Investigation of the mineral profile of *Fucus serratus* Linneaus aqueous extracts and examination of the routes of uptake of minerals both *in vivo* and *in vitro*

Author Tarha Westby

Supervisors Dr. Aodhmar Cadogan

Ms. Geraldine Duignan

Submitted for the award of Doctor of Philosophy at Institute of Technology Sligo

Table of Contents

Abstract

Seaweed baths are used to promote health and well-being. Seaweed typically contains water, fibre, carbohydrate, protein, lipid, vitamins and minerals. Minerals can account for up to 40% of its composition. Iodine is vital for normal growth and development. Literature indicates that the Irish adult population is iodine deficient (82 µg L^{-1}). Diet is its main source however inhalation of gaseous iodine also occurs. Simulated seaweed baths were prepared throughout the research under varying conditions of seaweed mass and temperature. Spectrophotometric methods, i.e. Sandell-Kolthoff, AAS, FES and ICP-MS, were used to determine the mineral profile of *Fucus serratus* L. and seaweed bathwater. The *Fucus serratus* L. mineral content is approximately 2% dry weight based on the minerals measured. On addition of *Fucus serratus* L. the bathwater pH ranges from 5.29 to 6.36 with an average pH of 5.88±0.26. Lower pH influences the mineral species present; molecular iodine predominates under acidic conditions. The bathwater mineral concentrations increase with increasing mass of seaweed at constant volume however, per gram of seaweed, the relative mineral concentrations decrease. Increasing temperature (20-80 ˚C) leads to a higher efflux of minerals, most significantly between 20 and 40 ˚C. All the minerals studied show a significant difference in concentration over 12 months. Iodine levels are lowest in summer and highest in winter while all other minerals indicate highest levels in summer. The mineral contribution from seawater to the overall bathwater concentration is minimal. Iodine uptake (via inhalation) was studied *in vivo* with 30 subjects, bathers and non-bathers. Their Urinary Iodine Concentration (UIC), analysed pre and post seaweed bath, indicate a significant increase (p = 0.015) from 76 to 95 µg L^{-1} following exposure to seaweed baths. *In vitro* studies indicate that aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc may have the potential to be taken up by a bather via dermal absorption.

Declaration

I declare that this thesis was composed solely by myself, that the work contained herein is my own except where explicitly stated otherwise and that this work has not been submitted for any other qualification.

Sections of this work have been published in Botanica Marina (July 2016) and Environmental Geochemistry and Health (September 2017).

Signed: _____

Acknowledgements

I am deeply grateful to all who helped in any way along my journey, both personally and professionally.

When I reflect on this in years to come it will be your kindness that will make me smile.

We ourselves feel that what we are doing is just a drop in the ocean

But, the ocean would be less because of that missing drop

Mother Teresa

For

my

cheer

squad

#light

#blessed

#gratitude

List of abbreviations

AAS	Atomic Absorption Spectrophotometry
ACS	American Chemical Society
ADD	Attention Deficit Disorder
AMU	Atomic Mass Unit
ANOVA	Analysis of Variance
AP	Ammonium Persulphate
ATSDR	Agency for Toxic Substances and Disease Registry
CAS	Ceric Ammonium Sulphate
CRM	Certified Reference Material
CV	Coefficient of Variation
DW	Dry Weight
EC	European Commission
EPA	Environmental Protection Agency
FES	Flame Emission Spectrophotometry
GI	Gastrointestinal
ICH	International Council for Harmonisation
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDD	Iodine Deficiency Disorders
I^-	Iodide
I_2	Iodine
IO_3^-	Iodate
LC-PUFA	Long Chain Polyunsaturated Fatty Acids
NAA	Neutron Activation Analysis
NC	Not Computable
ND	Not Detected
NIST	National Institute of Standards and Technology
PBS	Phosphate Buffered Saline
RDA	Recommended Daily Allowance
RF	Radio Frequency

ROS	Reactive Oxygen Species
RPM	Revolutions per Minute
SD	Standard Deviation
SK	Sandell-Kolthoff
SRM	Standard Reference Material
TMAH	Tetramethylammonium Hydroxide
UIC	Urinary Iodine Concentration
WHO	World Health Organisation

1 Introduction

Seaweed Baths: an underexplored resource

Thalassotherapy describes the use of seawater, marine products or sea air to benefit the body (Routh and Bhowmik (1996)). This concept of bathing in seawater has existed since the Roman Empire. Various cultures e.g. Roman, Turkish, French and Arabic have indigenous bathing rituals using thermal waters for their mineromedicinal properties (Ledo (1996)). Bathing waters unique to the maritime hydro-mineral province of northern Bulgaria are characterized by a high content of dissolved mineral substances notably sodium, chloride, iodine and bromine (Vassileva (1996)). The Dead Sea is also renowned for its therapeutic value; attributed to the high concentrations of bromides, calcium, chlorides, magnesium, potassium, sodium and strontium it contains (Moses et al (2006)). One thalassotherapy, indigenous to Ireland, is a seaweed bath prepared using hot fresh water, seawater and fresh brown seaweed such as *Fucus serratus* Linneaus (L.).

The health and well-being industry in Ireland suggests that seaweed baths can aid the body in various ways including; the iodine released helps to regulate the body's metabolism; the alginates, which have been used historically to treat burns and wounds, can also reduce skin irritations and conditions such as psoriasis, eczema and dermatitis; the antioxidants contained within the seaweed can protect the body from oxygen free radicals which cause ageing and disease and the essential fatty acids can help lock moisture within the skin to reduce drying and hardening and maintain its elasticity (Voya (2017)).

These claims are loosely supported by the documented composition of seaweeds (Armisen (1995), van Netten et al (2000), Nishizawa (2002), Dawczynski et al (2007), Skulas-Ray et al (2008), Matanjun et al (2009), Polat and Ozogul (2009), Wijesinghe et al (2012)). Claims of the therapeutic properties of *Fucus serratus* L. include antioxidant, anti-inflammatory, thyroid stimulating, antimicrobial and tissue healing (Pesando and Caram (1984), Jiménez-Escrig et al (2001), Bergé et al (2002), Ruperez et al (2002), Sezer et al (2008) and Teas et al (2009)). Pesando and Caram (1984)

examined the antimicrobial activity of seaweed extracts on gram positive and negative bacteria on agar plates. Both Jiménez-Escrig et al (2001) and Rupérez et al (2002) established the antioxidant activity of seaweed isolates using standard methods i.e. free redical scavenging capacity, polyphenol content and ferric reducing power assays. Bergé et al (2002) investigated the anti-inflammatory and anti-proliferative properties *in vitro*. Sezer et al (2008) used seaweed derived fucoidan hydrogels to treat burns while Teas et al (2009) carried out an *in vivo* study investigating the effects of seaweed supplementation. It was also shown, in an animal study by Nolan et al (2000) that iodine normalizes elevated adrenal corticosteroid hormone secretion related to stress. They established the correlation between iodine deficiency and lower corticosteroid secretion in response to stresses. Indeed, the established antioxidant effect of iodine from seaweed based diets (Smyth (2003)) could also provide supportive evidence for a therapeutic effect of iodine from seaweed baths.

A review of the literature reveals that while extensive accounts of research into various species of seaweed and their attributes in cooking and cosmetic preparations have been documented; no published research explores a seaweed bath setting. This research set out to examine the bathwater matrix more closely and establish if there is any evidence to support claims of health benefits associated with bathing which are used to promote this holistic ritual.

The question of whether natural chemicals found in seaweed bathwater can provide measurable health benefits or risks for the bather was the central concept from which the research developed. This research exploits the setting of the traditional seaweed bath to investigate and quantify the release of iodine and other minerals from the brown seaweed *Fucus serratus* L. into the bathwater and the subsequent uptake of such minerals by a bather. There are four main routes by which a substance may enter the body: inhalation, ingestion, injection and dermal (skin) contact. In the context of this study, and the environment of a seaweed bath, uptake refers to entry via inhalation of volatile components from the seaweed or dermal contact with the seaweed or seaweed bathwater.

A better understanding of the seaweed and bathwater is required, specifically their chemistry before attributing benefits to bathing. In the health and well-being industry, claim substantiation plays a major role as effective marketing depends on it. It is essential that links, however tenuous, between use and efficacy of a product or service can be observed. In the absence of such links, there is scope for rigorous critique.

1.1 Seaweed and the Global Context

Seaweeds have been used as sea vegetables, medicines and fertilizers for centuries (Kiuru et al (2014)). Seaweed is an integral part of the lifestyle of various cultures globally e.g. Asian diet, French cosmetics, Irish agriculture. It is a resource which is harvested and exploited commercially for a global market which was estimated to be worth $11.34 billion in 2016 and this is expected to reach $17.59 billion by 2021 (Research and Markets (2016)).

In Ireland, the seaweed industry is worth €18 million per year. The main sectors include agriculture, algin isolation, bioactive isolation for use in cosmetics and pharmaceuticals, biofuel production and food (Tiwari and Troy (2015)). These diverse sectors exploit a variety of molecules including polysaccharides, minerals and antioxidants found in seaweed (Seghetta et al (2017)).

Chondrus crispus, *Codium sp.*, *Gigartina pistillata*, *Himanthalia elongata*, *Laminaria ochroleuca*, *Laminaria saccharina*, *Mastocarpus stellatus*, *Porphyra sp.*, *Ulva lactuca* and *Undaria pinnatifida* (***Figures 1.1-1.9***) are harvested, under licence, for use in the food sector. *Chondrus crispus*, *Mastocarpus stellatus* and *Gelidium sesquipedale* (***Figures 1.1, 1.6*** and ***1.10***) are harvested for agar and carageenan production. *Ascophyllum nodosum* and *Fucus serratus* L. (***Figures 1.11*** and ***1.12***) are harvested for use in the cosmetic industry (Walsh and Watson (2011)). *Fucus serratus* L. (200 tonnes) is harvested annually in Ireland for commercial use including use in seaweed baths (Walsh and Watson (2011)). One seaweed bath provider in the North West of Ireland can cater for up to 35, 000 clients annually.

Figure 1.1 *Chondrus crispus* in situ (AlgaeBase (2017))

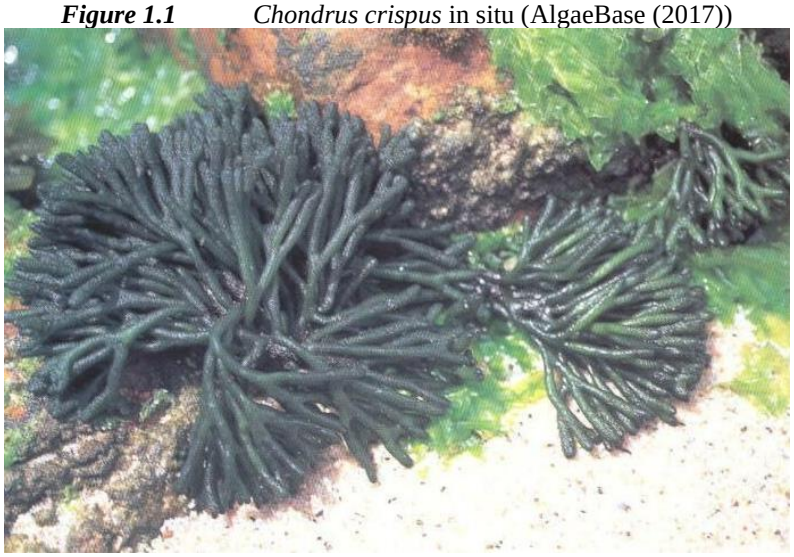


Figure 1.2 *Codium sp.*in situ (Morrissey et al (2001))

Figure 1.3 *Gigartina pistillata* in situ (AlgaeBase (2017))

Figure 1.4 *Himanthalia elongata* in situ (AlgaeBase (2017))

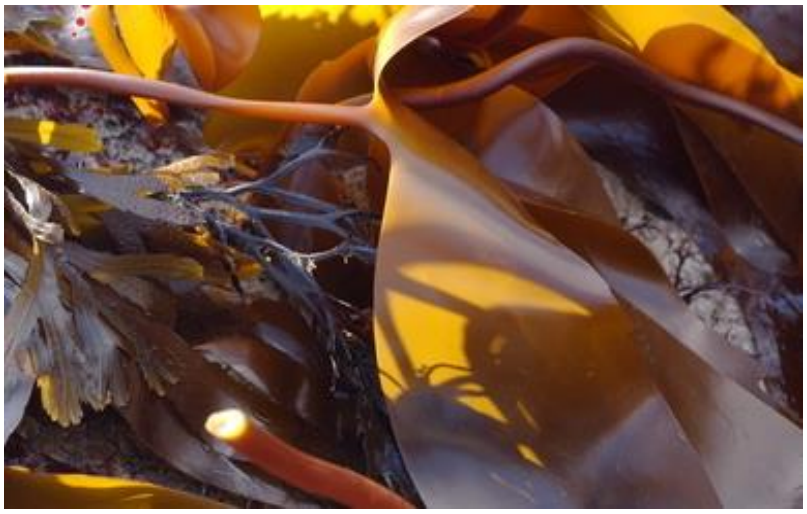


Figure 1.5 *Laminaria* in situ (AlgaeBase (2017))

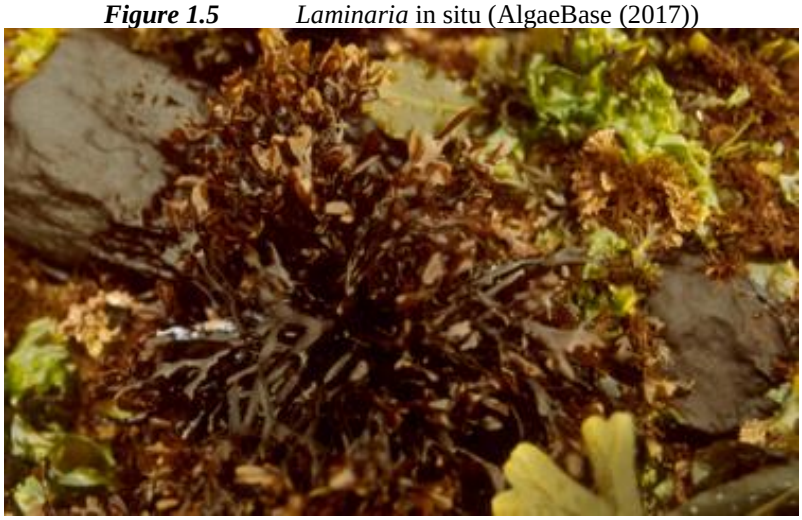


Figure 1.6 *Mastocarpus stellatus* in situ (AlgaeBase (2017))

Figure 1.7 *Porphyra sp*. in situ (AlgaeBase (2017))

Figure 1.8 *Ulva lactuca* in situ (AlgaeBase (2017))

Figure 1.9 *Undaria pinnatifida* in situ (AlgaeBase (2017))

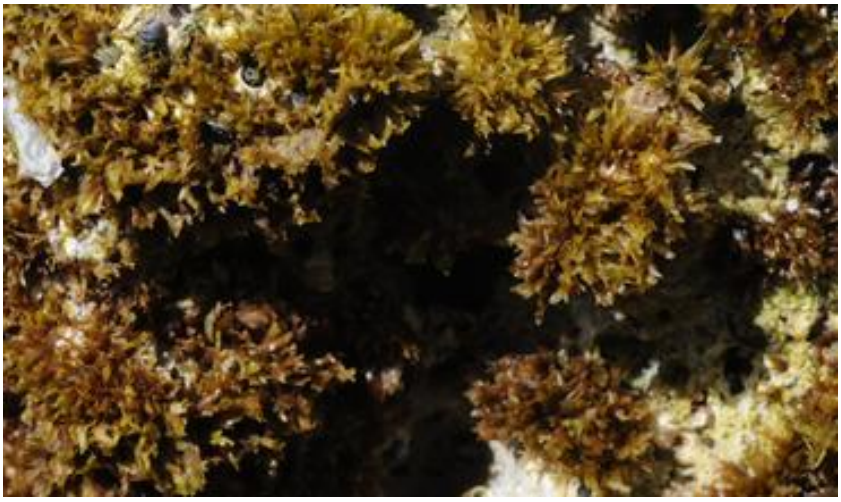


Figure 1.10 *Gelidium sesquipedale* in situ (AlgaeBase (2017))

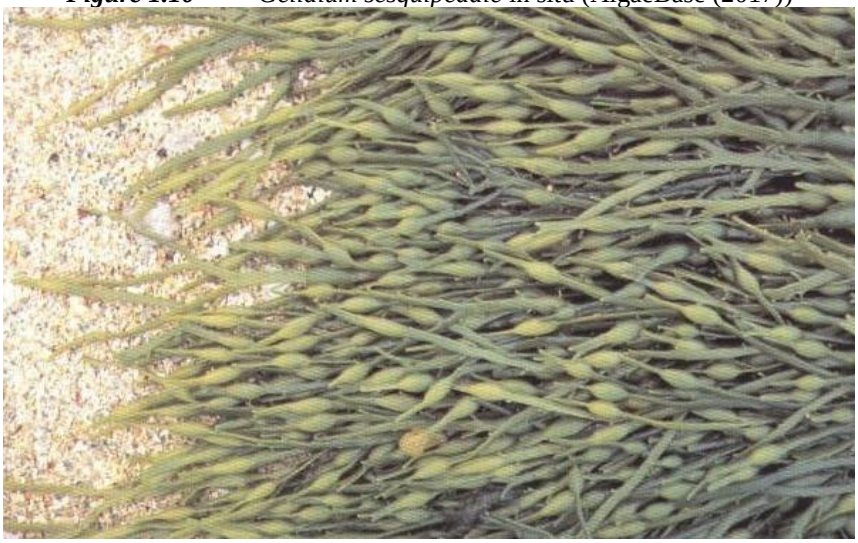


Figure 1.11 *Ascophyllum nodosum* in situ (AlgaeBase (2017))

Figure 1.12 *Fucus serratus* L.in situ (AlgaeBase (2017))

1.1.2 Agriculture

The use of seaweed as a fertiliser has a long history in coastal regions with sandy textured soils which have low levels of organic matter. The improved nutrient content of the soil is associated with biological mineralisation of the seaweed and the interaction between the soil itself and organic compounds derived from the seaweed. The presence of polysaccharides in the seaweed also aid soil binding (Haslam and Hopkins (1996)).

Seaweed supplementation of livestock diet is evident in writings from ancient Greece. Due to its high mineral content *Ascophyllum nodosum* is the seaweed most frequently used as animal feed (Evans and Critchley (2014)) and represents over 75% of the seaweed harvested in Ireland (Walsh and Watson (2011)). Using seaweed as an agricultural food source can prevent mineral deficiency in the food chain. The health benefits associated with seaweed consumption i.e. anti-oxidant, anti-microbial also applies to animals and results in healthier livestock (Evans and Critchley (2014)).

1.1.3 Algin isolation

Algin is a phycocolloid (algal polysaccharide) which is extracted from seaweed. Its form is either acidic or salt. Brown seaweeds in particular, produce highly viscous bioabsorbable alginic acid (Dawczynski et al (2007)). The alginate salt, a mixed salt of calcium, magnesium, potassium or sodium, is a key component of the cell wall (40–50% dry weight (DW)) (Bruneton (1995)). The latest data on global alginate markets show that approximately 30,000 tonnes of alginate is produced annually (Abreu et al (2014)). It is routinely used as a gelling agent, thickener, stabiliser and emulsifier in food e.g. ice-cream and mayonnaise (Brownlee et al (2009)) but is also suitable in medicine and for tissue culture (Adams (2016)) as well as pharmaceutical, cosmetic and food industries (Armisen (1995)).

1.1.4 Bioactive isolation

Biologically active metabolites, which possess benefits akin to traditional drugs, are also isolated from seaweed (Wijesinghe et al (2012)). As seaweeds are exposed to environmental stressors, they adapt by developing compounds for protection. A number of bioactive isolates of seaweed, with documented *in vivo* efficacy (***Table 1.1***) are regularly incorporated into topical cosmetic and pharmaceutical products e.g. skin moisturizers, toothpaste, colour cosmetics and gels (Lewington (2003), Rupérez (2002), Dhargalkar and Pereira (2005), Makkar et al (2015)).

Table 1.1	**Bioactive components isolated from seaweed**	
Agar	Anti-coagulant Anti-oxidant Gelling agent	Dawczynski et al (2007) Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Zanella et al (2014) Wang et al (2015)
Algin	Gelling agent Skin regeneration	Dawczynski et al (2007) Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Zanella et al (2014) Wang et al (2015)
Carrageenan	Anti-coagulant Anti-inflammatory Anti-microbial Anti-tumour Gelling agent	Dawczynski et al (2007) Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Wang et al (2015)
Fatty acid e.g. omega-3	Anti-ageing Anti-inflammatory Anti-oxidant Cell structure Lowering cholesterol	Kim et al (1996) Khotimchenko (1998) Narayan et al (2006) Spolaore (2006) Dawczynski et al (2007)

Table 1.1	**Bioactive components isolated from seaweed**	
Fatty acid **e.g. omega-3**	Photo protective Skin regeneration	Kim et al (2008) Skulas-Ray et al (2008) Mohamed et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Zanella et al (2014) Wang et al (2015)
Phenolic **e.g. phlorotannin**	Anti-ageing Anti-allergic Anti-inflammatory Anti-microbial Anti-oxidant Anti-tumour Free radical scavenger Photo protective Wound healing	Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Wang et al (2015) Leyton et al (2016)
Pigment **e.g. fucoxanthin (carotenoid)**	Anti-oxidant Photo protective Skin regeneration	Narayan et al (2006) Spolaore (2006) Kim et al (2008) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Zanella et al (2014) Wang et al (2015) Leyton et al (2016) Galasso et al (2017)
Polysaccharide **e.g. fucoidan**	Anti-ageing Anti-coagulant Anti-inflammatory Anti-microbial Anti-tumour Lower cholesterol	Spolaore (2006) Dawczynski et al (2007) Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Zanella et al (2014) Wang et al (2015) Leyton et al (2016)
Terpenoid	Photo protective	Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013)

Table 1.1	Bioactive components isolated from seaweed	
Terpenoid	Photo protective	Zanella et al (2014)
Vitamin **e.g. tocopherol**	Photo protective	Narayan et al (2006) Spolaore (2006) Dawczynski et al (2007) Kim et al (2008) Mohamed et al (2012) Wijesinghe et al (2012) Agatonovic-Kustrin and Morton (2013) Borowitzka (2013) Zanella et al (2014) Wang et al (2015)

1.1.5 Biofuel

As alternatives to fossil fuels are investigated; it has been shown that biogas and biomethane are produced by anaerobic digestion of seaweed (Vanegas and Bartlett (2013)). The seaweed-derived biogas is a sustainable source of fuel and a replacement of natural gas since seaweeds have a higher growth rate and unlike terrestrial plants, do not compete for arable land, fertilizer or fresh water (Gao and Mc Kinley (1994)). As cultivation and harvesting requires minimal human labour, fuel production is inexpensive compared to current fuel production costs.

1.1.6 Food

In Western cultures seaweeds have most commonly been used as a source of polysaccharides for the food industry. This contrasts with Asian cultures in which, seaweed forms a staple part of the diet, incorporated into soups, salads and sushi (Rupérez (2002)). Algin is extracted from the plant and used in food production e.g. as a bulking or gelling agent (Bocanegra et al (2009)). In Ireland, Dulse (*Palmaria palmata*) has been traditionally eaten raw however more recently is being incorporated into the diet as food is prepared e.g. bread and stew (Mouritsen (2013)). Globally, algae is increasingly regarded as a rich source of vitamins and minerals, which can be lacking in terrestrial plants, and as a result is used to enrich the diet (Bocanegra et al (2009)).

1.1.7 Seaweed Baths

Seaweed baths are a traditional therapy in Ireland where baths containing seaweed e.g. *Fucus serratus* L. (***Figure 1.13***) are used to promote health and well-being. Typical commercial baths prepared using approximately 5 kg of fresh *Fucus serratus* L. in 100 litres of water at 40–50 ˚C. Anecdotally, seaweed baths have many beneficial properties attributed to the various minerals, vitamins, complex polysaccharides and proteins present in high concentrations in the seaweed (***Figure 1.14***).

Figure 1.13 Typical *Fucus serratus* L. seaweed bath

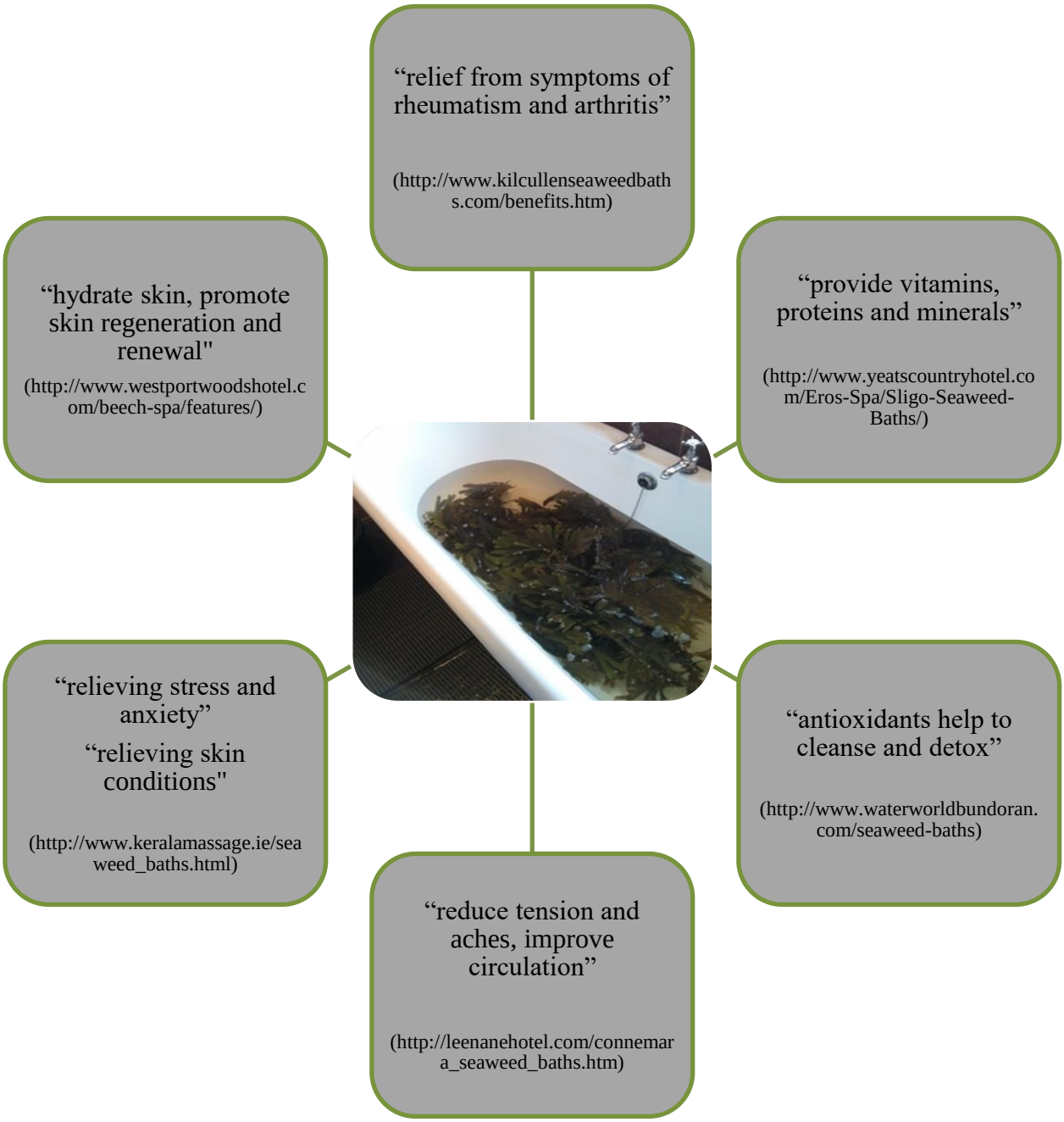


Figure 1.14 Claims of health benefits of seaweed baths

1.2 Seaweed

The term "seaweed" does not have any taxonomic value rather it is a popular term used to describe the common large marine algae (Makkar et al (2015)). Algae can be classified into two groups; microalgae and macroalgae (seaweeds). Seaweeds are macroscopic, multicellular marine plants which are also classed by pigmentation as red (Rhodophyta), brown (Phaeophyta) or green (Chlorophyta) depending on nutrient and chemical composition. There are approximately 72,500 species of seaweeds worldwide (Guiry (2012)).

Intertidal rocky shores are covered with this macrovegetation as they can anchor to the rock by a holdfast (***Figure 1.15***) and grow. Muddy or sandy shores have less seaweed as there is no permanent surface to anchor securely to. Multicellular seaweed often grow vertically bringing them closer to light allowing them to grow without competition for space thus enabling them to harvest nutrients from a larger volume of seawater. Every species has its own requirements for water salinity, nutrients, water movement, temperature and light (Mc Hugh (2003)). Nutrient uptake and photosynthesis take place across the entire surface of the plant (Hurd et al (2014)).

Figure 1.15 Seaweed holdfast in situ (AlgaeBase (2017))

1.2.1 Seaweed composition

It is noted that seaweeds have a highly variable chemical composition which is attributed to their natural environments and the variation in conditions over time (Guiry (2017)); therefore, the main components of the plant are described however these vary with respect to species, geographical location and surrounding waters. One common feature of fresh seaweeds is that they contain very large amounts of water (70-90%) (Makkar et al (2015)). In general, they contain a complex array of compounds including fibre, carbohydrate, protein, lipid, vitamins and minerals.

1.2.2 Fibre

Seaweed can contain up to 62% total fibres dry weight (Dawczynski et al. 2007) which exceeds that of terrestrial foodstuffs e.g. cereal and vegetables (Holdt and Kraan (2011)). Specifically Fucales contain approximately 30% (Murata and Nakazoe (2001)). There are two classes of dietary fibres in algae; insoluble i.e. cellulose and water soluble i.e. agar, alginate, fucoidan (Holdt and Kraan (2011)).

1.2.3 Carbohydrate

The total carbohydrate content of seaweed can range from 4–76% (Holdt and Kraan (2011)). The type and concentration of carbohydrates varies significantly with species (Dawczynski et al (2007)) and their synthesis is favoured by increased light intensity, temperature and decrease of nitrogen (Marinho-Soriano et al (2006)). The typical carbohydrates found in (the cell wall of) brown algae are fucoidan, laminaran, cellulose, algin and mannitol. The cell walls of brown seaweeds, particularly of Fucales and Laminariales, consist mainly of fucoidan, which is composed of variable amounts of saccharide units with varying degrees of sulphation (Mišurcová (2012)). Carbohydrates found in seaweed also contain carboxyl, sulphate and phosphate groups which also aid the binding of metals e.g. calcium and potassium (Ródenas de la Rocha et al (2009), Wijesinghe et al (2012)).

1.2.4 Protein

The protein content of seaweed varies with season and environmental conditions (Dawczynski et al (2007)) but contains all essential amino acids which are available in all seasons. Brown seaweeds are rich sources of alanine, cysteine, glycine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine and valine which are the building blocks of proteins within the cell (Wu (2013)). In Fucales, the protein content is 17–21% dry weight. In contrast to carbohydrate synthesis the favourable environmental conditions for protein synthesis are an increase of nitrogen, reduced light and temperature i.e. colder, winter months (Marinho-Soriano et al (2006)).

1.2.5 Lipid

The lipid component of seaweed accounts for up to 4.5% dry weight. In particular, the total lipids found in *Fucus serratus* L. account for 0.4-2.1% of its dry weight and are most abundant in warmer months and lowest in colder months (Kim et al (1996), Khotimchenko (1998), (Holdt and Kraan (2011)). Dawczynski et al (2007) found that fatty acid composition varies with species and changes depending on temperature, light intensity, mineral availability and salinity of the surrounding seawater, nitrogen compounds and local pollution. Sánchez-Machado et al (2004) reported that 90% of the total fatty acids in brown algae are long chain polyunsaturated fatty acids (LC-PUFA). The two main classes of PUFAs are omega-3 and omega-6. The predominant omega-3 fatty acid in seaweed is eicosapentaenoic acid (Dawczynski et al (2007)).

1.2.6 Vitamin and Mineral

All seaweeds are excellent sources of vitamin A precursors, vitamin B-complex and vitamin C (Mohamed et el (2012)). Brown seaweeds, especially Fucales, have the highest levels of vitamin E (Bocanegra et al (2009)). Seaweeds are also characterized by a high concentration of minerals as they concentrate minerals from surrounding waters,

10-20 times that of terrestrial plants (Moreda-Piñeiro et al (2012)). This is due to their ability to store sea salts as a result of their cell wall structure, more specifically as a result of the carboxyl functional group (-COOH) which is most abundant in brown algae alginates e.g. sodium alginate (***Figure 1.16***). The mechanisms involved in sorption and storage of salts are both chemical and physical in nature. Both electrostatic attraction and complexation of metal take place on the surface of the cell wall (Pennesi et al (2015)).

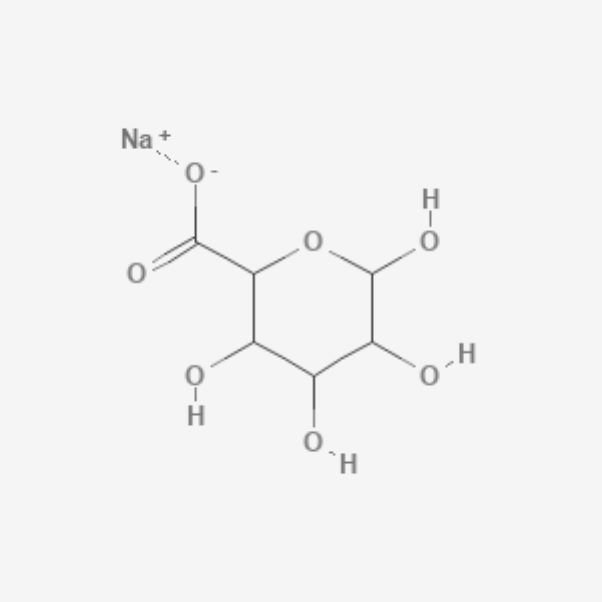


Figure 1.16 Sodium alginate (US National Library of Medicine (2018))

The mineral content of seaweed can account for up to 40% of its composition and the variation is attributed to seaweed phylum, stage of growth, geographical origin and location along with seasonal, environmental and physiological variations ocean residence time and wave exposure (van Netten et al (2000), Rupérez (2002) Marinho-Soriano et al (2006)).

Seaweeds contain calcium, iodine, magnesium, manganese, phosphorous, potassium, sodium and zinc (Matanjun et al (2009), Polat and Ozogul (2009)). Analysis of the ash of various seaweeds by Rupérez (2002) indicated that the samples contained higher amounts of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc

than edible terrestrial plants. Mohamed at el (2012) also found samples to have a higher content of calcium and phosphorous than apples, oranges, carrots and potatoes. Nishizawa (2002) found ashed samples also contained an abundance of bromine, chloride, cobalt, fluorine, iodine, phosphorous, selenium, sulfur and vanadium. Furthermore, van Netten et al (2000) detected aluminium, chromium, cobalt, molybdenum and nickel in samples. As seaweed is not specific in mineral uptake, heavy metals arsenic, cadmium, mercury and lead as well as radium and uranium have also been detected in samples (van Netten et al (2000), Nishizawa (2002), Rupérez (2002)).

1.3 Brown seaweed

The brown algae group of seaweeds (Phaeophyta) contains approximately 2000 species (Ginneken et al (2011)), 265 orders and over 1500 genera (Khotimchenko (1998)). They live primarily in shallow waters or on shoreline rocks and have very flexible stems that allow them to withstand the constant pounding of the waves (Ghosh et al (2012)). *Fucus serratus* L. is an intertidal seaweed (Chance et al (2009)) found in the north east Atlantic from Norway to Northern Portugal, the North Sea and West Baltic (Edwards et al (2012)). It is a brown seaweed from the order Fucales is found growing on rock and regularly grows together with *Fucus vesiculosus* L. on the shore (***Figure 1.17***).

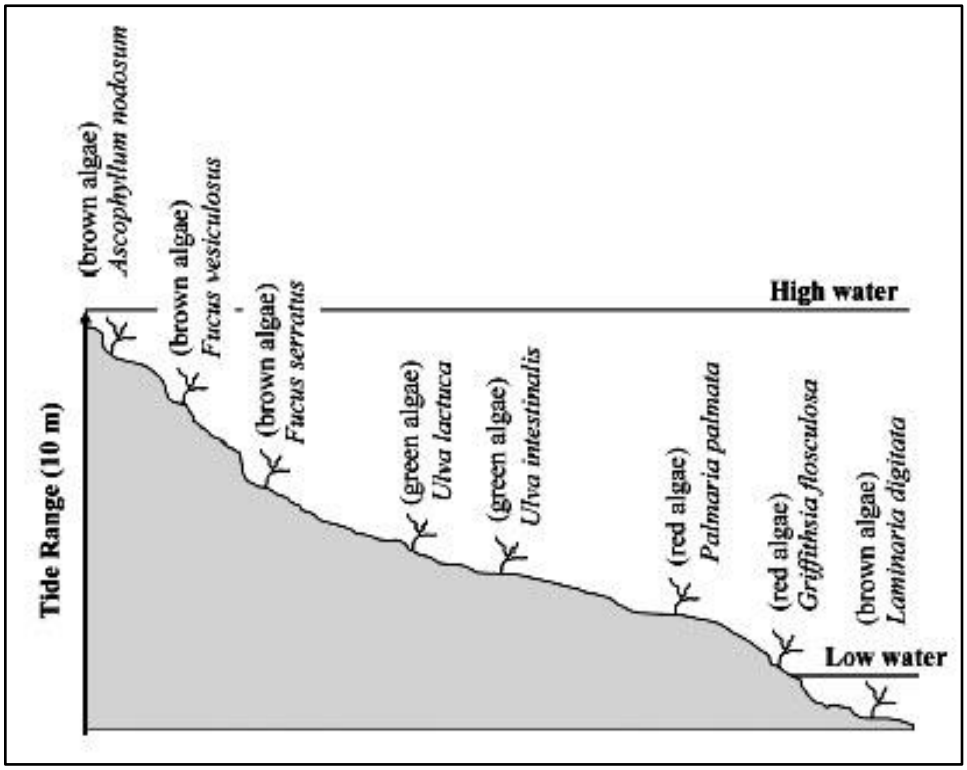


Figure 1.17 Fucales distribution (Bravo-Linares et al (2010))

It has an irregular branched frond and distinct midrib with leaves that are flat and smooth with serrated edges hence the common name 'serrated wrack' (Edwards et al (2012)) (***Figure 1.18***). It is olive/yellow/brown in colour, caused by the pigment fucoxanthin which masks other pigments i.e. beta-carotene and chlorophyll (Guiry (2017)).

Figure 1.18 *Fucus serratus* L. frond in situ (AlgaeBase (2017))

The documented mineral concentration of *Fucus serratus* L. and *Fucus vesiculosus* L. is illustrated in ***Table 1.2*** while ***Figure 1.19*** outlines the overall general composition of a brown seaweed *Fucus vesiculosus* L.

Table 1.2 Mineral concentration of Fucales (µg g^{-1} DW)			
Mineral	**Literature value (µg g^{-1} DW)**		**Reference**
	Fucus serratus	***Fucus Vesiculosus***	
Aluminium	1184	228	Coquery et al (1997) Van Netten et al (2000)
Arsenic	44.3	ND	Coquery et al (1997)
Cadmium	0.5	0.3 1.7	Coquery et al (1997) Van Netten et al (2000) Balina et al (2016)
Calcium	12730 12840	9380 21500	Coquery et al (1997) Ruperez (2002) Balina (2016) Cabrita et al (2016)
Chromium	10.4	9.6	Coquery et al (1997) Balina et al (2016)
Cobalt	0.88 1.96	0.39	Coquery et al (1997) Van Netten et al (2000) Cabrita et al (2016)
Copper	2.69 5.1	1.4 12.7	Coquery et al (1997) Van Netten et al (2000) Balina et al (2016) Cabrita et al (2016)
Iodine	1400-2500	ND	Nitschke and Stengel (2015)
Lead	0.5 2.2	ND	Coquery et al (1997) Cabrita et al (2016)
Magnesium	7240 9070	ND	Coquery et al (1997) Cabrita et al (2016)
Manganese	56.1 149.6	ND	Coquery et al (1997) Cabrita et al (2016)
Mercury	0.1 0.4	ND	Coquery et al (1997) Cabrita et al (2016)
Molybdenum	0.3 2.7	ND	Coquery et al (1997) Cabrita et al (2016)

Table 1.2 Mineral concentration of Fucales (µg g^{-1} DW)			
Mineral	**Literature value (µg g^{-1} DW)**		**Reference**
	Fucus serratus	***Fucus Vesiculosus***	
Nickel	3.8	15.3	Coquery et al (1997) Van Netten et al (2000)
Potassium	ND	2000-7000 11000	Truus et al (2001) Balina (2016)
Selenium	0.1 1.2	ND	Coquery et al (1997) Cabrita et al (2016)
Sodium	ND	460-510	Truus et al (2001)
Zinc	47.3 52.8	37	Coquery et al (1997) Ruperez (2002) Cabrita et al (2016)

ND = No data

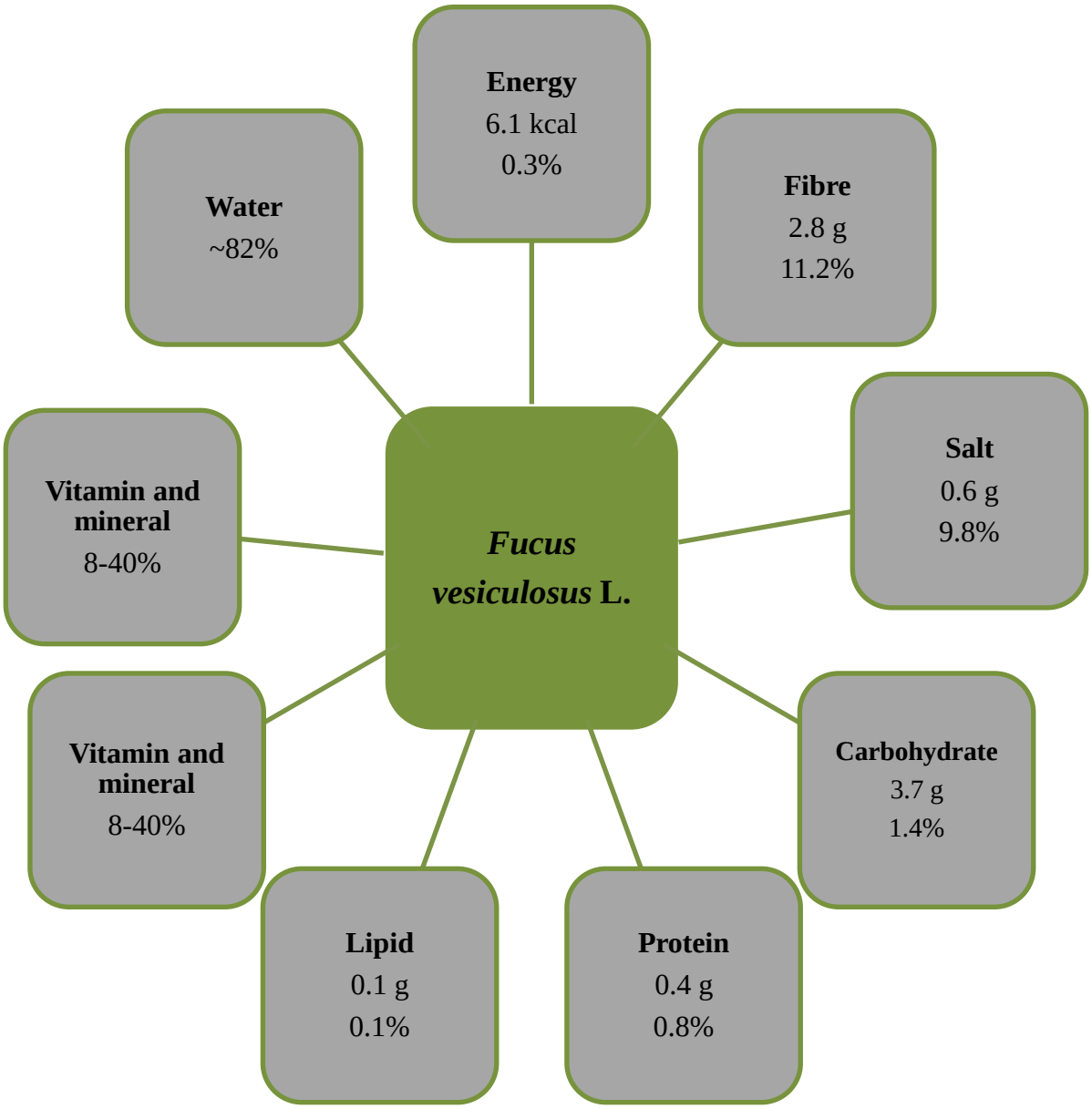


Figure 1.19 *Fucus vesiculosus* L. composition (Abreu et al (2014))

1.4 Iodine

Iodine, a halogen, is a naturally occurring trace element found in sea sediments (high concentration), on land in rocks, soils, vegetation and water sources, and in seawater and sea air (low concentration) (Fuge and Johnson (1986), Muramatsu and Wedepohl (1998)). In seawater, iodine can exist as molecular iodine (I_2), iodide ions (I^-), iodate ions (IO_3^-) and organic iodine (Li et al (2009)). Most forms of iodine dissolve readily in water and alcohol (ATSDR (2004)). Iodine speciation is restricted to species with a stable valance state, iodide (-1) and iodate (+5) (Bruchertseifer et al (2003)). It also exists as iodo-organic compounds such as *p*-iodobenzoic acid, *m*-iodophenol, 2-iodophenol and 3-iodotyrosine (Ohashi et al (2000)). In aqueous media, molecular iodine predominates under acidic conditions whereas iodide and iodate can only co-exist in neutral or basic solutions (***Figure 1.20***). In very acidic solutions iodide cannot co-exist with iodate due to oxidation of iodide by iodate (Fuge and Johnson (1986), Bruchertseifer et al (2003)). The pH of seaweed bathwater has been determined to be in the range of 5.29 to 6.36. At this pH the species most likely to predominate are iodate and molecular iodine.

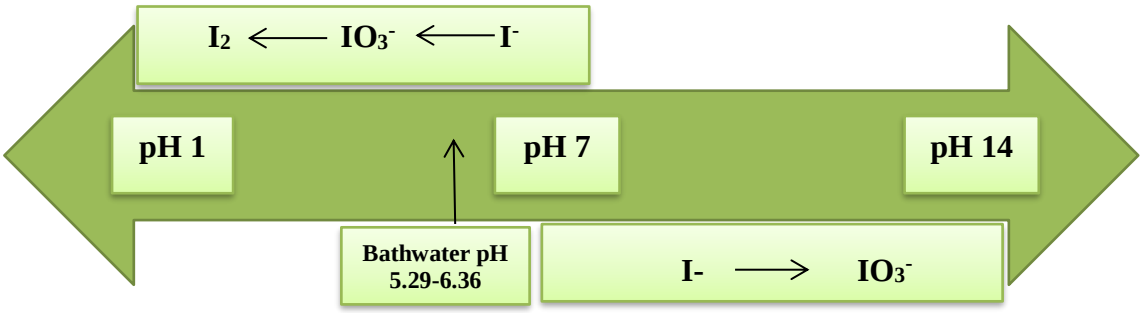


Figure 1.20 Schematic diagram of iodine speciation in an aqueous environment relative to the prevailing pH

Iodine is accumulated by algae from seawater and used by the plant as an anti-oxidant. Molecular iodine diffuses into brown algae where it is reduced to iodide and other iodinating molecules. During steady state conditions iodine is accumulated by the algae

however under oxidative stress e.g. change in temperature, light, UV radiation an efflux of iodine occurs (Küpper et al (1998)).

While submerged in seawater, the uptake and release of iodine species by seaweeds impacts the speciation of dissolved iodine in surrounding waters. Iodide accumulates in the cell wall and is released from there when the algae is under oxidative stress. In surface and coastal waters, up to 50% of dissolved iodine is found as I^- (Chance et al (2009)). Chance et al (2009) determined that in rock pools isolated from outgoing tides, the presence of *Fucus serratus* L. leads to an increase in iodide levels due to the release of stored iodide and the reduction of iodate to iodide in seawater. This is further illustrated in ***Figure 1.21*****.** When submerged and unstressed (a) iodide is accumulated from the surrounding water. Under oxidative stress (indicated in red) iodide is released to counteract the effect of hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS). Conversely, at low tide (b) molecular iodine is released as aerosol directly into the atmosphere (Küpper et al (2008)). Iodine is transferred from the sea to the food chain by a complex set of chemical reactions. ***Figure 1.22*** illustrates the pathway of iodine from source to animal.

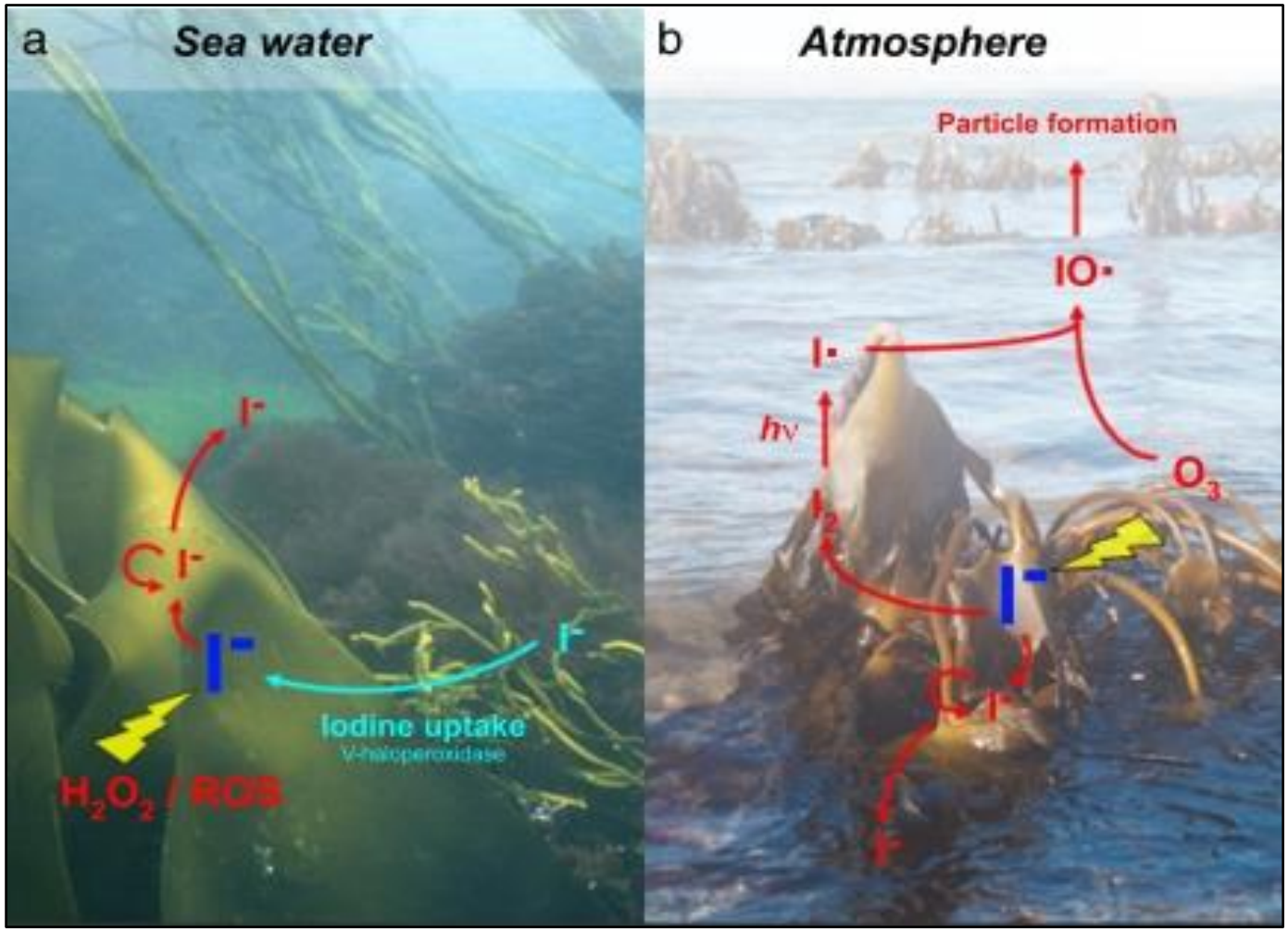


Figure 1.21 Iodine metabolism of brown alga (Küpper et al (2008))

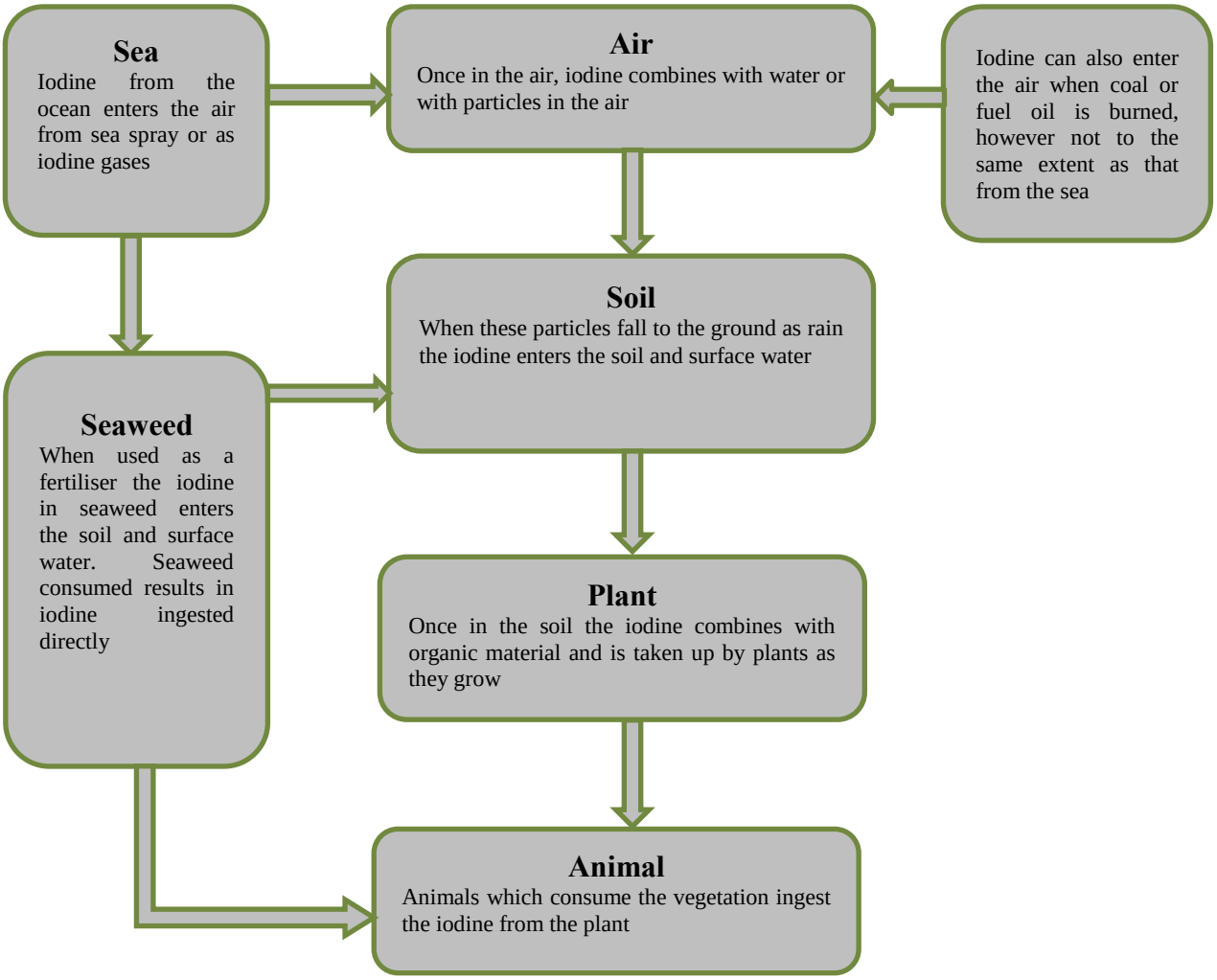


Figure 1.22 Pathway of iodine from source to animal (Li et al (2009), Smyth (2011))

1.4.1 Recommended Daily Allowance (RDA)

The World Health Organisation (WHO) recommends an optimal daily intake of 150-299 µg of iodine per day for adults to achieve optimal iodine nutrition (***Table 1.3***). From a worldwide perspective, insufficient iodine intake is a significant public health issue. If the diet is deficient the easiest and most commonly used mode of supplementation is through iodisation of salt with potassium iodate (KIO_3) or potassium iodide (KI), KIO_3 being more stable (Khazan et al (2013)).

Table 1.3 **Iodine nutrition**		
Iodine intake (µg/day) for adults	**Iodine nutrition**	**Urinary Iodine Concentration (UIC) (µg L^{-1})**
<30	Severe deficiency	<20
30-74	Moderate deficiency	20-49
75-149	Mild deficiency	50-99
150-299	Optimal	100-199
300-449	More than adequate	200-299
>449	Possible excess	>299

1.4.2 Sources

Diet is the main source of iodine with the most common foodstuffs being bread, dairy products, soy milk, infant formula, water, eggs, dried seaweed, saltwater fish, shellfish, iodized table salt, and soy sauce, baked potato with peel, vegetables and turkey breast (Haddow et al (1999), Jootse and Strydom (2010)). Adequate dietary sources are dependent upon the iodine content of water and soil. 30% of the world's population lives in areas with iodine deficient soil (Khazan et al (2013)). Flechas (2011) discusses the issue of iodine deficient soil leading to an iodine deficient diet as iodine, along with other nutrients, can be washed from the soil. He also remarks that 80% of vegans are iodine deficient as their diet is plant based but this does not necessarily include marine plants (Krajčovičová-Kudláčková et al (2003)). In Northern Europe, indoor feeding of cattle in winter with mineral rich supplements leads to an increased UIC (Demers and Spencer (2002)). In Malaysia, iodisation of water has been implemented in areas of iodine deficiency (Hussain and Wan Mohamud (2006)).

Iodine enters the body mainly through the diet however other possible routes are inhalation of gaseous iodine from the air and through the skin (ATSDR (2004)). Molecular iodine in particular is readily and extensively absorbed by inhalation (Morgan et al (1968), Risher and Keith (2009)). Smyth et al (2011 and 2016) found that gaseous iodine from iodine rich coastal areas is a potential contributor to iodine intake in an Irish

study. Comparing populations from coastal and inland areas they determined that the UIC was higher for the population living in a coastal area.

1.4.3 Health Impact

Iodine is a vital micronutrient for normal thyroid function, growth and development of humans and animals (Ahad and Ganie (2010)). It is an essential constituent of the structure of thyroid hormones thyroxine and triiodothyroxine that help the body control metabolism (Rendl et al (1998)). As the thyroid is involved in various activities throughout the body including reproduction, nerve and muscle functioning, digestion, hair and nail growth iodine sufficiency is therefore critical for their correct functioning (Haddow et al (1999)).

Iodine (I_2) will diffuse into cells whereas iodide (I^-) needs to be transported into the cell. Iodine trapping is the first step in the metabolism of iodine. The process starts with the uptake of iodide from a capillary into a follicular cell of the thyroid via the sodium iodide symporter NIS transport system (Ahad and Ganie (2010)). NIS is the transport mechanism which pumps iodide into a cell or tissue. NIS works against a gradient pushing iodide from a low concentration outside the cell to a high concentration inside the cell. It is involved in the accumulation of iodine in the thyroid gland for the synthesis of thyroid hormone. It is also found in the salivary gland (without iodine saliva is not produced), the stomach, pancreas, breast, cerebral spinal fluid, the skin (lack of iodine to the skin means that the skin cannot sweat), brain (iodine makes the brain more alert), thymus and ovaries (Flechas (2011)).

Iodine and iodide are concentrated preferentially at different areas of the body; iodine at the breast, the prostate and the stomach, iodide at the thyroid, the breast, the skin and the salivary glands (Flechas (2011), Gulaboglu et al (2012)). Ingested iodine is mainly absorbed through the gastrointestinal (GI) tract as iodide (Soldin (2002)).

1.4.4 Deficiency and Excess

Almost 30% of the worldwide population have insufficient iodine intake (i.e. median UIC <100 µg L^{-1}) and consequently suffer from Iodine Deficiency Disorders (IDD) (Mina et al (2011)). This is the leading cause of preventable brain damage with symptoms that include spontaneous abortion, infant mortality mal-development of the foetal brain, delayed intellectual and physical development, low IQ, mental retardation and growth retardation (cretinism) (May et al (1990), WHO (2004), De Benoist et al (2008), Zimmerman (2010), Flechas (2011)). It is required at all stages of life but deficiency in critical periods of life i.e. prenatal and neonatal can result in metabolic deceleration and permanent brain damage due to hypothyroxinaemia (low concentration of thyroxine in the blood) (Khazan et al (2013)). Mild deficiency in early foetal life can lead to attention deficit disorder (ADD) (Flechas (2011)).

It is essential for normal growth and development of children and critical for their central nervous system development and IQ. Haddow et al (1999) found that iodine deficiency in pregnant women can adversely affect their children's performance on neuropsychological tests and that iodine supplementation to hypothyroid pregnant women can increase the IQ score of their children. According to Flechas (2011), 'giving iodine to a pregnant mother results in a baby with an IQ 20 to 30 points higher than the parents'.

Flechas (2011) also discussed National Health and Nutrition Examination Surveys (NHANES) (1971-2000) which showed a 50% decrease in iodine levels and an increase in thyroid illness, breast, prostate, endometrium and ovarian cancer; all of these associated with goiter caused by iodine deficiency. Deficiency in women specifically can result in infertility due to lack of ovulation (Haddow et al (1999)).

It affects thyroid hormone production and increases thyrocyte growth and mutation (thyrocytes secrete thyroid hormones) and, as a consequence, nodules develop on the thyroid (Delange (1998)). Available research suggests that the Irish population is

deficient in iodine at 82 µg L^{-1} (WHO (2004)). The deficiency can be easily prevented through adequate dietary intake of iodine, most commonly achieved by use of iodised salt. However, iodisation of salt has not been adopted as a public health policy and is not common in Ireland.

It must be noted that too much iodine i.e. over 449 µg per day can also be damaging to health. While the main cause of goiter is iodine deficiency, over production of thyroid hormones can also lead to over stimulation of the thyroid which can result in enlargement of the thyroid or nodules forming on it (Haddow et al (1999)). Consumption of too much iodine, particularly following supplementation, can lead to iodine induced hypothyroidism i.e. the Wolff-Chaikoff effect, whereby excessive quantities of iodine consumed inhibit thyroid function temporarily (Markou (2001), Demers and Spencer (2002)). Symptoms of this excessive consumption include irritation, nervousness, poisoning due to nitrogen retention and anuria (non-passage of urine) (Agrawal et al (1999)).

1.4.5 Urinary Iodine (UI)

UI is a good indicator of recent intake of iodine as approximately 90% of the daily intake is excreted in the urine (Zimmerman (2010)). UI has a diurnal variation i.e. daily pattern, with the median value 8-12 hours after the last meal (Demers and Spencer (2002)). A spot urine sample i.e. first morning sample is indicative of how much iodine has been consumed in the previous 24 hours (Flechas (2011)). If the body is iodine sufficient more than 90% of iodine consumed is excreted in the urine therefore UI analysis gives an approximation of *recent* dietary intake (Jooste and Strydom (2010)) and the iodine status of a population. If the body is deficient iodine consumed will bind to tissues in the body and less will be excreted. Iodine deficiency, or excess, is determined based on the median and distribution of the UIC of a group rather than an individual (Rendl et al (1998), Jooste and Strydom (2010)) as there is considerable day to day variation in iodine excretion within and between individuals due to hydration, diet

and metabolism; long term iodine status however cannot be determined from a single (24 hour) sample (Haddow et al (2007)).

1.5 Other minerals of interest

Minerals are components that do not contain carbon. They are divided into 'macrominerals' and 'micro' or 'trace' minerals. Macrominerals e.g. calcium, magnesium, phosphorus, potassium and sodium are required in larger quantities in the diet whereas microminerals or trace minerals e.g. copper, iron, manganese and zinc are required in much smaller amounts. If it can be shown that these minerals can be absorbed by the body in the context of a seaweed bath, this will impact on health beyond topical relief of skin conditions aiding proper functioning of the body supporting enzyme and immune function, bone formation and overall homeostasis (balance of physiological processes). ***Table 1.4*** illustrates the recommended daily allowance (RDA), sources and health impact of the minerals under investigation**.**

Table 1.4 **Minerals present in *Fucus serratus* L., RDA sources and associated health impact**				
Mineral	**RDA**	**Sources**	**Health impact**	**Reference**
Aluminium	0.2 mg L^{-1}	Antacid products Drinking water Food additives	Potential neurotoxicity Development of onset of Alzheimer disease	WHO (2003)
Arsenic	0.01 mg L^{-1}	Groundwater Marine organisms By-product of mining, smelting and burning of fossil fuels Smoking Meat, poultry, dairy, fish and cereals, drinking water	Increased risk of cancer e.g. skin, lung, bladder, kidney Skin lesions and pigmentation changes Poison Gastro-intestinal symptoms, Cardiovascular and kidney failure, Disturbances of nervous system functions Death	Gartrell et al (1986a) Gartrell et al (1986b) Civantos et al (1995) WHO (2001)
Cadmium	0.003 mg L^{-1}	Cereals Fruit and vegetables Meat, fish and offal (kidney and liver) Mussels, oysters, scallops	Toxic to kidney Lung, endometrial, bladder and breast cancer Bone demineralisation Skeletal changes	EFSA (2009) FSAI (2009)
Calcium	1000 mg	Dairy produce in particular milk and cheese Cabbage, kale, broccoli, beans, raisins	Bone and teeth structure and function and strength Vascular contraction Vasodilation Muscle function Nerve transmission Intracellular signalling Hormonal secretion Vitamin D synthesis Regulation of blood clotting	IOM (2010) Anderson and Garner (2011) Pérez-López et al (2012) Stipanuk and Caudill (2013)
Chromium	120 µg	Whole grains, cheese, meat, calf liver, brewer's yeast, peas, beans, processed meat	Aids insulin function Metabolism of carbohydrate and fat	Mertz (1993) Vincent (2003) Stipanuk and Caudill (2013) Wong (2012)

Table 1.4 **Minerals present in *Fucus serratus* L., RDA sources and associated health impact**				
Mineral	**RDA**	**Sources**	**Health impact**	**Reference**
Cobalt	5-8 mg	Milk, cheese, meat, offal green leafy vegetables, fish, eggs, cereals	Vitamin B_{12} synthesis Neurotransmitters production Formation of erythrocytes in bone marrow	Underwood (1977) Battaglia et al (2009) Ortega et al (2009) Simonsen et al (2011)
Copper	2 mg	Oysters, shell-fish, whole grains, chick peas, sunflower seeds, beans, Brazilian nuts, cashews, potatoes, offal, dark leafy greens, dried fruits, cocoa, black pepper, yeast	Forms a critical part of most enzymes Cardiovascular and bone health, immune function and cholesterol metabolism Deficiency can cause oxidative stress in cells and during pregnancy can cause foetal malformations	Harris (2001) IOM (2001) Uriu-Adams and Keen (2005) Araya et al (2007)
Lead	0.01 mg	Inhalation during smelting, recycling, stripping leaded paint, leaded gasoline or leaded aviation fuel, ingestion of contaminated dust, water and food	Toxic to nervous system Interferes with calcium and vitamin D metabolism High blood pressure Kidney damage Exposure during pregnancy can cause foetal malformations Liver, gastrointestinal and renal damage	WHO (2003)
Magnesium	400 mg	Spinach, beans, peas, soy nuts, unrefined grains, pumpkin seeds, sunflower seeds, peanuts, cashew nuts, corn, tofu	Cofactor in over 300 enzymes which regulate protein synthesis, muscle and nerve function Control blood glucose and blood pressure Energy production, oxidative phosphorylation, and glycolysis Structural	Rude (2010) Rude (2012) Stipanuk and Caudill (2013)
Magnesium	400 mg	Spinach, beans, peas, soy nuts, unrefined grains, pumpkin seeds, sunflower seeds, peanuts, cashew nuts, corn, tofu	development of bone DNA, RNA and glutathione synthesis Active transport of ions across cell membranes Nerve impulse conduction, muscle contraction and heart rhythm	Rude (2010) Rude (2012) Stipanuk and Caudill (2013)

Table 1.4 **Minerals present in *Fucus serratus* L., RDA sources and associated health impact**				
Mineral	**RDA**	**Sources**	**Health impact**	**Reference**
Manganese	2 mg	Blueberries, ginger, egg yolks, green vegetables, legumes, nuts, bananas, olives, avocados	Brain functioning Required for normal amino acid, lipid, protein, and carbohydrate metabolism Bone and cartilage development Wound healing Excessively ingested quantities can cause neurotoxicity, hepatotoxicity, pulmonary toxicity and reproductive and developmental toxicity	Schafer and Anke (2005) Erikson et al (2007) Bornhorst et al (2010) Schafer and Anke (2005) Erikson et al (2007) Bornhorst et al (2010)
Mercury	0.006 mg L^{-1}	Mining operations, industrial processes, combustion of fossil fuels (especially charcoal), production of cement, incineration of municipal, chemical and medical wastes	Haemorrhagic gastritis Colitis Damage to the kidney	WHO (2003)
Molybdenum	75 µg	Meats, whole grains, buck-wheat, barley, wheat germ, lima beans, peas, lentils, peanuts, beans, sunflower seeds, milk, green leafy veg.	Breaks down proteins for cell renewal Cofactor for a number of enzymes Prevents anaemia Enhances the feeling of well-being Anti-cancer of stomach and oesophagus	IOM (2010) Gupta and Gupta (2014)
Nickel	0.07 mg L^{-1}	Oatmeal, dried peas, beans, nuts, chocolate	Aids iron absorption, adrenaline and glucose metabolism Improves bone strength Can cause allergic contact dermatitis	Lidén and Johnsson (2001) Stipanuk and Caudill (2013)
Potassium	3500 mg	Broccoli, peas, lima beans, tomatoes, potatoes, citrus fruits, cantaloupe, bananas, squash, tomatoes, carrots, kiwi, prunes, dried apricots,	Moves nutrients and waste around the cell Nerve functioning and muscle contraction Regular heartbeat	Haas (2000) Mc Gill et al (2008)

Table 1.4 **Minerals present in *Fucus serratus* L., RDA sources and associated health impact**				
Mineral	**RDA**	**Sources**	**Health impact**	**Reference**
		milk, yogurt, nuts		
Selenium	70 µg	Lentils Meat, seafood, bread, Brazilian nut	Reproduction. Thyroid hormone metabolism DNA synthesis Immune function Protection from oxidative damage which can result in chronic disease e.g. cancer, cognitive deficit, mood and behavioural disorders	Thavarajah et al (2011) Sunde (2012) Gashu and Stoecker (2017)
Sodium	2400 mg	Salt processed meats, canned foods, cheese, bread, sauces, pickled food, rice, pasta.	Blood pressure	Dietitians of Canada (2011) Huggins et al (2011)
Zinc	15 mg	Meat, poultry, oysters, seafood, milk products, nuts, seeds, whole grains, legumes, pumpkins and cereals	Immune system functioning DNA synthesis RNA transcription Cell division, activation and apoptosis Enzyme function Antioxidant Anti-inflammatory	Rangan and Samman (2007) Prasad (2009) Prasad (2014)

1.6 Skin Architecture

The skin, the largest organ of the body, is a multi-layered bio membrane consisting of the epidermis, dermis and subcutaneous layer. Its complex structure lends itself to immune and barrier functions which include UV protection, environmental protection, thermal protection, anti-microbial protection, physical protection but also permeability i.e. controlling the passage of water and other substances through the skin (Pillai et al (2009)). Each layer is unique in its composition and serves different functions to the next (***Figure 1.23***).

