

# An assessment of a sub-tropical seagrass, *Zostera muelleri*, as a potential bioindicator of trace elements

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Presented for the degree of Masters of Applied Science

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December 2019



## Abstract

Semi-enclosed estuarine areas along the east coast of Australia accommodate industrial and shipping activities, but are also often areas of suitable seagrass habitat. Port Curtis is one such semi-enclosed estuary located in Gladstone, Queensland, and accommodates Australia's fifth largest multi-commodity port. The local consortium, Port Curtis Integrated Monitoring Program Inc. (PCIMP) were looking for a local ecologically relevant trace element (TE) bioindicator to complement current sediment and water quality monitoring. The overarching aim of this research was to ascertain whether the locally predominant seagrass species, *Zostera muelleri*, could be a potential TE bioindicator. *Zostera muelleri* already meets some TE bioindicator criteria in that it is present where PCIMP monitors and is abundant enough to sample; however, further investigation of the ecology of *Z. muelleri* with respect to TE exposure was required to ascertain if the species was suitable. Specifically, the study examined *Z. muelleri*'s capacity to accumulate, partition and translocate TEs in relation to environmental TE concentrations over the spatial and temporal scale within the field and under manipulated experimental conditions.

Spatial assessments were undertaken by assessing whole *Z. muelleri* TE concentration variability (Al, As, Cr, Cd, Cu, Fe, Pb, Mn, Ni and Zn) between and within five locations across Port Curtis during the peak growing period. It was expected that if *Z. muelleri* was a good indicator of differences in environmental TE exposure, TE concentrations would vary between locations more than within locations. Additionally, other factors such as plant morphology, sediment characteristics and epiphyte cover could drive location variation. Results indicated that each seagrass TE (except Zn) had significantly different spatial variability, suggesting that different natural or anthropogenic TE sources exist within Port Curtis. Additionally, localised meadow influences created significant within-meadow effects for seagrass As, Cu, Fe and Ni concentrations. Seven of the ten TEs analysed in *Z. muelleri* had strong relationships with sediment TEs; however, no comparison to water TEs could be made due to low concentrations in water samples tested. Percent silt and % epiphyte cover explained the greatest variation in seagrass TE concentrations. *Zostera muelleri* TE concentrations demonstrated different location TE exposures, suggesting that it would be a good bioindicator of TEs.

*Zostera muelleri* TE concentrations (Al, As, Cr, Cd, Cu, Fe, Pb, Mn, Ni and Zn) were observed over the active growing season of the Austral spring to summer. It was expected that seagrass TE concentrations within seagrass compartments would change

over time due to either seasonal growth or external environmental TE exposures. Trace element concentrations in *Z. muelleri* were variable between seagrass compartments (e.g., Cu was greater in the above-ground compartment than in the below-ground compartment) and over time. Variations in seagrass TE concentrations over time were grouped and explained by either biological characteristics such as growth, or by external summer influences and did not appear to be due to environmental TE concentrations. It is evident that TE concentrations in *Z. muelleri* are influenced by season, limiting when and how often to sample *Z. muelleri* as a bioindicator.

Light and salinity are two environmental variables that are dynamic within estuarine areas. It was suspected that these variables could influence the capacity of *Z. muelleri* to accumulate TEs and therefore its recommendation as a bioindicator. Salinity and light were manipulated within two individual laboratory experiments with exposure to one element, Cu, due to known effects on *Z. muelleri*. Copper exposures were control, low ( $5 \mu\text{g L}^{-1}$ ) and high ( $50 \mu\text{g L}^{-1}$ ) and the manipulated experiments were 1) variable salinities (normal  $54 \text{ mS cm}^{-1}$  and reduced  $44 \text{ mS cm}^{-1}$ ) and 2) low light ( $15.3 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Results of the experiments demonstrated that initial (24 h) leaf Cu accumulation was in proportion to exposure concentrations, irrespective of manipulated environmental conditions. This suggests that *Z. muelleri* leaves could act as a Cu bioindicator at times of reduced light and salinity (e.g., during a flood or along an estuarine gradient). During the low light experiment, the Cu concentrations in the below-ground compartment of the seagrass significantly increased over time, suggesting active Cu accumulation to supply *Z. muelleri* with new Cu for metabolic requirements. Active Cu accumulation could influence the use of *Z. muelleri* as a Cu bioindicator in that *Z. muelleri* would not be displaying steady state Cu concentrations.

The study provided new knowledge of *Z. muelleri* in relation to its use, partitioning and accumulation of a selection of analysed TEs, which was used to assess whether *Z. muelleri* can be proposed as a bioindicator. The results demonstrated that *Z. muelleri* can be a strong temporal and spatial accumulator of certain TEs from the environment. However, the interaction of age, growth, compartment tested, and specific TE uptake mechanisms influenced overall TE concentrations, and should be measured and used to interpret bioindicator results. Environmental variables such as light and salinity did not influence TE accumulation by *Z. muelleri* in an experimental environment. The results of the field study, however, showed that some environmental variables that vary between locations, such as silt and epiphytes, can contribute to TE concentrations in seagrass samples.

## **Acknowledgements**

Firstly, I would like to thank my supervisors, Emma Jackson, Amie Anastasi, Nicole Flint, and Gordon Dwane for their insight, constructive comments and thoughts on guiding this research to the present outcome. I would also like to thank the following people for their assistance with field or laboratory work, thesis review, general assistance or conversations: Catherine Jones, Megan Ellis, Rachel Manassa, Jacquie Hindle, Julie-ann Malan, Matt Misselbach, Harry Misselbach, Morgan Parker, Dylan Charlesworth, Gidargil staff, AB marine coxswains and other co-workers in building 604.

To my family and local Gladstone and Rockhampton friends thank you for supporting me over the two years and in trying to help me find my feet in this town and this project.

I gratefully acknowledge the funding received from Port Curtis Integrated Monitoring Program Inc. that has supported this research. This research was undertaken using data by PCIMP and Gladstone Ports Corporation for parameter (salinity and light) and trace element clarification. This research higher degree candidature was supported by a Scholarship from the Australian Government's Research Training Scheme. I gratefully acknowledge the financial support provided by the Australian Government.

## ACKNOWLEDGEMENT OF PROFESSIONAL SERVICES

Professional editor, Leonie Barnett, provided copyediting and proof-reading services, according to the guidelines laid out in the University-endorsed national 'Guidelines for Editing Research Theses'.

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## Commonly used abbreviations and units

BCF = Bioconcentration Factor

CQUniversity = Central Queensland University

LoR = Limit of Reporting

MQY = maximum quantum yield

PCIMP = Port Curtis Integrated Monitoring Program Inc.

SE = standard error

SD = standard deviation

TE = Trace elements

°C = temperature Celsius

cm = centimetres

d = days

DW = dry weight

g = grams

H = height

h = hours

Km = kilometres

L = length

m = metres

m<sup>2</sup> = metres squared

mg L<sup>-1</sup> = milligrams per litre

mg kg<sup>-1</sup> = milligrams per kilogram

mol photons m<sup>-2</sup> d<sup>-1</sup> = mol photons per metre per day

mS cm<sup>-1</sup> = milli Siemens per centimetre

n = number

µg L<sup>-1</sup> = micrograms per litre

µM = micromolar

µm = micrometres

µmol photons m<sup>-2</sup> s<sup>-1</sup> = micromol of photons per metre per second

W = width

WW = wet weight

## Element abbreviations

Al = aluminium

Ag = silver

As = arsenic

B = boron

Ba = barium

Be = beryllium

Bi = bismuth

Br = bromine

Ca = calcium

Cd = cadmium

Cr = chromium

Co = cobalt

Cu = copper

Fe = iron

Hg = mercury

K = potassium

Mg = magnesium

Mn = manganese

Mo = molybdenum

Na = sodium

Ni = nickel

Pb = lead

Rb = rubidium

Sb = antimony

Se = selenium

Sn = tin

Sr = strontium

Tl = thallium

U = uranium

V = vanadium

Zn = zinc

## **Related publications and presentations**

### **Conference poster presentation**

Skillington A, Flint N, Anastasi A, Dwane G, Jackson EL (2017) A tropical seagrass, *Zostera muelleri*, as a potential metal bioindicator. Estuarine and Coastal Shelf Association, Perth, Western Australia, Australia.

### **Report**

Skillington A, Jackson EL (2019) Port Curtis Integrated Monitoring Program report on seagrass trace metal sampling November 2018. CQUniversity, Gladstone.

# Chapter 1. **General introduction and project background**

## 1.1 Introduction

The coastal zone is an area of significant interaction between natural marine ecosystems and anthropogenic activities, and requires the sustainable management of both. One aspect of managing coastal ecosystems is the monitoring and detection of pollutants to avoid negative effects. This can involve significant time and financial cost, due to the requirement to monitor a large number of parameters replicated spatially and temporally (Dafforn et al. 2012; Rainbow 2006). All this monitoring is then followed up with interpretation of results to determine whether human activities (e.g., coastal development, shipping or tourism) and their associated pressures (e.g., pollutants such as excessive trace elements or, nutrients) have an effect on the environment and whether the management of pollutant sources is effective (Elliott et al. 2017). However, measuring one pollutant, such as bioavailable trace elements (TE), within the marine environment can be problematic as concentrations may be below detectable analytical limits or in a non-bioavailable phase, and requiring expert interpretation (Dafforn et al. 2012). To overcome this issue, local ecologically relevant bioindicators are often used to supplement water and sediment quality analysis (Rainbow 2006). This research investigates the use of seagrass as a bioindicator within Port Curtis, Gladstone, Australia. Seagrass meadows are an important coastal ecosystem that are at threat from loss and ecosystem degradation and utilising them as a TE bioindicator could help prevent their decline and assist with the monitoring of TE sources.

Bioindicators (also termed biomonitors, but for this thesis the term bioindicator is used to indicate TE change) readily accumulate TEs and are therefore indicative of exposure over time and space (Rainbow 2006). According to Rainbow (2006), a suitable bioavailable TE bioindicator species should be:

- able to represent the contaminant over a measurable period of time,
- abundant and adequate for analysis,
- sedentary,
- easy to identify,
- able to net accumulate TEs, and
- sensitive to TE changes within the environment.

Being sensitive to changes in environmental TE concentrations is an important aspect of a bioindicator as it can assist in identifying the source of the TEs (anthropogenic or natural) over temporal and spatial scales. In addition, an indicator's status (TE concentration) also needs to meet management requirements (e.g., thresholds or absence/presence) where decisions are made in respect to reducing the pollutant (Elliott

2011; Elliott et al. 2017). Essentially, an indicator for pollutant management should have the following attributes and should be:

- able to show a measurable and interpretable response,
- relevant,
- repeatable,
- predictable, and
- based on rigorous science.

Other aspects of indicator selection that will not be analysed within this study, but require consideration, are the social, financial and appropriate timing for management decisions (Elliott 2011; Elliott et al. 2017; McMahon, Collier & Lavery 2013).

Seagrasses meet many of these bioindicator requirements as they are abundant, sedentary, easy to identify and accumulate TEs from the sediment and water environments (Pergent-Martini & Pergent 2000). Seagrasses have been used as a bioindicator for a variety of water or sediment pollutants, including nutrients, herbicides, polycyclic aromatic hydrocarbons (PAHs) and TEs (Lewis & Devereux 2009), and activities and pressures, such as physical disturbance (e.g., caused by anchoring or coastal development) (Herrera-Silveira et al. 2010; Montefalcone et al. 2008), light limitation (McMahon, Collier & Lavery 2013), aquaculture (Holmer et al. 2008) and sewage or saline outlets (Cambridge et al. 2017; Connolly et al. 2013). Seagrasses as bioindicators of TE contamination have a long proven history, but with a predominant focus on European waters and the species *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Ascherson and *Zostera marina* L. (Bonanno & Orlando-Bonaca 2017; Govers et al. 2014; Lewis & Devereux 2009; Pergent-Martini & Pergent 2000; Vonk et al. 2018).

Variables measured in seagrass as bioindicators of TE uptake or effects include everything from biomarkers at the cellular level (photosynthetic response) to ecosystem (meadow) level changes (Pergent-Martini & Pergent 2000). The endpoints measured can also reflect the response time; for example, indicators at the cellular level are early-warning indicators, taking only days to change, whereas indicators at the meadow scale provide a later warning, with a lag time of months (Elliott 2011; McMahon, Collier & Lavery 2013). However, the predominant variable measured in seagrass as a bioindicator is the accumulation or bioconcentration of selected TEs of concern (Pergent-Martini & Pergent 2000). Combining accumulation results with other seagrass metrics into a multi-metric index can supplement the understanding of the ecosystem. The

purpose of an index is to numerically simplify and quantify the changes observed within the environment for easier interpretation (Orfanidis, Panayotidis & Stamatis 2003). Emerging indices for reporting TE seagrass bioindicators are the Trace Element Pollution Index (TEPI) and the Trace Element Spatial Variation Index (TESVI) (Richir & Gobert 2014). These indices quantify the overall levels of TEs and the variability of the element in the environment, and subsequently identify local hotspots of TE contamination (Richir & Gobert 2014).

Seagrasses, like terrestrial angiosperms, require small quantities of TEs to meet their metabolic requirements for photosynthesis and growth (Kabata-Pendias 2001; Macinnis-Ng & Ralph 2004). Trace elements can be passively or actively accumulated and regulated through processes of desorption (release of TEs), exclusion (actively not accumulated) and translocation to the required compartment (leaf, rhizome or root) to be stored or immediately metabolised (Pergent-Martini & Pergent 2000; Prange & Dennison 2000). These metabolic processes and the inclusion of seagrass leaf senescence and natural shoot turnover are factors that can influence the net accumulation and retention of TEs (Pergent-Martini & Pergent 2000). Previous studies have demonstrated that seagrasses accumulate excessive essential (Cu and Zn) and non-essential (Cd and Pb) TEs, with a range of toxic effects due to exposure, including senescence, exclusion and reduced photosynthetic capacity (Buapet et al. 2019; Macinnis-Ng & Ralph 2004; Prange & Dennison 2000; Ralph & Burchett 1998). The accumulation of a TE is dependent on the TE studied, the TE concentration, exposure time and competing ions (Pergent-Martini & Pergent 2000; Wang & Lewis 1997). Additionally, external environmental factors such as temperature, light, pH and salinity can affect the uptake of the TE (Bond et al. 1988; Wang & Lewis 1997). Environmental factors in combination with TE uptake in controlled seagrass uptake experiments are limited and require further research (Vonk et al. 2018). Seagrass biology and physiology, such as age, life history (seasonality), metabolic rates, tolerance of TEs, uptake route and TE compartmentalisation/translocation are all species-dependent (Macinnis-Ng & Ralph 2004; Pergent-Martini & Pergent 2000; Rainbow 2006; Vonk et al. 2018; Wang & Lewis 1997). Rainbow (2006) states that before using a particular species as a bioindicator, it is critical to understand the biology and the chemical kinetics of the species. There remains a knowledge gap regarding the fates and effects of TE accumulation for many seagrass species (Lewis & Devereux 2009).

The lack of studies on seagrasses as TE bioindicators is most pronounced in tropical areas and for tropical/sub-tropical species, especially within Australasia (Govers et al.

2014). This could be due to seagrass researchers in tropical areas primarily focusing on higher risk pressures; for example, light limiting activities such as turbidity and sedimentation (McMahon, Collier & Lavery 2013). Another possible reason why some tropical seagrasses have not been used as TE bioindicators is because their small size does not meet the biomass required for chemical analysis without being destructive of the whole plant or meadow. For example, *Zostera muelleri* Irmisch ex Ascherson<sup>1</sup> is defined as an opportunistic to colonising, tropical/sub-tropical species with a high shoot turnover, in comparison to *Posidonia* spp., which are long lived, temperate and have a slow shoot turnover (Kilminster et al. 2015). These contrasting traits influence the timeframe within which the two species integrate TEs and, from a practical perspective, the number of shoots required to meet biomass requirements for sample analysis (Pergent-Martini & Pergent 2000). While these opportunistic species traits of low biomass and high shoot turnover may seem undesirable for a bioindicator, as they may not have a long time to bioaccumulate toxins, they could potentially reflect the short term (months) variable water quality. Therefore, the use of *Z. muelleri* as a bioindicator of TEs is worthy of further investigation.

## 1.2 Background and Study Location

The Port of Gladstone is situated within the estuarine complex of Port Curtis and accommodates a variety of heavy industries along the coastline including coal export, coal fired power production, chemical manufacture, aluminium smelting and liquefied natural gas production (Flint et al. 2015). The harbour's mid to far-field (ambient) environment is monitored extensively by a consortium partnership of industry members through the Port Curtis Integrated Monitoring Program Inc. (PCIMP, [www.pcimp.aims.gov.au](http://www.pcimp.aims.gov.au)). In addition to quarterly water monitoring and annual sediment sampling, the program includes biological monitoring in the form of *in-situ* exposures of deployed oysters as a TE bioaccumulator. However, PCIMP is considering alternatives to oysters that are more representative of the marine TE conditions occurring in Port Curtis (Mr Gordon Dwane 2017, pers.comm., 12 June 2017). This prompted the search for a bioindicator of water and sediment TEs. A pilot study of seagrass bioaccumulation highlighted the potential use of *Z. muelleri* as a bioindicator of TEs (Jackson et al. 2016). Within Port Curtis the local seagrass, *Z. muelleri*, can be found in large monospecific and mixed species stands of up to 40 km<sup>2</sup> and provides important local ecosystem services such as habitat and food sources (Chartrand et al. 2016). In order to utilise the local

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<sup>1</sup> *Zostera muelleri* will be used within the thesis where previous research used other synonym names of *Z. muelleri* subsp. *capricorni*. or *Z. capricorni*.

seagrass, *Z. muelleri*, as a TE bioindicator for the Port Curtis area, more information on its capability to translocate, use and accumulate TEs from the environment is required.

### 1.3 Thesis Scope and Objectives

The overarching aim of the project was to determine if a sub-tropical fast growing seagrass, *Z. muelleri*, could be utilised as a bioindicator of bioavailable TEs in the water and sediment environment. The aim was to be addressed through the following objectives:

- Identify within the literature the use of seagrass as bioindicators and how and why seagrass accumulate and utilise TEs and the variability that occurs (Chapter 2). This review feeds into the methods selected for the following chapters.
- Understand if *Z. muelleri* can be used as a TE bioindicator of Port Curtis water and sediment TEs through the interpretation of spatial and temporal variability:
  - Spatial variability (meadow scale versus Port Curtis scale) of seagrass TEs concentration and the relationship to the TE concentrations in the environment, and the environmental drivers of variability. These results were compared to other seagrass TE indices to assess their applicability and relevance to Port Curtis as potential indices (Chapter 3).
  - Temporal variability of TE concentrations within the above- and below-ground seagrass compartments to ascertain whether they correlated to the environment (water and sediment) TEs or were due to natural variation (Chapter 4).
- Investigate *Z. muelleri* uptake, accumulation and partitioning of, and response (morphology and physiology) to, a specific TE (Cu) under varying environmental conditions through the use of manipulative laboratory experiments (Chapters 5 and 6).
- Compare results against broad TE bioindicator criteria to ascertain whether *Z. muelleri* meets the requirements for management use (Chapter 7).

## **Chapter 2. Seagrass as bioindicators and their trace element use: a review**

## 2.1 Seagrasses and Trace Elements Literature Review

### 2.1.1 Trace element use

Seagrasses, like other angiosperms, require essential macro- and micronutrients for growth and development. The requirement and utilisation of elements determines the concentration of elements within the plant; however, elements can also be passively absorbed even when they are not required. Macronutrients such as H, C, N, P, K, S, Ca and Mg are found in large concentrations and accumulate and bind in structural components due to fundamental metabolic processes (Brix & Lyngby 1983; Malea 1994a). Micronutrients include elements that are required or are essential in small or 'trace' amounts for specific biochemical processes; these include, but are not limited to, Co, Cu, Cr, Fe, Mn, Mo, Na, Ni, Se, Sn, V and Zn (Kabata-Pendias 2001; Richir & Gobert 2016). There are also TEs that are not essential but that can accumulate, such as Ag, Al, As, Au, Cd, Ga, Hg, Pb and Ti, many of which are very toxic in small quantities (Malea & Kevrekidis 2013; Richir & Gobert 2016). Knowledge of the use of elements by seagrasses is oriented towards macronutrients such as N, P and C due to numerous nutrient impact and carbon studies, and little is known about micronutrient or TE use and regulation (Lewis & Devereux 2009). Factors that influence the total TE concentration within seagrasses are determined by the binding sites of the seagrass and element kinetics and behaviour such as antagonistic, synergistic, absorption (passive or active), regulation and translocation capacity (Greco et al. 2019; Malea & Haritonidis 1995b; Sanchiz, García-Carrascosa & Pastor 1999).

The binding sites for TEs within seagrasses are within the cells' thin cuticle of the leaf or the fine roots (Malea 1994b). The uptake process of TEs is described in three stages: 1. initial uptake by adsorption passive processes; 2. crosses from the plasmalemma into the protoplasm; and 3. the active accumulation or absorption into the cell (Malea 1994b). Mobilisation of TEs can be reversed and desorb where the plant releases TEs from or through the plant surface (Penello & Brinkhuis 1980). The root system has special mechanisms where the root can assist in the release and mobilisation of sediment bound TEs for use by the seagrass (Brodersen et al. 2017). Synergistic and antagonistic behaviours of TEs also need to be considered. Greco et al. (2019) found that Cd inhibited Cu uptake within *Z. marina* as an example of antagonistic TE behaviour, while As within seagrass species below-ground compartment appears to be dependent on Fe uptake (Maher et al. 2011; Thomson, Maher & Foster 2007). There is a deficiency of knowledge about the relationship between seagrasses and TEs. Table 2.1 provides a summary of TEs and their roles within terrestrial plants and seagrasses, where known.

**Table 2.1. Trace elements and their roles within plants; adapted from Pais and Benton Jones (1997), Kabata-Pendias (2001) and Gerendas et al. (1999) with known seagrass references.**

	<b>Constituent of</b>	<b>Involved in</b>	<b>Within seagrass</b>
<b>Al</b>	-	Control colloidal properties in the cell, dehydrogenases and oxidases	-
<b>As</b>	Phospholipid (algae)	Metabolism of carbohydrates in algae and fungi	-
<b>B</b>	Phosphogluconates	Metabolism and transport of carbohydrates, flavonoid synthesis, phosphate utilisation, polyphenol production, RNA formation and cellular activities such as respiration and growth	-
<b>Co</b>	Cobamide coenzyme	N <sub>2</sub> fixation and stimulation, synthesis of chlorophyll and proteins	-
<b>Cu</b>	Oxidases, chloroplast protein plastocyanins, and ceniloplasmin	Oxidation, photosynthesis, protein and carbohydrate metabolism, N <sub>2</sub> fixation and valence changes, cell wall metabolism, desaturation and hydroxylation of fatty acids	Electron transport for photosystem II enzymes, metabolism, protein, mitochondria (Macinnis-Ng & Ralph 2004; Ralph & Burchett 1998)
<b>Fe</b>	Haem-proteins and nonhaem iron proteins, dehydrogenases, and ferredoxins	Photosynthesis, N <sub>2</sub> fixation, enzyme systems, nitrate and sulphate reduction and energy NADP production	Deficiency (Duarte, Martín & Margarita 1995) and toxicity (Prange & Dennison 2000)
<b>Mn</b>	Many enzyme systems	Photoproduction of oxygen in chloroplasts and indirectly in nitrate reduction, oxidation-reduction processes within the photosynthetic electron transport system	-
<b>Mo</b>	Nitrate reductase, nitrogenases, oxidases and molybdoferredoxin	N <sub>2</sub> fixation, nitrate reduction and valence changes. Requirement for Mo is reduced by the availability and utilisation of ammonia	-
<b>Ni</b>	Urease apoprotein	Possibly in action of hydrogenase and translocation of N. Component of urease	-
<b>V</b>	Porphyrins, haemoproteins	Lipid metabolism, photosynthesis (green algae) and possibly in N <sub>2</sub> fixation	-
<b>Zn</b>	Anhydrases, dehydrogenases, proteinases and peptidases	Carbohydrate, nucleic acid and lipid metabolism, carbonic anhydrase activation. Similar to Mn and Mg enzyme functions	Enzyme activity for plant growth, respiration (Macinnis-Ng & Ralph 2004; Ralph & Burchett 1998)

Copper is an essential TE that is found in higher concentrations in areas of new growth and can increase growth if available in low concentrations, suggesting that Cu is required for growth or metabolism (Brix & Lyngby 1982; Lyngby & Brix 1984). However, Cu in sub-lethal to excessive amounts (0.25 to 10 mg L<sup>-1</sup>) has been found to disturb electron transport for Photosystem II (PSII) and therefore causes chlorophyll degradation, reduced growth and other toxic effects such as leaf senescence, oxidative stress and necrosis (Buapet et al. 2019; Llagostera et al. 2016; Lyngby & Brix 1984; Macinnis-Ng & Ralph 2004; Ralph & Burchett 1998; Zheng et al. 2018). Ralph and Burchett (1998) also found Zn in excessive amounts (10 mg L<sup>-1</sup>) to be quite toxic in terms of photosynthetic response in *Halophila ovalis* (R.Brown) J.D.Hooker. However, Macinnis-Ng and Ralph (2004) found Zn to be less toxic than Cu for *Z. muelleri* in regards to photochemical responses. Iron is an important TE that is used in photosynthetic and respiration processes, and Malea and Haritonidis (1995b) observed greater uptake of Fe in summer due to higher photosynthetic requirements. In addition, leaf Fe deficiency (<100 µg Fe g DW<sup>-1</sup>) has occurred within *Thalassia testudinum* K.D.Koenig and *Syringodium filiforme* Kützting at sites with carbonate sediments (Duarte, Martín & Margarita 1995).

The non-essential TE, Cd, is rapidly absorbed by the roots and leaf of *Halophila stipulacea* (Forsskål) Ascherson and *H. ovalis*, yet is phytotoxic (Malea 1994b; Ralph & Burchett 1998). Malea, Adamakis and Kevrekidis (2013a) found that the initial uptake rate of Cd has a greater effect on toxicity than the overall concentration accumulated, and recorded microtubule disturbance occurring at day three and cell death at day seven for *C. nodosa*. The authors suggested that the toxic effects were due to the cell's detoxification mechanisms being overwhelmed by TEs, resulting in incomplete detoxification and cell death. Other non-essential TEs such as Pb and Hg can also become toxic to seagrasses due to their potential to bioaccumulate within cells (Bonanno & Di Martino 2016; Pergent-Martini 1998; Tupan & Azrianingsih 2016). There is an inverse relationship between TE toxicity and the accumulated concentration at which a toxic effect is observed. For example, only a small amount of Hg is required to produce toxic effects. For *Z. marina*, the order of TE toxicity effect upon growth was Hg≥Cu>Cd≥Zn>Cr(III),Pb (Lyngby & Brix 1984).

### 2.1.2 Bioaccumulation

In order to understand the accumulation and translocation of TEs by seagrasses, a number of unique laboratory experiments have been conducted. These were typically run with one species and one TE added to the water and only a few have attempted to

spike sediment with a TE (Fabris, Harris & Smith 1982; Nielsen et al. 2017). Experiments were conducted either with whole shoots in aquaria with water, or with seagrass shoots in a two-compartment system to separate the above- and below-ground parts (Lyngby, Brix & Schierup 1982; Malea, Adamakis & Kevrekidis 2013b). The variables measured included from actual concentration uptake, growth, translocation or physiological effects such as fluorescence. Only a few studies tested the influence of environmental variables such as salinity and temperature on Cd, Cu, Mn and Pb uptake (Bond et al. 1988; Brinkhuis, Penello & Churchill 1980; Gamain et al. 2018; Nielsen et al. 2017). Salinity had different results. Bond et al. (1988) found that there was increased Pb uptake with decreased salinity, possibly due to less ionic site competition. However, Nielsen et al. (2017) found greater Cu uptake within the leaves under higher salinities. In light of these different results and future predictions of extreme weather events, where increased TE loadings are expected, there is pertinence in understanding TE uptake under different scenarios of variable environmental levels (light and salinity) and in different seagrass species (Vonk et al. 2018). Accumulation observations from field and laboratory experiments are summarised in Table 2.2, with the focus on *Zostera* spp. for relevance to this project, and note that examples are from sub-tropical (e.g., Sydney) and temperate (e.g., Melbourne or Denmark) areas.

**Table 2.2. Examples from laboratory and field observations of trace element uptake and mobilisation within *Zostera* spp. Basipetal translocation = leaf to root-rhizome, and acropetal translocation = root-rhizome to leaf. Na = not applicable.**

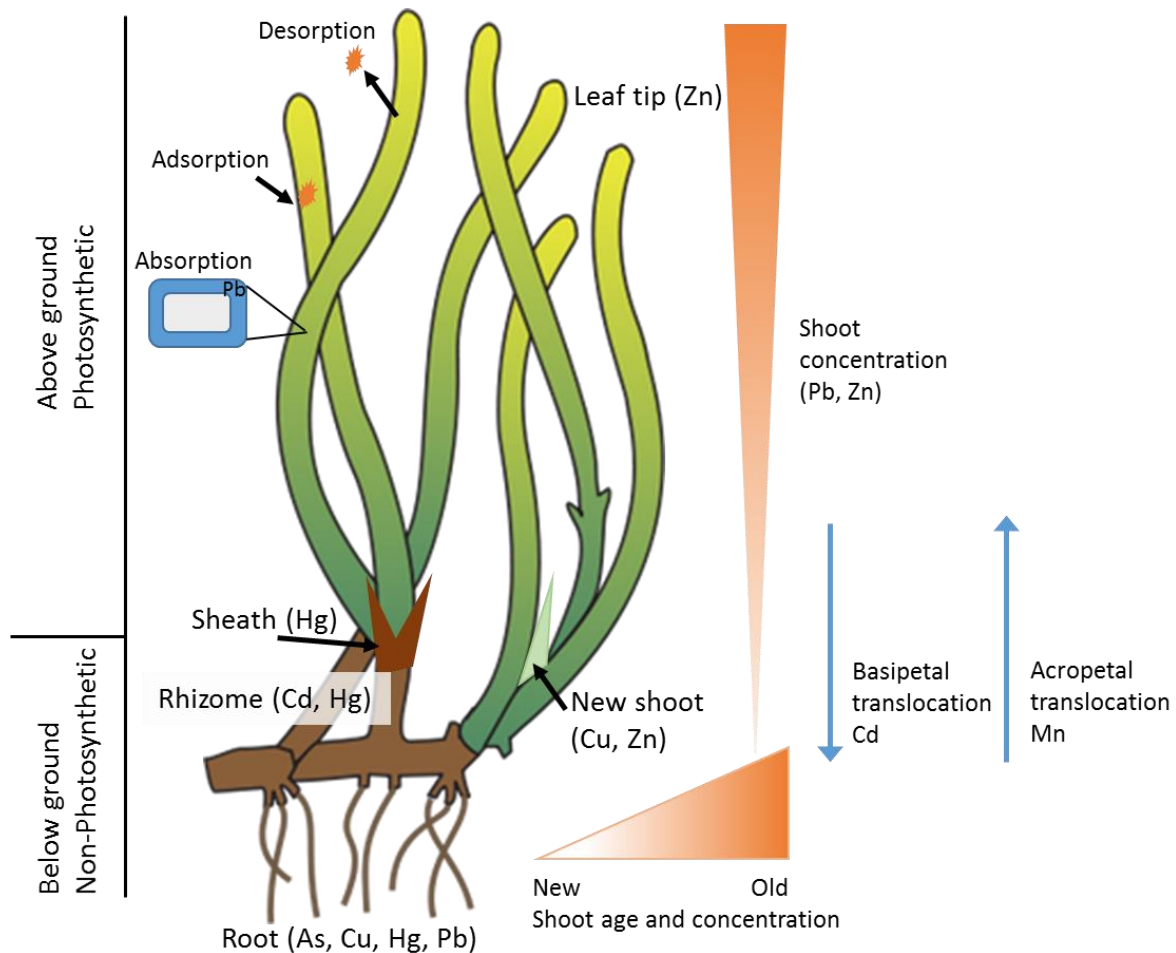
Species	Element	Above/ Below	Uptake	Desorption	Translocation	Other	Article
<i>Zostera marina</i>	Cd	leaf<root	Yes	Yes through leaf	Bidirectional	Basipetal translocation enhanced with salt gradient. Transported across cell cytoplasm or into vascular tissues.	Brinkhuis, Penello and Churchill (1980)
<i>Zostera marina</i>	Cd	leaf>root	Yes	Na	Basipetal Yes Acropetal No	Reflected water concentration. Active transport across cell membranes and not passive diffusion.	Faraday and Churchill (1979)
<i>Zostera marina</i>	Cu	Na	Yes	Na	Acropetal	Uptake from sediment and translocation greater effect on growth.	Nielsen et al. (2017)
<i>Zostera marina</i>	Mn	leaf>root	Variable	Na	Acropetal	Mn fixed in leaf cell cytoplasm.	Brinkhuis, Penello and Churchill (1980)
<i>Zostera marina</i>	Zn	Na	Yes	Na	Insignificant acropetal	Zn movement was due to new growth.	Lyngby, Brix and Schierup (1982)
<i>Zostera marina</i>	Zn	leaf>root	Yes	Na	Bidirectional	-	Drifmeyer (1980)
<i>Zostera muelleri</i>	Cu	leaf>root	Yes	Na	Not significant	-	Carter and Eriksen (1992)
<i>Zostera muelleri</i>	Pb	Na	Yes, dead and green leaves	Leaf surface Pb loss possible with EDTA	Na	Green and dead leaves Pb uptake: greater passive adsorption to leaf surface than active adsorption.	Bond et al. (1985)
<i>Zostera muelleri</i>	As	leaf<root root>rhizome	Yes	Na	Na	Fe assisted uptake.	Maher et al. (2011)
<i>Zostera muelleri</i>	Cu	leaf>root	Yes	Not apparent	Na	Field test, great variation between sites.	Macinnis-Ng and Ralph (2004)
<i>Zostera muelleri</i>	Zn	leaf>root	Yes	Not apparent	Na	Field test, great variation within sites.	Macinnis-Ng and Ralph (2004)

### 2.1.2.1 Trace element accumulation

Determination of whether the local seagrass can be a TE bioindicator requires an understanding of the capability and requirements of seagrass to regulate or accumulate and reflect environmental TEs (Bonanno & Orlando-Bonaca 2018; Prange & Dennison 2000). Previous studies found that *Zostera* spp. readily accumulate As, Cd, Cu, Pb and Zn, while Mn uptake was variable over time (Table 2.2). The initial uptake rate for Cu, Cd, Hg, Pb and Zn was quite rapid within the first half day to two days and plateaus out from either day two or five onwards, pending on the TE and the part of the seagrass (Lyngby & Brix 1984). Uptake rate is dependent on species as Bond et al. (1988) observed that *Halophila ovalis* subsp. *australis* (Doty and B.C.Stone) den Hartog had slower uptake of Pb than *Zostera* spp., but reached the same maximum concentration. The greatest determinant of uptake is the concentration of the TE within the medium (Faraday & Churchill 1979). For example, Carter and Eriksen (1992) found Cu concentration in seagrass reflected the water concentration. However, Al concentrations in water were not correlated with concentrations in *H. stipulacea* tissue, confirming that non-essential TEs can be inhibited by protoplasmic resistance for this specie (Malea & Haritonidis 1996). The uptake process appears to be a passive process as it has been observed that already dead or older leaves can accumulate more than the living younger leaves (Bond et al. 1985; Lyngby & Brix 1984). This is typically due to the TE; for example, Cu, Hg or Zn cause cell deterioration and therefore provides more sites for absorption (Malea & Haritonidis 1995a).

Accumulation preference between above- or below-ground biomass or partitioning of TEs within compartments is not consistent between species or TE (Table 2.2, Fig. 2.1) (Pergent-Martini & Pergent 2000). Differences between compartment maximum TE concentration can be seen as from leaves>root>rhizome or root>leaf>rhizome, no difference between compartments (Bonanno & Di Martino 2017), or a gradual increase in concentration from the basal area to the tip as observed in *P. oceanica* (Fig. 2.1) (Conti et al. 2010). However, Pergent-Martini and Pergent (2000) suggest that, in general, Cd, Cu, K, Mg and Zn tend to be found at higher concentrations in above-ground compartments than in below-ground compartments. An example of accumulation preference for below-ground compartments is Hg and Pb in *P. oceanica* (Fig. 2.1) (Maserti, Ferrara & Paterno 1988; Pergent & Pergent-Martini 1999; Sanchiz, García-Carrascosa & Pastor 1999). Both Faraday and Churchill (1979) and Brinkhuis, Penello and Churchill (1980) agreed that the root system of *Z. marina* was a sink for Cd. Accumulation within the root system is also due to the root-rhizome system being older

and a slower turnover and subject to a longer term of accumulation, unlike leaves that are younger and observe a seasonal turnover (Pergent-Martini & Pergent 2000). Therefore, the selection of a certain compartment or bundling together of parts for analysis could influence the interpretation of TE sources.



**Figure 2.1. Diagrammatic representation of a fictional seagrass with generalised examples of trace element accumulation processes (adsorption, absorption, new growth) and organs with highest concentrations (old versus new leaves, below-ground versus above-ground or within leaf differences, Pb deposit within blue cell walls). Seagrass image: courtesy of the integration and application network, University of Maryland Center of Environmental Science ([ian.umes.edu/symbols/](http://ian.umes.edu/symbols/)).**

#### **2.1.2.2 Accumulation species variability**

The factors that drive differences in TE bioaccumulation within seagrasses and that are not dependent on the external environment include the species tested, growth cycle, age, tissue tested and tolerance (Pergent-Martini & Pergent 2000; Vonk et al. 2018). For example, *P. oceanica* accumulated more Ni and Cu than *C. nodosa*, which accumulated

more Cr from the same site (Bonanno, Borg & Di Martino 2017; Catsiki & Panayotidis 1993). In a comparison between *P. oceanica*, *C. nodosa* and *Zostera (Zosterella) noltei*<sup>2</sup> Hornemann, Sanchiz, García-Carrascosa and Pastor (1999) identified different Zn concentrations within different compartments for these species, but similar Pb concentrations between species. A recent review and meta-analysis found evidence of colonising species (e.g., *Zostera*) having significantly higher leaf concentrations for Al, Fe, Mn and Si and significantly lower concentrations of Zn in comparison to climax species (e.g., *Posidonia*) (Vonk et al. 2018). In contrast, Nienhuis (1986) found only one significant difference between metal accumulation in plant parts across nine different tropical species (no *Zostera* spp. included), with *Thalassodendron ciliatum* (Forsskål) den Hartog displaying 3–4 times higher Cd in the leaves, shoots and rhizomes than in the other species. In the same study, *H. ovalis* appeared to accumulate more Zn compared to the other eight species (Nienhuis 1986).

### **2.1.2.3 Accumulation seasonal variability**

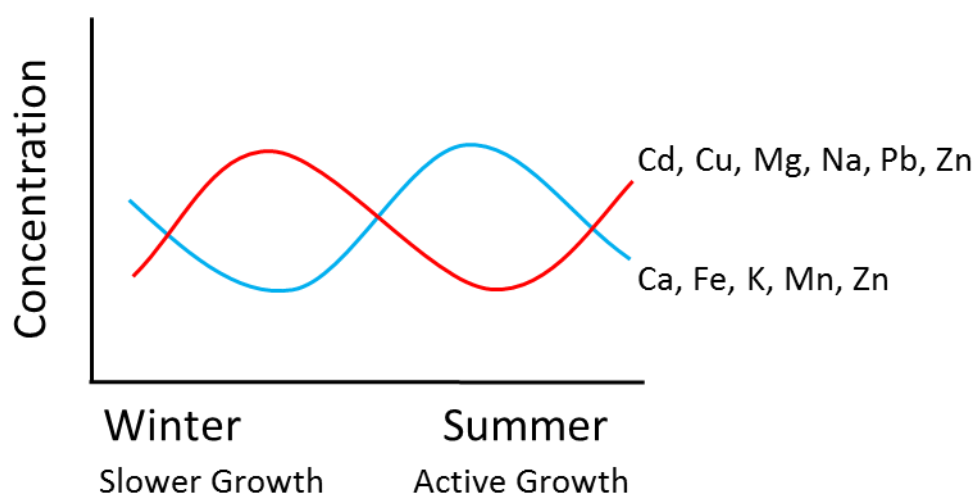
A major aspect affecting observed TE concentration over time is seasonal influence such as those involving the species life cycle or external environmental factors. Malea and Kevrekidis (2013) observed that seasonal weather patterns (elevated rainfall in spring) and the subsequent delivery of TEs by runoff to seagrasses was reflected in the root-rhizome concentration of TEs. Other external factors that contributed to bioaccumulation of TEs included: the physico-chemical properties of the water and sediment (e.g., pH or temperature), local disturbances or resuspension (excavation) of sediment (Prange & Dennison 2000), hydrology/oceanography (Chernova, Khristoforova & Vyshkvartsev 2002; Gosselin et al. 2006), historical land-use (Díaz et al. 2018) and other sources, such as groundwater (Avelar et al. 2013; Whelan et al. 2005). There is no single generalised pattern for the seasonal TE concentration within seagrasses and where differences occur between seasonal concentrations, they are at times not significantly different (Pergent-Martini & Pergent 2000). Nevertheless, some general patterns are observed in regard to seasons that require consideration.

Two general seasonal patterns in growth and the accumulation of TEs have been observed, irrespective of external seasonal weather influences (e.g., runoff) on TE concentrations (Fig. 2.2). One pattern observed was the increase in concentration from the uptake of TEs that are required for seagrass growth metabolic requirements in spring

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<sup>2</sup> *Zostera noltei* will be used throughout the thesis for naming consistency even though articles refer to *Z. noltei* with alternative synonymised name of *Z. noltii*.

or summer (Fig. 2.2) (Pergent-Martini & Pergent 2000). The other pattern was the decrease of TE concentration due to the dilution factor of greater biomass of the seagrass (Fig. 2.2). Malea (1994a) found seasonal differences for *H. stipulacea* with Fe, K and Zn increasing in summer and decreasing in winter, while Cu, Na and Mg concentrations were lower in summer and higher in winter. The Fe increase was due to the requirement of the seagrass for this TE, while low Cu concentrations in summer were due to biomass dilution (Malea 1994a). Another explanation of Cu seasonality was that Cu was predominantly found in new shoots of *Z. marina* and lower in older leaves, and therefore the Cu peak would coincide with seasons of maximum new shoot development such as during spring (Lyngby & Brix 1982). Ward, Correll and Anderson (1986) also observed the same seasonal differences in *Posidonia australis* J.D.Hooker, with Cu concentrations at a minimum in autumn/winter and maximum in spring; this was also observed for Cd and Zn.



**Figure 2.2. A generalised example of seasonal difference in trace element concentrations in seagrass irrespective of seasonal weather.**

One important deviation to the seasonality of TEs is the influence of location, particularly whether or not it is polluted. Richir and Gobert (2014) found that at unpolluted locations, seagrass TEs displayed seasonal variations that correlated with leaf growth, while the same TEs at a polluted site did not follow growth patterns; however, no statistical analyses were performed to determine if this difference was significant.

To address the issue of sampling interference and seasonal dynamics when using seagrass as bioindicators of TEs, it is strongly suggested to sample in a certain season

and to be consistent. For example, Malea and Haritonidis (1999) suggested that collection of *C. nodosa* as bioindicators of Mn and Cu should occur in autumn when concentrations are the highest. Ward (1987) confirms the requirement for collection standardisation, suggesting that leaves should either be collected at the same age, or that inter-location/time comparisons should be used to eliminate leaf-age variables. Richir and Gobert (2014) also suggest sampling should occur during key phases of the growth cycle, such as during the months of peak growth instead of at set times.

#### **2.1.2.4 Regulation**

Knowledge of the ability of seagrasses to regulate the uptake and retention of TEs is important for understanding if the seagrass is a true net accumulator or bioindicator of environmental TEs (Pergent-Martini & Pergent 2000). Examples of TE regulation processes include metabolism, desorption, translocation and loss through death of compartments (leaf or roots) (Fig. 2.1) (Pergent-Martini & Pergent 2000; Rainbow 2006). The process of desorption, such as the loss of TEs from the leaf surface, occurs when the TE may only be adsorbed to the surface and released due to equilibration with the surrounding medium concentration (Carter & Eriksen 1992). This was observed in the field by Fabris, Harris and Smith (1982) where *Heterozostera tasmanica* Martens ex Ascherson from a polluted site was exchanged with seagrass from an unpolluted site, and Cd was observed to desorb from the polluted plants in unpolluted waters and vice versa. A laboratory study by Penello and Brinkhuis (1980) found that high uptake and high initial loss rates of Cd from *Z. marina* was dependent on time and the concentration of Cd in the water. Of the few experiments that included a recovery period after uptake, Malea and Haritonidis (1995a) found that Zn concentrations in *H. stipulacea* decreased from leakage during the recovery phase; however, analysis of toxicity was not performed.

It is possible that the ability of seagrasses to translocate and redistribute TEs could confound the use of seagrass as a bioindicator of environmental concentrations, as the seagrass compartment and environmental TE concentrations may never correlate. Translocation or the mobilisation of TEs occurs within seagrass to redistribute TEs throughout the seagrass for metabolic processes or as a protective measure (Faraday & Churchill 1979; Lyngby, Brix & Schierup 1982). The process of translocation for protection has been observed where non-essential TEs are stored away from photosynthetic parts; for example Pb stored between cells within *Thalassia hemprichii* (Ehrenberg) Ascherson (Fig. 2.1; refer to blue cell representation) (Tupan & Azrianingsih

2016). A study of *Z. marina* found that Mn accumulated and remained immobile within the adult leaves and did not translocate to other compartments (Penello & Brinkhuis 1980). There does not appear to be a consistent pattern of Cu translocation in seagrass, with observations showing either no significant translocation (Carter & Eriksen 1992) or strong acropetal (upward) translocation from root to leaf in *Z. marina* (Fig. 2.1) (Nielsen et al. 2017). Another method applied to infer translocation from the environment to seagrass is the exploration of TE concentration correlations between compartments (e.g., seagrass and environment). Malea (1994b) proposed that a significant correlation between leaf Cu concentration and sediment Cu may suggest a root to leaf translocation in *H. stipulacea*. In contrast, Lyngby, Brix and Schierup (1982) calculated the percentage of acropetal translocation of Zn within *Z. marina*, and observed minimal translocation after 21 days (0.28%); the translocated Zn was observed in the new leaves or roots (Fig. 2.1).

Horizontal translocation along a rhizome has not been addressed in detail as the majority of tests were conducted using one shoot of multiple leaves, or slow growing temperate species. Brinkhuis, Penello and Churchill (1980) observed that *Z. marina* transported radionuclide  $^{109}\text{Cd}$  from old shoots to new tissues in field depuration experiments, although the total concentration of Cd per weight was not significantly different between parts. The study did not include potential uptake of natural sources of Cd in addition to the radionuclide Cd. This area of study requires more research before any statement can be made regarding horizontal translocation.

## 2.2 Systematic Review

### 2.2.1 Systematic review method

To address the question of ‘Where and how has seagrass been used as a bioindicator?’ a systematic review was undertaken. The systematic review method is a methodical process of obtaining information in a comprehensive and repeatable way (Neyeloff, Fuchs & Moreira 2012). The search engines Scopus and ScienceDirect were used on 20 April 2017. The fields Abstract, Title and Keywords were searched with the use of Boolean operators and wildcards where needed, using the words bioindicator, bio-indicator, biomonitor, bio-monitor, biological indicator, ecological indicator, seagrass, eelgrass and all seagrass genus names: *Amphibolis*, *Cymodocea*, *Enhalus*, *Halophila*, *Halodule*, *Phyllospadix*, *Posidonia*, *Syringodium*, *Thalassia*, *Thalassodendron* and *Zostera*.

The results of the search were sorted by reviewing the article title, then the abstract, followed by the article content. Only articles that included manipulative and field experiments of seagrasses focussed on essential and non-essential TEs were included in the systematic review. Papers were limited to seagrass; other aquatic plants such as macrophytes or saltmarsh respond differently to metal exposure (different abilities to accumulate and detoxify metals) and as a result were excluded (Bonanno, Borg & Di Martino 2017). Papers were excluded from the review if they were written in a language other than English or were not peer reviewed. No date constraint was applied and duplicates were removed.

Results of the initial search produced a total of 460 papers, and 225 remained after exclusion criteria were applied. The resulting papers were categorised into three groups covering seagrasses as bioindicators of other pressures on the ecosystem (116), seagrasses as bioindicators of TEs from field observations and experiments (59) and seagrass indices (50). The reference lists of included papers were scanned and additional papers were added if they fitted the inclusion criteria and contributed to overall knowledge. Reviews were referred to but were not included in analysis of studies.

### **2.2.2 Seagrass bioindicators**

Seagrasses have been used as bioindicators for a variety of pressures, including poor water quality (reduced light and pollutants such as nutrients and herbicides), development, climate change and coastal management (Table 2.3). The use of seagrasses as bioindicators has predominantly focussed on the effect on, or responses of, seagrasses to a pressure, measured from the cellular DNA level, to species morphological and physiological response, to meadow community scale responses (Abal et al. 1994; Franssen et al. 2014; Fyfe & Davis 2007). These studies focussed on the interpretation of the effect of the pressure (e.g., the effect of excessive nutrients on the physiology of seagrass), as a bioindicator of a specific pressure or as a bioindicator of the ecosystem health. In comparison, another interpretation of a bioindicator is that the species of flora or fauna can spatially and temporally display the total concentration of TE without toxic effects and can therefore provide a relative measure or reflection of the TE within the environment and can aid in identifying the source (Rainbow 2006; Ward 1987). The benefit of this type of bioindicator for the management of coastal pollutant concentrations is that it can enhance or replace the use of spot sampling of water and sediment TE concentrations.

**Table 2.3. Examples of seagrass as a bioindicator of pressures other than trace element concentrations.**

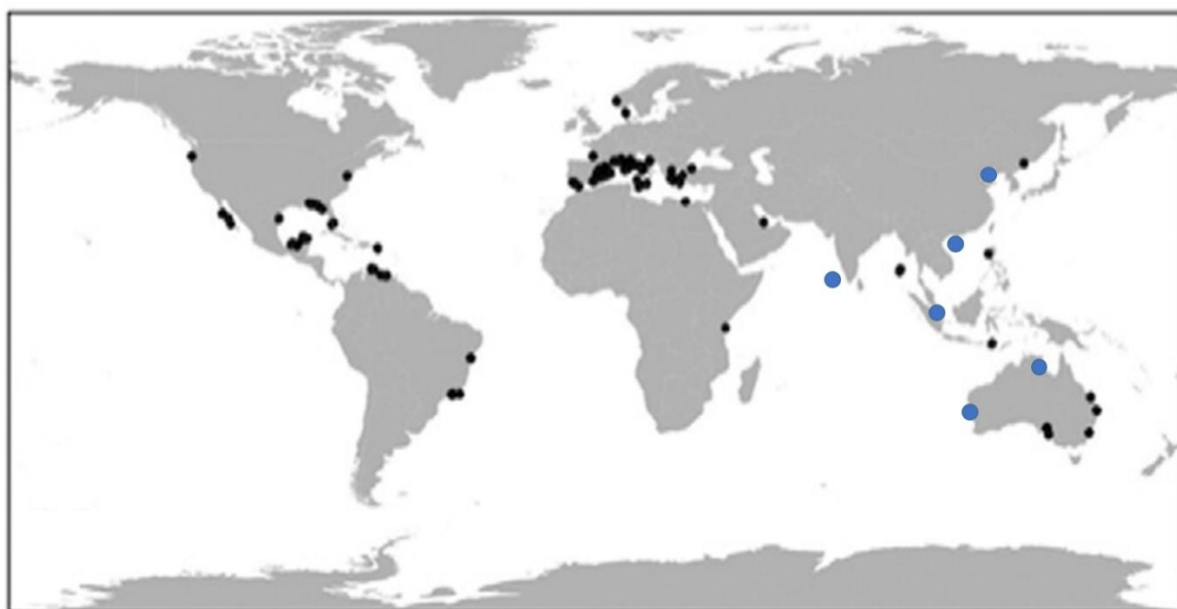
<b>Bioindicator example</b>	<b>Reference examples</b>
Physical disturbance (e.g. construction, development, dredging, tourism, restoration, anchoring)	Bach, Jensen and Lyngby (1997), Capello et al. (2014), Fyfe and Davis (2007), Herrera-Silveira et al. (2010), Montefalcone et al. (2008)
Light: limitation or stress	Cozza et al. (2004)
Ecosystem management (Integrated Coastal Zone Management effectiveness, conservation)	Kilminster et al. (2015), Orfanidis et al. (2010)
Aquaculture	Holmer et al. (2008)
Nutrient pollutants	Benson, Schlezinger and Howes (2013)
Polycyclic aromatic hydrocarbon	Apostolopoulou et al. (2014)
Radionuclides	Calmet et al. (1991)
Herbicides, pesticides	Fernandez and Gardinali (2016), Haynes, Müller and Carter (2000)
Sewage outlets	Cabaço et al. (2008), Connolly et al. (2013)
Water quality	Waycott, Longstaff and Mellors (2005)
Saline outlet (hypersalinity)	Cambridge et al. (2017)
Climate change (El Niño, warming water)	Carlson Jr et al. (2003), Diaz-Almela, Marbà and Duarte (2007)
Thermal stress	Abe et al. (2009)
Natural (hydromorphological stressors)	Recio et al. (2013)
Urban runoff	Boumaza et al. (2014)
Organic matter	Elliott, Spear and Wyllie-Echeverria (2006)

## **2.2.3 Seagrass bioindicators of trace elements**

### **2.2.3.1 History and locality of seagrass bioindicators of trace elements**

The assessment of seagrasses as TE bioindicators started in the 1980s with early work from Denmark, the Mediterranean and Australia (Brix, Lyngby & Schierup 1983; Maserti, Ferrara & Paterno 1988; Ward, Correll & Anderson 1986). These studies focussed on seagrass adjacent to sites of coastal pressures of urbanisation and heavy industry (Lyngby & Brix 1987; Ward 1987). Reported results from these studies showed that the local seagrasses accumulated the TEs, with results strongly reflecting the adjacent land

use. Seagrasses have since been used as TE bioindicators worldwide; however, the majority of studies have focussed on the temperate Mediterranean (Fig. 2.3) (Govers et al. 2014). A global meta-analysis by Govers et al. (2014) reported the concentrations of TEs within seagrass leaves as indicators of TEs in the environment and noted the absence of studies from tropical areas such as the Caribbean, northern Australia, southern Africa and Asia. Studies within these aforementioned areas have been increasing, with the most recent meta-analysis by Sánchez-Quiles, Marbà and Tovar-Sánchez (2017) including more studies.

