-	-	-	3.40 (Neanthes)
Lead	30.2	46.7	112	218	400 (Bivalve)

Table 5.10. Sediment quality guidelines (SQG) for metals in coastal sediments (Araújo et al., 2010). All concentrations in mg kg^{-1}.

Analyte	No observable biological effect	Area of uncertainty	Observable biological effects
Copper	60	60-143	143
Lead	50	50-218	218
Zinc	143	143-152	152

Table 5.11. Analyte concentrations (mg kg^{-1}) in Ribble sediment in May 1999 (Ridgway et al., 2001) and at low tidal frame height in the current study

	Ridgeway et al (2001)		Current Study (low tidal frame)	
Analyte	**"Ribble 3"**	**Background signature**	**September 2014**	**April 2015**
Aluminium (%)	-	-	0.20 ± 0.04	0.26 ± 0.08
Vanadium	26	82	6.8 ± 0.57	8.3 ± 1.6
Chromium	98	115	1.6 ± 0.83	1.7 ± 1.1
Manganese	-	2097	370 ± 56	410 ± 75
Iron (%)	-	-	0.72 ± 0.08	0.93 ± 0.23
Cobalt	-	-	2.5 ± 0.45	3.3 ± 0.83
Nickel	10	54	5.4 ± 1.0	7.0 ± 2.2
Copper	5	47	11 ± 2.1	9.1 ± 3.1
Zinc	4	17	40 ± 12	52 ± 18
Arsenic	5	3	5.0 ± 0.82	6.2 ± 2.1
Silver	-	-	0.08 ± 0.02	0.10 ± 0.05
Cadmium	-	-	0.12 ± 0.14	0.24 ± 0.11
Tin	2	5	0.62 ± 0.19	0.58 ± 0.21
Lead	16	53	12 ± 4.8	19 ± 7.2

5.4.2. Pore water

Unsurprisingly, concentrations of all analytes were found to be lower in pore water than sediments. Mn, Fe, Co, Zn and Cd all had higher concentrations in pore water than overlying water (Tables 5.1, 5.3 and 5.4).

Concentrations of analytes in pore water may indicate greater bioavailability to algae and other organisms and it has been suggested that pore water be used as a surrogate test fraction where toxicity testing using sediment is difficult (Ankley and Schubauer-Berigan, 1994). Unfortunately pore water is difficult to extract from the sediment (Chapman et al., 2002) and where the sediment had a larger particle size (i.e. at site 3) it was not available in quantities large enough for analysis. Additionally, concentrations of analytes in pore water were often below the detection limit (e.g. Sn, Pb and Cu).

Unexpectedly, no relationships were observed between pore water and diatom metal concentrations for any analytes except As, which had a decreasing uptake to diatoms as pore water concentration increased (Fig. 5.33). Analytes which were bioconcentrated in diatoms in comparison to sediment (e.g. Cd, Sn and Pb, Table 5.6) had low concentrations in pore water.

In fact, concentrations of these metals were below the LoD (the lowest concentration at which detection is feasible) in pore water in September 2014 (Table. 5.3) when bioconcentration in diatoms was greatest. Conversely, although Fe was present in pore water, there was no bioconcentration.

Although pore water has often been seen as an important uptake route for sediment dwelling organisms (Adams et al., 1992, Chapman et al., 2002) and may represent the first bioavailable fraction, caution in assuming chemical toxicity is correlated to pore water concentration has been advised (Burton Jr., 2010). As pointed out by Chapman et al. (2002) organisms within the sediment often live in an environment of their own making; they modify the environment through their presence by, for example, the creation of burrows or modifying oxygen levels. This is the case with benthic diatoms which inhabit a biofilm with other organisms (algae and bacteria) and extracellular polymeric substances (EPS) (De Brouwer and Stal, 2002). EPS, secreted by the organisms of the biofilm including the diatoms, changes biochemistry and acts as a chelator, binding metals (Bhaskar and Bhosle, 2005). In addition, extraction of pore water from the sediment may also change its properties and, thereby, the speciation and bioavailability of metals (Chapman et al., 2002).

An additional issue may be due to pore water extraction techniques. Although care was taken through the use of cores not to disturb the sediment structure prior to pore water extraction, it is possible that partitioning of metals between sediment and pore water was altered (Chapman et al., 2002). A possible solution to this problem is to test pore water in situ using microelectrodes (Chapman et al., 2002).

5.4.3. Overlying water

A comparison of overlying water concentrations (Table 5.4) with SQUIRT (Table 5.12) (Buchman, 2008) showed that Cu, in April 2015, and Pb, in September 2014, were present in overlying water in concentrations greater than those which are described as producing chronic effects. Additionally, concentrations of Cu in the April 2015 sediment samples were close to levels described as producing acute effects whilst As was present at both time points in concentrations greater than those described as producing acute effects (this was the case in the overlying water compartment during the mesocosm experiment, Chapter 4, Sections 4.3.4 and 4.4.2).

Those analytes which had levels close to or above effects levels in overlying water (Cu, As and Pb) also had concentrations close to or above effects levels in the sediment (Tables 5.1 and 5.9). Reports of effects levels of metals in water vary widely dependent on diatom species. For example the IC_{50} (concentration which inhibits growth rate by 50%) for Cu is as low as 8 µg L^{-1}

for *Phaeodactylum tricornutum* (Levy et al., 2008), whilst for *Chaetoceros calcitrans* it is 450 µg L^{-1} (Anu et al., 2016) and *C. closterium* 7700 µg L^{-1} (Satoh et al., 2005). *C. closterium* has also been reported as having IC_{50} levels for both As and Pb of 8700 µg L^{-1} (Satoh et al., 2005), although this is also likely to vary as widely for other species.

Water samples are subject to high temporal and spatial variability (a recent study found trace metal concentrations in the coastal waters to vary over time scales of hours (Pinedo-Gonzalez et al., 2014)), this is particularly true in estuaries due to the tides and the changing influence of fluvial and offshore waters. Despite this, concentrations of metals in overlying water may provide information on metals inputs and explain bioavailability where sediment concentrations do not.

Table 5.12. Screening Quick Reference Table for effects levels of analytes in marine surface waters from SQuirRTs (Buchman, 2008). All concentrations in µg L^{-1}.

Analyte	Acute	Chronic
Aluminium	-	-
Vanadium	-	50
Chromium	-	-
Manganese	-	100
Iron	300	50
Cobalt	-	1
Nickel	74	8.2
Copper	4.8	3.1
Zinc	90	81
Arsenic	69	36
Silver	0.95	-
Cadmium	40	8.8
Tin	-	-
Lead	210	8.1

5.4.4. Benthic algae

Biomass

Algal biomass (using weight collected via the lens tissue method as a proxy) was found to be greater in September 2014 in the middle than in the lower section of the tidal frame. Additionally, during the field sampling a very obvious dark brown biofilm was observed across the mudflat at the level of the mid tidal frame on this date.

A range of physical factors including sediment particle size (MacIntyre et al., 1996) and emersion time (Orvain et al., 2012) control MPB distribution on the mudflat. In the lower tidal frame higher energy (which increases sediment resuspension), larger sediment particle size and shorter emersion times (which causes light limitation beneath turbid water) are likely to reduce MPB growth. In the higher tidal frame of the mudflat, although the emersion times are increased and energy is lower, microscopic algae growth might be limited due to the increased surface temperature and drying out of the sediment surface (Guarini et al., 1997). For these reasons and as observed in this study, peak MPB biomass is often in the mid tidal frame (Underwood and Kromkamp, 1999) although this is not without exception being site dependant (Jesus et al., 2009).

Whilst, anecdotally, higher diatom biomass was visually observed in the middle of the tidal frame in September 2014 compared to April 2015, evidence for a seasonal difference in diatom biomass in this study is limited. A higher biomass was not confirmed by the mass of diatoms collected in the field, however, the mass collected in the laboratory from sediment from the middle of the tidal frame in September was much greater than in April (Figs. 5.14 and 5.15). This discrepancy between the quantities of algae collected by the two methods (laboratory and field) was possibly due to the time available for migration and is discussed further below. A seasonal variation which resulted in biomass peaks in late spring and summer was observed by Montani et al. (2003), whilst Uncles et al. (2003) reported a pronounced variation in sediment chlorophyll *a* and EPS due to the presence of diatoms in late summer and early autumn (similar to what was observed in the current study), other studies have reported a lack of an obvious seasonal pattern at muddy sites (Jesus et al., 2009).

Collection method

Diatoms were collected from the sediment by two methods, in the laboratory and in the field. In the field lens tissue and gauze was placed on the sediment surface for an hour before removal and washing of diatoms from the surface. In the laboratory collected sediment was spread onto a plastic tray, before covering with layers of lens tissue and gauze in the same manner on site.

For laboratory samples lens tissue and gauze was left in situ for approximately 15 hours. See sections 5.2.1 and 5.2.2 for further details.

It should be acknowledged that there are potential problems with the lens tissue method. Cook et al. (2004) found that the cyanobacteria component became enriched relative to diatoms and green algae when this sampling method was used. It has also been found that the lens tissue method preferentially collects large motile epipelic diatoms and does not reflect the true species diversity (Ribeiro et al., 2012). Additionally, it must be recognised that the number of diatoms collected will depend on both the biomass of diatoms present and their migration strategy which, although it is controlled by light intensity, can vary with species (Paterson, 1986). However, for the purposes of this study, it is the comparison of the diatom communities that were present on the lens tissue at the different sites that is of interest, making the issues mentioned above less important.

Successful harvesting of epipelic diatoms is dependent on weather conditions. Whilst the pattern of diatom migration is commonly understood to be upward to the sediment surface as the tide recedes in the daytime, they may also be affected by weather conditions such as light intensity and rainfall (Consalvey et al., 2004). Whilst the weather was sunny with very light winds on both sampling occasions for this study, this might be considered lucky. Either rain or high winds could have distorted results with the possibility that diatoms would not migrate in sufficient quantities for analysis or that gauze would be contaminated with sediment. It is also feasible that high winds would make field collection by this method impossible. Additionally, it was also observed during fieldwork at the Lytham site in May 2014 that high light levels can also impede diatom sampling. Following removal of the gauze, lens tissue and plastic windshield used to sample diatoms in the field (Fig.5.5) a dark brown line was observed around the outside of the sampling area where the windshield had cast a shadow (Fig. 5.34). This indicated that, under the high light intensity, diatoms had migrated into the shadow rather than the light, probably to minimise photo-inhibition (Paterson et al., 2003).

Whilst unable to account for sample enrichment by cyanobacteria or larger diatom species, the potential to separate diatoms from the sediment under laboratory conditions would allow for migration over a longer time period under controlled light conditions so that optimum quantities could be collected.

It has been shown that collection of diatoms by the laboratory method can yield a higher quantity of diatoms (Fig. 5.14) and there is less chance of contamination due to wind-blown sediment or splashing of sediment onto the gauze surface. However, comparison of metal concentrations in the field and laboratory collected diatoms revealed a disparity in concentrations of Al, V, Fe and Sn, with higher concentrations in diatoms separated from sediment in the field (Figs. 5.16 to 5.19), whilst other analytes showed similar concentrations in

diatoms collected by either method. Change in uptake may be due to an alteration of the sediment properties, e.g. a change in redox potential, due to increased oxygen content in collecting and moving the sediment. Diatoms have been shown to take up available sediment contaminants quickly (within three hours, Chapter 3) and respond to changing sediment conditions (e.g. sediment disturbance, Chapter 4).

Whilst the laboratory separation method has several advantages over field collection, it would need to be further tested and refined if it was to be used in the future. At this stage it would be recommended that diatom collection for analysis of metal uptake be carried out in the field. However, if on arrival at field sites, weather conditions were found to be detrimental to sample collection (e.g. rain or high winds) the laboratory method could be utilised to avoid wasted effort. Sampling and separation in the laboratory remains useful for studies which are looking at species composition.

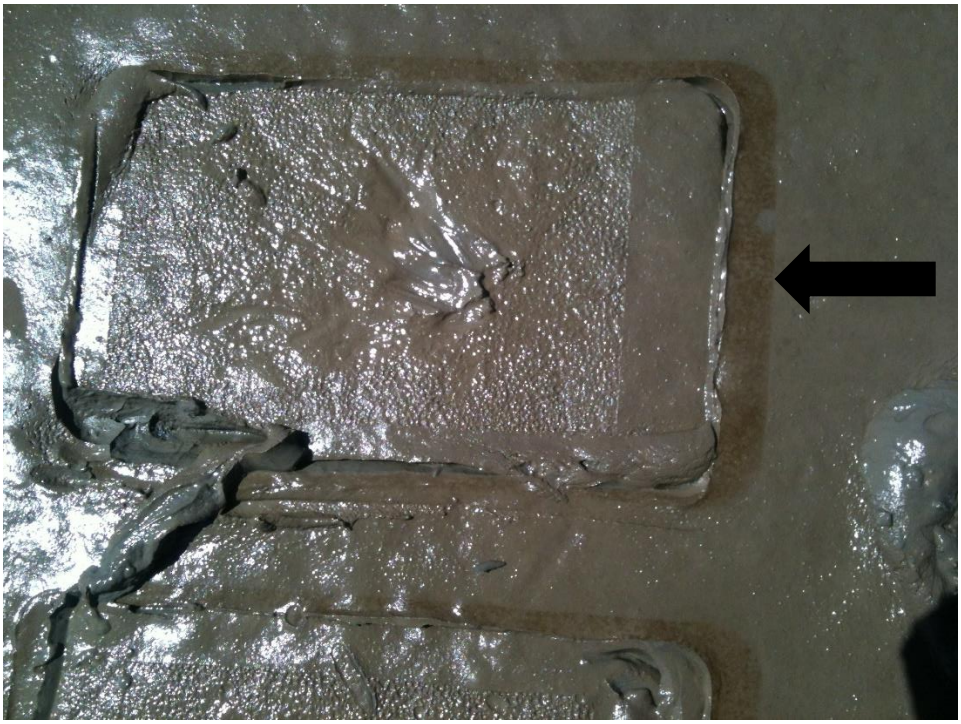


Figure 5.34. Diatom migration into shadow of plastic windshield during fieldwork in May 2014

Metal content

The only analyte for which no bioaccumulation was observed in this study on either sampling date was Fe. Diatom Al was in equilibrium with sediment in April 2015 with a BCF of 1.0. Bioconcentration of other analytes ranged from low (BCF of 1.1 for Mn and Co in April 2015) to high (BCF of 53 for Cd in September 2014) (Table 5.6).

Cd had low concentrations in sediment (less than 0.75 kg mg^{-1}) and concentrations in overlying water were below the LoD, but a comparatively high concentration was observed in diatom samples. This tallies with results from Chapter 3 (Table 3.3) in which BCF values from contaminated sediment ranged from 3.0 to 71.

Contrary to results found here, Stronkhorst et al. (1994), in a study on the Westerschelde estuary, found no bioconcentration of Cd, Cr or Zn in diatom samples and only limited bioconcentration of Cu. This was despite sediment concentrations of all these metals being higher in that study than the current one. It was concluded by Stronkhorst et al. (1994) that low bioconcentration was due to either low bioavailability of contaminants or the fast growth and short life of benthic algae which prevented equilibrium with pore water. The first of these is more likely as concentrations in benthic diatoms were reported as being in a similar range to concentrations in phytoplankton at the same location. It is well known from studies of planktonic algae and it has been shown here, that the growth and lifespan of diatoms do not prohibit uptake of metals and other contaminants.

A second study of metal uptake by benthic diatoms (Absil and van Scheppingen, 1996) reported BCFs for Cd, Pb, Cu, and Zn from eleven sites in the Westerschelde and Oosterschelde estuaries in Belgium and the Netherlands. In this case BCF values reported were much closer to those seen in this study, particularly in the Oosterschelde estuary with BCF ranges for Cd, Pb, Cu, and Zn of 1.4-21.7, 0.09-1.71, 1.4-14.9 and 1.1-4.4 respectively. Additionally, BCF values reported for the Westerschelde by Absil and van Scheppingen (1996) were in line with those reported by Stronkhorst (1994) supporting the theory of lower bioavailability at that location.

Concentrations of most analytes in diatoms grown in the mesocosm (Chapter 4, section 4.3.5) were similar to those observed in the field. Variations to this observation are Cr, As and Ag, which were all higher in diatoms from the mesocosm and Ni which had lower concentrations in mesocosm collected diatoms than those from the field. As the sediment used in the mesocosm study was collected from the field study site at Lytham St. Anne's, the similarity of results validate the use of the mesocosm for future studies into response of benthic algae to sediment contaminants.

In field samples metal concentrations internal to the algal cell were often similar to total concentrations (internal plus adsorbed metals) and were sometimes measured as higher.

Although in Chapter 3 internal Cd concentrations were shown to be greater than external (Section 3.3.7, Fig. 3.12), higher internal than total concentrations are not feasible. It is thought that this may have occurred due to the additional sample handling required to investigate internal concentrations.

Prior to normalisation of sediment analytes to organic matter, it appeared that, for metals where sediment concentration was related to diatom concertation (Al, V, Fe, Co, Zn and Pb), as sediment concentration of analytes increased, concentration in diatoms was reduced. There was an effect of date on concentration of metals in diatoms, with lower concentrations in April 2015. There was no effect of tidal frame height.

Higher metal concentrations in diatoms in September may be due to sediment pH. Sediment pH (Figs. 5.9 and 5.10) was higher in March and May 2014 than September 2014 and (although there were no data available for April 2015) this may indicate a seasonal variation. Araujo et al. (2008) observed seasonal pH variation at the Cávado estuary, Portugal, where pH values were also lower in winter months. It is possible that pH would have affected the bioavailability of metals (Burton Jr., 2010) as the sorption capacity of clay minerals increases with increasing pH and decreasing pH causes release of metals to pore water (Zhang et al., 2014).

The relationships found between sediment (prior to normalisation to organic matter content) and diatom concentrations were negative, i.e. as the metal concentration in the sediment increased, diatom concentrations decreased. This apparent negative relationship was previously reported by Absil and van Scheppingen (1996), although they did not speculate on possible causes. In light of the negative relationship observed in the current study, it is postulated that, due to the observed (Fig. 5.8) and previously documented influence of sediment organic matter on sediment metals concentration (Ridgway et al., 2001), this is an effect of sediment organic matter on both the sediment and diatom metal concentration. Organic matter increases sediment metal concentration and decreases uptake to benthic algae. This hypothesis is complicated by the fact that the benthic algae contributes to sediment organic matter directly through the presence of algal cells and indirectly through the production of EPS. In this study, where biofilms were observed (in this instance in the centre of the tidal frame in September 2014) percentage sediment organic matter and sediment metals both increased. This hypothesis therefore requires further testing, but could account for the observation. It must also be acknowledged that this relationship may not always hold true, McCormick et al. (2014) reported that although locations with an obvious biofilm had a higher percentage organic matter there was no increase in total metal concentrations.

In addition to observations in the field, it was previously observed (Chapter 3, section 3.3.5) that sediment with a higher organic matter content had a higher Cd concentration and that

uptake to *C. closterium* was reduced. This is despite the lower affinity of Cd to organic matter than other metals (Davies-Colley et al. (1984) and as shown above in Table 5.2 and Fig. 5.12).

It is proposed that diatoms may be protected from metal uptake in part by their self-created biofilm habitat. EPS excreted by diatoms has strong metal binding properties (Schlekat et al., 1998, Bhaskar and Bhosle, 2005) and contributes to the organic matter content of the sediment (Decho, 2000). It is therefore possible that EPS could account (at least in part) for the correlation between metal concentration and organic matter in the sediment (Fig. 5.12). This might also account for lower concentrations of metals in diatoms associated with these high organic matter, high metal concentration locations in that metals remained strongly bound within the sediment by the EPS and were therefore not taken up by the algae. Studies have shown that EPS offers a protective role to planktonic algae, for example a recent study demonstrated that the presence of EPS reduces internal concentrations of Ag nanoparticles in *Chlorella pyrenoidosa* (Zhou et al., 2016).

This study has shown that bioavailability of metals was influenced by both physio-chemical conditions and biotic factors. It is necessary to minimize the differences due to the bulk sediment characteristics, which was achieved here through normalisation of sediment metals to percentage organic matter with which sediment metal concentrations were shown to be highly correlated (Fig. 5.12 and Table 5.2). The use of normalisation broke the correlation which existed between certain sediment analytes and organic matter.

Following normalisation most of the metals (Al, Mn, Co, Zn, Ag and Pb) which previously showed a relationship between sampling date and concentration in diatoms (with higher concentrations in September 2014) continued to do so. The exceptions to this were Cu and Sn. Additionally, the metals which previously showed a relationship between sediment concentration and diatom concentration (V, Fe, Co and Pb) now showed a relationship between diatom concentration and tidal frame position. Exceptions here were Al and Zn. Ag, for which diatom concentrations were not previously correlated with sediment concentrations, also showed a relationship between position in the tidal frame and concentration in algae. There were no longer any relationships, positive or negative, observed between sediment metal concentrations and concentrations in diatoms. This may point to a low variability in bioavailable metals across the site and that uptake to benthic diatoms was controlled by other factors at play across the tidal frame. These were perhaps caused by differences in emersion time which could affect availability of metals to algae due to changes in redox conditions or concentrations of metals in overlying water.

Alternatively, variation in uptake by algae across the tidal frame may be affected by sediment disturbance levels. Sediment disturbance was shown to increase uptake of Al, V, Fe, Pb and Sn (Chapter 4, sections 4.3.5 and 4.4.4). Metal concentrations in diatoms were often greater in the

lower part of the tidal frame (Figs. 5.30 to 5.30) where disturbance was greatest due to higher energy in this part of the system. Higher energy results in larger sediment particles and reduced algae biomass. A reduced presence of epipelic diatoms could contribute to higher incidence of sediment resuspension (disturbance). Epipelic diatoms are implicated in sediment stabilisation through the production of EPS and biofilm formation which has cohesive properties (Underwood and Paterson, 2003, Stal, 2010). A lower biomass of epipelic diatoms could have resulted in greater disturbance releasing metals to the water column for uptake by the algae that are present.

5.5. Conclusion

It has been shown that sediment metal concentration varies with position in the tidal frame and with date. It is thought that this is related to particle size and organic matter content, both of which also vary with tidal frame height. Sediment organic matter is perhaps the more important of these relationships as it also varied with date and was shown to be highly correlated with sediment metal concentrations.

The concentration of some metals in diatoms were shown to be related to tidal frame position (V, Fe, Co, Ag and Pb) and others were related to sampling date (Al, Mn, Co, Zn Ag and Pb), which may indicate seasonality. Reasons for differences with height in the tidal frame and date are not fully understood and require further exploration, during which measurement of parameters such as redox potential and pore water salinity would be recommended.

Although diatom metal concentration varied due to the same parameters (tidal frame height and date) as sediment metal concentrations, there was initially a negative relationship between sediment and diatom concentrations of some metals (Al, V, Fe, Co, Zn and Pb). However, following normalisation of sediment metal concentrations to organic matter content no relationships between diatom and sediment concentrations were identified. It was postulated that this was due to some effect of percentage sediment organic matter on both sediment metal concentration and bioavailability, which may be mediated by EPS production by diatoms and would warrant further investigation. The use of sequential extraction techniques might be useful in clarifying these relationships.

There was only one relationship, with As, identified between metal concentrations in pore water and in diatoms. This may be due to extraction techniques which can cause changes in partitioning between sediment and pore water and should be investigated further.

Changes in diatom metal concentration with date could indicate some seasonality, but this would need to be further studied to identify whether either algae biomass or metal uptake are truly seasonal. If this is shown to be the case there could be some environmental impact, for

instance if concentrations in diatoms were higher in winter thereby coinciding with increased bird numbers feeding on the mudflat.

Although diatoms were successfully separated from sediment in the laboratory and in larger quantities than in the field, metal concentrations in diatoms were not always equivalent, probably due to a change in bioavailability and the quick response of diatoms to these changes. These quick responses were identified through comparisons of concentration factors (CFs) and BCFs of Cd in Chapter 3. This laboratory method of diatom collection may still be preferable to lost resource due to unsuitable collection conditions (e.g. high winds or rain) in the field and should be investigated further.

The mesocosm (Chapter 5) produced similar results, in terms of sediment and diatom concentrations, for most metals to those observed in the field and has therefore been shown to be a useful resource for manipulative/semi-controlled studies in the future.

The laboratory study (Chapter 3) was also verified to some extent by the field observations. Although Cd concentrations in field sediment were much lower than those used in the laboratory the BCFs for Cd were similar to those observed in the laboratory.

6. Conclusion

The research described aimed to gain an understanding of metal uptake to and from sediment and the role that the major primary producer of the intertidal mudflat, epipelic diatoms, plays in these processes. In part this work is driven by the potential impact of climate change on the diatoms, their biological processes and sediment (re)distribution (through for example the expected sea level rise and increased storminess). Sediment is known to be a sink for metal contaminants and the close affinity of metals to fine grained silts, clays and organic matter means that any (re)distribution of these components of the sediment in the estuaries may lead to increased bioavailability and new exposure pathways to wildlife and potentially humans. Consequently, there are likely to be changes to uptake, transfer and effects of metal contaminants. However, prior to this work, there was a paucity of research into uptake of metals from sediment to epipelic diatoms. The research here has shown that:

- diatoms mediate the partitioning of cadmium (Cd) (as an example metal) between sediment and water
- diatoms take up greater quantities of most metals than benthic macroalgae
- metal uptake to diatoms was mediated by sediment properties
- sediment disturbance can increase the uptake of metals to diatoms
- uptake of metals is affected by position on the mudflat/within the tidal frame

The following sections describe these overall findings in more detail but starts with a brief statement of prior knowledge about metal contamination, diatoms and their interactions.

Metal contaminants

As shown earlier, estuarine sediments, are a sink for contaminants derived from fluvial, marine and atmospheric sources. These include polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorines, metals (including radionuclides) etc., from multiple sources including waste disposal, farming and the chemical, nuclear, manufacturing and extractive industries. The metal contaminants were the focus for this study because they are naturally ubiquitous in aquatic systems and whilst many (e.g. zinc (Zn), copper (Cu)), are essential micronutrients, but are toxic at high concentrations, others (e.g. lead (Pb)) serve no biological function and can be toxic at low levels.

Whilst metal releases to estuaries have declined in recent years due to increased regulation and declining industrial activity, metals previously discharged and which are now locked up in saltmarsh sediments remain a concern. For example, there are indications that saltmarshes are already being eroded, due to climate change related sea level rise, in some locations (Beldowska et al., 2016). This erosion may result in the redistribution of historically contaminated sediment to locations, such as the mudflats, where it is more bioavailable to biota, such as the MPB. In addition to causing redistribution, climate change effects, such as increasing temperatures and storminess, may also alter the bioavailability of metals to MPB. Increased concentrations of metals within the MPB could potentially increase their transfer to higher organisms through the food chain with potential impacts for biota.

Whilst planktonic algae have been well studied with respect to metal uptake from the water column, there has been little research involving MPB and uptake of metals from sediment. The extent to which contaminant uptake by microphytobenthic algae occurs and under what conditions is therefore poorly understood.

Microphytobenthos

In chapters 1 and 2, it was shown that Microphytobenthos (MPB) is a mixed community of microscopic algae and bacteria, which inhabit the top few millimetres of bottom sediment, is a key component of intertidal mudflats. The literature indicates that MPB contributes to a significant proportion of estuarine primary production, forms the base of the food chain and has influences on sediment distribution and resuspension (for example, through production of extracellular polymeric substances (EPS)). EPS is a mucilage (composed mainly of sugars) excreted epipelic diatoms, other algae and bacteria. EPS is produced to help protect diatoms from desiccation and to promote mobility. MPB is visible as a biofilm which darkens the surface of the sediment (Fig 5.34) and can stabilise it although under high disturbance events the MPB 'mat' can be peeled off. Under these circumstances there can be more erosion than if the MPB had not been there.

The importance of the MPB as the basis of the food chain is illustrated by the fact that it has been estimated to supply up to 45% of the organic resources of an estuary (Meleder et al., 2010) supplementing the planktonic resource when resuspended due to tidal currents and wind action. As a food source to deposit feeders however, the importance of MPB is difficult to disentangle from the detritus of salt marsh and submerged aquatic vegetation, but it is thought that MPB may be preferred as it is nutritionally richer. MPB is consumed by deposit feeders (e.g. benthic infauna and detritivorous fish), suspension feeders (e.g. mussels and pelagic fish) and shorebirds.

Diatoms mediate the partitioning of cadmium (as an example metal) between sediment and water

Chapter 3 describes a laboratory experiment, conducted in a controlled environment facility, in which a single species of diatom, *Cylindrotheca closterium*, was exposed to Cd contaminated sediment and water. The results show that diatoms influenced metal partitioning between sediment and water.

The presence of diatoms slowed the transfer of Cd from the overlying water to the sediment and, in what appears to be a two stage process, the Cd was taken up first by the algae and then transferred to the sediment. Where sediment was initially contaminated the presence of diatoms increased the concentration of Cd in both the overlying and pore water.

These findings are important to our understanding of metal bioavailability in the estuarine system. Diatoms at the sediment surface will quickly sequester metal contaminants from the water with the potential that these will be passed up the food chain. Secondly, by increasing metal concentrations in the pore and overlying water diatoms may increase metal bioavailability to other sediment organisms.

Metal uptake to diatoms was mediated by sediment properties

The laboratory study described in chapter 3, which confirmed uptake of a metal (Cd) from sediment to diatoms, also showed that this was mediated by the sediment properties of particle size and organic matter content. This finding highlights the importance of their control on metal bioavailability and indicates that as well as metal concentration, it is important to consider other sediment properties when assessing the potential for uptake by algae.

Diatoms take up greater quantities of most metals than benthic macroalgae

In the experiment in an intertidal mesocosm, which is described in chapter 4, it was possible to increase the realism of the study system through the introduction of a tide and natural light levels, whilst controlling water temperature and levels of sediment disturbance (to mimic the sea water temperature and increased storminess predicted due to climate change). In this system it was also possible to increase the complexity by studying a natural MPB community as opposed to a single species.

The results show that although there was uptake of metals from the sediment to all the types of algae present (diatoms and filamentous and sheet macroalgae), the diatoms had higher concentrations of all the metals analysed, except tin (Sn). It is speculated that the higher

uptake of metals by diatoms is due to their smaller size (and consequentially larger surface to volume ratio) and closer association with the sediment than other algae. This finding highlights the importance of diatoms in mobilising sediment contaminants up the food chain.

Sediment disturbance can increase the uptake of metals to diatoms

The study in the mesocosm also showed that there was an increase in the uptake of some metals (iron, aluminium, vanadium and lead) to diatoms when the sediment was disturbed. This is important as predicted intensification in storminess could increase sediment disturbance and therefore potentially uptake of metals by diatoms.

There was also an indication that there may be some combined effect of temperature and disturbance as uptake of some metals (arsenic, manganese and Sn) decreases with increasing temperature during the disturbance phase of the experiment. This highlights the need to include multiple stressors in investigations of metal uptake as there are the potential for synergistic and/or antagonistic effects.

Uptake of metals is affected by position on the mudflat/within the tidal frame

A field study (chapter 5) carried out on the mudflats at Lytham St. Anne's in September 2014 and April 2015 sampled diatoms and sediment from three heights in the tidal frame. The tidal frame was identified as a variable because it can influence sediment organic matter and particle size distribution spatially as well as emersion/immersion time and was thought to influence metal concentration and bioavailability as well as diatom biomass.

The study showed that there was variation in metal uptake to diatoms in different positions in the tidal frame, with lower metal uptake from the middle of the tidal frame where organic matter content was greatest. Whilst the tidal frame can also act as a proxy for particle size there was no difference in percentage clay particles with tidal frame position, therefore it was concluded that the effect was most likely due to organic matter.

There was a difference in uptake of some metals with sampling date with higher concentrations observed in September indicating possible seasonal variation, which may be linked to changes in pH as this was correspondingly lower. Factors such as salinity and redox potential might also have influenced the metal concentrations with sampling date but were not examined here.

Laboratory and mesocosm v field studies

Overall the work here has demonstrated the usefulness of laboratory and mesocosm studies for research into MPB and its influence on metal contaminants in estuarine intertidal sediments. The laboratory and mesocosm studies both produced similar outcomes to those observed in the field but under more controlled and easily manipulated conditions. Field studies will of course continue to be vital in improving understanding of metals availability and transfer under natural conditions, however for improving understanding of the mechanisms underpinning these processes controlled experiments to isolate specific factors and investigate them are needed. The fundamental problem, which is perhaps the reason for the paucity of literature on this subject, remains our ability to isolate the different component parts (e.g. diatoms, cyanobacteria, euglenoids and dianoflagellates) of the MPB from sediment samples. This is likely to be easier to do under controlled conditions. The subproject within chapter 5, where the possible separation of algae from sediment collected in the field, showed that it was possible to extract algae successfully and these samples could be used to investigate species composition, community structure and so on in the future. However, as noted in chapter 5, there were potential changes in the metal speciation, perhaps as a result of redox changes, that affected the metal concentrations measured in the diatoms.