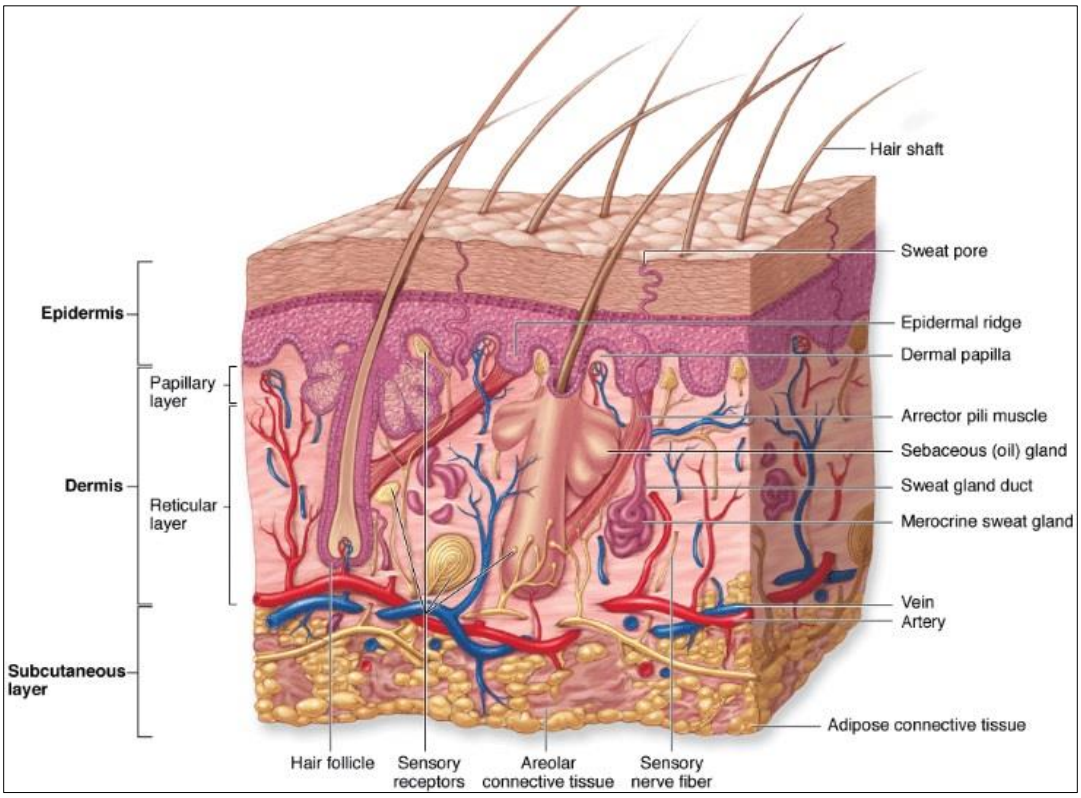


Figure 1.23 Skin architecture (Mescher (2016))

1.6.1 Epidermis

The various layers of the epidermis consist of the stratum corneum, stratum granulosum, stratum spinosum and stratum basale (***Figure 1.24***). The outer layer of the epidermis, the stratum corneum is a stratified layer which prevents entry of foreign substances and loss of water to the environment. The predominant cell type in this layer is the keratinocyte i.e. a flattened, anucleaete cell which produces keratin. It is more commonly referred to as a corneocyte. Corneocytes are

interlocked in vertical columns of up to 30 cells. They are embedded in a lipid matrix which consists of ceramides, fatty acids and cholesterol (Schurer and Elias (1991), (Weerheim and Ponec (2001)). The lipids form a rigid, crystalline structure which enhances the barrier function of the stratum corneum and controls penetration. In addition to corneocytes, the epidermis also contains melanocytes (which produce melanosomes, organelles which contain melanin), Langerhans cells (which communicate with the immune system) and Merkel cells (which are responsible for sensation/touch).

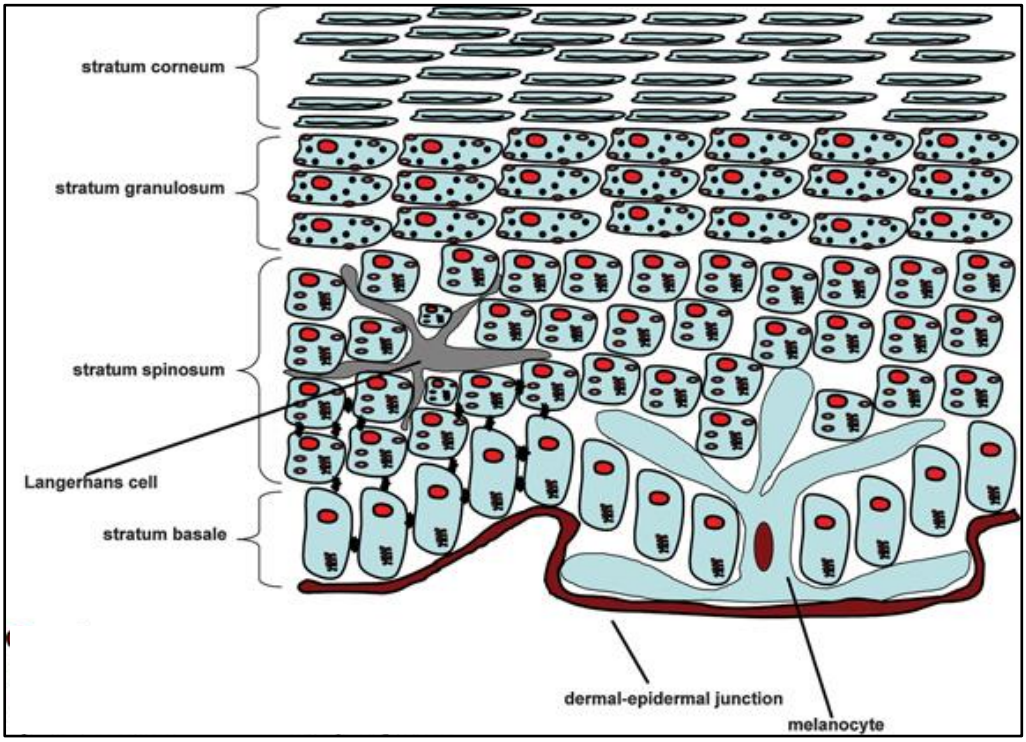


Figure 1.24 Epidermis (Allured Publishing (2013))

Beneath the stratum corneum lies the stratum granulosum which is 2-3 layers of cells thick. The cells in this layer are more viable than that of the stratum corneum and transform in this space to a cornified cell. The stratum basale is the deepest layer of the epidermis. The basement membrane lies at the dermal-epidermal junction. It is 0.5-1 μm in thickness. One of its key functions is to control the passage of substances between the epidermis and dermis. It can serve as a reservoir for prolonged release of substances applied to the skin (Iozzo (2005)).

The dermis constitutes the major component of the skins structure, approximately 90% of its total weight. It has 2 distinct layers, the papillary layer and the reticular

layer which combine to form the nerve and vascular system of the skin (***Figure 1.23***). The function of the dermis is to bind and hold water, regulate temperature and protect the body during impact. Fibroblasts are the main cell type in this layer and these produce structural proteins, collagen and elastin, which provide the skins support, tensile strength and elasticity (Menon (2015)).

Given the skins complex composition, there are many routes for absorption of a substance which include via intercellular lipids, via corneocytes or via follicles. Hair follicles are present all over the body with the exception of the soles of the feet and palms of the hands. Their distribution, however, is uneven as is their size which can vary between 70 and 170 μm (Otberg et al (2004)). Follicles contain sebaceous glands and as a result are filled with sebum which exudes from the follicle working against a substance applied topically. The structure of the follicle however can lend itself to the production of a reservoir of applied substance which also can allow for gradual, prolonged absorption (Lademann et al (2007)).

1.7 Analytical Methods

1.7.1 Sandell-Kolthoff (SK) method

This inexpensive, spectrophotometric method has been adapted for the analysis of iodine in various matrices i.e. serum, food, water, milk and soil (Shelor and Dasgupta (2011), Błażewicz (2012)). Samples are initially digested with an oxidising agent ammonium persulphate, to remove interfering substances and organic matter that can alter the reaction rate (May et al (1997)). In the case of urinary analysis this step also releases any iodine bound to urine excretory compounds (Khazan et al (2013)).

Iodine in the sample must be present in the form of iodide. Iodate present can be satisfactorily reduced to iodide by allowing the sample to stand for some time after the addition of sulphuric acid and arsenite solution (Sandell and Kolthoff (1937)). The SK reaction then involves the reduction of yellow ceric ammonium sulphate (CAS) by arsenic in the presence of iodide to colourless cerous ions and iodine (Jooste and Strydom (2010)) as follows:

$$As^{3+}_{(aq)} + I_{2\,(aq)} \longrightarrow As^{5+}_{(aq)} + 2I^{-}_{(aq)}$$

$$2Ce^{4+}_{(aq)}\ \textbf{(yellow)} + 2I^{-}_{(aq)} \longrightarrow 2Ce^{3+}_{(aq)}\ \textbf{(colourless)} + I_{2\,(aq)}$$

It is necessary to maintain an acidic environment in order to prevent the precipitation of ceric arsenate (Sandell and Kolthoff (1937)). The catalytic activity of the iodide (I^-) is directly proportional to its concentration in the sample.

In the original work by Sandell and Kolthoff (1937), they remarked that there were a number of substances which inhibit the reaction between CAS and arsenious acid solution. These include fluoride, mercury salts, cyanide and silver. Truesdale (2008) suspected a positive interference of the SK method by some other reducing agent in the form of organic material released by seaweed. May et al (1997) stated that ‘no digestion method can be guaranteed to completely remove all potential interferences from a particular matrix’. They investigated interferences from thiocyanate in urine samples of smokers which can give rise to an overestimation in UIC. They also noted that ions such as nitrite and ferrous iron have been shown to

act as reducing agents in the SK reaction. Ascorbic acid present in urine can also interfere with the SK reaction (Mina et al (2011)).

1.7.2 Spectroscopy

Spectroscopic techniques are based on electron transitions in the outer orbitals of atoms due to interaction of energy with matter. The electromagnetic spectrum extends from radio to x-ray and gamma rays (Potts (2014)). Electrons in the orbitals of an atom are excited by the application of light within the UV/visible region of the spectrum (200-750 nm). Electrons from inner orbitals are less likely to transition as they are more tightly bound to the nucleus. As the atom absorbs energy from a photon of light its energy increases. This excitation causes the electrons to move from a stable ground state to an excited state (***Figure 1.25***). This is an unstable state for an electron therefore it decays back to ground state i.e. the lowest energy state of an atom and in doing so emits energy which is detected and measured. This emission is equivalent to the difference between the orbitals in the transition and is characteristic of an atom. The concentration is based on a linear relationship with absorption of light.

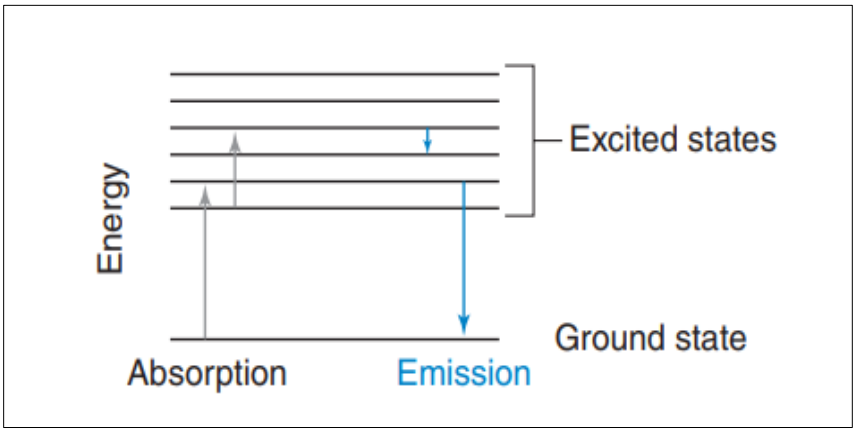


Figure 1.25 Absorption of light increases the energy of a molecule. Emission of light decreases its energy (Harris (2007))

The light source for atomic absorption spectrophotometry (***Figure 1.26***) is a hollow cathode lamp which consists of cathode made of the element being analysed and a tungsten anode. When a current is applied, gas e.g. argon within the lamp is ionized and cations accelerate towards the cathode. Metal atoms are released from the cathode which collide with electrons to emit a photon characteristic of the element (Harris (2007)). The emission is concentrated into a beam which is aligned with the flame. Fuel (acetylene), oxidant (air) and sample are mixed before being aspirated into a flame (2000–3000 ˚C). The liquid evaporates and the solid is atomized. This beam is absorbed by the ground state atoms in the sample (Bhanot (2014)). The concentration of analyte is determined based on the absorption of characteristic wavelengths of radiation.

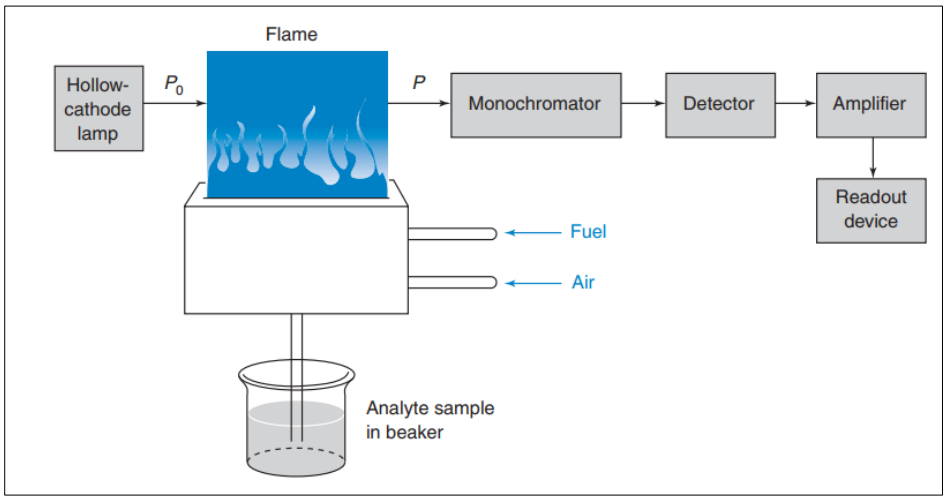


Figure 1.26 Atomic absorption spectrophotometry (Harris (2007))

This technique is most sensitive for elements with low or middle atomic numbers as they have a simpler electron configuration therefore absorption is concentrated across fewer orbitals unlike the more complex configuration of elements with higher atomic numbers (Potts (2014)). Unfortunately however, analysis is restricted to single element determination unlike ICP-MS.

AAS is typically used for the quantification of metallic elements in a sample. It has many applications across various industries including environmental (soil and water), food (nutrient analysis), forensic (drugs and gunshot residue), geochemistry,

marine, medical (biological fluids and tissues) and petroleum (Haswell (1991), Lim and Lewis (2013)).

1.7.3 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

A plasma is formed by the interactions of the magnetic field produced by radio frequency passing through a copper coil on a tangential flow of argon gas (***Figure 1.27***). It can reach temperatures of 4700-9700 ˚C and as a result allows for increased sensitivity with lower detection limits and the production of both electrons and ions within the plasma. Under pressure, the nebulizer gas pierces a hole in the base of the plasma and forms a channel through the centre of it into which it injects the sample aerosol. When the analyte enters the plasma it exists as ground state atoms and positively charged ions. The high energy of the plasma removes one or more electrons from an atoms outer orbital to generate a free ion as follows:

$$M \longrightarrow M^{+} + e^{-}$$

This plasma energy is high enough to ionize most of the elements in the periodic table (Thomas (2013)).

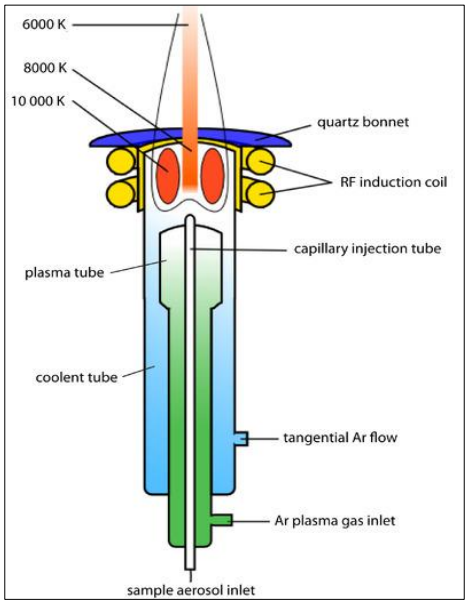


Figure 1.27 ICP-MS Plasma torch consisting of 3 concentric coaxial glass tubes with induction coils wrapped around the upper opening (Linn High Therm Gmbh. (2017))

As the sample passes through the different heating zones of the plasma it is dried, vaporized, atomised then ionized. A quadrupole separates analyte ions based on mass-to-charge (*m/z*) ratio (while filtering out any non-analytes or interfering ions) which results in a stream of ions of one mass passing through to the mass spectrometer where they are measured (***Figure 1.28***). The intensity of the signal is proportional to the population of the excited state electrons. Due to the higher temperatures of the plasma each atom emits many photons per second due to the continuous excitement of electrons (Harris (2007)).

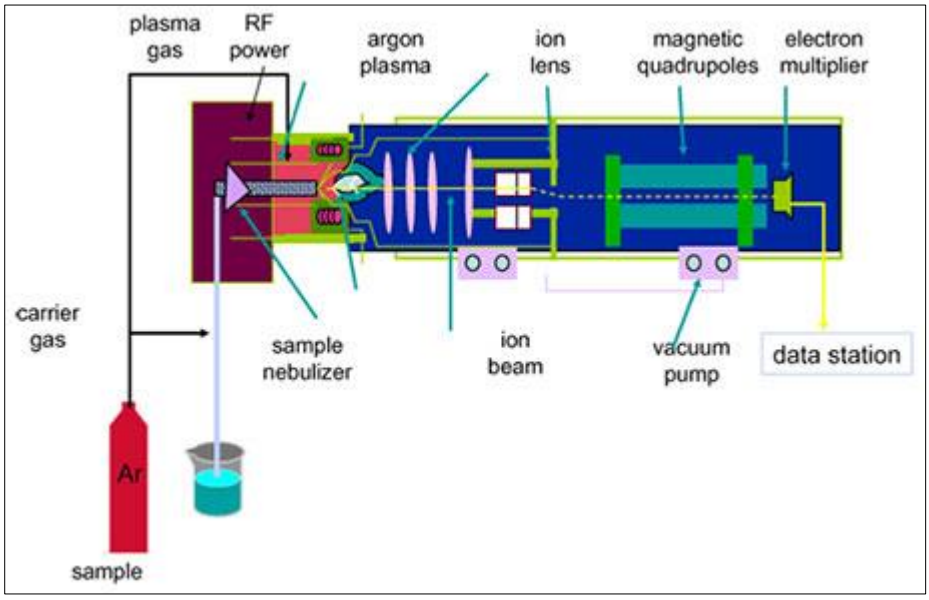


Figure 1.28 ICP-MS overview (Merck (2017))

ICP-MS allows for simultaneous determination of in excess of 30 elements (Goullé et al (2005)) in a sample. When used in conjunction with microwave digestion to eliminate, or at best control, interfering substances it has a detection limit in the range of ppt-ppb (Fernández-Sánchez et al (2007), Ródenas de la Rocha et al, 2009). It has been applied to elemental analysis of various matrices including seaweed (Coquery et al (1997), Caroli et al (1999), Ródenas de la Rocha et al (2009)), seawater (Lagerström et al (2013)) and biological samples ((Bravo et al (2007), Goullé et al (2005), Heitland and Köster (2006), Szpunar et al (1997a)). All minerals of interest to this study are routinely investigated in water samples by ICP-

MS as set out by a standard Environmental Protection Agency (EPA) method (200.8) (Environmental Monitoring Systems (1996)). Coquery et al (1997) carried out a thorough investigation of *Fucus* samples and compiled a detailed mineral composition as per ***Table 1.2***. Ródenas de la Rocha et al (2009) also determined the levels of cadmium, cobalt, chromium, lead, molybdenum, nickel and selenium in brown algae using ICP-MS. Lagerström et al (2013) detected cobalt, copper, manganese, nickel and zinc in seawater from the North Atlantic and North Pacific. Bravo et al (2007) detected aluminium, cadmium, chromium, cobalt, copper, magnesium, manganese, mercury, nickel, selenium and zinc in blood using ICP-MS. Goullé et al (2005) analysed blood, urine and hair for the presence of 30 elements including aluminium, arsenic, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium and zinc. Similarly, Heitland and Köster (2006) analysed urine for the same elements.

1.7.4 Enhancer cells

Enhancer cell technology is used to examine dermal absorption, *in vitro.* It uses the principles of franz diffusion cells whereby a sample, in this study an aliquot of fresh simulated bathwater, is applied to a donor chamber/enhancer cell body and its diffusion across a membrane into receptor fluid, which represents and mimics the body's circulatory system, is monitored and the results inferred to an *in vivo* setting (***Figure 1.29***). The amount of minerals permeating is typically determined as a function of time (WHO (2006)).

They operate by passive diffusion of a substance from an area of high concentration in the enhancer cell body to that of low concentration in a receptor fluid through a membrane which can be either artificial or biological. The WHO (2006) state that there is good correlation between *in vivo* and *in vitro* dermal absorption data and that *in vitro* measurements can be used to predict *in vivo* absorption.

The physical and chemical properties of a substance will impact its penetration through the skin. Such properties include but are not limited to its liposolubility, molecular weight, electronic structure and dissociation constant (European

Commission (EC) (2004)). Another influencing factor on dermal absorption is the location of contact between the product and the skin. This is not a major consideration for this study however, as the whole body is immersed in the bathwaters during the treatment (as treatment rooms are private bathers are not required to wear bathing suits); the surface area exposed is approximately 1.6m^2 for women and approximately 1.9 m^2 for men (Mostellar (1987)).

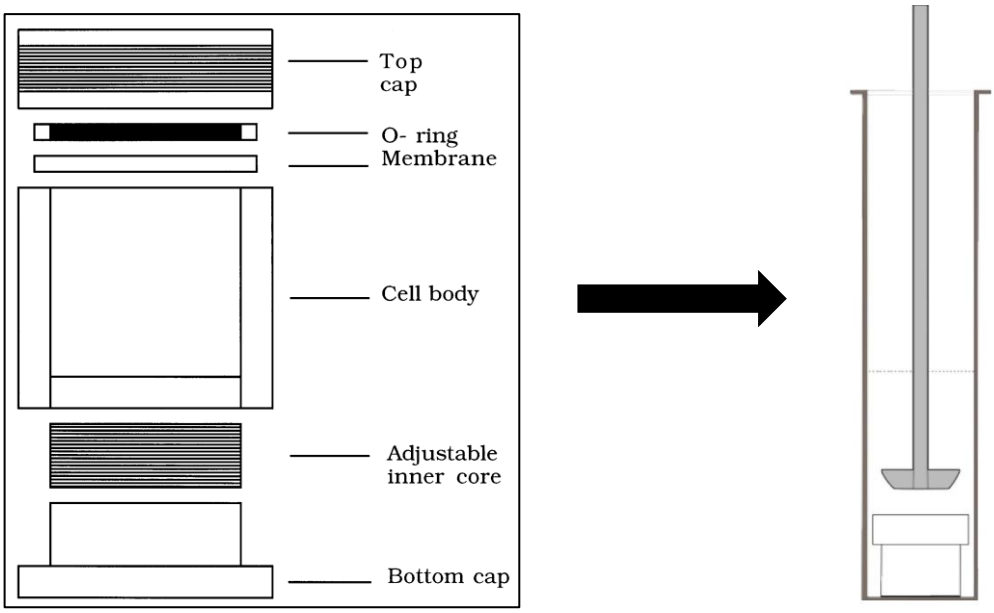


Figure 1.29 Enhancer cell technology and dissolution vessel (Agilent Technologies, Inc. (2013), Kregar et al (2015))

The permeation of minerals from the sample into the receptor fluid involves firstly partitioning within the membrane before diffusing through it. It is important to note that penetration of minerals into the membrane can occur without passing through it forming a depot/reservoir in the brick like structure of the epidermis (***Figure 1.24***). Diembeck et al (1999) distinguish dermal absorption from dermal penetration whereby stratum corneum absorption refers to the quantity of topically applied test substance which is found in the stratum corneum after experimentation. This quantity is not systemically available. Dermal absorption refers to the amount of test substance which is found in the epidermis and dermis after experimentation. This is

systemically available. Finally, dermal penetration refers to the amount of test substance that is found in the receptor fluid after experimentation and this too is systemically available.

Albèr et al (2014) discuss the composition of the skin and the large difference in water activity between the water rich inside of the body (which corresponds to 100% relative humidity) and dry ambient temperature outside the body. This difference leads to a continuous leakage through the skin which is required for the integrity and flexibility of the skin barrier. They explain that, due to the composition of the outermost layer of the skin this water leakage remains constant at over a wide range in ambient relative humidity. However, they note that at humidity above 90% (similar to the seaweed bath environment) the water loss is decreased due to the reduced driving force for water to evaporate from the skin. More interestingly, the overall permeability of the skin becomes significantly higher. This implies that increased hydration can alter the skin structure to facilitate the diffusion of most small molecules (Sparr and Wennerström (2001), Silva et al (2007)).

The mineral concentration of the bathwater applied to the donor chamber will be determined in the first instance. There are 4 potential results which can illustrate the behaviour of the minerals in a sample:

1. The concentration of minerals in the donor and receptor chambers remain constant; This implies that there is no movement of minerals across the membrane or loss of minerals to the surrounding atmosphere

2. The concentration of minerals in the donor chamber decrease but that of the receptor chamber remains constant; This implies that there is movement of minerals into the membrane or they are released to the surrounding environment

3. The concentration of minerals in the donor chamber decreases and the receptor increases by the same amount; This implies that there is movement of minerals directly across the membrane

4. The concentration of minerals in the donor chamber decrease to a greater extent that of the receptor chambers increase; This implies that there is movement of minerals across the membrane but it is a gradual process

1.8 Potential for dermal absorption from seaweed bathwater

Guy et al (1999) write that calcium, cobalt and copper penetrate the skin with calcium forming deposits of insoluble precipitate in dermal connective tissues. Tateo et al (2009) determined the uptake of aluminium, cadmium, calcium, chromium, copper, magnesium, manganese, nickel, potassium, selenium and zinc from mud application *in vitro.* Wester et al (1992) demonstrated the penetration of cadmium through the skin while Sun et al (2002) and Larese et al (2006) investigated the penetration of lead through the skin and found significant amounts can be absorbed. Hostýnek (2003) focussed on the investigation of mercury, lead, nickel and chromium. Current research indicates that there is potential for absorption of minerals through the skin (***Table 1.5***) however this has not yet been established in the context of a seaweed bath.

Table 1.5 **Potential for dermal absorption and penetration of minerals present in *Fucus serratus* L. seaweed bathwater matrix**		
Mineral	**Potential for dermal absorption from seaweed bathwater**	**Reference**
Aluminium	Yes	Tateo (2009)
Arsenic	Yes	Jang et al (2016)
Cadmium	Yes	Wester et al (1992) Tateo (2009)
Calcium	Yes	Guy et al (1999) Tateo (2009)
Chromium	Yes	Hostýnek (2003) Tateo (2009)
Cobalt	Yes	Larese et al (2007) Leggett (2008) Klasson et al (2017)
Copper	Yes	Guy et al (1999) Tateo (2009)
Iodine	Yes	Miller et al (1989) Boothman (2010)
Lead	Yes	Sun et al (2002) Hostýnek (2003) Larese et al (2006)
Magnesium	Yes	Tateo (2009)
Manganese	Yes	Tateo (2009)
Mercury	Yes	Hostýnek (2003)
Molybdenum	Yes	Guy et al (1999)
Nickel	Yes	Hostýnek (2003) Larese et al (2007) Tateo (2009)
Potassium	Yes	Tateo (2009)
Selenium	Yes	Tateo (2009)
Sodium	Yes	E.C. (2004)
Zinc	Yes	Tateo (2009)

1.9 Problem statement

While seaweed baths are a traditional bathing therapy that dates back to the start of the last century, there is no scientific data to suggest that bathing with seaweed has any impact on health. The behaviour of seaweed in hot water and the subsequent chemistry of the bathwater itself are unknown. Questions such as ‘why should someone take a seaweed bath?’ or ‘what does it do for the body?’ have guided this

study to establish what happens to the body during a seaweed bath. Specifically, the research set out to investigate and quantify the release of minerals from the brown seaweed *Fucus serratus* L. into the bathwater and their subsequent transdermal penetration.

1.10 Justification

Seaweed has been explored extensively, as previously detailed, however it has not been investigated in the context of bathing. There may be theoretical assumptions as to what could *potentially* be released into the bathwater and subsequently taken up by the body however it is important to establish its behaviour in this environment so that bathers are better informed with evidence of the health benefits associated with seaweed baths given that the treatment impacts directly on their health and well-being as minerals released may be beneficial but given that Fucales can also contain heavy metals such as arsenic, cadmium, mercury these may be harmful to a bather.

This research also has the potential to benefit other parties including the cosmetic science community as it provides new data on an established cosmetic treatment and the wider science community as it provides an application of standard spectroscopic methods to a new matrix. In terms of environmental issues and sustainability, this research can also aid in potential sustainability programs using spent seaweed.

1.11 Hypothesis

The various minerals found in *Fucus serratus* L. are illustrated in ***Table 1.2***. It is hypothesised that, under normal bathing conditions, some or all of these can potentially be released from the seaweed into the bathwater for absorption into the body or transferred to the skin during dermal contact throughout bathing.

1.12 Objectives

1.12.1 General objective

To explore *Fucus serratus* L. bathwater in terms of mineral content and the subsequent uptake of minerals by the body.

1.12.2 Specific objectives

The project consisted of 4 main phases

Phase 1 **Iodine analysis**

To collect and store samples of seawater used to prepare the baths and bathwater samples after 40 minutes.

To prepare simulated seaweed baths weekly for 12 months.

To examine the homogeneity and stability of bathwater samples.

To establish the pH of this matrix as it influences iodine speciation.

To optimize and validate the Sandell-Kolthoff (SK) micromethod for total iodine determination in *Fucus serratus* L. seaweed, seawater, bathwater and urine.

Phase 2 ***In vivo* study**

To carry out a survey of clients and staff of Voya Seaweed Baths.

To carry out a preliminary *in vivo* study to determine the potential uptake of iodine by the body during a seaweed bath.

To obtain Research Ethics Committee approval for a larger study to measure the iodine and creatinine levels in urine of subjects pre and post bathing in *Fucus serratus* L. seaweed baths to establish if iodine is taken up by the body during a seaweed bath.

To investigate *in vivo* if iodine is taken up by the body during a seaweed bath by immersion in or adjacent to a bath and to gain insight into potential routes of uptake by the body.

Phase 3 **Mineral analysis**

To optimise spectrophotometric methods (Atomic Absorption Spectrophotometry (AAS) and Flame Emission Spectrophotometry (FES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)) for determination of aluminium, cadmium, calcium, chromium, cobalt, copper, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, sodium and zinc in *Fucus serratus* L. seaweed, seawater and bathwater.

To examine the release of aluminium, cadmium, calcium, chromium, cobalt, copper, iodine, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, sodium and zinc from *Fucus serratus* L. seaweed into bathwater under lab controlled conditions of varying mass, temperature and season.

To determine the seasonal variation of aluminium, cadmium, chromium, cobalt, copper, iodine, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, sodium and zinc in seawater.

Phase 4 ***In vitro* study**

To optimise Enhancer cell technology for the determination of the dermal absorption *in vitro* of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc from *Fucus serratus* L. bathwater.

To analyse the dermal absorption *in vitro* of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc from *Fucus serratus* L. bathwater.

2 Methodology: Materials and Instrumentation

As set out in Chapter 1, the general objective for the research was to explore *Fucus serratus* L. bathwater in terms of mineral content and the subsequent uptake of minerals by the body. This chapter sets out the rationale for choice of techniques, the sourcing of materials, the details of the procedures used and the results of the optimisation and validation studies completed including background studies e.g. homogeneity and stability studies.

2.1 Choice of Analytical Methods

The seaweed *Fucus serratus* L. was chosen for all the studies as it is the main species used in seaweed baths in Ireland. It is chosen for commercial use, due to its abundance on the west coast, its ease of harvesting and its appealing aesthetic appearance. Occasionally *Fucus vesiculosus* L. is used. The latter seaweed grows in similar environments (***Figure 1.17***) and is comparable in chemical composition to *Fucus serratus* L. (Morrissey et al (2001))). Given the high concentration of iodine in seaweeds generally, this element was selected as the first analytical target. The fresh weight iodine content of *Fucus serratus* L. ranges from 1400-2500 µg g^{-1} (Nitschke and Stengel (2015)). Based on this data, the iodine release was predicted to be in the µg L^{-1} to low mg L^{-1} in the baths, to guide the selection of analytical method.

A review by Shelor and Dasgupta (2011) outlines the three commonly used methods for iodine analysis as the Sandell Kolthoff (SK) method, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Neutron Activation Analysis (NAA) and to a lesser extent other electrochemical and chromatographic methods. The SK method is a simple, rapid, cost effective spectrophotometric method which does not require expensive instrumentation and is routinely used for samples such as biological fluids, environmental samples and foodstuffs (Shelor and Dasgupta (2011), Błażewicz (2012)). NAA was not available and ICP-MS is a costly choice for a single element analysis, albeit it has excellent sensitivity in the case of low concentration samples. As sensitivity was not expected to be an issue the SK method was selected. In this chapter the validation of two methods for determination

of total iodine in seaweed bathwater are outlined. The first method is a well-established SK assay using higher volumes of sample and reagents. A second micromethod based on adaptation of the previous method to a 96 well micro plate was then developed. The two methods are compared by correlating results for seawater and bathwater samples.

Following iodine determination in the samples, a more detailed mineral profile of the matrix was carried out and as such ICP-MS was used due, in part, to its increased sensitivity but mostly as it allowed for simultaneous detection of many minerals in a single sample thus allowing for an increased throughput of samples. It is worth noting that iodine analysis by ICP-MS also requires a different sample preparation using tetramethylammonium hydroxide (TMAH) therefore the same extracted samples could not be used for general metal analysis as well as iodine measurement. AAS and FES were selected as more suitable alternatives to ICP-MS for detection of the more abundant minerals i.e. calcium, magnesium, potassium and sodium as they are present at much higher concentrations. Analysis of high concentration samples is not typically carried out using ICP-MS.

At the commercial level there will always be variation in the level of analyte in the bathwater due to choice of seaweed species, seasonal variation within the seaweed, the mix of seawater and freshwater used and individual bather preferences such as the temperature and duration of a bathing. To control the conditions of the study and to reduce the risk of possible contamination and safety considerations of sampling directly from the used baths, simulated baths were prepared for most of the studies. In this way the amount of seaweed, volume of water and temperature were controlled. The ratio of weight of seaweed to volume of water was at a greater ratio in the earlier studies compared to the commercial baths to ensure sufficient amount of analyte for detection. Analysis of typical baths were carried out and compared in later work.

Finally, having established a mineral profile of the bathwater it was important to ascertain whether there is (potential) uptake of minerals by a bather during a treatment. Documented literature suggests that this is certainly possible (***Table 1.5***) so *in vitro* testing allowed for its investigation. When investigating dermal absorption in an *in vitro* setting there are various parameters to consider. One of the

main considerations is the type of membrane to choose. Membranes can be either synthetic or biological including human tissue *ex vivo*, animal tissue e.g. mice or rabbit, pig or dog, polymeric membranes *in vitro* and human skin equivalents skin constructs engineered from human cells. There are many factors which influence the choice of membrane e.g. purpose of the study, availability of skin samples and cost. Although the use of human skin may best represent *in vivo* conditions, human skin samples were not readily available and, similar to the use of animal tissue, have significant ethical implications. As a result the synthetic membrane selected for this study was Cuprophan. Cuprophan is a cellulose membrane, 11.5 µm thick, with uniform porosity. It is routinely used for renal dialysis and increasingly for transdermal testing. Use of a synthetic membrane also eliminates *in vivo* variables such as skin age, race, sex and anatomical site (Ng et al (2010)).

Other instrumentation parameters to consider are paddle rotation speed (revolutions per minute (rpm)), receptor temperature and receptor volume. The receptor fluid is stirred continuously by paddles to ensure uniform distribution of analyte in the medium. Its temperature is maintained to replicate skin and body temperature. Receptor volume can vary depending on apparatus used; in this study it was possible to use a receptor volume up to 200 ml. Optimization of method involved variation of these parameters in order to establish the optimum conditions for *in vitro* determination of transdermal absorption of minerals from bathwater.

This chapter sets out the methods and materials for:

1. Seaweed sample collection, preparation, homogeneity studies, stability and pH procedures
2. Optimization and validation of the SK micromethod for total iodine determination in *Fucus serratus* L. seaweed, seawater, bathwater and urine
3. Optimization of ICP-MS for determination of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc in *Fucus serratus* L. seaweed, seawater and bathwater
4. Optimization of Atomic Absorption Spectrophotometry (AAS) and Flame Emission Spectrophotometry (FES) methods for determination of calcium, magnesium, potassium and sodium in *Fucus serratus* L. seaweed, seawater and bathwater
5. Optimization of Enhancer cell technology for the determination of dermal absorption of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc from *Fucus serratus* L. seaweed bathwater

2.2 Materials

Ammonium persulphate (98+% ACS Reagent), arsenic trioxide (99.995% metals basis), bismuth and indium standards (CRM TraceCERT®), ceric ammonium sulphate (ACS Reagent), hydrogen peroxide (Ultratrace), phosphate buffered saline (grade/purity not available), potassium iodate (ACS Reagent 99.5%), sodium alginate (grade/purity not available), sodium chloride (ACS Reagent), sodium hydroxide (grade/purity not available), sulphuric acid (95-98% ACS Reagent) and tetramethylammonium hydroxide (ACS Reagent) were purchased from Sigma Aldrich, Arklow, Ireland. Magnesium AAS standard ((1000 mg L^{-1} (CRM TraceCERT®)) in 0.5 M HNO_3) was purchased from Merck, Germany. Calcium, potassium and sodium AAS standards (1000 mg L^{-1} (99.99%)) were purchased from Lennox, Dublin, Ireland. A custom multi element standard 7% $^{v}/_{v}$ HNO_3 containing 1000 mg L^{-1} aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc and tuning solution containing 10 mg L^{-1} barium, beryllium, cerium, cobalt, indium, lead, magnesium, thallium and thorium in 7% $^{v}/_{v}$ HNO_3 was purchased from Aquilant Analytical Sciences, Dublin, Ireland for the ICP-MS (grade/purity not available). Buffers for pH calibration were purchased from Lennox Laboratory Supplies, Dublin, Ireland (SRM from NIST). Super Purity Nitric acid was purchased from ROMIL, Cambridge, United Kingdom. Sterile, polypropylene, flat bottom 96 well microplates were purchased from Eppendorf supplied by Fisher Scientific, Dublin, Ireland and Eppendorf sealing film was supplied by Mason Technology, Dublin, Ireland. Cuprophan membrane was supplied by Agilent, Cork, Ireland (11.5 µm thickness). Reagents were prepared using nitric acid (ACS Reagent) washed Grade A glassware. Ultrapure water (18.2 MΩ) was used for sample preparation throughout.

2.3 Optimization and validation of the SK micromethod for total iodine determination in *Fucus serratus* L. seaweed, seawater, bathwater and urine

2.3.1 SK method reagent preparation

Ammonium persulphate (AP) 1M was prepared in water and stored in the dark. Arsenious acid solution (0.05M) was prepared by adding arsenic trioxide, sodium chloride and 200 ml 5 N sulphuric acid to 500 ml water. This was heated gently to dissolve, cooled to room temperature and diluted with water to 1L, then filtered through Whatman membrane filter paper (150 mm) and stored in the dark at room temperature. Ceric ammonium sulphate (CAS) (0.075M) was prepared in 250 ml of 3.5N sulphuric acid and stored in the dark at room temperature. A stock standard solution, 1000 mg L^{-1} iodine, was prepared by dissolving 168.6 mg potassium iodate (KIO_3) in 100 ml water (0.0079M). Working standards were prepared by serial dilution of stock to the range of 20–600 µg L^{-1} and stored at room temperature in the dark prior to analysis. Solutions of sodium alginate were prepared, 0.25–1% $^{w}/_{w}$, in 0.1M sodium hydroxide.

2.3.2 SK method optimization

A 250 µl aliquot of each iodine standard and each sample i.e. seawater, bathwater or urine was pipetted into test tubes in duplicate for analysis (Eppendorf autopipette). A 1 ml aliquot of 1M ammonium persulphate was added to each test tube. All test tubes were heated in a water bath at 100 ˚C for 60-70 minutes then allowed to cool to room temperature. Once cooled 2.5 ml of arsenious acid solution was added to each test tube, mixed by vortex at 3000 rpm (Fisher Scientific, FB 15012 Topmix Evolution ZX) and allowed to stand at room temperature for 15 minutes. A 300 µl aliquot of CAS solution was added to each successive test tube at 20 second intervals and each test tube was vortexed immediately for 5 seconds following the addition. All standards and samples were left to stand at room temperature for exactly 30

minutes. Exactly 30 minutes after the addition of CAS solution to the first test tube its absorbance was read at 420 nm (Jenway 6305 Spectrophotometer). Successive test tubes were read at the same 20 second interval as was used for the addition of CAS solution to each sample. As the response concentration curve was exponential, a linear response standard curve was constructed by plotting the Log (Absorbance) *v* Iodine concentration (µg L^{-1}) (Dunn et al (1993), Ohashi et al (2000), Rendl et al (1998)).

2.3.3 SK micromethod

The above method was transferred to a microplate similar to Ohashi et al (2000), Hussain and Wan Mohamud (2006) and Mina et al (2011). A 50 µl aliquot (blank (ultrapure water) (n=2), iodine standard (n=2), seawater (n=3), diluted bathwater (n=3) or urine (n=3) were pipetted into the wells of a 96 well plate (***Figure 2.1***). For the digestion ammonium persulphate (100 µl) was added to each well and the contents mixed by drawing it into the pipette tip 5 times (Eppendorf Multichannel pipette). The microplate was sealed with sealing film and wrapped in tinfoil and incubated at 100 ˚C for 60 minutes (Carbolite Fan Convection Laboratory oven EUROTHERM 3216). It was removed from the oven after digestion and allowed to cool to room temperature. Aliquots of the digested sample (50 µl) were transferred to a clean microplate and 100 µl of arsenious acid solution was added to the well and mixed by drawing the contents of the well into the pipette tip 5 times. The microplate was allowed to stand for 15 minutes. Following this, 50 µl of CAS solution was added with 30 second intervals between additions to each row using a multichannel pipette and the contents mixed by drawing it into the pipette tip 5 times. The microplate was allowed to stand at room temperature for exactly 30 minutes. Exactly 30 minutes following the addition of CAS solution to the first row its absorbance was read at 420 nm (Fluostar Optima, BMG Labtech, Ortenberg, Germany). Successive rows were read at 30 second intervals as used for the addition of CAS solution to each row. To avoid the effect of temperature variations on the reaction all standards and samples were analysed within the same batch on the same day.

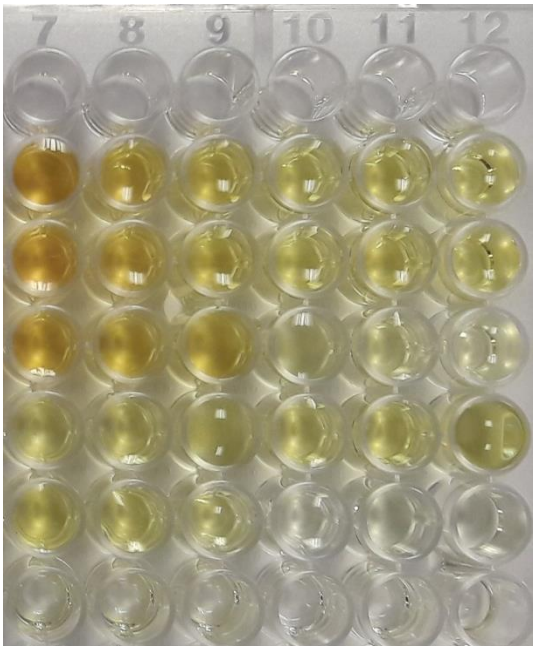


Figure 2.1 SK micromethod typical microplate (Rows 2 and 3 contain duplicate KIO_3 standards (0-300 µg L^{-1}), rows 4-6 contain 6 samples in triplicate)

2.3.4 SK method validations

Linearity and range, inter assay and intra assay precision, limit of detection (LOD), limit of quantitation (LOQ), recovery and robustness were carried out as per International Council for Harmonisation (ICH) guidelines (1996) when validating methods.

Linearity refers to a response that is directly proportional to concentration of analyte and was determined using standard solutions of potassium iodate. Accuracy is the conformity between a result and the conventional true value or accepted reference value. Commercial standards should provide the basis for accurate measurement and recovery tests (Khazan et al (2013)). Caldwell at al (2003) note the challenge in standard and sample preparation due to a lack of matrix-matched reference material for urinary iodine. A similar challenge is also presented due to the complex nature of the sample matrix (i.e. presence of algin and other polysaccharides, lipids and minerals at varying concentrations) and the unavailability of a standard sample devoid of iodine. There are no certified reference materials available to purchase that would artificially replicate the bathwater. It was not possible to test specifically

for accuracy, however recovery was used as an indication of the level of accuracy that could be obtained from the method. Precision is the closeness of agreement between a series of measurements. It is further divided into intra-assay, inter-assay precision and reproducibility. For this validation, intra-assay and inter-assay precision were examined. Intra-assay refers to a short time period, e.g. 1 day, using the same conditions (method, sample, and laboratory) whereas inter-assay precision examines the precision of a series of measurements over a longer period of time, e.g. 3 days, looking at variation in the day, laboratory and/or equipment. The LOD is the smallest amount of analyte that can be reliably detected with confidence that is statistically different from the blank. The LOQ is the lowest amount of analyte that can be quantitatively determined with precision and accuracy. The average log absorbance value for the blank of 12 runs was inserted into its equation of the line in order to determine its equivalent concentration. Statistically, the LOD was calculated as 3 times the standard deviation of the blank and the LOQ as 10 times the standard deviation of the blank. The methods for calculation of recovery and robustness varied slightly for the two methods and the specific details are outlined separately below. Specificity, or more specifically algin interference, was tested using standard solutions of sodium alginate. Standards of potassium iodate were prepared using a solution of sodium alginate and the total iodine concentration measured using the SK micromethod protocol.

2.3.5 SK method optimization

Linearity was determined using standard concentrations of potassium iodate in the range of 20-600 µg L^{-1} (run in duplicate) as shown in ***Table 2.1.*** A typical calibration curve for the method is plotted in ***Figure 2.2.***

Table 2.1 Optimization of SK method			
Linearity and Range of 2 typical calibrations (7 point calibration curve)			
Run	**Range (µg L^{-1})**	**Line of best fit**	**Correlation Coefficient**
1	20-600	y = -0.0029x + 0.2885	R^2 = 0.9975
2	20-600	y = -0.0024x + 0.1899	R^2 = 0.9981

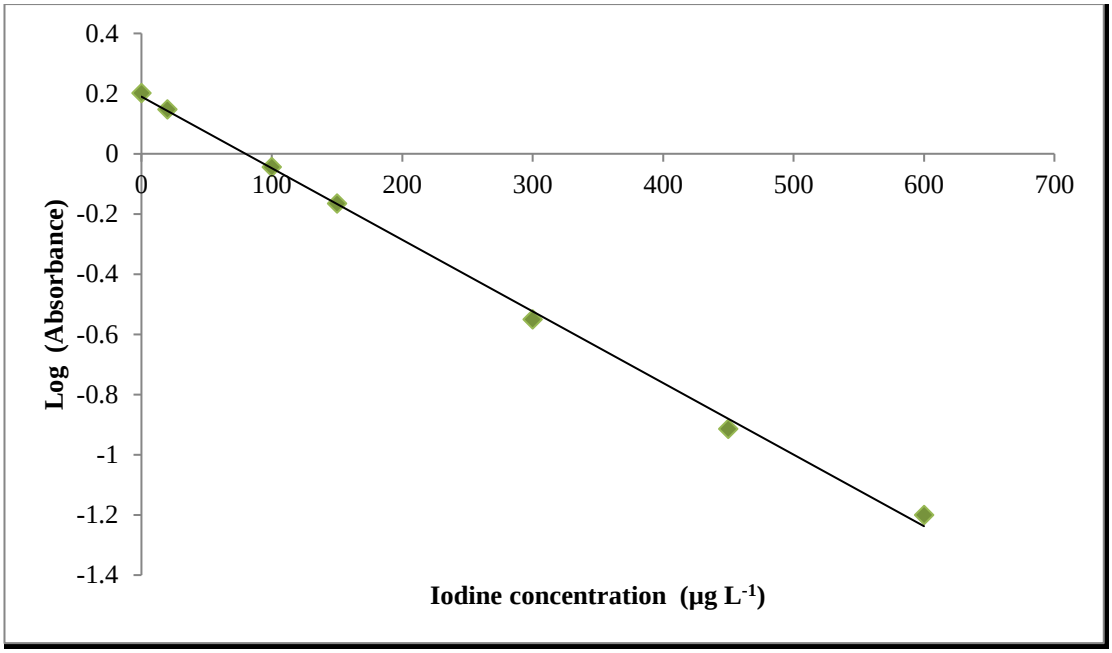


Figure 2.2 ***Optimization of SK method*** Linearity: Typical calibration curve of Log (Absorbance) *v* Iodine concentration (µg L^{-1}) $R^2 = 0.9981$ (Run 2 from ***Table 2.4***)

Precision was measured by replicate analysis of a 150 µg L^{-1} iodine standard (n=8/n=10), urine samples (n=6) and bathwater samples (n=6). Intra-assay indicates a coefficient of variation (%CV) no greater than 2.8%. The inter-assay precision was determined by replicate analysis over 3 days with a %CV of 8.0% (***Table 2.2***). Intra-assay was also determined using a bathwater sample the %CV of which was found to be 6.9% (n=6) and with analysis of a urine sample 9.5% (n=6) (***Table 2.3***). The LOD and LOQ were determined to be 18 µg L^{-1} and 60 µg L^{-1} respectively (***Table 2.4***).

Table 2.2 Optimization of SK method

Intra-assay and inter-assay precision based on 150 (µg L^{-1}) standard

Day	n	Mean (µg L^{-1})	SD	%CV	Inter-assay precision	
1	10	141	3.9	2.8	**Average Concentration**	149
2	10	163	2.8	1.7	**S.D.**	11.9
3	8	144	1.8	1.3	**% CV**	8.0

Table 2.3 Optimization of SK method

Intra-assay precision based on urine and bathwater samples (n=6)				
Sample	**n**	**Mean (µg L^{-1})**	**SD**	**%CV**
Urine	6	46	4.4	9.5
Bathwater	6	81	5.6	6.9

Table 2.4 Optimization of SK method		
Limit of Detection (LOD) and Limit of Quantitation (LOQ) over 12 runs where blanks were run in duplicate		
Run	**Line of best fit**	**Equivalent concentration (µg L^{-1})**
1	y = -0.0024x + 0.1899	-5.0
2	y = -0.004x + 0.287	6.8
3	y = -0.0021x + 0.2154	-2.9
4	y = -0.0018x + 0.2006	2.8
5	y = -0.0021x + 0.2069	-1.8
6	y = -0.0021x + 0.2154	-2.9
7	y = -0.0021x + 0.2202	-2.6
8	y = -0.0018x + 0.2105	-1.2
9	y = -0.0021x + 0.185	-0.1
10	y = -0.0025x + 0.1597	0.7
11	y = -0.0033x + 0.1753	11.4
12	y = -0.0016x + 0.2268	-12.6
Average		**-0.619**
SD		**5.97**
LOD		**18**
LOQ		**60**

Recovery is the detection of a *known* quantity of analyte which is added to the sample. Spiked samples were prepared by a 1 in 50 dilution of bathwater including a 5, 10 or 20 ml spike of 1 mg L^{-1} KIO_3 then brought to final volume, 100 ml, with ultrapure water. These volumes resulted in a KIO_3 spike of 50, 100 and 200 µg L^{-1} respectively. The unspiked sample concentration was determined to be 81 µg L^{-1}.

The concentration of the spiked samples was determined and the resulting spike expressed as a percentage of the known quantity added to the sample originally (***Table 2.5***). Recovery was determined to be 102-126%.

Table 2.5 Optimization of SK method **Recovery (%) based on standard addition (n=2)**		
Sample	**Average (µg L^{-1})**	**Recovery (%)**
Unspiked (n=6)	81	N/A
Sample + 50 µg L^{-1} spike	132	102
Sample + 100 µg L^{-1} spike	194	113
Sample + 200 µg L^{-1} spike	332	126

Robustness is the ability of the method to remain unaffected by deliberate variations in analytical parameters. The parameters varied to assess robustness were:

(i) Digestion Time with 1M ammonium persulphate

(ii) Time interval between adding CAS to sample and reading its absorbance

(iii) Time interval between adding CAS to successive samples

The digestion time with 1M ammonium persulphate was varied for 50, 60 and 70 minutes with the remainder of the method carried out as per protocol. The concentration of three standards 100, 300 and 600 µg L^{-1} were determined for each digestion and compared to their expected value.

To examine variation with respect to time interval between addition of CAS to a sample and reading its absorbance, standards were run in duplicate over 4 runs as per protocol, with the time interval varying from 25-40 minutes. The timing of this step was critical; it is specified in the literature that the absorbance should be read at exactly 30 minutes after the addition of CAS. The time interval between adding CAS to successive tubes was also varied between 10 and 40 seconds over 4 runs as per protocol.

Results indicate no significant difference in the recovery between the three digestion times or the four selected time intervals (***Table 2.6***). The data presented also supports a 20 second time interval between adding CAS to successive tubes allowing for comfortable adding of reagent and subsequent reading of absorbance.

Table 2.6 Optimization of SK method				
Robustness: Percentage of standard determined following variation in digestion time with 1M ammonium persulphate (50, 60 and 70 minutes), variation in time interval between adding CAS to sample and reading its absorbance (25, 30, 35 and 40 minutes) and variation in time interval between adding CAS to successive samples (10, 20, 30 and 40 second) (n=2)				
Digestion Time with 1M ammonium persulphate				
Digestion Time	**% of standard**			
	0 µg L^{-1}	**100 µg L^{-1}**	**300 µg L^{-1}**	**600 µg L^{-1}**
50 minutes	Off Scale	98	99	98
60 minutes	Off Scale	107	101	102
70 minutes	Off Scale	103	100	100
Time interval between adding CAS to sample and reading its absorbance				
Time interval	**% of standard**			
	0 µg L^{-1}	**100 µg L^{-1}**	**300 µg L^{-1}**	**600 µg L^{-1}**
25 minutes	Off Scale	99	103	100
30 minutes	Off Scale	100	103	102
35 minutes	Off Scale	97	101	100
40 minutes	Off Scale	96	92	100
Time interval between adding CAS to successive samples				
Time interval	**% of standard**			
	0 µg L^{-1}	**100 µg L^{-1}**	**300 µg L^{-1}**	**600 µg L^{-1}**
10 seconds	Off Scale	94	98	97
20 Seconds	Off Scale	93	104	99
30 Seconds	Off Scale	116	90	101
40 Seconds	Off Scale	91	105	99

2.3.6 SK micromethod validation

Linearity was determined using standard concentrations of potassium iodate in the range of 20-300 µg L^{-1} (run in duplicate) (***Table 2.7)***. While the first method indicated linearity from 20-600 µg L^{-1}, when transferred to micromethod the response was not linear between 300-600 µg L^{-1}. At higher concentrations, over 300 µg L^{-1}, the R^2 reduced to 0.9593. Therefore the upper end of the range was reduced to 300 µg L^{-1}. The difference in validated range between the methods was initially attributed to lower working volumes and the use of a different spectrophotometer. The reason for this was not fully determined at the time but has since been attributed to contamination within the laboratory from nearby use of Lugol's iodine which contributed to increased iodine in the air and subsequently reagents and equipment. The results below were generated following a move to a different laboratory.

The correlation coefficient (R^2) ranges from 0.9956 to 0.9994 based on n=11 calibration curves (average = 0.9985) (***Table 2.7***). A typical calibration curve is plotted in ***Figure 2.3.***

Table 2.7 Validation of SK micromethod

Linearity and Range of 4 calibrations (5 point calibration curve)			
Run	**Range (µg L^{-1})**	**Line of best fit**	**Correlation Coefficient**
1	20-300	y = -0.0028x + 0.4907	R^2 = 0.9970
2	20-300	y = -0.0021x + 0.4847	R^2 = 0.9956
3	20-300	y = -0.0022x + 0.4958	R^2 = 0.9987
4	20-300	y = -0.0027x + 0.5186	R^2 = 0.9994

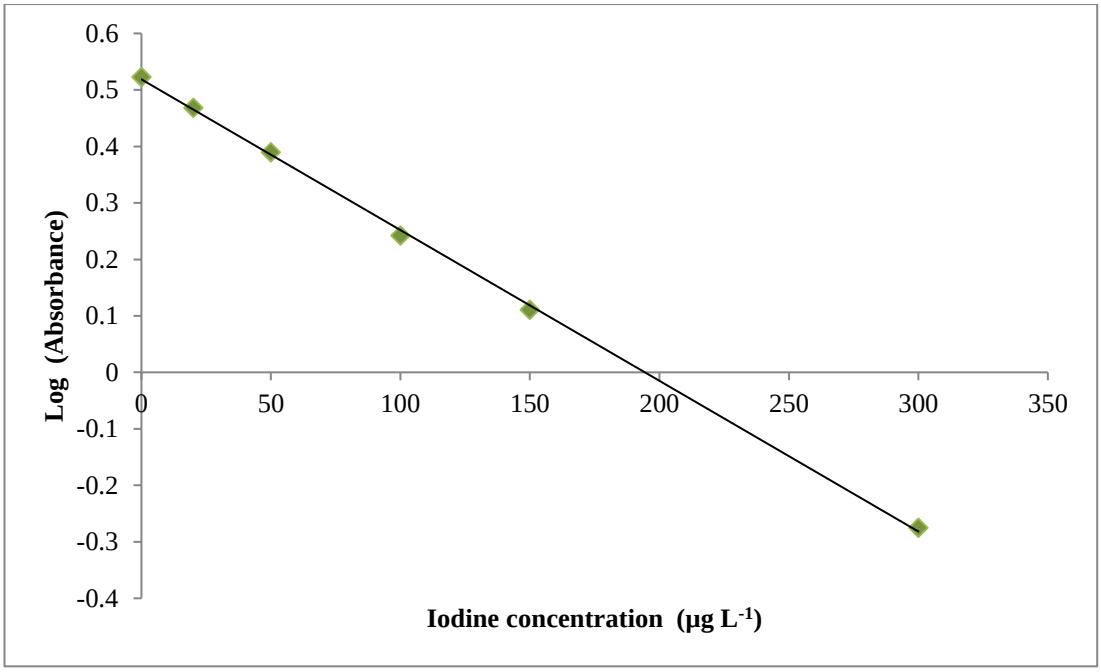


Figure 2.3 ***Validation of SK micromethod*** Linearity: Typical calibration curve of Log (Absorbance) *v* Iodine concentration (µg L^{-1}) $R^2 = 0.9994$ (Run 4 from ***Table 2.10***)

The intra-assay precision ranged from 0.4 to 5.1% (mean 2.5% CV) based on replicate analysis (n=3) of 6 separate 1:20 diluted bathwater samples carried out on 7 different days. The inter-assay precision was 6.7% CV based on the mean concentrations over 7 days (***Table 2.8***). A review by Shelor and Dasgupta (2011) outlines the range in intra and inter-assay precision recorded with SK method/SK micromethod for various matrices including biological fluids (2.8-13% and 4-15% respectively), food (0.031-6.8%) and environmental samples (2.9-9.5%). Ohashi et al (2000) report an intra-assay CV of 2.0% on a high concentration urine sample (319 µg L^{-1}). This CV increases to 10% on a lower concentration urine sample (37.4 µg L^{-1}). Similarly, Mina et al (2011) reported an intra-assay CV range from 7-11% in an SK micromethod for urine. Inter-assay precision by Ohashi et al (2000) ranged from 4.4-20%.

Based on the standard deviation (SD) of 12 blank concentrations the LOD was determined to be 9 µg L^{-1} (based on 3 SD) and the LOQ was determined to be 29 µg L^{-1} (based on 10 SD) (***Table 2.9***). This is comparable to Hussain and Wan Mohamud (2006) and Ohashi et al (2000) who determined an LOD of 14 µg L^{-1} with a working range of 0-200 µg L^{-1} and 40-400 µg L^{-1} respectively using an SK microplate method for urine samples. Based on the LOQ of 29 µg L^{-1} a working linear range of 30-300 µg L^{-1} is recommended.

Table 2.8 Validation of SK micromethod

Precision: Intra-assay precision of 6 separate 1 in 20 diluted bathwater samples analysed in triplicate inter-assay precision (n=7)			
	Mean concentration (µg L^{-1})±SD	**%CV**	**Mean %CV**
Intra-assay n=6	223.0±0.9	0.4	**2.5**
	186.9±5.0	2.6	
	213.3±5.85	2.7	
	198.2±10.1	5.1	
	197.8±2.8	1.4	
	223.8±8.2	3.0	
	205.9±3.0	1.5	
Inter-assay n=7 days	207.0±13.8		**6.7**

Table 2.9 Validation of SK micromethod		
Limit of Detection (LOD) and Limit of Quantitation (LOQ) over 12 runs where blanks were run in duplicate		
Run	**Line of best fit**	**Equivalent concentration (µg L^{-1})**
1	y = -0.0028x + 0.4907	-6.1
2	y = -0.0021x + 0.4847	-3.2
3	y = -0.0022x + 0.4958	0.3
4	y = -0.0027x + 0.5186	-1.6
5	y = -0.0024x + 0.5214	-0.12
6	y = -0.0024x + 0.5206	0.02
7	y = -0.0021x + 0.5158	-3.7
8	y = -0.0025x + 0.5058	-1.9
9	y = -0.003x + 0.513	-1.2
10	y = -0.0027x + 0.5391	5.5
11	y = -0.0024x + 0.5206	0.02
12	y = -0.0021x + 0.5158	-3.7
	Average	**-1.3**
	S.D.	**2.9**
	LOD	**9**
	LOQ	**29**

A spiked recovery procedure, adapted from Ohashi et al (2000), was used as an indication of the level of accuracy that can be obtained from this method. Bathwater samples were diluted 1:20 with ultrapure water (mean concentration 207.0 µg L^{-1}) An iodate spiked sample (50 µg L^{-1}) was prepared by adding 1 ml of 1 mg L^{-1} KIO_3 to a 20 ml volumetric flask and bringing it to volume with the diluted bathwater. A second sample (unspiked) was prepared in a similar way but using 1 ml of ultrapure water as the spike. The recovery was carried out over 3 days as per the following equation:

$$\%\text{Recovery} = \left(\frac{[KIO_3 \text{ spiked sample}] - [\text{Water spiked sample}]}{[\text{Added iodine}] \times \text{Dilution factor}}\right) \times 100$$

The recovery of an iodate spiked diluted bathwater sample ranged from 87 to 114% (***Table 2.10***). Ohashi et al (2000) investigated the effect of digestion times on recovery of iodate and found that digestion times between 50 and 70 minutes yielded the highest recovery. Rendl (1998) also investigated digestion time with ammonium persulphate and found consistent recoveries between 40 and 70 minutes.

Table 2.10 Validation of SK micromethod

Recovery: Recovery (%) of diluted 1:20 bathwater samples (mean concentration 207.0 (µg L^{-1}) with added water or 50 (µg L^{-1}) of potassium iodate

Day	n	Average iodine concentration (µg L^{-1})±S.D.		Iodine recovered (µg L^{-1})	Recovery (%)
		Added water	Added iodate		
1	6	233.7±6.8	290.7±7.1	57.0	114
2	6	211.0±2.6	254.5±5.7	43.5	87
3	6	192.1±2.4	238.4±2.2	46.3	93

The following analytical parameters were varied to establish the robustness of the micromethod: Digestion time with 1M ammonium persulphate, temperature during digestion with 1M ammonium persulphate, time interval between adding CAS to sample and reading its absorbance and time interval between adding CAS to successive samples. A 1:20 diluted bathwater sample (mean 207.0 µg L^{-1} iodine measured using final method parameters) was used as a reference concentration and all measurements were made in triplicate (***Table 2.11***).

To examine any variation with respect to digestion time with 1M ammonium persulphate the samples were digested for 50, 60 and 70 minutes in one run and the remainder of the method was carried out as per protocol. To examine any variation with respect to temperature during digestion with 1M ammonium persulphate the samples were digested for 60 minutes at 90, 100 and 110 ˚C over 3 runs and the remainder of the method was carried out as per protocol. To examine variation with respect to time interval between addition of CAS to a sample and reading its absorbance samples were analysed over 4 runs as per protocol. The absorbance was read 25, 30, 35 and 40 minutes after the addition of CAS to the sample. To examine

variation in time interval between additions of CAS to successive samples the samples were analysed over 3 runs as per protocol. The CAS was added at 20, 30 and 40 second intervals to successive samples in each individual run. This interval must be matched when the absorbance is read on the microplate reader. A minimum of 30 seconds is required for the plate reader to complete its reading accurately.

With respect to digestion time with 1M ammonium persulphate the results are not significantly different. The recovery ranges from 96-111% for different digestion times. A digestion time of 60 minutes was selected as the data illustrates the lowest error in recovery (-4%) and a low %CV The %CV, where n=6, for each set of samples (***Table 2.11***) indicates 0.2% variation between 50 and 60 minutes digestion time. As the standard method outlines a digestion time of 60 minutes, based on this data, this was set as the optimum digestion time.

Similar to digestion time, variation of temperature during digestion and the time interval between addition of CAS to a sample and reading its absorbance both are within the normal variation. Variation in temperature during digestion between 90 and 110 °C during digestion resulted in very little difference in the recovery of iodine; 96-105%. The optimum digestion temperature was 100 ˚C as it shows a %CV of 1.4, where n=6. As there was no difference in recovery when the interval between adding CAS to a sample and reading its absorbance was increased from 25 to 40 minutes, a 30 minutes interval was selected. The time interval between adding CAS to successive samples was also varied between 20 and 40 seconds. It is critical that this interval matches the interval at which the absorbance of each successive sample is read with the microplate reader. A 30 second interval was selected as it showed a low %CV and was practical to implement. An optimum digestion time of 60 minutes, temperature of 100 ˚C, time interval between adding CAS to sample of 30 minutes and time interval between adding CAS to successive samples of 30 seconds were selected as the final assay parameters.

Table 2.11 Validation of SK micromethod			
Robustness: Recovery (%) of diluted bathwater samples (mean concentration 207.0 (µg L^{-1}) determined following variation in digestion time with 1M ammonium persulphate (50, 60 and 70 minutes), variation in digestion temperature (90, 100 and 110 ˚C), variation in time interval between adding CAS to sample and reading its absorbance (25, 30, 35 and 40 minutes) and variation in time interval between adding CAS to successive samples (10, 20, 30 and 40 second)			
	Sample Concentration (µg L^{-1})±SD	**%CV**	**Recovery (%)**
Digestion time with 1M ammonium persulphate			
50 minutes	229.9±11.3	4.9	111
60 minutes	198.1±10.1	5.1	96
70 minutes	217.7±19.9	9.1	105
Temperature during digestion with 1M ammonium persulphate			
90 ˚C	216.7±16.3	7.5	105
100 ˚C	197.8±2.8	1.4	96
110 ˚C	215.8±11.1	5.2	104
Time interval between adding CAS to sample and reading its absorbance			
25 minutes	203.2±15.0	7.4	98
30 minutes	203.5±15.0	7.4	98
35 minutes	200.9±14.9	7.4	97
40 minutes	201.5±15.0	7.4	97
Time interval between adding CAS to successive samples			
20 Seconds	164.8±5.6	3.4	80
30 Seconds	232.3±2.5	2.5	112
40 Seconds	216.8±3.9	3.9	105

2.3.7 Specificity: Alginate interference

Mineralisation of a sample is necessary to digest any organic material which may interfere with the SK reaction by chelation of Ce (IV) or Ce (III) or otherwise affect the reaction rate. The possibility that some of the components of the bathwater matrix, released from the seaweed, may contribute to the variability in precision and recovery cannot be excluded. Four sets (n=2) of potassium iodate standards (0, 100, 200 and 300 µg L^{-1}) were spiked with 2% sodium alginate to give alginate concentrations 0–1% $^{w}/_{v}$. The iodine concentrations determined were compared and a *p*-value = 0.804 indicates that there is no significant difference between the data sets. Therefore, the presence of alginate in the matrix does not negatively impact the precision of the assay.

2.3.8 Correlation between methods

In order to correlate the SK method and its SK micromethod version, samples of seawater and bathwater from the same time period (February-April 2013) were analysed in duplicate by both methods and the results compared (***Table 2.12***).

Table 2.12 **Correlation between SK method and micromethod**			
Analysis of iodine concentration (µg L^{-1}) in seawater and bathwater			
		SK method	**SK micromethod**
Seawater	**n**	10	9
	Minimum	37	32
	Maximum	69	55
	Average	53	46
Bathwater	**n**	10	10
	Minimum	2425	2247
	Maximum	12627	14750
	Average	8693	8787

A one way ANOVA was carried out to compare the iodine concentration of both seawater and bathwater obtained with each method. The *p*-values were 0.148 and 0.954 respectively which show good correlation between the results for each method. ***Figure 2.4*** further illustrates this correlation between both methods for bathwater with an $R^2 = 0.9240$.

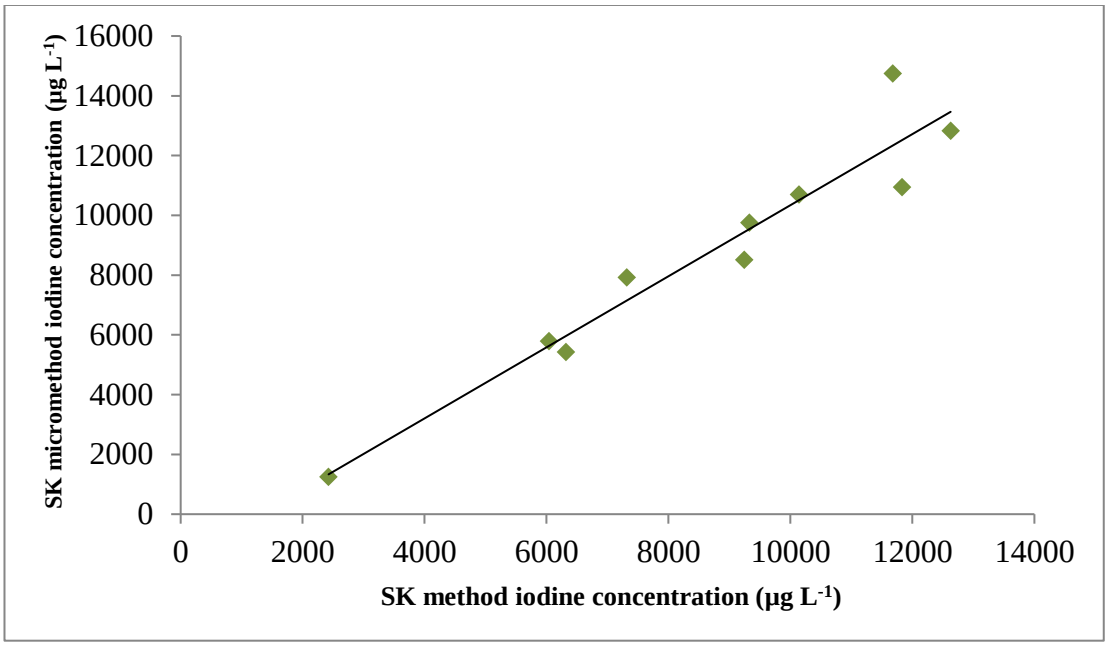


Figure 2.4 **Correlation between SK method and micromethod for bathwater** Average iodine concentration (µg L^{-1}) of bathwater samples (n=10) by SK method and micromethod

2.3.9 Comparison of methods

Overall, results for both methods are comparable, as outlined in ***Table 2.13.*** Comparison of the data sets using ANOVA indicates no significant difference for either sample type (p=0.148 for seawater, p=0.954 for bathwater). The micromethod was selected as the method for future analysis given its reduced costs, potential for increased throughput of samples with reduced waste and reduced negative environmental impact.

Table 2.13 **Validation Summary**		
Parameters for SK method and micromethod		
	SK Method	**SK Micromethod**
Linearity	Minimum R^2 = 0.9910 Maximum R^2 = 0.9981	Minimum R^2 = 0.9956 Maximum R^2 = 0.9994
Range	20-600 µg L^{-1}	20-300 µg L^{-1}

Table 2.13 **Validation Summary**		
LOD	18 µg L^{-1}	9 µg L^{-1}
LOQ	60 µg L^{-1}	29 µg L^{-1}
Intra-assay	≤ 2.8% n=10	≤ 2.5% n=6
Inter-assay Precision	8.0% n=6	6.7% n=7
Recovery	102-126%	87-113%
Robustness Optimum conditions	70 minute digestion 20 second interval between additions of CAS 30 minute incubation with CAS	60 minute digestion 30 second interval between additions of CAS 30 minute incubation with CAS 100˚C digestion temperature

2.3.10 UIC

The iodine concentration of urine samples was determined using the SK micromethod. The creatinine concentration of the samples was also determined in order to calculate the corrected iodine concentration. This was carried out to eliminate erroneous iodine concentrations due to dehydration or malnutrition as creatinine can act as an 'internal standard' in the urine sample. It is noted however that there is much debate surrounding the use of the iodine creatinine ratio. While Konno et al (1993), Soldin (2002) and Barr et al (2005) discuss that using the ratio may not be as reliable as originally outlined, Knudsen et al (2000), Haddow et al (2007) and, more recently, Li et al (2016) indicate that using a corrected iodine concentration provides a better reflection of UIC. As a result of the conflicting literature, two data sets are presented the first including the creatinine adjustment, the second without creatinine adjustment.

Creatinine analysis was carried out using the Jaffé method on the Roche Modular analyser at the Biochemistry Department, Sligo University Hospital. This is a kinetic colorimetric assay where sodium hydroxide (0.2 mol L^{-1}) is added to a sample followed by picric acid (25 mmol L^{-1}). Under alkaline conditions creatinine forms a yellow-orange complex with picrate. The rate of colour formation is directly

proportional to the creatinine concentration in the sample. The corrected iodine value of each sample was then determined using the following equation (Zimmermann (2010)):

$$\text{Corrected iodine} = \left(\frac{\text{Iodine}\ (\mu\text{g}\ L^{-1})}{\text{Creatinine}\ (\text{mmol}\ L^{-1})}\right) \times 8.85$$

As urinary iodine values tend not to be normally distributed (WHO (2013) the population median is the preferred measure of central tendency, and percentiles, rather than standard deviations, were used to describe the distribution of data.