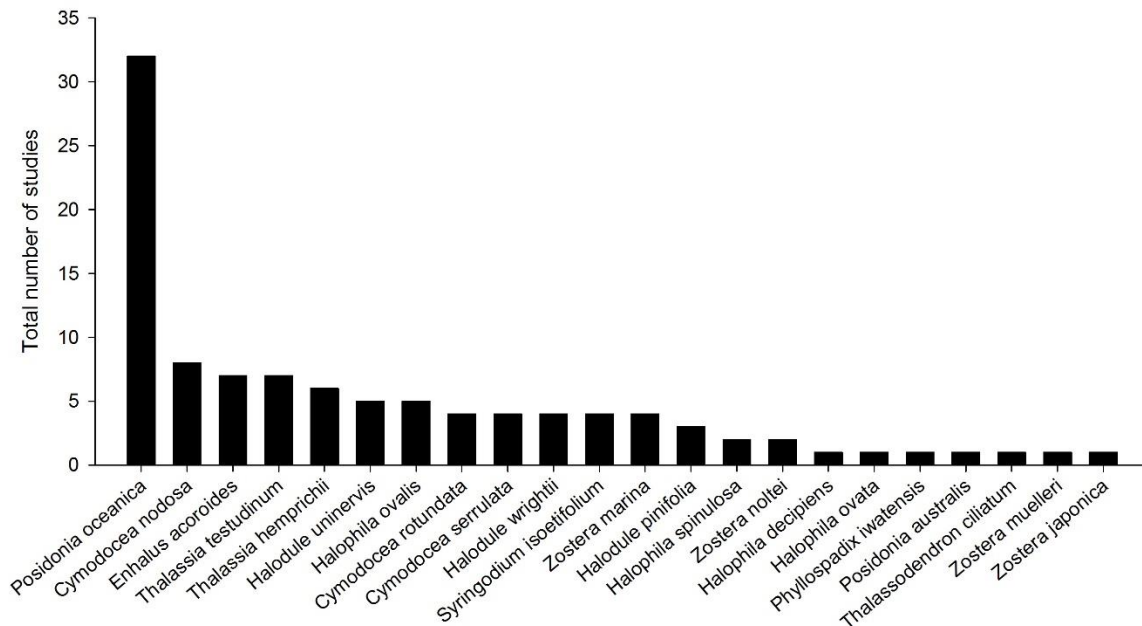


**Figure 2.3. Global map of seagrass as trace element bioindicators, adapted from Sánchez-Quiles, Marbà and Tovar-Sánchez (2017). Additional sites (blue dots) represent the following studies not included in the Sánchez-Quiles, Marbà and Tovar-Sánchez (2017) review (Ahmad et al. 2015; Kilminster 2013; Li & Huang 2012; Lin et al. 2016; Munksgaard, Moir & Parry 2002; Thangaradjou et al. 2013).**

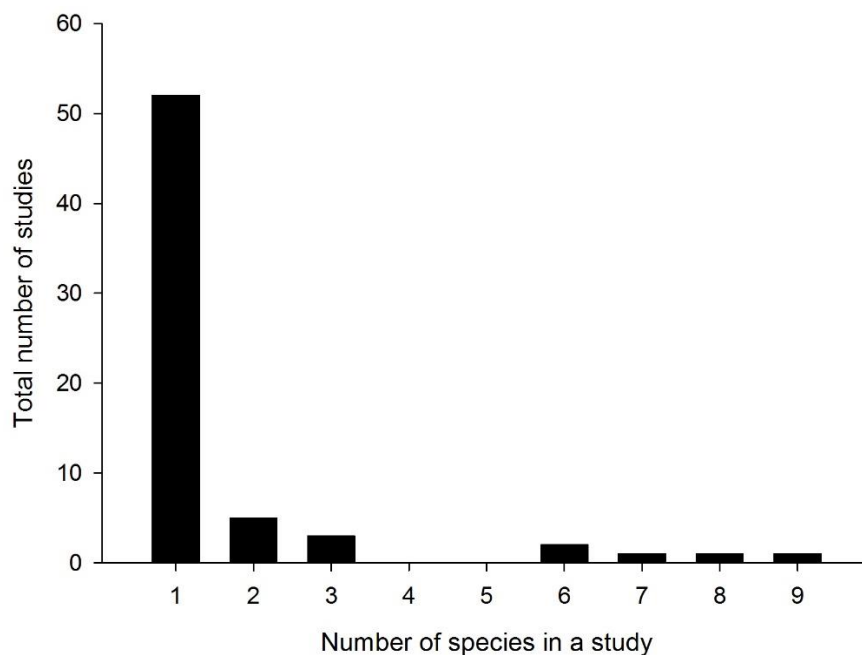
### **2.2.3.2 Seagrass species used as trace element bioindicators**

The predominant species of seagrass used as bioindicators, as identified by this review, was *P. oceanica* followed by *C. nodosa*, *T. testudinum* and *Enhalus acoroides* (Linnaeus f.) Royle (Fig. 2.4). The remainder of the studies represented additional species of the genera *Halophila*, *Halodule*, *Phyllospadix*, *Syringodium*, *Thalassia*, *Thalassodendron* and *Zostera*. There was only one TE study for *Z. muelleri*. The number of species within a study reflected the climate zones. For example, within temperate areas, where diversity is low and meadows homogenous, research focussed on one species such as *P. oceanica* (Fig. 2.5). In tropical areas where diversity of seagrass species is high and meadows are poly-specific, research tended to use six to nine different species (Fig.

2.5). The use of multiple species in tropical studies was because the local area had spatial variation of mono-specific meadows over the depth gradient (intertidal versus subtidal variation) or variation of species between areas as not every species was found at every site (Govers et al. 2014; Nienhuis 1986).



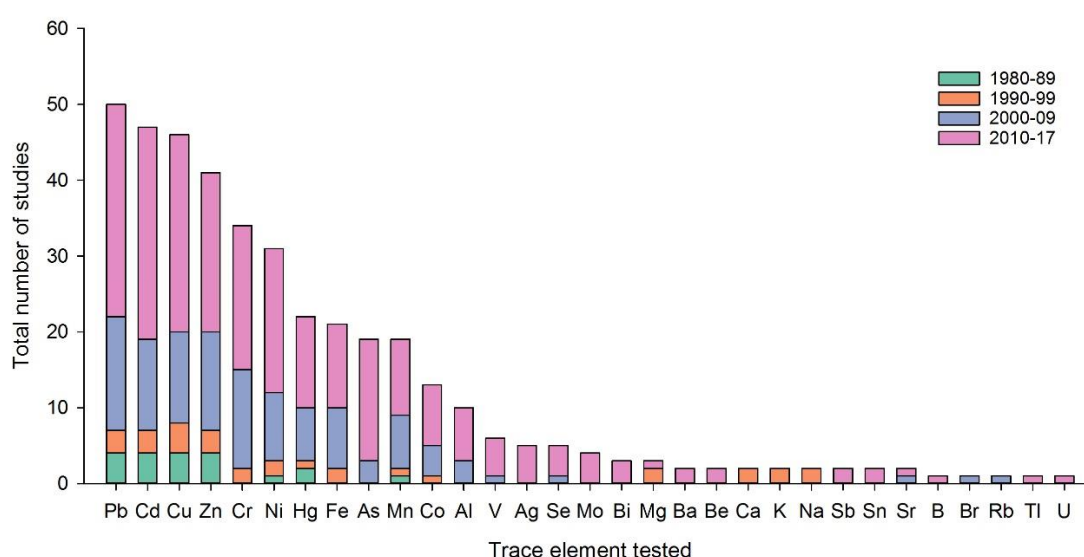
**Figure 2.4.** The total number of studies identified in this review for each seagrass species.



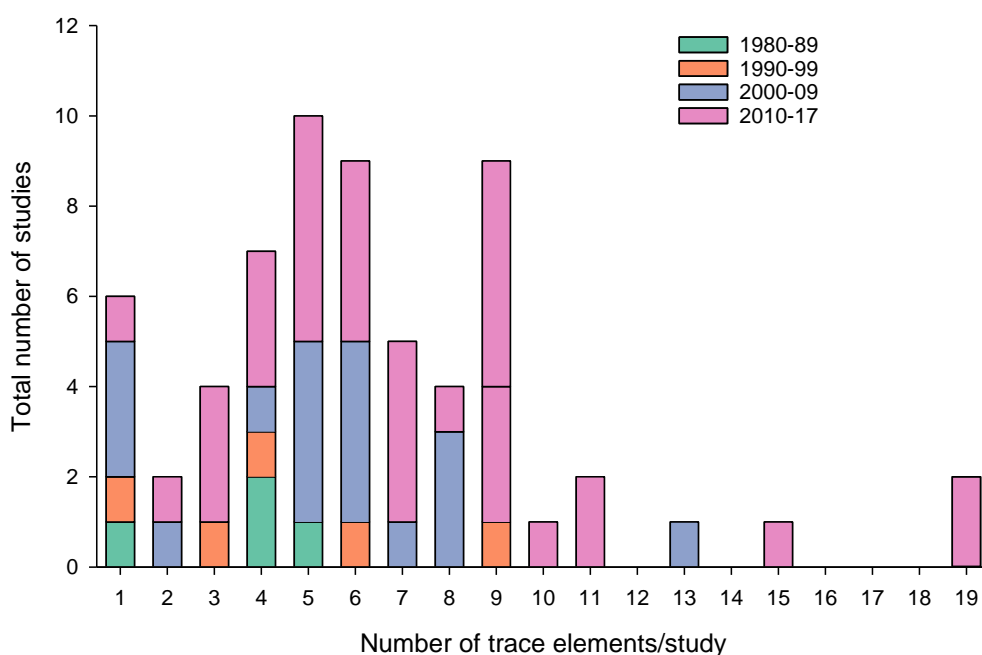
**Figure 2.5.** The total number of studies by the number of seagrass species studied as a trace element bioindicator.

### 2.2.3.3 Trace elements analysed using seagrass as bioindicators

Historically seagrasses have been studied as bioindicators of typical heavy industry pollutants such as Cd, Cr, Cu, Ni, Pb and Zn (Fig. 2.6). While some studies focussed on just one TE, studies often examined five to six of these metals at a time (Fig. 2.7). The next most commonly studied TEs were As, Al, Co, Fe, Hg and Mn (Fig. 2.6). A minority of recent studies now expand their list of TEs to other rarely reported or emerging TEs such as Ag, B, Ba, Be, Bi, Br, Ca, K, Mg, Mo, Na, Rb, Sb, Se, Sn, Sr, Tl, U and V (Fig. 2.6 and Fig. 2.7) (Luy et al. 2012; Malea & Haritonidis 1999; Malea & Kevrekidis 2013; Richir et al. 2013; Solís et al. 2008).



**Figure 2.6. Occurrence of elements studied within seagrass trace element bioindicator studies by decade.**



**Figure 2.7. The frequency of trace elements tested per study by decade.**

The recent increase in the range of TEs studied could be due to analytical instrument advancement or, most likely, an increase in knowledge of TE sources (Luy et al. 2012). For example, it is now recognised that V is often sourced from boat harbours and petrol use (Luy et al. 2012; Richir & Gobert 2014). The TEs in a study would be selected for analysis based on the research question; for example, if the aim is to indicate impacts of coastal land use over a broad spatial scale (hundreds of kilometres), a broad suite of TEs could be utilised; however, at a local scale only known TEs of concern may be examined.

Isotopic studies are frequently undertaken to identify the difference between anthropogenic and non-anthropogenic sources of TEs, such as for Pb (Munksgaard, Moir & Parry 2002). The only isotopic studies identified through the systematic review investigated Pb bioaccumulation (Hoven, Gaudette & Short 1999; Munksgaard, Moir & Parry 2002). Both studies manipulated the seagrass in some way, by either spiking or deploying clean seagrass in polluted areas to observe the industrial or ore-derived Pb isotope accumulation. In addition to the choice of TE tested, it is necessary to understand accumulation and metabolic use of the TE under different conditions by different species.

#### **2.2.3.4 Seagrass trace element bioindicator studies**

The majority of studies that have examined the ability of local seagrass species to act as a TE bioindicator looked at the total concentration of TEs within the seagrasses. Assessments were typically conducted by field studies where specimens that had been exposed to local pollutant conditions were collected, and TE concentration recorded. Few studies manipulated pollutant concentrations under field conditions with experimental chambers to observe accumulation of added TEs by seagrass meadows (Macinnis-Ng & Ralph 2004; Munksgaard, Moir & Parry 2002; Richir et al. 2013).

To understand the accumulation of TEs from the different routes of uptake that is, sediment or water, the concentration was measured in the leaves, root or rhizome separately (Bonanno, Borg & Di Martino 2017), in the whole plant (Brito et al. 2016) or in a single compartment, such as the leaves (Conti, Mecozzi & Finoia 2015). Examples of seagrass partitioned for testing included from within leaf partitioning (leaf tip, blade and base of blade), leaf age (3<sup>rd</sup> intermediate leaf), sheaths, rhizomes and roots, or divided as above- or below-ground, or photosynthetic parts vs non-photosynthetic parts (Bonanno & Di Martino 2017; Brix, Lyngby & Schierup 1983; Conti et al. 2010). The morphology of a seagrass species predetermines how it will be analysed; for example, small species are often kept whole (*H. ovalis*), or a species may be protected and therefore require a less destructive sampling method (e.g., only leaves taken) (Nienhuis 1986; Zakhama-Sraieb et al. 2016). One specialised method of analysis for seagrasses and TE accumulation is the process of lepidochronology (the study of rhizome and sheath age), which is a commonly used method for historical determining (decadal) concentrations of Hg within the sheaths of *P. oceanica* (Gosselin et al. 2006; Lafabrie et al. 2007a). Lepidochronology can only be applied to long-lived larger seagrass species (e.g., *Posidonia* spp.) where enough volume within the sheath material is found, and subsequently may not be applied to *Zostera* spp. as it has smaller sheaths.

#### **2.2.3.5 Accumulation and translocation factors**

Examination of the total concentration of TEs in seagrasses may give some information on the availability of TEs in the environment, but assessment of accumulation and translocation rates can provide a more comprehensive understanding of the TE source. One common formula used to understand the accumulation or translocation of TEs in seagrass is the ratio between the sink (e.g., leaf, rhizome or root) and the source (e.g., water, sediment, leaf, rhizome or root). Throughout the literature, the same formula is used and different authors have assigned their own Factor name (Table 2.4).

**Table 2.4. Examples of Factors used when assessing seagrass trace element concentrations.**

Factor	Formula	Reference
Biosediment Concentration Factor	$C_{\text{organism}} / C_{\text{sediment}}$	Lafabrie et al. (2007b)
Bioconcentration Factor (BCF)	$C_{\text{root}} / C_{\text{sediment}}$	Bonanno and Di Martino (2017)
Biotransference Factor (BTF)	$C_{\text{sink}} / C_{\text{source}}$	Maher et al. (2011)
Translocation Factor (TF)	$C_{\text{rhizome}} / C_{\text{root}},$ $C_{\text{leaf}} / C_{\text{root}},$ $C_{\text{leaf}} / C_{\text{rhizome}}$	Bonanno and Di Martino (2017)

C = concentration of TE as dry weight.

Whereas calculating the Bioconcentration Factor (BCF) can indicate the potential environmental sources of TEs, consideration needs to be given to how environmental TEs are measured (snapshot versus diffusive gradient thin films) and whether all environments or sources of TEs are measured (e.g., pore water) to test for correlation. Some studies included the collection of potential environmental sources of TEs such as overlying water and sediment to provide context. However, correlation between water and seagrass TE concentrations were not always clear, and this could be due to inappropriate one-off water sampling methods (not time integrated), water concentrations less than detection or due to the species uptake mechanisms (Bonanno & Di Martino 2017) .

### **2.2.3.6 Accumulation patterns**

From the literature it is evident that seagrasses do accumulate TEs, yet no global statement can be made in regards to the accumulation of a particular TE within a specific seagrass. Accumulation of TEs varies with the species of seagrass, the part of the seagrass analysed (referred to here as compartment), the TE tested, the concentration of the TE in the environment, seasonality and the physiological requirements of the seagrass for the TE tested (Malea & Haritonidis 1995b). The accumulation pattern of TEs reflects the plant's requirement for the TE, with macronutrients tending to have a higher concentration and micronutrients tending to have a smaller concentration. For example, in *H. stipulacea* and *C. nodosa* the whole plant accumulation pattern was  $\text{Na} > \text{Ca} > \text{K} > \text{Mg} > \text{Fe} > \text{Pb} > \text{Zn} > \text{Cu} \sim \text{Cd}$  with macronutrients (Na, Ca, K, Mg, and Fe)

accumulated at higher concentrations than micronutrients (Zn and Cu) (Malea 1994a; Malea & Haritonidis 1995b). Deviations from a pattern could explain external natural sources of pollution when a suite of TEs are tested (Richir & Gobert 2014).

The systematic review identified some general trends in accumulation between seagrass compartments for one TE, or different orders of TE concentrations within a single compartment. For example, in *P. oceanica*, TE concentrations differed between compartments for example leaf>root>rhizome (Cd, Ni, Zn) or root>leaf>rhizome (As, Cr, Cu, Pb) (Bonanno & Di Martino 2017). Other studies found no difference between compartments, that is leaf = rhizome = root, as seen for Cu concentrations within *C. nodosa* (Bonanno & Di Martino 2016). Other studies have found the general TE accumulation pattern to be Zn>Cu>Cd>Pb>Cr within the leaves of *P. oceanica* (Campanella et al. 2001; Conti et al. 2010; Conti, Iacobucci & Cecchetti 2007), slight variations where Pb was exchange for Cd (Gosselin et al. 2006; Schlacher-Hoenlinger & Schlacher 1998a). Chromium is usually low within *P. oceanica* but Mallezi et al. (2012) found it to be in higher concentration (Cr>Cu>Pb>Cd) due to a naturally high geological source. However, when grouping all the seagrass species leaf material and TEs from Govers et al. (2014), the meta-analysis results produced a pattern of Fe>Zn>Ni>Cu>Pb>Cr>Co>Cd>Hg.

### **2.2.3.7 Habitat coverage**

The majority (86%) of studies from the systematic review were subtidal (or assumed to be subtidal due to the species reported), with the remainder (14%) of studies representing intertidal areas and studies where depth of collection was not reported. The depth a species is sampled from potentially reflects the external environmental influences on the uptake of TEs, such as light, wave exposure and exposure time to concentrations within the medium (e.g., water). Exposure time to water could potentially influence the actual concentration within the leaves as the time of uptake is reduced by being intermittently submerged, and testing for this influence has not been explicitly reported. Consideration of depth (vertical gradient within a meadow), even when fully submersed, requires consideration on TE concentrations due to other factors such as light availability for photosynthesis being variable over depth and potentially influencing TE requirements. Subtidal studies that did consider depth when sampling often found no difference in TE uptake over depth within a small area (Bravo et al. 2016; Malea, Haritonidis & Kevrekidis 1994). However, over the horizontal spatial scale of kilometres there were significant differences in TE concentrations within seagrasses. These

differences reflected the distance from the source of coastal land use and/or the natural geology (Malltezi et al. 2012; Richir et al. 2015; Ward 1987). This systematic review has established that intertidal areas require further research.

#### **2.2.3.8 Temporal coverage**

Temporal coverage of studies varied from single sampling events (Bravo et al. 2016) to multi-year monitoring (Roca et al. 2017), while frequency of sampling varied from monthly, to over a year, to once a year at set times. Temporal studies typically attempted to understand the reasons for seagrass TE concentrations to change over time and found that seagrass as a bioindicator of TEs was influenced by seasonal differences of uptake due to seagrass physiology and external seasonal influences (water temperature and rain runoff, explained in section 2.1.2.3) (Bonanno & Di Martino 2016; Malea & Haritonidis 1999; Schlacher-Hoenlinger & Schlacher 1998a; Ward 1987). External loadings and timing with water physico-chemical seasonality and growth cycle of the species is important to understand and therefore interpret what the bioindicator is actually representing (see section 2.1.2 for detailed accumulation variability examples). Other temporal influences that could change the TE loading in the environment include the timing of local anthropogenic disturbances such as dredging, construction work or ceased mining in relation to sampling times (Filho et al. 2004; Lafabrie, Pergent-Martini & Pergent 2008; Prange & Dennison 2000).

#### **2.2.3.9 Seagrass indices**

The development of an index is a way of representing complex information as a single metric that can be meaningfully interpreted for communication or management. The development of a seagrass index requires the measurement of variables using either destructive (e.g., leaf area, epiphytic cover) or non-destructive techniques (e.g., meadow area coverage), and can range from the cellular level to the community level (Montefalcone 2009). Due to the different types of stressors and reporting requirements by agencies, such as the European Water Directive, there are many seagrass indices. Examples of seagrass indices that are multivariate and include TE variables are single species indices such as the *P. oceanica* multivariate index (POMI) (Romero et al. 2007) and CYMOX for *C. nodosa* (Oliva et al. 2012).

Two *P. oceanica* indices that focused on coastal TE pollution were developed by Richir and Gobert (2014) and applied over the whole Mediterranean area (Richir et al. 2015). Firstly, the Trace Element Spatial Variation Index (TESVI, Table 2.5) was developed to

compare the variability of TE concentrations in the seagrass leaf over a large spatial scale. For example, the TEVSI value of Mn was 0.5 and Mn concentration was therefore not spatially variable, whereas the TESVI value for V was 12.3 and therefore V concentrations were highly variable throughout the area. The second calculation was the Trace Element Pollution Index (TEPI, Table 2.5), which is a modified weighted Metal Pollution Index (MPI) (Richir & Gobert 2014). The TEPI refers to the overall level of TE contamination in seagrass leaves at one site in reference to all the other sites, where higher values indicate greater pollution.

**Table 2.5. Examples of Indices used with seagrass trace element concentrations.**

Index	Formula	Reference
Metal Pollution Index (MPI, actual concentration of the metal)	$(C_1 * C_2 \dots C_n)^{1/n}$	Copat et al. (2012), Lafabrie, Pergent-Martini and Pergent (2008)
Trace Element Pollution Index (TEPI mean normalised concentration)	$(Cf_1 * Cf_2 \dots Cf_n)^{1/n}$	Richir and Gobert (2014)
Trace Element Spatial Variation Index (TESVI)	$[(X_{\max} / X_{\min}) / (\Sigma(X_{\max} / X_i) / n)] * SD$	Richir and Gobert (2014)

C = concentration of element as dry weight.

Cf = mean normalised concentration of element as dry weight

$X_{\max}$  and  $X_{\min}$  = the maximum and minimum mean concentrations recorded among the  $n$  sites

$X_i$  = the mean concentrations recorded in each of the  $n$  sites

SD = standard deviation of the mean ratio  $\Sigma(X_{\max}/X_i)/n$ .

Considerations for developing an index and sampling program need to include whether the sample location is representative, whether results correlate with contamination and the selection of other variables (Richir & Gobert 2014). It is also important to understand that each species, whether a bivalve or seagrass, has their own unique bioaccumulation behaviour which must be understood before utilising it as a bioindicator.

## 2.3 Application to Project

The systematic review of the literature identified that there was a lack of research on seagrass use of TEs in tropical and sub-tropical subtidal areas, and on the use of colonising and opportunistic seagrass species, such as *Z. muelleri*, as TE bioindicators. Trace element uptake and metabolic requirements are unique for each seagrass species. Laboratory experiments using local species are warranted, as limited uptake and partitioning experiments have been conducted on *Z. muelleri* within tropical/sub-tropical areas. In addition, very few studies have looked at the influence of environmental variables (e.g., salinity or light) on TE uptake and therefore this knowledge gap will be addressed within this study. It is clear from the literature that a standardised sampling method will be required to allow for variation in the growth cycle of seagrass in field assessments. The possible influences on TE concentrations, such as through seasonal weather (unplanned) and anthropogenic disturbances (planned) will also need to be considered in the design of the field assessments. The methods selected will address the lack of knowledge of sub-tropical seagrasses as a TE bioindicator for the management of coastal waters.

### **Chapter 3. Trace element variability between and within *Zostera muelleri* meadows and their environmental links**

### 3.1 Introduction

Globally, coastal areas are zones of increasing 'Blue Growth' activities (the sustainable growth of marine and maritime sectors), which increase pressures and changes to the coastal environment (Fowles et al. 2018; Islam & Tanaka 2004). Marine environmental management requires an understanding of how these activities influence the local ecology and environmental conditions (e.g., pollutant loads) in order to initiate an appropriate response (Elliott et al. 2017). Excessive TEs are a pollutant that is associated with Blue Growth, industrial activities and catchment runoff (Birch & McCready 2009; Islam & Tanaka 2004), and some can have toxic effects on biota, or bioaccumulate (Schneider et al. 2018) or biomagnify within other biota (Schneider et al. 2015). Measuring the concentrations of bioavailable TEs within the environment is difficult as one-off sampling events may not be representative over time, measured concentrations can be below or close to laboratory limits of detection, or the concentration of sediment bound TEs can be misinterpreted (Dafforn et al. 2012; Rainbow 2006). Local TE bioindicators are a useful tool in addressing these issues as they accumulate and amplify the bioavailable TEs that are within the environment and with appropriate interpretation of results can distinguish sources of pollutants over different spatial scales (Rainbow 2006).

Along the east coast of Australia there are small to large industrial and shipping areas operating within enclosed to semi-enclosed estuaries (Ambo-Rappe, Lajus & Schreider 2007; Howley 2001). These semi-enclosed estuaries are also prime habitats for seagrass, with the predominant species along the Australian east coast being *Z. muelleri* (Ferguson et al. 2018; Green & Short 2003). Trace element concentrations in *Z. muelleri* have been examined over a spatial gradient within a few of these industrially occupied temperate estuaries, with examples from Lake Macquarie (Ambo-Rappe, Lajus & Schreider 2007), Lake Illawarra (Howley 2001) and the Derwent estuary (Farias et al. 2018). These studies found that *Z. muelleri* can display a gradient of Cu, Cd, Pb and Zn away from pollution point sources. Locally, within Port Curtis, *Z. muelleri* has been analysed for TEs and found to display spatial differences (Jones et al. 2005; Prange & Dennison 2000), although the implications of these findings for using seagrass as a spatial bioindicator are unclear. The majority of studies that report concentrations of TEs in seagrass assume that the TEs present within the seagrass reflect what is in the environment, and therefore do not test environmental TEs. Of the two available studies from Port Curtis, only Jones et al. (2005) attempted to compare seagrass TEs with environmental TEs (water and sediment). However, both local studies (Jones et al.

2005; Prange & Dennison 2000) concluded that it was difficult to identify TE sources due to: a) Port Curtis's complex hydrological circulations, b) diffuse sources such as the air, c) background levels of natural elements, or d) local disturbances.

Distance from the source, external local environmental (e.g., wave energy, pH and temperature) and biological variables (e.g., seagrass leaf area or abundance of epiphytes) are known to influence the bioavailability and concentrations of seagrass TEs (Ambo-Rappe, Lajus & Schreider 2007; El-Hacen et al. 2019; Pergent-Martini & Pergent 2000; Sanz-Lázaro et al. 2012). Seagrasses grow in variable environments that cover the estuarine to oceanic gradient where the physical and chemical properties of water and sediments (e.g., temperature, salinity or pH) are variable and these could influence the localised TE bioavailability (Angel et al. 2010; Ferguson et al. 2018; Hatje et al. 2003; Lewis & Devereux 2009). These aspects of variable localised water physico-chemistry and wave energy can also influence seagrass phylogenetic morphology (e.g., leaf area and biomass), and therefore result in greater differences between sites than within a location (El-Hacen et al. 2019; Ferguson et al. 2018; Maxwell et al. 2014). This is relevant as these morphological differences could influence TE concentrations; for example, a location with low light may cause seagrass to develop larger leaf surface area to increase the rate of photosynthesis (Maxwell et al. 2014), but the larger surface area also allows for increased TE accumulation (Malea & Kevrekidis 2013; Richir & Gobert 2016).

Seagrass physiology and epiphytes are also potential factors causing variability in TE concentration within seagrasses (De Casabianca et al. 2004; Pergent-Martini & Pergent 2000; Richir et al. 2013). It is important to understand these normal variations in TE concentrations in order to understand the relationship between the bioindicator and changes in the environment (Rainbow 2006). For example, if variability in Cr concentrations was higher within a seagrass meadow than between meadows, it would be difficult to interpret a single external source of Cr. Biological variables that influence differences in the concentration of TEs in seagrass include the species tested, leaf age, epiphytes and growth patterns (colonising/opportunistic vs established) (Pergent-Martini & Pergent 2000; Sanz-Lázaro et al. 2012; Vonk et al. 2018). For example, epiphytes on *P. oceanica* can accumulate certain TEs at concentrations 4.5 to 18.4 times greater than the leaves of *P. oceanica*, and therefore influence the overall leaf concentrations if they are not separated (Richir et al. 2013). Location variables such as leaf area, epiphyte cover, water chemistry and sediment particle size have not previously been used to try to

explain *Z. muelleri* TE concentration variability within Australia or Port Curtis or subsequently determine the utility of *Z. muelleri* as a bioindicator.

With the advancement of analytical technology and increasing understanding of emerging TEs (e.g., V), further TEs are being tested in addition to the typical TEs such as Cu, Cd, Cr, Pb, Ni and Zn (Richir & Gobert 2016). This is advantageous as relationships of antagonistic or synergistic chemical behaviour can be observed as a broader suite of TEs are tested. For example, Maher et al. (2011) observed a non-significant relationship between As and Fe concentrations within *Z. muelleri* root system, however, there was a significant correlation of As and Fe within the rhizome and leaf compartments, concluding that there was still evidence to support the theory that Fe assisted As uptake. To aid interpretation of TE concentrations across multiple locations, indices have been developed for assessing seagrass and TE pollution. The TEPI (Richir & Gobert 2014), is a weighted version of the MPI, but due to the variability of the MPI data (highly polluted versus minor pollution) the process of mean normalising the data reduces the resultant number variability. The TESVI calculates the variability of a single TE in comparison to all locations (Richir & Gobert 2014). The interpretation of the accumulation by seagrass of bioavailable TEs from the environment can also be aided using calculations such as the BCF. Values of BCF increase when there is greater accumulation of TEs from the environment (Kilminster 2013). Finally, simply ranking elements by concentration at each location, and then comparing similarities and differences can help to identify spatial patterns in TEs (Pergent-Martini & Pergent 2000). Understanding the spatial differences in seagrass accumulation and correlation to the environmental TEs aids in a seagrasses use as a bioindicator of different environments and subsequently the management of those environments. For example, variable BCF Pb values reported by Birch, Cox and Besley (2018) demonstrated differences in accumulation due to location (higher BCF at polluted sites) and seagrass compartments (higher BCF within leaves than in the rhizome and root compartments).

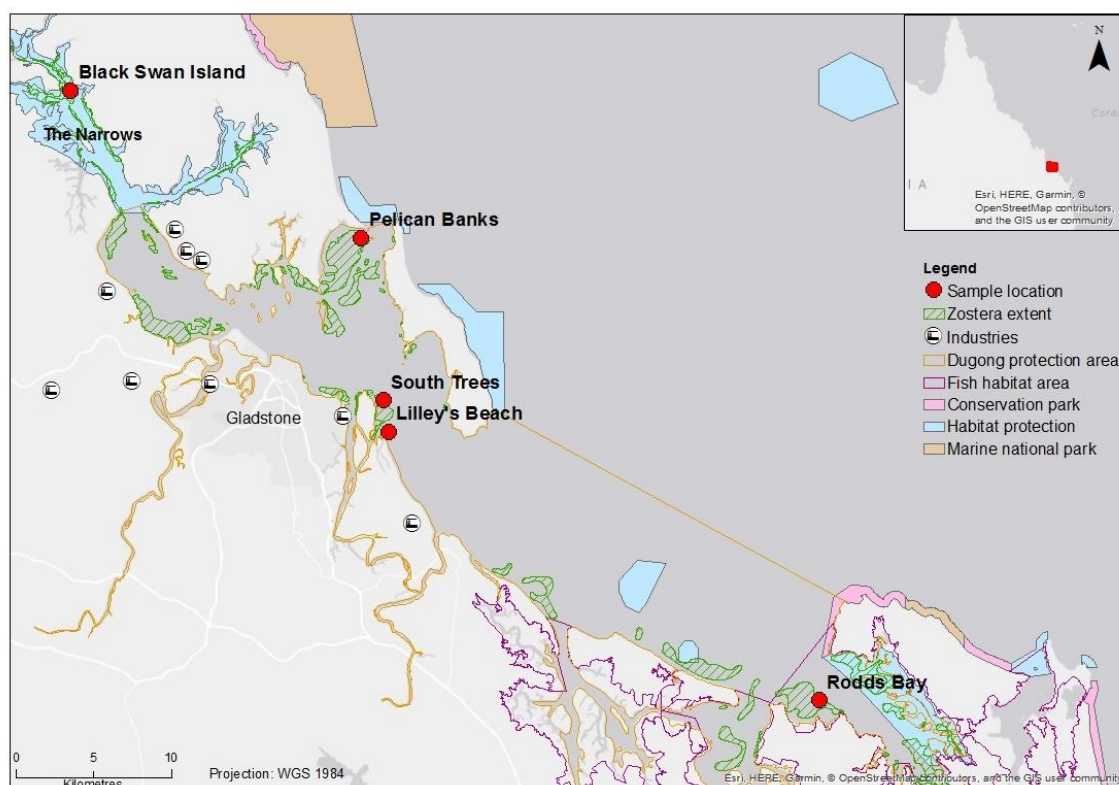
While *Z. muelleri* has been analysed for TEs spatially within Port Curtis, these studies were limited. Prange and Dennison (2000) tested five TEs over a large spatial scale and Jones et al. (2005) tested ten TEs but at only two locations. Further knowledge is required to determine whether seagrass can be an effective bioindicator of spatial differences in TE concentrations. It is expected that *Z. muelleri* TE concentrations across Port Curtis will be location specific, unless TE bioavailability and/or regulation are influenced by external environmental or internal seagrass biological factors. However, estuarine environmental variability could drive the differences in seagrass TE

concentrations to a greater extent than the distance to the source. This project aims to further the knowledge of local *Z. muelleri* as a TE bioindicator by assessing whether: 1) whole *Z. muelleri* had different TE concentrations within or between locations throughout Port Curtis, and whether there is a relationship (accumulation or correlation) between the seagrass TE concentrations and water and sediment sample TE concentrations; and 2) the measured environmental drivers such as sediment particle size, water quality (physico-chemistry and TEs) and seagrass morphometrics explains the variability in seagrass TE concentrations. Additionally a further aim will assess the application of internationally developed indices to local data.

## **3.2 Methods**

### **3.2.1 Site selection**

Port Curtis is characterised as a macro-tidally dominated subtropical estuary that is fed by the Calliope and Boyne rivers with predominant rainfall occurring in summer (Fig 3.1) (Flint et al. 2015; Herzfeld et al. 2004). Modelled hydrological flows of Port Curtis indicate that the predominant water movement is from the south to the north with a flushing time of 22–26 days (Herzfeld et al. 2004). Physico-chemical variables delineate different habitat areas, with the western shores being predominantly estuarine, and mixing with oceanic waters on the eastern boundary of the fringing islands (Angel et al. 2010). The main township within Port Curtis is Gladstone, which has a resident population of 55 616 (year 2016, [www.censusdata.abs.gov.au](http://www.censusdata.abs.gov.au)). The Port of Gladstone is Australia's fifth largest multi-commodity port and supports an array of industries such as coal, liquefied natural gas, ammonium nitrate, alumina and aluminium production (Flint et al. 2015). The water quality reference zone used by the PCIMP monitoring program is Rodds Bay, which lies to the south of Gladstone and consists of a small township (Turkey Beach, <200 people in 2016, [www.censusdata.abs.gov.au](http://www.censusdata.abs.gov.au)) and a catchment of small rural agricultural properties and national parks. Port Curtis is adjacent to the Great Barrier Reef Marine Park and within the boundary of the Great Barrier Reef World Heritage Area. The waters of Port Curtis support an array of important ecosystems that require management and this is reflected in the array of jurisdictional zoning which includes the Gladstone Harbour, Great Barrier Reef Marine Park zones, Fish habitat and Dugong protection areas (Fig. 3.1).



**Figure 3.1. Map of Gladstone indicating locations of sample locations, industrial activity, and past *Zostera muelleri* extent (seagrass extent supplied through a joint partnership of Gladstone Ports Corporation and TropWater). Additional zoning of: Great Barrier Reef Marine Park (conservation, habitat protection and marine national park zones), dugong protection area and fish habitat area.**

One predominant marine ecosystem found throughout Port Curtis is the intertidal and deep seagrass meadows of *Halodule uninervis* (Forsskål) Ascherson, *Halophila decipiens* Ostenfeld, *H. ovalis*, *Halophila spinulosa* (R.Brown) Ascherson and *Z. muelleri* (Chartrand, Rasheed & Carter 2018). These meadows are a predominant food source to local megaherbivore populations of endangered green turtles *Chelonia mydas* and vulnerable *Dugong dugon* (Prior, Booth & Limpus 2015; Rasheed et al. 2017). The large permanent meadows are dominated by *Z. muelleri*, a strappy leaf, opportunistic seagrass species that displays seasonal biomass variation due to natural growth cycles, with peak growth occurring in the late Austral spring of November (Fig. 3.1) (Chartrand et al. 2016).

Sampling locations for *Z. muelleri* across Port Curtis were selected using past knowledge of seagrass presence, then visually assessed for % seagrass cover (>15%) upon arrival and were additionally limited to areas where *H. ovalis* and *H. uninervis* coverage was <10%. Using this assessment, one potential location within Shoal Bay on the western

side of Facing Island was not sampled due to low <15% *Z. muelleri* seagrass cover. The five locations that were selected and sampled varied in distance to potential point sources (approximate distance from Gladstone city centre in parentheses): South Trees (8.5 km), Lilley's Beach (10 km), Pelican Banks (10 km), Black Swan Island (20 km), and Rodds Bay (40 km) (Fig. 3.1). Air temperature during sampling ranged from 19.5°C to 29.3°C. Recorded rainfall at the Gladstone airport weather station in the week preceding sampling was 16.2 mm and during the sampling period was 0 mm (Bureau of Meteorology Australia, [www.bom.gov.au](http://www.bom.gov.au)).

### 3.2.2 Sample collection

*Zostera muelleri* was collected at intertidal locations on low spring tides, over five consecutive days at the beginning of November 2017, under permit (CQU GBRMPA approval permit reference number G17/10-028) and notification (Department of Agriculture and Fisheries) conditions. At each location, seagrass sample collection avoided the meadow edge, low water mark and tidal pools to reduce the influence of epiphytes and the time of submersion on TE concentrations. Sampling design at the five locations consisted of three sites at 50–200 m apart with each site having three random replicate samples at 3–5 m apart. Each replicate sample included whole seagrass material (leaves, rhizomes, roots, epiphytes and flowers) for TE analysis, recordings of seagrass morphometrics, and sediment for particle size and TE analysis. Seagrass morphometrics and meadow properties (% seagrass cover, % species composition, % algae, % epiphyte, leaf length and width, and general observations of grazing and flowering) were visually observed by the researcher within a randomly placed 0.5 x 0.5 m quadrat (McKenzie, Campbell & Roder 2003). Assessment of % seagrass cover was compared to previously produced percent cover photo standards and % epiphyte was assessed as the percent of the seagrass leaf surface area that was covered in epiphytes within the quadrat (McKenzie, Campbell & Order 2003). Leaf length and width were determined by collecting five leaves from within the quadrat and photographing them in the field on a white background with a variable scale bar for calculations. These photographs were digitally analysed later with the aid of ImageJ software ([www.imagej.nih.gov/ij/](http://www.imagej.nih.gov/ij/)). Whole seagrass for TE determination were collected by pooling six cores (plastic core: 9 cm diameter x 10 cm depth) that were collected from within a 2 m radius around the quadrat. The rhizosphere sediment was removed from the seagrass using ambient seawater. Seagrass samples were then placed in plastic bags and kept on ice until return to the laboratory where they were stored frozen until sample processing, which occurred within two months of sampling. Sediment samples

were collected, minimising seagrass material, using a plastic corer (9 cm diameter x 10 cm depth), placed into plastic bags and treated in the same manner as the seagrass regarding transportation and handling.

### **3.2.3 Sample preparation and analysis**

Whole seagrass samples were prepared for TE analysis by rinsing the seagrass with Milli-Q water and removing non-seagrass biotic and abiotic material. The seagrass was then patted dry with paper towel, weighed (wet weight), placed into a new clean plastic bag and frozen. Whole seagrass samples were then freeze-dried and hand agitated within the plastic bag to homogenise the sample. Sediment was wet sieved through a 2 mm sieve and the 2 mm retained fraction was dried at 60°C for 24 h and ground by mortar and pestle. The sediment particle size of silt (<63 µm) was determined from a <1 mm sieved subsample and measured by laser particle size analysis using a Malvern MasterSizer 3000, Hydro EV.

Seagrass and sediment samples were analysed at the Australian Government National Measurement Institute (NMI) laboratory, Sydney, for total recoverable Al, As, Cd, Cu, Cr, Fe, Mn, Pb, Ni and Zn. The NATA accredited NMI in-house analysis methods NT2.46 and NT2.49 were used for seagrass and sediment respectively. Samples were digested in high purity nitric and hydrochloric acids by heating on a hot block at 95–100°C for two hours. Seagrass and sediment element concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900) and results were reported as dry weight. Recovery for each TE was 97–111% for seagrass, and 84–116% for sediment (Appendix A, Table A1 and Table A2). Values below the analytical limit of reporting (LoR) were recorded as the LoR value for reporting and calculations. Dissolved seawater TEs and physico-chemical parameters were supplied by PCIMP from their November 2017 sampling event from sites adjacent to sampled meadows.

### **3.2.4 Data analysis**

To assess whether differences in TE concentrations (in seagrass and sediment) and seagrass morphometric and environmental descriptors (e.g., % seagrass cover, % silt) were significantly different between locations than within locations a general linear model univariate analysis of variance (ANOVA) was applied with the effects of location (fixed, five levels: Black Swan Island (BS), Pelican Banks (PB), South Trees (ST), Lilley's Beach (LB) and Rodds Bay (RB)) and the effect of sites (nested, random, three levels; 1, 2 and 3). Data was checked for normality and homogeneity of variances, and

transformations were performed where required. Tukey HSD post-hoc tests were performed where significant differences were found between locations. The ANOVAs were carried out using SPSS v. 24 (IBM corp., Armond, NY).

Bioconcentration Factors (Equation 1) were applied to the seagrass TEs for each location and compared to the sediment and dissolved seawater TEs:

**Equation 1.** Bioconcentration Factor = 
$$\frac{\text{Whole seagrass concentration (mg kg}^{-1} \text{ DW)}}{\text{Environment concentration (mg kg}^{-1} \text{ DW)}}$$

Bioconcentration Factor indicates the amount of accumulation, with values greater than 1 indicating accumulation within the seagrass from the environment (Bonanno & Borg 2018; Kilminster 2013). Pearson correlations between sediment and seagrass TEs were performed to test for a relationship between biological and environmental TE sources. Correlations were not performed to test for relationships between seagrass and dissolved TE concentrations in water samples, due to insufficient water sample replicates.

Primer v.7 and the Permutational Multivariate Analysis of Variance (Permanova) + software package (Anderson 2008) was used to explore the multivariate aspects of the dataset. To meet the first aim of seagrass TE variability, Non-metric Multidimensional Scaling (nMDS) was utilised to assess the similarity of seagrass TEs by location (data was normalised and Euclidean distance was applied). Principal component analysis (PCA) was performed on all sediment TEs (normalised) to address aim one to describe the sediment TEs. Another PCA on the environmental (e.g., physico-chemistry, % silt) and seagrass morphometric data was performed to visualise the environmental data that was to be used in aim two. The BIOENV procedure was applied to find the best possible rank order match between all seagrass TEs and the environmental data (e.g., physico-chemistry, sediment TE, % silt, seagrass morphometrics) that could explain possible environmental drivers to seagrass TE variability (aim two). Data for % silt and % epiphyte cover were square root transformed and the Euclidean distance was applied before producing the resemblance matrix. Following determination of the best match of environmental variables to all seagrass TE concentrations (from the BIOENV outputs), the best explaining predictors (% silt and % epiphyte cover) were compared to each seagrass TE by the distance-based linear models (DistLM) sequential test to explain the relationship of those predictors. The DistLM selection criterion was adjusted  $R^2$ , selection procedure was specified and Euclidean distance was applied. Results of

DistLM were used to demonstrate the proportion of the applied variables in explaining the seagrass TE location variability.

Two indices were applied to the seagrass TE concentrations to ascertain their levels of contamination and TE variability. Higher TEPI values indicate a site with overall higher concentrations of TEs in comparison to the other locations (Richir & Gobert 2014). The TEPI is calculated for each location by Equation 2, where  $Cf_n$  is the mean normalised concentration of each TE ( $n$ ) in a given location.

**Equation 2.** 
$$TEPI = (Cf_1 * Cf_2 \dots Cf_n)^{1/n}$$

The second index applied was TESVI (Richir & Gobert 2014), which compares the variability of a single TE throughout all locations and is calculated by Equation 3 where for a single TE the  $x_{max}$  and  $x_{min}$  are the maximum and minimum average concentration from among the five locations,  $X_i$  is the average of the TE within each location ( $n$ ) and SD is the standard deviation of the mean ratio  $\Sigma(x_{max}/x_i/n)$ .

**Equation 3.** 
$$TESVI = [(x_{max}/x_{min})/(\Sigma (x_{max}/x_i)/n)] * SD$$

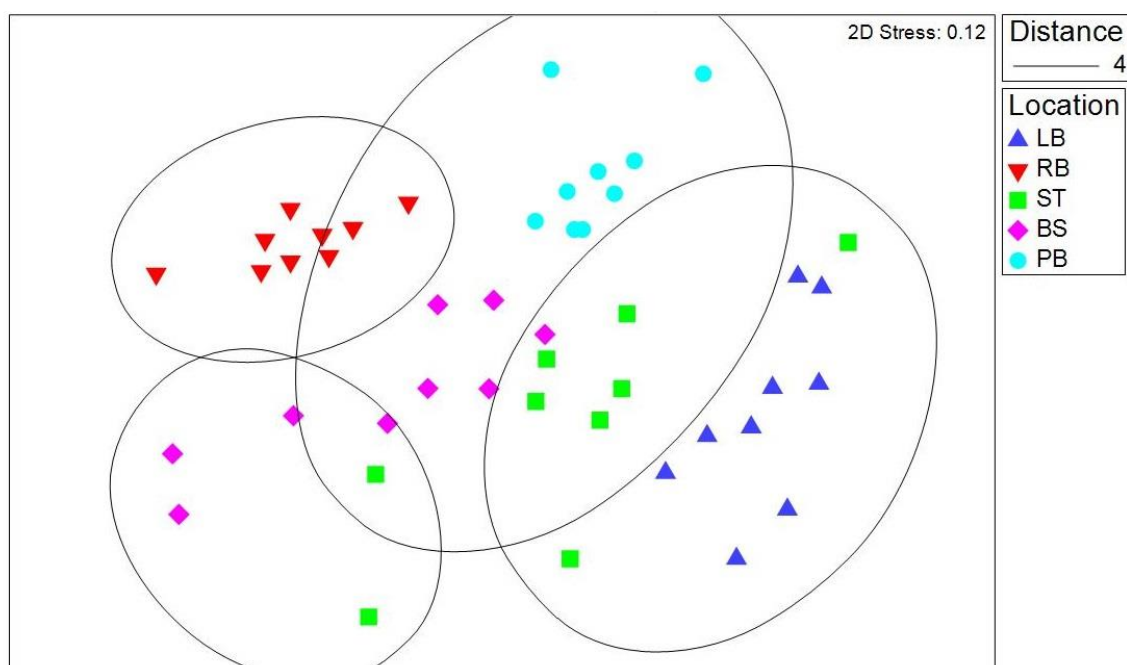
A high TESVI value indicates higher variability between locations for that seagrass TE concentration, indicating a potential source of that TE or variable seagrass accumulation. A lower TESVI value indicates low variability in seagrass TE concentrations, indicating either no source of pollution or no difference in accumulation due to self-regulation by the seagrass.

## 3.3 Results

### 3.3.1 Spatial relationships in trace element concentrations

#### 3.3.1.1 Seagrass trace elements

Spatial variation of seagrass TE concentrations was location and TE specific. Overall seagrass TE composition was dissimilar between locations, with Rodds Bay and Pelican Banks being most dissimilar to the other locations (Fig. 3.2).



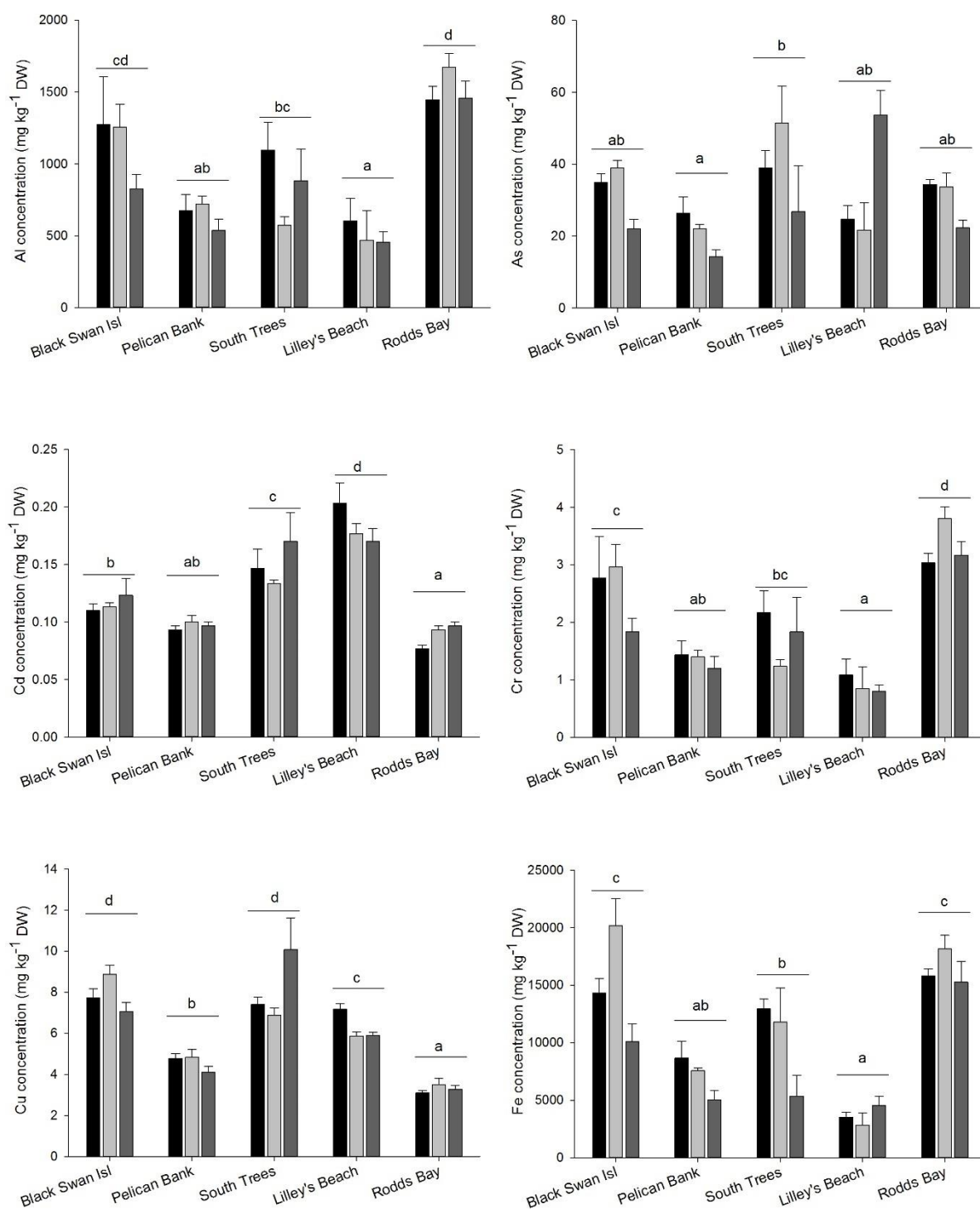
**Figure 3.2. Non-Metric dimensional scaling of all seagrass trace element concentrations. Location abbreviations: Lilley's Beach (LB), Rodds Bay (RB), South Trees (ST), Black Swan Island (BS), and Pelican Banks (PB).**

Significant differences between locations and variability within location at the meadow scale were observed for most TEs, with the exception of seagrass Zn concentrations, which showed no significant difference between or within locations or sites, with an overall mean of  $26.6 \pm 4.83 \text{ mg kg}^{-1}$  (Lo:  $F_{4,30} = 1.477$ ,  $p = 0.234$ , Si(lo)  $F_{10,30} = 1.958$ ,  $p = 0.076$ , Table 3.1, Fig. 3.3). Seagrass TEs that were significantly different between locations with no significant site effect were Al, Cd, Cr, Mn and Pb (Al Lo:  $F_{4,30} = 305.07$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 1.362$ ,  $p = 0.245$ ; Cd Lo:  $F_{4,30} = 313.56$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 1.39$ ,  $p = 0.232$ ; Cr Lo:  $F_{4,30} = 87.13$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 1.39$ ,  $p = 0.232$ ; Mn Lo:  $F_{4,30} = 80.84$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 1.78$ ,  $p = 0.105$ ; Pb Lo:  $F_{4,30} = 137.67$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 1.14$ ,  $p = 0.369$ , Table 3.1, Fig. 3.3). Significant location variability of these TEs (Al, Cd, Cr, Mn and Pb) demonstrated Rodds Bay seagrass to have significantly higher mean concentrations of Al  $1525.6 \pm 191.25 \text{ mg kg}^{-1}$ , Cr  $3.33 \pm 0.47 \text{ mg kg}^{-1}$  and Pb  $2.79 \pm 0.42 \text{ mg kg}^{-1}$  than other locations and a significantly lower mean of Cd  $0.09 \pm 0.01 \text{ mg kg}^{-1}$  (Fig. 3.3). Seagrass collected from Lilley's Beach had significantly lower concentrations of some TEs than other locations with means of Al  $510 \pm 243 \text{ mg kg}^{-1}$ , Cr  $0.91 \pm 0.44 \text{ mg kg}^{-1}$  and Pb  $0.76 \pm 0.24 \text{ mg kg}^{-1}$  (Fig. 3.3). Seagrass from Lilley's Beach also had significantly higher mean concentrations of Cd  $0.18 \pm 0.03 \text{ mg kg}^{-1}$  and Mn  $290 \pm 54.8 \text{ mg kg}^{-1}$  than seagrass from other locations (Fig. 3.3). The lowest mean of Mn

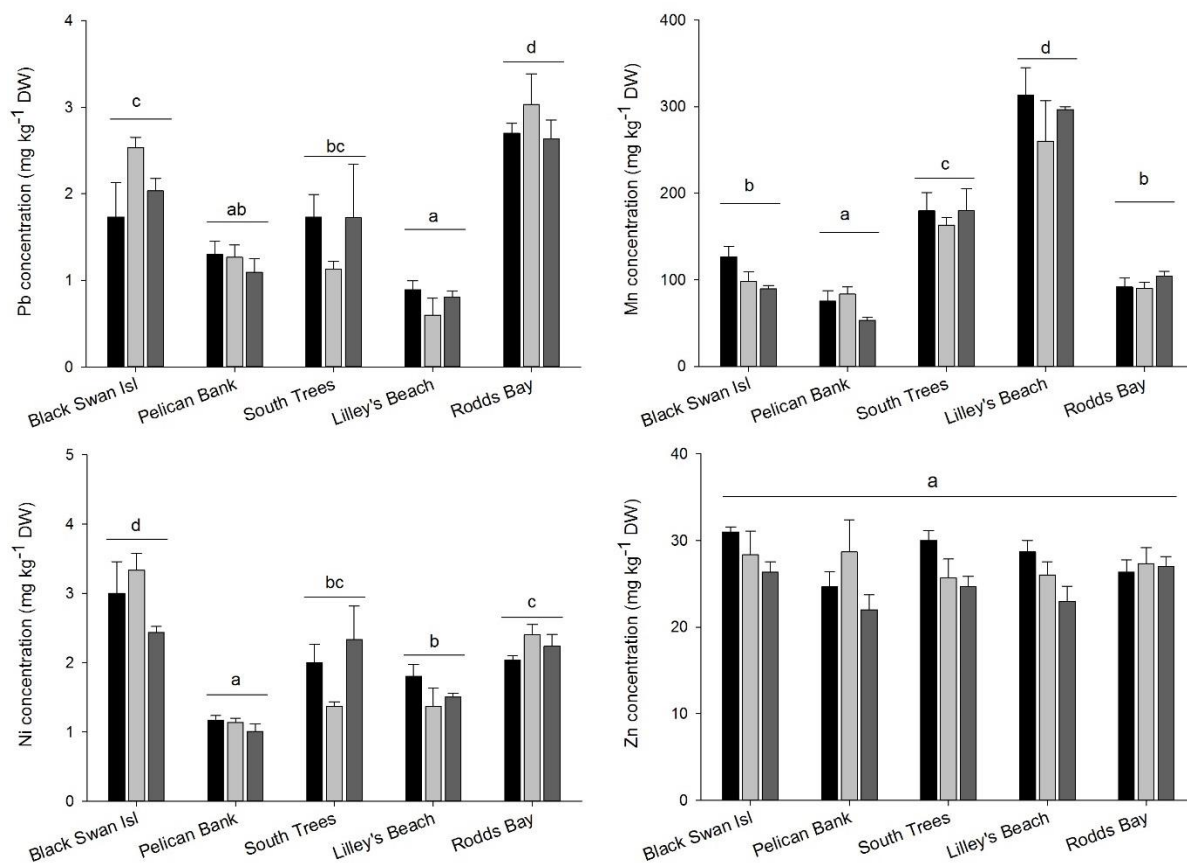
70.8 ± 18.9 mg kg<sup>-1</sup> in seagrass collected from Pelican Banks was significantly different to other locations (Fig. 3.3).

