The effects of Contaminated Rena Sediments on Juvenile Paua (*Haliotis iris*)

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Abstract

The grounding of the MV Rena on Otaiti resulted in the release of heavy fuel oil and container debris contaminants into the surrounding environments including the rocky shores of the adjacent Mōtītī Island. This is the habitat where significant populations of benthic paua reside. Paua (*Haliotis iris*) are a staple and consistent food source for Mōtītī Island. Being an offshore island with no amenities, Mōtītī Island residents are reliant on the ocean as a pataka kai (food cupboard) and are therefore acutely aware of environmental influences to the harvest of kaimoana.

This thesis aimed to address concerns relating to the effects of contaminated boundary layer water emanating from contaminated 'Rena' sediment on juvenile paua. Research was focused in two areas: 1) the sublethal behavioural effects of contaminated Rena sediment to Paua and 2) the accumulation of trace metals in the edible tissue and viscera mass. The experiments were carried out with the use of a close circuit aquaria in a laboratory environment, followed by a field experiment.

In all experiments, paua in control treatments were healthy by comparison to paua exposed to treatments with Rena contaminated sediments and copper as judged by survivorship and behaviour. The most likely cause of of behavioural aberrations and mortality observed was deemed to be copper as demonstrated by Diffusive Gradient in thin film (DGT) and ambient water analyses in both experiments. Copper that is bioavailable can increase quickly in the edible tissue and viscera mass as was identified as the visceral mass of Rena and copper exposed paua had a higher mean concentration of this and other trace metals.

On Otaiti, the effects to paua from the Rena ship wreck and lost container contents, know to include a medly of metals and other contaminants, is not likely to be limited to copper alone. Results demosntrate the relevance of examining the effects of water borne contaminated plumes emanating from complex mixtures of contaminants. This is rarely done in ecotoxicological studies which tend to focus on individual contaminant compounds.

Acknowledgements

Firstly I would like to thank my supervisors Chris Battershill and Adam Hartland, for all of their assistance and advice throughout this project. Chris, your vison, enthusiasm and “can do” attitude has made this all the more enjoyable. Thanks to both of you for sharing with me your wisdom, for showing me patience and for exposing me to great opportunities. Its greatly appreciated so cheers.

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Cheers to all the whanau and friends who have wished me luck along the way. The rounds of golf, dive missions or casual beers were mean for wind downs. And to Mum, G and the girls; thanks for looking out for things when we weren’t home. Really appreciate all pressure you took off us when we had to knuckle down.

And high five to my beautiful girlfriend, Te Puea Dempsey, for all the trials and tribulations. We got into this craziness together and now we’re there. Mean!

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My Journey

Tangaroa wainoa, Tangaroa waitapu.
Nōu ko te ngāwari, nōu ko te marino,
Nōu ko te hōhonu, nōu ko te wātea,
Nōu ko te waitapu, nōu ko te wainoa
Whakanoatia me whakatapua e
Haumi e, hui e, Taiki e!

Ko Te Moana a Toi te Moana
Ko Wairanaki te Awa
Ko Mōtītī te Motu
Ko Mataatua te Waka
Ko Ngāti Awa te Iwi
Ko Patuwai te Hapū
Ko Tamateapokaiwhenua raua ko Te Hinga o Te Ra ngā Marae
He uri ahau ō te whanau Faulkner
Ko Amelia Burrell (nee Faulkner) raua ko Maurice McSweeney oku mātua
No reira, tena koutou katoa.

This journey begins with a child that lived on a small offshore island.

The island was a magnificent paradise for some; but for me, it was known simply as home. When I was young it seemed like such a noisy place. The wave's crashed on the rocks, the wind would blow over the cliffs, a tractor or motorbike could be heard driving up the roads leaving nothing but dust trails, and the planes would chase the cows off the airstrip. To the my cousins and I, the island was their place to learn, play and explore. The rules were simple; have respect; be back before dark; behave yourself if you go to your Uncle and Aunties; and call home if it gets late. From the moment the I was born, the moana (ocean) and taiao (environment) became a part of him. I knew a wind from the northeast meant you go west for a kai and shelter; to be careful around the rocky cliffs; and the cows need to stay away from the garden. These foundations were instilled by the Kaumatua (elders)

to become intrinsically known, allowing the mokopuna (children) to always be safe, alert and respectful.

As a child I was lucky enough to have my first memories on Mōtītī Island. These memories were of a simple, but full life. Every day was an adventure and I was only limited by my imagination. We had to be resourceful and our subsistence lifestyle meant the bays around the island were our main food cupboard. Igrew up on delicate taste of kina, paua, fish and fresh vegetables from our garden.

There is one story of when my mum, dad and sister went to the beach to go for a dive and to gather food for dinner. I was about 2 years old and was placed on a rock with a fresh paua to chew on. This was normal for all the babies so the older ones could go out in the water. Mum would always keep one eye on me to make sure I was safe. One day she gave me a bigger paua and stuck me on a bigger rock to keep me away from a cake she has brought down for lunch. She kept an eye on me while she was fishing and then had a moment of panic when she couldn't see me on the rock. She rushed back and found I had climb down the big rock, over the rocky shore, and eaten all the cake!

I stayed on the island until I outgrew the school there and my parents thought it was time for me to go to the mainland (Tauranga) to carry on my schooling. It was from that moment, Mōtītī Island started to become a distant paradise. I missed the island for a long time. The more you miss something the more beautiful it becomes.

I never finished high school and worked heaps of different jobs trying to find that one that made me tick. I tried all sorts: labouring, retail and sales, working the mines in Australia, events, and landscaping. Throughout all of my travels, I always needed to be close to the sea and have access to fresh seafood or I would get sick – an indescribable type of sick. There was one job in Australia that took us inland to a mine for a few of weeks. I had been Brisbane based but we came back out via Melbourne. I has missed the coast so much I jumped straight into the Geelong for a swim and to

reconnect with the ocean. Unfortunately I got a bad ear infection from that water, but at least I was back on the coast.

Eventually I realised I need to work around the moana. I've spent the last few years making that possible, and this mahi rangahau (research) is about something important to me and my whanau. It was staple food stock growing up and the islands signature taonga kai (precious food stock). paua and other seafood don't grow like they used to. I dearly want my uri (offspring) to be able to interact with the moana the same way I did as a child, but it's dependant on the longevity of our kaimoana and culture.

My niece's first trip to the island. My sister was craving a paua on the fire so we all went home. This is what it's all about

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Glossary

Māori	English
Aotearoa	"The land of the long white cloud", New Zealand
Haka and Poi	Customary dance styles; form of expression
Hapū	Subtribe
Hawaiki	Ancient homeland
Iwi	Tribe
Kai	Food; sustenance
Kaimoana	Seafood
Kaitiaki	Custodian; guardian; caretaker
Kaitiakitanga	The action of guardianship
Karakia	Prayer or incantation
Kaumātua	Elder
Kawa	Customary protocol
Mana	Prestige
Manaakitanga	To show hospitality
Manuhiri	Visitor; guest
Māori	Indigenous people of New Zealand
Mātauranga [Māori]	Māori knowledge and way of life
Mauri	Essential life force
Moana	Ocean or sea
Mōtītī	An island within the Bay of Plenty region
Mōtītītanga	Mōtītī identity and way of life
Ngā uri o Mōtītī	The descendants of Mōtītī
Ngātoroirangi	Distinguished Te Arawa priest
Otaiti	Astrolabe Reef
Pakiwaitara	Legends; old stories
Pataka kai	Food cupboard
Pepeha	Tribal identifier
Powhiri	Formal welcome
Rohe Moana	Customary fishing area
Tangaroa	God of the sea
Tangata Whenua	Local people

Tangi	Grieve; morn
Taonga	Prized or treasured item or resource
Tapu	Sacred; restriction
Te Arawa	Tribe from central north island to east coast
Te Ika-a-Māui	North Island
Te Moana-a-Toi-Te-Huatahi	The Bay of Plenty region
Te Reo Rangatira	The language of chiefs
Tikanga	Custom
Tohunga	Expert; agent of the spiritual realm
Tūhua	Mayor Island
Waharoa	Gateway
Wāhi tapu	Sacred site; site of cultural significance
Waiata	Song
Whakapapa	Genealogy
Whanau	Family; kin
Whenua	Land

Chapter 1

General Introduction

1.1 Otaiti and Mōtītī Island

Otaiti (Astrolabe Reef) is located within Te Moana-a-Toi-Te-Huatahi. It is approximately 21 km north-east of Tauranga Harbour, 9 km from Papamoa and 7 km from Mōtītī Island (Ministry for the Environment, 2011) off the eastern coast of Te Ika-a-Māui in Aotearoa. The full name for Otaiti is "Te Tau O Taiti" which refers to the waharoa to Mōtītī. Oral history tells us that when Ngātoroirangi; a distinguished Te Arawa tohunga, was on his voyage from the ancestral homeland of Hawaiki, he stopped at Otaiti to perform karakia before landing on Mōtītī, where he spent the remainder of his days. Because of this, Otaiti is a wāhi tapu to the tangata whenua of Mōtītī Island (Ngāi Te Hapū Incorporated, 2014).

Mōtītī Island is a private island occupied by the local hapū Te Patuwai. Patuwai, meaning slain in the water, is the name acquired after a battle at sea between Whakatōhea and Ngāi Te Hapū. Ngāi Te Hapū and Te Patuwai share the same ancestral lines, so are one and the same people. Ngāi Te Hapū/Patuwai occupation on Mōtītī Island has been long standing. The use of the resources from Mōtītī Island, and the surrounding reefs, rocks and islets has sustained the Hapū since the mid-17th century, with cultivation of crops developing in more recent times (Ngāi Te Hapū Incorporated, 2014). The Wills family and Sunchaser Avocados Limited share occupation of the southern section of the island, with Ngāi Te Hapū/Patuwai living at the northern end.

1.1.1 Cultural Values of Mōtītī Island

The cultural values important to Mōtītī have been identified by Ngāi Te Hapū Incorporated (2014). Respect is upmost for the hapū; for the people,

whenua, moana, other waterways, reefs, rocks, islands on and surrounding Mōtītī and all tradition sites inherited. The maintenance of cultural practises through the observance of proper tikanga which include the rituals of karakia, pōwhiri and tangi, the use of te reo rangatira, pepeha, whakapapa, waiata, pakiwaitara, haka and poi are also of importance (Ngāi Te Hapū Incorporated, 2014).

Some of the importance behind the act of karakia is to ensure safety for the people, success for the activities that lay ahead, to pay respect to the taonga and to pay respect to Otaiti. It is believed that when you leave this life, Otaiti is the stepping stone to the ancestors and the ancestral homeland (Ngāi Te Hapū Incorporated, 2014).

Cultural values are a reminder to the people of who they are and illustrate their place in the world. It is important to be actively practising kaitiakitanga by maintaining the connection with the whenua and moana as a resource base. The practise of manaakitanga to manuhiri and to each other preserves the mana of the hapū. Deterioration of the moana has the potential to unknowingly dilute their intergenerational relationship between Mōtītī, Kaumātua and whanau. This can lead to the loss of Mōtītītanga (Ngāi Te Hapū Incorporated, 2014).

1.1.2 Customary Fishing

Māori were historically heavily reliant on an ocean sourced diet (Dick, 2013) and took their responsibility and obligation as kaitiaki very seriously (Booth & Cox, 2003). Traditional resource management included the enhancement of taonga fisheries stock by transplantation, protection of nursery area or removal of predator species in an area; and harvest pressures were carefully controlled according to tikanga and tapu (Booth & Cox, 2003; Dick, 2013). Mollusc species were commonly managed in this manner. Examples of this type of traditional resource management are also evident throughout the South Pacific. In the Cook Islands, villagers would care for or relocate the giant clam, pa'ua (*Tridacna gigas*) closer to shore for protection and care

from storms, floods or high winds that could damage crops on land. These pa'ua farms were treated as a food reserve (Hickey, 2001).

Mōtītī kaitiaki are responsible for the seascape with the Mōtītī rohe moana (customary fishing area) (Fig 1.1) (Ngāi Te Hapū Incorporated, 2014). This area has always provided for the people of Mōtītī Island. Many fish species inhabit the reefs and islands that surround Mōtītī such as kahawai (*Arripis trutta*), trevally (*Caranx georgianus*), snapper (*Chrysophrys auratus*), kingfish (*Seriola lalandi*), jack mackerel (*Trachurus novaezelandiae*), hapūka (*Polyprion oxygeneios* or *Polyprion moene*) and marlin (*Kajikia audax*). Care has always been placed on the taonga. The larger fish would only be taken within the summer months when the waters are warmer. There was a time when the big game fish in the northern water near Otaiti reef, rivalled that of Tuhua (Mayor Island). Other fish that are common to the area of Otaiti are blue (*Scorpis violacea*) and pink maomao (*Carprodon longimanus*), demoiselles (*Chromis dispilus*), perch (*Helicolenus percoides*), and long finned boar fish (*Zaclistius elevatus*). In traditional times, seals were also taken from the reef at low tide. Barracuda (*Sphyraena acutipinnis*) was once taken in large quantities with the use of nets or wooden lure (Ngāi Te Hapū Incorporated, 2014) that worked in a similar way to the modern day surface lures. Otaiti has provided kina (*Evechinus chloroticus*), paua (*Haliotis iris*) and crayfish (*Jasus sp*) and to a lesser extent *kotore moana* (sea anemones) and seaweeds (Ngāi Te Hapū Incorporated, 2014).

The act of fishing not only supplies kai but also allows intergenerational connections to be maintained. Mōtītī Kaumatua have experiences with Otaiti since they were children, going on trips with their kaumatua. These experiences involved performing karakia before fishing, releasing the first fish caught to give thanks to Tangaroa, and the practise of giving away the first fish kept to other people or families under the banner of manaakitanga.

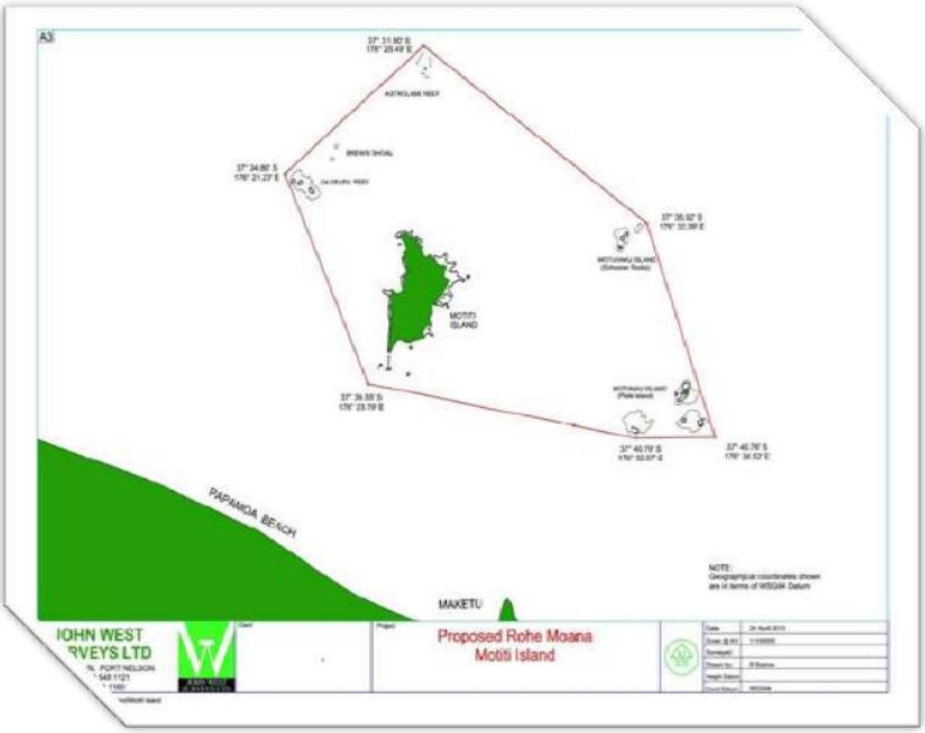

Fig 1-1. Proposed Rohe Moana for Mōtītī Island Sourced from Ngāi Te Hapū Incorporated (2014)

1.1.3 Cultural Value of Paua

Paua are a staple and consistent food source for Mōtītī Island. Though the previously mentioned species are taonga (treasured), they are seasonal. Paua are in close proximity to the shoreline which gives people of all ages and abilities the opportunity to collect them. Being an offshore island, Mōtītī Island residents are still hugely reliant on the ocean as a pataka kai (food cupboard) and are therefore acutely aware of environmental influences to the harvest of kaimoana, for example the wind or tides.

Paua are a delicacy, and harvest pressures in the Bay of Plenty make it difficult to find at legal size in most areas of the north island (Hooker *et al.,* 1997; Dick, 2013). Taonga kaimoana is often what brings whanau (family) home (Dick, 2013) as is the case with ngā uri o Mōtītī (the descendants of Mōtītī) to have a taste of paua. This maintains the connection with the whenua, moana and most importantly, the Kaumatua (elders). Kaumatua are the holders of cultural knowledge relating to the harvest and utilisation

of kaimoana products which has sustained the community for centuries. The transmission of knowledge is fundamental to Mōtītī kawa so that next generation understands what it means to be Kaitiaki of Mōtītī. The preservation and protection of the moana is therefore vital for ensuring the well-being of the hapū currently on Mōtītī.

1.1.4 Paua Biology

The *Haliotis* species is commonly referred to globally as abalone. *Haliotis* means sea ear in Greek, due to the shape of the shell. The Māori name for *H. iris* is ‘paua’ (Poore, 1969). Paua have a large muscular foot which attaches them to the hard rocky substrate. They can range in size from juveniles of a few millimetres up to 200 mm. The muscular body attaches to the shell which can be pulled down as protection against predators (Poore, 1969). It is the muscular foot that is eaten.

Water current is drawn under the shell through the gills in the mantle cavity on the left side of the body and is expelled out via the respiratory pores located on the top of the shell (Poore, 1969). Paua have numerous sensory organs such as tactile tentacles that surround the shells edge and paired head tentacle. The tentacle allows the paua to orientate themselves with the currents to allow water flow over the gills, this allows them to detect chemical signals from other paua for aggregation, for the purposes of food sharing, to allow group defence and spawning success (Selvamani *et al.,* 2000).

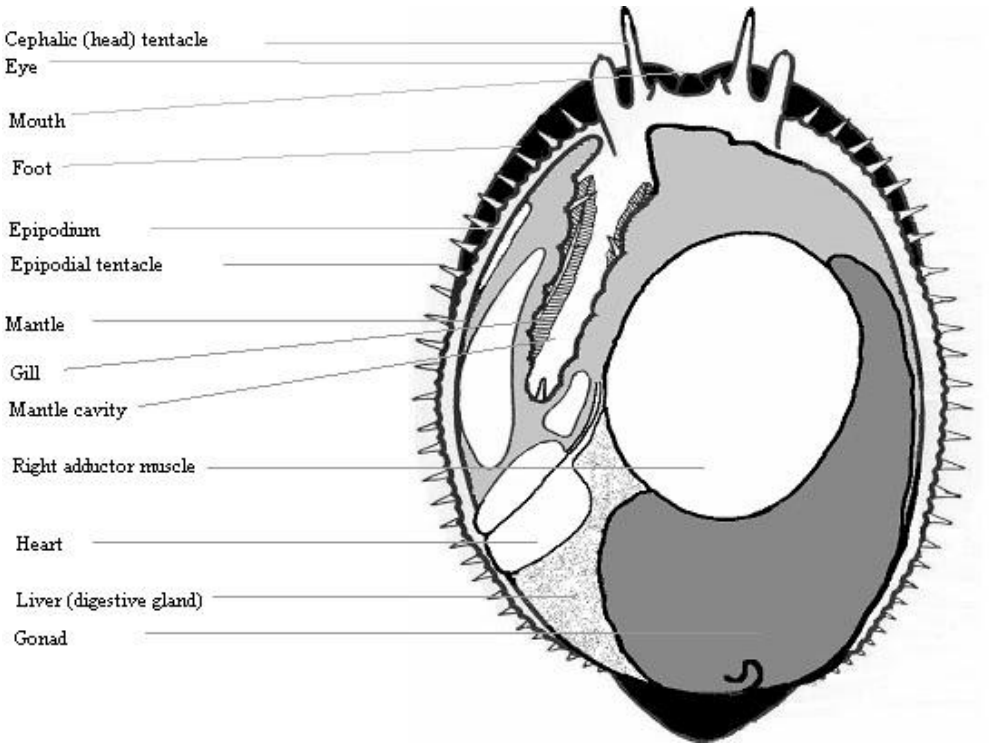

Fig 1-2. Picture identifying different internal organs in a paua. Source (Moss *et al.*, 1995)

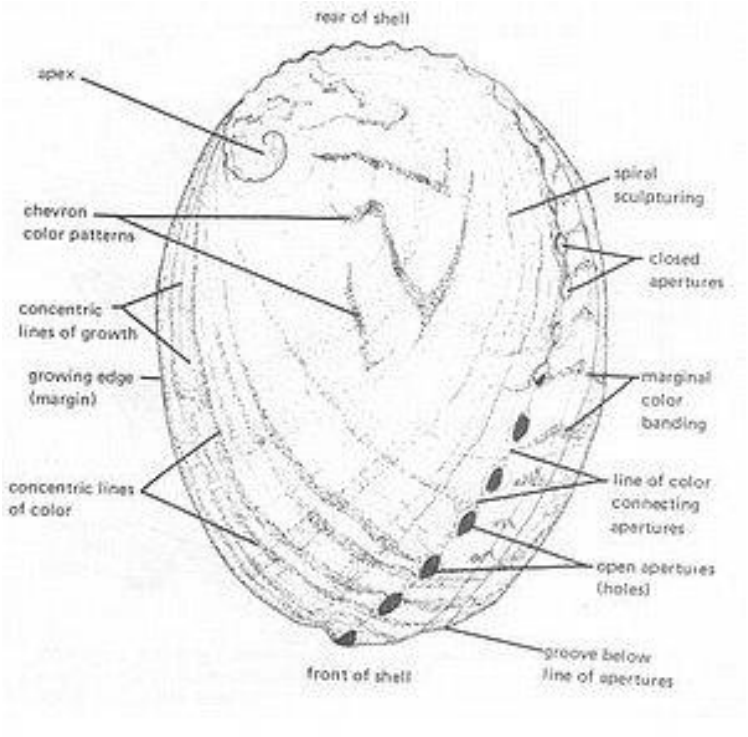

Fig 1-3. Picture identifying different external characteristics of a paua.

Paua are commonly found between below the low tide mark to 12 m water depth (Poore, 1969). Juvenile paua (<5 mm) are found in the shallow sub-littoral zone. From about 5-10 mm juvenile's move into to the intertidal zone and can been seen under rocks and boulders. When juveniles reach sexual maturity at 70-90 mm begin moving into the intertidal zone into deeper water into adult aggregations (Sainsbury, 1982).

Reproduction occurs through broadcast spawning were the fertilised eggs spend 2-5 days within the water column. Larvae can then respond to chemical cues released from crustose coralline algae which then trigger settlement and metamorphosis. Paua do settle and metamorphose onto substrates covered in biofilm, however there is less chance of survival (Roberts *et al.,* 2010).

Of the various types of macroalgae, paua prefer *Lessonia variegate* however they will consume other macroalgae if more easily accessible. Although feeding on fresh algae is more beneficial, water movement appears to inhibit consumption. As a result paua feed equally on fresh and aged algae. Drift algae is caught by the paua by trapping it under the shell. Feeding on drift algae appears to be the preferred method compared with grazing and therefore there is more chance of accessing food in high water flow environments of their common habitat (Poore, 1972; Cornwall *et al.,* 2009).

1.2 The MV Rena Grounding

On the 5th night of October 2012, at 2:19am the MV Rena ran aground on Otaiti in clear weather from Napier to Tauranga Harbour. The vessel was travelling at 17 knots (31km/h) when the Rena collided with Otaiti which then penetrated the hull of the vessel imbedding it on the reef. At the time of the grounding the Rena had 1733 tonnes of fuel oil and 1368 containers of goods on board There were a variety of contents within the containers, including; 121 containing perishable food stuff, and 32 containers containing dangerous goods (Ministry for the Environment, 2011).

The grounding on Otaiti resulted in the release of heavy fuel oil and contaminants into the surrounding environments (Battershill *et al.,* 2013; Ross & Battershill 2013; BECA, 2014) including the rocky shores of Mōtītī Island within the habitat where benthic paua reside. Timber and recycled plastic bundles washed onto the shores of Mōtītī. Some of this material was covered in oil. There were several containers with various metal contents such as copper, aluminium, phosphate, cadmium, zinc, chromium and boron. Many metals in trace form are necessary in biological function, but are toxic in excess and can cause effects on the immunomodulatory activities of haemocytes (Phillips, 1994; Silva-Aciares *et al.,* 2013). Key concerns remain around a container housing 23.3t of copper fillings (Maritime New Zealand, 2012) which is still present on Otaiti (Elvines *et al.,* 2014). Trace metals can have significant adverse effects to marine organisms such as behaviour abnormalities and their physiological processes (Gorski & Nugegoda, 2006).

Given the importance of paua to the ecology and the people of Mōtītī, it is important to understand how metals such as copper affect paua behaviour. The suite of Rena contaminants has the potential to cause adverse effect on the marine food web and survival of key kaimoana species. Due to the ongoing concerns of Iwi and the general public, there has been a focus of inquiry into how kaimoana species may have been affected by Rena event. Most work carried out during the initial phase of the response focused on biota such as coastal tuatua (*Paphies subtriangulata*) (Battershill *et al.,* 2013; Ross *et al.,* in press). However, tuatua are not present around Mōtītī so it is not a relevant species for the community that reside on the Island.

For the purposes of this study, paua have been chosen as the species of focus. This is because of their stated importance (above) and due to the fact that there is relatively limited information on the ecotoxicity of contaminants to sedentary invertebrates especially abalone species, and even more rare is information on mixtures of contaminants such as heavy fuel oils (HFO) and metals (copper).

1.3 Paua as a test organism

The relevance of paua as a target species for examining the effects of Rena contamination is substantial. Paua are the staple year round diet for the people at Mōtītī Island with significant cultural connection associated with them. Paua can be considered vulnerable so it is imperative that this be addressed. Paua can encapsulate what mauri is about, through their cultural, generational and dietary connection with the moana, whenua and the people.

Paua are a useful test organism because there is substantial background literature from other *Haliotis* species that can be used for comparative analysis, the ease of maintenance within aquaria and environment, and cultural relevance to the ecology in New Zealand and for the Rena grounding. Very little research has been done to date on the effects to paua caused by the Rena grounding.

The effect of trace metals on abalone species have been examined throughout the world (Gorski, 2006 and references therein). However, there is no ecotoxicology literature available for the New Zealand blackfoot species *Haliotis iris*. Paua are a benthic species with a small seasonal migratory distribution (Poore, 1972) which makes them vulnerable to disturbances.

1.4 Thesis Objectives

The purpose of this study is to investigate how paua respond behaviourally and physiologically when confronted with contaminated substances relevant to the Rena event.

This thesis originally set out to incorporate Mātauranga Māori due to the need to examine the concept of mauri and how it has been affected by the Rena grounding and breakup. This approach was taken to meet the challenges expressed in the Ministry for the Environment Rena Long Term Environment recovery plan where Minister Nick Smith indicated that the effects of and the recovery of mauri of the moana needed to be examined

(Ministry for the Environment, 2011). It is the intention of this thesis to give some understanding of the effects of the Rena as it may influence the recovery of the mauri, for Moana a Toi Iwi in general and Mōtītī Island in particular. However, from discussion at Mōtītī Island with Kaumatua and the people from Mōtītī, it was decided that the mātauranga should stay at Mōtītī. So, for that reason this thesis includes Mōtītī knowledge that is available to the public through online sources or other documents that have been made available to all.

As paua are the staple food source for Mōtītī it is important that it is protected and any effects that could be caused by the Rena contaminants are known. The objective of the thesis is to investigate whether paua (*Haliotis Iris*) are affected by contaminants (on-reef sediment) of concern from the Rena grounding. This will be achieved by;

1. Investigating the effect of on-reef sediment influenced by Rena contaminants to juvenile paua in a laboratory based experiment

2. Investigating the effect of on-reef sediment influenced by Rena contaminants to juvenile paua in a field based experiment

A synopsis of key findings from these two research objectives, coupled with an in depth literature review will provide a greater understanding of the impact of the Rena and its associated debris to a kaimoana species of cultural, recreational, commercial and ecological importance.

Chapter 2

Laboratory Experiment

2.1 Introduction

2.1.1 Trace Metal Ecotoxicity

Trace metals accumulate within aquatic invertebrates whether they are essential or not and accumulate in different invertebrates at different concentrations (Phillips, 1994b). The amount of accumulation varies depending on the taxa. Species that are living within the same habitat can have varying concentrations of trace metals which can also vary within the organism dependant on the tissue or organ sampled. Therefore, although one species may have a high concentration of trace metals, this could be considered to be low for a given species (Rainbow 1996). For example, a low Zn concentration within an oyster would be considered high for a mussel (Phillips & Rainbow 1994), and a high presence of zinc within a caridean decapods would be below that of a barnacle (Rainbow 1998). It is therefore important to identify what is relevant to the species of interest and how this relates to its ecology.

With essential trace metals there is a minimum requirement needed for metabolic processes. Zinc is key for many enzymes, such as carbonic anhydrase. Copper is required for respiratory protein haemocyanin which can be found in molluscs and arthropods. However, increases in essential trace metals above that needed for metabolism has the potential to induce toxic effects (Rainbow 1993). Non-essential trace metals such as aluminium, cadmium and lead have no required minimum and therefore need to be excreted or detoxified (Cullen *et al.,* 1999).

The edible tissue of snapper (*Pagrus auratus*), abalone (*Haliotis rubra*), and lobster (*Jasus edwardsii*) were analysed for trace metal accumulation in and around Port Phillip Bay in Victoria, Australia (Fabris, *et al.,* (2006). Considering the close proximity to Melbourne and Geelong with a

population of 3 million, all species were found to have trace metal concentration below that recommended by the Food Standards Australia New Zealand (1991). However, Fabris, *et al.,* (2006) found that abalone (*Haliotis rubra*) were not regulating copper as well as other species. The mean concentration of copper and zinc within Port Phillip Bay was 0.47µg/L and the maximum concentration to be 0.63 µg/L and 1.05 µg/L respectively. The worldwide background water quality range for is *Haliotis* sp. is 0.47-76 µg/L for copper and 0.47-3000 µg/L for zinc (Stauber *et al.,* 2005). The New Zealand background water quality in marine waters is 0.1-0.2 µg/L Cu, 0.005-0.02 µg/L Zn and 0.33 µg/L Ni ,which is below the water quality guidelines with a trigger value at 99% protection of 0.3 µg/L Cu, 7 µg/L Zn and 0.33 µg/L Ni (Dickson & Hunter, 1981).