2.4 Seaweed sample collection, preparation, homogeneity studies, stability and pH procedures

2.4.1 Sample collection

Fresh, complete *Fucus serratus* L. (fronds) and seawater samples were collected from Voya Seaweed Baths, Strandhill, Co. Sligo, Ireland (54°16'13.2"N 8°36'39.9"W). The *Fucus serratus* L. was hand harvested from Easkey, Co. Sligo, Ireland (54°17′19″N 8°57′42″W). Harvesting was carried out every 1 to 3 days. The fresh samples were typically rinsed in potable water to remove epiphytes, crustaceans and sand and then stored in potable water before use. In order to mimic the commercial seaweed baths storage conditions for this study, freshly harvested samples were transported to the lab in potable water. They were kept at ambient temperature (15-25 °C) for 18-25 hours (with the exception of one sample 40 hours) until processing. Seawater samples were collected concomitantly in a 1L polypropylene bottle. When baths were prepared in duplicate or triplicate, in order to minimise variation, seaweed samples were collected on the same day from the same harvest and were visually similar pieces (in terms of colour, shape and length).

In order to assess seasonal variation in pH, seawater and bathwater mineral levels samples were collected weekly from February 2013 to January 2014 and prepared as below. These were stored at -20 °C and analysed together at the end of the sampling

period. Samples were collected for individual studies at other times of the year at outlined in ***Table 2.14.***

***Table 2.14 Fucus serratus* L. (collection times for individual studies)**	
Study	**Collection and preparation**
Sample homogeneity	January 2015
Sample stability	December 2014
Seawater pH	June 2015
SK method optimization	July 2013
SK micromethod validation	April–May 2014
Effect of increasing time on iodine concentration	July 2014
Effect of increasing temperature on iodine concentration	August 2014
Urinary Iodine Study	June 2015
Effect of increasing mass and temperature on bathwater mineral concentration	August 2015
Microwave, AAS and FES optimization and ICP-MS optimization	November 2015
Dermal absorption *in vitro*	August 2016

2.4.2 Simulated Bath preparation

A simulated bath was prepared by heating 500 ml of ultrapure water to 70 °C in a 2 litre beaker (borosilicate) on a hotplate (BIBBY HB502, BIBBY Scientific, Staffordshire, UK). The seaweed sample, approximately 20 cm length, complete, weighing 150±0.5 g was added followed by 500 ml of seawater. The simulated bath was maintained at 55 °C for 40 minutes. After 40 minutes the intact seaweed and any visible fragments were removed using a plastic tongs and 50 ml aliquots of bathwater were transferred to 50 ml polypropylene labelled sample tubes. A 50 ml sample of the seawater was collected concurrently, filtered through a 0.45 µm Whatmann membrane filter and stored as above. Samples were thawed overnight at room temperature prior to analysis. This sample preparation procedure was modified for individual studies with respect to matrix composition and temperature as outlined in ***Table 2.15.***

***Table 2.15* Modified sample preparation conditions for individual studies**	
Study	**Modified conditions**

Effect of increasing time on iodine concentration	Samples were collected after 15 seconds and at 5 minute intervals up to 50 minutes
Effect of increasing temperature on bathwater mineral concentration	Baths (n=3 for iodine, n=2 for other minerals) were prepared at each temperature 20 ˚C, 40 ˚C, 60 ˚C and 80 ˚C Seaweed was added to each bath and the baths were maintained at the respective temperature for 40 minutes Samples were collected after 15 seconds and at 10 minute intervals up to 40 minutes
Effect of increasing mass of seaweed on bathwater mineral concentration	Baths (n=3) were prepared at 70 ˚C Seaweed (25 g, 50 g, 75 g or 100 g) was added to each bath and the baths were maintained at 50-55 ˚C for 40 minutes
AAS, FES, ICP-MS and Dermal absorption/penetration *in vitro*	Ultrapure water used instead of seawater to reduce the potential loss of ICP-MS sensitivity due to high levels of dissolved solids in the seawater which originally constituted 50% of the bathwater matrix

Statistical analysis

Statistical analyses were carried out using a one-way Analysis of Variance (ANOVA) in Minitab 17 where a p-value < 0.05 is considered significantly different.

2.4.3 Sample homogeneity

When *Fucus serratus* L. is placed in a hot aqueous environment it stimulates the release of alginate, a highly abundant polysaccharide present in the cell wall (Nishide et al (1984)). This increases the viscosity of the sample. To analyse the homogeneity of samples, a fresh simulated bath was prepared. After 40 minutes 15 ml (n=4) aliquots were taken from the top, middle and bottom of the bath respectively. A magnetic stirring bar (75 mm x 15 mm) was then added and the bathwater was stirred at low (450 rpm), medium (900 rpm) and high speed (1350 rpm). Aliquots (n=4) (15 ml) were taken from the top, middle and bottom at each speed.

Results of the iodine concentration from all sampling locations were analysed using a one way ANOVA to establish the statistical difference in concentration between unstirred and stirred baths and also between the speeds of stirring (significant difference *p*-value < 0.05). ***Table 2.16*** illustrates the average concentration ± standard deviation (SD) (where n=4) for each sampling location and the *p*-value for each bath.

Table 2.16 **Iodine concentration (µg L^{-1})±SD of unstirred and stirred simulated seaweed baths (average value where n=4)**

Sampling location	**Iodine Concentration (µg L^{-1})±SD**			
	Stirring speed (rpm)			
	Unstirred	**Low (450)**	**Medium (900)**	**High (1350)**
Top	1025±43	1060±66	951±49	1182±98
Middle	1140±44	1041±62	973±102	1159±82
Bottom	1089±51	1054±94	1100±115	1233±146
p-value	0.0192	0.9343	0.1019	0.6453

The average iodine concentration from each location of an unstirred bath is 1025, 1140 and 1089 µg L^{-1} for the top, middle and bottom respectively. A one way ANOVA indicates that there is a significant difference between the iodine concentration of samples taken from these sites in the bath where the *p*-value = 0.0192. As the highest concentration was recorded for the middle of the bath it implies that this is the optimum sampling location in order to best represent the iodine levels in the bath. There was no significant difference between the iodine concentrations recorded at each location for the stirred baths (*p*-value 0.9343, 0.1019 and 0.6453 for low, medium and high stirring respectively). Neither the stirring speed nor the sampling locations were significant.

A t-test was carried out to compare the results from the middle of the unstirred bath and the bottom of the high stirred bath (highest recorded concentrations for unstirred and stirred baths). The results indicate that there is also no significant difference between an unstirred and stirred bath (*p*-value = 0.144) however the location of sampling is significant. While stirring is not compulsory to ensure a homogenous matrix but it is recommended as good practice. It is necessary however, that samples are extracted centrally so that the highest analyte concentration is established.

2.4.4 Sample stability

A stability study was carried out from December 2014 to November 2015 to determine the effect of storage conditions at -20 ˚C on the concentration of iodine in the stored bathwater samples. This included both long term storage of samples and the effect of repeated freezing and thawing on an individual sample.

2.4.4.1 Long term stability

A simulated bath was prepared and 15 ml aliquots of bathwater were collected (n=12). These samples were stored at -20 ˚C and thawed overnight at room temperature prior to analysis. All samples were mixed by shaking before an aliquot was taken for dilution (1 in 20). The initial iodine concentration of the bathwater sample was determined to be 2015 µg L^{-1} (December 2014). The first sample was analysed in January 2015 and replaced in storage. The second sample was analysed in February 2015 and replaced in storage and so on each month until November 2015.

The iodine concentration ranged from 1669 to 2237 µg L^{-1} with an average of 1941±174 µg L^{-1} (***Figure 2.5***). The data was grouped by quarter (Q1-Q4) and analysed using a one way ANOVA. A *p*-value of 0.873 indicates that there is no significant difference in iodine concentration during long term storage. While the concentration can vary over time it does not consistently increase or decrease.

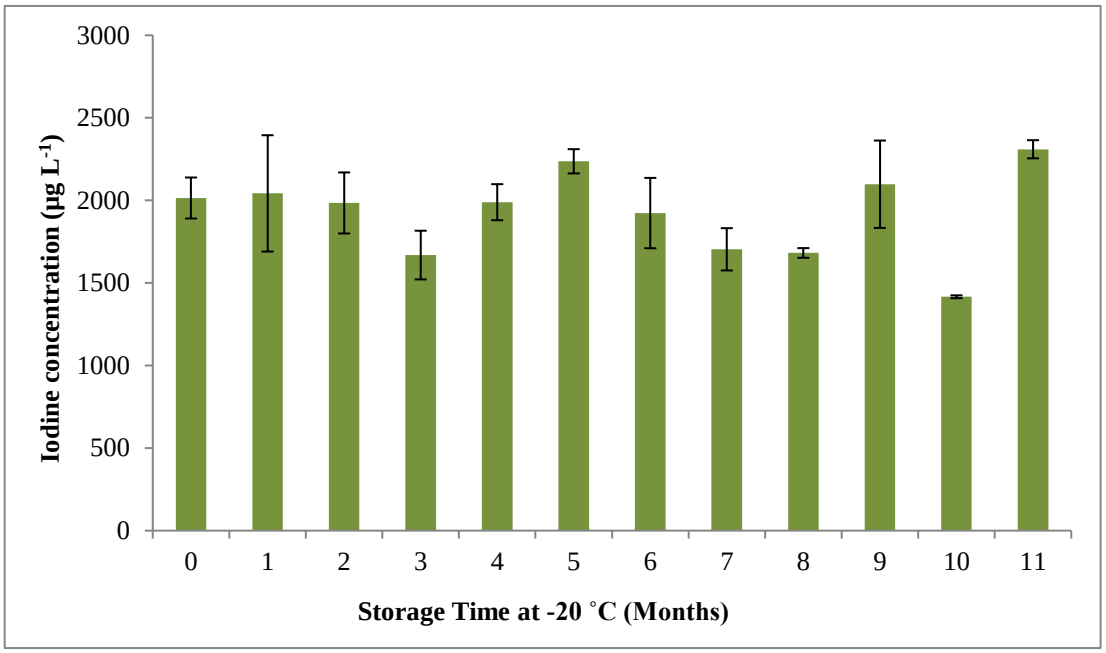


Figure 2.5 Long term stability of iodine concentration in frozen seaweed bathwater samples over 12 months December 2014 – November 2015 measured by the Sandell-Kolthoff micromethod (1941±174 µg L^{-1} (n=3)); *p*-value = 0.873 indicates no significant difference in iodine concentration

2.4.4.2 Freeze Thaw stability

A second stability study was designed to examine the freeze thaw stability of one discrete sample. The second sample, February 2015, was replaced in storage following analysis of the iodine concentration and re-analysed at the same 1 month interval as that used for long term stability analysis until November 2015. This sample had an initial iodine concentration of 1984 µg L^{-1} when analysed following ~8 weeks storage at -20 ˚C. Over 10 months the iodine concentration ranged from 1782 to 2282 µg L^{-1} with an average of 2002±163 µg L^{-1} (***Figure 2.6***). A *p*-value of 0.130 indicates that there is no significant difference in iodine concentration during freeze thaw cycles. Similarly an ANOVA was carried out for the January, March and April samples and their *p*-values were 0.298 and 0.940 and 0.720 respectively.

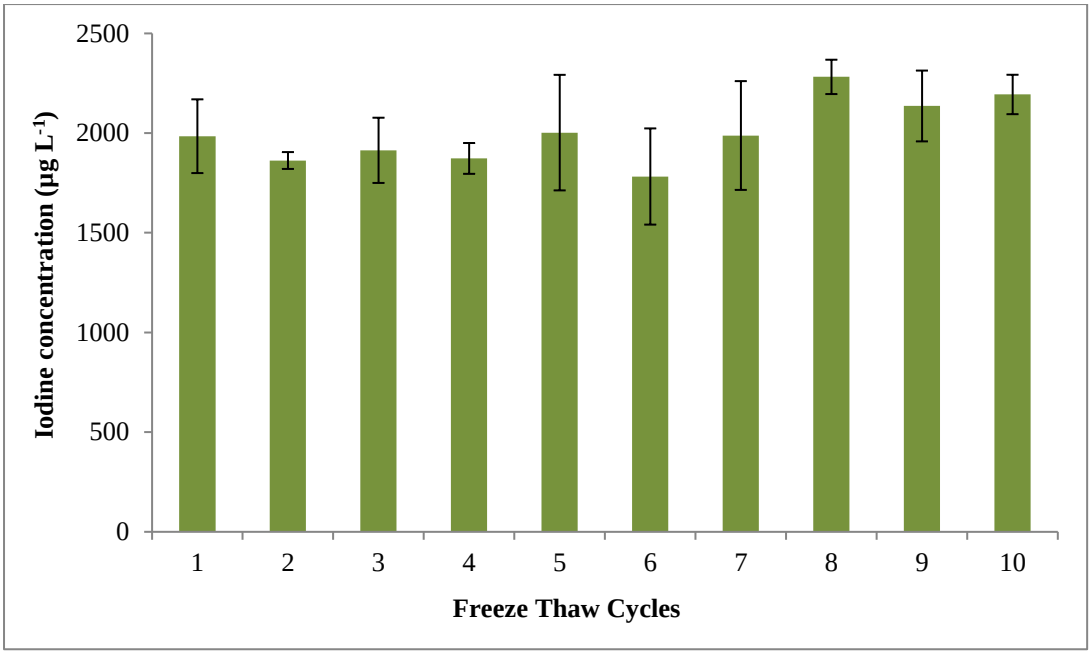


Figure 2.6 Freeze thaw stability of iodine concentration in individual frozen seaweed bathwater sample over 10 months February 2015-November 2015 measured by the Sandell Kolthoff micromethod (2002±163 µg L^{-1} (n=3)); *p*-value 0.130 indicates no significant difference in iodine concentration following 10 cycles of repeated freezing and thawing

Results indicate that storage at -20 ˚C is suitable for the bathwater samples over 12 months and that repeated freezing and thawing does not affect the iodine concentration of samples significantly.

2.4.5 Sample pH

The pH of seawater and bathwater samples was determined using a Eutech standard electrode with internal silver/silver chloride reference in combination with a Eutech pH 700 pH meter (Thermo Scientific (Ireland)). This was calibrated with buffer solutions pH 4.01 and 10.01.

2.5 Optimization of ICP-MS for determination of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc in *Fucus serratus* L. seaweed, seawater and bathwater.

2.5.1 Microwave digestion optimization

A Milestone ETHOS D Labstation microwave digester was used to digest aqueous and solid samples. The digestion procedure (Milestone S.r.l. (2001)) was optimized prior to analysis using an aqueous simulated bathwater sample digested as per standard operating conditions (***Table 2.17***). The volume of internal standard (bismuth and indium (CRM TraceCERT®), Sigma Aldrich, Arklow, Ireland) was maintained (0.2 ml) while the ratio of sample to nitric acid (Super Purity, ROMIL, Cambridge, United Kingdom) was adjusted e.g. 8.8 ml: 1 ml, 6.8 ml: 3 ml, in order to establish the optimum digestion condition (***Table 2.18***). Samples were run in triplicate and the average %CV for across all concentrations at each condition was determined.

Table 2.17 **Microwave digestion programme for acid digestion of samples**

Step	Power (W)	Initial temperature (˚C)	Final temperature (˚C)	Ramp time (min)	Hold time (min)
1	1000	22	180	10	0
2	1000	180	180	0	10
3	Cooling				30

Table 2.18 **Microwave digestion optimization. Ratio of sample volume (ml) to nitric acid volume (ml)**

Sample volume (ml)	Nitric acid volume (ml)	Internal standard volume (ml)
1.0	8.8	0.2
2.0	7.8	0.2
3.0	6.8	0.2
5.0	4.8	0.2

The typical mineral yield pattern with increasing acid volume is illustrated in ***Figure 2.7*** for manganese and zinc. Digestion with 5 ml of nitric acid yielded the highest concentration for most minerals with a %CV of 5.6. A ratio of 4.8 ml sample: 5 ml nitric acid was therefore set as the optimum digestion condition for sample digestion to allow for a total digestion volume of 10 ml.

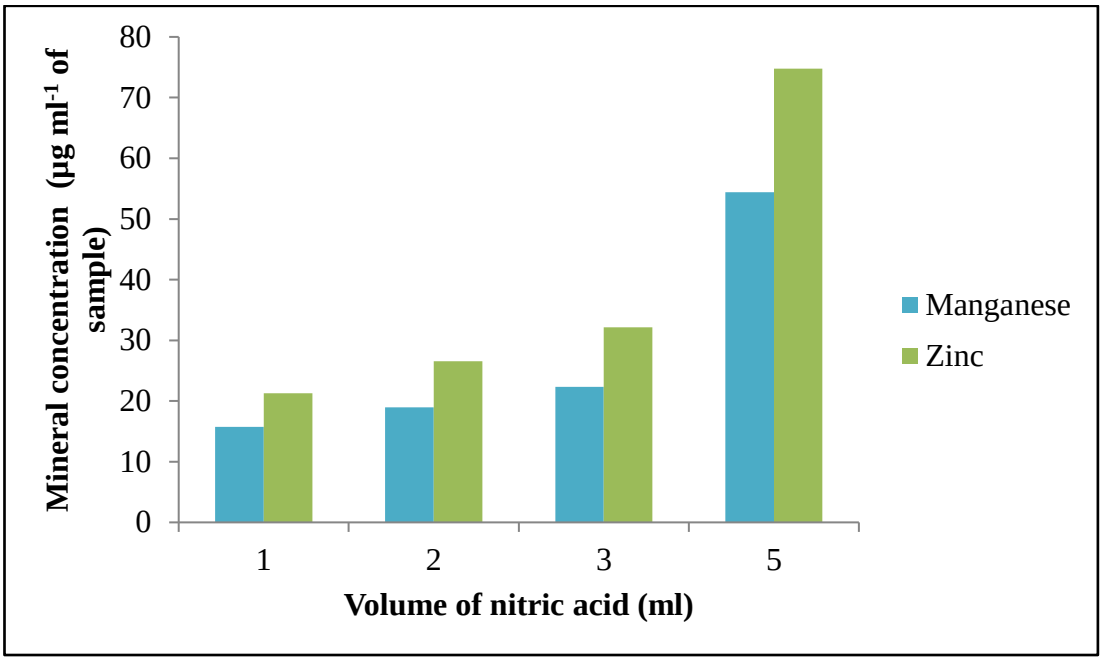


Figure 2.7 Microwave digestion with increasing volume of nitric acid (1–5 ml) and manganese and zinc yield

Solid samples of *Fucus serratus* L. were dried to constant weight in a conventional oven at 100 ˚C. The average water loss was 82% (n=10). Following drying, samples were ground using a porcelain pestle and mortar. Aliquots of 0.25 g were transferred to digestion vessels followed by 0.2 ml internal standard (20 µg L^{-1} bismuth and indium), 2 ml hydrogen peroxide and 9 ml nitric acid (Milestone S.r.l. (2001)).

Aqueous samples (seawater and bathwater) (4.8 ml) were digested with 0.2 ml internal standard and 5 ml ultra-pure concentrated nitric acid. The digestion vessels were sealed and digestion was carried out as per ***Table 2.17.*** Following digestion samples were transferred to nitric acid washed glass vials for analysis.

2.5.2 ICP-MS set up

The Varian 820-MS ICP-MS was used for the analysis of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc in digested samples. Plasma alignment and mass calibration were carried out using tuning solution (10 µg L^{-1}). Working standards were prepared by serial dilution of the stock multi-element standard in the range of 0.5-100 µg L^{-1} (in 1% nitric acid). All working standards also contained both bismuth and indium (20 µg L^{-1}). Samples were introduced to the plasma followed by 1% nitric acid between each sample to reduce potential interference from sample carryover and build up. Samples were diluted where minerals were detected above the calibration curve. Instrument settings are set out in ***Table 2.19*****.**

2.5.3 ICP-MS optimization

Linearity was determined using standard concentrations of the multi-element standard in the range of 0.5-100 µg L^{-1}. The LOD and LOQ for all elements were determined by replicate analysis (n=10) of the blank (1% nitric acid). The LOD and LOQ were calculated as 3 times and 10 times the standard deviation of the blank respectively. Precision was determined by taking five replicate measurements of one bathwater sample in one day (intra-assay) and calculating the %CV across the measurements for each mineral. Instrument precision was also calculated for each mineral across 5 replicate measurements of each standard concentration. Recovery was determined by spiking samples of known concentration with multi-element standard (10 and 50 µg L^{-1}). The spike detected was expressed as a percentage of the known concentration added to the sample.

Table 2.19 **Varian 820-MS ICP-MS operating conditions**	
R.F. power	1.40 kW
Plasma flow rate	17.5 L min^{-1}
Auxiliary flow rate	1.65 L min^{-1}
Nebulizer flow rate	1.00 L min^{-1}
Mass analyser	Quadrupole
High resolution	0.68 AMU
Low resolution	0.63 AMU
Scanning mode	Peak hopping
Points/Peak	1
Scans/Replicate	20
Replicates/Sample	5
Sample uptake delay	40 sec
Stabilization delay	10 sec
Analysis time	100 sec
Internal Standard	In^{115} Bi^{209}
Isotopes measured	Al^{27} Cr^{52} Cr^{53} Mn^{55} Co^{59} Ni^{60} Ni^{62} Cu^{63} Cu^{65} Zn^{66} Zn^{68} Se^{77} Se^{78} Se^{82} Mo^{95} Mo^{97} Mo^{98} Cd^{111} Cd^{114} Hg^{199} Hg^{200} Hg^{201} Hg^{202} Pb^{206} Pb^{207} Pb^{208}

Data analysis of signals detected by software first required determination of the ratio of analyte counts per second to internal standard counts per second (c/s). The equivalent concentration of this ratio was then established from the equation of the line for each analyte.

Linearity was observed in the range of 0.5-100 µg L^{-1} for all minerals with an average correlation coefficient of 0.9997. Typical calibration curves for 4 minerals are plotted in ***Figure 2.8.*** The method was shown to be suitable for all minerals with both LOD and LOQ set at part per trillion which is typical for ICP-MS analysis. The lower range of the calibration curve was x100–x1000 above the typical detection limit of the instrument per the Varian 820-MS specification, sensitivity was not determined.

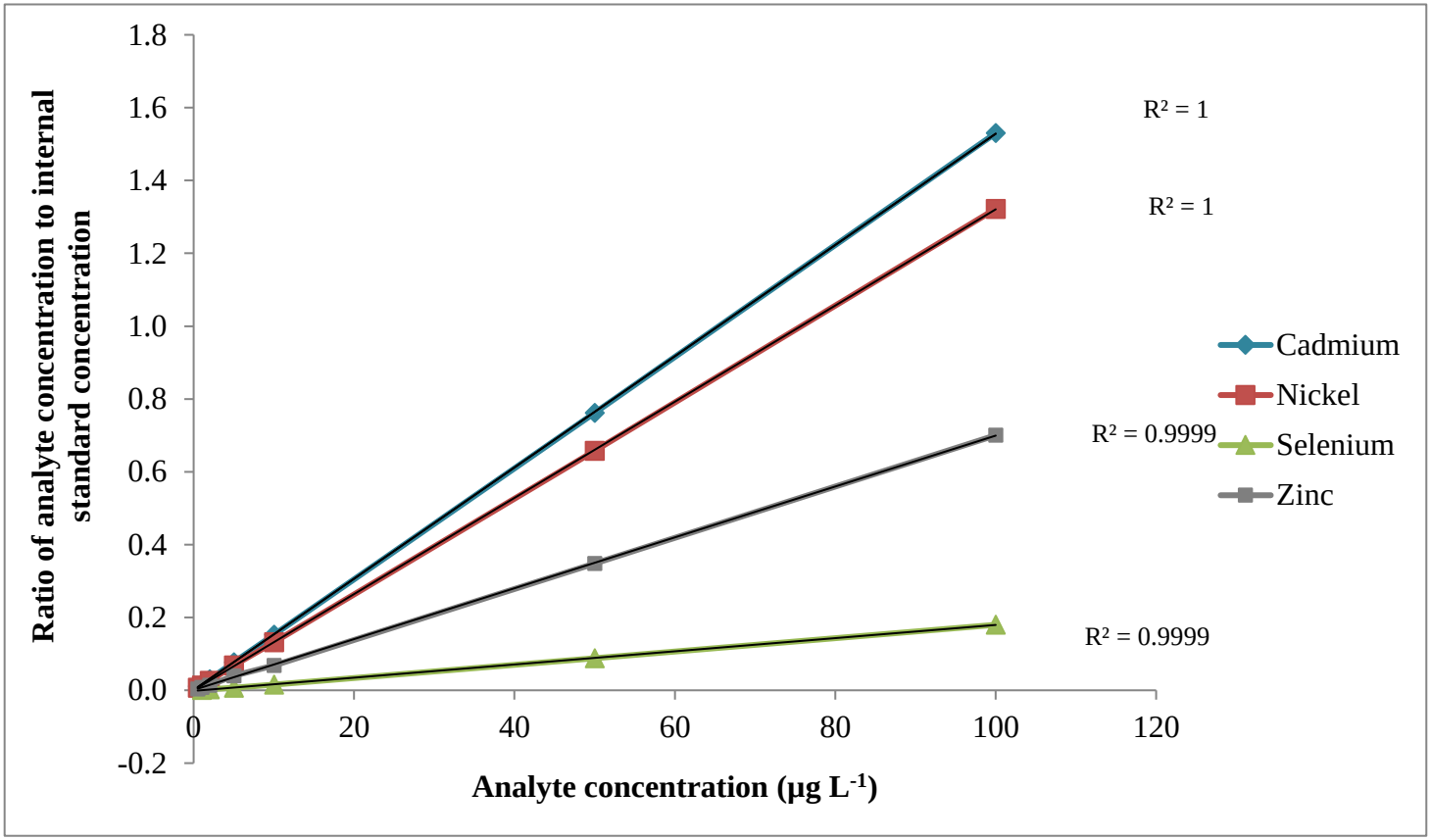


Figure 2.8 **Optimization of ICP-MS** Typical calibration curve of ratio of analyte concentration to internal standard concentration *v* analyte concentration (µg L^{-1}) for cadmium, nickel, zinc and selenium

Intra-assay precision ranged from 1.7–5.3% based on replicate analysis (n=6) of a bathwater sample. Instrument precision ranged from 1.5–3.1% based on replicate analysis (n=5) of each standard concentration in a 7 point calibration. It can be expected that there would be more variation in bathwater samples than standards however the% CV ranges are comparable, albeit that of bathwater is marginally higher, despite the differences in sample matrices. Lagerström et al (2013) determined the precision of cobalt, copper, manganese, nickel and zinc detection in seawater to be 4, 4, 2, 3 and 3% respectively. Yang et al (2009) report a %CV of 4.5 for chromium in seawater while other studies by Field et al (2007) and Kira et al (2014) report a %CV of 2.2 for molybdenum in seawater and 2.6 for selenium in biological fluid. Cheng et al (2004) record precision of aluminium detection to be 1% in groundwater and Milne et al (2010) detected cadmium and lead in seawater with assay precision of 1.3 and 5.1%.

Recovery of spiked samples ranged from 94–151%. With the exception of chromium, cobalt and copper (143–151%) all minerals were within the acceptable range of 70–130% (Environmental Monitoring Systems (1996)). The higher recoveries are attributed to the behaviour of chromium, cobalt and copper during the run. Satyanarayanan et al (2007) detail the recovery of chromium, cobalt and copper from seawater and found that pH impacted significantly on their recovery. This may explain the higher recoveries for chromium, cobalt and copper and as such results presented are an over estimation of their concentration in samples.

Overall, the data suggests that the method is suitable for mineral analysis of acid digested bathwater samples as it allows for simultaneous detection of minerals at trace levels (***Table 2.20***). It is not however suitable for detection of minerals at high concentrations e.g. calcium, magnesium, potassium or sodium. AAS and FES are more suitable alternatives for detection of these metals which are present at high levels (mg L^{-1}) in seawater, seaweed and bathwater.

Table 2.20 **Optimization of ICP-MS**						
Linearity, range (0.5–100 µg L^{-1}), LOD, LOQ, precision and recovery of aluminium, cadmium, chromium, cobalt, copper lead, manganese, molybdenum, nickel, selenium and zinc						
Mineral	**Correlation coefficient**	**LOD (µg L^{-1})**	**LOQ (µg L^{-1})**	**Intra-assay Precision**	**Instrument Precision**	**Recovery (%)**
Aluminium	$R^2 = 0.9998$	0.29	0.98	2.3	2.5	125
Cadmium	$R^2 = 1.0000$	0.01	0.02	5.3	2.9	99
Chromium	$R^2 = 0.9999$	0.11	0.37	3.1	2.2	143
Cobalt	$R^2 = 0.9997$	0.003	0.01	4.2	2.0	151
Copper	$R^2 = 0.9997$	0.01	0.04	4.5	1.9	148
Lead	$R^2 = 1.0000$	0.01	0.03	1.7	2.5	99
Manganese	$R^2 = 0.9998$	0.01	0.03	2.3	1.5	ND
Molybdenum	$R^2 = 0.9999$	0.15	0.51	4.7	2.9	114
Nickel	$R^2 = 0.9994$	0.02	0.07	3.3	1.9	131
Selenium	$R^2 = 0.9996$	1.45	4.85	4.8	3.1	94
Zinc	$R^2 = 0.9994$	0.15	0.50	2.6	1.9	131

2.6 Optimization of Atomic Absorption Spectrophotometry (AAS) and Flame Emission Spectrophotometry (FES) methods for determination of calcium, magnesium, potassium and sodium in *Fucus serratus* L. seaweed, seawater and bathwater

2.6.1 AAS optimization

The Agilent 280 FS atomic absorption spectrophotometer was used for the analysis of magnesium and potassium in digested samples. Working standards (Magnesium AAS standard ((1000 mg L^{-1} (CRM TraceCERT®)) in 0.5 M HNO_3), Merck, Germany; Potassium AAS standard (1000 mg L^{-1} (99.99%)), Lennox, Dublin, Ireland) in the range of 0.1–5 mg L^{-1} were used to optimize the AAS signal with typical correlation coefficients of 0.9995 and 0.9999 respectively (***Figures 2.9*** and ***2.10***). Analysis was carried out as per ***Table 2.21.***

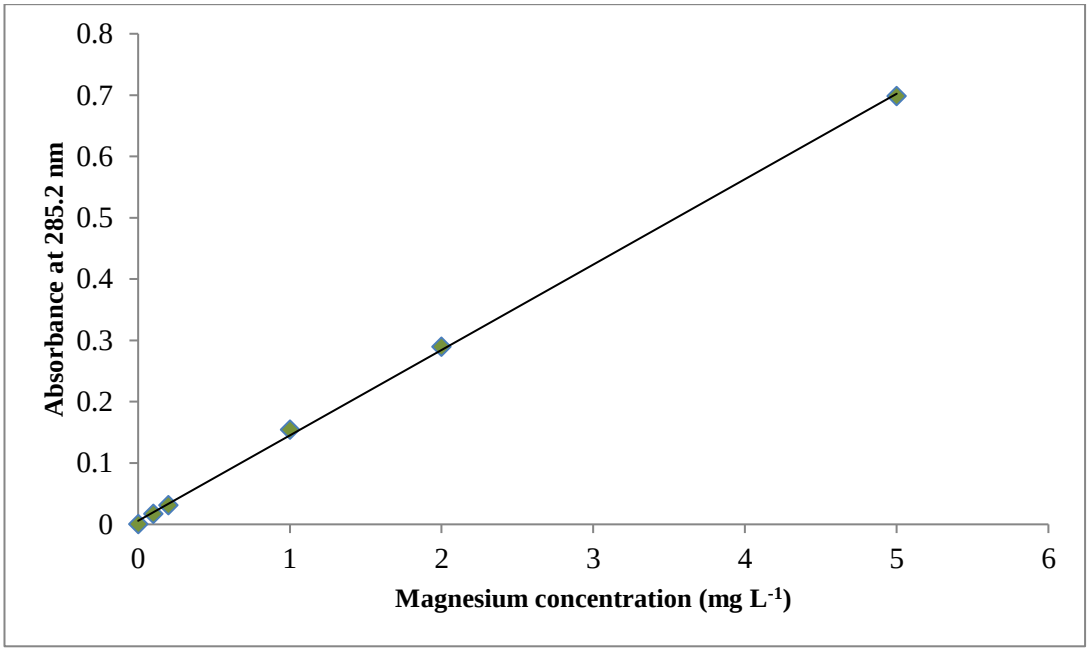


Figure 2.9 **Optimization of AAS** Linearity: Typical calibration curve of Absorbance at 285.2 nm *v* Magnesium concentration (mg L^{-1}) $R^2 = 0.9995$

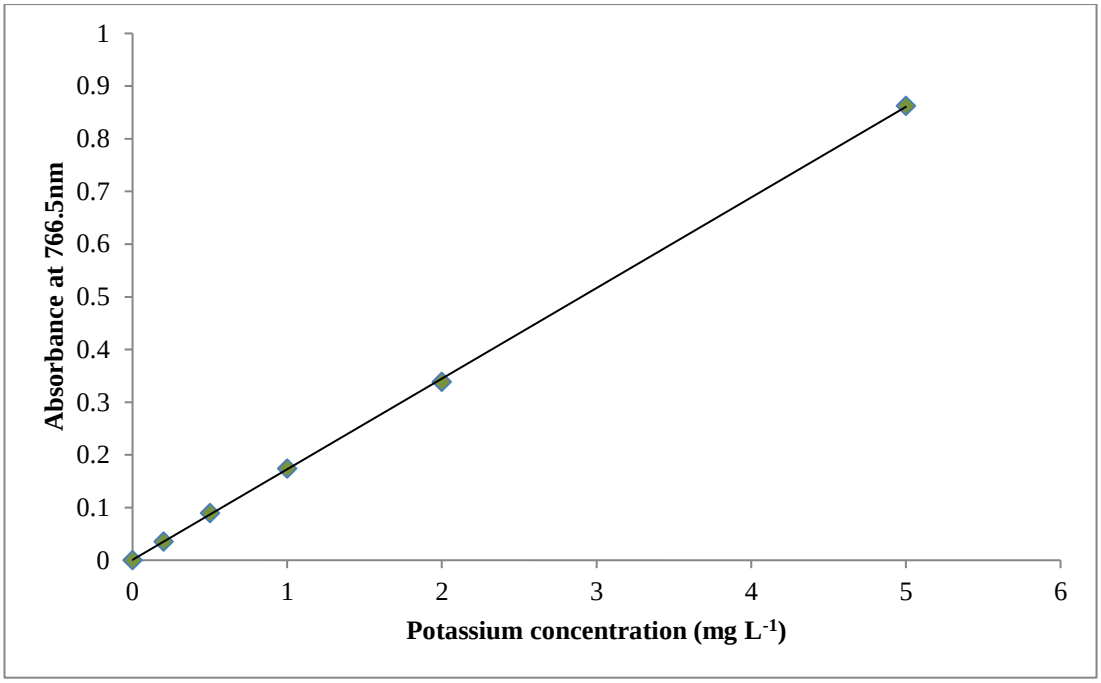


Figure 2.10 **Optimization of AAS** Linearity: Typical calibration curve of Absorbance at 766.5 nm *v* Potassium concentration (mg L^{-1}) $R^2 = 0.9999$

Table 2.21 **Agilent 280 FS AAS operating conditions**		
	Magnesium	**Potassium**
Wavelength (nm)	285.2	766.5
Slit Width (nm)	0.5	1.0
Lamp Current (mA)	4.0	5.0
Flame Type	Air/Acetylene	
Air Flow (L min^{-1})	13.50	
Acetylene Flow (L min^{-1})	2.00	
Burner Height (mm)	13.5	

It was not possible to analyse calcium with this instrument as there was no nitrous oxide gas connected therefore an alternative AAS (Perkin Elmer 2380) was used for the detection of calcium in *Fucus serratus* L. samples only, not aqueous samples. Working standards in the range of 0.1–10 mg L^{-1} were used to optimize the signal with correlation coefficients of 0.9976 (***Figure 2.11***). Analysis was carried out as per ***Table 2.22.***

Table 2.22 **Perkin Elmer 2380 AAS operating conditions**	
	Calcium
Wavelength (nm)	422.7
Slit Width (nm)	0.7
Lamp Current (mA)	15
Flame Type	Nitrous Oxide/Acetylene
Air Flow (ml min^{-1})	40.00
Fuel Flow (ml min^{-1})	20.00
Burner Height (mm)	13.5

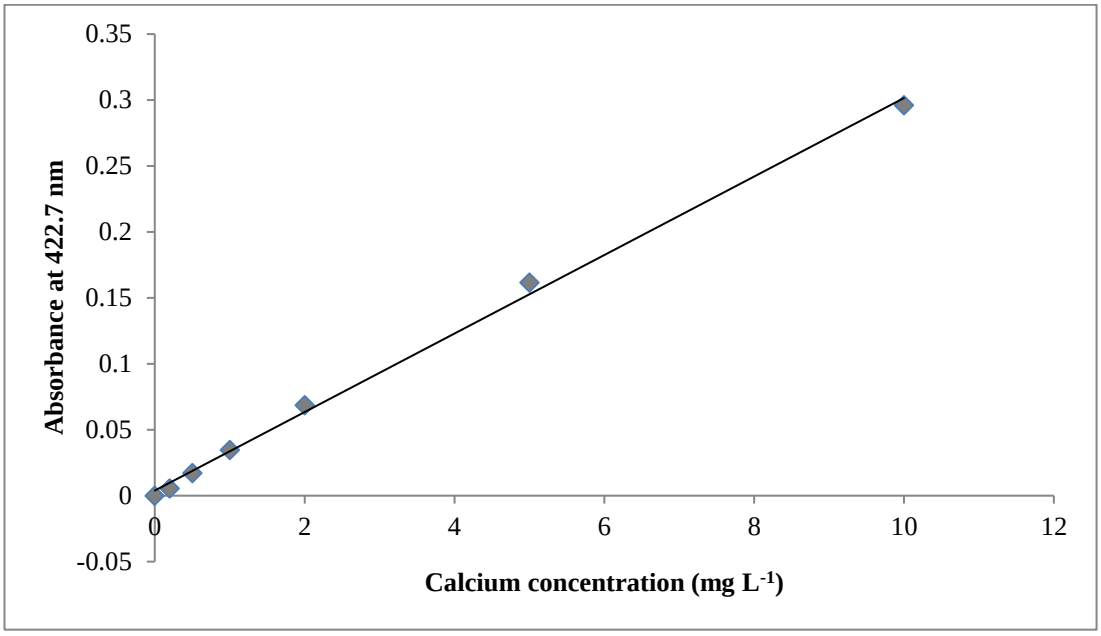


Figure 2.11 **Optimization of AAS** Linearity: Typical calibration curve of Absorbance at 422.7 nm *v* Calcium concentration (mg L^{-1}) $R^2 = 0.9976$

2.6.2 FES optimization

The Sherwood 360 flame emission spectrophotometer was used for the analysis of sodium in digested samples. Working standards prepared in the range of 1–10 mg L^{-1} were used to optimize the signal with a maximum correlation coefficient of 1 (***Figure 2.12***). Ultrapure water was aspirated for 30 minutes prior to analysis to stabilise the signal. Blanks, standards and samples were aspirated for 20 seconds before recording the signal intensity. Nitric acid (1%) was flushed through the instrument after each sample set.

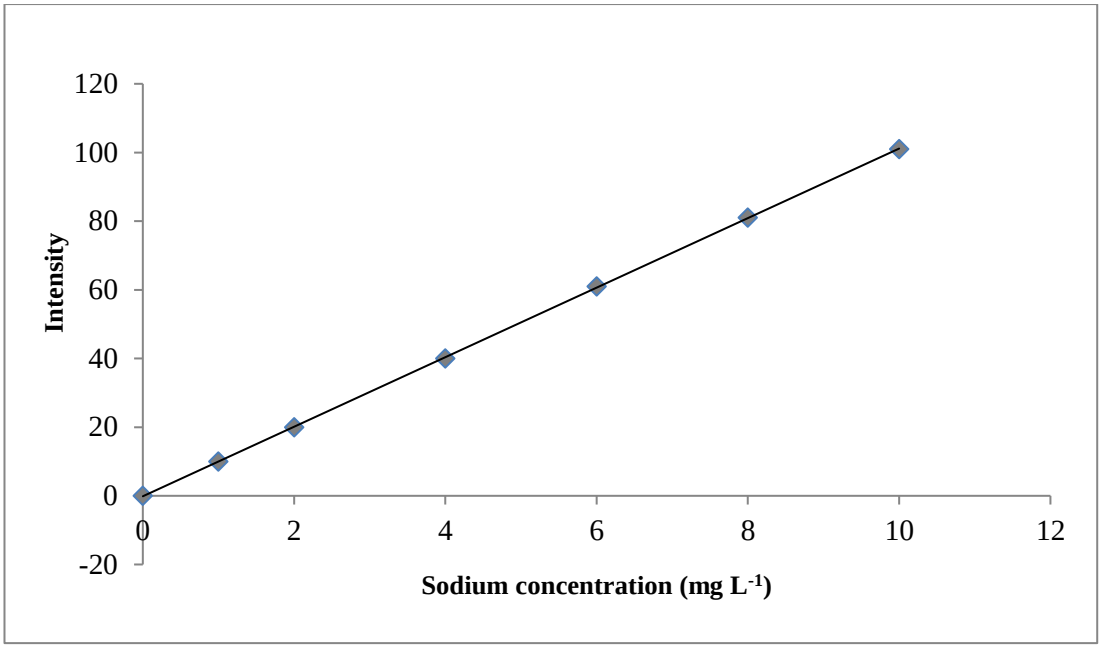


Figure 2.12 **Optimization of FES** Linearity: Typical calibration curve of Absorbance *v* Sodium concentration (mg L^{-1}) $R^2 = 1$

Although these methods are slower in terms of sample throughput, as they only allow for single element detection in a sample, they were shown to be linear up to 10 mg L^{-1} with correlation coefficients of 0.9995 to 1.

2.7 Optimization of Enhancer cell technology for the determination of dermal absorption of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc from *Fucus serratus* L. seaweed bathwater

2.7.1 Enhancer cell technology and dissolution apparatus optimization

The enhancer cell body was filled with 5 ml of the multi-element standard (1000 mg L^{-1} (Aquilant Analytical Sciences, Dublin, Ireland)) and the base adjusted until the sample was level with the opening of the cell body. The cuprophan membrane (11.5 µm thickness, 5.5 cm diameter) was applied; this provided an absorption surface area of 4 cm^2 (Agilent, Cork, Ireland). The membrane was hydrated for 24 hours as per the manufacturer's guideline prior to the start of the experiment by immersion in receptor fluid Phosphate Buffered Saline (PBS) (grade/purity not available Sigma Aldrich, Arklow, Ireland) in order to remove glycerol. As glycerol is incorporated into the cellulose membrane to enhance its texture and reduce brittleness it can also block the membrane pores (Ng et al (2010)). The enhancer cell was sealed ensuring no air bubbles were present under the membrane. The sealed enhancer cell (***Figure 1.29***) was placed in the dissolution vessel then placed in the dissolution apparatus (Vankel VK7010 supplied by Agilent, Cork, Ireland) (***Figure 2.13***).

The paddle height was adjusted as per manufacturers' guidelines. A cover was placed over the vessel to avoid evaporation of the receptor fluid (PBS). A water bath was used to maintain the temperature of the receptor fluid and membrane throughout. The apparatus was allowed to equilibrate for 30 minutes prior to starting the experiment.

The paddle rotation speed was varied at 50, 100 and 200 rpm. Similarly the receptor temperature was varied at 32, 37 and 50 °C and the receptor volume was varied at 50, 100 and 200 ml PBS. Each test run was 50 minutes and samples (1 ml) collected after 50 minutes were analysed by ICP-MS.

Figure 2.13 Varian VK 7010 Dissolution Apparatus Varian Inc. (2006)

Optimization required variation in receptor temperature (32, 37 and 50 ˚C), paddle rotation speed (50, 100 and 200 rpm) and receptor volume (50, 100 and 200 ml) to establish the optimum conditions for *in vitro* analysis (***Figure 2.14***). The typical changes in cumulative mineral concentration relative to receptor temperature, paddle rotation speed and receptor volume are illustrated for zinc in ***Figures 2.15*** to ***2.17***.

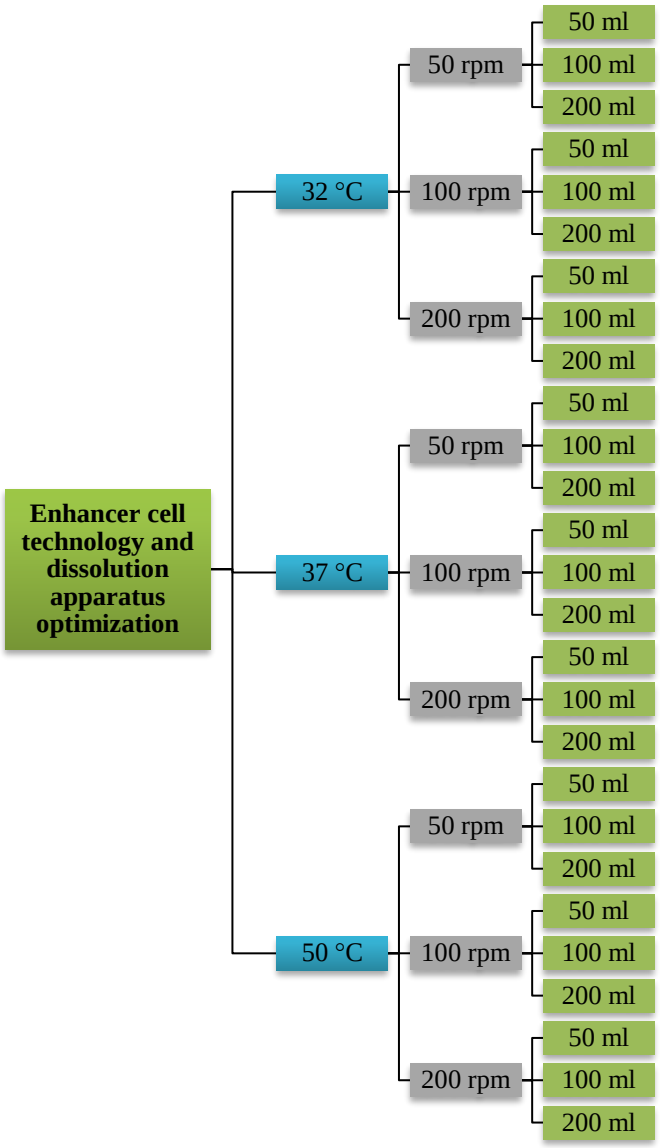


Figure 2.14 **Optimization of enhancer cell technology and dissolution apparatus** Conditions for *in vitro* analysis of dermal absorption

2.7.2 Receptor temperature

The cumulative concentration of zinc increased with increasing temperature from a minimum absorption of 12870 µg L^{-1} at 32 ˚C to a maximum of 27786 µg L^{-1} at 50 ˚C (***Figures 2.15***to ***2.17***). This increase was also evident for the other minerals analysed. While this indicates increased absorption with increased temperature it is standard practice to carry out analysis with the receptor temperature at body temperature (37 ˚C).

2.7.3 Paddle rotation speed

The data also indicates that a higher paddle rotation speed (200 rpm) leads to higher concentration of zinc absorbed compared to medium and lower rotation speed. At 32 ˚C 2016-7493 µg L^{-1} is absorbed at 50–100 rpm; this increases to 12870 µg L^{-1} at 200 rpm. These increase further with increase in temperature at 37 ˚C to 2415-15178 µg L^{-1} at 50-100 rpm and up to 22451 µg L^{-1} at 200 rpm. At 50 ˚C, 1742-20089 µg L^{-1} is absorbed at 50-100 rpm reaching a maximum of 27786 µg L^{-1} at 200 rpm. ***Figures 2.15*** to ***2.17*** show that the concentration decreases with increased receptor volume however the higher rotation speed yielding higher concentration remains consistent.

2.7.4 Receptor volume

It is also evident from ***Figures 2.15*** to ***2.17*** that zinc concentration decreases with increasing receptor volume (50–200 ml). The trend is consistent irrespective of change in temperature and paddle rotation speed.

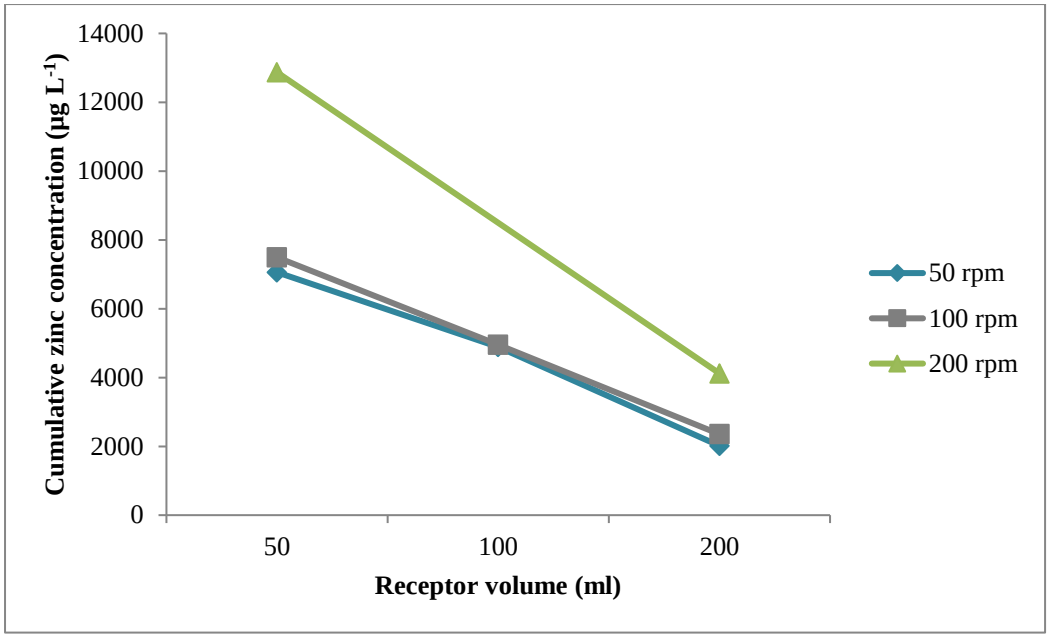


Figure 2.15 **Optimization of enhancer cell technology and dissolution apparatus** Zinc absorption (µg L^{-1}) from multi-element standard over 50 minutes at 32 ˚C

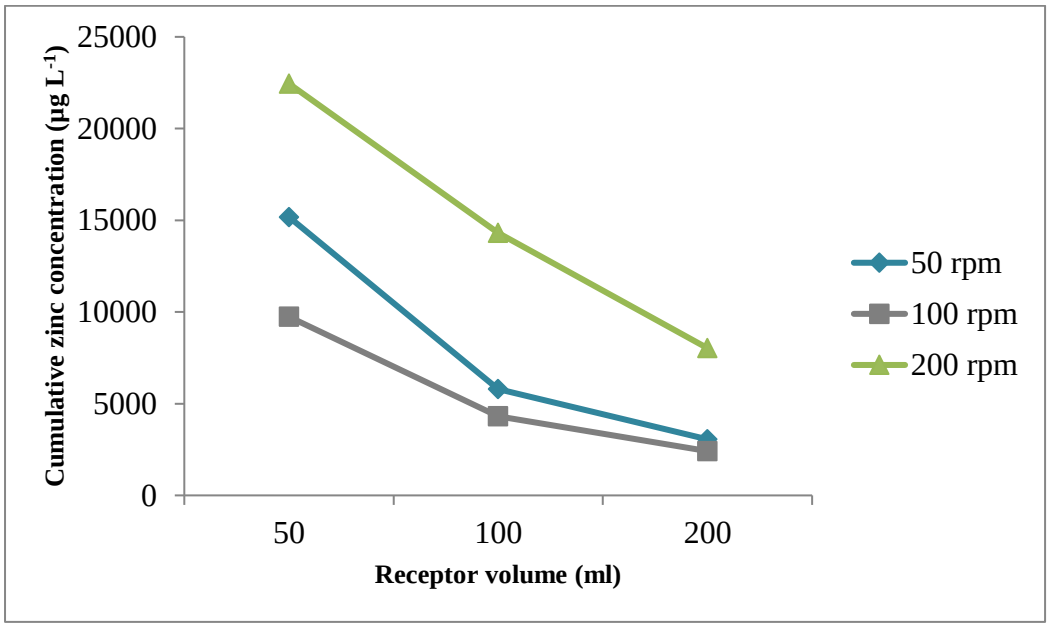


Figure 2.16 **Optimization of enhancer cell technology and dissolution apparatus** Zinc absorption (µg L^{-1}) from multi-element standard over 50 minutes at 37 ˚C

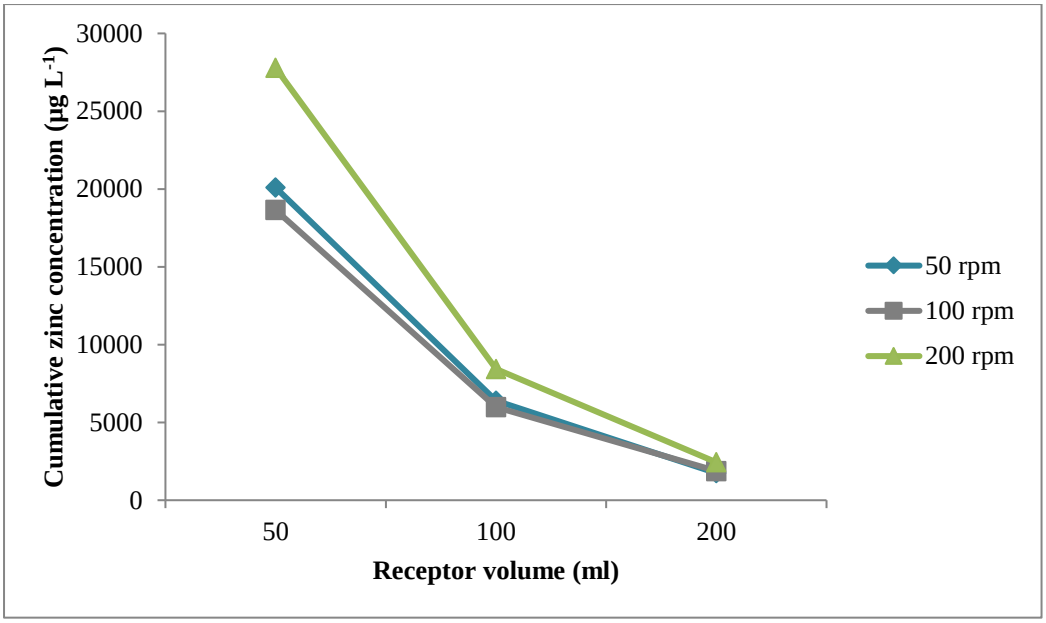


Figure 2.17 **Optimization of enhancer cell technology and dissolution apparatus** Zinc absorption (µg L^{-1}) from multi-element standard over 50 minutes at 50 ˚C

As all target minerals were detected in the receptor cell following immersion of the enhancer cell this technique was shown to be suitable for *in vitro* analysis as both membrane and system were shown to be acceptable. Based on the intended purpose of the study the final condition selected for analysis were a receptor temperature of 37 °C, to replicate body temperature, a paddle rotation speed of 200 rpm to ensure optimum transdermal absorption of minerals from the enhancer cell, as well as uniform distribution of minerals in the receptor cell and a receptor volume of 50 ml to capture minerals at lower concentrations (***Table 2.23***).

Table 2.23 **Chosen conditions for *in vitro* dermal absorption analysis using enhancer cell technology and dissolution apparatus**	
Parameter	**Chosen condition**
Receptor temperature (˚C)	37
Paddle rotation speed (rpm)	200
Receptor volume (ml)	50

2.7.5 *In vitro* study

The enhancer cell body was filled with 5 ml of simulated bathwater sample and the hydrated membrane was applied and sealed. This was immersed in the dissolution vessel containing 50 ml of PBS receptor fluid then placed in the dissolution apparatus with the cover in place to avoid its evaporation. The enhancer cells were prepared in triplicate. The water bath was set at 37 °C to maintain the temperature of the receptor fluid throughout and to ensure a membrane temperature of 37 °C and the paddles were set to spin at 200 rpm.

The first sample (1 ml) was extracted from the receptor fluid using an auto pipette into a disposable eppendorf (Eppendorf) after ~10 seconds. The receptor volume was maintained following sample collection by addition of fresh PBS (1 ml) at 37 °C. Further samples (1 ml) were collected at 10 minute intervals for the first 6 hours then every 30 minutes for 4 hours with the final sample collected after 18 hours. The study was extended over 18 hours in order to capture data on the behaviour of minerals in the time following a seaweed bath treatment. A recommendation by bath providers following bathing is to leave the bathwater extracts on the skin rather than removing by washing therefore it was expected that prolonged exposure to the bathwater matrix may lead to increased absorption of minerals. It is necessary to collect multiple samples to generate an adequate release profile of the minerals and to establish a burst effect whereby there is a high initial release of minerals into the receptor fluid. Samples were prepared for ICP-MS analysis by addition of 10 µl of 1% nitric acid and 20 µl internal standards (bismuth and indium) to each eppendorf which was vortexed before analysis.

The cumulative amount of mineral penetrating was calculated as follows:

$$\textbf{Cumulative amount of mineral (µg) per cm}^2 = \frac{\textbf{(Amount of mineral}^a \textbf{ (µg) in 50 ml PBS} + \sum \textbf{Amount of mineral (µg) in previous 1 ml sample aliquots}^b\textbf{)}}{4^c}$$

Note : [a]the amount of mineral (µg) in 50 ml PBS was the concentration in any 1ml sample removed

[b]the sum of the amounts of mineral (µg) in previous 1 ml sample aliquots i.e. the total concentration collected and removed prior to that sample. The sum of these 2 concentrations was divided by [c] to provide a cumulative amount of mineral (µg) penetration per cm^2 of membrane

[c]exposed surface area of membrane i.e. 4cm

Following optimization and validation of all the above methods they were then used for analysis of *Fucus serratus* L., bathwater and seawater samples to analyse the chemistry of the seaweed baths under various conditions of mass, temperature and season.

3 Mineral profile of *Fucus serratus* L., seawater and bathwater

Using the analytical techniques optimize and validated in Chapter 2, this chapter sets out the application of these methods to solid (*Fucus serratus* L.) and aqueous samples (seawater and bathwater) in order to determine their mineral profiles and pH characteristics.

The levels of aluminium, cadmium, calcium, chromium, cobalt, copper, iodine, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, sodium and zinc are analysed in a range of experiments. Firstly their concentration in *Fucus serratus* L. is measured and compared to literature values. The release of these minerals from the seaweed into the bathwater is then examined under laboratory controlled conditions of varying mass and temperature. The effect of increasing mass of *Fucus serratus* L. (25-100 g L^{-1}) on mineral release is examined to establish the optimum seaweed: water ratios for bathing which might maximise exposure to beneficial minerals while limiting exposure to those which are more harmful. The effect of increasing temperature (20-80 ˚C) on mineral release is also examined in order to identify the optimum temperature for bathing to ensure maximum exposure to beneficial components released from the seaweed. In the case of iodine, a time release profile at a constant temperature is also studied. The pH range of seawater and bathwater is also determined as the pH influences the mineral species present in the bath.

Finally the seasonal variation of aluminium, cadmium, chromium, cobalt, copper, iodine, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, sodium and zinc concentrations in seawater and bathwater over 12 months is also studied to ascertain whether or not there is an optimal season for bathing relative to mineral content.

3.1 *Fucus serratus* L. mineral profile

The concentration of the minerals detected in *Fucus serratus* L. by FES, AAS and ICP-MS is illustrated in ***Table 3.1***. Literature values for Fucales are also included for comparison. An experiment was carried out to adapt the SK method for the measurement of iodine in dried seaweed. Initially samples were digested using ammonium persulphate however visual inspection indicated that samples were not digested sufficiently. An alternative digestion using Tetramethylammonium Hydroxide (TMAH) was carried out (Tagami et al (2006), Zheng et al (2012)). Following digestion the samples were processed per the SK method protocol (2.3.3), however the samples remained opaque at the end of the assay and their iodine concentration could not be determined. Instead literature values using other methods are shown for iodine.

The measured mineral concentrations are, for the most part, in line with literature values. Given that algae accumulate minerals from surrounding seawater the ranges of minerals identified are in line with documented values for seawater (Stanford University (2015)). Heavy metals lead, mercury, cadmium and chromium were detected at low levels (0.5, 0.3, 1.6 and 6.2 µg g^{-1} respectively). Macrominerals were present in much higher concentrations (137.3-6493.9 µg g^{-1} (calcium and potassium n=1; magnesium n = 2)).

Selenium was detected at a higher concentration (7.4±0.9 µg g^{-1}) than the literature value (1.2 µg g^{-1}). It is notably higher in bathwater samples (***Figure 3.29***) in August which is also when samples were collected for analysis. Conversely, aluminium is considerably lower (17.1±1.5 µg g^{-1}) than its documented concentration (228 µg g^{-1}). While the variation in selenium can be attributed to seasonal factors, variation in aluminium can be attributed to variation in geographical location: the documented value for aluminium is based on *Fucus vesiculosus* samples from Norway.

Overall, the sum total of the mineral concentrations detected in the samples was estimated to be approximately 2% dry weight (approximately 20 mg g^{-1}D.W.) which is mainly due to the presence of magnesium, calcium, potassium and sodium.

Table 3.1 **Mineral analysis of *Fucus serratus* L. determined by FES, AAS and ICP-MS, with comparison to documented data for Fucales where n=3, **n=2, * n=1**				
Mineral	***Fucales* concentration (μg g^{-1} DW)**			**Reference**
	Measured	**Literature value**		
	Fucus serratus	***Fucus serratus***	***Fucus vesiculosus***	
Aluminium	17.1±1.5	1184	228	Coquery et al (1997) Van Netten et al (2000)
Cadmium	1.6±0.7	0.5	0.3 1.7	Coquery et al (1997) Van Netten et al (2000) Balina et al (2016)
*Calcium	9761.4	12730 12840±1	9380±70 21500	Coquery et al (1997) Ruperez (2002) Balina (2016) Cabrita et al (2016)
Chromium	6.2±1.9	10.4	9.6	Coquery et al (1997) Balina et al (2016)
Cobalt	1.0±0.3	0.88 1.96	0.39	Coquery et al (1997) Van Netten et al (2000) Cabrita et al (2016)
Copper	7.3±1.6	5.1 2.69±0.2	1.4 12.7	Coquery et al (1997) Van Netten et al (2000) Balina et al (2016) Cabrita et al (2016)
Iodine	ND	1400-2500	NR	Nitschke and Stengel (2015)
Lead	0.5±0.2	2.2 0.5	NR	Coquery et al (1997) Cabrita et al (2016)
**Magnesium	6493.9±17.3	9070 7240±31	NR	Coquery et al (1997) Cabrita et al (2016)
Manganese	137.3±12.8	56.1 149.6±0.4	55±1.1	Coquery et al (1997) Ruperez (2002) Cabrita et al (2016)
Mercury	0.3±0.1 0.3±0.1	0.4 0.1	NR NR	Coquery et al (1997) Cabrita et al (2016)
Molybdenum	0.4±0.1	2.7 0.3	NR	Coquery et al (1997) Cabrita et al (2016)

Table 3.1 **Mineral analysis of *Fucus serratus* L. determined by FES, AAS and ICP-MS, with comparison to documented data for Fucales where n=3, **n=2, * n=1**				
Mineral	***Fucales* concentration (µg g^{-1} DW)**			**Reference**
	Measured	**Literature value**		
	Fucus serratus	***Fucus serratus***	***Fucus vesiculosus***	
Nickel	18.5±4.2	3.8	15.3	Coquery et al (1997) Van Netten et al (2000)
*Potassium	2419.7	NR	2000-7000 11000	Truus et al (2001) Balina (2016)
**Selenium	7.4±0.9	0.1 1.2±0.4	NR	Coquery et al (1997) Cabrita et al (2016)
Sodium	789.3±157.6	NR	460-510	Truus et al (2001)
Zinc	34.1±5.4	47.3 52.75±0.4	37±3.7	Coquery et al (1997) Ruperez (2002) Cabrita et al (2016)
Total mineral concentration	19696±204.6	N/A	N/A	N/A

ND = Not Determined; NR Not reported.

3.2 Mineral release from *Fucus serratus* L. seaweed into ultrapure water under laboratory controlled conditions of varying mass, temperature and time

3.2.1 Effect of increasing mass of *Fucus serratus* L. (50-200 g L^{-1}) on bathwater mineral concentration

The mass dependent mineral release profile at constant temperature (50-55 ˚C) of aqueous samples is illustrated in ***Table 3.2*****.** The change in mineral concentration relative to change in seaweed mass is shown graphically by range in ***Figures 3.1*** to ***3.5***. This study was carried out to establish whether a linear increase in algal mass resulted in a directly proportional increase in aqueous mineral concentration. In general the mineral concentration increased as the ratios of mass of seaweed:volume of water increased from 50-200 g of seaweed per litre for all minerals. However, the rate of increase was not uniform across all minerals. Cadmium, magnesium and manganese are the only metals for which proportional release is demonstrated i.e. a 4 fold increase in seaweed mass led to an approximate 4 fold increase in concentration ***Figures 3.1***, ***Figure 3.5*** and ***Figure 3.4*** respectively. In response to a 4 fold increase in mass of seaweed, the concentration of the macro elements sodium and potassium and micro elements chromium, copper, lead and nickel in the aqueous solution increased approximately ~2 fold and cobalt and zinc increased by ~ 2-3 fold. Aluminium, molybdenum and selenium showed only a marginal increase in concentration with increased mass. While investigation of the behaviour of individual minerals e.g. ion solubility or mineral saturation was beyond the scope of this research, results indicate that the relationship between seaweed mass and bathwater concentration is complex and not simply mass dependent.

Table 3.2 **Multielement analysis of aqueous samples with increasing *Fucus serratus* L. mass: volume ratios and overall% increase in concentration of test element following four fold increase in mass/volume. Test condition prepared in triplicate, analysis of duplicate samples (n=6)**				
Mineral	**Mass of *Fucus serratus* L. (g) per litre of ultrapure water**			
	50	**100**	**150**	**200**
Aluminium (µg L^{-1})	355.50±7.43	373.83±24.05	390.42±24.06	391.00±23.90
Cadmium (µg L^{-1})	0.29±0.09	0.90±0.06	0.83±0.22	1.56±0.16
Chromium (µg L^{-1})	14.52±0.89	23.13±2.27	20.04±3.72	25.50±1.87
Cobalt (µg L^{-1})	3.25±0.38	7.42±0.54	6.69±1.40	11.56±1.01
Copper (µg L^{-1})	28.90±3.48	53.58±8.65	47.79±4.71	46.96±11.18
Lead (µg L^{-1})	0.40±0.07	0.46±0.04	0.52±0.04	0.94±0.08
Magnesium (mg L^{-1})	17.13±4.43	42.27±13.33	85.13±50.67	100.88±28.83
Manganese (mg L^{-1})	2.58±0.22	6.42±1.77	8.52±2.48	16.69±2.33
Molybdenum (µg L^{-1})	11.46±0.65	15.42±1.27	14.58±1.37	18.75±0.80
Nickel (µg L^{-1})	56.00±6.15	91.52±21.91	75.06±10.44	116.31±5.20
Potassium (mg L^{-1})	4.06±0.79	4.17±1.24	5.92±1.98	9.65±0.33
Selenium (µg L^{-1})	120.73±16.75	172.02±14.66	124.40±16.35	164.02±6.72
Sodium (mg L^{-1})	295.98±54.56	430.29±77.56	462.29±12.63	566.27±15.25
Zinc (µg L^{-1})	129.63±10.44	253.56±24.09	270.88±47.93	399.98±35.20

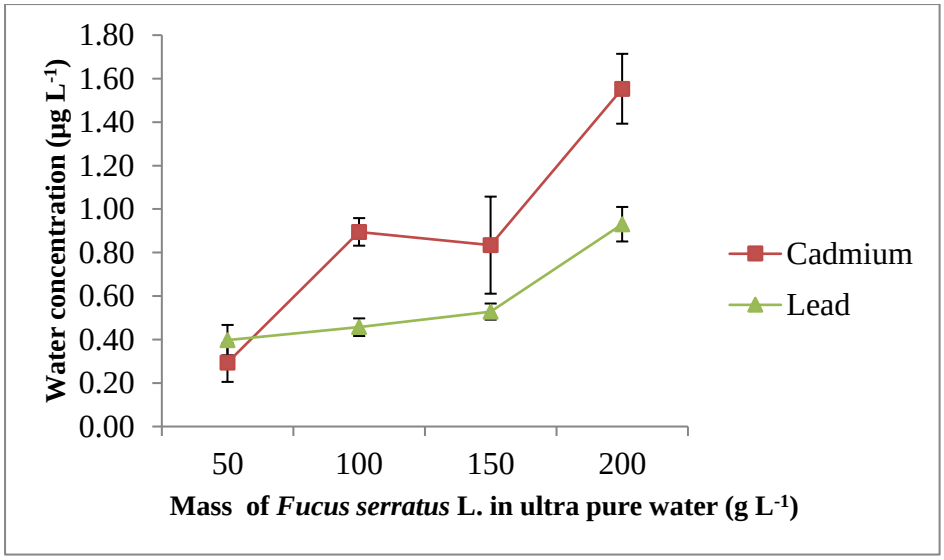


Figure 3.1 Cadmium and lead concentration of aqueous samples with increasing mass seaweed/volume ratios *Fucus serratus* L. (50–200 g L^{-1}); minerals in low µg L^{-1} range. Test condition prepared in triplicate, analysis of duplicate samples (n=6)

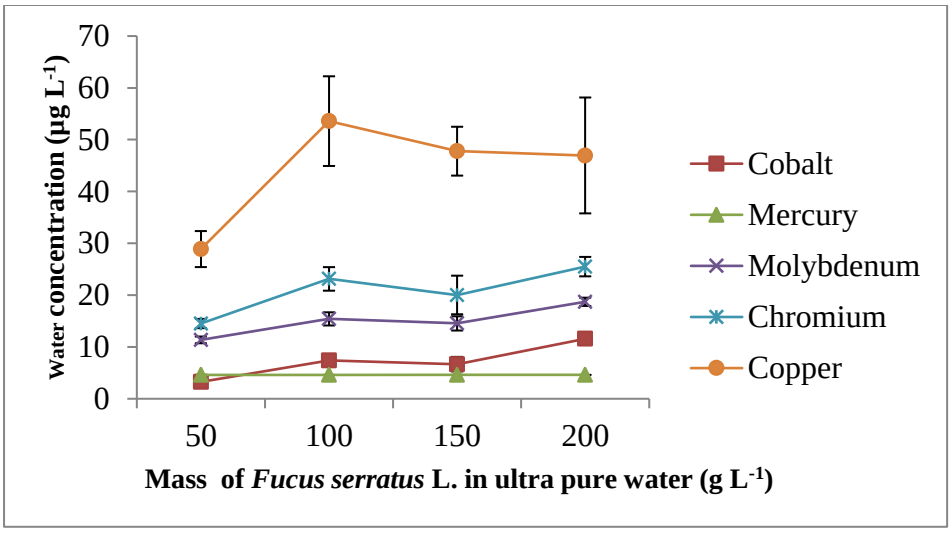


Figure 3.2 Cobalt, mercury, molybdenum, chromium and copper concentration of aqueous samples with increasing mass seaweed/volume ratios *Fucus serratus* L. (50–200 g L^{-1}); minerals in 0-100 µg L^{-1} range. Test condition prepared in triplicate, analysis of duplicate samples (n=6)

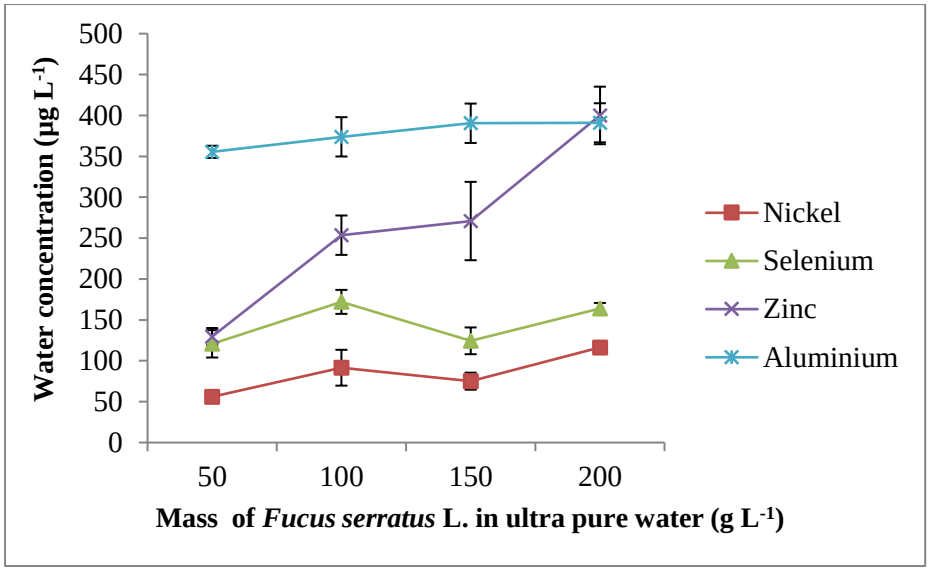


Figure 3.3 Nickel, selenium, zinc and aluminium concentration of aqueous samples with increasing mass seaweed/volume ratios *Fucus serratus* L. (50–200 g L^{-1}); minerals in 50-500 µg L^{-1} range. Test condition prepared in triplicate, analysis of duplicate samples (n=6)

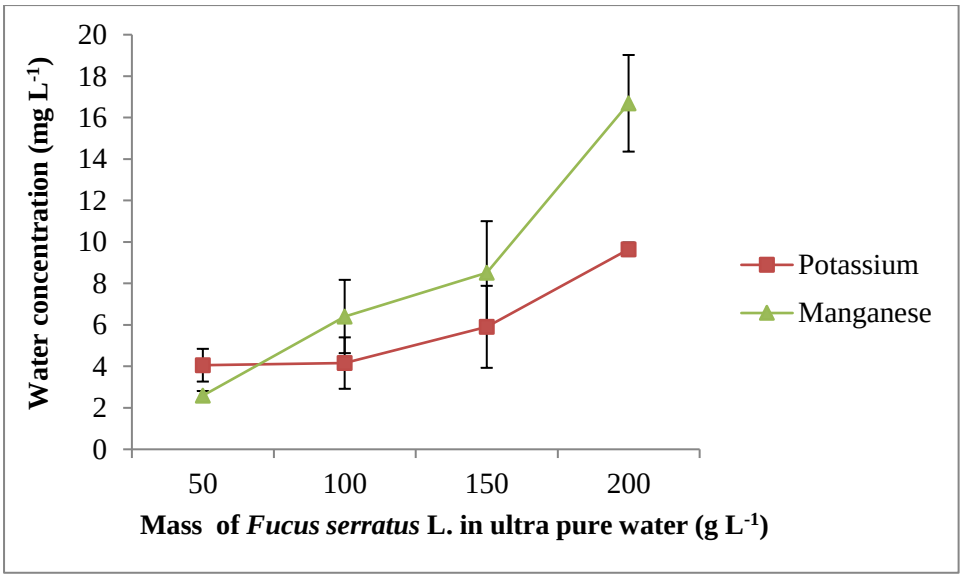


Figure 3.4 Potassium and manganese concentration of aqueous samples with increasing mass seaweed volume ratios *Fucus serratus* L. (50–200 g L^{-1}); minerals in low mg L^{-1} range. Test condition prepared in triplicate, analysis of duplicate samples (n=6)

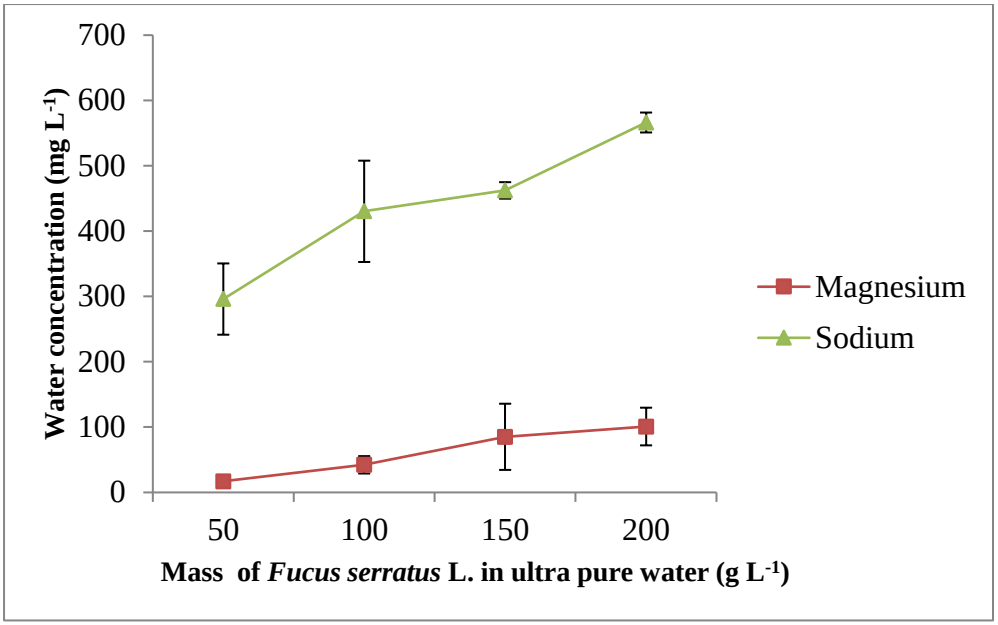


Figure 3.5 Magnesium and sodium concentration of aqueous samples with increasing mass seaweed/volume ratios *Fucus serratus* L. (50–200 g L^{-1}); minerals in 0-1000 mg L^{-1} range. Test condition prepared in triplicate, analysis of duplicate samples (n=6)

Given the variation in mineral behaviour with increasing mass i.e. range in total aqueous concentration and non-linear responses; the data was reprocessed for each mineral where the aqueous mineral concentration was divided by the mass of seaweed used for each test condition to compare the mineral concentration of each mineral per gram of fresh seaweed. This is shown in ***Figures 3.6*** to ***3.11***. Interestingly, results show a trend towards a decrease in aqueous concentration with increasing algal mass to volume ratios for almost all minerals. The concentration of aluminium, chromium, copper, lead, mercury, molybdenum, nickel, potassium, selenium, sodium and zinc per gram of seaweed deceases as mass increases although this is not a fourfold decrease. While the concentrations of cadmium and cobalt appear to fluctuate, it has been noted that results may be unreliable (**section 2.5.3**). The effects of diffusion can be discounted as the aqueous concentration is significantly less than that present in the seaweed. In general it might be expected that minerals are released into solution in proportion to the amount available i.e. the mass of seaweed present however these results further illustrate that complex

kinetics and mechanisms exist within the simulated matrix. Although not examined specifically this variation may be related to the different mechanisms of biosorption for individual minerals in the macroalgae.