**Table 3.1. Univariate ANOVA results for each trace element concentration in whole seagrass samples, by location and site (site nested within location). Values in bold are significant  $p < 0.05$ .**

		<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<b>Aluminium</b>	Location	4	8199.8	305.07	<b>0.000</b>
	Site (Location)	10	26.88	1.36	0.245
	Error	30	19.74		
<b>Arsenic</b>	Location	4	9006.2	24.06	<b>0.000</b>
	Site (Location)	10	374.3	3.9	<b>0.002</b>
	Error	30	95.5		
<b>Cadmium</b>	Location	4	0.156	313.56	<b>0.000</b>
	Site (Location)	10	0.0	1.39	0.232
	Error	30	0.0		
<b>Chromium</b>	Location	4	41.67	87.13	<b>0.000</b>
	Site (Location)	10	0.478	1.39	0.232
	Error	30	0.344		
<b>Copper</b>	Location	4	359.15	132.4	<b>0.000</b>
	Site (Location)	10	2.71	3.54	<b>0.003</b>
	Error	30	0.77		
<b>Iron</b>	Location	4	1175986760	39.99	<b>0.000</b>
	Site (Location)	10	29405833	4.61	<b>0.001</b>
	Error	30	6378468		
<b>Manganese</b>	Location	4	2.83	80.84	<b>0.000</b>
	Site (Location)	10	0.063	1.78	0.105
	Error	30	0.035		
<b>Lead</b>	Location	4	29.88	137.67	<b>0.000</b>
	Site (Location)	10	0.217	1.14	0.369
	Error	30	0.191		
<b>Nickel</b>	Location	4	1.21	37.89	<b>0.000</b>
	Site (Location)	10	0.077	2.4	<b>0.031</b>
	Error	30	0.032		
<b>Zinc</b>	Location	4	14.967	1.48	0.234
	Site (Location)	10	19.84	1.96	0.076
	Error	30	10.13		



**Figure 3.3. Seagrass trace element concentrations (mean  $\pm$  SE,  $n = 3$ , mg kg<sup>-1</sup> dry weight) by location. Bar colour indicates: black, site 1; light grey, site 2; dark grey, site 3. Similar letters indicate no significant differences between location.**



**Figure 3.3 (continued) Seagrass trace element concentrations (mean  $\pm$  SE,  $n = 3$ ,  $\text{mg kg}^{-1}$  dry weight) by location. Bar colour indicates: black, site 1; light grey, site 2; dark grey, site 3. Similar letters indicate no significant differences between location.**

When seagrass TE concentrations were significantly different at the site level (nested within location), it suggests larger variation at the meadow scale. Significant differences at the site level were observed for As, Cu, Fe and Ni (As Lo:  $F_{4,30} = 24.06$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 3.9$ ,  $p < 0.05$ ; Cu Lo:  $F_{4,30} = 132.4$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 3.54$ ,  $p < 0.01$ ; Fe Lo:  $F_{4,30} = 39.99$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 4.61$ ,  $p < 0.001$ ; Ni Lo:  $F_{4,30} = 37.89$ ,  $p < 0.001$ , Si(lo)  $F_{10,30} = 2.4$ ,  $p < 0.05$ ; Table 3.1, Fig. 3.3). Site mean concentrations of As at South Trees ranged from 26.8 to 51.3  $\text{mg kg}^{-1}$  and Fe at South Trees ranged from 5343 to 12933  $\text{mg kg}^{-1}$ . Concentrations of As in seagrass were significantly different between locations, with the lowest mean As concentration recorded at Pelican Banks  $20.89 \pm 6.88 \text{ mg kg}^{-1}$  and the highest at South Trees  $39.06 \pm 18.19 \text{ mg kg}^{-1}$  (Fig. 3.3). Seagrass Fe concentrations varied with location, with Lilley's Beach having the lowest mean concentration  $3634 \pm 1420 \text{ mg kg}^{-1}$ , and Rodds Bay ( $16400 \pm 2346 \text{ mg kg}^{-1}$ ) and Black Swan Island ( $15124 \pm 5123 \text{ mg kg}^{-1}$ ) having significantly higher mean concentrations (Fig. 3.3). Seagrass Cu and Ni concentrations had a significant site influence in addition

to the significant location differences, although the significant differences between sites within a location appear to have been driven by a difference in only one location of South Trees (Fig. 3.3). Mean seagrass Cu concentrations were significantly lower at Rodds Bay with  $3.29 \pm 0.37 \text{ mg kg}^{-1}$  and significantly higher at Black Swan Island  $7.89 \pm 1.03 \text{ mg kg}^{-1}$  and South Trees  $8.11 \pm 2.05 \text{ mg kg}^{-1}$  (Fig. 3.3). Mean seagrass Ni concentrations ranged from  $1.10 \pm 0.15 \text{ mg kg}^{-1}$  at Pelican Banks to significantly higher concentrations at Black Swan Island  $2.92 \pm 0.6 \text{ mg kg}^{-1}$ .

The order of seagrass TEs by concentration was the same at all locations for the first five TEs (Fe>Al>Mn>As>Zn) and Cd was consistently the lowest (Table 3.2). Copper, Ni, Cr and Pb varied in their order depending on the location (Table 3.2).

**Table 3.2. Order of trace elements by concentration for each location.**

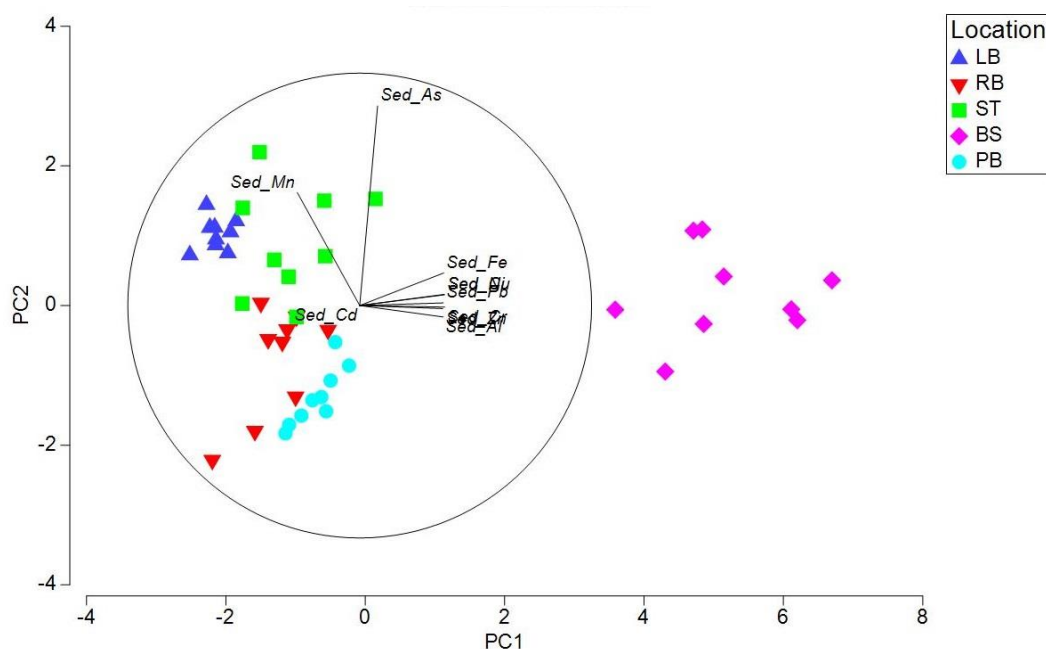
	Element order
<b>Black Swan Island</b>	Fe>Al>Mn>As>Zn>Cu>Ni>Cr>Pb>Cd
<b>Pelican Banks</b>	Fe>Al>Mn>As>Zn>Cu>Cr>Pb>Ni>Cd
<b>South Trees</b>	Fe>Al>Mn>As>Zn>Cu>Ni>Cr>Pb>Cd
<b>Lilley's Beach</b>	Fe>Al>Mn>As>Zn>Cu>Ni>Cr>Pb>Cd
<b>Rodds Bay</b>	Fe>Al>Mn>As>Zn>Cr≈Cu>Pb>Ni>Cd

### **3.3.1.2 Sediment and dissolved water trace elements**

Total recoverable sediment TEs did not display the same significant differences between locations or within site variability as observed for *Z. muelleri* TE concentrations.

Sediment Cd was below the LoR at all locations. The PCA of sediment TE concentrations clearly demonstrates that Black Swan Island is distinctly different from the other locations by its TE composition (Fig. 3.4). PC1 was 81.8%, explained by sediment Al, Cr, Cu, Fe, Pb, Mn, Ni, and Zn, and PC2 was 13.5%, explained by sediment As and Mn. This is confirmed statistically by sediment concentrations of eight TEs at Black Swan Island being significantly higher than at other locations (Al, As, Cr, Cu, Fe, Pb, Ni and Zn, Table 3.3, Appendix B Table B1). The location with the lowest mean sediment concentrations was Lilley's Beach (Al, Cr, Cu, Fe, Pb, and Zn, Table 3.3). Sediment Zn concentrations, unlike the seagrass results, had significant differences ( $p < 0.001$ , Appendix B Table B1) between locations with location means ranging from 10.8 to 26.9

mg kg<sup>-1</sup> (Table 3.3). Dissolved (0.45 µm filtered) TE concentrations in seawater samples were below the LoR for Cd, Cr, Cu, Pb and Ni (Table 3.3) with Al, As, Fe, Mn and Zn having measurable results. Dissolved Al, in comparison to the other locations, was higher within the estuarine areas of Black Swan Island and Rodds Bay (Table 3.3). Dissolved Mn was highest around South Trees and Lilley's Beach (Table 3.3). No statistics were performed due to the low and uneven weighting.



**Figure 3.4. Principal Component Analysis of all sediment trace element concentrations. Location abbreviations: Lilley's Beach (LB), Rodds Bay (RB), South Trees (ST), Black Swan Island (BS), and Pelican Banks (PB).**

Table 3.3. Sediment (mean  $\pm$  SD, n = 9, mg kg<sup>-1</sup> dry weight) trace element concentrations at each location and dissolved (0.45  $\mu$ m filtered) seawater concentrations obtained from PCIMP (mean  $\pm$  SD, n = 2–3,  $\mu$ g L<sup>-1</sup>) for each trace element at each location. Bold indicates maximum mean, italics indicates minimum mean. Similar letters indicate no significant difference between locations. Full F table within Appendix B Table B1.

	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
<b>Sediment</b>										
<b>Black Swan Island</b>	<b>8237</b> (1578) <sup>c</sup>	<b>9.47</b> (1.04) <sup>b</sup>	<0.5 (0)	<b>21.4</b> (2.19) <sup>c</sup>	<b>9.22</b> (1.28) <sup>c</sup>	<b>14311</b> (1343) <sup>c</sup>	<b>5.63</b> (0.32) <sup>d</sup>	76 (8.44) <sup>a</sup>	<b>9.58</b> (1.46) <sup>c</sup>	<b>26.9</b> (1.05) <sup>d</sup>
<b>Pelican Banks</b>	3866 (287) <sup>b</sup>	7.06 (0.49) <sup>a</sup>	<0.5 (0)	10.9 (0.78) <sup>b</sup>	2.82 (0.28) <sup>b</sup>	7644 (514) <sup>ab</sup>	2.31 (0.17) <sup>b</sup>	131 (13.6) <sup>b</sup>	3.56 (0.27) <sup>b</sup>	15.4 (1.13) <sup>c</sup>
<b>South Trees</b>	3715 (663) <sup>b</sup>	9.17 (1.04) <sup>b</sup>	<0.5 (0)	10.5 (1.72) <sup>b</sup>	2.79 (0.54) <sup>b</sup>	8500 (966) <sup>b</sup>	2.51 (0.41) <sup>b</sup>	<b>238</b> (22.0) <sup>e</sup>	3.70 (0.55) <sup>b</sup>	13.6 (1.88) <sup>b</sup>
<b>Lilley's Beach</b>	2337 (157) <sup>a</sup>	9.90 (0.23) <sup>b</sup>	<0.5 (0)	8.17 (0.52) <sup>a</sup>	1.48 (0.20) <sup>a</sup>	6800 (369) <sup>a</sup>	1.81 (0.12) <sup>a</sup>	206 (22.4) <sup>d</sup>	2.78 (0.12) <sup>a</sup>	10.2 (0.16) <sup>a</sup>
<b>Rodds Bay</b>	3505 (460) <sup>b</sup>	7.47 (0.98) <sup>a</sup>	<0.5 (0)	10.4 (1.20) <sup>b</sup>	1.69 (0.28) <sup>a</sup>	7164 (850) <sup>a</sup>	2.79 (0.31) <sup>c</sup>	165 (21.0) <sup>c</sup>	3.46 (0.41) <sup>b</sup>	10.8 (1.30) <sup>a</sup>
<b>Dissolved (0.45<math>\mu</math>m filtered) seawater</b>										
<b>Black Swan Island</b>	<b>6.50</b> (2.60)	1.30 (0.10)	<0.1 (0)	<1 (0)	<1 (0)	6.97 (1.08)	<1 (0)	2.07 (0.70)	<1 (0)	1.17 (0.30)
<b>Pelican Banks</b>	5.00 (0.00)	1.50 (0.00)	<0.1 (0)	<1 (0)	<1 (0)	<b>10.9</b> (2.90)	<1 (0)	1.00 (0.00)	<1 (0)	1.00 (0.00)
<b>South Trees and Lilley's Beach*</b>	5.07 (0.12)	1.53 (0.06)	<0.1 (0)	<1 (0)	<1 (0)	5.00 (0.00)	<1 (0)	<b>4.63</b> (5.53)	<1 (0)	1.47 (0.57)
<b>Rodds Bay</b>	6.20 (1.04)	<b>1.63</b> (0.60)	<0.1 (0)	<1 (0)	<1 (0)	5.97 (0.75)	<1 (0)	2.10 (0.82)	<1 (0)	<b>2.03</b> (1.79)

\* South Trees and Lilley's Beach water quality are the same as the meadows are adjacent to the same water body.

### **3.3.1.3 Bioconcentration Factors and correlations**

Correlations between the concentrations of TEs in the environment and in whole seagrass samples were tested as a means of observing potential accumulation by seagrass. The BCF indicated that there is a difference in seagrass TE accumulation in regard to the sources (water or sediment) and that this was variable between locations (Table 3.4). The BCFs of seagrass to sediment show that Rodds Bay has some of the highest BCFs (accumulation) for six elements (Al 0.44, As 4.03, Cr 0.32, Fe 2.29, Pb 1.0, Ni 0.64 and Zn 2.48) with Al, Cr, Fe, Pb, Ni and Zn BCF values being almost double or triple in enrichment than at the other locations (Table 3.4). The highest sediment BCF values were recorded for As, with values >3 at all locations, suggesting greater As accumulation from the sediment in comparison to other TEs, irrespective of location (Table 3.4).

Bioconcentration Factors of seagrass to dissolved water, where calculable, were higher for Al, As and Fe at Black Swan Island, South Trees and Rodds Bay than at the other locations (Table 3.4). Zinc BCFs displayed the reverse location accumulation in respect to the source (water or sediment) of Zn, with South Trees, Lilley's Beach, and Rodds Bay having higher sediment BCFs while Black Swan Island and Pelican Banks had greater dissolved BCFs, suggesting potential different sources of Zn (Table 3.4). Pearson correlations demonstrated significant ( $p < 0.05$ ) positive relationships between whole seagrass and sediment concentrations of Al, Cd, Cr, Cu, Fe, Mn, Ni and Pb (Table 3.5).

Table 3.4. Bioconcentration Factors for whole seagrass to sediment (top rows) and whole seagrass to dissolved (0.45 µm filtered) seawater (bottom rows). Cells with '-' indicate that BCF could not be calculated because trace element concentrations were below the limit of reporting. Values highlighted in blue are greater than the average of that trace element.

Sediment	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
Black Swan Island	0.14	3.38	-	0.12	0.86	1.04	0.37	1.38	0.31	1.06
Pelican Banks	0.17	2.96	-	0.12	1.62	0.93	0.53	0.54	0.31	1.63
South Trees	0.23	4.26	-	0.17	2.91	1.18	0.61	0.73	0.51	1.98
Lilley's Beach	0.22	3.37	-	0.11	4.27	0.53	0.42	1.40	0.56	2.53
Rodds Bay	0.44	4.03	-	0.32	1.95	2.29	1.00	0.58	0.64	2.48
Dissolved seawater	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
Black Swan Island	172	24.6	-	-	-	2136	-	50.8	-	24.5
Pelican Banks	129	13.9	-	-	-	647	-	70.8	-	25.1
South Trees	168	25.5	-	-	-	2004	-	37.7	-	18.3
Lilley's Beach	101	21.7	-	-	-	727	-	62.6	-	17.7
Rodds Bay	246	18.4	-	-	-	2749	-	45.5	-	13.2

Table 3.5. Pearson correlations between whole *Zostera muelleri* and sediment trace element concentrations. Values in bold are significant,  $p < 0.05$ . '-' indicates no value due to sediment Cd < limit of reporting.

	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
Correlation	0.322	0.256	-	0.335	0.458	0.405	0.405	0.591	0.639	0.289
$p$	<b>0.031</b>	0.089	-	<b>0.024</b>	<b>0.002</b>	<b>0.006</b>	<b>0.006</b>	<b>0.000</b>	<b>0.000</b>	0.054

### 3.3.2 Environmental and biological drivers

#### 3.3.2.1 Seagrass morphometrics

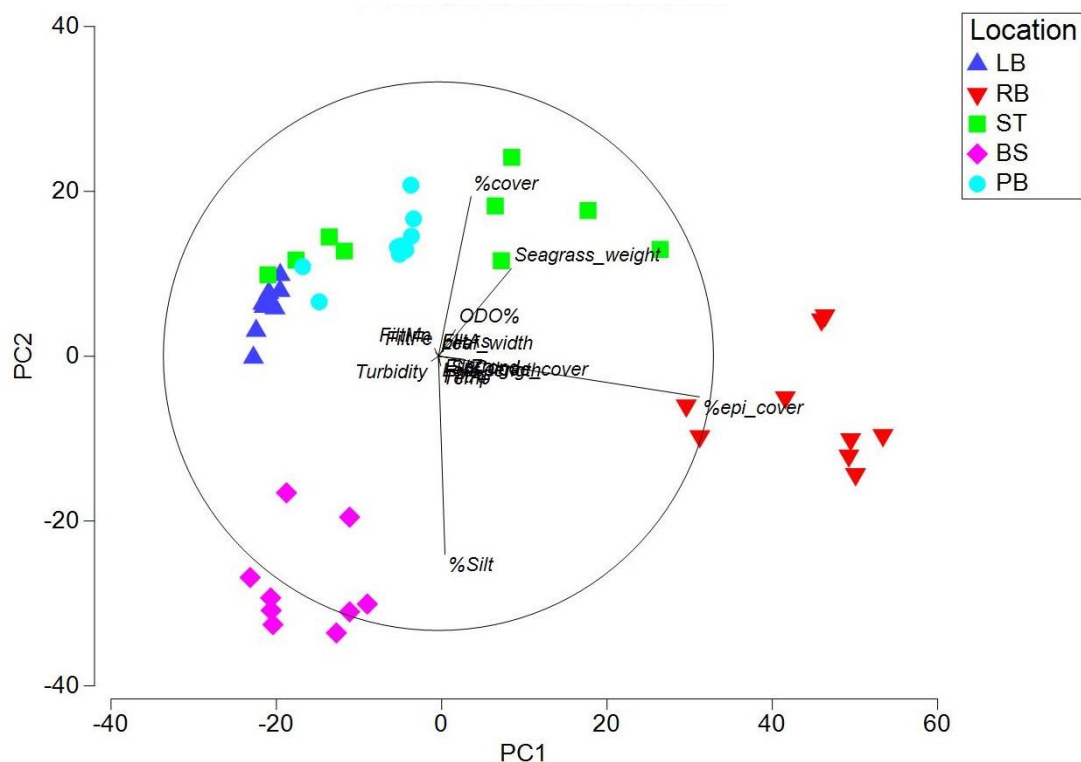
*Zostera muelleri* was the dominant seagrass species at the meadows sampled, with *H. ovalis* also present at Lilley's Beach, South Trees and Pelican Banks and *H. uninervis* present at Lilley's Beach. The mean biomass (wet weight) of *Z. muelleri* present at each location varied, with Black Swan Island (10.7 g) and Lilley's Beach (13.5 g) having significantly lower biomass than the other locations of >27.7 g (Lo:  $F_{4,30} = 27.75$ ,  $p < 0.001$ , Table 3.6, Appendix B Table B2). Seagrass morphometrics were significantly different between locations for each metric; for example, % seagrass cover was significantly higher at Pelican Banks (mean 62.2%) and South Trees (mean 68.3%) than at the other locations with means of <50% (Lo:  $F_{4,30} = 33.6$ ,  $p < 0.001$ , Table 3.6, Appendix B Table B2). Rodds Bay had significantly higher % algae cover with a mean of 6% (Lo:  $F_{4,30} = 44.4$ ,  $p < 0.001$ ) and significantly higher % epiphyte cover with a mean of 62.2% (Lo:  $F_{4,30} = 76.5$ ,  $p < 0.001$ ) compared to the other locations (Table 3.6, Appendix B Table B2). Mean leaf length was variable between locations. Mean leaf length was significantly longer at Lilley's Beach (6.4 cm) and shortest at Pelican Banks (2.33 cm) (Lo:  $F_{4,30} = 19.5$ ,  $p < 0.001$ , Appendix B Table B2). Mean leaf width was also significantly different between locations, with significantly narrower leaves at Lilley's Beach (0.07 cm) and wider leaves at Pelican Banks (0.14 cm) and Rodds Bay (0.13 cm) (Lo:  $F_{4,30} = 36.6$ ,  $p < 0.001$ , Appendix B Table B2). Sediment grain size distribution showed significant difference of % silt fraction (<63  $\mu\text{m}$ ) between locations (Lo:  $F_{4,30} = 222.63$ ,  $p < 0.001$ , Appendix B Table B2) with Black Swan Island having the highest mean silt content (42.9%) and Lilley's Beach the lowest silt content (2.3%) (Table 3.6).

**Table 3.6. Seagrass metrics for each location (mean  $\pm$  SD, n = 9). Similar letters indicates no significant differences between locations. WW = wet weight of an area 381.72 cm<sup>2</sup>.**

	Seagrass cover	Algae cover	Epiphyte cover	Leaf length	Leaf width	Whole seagrass	Silt
Unit	%	%	%	cm	cm	g WW	%
<b>Black Swan Island</b>	40.0 <sup>a</sup> (8.66)	0.00 <sup>a</sup> (0.00)	5.33 <sup>b</sup> (4.47)	4.61 <sup>b</sup> (0.93)	0.10 <sup>b</sup> (0.03)	10.7 <sup>a</sup> (2.54)	42.9 <sup>d</sup> (7.67)
<b>Pelican Banks</b>	62.2 <sup>c</sup> (6.67)	0.33 <sup>a</sup> (1.00)	8.00 <sup>b</sup> (4.00)	2.33 <sup>a</sup> (0.70)	0.14 <sup>c</sup> (0.03)	27.7 <sup>b</sup> (4.01)	12.2 <sup>b</sup> (1.35)
<b>South Trees</b>	68.3 <sup>c</sup> (8.29)	0.78 <sup>a</sup> (1.72)	15.4 <sup>b</sup> (14.1)	4.80 <sup>b</sup> (0.96)	0.09 <sup>b</sup> (0.02)	26.4 <sup>b</sup> (11.23)	12.2 <sup>b</sup> (5.79)
<b>Lilley's Beach</b>	43.9 <sup>ab</sup> (4.17)	0.00 <sup>a</sup> (0.00)	0.00 <sup>a</sup> (0.00)	6.40 <sup>c</sup> (1.02)	0.07 <sup>a</sup> (0.01)	13.5 <sup>a</sup> (2.99)	2.30 <sup>a</sup> (1.06)
<b>Rodds Bay</b>	50.0 <sup>b</sup> (7.50)	6.11 <sup>b</sup> (2.20)	62.2 <sup>c</sup> (8.33)	5.01 <sup>bc</sup> (1.63)	0.13 <sup>c</sup> (0.02)	28.6 <sup>b</sup> (6.97)	18.5 <sup>c</sup> (3.88)

### 3.3.2.2 Environmental drivers

The PCA of environmental variables (e.g. % silt, dissolved TEs and seagrass morphometrics) demonstrated that Rodds Bay was distinct to the other locations (Fig. 3.5). The PC1 of 58% was explained by % epiphyte cover, seagrass wet weight, % seagrass cover and PC2 of 35.8% was explained by % silt, % seagrass cover, seagrass wet weight and % epiphyte cover. The best matched similarity from the BIOENV procedure was 0.561 correlation and was best explained by % silt and % epiphyte cover.



**Figure 3.5. Principal Component Analysis of all environmental variables including % silt, dissolved water Al, As, Fe, Mn, Zn, water physico-chemistry (temperature, specific conductivity, dissolved oxygen % saturation) and seagrass morphometrics (leaf length, leaf width, % algae cover, % epiphyte cover, % seagrass cover, seagrass weight). Location abbreviations: Lilley's Beach (LB), Rodds Bay (RB), South Trees (ST), Black Swan Island (BS), and Pelican Banks (PB).**

Following on from the BIOENV and applying the best similarity of the two environmental variables (% silt and % epiphyte cover) to each seagrass TE through the use of DistLM demonstrated different significant relationships (Table 3.7, Appendix B Table B3). Seagrass As concentrations were not significantly ( $p > 0.05$ ) related to either % silt or % epiphyte cover (Table 3.7), and only a very low proportion of variability in Zn was significantly ( $p < 0.01$ ) explained by % silt (14%, Table 3.7). Seagrass Cu concentrations were significantly explained by only % epiphyte cover ( $p < 0.00$ , 28%, Table 3.7). The seagrass TEs that had significant ( $p < 0.00$ ) equal proportion (~30%) of their concentrations explained by % silt and % epiphyte cover were Cr and Pb (Table 3.7). Seagrass Al cumulative results of 60% were significantly ( $p < 0.00$ ) explained more by a greater proportion of % epiphyte cover (34%) than % silt (26%) (Table 3.7). Seagrass TEs that had their concentration variation explained by a greater proportion of % silt to % epiphyte cover were Cd (34:20), Fe (49:19), Mn (38:8) and Ni (36:0) (Table 3.7).

**Table 3.7. Distance-based linear model sequential test significant results for each seagrass trace element and the variables of % silt and % epiphyte cover. Full statistical table within Appendix B Table B3.**

	Variable	Pseudo-F	<i>p</i>	Proportion %	Cumulative %
<b>Aluminium</b>	% silt	15.36	0.00	26	26
	% epiphyte cover	36.26	0.00	34	60
<b>Arsenic</b>	% silt	0.13	0.73	0	0
	% epiphyte cover	0.06	0.80	0	0
<b>Cadmium</b>	% silt	21.70	0.00	34	34
	% epiphyte cover	18.12	0.00	20	54
<b>Chromium</b>	% silt	19.12	0.00	31	31
	% epiphyte cover	36.89	0.00	32	63
<b>Copper</b>	% silt	0.40	0.53	1	1
	% epiphyte cover	16.17	0.00	28	28
<b>Iron</b>	% silt	42.09	0.00	49	49
	% epi	25.64	0.00	19	69
<b>Lead</b>	% silt	18.26	0.00	30	30
	% epiphyte cover	37.40	0.00	33	63
<b>Manganese</b>	% silt	26.89	0.00	38	38
	% epiphyte cover	5.91	0.02	8	46
<b>Nickel</b>	% silt	24.38	0.00	36	36
	% epiphyte cover	0.05	0.81	0	36
<b>Zinc</b>	% silt	6.93	0.01	14	14
	% epiphyte cover	0.18	0.69	0	14

### 3.3.3 Seagrass pollution indices

Seagrass from Black Swan Island had the highest TEPI value (1.15) and Pelican Banks had the lowest TEPI value of 0.69 (Table 3.8). The TESVI values for each seagrass TE were unique, with Zn having the lowest value of 0.01, followed by As with 0.09, suggesting that Zn and As concentrations in seagrass were similar between locations (Table 3.9). The highest TESVI value of 0.61 was recorded for Fe, demonstrating that Fe seagrass concentrations show the greatest variability between locations (Table 3.9). The TEVSI values of the other TEs (Al, Cd, Cr, Cu, Pb, Mn and Ni) fell between the values of 0.12 and 0.39, suggesting a degree of location variability (Table 3.9).

**Table 3.8. Trace element pollution index values for each location.**

Location	TEPI
Black Swan Island	1.15
Pelican Banks	0.69
South Trees	1.05
Lilley's Beach	0.79
Rodds Bay	1.07

**Table 3.9. Trace element spatial variation index seagrass values of each trace element and associated calculations. Location  $X_{\max}$  is the location that displayed the maximum mean of that trace element.**

	n	$x_{\max}/x_{\min}$	$\Sigma(x_{\max}/x_i)/n$ $\pm$ SD	TESVI	Location $x_{\max}$
Aluminium	5	2.99	0.38 (0.16)	0.25	Rodds Bay
Arsenic	5	1.87	0.26 (0.07)	0.09	South Trees
Cadmium	5	2.06	0.31 (0.09)	0.12	Lilley's Beach
Chromium	5	3.66	0.41 (2.07)	0.37	Rodds Bay
Copper	5	2.47	0.30 (1.51)	0.20	South Trees
Iron	5	4.51	0.42 (2.11)	0.61	Rodds Bay
Lead	5	3.65	0.40 (2.02)	0.37	Rodds Bay
Manganese	5	4.10	0.50 (0.39)	0.39	Lilley's Beach
Nickel	5	2.65	0.34 (0.20)	0.20	Black Swan Island
Zinc	5	1.14	0.21 (0.01)	0.01	Black Swan Island

## 3.4 Discussion

### 3.4.1 Trace element concentrations and relationship with environment

To assess the potential use of *Z. muelleri* as a TE bioindicator within the intertidal seagrass meadows of Port Curtis, it was important to first gain an understanding of the variability of TEs and potential relationships between seagrass and the environment. This involved exploring *Z. muelleri* TE composition and its relationship with environmental TEs, and whether other environmental drivers influenced TE accumulation. This study is a snapshot of one event at the time of maximum seagrass growth, from across a natural estuarine gradient, where locations encompassed variable sediment particle size and physico-chemistry readings. Trace element concentrations found throughout the bay are similar to or less than previous Port Curtis studies by Prange and Dennison (2000) and Jones et al. (2005) (dry weight conversion by Apte et al. (2005) is used) and another tropical study by Denton et al. (1980) (Table 3.10). In comparison to other polluted estuaries within Australia, the results of this study showed quite low concentrations of typical TEs, including Cu, Pb and Zn, compared to Farias et al. (2018), Ambo-Rappe, Lajus and Schreider (2007) and Birch, Cox and Besley (2018) (Table 3.10). This result suggests that the local *Z. muelleri* in Port Curtis as a bioindicator is not displaying any local elevated sources of these pollutants to the extent observed elsewhere.

**Table 3.10. Results from other trace element studies and *Zostera muelleri* from within Australia. Ranges are the mean minimum and maximum except for this study where absolute minimum and maximum values are given. Seagrass part analysed is abbreviated as W = whole, L = leaf, RR = root-rhizome, Ro = root, Rh = rhizome. Units mg kg<sup>-1</sup> dry weight. '-' indicates no data.**

Part	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn	Where	Study
W	250–1930	3.5–70	0.07–0.23	0.43–4.2	2.9–13.0	1540–24800	0.35–3.7	46–350	0.81–3.9	19–36	Port Curtis	This study
W	-	-	0.2	0.9–1.9	2.8–3.0	3500–5250	0.4	44–70	0.6–1.8	14–18	North Queensland	Denton et al. (1980)
L	625–1794	-	-	5.0–30.6	7.9–12.3	2089–7592	-	-	-	23.7–74.7	Port Curtis	Prange and Dennison (2000)
RR	422–2206	-	-	4.7–29.7	2.1–14.4	1829–17889	-	-	-	7.7–60.2	Port Curtis	Prange and Dennison (2000)
L	832–2410	1.5–12.1	0.09–0.2	4.0–9.4	3.0–19.0	880–5560	0.6–1.3	-	1.6–4.8	15–20	Port Curtis	Apte et al. (2005) DW conversion of Jones et al. (2005)
W	-	11–18	-	-	23.0–27.0	-	68–111	-	-	338–424	Derwent River	Farias et al. (2018)
L	-	-	2.1–6.1	-	13.5–52.1	-	3.4–148.4	-	-	115.4–397	Lake Macquarie	Ambo-Rappe, Lajus and Schreider (2007)
Ro	-	-	3.0–20.2	-	15.3–84.1	-	4.1–211.7	-	-	63.9–592	Lake Macquarie	Ambo-Rappe, Lajus and Schreider (2007)
L	-	0.85–1.15	0.24–0.96	-	5.8–15.2	-	1.99–3.51	307–1292	<1–3.54	41.2–133	Lake Illawarra	Howley (2001)
Rh	-	0.89–4.75	0.1–0.41	-	2.2–8.02	-	1.39–11	13–516	<1–5.16	19.4–54	Lake Illawarra	Howley (2001)
L	-	1.9–5.9	-	0.6–5.6	5.4–73.0	-	1.4–48	13–465	-	68–247	Sydney	Birch, Cox and Besley (2018)
Rh	-	3.7–58	-	2.4–15	3.8–93.0	-	4.5–152	7.1–331	-	70–455	Sydney	Birch, Cox and Besley (2018)
Ro	-	5.0–100	-	0.3–9.5	2.5–42.0	-	0.5–66	1.9–145	-	18–184	Sydney	Birch, Cox and Besley (2018)

The majority of *Z. muelleri* TE concentrations were TE and location specific, as each TE had location variability, except for Zn that had no variation between locations; this is supported by the variable TESVI values (Table 3.9). The nMDS (Fig. 3.2) demonstrated that there was a degree of similarity between locations but clearly showed that during the period of study seagrass collected from Rodds Bay had a TE composition that was dissimilar to the other locations. The similarity of seagrass TE concentrations between locations may be due to the unique composition of the overall TEs for each location with not one location having every TE maximum or minimum. Overall seagrass TE composition was separated by location with samples from the estuarine, western part of the bay having higher overall TE concentrations and TEPI values than Pelican Banks, which is closer to oceanic influences (Table 3.8). The location with the highest seagrass TE concentrations according to TEPI values was Black Swan Island, while Rodds Bay was the location displaying the highest means for most of the analysed TEs. The stoichiometric order of TE concentrations was the same at all locations for the five TEs recorded at the highest concentrations (Fe>Al>Mn>As>Zn), suggesting that there was no clear point source of these TEs (Table 3.2). The order of elements identified in this study is confirmed by Vonk et al. (2018), who conducted a meta-analysis and found that opportunistic species (*Zostera*) have higher Fe and Al leaf content than established (*Posidonia*) species. The order of the five TEs recorded at the lowest concentrations in this study (Cd, Cu, Cr, Ni and Pb) changed depending on the location and therefore can demonstrate potential external sources of TEs when compared to other locations. The only difference in element order between this study and the previous study by Prange and Dennison (2000) is that the earlier study found Cr > Cu, whereas in this study Cu > Cr (Table 3.10). These deviations suggest that the order and TE concentrations within *Z. muelleri* can change over time and spatially, and that *Z. muelleri* may be sensitive to such TE changes and therefore can be recommended as a bioindicator.

Zinc concentrations in Port Curtis seagrass samples had a low TESVI value (and therefore low variation between locations), low concentrations and no significant difference between locations or sites. However, *Z. muelleri* has demonstrated strong accumulation of Zn (higher BCF values) but with no correlation to sediment Zn concentrations; this suggests that *Z. muelleri* is regulating its Zn accumulation to a possible steady state. This result suggests *Z. muelleri* may not be a useful bioindicator of Zn. Previous studies of Zn concentrations in *Z. muelleri* in Port Curtis demonstrated consistent Zn concentrations (Prange & Dennison 2000), while other locations in Australia (Table 3.10) have demonstrated that the species can accumulate Zn up to 424 mg kg<sup>-1</sup> and it has been recommended as a bioindicator of this TE (Farias et al. 2018). Possible explanations for why this study did not see variable Zn concentrations could be that the study was conducted at only one time point or that the

analysed seagrass sample was whole (not separate compartments), or could be a reflection of low bioavailable Zn concentrations across the bay or as a result of other TE behaviour that limited Zn accumulation.

Arsenic had the second lowest TESVI value (Table 3.9) and lower significant difference between and within locations, but also had a large range of concentrations (minimum 3.5 mg kg<sup>-1</sup> to maximum 70 mg kg<sup>-1</sup>). Seagrass As concentrations recorded in this study were 3 to 4 times higher than other studies. The source of the higher As concentrations is most likely from the sediment, as sediment BCF values were high (>3) indicating that the seagrass is actively accumulating As from the environment. *Zostera muelleri* accumulation of As can be stronger with a BCF value of 8.3 (sediment to seagrass roots) (Maher et al. 2011). However, *Z. muelleri* As concentrations demonstrated a non-significant correlation to sediment. The lack of a correlation between sediment and seagrass As concentrations is most likely due to the ability of *Z. muelleri* to not inhibit As uptake within the below-ground compartment (Maher et al. 2011). The suitability of *Z. muelleri* as a bioindicator of As is mixed as it does clearly accumulate As from the environment but does not correlate to the environmental concentrations and therefore identify sources of anthropogenic As. Additionally, further investigation between compartments will need to be made before a recommendation is made, and this will be addressed in Chapter 4.

Seagrass Fe concentrations had the highest variation between locations with the lowest concentrations recorded in seagrass from Lilley's Beach (<5000 mg kg<sup>-1</sup>) and the highest concentrations from Black Swan Island and Rodds Bay (>15000 mg kg<sup>-1</sup>). Seagrass Fe concentrations were also significantly variable within a meadow. *Zostera* spp. are known to create spatially heterogeneous sediment Fe concentrations as their root system reduces the Fe to a bioavailable form in local patches (Deborde et al. 2008; Pagès et al. 2012). This localised Fe variability will most likely also explain the within meadow As variability. This example of sediment driven seagrass Fe variability is supported within this study by the strong correlation between seagrass and sediment Fe concentrations and all samples showed a degree of accumulation through the interpretation of the BCF values. From this study, seagrass Fe concentrations displayed some of the highest Fe concentrations in comparison to other studies (Table 3.10). *Zostera muelleri* has demonstrated that it can be used as a spatially localised Fe bioindicator.

Seagrass Al, Cr, Ni and Pb were also significantly lower at Lilley's Beach and/or Pelican Banks and higher at Rodds Bay and/or Black Swan Island (Figure 3.3). Seagrass concentrations of Al, Cr, Ni and Pb in this study were similar to the concentrations recorded

by previous studies in Port Curtis (Apte et al. 2005; Denton et al. 1980; Prange & Dennison 2000) and lower than those reported from the polluted Derwent estuary (Farias et al. 2018). These four TEs were all spatially variable, with mid-range TESVI values, significant correlation to sediment and a small degree of accumulation with low BCF values. The results suggest that the seagrass is accumulating these TEs from the sediment and potentially from the water and that this can explain the spatial differences observed. *Zostera muelleri* has demonstrated elsewhere to be an accumulator of Cr, with higher BCFs (1.7–2.3) observed by Birch, Cox and Besley (2018) and elevated values previously seen within Port Curtis (Prange & Dennison 2000). Uptake of Pb by *Zostera* spp. within other studies has demonstrated a strong accumulation and spatial variation within leaf material (Table 3.9) and could be a potential bioindicator (Birch, Cox & Besley 2018; Bond et al. 1988; Lyngby & Brix 1984). *Zostera muelleri* Ni concentrations were found to be significantly higher at Black Swan Island, which is a location away from industrial sources. In this study, dissolved (0.45 µm filtered) Ni in water samples was below the limit of reporting but a previous ultra-low water TE concentration study by Angel et al. (2010) found elevated dissolved Ni (~0.8 µg L<sup>-1</sup>) within the area of the Black Swan Island meadow in comparison to inner harbour concentrations (<0.3 µg L<sup>-1</sup>), suggesting that *Z. muelleri* is capable of reflecting these small spatial differences in Ni concentrations. The source of Ni within this area is suggested by Angel et al. (2010) to be a result of natural chemical processes such as lower pH increasing the reduction of manganese hydroxides.

Sediment BCF values of Al, Cr, Ni and Pb were higher at Rodds Bay than at the other locations sampled. This result is surprising because Rodds Bay is also considered to have lower concentrations of pollutants, being more distant from industrial activities. It is possible that concentrations are higher at Rodds Bay than the other sites because of the higher % epiphyte cover on seagrass leaves, which could contribute to the overall concentrations measured, or because the location of seagrass collection was towards the lower water edge and that the seagrass may be inundated for longer than at other locations. *Zostera muelleri* as a bioindicator of Al, Cr, Ni and Pb concentrations is recommended as it has displayed accumulation and spatial variability.

In contrast, the remaining TEs Cu, Cd and Mn showed different spatial patterns. Copper, Cd and Mn concentrations in seagrass samples were significantly lower at Rodds Bay, followed by Pelican Banks. Significantly higher concentrations of Cd and Mn were recorded at South Trees and Lilley's Beach, and Cu at Black Swan Island. In this study concentrations of Cu and Cd were similar to previous Port Curtis and other study results (Table 3.10). Concentrations of Mn in *Z. muelleri* have not been previously measured within Port Curtis,

but values in this study were similar to a study by Birch, Cox and Besley (2018) in Sydney Harbour and lower than concentrations recorded by Howley (2001) at Lake Illawarra (Table 9). From laboratory studies, *Z. marina* was shown to be an accumulator of Cd and Mn and the below-ground compartment was a sink for Cd, while the leaf material accumulated and locked up Mn (Brinkhuis, Penello & Churchill 1980). The spatial pattern of higher Cd and Mn concentrations at South Trees and Lilley's Beach, which are 2 km apart, suggests that there was a local source of these TEs. A previous history of Mn mining within Gladstone, in combination with the local natural geology, could be contributing to the local Mn variability (Anastasi & Wilson 2010). In this study, Cu and Mn concentrations in *Z. muelleri* were significantly correlated to sediment concentrations, and displayed mid-range TESVI values and strong BCF accumulation at different locations. These results indicate that *Z. muelleri* accumulates Cu and Mn according to the location and that it could be a bioindicator of these TEs. For example, seagrass at Black Swan Island and South Trees, followed by Lilley's Beach, displayed the significantly highest Cu concentrations, yet this is not reflected within the local sediment Cu BCF values. Differences in seagrass Cu concentrations could be due to the dissolved Cu concentrations as the data supplied from PCIMP had concentrations < LoR, however, Angel et al. (2010) found dissolved Cu to be more elevated within Port Curtis harbour than the nearby oceanic waters. Angel et al. (2010) suggested that Cu within Port Curtis was anthropogenically sourced and knowing this could possibly explain the seagrass spatial observations observed within our study. For example, locations away from industry (Pelican Banks and Rodds Bay) had lower Cu concentrations than the other locations. *Zostera muelleri* displayed spatial variability in Cd concentrations and these were higher than the environmental Cd concentrations (water and sediment) that were below the LoR, suggesting that *Z. muelleri* could be used as a Cd bioindicator. Additionally, *Z. muelleri* could be a good spatial bioindicator of anthropogenic and natural sources of Cu and Mn.

### 3.4.2 Environmental drivers

Seagrass was collected over a large semi-enclosed estuary; natural physico-chemical variation and the seagrass morphodynamics were also different between the locations as previously demonstrated for *Z. muelleri* in Moreton Bay (Maxwell et al. 2014). It has been suggested in other studies that TE concentrations can be linked to leaf surface area or leaf age (Malea & Kevrekidis 2013), yet this study found no link to leaf dynamics. This difference is most likely due to two factors: 1) the sample was analysed as whole plant and the leaf concentrations were combined with the root-rhizome compartment, or 2) differences in localised grazing by herbivores, illustrated by shorter leaf lengths found at Pelican Banks. Samples were collected in November, which overlaps with the season for green turtle mating

within Port Curtis, and therefore greater grazing pressure is likely (Prior, Booth & Limpus 2015).

Results from this study have shown evidence for spatial variation in seagrass TE concentrations, and there appears to be a strong link to the local sediment concentrations for certain TEs. A remaining question to address is whether other drivers that are not the TEs contribute to the variation in accumulation by *Z. muelleri*. Results of the environmental variables PCA (Fig. 3.5) showed separation of locations due to % epiphyte cover, biomass (wet weight), % silt and % seagrass cover. These results confirm that *Z. muelleri* meadows are distinct to each other due to the local conditions of silt and the growth of epiphytes. The BIOENV results demonstrated similar results to the environmental variables PCA with % silt and % epiphyte cover explaining the seagrass TE concentration variability. These variables of silt and epiphytes are important to understand in TE studies as they have demonstrated that they can influence seagrass TE concentration (Bravo et al. 2016; Richir et al. 2013; Schlacher-Hoenlinger & Schlacher 1998b). Percentage silt and % epiphyte cover through the use of DistLM (Table 3.7) demonstrated the different contribution of % silt and % epiphyte cover to the TE concentration variability, with overall cumulative results ranging from 0% for As to 69% for Fe.

Understanding epiphyte presence in the cycling of TEs is important as they are a dominant compartment within seagrass meadows (Sanz-Lázaro et al. 2012). The limited studies which have previously analysed epiphytes separately to seagrass found epiphytes to have significantly higher concentrations of certain TEs (e.g., As, Al, Bi, Cr, Cu, Fe, Pb, V and Zn) than the seagrass (Maher et al. 2011; Richir et al. 2013; Sanz-Lázaro et al. 2012). The seagrass TEs within this study that had a significant relationship and a greater proportion of variability explained by % epiphyte cover than % silt were Al, Cr, Cu, and Pb (Table 3.7). These TEs correspond to the locations with higher % epiphyte cover: Rodds Bay > South Trees > Pelican Banks > Black Swan Island. While this study did not separate out the epiphyte material from the seagrass, the results can only suggest that % epiphyte cover has the potential to influence overall TE concentrations and requires further investigation. In this study, the seagrass was sampled whole and the contribution by biomass was predominantly the root-rhizome compartment, suggesting that below-ground factors such as particle size could be a stronger driver for certain seagrass TE concentrations.

Sediment particle size is the other environmental driver that can explain the variability in seagrass TE concentrations, as bioavailable TEs within the environment are found to be in higher concentrations on the finer (silt) particles (Bravo et al. 2016). Trace elements that

had a greater proportion (>46%) of the TE variability explained by % silt more than % epiphyte cover were Cd, Fe, Mn, and Ni (Table 3.7). Locations with lower levels of silt equate to explaining the higher seagrass concentrations observed at South Trees and Lilley's beach of Cd and Mn, while higher Fe and Ni seagrass concentrations were found at locations of higher % silt, such as Black Swan Island and Rodds Bay. These two contrasting explanations of % silt in relationship to seagrass TEs demonstrates that % silt could be TE specific and not the only influence on *Z. muelleri* TE concentrations. The analysis of sediment TEs within this study was a total recoverable digest on the <2 mm fraction, which could have overestimated the bioavailable fraction of sediment TE to the seagrass as the strong acid digest would release all sediment bound mineralised TEs. Further understanding of silt (<63 µm) contribution to this study's seagrass TE bioavailability could be assessed by doing a weak acid digest on the <63 µm fraction. The silt fraction was not analysed separately, or solely, due to making a decision to select only one fraction to analyse that encompassed the wide range of particle size across the bay. Other studies have looked at different sediment fractions, sediment depths and digest procedures and found different relationships between sediment TEs and the seagrass TEs but with no consistent pattern (Bravo et al. 2016; Kilminster 2013). The potential influence of % silt as a driver of *Z. muelleri* TEs means that seagrass concentrations could change over time due to changing proportions of silt, and therefore the seagrass can be a good long term bioindicator of changes of sediment within a location. Sediment samples would need to be analysed for particle size each time seagrass is sampled, to assist in the interpretation of results.

### 3.4.3 Indices

The use of developed indices assisted in demonstrating the variation in seagrass TE concentrations between and within locations. The TEPI spatially demonstrated the sum of all TEs at a location and clearly showed that the western locations along the coast and within estuarine areas had higher levels of TEs and higher TEPI values than the eastern location of Pelican Banks. The variation between values in this study was small at 0.69–1.15 and within the same range (0.251–1.799) as values reported by Richir et al. (2015) for *P. oceanica* in the Mediterranean, and within the same range (0.56–1.27) reported by Wilkes et al. (2017) for *Z. noltei* throughout Ireland. The TEPI values reported by Richir et al. (2015) demonstrated a wider range of TE concentrations, as their study locations varied from highly polluted to less impacted locations. In this study, the variation in TEPI values appears to be due to the local estuarine influences at the estuarine locations of Black Swan Island and Rodds Bay. The seagrass meadow at Black Swan Island is situated in a channel called the Narrows and, as discussed previously, Angel et al. (2010) found that the source of some of

these TEs (Ni and Mn) within this area was due to the local biogeochemistry, but that other TEs such as Cu and Zn were anthropogenically sourced. Knowing that TEs are locally sourced suggests that the seagrass at Black Swan Island is indicating the environmental TE concentrations potentially due to low level pollution (Cu) and to natural sources (Ni). Understanding this in relation to the other locations for *Z. muelleri* as a bioindicator may require careful interpretation and whether like-for-like locations are compared (e.g., locations of similar environmental variables). Careful assessment of long term studies of low levels of other dissolved TEs within Port Curtis is required to ascertain whether changes occur due to external pollution from the water environment.

The TESVI index displayed the variability in TE accumulation in seagrass from the environment across the greater spatial scale. In this study, TESVI demonstrated the TEs of high variability (Fe, Mn, Cr, Pb and Al) to be spatially different between locations. The primary function of TESVI is usually to assist in identifying problematic TEs; however, this study was able to use the obtained values to see that *Z. muelleri* appears to either not regulate As or regulate Zn concentrations, as very low TESVI values were recorded in areas where environmental TEs were variable. The TEPI and TESVI indices were originally created for a Mediterranean species of seagrass, but this study has shown that these indices were applicable to the assessment of TE concentrations in *Z. muelleri*, in that they assisted in the interpretation of TE variability, and gave weight to the assessment of *Z. muelleri* as a bioindicator. While TESVI and TEPI have yet to be used in a longitudinal study they may have the potential to be used to assess TE changes over time within a location and further the knowledge of TE variability and regulation by the seagrass (Richir & Gobert 2014; Richir et al. 2015).

### 3.5 Conclusion

*Zostera muelleri* has displayed greater spatial variability in TE concentrations between meadows than within meadows due to localised sources of bioavailable TEs and has been demonstrated as a spatial bioindicator for certain natural or anthropogenically sourced TEs. Within meadow differences may be explained by local influences of sediment or seagrass composition and differences between meadows may be explained by local sources, silt and epiphytes. Accumulation of TEs by *Z. muelleri* was TE specific, ranging from greater accumulation of As to the possible regulation of Zn. As a bioindicator, *Z. muelleri* did show a strong relationship with the sediment environment and potentially the water where values were above LoR. The proportion of environmental drivers of % silt and % epiphyte cover can explain some of the variation in seagrass TE concentrations (e.g., Cd, Mn and Pb).

However, it is noted that these may not be the only drivers of TE variability. Further study into the contribution of silt and epiphytes on *Z. muelleri* TE concentrations is required and until then epiphyte cover and silt should be noted when collected. Knowing the full contribution of silt and epiphyte cover to measured seagrass TE concentrations should not influence the decision to use *Z. muelleri* as a bioindicator, as epiphytes are a predominant component of seagrass ecosystems. The use of internationally derived seagrass TE indices were applicable to this study and helped interpret the variation of TEs in the environment. As a coastal management tool for the Port Curtis waters, it is recommended that *Z. muelleri* could be used as a bioindicator for all TEs tested, especially for Cd that is not found in the environment (< LoR), and to include Zn in analysis because of past examples throughout Australia. Future use of *Z. muelleri* as a bioindicator should consider that TEs can change over time and with greater longitudinal studies will come greater understanding of the TE behaviour within Port Curtis.

## **Chapter 4. Temporal variation of trace elements in *Zostera muelleri* as a potential bioindicator**

## 4.1 Introduction

Seagrasses along the east Australian coast predominantly grow within the estuarine coastal area, which is the interface between the offshore clean oligotrophic waters and the coastal waters influenced by activities such as industry, agriculture, tourism and urbanisation and their associated contaminants and stressors (Ferguson et al. 2018; Orth et al. 2006). It is also within this coastal zone that most global seagrass loss has occurred, with up to 30% loss due to different pressures such as poor water and sediment quality, coastal development and global impacts of climate change (Fraser & Kendrick 2017; Waycott et al. 2009). The resilience of seagrass to environmental perturbations can be enhanced by improving the biophysical environment, such as having reduced concentrations of nutrients, TEs and pesticides (Unsworth et al. 2015). Seagrasses have a unique growth form, being a rooted vascular plant, making them a unique bioindicator with the potential to reflect the water and sediment TE quality across the estuarine coastal gradient (Pergent-Martini & Pergent 2000; Richir & Gobert 2016). In this study, the bioindicator potential of *Z. muelleri* within Port Curtis is being investigated, and this requires an understanding of the temporal variability in TE concentrations in this species.