Recommendations for future work

There is scope for further research with metals and to extend the work described here to other contaminants of concern. The key areas for further investigation arising from this research are to:

- better understand diatom-mediated changes to parameters such as pH and redox potential in the surface sediment to explore the influence of these on the availability of metals to the diatoms and potentially other biota
- establish the relative contribution of the diatoms within the MPB, the MPB itself and EPS to the organic matter content of sediment because the results in this project showed the strong influence of organic matter on sediment metal concentrations and the uptake by diatoms
- identify the differences in the diatom community structures found in sediments with differing levels of disturbance. The mesocosm disturbance experiment clearly showed the impact on macroalgae and it is postulated that there may be similar, but more difficult to observe, changes in the diatom community. Similarly changes to diatom community structures under the predicted temperature increases should be considered. Given the changes in metals in the diatoms present in the mesocosm under varying

temperature and disturbance levels, it is anticipated that the diatom community structure will change given that diatom species are known to differ in their sensitivities to metal toxicity

- translate the findings of this study to the wider landscape scale using remote sensing techniques to better understand MPB suspension and redistribution due to tides

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Appendix 3-1. f/2 Medium plus sodium metasilicate (f/2+Si) (Guillard and Ryther, 1962)

Make up five stock solutions in Milli-Q® water as follows:

1. Nitrate stock solution

Add 37.5g $NaNO_3$ to 250ml Milli-Q® water in a 500ml volumetric flask (if difficult to dissolve, swirl flask under hot water). Once dissolved fill to 500ml with Milli-Q® water. Insert stopper and mix by inversion and shaking.

2. Phosphate stock solution

Dissolve 2.5g $NaH_2PO_4.H_2O$ in 250ml Milli-Q® water in a 500ml volumetric flask. Once dissolved fill to 500ml with Milli-Q® water.

3. Trace metals stock solution

Make up 100 ml of each of the following in Milli-Q® water

- $CuSO_4.5H_2O$ 0.98g
- $Na_2MoO_4.2H_2O$ 0.63g
- $CoCl_2.6H_2O$ 1g

Make up 50 mL of each of the following in Milli-Q® water

- $MnCl_2.4H_2O$ 9g
- $ZnSO_4.7H_2O$ 1.1g

Dissolve 1.575g $FeCl_3.6H_2O$ and 2.18g $Na_2EDTA.2H_2O$ in 250 ml Milli-Q® water and add 0.5ml of each of the trace metal solutions. Make up to 500 ml with Milli-Q® water and mix thoroughly.

4. Silicate stock solution

15g $Na_2SiO_3.9H_2O$ is dissolved in 250 ml Milli-Q® water, then made up to 500ml and mixed.

Autoclave all the nitrate, phosphate and trace metal stock and ~5ml of the silicate stock at 121°C and 105kPa for 20 minutes.

5. Vitamin stock solution

- Add 100mg biotin to 50ml Milli-Q® water and make up to 100ml.
- Add 100mg cyanocobalamin to 50ml Milli-Q® water and make up to 100ml.
- Dissolve 50mg thiamine HCl in 200ml Milli-Q® water

Add 0.25ml of each of the vitamin solutions to 150ml Milli-Q® water and make up to 250ml. Then filter sterilize into a sterilized bottle and store in the refrigerator for up to 3 months or decant into sterile 15ml centrifuge tubes which can then be stored frozen.

Finally, make up 1L artificial seawater using Tropic Marin® synthetic sea salt in Milli-Q® water to salinity of 22 and add 1ml of each stock solution except the vitamins. Check pH and adjust to 8.0 with 1M NaOH or HCl if necessary. Autoclave at 121°C and 105kPa for 20 minutes. When solutions are cool and pH has returned to normal (1-2 days) add 0.5ml vitamin stock.

To make a "light" media (i.e. excluding EDTA for experiment purposes) omit the trace metals stock.

Stock solutions should be stored refrigerated at 4°C in bottles covered with aluminium foil to exclude light.

Appendix 3-2. Example R Model Output

candidate model (lmer) to investigate effect of time and sediment type on Cd uptake to *C. closterium*

DTsed2<-lmer(log(CdPPM)~Time+Sediment+(1|Run),data=DSCon1, REML=TRUE, verbose=FALSE)

Linear mixed model fit by REML t-tests use Satterthwaite approximations to degrees of freedom [lmerMod]

Formula: log(CdPPM) ~ Time + Sediment + (1 | Run)
Data: DSCon1

REML criterion at convergence: 24.4

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.49319	-0.63752	-0.00659	0.71446	1.45871

Random effects:

Groups	Name	Variance	Std. Dev.
Run	(Intercept)	0.2322	0.4818
Residual		0.1465	0.3828

Number of obs: 18, groups: Run, 3

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	5.5214	0.3316	3.2610	16.651	0.000288 ***
Timetwenty four	0.1672	0.2210	12.0000	0.756	0.463993
Timeninety six	-0.2847	0.2210	12.0000	-1.288	0.221938
SedimentUnprocessed	-1.2531	0.1804	12.0000	-6.945	1.55e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:

	(Intr)	Tmtwnf	Tmnnts
Timetwntyfr	-0.333		
Timenintysx	-0.333	0.500	
SdmntUnprcs	-0.272	0.000	0.000

#Calculation of p and chi-sq value for effect of time

Data: DSCon1

Models:

..1: log(CdPPM) ~ Sediment + (1 | Run)

object: log(CdPPM) ~ Time + Sediment + (1 | Run)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	4	31.574	35.136	-11.7872	23.574			
object	6	31.004	36.346	-9.5018	19.004	4.5709	2	0.1017

#Calculation of p and chi-sq value for effect of sediment type

Data: DSCon1

Models:

..1: log(CdPPM) ~ (1 | Run)

object: log(CdPPM) ~ Sediment + (1 | Run)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	3	50.231	52.902	-22.115	44.231			
object	4	31.574	35.136	-11.787	23.574	20.656	1	5.496e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix 4-1. Arduino code for mesocosm temperature controllers

```
//File: DataLogger.ino
// Amani 19/08/2015

//Use a voltage divider to indicate electrical resistance of a thermometer
//Measurements and conversions are managed via objects
//Include time and date stamp
//Save to SD card

#include <Thermistor.h>
#include <SD.h>
#include <SPI.h>
#include <Wire.h>
#include "RTClib.h"

#define LOG_INTERVAL 5000

//temp is a thermistor object
Thermistor temp0(0);
Thermistor temp1(1);
Thermistor temp2(2);

// for the data logging shield, we use digital pin 10 for the SD cs line
const int chipSelect = 10;

//the logging file
File logFile;

//the clock
RTC_DS1307 rtc;

void setup() {
```

```
 Serial.begin(9600);

// initialize RTC
  Wire.begin();
  rtc.begin();

  if (! rtc.isrunning()) {
  Serial.println("RTC is NOT running!");
  }

//initialize the SD card
Serial.println("Initializing the SD card...");
pinMode(10, OUTPUT);

// see if the card is present and can be initialized:
if (!SD.begin(chipSelect)) {
Serial.println("Card failed, or not present");
// don't do anything more:
return;
}
Serial.println("card initialized.");

// create a new file
char filename[] = "LOGGER00.CSV";
for (uint8_t i = 0; i < 100; i++) {
filename[6] = i/10 + '0';
filename[7] = i%10 + '0';
if (! SD.exists(filename)) {
// only open a new file if it doesn't exist
logFile = SD.open(filename, FILE_WRITE);
break; // leave the loop!
}
}
```

```
if(logFile){
Serial.print("Logging to:");
Serial.println(filename);
logFile.println("millis,Date,Time,T1(C),T2(C),T3(C)");
}

Serial.println("millis  Date      Time  T1(C)  T2(C)  T3(C)");

 temp0.fixedResistance(2200);
 temp1.fixedResistance(2200);
 temp2.fixedResistance(2200);
 temp0.coefficients(1.498872e-03, 2.379047e-04, 1.066953e-07);
 temp1.coefficients(1.498872e-03, 2.379047e-04, 1.066953e-07);
 temp2.coefficients(1.498872e-03, 2.379047e-04, 1.066953e-07);


 for(int pinNumber = 2; pinNumber<5; pinNumber++){  //set up output pins
 pinMode(pinNumber, OUTPUT);                        //the "for" loop sets up all the pins
 digitalWrite(pinNumber, LOW);
 }
}

//loop is repeated indefinately
void loop() {

// log milliseconds since starting
uint32_t m = millis();
logFile.print(m); //milliseconds since start
logFile.print(",");
Serial.print(m);
Serial.print("  ");
```

```
//log time
   DateTime now = rtc.now();


  Serial.print(now.day(), DEC);
  Serial.print('/');
  Serial.print(now.month(), DEC);
  Serial.print('/');
  Serial.print(now.year(), DEC);
  Serial.print(' ');
  Serial.print(now.hour(), DEC);
  Serial.print(':');
  Serial.print(now.minute(), DEC);
  Serial.print(':');
  Serial.print(now.second(), DEC);
  Serial.print("  ");


    logFile.print(now.day(), DEC);
    logFile.print('/');
    logFile.print(now.month(), DEC);
    logFile.print('/');
    logFile.print(now.year(), DEC);
    logFile.print(',');
    logFile.print(now.hour(), DEC);
    logFile.print(':');
    logFile.print(now.minute(), DEC);
    logFile.print(':');
    logFile.print(now.second(), DEC);
    logFile.print(",");

double temperature0 = temp0.getTemp();
double temperature1 = temp1.getTemp();
double temperature2 = temp2.getTemp();
```

```
Serial.print(temperature0); Serial.print("  ");
Serial.print(temperature1); Serial.print("  ");
Serial.println(temperature2);



logFile.print(temperature0); logFile.print(",");
logFile.print(temperature1); logFile.print(",");
logFile.println(temperature2);



if ((temperature1-temperature0)<1.5){ //if temperature to sensor 2 less than 1.5 degrees more than sensor 1
  digitalWrite(2, HIGH);             //switch heater on
}
else if ((temperature1-temperature0)>=1.5){ //if temperature to sensor 2 is 1.5 degrees or more than sensor 1
  digitalWrite(2, LOW);                 //switch heater off
}
if ((temperature2-temperature0)<4){ //if temperature to sensor 3 less than 4 degrees more than sensor 1
  digitalWrite(3, HIGH);             //switch heater on
}
else if ((temperature2-temperature0)>=4){ //if temperature to sensor 3 is 4 degrees  or more than to sensor 1
  digitalWrite(3, LOW);                 //switch heater off
}


delay(5000);   //delay all readings by 5 seconds



logFile.flush();



}
```

Appendix 4-2. Example R Model Output

candidate model (lmer) to investigate effect of sediment depth, percentage organic matter, temperature and disturbance treatments on Mn concentration in the sediment at sampling point D01

MnD01a<-lmer(data=D01Sed, Mn~Level+Organic+Temperature+Disturbance+(1|Tank))

Linear mixed model fit by REML t-tests use Satterthwaite approximations to degrees of freedom [lmerMod]

Formula: Mn ~ Level + Organic + Temperature + Disturbance + (1 | Tank)

Data: D01Sed

REML criterion at convergence: 472.8

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.91631	-0.64573	-0.08723	0.67501	2.83322

Random effects:

Groups	Name	Variance	Std. Dev.
Run	(Intercept)	112.0	10.58
Residual		882.7	29.71

Number of obs: 54, groups: Tank, 9

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	403.880	47.305	44.470	8.538	6.33e-11 ***
LevelLower	-142.658	11.433	46.040	-12.478	2.22e-16 ***
Organic	37.225	15.779	46.100	2.359	0.0226 *
Temperature1.5	19.816	13.153	4.650	1.507	0.1965
Temperature4	2.124	13.281	4.810	0.160	0.8794
Disturbancetwice	-22.315	9.946	39.860	-2.244	0.0305 *
Disturbancethree	-23.267	9.929	39.810	-2.343	0.0242 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:

	(Intr)	LvlLwr	Organc	Tmp1.5	Tmprt4	Dstrbnctw
LevelLower	-0.746					
Organic	-0.969	0.707				
Tempertr1.5	-0.176	0.027	0.038			
Temperatur4	-0.276	0.101	0.143	0.500		
Distrbnctwc	-0.015	-0.065	-0.093	-0.004	-0.013	
Distrbncthr	-0.175	0.051	0.072	0.003	0.010	0.490

#Calculation of p and chi-sq value for effect of disturbance

Data: D01Sed

Models:
object: Mn ~ Level + Organic + Temperature + (1 | Tank)
..1: Mn ~ Level + Organic + Temperature + Disturbance + (1 | Tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
object	7	537.57	551.50	-261.79	523.57			
..1	9	534.54	552.44	-258.27	516.54	7.0304	2	0.02974 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

#Calculation of p and chi-sq value for effect of temperature

Data: D01Sed
Models:
..1: Mn ~ Level + Organic + (1 | Tank)
object: Mn ~ Level + Organic + Temperature + (1 | Tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	5	537.29	547.24	-263.64	527.29			
object	7	537.57	551.50	-261.79	523.57	3.7179	2	0.1558

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

#Calculation of p and chi-sq value for effect of percentage organic matter

Data: D01Sed
Models:
object: Mn ~ Level + (1 | Tank)
..1: Mn ~ Level + Organic + (1 | Tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
object	4	540.33	548.29	-266.17	532.33			
..1	5	537.29	547.24	-263.64	527.29	5.0386	1	0.02479 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Calculation of p and chi-sq value for effect of sediment depth

Data: D01Sed
Models:
..1: Mn ~ 1 + (1 | Tank)
object: Mn ~ Level + (1 | Tank)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	3	642.73	648.70	-318.37	636.73			
object	4	540.33	548.29	-266.16	532.33	104.4	1	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4-3. Temperatures in air, sediment and water in the week prior to each sampling point

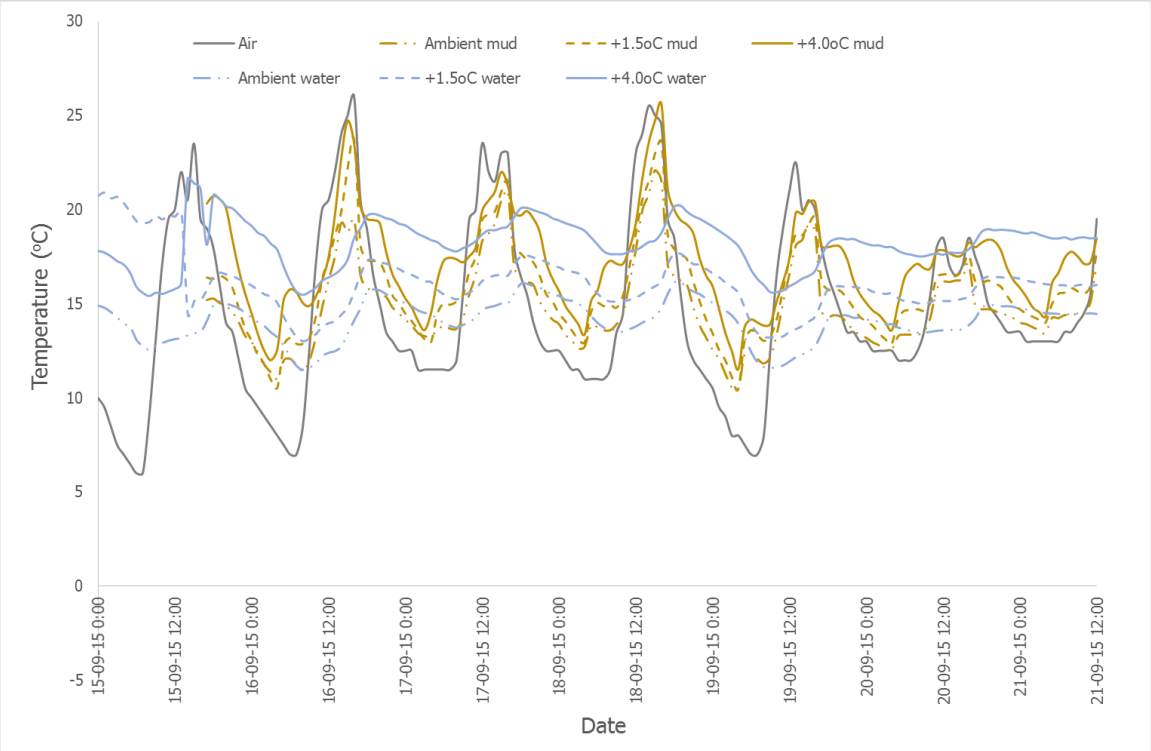

Fig. A4-3.1. Air, sediment and water temperatures in the week prior to sampling point M01

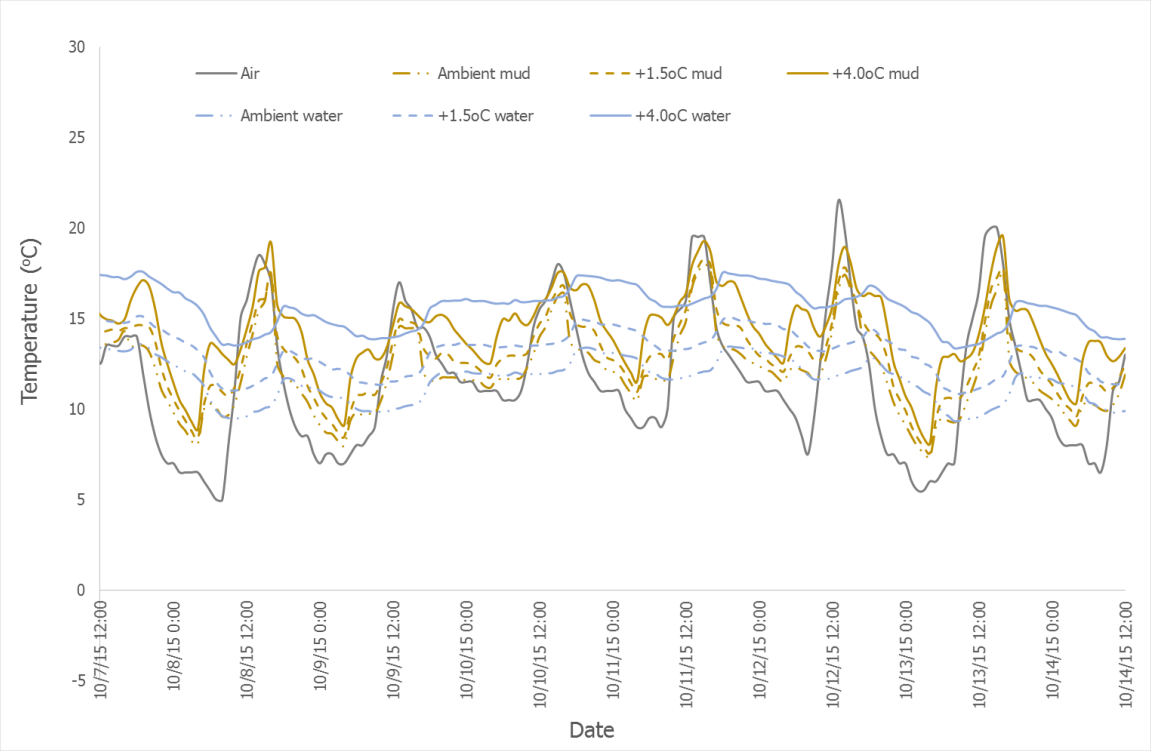

Fig. A4-3.2. Air, sediment and water temperatures in the week prior to sampling point M03

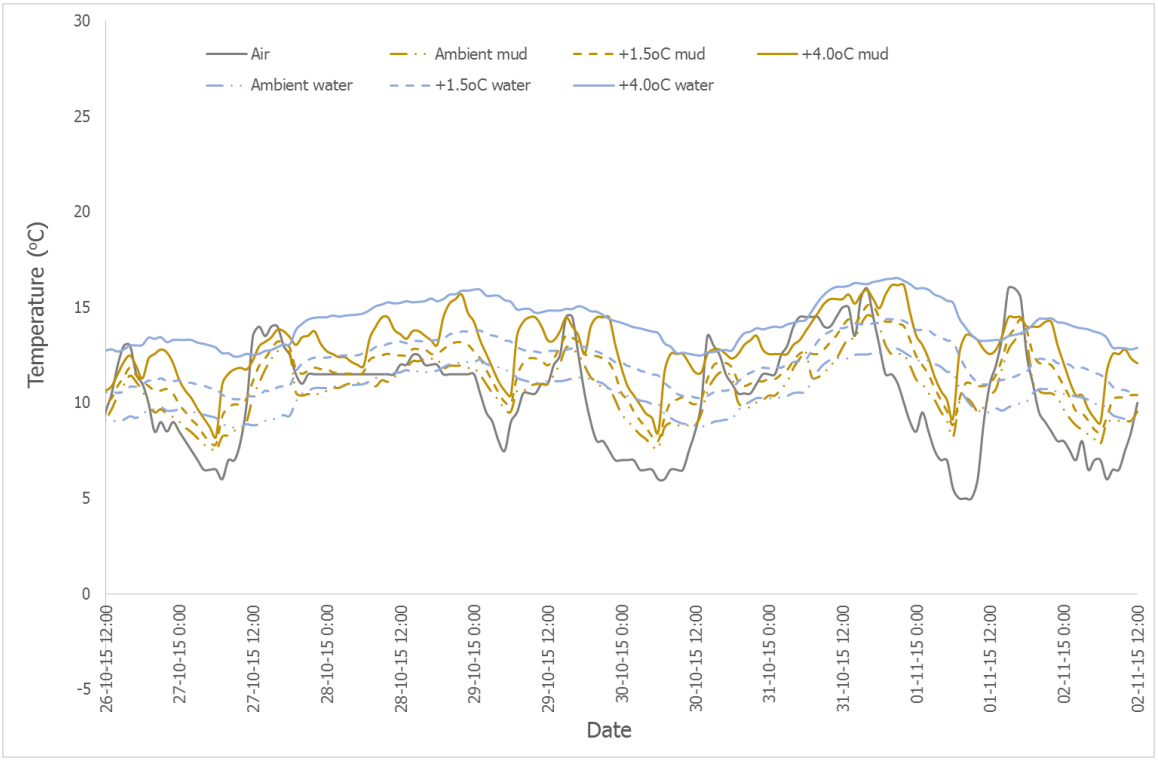

Fig. A4-3.3. Air, sediment and water temperatures in the week prior to sampling point M04

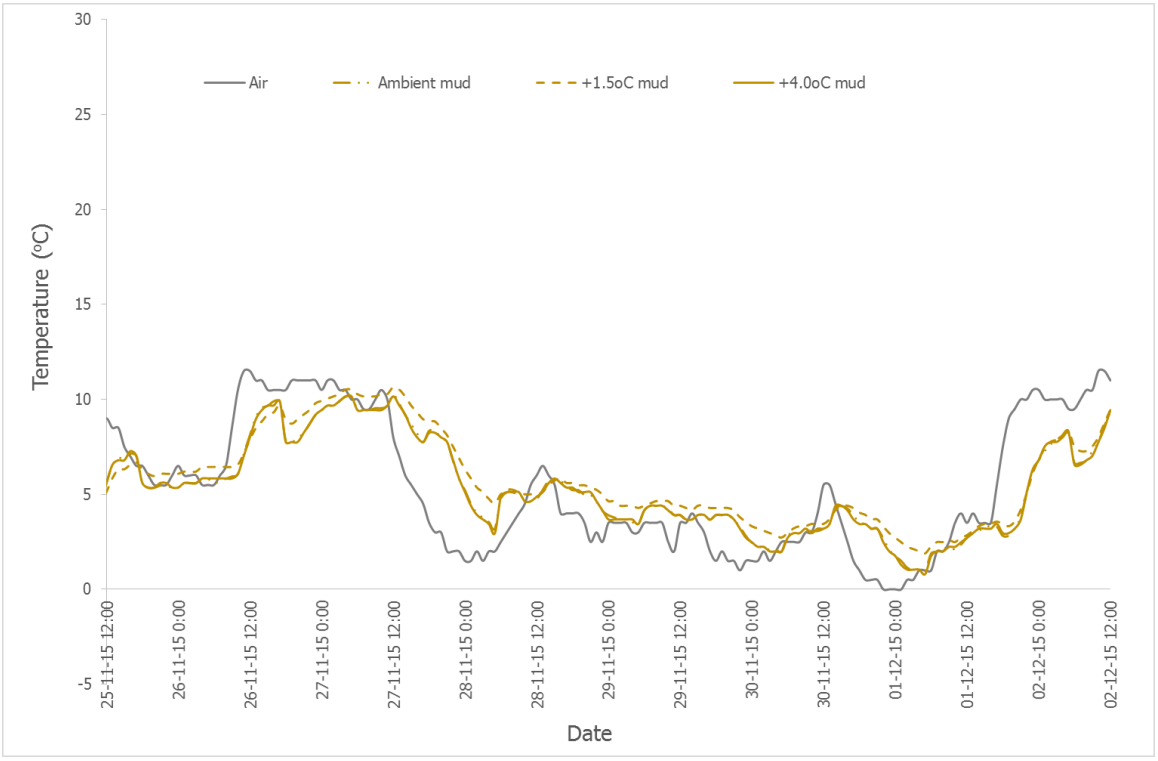

Fig. A4-3.4. Air and sediment temperatures in the week prior to sampling point M05

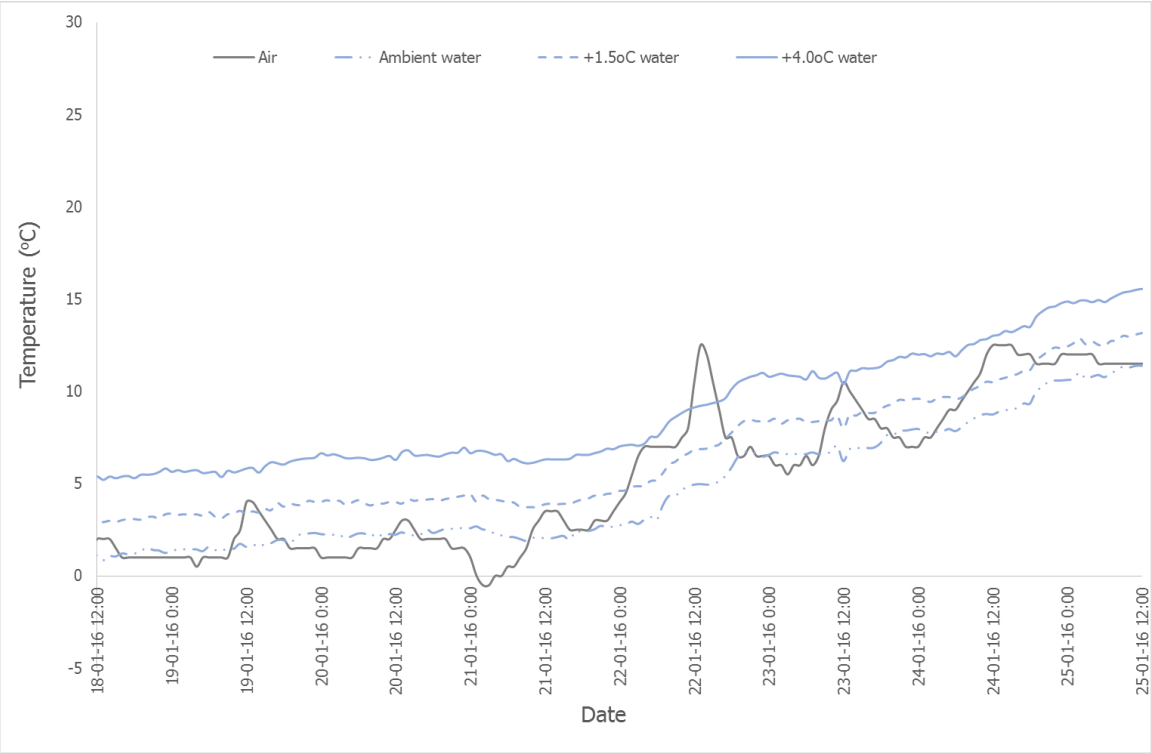

Fig. A4-3.5. Air and water temperatures in the week prior to sampling point M07

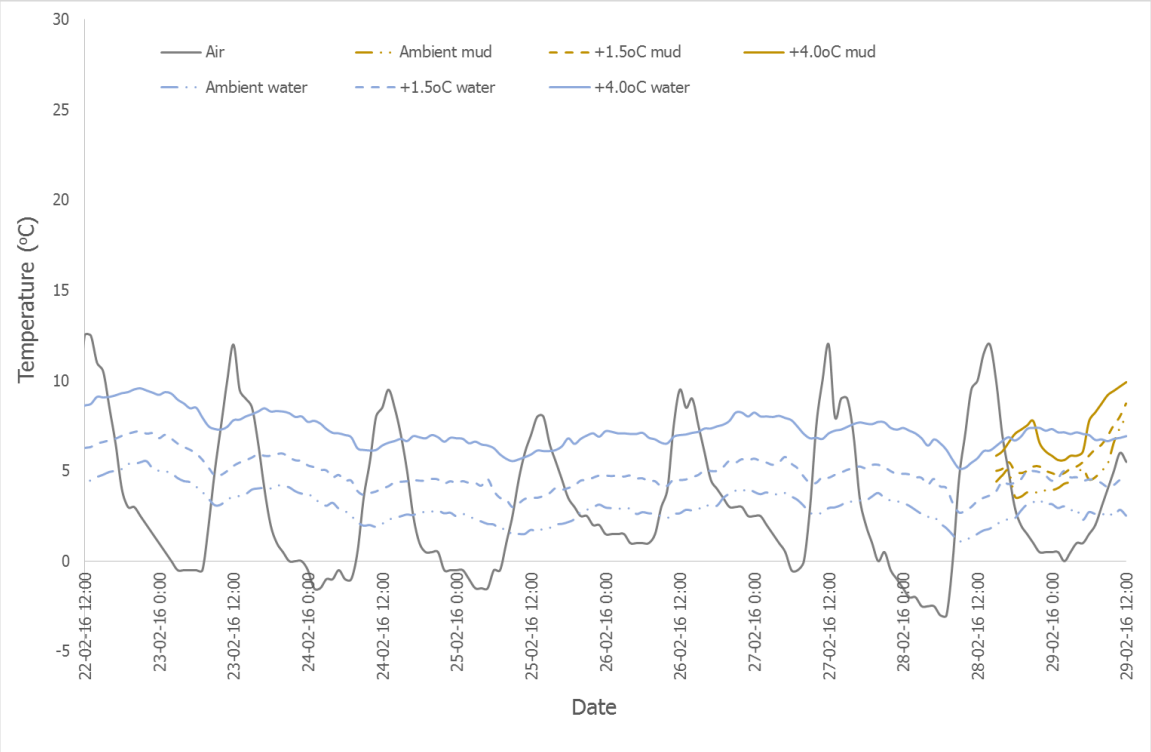

Fig. A4-3.6. Air, sediment and water temperatures in the week prior to sampling point M08

