Metals in New Zealand *Undaria pinnatifida* (Wakame)

Leo Hau

A thesis submitted to the

Auckland University of Technology

in partial fulfillment of the requirements for the degree of

Master of Applied Science (MAppSc)

School of Applied Science, Faculty of Health and Environmental Sciences

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no materials previously published or written by another person (except where explicitly defined in the acknowledgments), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed: _____ Date: _____

Leo Hau

Acknowledgements

I would like to express my thanks to my supervisor Dr. Lindsey White of Auckland University of Technology. His guidance and patience had given me strength to complete my thesis year.

Also I would like to thank Dr. John Robertson useful technical comments and assistance during the project.

Thanks to Chris Whyburd, Wang Yan, and Percy Perera, for their help with preparing laboratory equipments, which allowed me to work freely and achieved the best results. Thanks to Lea and Neil Bramley for their efforts for sampling in the Wellington region and their enthusiasm for the seaweed industry and my project. Thanks to Mark Allsopp, and other staff at Wakatu Inc. for arranging the collection trips in Port Underwood. Also thanks to Weiwei Chen, Glenn Farrington and Savisene Boulom for seaweed collection in Port Underwood.Last, but not least, I am grateful to my parents, my girl friend and my friends for their moral support.

Abstract

Undaria pinnatifida, Wakame is a popular edible seaweed in Asia (Yamanaka & Akiyama, 1993). Wakame has been recognized as a food rich in minerals, fiber and bioactive compounds such as proteins, vitamins, carotenoids such as fucoxanthin, and polyunsaturated fatty acids (Murata & Nakazoe, 2001).

U. pinnatifida was first recorded in New Zealand in Wellington Harbor in 1987. (Hay & Luckens, 1987) It was classified as an unwanted species according to the Biosecurity Act 1993 under section 164c however, when it was clear that it could not be eradicated a new policy was applied in April 2010, which allowed greater freedom to use *U. pinnatifida* commercially.

The primary aim for this study was to evaluate the concentrations of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), calcium (Ca), mercury (Hg), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), potassium (K), lead (Pb), selenium (Se) and zinc (Zn) in *U. pinnatifida* and to compare the metal concentrations between the blade and sporophyll tissue. These data were compared with nutrient reference values for Australia and New Zealand and WHO/FAO guidelines to determine the safety and suitability of harvesting *U. pinnatifida* to manufacture edible wakame products.

U. pinnatifida was collected from two mussel farms, PE 327 and 106 from Port Underwood, South Island, New Zealand. Sampling of PE 327 was carried out on a monthly basis from April 2011 to October 2011. Sampling of 106 was carried on monthly basis from July 2011 to October 2011. Two additional sites on the eastern and western side of Miramar Peninsula in Wellington Harbor; Shelley Bay (site A) and Worser Bay (site B) were integrated into the study from August 2011 to November 2011.

Harvested samples were dried by oven or freeze dried method then ground to a powder using a blade mill. The dried *U pinnatifida* was digested with nitric acid and perchloric

acid and the resulting solutions then analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

In brief, the highest monthly mean concentration of metals found in New Zealand wild *U. pinnatifida* were Ca (16.97 g kg^{-1}), K (48.48 g kg^{-1}), Mg (9.47 g kg^{-1}), Na (62.55 g kg^{-1}), P (12.05 g kg^{-1}), Cr (1.04 mg kg^{-1}), Cu (3.78 mg kg^{-1}), Mn (14.61 mg kg^{-1}), Ni (2.78 mg kg^{-1}), Se (0.83 mg kg^{-1}), Zn (35.03 mg kg^{-1}), As (46.71 mg kg^{-1}), Cd (2.91 mg kg^{-1}), Hg (0.042 mg kg^{-1}) and Pb (0.31m g kg^{-1}).

The results showed that New Zealand *U. pinnatifida* is a good source of the nutritionally important minerals calcium, sodium, magnesium, potassium and phosphorus. They also contained trace amounts of minerals such as chromium, copper, manganese, nickel, selenium and zinc. Contaminants such as arsenic, cadmium, mercury and lead were found at very low, safe, levels.

Chapter 1 Introduction

There is a lot of interest in the use of seaweeds, either as whole foods or refined for their active components (McHugh, 2003). These interests have driven academic research programs and government funded projects, as well as private commercial new product development initiatives. The majority of these efforts has targeted commonly available seaweed genera and is focused on whole plants as functional foods, or targets specific refined compounds with demonstrated bioactivity. Another major global focus has been the collection of seaweeds from specific regions and then the screening of these seaweeds for specific bio activity, food safety and their content of various compounds of interest.

Seaweeds have been employed as food and medicines in many Asian countries such as Japan, Korea, China, Vietnam, Indonesia and Taiwan for a long period of time (McHugh, 2002; Barsanti & Gualtieri, 2006.) . Wakame, *U. pinnatifida*, is the most popular edible seaweed in Asia. It has a sweet flavour and is most often served in soups and salads (Murata & Nakazoe, 2001). Asian countries, especially Japan and Korea are the main suppliers and use the most *U. pinnatifida* and related products and have already successfully developed cultivation techniques and commercialisation of *U. pinnatifida* related products.

Seaweed consumption and usage has existed in New Zealand for a very long period of time. In the early 1800s, long before the European settlement, the traditional Maori diet and medicine had often included a number of seaweeds (Brooker, Cambie, & Cooper, 1981). Seaweed such as *Ulva* spp. *Porphyra* spp. and *Gigartina* spp. were often included (Crowe, 1981). Brown seaweeds such as *Durvillaea antarctica* (rimuroa), were roasted and eaten as a curative for eczema and intestinal upsets (Brooker *et al*., 1981; Crowe, 1981). European immigrants consumed *Porphyra* spp. as food and made milk puddings using carrageenan extracted from seaweeds such as *Gigartina* spp. and more recently *Porphyra* spp. was sent to New Zealand troops in World War II as a replacement of chewing gum (Brooker *et al*., 1981).

1.1 Introduction of *Undaria pinnatifida* in New Zealand

Undaria pinnatifida was first recorded in New Zealand in Wellington Harbor in 1987 (Hay & Luckens, 1987). The gametophytes were transported to New Zealand in the ballast of foreign fishing vessels (Neill, Heesch, & Nelson, 2009). At present, *U. pinnatifida* in New Zealand has been reported from Great Barrier Island, Auckland (Waitemata Harbor), Coromandel, Tauranga, Gisborne, Napier, Port Taranaki, Wellington and the Wellington region of Cook Strait in the North Island, in the Marlborough Sounds, Nelson, Golden Bay, Kaikoura, Lyttelton, Akaroa, Timaru, Oamaru, Dunedin Harbor, Bluff in the South Island and also from Stewart Island and the Snares Islands (Neill *et al*., 2009). Unlike more tropical climates where there is significant dieback in warm conditions, *U. pinnatifida* has displayed an annual life cycle in New Zealand waters (Neill *et al*., 2009).

In 2000 *U. pinnatifida* is classified as an unwanted species according to the Biosecurity Act 1993 under section 164c (MAF, 2009). However, by 2004 a policy was developed that allowed the commercial harvest of the seaweed in two situations: where it was taken as a by-product of another activity, for example, the clearing of mussel farming lines or as part of a control or eradication programme (MAF, 2009). In 2009 to 2010 the government had reviewed the 2004 policy related to limited commercialisation of *U. pinnatifida* and had revised a new policy in April 2010 allowing greater freedom for the marine industry to use this seaweed commercially (MAF, 2010).

The new 2010 policy was summerised into four main points (MAF, 2010).

1. The farming of *U. pinnatifida* is to be allowed in selected infested areas.
2. Harvest of *U. pinnatifida* can be carried out on artificial surfaces such as marina and sea farm.
3. Harvest can be carried out in areas not vulnerable or sensitive to commercial harvest techniques if the *U. pinnatifida* is casted ashore.
4. Harvest is prohibited from natural surfaces but except when part of a programme specifically designed to control *U. pinnatifida*.

1.2 Biology of *Undaria pinnatifida*

The laminarian kelp *Undaria pinnatifida* (Laminariales, Phaeophyta) has a biphasic life cycle, the sporophyte (diploid) stage which is macroscopic and is visible to the naked eye and its gametophyte (haploid) stage which is microscopic in size (Saito, 1975). Although the durability of its sporophytes stage is approximately six months, the gametophyte stage is able to remain viable for more than 24 months (Stuart, 2003).

In the sporophyte state colour can vary from yellowish to dark brown and the size can range up to two metres in length. In its mature state and it can be up to three metres (Lobban & Harrison, 1996). Mature sporophytes of *U. pinnatifida* have holdfasts which act as anchorage for the sporophytes and give rise to the stipe (Lobban & Harrison, 1996). Hay (1990) further described *U. pinnatifida* structure as follows, a strap-like midrib (1-3 cm wide), which runs the full length of the thallus with edges of the midrib expanded as a thin, membranous, pinnatifid blade with pinnae (50-80 cm long).

When *U. pinnatifida* reaches its mature state, the sporophylls develop on bilateral sides of the stipe (Hay, 1990; Gibbs & Hay, 1998). Reproduction occurs by the annual release of asexual zoospores by the mature sporophyll (Parsons, 1994; Oh & Koh, 1996). Millions of haploid zoospores drift with the seawater until they reach a suitable site for attachment (Oh & Koh, 1996). Attached zoospores germinate into microscopic male and female gametophytes (Stuart, 2003). These gametophytes are able to remain viable for up to three years in their dormant state before they germinate (Ohno & Matsuoka, 1993). Male gametophytes release mobile sperm into the surrounding water while female gametophytes produce eggs which remain on the gametophyte (Saito, 1975). Mobile sperm fertilises the egg, which begins to form a germling which develops into new sporophytes (Saito, 1975).

U. pinnatifida is an annual seaweed (Saito, 1975; Hay, 1990) . In late summer and early autumn mature seaweeds degenerate and new sporophyte become established (Hay &

Villouta, 1993). In Japan, sporophytes of *U. pinnatifida* are completely dieback during autumn when water temperatures drop below 20°C (Saito, 1975; Ohno & Matsuoka, 1993). However some New Zealand populations, for example in the Wellington harbour, exhibit overlapping generations and sporophytes can be found year-round. (Hay & Villouta, 1993). This phenomenon might be attributed to the narrower range of annual sea temperature of the New Zealand water when compared to those in Japan and Korean (Hay & Villouta, 1993).

1.3 Economic values and applications of seaweed

The aquaculture industry produced 15.8 million tonnes of aquatic plants in 2008, which has an estimated value of US$ 7.4 billion. The industry has enjoyed a consistent production growth rate of 7.7% annually (FAO, 2010). The production of aquatic plants was dominated by the production of seaweeds, 99.6 % by quantity and 99.3 % by value in 2008 (FAO, 2010).

East and Southeast Asian countries dominate seaweed culture, 99.8 % by quantity and 99.5 % by value in 2008, with almost all the seaweed species in these areas cultured for human consumption. (FAO, 2010). In 2008, China produced 62.8% of the world's aquaculture production of seaweeds by quantity followed by Indonesia (13.7 %), the Philippines (10.6 %), the Republic of Korea (5.9 %), Japan (2.9 %) and the Democratic People's Republic of Korea (2.8 %) (FAO, 2010). However Japan is the second-most important aquatic plant producing country in terms of value (US$ 1.1 billion), because of to its high-priced Nori production (FAO, 2008, 2010). Other use of seaweed include *Eucheuma* seaweed which is used as the major species for carrageenan extraction and Japanese kelp which is used as a raw material for the extraction of iodine and alginate (Barsanti & Gualtieri, 2006.). Chile was the most important seaweed culturing country outside Asia, producing 21,700 tonnes in 2008 while 14,700 tonnes produced in Africa (FAO, 2010).

The highest production of cultured seaweed in 2008 was of Japanese kelp (*Laminaria japonica*, 4.8 million tonnes), followed by *Eucheuma* seaweeds (*Kappaphycus alvarezii* and *Eucheuma* spp., 3.8 million tonnes), Wakame (*Undaria pinnatifida*, 1.8 million tonnes), *Gracilaria* spp. (1.4 million tonnes) and Nori (*Porphyra* spp., 1.4 million tonnes) (FAO, 2010).

1.4 Economic values and application of *Undaria pinnatifida*

U. pinnatifida has been cultured and collected from natural habitats for centuries. It is one of the main commercially harvested and cultivated species in Asia, and its range has been extended by intentional introductions and translocations for aquaculture from China and to Atlantic France and Mediterranean France however most movement of *U. pinnatifida* has been by unintentional introductions to Europe, USA, Australia, New Zealand, Mexico and Argentina (McHugh, 2003).

Wakame is more popular in the Republic of Korea than in Japan, although the market in Japan had expanded (McHugh, 2003). The current harvest is between 450,000 and 500,000 tonnes in Japan and Korea respectively with China producing a few hundred tonnes (FAO, 2012a). The global production harvest of wild *U. pinnatifida* was 4783 tonnes in 2010 (FAO, 2012b).

Wakame has high total dietary fiber content, higher than Nori or Kombu. Like the other brown seaweeds, the fat content of Wakame is quite low. Air-dried Wakame has similar vitamin content to the wet seaweed and is relatively rich in the vitamin B group, especially niacin (McHugh, 2003; Kolb, Vallorani, Milanovic, & Stocchi, 2004) . Raw Wakame contains substantial amounts of essential trace elements such as manganese, copper, cobalt, iron, nickel and zinc, similar to Kombu and Hijiki (McHugh, 2003). Processed Wakame is a very convenient form, used for various instant foods such as noodles and soups (Murata & Nakazoe, 2001; McHugh, 2003). The most common dried Wakame product is made from blanched and salted Wakame which is washed with freshwater to remove salt, cut into small pieces, dried in a flow-through dryer and passed through sieves to sort the different sized pieces (Watanabe & Nısizawa, 1984; McHugh, 2003). It has a long storage life and has a fresh green colour when rehydrated (Murata & Nakazoe, 2001; McHugh, 2003).

In addition to human consumption as a regular food item, there is growing interest of *U. pinnatifida* in the health food and pharmaceutical markets (Hwang, Gong, & Park, 2011).

U. pinnatifida has also proved to be an very useful source of Fucoidan, a fucose-containing sulfated poly-saccharide found in brown algae and proven to have anticoagulant and antiviral activities (Noda, Amano, Arashima, & Nisizawa, 1990; Lee, Hayashi, Hashimoto, & Nakano, 2004). Antioxidant compounds such as Fucoxanthin, have been extracted from *U. pinnatifida* (Yan, Chuda, Suzuki, & Nagata, 1999). Antiviral activities from *U. pinnatifida* had also been confirmed to inhibit the Herpes simplex virus (Khan & Satam, 2003).

The commercial value of *U. pinnatifida* varies according to the quality, origin of the product and end use (MAF, 2009). Aquaculture New Zealand estimated that *U. pinnatifida* could return between NZ$ 500/tonne as bulk seaweed for use in agricultural products (Aquaculture New Zealand, 2008). Estimates of more than NZ$ 1000/tonne for premium grade food *U. pinnatifida* uses has also been suggested (Aquaculture New Zealand, 2008). Aquaculture New Zealand estimated that in the Marlborough Sounds there is, on average, 5 tonnes of wild *U. pinnatifida* per long-line and note that there are thousands of long-lines in the Marlborough Sounds (Aquaculture New Zealand, 2008).

1.6 Metals in *Undaria pinnatifida*

Given that *Undaria pinnatifida* is regularly consumed by large number of humans and *U. pinnatifida* is now able to be harvested as a commercial product in New Zealand, it is important to examine the nutritional quality of New Zealand *U. pinnatifida.* This thesis focuses on metals components in *U. pinnatifida* as these metals have been shown to have impact on human health (Hunter, Simpson, & Strank, 1980; Almela *et al.*, 2002; Rupérez, 2002; Almela, Jesus Clemente, Velez, & Montoro, 2006; MacArtain, Gill, Brooks, Campbell, & Rowland, 2007; Rose *et al.*, 2007; Besada, Andrade, Schultze, & González, 2009; Hwang, Park, Park, Choi, & Kim, 2010; Smith, Summers, & Wong, 2010). Fifteen metals were chosen in this study

Table 1.Elements targeted in this thesis

Heavy Metals	**Chemical symbols**
Arsenic	**As**
Cadmium	**Cd**
Mercury	**Hg**
Lead	**Pb**
Minerals	
Calcium	**Ca**
Potassium	**K**
Magnesium	**Mg**
Sodium	**Na**
Phosphorus	**P**
Chromium	**Cr**
Copper	**Cu**
Manganese	**Mn**
Nickel	**Ni**
Selenium	**Se**
Zinc	**Zn**

1.7 Effect of metals on human health

Heavy metals are members of a loosely-defined subset of elements that exhibit metallic properties, which include the transition metals, some metalloids, lanthanides, and actinides (Hunter *et al*., 1980).

Common metals are all naturally occurring substances that are often present in the environment at low levels. They can be dangerous to humans if they are exposed in large amounts to these metals by ingestion (drinking or eating) or inhalation (Singh, Gautam, Mishra, & Gupta, 2011). Heavy metals become toxic when they are not metabolised by the body and accumulate in the tissues and organs. Various food poisoning cases, due to heavy metal contamination of the coastal environment had been reported internationally (Phillips & Rainbow, 1992). Different heavy metals have different effects on human health. For example, elements such as cadmium, lead and mercury are more harmful than the other metal compounds (Manahan, 1993). Mercury poisoning was reported in Minimata Bay Japan, in the eastern Shiranui sea in 1953, where fish and shellfish were contaminated with mercury (Phillips & Rainbow, 1992). Mercury poisoning due to aquatic contamination had also been reported from several other parts of the world, including Sweden, Canada and the USA (Phillips & Rainbow, 1992).

Calcium is an important mineral for human bone development (Heaney, 1986; Anonymous, 2005). It plays a minor role in the body, such as some exocytosis, neurotransmitter release, and muscle contraction (Heaney, Saville, & Recker, 1975; WHO, 2004). Compared with other metals, calcium and most calcium compounds have low toxicity. This is expected as it has very high natural abundance in the environment and in organisms (WHO, 2004). Calcium poses few serious environmental problems and acute calcium poisoning is rare, and difficult to achieve unless calcium compounds are administered intravenously (WHO, 2004).

Potassium ions are important in neuron function and in influencing osmotic balance between cells and the interstitial fluid (Whelton *et al.*, 1997; Anonymous, 2005; WHO,

2009). This element also controls muscle contraction and the sending of all nerve impulses through action potentials (Whelton *et al.*, 1997; Anonymous, 2005; WHO, 2009). The primary source of K for the general population is the diet, as K is found in all foods, particularly vegetables and fruits (Holbrook *et al*., 1984). Potassium intoxication by ingestion is rare, because high level potassium is rapidly excreted in healthy kidney and caused vomiting (Wetli & Davis, 1978; Holbrook *et al.*, 1984; WHO, 2009).

Magnesium is essential to all cells of all known living organisms. Mg is used as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes (Schroeder, Nason, & Tipton, 1969; Al-Ghamdi, Cameron, & Suton, 1994). It is important to monitor magnesium levels carefully as this element regulates potassium fluxes and its involvement in the metabolism of calcium in humans (Classen, 1984; WHO, 2004). Over dose of Mg is rare, as excess magnesium in the body can be cleared by healthy kidneys easily (Quarme & Disks, 1986).

Sodium is an essential nutrient that regulates blood volume, blood pressure, osmotic equilibrium and pH. Sodium is the primary electrolyte which regulates the extracellular fluid levels in the body (Fregly, 1984). Na is essential for hydration because this mineral pumps water into the cell (Fregly, 1984). Excessive consumption of Na on a regular basis is often associated with hypertension and edema, further high intakes of sodium could lead to osteoporosis because sodium may increase urinary lost of calcium (Fuchs *et al*., 1987).

The main sources of phosphorus for humans are foods containing protein (Nordin, 1989). Inorganic phosphorus in the form of the phosphate PO_4^{3-} is required for all known forms of life playing a major role in molecules such as DNA and RNA where it is involved in structural construction. Living cells also use P to transport cellular energy in the form of adenosine triphosphate (ATP) (Nordin, 1989). Deficiency of P can lead to symptoms of hypophosphatemia, muscle and neurological dysfunction, and disruption of muscle and blood cells due to lack of ATP (Lotz, Zisnman, & Bartter, 1968; Nordin, 1989). Too

much P could lead to diarrhoea, calcification of organs and soft tissue, and could interfere with the body's ability to use element such as calcium (Spencer, Menczel, Lewin, & Samachson, 1965).

Chromium is often found in rocks, animals, plants, and soil and could be a liquid, solid, or gas. Chromium (VI) compounds are toxins and known human carcinogens, whereas Chromium (III) is an essential nutrient at moderate level (Lim, Sargent, & Kusubov, 1983; Das, Grewal, & Banerjee, 2011). Breathing high levels of Cr can cause irritation to the lining of the nose and breathing problems, such as asthma (Das *et al*., 2011). High chromium intakes may cause renal failure, genotoxicity, and are carcinogenic to human (Stearns, Wise, Patierno, & Wetterhahn, 1995; Loubieres *et al.*, 1999).

Copper in the environment occurs mainly though electroplating industries and sewage effluents (Hickey, 1992; Donohue, 2004). Copper is also a component of a number of metalloenzymes including diamine oxidase and monoamine oxidase (Turnlund, 1998). Copper is widely distributed in foods with organ meats, seafood, nuts and seeds being major contributors (Harris, 1997). Long term exposures of Cu cause cirrhosis of the liver and jaundice (Harris & Gitlin, 1996). Whereas deficiency of Cu in the body could cause symptoms such as weight loss, bone disorders and microcytic hypochromic anaemia (Higuchi, Higashi, Nakamura, & Matsuda, 1988; Singh *et al*., 2011).

Manganese is used principally in the manufacture of iron and steel alloys (Du, 2011). Compounds containing manganese have also been used as an ingredient in various products such as batteries, glass, fertilizers and livestock feeding supplements (Du, 2011). Mn is an essential element for many living organisms, including humans. For example, some enzymes require manganese e.g. manganese superoxide dismutase, and some are activated by the element e.g. kinases, decarboxylases (Finley, Johnson, & Johnson, 1994; Williams-Johnson, 1999). Inadequate intake or overexposure of Mn could lead to neurological impairment (Greger, 1998; Du, 2011; Singh *et al.*, 2011). Manganese deficiency in humans appears to be rare, because many common foods have sufficient amount of Mn (Du, 2011).

Nickel is used mainly in the production of stainless steels, non-ferrous alloys, and super alloys (Fawell, 2005). Other uses of Ni and Ni salts include electroplating and as catalysts. Acute absorption of Nickel can cause effects on kidney function, including tubular and glomerular lesions and it is also a possible carcinogen (Sunderman Jr, Dingle, Hopfer, & Swift, 1988; Fawell, 2005).

Selenium is a trace mineral widely distributed in most rocks and soils (Das *et al*., 2011). Overdose of Se leads to selenosis (Helzlsouer, Jacobs, & Morris, 1985; Das *et al.*, 2011). Deficiency of Se leads to Keshan Disease (Keshan Disease Research Group, 1979). In humans, selenium is a trace element nutrient that functions as cofactor for reduction of antioxidant enzymes, such as glutathione peroxidase and thioredoxin reductase which involves in controlling tissue concentrations of highly reactive oxygen-containing metabolites (Whanger, 1998; Holben & Smith, 1999; WHO, 2004). These metabolites are essential at low concentrations for maintaining cell-mediated immunity against infections but highly toxic if produced in excess (Whanger, 1998; WHO, 2004).

Zinc is an essential component to over three hundred enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients (King & Keen, 1999). Zn also stabilises the molecular structure of cellular components and membranes, and as a result integrity of cells and organs is achieved (King & Keen, 1999; Das *et al.*, 2011). However over absorption of Zn could cause damage in the nervous system (WHO, 2004; Das *et al.*, 2011).

Arsenic can be released in large quantities through volcanic activity, erosion of rocks, forest fires and human activity. Arsenic is odorless and tasteless (Das *et al*., 2011). Inorganic arsenic is a known carcinogen and could cause cancer of the skin, lungs, liver and bladder (Rose *et al*., 2007). Very high levels can possibly result in death (Das *et al*., 2011). Long-term low level exposure can cause a darkening of the skin (Das *et al*., 2011) .

Cadmium is a very toxic metal, which can be found in all soils and rocks, welding, electroplating, fertilizers and pesticides (Singh *et al*., 2011). Cadmium and cadmium compounds are known human carcinogens (Das *et al*., 2011; Singh *et al*., 2011). Ingesting very high levels severely irritate the stomach, leading to vomiting and diarrhea. Long-term exposure of Cd leads to possible kidney disease, lung damage, increase of blood pressure and Ca in bone could also be replaced by cadmium causing brittleness of the bones (Abbe & Riedel, 2000; Das *et al*., 2011; Singh *et al*., 2011).

Mercury combines with other elements to form organic and inorganic mercury compounds. The United States Environmental Protection Agency (EPA) have determined that mercuric chloride and methyl mercury are possible human carcinogens (Das *et al*., 2011). Human exposure to high levels of mercury could permanently damage the brain, kidneys, developing fetuses and nervous system (Das *et al*., 2011). Effects on brain functioning may result in irritability, shyness, tremors, changes in vision or hearing, and memory problems (Das *et al*., 2011; Singh *et al*., 2011).

Lead is a probable human carcinogen (Das *et al*., 2011). Which can affect every organ and system in the body (Singh *et al*., 2011). Exposure to high lead levels could severely damage the brain, kidneys and cause miscarriage in pregnant women (Das *et al*., 2011).

1.8 Heavy metals in the marine environment

Pollutants in the aquatic environment that are not degraded by biological or chemical processes have the ability to accumulate in high concentrations in water and sediments of aquatic habitats (Clark, 1997). Heavy metals are non-degradable pollutants in the aquatic environment and occur both in sediments and water (Clark, 1997).

Natural processes such as gaseous state and aerosols might cause some heavy metals to enter the marine environment (Kennish, 1992). It is also possible metals may reach the sea surface by dry deposition, precipitation, or by gaseous exchange (Kennish, 1997). Hydrothermal activity in deep seawater is another natural source of heavy metals, particularly arsenic and mercury (Kennish, 1992). Heavy metals are normally supplied to the sea by river water or as windborne materials following the weathering of soil in coastal areas (Penny, 1984). Heavy metals could also be transported by river waters sewage and water ways systems to coastal environments followed by accumulation in high concentrations in oceanic environments, where they are presented in particulate and dissolved forms (Kennish, 1997). Rainwater that contacts impervious surfaces such as roofs, roads, and concrete surfaces is referred to as stormwater and acts as a major nonpoint source of heavy metals in estuarine and coastal water (Patin, 1982). Different contaminants or heavy metals from inland areas can be transported directly or indirectly to coastal waters in stormwater.

Coastal pollution poses a potential health risk for humans because people all over the world use coastal organisms as food sources and coastal water for various recreational purposes (Edwards & Edyvane, 2001). However, the most noticeable health risk is associated with consumption of seafood in which organic and inorganic pollutants are often accumulated in the seaweed tissues and marine organisms. (Han & Jeng, 1998).

1.9 Metals in brown seaweed -metal accumulation pathways

Accumulation of metals in seaweeds depends on two main factors, the bioavailability of metals in the surrounding water and the uptake capability of metal by the seaweed (Davis, Volesky, & Mucci, 2003). Cell walls in seaweeds contain polysaccharides and proteins, which play an important role in metal retention. The uptake of metals can occur in two ways. The first is passive uptake, a surface reaction, which metals are absorbed by algal surfaces through electrostatic attraction to negatives sites (Ishak & Hamzah, 2010). This is independent on factors which influence the metabolism such as temperature, light, pH or age of the plant, but it is also influenced by the relative abundance of elements in the surrounding water (Besada *et al*., 2009). With passive uptake metal ions adsorb onto the cell surface within a relatively short span of time, normally within few seconds or minutes (Besada *et al*., 2009; Ishak & Hamzah, 2010). The second way metals can be taken up into seaweeds is a slower active uptake in which metal ions are transported across the cell membrane into the cytoplasm. This form of uptake is more dependent upon metabolic processes (Mehta & Gaur, 2005; Ishak & Hamzah, 2010).