Other metals in the marine water quality requirements under the ANZEEC Water Quality Guidelines (2000) at 99% level of protection are: cobalt (0.005 µg/L), cadmium (0.7 µg/L), chromium lll (7.7µg/L), chromium Vl (0.14 µg/L), nickel (7 mg/L), mercury (0.1 µg/L) and lead (2.2 µg/L). However these metals have no background marine water quality information for New Zealand due to insufficient data (ID) (ANZEEC, 2000). So for this reason Australia and the world background levels are used as a reference in this study (Appendix l). Some metals such as manganese and iron currently do not have a trigger value for 99% protection. In the world the background marine concentration for manganese is 0.003-0.38 µg/L and iron is <0.006-0.14 µg/L (ANZEEC, 2000).

Most metal toxicology research is based on single trace metal effects rather a mixture of metals. The Rena has mixture of contaminants so it provides an opportunity to investigate a real world contaminant mixture.

2.1.2 MV Rena

When the MV Rena ran aground, a variety of contaminants were on board. A contaminant of concern was 23.3t of copper in a container in the stern section and the copper based antifouling paint organotins such as tributyltin (TBT) base (Elvines *et al.,* 2014). The container containing copper was

found breached in 2012 when divers observed an isolated area of sediment containing copper fillings.

Sediment analysis revealed elevated levels of copper, zinc, chromium and aluminium as well as TBT and other organotins (Don *et al., 2014;* Ross *et al.,* 2014). Trace metals (Martin *et al.,* 1977; Ikuta, 1987; Tsai *et al.,* 2004; Fabris*, et al.,* 2006; Gorski, 2006; Silva-Aciares*, et al.,* 2013), organotins (Gopalakrishnan *et al.,* 2011) and PAHs (Gopalakrishnan *et al.,* 2009) have the potential to cause adverse effects to *Haliotis* species. Different organisms accumulate different contaminants at different rates (Phillips, 1994b). So it is unclear as to what effect this could have to *Haliotis iris.* Traditionally paua and other kaimoana species have been collected from Otaiti reef (Ngāi Te Hapū Incorporated, 2014), however since the grounding of the Rena, very few paua have been observed there (Ross & Battershill, 2013). It has been indicated that abalone are more sensitive to contaminants then other organisms (Ikuta, 1987), hence there may be some relationship with the absence of paua on Otaiti following the Rena incident, but this will be difficult to verify given the lack of quantitative information on paua abundance prior to the ship wreck.

There were many contaminants on board the Rena (Refer to Appendix ll) and within the hull paint that have been addressed as a concern to the surrounding environment (Don, 2014; Safinah, 2014; Ross *et al., in press*). It is for this reason that the effects of contaminated sediment on paua (*Haliotis iris*) are investigated in this study. The question that will be investigated is: are paua adversely affected by on-reef sediment on Otaiti reef containing copper released from the container onboard the vessel? This will be achieved by examining the effect of Rena contaminated on reef sediment to juvenile paua within a closed circuit aquaria will be investigated. The null hypothesis tested will be that the Rena contaminated sediment will have no impact to the behaviour or survivorship of juvenile paua.

Upon completion of the experiment, each paua was separated and analysed in two areas; 1) the edible tissue 2) viscera mass. The edible tissue was used as it is important for human consumption as well as containing foot,

tentacles, adductor muscle, etc. The viscera mass contains the body organs such as digestive tract, gonads, kidney, heart, stomach, etc.

2.2 Methods

2.2.1 Collection of Test Animals and Experimental Aquaria

Juvenile paua (25-63mm) were collected by hand from the Coromandel region under MPI special permit (560) and placed into a bucket of fresh seawater lined with a plastic bag. Animals were quickly transported to the University of Waikato Coastal Marine Field Station in Tauranga, New Zealand where experiments were to be conducted. Paua were placed into holding tanks to recover from any stress caused by collection and to acclimatise to test conditions. Natural seawater was obtained from the Sulphur Point boat ramp in Tauranga on an incoming tide and stored onsite in a 1000L storage tank until needed.

A series of connected chill baths were utilised to regulate experimental temperature whilst ensuring the isolation of each 40L test aquaria (Fig 2.1, 2.2). Each aquarium was individually aerated from a central air hose, with air flow regulated by a tap. Ambient water temperature ranged between 14.7 - 15.9°C with an average of 15.38±0.54°C, pH was 8.0 for all aquaria, and dissolved oxygen ranged between 8.5 - 9.46 mg/L with an average of 9.12mg/L as test conditions. Natural daylight was used. The photoperiod at the time of the experiment was 11:30h daylight. The chill bath system had a cover top as seen in Fig 2.2 which reduced the light intensity and minimised dust from entering the system. Glass lids were placed on all the test aquaria also to minimise dust.

2.2.2 Experimental Design

As copper is known to be prevalent in the contaminated sediment (Ross, P. *pers* comm.), copper flakes were used as a comparative positive control. Three aquaria were used to replicate each of the four treatments; Otaiti sediment (OS), copper flakes positive control (CP), non-contaminant sediment control (C2), and no sediment control (C1). OS was collected by divers from between the Rena hull at 37°32’40.38”S, 176°25’45011”E

during monitoring of Otaiti (Elvines*, et al.,* 2014). Copper flakes for CP treatment were obtained from an industrial supplier in Hamilton to compare to the OS mixture. Non contaminant sediment was collected from between Moturiki and Motuotau near Mount Maunganui (GPS 37°37’53”S, 176°11’10”E).

Four treatment bags of sediment were placed into each aquarium and there were 3 replicate aquaria per treatment. The contents of each test bag was 50g OS contaminant mixed with 50g of non-contaminant sediment; 50g CP contaminant mixed with 50g of non-contaminant sediment; and 100g non contaminant sediment in C2 (Table 2.1). This gave a total overall weight of 400g of sediment per aquaria. Control 1 was to control against the non-contaminant sediment so no treatment bags were used. The sediment bags were placed into the aquaria following acclimatisation and attachment of Diffusive Gradient in Thin Film (DGT) samplers. DGT measure the amount of dissolved cations in solution. Water and ions diffuse through the filter membrane and diffusive gel with the trace metal then binds to the resin layer selected for trace metals (Chelax 100) (Davidson and Zhang, 1994; Hartland *et al.,* 2011; Schintu *et al.,* 2008).

Table 2-1: Sediment types within controls and treatments. Each was undertaken in triplicates; treatment group’s juvenile paua are exposed to during the 48hr period.

Treatments	Control 1	Control 2	CP	OS
Sediment Types	No sediment	Non-contaminant sediment 4x 100g per aquaria	4 bags per aquaria (1 bag = 50g copper flakes mixed with 50g non contaminant sediment)	4 bags per aquaria (1 bag = 50g Otaiti sediment mixed with 50g non contaminant sediment)

Before the commencement of the experiment, paua were measured, weighed and randomly designated to 1 of 12 40L test aquaria. They spent a further 24hrs to recover from any stress from handling and moving from the stock aquaria. Diffusive Gradient in thin film (DGT's) pre-loaded with a 0.75mm chelex-100 resin (Fig 2.6) was installed to one aquaria per treatment group. The 4 DGT samplers were used to measure the total amount of dissolved trace metals available in the water column over the experimental duration (Fig. 2.7). Bulk water samples were taken from each aquaria in a sterilized 100ml container with the time and aquaria code recorded on each. These were taken at 6 and 48hrs.

2.2.3 Behavioural Analysis

Paua behaviour was monitored every hour for the first three hours, then every three hours until 12 hours, and then every 12 hours thereafter. The behavioural characteristics monitored followed that of Gorski (2006) which were; tentacle presence and their sensitivity to stimuli, surface adhesion by the foot, mucus secretion, righting reflex and movements within the aquaria. Adhesion was tested with a gentle prod and the shell movement and adhesion was recorded.

Fig 2-1: Temperature control 'chill' bath system containing six 40L glass aquaria within each of three black chill baths. The two highest chill baths "left and centre" were used for the experiment. The chill bath on the right was used as a holding tank.

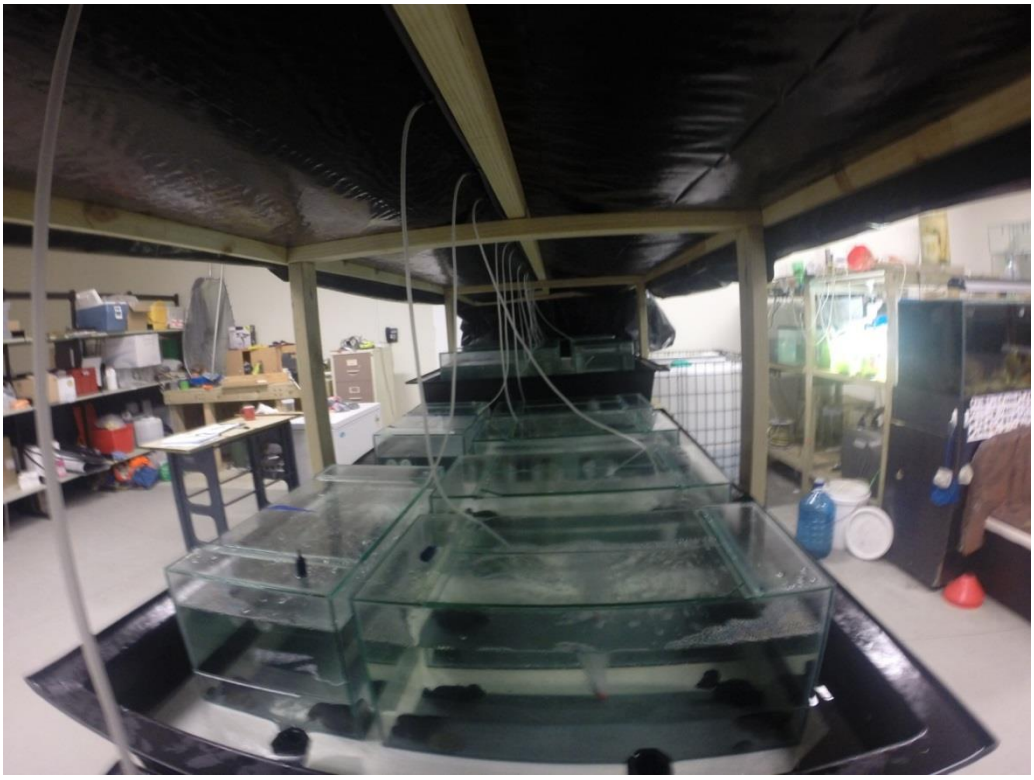

Fig 2-2: Aquaria placement and airline setup

Fig 2-3: Paua with normal tentacle protruding

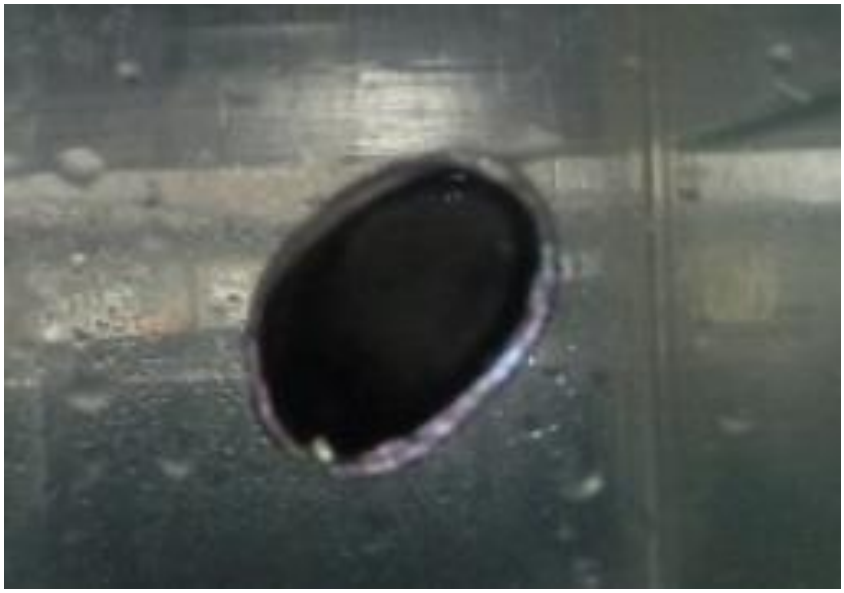

Fig 2-4: Paua with skirt and head tentacles retracted within the shell

Fig 2-5: Paua losing adhesion on the side of the aquaria. All tentacles retracted

2.2.4 Tissue analysis

As paua died throughout the study, their length and weight was recorded prior to placing them in the -20°C freezer. At the conclusion of the 48hr exposure period, control paua were removed from the aquaria, photographed, weighed and length was recorded and then placed in the -20°C freezer. When ready paua were shucked, dissected (edible tissue and viscera mass) and air dried at 51°C for 72hrs.

Samples were prepared for ICP-MS analysis by first being homogenised to a fine powder with a pestle and mortar. Samples were weighed out to 0.2g and placed into a 50ml Falcon tube. These were left to digest in 1ml HNO_3 and 0.33ml HCl overnight, then placed on a heating block for 1 hour at 50°C. Once cooled, the volume of the falcon tube was topped up to 50mL with de-ionised water (DI) and then placed into a centrifuge at 4000rpm for 10mins. A 5ml sample was taken from the 50ml solution that was centrifuged. It was syringe filtered at 0.45 μm and 2.5g was weighed into a 15ml falcon tube and the weight was recorded. This was then topped up to 10mls with DI water and the weight was recorded.

After ICP-MS analysis the mass of the metals accumulated in the tissue was calculated using equation (1):

$$C_t = (C_s \times V_s \times DF) / W_t \qquad (1)$$

C_t = metal concentration in the tissue sample (micrograms per gram)

C_s = metal concentration in acid digested solution (micrograms per litre)

V_s = volume of acid digested sample solution (litres)

DF = dilution factor of analysed acid digest

W_t = dry weight of tissue (grams)

2.2.5 Water analysis

Water samples were collected from tanks and filtered (0.45 um) in a 50mL falcon tube. For processing, an aliquot (0.4mL) of sample water was combined with 9.4mL to achieve 25% dilution. 0.2mL of HNO_3 was added to acidify sample for 24 hours before being run through ICP-MS. Post analysis, ICP-MS values were multiplied by 25 to account for the dilution factor for statistical comparison.

2.2.6 DGT Analysis

At the conclusion of the experiment, DGT samplers were removed from the water and rinsed with de-ionised water. They were then placed individually into zip lock bags, labelled and refrigerated until analysed.

The processing of DGT samplers required the resin layer to be removed by inserting a screw driver into the groove and twisting, to break the cap on the piston. Using plastic tweezers, the membrane filter and diffusive gel were removed to expose the resin gel. The resin gel was then placed into a clean falcon tube and 1ml of 1M HNO_3 solution was added, ensuring that the resin was completely immersed for a minimum of 24 hours. Solutions were then topped up with 4 ml of de-ionised water bring the total amount of solution to 5 ml or a dilution factor of 5 prior to ICP-MS analysis.

After ICP-MS analysis the mass of the metals accumulated in the resin gel was calculated using the equation (2):

$$M=Ce\ (V_{HNO3}+Vgel)/fe \qquad (2)$$

Ce = concentration of metals in 1M HNO3 solution (in µg/L)

V_{HNO3} = volume of HNO3 added to the resin

V gel = volume of the resin gel (typically 0.16 ml)

Fe =elution factor of the metal (typically 0.8)

Equation 3 was used to calculate the concentration of metals measured by the DGT's is:

$$C_{DGT} = M\Delta g/(DtA) \qquad (3)$$

Δg = thickness of the diffusive gel (0.4) plus the thickness of the filter membrane (0.13)

D = diffusion coefficient of the metal in the gel

T = deployment time in seconds

A = exposure area (A=3.14 cm^2)

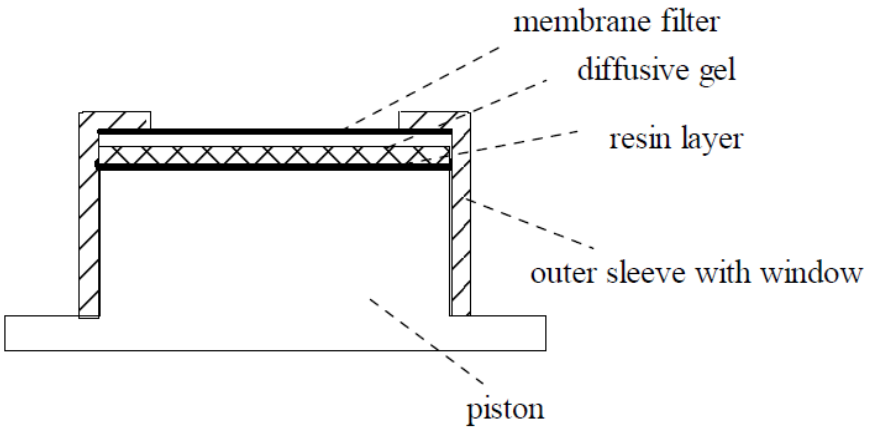

Fig 2-6: Diagram of a DGT solution unit identifying the piston housing, and the outer sleeve that secures the membrane filter, diffusive gel and resin layer.

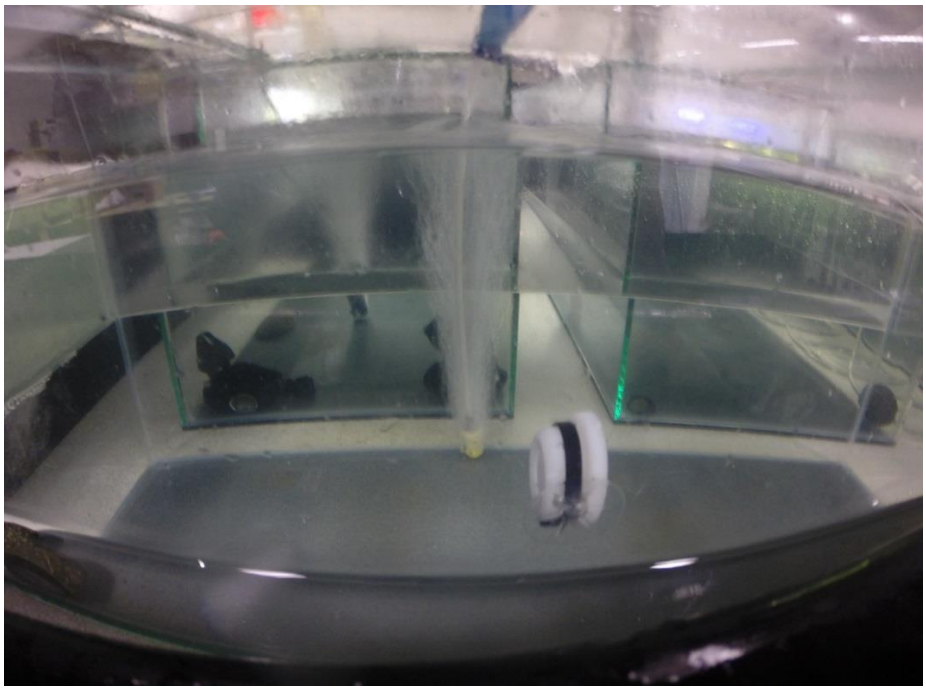

Fig 2-7: Position of the DGT within the aquaria of control 1 with no sediment

2.3 Results

2.3.1 Behaviour

Control survival was 100% throughout the duration of the experiment. Normal behaviour was exhibited by test animals as observed prior to the commencement of the experiment which includes; head and skirt tentacles protruding from the shell (Fig 2.3, 2.9); the foot fills the entire cavity of the mantle (Fig 2.4); no visual presence of mucus; adequate surface adhesion; ability to pull the shell down tightly and efficient righting reflex. Behavioural changes and 100% mortality was observed in both OS and CP treatments. Paua in OS and CP treatments showed similar trends of behavioural changes (Fig 2.5, 2.10) though paua within the OS treatment appeared to be affected sooner that paua in CP.

Between 0-6 hours, CP treatments had reduced tentacle protrusion whereas OS treatment animals showed signs of delayed response, the head and skirt tentacles weren't visible, adhesion was reduced and two animals tried to climb out of treatment aquaria. Surface bubbles with a rainbow sheen began forming in OS aquariums and were no longer present by 9hrs (Fig 2.8, 2.11). This could be an indicator of the range of contaminants that have been released from the Rena as the same bubbles weren't present in the other treatment tanks. At 9 hours the CP treatments started to show effects more prominently as paua presented with a raised shell, no skirt tentacles visible and delayed response.

At 12 hours into the experiment, adhesion was further reduced in both treatments, with the righting reflex impaired in OS treatment animals. Mortality was recorded at 24 hours for the smallest test subject in both OS (55% or n=5) and CP (33% or n=3). This was accompanied with further reduction in adhesion and the retraction of tentacles.

Mucus began to develop around the gills at 30 hours, with black mantel pigment cells visibly sloughing off at 36 hours in both treatment groups (Fig 2.12). Further reduction in adhesion, response and mortality continued until the experiment ceased at 48 hours (Table 2.2, 2.3; Fig 2.13, 2.14).

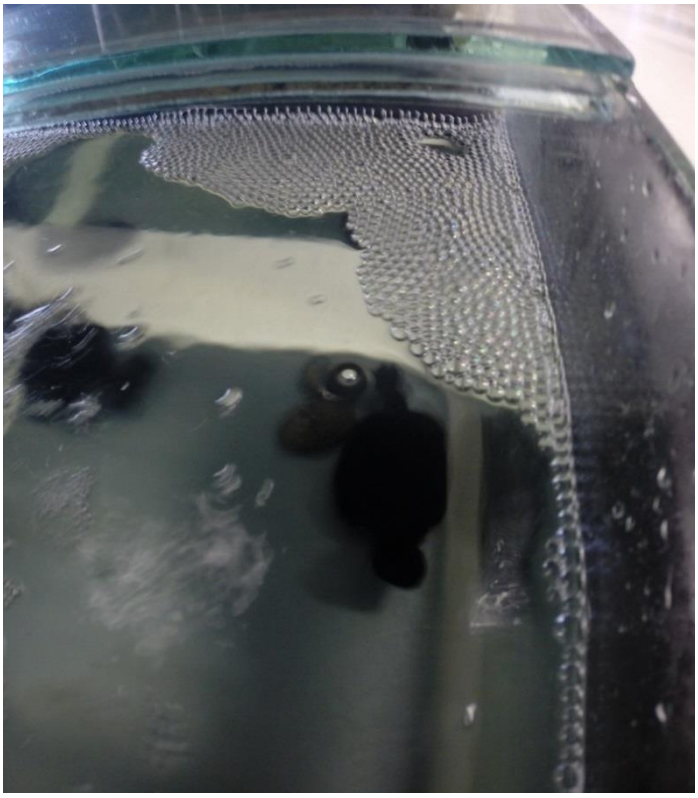

Fig 2-8: Bubbles forming in the OS aquaria at 6hrs

Fig 2-9: Paua exhibiting normal behaviour, with Head and Skirt (epipodial) tentacle extended. The paua on the side of the aquaria is showing how the body fills the entirety of the shell during normal behaviour.

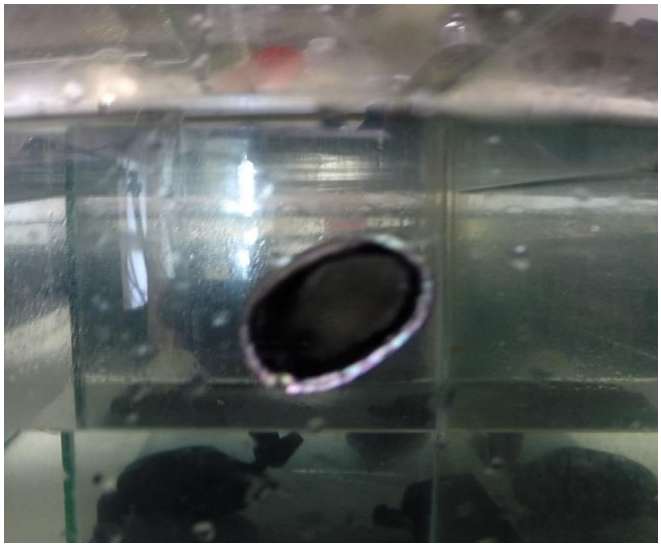

Fig 2-10: Paua from OS treatment group showing abnormal foot retraction within the shell. Paua also seen with no skirt tentacle protruding from the body, compared to the control.

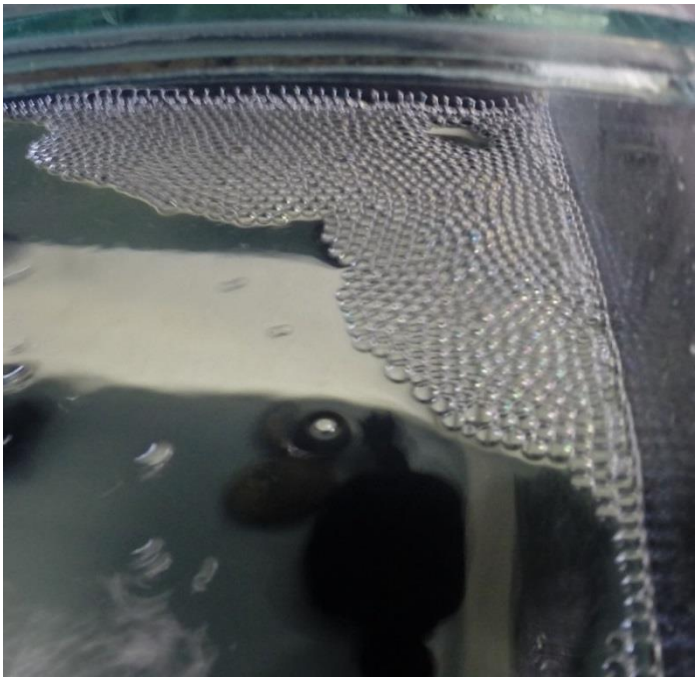

Fig 2-11: Surface bubbles that began forming on OS treatment at approximately 5-6 hrs.

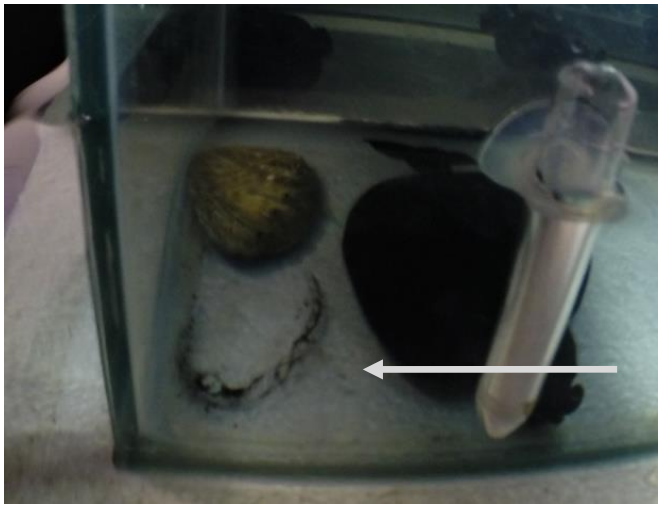

Fig 2-12: Paua that was no longer responsive. The paua was moved back to show the mantle pigment cells within the mucus.

Table 2-2: Paua sizes, weights and time at mortality for copper positive control (CP) and Otaiti sediment (OS) treatment group.

Time Period	CP (mm)	CP (Grams)	OS (mm)	OS (Grams)
24hr	26	1.863	21	0.944
	30	2.541	24	1.542
	32	2.918	26	1.585
			27	2.1
			46	9.489
30hr	26	1.594		
	63	29.123		
36hr	55	15.761		
48hr			25	1.427
	50	13.43	51	13.815
	54	15.782	52	15.082
	55	17.667	63	26.352

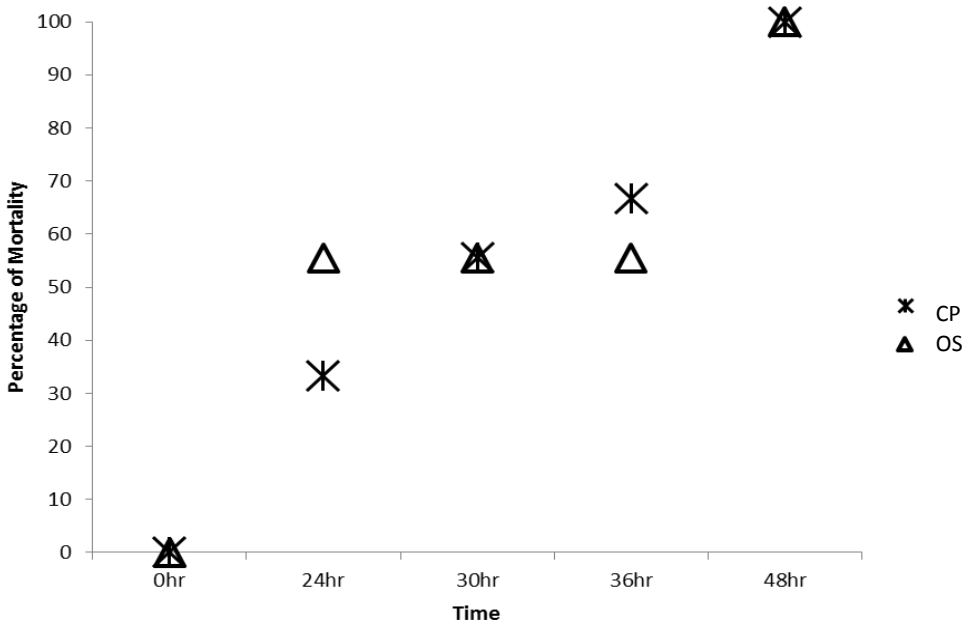

Fig 2-13: Percentage of mortality over the duration of the experiment during the allotted times

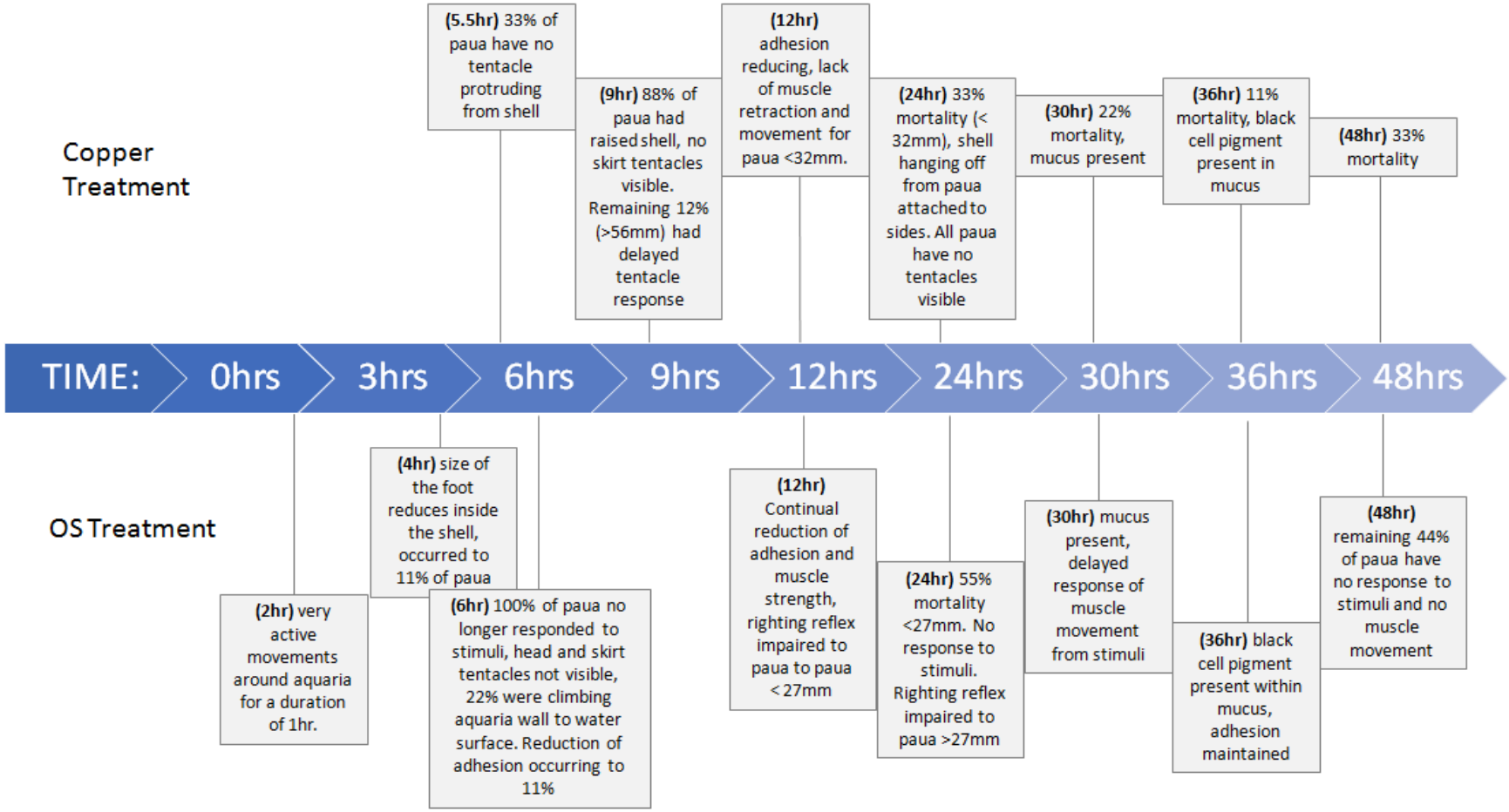

Fig 2-14: Timeline of observed behavioural changes in OS and CP treatments

2.3.2 DGT results

DGT samplers were in the aquariums for 3.06 days, therefore integrating metal concentrations over a slightly longer time window than the paua exposure time. As there was no significant difference observed with the bulk water samples from the control 1 and 2 aquaria over 48hrs, the dissolved concentrations of trace metals could be conservatively compared to the bulk water (Table 2.4).