Research on seagrasses as TE bioindicators has demonstrated that each seagrass species displays a unique pattern of TE accumulation (Vonk et al. 2018). Therefore, before a seagrass can be interpreted as a TE bioindicator, further knowledge on the natural variation in TE concentrations and the temporal relationship between environmental TEs and that species is required. Current knowledge of temporal patterns in TE accumulation by *Z. muelleri* in Australia is very limited (Prange & Dennison 2000). Temporal changes can occur due to seagrass physiology that affects TE concentrations or due to changes in environmental TE concentrations. Some examples of the causes of temporal changes in external environmental loadings can include seasonal influx of freshwater (Malea & Kevrekidis 2013; Schlacher-Hoenlinger & Schlacher 1998b), inputs from anthropogenic activities such as industries, marinas, cessation of mining operations (decrease in TEs) or coastal development (temporary increase in TEs) (Brito et al. 2016; Lafabrie, Pergent & Pergent-Martini 2009; Prange & Dennison 2000). However, evidence attributing increases in environmental TEs to increasing seagrass TE concentrations is scarce, with varying degrees of correlation. Explanations for the observed variation in correlation have been linked to TE concentrations in the water being too low to measure, or too infrequent testing of environmental TE concentrations in comparison to the period of seagrass exposure (Bonanno & Di Martino 2016). Knowing

that there are seasonal changes in environmental TE concentrations can assist in the interpretation of temporal changes in seagrass due to anthropogenic influences versus natural, and can therefore assist in the assessment of its use as a bioindicator.

Another reason for a lack of correlation between seagrass and environmental TE concentrations is the natural seasonal growth cycle of seagrasses. Examples of leaf temporal TE patterns in seagrass were higher Cu and Zn within *Z. marina* when growing had ceased (winter) and higher Cu in *C. nodosa* in autumn when biomass was low and decreased Cu during the growing season (Lyngby & Brix 1982; Malea & Haritonidis 1999). *Zostera noltei* demonstrated a significant seasonal release of sediment bioavailable Fe and P during the below-ground compartment active growth phase (May to September) (Deborde et al. 2008). Additionally, seagrass is exposed to two environments, that of the water and sediment, and has the potential to vertically translocate TEs away from the adjacent environment and therefore not correlate with environmental concentrations (Bonanno & Di Martino 2016). Some studies of translocation within *Z. marina* demonstrated minimal acropetal (upwards) translocation of Cu and Zn, which was only found within new growth (Lyngby, Brix & Schierup 1982; Nielsen et al. 2017), whereas Brinkhuis, Penello and Churchill (1980) found that *Z. marina* translocated Cd both up and down but did not translocate Mn between compartments. By understanding the degree of seasonal accumulation and translocation of TEs within the chosen seagrass bioindicator species, interpretation of the environmental TEs can be improved.

Other biological traits such as leaf and root-rhizome age and growth could influence seagrass as a temporal TE bioindicator. Malea and Kevrekidis (2013) suggested that the older, larger leaves of *C. nodosa* had more time to accumulate TEs in comparison to the younger leaves. Furthermore, seasonal variation in pooled TE leaf concentrations may be due to the loss (excision) of the adult leaves, producing a decline in TE concentrations (Malea & Kevrekidis 2013). Whether the percentage of adult to younger leaves within a *Zostera* meadow influences TE overall concentrations is unknown, but higher turnover of shoots in *Zostera* could influence variations in temporal TE concentrations. This variability could possibly explain *Z. muelleri* leaf Cu and Zn variability within a meadow (Macinnis-Ng & Ralph 2004). Temporal TE concentrations in below-ground components of *Z. marina* appear to present less of a seasonal change in comparison to the above-ground components (Lyngby & Brix 1982). Alternative hypotheses for changing TE concentrations in leaf and root-rhizome, with age within a *Zostera* meadow are patch disturbance (therefore different exposure time) due to grazing

by megaherbivores, human physical disturbance (bait digging, anchor scars), wave disturbances or within site sediment heterogeneity (Aragones et al. 2006; Deborde et al. 2008; Diedrich et al. 2013; El-Hacen et al. 2019). Knowing that leaf age, growth and within meadow disturbances have the potential to influence TE variability is relevant to understanding and interpreting seagrass TE concentrations as a bioindicator.

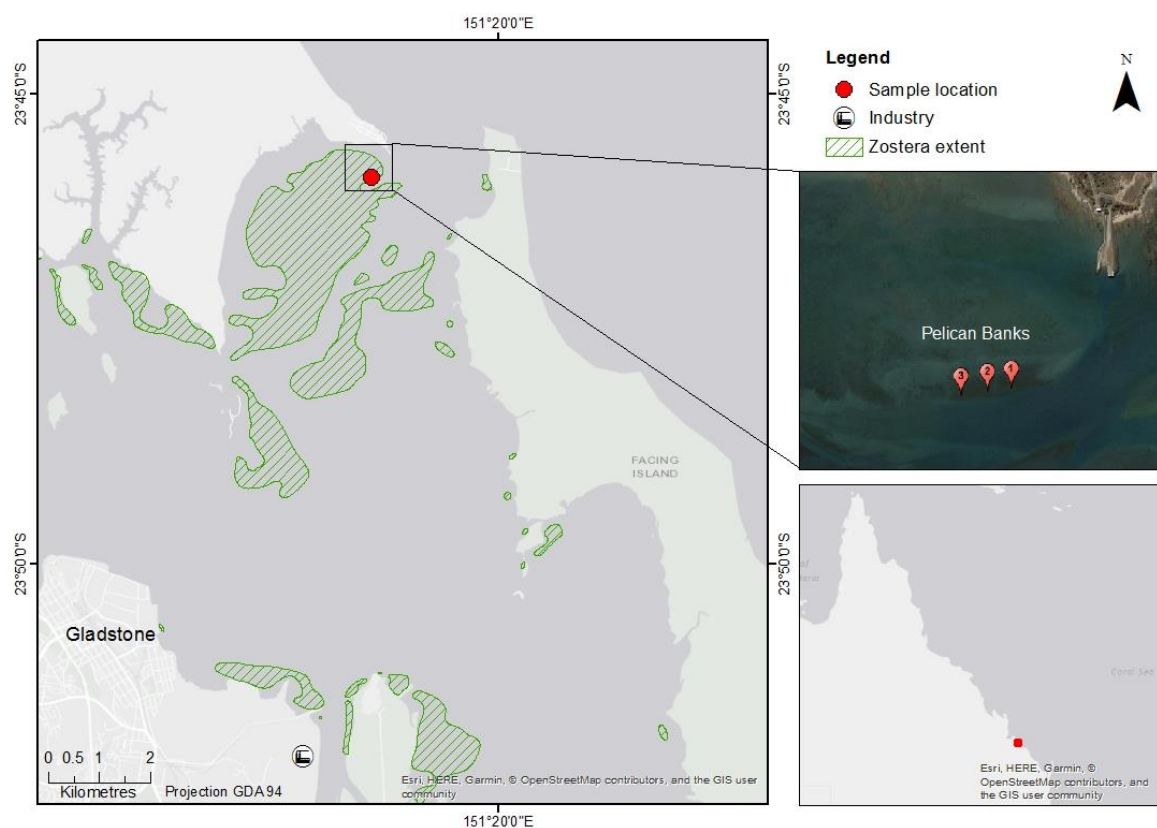
From the assessment of current knowledge, it is expected that the above- and below-ground compartments of *Z. muelleri* will show temporal differences in TE concentration due to changes in the TEs in their respective environments (water and sediment), or seasonal growth. The aim of this study is to understand temporal variability of *Z. muelleri* as a bioindicator from observations of seagrass accumulation, partitioning and relationship with the environmental TEs. The specific aims of this study were to determine; 1) *Z. muelleri* TE concentrations in above- and below-ground compartments across the seagrass growing cycle, which encompasses natural seasonal weather events, and 2) whether the TE concentrations in above- and below-ground seagrass compartments correlated to the TE concentrations in environmental sources (water and sediment) or to each other.

## 4.2 Methods

### 4.2.1 Study location

A southerly exposed, intertidal *Z. muelleri* meadow at Pelican Banks North (23.76475 S 151.31090 E) on the eastern side of Port Curtis was sampled (Fig. 4.1). The site was chosen as it is at least 10 km away from the predominant estuarine and anthropogenic influences of Gladstone. The Pelican Banks seagrass meadow is the largest persistent meadow within Port Curtis, and supports local mega-grazers such as dugong and green turtles (Prior, Booth & Limpus 2015; Rasheed et al. 2017). Port Curtis seagrass meadows are extensively monitored, with results demonstrating that over the years seagrass coverage has fluctuated due to flooding and recovery (Chartrand, Rasheed & Carter 2018; McKenzie et al. 2017). Monitoring of the seasonal growth cycle of *Z. muelleri* within Port Curtis has demonstrated an increase in biomass over August to December and then a decrease in the later summer months of January and February, coinciding with the local higher rainfall and temperatures (Chartrand et al. 2016). During this sampling period (August 2017 to 31 January 2018) Port Curtis water temperatures increased from 21°C in August 2017 to 29°C in March 2018 and specific conductivity decreased from 54.8 mS cm<sup>-1</sup> in August 2017 to 51.2 mS cm<sup>-1</sup> and 52.58 mS cm<sup>-1</sup> in November 2017 and March 2018, respectively (unpublished data, PCIMP, provided

2018). Rainfall from July 2017 to September 2017 was 48 mm and from October 2017 to late January 2018 was 452.8 mm (Bureau of Meteorology Australia, [www.bom.gov.au](http://www.bom.gov.au), Appendix C, Table C1 and Figure C1).



**Figure 4.1. Map of Pelican Banks sampling location with previous *Zostera muelleri* extent displayed (seagrass extent supplied through a joint partnership of Gladstone Ports Corporation and TropWater). Inset Google earth image of sites in relation to Curtis Island boat ramp and indication of seagrass meadows and sand banks.**

## 4.2.2 Seagrass collection

To assess temporal variation in TE concentrations in *Z. muelleri*, replicate samples were collected six times on low spring tides over the growing period between August 2017 and the end of January 2018 (sample dates provided in Appendix C, Figure C1). The sampling design consisted of three sites at ~60 m apart with three replicates at each site at ~5 m apart. For each replicate, the following sample and information was taken: seagrass samples for analysis of TEs, sediment samples for analysis of TEs and seagrass morphometrics. To achieve minimum biomass requirements for analysis, seagrass material was collected from pooling six cores (plastic, 9 cm diameter x 10 cm depth) for each replicate. Rhizosphere sediment was gently washed off the seagrass

with the use of ambient seawater and a plastic sieve, and the seagrass was then placed in a clean plastic bag. A sediment sample was collected using a plastic corer (9 cm diameter x 10 cm depth) from near each sampled seagrass replicate and placed in a plastic bag. Seagrass and sediment were kept on ice until return to the laboratory where they were frozen until further processing (<2 months). Seagrass morphometrics were observed by the random placement of a 0.5 x 0.5 m quadrat and were recorded for the purpose of describing the environment at the time of collection. Percent seagrass cover, % species composition, % algae, % epiphyte cover, leaf length, leaf width and general notes, such as site disturbances, were recorded by the researcher (McKenzie, Campbell & Roder 2003). Assessment of % seagrass cover was compared to previously produced percent cover photo standards and % epiphyte was assessed as the percent of the seagrass leaf surface area that was covered in epiphytes within the quadrat (McKenzie, Campbell & Order 2003). Leaf length and width were determined by taking a photo of five leaves per site in the field on a white background with a variable scale bar for calculations and later digitally measured using ImageJ software ([www.imagej.nih.gov/ij/](http://www.imagej.nih.gov/ij/)).

#### **4.2.3 Sample preparation and analysis**

Preparation of the seagrass for TE analysis involved removal of the extraneous material and the representative sample was then rinsed with Milli-Q water. The above-ground material (leaves and flowers) was separated from the below-ground material (roots and rhizomes) and analysed as two separate compartments. The number of flowers that were included in a sample to be analysed for TEs was counted and recorded. Samples were then patted dry with paper towel and stored frozen until subsequent freeze drying whereupon smaller seagrass particles ready for digestion were formed by agitation in a plastic bag. Sediment samples for TE analysis were wet sieved through a 2 mm sieve and dried at 60°C for 24 h and then ground using a mortar and pestle. Percent silt (<63 µm) was analysed on a sediment subsample and measured by laser particle size analysis using a Malvern MasterSizer 3000, Hydro EV.

Seagrass and sediment samples were analysed at the NATA-accredited Australian Government NMI laboratory, Sydney, by their in-house methods of NT2.46 and NT2.49 for seagrass and sediment, respectively. Total recoverable Al, As, Cd, Cu, Cr, Fe, Mn, Pb, Ni and Zn for sediment and seagrass was digested in high purity nitric and hydrochloric acids by heating on a hot block at 95–100°C for 2 hours. Trace element concentrations were determined by ICP-MS (Agilent 7900) and results reported on a dry

weight basis. Matrix spikes and laboratory control sample recoveries for all TEs for seagrass were 93–104 % and 84–116 % for all sediment TEs (Appendix A, Table A1, Table A2). Concentrations of dissolved (<0.45 µm filtered) TEs in water samples and physico-chemical data were provided by PCIMP.

#### **4.2.4 Data analysis**

Temporal (sampling event) and site differences for all seagrass (above- and below-ground) TEs, sediment TEs and seagrass morphometrics were analysed by a General Linear Model univariate two-way ANOVA with the factors of sampling event (fixed, six levels: August, September, November, December, early January and late January) and site (fixed, orthogonal, three levels: 1, 2 and 3). Data were transformed where required to meet homogeneity of variance and normality requirements for ANOVA. The below-ground seagrass Mn concentrations contained an outlier that was more than three times the average and subsequently the value was removed for statistical and graphing purposes. Where significant differences were observed for the effect of sampling event or site, a Tukey HSD post-hoc test was performed. SPSS v. 24 (IBM corp., Armonk, NY) was used for all statistical analyses. Cluster analyses were performed using the Primer software v. 7 (Anderson 2008) on a data matrix of TE compartments as variables (column) and individual samples were samples (rows); Euclidean distance was applied.

To assess the relationship between environmental and seagrass TE concentrations the following calculations were applied. Bioconcentration Factors for each TE were calculated as the ratio between seagrass compartments and sediment (Bonanno & Borg 2018; Kilminster 2013). A resulting BCF value greater than 1 indicates greater accumulation from the environment, suggesting that the seagrass accumulates a TE more than its environmental concentrations. A one-way ANOVA was performed on all BCF values to determine if accumulation significantly changed between sampling events. Pearson's correlations were performed to explore the relationships between the seagrass compartments and the sediment and water TEs and between the above- and below-ground compartments.

### **4.3 Results**

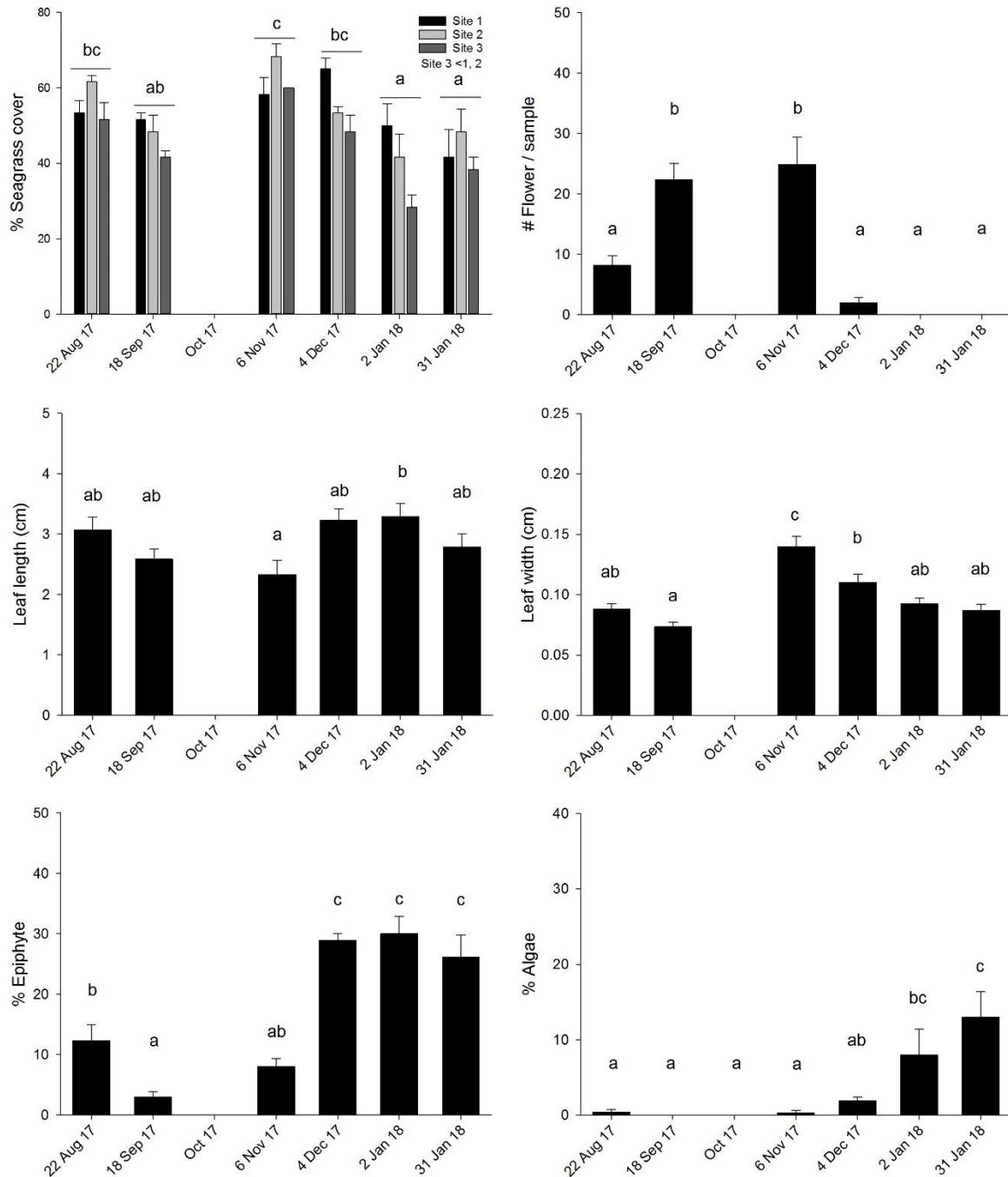
#### **4.3.1 Meadow description**

Percentage seagrass cover showed no significant interaction between sampling event and site ( $F_{5,10} = 1.78$ ,  $p = 0.100$ ); however, post-hoc tests of a significant difference

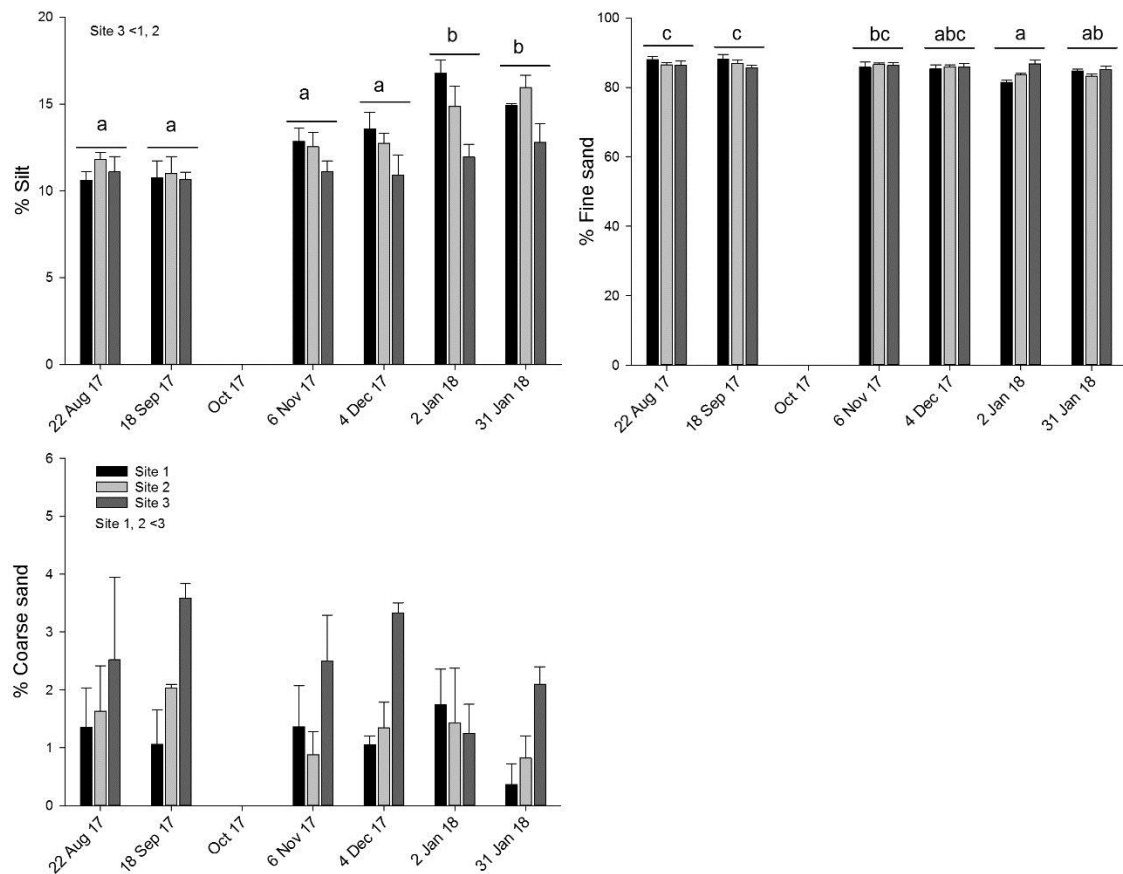
between sites ( $F_{2,10} = 9.19$ ,  $p < 0.001$ ) showed that site 3 had significantly less *Z. muelleri* coverage compared to other sites across sampling events (Table 4.1). Additionally, seagrass cover had a significant sampling event effect ( $F_{5,10} = 13.29$ ,  $p < 0.001$ , Table 4.1) with significantly higher mean % seagrass cover in November ( $62.2 \pm 6.67\%$ ) than in January ( $41.4 \pm 10.8\%$ ) across all sites (Table 4.1, Fig. 4.2). The number of flowers per sample also demonstrated this seasonal pattern, showing a significant sampling event effect ( $F_{5,10} = 23.13$ ,  $p < 0.001$ , Table 4.1), with significantly higher mean number of flowers per sample in September and November ( $23.6 \pm 11.0$ ) than the other months, during which low ( $2 \pm 2.74$ ) to zero flowers were observed (Fig. 4.2). There was a significant effect of sampling event for both leaf length ( $F_{5,10} = 3.21$ ,  $p < 0.05$ ) and width ( $F_{5,10} = 16.9$ ,  $p < 0.001$  Table 4.1, Fig. 4.2). Mean leaf dimensions were significantly shorter ( $2.33 \pm 0.7$  cm) and wider ( $0.14 \pm 0.03$  cm) in November than in the other sampling months (Fig 4.2). Percentage of epiphyte cover was significantly different between sampling events with a higher mean % epiphyte cover between December and January ( $28.3 \pm 8.2\%$ ,  $F_{5,10} = 29.13$ ,  $p < 0.001$ , Table 4.1, Fig. 4.2). There was a significant interaction between sampling event and site for % algae ( $F_{10,36} = 4.63$ ,  $p < 0.001$ , Table 4.1); however, the site influence was not significant ( $F_{2,10} = 3.06$ ,  $p = 0.059$ , Table 4.1). Seasonal % algae was significantly higher in the austral summer months of December to January  $7.63 \pm 9.3\%$  ( $F_{5,10} = 13.52$ ,  $p < 0.001$ , Table 4.1, Fig. 4.2).

**Table 4.1. Results of Univariate two-way ANOVAs for each measurement by sampling event (event) and site. Post-hoc numbers indicate month of sampling: 1 = August 2017, 2 = September 2017, 3 = November 2017, 4 = December 2017, 5 = 2 January 2018, 6 = 31 January 2018. Sites are coded as 1, 2 and 3. Values in bold are significant  $p < 0.05$ .**

		df	MS	F	<i>p</i>	Post - hoc
% Seagrass cover	Event	5	664.4	13.29	<b>0.000</b>	5,6,2<2,1,4<1,4,3
	Site	2	459.7	9.19	<b>0.001</b>	3<1,2
	Event*site	10	89.2	1.78	0.100	
	Error	30	50.0			
# Flowers / sample	Event	5	1151.75	23.13	<b>0.000</b>	5,6,4,1<2,3
	Site	2	36.07	0.72	0.492	
	Event*site	10	38.76	0.78	0.649	
	Error	30	49.80			
Leaf length	Event	5	1.29	3.21	<b>0.017</b>	3,2,6,1,4<2,6,1,4,5
	Site	2	0.94	2.33	0.112	
	Event*site	10	0.22	0.56	0.837	
	Error	30	0.4			
Leaf width	Event	5	0.005	16.9	<b>0.000</b>	2,6,1,5<6,1,5,4<3
	Site	2	0.000	1.37	0.266	
	Event*site	10	0.000	0.98	0.474	
	Error	30	0.000			
% Epiphyte	Event	5	1242.15	29.13	<b>0.000</b>	2,3<3,1<6,4,5
	Site	2	3.24	0.08	0.927	
	Event*site	10	82.61	1.94	0.072	
	Error	30	42.65			
% Algae	Event	5	258.34	13.52	<b>0.000</b>	2,3,1,4<4,5<5,6
	Site	2	58.39	3.06	0.059	
	Event*site	10	88.43	4.63	<b>0.000</b>	
	Error	30	19.11			
% Silt	Event	5	23.31	12.14	<b>0.000</b>	2,1,3,4<5,6
	Site	2	18.97	9.88	<b>0.000</b>	3<1,2
	Event*site	10	3.17	1.65	0.132	
	Error	30	1.92			
% Fine sand	Event	5	14.84	6.11	<b>0.000</b>	5,6,4<6,4,3<4,3,2,1
	Site	2	1.47	0.61	0.551	
	Event*site	10	6.14	2.53	<b>0.020</b>	
	Error	30	2.43			
% Coarse sand	Event	5	1.39	1.20	0.329	
	Site	2	10.15	8.77	<b>0.001</b>	1,2<3
	Event*site	10	1.02	0.88	0.561	
	Error	30	1.16			



**Figure 4.2. Temporal seagrass meadow measurements (mean  $\pm$  SE, n = 9 per sampling event, n = 3 per site). Bar colour within % seagrass cover indicate: black site 1, light grey site 2, dark grey site 3. Similar letters indicates no significant differences between sampling events.**

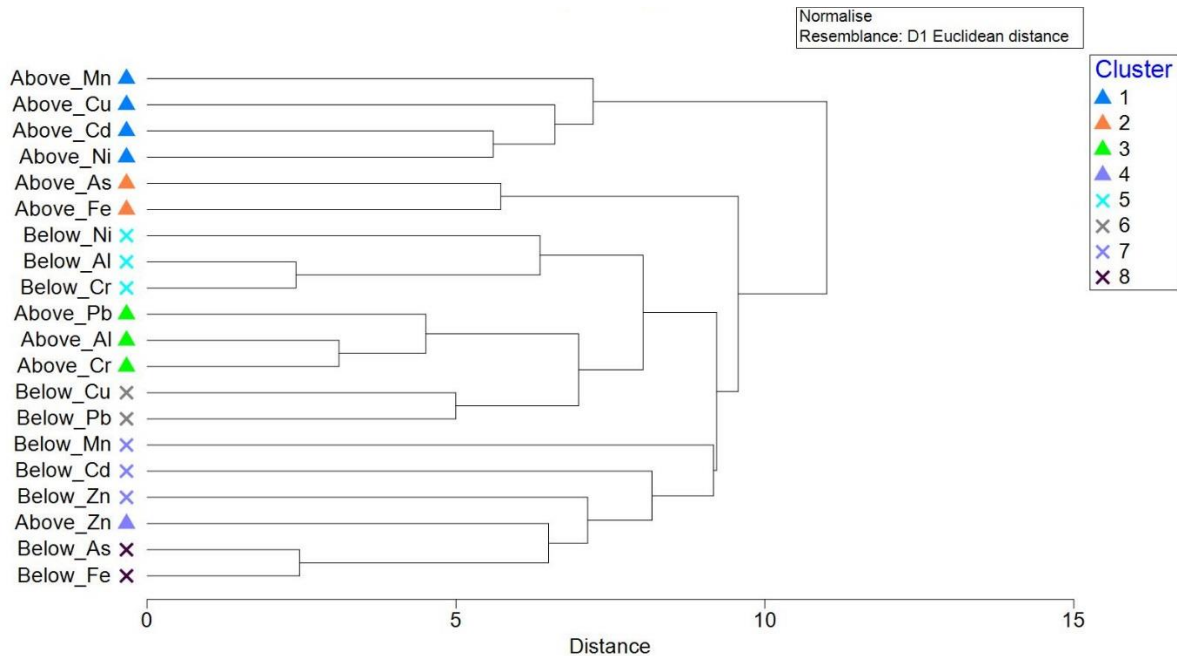


**Figure 4.3. Percentage of sediment particle size by site for each sampling event and site (mean  $\pm$  SE,  $n = 3$ , black site 1, light grey site 2 and, dark grey site 3). Similar letters indicates no significant differences between sampling event.**

The temporal *Z. muelleri* meadow sediment particle size was predominantly fine sand ( $85 \pm 2.06\%$ ) followed by silt ( $12 \pm 2.19\%$ ) and a small fraction of coarse sand ( $1.69 \pm 1.22\%$ , Fig. 4.3). There was no interaction between sampling event and site for silt and coarse sand but there was an interaction for fine sand ( $F_{5,36} = 2.53$ ,  $p = 0.20$ , Table 4.1). Percentage of fine sand showed a significant sampling event effect ( $F_{5,10} = 6.11$ ,  $p < 0.001$ ) with significantly less percent fine sand in January (84%) than August (87%, Table 4.1). The percentage of silt and coarse sand were significantly different between sites, with site 3 having significantly less silt than sites 1 and 2 ( $F_{2,10} = 9.88$ ,  $p < 0.001$ ), whereas coarse sand was the opposite distribution with site 1 and 2 having less silt than site 3 ( $F_{2,10} = 8.77$ ,  $p < 0.001$ , Table 4.1). Mean percentage of silt at all of the sites significantly increased over the sampling event ( $F_{5,10} = 12.14$ ,  $p < 0.001$ ) from  $11.6 \pm 1.5\%$  in August–December to a mean percentage of  $14.5 \pm 2.12\%$  in January (Fig. 4.3).

### 4.3.2 Seagrass trace element concentrations

Above- and below-ground individual TE concentrations were variable over the six month sampling period. However, similar temporal TE trends occurred in each compartment and were clustered by their TE association (e.g., below-ground As and Fe, Fig. 4.4) and not clustered by TE (e.g., above- and below-ground As). Concentrations of TEs within each compartment are presented by their cluster to explain their similarity over time.



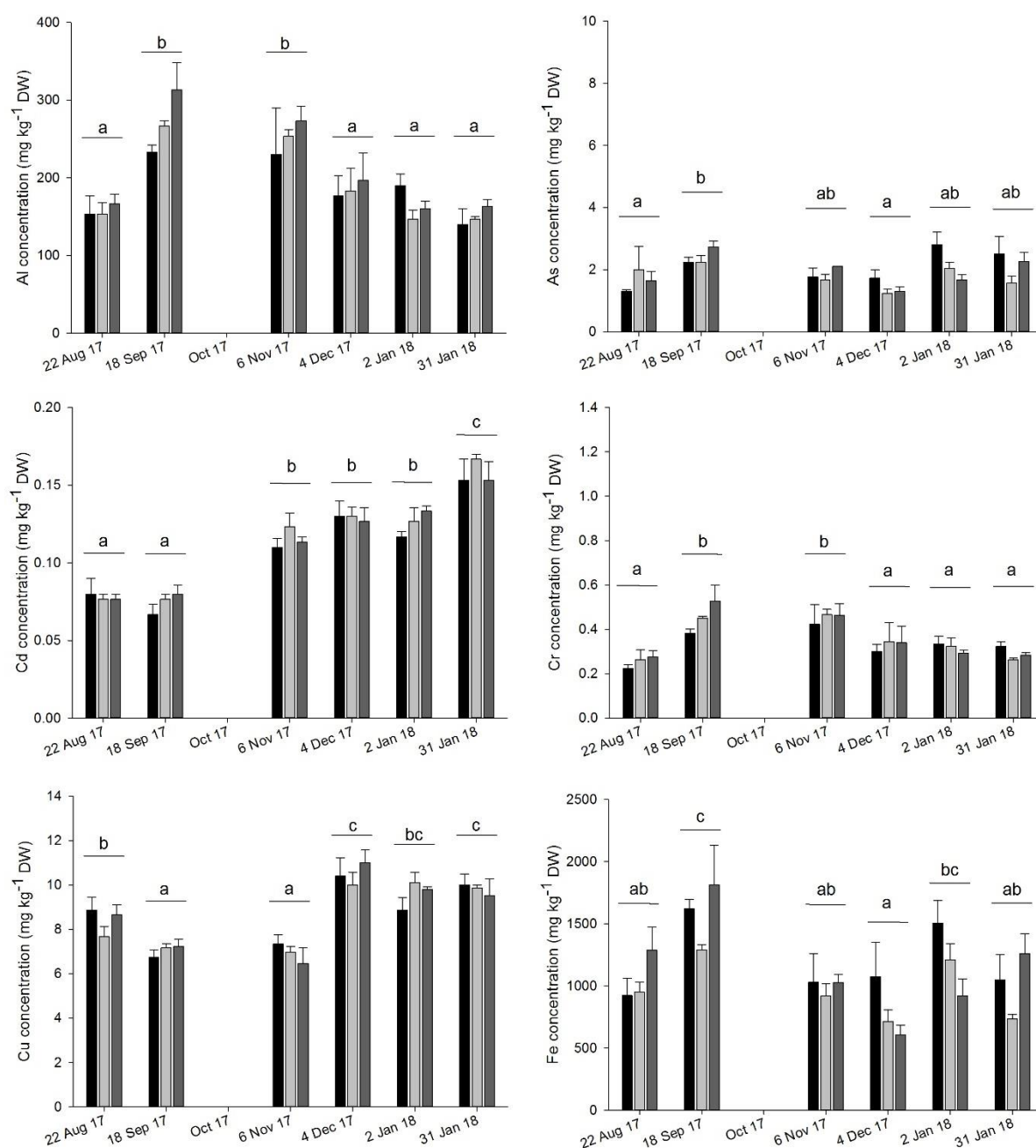
**Figure 4.4. Hierarchical cluster analysis of all seagrass trace elements by compartment over the sampling period. Clusters grouped closer to 1 indicate stronger similarity. Cluster symbols: ▲ = above-ground, x = below-ground. Colours indicate cluster groups by compartment.**

#### 4.3.2.1 Above-ground trace element concentrations

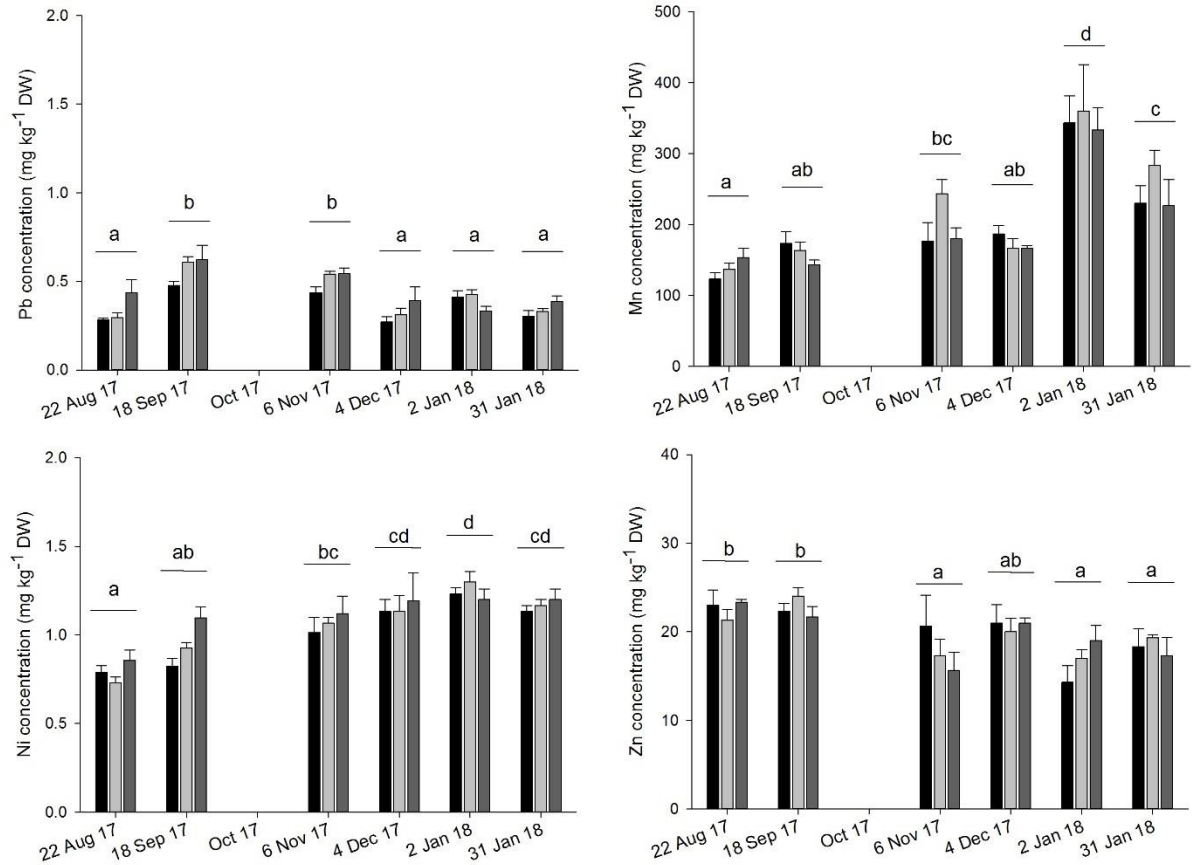
Within the above-ground compartment there was no significant interaction of sampling event and site for any TE (Table 4.2). All TEs within the above-ground compartment were significantly different between sampling events (Table 4.2) and above-ground Fe and Pb were the only TEs that were significantly different between sites (Fe  $F_{2,10} = 3.41$ ,  $p < 0.05$ ; Pb  $F_{2,10} = 7.39$ ,  $p < 0.01$ , Table 4.2). Post-hoc tests showed that the above-ground Fe (site 2,3 < 3,1) and Pb (1,2 < 2,3) site effects did not occur within every sampling event (Fig. 4.5), suggesting that there were at times localised temporal meadow differences for these TEs. Above-ground Pb concentrations demonstrated a general (five of the six sampling months) increasing gradient from the east (site 1) to the west (site 3, Fig. 4.5).

**Table 4.2. Univariate two-way ANOVAs by sampling event (event) and site of above- and below-ground seagrass for each trace element. Values in bold are significant  $p < 0.05$ . Full ANOVA table is provided in Appendix C Table C2, Table C3.**

		Above-ground			Below-ground	
		df	F	P	F	p
<b>Al</b>	Event	5	14.45	<b>0.000</b>	10.95	<b>0.000</b>
	Site	2	1.92	0.162	4.12	<b>0.025</b>
	Event*site	10	0.65	0.759	1.47	0.191
<b>As</b>	Event	5	4.17	<b>0.004</b>	19.27	<b>0.000</b>
	Site	2	1.15	0.329	0.71	0.497
	Event*site	10	1.68	0.124	1.22	0.314
<b>Cd</b>	Event	5	56.86	<b>0.000</b>	3.69	<b>0.008</b>
	Site	2	1.47	0.244	1.39	0.263
	Event*site	10	0.57	0.830	1.11	0.380
<b>Cr</b>	Event	5	10.07	<b>0.000</b>	7.67	<b>0.000</b>
	Site	2	0.79	0.464	2.79	0.075
	Event*site	10	0.65	0.763	0.87	0.570
<b>Cu</b>	Event	5	26.77	<b>0.000</b>	10.27	<b>0.000</b>
	Site	2	0.15	0.865	0.41	0.666
	Event*site	10	1.10	0.391	1.42	0.211
<b>Fe</b>	Event	5	8.09	<b>0.000</b>	9.79	<b>0.000</b>
	Site	2	3.41	<b>0.044</b>	0.98	0.385
	Event*site	10	1.86	0.084	1.13	0.371
<b>Pb</b>	Event	5	19.06	<b>0.000</b>	22.33	<b>0.000</b>
	Site	2	7.39	<b>0.002</b>	3.97	<b>0.028</b>
	Event*site	10	1.63	0.138	2.14	<b>0.046</b>
<b>Mn</b>	Event	5	30.33	<b>0.000</b>	12.65	<b>0.000</b>
	Site	2	1.63	0.209	3.85	<b>0.031</b>
	Event*site	10	1.13	0.370	1.89	0.080
<b>Ni</b>	Event	5	18.41	<b>0.000</b>	7.65	<b>0.000</b>
	Site	2	2.76	0.076	1.51	0.234
	Event*site	10	0.82	0.616	1.19	0.327
<b>Zn</b>	Event	5	6.77	<b>0.000</b>	5.08	<b>0.001</b>
	Site	2	0.04	0.959	0.72	0.496
	Event*site	10	1.13	0.368	0.58	0.817



**Figure 4.5.** Trace element concentrations of above-ground samples (mean  $\pm$  SE,  $n = 3$ , mg kg<sup>-1</sup> dry weight) over the sampling period and site (black site 1, light grey site 2 and, dark grey site 3). Similar letters indicates no significant differences between sampling events.



**Figure 4.5 (continued).** Trace element concentrations of above-ground samples (mean  $\pm$  SE,  $n = 3$ , mg kg<sup>-1</sup> dry weight) over the sampling period and site (black site 1, light grey site 2 and dark grey site 3). Similar letters indicates no significant differences between sampling events.

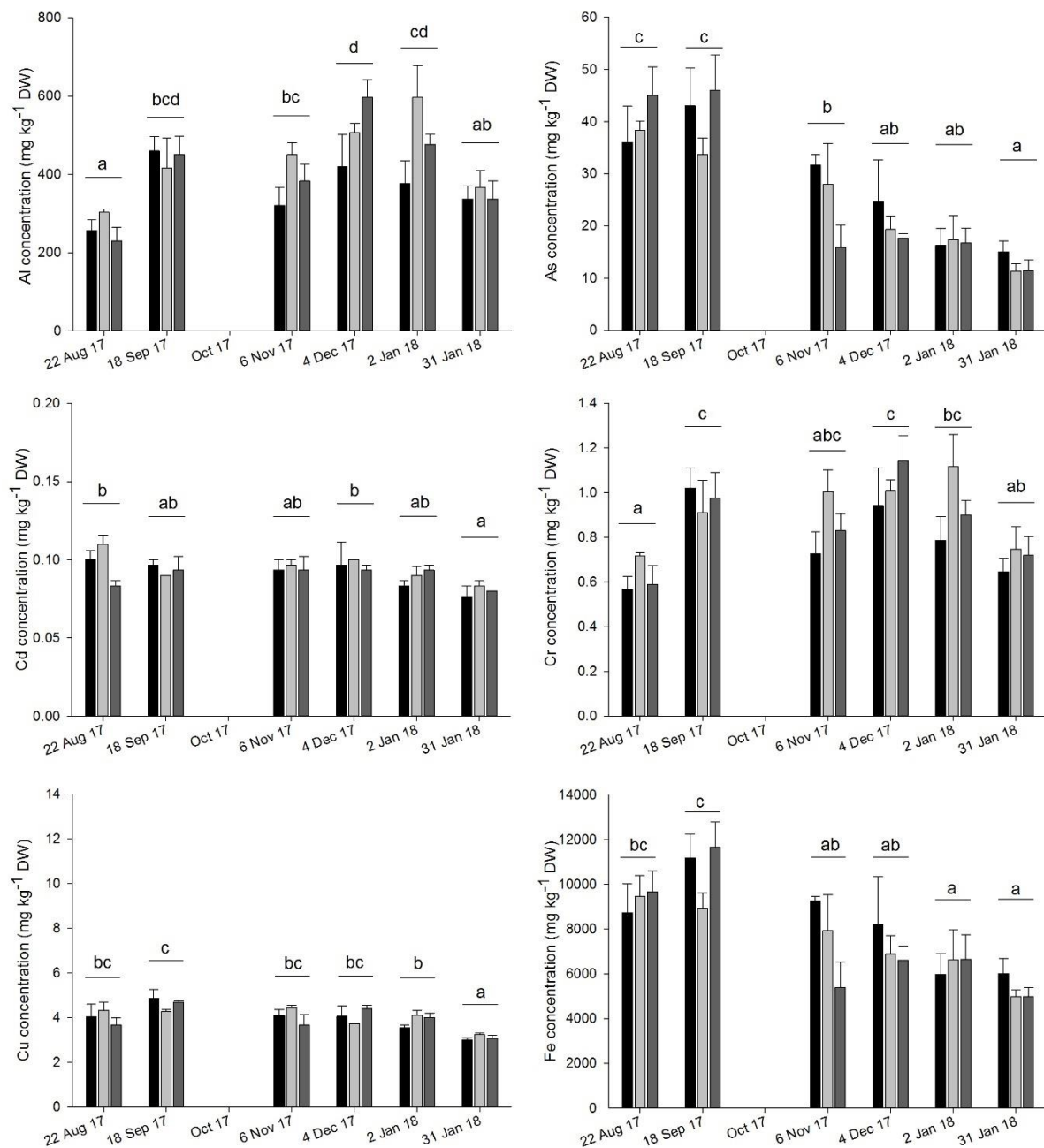
The cluster analysis and ANOVA results illustrated some notable seasonal patterns for different TEs within the above-ground compartment (Table 2, Fig. 4.5). The first pattern (Fig. 4.4) of seasonal differences is the grouping of above-ground Cu, Cd, Mn and Ni; these TEs were present in significantly higher concentrations towards the end of the growing season (December to end of January) than in August to November. Above-ground Cu mean concentrations were significantly ( $F_{5,10} = 26.77$ ,  $p < 0.001$ , Table 4.2) higher in December to late January ( $9.95 \pm 0.97$  mg kg<sup>-1</sup>) than in September ( $7.04 \pm 0.5$  mg kg<sup>-1</sup>) and November ( $6.92 \pm 0.83$  mg kg<sup>-1</sup>) (Fig. 4.5). Mean Cd mean concentrations in the above-ground compartment significantly ( $F_{5,10} = 56.86$ ,  $p < 0.001$ , Table 4.2) increased from  $0.08 \pm 0.01$  mg kg<sup>-1</sup> in August and September to  $0.16 \pm 0.02$  mg kg<sup>-1</sup> in late January (Fig. 4.5). Mean Mn concentrations in the above-ground compartment significantly ( $F_{5,10} = 30.33$ ,  $p < 0.001$ , Table 4.2) increased more than two fold from  $137.8 \pm 19.3$  mg kg<sup>-1</sup> in August to  $345.6 \pm 72.3$  mg kg<sup>-1</sup> in early January (Fig. 4.5). Mean Ni

concentrations significantly ( $F_{5,10} = 18.41$ ,  $p < 0.001$ , Table 4.2) increased from  $0.79 \pm 0.08 \text{ mg kg}^{-1}$  in August to  $1.18 \pm 0.12 \text{ mg kg}^{-1}$  in December to January (Fig. 4.5).

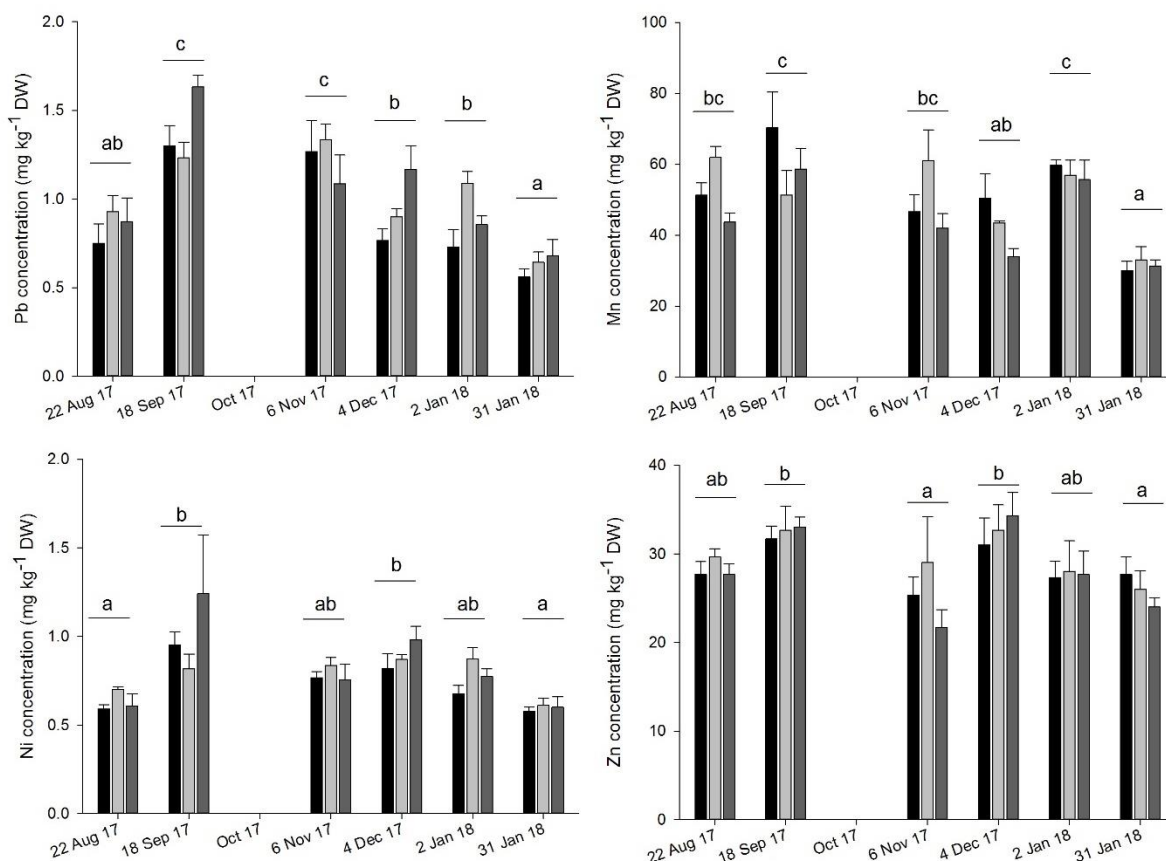
The second cluster (Fig. 4.4) formed due to significantly higher Al, Cr and Pb concentrations (Al  $F_{5,10} = 14.5$ ,  $p < 0.001$ ; Cr  $F_{5,10} = 10.7$ ,  $p < 0.001$ ; Pb  $F_{5,10} = 19.06$ ,  $p < 0.001$ , Table 4.2) in September and November (Al  $261.7 \pm 52.3 \text{ mg kg}^{-1}$ ; Cr  $0.45 \pm 0.09 \text{ mg kg}^{-1}$ ; Pb  $0.54 \pm 0.09 \text{ mg kg}^{-1}$ ) than in the other sampling events (Al  $164.7 \pm 33.3 \text{ mg kg}^{-1}$ ; Cr  $0.30 \pm 0.07 \text{ mg kg}^{-1}$ ; Pb  $0.35 \pm 0.08 \text{ mg kg}^{-1}$ , Fig. 4.5). The third cluster (Fig. 4.4) is a result of significantly different above-ground concentrations of As and Fe between sampling events (As  $F_{5,10} = 4.17$ ,  $p < 0.01$ ; Fe  $F_{5,10} = 8.09$ ,  $p < 0.001$ , Table 4.2); ranging from a maximum in September ( $2.4 \pm 0.4 \text{ mg kg}^{-1}$  and  $1574 \pm 365 \text{ mg kg}^{-1}$ , respectively) to a minimum in December ( $1.42 \pm 0.37 \text{ mg kg}^{-1}$  and  $797 \pm 335 \text{ mg kg}^{-1}$ , respectively, Fig. 4.5). Above-ground Zn concentrations were significantly different between sampling events ( $F_{5,10} = 6.77$ ,  $p < 0.001$ , Table 4.2) and concentrations decreased from  $22.6 \pm 2.07 \text{ mg kg}^{-1}$  in September to  $16.8 \pm 3.11 \text{ mg kg}^{-1}$  in early January (Fig. 4.5).

#### **4.3.2.2 Below-ground trace element concentrations**

Within the below-ground compartment, Pb was the only TE to have a significant, albeit weak, interaction between sampling event and site ( $F_{10,36} = 2.14$ ,  $p < 0.05$ , Table 4.2) and significant effects of site ( $F_{2,10} = 3.97$ ,  $p < 0.05$ ) and sampling event ( $F_{5,10} = 22.33$ ,  $p < 0.001$ , Table 4.2). The significant interaction observed within the below-ground Pb concentrations was that sites were 1, 2 < 3 within the majority of the sampling events (Fig. 4.6). Below-ground concentrations of Al and Mn were also significantly different between sites (Al  $F_{2,10} = 4.12$ ,  $p < 0.05$ , post-hoc 1,3 < 3,2; Mn  $F_{2,10} = 3.85$ ,  $p < 0.05$ , post-hoc 3,1 < 1,2, Table 4.2, Fig. 4.6).



**Figure 4.6.** Trace element concentrations of below-ground samples (mean  $\pm$  SE,  $n = 3$ ,  $\text{mg kg}^{-1}$  dry weight) over the sampling period and site (black site 1, light grey site 2 and dark grey site 3). Similar letters indicates no significant differences between sampling events.



**Figure 4.6 (continued). Trace element concentrations of below-ground samples (mean  $\pm$  SE,  $n = 3$ , mg kg<sup>-1</sup> dry weight) over the sampling period and site (black site 1, light grey site 2 and dark grey site 3). Similar letters indicates no significant differences between sampling events.**

All below-ground concentrations of TEs were significantly different between sampling events (Table 4.2), with each TE showing different seasonal patterns to each other and to the above-ground compartment. The first below-ground cluster (Fig. 4.4) formed as a result of significantly lower Al, Cr and Ni concentrations before the growing season in August and in late January (Al  $F_{5,10} = 10.95$ ,  $p < 0.001$ , August  $263 \pm 50.5$  mg kg<sup>-1</sup>; Cr  $F_{5,10} = 7.67$ ,  $p < 0.001$ , August  $0.63 \pm 0.11$  mg kg<sup>-1</sup>; Ni  $F_{5,10} = 7.65$ ,  $p < 0.001$ , August  $0.63 \pm 0.08$  mg kg<sup>-1</sup>, Table 4.2) than in the other sampling events. Mean Al, Cr and Ni concentrations were highest in either September or December (Fig. 4.6). The next cluster (Fig. 4.4) forms due to significantly higher below-ground concentrations of Cu and Pb in September (Cu  $F_{5,10} = 10.27$ ,  $p < 0.001$ ,  $4.61 \pm 0.44$  mg kg<sup>-1</sup>; Pb  $F_{5,10} = 22.33$ ,  $p < 0.001$ ,  $1.39 \pm 0.23$  mg kg<sup>-1</sup>, Table 4.2) than in January (Cu  $3.1 \pm 0.19$  mg kg<sup>-1</sup>; Pb  $0.63 \pm 0.12$  mg kg<sup>-1</sup> Fig. 4.6). Similarly, in the next cluster (Fig. 4.4), below-ground mean concentrations of Cd, Mn and Zn were significantly lower in late January (Cd  $F_{5,10} = 3.69$ ,  $p < 0.01$ ,  $0.08 \pm 0.007$  mg kg<sup>-1</sup>; Mn  $F_{5,10} = 12.65$ ,  $p < 0.001$ ,  $31.4 \pm 4.48$  mg kg<sup>-1</sup>; Zn  $F_{5,10}$

= 5.08,  $p < 0.001$ ,  $25.89 \pm 3.10 \text{ mg kg}^{-1}$ , Table 4.2), with maximum means falling in other sampling events (Fig. 4.6). The final cluster (Fig. 4.4) forms as below-ground concentrations of As and Fe were higher in September (As  $F_{5,10} = 19.27$ ,  $p < 0.001$ ,  $40.9 \pm 0.4 \text{ mg kg}^{-1}$ ; Fe  $F_{5,10} = 9.79$ ,  $p < 0.001$ ,  $10593 \pm 1932 \text{ mg kg}^{-1}$ ) and significantly lower in late January (As  $12.6 \pm 3.37 \text{ mg kg}^{-1}$ ; Fe  $5321 \pm 896 \text{ mg kg}^{-1}$ , Table 4.2, Fig. 4.6).