The cellular biology of brown seaweeds plays an important role in the metal accumulation pathway. More specifically, it is the properties of cell wall constituents, such as alginate and fucoidan, which are solely responsible for metal binding and accumulations (Davis *et al*., 2003). Lobban & Harrison (1996) described the structure of alga cell walls in the following manner; the brown algae cell wall is constructed by at least two different layers. The inner layer consists of a microfibrillar skeleton which contributes to the rigidity of the wall. The outer layer is an amorphous embedding matrix. The amorphous matrix is attached to the microfibrillar skeleton layer by hydrogen bonds and does not penetrate the fibers. The inner, rigid fibrillar layer of brown algae is mainly comprised of the uncharged cellulose polymer with β(1-4)-linked unbranched glucan..

The biosorption mechanism of metals is very closely related to the chemistry of the components of the cell wall. The cell wall properties such as electrostatic attraction and

complexation could also influence the absorption of metals. The Brown algal embedding matrix contains predominately alginic acid or alginate, the salt of alginic acid with a smaller amount of sulfated polysaccharide (Fucoidan) (Graham & Wilcox, 2000). Alginic acid or alginate, is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M residue) or α-L- guluronic acid (G residue) residues arranged by covalent bond linked together in different unregular sequences or blocks (Lobban & Harrison, 1996; Graham & Wilcox, 2000). The monomers appears as homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks) (Haug, Larsen, & Smidsrod, 1966). The carboylic acid dissociation constants of M and G had been determined as pKa = 3.38 and pKa = 3.65; respectively, with similar pKa values for the polymers(Haug, 1961). The main function of alginate is to maintain the strength and flexibility of the cell wall in brown algae. Alginates made up to around 20%-40% of the dry weight of brown seaweed (Lobban & Harrison, 1996; Graham & Wilcox, 2000).

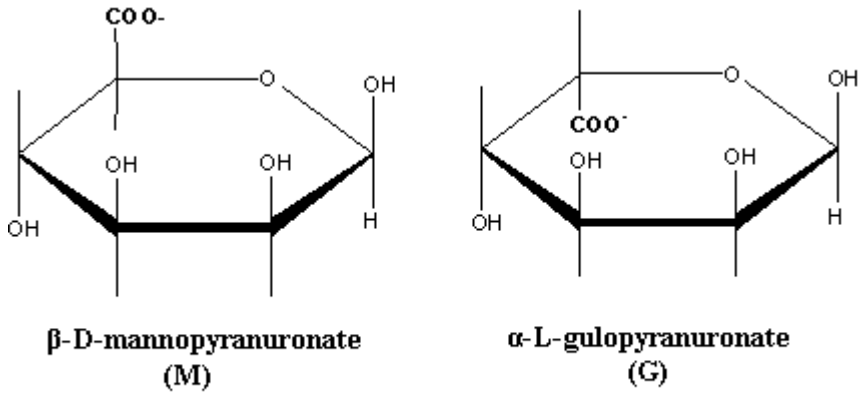

Figure 1. Alginate monomers.

M- and G-block sequences have shown significant structural differences and their proportions in the alginate and contribute to the physical properties and reactivity of the polysaccharide (Figure 3) (Haug, Myklestad, Larsen, & Smidsrod, 1967). Polymannuronic acid has flat ribbon-like chain with molecular repeat of 10.35 Å (Atkins, Mackie, Nieduszynski, Parker, & Smolko, 1973a). It is constructed with two

diequatorially (1e-4e) linked β-D-mannuronic acid residues in the chair conformation (Figure 4) (Atkins *et al.*, 1973a; Graham & Wilcox, 2000) . Whereas, polyguluronic acid contains two diaxially (1a-4a) linked α-L-guluronic acid residues in the chair conformer which creates a rod-like polymer with a molecular repeat of 8.7 Å (Figure 4) (Atkins, Mackie, Nieduszynski, Parker, & Smolko, 1973b; Graham & Wilcox, 2000). This key difference in molecular conformation between the two homopolymeric blocks is believed to be chiefly responsible for the variable affinity of alginates for metals. The polymer conformations of the two different blocks in alginate are different. But this difference also depends on the genus of the algae and from which part of the plant it comes from (Davis *et al*., 2003).

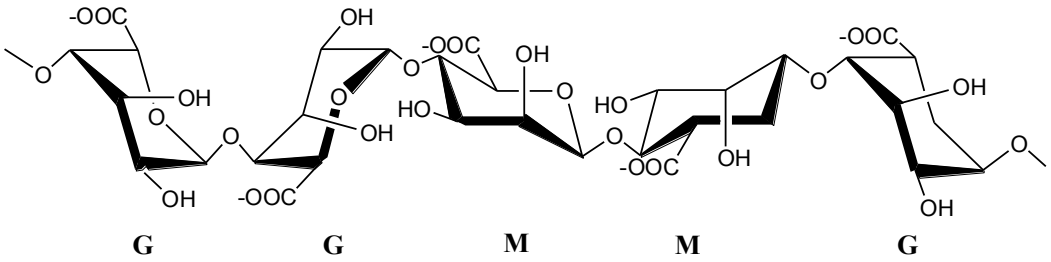

Figure 2. Chain sequences of the alginate polymer.

The variation of the M: G block ratio is depended on species and possible geographical factors, which have not been studied in detail (Graham & Wilcox, 2000). Variation in the affinity of some divalent metals to alginates with different M: G ratios have been demonstrated (Haug, 1961). Haug (1961) showed that the affinity of alginates for divalent cations such as Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+}, etc. increased with the guluronic acid content.

The alginates have an ordered network and adapt an inter-chain dimerization of the polyguluronic sequences in the presence of calcium or other divalent cations of similar size (Lobban & Harrison, 1996). The poly-L-guluronic sections have rod like shapes and alignment of two chains create an array of coordination sites (Lobban & Harrison, 1996; Davis *et al.*, 2003). These cavities are suitable for divalent cations for example Ca^{2+}.

These divalent ions are bound with the carboxylate oxygen and other oxygen atoms of G residues, described as the ‘‘egg-box’’ model (Figure 5) (Lobban & Harrison, 1996; Graham & Wilcox, 2000; Davis *et al.*, 2003). In the end the region of dimerization are terminated by chain sequences of polymannuronic acid residues (Davis *et al*., 2003). As a result, several different chains become interconnected and this contributes to the gel network formation (Davis *et al*., 2003).

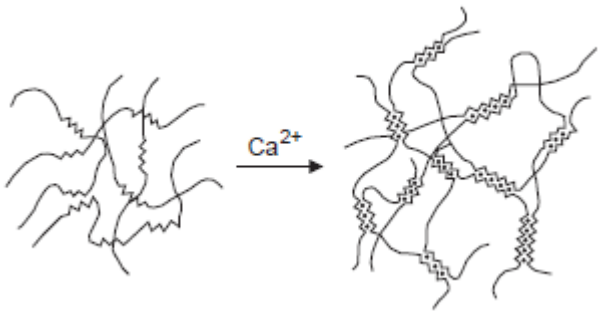

Figure 3. Ca binding in alginate associated with the “Egg box” model (Davis *et al*., 2003).

Brown algae also contain 5 to 20% sulfated polysaccharide fucoidan, about 40% of which is sulfate esters (Davis *et al*., 2003). Fucoidan can be found in the matrix but also within the inner cell wall (Davis *et al*., 2003). Fucoidan is a branched polysaccharide sulfate ester with L-fucose building blocks, which are predominantly $\alpha(1\rightarrow2)$ linked. Trivalent cations mainly bind to sulfated polysaccharides in low pH environments (Davis *et al*., 2003).

The algal cell wall also has many functional groups, such as, hydroxyl (OH), phosphoryl (PO_3O_2), amino (NH_2), and sulphydryl (SH), etc. These functional groups can be found in various cell wall components, e.g., peptidoglycan, teichouronic acid, teichoic acids, polysaccharides and proteins (Davis *et al*., 2003; Mehta & Gaur, 2005). They have the ability to confer a negative charge to the cell surface. In general, metal ions in water are in the form of cations and could be easily absorbed onto the call surface (Graham & Wilcox, 2000; Davis *et al.*, 2003). Each functional group has specific pKa (dissociation

constant), and it dissociates into particular anions and protons at a specific pH conditions (Davis *et al*., 2003).

It is noteworthy that the distribution and abundance of cell wall components vary among different algal groups, as to the number and kinds of functional groups (Lobban & Harrison, 1996). Among different cell wall components, polysaccharides and proteins have most of the metal binding sites (Lobban & Harrison, 1996). When metals are inside the cell, they may bind to cytoplasmic ligands, phytochelatins and metallothioneins, and other intracellular molecules or precipitate (Davis *et al*., 2003). Metal concentration can play a role in controlling biological macromolecules and enzymes as they contain appropriate functional groups or metal co-factors to achieve particular activity (Lobban & Harrison, 1996).

Brown seaweeds cellular structure in relation in metal binding has been studied and resulted in more economic benefits. Biosorption is a term that describes the removal of heavy metals by the passive binding to nonliving biomass from aqueous solution (Mehta & Gaur, 2005; Wang & Chen, 2009 ; Ishak & Hamzah, 2010). Various seaweeds and especially brown algae have been used as a raw material to produce biosorbents for the removal of heavy metals in contaminated areas. For example *U. pinnatifida* and *Sargassum* sp were also proved to be a excellent raw seaweed to be used as biosorbent for heavy metals (Kim, Yoo, & Lee, 1995; Bina, Kermani, Movahedian, & Khazaei, 2006). More recently Kim *et al* (1999) demonstrated that the further introduction of sulphur groups onto the cell surface of *U pinnatifida* increased the bio-sorption capacity of lead ions. The total sulphur content of the cell increased to 13.8% (w:w) through xanthation (Kim, Park, Yoo, & Kwak, 1999). Xanthate groups introduced onto the cell wall of *U pinnatifida* enabled the biomass to adsorb lead ions (Kim *et al*., 1999).

1.10 Study aims

The main aim of this study was to evaluate the concentration of metals (Table 1) in samples of *Undaria pinnatifida* from New Zealand's South Island, (Port Underwood) and North Island, (Wellington) to determine the overall suitability to use *Undaria pinnatifida* to manufacture food products in terms of heavy metal safety. The study also aimed to compare the concentration of metals between the blade and sporophyll tissue and to investigate the possible seasonal variations of metals in the two locations.

Chapter 2 Methodology

2.1 Sample collection

Sampling for this research focused on four different sites. The four sites were believed to be unaffected by pollutions and provided consistent population of *Undaria pinnatifida* and possibility of being developed as commercial farming or harvesting site of such seaweed.

2.1.1 South Island locations

Undaria pinnatifida was collected from two mussel farms from Port Underwood, South Island, New Zealand. The two farms were designated as PE 327 (41° 20 36.89 S, 174° 07 50.17 E) and 106 (41° 19 35.05 S, 174° 08 56.71 E). Sampling of PE 327 was carried out on a monthly basis from April to October 2011. Whereas sampling of 106 was carried on monthly basis from July to October 2011. Every month six mature plants were collected from each farm. The license to harvest the *U. pinnatifida* was issued by MAF Biosecurity New Zealand, Biosecurity Act 1993 Section 52 Permission granted to Wakatu Seafoods.

2.1.2. North Island Locations

The two additional sites were integrated into this study from August to November 2011. They were located on the eastern and western side of Miramar Peninsula in Wellington Harbour, New Zealand. The eastern sampling site was designated as Wellington site A, located in Shelley Bay (41° 17 38.082 S, 174° 49 16.110 E), the western sampling site is designated as Wellington site B, located in Worser Bay (41° 18 46.207 S, 174° 49 49.678 E). Six mature replicate plants were collected from each farm. The license to harvest the *U. pinnatifida* was issued by MAF Biosecurity New Zealand Biosecurity Act 1993 Section 52 Permission granted to Sustainable Seafood NZ Ltd.

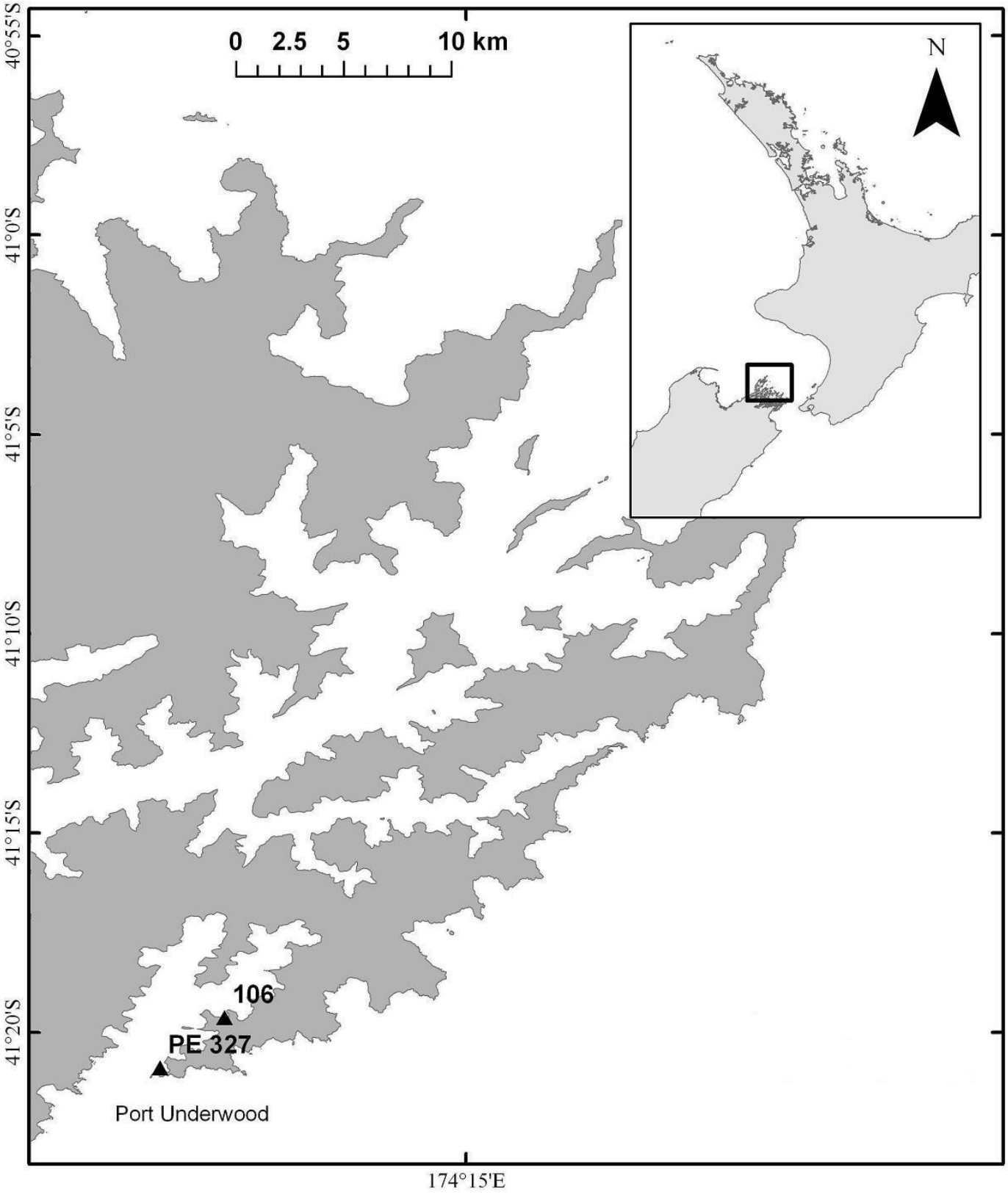

Figure 4. The location of Port Underwood sampling sites.

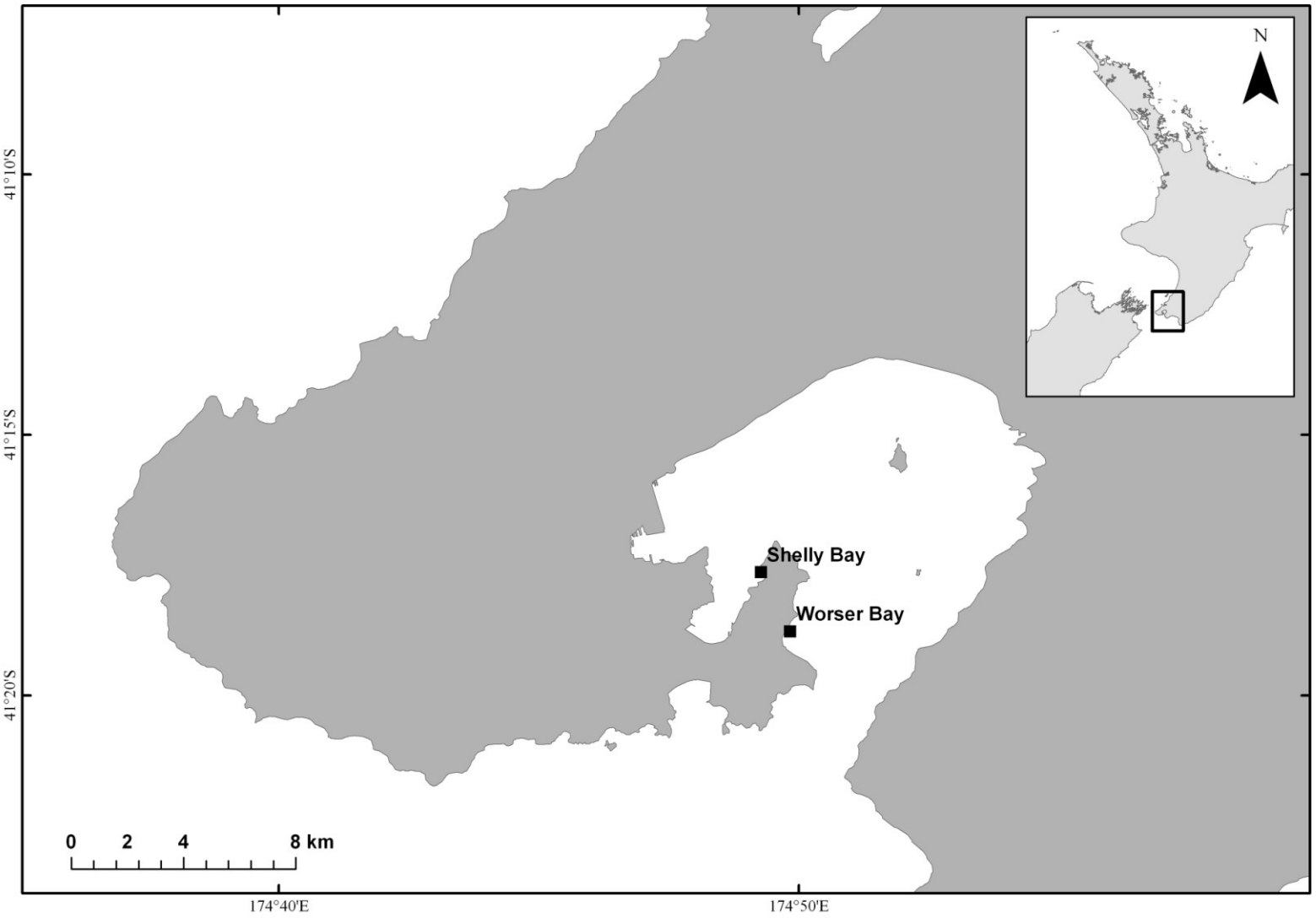

Figure 5. The location of Wellington sampling sites.

2.1.3 Commercial samples

Three bags of three different imported commercial Wakame products were purchased from a supermarket.

Table 2. Information of commercial product samples

Product name	Manufacturer	Package weight	Origins of seaweed, claimed by the label.
Katto Wakame	Daichu Shokuhin Ltd	22g	Japan
Fue Fue Wakame Gureeto	Daichu Shokuhin Ltd	18g	China
Maejima Tabetaro Cut Wakeme	Maejima Shokuhin Co. Ltd	30g	Korea

2.1.4 Seaweed pre-treatment

The seaweed samples collected from PE 327 and 106 were first rinsed with seawater to remove debris and epiphytic organisms from the thallus. The blade was separated from the sporophyll and both placed in separate zip-lock bags in a chilli-bin. They were frozen and flown to the Vitaco Health New Zealand Limited freeze drying plant located in Blockhouse Bay, Auckland, New Zealand. The samples were freeze dried at -18°C to remove all moisture. The samples were then shipped to AUT laboratory, grounded to fine powder by blender and stored in clean polyethyene bottles to await analysis.

The *U. pinnatifida* samples obtained from Wellington harbor were rinsed with fresh water to remove debris and epiphytic organisms from the thallus. The blades were separated from the sporophylls and both placed in separate zip-lock bags and packed in a box and flown to the laboratory at Auckland University of Technology, Auckland, New Zealand. The samples were then briefly washed again with de-ionized water to remove possible

remaining debris. The samples were dried to constant weight at 60°C in a Sanyo MOV-112 laboratory oven. They were then ground to fine powder by blender and stored in clean polystyrene bottles to await analysis.

2.2 Metals analysis

The concentrations of metals in the *U. pinnatifida* samples were determined by a modified method of Denton & Burdon-Jones (1986) and Qari & Siddiqui (2010). Briefly, the dried, ground samples were digested in acid, filtered, diluted and measured on an inductively coupled plasma atomic emission spectroscopy (ICP-AES) machine (Figure 6).

Figure 6. Varian Liberty ICP AX Sequential Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

2.2.1 Acid digestion

Acid digestion has been widely used in elemental analysis in many organic samples such as plant, food, and animal tissues. Acid digestion can be described as mechanical sample preparation to completely transfer the analytes into solution so they can be introduced into the determination step, e.g. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), Inductively coupled plasma mass spectrometry (ICP-MS) or

Atomic absorption spectroscopy (AAS) (Worsfold, Townshend, & Poole, 2005). The goal of every digestion process is therefore the complete solution of the analytes and the complete decomposition of the solid or matrix while avoiding loss or contamination of the analyte (Worsfold *et al*., 2005).

Microwave assisted digestion with a Teflon reactor and nitric acid (HNO_3) are the most common method used in metal analysis of seaweeds (Villares, Puente, & Carballeira, 2001; Mohamed & Khaled, 2005; Al-Shwaf & Rushdi, 2008; Cofrades *et al.*, 2010; Domínguez-González *et al.*, 2010; Hwang *et al.*, 2010). These methods speed up and achieve the digestion more effectively (Balcerzak, 2002). The drawbacks of this method are the slow cool down time needed and relatively high operational cost.

Acid digestion using HNO_3 followed by additional perchloric acid ($HClO_4$) has been used in metal analysis (McQuaker, Brown, & Kluckner, 1979; Shaibur, Shamim, Huq, & Kawai, 2010). Including metal analysis of seaweeds (Denton & Burdon-Jones, 1986 ; Qari & Siddiqui, 2010). $HClO_4$ prevents excessive frothing which occurs when HNO_3 alone was used (Shaibur *et al*., 2010). It also acts as a helper to complete the digestion of the materials (Namieśnik, Chrzanowski, & Szpinek, 2003).

As reviewed above, recent research related to metal concentrations in seaweed applied pressurize and microwave assisted acid digestion methods involving Nitric Acid. This method, in conjunction with ICP AES was reported as early as 1979 (McQuaker., *et al*). Therefore, for both financial and technical reasons acid digestion with HNO_3 and $HClO_4$ was chosen as the digestion method in this study.

Acid digestions were carried out by adding an 0.5 g of sample to 10 mL of concentrated Laboratory Analytical Grade 70% HNO_3 in acid digestion block (VELP Scientifica DK20 heating digester – Figure 7). The reaction mixture was heated at 90 °C for 30 minutes and then 110°C for 2 hours. 5 mL of 80% $HClO_4$ was then added and heating discontinued when dense white fumes appeared. After cooling, the mixture was filtered through

Whatman number 42 filter paper. The resulting solution was finally made up to 50 mL with deionized water in a volumetric flask.

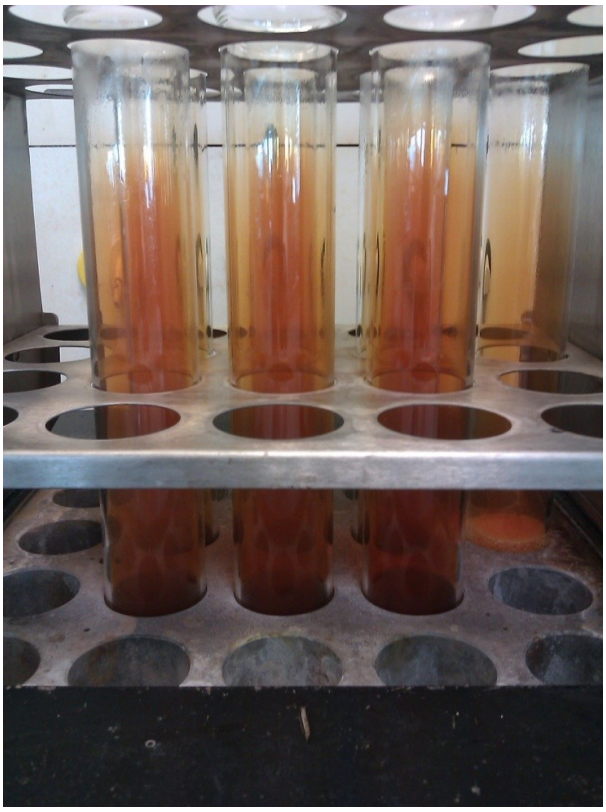

Figure 7. Acid Digestion on VELP Scientifica DK20 heating digester, the brown fumes indicated the formation of NO_2 as pulverized samples were being digested by HNO_3.

2.2.2 Advantage of Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

An ICP-AES was chosen for the determination of metals as ICP-AES is capable of analysing multiple elements simultaneously and is more sensitive to some elements than atomic absorption spectroscopy (AAS). ICP-AES is able to handle both simple and complex sample matrices with high productivity. ICP-AES has the ability to detect most of the elements in the periodic table, which makes it an ideal tool in metal detections. There are four major advantages of ICP AES over AAS.

1. ICP AES has a wide working range, usually from 0.1 to 1000 μg mL^{-1}. Whereas

AAS is ranged from 1 to 10 μg mL^{-1} (Mendham, Denney, Barnes, & Thomas, 2000).

2. ICP AES is able to perform simultaneous multi element analyses and rapid sequential analyses (Mendham *et al*., 2000).
3. ICP AES has precision over AAS by using an internal standard, usually 0.1-1% relative standard deviation (RSD). With flame AAS the precision is usually 1-2% RSD and with furnace AAS it is 1-3% RSD (Mendham *et al*., 2000).
4. Quick measurement of samples can be achieved with ablation and other vaporization methods (Mendham *et al*., 2000).

2.2.3 Chemistry of Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

The Inductively coupled plasma atomic emission spectroscopy (ICP-AES) consists of two main parts, the ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes (Manning & Grow, 1997). The coil of the radio frequency (RF) generator surrounds part of this quartz torch. When the torch is in operation, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil (Manning & Grow, 1997). This RF signal is created by the RF generator. Pure inert argon gas is then used to ignite the plasma (Manning & Grow, 1997).

The argon gas ionizes in the intense electromagnetic field. The ionized argon gas flows in a rotationally symmetrical pattern towards the magnetic field of the RF coil. Eventually high temperature plasma of about 7000 K is generated due to the collisions created between the neutral argon atoms and the charged particles (Manning & Grow, 1997; Thomas, 2001). The peristaltic pump is designed to deliver an aqueous sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame where an immediate collision between the sample and the plasma occurs (Manning & Grow, 1997). The plasma thermally excites the outer-shell electrons of the elements in the sample (Thomas, 2001). This is followed by the relaxation process, in which the

excited electrons are returned to the ground state with the emission of photons of light with an energy characteristic of the element (Figure 8) (Thomas, 2001).