Table 2-3: Concentrations of trace metals (µg/L). DGT data from the treatment groups for Control 1 with no sediment; Control 2 with non-contaminant sediment; Copper positive sediment and Otaiti Sediment with copper fillings collected from Otaiti reef. (<DL= less than detection limits).

Treatments	C1	C2	CP	OS
Al	0.0194	0.0057	0.0009	0.0061
Cr	0.0025	0.0028	0.0035	0.003
Fe	<DL	<DL	<DL	<DL
Mn	0.008	0.016	0.288	0.369
Co	0.002	0.0017	0.0045	0.0058
Ni	0.017	0.015	0.028	0.056
Cu	0.1	0.09	78.98	88.61
Zn	3.93	3.44	3.63	3.52
Cd	0.0202	0.0075	0.0082	0.0087
Pb	0.078	0.048	0.098	0.042

2.3.3 Ambient water and metals concentrations in paua tissue

Statistical comparisons were determined using Tukey HSD test (± SE). Tukey HSD (Honest significance test) identifies differences between two means that are greater than the expected standard error. Tukeys test is more suitable when multiple comparisons are made, reducing type 1 error.

P values where significance is stated as P<0.05, are reported for ambient water and paua tissue for each trace metal analysed. Statistical comparisons made were between edible tissue and viscera mass of the same replicate, however comparisons between different tissues from different replicates are not reported here. Breakdown Table of Descriptive Statistic for ambient water and trace metals in paua tissues are attached in Appendix III.

2.3.4 Copper

2.3.4.1 Ambient water Concentration

There was no significant difference between control 1 and 2 (p>0.05) (Fig 2.15 top). CP and OS at 6hrs was significantly different to both controls at 6 and 48hrs (p<0.05). CP and OS were not significant to each other at 6hrs. The same can be said for 48hrs. Both CP and OS at 6hrs were significantly different compared to 48hrs (p<0.05).

2.3.4.2 Paua Tissues

There was no significant difference between the control 1 and 2 and the edible tissue and viscera mass of these controls (p<0.05) (Fig 2.15 bottom). CP and OS were significantly different from the controls for edible tissue and viscera mass (p<0.05).

The CP and OS edible tissue was significantly different to the viscera mass (p<0.05). There was no significant difference between the edible tissues of CP compared with OS. The same can be said for the viscera mass of CP and OS (p>0.05). The viscera mass mean concentration was greater than the edible tissue for all treatments.

Assuming $Cu^{2+}_{(aq)}$ solubility in pH ~8 ocean water was controlled by $Cu(OH)_{2(s)}$, the maximum $Cu^{2+}_{(aq)}$, concentration was calculated in the geochemical model PHREEQC using the wateq4.dat thermodynamic database. Therefore, assuming $Cu(OH)_{2(s)}$ SI = 0 the maximum probable $Cu^{2+}_{(aq)}$ was calculated at 1.3 mg L^{-1}. Therefore, the recorded values of Cu(aq) in the aquaria were well within the expected range given the treatment bag dosages (*Hartland pers. comm*.).

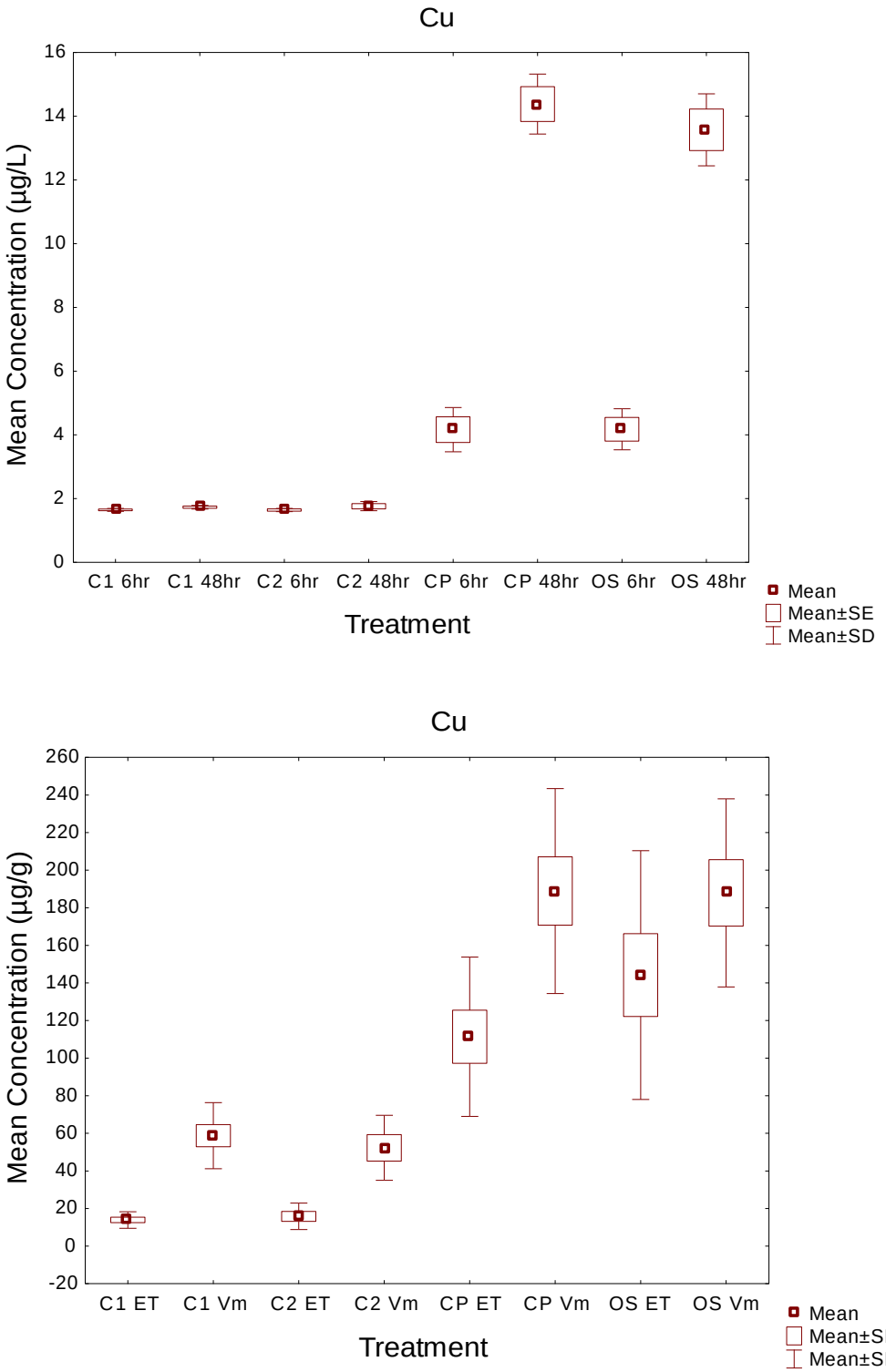

Fig 2-15. Mean concentration of copper in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass

2.3.5 Manganese (Mn)

2.3.5.1 Ambient water Concentration

There was no significant difference between control 1 and 2 ($p>0.05$) (Fig 2.16 top). CP and OS at 6hrs was significant different to both controls at 6 and 48hrs ($p<0.05$). CP and OS were not significant to each other at 6hrs, however they were significant to each other at 48hrs ($p<0.05$). CP at 6hrs had no significant difference to CP at 48hrs ($p>0.05$). OS at 6hr was significant to OS at 48hrs ($p<0.05$).

There was no significant difference between control 1 and 2 ($p>0.05$). CP and OS at 6hrs was significantly different to both controls at 6 and 48hrs ($p<0.05$). CP and OS were not significant to each other at 6hrs. The same can be said for 48hrs. Both CP and OS at 6hrs were significantly different compared to 48hrs ($p<0.05$).

2.3.5.2 Paua Tissues

Control 1 and 2 edible tissue was not significant to the edible tissue in CP and OS (Fig 2.16 bottom). The same can be said for the viscera mass.

The edible tissue in control 1 and 2 was not significant to each other. The same can be said for the viscera mass. The edible tissue in control 1 was significant to control 1 viscera mass. The edible tissue in control 2 was not significant to the viscera mass in control 2.

The edible tissue in CP was significant to the viscera mass and the same can be said for OS. The viscera mass mean concentration was greater than the edible tissue for all treatments.

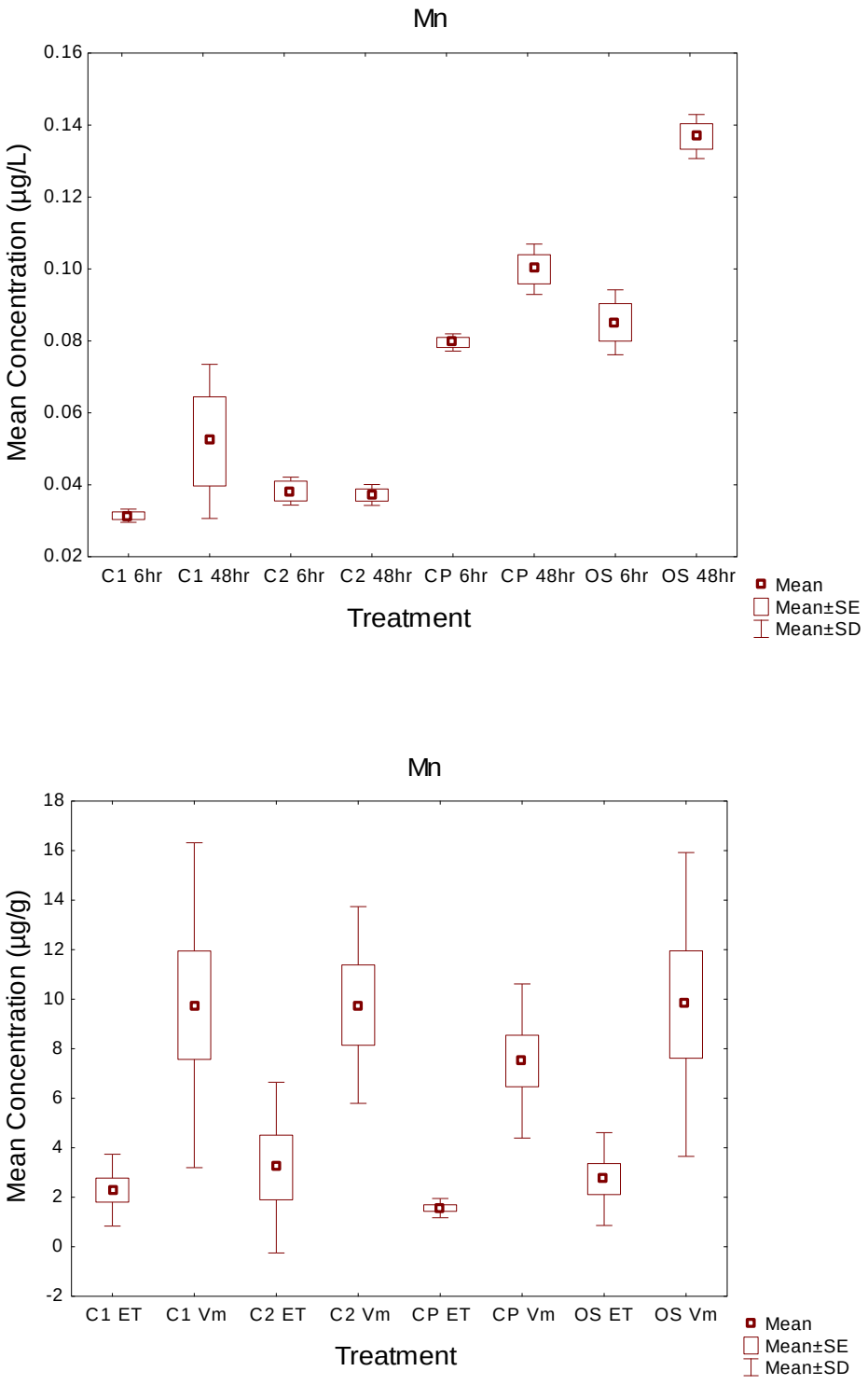

Fig 2-16: Mean concentration of manganese in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass

2.3.6 Aluminium (Al)

2.3.6.1 Ambient water Concentration

The only significant difference observed was between control 1 at 6hrs with OS at 48hrs ($p<0.05$). CP mean decreased in concentration from 6 compared to 48hrs (Fig 2.17 top).

2.3.6.2 Paua Tissues

No relevant significant differences were observed for Aluminium ($p>0.05$). The viscera mass mean concentration was greater than the edible tissue for all treatments (Fig 2.17 bottom).

2.3.7 Cobalt (Co)

No significant differences were observed in ambient water for any time period or group ($p>0.05$) Fig 2.18 top).

2.3.7.1 Paua Tissues

Control 1 and 2 edible tissue was not significant to the edible tissue of CP and OS (p.0.05) Fig 2.18 bottom). The same can be said for the viscera mass.

The edible tissue of control 1 and 2 and CP and OS were significantly different to the viscera mass of the respective groups ($p<0.05$). The viscera mass mean concentration was greater than the edible tissue for all treatments.

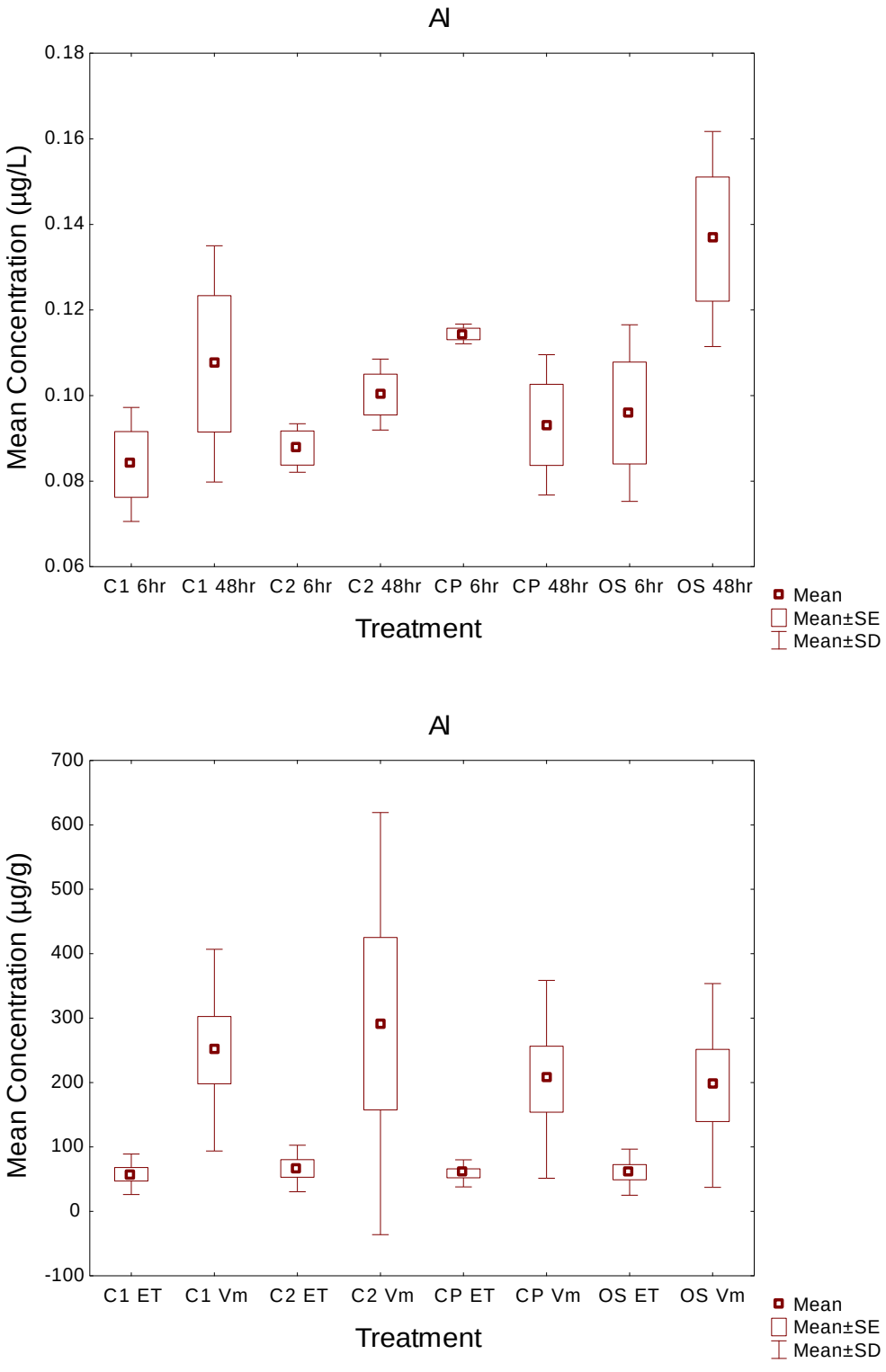

Fig 2-17: Mean concentration of Aluminium in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass.

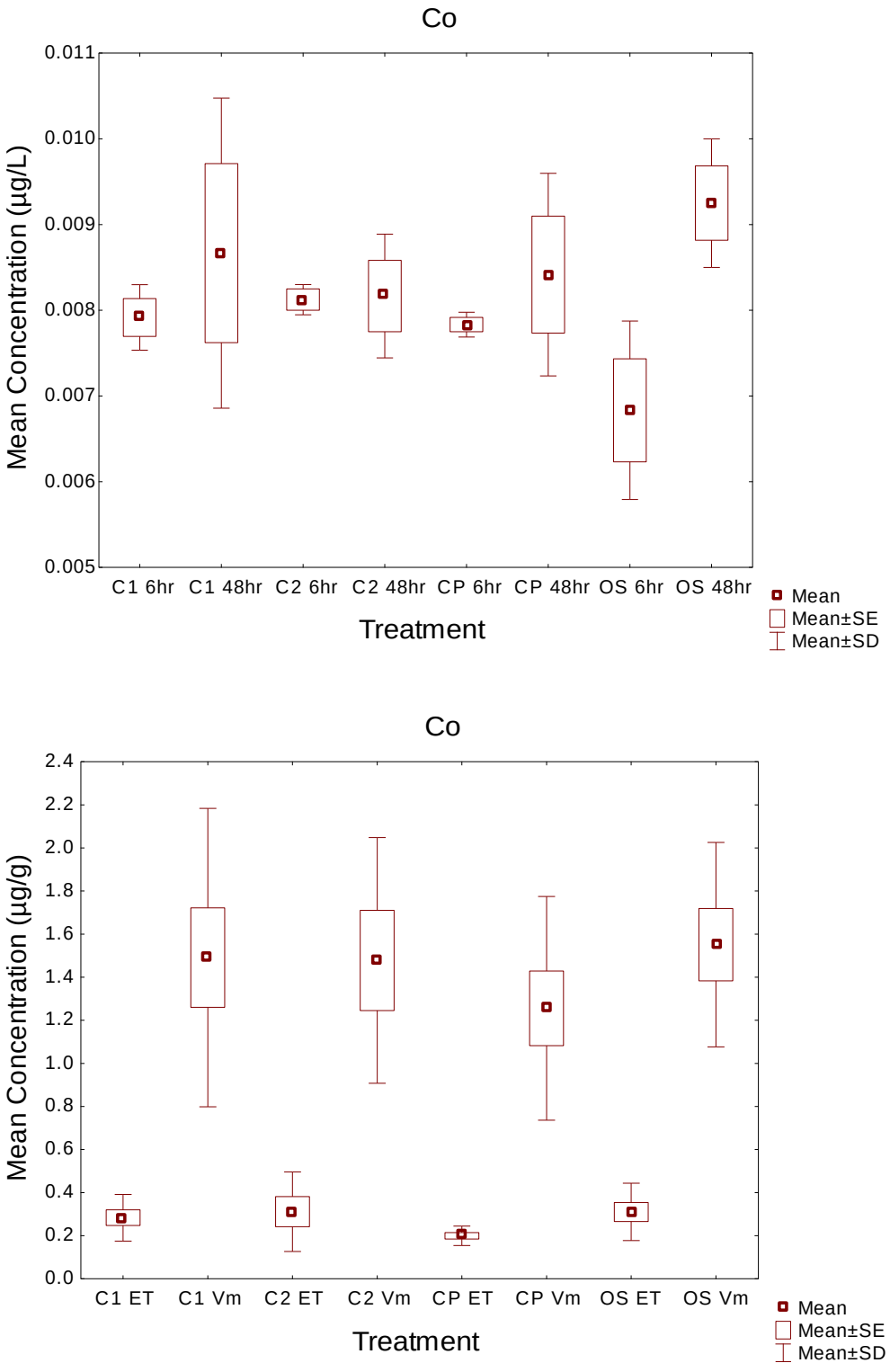

Fig 2-18: Mean concentration of cobalt in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass.

2.3.8 Cadmium (Cd)

2.3.8.1 Ambient water Concentration

There were no observed significant differences for any time period or group (p>0.05) (p>0.05) (Fig 2.19, top). Control 1 was below detection limits and Overtime the mean concentration in control 2, CP and OS decreased below detection limits.

2.3.8.2 Paua Tissues

Control 1 and 2 edible tissue was not significant to the edible tissue of CP and OS (Fig 2.19, bottom). The same can be said for the viscera mass (p>0.05). The edible tissue of control 1 was significant to the viscera mass of control 1. The same can be said for control 2 and CP (p<0.05).

There was no significant difference between the edible tissues of CP with OS. The same can be said for the viscera mass (p>0.05). There was no significant difference between the edible tissues of OS with the viscera mass of OS. The viscera mass mean concentration was greater than the edible tissue for all treatments.

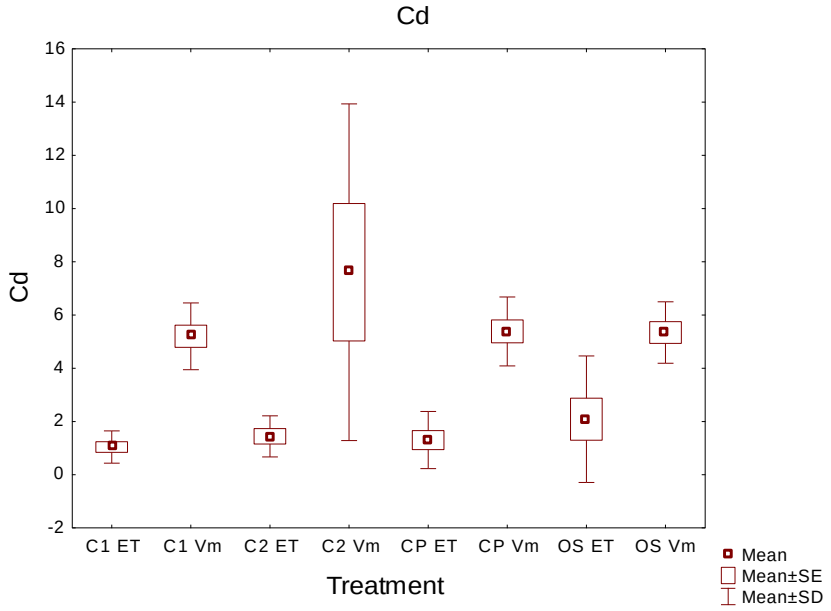

Fig 2-19: Mean concentration of cadmium in tissue samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

2.3.9 Zinc (Zn)

2.3.9.1 Ambient water Concentration

Controls 1 and 2 had no relevant significant difference compared to CP and OS ($p>0.05$) (Fig 2.20 top. The mean concentrations decreased from 6 to 48hrs for control 2, CP and OS groups. Control remained consistent.

2.3.9.2 Paua Tissues

Control 1 and 2 edible tissue was not significantly different to CP or OS edible tissue ($p>0.05$) (Fig 2.20 bottom). The same can be said about viscera mass. Control 1 edible tissue was significantly different to the viscera mass ($p<0.05$). Control 2 edible tissue was not significantly different to the viscera mass ($p>0.05$).

CP edible tissue was significantly different to the viscera mass ($p<0.05$). OS edible tissue was not significantly different to the viscera mass ($p>0.05$). The viscera mass mean concentration was greater than the edible tissue for all treatments.

2.3.10 Chromium (Cr)

2.3.10.1 Ambient water Concentration

There were no observed significant differences for any time period or group ($p>0.05$) (Fig 2.21 top).

2.3.10.2 Paua Tissues

Control 1 and 2 edible tissue was not significantly different to CP and OS edible tissues (Fig 2.21 bottom). The same can be said of viscera mass ($p>0.05$). In most cases, the edible tissues were significantly different to the viscera mass of all groups ($p<0.05$) apart from control 2. Control 2 edible showed a weak non-significant difference to the viscera mass of CP and OS ($p>0.05$).