#### **4.3.2.3 Environment trace element concentrations**

All sediment TEs differed significantly between sites (Table 3,  $p < 0.05$ ) with the same post-hoc test result for all TEs (3,1<1,2, Table 4.3). Concentrations of TEs in sediment samples were relatively consistent over time and significant sampling event effects were only detected for three TEs (Al, Cr and Zn), with slightly higher mean concentrations in August (Al  $4140 \pm 292 \text{ mg kg}^{-1}$ ; Cr  $11.8 \pm 0.83 \text{ mg kg}^{-1}$ ; Zn  $16.3 \pm 1.00 \text{ mg kg}^{-1}$ ) than December (Al  $3694 \pm 349 \text{ mg kg}^{-1}$ ; Cr  $10.5 \pm 0.9 \text{ mg kg}^{-1}$ ) or January (Zn  $14.8 \pm 1.20 \text{ mg kg}^{-1}$ , Table 4.3, Table 4.4). Dissolved (0.45  $\mu\text{m}$  filtered) TE concentrations in water samples (data provided by PCIMP) included low levels of As  $1.4\text{--}1.7 \mu\text{g L}^{-1}$ , Fe  $5\text{--}11 \mu\text{g L}^{-1}$ , Mn  $1.0\text{--}2.25 \mu\text{g L}^{-1}$  and Ni  $1.0\text{--}1.15 \mu\text{g L}^{-1}$  with variable concentrations over the sampling period (Table 4.4).

**Table 4.3. Univariate two-way ANOVAs by sampling event (event) and site of sediment for each trace element. No Cd as < limit of reporting. Values in bold are significant  $p < 0.05$ .**

		df	MS	F	p	Post - hoc
<b>Al</b>	Event	5	252926	2.751	<b>0.033</b>	4,5,6,3,2<5,6,3,2,1
	Site	2	403401	4.388	<b>0.020</b>	3,1<1,2
	Event*site	10	55448	0.603	0.801	
	Error	36	91929			
<b>As</b>	Event	5	0.562	2.379	0.058	
	Site	2	0.994	4.206	<b>0.023</b>	3,1<1,2
	Event*site	10	0.356	1.508	0.177	
	Error	36	0.236			
<b>Cr</b>	Event	5	1.982	3.362	<b>0.014</b>	4,6,5,3,2<5,3,2,1
	Site	2	2.891	4.902	<b>0.013</b>	3,1<1,2
	Event*site	10	0.467	0.793	0.636	
	Error	36	0.590			
<b>Cu</b>	Event	5	0.103	1.497	0.215	
	Site	2	0.234	3.395	<b>0.045</b>	3,1<1,2
	Event*site	10	0.037	0.537	0.852	
	Error	36	0.069			
<b>Fe</b>	Event	5	479506	1.670	0.167	
	Site	2	994316	3.464	<b>0.042</b>	3,1<1,2
	Event*site	10	279563	0.974	0.482	
	Error	36	287044			
<b>Pb</b>	Event	5	0.032	0.952	0.460	
	Site	2	0.170	5.106	<b>0.011</b>	3,1<1,2
	Event*site	10	0.036	1.092	0.394	
	Error	36	0.033			
<b>Mn</b>	Event	5	238.89	2.224	0.073	
	Site	2	355.56	3.310	<b>0.048</b>	3,1<1,2
	Event*site	10	177.78	1.655	0.130	
	Error	36	107.41			
<b>Ni</b>	Event	5	0.127	1.625	0.178	
	Site	2	0.359	4.584	<b>0.017</b>	3,1<1,2
	Event*site	10	0.049	0.624	0.784	
	Error	36	0.078			
<b>Zn</b>	Event	5	3.022	2.720	0.035	5,4,6,3,2<4,6,3,2,1
	Site	2	7.389	6.650	<b>0.003</b>	3,1<1,2
	Event*site	10	1.011	0.910	0.534	
	Error	36	1.111			

**Table 4.4. Sediment and dissolved trace elements (mean  $\pm$  SD, n = 9 sediment, mg kg<sup>-1</sup> dry weight, n = 2 dissolved water,  $\mu$ g L<sup>-1</sup>) for each sampling event. Similar letters indicates no significant differences between sampling events. Env. = environment tested. Sed = sediment, Diss = dissolved (0.45  $\mu$ m filtered) TE concentrations. '-' indicates no data within those months.**

TE	Env.	Aug	Sept	Nov	Dec	2 Jan	31 Jan
<b>Al</b>	<b>Sed</b>	4140	3917	3866	3694	3724	3742
		(292) <sup>b</sup>	(368) <sup>ab</sup>	(287) <sup>ab</sup>	(349) <sup>a</sup>	(323) <sup>ab</sup>	(230) <sup>ab</sup>
<b>As</b>	<b>Sed</b>	7.36	6.94	7.06	6.76	6.69	6.77
		(0.59)	(0.67)	(0.49)	(0.58)	(0.52)	(0.33)
	<b>Diss</b>	1.4	-	1.5	-	-	1.7
		(0.0)		(0.0)			(0.0)
<b>Cd</b>	<b>Sed</b>	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
		(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
<b>Cr</b>	<b>Sed</b>	11.8	11.2	10.9	10.5	10.86	10.8
		(0.83) <sup>b</sup>	(0.87) <sup>ab</sup>	(0.78) <sup>ab</sup>	(0.90) <sup>a</sup>	(0.94) <sup>ab</sup>	(0.50) <sup>a</sup>
<b>Cu</b>	<b>Sed</b>	2.98	2.81	2.82	2.64	2.78	2.83
		(0.19)	(0.26)	(0.28)	(0.34)	(0.3)	(0.17)
<b>Fe</b>	<b>Sed</b>	8023	7683	7644	7357	7450	7581
		(552)	(662)	(514)	(648)	(567)	(374)
	<b>Diss</b>	5.0	-	11.0	-	-	5.0
		(0.0)		(2.9)			(0.0)
<b>Pb</b>	<b>Sed</b>	2.43	2.36	2.31	2.26	2.31	2.34
		(0.22)	(0.19)	(0.17)	(0.23)	(0.20)	(0.17)
<b>Mn</b>	<b>Sed</b>	137	129	131	127	122	124
		(12.3)	(11.7)	(13.6)	(13.2)	(9.72)	(7.26)
	<b>Diss</b>	2.25	-	1.00	-	-	1.70
		(0.35)		(0.00)			(0.00)
<b>Ni</b>	<b>Sed</b>	3.78	3.6 (0.31)	3.56	3.43	3.49	3.53
		(0.30)		(0.32)	(0.32)	(0.33)	(0.19)
	<b>Diss</b>	1.00	-	1.00	-	-	1.15
		(0.00)		(0.00)			(0.21)
<b>Zn</b>	<b>Sed</b>	16.3	15.6	15.4	14.9	14.8	15.0
		(1.00) <sup>b</sup>	(1.24) <sup>ab</sup>	(1.13) <sup>ab</sup>	(1.45) <sup>ab</sup>	(1.20) <sup>a</sup>	(0.87) <sup>ab</sup>

### 4.3.3 Seagrass accumulation and correlation

The order of TE concentrations within each compartment was similar at the higher (Al, Fe, Mn and Zn) and lower (Ni, Pb, Cr and Cd) ranks but order changed dependent on the compartment. Trace element concentrations within the three materials analysed decreased as follows:

Fe>Mn>Al>Zn>Cu>As>Ni>Pb>Cr>Cd above-ground seagrass

Fe>Al>Mn>Zn>As>Cu>Pb>~Cr>~Ni>Cd below-ground seagrass

Fe>Al>Mn>Zn>Cr>As>Ni>Cu>Pb>Cd sediment.

Trace element accumulation between seagrass compartments and sediment (dissolved TEs always had lower concentrations than the other materials tested and therefore were excluded from Table 4.5) was different for each TE. Higher concentrations of Cu, Cd and Mn were recorded in the above-ground compartment than in the below-ground compartment or the sediment (Table 4.5). Arsenic, Fe and Zn were found in higher concentrations within the below-ground compartment than in the sediment or above-ground compartment (Table 4.5). Sediment had higher concentrations than both seagrass compartments of Al, Cr, Pb and Ni (Table 4.5).

**Table 4.5. Compartment order by trace element concentration.**

Trace elements	Compartment order
Cu, Cd	Above > below > sediment
Mn	Above > sediment > below
As, Fe	Below > sediment > above
Zn	Below > above > sediment
Al, Cr, Pb	Sediment > below > above
Ni	Sediment > above > below

Correlations between seagrass compartments and the environment were tested to ascertain possible environmental TE sources. No significant correlations were observed between any part of the seagrass and dissolved (<0.45 µm filtered) TEs in water samples (Table 4.6). The only significant correlation between sediment TE concentration and any seagrass compartment was a significant ( $p < 0.05$ ) weak negative correlation to above-ground Ni (Table 4.6). Relationships between the above- and below-ground seagrass compartments demonstrated that Cr, Fe, Pb and Zn had positive medium to strong significant ( $p < 0.01$ ) correlations (Table 4.6).

**Table 4.6. Pearson's correlations for each trace element between the above- and below-ground compartment to either sediment or dissolved trace element concentrations and between the above- and below-ground compartment. Na indicates not applicable as dissolved trace elements were < limit of reporting. \* Correlation is significant at the 0.05 level (2-tailed), \*\* Correlation is significant at the 0.01 level (2-tailed). Significant values provided in Appendix C Table C4.**

	<b>Above - sediment</b>	<b>Below - sediment</b>	<b>Above - dissolved</b>	<b>Below - dissolved</b>	<b>Above - below</b>
<b>Aluminium</b>	0.038	-0.087	Na	Na	0.158
<b>Arsenic</b>	-0.125	0.255	0.666	-0.742	0.138
<b>Cadmium</b>	Na	Na	Na	Na	-0.256
<b>Chromium</b>	0.01	-0.053	Na	Na	0.335*
<b>Copper</b>	-0.074	0.127	Na	Na	-0.212
<b>Iron</b>	-0.077	0.154	0.505	0.509	0.521**
<b>Lead</b>	0.033	-0.002	Na	Na	0.713**
<b>Manganese</b>	-0.196	0.191	-0.152	0.333	0.108
<b>Nickel</b>	-0.269*	-0.092	Na	Na	0.148
<b>Zinc</b>	0.25	0.038	Na	Na	0.504**

Bioconcentration Factors from the sediment to the above- and below-ground compartment varied significantly ( $p < 0.01$ ) with time for all TEs except Mn in the below-ground compartment (Table 4.7, ANOVA tables are provided in Appendix C Table C5, Table C6). Above-ground BCF values indicate stronger accumulation of Cu, Mn and Zn from sediment (Table 4.7). Above-ground Cu, Mn and Ni BCF values significantly increased over the sampling period, while the other TEs were variable between months (Table 4.7, Appendix C Table C5). Below-ground BCF values indicate stronger accumulation of As, Cu and Zn and occasionally of Fe (Table 4.7). Over time, below-ground BCF values for As and Fe significantly decreased while the rest of the TEs were variable between months (Table 4.7).

**Table 4.7. Bioconcentration Factors between above-ground and sediment (top section) and below-ground and sediment (below section). Significant *p* value for one-way ANOVA for each trace element. Shaded cells indicate Bioconcentration Factors >1. Similar letters indicates no significant differences between sampling events. Full F tables provided in Appendix C Table C5, Table C6.**

Above	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
<i>p</i>	0.000	0.006	-	0.000	0.000	0.001	0.000	0.000	0.000	0.004
Aug	0.04 <sup>a</sup>	0.22 <sup>a</sup>	-	0.02 <sup>a</sup>	2.83 <sup>a</sup>	0.13 <sup>a</sup>	0.14 <sup>a</sup>	1.02 <sup>a</sup>	0.21 <sup>a</sup>	1.38 <sup>ab</sup>
Sept	0.07 <sup>c</sup>	0.35 <sup>b</sup>	-	0.04 <sup>bc</sup>	2.53 <sup>a</sup>	0.21 <sup>b</sup>	0.24 <sup>c</sup>	1.25 <sup>ab</sup>	0.27 <sup>ab</sup>	1.46 <sup>b</sup>
Nov	0.07 <sup>bc</sup>	0.26 <sup>ab</sup>	-	0.04 <sup>c</sup>	2.46 <sup>a</sup>	0.13 <sup>a</sup>	0.22 <sup>bc</sup>	1.53 <sup>bc</sup>	0.30 <sup>bc</sup>	1.16 <sup>a</sup>
Dec	0.05 <sup>ab</sup>	0.21 <sup>a</sup>	-	0.03 <sup>ab</sup>	4.00 <sup>b</sup>	0.11 <sup>a</sup>	0.15 <sup>a</sup>	1.38 <sup>ab</sup>	0.34 <sup>c</sup>	1.40 <sup>ab</sup>
2 Jan	0.04 <sup>a</sup>	0.32 <sup>ab</sup>	-	0.03 <sup>a</sup>	3.50 <sup>b</sup>	0.16 <sup>ab</sup>	0.17 <sup>ab</sup>	2.84 <sup>d</sup>	0.36 <sup>c</sup>	1.15 <sup>a</sup>
31 Jan	0.04 <sup>a</sup>	0.31 <sup>ab</sup>	-	0.03 <sup>a</sup>	3.47 <sup>b</sup>	0.13 <sup>a</sup>	0.15 <sup>a</sup>	1.98 <sup>c</sup>	0.33 <sup>c</sup>	1.22 <sup>ab</sup>
<b>Below</b>										
<i>p</i>	0.000	0.000	-	0.000	0.000	0.000	0.000	0.152	0.000	0.001
Aug	0.06 <sup>a</sup>	5.43 <sup>b</sup>	-	0.05 <sup>a</sup>	1.35 <sup>ab</sup>	1.16 <sup>bc</sup>	0.35 <sup>ab</sup>	0.39 <sup>a</sup>	0.17 <sup>a</sup>	1.74 <sup>ab</sup>
Sept	0.11 <sup>bcd</sup>	5.96 <sup>b</sup>	-	0.09 <sup>bc</sup>	1.65 <sup>c</sup>	1.39 <sup>c</sup>	0.59 <sup>d</sup>	0.46 <sup>a</sup>	0.28 <sup>b</sup>	2.10 <sup>bc</sup>
Nov	0.10 <sup>bc</sup>	3.55 <sup>a</sup>	-	0.08 <sup>bc</sup>	1.44 <sup>bc</sup>	0.99 <sup>ab</sup>	0.53 <sup>cd</sup>	0.38 <sup>a</sup>	0.22 <sup>ab</sup>	1.64 <sup>a</sup>
Dec	0.14 <sup>d</sup>	3.06 <sup>a</sup>	-	0.10 <sup>c</sup>	1.55 <sup>bc</sup>	0.99 <sup>ab</sup>	0.42 <sup>bc</sup>	0.50 <sup>a</sup>	0.26 <sup>b</sup>	2.21 <sup>c</sup>
2 Jan	0.13 <sup>cd</sup>	2.56 <sup>a</sup>	-	0.09 <sup>bc</sup>	1.42 <sup>bc</sup>	0.88 <sup>ab</sup>	0.39 <sup>ab</sup>	0.47 <sup>a</sup>	0.22 <sup>ab</sup>	1.89 <sup>abc</sup>
31 Jan	0.09 <sup>ab</sup>	1.85 <sup>a</sup>	-	0.07 <sup>ab</sup>	1.10 <sup>a</sup>	0.70 <sup>a</sup>	0.27 <sup>a</sup>	0.25 <sup>a</sup>	0.17 <sup>a</sup>	1.73 <sup>ab</sup>

## 4.4 Discussion

The knowledge of temporal changes in TE concentrations within a potential bioindicator can influence how and when the bioindicator can be used or interpreted correctly, and whether it is a true time integrated bioindicator (Rainbow 2006). Results from this study have demonstrated that partitioned *Z. muelleri* TE concentrations do change through time and therefore possibly influence when to use this species as a bioindicator. The growth cycle of *Z. muelleri* over the six month period was characterised by maximum seagrass cover and flowering in November and a summer biomass decrease. This cycle is similar to the previously reported growth cycle of *Z. muelleri* within Port Curtis and north Queensland (Chartrand et al. 2016; McKenzie 1994). Knowing that this cycle occurs in a predictable manner can assist in determining when to sample within the

growth cycle, and the same theory could potentially be applied to other *Z. muelleri* meadows that display this growth cycle.

#### **4.4.1 *Zostera muelleri* compartments**

*Zostera muelleri* had detectable concentrations of all analysed TEs, and TEs had accumulated in concentrations higher than the environmental concentrations. *Zostera muelleri* TE concentrations within this study when compared to previous Port Curtis results were either similar (Apte et al. 2005) or distinctly lower for Al, Cr, Cu Fe and Zn (Table 4.8) by a factor of 1.5–47 depending on the compartment (Prange & Dennison 2000). Both *Z. muelleri* compartments (above- and below-ground) had higher Fe, Al and Mn concentrations than other TEs and this order of TEs is the same as has previously been recorded from other locations (Sydney, Lake Illawarra, Table 4.8) (Birch, Cox & Besley 2018; Howley 2001). The higher concentrations of Fe and Al observed within *Z. muelleri* concurs with a meta-analysis by Vonk et al. (2018), who found that colonising species have higher concentrations of these TEs than persistent species. Changes in the order of the more toxic TEs, such as Pb and Cr, can indicate localised pollution. *Zostera muelleri* has demonstrated variable order of these TEs with leaf compartment Pb>As, Cr (Birch, Cox & Besley 2018) or leaf Cr>Cu (Table 4.8) (Prange & Dennison 2000). In the present study, the above-ground order Ni>Pb>Cr>Cd was different to the below-ground order Pb>~Cr>~Ni>Cd, suggesting variable accumulation and storage of these TEs. Concentrations of TEs in *Z. muelleri* from past studies in Port Curtis and other areas suggests that this meadow is far enough away from anthropogenic influences that the results can be interpreted as possible natural changes.

**Table 4.8. Results from other trace element studies and *Zostera muelleri* from within Australia. Seagrass part analysed is abbreviated as A = above, B = below, L = leaf, RR = root-rhizome, Ro = root, Rh = rhizome. Ranges are the mean minimum and maximum except for this study where absolute minimum and maximum values are given. Units mg kg<sup>-1</sup> dry weight. '-' indicates no data.**

Part	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn	Where	Study
A	120–380	1.1–3.6	0.06–0.18	0.2–0.65	5.2– 12	510–2370	0.22–0.74	110–440	0.68–1.5	12–27	Port Curtis	This study (range)
B	170–760	7.3–59	0.07–0.12	0.45–1.4	2.8– 5.3	3910–13900	0.48–1.7	26–85	0.48–1.9	18–39	Port Curtis	This study (range)
L	625–1794	-	-	5.0–30.6	7.9–12.3	2089–7592	-	-	-	23.7–74.7	Port Curtis	Prange and Dennison (2000)
RR	422–2206	-	-	4.7–29.7	2.1–14.4	1829–17889	-	-	-	7.7–60.2	Port Curtis	Prange and Dennison (2000)
L	832–2410	1.5–12.1	0.09–0.2	4.0–9.4	3.0–19.0	880–5560	0.6–1.3	-	1.6–4.8	15–20	Port Curtis	Apte et al. (2005)
L	-	-	2.1–6.1	-	13.5–52.1	-	3.4–148.4	-	-	115.4–397	Lake Macquarie	Ambo-Rappe, Lajus and Schreider (2007)
Ro	-	-	3.0–20.2	-	15.3–84.1	-	4.1–211.7	-	-	63.9–592	Lake Macquarie	Ambo-Rappe, Lajus and Schreider (2007)
L	-	0.85–1.15	0.24–0.96	-	5.8–15.2	-	1.99–3.51	307–1292	1.5–2.0	41.2–133	Lake Illawarra	Howley (2001)
Rh	-	0.89–4.75	0.1–0.41	-	2.2–8.02	-	1.39–11.0	13–516	<1.0	19.4– 54	Lake Illawarra	Howley (2001)
L	-	1.9–5.9	-	0.6–5.6	5.4–73.0	-	1.4–48.0	13–465	-	68–247	Sydney	Birch, Cox and Besley (2018)
Rh	-	3.7–58	-	2.4– 15	3.8–93.0	-	4.5–152	7.1–331	-	70–455	Sydney	Birch, Cox and Besley (2018)
Ro	-	5.0–100	-	0.3–9.5	2.5–42.0	-	0.5–66.0	1.9–145	-	18–184	Sydney	Birch, Cox and Besley (2018)

Different TE accumulation patterns were identified between compartments of *Z. muelleri*, and these are a reported phenomenon in seagrass bioindicators (Bonanno & Borg 2018; Pergent-Martini & Pergent 2000). Higher concentrations of TEs within the above-ground material is likely due to either differences in accumulation from the immediate environment or upward translocation to remove TEs from the seagrass through leaf loss (Bonanno & Di Martino 2017; Pergent-Martini & Pergent 2000). The above-ground compartment had higher mean concentrations of Cd ( $0.11 \text{ mg kg}^{-1}$ ), Cu ( $8.7 \text{ mg kg}^{-1}$ ), Mn ( $210 \text{ mg kg}^{-1}$ ) and Ni ( $1.06 \text{ mg kg}^{-1}$ ) than the below-ground compartment Cd ( $0.09 \text{ mg kg}^{-1}$ ), Cu ( $3.95 \text{ mg kg}^{-1}$ ), Mn ( $49.1 \text{ mg kg}^{-1}$ ) and Ni ( $0.78 \text{ mg kg}^{-1}$ , Fig. 4.4, Table 4.5). *Zostera* spp. has demonstrated this same compartmentation with higher Cu, Ni and Mn and to a lesser degree Cd in the leaf or above-ground material than in the below-ground material (Table 4.8 and other studies) (Birch, Cox & Besley 2018; Brix & Lyngby 1983; Howley 2001; Lin et al. 2018; Lyngby & Brix 1982; Prange & Dennison 2000). Lin et al. (2018) also observed *Zostera japonica* Ascherson and Graebner to have higher Zn in addition to Cu, Cd and Mn in above-ground material than in below-ground material, unlike the findings within this study where Zn was greater in the below-ground compartment than in the above-ground. The above-ground compartment by itself may not be recommended as a sole bioindicator as very few TEs (Cu, Cd, Mn and Ni) were found in higher concentrations.

The below-ground compartment had higher concentrations of Al, As, Cr, Fe, Pb and Zn than the above-ground material and this can be observed from other *Z. muelleri* studies (Table 4.8) (Ambo-Rappe, Lajus & Schreider 2007; Birch, Cox & Besley 2018; Maher et al. 2011; Prange & Dennison 2000). Storage of non-essential TEs within the below-ground compartment is a common occurrence in seagrass, with Pb and Hg preferentially found in *P. oceanica* roots or rhizomes (Bonanno & Di Martino 2017; Pergent-Martini & Pergent 2000). Greater accumulation of As and Fe within the below-ground compartment of *Z. muelleri* is possibly due to the lack of an As uptake system (Maher et al. 2011). In this study, concentration of Cd appears to be higher within the above-ground material, whereas other studies have observed different patterns including leaf > rhizome (Howley 2001) and root > leaf (Ambo-Rappe, Lajus & Schreider 2007). This compartment preference variability can be explained by the evidence of bidirectional translocation of Cd within *Z. marina*; however, the root-rhizome overall appears to be a Cd sink (Brinkhuis, Penello & Churchill 1980). Below-ground Zn concentrations observed within this study were greater than the above-ground concentrations; however, this pattern is not commonly observed in *Zostera* spp. (Table 4.8). The predominant pattern for Zn in *Zostera* spp. is leaf > root-rhizome (Howley 2001; Lin et al. 2018; Lyngby & Brix 1982; Prange & Dennison 2000). One possible reason that *Z. muelleri* did not demonstrate this previously reported pattern (leaf > root-

rhizome) in the present study may include low concentrations of bioavailable Zn in the study area. The below-ground compartment of *Z. muelleri* could be a good sole bioindicator of TE concentrations as higher concentrations for six elements (Al, As, Cr, Fe, Pb and Zn) were recorded.

This study identified a significant positive relationship between seagrass compartments for Cr, Fe, Pb and Zn, suggesting upwards translocation. Strong above- and below-ground compartment correlations have been observed for Pb in *C. nodosa*, *P. oceanica* and *Z. marina* (Brix & Lyngby 1984; Malea & Haritonidis 1999; Malea, Mylona & Kevrekidis 2019). Malea, Mylona and Kevrekidis (2019) confirming in their latest research that *P. oceanica* Pb concentrations were primarily correlated to the sites sediment Pb concentrations and internal upwards translocation and not from proportional uptake from the environment as previously suggested (Malea & Haritonidis 1999). Lead concentrations in both compartments of *Z. muelleri* also at times displayed a spatial gradient, with higher concentrations recorded at the site that was furthest away from a boat ramp (site 3, Fig. 4.1). This gradient is contradictory to what would be expected, in that higher Pb could be found at site 1 closer to the boat ramp. It is possible that site 3 is near a tidal rivulet, or the hydrodynamics of the area could be influencing sediment and water movement and therefore TE concentrations. *Zostera muelleri* could be utilised as a localised spatial and temporal bioindicator of Pb concentrations.

#### **4.4.2 Temporal observations**

Temporal patterns observed within the two seagrass compartments suggested both natural biological changes and possible external influences on seagrass TEs (e.g., Mn). Temporal TE patterns were different between above- and below-ground seagrass compartments, with the exception of Pb, which showed similar temporal changes over time in both compartments.

##### **4.4.2.1 Temporal above-ground patterns**

Concentrations of Cu, Cd, Ni and Mn tended to be higher from December to end of January than the preceding sampling events (Fig. 4.5). The later sampling events (December to January) correspond to higher air and water temperatures, rainfall, completion of flowering, higher epiphyte cover, algal growth, higher % silt and decreasing seagrass cover (Fig. 4.2, Fig. 4.3). Prange and Dennison (2000) reported non-significant seasonal Cu concentrations that were higher in September and January (summer), while other studies of *Z. marina* reported peak Cu concentrations in spring that were possibly attributed to a greater

proportion of younger leaves that are higher in Cu (Brix & Lyngby 1982; Lyngby & Brix 1982). Due to the lack of temporal studies of TEs in *Z. muelleri*, Cd, Ni and Mn temporal trends identified in this study will be compared to other seagrass species. In previous studies, concentrations of Cd within *Z. marina* leaves peaked in winter and had lower concentrations in summer, a pattern that is converse to the above-ground pattern identified in the present study. Additionally, Mn concentrations in *Z. marina* and *P. australis* leaves and Ni concentrations in *C. nodosa* leaves peaked in late summer, with Mn and Ni concentrations attributed to be due to external seasonal environmental concentrations (Lyngby & Brix 1983; Malea & Kevrekidis 2013; Ward 1987). In this study, the higher concentrations of some TEs in summer could be attributed to external influences such as higher epiphyte growth, seasonal rainfall and inputs into Port Curtis, and *Z. muelleri* affinity to accumulate certain TEs. These explanations can possibly explain why this study observed higher Cu in summer and not in spring at the time of new growth, as other studies have observed. Additionally, the peak in above-ground Mn is most likely due to external environmental conditions of higher rainfall (100 mm, 1–2 January 2017), contributing an increase in TEs as *Zostera* leaves have a great affinity in accumulating Mn (Brinkhuis, Penello & Churchill 1980). However, attributing the higher concentrations of Cu, Cd, Ni and Mn to dissolved (0.45 µm filtered) TE concentrations in water samples will be difficult as dissolved values for some TEs are usually below the limit of reporting and therefore cannot be used for comparisons. If *Z. muelleri* is reflecting seasonal changes in water concentration of TEs, the above-ground compartment could be used as a bioindicator of the variable water environment and potentially could be sampled after summer to understand the seasonal TE environmental loads during that period.

Higher concentrations of Al, As, Cr, Fe, Pb and Zn were observed in the above-ground compartment at the start of the growth season (spring) and lower concentrations over summer (December to January). The period from September to November (spring) corresponded to lower rainfall, silt, epiphyte cover and algae cover, higher seagrass cover and peak flowering (Fig. 4.2, Fig. 4.3). A temporal study by Prange and Dennison (2000) found Al concentrations to be higher in seagrass leaves in winter, and Cr, Fe and Zn to be higher in spring, with only Fe being significantly different. Elsewhere, *Z. marina* demonstrated having higher Pb and Zn leaf concentrations in winter (Lyngby & Brix 1982). and *C. nodosa* leaves had higher As and Fe concentrations in summer and Al peaked in late autumn-early winter (Malea & Haritonidis 1995b; Malea & Kevrekidis 2013). The higher concentrations observed in the previous European examples are explained by winter runoff (higher Pb is associated with winter runoff) or cessation of growth (Lyngby & Brix 1982; Malea & Haritonidis 1999). *Cymodocea nodosa* leaves in winter are at the end of their

growing cycle, where increased biomass diluted As and Fe concentrations, but increased Al concentrations due to increase of leaf area (greater area for binding) before leaf loss occurred (Malea & Kevrekidis 2013). The higher concentrations in the above-ground compartment identified within September (spring) in this study could be because concentrations were higher before being diluted by newer leaves that have had less time to accumulate TEs. Additionally, the higher concentrations could be due to the inclusion of the flowers in the above-ground samples and future work could assess their TE contribution, as this has not previously been studied. These results are from one location and different seasonal patterns have been found to occur between locations, even for one species (Richir & Gobert 2014). A thorough understanding of *Z. muelleri* seasonal concentrations will only come from additional temporal and longitudinal studies. Additionally, an understanding of the contribution of shoot age distribution (old:young leaves) at the time of sampling could assist in TE accumulation patterns and therefore further decisions of when to sample (e.g., peak biomass when higher concentrations of certain TEs occur).

#### **4.4.2.2 Temporal below-ground patterns**

Temporal trends in below-ground TE concentrations were dissimilar to the above-ground compartment, suggesting that accumulation is not a function of the above-ground concentration for some TEs. The majority of the below-ground concentrations of TEs, except Al, Cr and Ni, were significantly higher in September at the start of spring and had lower concentrations in late January. Conversely, below-ground Al, Cr and Ni had higher concentrations in early summer. Prange and Dennison (2000) found *Z. muelleri* below-ground Cr, Fe and Zn to be higher in September (spring) and below-ground Al and Cu were higher in winter, with only Fe being significantly different than the other months of July and January. However, *Z. marina* below-ground compartment Cd, Cu and Mn had no significant temporal patterns, but Pb and Fe had significantly higher concentrations occurring in summer due to the growth phase (Lyngby & Brix 1982, 1983). The growth phase of *Z. muelleri* has previously been reported, and higher below-ground biomass was recorded in the lead in to November (McKenzie 1994), with older root-rhizome in August and September and fresher looking rhizomes and lower biomass in summer (personal observation). Below-ground concentrations of TEs could be explained by the growth cycle of the seagrass.

Seasonal rhizosphere sediment P and Fe cycling has been linked with the growth cycle of *Z. noltei* where higher concentrations are found in the active growing season (Deborde et al. 2008). The seasonal variability is due to oxygenation of the anoxic sediment by new roots, subsequently changing the redox state of the sediment TEs and releasing sediment bound

Fe and P for the seagrass to accumulate (Deborde et al. 2008; Pagès et al. 2012). This sediment seasonal variation was observed in the present study, within the seagrass where higher concentrations of Fe and other TEs occurred in the growing months and subsequently decreased in concentration after the active growing season. Confirming this seasonal TE variation in concentrations in the *Z. muelleri* below-ground compartment would require further investigation over a full year, with additional measurements such as rhizome distance and decomposition state to aid in interpretation and therefore the potential decision to use *Z. muelleri* as a bioindicator. This study has demonstrated that the below-ground compartment of *Z. muelleri* could be used as a temporal bioindicator, but that changes with age could influence when to sample. For example, an optimal time to sample may occur after the new root development occurred and at the time of higher above-ground biomass in November. Results of this study agree with other seagrass TE bioindicator studies that recommend using the below-ground compartment for longer timescale (decadal) monitoring, and the above-ground compartment for short term monitoring (weather, seasonal, fluctuating point sources) (Pergent-Martini & Pergent 2000). While this study occurred over the growing period, further sampling in the other months (February to August) could assist to understand the temporal changes observed and whether they were significant due to age (older vs younger).

#### **4.4.3 Environment relationship**

One criterion of a successful bioindicator is that they correlate with or indicate the presence of TEs within the environment (Rainbow 2006). This study demonstrated that *Z. muelleri* does accumulate TEs at higher concentrations than its environment, with BCF values >1 for below-ground As, Cu, Fe and Zn and above-ground Cu, Mn and Zn (Table 4.7). However, correlation analysis between the seagrass compartments and the environment showed a relationship between the above-ground material and sediment for Ni, with a weak negative correlation (-0.269, Table 4.6), which is not a common seagrass correlation. Other seagrass TE bioindicator studies have reported limited significant correlations to environmental TE concentrations (Bonanno & Di Martino 2017; De Casabianca et al. 2004; Kilminster 2013; Lin et al. 2018; Malea & Haritonidis 1999). For example, *Z. japonica* variable TE accumulation over time did not correlate to the constant sediment TEs and therefore conclusions about TE accumulation by seagrass is complicated (Lin et al. 2018). The lack of correlations between seagrass and the environment is most likely due to seagrass regulation of TE concentrations by accumulation of the TE actively (Cu), or passively (As), with the added effect of compartment age, metabolism, relationship and growth (Brix & Lyngby 1982; Pergent-Martini & Pergent 2000). Understanding a time integration of a TE under controlled

kinetic uptake experiments for a seagrass can assist in its use as a bioindicator, as seen by *C. nodosa* and Cd uptake (Malea et al. 2018). Additionally, long term monitoring will tease apart the seagrass and environment relationships as the results will demonstrate how consistent or repeatable TE concentrations are over time.

## 4.5 Conclusion

*Zostera muelleri* accumulated each TE from the environment to varying degrees, and this suggests that it has the potential to be a bioindicator of environmental TEs. However, differences in TE concentrations were variable between seagrass compartments and over time, suggesting that the different compartments behave differently in accumulating TEs and that the biological characteristics of a seagrass sample (age and growth status) can influence its use as a bioindicator. The knowledge developed from this study will be enhanced by future longitudinal sampling that will elucidate *Z. muelleri* natural TE range due to growth, and therefore its use as a bioindicator. As a bioindicator, *Z. muelleri* meets some criteria, as it does reflect concentrations that are not measurable within the environment. However, active and passive accumulation of some TEs makes interpretation difficult. It also appears that *Z. muelleri* could be used as a spatial and temporal bioindicator of Pb, with a site gradient observed.

Sampling over the growth cycle of *Z. muelleri* has provided more information on when to take representative below-ground samples for the majority of TEs. Sampling of the below-ground compartment is recommended to be undertaken during the period of maximum seagrass cover between September and November (or whenever it occurs year to year) as to represent concentrations after the below-ground new growth has occurred. In contrast, it is difficult to recommend when to sample the above-ground compartment as many different trends in TE concentration were identified in this compartment. Sampling during the months when maximum seagrass cover is recorded will allow for collection of results that are not impacted by summer leaf loss and may result in greater accumulation records for certain TEs. Future research into the role of flowers in accumulation could tease out what *Z. muelleri* leaves are accumulating.

**Chapter 5. Influence of variable specific  
conductivities on Cu and other trace  
elements uptake and partitioning in *Zostera  
muelleri***

## 5.1 Introduction

Over the past few decades coastal management of persistent pollutants such as TE has evolved from snap-shot monitoring of the water and sediment environments, to using time-integrated bioindicators or biomonitors (biological components of the ecosystem used to detect change), or bioaccumulators (accumulators of TE contamination). Some overarching frameworks driving the use of bioindicators or bioaccumulators include the Water Framework Directive within European Union waters or sediment toxicity assessment for dredging consideration or as a weight of evidence ecosystem approach within Australia (Anastasi & Wilson 2010; ANZG 2018; Borja et al. 2013; Simpson & Batley 2016). Bioindicators can be used to ascertain whether the water and sediment quality is poor and to spatially or temporally identify bioavailable harmful natural and anthropogenic TE sources. Often, bioindicators only reflect TEs from one environment, such as for bivalves reflecting water particulate TEs and not necessarily the sediment environment TEs (Rainbow 2006). Therefore, using an indicator of multiple environments (water and sediment), such as seagrasses, has the potential of being a particularly ecologically relevant bioindicator. However, aspects of how seagrasses use, compartmentalise and store TEs could also confound the use of seagrass as a bioindicator of two environments (Bonanno & Di Martino 2017; Pergent-Martini & Pergent 2000). Controlled experiments are needed to address this knowledge gap for local seagrass and assess the potential for seagrass to be used as a water and sediment TE bioindicator.

Seagrasses are known to be effective bioindicators and bioaccumulators of TE for use within coastal management (Bonanno & Di Martino 2017; Lyngby & Brix 1987; Malea et al. 2018; Roca et al. 2017). However, the knowledge of seagrass TE requirements and use is currently poor (Lewis & Devereux 2009). Laboratory and field research in seagrass TE uptake and effects are increasing with the majority of studies typically focussed on temperate seagrass species such as *P. oceanica*, *Z. marina* and *C. nodosa* within Europe (Lewis & Devereux 2009; Llagostera et al. 2016; Lyngby & Brix 1984). Of the limited studies conducted within Australia, TE laboratory or field manipulated studies of TE concentrations (Cd, Cu, Fe, Pb and Zn) in *Z. muelleri* have previously been assessed within sub-tropical (Prange & Dennison 2000) and temperate areas (Bond et al. 1985; Bond et al. 1988; Carter & Eriksen 1992; Macinnis-Ng & Ralph 2004). These studies found that *Z. muelleri* leaves accumulated Cu and Pb (Bond et al. 1988; Buapet et al. 2019; Carter & Eriksen 1992), and were photosynthetically sensitive to elevated Cu and Zn, and to a lesser extent, photosynthetically sensitive to Cd and Pb (Buapet et al.

2019; Macinnis-Ng & Ralph 2002; Prange & Dennison 2000). In the tropics, the photosynthetic inhibiting effects of herbicides on *Z. muelleri* have been examined (Flores et al. 2013; Negri et al. 2015) but the effects of TE exposure on tropical/sub-tropical *Z. muelleri* have not yet been established.

To further understand the potential of *Z. muelleri* as a TE bioindicator in the sub-tropics, it is important to understand the potential uptake and effects under controlled experimental conditions. Bioaccumulation of TEs differs between seagrass species, and is also dependent on physico-chemical variables such as pH, temperature, salinity, organic matter, element concentration gradient, redox potential and the medium that the element is within (water or sediment) (Lewis & Devereux 2009; Pergent-Martini & Pergent 2000; Wang & Lewis 1997). At their extremes, environmental variables such as salinity are natural stressors to seagrass physiology, growth and metabolism of carbon and the uptake of nutrients (Collier et al. 2014; Touchette 2007). Another reason for choosing salinity as a controlled driver is that the seagrass being studied grows in an estuarine environment, an area of variable salinity. This study aimed to assess whether different salinity treatments influenced Cu accumulation.

Summer salinity regimes along the Queensland coast can range from hypersaline concentrations of >35, to hyposaline concentrations of around 0 after intense rainfall, with varying freshwater plume persistence time ranging from weeks to months (Howley, Devlin & Burford 2018; Jones & Berkelmans 2014). At times of high rainfall, the low salinity floodwater also carries increased total suspended solids, nutrients, pesticides and TEs (Brodie & Pearson 2016). In addition to seasonal salinity variability, natural spatial and temporal dynamic salinity regimes are observed within semi-enclosed estuaries such as Port Curtis (Angel et al. 2010). The response of seagrass to reduced water quality is typically negative, and includes reduced meadow cover due to reduced light penetration and inhibited photosynthetic processes as a result of the exposure to pollutants such as herbicides, pesticides or TEs (Unsworth et al. 2015). In the face of future wide-reaching impacts such as climate change (increased flood events, heat stress and storm events) and local direct anthropogenic pressures (dredging, reclamation and anchoring) the management of water and sediment environmental pollutants could aid in improving seagrass resilience to these impacts (Brodie & Pearson 2016; Fraser & Kendrick 2017; Unsworth et al. 2015).

Few studies have investigated how environmental variables such as salinity influence TE uptake and translocation by seagrass. Bond et al. (1988) observed that excised

*Z. muelleri* leaves had higher Pb concentrations when exposed to a salinity of 0 (distilled water) compared to exposure to ambient seawater salinity (~35), and high salinity (twice that of seawater), but proposed that this uptake was due to ion-exchange processes. Brinkhuis, Penello and Churchill (1980) found that the uptake and movement of elements by *Z. marina* was element-dependent, and showed that Cd freely moved against salinity gradients, whereas Mn was more immobile within compartments. In contrast, and more recently, Nielsen et al. (2017) found, within a controlled experiment, a weak significant effect of salinity on Cu uptake in *Z. marina* with higher accumulation at higher salinities (34) than reduced salinity (5). These contrasting scenarios of Cu uptake under differing salinity concentrations warrants further investigation as times of increased runoff are usually associated with increased TEs. One requirement of a TE bioindicator is that it is tolerant to other stresses such as salinity changes and has the capability to reflect TE water quality irrespective of salinity exposure (Rainbow 2006). This will be addressed within this experiment where there are two salinity scenarios in addition to the element exposure.

Previous research into understanding the fates and effects of seagrass to TE exposure has been explored by a wide variety of methodologies (Lewis & Devereux 2009). The lack of method standardisation is primarily due to the natural breadth of seagrass species biomass and size (e.g. small *Halophila* to large *Posidonia*), which determines the element effect approach being either destructive (Lyngby & Brix 1984) or non-destructive (Ralph & Burchett 1998). Measured variables to assess the influence and effects of TE exposure on seagrasses include uptake and/or desorption kinetics (Malea & Haritonidis 1995a; Prange & Dennison 2000), microtubule effects (Malea, Adamakis & Kevrekidis 2014), leaf necrosis (Llagostera et al. 2016), genetic expression (Buapet et al. 2019; Greco et al. 2019) and translocation between compartments (Lyngby, Brix & Schierup 1982).

While it is important to understand the effects of TEs on seagrass, the study presented here aimed to understand whether seagrass had the potential to accumulate and reflect environmental concentrations, and therefore indicate bioavailable TE concentrations, under different salinity scenarios. The method applied for this study required a destructive approach in order to observe possible accumulation and translocation of the element between seagrass compartments (leaves, rhizome and roots). The majority of previous laboratory studies have focussed on TE uptake from water as either a pulse event (Llagostera et al. 2016) or as a sustained concentration to assess uptake kinetics (Malea et al. 2018). In general, seagrass takes up and accumulates TEs with increasing

concentration over exposure time, followed by a plateau in uptake. The initial rate of uptake is often rapid and disruptive to cells (Malea, Adamakis & Kevrekidis 2014). However, most studies have used water exposure concentrations that are significantly greater ( $\text{mg L}^{-1}$ ) than would usually be found within the environment ( $\mu\text{g L}^{-1}$ ) (Llagostera et al. 2016). Although producing an effect can be important in some circumstances, an exposure concentration close to environmental concentrations allows a realistic assessment of whether negative impacts or uptake are likely within the natural range the seagrass is exposed to (Llagostera et al. 2016), and therefore assesses the potential to use the seagrass as a bioindicator. Therefore, this study aimed to test an element concentration close to what was observed within Port Curtis waters, as well as an elevated concentration that was not excessive but elevated enough to produce an observable effect.

Copper was chosen as the TE for exposure, due to evidence of Cu exceeding 99% trigger value guidelines throughout the local Port Curtis waters (Angel et al. 2010) and previous evidence of *Z. muelleri* exposed to Cu within laboratory experiments (Prange & Dennison 2000). Within Port Curtis, the overall assessment of water TEs meet the determined environmental levels; however, dissolved Cu is sometimes present in higher values (Angel et al. 2010; Gladstone Healthy Harbour Partnership 2017; unpublished data, PCIMP). Copper is an essential element of the plastocyanin protein that is involved in electron transport processes and other metabolic processes, and at elevated concentrations it inhibits PSII activity and induces senescence (Macinnis-Ng & Ralph 2002; Papathanasiou, Orfanidis & Brown 2015). However, different seagrass species behave differently to Cu toxicity. Prange and Dennison (2000) and Llagostera et al. (2016) both found *Cymodocea* spp. to be tolerant to Cu ( $1 \text{ mg L}^{-1}$ ) with no significant PSII response, whereas Cu concentrations of  $1 \text{ mg L}^{-1}$  were found to be toxic to *Z. muelleri* cells by reducing its photosynthetic efficiency, and to cause premature leaf abscission in *H. ovalis* (Prange & Dennison 2000; Ralph & Burchett 1998).

The local consortium partnership of Gladstone organisations and industries, PCIMP, are seeking another locally relevant TE bioindicator that will support the continual improvement of coastal management of TEs. This experiment aimed to gain further understanding of the potential use of a local dominant seagrass species *Z. muelleri* as a bioindicator of local water quality within closed conditions, by determining if *Z. muelleri* reflected the water Cu concentration (three levels) under two levels of salinity. If Cu uptake is influenced by salinity then this will influence the interpretation of the seagrass as a bioindicator for Cu in the tropics and sub-tropics, where salinity can vary following

flood events in the summer and over the estuarine spatial scale. The specific aims of this study were to determine whether the seagrass leaf and below-ground compartments (rhizome and roots) reflected the water Cu concentration and whether these would be different between salinity levels and over time. Final aim was to determine whether the photosynthetic rate changed over time due to the different Cu and salinity exposures.

## 5.2 Methods

### 5.2.1 Experimental approach

Two nominal concentrations of Cu were assessed in the current experiment. One exposure concentration ( $5 \mu\text{g L}^{-1}$ ) reflected the marine and estuarine Cu concentrations measured within Port Curtis waters (dissolved Cu range  $<1\text{--}2.1 \mu\text{g L}^{-1}$ , mean  $1.06 \mu\text{g L}^{-1}$ ; total Cu range  $<1\text{--}11 \mu\text{g L}^{-1}$ , mean  $1.5 \mu\text{g L}^{-1}$ , unpublished data PCIMP). While this proposed value of  $5 \mu\text{g L}^{-1}$  is higher than the observed dissolved Cu concentrations within Port Curtis harbour waters, it is a value that is measurable and is herein referred to as low Cu exposure. The second Cu exposure concentration ( $50 \mu\text{g L}^{-1}$ ) was selected to produce an observable change in seagrass uptake and health without being excessive, and is herein referred to as high Cu exposure. Finally, a control with no Cu addition was included, with collected experimental water Cu concentrations below the LoR of  $1 \mu\text{g L}^{-1}$ .

The two levels of salinity for the experiment were 'normal' and 'reduced' salinity values that are experienced within Port Curtis, Gladstone. Salinity will be referred to from herein as specific conductivity as it is a measurable parameter. The specific conductivity values were selected from past Port Curtis water quality data recorded adjacent to a seagrass meadow (Gladstone Ports Corporation, unpublished data, 2017). The average 'normal' specific conductivity throughout Port Curtis is typically  $\sim 54 \text{ mS cm}^{-1}$  (salinity equivalent  $\sim 35$ ). After a flood event, specific conductivity within Port Curtis reduces to concentrations as low as  $20 \text{ mS cm}^{-1}$ , or even lower temporarily, dependent on rainfall, but may remain at concentrations of  $\sim 40 \text{ mS cm}^{-1}$  (salinity equivalent  $\sim 25$ ) for a period of a few weeks (Gladstone Ports Corporation, unpublished data, 2017). Normal specific conductivity was defined as the ambient specific conductivity of collected water,  $\sim 54 \text{ mS cm}^{-1}$ , and reduced specific conductivity refers to a specific conductivity of  $\sim 44 \text{ mS cm}^{-1}$ .

### 5.2.2 Seagrass collection

*Zostera muelleri* cores were obtained from an intertidal meadow at Pelican Banks (24° 45.974'S, 151° 18.873'E) in March 2018. Samples were collected under a Notification form for Accepted Development from Fisheries Queensland, Department of Agriculture and Fisheries. Whole seagrass and rhizome sediment cores were collected using 15 cm diameter PVC corers to a depth of 10 cm and placed within paper lined pots. Cores were collected from across an area of 50 m<sup>2</sup>. Seagrass meadow edge, low water mark and tidal pools were avoided as these areas showed greater epiphytic cover that may influence uptake. No flowering was observed at time of collection. Cores were transported back to the CQUniversity Gladstone Marina Campus laboratory within 2 h of collection. Upon arrival, pots were washed with filtered seawater, and the seagrass leaves and sediment surface were gently wiped to remove any excess epiphytes and algae, and randomly placed within glass tanks. To determine whether Cu concentrations changed between field collection and the start of the experiment an extra nine cores (three cores pooled for three samples) were taken from close to where the experimental cores were collected, and these are referred to as field samples. These samples were rinsed of sediment through a plastic sieve with ambient seawater and kept chilled until return to the laboratory where they were kept frozen until processing.

Seagrass cores were not re-potted to remove biota and to homogenise the sediment to avoid potential damage to seagrass roots. To address the issue of not repotting the seagrass and other potential sources or sinks of Cu, the analyses of the below-ground compartment (rhizome and roots) and sediment were carried out. The additional biota not removed from the seagrass cores also have the potential to accumulate the dosed Cu. The advantage of using whole seagrass cores is that they represent field conditions and include biota, such as worms and bivalves, and the sediment biogeochemical processes would be closer to natural conditions than for homogenised sediment that has not reached equilibration biogeochemically.

### 5.2.3 Experimental design

Experimental glass tanks (30 cm W x 38 cm H x 60 cm L) were washed with 10% nitric acid and rinsed with reverse osmosis water. Tanks were housed within an air-conditioned temperature-controlled room and each tank individually aerated gently. Treatments were randomly distributed across tanks throughout the temperature-controlled room to account for potential differences in light and temperature. The light source was fluorescent tubes and light intensity was measured using three Odyssey light

integrating loggers for the duration of the experiment. Variation in light irradiance throughout the tanks due to the tank and pot position ranged between ~ 89.5–129  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  over a 12:12 h light:dark cycle. Individual tanks contained 25 L of 0.5  $\mu\text{m}$  filtered seawater obtained from Rodds Bay, a site of lower recorded concentrations of TEs in water samples (24° 3.060'S, 151° 37.331'E). Milli-Q water was added to the filtered seawater to produce the 'reduced' specific conductivity treatment. Halfway through the experiment, water specific conductivity was checked as evaporation was not controlled and 1.5 L of Milli-Q water was added to all of the tanks to reduce specific conductivity to original concentrations. The experiment ran for 12 days with Cu addition at time point T0 and the completion of the experiment at time point T11 (Table 5.1). The seagrass was kept in experimental aquaria for two days to acclimatise to water and temperature conditions before Cu addition (Table 5.1).

**Table 5.1. A timeline of when sampling occurred, the number of samples taken (n) and pulse amplitude modulator (PAM) readings taken during the experiment. T = time point.**

Time point	Day	Above	Below	Dissolved water	Sediment	PAM	Notes
-T2							Seagrass collected and placed in tanks
Baseline (T0)	1	3	3	18	3	18	Cu addition after baseline and PAM analysis.
T1	2	18	18		18	18	
T3	4					18	
T5	6					18	
T6	7						Milli-Q water added
T7	8					18	
T9	10					18	
T10	11			18		18	
T11	12	18	18		18		

Copper (control, low 5  $\mu\text{g L}^{-1}$  and high 50  $\mu\text{g L}^{-1}$ ) and specific conductivity (normal and reduced) treatments were fully crossed with three replicate tanks of each treatment. Each tank initially started with six seagrass cores, with three to be used for initial and three for final Cu assessment. It was necessary to use three pots for each assessment due to the low biomass of leaf material and to meet the requirements of minimum weight for TE digestion. An additional nine pots for baseline samples (three pots pooled for one sample,  $n = 3$ ) were spread at random throughout the tanks and were analysed before Cu addition. Copper chloride ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) was added at the calculated pre-determined concentrations of 5  $\mu\text{g L}^{-1}$  and 50  $\mu\text{g L}^{-1}$  and after a brief period of time ( $<0.5$  h) initial (T0) filtered (0.45  $\mu\text{m}$ ) water samples were taken and acidified to  $\text{pH} < 2$  with analytical grade 70% nitric acid. Final water samples, pulse-amplitude modulation (PAM) fluorescence and specific conductivity readings were taken at time point T10, the day before seagrass and sediment analyses (time point T11) due to processing time constraints (Table 5.1). Seagrass compartments and sediment were analysed for Cu at baseline (T0,  $n = 3$ ), within 24 h after Cu addition (T1,  $n = 18$ ), and at the completion of the experiment (T11,  $n = 18$ ) (Table 5.1).

At each time point where seagrass was analysed (T0, T1 and T11), each sample was treated as follows. Three pots were randomly selected from each tank and were pooled to form one sample. Leaf material was collected from all three pots by gloved hands and plastic spatula and rinsed with Milli-Q water, blotted dry and stored frozen in a plastic container. The three cores were then split into quarters, and one quarter from each pot was removed for sediment analysis (root and rhizome removed later) and pooled with the remaining three quarters for analysis of roots and rhizomes as the below-ground compartment. Roots and rhizomes from the three pots were then sieved from the remaining three quarters of the core through a plastic sieve with reverse osmosis water and pooled together. All samples were placed in clean plastic bags and stored at  $-20^\circ\text{C}$  until further processing within one month. Roots and rhizomes were later sorted to remove excess shell and non-seagrass biotic material, rinsed with Milli-Q water and re-frozen, then freeze-dried and hand agitated within the plastic bag to form small particles. Leaf material was freeze-dried and weighed to ensure that enough material was available for TE digestion ( $> 0.1$  g dry weight) and hand agitated within the plastic bag to form small particles for digestion. Sediment was wet sieved through a 2 mm sieve to remove root-rhizomes and other biotic material and then oven dried at  $60^\circ\text{C}$  for 24 h before being ground using a mortar and pestle.

Maximum quantum yield ( $F_v/F_m$ ) was measured prior to Cu addition (T0) and then on days T1, T3, T5, T7, T9 and T10 with a diving PAM fluorometer (diving-PAM, Walz, Germany). After 30 min of dark adaptation, five measurements were taken in each aquarium from mid-way along adult leaves. Maximum quantum yield was calculated using Equation 1, where  $F_m$  is the maximum fluorescence and  $F_o$  is the initial fluorescence in dark adapted samples.

**Equation 1.**  $F_v/F_m = (F_m - F_o) / F_m$

The total amount (mg) of Cu in each compartment (leaf, below-ground, sediment and dissolved water) was estimated by multiplying the concentration of Cu in the compartment by its mass. A comparison of the Cu weight by compartment was then made between the baseline and control at T0 to the average of T1 and T11 for both low and high Cu exposures. This calculation provides information of Cu concentration in regards to the biomass of the compartment; for example, the below-ground (root-rhizome) compartment had greater biomass than the leaf compartment and therefore could potentially have had more or less Cu when compared to other compartments.