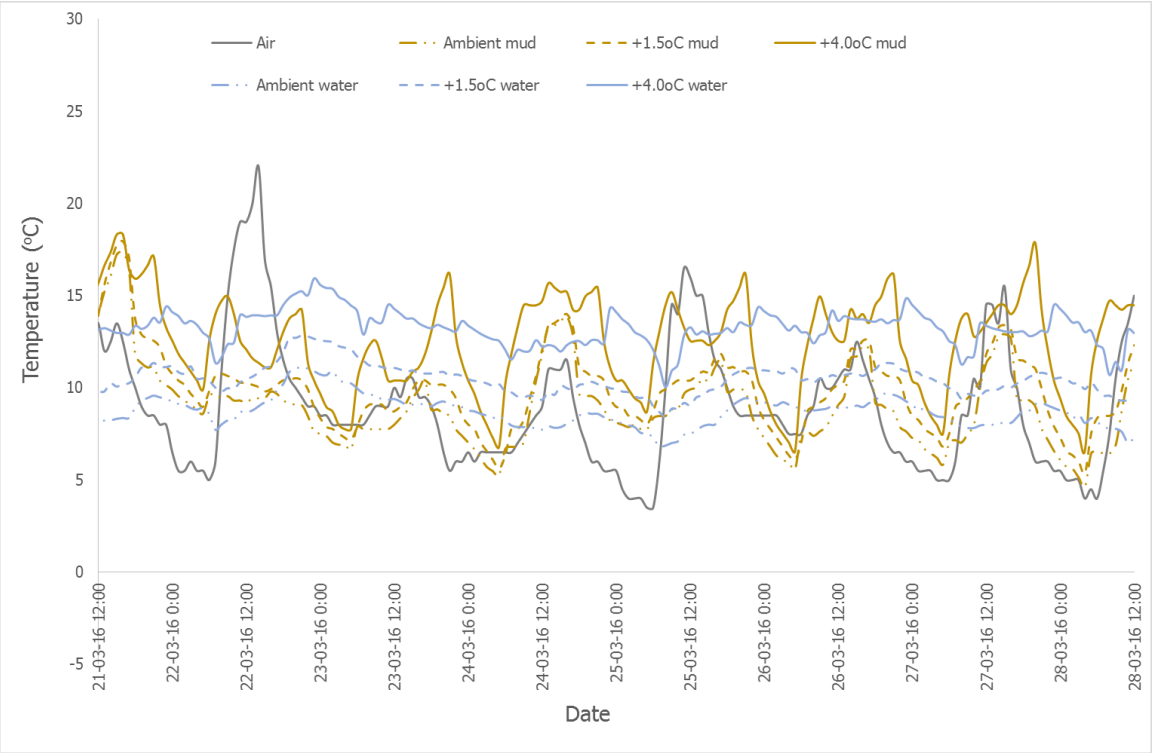

Fig. A4-3.7. Air, sediment and water temperatures in the week prior to sampling point M09

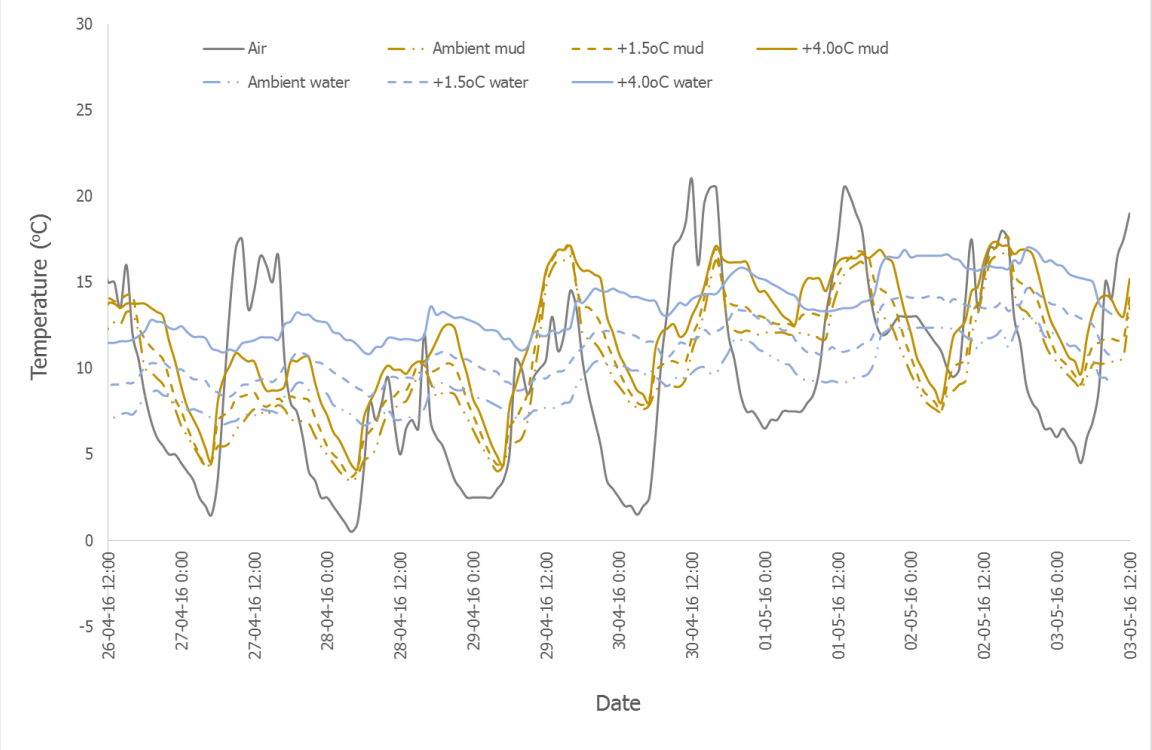

Fig. A4-3.8. Air, sediment and water temperatures in the week prior to sampling point D01

Appendix 4-4. Analyte concentrations in sediment at sampling points M00, M05, M09 and D01

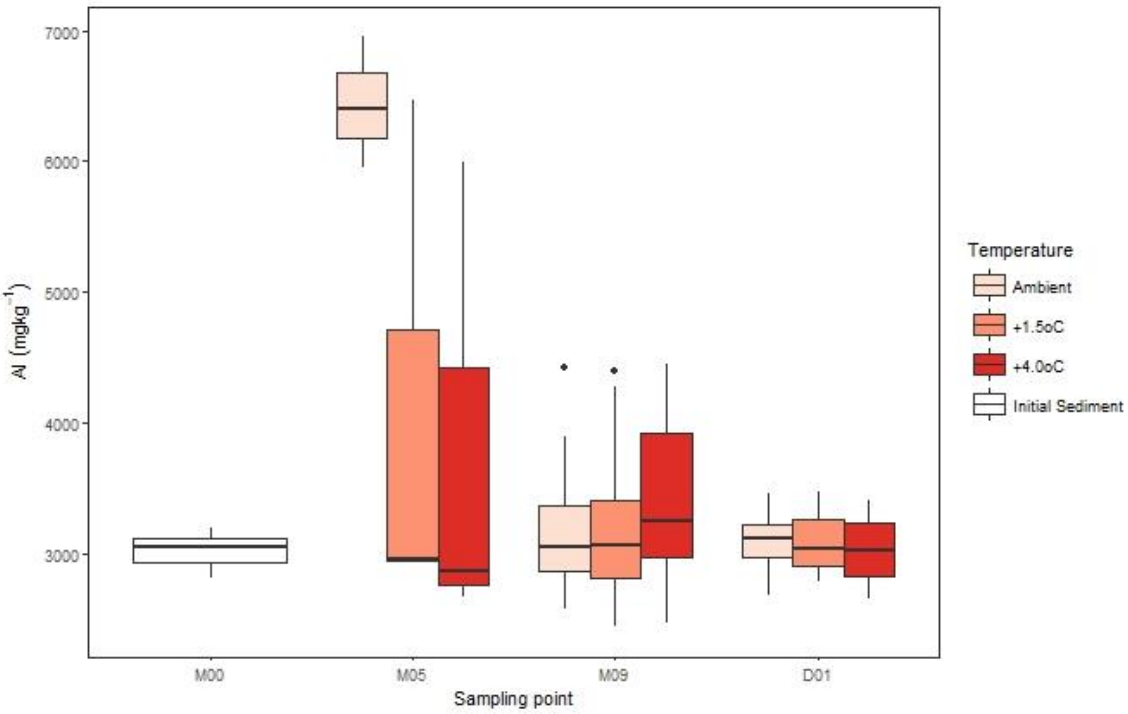

Fig. A4-4.1. Sediment concentrations (mg kg^{-1} DW) of aluminium in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

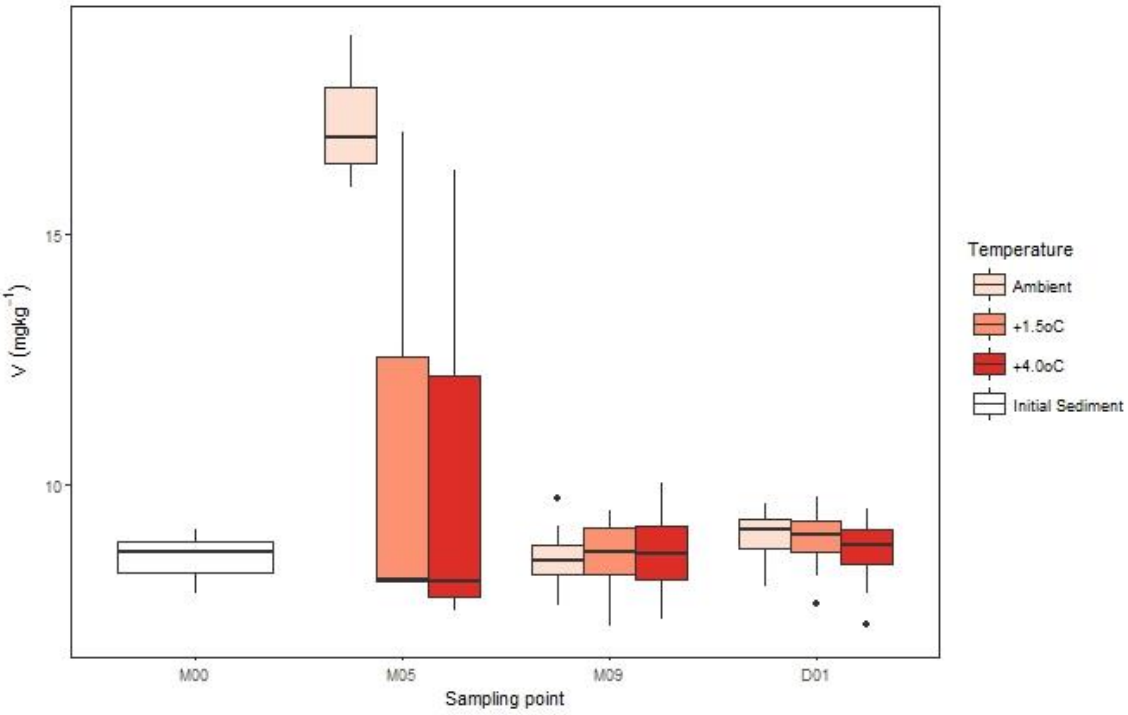

Fig. A4-4.2. Sediment concentrations (mg kg^{-1} DW) of vanadium in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

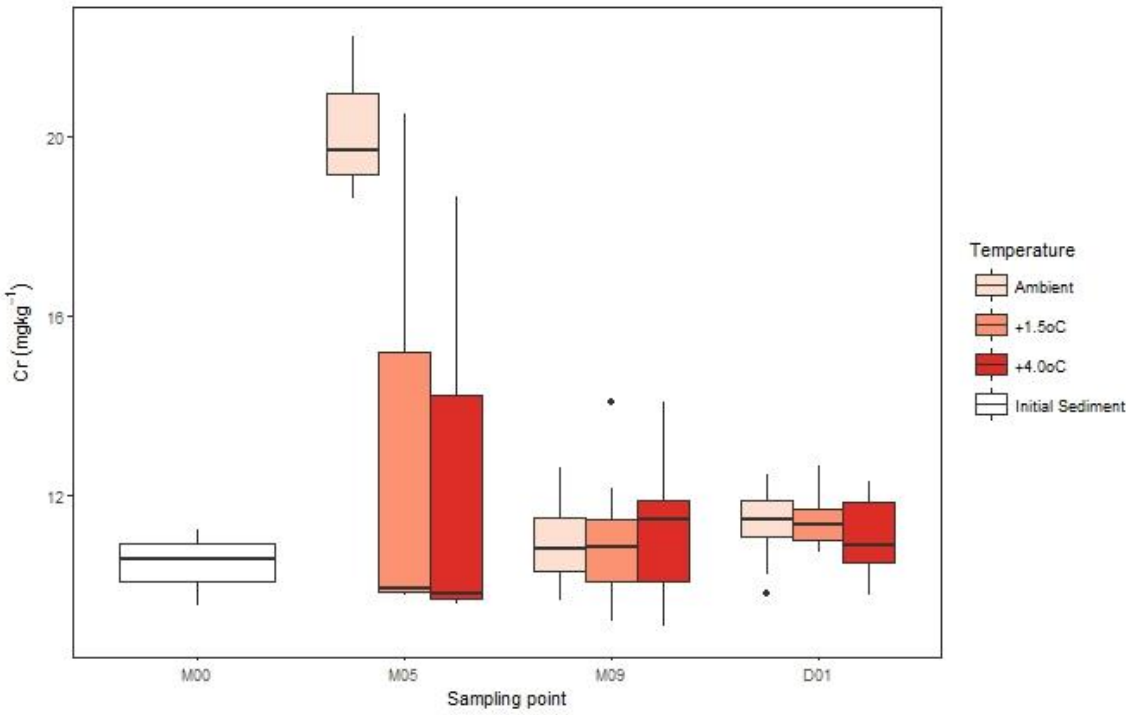

Fig. A4-4.3. Sediment concentrations (mg kg^{-1} DW) of chromium in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

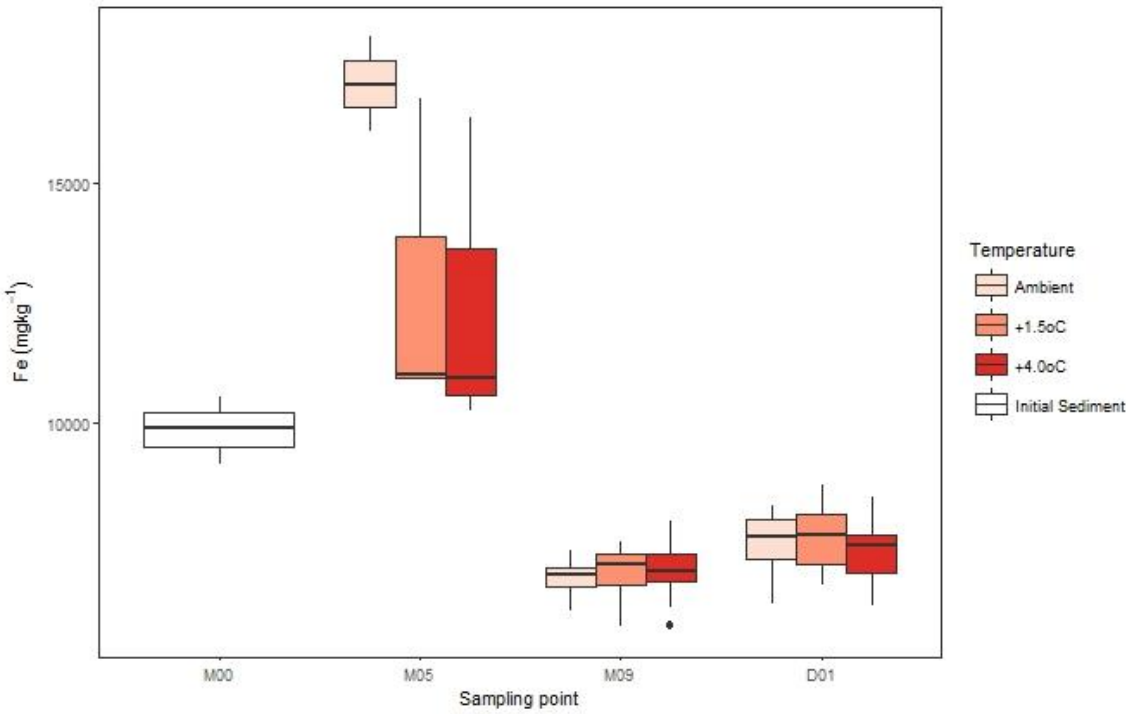

Fig. A4-4.4. Sediment concentrations (mg kg^{-1} DW) of iron in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

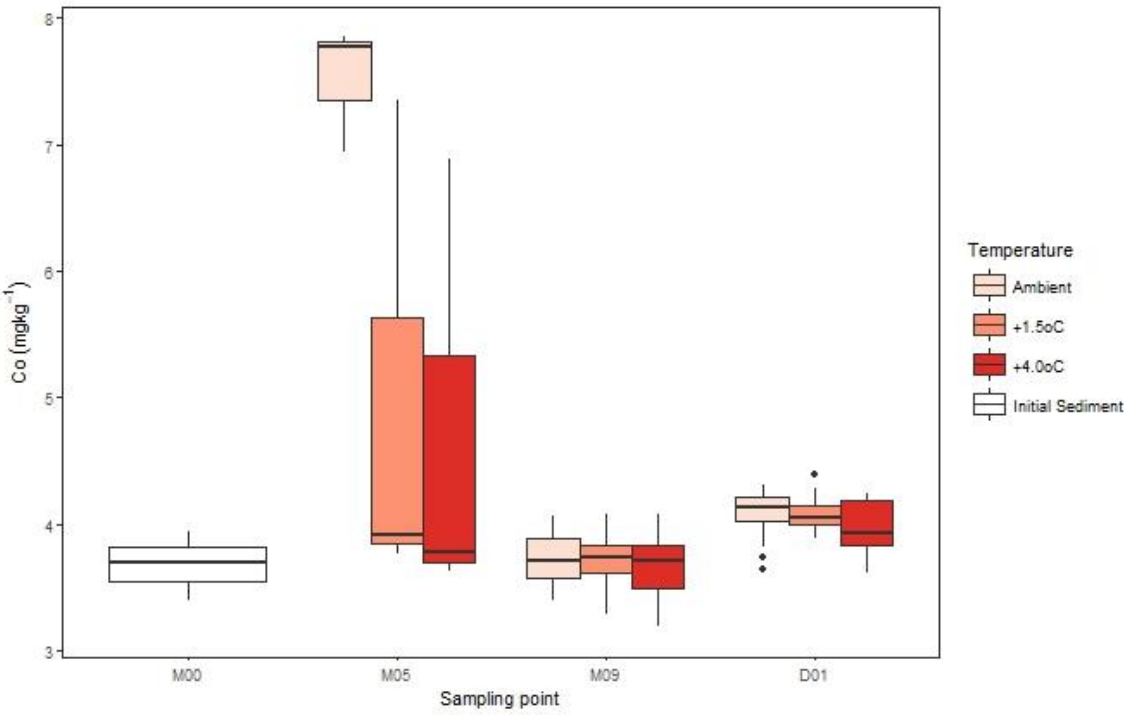

Fig. A4-4.5. Sediment concentrations (mg kg^{-1} DW) of cobalt in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

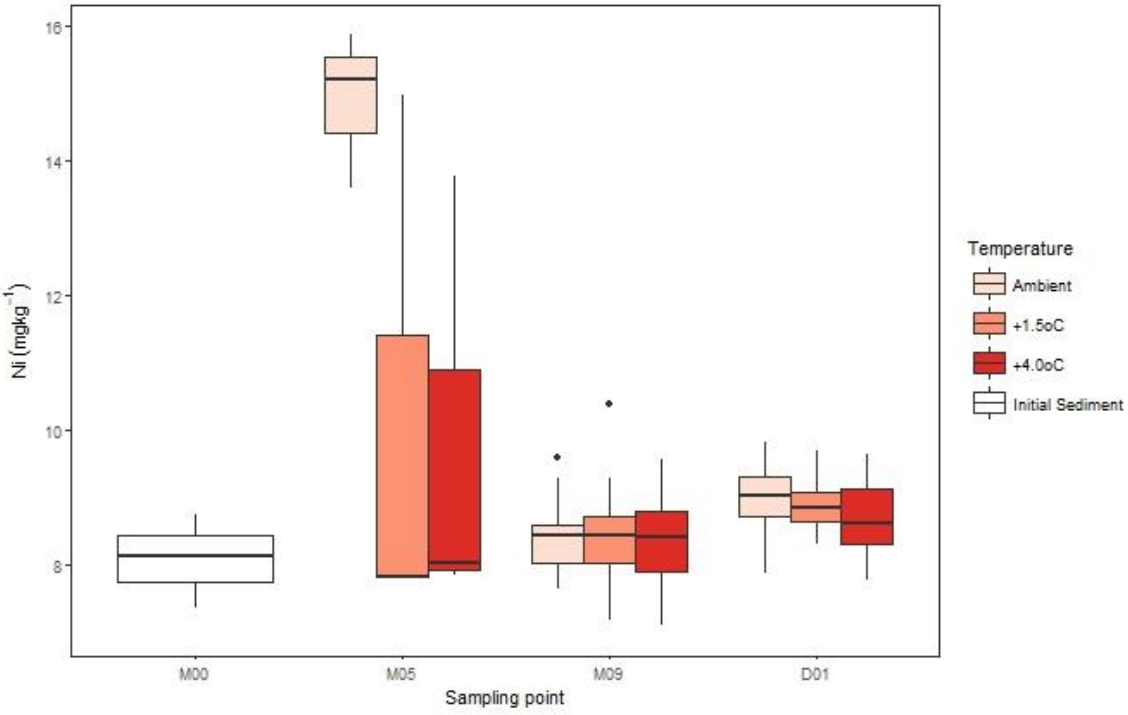

Fig. A4-4.6. Sediment concentrations (mg kg^{-1} DW) of nickel in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

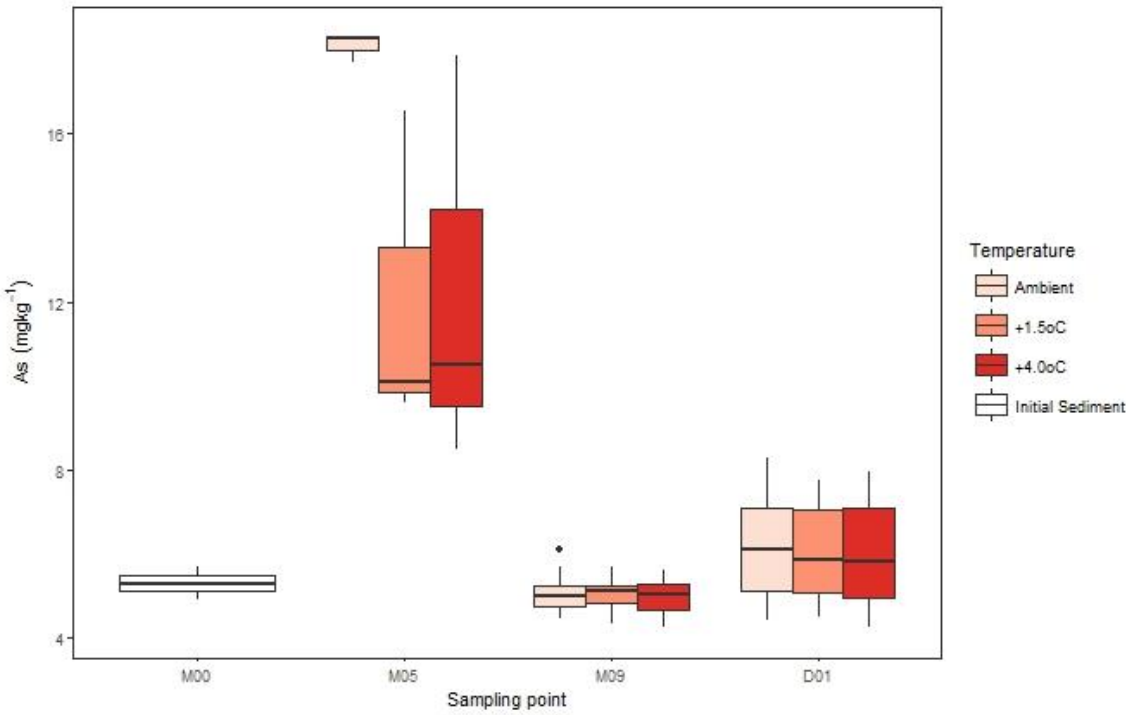

Fig. A4-4.7. Sediment concentrations (mg kg^{-1} DW) of arsenic in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

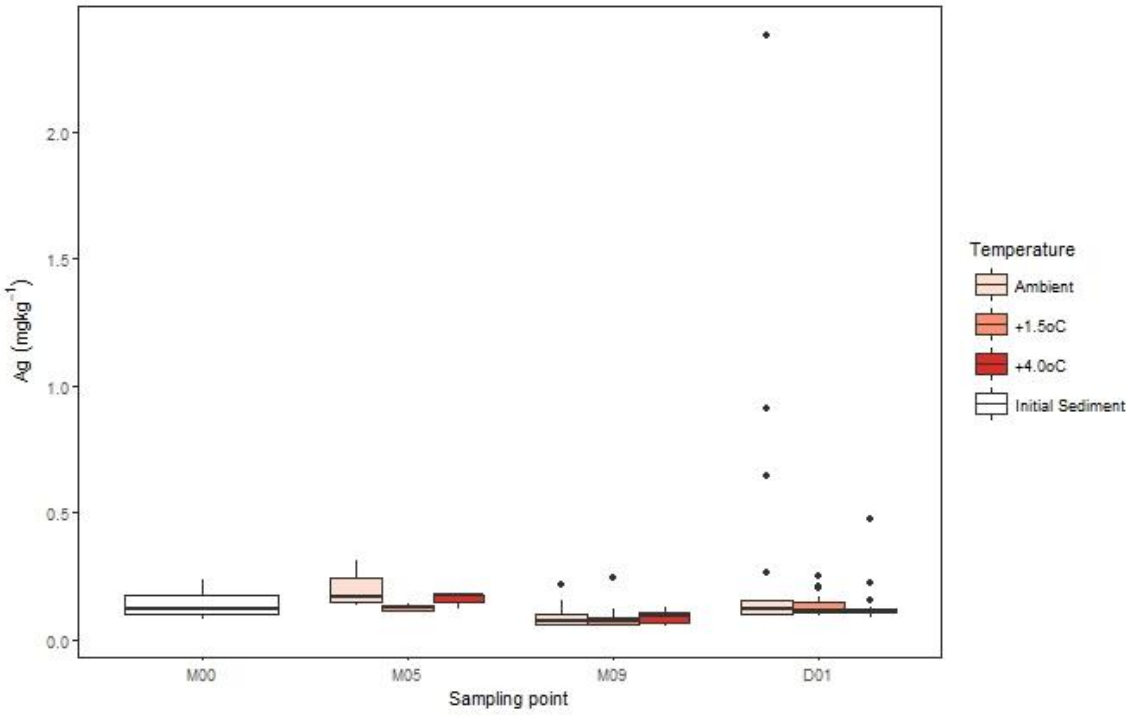

Fig. A4-4.8. Sediment concentrations (mg kg^{-1} DW) of silver in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

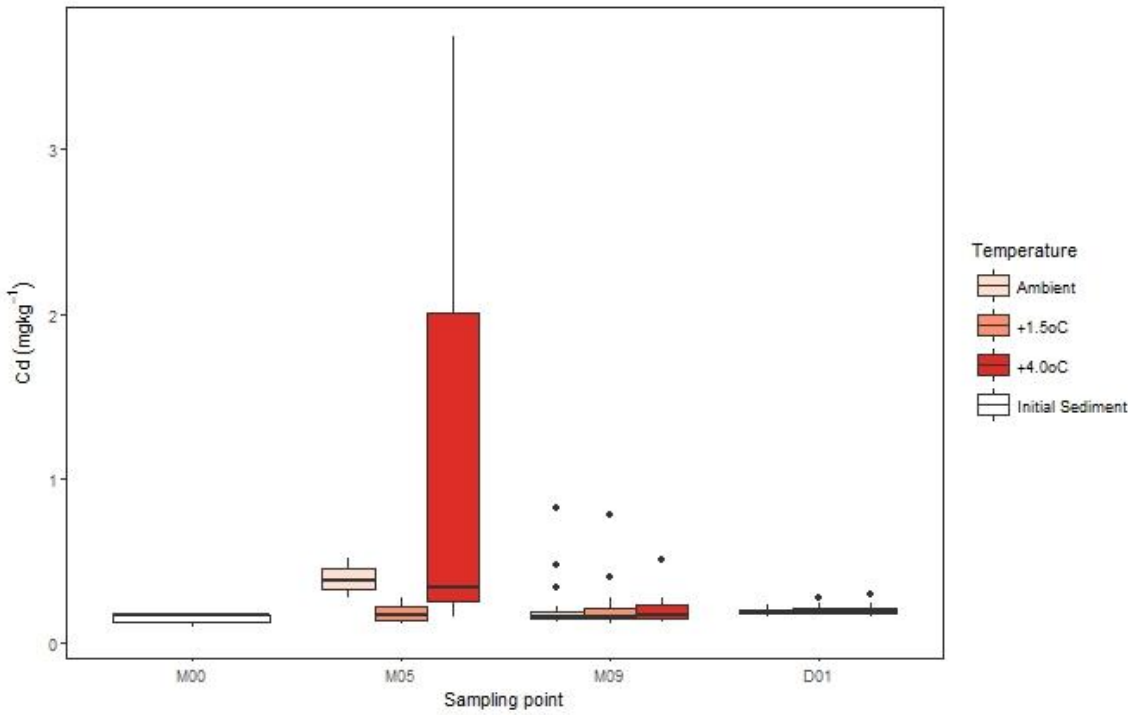

Fig. A4-4.9. Sediment concentrations (mg kg^{-1} DW) of cadmium in the initial sediment (M00) and at three sampling points (M00 and M05 n=3, M09 and D01 n=18)

Appendix 4-5. Analyte concentrations in water at sampling points M02 to M09 and D01

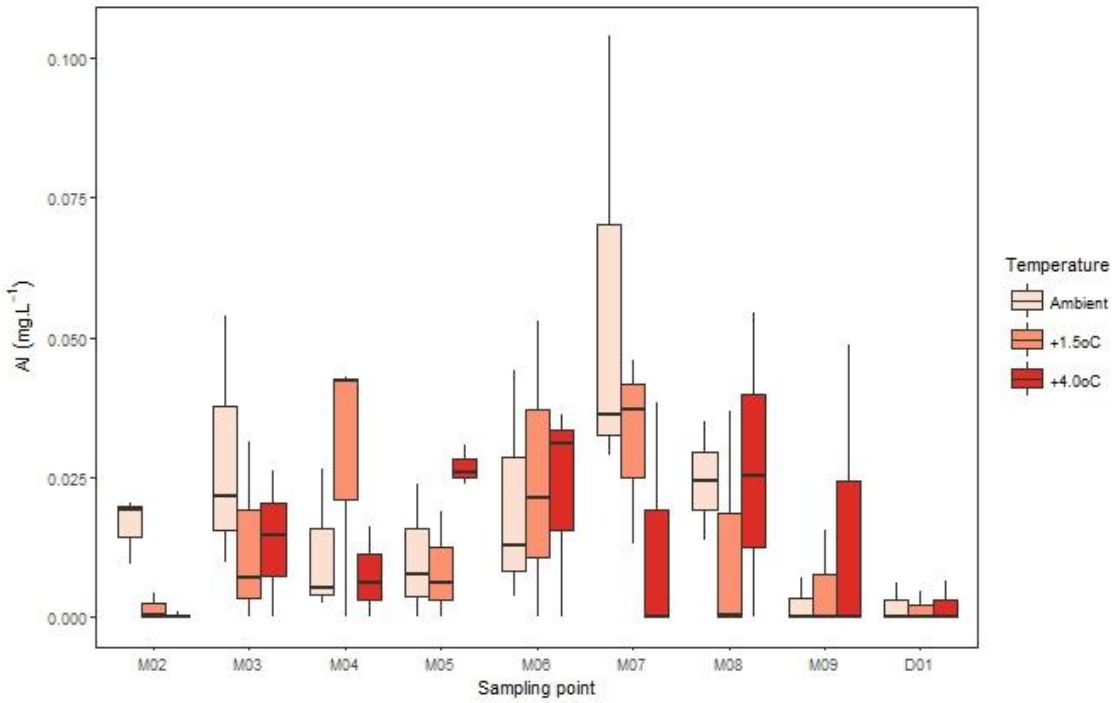

Fig. A4-5.1. Concentrations of aluminium (mg L^{-1}) in overlying water at nine sampling points (n=3)