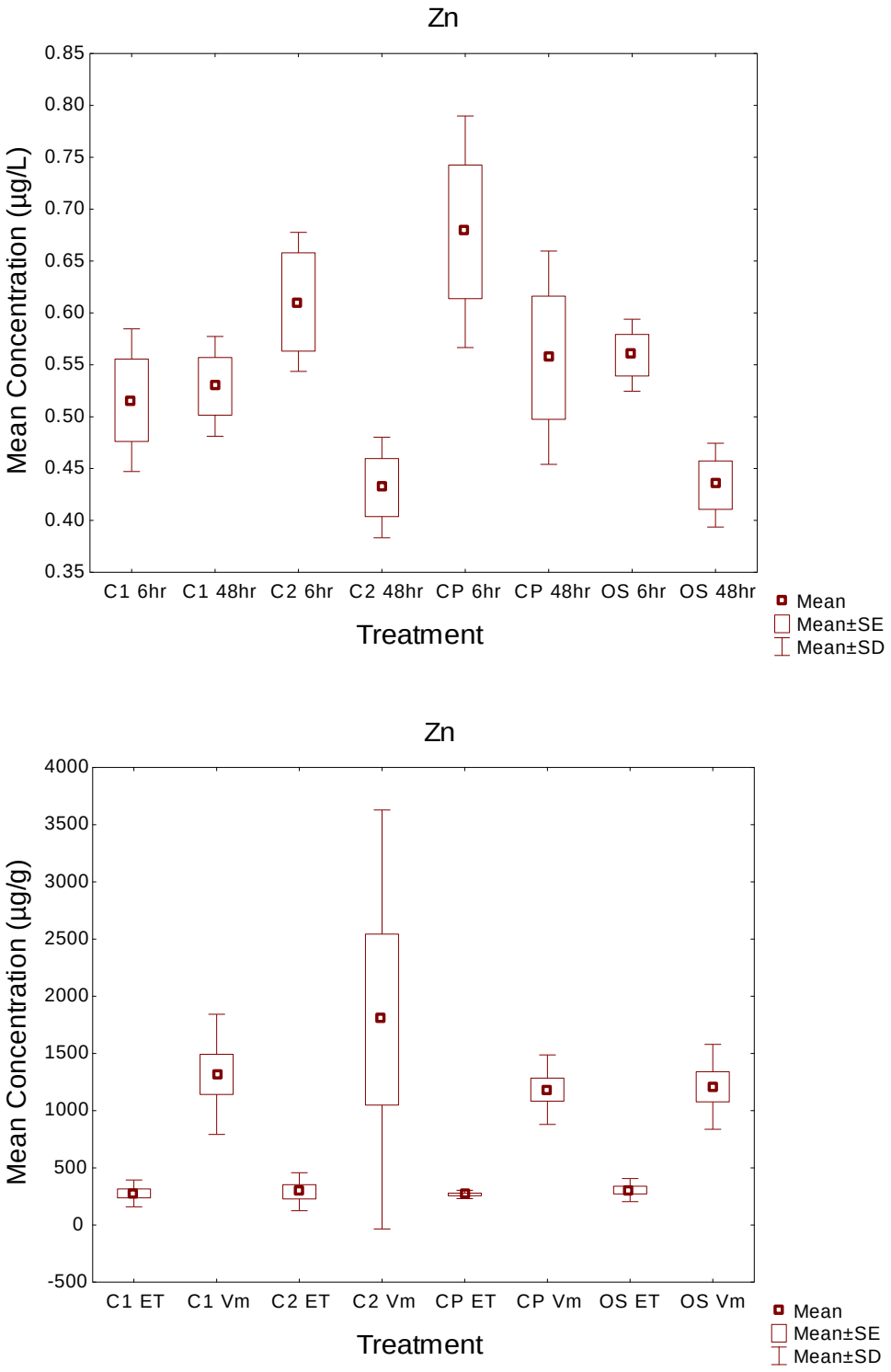

Fig 2-20: Mean concentration of zinc in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass

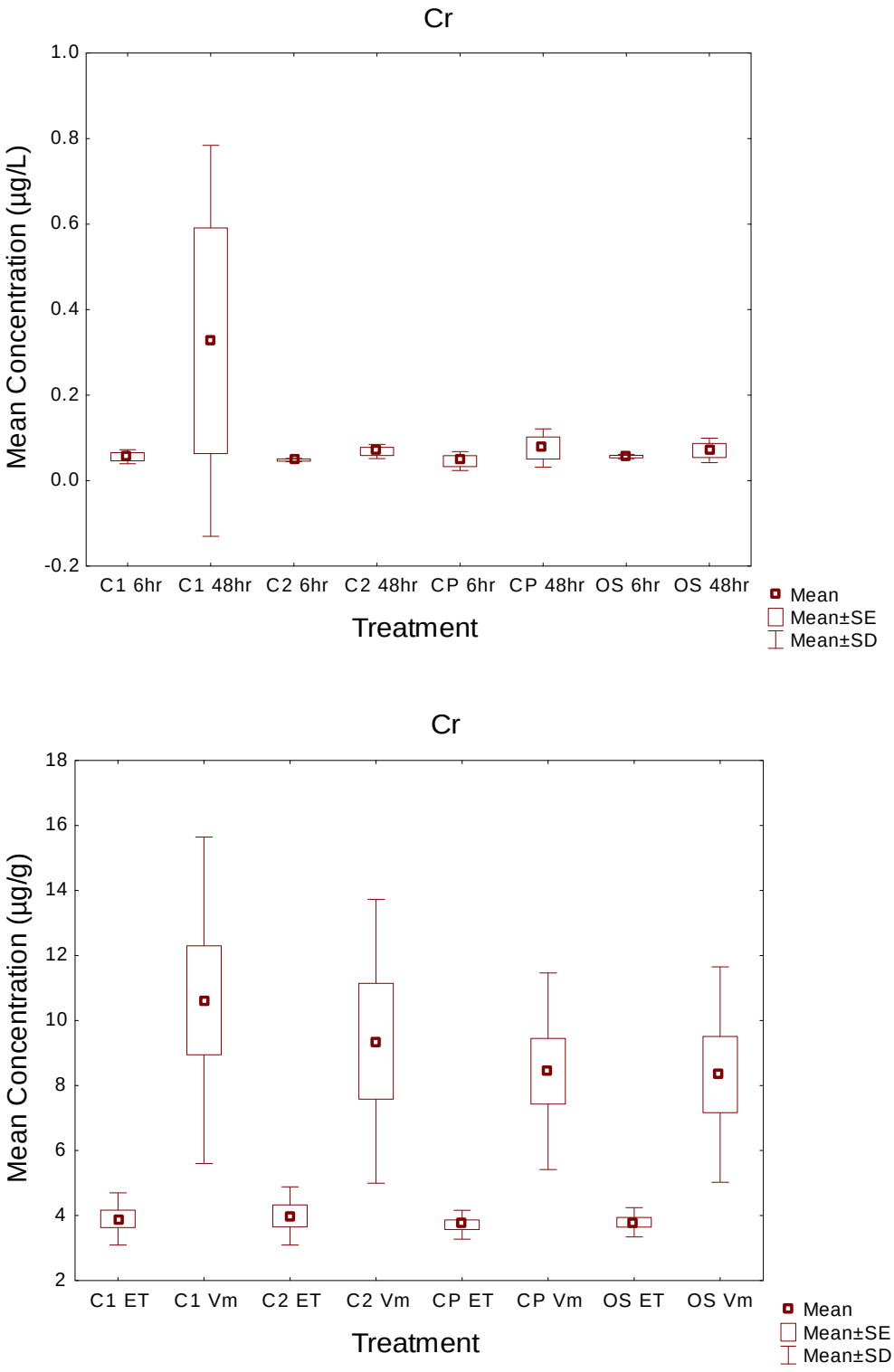

Fig 2-21: Mean concentration of chromium in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass

2.3.11 Iron (Fe)

2.3.11.1 Ambient water Concentration

There were no observed significant differences for any time period or group ($p>0.05$) (Fig 2.22 top).

2.3.11.2 Paua Tissues

Control 1 and 2 edible tissue was not significantly different to the edible tissue of CP and OS. The same can be said for the viscera mass ($p>0.05$) (Fig 2.22 bottom).

The edible tissue of control 1 and 2 was not significantly different to the viscera mass of their groups ($p>0.05$). The edible tissue of CP and OS was significantly different to the viscera mass of their groups ($p<0.05$). The viscera mass mean concentration was greater than the edible tissue for all treatments.

2.3.12 Nickel

2.3.12.1 Ambient water Concentration

There were no observed significant differences for any time period or group ($p>0.05$) (Fig 2.23 top).

2.3.12.2 Paua Tissues

There was no significant differences of relevance between all groups and tissue compartments ($p>0.05$) (Fig 2.23 bottom). The viscera mass mean concentration was greater than the edible tissue for all treatments.

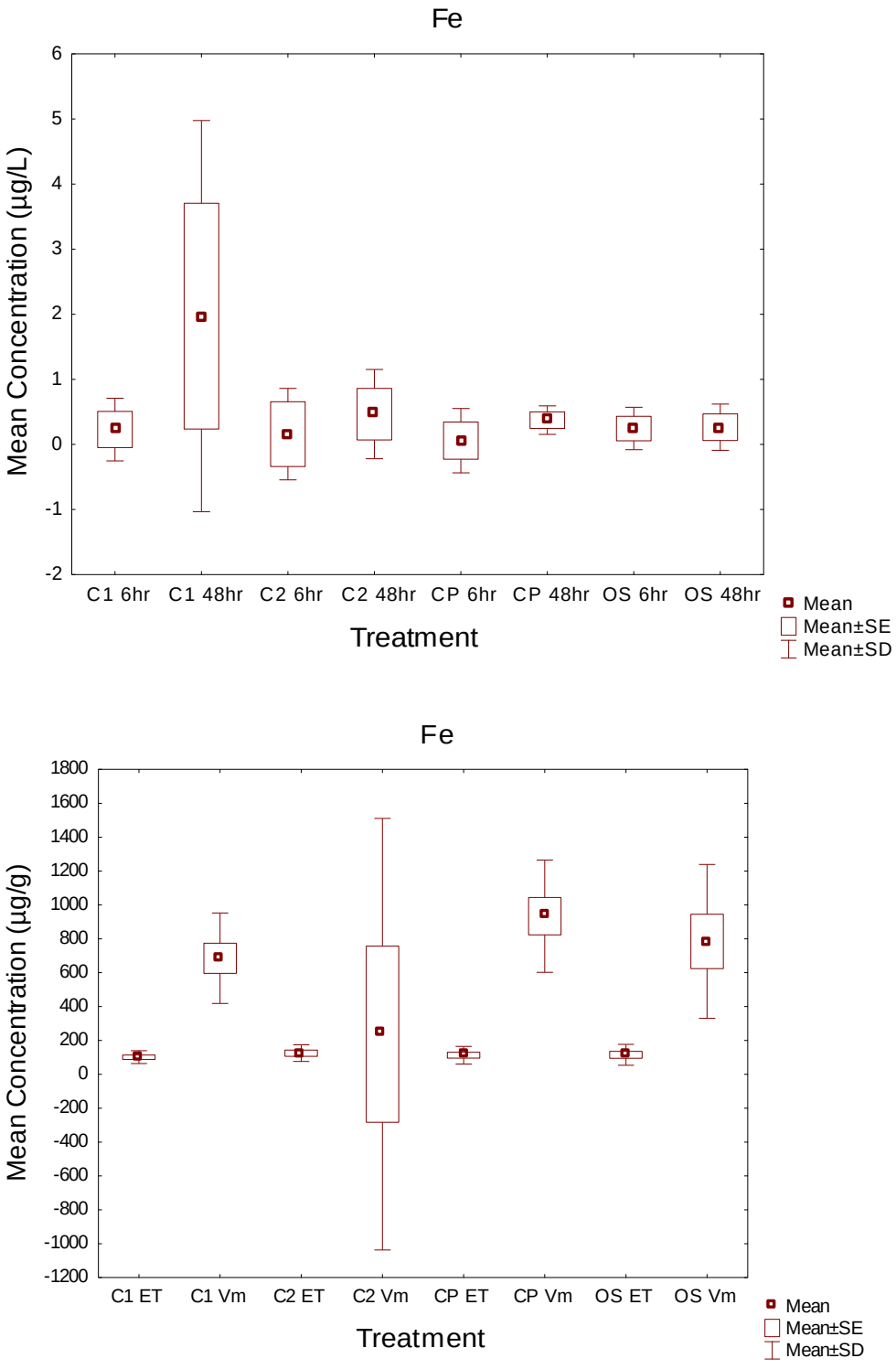

Fig 2-22: Mean concentration of iron in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass.

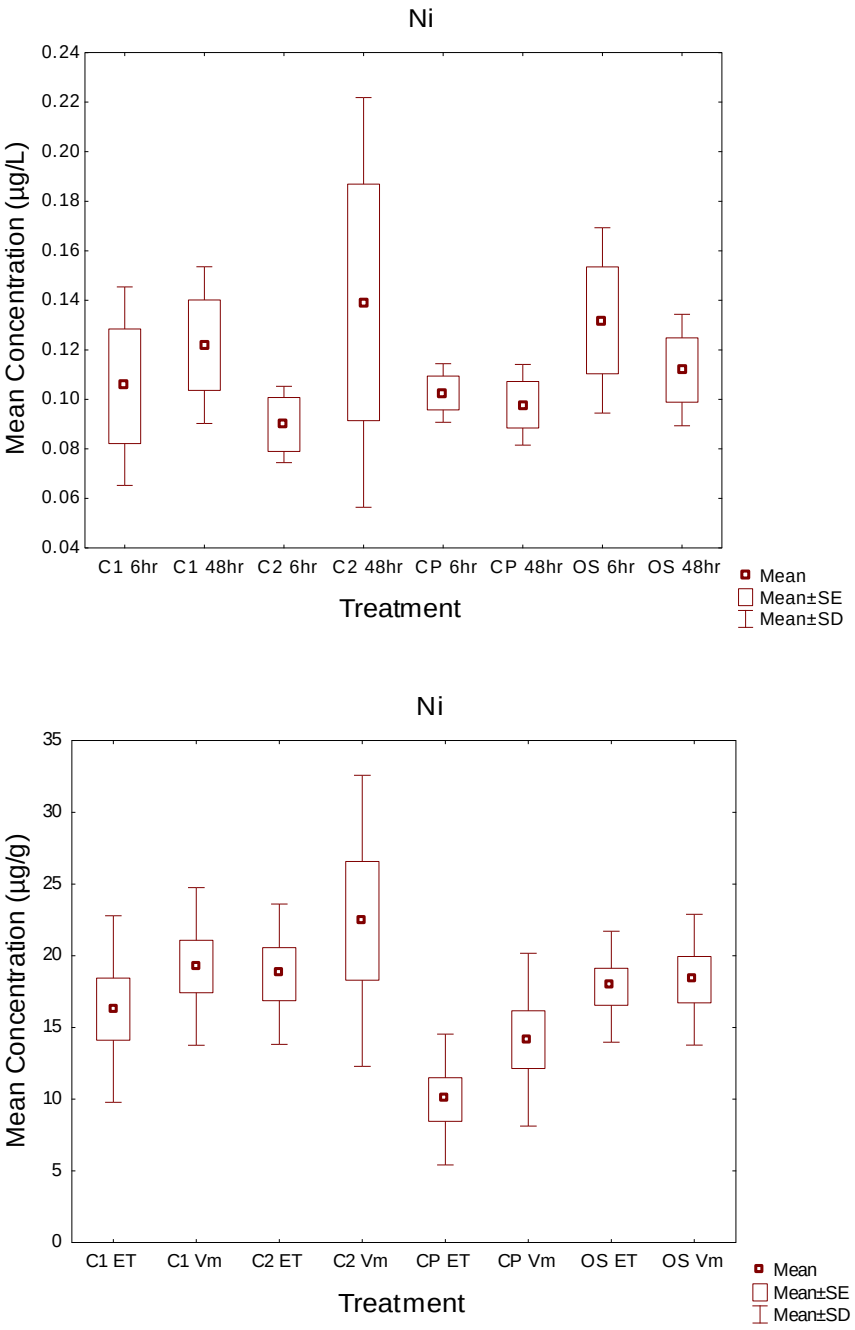

Fig 2-23: Mean concentration of copper in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass.

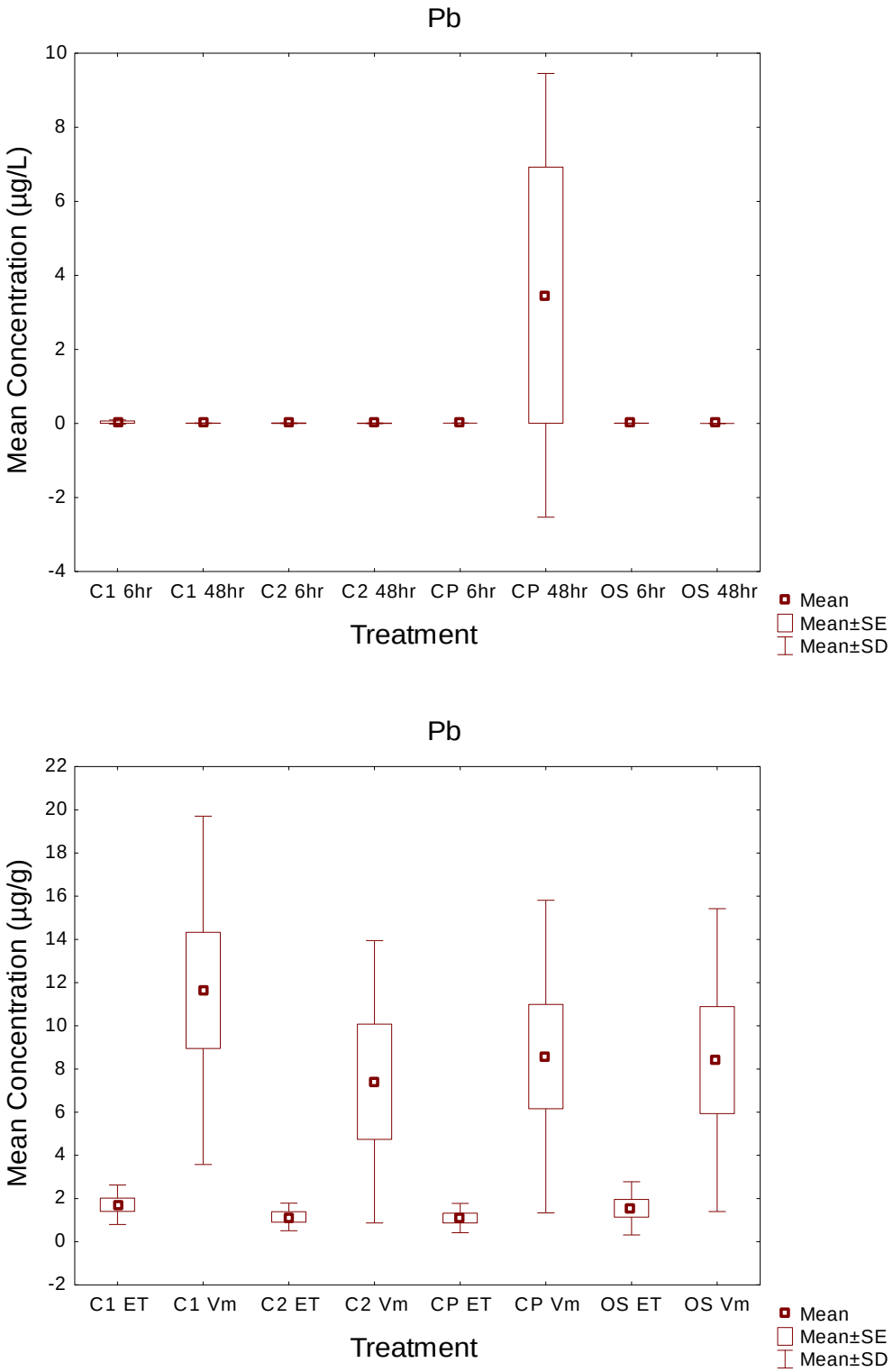

Fig 2-24: Mean concentration of lead in ambient water at 6hrs and 48hrs (top) and tissue (bottom) samples for control no sediment (C1), control with sediment (C2), copper positive (CP) and on-reef sediment (OS). Water samples taken at 6hr and 48hrs. Tissue analysed ET = edible tissue, Vm=Viscera mass.

2.3.13 Lead

There were no observed significant differences for ambient water concentrations at any time period for any group ($p>0.05$) (Fig 2.24 top).

2.3.13.1 Paua Tissues

Control 1 and 2 edible tissue was not significantly different to CP and OS edible tissue Fig 2.24 bottom). The same can be said for the viscera mass ($p>0.05$). There was no significant difference between the edible tissue and the viscera mass of all groups.

Table 2-4: Mean concentration of metals of CP (copper positive) and OS (Otaiti sediment) above, below, + and – or unchanged from the control for ambient water and tissues

Lab Experiment				
Trace Metals	**CP Water**	**OS Water**	**CP Tissue**	**OS Tissue**
Cu				
Mn				
P				
Al				
Co				
Cd				
Zn				
Cr				
Fe				
Ni				
Pb				
KEY: Differences from the controls				
Increase (+)			**Decrease (-)**	
+ and -			**Unchanged**	

2.4 Discussion

This study looked at the effect of sediment from Otaiti reef on the behaviour of paua and the accumulation of trace metals in the edible tissue and viscera mass of these animals. Behavioural effects were noted sooner in the Otaiti sediment treatments than all other treatments and these effects were severe. It is hypothesised that this was due to copper contamination as Cu was by far the most dominant trace metal seen in this study across all mediums analysed. In addition, the effects of positive copper control experiments matched those of Otaiti sediment treatments, albeit in a slightly delayed time frame.

Paua in controls 1 and 2 had normal behavioural response to stimuli with good tentacle presence. Paua also held their shell down securely when prodded and there was minimal mucus presence. Control 1 and 2 survival was 100% for the entirety of the study. All elements analysed had no significant difference between 6 and 48hr for controls 1 and 2 (Section 2.3.3, table 2.5). Within 6hrs the copper concentration in the water of Otaiti sediment treatment was 417.8±37.3 µg/L Cu compared to 164.5±3.54 µg/L Cu within the inert sediment control. Both values are within the expected range for Cu solubility assuming control by Cu hydroxides.

Behavioural abnormalities observed were reduced tentacle presences and delayed response to stimuli, followed by a retraction of the foot size in the mantle cavity (Fig 2.4). As the foot began to reduce in surface area with the substrate, surface adhesion reduced and the muscle strength appeared to weaken. This may be caused by blood moving away from the foot, adductor muscle and tentacles to areas more dependent on oxygen (Donovan, 1999). Paua <32mm appear to be affected earlier in the copper positive and Otaiti sediment treatments than those of the other size classes (Table 2.2). From 24-48hrs behaviour quickly deteriorated for the other size classes (<63mm) leading to 100% mortality at 48hrs for both Otaiti and copper positive treatment groups.

Increase of copper in solution increased the concentration in the edible tissue by 8 fold and the viscera mass by 3 fold in OS and CP from the controls. It is suggested that copper was likely to be a major contributing factor to the early aberrant responses in behaviour, as the concentration of copper for the same respective time or tissue type for copper positive and Otaiti sediment had no significant difference ($p< 0.05$). However, if copper was solely responsible for the affects seen here, it would be expected that the concentration of copper in the copper positive treatment would be higher than that of the Otaiti reef mixture, whereas the concentration of copper in the Otaiti treatment was similar to the positive copper treatment. Hence it is likely that other contaminants in the Otaiti sediment treatments influenced the resultant toxicity.

Water chemistry showed that manganese increased significantly from the controls in both copper positive and Otaiti treatment groups ($p<0.05$). Otaiti treatment was not significantly different at 6hrs compared to that of copper positive treatment for the same time period, although Otaiti treatment at 48hr was significantly different to all groups and times. This does not however indicate why the paua behaviour in the Otaiti treatment group was affected earlier than copper positive treatment group.

The viscera mass maintained a higher concentration of metals than that seen in the edible tissue, for all metals analysed, which is consistent with other studies (Ikuta, 1987; Hyne *et al.,* 1992). Gorski (2006) found that *Haliotis rubra* exposed to copper at 1, 5 and 25 µg/L was significantly different from the controls within two days of exposure. Concentrations as low as 1 and 5 µg/L reached concentration of 95.6 and 159.7 µg/g within the tissues in 28 days (Gorski, 2006). Considering concentrations in the edible tissue and viscera in this study were higher than that seen by Gorski (2006) after 48hr, it can be suggested that paua can effectively bioaccumulate copper within a short period of time.

The weight of Otaiti sediment and ‘copper positive’ used within treatments was equal. The Otaiti sediment contained a mixture of pebbles and various other materials in the sediments, whereas the copper positive control was

purely copper. Previous Rena research shows biota in close proximity to the seafloor have consistently shown elevated levels of accumulated metals such as copper, zinc and tin as well as PAHs and organotins (Ross *et al.,* 2015). Although PAHs and TBT were not included in this study, surface bubbles with a rainbow sheen consistent with fuel oil was observed in the Otaiti treatment group at 6hrs. This could suggest that there are hydrocarbons present in the sediment as recorded by Ross *et al.,* (2015).

A report by Ross & Battershill (2013), found that there were very few paua within the sites at Otaiti. PAHs in paua analysed at Otaiti, Mōtītī and the East Cape ranged from 0.003 to 0.0571 mg/kg. One individual black-foot paua was found at Astro-6 and Astro-7 had a muscle total PAH concentration of 0.0198 and 0.0571 mg/kg respectively, while Mōtītī and East Cape had a total PAH concentration of 0.007±0.003 and 0.016±0.006 mg/kg (±se) respectively.

Literature related to total PAHs and the effects to *Haliotis spp* is limited, however, Benzo(a)pyrene (B(a)P) a polycyclic aromatic hydrocarbon exposed to *Haliotis diversicolor* has been investigated. Gopalakrishnan*, et al.,* (2009) found a relationship between B(a)P and the immunological parameters at concentrations of 0.01 to 3.2 mg/L. B(a)P was found to significantly decreased the total number of circulating haemocytes. The paua analysed by Ross & Battershill (2013) had a B(a)P of <0.0008 mg/kg within the total muscle tissue (*pers comm*.). This is below concentration exposed to *Haliotis diversicolor* (Gopalakrishnan*, et al.,* 2009). However as seen in the results, the viscera mass can accumulate metals at higher elevations then the edible tissue, whether this is the case with hydrocarbons is unknown.

TBT concentrations within the tissue of biota on Otaiti have also been shown to be elevated (Ross *et al.,* 2015). Concentrations of 0.35 µg/L of TBT exposed to *Haliotis diversiocolor* for 21 days have shown that there was no observed recovery after 14 days to 21days exposure. This was determined from the intra cellular superoxide and nitrite production and a decrease in

total hemocyte count, membrane stability and lysozyme activity (Gopalakrishnan*, et al.,* 2011).

Bulk water samples from this study show the decrease in concentration of cadmium and zinc overtime. Although these changes in concentration were not significant within the 48 hrs of the experiment, it does show an interesting trend. Ross *et al.,* (2015) found elevated levels of cadmium and zinc in the sediment at Otaiti. The control without sediment for cadmium and zinc was the only treatment not to show a decrease in concentrations of these metals. No significant differences (>0.05) were observed indicating that metal uptake was occurring in the tissues within 48hrs of this study. Gorski (2006) found that *Haliotis rubra* did not significantly accumulate zinc until after 7 days of exposure to 20 µg/L in the edible tissue while viscera mass decreased in concentration prior to 7days and then increase significantly from 14 days until reaching a maximum concentration at 28 days of 437.8 µg/g. Gorski (2006) found that exposure to cadmium at concentration of 4 µg/L was significantly different in the edible tissue following initial exposure. Significant accumulation in the visceral mass did not occur until 21 days after exposure. Furthermore the viscera mass had the highest accumulation of cadmium compared to the edible tissue (Gorski, 2006), which also was seen in this study.

Different organisms accumulate contaminants at different rates (Phillips, 1994b), however it can be seen in this study that after 48hrs of exposure to Otaiti sediment, concentrations in paua edible tissue and viscera mass elevated to 144.20±22.06 µg/L Cu and 187.93 ±17.68 µg/L Cu respectively while the inert sediment control was 15.84±2.66 µg/L Cu in the edible tissue and 52.84±7.07 µg/L Cu in the viscera mass. The tissue analysis in the study corroborates that of Ikuta (1987). The most likely way for metals to accumulate in the tissues is via the blood stream. Abalone obtains oxygen and essential trace metals from water flow over the gills. Haemocyanin found in the blood, transports essential trace metals such as copper throughout the body to different tissue groups making it available for accumulation.

A cocktail of trace metals, oil and other contaminants that were not analysed, could be a contributing factor for the early effects seen from the paua in OS treatment group. The range of contaminants released from the Rena could also influence observed early effects to behaviour. The mixture of the trace metals makes it difficult to predict whether or not it is a single trace metal or a combination of metals that is causing the effects to the paua. For instance, Cadmium and manganese were the only trace metals that had a higher concentration within the water of the Rena sediment treatment than that in Cu treatment and the controls 1 and 2 (0.24±0.0 µg/L, 0.11±0.03 µg/L, 0.00 µg/L and 0.13±0.08 µg/L respectively). This is presumably due to the additional contamination from other sources in the Rena sediments. Other pollutants that are of concern due to high levels seen within the Otaiti sediment include PAHs and organotins (Ross *et al.,* 2015) though those analyses are not included in this study. Surface bubbles with a rainbow sheen began forming in OS aquariums could be an indicator of the range of contaminants that have been released from the Rena as the same bubbles weren't present in the other treatment tanks.

Hence the effects described here are not likely to be limited to copper and the other trace metal alone. The results do however indicated that it is relevant to examine the reality of complex mixtures of contaminants with appropriate controls. This is rarely done in ecotoxicological studies which tend to focus on individual contaminant compounds. While it is beyond the scope of this thesis to tease apart the interacting chemistry, it is clear that the Otaiti sediments are having a significant effect more than equal to pure copper contamination at high levels.

2.4.1 Limitations of the research

The size of the paua limited the amount of trace metal analysis that could be achieved. For that reason PAH and organotins could not be analysed. The continual release of trace metals within the aquaria caused increasing concentrations over time. In the environment the concentrations would not accumulate to such levels in the water as quickly resulting in more of a biological accumulation effect at lower concentrations over time.

Chapter 3

Field Experiment

3.1 Introduction

With the continuing growth of the human population and need of resources to supply the demand, there is a consistent pressure being placed on the environment (He *et al.,* 2013). The need for resources to be exported from one part of the world to another is a way for countries to fulfil this demand. Container ships are the most effective way of transporting large quantities goods globally. Container ship capacity is measured in twenty-foot equivalent units (TEU). However the loads can be a mix of 20 and 40 foot (2-TEU) ISO standard containers (WSC, 2014). In 2013, approximately 120 million containers packed with cargo were transported over the oceans globally. For 2011, 2012 and 2013 it was estimated that the average annual loss of containers over board was 2,683 containers, this includes catastrophic losses (WSC, 2014). However, the WSC considers this average to be enlarged due to two factors. The first was in 2013 when the *MOL Comfort* lost all 4,293 containers with the vessel in the Indian Ocean and the second was the grounding of the MV Rena on Otaiti (Astrolabe) reef which lost roughly 900 containers over board off the Bay of Plenty, New Zealand (WSC, 2014).

The Rena was 236m long and had a dead weight of 47,231 tonnes. The vessel container capacity was 3351 TEU and at the time of the grounding, was carrying 1,368 containers. The grounding was on the 5th October 2011 and has been called New Zealand's worst maritime environmental disaster by Nick Smith (Harper, 2011). Just over 3 years on from the grounding of the Rena, sediment analysed from around the reef has shown elevated levels of heavy metals such as cadmium, copper, chromium, lead, nickel, tin and zinc, while organotins and PAHs appear to be more widespread (Don, 2014; Ross*, et al., in press*). Of the biota that has been found in large enough quantities for analysis to be performed, urchins (*Evechinus*

chloroticus), gastropods, benthic predatory fish such as sea perch (*Helicolenus percoides*) and scorpion fish (*Scorpaena papillosa*) have recorded elevated levels of these contaminants (Ross & Battershil, 2014, *in press*). This highlights the likelihood that species associated with the seafloor are more likely to accumulate metals, organotins and PAHs through trophic interactions.

Paua are an important and indeed iconic species to New Zealand and have special significance to iwi. This is certainly the case for the Bay of Plenty and to Mōtītī islanders in particular. Paua are important ecologically characterising shallow reef environments. However, very little research has been conducted on paua (*Haliotis iris*) in regards to environmental contamination in general and to the Rena grounding in particular. Paua are a benthic species that reside commonly between 0-10m water depths where oil and debris released from Otaiti reef coincided before being washed up on the surrounding shores of Mōtītī and surround Bay of Plenty region.

Rena related monitoring conducted in 2012 found that the total PAH ranges in the muscle of paua from areas affected by Rena debris on Otaiti was between 0.0198 and 0.0571 mg/kg. Paua analysed for total PAH from Mōtītī and the East Cape ranged between 0.007±0.003 and 0.016±0.006 mg/kg (Ross & Battershill, 2013). No other Rena related analysis has been done for paua.

Paua are important culturally, recreationally and commercially, so it is important to understand whether they have been affected by the debris released from the Rena. It has been identified in the previous chapter that paua are affected by Otaiti sediment in a laboratory situation.

The purpose of this chapter is to investigate whether paua are affected by Otaiti sediment in regard to the accumulation of trace metals in the edible tissue and viscera mass as well as survival in a field manipulation experiment. Due to resource limitations, organotins and PAHs were not analysed.

3.2 Methods

3.2.1 Study Animals

Paua were collected and maintained as described in the previous chapter. Paua remained in aquaria until the 29th October 2014 as an acclimatisation step, at which time they were deployed into the field experiment. Paua were retrieved on the 1st December 2014 for trace metal analysis.

3.2.2 Field Cage Construction

Paua cages were constructed using PVC stormwater pipe and secured in place with two star pickets (Fig 3-1). Each pipe was 400mm in length with a 255mm diameter opening at each end. A 200mm x 255mm diameter section was removed from the centre portion leaving 100mm either side of the pipe. The openings allowed water to flow in and out of the cage while still maintaining structural strength within a high energy environment and also gave the paua areas for shelter.

Plastic 15mm mesh (from corner to corner) was fixed with cable ties to each end and over the central portion of the cages to prevent paua becoming lost to the environment. Four holes were drilled into the bottom centre of the cage to secure the treatment sediment and also allow any sediment to flush out that may build up.