Values reported for initial control concentrations within leaf, root-rhizome and sediment were the averages at T1 for six pots. Dissolved Cu concentrations reported for the initial low and high Cu exposures were the averages of the Cu at the time of addition. These starting values were then compared to the average of T1 and T11 for the leaf, root-rhizome and sediment compartments for three pots and the final T10 dissolved Cu concentration. The average of the T1 and T11 compartments was calculated to account for the variation of Cu concentration within the leaf and below-ground material over time. The resulting values describe the amount of Cu observed within a tank for each compartment when adjusted for biomass and volume, for the purpose of observing whether all of the dissolved Cu was accumulated.

#### **5.2.4 Trace element determination**

Samples were analysed by the NATA-accredited Australian Government NMI laboratory, Sydney, by their in-house methods of NT2.47 (water), NT2.49 (sediment) and NT2.46 (seagrass). Digestion of sediment and seagrass was with high purity nitric and hydrochloric acids by heating on a hot block at 95–100°C for 2 h. Trace element concentrations were determined by ICP-MS (Agilent 7900) and seagrass (leaf and

below-ground) and sediment Cu concentrations were reported on a dry weight basis. Filtered (0.45 µm) water was tested for a suite of elements (Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Ni and Zn) to assess if any other elements may have confounded Cu uptake. Upon seeing the initial and final TE water results, further exploration of Fe and Mn was required of the seagrass and sediment compartments and the subsequent data was requested from NMI. The quality control and assurance of analytical methods was checked using sample duplicates and laboratory control samples, with recoveries within acceptable limits for sediment Cu (90–116% recovery, Appendix A, Table A1), seagrass Cu (96–99% recovery Appendix A, Table A2), and all filtered water elements (97–115% recovery, Appendix D Table D1). Supplied water quality control checks of duplicates, blanks and nominal concentrations were within 10% of nominal values. Where TE concentrations were less than the limit of reporting, the limit of reporting values were used for reporting and calculations.

### **5.2.5 Data analysis**

Field and baseline (T0) seagrass samples were collected for comparative purposes and were not included in statistical analyses. A three-way ANOVA was used to determine if the dependent variables of Cu concentration within leaf, root-rhizome material and sediment were significantly different between three factors: time (fixed, two levels: T1 and T11), copper exposure treatment (fixed, orthogonal three levels: control, low 5 µg L<sup>-1</sup> and high 50 µg L<sup>-1</sup>) and specific conductivity (fixed, orthogonal, two levels: normal and reduced). The independent variables were tested for meeting the requirements of homogeneity of variance and normality, and as a result leaf Cu concentrations were natural log transformed. The difference in dissolved Cu concentrations in the water for each specific conductivity and time group was tested using a one-way ANOVA (four levels: T0 Normal, T0 Reduced, T10 Normal and T10 Reduced). Significant differences were explored with a post-hoc comparison of means test (Tukey HSD test). Concentrations of Fe in water could not be transformed to meet assumptions but an ANOVA was still carried out with  $\alpha$  set to 0.01 to compensate for the increased likelihood of Type II error (Underwood 1997). To determine differences of dissolved element results, significant results of time simple effect were determined by independent T tests for each level of Cu treatment.

Maximum quantum yield data was tested by a one-way repeated measures ANOVA with time as the repeated measure (seven levels) and a treatment factor (six levels: control normal, control reduced, low normal, low reduced, high normal, high reduced).

Sphericity was adjusted where assumption was not met and contrasts between time levels were assessed. Statistical analyses were performed using SPSS v. 24 (IBM corp., Armonk, NY).

## 5.3 Results

### 5.3.1 Aquaria conditions

Specific conductivity concentrations were measured at  $54.9 \pm 0.49 \text{ mS cm}^{-1}$  (normal treatment) and  $44.87 \pm 0.6 \text{ mS cm}^{-1}$  (reduced treatment) and readings varied less than 5% during experimental period (Table 5.2). Mean pH ranged from 7.74 to 8.06 with higher pH at the completion of the experiment (Table 5.2). Water temperature over the experimental period and between tanks was  $25.38 \pm 0.38^{\circ}\text{C}$ .

**Table 5.2. Summary values (mean  $\pm$  SD, n = 3) of specific conductivity (sp. cond.) and pH at the beginning (T0) and end of the experiment (T10) by Cu and specific conductivity treatment.**

Cu	Sp. Cond.	Initial (T0)		Final (T10)	
		Sp. Cond. $\text{mS cm}^{-1}$	pH	Sp. Cond. $\text{mS cm}^{-1}$	pH
Control	Normal	53.71 (0.46)	7.74 (0.05)	56.00 (0.44)	7.86 (0.10)
Low (5)	Normal	54.00 (0.04)	7.87 (0.04)	55.88 (0.16)	8.03 (0.04)
High (50)	Normal	54.06 (0.10)	7.88 (0.04)	55.87 (0.47)	8.00 (0.06)
Control	Reduced	44.04 (0.08)	7.92 (0.03)	45.89 (0.29)	8.06 (0.02)
Low (5)	Reduced	43.82 (0.23)	7.93 (0.05)	45.60 (0.56)	8.05 (0.08)
High (50)	Reduced	43.97 (0.29)	7.93 (0.02)	45.95 (0.21)	8.04 (0.04)

Dissolved Cu concentrations for control tanks were below or close to the LoR of  $1 \mu\text{g L}^{-1}$  (Table 5.3). Initial (T0) dissolved Cu concentrations for low Cu exposure (range  $4.3\text{--}5 \mu\text{g L}^{-1}$ ) was 88–94% of nominal concentration ( $5 \mu\text{g L}^{-1}$ ) and high Cu exposure (range  $41\text{--}53 \mu\text{g L}^{-1}$ ) was within 95% of nominal concentration ( $50 \mu\text{g L}^{-1}$ , Table 5.3). Additional measured water elements were either below the limit of reporting (Al  $<5 \mu\text{g L}^{-1}$ , Cd  $<0.1 \mu\text{g L}^{-1}$ , Pb  $<1 \mu\text{g L}^{-1}$  and Ni  $<1 \mu\text{g L}^{-1}$ ) or close to the limit of reporting (Cr  $1 \mu\text{g L}^{-1}$  and Zn  $1 \mu\text{g L}^{-1}$ ) and are unlikely to have influenced Cu uptake at these concentrations (Table 5.3). Mean As concentrations by treatment ranged from  $2.73\text{--}3.60 \mu\text{g L}^{-1}$  (Table 5.3). There was no significant interaction between time, Cu exposure or specific conductivity

for dissolved As concentrations. However, for As dissolved concentrations there was a significant specific conductivity effect ( $F_{1,24} = 9.26$ ,  $p < 0.01$ , Table 5.4) and a time effect ( $F_{1,24} = 25.7$ ,  $p < 0.001$ , Table 5.4, Appendix D Table D2). All of the initial (T0) reduced specific conductivity treatments (nine tanks) had lower As concentrations than the normal specific conductivity treatments (nine tanks) and lower As concentrations than all of the final (T10) specific conductivity treatments (Table 3, Appendix D Table D3).

Dissolved Fe concentrations significantly increased ( $F_{1,24} = 56.6$ ,  $p < 0.001$ , Table 5.3, Appendix D Table D4) over time, with concentrations  $< 8 \mu\text{g L}^{-1}$  at T0 and increasing to a mean of  $28 \mu\text{g L}^{-1}$  at T10. Mean dissolved Mn concentrations decreased significantly ( $F_{1,24} = 51.0$ ,  $p < 0.001$ , Table 5.4, Appendix D Table D5) over time from  $14.0\text{--}23.7 \mu\text{g L}^{-1}$  at T0 to  $5.9\text{--}10.9 \mu\text{g L}^{-1}$  at T10 (Table 5.3), irrespective of Cu or specific conductivity treatment.

**Table 5.3. Nominal and measured dissolved trace element concentrations (mean  $\pm$  SD, n = 3) across Cu (Control, Low 5  $\mu\text{g L}^{-1}$ , High 50  $\mu\text{g L}^{-1}$ ) and Specific Conductivity (N = normal, R = reduced) treatments at the beginning (T0) and end of the experiment (T11). Similar letters represent no significant differences between time and specific conductivity treatments.**

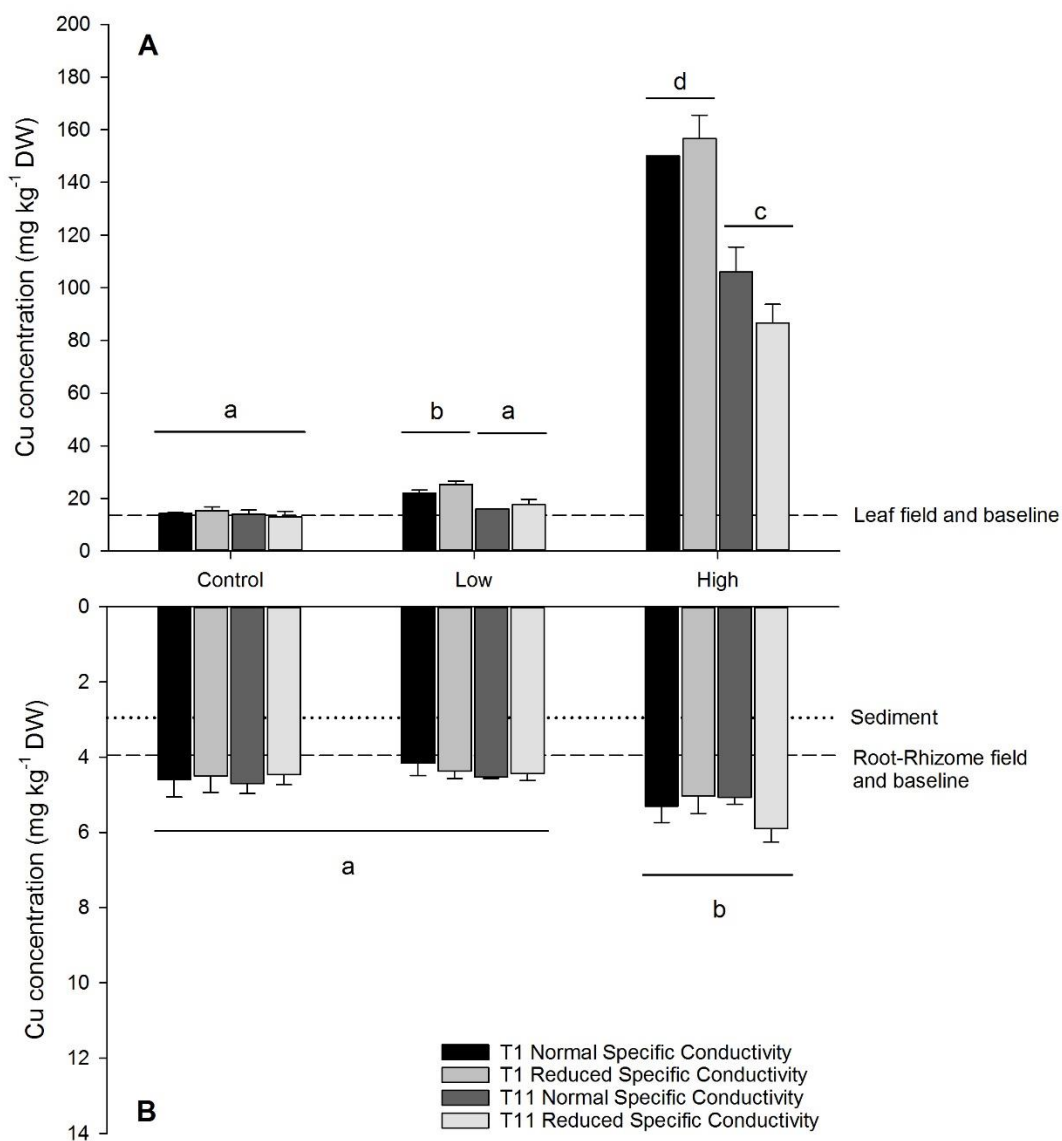
		Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
		$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$	$\mu\text{g L}^{-1}$
<b>Initial (T0)</b>											
Control	N	<5.0 (0.0)	3.17 (0.23) <sup>a</sup>	<0.1 (0.0)	<1.0 (0.0)	1.10 (0.17)	7.43 (3.15) <sup>a</sup>	18.3 (1.53) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	2.30 (2.25)
Control	R	<5.0 (0.0)	2.80 (0.26) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	<1.0 (0.00)	<5.0 (0.00) <sup>a</sup>	17.7 (6.43) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	2.23 (1.72)
Low	N	<5.0 (0.0)	3.03 (0.32) <sup>a</sup>	<0.1 (0.0)	<1.0 (0.0)	4.40 (0.17)	8.00 (5.20) <sup>a</sup>	23.7 (5.86) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Low	R	<5.0 (0.0)	2.67 (0.06) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	4.70 (0.30)	<5.0 (0.00) <sup>a</sup>	14.0 (3.00) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	1.33 (0.58)
High	N	<5.0 (0.0)	3.37 (0.21) <sup>a</sup>	<0.1 (0.0)	<1.0 (0.0)	47.3 (6.03)	<5.0 (0.00) <sup>a</sup>	18.7 (9.61) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
High	R	<5.0 (0.0)	2.73 (0.06) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	47.7 (2.31)	5.43 (0.75) <sup>a</sup>	16.0 (3.46) <sup>a</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
<b>Final (T10)</b>											
Control	N	<5.0 (0.0)	3.50 (0.17) <sup>b</sup>	<0.1 (0.0)	1.03 (0.06)	<1.0 (0.0)	53.0 (16.1) <sup>b</sup>	10.9 (2.10) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Control	R	<5.0 (0.0)	3.37 (0.31) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	<1.0 (0.0)	13.3 (8.12) <sup>b</sup>	6.1 (3.65) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	1.17 (0.29)
Low	N	<5.0 (0.0)	3.27 (0.61) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	<1.0 (0.0)	27.0 (11.8) <sup>b</sup>	5.9 (1.40) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Low	R	<5.0 (0.0)	3.17 (0.15) <sup>b</sup>	<0.1 (0.0)	<1.0 (0.0)	<1.0 (0.0)	20.3 (12.4) <sup>b</sup>	8.7 (2.12) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
High	N	<5.0 (0.0)	3.70 (0.10) <sup>b</sup>	<0.1 (0.0)	1.10 (0.17)	2.07 (0.25)	22.7 (15.3) <sup>b</sup>	9.3 (3.20) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
High	R	<5.0 (0.0)	3.60 (0.36) <sup>b</sup>	<0.1 (0.0)	1.10 (0.17)	1.30 (0.44)	32.7 (7.02) <sup>b</sup>	4.7 (2.27) <sup>b</sup>	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)

**Table 5.4. Results of three-way ANOVA of Cu, time and specific conductivity (Sp. Cond.) treatment for selected dissolved trace elements. Significant effects indicated in bold where  $p < 0.05$ . Full F table within Appendix D Tables D2, D4 and D5.**

	df	As		Fe		Mn	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Cu	2	3.76	0.035	0.86	0.437	0.21	0.810
Time	1	25.7	<b>0.000</b>	56.6	<b>0.000</b>	51.02	<b>0.000</b>
Sp. Cond.	1	9.26	<b>0.006</b>	5.46	<b>0.028</b>	5.03	<b>0.035</b>
Cu * Time	2	0.54	0.591	0.92	0.414	0.16	0.851
Cu * Sp. Cond.	2	0.20	0.818	6.74	<b>0.005</b>	0.04	0.966
Time * Sp. Cond.	1	3.42	0.077	3.14	0.089	0.52	0.477
Cu * Time * Sp. Cond.	2	0.26	0.773	5.58	<b>0.010</b>	3.20	0.059
Error	24						

### 5.3.2 Copper concentrations

Leaf Cu concentrations for field and baseline samples were  $14 \pm 0.0 \text{ mg kg}^{-1}$  and  $13 \pm 0.0 \text{ mg kg}^{-1}$ , respectively. There was no significant interaction between time, Cu exposure and specific conductivity treatment ( $F_{2,24} = 0.308$ ,  $p = 0.74$ , Table 5.5). However, there was a significant interaction for leaf Cu concentrations between Cu exposure and time ( $F_{1,24} = 5.08$ ,  $p < 0.05$ , Table 5); initial mean leaf Cu concentration from the high ( $50 \mu\text{g L}^{-1}$ ) Cu exposure was significantly higher ( $153.3 \pm 10.3 \text{ mg kg}^{-1}$ ) than the final Cu concentration ( $96.3 \pm 16.7 \text{ mg kg}^{-1}$ ). In addition, the initial mean leaf Cu concentration from the low ( $5 \mu\text{g L}^{-1}$ ) Cu exposure was significantly higher ( $23.7 \pm 2.58 \text{ mg kg}^{-1}$ ) than the final Cu concentration ( $16.8 \pm 2.40 \text{ mg kg}^{-1}$ , Fig. 5.1). Mean control leaf Cu concentration ( $14.17 \pm 0.96 \text{ mg kg}^{-1}$ ) was similar to Cu concentrations in field and baseline samples ( $13\text{--}14 \text{ mg kg}^{-1}$ ) and throughout the experiment concentrations remained within the range of  $10\text{--}18 \text{ mg kg}^{-1}$  (Fig. 5.1). The mean dry weight of leaf material increased from  $0.26 \pm 0.02 \text{ g}$  at T1 to  $0.34 \pm 0.34 \text{ g}$  at T11.

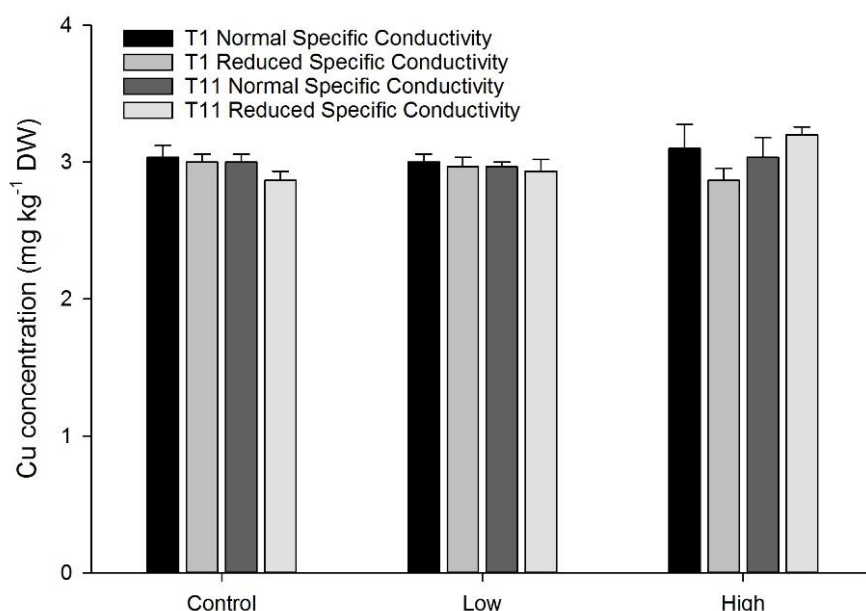


**Figure 5.1. Copper concentration (mean  $\pm$  SE, n = 3, dry weight) within seagrass leaf (A) and below-ground (B) compartments for each treatment (Cu: Control, Low 5  $\mu\text{g L}^{-1}$ , High 50  $\mu\text{g L}^{-1}$ ; Specific conductivity: normal and reduced) and time point (T1 initial, T11 final). Note vertical axis scales are different. Reference lines are means of the field and baseline samples for the leaf and root-rhizome compartments and sediment mean of baseline and control samples. Similar letters indicate no significant difference.**

**Table 5.5. Results of a three-way ANOVA of Cu and specific conductivity (Sp. Cond.) treatment and time for Cu concentrations by compartment. Significant effects indicated in bold where  $p < 0.05$ . Full F table within Appendix D Table D6.**

	df	Leaf		Root-Rhizome		Sediment	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Cu	2	782.8	<b>0.000</b>	9.36	<b>0.001</b>	1.03	0.371
Time	1	41.88	<b>0.000</b>	0.99	0.329	0.01	0.916
Sp. Cond.	1	0.02	0.892	0.09	0.772	0.92	0.347
Cu * Time	2	5.08	<b>0.014</b>	0.19	0.827	1.58	0.227
Cu * Sp. Cond.	2	1.41	0.263	0.47	0.631	0.10	0.903
Time * Sp. Cond.	1	2.39	0.136	0.34	0.563	0.92	0.347
Cu * Time * Sp. Cond.	2	0.31	0.738	1.36	0.277	2.15	0.139
Error	24						

Mean below-ground (roots and rhizomes) Cu concentrations for field and baseline samples were  $3.4 \pm 0.9 \text{ mg kg}^{-1}$  and  $4.46 \pm 0.59 \text{ mg kg}^{-1}$ , respectively. Copper concentrations within the below-ground compartments during the experiment ranged from 3.7 to 6.6  $\text{mg kg}^{-1}$  (Fig. 5.1). There was no significant interaction of time, Cu exposure and specific conductivity treatments for below-ground Cu concentrations ( $F_{2,24} = 1.36$ ,  $p = 0.28$ , Table 5). However, there was a significant difference between Cu exposure treatment ( $F_{2,24} = 9.36$ ,  $p < 0.001$ , Table 5.5), with mean high Cu exposure below-ground compartments being ( $5.3 \pm 0.67 \text{ mg kg}^{-1}$ ) higher than mean control and low Cu exposure concentrations ( $4.47 \pm 0.46 \text{ mg kg}^{-1}$ , Fig. 5.1). Total recoverable sediment Cu concentrations were not significantly different between time, Cu exposure or specific conductivity treatment or any other single effects of time, Cu exposure or specific conductivity ( $F_{2,24} = 2.148$ ,  $p = 0.139$ , Table 5.5). Mean sediment Cu concentration including baseline samples was  $2.98 \pm 0.15 \text{ mg kg}^{-1}$  (Fig. 5.2).



**Figure 5.2. Sediment Cu concentrations (mean  $\pm$  SE,  $n = 3$ , dry weight) for each treatment (Cu: Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ; Specific conductivity: normal and reduced) and time point (T1 initial, T11 final).**

### 5.3.3 Copper biomass correction

Concentrations of dissolved Cu in water by weight decreased from  $0.114 \text{ mg}$  to  $0.025 \text{ mg}$  for low Cu exposure and  $1.188 \text{ mg}$  to  $0.042 \text{ mg}$  for high Cu exposure (Table 5.6). In the low Cu exposure, the concentration of Cu in the below-ground compartment ( $0.021 \text{ mg}$ ) was greater than the leaf Cu compartment ( $0.012 \text{ mg}$ , Table 5.6) by the end of the experiment. In contrast, in the high Cu exposure, the concentration of Cu in the leaf compartment ( $0.07 \text{ mg}$ ) was higher than in the below-ground compartment ( $0.025 \text{ mg}$ , Table 5.7). In both exposures, the values of dissolved Cu concentrations observed at the end of the experiment do not equate to the sum of final values for each compartment (excluding sediment), and this suggests that the seagrass did not accumulate all of the Cu from the dissolved water fraction.

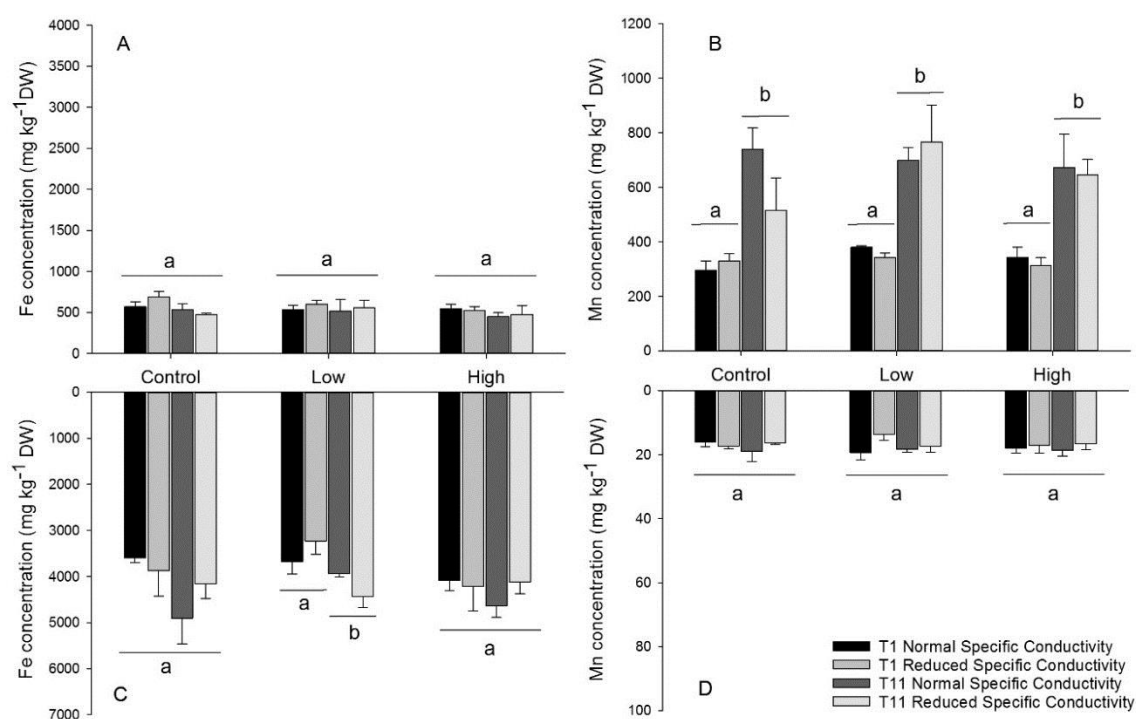
**Table 5.6. Calculated Cu distribution between compartments within a single tank at time of Cu addition (T0), and the mean of 24 h (T1) and at the completion of the experiment (T11) for both Low (5 µg L<sup>-1</sup>) and High (50 µg L<sup>-1</sup>) Cu exposure treatments. Initial amount of Cu addition to water (starting concentration) indicated by italics. Note values given at each time point are for different weights of seagrass.**

Time	T0	T1, T11 Avg.	T0	T1, T11 Avg.
Treatment	Low	Low	High	High
	(6 pots)	(3 pots)	(6 pots)	(3 pots)
Compartment	mg	mg	mg	mg
Water	<i>0.114</i>	0.025	<i>1.188</i>	0.042
Leaf	0.016	0.012	0.016	0.070
Root-Rhizome	0.039	0.021	0.039	0.025
Sediment	53.46	26.69	53.46	27.48
Sum of Cu (excluding sediment)		0.058		0.137

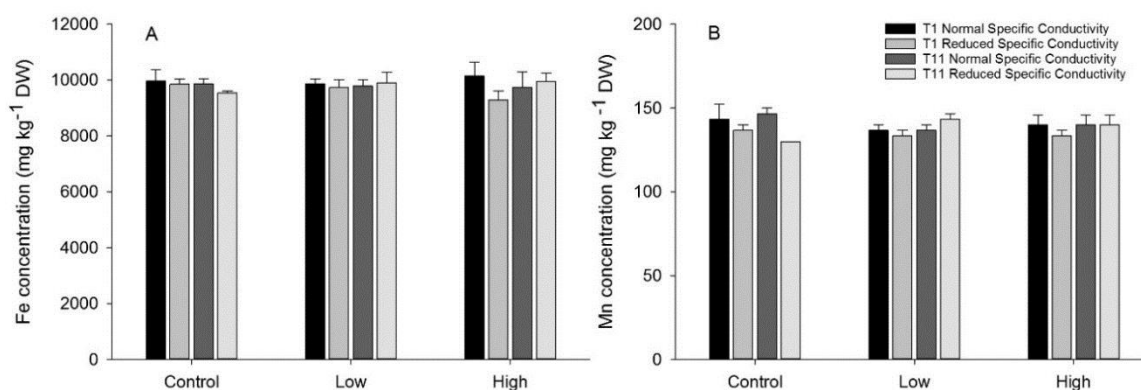
### 5.3.4 Iron and manganese concentrations

Concentrations of Fe in the seagrass leaves ranged from 310 to 790 mg kg<sup>-1</sup> over the duration of the experiment. There was no significant interaction between time, Cu exposure or specific conductivity treatment ( $F_{2,24} = 0.617$ ,  $p = 0.55$ , Table 8, Fig. 5.3a) or any other single effect (time, Cu exposure or specific conductivity). Concentrations of Fe in the below-ground seagrass compartment (roots and rhizomes) were eight times greater than the leaf compartment and ranged from 2840 to 5970 mg kg<sup>-1</sup>. For below-ground Fe concentrations, there was no significant interaction between time, Cu exposure or specific conductivity treatment ( $F_{2,24} = 2.34$ ,  $p = 0.12$ , Table 8, Fig. 5.3c). However, there was a significant effect of time ( $F_{1,24} = 8.75$ ,  $p < 0.01$ , Table 5.8) on below-ground Fe concentrations with a significant increase from 3455 mg kg<sup>-1</sup> at T0 to 4185 mg kg<sup>-1</sup> at T11 ( $t(10) = -2.89$ ,  $p = 0.016$ ) for the low Cu exposure treatment (Fig. 5.3c). Mean leaf Mn concentrations, irrespective of specific conductivity or Cu treatments, were significantly different between T1 and T11 (increasing from 334 mg kg<sup>-1</sup>  $\pm$  47.5 at T1 to 674 mg kg<sup>-1</sup>  $\pm$  166.4 at T11,  $F_{1,24} = 65.61$ ,  $p < 0.001$ , Fig. 5.3b). Below-ground Mn concentrations were 25 times lower than leaf material concentrations and

were constant throughout the experiment with an overall mean of  $17.3 \text{ mg kg}^{-1} \pm 3.07$  ( $F_{2,24} = 1.4$ ,  $p = 0.27$ , Fig 5.3d). Throughout the experiment sediment Fe concentrations ranged from 8650 to 11100  $\text{mg kg}^{-1}$  (Fig. 5.4a) and Mn sediment concentrations ranged from 130 to 160  $\text{mg kg}^{-1}$  (Fig. 5.4b). There was no significant difference of time, Cu exposure or specific conductivity for Fe and Mn sediment concentrations (Fe  $F_{2,24} = 1.35$ ,  $p = 0.28$ ; Mn  $F_{2,24} = 0.97$ ,  $p = 0.39$ , Table 5.8).



**Figure 5.3. Fe (left) and Mn (right) seagrass concentration (mean  $\pm$  SE,  $n = 3$ , dry weight) within leaf (A, B) and below-ground (C, D) compartments for each treatment (Cu: Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ; Specific conductivity: normal and reduced) and time point (T1 initial, T11 final). Note vertical axis scales are different. Similar letters indicate no significant difference.**



**Figure 5.4. Fe (A) and Mn (B) sediment concentrations (mean  $\pm$  SE,  $n = 3$ , dry weight) for each treatment: (Cu: Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ; Specific conductivity: normal and reduced) and time point (T1 initial, T11 final). Note vertical axis scales are different.**

**Table 5.7. Results of three-way ANOVA of Fe and Mn by Cu, time and specific conductivity (Sp. Cond.) treatment for each compartment. Significant effects indicated in bold where  $p < 0.05$ . Full F table within Appendix D Table D4, Table D5.**

Fe	Leaf			Root-Rhizome		Sediment	
	df	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Cu	2	0.97	0.394	1.74	0.197	0.02	0.982
Time	1	3.16	0.088	8.75	<b>0.007</b>	0.003	0.956
Sp. Cond.	1	0.43	0.520	0.46	0.506	0.87	0.361
Cu * Time	2	0.38	0.689	0.82	0.452	0.27	0.765
Cu * Sp. Cond.	2	0.12	0.891	0.17	0.847	0.23	0.793
Time * Sp. Cond.	1	0.39	0.536	0.40	0.535	0.93	0.344
Cu * Time * Sp. Cond.	2	0.62	0.548	2.34	0.118	0.97	0.392
Error	24						

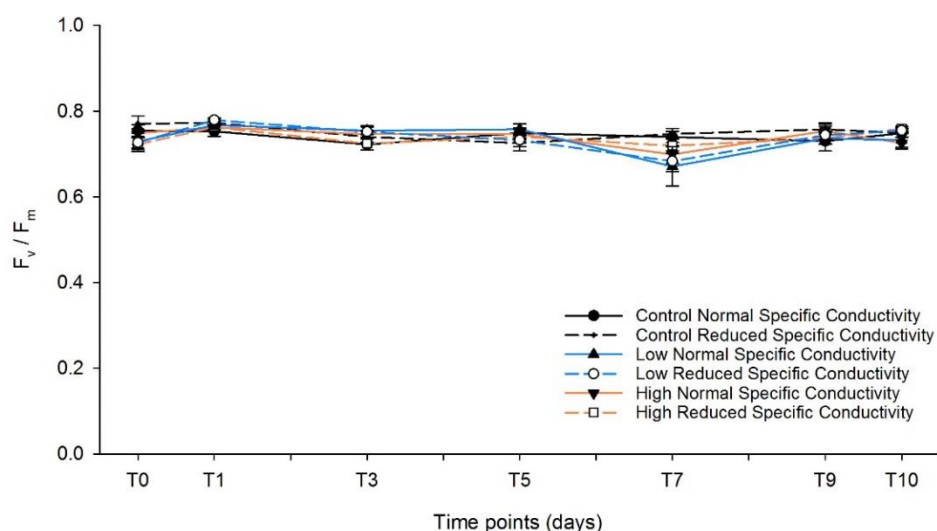
  

Mn	Leaf			Root-Rhizome		Sediment	
	df	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Cu	2	1.17	0.327	0.07	0.936	0.13	0.878
Time	1	65.6	<b>0.000</b>	0.60	0.446	0.70	0.412
Sp. Cond.	1	0.74	0.397	2.91	0.101	2.78	0.108
Cu * Time	2	0.16	0.852	0.10	0.901	0.57	0.576
Cu * Sp. Cond.	2	0.58	0.566	0.54	0.591	2.13	0.141
Time * Sp. Cond.	1	0.36	0.556	0.003	0.959	0.17	0.680
Cu * Time * Sp. Cond.	2	1.64	0.215	1.40	0.266	1.35	0.279
Error	24						

### 5.3.5 Photosynthetic effect

Maximum Quantum Yield (MQY) of seagrass did not indicate photosynthetic inhibition due to Cu exposure or specific conductivity treatment (Fig. 5.5). Mauchly's Test of Sphericity showed that the assumption of sphericity had been violated (i.e., the variances of the differences were not equal), and therefore a Greenhouse-Geisser correction ( $\epsilon = 0.46$ ) was used (Appendix D Table D7). The repeated measures ANOVA found no significant interaction between subject effects for Cu and specific conductivity treatments and time ( $F_{13.9, 33.3} = 1.17$ ,  $p = 0.344$ , Appendix D Table D8). However, there was a significant effect of time on MQY ( $F_{2.77, 33.26} = 6.47$ ,  $p < 0.01$ , Appendix D Table D8). The within subject effect of time revealed significant differences between T0 and T1 ( $F_{1,12} =$

18.46,  $p < 0.001$ ), T1 and T3 ( $F_{1,12} = 13.84$ ,  $p < 0.01$ ), T5 and T7 ( $F_{1,12} = 6.09$ ,  $p < 0.05$ ) and T7 and T9 ( $F_{1,12} = 5.15$ ,  $p < 0.05$ , Appendix D Table D9). This is seen in Figure 5.5 where T1 was higher and less variable, and T7 was lower, than readings taken either side of that day, and greatly variable across treatments. There was no significant difference of the between-subject effect for the treatment factor of Cu and specific conductivity treatments ( $F_{5,12} = 0.752$ ,  $p = 0.6$ , Appendix D Table D10).



**Figure 5.5. Maximum quantum yield  $F_v/F_m$  (mean  $\pm$  SE,  $n = 15$ ) from before Cu addition (T0) to prior to project completion (T10) for each crossed treatment: (Cu: Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ; Specific conductivity: normal and reduced).**

## 5.4 Discussion

*Zostera muelleri* demonstrated Cu accumulation that was strongly dependent on water Cu concentration and compartments tested. Accumulation was greater in the leaves than in below-ground compartments, with seagrass in high Cu exposure ( $50 \mu\text{g L}^{-1}$ ) treatments showing the greatest mean Cu accumulation of  $153 \text{ mg kg}^{-1}$ , irrespective of specific conductivity. Prange and Dennison (2000) and Macinnis-Ng and Ralph (2004) also observed *Z. muelleri* to be a strong accumulator within the leaves followed by roots when exposed to high Cu in the water. The rate of accumulation observed within this study was rapid; within 24 h seagrass leaves in both low and high Cu exposures had significantly higher concentrations of Cu than control seagrass leaves. This rapid uptake of Cu is similar to that identified by Macinnis-Ng and Ralph (2004), where Cu uptake by *Z. muelleri* leaves was 200–1120% higher after 10 h exposure to nominal  $1 \text{ mg L}^{-1}$  Cu

(actual Cu 0.4–0.5 mg L<sup>-1</sup>) than in controls. The rapid accumulation of Cu by *Z. muelleri* identified by Macinnis-Ng and Ralph (2004) occurred at much higher water concentrations of Cu in comparison to the present study, which has shown that *Z. muelleri* is also able to accumulate Cu at lower Cu concentrations of 4.5 µg L<sup>-1</sup> and 50 µg L<sup>-1</sup>. The ability of *Z. muelleri* to accumulate Cu at lower water concentrations suggests that it meets the criteria of a bioindicator in that it can accumulate the element irrespective of the environmental concentration. There was no effect of normal or reduced specific conductivity on Cu accumulation in this study. *Zostera muelleri* is naturally tolerant to a wide range of specific conductivities (Collier et al. 2014). The two specific conductivities tested were within the natural range of the species tolerance, which may explain why element uptake was not affected by specific conductivity. Therefore, at times or at locations with variable specific conductivity (e.g., during a flood plume or spatial differences along an estuarine gradient), uptake of elements will likely occur regardless of specific conductivity interactions. This suggests that over a temporal or spatial scale of varying dissolved Cu concentration and varying specific conductivity, seagrass leaf Cu concentrations could reflect the local water quality and be a useful bioindicator.

Background dissolved Fe and Mn concentrations were noted to significantly change over the period of the experiment in all treatments. Analyses of the concentrations of Fe and Mn within the seagrass and sediment compartments were carried out to assess whether they were an element source or sink. Manganese is known to oxidise and reduce in concentration over time, which may explain the variations in water concentrations, but within this experiment the concentration of Mn in leaf material significantly increased, irrespective of Cu concentration exposure or specific conductivity treatments. This suggests that this element is actively taken up by the seagrass and could be utilised as an indicator of dissolved Mn. Manganese uptake by seagrass leaves has been observed in *Z. marina* (Brinkhuis, Penello & Churchill 1980). Brinkhuis, Penello and Churchill (1980) do not discuss the reasons for the passive accumulation of Mn but this accumulation is most likely due to Mn being involved in the light photochemical processes (Kirk 1994). Leaf Fe concentrations did not change significantly over time even with the increase in dissolved Fe concentrations in the tank water, and this has also been observed by Prange and Dennison (2000) who found that *Z. muelleri* leaf tissue did not accumulate Fe after addition of 1 mg L<sup>-1</sup> of Fe. The increase of dissolved Fe concentration within the water could be due to the sediment mobilising Fe from the anoxic sediment within the aerated tanks. The lack of Fe accumulation suggests that the *Z. muelleri* leaf compartment would not be a good indicator of Fe, either because it

potentially controls the uptake or because the plant was not Fe deficient during the experiment. Iron, Mn and Cu are all essential elements involved in photosynthesis and therefore it is assumed that *Z. muelleri* would accumulate these TEs, but the passive accumulation of Mn and no accumulation of Fe appears to be opposing to this assumption. While these observations are secondary to the main research question, they further the limited knowledge of *Z. muelleri* and element concentrations under experimental conditions, and indicate that Mn was passively accumulated whereas Fe was not accumulated in the leaves.

The high Cu exposure below-ground concentrations were significantly higher than the other Cu exposures at both the beginning and the end of the experiment. This could have occurred from basipetal (leaf to root) translocation or accumulation from the sediment as that was not controlled for (TE removed from sediment). Basipetal translocation of Cu and other essential elements has been shown to occur at a slower rate than uptake. This has been observed by Richir et al. (2013) where they noted that *P. oceanica* continued to slowly translocate essential elements from the exposed leaves to the below-ground compartment during the recovery period, when exposures to higher concentrations of essential elements were removed. *Zostera* spp. have also displayed weak basipetal translocation of Cu after water Cu exposure (Macinnis-Ng & Ralph 2004; Nielsen et al. 2017). Richir et al. (2013) explained that basipetal translocation occurred due to the imbalance between the above- and below-ground compartments and was not due to the elevated water element concentration. In the Richir et al. (2013) study, they could not exclusively state that the below-ground accumulation did not accumulate TEs from the sediment (or elsewhere) in addition to basipetal translocation. It is possible that below-ground Cu accumulation in this study and for Richir et al. (2013) could possibly have occurred by the seagrass sourcing Cu from the sediment or pore water to offset the above-ground concentrations. In the current study, control and low Cu exposure Cu concentrations in the below-ground and above-ground compartments did not indicate that translocation was occurring (either basipetal or acropetal: roots to leaves), suggesting that at low dissolved Cu water concentrations it is not necessary for Cu to be translocated to or accumulated by below-ground compartments or to above-ground compartments. It is possible that elements sourced from the water would have been translocated if the experimental period was longer than 12 d. Therefore, in application to field assessments of below-ground Cu concentrations and understanding the source of the element (water or sediment), this research suggests that below-ground concentrations are primarily due to the site's steady state sediment concentration. Therefore, as a bioindicator, *Z. muelleri* below-ground compartment is reflecting the long-

term sediment Cu concentration due to its slower growth. Whereas, the leaves are reflecting the short-term water Cu concentrations, and as a bioindicator, the seagrass compartments are indicating the environment's temporal and variable Cu concentrations.

The apportionment of Cu between the above- and below-ground compartments, when adjusted for compartment biomass, demonstrated that under low Cu exposure, the root-rhizome compartments held greater amounts of Cu than the leaf material, but under high Cu exposure, the leaf compartment held more Cu. It was also observed that not all of the Cu was accumulated by the seagrass as the final amounts of Cu did not equate to the initial dissolved spiked Cu. While this experiment was not designed to assess the full Cu mass balance, it is interesting to note that there could be a limit to the *Z. muelleri* uptake of Cu or that the Cu was absorbed and lost to the other sinks within the tank. Possible sinks of Cu within the tank include fauna (decapods, bivalves and polychaetes), sediment pore water, glass tank or plastic pot surface (including biofilms), sediment surface (lost due to pot removal from the tank), organic material or epiphytes.

Seagrass elemental concentrations under natural conditions change over time due to availability and metabolic requirements and processing of elements. This study statistically investigated two time points for seagrass exposure to elevated Cu concentrations. It is possible that maximum leaf Cu accumulation and the subsequent decrease in dissolved water Cu could have occurred at any time before or after 24 h, and that the release of Cu from the leaf material could potentially have occurred after the Cu was significantly reduced within the water. In this study, the concentration of Cu in leaf material was significantly lower at the completion of the experiment than the initial Cu concentrations, for both of the low and high Cu exposures. Macinnis-Ng and Ralph (2004) also observed that Cu concentrations in leaves of *Z. muelleri* returned to near or at control background concentrations within 96 h. Lyngby and Brix (1984) exposed *Z. marina* to Cu and tested more time points, and observed an immediate slow uptake that peaked around day five, before concentrations proceeded to decrease or plateau dependent on the water Cu concentration.

There are multiple potential explanations for a decrease in concentration of the element in the plant after a period of time. The first explanation is that the element concentrations are diluted with growth (increase in biomass) or leaf age (Brix & Lyngby 1982; Malea, Haritonidis & Kevrekidis 1994). The current study did not standardise or measure growth from either leaf length or the number of shoots per pot to allow the calculation of growth differences between treatments and therefore dilution of

accumulated Cu; however, from personal observations, the quantity of seagrass material was higher at the final time point, as were the dry weights of leaf material. Measuring leaf length, area and age could contribute to an understanding of accumulation and the effect of Cu on *Z. muelleri*, as Lyngby and Brix (1984) found *Z. marina* to have positive growth with lower 0.1–0.5  $\mu\text{M}$  (0.6–31.8  $\mu\text{g L}^{-1}$  equivalent) Cu concentrations and inhibited growth at higher Cu concentrations. However, previous experiments of Cu uptake often lack a control over the exposure time. While a significant decrease in Cu in leaves was observed in the high Cu exposure treatment and an apparent decrease in the low Cu exposure treatment, control leaf concentrations did not change over time, and if the dilution theory is to be applied, it should also apply to control samples.

Another possible explanation for a reduction in Cu concentrations in the leaves after a period of time in the high exposure treatments, but not the control treatments, is that *Z. muelleri* is able to self-regulate (uptake, desorb and translocate) its Cu requirements and was releasing excess Cu to the water to equilibrate with background concentrations (Brix & Lyngby 1982; Richir & Gobert 2016). Therefore, the leaf Cu concentrations in the high Cu exposure decreased as the seagrass regulated concentrations to return to a steady state (homeostasis) by either actively releasing any adsorbed Cu back into the water or translocating it to the roots. The negative effects of excessive Cu within leaf compartments could explain why the leaves were actively either releasing and or translocating Cu away from the leaves in order to protect metabolic processes. If experiments were run long enough, both leaf and below-ground compartments may display Cu homeostasis subject to metabolic requirements and the environmental concentrations. Understanding that *Z. muelleri* possibly displays homeostatic behaviour with Cu and applying this knowledge to *Z. muelleri* as a field bioindicator signifies that Cu concentrations will be site dependent due to the ambient water Cu concentrations, and therefore have the potential to display differences between contaminated and uncontaminated sites. However, Cu within seagrass is highly seasonal (Chapter 3), and is irrespective of external loadings (Lyngby & Brix 1982) and further field experiments are required to understand *Z. muelleri* and Cu use.

Excessive Cu (0.1–1  $\text{mg L}^{-1}$ ) has proven to be toxic to *Z. muelleri* in its inhibition of photosynthetic processes (Buapet et al. 2019; Macinnis-Ng & Ralph 2002, 2004; Prange & Dennison 2000). In this study MQY did not demonstrate any significant toxic effects due to treatment. This non-significant toxic effect has previously been observed for *C. nodosa* exposed to 8.4 and 84  $\mu\text{g mL}^{-1}$  Cu, and this suggests that a non-significant impact can occur (Nielsen et al. 2017). The levels of MQY readings observed over time

were only slightly variable even though significant. The lack of a significant response to Cu exposure, as has been observed by previous research, could be due to the concentrations being significantly lower than other exposure levels of 100–1000  $\mu\text{g L}^{-1}$  Cu. The slight decrease at T7 was most likely due to the addition of new freshwater to all tanks the day before and that this addition produced a synergistic effect with the Cu accumulation. While we did not observe significant negative effects due to Cu treatment, it is possible that the timing of measurements missed the effects. Macinnis-Ng and Ralph (2004) found effective quantum yield (EQY) to decrease within the first 2–10 h and then, after removal of Cu, EQY readings returned to background levels at 24 h. Therefore, our seagrass could have displayed toxic effects within the first 24 h when initial uptake occurred. This initial uptake of an element is crucial in understanding a seagrasses response to TE accumulation as the greatest disruption to microtubule cells is due to the rapid rate of initial uptake and not the total element accumulated (Malea, Adamakis & Kevrekidis 2013a, 2014). Therefore, the time of most harm to seagrass is during the initial uptake or at the time of maximum accumulation. Another explanation within the literature of opposing EQY responses of Cu exposure is dependent on the source of seagrass for experiments. Macinnis-Ng and Ralph (2004) found that naïve seagrass was more sensitive to Cu than polluted seagrass while Papathanasiou, Orfanidis and Brown (2015) found that *C. nodosa* from a polluted site was more sensitive to Cu than less polluted seagrass. While the primary focus of this study was to ascertain whether *Z. muelleri* could be a bioindicator of low Cu concentrations, we were able to see that it is tolerant in its effects to low Cu exposure after 24 h. The knowledge that *Z. muelleri* is tolerant of realistic Cu concentrations that are observed within Port Curtis (dissolved Cu  $<2.1 \mu\text{g L}^{-1}$ , total Cu  $<11 \mu\text{g L}^{-1}$ , unpublished data, PCIMP), indicates that *Z. muelleri* meets the criteria of a bioindicator by being tolerant of realistic concentrations of potential contaminants.

## 5.5 Conclusion

This experiment has furthered the understanding of the local ecologically relevant seagrass *Z. muelleri* as a bioindicator of water Cu and contributed to the limited knowledge of seagrass and TE use. *Zostera muelleri* displayed its ability to accumulate low levels of Cu and to metabolise and process Cu for its requirements by actively excluding excessive leaf Cu by possible weak translocation to below-ground compartments. The understanding that *Z. muelleri* leaf material is sensitive to accumulating low levels of Cu, irrespective of specific conductivity variability, warrants *Z. muelleri* to be a potential spatial and temporal water Cu bioindicator. Therefore, this

suggests that, at times of high freshwater runoff, *Z. muelleri* could accumulate and reflect the freshwater bioavailable Cu loading or reflect the spatially specific conductivity variable estuarine differences in Cu. Further research to confirm this phenomenon of Cu accumulation regardless of specific conductivity within the field of a pulse event would be beneficial for understanding what occurs in addition to other environmental influences at different times in the *Z. muelleri* growth cycle.

**Chapter 6. Low light effect on Cu accumulation  
and partitioning by *Zostera muelleri***

## 6.1 Introduction

The accumulation, utilisation, translocation and effects of elements on and by seagrasses are poorly understood, although it is becoming increasingly apparent that element accumulation differs at the species level (Lewis & Devereux 2009; Vonk et al. 2018). The knowledge that TE accumulation is species-specific justifies the use of manipulative experiments of TEs on a potential local seagrass as a bioindicator species where knowledge is deficient. Another research gap is the limited knowledge of how TE accumulation in seagrass may vary with environmental stressors such as salinity or light (Lewis & Devereux 2009; Vonk et al. 2018). This research gap is important to address as environmental variables have the potential to influence TE uptake directly, or to stress the seagrass and subsequently indirectly influence TE uptake (Bond et al. 1988; Wang & Lewis 1997). Understanding the potential effects of environmental variables on TE uptake is important in terms of using seagrass as a TE bioindicator, as the bioindicator is required to demonstrate accumulation irrespective of environmental variation and to be resilient to the natural variation of environmental variables such as light.

Manipulative laboratory experiments provide an opportunity to expose seagrass to excessive TEs or environmental variables to produce measurable effects and subsequently observe the underlying metabolic functions (e.g., growth, photosynthetic response, gene regulation) that may not be apparent under low or natural exposure levels. Reduced light is a known stressor for seagrasses, with effects including growth inhibition and reduction in above- (leaf loss) and below-ground biomass (roots and rhizome), but an increase in photosynthetic efficiency (Abal et al. 1994; Collier, Waycott & Ospina 2012; Ralph et al. 2007; York et al. 2013). One mechanism to enhance photosynthesis under reduced light conditions is an increase in chlorophyll *a* to capture more light (Abal et al. 1994; Lee, Park & Kim 2007). However, this may not be the normal response as other factors such as leaf depth and area may influence chlorophyll content under low light conditions (Collier, Waycott & Ospina 2012). Either way, chlorophyll usage within the PSII and PSI photosynthetic process requires the essential TEs of Cu, Fe, and Mn (Kirk 1994; Macinnis-Ng & Ralph 2002). The physiological processes and requirements for TEs can influence the use of a bioindicator for a particular TE. For instance, the seagrass may be actively accumulating TEs that are involved in photochemical processes (such as Cu, Mn and Fe) to meet the metabolic processes and not necessarily because of the surrounding environment's concentrations (Pergent-Martini & Pergent 2000).

Reduced light on seagrass also influences below-ground physiological responses such as reallocation of stored carbohydrates and supply of macronutrients to the plant (Ralph et al. 2007). Low light has been reported to reduce the oxygenation of the sediment and additionally decrease the release and availability of nutrients and TEs and the flux of those TEs to the above-ground compartments (Schrammeyer et al. 2018). These effects of reduced light to the above- and below-ground compartments could therefore potentially confound the use of seagrass as an environmental bioindicator of TE concentrations, as the seagrass could be regulating its uptake of the TE and therefore not truly reflecting environmental concentrations. To date, research has been conducted on the effects of reduced light on macro-element uptake (C, N and P) and translocation (Collier, Prado & Lavery 2010; Pérez-Lloréns et al. 1993), but not the effects of reduced light on uptake and translocation of TEs. This study will address the joint effects of reduced light and TE exposure on *Z. muelleri* TE accumulation.

Copper is an essential TE for vascular plants as it is important for photosynthetic processes, and can be found in higher concentrations in younger *Z. marina* leaves (Brix & Lyngby 1982; Ralph & Burchett 1998). However, excess Cu ( $0.1\text{--}10\text{ mg L}^{-1}$ ) inhibits photosynthetic processes and causes leaf abscission (Macinnis-Ng & Ralph 2002; Ralph & Burchett 1998). When seagrass is exposed to high ( $2800\text{ mg kg}^{-1}$ ) sediment Cu concentrations, the root-rhizome compartment readily accumulates and acropetally translocates the Cu to the leaves, where significant effects of decreased leaf growth rates, leaf numbers, and increased leaf mortality are observed (Nielsen et al. 2017). Whilst Cu can cause photosynthetic inhibition, reduced light scenarios, however, increase photosynthetic efficiency (Prange & Dennison 2000; Ralph et al. 2007; York et al. 2013). These contrasting physiological behaviours have the potential to influence Cu accumulation to assist in photosynthetic efficiency and therefore the effectiveness of a seagrass bioindicator. Field observations of *Z. muelleri* demonstrated that it meets the requirement of a bioindicator by displaying a spatial gradient of Cu within a semi-enclosed estuary and therefore suggest that *Z. muelleri* can passively accumulate Cu and reflect the environmental Cu, irrespective of metabolic requirements (Ambo-Rappe, Lajus & Schreider 2007).

Understanding how a seagrass species behaves under different environmental conditions can help determine the potential effectiveness of the seagrass as a bioindicator of TEs of concern. It is postulated that the Cu uptake by *Z. muelleri* could be independent of light, as leaf accumulation of Cu appears to be a passive process required for new growth and photosynthesis. In addition, the effect of low light on the

below-ground compartment and Cu concentrations is unknown, but it is hypothesised that reallocation of Cu through translocation could occur to support leaf requirements, as seen with redistribution of carbohydrates from rhizomes under low light. Copper was chosen as the exposure TE for this experiment due to its potential effects upon *Zostera* spp. and because the study site (Port Curtis) has previously been reported by Angel et al. (2010) to have anthropogenically sourced dissolved Cu concentrations. This study aimed to understand if the accumulation, translocation and effects of a range of Cu exposures on a local seagrass species, *Z. muelleri*, was influenced by reduced light conditions. The specific aims of this study were to determine whether the low light conditions affected the leaf and below-ground compartments in reflecting the water Cu concentrations and over time. Final aim was to determine whether the photosynthetic rate changed over time due to the different Cu and low light conditions.

## 6.2 Methods

### 6.2.1 Experiment approach

Nominal Cu concentrations used during the exposure experiment were control, low ( $5 \mu\text{g L}^{-1}$ ) and high ( $50 \mu\text{g L}^{-1}$ ). The selection of these concentrations is further justified in Chapter 5, section 5.2.1. The reduced light scenario for this study was  $0.73 \text{ mol photons m}^{-2} \text{ d}^{-1}$  (fluorescent tubes, Sylvania Gro-lux, F18W). This level of light was well below the light threshold of  $6 \text{ mol photons m}^{-2} \text{ d}^{-1}$  for the local *Z. muelleri* in Port Curtis (Chartrand et al. 2016). This threshold was introduced to protect the local dominant seagrass species, *Z. muelleri*, from reduced light activities and processes of sediment resuspension or turbid waters from dredging (Chartrand et al. 2016). All seagrasses used in the experiment were exposed to the same reduced light level. This experiment was therefore not a comparison of reduced light and normal light, and interpretation of the effect of reduced light was made in recognition of this.