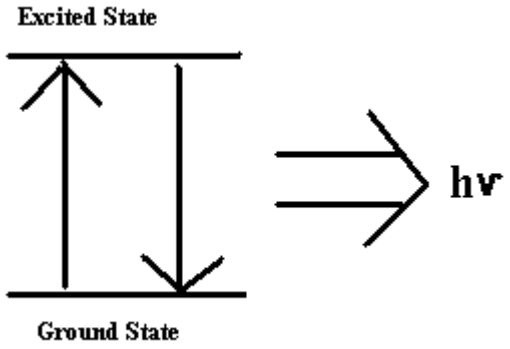

Figure 8. Emission of radiation occurred when electron return to the ground state from excited state.

A spectrum of light wavelengths is emitted simultaneously due to the presence of a mixture of elements in the sample. Therefore the spectrometer is designed to use an optical device called a grating to disperse the light, separating the particular element emissions (Manning & Grow, 1997). The separated emissions are then directed to a dedicated photomultiplier tube detector which detects the specific wavelength for each element line (Manning & Grow, 1997). The intensity of each line is compared with the measured intensities of the standards with known concentrations (Manning & Grow, 1997). The sample elements concentrations are then computed by interpolation along the calibration lines. The more intense this light, the more concentrated the element (Manning & Grow, 1997).

2.2.4 Measurement of metals

Each blade and sporophyll sample from a single plant harvested was subjected to two replicate metals analysis experiments. This allowed comparisons of metal concentration between plants, as well as the identification of possible experimental errors.

The ICP AES was running at Power of 1.2 kW, plasma flow at 15.0 L/min, auxiliary flow at 1.5 L/min, nebulizer Pressure at 200 kPa, replicate time at 1 second, stability time of 15 seconds and PMT Voltage of 650 V. The ICP AES sample introduction settings was set at default, sample uptake of 30 seconds, rinse time of 10 seconds and pump rate at 15 rpm.

Different wavelengths were assigned for the ICE AES for measuring concentration of particular metal; the wavelengths were as followed: As - 193.696 nm, Ca - 396.847 nm, Cd - 228.803 nm, Cr - 267.716 nm, Cu - 224.700 nm, Hg - 253.652 nm, K - 766.490 nm, Mg - 285.213 nm, Mn - 260.569 nm, Na - 330.237 nm, Ni - 231.604 nm, P - 213.618 nm, Pb - 220.353 nm, Se - 196.026 nm and Zn - 206.200 nm. The software applied in controlling the ICP AES was ICP Expert 4.0 on a Windows Me platform system.

2.2.5 Metal element standards

Commercial standards of 1000 ppm of Ca, Cr, Mg, Mn, Na and Se manufactured by BDH Ltd and 1000 ppm of As, K, P, Pb and Ni manufactured by Merck Ltd were used. 1000 ppm standard of Cu was made by dissolving 1.000 g of AR graded copper metal in 3mL of concentrated nitric acid, and then diluted with deionised water to 1 litre in a volumetric flask. 1000 ppm Zn standard was also prepared with the same method, 1 g of AR graded pure Zn metal was dissolved in 3 mL of concentrated nitric acid, and then diluted with deionised water to 1 litre in a volumetric flask. 1000 ppm of Hg standard was made by dissolving 1.3540 g of $HgCl_2$ in 10 mL of HNO_3 followed by dilution to 1 litre in a volumetric flask with deionised water. 1000 ppm of Cd standard was made by dissolving 2.2819 g of $3CdSO_4 \cdot 8H_2O$ in 250 mL of deionised water and diluted to 1 litre in a volumetric flask with deionised water.

The above fifteen 1000 ppm metal standards were used to make multi-elements standards and applied for ICP AES calibrations.

2.2.6 Quality control

To maintain the ICP AES reproducibility and standard of the machine wavelength calibrations and torch cleaning were routinely carried out.

Wavelength calibrations were performed before each month's analysis. The ICP Expert program had programmed to calibrate wavelength with the mercury line at first order with 194.163 nm, 252.652 nm, 365.015 nm, 404.656 nm, 435.833 nm, 546.073 nm, 2^{nd} order with, 365.015 nm, 404.656 nm, 435.833 nm and 3^{rd} order with 312.567 nm.

Due to heavy usage of the ICP AES, the torch was cleaned when deposits were noticed on the surface of the outer cone of the torch. This aimed to prevent torch melt down. To remove other deposits or stains, the torch was soaked in aqua regia (concentrated nitric acid: concentrated hydrochloric acid, 1:3 by volume) overnight. Then rinsed well with water and dried before it was connected back in to the machine. This was followed by torch alignment programmed in the ICP Expert software.

2.3 Pilot studies

Pilot studies were carried out to determine the most timely and economic method with the available resources.

2.3.1 Comparison of fresh and dried of *Undaria pinnatifida* digestion

Fresh tissue sample digestion had been adapted to different metal concentration studies in plant, meat and other organic samples. However a majority of research of metal concentration in seaweed had been carried out with dried samples. This trial was aimed to access the possibility of using fresh samples in this research.

Two replicate seaweed samples were (5g wet weight) were cut out from an individual plant harvested from farm 233 from Pelorus Sound, South Island, New Zealand (41º 09 22.88" S, 173º 51 12.65 E) obtained in May 2011. The two samples were digested in 10 mL of HNO_3 for 120 minutes followed by addition of 5 mL $HClO_4$. The experiment was repeated with the same conditions but with 20 mL HNO_3.

2.3.2 Comparison of sample size

Most previous studies used 1g of dried seaweed sample for metal concentration measurements. In this study, it was important to investigate the optimal amount of samples to be used to obtain stable reproducible results and to take into consideration that the sample size might be unpredictable during the sampling period.

Homogenized samples from farm 327 obtained in April 2011 were used. The testing weights were 0.1 g, 0.25 g, 0.5 g and 1 g. Four sub samples of each trial weight were taken for digestion. The digestion parameters were identical as the previous trial.

2.3.3 Comparison of freeze dried and oven dried samples

While previous studies have shown that there is no difference in metal analyses (Hossain, N. Canha, M. C. Freitas, Santa Regina, & A. Garcia-Sanche, 2011), whether the samples are oven dried or freeze dried, as both methods were used in this study, comparison of these methods on *Undaria pinnatifida* were carried out.

Three plants from Wellington site B, were used in this trial. The samples were briefly washed with de-ionized water to remove possible remaining debris. The two sides of the blade of each plant were separated from the mid stipe. The two half blades of each plant were then subjected to oven dry and freeze dry pretreatment respectively. The oven drying process was carried out at 60°C in a Sanyo MOV-112 laboratory oven for 72 hours and the freeze drying process was carried in a Christ Alpha 2-4 freeze drier at -20°C for 72 hours. The samples were grounded to fine powder by a blender and stored in clean polystyrene bottles to await analysis. Each sample was subject to two metal analysis experiments. The digestion and analysis procedures used were identical to section 2.2.1 and 2.2.2.

2.4 Statistical analysis

All statistical analysis were carried out with Minitab 14 software and graphs were created with Microsoft excel 2007 software.

2.4.1 Statistical analysis for pilot studies

One way ANOVA was used to compare sample size experiments to determine the appropriate sample size to be used in the acid digestion. A Paired T test was used in the comparison of freeze dried versus oven dried experiment to determine differences.

2.4.2 Statistical analysis for main study

A one way ANOVA analysis of farm PE 327 was employed to examine seasonal differences in metal concentrations, as this site contained the most amount and frequent data. Comparisons between site and time employed a two way ANOVA for data from August to October as these three months had contained data from all four locations. All fifteen metals analysis data from PE 327 were also used to perform paired T- tests to determine the difference in metal concentrations between the blade and sporophyll tissue.

Chapter 3 Results

3.1 Pilot studies results

3.1.1 Comparison of fresh versus dried sample digestion

The digestion could not produce an ideal effect to dissolve the sample. The reaction did not appear to be violent as expected with very little brown fume released from the mixtures. Also during the filtering process fragments of tissue believed to be un-dissolved fiber were identified. These phenomena indicated the oxidizing ability of the nitric acid did not take place as expected. This is caused by large water content in the fresh tissue of the seaweed which diluted the acidity of the solution. Fresh *Undaria pinnatifida* has water content of more than 80%.

The experiment with 20 mL HNO_3 caused digestions that were more violent with reasonable amount of brown fume produced. But un-dissolved fibers were also observed in the mixture. Therefore fresh samples were not used in this study due to the amount of HNO_3 needed and the amount of un-dissolved tissues. Dried pulverized samples were used, because dried sample had most of their organic structures destroyed in the drying process and dried powder provided more surface area for the acid to digest the sample.

3.1.2 Comparison of sample size

The one way ANOVA tests showed significant difference for all metals between the four sample sizes. Tukey's comparison of means indicated that there was no difference between 1g and 0.5g of sample in the acid digestion step (Appendix 1). So for the remaining analysis 0.5 g was used.

3.1.3 Comparison of freeze dried versus oven dried samples

Paired T-test of all metals showed no significant difference between the results obtained from digestions of freeze dried and oven dried samples (Appendix 2).

3.2 Spatial and temporal variation of calcium concentrations in *Undaria pinnatifida*

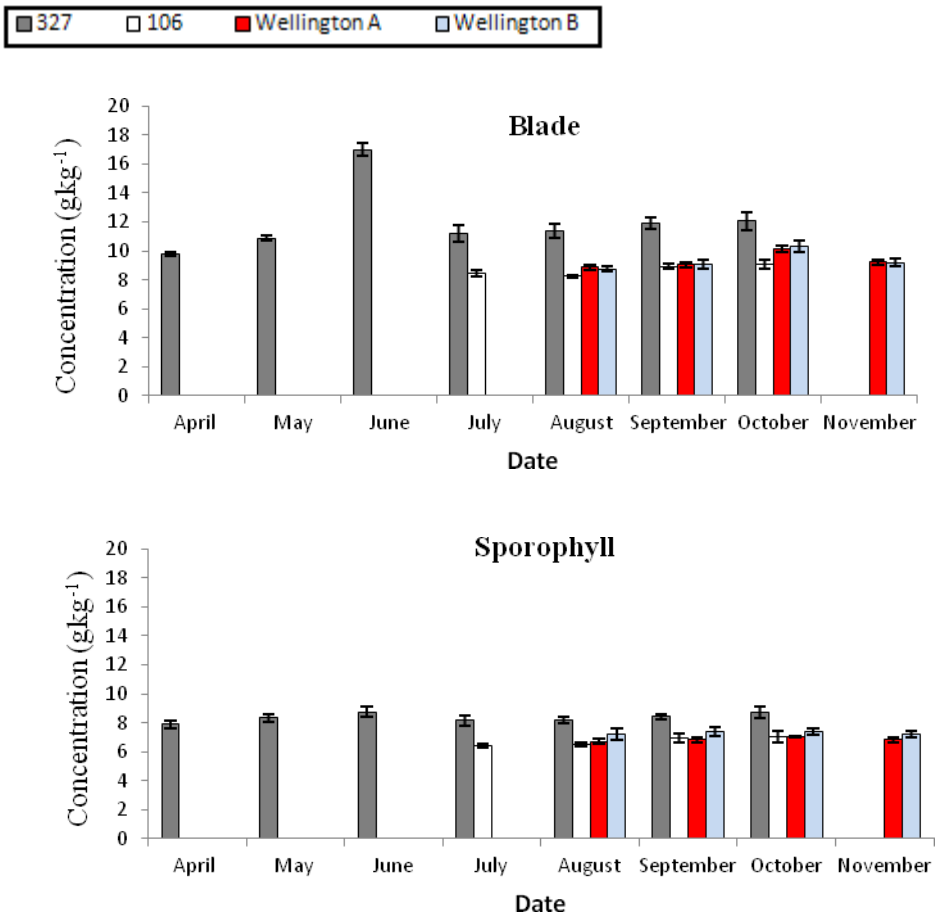

Figure 9. Monthly calcium concentrations in *Undaria pinnatifida*.

There was an increase in the blade tissue content of calcium in 327 between May and June, and then it became relatively stable. In general the blade tissue contents of Ca in 327 were slightly higher than the other three sites. A similar pattern of fluctuations for the sporophyll tissue content of Ca had been observed for all for sites. In farm PE 327 had slightly higher sporophyll tissue content of Ca than the other three sites.

Table 3. Results of One-way ANOVA testing for differences in farm PE327 for calcium concentrations between months for both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	134.690	22.448	62.41	0.000
Error	53	19.063	0.360		
Total	59	153.753			

S = 0.5997 R-Sq = 87.60% R-Sq(adj) = 86.20%

Sporophyll

Source	DF	SS	MS	F	P
time	6	3.679	0.613	4.38	0.001
Error	53	7.425	0.140		
Total	59	11.103			

S = 0.3743 R-Sq = 33.13% R-Sq(adj) = 25.56%

There was a significant difference ($P < 0.001$) between the blade tissue content of calcium in farm PE 327 across the seven months study (Table 3). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade content of Ca in PE 327. However this was not enough to suggest that clear seasonal trend.

There was a significant difference ($P < 0.05$) between the sporophyll tissue content of Ca in farm PE 327 across the seven months study (Table 3). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Ca in PE 327. However this was not enough to suggest a clear seasonal trend.

Table 4. Two way analysis of Variance of calcium in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	193.529	64.5096	295.79	0.000
time	2	28.490	14.2451	65.32	0.000
Interaction	6	6.504	1.0840	4.97	0.000
Error	132	28.788	0.2181		
Total	143	257.311			

S = 0.4670 R-Sq = 88.81% R-Sq(adj) = 87.88%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	59.8962	19.9654	133.22	0.000
time	2	3.5724	1.7862	11.92	0.000
Interaction	6	0.6656	0.1109	0.74	0.618
Error	132	19.7830	0.1499		
Total	143	83.9172			

S = 0.3871 R-Sq = 76.43% R-Sq(adj) = 74.46%

Table 4 shows that there were significant differences between both time and site for the blade tissue content of calcium. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner. A difference pattern was found for the sposophyll where there were significant differences between both site and time but no interaction between the two parameters.

Table 5. Comparison of the blade and sporophyll tissue content of calcium

Paired T for Ca(g/kg) Blade - Ca(g/kg) Sporophyll

	N	Mean	StDev	SE Mean
Ca(g/kg) Blade	60	11.8137	1.6143	0.2084
Ca(g/kg) Sporoph	60	8.3511	0.4338	0.0560
Difference	60	3.46253	1.48283	0.19143

95% CI for mean difference: (3.07948, 3.84559)
T-Test of mean difference = 0 (vs not = 0): T-Value = 18.09 P-Value = 0.000

Table 5 shows that there was significant difference of calcium content between the blade and sporophyll tissue from farm 327. In farm 327, the blade tissue had content of Ca on average between 3.07 and 3.84 g kg^{-1}higher than the sporophyll tissue.

3.3 Spatial and temporal variation of potassium concentrations in *Undaria pinnatifida*

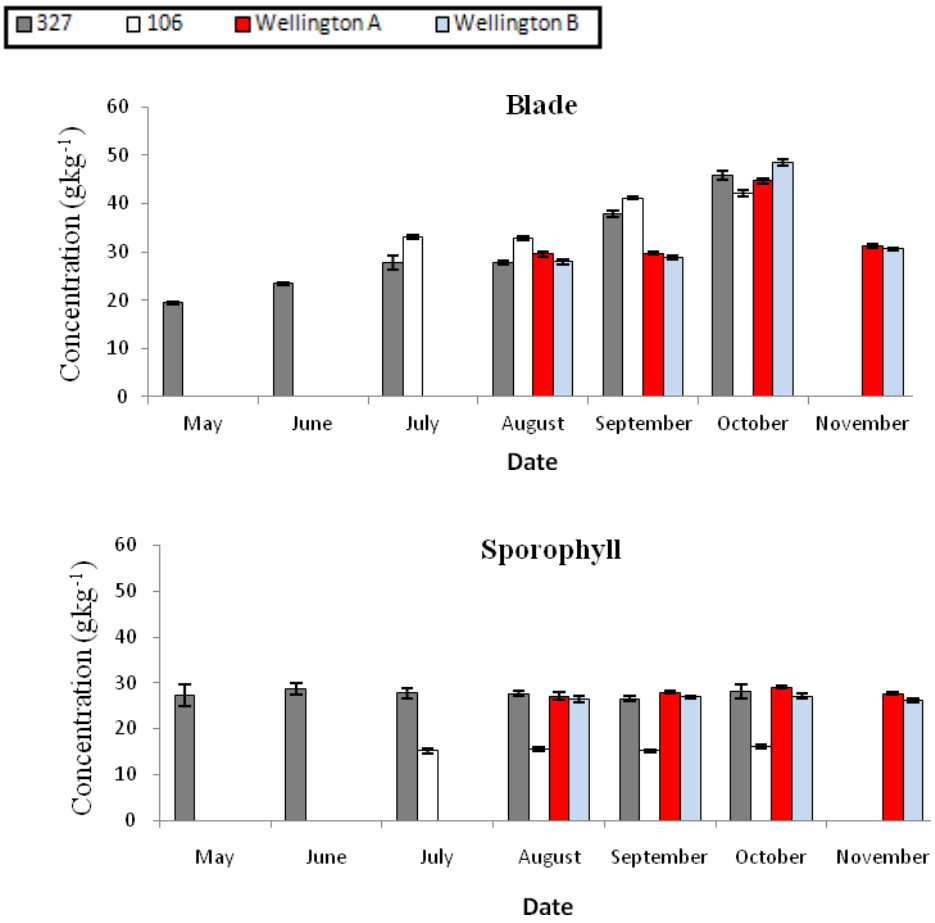

Figure 10. Monthly potassium concentrations in *Undaria pinnatifida*.

There were steady increases of blade content of potassium in PE 327 from April to October, a similar trend also observed along the sampling period in farm 106 and both wellington sites between August and October. A similar pattern of fluctuations of the sporophyll tissue content of K had been observed for all for sites but the sporophyll content of K in 106 had noticeable lower concentration than the other three sites.

Table 6. Results of a One-way ANOVA testing for differences in farm PE327 for potassium concentrations between months in both blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
time	5	4026.94	805.39	503.43	0.000
Error	50	79.99	1.60		
Total	55	4106.93			

S = 1.265 R-Sq = 98.05% R-Sq(adj) = 97.86%

Sporophyll

Source	DF	SS	MS	F	P
time	5	22.465	4.493	5.43	0.000
Error	50	41.404	0.828		
Total	55	63.869			

S = 0.9100 R-Sq = 35.17% R-Sq(adj) = 28.69%

There was significant difference ($P < 0.001$) between the blade tissue content of potassium in farm PE 327 across the seven months study (Table 6). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of K in farm PE 327. This suggested that there was a possible seasonal trend

There was significant difference ($P < 0.001$) between the sporophyll tissue content of K in farm PE 327 across the seven months study (Table 6). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll content of K in farm PE 327. However this evidence was not significant to suggest that there was a clear seasonal trend.

Table 7. Two way analysis of Variance of potassium in the period between August and October for blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
Site id	3	392.28	130.76	210.35	0.000
time	2	6270.62	3135.31	5043.64	0.000
Interaction	6	1393.89	232.31	373.72	0.000
Error	132	82.06	0.62		
Total	143	8138.85			

S = 0.7884 R-Sq = 98.99% R-Sq(adj) = 98.91%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	3802.47	1267.49	2187.61	0.000
time	2	26.52	13.26	22.88	0.000
Interaction	6	18.22	3.04	5.24	0.000
Error	132	76.48	0.58		
Total	143	3923.68			

S = 0.7612 R-Sq = 98.05% R-Sq(adj) = 97.89%

Table 7 shows that there was significant differences for both time and site for the blade tissue content of potassium. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner. A similar pattern was found for sporophyll, where there were signifcant differences for both site and time for the sporophyll content of K. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 8. Comparison of the blade and sporophyll tissue content of potassium

Paired T for K(g/kg) Blade - K(g/kg) Sporophyll

	N	Mean	StDev	SE Mean
K(g/kg) Blade	56	32.9044	8.6413	1.1547
K(g/kg) Sporophy	56	27.5811	1.0776	0.1440
Difference	56	5.32329	8.75369	1.16976

95% CI for mean difference: (2.97903, 7.66754)
T-Test of mean difference = 0 (vs not = 0): T-Value = 4.55 P-Value = 0.000

Table 8 shows that there was significant difference of potassium content between the blade and sporophyll tissue from farm 327. In farm 327, the blade tissue had content of K on average between 2.98 and 7.67 g kg^{-1} higher than the sporophyll tissue.

3.4 Spatial and temporal variation of magnesium concentrations in *Undaria pinnatifida*

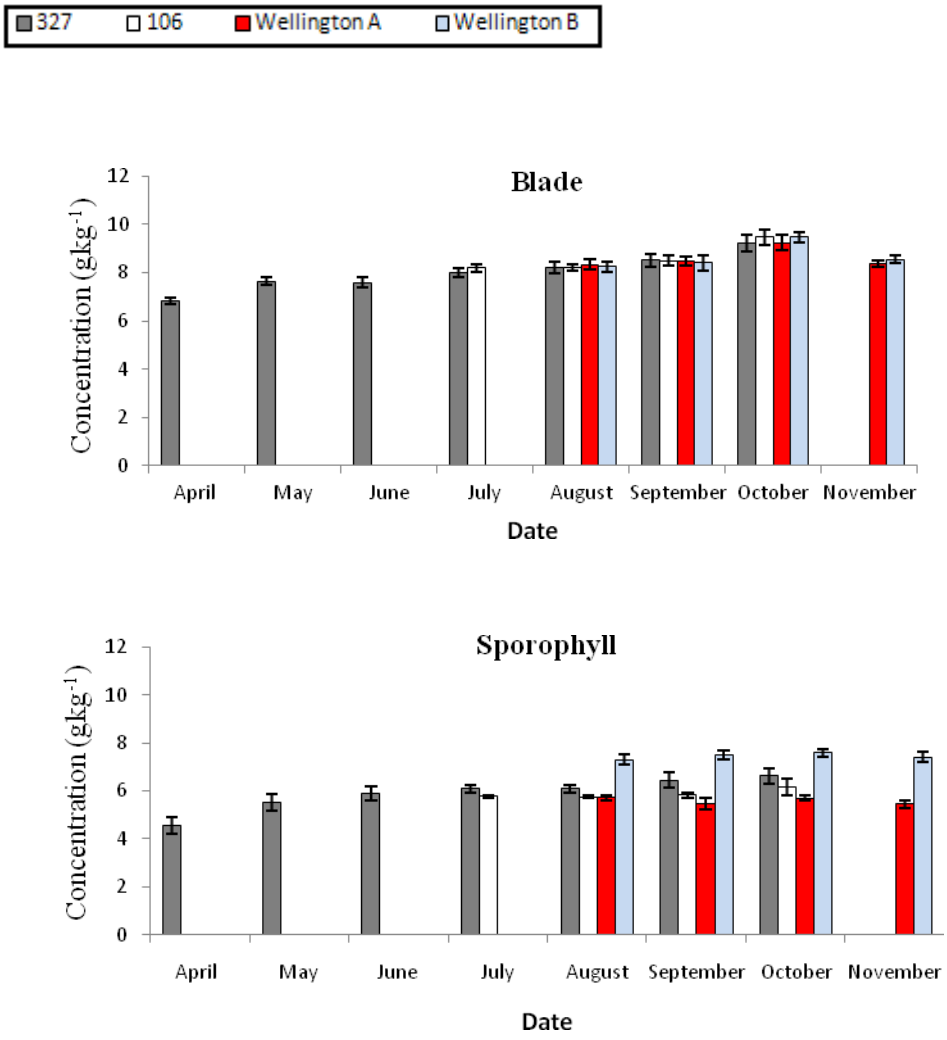

Figure 11. Monthly magnesium concentrations in *Undaria pinnatifida*.

There was an increasing trend for the blade tissue content of magnesium in PE 327 between April and October. The blade tissue contents of Mg in August to October were very similar between the four sites. There was also an increasing trend between April and October for the sporophyll tissue content of Mg in PE 327. The other three sites had shown some fluctuations of sporophyll tissue content of Mg, in which Wellington B site

had slightly higher concentration than the other three sites between August and November.

Table 9. Results of a One-way ANOVA testing for differences in farm PE327 for magnesium concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	23.9224	3.9871	39.94	0.000
Error	53	5.2902	0.0998		
Total	59	29.2125			

S = 0.3159 R-Sq = 81.89% R-Sq(adj) = 79.84%

Sporophyll

Source	DF	SS	MS	F	P
time	6	15.8459	2.6410	31.96	0.000
Error	53	4.3790	0.0826		
Total	59	20.2249			

S = 0.2874 R-Sq = 78.35% R-Sq(adj) = 75.90%

There was a significant difference ($P < 0.001$) between the blade tissue contents of magnesium in farm PE 327 across the seven months this study (Table 9). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Mg in PE 327. This evidences suggested that there was a possible seasonal trend.

There was a significant difference ($P < 0.001$) between the sporophyll tissue content of Mg from farm PE 327 across the seven months this study (Table 9). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Mg in PE 327. However this evidences was not significant to suggest that there was a clear seasonal trend.

Table 10. Two way analysis of Variance for magnesium in the period between August and October for blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
Site id	3	0.1606	0.0535	0.45	0.714
time	2	32.1725	16.0863	136.63	0.000
Interaction	6	0.8020	0.1337	1.14	0.345
Error	132	15.5412	0.1177		
Total	143	48.6764			

S = 0.3431 R-Sq = 68.07% R-Sq(adj) = 65.41%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	69.8328	23.2776	311.27	0.000
time	2	2.4171	1.2086	16.16	0.000
Interaction	6	1.5500	0.2583	3.45	0.003
Error	132	9.8712	0.0748		
Total	143	83.6711			

S = 0.2735 R-Sq = 88.20% R-Sq(adj) = 87.22%

Table 10 shows that there was significant differences between time but not site for the blade tissue content of magnesium. There was also no significant interaction between these two parameters A different pattern was found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Mg. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 11. Comparison of the blade and sporophyll tissue content of magnesium

Paired T for Mg (g/kg) Blade - Mg (g/kg) Sporophyll

	N	Mean	StDev	SE Mean
Mg (g/kg) Blade	60	8.25620	0.70365	0.09084
Mg (g/kg) Sporop	60	6.11930	0.58549	0.07559
Difference	60	2.13690	0.49611	0.06405

95% CI for mean difference: (2.00874, 2.26506)
T-Test of mean difference = 0 (vs not = 0): T-Value = 33.36 P-Value = 0.000

Table 11 shows that there was significant difference of magnesium content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissues had content of Mg on average between 2.00 and 2.26 g kg^{-1}higher than the sporophyll tissue.