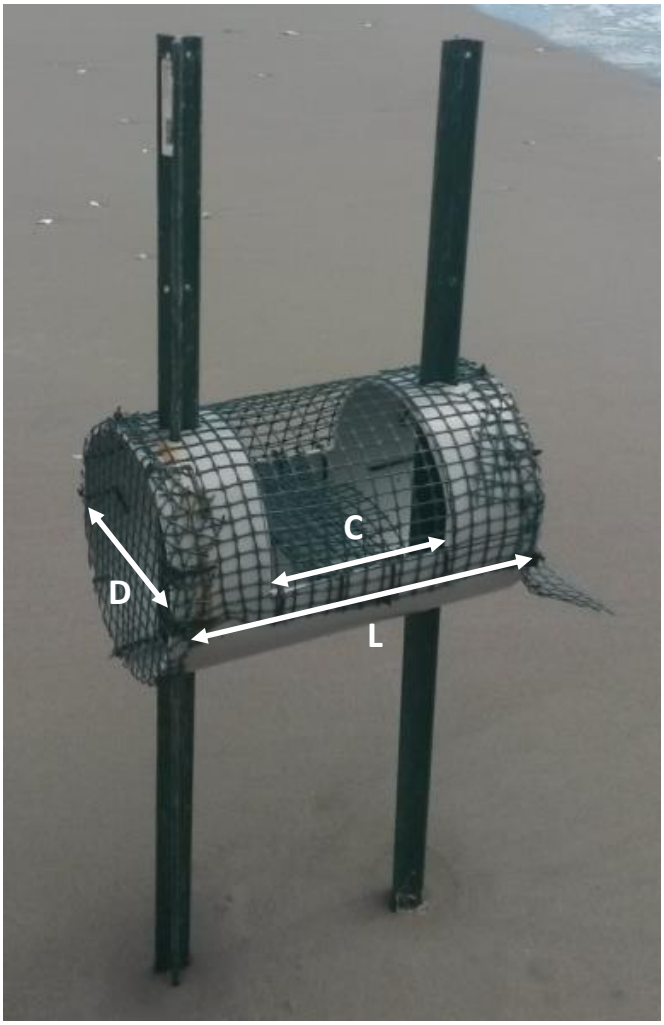

Fig 3-1: Paua Cage fixed in position on two 1.8m star pickets. (L)ength=400mm, (D)iameter= 255mm, (C)entre cut out=200mm.

3.3.3 Location of Deployment

The experiment was located off Moturiki Island at Omanu Beach near Mount Maunganui GPS 37 37.886ºE, 176 11.186ºS (Fig 3.2).

Two treatment groups and a control were deployed (control, copper filing and Rena sediment) with each cage containing one bag of sediment weighing 200g. Three replicates per treatment were installed. The experiment covered an approximate area of 20m x 4m. Each treatment was placed 10m apart, with each replicates 2m from each other.

The cages were orientated so that the star pickets were on a north to south bearing in order to ensure moderate (not extreme) water flow through the tubes (prevailing swells ran northeast to southwest, also providing the structure with more stability.

Paua were allocated randomly into cages at 10 animals per cage. The experiment had a soak time of 33 days. Weather was moderate in this period and there was no significant surf.

Fig 3-2: Area where the experiment took place. Each star represents approximate location and position of each treatment group and cage.

3.3.4 DGT Analysis

Three DGTs were attached to each cage. DGTs were attached to each end of the cage in the centre portion of the openings and one attached to the top opening in the centre. This was to minimise the chances of paua crawling over the DGTs limiting the amount of metals diffusion into the resin layer and to also inhibit mucus presence on the DGTs, thereby affecting the accumulation of metal and impacting the overall result. DGTs were attached by cutting the mesh so that the outer sleeve window could protrude into the enclosure. A piece of mesh was placed over the bottom end of the piston, securing it from being dislodged and lost to the environment. The cages were orientated on a North-South bearing and each end of the cage was

marked accordingly. Orientating the cages on a North-South bearing minimised the amount wave energy exerted on the cages reducing the chances of dislodgment. This also allowed comparisons to be made of which end of the cage contains the highest amount of trace metals.

Upon retrieval, the securing mesh was clipped off allowing the DGT to be removed and bagged. The time was taken of when the DGT's were removed from the water. They were then rinsed with De-ionised water to remove any deposited sediment and bio-foul off the membrane and piston. They were then placed individually into zip lock bags, labelled and chilled on ice until returning to the Coastal Field Station. Once there, DGT's were refrigerated until analysis was undertaken. DGT were prepared for analysis as described in chapter 2.

3.3.5 Tissue Preparation

Paua were removed from their cages on site and placed in zip lock bags, labelled and chilled until arrival at the Coastal Field Station (15 minutes away). Once there, paua were shucked, the edible tissue was separated from the viscera mass. Each tissue sample was then coded and placed individually into the oven to dry at 51°C for 72hrs. Samples were then homogenised with a pestle and mortar.

Due to resource limitations, five edible tissues were amalgamated into one sample per cage. The same was done for the viscera mass. As there were three replicates per treatment, this gave a sample size three for statistical analysis. The preparation of the tissue followed that described in the previous chapter prior to ICP-MS analysis.

3.3 Results

The duration of the experiment was 33 days with a temperature of 14.6 ± 1C°, pH 8.0, O_2 was 91.2%/7.61mg/L and conductivity was 39.04 and 48.64.

There was one paua from the Rena treatment cages that perished 8th November. It cannot be determined whether the paua died from an accumulation of trace metals from the Rena sediment, whether it had been

preyed upon by other species such as octopus, hermit crabs or baby crayfish, a combination of these, or another factor due to no tissue being present and only the shell remaining. The 15mm gap in the mesh could not restrict all biota from the cage. The main purpose for the mesh was to prevent the treatment sediment and the paua from being lost to the environment. There was one paua in each of the treatment groups (copper and Rena treatments) that show signs of lethargy with minimal response to stimuli on the foot.

There were an estimated total of 48 hermit crabs and 15 juvenile crayfish across the control cages, 40 hermit crabs and 20 juvenile crayfish across the Rena treatment cages and 33 juvenile crayfish and 70 hermit crabs and across the copper treatment cages. Juvenile crayfish observed were in the puerulus stage in all of the cages and triple fin were also observed in large numbers. However as the occurrence of these species was not expected these numbers are only estimates as some of the hermit crabs, crayfish and triple fins escaped upon retrieval. It is recommended for future work of this kind, that the cages be bagged before removal to allow an accurate record of the species that may inhabit such areas and allow statistical analysis be performed to determine whether or not these species are attracted to the structure or the contaminant.

3.3.1 DGT and Tissue Trace Metals

There is limited data on the diffusive coefficient of metals for DGT analysis. The following trace metals relate to those which have known diffusion coefficients:

3.3.2 Copper (Cu)

3.3.2.1 DGT's

CP and OS were not significantly different from each other ($p>0.05$) (Fig 3.3 top). CP and OS were significantly different from the control; however they were not significantly different from each other. CP had the greater mean concentration of copper followed by OS.

3.3.2.2 Edible Tissues and Viscera Mass

There were no significant differences in the tissue data (p>0.05) (Fig 3.3 bottom). CP and OS tissues were greater than the mean concentration of the control. CP and OS viscera mass had a greater mean concentration then the edible tissue. This trend was not seen in the control.

3.3.3 Manganese (Mn)

3.3.3.1 DGT's

There was no significant differences observed (p>0.05) (Fig 3.4 top). The mean concentration was greater in OS than the other groups.

3.3.3.2 Edible Tissues and Viscera Mass

There were no significant differences in the tissues (p>0.05) (Fig 3.4 bottom). CP and OS had a lower mean concentration than the control. The mean concentration in the viscera mass was greater than the edible tissue for all groups.

3.3.4 Aluminium (Al)

3.3.4.1 DGT's

There was no significant differences observed (p>0.05) (Fig 3.5 top). The mean concentration was greater in OS than the other groups.

3.3.4.2 Edible Tissues and Viscera Mass

There were no significant differences in the tissues (p>0.05) (Fig 3.5 bottom). OS and CP had lower mean concentrations then the control. The viscera mass mean concentration was higher than the edible tissue for all groups.

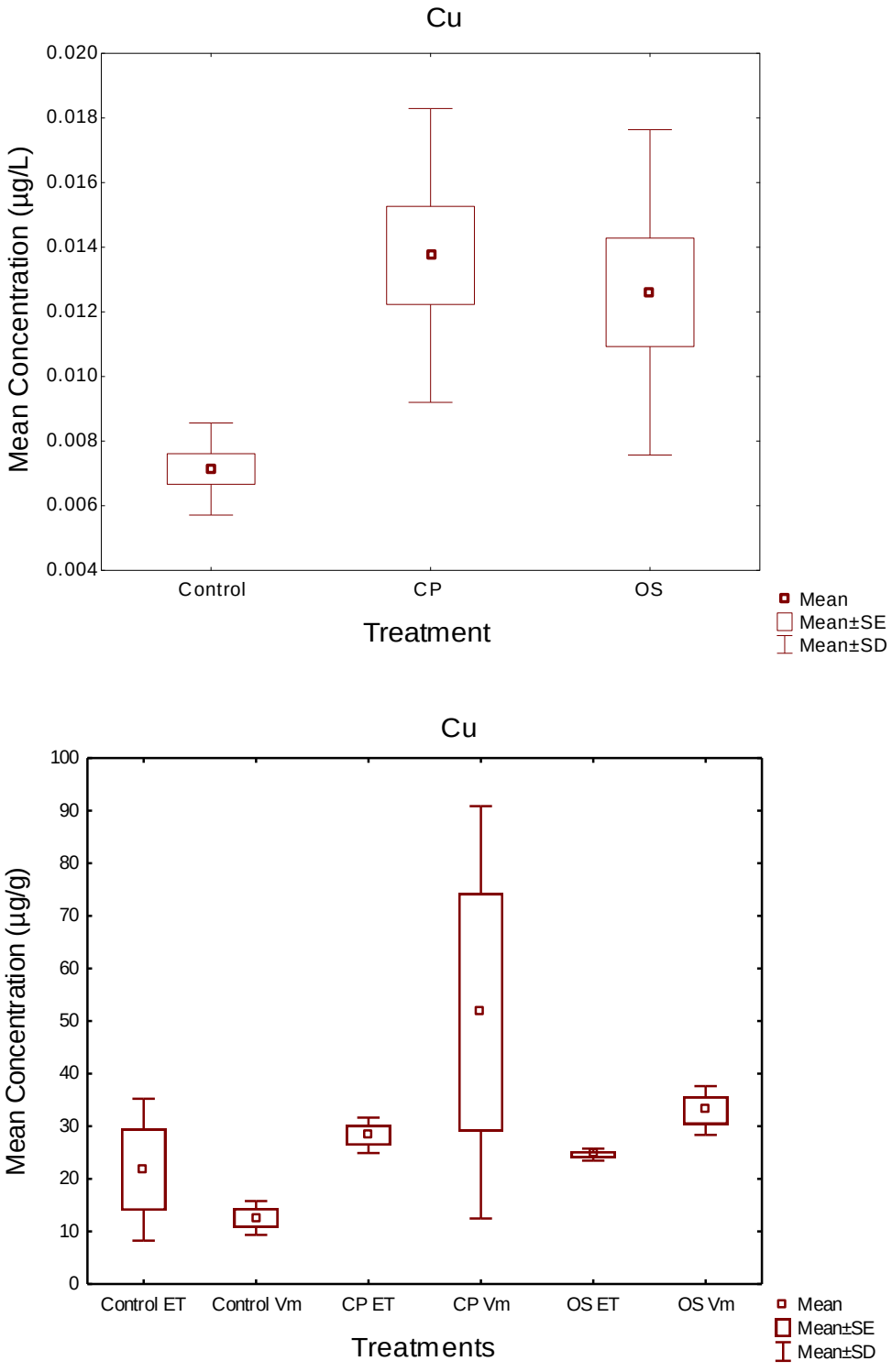

Fig 3-3. Mean concentration of copper in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

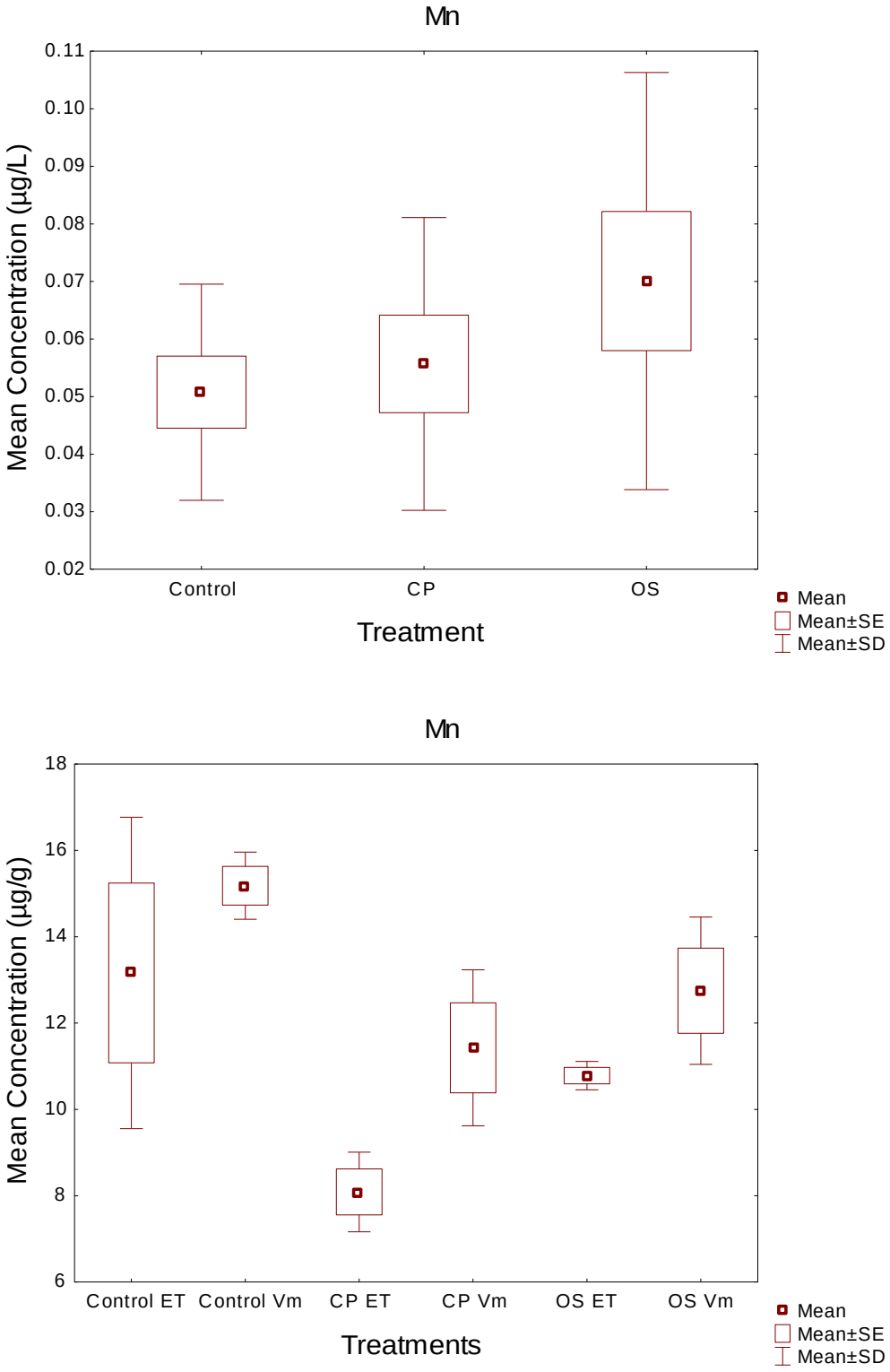

Fig 3-4: Mean concentration of manganese in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

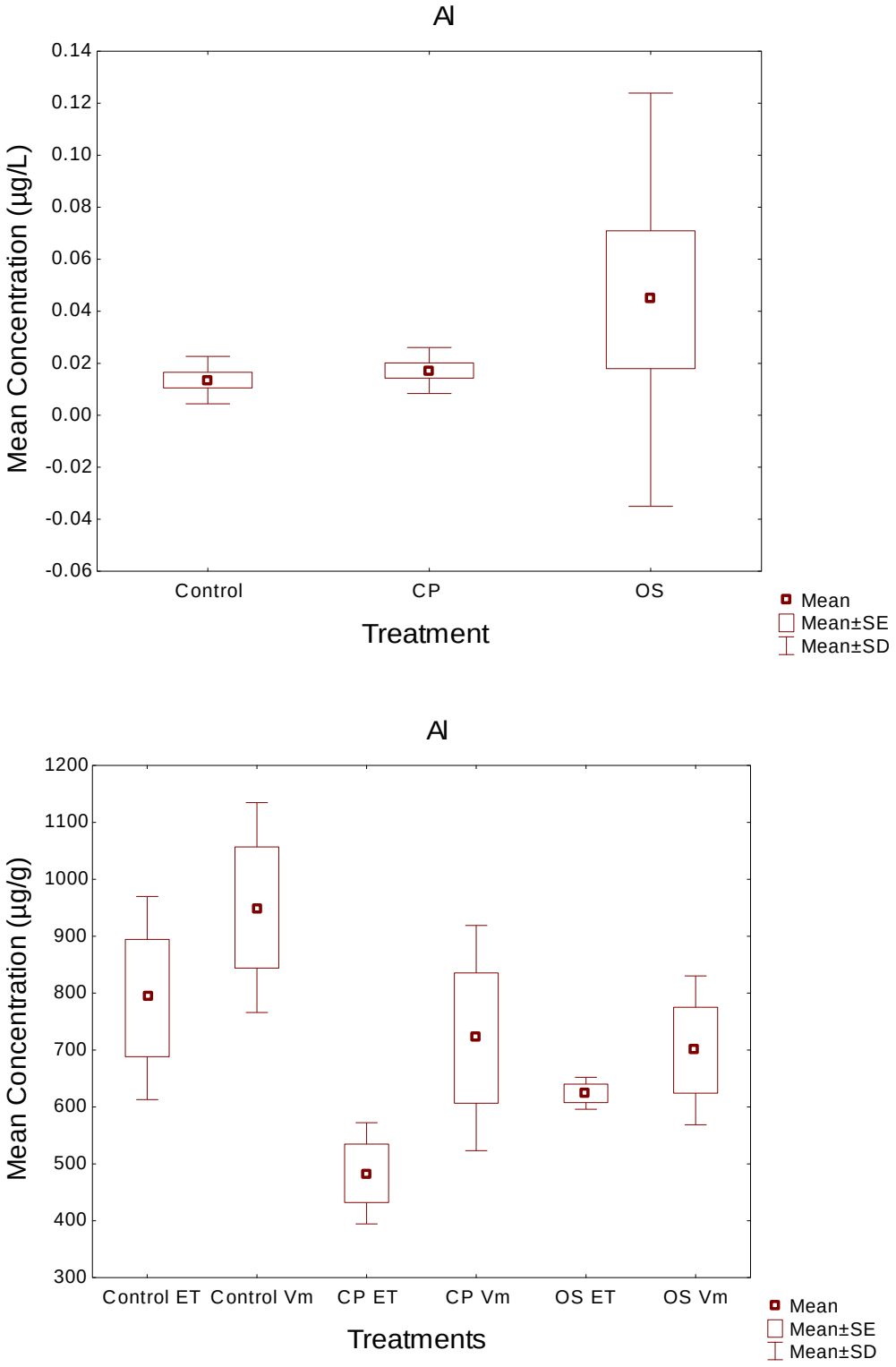

Fig 3-5: Mean concentration of aluminium in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

3.3.5 Cobalt (Co)

3.3.5.1 DGT's

There was no significant difference observed ($p>0.05$) (Fig 3.6 top). The mean concentration was greater in OS than the other groups. The north DGT in OS was significantly different to the controls and the south DGTs of OS and CP.

3.3.5.2 Edible Tissues and Viscera Mass

OS was not significantly different from CP for either the edible tissue or viscera mass ($p>0.05$) (Fig 3.6 bottom). The viscera mass was significantly different to the edible tissue in OS and CP ($p<0.01$). The viscera mass mean concentration was greater than the controls while the edible tissue in OS and CP was less in the control.

3.3.6 Cadmium (Cd)

3.3.6.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.7 top). The mean concentration was greater in OS than the other groups.

3.3.6.2 Edible Tissues and Viscera Mass

OS was not significantly different from CP for either the edible tissue or viscera mass ($p>0.05$) (Fig 3.7 bottom). The viscera mass was significantly different to the edible tissue in OS and CP ($p<0.05$). The viscera mass mean concentration was greater than the controls while the edible tissue in OS and CP was less than in the control.

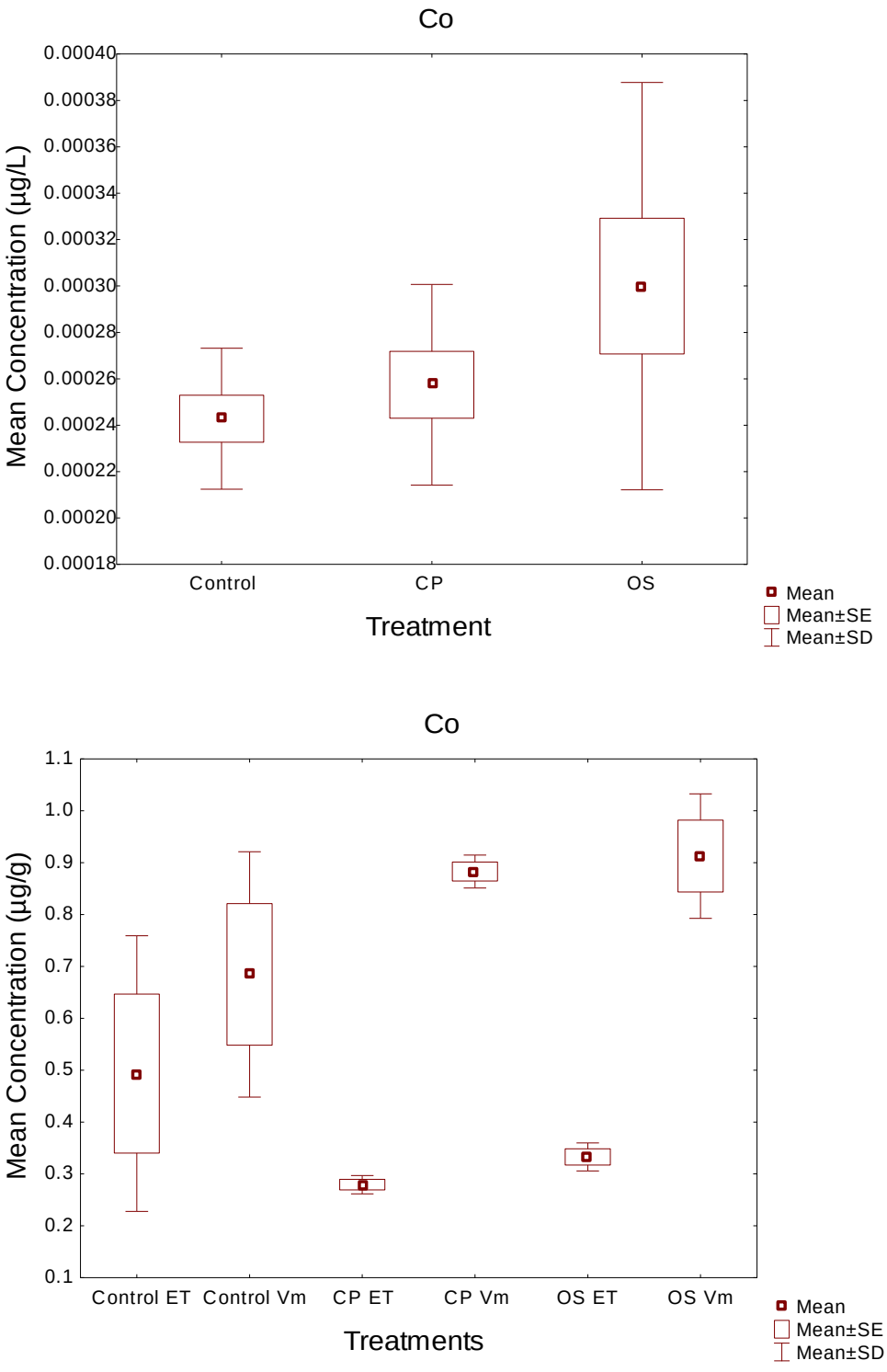

Fig 3-6: Mean concentration of cobalt in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

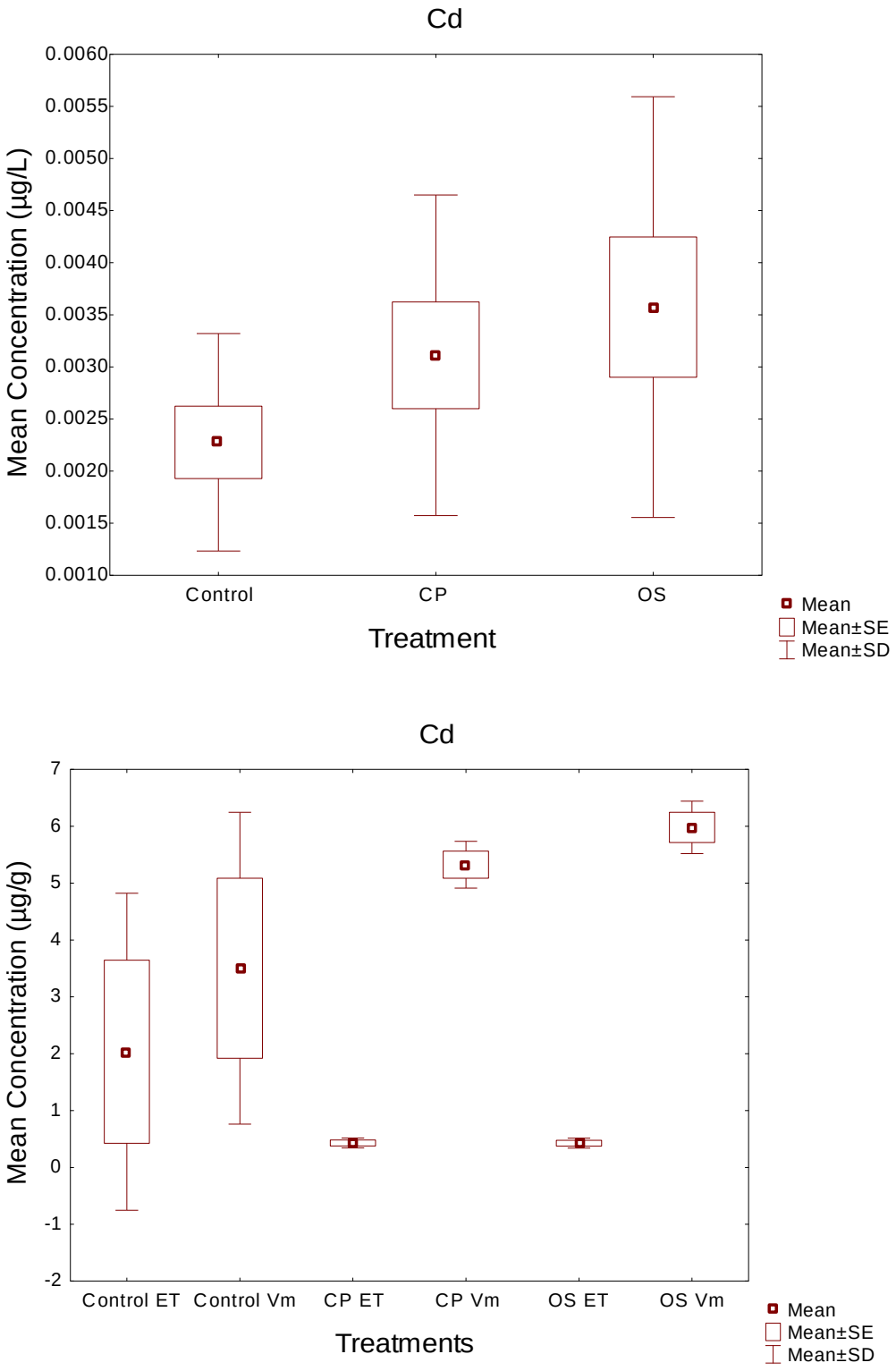

Fig 3-7: Mean concentration of cadmium in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

3.3.7 Zinc (Zn)

3.3.7.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.8 top). The mean concentration was greater in OS than the other groups.

3.3.7.2 Edible Tissues and Viscera Mass

OS was not significantly different from CP for either the edible tissue or viscera mass ($p>0.05$) (Fig 3.8 bottom). The viscera mass was significantly different to the edible tissue in OS and CP ($p<0.05$). The viscera mass mean concentration was greater than the controls while the edible tissue in OS and CP was less than in the control.

3.3.8 Chromium (Cr)

3.3.8.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.9 top). The mean concentration was greater in OS than the other groups.

3.3.8.2 Edible Tissues and Viscera Mass

OS was not significantly different from CP for either the edible tissue or viscera mass ($p>0.05$) (Fig 3.9 bottom). The edible tissue had a greater mean concentration than the viscera mass. OS viscera mass was lower than the controls while the edible tissue was greater.