### 6.2.2 Experimental design

Whole *Z. muelleri* cores were collected in January 2018 in the same manner as described in Chapter 5 section 5.2.2, from the same location under permit conditions. Unlike the previous chapter, no field seagrass samples were taken, but additional seagrass samples were taken for baseline values. Tank handling and experimental room setup is described in Chapter 5 section 5.2.3. Filtered seawater ( $0.5 \mu\text{m}$  inline filter) for use in the experiment was collected within 500 m of the location from where the seagrass was collected. The filtered water was placed in glass tanks to equilibrate to

ambient conditions over-night before seagrass was added the following day. There were four replicate tanks of each Cu exposure (control, low 5  $\mu\text{g L}^{-1}$  and high 50  $\mu\text{g L}^{-1}$ ), and each tank started the experiment containing six cores (three cores for initial sampling period T1 and three for final T11, Table 6.1). An additional nine pots were individually spread throughout nine tanks and analysed as three baseline samples (3 pots = 1 sample) before Cu addition. Seagrasses had one day of acclimation to tank conditions before the addition of Cu. The experiment ran for 12 d (Table 6.1).

**Table 6.1. A timeline of when sampling occurred, the number of samples taken (n) and pulse amplitude modulator (PAM) readings taken during the experiment. T = time point**

Time point	Day	Above	Below	Dissolved TEs	Sediment	PAM	Notes
-T1							Seagrass collected and placed in tanks
Baseline (T0)	1	3	3	12	3	12	Cu addition after baseline and PAM analysis
T1	2	12	12		12	12	
T3	4					12	
T5	6					12	
T7	8					12	
T9	10					12	
T10	11					12	
T11	12	12	12	12	12		Photos taken

Seagrass compartments (leaf, below-ground and sediment) were analysed for Cu concentrations at baseline (T0), T1 and T11 (Table 6.1), by pooling three pots to form one sample (see details of sample preparation in Chapter 5 section 5.2.3). Leaf weight was measured prior to freezing and was reported as wet weight (g). Within this experiment, it was noted that the seagrass was losing green leaves and leaf colour was changing from green to brown. The leaf material that was collected and pooled at the

end of the experiment included both brown and green leaves. To capture the proportion of leaf colour change between Cu exposure treatments, photographs were taken of each of the remaining three pots within each tank on the last day (T11). The proportion of brown and green leaves was assessed by applying eight dots at random to the computer screen, then opening the image and counting the number of green leaves and brown leaves that were beneath a dot. If a dot was not touching a leaf the nearest leaf to the dot was counted, and where there were two dots on one leaf the nearest leaf to the dotted leaf was counted. The process was repeated three times for each image with a different dot arrangement each time and the average of the three was used for analysis.

Filtered (0.45  $\mu\text{m}$ ) water samples were taken just after Cu addition (<0.5 h) to determine the actual Cu concentrations in each tank, and at the completion of the experiment (T11) to observe the remaining Cu within the water. Filtered samples were acidified to pH <2 with analytical grade 70% nitric acid and stored at 4°C until analysis. To assess relative differences in photosynthetic rate MQY ( $F_v/F_m$ ) measurements were taken from five green leaves from each tank using a PAM fluorometer (Diving-PAM, Walz, Germany) (Table 6.1, and see Chapter 5 section 5.2.4).

The total amount (mg) of Cu in each compartment (leaf, below-ground, sediment and dissolved water) was estimated by multiplying the concentration of Cu in the compartment by its mass. A comparison of the Cu weight by compartment was then made between the baseline and control at T0 to the average of T1 and T11 for both low and high Cu exposures. This calculation provides information of Cu concentration in regards to the biomass of the compartment; for example, the below-ground (root-rhizome) compartment had greater biomass than the leaf compartment and therefore could potentially have had more or less Cu when compared to other compartments.

### **6.2.3 Trace element determination**

Seagrass and sediment samples were sent as dried material (sample preparation further described in Chapter 5 section 5.2.3) to be analysed by the certified Australian Government NMI laboratory, Sydney, by their in-house methods of NT2.47 (water), NT2.49 (sediment) and NT2.46 (seagrass). Seagrass leaf, roots-rhizome and sediment were digested by NMI with the application of high purity nitric and hydrochloric acids by heating on a hot block at 95–100°C for 2 h. Trace element concentrations were determined by ICP-MS (Agilent 7900) with seagrass (leaf and below-ground) and sediment Cu concentrations reported on a dry weight basis. Filtered (0.45  $\mu\text{m}$ ) water

samples were tested for a suite of TEs (Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Ni, and Zn) to determine Cu concentrations and whether any other TEs were present, as these may confound Cu uptake. The quality assurance and quality control (QAQC) practice of duplicates and blanks was applied to the dissolved water samples with QAQC results being <10% of nominal values and therefore meeting the QAQC requirements. Sample spike and laboratory control sample recoveries were within acceptable limits with recovery of 96–103% for seagrass Cu, 97–109% for sediment Cu and 91–112% for all filtered water TEs (Appendix E Tables E1, Table E2, and Table E3). Where values were below the limit of reporting, the limit of reporting value was used in calculations and the less than symbol (<) was used for reporting.

## **6.2.4 Data analysis**

General Linear Model univariate two-way ANOVAs were conducted to assess differences between Cu exposure (fixed, three levels: nominal concentrations control, low 5  $\mu\text{g L}^{-1}$  and high 50  $\mu\text{g L}^{-1}$ ) and time (fixed, two levels: T1 and T11) for the independent variables of leaf, below-ground and sediment compartment Cu concentrations. A one-way ANOVA was used to assess the significant differences in % leaf colour (brown and green) between Cu exposure treatment (fixed, three levels: control, low 5  $\mu\text{g L}^{-1}$  and high 50  $\mu\text{g L}^{-1}$ ) at T11. Data was natural log transformed where required to meet homogeneity of variance and normality requirements of the analysis. Tukey HSD post-hoc tests were performed where significant differences were found. Differences in MQY from the same plants throughout the experimental period were analysed using a one-way repeated measures ANOVA with time as the within repeated measure (seven levels: T0, T1, T3, T5, T7, T9 and T10) and the between-subject factor of Cu exposure (three levels: control, low 5  $\mu\text{g L}^{-1}$  and high 50  $\mu\text{g L}^{-1}$ ). Mauchly's Test of Sphericity was used, and results adjusted where assumption of sphericity was not met. SPSS v. 24 (IBM corp., Armonk, NY) was used for statistical analysis.

## **6.3 Results**

### **6.3.1 Aquaria conditions**

The tank conditions during the experiment are summarised in Table 6.2. Light conditions for the experiment were low, with a mean of 15.3  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  during the 14 h light period (equivalent to 0.73  $\text{mol photons m}^{-2} \text{d}^{-1}$ ). Mean specific conductivity increased by ~5% from 54631  $\mu\text{S cm}^{-1}$  to 57701  $\mu\text{S cm}^{-1}$  over the experimental period due to evaporation from all tanks. Copper concentrations in control tanks were at or

near the limit of reporting of  $1 \mu\text{g L}^{-1}$ . Initial (T0) dissolved Cu concentration in the low Cu exposure treatment ( $5 \mu\text{g L}^{-1}$ ) ranged from  $4.7\text{--}6.1 \mu\text{g L}^{-1}$  and in the high Cu exposure treatment ( $50 \mu\text{g L}^{-1}$ ) the Cu concentration range was  $42\text{--}57 \mu\text{g L}^{-1}$ . Copper exposure means were within 5% of nominal values, and therefore reference to the nominal values can be inferred as the actual values. Dissolved Cu concentrations decreased over time, with control and low exposure treatments showing values at or around the LoR of  $1.0 \mu\text{g L}^{-1}$  at T11, while the mean of the high Cu exposure treatment was  $2.48 \mu\text{g L}^{-1}$  Cu at T11. The nine supplementary analysed TEs were not considered to be high enough to confound or inhibit Cu uptake, as all recorded concentrations were lower than has previously been observed within Port Curtis (Table 6.2).

**Table 6.2. Water quality in experimental tanks over the period of the experiment (mean  $\pm$  SD), and dissolved trace element concentrations at time of Cu addition (T0) and at the completion of the experiment (T11). Copper concentrations for the three nominal Cu treatments (mean  $\pm$  SD, n = 4). Full table of trace elements by treatment is provided in Appendix E Table E4.**

	Initial (T0)	Final (T11)
Temperature °C <sup>a</sup>	25.3 (0.38)	25.5 (0.40)
Dissolved Oxygen % <sup>a</sup>	90.33 (5.53)	94.65 (1.63)
Specific Conductivity $\mu\text{S cm}^{-1\text{a}}$	54631 (262.8)	57701 (475)
Salinity <sup>a</sup>	36.2 (0.13)	38.4 (0.35)
pH <sup>a</sup>	7.73 (0.13)	7.91 (0.06)
PAR $\mu\text{mol photons m}^{-2} \text{s}^{-1\text{b}}$	15.3 (0.45)	
Light h <sup>c</sup>	14	
Control Cu <sup>d</sup>	1.03 (0.05)	1.15 (0.3)
Low Cu (5 $\mu\text{g L}^{-1}$ ) <sup>d</sup>	5.15 (0.65)	1.0 (0.0)
High Cu (50 $\mu\text{g L}^{-1}$ ) <sup>d</sup>	49.5 (6.14)	2.48 (0.22)
Aluminium $\mu\text{g L}^{-1} \text{e}$	6.18 (1.2)	5.12 (0.4)
Arsenic $\mu\text{g L}^{-1} \text{e}$	2.48 (0.3)	4.94 (0.86)
Cadmium $\mu\text{g L}^{-1} \text{e}$	<0.1 (0.0)	<0.1 (0.0)
Chromium $\mu\text{g L}^{-1} \text{e}$	<1.0 (0.0)	<1.0 (0)
Iron $\mu\text{g L}^{-1} \text{e}$	11.4 (4.01)	16.0 (4.66)
Lead $\mu\text{g L}^{-1} \text{e}$	<1.0 (0.0)	<1.0 (0.0)
Manganese $\mu\text{g L}^{-1} \text{e}$	57.5 (30.6)	10.8 (2.95)
Nickel $\mu\text{g L}^{-1} \text{e}$	<1.0 (0.0)	<1.0 (0.0)
Zinc $\mu\text{g L}^{-1} \text{e}$	2.87 (0.59)	1.58 (0.62)

<sup>a</sup> Mean physico-chemical parameters (n = 12) at the start of the of the experiment before Cu addition and the completion of the experiment (n = 4).

<sup>b</sup> Mean of three light loggers over the acclimation and experimental period.

<sup>c</sup> Number of light hours over the acclimation and experimental period.

<sup>d</sup> Mean of each Cu treatment (n = 4) at the time of Cu addition (T0) and at the completion of the experiment (T11).

<sup>e</sup> Mean concentrations of all tanks (n = 12) at the time of Cu addition (T0) and at the completion of the experiment (T11).

### 6.3.2 Copper concentrations

*Zostera muelleri* leaf Cu concentrations in baseline and the control treatment ranged from 9.2 to 14 mg kg<sup>-1</sup> (Fig. 6.1). There was no significant interaction between time and Cu exposures for leaf Cu concentrations ( $F_{2,18} = 0.33$ ,  $p = 0.72$ , Table 6.3). However,

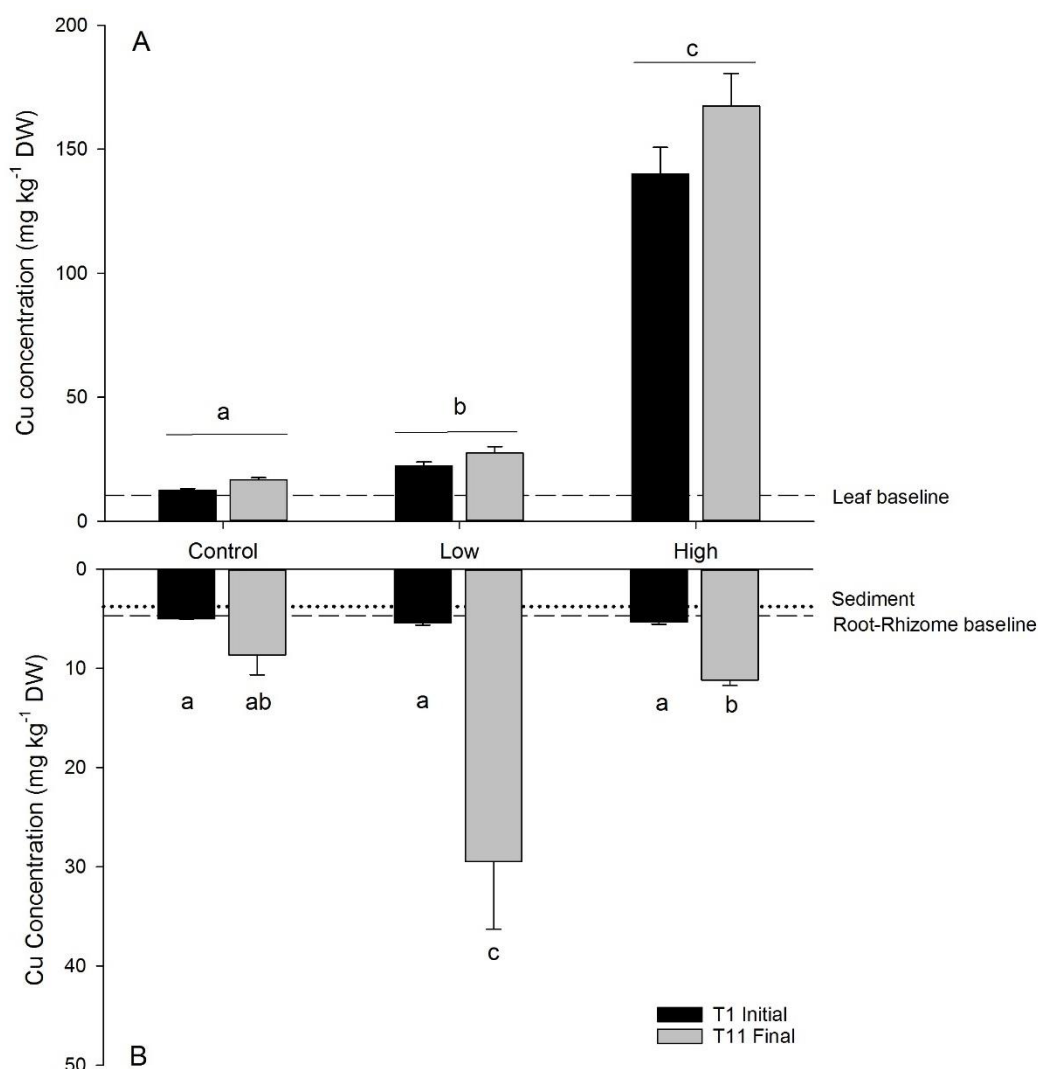
there was a significant effect of Cu exposure on leaf Cu concentrations ( $F_{1,18} = 568.23$ ,  $p < 0.001$ , Table 6.3). The significant Cu exposure on the leaf material resulted in the following mean overall leaf Cu concentrations: control  $14.63 \text{ mg kg}^{-1}$  < low  $24.88 \text{ mg kg}^{-1}$  < high  $153.8 \text{ mg kg}^{-1}$  (Fig. 6.1). Mean wet weight of leaf material increased from  $1.89 \pm 0.3 \text{ g}$  at T1 to  $2.36 \pm 0.5 \text{ g}$  at T11.

**Table 6.3. Two-way ANOVA results for the different compartments Cu concentrations and time. Significant effects indicated in bold where  $p < 0.05$ .**

	df	Leaf			Root-Rhizome		
		MS	F	<i>p</i>	MS	F	<i>p</i>
Time	1	0.306	14.25	<b>0.001</b>	5.32	68.18	<b>0.000</b>
Cu	2	12.199	568.23	<b>0.000</b>	0.917	11.75	<b>0.001</b>
Time * Cu	2	0.007	0.33	0.720	0.725	9.29	<b>0.002</b>
Error	18	0.021			0.078		

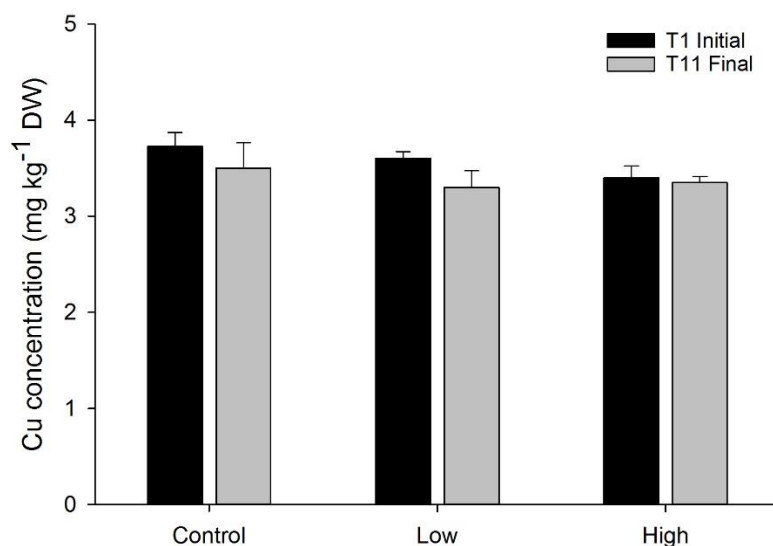
Sediment				
	df	MS	F	<i>p</i>
Time	1	0.220	2.283	0.148
Cu	2	0.118	1.222	0.318
Time * Cu	2	0.033	0.341	0.716
Error	18	0.097		



**Figure 6.1.** Cu concentrations (Control, Low 5  $\mu\text{g L}^{-1}$ , High 50  $\mu\text{g L}^{-1}$ , mean  $\pm$  SE,  $n = 4$ , dry weight) within (A) leaf and (B) root-rhizome compartments. Reference lines are the means of the baseline samples for the leaf and root-rhizome compartments and sediment mean of baseline and control samples. Similar letters indicate no significant difference between treatments and over time.

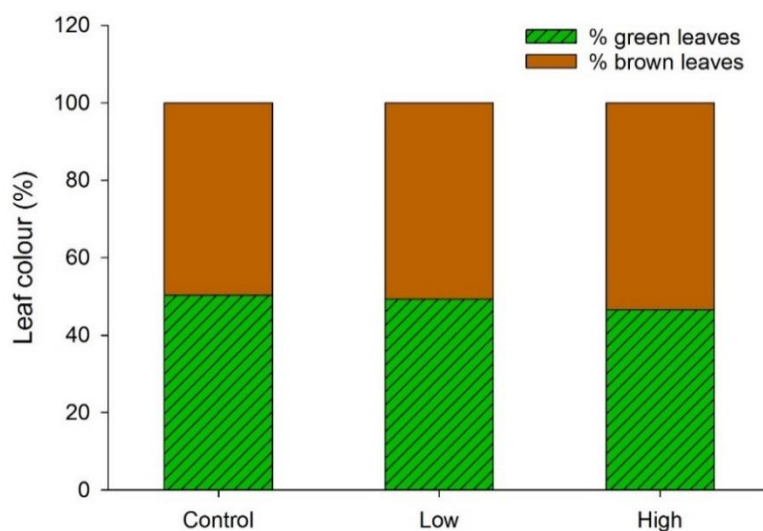
Below-ground (root-rhizomes) Cu concentrations for baseline and all initial (T1) values across treatments ranged from 4.0 to 5.9  $\text{mg kg}^{-1}$  (Fig. 6.1). There was a significant interaction between time and Cu exposure treatment for the below-ground Cu concentrations ( $F_{2,18} = 9.29$ ,  $p < 0.01$ , Table 6.3). All below-ground Cu concentrations at T1 (mean  $\pm$  SD: control  $4.97 \pm 0.1 \text{ mg kg}^{-1}$ , low  $5.42 \pm 0.42 \text{ mg kg}^{-1}$  and high  $5.32 \pm 0.45 \text{ mg kg}^{-1}$ ) significantly increased over time, with low Cu exposure increasing up to five times ( $29.5 \pm 13.6 \text{ mg kg}^{-1}$ ), and control and high exposures doubling (control  $8.65 \pm 4.0$ , high  $11.18 \pm 1.09$ , Fig. 6.1). However, final (T11) below-ground concentrations in the controls were not significantly different to initial (T1) Cu concentrations (Fig. 6.1). Sediment Cu concentrations ranged from 2.8 to 4.0  $\text{mg kg}^{-1}$  for all samples and there

was no significant interaction between time and Cu exposure ( $F_{2,18} = 0.716$ ,  $p = 0.716$ ) or a significant main effect of time or Cu treatment (Fig. 6.2, Table 6.3).



**Figure 6.2.** Sediment Cu concentrations (mean  $\pm$  SE,  $n = 4$ , dry weight) at the beginning (T1) and the end of the experiment (T11) by Cu exposure (Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ).

Leaf colour at the end of the experiment was approximately 50% green and 50% brown in all Cu exposure treatments (Fig. 6.3). There was no significant difference of % leaf colour between Cu exposure treatments (Table 6.4). Green leaf abscission was noted to increase towards the end of the experiment and was observed primarily in one tank, and therefore the effect was not deemed as a Cu exposure effect but more likely to be a tank effect.



**Figure 6.3.** Percentage of leaf colour at the end of the experiment (T11) by Cu exposure treatment (Control, Low  $5 \mu\text{g L}^{-1}$ , High  $50 \mu\text{g L}^{-1}$ ).

**Table 6.4. One-way ANOVA results for the percentage of leaf colour at the end of the experiment by Cu treatment.**

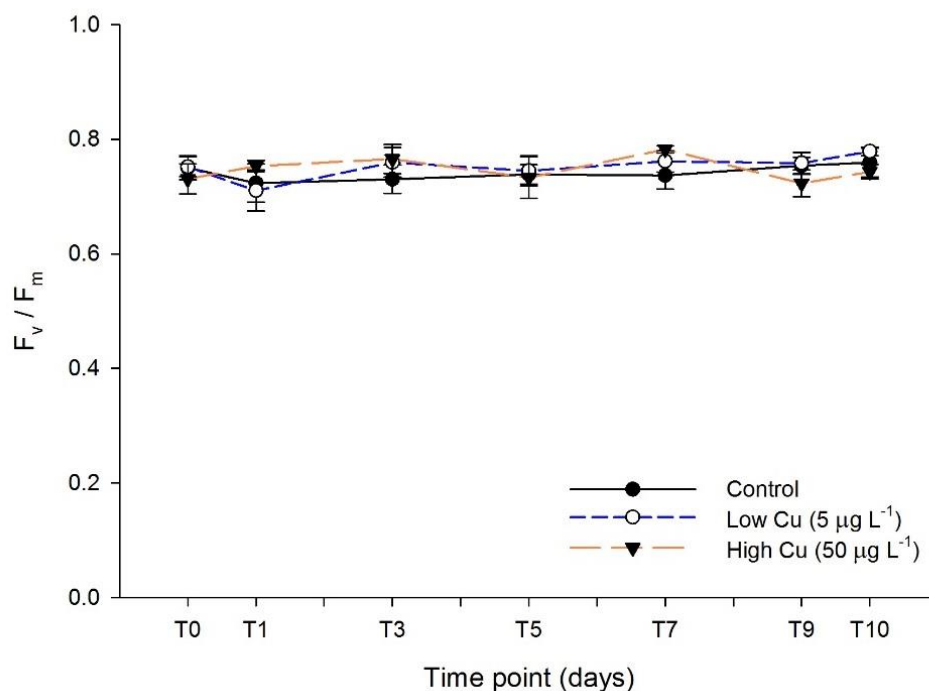
	Green				Brown		
	df	MS	F	<i>p</i>	MS	F	<i>p</i>
Cu exposure	2	0.299	0.419	0.661	0.299	0.419	0.661
Error	35				35		

The total estimated weight of Cu was greater in the below-ground compartment than in leaf material for the low Cu exposure treatment. In the high Cu exposure treatment, the Cu concentration in the leaf compartment was greater than in the below-ground compartment over the entire experimental period (Table 6.5).

**Table 6.5. Calculated Cu distribution between compartments within a single tank at time of Cu additions (T0) and the means of after 24 h (T1) and at the completion of the experiment (T11).**

Time	T0	T1, T11 mean	T0	T1, T11 mean
Treatment	Low	Low	High	High
	(6 pots)	(3 pots)	(6 pots)	(3 pots)
Compartment	mg	mg	mg	mg
Water	0.130	0.025	1.240	0.062
Leaf	0.013	0.014	0.013	0.094
Root-Rhizome	0.045	0.080	0.045	0.051
Sediment	67.86	31.05	67.86	30.15
Sum of Cu (excluding sediment)		0.119		0.207

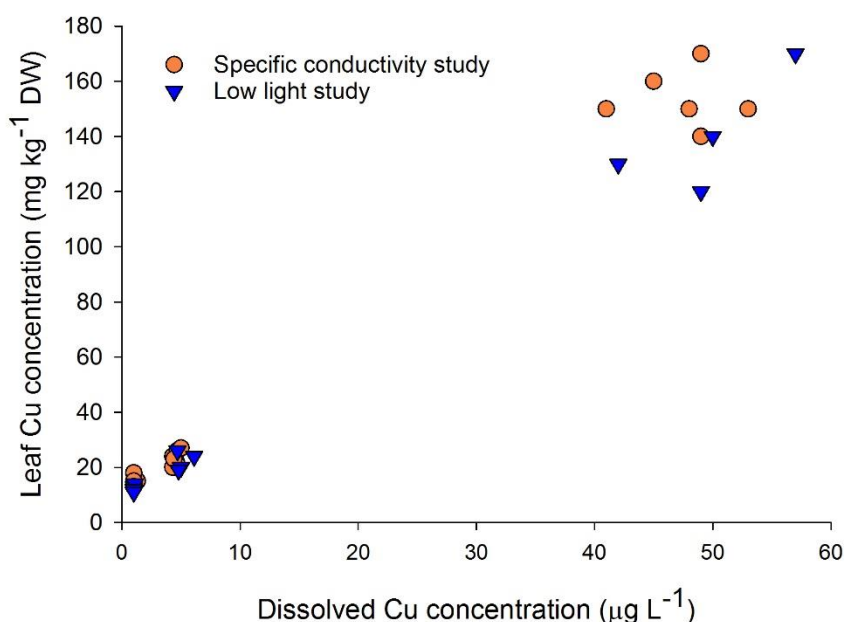
Maximum quantum yield ( $F_v/F_m$ ) on the green leaves during the experimental period did not display any significant effects due to Cu exposure (Fig. 6.4). Mauchly's Test of Sphericity was not violated and normal assumptions were made (Appendix E Table E5). Repeated measures ANOVA found no significant difference within subject interaction of time and Cu on MQY ( $F_{12,24} = 0.447$ ,  $p = 0.936$ , Appendix E Table E6). Furthermore, the between subject effect (Cu exposure) had no significant difference on MQY values ( $F_{2,9} = 0.265$ ,  $p = 0.773$ , Appendix E Table E6).



**Figure 6.4.** Maximum quantum yield (mean  $\pm$  SE,  $n = 20$ ) from before Cu addition (T0) to prior the project completion (T10) for each Cu exposure (Control, Low 5  $\mu\text{g L}^{-1}$ , High 50  $\mu\text{g L}^{-1}$ ).

## 6.4 Discussion

The utilisation of a local seagrass as a TE bioindicator requires the understanding of whether TE uptake is influenced by environmental variables, such as light, and therefore influences the suitability of the seagrass for use as a bioindicator. The leaf compartment of *Z. muelleri* displayed passive Cu uptake from the water for each Cu exposure, irrespective of the extremely reduced light conditions. The amount accumulated was comparable to that reported in Chapter 5 (Fig. 6.5). This suggests that Cu accumulation occurs irrespective of light and specific conductivity and that accumulation in this study is consistent with other Cu accumulation studies using *Z. muelleri* (Buapet et al. 2019; Macinnis-Ng & Ralph 2004; Prange & Dennison 2000).



**Figure 6.5. Scatterplot of dissolved Cu concentrations (Control, Low 5 µg L<sup>-1</sup>, High 50 µg L<sup>-1</sup>) at T0 and initial (T1) leaf Cu concentrations of this low light study (▼) and the specific conductivity experiment (● Chapter 5).**

In this study, half of the leaves had turned brown by the end of the study, suggesting senescence over the experimental period that has the potential to influence Cu accumulation and concentrations. Brown leaves could influence Cu concentrations in the sense that the brown leaves cannot grow to dilute Cu concentrations and that Cu within the dead leaves may not be released. In a study of *Z. muelleri* and Pb uptake, a comparison of green and dead leaves demonstrated that the initial uptake by green leaves was 11% more than dead leaves but the final concentration of both was the same after six days, suggesting that accumulation is a process that does not require the plant to be alive, indicating passive uptake (Bond et al. 1985). This ability to passively accumulate TEs meets the requirements of a bioindicator in that it can accumulate TEs and potentially reflect the environment. While direct comparison of light levels (e.g., normal and reduced) were not made in this study, the results of this experiment and the previous experiment (full light, Chapter 5) suggest that *Z. muelleri* has the potential to accumulate Cu even under lower light conditions. There are no previous or current published studies that investigated multiple stressors of reduced light and TE accumulation (as opposed to studies that focussed solely on physiological effects on seagrass), and so no comparison to other studies can be made. However, recent multi-stressor experiments researched *Z. noltei*'s Cu uptake under variable temperature or pH conditions (de los Santos et al. 2019; Gamain et al. 2018). Both *Z. noltei* multi-stressor

experiments found no significant difference in Cu accumulation due to variable temperature or pH but other physiological effects such as reduced growth rates and reduced photosynthesis did occur due to the stressors and higher Cu dosages (10 – 300  $\mu\text{g L}^{-1}$ ) (de los Santos et al. 2019; Gamain et al. 2018). This current study and the experiment described in Chapter 5 suggest that Cu accumulation by *Z. muelleri* is a passive process; however, there may be a limit to accumulation as the high Cu exposure treatments had residual dissolved Cu within the tanks. This could be due to *Z. muelleri* having a reduction in binding sites within the leaf material for all of the Cu to bind (Malea et al. 2018). This passive process of accumulation suggests that *Z. muelleri* leaves could uptake Cu under any conditions and this aspect meets the requirement of a bioindicator that has the capability to uptake the TE under natural variable light environments, such as at deep depths or within a turbid estuary.

Reduced light causes significant negative effects to *Z. muelleri*, such as reduced leaf growth, reduced carbohydrates and leaf shedding from the increase in upregulated abscisic acid genes (Collier, Waycott & Ospina 2012; Davey et al. 2018). However, reduced light causes increased photosynthetic efficiency to increase the photosynthetic capability of the seagrass (Ralph et al. 2007). Within this study MQY results from the green leaves indicated that there was no significant effect of Cu treatment during the experiment and no assumption can be made in regards to the effect of the light exposure on MQY. No significant effect of Cu exposure on photosynthetic efficiency has been observed with *Z. marina* (Nielsen et al. 2017). Leaf senescence and shedding is a common response to low light as a protective mechanism to reduce the amount of the plant to be maintained for photosynthetic metabolic processes (Collier, Waycott & Ospina 2012). During the experiment, the abscission of green leaves was observed. This was especially noted in one tank in the low Cu exposure treatment, but also increased in occurrence throughout additional tanks towards the end of the experiment. All tanks had a notable increase of brown leaves by the end of the experiment. These effects of leaf loss and colour change are most likely due to the reduced light conditions, as leaf colour change was observed in all tanks, but a comparison of lighting (normal and reduced) could tease apart the light and Cu effects. Within other studies, Cu induced seagrass leaf loss due to the suspected increase of abscisic acid has been observed to occur with *Halophila* spp. but not the Cu tolerant *Z. muelleri* (Prange & Dennison 2000; Ralph & Burchett 1998). Reduced light effects (resulting in changed leaf colour) appeared to be the main driver on *Z. muelleri* appearance than leaf Cu accumulation. Similar results were observed by Gamain et al. (2018), where temperature effects appeared to be greater than the effects of Cu accumulation. Future studies investigating

other effects such as gene expression and leaf growth rate may help to determine whether there are interactive effects on *Z. muelleri* of the combination of reduced light and Cu accumulation. The present study investigated whether light influenced Cu accumulation and it appears that Cu accumulation occurs irrespective of light and that *Z. muelleri* meets the requirements of a bioindicator.

The strongest change in Cu concentrations was observed within the below-ground compartments that displayed significant enrichment of Cu at the end of the experiment. A proposed cause of this overarching effect is due to low light conditions across all treatments. Seagrass translocation of TEs or nutrients under dark or light limiting scenarios is dependent on metabolic requirements (Ralph et al. 2007). For example, *Z. noltei* under dark conditions displayed greater uptake and storage of P within the rhizomes from the rhizosphere P exposure than under normal light conditions ( $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) (Pérez-Lloréns et al. 1993). In this study, the response of root-rhizomes actively accumulating bioavailable Cu from the sediment (or pore water, or another unknown source) is proposed to be for either immediate use for new growth, or to be stored within the rhizome for later use when normal light conditions resume. Copper concentrations in leaf material increased over time but the result was not significant. This increase could reflect a situation where the newly acquired below-ground Cu is redirected to new above-ground growth. Within the literature, younger leaves from *Zostera* spp. have been observed to have higher Cu, P and Zn than older leaves, not due to accumulation or internal translocation (adult to young leaves), but due to new growth requiring these TE to meet their metabolic requirements (Brix & Lyngby 1982; Lyngby, Brix & Schierup 1982; Pérez-Lloréns et al. 1993). In this present study, below-ground accumulation and redistribution to new leaves still occurred even though there was ample Cu to be sourced from the newly accumulated Cu within the leaf material, suggesting that translocation from adult and dead leaves to new leaves is not a predominant function within *Z. muelleri*. This is unlike another seagrass species such as *Posidonia sinuosa* Cambridge and Kuo in which macronutrients can redistribute between adult to younger leaves (Collier, Prado & Lavery 2010). Further tests such as two-compartment studies (Lyngby, Brix & Schierup 1982) while observing leaf length and shoot development could assist in understanding where the below-ground accumulated Cu redistributes to.

The implications of observing a below-ground response of active Cu accumulation in addition to the above-ground passive Cu accumulation requires further consideration when utilising seagrass as a bioindicator. For example, the variable below-ground Cu

concentrations at the end of the experiment across all treatments would not correlate to the sediment Cu concentrations. This lack of a correlation or relationship between the bioindicator and the environment is not meeting the assumption of a bioindicator in that an indicator is to provide a time integrated consistent measure of the TE in the environment (Rainbow 2006). This was observed in Chapter 4 where above- and below-ground Cu concentrations significantly changed temporally, due to growth, and therefore concentrations were decoupled and not correlated to the sediment or water environments. This experiment confirms that results of *Z. muelleri* as a Cu bioindicator needs to consider growth factors that may inform when to sample.

## 6.5 Conclusion

*Zostera muelleri* leaves accumulated Cu under low light conditions. Leaf Cu accumulation was directly related to the water Cu concentration, with leaf concentrations not significantly changing over time. However, a notable response not recorded before in the literature was the active uptake of Cu by the root-rhizome compartment from the sediment rhizosphere due to the low light conditions, causing *Z. muelleri* to accumulate Cu for storage or immediate use by new leaves. These different accumulation methods suggest that *Z. muelleri* can control its Cu accumulation to meet its metabolic requirements when required. However, the active uptake process observed could confound the use of *Z. muelleri* as a Cu bioindicator as it may not reflect the environment. This below-ground result was due to a sustained extremely reduced light condition ( $0.73 \text{ mol photons m}^{-2} \text{ d}^{-1}$ ) and may not occur naturally (previous Port Curtis readings for healthy seagrass was a 14 day rolling average  $>6 \text{ mol photons m}^{-2} \text{ d}^{-1}$ , Chartrand et al. (2016)), and if these results were to occur then careful interpretation could be applied from this new knowledge. This study has demonstrated that *Z. muelleri* accumulates Cu in the leaf compartment irrespective of low light conditions, meaning that accumulation could occur at times of decreased light conditions from either natural or anthropogenic increases in turbidity (sediment resuspension or particulate laden floodwaters). Therefore, *Z. muelleri* is a potentially effective bioindicator of the bioavailable Cu concentrations in the environment and could potentially be used to identify spatial or temporal sources of TE contamination.

**Chapter 7. Discussion and conclusion of *Zostera muelleri* as a potential trace element bioindicator**

## 7.1 Potential of *Zostera muelleri* as a trace element bioindicator

Bioindicators are used to demonstrate the changes in pollutants or pressures occurring within the environment and are ecologically relevant to the health of the system.

Seagrasses are known bioindicators of ecosystem stresses as they are abundant over large areas, sessile, sensitive to disturbances and accumulate pollutants (Lewis & Devereux 2009; Pergent-Martini & Pergent 2000). Seagrasses are also a key primary producer for many food webs and are directly consumed by micro- and mega-grazers (Nowicki, Fourqurean & Heithaus 2018). Therefore, using seagrasses as a bioindicator for TEs has the advantage in that they are a local ecologically relevant bioindicator. *Zostera muelleri* already meets some aspects of a bioindicator in that it is sessile and present in areas of monitoring; however, further knowledge of TE utilisation was required.

This study investigated the potential use of *Z. muelleri* as a TE bioindicator, based on the results of field and laboratory assessments to understand *Z. muelleri*'s capability of TE accumulation, regulation, and partitioning in relation to the water and sediment TE concentrations. It was expected that *Z. muelleri* TE concentrations throughout Port Curtis would be different across locations due to factors such as location specific TE concentrations, seagrass regulation or external environmental drivers. Therefore, this study firstly examined whether there was a greater difference in *Z. muelleri* TE concentrations at a location compared to between locations throughout Port Curtis, and whether seagrass TE variability was due to environmental drivers such as sediment particle size, water quality and seagrass morphometrics (Chapter 3). Secondly, this study investigated whether seagrass TE concentrations changed temporally over the active growing period due to growth or natural seasonal weather events (Chapter 4). Lastly, *Z. muelleri* was exposed to one specific TE (Cu) under controlled laboratory conditions, with varying salinity and light scenarios to assess the influence of environmental conditions on the rate, and effect, of uptake (Chapters 5 and 6). This chapter considers these results in the context of using *Z. muelleri* as a bioindicator of TEs in Port Curtis. The research results are assessed against broad bioindicator criteria such as practicality, relevance and response (e.g., interpretable, spatial and temporal), to establish if *Z. muelleri* meets the aspects of a TE bioindicator.

## 7.2 Practicality

Prior to the experimental components of this study it was already possible to establish from previous research that *Z. muelleri* met some of the practical aspects of a bioindicator, as outlined by Rainbow (2006) and explained in Chapter 1, in that the species is:

- abundant (sufficient to sample);
- sessile with permanent meadows (seagrass is present throughout the year to integrate TEs and to be sampled throughout the year);
- easy to identify;
- cosmopolitan (Australian east coast and New Zealand);
- resistant to handling stress;
- tolerant of a range of water physico-chemical parameters across the estuarine gradient; and
- tolerant to typical TE exposures.

The results of the current study indicated that, at least for Cu, the accumulation by seagrass leaves was independent of the environmental variables examined (i.e., specific conductivity and light, Chapters 5 and 6). The practical implication of this for the use of *Z. muelleri* as a bioindicator is that for sampling along an estuarine spatial gradient or at times of reduced specific conductivity and reduced light (e.g., a flooding event), *Z. muelleri* would still reflect environmental sources of TEs. Additionally, from the laboratory experiments (Chapters 5 and 6), *Z. muelleri* demonstrated itself be tolerant to low ( $5 \mu\text{g L}^{-1}$ ) and high ( $50 \mu\text{g L}^{-1}$ ) dissolved Cu exposure by not displaying any significant decrease in photosynthetic efficiency due to Cu, and demonstrated that it is tolerant to realistic concentrations of Cu exposures in Port Curtis.

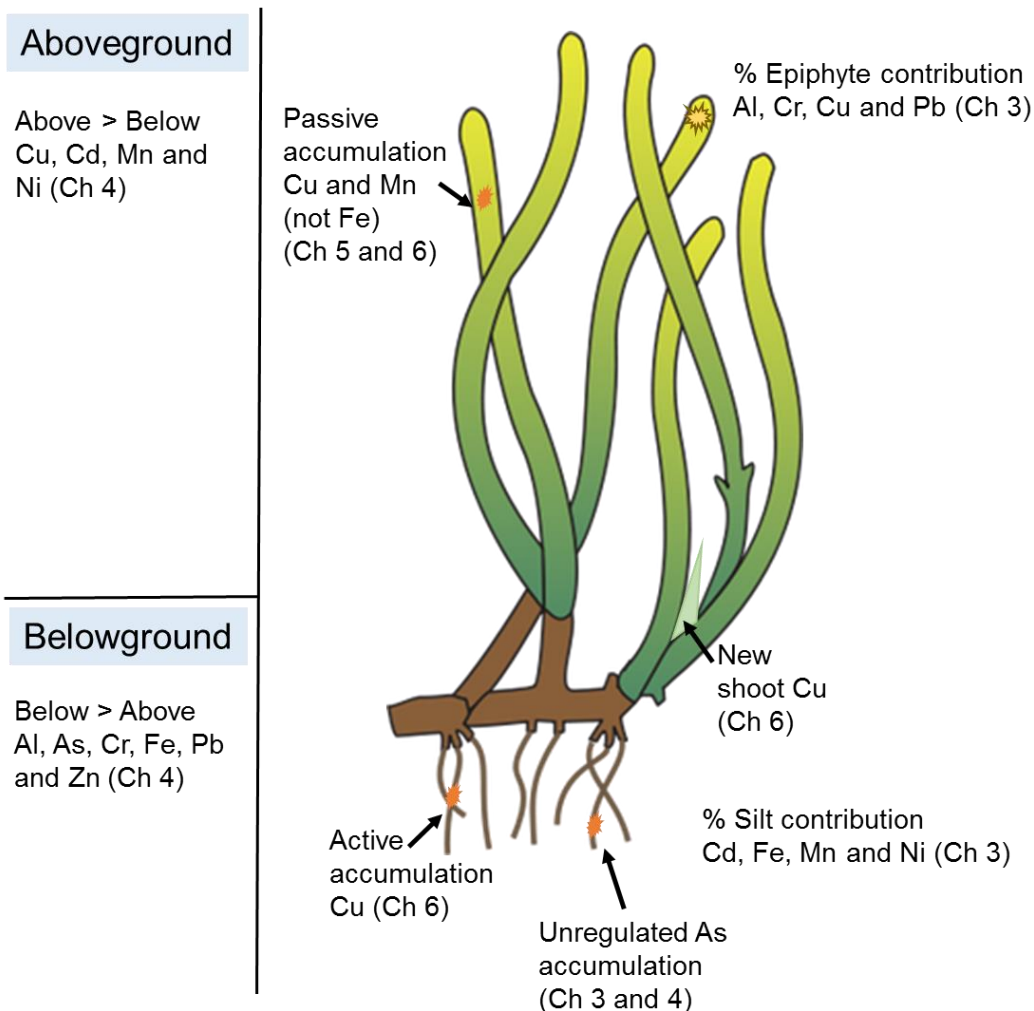
## 7.3 Relevance

Bioindicators should be local and ecologically relevant to the environment being measured and use of individual indicator species is therefore restricted to their natural geographical distributions (Flint et al. 2017; Oliva et al. 2012). *Zostera muelleri* is a local ecologically relevant seagrass species in Port Curtis and in other geographic locations, where it can be a source of food for a range of animals, provide habitat, improve meadow stability and influence localised biogeochemical cycling (Larkum, Kendrick & Ralph 2018; Prior, Booth & Limpus 2015). An understanding of *Z. muelleri* TE concentrations is ecologically relevant in Port Curtis due to the potential bio-transfer of

TEs to endangered species that graze on seagrass, such as dugong and green turtles. Previous studies, inside and outside Port Curtis have suggested that poor turtle health is linked with accumulation of pollutants such as TEs (Gaus et al. 2012; Gaus et al. 2019). While a direct link between seagrass TEs and green turtle TEs is yet to be made, the monitoring and understanding of *Z. muelleri* TE concentrations as an indicator of TEs within the environment could assist in understanding turtle health. Given its broad distribution (Ferguson et al. 2018; Green & Short 2003), *Z. muelleri* could be applied as an Australian east coast and New Zealand cosmopolitan bioindicator and be relevant and applicable to other coastal areas beyond Port Curtis.

## 7.4 Response

Through the interpretation of the results, a bioindicator is to be sensitive in indicating the presence and changes of TEs over time and space in relation to environmental TEs (Rainbow 2006). Firstly, in the current study *Z. muelleri* demonstrated different capabilities of accumulation and regulation for each TE with examples summarised in Figure 7.1. For example, Zn concentrations appeared to be regulated by the whole plant (Chapters 3 and 4), whilst increased concentrations of As in the root and rhizomes suggest the lack of an exclusion mechanism (Chapters 3 and 4). The laboratory experiments provided evidence of different accumulation mechanisms with passive leaf Cu (Chapters 5 and 6) and Mn uptake (Chapter 5), regulated leaf Fe uptake (Chapter 5) and active below-ground Cu uptake (Chapter 6, Fig. 7.1).



**Figure 7.1. Diagrammatic summary of *Zostera muelleri* and its pattern of trace element accumulation as derived from each chapter. Seagrass image: courtesy of the integration and application network, University of Maryland Center of Environmental Science ([ian.umes.edu/symbols/](http://ian.umes.edu/symbols/)).**

#### 7.4.1 Spatial variability of TE concentrations in *Zostera muelleri*

A good spatial bioindicator should reflect location specific TE concentrations. Variability across the spatial scale was due to the localised meadow sediment heterogeneity, water physico-chemistry (e.g., pH), TE behaviour and the Port Curtis scale environmental variables (Chapter 3). The two pollution indices (TEPI and TESVI, Chapter 3) clearly demonstrated that *Z. muelleri* TE concentrations at each location were different to each other (TEPI). Additionally, TESVI values for each individual TE concentration in *Z. muelleri* samples varied across Port Curtis from no variability (Zn) to high variability (Fe, Chapter 3). *Zostera muelleri* TE results demonstrated that it was indicating each locations different TE concentrations either due to natural or anthropogenic sources

(Chapter 3). For example, as described in Chapter 3, seagrass TEs that displayed a spatial pattern due to possible natural localised sources were Ni within the Narrows (Black Swan Island), possibly due to the lower pH that assisted in the release of bioavailable Ni (Angel et al. 2010). Cadmium and Mn were found in elevated concentrations at neighbouring seagrass meadows of South Trees and Lilley's Beach, most likely due to the localised release and sourcing of natural Mn (Anastasi & Wilson 2010). The other TE of note that could be displaying low concentrations of an anthropogenic source is Cu, as higher concentrations were found at the locations closer to Gladstone and therefore *Z. muelleri* could be recommended as a good spatial bioindicator for monitoring the local low Cu concentrations. The understanding of sources and patterns of TEs, and potentially the hydrology of Port Curtis, could be enhanced by the addition of other meadows throughout Port Curtis, especially those away from estuarine influences.

Examples of where *Z. muelleri* may not be a good spatial TE bioindicator include for As, due to high variability within meadows, and Zn, due to the lack of a significant difference between and within meadows (Chapter 3). However, BCF values (Chapter 3 and 4) demonstrated that seagrass had high accumulation of As and Zn from the environment, suggesting that other uptake or regulation mechanisms must exist within *Z. muelleri* to control the overall concentrations, such as homeostasis for Zn or lack of regulation for As. The lack of significant differences between and within locations for As and Zn could be due to the seagrass being analysed as whole samples, and significant differences may be found when separating the above-ground and below-ground compartments. Elucidating the differences within a location for each compartment (Chapter 4) demonstrated that there was no significant site effect for As and Zn within the below-ground compartment, suggesting that at up to 120 m (the maximum distance between samples collected in Chapter 4) concentrations should be representative of the area (meadow) unless there is significant local sediment influence. *Zostera muelleri* as a bioindicator of As accumulation will potentially not demonstrate localised sources of As, and is therefore not recommended as a bioindicator as interpretation of results is not fully understood and further research would be required. *Zostera muelleri* as a Zn bioindicator from this study is partially recommended as it does accumulate Zn, but a regulation process may exist and it is only from interpreting other existing studies that *Z. muelleri* can be fully recommended as a spatial Zn environmental bioindicator.

The link between TE concentrations in whole samples of *Z. muelleri* and concentrations of TEs in sediment samples was strong for seven of the ten analysed TEs (Al, Cr, Cu,

Fe, Pb, Mn and Ni); however, there was no link between seagrass samples and the concentration of dissolved TEs in water samples as the majority were below the limit of reporting (Chapter 3). However, when all of the environmental variables and seagrass morphometrics (including sediment TEs) were considered, spatial variability in *Z. muelleri* TE concentrations were partly explained by % silt and % epiphyte cover. These factors were TE specific; for example, Cu concentrations within whole seagrass was explained by epiphytes and Ni was explained by silt (Chapter 3). As silt and epiphyte cover can influence overall seagrass TE concentrations, continuing to measure these when monitoring in the future will be important for appropriate interpretation of results. Silt is an important variable to understand as the amount of silt can increase or decrease at a location, and therefore increase or decrease the bioavailable amounts of TE to the seagrass. Manipulative experiments would provide empirical evidence of the relationships between TE concentrations in the seagrass and these and other environmental variables or seagrass morphometrics.

#### **7.4.2 *Zostera muelleri* trace element temporal variability**

A good TE bioindicator would be able to represent TE concentrations over a given period of time (Rainbow 2006). However, a seagrass growth cycle (shoot turnover) and its variable uptake mechanisms can lead to differences over time (months or years) in TE integration. Evidence from the temporal field assessment (Chapter 4) demonstrates that over the growth cycle the two seagrass compartments are independent of each other for certain TEs suggesting different time integration. Additionally, it is evident that different TEs had greater accumulation and preference between compartments (e.g., Cu above>below) or a proportional relationship between compartments (e.g., Cr, Fe, Pb and Zn, Chapter 4) due to possible upwards translocation of TEs through the seagrass. Biological reasons for compartmentation or upwards translocations of TEs are for either metabolic requirements (e.g., Fe for photosynthesis) or as a method of removal for non-essential TEs (e.g., Pb) through leaf turnover (Pergent-Martini & Pergent 2000). In terms of sampling seagrass as a bioindicator, this means that consideration must be given to the timing of sampling, by either multiple sampling throughout the year and seasons, or by standardising sampling to the peak growth period each year, noting that this period may or may not fall on the same calendar dates.

##### **7.4.2.1 Above-ground accumulation**

As seen within Chapter 4, the above-ground compartment TE patterns were overall seen as a shorter term (months) bioindicator due to seasonal growth with no interpretable

significant correlation to environmental TEs. The influence of seasonal growth on the above-ground variable TE concentrations and the lack of a correlation to environmental TEs is due to the turnover changes in composition of the leaf/shoot material within the area; that is, more older leaves means longer to integrate TEs. This study investigated leaf area as a possible function of age but found that there was no relationship between TE concentrations and leaf area. This lack of a significant relationship was most likely due to heavy local grazing noted in November and possibly from other localised influences such as wind wave driven disturbance. However, knowing that *Zostera* spp. new leaves have higher concentrations of Cu (possibly observed within Chapter 6) leads to the interpretation that the leaf age ratio (young:old) could contribute to the interpretation of results of a bioindicator, as temporal changes are due to internal metabolic requirements. This seasonality of new leaves could explain the seasonal Cu concentrations within the above-ground material in addition to external loadings (Chapter 4). The above-ground accumulation over time demonstrated that Mn could be reflecting the seasonal concentrations from higher rainfall (Chapter 4). As evidenced from the laboratory experiment (Chapter 5), Mn was observed to be rapidly taken up and this supports the idea that the field leaf material can rapidly absorb local Mn, suggesting that *Z. muelleri* could be an environmental temporal (and spatial) Mn bioindicator. However, this rapid uptake was not observed for other TEs in the leaves and suggests that the other TEs have their own uptake mechanisms or that their integration is slower, such as from a steady state of a local source rather than immediately after rain or a local disturbance (Chapter 4). This study was conducted over one growing period and decisions should be made in light of this; further justification of when to sample and knowledge of TE temporal variability would be strengthened by sampling over a longer period of time.

The results presented in Chapters 5 and 6 demonstrated that *Z. muelleri* displayed different aspects of sensitivity to timing with leaf material demonstrating rapid (<24 h) Cu and slower Mn accumulation that was in proportion to exposure concentrations. However, over time the leaves displayed different behaviour, with Cu concentrations decreasing, possibly due to dilution from Cu induced growth stimulation (Chapter 5) or non-significantly increasing due to new shoot growth (Chapter 6). These outcomes support the evidence that Cu is an essential TE and concentrations are related to growth and leaf age (Brix & Lyngby 1982; Malea & Haritonidis 1999). When using *Z. muelleri* as a bioindicator, interpretation may therefore require an understanding of the factor of growth over the season and the composition of the sample with differing leaf ages or another form of standardisation such as selecting growing ends.

#### **7.4.2.2 Below-ground accumulation**

Below-ground temporal changes as seen within Chapter 4 were similar to other seagrass species in that overall most TE concentrations did not change to the same extent as the above-ground compartment. For this reason, the below-ground compartment is recommended as a long term (years) bioindicator, and is more relevant to environmental concentrations in the sediment. However, correlation relationships between constant sediment TEs and variable below-ground TEs were non-significant, with variable seagrass TEs to be driven by *Z. muelleri* seasonal growth. For example, As and Fe concentrations in the below-ground seagrass compartments markedly decreased over the growing period. One explanation for this seasonal change is that new root development at the beginning of the growth period (August–September) caused the sediment Fe to oxidise and reduce to a more bioavailable form that could be accumulated (Chapter 4). The majority of the other below-ground seagrass TEs were recorded at maximum concentrations in September, just prior to the maximum above-ground growth season, and lower concentrations were recorded in summer. This seasonal below-ground TE pattern is most likely due to the age of the root-rhizome system as the older compartments have had more time to accumulate sediment TEs. Additionally, the maximum above-ground growth was past the active growing season and not requiring the below-ground compartment to actively or passively accumulate new TEs. Therefore, it is possible that the rate of below-ground growth could possibly determine the rate of accumulation of TEs. This possibility requires further investigation and application of the found knowledge to the use of a bioindicator.

The greatest effect over time of Cu accumulation that has not been demonstrated before was the slower active accumulation of Cu by the below-ground compartment (Chapter 6, Fig. 7.1). However, as discussed in Chapter 6, the degree of active accumulation may not occur under natural conditions as the levels of light were lower than would normally occur in the field, but knowing that the root-rhizomes can actively accumulate Cu is relevant. In Chapter 5 the below-ground Cu concentrations for the high ( $50 \mu\text{g L}^{-1}$ ) Cu treatment were significantly higher than the other treatments and it was hypothesised in the discussion that the increase was due to possible downwards Cu translocation or accumulation from other sources. However, stronger evidence of active accumulation and not translocation is seen in Chapter 6 where *Z. muelleri* appeared to actively accumulate Cu to either offset the imbalance of the above-ground concentrations or for the requirement of new Cu for new above-ground growth. Understanding the internal

translocation (upwards or downwards) of Cu within *Z. muelleri* could be assisted by a two compartment study similar to that described by Lyngby, Brix and Schierup (1982). Knowing that *Z. muelleri* as a bioindicator species can actively accumulate and regulate certain TEs could potentially influence its use as a bioindicator of that TE in that TE concentrations could be elevated during the period of growth, a possible period of higher accumulation rates. It could be possible to navigate the degree of accumulation by understanding the seasonal steady state of TEs at a location. This could be tested through longitudinal studies or by comparing accumulation rates between locations.