3.5 Spatial and temporal variation of sodium concentrations in *Undaria pinnatifida*

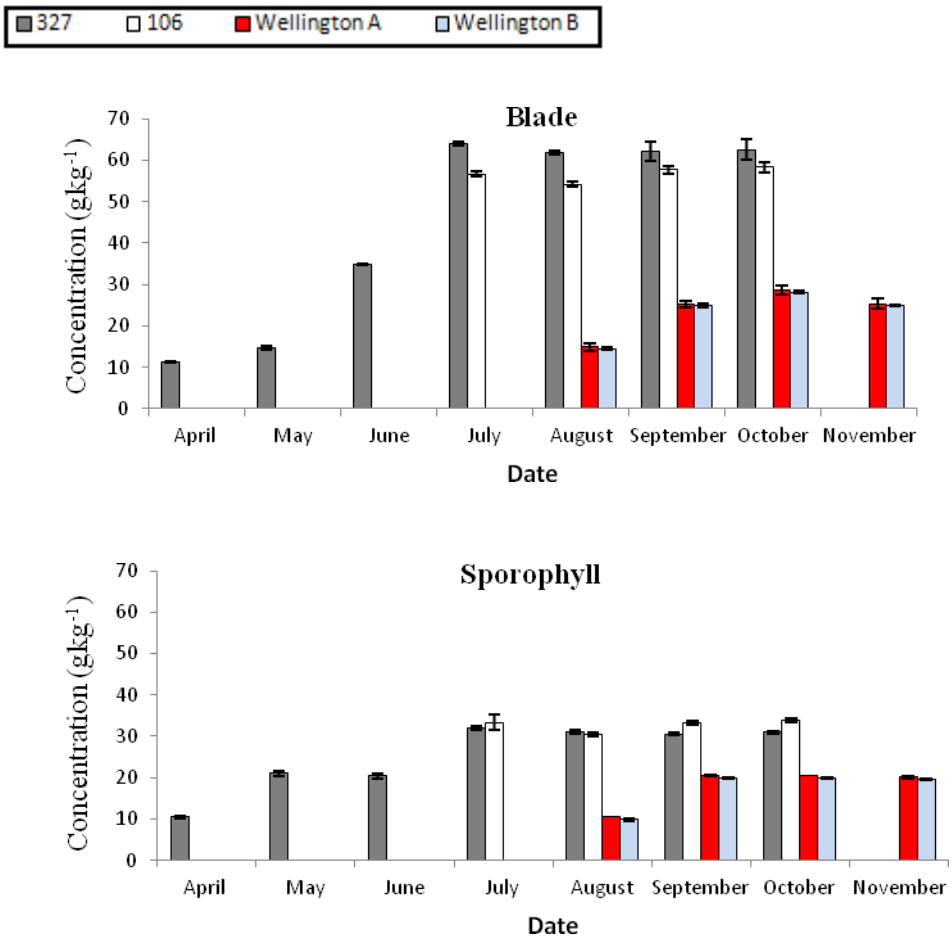

Figure 12. Monthly sodium concentrations in *Undaria pinnatifida*.

There was a steady increase of the blade content of sodium between April and July in PE 327, and followed by small fluctuations near the end of the sampling period. The blade contents of Na from Port Underwood were higher than sites from Wellington. There was also a steady increase of the sporophyll content of Na between April and July from PE 327 and followed by small fluctuations toward end of the sampling period. The sporophyll Na levels from Port Underwood were higher than sites from Wellington.

Table 12. Results of One-way ANOVA testing for differences in farm PE327 for sodium concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	18542.20	3090.37	3090.51	0.000
Error	53	53.00	1.00		
Total	59	18595.19			

S = 1.000 R-Sq = 99.71% R-Sq(adj) = 99.68%

Sporophyll

Source	DF	SS	MS	F	P
time	6	2148.485	358.081	1112.94	0.000
Error	53	17.052	0.322		
Total	59	2165.538			

S = 0.5672 R-Sq = 99.21% R-Sq(adj) = 99.12%

There was significant difference ($P < 0.001$) between the blade tissue content of sodium in farm PE 327 across the seven months study (Table 12). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Na in farm PE 327. This was not enough to suggest a clear seasonal trend.

There was significant difference ($P < 0.001$) between the sporophyll tissue content of Na in farm PE 327 across the seven months study (Table 12). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Na in farm PE 327. This was not enough to suggest a clear seasonal trend.

Table 13. Two way analysis of Variance for sodium in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	40938.6	13646.2	26624.03	0.000
time	2	239.0	119.5	233.18	0.000
Interaction	6	93.8	15.6	30.51	0.000
Error	132	67.7	0.5		
Total	143	41339.1			

S = 0.7159 R-Sq = 99.84% R-Sq(adj) = 99.82%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	4887.96	1629.32	5812.30	0.000
time	2	15.41	7.70	27.48	0.000
Interaction	6	57.33	9.55	34.09	0.000
Error	132	37.00	0.28		
Total	143	4997.70			

S = 0.5295 R-Sq = 99.26% R-Sq(adj) = 99.20%

Table 13 shows that there was significant differences for both time and site for the blade tissue content of sodium. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner. A similar pattern was also found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Na. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 14 shows that there was significant difference of sodium content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Na on average between 22.64 and 28.92 g kg^{-1}higher than the sporophyll tissue.

Table 14. Comparison of blade and sporophyll tissue content of sodium

```
Paired T for Na (g/kg) Blade - Na (g/kg) Sporophyll

                      N     Mean    StDev  SE Mean
Na (g/kg) Blade      60  54.1961  17.7531   2.2919
Na (g/kg) Sporop     60  28.4114   6.0584   0.7821
Difference           60  25.7847  12.1730   1.5715

95% CI for mean difference: (22.6401, 28.9293)
T-Test of mean difference = 0 (vs not = 0): T-Value = 16.41  P-Value = 0.000
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3.6 Spatial and temporal variation of phosphorus concentrations in *Undaria pinnatifida*

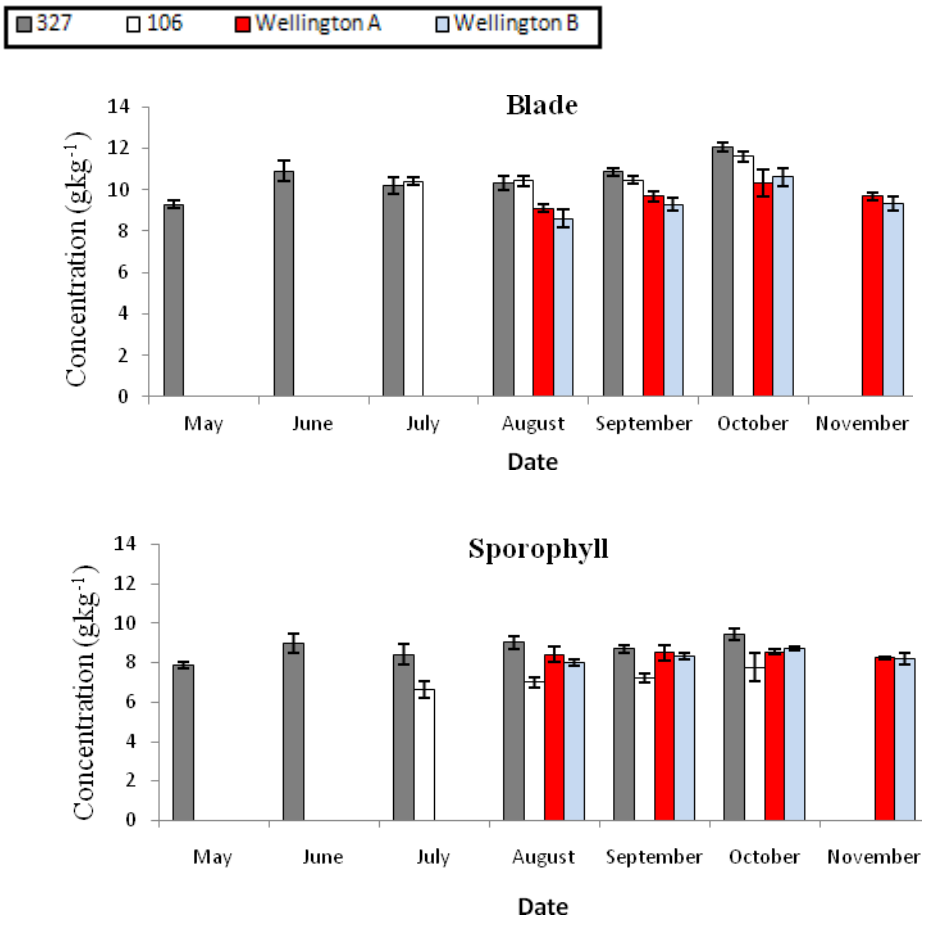

Figure 13. Monthly phosphorus concentrations in *Undaria pinnatifida*.

A pattern of fluctuations without noticeable trend for the blade tissue content of phosphorus had been observed from all four sites. A similar pattern was also observed for the sporophyll tissue content of P.

Table 15. Results of a One-way ANOVA testing for differences in farm PE327 for phosphorus concentrations between months in both blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
time	5	35.558	7.112	34.48	0.000
Error	50	10.313	0.206		
Total	55	45.871			

S = 0.4542 R-Sq = 77.52% R-Sq(adj) = 75.27%

Sporophyll

Source	DF	SS	MS	F	P
time	5	10.602	2.120	12.68	0.000
Error	50	8.360	0.167		
Total	55	18.963			

S = 0.4089 R-Sq = 55.91% R-Sq(adj) = 51.50%

There was significant difference ($P < 0.001$) between the blade tissue content of phosphorus in farm PE 327 across the seven months study (Table 15). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of P in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

There was significant difference ($P < 0.001$) between the sporophyll tissue content of P in farm PE 327 across the seven months study (Table 15). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of P in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 16. Two way analysis of Variance of phosphorus in the period between August and October for blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
Site id	3	68.730	22.9100	97.33	0.000
time	2	59.909	29.9544	127.26	0.000
Interaction	6	4.078	0.6796	2.89	0.011
Error	132	31.070	0.2354		
Total	143	163.787			

S = 0.4852 R-Sq = 81.03% R-Sq(adj) = 79.45%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	55.5153	18.5051	159.52	0.000
time	2	7.1377	3.5688	30.76	0.000
Interaction	6	3.0148	0.5025	4.33	0.001
Error	132	15.3129	0.1160		
Total	143	80.9807			

S = 0.3406 R-Sq = 81.09% R-Sq(adj) = 79.51%

Table 43 shows that there was significant differences for both time and site for the blade tissue content of phosphorus. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner. A similar pattern was found for sporophyll, where there were signifcant differences for both site and time for the sporophyll content of P. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 17. Comparison of the blade and sporophyll tissue content of phosphorus

Paired T for P(g/kg) Blade - P(g/kg) Sporophyll

	N	Mean	StDev	SE Mean
P(g/kg) Blade	56	10.7452	0.9132	0.1220
P(g/kg) Sporophy	56	8.8129	0.5872	0.0785
Difference	56	1.93232	0.75554	0.10096

95% CI for mean difference: (1.72999, 2.13466)
T-Test of mean difference = 0 (vs not = 0): T-Value = 19.14 P-Value = 0.000

Table 17 shows that there was significant difference of phosphorus content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of P on average between 1.72 and 2.13 g kg^{-1}higher than the sporophyll tissue.

3.7 Spatial and temporal variation of chromium concentrations in *Undaria pinnatifida*

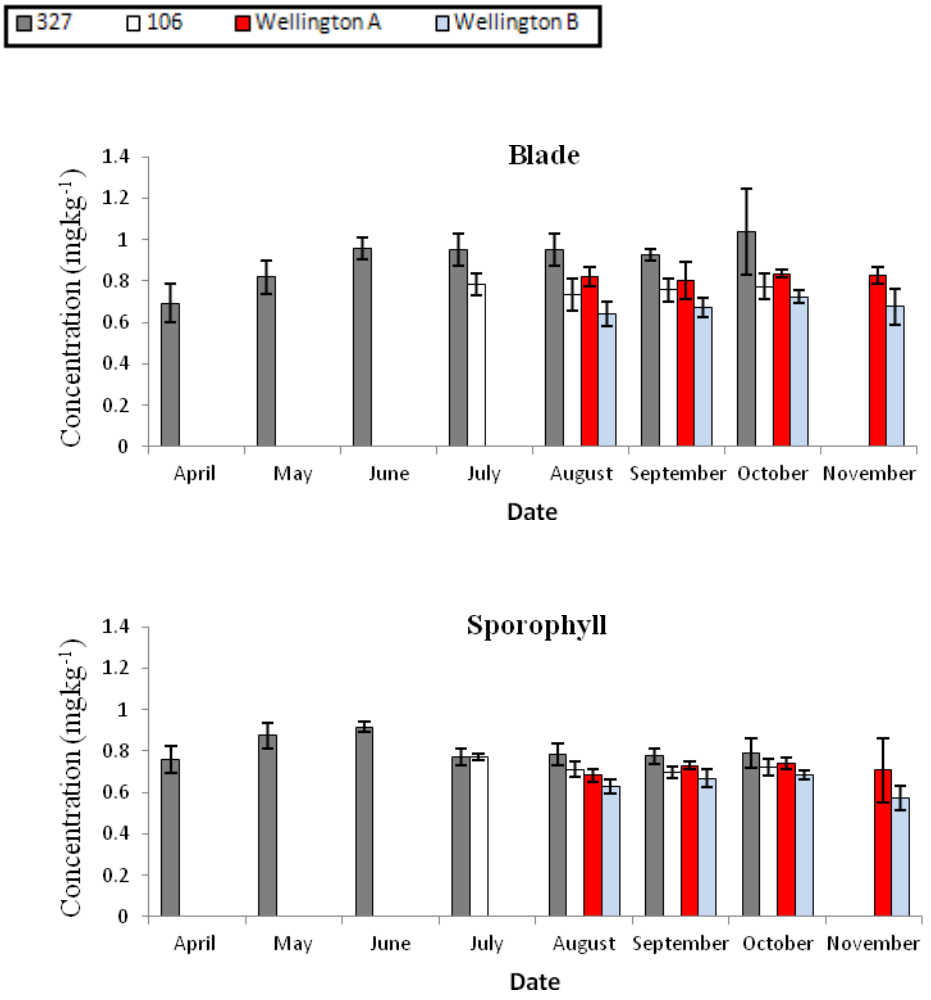

Figure 14. Monthly chromium concentrations in *Undaria pinnatifida*.

There was an increasing trend between April and June for the blade tissue content of chromium in farm PE 327, it became relative stable till September and ended with another small increase in October. The blade tissue content of Cr from the other three sites had shown some fluctuations during their sampling period. There was also an increasing trend between April and June for the sporophyll tissue content of Cr in farm PE 327, it became relative stable to the end of its sampling period. The sporophyll tissue

contents of Cr in the other three sites were steady and had shown some fluctuations during their sampling periods.

Table 18. Results of a One-way ANOVA testing for differences in farm PE327 for chromium concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	0.4246	0.0708	2.96	0.014
Error	53	1.2674	0.0239		
Total	59	1.6921			

S = 0.1546 R-Sq = 25.10% R-Sq(adj) = 16.62%

Sporophyll

Source	DF	SS	MS	F	P
time	6	0.10179	0.01696	5.30	0.000
Error	53	0.16965	0.00320		
Total	59	0.27144			

S = 0.05658 R-Sq = 37.50% R-Sq(adj) = 30.42%

There was a significant difference ($P < 0.05$) between the blade tissue content of chromium in farm PE 327 across the seven months study (Table 18). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Cr in farm PE 327. However this evidence was not significant to suggest that there was a clear seasonal trend.

There was a significant difference ($P < 0.001$) between the sporophyll tissue content of Cr in farm PE 327 across the seven months study (Table 18). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissues content of Cr in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 19. Two way analysis of Variance for chromium in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	1.67301	0.557669	44.69	0.000
time	2	0.09784	0.048918	3.92	0.022
Interaction	6	0.04333	0.007221	0.58	0.747
Error	132	1.64712	0.012478		
Total	143	3.46129			

S = 0.1117 R-Sq = 52.41% R-Sq(adj) = 48.45%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	0.272493	0.0908309	33.16	0.000
time	2	0.024584	0.0122919	4.49	0.013
Interaction	6	0.021059	0.0035098	1.28	0.270
Error	132	0.361551	0.0027390		
Total	143	0.679686			

S = 0.05234 R-Sq = 46.81% R-Sq(adj) = 42.37%

Table 19 shows that there were significant differences between for both site but time for the blade tissue content of chromium but no interaction between the two parameters. A similar pattern was also found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Cr but no interaction between the two parameters.

Table 20 shows that there was significant difference of chromium content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissues had content of Cr on average between 0.098 and 0.188 mg kg^{-1}higher than the sporophyll tissue.

Table 20. Comparison of the blade and sporophyll tissue content of chromium

```
Paired T for Cr Blade - Cr Sporophyll

                N      Mean     StDev   SE Mean
Cr Blade       60  0.938550  0.169348  0.021863
Cr Sporophyll  60  0.795067  0.067828  0.008757
Difference     60  0.143483  0.172580  0.022280


95% CI for mean difference: (0.098901, 0.188066)
T-Test of mean difference = 0 (vs not = 0): T-Value = 6.44  P-Value = 0.000
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3.8 Spatial and temporal variation of copper concentrations in *Undaria pinnatifida*

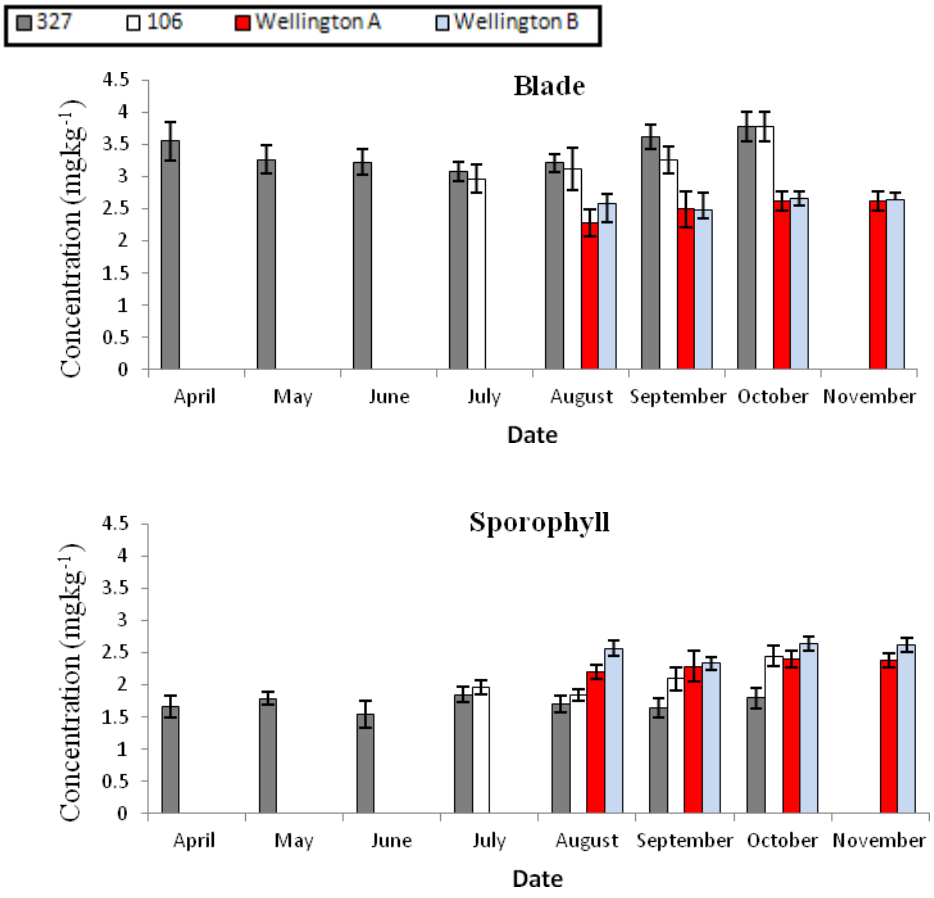

Figure 15. Monthly copper concentrations in *Undaria pinnatifida*.

The blade tissue contents of copper in the sites of Port Underwood were similar and were higher than the two sites from wellington. There was a clear decreasing trend of the blade tissue content of Cu between April and July in farm PE 327, and the trend became positive between July and October. The blade tissue content of Cu in the other three sites had small fluctuations and relative stable during their sampling period. The sporophyll tissue content of Cu from all four sites had shown some fluctuations during their sampling period with no noticeable trend observed.

Table 21. Results of a One-way ANOVA testing for differences in farm PE327 for copper concentrations between months in both blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
time	6	4.2047	0.7008	9.42	0.000
Error	53	3.9408	0.0744		
Total	59	8.1455			

S = 0.2727 R-Sq = 51.62% R-Sq(adj) = 46.14%

Sporophyll

Source	DF	SS	MS	F	P
time	6	0.4961	0.0827	2.61	0.027
Error	53	1.6769	0.0316		
Total	59	2.1730			

S = 0.1779 R-Sq = 22.83% R-Sq(adj) = 14.09%

There was a significant difference ($P < 0.001$) between the blade tissue content of copper in farm PE 327 across the seven months of study (Table 21). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Cu in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

There was a significant difference ($P < 0.05$) between the sporophyll tissue content of Cu in farm PE 327 across the seven months of study (Table 21). The Tukey's comparison of means did not show that there were statistical differences between the monthly means of the sporophyll tissue content of Cu in PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 22. Two way analysis of Variance for copper in the period between August and October for blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
Site id	3	32.3905	10.7968	117.31	0.000
time	2	4.1339	2.0669	22.46	0.000
Interaction	6	1.6708	0.2785	3.03	0.008
Error	132	12.1490	0.0920		
Total	143	50.3441			

S = 0.3034 R-Sq = 75.87% R-Sq(adj) = 73.86%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	12.7306	4.24352	79.77	0.000
time	2	1.4414	0.72069	13.55	0.000
Interaction	6	0.8850	0.14749	2.77	0.014
Error	132	7.0219	0.05320		
Total	143	22.0788			

S = 0.2306 R-Sq = 68.20% R-Sq(adj) = 65.55%

Table 22 shows that there were significant differences between for both site but time for the blade tissue content of copper. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner. A similar pattern was also found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Cu. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 23. Comparison of the blade and sporophyll tissue content of copper

Paired T for Cu Blade - Cu Sporophyll

	N	Mean	StDev	SE Mean
Cu Blade	60	3.40518	0.37156	0.04797
Cu Sporophyll	60	1.73138	0.19191	0.02478
Difference	60	1.67380	0.43096	0.05564

95% CI for mean difference: (1.56247, 1.78513)
T-Test of mean difference = 0 (vs not = 0): T-Value = 30.08 P-Value = 0.000

Table 23 shows that there was significant difference of copper content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Cu on average between 1.56 and 1.78 mg kg^{-1}higher than the sporophyll tissue.

3.9 Spatial and temporal variation of manganese concentrations in *Undaria pinnatifida*

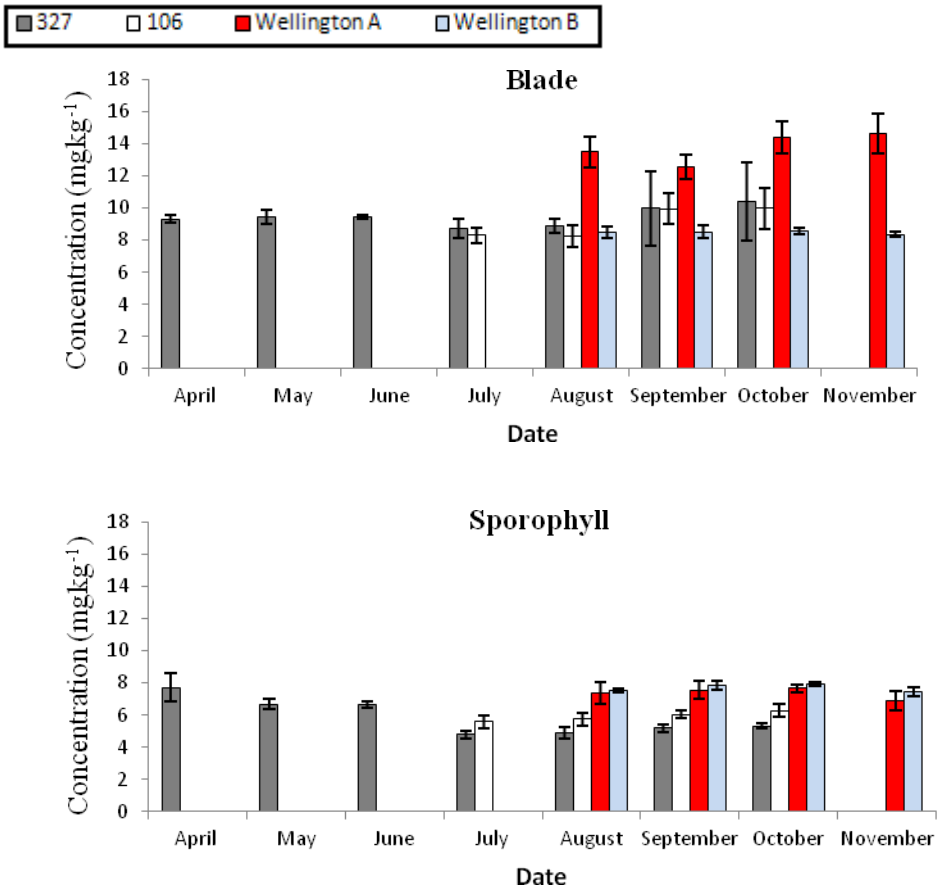

Figure 16. Monthly manganese concentrations in *Undaria pinnatifida.*

There were small fluctuations for the blade tissue content of manganese in all four sites during the sampling period. However the blade tissues in Wellington site A had higher Mn content than the other three sites. All four sites showed fluctuations for the sporophyll

tissue content of Mn. It is noteworthy the sporophyll content of Mn in Wellington sites were higher than the Port Underwood sites.

Table 24. Results of One-way ANOVA testing for differences in farm PE327 for manganese concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	23.99	4.00	0.83	0.555
Error	53	256.54	4.84		
Total	59	280.53			

S = 2.200 R-Sq = 8.55% R-Sq(adj) = 0.00%

Sporophyll

Source	DF	SS	MS	F	P
time	6	42.341	7.057	31.06	0.000
Error	53	12.043	0.227		
Total	59	54.384			

S = 0.4767 R-Sq = 77.86% R-Sq(adj) = 75.35%

There was no difference ($P > 0.10$) between the blade tissue content of manganese in farm PE 327 across the seven months study (Table 24). The Tukey's comparison of means did not show that there were statistical differences between the monthly means of the blade tissue content of Mn in PE 327. This evidence was not significant to suggest that there was a clear seasonal trend.

There was a significant difference ($P < 0.001$) between the sporophyll tissue content of Mn in farm PE 327 across the seven months study (Table 24). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Mn in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 25. Two way analysis of Variance for manganese in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	520.10	173.367	48.17	0.000
time	2	27.21	13.604	3.78	0.025
Interaction	6	30.69	5.115	1.42	0.211
Error	132	475.05	3.599		
Total	143	1053.05			

S = 1.897 R-Sq = 54.89% R-Sq(adj) = 51.13%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	169.195	56.3982	186.75	0.000
time	2	4.298	2.1490	7.12	0.001
Interaction	6	0.168	0.0280	0.09	0.997
Error	132	39.864	0.3020		
Total	143	213.525			

S = 0.5495 R-Sq = 81.33% R-Sq(adj) = 79.77%

Table 25 shows that there was significant differences for both time and site for the blade tissue content of manganese There was no significant interaction between these two parameters A similar pattern was also found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Mn. There was also no significant interaction between these two parameters.

Table 26. Comparison of the blade and sporophyll tissue content of manganese

Paired T for Mn Blade - Mn Sporophyll

	N	Mean	StDev	SE Mean
Mn Blade	60	9.47908	2.18055	0.28151
Mn Sporophyll	60	5.44703	0.96009	0.12395
Difference	60	4.03205	2.31623	0.29902

95% CI for mean difference: (3.43370, 4.63040)
T-Test of mean difference = 0 (vs not = 0): T-Value = 13.48 P-Value = 0.000

Table 26 shows that there was significant difference of manganese content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Mn on average between 2.32 and 4.63 mg kg^{-1}higher than the sporophyll tissue.