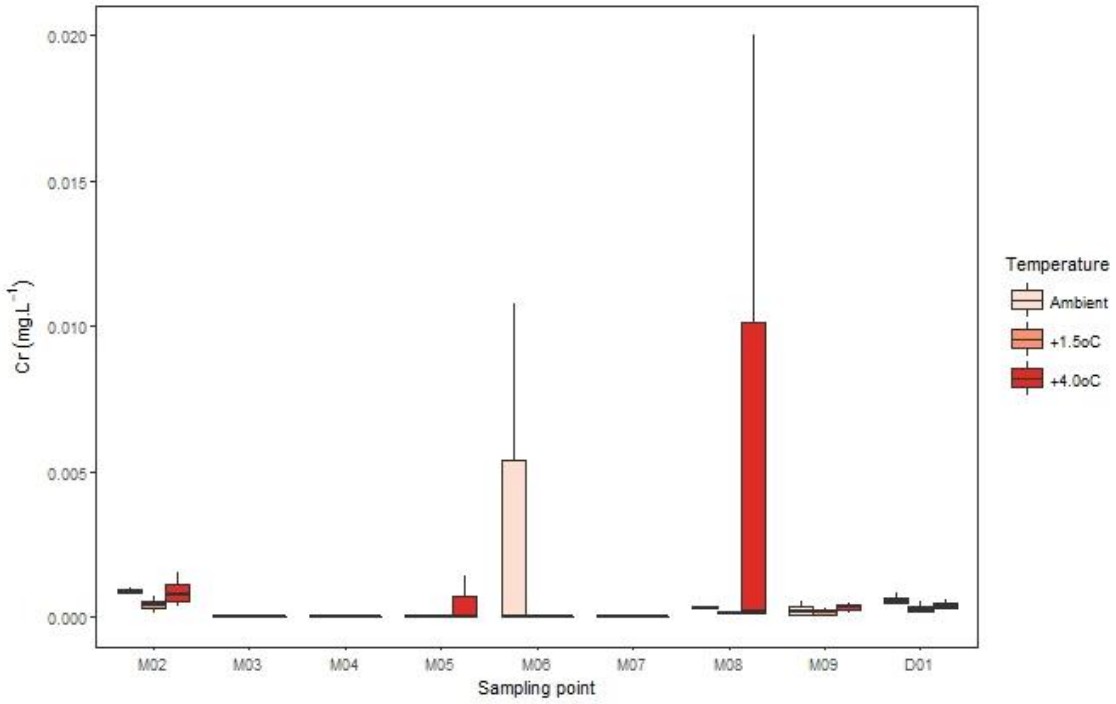

Fig. A4-5.2. Concentrations of chromium (mg L^{-1}) in overlying water at nine sampling points (n=3)

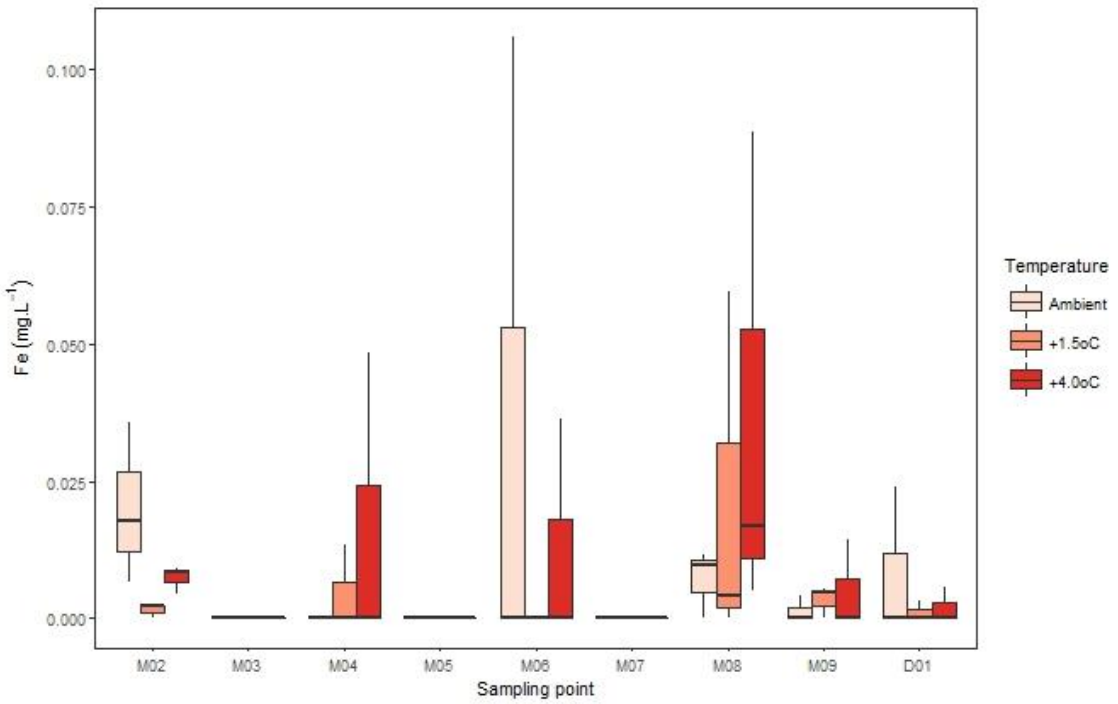

Fig. A4-5.3. Concentrations of iron (mg L^{-1}) in overlying water at nine sampling points (n=3)

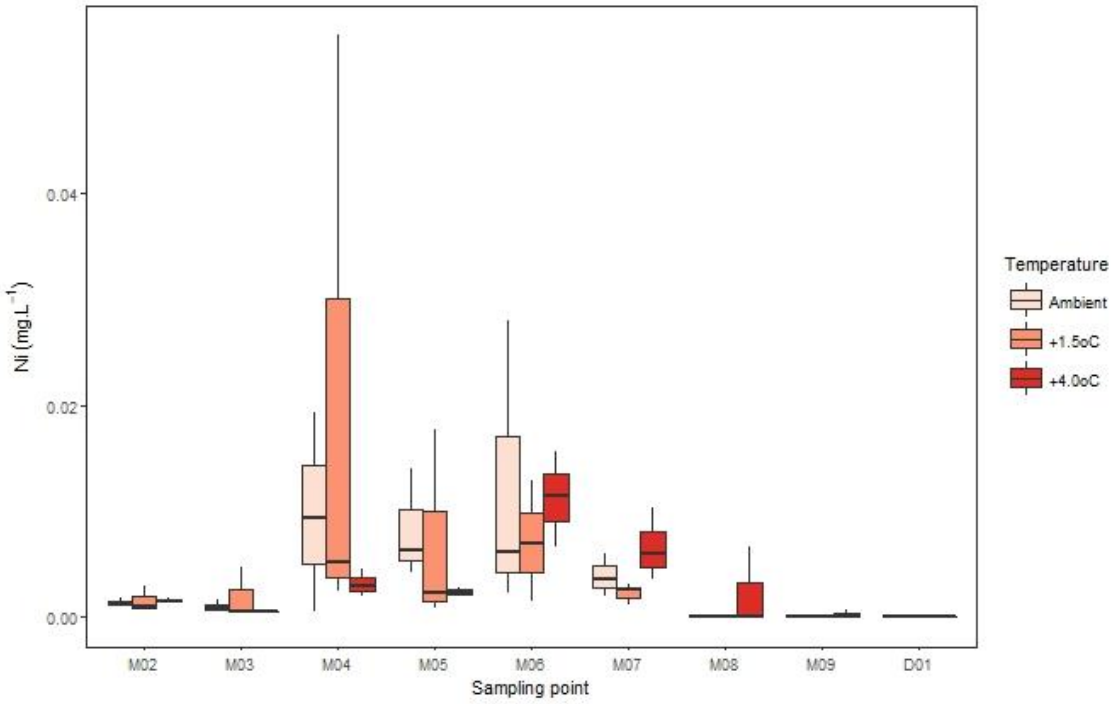

Fig. A4-5.4. Concentrations of nickel (mg L^{-1}) in overlying water at nine sampling points (n=3)

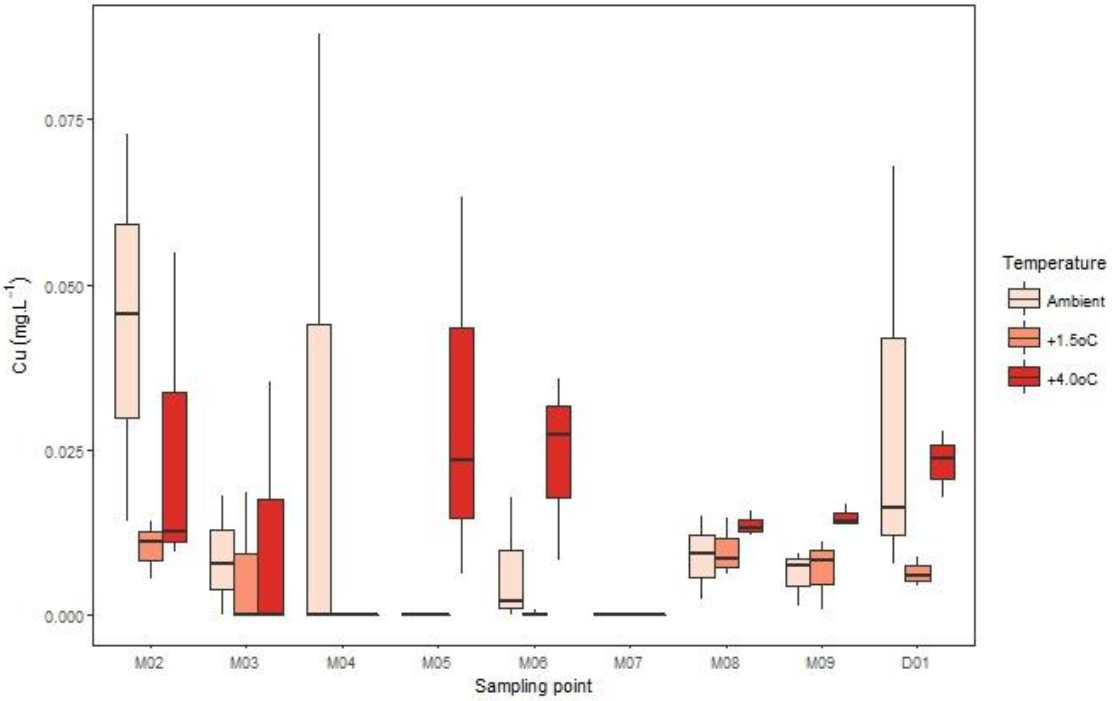

Fig. A4-5.5. Concentrations of copper (mg L^{-1}) in overlying water at nine sampling points (n=3)

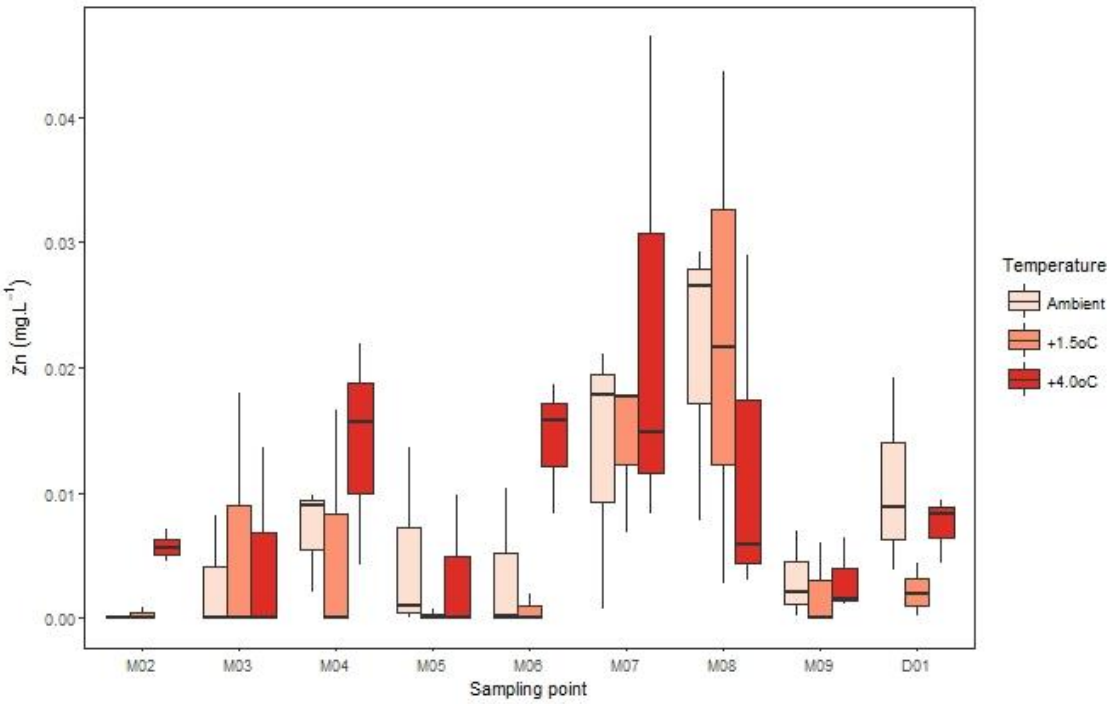

Fig. A4-5.6. Concentrations of zinc (mg L^{-1}) in overlying water at nine sampling points (n=3)

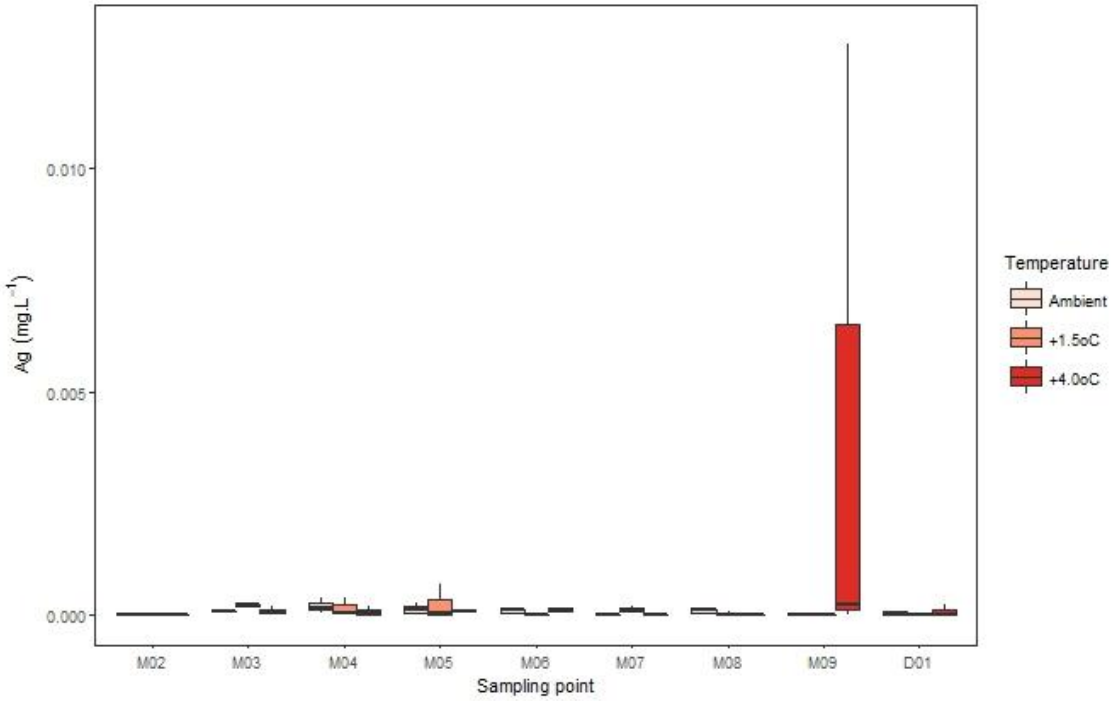

Fig. A4-5.7. Concentrations of silver (mg L^{-1}) in overlying water at nine sampling points (n=3)

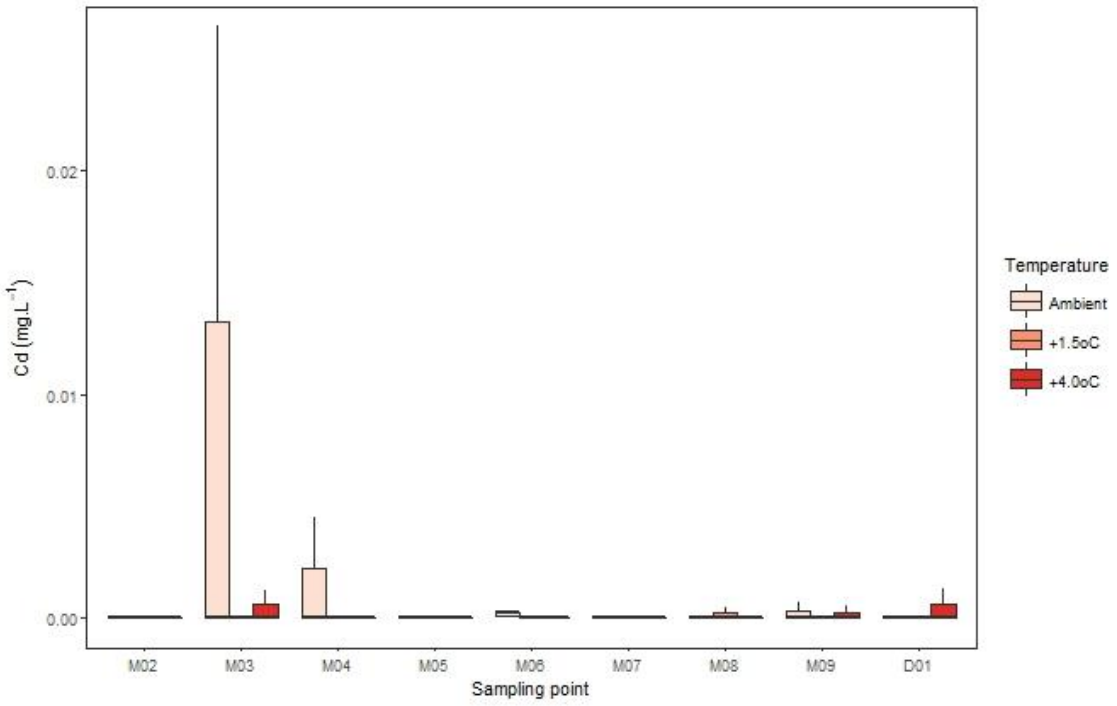

Fig. A4-5.8. Concentrations of cadmium (mg L^{-1}) in overlying water at nine sampling points (n=3)

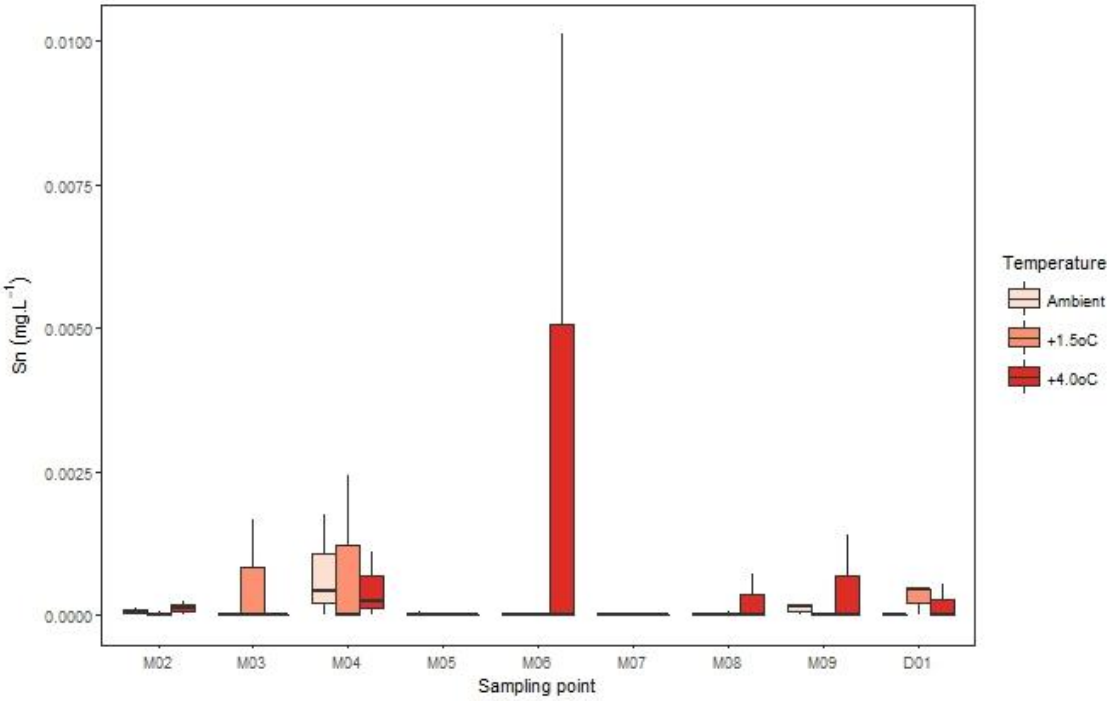

Fig. A4-5.9. Concentrations of tin (mg L^{-1}) in overlying water at nine sampling points (n=3)

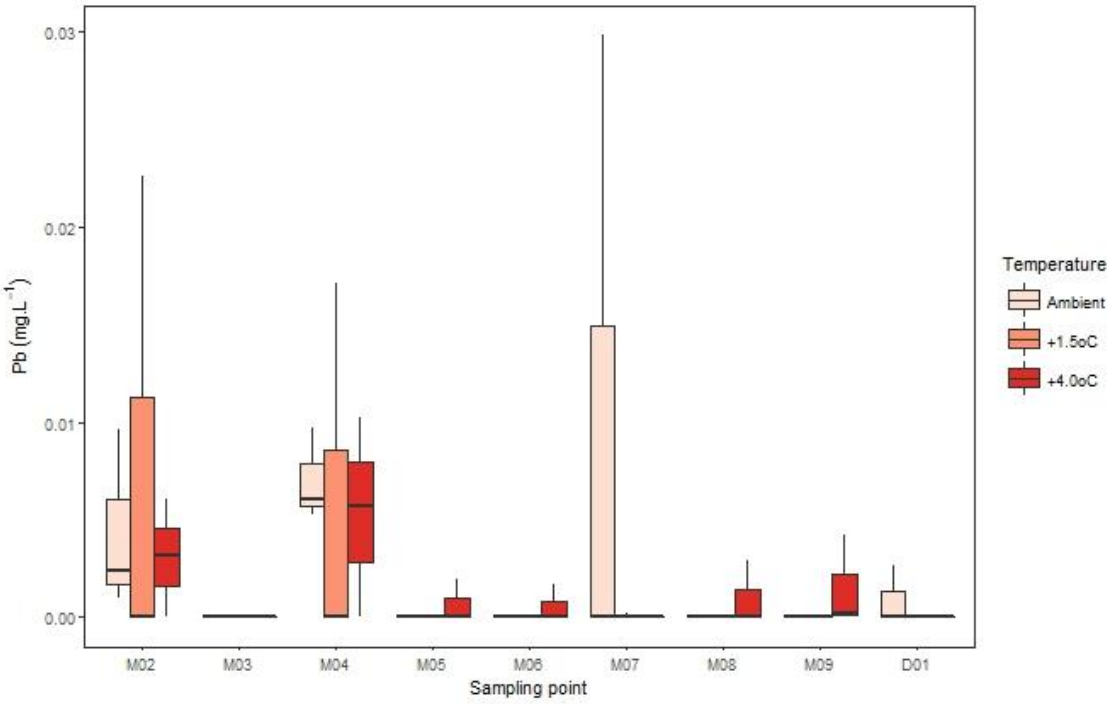

Fig. A4-5.10. Concentrations of lead (mg L^{-1}) in overlying water at nine sampling points (n=3)

Appendix 4-6. Analyte concentrations in different types of algae at sampling points M02 to M09

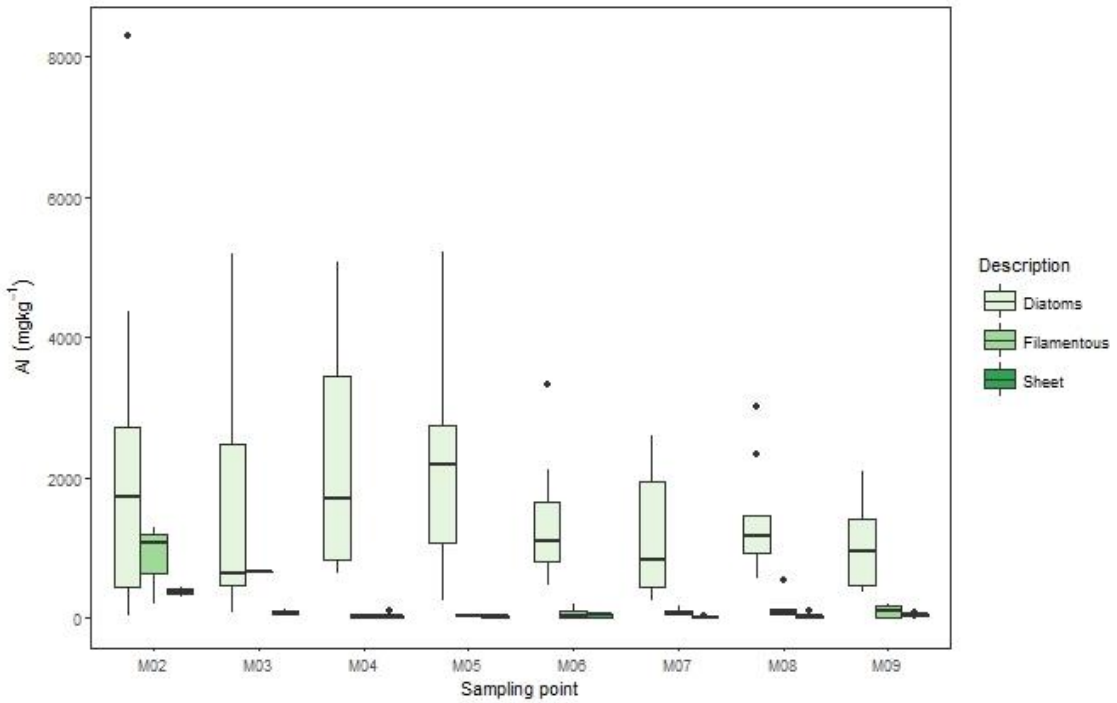

Fig. A4-6.1. Concentrations of aluminium in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

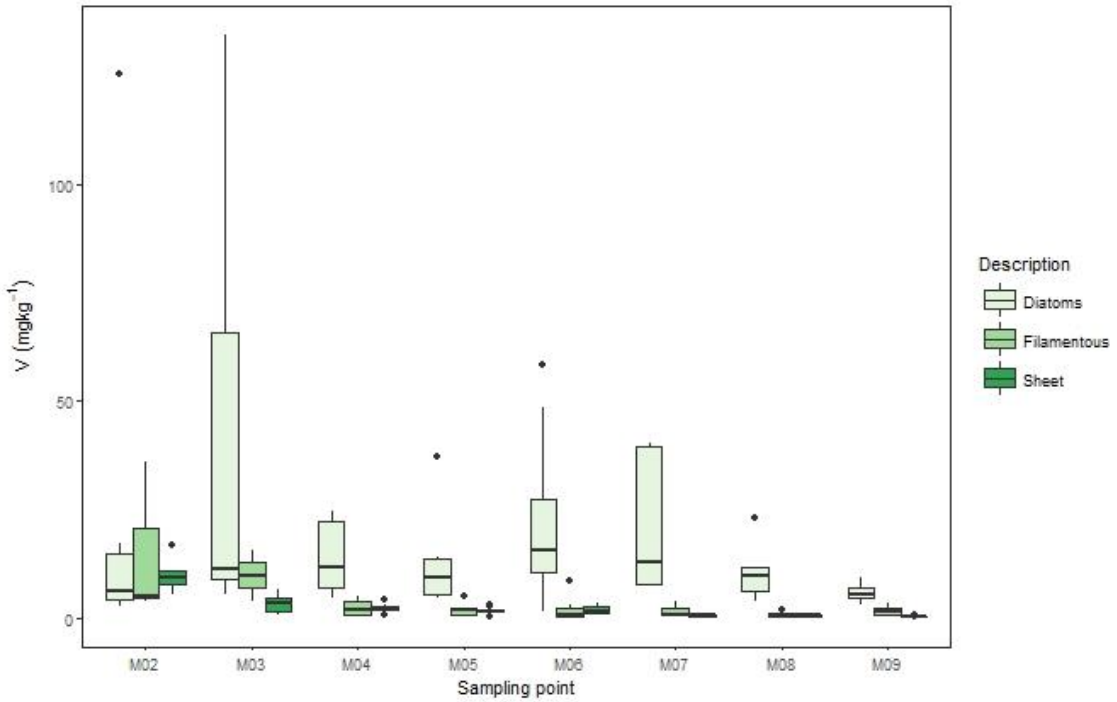

Fig. A4-6.2. Concentrations of vanadium in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

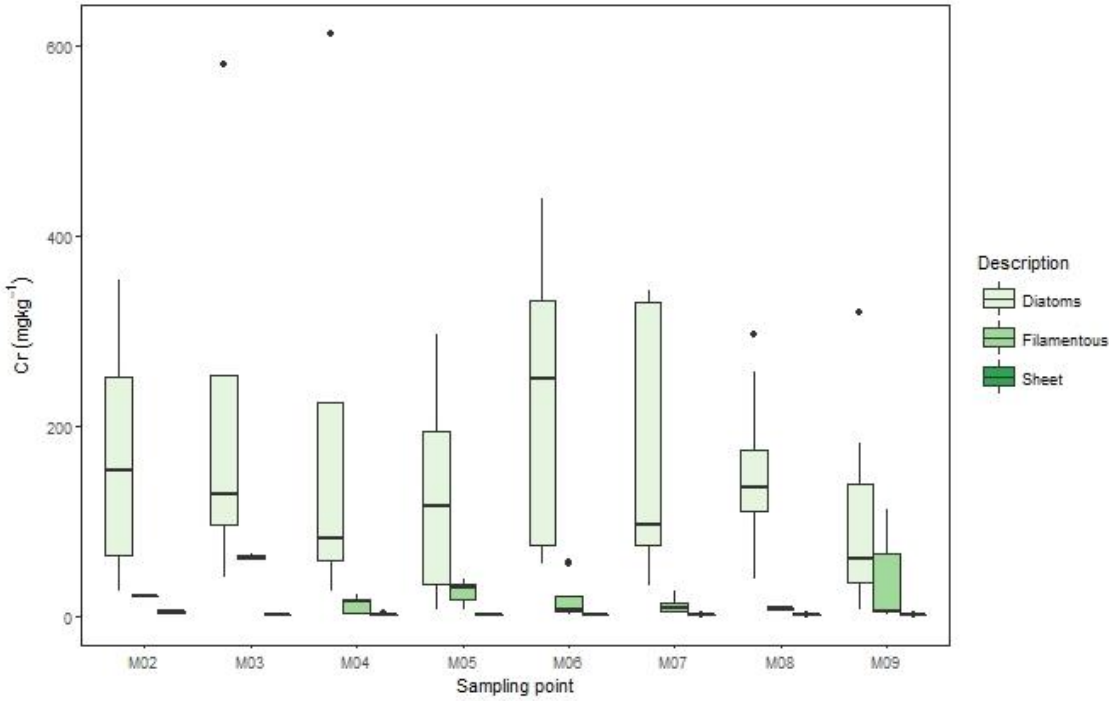

Fig. A4-6.3. Concentrations of chromium in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

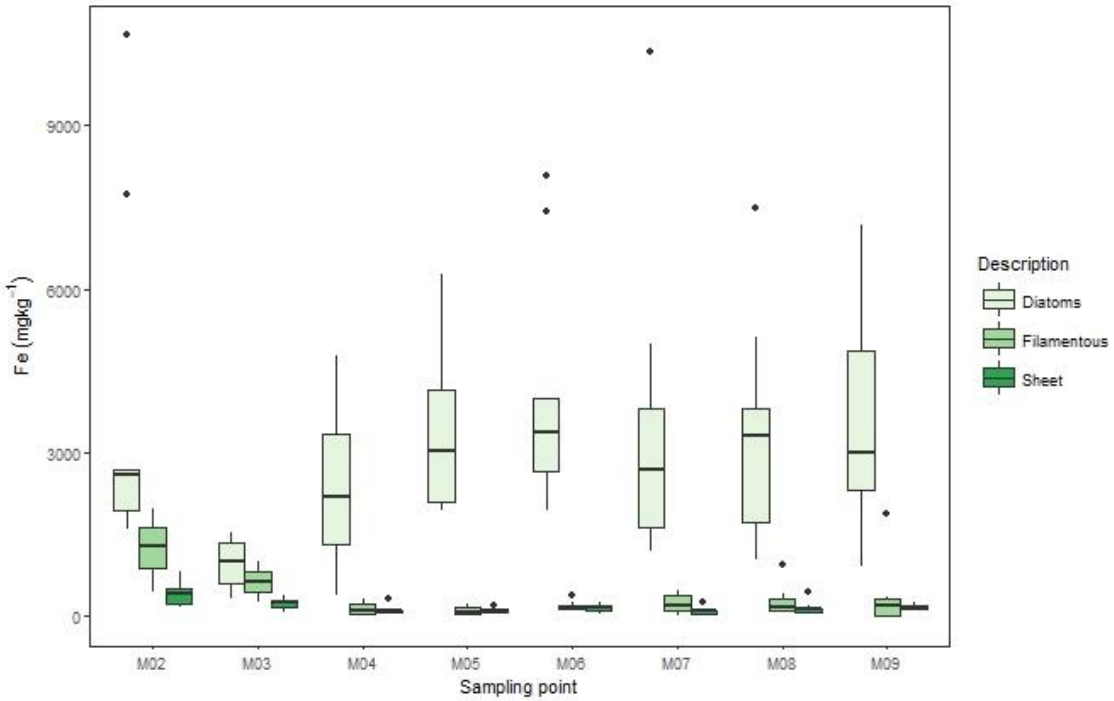

Fig. A4-6.4. Concentrations of iron in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