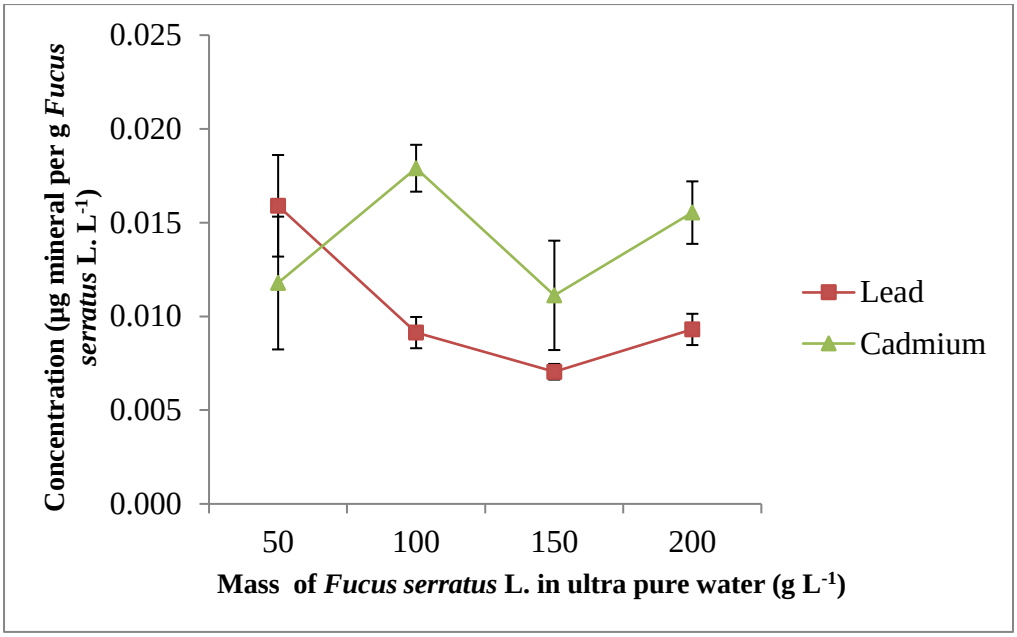


Figure 3.6 Lead and cadmium concentration (µg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

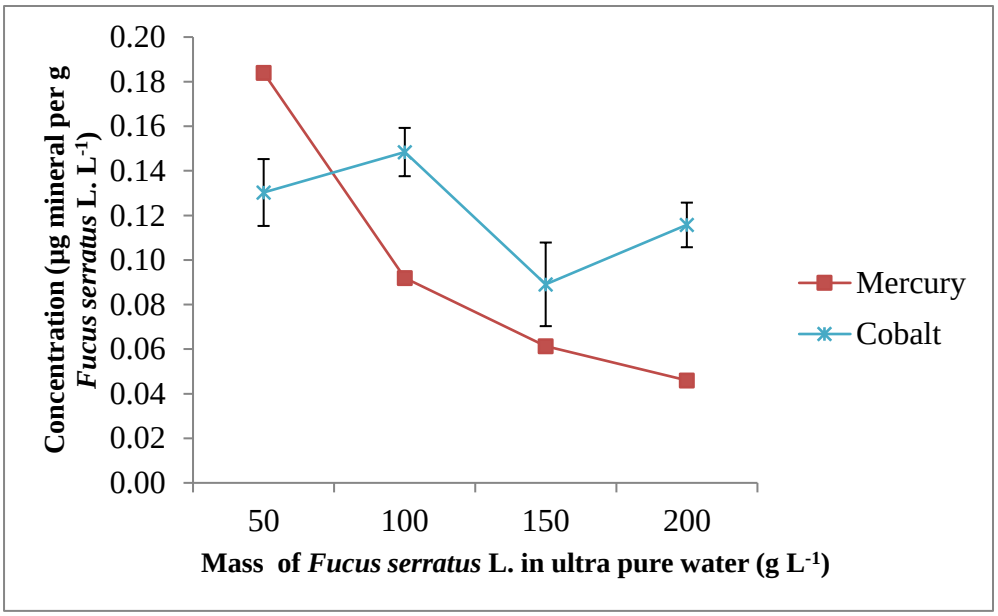


Figure 3.7 Mercury and cobalt concentration (µg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

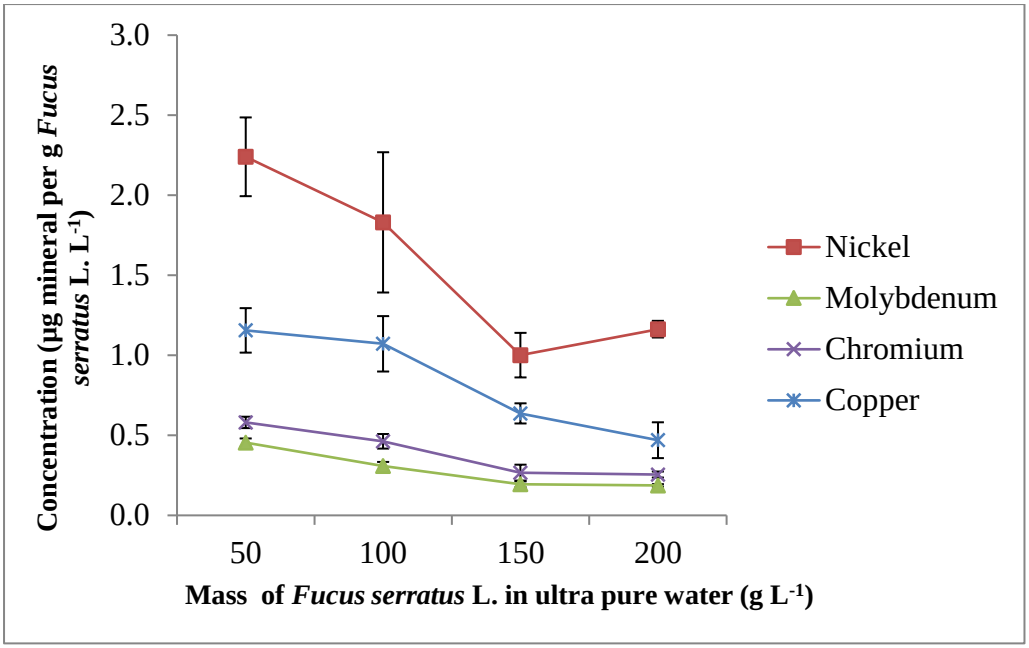


Figure 3.8 Nickel, molybdenum, chromium and copper concentration (µg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

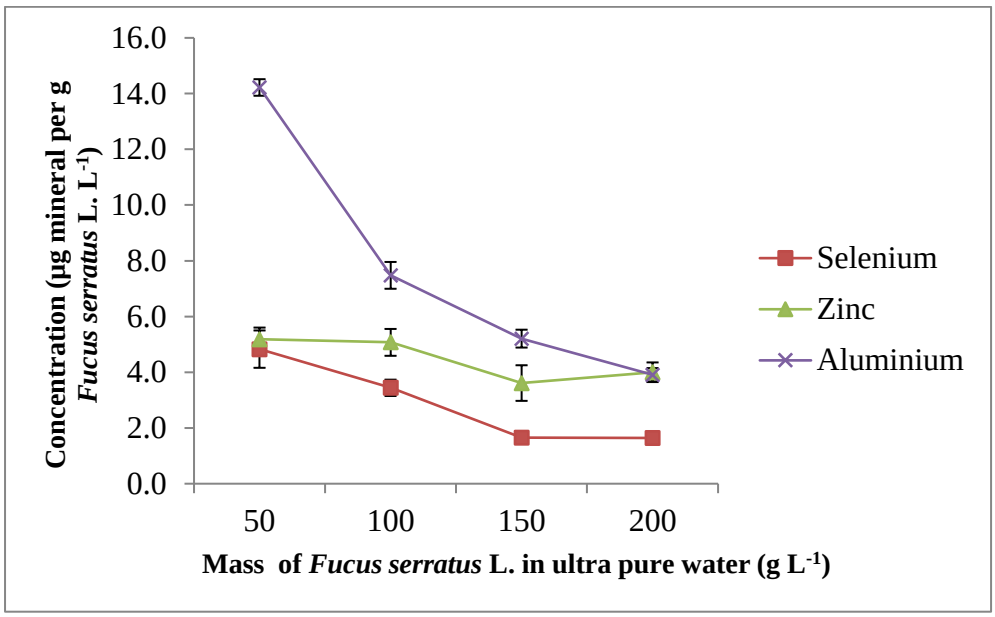


Figure 3.9 Selenium, zinc and aluminium concentration (µg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

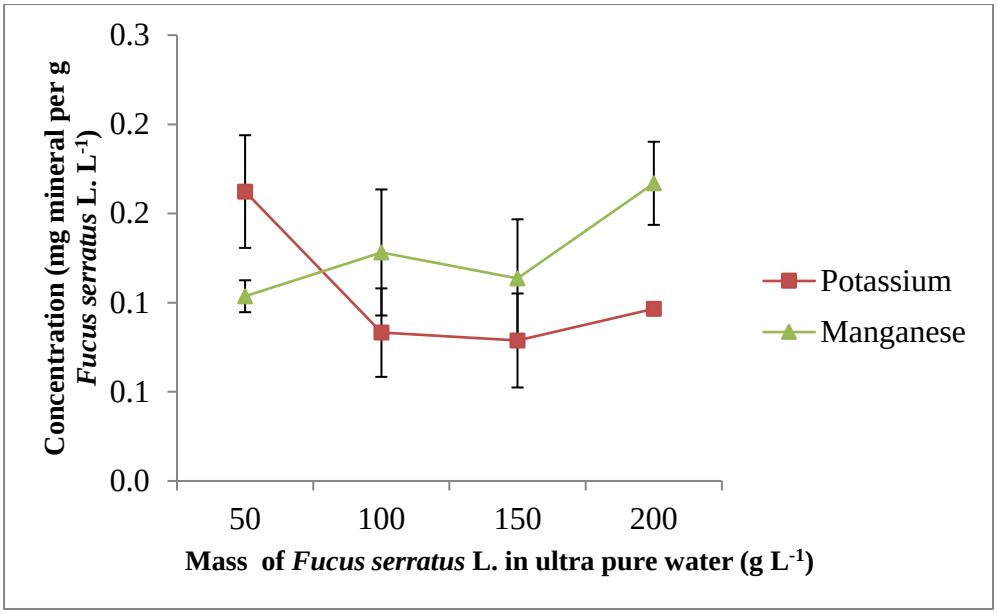


Figure 3.10 Potassium and manganese concentration (mg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

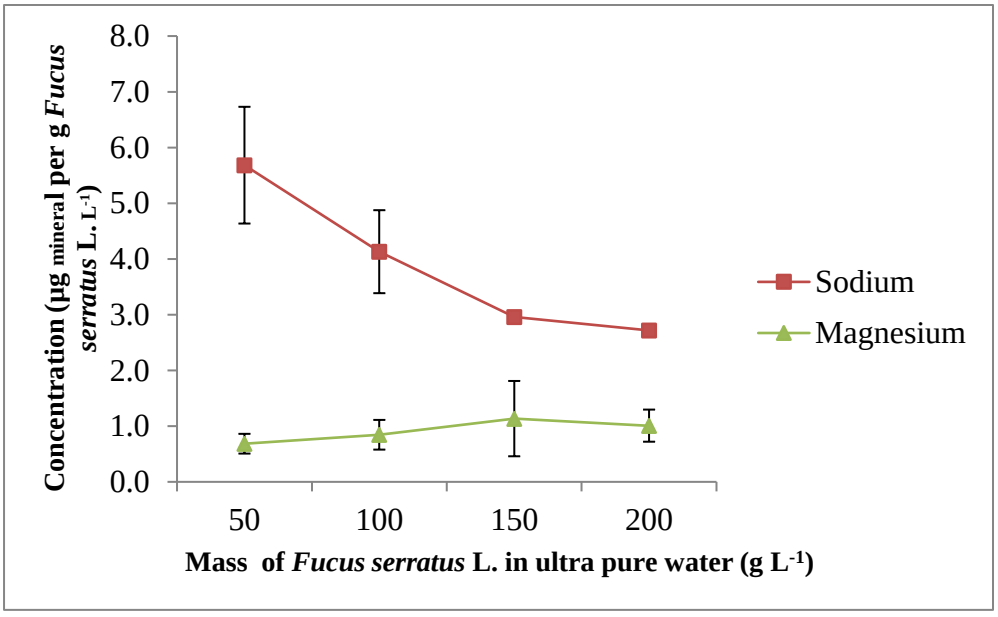


Figure 3.11 Sodium and magnesium concentration (mg mineral per gram *Fucus serratus* L.) with increasing mass of seaweed (50-200 g L^{-1} ultrapure water)

The algal cell wall structure lends itself to binding of metals from solution (Mata et al (2008)). The components of the cell wall, in particular alginic acid, provide an embedding matrix which anchors the free metal ions in solution to the algal biomass (Abdel-Ghani and El-Chaghaby (2014), He and Chen (2014)). Due to the increased surface area available with increasing mass more binding sites are available for metal sorption therefore reducing the overall metal concentration of the water. González et al (2012) and Ahmady-Asbchin et al (2013) report the uptake of chromium, copper and nickel by *Sargassum muticum* and S*argassum angustifolium.* Sulaymon et al (2013) carried out experiments to investigate the bio sorption of lead, cadmium, copper and arsenic using Chlorophyta and Cyanophyta. They found that increasing mass (from 0.05–3 g) led to increased bio sorption of metal ions. Furthermore, Abdel –Aty et al (2013) concurs that increasing the adsorbent dose, i.e. *Anabaena sphaerica* mass, provides greater surface area for cadmium and lead uptake.

The pH can also affect metal ion solubility and biosorbent charge. Farooq et al (2010) demonstrated that at low pH the removal of metal ions from solution is almost negligible due to a positive surface charge; however, as pH increases functional groups on the cell wall undergo deprotonation and become negatively charged which in turn attracts positively charged metal ions. Although the pH of these samples was not measured, based on the pH results in ***Table 3.4*** the pH is likely to be in the same range (5.29-6.36) i.e. acidic.

Results indicate that there may be various mechanisms of biosorption and release of minerals occurring simultaneously. These are likely to be affected by the parameters including ion-solubility, saturation, diffusion, ion-complexation and pH. Further investigations of the specific mechanisms for each mineral were beyond the scope of this study. However, it is evident that in the context of a bather, increasing the seaweed mass will not necessarily lead to increased minerals in the bath.

3.2.2 Effect of increasing temperature (20-80 ˚C) on mineral concentrations in bathwater

Once macroalgae are removed from their natural environment they are at risk of environmental stresses, including atmospheric oxidants e.g. ozone, heat and desiccation. For example, iodine release by macro algae occurs in response to oxidative stress but stress can also occur during normal development prior to harvesting (Küpper et al (2008), Chance (2009)). Given the relatively high temperatures of the bath it is likely that heat is the predominant driver for the release of minerals into the bathwater as the application of heat is well known to disrupt the cell wall architecture. Cell membranes become more permeable and break down with increased temperature (at temperatures above 54 ˚C) which results in the contents of the cell being disrupted. In turn, minerals are released from the cell wall structure (Daniell (1969)).

3.2.2.1 Iodine

The effect of increasing temperature on iodine concentration is shown in ***Figure 3.12***. There is a sharp increase in iodine concentration within the first 10 minutes of addition of seaweed. Thereafter, the iodine concentration of the baths maintained at 20–60 ˚C remains relatively stable. At 20 ˚C the total iodine rose slowly over the 40 minutes to an average maximum of 477 µg L^{-1} (11.8% CV) over the three baths. There is a sharp increase in the amount of iodine released when the temperature of the water is raised to 40 ˚C. The iodine concentration at 40 ˚C and 60 ˚C shows a stable maximum after 30 minutes of between 2200 to 2600 µg L^{-1}. At the highest temperature (80 ˚C) there is a further increase in iodine up to the average maximum of 3756 µg L^{-1} (6.6% CV) across the three baths. The bath maintained at the highest temperature (80 ˚C) continues to increase in iodine concentration to up the final time point of 40 minutes. The very high or low temperatures are not realistic bathing temperatures as typical baths are 40-60 ˚C, however the data does demonstrate that increased temperature stimulates higher efflux of iodine.

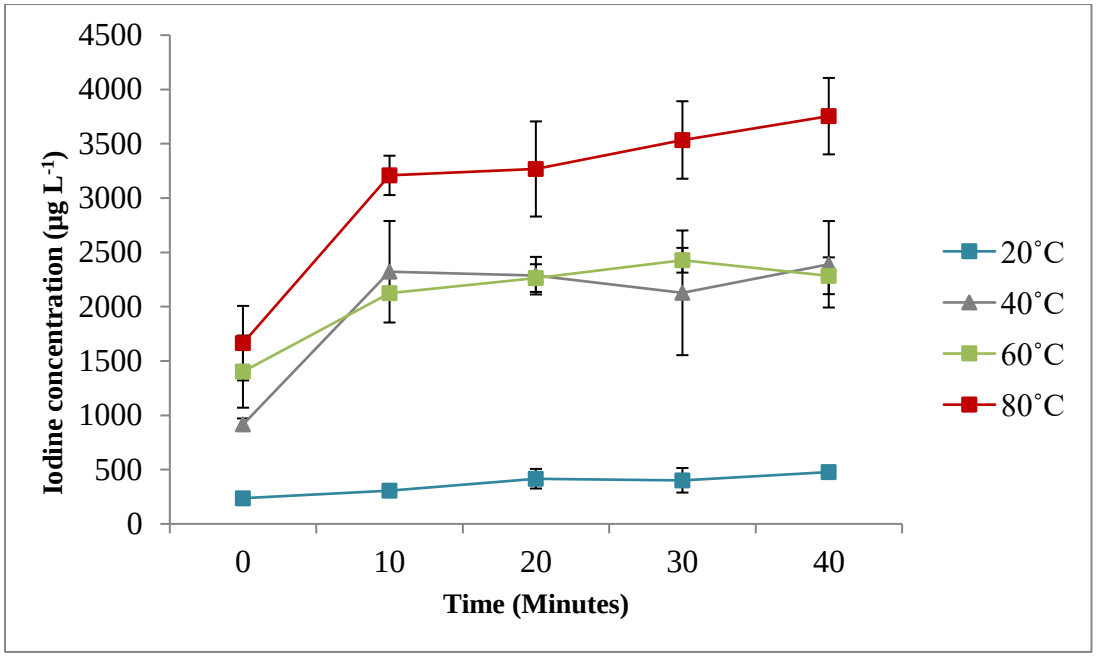


Figure 3.12 Comparison of total iodine concentration (µg L^{-1}) in baths prepared at different temperatures from 20-80 ˚C. Average and SD of three baths plotted at each temperature

3.2.2.2 Other minerals

The effect of increasing temperature on other mineral concentrations is shown in ***Table 3.3*** and ***Figures 3.13 to 3.17.*** Variation in mineral release with respect to temperature was tested using one-way ANOVA, comparing the results at each temperature setting. Increased release of minerals is most evident and most significant between 20 and 40 ˚C (***Table 3.3***). Based on the mineral concentration of both seaweed samples (n=3) (***Table 3.1***) and aqueous samples (n=24) (***Table 3.3***), at 20 ˚C approximately 0.64% of the available mineral content is released and increases to 1.1% at 40 ˚C, 1.04% at 60 ˚C and 1.03% at 80 ˚C.

Table 3.3 **Mineral concentration of *Fucus serratus* L. aqueous samples (75g in 500 ml) with increasing temperature (20–80 ˚C); test condition prepared in duplicate, analysis of duplicate samples (n=4), significant difference indicated with *p*-value where $p < 0.05$ is significant. ND = not detected, NC = not computable**

	Temperature (˚C)					
Mineral	**20**	**40**	**60**	**80**	**20–40 *p*-value**	**40–80 *p*-value**
Aluminium (µg L^{-1})	392.16±1.10	457.05±27.15	489.35±11.15	416.89±23.02	0.003	0.003
Cadmium (µg L^{-1})	ND	0.75±0.08	1.83±0.25	3.58±1.07	NC	0.000
Chromium (µg L^{-1})	15.86±0.84	41.26±4.85	44.67±4.90	48.88±11.56	0.000	0.417
Cobalt (µg L^{-1})	0.75±0.04	8.09±0.58	10.20±0.42	9.70±0.64	0.000	0.001
Copper (µg L^{-1})	18.57±1.64	25.24±4.66	19.28±1.70	22.44±1.62	0.002	0.520
Lead (µg L^{-1})	0.18±0.07	0.80±0.65	0.68±0.06	0.38±0.12	0.107	0.320
Magnesium (mg L^{-1})	34.33±17.58	229.64±74.72	203.47±35.71	166.52±49.26	0.002	0.319
Manganese (mg L^{-1})	0.30±7.47	9.80±2.12	9.78±1.43	10.06±0.78	0.000	0.960
Molybdenum (µg L^{-1})	7.54±0.17	26.99±6.45	31.85±3.24	28.87±2.35	0.001	0.332
Nickel (µg L^{-1})	21.83±4.33	153.09±26.81	149.38±17.74	126.35±13.68	0.000	0.183
Potassium (mg L^{-1})	3.26±0.92	10.99±2.26	10.63±1.30	12.98±1.06	0.001	0.143
Selenium (µg L^{-1})	86.80±4.81	344.05±96.28	348.02±54.16	293.46±36.50	0.002	0.471
Sodium (mg L^{-1})	1055.07±255.35	1254.65±361.51	1074.40±96.64	1239.19±369.73	0.402	0.661
Zinc (µg L^{-1})	36.06±1.31	321.98±17.61	315.58±28.80	319.07±37.96	0.000	0.954

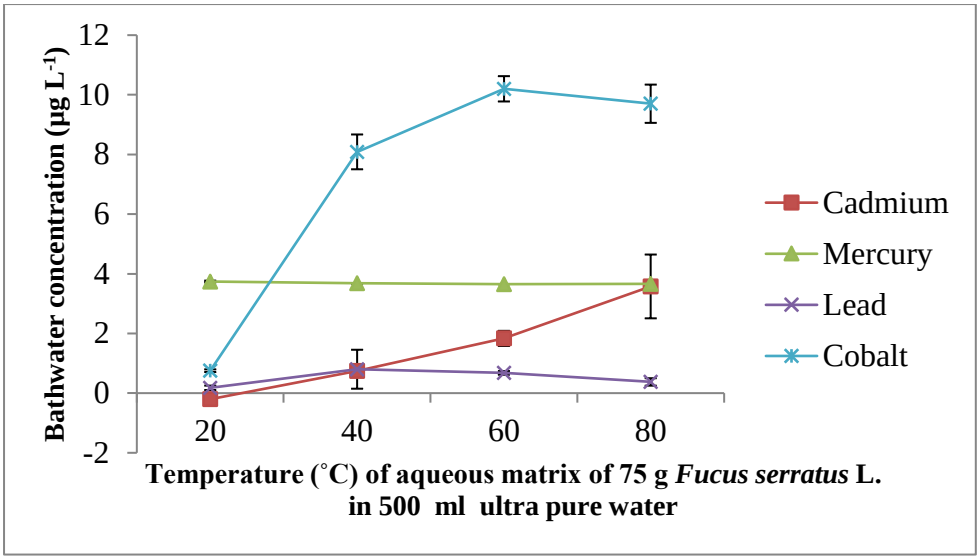


Figure 3.13 Cadmium, mercury, lead and cobalt concentration of aqueous samples with increasing temperature (20–80 ˚C) at constant mass (*Fucus serratus* L.) and volume (ultrapure water); minerals in low µg L^{-1} range. Test condition prepared in duplicate, analysis of duplicate samples (n=4)

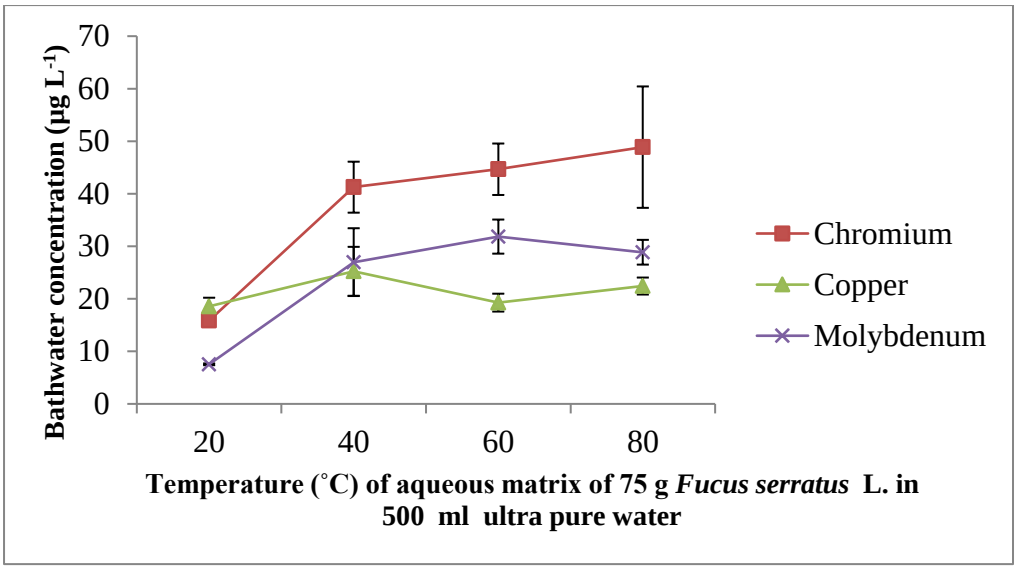


Figure 3.14 Chromium, copper and molybdenum concentration of aqueous samples with increasing temperature (20–80 ˚C) at constant mass (*Fucus serratus* L.) and volume (ultrapure water); minerals in 5-50 µg L^{-1} range. Test condition prepared in duplicate, analysis of duplicate samples (n=4)

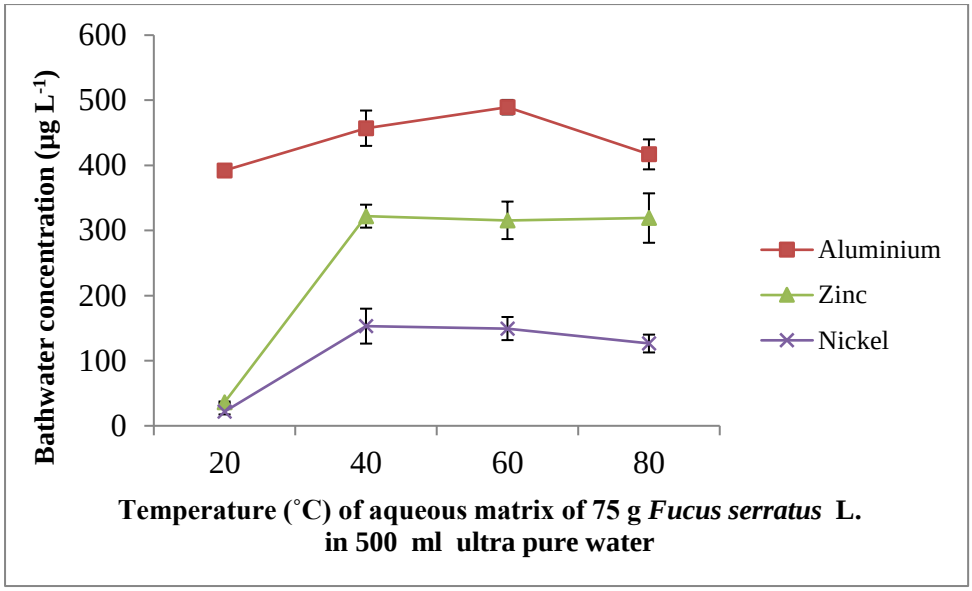


Figure 3.15 Aluminium, zinc and nickel concentration of aqueous samples with increasing temperature (20–80 ˚C) at constant mass (*Fucus serratus* L.) and volume (ultrapure water); minerals in 0-500 µg L^{-1} range. Test condition prepared in duplicate, analysis of duplicate samples (n=4)

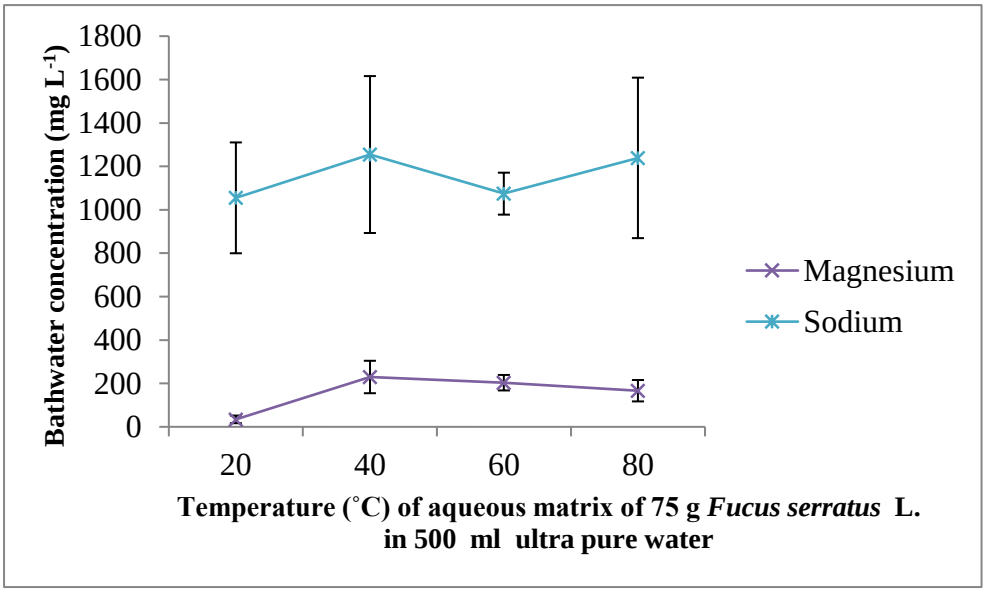


Figure 3.16 Magnesium and sodium concentration of aqueous samples with increasing temperature (20–80 ˚C) at constant mass (*Fucus serratus* L.) and volume (ultrapure water); minerals in 30-1300 mg L^{-1} range. Test condition prepared in duplicate, analysis of duplicate samples (n=4)

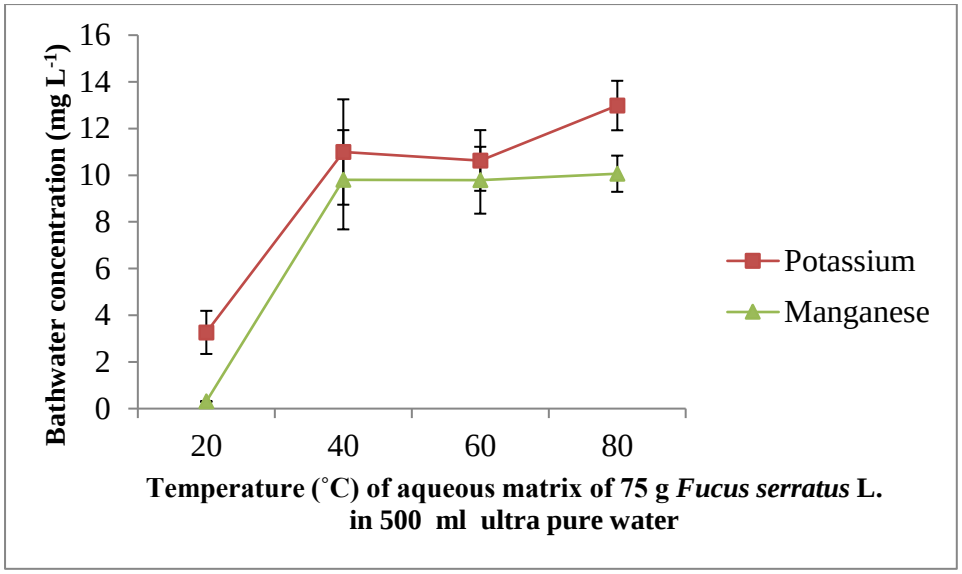


Figure 3.17 Potassium and manganese concentration of aqueous samples with increasing temperature (20–80 ˚C) at constant mass (*Fucus serratus* L.) and volume (ultrapure water); minerals in 0-15 mg L^{-1} range. Test condition prepared in duplicate, analysis of duplicate samples (n=4)

Results indicate that increasing temperature leads to a higher efflux of minerals (***Table 3.3, Figures 3.13*** to ***3.17***). However, the relative increase varies with each mineral (constant ratio of 150 g seaweed L^{-1}). With the exception of lead (0.18–0.4 µg L^{-1}) and sodium (1055.07–1239.09 mg L^{-1}) there is a significant difference between the concentration of each metal at 20 ˚C and its concentration at 40 ˚C. Conversely, with the exception of aluminium (392.16–416.89 µg L^{-1}), cadmium (0.75–3.58 µg L^{-1}) and cobalt (0.75–9.7 µg L^{-1}) there is no significant difference between 40 ˚C and 80 ˚C for the remaining minerals. The lack of uniformity of mineral efflux may be due, in part, to specific binding mechanisms of lead and sodium whereby they are more readily released, even at lower temperatures; therefore there is no significant difference in their concentrations with increasing temperature. The significant difference between 40 and 80 ˚C for aluminium, cadmium and cobalt suggests that they are more tightly bound to the algae. Unlike the other minerals, these concentrations continue to increase up to 80 ˚C which results in significant difference rather than plateau in concentration.

3.2.3 Effect of increasing time (0-50 minutes) on iodine concentration in bathwater

In the commercial setting, the conditions in seaweed baths are highly variable but typically contain 4±0.5 kg of seaweed in 120±20 L of water giving a fresh seaweed estimate of 25–50 g L^{-1}. The bath is prepared by dispensing the hot water first and allowing the seaweed to soak before adding cold seawater to adjust to a comfortable temperature, the typical bath duration is 40–50 minutes. This stimulates the release of components from the seaweed.

In this time release study a modified bath was prepared where the seaweed was added to the hot water/seawater mix and samples were taken immediately and at short time intervals. The time release profile of iodine from seaweed into the aqueous environment is shown in ***Figure 3.18***. The initial iodine concentration, prior to addition of the *Fucus serratus* L., was 25 µg L^{-1} due to the iodine content of the seawater. The total iodine concentration reached a maximum of almost 4 mg L^{-1} after 30 minutes, with no significant increase in the concentration between 30–50 minutes at 50-55 ˚C. Almost 80% of the final average concentration was reached after 5 minutes. The amount of iodine released is estimated to be 26.7 µg g^{-1} of fresh seaweed, based on the final concentration of 4 mg L^{-1} iodine. Based on an average iodine concentration of 300 µg g^{-1} fresh weight (Nitschke and Stengel (2015)), approximately 10% of the available iodine is released into a seaweed bath. From a bathers perspective these results indicate that the iodine is available from the beginning of the bathing period and that within the typical bathing period the iodine has reached its maximal concentration.

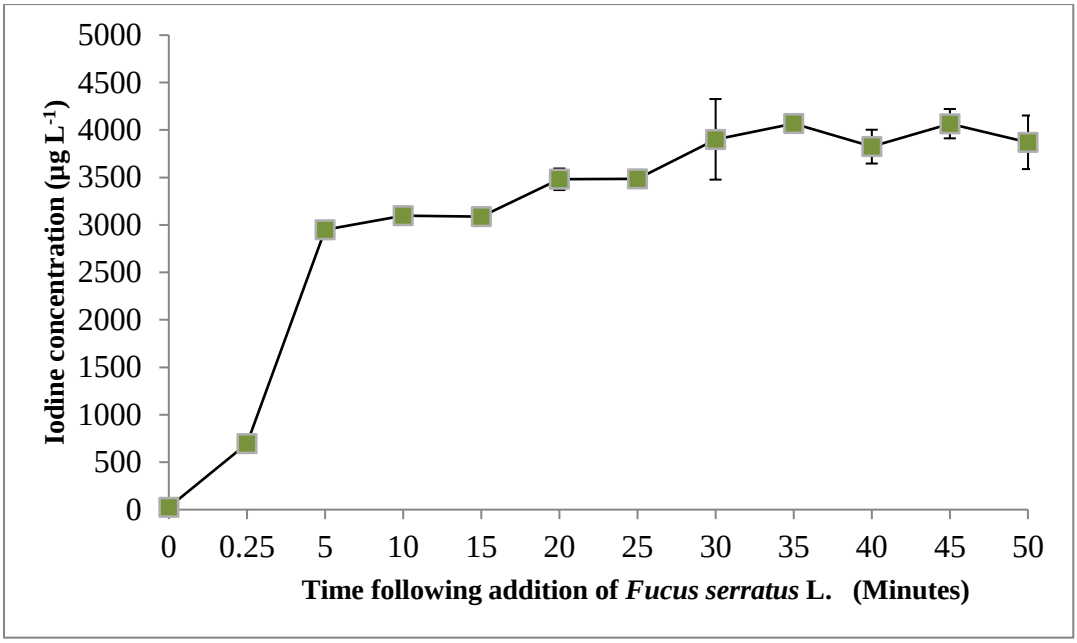


Figure 3.18 Iodine concentration over 50 minutes following addition of *Fucus serratus* L. to a 50:50 seawater: ultrapure water mix based on one simulated seaweed bath

3.2.4 Measurement of pH

The pH of the stored seawater and bathwater samples are shown in ***Table 3.4.*** As expected the seawater is slightly basic- the pH ranged from 7.92 to 8.81 with an average of 8.48±0.25 and there is no significant seasonal variation (p = 0.375).

The bathwater pH ranged from 5.29 to 6.36 with an average of 5.88±0.26 thus demonstrating that the addition of the seaweed *Fucus serratus* L. lowers the pH of the bathwater. There is no significant difference in pH over the 43 samples (p = 0.461). The seasonal variation in mineral content of seawater or bathwater has no impact on the effective pH. The alginate is thought to be responsible for the acidity of the bath the main contributing factor to the stable pH of the bathwater. While there is a seasonal pattern in the levels of alginate released by brown algae i.e. higher in summer and lower in winter (Rosell and Srivastava (1984)), given its very low pH (~3) (Lee and Mooney (2012)) the variation in quantity being released does not appear to impact the overall pH.

Table 3.4 **pH of seawater and bathwater samples (where n=22 and n=43 respectively); *p*-values 0.375 and 0.461 indicate no significant difference**		
	Seawater	**Bathwater**
Average	8.48± 0.25	5.88 ± 0.26
Minimum	7.92	5.29
Maximum	8.81	6.36
p-value	0.375	0.461
n	22	43

3.3 Seasonal variation of minerals in seawater and bathwater

Stored samples prepared from February 2013 to January 2014 were used to investigate the seasonal profile of minerals aluminium, cadmium, chromium, cobalt, copper, iodine, lead, manganese, molybdenum, nickel, selenium and zinc therein.

3.3.1 Iodine

The iodine concentration of seawater samples ranged from a minimum of 22 µg L^{-1} to a maximum of 105 µg L^{-1} with an average of 52 µg L^{-1}± 19 µg L^{-1} (n= 40) (***Table 3.5*** and ***Figure 3.19b***). This is in line with the documented range for iodine in seawater (Chance et al (2009), Ito and Hirokawa (2009), Zheng et al (2012)) which is 40 to 80 µg L^{-1}. Data was grouped by season and a one-way ANOVA showed no significant seasonal variation (p = 0.178).

Table 3.5 **Average monthly iodine concentration (µg L^{-1}) of seawater and bathwater (*Fucus serratus* L. + 50:50 seawater: ultrapure water mix) (n=40).**

	Iodine concentration (µg L^{-1})	
Month	**Seawater**	**Bathwater**
January	61	9224
February	46	8144
March	46	6567
April	57	5037
May	50	4010
June	54	2522
July	41	5875
August	46	3393
September	46	2976
October	53	7669
November	72	4566
December	44	8385
***p* value**	**0.178**	**0.02**

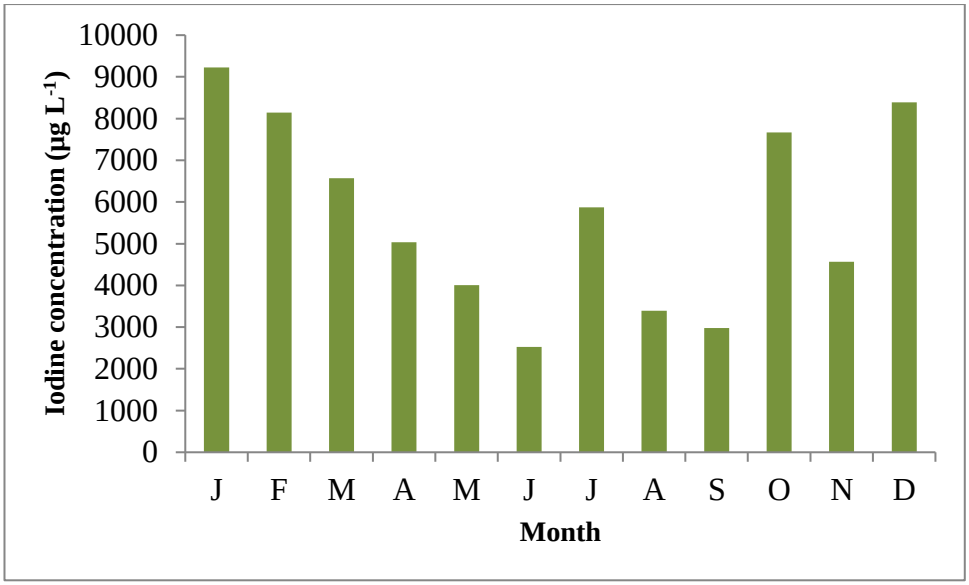


Figure 3.19a Average monthly iodine concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as $p = 0.02$

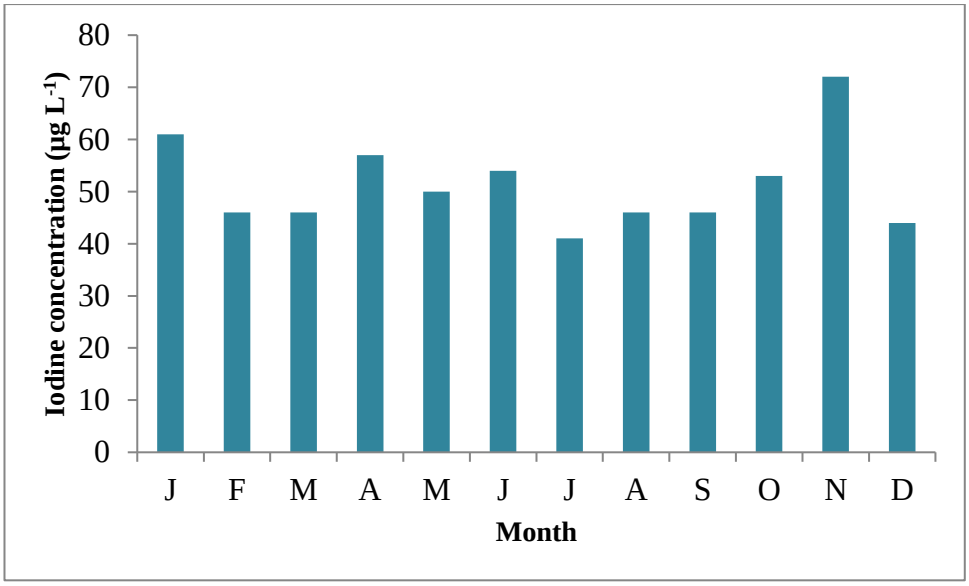


Figure 3.19b Average monthly iodine concentration of seawater (µg L^{-1}) indicating no significant seasonal variation as $p = 0.178$

The iodine concentration of bathwater samples ranged from 808-13734 µg L^{-1} with an average of 5877 µg L^{-1}± 4028 µg L^{-1} (n=40) (***Table 3.5*** and ***Figure 3.19a***). There is significant seasonal variation in the bathwater iodine concentration (p = 0.02) across the full data set. There is significant difference between spring and summer levels (p = 0.06) and spring and autumn levels (p =0.03). There is also significant difference between winter and summer (p = 0.04) and winter and autumn (p = 0.035). Conversely, there is no significant difference between summer and autumn (p = 0.971) or winter and spring (p = 0.930). This pattern is similar to that discussed by Patti et al (1990) and Gall et al (2004) in their investigation into brown algae as they state that iodine contents were lowest in summer and highest in late autumn and winter.

3.3.2 Other minerals in seawater

The average monthly concentration for each mineral studied in seawater is presented in ***Table 3.6*****.** Minerals present in concentrations greater than 1 mg kg^{-1} e.g. calcium, magnesium, potassium or sodium tend to remain constant across the major oceans (Pilson (1998)). However, the exact composition of seawater varies with location and climate (Murray (2000)). As the sampling site is on the exposed western seaboard, which is not likely to be affected by local conditions e.g. estuaries/salt flats the analysis focussed on the minor elements where some variation might be present due to local factors e.g. industry effluent. Minerals analysed were aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc

The data was also grouped several ways, but the only grouping that indicated any trends was when the weekly data was grouped as follows;

Sampling period 1; January–April

Sampling period 2; May–August

Sampling period 3; September–December

and analysed by one way ANOVA (***Table 3.7***).

Even with this grouping aluminium, cadmium, chromium, lead, manganese, selenium and zinc concentrations also remained constant over one year (February 2013–January 2014) with no significant difference between sampling period ($p > 0.05$). Cobalt, copper, molybdenum and nickel showed more variation across the same time ($p < 0.05$). Specifically, their concentrations were higher at the beginning of the year. The most significant difference in concentration occurs between sampling periods 1 and 3 and 2 and 3.

Results indicate that minerals present at lower concentrations tend to show more variation over time as expected. This may be due in part to local climate and environmental factors.

Table 3.6 **Average monthly and average annual mineral concentration (µg L^{-1}) of seawater measured by ICP-MS**											
	Average Monthly Concentration in seawater (µg L^{-1})										
Month	**Al**	**Cd**	**Cr**	**Co**	**Cu**	**Pb**	**Mn**	**Mo**	**Ni**	**Se**	**Zn**
January	373.10	0.21	70.09	0.82	9.54	22.28	5.91	0.48	136.05	175.14	309.75
February	425.20	0.26	73.34	1.40	34.86	35.16	8.45	0.76	694.72	162.05	289.78
March	433.52	0.22	79.90	1.21	29.65	26.65	9.15	0.63	608.19	154.73	769.72
April	478.76	0.22	71.59	1.13	20.09	18.97	6.96	0.62	354.45	139.85	491.66
May	547.73	0.23	78.47	1.22	16.30	21.05	7.63	0.56	294.29	195.89	484.11
June	478.45	0.22	79.17	1.22	17.01	17.58	7.24	0.59	297.38	134.19	442.90
July	413.23	0.23	70.20	1.29	15.86	11.23	6.49	0.56	241.63	156.40	176.64
August	367.19	0.21	73.33	0.97	14.85	10.69	6.38	0.56	219.99	158.15	294.55
September	426.73	0.22	78.23	0.90	13.66	10.72	6.48	0.52	217.43	184.10	343.67
October	344.54	0.21	53.87	0.87	10.72	5.59	4.88	0.49	145.80	102.51	166.73
November	481.74	0.23	67.27	0.92	11.24	26.49	7.99	0.49	183.08	150.82	575.65
December	380.09	0.22	71.63	0.86	10.28	24.24	6.27	0.49	162.79	174.11	404.26
Average	**429.19**	**0.22**	**72.26**	**1.07**	**17.01**	**19.22**	**6.99**	**0.56**	**296.32**	**157.33**	**395.79**

Table 3.7 **Significant differences in mineral concentrations of seawater with 3 separate sampling periods** **Sampling period 1; January–April,** **Sampling period 2; May–August,** **Sampling period 3; September–December**				
	Sampling period 1:2 ***p*-value**	**Sampling period 2:3** ***p*-value**	**Sampling period 1:3** ***p*-value**	**Across entire sampling period** ***p*-value**
Aluminium	0.829	0.917	0.934	0.981
Cadmium	0.693	0.598	0.496	0.731
Chromium	0.939	0.131	0.173	0.257
Cobalt	0.903	0.000	0.000	0.000
Copper	0.04	0.000	0.007	0.003
Lead	0.884	0.422	0.107	0.528
Manganese	0.115	0.678	0.086	0.12
Molybdenum	0.025	0.000	0.000	0.000
Nickel	0.045	0.000	0.012	0.006
Selenium	0.536	0.783	0.777	0.846
Zinc	0.079	0.140	0.829	0.262

3.3.3 Other minerals in bathwater

The average monthly concentration for each mineral studied in bathwater is presented in ***Table 3.9***. All minerals showed a significant difference in concentration over the year (***Table 3.8***). Specifically, the most significant difference in concentration of all minerals is between sampling periods 2 and 3 i.e. between summer and winter. There is no significant difference in the concentration of aluminium, cobalt or manganese between sampling periods 1 and 2. Similarly, there is no significant difference in the concentration of aluminium, cadmium or selenium between sampling periods 1 and 3.

Table 3.8 **Significant differences in mineral concentrations of bathwater with 3 separate sampling periods**

Sampling period 1; January–April,
Sampling period 2; May–August,
Sampling period 3; September–December

	Sampling period 1:2 ***p*-value**	**Sampling period 2:3** ***p*-value**	**Sampling period 1:3** ***p*-value**	**Across entire sampling period** ***p*-value**
Aluminium	0.094	0.019	0.130	0.019
Cadmium	0.015	0.001	0.065	0.000
Chromium	0.045	0.001	0.023	0.001
Cobalt	0.384	0.007	0.009	0.008
Copper	0.000	0.000	0.049	0.000
Lead	0.255	0.000	0.000	0.000
Manganese	0.305	0.003	0.008	0.005
Molybdenum	0.000	0.000	0.028	0.000
Nickel	0.000	0.000	0.002	0.000
Selenium	0.004	0.02	0.709	0.004
Zinc	0.076	0.026	0.000	0.016

Table 3.9 **Average monthly mineral concentration (µg L^{-1}) of bathwater measured by ICP-MS**											
	Average Monthly Concentration in bathwater (µg L^{-1})										
Month	Al	Cd	Cr	Co	Cu	Pb	Mn	Mo	Ni	Se	Zn
January	1246.84	4.51	451.74	9.78	260.21	2.33	1552.05	10.60	633.82	539.75	229.38
February	791.31	12.71	1613.71	31.13	237.10	7.10	2979.57	37.68	312.92	940.66	328.88
March	865.87	15.40	1822.98	34.40	274.41	8.44	4593.14	36.73	228.99	1195.84	360.63
April	785.09	19.63	1434.32	24.99	345.21	5.71	2855.03	35.62	297.66	1024.95	355.04
May	733.45	16.00	1748.61	23.30	1099.05	7.69	2879.97	68.40	3079.14	1565.67	320.37
June	642.76	21.49	1571.45	22.04	2191.23	5.28	2371.90	72.83	6599.39	1540.20	386.56
July	1513.13	23.90	1792.00	28.76	2104.66	8.69	3838.40	56.14	5699.01	1999.98	546.83
August	1813.50	44.07	3382.52	51.55	1598.12	11.02	6360.91	64.83	3888.22	4550.94	1931.23
September	939.85	16.37	1263.69	24.23	1149.43	4.78	2947.73	34.36	3010.04	1864.86	493.45
October	422.66	4.92	435.86	11.46	501.58	1.76	940.08	13.28	1367.21	812.60	190.31
November	408.03	3.36	627.25	12.01	371.55	2.08	1159.92	15.18	955.82	900.71	229.51
December	652.61	3.29	417.62	8.31	230.83	1.71	1198.58	11.74	539.96	560.68	145.41
Average	**901.26**	**15.47**	**1380.15**	**23.50**	**863.61**	**5.55**	**2806.44**	**38.12**	**2217.68**	**1458.07**	**459.80**

The seasonal variation in mineral concentration is further illustrated in ***Figures 3.20*** to ***3.30***. Stacked graphs (***Figures 3.20***, ***3.23***, ***3.25***, ***3.28***, ***3.29*** and ***3.30***) show the total concentration and the contribution from seawater to the total. Where the seawater concentration is significantly lower than the total bathwater concentration the data is presented across two separate graphs ***a*** and ***b*** (***Figures 3.21***, ***3.22***, ***3.24***, ***3.26*** and ***3.27***). With the exception of aluminium, lead and zinc, the contribution from seawater to the overall mineral concentration is almost negligible. The data shows that the main contribution of minerals to the bath comes from the seaweed itself. As such, the use of seawater in bath preparation could be regarded as redundant.

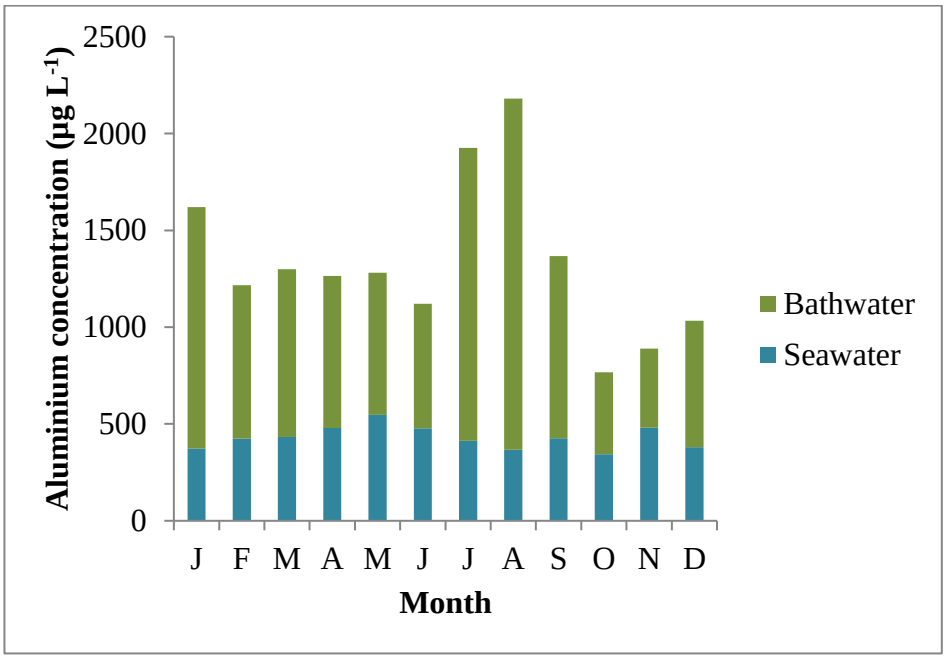


Figure 3.20 Average monthly aluminium concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as $p = 0.981$. Significant seasonal variation in simulated bathwater as $p = 0.019$

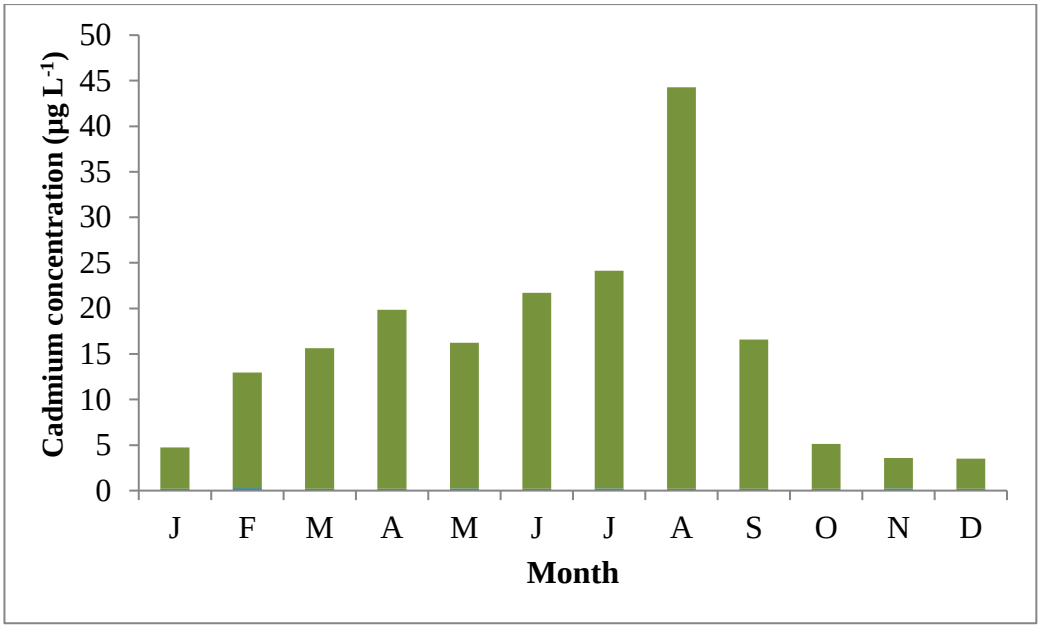


Figure 3.21a Average monthly cadmium concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as $p = 0.000$

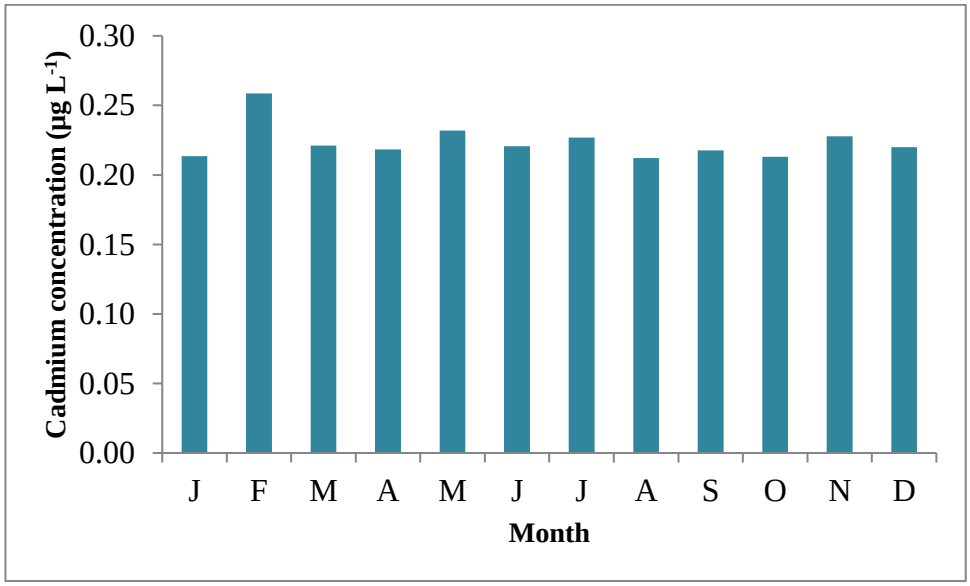


Figure 3.21b Average monthly cadmium concentration of seawater (µg L^{-1}) indicating no significant seasonal variation as $p = 0.003$

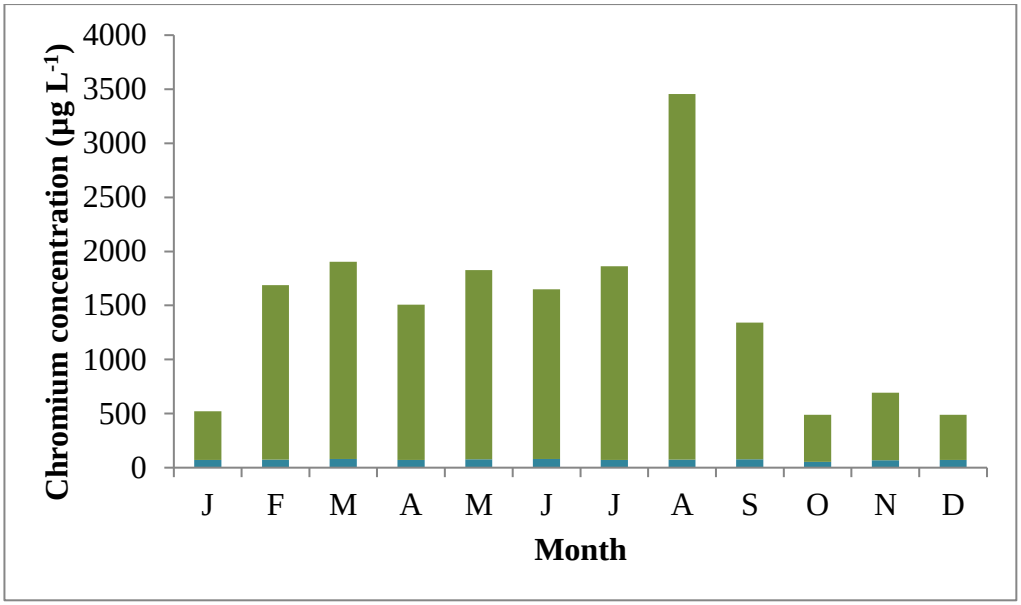


Figure 3.22a Average monthly chromium concentration of seawater and simulated bathwater (µg L^{-1}) indicating significant seasonal variation as $p = 0.001$

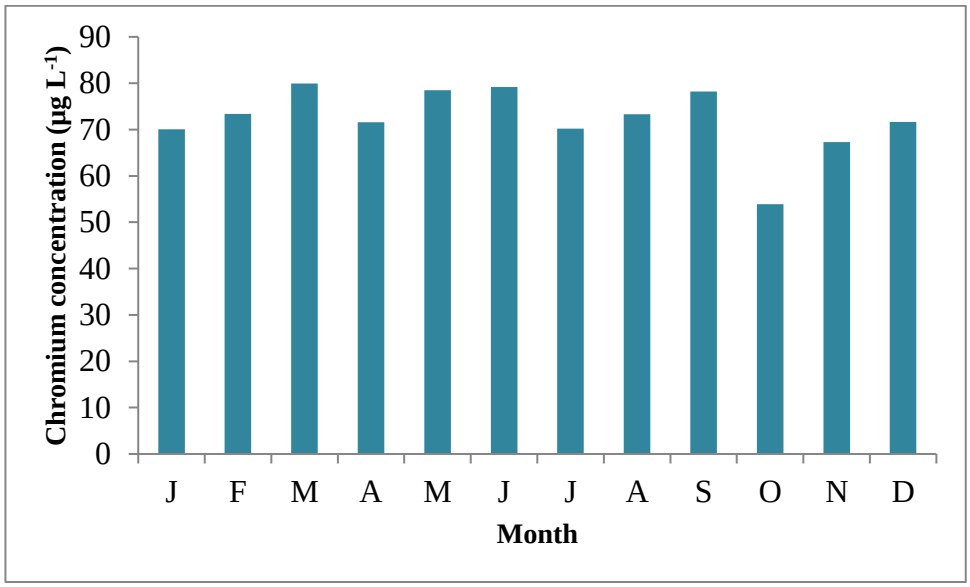


Figure 3.22b Average monthly chromium concentration of seawater (µg L^{-1}) indicating no significant seasonal variation as $p = 0.257$

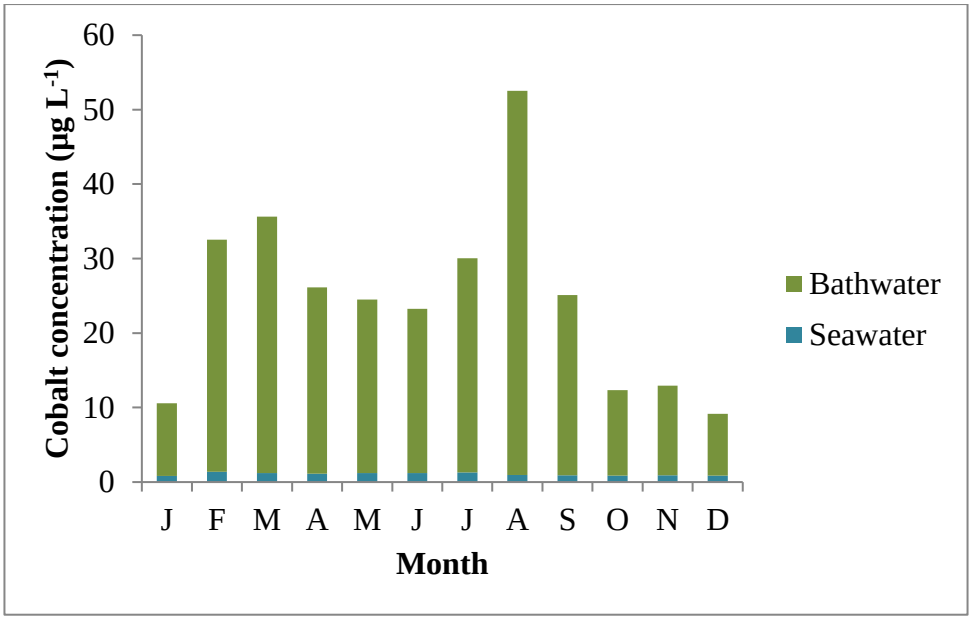


Figure 3.23 Average monthly cobalt concentration of seawater and simulated bathwater (µg L^{-1}). Significant seasonal variation in seawater as p = 0.000. Significant seasonal variation in simulated bathwater as p = 0.008

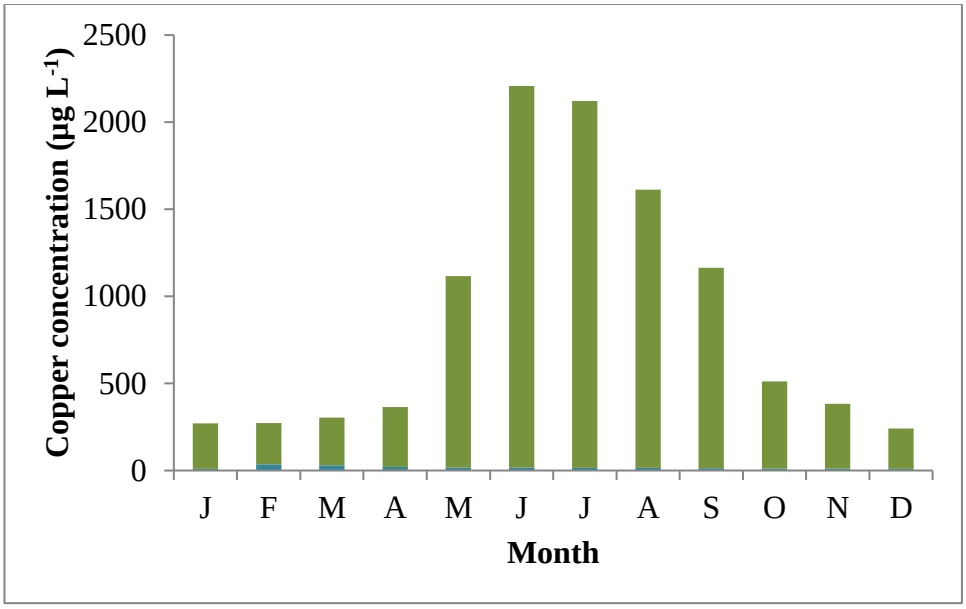


Figure 3.24a Average monthly copper concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as p = 0.000

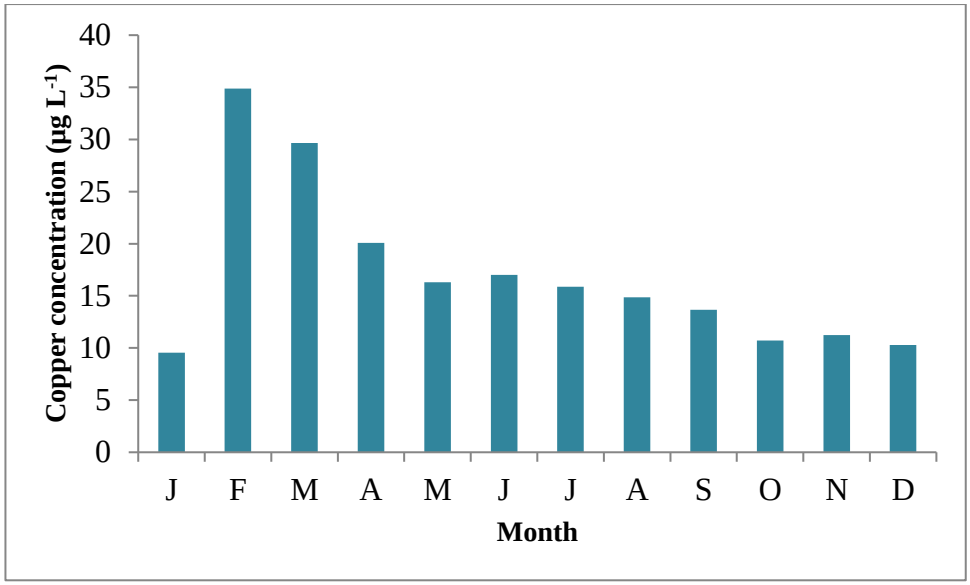


Figure 3.24b Average monthly copper concentration of seawater (µg L^{-1}) indicating significant seasonal variation in seawater as $p = 0.003$