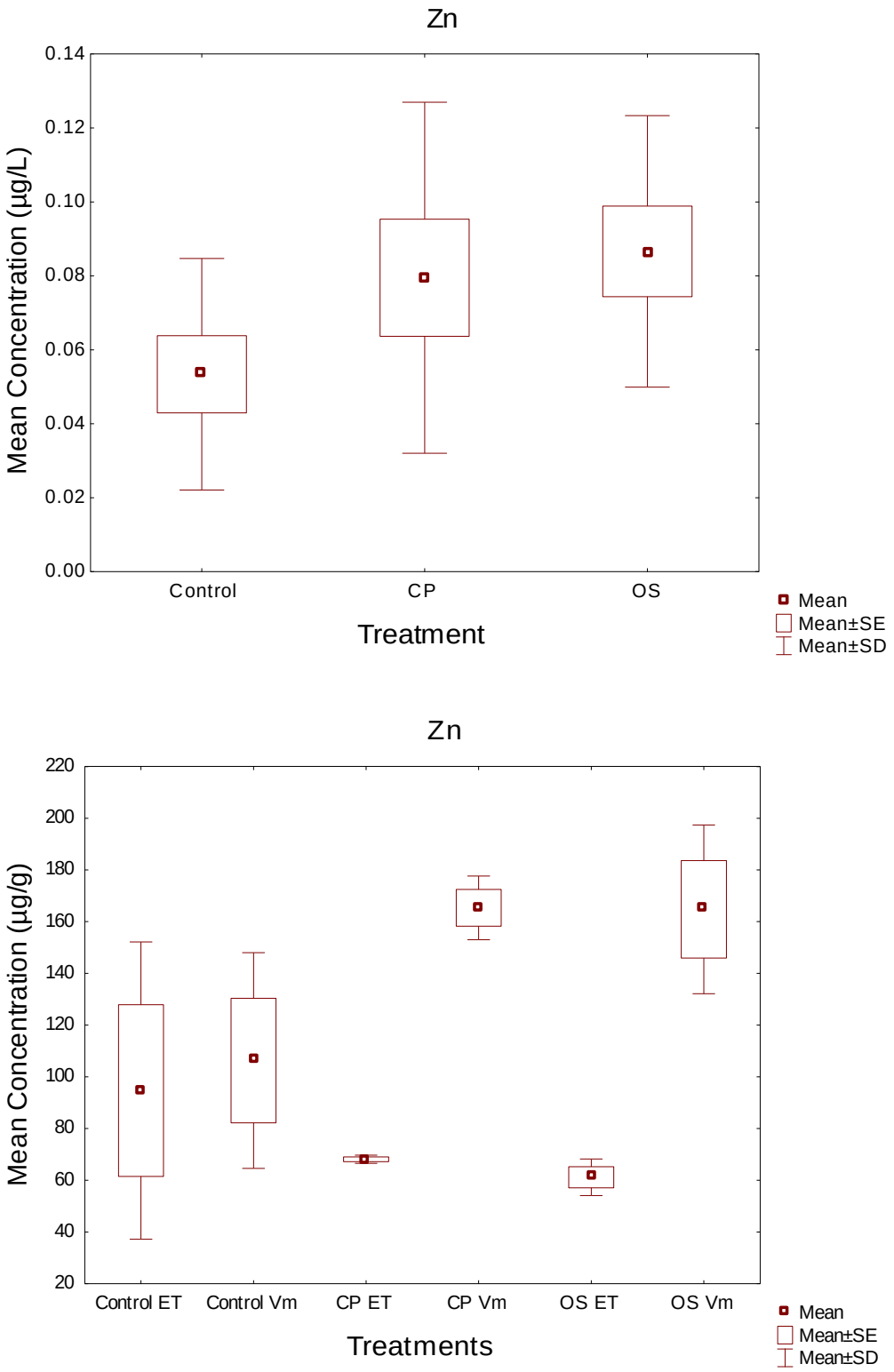

Fig 3-8: Mean concentration of zinc in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

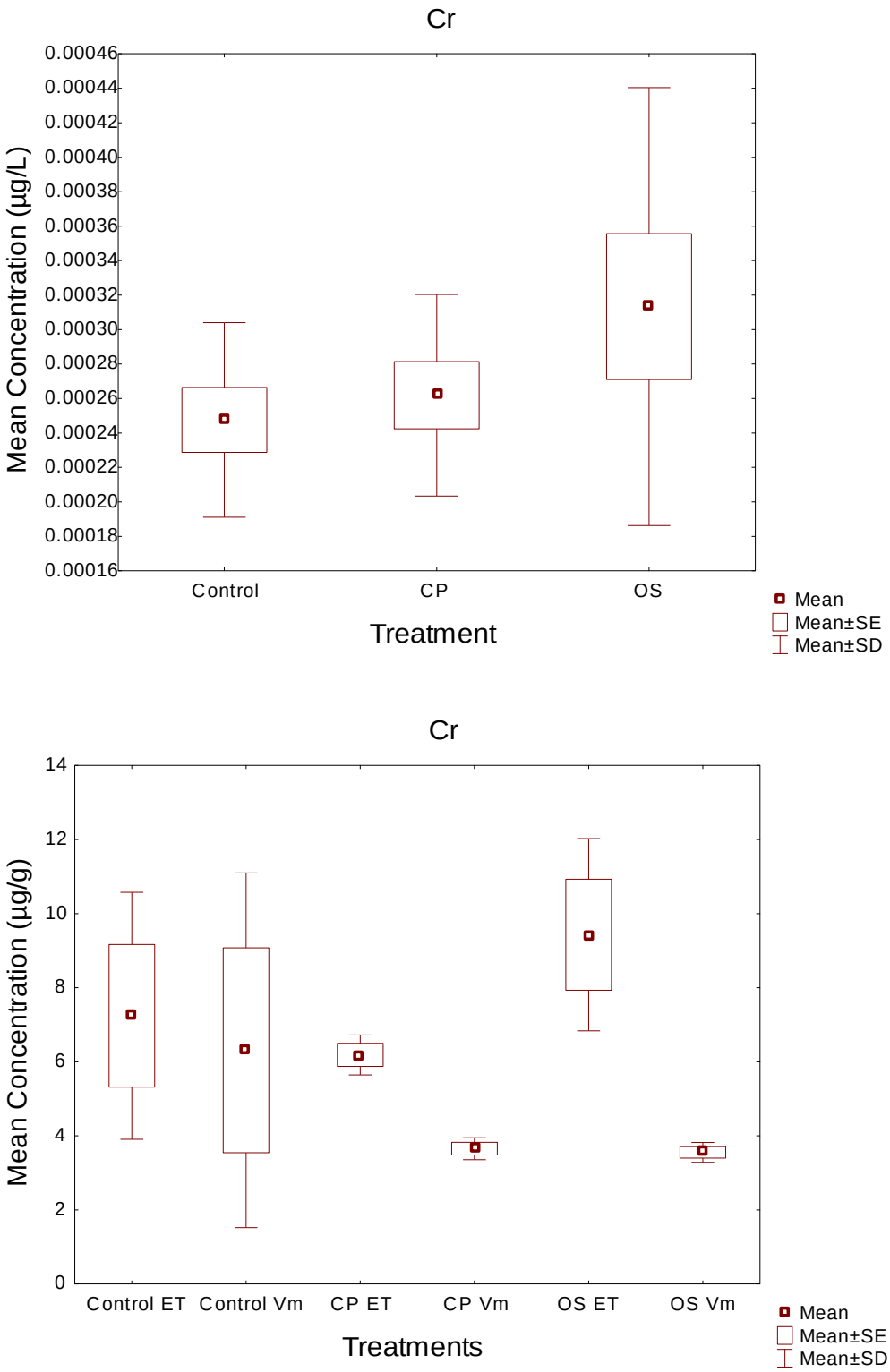

Fig 3-9: Mean concentration of chromium in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

3.3.9 Iron (Fe)

3.3.9.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.10 top). The mean concentration was greater in OS than the other groups.

3.3.9.2 Edible Tissues and Viscera Mass

OS was not significantly different from CP for either the edible tissue or viscera mass ($p>0.05$). CP and OS edible tissue was significantly different from the control ($p<0.05$) (Fig 3.10 bottom), however the viscera mass was not significant ($p>0.05$). The edible tissue of CP and OS was significantly different from the viscera mass ($p<0.01$).

3.3.10 Nickel (Ni)

3.3.10.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.11 top). The mean concentration was greater in OS than the other groups.

3.3.10.2 Edible Tissues and Viscera Mass

There was no significant differences observed in the tissues ($p>0.05$) (Fig 3.11 bottom). CP and OS viscera mass mean concentration was greater than the edible tissue. The control had a lower mean concentration in the viscera mass than the edible tissue.

3.3.11 Lead (Pb)

3.3.11.1 DGT's

There was no significant differences observed ($p>0.05$) (Fig 3.12 top). The mean concentration was greater in OS than the other groups.

3.3.11.2 Edible Tissues and Viscera Mass

OS was not significantly different from either the edible tissue or viscera mass of CP or the control ($p>0.05$) (Fig 3.12 bottom). The mean concentration of the viscera mass was significantly greater in than the edible tissue for CP and OS ($p<0.05$).

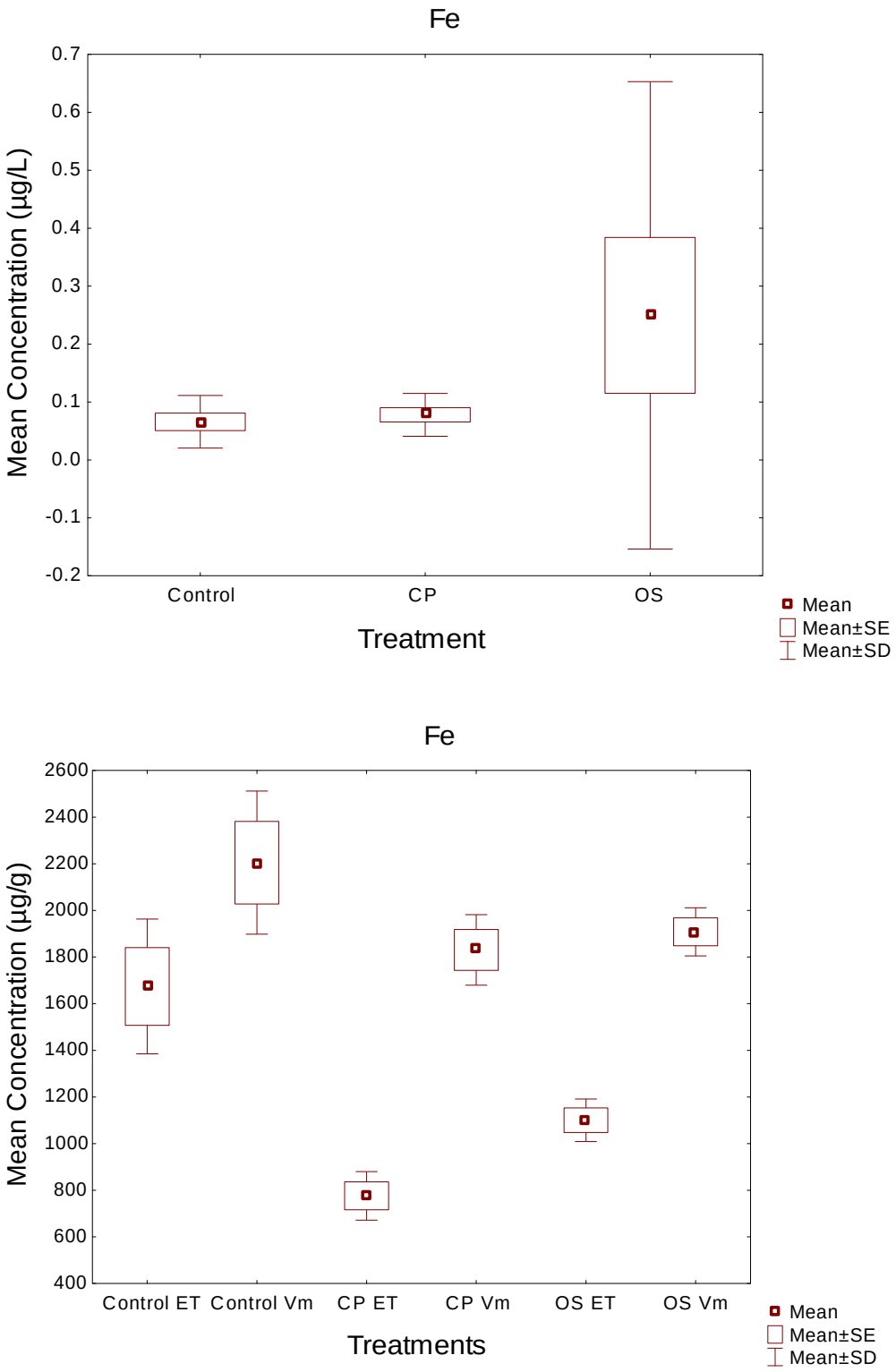

Fig 3-10: Mean concentration of iron in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

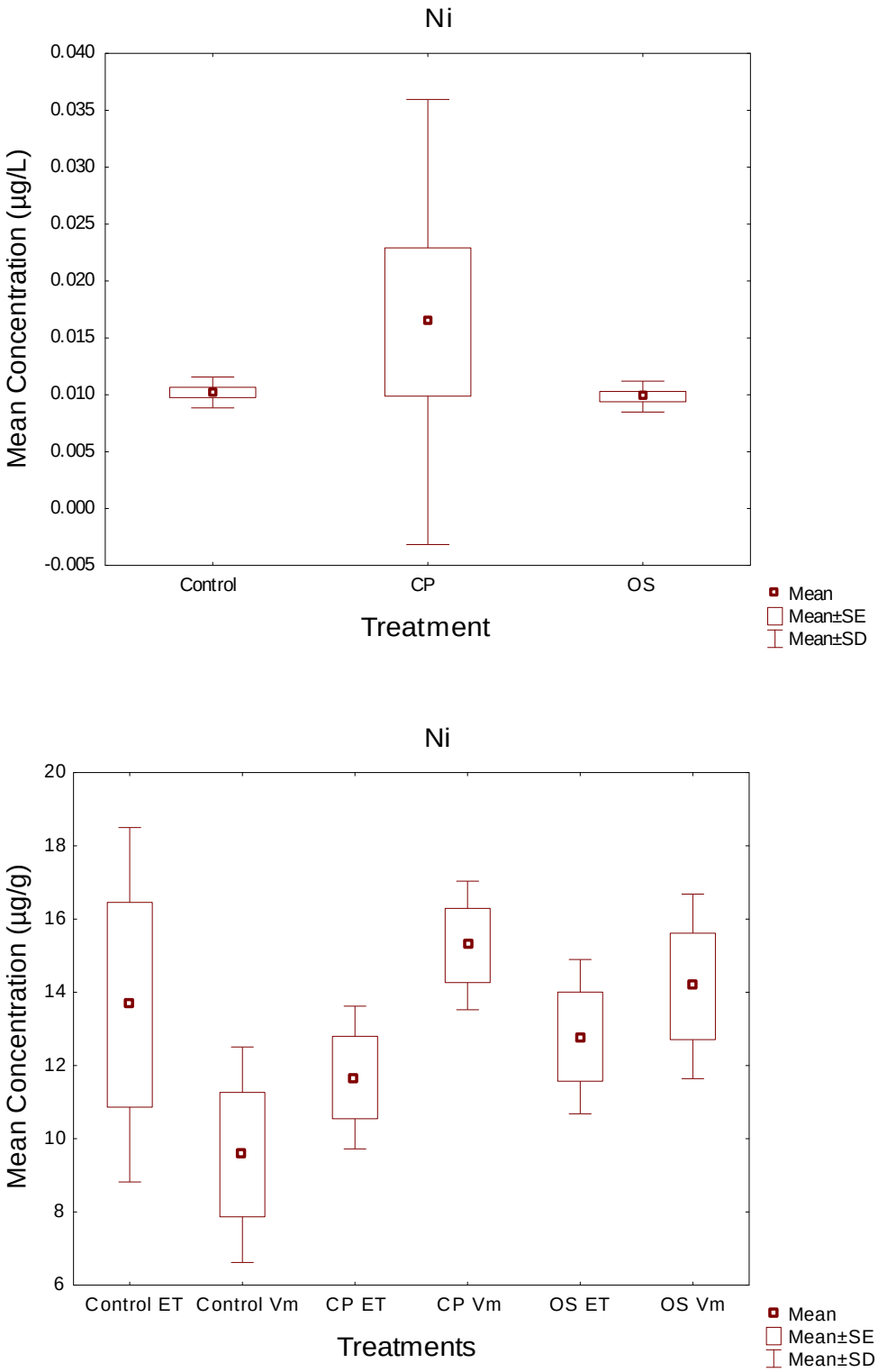

Fig 3-11: Mean concentration of nickel in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

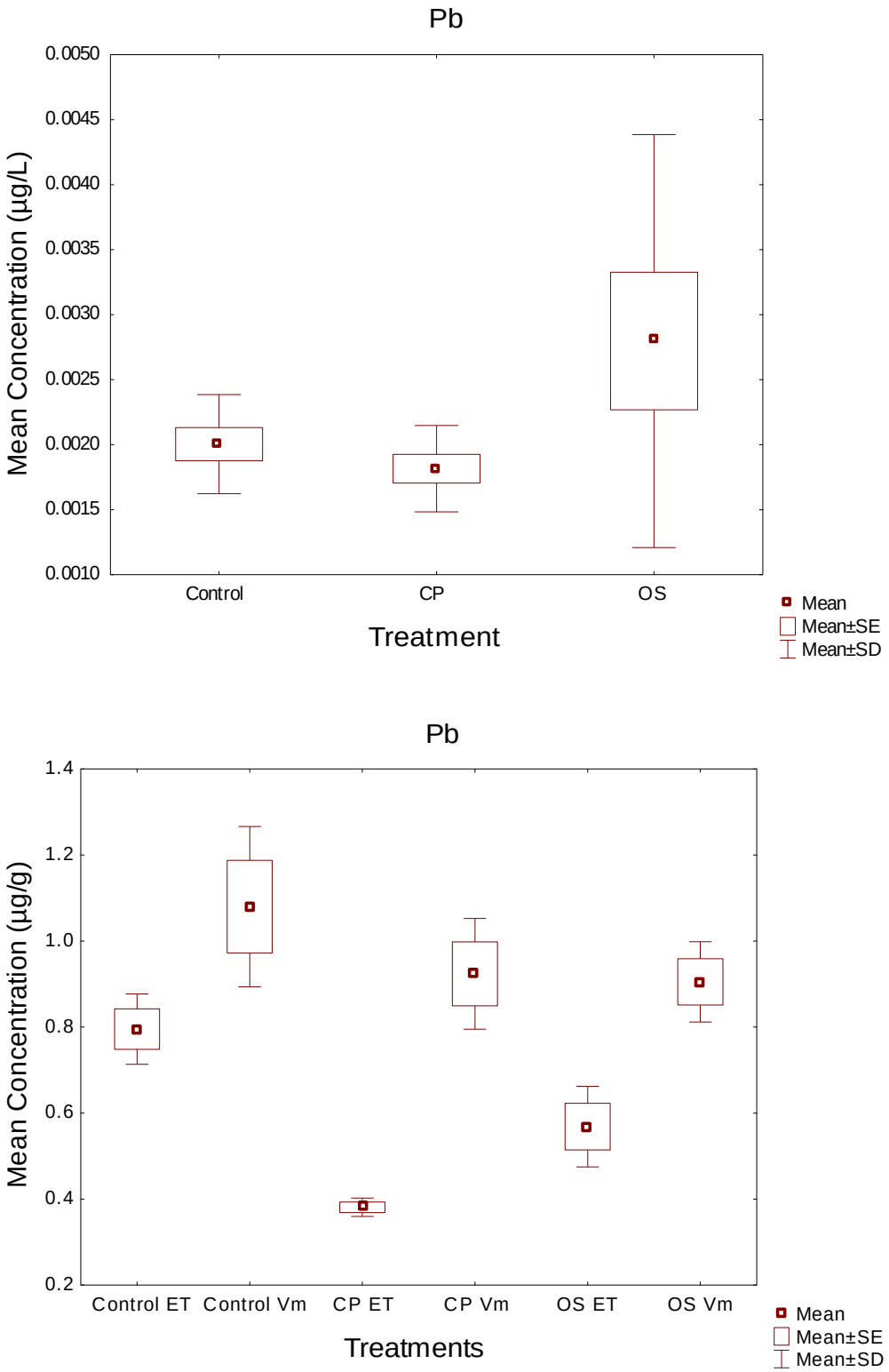

Fig 3-12: Mean concentration of lead in the DGT's (top) and tissue (bottom) samples for control, copper positive (CP) and on-reef sediment (OS). Tissue analysed ET = edible tissue, Vm=Viscera mass.

Table 3-1: Mean concentration of metals of CP (copper positive) and OS (Otaiti sediment) above, below, + and – or unchanged from the control for DGT and tissues.

In Situ				
Trace Metals	CP DGT	OS DGT	CP Tissue	OS Tissue
Cu				
Mn				
Al				
Co				
Cd				
Zn				
Cr				
Fe				
Ni				
Pb				
KEY: Differences from the controls				
Increase (+)			Decrease (-)	
+ and -			Unchanged	

3.4 Discussion

The purpose of this study was to determine whether sediment from Otaiti reef influenced by the MV Rena container debris, releases trace metals into the surrounding water, therefore making metals biologically available to paua. This could lead to the accumulation of metals into tissues, in turn causing an effect to paua health. This chapter addresses this aim with a field manipulation experiment.

DGT's were used to assess the levels of metal contaminants coming off the treatments (clean and contaminated sand bags) in a realistic situation to establish the concentration gradients inside the experimental chambers deployed in the field. The pooled mean concentration of the DGTs show a mean elevation of trace metals originating from OS relative to the controls apart from nickel, which was lower. This trend shows that in the field the DGTs are still registering increases in concentration for these trace metals.

The DGT data show certain trace metals being significantly enriched within the Northern positioned DGT inside the cages suggesting some inside cage diffusion gradient exists, even though the field conditions were relatively exposed to current and swell water movement. It was observed that paua were commonly attached to the upper southern portion of the cage enclosures (*pers.obs*.).

There was no significant differences in the tissue accumulation in the edible tissue or the viscera mass compared to the non-contaminated control. However trends show that both the edible tissue and viscera mass were increasing in concentration for copper steadily within these tissues (Appendix III). The non-significance seen here may also be due to the sample size and amalgamation used within this study. The viscera mass had increased concentration of trace metals such as; cobalt, cadmium, zinc and nickel while the edible tissue decreased. The opposite trend was seen for chromium and lead.

When DGTs were pooled into their treatments however, (in order to block within cage variability), copper was the only metal significantly different to

the non-contaminated control. There was no significant difference between CP and OS, consistent with comparable Cu dissolution kinetics in CP and OS.

Overall, there was a single mortality from this study which occurred in the OS treatment group. Analysis of the tissue of the paua could not be conducted because the shell was empty on observation. It is assumed that when the paua died, the tissue was consumed by the hermit crabs, crayfish, triple fins or octopus or a combination of these species. There were two lethargic paua observed, one in OS and one in CP treatment, upon retrieval of the cages. The lethargic paua had delayed muscle movement and showed a slow response to stimuli to the underside of the foot. This could be the start of blood flow moving away from the foot area (Donovan, 2008). This is similar behavioural observations seen in the laboratory experiments (previous chapter). Control cage paua were by comparison healthy as judged by survivorship and behaviour on retrieval.

DGTs showed copper was significantly different in OS compared to the controls. This was not seen in the tissue of sacrificed animals at the end of the experiment, with no significant differences occurring within the 33 days of the field study. This would suggest that copper in CP and OS were equally available for accumulation in paua. It would also suggest that the concentration of copper that paua are exposed to here, is lower enough for effective regulation within the tissues (Rainbow, 2007). Ikuta (1987) found at 60 days exposure to 20 and 25 µg/l of copper resulted in 24 and 29.7 µg/g respectively in the edible tissue, while the viscera mass had a concentration of 41.8 and 51.1 µg/g respectively. These findings are similar to that seen in this study for CP (edible tissue 28.28 µg/g, viscera mass 51.66 µg/g) and OS (edible tissue 24.60 µg/g, viscera mass 32.99 µg/g) treatments.

The viscera mass had significantly higher concentration than the edible tissue for cadmium, cobalt, iron, zinc, and lead. The same trend could be seen for manganese, nickel, copper and aluminium, however there was no significant difference for these metals which supports the findings of Ikuta

(1987). The opposite was seen for chromium with the edible tissue having a higher level than the viscera mass with no significance occurring.

Lead concentrations in this study were below all concentrations and times seen by Ikuta (1987). However, it was reported that concentration in the viscera mass continued to increase while edible tissue decreased overtime Ikuta (1987). This could be positive for human consumption, however, because the reproduction organs are in the viscera mass, the reproduction of paua may be negatively impacted.

Manganese in this study exceeded all concentrations and exposure times recorded by Ikuta (1987). This may indicate that *H. iris* can uptake manganese faster than *H. discus.* Cadmium concentration in the viscera mass was similar to that seen by Ikuta (1987) at 15 days exposure to 5 µg/l while the edible tissue exposed to the same concentration took 60 days in that study (Ikuta, 1987).

The DGT data indicates that there were elevations within the concentrations of trace metals (Table 3-1). The changes in concentration from the controls that were observed within the DGT data, was not consistent within the tissue trace metals (Table 3-1). Copper was the only consistent trace metal to increase within the tissue and the DGTs. Nickel also increased in the tissue however elevations were only seen within the Cu treatment group while the concentration in the Rena treatment remained consistent with the control.

Increases in concentrations within both the CP and OS for cobalt, cadmium and zinc seen from the DGTs showed variations in accumulation for the same metals within the tissues. Aluminium, iron and lead all show decrease from the controls for both CP and OS treatments although the concentration seen within the DGT data was either constant or had increased.

The findings of this study show that the weathered Otaiti sediment over three years on from the grounding is still releasing copper into the water column which can be detected by the DGTs. It also indicates that Otaiti contaminated sediments have a deleterious effect on paua health. There is an elevated trend showing from the tissue analysis, however further work is

required to make underlining conclusions. Organotins and PAH need to be included in any further analysis as there is still significant elevations in concentration relative to the surrounding reef of the Otaiti.

Chapter 4

General Discussion

The grounding of the Rena has highlighted vulnerabilities within New Zealand's marine ecosystem and its response to a maritime catastrophe. Since the grounding of the Rena there has been review of the response to the Rena incident which has highlighted areas for improvement (Murdoch, 2013). The affected environment is still recovering from the impacts caused. A report assessing the feasibility of full wreck removal highlights the MV Rena total lightweight to be ~14,500 tonnes (Barker, 2014). A recent media release highlighted the removal of approx 23% of the ship and debris (Insurers respond to toxic Rena claims, 2015). The remaining ~11,000 tonnes is comprised of ship structure and container debris.

Copper and other contaminants that remain on the reef are still of concern to the surrounding environment. There are reports that highlight metals, PAHs and organotins such as TBT are still at elevated levels surrounding Otaiti. Furthermore, there is no evidence of a decrease in concentrations occurring three years on from the initial grounding (Don, 2014; Ross*, et al., in press*).

The local hapū on Mōtītī have identified their cultural concerns and impacts to the mauri are on-going (Steiger, 2012; Ngāi Te Hapū Incorporated, 2014). Kaimoana plays an important role in the identity of the people of Mōtītī and Otaiti is part in parcel of who they are (Ngāi Te Hapū Incorporated, 2014). More specifically, paua (*Haliotis iris*) reside on the rocky shore within Mōtītī's rohe. Paua have been of cultural importance for generations and since the colonisation of Aotearoa; have pulled the whanau back to Mōtītī time and time again, insuring the next generation of kaitiaki are ready to take over (Ngāi Te Hapū Incorporated, 2014). Since the grounding of the Rena, there has been a catastrophic impact to Mōtītī's rohe moana. For effective kaitiaki management, understanding of the impacts to taonga species such as paua needs to be investigated.

For this reason it is important to assess whether the contaminated copper sediment on Otaiti reef has the potential to affect this benthic and culturally important species. Water monitoring carried out around Otaiti and Mōtītī have shown elevated levels of some metals (Dempsey, 2015 *in prep*). Trace metals that are essential or non-essential have the ability to become toxic at a species dependant threshold concentration (Rainbow, 2007). This can be lethal or be incorporated in the cellular process and cause detriment to the cells causing acute affects to an organism (Gorski & Nugegoda, 2006). Very few paua have been observed or analysed around Otaiti of quantitative significance. The cultural report by Ngāi Te Hapū Incorporated, (2014) has brought to reference that this species is of importance to the hapū at Mōtītī.

This thesis aimed to address concerns relating to the effects of water borne pollution emanating from contaminated Rena sediment on juvenile paua. Research was focused in two areas: 1) the behavioural effects to the contaminated Rena sediment and 2) the accumulation of trace metals in the edible tissue and viscera mass. This was determined by use of a close circuit aquaria and field experiment.

To the author's knowledge, this is the first contaminated sediment experiment of its kind.. The OS and CP treatments had a consistently higher mean concentration for copper in the water and paua tissues than the control treatment. Trace metal concentrations were picked up by the DGT samplers in marine waters which further adds validity to use of DGT's as a chemical alternative to bio monitoring.

When trace metals are biologically available for accumulation, paua have shown the ability to accumulate these effectively in the edible tissue and viscera mass at concerning concentrations. The contaminated sediment on Otaiti has shown to still be releasing significant amounts of copper into the water column (Ross *et al.,*, in press). This has shown effect the behaviour, survivorship and physiology of the New Zealand paua (*H.iris*) in a similar manner to overseas *Haliotis* species (Martin, 1977; Ikuta, 1987; Arai, 2003, Tsai, 2004; Fabris, 2006; Gorski, 2006; Silva-Aciares, 2013).

As the concentration of contaminants increase, the vulnerability of the organism to predation becomes more prevalent. There antennae begin to retract within their shell, limiting their sensory ability for detection of predators. This can then lead to a delayed response to movements within close proximity to the organism. Depending on the contaminant concentration, paua may shunt blood flow away from areas considered less important for survivorship (Gorski, 2006). If this behaviour continues, paua can then lose their ability to hold fast and securely to the substrate. This behaviour can occur quickly in the presence of one contaminant and even faster in the presence of multiple as seen in this study. Metals and contaminants accumulate predominately in the viscera mass. This could have further implications to the long-term physiology to paua.

There is still ongoing concern as to what effects the grounding of the Rena has caused to the surrounding marine environment. Don (2014) and Ross*, et al.,.* (*in press*) have both reported enrichment of sediment with metals, PAHs and organotins. Don (2014) indicated that its less than likely for adverse effects to impact reef biota at 500-1500m from the reef, although TBT is detectable in sediments 500-1000m away from the reef. Research by Horiguchi (2002) has shown small concentrations of Tributyltin and triphenyltin as low as 0.0001 mg/L or 0.1 µg/L causes significant spermatogenesis in the ovaries of female *Haliotis gigantean.* With an approximate range of 0.002 to 9mg/kg recorded in the sediment around Otaiti reef (Don, 2014), there are still significant concern of the health of the ecosystem at Otaiti. For this reason it is recommended that PAH and organotins be included in any future work. Low concentrations of TBT can affect the spermatogenesis in the ovaries of female *Haliotis gigantean* (Horiguchi, 2002). This could have long term effects to the population dynamics of key kai moana species.

This study highlights the effect of Rena contaminants to a key taonga species. Paua are ecologically, culturally and recreationally important. Further research is needed to better understand the direct and indirect long

term impacts of Rena derived pollutant mixtures and contaminants to a once thriving reef ecosystem.