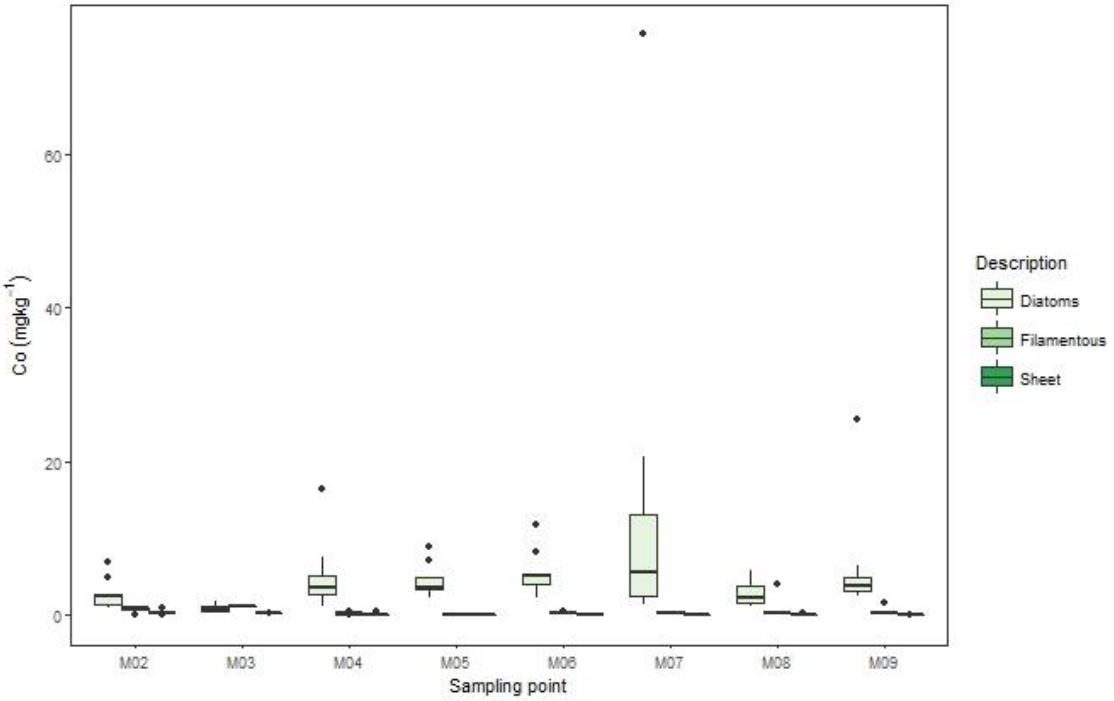

Fig. A4-6.5. Concentrations of cobalt in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

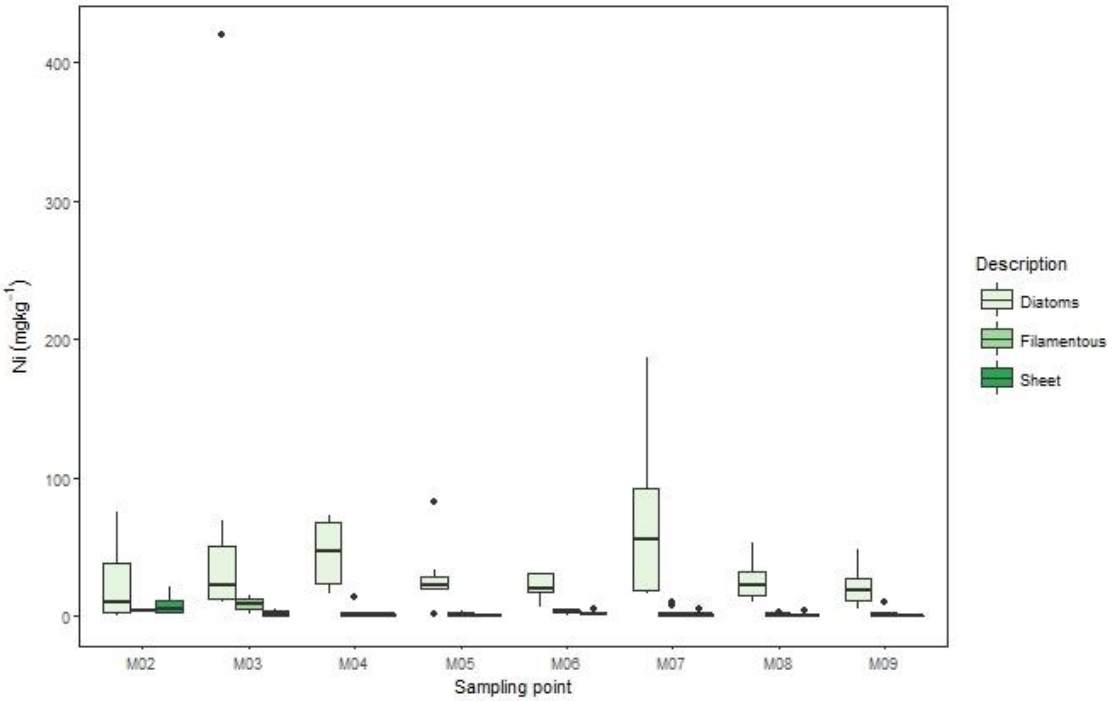

Fig. A4-6.6. Concentrations of nickel in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

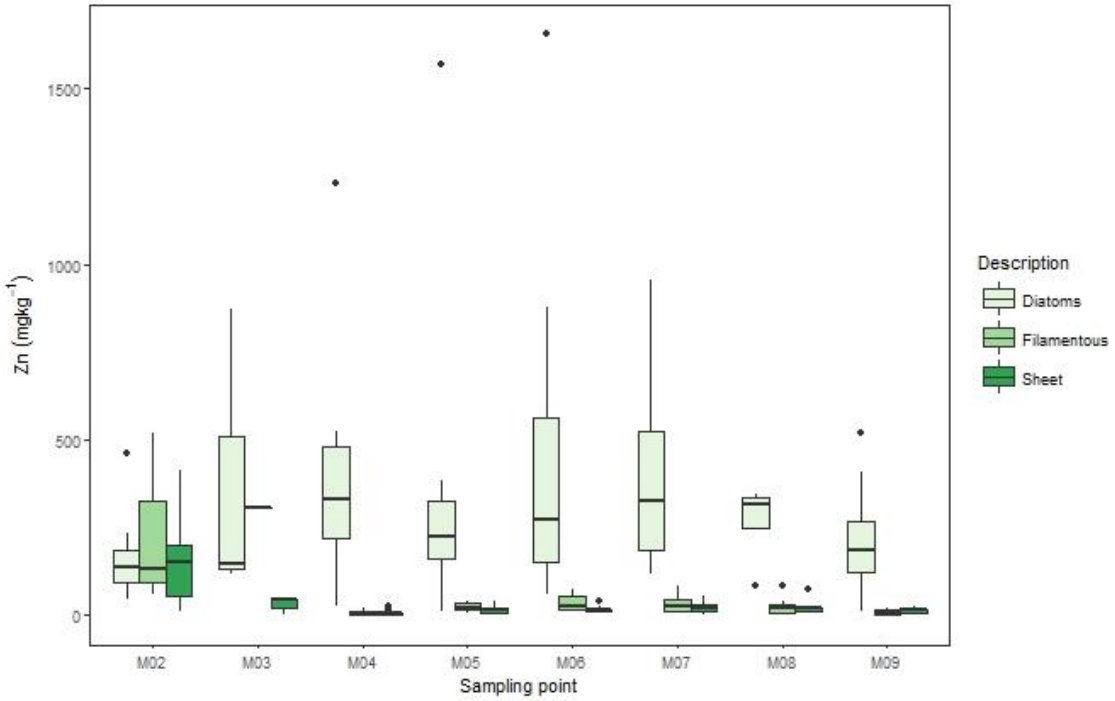

Fig. A4-6.7. Concentrations of zinc in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

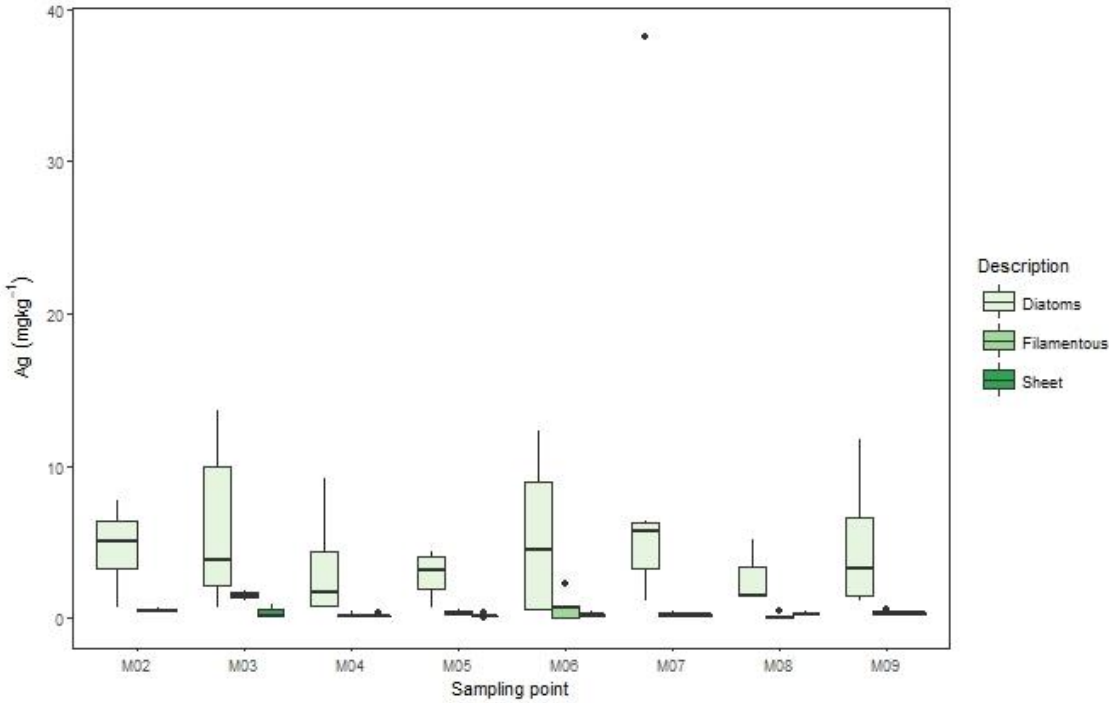

Fig. A4-6.8. Concentrations of silver in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

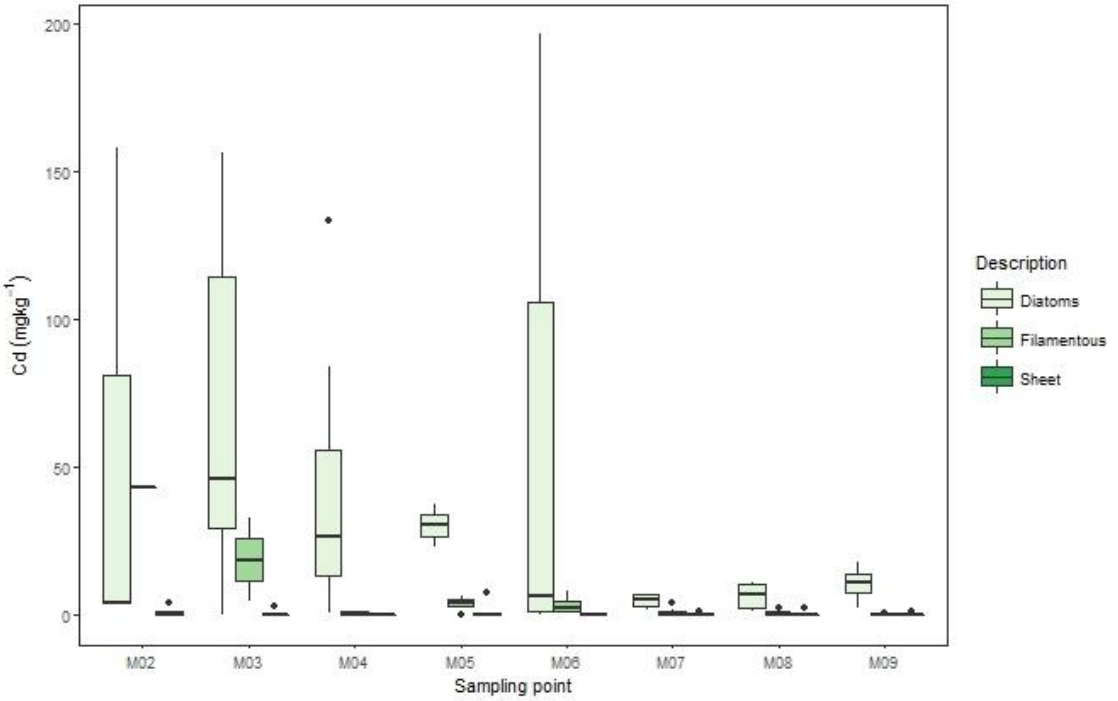

Fig. A4-6.9. Concentrations of cadmium in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

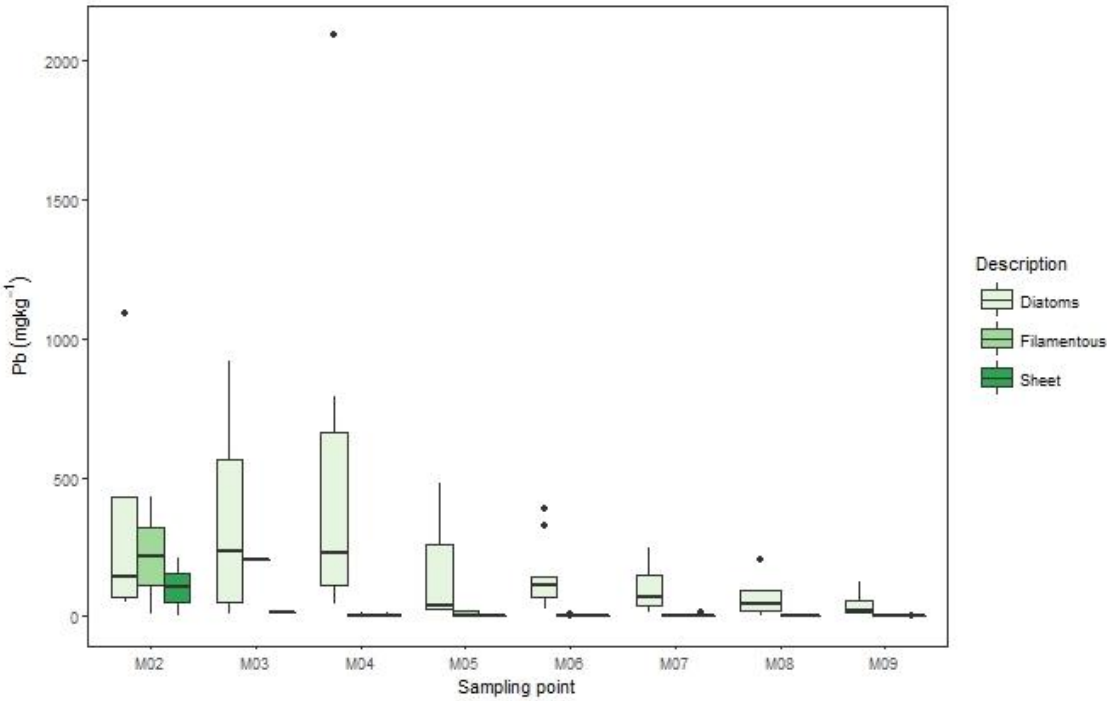

Fig. A4-6.10. Concentrations of lead in three algal groups (mg kg^{-1} DW) at sampling points M02 to M09 (n=3)

Appendix 4-7. Analyte concentrations in diatoms types of algae at sampling points M01 to M09 and D01

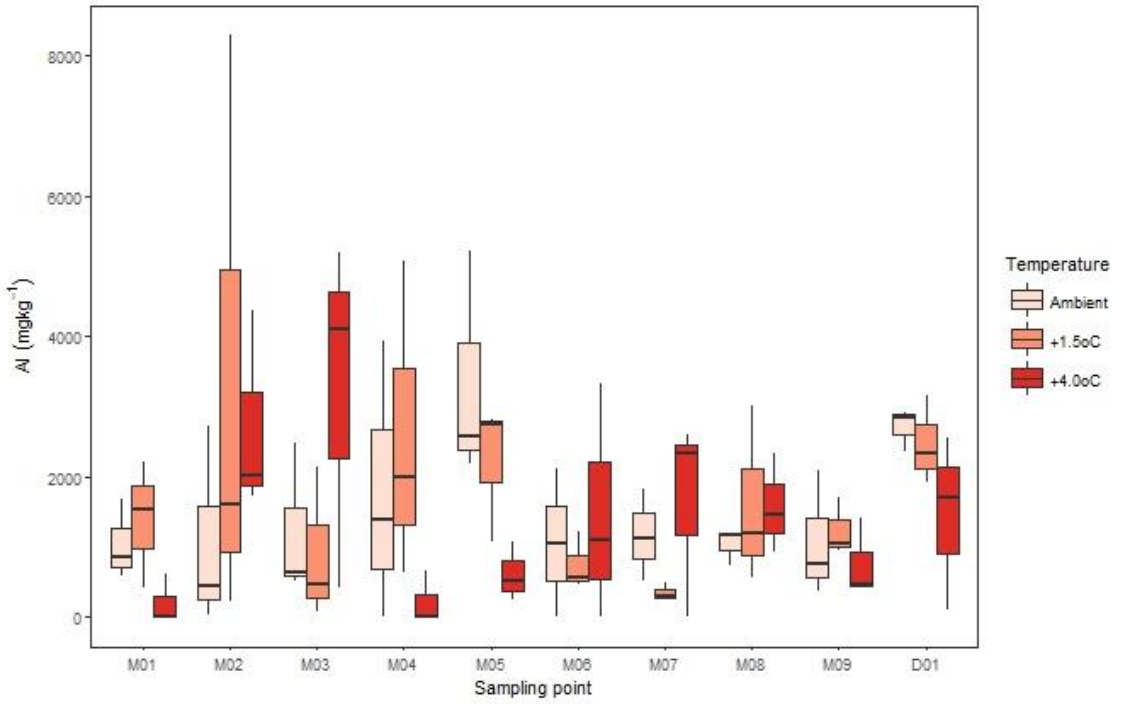

Fig. A4-7.1. Concentrations of aluminium in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

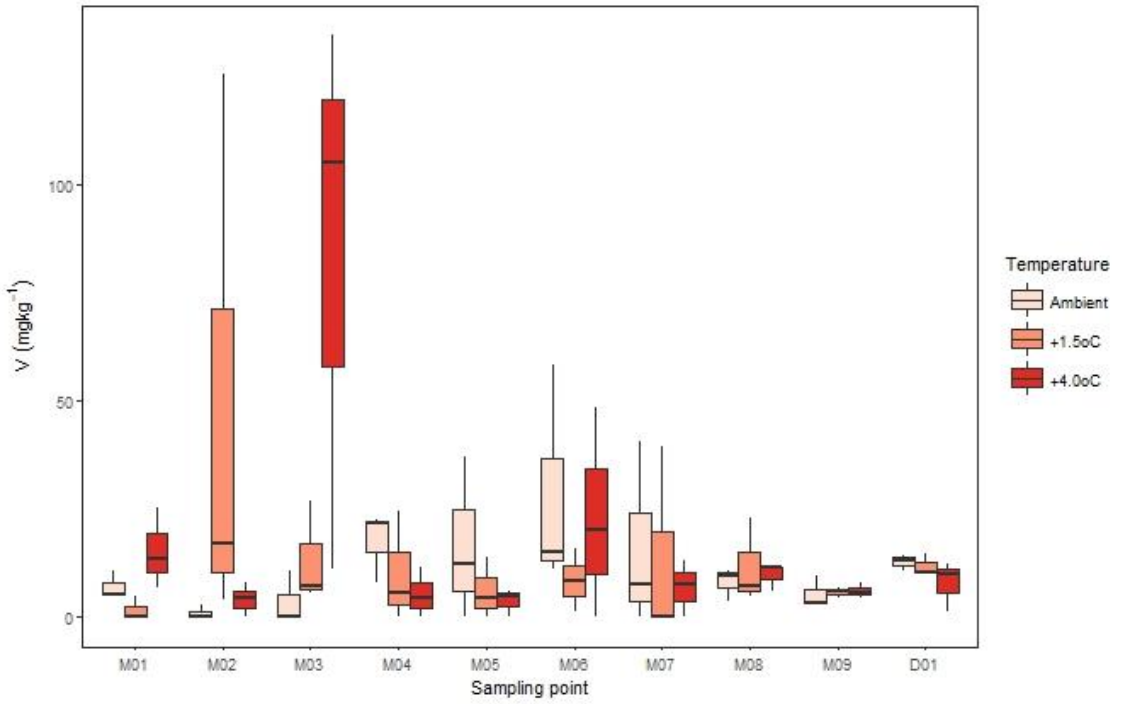

Fig. A4-7.2. Concentrations of vanadium in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

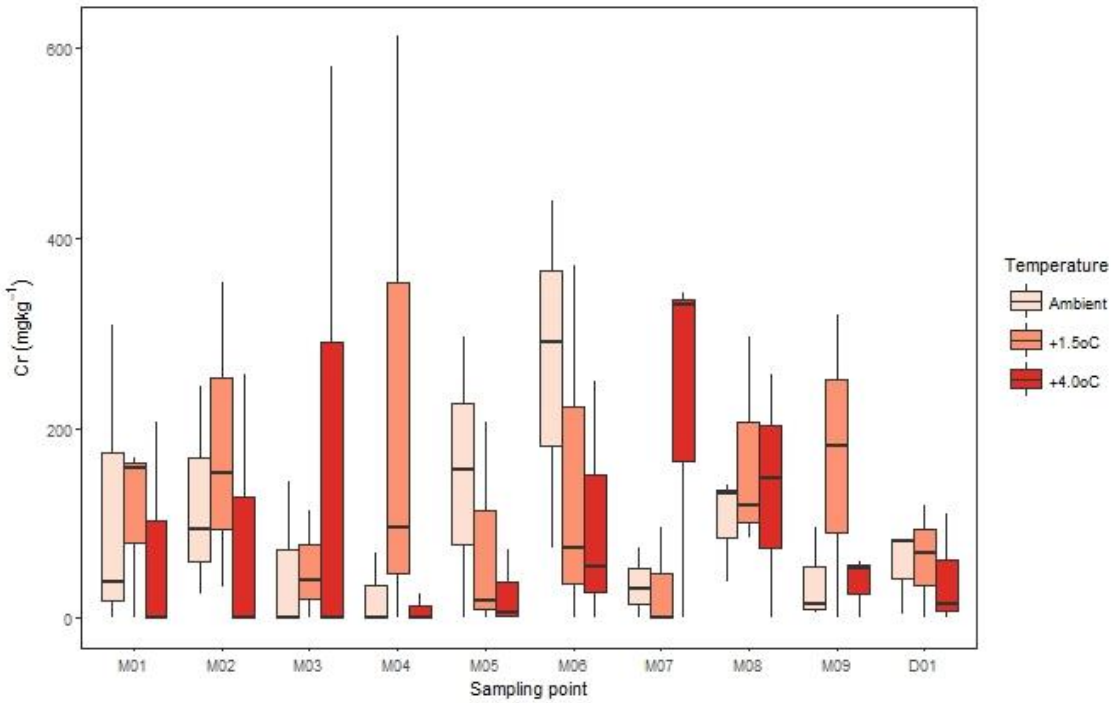

Fig. A4-7.3. Concentrations of chromium in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

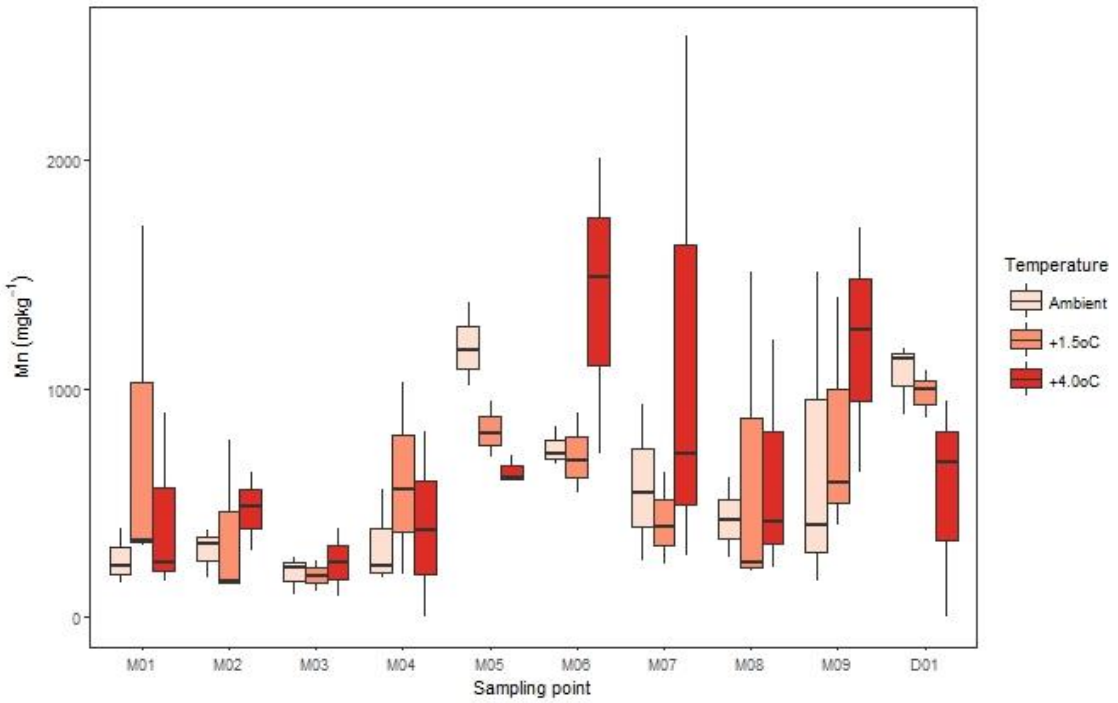

Fig. A4-7.4. Concentrations of manganese in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

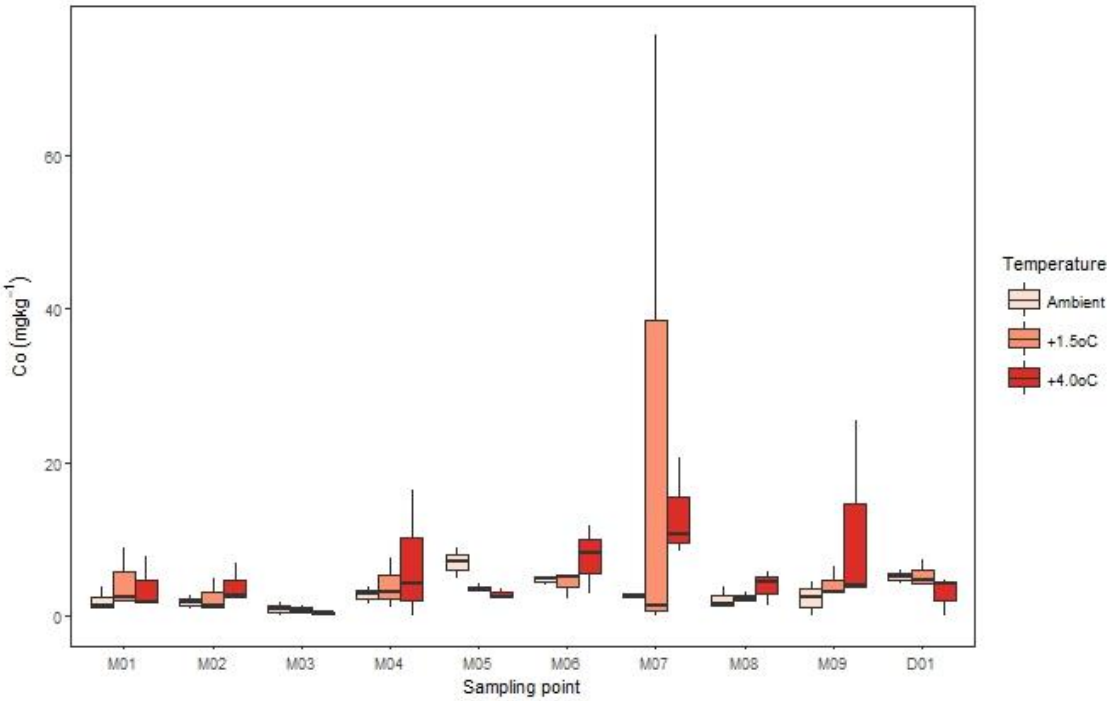

Fig. A4-7.5. Concentrations of cobalt in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

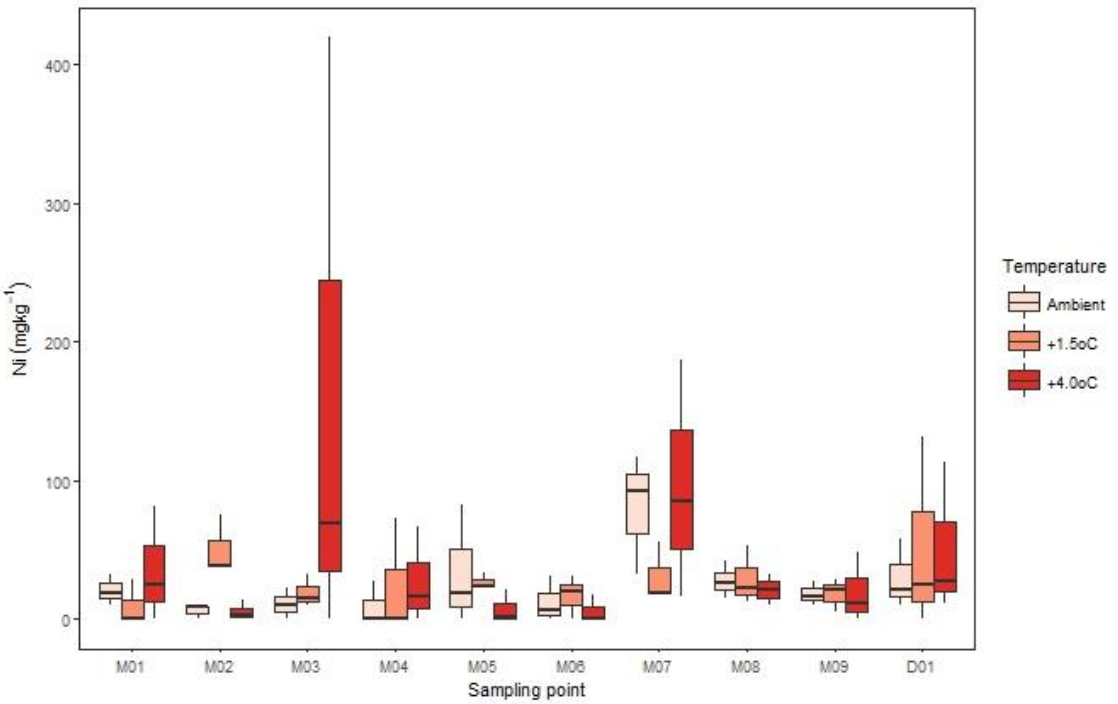

Fig. A4-7.6. Concentrations of nickel in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