3.10 Spatial and temporal variation of nickel concentrations in *Undaria pinnatifida*

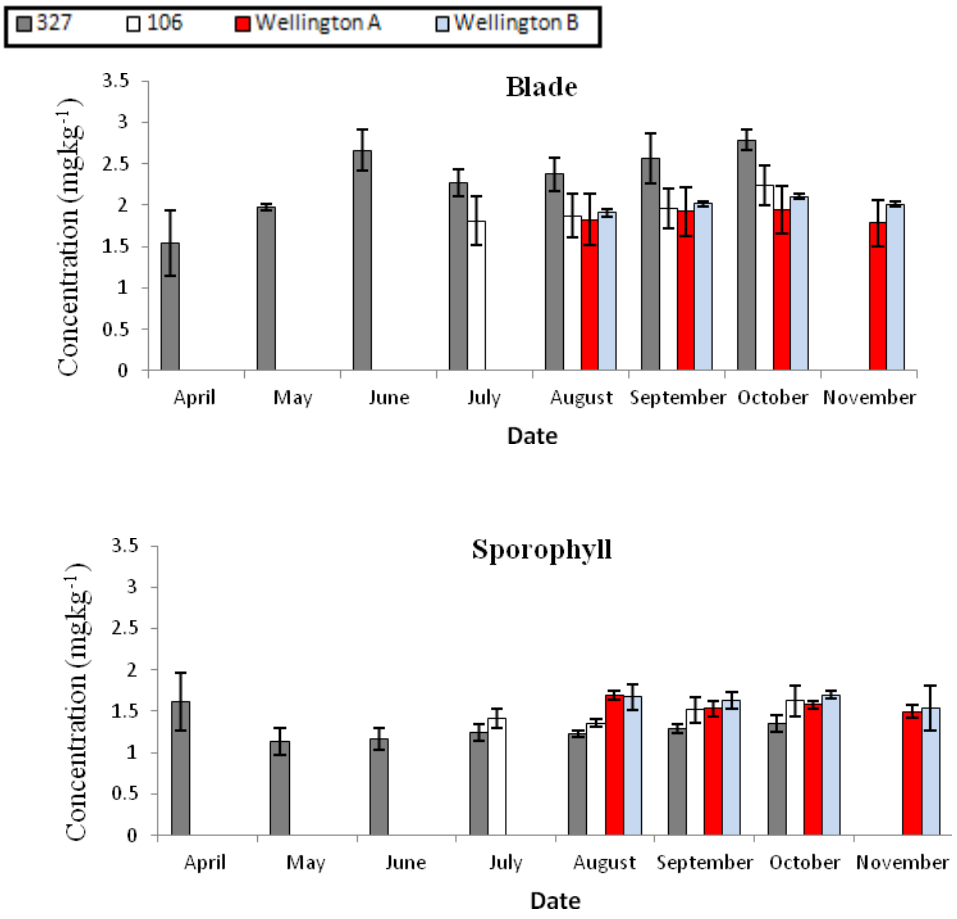

Figure 17. Monthly nickel concentrations in *Undaria pinnatifida*.

In farm PE 327, there was a steady increase of the blade tissue content of nickel between April and June, which was followed by small drop between June and July, and ended with another small increasing trend. The blade tissue contents of Ni in Port Underwood sites were slightly higher than sites from wellington. The sporophyll tissue contents of Ni in all four sites were relatively stable with some fluctuations and no noticeable trend.

Table 27. Results of One-way ANOVA testing for differences in farm PE327 for nickel concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	6.2898	1.0483	14.53	0.000
Error	53	3.8247	0.0722		
Total	59	10.1145			

S = 0.2686 R-Sq = 62.19% R-Sq(adj) = 57.91%

Sporophyll

Source	DF	SS	MS	F	P
time	6	0.6938	0.1156	5.12	0.000
Error	53	1.1963	0.0226		
Total	59	1.8901			

S = 0.1502 R-Sq = 36.71% R-Sq(adj) = 29.54%

There was significant difference ($P < 0.001$) between the blade tissue content of nickel in farm PE 327 across the seven months study (Table 27). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Ni in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

There was significant difference ($P < 0.001$) between the sporophyll tissue content of nickel in farm PE 327 across the seven months study (Table 27). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Ni in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 28. Two way analysis of Variance of nickel in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	10.0308	3.34360	78.92	0.000
time	2	1.7838	0.89188	21.05	0.000
Interaction	6	0.4122	0.06871	1.62	0.146
Error	132	5.5922	0.04237		
Total	143	17.8190			

S = 0.2058 R-Sq = 68.62% R-Sq(adj) = 66.00%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	2.89268	0.964226	34.24	0.000
time	2	0.16655	0.083275	2.96	0.055
Interaction	6	0.54317	0.090528	3.21	0.006
Error	132	3.71739	0.028162		
Total	143	7.31979			

S = 0.1678 R-Sq = 49.21% R-Sq(adj) = 44.98%

Table 28 shows that there was significant differences for both time and site for the blade tissue content of nickel. However there was no significant interaction between these two parameters. A different pattern was also found for sporophyll, where there were signifcant differences between site but not time for the sporophyll content of Ni. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 29. Comparison of the blade and sporophyll tissue content of nickel

Paired T for Ni Blade - Ni Sporophyll

	N	Mean	StDev	SE Mean
Ni Blade	60	2.41158	0.41404	0.05345
Ni Sporophyll	60	1.28592	0.17899	0.02311
Difference	60	1.12567	0.48383	0.06246

95% CI for mean difference: (1.00068, 1.25065)
T-Test of mean difference = 0 (vs not = 0): T-Value = 18.02 P-Value = 0.000

Table 29 shows that there was significant difference of nickel content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Ni on average between 1.00 and 1.25 mg kg^{-1} higher than the sporophyll tissue.

3.11 Spatial and temporal variation of selenium concentrations in *Undaria pinnatifida*

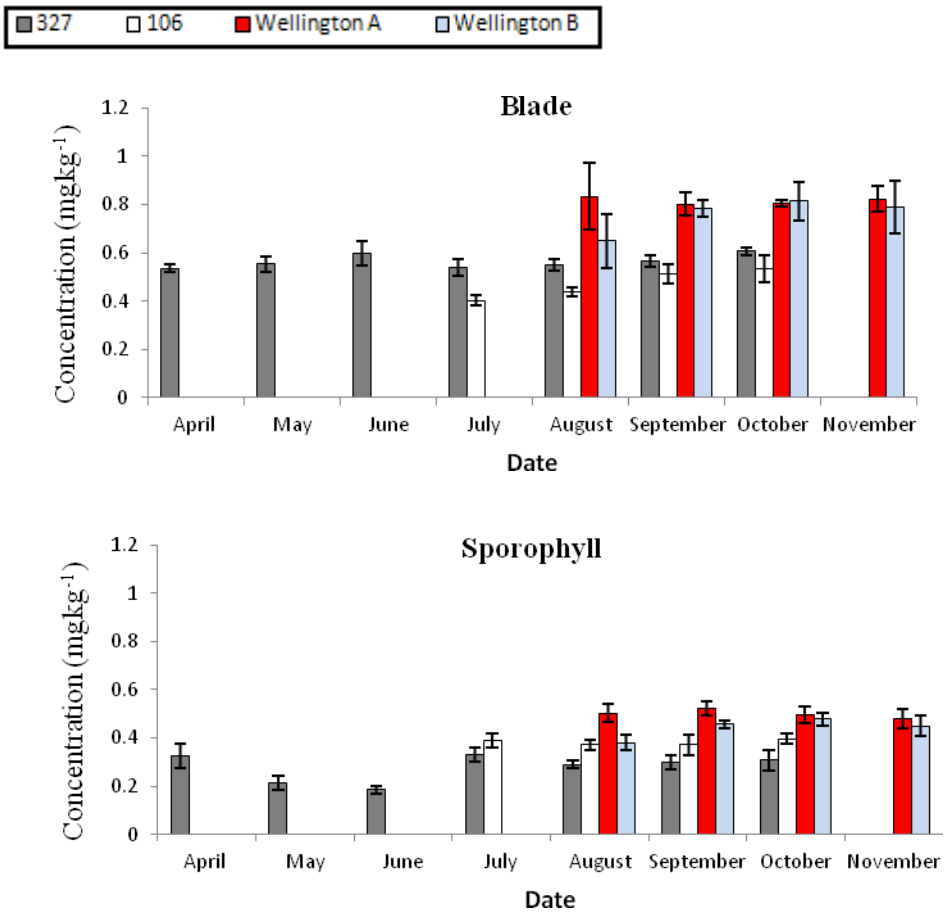

Figure 18 Monthly selenium concentrations in *Undaria pinnatifida.*

The blade tissue contents of selenium in all four sites showed patterns of fluctuation. It is noticeable that blade tissue in Wellington sites had higher Se content than Port Underwood sites. The sporophyll tissue content of Se from all four sites also behaved in a similar manner.

Table 30. Results of One-way ANOVA testing for differences in farm PE327 for selenium concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	0.03809	0.00635	5.12	0.000
Error	53	0.06570	0.00124		
Total	59	0.10379			

S = 0.03521 R-Sq = 36.70% R-Sq(adj) = 29.53%

Sporophyll

Source	DF	SS	MS	F	P
time	6	0.09791	0.01632	8.92	0.000
Error	53	0.09698	0.00183		
Total	59	0.19489			

S = 0.04278 R-Sq = 50.24% R-Sq(adj) = 44.60%

There was significant difference ($P < 0.001$) between the blade tissue content of selenium in farm PE 327 across the seven months study (Table 30). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Se in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

There was significant difference ($P < 0.001$) between the sporophyll tissue content of selenium in farm PE 327 across the seven months study (Table 30). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Se in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 31. Two way analysis of Variance of selenium in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	2.37283	0.790945	116.33	0.000
time	2	0.12754	0.063770	9.38	0.000
Interaction	6	0.13815	0.023026	3.39	0.004
Error	132	0.89749	0.006799		
Total	143	3.53602			

S = 0.08246 R-Sq = 74.62% R-Sq(adj) = 72.50%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	0.84878	0.282928	123.13	0.000
time	2	0.02951	0.014753	6.42	0.002
Interaction	6	0.04462	0.007437	3.24	0.005
Error	132	0.30330	0.002298		
Total	143	1.22622			

S = 0.04793 R-Sq = 75.27% R-Sq(adj) = 73.20%

Table 31 shows that there was significant differences for both time and site for the blade tissue content of selenium. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner. A similsr pattern was also found for sporophyll, where there were signifcant differences for both site and time for the sporophyll content of Se. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 32 Comparison of the blade and sporophyll tissue content of Selenium

Paired T for Se Blade - Se Sporophyll

	N	Mean	StDev	SE Mean
Se Blade	60	0.565167	0.041943	0.005415
Se Sporophyll	60	0.293650	0.057474	0.007420
Difference	60	0.271517	0.077979	0.010067

95% CI for mean difference: (0.251373, 0.291661)
T-Test of mean difference = 0 (vs not = 0): T-Value = 26.97 P-Value = 0.000

Table 32 shows that there was significant difference of selenium content between the blade and the sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Se on average between 0.25 and 0.29 mg kg^{-1}higher than the sporophyll tissue.

3.12 Spatial and temporal variation of zinc concentrations in *Undaria pinnatifida*

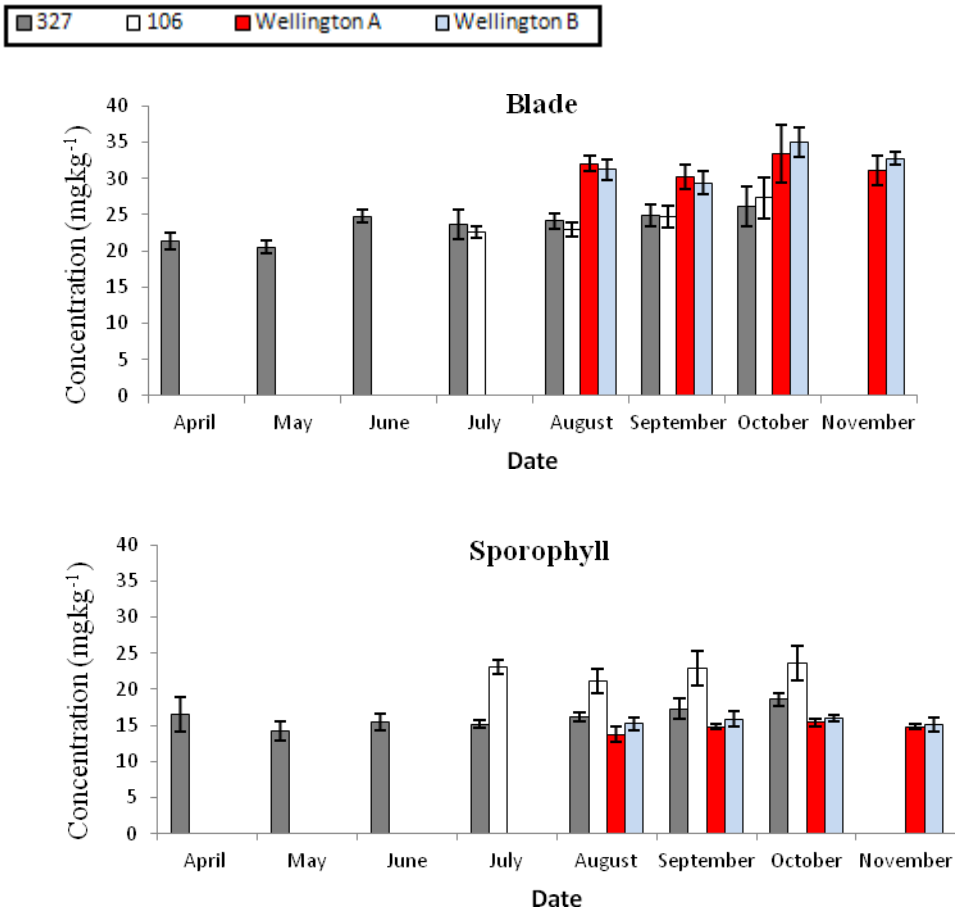

Figure 19. Monthly zinc concentrations in *Undaria pinnatifida*.

The blade tissue contents of zinc in all four sites showed small fluctuations. Wellington sites had slightly higher content of blade tissue Zn than Port Underwood sites. The sporophyll tissue content of Zn in all four sites also showed small flcutuations. It was noticeable that farm 106 had slightly higher sporophyll tissue content of Zn than the other three sites.

Table 33. Results of One-way ANOVA testing for differences in farm PE327 for zinc concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	140.68	23.45	3.99	0.002
Error	53	311.74	5.88		
Total	59	452.42			

S = 2.425 R-Sq = 31.09% R-Sq(adj) = 23.29%

Sporophyll

Source	DF	SS	MS	F	P
time	6	109.17	18.19	8.14	0.000
Error	53	118.42	2.23		
Total	59	227.59			

S = 1.495 R-Sq = 47.97% R-Sq(adj) = 42.08%

There was significant difference ($P < 0.05$) between the blade tissue content of zinc in farm PE 327 across the seven months this study (Table 33). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Zn in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

There was significant difference ($P < 0.001$) between the sporophyll tissue content of zinc in farm PE 327 across the seven months this study (Table 33). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Zn in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 34. Two way analysis of Variance of zinc in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	1713.58	571.193	77.46	0.000
time	2	296.34	148.170	20.09	0.000
Interaction	6	105.56	17.593	2.39	0.032
Error	132	973.41	7.374		
Total	143	3088.89			

S = 2.716 R-Sq = 68.49% R-Sq(adj) = 65.86%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	1323.59	441.196	126.02	0.000
time	2	83.80	41.900	11.97	0.000
Interaction	6	12.96	2.160	0.62	0.716
Error	132	462.14	3.501		
Total	143	1882.49			

S = 1.871 R-Sq = 75.45% R-Sq(adj) = 73.40%

Table 34 shows that there was significant differences for both time and site for the blade tissue content of zinc. There was also a significant interaction between these two parameters indicated that they did not vary in a systematic manner. A different pattern was found for sporophyll, where there were signifcant differences for both site and time for the sporophyll content of Zn. However there was no significant interaction between these two parameters.

Table 35. Comparison of the blade and sporophyll tissue content of zinc

Paired T for Zn Blade - Zn Sporophyll

	N	Mean	StDev	SE Mean
Zn Blade	60	24.1833	2.7691	0.3575
Zn Sporophyll	60	16.5116	1.9640	0.2536
Difference	60	7.67167	2.94057	0.37963

95% CI for mean difference: (6.91204, 8.43130)
T-Test of mean difference = 0 (vs not = 0): T-Value = 20.21 P-Value = 0.000

Table 35 shows that there was significant difference of zinc content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Zn on average between 6.91 and 8.43 mg kg^{-1}higher than the sporophyll sample.

3.13 Spatial and temporal variation of arsenic concentrations in *Undaria pinnatifida*

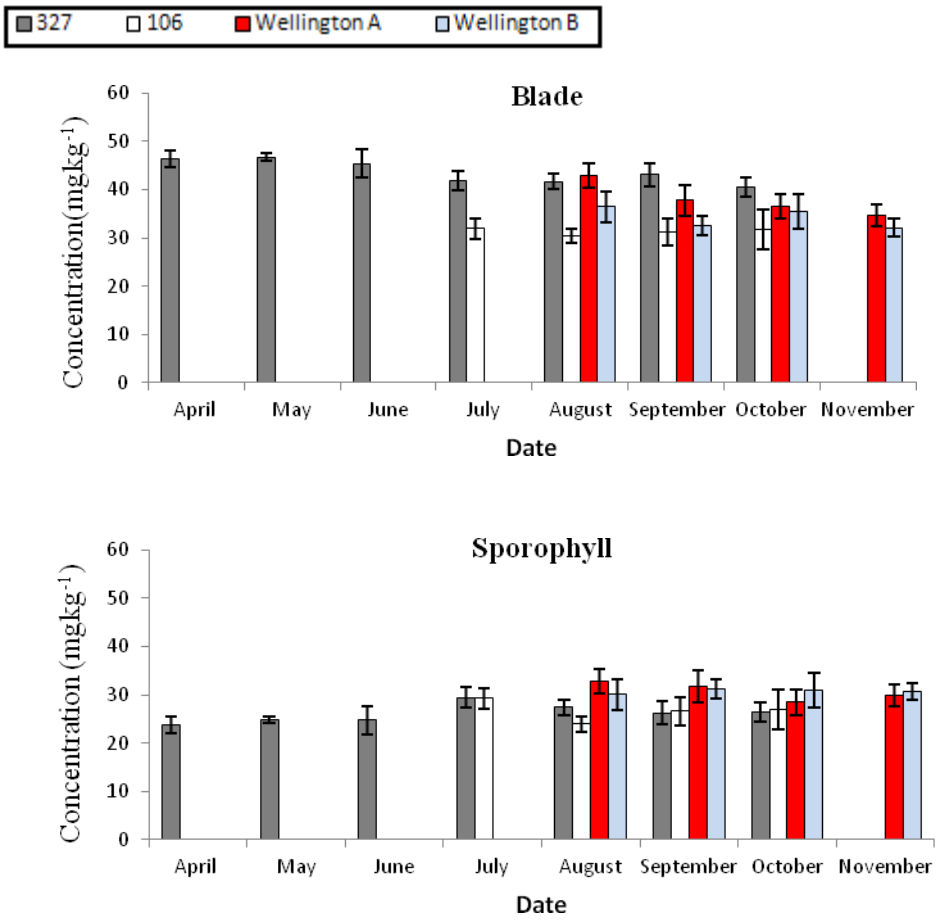

Figure 20. Monthly arsenic concentrations in *Undaria pinnatifida*.

The blade tissue content of arsenic between May to October in farm PE 327 showed a slow decreasing trend and a similar trend also been noticed in Wellington Site A between August and November. The blade tissue contents of As from the other two sites showed small fluctuations and were relative stable during their sampling period. The sporophyll tissue content of arsenic between April and July in farm PE 327 showed a slow increasing trend and small fluctuations had been identified in the other three sites.

Table 36. Results of a One-way ANOVA testing for differences in farm PE327 for arsenic concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	220.97	36.83	5.52	0.000
Error	53	353.68	6.67		
Total	59	574.65			

S = 2.583 R-Sq = 38.45% R-Sq(adj) = 31.49%

Sporophyll

Source	DF	SS	MS	F	P
time	6	162.3	27.1	2.15	0.063
Error	53	667.3	12.6		
Total	59	829.6			

S = 3.548 R-Sq = 19.56% R-Sq(adj) = 10.46%

There was a significant difference ($P < 0.001$) between the blade tissue content of arsenic in farm PE 327 across the seven months study (Table 36). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade content of As from PE 327. This evidence was not significant to suggest that there was a clear seasonal trend.

Table 37. Two way analysis of Variance of arsenic between sites and time in the period between August to October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	2374.77	791.589	58.60	0.000
time	2	95.83	47.917	3.55	0.032
Interaction	6	329.96	54.994	4.07	0.001
Error	132	1783.02	13.508		
Total	143	4583.59			

S = 3.675 R-Sq = 61.10% R-Sq(adj) = 57.86%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	786.27	262.089	15.73	0.000
time	2	15.30	7.648	0.46	0.633
Interaction	6	193.53	32.255	1.94	0.079
Error	132	2198.86	16.658		
Total	143	3193.96			

S = 4.081 R-Sq = 31.16% R-Sq(adj) = 25.42%

There was no significant difference (P > 0.05) between the sporophyll tissue content of As in farm PE 327 across the seven months study (Table 36). This evidence was not significant to suggest that there was a clear seasonal trend.

Table 37 shows that there were significant differences between both time and site for the blade tissue content of arsenic. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner. A difference patter was found for the sposophylll where there was a difference between site but not time.

Table 38. Comparison of the blade and sporophyll tissue content of arsenic

Paired T for As Blade - As Sporophyll

	N	Mean	StDev	SE Mean
As Blade	60	42.6697	3.1209	0.4029
As Sporophyll	60	26.7862	3.7498	0.4841
Difference	60	15.8835	5.3085	0.6853

95% CI for mean difference: (14.5121, 17.2548)
T-Test of mean difference = 0 (vs not = 0): T-Value = 23.18 P-Value = 0.000

Table 38 shows that there was significant difference of arsenic content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissues had content of As on average between 14.51and 17.25 mg kg^{-1} higher than the sporophyll tissue.

3.14 Spatial and temporal variation of cadmium concentrations in *Undaria pinnatifida*

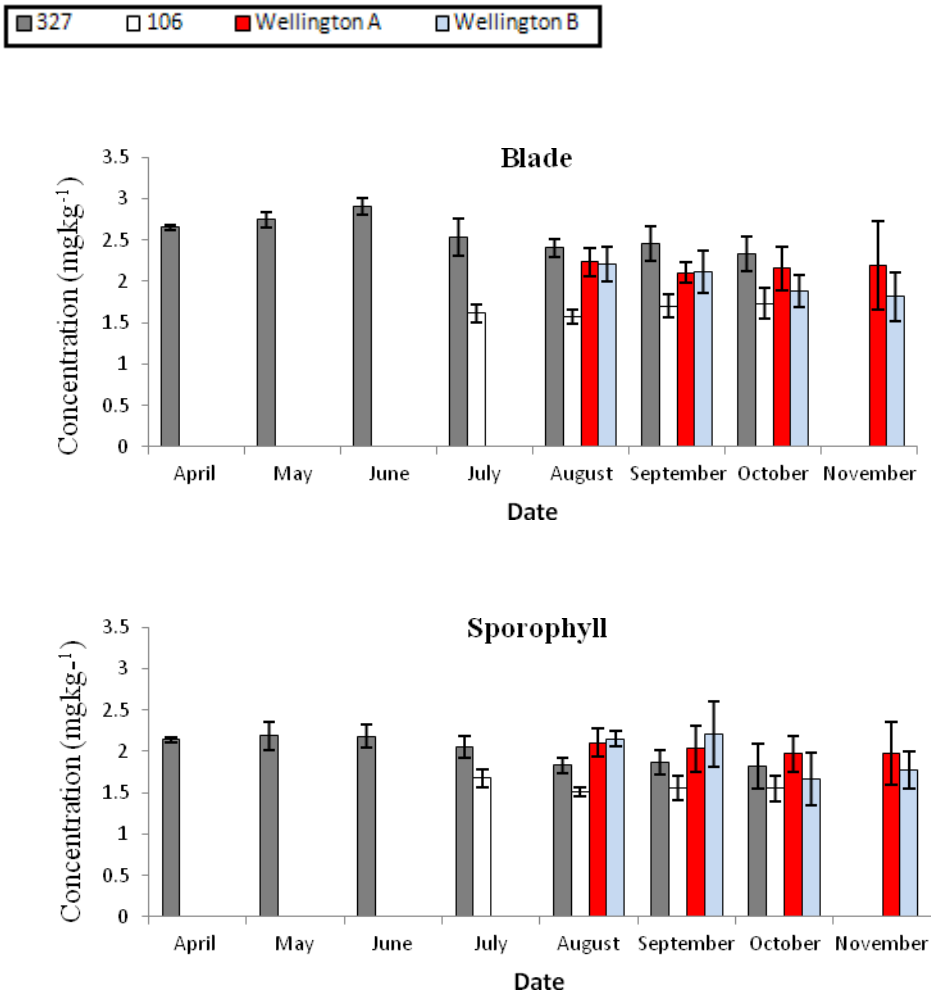

Figure 21. Monthly cadmium concentrations in *Undaria pinnatifida*.

Both the blade and sporophyll tissue contents of cadmium in all four sites showed small fluctuations with no clear trends identified.

Table 39. Results of a One-way ANOVA testing for differences in farm PE327 for cadmium concentrations between months in both blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
time	6	1.4505	0.2418	3.53	0.005
Error	53	3.6268	0.0684		
Total	59	5.0773			

S = 0.2616 R-Sq = 28.57% R-Sq(adj) = 20.48%

Sporophyll

Source	DF	SS	MS	F	P
time	6	1.1803	0.1967	3.59	0.005
Error	53	2.9005	0.0547		
Total	59	4.0808			

S = 0.2339 R-Sq = 28.92% R-Sq(adj) = 20.88%

There was a significant difference ($P < 0.05$) between the blade tissue content of cadmium in farm PE 327 across the seven months study (Table 39). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Cd in farm PE 327. However this evidence was not significant to suggest that there was a clear seasonal trend.

There was a significant difference ($P < 0.05$) between Cd concentrations in sporophyll tissue from farm PE 327 across the seven months study (Table 39). The Tukey's comparison of means did not show that there were statistical differences between the monthly means of the sporophyll tissue content of Cd in farm PE 327. This evidence suggested no clear seasonal trend.

Table 40 shows that there were significant differences between site but not time for the blade tissue content of cadmium but no interaction between the two parameters. But there a different pattern was found for sporophyll, where there were signifcant differences between both site and time for the sporophyll content of Cd. There was also significant interaction between these two parameters indicated that they did not vary in a systematic manner.

Table 40. Two way analysis of Variance of cadmium in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	10.0106	3.33687	45.86	0.000
time	2	0.1661	0.08306	1.14	0.322
Interaction	6	0.8679	0.14465	1.99	0.072
Error	132	9.6039	0.07276		
Total	143	20.6485			

S = 0.2697 R-Sq = 53.49% R-Sq(adj) = 49.61%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	5.5950	1.86501	19.13	0.000
time	2	0.7780	0.38899	3.99	0.021
Interaction	6	1.4682	0.24469	2.51	0.025
Error	132	12.8692	0.09749		
Total	143	20.7104			

S = 0.3122 R-Sq = 37.86% R-Sq(adj) = 32.68%

Table 41. Comparison of the blade and sporophyll tissue content of cadmium

Paired T for Cd Blade - Cd Sporophyll

	N	Mean	StDev	SE Mean
Cd Blade	60	2.49925	0.29335	0.03787
Cd Sporophyll	60	1.94915	0.26299	0.03395
Difference	60	0.550100	0.339458	0.043824

95% CI for mean difference: (0.462409, 0.637791)
T-Test of mean difference = 0 (vs not = 0): T-Value = 12.55 P-Value = 0.000

Table 41 shows that there was significant difference of cadmium content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissues had content of Cd on average between 0.46 and 0.63 mg kg^{-1}higher than the sporophyll tissue.