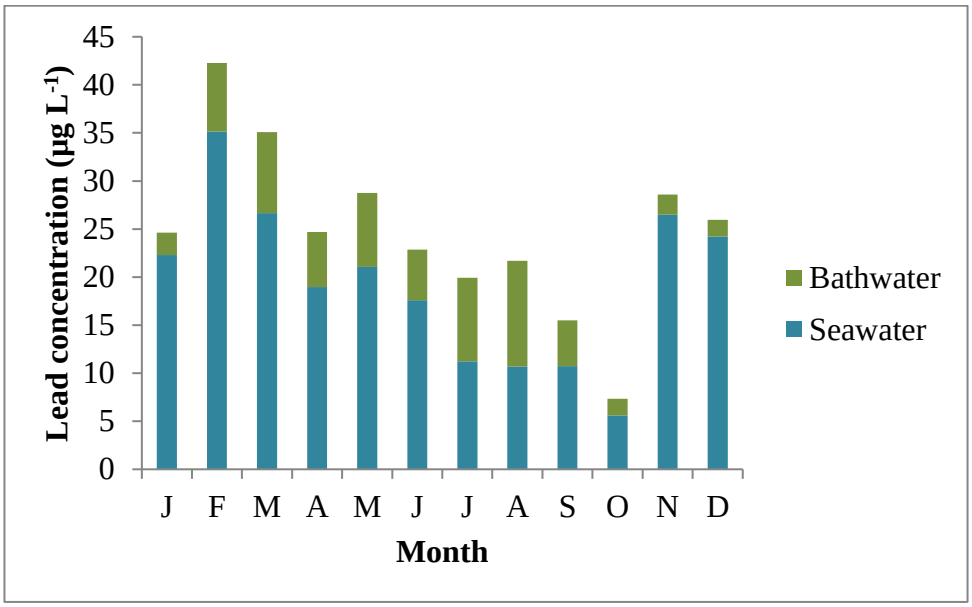


Figure 3.25 Average monthly lead concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as $p = 0.528$. Significant seasonal variation in simulated bathwater as $p = 0.000$

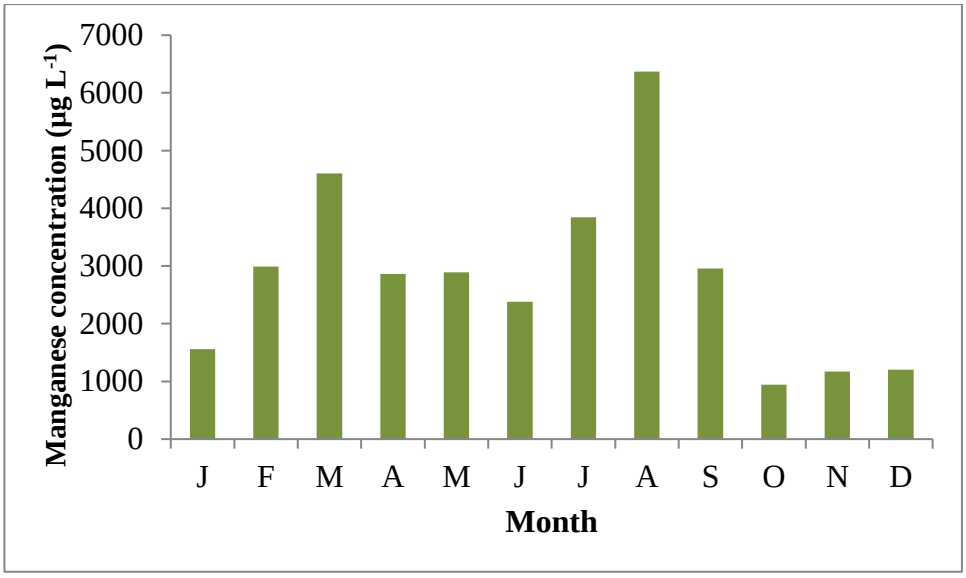


Figure 3.26a Average monthly manganese concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as $p = 0.005$

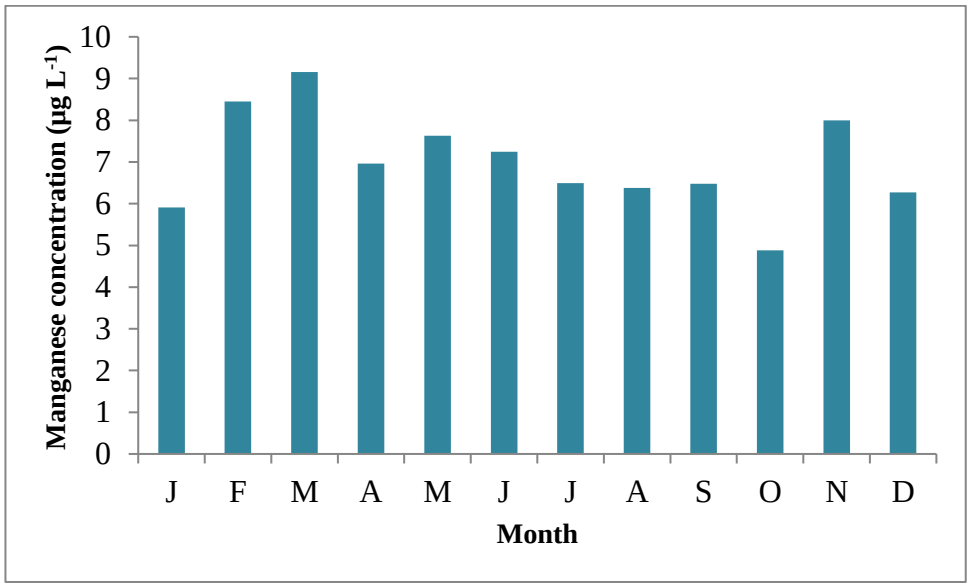


Figure 3.26b Average monthly manganese concentration of seawater (µg L^{-1}) indicating no significant seasonal variation in seawater as $p = 0.12$

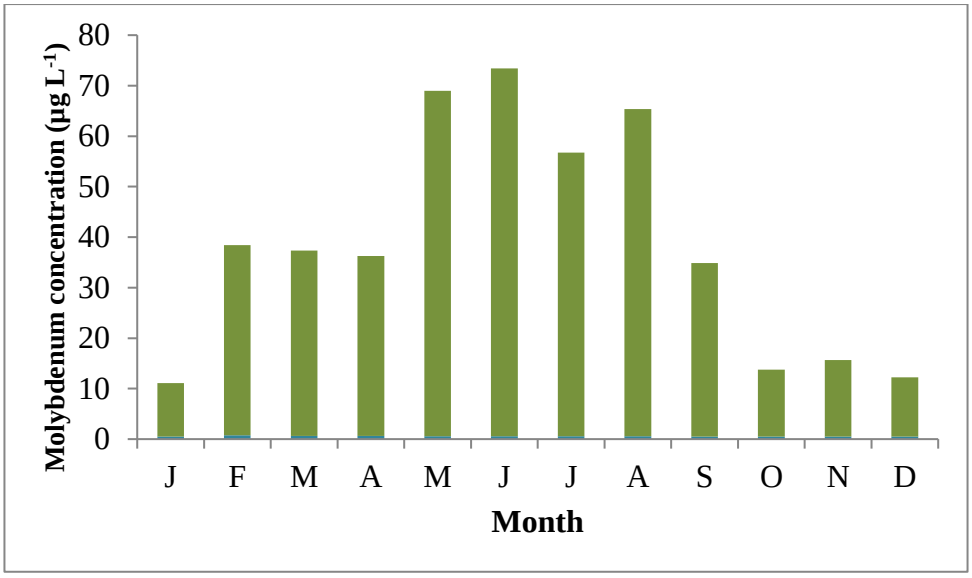


Figure 3.27a Average monthly molybdenum concentration simulated bathwater (µg L^{-1}) indicating significant seasonal variation as $p = 0.000$

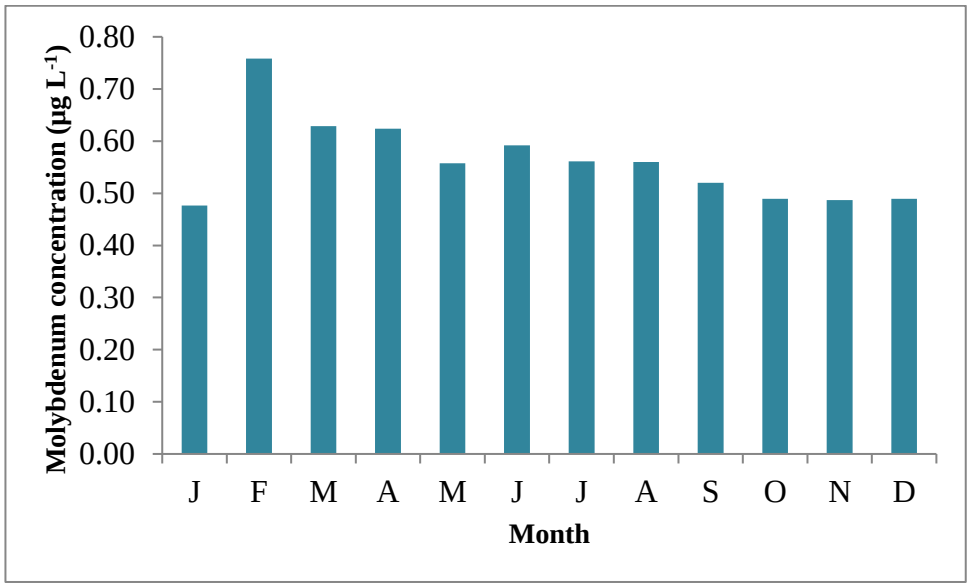


Figure 3.27b Average monthly molybdenum concentration of seawater (µg L^{-1}) indicating significant seasonal variation as $p = 0.000$

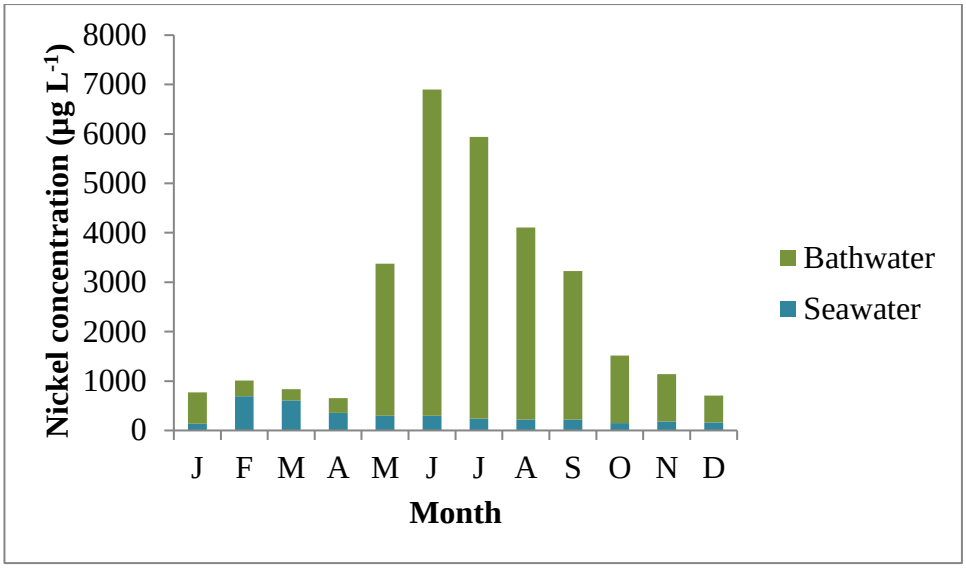


Figure 3.28 Average monthly nickel concentration of seawater and simulated bathwater (µg L^{-1}). Significant seasonal variation in both samples as p = 0.006 and p = 0.000 respectively

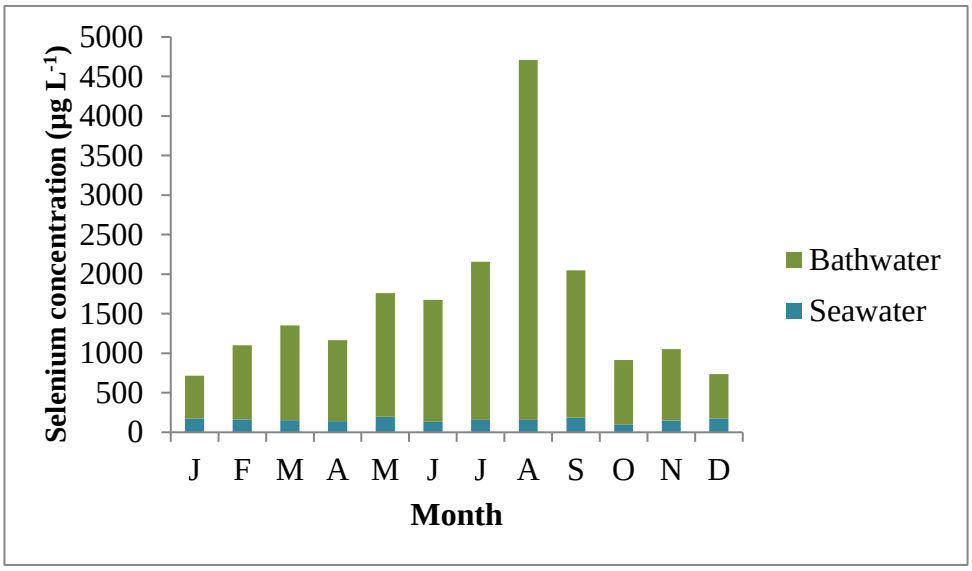


Figure 3.29 Average monthly selenium concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as p = 0.846. Significant seasonal variation in simulated bathwater as p = 0.004

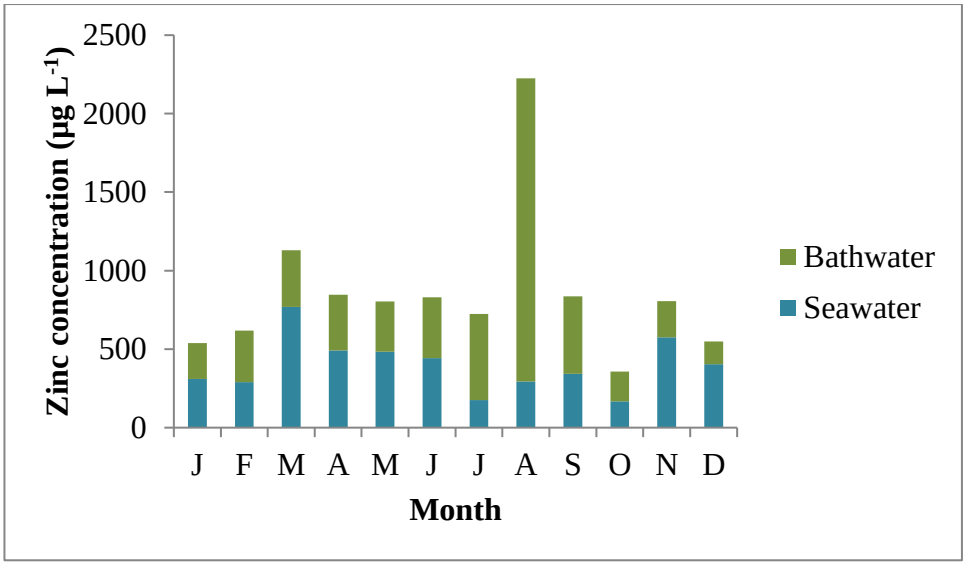


Figure 3.30 Average monthly zinc concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as p = 0.262. Significant seasonal variation in simulated bathwater as p = 0.016

While accumulation of minerals throughout the year occurs at different rates the highest concentrations are evident in the sampling period 2 (May-August) for all minerals except lead. This is attributed to the higher abundance of alginate in summer (Rosell and Srivastava (1984)). These polysaccharides in the cell wall aid the anchoring of free metals from seawater to the algal mass. Cellulose in the cell wall also contains functional amino, carboxyl, sulphate and phosphate groups which aid the binding of metals (Davis (2003), Romera et al (2007), Ibrahim (2011), Wijesinghe et al (2012)). Interestingly, Malea et al (2015) investigated the seasonal trend of cadmium, cobalt, chromium, copper, manganese, molybdenum, nickel, lead, selenium and zinc in green algae on the coast of Greece and found the opposite trend where their levels were lowest in spring-summer and peaked in winter. This can be attributed to the behaviour of a different species growing in a different location in a different climate.

Overall, results firstly indicate that although increasing the mass of seaweed in a bath leads to increased mineral concentration having more seaweed does not imply increased exposure to minerals therefore a seaweed:water ratios of 50 g L^{-1} is most suitable for optimum exposure in this study. Secondly, while higher temperatures lead to an increase in mineral concentration, baths are typically prepared and maintained at 40-50 ˚C. It appears that there is a sharp increase in mineral concentration within the first 5-10 minutes following addition of seaweed consequently minerals are available for uptake by a bather from the start of a treatment. It is also interesting to note that the pH of the bathwater is close to skin pH of 5.5 thus maintaining its acid mantle. It is not possible to conclude the optimum time of year for bathing as it is bather specific however it is useful to note that most minerals are present at higher levels in summer-autumn therefore at maximum levels for dermal uptake. Conversely, more iodine is available during the winter. While iodine is the most abundant mineral detected, other minerals are also present at high levels i.e. aluminium (1.8 mg L^{-1}), chromium (3.4 mg L^{-1}), copper (2.2 mg L^{-1}), manganese (6.4 mg L^{-1}), nickel (6.6 mg L^{-1}), selenium (4.5 mg L^{-1}) and zinc (1.9 mg L^{-1}) while heavy metals cadmium and lead are present at lower levels i.e. 44 and 11 μg L^{-1} respectively. Finally, in terms of bath preparation, it is not compulsory that baths are prepared using seawater as its mineral contribution is negligible compared to that of the seaweed itself.

Having established the mineral profile of the seawater and bathwater, along with the optimum bath preparation conditions in terms of seaweed: water ratios and temperature the next phase was to determine the uptake of these minerals by a bather during a typical seaweed bath treatment both *in vivo* and *in vitro*. Chapter 4 is focussed on the *in vivo* uptake of iodine through a UIC study while Chapter 5 further investigates the transdermal uptake of other minerals *in vitro*.

4 *In vivo* uptake of iodine from a *Fucus serratus* L. seaweed bath

The studies of *in vivo* uptake of iodine were completed in three phases. The initial phase was to carry out a survey of clients and staff of Voya Seaweed Baths to ascertain their views in relation to seaweed, seaweed baths and potential benefits of bathing. In particular, it was of interest whether they associated bathing with any health benefits including iodine uptake.

In the second phase, a preliminary *in vivo* study was carried out on a small sample population to determine the potential uptake of iodine by the body during a seaweed bath and the feasibility of testing iodine uptake in this context. Based on the promising results generated, a third phase larger study was designed which required rigorous ethical review. Following Research Ethics Committee approval through IT Sligo, the study to measure urinary iodine and creatinine levels of subjects' pre and post-treatment in *Fucus serratus* L. seaweed baths was undertaken.

The purpose of this study was to determine if iodine, present in seaweed bathwater, is taken up by the body during a seaweed bath treatment, thus leading to an increase in UIC and to gain some insight into the dominant route of uptake; transdermal *v* inhalation. Specifically, the study aimed to establish whether bathing/immersion (unclothed) in or simply sitting beside a seaweed bath (clothed) caused an increase in UIC. *In vivo* analysis allowed for comparison between pre-treatment and post-treatment UIC profiles of subjects following exposure to a seaweed bath.

Given the acidity of the bathwater matrix, pH 5.88±0.26, it is expected that the species of iodine present includes molecular iodine. Gaseous iodine from iodine rich coastal areas in Ireland has been identified as a contributor to iodine intake (Smyth et al (2011)). When iodine vapour is introduced to the body via inhalation it diffuses directly into the bloodstream (Dessanges (2001)). Given its volatility in this environment it is expected to transfer to humans via a combination of inhalation (Smyth et al (2011)) and potentially dermal absorption (Boothman (2010)). Non-volatile species (I^-, IO_3^- or organic iodine) within the bathwater could also be available for dermal absorption.

4.1 Survey of clients and staff of Voya Seaweed Baths

A survey of clients and staff was compiled and carried out in January 2015 in order to establish their perception of the baths and associated health benefits (Appendices C and D). The survey mainly consisted of questions to target specific characteristics of seaweed baths and the seaweed itself i.e. the colour of the seaweed used in the baths, the consistency/texture of the bathwater during the year, the variation in the bathwater colour throughout the year and why clients use the baths. In particular, certain questions were asked in order to determine if the groups were aware of iodine and its effects in the context of seaweed baths and also the potential benefits of taking seaweed baths. Of the 18 clients who responded to the survey, 5 were first time users of the seaweed baths and 13 were regular users. Both staff and clients indicated that relaxation was the most common reason for using seaweed baths as opposed to other associated health benefits. It would appear that clients are unaware of any associated health benefits of using seaweed baths i.e. that iodine, or other minerals, present in the bathwater could potentially be taken up by the body however one third of clients felt that it was of most benefit to use the seaweed baths in winter. Interestingly, both groups commented that the bathwater was thicker post-treatment in summer/autumn. When asked if at the end of a treatment they leave the bathwater extracts on the skin or do they shower it off, 77% of respondents stated that they leave the extracts on their skin while 8% shower it off: the remaining 15% did not comment. It is therefore possible that this may lead to prolonged exposure to the minerals deposited on the skin from the bathwater following bathing.

4.2 Preliminary *in vivo* feasibility study

This feasibility study built on a previous unpublished work on one subject by Professor Peter Smyth which suggested that UIC increased following a seaweed bath. The aim of this study was to establish the feasibility of an *in vivo* study to assess iodine uptake by the body during a seaweed bath treatment and to explore possible routes of uptake. As 90% of iodine in the body is excreted in urine (Jooste and Strydom (2010)) subjects were required to collect pre-treatment and post-treatment urine samples over 32 hours so that their UIC profiles could be compiled and compared.

A small sample population of 8 subjects was recruited. Bathing subjects (2 male and 1 female (aged 32-46)) immersed themselves in the seaweed bath for 40 minutes (a typical bathing time). As treatment rooms are private bathing suits were not required. Non-bathing subjects, fully clothed (5 female (aged 26-47)) were requested to sit adjacent to the seaweed bath for the same duration and not to have dermal contact with the seaweed or bathwater. The seaweed bath was prepared using cold seawater, hot tap water and a bucket of *Fucus serratus* L. seaweed. Subjects were requested to refrain from using steam or shower facilities prior to or after bathing. As there is potential for dermal absorption of iodine by bathers it was requested that bathing subjects would not shower after their treatment as it is possible that algin present in the bathwater may trap minerals by forming an occlusive cover keeping these minerals in contact with the skin post-treatment. This may lead to dermal absorption over time. They were also provided with 1.5L of drinking water to keep hydrated throughout. All subjects collected a urine sample immediately prior to the seaweed bath treatment. Their second urine sample was collected immediately after and a further 6 samples were collected over the following 32 hours at 12.00, 18.00, bedtime, morning, 12.00 and 18.00. One subject (female, 31), having had no exposure to seaweed, seaweed baths etc., provided a baseline UIC for comparison by collecting samples at the same designated times on 2 separate occasions. Samples were stored at -20 °C and allowed to reach room temperature prior to analysis.

The median UIC profile for all subjects is shown in ***Figure 4.1***. The UIC was measured using the SK micromethod with no dilution of the samples. Results indicated that the

UIC of almost all subjects increased following exposure to a seaweed bath, whether immersed in the water or sitting adjacent to the bath. Of the 8 subjects tested, 7 showed a peak in UIC between 8 and 22 hours post-treatment. The UIC of bathing subjects spiked (128 µg L^{-1}) at 22 hours post-treatment. That of non-bathing subjects spiked (119 µg L^{-1}) at 8 hours post-treatment. The UIC profile of 4 subjects showed a gradual increase before spiking at 12-22 hours post-treatment. The UIC for 3 subjects spiked at approximately 12 hours post-treatment (night time samples) while 2 display a peak at 22 hours post-treatment (morning samples). Finally, 2 subjects showed a peak at 26 hours post-treatment. Initially it was considered that the iodine in the bathwater may deposit in the skin of bathers and would take longer to appear in the urine having penetrated the skin first which may explain the later increase in UIC post-bathing however 2 of these subjects were control subjects with no dermal exposure to seaweed or the bathwater therefore it was deduced that the increase in UIC was attributed to the uptake of iodine via inhalation of the volatile species (I_2) during the treatment.

Furthermore, the baseline UIC of one subject ranged from 6 to 67 µg L^{-1} (average 39 µg L^{-1}) in the first collection and 21 to 57 ppb (average 37 µg L^{-1}) in the second. Their UIC increased to 133 µg L^{-1} during the *in vivo* study which is almost double their typical daily UIC which further supports that iodine while not absorbed through the skin during the treatment is still taken up by the body.

These results suggest that in order to increase iodine in the body it is not necessary to be immersed in the bath as volatile iodine contributes greatly to the increase in UIC for almost all subjects. The increase in UIC of non-bathers can only be attributed to inhalation of volatile iodine released by the seaweed as there was minimal dermal exposure to iodine for the fully clothed control subjects. Based on these findings, a larger study was proposed to investigate this further.

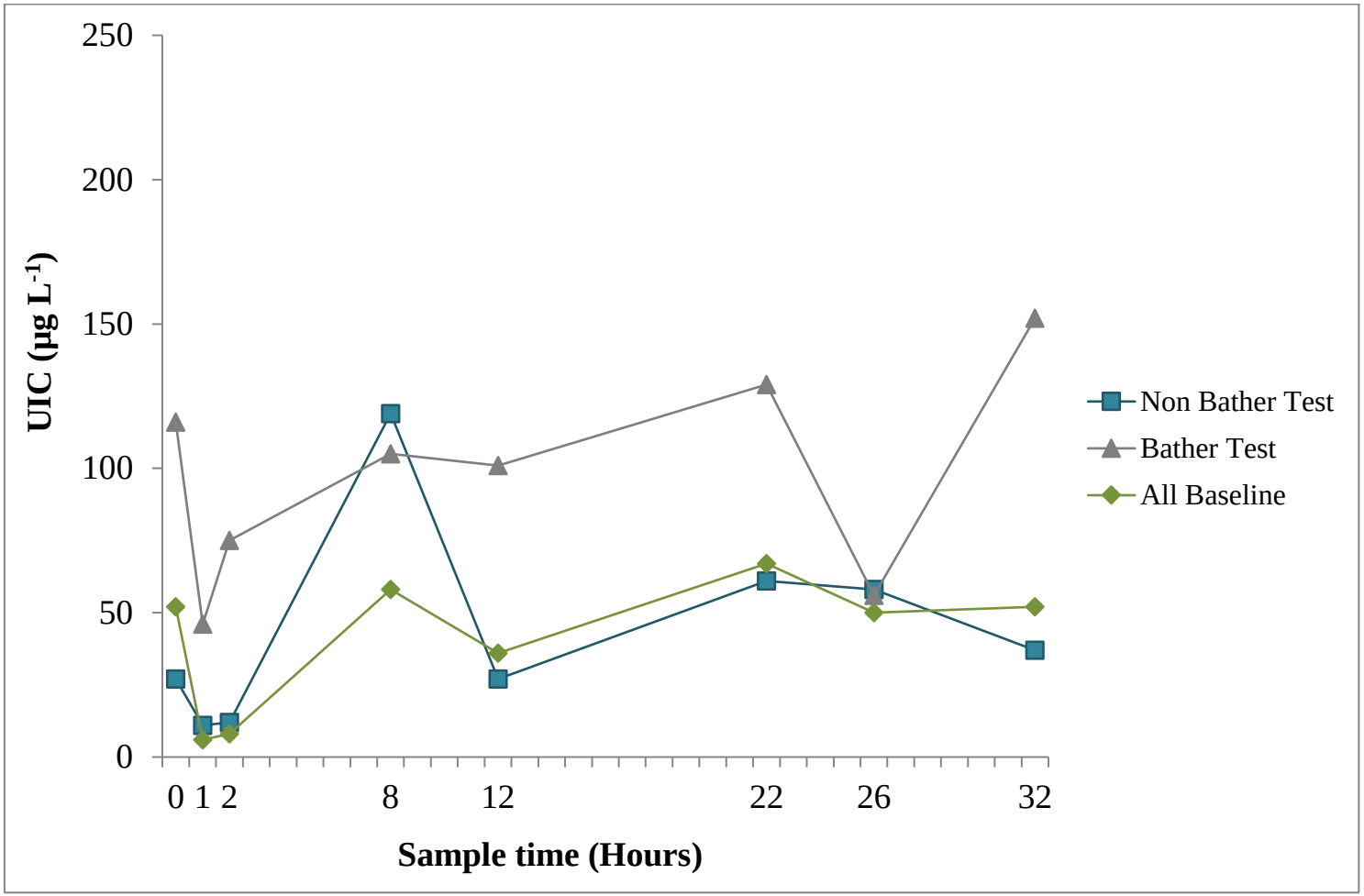


Figure 4.1 Median UIC of bathers and non bathers and baseline over 32 hours post seaweed bath.

4.3 Pilot *in vivo* study: to measure changes in population median UIC following exposure to seaweed bath

4.3.1 Research Ethics Committee approval

The first stage of the larger, more detailed study required ethics approval in order to measure a subject's urinary iodine and creatinine levels pre and post-bathing in *Fucus serratus* L. seaweed bath. This approval also required consideration of any potential risks to subjects' i.e. adverse reaction to the treatment, slips, trips; these were assessed and minimised as part of periodic risk assessment for public liability purposes with the seaweed bath provider (Appendix E). Prior to taking part in the study, all participants were required to complete a questionnaire (Appendix H) and Certificate of Consent (Appendix J). It was also explicit that the identity of subjects and any personal information collected related to them was kept confidential on file and that all personal data will be destroyed after three years.

4.3.2 Subject recruitment

Thirty adult subjects were recruited through advertisement in IT Sligo and Voya Seaweed Baths, Sligo, Ireland (Appendix F). A total maximum of 30 subjects was recruited in order to obtain a minimum of 10 subjects in each of the two study groups;

Bathing/bather (n=15) (immersed in the seaweed bath for 40 minutes)

Non-bathing/non-bather (n=15) (sat adjacent to the seaweed bath for the same duration for 40 minutes).

The additional recruitment was to allow for drop out from the study or subjects who collect insufficient urine samples to determine any trend. Both male and female subjects in the age bracket (20-50 years) were selected randomly from the population. In line with standard protocol by the Voya Seaweed Baths and certain study requirements the following exclusions also applied: not pregnant, no history of underactive or overactive thyroid or sensitivity/intolerance to seaweed/seaweed containing products, no surgical procedures in the previous 6 months, not taking iodine

supplements and not taken a seaweed bath in the week prior to the study. The baths were provided free of charge by Voya Seaweed Baths.

4.3.3 Study protocol

On completion of their certificate of consent and questionnaire, each subject was assigned randomly to 2 study groups; bathing (n=15) (immersed in the seaweed bath for 40 minutes) and non-bathing (n=15) (sat adjacent to the seaweed bath for the same duration for 40 minutes). The study was carried out over four days; 2 pre-treatment days and 2 post-treatment days within a two week period. The pre-treatment phase of the study required subjects to collect urine samples using pre-labelled specimen bottles at the time intervals outlined in ***Table 4.1*** to establish their baseline UIC. The post-treatment phase of the study required subjects to collect samples at the same time intervals however in this instance the bath treatment was provided between sample 1 and 2. Both groups were exposed to seaweed baths, consisting of cold seawater, hot tap water (total volume ~100±20 L) and 4±0.8 kg *Fucus serratus* L.

While in clinical pharmacology studies, it is advantageous to collect all urine over a 24 hour period, this was not deemed necessary in this instance as the objective of the study was not to determine the total iodine uptake, rather to establish if there is a change in UIC profile following a seaweed bath which can be established with this protocol. Also, 24 hour sampling was not feasible in terms of cost, analysis and practicality of collection. Spot sampling, at specific time points which are uniform between all subjects, is routinely carried out in urinary analysis and can be used when 24 hour collection is not feasible (García-Villalba et al (2010), Chetwynd et al (2017)).

Sampling times (***Table 4.1***) were selected to capture a comprehensive UIC profile. Sample 1 was the first morning sample after which the treatment was taken. Sample 2 was immediately after the treatment to capture any instant increase in UIC. Samples 3, 4 and 5 were at midday, evening and night time the same day. Sample 6 was the first morning sample on day 2 which was followed by another midday and evening sample (7 and 8). Feedback from subjects in the preliminary *in vivo* study indicated that they

found it difficult to take samples as often as required by the protocol. Results from that study also indicated that it was possible to establish the UIC profile with these designated sampling times therefore the timeline was maintained for the pilot study. Extra samples were provided for any subjects who needed to urinate more frequently.

Table 4.1 **Timeline for urine sample collection for baseline and treatment groups**

Urine Sample Number	Day	Approximate Sample Time
1	1	10.00
2	1	11.00
3	1	12.00
4	1	18.00
5	1	Night i.e. bedtime
6	2	Morning
7	2	12.00
8	2	18.00

4.3.4 Food Log

As diet is the main source of iodine subjects were requested to avoid large quantities of iodine rich foods (especially seafood (fish, shellfish, and seaweed), dairy (egg yolks, milk, yogurt, cheese, butter, ice cream, sour cream, milk chocolate), soy (soybeans, soy sauce, and tofu) and beans (red kidney beans, lima beans, pinto beans or cowpeas)) on the day before the study and the two days of the study as it may give rise to an increased UIC It was also requested that they fill in a food log provided related to their food and drink consumption over the urine collection days (Appendix I). The purpose of this food log was

(i) to record information which may indicate the cause of a particularly low or high UIC and

(ii) to potentially analyse the contribution to UIC from diet.

It was designed to capture the types of foods eaten including their quantities and the types of drinks including their volume. Serving portion diagrams were also included as a guide.

Although subjects were requested to include as much detail as possible their compliance was not uniform. For example, one subject recorded their dinner as 6 ounces of roasted beef with ½ cup of potatoes while another recorded theirs as pasta. Similarly, one subject recorded their drink log as coffee while another detailed 1.5 litres of water.

Unfortunately, adherence to the protocol was poor and analysis of the contribution of iodine from food has been limited. Overall, the information provided was not negligible and the food logs were used more as a reference for each individual subject. In particular, where any UIC recorded was unusually low or high the food log for that subject was consulted to establish if they had eaten any foods rich in iodine or if they had drank a large volume over the study days which may have diluted their urine samples. There was no correlation found between food intake and high UIC or drink intake and low UIC for any individual subject. It was, however, beyond the scope of the project and ethical approval to control/restrict types and quantities of food/drink consumed. While diet was monitored as much as possible other factors such as weight, fitness level and thyroid function were not monitored as part of the study.

4.3.5 Bath data

Descriptive characteristics (the amount of seaweed used, the iodine concentration and the temperature before and after 40 minutes bathing period) of the 22 commercial baths used for the *in vivo* study are presented in ***Table 4.2***. No correlation exists between bath characteristics and individual UIC for any subject.

Table 4.2 **Composition of commercial seaweed baths (n=22) used in the *in vivo* study showing the total weight of seaweed, estimated volume of water, iodine composition and temperature before (t_0) and after (t_{40}) an average bathing period of 40 minutes**		
	Average	**Range**
Wet mass of *Fucus serratus* L. (kg) per bath	4.04±0.8	2.92-5.66
Seawater iodine concentration (µg L^{-1})	18.1±7.9	6-33
Bathwater iodine concentration @ t_0 (µg L^{-1})	581.3±333.9	257-1353
Bathwater iodine concentration @ t_{40} (µg L^{-1})	954.1±440.4	320-2022
Temperature @ t_0 (˚C)	44.1±2.5	40-51
Temperature @ t_{40} (˚C)	38.8±1.5	37-42
Volume (L)	100	80-120

4.3.6 Urine testing

The SK micromethod was used directly for UIC following inter lab comparisons with two EQUIP (Ensuring the Quality of Iodine Procedures) accredited external labs:

1. Human Nutrition Laboratory, Institute of Food, Nutrition and Health, University of Zurich (n=8) correlation coefficient = 0.9951 (***Figure 4.2***)

2. Centre for Public Health, Queen's University Belfast (n=16) correlation coefficient = 0.9914 (***Figure 4.3***)

Although the method was not validated specifically for urine, it is originally designed for urine analysis (Sandell and Kolthoff (1937)) and the interlaboratory comparisons indicate it is suitable.

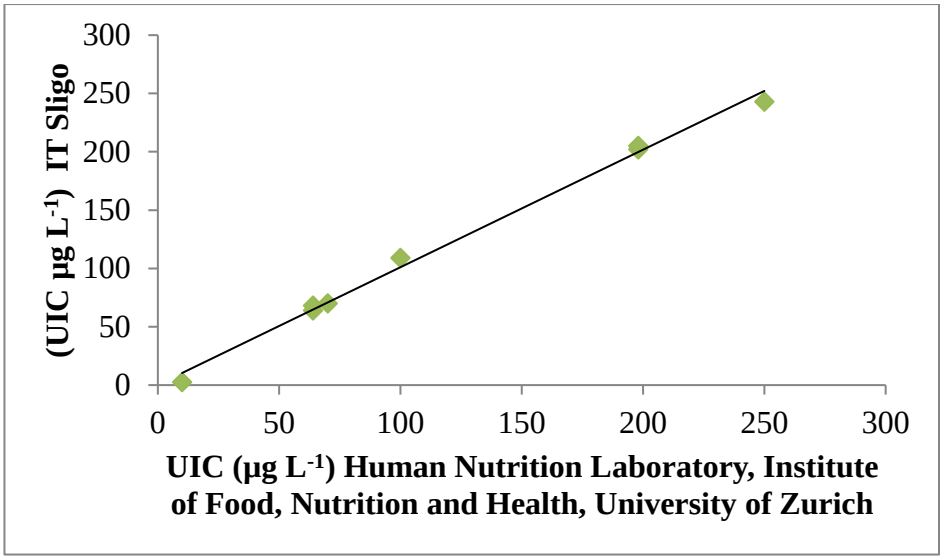


Figure 4.2 Correlation between IT Sligo and Human Nutrition Laboratory, Institute of Food, Nutrition and Health, University of Zurich for control samples (n=8) UIC (µg L^{-1}) correlation coefficient = 0.9951

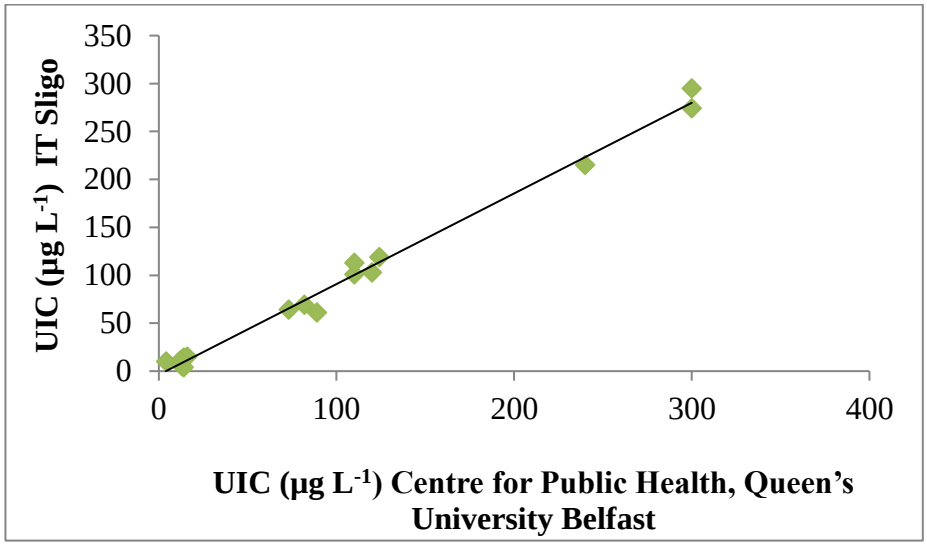


Figure 4.3 Correlation between IT Sligo and Centre for Public Health, Queen's University Belfast for control samples (n=16) UIC (µg L^{-1}) correlation coefficient = 0.9914

4.3.7 UIC

Samples were collected, stored at -20 ˚C and analysed together at the end of the study (both SK analysis and creatinine analysis). Despite the UIC profile for some subjects being atypical, findings are relative to the population as a whole. The population median was calculated (as urinary iodine values tend not to be normally distributed (WHO (2013)) at each sampling time across 30 subjects for the baseline results and across 15 subjects for each test group, bather and non-bather. The baseline, bather and non-bather UIC profiles are illustrated in ***Figures 4.4*** and ***4.5*** where ***Figure 4.4*** is presented with creatinine adjustment and ***Figure 4.5*** is presented with no creatinine adjustment (the creatinine adjusted data provides a more conservative representation of the population UIC and the increase post treatment).

The pre-treatment creatinine adjusted median UIC of all subjects' of 76 µg L^{-1} and non-adjusted of 60 µg L^{-1} (range 60-94 µg L^{-1}) is in line with the findings of the WHO (2004) which classes the Irish population (adults 22-61) as mildly deficient (UIC 82 µg L^{-1}) in iodine. As expected, there was no significant difference between the pre-treatment UIC of both groups (p = 0.479) where bathers pre-treatment creatinine adjusted and non-adjusted median was 72 µg L^{-1} and non-bathers pre-treatment creatinine adjusted median was 75 µg L^{-1} and non-adjusted median was 65 µg L^{-1} (***Table 4.3***).

Following the treatment, there was a significant increase (p = 0.015) in UIC for the entire population (***Figures 4.4*** and ***4.5*** and ***Table 4.3***). The median UIC of all subjects (of both bather and non-bather) increased from pre-treatment 76 µg L^{-1} and non-adjusted of 60 µg L^{-1} to 95 µg L^{-1} and non-adjusted of 70 µg L^{-1} respectively. Both the creatinine adjusted UIC and non-adjusted UIC of bathers increased from 72 µg L^{-1} to 86 µg L^{-1} post treatment representing ~ 20% increase from pre-treatment UIC and is significantly different where p = 0.016. The creatinine adjusted UIC of non-bathers increased from 75 µg L^{-1} to 105 µg L^{-1} and their non-adjusted UIC increased from 65 µg L^{-1} to 105 µg L^{-1} post treatment representing ~ 40% and 60% increase respectively from pre-treatment UIC and are significantly different where p = 0.006.

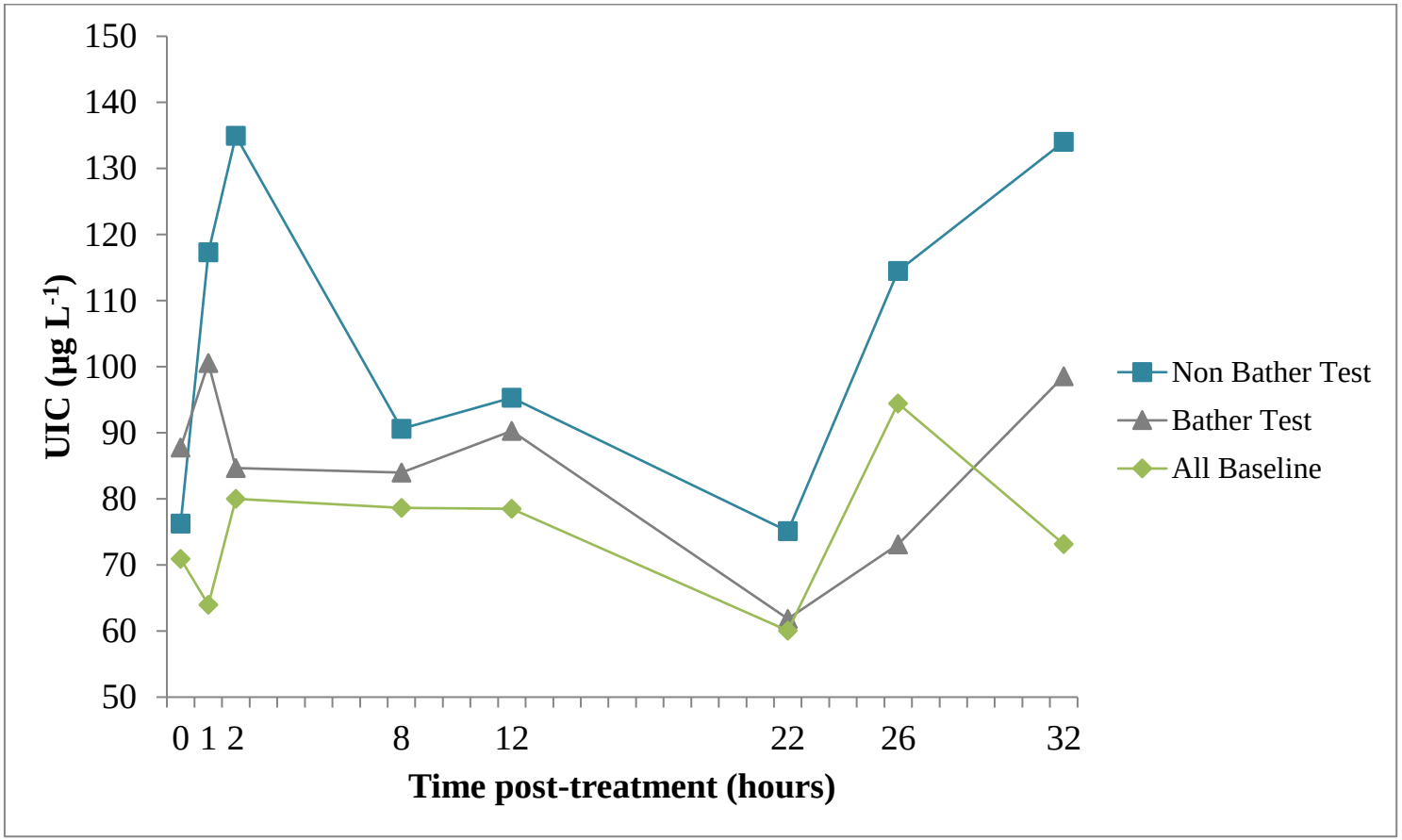


Figure 4.4 Comparison of pre-treatment UIC and post-treatment UIC for all subjects (n = 30, 15 bathers, 15 non-bathers). Pre-treatment median: 76 µg L^{-1} and post-treatment median: 95 µg L^{-1}

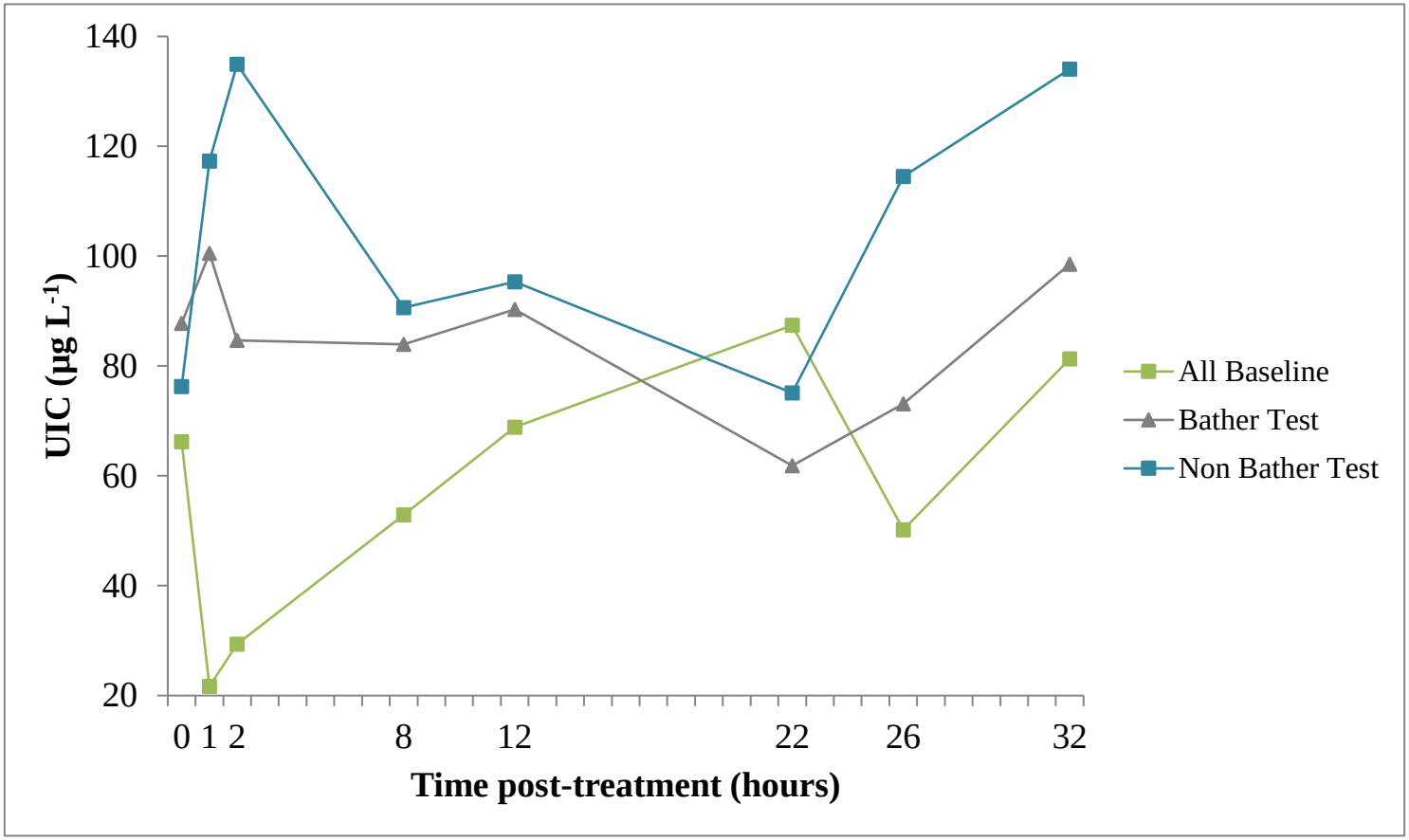


Figure 4.5 Comparison of pre-treatment UIC and post-treatment UIC for all subjects with no creatinine adjustment (n = 30, 15 bathers, 15 non-bathers). Pre-treatment median: 76 µg $L^{-1}$and post-treatment median: 95 µg L^{-1}

Table 4.3 **Significant difference (*p*-value) between pre-treatment and post-treatment UIC for both groups and the entire study population. No significant difference across entire study population baseline UIC; significant difference between bather and non-bather post-treatment UIC**			
Subject	**Pre-treatment *v* Post-treatment**	**Bather *v* Non-bather pre-treatment**	**Bather *v* Non-bather post-treatment**
Bather	0.016	0.479	0.015
Non-bather	0.006		
All	0.015		

4.3.8 Route of iodine uptake

While the study design does not allow categorical claims for the route of uptake, the significant differences between the pre-treatment and post-treatment UIC for the individual groups (***Table 4.3***) strongly suggest that inhalation of iodine vapour is the dominant contributor to the increase in UIC for all subjects; there was significantly less although not negligible possibility of dermal absorption for the non-bathers. For bathers, there was a 14 µg L^{-1} increase in UIC. This is approximately a 20% increase from baseline UIC and is significant where $p = 0.016$. For non-bathers, there was a 30 µg L^{-1} increase in UIC (40 µg L^{-1} increase non-adjusted). This is represents a 40% increase from baseline UIC and is significant where $p = 0.006$.

The higher UIC for non-bathers (significantly different to bathers where $p = 0.015$) may be attributed to the difference in position during the treatment for both groups. Normal breathing rates vary with activity and are lower when lying (0.32-0.45 $m^3 h^{-1}$) compared with sitting (0.39-0.54 m^3h^{-1}) (Kubota et al (2013), Brudecki et al (2017)). Under the study conditions the seated position of the non-bathing group may have led to increased respiration rates.

Despite bathers having the potential for uptake via inhalation and dermal absorption results also suggest that the bioavailability of iodine via dermal routes is low. This is most likely due to the predominant species of iodine present i.e. iodine gas (I_2) which is inhaled and iodate (IO_3^-) which does not cross the skin readily (Goldsmith (1983)).

Also, given that bathers are warm post-treatment, any iodine deposits on the skin are potentially lost due to perspiration.

Aside from the holistic therapeutic value of this form of thalassotherapy it has been shown that there is also an added health benefit in the uptake of iodine during the treatment. Results indicate that inhalation of volatile iodine may be the dominant route of uptake during a seaweed bath contributing up to 14% of the RDA of iodine in one treatment (19 µg L^{-1} increase in UIC represents 90% of the excreted iodine taken in). The average total iodine levels in commercial seaweed baths is 768 µg L^{-1}. Studies on seasonal variation in Chapter 3 indicate that the optimum bathing time for maximum exposure to volatile iodine is winter (November-January). This is also the time when clients indicated that they felt was of most benefit to them to use seaweed baths. While it is not suggested that the use of seaweed bath treatments could be an efficient mechanism to supplement iodine it may however be an effective mechanism. Following the confirmation of iodine uptake during a treatment, further investigations, *in vitro,* explore the potential for dermal uptake of other minerals by a bather.

5 *In vitro* uptake of minerals from a *Fucus serratus* L. seaweed bath

5.1 Introduction

While results of the *in vivo* study demonstrate that iodine is taken up by a bather via inhalation, it was unknown whether the other minerals present in the bathwater might be absorbed through the skin as a consequence of the same bathing treatment. Literature indicates that aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc can be absorbed through the skin albeit in different contexts e.g. from mud baths, dermal patches or wound dressings (***Table 1.5***).

Following the determination of the mineral profile of the bathwater matrix presented in chapter 3 the potential for dermal absorption of minerals in an *in vitro* setting using Enhancer cell technology with ICP-MS detection is explored in this chapter. As there is good correlation between *in vivo* and *in vitro* dermal absorption data, *in vitro* measurements can be used to predict *in vivo* absorption (WHO (2006)).

The aim of this study was to use Enhancer cell technology and dissolution apparatus (***Figure 1.29*** and ***2.13***) to establish the dermal absorption/penetration of minerals up to 18 hours post exposure of a membrane (Cuprophan) to a simulated bathwater sample and subsequently infer this data to an *in vivo* setting. Cuprophan was used in this study is increasingly used for *in vitro* transdermal testing. This synthetic membrane also eliminates variables such as skin age, race, sex and anatomical site associated with *in vivo* settings. Typically in these types of studies, the cumulative amount of minerals absorbed (per unit surface area) is determined as a function of time (WHO (2006)).

A post treatment recommendation by seaweed bath providers is to refrain from showering in order to maximise exposure to beneficial components from the bathwater that may remain on the skin following bathing. As a result, of this recommendation the study was run for 18 hours. The enhancer cells (n=3) were filled with simulated bathwater and the membrane applied. The first receiver vessel samples, at t_0, were collected immediately and at 10 minute intervals up to 6 hours then at 30 minute intervals for 4 hours with the final samples collected after 18 hours. The cumulative

amount of mineral crossing the membrane, µg per cm^2, into the receiver vessel was then calculated as per section 2.7.5. The first study was to establish the amount of mineral crossing the membrane over 40 minutes which reflects a typical bathing time. In line with recommendation to refrain from showering post treatment the second study was to establish the amount of mineral crossing the membrane up to 18 hours contact time. Finally, the data was used to establish the theoretical dermal uptake by a bather *in vivo* using:

(i) the standard seaweed bath preparation i.e. ~5 kg *Fucus serratus* L. + 100 L water

(ii) the exposed surface area during a treatment i.e. 1.6 m^2 for women and 1.9 m^2 for men (Mostellar (1987))

5.2 *In vivo* setting results up to 40 minutes

The cumulative amount of mineral absorbed per cm^2 during a typical 40 minute treatment is displayed in ***Figures 5.1*** to ***5.3,*** for different concentration ranges**.** It is shown that the absorption across the membrane is instantaneous as there was already a measureable concentration in the receiving vessel in the first sample (~10 seconds). Most minerals reach their maximum in the cumulative concentration within 20 minutes.

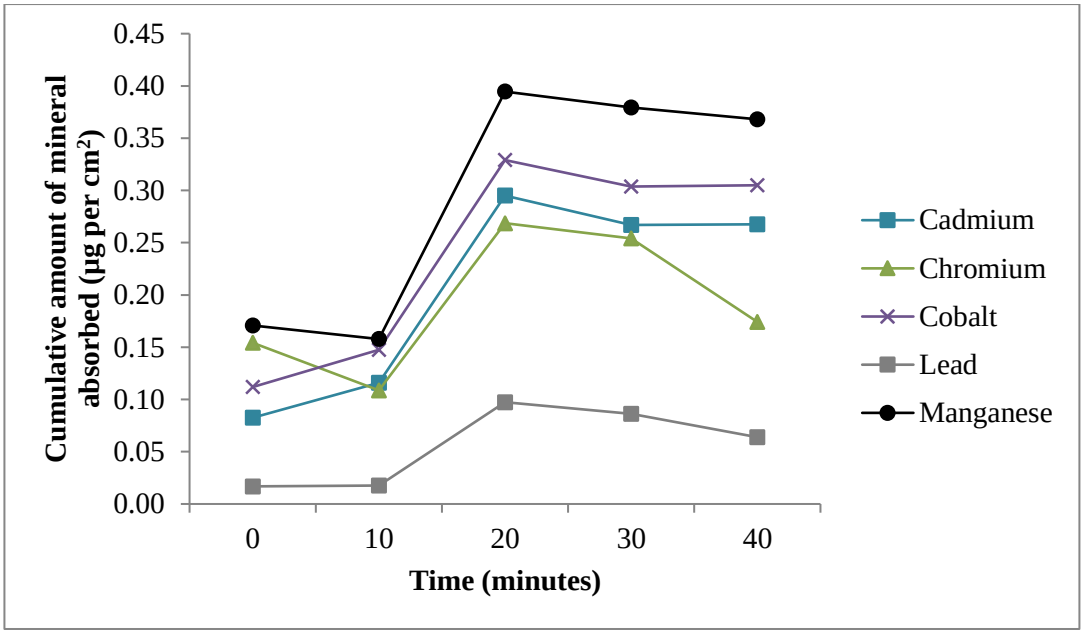


Figure 5.1 Cumulative amount of cadmium, chromium, cobalt, lead and manganese absorbed (maximum 0.45 µg) per cm^2 over 40 minutes

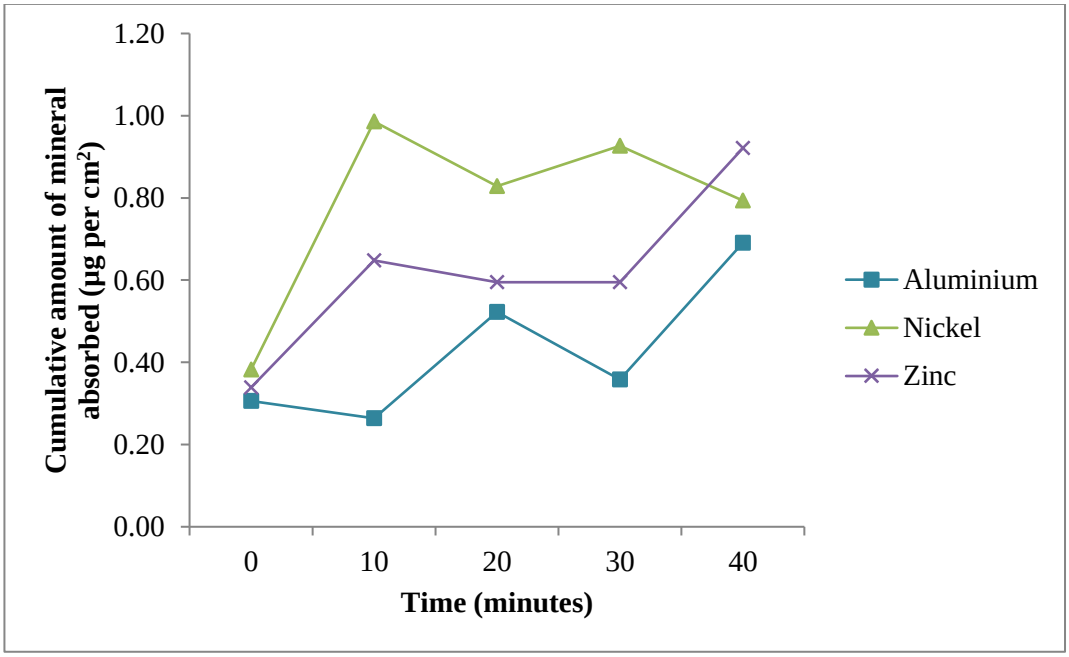


Figure 5.2 Cumulative amount of aluminium, nickel and zinc absorbed (maximum 1 µg) per cm^2 over 40 minutes

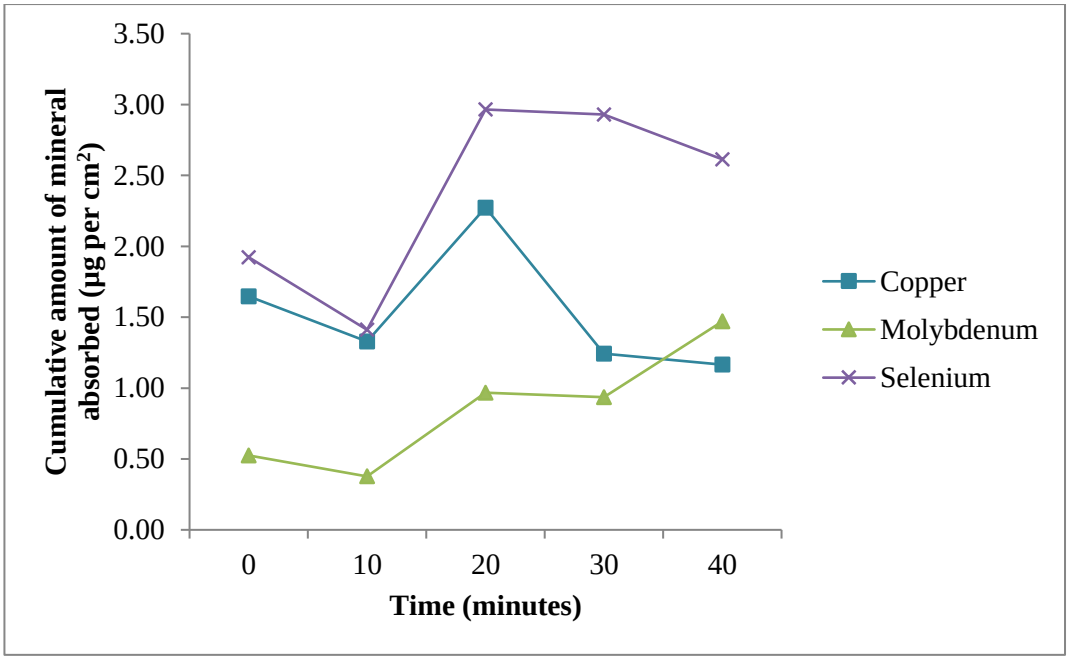


Figure 5.3 Cumulative amount of copper, molybdenum and selenium absorbed (maximum 3 µg) per cm^2 over 40 minutes

5.3 *In vivo* setting results up to 18 hours

Although the study was run for 18 hours the set up does not replicate *in vivo* conditions for example, minerals may be lost to the environment via perspiration or friction with clothing following bathing. Also, it is noted that the study conditions allow for continuous contact with the bathwater matrix as opposed to an algin residue post treatment. However despite these factors the cumulative amount of mineral absorbed (µg) per cm^2 over 18 hours are illustrated in ***Figures 5.4*** to ***5.8.*** These figures are split into

a illustrating the cumulative amount of mineral absorbed per cm^2 over 3 hours and

b illustrating the cumulative amount of mineral absorbed per cm^2 between 3 and 18 hours.

Results show that all minerals tested cross the membrane into the receiver vessel however the rate of absorption is not uniform across all minerals. The cumulative

concentration per mineral increased as follows: lead < chromium < cadmium < cobalt < zinc < manganese < molybdenum < aluminium < selenium < nickel < copper. While none of the minerals display a linear response relative to time, the absorbed amount continues to increase over 18 hours. Given this continuous increase in absorbed concentration, it also suggests that neither the membrane nor the receiving buffer becomes saturated with applied dose. Chromium, lead and selenium display the most stable profiles which peak after 2 hours then plateau unlike cadmium, cobalt, manganese, molybdenum or nickel which indicate burst followed by sink over the 18 hours.