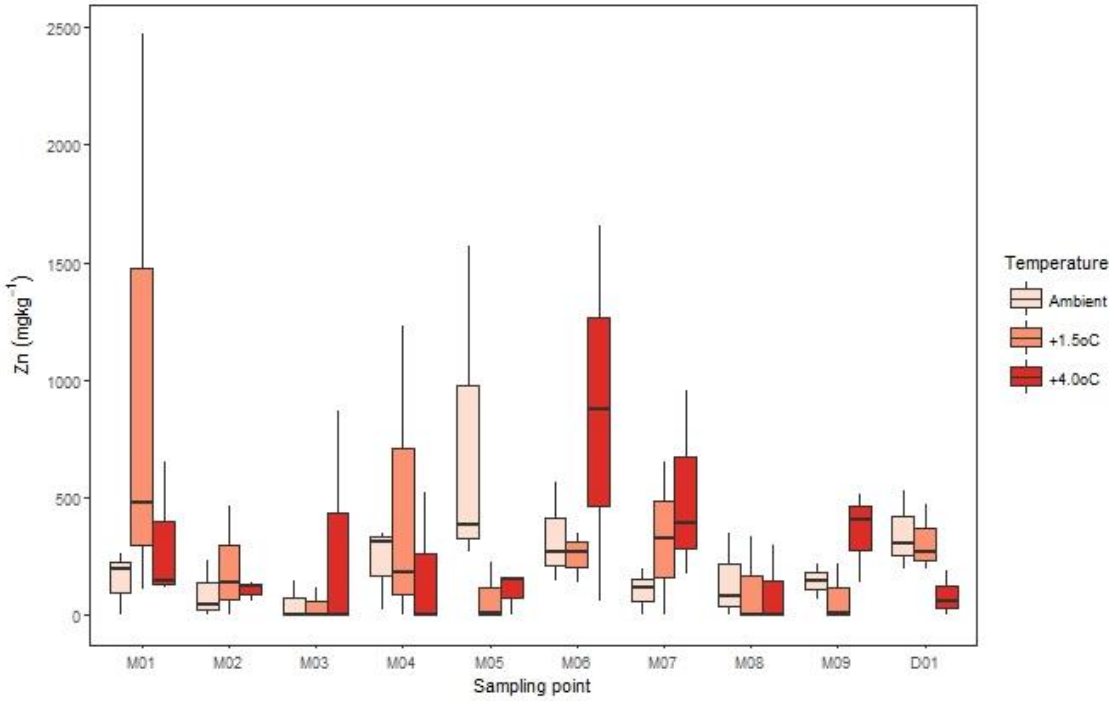

Fig. A4-7.7. Concentrations of zinc in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

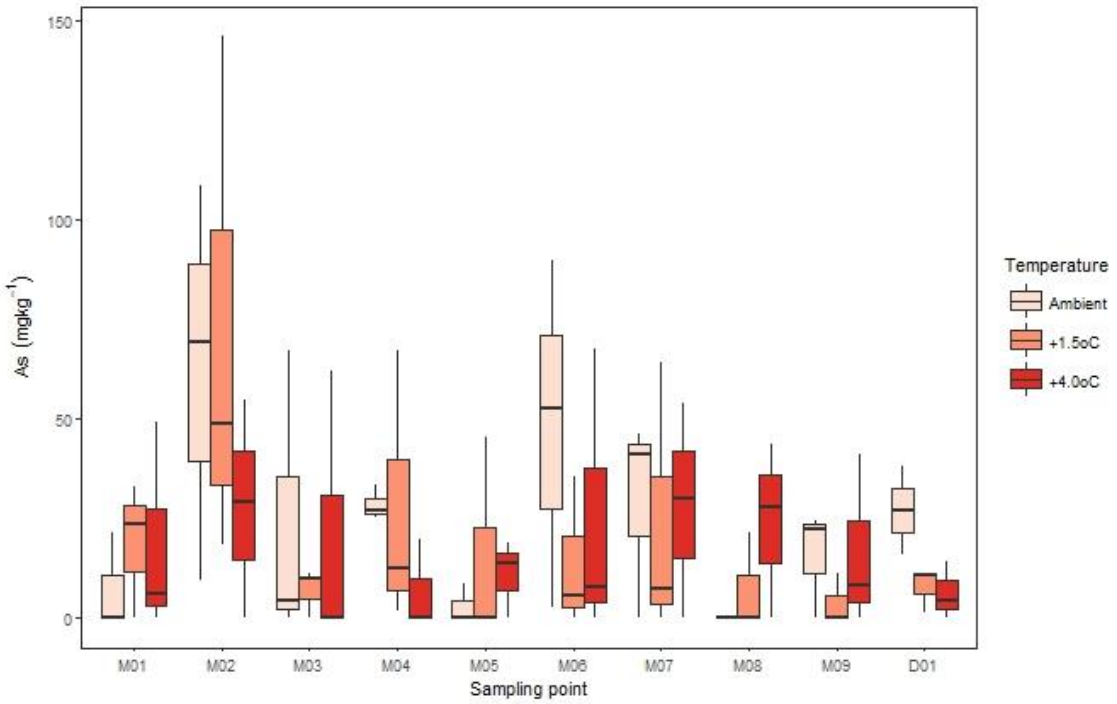

Fig. A4-7.8. Concentrations of arsenic in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

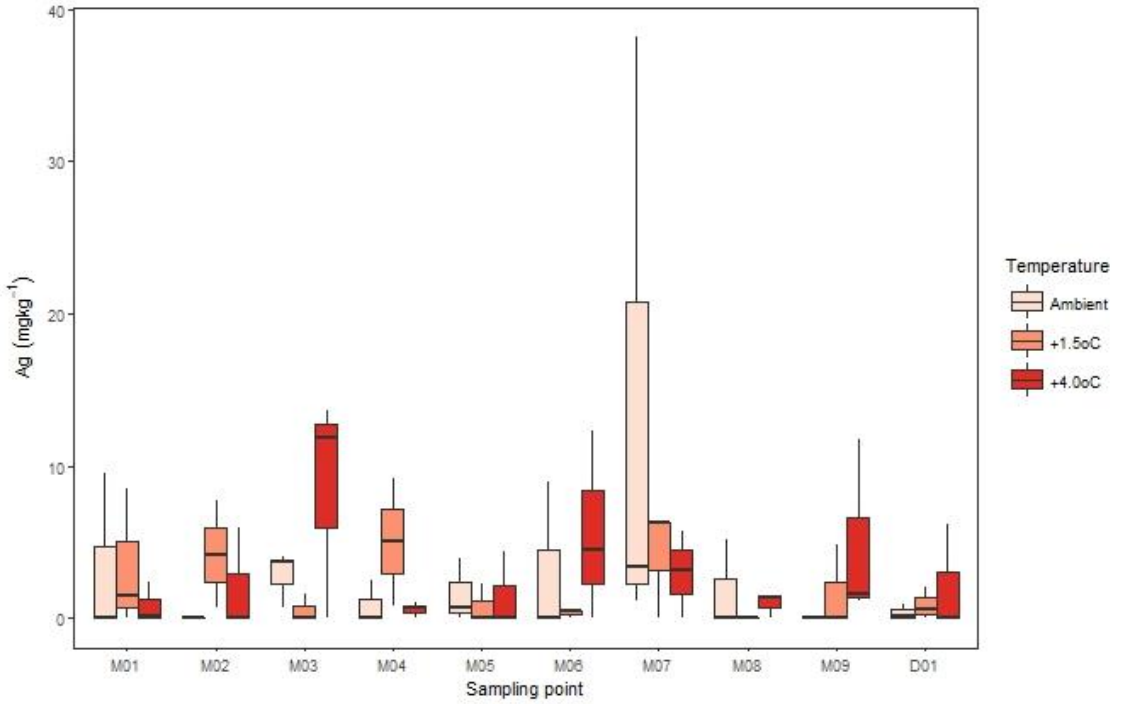

Fig. A4-7.9. Concentrations of silver in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

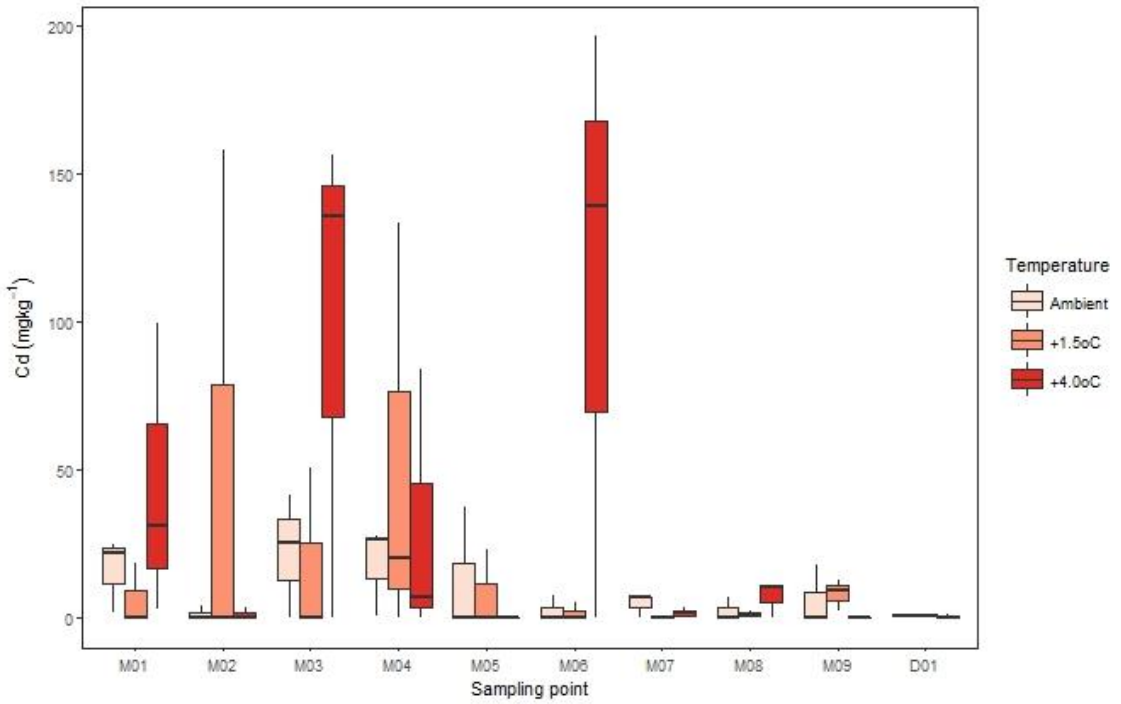

Fig. A4-7.10. Concentrations of cadmium in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

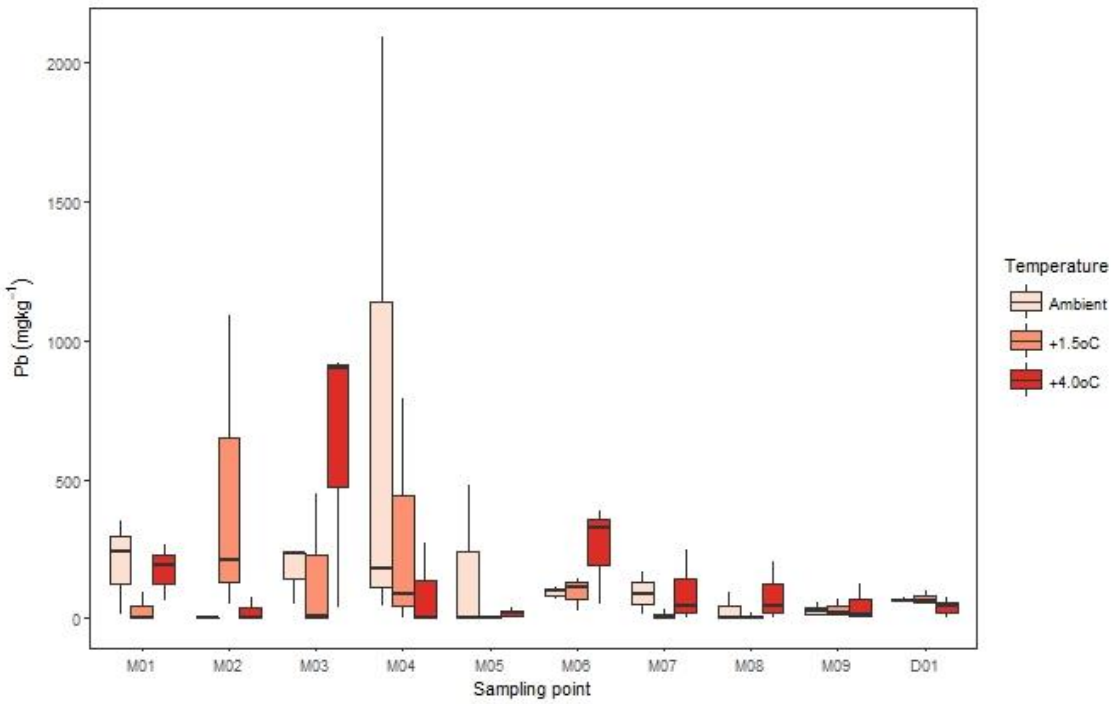

Fig. A4-7.11. Concentrations of lead in diatoms (mg kg^{-1} DW) at all sampling points (n=3)

Appendix 4-8. Analyte concentrations in diatoms at sampling points M01 to M06 and M09 against average sediment temperature

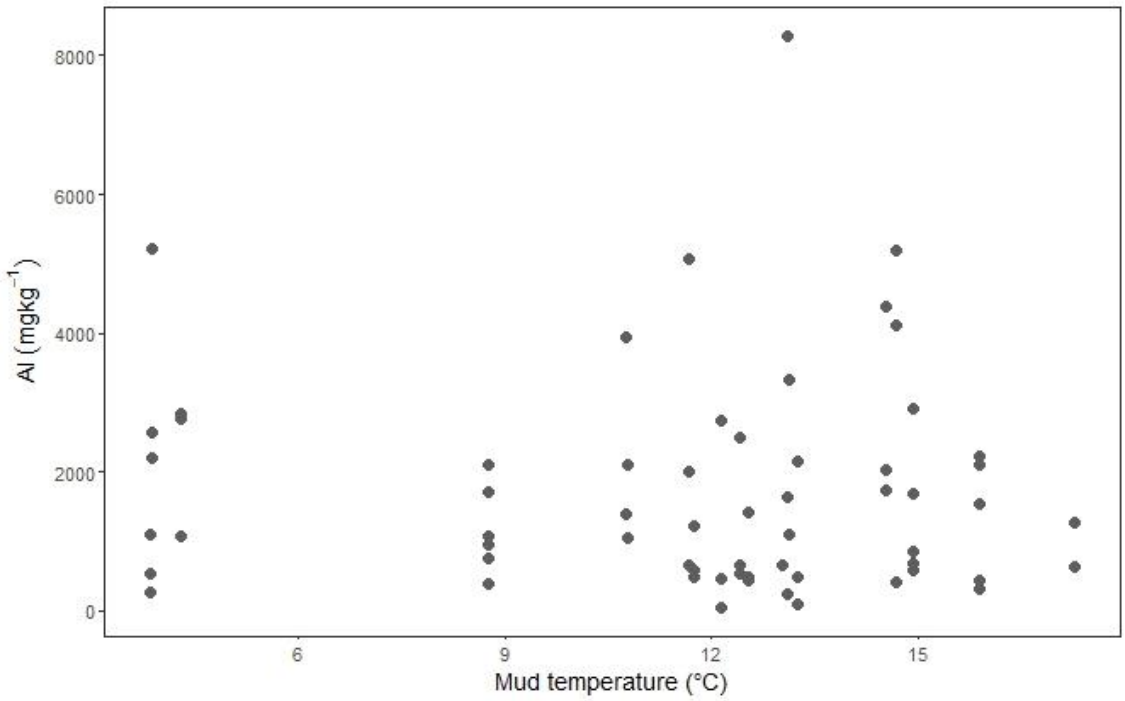

Fig. A4-8.1. Concentrations of aluminium in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

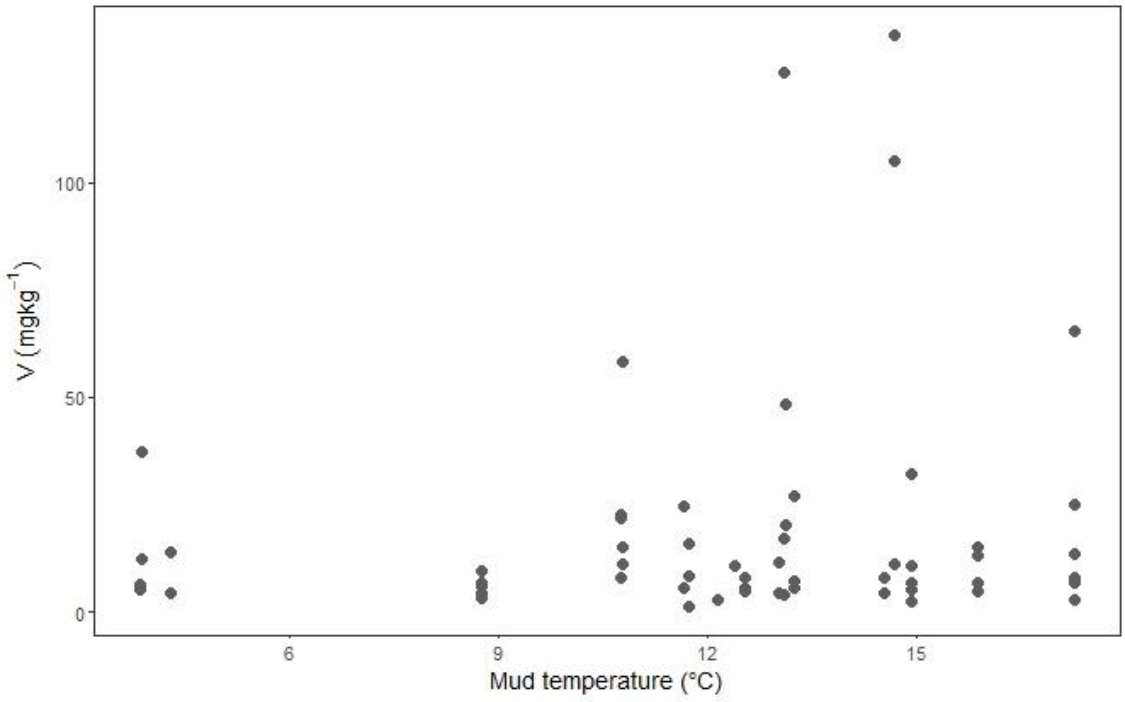

Fig. A4-8.2. Concentrations of vanadium in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

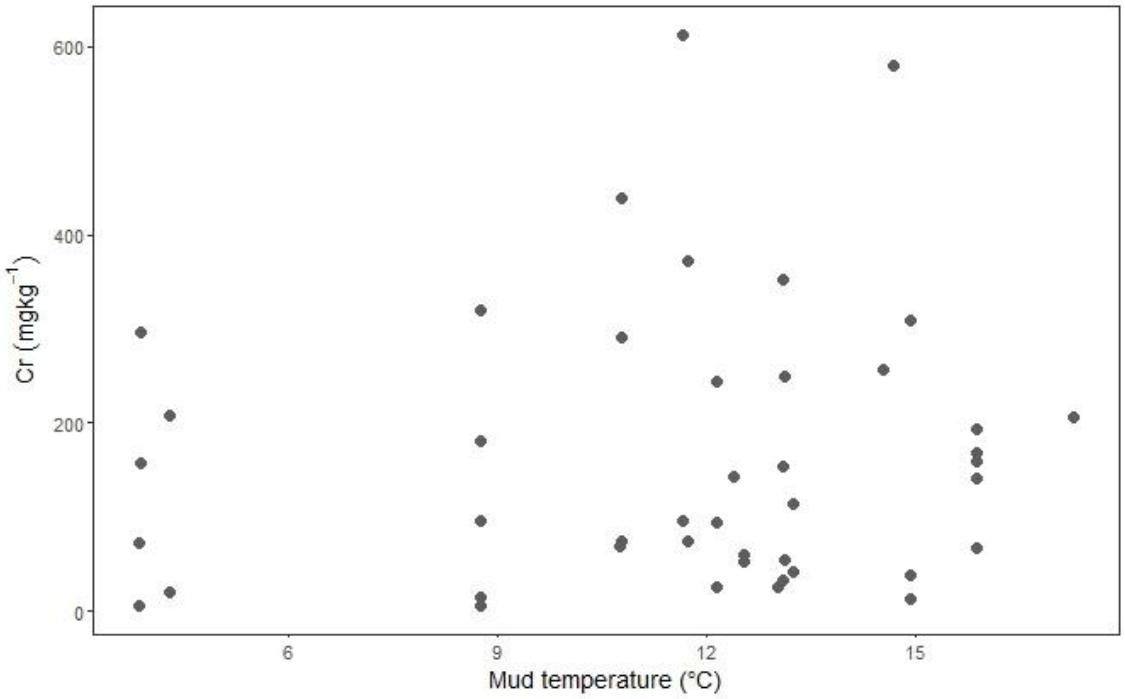

Fig. A4-8.3. Concentrations of chromium in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

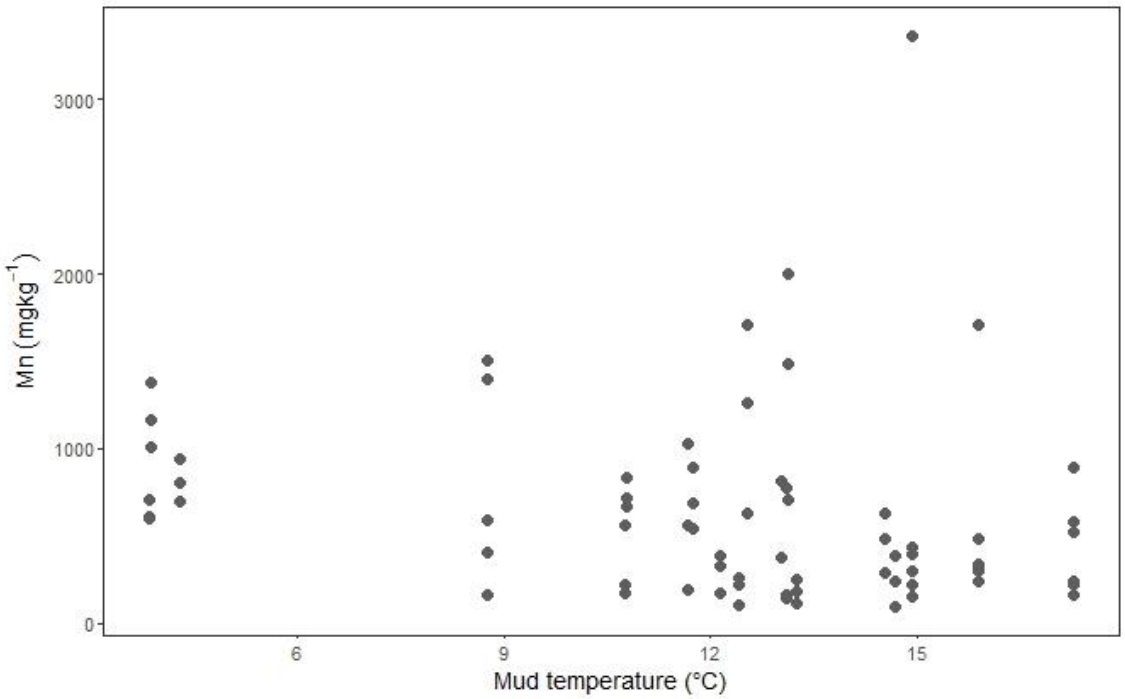

Fig. A4-8.4. Concentrations of manganese in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

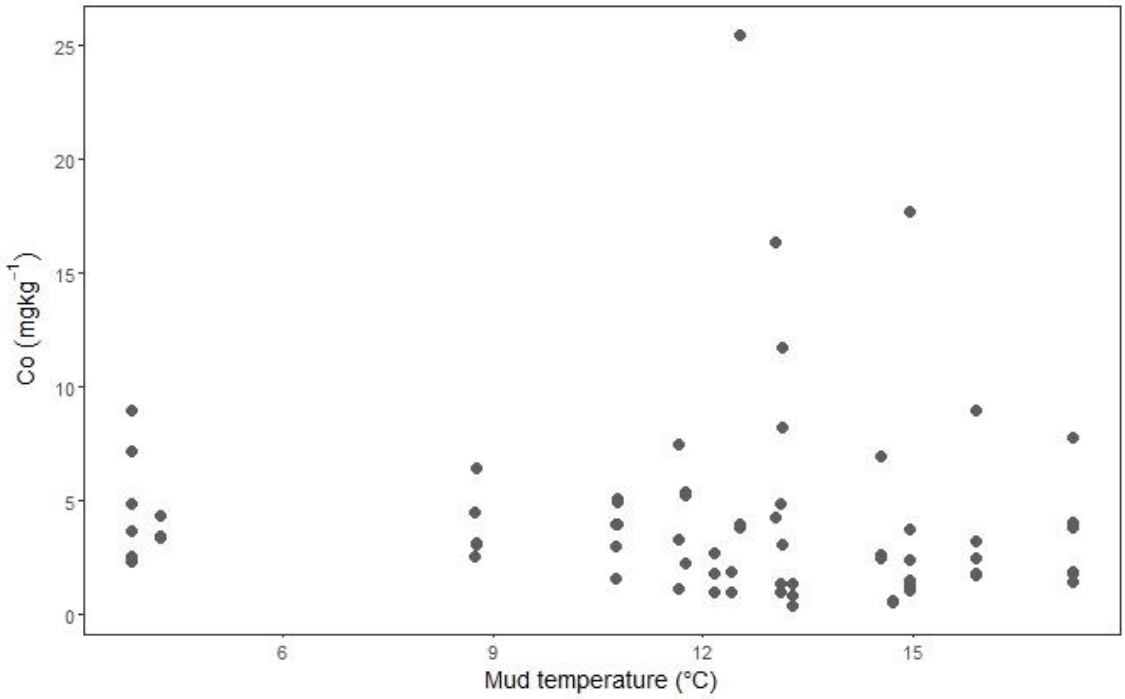

Fig. A4-8.5. Concentrations of cobalt in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

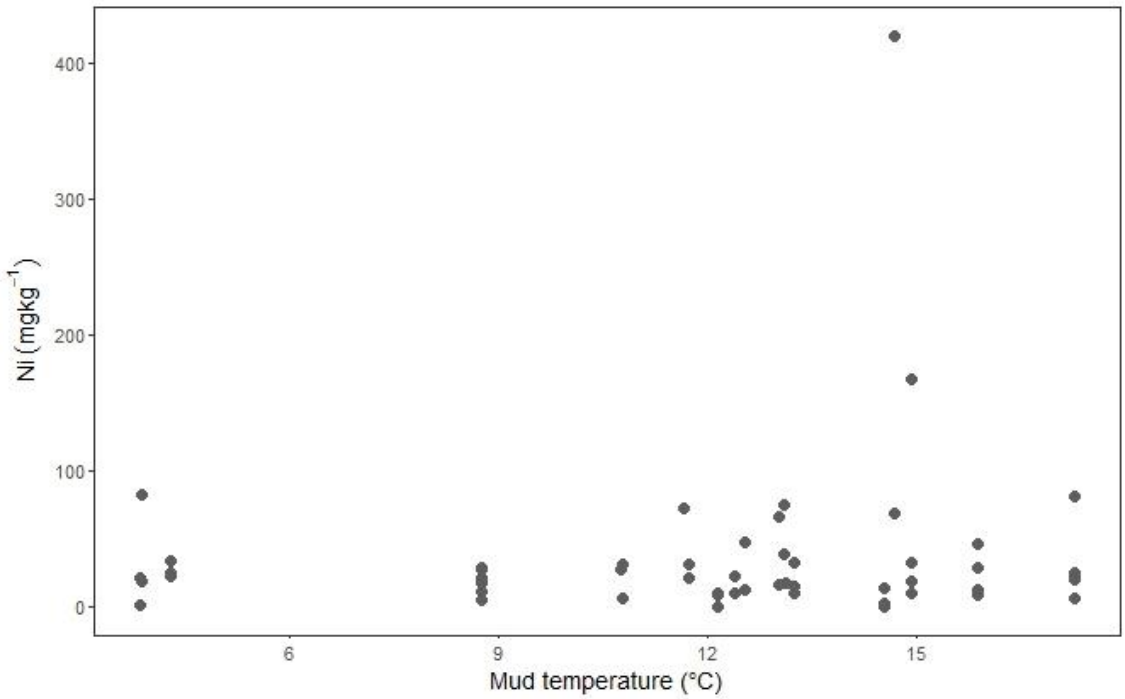

Fig. A4-8.6. Concentrations of nickel in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

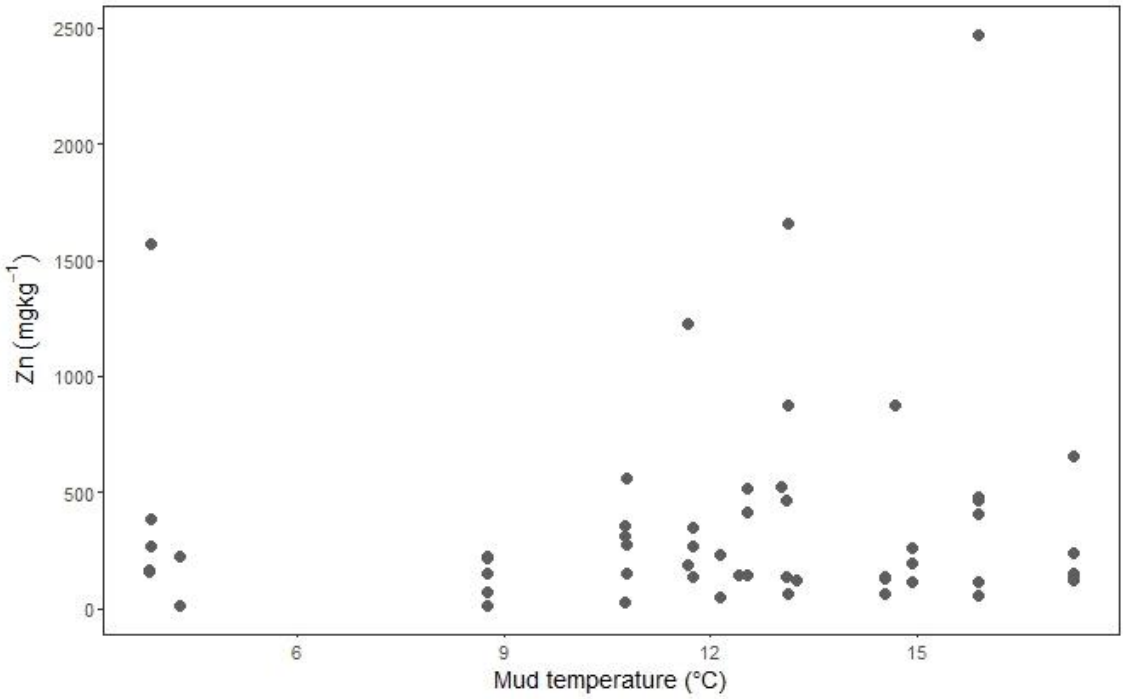

Fig. A4-8.7. Concentrations of zinc in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

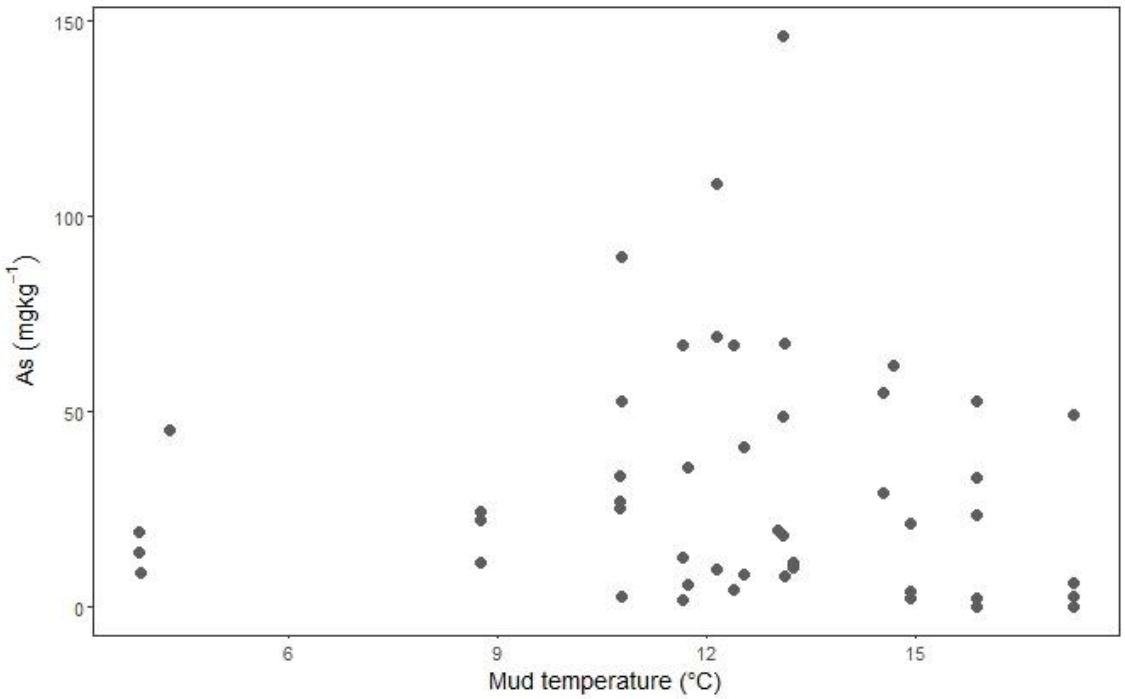

Fig. A4-8.8. Concentrations of arsenic in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