3.15 Spatial and temporal variation of mercury concentrations in *Undaria pinnatifida*

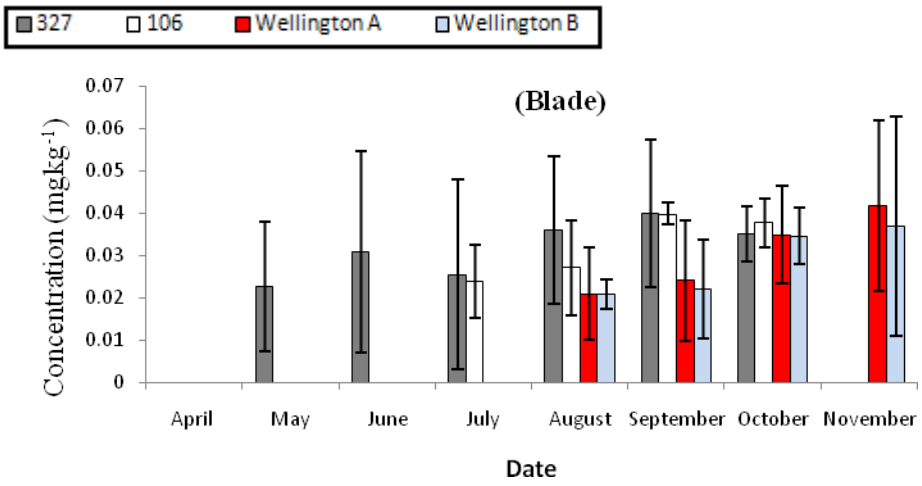

Figure 22. Monthly mercury concentrations in *Undaria pinnatifida*.

The blade tissue content of mercury in all four sites showed small fluctuations with no clear trend idenified. There was no detectable Hg recorded in the blade samples in farm PE 327 in April 2011. There were no statistical analyses performed for sporophyll tissue content Hg. This was because no reliable values recorded. This was probably caused by the decline of the sensitivity and the age of the ICP AES to detect the extremely small concentration.

Table 42. Results of a One-way ANOVA testing for differences in farm PE327 for mercury concentrations between months in blade tissue.

Blade

Source	DF	SS	MS	F	P
time	6	0.001365	0.000228	0.55	0.768
Error	53	0.021932	0.000414		
Total	59	0.023297			

S = 0.02034 R-Sq = 5.86% R-Sq(adj) = 0.00%

There was no difference (P > 0.10) between the blade tissue content of mercury in farm PE 327 across the seven months study (Table 42). The Tukey's comparison of means did not show statistical differences between the monthly means of the blade tissue content of Hg in farm PE 327. This evidence was not significant to suggest that there was a seasonal trend.

Table 43. Two way analysis of Variance for mercury in the period between August and October for blade tissue.

Blade

Source	DF	SS	MS	F	P
time	2	0.0015044	0.0007522	2.34	0.101
Site id	3	0.0002215	0.0000738	0.23	0.876
Interaction	6	0.0003927	0.0000654	0.20	0.975
Error	132	0.0425041	0.0003220		
Total	143	0.0446227			

S = 0.01794 R-Sq = 4.75% R-Sq(adj) = 0.00%

Table 43 shows that there were no significant differences between for both site but time for the blade tissue content of mercury. There was no significant interaction between these two parameters.

3.16 Spatial and temporal variation of lead concentrations in *Undaria pinnatifida*

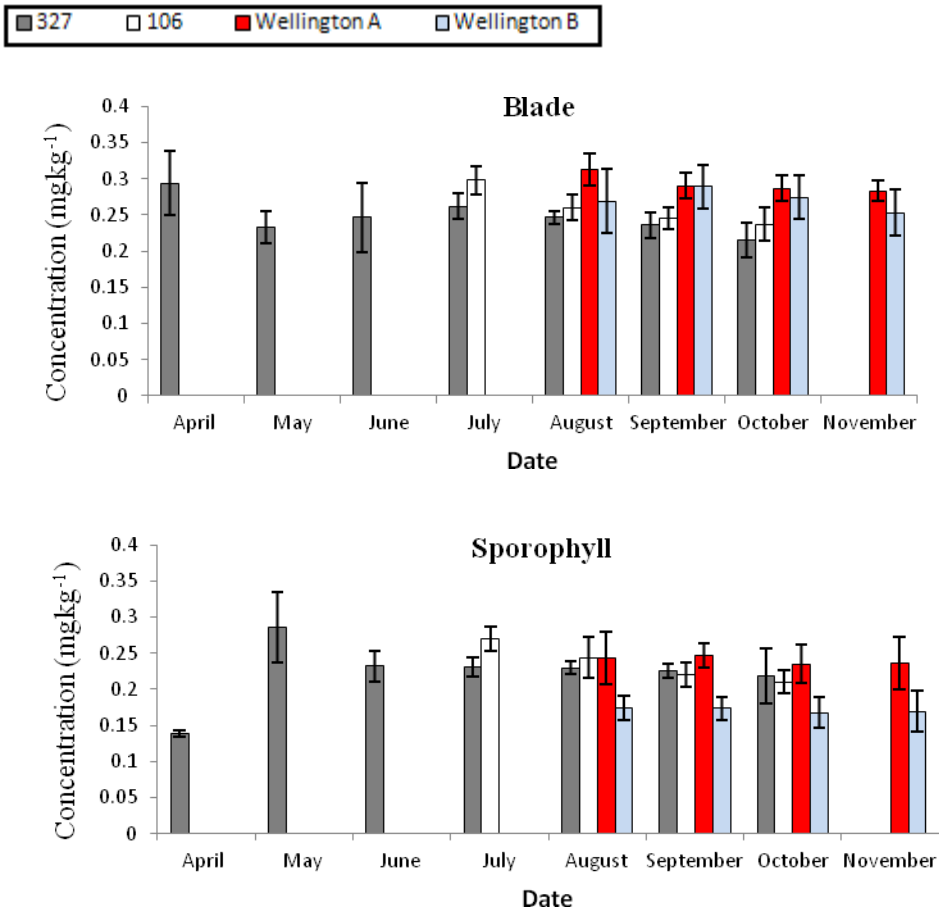

Figure 23. Monthly lead concentrations in *Undaria pinnatifida*.

The blade tissue content of mercury in all four sites showed small fluctuations.

There was an increase of the sporophyll tissue content of Pb level between April and May in farm PE 327, the level approached steady with small fluctuations for the rest of the sampling period. The sporophyll tissue content of mercury in all four sites showed small fluctuations.

Table 44. Results of a One-way ANOVA testing for differences in farm PE327 for lead concentrations between months in both blade and sporophyll tissue.

Blade

Source	DF	SS	MS	F	P
time	6	0.025913	0.004319	4.52	0.001
Error	53	0.050676	0.000956		
Total	59	0.076589			

S = 0.03092 R-Sq = 33.83% R-Sq(adj) = 26.34%

Sporophyll

Source	DF	SS	MS	F	P
time	6	0.04628	0.00771	6.39	0.000
Error	53	0.06398	0.00121		
Total	59	0.11026			

S = 0.03474 R-Sq = 41.97% R-Sq(adj) = 35.40%

There was significant difference ($P < 0.05$) between the blade tissue content of lead in farm PE 327 across the seven months study (Table 44). The Tukey's comparison of means showed that there were statistical differences between the monthly means of the blade tissue content of Pb in farm PE 327. This evidence was not significant to suggest that there was a clear seasonal trend.

Table 45. Two way analysis of Variance of lead in the period between August and October for blade and sporophyll tissue

Blade

Source	DF	SS	MS	F	P
Site id	3	0.090615	0.0302051	27.04	0.000
time	2	0.008935	0.0044675	4.00	0.021
Interaction	6	0.008394	0.0013990	1.25	0.284
Error	132	0.147477	0.0011172		
Total	143	0.255421			

S = 0.03343 R-Sq = 42.26% R-Sq(adj) = 37.45%

Sporophyll

Source	DF	SS	MS	F	P
Site id	3	0.099011	0.0330038	32.71	0.000
time	2	0.005525	0.0027625	2.74	0.068
Interaction	6	0.003709	0.0006182	0.61	0.720
Error	132	0.133182	0.0010090		
Total	143	0.241428			

S = 0.03176 R-Sq = 44.84% R-Sq(adj) = 40.24%

There was significant difference ($P < 0.001$) between the sporophyll tissue content of lead in farm PE 327 across the seven months study (Table 44). The Tukey's comparison of

means showed that there were statistical differences between the monthly means of the sporophyll tissue content of Pb in farm PE 327. However, this evidence was not significant to suggest that there was a clear seasonal trend.

Table 45 shows that there was significant differences for both time and site for the blade tissue content of lead. However there was no significant interaction between these two parameters. A different pattern was also found for sporophyll, where there were signifcant differences for site but not time for the sporophyll content of Pb. There was also no significant interaction between these two parameters.

Table 46. Comparison of the blade and sporophyll tissue content of lead

Paired T for Pb Blade - Pb Sporophyll

	N	Mean	StDev	SE Mean
Pb Blade	60	0.243300	0.036029	0.004651
Pb Sporophyll	60	0.224617	0.043229	0.005581
Difference	60	0.018683	0.063842	0.008242

95% CI for mean difference: (0.002191, 0.035175)
T-Test of mean difference = 0 (vs not = 0): T-Value = 2.27 P-Value = 0.027

Table 46 shows that there was significant difference of lead content between the blade and sporophyll tissue in farm 327. In farm 327, the blade tissue had content of Pb on average between 0.0022 and 0.035 mg kg^{-1}higher than the sporophyll tissue.

Chapter 4 Discussion

4.1 Evaluation of New Zealand *Undaria pinnatifida* mineral contents

The result from this research has identified that the *Undaria pinnatifida* contained variety of minerals or heavy metals. These metals vary in content and shown different patterns. Essential minerals for human health such as Ca, K, Na, Mg and P were identified to be the most abundant minerals in the wild *U. pinnatifida* sampled from both South and North Island of New Zealand. On the other hand Cr, Cu, Mn, Ni, Se and Zn contents were much smaller in the wild *U. pinnatifida.* These minerals existed in trace amounts but are still significant to humans diet and health.

Various agencies e.g. the World Health Organisation (WHO) and the National Health and Medical Research Council of Australia (NHMRC) have recommended daily intake (RDI), upper level of intake (UI), tolerable daily intake (TDI) and adequate intake (AI) for some of the metals in this study. In addition, the WHO has also provided guidelines of provisional tolerable weekly intakes (TWI) for some of the more harmful heavy metals in this study. Table 47 compares the values obtained in this study with these limits. For the purposes of this discussion the amount of each metal in a 40 g serving of wild *Undaria pinnatifida* was compared, this is an approximate amount of wakame one might consume as seaweed salad ordered in a Japanese restaurant. Mean data across all sites from October 2011 was used, because most metals displayed the highest concentration in that month and October is the most likely harvesting period due to the large size of the plants in that month. A comparison of arsenic was not carried out, because available guidelines only govern inorganic arsenic levels, while total arsenic level was measured in this study. Table 47 shows that there were no significant differences on the health effect between consumption of wild *U. pinnatifida* obtained in October from Port Underwood and Wellington, but except for the result obtained from sodium analysis.

Table 47. Consumption of 40g of wild *Undaria pinnatifida* obtained in October 2011. RDI = recommended daily intake; AI = adequate intake; UI = upper level of intake; TDI = tolerable daily intake (per 70 kg body weight); TWI = tolerable weekly intake (per 70 kg body weight).

Metal	WHO/FAO guidelines	NHMRC guidelines	% of WHO/FAO guidelines in consumption of 40g of wild *U. pinnatifida* Port Underwood	% of NHMRC guidelines in consumption of 40g of wild *U. pinnatifida* from Port Underwood	% of WHO/FAO guidelines in consumption of 40g of wild *U. pinnatifida* from Wellington	% of NHMRC guidelines in consumption of 40g of wild *U. pinnatifida* from Wellington
Calcium (Ca)	1-1.3 g RDI	1 g RDI	28% of RDI	36% of RDI	31% of RDI	41% of RDI
Potassium (K)		2.8-3.8 g AI		48% of AI		51% of AI
Magnesium (Mg)		0.32-0.42 g RDI		90% of RDI		90% of RDI
Sodium (Na)		2.3 g UI		108% of UI		50% of UI
Phosphorus (P)		1 g RDI		48% of RDI		42% of RDI
Chromium (Cr)		0.025-0.035 mg AI		118% of AI		96% of AI
Copper (Cu)		10mg UI		1.5% of UI		1.1% of UI
Manganese (Mn)		5-5.5 mg AI		8% of AI		10.6% of AI
Nickel (Ni)	0.84 mg TDI		13% of TDI			10% of TDI
Selenium (Se)	0.026-0.034 mg RDI	0.06-0.07 mg RDI	71% of RDI	35% of RDI	95% of RDI	46% of RDI
Zinc (Zn)		8-14 mg RDI		7.8% of RDI		10% of RDI
Cadmium (Cd)	0.49 mg TWI		19% of TWI			18% of TWI
Mercury (Hg)	0.112 mg TWI		1.32% of TWI			1.25% of TWI
Lead (Pb)	1.75 mg TWI		0.54% of TWI			0.64% of TWI

4.1.1 Calcium

The highest monthly mean of the blade tissue content of calcium were 16.97 g kg^{-1} ± 0.45 SD (June), 9.07 g kg^{-1} ± 0.34 SD (October), 10.13 g kg^{-1} ± 0.20 SD (October) and 10.31 g kg^{-1} ± 0.41 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Ca concentrations were 8.75 g kg^{-1} ± 0.35 SD (June), 7.03 g kg^{-1} ± 0.38 SD (October), 7.03 g kg^{-1} ± 0.054 SD (October) and 7.41 g kg^{-1} ± 0.45 SD (June) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were comparable with previous research of *U. pinnatifida* e.g. 12.8 g kg^{-1} from New Zealand (Smith *et al*., 2010), 9.31 g kg^{-1} recorded in Spain (Rupérez, 2002) and 9.5 g kg^{-1} (Kolb *et al*., 2004). The content of Ca in commercial samples were 7.17 g kg^{-1} ± 0.36 SD, 8.19 g kg^{-1} ± 0.48 SD and 6.78 g kg^{-1} ± 0.29 SD for Japan, China and South Korea respectively. The World Health Organisation recommends the daily intake (RDI) of Ca is between 1g/day and 1.3 g/day for adult (WHO, 2004). Whereas the nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) for Ca is 2.5g/day and the recommended daily intake for adult is 1g/day(Anonymous, 2005). The Australia New Zealand food standards code suggests the Ca recommended dietary intake for adult is 0.8g (FSANZ, 2011a). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood would contribute 28% and 36% of the RDI by WHO/FAO and NHMRC respectively. Also, consumption of 40 g of the wild *U. pinnatifida* obtained in October from Wellington would contribute 31% and 41% of the RDI by WHO/FAO and NHMRC respectively.

4.1.2 Potassium

The highest monthly mean of the blade tissue content of potassium were 45.86 g kg^{-1} ± 0.91 SD (October), 42.14 g kg^{-1} ± 0.59 SD (October), 44.68 g kg^{-1} ± 0.52 SD (October) and 48.48 g kg^{-1} ± 0.56 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of K

were 28.69 g kg^{-1} ± 0.85 SD (June), 16.10 g kg^{-1} ± 0.44 SD (October), 28.97 g kg^{-1} ± 0.29 SD (October) and 27.08 g kg^{-1} ± 0.52 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were lower than with what had been found in previous research of *U. pinnatifida* e.g. 71.2 g kg^{-1} from New Zealand (Smith *et al*., 2010), 86.99 g kg^{-1} recorded in Spain (Rupérez, 2002) and 56.91 g kg^{-1} (Kolb *et al*., 2004). The content of K in commercial samples were 72.72 g kg^{-1} ± 0.78 SD, 38.61 g kg^{-1} ± 0.21 SD and 84.77 g kg^{-1} ± 1.26 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation do not have a recommended intake of K but the nutrient reference values for Australia and New Zealand states that the adequate intake (AI) for K is 2.8g/day and 3.8g/day for adult women and men respectively (Anonymous, 2005). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 48% and 51 % of the AI recommended by NHMRC respectively.

4.1.3 Sodium

The highest monthly mean of the blade tissue content of sodium were 62.55 g kg^{-1} ± 0.67 SD (October), 58.31 g kg^{-1} ± 0.54 SD (October), 28.60 g kg^{-1} ± 0.50 SD (October) and 28.18 g kg^{-1} ± 0.28 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Na were 32.10 g kg^{-1} ± 0.46 SD (July), 33.83 g kg^{-1} ± 0.13 SD (October), 20.42 g kg^{-1} ± 0.16 SD (September) and 19.95 g kg^{-1} ± 0.15 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The Post Underwood blade values were higher than what had been found in previous research of *U. pinnatifida* e.g. 36.1 g kg^{-1} from New Zealand (Smith *et al*., 2010), but lower than 76.64 g kg^{-1} recorded in Spain (Rupérez, 2002) and 64.94 g kg^{-1} (Kolb *et al*., 2004). The content of Na in commercial samples were 75.68 g kg^{-1} ± 0.31 SD, 55.25 g kg^{-1} ± 0.95 SD and 69.26 g kg^{-1} ± 0.55 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation do not have a recommended intake of Na but the Nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) for Na is 2.3

g/day for adult and the AI is 0.460-0.92 g/day (Anonymous, 2005). Consumption of 40 g of the wild *U. pinnatifida* obtained from Port Underwood and Wellington in October would contribute 108% and 50% of the NHMRC recommended UI respectively.

The sodium concentrations from 327 and 106 between July and October were significant higher than results from Wellington, this was caused by mistakes with the sampling students who washed the sample with sea water instead of fresh water, and the samples were transported straight to the frozen dry plant. As a result salt residues mixed with the sample and elevated the Na concentration.

The highest blade sodium/potassium ratios were 1.36, 1.38, 0.64 and 0.58 respectively for farm 327, 106, Wellington site A and Wellington site B. The highest sporophyll Na/K ratio were 1.11, 2.10, 0.70 and 0.74 respectively for farm 327, 106, Wellington site A and Wellington site B. These results were comparable with Na/K ratio 0.33-1.34 for seaweeds reported previous (Rupérez, 2002). High level of Na had been associated with high blood pressure and heart diseases, as a result the intake of sodium chloride and diets with a high Na/K ratio had been is not recommended (Grimm *et al.*, 1988; Cofrades *et al.*, 2010). Na/K ratios in olives and sausages were 43.63 and 4.89, respectively (Ortega-Calvo, Mazuelos, Hermosın, & Saiz-Jimenez, 1993). In the case of seaweed, the role of Na had been associated with other minerals such as potassium, with which it forms a balanced (Cofrades *et al*., 2010). The concentration Na and K in seaweeds was high and higher than the value reported for land vegetables (USDA, 2001). However the ratio of sodium to potassium was low and this helped to combat fluid retention and high blood pressure without the risk of compromised the potassium balance (Rupérez, 2002) . The above findings corresponded well with other literatures reported and proved the *U. pinnatifida* in New Zealand had a beneficial effect in preventing heart diseases.

4.1.4 Magnesium

The highest monthly mean of the blade tissue content of magnesium were 9.21 g kg^{-1} ± 0.35 SD (October), 9.47 g kg^{-1} ± 0.31 SD (October), 9.23 g kg^{-1} ± 0.33 SD (October) and 9.47 g kg^{-1} ± 0.22 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Mg were 6.64 g kg^{-1} ± 0.32 SD (October), 6.16 g kg^{-1} ± 0.37 SD (October), 5.72 g kg^{-1} ± 0.11 SD (August) and 7.59 g kg^{-1} ± 0.14 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were similar to what had been found in previous research of *U. pinnatifida* e.g. 8.33 g kg^{-1} (Cofrades *et al*., 2010) but lower than 11.81 g kg^{-1} (Rupérez, 2002). The content of Mg in commercial samples were 7.10 g kg^{-1} ± 0.21SD, 3.86 g kg^{-1} ± 0.22SD and 8.47 g kg^{-1} ± 0.13 SD for product harvested from Japan, China and South Korea respectively. World Health Organisation has recommends the daily intake (RDI) of Mg were 0.22g/day and 0.26 g/day for adult women and man respectively. Whereas the nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) for adult Mg is 0.35g/day and the RDI for adult is between 0.31 and 0.42 g/day.(Anonymous, 2005). The Australia New Zealand food standards code suggests the Mg recommended dietary intake for adult is 0.32g (FSANZ, 2011a). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would both contribute 90% of the NHMRC RDI. Magnesium is a calcium regulator, and hypomagnesemia is one of the causes of hypocalcemia (Anonymous, 2005). It is important to maintain certain balanced between magnesium and calcium, and the results proved that New Zealand is a good dietary option to achieve this.

4.1.5 Phosphorus

The highest monthly mean of the blade tissue content of phosphorus were 12.05 g kg^{-1} ± 0.33 SD (October), 11.62 g kg^{-1} ± 0.26 SD (October), 10.31 g kg^{-1} ± 0.66 SD (October) and 10.61 g kg^{-1} ± 0.43 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of P were 9.41 g kg^{-1} ± 0.29 SD (October), 7.76 g kg^{-1} ± 0.74 SD (October), 8.54 g kg^{-1} ± 0.135 SD (October) and 8.71 g kg^{-1} ± 0.12 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were higher than what had been found in previous research of *U. pinnatifida* e.g. 4.79 g kg^{-1} (Smith *et al*., 2010) but lower than 4.50 g kg^{-1} (Kolb *et al*., 2004). The content of P in commercial samples were 12.43 g kg^{-1} ± 0.78 SD, 11.08 g kg^{-1} ± 0.15 SD and 7.71 g kg^{-1} ± 0.81 SD for product harvested from Japan, China and South Korea respectively. The nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) of P are 4 g/day for adult between 19 to 70 years old and 3 g/kg for adult above 70 years old and the recommended dietary intake (RDI) for adult is 1 g/day (Anonymous, 2005). The Australia New Zealand food standards code suggests the P recommended dietary intake for adult is 1 g (FSANZ, 2011a). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 48% and 42% of the NHMRC RDI respectively.

4.1.6 Chromium

The highest monthly mean of the blade tissue content of chromium were 1.04 mg kg^{-1} ± 0.21 SD (October), 0.78 mg kg^{-1} ± 0.053 SD (July), 0.84 mg kg^{-1} ± 0.062 SD (October) and 0.73 mg kg^{-1} ± 0.0293 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Cr were 0.92 mg kg^{-1} ± 0.024 SD (July), 0.77 mg kg^{-1} ± 0.016 SD (July), 0.74 mg kg^{-1} ± 0.026 SD (October) and 0.69 mg kg^{-1} ± 0.019 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were similar to what had

been found in previous research of *U. pinnatifida* e.g. 0.74 mg kg^{-1} (Smith *et al*., 2010) and 0.72 mg kg^{-1} (Kolb *et al*., 2004). The content of Cr in commercial samples 1.08 mg kg^{-1} ± 0.061 SD, 0.72 mg kg^{-1} ± 0.048 SD and 0.85 mg kg^{-1} ± 0.039 SD for product harvested from Japan, China and South Korea respectively. The nutrient reference values for Australia and New Zealand states that the adequate intake (AI) are 0.035 mg/day and 0.025 mg/day for adult men and women respectively(Anonymous, 2005). The Australia New Zealand food standards code suggests the Cr estimated safe and adequate daily dietary intake recommended for adult is 0.2 mg (FSANZ, 2011a). Therefore consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 118% and 96% of the NHMRC recommended AI respectively.

4.1.7 Copper

The highest monthly mean of the blade tissue content of copper were 3.78 mg kg^{-1} ± 0.23 SD (October), 3.77 mg kg^{-1} ± 0.23 SD (October), 2.62 mg kg^{-1} ± 0.15 SD (October) and 2.66 mg kg^{-1} ± 0.12 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Cu were 1.85 mg kg^{-1} ± 0.12 SD (July), 2.44 mg kg^{-1} ± 0.16 SD (October), 2.40 mg kg^{-1} ± 0.12 SD (October) and 2.64 mg kg^{-1} ± 0.11 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were lower than what had been found in previous research of *U. pinnatifida* e.g. 9.76 mg kg^{-1} (Smith *et al*., 2010) but high than 1.8 mg kg^{-1} (Kolb *et al*., 2004). The content of Cu in commercial samples were 1.87 mg kg^{-1} ± 0.12 SD, 1.06 mg kg^{-1} ± 0.035 SD and 1.13 mg kg^{-1} ± 0.047 SD for product harvested from Japan, China and South Korea respectively. The nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) for adult of Cu is 10 mg/day and the AI is 1.7 and 1.2 mg/day for adult men and women respectively (Anonymous, 2005). Therefore consumption of less than 1 kg of wild *U. pinnatifida* would enough to delivery adequate amount of Cu to human. Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 1.5% and 1.1% of the NHMRC recommended UI respectively.

4.1.8 Manganese

The highest monthly mean of the blade tissue content of manganese were 10.39 mg kg^{-1} ± 2.45 SD (October), 9.99 mg kg^{-1} ± 1.26 SD (October), 14.61 mg kg^{-1} ± 1.23 SD (November) and 8.57 mg kg^{-1} ± 1.95 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Mn were 7.72 mg kg^{-1} ± 0.85 SD (April), 6.26 mg kg^{-1} ± 0.41 SD (October), 7.68 mg kg^{-1} ± 0.25 SD (October) and 7.93 mg kg^{-1} ± 0.13 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values did not have a huge difference when compared to what had been found in previous research of *U. pinnatifida* e.g. 10.1 mg kg^{-1} (Smith *et al*., 2010) and 8.7 mg kg^{-1} (Rupérez, 2002). The content of Mn in commercial samples were 7.61 mg kg^{-1} ± 1.58 SD, 13.62 mg kg^{-1} ± 0.77 SD and 5.98 mg kg^{-1} ± 0.21 SD for product harvested from Japan, China and South Korea respectively. The Australia New Zealand food standards code for Mn states estimated safe and adequate daily dietary intake recommended for adult is 5 mg (FSANZ, 2011a). The nutrient reference values for Australia and New Zealand states that the adequate intakes (AI) are 5.5mg/day and 5mg/day for adult men and women respectively (Anonymous, 2005). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 8% and 10.6% of the NHMRC recommended AI respectively.

4.1.9 Nickel

The highest monthly mean of the blade tissue content of nickel were 2.78 mg kg^{-1} ± 0.12 SD (October), 2.24 mg kg^{-1} ± 1.12 SD (October), 1.95 mg kg^{-1} ± 0.067 SD (October) and 2.10 mg kg^{-1} ± 0.057 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Ni were 1.35 mg kg^{-1} ± 0.11 SD (April), 1.62 mg kg^{-1} ± 0.18 SD (October), 1.69 mg kg^{-1} ± 0.056 SD (August) and 1.70 mg kg^{-1} ± 0.050 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values did not have a huge difference

when compared to what had been found in previous researches of *U. pinnatifida* e.g. 2.65 mg kg^{-1} (Kolb *et al*., 2004). The content of Ni in commercial samples were 0.72 mg kg^{-1} ± 0.036 SD, 0.32 mg kg^{-1} ± 0.021 SD and 2.07 mg kg^{-1} ± 0.16 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation/Food and Agriculture Organization of the United Nations (WHO/FAO) state that the Ni tolerable daily intake (TDI) is 12 µg/kg of body weight(WHO, 2005). Assuming an adult with 70kg the level would be 0.84 mg per 70 g person per day. Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 13% and 10% of the WHO/FAO recommended TDI respectively.