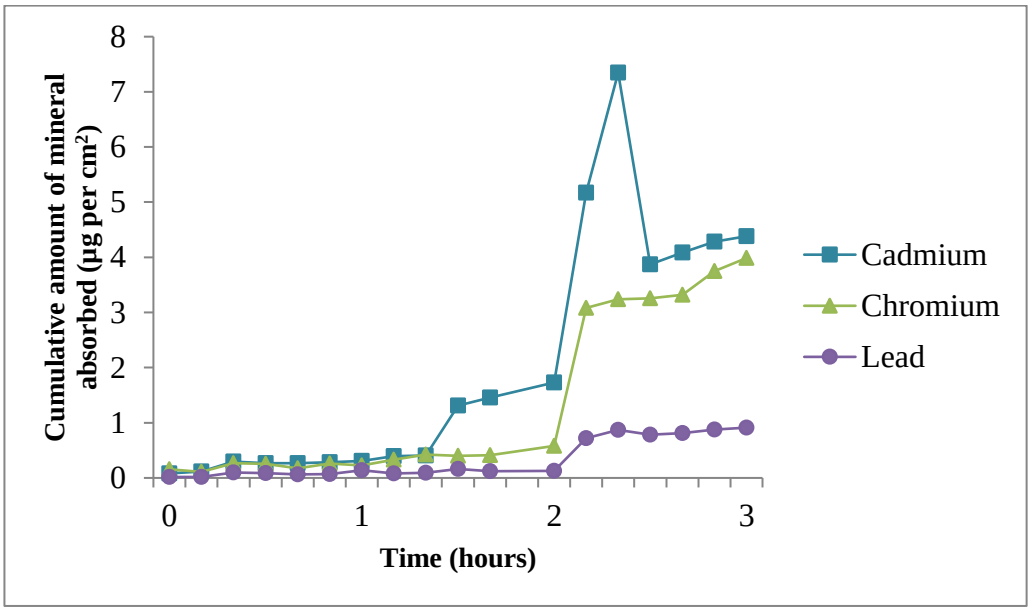


Figure 5.4a Cumulative amount of cadmium, chromium and lead absorbed (up to 8 µg) per cm^2 over 3 hours

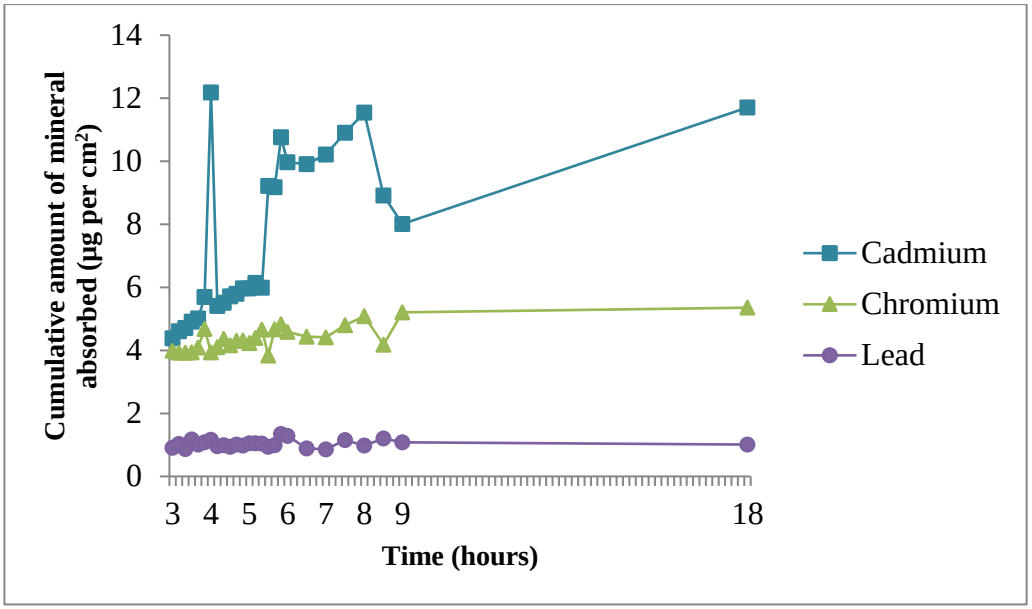


Figure 5.4b Cumulative amount of cadmium, chromium and lead absorbed (up to 12 µg) per cm^2 between 3 and 18 hours

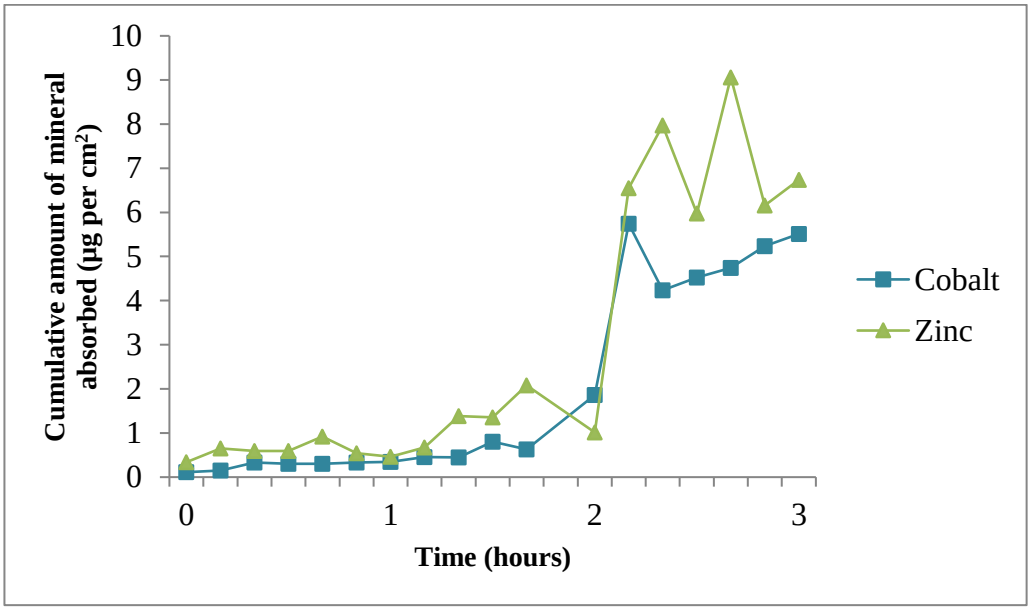


Figure 5.5a Cumulative amount of cobalt and zinc absorbed (up to 9 µg) per cm^2 over 3 hours

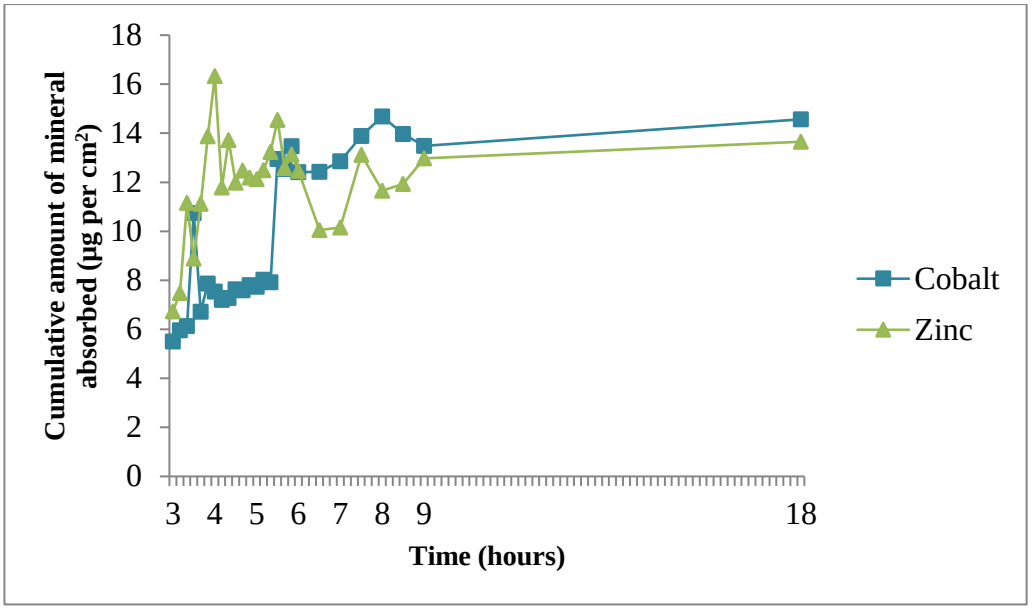


Figure 5.5b Cumulative amount of cobalt and zinc absorbed (up to 17 µg) per cm^2 between 3 and 18 hours

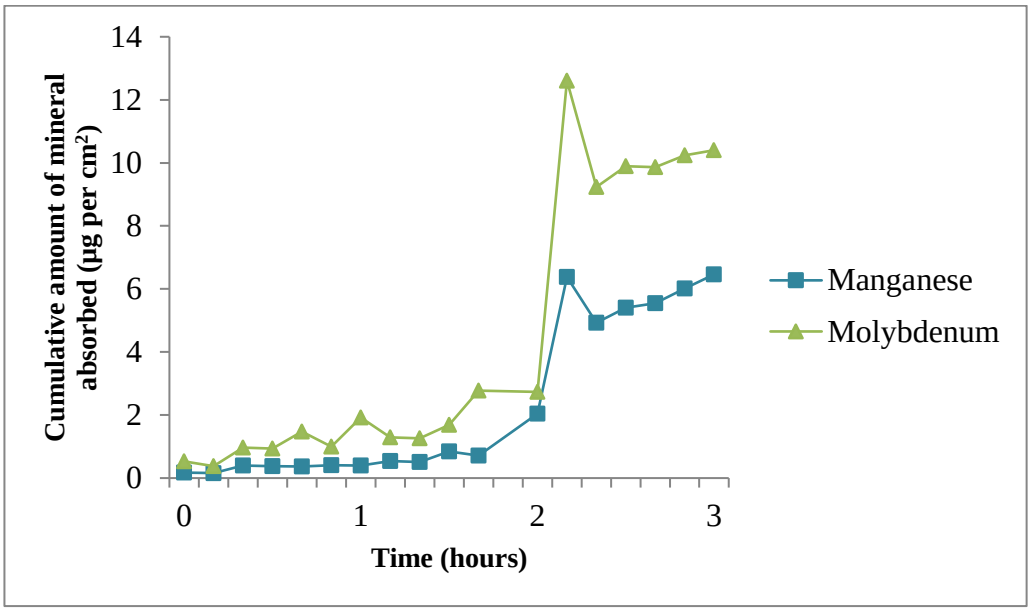


Figure 5.6a Cumulative amount of manganese and molybdenum absorbed (up to 13 µg) per cm^2 over 3 hours

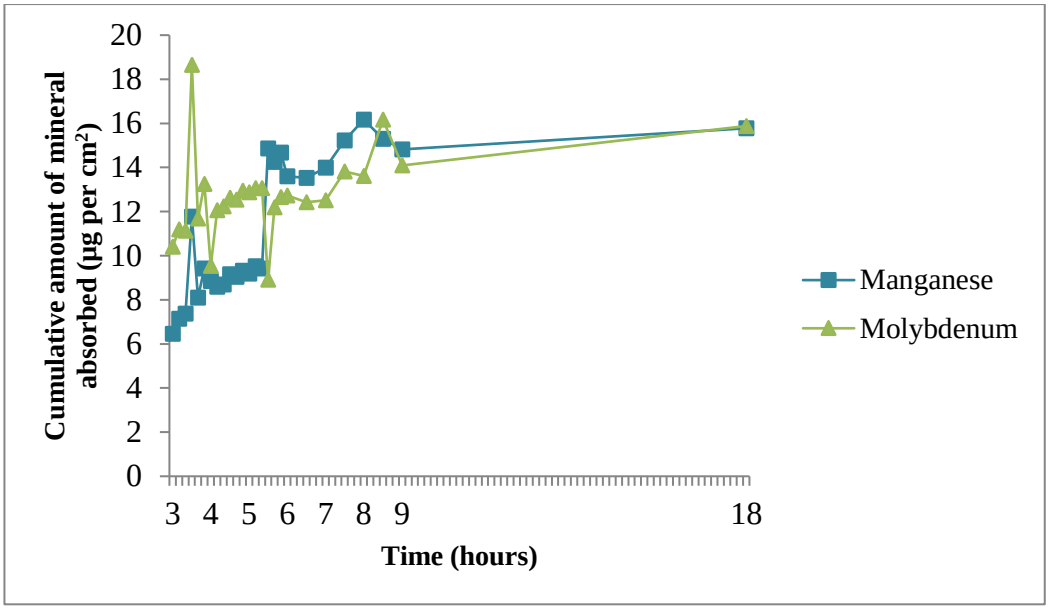


Figure 5.6b Cumulative amount of manganese and molybdenum absorbed (up to 19 µg) per cm^2 between 3 and 18 hours

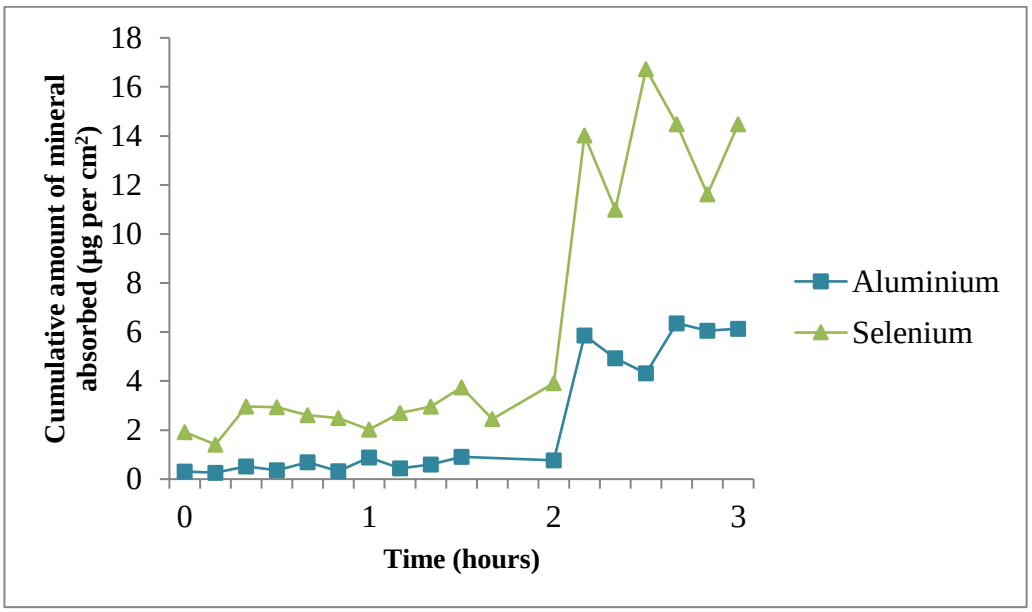


Figure 5.7a Cumulative amount of aluminium and selenium absorbed (up to 17 µg) per cm^2 over 3 hours

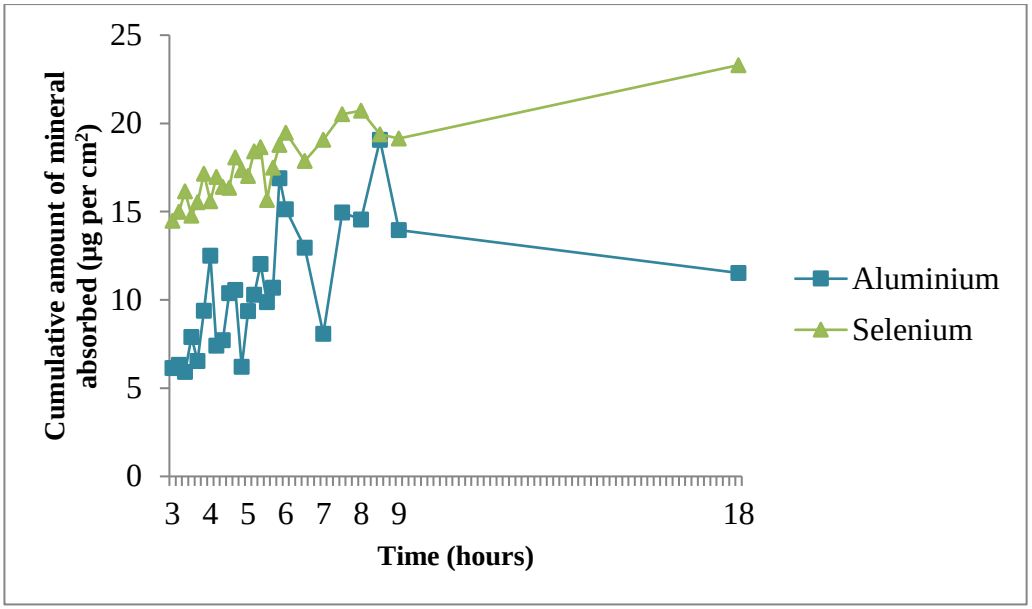


Figure 5.7b Cumulative amount of aluminium and selenium absorbed (up to 23 µg) per cm^2 between 3 and 18 hours

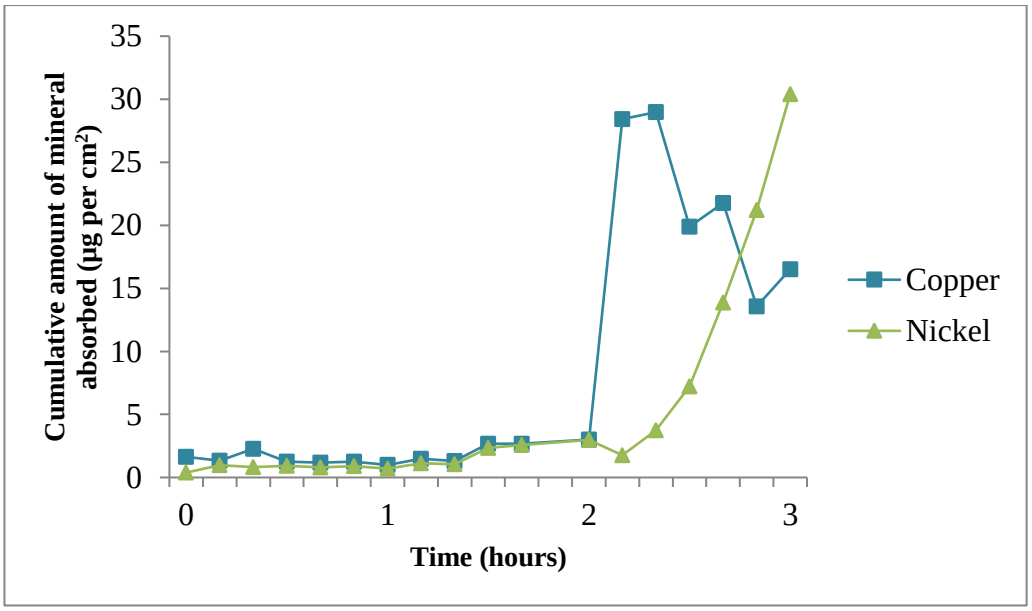


Figure 5.8a Cumulative amount of copper and nickel absorbed (up to 32 µg) per cm^2 over 3 hours

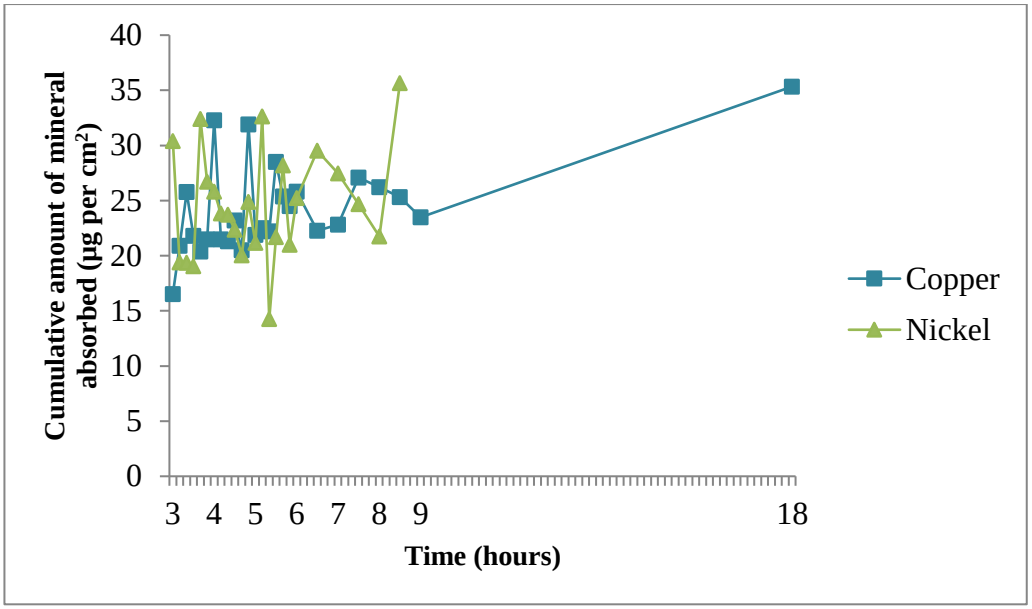


Figure 5.8b Cumulative amount of copper and nickel absorbed (up to 40 µg) per cm^2 between 3 and 18 hours

In order to establish the cumulative amount of mineral which could *theoretically* be absorbed during a commercial bath using ~5 kg *Fucus serratus* L. + 100 L water the absorbed mineral concentrations per cm^2 were initially reduced by a factor of 3 as typical commercial baths use a seaweed: water ratio of 50 g L^{-1} whereas the simulated bathwater samples was prepared using 150 g L^{-1}. This was then multiplied by the standard surface area for both female and male bathers i.e. 1.6 m^2 for female and 1.9 m^2 for male (Mostellar (1987)). Results are compared in ***Table 5.1***which also indicates the RDA for each mineral. ***Table 1.4*** outlines the health impact of all minerals tested. It is important to note that the comparison is limited as the data set is small and has not taken into account variation within the bath in terms of seasonal fluctuation of mineral composition and individual bather preference of temperature and water volume.

With the exception of cobalt, manganese (for females) and zinc the potential mineral absorption could lead to levels in excess of RDA during the 40 minute treatment time. Specifically, higher levels of aluminium may lead to neurotoxicity while cadmium can be toxic to most organs. Similarly, lead is toxic to the body as it interferes with

metabolism and can lead to organ damage. Conversely, higher levels of chromium, copper and nickel are beneficial to health as they aid metabolism. Interestingly, both molybdenum and selenium are associated with immune function and well-being therefore higher levels are of benefit to bathers especially given their inclination to use seaweed baths for relaxation.

Table 5.1 **Theoretical cumulative mineral absorption (µg) of aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc from *Fucus serratus* L. seaweed bathwater (50 g L^{-1}) over 40 minutes for female (F) and male (M) bathers with exposed surface area of 1.6 m^2 and 1.9 m^2 respectively**

	Aluminium		**Cadmium**		**Chromium**		**Cobalt**		**Copper**		**Lead**		**Manganese**		**Molybdenum**		**Nickel**		**Selenium**		**Zinc**	
Time (minutes)	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
0	1632	1938	440	522	822	976	597	709	8779	10426	90	107	911	1081	2797	3321	2038	2420	10249	12171	1806	2145
10	1407	1671	619	735	580	688	787	935	7081	8409	94	112	842	999	2011	2388	5258	6244	7526	8937	3456	4104
20	2787	3309	1574	1869	1433	1701	1755	2084	12125	14398	519	617	2104	2499	5161	6129	4420	5248	15813	18778	3175	3770
30	1909	2267	1423	1690	1354	1608	1619	1923	6634	7878	459	546	2023	2402	4992	5928	4942	5869	15625	18555	3171	3766
40	3684	4375	1427	1694	929	1103	1627	1932	6215	7380	340	404	1963	2331	7842	9313	4232	5026	13933	16545	4912	5833
RDA (µg)	**200**		**3**		**120**		**5000-8000**		**2000**		**10**		**2000**		**75**		**70**		**70**		**15000**	

The mineral concentration of the simulated bathwater applied to the membrane was also determined. This was based on a seaweed:water ratio of 150 g L^{-1}. The theoretical concentration based on the typical commercial bath seaweed:water ratio (***Table 4.2***) was determined and used to establish theoretical% absorption of each mineral *in vivo (**Table 5.2**)*. The % absorbed is generally in line with the Guidance Document on Dermal Absorption (2000) which states that there is an inverse relationship between mineral concentration and percentage absorption. Although there are some exceptions (zinc and lead) results show that a higher applied dose/concentration does not imply higher absorbed concentration nor does it increase the rate of absorption.

Table 5.2 **Theoretical % mineral absorption from *Fucus serratus* L. seaweed bathwater (50 g L^{-1}) over 40 minutes for female (F) and male (M) bathers with exposed surface area of 1.6 m^2 and 1.9 m^2 respectively**

Mineral	Theoretical bathwater concentration (µg L^{-1})	% mineral absorption	
		F	M
Aluminium	5000000	0.04	0.04
Manganese	958333	0.18	0.22
Copper	500000	1.15	1.36
Zinc	366667	0.69	0.82
Nickel	250000	1.67	1.98
Selenium	225000	3.65	4.33
Chromium	58333	2.88	3.41
Lead	33333	0.99	1.17
Molybdenum	33333	14.22	16.88
Cobalt	16667	9.58	11.38

Overall, the preliminary data in this study allowed for further exploration of the dermal absorption of minerals from *Fucus serratus* L. seaweed bathwater. The cell enhancer system proved to be a suitable for exploring mineral transdermal absorption. The macro minerals e.g. sodium, magnesium, potassium, calcium were not tested due to the limited sample volume available. However, from a qualitative perspective, it was shown that all target minerals were absorbed through the membrane and this occurred immediately on immersion of the enhancer cell in the receiver vessel. While this is in line with literature (Miller et al (1989), Wester et al (1991), Ledo (1996), Burks (1998), Sun et al (2002), Hostýnek (2003), EC (2004), Abraham (2005), Guy et al (2005), Larese et al (2006), Larese et al (2007), Leggett (2008), Tateo (2009), Boothman (2010), Jang et al (2016) and Klasson et al (2017)) it has not been previously shown in the context of a seaweed bath.

Although all minerals were absorbed through the membrane, their absorption profiles were not uniform. The minerals exhibited different absorption behaviour patterns and rates of absorption. It is noted that there are *in vivo* factors which could also affect absorption. For instance, there may be cycles of absorption which are dependent on the natural barrier function mechanism in the skin which prevents everything which comes in contact with the skin being absorbed instantaneously. Irrespective of applied concentration, absorption may occur to a point after which further application is redundant until the initial application is metabolised. To that end, the recommendation to refrain from showering post treatment may allow for mineral reservoirs to form under an algin residue which provides an occlusive layer allowing for prolonged exposure and release of minerals over time after bathing. However, this is not indefinite as after some time friction from clothes and movement as well as sweating could reduce the absorption of minerals. While an increased bathing time would lead to increased exposure to and absorption of beneficial minerals it must be noted that this would also lead to increased exposure to and absorption of more hazardous components e.g. cadmium and lead. Further investigation would be required to ensure that bathers are not exposed to harmful, toxic metals.

6 Discussion, Conclusions and Recommendations

This research aimed to address the knowledge gap relative to the mineral constituents of seaweed baths and their health effects on bathers. It is important to note that this is based on one seaweed bath provider (Voya Seaweed Baths, Strandhill, Sligo, Ireland), the specific type of seaweed used at this facility (*Fucus serratus* L.) and its mineral composition. This seaweed bath provider claims that bathing can detoxify the body, renew damaged skin cells, protect against cell damage and aging, improve suppleness and elasticity of the skin, treat dry skin conditions e.g. eczema and lead to a 'measurable uptake' of minerals, in particular iodine. Following a literature review, it was deduced that these claims were based on links, some very tenuous, to associated health benefits of seaweed rather than bathing. Regular clients of Voya Seaweed Baths most commonly cited relaxation as their main reason for having a seaweed bath, rather than a desire to boost health in any particular way. They did not associate bathing with specific health benefits or risks. The research presented has characterised the specific mineral content of seaweed bathwater to which seaweed bath users are exposed and provides evidence to enhance understanding of its significance *in vivo* for health.

The preparation of seawater and simulated bathwater weekly for twelve months provided a substantial collection of samples in order to thoroughly explore mineral behaviour, particularly over time. Both long term storage (-20 ˚C) and repeated freezing and thawing of samples was determined to be adequate and neither affected the concentration of iodine significantly.

The SK micromethod was suitable for iodine determination in all sample types tested, i.e. seawater, bathwater and urine with an intra-assay precision of 2.5% and an inter-assay precision of 6.7%. The high levels of alginate in the matrix, particularly in summer-early autumn do not negatively impact the method. It is essential however that it is carried out in an iodine free environment given that the target concentrations are very low (up to 300 µg L^{-1}). It is also noted that it determines the amount of total iodine in samples, given that the method converts all iodine species present to iodide. This limitation of the method meant that the exact species of iodine in the bathwater was not determined. It was most useful in terms of

its reduced reagent costs; increased throughput of samples generating less waste hence reducing the negative environmental impact compared to the larger scale test tube method.

There was no significant difference in seawater pH ($p = 0.375$) or bathwater pH ($p = 0.461$) however it was shown that the addition of the seaweed *Fucus serratus* L. lowers the pH of the bathwater. Although there is a significant difference in mineral concentration over 12 months the pH does not fluctuate to the same extent which suggests that the mineral content is not the main contributor to its acidity. The mass of seaweed remained constant therefore the stable pH is due to the presence of a stronger over riding element. While alginate content is also documented to be seasonal its prevailing pH is 2-3 therefore, irrespective of season, the bathwater pH remains acidic. The low pH of the bath is particularly suitable for seaweed bath users who may suffer with dry and sensitive skin disorders e.g. eczema which can be attributed to higher skin pH. As healthy skin has a pH of 5.5, many cosmeceutical products are manufactured to alleviate the symptoms of dry skin conditions therefore bathing in an acidic environment may lead to easing the symptoms of dry skin conditions.

The matrix pH does affect the mineral speciation. The species of iodine most likely to be present have been hypothesised however further analysis to determine the species of iodine and indeed other minerals is of interest as some species of minerals are more harmful than others. Also, certain species of an element are more likely to be absorbed through the skin than others e.g. iodine (I_2) can cross the skin whereas iodate (IO_3^-) and iodide (I^-) cannot.

Acid digestion using a microwave was shown to be a suitable digestion method for mineral analysis of samples. With low LODs and LOQs (part per trillion) ICP-MS proved to be a useful technique for mineral determination (up to 100 µg L^{-1}) with an instrument precision of 1.5–3.1%. Simultaneous detection of minerals in one sample in one run provided much more information on the bathwater matrix than other single element analyses. Despite the large quantities of data that can be generated by ICP-MS, in practice this technique is limited by the cost of argon. This cost was also a deterrent when considering methods of analysis for iodine; iodine can be detected using ICP-MS however the cost for a single element analysis was not

practical as an alkaline digestion procedure was also required. Investigation of low levels of arsenic and mercury can be problematic as arsenic can provide false positive results due to interference from argon chloride while mercury can be difficult to determine due to carryover of samples. Also, it is not suitable for detection of minerals at high concentrations (mg L^{-1}) e.g. magnesium, potassium or sodium. While AAS and FES sufficed for this section of analyses, they are slower methods as they only allow for single element detection.

The Enhancer cell technology was a modified version of Franz Diffusion cells which are routinely used in transdermal testing. The method requires specialised glassware yet the method set up is simple and easy to use. Use of the synthetic membrane Cuprophan, which is increasingly used for transdermal absorption testing, was the preferred option as it is more cost effective than human derived membranes with no ethical implications and eliminates *in vivo* variables such as skin age, race, sex and anatomical site. Initially used to establish qualitative transdermal absorption, results were robust enough to quantify the absorption profiles of most minerals. However, unlike Franz Diffusion cells the applied dose is limited, in this case 5 ml, therefore the profile represents a finite dose application which is not always suitable for certain applications.

The concentration of the minerals detected in *Fucus serratus* L. were mostly in line with literature values and accounted for approximately 2% $^{w}/_{w}$ of the mass sampled due to the higher abundance of magnesium, calcium, potassium and sodium. Heavy metals lead, mercury, cadmium and chromium were present at low levels (below 6.2 µg g^{-1}) while macrominerals magnesium, calcium, potassium and sodium were present in much higher concentrations (up to approximately 6.5 mg g^{-1}). The bathwater mineral concentrations increased with increasing mass of seaweed at constant volume. The increase was not linear nor was it constant across all minerals. It is suggested that this is due to specific binding mechanisms of minerals to the algae however further investigation is required as it is beyond the scope of the current study. It was also shown that per gram of seaweed the relative mineral concentrations decrease. This process of biosorption is documented, in particular in the context of water bioremediation where algae is used to 'mop up' contaminants. It is due to the structure of the cell wall which aids the binding and anchoring of

minerals from aqueous solution. The low levels of heavy metals detected in the solid *Fucus serratus* L. sample suggests that their levels in surrounding seawater were low at the time of harvesting.

It has also been shown that increasing temperature leads to a higher efflux of minerals. This is most significant between 20 ˚C and 40 ˚C while there is no significant difference in mineral concentration between 40 ˚C and 80 ˚C. Temperature was identified as a critical parameter for optimum efflux of minerals from algae where increased temperature i.e. above 40 ˚C at constant volume and mass of seaweed leads to higher release.

Despite relatively stable, constant, lower mineral concentrations in seawater, their concentrations in seaweed are significantly higher and this higher concentration varies with time (aluminium p=0.019, cadmium p=0.000, chromium p=0.001, cobalt p=0.008, copper p=0.000, lead p=0.000, manganese p=0.005, molybdenum p=0.000, nickel p=0.00, selenium p=0.004 and zinc p=0.016). In fact, as it was shown that the main contribution of minerals to the bath comes from the seaweed itself, the use of seawater in bath preparation is not necessary to ensure optimum exposure to minerals. The higher concentration of minerals is due to the accumulation (biosorption) of minerals from seawater by algae; however they are not accumulated at the same rate, as they do not appear to have equal affinity for algae. The results also suggest that the algae itself can differentiate between useful and harmful minerals. While in the sea, it acts like a sponge and can potentially contain any chemicals that are present in surrounding water, it can differentiate, to some extent, good from bad so as not to accumulate very high levels of mercury, lead, cadmium etc. This is highlighted for example with chromium and iodine whereby their seawater concentrations are similarly low, 72 and 60 µg L^{-1} respectively, however their concentrations in the seaweed (6.2±1.9 and 1400-2500 µg g^{-1} DW respectively) and subsequently the bathwater (20.04±3.72 µg L^{-1} and 4 mg L^{-1} respectively) vary substantially.

While it has been noted that the pattern of accumulation is similar to the documented pattern of alginate content, the increased alginate occurs at a critical point in the reproductive cycle of the plant where alginate is used to release and carry spores from the parent plant. The simultaneous accumulation of minerals may be attributed

to the same reproductive cycle. Furthermore, iodine levels are lowest in summer but all other minerals (except lead) are highest at this time of the year. It is interesting to note that one third of clients felt that it was of most benefit to use the seaweed baths in winter albeit they did not specify why they felt this way.

While it may be assumed that increased seaweed mass leads to higher concentrations of minerals, results suggest that this is not necessarily the case. In fact, the optimum seaweed: water ratio is 50 g per litre of water. Thereafter, the increased surface area of the algal mass provides an environment for adsorption of minerals from the aqueous matrix. As a typical bath contains approximately 100 litres of water, up to 5 kg of seaweed is sufficient to provide exposure to beneficial minerals. The results also indicate that a bathing temperature above 40 ˚C will also maximise exposure to most minerals. It is important to note also that despite the mineral release into the hot aqueous environment results indicate that most of the minerals are retained within the plant. It is not possible to determine the optimal season for bathing relative to mineral content however as peak concentrations of minerals vary with season.

The aim of the UIC was to establish a change, if any, in UIC profile following a seaweed bath treatment. The UIC of the study population was shown to increase from baseline 76 µg L^{-1} and non-adjusted of 60 µg L^{-1} to 95 µg L^{-1} and non-adjusted of 70 µg L^{-1} respectively (p = 0.015) following exposure to *Fucus serratus* L. seaweed baths. Results indicated that inhalation of iodine vapour may be a significant contributor to increased UIC given its volatility. While iodine uptake via respiration has been investigated previously, it has not been explored in the context of a seaweed bath. *In vitro* testing indicated instant movement of minerals across the membrane. The warm, moist environment (when the skin is more permeable) coupled with the bathwater pH is conducive to dermal absorption. Just as the minerals are not uniform in their release from the algae into the bath, they are not uniform in their absorption from the bath. Unfortunately, it is not possible to state conclusively whether bathers are exposed to harmful levels of heavy metals as the data indicates that mineral levels can reach in excess of their RDA during the a treatment. It is important to note that this is based on theoretical assumptions.

Further *in vivo* studies would confirm the levels of these heavy metals post treatment.

Summary of key findings:

- *Fucus serratus* L. seaweed baths are acidic on addition of seaweed (5.88±0.26)
- This pH affects mineral speciation within the bathwater where molecular iodine predominates under acidic conditions
- The mineral content of the *Fucus serratus* L. used is approximately 2% dry weight
- Mineral release from the seaweed into the bathwater is mass and temperature dependent at constant volume where an increase in both leads to increased bathwater concentration
- Mineral concentration also varies significantly relative to season with a higher concentration of iodine in winter and of other minerals in summer
- The study population was shown to be iodine deficient which is in line with WHO literature
- Inhalation of iodine during a seaweed bath is a major contributor to the increase in UIC
- Aluminium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium and zinc may also be taken up by a bather via dermal absorption

So, why should someone take a seaweed bath and what does it do for the body?

This form of thalassotherapy while in part is a holistic bathing ritual the bathwater itself is an abundant reservoir of algin and minerals. To date, the associated health benefits of bathing have been solely based on the reported composition and positive attributes of seaweeds in general. Aside from the therapeutic value of an hour of relaxation, there is uptake of minerals by a bather during a treatment. It is not suggested that the use of seaweed bath treatments is an efficient mechanism to

supplement minerals it may however be an effective mechanism. Indeed, the more efficient methods include diet or supplements. Moreover, results indicate that most of the minerals are retained within the plant therefore the use of spent seaweed on the soil results in mineral rich soil which in turn benefits the food chain.

Recommendations for further research

Following on from this study there are some key areas which are recommended for further research:

1. Samples are stable and available for further chemical profiling of other minerals and vitamins that are present in order to compile a complete annual profile of the bathwater. This information may be useful to other areas of the seaweed industry which could exploit this seasonality when harvesting in order to maximise use of its benefits. For example, cosmetic companies may require specific ingredients which are more abundant at certain times of the year. Similarly, when used as agricultural feed, in order to ensure nutrient sufficiency for animals, further information on its chemical profile over time is of interest.

2. As mentioned, it is also of interest to establish the species of certain minerals present as some species are more harmful than others. Also, species may differ in their uptake by a bather.

3. Further exploration of the biosorption process i.e. the specific binding mechanisms of minerals to the algae would be of interest as it would be possible to correlate this with the seasonal variation in mineral concentration while examining the potential use of *Fucus serratus* L. in marine bioremediation.

4. Algin is known to aid skin regeneration and hydration. Sodium and calcium alginate have been used in wound dressings to promote skin healing and renewal (Choi et al (1999), Khotimchenko et al (2001)). Although the concentration of algin was not explored specifically, given the seasonal variation in mineral concentration it is deduced that align plays a key role. The use of seaweed baths, particularly in late summer early autumn, therefore could help skin conditions. It is recommended that further analysis of stored samples would allow for the seasonal variation of algin to be determined and correlated with the seasonal variation in mineral concentration.

5. An *in vivo* study comparing commonly used pharmaceutical preparations and seaweed baths in the treatment of eczematic lesions may support the claim that seaweed baths help treat dry skin conditions improving suppleness and elasticity of the skin

6. Even though the enhancer cell technology is a suitable system for exploration of mineral absorption, a larger, controlled *in vivo* study to examine the uptake of more/all minerals would be useful to (i) correlate with *in vitro* results and (ii) confirm the levels of heavy metals absorbed during a treatment

7. Based on the preliminary data from the pilot *in vivo* study a larger more controlled clinical trial, with larger subject numbers, would be of interest in order to establish:

    a. The total iodine vapour concentration released from the seaweed bath
    b. The total iodine vapour inhaled by a subjects (this must exclude any possibility of dermal uptake)

It is also noted following this research that adherence to documenting food intake is critical as diet is a major contributor to UIC. This should be addressed in the ethical approval process and rigorously controlled in terms of diet and hydration. Also, all urine passed should be collected at 2 hour intervals and analysed for total UIC. This type of sampling will also allow for plotting of a UIC profile over the study time.

Bibliography

Abalovich, M. and Glinoer, D. (2009) 'Pregnancy and Hypothyroidism: Autoimmune Thyroiditis and Iodine Deficiency Complicating Mother and Fetus'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Aceves, C. and Anguiano, B. (2009) 'Is Iodine an Antioxidant and Antiproliferative Agent for the Mammary and Prostate Glands?'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Ajjan, R.A., Kamaruddin, N.A., Crisp, M., Watson, P.F., Ludgate, M. and Weetman, A.P. (1998) 'Regulation and tissue distribution of the human sodium iodide symporter gene', *Clinical Endocrinology*, 49, pp. 517–523.

Akomeah, F., Nazir, T., Martin, G.P. and Brown, M. B. (2004) 'Effect of heat on the percutaneous absorption and skin retention of three model penetrants', *European Journal of Pharmaceutical Sciences,* 21 (2–3), pp. 337-345.

Ali-Mohamed, A. Y. and Jamali, S. G. A. (1989) 'Potentiometric determination of fluoride and iodide in natural and processed waters of Bahrain using ion-selective electrodes', *Water Research,* 23 (5), pp. 659-662.

Albalasmeh, A.A., Berhe, A.A. and Ghezzehei, T.A. (2013) 'A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry', *Carbohydrate Polymers*, 97 (2), pp. 253-261.

Andersen, S. and Laurberg, P. (2009) 'The Nature of Iodine in Drinking Water'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Andersson, M., De Benoist, B. and Rogers, L. (2010) 'Epidemiology of iodine deficiency: Salt iodisation and iodine status', *Best Practice & Research Clinical Endocrinology & Metabolism*, 24 (1), pp. 1-11.

Andrade, T.A., Aguiar, A.F., Guedes, F.A., Leite, M.N., Caetano, G.F., Coelho, E.B., Das, P.K. and Frade, M.A. (2015) *'Ex vivo* Model of Human Skin (hOSEC) as

Alternative to Animal use for Cosmetic Tests', *Procedia Engineering*, 110, pp. 67-73.

Antignac, E., Nohynek, G.J., Re, T., Clouzeau, J. and Toutain, H. (2011) 'Safety of botanical ingredients in personal care products/cosmetics', *Food and Chemical Toxicology*, 49 (2), pp. 324-341.

Ares, S., Quero, J. and Morreale De Escobar, G. (2009) 'Iodine Nutrition and Iodine Deficiency in Term and Preterm Newborns: Iodine Nutrition in Newborns'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Baert, B., Boonen, J., Burvenich, C., Roche, N., Stillaert, F., Blondeel, P., Van Bocxlaer, J and De Spiegeleer, B. (2010) 'A New Discriminative Criterion for the Development of Franz Diffusion Tests for Transdermal Pharmaceuticals', *Journal of Pharmacy and Pharmaceutical Science*, 13(2), pp. 218 – 230.

Barry, B. W. (2002) 'Drug delivery routes in skin: a novel approach', *Advanced Drug Delivery Reviews*, 54, Supplement (0), pp. S31-S40.

Bernal, J., Mendiola, J.A., Ibáñez, E. and Cifuentes, A. (2011) 'Advanced analysis of nutraceuticals', *Journal of Pharmaceutical and Biomedical Analysis*, 55 (4), pp. 758-774.

Bhagat, P.R., Acharya, R., Nair, A.G.C., Pandey, A.K., Rajurkar, N.S. and Reddy, A.V.R. (2009) 'Estimation of iodine in food, food products and salt using ENAA',*Food Chemistry*, 115 (2), pp. 706-710.

Black, W.A.P. and Mitchell, R.L. (1952) 'Trace metals in the common brown algae and in seawater', *Journal of the Marine Biological Association of the United Kingdom*, 30, pp. 575-584.

Bocca, B., Pino, A., Alimonti, A. and Forte, G. (2014) 'Toxic metals contained in cosmetics: A status report', *Regulatory Toxicology and Pharmacology*, 68 (3), pp. 447-467.

Bruggeman, V. and Darras, V. (2009) 'Dioxins, PCBs and Iodine: Effects on Synthesis and Metabolism of the Iodine Containing Thyroid Hormones'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Buist, H. E., Van Burgsteden, J. A., Freidig, A. P., Maas, W. J. M. and Van De Sandt, J. J. M. (2010) 'New *in vitro* dermal absorption database and the prediction of dermal absorption under finite conditions for risk assessment purposes', *Regulatory Toxicology and Pharmacology*, 57 (2–3), pp. 200-209.

Bravo, I.D.B, Sanz Castro, R., López Riquelme, N., Tormo Díaz, C. and Apraiz Goyenaga, D. (2007) 'Optimization of the trace element determination by ICP-MS in human blood serum', *Journal of Trace Elements in Medicine and Biology*, 21, Supplement 1(0), pp. 14-17.

Bürgi, H. (2010) 'Iodine excess', *Best Practice & Research Clinical Endocrinology & Metabolism*, 24 (1), pp. 107-115.

Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcão, V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P. and Pinto, E. (2007) 'Metabolites from algae with economical impact', *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146 (1–2), pp. 60-78.

Chandini, S. K., Ganesan, P. and Bhaskar, N. (2008) *'In vitro* antioxidant activities of three selected brown seaweeds of India', *Food Chemistry*, 107 (2), pp. 707-713.

Chattaraj, S.C., Swarbrick, J. and Kanfer, I. (1995) 'A simple diffusion cell to monitor drug release from semi-solid dosage forms', *International Journal of Pharmaceutics*, 120 (1), pp. 119-124.

Cherfi, A., Abdoun, S. and Gaci, O. (2014) 'Food survey: Levels and potential health risks of chromium, lead, zinc and copper content in fruits and vegetables consumed in Algeria', *Food and Chemical Toxicology*, 70 (0), pp. 48-53.

Chetwynd, A.J., Dunn, W.B., Rodriguez-Blanco, G. (2017) 'Collection and Preparation of Clinical Samples for Metabolomics'. *In*: Sussulini, A. (ed)

Metabolomics: From Fundamentals to Clinical Applications. Advances in Experimental Medicine and Biology, Cham: Springer.

Chevallier, E., Chekri, R., Zinck, J., Guérin, T. and Noël, L. (2015) 'Simultaneous determination of 31 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation based on the accuracy profile', *Journal of Food Composition and Analysis,* 41 (0), pp. 35-41.

Coquery, M., Carvalho, F., Sabine, A. and Horvat, M. (1999) *The IAEA worldwide intercomparison exercises (1990-1997): Determination of trace elements in marine sediments and biological samples.*

Creed, J.C., Norton, T.A. and Kain, J.M. (1997) 'Intraspecific competition in *Fucus serratus* germlings: The interaction of light, nutrients and density', *Journal of Experimental Marine Biology and Ecology,* 212 (2), pp. 211-223.

Dembitsky, V.M., Rozentsvet, O.A. and Pechenkina, E.E. (1990) 'Glycolipids, phospholipids and fatty acids of brown algae species', *Phytochemistry,* 29 (11), pp. 3417-3421.

Domínguez-González, R., Romarís-Hortas, V., García-Sartal, C., Moreda-Piñeiro, A., Barciela-Alonso, M.D.C. and Bermejo-Barrera, P. (2010) 'Evaluation of an *in vitro* method to estimate trace elements bioavailability in edible seaweeds', *Talanta,* 82 (5), pp. 1668-1673.

Dyke, J.V., Dasgupta, P.K. and Kirk, A.B. (2009) 'Trace iodine quantitation in biological samples by mass spectrometric methods: The optimum internal standard', *Talanta,* 79 (2), pp. 235-242.

Elias, P.M. and Choi, E.H. (2005) 'Interactions among stratum corneum defensive functions'. *Experimental Dermatology*, 14, pp. 776-776.

Eom, S.H., Kim, Y.M. and Kim, S.K. (2012) 'Antimicrobial effect of phlorotannins from marine brown algae', *Food and Chemical Toxicology,* 50 (9), pp. 3251-3255.

Espín, J.C., García-Conesa, M.T. and Tomás-Barberán, F.A. (2007) 'Nutraceuticals: Facts and fiction', *Phytochemistry,* 68 (22–24), pp. 2986-3008.

Feldstein, M.M., Raigorodskii, I.M., Iordanskii, A.L. and Hadgraft, J. (1998) 'Modelling of percutaneous drug transport *in vitro* using skin-imitating Carbosil membrane', *Journal of Controlled Release,* 52 (1–2), pp. 25-40.

Fields, C. and Borak, J. (2009) 'Iodine Deficiency in Vegetarian and Vegan Diets: Evidence-Based Review of the World's Literature on Iodine Content in Vegetarian Diets'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Fleurence, J. (1999) 'Seaweed proteins: biochemical, nutritional aspects and potential uses', *Trends in Food Science & Technology,* 10 (1), pp. 25-28.

Foley, R. (2014) 'The Roman–Irish Bath: Medical/health history as therapeutic assemblage', *Social Science & Medicine,* 106 (0), pp. 10-19.

Freeland-Graves, J.H., Sanjeevi, N. and Lee, J.J. (2015) 'Global perspectives on trace element requirements', *Journal of Trace Elements in Medicine and Biology,* 31 (0), pp. 135-141.

Gamer, A.O., Leibold, E. and Van Ravenzwaay, B. (2006) 'The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin', *Toxicology in Vitro,* 20 (3), pp. 301-307.

García-Villalba, R., Carrasco-Pancorbo, A., Nevedomskaya, E., Mayboroda, O.A., Deelder, A.M., Segura-Carretero, A. and Fernández-Gutiérrez, A. (2010). 'Exploratory analysis of human urine by LC–ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols', *Analytical and Bioanalytical Chemistry*, 398(1), pp. 463–475.

Geiszinger, A. (2001) 'Arsenic biotransformation by the brown macroalga', *Environmental toxicology and chemistry,* 20 (10), pp. 2255-2262.

Ghersetich, I. and Lotti, T.M. (1996) 'Immunologic aspects: Immunology of mineral water spas', *Clinics in Dermatology,* 14 (6), pp. 563-566.

Gochfeld, M. (2003) 'Cases of mercury exposure, bioavailability, and absorption', *Ecotoxicology and Environmental Safety,* 56 (1), pp. 174-179.

González, A.G., Herrador, M.Á. and Asuero, A.G. (2010) 'Intra-laboratory assessment of method accuracy (trueness and precision) by using validation standards', *Talanta,* 82 (5), pp. 1995-1998.

Gupta, S. and Abu-Ghannam, N. (2011) 'Bioactive potential and possible health effects of edible brown seaweeds', *Trends in Food Science & Technology,* 22 (6), pp. 315-326.

Gustavo González, A. and Ángeles Herrador, M. (2007) 'A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles', *TrAC Trends in Analytical Chemistry,* 26 (3), pp. 227-238.

Haas, E. and Levin, B. (2012) *Staying Healthy with Nutrition, rev: The Complete Guide to Diet and Nutritional Medicine.* United States of America: Potter/TenSpeed/Harmony.

Haldimann, M., Alt, A., Blanc, A. and Blondeau, K. (2005) 'Iodine content of food groups', *Journal of Food Composition and Analysis,* 18 (6), pp. 461-471.

Herrmann, C., FitzGerald, J., O'Shea, R., Xia, A., O'Kiely, P. and Murphy, J.D. (2015) 'Ensiling of seaweed for a seaweed biofuel industry', *Bioresource Technology*, 196, pp. 301–313.

Hetzel, B. S. (2009a) 'Iodine Deficiency and the Brain: An Overview'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine,* San Diego: Academic Press.

Hill, J.R. and Wertz, P.W. (2003) 'Molecular models of the intercellular lipid lamellae from epidermal stratum corneum', *Biochimica et Biophysica Acta (BBA)-Biomembranes,* 1616 (2), pp. 121-126.

Hou, X., Chai, C., Qian, Q., Yan, X. and Fan, X. (1997) 'Determination of chemical species of iodine in some seaweeds ', *Science of The Total Environment*, 204 (3), pp. 215-221.

Hou, X. and Yan, X. (1998) 'Study on the concentration and seasonal variation of inorganic elements in 35 species of marine algae', *Science of The Total Environment*, 222 (3), pp. 141-156.

Hursh, J.B., Clarkson, T.W., Miles, E.F. and Goldsmith, L.A. (1989) 'Percutaneous absorption of mercury vapor by man', *Archives of Environmental Health*, 44, pp. 120–127.

Huynh, D., Zhou, S.J., Gibson, R., Palmer, L. and Muhlhausler, B. (2015) 'Validation of an optimized method for the determination of iodine in human breast milk by inductively coupled plasma mass spectrometry (ICPMS) after tetramethylammonium hydroxide extraction', *Journal of Trace Elements in Medicine and Biology, 29, pp. 75-82.*

Hyde, M.B. (1938) 'Observations on *Fucus serratus* L. kept under laboratory conditions', *Journal of Ecology*, 26 (2), pp. 316-327.

Jacobi, U., Taube, H., Schäfer, U. F., Sterry, W. and Lademann, J. (2005) 'Comparison of four different *in vitro* systems to study the reservoir capacity of the stratum corneum', *Journal of Controlled Release*, 103 (1), pp. 61-71.

Jain, R.B. (2016) 'Trends and variability in the levels of urinary thiocyanate, perchlorate, and nitrate by age, gender, race/ethnicity, smoking status, and exposure to environmental tobacco smoke over 2005–2012', *Science of The Total Environment*, 557–558, pp. 221-230.

Jeon, Y.J., Wijesinghe, W.A.J.P. and Kim, S.K. (2011) Enzyme-assisted Extraction and Recovery of Bioactive Components from Seaweeds. *In*: Kim, S.K. (ed) *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*. Chichester: John Wiley and Sons Ltd.

Jiménez-Escrig, A. and Sánchez-Muniz, F.J. (2000) 'Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism', *Nutrition Research,* 20 (4), pp. 585-598.

Jooste, P. L. (2009) 'Knowledge of Iodine Nutrition'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Josefsson, M. and Ekblad, E. (2009) 'Sodium Iodide Symporter (NIS) in Gastric Mucosa: Gastric Iodide Secretion'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Kadam, S.U., Álvarez, C., Tiwari, B.K. and O'Donnell, C.P. (2015b) 'Extraction of biomolecules from seaweeds', *Seaweed Sustainability.* San Diego: Academic Press.

Katz, U., Shoenfeld, Y., Zakin, V., Sherer, Y. and Sukenik, S. (2012) 'Scientific Evidence of the Therapeutic Effects of Dead Sea Treatments: A Systematic Review', *Seminars in Arthritis and Rheumatism,* 42 (2), pp. 186-200.

Kazandjieva, J., Grozdev, I., Darlenski, R. and Tsankov, N. (2008) 'Climatotherapy of psoriasis', *Clinics in Dermatology,* 26 (5), pp. 477-485.

Keogh, S.M., Aldahan, A., Possnert, G., Finegan, P., León Vintró, L. and Mitchell, P. I. (2007) 'Trends in the spatial and temporal distribution of ^{129}I and ^{99}Tc in coastal waters surrounding Ireland using *Fucus vesiculosus* as a bio-indicator', *Journal of Environmental Radioactivity,* 95 (1), pp. 23-38.

Khotimchenko, S.V. and Titlyanova, T.V. (1996) 'Distribution of an amino acid-containing phospholipid in brown algae', *Phytochemistry,* 41 (6), pp. 1535-1537.

Kim, S.K. and Pangestuti, R. (2011) 'Potential Role of Marine Algae on Female Health, Beauty, and Longevity'. *Advances in Food and Nutrition Research*, 64, pp. 41-55.

Korevaar, T.I.M., Muetzel, R., Medici, M., Chaker, L., Jaddoe, V.W.V., De Rijke, Y.B., Steegers, E.A. P., Visser, T.J., White, T., Tiemeier, H. and Peeters, R.P. (2016) 'Association of maternal thyroid function during early pregnancy with offspring IQ

and brain morphology in childhood: a population-based prospective cohort study', *The Lancet Diabetes & Endocrinology*, 4 (1), pp. 35-43.

Kroner, Z. (2011) *Vitamins and Minerals.* California: ABC-CLIO, LLC

Kumar, D. and Gaur, J.P. (2011) 'Metal biosorption by two cyanobacterial mats in relation to pH, biomass concentration, pretreatment and reuse', *Bioresource Technology*, 102 (3), pp. 2529-2535.

Larrea-Marín, M.T., Pomares-Alfonso, M.S., Gómez-Juaristi, M., Sánchez-Muniz, F.J. and De La Rocha, S. R. (2010) 'Validation of an ICP-OES method for macro and trace element determination in Laminaria and Porphyra seaweeds from four different countries', *Journal of Food Composition and Analysis*, 23 (8), pp. 814-820.

Ledo, E. (1996) 'Mineral water and spas in Spain', *Clinics in Dermatology*, 14 (6), pp. 641-646.

Lee, P.H., Conradi, R. and Shanmugasundaram, V. (2010) 'Development of an *in silico* model for human skin permeation based on a Franz cell skin permeability assay', *Bioorganic & Medicinal Chemistry Letters*, 20 (1), pp. 69-73.

Le Tutour, B., Benslimane, F., and Gouleau, M.P., Gouygou, J.P., Saadan, B. and Quemeneur, F. (1998) 'Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata*, *Himanthalia elongata*, *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum*'. *Journal of Applied Phycology*, 10 (2), pp.121–129.

Lewis, S.W. and Lim, K.F. (2013) 'Spectroscopy: Basic Principles A2-Siegel, Jay A'. *In:* Saukko, P.J. and Houck, M.M. (eds.) *Encyclopedia of Forensic Sciences.* Waltham: Academic Press.

Lightowler, H.J. (2009) 'Assessment of Iodine Intake and Iodine Status in Vegans'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Mæhre, H.K., Malde, M.K., Eilertsen, K.E. and Elvevoll, E.O. (2014) 'Characterization of protein, lipid and mineral contents in common Norwegian

seaweeds and evaluation of their potential as food and feed', *Journal of the Science of Food and Agriculture*, 94 (15), pp. 3281-3290.

Mabeau, S. and Fleurence, J. (1993) 'Seaweed in food products: biochemical and nutritional aspects', *Trends in Food Science & Technology*, 4 (4), pp. 103-107.

Mackerras, D., Singh, G., Davison, B., Ma, G. and Eastman, C.J. (2017) 'Has mandatory fortification with iodine made a difference? Results from two cohort studies in the Darwin area', *Journal of Nutrition and Intermediary Metabolism*, 8, pp. 71-72.

Macours, P., Aubry, J.C., Hauquier, B., Boeynaems, J.M., Goldman, S. and Moreno-Reyes, R. (2008) 'Determination of urinary iodine by inductively coupled plasma mass spectrometry', *Journal of Trace Elements in Medicine and Biology*, 22 (2), pp. 162-165.

Malea, P. and Kevrekidis, T. (2014) 'Trace element patterns in marine macroalgae', *Science of The Total Environment*, 494–495 (0), pp. 144-157.

Marsham, S., Scott, G.W. and Tobin, M.L. (2007) 'Comparison of nutritive chemistry of a range of temperate seaweeds', *Food Chemistry*, 100 (4), pp. 1331-1336.

Martin, M.H., Nickless, G. and Stenner, R.D. (1997) 'Concentrations of cadmium, copper, lead, nickel and zinc in the alga *Fucus serratus* in the Severn estuary from 1971 to 1995', *Chemosphere*, 34 (2), pp. 325-334.

Martín-Cameán, A., Jos, A., Calleja, A., Gil, F., Iglesias-Linares, A., Solano, E. and Cameán, A. M. (2014) 'Development and validation of an inductively coupled plasma mass spectrometry (ICP-MS) method for the determination of cobalt, chromium, copper and nickel in oral mucosa cells', *Microchemical Journal*, 114 (0), pp. 73-79.

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.I. and Lee, Y. C. (2005) 'Carbohydrate analysis by a phenol–sulfuric acid method in microplate format', *Analytical Biochemistry*, 339 (1), pp. 69-72.

Mecozzi, M. (2005) 'Estimation of total carbohydrate amount in environmental samples by the phenol–sulphuric acid method assisted by multivariate calibration', *Chemometrics and Intelligent Laboratory Systems,* 79 (1–2), pp. 84-90.

Melse-Boonstra, A., Gowachirapant, S., Jaiswal, N., Winichagoon, P., Srinivasan, K. and Zimmermann, M.B. (2012) 'Iodine supplementation in pregnancy and its effect on child cognition', *Journal of Trace Elements in Medicine and Biology,* 26 (2–3), pp. 134-136.

Millour, S., Noël, L., Kadar, A., Chekri, R., Vastel, C. and Guérin, T. (2011) 'Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation', *Journal of Food Composition and Analysis,* 24 (1), pp. 111-120.

Momoh, F.U., Boateng, J.S., Richardson, S.C.W., Chowdhry, B.Z. and Mitchell, J.C. (2015) 'Development and functional characterization of alginate dressing as potential protein delivery system for wound healing', *International Journal of Biological Macromolecules,* 81, pp. 137-150.

Moss, G.A. (2010) 'Water and Health: a forgotten connection?', *Perspectives in Public Health* , 130 (5), pp. 227-232.

Müssig, K. (2009) 'Chapter 93-Iodine-Induced Toxic Effects due to Seaweed Consumption'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine,* San Diego: Academic Press.

Nauman, J. and Wolff, J. (1993) 'Iodide Prophylaxis in Poland after the Chernobyl Reactor Accident: Benefits and Risks', *American Journal of Medicine,* 94, pp. 524-532.

Neubert, A. and Remer, T. (1996) 'Measurement of urinary iodine concentration', *The Lancet,* 347 (9012), pp. 1414-1415.

Nimer, M., Ansaloni, J.A. and Silva, M.E. (2009) 'Iodization of Salt: The Control of Iodine Deficiency Disorders in Brazil'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine,* San Diego: Academic Press.

Nóbrega, J.A., Pirola, C., Fialho, L.L., Rota, G., De Campos Jordão, C.E.K.M.A. and Pollo, F. (2012) 'Microwave-assisted digestion of organic samples: How simple can it become?', *Talanta*, 98 (0), pp. 272-276.

O'Sullivan, A. M., O'Callaghan, Y. C., O'Grady, M. N., Queguineur, B., Hanniffy, D., Troy, D. J., Kerry, J. P. and O'Brien, N. M. (2011) *'In vitro* and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland', *Food Chemistry*, 126 (3), pp. 1064-1070.

Pasquet, V., Chérouvrier, J.R., Farhat, F., Thiéry, V., Piot, J.M., Bérard, J.B., Kaas, R., Serive, B., Patrice, T., Cadoret, J.P. and Picot, L. (2011) 'Study on the microalgal pigments extraction process: Performance of microwave assisted extraction', *Process Biochemistry*, 46 (1), pp. 59-67.

Pearce, E., Pino, S., He, X., Bazrafshan, H.R., Lee, S.L. and Braverman, L.E. (2004) 'Sources of Dietary Iodine: Bread, Cows' Milk, and Infant Formula in the Boston Area', *Journal of Clinical Endocrinology and Metabolism*, 89 (7), pp. 3421–3424.

Peinado, I., Girón, J., Koutsidis, G. and Ames, J.M. (2014). 'Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds', *Food Research International*, 66, pp. 36-44.

Pennesi, C., Rindi, F., Totti, C., and Beolchini F. (2015) Marine Macrophytes: Biosorbents. *In*: Kim, S.K. (ed) *Handbook of Marine Biotechnology*. Berlin: Springer.

Provenzano, M.J., Domann, F. and Hefley, L. (2009) 'Cellular Iodine Transport: Body Distribution of the Human Sodium Iodide Symporter'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Rasmussen, L.B., Andersen, S., Ovesen, L. and Laurberg, P. (2009a) 'Iodine Intake and Food Choice'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Rege, P.R., Vilivalam, V.D. and Collins, C.C. (1998) 'Development in release testing of topical dosage forms: use of the Enhancer Cell™ with automated sampling', *Journal of Pharmaceutical and Biomedical Analysis,* 17 (8), pp. 1225-1233.

Richard, D., Kefi, K., Barbe, U., Bausero, P. and Visioli, F. (2008) 'Polyunsaturated fatty acids as antioxidants', *Pharmacological Research,* 57 (6), pp. 451-455.

Rioux, L.E., Turgeon, S.L. and Beaulieu, M. (2007) 'Characterization of polysaccharides extracted from brown seaweeds', *Carbohydrate Polymers,* 69 (3), pp. 530-537.

Romarís-Hortas, V., Moreda-Piñeiro, A. and Bermejo-Barrera, P. (2009) 'Microwave assisted extraction of iodine and bromine from edible seaweed for inductively coupled plasma-mass spectrometry determination', *Talanta,* 79 (3), pp. 947-952.

Routh, H.B., Bhowmik, K.R., Parish, L.C. and Witkowski, J.A. (1996) 'Balneology, mineral water, and spas in historical perspective', *Clinics in Dermatology,* 14 (6), pp. 551-554.

Sarkar, A. and Paul, B. (2016) 'The global menace of arsenic and its conventional remediation-A critical review', *Chemosphere,* 158 pp. 37-49.

Sertori, T. (2008) *Vitamins and Minerals*. United States of America: Marshall Cavendish

Smith, K.I., Douce, R. and Harwood, J.L. (1982) 'Phospholipid metabolism in the brown alga, *Fucus serratus'*, *Phytochemistry,* 21 (3), pp. 569-573.

Sparr, E. and Wennerström, H. (2001) 'Responding Phospholipid Membranes—Interplay between Hydration and Permeability', *Biophysical Journal,* 81 (2), pp. 1014-1028.

Spitzweg, C. and Morris, J.C. (2009) 'The Sodium–Iodide Symporter (NIS): NIS-Mediated Radioiodide Therapy'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Stengel, D.B., Connan, S. and Popper, Z.A. (2011) 'Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application', *Biotechnology Advances*, 29 (5), pp. 483-501.

Stengel, D.B., Macken, A., Morrison, L. and Morley, N. (2004) 'Zinc concentrations in marine macroalgae and a lichen from western Ireland in relation to phylogenetic grouping, habitat and morphology', *Marine Pollution Bulletin*, 48 (9–10), pp. 902-909.

Sukenik, S., Flusser, D. and Abu-Shakra, M. (1999) 'the role of spa therapy in various rheumatic diseases', *Rheumatic Disease Clinics of North America*, 25 (4), pp. 883-897.

Tagami, K. and Uchida, S. (2006) 'Concentrations of chlorine, bromine and iodine in Japanese rivers', *Chemosphere*, 65 (11), pp. 2358-2365

Thomas, N.V. and Kim, S.K. (2011) 'Potential pharmacological applications of polyphenolic derivatives from marine brown algae', *Environmental Toxicology and Pharmacology*, 32 (3), pp. 325-335.

Trumbo, P., Yates, A.A., Schlicker, S. and Poos, M. (2001) 'Dietary Reference Intakes', *Journal of the American Dietetic Association*, 101 (3), pp. 294-301.

Trumpff, C., De Schepper, J., Tafforeau, J., Van Oyen, H., Vanderfaeillie, J. and Vandevijvere, S. (2013) 'Mild iodine deficiency in pregnancy in Europe and its consequences for cognitive and psychomotor development of children: A review', *Journal of Trace Elements in Medicine and Biology*, 27 (3), pp. 174-183.

Van Zyl, L., Du Preez, J., Gerber, M., Du Plessis, J. and Viljoen, J. (2016) 'Essential Fatty Acids as Transdermal Penetration Enhancers', *Journal of Pharmaceutical Sciences*, 105 (1), pp. 188-193.

Venter, J.P., Müller, D.G., Du Plessis, J. and Goosen, C. (2001) 'A comparative study of an in situ adapted diffusion cell and an in vitro Franz diffusion cell method for transdermal absorption of doxylamine', *European Journal of Pharmaceutical Sciences*, 13 (2), pp. 169-177.

Watson, S. (2010) *Vitamins and Minerals: Getting the Nutrients Your Body Needs.* New York: The Rosen Publishing Group

Yamamoto, T., Otsuka, Y., Okazaki, M., and Okamoto, K. (1979) 'The distribution of chemical elements in algae' *Marine algae in pharmaceutical science,* pp. 569–607

Yang, B., Patsavas, M.C., Byrne, R.H. and Ma, J. (2014) 'Seawater pH measurements in the field: A DIY photometer with 0.01 unit pH accuracy', *Marine Chemistry,* 160, pp. 75-81.

Yun, A.J. and Doux, J.D. (2009) 'Iodine in the Ecosystem: An Overview'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Zhang, R., Ma, H., Li, H., Xu, J., Zhang, J., Zhang, Y. and Wang, Q. (2013) 'An improved candidate reference method for serum potassium measurement by inductively coupled plasma-mass spectrometry', *Clinica Chimica Acta*, 420 (0), pp. 146-149.

Zimmermann, M.B. (2008) 'Iodine requirements and the risks and benefits of correcting iodine deficiency in populations', *Journal of Trace Elements in Medicine and Biology*, 22 (2), pp. 81-92.

Zimmermann, M.B., Jooste, P.L. and Pandav, C.S. 'Iodine-deficiency disorders', *The Lancet*, 372 (9645), pp. 1251-1262.

Zvyagintseva, T.N., Shevchenko, N.M., Popivnich, I.B., Isakov, V.V., Scobun, A.S., Sundukova, E.V. and Elyakova, L.A. (1999) 'A new procedure for the separation of water-soluble polysaccharides from brown seaweeds', *Carbohydrate Research*, 322 (1–2), pp. 32-39.

References

Abdel-Aty, A.M., Ammar, N.S., Abdel Ghafar, H.H. and Ali, R.K. (2013) 'Biosorption of cadmium and lead from aqueous solution by fresh water alga Anabaena sphaerica biomass'. *Journal of Advanced Research,* 4 (4), pp. 367-374.

Abdel-Ghani, N.T. and El-Chaghaby, G.A. (2014) 'Biosorption for metal ions removal from aqueous solutions: a review of recent studies', *International Journal of Latest Research in Science and Technology,* 3 (1), pp. 24-42.

Abreu, M.H., Pereira, R. and Sassi, J.F. (2014) 'Marine Algae and the Global Food Industry'. *In*: Pereira, L. and Magalhaes Neto, J. (eds.) *Marine Algae: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology.* Florida: CRC Press.

Adams, J.M.M. (2016) 'A European and personal perspective on seaweed uses: historical, present and future', Available at http://www.nrn-lcee.ac.uk/documents/Adams2016seaweedresources_P16-P21only.pdf, Last accessed Last accessed 28 November 2017.

Agatonovic-Kustrin, S. and Morton, D.W. (2013) 'Cosmeceuticals derived from bioactive substances found in marine algae'. *Oceanography,* 1 (2), pp. 106–117.

Agency for Toxic Substances and Disease Registry (A.T.S.D.R.) Division of Toxicology. (2004). 'Public Health Statement: Iodine'. Available at http://www.atsdr.cdc.gov/ToxProfiles/tp158-c1-b.pdf, Accessed 22 November 2017.

Agilent Technologies, Inc. (2013) 'Agilent Enhancer Cell Operator's Manual' California: Agilent Technologies, Inc.

Agilent Technologies, Inc. (2016) 'Agilent 240/280 Series AA User's Guide'. Australia: Agilent Technologies Australia [M] Pty Ltd.

Agrawal, O., Sunita, G. and Gupta, V.K. (1999) 'A sensitive colorimetric method for the micro determination of iodine in marine water'. *Talanta,* 49 (4), pp. 923-928.

Ahad, F. and Ganie, S.A. (2010) 'Iodine, Iodine metabolism and Iodine deficiency disorders revisited'. *Indian Journal of Endocrinology and Metabolism*. 14 (1), pp. 13-17.

Ahmady-Asbchin, S., Tabaraki, R., Jafari, N., Allahverdi, A. and Azhdehakoshpour, A. (2013) 'Study of nickel and copper biosorption on brown algae Sargassum angustifolium: application of response surface methodology (RSM)'. *Environmental Technology*, 34 (16), pp. 2423-2431.

Albèr, C., Buraczewska-Norin, I., Kocherbitov, V., Saleem, S., Lodén, M. and Engblom, J. (2014) 'Effects of water activity and low molecular weight humectants on skin permeability and hydration dynamics – a double-blind, randomized and controlled study'. *International Journal of Cosmetic Science*, 36, pp. 412–418.

AlgaeBase. (2017) *Ascophyllum nodosum* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Chondrus crispus* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Fucus serratus* L. [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Fucus serratus* L. frond [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Gelidium sesquipedale* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Gigartina pistillata* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Himanthalia elongata* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Laminaria* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Mastocarpus stellatus* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Porphyra sp.* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) Seaweed holdfast [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Ulva lactuca* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

AlgaeBase. (2017) *Undaria pinnatifida* [jpeg], Available at: http://www.algaebase.org, Last accessed 28 November 2017

Allured Publishing. (2013) Diagram of the epidermis [jpeg]. Available at: http://www.thecosmeticchemist.com/education/skin_science/epidermis.html, [Last accessed 28 November 2017]

Anderson, J.J.B. and Garner, S.C. (2011) 'Calcium and bone'. *In*: Anderson, J.J.B., Garner, S.C. and Klemmer, P.J. (eds.) *Diet, Nutrients, and Bone Health*. Florida: CRC Press.

Araya, M., Olivares, M. and Pizarro, F. (2007) 'Copper in human health'. *International Journal of Environment and Health*, 1, pp. 608–620.

Armisen, R. (1995) 'World-wide use and importance of *Gracilaria*'. *Journal of Applied Phycology*, 7, pp. 231–243.

Balina, K., Romagnoli, F. and Blumberga, D. (2016) 'Chemical Composition and Potential Use of *Fucus vesiculosus* from Gulf of Riga', *Energy Procedia*, 95, pp. 43-49.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L. (2005) 'Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements'. *Environmental Health Perspectives*, 113(2), pp.192-200.

Battaglia, V., Compagnone, A., Bandino, A., Bragadin, M., Rossi, C. A., Zanetti, F., Colombatto, S., Grillo, M. A. and Toninello, A. (2009) 'Cobalt induces oxidative stress in isolated liver mitochondria responsible for permeability transition and intrinsic apoptosis in hepatocyte primary cultures', *The International Journal of Biochemistry & Cell Biology*, 41 (3), pp. 586-594.

Bergé, J.P., Debiton, E., Dumay, J., Durand, P. and Barthomeuf, C. (2002) '*In vitro* anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga *Porphyridium cruentum'*. *Journal of Agriculture and Food Chemistry*, 50 (21), pp. 6227-6232.

Bhanot, D. (2014) *Component diagram of AAS* [jpeg]. Available at: http://lab-training.com/2014/11/28/launch-certificate-program-atomic-absorption-spectroscopy/ [Last accessed 28 November 2017]

Błażewicz, A. (2012) 'A Review of Spectrophotometric and Chromatographic Methods and Sample Preparation Procedures for Determination of Iodine in Miscellaneous Matrices'. *In*: Uddin, J. (ed.) *Macro To Nano Spectroscopy*. Croatia:InTech.

Bocanegra, A., Bastida, S., Bened, J., Rodenas, S., and Sanchez-Muniz, F. J. (2009) 'Characteristics and nutritional and cardiovascular-health properties of seaweeds', *Journal of Medicinal Food*, 12 (2), pp. 236-258.

Boothman, S. (2009) 'Iodine White Paper: The Use of Iodine in Wound Therapy'. Available at: https://www.yumpu.com/en/document/view/27008550/iodine-white-paper-systagenix, Last accessed 10 November 2017.

Bornhorst, J., Ebert, F., Hartwig, A., Michalke, B. and Schwerdtle, T. (2010) 'Manganese inhibits poly(ADP-ribosyl)ation in human cells: a possible mechanism behind manganese induced toxicity?'. Journal of Environmental Monitoring, 12, pp. 2062–2069.

Borowitzka, M.A. (2013) 'High-value products from microalgae-their development and commercialisation'. Journal of Applied Phycology, 25, pp. 743-756.

Bravo, I.D.B, Sanz Castro, R., López Riquelme, N., Tormo Díaz, C. and Apraiz Goyenaga, D. (2007) 'Optimization of the trace element determination by ICP-MS in human blood serum'. *Journal of Trace Elements in Medicine and Biology*, 21, Supplement 1 (0), pp. 14-17.

Bravo-Linares, C.M., Mudge, S.M. and Loyola-Sepulveda, R.H. (2010) Intertidal profile showing the relative position of the macroalgae collected for the experiments

(tidal range 10 m). [jpeg]. ‘Production of volatile organic compounds (VOCs) by temperate macroalgae: the use of solid phase microextraction (SPME) coupled to GC-MS as method of analysis’, p. 228.

Brownlee, I.A., Seal, C.J., Wilcox, M., Dettmar, P.W. and Pearson, J.P. (2009) ‘Applications of Alginates in Food’, A*lginates: Biology and Applications*, 13, pp. 211-228.

Bruchertseifer, H., Cripps, R., Guentay, S. and Jaeckel, B. (2003) ‘Analysis of iodine in aqueous solutions’. *Analytical and Bioanalytical Chemistry*. 375 (8), pp. 1107-1110.

Brudecki, K., Szufa, K. and Mietelsk, J. W. (2017) ‘^{131}I age-dependent inhalation dose in Southern Poland from Fukushima accident’, *Radiation and Environmental Biophysics,* 56, pp. 9–17.

Bruneton, J. (1995) ‘Pharmacognosy, phytochemistry, medicinal plants’. Paris: Lavoisier.

Burks, R.I. (1998) ‘Povidone-Iodine Solution in Wound Treatment’, *Physical Therapy*, 78 (2), pp. 212-218.

Cabrita, A.R.J., Maia, M.R.G., Oliveira, H.M., Sousa-Pinto, I., Almeida, A.A., Pinto, E. and Fonseca, A.J.M. (2016) ‘Tracing seaweeds as mineral sources for farm-animals’, Journal of Applied Phycology, 28, pp. 3135–3150.

Caldwell, K.L., Maxwell, B., Makhmudov, A., Pino, S., Braverman, L.E., Jones, R.L., and Hollowell, J.G. (2003) ‘Use of Inductively Coupled Plasma Mass Spectrometry to Measure Urinary Iodine in NHANES 2000: Comparison with Previous Method’ *Clinical Chemistry,* 49 (6), pp. 1019-1021.

Caroli, S., Forte, G. and Iamiceli, A.L. (1999) ‘ICP-AES and ICP-MS Quantification of Trace Elements in the Marine Macroalga *Fucus* sample, a New Candidate Certified Reference Material’. *Microchemical Journal,* 62 (2), pp. 244-250.

Chance, R., Baker, A.R., Küpper, F.C., Hughes, C., Kloareg, B. and Malin, G. (2009) ‘Release and transformations of inorganic iodine by marine macroalgae’. *Estuarine, Coastal and Shelf Science,* 82 (3), pp. 406-414.

Cheng, Z., Zheng, Y., Mortlock, R. and van Geen, A. (2004) 'Rapid multi-element analysis of groundwater by high-resolution inductively coupled plasma mass spectrometry', *Analytical and Bioanalytical Chemistry*, 379, pp. 512–518.

Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H. and Nam, Y.S. (1999) 'Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge', *Biomaterials*, 20 (5), pp. 409-417.

Civantos D.P., Lopez Rodriguez, A., Aguado-Borruey, J.M., and Narvaez, J.A. (1995) 'Fulminant malignant arrythmia and multiorgan failure in acute arsenic poisoning'. *Chest*, 108, pp. 1774–1775.

Coquery, M., Carvalho, F.P., Horvat, M., and Azemard, S. (1997). 'World-wide intercomparison exercise for the determination of trace elements in *Fucus* sample IAEA-140 Report no 64'. International Atomic Energy Agency (IAEA), pp. 93

Daniell, J.W., Chappell, W.E. and Couch, H. B. (1969) 'Effect of Sublethal and Lethal Temperatures on Plant Cells', *Plant Physiology*, 44, pp. 1684-1689.

Davis, T. A., Volesky, B. and Mucci, A. (2003) 'A review of the biochemistry of heavy metal biosorption by brown algae'. *Water Research*, 37 (18), pp. 4311-4330.

Dawczynski, C., Schubert, R. and Jahreis, G. (2007) 'Amino acids, fatty acids, and dietary fibre in edible seaweed products'. *Food Chemistry*, 103, pp. 891–899.

De Benoist, B., McLean, E., Andersson, M. and Rogers L. (2008). 'Iodine deficiency in 2007: Global progress since 1993'. *Food and Nutrition Bulletin*, 29 (3), pp. 195-202.

Delange, F. (1998) 'Risks and benefits of iodine supplementation'. *The Lancet*, 351 (9107), pp. 923-924.

Demers, L.M. and Spencer, C.A. (2002) 'NACB: Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease'. National Academy of Clinical Biochemistry, Available at: https://www.aacc.org/science-and-practice/practice-guidelines/thyroid-disease. Last accessed 20 November 2017.

Dessanges, J.F. (2001) ‘A History of Nebulization’. *Journal of Aerosol Medicine,* 14 (1), pp. 65–71.

Dhargalkar, V.K. and Pereira, N. (2005) ‘Seaweeds: Promising plants of the millennium’. *Science and Culture,* 7 (3-4), pp. 60-66.

Diembeck, W., Beck, H., Benech-Kieffer, F., Courtellemont, P., Dupuis, J., Lovell, W., Paye, M., Spengler, J. and Steiling, W. (1999) ‘Test guidelines for in vitro assessment of dermal absorption and percutaneous penetration of cosmetic ingredients’, *Food and Chemical Toxicology,* 37, pp. 191-205.

Dietitians of Canada (2011) ‘Food sources of Calcium’, Available at https://www.healthstandnutrition.com/wp-content/uploads/2011/09/NEW-Food-Sources-of-Calcium.pdf, Last accessed 28 November 2017.

Dunn, J.T., Crutchfield, H.E., Gutekunst, R. and Dunn, A.D. (1993) ‘Two simple methods for measuring iodine in urine’. *Thyroid*, 3 (2), pp. 119-123.

Edwards, M., Hanniffy, D., Heesch, S., Hernandez-Kantun, J., Moniz, M., Queguineur, B., Ratcliff, J., Soler-Vila, A. and Wan, A. (2012) ‘Macroalgae Fact-sheets’, Available at http://www.seaweed.ie/irish_seaweed_contacts/doc/FactSheets.pdf, Last accessed 10 November 2017.

European Food Safety Authority (E.F.S.A.) (2009) ‘Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food’. *EFSA Journal* 980, pp. 1-139.

Environmental Monitoring Systems (1996) ‘Method 200.8:Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry’. *Methods for the Determination of Metals in Environmental Samples.* New Jersey: William Andrew Publishing.

Erikson, K.M., Thompson, K., Aschner, J. and Aschner, M. (2007) ‘Manganese neurotoxicity: a focus on the neonate’. *Pharmacology and Therapeutics,* 113, pp. 369–377.

European Commission (E.C.) (2004) ‘Guidance Document on Dermal Absorption’. Available at: https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_tox_dermal-absorp-2004.pdf, Last accessed 28 November 2017

Evans, F.D. and Critchley, A.T. (2014) ‘Seaweeds for animal production use’. *Journal of Applied Phycology*. 26 (2), pp. 891-899.

Farooq, U., Kozinski, J.A., Khan, M.A. and Athar, M. (2010) ‘Biosorption of heavy metal ions using wheat based biosorbents – A review of the recent literature’. *Bioresource Technology,* 101 (14), pp. 5043-5053.

Fernández-Sánchez, L.M., Bermejo-Barrera, P., Fraga-Bermudez, J.M., Szpunar, J. and Lobinski, R. (2007) ‘Determination of iodine in human milk and infant formulas’. *Journal of Trace Elements in Medicine and Biology,* 21 (1), pp. 10-13.

Field, T., Diego, M. and Hernandez-Reif, M. (2007) ‘Massage therapy research’. *Developmental Review,* 27 (1), pp. 75-89.