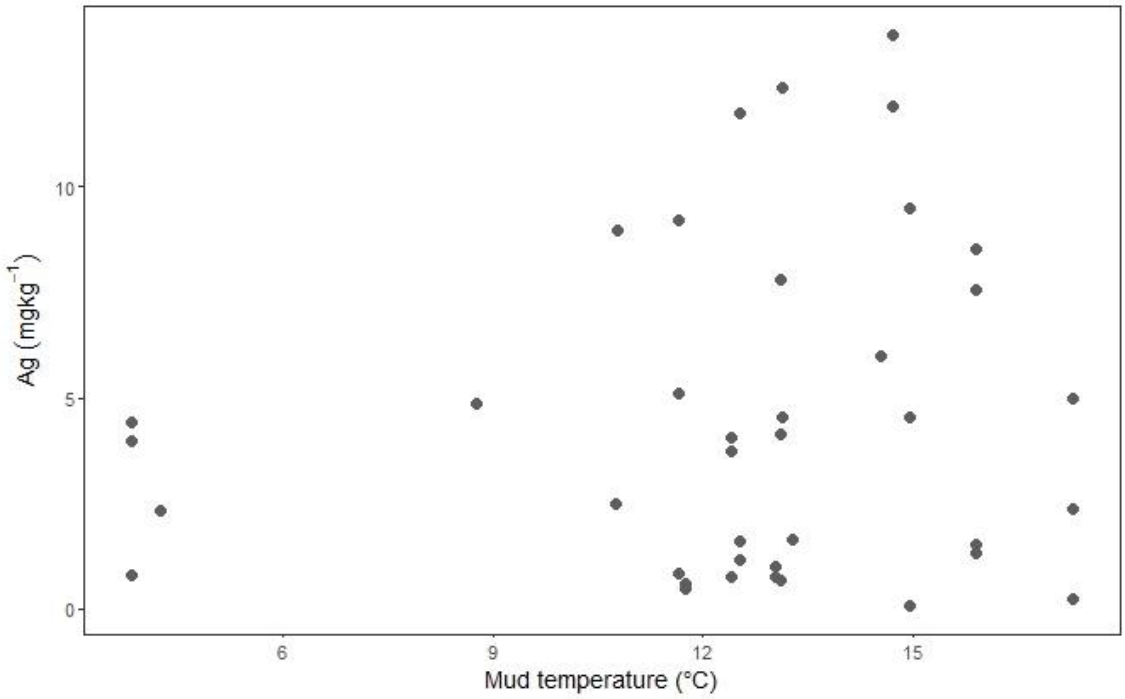

Fig. A4-8.9. Concentrations of silver in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

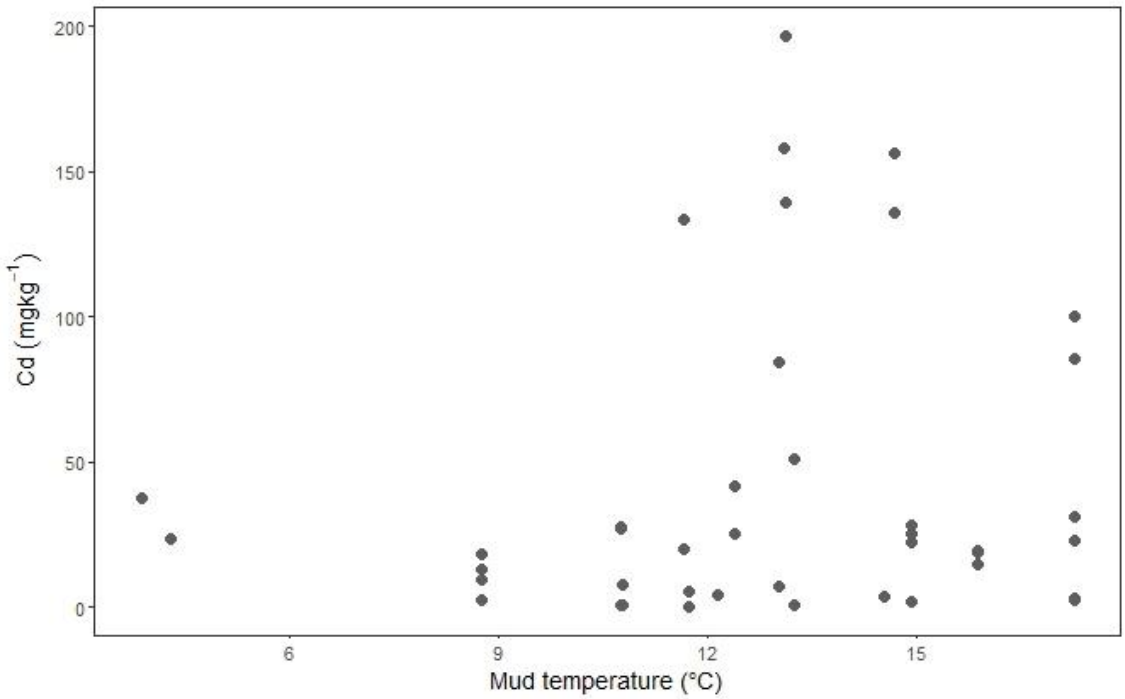

Fig. A4-8.10. Concentrations of cadmium in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

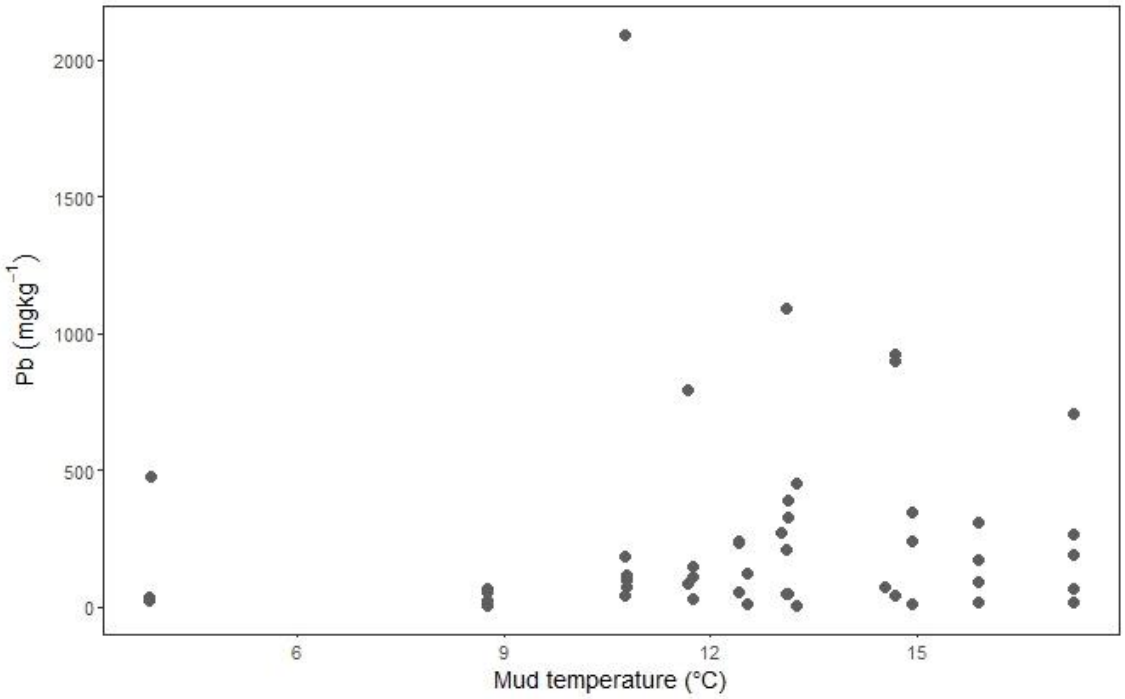

Fig. A4-8.11. Concentrations of lead in diatoms (mg kg^{-1} DW) at sampling points M01 to M06 and M09 against average sediment temperature

Appendix 4-9. Photographs of middle tray (tray 2) of all tanks on six dates

Fig. A4-9.1. Photographs of middle tray (tray 2) of all tanks on 22/10/15, 36 days after start of temperature experiment

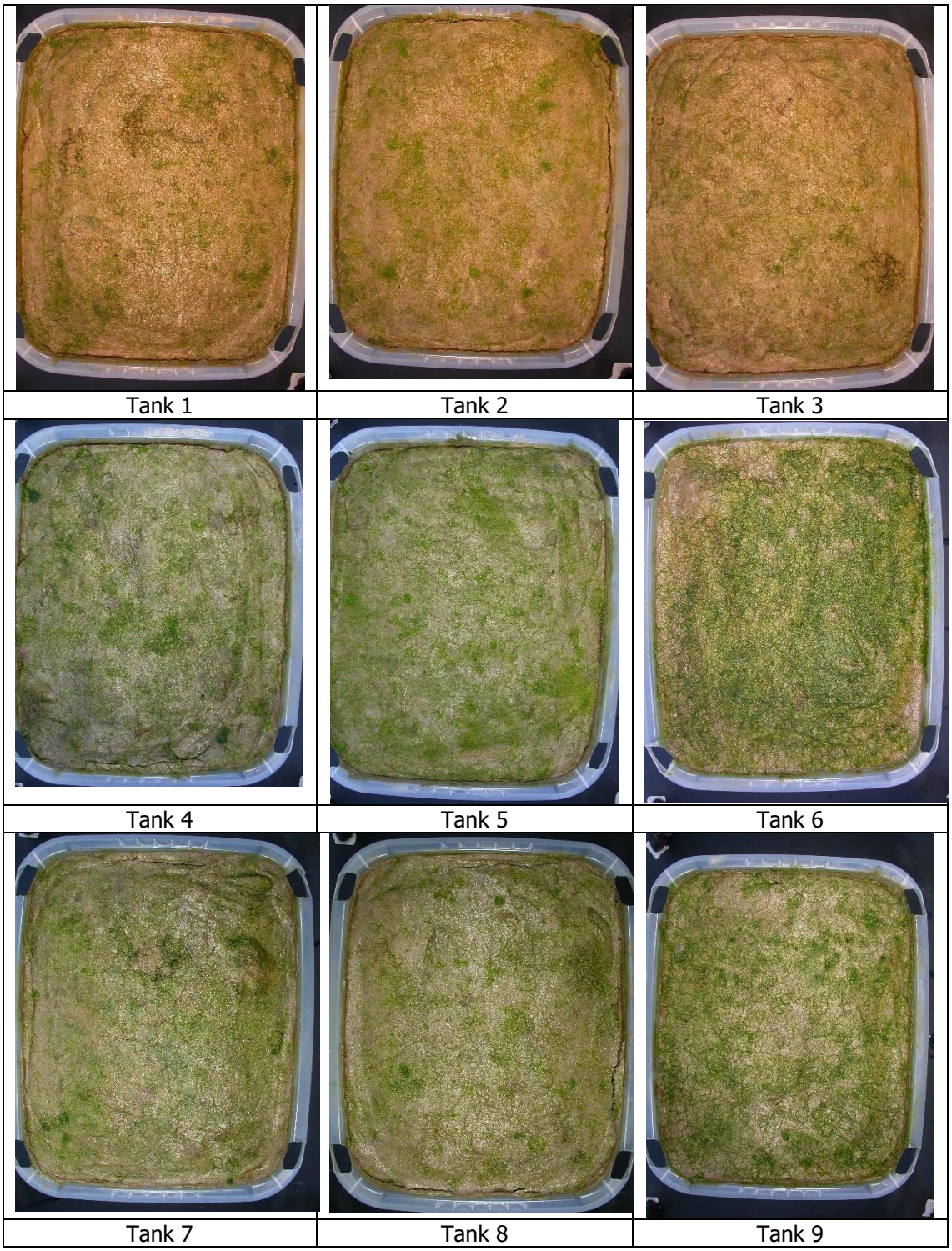

Fig. A4-9.2. Photographs of middle tray (tray 2) of all tanks on 17/11/15, 62 days after start of temperature experiment

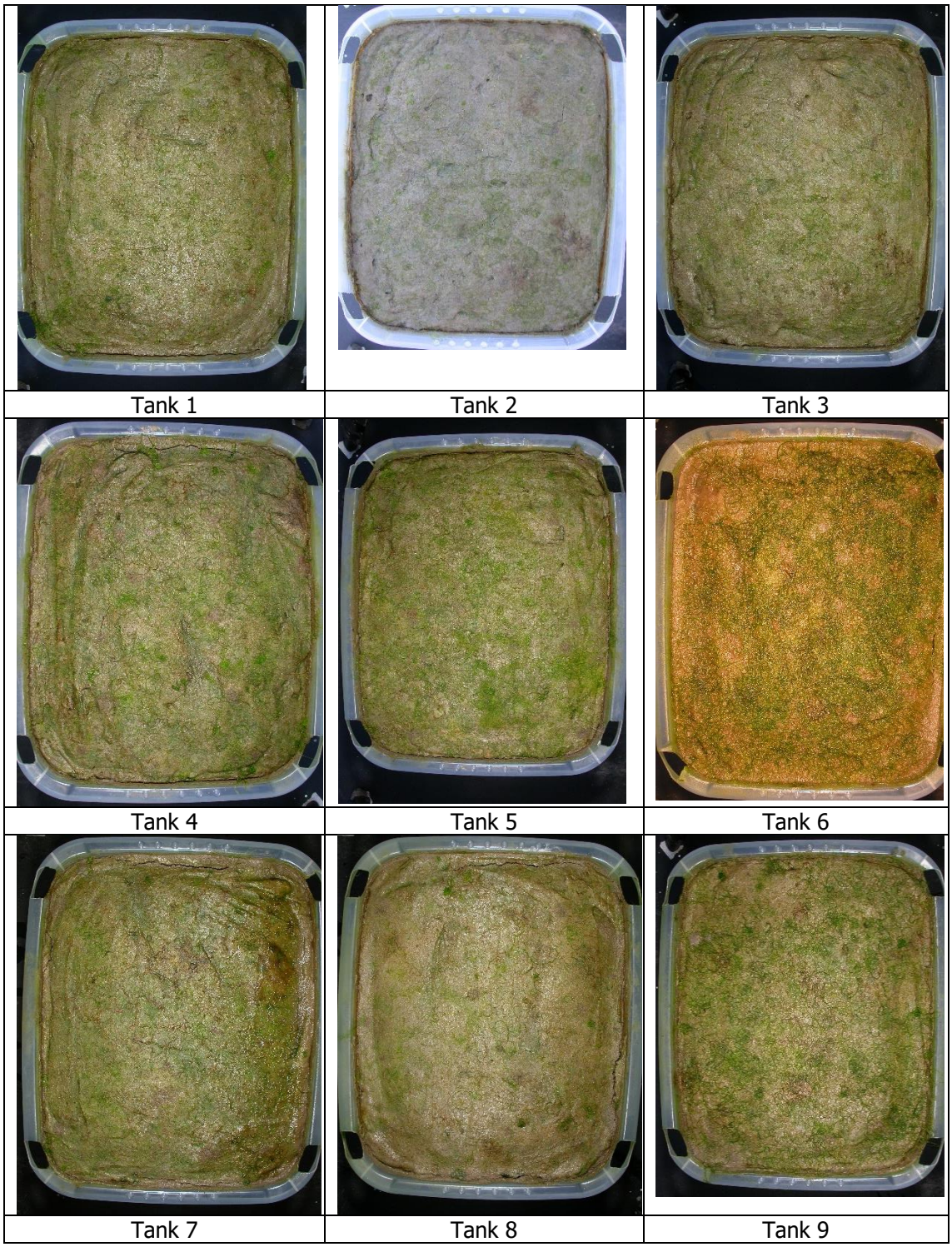

Fig. A4-9.3. Photographs of middle tray (tray 2) of all tanks on 22/12/15, 97 days after start of temperature experiment

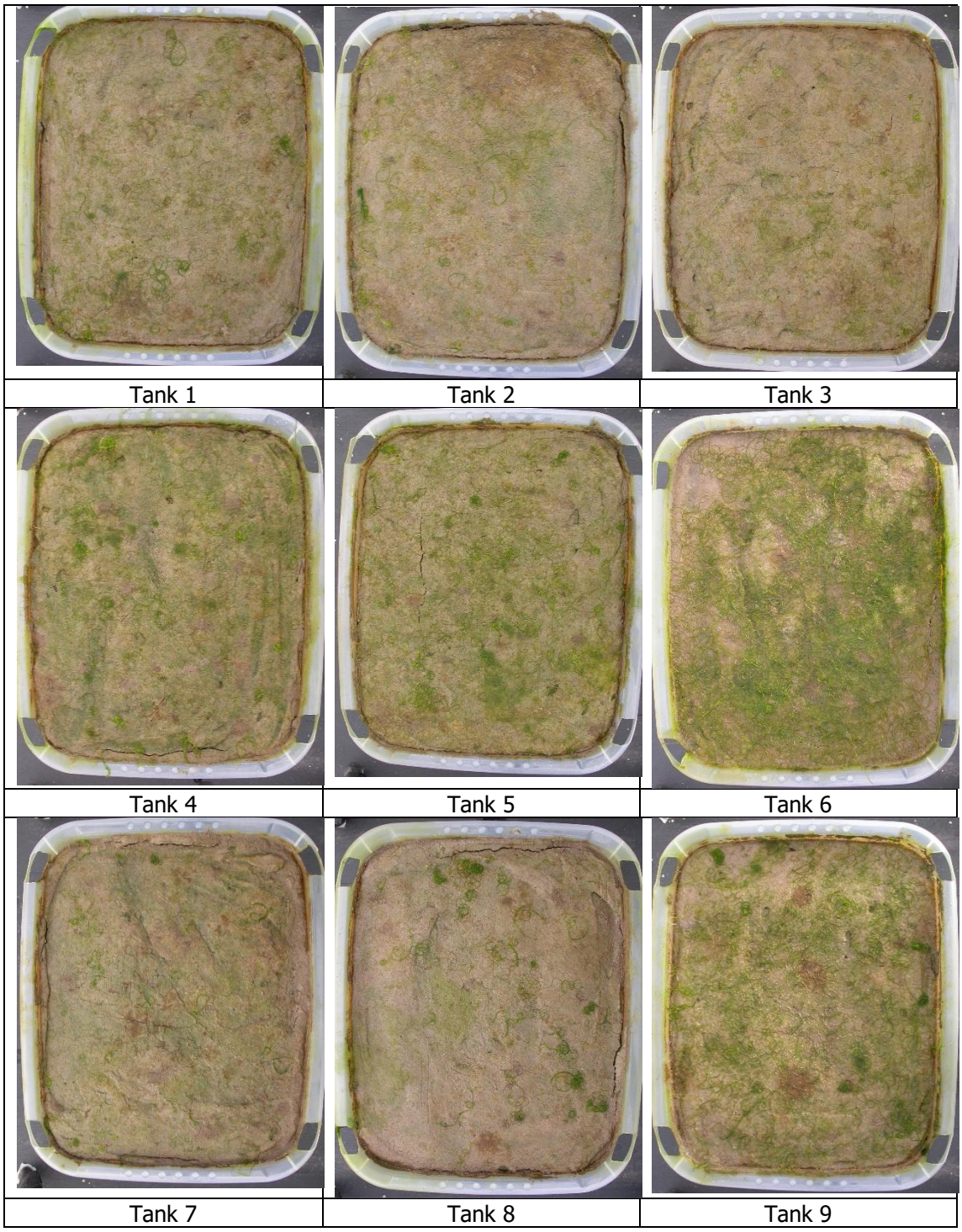

Fig. A4-9.4. Photographs of middle tray (tray 2) of all tanks on 22/1/16, 128 days after start of temperature experiment

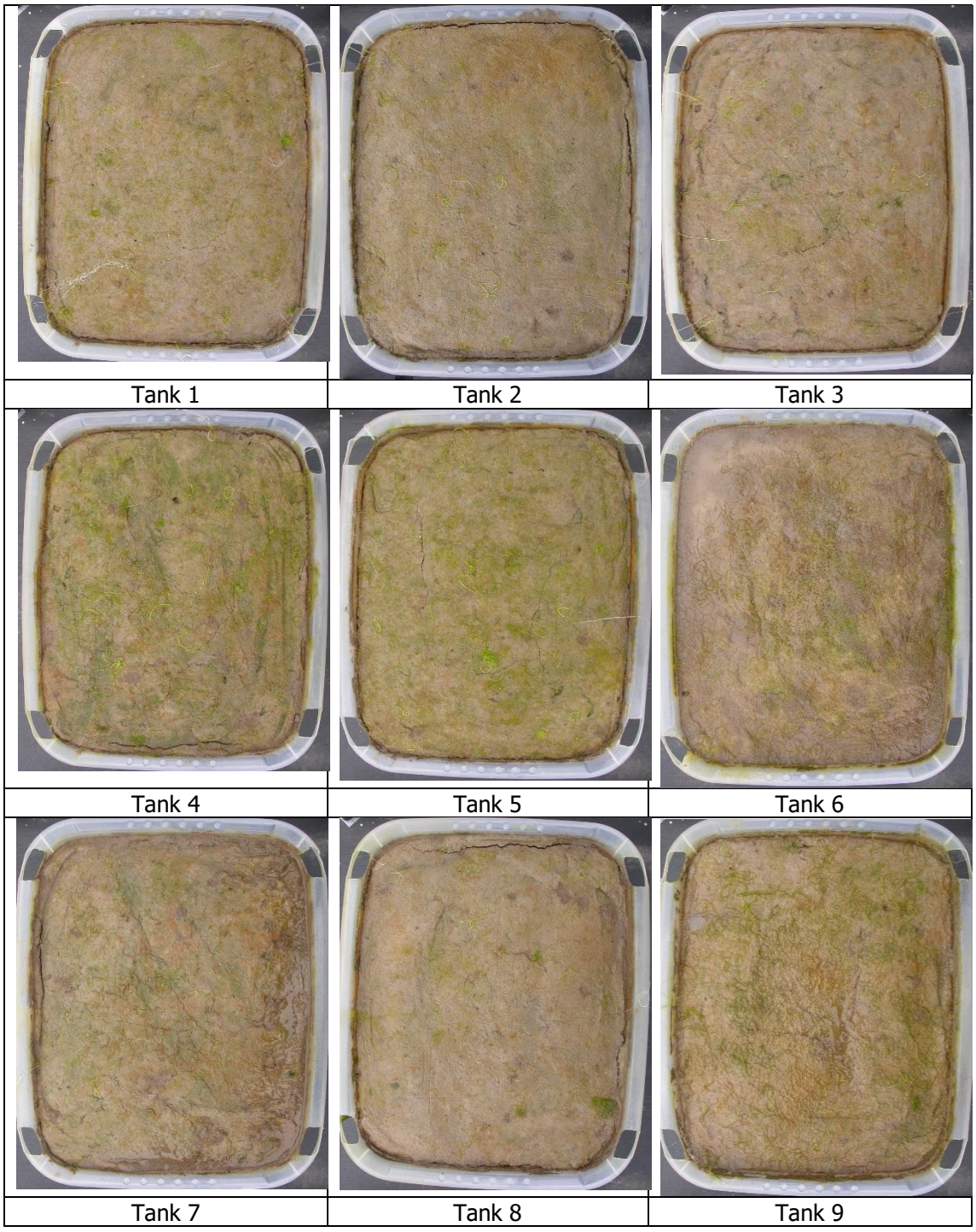

Fig. A4-9.5. Photographs of middle tray (tray 2) of all tanks on 17/3/16, 182 days after start of temperature experiment

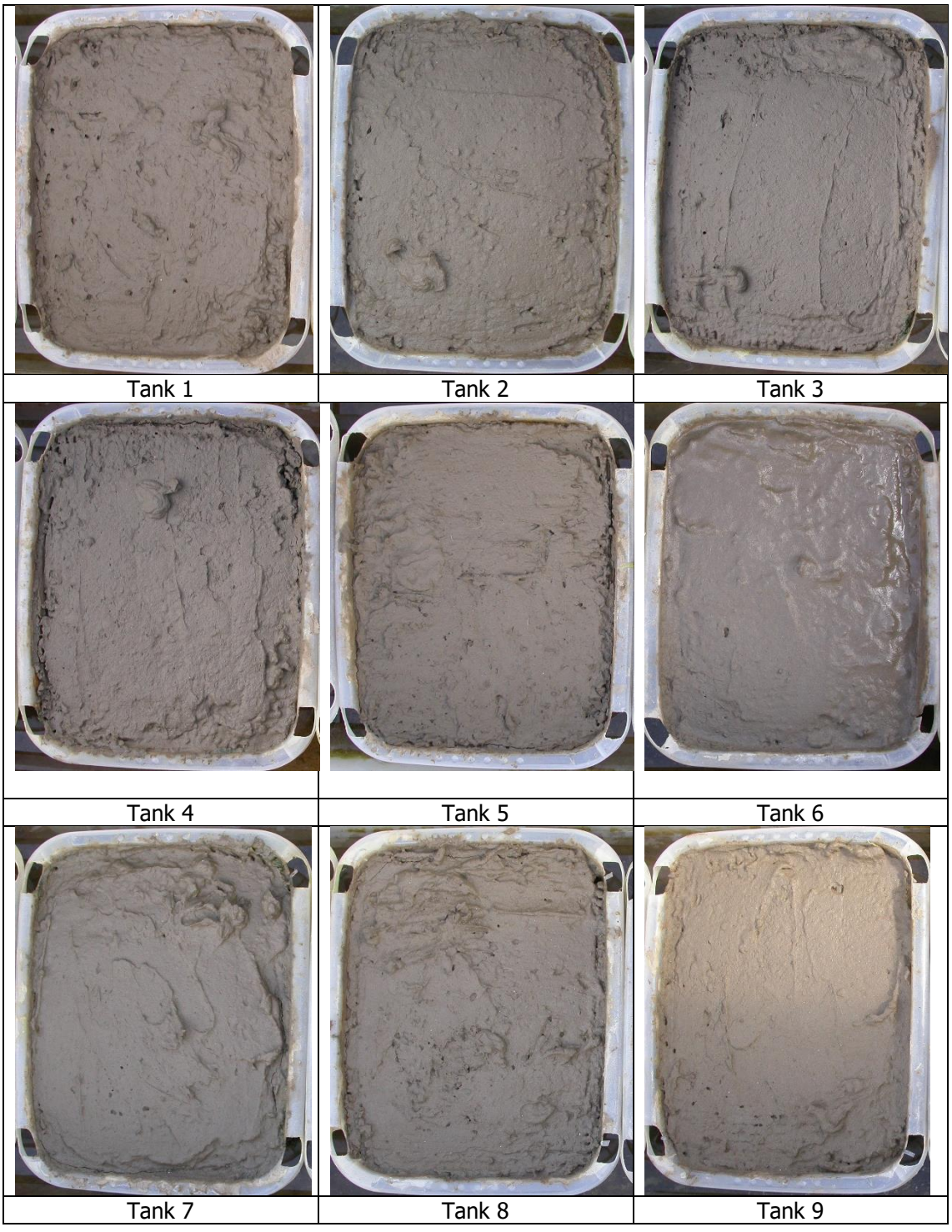

Fig. A4-9.6. Photographs of middle tray (tray 2) of all tanks on 27/4/16, 19 days after start of disturbance experiment

Appendix 5-1. Instrument limit of detection (LoD) for all analytes

Table A5-1.1. Instrument limit of detection of analytes in mg kg^{-1}

Analyte	LoD (mg kg^{-1})
Aluminium	2.24E-02
Vanadium	5.84E-06
Chromium	7.95E-05
Magnesium	1.44E-04
Iron	7.31E-04
Cobalt	4.40E-06
Nickel	2.01E-04
Copper	7.59E-03
Zinc	8.32E-04
Arsenic	3.74E-04
Silver	8.44E-06
Cadmium	1.33E-04
Tin	4.78E-05
Lead	1.19E-04

Appendix 5-2. Percentage of values below the detection limit

Table A5-2.1. Percentage of values which were below the detection limit for three compartments (diatoms, pore water and overlying water) on both sampling dates and therefore given zero values for analysis

Analyte	Diatoms		Pore water		Overlying water	
	Sep-14	Apr-15	Sep-14	Apr-15	Sep-14	Apr-15
Aluminium	0	0	10	20	33	33
Vanadium	0	0	10	10	0	0
Chromium	25	25	10	10	0	0
Manganese	0	0	10	10	66	0
Iron	0	0	10	10	0	0
Cobalt	0	0	10	10	0	0
Nickel	10	15	10	10	33	0
Copper	5	60	10	30	0	100
Zinc	0	25	10	10	0	0
Arsenic	20	25	10	10	0	0
Silver	15	50	10	10	0	100
Cadmium	10	40	30	10	100	100
Tin	0	30	70	30	100	100
Lead	5	5	80	10	100	0

Appendix 5-3. Example R Model Output

candidate model (lmer) to investigate effect of collection method (field or laboratory), date and tidal frame position on the mass of diatoms (mg) collected

mass1<-lmer(data=Mass, Mass..mg.~Collection+Date+tidal.frame+(1|Date), REML=TRUE)

Linear mixed model fit by REML t-tests use Satterthwaite approximations to degrees of freedom [lmerMod]

Formula: Mass..mg. ~ Collection + Date + tidal.frame + (1 | Date)

Data: Mass

REML criterion at convergence: 397.3

Scaled residuals:

Min	1Q	Median	3Q	Max
-0.9717	-0.5109	-0.0383	0.1096	4.8953

Random effects:

Groups	Name	Variance	Std. Dev.
Run	(Intercept)	226.2	15.04
Residual		3505.4	59.21

Number of obs: 40, groups: Date, 2

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	10.395	24.911	0.000	0.417	1.0000
Collectionlab	33.916	18.723	35.000	1.811	0.0787 .
DateApr-15	-32.053	28.337	0.000	-1.131	1.0000
tidal.frameMid	53.537	22.610	35.000	2.368	0.0235 *
tidal.frameLow	-3.752	22.610	35.000	-0.166	0.8692

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:

	(Intr)	Cllctn	DtA-15	tdl.fM
Collectinlb	-0.376			
DateApr -15	-0.569	0.000		
tidal.frmMd	-0.389	0.000	0.000	
tidal.frmLw	-0.389	0.000	0.000	0.429

#Calculation of p and chi-sq value for effect of tidal frame position

Data: Mass

Models:
..1: Mass..mg. ~ Collection + Date + (1 | Date)
object: Mass..mg. ~ Collection + Date + tidal.frame + (1 | Date)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	5	452.25	460.69	-221.12	442.25			
object	7	448.66	460.48	-217.33	434.66	7.5915	2	0.02247 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

#Calculation of p and chi-sq value for effect of date

Data: Mass
Models:
object: Mass..mg. ~ Collection + (1 | Date)
..1: Mass..mg. ~ Collection + Date + (1 | Date)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
object	4	452.85	459.60	-222.42	444.85			
..1	5	452.25	460.69	-221.12	442.25	2.601	1	0.1068

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

#Calculation of p and chi-sq value for effect of collection method

Data: Mass

Models:

..1: Mass..mg. ~ 1 + (1 | Date)

object: Mass..mg. ~ Collection + (1 | Date)

	Df	AIC	BIC	logLik	deviance	Chisq	Chi Df	Pr(>Chisq)
..1	3	453.69	458.75	-223.84	447.69			
object	4	452.85	459.60	-222.42	444.85	2.8382	1	0.09205 .