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Appendix I

	Marine Water quality guidelines	Background Metal Concentrations		
Trace Metal	**Trigger Values. Level of Protection 99% (ANZEEC)**	**Australia**	**New Zealand**	**World**
Cu	**0.3**	0.025-0.38	0.1-0.2	0.003-0.37
Mn	ID			0.003-0.38
Al	ID			
Co	**0.005**			
Cd	**0.7**	0.002-0.7		0.001-1.1
Zn	**7**	<0.022-0.1	0.005-0.02	0.003-0.59
Cr lll	**7.7**	0.062-0.1		
Cr Vl	**0.14**			
Fe	ID			<0.006-0.14
Ni	**7**	0.13-0.5	0.33	0.12-0.7
Pb	**2.2**	<0.006-0.03		

Appendix II

MANIFEST DETAILS		
Cargo	**Total WT TONNES**	**n# of containers**
Aluminium	**2216.8**	**75**
Asphalt	**22**	**1**
Auto Parts	**11.9**	**2**
Baling Twine	**27.7**	**1**
Black Tea	**12.7**	**1**
Butter	**321.3**	**14**
Car	**3.2**	**1**
Car Bundle	**208.7**	**10**
Car Seat Covers	**5.1**	**1**
Caustic Calcined Magnesia	**132.5**	**5**
Cement	**29.5**	**1**

Ceramics Proppant Mesh	**275.2**	**11**
Choc Malt	**70.5**	**4**
Copper Scrap	**23.3**	**1**
Cryolite	**542.5**	**21**
DA-HFP	**27.3**	**1**
Decking	**37.7**	**2**
Empty	**1698**	**477**
Energy Cable	**34.8**	**2**
Fabric	**15.5**	**1**
Ferro Silicon	**96**	**4**
Filters	**8**	**1**
Folding Door	**9**	**1**
Food Stuff	**388.2**	**18**
Furniture	**195.5**	**18**
Fzn Fish	**480.4**	**19**
Fzn Fries	**260.1**	**12**
Fzn MDM Blocks	**502.9**	**16**
Fzn Meat	**940.4**	**37**
Fzn Meat Pies	**24..4**	**1**
Fzn Offal	**45.6**	**2**
Fzn Pasta Meals	**102**	**5**
Fzn Pastry	**18**	**1**
Fzn Seafood	**149.1**	**7**
Fzn Vegetables	**157.3**	**8**
Galvanised Pipes	**26.4**	**1**
Garage Doors	**9.4**	**1**
General	**57.4**	**3**
Glass Bottles	**113.4**	**5**
Grinding Media	**46**	**2**
Home Brew Kits	**36**	**2**
Hydraulic Machinery	**34**	**2**
Ice Cream	**15.3**	**1**
Laser Paper	**66**	**3**
Machinary	**9**	**1**
Malflute	**15**	**1**
MDF	**1140.6**	**41**
Meal	**199**	**9**
Metal Boxes	**51.3**	**2**
Metal Scrap	**29.9**	**1**
Metallugical Coke	**188.9**	**8**
Milk Fat	**261.3**	**11**
Milk Powder	**3722.8**	**143**
Motor Car	**28**	**2**

Paint	**8**	**1**
Peat	**15**	**1**
Pebbles	**87.7**	**3**
Pentaerythritol Mono	**22.6**	**1**
Personnal Effects	**45.3**	**7**
Plastic Beads	**98.2**	**4**
Plastic Packaging	**24.3**	**3**
Plastic scrap	**23.4**	**1**
Plastic Storage Racks	**9.6**	**1**
Plywood	**83.9**	**3**
Pool Tablets	**11.9**	
Potassium Nitrate	**26.4**	**1**
Pottary Wares	**23**	**1**
Poultry Keeping Equipment	**21.8**	**1**
Printing Paper	**19.1**	**1**
Pulp	**598.3**	**30**
Scrap Aluminium	**25.1**	**1**
Shop Fittings	**30.5**	**4**
Skins	**380.4**	**18**
Snell Wipes	**5.9**	**1**
Steel Castings	**14**	**1**
Steel Scrap	**1364.2**	**56**
Stockfeed	**424**	**20**
Timber	**3211.7**	**123**
Titanium Dioxide	**69.7**	**3**
Trampolines	**20.1**	**1**
Tyres	**105.7**	**7**
UHT Milk	**23.5**	**1**
Vinyl Gloves	**10.1**	**1**
Waste Paper	**585.2**	**21**
Welding Electrodes	**46.2**	**2**
Wheel Barrows	**14.7**	**1**
Wine	**140.2**	**6**
Wire Rod	**200.8**	**9**
Wool	**196.1**	**11**

Appendix III

3.5 Laboratory Experiment

3.5.1 Copper

Breakdown Table of Descriptive Statistics (Snap shot water Adam input)
N=23 (No missing data in dep. var. list)

Treatment	Cu Means	Confidence -95.000 %	Confidence +95.000 %	Cu N	Cu Sum	Cu Std.Dev.	Cu Variance	Cu Std.Err.
C1 6hr	1.649	1.534	1.763	3	4.95	0.0462	0.002	0.0267
C1 48hr	1.734	1.593	1.874	3	5.20	0.0566	0.003	0.0327
C2 6hr	1.645	1.195	2.094	2	3.29	0.0500	0.003	0.0354
C2 48hr	1.765	1.411	2.120	3	5.30	0.1427	0.020	0.0824
CP 6hr	4.165	2.436	5.894	3	12.50	0.6961	0.485	0.4019
CP 48hr	14.383	12.039	16.727	3	43.15	0.9436	0.890	0.5448
OS 6hr	4.178	2.576	5.780	3	12.53	0.6448	0.416	0.3723

3.5.2 Manganese

Breakdown Table of Descriptive Statistics
N=23 (No missing data in dep. var. list)

Treatment	Mn Means	Confidence -95.000	Confidence +95.000	Mn N	Mn Sum	Mn Std.Dev.	Mn Variance	Mn Std.Err.
C1 6hr	0.03142	0.02684	0.03599	3	0.09425	0.00184	0.00000	0.00106
C1 48hr	0.05208	-0.00115	0.10531	3	0.15625	0.02143	0.00046	0.01237
C2 6hr	0.03825	0.00331	0.07319	2	0.07650	0.00389	0.00002	0.00275
C2 48hr	0.03717	0.02997	0.04436	3	0.11150	0.00290	0.00001	0.00167
CP 6hr	0.07958	0.07355	0.08562	3	0.23875	0.00243	0.00001	0.00140
CP 48hr	0.09992	0.08252	0.11731	3	0.29975	0.00700	0.00005	0.00404
OS 6hr	0.08517	0.06274	0.10760	3	0.25550	0.00903	0.00008	0.00521
OS 48hr	0.13683	0.12159	0.15208	3	0.41050	0.00614	0.00004	0.00354

Tukey HSD test; Variable: Mn
Marked differences are significant at p < .05000

Treatment	{1} M=.03142	{2} M=.05208	{3} M=.03825	{4} M=.03717	{5} M=.07958	{6} M=.09992	{7} M=.08517	{8} M=.13683
C1 6hr {1}		0.1918	0.9903	0.9932	0.0004	0.0002	0.0002	0.0002
C1 48hr {2}	0.1918		0.7303	0.5364	0.0404	0.0004	0.0103	0.0002
C2 6hr {3}	0.9903	0.7303		1.0000	0.0041	0.0002	0.0013	0.0002
C2 48hr {4}	0.9932	0.5364	1.0000		0.0012	0.0002	0.0004	0.0002
CP 6hr {5}	0.0004	0.0404	0.0041	0.0012		0.2055	0.9943	0.0002
CP 48hr {6}	0.0002	0.0004	0.0002	0.0002	0.2055		0.5491	0.0041
OS 6hr {7}	0.0002	0.0103	0.0013	0.0004	0.9943	0.5491		0.0003

3.5.3 Aluminium

Breakdown Table of Descriptive Statistics
N=23 (No missing data in dep. var. list)

Treatment	Al Means	Confidence -95.000%	Confidence +95.000%	Al N	Al Sum	Al Std.Dev.	Al Variance	Al Std.Err.
C1 6hr	0.08392	0.05084	0.11700	3	0.25175	0.01332	0.00018	0.00769
C1 48hr	0.10742	0.03886	0.17597	3	0.32225	0.02760	0.00076	0.01593
C2 6hr	0.08775	0.03693	0.13857	2	0.17550	0.00566	0.00003	0.00400
C2 48hr	0.10025	0.07966	0.12084	3	0.30075	0.00829	0.00007	0.00478
CP 6hr	0.11442	0.10868	0.12015	3	0.34325	0.00231	0.00001	0.00133
CP 48hr	0.09317	0.05242	0.13392	3	0.27950	0.01640	0.00027	0.00947
OS 6hr	0.09592	0.04468	0.14715	3	0.28775	0.02063	0.00043	0.01191
OS 48hr	0.13658	0.07417	0.19900	3	0.40975	0.02513	0.00063	0.01451

Tukey HSD test; Variable: Al
Marked differences are significant at p < .05000

Treatment	{1} M=.08392	{2} M=.10742	{3} M=.08775	{4} M=.10025	{5} M=.11442	{6} M=.09317	{7} M=.09592	{8} M=.13658
C1 6hr {1}		0.73	1.00	0.94	0.45	1.00	0.99	0.04
C1 48hr {2}	0.73		0.91	1.00	1.00	0.97	0.99	0.50
C2 6hr {3}	1.00	0.91		0.99	0.72	1.00	1.00	0.12
C2 48hr {4}	0.94	1.00	0.99		0.97	1.00	1.00	0.26
CP 6hr {5}	0.45	1.00	0.72	0.97		0.81	0.89	0.78
CP 48hr {6}	1.00	0.97	1.00	1.00	0.81		1.00	0.12
OS 6hr {7}	0.99	0.99	1.00	1.00	0.89	1.00		0.16

3.5.4 Cobalt

Breakdown Table of Descriptive Statistics
N=23 (No missing data in dep. var. list)

Treatment	Co Means	Confidence -95.000%	Confidence +95.000%	Co N	Co Sum	Co Std.Dev.	Co Variance	Co Std.Err.
C1 6hr	0.0079	0.0070	0.0089	3	0.0238	0.0004	0.0000	0.0002
C1 48hr	0.0087	0.0042	0.0132	3	0.0260	0.0018	0.0000	0.0010
C2 6hr	0.0081	0.0065	0.0097	2	0.0163	0.0002	0.0000	0.0001
C2 48hr	0.0082	0.0064	0.0100	3	0.0245	0.0007	0.0000	0.0004
CP 6hr	0.0078	0.0075	0.0082	3	0.0235	0.0001	0.0000	0.0001
CP 48hr	0.0084	0.0055	0.0114	3	0.0253	0.0012	0.0000	0.0007
OS 6hr	0.0068	0.0042	0.0094	3	0.0205	0.0010	0.0000	0.0006

Tukey HSD test; Variable: Co
Marked differences are significant at p < .05000

Treatment	{1} M=.00792	{2} M=.00867	{3} M=.00812	{4} M=.00817	{5} M=.00783	{6} M=.00842	{7} M=.00683	{8} M=.00925
C1 6hr {1}		0.98	1.00	1.00	1.00	1.00	0.86	0.69
C1 48hr {2}	0.98		1.00	1.00	0.96	1.00	0.34	0.99
C2 6hr {3}	1.00	1.00		1.00	1.00	1.00	0.81	0.89
C2 48hr {4}	1.00	1.00	1.00		1.00	1.00	0.69	0.86
CP 6hr {5}	1.00	0.96	1.00	1.00		0.99	0.90	0.63
CP 48hr {6}	1.00	1.00	1.00	1.00	0.99		0.51	0.96
OS 6hr {7}	0.86	0.34	0.81	0.69	0.90	0.51		0.11

3.5.5 Cadmium

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Cd Means	Confidence -95.000%	Confidence +95.000%	Cd N	Cd Sum	Cd Std.Dev.	Cd Variance	Cd Std.Err.
C1 6hr	-0.000583	-0.005603	0.004436	3	-0.001750	0.002021	0.000004	0.001167
C1 48hr	0.000167	-0.003020	0.003354	3	0.000500	0.001283	0.000002	0.000741
C2 6hr	0.001250	-0.008280	0.010780	2	0.002500	0.001061	0.000001	0.000750
C2 48hr	-0.001000	-0.003846	0.001846	3	-0.003000	0.001146	0.000001	0.000661
CP 6hr	0.001083	-0.000209	0.002376	3	0.003250	0.000520	0.000000	0.000300
CP 48hr	-0.001417	-0.003768	0.000935	3	-0.004250	0.000946	0.000001	0.000546
OS 6hr	0.002417	-0.001811	0.006644	3	0.007250	0.001702	0.000003	0.000982
OS 48hr	0.000500	-0.003847	0.004847	3	0.001500	0.001750	0.000003	0.001010

	Tukey HSD test; Variable: Cd Marked differences are significant at p < .05000							
Treatment	{1} M=-.0006	{2} M=.00017	{3} M=.00125	{4} M=-.0010	{5} M=.00108	{6} M=-.0014	{7} M=.00242	{8} M=.00050
C1 6hr {1}		1.00	0.83	1.00	0.82	0.99	0.22	0.98
C1 48hr {2}	1.00		0.99	0.96	0.99	0.85	0.53	1.00
C2 6hr {3}	0.83	0.99		0.65	1.00	0.46	0.98	1.00
C2 48hr {4}	1.00	0.96	0.65		0.62	1.00	0.12	0.88
CP 6hr {5}	0.82	0.99	1.00	0.62		0.41	0.93	1.00
CP 48hr {6}	0.99	0.85	0.46	1.00	0.41		0.06	0.70
OS 6hr {7}	0.22	0.53	0.98	0.12	0.93	0.06		0.70

3.5.6 Zinc

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Zn Means	Confidence -95.000%	Confidence +95.000%	Zn N	Zn Sum	Zn Std.Dev.	Zn Variance	Zn Std.Err.
C1 6hr	0.516	0.345	0.687	3	1.55	0.069	0.005	0.040
C1 48hr	0.529	0.410	0.649	3	1.59	0.048	0.002	0.028
C2 6hr	0.611	0.009	1.213	2	1.22	0.067	0.004	0.047
C2 48hr	0.432	0.311	0.552	3	1.30	0.048	0.002	0.028
CP 6hr	0.678	0.401	0.955	3	2.03	0.112	0.012	0.064
CP 48hr	0.557	0.301	0.812	3	1.67	0.103	0.011	0.059
OS 6hr	0.559	0.473	0.645	3	1.68	0.035	0.001	0.020

Treatment	Tukey HSD test; Variable: Zn Marked differences are significant at p < .05000							
	{1} M=.51592	{2} M=.52925	{3} M=.61063	{4} M=.43175	{5} M=.67825	{6} M=.55683	{7} M=.55933	{8} M=.43408
C1 6hr {1}		1.00	0.81	0.82	0.16	1.00	0.99	0.84
C1 48hr {2}	1.00		0.90	0.69	0.24	1.00	1.00	0.72
C2 6hr {3}	0.81	0.90		0.17	0.96	0.99	0.99	0.19
C2 48hr {4}	0.82	0.69	0.17		0.01	0.42	0.40	1.00
CP 6hr {5}	0.16	0.24	0.96	0.01		0.45	0.48	0.01
CP 48hr {6}	1.00	1.00	0.99	0.42	0.45		1.00	0.44
OS 6hr {7}	0.99	1.00	0.99	0.40	0.48	1.00		0.42

3.5.7 Chromium

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Cr Means	Confidence -95.000%	Confidence +95.000%	Cr N	Cr Sum	Cr Std.Dev.	Cr Variance	Cr Std.Err.
C1 6hr	0.05592	0.01524	0.09659	3	0.16775	0.01638	0.00027	0.00945
C1 48hr	0.32708	-0.80874	1.46291	3	0.98125	0.45723	0.20906	0.26398
C2 6hr	0.04825	0.01648	0.08002	2	0.09650	0.00354	0.00001	0.00250
C2 48hr	0.06825	0.02704	0.10946	3	0.20475	0.01659	0.00028	0.00958
CP 6hr	0.04583	-0.00931	0.10098	3	0.13750	0.02220	0.00049	0.01282
CP 48hr	0.07625	-0.03493	0.18743	3	0.22875	0.04476	0.00200	0.02584
OS 6hr	0.05642	0.04374	0.06909	3	0.16925	0.00510	0.00003	0.00295
OS 48hr	0.07067	-0.00043	0.14176	3	0.21200	0.02862	0.00082	0.01652

Treatment	Tukey HSD test; Variable: Cr Marked differences are significant at p < .05000							
	{1} M=.05592	{2} M=.32708	{3} M=.04825	{4} M=.06825	{5} M=.04583	{6} M=.07625	{7} M=.05642	{8} M=.07067
C1 6hr {1}		0.53	1.00	1.00	1.00	1.00	1.00	1.00
C1 48hr {2}	0.53		0.62	0.58	0.49	0.62	0.53	0.59
C2 6hr {3}	1.00	0.62		1.00	1.00	1.00	1.00	1.00
C2 48hr {4}	1.00	0.58	1.00		1.00	1.00	1.00	1.00
CP 6hr {5}	1.00	0.49	1.00	1.00		1.00	1.00	1.00
CP 48hr {6}	1.00	0.62	1.00	1.00	1.00		1.00	1.00
OS 6hr {7}	1.00	0.53	1.00	1.00	1.00	1.00		1.00

3.5.8 Iron

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Fe Means	Confidence -95.000 %	Confidence +95.000 %	Fe N	Fe Sum	Fe Std.Dev.	Fe Variance	Fe Std.Err.
C1 6hr	0.230	-0.97	1.427	3	0.69	0.482	0.232	0.278
C1 48hr	1.972	-5.49	9.436	3	5.92	3.004	9.026	1.735
C2 6hr	0.159	-6.15	6.473	2	0.32	0.703	0.494	0.497
C2 48hr	0.467	-1.24	2.171	3	1.40	0.686	0.471	0.396
CP 6hr	0.059	-1.17	1.287	3	0.18	0.494	0.244	0.285
CP 48hr	0.375	-0.17	0.921	3	1.13	0.220	0.048	0.127
OS 6hr	0.246	-0.56	1.053	3	0.74	0.325	0.106	0.188

	Tukey HSD test; Variable: Fe Marked differences are significant at p < .05000							
Treatment	{1} M=.23033	{2} M=1.9724	{3} M=.15937	{4} M=.46667	{5} M=.05875	{6} M=.37517	{7} M=.24600	{8} M=.26567
C1 6hr {1}		0.63	1.00	1.00	1.00	1.00	1.00	1.00
C1 48hr {2}	0.63		0.70	0.77	0.52	0.71	0.64	0.65
C2 6hr {3}	1.00	0.70		1.00	1.00	1.00	1.00	1.00
C2 48hr {4}	1.00	0.77	1.00		1.00	1.00	1.00	1.00
CP 6hr {5}	1.00	0.52	1.00	1.00		1.00	1.00	1.00
CP 48hr {6}	1.00	0.71	1.00	1.00	1.00		1.00	1.00
OS 6hr {7}	1.00	0.64	1.00	1.00	1.00	1.00		1.00

3.5.9 Nickel

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Ni Means	Confidence -95.000	Confidence +95.000	Ni N	Ni Sum	Ni Std.Dev.	Ni Variance	Ni Std.Err.
C1 6hr	0.11	0.01	0.20	3	0.32	0.04	0.00	0.02
C1 48hr	0.12	0.04	0.20	3	0.37	0.03	0.00	0.02
C2 6hr	0.09	-0.05	0.23	2	0.18	0.02	0.00	0.01
C2 48hr	0.14	-0.07	0.34	3	0.42	0.08	0.01	0.05
CP 6hr	0.10	0.07	0.13	3	0.31	0.01	0.00	0.01
CP 48hr	0.10	0.06	0.14	3	0.29	0.02	0.00	0.01
OS 6hr	0.13	0.04	0.22	3	0.40	0.04	0.00	0.02
OS 48hr	0.11	0.06	0.17	3	0.34	0.02	0.00	0.01

	Tukey HSD test; Variable: Ni Marked differences are significant at p < .05000							
Treatment	{1} M=.10533	{2} M=.12192	{3} M=.08987	{4} M=.13917	{5} M=.10258	{6} M=.09783	{7} M=.13192	{8} M=.11183
C1 6hr {1}		1.00	1.00	0.96	1.00	1.00	0.99	1.00
C1 48hr {2}	1.00		0.98	1.00	1.00	0.99	1.00	1.00
C2 6hr {3}	1.00	0.98		0.86	1.00	1.00	0.93	1.00
C2 48hr {4}	0.96	1.00	0.86		0.94	0.90	1.00	0.99
CP 6hr {5}	1.00	1.00	1.00	0.94		1.00	0.98	1.00
CP 48hr {6}	1.00	0.99	1.00	0.90	1.00		0.96	1.00
OS 6hr {7}	0.99	1.00	0.93	1.00	0.98	0.96		1.00

3.5.10 Lead

Breakdown Table of Descriptive Statistics N=23 (No missing data in dep. var. list)								
Treatment	Pb Means	Confidence -95.000%	Confidence +95.000%	Pb N	Pb Sum	Pb Std.Dev	Pb Variance	Pb Std.Err.
C1 6hr	0.03950	-0.098	0.1771	3	0.1185	0.05540	0.0031	0.03199
C1 48hr	0.00708	-0.006	0.0198	3	0.0213	0.00513	0.0000	0.00296
C2 6hr	0.00713	-0.068	0.0818	2	0.0143	0.00831	0.0001	0.00588
C2 48hr	0.00683	-0.016	0.0298	3	0.0205	0.00923	0.0001	0.00533
CP 6hr	0.00983	0.002	0.0176	3	0.0295	0.00313	0.0000	0.00180
CP 48hr	3.46425	-11.420	18.3487	3	10.3928	5.99181	35.9018	3.45938
OS 6hr	0.00775	-0.001	0.0169	3	0.0233	0.00370	0.0000	0.00214
OS 48hr	0.00275	0.001	0.0049	3	0.0083	0.00087	0.0000	0.00050

	Tukey HSD test; Variable: Pb Marked differences are significant at p < .05000							
Treatment	{1} M=.03950	{2} M=.00708	{3} M=.00713	{4} M=.00683	{5} M=.00983	{6} M=3.4643	{7} M=.00775	{8} M=.00275
C1 6hr {1}		1.00	1.00	1.00	1.00	0.56	1.00	1.00
C1 48hr {2}	1.00		1.00	1.00	1.00	0.55	1.00	1.00
C2 6hr {3}	1.00	1.00		1.00	1.00	0.67	1.00	1.00
C2 48hr {4}	1.00	1.00	1.00		1.00	0.55	1.00	1.00
CP 6hr {5}	1.00	1.00	1.00	1.00		0.55	1.00	1.00
CP 48hr {6}	0.56	0.55	0.67	0.55	0.55		0.55	0.55
OS 6hr {7}	1.00	1.00	1.00	1.00	1.00	0.55		1.00

3.6 Tissue Tables

3.6.1 Copper

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatmen	Cu Means	Confidence -95.000	Confidence +95.000	Cu N	Cu Sum	Cu Std.Dev.	Cu Variance	Cu Std.Err.
C1 ET	13.9	10.5	17.3	9	125	4.38	19	1.46
C1 Vm	58.7	45.2	72.3	9	529	17.58	309	5.86
C2 ET	15.8	9.3	22.3	7	111	7.03	49	2.66
C2 Vm	52.3	34.1	70.5	6	314	17.31	300	7.07
CP ET	111.4	78.8	144.0	9	1002	42.41	1799	14.14
CP Vm	188.9	147.0	230.8	9	1700	54.50	2970	18.17
OS ET	144.2	93.3	195.1	9	1298	66.17	4379	22.06
OS Vm	187.9	146.1	229.7	8	1503	50.01	2501	17.68

	Tukey HSD test; Variable: Cu Marked differences are significant at p < .05000							
Treatment	{1} M=13.904	{2} M=58.744	{3} M=15.841	{4} M=52.289	{5} M=111.38	{6} M=188.91	{7} M=144.20	{8} M=187.93
C1 ET {1}		0.2860	1.0000	0.6236	0.0002	0.0001	0.0001	0.0001
C1 Vm {2}	0.2860		0.4258	1.0000	0.1266	0.0001	0.0010	0.0001
C2 ET {3}	1.0000	0.4258		0.7378	0.0006	0.0001	0.0001	0.0001
C2 Vm {4}	0.6236	1.0000	0.7378		0.1234	0.0001	0.0017	0.0001
CP ET {5}	0.0002	0.1266	0.0006	0.1234		0.0036	0.6749	0.0061
CP Vm {6}	0.0001	0.0001	0.0001	0.0001	0.0036		0.2894	1.0000
OS ET {7}	0.0001	0.0010	0.0001	0.0017	0.6749	0.2894		0.3542

3.6.2 Manganese

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Mn Means	Confidence -95.000 %	Confidence +95.000%	Mn N	Mn Sum	Mn Std.Dev.	Mn Variance	Mn Std.Err.
C1 ET	2.292	1.176	3.41	9	20.6	1.452	2.11	0.484
C1 Vm	9.759	4.714	14.80	9	87.8	6.563	43.08	2.188
C2 ET	3.203	0.013	6.39	7	22.4	3.449	11.90	1.304
C2 Vm	9.768	5.599	13.94	6	58.6	3.973	15.78	1.622
CP ET	1.566	1.267	1.87	9	14.1	0.389	0.15	0.130
CP Vm	7.505	5.111	9.90	9	67.5	3.114	9.70	1.038
OS ET	2.736	1.295	4.18	9	24.6	1.876	3.52	0.625

Treatment	Tukey HSD test; Variable: Mn Marked differences are significant at p < .05000							
	{1} M=2.2921	{2} M=9.7594	{3} M=3.2029	{4} M=9.7680	{5} M=1.5659	{6} M=7.5052	{7} M=2.7365	{8} M=9.7884
C1 ET {1}		0.004	1.000	0.013	1.000	0.106	1.000	0.005
C1 Vm {2}	0.004		0.030	1.000	0.001	0.921	0.007	1.000
C2 ET {3}	1.000	0.030		0.067	0.990	0.373	1.000	0.037
C2 Vm {4}	0.013	1.000	0.067		0.004	0.954	0.024	1.000
CP ET {5}	1.000	0.001	0.990	0.004		0.040	0.998	0.002
CP Vm {6}	0.106	0.921	0.373	0.954	0.040		0.179	0.927
OS ET {7}	1.000	0.007	1.000	0.024	0.998	0.179		0.010

3.6.3 Aluminium

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Al Means	Confidence -95.000%	Confidence +95.000%	Al N	Al Sum	Al Std.Dev.	Al Variance	Al Std.Err.
C1 ET	57.57	33.38	81.75	9	518.1	31.46	990	10.49
C1 Vm	250.31	129.91	370.70	9	2252.8	156.63	24533	52.21
C2 ET	66.70	33.31	100.10	7	466.9	36.11	1304	13.65
C2 Vm	291.54	-52.27	635.35	6	1749.2	327.62	107332	133.75
CP ET	59.22	43.07	75.37	9	533.0	21.01	442	7.00
CP Vm	205.16	87.11	323.20	9	1846.4	153.57	23584	51.19
OS ET	61.00	33.56	88.43	9	549.0	35.69	1274	11.90
OS Vm	195.61	63.39	327.83	8	1564.9	158.15	25013	55.92

Treatment	Tukey HSD test; Variable: Al Marked differences are significant at p < .05000							
	{1} M=57.567	{2} M=250.31	{3} M=66.704	{4} M=29 1.54	{5} M=59.217	{6} M=205.16	{7} M=60.996	{8} M=195.61
C1 ET {1}		0.08	1.00	0.04	1.00	0.34	1.00	0.47
C1 Vm {2}	0.08		0.17	1.00	0.09	1.00	0.10	0.99
C2 ET {3}	1.00	0.17		0.09	1.00	0.51	1.00	0.63
C2 Vm {4}	0.04	1.00	0.09		0.05	0.94	0.05	0.90
CP ET {5}	1.00	0.09	1.00	0.05		0.35	1.00	0.48
CP Vm {6}	0.34	1.00	0.51	0.94	0.35		0.37	1.00
OS ET {7}	1.00	0.10	1.00	0.05	1.00	0.37		0.50

3.6.4 Cobalt

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Co Means	Confidence -95.000%	Confidence +95.000	Co N	Co Sum	Co Std.Dev.	Co Variance	Co Std.Err.
C1 ET	0.28	0.20	0.37	9	2.6	0.11	0.01	0.04
C1 Vm	1.49	0.96	2.02	9	13.4	0.69	0.48	0.23
C2 ET	0.31	0.14	0.48	7	2.2	0.18	0.03	0.07
C2 Vm	1.48	0.88	2.08	6	8.9	0.57	0.33	0.23
CP ET	0.20	0.17	0.23	9	1.8	0.05	0.00	0.02
CP Vm	1.26	0.86	1.65	9	11.3	0.52	0.27	0.17
OS ET	0.31	0.21	0.41	9	2.8	0.13	0.02	0.04
OS Vm	1.55	1.15	1.95	8	12.4	0.47	0.23	0.17

	Tukey HSD test; Variable: Co Marked differences are significant at p < .05000							
Treatment	{1} M=.28371	{2} M=1.4911	{3} M=.31165	{4} M=1.4777	{5} M=.20000	{6} M=1.2555	{7} M=.31043	{8} M=1.5513
C1 ET {1}		0.0001	1.0000	0.0001	0.9999	0.0002	1.0000	0.0001
C1 Vm {2}	0.0001		0.0001	1.0000	0.0001	0.9211	0.0001	1.0000
C2 ET {3}	1.0000	0.0001		0.0002	0.9994	0.0007	1.0000	0.0001
C2 Vm {4}	0.0001	1.0000	0.0002		0.0001	0.9673	0.0002	1.0000
CP ET {5}	0.9999	0.0001	0.9994	0.0001		0.0001	0.9991	0.0001
CP Vm {6}	0.0002	0.9211	0.0007	0.9673	0.0001		0.0003	0.8087
OS ET {7}	1.0000	0.0001	1.0000	0.0002	0.9991	0.0003		0.0001

3.6.5 Cadmium

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Cd Means	Confidence -95.000%	Confidence +95.000%	Cd N	Cd Sum	Cd Std.Dev.	Cd Variance	Cd Std.Err.
C1 ET	1.0398	0.5731	1.507	9	9.36	0.6072	0.369	0.2024
C1 Vm	5.2027	4.2388	6.167	9	46.82	1.2540	1.572	0.4180
C2 ET	1.4416	0.7289	2.154	7	10.09	0.7706	0.594	0.2913
C2 Vm	7.6098	0.9724	14.247	6	45.66	6.3247	40.002	2.5821
CP ET	1.3026	0.4778	2.128	9	11.72	1.0731	1.152	0.3577
CP Vm	5.3858	4.3906	6.381	9	48.47	1.2947	1.676	0.4316
OS ET	2.0863	0.2612	3.911	9	18.78	2.3744	5.638	0.7915
OS Vm	5.3426	4.3781	6.307	8	42.74	1.1537	1.331	0.4079

Treatment	Tukey HSD test; Variable: Cd Marked differences are significant at p < .05000							
	{1} M=1.0398	{2} M=5.2027	{3} M=1.4416	{4} M=7.6098	{5} M=1.3026	{6} M=5.3858	{7} M=2.0863	{8} M=5.3426
C1 ET {1}		0.0057	1.0000	0.0001	1.0000	0.0034	0.9753	0.0056
C1 Vm {2}	0.0057		0.0330	0.4771	0.0120	1.0000	0.0860	1.0000
C2 ET {3}	1.0000	0.0330		0.0003	1.0000	0.0212	0.9992	0.0302
C2 Vm {4}	0.0001	0.4771	0.0003		0.0002	0.5781	0.0006	0.5845
CP ET {5}	1.0000	0.0120	1.0000	0.0002		0.0072	0.9955	0.0114
CP Vm {6}	0.0034	1.0000	0.0212	0.5781	0.0072		0.0564	1.0000
OS ET {7}	0.9753	0.0860	0.9992	0.0006	0.9955	0.0564		0.0781