### **7.4.3 Summary of considerations for utilising *Zostera muelleri* as a bioindicator**

Seasonal TE patterns caused by cyclical growth can influence the concentrations of an opportunistic seagrass species and its utility as a bioindicator. An understanding of when concentrations change can assist in deciding when to sample, the number of samples to collect, and how often to sample the seagrass and therefore what it is representing. The active growing months of August–September coming into maximum surface biomass during the period of September–November was an optimal time to analyse the effects of growth on TE concentrations. However, the other six months of the slower growth cycle (February–July) for *Z. muelleri* could elucidate more information on the cyclical nature of TE concentrations due to growth. If comparisons of *Z. muelleri* TE concentrations at each location were to be made between years the growing period of September–November would still be recommended as an optimal time to sample as there would be ample seagrass to collect. Secondly, the TE concentrations at the meadow could be a mix of old and new growth and therefore a mixed integration of the steady state of the local environmental TEs. Representative sampling of a meadow will determine the number of samples and the distance between sites, replicates or cores to be taken. Concentrations of TEs in the below-ground compartment of seagrass were affected by site, and more samples are required to understand within-site variability. Alternatively, a number of cores could be pooled together to represent (on average) the entire seagrass meadow. Conversely, if a meadow was to be sampled at only one site, the distance between the replicates could be increased to >5m, to be representative of the meadow. The design of the sampling regime will be dependent on the question and budget requirements. The other timeframe not considered in this study is decadal changes in *Z. muelleri* TE concentrations. Nonetheless, there is possible evidence that *Z. muelleri* could be a good long term bioindicator, as concentrations from Pelican Banks in this study were markedly lower than those reported by Prange and Dennison (2000)

(Chapter 4). However, justifying *Z. muelleri* as a long-term bioindicator (as opposed to a bioindicator for short term seasonal changes) can only be ascertained by long-term monitoring of *Z. muelleri*'s TE concentrations. This justification could be further addressed by observing TE concentrations over time at multiple sites to observe whether all sites increase or decrease and therefore tease out the site and TE specific long-term patterns of *Z. muelleri* as a bioindicator.

As discussed throughout this thesis, the capacity of each TE to be accumulated by *Z. muelleri* was different, and therefore different recommendations are made for the TEs analysed. Table 7.1 is a summary of *Z. muelleri* and observations made from the different aspects of compartments and time and spatial integrations. In Table 7.1, values are given that indicate the confidence in the recommendation of that TE within a compartment and this confidence is drawn from this study and evidence from other *Zostera* studies that strengthen a recommendation, while the different colours indicate the recommendation for each TE and compartment. At this stage a few elements can be recommended, such as Cu due to the laboratory experiments, or Cd as environmental TEs are < LoR. Conversely, the field assessments suggest that the below-ground compartment of *Z. muelleri* is not a good indicator of environmental As concentrations and the above-ground compartment is not a good indicator of environmental Fe concentrations (Table 7.1). Greater confidence in the ability of *Z. muelleri* to accumulate TEs, and subsequently its use as a bioindicator, would be enhanced by longitudinal studies and observations of higher field TE exposures, or from simple laboratory tests.

**Table 7.1. Summary table of *Zostera muelleri* behaviour with trace elements studied over the spatial and temporal scales and within the above- and below-ground compartments. Differences in colour indicate the recommendation made for the trace element, while the numbers given is the level of confidence for the recommendation that was drawn from this thesis and journal article evidence. Colour coding: red = not recommended, yellow = does accumulate but further information needed, green = can be used as a bioindicator. Confidence in recommendation: 1 = not recommended, 2 = unsure, 3 = can be recommended.**

	Spatial	Temporal	Above	Below	Comments
<b>Al</b>	3	3	3	3	Above- and below-ground could be recommended as a localised spatial and temporal bioindicator, but silt and epiphytes could influence results.
<b>As</b>	2	2	2	2	Below-ground not recommended as a bioindicator as accumulation is not controlled. Above-ground maybe be a poor bioindicator.
<b>Cd</b>	2	2	2	2	Low accumulation of Cd from the environment but temporal and spatial results could be due to external factors such as dissolved Cd, epiphytes and silt. Above-ground recommended as accumulation was greater than below-ground.
<b>Cr</b>	2	2	2	2	Low accumulation of Cr and spatial and temporal variability could be due to silt and epiphyte cover.
<b>Cu</b>	3	3	3	3	Leaves could indicate dissolved Cu over time and spatially. However active growth could influence below-ground concentrations.
<b>Fe</b>	2	2	2	2	Leaves appeared to not accumulate dissolved Fe and possibly sourced from upwards translocation. Below-ground can be a good spatial bioindicator; however, new growth could influence temporal concentrations.
<b>Pb</b>	3	3	3	3	Low accumulation by both compartments but has demonstrated a degree of temporal and spatial variability.
<b>Mn</b>	3	3	3	2	Leaves passively accumulate dissolved Mn and could be a good spatial and temporal bioindicator of Mn sources. Below-ground temporal and spatial variability due to local sources requires further research.
<b>Ni</b>	3	2	3	2	Low accumulation of Ni, but spatial differences were due to natural TE sources. Above-ground recommended as bioindicator.
<b>Zn</b>	2	2	3	3	Both compartments accumulate Zn but there appears to be a regulation process occurring. Long term studies should elucidate temporal and spatial variability

## 7.5 Conclusion

Measuring TE presence and impacts within the coastal environment requires the use of bioindicators to understand the risk of bioavailable TE concentrations. This study has demonstrated the potential use of the local ecologically relevant intertidal seagrass *Z. muelleri* as a TE bioindicator for coastal TE management. This research has demonstrated that *Z. muelleri* has the capacity to be a temporal and spatial accumulator of certain TEs from the environment; however, the interaction of age, growth, compartment tested, and other TE specific uptake mechanisms influenced overall TE concentrations. Additionally, research of environmental variables such as light and salinity has shown the potential of *Z. muelleri* to bioaccumulate TEs irrespective of variable light and salinity levels. Within the field, however, environmental variables at a location such as silt and epiphyte cover can contribute to certain TE concentrations in *Z. muelleri*. Overall, this research has demonstrated the potential of *Z. muelleri* to be a TE bioindicator; however, concentrations of each TE within the seagrass is TE specific and knowledge of its behaviour may come from further long-term or laboratory studies.

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# Appendix A

**Table A1. National Measurement Institute laboratory quality assurance report for sediment samples for analysed trace elements.**

Page 1 of 2



Australian Government  
National Measurement Institute

## QUALITY ASSURANCE REPORT

Client: Central Queensland University

NMI QA Report No: CQU01/180518

Sample Matrix: Sediment

Analyte	Method	LOR	Blank	Duplicates		RPD	Recoveries	
				Sample	Duplicate		LCS	Matrix Spike
		mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
<b>Inorganics Section</b>				N18/015155				N18/015155
Aluminium	NT2.49	0.5	<0.5	3950	4350	10	115	99
Arsenic	NT2.49	0.5	<0.5	7.6	8.1	6	109	102
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	101
Chromium	NT2.49	0.5	<0.5	11	12	9	115	95
Copper	NT2.49	0.5	<0.5	3.0	3.2	6	116	90
Iron	NT2.49	0.5	<0.5	7980	8430	5	102	99
Lead	NT2.49	0.5	<0.5	2.5	2.6	4	110	84
Manganese	NT2.49	0.5	<0.5	150	160	6	108	91
Nickel	NT2.49	0.5	<0.5	3.7	3.9	5	116	94
Zinc	NT2.49	0.5	<0.5	16	17	6	113	86
<b>Inorganics Section</b>				N18/015175				N18/015175
Aluminium	NT2.49	0.5	<0.5	3910	3610	8	115	84
Arsenic	NT2.49	0.5	<0.5	6.6	6.6	0	109	96
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	100
Chromium	NT2.49	0.5	<0.5	11	11	0	115	92
Copper	NT2.49	0.5	<0.5	2.7	2.8	4	116	89
Iron	NT2.49	0.5	<0.5	7500	7590	1	102	88
Lead	NT2.49	0.5	<0.5	2.4	2.4	0	110	84
Manganese	NT2.49	0.5	<0.5	120	120	0	108	85
Nickel	NT2.49	0.5	<0.5	3.5	3.5	0	116	89
Zinc	NT2.49	0.5	<0.5	15	15	0	113	86
<b>Inorganics Section</b>				N18/015240				N18/015240
Aluminium	NT2.49	0.5	<0.5	2090	2430	15	115	87
Arsenic	NT2.49	0.5	<0.5	9.2	11	18	109	101
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	100
Chromium	NT2.49	0.5	<0.5	7.5	8.9	17	115	94
Copper	NT2.49	0.5	<0.5	1.2	1.3	8	116	89
Iron	NT2.49	0.5	<0.5	6210	7320	16	102	97
Lead	NT2.49	0.5	<0.5	1.8	2.0	11	110	84
Manganese	NT2.49	0.5	<0.5	170	210	21	108	102
Nickel	NT2.49	0.5	<0.5	2.5	2.9	15	116	92
Zinc	NT2.49	0.5	<0.5	8.5	10	16	113	85
<b>Inorganics Section</b>				N18/015260				N18/015260
Aluminium	NT2.49	0.5	<0.5	8480	7050	18	115	87
Arsenic	NT2.49	0.5	<0.5	11	9.5	15	109	101
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	100
Chromium	NT2.49	0.5	<0.5	22	20	10	115	94
Copper	NT2.49	0.5	<0.5	10	8.9	12	116	91
Iron	NT2.49	0.5	<0.5	15300	13300	14	102	106
Lead	NT2.49	0.5	<0.5	6.1	5.3	14	110	86
Manganese	NT2.49	0.5	<0.5	70	60	15	108	92
Nickel	NT2.49	0.5	<0.5	10	8.8	13	116	101
Zinc	NT2.49	0.5	<0.5	28	25	11	113	88
<b>Inorganics Section</b>				N18/015328				N18/015328
Aluminium	NT2.49	0.5	<0.5	4130	4790	15	115	90
Arsenic	NT2.49	0.5	<0.5	8.6	9.4	9	109	100
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	99
Chromium	NT2.49	0.5	<0.5	13	14	7	115	94
Copper	NT2.49	0.5	<0.5	2.8	3.2	13	116	90
Iron	NT2.49	0.5	<0.5	9250	10300	11	102	109
Lead	NT2.49	0.5	<0.5	2.4	2.9	19	110	83
Manganese	NT2.49	0.5	<0.5	130	140	7	108	92
Nickel	NT2.49	0.5	<0.5	4.1	4.4	7	116	93
Zinc	NT2.49	0.5	<0.5	19	21	10	113	84



### QUALITY ASSURANCE REPORT

Inorganics Section				N18/015384				N18/015384
Aluminium	NT2.49	0.5	<0.5	4220	4080	3	115	83
Arsenic	NT2.49	0.5	<0.5	8.7	8.6	1	109	102
Cadmium	NT2.49	0.5	<0.5	<0.5	<0.5	NA	104	99
Chromium	NT2.49	0.5	<0.5	13	12	8	115	94
Copper	NT2.49	0.5	<0.5	2.9	2.7	7	116	91
Iron	NT2.49	0.5	<0.5	9370	9210	2	102	108
Lead	NT2.49	0.5	<0.5	2.5	2.4	4	110	84
Manganese	NT2.49	0.5	<0.5	140	130	7	108	99
Nickel	NT2.49	0.5	<0.5	4.0	3.9	3	116	92
Zinc	NT2.49	0.5	<0.5	19	19	0	113	86

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**Legend:**

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data cannot be reported.

\*\*: reference value not available

\* sample was not spiked for this element

**Comments:**

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

**Signed:**

Dr Andrew Evans  
Inorganics Section, NMI-North Ryde  
15/06/2018

**Date:**

Table A2. National Measurement Institute laboratory quality assurance report of seagrass samples supplied for Chapter 3, 4 and 5.



Australian Government  
National Measurement Institute

Page 1 of 1

Client: CENTRAL QUEENSLAND UNI  
NMI QA Report No: CQU01/180518 T1  
Sample Matrix: seagrass

QUALITY ASSURANCE REPORT

Analyte	Method	LOR	Blank	Recoveries																
				Sample mg/kg	Duplicate mg/kg	RPD %	Sample mg/kg	Duplicate mg/kg	RPD %	Sample mg/kg	Duplicate mg/kg	RPD %	LCS	Matrix Spike	Matrix Spike	Matrix Spike				
													%	%	%	%				
Inorganics Section				N18/015123			N18/015197			N18/015217			N18/015197							
Aluminum	NT2.46	0.5	<0.5	470	560	17.5	1410	1150	20.3	790	830	4.9	101	104						
Arsenic	NT2.46	0.05	<0.05	22	21	4.7	19	19	0.0	26	27	3.8	102	105						
Cadmium	NT2.46	0.01	<0.01	0.10	0.10	0.0	0.082	0.093	12.6	0.12	0.12	0.0	100	100						
Chromium	NT2.46	0.05	<0.05	0.91	1.0	9.4	3.2	2.4	28.6	1.8	2.0	10.5	99	102						
Copper	NT2.46	0.01	<0.01	4.1	4.4	7.1	2.9	2.9	0.0	6.1	6.7	9.4	97	102						
Iron	NT2.46	0.5	<0.5	8450	8990	6.2	13000	11400	13.1	12500	13800	9.9	103	111						
Lead	NT2.46	0.01	<0.01	0.89	1.0	11.6	2.4	2.0	18.2	1.7	2.0	16.2	101	101						
Manganese	NT2.46	0.01	<0.01	83	86	4.7	110.0	81.0	30.4	79	88	10.8	104	100						
Mercury	NT2.46	0.01	<0.01	<0.01	<0.01	NA	0.01	<0.01	NA	<0.01	<0.01	NA	101	94						
Nickel	NT2.46	0.01	<0.01	0.84	0.88	4.7	2.0	1.8	10.5	2.2	2.5	12.8	98	100						
Selenium	NT2.46	0.05	<0.05	0.13	0.14	7.4	NA	NA	NA	NA	NA	NA	91	NA						
Zinc	NT2.46	0.01	<0.01	32	33	3.1	24	27	11.8	23	25	8.3	98	103						
Inorganics Section				N18/015042			N18/015079			N18/015103			N18/015042				N18/015079		N18/015103	
Aluminum	NT2.46	0.5	<0.5	390	380	2.6	150	160	6.5	480	390	20.7	101	99		95		99		
Arsenic	NT2.46	0.05	<0.05	3.4	2.8	19.4	1.9	1.8	5.4	25	28	11.3	102	104		103		90		
Cadmium	NT2.46	0.01	<0.01	0.081	0.083	2.4	0.13	0.12	8.0	0.091	0.083	9.2	100	98		98		99		
Chromium	NT2.46	0.05	<0.05	0.69	0.62	10.7	0.28	0.25	11.3	1.0	0.79	23.5	99	98		97		100		
Copper	NT2.46	0.01	<0.01	6.7	7.0	4.4	8.8	8.5	1.2	4.4	3.9	12.0	97	98		97		100		
Iron	NT2.46	0.5	<0.5	2380	2350	1.3	1270	1290	1.6	7260	7090	2.4	103	102		101		94		
Lead	NT2.46	0.01	<0.01	0.72	0.75	4.1	0.44	0.44	0.0	1.3	1.2	8.0	101	97		95		101		
Manganese	NT2.46	0.01	<0.01	150	160	6.5	110	200	16.2	76	76	2.6	104	97		97		93		
Mercury	NT2.46	0.01	<0.01	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	101	97		97		100		
Nickel	NT2.46	0.01	<0.01	1.2	1.2	0.0	1.0	1.1	9.5	0.80	0.70	13.3	98	97		98		100		
Selenium	NT2.46	0.05	<0.05	0.47	0.45	4.3	0.24	0.27	11.8	0.18	0.16	11.8	91	97		99		103		
Zinc	NT2.46	0.01	<0.01	20	20	0.0	15	14	6.9	30	26	6.9	98	97		96		99		
Inorganics Section				N18/015273			N18/015316			N18/015373			N18/015273				N18/015316		N18/015373	
Aluminum	NT2.46	0.5	<0.5	340	260	26.7	420	430	2.4	390	360	8.0	101	97		98		98		
Arsenic	NT2.46	0.05	<0.05	7.2	7.5	4.1	6.1	5.7	6.8	8.2	8.9	8.2	102	108		102		96		
Cadmium	NT2.46	0.01	<0.01	0.11	0.11	0.0	0.10	0.10	0.0	0.12	0.11	8.7	100	100		100		96		
Chromium	NT2.46	0.05	<0.05	0.76	0.64	17.1	0.79	0.89	11.9	0.82	0.74	10.3	99	98		99		96		
Copper	NT2.46	0.01	<0.01	4.0	3.8	10.5	5.3	5.2	1.9	5.6	5.1	9.3	97	99		96		99		
Iron	NT2.46	0.5	<0.5	3660	3560	2.8	3620	3610	5.1	3830	3600	6.2	103	100		100		96		
Lead	NT2.46	0.01	<0.01	0.71	0.53	29.0	0.72	0.78	5.4	0.65	0.61	6.3	101	100		99		96		
Manganese	NT2.46	0.01	<0.01	15	14	6.9	16	16	0.0	16	13	20.7	104	100		98		96		
Mercury	NT2.46	0.01	<0.01	<0.01	<0.01	NA	<0.01	<0.01	NA	<0.01	<0.01	NA	101	100		106		102		
Nickel	NT2.46	0.01	<0.01	0.97	0.81	18.0	0.70	0.77	9.5	0.70	0.64	9.0	98	101		99		97		
Selenium	NT2.46	0.05	<0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	91	NA		NA		NA		
Zinc	NT2.46	0.01	<0.01	22	21	4.7	31	28	10.2	28	27	3.6	98	100		99		96		

Filename =

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Legend:

Acceptable recovery is 75-120%

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

*Dr Andrew Evans*

Dr Andrew Evans  
Inorganics, NMI-North Ryde  
27/06/2018

Date:

## Appendix B

**Table B1. Univariate two-way ANOVA results for each trace element concentration in sediment samples, by location and site (site nested within location). No Cadmium as results were < limit of reporting. Values in bold are significant  $p < 0.05$ . Tukey Post-hoc results between locations with location abbreviations: BS = Black Swan Island, LB = Lilley's Beach, PB = Pelican Banks, RB = Rodds Bay and ST = South Trees.**

	df	MS	F	p	Post-hoc	MS	F	p	Post-hoc
<b>Aluminium</b>						<b>Arsenic</b>			
Location	4	205868828.89	101.33	<b>0.000</b>	LB<RB,ST,PB<BS	678.94	815.81	<b>0.000</b>	PB,RB<ST,BS, LB
Site (Lo)	10	2031702.22	10.74	<b>0.000</b>		0.83	1.31	0.27	
Residual	30	189204.44							
<b>Chromium</b>						<b>Copper</b>			
Location	4	1553.53	277.6	<b>0.000</b>	LB<RB,ST,PB<BS	4.70	303.87	<b>0.000</b>	LB,RB<ST,PB<BS
Site (Lo)	10	5.6	6.86	0.000		0.04	2.62	0.020	
Residual	30								
<b>Iron</b>						<b>Lead</b>			
Location	4	779516453.3	437.15	<b>0.000</b>	LB,RB, PB< PB=ST< BS	2.364	26.13	<b>0.000</b>	LB<PB=ST<ST=BS<BS=RB
Site (Lo)	10	1783193.3	4.1	<b>0.001</b>		0.1	1.12	0.383	
Residual	30								
<b>Manganese</b>						<b>Nickel</b>			
Location	4	2.83	80.84	<b>0.000</b>	PB<RB, BS<ST< LB	1.21	37.89	<b>0.000</b>	PB<LB, ST< ST, RB< BS
Site (Lo)	10	0.063	1.78	0.105		0.077	2.40	<b>0.031</b>	
Residual	30								
<b>Zinc</b>									
Location	4	2460.85	546.67	<b>0.000</b>	LB, RB< ST<PB< BS				
Site (Lo)	10	4.502	5.09	<b>0.000</b>					
Residual	30								

**Table B2. Univariate two-way ANOVA results for each seagrass variable and % silt, by location and site (site nested within location). Values in bold are significant  $p < 0.05$ . Tukey Post-hoc results between locations with location abbreviations: BS = Black Swan Island, LB = Lilley's Beach, PB = Pelican Banks, RB = Rodds Bay and ST = South Trees.**

	df	MS	F	p	Post-hoc	MS	F	p	Post-hoc
<b>% Seagrass cover</b>					<b>Leaf length</b>				
Location	4	1307.5	33.6	<b>0.000</b>	BS, LB < LB, RB < PB, ST	19.3	19.5	<b>0.000</b>	PB < BS, ST, RB < LB
Site (Lo)	10	92.8	2.4	<b>0.032</b>		1.8	1.8	0.101	
Residual	30								
<b>Leaf width</b>					<b>% Algae cover</b>				
Location	4	0.758	36.6	<b>0.000</b>	LB < ST, BS < RB, PB	62.18	44.4	<b>0.000</b>	LB, BS, PB, ST < RB
Site (Lo)	10	0.059	2.84	0.013		2.84	2.03	0.065	
Residual	30								
<b>% Epiphyte cover</b>					<b>Biomass (wet weight)</b>				
Location	4	76.03	76.5	<b>0.000</b>	LB < BS, PB, ST < RB	1.84	27.75	<b>0.000</b>	BS, LB < ST, PB, RB
Site (Lo)	10	3.07	3.1	<b>0.008</b>		0.19	2.8	<b>0.014</b>	
Residual	30								
<b>% Silt</b>									
Location	4	29.96	222.6	<b>0.000</b>	LB < ST, PB < RB < BS				
Site (Lo)	10	0.788	5.86	<b>0.000</b>					
Residual	30								

**Table B3. Results of the distance based linear model (DistLM) sequential test result for each seagrass TE. % epi = % epiphyte cover.**

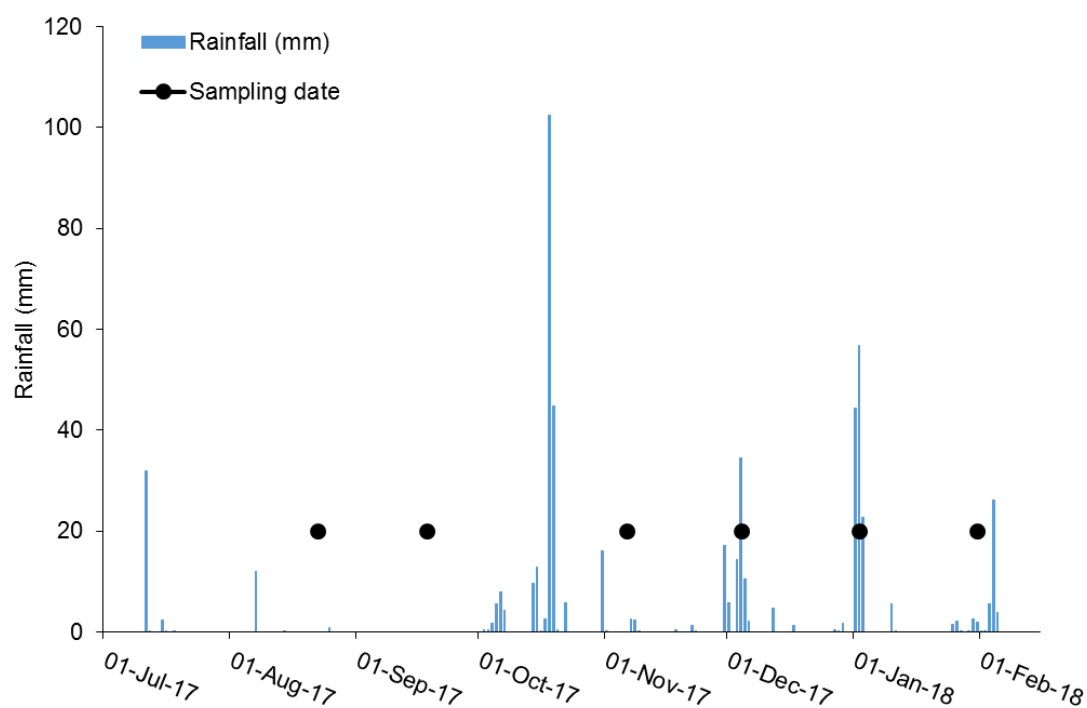
	Variable	Adjusted R <sup>2</sup>	SS (trace)	Pseudo-F	p	Proportion	Cumulative	Residual df
<b>Aluminium</b>	% silt	0.25	2380800.00	15.36	0.00	0.26	0.26	43
	% epi	0.59	3088300	36.26	0.00	0.34	0.60	42
<b>Arsenic</b>	% silt	-0.02	25.42	0.13	0.73	0.00	0.00	43
	% epi	-0.04	11.73	0.06	0.80	0.00	0.00	42
<b>Cadmium</b>	% silt	0.32	0.02	21.70	0.00	0.34	0.34	43
	% epi	0.51	0.01	18.12	0.00	0.20	0.54	42
<b>Chromium</b>	% silt	0.29	14.98	19.12	0.00	0.31	0.31	43
	% epi	0.61	15.75	36.89	0.00	0.32	0.63	42
<b>Copper</b>	% silt	-0.01	1.92	0.40	0.53	0.01	0.01	43
	% epi	0.25	57.22	16.17	0.00	0.28	0.28	42
<b>Iron</b>	% silt	0.48	741920000	42.09	0.00	0.49	0.49	43
	% epi	0.67	287260000	25.64	0.00	0.19	0.69	42
<b>Lead</b>	% silt	0.28	9.00	18.26	0.00	0.30	0.30	43
	% epi	0.61	9.99	37.40	0.00	0.33	0.63	42
<b>Manganese</b>	% silt	0.37	124100.00	26.89	0.00	0.38	0.38	43
	% epi	0.44	24495.00	5.91	0.02	0.08	0.46	42
<b>Nickel</b>	% silt	0.35	9.02	24.38	0.00	0.36	0.36	43
	% epi	0.33	0.02	0.05	0.81	0.00	0.36	42
<b>Zinc</b>	% silt	0.12	78.02	6.93	0.01	0.14	0.14	43
	% epi	0.10	2.08	0.18	0.69	0.00	0.14	42

## Appendix C

**Table C1. Recorded monthly and mean daily rainfall from the Gladstone airport weather station (Bom.gov.au).**

	Rainfall Monthly Total	Rainfall Daily Mean
	mm	mm
July 2017	35.00	1.13
August 2017	13.00	0.42
September 2017	0.00	0.00
October 2017	214.80	7.16
November 2017	24.40	0.81
December 2017	75.20	2.43
January 2018	138.40	4.46
February 2018	212.00	6.84

**Figure C2. Recorded daily rainfall from July 2017 to February 2018. Sourced from Gladstone airport weather station (www.bom.gov.au). Sampling dates: 22 August 2017, 18 September 2017, 6 November 2017, 4 December 2017, 2 January 2018 and 31 January 2018.**



**Table C2. Two-way ANOVA and post-hoc results for the above-ground compartment for all trace elements. Month: 1 = August 17, 2 = September 17, 3 = November 2017, 4 = December, 5 = 2 January 2018, and 6 = 31 January 2018. Site: 1, 2 and 3. Bold indicates significance  $p < 0.05$ .**

	df	MS	F	p	Post-hoc	MS	F	p	Post-hoc
<b>Aluminium</b>					<b>Arsenic</b>				
Month	5	24136	14.5	<b>0.00</b>	1, 4, 5, 6 < 2, 3	1.182	4.17	<b>0.004</b>	4,1,3,5,6,<,3,5,6,2
Site	2	3201	1.92	0.16		0.325	1.15	0.329	
Month*site	10	1090	0.65	0.76		0.476	1.68	0.124	
Error	36	1670				0.283			
<b>Cadmium</b>					<b>Chromium</b>				
Month	5	0.009	56.9	<b>0.00</b>	2,1 < 3,5,4 < 6	0.063	10.1	<b>0.00</b>	1,6,5,4<3,2
Site	2	0.000	1.47	0.24		0.005	0.79	0.46	
Month*site	10	0.000	0.57	0.83		0.004	0.65	0.76	
Error	36	0.000				0.006			
<b>Copper</b>					<b>Iron</b>				
Month	5	20.0	26.8	<b>0.00</b>	2,3<1,5<5,6,4	629620	8.09	<b>0.000</b>	4,3,6,1<3,6,1,5<5,2
Site	2	0.11	0.15	0.87		265612	3.41	<b>0.044</b>	2,3<3,1
Month*site	10	0.82	1.1	0.39		144872	1.86	0.084	
Error	36	0.75				77840			
<b>Lead</b>					<b>Manganese</b>				
Month	5	0.09	19.1	<b>0.000</b>	4,1,6,5<3,2	0.96	30.3	<b>0.000</b>	1,24<2,4,3<3,6<5
Site	2	0.036	7.39	<b>0.002</b>	1,2<2,3	0.05	1.63	0.209	
Month*site	10	0.008	1.63	0.14		0.04	1.13	0.370	
Error	36	0.005				0.03			
<b>Nickel</b>					<b>Zinc</b>				
Month	5	0.249	18.4	<b>0.000</b>	1,2<2,3<3,4,6<4,5,6	56.7	6.77	<b>0.000</b>	5,3,6,4<4,1,2,
Site	2	0.037	2.76	0.076		0.35	0.04	0.959	
Month*site	10	0.011	0.82	0.616		9.46	1.13	0.368	
Error	36	0.014				8.37			

**Table C3. Two-way ANOVA and post-hoc results for the below-ground compartment for all trace elements. Month: 1 = August 17, 2 = September 17, 3 = November 2017, 4 = December, 5 = 2 January 2018, and 6 = 31 January 2018. Site: 1, 2 and 3. Bold indicates significance  $p < 0.05$ .**

	df	MS	F	p	Post-hoc	df	MS	F	p	Post-hoc
<b>Aluminium</b>						<b>Arsenic</b>				
Month	5	75561	10.95	<b>0.000</b>	1,6<6,3,2<3,2,5<2,5,4	5	1272	19.3	<b>0.000</b>	6,5,4<5,4,3<1,2
Site	2	28390	4.15	<b>0.025</b>	1,3<3,2	2	47.06	0.71	0.497	
Month*site	10	10135	1.49	0.19		10	80.24	1.22	0.314	
Error	36	6900				36	66.02			
<b>Cadmium</b>						<b>Chromium</b>				
Month	5	0.000	3.69	<b>0.008</b>	6,5,2,3<2,3,4,1	5	0.225	7.67	<b>0.000</b>	1,6,3<6,3,5<3,5,2,4
Site	2	0.000	1.39	0.263		2	0.082	2.79	0.075	
Month*site	10	0.000	1.11	0.380		10	0.026	0.87	0.570	
Error	36	0.000				36	0.029			
<b>Copper</b>						<b>Iron</b>				
Month	5	0.149	10.3	<b>0.000</b>	6<5,1,3,4,<1,3,4,2	5	33236616	9.79	<b>0.000</b>	6,5,4,3<4,3,1<1,2
Site	2	0.006	0.41	0.666		2	3325038	0.98	0.385	
Month*site	10	0.021	1.42	0.211		10	3820465	1.13	0.371	
Error	36	0.015				36	3394770			
<b>Lead</b>						<b>Manganese</b>				
Month	5	0.681	22.34	<b>0.000</b>	6,1,<1,5,4<3,2	5	989	12.7	<b>0.000</b>	6,4<4,3,1<3,1,5,2
Site	2	0.121	3.97	<b>0.028</b>	1,2<2,3	2	300	3.85	<b>0.031</b>	3,1<1,2
Month*site	10	0.065	2.14	<b>0.046</b>		10	147	1.89	0.080	
Error	36	0.030				35	78.2			
<b>Nickel</b>						<b>Zinc</b>				
Month	5	0.211	7.65	<b>0.000</b>	6,1,5,3<5,3,4,2	5	90.3	5.08	<b>0.001</b>	3,5,6,1<5,1,2,4
Site	2	0.042	1.51	0.234		2	12.7	0.72	0.496	
Month*site	10	0.033	1.19	0.327		10	10.4	0.58	0.817	
Error	36	0.028				36	17.8			

Table C4. Pearson's correlation (r) for each trace element between different above- and below-ground and sediment and dissolved trace elements. Correlation between above- and below-ground. Significant  $p < 0.05$  values in bold. Na = not applicable. Above = above-ground seagrass, below = below-ground seagrass.

	Above - sediment		Below - sediment		Above - dissolved		Below - dissolved		Above - below	
	r	p	r	p	r	p	r	p	r	p
Aluminium	0.038	0.786	-0.087	0.534	Na		Na		0.158	0.253
Arsenic	-0.125	0.369	0.255	0.063	0.666	0.148	-0.742	0.091	0.138	0.319
Cadmium	Na		Na		Na		Na		-0.256	0.062
Chromium	0.01	0.944	-0.053	0.704	Na		Na		0.335*	<b>0.013</b>
Copper	-0.074	0.593	0.127	0.358	Na		Na		-0.212	0.124
Iron	-0.077	0.582	0.154	0.266	0.505	0.306	0.509	0.302	0.521**	<b>0.000</b>
Lead	0.033	0.812	-0.002	0.990	Na		Na		0.713**	<b>0.000</b>
Manganese	-0.196	0.156	0.191	0.171	-0.152	0.774	0.333	0.519	0.108	0.443
Nickel	-0.269*	<b>0.049</b>	-0.092	0.509	Na		Na		0.148	0.287
Zinc	0.25	0.068	0.038	0.783	Na		Na		0.504**	<b>0.000</b>

**Table C5. Results of one-way ANOVA for BCF values of above-ground to sediment for all trace elements except Cd as sediment Cd was < limit of reporting. Significant  $p < 0.05$  values in bold.**

		<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
<b>Aluminium</b>	Between Groups	0.008	5	0.002	11.454	<b>0.000</b>
	Within Groups	0.007	48	0.000		
	Total	0.015	53			
<b>Arsenic</b>	Between Groups	0.143	5	0.029	3.767	<b>0.006</b>
	Within Groups	0.364	48	0.008		
	Total	0.507	53			
<b>Chromium</b>	Between Groups	0.003	5	0.001	10.145	<b>0.000</b>
	Within Groups	0.003	48	0.000		
	Total	0.005	53			
<b>Copper</b>	Between Groups	17.041	5	3.408	21.105	<b>0.000</b>
	Within Groups	7.752	48	0.161		
	Total	24.793	53			
<b>Iron</b>	Between Groups	0.055	5	0.011	5.058	<b>0.001</b>
	Within Groups	0.105	48	0.002		
	Total	0.160	53			
<b>Lead</b>	Between Groups	0.085	5	0.017	11.460	<b>0.000</b>
	Within Groups	0.071	48	0.001		
	Total	0.156	53			
<b>Manganese</b>	Between Groups	19.396	5	3.879	33.308	<b>0.000</b>
	Within Groups	5.590	48	0.116		
	Total	24.986	53			
<b>Nickel</b>	Between Groups	0.135	5	0.027	15.221	<b>0.000</b>
	Within Groups	0.085	48	0.002		
	Total	0.219	53			
<b>Zinc</b>	Between Groups	0.823	5	0.165	4.006	<b>0.004</b>
	Within Groups	1.972	48	0.041		
	Total	2.795	53			

**Table C6. Results of one-way ANOVA for BCF values of below-ground to sediment for all trace elements except Cd as sediment Cd was < limit of reporting. Significant  $p < 0.05$  values in bold.**

		<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
<b>Aluminium</b>	Between Groups	0.033	5	0.007	11.085	<b>0.000</b>
	Within Groups	0.029	48	0.001		
	Total	0.062	53			
<b>Arsenic</b>	Between Groups	119.183	5	23.837	14.972	<b>0.000</b>
	Within Groups	76.420	48	1.592		
	Total	195.603	53			
<b>Chromium</b>	Between Groups	0.012	5	0.002	9.317	<b>0.000</b>
	Within Groups	0.013	48	0.000		
	Total	0.025	53			
<b>Copper</b>	Between Groups	1.605	5	0.321	8.318	<b>0.000</b>
	Within Groups	1.853	48	0.039		
	Total	3.458	53			
<b>Iron</b>	Between Groups	2.526	5	0.505	7.308	<b>0.000</b>
	Within Groups	3.318	48	0.069		
	Total	5.844	53			
<b>Lead</b>	Between Groups	0.638	5	0.128	13.584	<b>0.000</b>
	Within Groups	0.451	48	0.009		
	Total	1.090	53			
<b>Manganese</b>	Between Groups	0.379	5	0.076	1.702	0.152
	Within Groups	2.140	48	0.045		
	Total	2.519	53			
<b>Nickel</b>	Between Groups	0.096	5	0.019	6.458	<b>0.000</b>
	Within Groups	0.143	48	0.003		
	Total	0.239	53			
<b>Zinc</b>	Between Groups	2.309	5	0.462	5.383	<b>0.001</b>
	Within Groups	4.118	48	0.086		
	Total	6.427	53			

# Appendix D

**Table D1. National Measurement Institute quality assurance report for dissolved water samples associated with Chapter 5 Cu exposure experiment.**



Australian Government  
National Measurement Institute

Page 1 of 1

## QUALITY ASSURANCE REPORT

**Client:** Central Queensland University

**NMI QA Report No:** CQU01/180518/1 T1

**Sample Matrix:** Water

Analyte	Method	LOR	Blank	Duplicates			Recoveries		
		ug/L	ug/L	Sample	Duplicate	RPD	LCS	Matrix Spike	
				ug/L	ug/L	%	%	%	
Inorganics Section				N18/015416/1				N18/015416/	
Aluminium Filtered	NT2.47	5	<5	<5	<5	NA	115	NA	
Arsenic Filtered	NT2.47	1	<1	3.2	3.3	2	101	NA	
Cadmium Filtered	NT2.47	0.1	<0.1	<0.1	<0.1	NA	97	NA	
Chromium Filtered	NT2.47	1	<1	<1	<1	NA	100	NA	
Iron Filtered	NT2.47	5	<5	27	27	0	105	NA	
Lead Filtered	NT2.47	1	<1	<1	<1	NA	99	NA	
Manganese Filtered	NT2.47	1	<1	11	11	0	100	NA	
Nickel Filtered	NT2.47	1	<1	<1	<1	NA	101	NA	
Zinc Filtered	NT2.47	1	<1	<1	<1	NA	97	NA	

Filename =

K:\Inorganics\Quality System\QA Reports\TE\QAR2018\Water\

Legend:

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

<

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data is not reliable.

Comments:

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

Signed:

Dr Andrew Evans  
Inorganics, NMI-North Ryde  
24/07/2018

Date:



# QUALITY ASSURANCE REPORT

**Client:** Central Queensland University

**NMI QA Report No:** CQU01/180518 T1

**Sample Matrix:** Water

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
		ug/L	ug/L	Sample ug/L	Duplicate ug/L	RPD %	LCS %	Matrix Spike %
Inorganics Section				N18/015416				N18/015416
Copper Filtered	NT2.47	1	<1	<1	<1	NA	100	99

Filename =

K:\Inorganics\Quality System\QA Reports\TE\QAR2018\Water\

## Legend:

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

<

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data is not reliable.

## Comments:

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

**Signed:**

Dr Andrew Evans  
Inorganics , NMI-North Ryde  
8/06/2018

**Date:**

**Table D2. Results of three-way ANOVA of Cu, time and specific conductivity (Sp. Cond.) treatment for As dissolved concentrations. Significant effects indicated in bold where  $p < 0.05$ .**

	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Cu	2	0.302	3.757	<b>0.035</b>
Time	1	2.007	25.712	<b>0.000</b>
Sp. Cond.	1	0.723	9.256	<b>0.006</b>
Cu * Time	2	0.042	0.537	0.591
Cu * Sp. Cond.	2	0.016	0.203	0.818
Time * Sp. Cond.	1	0.267	3.42	0.077
Cu * Time * Sp. Cond.	2	0.02	0.26	0.773
Error	24	0.078		

**Table D3. Results of a one-way ANOVA post-hoc test for dissolved As concentrations by specific conductivity (normal and reduced) and time (T1 = 24 h, T11 = completion of experiment) treatments.**

	N	Subset for alpha = 0.05	
		1	2
T1 Reduced	9	2.7333	
T1 Normal	9		3.1889
T11 Reduced	9		3.3778
T11 Normal	9		3.4889

**Table D4. Results of three-way ANOVA of Cu, time and specific conductivity (Sp. Cond.) treatment for Fe concentrations by separate compartments. Significant effects indicated in bold where  $p < 0.05$ .**

<b>Leaf</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	15744.444	0.968	0.394
Time	1	51377.778	3.158	0.088
Sp. Cond.	1	6944.444	0.427	0.520
Cu * Time	2	6144.444	0.378	0.689
Cu * Sp. Cond.	2	1877.778	0.115	0.891
Time * Sp. Cond.	1	6400.000	0.393	0.536
Cu * Time * Sp. Cond.	2	10033.333	0.617	0.548
Error	24	16269.444		
<b>Root Rhizome</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	611908.333	1.743	0.197
Time	1	3074177.778	8.754	<b>0.007</b>
Sp. Cond.	1	160000.000	0.456	0.506
Cu * Time	2	288502.778	0.822	0.452
Cu * Sp. Cond.	2	58608.333	0.167	0.847
Time * Sp. Cond.	1	139377.778	0.397	0.535
Cu * Time * Sp. Cond.	2	823102.778	2.344	0.118
Error	24	351158.333		
<b>Sediment</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	5769.444	0.018	0.982
Time	1	1002.778	0.003	0.956
Sp. Cond.	1	282669.444	0.866	0.361
Cu * Time	2	88252.778	0.270	0.765
Cu * Sp. Cond.	2	76302.778	0.234	0.793
Time * Sp. Cond.	1	304336.111	0.933	0.344
Cu * Time * Sp. Cond.	2	317986.111	0.974	0.392
Error	24	326322.222		
<b>Water</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	67.027	0.856	0.437
Time	1	4428.903	56.563	<b>0.000</b>
Sp. Cond.	1	427.800	5.464	<b>0.028</b>
Cu * Time	2	71.701	0.916	0.414
Cu * Sp. Cond.	2	527.669	6.739	<b>0.005</b>
Time * Sp. Cond.	1	245.967	3.141	0.089
Cu * Time * Sp. Cond.	2	437.180	5.583	0.010
Error	24	78.300		

**Table D5. Results of three-way ANOVA of Cu, time and specific conductivity (Sp. Cond.) treatment for Mn concentrations by separate compartments. Significant effects indicated in bold where  $p < 0.05$ .**

<b>Leaf</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><math>p</math></b>
Cu	2	18533.333	1.173	0.327
Time	1	1037002.778	65.610	<b>0.000</b>
Sp. Cond.	1	11736.111	0.743	0.397
Cu * Time	2	2544.444	0.161	0.852
Cu * Sp. Cond.	2	9211.111	0.583	0.566
Time * Sp. Cond.	1	5625.000	0.356	0.556
Cu * Time * Sp. Cond.	2	25900.000	1.639	0.215
Error	24	15805.556		
<b>Root Rhizome</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><math>p</math></b>
Cu	2	0.694	0.067	0.936
Time	1	6.250	0.602	0.446
Sp. Cond.	1	30.250	2.912	0.101
Cu * Time	2	1.083	0.104	0.901
Cu * Sp. Cond.	2	5.583	0.537	0.591
Time * Sp. Cond.	1	0.028	0.003	0.959
Cu * Time * Sp. Cond.	2	14.528	1.398	0.266
Error	24	10.389		
<b>Sediment</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><math>p</math></b>
Cu	2	8.333	0.130	0.878
Time	1	44.444	0.696	0.412
Sp. Cond.	1	177.778	2.783	0.108
Cu * Time	2	36.111	0.565	0.576
Cu * Sp. Cond.	2	136.111	2.130	0.141
Time * Sp. Cond.	1	11.111	0.174	0.680
Cu * Time * Sp. Cond.	2	86.111	1.348	0.279
Error	24	63.889		
<b>Water</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><math>p</math></b>
Cu	2	4.101	0.213	0.810
Time	1	981.778	51.018	<b>0.000</b>
Sp. Cond.	1	96.694	5.025	0.035
Cu * Time	2	3.129	0.163	0.851
Cu * Sp. Cond.	2	0.674	0.035	0.966
Time * Sp. Cond.	1	10.028	0.521	0.477
Cu * Time * Sp. Cond.	2	61.590	3.201	0.059
Error	24	19.244		

**Table D6. Results of three-way ANOVA of Cu, time and specific conductivity (Sp. Cond.) treatment for Cu concentrations by separate compartments. Significant effects indicated in bold where  $p < 0.05$ .**

<b>Leaf</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	16.057	782.824	<b>0.000</b>
Time	1	0.859	41.877	<b>0.000</b>
Sp. Cond.	1	0.000	0.019	0.892
Cu * Time	2	0.104	5.079	<b>0.014</b>
Cu * Sp. Cond.	2	0.029	1.411	0.263
Time * Sp. Cond.	1	0.049	2.386	0.136
Cu * Time * Sp. Cond.	2	0.006	0.308	0.738
Error	24	0.021		
<b>Root Rhizome</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	3.029	9.359	<b>0.001</b>
Time	1	0.321	0.992	0.329
Sp. Cond.	1	0.028	0.086	0.772
Cu * Time	2	0.062	0.191	0.827
Cu * Sp. Cond.	2	0.152	0.470	0.631
Time * Sp. Cond.	1	0.111	0.343	0.563
Cu * Time * Sp. Cond.	2	0.439	1.355	0.277
Error	24	0.324		
<b>Sediment</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
Cu	2	0.025	1.034	0.371
Time	1	0.000	0.011	0.916
Sp. Cond.	1	0.022	0.920	0.347
Cu * Time	2	0.039	1.580	0.227
Cu * Sp. Cond.	2	0.003	0.102	0.903
Time * Sp. Cond.	1	0.023	0.920	0.347
Cu * Time * Sp. Cond.	2	0.053	2.148	0.139
Error	24	0.024		

**Table D7. Results of the one-way repeated measure ANOVA Maximum Quantum Yield Mauchly's test of sphericity.**

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	<i>p</i>	Epsilon <sup>b</sup>		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Time	0.030	34.335	20	<b>0.030</b>	0.462	0.864	0.167

**Table D8. Results of the one-way repeated measure ANOVA Maximum Quantum Yield, within subject effects. Factor variable equates to the following: normal and reduced; control normal, control reduced, low normal, low reduced, high normal, high reduced. Sphericity was not assumed so Greenhouse-Geisser was used.**

	df	MS	F	<i>p</i>
Time	2.770	0.011	6.468	0.002
Time * Factor	13.849	0.002	1.166	0.344
Error(time)	33.238	0.002		

**Table D9. Results of the one-way repeated measure ANOVA Maximum Quantum Yield tests of within subject contrasts. Factor variable equates to the following: normal and reduced; control normal, control reduced, low normal, low reduced, high normal, high reduced.**

		<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
time	T0 vs. T1	1	0.011	18.459	0.001
	T1 vs. T3	1	0.012	13.837	0.003
	T3 vs. T5	1	6.753E-05	0.064	0.805
	T5 vs. T7	1	0.018	6.090	0.030
	T7 vs. T9	1	0.020	5.148	0.043
	T9 vs. T10	1	5.367E-05	0.068	0.798
time * factor	T0 vs. T1	5	0.001	2.579	0.083
	T1 vs. T3	5	0.000	0.330	0.885
	T3 vs. T5	5	0.001	0.877	0.525
	T5 vs. T7	5	0.004	1.389	0.296
	T7 vs. T9	5	0.003	0.750	0.601
	T9 vs. T10	5	0.001	1.358	0.306
Error(time)	T0 vs. T1	12	0.001		
	T1 vs. T3	12	0.001		
	T3 vs. T5	12	0.001		
	T5 vs. T7	12	0.003		
	T7 vs. T9	12	0.004		
	T9 vs. T10	12	0.001		

**Table D10. Results of the one-way repeated measure ANOVA Maximum Quantum Yield tests of between subject effects.**

	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Cu	5	9.975E-05	0.752	0.600
Error	12	0.000		

# Appendix E

**Table E1. National Measurement Institute laboratory quality assurance report for seagrass samples for trace elements tested.**

Page 1 of 1



Australian Government  
National Measurement Institute

## QUALITY ASSURANCE REPORT

Client: CENTRAL QUEENSLAND UNI

NMI QA Report No: CQU01/180801

Sample Matrix: Food

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
		mg/kg	mg/kg	Sample mg/kg	Duplicate mg/kg	RPD %	LCS %	Matrix Spike
Inorganics Section				N18/022127				N18/022127
Copper	NT2.46	0.01	<0.01	5.4	4.7	13.9	96	100
Inorganics Section				N18/022137				N18/022137
Copper	NT2.46	0.01	<0.01	4.9	4.4	10.8	96	100
Inorganics Section				N18/022173				N18/022173
Copper	NT2.46	0.01	<0.01	10.0	8.8	12.8	96	103

Filename = K:\Inorganics\Quality System\QA Reports\TE\QAR2018\Food & Misc\

Legend:

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data is not reliable.

\*\*: reference value not available

Comments:

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

Signed:

Dr Andrew Evans  
Inorganics Manager, NMI-North Ryde

Date:

15/08/2018

**Table E2. National Measurement Institute laboratory quality assurance report for seagrass samples for trace elements tested.**

Page 1 of 1



**Australian Government**  
National Measurement Institute

**QUALITY ASSURANCE REPORT**

**Client:** Central Queensland University

**NMI QA Report No:** CQU01/180801 T1

**Sample Matrix:** Sediment

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
				Sample	Duplicate	RPD	LCS	Matrix Spike
		mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
Inorganics Section				N18/022177				N18/022177
Copper	NT2.49	0.5	<0.5	3.7	4.4	17	109	99
Inorganics Section				N18/022185				N18/022185
Copper	NT2.49	0.5	<0.5	3.5	3.4	3	109	97

Filename = K:\Inorganics\Quality System\QA Reports\TE\QAR2018\Soil\

**Legend:**

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data cannot be reported.

\*\*: reference value not available

\* sample was not spiked for this element

**Comments:**

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

**Signed:**

Dr Andrew Evans  
Inorganics Section, NMI-North Ryde  
14/08/2018

**Date:**

**Table E3. National Measurement Institute laboratory quality assurance report for seagrass samples for trace elements tested.**

Page 1 of 1



Australian Government  
National Measurement Institute

**QUALITY ASSURANCE REPORT**

**Client:** Central Queensland University

**NMI QA Report No:** CQU01/180801 T1

**Sample Matrix:** Water

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
		ug/L	ug/L	Sample ug/L	Duplicate ug/L	RPD %	LCS %	Matrix Spike %
Inorganics Section				N18/022194			N18/022194	
Aluminium Filtered	NT2.47	5	<5	7.5	9.3	21.4	116	104
Arsenic Filtered	NT2.47	1	<1	2.9	2.6	10.9	91	99
Cadmium Filtered	NT2.47	0.1	<0.1	<0.1	<0.1	NA	99	100
Chromium Filtered	NT2.47	1	<1	<1	<1	NA	111	100
Copper Filtered	NT2.47	1	<1	1.0	<1	NA	91	101
Iron Filtered	NT2.47	5	<5	19	25	27.3	112	100
Lead Filtered	NT2.47	1	<1	<1	<1	NA	98	100
Manganese Filtered	NT2.47	1	<1	86	87	1.2	111	100
Nickel Filtered	NT2.47	1	<1	<1	<1	NA	92	103
Zinc Filtered	NT2.47	1	<1	3.6	3.1	14.9	99	102

Filename =

K:\Inorganics\Quality System\QA Reports\TE\QAR2018\Food & Misc\

**Legend:**

Acceptable recovery is 75-120%.

Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.

LOR = Limit Of Reporting

ND = Not Determined

RPD = Relative Percent Difference

NA = Not Applicable

<

LCS = Laboratory Control Sample.

#: Spike level is less than 50% of the sample's concentration, hence the recovery data is not reliable.

**Comments:**

Results greater than ten times LOR have been rounded to two significant figures.

This report shall not be reproduced except in full.

**Signed:**

Dr Andrew Evans  
Inorganics, NMI-North Ryde  
7/08/2018

**Date:**

**Table E4. Maximum Quantum Yield one-way repeated measures ANOVA Mauchly's test of sphericity output.**

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	p	Epsilon <sup>b</sup>		
					Greenhouse- Geisser	Huynh-Feldt	Lower-bound
Time	0.040	21.791	20	0.406	0.595	1.000	0.167

**Table E5. Maximum Quantum Yield one-way repeated measures ANOVA, tests of within and between subjects effects.**

Within	df	MS	F	p
Time	6	0.002	0.521	0.790
Time * Cu	12	0.001	0.447	0.936
Error(Time)	54	0.003		

Between	df	MS	F	p
Cu	2	0.000	0.265	0.773
Error	9	0.000		

**Table E6. Water quality in experimental tanks (mean  $\pm$  SD in parenthesis, n = 4) by Cu treatment (Control, Low 5  $\mu\text{g L}^{-1}$  and High 50  $\mu\text{g L}^{-1}$ ) at time of Cu addition (T0) and at the completion of the experiment (T11).**

	Control (T0)	Control (T11)	Low (T0)	Low (T11)	High (T0)	High (T11)
Temperature*	25.3 (0.38)	25.5 (0.40)				
Dissolved Oxygen %	88.25 (7.85)	94.65 (1.63)	91.55 (4.48)		91.16 (4.69)	
Specific Conductivity $\mu\text{S cm}^{-1}$	54582 (342)	57701 (475)	54713 (52)		54598 (345)	
Salinity	36.24 (0.04)	38.44 (0.35)	36.21 (0.02)		36.13 (0.23)	
pH	7.71 (0.07)	7.91 (0.06)	7.79 (0.03)		7.68 (0.21)	
Aluminium $\mu\text{g L}^{-1}$	6.58 (1.68)	<5.0 (0.0)	5.3 (0.6)	5.35 (0.7)	6.65 (0.75)	<5.0 (0.0)
Arsenic $\mu\text{g L}^{-1}$	2.58 (0.21)	4.95 (1.07)	2.33 (0.22)	4.6 (0.45)	2.55 (0.44)	5.28 (1.02)
Cadmium $\mu\text{g L}^{-1}$	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)	<0.1 (0.0)
Chromium $\mu\text{g L}^{-1}$	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Copper $\mu\text{g L}^{-1}$	1.03 (0.05)	1.15 (0.3)	5.15 (0.65)	<1.0 (0.0)	49.5 (6.14)	2.48 (0.22)
Iron $\mu\text{g L}^{-1}$	13.0 (6.98)	13.4 (4.03)	10.8 (1.21)	16.5 (5.52)	10.4 (1.89)	18.0 (4.24)
Lead $\mu\text{g L}^{-1}$	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Manganese $\mu\text{g L}^{-1}$	58.25 (32.9)	11.4 (4.49)	54.5 (33.1)	11.7 (1.78)	59.8 (35.2)	9.18 (1.89)
Nickel $\mu\text{g L}^{-1}$	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)	<1.0 (0.0)
Zinc $\mu\text{g L}^{-1}$	3.15 (0.58)	1.93 (1.01)	2.45 (0.35)	1.58 (0.22)	3.0 (0.68)	1.25 (0.17)

\* Temperature is reported here from the logger (one tank) over the period of the experiment and averaged with the YSI logger readings from every tank.