Flechas, J. (2011) ‘Total Body Iodine Sufficiency’, *Restorative Medicine.* Portland Oregon. Available at: http://articles.mercola.com/sites/articles/archive/2013/05/04/iodine-deficiency-affect-childs-brain-function.aspx, Last accessed 10 November 2017.

Food Safety Authority of Ireland (F.S.A.I.) (2009) ‘Mercury, Lead, Cadmium, Tin and Arsenic in Food’. Ireland: Food Safety Authority of Ireland.

Fuge, R. and Johnson, C.C. (1986) ‘The geochemistry of iodine-a review’. *Environmental Geochemistry and Health*. 8, pp. 31-54.

Galasso, C., Corinaldesi, C. and Sansone, C. (2017) ‘Carotenoids from Marine Organisms: Biological Functions and Industrial Applications’, *Antioxidants,* 6 (96), pp. 1-33.

Gall, E.A, Küpper, F.C. and Kloareg, B. (2004) ‘A survey of iodine content in *Laminaria digitata*’, *Botanica Marina,* 47, pp. 30–37.

Gao, K. and Mc Kinley, K. (1994) ‘Use of macroalgae for marine biomass production and CO_2 remediation: a review’. Journal of Applied Phycology, 6, pp. 45-60.

Gartrell, M.J., Craun, J.C., Podrebarac, D.S. and Gunderson, E.L. (1986a) ‘Pesticides, selected elements, and other chemicals in adult total diet samples, October 1980–March 1982’. *Journal of the Association of Official Analytical Chemists*, 69, pp. 146–159.

Gartrell, M.J., Craun, J.C., Podrebarac, D.S. and Gunderson, E.L. (1986b) ‘Pesticides, selected elements, and other chemicals in infant and toddler total diet samples, October 1980–March 1982’. *Journal of the Association of Official Analytical Chemists*, 69, pp. 123–145.

Gashu, D. and Stoecker, B.J. (2017) ‘Selenium and Cognition: Mechanism and Evidence’. *In*: Preedy, V.R. and Patel, V.B. (eds.) *Handbook of Famine, Starvation, and Nutrient Deprivation: From Biology to Policy*. Switzerland: Springer International Publishing.

Ghosh, R., Banerjee, K., and Mitra, A. (2012) ‘Seaweeds in the Lower Gangetic Delta’. *In*: Kim, S.K. (ed.), *Handbook of marine macroalgae: biotechnology and applied phycology*. New Jersey: John Wiley and Sons.

Ginneken, V., Helsper, J., de Visser, W., van Keulen, H. and Brandenburg, W.A. (2011) ‘Polyunsaturated fatty acids in various macroalgal species from north Atlantic and subtropical seas’. *Lipids in health and disease*, 10 (104), pp. 1-8.

Goldsmith, L.A. (1983) ‘*Biochemistry and Physiology of the Skin’*. Oxford: Oxford University Press.

González Bermúdez, Y., Rodríguez Rico, I.L., Guibal, E., Calero De Hoces, M. and Martín-Lara, M.Á. (2012) ‘Biosorption of hexavalent chromium from aqueous solution by Sargassum muticum brown alga. Application of statistical design for process optimization’. *Chemical Engineering Journal*, 183 pp. 68-76.

Goullé, J.-P., Mahieu, L., Castermant, J., Neveu, N., Bonneau, L., Lainé, G., Bouige, D. and Lacroix, C. (2005) 'Metal and metalloid multi-elementary ICP-MS validation

in whole blood, plasma, urine and hair: Reference values', *Forensic Science International*, 153 (1), pp. 39-44.

Guiry, M.D. (2012) 'How many species of algae are there?'. *Journal of Phycology*, 48, pp. 1057–1063.

Guiry, M.D. (2017) 'The Seaweed Site: information on marine algae'. Available at: http://www.seaweed.ie/, Last accessed 29 November 2017

Gulaboglu, M., Akgul, H.M., Akgul, N. and Cetin, M. (2012) 'Urine and saliva iodine levels in patients with dental caries and normal healthy volunteers'. *Trace Elements & Electrolytes.* 29 (1), pp. 28-33.

Gupta, U.C. and Gupta, S.C. (2014) 'Sources and Deficiency Diseases of Mineral Nutrients in Human Health and Nutrition: A Review'. *Pedosphere*, 24 (1), pp. 13-38.

Guy, R.H., Hostýnek, J.J., Hinz, R.S. and Lorence, C.R. (1999) *Metals and the Skin: Topical Effects and Systemic Absorption.* New York: Marcel Dekker.

Haas, E.M. (2000) 'Role of potassium in maintaining health', Available at http://hkpp.org/patients/potassiumhealth, Last accessed 28 November 2017.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D. and Klein, R.Z. (1999) 'Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child', *The New England Journal of Medicine*, 341 (8), pp. 549-555.

Haddow, J.E., McClain, M.R., Palomaki, G.E. and Hollowell, J.G. (2007) 'Urine Iodine Measurements, Creatinine Adjustment, and Thyroid Deficiency in an Adult United States Population'. *Journal of Clinical Endocrinology & Metabolism.* 92 (3), pp. 1019–1022.

Harris, E.D. (2001) 'Copper homeostasis: the role of cellular transporters'. *Nutrition Reviews*, 59, pp. 281-285.

Harris, D.C. (2007) Absorption of light increases the energy of a molecule. Emission of light decreases its energy. [pdf] *Quantitative Chemical Analysis* 7th edition, pp. 380.

Harris, D.C. (2007) Atomic absorption experiment. [pdf] *Quantitative Chemical Analysis* 7th edition, pp. 455.

Harris, D.C. (2007) Quantitative Chemical Analysis. New York: W. H. Freeman.

Haslam, S.F.I. and Hopkins, D.W. (1996) 'Physical and biological effects of kelp (seaweed) added to soil'. *Applied Soil Ecology.* 3 (3), pp. 257-261.

Haswell, S.J. (ed.). (1991). *Atomic absorption spectrometry*. Netherlands: Elsevier.

He, J. and Chen, J.P. (2014) 'A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modelling simulation tools', *Bioresource Technology*, 160, pp. 67-78.

Heitland, P. and Köster, H.D. (2006) 'Biomonitoring of 30 trace elements in urine of children and adults by ICP-MS'. *Clinica Chimica Acta*, 365 (1–2), pp. 310-318.

Holdt, S.L. and Kraan, S. (2011) 'Bioactive compounds in seaweed: Functional food applications and legislation'. *Journal of Applied Phycology.* 23, pp. 543–597.

Hostýnek, J.J. (2003) 'Factors determining percutaneous metal absorption'. *Food and Chemical Toxicology,* 41 (3), pp. 327-345.

Huggins, C.E., O'Reilly, S., Brinkman, M., Hodge, A., Giles, G.G., English, D.R. and Newson, C.A. (2011) 'Relationship of urinary sodium and sodium-to-potassium ratio to blood pressure in older adults in Australia'. *Medical Journal of Australia*, 195, pp. 128–132.

Hurd, C.L., Harrison, P.J., Bischof, K. and Lobban, C.S. (2014) 'Seaweed Ecology and Physiology'. Cambridge: Cambridge University Press.

Hussain, H. and Wan Mohamud, W.N. (2006) 'A cost-effective modified micromethod for measuring urine iodine'. *Tropical Biomedicine.* 23 (1), pp. 109–115.

Ibrahim, W.M. (2011) ‘Biosorption of heavy metal ions from aqueous solution by red macroalgae’, *Journal of Hazardous Materials*, 192 (3), pp. 1827-1835.

Institute of Medicine (I.O.M.) (2010) ‘Dietary Reference Intakes for Calcium and Vitamin D’. Washington DC: National Academy Press.

Institute of Medicine (I.O.M.) (2010) ‘Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc’. Washington DC: National Academy Press.

International Council for Harmonisation (I.C.H.) (1996) ‘Harmonised Tripartite Guideline Q2 (R1): Validation of Analytical Procedures: Text and Methodology’. Geneva: International Council for Harmonisation.

Iozzo, R.V. (2005) ‘Basement membrane proteoglycans: from cellar to ceiling’. *Nature Reviews Molecular Cell Biology*, 6, pp. 646-656.

Ito, K. and Hirokawa, T. (2009) ‘Iodine and Iodine Species in Seawater: Speciation, Distribution, and Dynamics’. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Jang, Y., Somanna, Y. and Kim, H. (2016) ‘Source, Distribution, Toxicity and Remediation of Arsenic in the Environment – A review’. *International Journal of Applied Environmental Sciences.* 11 (2), pp. 559-581.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R. and Saura-Calixto, F. (2001) ‘Antioxidant activity of fresh and processed edible seaweeds’. *Journal of the Science of Food and Agriculture*, 81 (5), pp. 530-534.

Jooste, P. L. and Strydom, E. (2010) 'Methods for determination of iodine in urine and salt'. *Best Practice & Research Clinical Endocrinology & Metabolism*, 24 (1), pp. 77-88.

Kerala (2015) ‘Seaweed Baths’, Available at http://www.keralamassage.ie/seaweed_baths.html, Last accessed 10 November 2017.

Khazan, M., Azizi, F. and Hedayati, M. (2013) ‘A Review on Iodine Determination Methods in Salt and Biological Samples’, *International Journal of Medical Sciences.* 1 (1), pp. 1-9.

Khotimchenko, S.V. (1998) ‘Fatty acids of brown algae from the Russian far east’. *Phytochemistry,* 49 (8), pp. 2363-2369.

Khotimchenko, Y.S., Kovalev, V.V, Savchenko, O.V. and Ziganshina, O.A. (2001) ‘Physical–chemical properties, physiological activity, and usage of alginates, the polysaccharides of brown algae’, *Russian Journal of Marine Biology,* 27 (6), pp. S53-S64.

Kilcullens Seaweed Baths (2017) ‘The Benefits of Seaweed Baths’ Available at https://www.kilcullenseaweedbaths.net/about, Last accessed 10 November 2017.

Kim, M.K., Dubacq, J.P., Thomas, J.C. and Giraud, G. (1996) ‘Seasonal variations of triacylglycerols and fatty acids in *Fucus serratus’. Phytochemistry,* 43 (1), pp. 49-55.

Kim, S.K., Ravichandran, Y.D., Khan S.B. and Kim, Y.T. (2008) ‘Prospective of the cosmeceuticals derived from marine organisms’. *Biotechnology and Bioprocess Engineering,* 13, pp. 511-523.

Kira, C.S., Sakuma, A.M. and da Cruz Gouveia, N. (2014) ‘Fast and simple multi-element determination of essential and toxic metals in whole blood with quadrupole ICP-MS’, *Journal of Applied Pharmaceutical Science*, 4 (5), pp. 39-45.

Kiuru, P., D'Auria, M. V., Muller, C. D., Tammela, P., Vuorela, H. and Yli-Kauhaluoma, J. (2014) ‘Exploring marine resources for bioactive compounds’. *Planta Med,* 80 (14), pp. 1234-1246.

Klasson, M., Lindberg, M., Bryngelsson, I.L., Arvidsson, H., Pettersson, C., Husby, B. and Westberg, H. (2017) ‘Biological monitoring of dermal and air exposure to cobalt at a Swedish hard metal production plant: does dermal exposure contribute to uptake?’ *Contact Dermatitis,* 77 (4), pp. 201–207.

Knudsen, N., Christiansen, E., Brandt-Christensen, M., Nygaard, B. and Perrild, H. (2000) ‘Age- and sex-adjusted iodine/creatinine ratio. A new standard in

epidemiological surveys? Evaluation of three different estimates of iodine excretion based on casual urine samples and comparison to 24 h values'. *European Journal of Clinical Nutrition*, 54(4), pp. 361-363.

Konno, N., Yuri, K., Miura, K., Ku,agai, M. and Murakami, S. (1993) 'Clinical Evaluation of the lodide/Creatinine Ratio of Casual Urine Samples as an Index of Daily Iodide Excretion in a Population Study'. Endocrine Journal, 40(1), pp.163-169

Krajčovičová-Kudláčková, M., Bucková, K., Klimes, I. and Seboková, E. (2003) 'Iodine deficiency in vegetarians and vegans'. *Annals of Nutrition and Metabolism*. 47 (5), pp. 183-185.

Kregar, M.L., Dürrigl, M., Rožman, A., Jelčić, Ž., Cetina-Čižmek, B. and Filipović-Grćić, J. (2015) 'Development and validation of an in vitro release method for topical particulate delivery systems'. International Journal of Pharmaceutics. 485 (1-2), pp. 202-214.

Kubota, S., Endo, Y. and Kubota, M. (2013) 'Effect of upper torso inclination in Fowler's position on autonomic cardiovascular regulation'. *Journal of Physiological Sciences*, 63, pp. 369-376.

Küpper, H., Küpper, F. and Spiller, M. (1998)'*In situ* detection of heavy metal substituted chlorophylls in water plants', *Photosynthesis Research*, 58, pp. 125–133.??

Küpper, F.C., Schweigert, N., Ar Gall, E. Legendre, J.M., Vilter, H. and Kloareg, B. (1998) 'Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide'. *Planta*, 207 (2), pp. 163-171.

Küpper, F.C., Carpenter, L.J., McFiggans, G.B., Palmer, C.J., Waite, T.J., Boneberg, E.-M., and Feiters, M.C. (2008) 'Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry'. *Proceedings of the National Academy of Sciences of the United States of America*, 105 (19), pp. 6954–6958.

Lademann, J., Richter, H., Teichmann, A., Otberg, N., Blume-Peytavi, U., Luengo, J., Weiß, B., Schaefer, U. F., Lehr, C.M., Wepf, R. and Sterry, W. (2007)

'Nanoparticles – An efficient carrier for drug delivery into the hair follicles', *European Journal of Pharmaceutics and Biopharmaceutics,* 66 (2), pp. 159-164.

Lagerström, M.E., Field, M.P., Séguret, M., Fischer, L., Hann, S. and Sherrell, R.M. (2013) 'Automated on-line flow-injection ICP-MS determination of trace metals (Mn, Fe, Co, Ni, Cu and Zn) in open ocean seawater: Application to the GEOTRACES program'. *Marine Chemistry*, 155 (0), pp. 71-80.

Larese, F.F., Boeniger, M., Maina, G., Adami, G., Spinelli, P. and Damian, A. (2006) 'Skin absorption of inorganic lead (PbO) and the effect of skin cleansers', *Journal of Occupational and Environmental Medicine,* 48 (7), pp. 692-699.

Larese, F., Gianpietro, A., Venier, M., Maina, G. and Renzi, N. (2007) '*In vitro* percutaneous absorption of metal compounds'. *Toxicology Letters,* 170 (1), pp. 49-56.

Lee, K.Y. and Mooney, D.J. (2012) 'Alginate: properties and biomedical applications', *Progress in Polymer Science*, 37 (1), pp.106-126.

Leenane Hotel (2017) 'Connemara Seaweed Baths', Available at http://leenanehotel.com/connemara-seaweed-baths/, Last accessed 10 November 2017.

Leggett R.W. (2008) 'The biokinetics of inorganic cobalt in the human body'. *Science of the Total Environment*, 389, pp. 259-269.

Leyton, A., Pezoa-Conte, R., Barriga, A., Buschmann, A.H., Mäki-Arvela, P., Mikkola, J.P. and Lienqueo, M.E. (2016) 'Identification and efficient extraction method of phlorotannins from the brown seaweed *Macrocystis pyrifera* using an orthogonal experimental design', *Algal Research,* 16 (Supplement C), pp. 201-208.

Lewington, A. (2003) Plants for People. Cornwall: Eden Project Books.

Li, H.B., Xu, X.R. and Chen, F. (2009) 'Determination of Iodine in Seawater: Methods and Applications'. *In*: Watson, E.b.R.P.N.B. (ed) *Comprehensive Handbook of Iodine*, San Diego: Academic Press.

Li. C., Peng, S., Zhang, X., Xie, X., Wang, D., Mao, J., Teng, X., Shan, Z. and Teng, W. (2016) 'The Urine Iodine to Creatinine as an Optimal Index of Iodine During Pregnancy in an Iodine Adequate Area in China'. *The Journal of Clinical Endocrinology & Metabolism*, 101(3), pp. 1290–1298.

Lidén, C. and Johnsson, S. (2001) 'Nickel on the Swedish market before the nickel directive'. *Contact Dermatitis*, 44, pp. 7–12.

Lim, K.F. and Lewis, S.W. (2013) 'Spectroscopic Techniques'. *In*: Siegel, J. A. and Saukko, P. J. (eds) *Encyclopedia of Forensic Sciences*, Australia: Academic Press.

Linn High Therm Gmbh. (2017) Plasma torch. [jpeg]. Available at: https://www.linn-high-therm.de/products/details/pid/icp-generator.html, Last accessed 29 November 2017

Makkar, H.P.S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. and Ankers, P. (2015) 'Seaweeds for livestock diets: A review', *Animal Feed Science and Technology*, 212, pp. 1-17.

Malea, P., Chatziapostolou, A. and Kevrekidis, T. (2015) 'Trace element seasonality in marine macroalgae of different functional-form groups', *Marine Environmental Research*, 103 (0), pp. 18-26.

Marinho-Soriano, E., Fonseca, P.C., Carneiro, M.A.A. and Moreira, W.S.C. (2006) 'Seasonal variation in the chemical composition of two tropical seaweeds'. *Bioresource Technology*. 97 (18), pp. 2402-2406.

Markou, K., Georgopoulos, N., Kyriazopoulou, V. and Vagenakis, A.G. (2001) 'Iodine-Induced hypothyroidism'. *Thyroid*, 11 (5), pp. 501-510.

Mata, Y.N., Blázquez, M.L., Ballester, A., Gonzalez , F. and Muñoz, J.A. (2008) 'Characterization of the biosorption of cadmium, lead and copper with the brown alga *Fucus vesiculosus* ', *Journal of Hazardous Materials*, 158, pp. 316–323.

Matanjun, P., Mohamed, S., Mustapha, N. M., and Muhammad, K. (2009) 'Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*'. *Journal of Applied Phycology*, 21, pp. 75-80.

May, W., Wu, D., Eastman, C., Bourdoux, P and Maberly, G., (1990) 'Evaluation of automated urinary iodine methods: problems of interfering substances identified'. *Clinical Chemistry*, 36 (6), pp. 865-869.

May, S.L., May, W.A., Bourdoux, P.P., Pino, S., Sullivan, K.M., and Maberly, G.F. (1997) 'Validation of a simple, manual urinary iodine method for estimating the prevalence of iodine-deficiency disorders, and interlaboratory comparison with other methods', *American Journal of Clinical Nutrition,* 65, pp. 1441-1445.

Mc Gill, C.R., Fulgoni, V.L., Di Rienzo, D., Huth, P.J., Kurilich, A.C. and Miller, G.D. (2008) 'Contribution of dairy products to dietary potassium intake in the United States population'. *Journal of the American College of Nutrition*, 27, pp. 44–50.

Mc Hugh, D. (2003) 'A guide to the seaweed industry', Rome: Food and Agriculture Organization of the United Nations.

Menon, G.K. (2015) 'Skin Basics; Structure and Function'. *In:* Pappas, A. (ed) *Lipids and Skin Health.* Switzerland: Springer International Publishing.

Merck. (2017) ICPMS Overview [jpeg]. Available at: http://www.merckmillipore.com/IE/en/water-purification/learning-centers/applications/inorganic-analysis/icp-ms/_e2b.qB.s7QAAAFAniQQWTtN,nav?ReferrerURL=https%3A%2F%2Fwww.google.ie%2F&bd=1, [Last accessed 28 November 2017]

Mertz, W. (1993) 'Chromium in human nutrition: a review'. *Journal of Nutrition*, 123, pp. 626-633.

Mescher, A.L. (2016) Skin architecture. [gif] *Junqueira's Basic Histology: Text and Atlas*.14th edition, pp.372.

Milestone S.r.l. (2001) 'ETHOS D User Manual' Italy: Milestone S.r.l.

Miller, K.L., Coen, P.E., White, W.J., Hurst, W. J., Achey, B.E. and Max Lang, C. (1989) 'Effectiveness of skin absorption of tincture of I in blocking radioiodine from the human thyroid gland'. *Health Physics,* 56 (6), pp. 911-914.

Milne, A., Landing, W., Bizimis, M. and Morton, P. (2010) 'Determination of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in seawater using high resolution magnetic sector inductively coupled mass spectrometry (HR-ICP-MS)', *Analytica Chimica Acta,*665 (2), pp. 200-207.

Mina, A., Favaloro, E. J. and Koutts, J. (2011) 'A robust method for testing urinary iodine using a microtitre robotic system'. *Journal of Trace Elements in Medicine and Biology*. 25 (4), pp. 213-217.

Mišurcová, L., (2012) 'Chemical composition of seaweeds'. *In*: Kim, S.K. (ed.), *Handbook of marine macroalgae: biotechnology and applied phycology*. New Jersey: John Wiley and Sons.

Mohamed, S., Hashim, S.N. and Rahman, H.A. (2012) 'Seaweeds: A sustainable functional food for complementary and alternative therapy'. *Trends in Food Science and Technology,* 23 (2), pp. 83-96.

Moreda-Piñeiro, A., Peña-Vásquez, E., Bermejo-Barrera, P., (2012) 'Significance of the presence of trace and ultratrace elements in seaweeds'. *In*: Kim, S.K. (ed.), *Handbook of marine macroalgae: biotechnology and applied phycology*. New Jersey: John Wiley and Sons.

Morgan, A., Morgan, D.J. and Black, A. (1968) 'A Study of the Deposition, Translocation and Excretion of Radioiodine Inhaled As Iodine Vapour'. *Health Physics.* 15, pp. 313-322.

Morrissey, J., Kraan, S. and Guiry, M.D. (2001) Codium fragile. [jpeg] 'A guide to commercially important seaweeds on the Irish coast', p.61.

Moses, S.W., David, M., Goldhammer, E., Tal, A. and Sukenik, S. (2006) 'The Dead Sea, a Unique Natural Health Resort'. *Israel Medical Association Journal*, 8, pp. 483-488.

Mostellar, R.D. (1987) 'Simplified calculation of body-surface area', *New England Journal of Medicine,* 317 (17), pp. 1098.

Mouritsen, O.G., Dawczynski, C., Duelund, L., Jahreis, G., Vetter, W. and Schröder, M. (2013) 'On the human consumption of the red seaweed Dulse (*Palmaria palmata* (L.)', *Journal of Applied Phycology*, 25 (6), pp. 1777-1791.

Muramatsu, Y. and Hans Wedepohl, K. (1998) 'The distribution of iodine in the earth's crust'. *Chemical Geology*. 147 (3–4), pp. 201-216.

Murata, M. and Nakazoe, J. (2001) 'Production and use of marine algae in Japan'. *Japan Agricultural Research Quarterly*, 35, pp. 281-290.

Murray, J.W. (2000) 'The Oceans'. *International Geophysics*, 72, pp. 230-278.

Narayan, B., Miyashita, K. and Hosakawa, M. (2006) 'Physiological Effects of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA)—A Review', *Food Reviews International*, 22 (3), pp. 291-307.

Ng, S.F., Rouse, J.J., Sanderson, F.D., Meidan, V., and Eccleston, GM. (2010) 'Validation of a Static Franz Diffusion Cell System for *In Vitro* Permeation Studies'. *American Association of Pharmaceutical Scientists*, 11 (3), pp. 1432-1441.

Nishide, E., Tsukayarna, K., Uchida, N. and Nisizawa, K. (1984) 'Isolation of water-soluble alginate from brown algae', *Hydrobiologia*, 116 (1), pp. 557-562.

Nishizawa, K. (2002) *Seaweeds Kaiso: Bountiful Harvest From The Sea: Sustenance For Health and Well-Being by preventing common life-style related diseases*. Japan: Japan Seaweed Association.

Nitschke, U. and Stengel, D.B. (2015) 'A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products'. *Food Chemistry*, 172, pp. 326-334.

Nolan, L.A., Windle, R.J., Wood, S.A., Kershaw, Y.M., Lunness, H.R., Lightman, S.L., Ingram, C.D. and Levy, A. (2000) 'Chronic iodine deprivation attenuates stress-induced and diurnal variation in corticosterone secretion in female Wistar rats'. *Journal of Neuroendocrinology*. 12, pp. 1149-1159.

Ohashi, T., Yamaki, M., Pandav, C.S., Karmarkar, M.G. and Minoru, I., (2000) 'Simple Microplate Method for Determination of Urinary Iodine'. *Clinical Chemistry*. 46 (4), pp. 529–536.

Ortega, R., Bresson, C., Fraysse, A., Sandre, C., Devès, G., Gombert, C., Tabarant, M., Bleuet, P., Seznec, H., Simionovici, A., Moretto, P. and Moulin, C. (2009) 'Cobalt distribution in keratinocyte cells indicates nuclear and perinuclear accumulation and interaction with magnesium and zinc homeostasis', *Toxicology Letters*, 188 (1), pp. 26-32.

Otberg, N., Richter, H, Schaefer, H., Blume-Peytavi, U., Sterry, W. and Lademann, J. (2004) 'Variations of hair follicle size and distribution in different body sites'. *Journal of Investigative Dermatology*, 122 (1), pp. 14-19.

Patti, F., Jeanmaire, L., Masson, M., Pinton, P. and Garcet M. (1990) 'Temporal variations of iodine^{129}, potassium^{40} and technetium^{99} concentrations in *Fucus serratus* in the English Channel'. *Journal of Radioanalytical and Nuclear Chemistry*, 142 (2), pp. 467-480.

Pérez-López, F.R., Brincat, M., Erel, C.T., Tremollieres, F., Gambacciani, M., Lambrinoudaki, I., Moen, M.H., Schenck-Gustafsson, K., Vujovic, S., Rozenberg, S. and Rees, M. (2012) 'EMAS position statement: Vitamin D and postmenopausal health'. *Maturitas*, 71, pp. 83–88.

Pesando, D. and Caram, B. (1984) 'Screening of marine algae from the French Mediterranean coast for antibacterial and antifungal activity'. *Botany*, 27 (8), pp. 3281-3386.

Pillai, S., Cornell, M. and Oresajo, C. (2009) 'Epidermal Barrier'. *In*: Draelos, Z.D. (ed) *Cosmetic Dermatology*. Oxford: Wiley-Blackwell.

Pilson, M.E.Q. (1998) *An Introduction to the Chemistry of the Sea.* Cambridge: Cambridge University Press.

Polat, S. and Ozogul, Y. (2009) 'Fatty acid, mineral and proximate composition of some seaweeds from the northeastern Mediterranean coast'. *Italian Journal of Food Science*, 21 (3), pp. 317-324.

Potts, P.J. (2014) 'Principles of Atomic Spectroscopy' *In:* Turekian, K.K. (ed.) *Treatise on Geochemistry (Second Edition).* Oxford: Elsevier.

Prasad, A.S. (2009) ‘Zinc: role in immunity, oxidative stress and chronic inflammation’. *Current Opinion in Clinical Nutrition and Metabolic Care,* 12 (6), pp. 646-652.

Prasad, A.S. (2014) ‘Impact of the discovery of human zinc deficiency on health’. *Journal of Trace Elements in Medicine and Biology,* 28 (4), pp. 357-363.

Rangan, A.M. and Samman, S. (2007) ‘Zinc intake and its dietary sources: results of the 2007 Australian national children’s nutrition and physical activity survey’. *Nutrients,* 4, pp. 611–624.

Rendl, J., Bier, D. and Reiners, C. (1998) ‘Methods for measuring iodine in urine and serum’. *Journal of Experimental and Clinical Endocrinology and Diabetes.* 106 (4), pp. 34-41.

Research and Markets (2016) ‘Commercial Seaweeds Market-Global Forecasts to 2021 by Type (Red, Brown, Green), Form (Liquid, Powdered, Flakes), Application (Agriculture, Animal Feed, Human Food, and Others), and by Region’. Illinois: Markets and Markets.

Risher, J.F. and Keith, L.S. (2009) Iodine and inorganic iodides: human health aspects. Geneva: World Health Organization.

Ródenas de la Rocha, S., Sánchez-Muniz, F.J., Gómez-Juaristi, M. and Marín, M.T.L. (2009) ‘Trace elements determination in edible seaweeds by an optimized and validated ICP-MS method’. *Journal of Food Composition and Analysis.* 22 (4), pp. 330-336.

Romera, E., González, F., Ballester, A., Blázquez, M.L. and Muñoz, J.A. (2007) ‘Comparative study of biosorption of heavy metals using different types of algae’, *Bioresource Technology,* 98, pp. 3344-3353.

Rosell, K.G. and Srivastava, L. (1984) ‘Seasonal variation in the chemical constituents of the brown algae *Macrocystis integrifolia* and *Nereocystis luetkeana*’, Canadian Journal of Botany, 62, pp. 2229-2236.

Routh, H. B. and Bhowmik, K. R. (1996) 'A glossary of concepts relating to balneology, mineral water, and the spa'. *Clinics in Dermatology,* 14 (6), pp. 549-550.

Rude, R.K. (2010) Magnesium. *In*: Coates, P.M., Betz, J.M., Blackman, M.R., Cragg, G.M., Levine, M., Moss, J., White, J.D. (eds) *Encyclopedia of Dietary Supplements*. New York: Informa Healthcare.

Rude, R.K. (2012) Magnesium. *In*: Ross, A.C., Caballero, B., Cousins, R.J., Tucker, K.L., Ziegler, T.R. (eds) *Modern Nutrition in Health and Disease.* Baltimore: Lippincott, Williams and Wilkins.

Rupérez, P. (2002) 'Mineral content of edible marine seaweeds'. *Food Chemistry,* 79 (1), pp. 23-26.

Rupérez, P., Ahrazem, O. and Leal, J. A. (2002) 'Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus'. Journal of Agriculture and Food Chemistry*, 50 (4), pp. 840-845.

Sánchez-Machado, D.I., López-Cervantes, J., López-Hernández, J. and Paseiro-Losada, P. (2004) 'Fatty acids, total lipid, protein and ash contents of processed edible seaweeds'. *Food Chemistry,* 85 (3), pp. 439-444.

Sandell, E.B. and Kolthoff, I.M. (1937) 'Micro Determination of Iodine by a Catalytic Method', *Microchimica Acta*, 1, pp. 9-25.

Satyanarayanan, M., Balaram, V., Rao, T.G., Dasaram, B., Ramesh, S.L., Mathur, R. and Drolia, R.K. (2007) 'Determination of trace metals in seawater by ICP-MS after preconcentration and matrix separation by dithiocarbamate complexes', *Indian Journal of Marine Sciences*, 36 (1), pp. 71-75.

Schafer, U. and Anke, M. (2005) 'Is there cause for concern about the nutritional manganese supply?' *Journal of Elementology*, 10, pp. 1025 –1034.

Schurer, N.Y. and Elias, P.M. (1991) 'The biochemistry and function of stratum corneum lipids'. *Advances in Lipid Research*, 24, pp. 27-56.

Seghetta, M., Romeo, D., D'Este, M., Alvarado-Morales, M., Angelidaki, I., Bastianoni, S. and Thomsen, M. (2017) 'Seaweed as innovative feedstock for energy and feed-evaluating the impacts through a Life Cycle Assessment'. *Journal of Cleaner Production*. 150, pp. 1-15.

Sezer, A.D., Cevher, E., Hatipoğlu, F., Oğurtan, Z., Baş, A.L. and Akbuğa, J. (2008) 'Preparation of fucoidan-chitosan hydrogel and its application as burn healing accelerator on rabbits'. *Biological and Pharmaceutical Bulletin*, 31 (12), pp. 2326-2333.

Shelor, C.P. and Dasgupta, P.K. (2011) 'Review of analytical methods for the quantification of iodine in complex matrices'. *Analytica Chimica Acta,* 702 (1), pp. 16-36.

Sherwood Scientific Ltd. (2010) 'Model 360 Flame Photometer Operator Manual'. United Kingdom: Sherwood Scientific Ltd.

Silva, C.L., Topgaard, D., Kocherbitov, V., Sousa, J.J.S., Pais, A.A.C.C. and Sparr, E. (2007) 'Stratum corneum hydration: Phase transformations and mobility in stratum corneum, extracted lipids and isolated corneocytes'. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1768 (11), pp. 2647-2659.

Simonsen, L. O., Brown, A. M., Harbak, H., Kristensen, B. I. and Bennekou, P. (2011) 'Cobalt uptake and binding in human red blood cells', *Blood Cells, Molecules, and Diseases,* 46 (4), pp. 266-276.

Skulas-Ray, A.C., West, S.G., Davidson, M.H., and Kris-Etherton, P.M. (2008) 'Omega-3 fatty acid concentrates in the treatment of moderate hypertriglyceridemia'. *Expert Opinion on Pharmacotherapy*, 9 (7), pp. 1237-1248.

Smyth, P.A. (2003) 'Role of iodine in antioxidant defense in thyroid and breast disease'. *Biofactors*. 19, pp. 121–130.

Smyth, P.P., Burns, R., Huang, R.J., Hoffman, T., Mullan, K., Graham, U., Seitz, K., Platt, U. and O'Dowd, C. (2011) 'Does iodine gas released from seaweed contribute to dietary iodine intake?'. *Environmental Geochemistry and Health*, 33 (4), pp. 389-97.

Smyth, P., Burns, R., Casey, M., Mullan, K., O'Herlihy, C. and O'Dowd, C. (2016) 'Iodine Status over Two Decades: Influence of Seaweed Exposure'. *Irish Medical Journal*. 109 (6), pp. 422.

Soldin, O.P. (2002) ‘Controversies in urinary iodine determinations’. *Clinical Biochemistry.* 35 (8), pp. 575-579.

Sparr, E. and Wennerström, H. (2001) ‘Responding Phospholipid Membranes—Interplay between Hydration and Permeability’. *Biophysical Journal,* 81 (2), pp. 1014-1028.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006) ‘Commercial applications of microalgae’. *Journal of Bioscience and Bioengineering,* 101 (2), pp. 87-96.

Stipanuk, M.H. and Caudill, M.A. (2013) ‘*Biochemical, Physiological, and Molecular Aspects of Human Nutrition*’. Philadelphia: Saunders Publishing.

Stanford University (2015) ‘Mineral Makeup of Seawater’, Available at https://web.stanford.edu/group/Urchin/mineral.html, Last accessed 22 November 2017.

Sulaymon, A.H., Mohammed, A.A. and Al-Musawi, T.J. (2013) ‘Competitive biosorption of lead, cadmium, copper and arsenic ions using algae’, *Environmental Science and Pollution Research*, 20, pp. 3011–3023.

Sun, C.C., Wong, T.T., Hwang, Y.H., Chao, K.Y., Jee, S.H. and Wang, J.D. (2002) ‘Percutaneous absorption of inorganic lead compounds’, *American Industrial Hygiene Association*, 63 (5), pp. 641–646.

Sunde, R.A. (2012) Selenium. *In*: Ross, A.C., Caballero, B., Cousins, R.J., Tucker, K.L., Ziegler, T.R. (eds) *Modern Nutrition in Health and Disease.* Philadelphia: Lippincott, Williams and Wilkins.

Szpunar, J., Bettmer, J., Robert, M., Chassaigne, H., Cammann, K., Lobinski, R. and Donard, O. F. X. (1997a) 'Validation of the determination of copper and zinc in blood plasma and urine by ICP MS with cross-flow and direct injection nebulization', *Talanta*, 44(8), pp. 1389-1396.

Tagami, K. and Uchida, S. (2006) 'Concentrations of chlorine, bromine and iodine in Japanese rivers', *Chemosphere*, 65(11), pp. 2358-2365.

Tateo, F., Ravaglioli, A., Andreoli, C., Bonina, F., Coiro, V., Degetto, S., Giaretta, A., Menconi Orsini, A., Puglia, C. and Summa, V. (2009) 'The *in-vitro* percutaneous migration of chemical elements from a thermal mud for healing use'. *Applied Clay Science*, 44 (1–2), pp. 83-94.

Teas, J., Hurley, T.G., Herbert, J.R., Franke, A.A., Sepkovic, D.W. and Kurzer, M.S. (2009) 'Dietary seaweed modifies estrogen and phytoestrogen metabolism in healthy postmenopausal women'. *Journal of Nutrition*, 139 (5), pp. 939-944.

Thavarajah, D., Thavarajah, P., Sarker, A., Materne, M., Vandemark, G. and Shrestha, R.A. (2011) 'A global survey of effects of genotype and environment on selenium concentration in lentils (*Lens culinaris* L.): Implications for nutritional fortification strategies'. *Food Chemistry*, 125, pp. 72–76.

Thomas, R. (2013) 'Practical Guide to ICP-MS: A Tutorial for Beginners', Florida: CRC Press.

Tiwari, B.K. and Troy, D.J. (2015) 'Seaweed sustainability – food and nonfood applications'. *Seaweed Sustainability.* San Diego: Academic Press.

Truesdale, V.W. (2008) 'The biogeochemical effect of seaweeds upon close-to natural concentrations of dissolved iodate and iodide in seawater – Preliminary study with *Laminaria digitata* and *Fucus serratus*'. *Estuarine, Coastal and Shelf Science,* 78 (1), pp. 155-165.

Truus, K. and Vaher, M. and Taure, I. (2001) 'Algal biomass from *Fucus vesiculosus* (Phaeophyta): Investigation of the mineral and alginate components', Proceeding of the Estonian Academy of Sciences, 50 (2), pp. 95–103.

Underwood, E.J. (1977) *Trace elements in human and animal nutrition*. New York: Academic Press.

Uriu-Adams, J.Y. and Keen, C.L. (2005) 'Copper, oxidative stress and human health'. *Molecular Aspects of Medicine,* 26, pp. 268–298.

U.S. Food and Drug Administration (U.S.F.D.A.) (2009) 'Guidance for industry: A food labelling guide'. Maryland: Food and Drug Administration.

US National Library of Medicine (NLM) Pubchem Open Chemistry Database Compound summary (2018) *Sodium Alginate* [png], Available at: https://pubchem.ncbi.nlm.nih.gov/compound/5102882#section=Top, Last accessed 20 September 2018

Van Netten, C., Hoption Cann, S.A., Morley, D.R. and van Netten, J.P. (2000) 'Elemental and radioactive analysis of commercially available seaweed'. *Science of The Total Environment*, 255 (1–3), pp. 169-175.

Vanegas, C.H. and Bartlett, J. (2013) 'Green energy from marine algae: biogas production and composition from the anaerobic digestion of Irish seaweed species'. *Environmental Technology*, 34 (15), pp. 2277-2283.

Varian (2005) 'Varian 810-MS and 820-MS ICP Mass Spectrometers', Available at http://chemistry.unt.edu/~verbeck/LIMS/Manuals/820_Specifications.pdf, Last accessed 22 November 2017.

Varian Inc. (2006) 'VK 7000/7010 Dissolution Apparatus Operator's Manual'. North Carolina: Varian Inc.

Vassileva, S. (1996) 'Mineral water and spas in Bulgaria'. *Clinics in Dermatology*, 14 (6), pp. 601-605.

Vincent, J.B. (2003) 'The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent'. *Sports Medicine*, 33, pp. 213-230.

Voya Seaweed Baths (2017) 'Health Benefits of Seaweed', Available at http://www.voyaseaweedbaths.com/seaweed-baths/health-benefits/, Last accessed 22 November 2017.

Walsh, M. and Watson., L. (2011) 'Part 1: A market analysis towards the further development of seaweed aquaculture in Ireland'. Bord Iascaigh Mhara. pp. 1-50.

Wang, H.M.D., Chen, C.C., Huynh, P. and Chang, J.S. (2015) 'Exploring the potential of using algae in cosmetics', Bioresource Technology, 184, pp. 355-362.

Water World (2016) ‘Bundoran Seaweed Baths: The Benefits’, Available at http://www.waterworldbundoran.com/seaweedbaths/, Last accessed 10 November 2017.

Weerheim, A. and Ponec, M. (2001) ‘Determination of stratum corneum lipid profile by tape stripping in combination with high-performance thin-lay chromatography’, *Archives of Dermatological Research,* 293, pp. 191-199.

Wester, R.C., Maibach, H.I., Sepik, L., Melendres, J., DiZio, S. and Wade, M. (1992) ‘*In vitro* percutaneous absorption of cadmium from water and soil into human skin’, *Fundamental and Applied Toxicology,* 19, pp. 1-5.

Westport Woods Hotel (2017) ‘Seaweed Bath’, Available at https://www.westportwoodshotel.com/Features.html, Last accessed 10 November 2017.

Wijesinghe, W.A.J.P. and Jeon, Y.J. (2012) ‘Enzyme-assistant extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: A review’. *Fitoterapia,* 83 (1), pp. 6-12.

Wong, C. (2012) ‘Chromium picolinate side effects’, Available at http://altmedicine.about.com/od/herbsupplementguide/a/chromiumsideeff.htm, Last accessed 28 November 2017.

World Health Organisation (WHO) (2001) ‘Arsenic and arsenic compounds’. Geneva: World Health Organization.

World Health Organisation (WHO) (2003) ‘Aluminium in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality’. Geneva: World Health Organization.

World Health Organisation (WHO) (2003) ‘Elemental mercury and inorganic mercury compounds: human health aspects’. Geneva: World Health Organization.

World Health Organisation (WHO) (2003) ‘Lead in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality’. Geneva: World Health Organization.

World Health Organisation (WHO) (2004) ‘Iodine status worldwide WHO Global Database on Iodine Deficiency’. Geneva: World Health Organization.

World Health Organisation (WHO) (2006), ‘Environmental Health Criteria 235 Dermal Absorption’. Geneva: World Health Organization.

World Health Organisation (WHO) (2007) ‘Vitamin and Mineral Nutrition Information System (VMNIS), Global Database on Iodine Deficiency’. Geneva: World Health Organization.

World Health Organisation (WHO) (2013) ‘Urinary iodine concentrations for determining iodine status in populations: VMNIS | Vitamin and Mineral Nutrition Information System’. Geneva: World Health Organization.

Wu, G. (2013) ‘Amino Acids: Biochemistry and Nutrition’. Florida: CRC Press.

Yang, L., Willie, S. and Sturgeon, R.E. (2009) ‘Determination of total chromium in seawater with isotope dilution sector field ICP-MS following on-line matrix separation’, Journal of Analytical Atomic Spectrometry, 24, pp. 958–963.

Yeats Country Hotel Eros Spa (2017) ‘World Famous Seaweed Baths & Quality Irish Seaweed Products’, Available at https://www.yeatscountryhotel.com/seaweed-bath.html, Last accessed 10 November 2017.

Zanella, L., Pertile, P., Massironi, M. and Caviola, E. (2014). *Extracts of Microalgae and Their Application.* Google Patents US 13/883.

Zava, T.T., Kapur, S. and Zava, T.D. (2013) ‘Iodine and creatinine testing in urine dried on filter paper’. *Analytica Chimica Acta*. 974, pp. 64-69.

Zheng, J., Takata, H., Tagami, K., Aono, T., Fujita, K. and Uchida, S. (2012) ‘Rapid determination of total iodine in Japanese coastal seawater using SF-ICP-MS’. *Microchemical Journal,* 100 (0), pp. 42-47.

Zimmermann, M.B. (2010) ‘Iodine deficiency in industrialised countries’. *Proceedings of the Nutrition Society*, 69, pp. 133–143.

Appendix A List of figures

Figure 3.20 Average monthly aluminium concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as p = 0.981. Significant seasonal variation in simulated bathwater as p = 0.019

Figure 3.21a Average monthly cadmium concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as p = 0.000

Figure 3.21b Average monthly cadmium concentration of seawater (µg L^{-1}) indicating no significant seasonal variation as p = 0.003

Figure 3.22a Average monthly chromium concentration of seawater and simulated bathwater (µg L^{-1}) indicating significant seasonal variation as p = 0.001

Figure 3.22b Average monthly chromium concentration of seawater (µg L^{-1}) indicating no significant seasonal variation as p = 0.257

Figure 3.23 Average monthly cobalt concentration of seawater and simulated bathwater (µg L^{-1}). Significant seasonal variation in seawater as p = 0.000. Significant seasonal variation in simulated bathwater as p = 0.008

Figure 3.24a Average monthly copper concentration of simulated bathwater (µg L^{-1}) indicating significant seasonal variation as p = 0.000

Figure 3.24b Average monthly copper concentration of seawater (µg L^{-1}) indicating significant seasonal variation in seawater as p = 0.003

Figure 3.25 Average monthly lead concentration of seawater and simulated bathwater (µg L^{-1}). No significant seasonal variation in seawater as p = 0.528. Significant seasonal variation in simulated bathwater as p = 0.000

Appendix B List of tables

(50 g L^{-1}) over 40 minutes for female (F) and male (M) bathers with exposed surface area of 1.6 m^2 and 1.9 m^2 respectively

Table 5.2 Theoretical *%* mineral absorption from *Fucus serratus* L. seaweed bathwater (50 g L^{-1}) over 40 minutes for female (F) and male (M) bathers with exposed surface area of 1.6 m^2 and 1.9 m^2 respectively

Appendix C Client Questionnaire

Client Questionnaire **Voya Seaweed Baths**			
Please the appropriate answer			
1	**First Time Client**	**Please continue to question 9**	
2	**Regular Client**		
	If regular, how often do you use the seaweed baths?		
2a	**Weekly**		
2b	**Monthly**		
2c	**Annually**		
		Yes	**No**
3	**Is there a particular time of year when you get more benefit from taking a seaweed bath?**		**Please continue to question 4**
3a	**If so,**	**When**	**Why**
4	**Is there a particular time of year when you prefer to have a seaweed bath?**		**Please continue to question 5**
4a	**If so,**	**When**	**Why**

		Yes	**No**
5	**Do you notice any changes in the seaweed *Fucus serratus* L. used in the seaweed baths during the year?**		**Please continue to question 6**
If yes,			
5a	**How would you describe the colour of *Fucus serratus* L. at different times of the year (Spring/Summer/Autumn/Winter)?**		
5b	**How would you describe the texture of *Fucus serratus* L. at different times of the year (Spring/Summer/Autumn/Winter)?**		

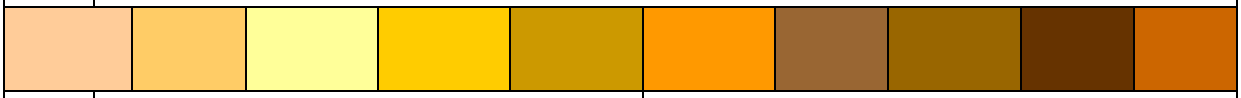
		Yes	**No**	**Don't know**
6	**Do you notice if the bathwater colour varies throughout the year?**		**Please continue to question 7**	**Please continue to question 7**
6a	**Is there a time of year when the bathwater is darker (brown) at the end of a treatment?**			
6b	**Please □ the colour from the following which best represents the bathwater when it is darkest in your opinion**			

6c	**In your opinion, what causes the colour of the bathwater to change (from straw yellow to brown)?**			
7	**Do you notice if the bathwater texture/consistency varies throughout the year?**		**Please continue to question 8**	**Please continue to question 8**
7a	**What time of year is the bathwater thicker at the end of a treatment?**			
8	**At the end of a treatment do you leave the seaweed bathwater extracts on the skin or do you shower it off?**	**Leave it on the skin**	**Shower it off**	
9	**What is the main reason you have for using seaweed baths?**	**Please the appropriate answer**		
9a	**Skin conditions (such as eczema, psoriasis, dermatitis)**			
9b	**Detox**			
9c	**Muscular aches and pains**			
9d	**Arthritis**			
9e	**Relaxation and stress relief**			
9f	**Other** **Please specify**			
		Yes	**No**	

10	**Did you know that iodine can potentially be absorbed by the body during a seaweed bath?**		
Any other comments?			
Thank you for taking the time to fill out this questionnaire			

Appendix D Staff Questionnaire

Staff Questionnaire **Voya Seaweed Baths**				
Please the appropriate answer				
Duration at Voya				
		Yes	**No**	
1	**Do you notice any changes in the seaweed *Fucus serratus* L. used in the seaweed baths during the year?**		**Please continue to question 2**	
If yes,				
1a	**How would you describe the colour of *Fucus serratus* L. at different times of the year (Spring/Summer/Autumn/Winter)?**			
1b	**How would you describe the texture of *Fucus serratus* L. at different times of the year (Spring/Summer/Autumn/Winter)?**			
		Yes	**No**	**Don't know**
2	**Do you notice if the bathwater colour varies throughout the year?**		**Please continue to question 3**	**Please continue to question 3**
2a	**Is there a time of year when the bathwater is darker (brown) at the**			

	end of a treatment?			
2b	**Please the colour from the following which best represents the bathwater when it is darkest in your opinion**			
				
2c	**In your opinion, what causes the colour of the bathwater to change (from straw yellow to brown)?**			
3	**Do you notice if the bathwater texture/consistency varies throughout the year?**		**Please continue to question 4**	**Please continue to question 4**
3a	**What time of year is the bathwater thicker at the end of a treatment?**			
4	**What is the main reason clients have for using seaweed baths?**	**Please the appropriate answer**		
4a	**Skin conditions (such as eczema, psoriasis, dermatitis)**			
4b	**Detox**			
4c	**Muscular aches and pains**			
4d	**Arthritis**			
4e	**Relaxation and stress relief**			
4f	**Other** **Please specify**			
		Yes	**No**	

5	**Is there a particular time of year when clients get more benefit from taking a seaweed bath?**		**Please continue to question 6**
5a	**If so,**	**When**	**Why**
6	**Is there a particular time of year when clients prefer to have a seaweed bath?**		**Please continue to question 7**
6a	**If so,**	**When**	**Why**
		Yes	**No**
7	**Did you know that iodine can potentially be absorbed by the body during a seaweed bath?**		
Any other comments?			
Thank you for taking the time to fill out this questionnaire			

Appendix E Ethics Proposal

Ethics Proposal

Title of the Study Urinary Iodine Study

Principal Investigator

Tara Westby

Signature _____

Qualification: BSc (Hons) Forensic Investigation and Analysis, & B.A. (Hons.) in Legal Science and French, minor in Spanish & Sociology and Politics.

Position: Research Student, Department of Life Science, Institute of Technology Sligo.

Address: Ballincar, Rosses Point Road, Sligo

Study Site: Research laboratory, School of Science, Institute of Technology Sligo and Voya seaweed Baths, Strandhill, Sligo.

Supervisors/Co-Investigators

First Supervisor: Dr Aodhmar Cadogan, Lecturer, Department of Life Science, IT Sligo. cadogan.aodhmar@itsligo.ie

Co-Investigator: Geraldine Duignan, Lecturer, Department of Life Science, IT Sligo. duignan.geraldine@itsligo.ie

Purpose of the study

The purpose of this study is to gather preliminary data on whether or not iodine present in *Fucus serratus* L. seaweed is absorbed, either transdermally or by inhalation, during a seaweed bath treatment, leading to an increase in urinary iodine concentration (UIC). The seaweed bath treatments will be provided by Voya

Seaweed baths, Sligo. The study participants will be asked to provide control urine samples prior to the study to establish baseline levels of iodine in their urine in the absence of exposure to seaweed. The first study group will be asked to then take a seaweed bath for 40 mins which is the normal duration of a treatment. The second study group will be asked to remain seated beside the seaweed bath and not have dermal contact with the seaweed or bathwater for 40 mins. Both study groups will be asked to collect urine samples before and up to 30 hours following the bath treatment. The purpose of the first group is to determine if there is a measureable increase in urinary iodine following the seaweed bath treatment due to a combination of inhalation and transdermal absorption. The purpose of the second group is to determine if volatile iodine (I_2) from the seaweed *Fucus serratus* L. and seaweed bathwater is present in high enough concentrations, to increase the urinary iodine concentration (UIC) when inhaled during a seaweed bath treatment without dermal contact.

Background to the study

Bathing in warm baths containing seaweeds e.g. *Fucus serratus* L. is a traditional therapy indigenous to Ireland and the baths are used to promote health and well-being. Anecdotally, these seaweed bath treatments are beneficial to human health; however there is no published literature that documents the beneficial components of seaweed baths or links health outcomes to bathing in seaweed baths.

It is well known that seaweeds contain many different chemical components including high levels of iodine (130ppm in *Fucus vesiculosus*-very similar seaweed to *Fucus serratus*). Research by the study investigator to date has involved the measurement of the concentration of iodine in seawater and seaweed bathwater. Following testing of simulated bathwater samples over a one year period the range of iodine concentration in bathwater has been established as 1-14ppm total iodine, thus confirming that iodine is released into the bathwater in substantial amounts.

Iodine is an important element for the control of a healthy metabolism and available research (World Health Organisation (WHO) (2007)) suggests that the Irish population are borderline deficient in iodine. Previous unpublished studies, Smyth

(2014), on the transdermal uptake of iodine from seaweed baths suggest that there is a ‘measurable uptake of minerals by the skin from seaweed baths’.

Diet is the main source of iodine (Jooste & Strydom (2010)) with the most common iodine rich foodstuffs being seafood, seaweed based foods and iodine supplemented salt. Subjects can eat and drink normally throughout the study days but are requested to avoid eating these iodine rich foods on the day before the study and the two days of the study. It will also be requested that they fill in a questionnaire related to their food and drink consumption over the urine collection days. As 90% of iodine in the body is excreted in urine (Jooste & Strydom (2010)) this study will require the collection and sampling of subject’s urine.

Study Design / Methodology

Type of Research Intervention

This study will involve the subject completing a Certificate of Consent, collecting 8 baseline urine samples over a 2 day period, completing a study questionnaire, and then collecting a further 8 urine samples prior to, and at designated times over 2 days following a seaweed bath treatment. The bath treatment will take place at Voya Seaweed Baths and takes approximately one hour.

Subject Selection

Urinary iodine population studies generally require very large numbers of subjects to establish median iodine levels; however as this is a preliminary study the number of research subjects is much smaller. Following consultation with Dr. Peter Smyth and discussion of sample number it was concluded that 10 subjects in each of the two study groups will be sufficient. Hence a total maximum of 30 subjects will be recruited in order to obtain a minimum of 10 subjects in each of the two study groups. The additional recruitment is to allow for drop out from the study or subjects who collect insufficient urine samples to determine any trend.

Approximately equal numbers of male and female subjects will be selected although a strict 50/50 male female split is not necessary for this preliminary study. The subjects will be selected randomly from the population, will be in the age bracket

(20-50 years), who have not taken a seaweed bath in the preceding week and are not regular users of iodine supplements. The subjects will be assigned to alternate treatment groups on a first come basis by the principal investigator. As this is a preliminary study factors such as weight, fitness level will not be controlled in the study subjects. There is no requirement to inform the subjects' GP of their participation in the study as Seaweed baths could be considered a normal recreational activity. GP contact details will not be requested. As the seaweed baths are open to all members of the public and are used by a wide range of people with differing health status only the following restrictions will apply. These are based on a combination of the study requirements and the normal restrictions on bath users:

1. Be at least 18 years of age and able to consent for yourself
2. No history of underactive or overactive thyroid
3. No sensitivity or intolerance to seaweed or seaweed containing products.
4. No surgical procedures in the past 6 months
5. Not pregnant
6. Not currently taking Iodine Supplements
7. Not taken a seaweed bath in the week prior to the study
8. Complete a pre-bath health consultation with Voya Staff. This is a general health screening carried out with all new users of the baths to exclude people with certain conditions e.g. heart problems. This will apply to all the study participants, even the non-bathers.

Subject Recruitment

Subjects will be recruited through informal and formal advertisement in IT Sligo and Voya Seaweed baths. The IT Sligo recruitment is targeted at academic and non-academic staff and postgraduate students. The advertisement at Voya seaweed baths is targeted at clients and locals. The advertisement is outlined in Appendix I

Procedure

The study will be carried out over approximately four days (two baseline days and two treatment days). The four days do not have to be consecutive but will be completed within an approximate two week period. The subjects will be given the Study Information sheet, consent form and the questionnaire at least one day before commencement of the study. They will be asked to complete the questionnaire in advance of meeting the Principal Investigator. They will have an opportunity to have any questions answered before agreeing consent.

Baseline Sample Collection

The study subjects will be asked to collect 8 of their own urine samples over the course of 2 days at the time intervals outlined in the table below. All urine samples in the study will be collected using pre-labelled specimen bottles which will be provided. The bottles will be labelled with a subject number only. Two spare bottles will also be provided. The samples will be collected by the investigator at several stages throughout the 2 days. They will be kept refrigerated and /or frozen until analysis.

Treatment Sample Collection

The following week the subjects will be asked to collect their own urine samples again, at the same time intervals as the baseline samples but this time prior to and following the seaweed bath treatment. They will be requested to collect 1 sample pre-treatment and 7 samples over the ~30 hour period following the treatment-as outlined in Table 1 below. The samples will be collected by the investigator at several stages throughout the 2 days. They will be kept refrigerated and /or frozen until analysis.

Bathing subjects will be requested to immerse themselves in the seaweed bath for 40 minutes (a typical bathing time). They are requested not to use the steam facilities during the treatment and not to shower after the treatment but to towel dry. Non Bathing subjects will be requested to sit beside the seaweed bath for the same duration and not to have dermal contact with the seaweed or bathwater. They are also requested to not use the steam or shower facilities. 1.5 litres of drinking water

will be provided to keep all subjects to keep hydrated during the treatment. The subjects can opt to partake in both the non-bathing and bathing treatments if they wish.

Table 1 Timelines for urine sample collection

Urine Sample Number	Day	Approximate Sample Time	Approximate collection time by the investigator
Baseline Samples			
Baseline sample 1	Day 1	10.00	Day 1-lunchtime
Baseline sample 2	Day 1	11.00	Day 1-lunchtime
Baseline sample 3	Day 1	12.00	Day 1-lunchtime
Baseline sample 4	Day 1	18.00	Day 1-evening ~18:00hr
Baseline sample 5	Day 1	Night i.e. bedtime	Day 2-morning ~10.00hr
Baseline sample 6	Day 2	Morning	Day 2-evening ~18:00hr
Baseline sample 7	Day 2	12.00	Day 2-evening ~18:00hr
Baseline sample 8	Day 2	18.00	Day 2-evening ~18:00hr

Urine Sample Number	Day	Approximate Sample Time	Approximate collection time by the investigator
Treatment Samples			
Treatment sample 1	Day 1	10.00 pre treatment	Day 1-lunchtime
Treatment sample 2	Day 1	11.00 post treatment	Day 1-lunchtime
Treatment sample 3	Day 1	12.00	Day 1-lunchtime
Treatment sample 4	Day 1	18.00	Day 1-evening ~18:00hr
Treatment sample 5	Day 1	Night i.e. bedtime	Day 2-morning ~10.00hr
Treatment sample 6	Day 2	Morning	Day 2-evening ~18:00hr
Treatment sample 7	Day 2	12.00	Day 2-evening ~18:00hr
Treatment sample 8	Day 2	18.00	Day 2-evening ~18:00hr

Analysis

The samples will be stored in a fridge and then freezer until all the samples have been collected. All samples will be tested for total iodine concentration using the Sandell-Kolthoff Method. The Sandell-Kolthoff Method is a standard method used for urinary iodine (UI) analysis (Khazan et al (2013), Jooste & Strydom (2010), Mina et al (2011) & Shelor and Dasgupta (2011)) and has been adapted for use at IT Sligo research laboratory. The creatinine concentration of the samples will also be determined to calculate the UI/Creatinine ratio. This is recommended estimation of recent iodine intake as the ratio of iodine/creatinine (UI/Cr) corrects for dilution of

the urine in spot sample (Zava 2013). The IT Sligo laboratory is not a certified testing laboratory. An increase of >50ppb total urinary iodine over 2-4 hour time period is expected to be a significant increase (P. P. A. Smyth 2014). No other measurements will be made. Once tested, the samples will be disposed in a safe manner.

Results

The result will be given to each participant following analysis by email or letter. Any incidental findings will be brought to the attention of the participant and they may be advised to consult with a healthcare professional and possible repeat testing may be advised.

Potential Harms

No adverse effects have been reported in relation to taking a seaweed bath treatments, it is considered a normal recreational activity. Any subjects who do not meet the normal health restrictions of Voya will be excluded from the study. Iodine is a normal constituent of diet and sensitivity or intolerance is rare. Any unlikely reaction to the bathwater which the participants may experience will be dealt with immediately. Any potential risks, e.g. slips, trips have been assessed and minimised by Voya as part of their periodic risk assessment for public liability purposes. No additional risks are foreseen.

Potential Benefits

Study participants will not receive any direct medical or financial benefit from participating and there are no anticipated expenses for their participation in this study. The researcher carrying out the study will benefit upon its completion due to the attainment of a PhD qualification.

Participation in the study is completely voluntary. If a subject does participate, they may freely withdraw from it at any time.

Data Management & Confidentiality

This study is part of a larger research project which will involve a PhD thesis submitted for examination to the Exam Board at the Institute of Technology Sligo. Each urine sample will be identified by a coded numbering system known to the investigator and first supervisor. Only the principal investigator and first supervisor will have access to the link between the sample number and the subject identity. The identity of those participating in the study and any information collected related to them i.e. personal details will be kept confidential and will not be shared, published or disseminated in any way. A hard copy of this information will be kept locked in a filing cabinet by the first supervisor Dr. Aodhmar Cadogan at the School of Science for a period of no greater three years after which all personal data will be destroyed (hard copy) or deleted (electronic copy).

The results obtained will be shared with subjects on an individual basis. Following this study, irrevocably anonymised data on the UI or UI/Cr concentration **only** may be shared through publications, conferences and other forms of media in order that other interested people may learn from the study. All electronic data related to the results will be stored in a password protected specific research study file, with limited and controlled access.

References

Jooste, P. L. and Strydom, E. (2010) 'Methods for determination of iodine in urine and salt'. *Best Practice & Research Clinical Endocrinology & Metabolism*, 24 (1), pp. 77-88.

Khazan, M., Azizi, F. and Hedayati, M. (2013) 'A Review on Iodine Determination Methods in Salt and Biological Samples', *International Journal of Medical Sciences.* 1 (1), pp. 1-9.

Mina, A., Favaloro, E. J. and Koutts, J. (2011) 'A robust method for testing urinary iodine using a microtitre robotic system'. *Journal of Trace Elements in Medicine and Biology*. 25 (4), pp. 213-217.

Shelor, C.P. and Dasgupta, P.K. (2011) ‘Review of analytical methods for the quantification of iodine in complex matrices’. *Analytica Chimica Acta,* 702 (1), pp. 16-36.

Smyth, P. P. A, Personal communications, November 2014. Dr Peter P. A. Smyth is an adjunct Professor in the School of Physics and Environmental Change Institute, National University of Ireland, Galway, Ireland and formerly of UCD Conway Institute of Biomolecular and Biomedical Research.

World Health Organisation (WHO) (2007) ‘Vitamin and Mineral Nutrition Information System (VMNIS), Global Database on Iodine Deficiency’. Geneva: World Health Organization.

Zava, T.T., Kapur, S. and Zava, T.D. (2013) ‘Iodine and creatinine testing in urine dried on filter paper’. *Analytica Chimica Acta*. 974, pp. 64-69.

Appendix F Subject recruitment advertisement

May 2015

Volunteers Required

To participate in a Urinary Iodine Study
in conjunction with the School of Science, IT Sligo and Voya Seaweed baths.

A study is being carried out to gather preliminary data on whether or not iodine present in *Fucus serratus L.* seaweed is absorbed, either transdermally or by inhalation, during a seaweed bath treatment, leading to an increase in urinary iodine concentration (UIC). The seaweed bath treatments will be provided by Voya Seaweed baths, Sligo.

If you are interested in participating in this study please contact the Study Co-ordinator Tarha Westby at 087 2980695 / 071 9305481 or email tarha.westby@gmail.com

Appendix G Study Information for subjects

Study Information

Hello and thank you for expressing an interest in partaking in our study. You are being invited to partake in a study to measure urinary iodine in persons before, during and after taking a Seaweed bath. This Information Sheet aims to provide you with any information that you may need in relation to the study and what is being asked of you. If you have any questions please feel free to contact us at any time using the contact details at the end of this section.

Before you decide if you will participate, you may discuss the research with family and friends and ask any questions which may be of concern to you.

The study is being conducted by researchers at Institute of Technology Sligo, in conjunction with Voya Seaweed Baths. The research will form part of a PhD qualification by postgraduate student Tarha Westby.

What does the study aim to do?

The aim of this study is to gather preliminary data on whether or not iodine present in *Fucus serratus* L. seaweed baths is absorbed, either trans-dermally or by inhalation, during a seaweed bath treatment leading to an increase in urinary iodine concentration (UIC).

What is the purpose of the research?

The purpose of this study is to determine if iodine (I_2) present in the seaweed *Fucus serratus* L. and seaweed bathwater is inhaled or absorbed during a seaweed bath treatment. Iodine is an important element for the control of a healthy metabolism and available research suggests that the Irish population can be deficient in iodine. Following testing of simulated bathwater samples over a one year period the bathwater concentration of iodine has been found to range from 1-14ppm iodine which is lower than that in seaweed but still a substantial amount.

The concentration of 'iodine' will be determined by analysis of the iodine to creatinine ratio (IU/Cr) in the urine of the study subjects. The 'iodine concentration' of urine will be measured prior to and after a seaweed bath treatment. The purpose of the study is gather preliminary data on (i) whether bathing or simply sitting beside a bath causes an increase in the urinary iodine level and (ii) how quickly this returns to the baseline level.

What are you being asked to do?

In the first part of the study, you will be required to take 8 of your own urine samples over 2 days before the bathwater treatment to establish a baseline urinary iodine level. Then on the day of the treatment you will be asked to take your own urine sample before a seaweed bath, immediately after the bath and a further 6 samples during the following 30 hours. There will be two study groups. The first group will be immersed in the seaweed bath for 40mins which is the normal bathing time. The second group will be siting adjacent to, but not in the bath. You may be assigned to either of the study groups. The urine samples will be taken by you during the course of your normal day to day activities e.g. at home, work, leisure etc. The samples will be collected by the researcher at agreed times and stored in a fridge and later a freezer until analysis. You are asked to avoid some foods during the study days that have very high iodine content.

Where will the study take place?

The researcher will meet with you at IT Sligo or in Voya Seaweed baths prior to the study to obtain your consent and go through any questions you may have. You will take your own urine sample at predetermined intervals prior to the bath treatment and after the treatment as part of your normal daily activities.

The seaweed baths will be provided free of charge at Voya Seaweed baths, Strandhill. The baths are located on the seafront. You will be greeted by the Staff there who will explain the use of the baths and any routine safety information. Any contraindications will be highlighted by Staff. You will be provided with drinking water. Toilet facilities are also available.

What exactly will the samples by tested for?

The **total iodine** in your urine sample will be measured using a standard analysis called the Sandell-Kolthoff Method. This is a standard method for urinary iodine analysis and has been adapted for use at an IT Sligo research laboratory. The creatinine in the urine will also be measured to adjust the iodine results for changes in urine dilution throughout the day. The IT Sligo laboratory is not a certified testing laboratory. This testing is not intended to replace any similar testing which would provide you with medical information or diagnosis.

Will you find out the results of the study?

Your individual results will be sent to you by email or by letter following analysis. If you are concerned about the result you may choose to consult with your doctor or healthcare professional and possible repeat testing may be advised.

Who will know about the results?

The results obtained will be shared with you on an individual basis. Following this study, anonymised data on the urinary iodine or iodine/creatinine concentration **only** may be shared through publications and conferences and other forms of media in order that other interested people may learn from the study. Confidential information i.e. personal details will not be shared, published or disseminated in any way. This information will be secured on file by Dr. Aodhmar Cadogan at the School of Science and will be destroyed after three years.

What will happen to the samples?

Your sample will only be identified through a code. Your sample will be stored securely in a fridge and then a freezer until all the samples have been collected. Once tested, the samples will be disposed in a safe manner.

Will my personal details be kept confidential?

The identity of those participating in the study and any personal information collected related to you will be regarded as highly confidential and will be kept in a secure locked location. Your urine sample will be identified only by a coded

numbering system. All final results will be irrevocably anonymised; this means any information that could identify you will be removed after the testing.

Do I have to take part?

No, it is up to you. Your alternative is to not participate in this study. You may withdraw from the study at any time and for any reason, without having to give an explanation. Contact Tarha Westby to inform her of your decision. It is your choice and all of your rights will still be respected

Are there are restrictions on who can take part?

To take part you must meet the following

1. Be at least 18 years of age and able to consent for yourself
2. No history of underactive or overactive thyroid
3. No sensitivity or intolerance to seaweed or seaweed containing products.
4. No surgical procedures in the past 6 months
5. Not pregnant
6. Not currently taking Iodine Supplements
7. Not taken a seaweed bath in the week prior to the study.
8. Complete a pre-bath health consultation with Voya Staff. This is a general health screening carried out with all new users of the baths to exclude people with certain conditions e.g. Heart problems. This will apply to all the study participants, even the non-bathers.

What are the Potential Risks?

There are no anticipated risks to subjects. Iodine is a normal constituent of diet and sensitivity or intolerance is rare. Any unlikely reaction to the bathwater which you may experience will be dealt with immediately. Any potential risks, e.g. slips, trips

have been assessed and minimised by Voya as part of their periodic risk assessment for public liability purposes. No additional risks are foreseen.

Who will benefit from the Study?

You will not receive any direct medical or financial benefit from participating in this study but your participation is likely to help find an answer to a research question. The bath will be provided free of charge and a pack containing all the necessary collection bottles will be provided. There are no anticipated expenses for your participation in this study.

How should I collect the urine sample?

Here are some simple instructions for collecting a Urine Sample

1. Wash hands with soap and warm water.

2. As you start to urinate, allow a small amount of urine to fall into the toilet bowl. (This clears the urethra of contaminants) Do not touch the inside of the collection bottle.

4. After the urine stream is well established, urinate into the bottle. Once an adequate amount of urine fills the bottle (the bottle only needs to be half-full), remove the bottle from the urine stream.

5. Pass the remaining urine into the toilet.

6. Screw the lid on the bottle tightly (do not touch the inside of the bottle or lid).

7. Finish toilet as normal and wash hands again with soap and warm water.

What do I need to do if I am selected as a bather?

Bathing subjects will be requested to immerse themselves in the seaweed bath for 40 minutes (a typical bathing time). You are requested not to use the steam facilities

during the treatment and not to shower after the treatment but to towel dry. You will be provided with drinking water and towels.

What do I need to do if I am selected as a non-bather?

Non Bathing subjects will be requested to sit beside the seaweed bath for the same duration and not to have dermal contact with the seaweed or bathwater. You are also requested to not use the steam or shower facilities. You will be provided with drinking water and you are advised to bring some reading material.

Who do I contact if I have any questions?

Do not sign the Certificate of Consent unless you have had a chance to ask questions and have received satisfactory answers to all of your questions. You can contact the principal investigator Tarha Westby at G2018 Innovation Centre, IT Sligo, Sligo. Email tarha.westby@gmail.com

Phone: 087 2980695 / 071 9305481 (office hours)

Alternatively you can contact:

Dr Aodhmar Cadogan, Department of Life Science, School of Science, IT Sligo, Sligo.

Telephone 086 2372324 or 071 9305626(office hours), email cadogan.aodhmar@itsligo.ie

Appendix H Participant Questionnaire

Participant Questionnaire

Name _____ Age _____

Contact Number _____ Sex _____

Address for correspondence
_____
(email or post)
_____

Emergency contact _____

Do you smoke? Yes ☐ No ☐
If yes, how many cigarettes do you smoke per day? _____

Have you taken a seaweed bath previously? _____
Have you taken one in the last week? Yes ☐ No ☐

Urine Sample Number	Day	Approximate Sample Time	Actual sample time / comments
Baseline sample 1	Day 1	10.00	
Baseline sample 2	Day 1	11.00	
Baseline sample 3	Day 1	12.00	
Baseline sample 4	Day 1	18.00	
Baseline sample 5	Day 1	Night i.e. bedtime	
Baseline sample 6	Day 2	Morning	
Baseline sample 7	Day 2	12.00	
Baseline sample 8	Day 2	18.00	
Treatment sample 1	Day 1	10.00 pre treatment	
Treatment sample 2	Day 1	11.00 post treatment	
Treatment sample 3	Day 1	12.00	
Treatment sample 4	Day 1	18.00	
Treatment sample 5	Day 1	Night i.e. bedtime	
Treatment sample 6	Day 2	Morning	
Treatment sample 7	Day 2	12.00	
Treatment sample 8	Day 2	18.00	

List of Iodine Rich foods-please avoid large quantities of these foods particularly the seafood.

- seafood (anything from the ocean should be avoided, including fish, shellfish, and seaweed containing products like kelp supplements)
- dairy (egg yolks, milk, yogurt, cheese, butter, ice cream, sour cream)
- soy (includes soybeans, soy sauce, tofu, and any other food with soy)
- chocolate particularly milk chocolate which contains dairy
- beans (e.g. avoid red kidney beans, lima beans, pinto beans or cowpeas)

Appendix I (i) Food Frequency Questionnaire

FOOD FREQUENCY QUESTIONNAIRE **BASE LINE URINE SAMPLES**			
Meal B = Breakfast L = Lunch D = Dinner S = Snacks Place Prepared H = Home R = Restaurant O = Other Sample size S = Small M= Medium L = Large (see Serving Size Pictures)			
	Meal **B/L/D/S**	**Food Log** **List as much detail as you can and include the 'Place Prepared codes' and 'Sample Size' codes.**	**Drink Log**
Day Before Study			
Day 1 of study			
Day 2 of study			

Return this questionnaire at the end of the study

Appendix I (ii) Food Frequency Questionnaire

FOOD FREQUENCY QUESTIONAIRE **BATHING / NON BATHING URINE SAMPLES**			
Meal = B = Breakfast L = Lunch D = Dinner S = Snacks Place Prepared H = Home R = Restaurant O = Other Sample size S = Small M= Medium L = Large (see Serving Size Pictures)			
	Meal **B/L/D/S**	**Food Log** **List as much detail as you can and include the 'Place Prepared codes' and 'Sample Size' codes.**	**Drink Log**
Day Before Study			
Day 1 of study			
Day 2 of study			

Return this questionnaire at the end of the study

Appendix J Certificate of Consent

Certificate of Consent

If you agree to participate in this study, you will receive a signed and dated copy of this Certificate of Consent for your records and the original will be retained by Dr. Aodhmar Cadogan.