3.6.6 Zinc

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Zn Means	Confidence -95.000%	Confidence +95.000%	Zn N	Zn Sum	Zn Std.Dev.	Zn Variance	Zn Std.Err.
C1 ET	276.7	186.3	367.0	9	2490	117.5	13813	39.18
C1 Vm	1316.9	912.7	1721.1	9	11852	525.8	276506	175.28
C2 ET	290.6	137.3	443.8	7	2034	165.7	27458	62.63
C2 Vm	1796.1	-126.7	3719.0	6	10777	1832.3	3357297	748.03
CP ET	266.8	238.8	294.7	9	2401	36.3	1320	12.11
CP Vm	1183.2	949.6	1416.9	9	10649	303.9	92385	101.32
OS ET	305.6	228.0	383.3	9	2751	101.0	10198	33.66
OS Vm	1208.6	898.3	1519.0	8	9669	371.2	137800	131.24

Treatment	Tukey HSD test; Variable: Zn Marked differences are significant at p < .05000							
	{1} M=276.6	{2} M=1316.	{3} M=290.5	{4} M=1796.	{5} M=266.7	{6} M=1183.	{7} M=305.6	{8} M=1208.
C1 ET {1}		0.0120	1.0000	0.0004	1.0000	0.0444	1.0000	0.0454
C1 Vm {2}	0.0120		0.0268	0.7997	0.0109	0.9998	0.0162	1.0000
C2 ET {3}	1.0000	0.0268		0.0010	1.0000	0.0834	1.0000	0.0825
C2 Vm {4}	0.0004	0.7997	0.0010		0.0004	0.5371	0.0005	0.6195
CP ET {5}	1.0000	0.0109	1.0000	0.0004		0.0405	1.0000	0.0415
CP Vm {6}	0.0444	0.9998	0.0834	0.5371	0.0405		0.0576	1.0000
OS ET {7}	1.0000	0.0162	1.0000	0.0005	1.0000	0.0576		0.0584
OS Vm {8}	0.0454	1.0000	0.0825	0.6195	0.0415	1.0000	0.0584	

3.6.7 Chromium

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatmen	Cr Means	Confidenc e -95.000%	Confidenc e +95.000%	Cr N	Cr Sum	Cr Std.Dev.	Cr Variance	Cr Std.Err.
C1 ET	3.899	3.2820	4.516	9	35.09	0.8028	0.644	0.2676
C1 Vm	10.624	6.7627	14.486	9	95.62	5.0240	25.240	1.6747
C2 ET	3.991	3.1615	4.820	7	27.94	0.8967	0.804	0.3389
C2 Vm	9.363	4.7816	13.945	6	56.18	4.3658	19.060	1.7823
CP ET	3.720	3.3774	4.062	9	33.48	0.4453	0.198	0.1484
CP Vm	8.441	6.1172	10.766	9	75.97	3.0238	9.143	1.0079
OS ET	3.795	3.4471	4.142	9	34.15	0.4523	0.205	0.1508
OS Vm	8.339	5.5696	11.109	8	66.71	3.3128	10.974	1.1712

	Tukey HSD test; Variable: Cr Marked differences are significant at p < .05000							
Treatment	{1} M=3.899 0	{2} M=10.62 4	{3} M=3.990 7	{4} M=9.363 2	{5} M=3.719 6	{6} M=8.441 5	{7} M=3.794 7	{8} M=8.3391
C1 ET {1}		0.0002	1.0000	0.0114	1.0000	0.0239	1.0000	0.0387
C1 Vm {2}	0.0002		0.0006	0.9893	0.0002	0.7222	0.0002	0.7064
C2 ET {3}	1.0000	0.0006		0.0235	1.0000	0.0513	1.0000	0.0751
C2 Vm {4}	0.0114	0.9893	0.0235		0.0079	0.9985	0.0092	0.9975
CP ET {5}	1.0000	0.0002	1.0000	0.0079		0.0164	1.0000	0.0272
CP Vm {6}	0.0239	0.7222	0.0513	0.9985	0.0164		0.0192	1.0000
OS ET {7}	1.0000	0.0002	1.0000	0.0092	1.0000	0.0192		0.0315

3.6.8 Iron

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatme nt	Fe Means	Confider ce -95.000	Confidenc e +95.000%	Fe N	Fe Sum	Fe Std.Dev.	Fe Variance	Fe Std.Err.
C1 ET	101.22	72	130.3	9	911	37.8	1430	12.60
C1 Vm	685.18	480	890.4	9	6167	267.0	71267	88.99
C2 ET	125.14	80	170.1	7	876	48.7	2368	18.39
C2 Vm	237.04	-1099	1573.5	6	1422	1273.5	1621907	519.92
CP ET	113.21	73	153.3	9	1019	52.2	2723	17.39
CP Vm	933.67	679	1188.5	9	8403	331.5	109867	110.49
OS ET	115.19	68	162.5	9	1037	61.6	3793	20.53
OS Vm	784.50	405	1164.2	8	6276	454.1	206246	160.56

Treatment	Tukey HSD test; Variable: Fe Marked differences are significant at p < .05000							
	{1} M=101.22	{2} M=685.18	{3} M=125.14	{4} M=237.04	{5} M=113.21	{6} M=933.67	{7} M=115.19	{8} M=784.50
C1 ET {1}		0.106	1.000	0.999	1.000	0.004	1.000	0.041
C1 Vm {2}	0.106		0.199	0.527	0.121	0.927	0.124	1.000
C2 ET {3}	1.000	0.199		1.000	1.000	0.012	1.000	0.088
C2 Vm {4}	0.999	0.527	1.000		0.999	0.068	0.999	0.301
CP ET {5}	1.000	0.121	1.000	0.999		0.005	1.000	0.048
CP Vm {6}	0.004	0.927	0.012	0.068	0.005		0.005	0.997
OS ET {7}	1.000	0.124	1.000	0.999	1.000	0.005		0.049

3.6.9 Nickel

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Ni Means	Confidence -95.000%	Confidence +95.000%	Ni N	Ni Sum	Ni Std.Dev.	Ni Variance	Ni Std.Err.
C1 ET	16.282	11.283	21.281	9	146.5	6.503	42.29	2.1678
C1 Vm	19.254	15.031	23.476	9	173.3	5.493	30.18	1.8311
C2 ET	18.714	14.191	23.238	7	131.0	4.891	23.92	1.8486
C2 Vm	22.437	11.790	33.083	6	134.6	10.145	102.92	4.1416
CP ET	9.980	6.474	13.486	9	89.8	4.561	20.80	1.5204
CP Vm	14.149	9.522	18.776	9	127.3	6.019	36.23	2.0064
OS ET	17.837	14.864	20.811	9	160.5	3.868	14.96	1.2895
OS Vm	18.333	14.518	22.149	8	146.7	4.564	20.83	1.6135

Treatment	Tukey HSD test; Variable: Ni Marked differences are significant at p < .05000							
	{1} M=16.282	{2} M=19.254	{3} M=18.714	{4} M=22.437	{5} M=9.9800	{6} M=14.149	{7} M=17.837	{8} M=18.333
C1 ET {1}		0.958	0.991	0.485	0.311	0.994	0.999	0.996
C1 Vm {2}	0.958		1.000	0.966	0.026	0.581	1.000	1.000
C2 ET {3}	0.991	1.000		0.942	0.075	0.772	1.000	1.000
C2 Vm {4}	0.485	0.966	0.942		0.004	0.142	0.804	0.893
CP ET {5}	0.311	0.026	0.075	0.004		0.793	0.099	0.080
CP Vm {6}	0.994	0.581	0.772	0.142	0.793		0.877	0.814
OS ET {7}	0.999	1.000	1.000	0.804	0.099	0.877		1.000

3.6.10 Lead

Breakdown Table of Descriptive Statistics N=66 (No missing data in dep. var. list)								
Treatment	Pb Means	Confidence -95.000%	Confidence +95.000	Pb N	Pb Sum	Pb Std.Dev.	Pb Variance	Pb Std.Err.
C1 ET	1.7	1.01	2.4	9	15	0.91	0.8	0.30
C1 Vm	11.6	5.44	17.8	9	105	8.06	65.0	2.69
C2 ET	1.2	0.56	1.7	7	8	0.64	0.4	0.24
C2 Vm	7.4	0.55	14.3	6	44	6.54	42.8	2.67
CP ET	1.1	0.57	1.6	9	10	0.68	0.5	0.23
CP Vm	8.6	3.02	14.1	9	77	7.24	52.4	2.41
OS ET	1.6	0.60	2.5	9	14	1.23	1.5	0.41
OS Vm	8.4	2.55	14.3	8	67	7.01	49.2	2.48

	Tukey HSD test; Variable: Pb Marked differences are significant at p < .05000							
Treatment	{1} M=1.7139	{2} M=11.641	{3} M=1.1509	{4} M=7.4097	{5} M=1.0964	{6} M=8.5779	{7} M=1.5507	{8} M=8.4118
C1 ET {1}		0.003	1.000	0.421	1.000	0.105	1.000	0.146
C1 Vm {2}	0.003		0.004	0.768	0.001	0.907	0.002	0.896
C2 ET {3}	1.000	0.004		0.370	1.000	0.097	1.000	0.132
C2 Vm {4}	0.421	0.768	0.370		0.292	1.000	0.385	1.000
CP ET {5}	1.000	0.001	1.000	0.292		0.056	1.000	0.083
CP Vm {6}	0.105	0.907	0.097	1.000	0.056		0.089	1.000
OS ET {7}	1.000	0.002	1.000	0.385	1.000	0.089		0.127

Field Experiment

3.7 DGT's Tables

3.7.1 Copper

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Cu Means	Confidence -95.000	Confidence +95.000	Cu N	Cu Sum	Cu Std.Dev.	Cu Variance	Cu Std.Err.
Control	0.00714	0.00604	0.00823	9	0.06425	0.00143	0.00000	0.00048
CP	0.01375	0.01025	0.01724	9	0.12374	0.00455	0.00002	0.00152
OS	0.01261	0.00874	0.01648	9	0.11347	0.00503	0.00003	0.00168

Tukey HSD test; Variable: Cu (Caleb DGT real
Marked differences are significant at p < .0500

Treatment	{1} M=.00714	{2} M=.01375	{3} M=.01261
Control {1}		0.005	0.021
CP {2}	0.005		0.819
OS {3}	0.021	0.819	

3.7.2 Manganese

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Mn Means	Confidence -95.00	Confidence +95.00	Mn N	Mn Sum	Mn Std.Dev	Mn Variance	Mn Std.Err
Control	0.0508	0.0363	0.0652	9	0.4569	0.0188	0.0004	0.0063
CP	0.0557	0.0361	0.0752	9	0.5011	0.0254	0.0006	0.0085
OS	0.0701	0.0422	0.0979	9	0.6307	0.0362	0.0013	0.0121

Tukey HSD test; Variable: Mn (Caleb DGT real
Marked differences are significant at p < .0500

Treatment	{1} M=.05077	{2} M=.05568	{3} M=.07008
Control {1}		0.93	0.32
CP {2}	0.93		0.52
OS {3}	0.32	0.52	

3.7.3 Aluminium

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Al Means	Confidence -95.000	Confidence +95.000	Al N	Al Sum	Al Std.Dev.	Al Variance	Al Std.Err.
Control	0.0135	0.0066	0.0205	9	0.1219	0.0091	0.0001	0.0030
CP	0.0172	0.0104	0.0240	9	0.1550	0.0089	0.0001	0.0030
OS	0.0445	-0.0167	0.1056	9	0.4001	0.0795	0.0063	0.0265

	Tukey HSD test; Variable: Al (Caleb DGT real d Marked differences are significant at p < .0500		
Treatment	{1} M=.01354	{2} M=.01722	{3} M=.04445
Control {1}		0.98	0.35
CP {2}	0.98		0.44
OS {3}	0.35	0.44	

3.7.4 Cobalt

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Co Means	Confidence -95.000%	Confidence +95.000%	Co N	Co Sum	Co Std.Dev.	Co Variance	Co Std.Err.
Control	0.0002	0.0002	0.0003	9	0.0022	0.00003	0.0000	0.00001
CP	0.0003	0.0002	0.0003	9	0.0023	0.00004	0.0000	0.00001
OS	0.0003	0.0002	0.0004	9	0.0027	0.00009	0.0000	0.00003

	Tukey HSD test; Variable: Co (Caleb DGT real Marked differences are significant at p < .0500		
Treatment	{1} M=.00024	{2} M=.00026	{3} M=.00030
Control {1}		0.86	0.12
CP {2}	0.86		0.30
OS {3}	0.12	0.30	

3.7.5 Cadmium

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Cd Means	Confidence -95.000	Confidence +95.000	Cd N	Cd Sum	Cd Std.Dev.	Cd Variance	Cd Std.Err.
Control	0.002	0.001	0.003	9	0.020	0.00104	0.000001	0.00034
CP	0.003	0.002	0.004	9	0.028	0.00153	0.000002	0.00051
OS	0.004	0.002	0.005	9	0.032	0.00202	0.000004	0.00067

	Tukey HSD test; Variable: Cd (Caleb DGT real Marked differences are significant at p < .0500		
Treatment	{1} M=.00228	{2} M=.00311	{3} M=.00357
Control {1}		0.51	0.21
CP {2}	0.51		0.81
OS {3}	0.21	0.81	

3.7.6 Zinc

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Zn Means	Confidence -95.000%	Confidence +95.000	Zn N	Zn Sum	Zn Std.Dev.	Zn Variance	Zn Std.Err.
Control	0.053	0.029	0.077	9	0.481	0.031	0.001	0.010
CP	0.079	0.043	0.116	9	0.715	0.047	0.002	0.016
OS	0.087	0.058	0.115	9	0.780	0.037	0.001	0.012

Tukey HSD test; Variable: Zn (Caleb DGT real data)
Marked differences are significant at p < .05000

Treatment	{1} M=.05341	{2} M=.07949	{3} M=.08662
Control {1}		0.35	0.19
CP {2}	0.35		0.92
OS {3}	0.19	0.92	

3.7.7 Chromium

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Cr Means	Confidence -95.000%	Confidence +95.000%	Cr N	Cr Sum	Cr Std.Dev.	Cr Variance	Cr Std.Err.
Control	0.00025	0.00020	0.00029	9	0.002	0.00006	0.00000	0.00002
CP	0.00026	0.00022	0.00031	9	0.002	0.00006	0.00000	0.00002
OS	0.00031	0.00022	0.00041	9	0.003	0.00013	0.00000	0.00004

Tukey HSD test; Variable: Cr (Caleb DGT real d
Marked differences are significant at p < .05000

Treatment	{1} M=.00025	{2} M=.00026	{3} M=.00031
Control {1}		0.94	0.26
CP {2}	0.94		0.44
OS {3}	0.26	0.44	

3.7.8 Iron

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Fe Means	Confidence -95.000%	Confidence +95.000	Fe N	Fe Sum	Fe Std.Dev.	Fe Variance	Fe Std.Err.
Control	0.066	0.031	0.101	9	0.592	0.045	0.002	0.015
CP	0.078	0.049	0.106	9	0.700	0.037	0.001	0.012
OS	0.250	-0.060	0.560	9	2.247	0.403	0.163	0.134

	Tukey HSD test; Variable: Fe (Caleb DGT real d Marked differences are significant at p < .05000		
Treatment	{1} M=.06578	{2} M=.07781	{3} M=.24967
Control {1}		0.99	0.24
CP {2}	0.99		0.29
OS {3}	0.24	0.29	

3.7.9 Nickel

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Ni Means	Confidence -95.000%	Confidence +95.000	Ni N	Ni Sum	Ni Std.Dev.	Ni Variance	Ni Std.Err.
Control	0.010205	0.009161	0.011250	9	0.091846	0.001359	0.000002	0.000453
CP	0.016395	0.001361	0.031429	9	0.147559	0.019558	0.000383	0.006519
OS	0.009840	0.008797	0.010883	9	0.088563	0.001357	0.000002	0.000452

	Tukey HSD test; Variable: Ni (Caleb DGT real d Marked differences are significant at p < .05000		
Treatment	{1} M=.01021	{2} M=.01640	{3} M=.00984
Control {1}		0.49	1.00
CP {2}	0.49		0.45
OS {3}	1.00	0.45	

3.7.10 Lead

Breakdown Table of Descriptive Statistics (Caleb DGT real data)
N=27 (No missing data in dep. var. list)

Treatment	Pb Means	Confidence -95.000	Confidence +95.000	Pb N	Pb Sum	Pb Std.Dev.	Pb Variance	Pb Std.Err.
Control	0.0020	0.0017	0.0023	9	0.0180	0.0004	0.000000	0.00013
CP	0.0018	0.0016	0.0021	9	0.0163	0.0003	0.000000	0.00011
OS	0.0028	0.0016	0.0040	9	0.0252	0.0016	0.000003	0.00053

	Tukey HSD test; Variable: Pb (Caleb DGT real d Marked differences are significant at p < .05000		
Treatment	{1} M=.00200	{2} M=.00182	{3} M=.00280
Control {1}		0.91	0.21
CP {2}	0.91		0.10
OS {3}	0.21	0.10	

3.8 Tissues Tables

3.8.1 Copper

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Cu Means	Confidence -95.000	Confidence +95.000	Cu N	Cu Sum	Cu Std.Dev.	Cu Variance	Cu Std.Err.
Control ET	21.75	-11.7	55.2	3	65.2	13.48	182	7.78
Control Vm	12.56	4.6	20.6	3	37.7	3.22	10	1.86
CP ET	28.28	19.9	36.6	3	84.8	3.37	11	1.94
CP Vm	51.66	-45.7	149.1	3	155.0	39.21	1537	22.64
OS ET	24.60	21.8	27.4	3	73.8	1.13	1	0.65
OS Vm	32.99	21.4	44.5	3	99.0	4.65	22	2.68

Tukey HSD test; Variable: Cu (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=21.74	{2} M=12.55	{3} M=28.28	{4} M=51.65	{5} M=24.60	{6} M=32.99
Control ET {1}		0.98	1.00	0.33	1.00	0.96
Control Vm {2}	0.98		0.86	0.13	0.95	0.69
CP ET {3}	1.00	0.86		0.57	1.00	1.00
CP Vm {4}	0.33	0.13	0.57		0.43	0.76
OS ET {5}	1.00	0.95	1.00	0.43		0.99
OS Vm {6}	0.96	0.69	1.00	0.76	0.99	

3.8.2 Manganese

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Mn Means	Confidence -95.000%	Confidence +95.000	Mn N	Mn Sum	Mn Std.Dev.	Mn Variance	Mn Std.Err.
Control ET	13.16	4.20	22.12	3	39.5	3.607	13.01	2.082
Control Vm	15.18	13.25	17.11	3	45.6	0.776	0.60	0.448
CP ET	8.09	5.80	10.38	3	24.3	0.922	0.85	0.532
CP Vm	11.43	6.94	15.91	3	34.3	1.806	3.26	1.043
OS ET	10.78	9.96	11.60	3	32.4	0.330	0.11	0.190
OS Vm	12.75	8.51	16.99	3	38.3	1.707	2.91	0.986

Tukey HSD test; Variable: Mn (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=13.162	{2} M=15.184	{3} M=8.0881	{4} M=11.428	{5} M=10.783	{6} M=12.751
Control ET {1}		0.763	0.052	0.855	0.633	1.000
Control Vm {2}	0.763		0.006	0.206	0.107	0.612
CP ET {3}	0.052	0.006		0.305	0.514	0.081
CP Vm {4}	0.855	0.206	0.305		0.998	0.946
OS ET {5}	0.633	0.107	0.514	0.998		0.782
OS Vm {6}	1.000	0.612	0.081	0.946	0.782	

3.8.3 Aluminium

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Al Means	Confidence -95.000%	Confidence +95.000	Al N	Al Sum	Al Std.Dev.	Al Variance	Al Std.Err.
Control ET	791.46	347.99	1234.9	3	2374	178.52	31870	103.07
Control Vm	950.44	492.27	1408.6	3	2851	184.44	34018	106.49
CP ET	483.48	262.76	704.2	3	1450	88.85	7895	51.30
CP Vm	721.05	229.31	1212.8	3	2163	197.95	39186	114.29
OS ET	624.01	554.35	693.7	3	1872	28.04	786	16.19
OS Vm	699.59	374.74	1024.4	3	2099	130.77	17101	75.50

Tukey HSD test; Variable: Al (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=791.46	{2} M=950.44	{3} M=483.48	{4} M=721.05	{5} M=624.01	{6} M=699.59
Control ET {1}		0.77	0.18	0.99	0.73	0.97
Control Vm {2}	0.77		0.02	0.45	0.14	0.36
CP ET {3}	0.18	0.02		0.41	0.84	0.50
CP Vm {4}	0.99	0.45	0.41		0.96	1.00
OS ET {5}	0.73	0.14	0.84	0.96		0.99
OS Vm {6}	0.97	0.36	0.50	1.00	0.99	

3.8.4 Cobalt

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Co Means	Confidence -95.000%	Confidence +95.000	Co N	Co Sum	Co Std.Dev.	Co Variance	Co Std.Err.
Control ET	0.494	-0.166	1.153	3	1.48	0.266	0.071	0.153
Control Vm	0.685	0.097	1.272	3	2.05	0.237	0.056	0.137
CP ET	0.280	0.236	0.324	3	0.84	0.018	0.000	0.010
CP Vm	0.883	0.804	0.962	3	2.65	0.032	0.001	0.018
OS ET	0.333	0.266	0.400	3	1.00	0.027	0.001	0.016
OS Vm	0.913	0.615	1.211	3	2.74	0.120	0.014	0.069

Tukey HSD test; Variable: Co (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=.49359	{2} M=.68478	{3} M=.27952	{4} M=.88303	{5} M=.33297	{6} M=.91286
Control ET {1}		0.661	0.557	0.078	0.793	0.053
Control Vm {2}	0.661		0.064	0.629	0.127	0.495
CP ET {3}	0.557	0.064		0.005	0.998	0.003
CP Vm {4}	0.078	0.629	0.005		0.009	1.000
OS ET {5}	0.793	0.127	0.998	0.009		0.006
OS Vm {6}	0.053	0.495	0.003	1.000	0.006	

3.8.5 Cadmium

Breakdown Table of Descriptive Statistics (In Situ) N=18 (No missing data in dep. var. list)								
Treatments	Cd Means	Confidence -95.000%	Confidence +95.000	Cd N	Cd Sum	Cd Std.Dev.	Cd Variance	Cd Std.Err.
Control ET	2.035	-4.89	8.96	3	6.10	2.789	7.778	1.610
Control Vm	3.504	-3.31	10.31	3	10.51	2.742	7.518	1.583
CP ET	0.430	0.21	0.65	3	1.29	0.089	0.008	0.051
CP Vm	5.324	4.30	6.35	3	15.97	0.412	0.170	0.238
OS ET	0.427	0.21	0.65	3	1.28	0.088	0.008	0.051
OS Vm	5.980	4.83	7.12	3	17.94	0.461	0.212	0.266

	Tukey HSD test; Variable: Cd (In Situ) Marked differences are significant at p < .05000					
Treatments	{1} M=2.0349	{2} M=3.5035	{3} M=.42999	{4} M=5.3244	{5} M=.42659	{6} M=5.9797
Control ET {1}		0.87	0.82	0.20	0.82	0.09
Control Vm {2}	0.87		0.26	0.74	0.25	0.46
CP ET {3}	0.82	0.26		0.03	1.00	0.01
CP Vm {4}	0.20	0.74	0.03		0.03	1.00
OS ET {5}	0.82	0.25	1.00	0.03		0.01
OS Vm {6}	0.09	0.46	0.01	1.00	0.01	

3.8.6 Zinc

Breakdown Table of Descriptive Statistics (In Situ) N=18 (No missing data in dep. var. list)								
Treatments	Zn Means	Confidence -95.000%	Confidence +95.000	Zn N	Zn Sum	Zn Std.Dev.	Zn Variance	Zn Std.Err.
Control ET	94.7	-48.1	237.5	3	284	57.50	3306	33.20
Control Vm	106.3	2.8	209.9	3	319	41.68	1738	24.07
CP ET	68.1	64.2	72.1	3	204	1.60	3	0.93
CP Vm	165.3	134.8	195.9	3	496	12.31	152	7.11
OS ET	61.2	43.6	78.8	3	184	7.09	50	4.10
OS Vm	164.8	83.8	245.8	3	494	32.62	1064	18.83

	Tukey HSD test; Variable: Zn (In Situ) Marked differences are significant at p < .05000					
Treatments	{1} M=94.695	{2} M=106.31	{3} M=68.133	{4} M=165.35	{5} M=61.180	{6} M=164.79
Control ET {1}		1.00	0.91	0.15	0.80	0.16
Control Vm {2}	1.00		0.70	0.29	0.55	0.30
CP ET {3}	0.91	0.70		0.03	1.00	0.03
CP Vm {4}	0.15	0.29	0.03		0.02	1.00
OS ET {5}	0.80	0.55	1.00	0.02		0.02
OS Vm {6}	0.16	0.30	0.03	1.00	0.02	

3.8.7 Chromium

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Cr Means	Confidence -95.000%	Confidence +95.000%	Cr N	Cr Sum	Cr Std.Dev.	Cr Variance	Cr Std.Err.
Control ET	7.242	-1.04	15.52	3	21.7	3.333	11.11	1.924
Control Vm	6.310	-5.59	18.21	3	18.9	4.789	22.93	2.765
CP ET	6.186	4.85	7.53	3	18.6	0.539	0.29	0.311
CP Vm	3.655	2.92	4.39	3	11.0	0.296	0.09	0.171
OS ET	9.430	2.98	15.88	3	28.3	2.595	6.73	1.498
OS Vm	3.555	2.89	4.22	3	10.7	0.269	0.07	0.156

Tukey HSD test; Variable: Cr (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=7.2421	{2} M=6.3103	{3} M=6.1857	{4} M=3.6551	{5} M=9.4299	{6} M=3.5547
Control ET {1}		1.00	1.00	0.57	0.90	0.54
Control Vm {2}	1.00		1.00	0.81	0.69	0.79
CP ET {3}	1.00	1.00		0.84	0.66	0.82
CP Vm {4}	0.57	0.81	0.84		0.15	1.00
OS ET {5}	0.90	0.69	0.66	0.15		0.14
OS Vm {6}	0.54	0.79	0.82	1.00	0.14	

3.8.8 Iron

Breakdown Table of Descriptive Statistics (In Situ)
N=18 (No missing data in dep. var. list)

Treatments	Fe Means	Confidence -95.000	Confidence +95.00	Fe N	Fe Sum	Fe Std.Dev.	Fe Variance	Fe Std.Err.
Control ET	1673.9	956.1	2391.6	3	5022	288.94	83486	166.82
Control Vm	2204.7	1442.7	2966.8	3	6614	306.76	94102	177.11
CP ET	776.2	518.0	1034.4	3	2329	103.94	10804	60.01
CP Vm	1830.8	1454.9	2206.6	3	5492	151.30	22891	87.35
OS ET	1100.1	873.4	1326.8	3	3300	91.25	8326	52.68
OS Vm	1908.3	1651.9	2164.7	3	5725	103.22	10654	59.59

Tukey HSD test; Variable: Fe (In Situ)
Marked differences are significant at p < .05000

Treatments	{1} M=1673.9	{2} M=2204.7	{3} M=776.21	{4} M=1830.8	{5} M=1100.1	{6} M=1908.3
Control ET {1}		0.0536	0.0013	0.9156	0.0341	0.6902
Control Vm {2}	0.0536		0.0002	0.2515	0.0003	0.4714
CP ET {3}	0.0013	0.0002		0.0004	0.3834	0.0003
CP Vm {4}	0.9156	0.2515	0.0004		0.0066	0.9959
OS ET {5}	0.0341	0.0003	0.3834	0.0066		0.0031
OS Vm {6}	0.6902	0.4714	0.0003	0.9959	0.0031	

3.8.9 Nickel

Breakdown Table of Descriptive Statistics (In Situ) N=18 (No missing data in dep. var. list)								
Treatments	Ni Means	Confidence -95.000%	Confidence +95.000	Ni N	Ni Sum	Ni Std.Dev.	Ni Variance	Ni Std.Err.
Control ET	13.660	1.636	25.684	3	40.98	4.8402	23.428	2.7945
Control Vm	9.565	2.256	16.875	3	28.70	2.9424	8.658	1.6988
CP ET	11.671	6.829	16.513	3	35.01	1.9491	3.799	1.1253
CP Vm	15.278	10.914	19.642	3	45.83	1.7568	3.086	1.0143
OS ET	12.788	7.550	18.026	3	38.36	2.1085	4.446	1.2174
OS Vm	14.161	7.901	20.421	3	42.48	2.5200	6.350	1.4549

	Tukey HSD test; Variable: Ni (In Situ) Marked differences are significant at p < .05000					
Treatments	{1} M=13.660	{2} M=9.5652	{3} M=11.671	{4} M=15.278	{5} M=12.788	{6} M=14.161
Control ET {1}		0.53	0.95	0.98	1.00	1.00
Control Vm {2}	0.53		0.94	0.22	0.74	0.42
CP ET {3}	0.95	0.94		0.65	1.00	0.89
CP Vm {4}	0.98	0.22	0.65		0.89	1.00
OS ET {5}	1.00	0.74	1.00	0.89		0.99
OS Vm {6}	1.00	0.42	0.89	1.00	0.99	

3.9 Lead

Breakdown Table of Descriptive Statistics (In Situ) N=18 (No missing data in dep. var. list)								
Treatments	Pb Means	Confidence -95.000%	Confidence +95.000%	Pb N	Pb Sum	Pb Std.Dev.	Pb Variance	Pb Std.Err.
Control ET	0.795	0.592	0.998	3	2.39	0.082	0.007	0.047
Control Vm	1.080	0.617	1.543	3	3.24	0.186	0.035	0.108
CP ET	0.381	0.328	0.434	3	1.14	0.021	0.000	0.012
CP Vm	0.924	0.604	1.244	3	2.77	0.129	0.017	0.074
OS ET	0.569	0.336	0.801	3	1.71	0.094	0.009	0.054
OS Vm	0.905	0.674	1.137	3	2.72	0.093	0.009	0.054

	Tukey HSD test; Variable: Pb (In Situ) Marked differences are significant at p < .05000					
Treatments	{1} M=.79526	{2} M=1.0799	{3} M=.38107	{4} M=.92385	{5} M=.56859	{6} M=.90523
Control ET {1}		0.0771	0.0073	0.7269	0.2085	0.8303
Control Vm {2}	0.0771		0.0002	0.5568	0.0014	0.4455
CP ET {3}	0.0073	0.0002		0.0009	0.3756	0.0012
CP Vm {4}	0.7269	0.5568	0.0009		0.0213	0.9999
OS ET {5}	0.2085	0.0014	0.3756	0.0213		0.0300
OS Vm {6}	0.8303	0.4455	0.0012	0.9999	0.0300	