4.1.10 Selenium

The highest monthly mean of the blade tissue content of selenium were 0.61 mg kg^{-1} ± 0.0164 SD (October), 0.53 mg kg^{-1} ± 0.056 SD (October), 0.83 mg kg^{-1} ± 0.138 SD (August) and 0.81 mg kg^{-1} ± 0.078 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Se were 0.33 mg kg^{-1} ± 0.030 SD (July), 0.39 mg kg^{-1} ± 0.022 SD (October), 0.52 mg kg^{-1} ± 0.030 SD (September) and 0.48 mg kg^{-1} ± 0.025 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values were higher than what had been found in previous research of *U. pinnatifida* e.g. 0.070 mg kg^{-1} (Smith *et al*., 2010) and 0.5 mg kg^{-1} (Kolb *et al*., 2004). The content of Se in commercial samples were 0.11 mg kg^{-1} ± 0.015 SD, 0.36 mg kg^{-1} ± 0.0063 SD and 0.22 mg kg^{-1} ± 0.0079 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation has RDI for adult of Se are 0.026 and 0.034 mg/day respectively for adult women and men (WHO, 2004). Whereas the nutrient reference values for Australia and New Zealand states that upper level of intake (UI) for adult of Se is 0.4 mg/day and the recommended daily intake (RDI) are 0.06 and 0.07 mg/day for women and men respectively (Anonymous, 2005). The Australia New Zealand food standards code suggests the Se recommended dietary intake for adult is 0.07 mg (FSANZ, 2011a). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood would contribute 71% and 35% of the WHO/FAO and

NHMRC RDI respectively. Also Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Wellington would contribute 95% and 46% of the WHO/FAO and NHMRC RDI respectively

4.1.11 Zinc

The highest monthly mean of the blade tissue content of zinc were 26.11 mg kg^{-1} ± 2.72 SD (October), 27.30 mg kg^{-1} ± 2.77 SD (October), 33.39 mg kg^{-1} ± 3.99 SD (October) and 35.03 mg kg^{-1} ± 2.05 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Zn were 18.60 mg kg^{-1} ± 0.92 SD (October), 23.60 mg kg^{-1} ± 2.33 SD (October), 15.41 mg kg^{-1} ± 0.53 SD (October) and 16.01 mg kg^{-1} ± 0.49 SD (October) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values had some differences when compared to what had been found in previous research of *U. pinnatifida* e.g. 22.9 mg kg^{-1} (Smith *et al*., 2010), 17.4 mg kg^{-1} (Rupérez, 2002) and 9.44 mg kg^{-1} (Kolb *et al*., 2004). The content of Zn in commercial samples were 28.51 mg kg^{-1} ± 1.26 SD, 30.45 mg kg^{-1} ± 2.05 SD and 30.50 mg kg^{-1} ± 1.66 SD for product harvested from Japan, China and South Korea respectively. The nutrient reference values for Australia and New Zealand states that the upper level of intake (UI) for Zn is 40 mg/day and the recommended daily intake (RDI) are 14 and 8 mg/day for adult men and women respectively(Anonymous, 2005). The Australia New Zealand food standards code suggests the Zn recommended dietary intake for adult is 12 mg (FSANZ, 2011a). Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Post Underwood and Wellington would contribute 7.8% and 10% of the NHMRC RDI respectively.

In conclusion, *U. pinnatifida* from New Zealand can be considered as a good source to provide humans with enough trace minerals. Consumption with 1g of *U. pinnatifida* would only contribute a microgram of Cr, Cu, Mn, Ni, Se and Zn, to the diet and normal dietary intake amount of seaweed would not cause any adverse effects or overdose.

4.2 Evaluation of possible heavy metals contaminations in New Zealand *Undaria pinnatifida*

As mentioned above, arsenic, cadmium, mercury and lead are potentially harmful to humans and overdose often cause by oral consumption of food products contaminated by these metals. This section focuses on the safety of the consumption of wild *Undaria pinnatifida.*

4.2.1 Arsenic

The highest monthly mean of the blade tissue content of arsenic were 46.71 mg kg^{-1} ± 0.75 SD (May), 31.89 mg kg^{-1} ± 2.03 SD (July), 42.88 mg kg^{-1} ± 2.56 SD (August) and 36.41 mg kg^{-1} ± 3.30 SD (August) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of As were 29.47 mg kg^{-1} ± 1.75 (July), 29.23 mg kg^{-1} ± 2.27 SD (July), 32.84 mg kg^{-1} ± 2.30 SD (August) and 31.27 mg kg^{-1} ± 1.08 SD (September) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values had some differences when compared with what had been found in previous research of *U. pinnatifida* e.g.35.62 mg kg^{-1}(Smith *et al*., 2010) and seaweed product in Spain contained total As could ranged from 0.031-149 mg kg^{-1} (Almela *et al.*, 2006). The content of As in commercial samples were 23.84 mg kg^{-1} ± 1.76 SD, 18.11 mg kg^{-1} ± 0.77 SD and 34.67 mg kg^{-1} ± 1.56 SD for product harvested from Japan, China and South Korea respectively. It had been concluded that Marine algae could contain high levels of arsenic, but most were bound into organic molecules such as arsenosugars, which were not acutely toxic like the inorganic forms (Andrewes *et al*., 2004). In New Zealand, the only regulation applying to seaweed foods is inorganic arsenic. In the New Zealand Food Standards Code, the limit for inorganic arsenic in seaweeds is 1 mg kg^{-1} where the material is adjusted to 85% moisture (FSANZ, 2011b). However, there was no evidence that consumption of organic arsenic at levels up to 50 mg/kg/bw per day, through high levels of fish consumption had led in adverse effects (COT, 2003). Therefore, the total arsenic detected in seaweeds was unlikely to contribute health problems.

4.2.2 Cadmium

The highest monthly mean of the blade tissue content of cadmium were 2.91 mg kg^{-1} ± 0.097 SD (June), 1.73 mg kg^{-1} ± 0.19 SD (October), 2.24 mg kg^{-1} ± 0.16 SD (August) and 2.21 mg kg^{-1} ± 0.21 SD (August) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Cd were 2.19 mg kg^{-1} ± 0.17 SD (May), 1.68 mg kg^{-1} ± 0.11 SD (July), 2.10 mg kg^{-1} ± 0.17 SD (August) and 2.20 mg kg^{-1} ± 0.40 SD (September) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values had some differences when compared with what had been found in previous research of *U. pinnatifida* e.g.0.13 to 1.9 mg kg^{-1}(Almela *et al.*, 2002). The content of Cd in commercial samples were 1.87 mg kg^{-1} ± 0.015 SD, 1.89 mg kg^{-1} ± 0.15 SD and 1.65 mg kg^{-1} ± 0.023 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation/Food and Agriculture Organization of the United Nations (WHO/FAO) states that the Cd provisional tolerable weekly intake (TWI) is 7 µg/kg of body weight (WHO, 2003a). Assuming an adult with 70kg the level would be 0.49 mg per 70 g person per week. Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 19% and 18% of the WHO/FAO recommended TWI respectively.

4.2.3 Mercury

The highest monthly mean of the blade tissue content of mercury were 0.04 mg kg^{-1} ± 0.017 SD (September), 0.04 mg kg^{-1} ± 0.0025 SD (September), 0.042 mg kg^{-1} ± 0.020 SD (November) and 0.037 mg kg^{-1} ± 0.026 SD (November) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values had little differences when compared with previous research of *U. pinnatifida* e.g. 0.03 mg/kg (Smith *et al*., 2010) . The content of Hg in commercial samples were 0.045 mg kg^{-1} ± 0.015 SD, 0.022 mg kg^{-1} ± 0.021 SD and 0.044 mg kg^{-1} ± 0.011 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation/Food and Agriculture

Organization of the United Nations (WHO/FAO) states the Hg provisional tolerable weekly intake (TWI) is 1.6 µg/kg of body weight (JECFA, 2004). Assuming an adult with 70 kg the level would be 0.112 mg per 70 g person per week. Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 1.32% and 1.25% of the WHO/FAO recommended TWI respectively.

4.2.4 Lead

The highest monthly mean of the blade tissue content of lead were 0.29 mg kg^{-1} ± 0.044 SD (April), 0.30 mg kg^{-1} ± 0.02 SD (July), 0.31 mg kg^{-1} ± 0.022 SD (August) and 0.29 mg kg^{-1} ± 0.029 SD (September) respectively for farm 327, 106, Wellington site A and Wellington site B. The highest monthly mean of the sporophyll tissue content of Pb were 0.29 mg kg^{-1} ± 0.048 SD (May), 0.27 mg kg^{-1} ± 0.017 SD (July), 0.25 mg kg^{-1} ± 0.016 SD (September) and 0.174 mg kg^{-1} ± 0.016 SD (August) respectively for farm 327, 106, Wellington site A and Wellington site B. The blade values had some differences when compared with what had been found in previous research of *U. pinnatifida* e.g. 0.23 mg kg^{-1}(Smith *et al*., 2010) and 0.79 mg kg^{-1} (Kolb *et al*., 2004). The content of Pb in commercial samples were 0.82 mg kg^{-1} ± 0.043 SD, 0.84 mg kg^{-1} ± 0.028 SD and 0.69 mg kg^{-1} ± 0.060 SD for product harvested from Japan, China and South Korea respectively. The World Health Organisation/Food and Agriculture Organization of the United Nations (WHO/FAO) states the Pb provisional tolerable weekly intake (TWI) is 25 µg/kg of body weight (WHO, 2003b). Assuming an adult with 70kg the level would be 1.75 mg per 70 g person per week. Consumption of 40 g of the wild *U. pinnatifida* obtained in October from Port Underwood and Wellington would contribute 0.54% and 0.64% of the WHO/FAO recommended TWI respectively.

The comparison between the World Health Organisation / Food and Agriculture Organization of the United Nations (WHO/FAO) standards and the above results suggested that normal dietary consumptions of New Zealand *U. pinnatifida* will not deliver harmful amounts of these contaminant metals. The discussion above also concludes that most metals analysis did not show any significant difference caused by

fresh water and sea water rinsing. However the washing procedures had an important role regarding sodium analysis.

4.3 Distribution of metals between the blade and sporophyll tissue of *Undaria pinnatifida*.

There were significant differences between metal contents in the blade and sporophyll tissues, with the blade often containing higher concentration of metals than the sporophyll. This distribution may be able to be explained by the following mechanism. Absorption of elemental ions into the algal cells first occurred in the blade when the division and enlargement of the cells occurred, and the elements can be secondarily transferred to the sporophyll by active transport though inner hyphae in kelp species (Pfister, 1992; Wu & Meng, 1997; Kumura, Yasui, & Mizuta, 2006). Therefore, the lower metal concentrations in the sporophyll can be explained by the difference of transfer tendency of the metals through the transport system.

4.4 Temporal variation of metals in *Undaria pinnatifida*

All metals showed different variation across the seven months of this study. There were no clear trends identified for the metals analysis except the blade tissue content of magnesium and potassium. Villares (2002) suggested that the reasons for seasonal differences might include: environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH, etc.; metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors.

That metal concentrations decrease in seaweed during periods of growth and increase during dormant periods has been reported (Riget, Johansen, & Asmund, 1995). Other factors such as the age of the tissue and environmental factors such as salinity, temperature and as variation in metal concentrations in the environment also have an

important role in metals content in seaweed (Haritonidis & Malea, 1995). Higher concentrations of metals were found during growth periods in some studies. This is because higher rates of photosynthesis and respiration would favour the assimilation of metals (Catsiki & Papathanassiou, 1993). Weather patterns could also play a role with higher metal content in seaweed during the rainy season because potentially higher concentrations of metals in water was caused by an increase in terrestrial inputs (Lacerda, Teixeira, & Guimaraes, 1985) .

The above parameters could all be possible factors which caused the the seasonal variation of metals in blade and sporophyll of *Undaria pinnatifida* in farm PE 327. However it is inconclusive to specifically identify which parameters caused such variation. This is because this study did not include measurements of plant size, age, growth rate and weather pattern in the sampling sites and metals content in the seawater.

4.5 Evaluation of *Undaria pinnatifida* harvesting activities in Port Underwood and Wellington

Environmental aspects of the collection sites plays an important role in the heavy metal safety of the seaweeds (Hou & Yan, 1998). Wellington Harbour is a small (76 km^2) enclosed harbour at the southern end of the North Island of New Zealand (Booth, 1974). The greatest depth of water is 31 m, south-west of Somes Island (NZ Hydrographic Office 1969), but the average depth is 20 m (Gilmour, 1960). The total catchment area of the harbour is 725 km^2 (Brodie, 1958). The main freshwater source is the Hutt River with a catchment area of 630 km^2 (Johannesson, 1955). The minimum and maximum daily freshwater discharges of the Hutt River are approximately 2.6 X 10^6 m^3 and 180 X $10^6 m^3$ respectively (Maxwell, 1956). The tidal currents in the harbour; in its simplest form, the tide floods in a clockwise direction and ebbs in an anticlockwise direction, with current speeds varying from a maximum of 0.25 m/s at the harbour entrance channel to 0.10 m/s or less in the inner harbour (Brodie, 1958).

The channel connecting Wellington Harbour to the open sea is large enough to ensure good mixing of the harbour water with that outside (Maxwell, 1956; Gilmour, 1960). It would therefore be expected that although some special hydrological characteristics would be generated within the harbour, these would soon be assimilated by the circulation system, and would be reduced by the exchange with waters from outside the harbor (Maxwell, 1956; Gilmour, 1960). As a result areas near the harbour mouth and in central and western regions of the harbour would undergo the most regular exchange with Cook Strait waters (Brodie, 1958).

Concentrations of lead, mercury, and to a lesser extent copper and zinc, were presented above sediment quality guidelines in the subtidal sediments of various parts of Wellington Harbour, especially those adjacent to Wellington City (Stephenson, Milne, & Sorensen, 2008). However the sea water data of metal concentrations was lacking to demonstrate how the current would had an effect on the ambient sea water heavy contents and to conclude an actual environmental condition in wellington sites.

According to Port Underwood Sanitary Survey Report, Port Underwood is a double re entrant embayment located at the SE edge of the Marlborough Sounds at the north east tip of the South Island (Shearer, 2001). Shearer (2001) described the nature of Port Underwood as follow, it covers an area approximately 9km long and 3km wide with an alignment opening located in the SW to the periphery of Cloudy bay and Cook Strait. There is a 250-400+ m high range formed the eastern boundary of the port which shields this area from turbulence of Cook Strait and an approximately 3km long isthmus separates the inner port into two embayments. The water depth is shallow around 12-17m with little tidal range so wave action and coastal erosion are limited.

Wairau River is the primary source for the sediments on the floor and the sampling sites in Port Underwood are well away from the relatively large Wairau River that discharge to the Cloudy Bay at some point 10 km south (Shearer, 2001). Shearer (2001) suggested that the remoteness of this locations and the turbulence of the intervening waters suggest that any contaminants from this river and the intermediary catchments are usually well

dispersed and diluted before reaching Port Underwood sites, therefore heavy metal pollution source like stormwater system or drainage are not considered to be a threat as the closest town of Blenheim which is 15 km south from the sites. There are also no industries near the immediate or remote catchments that could produce heavy metal to the sites (Shearer, 2001).

As mentioned above both areas have their unique natural geographic characteristics and should allow *Undaria pinnatifida* to grow healthily without absorbing exceed amount of pollutants. However, close monitoring of the surrounding environment, especially the sea water condition, is needed to predict any possible pollution.

4.6 Conclusion

This thesis investigated the metal contents of *Undaria pinnatifida* harvested from New Zealand waters. It was found that *U. pinnatifida* is rich in Ca, Mg, Na, K and P with small amounts of Cr, Cu, Mn, Ni, Se and Zn. The concentrations of the above elements when compared to World Health Organisation / Food and Agriculture Organization of the United Nations (WHO/FAO) guidelines and nutrient reference values for Australia and New Zealand, show that (not surprisingly) the *U. pinnatifida* is safe for human consumption and the results for As, Cd, Hg and Pb when compared with WHO/FAO guidelines show that New Zealand *Undaria pinnatifida* contains no heavy metals in levels that would be of any concern.

The environmental factors and metal analysis results suggests that both Wellington and Port Underwood sites have potential for farming or harvesting *U. pinnatifida,* as the samples are not contaminated by any of the heavy metals investigated.

The seafood industry in Port Underwood is following the Marlborough Shellfish Quality Programme (MSQP) in managing the environmental and seafood quality (MFA, 2005). Therefore there is an existing operating system which can be applied to monitor the seaweed industry in that area. However it would be ideal if a similar program was

initiated in Wellington to focus on marine foods and to provide a means of sharing between the companies within the industry, rather than rely on council and government led programs, as these programs often focus on recreational water use or on purely scientific studies and are not ideal for the seafood industries.

This study acts as a precursor for future research related to bio indicators and the food science of *U. pinnatifida* in New Zealand. There are a few recommendations if future research is to focus on inorganic bio indication. A study area of suspected or proven heavy metals contamination should be chosen. The contaminated area will allow the maximum potential for accumulation of metals in the study species. A multi species scenario should also be applied in such research and the accumulation potential of different species assessed. A season long sampling plan should also be used so an evaluation of temporal trends of metal abundance can be made.

There are some improvements that could be introduced to the analytical chemistry procedures. The problem experienced with results of sodium was caused by washing the samples with sea water instead of fresh water, which results with inconsistent result. Therefore if a similar study is going to be carried out in the future a strict unified protocol should be followed for sample pre-treatment, washing and drying techniques. With respect to the drying process, both oven and freeze drying showed no impact on the final results. If resources allowed, microwave assisted digestion system and ICP-MS would give more sensitive and more consistent results. An introduction of an authentic certified reference material would improve the certainty of recoveries and interferences. In this study only total arsenic level was included but more detailed study of toxic inorganic arsenic species could be added to future research, even though most arsenic was in the form of organic arsenic in seaweeds. Because it is more toxic, there are regulations in New Zealand Food standard Code and WHO/FAO standards for inorganic arsenic species in foods. Finally there is no study in New Zealand which focused on the location of metals in the cellular structures of *U. pinnatifida* or other types of seaweeds. Therefore such area of research in the local species will provide us more knowledge about how metals are stored and accumulate in the cells of local seaweeds.

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Appendix 1: Statistical outputs for the comparison of sample size

One-way ANOVA: Ca g/kg versus amount

```
Source  DF     SS     MS     F      P
amount   3  1.249  0.416  1.52  0.261
Error   12  3.295  0.275
Total   15  4.543

S = 0.5240   R-Sq = 27.48%   R-Sq(adj) = 9.35%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
0.1g   4  10.729  0.582  (-----------*----------)
0.25g  4  10.990  0.647       (-----------*----------)
0.5g   4  11.446  0.362                 (----------*----------)
1g     4  11.310  0.458              (----------*----------)
                         -------+---------+---------+---------+--
                             10.50     11.00     11.50     12.00

Pooled StDev = 0.524


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.25g   -0.8391  0.2612  1.3616          (----------*----------)
0.5g    -0.3833  0.7171  1.8175              (----------*----------)
1g      -0.5194  0.5810  1.6814            (----------*----------)
                                 ------+---------+---------+---------+---
                                    -1.0       0.0       1.0       2.0


amount = 0.25g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.5g    -0.6445  0.4558  1.5562           (----------*----------)
1g      -0.7806  0.3198  1.4201          (----------*----------)
                                 ------+---------+---------+---------+---
                                    -1.0       0.0       1.0       2.0


amount = 0.5g subtracted from:

amount    Lower   Center   Upper  ------+---------+---------+---------+---
1g      -1.2365  -0.1361  0.9643      (----------*----------)
                                  ------+---------+---------+---------+---
                                     -1.0       0.0       1.0       2.0
```

One-way ANOVA: K g/kg versus amount

```
Source  DF      SS      MS      F      P
amount   3  312.54  104.18  54.77  0.000
Error   12   22.83    1.90
Total   15  335.37

S = 1.379   R-Sq = 93.19%   R-Sq(adj) = 91.49%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
0.1g   4  13.144  2.497    (----*---)
0.25g  4  12.275  1.081  (---*---)
0.5g   4  21.882  0.272                            (----*---)
1g     4  21.143  0.362                          (---*----)
                          ---------+---------+---------+---------+
                                14.0      17.5      21.0      24.5

Pooled StDev = 1.379


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center   Upper  --------+---------+---------+---------+-
0.25g   -3.765  -0.869   2.028              (---*---)
0.5g     5.841   8.738  11.634                          (---*----)
1g       5.103   7.999  10.895                        (---*----)
                        --------+---------+---------+---------+-
                             -7.0       0.0       7.0      14.0


amount = 0.25g subtracted from:

amount  Lower  Center   Upper  --------+---------+---------+---------+-
0.5g    6.710   9.606  12.502                            (---*---)
1g      5.971   8.868  11.764                          (---*---)
                       --------+---------+---------+---------+-
                            -7.0       0.0       7.0      14.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  --------+---------+---------+---------+-
1g      -3.635  -0.739  2.158              (---*---)
                       --------+---------+---------+---------+-
                            -7.0       0.0       7.0      14.0
```

One-way ANOVA: Mg g/kg versus amount

```
Source  DF      SS      MS      F      P
amount   3  35.159  11.720  14.90  0.000
Error   12   9.437   0.786
Total   15  44.596

S = 0.8868   R-Sq = 78.84%   R-Sq(adj) = 73.55%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
0.1g   4  4.1674  1.5812  (------*-----)
0.25g  4  6.8568  0.1965                   (------*-----)
0.5g   4  7.1869  0.5681                     (------*-----)
1g     4  8.1691  0.5331                            (-----*------)
                          ---------+---------+---------+---------+
                                 4.5       6.0       7.5       9.0

Pooled StDev = 0.8868


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center   Upper  ---+---------+---------+---------+------
0.25g   0.8271  2.6893  4.5516                (-------*------)
0.5g    1.1572  3.0195  4.8817                  (------*-------)
1g      2.1394  4.0017  5.8639                      (------*------)
                           ---+---------+---------+---------+------
                          -2.5       0.0       2.5       5.0


amount = 0.25g subtracted from:

amount    Lower  Center   Upper  ---+---------+---------+---------+------
0.5g    -1.5321  0.3302  2.1924         (------*-------)
1g      -0.5499  1.3123  3.1746             (------*-------)
                           ---+---------+---------+---------+------
                           -2.5       0.0       2.5       5.0


amount = 0.5g subtracted from:

amount    Lower  Center   Upper  ---+---------+---------+---------+------
1g      -0.8801  0.9822  2.8445          (-------*------)
                           ---+---------+---------+---------+------
                           -2.5       0.0       2.5       5.0
```

One-way ANOVA: Na g/kg versus amount

```
Source  DF     SS    MS     F      P
amount   3   1.49  0.50  0.25  0.863
Error   12  24.31  2.03
Total   15  25.81

S = 1.423   R-Sq = 5.78%   R-Sq(adj) = 0.00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
0.1g   4  11.585  2.554     (---------------*--------------)
0.25g  4  11.399  0.871   (---------------*--------------)
0.5g   4  12.141  0.846           (--------------*---------------)
1g     4  12.024  0.331         (--------------*---------------)
                          --+---------+---------+---------+-------
                         10.0      11.0      12.0      13.0

Pooled StDev = 1.423


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center  Upper  ---------+---------+---------+---------+
0.25g   -3.175  -0.186  2.803     (--------------*--------------)
0.5g    -2.433   0.556  3.545         (--------------*--------------)
1g      -2.550   0.439  3.428        (--------------*--------------)
                           ---------+---------+---------+---------+
                                 -2.0       0.0       2.0       4.0


amount = 0.25g subtracted from:

amount   Lower  Center  Upper  ---------+---------+---------+---------+
0.5g    -2.247   0.742  3.732          (--------------*--------------)
1g      -2.364   0.625  3.614         (--------------*--------------)
                           ---------+---------+---------+---------+
                                 -2.0       0.0       2.0       4.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  ---------+---------+---------+---------+
1g      -3.106  -0.117  2.872     (--------------*--------------)
                           ---------+---------+---------+---------+
                                 -2.0       0.0       2.0       4.0
```

One-way ANOVA: P g/kg versus amount

```
Source  DF      SS     MS      F      P
amount   3  23.145  7.715  51.55  0.000
Error   12   1.796  0.150
Total   15  24.940

S = 0.3869   R-Sq = 92.80%   R-Sq(adj) = 91.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
0.1g   4  6.3261  0.2656   (---*---)
0.25g  4  8.5853  0.6982                        (---*---)
0.5g   4  9.2752  0.1878                               (---*---)
1g     4  9.2325  0.0734                             (---*----)
                           -+---------+---------+---------+--------
                          6.0       7.0       8.0       9.0

Pooled StDev = 0.3869


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center   Upper     +---------+---------+---------+---------
0.25g   1.4468  2.2592  3.0716                       (----*----)
0.5g    2.1367  2.9491  3.7615                           (-----*----)
1g      2.0940  2.9064  3.7188                           (----*-----)
                           +---------+---------+---------+---------
                        -1.5       0.0       1.5       3.0


amount = 0.25g subtracted from:

amount    Lower  Center   Upper     +---------+---------+---------+---------
0.5g    -0.1225  0.6899  1.5023              (-----*----)
1g      -0.1652  0.6472  1.4596              (----*-----)
                           +---------+---------+---------+---------
                        -1.5       0.0       1.5       3.0


amount = 0.5g subtracted from:

amount    Lower   Center   Upper     +---------+---------+---------+---------
1g      -0.8551  -0.0427  0.7697         (-----*----)
                           +---------+---------+---------+---------
                        -1.5       0.0       1.5       3.0
```

One-way ANOVA: Cr versus amount

```
Source  DF     SS    MS     F      P
amount   3  10.96  3.65  3.52  0.049
Error   12  12.44  1.04
Total   15  23.40

S = 1.018   R-Sq = 46.84%   R-Sq(adj) = 33.54%


                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level  N   Mean  StDev   -+---------+---------+---------+--------
0.1g   4  0.978  1.955   (--------*--------)
0.25g  4  2.161  0.138             (--------*--------)
0.5g   4  3.050  0.412                    (--------*---------)
1g     4  2.941  0.369                   (---------*--------)
                        -+---------+---------+---------+--------
                       0.0       1.2       2.4       3.6

Pooled StDev = 1.018


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center  Upper  -----+---------+---------+---------+----
0.25g   -0.955   1.184  3.322            (----------*----------)
0.5g    -0.066   2.072  4.210                 (---------*----------)
1g      -0.174   1.964  4.102                (----------*----------)
                           -----+---------+---------+---------+----
                             -2.0       0.0       2.0       4.0


amount = 0.25g subtracted from:

amount   Lower  Center  Upper  -----+---------+---------+---------+----
0.5g    -1.250   0.889  3.027          (---------*----------)
1g      -1.358   0.780  2.918         (----------*----------)
                           -----+---------+---------+---------+----
                             -2.0       0.0       2.0       4.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  -----+---------+---------+---------+----
1g      -2.246  -0.108  2.030      (---------*----------)
                           -----+---------+---------+---------+----
                             -2.0       0.0       2.0       4.0
```

One-way ANOVA: Cu versus amount

```
Source  DF     SS    MS     F      P
amount   3  25.97  8.66  4.04  0.034
Error   12  25.74  2.14
Total   15  51.71

S = 1.465   R-Sq = 50.22%   R-Sq(adj) = 37.78%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  -----+---------+---------+---------+----
0.1g   4  0.816  1.633  (---------*---------)
0.25g  4  1.984  2.296         (---------*---------)
0.5g   4  3.996  0.354                    (---------*---------)
1g     4  3.591  0.720                  (---------*---------)
                        -----+---------+---------+---------+----
                           0.0       1.6       3.2       4.8

Pooled StDev = 1.465


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center  Upper  -------+---------+---------+---------+--
0.25g   -1.908   1.168  4.243             (---------*---------)
0.5g     0.104   3.180  6.255                   (----------*---------)
1g      -0.300   2.775  5.851                 (---------*----------)
                        -------+---------+---------+---------+--
                            -3.0       0.0       3.0       6.0


amount = 0.25g subtracted from:

amount   Lower  Center  Upper  -------+---------+---------+---------+--
0.5g    -1.064   2.012  5.087              (----------*---------)
1g      -1.468   1.607  4.683             (---------*----------)
                        -------+---------+---------+---------+--
                            -3.0       0.0       3.0       6.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  -------+---------+---------+---------+--
1g      -3.480  -0.404  2.671       (----------*---------)
                        -------+---------+---------+---------+--
                            -3.0       0.0       3.0       6.0
```