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix 5-4. Analyte concentrations in sediment

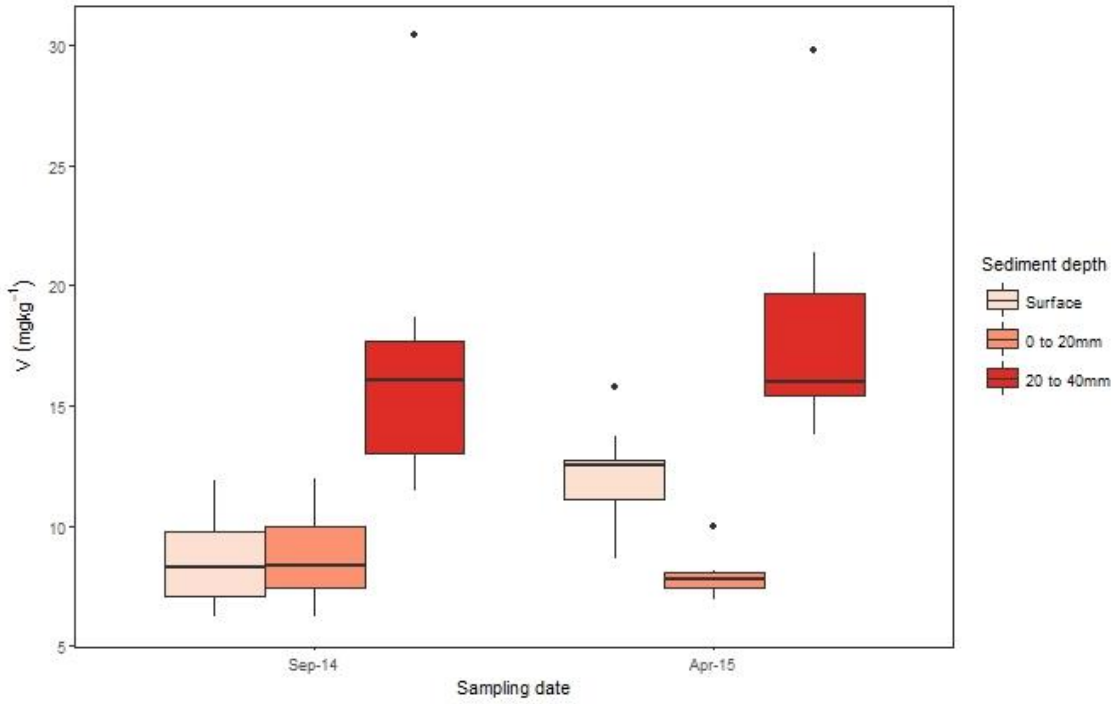

Fig. A5-4.1. Vanadium concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

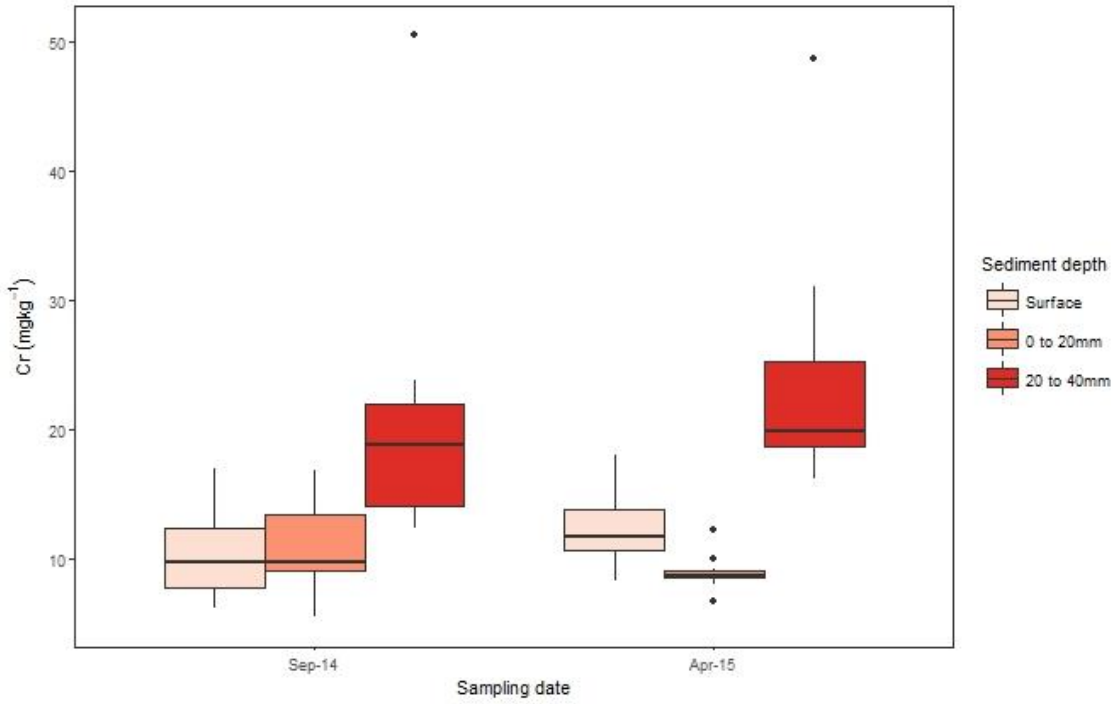

Fig. A5-4.2. Chromium concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

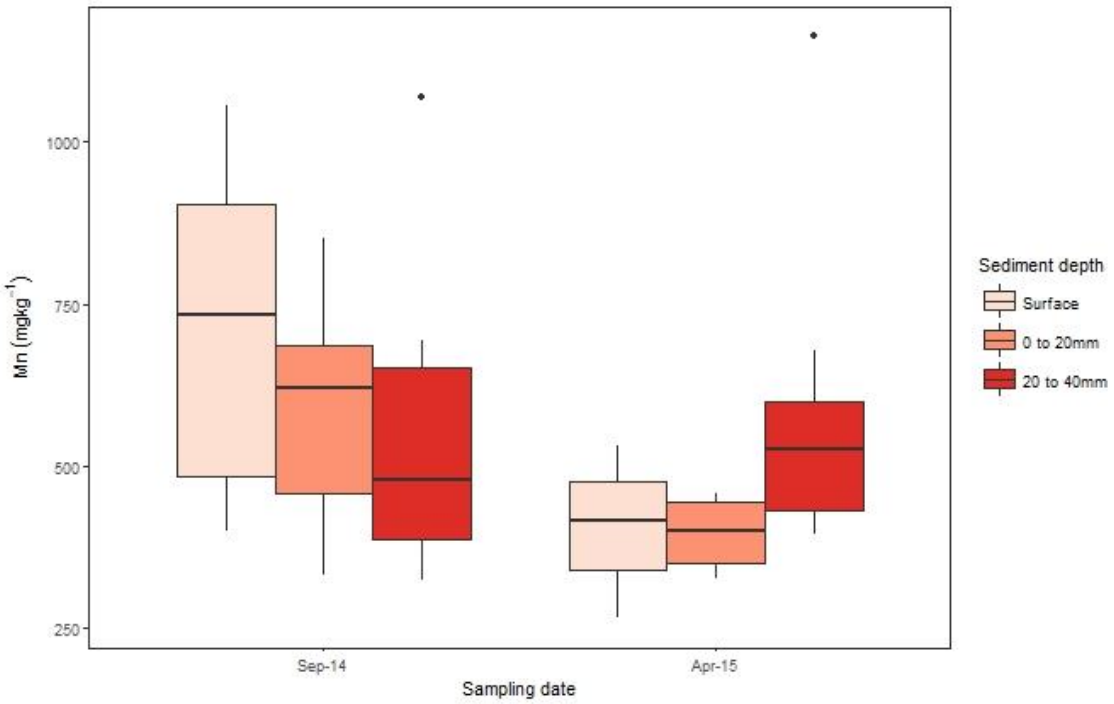

Fig. A5-4.3. Manganese concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

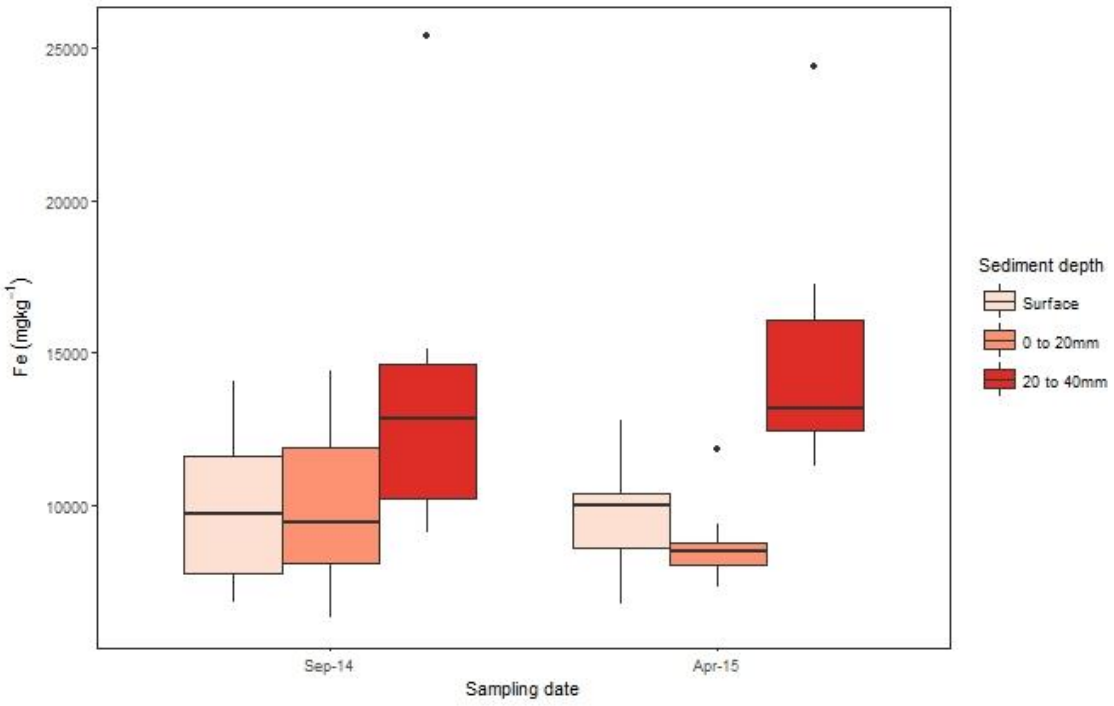

Fig. A5-4.4. Iron concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

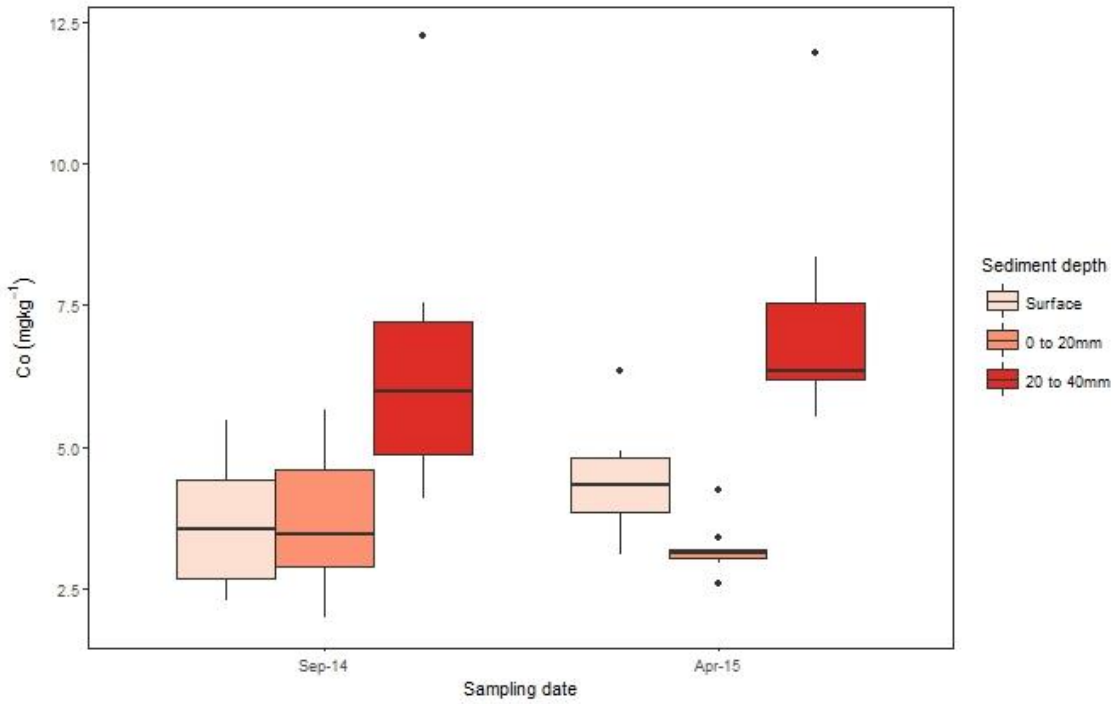

Fig. A5-4.5. Cobalt concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

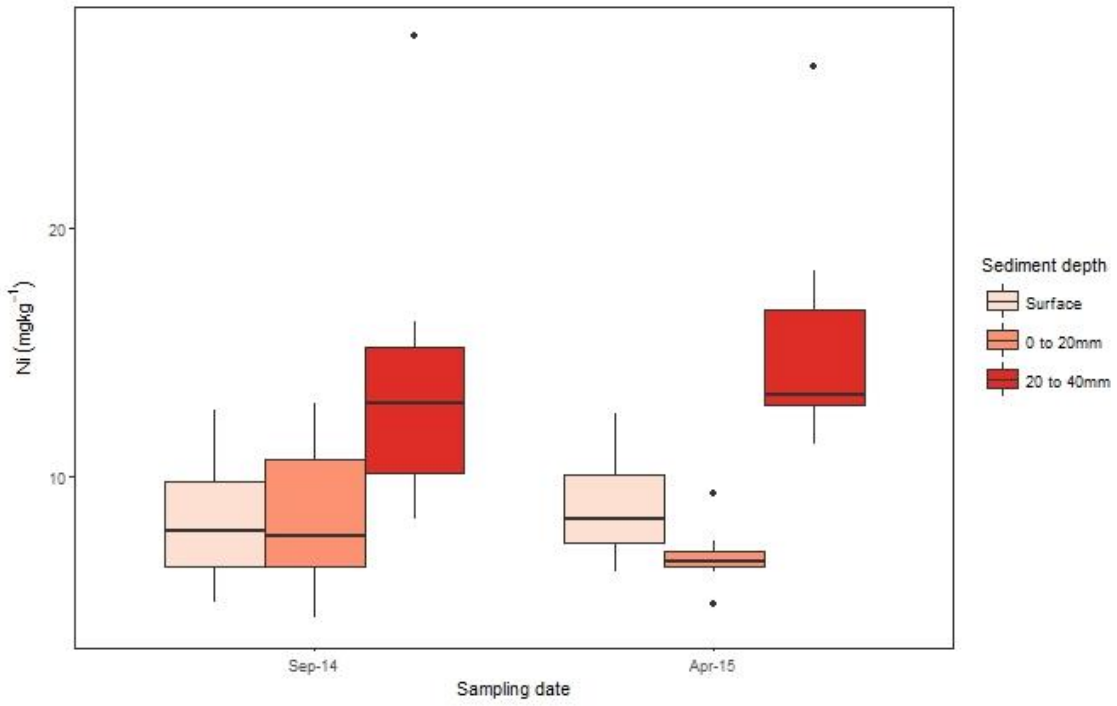

Fig. A5-4.6. Nickel concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

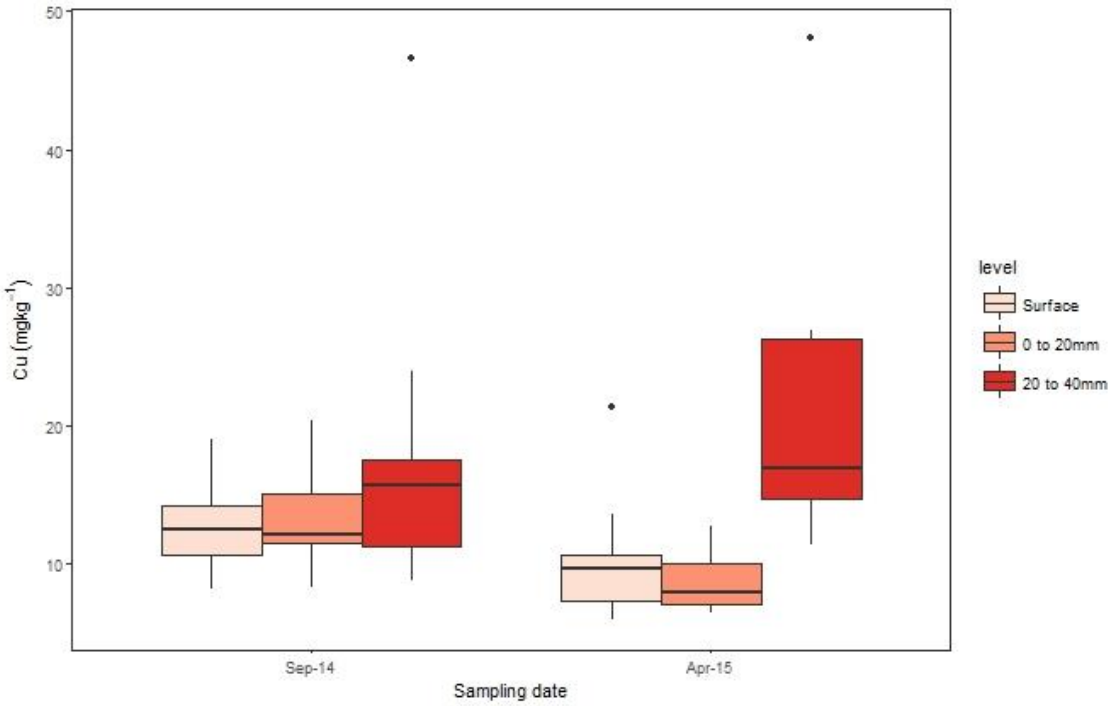

Fig. A5-4.7. Copper concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

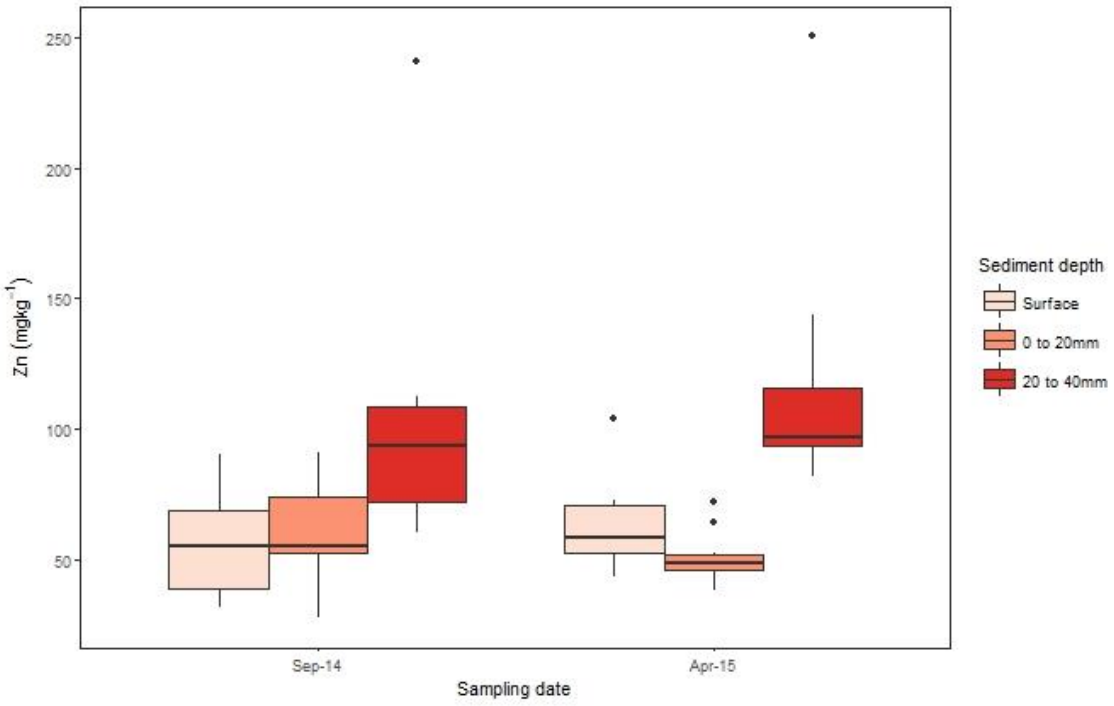

Fig. A5-4.8. Zinc concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

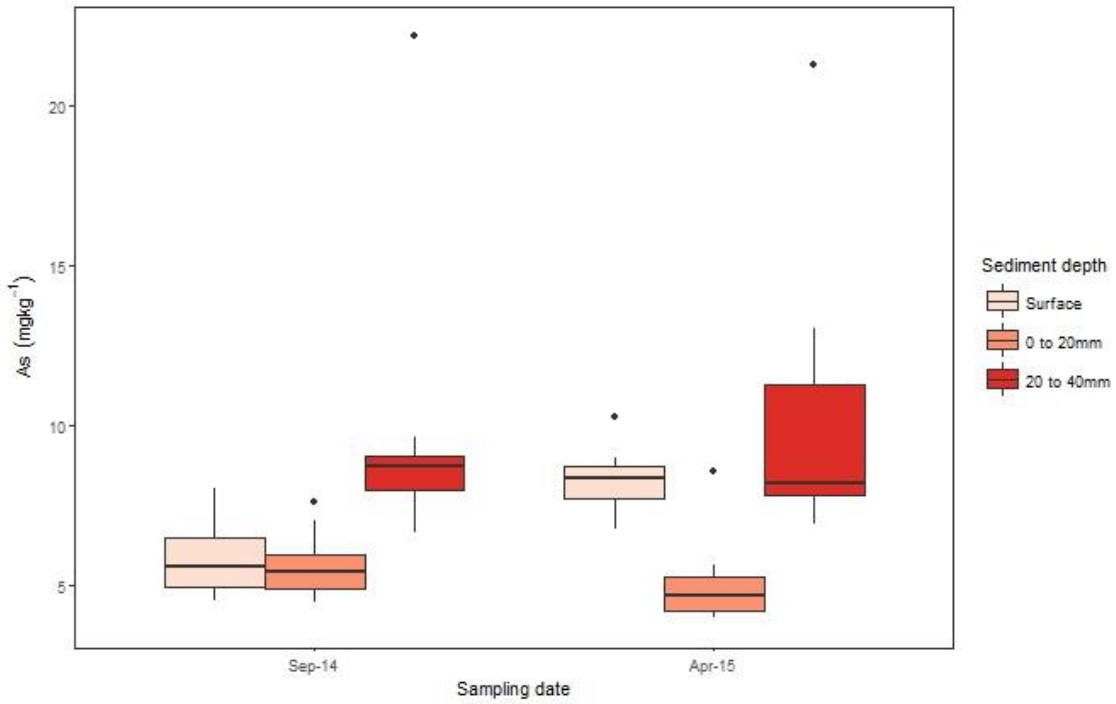

Fig. A5-4.9. Arsenic concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

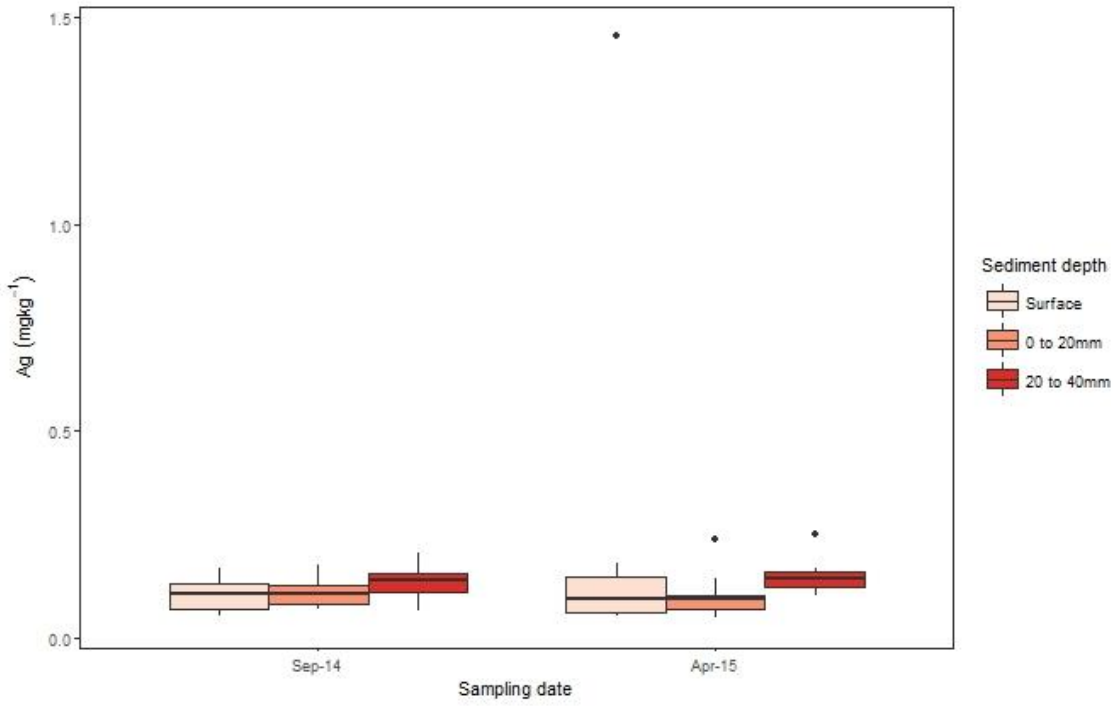

Fig. A5-4.10. Silver concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

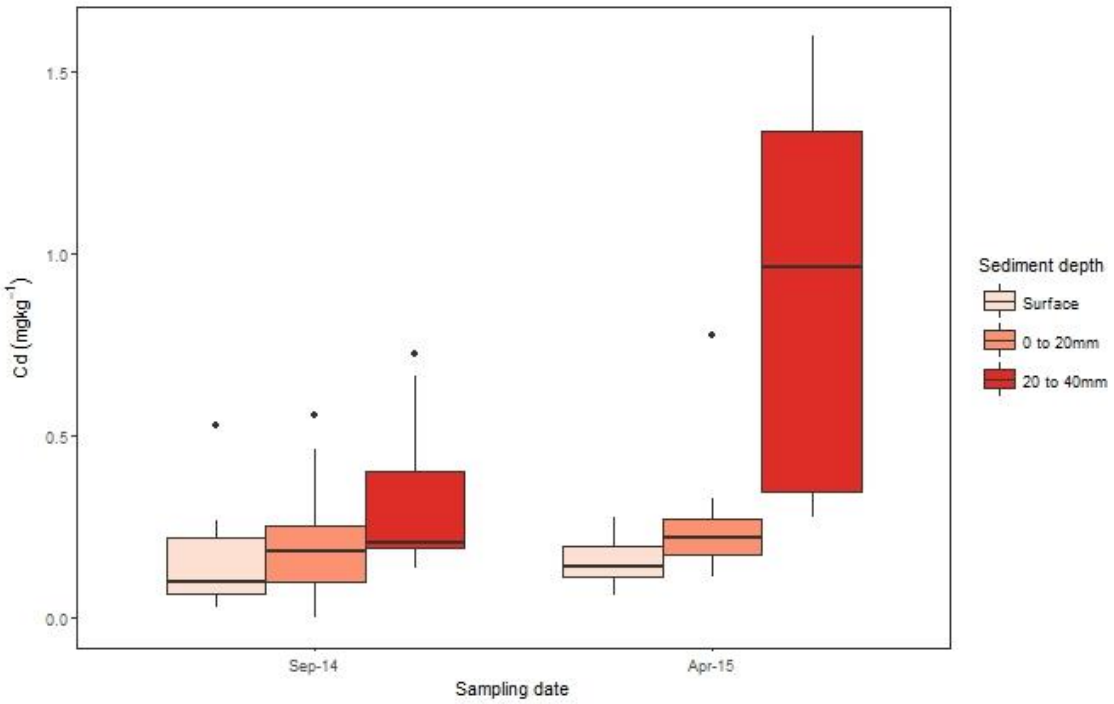

Fig. A5-4.11. Cadmium concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

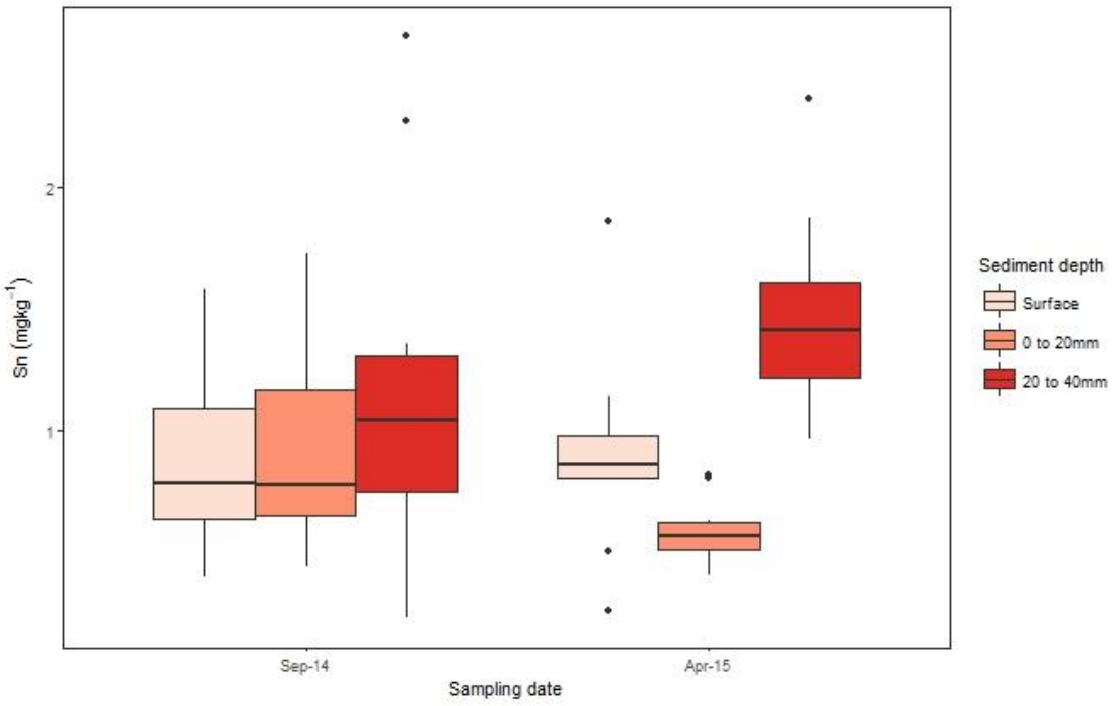

Fig. A5-4.12. Tin concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)

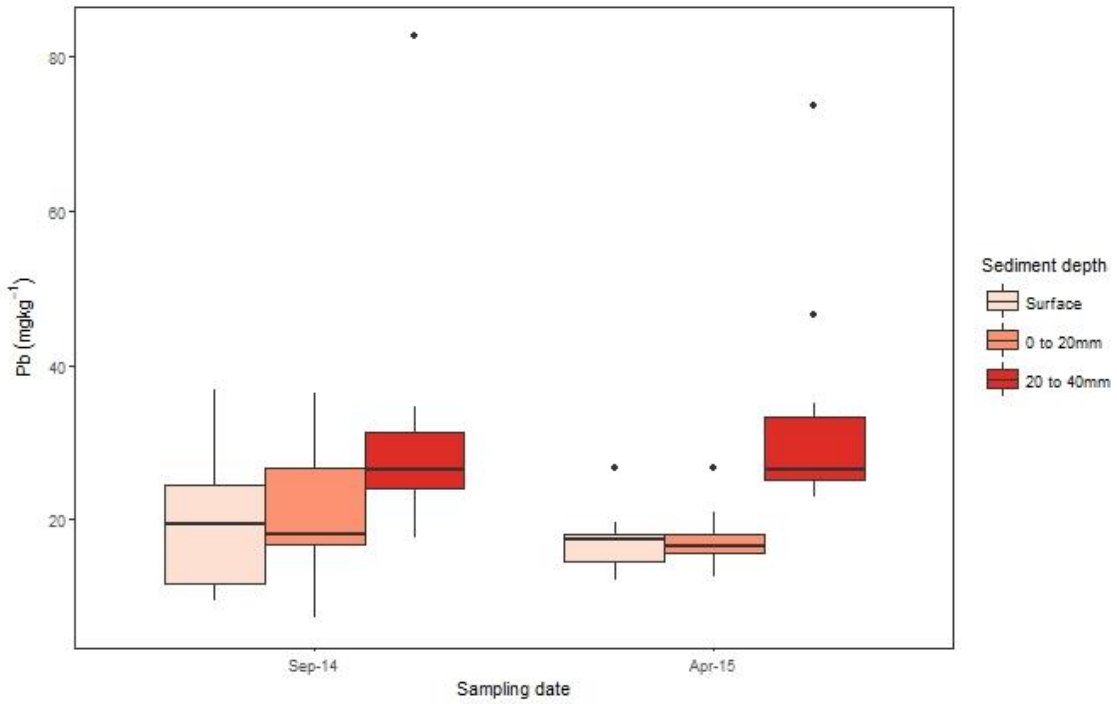

Fig. A5-4.13. Lead concentrations in sediment (mg kg^{-1} DW) in September 2014 and April 2015 at three depths, samples for all tidal frame heights combined (n=10)