One-way ANOVA: Mn versus amount

```
Source  DF     SS     MS      F      P
amount   3  44.39  14.80  10.06  0.001
Error   12  17.65   1.47
Total   15  62.04

S = 1.213   R-Sq = 71.55%   R-Sq(adj) = 64.44%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  --+---------+---------+---------+-------
0.1g   4   8.878  1.213  (-----*------)
0.25g  4   9.319  1.544    (------*-----)
0.5g   4  13.060  1.216                      (-----*------)
1g     4  11.266  0.741             (-----*------)
                         --+---------+---------+---------+-------
                         8.0      10.0      12.0      14.0

Pooled StDev = 1.213


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center  Upper  --------+---------+---------+---------+-
0.25g   -2.106   0.441  2.988             (------*-------)
0.5g     1.635   4.182  6.729                      (------*------)
1g      -0.158   2.388  4.935                   (------*------)
                         --------+---------+---------+---------+-
                              -3.5       0.0       3.5       7.0


amount = 0.25g subtracted from:

amount   Lower  Center  Upper  --------+---------+---------+---------+-
0.5g     1.194   3.741  6.288                     (-------*------)
1g      -0.599   1.947  4.494                  (-------*------)
                         --------+---------+---------+---------+-
                              -3.5       0.0       3.5       7.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  --------+---------+---------+---------+-
1g      -4.340  -1.794  0.753       (------*------)
                         --------+---------+---------+---------+-
                              -3.5       0.0       3.5       7.0
```

One-way ANOVA: Ni versus amount

```
Source  DF      SS     MS     F      P
amount   3  12.079  4.026  9.08  0.002
Error   12   5.322  0.444
Total   15  17.401

S = 0.6660   R-Sq = 69.41%   R-Sq(adj) = 61.77%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
0.1g   4  0.6925  0.8717    (------*------)
0.25g  4  1.9135  0.9364              (------*------)
0.5g   4  3.0203  0.1957                        (------*------)
1g     4  2.5165  0.3148                  (------*------)
                            +---------+---------+---------+---------
                          0.0       1.0       2.0       3.0

Pooled StDev = 0.6660


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.25g   -0.1775  1.2210  2.6195               (--------*-------)
0.5g     0.9292  2.3278  3.7263                      (--------*-------)
1g       0.4255  1.8240  3.2225                  (-------*--------)
                                 ------+---------+---------+---------+---
                                    -1.6       0.0       1.6       3.2


amount = 0.25g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.5g    -0.2918  1.1067  2.5053               (--------*--------)
1g      -0.7955  0.6030  2.0015           (--------*--------)
                                 ------+---------+---------+---------+---
                                    -1.6       0.0       1.6       3.2


amount = 0.5g subtracted from:

amount    Lower   Center   Upper  ------+---------+---------+---------+---
1g      -1.9023  -0.5038  0.8948      (--------*--------)
                                  ------+---------+---------+---------+---
                                     -1.6       0.0       1.6       3.2
```

One-way ANOVA: Se versus amount

```
Source  DF      SS     MS      F      P
amount   3  15.294  5.098  12.01  0.001
Error   12   5.096  0.425
Total   15  20.390

S = 0.6517   R-Sq = 75.01%   R-Sq(adj) = 68.76%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
0.1g   4  0.3973  0.7945  (------*------)
0.25g  4  0.5695  0.8936    (------*------)
0.5g   4  2.5098  0.3917                      (------*------)
1g     4  2.3540  0.3398                    (-------*------)
                          ---+---------+---------+---------+------
                           0.0       1.0       2.0       3.0

Pooled StDev = 0.6517


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount    Lower  Center   Upper  -------+---------+---------+---------+--
0.25g   -1.1962  0.1722  1.5407             (------*------)
0.5g     0.7440  2.1125  3.4810                      (------*-----)
1g       0.5882  1.9567  3.3252                    (------*------)
                                -------+---------+---------+---------+--
                                    -2.0       0.0       2.0       4.0


amount = 0.25g subtracted from:

amount   Lower  Center   Upper  -------+---------+---------+---------+--
0.5g    0.5718  1.9403  3.3087                    (------*------)
1g      0.4160  1.7845  3.1529                   (------*------)
                               -------+---------+---------+---------+--
                                   -2.0       0.0       2.0       4.0


amount = 0.5g subtracted from:

amount    Lower   Center   Upper  -------+---------+---------+---------+--
1g      -1.5243  -0.1558  1.2127           (------*------)
                                -------+---------+---------+---------+--
                                    -2.0       0.0       2.0       4.0
```

One-way ANOVA: Zn versus amount

```
Source  DF      SS     MS     F      P
amount   3   56.58  18.86  4.90  0.019
Error   12   46.23   3.85
Total   15  102.81

S = 1.963   R-Sq = 55.03%   R-Sq(adj) = 43.79%


                        Individual 95% CIs For Mean Based on Pooled
                        StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
0.1g   4  17.240  2.597     (--------*--------)
0.25g  4  19.602  2.085               (--------*--------)
0.5g   4  21.710  1.711                       (--------*--------)
1g     4  21.868  1.179                        (--------*--------)
                           +---------+---------+---------+---------
                        15.0      17.5      20.0      22.5

Pooled StDev = 1.963


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center  Upper  ------+---------+---------+---------+---
0.25g   -1.760   2.362  6.484              (---------*---------)
0.5g     0.348   4.469  8.591                   (---------*---------)
1g       0.506   4.628  8.750                   (----------*---------)
                           ------+---------+---------+---------+---
                              -4.0       0.0       4.0       8.0


amount = 0.25g subtracted from:

amount   Lower  Center  Upper  ------+---------+---------+---------+---
0.5g    -2.014   2.108  6.230             (---------*----------)
1g      -1.855   2.267  6.389             (----------*---------)
                           ------+---------+---------+---------+---
                              -4.0       0.0       4.0       8.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper  ------+---------+---------+---------+---
1g      -3.963   0.159  4.281        (---------*----------)
                           ------+---------+---------+---------+---
                              -4.0       0.0       4.0       8.0
```

One-way ANOVA: As versus amount

```
Source  DF     SS    MS     F      P
amount   3  197.9  66.0  5.09  0.017
Error   12  155.5  13.0
Total   15  353.4

S = 3.600   R-Sq = 56.00%   R-Sq(adj) = 45.00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
0.1g   4  50.980  4.626                   (-------*-------)
0.25g  4  47.380  4.471              (-------*-------)
0.5g   4  43.781  3.063     (-------*------)
1g     4  41.775  1.031  (-------*------)
                         ----+---------+---------+---------+-----
                          40.0      45.0      50.0      55.0

Pooled StDev = 3.600


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount    Lower  Center   Upper     -+---------+---------+---------+--------
0.25g   -11.160  -3.601   3.959            (--------*---------)
0.5g    -14.759  -7.200   0.360         (--------*--------)
1g      -16.764  -9.205  -1.646      (--------*---------)
                                    -+---------+---------+---------+--------
                                  -16.0      -8.0       0.0       8.0


amount = 0.25g subtracted from:

amount    Lower  Center  Upper     -+---------+---------+---------+--------
0.5g    -11.158  -3.599  3.960            (---------*--------)
1g      -13.164  -5.604  1.955         (--------*--------)
                                    -+---------+---------+---------+--------
                                  -16.0      -8.0       0.0       8.0


amount = 0.5g subtracted from:

amount   Lower  Center  Upper     -+---------+---------+---------+--------
1g      -9.565  -2.005  5.554            (--------*---------)
                                    -+---------+---------+---------+--------
                                  -16.0      -8.0       0.0       8.0
```

One-way ANOVA: Cd versus amount

```
Source  DF      SS     MS     F      P
amount   3  13.253  4.418  9.37  0.002
Error   12   5.659  0.472
Total   15  18.913

S = 0.6867   R-Sq = 70.08%   R-Sq(adj) = 62.59%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---+---------+---------+---------+------
0.1g   4  0.4963  0.5847  (-------*------)
0.25g  4  1.0410  1.2021        (------*-------)
0.5g   4  2.7073  0.2269                       (------*-------)
1g     4  2.3525  0.2195                  (-------*------)
                          ---+---------+---------+---------+------
                           0.0       1.0       2.0       3.0

Pooled StDev = 0.6867


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.25g   -0.8974  0.5448  1.9869             (------*------)
0.5g     0.7688  2.2110  3.6532                      (------*------)
1g       0.4141  1.8563  3.2984                 (------*------)
                                ------+---------+---------+---------+---
                                   -2.0       0.0       2.0       4.0


amount = 0.25g subtracted from:

amount    Lower  Center   Upper  ------+---------+---------+---------+---
0.5g     0.2241  1.6663  3.1084                (------*-------)
1g      -0.1307  1.3115  2.7537              (-------*------)
                                ------+---------+---------+---------+---
                                   -2.0       0.0       2.0       4.0


amount = 0.5g subtracted from:

amount    Lower   Center   Upper  ------+---------+---------+---------+---
1g      -1.7969  -0.3548  1.0874        (------*------)
                                ------+---------+---------+---------+---
                                   -2.0       0.0       2.0       4.0
```

One-way ANOVA: Hg mg/kg versus amount

```
Source  DF         SS         MS     F      P
amount   3  0.0003998  0.0001333  3.53  0.049
Error   12  0.0004534  0.0000378
Total   15  0.0008532

S = 0.006147   R-Sq = 46.86%   R-Sq(adj) = 33.57%

                             Individual 95% CIs For Mean Based on Pooled
                             StDev
Level  N      Mean     StDev     +---------+---------+---------+---------
0.1g   4  0.000000  0.000000     (---------*---------)
0.25g  4  0.002500  0.005000         (---------*--------)
0.5g   4  0.009725  0.006912                   (---------*--------)
1g     4  0.012153  0.008852                       (--------*---------)
                                +---------+---------+---------+---------
                             -0.0070    0.0000    0.0070    0.0140

Pooled StDev = 0.006147


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%

amount = 0.1g subtracted from:

amount      Lower    Center     Upper
0.25g   -0.010409  0.002500  0.015409
0.5g    -0.003184  0.009725  0.022634
1g      -0.000756  0.012153  0.025061

amount  ---------+---------+---------+---------+
0.25g             (----------*----------)
0.5g                    (----------*----------)
1g                        (----------*----------)
        ---------+---------+---------+---------+
              -0.012     0.000     0.012     0.024


amount = 0.25g subtracted from:

amount      Lower    Center     Upper
0.5g    -0.005684  0.007225  0.020134
1g      -0.003256  0.009653  0.022561

amount  ---------+---------+---------+---------+
0.5g                (----------*----------)
1g                    (----------*----------)
        ---------+---------+---------+---------+
              -0.012     0.000     0.012     0.024

amount = 0.5g subtracted from:

amount      Lower    Center     Upper
1g      -0.010481  0.002428  0.015336

amount  ---------+---------+---------+---------+
1g                (----------*----------)
        ---------+---------+---------+---------+
              -0.012     0.000     0.012     0.024
```

One-way ANOVA: Pb versus amount

```
Source  DF      SS     MS     F      P
amount   3   7.283  2.428  9.71  0.002
Error   12   3.000  0.250
Total   15  10.283

S = 0.5000   R-Sq = 70.83%   R-Sq(adj) = 63.53%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
0.1g   4  0.7475  0.8742  (-----*------)
0.25g  4  1.8495  0.4534              (------*------)
0.5g   4  2.4640  0.1313                      (------*------)
1g     4  2.3278  0.1137                    (------*------)
                          -------+---------+---------+---------+--
                               0.80      1.60      2.40      3.20

Pooled StDev = 0.5000


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of amount

Individual confidence level = 98.83%


amount = 0.1g subtracted from:

amount   Lower  Center   Upper  ----+---------+---------+---------+-----
0.25g   0.0520  1.1020  2.1520               (--------*--------)
0.5g    0.6665  1.7165  2.7665                     (--------*--------)
1g      0.5303  1.5803  2.6302                   (--------*--------)
                          ----+---------+---------+---------+-----
                           -1.2       0.0       1.2       2.4


amount = 0.25g subtracted from:

amount    Lower  Center   Upper  ----+---------+---------+---------+-----
0.5g    -0.4355  0.6145  1.6645           (--------*--------)
1g      -0.5717  0.4783  1.5282          (--------*--------)
                          ----+---------+---------+---------+-----
                           -1.2       0.0       1.2       2.4


amount = 0.5g subtracted from:

amount    Lower   Center   Upper  ----+---------+---------+---------+-----
1g      -1.1863  -0.1363  0.9137      (--------*--------)
                          ----+---------+---------+---------+-----
                           -1.2       0.0       1.2       2.4
```

Appendix 2: Statistical outputs for the comparison of freeze dried versus oven dried samples

(Ca) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   8.73597   0.19801   0.08084
freeze dry  6   8.59121   0.26967   0.11009
Difference  6  0.144757  0.347025  0.141672


95% CI for mean difference: (-0.219424, 0.508937)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.02  P-Value = 0.354
```

(K) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   30.3135    0.3498    0.1428
freeze dry  6   29.7071    0.7956    0.3248
Difference  6  0.606393  0.782443  0.319431


95% CI for mean difference: (-0.214730, 1.427516)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.90  P-Value = 0.116
```

(Mg) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   8.51619   0.15767   0.06437
freeze dry  6   8.49138   0.27662   0.11293
Difference  6  0.024803  0.269298  0.109940


95% CI for mean difference: (-0.257807, 0.307414)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.23  P-Value = 0.830
```

(Na) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   24.4314    0.7267    0.2967
freeze dry  6   24.1623    0.4860    0.1984
Difference  6  0.269087  0.799757  0.326499


95% CI for mean difference: (-0.570206, 1.108381)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.82  P-Value = 0.447
```

(P) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   8.67344   0.17136   0.06996
freeze dry  6   8.54040   0.34882   0.14240
Difference  6  0.133038  0.278679  0.113770


95% CI for mean difference: (-0.159417, 0.425494)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.17  P-Value = 0.295
```

(Cr) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N       Mean     StDev   SE Mean
oven dry    6   0.571591  0.059688  0.024367
freeze dry  6   0.597320  0.070175  0.028649
Difference  6  -0.025730  0.032425  0.013237


95% CI for mean difference: (-0.059758, 0.008298)
T-Test of mean difference = 0 (vs not = 0): T-Value = -1.94  P-Value = 0.110
```

(Cu) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   2.72751   0.23183   0.09464
freeze dry  6   2.67054   0.25347   0.10348
Difference  6  0.056969  0.100450  0.041009


95% CI for mean difference: (-0.048447, 0.162385)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.39  P-Value = 0.223
```

(Mn) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   7.55316   0.32278   0.13177
freeze dry  6   7.45826   0.23257   0.09495
Difference  6  0.094896  0.353980  0.144512


95% CI for mean difference: (-0.276583, 0.466376)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.66  P-Value = 0.540
```

(Ni) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   2.17741   0.20342   0.08305
freeze dry  6   1.87468   0.42105   0.17189
Difference  6  0.302728  0.570458  0.232888


95% CI for mean difference: (-0.295931, 0.901386)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.30  P-Value = 0.250
```

(Se) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6  0.802939  0.047353  0.019332
freeze dry  6  0.798272  0.056416  0.023032
Difference  6  0.004667  0.080653  0.032926


95% CI for mean difference: (-0.079973, 0.089307)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.14  P-Value = 0.893
```

(Zn) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   30.2543    0.7433    0.3035
freeze dry  6   30.1419    2.1486    0.8772
Difference  6  0.112435  2.278551  0.930215


95% CI for mean difference: (-2.278757, 2.503628)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.12  P-Value = 0.909
```

(As) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6   33.4294    3.5358    1.4435
freeze dry  6   33.2011    3.5007    1.4292
Difference  6  0.228259  5.361765  2.188931


95% CI for mean difference: (-5.398568, 5.855086)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.10  P-Value = 0.921
```

(Cd) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N       Mean     StDev   SE Mean
oven dry    6    1.77880   0.14469   0.05907
freeze dry  6    1.78947   0.16042   0.06549
Difference  6  -0.010668  0.075704  0.030906


95% CI for mean difference: (-0.090115, 0.068778)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.35  P-Value = 0.744
```

(Hg) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6  0.046758  0.040513  0.016539
freeze dry  6  0.037418  0.042865  0.017499
Difference  6  0.009340  0.042839  0.017489


95% CI for mean difference: (-0.035617, 0.054296)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.53  P-Value = 0.616
```

(Pb) Paired T-Test and CI: oven dry, freeze dry

```
Paired T for oven dry - freeze dry

            N      Mean     StDev   SE Mean
oven dry    6  0.267955  0.026664  0.010886
freeze dry  6  0.247316  0.019874  0.008113
Difference  6  0.020639  0.022149  0.009042


95% CI for mean difference: (-0.002604, 0.043883)
T-Test of mean difference = 0 (vs not = 0): T-Value = 2.28  P-Value = 0.071
```

Appendix 3: Table of metal contents of *Undaria pinnatifida* collected from four different sites in New Zealand.

Values are the range of the monthly means ± standard error

Metal	Site	Blade	Sporophyll
Ca (g/kg)	PE327	9.77 ± 0.148(April) – 16.97 ± 0.45 (June)	7.88 ± 0.24(April) – 8.75 ± 0.35(June)
Ca (g/kg)	106	8.26 ± 0.08 (August) – 9.07 ± 0.34 (October)	6.37 ± 0.13(July) – 7.03 ± 0.39(October)
Ca (g/kg)	Wellington Site A	8.89 ± 0.17(August) – 10.13 ± 0.20 (October)	6.72 ± 0.17(August) – 7.03 ± 0.055(October)
Ca (g/kg)	Wellington Site B	8.76 ± 0.20 (August) – 10.31 ± 0.40(October	7.20 ± 0.39(August) – 7.41 ± 0.23(October)
K (g/mg)	PE327	19.42 ± 0.26 (May) – 45.86 ± 0.91 (October	27.25 ± 0.27(May) – 28.69 ± 0.86(June)
K (g/mg)	106	32.85 ± 0.33 (August) – 42.14 ± 0.59 (October)	15.18 ± 0.54(July) – 16.10 ± 0.44(October)
K (g/mg)	Wellington Site A	29.49 ± 0.62(August) – 44.68 ± 0.52(October)	27.12 ± 0.88(July) – 28.97 ± 0.29(October)
K (g/mg)	Wellington Site B	27.92 ± 0.62(August) – 48.48 ± 0.56(October)	26.21 ± 0.49(November) – 27.08 ± 0.52(October)
Mg (g/kg)	PE327	6.83 ± 0.14 (April) – 9.21 ± 0.36 (October)	4.58 ± 0.36(April) – 6.64 ± 0.32(October)
Mg (g/kg)	106	8.20 ± 0.16(July) – 9.47 ± 0.31(October)	5.75 ± 0.045(August) – 6.16 ± 0.37(October)
Mg (g/kg)	Wellington Site A	8.34 ± 0.32(August) – 9.23 ± 0.33(October)	5.45 ± 0.14(November) – 5.72 ± 0.11(August)
Mg (g/kg)	Wellington Site B	8.26 ± 0.21(August)– 9.47 ± 0.22(October)	7.30 ± 0.22(August) – 7.59 ± 0.14(October)
Na (g/mg)	PE327	11.26 ± 0.19(April) – 62.55 ± 0.68(October)	10.46 ± 0.21(April) – 32.10 ± 0.46(July)
Na (g/mg)	106	54.24 ± 0.48(August) – 58.31 ± 0.55(October)	30.62 ± 0.47(August) – 33.83 ± 0.50(October)
Na (g/mg)	Wellington Site A	24.83 ± 0.33(August) – 28.60 ± 0.51(October)	10.45 ± 0.14(August) – 20.42 ± 0.16(September)
Na (g/mg)	Wellington Site B	24.15 ± 0.95(August)– 28.18 ± 0.28(October)	9.82 ± 0.35(August) – 19.95 ± 0.15(October)
P (g/kg)	PE327	9.28 ± 0.19(July) – 12.05 ± 0.23(October	7.89 ± 0.16(May) – 9.41 ± 0.30(October)
P (g/kg)	106	10.41 ± 0.19(July) – 11.62 ± 0.26(October)	6.60 ± 0.42(July) – 7.76 ± 0.73(October)
P (g/kg)	Wellington Site A	9.09 ± 0.19(August) – 10.31 ± 0.66(October)	8.24 ± 0.053(November) – 8.54 ± 0.13(October)
P (g/kg)	Wellington Site B	8.60 ± 0.43(August) – 10.61 ± 0.43(October)	7.99 ± 0.16(August) – 8.71 ± 0.11(October)

Cr (mg/kg)	PE327	0.69 ± 0.094(April) – 1.04 ± 0.21(October)	0.76 ± 0.065(April) – 0.92 ± 0.024(June)
Cr (mg/kg)	106	0.74 ± 0.077(August) – 0.78 ± 0.053(July)	0.70 ± 0.027(September) – 0.77 ± 0.016(July)
Cr (mg/kg)	Wellington Site A	0.80 ± 0.093(September) – 0.84 ± 0.020(October)	0.68 ± 0.031(August) – 0.74 ± 0.026(October)
Cr (mg/kg)	Wellington Site B	0.64 ± 0.060(August) – 0.73 ± 0.029(October)	0.57 ± 0.06(November) – 0.69 ± 0.019(October)
Cu (mg/kg)	PE327	3.08 ± 0.16(July) – 3.78 ± 0.23(October)	1.64 ± 0.16(September) – 1.85 ± 0.11(July)
Cu (mg/kg)	106	2.97 ± 0.22(July) – 3.77 ± 0.23(October)	1.84 ± 0.092(August) – 2.44 ± 0.16(October)
Cu (mg/kg)	Wellington Site A	2.28 ± 0.21(August) – 2.62 ± 0.15(October)	2.21 ± 0.10(August) – 2.40 ± 0.12(October)
Cu (mg/kg)	Wellington Site B	2.48 ± 0.27(September) – 2.66 ± 0.12(October)	2.33 ± 0.091(September) – 2.64 ± 0.11(October)
Mn (mg/kg)	PE327	8.73 ± 0.58(July) – 10.39 ± 2.45(October)	4.79 ± 0.26(July) – 7.72 ± 0.85(April)
Mn (mg/kg)	106	8.25 ± 0.67(August) – 9.99 ± 1.26(October)	5.59 ± 0.38(July) – 6.26 ± 0.40(October)
Mn (mg/kg)	Wellington Site A	12.57 ± 0.78(September)– 14.61 ± 1.23(October)	6.86 ± 0.59(November) – 7.68 ± 0.24(October)
Mn (mg/kg)	Wellington Site B	8.36 ± 0.17(November)– 8.57 ± 0.19(October)	7.46 ± 0.28(November) -7.93 ± 0.13(October)
Ni (mg/kg)	PE327	1.54 ± 0.39(April) – 2.78 ± 0.12(October)	1.14 ± 0.16(May) – 1.62 ± 0.35(April)
Ni (mg/kg)	106	1.81 ± 0.13(July) – 2.24 ± 0.12(October)	1.36 ± 0.045(August) – 1.62 ± 0.18(October)
Ni (mg/kg)	Wellington Site A	1.78 ± 0.11(November) – 1.95 ± 0.067(October)	1.50 ± 0.078(November) – 1.69 ± 0.056(August)
Ni (mg/kg)	Wellington Site B	1.91 ± 0.12(August) – 2.10 ± 0.057(October)	1.53 ± 0.27(November) – 1.70 ± 0.050(October)
Se (mg/kg)	PE327	0.54 ± 0.017(April) – 0.61 ± 0.016(October)	0.18 ± 0.0173(June) – 0.33 ± 0.030(July)
Se (mg/kg)	106	0.40 ± 0.02(July) – 0.53 ± 0.056(October)	0.37 ± 0.041(September) – 0.40 ± 0.021(October)
Se (mg/kg)	Wellington Site A	0.80 ± 0.049(September) – 0.83 ± 0.14(August)	0.48 ± 0.039(November) – 0.52 ± 0.03(September)
Se (mg/kg)	Wellington Site B	0.65 ± 0.11(August) – 0.81 ± 0.078(October)	0.38 ± 0.03(August) – 0.48 ± 0.025(October)
Zn (mg/kg)	PE327	20.52 ± (May) – 26.11 ± 2.71(October)	14.18 ± 1.28(May) – 18.60 ± 0.92(October)
Zn (mg/kg)	106	22.60 ± 0.76(July) – 27.30 ± 2.78(October)	21.16 ± 1.70(August) – 23.60 ± 2.33(October)
Zn (mg/kg)	Wellington Site A	30.24 ± 1.64(September) – 33.39 ± 3.99(October)	13.70 ± 1.06(August) – 15.41 ± 0.53(October)
Zn (mg/kg)	Wellington Site B	29.41 ± 1.66(September) – 35.03 ± 2.05(October)	15.10 ± 1.03(November) – 16.01 ± 0.49(October)

As (mg/kg)	PE327	40.54 ± 2.00(October) - 46.71 ± 0.75(May)	23.84 ± 1.49(April) – 29.47 ± 1.75(July)
As (mg/kg)	106	30.41 ± 1.52(August) – 31.89 ± 2.22(July)	23.94 ± 1.45(August) - 29.23 ± 2.27(July)
As (mg/kg)	Wellington Site A	34.79 ± 2.25(November) – 42.88 ± 2.56(August)	28.46 ± 5.87(October) – 32.84 ± 2.30(August)
As (mg/kg)	Wellington Site B	32.12 ± 1.77(November) – 36.41 ± 3.30(August)	30.03 ± 2.40(August) – 31.27 ± 7.38(September)
Cd (mg/kg)	PE327	2.33 ± 0.21(October) – 2.91 ± 0.097(June)	1.82 ± 0.26(October) – 2.19 ± 0.17(May)
Cd (mg/kg)	106	1.57 ± 0.088(August) – 1.74 ± 0.19(October)	1.51 ± 0.059(August) – 1.68 ± 0.11(July)
Cd (mg/kg)	Wellington Site A	2.11 ± 0.13(September) – 2.24 ± 0.17(August)	1.97 ± 0.21(October) – 2.10 ± 0.17(August)
Cd (mg/kg)	Wellington Site B	1.82 ± 0.29(November) – 2.21 ± 0.21(August)	1.67 ± 0.32(October) – 2.20 ± 0.4(September)
Hg (mg/kg)	PE327	0.023 ± 0.015(May) – 0.040 ± 0.017(September)	No values
Hg (mg/kg)	106	0.024 ± 0.0086(July) – 0.040 ± 0.0026(September)	No values
Hg (mg/kg)	Wellington Site A	0.021 ± 0.010(August) – 0.042 ± 0.020(November)	No values
Hg (mg/kg)	Wellington Site B	0.021 ± 0.0034(August) – 0.037 ± 0.026(November	No values
Pb (mg/kg)	PE327	0.22 ± 0.024(October) – 0.29 ± 0.044(April)	0.14 ± 0.0047(April) – 0.29 ± 0.048(May)
Pb (mg/kg)	106	0.24 ± 0.022(October) – 0.30 ± 0.019(July)	0.21 ± 0.015(October) – 0.27 ± 0.017(July)
Pb (mg/kg)	Wellington Site A	0.28 ± 0.018(September) – 0.31 ± 0.022(August)	0.23 ± 0.026(October) – 0.25 ± 0.016(September)
Pb (mg/kg)	Wellington Site B	0.25 ± 0.032(November) – 0.29 ± 0.029(September)	0.167 ± 0.021(October) – 0.174 ± 0.0167(August)