I have read and understood the Information Sheet about this research study.

I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction.

I consent voluntarily to participate in this study

I consent voluntarily to the publication of results related **only** to my urine samples submitted as part of the study carried out by the Principal Investigator aimed at determining the concentration of urinary iodine pre and post bathing in a typical seaweed bath.

By signing this consent form, I have not waived any of the legal rights which I otherwise would have as a subject in a research study

Subject (Print Name)	Subject (Sign and Date)
Investigator (Print Name)	Investigator (Sign and Date)

Appendix K List of presentations and publications

Westby, T., Cadogan, A., Duignan, G., Kirk, L. and Montgomery, N. Iodine uptake following use of seaweed baths: inhalation or dermal absorption? Poster presented at: 18th Annual Research Conference; 2017; Sligo University Hospital.

Westby, T., Cadogan, A. and Duignan, G. (2017) *In vivo* uptake of iodine from a *Fucus serratus* Linnaeus seaweed bath: does volatile iodine contribute? *Environmental Geochemistry and Health.* 1-9.

Westby, T., Duignan, G. Smyth, T. and Cadogan, A. (2016) Method validation and determination of total iodine in seaweed bathwater. *Botanica Marina*. 59(4):241-249

Westby, T., Duignan, G. and Cadogan, A. Investigation of the uptake of iodine following exposure to *Fucus serratus* L. seaweed bathwater. Poster presented at: International Federation of Societies of Cosmetic Chemists (IFSCC) Conference; 2015; Zurich, Switzerland

Westby, T., Duignan, G. and Cadogan, A. Analysis of iodine in *Fucus serratus* L. seaweed bathwater and its potential for uptake by the body. Poster presented at: European Conference on Marine Natural Products (ECMNP); 2015; Glasgow, Scotland

Westby, T., Duignan, G. and Cadogan, A. Analysis of iodine in *Fucus serratus* L. seaweed bathwater. Presented at: ENVIRON; 2015; IT Sligo

Westby, T., Duignan, G. and Cadogan, A. Determination of iodine in *Fucus serratus* L. seaweed bathwater. Presented at: ENVIRON; 2014; Trinity College, Dublin

Westby, T., Duignan, G. and Cadogan, A. Analysis of Iodine in biological fluids following dermal exposure to seaweed. Poster presented at: S.T.E.M. Conference; 2013; IT Sligo

Appendix L Seminar and Workshop attendance

British Association of Chemical Specialities (BACS) Personal Care Group and Society of Cosmetic Scientists (SCS) Meeting, 'Is beauty only skin deep? A focus on chemistry in skin care' (2015) Manchester

International Federation of Societies of Cosmetic Chemists (IFSCC) Conference (2014) France

Statistics and Data Handling workshop (2014) Research Alliance IT Sligo

Gas Chromatography and High Performance Liquid Chromatography User Meeting (2013) Thermo Scientific

Academic writing and publishing workshop (2013) Sligo Regional Hospital

Ion Chromatography User Meeting (2012) Thermo Scientific

DE GRUYTER Botanica Marina 2016; 59(4): 241–249

Tarha Westby, Geraldine Dulgnan, Thomas Smyth and Aodhmar Cadogan*

Method validation and determination of total iodine in seaweed bathwater

DOI 10.1515/bot-2016-0029
Received 6 April, 2016; accepted 28 June, 2016; online first 26 July, 2016

Abstract: Seaweeds are a well-known natural source of iodine, although the quantity present varies depending on species, geographical location and season. Traditional seaweed baths contain hot water and seaweed but to date this bathwater matrix has not been studied. The objective of this study was to validate and apply the classical spectrophotometric Sandell-Kolthoff (SK) method for the analysis of total iodine in simulated seaweed bathwater. The principle of the method is based on the catalytic activity of iodide in the reduction of ceric ammonium sulphate being directly proportional to its concentration in the sample. Linear working range was from 30 µg l^{-1} to 300 µg l^{-1} iodine. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 9 µg l^{-1} and 29 µg l^{-1}, respectively. The intra-assay repeatability indicated a mean coefficient of variation (CV) <2.5%. Inter-assay precision over 7 days indicated a CV of 6.7%. Recovery ranged from 87% to 114%. Addition of seaweed to simulated bathwater results in an increase in iodine which was found to be dependent on the length of contact time and on the temperature, with higher amounts released at longer immersion times and higher temperatures. The data confirms that traditional seaweed baths containing *Fucus serratus* stimulate the release of iodine into bathwater.

Keywords: *Fucus serratus*; iodine; Sandell-Kolthoff (SK); seaweed bath.

Introduction

Traditional seaweed baths, which are indigenous to Ireland, are hot baths prepared using fresh water and (occasionally) seawater along with the seaweed *Fucus serratus* L. These baths are holistic treatments during which a bather is immersed in the bathwater for approximately 40 min. *Fucus serratus* is an intertidal brown algae (Arrontes 2002) found in the Northeast Atlantic from Norway to northern Portugal, along the west coast of Ireland, the North Sea and West Baltic (Edwards et al. 2012). *Fucus serratus* is the main species used in seaweed baths in Ireland, chosen for its abundance on the west coast, ease of harvesting from the intertidal area and its appealing aesthetic appearance. Occasionally, *Fucus vesiculosus* L., which grows in similar environments and is comparable in chemical composition to *F. serratus*, is employed. *Fucus* species are a rich source of iodine, bromine, calcium, cobalt, copper, iron, manganese, molybdenum, nickel, phosphorus, potassium, titanium and zinc (Caroli et al. 1999, Morrissey et al. 2001). When *F. serratus* is placed in a hot aqueous environment it stimulates the release of alginate, a highly abundant polysaccharide present in the cell wall along with other chemical components (Nishide et al. 1984). Seaweeds have been used as sea vegetables, medicines and fertilisers for centuries (Kiuru et al. 2014) and it is estimated that the seaweed production and processing sector in Ireland will be worth €30 million per annum by 2020 (Sea Change 2006). One seaweed bath provider caters for up to 20,000 bathers per year in its facility on the Northwest coast of Ireland (N. Walton, Voya Seaweed Baths, Sligo, pers. comm.).

Seaweeds contain varying amounts of compounds with potential therapeutic properties including antioxidant, anti-inflammatory, thyroid-stimulating, antimicrobial and tissue-healing (Pesando and Caram 1984, Jiménez-Escrig et al. 2001, Bergé et al. 2002, Rupérez et al. 2002, Sezer et al. 2008, Teas et al. 2009). While there are countless references to seaweed and its various applications and industrial uses, there is no documented research discussing seaweed baths, their chemical composition and their advantages or disadvantages. Some of the therapeutic components present in seaweed may be available for uptake by the body during a seaweed bath treatment. However, the properties of seaweed and specifically *F. serratus* are used to infer (anecdotally) the benefits of seaweed baths but have not been scientifically examined.

Iodine is a vital micronutrient for normal thyroid function, growth and development (Ahad and Ganie 2010). It

*Corresponding author: Aodhmar Cadogan, Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland, e-mail: cadogan.aodhmar@itsligo.ie
Tarha Westby, Geraldine Dulgnan and Thomas Smyth: Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland. http://orcid.org/0000-0003-3259-7318 (T. Westby)

has also demonstrated anti-infective properties as it is commonly used in the treatment of wounds (Burks 1998) and iodine-rich solutions were historically used to treat many diseases. Iodine also normalises elevated adrenal corticosteroid hormone secretion related to stress (Ahad and Ganie 2010). The WHO (2004) describes the Irish population as mildly iodine-deficient. While it is not suggested that the use of seaweed bath treatments could be an efficient mechanism to treat iodine deficiency, it may however be an effective mechanism. In terms of iodine, given its volatility (as I_2 or volatile organic iodine), it could be expected to transfer to humans via a combination of inhalation (Smyth et al. 2011) and dermal absorption (Boothman 2009). Non-volatile species (I^-, IO_3^- or organic iodine) within the bathwater could be available for dermal absorption. The levels of iodine in seaweeds are known to vary depending on species, geographical location and season. The fresh-weight iodine content of *F. serratus* was recently reported as 320±77 µg g^{-1} (meristematic tip thallus) and 275±180 µg g^{-1} (mid thallus) (Nitschke and Stengel 2015).

The Sandell-Kolthoff (SK) method is a commonly employed spectrophotometric method used in the determination of iodine. It has been applied to various matrices including biological fluids, environmental samples and foodstuffs (Shelor and Dasgupta 2011, Błażewicz 2012). Prior to analysis, an acid digestion is carried out in order to break down polysaccharides and other cellular material. All iodine species present in the matrix are converted to iodide by arsenious acid solution. The assay is based on the catalytic effect of iodide (I^-) in the redox reaction between the yellow cerium (IV) and arsenic (III), which yields the colourless cerium (III) and the arsenic (V). It is a two-step reaction where the rate of reaction is measured during the reduction of the yellow-coloured ceric ions (Ce^{4+}) by arsenic (As^{3+}) in the presence of iodide (I^-) to form cerous ions (Ce^{3+}) and elemental iodine (I_2).

$$As^{3+} + I_2 \rightarrow As^{5+} + 2I^-$$

$$2Ce^{4+} + 2I^- \rightarrow 2Ce^{3+} + I_2$$

The catalytic activity of the iodine is directly proportional to its concentration in the sample (Sandell and Kolthoff 1936).

Before the potential inhalation or transdermal uptake of iodine can be explored, it is necessary to understand the composition of the baths themselves and the availability of the various components. While more sophisticated instrumental methods such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and neutron activation analysis (NAA) have been used for iodine measurement, the objective of this study is to establish a simple, rapid, cost-effective method to measure total iodine in a sample. This study sets out to assess the analytical performance of the SK method adapted to a microplate and its suitability for use in the determination of total iodine released from *F. serratus* into hot bathwater. Following validation the method is applied to simulated bathwater samples under different conditions of time and temperature to confirm the release of iodine into seaweed bathwater.

Materials and methods

Reagents

All reagents were purchased from Sigma Aldrich, Arklow, Ireland and prepared using Grade A glassware. Sterile, polypropylene, flat-bottom 96-well microplates were purchased from Eppendorf supplied by Fisher Scientific, Dublin, Ireland and Eppendorf sealing film was supplied by Mason Technology, Dublin, Ireland. Ultrapure water was used throughout.

Ammonium persulphate (1 M) was prepared in water and stored in the dark.

Arsenious acid solution (0.05 M) was prepared by adding arsenic trioxide, sodium chloride and 200 ml of 5 N sulphuric acid to 500 ml water. This was heated gently to dissolve, cooled to room temperature and diluted with water to 1 l, then filtered through Whatman filter paper (150 mm) and stored in the dark at room temperature.

Ceric ammonium sulphate (0.075 M) was prepared in 250 ml of 3.5 N sulphuric acid and stored in the dark at room temperature.

A stock standard solution, 1000 mg l^{-1} iodine, was prepared by dissolving 168.6 mg potassium iodate (KIO_3) in 100 ml water (0.0079 M). Working standards were prepared by serial dilution of stock to the range of 20–300 µg l^{-1} and stored at room temperature in the dark prior to analysis.

Sample preparation

Fucus serratus (fronds) and seawater samples were collected from Voya Seaweed Baths, Strandhill, Co. Sligo, Ireland. The *F. serratus* was hand-harvested from Easkey, Co. Sligo, Ireland (Spring 2013). Harvesting is carried out every 1–3 days. The fresh samples are typically rinsed in freshwater to remove epiphytes, crustaceans and sand, and then stored in freshwater before use in baths. In order to mimic the commercial seaweed baths storage conditions for this study, freshly

harvested samples were transported to the lab in freshwater. They were kept at ambient temperature for 18–25 h until processing. Seawater samples were collected in a 1-l polypropylene bottle. This seawater was collected from the shore at Strandhill, Co. Sligo, Ireland. In practice there is a high degree of variability in the heat, volume of water and amount of seaweed used in a bath due to individual user preferences. To control the conditions of the study and to circumvent possible contamination and safety considerations of sampling directly from the used baths, simulated baths were prepared. For the validation, one bath was prepared by heating 500 ml of ultrapure water to 70°C in a 2-l beaker on a hotplate (BIBBY HB502, BIBBY Scientific, Staffordshire, UK). The seaweed sample, approximately 20 cm in length, complete, weighing 150±0.5 g was added followed by 500 ml of seawater. The simulated bath was maintained at 55°C for 40 min. After 40 min the intact seaweed and any visible fragments were removed using plastic tongs and 50-ml aliquots of bathwater were transferred to 50-ml polypropylene labelled sample tubes. All samples were stored at -20°C, until analysis. A 1:20 dilution of this sample was used for the validation.

For the time release study a bath was prepared, similar to the validation study, with 150±0.5 g of seaweed added to 500 ml ultrapure water at ~70°C followed by 500 ml seawater at ~20°C. The temperature was maintained at 55°C. Samples were collected after ~15 s and at 5-min intervals up to 50 min. For the temperature studies, baths (n=3) containing 500 ml seawater and 500 ml ultrapure water were prepared at each temperature 20°C, 40°C, 60°C and 80°C. Seaweed (150±0.5 g) was added to each bath and the baths were maintained at the respective temperature for 40 min. Samples were collected after addition of the seaweed (~15 s) and at 10-min intervals and analysed in triplicate following dilution 1:20, 1:25 or 1:50. In order to minimise variation, the seaweed samples for the temperature experiments were collected on the same day from the same harvest and were visually similar pieces (in terms of colour, shape and length).

Sandell-Kolthoff (SK) micromethod

The analytical method was adapted from Dunn et al. (1993) and modified for use on a microplate similar to Hussain and Wan Mohamud (2006), Mina et al. (2011) and Ohashi et al. (2000). The bathwater samples were diluted with ultrapure water prior to analysis. A 50-µl aliquot (blank of ultrapure water, n=2; iodine standard, n=2; or diluted bathwater, n=3) were pipetted into the wells of a 96-well plate. Ammonium persulphate (100 µl) was added to each well for the digestion. The microplate was sealed with sealing film, wrapped in tinfoil and incubated at 100°C for 60 min. It was removed from the oven after digestion and allowed to cool to room temperature. Aliquots of the digested sample (50 µl) were transferred to a clean microplate and 100 µl of arsenious acid solution was added to the well and mixed by drawing the contents of the well into the pipette tip five times. The microplate was allowed to stand for 15 min. Following this, 50 µl of ceric ammonium sulphate solution was added with 30-s intervals between additions to each row using a multichannel pipette and allowed to stand at room temperature for 30 min. Exactly 30 min following the addition of ceric ammonium sulphate solution to the first row, its absorbance was read at 420 nm (Fluostar Optima, BMG Labtech, Ortenberg, Germany). Successive rows were read at 30-s intervals as used for the addition of ceric ammonium sulphate solution to each row. To avoid the effect of temperature variations on the reaction, all standards and samples were analysed within the same batch on the same day.

Linearity and range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), recovery, robustness were carried out as per ICH Harmonised Tripartite Guidelines (1994). Linearity refers to a response that is directly proportional to concentration of analyte.

Commercial standard reference materials should provide the basis for accurate measurement and recovery tests (Khazan et al. 2013); however Caldwell et al. (2003) note that challenges can arise in standard and sample preparation when there is a lack of matrix-matched reference material. A similar challenge is presented in this validation due to the complex nature of the sample matrix (i.e. presence of protein, lipids and polysaccharides at varying concentrations) and the unavailability of a standard sample devoid of iodine. As a result, spiked recovery is used as an indication of the level of accuracy that can be obtained from the method. Bathwater samples were initially diluted 1 in 20 with ultrapure water. The iodate-spiked sample (50 µg l^{-1}) was prepared by adding 1 ml of 1 mg l^{-1} KIO_3 to a 20-ml volumetric flask and bringing it to volume with the diluted bathwater. A second sample (unspiked) was prepared in a similar way but using 1 ml of ultrapure water as the spike. The recovery was carried out over 3 days as per the following equation:

$$\%\,\text{Recovery} = \left(\frac{[KIO_3\ \text{spiked sample}]-[\text{water spiked sample}]}{[\text{added iodine}]\times\text{dilution factor}}\right)\times 100$$

The following analytical parameters were varied in order to establish the robustness of the method: Digestion time with 1 M ammonium persulphate, temperature during digestion with 1 M ammonium persulphate, time interval

between adding ceric ammonium sulphate to sample and reading its absorbance and time interval between adding ceric ammonium sulphate to successive samples. Specificity was not determined as it is documented that the method is specific for iodine (Sandell and Kolthoff 1936, Dunn et al. 1993, Ohashi et al. 2000, Hussain and Wan Mohamud 2006, Jooste and Strydom 2010, Mina et al. 2011, Khazan et al. 2013).

Results

Linearity and range

The calibration series in the range 20–300 µg l^{-1} indicated a linear response to the logarithm of absorbance. The correlation coefficient R^2 ranged from 0.9956 to 0.9997 based on n=11 calibration curves (average=0.9985). A typical calibration response is shown in Figure 1.

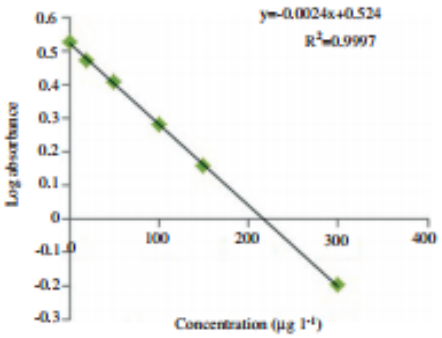


Figure 1: Validation of SK micromethod linearity. Typical calibration curve of log absorbance vs. concentration iodine (µg l^{-1}).

Limit of detection (LOD) and limit of quantification (LOQ)

The average log absorbance value for the blank of each run (n=12) was inserted into its equation of the line in order to determine its equivalent concentration. Based on the standard deviation (SD) of these 12 blank concentrations, the LOD was determined to be 9 µg l^{-1} (based on 3 SD) and the LOQ was determined to be 29 µg l^{-1} (based on 10 SD).

Precision

The intra assay precision ranged from 0.4 to 5.1% (mean 2.5% CV) based on replicate analysis (n=3) of six separate 1:20 diluted bathwater samples carried out on seven different days (Table 1). The inter-assay precision was 6.7% CV based on the mean concentrations over 7 days.

Accuracy

The recovery method was adapted from Ohashi et al. (2000). Diluted bathwater samples (mean 207.0 µg l^{-1}) were spiked with 1 mg l^{-1} potassium iodate to give a spike concentration of 50 µg l^{-1}. The recovery of an iodate-spiked diluted bathwater sample ranged from 87% to 114% (Table 2).

Robustness

A 1:20 diluted bathwater sample (mean 207.0 µg l^{-1} iodine measured using final method parameters) was used as a reference concentration. Variations were made to the

Table 1: Validation of Sandell-Kolthoff (SK) micromethod precision.

	Mean concentration (µg l^{-1})±SD	CV (%)	Mean CV (%)
Intra-assay n=6	223.0±0.9	0.4	2.5
	186.9±5.0	2.6	
	213.3±5.85	2.7	
	198.2±10.1	5.1	
	197.8±2.8	1.4	
	223.8±8.2	3.0	
	205.9±3.0	1.5	
Inter-assay n=7 assays	207.0±13.8		6.7

Intra-assay precision of six separate 1:20 diluted bathwater samples analysed in triplicate, and inter-assay precision (n=7).
CV, Coefficient of variation.

Table 2: Validation of Sandell-Kolthoff (SK) micromethod recovery.

Day	n	Average iodine concentration (µg l^{-1})±SD		Iodine recovered (µg l^{-1})	% Recovery
		Added water	Added iodate		
1	6	233.7±6.8	290.7±7.1	57.0	114
2	6	211.0±2.6	254.5±5.7	43.5	87
3	6	192.1±2.4	238.4±2.2	46.3	93
Average					98

% Recovery of diluted 1:20 bathwater samples (mean concentration 207.0 µg l^{-1}) with added water or 50 µg l^{-1} of potassium iodate.

Table 3: Validation of Sandell-Kolthoff (SK) micromethod robustness.

	Sample concentration (µg l^{-1})±SD	CV (%)	% Recovery
Digestion time with 1M ammonium persulphate			
50 min	229.9±11.3	4.9	111
60 min	198.1±10.1	5.1	96
70 min	217.7±19.9	9.1	105
Temperature during digestion with 1M ammonium persulphate			
90°C	216.7±16.3	7.5	105
100°C	197.8±2.8	1.4	96
110°C	215.8±11.1	5.2	104
Time interval between adding ceric ammonium sulphate to sample and reading its absorbance			
25 min	203.2±15.0	7.4	98
30 min	203.5±15.0	7.4	98
35 min	200.9±14.9	7.4	97
40 min	201.5±15.0	7.4	97
Time interval between adding ceric ammonium sulphate to successive samples			
20 s	164.8±5.6	3.4	80
30 s	232.3±2.5	2.5	112
40 s	216.8±3.9	3.9	105

% Recovery of diluted bathwater samples (mean iodine concentration 207.0 µg l^{-1}) determined at different digestion parameters and reading intervals (n=6).

CV, Coefficient of variation.

digestion time, digestion temperature and timing intervals measured in triplicate to check the robustness of the assay; results are presented in Table 3. The recovery ranged from 96% to 111% for different digestion times. A digestion time of 60 min was selected as the data demonstrated the lowest error in recovery (less 4%) and a low % CV. Variation in temperature during digestion between 90°C and 110°C during digestion resulted in very little difference in the % recovery of iodine: from 96% to 105%. As there was no difference in recovery when the interval between adding ceric ammonium sulphate to sample and reading its absorbance was increased from 25 to 40 min, a 30-min interval was selected. The time interval between adding ceric ammonium sulphate to successive samples was also varied between 20 and 40 s. It is critical that this interval matches the interval at which the absorbance of each successive sample is read with the microplate reader. A 30-s interval was selected as it showed a low CV and was practical to implement.

Effect of time and temperature on iodine release

The time release profile of iodine from seaweed into the aqueous environment is shown in Figure 2. The initial iodine concentration prior to addition of the seaweed was 25 µg l^{-1} due to the iodine content of the seawater. The total iodine concentration reached a maximum of almost 4000 µg l^{-1} after 30 min, with no significant increase in the concentration between 30 and 50 min. Almost 80% of the final average concentration was reached after 5 min.

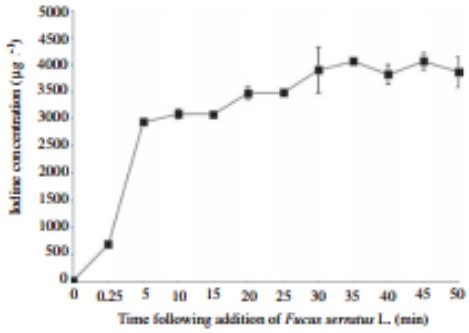


Figure 2: Effect of time on release of iodine. Iodine concentration of 1:1 mixture of seawater and deionised water over 50 min following addition of seaweed. Aqueous matrix held at 55°C (n=3 replicate measurements).

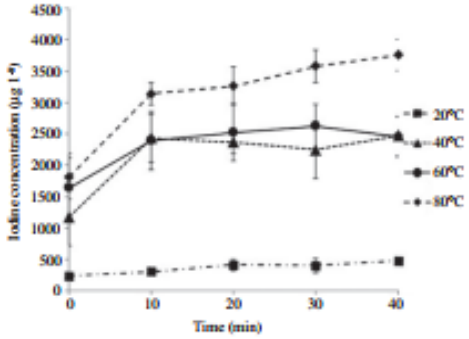


Figure 3: Effect of temperature on release of iodine. Comparison of total iodine concentration (µg l⁻¹) in baths prepared at different temperatures from 20°C to 80°C. Average and standard deviation (SD) of three baths plotted at each temperature.

The effect of the temperature on the iodine release concentration is shown in Figure 3. At 20°C the total iodine rose slowly over the 40 min to an average maximum of 477 µg l⁻¹ (11.8% CV) over the three baths. There was a sharp increase in the amount of iodine released when the temperature of the water was raised to 40°C. The iodine concentration at 40°C and 60°C showed a stable maximum of between 2200 and 2600 µg l⁻¹ after 30 min. At the highest temperature studied, 80°C, there was a further increase in iodine up to an average maximum of 3756 µg l⁻¹ (6.6% CV) across the three baths. Similar to the time study there was a sharp increase in iodine concentration within the first 10 min of addition of seaweed. Thereafter the iodine concentration of the baths maintained at 20–60°C remained relatively stable. The bath maintained at the highest temperature (80°C) continued to increase in iodine concentration to up the final time point of 40 min. The very high or low temperatures in Figure 3 are not realistic bathing temperatures as typical baths are from 40 to 60°C, however the data does demonstrate that increased temperature stimulated higher efflux of iodine.

Discussion

This study set out to assess and validate the SK method to measure the total iodine in bathwater and to establish typical concentration levels of iodine released by *Fucus serratus* in a heated aqueous environment. The microplate adaptation of the standard SK method was selected as the analytical method due to its ease of use, reduced costs and waste associated with the microplate adaptation, high throughput of samples and ease of adaptation to urine samples for future work.

Assay validation

Linearity was observed on the log scale from 30 to 300 µg l⁻¹ with an average correlation coefficient of 0.9985 (Figure 1). The SK method is typically used for the analysis of urine and has a documented working range of 20–300 µg l⁻¹. The method is sensitive for iodine in this matrix with an LOD of 9 µg l⁻¹. This is comparable to Hussain and Wan Mohamud (2006) and Ohashi et al. (2000) who determined an LOD of 14 µg l⁻¹ with a working range of 0–200 µg l⁻¹ and 40–400 µg l⁻¹, respectively, using an SK microplate method for urine samples. Based on the LOQ of 29 µg l⁻¹, a working linear range of 30–300 µg l⁻¹ is recommended. The intra-assay precision was 2.5% and the inter-assay precision was 6.7% (Table 1). A review by Shelor and Dasgupta (2011) outlines the range in intra and inter-assay precision recorded with SK method/SK micromethod for various matrices including biological fluids (2.8–13% and 4–15%, respectively), food (0.031–6.8%) and environmental samples (2.9–9.5%). Ohashi et al. (2000) reported an intra-assay CV of 2.0% on a high concentration urine sample (319 µg l⁻¹). This CV increases to 10% on a lower concentration urine sample (37.4 µg l⁻¹). Similarly Mina et al. (2011) reported an intra-assay CV range from 7% to 11% in a similar SK micromethod for urine. Inter-assay precision by Ohashi et al. (2000) ranged from 4.4 to 20%. Recovery of added iodine was in the range from 87% to 114% (Table 2). Ohashi et al. (2000) document the

recovery of iodine at 89–109%. Overall the assay precision and recoveries in the bathwater matrix were comparable to these documented values.

Complete mineralisation of the sample is necessary to digest any organics that may interfere in the reaction by chelation of Ce(IV) or Ce(III) or otherwise affect the reaction rate. All iodate and iodine-containing species are converted to iodide. Ohashi et al. (2000) investigated the effect of digestion times on recovery of iodate and found that digestion times between 50 and 70 min yielded the highest recovery. Rendl et al. (1998) also investigated digestion time with ammonium persulphate in a urinary test tube method and found consistent recoveries between 40 and 70 min. An optimum digestion time of 60 min, temperature of 100°C, time interval between adding ceric ammonium sulphate to sample of 30 min and time interval between adding ceric ammonium sulphate to successive samples of 30 s were selected as the final assay parameters.

Iodine in simulated baths

In the commercial setting, the conditions in seaweed baths are highly variable but typically contain 4±0.5 kg of seaweed in 120±20 l of water giving a fresh seaweed estimate of 25–50 g l^{-1}. The bath is prepared by dispensing the hot water first and allowing the seaweed to soak before adding cold seawater to adjust to a comfortable temperature. This stimulates the release of components from the seaweed. The simulated baths were prepared using fresh seaweed (150 g l^{-1}) under more controlled conditions. The release profile (Figure 2) confirms that the iodine in the seaweed was rapidly released into the aqueous environment in significant amounts at 55°C. The contribution from the seawater (50 µg l^{-1}) was minor and not expected to affect the rate of efflux or the analytical method. Comparing Figures 2 and 3 a higher amount of iodine was seen when the seaweed was added to the hot water first and then cooled to 55°C (Figure 2), rather than bringing the bath to the set temperature and adding the seaweed as in the experimental conditions for Figure 3. From these preliminary experiments the amount of iodine released is estimated to be 26.7 µg g^{-1} fresh seaweed, based on typical final concentration of 4000 µg l^{-1} iodine. Using an average iodine concentration in *Fucus serratus* of 300 µg g^{-1} fresh weight (Nitschke and Stengel 2015), this would indicate that somewhere in the region of 10% of the available iodine is being released. It is also noted that some iodine may be lost during storage of seaweed prior to bath preparation. However, these observations are based on a small number of baths and do not yet reflect seasonal factors.

The application of heat is well-known to disrupt the cell wall architecture (Daniell et al. 1969). Once macroalgae are removed from their natural environment they are at risk of environmental stresses, including atmospheric oxidants (e.g. ozone), heat and desiccation. Iodine release by macroalgae occurs in response to oxidative stress, but stress can also occur during normal development prior to harvesting (Küpper et al. 2008, Chance et al. 2009). Given the relatively high temperatures of the bath it is likely that heat is the predominant factor for the release of iodine into the bathwater.

To date this matrix has remained unexplored, however in addition to the iodine it is likely that the bathwater contains other mineral salts and organics, including polysaccharides, released from the seaweed. The possibility that some of the components of the matrix may contribute to the variability in precision and recovery, cannot be excluded. It must also be pointed out that while certain components of selected seaweeds have been chemically characterised, the majority of studies only focus on a particular group of compounds. As the SK method is recommended for urinary iodide analysis, it could be applied directly to measure the urine samples of bathers to determine the uptake of iodine by the body during a seaweed bath treatment. Further investigation of the pH, seasonal variation of iodine concentration, polysaccharide composition and speciation of the released iodine in the context of the bathwater matrix, would be very useful additional knowledge to inform the potential availability of chemicals for transdermal adsorption and, in doing so, will seed a nascent body of knowledge of the potential health effects of seaweed baths.

References

Ahad, F. and S.A. Ganie. 2010. Iodine, Iodine metabolism and Iodine deficiency disorders revisited. *Indian J. Endocrinol. Metab.* 14: 13–17.

Arrontes, J. 2002. Mechanisms of range expansion in the intertidal brown alga *Fucus serratus* in northern Spain. *Mar. Biol.* 141: 1059–1067.

Bergé, J.P., E. Debiton, J. Dumay, P. Durand and C. Barthomeuf. 2002. In vitro anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga *Porphyridium cruentum*. *J. Agric. Food Chem.* 50: 6227–6232.

Blażewicz, A. 2012. A Review of spectrophotometric and chromatographic methods and sample preparation procedures for determination of iodine in miscellaneous matrices. *In*: (J. Uddin, ed.) *Macro To Nano Spectroscopy*. InTech Publishing, Croatia. pp. 371–398.

Boothman S. 2009. *"Iodine white paper: the use of iodine in wound therapy"* Available at: http://www.systagenix.co.uk/cms/uploads/1042_Iodine_White_Paper_A5_(INT)LP_003.pdf. Accessed 13 May 2016.

Burks, R.I. 1998. Povidone-iodine solution in wound treatment. *Physical Therapy 78*: 212–218.
Caldwell, K.L., B. Maxwell, A. Makhmudov, S. Pino, L.E. Braverman, R.L. Jones and J.G. Hollowell. 2003. Use of inductively coupled plasma mass spectrometry to measure urinary iodine in NHANES 2000: comparison with previous method. *Clin. Chem. 49*: 1019–1021.
Caroli, S., G. Forte and A.L. Iamiceli. 1999. ICP-AES and ICP-MS quantification of trace elements in the marine macroalga fucus sample, a new candidate certified reference material. *Microchem. J. 62*: 244–250.
Chance, R., A.R. Baker, F.C. Küpper, C. Hughes, B. Kloareg and G. Malin 2009 'Release and transformations of inorganic iodine by marine macroalgae'. *Estuarine, Coastal and Shelf Sci. 82*: 406–414.
Daniell, J.W., W.E. Chappell and H.B. Couch. 1969. Effect of sublethal and lethal temperatures on plant cells. *Plant Physiol. 44*: 1684–1689.
Dunn, J.T., H.E. Crutchfield, R. Gutekunst and A.D. Dunn. 1993. Methods for measuring iodine in urine. ICCIDD/UNICEF/WHO, Netherlands.
Edwards, M., D. Hanniffy, S. Heesch, J. Hernandez-Kantun, M. Moniz, B. Queguineur, J. Ratcliff, A. Soler-Vila and A. Wan. 2012. Macroalgae fact-sheets. Available at http://www.seaweed.ie/irish_seaweed_contacts/doc/FactSheets.pdf. Last accessed 13 May 2016.
Hussain, H and W.N. Wan Mohamud. 2006. A cost-effective modified micromethod for measuring urine iodine. *Trop. Biomed. 23*: 109–115.
International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 1994. ICH harmonised tripartite guideline, validation of analytical procedures: text and methodology Q2:R1. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed 13 May 2016.
Jiménez-Escrig, A., I. Jiménez-Jiménez, R. Pulido and F. Saura-Calixto. 2001. Antioxidant activity of fresh and processed edible seaweeds. *J. Sci.Food Agric. 81*: 530–534.
Jooste, P. L. and E. Strydom. 2010. Methods for determination of iodine in urine and salt. *Best Pract. Res. Clin. Endocrinol. Metab. 24*: 77–88.
Khazan, M., F. Azizi and M. Hedayati. 2013. A review on iodine determination methods in salt and biological samples. *Int. J. Med. Sci. 1*: 1–9.
Kiuru, P., M.V. D'Auria, C.D. Muller, P. Tammela, H. Vuorela and J. Yli-Kauhaluoma. 2014. Exploring marine resources for bioactive compounds. *Planta Med. 80*: 1234–46.
Küpper, F.C., L.J. Carpenter, G.B. McFiggans, C.J. Palmer, T.J. Waite, E.M. Boneberg, S. Woitsch, M. Weiller, R. Abela, D. Grolimund, P. Potin, A. Butler, G.W. Luther III, P.M.H. Kroneck, W. Meyer-Klaucke and M.C. Feiters. 2008. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proc. Natl. Acad. Sci. USA. 105*: 6954–6958.
Mina, A., E.J. Favaloro and J. Koutts. 2011. A robust method for testing urinary iodine using a microtitre robotic system. *J. Trace Elem. Med. Biol. 25*: 213–217.
Morrissey, J., S. Kraan and M.D. Guiry. 2001. *A guide to commercially important seaweeds on the Irish coast*. Bord Iascaigh Mhara/Irish Fisheries Board, Dublin. pp. 1–66.
Nishide, E., K. Tsukayama, N. Uchida and K. Nisizawa. 1984. Isolation of water-soluble alginate from brown algae. *Hydrobiologia 116*: 557–562.
Nitschke, U. and D.B. Stengel. 2015. A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products. *Food Chem. 172*: 326–334.
Ohashi, T., M. Yamaki, C.S. Pandav, M.G. Karmarkar and I. Minoru. 2000. Simple microplate method for determination of urinary iodine. *Clin. Chem. 46*: 529–536.
Pesando, D. and B. Caram. 1984. Screening of marine algae from the French Mediterranean coast for antibacterial and antifungal activity. *Botany 27*: 3281–3386.
Rendl, J., D. Bier and C. Reiners 1998. Methods for measuring iodine in urine and serum. *Exp. Clin. Endocrinol. Diabetes. 106*: S34–41.
Rupérez, P., O. Ahrazem and J.A. Leal. 2002. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food Chem. 50*: 840–845.
Sandell, E.B. and I.M. Kolthoff. 1936. Micro determination of iodine by a catalytic method. *Microchimica Acta. 1*: 9–25.
Sea Change. 2006. A marine knowledge, research and innovation strategy for Ireland, 2007–2013. *Marine Institute* 1–52.
Sezer, A.D., E. Cevher, F. Hatipoğlu, Z. Oğurtan, A.L. Baş and J. Akbuğa. 2008. Preparation of fucoidan-chitosan hydrogel and its application as burn healing accelerator on rabbits. *Biol Pharm Bull. 31*: 2326–2333.
Shelor, C.P. and P.K. Dasgupta. 2011. Review of analytical methods for the quantification of iodine in complex matrices. *Anal. Chim. Acta 702*: 16–36.
Smyth, P.P., R. Burns, R.J. Huang, T. Hoffman, K. Mullan, U. Graham, K. Seitz, U. Platt and C. O'Dowd. 2011. 'Does iodine gas released from seaweed contribute to dietary iodine intake?' *Environ. Geochem. Health. 33*: 389–97.
Teas, J., T.G. Hurley, J.R. Herbert, A.A. Franke, D.W. Sepkovic and M.S. Kurzer. 2009. Dietary seaweed modifies estrogen and phytoestrogen metabolism in healthy postmenopausal women. *J. Nutr. 139*: 939–944.
WHO. 2004. '*Iodine status worldwide WHO global database on iodine deficiency*'. World Health Organization, Geneva.

Bionotes

Tarha Westby
Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland.
http://orcid.org/0000-0003-3259-7318

Tarha Westby received her BSc in Forensic Investigation and Analysis from the Institute of Technology Sligo in 2011 and is currently carrying out her PhD in Analytical Chemistry. Her interests include marine and cosmetic chemistry.

Geraldine Duignan
Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland

Geraldine Duignan lectures in pharmaceutical and health sciences at the Institute of Technology Sligo, Ireland. She holds an MSc in Pharmaceutical Analysis from the University of Strathclyde, Glasgow and has 8 years' experience of analytical method development in the pharmaceutical industry including development of a chromatographic method for the European Pharmacopoeia. Geraldine also holds an MSc in Science Communication from Dublin City University. Her interests include analytical chemistry, pharmacognosy and science communication.

Thomas Smyth
Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland

Thomas Smyth received his PhD in Pharmaceutical Biotechnology from Ulster University. After working for several years in Teagasc he is currently a lecturer at the Institute of Technology Sligo, Ireland. He has more than 6 years' experience in the analysis of marine components and over 15 years' experience in natural product chemistry. His interests are analytical chemistry, mass spectrometry, marine and terrestrial natural products, food chemistry and nutraceuticals.

Aodhmar Cadogan
Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo, F91 YW50, Ireland,
cadogan.aodhmar@itsligo.ie

Aodhmar Cadogan is a lecturer of Analytical Chemistry and Forensic Science at the Institute of Technology Sligo, Ireland. She received her PhD in Electrochemistry and Sensors from Dublin City University, Ireland. Following her PhD she spent some time in the area of Residue Analysis in Food. This was followed by 12 years of public sector and industrial experience in the areas of Forensic Science and the Diagnostic Device Industry. More recent research has concentrated on the chemical analysis of marine bathwater, seaweed and forensic materials.

Appendix N Publication

Environ Geochem Health
DOI 10.1007/s10653-017-0015-6

ORIGINAL PAPER

In vivo uptake of iodine from a *Fucus serratus* Linnaeus seaweed bath: does volatile iodine contribute?

Tarha Westby · Aodhmar Cadogan · Geraldine Duignan

Received: 9 January 2017 / Accepted: 11 August 2017

Abstract Seaweed baths containing *Fucus serratus* Linnaeus are a rich source of iodine which has the potential to increase the urinary iodide concentration (UIC) of the bather. In this study, the range of total iodine concentration in seawater (22–105 μg L^{-1}) and seaweed baths (808–13,734 μg L^{-1}) was measured over 1 year. The seasonal trend shows minimum levels in summer (May–July) and maximum in winter (November–January). The bathwater pH was found to be acidic, average pH 5.9 ± 0.3. An in vivo study with 30 volunteers was undertaken to measure the UIC of 15 bathers immersed in the bath and 15 non-bathers sitting adjacent to the bath. Their UIC was analysed pre- and post-seaweed bath and corrected for creatinine concentration. The corrected UIC of the population shows an increase following the seaweed bath from a pre-treatment median of 76 μg L^{-1} to a post-treatment median of 95 μg L^{-1}. The pre-treatment UIC for both groups did not indicate significant difference ($p = 0.479$); however, the post-treatment UIC for both did ($p = 0.015$) where the median bather test UIC was 86 μg L^{-1} and the non-bather UIC test was 105 μg L^{-1}. Results indicate the bath has the potential to increase the UIC by a significant amount and that inhalation of volatile iodine is a more significant contributor to UIC than previously documented.

Keywords Seaweed bath thalassotherapy · *Fucus serratus* Linnaeus · pH · Iodine · Urinary iodine · Inhalation

Abbreviations

ANOVA	Analysis of variance
CV	Coefficient of variation
DW	Dry weight
IDD	Iodine deficiency disorders
IO_3^-	Iodate
I^-	Iodide
I_2	Iodine
SD	Standard deviation
SK	Sandell–Kolthoff
UIC	Urinary iodine concentration
WHO	World Health Organisation

T. Westby · A. Cadogan · G. Duignan (✉)
Department of Life Science, School of Science, Institute of Technology Sligo, Ash Lane, Sligo F91 YW50, Ireland
e-mail: duignan.geraldine@itsligo.ie

T. Westby
e-mail: tara.westby@mail.itsligo.ie

A. Cadogan
e-mail: cadogan.aodhmar@itsligo.ie

Published online: 02 September 2017

Introduction

Iodine and seaweed

Thalassotherapy describes the use of seawater, marine products or sea air to benefit the body (Routh and

Bhowmik 1996). One thalassotherapy, indigenous to Ireland, is a seaweed bath prepared using hot fresh water, seawater and fresh brown seaweed such as *Fucus serratus* Linnaeus (L.) (Fig. 1). Fucales such as *Fucus serratus* L. contain a variety of beneficial components including iodine and other elements, fatty acids and antioxidants (Kim et al. 1996; Marsham et al. 2007; Morrissey et al. 2001; Nitschke and Stengel 2015; O'Sullivan et al. 2011; Peinado et al. 2014; Rupérez 2002 and van Netten et al. 2000).

The documented iodine concentration of *Fucus serratus* L. and *Fucus vesiculosus* (similar chemical composition) ranges from 1400 μg g^{-1} to over 2500 μg g^{-1} dry weight (Table 1). Previous work examined the release of iodine from *Fucus serratus* L. into a heated aqueous environment (Westby et al. (2016)). The iodine concentration reached ~4 mg L^{-1} after 40 min at 50 °C. Almost 80% of the maximum concentration was reached after 5 min, and increasing temperature led to a higher efflux of iodine.

Iodine is found in sea sediments (high concentration), on land in rocks, soils, vegetation and water sources, and in seawater and sea air (low concentration; Fuge and Johnson 1986; Muramatsu and Wedepohl 1998). In seawater, iodine can exist as molecular iodine (I_2), iodide ions (I^-), iodate ions (IO_3^-) and organic iodine (Li et al. 2009). In aqueous media, molecular iodine predominates under acidic conditions, whereas iodide and iodate tend to coexist in neutral or basic solutions (Fig. 2). In strongly acidic solutions (pH 2), iodide cannot coexist with iodate due to oxidation of iodide by iodate (Fuge and Johnson 1986; Bruchertseifer et al. 2003).

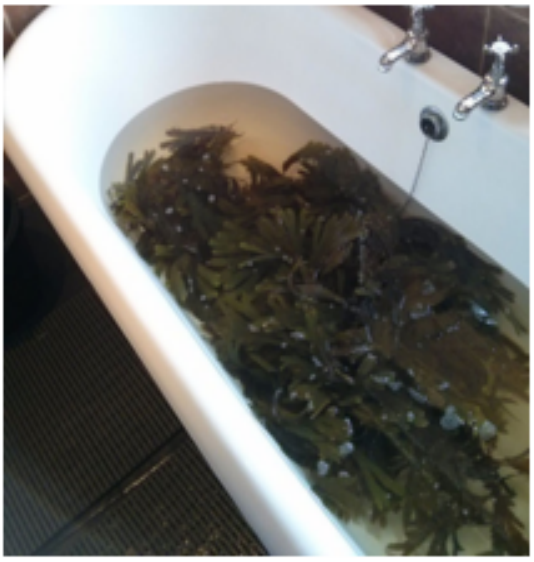


Fig. 1 Typical seaweed bath containing *Fucus serratus* L.

Iodine and health impact, uptake and UIC

Iodine is an essential component of the thyroid hormones which help the body control metabolism and normal growth and development. From a worldwide perspective, insufficient iodine intake is a significant public health issue leading to a range of iodine deficiency disorders (IDD) with mild to severe health impacts (WHO 2004; de Benoist et al. 2008; Zimmermann 2008). The WHO (2004) recommends a daily intake of 150–299 μg of iodine per day for adults to achieve optimal iodine nutrition (Table 2). Iodine enters the body mainly through the diet with other possible routes being drinking water, breathing gaseous iodine in the air and when placed on the skin (ATSDR 2004). In the context of a seaweed bath, intake is possible via dermal contact with the seaweed or seaweed bathwater and/or via inhalation of iodine gas.

Albèr et al. (2014) showed how the permeability of the skin barrier is highly dependent on the moisture level externally. They note that at humidity above 90% (similar to the seaweed bath environment) water loss is decreased due to the reduced driving force for water to evaporate from the skin. More interestingly, the overall permeability of the skin becomes significantly higher. This implies that increased hydration can alter the skin structure to facilitate the diffusion of most small molecules (Sparr and Wennerström 2001; Silva et al. 2007). Dermal application of slow release iodine preparations in surgical settings has been shown to lead to an increase in serum iodine concentration, and promote disinfection (Miller et al. 1989; Boothman 2009). These preparations contain molecular iodine which diffuses across the dermal layer more readily than ionic species. Intact skin can act as a barrier to iodide and iodate (Goldsmith 1983). In particular, iodide requires the sodium iodine symporter (NIS) in order to be transported through the dermis; the skin does not contain NIS (Ajjan et al. 1998).

Inhalation of iodine is also documented. Morgan et al. (1968) describe how iodine vapour is inhaled through the mouth and deposited in the respiratory tract, predominantly in the pharynx. From there, it is

Table 1 Reported iodine levels in two species of Fucales: *Fucus serratus* and *Fucus vesiculosus*

Seaweed species	Iodine concentration		References
	Fresh weight (FW) μg g^{-1}	Dry weight (DW) μg g^{-1}	
Fucus serratus	N/A	250–1400	Patti et al. (1990)
Fucus vesiculosus	N/A	732	Chance et al. (2009)
Fucus vesiculosus	276	N/A	Teas et al. (2004)
Fucus vesiculosus	245	1092	Nitschke and Stengel (2015)
Fucus serratus	595	2529	

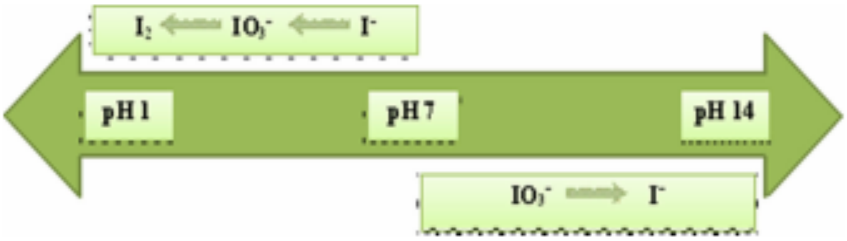


Fig. 2 Schematic diagram of iodine speciation in an aqueous environment relative to the prevailing pH

Table 2 Iodine nutrition status and equivalent daily intake (μg day^{-1}) according to WHO (2004)

UIC (μg L^{-1})	Iodine nutrition	Iodine intake (μg day^{-1}) for adults
<20	Severe deficiency	<30
20–49	Moderate deficiency	30–74
50–99	Mild deficiency	75–149
100–199	Optimal	150–299
200–299	More than adequate	300–449
>299	Possible excess	>449

carried to the digestive tract via saliva and absorbed via the intestines. Molecular iodine in particular is readily and extensively absorbed by inhalation (Risher and Keith 2009). While uptake of iodine via inhalation is mostly studied in the context of radiological pollution due to the release of radioactive iodine (Nauman and Wolff 1993), Smyth et al. (2011, 2016) investigated iodine respiration in coastal areas. Specifically, they compared the UIC of populations in seaweed-rich coastal areas, low seaweed abundance coastal areas and inland areas in Ireland. They found that those living in a seaweed-rich area displayed the highest urinary iodine concentration (UIC) which infers that gaseous iodine is a contributor to iodine intake.

Urinary analysis is a good indicator of recent intake of iodine as approximately 90% of the daily intake is excreted in the urine (Zimmermann 2008). Iodine deficiency, or excess, is determined based on the median UIC of a group as there is considerable day-to-day variation in iodine excretion between individuals due to hydration, diet and metabolism. Consequently, a large sample size is required to estimate the median UIC of a group (WHO 2007).

The aim of this paper was (i) to examine seaweed bathwater under laboratory-controlled conditions and establish the mean and seasonal iodine concentration of the seawater and bathwater used in the preparation of baths, (ii) to establish the pH of this matrix as it influences iodine speciation and (iii) to carry out an in vivo study to investigate whether a subjects' UIC increases by immersion in or adjacent to a bath and if so what is the mechanism involved in iodine uptake by the body.

Methods and materials

Sample preparation, storage and stability

Samples of *Fucus serratus* L. and seawater were collected weekly over a 12-month period (February 2013–January 2014) from Voya Seaweed Baths, Strandhill, Co. Sligo, Ireland (54°16′13.2″N, 8°36′39.9″W). The *Fucus serratus* L. samples were stored at ambient temperature for 18–25 h (with the exception of one sample 40 h) until processing. Details about the harvesting, rinsing and preparation of the samples have been reported previously (Westby et al. 2016). In summary, 500 ml water was heated to 70 °C. *Fucus serratus* L. (150 ± 0.05 g) and 500 ml water were added and maintained at 55 °C for 40 min. Aliquots (50 ml) were collected and stored at −20 °C. A 50-ml sample of the seawater was collected concurrently, filtered through a 0.45-µm Whatmann filter and stored as above. Samples were thawed at room temperature prior to analysis. Seawater samples were analysed directly, and bathwater samples were diluted 1:50. Ultrapure water (18.2 Ω) was used for sample preparation.

When the seaweed is placed in the hot aqueous environment, it stimulates the release of alginate, a highly abundant polysaccharide present in the cell wall. This increases the viscosity of the sample. Homogeneity of the simulated bathwater (1 L) was examined by taking samples at different stirring speeds (low, medium and high) and location (top, centre and bottom) following removal of the frond. Analysis of the total iodine concentration showed the matrix to be homogenous; samples were extracted centrally using a medium stir speed. Stability of the frozen samples (−20 °C) was examined by storing samples for 12 months and subjecting them to monthly freeze–thaw cycles. Analysis of the total iodine concentration indicated no significant difference across samples stored for 12 months at −20 °C ($p = 0.873$). Also, repeated freezing and thawing did not affect the iodine concentration significantly ($p = 0.130$ across ten freeze–thaw cycles of one sample). To examine the seasonal variation, the data were grouped by season (spring: February–April; summer: May–July; autumn: August–October; winter: November–January) and averaged.

Total iodine and pH measurement

The Sandell–Kolthoff (SK) micromethod was used for the measurement of total iodine as discussed previously (Westby et al. 2016). The pH of 22 seawater samples (collected in June 2015) and simulated bathwater samples ($n = 45$) was determined using a Eutech standard electrode with internal silver/silver chloride reference [supplied by Thermo Scientific (Ireland)] in combination with a Eutech pH 700 pH meter [supplied by Thermo Scientific (Ireland)]. This was calibrated with buffer solutions pH 4.01 and 10.01 (Lennox Laboratory Supplies, Dublin, Ireland).

In vivo study and UIC analysis

Following ethics approval from IT Sligo Research Ethics Committee, 30 adult subjects aged 20–50 years were selected randomly through informal and formal advertisement in IT Sligo and Voya Seaweed Baths. The following exclusions applied: not pregnant, no history of underactive or overactive thyroid or sensitivity/intolerance to seaweed/seaweed-containing products, no surgical procedures in the previous 6 months, not taking iodine supplements and not taken a seaweed bath in the week prior to the study. On completion of a certificate of consent and questionnaire, the 30 subjects were assigned randomly to two study groups: bathing ($n = 15$) and non-bathing ($n = 15$). Both groups would be exposed to seaweed baths, consisting of cold seawater/hot tap water (total volume ~100 ± 20 L) and 4 ± 0.5 kg *Fucus serratus* L. Bathing subjects were requested to immerse themselves in the seaweed bath for 40 min. Non-bathing subjects were requested to sit beside the seaweed bath, inhale normally for 40 min and not have any contact with the seaweed or bathwater. All subjects were requested to refrain from using steam facilities during the treatment, and bathers were requested not to shower after the treatment but to towel dry. Drinking water, 1.5 L, was provided to keep all subjects hydrated during the treatment. A food and drink log was kept during the study period. The study was carried out over 4 days: 2 pre-treatment days and 2 post-treatment days within a 2-week period. Pre-treatment urine samples were collected over 32 h at 10.00, 11.00, 12.00, 18.00 h, bedtime, morning, 12.00 and 18.00 h.

The pre-treatment sample was to establish a normal UIC profile for each subject. Post-treatment urine samples were collected the following week at the same time intervals as the pre-treatment samples; however, in this instance the first two samples were collected immediately prior to and following the seaweed bath. All urine samples were collected using pre-labelled 25-ml sterile plastic vials (Sarstedt). Iodine is stable in urine, provided that no evaporation takes place; therefore, a preservative was not required during sample collection. Samples were stored at −20 °C and allowed to reach room temperature prior to analysis. The SK method is a standard method used for UIC (Dunn et al. 1993) and has been adapted to a microplate method (Mina et al. 2011; Ohashi et al. 2000). The method has been validated for the bathwater matrix (Westby et al. 2016), and the same method was used directly for UIC following inter-laboratory comparisons with two external laboratories: Laboratory 1 ($n = 8$) correlation coefficient = 0.9951 and Laboratory 2 ($n = 16$) correlation coefficient = 0.9914. All samples were run in triplicate with no sample dilution.

The creatinine concentration of the samples was determined in order to calculate the corrected iodine concentration (Soldin 2002). This was carried out to eliminate erroneous iodine concentrations due to dehydration or malnutrition as creatinine acts as an internal standard in the urine sample. Creatinine analysis was carried out using the Jaffé method on the Roche Modular analyser at the Biochemistry Department, Sligo University Hospital. This is a kinetic colorimetric assay where sodium hydroxide (0.2 mol L^{-1}) is added to a sample followed by picric acid (25 mmol L^{-1}). Under alkaline conditions, creatinine forms a yellow-orange complex with picrate. The rate of colour formation is directly proportional to the creatinine concentration in the sample. The corrected iodine value of each sample was then determined using the following equation (Zimmermann 2008):

$$\text{Corrected iodine} = \left(\frac{\text{Iodine (mcg/L)}}{\text{Creatinine (mmol/L)}}\right) \times 8.85$$

Statistical analyses were carried out using a one-way ANOVA in Minitab where a p value <0.05 is considered significantly different.

Results

Seawater and seaweed bathwater

The iodine concentration of seawater samples taken across 1 year ranged from a minimum of 22 µg L^{-1} to a maximum of 105 µg L^{-1} with an average of 52 ± 19 µg L^{-1} SD ($n = 42$). A one-way ANOVA of the grouped data showed no significant seasonal trend ($p = 0.178$). The iodine concentration of simulated bathwater samples taken across the year ranged from 808 to 13,734 µg L^{-1} with an average of 5877 ± 4028 µg L^{-1} SD ($n = 45$). Figure 3 illustrates the mean weekly iodine concentration over the 1-year sampling period. There is a significant difference in iodine concentration ($p = 0.02$) across the full data set. Data were grouped by season and compared. There is significant difference between spring and summer ($p = 0.06$) and spring to autumn ($p = 0.03$). There is also significant difference between winter and summer ($p = 0.04$) and winter and autumn ($p = 0.035$). Conversely, there is no significant difference between summer and autumn ($p = 0.971$) or winter and spring ($p = 0.930$). The amount of seaweed in the 22 commercial baths used for the in vivo study, their iodine concentration and temperature before and after the 40-min bathing period were recorded as part of the study (Table 3). Analysis of 22 seawater samples showed an average pH of (8.48 ± 0.25 SD). Analysis of 45 bathwater samples (over 12 months) showed an average pH (5.88 ± 0.26 SD). ANOVA of the bathwater pH values indicated no significant difference across seasons ($p = 0.461$). It is suggested that approximately 10% of the available iodine was released during a simulated bath based on a limited number of baths (Westby et al. 2016). Similar calculations for this larger data set indicate ~13% release of available iodine in the simulated baths and ~8% release in the commercial bath based on an iodine concentration of ~ 300 µg g^{-1} fresh weight (Nitschke and Stengel 2015).

In vivo study

As diet is the main source of iodine, it was important to avoid any foods that may give rise to an increased UIC. Subjects were requested to avoid any iodine-rich foods (especially seafood (fish, shellfish and seaweed), dairy (egg yolks, milk, yogurt, cheese and butter), soy

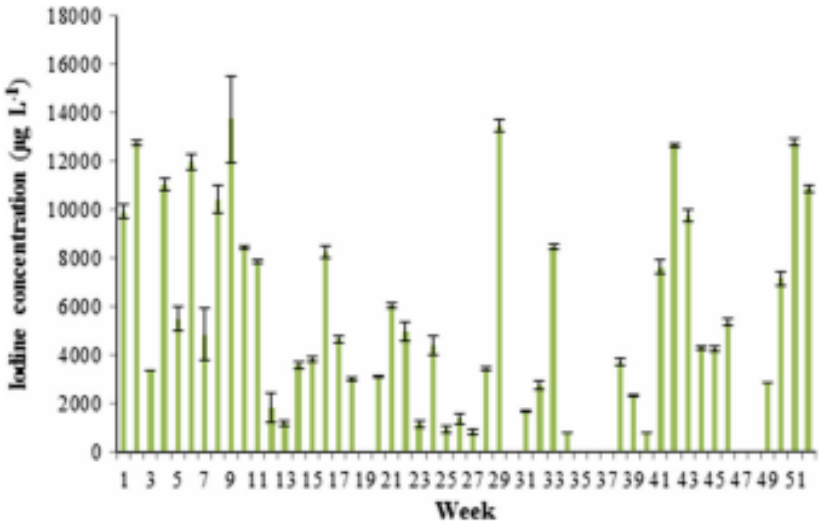


Fig. 3 Mean iodine concentration of simulated seaweed baths. *Error bar* indicates SD of three samples, week number relative to calendar year on x-axis

Table 3 Composition of a sample of commercial seaweed baths ($n = 22$) used in the in vivo study showing the total weight of seaweed, estimated volume of water, iodine composition and temperature before (t_0) and after (t_{40}) an average bathing period of 40 min

	Average ± SD	Range
Mass of *Fucus serratus* L. (kg)	4.04 ± 0.8	2.92–5.66
Seawater iodine concentration (μg L^{-1})	18.1 ± 7.9	6–33
Bathwater iodine concentration pre-treatment (μg L^{-1})	581.3 ± 333.9	257–1353
Bathwater iodine concentration post-treatment (μg L^{-1})	954.1 ± 440.4	320–2022
Temperature start (°C)	44.1 ± 2.5	40–51
Temperature end (°C)	38.8 ± 1.5	37–12
Volume (L)	100	80–120

(soybeans, soy sauce and tofu) and beans during the study and to document any food or drink consumed in the log provided. Unfortunately, adherence to this protocol was poor and analysis of the contribution of iodine from food has been limited. It must also be noted that factors such as weight, fitness level and thyroid function were not monitored during the study. Despite the UIC profile for some subjects being atypical, findings are relative to the population as a whole.

The UIC profile for the study population over 32 h is illustrated in Fig. 4. The UIC of subjects increased following the treatment from a pre-treatment median of 76 μg L^{-1} (range 60–94 μg L^{-1}) to a post-treatment median of 95 μg L^{-1} (range 64–104 μg L^{-1}). Pre-treatment values for each study group are not significantly different ($p = 0.479$). There is a significant difference between the pre-treatment UIC and post-treatment UIC for each study group ($p = 0.016$ and $p = 0.006$ for bathers and non-bathers, respectively). A p value of 0.015 represents a significant difference between the pre-treatment and post-treatment for the population as a whole. There is also a significant difference between the post-treatment UIC of the bathers and non-bathers ($p = 0.015$) where the post-treatment UIC of bathers was 86 μg L^{-1} and that of non-bathers was 105 μg L^{-1}.

Discussion

Bathwater

The average total iodine concentration of seawater, 52 μg L^{-1}, is within the documented range 40–80 μg L^{-1} (Ito and Hirokawa 2009; van Netten

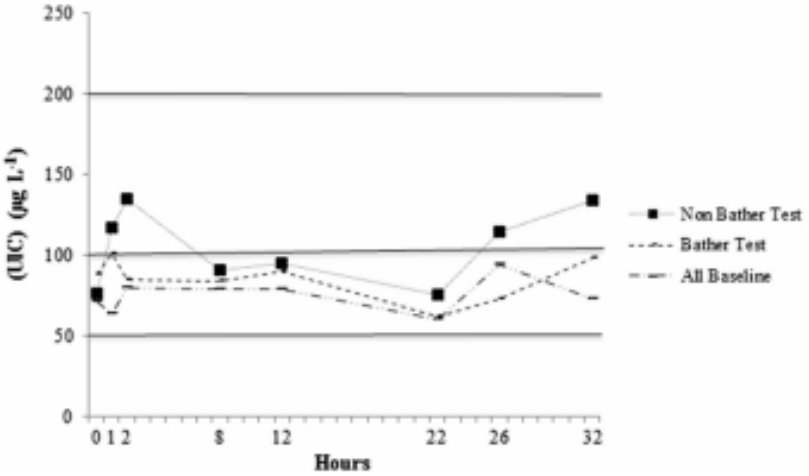


Fig. 4 Comparison of pre-treatment UIC and post-treatment UIC for all subjects (n = 30, 15 bathers, 15 non-bathers). Pre-treatment median: 76 μg L^{-1} and post-treatment median: 95 μg L^{-1}. The cut-off levels for iodine nutrition status, as illustrated in Table 2, are also indicated where 49 μg L^{-1} corresponds to moderate deficiency, 99 μg L^{-1} corresponds to mild deficiency and 199 μg L^{-1} corresponds to optimal levels

et al. 2000; Zheng et al. 2012) with no seasonal trend based on this data set. The iodine concentration of simulated baths (Fig. 3) and commercial-scale baths (Table 3) is significantly higher than that of seawater due to the efflux of iodine from the seaweed. It must be noted that the simulated bathwater was prepared with a higher ratio of seaweed to water than that of commercial-scale baths to allow for fluctuation in iodine levels over the sampling period.

Seasonal variation is evident where minimum levels are detected in summer (May–July) and maximum levels in winter (November–January; Fig. 3). This seasonal trend is similar to that discussed by Gall et al. (2004) and Patti et al. (1990) who state that iodine levels in brown algae are lowest in summer and highest in late autumn and winter. This in turn suggests that higher exposure to iodine for bathers occurs in winter.

While the seawater is alkaline, the bath itself is acidic, indicating that the addition of the seaweed *Fucus serratus* L. lowers the pH. This acidic environment affects the iodine species present in the bath (Fig. 2). It implies that while iodide (I^-) and iodate (IO_3^-) can coexist in (the) seawater, given the acidic nature of the seaweed bath, the iodine species present during a seaweed bath are most likely to be a high proportion of molecular iodine (I_2) along with smaller fractions of iodate and iodide.

In vivo study

The pre-treatment median UIC of subjects was determined to be 76 μg L^{-1}. This is in line with the findings of the WHO (2004) which classes the Irish population (adults 22–61) as mildly deficient (UIC 82 μg L^{-1}) in iodine. As expected, there was no significant difference found between the pre-treatment UIC of both groups ($p = 0.479$) where bathers pre-treatment median was 72 μg L^{-1} and non-bathers pre-treatment median was 75 μg L^{-1}.

Following the treatment, the median UIC (of both bather and non-bather) increased to 95 μg L^{-1} where bathers' post-treatment median was 86 μg L^{-1} and non-bathers post-treatment median was 105 μg L^{-1}. For the entire population, there was a significant increase in UIC post-treatment ($p = 0.015$).

Furthermore, results suggest that inhalation of iodine vapour contributes to the increase in UIC for all subjects as there was no possibility of dermal absorption for the non-bathers. This is illustrated by the significant differences between the pre-treatment and post-treatment UIC for the individual groups. For bathers, there was a 14 μg L^{-1} increase in UIC. This is approximately 20% increase from baseline UIC and is significant where $p = 0.016$. For non-bathers, there was a 30 μg L^{-1} increase in UIC. This represents a 40% increase from baseline UIC and is significant

where $p = 0.006$. The higher UIC for non-bathers might be attributed to the difference in position during the treatment for both groups. Normal breathing rates vary with activity and are lower when lying (0.32–0.45 $m^3\ h^{-1}$) compared with sitting (0.39–0.54 $m^3\ h^{-1}$; Kubota et al. 2013; Brudecki et al. 2017). Under the study conditions, the seated position of the non-bather group may have led to increased respiration rates.

Despite bathers having the potential for uptake via inhalation and dermal absorption, results suggest that the bioavailability of iodine via dermal routes is low. This is most likely due to the predominant species of iodine present, i.e. iodine gas (I_2) which is inhaled and iodate (IO_3^-) which does not cross the skin readily (Goldsmith 1983). Also, given that bathers are warm post-treatment, any iodine deposits on the skin are potentially lost due to perspiration.

Aside from the holistic therapeutic value of the bathing ritual, it has been shown that there is also a beneficial uptake of iodine during the treatment (up to 14% of the RDA of iodine in one treatment). The average total iodine levels in commercial seaweed baths was 768 μg L^{-1}. Seasonal variation is evident and suggests that the optimum bathing time for maximum exposure to volatile iodine is winter (November–January). The in vivo results confirm an increase in the UIC following exposure to the seaweed bath and point to inhalation of volatile iodine as the predominant route of uptake.

Acknowledgements This research was financially supported by a President's Bursary Award from the Institute of Technology Sligo. Seaweed samples and seaweed baths for the in vivo study were generously provided by Voya Seaweed Baths, Sligo. We acknowledge the technical assistance of Noreen Montgomery and Lydia Kirk of Sligo University Hospital and Dr James Murphy and Marcus Colon at Institute of Technology Sligo. We wish to also thank the volunteers who participated in the in vivo study.

References

Agency for Toxic Substances and Disease Registry (ATSDR) (2004). Public health statement iodine. Division of toxicology. http://www.atsdr.cdc.gov/ToxProfiles/tp158-c1-b.pdf, Accessed 29 Sept 2016.

Ajjan, R. A., Kamaruddin, N. A., Crisp, M., Watson, P. F., Ludgate, M., & Weetman, A. P. (1998). Regulation and tissue distribution of the human sodium iodide symporter gene. *Clinical Endocrinology, 49*, 517–523.

Albèr, C., Buraczewska-Norin, I., Kocherbitov, V., Saleem, S., Lodén, M., & Engblom, J. (2014). Effects of water activity and low molecular weight humectants on skin permeability and hydration dynamics—a double-blind, randomized and controlled study. *International Journal of Cosmetic Science, 36*, 412–418.

Boothman, S. (2009). Iodine white paper: The use of iodine in wound therapy. http://www.systagenix.co.uk/cms/uploads/1042_Iodine_White_Paper_A5_(INT)LP_003.pdf. Accessed 29 Sept 2016.

Bruchertseifer, H., Cripps, R., Guentay, S., & Jaeckel, B. (2003). Analysis of iodine in aqueous solutions. *Analytical and Bioanalytical Chemistry, 375*(8), 1107–1110.

Brudecki, K., Szufa, K., & Mietelsk, J. W. (2017). ^{131}I age-dependent inhalation dose in Southern Poland from Fukushima accident. *Radiation and Environmental Biophysics, 56*, 9–17.

Chance, R., Baker, A. R., Külpper, F. C., Hughes, C., Kloareg, B., & Malin, G. (2009). Release and transformations of inorganic iodine by marine macroalgae. *Estuarine, Coastal and Shelf Science, 82*(3), 406–414.

de Benoist, B., McLean, E., Andersson, M., & Rogers, L. (2008). Iodine deficiency in 2007: Global progress since 1993. *Food and Nutrition Bulletin, 29*(3), 195–202.

Dunn, J. T., Crutchfield, H. E., Gutekunst, R., & Dunn, A. D. (1993). *Methods for measuring iodine in urine*. Netherlands: ICCIDD/UNICEF/WHO.

Fuge, R., & Johnson, C. C. (1986). The geochemistry of iodine-a review. *Environmental Geochemistry and Health, 8*, 31–54.

Gall, E. A., Külpper, F. C., & Kloareg, B. (2004). A survey of iodine content in *Laminaria digitata*. *Botanica Marina, 47*, 30–37.

Goldsmith, L. A. (1983). *Biochemistry and physiology of the skin*. Oxford: Oxford University Press.

Ito, K., & Hirokawa, T. (2009). Iodine and iodine species in seawater: Speciation, distribution, and dynamics. In V. Preedy, G. Burrow & R. R. Watson (Eds.), *Comprehensive handbook of iodine* (pp. 83–91). San Diego: Academic Press.

Kim, M.-K., Dubacq, J.-P., Thomas, J.-C., & Giraud, G. (1996). Seasonal variations of triacylglycerols and fatty acids in *Fucus serratus*. *Phytochemistry, 43*(1), 49–55.

Kubota, S., Endo, Y., & Kubota, M. (2013). Effect of upper torso inclination in Fowler's position on autonomic cardiovascular regulation. *Journal of Physiological Sciences, 63*, 369–376.

Li, H.-B., Xu, X.-R., & Chen, F. (2009). Determination of iodine in seawater: Methods and applications. In V. Preedy, G. Burrow & R. R. Watson (Eds.), *Comprehensive handbook of iodine* (pp. 2–13). San Diego: Academic Press.

Marsham, S., Scott, G. W., & Tobin, M. L. (2007). Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chemistry, 100*(2007), 1331–1336.

Miller, K. L., Coen, P. E., White, W. J., Hurst, W. J., Achey, B. E., & Max Lang, C. (1989). Effectiveness of skin absorption of tincture of I in blocking radioiodine from the human thyroid gland. *Health Physics, 56*(6), 911–914.

Mina, A., Favaloro, E. J., & Koutts, J. (2011). A robust method for testing urinary iodine using a microtitre robotic system.

Journal of Trace Elements in Medicine and Biology, 25(4), 213–217.

Morgan, A., Morgan, D. J., & Black, A. (1968). A study of the deposition, translocation and excretion of radioiodine inhaled as iodine vapour. *Health Physics, 15*, 313–322.

Morrissey, J., Kraan, S., & Guiry, M. D. (2001). *A guide to commercially important seaweeds on the Irish coast*. Co. Dublin, Ireland: Bord Iascaigh Mhara/Irish Sea Fisheries Board.

Muramatsu, Y., & Wedepohl, K. H. (1998). The distribution of iodine in the earth's crust. *Chemical Geology, 147*(3–4), 201–216.

Nauman, J., & Wolff, J. (1993). Iodide prophylaxis in Poland after the Chernobyl reactor accident: Benefits and risks. *American Journal of Medicine, 94*, 524–532.

Nitschke, U., & Stengel, D. B. (2015). A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products. *Food Chemistry, 172*, 326–334.

O'Sullivan, A. M., O'Callaghan, Y. C., O'Grady, M. N., Queguineur, D., Hanniffy, D. J., Troy, D. J., et al. (2011). *In vitro* and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. *Food Chemistry, 126*, 1064–1070.

Ohashi, T., Yamaki, M., Pandav, C. S., Karmarkar, M. G., & Minoru, I. (2000). Simple microplate method for determination of urinary iodine. *Clinical Chemistry, 46*(4), 529–536.

Patti, F., Jeanmaire, L., Masson, M., Pinton, Ph, & Garcet, M. (1990). Temporal variations of iodine^{129}, potassium^{40} and technetium^{99} concentrations in *Fucus serratus* in the English Channel. *Journal of Radioanalytical and Nuclear Chemistry, 142*(2), 467–480.

Peinado, I., Girón, J., Koutsidis, G., & Ames, J. M. (2014). Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds. *Food Research International, 66*, 36–44.

Risher, J. F., & Keith, L. S. (2009). *Iodine and inorganic iodides: human health aspects*. Geneva: World Health Organization.

Routh, H. B., & Bhowmik, K. R. (1996). A glossary of concepts relating to balneology, mineral water, and the spa. *Clinics in Dermatology, 14*(6), 549–550.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry, 79*(1), 23–26.

Silva, C. L., Topgaard, D., Kocherbitov, V., Sousa, J. J. S., Pais, A. A. C. C., & Sparr, E. (2007). Stratum corneum hydration: Phase transformations and mobility in stratum corneum, extracted lipids and isolated corneocytes. *Biochimica et Biophysica Acta (BBA)—Biomembranes, 1768*(11), 2647–2659.

Smyth, P., Burns, R., Casey, M., Mullan, K., O'Herlihy, C., & O'Dowd, C. (2016). Iodine status over two decades: Influence of seaweed exposure. *Irish Medical Journal, 109*(6), 422.

Smyth, P. P., Burns, R., Huang, R. J., Hoffman, T., Mullan, K., Graham, U., et al. (2011). Does iodine gas released from seaweed contribute to dietary iodine intake? *Environmental Geochemistry and Health, 33*(4), 389–397.

Soldin, O. P. (2002). Controversies in urinary iodine determinations. *Clinical Biochemistry, 35*(8), 575–579.

Sparr, E., & Wennerström, H. (2001). Responding phospholipid membranes—interplay between hydration and permeability. *Biophysical Journal, 81*(2), 1014–1028.

Teas, J., Pino, S., Critchley, A., & Braverman, L. E. (2004). Variability of iodine content in common commercially available edible seaweeds. *Thyroid, 14*(10), 836–841.

van Netten, C., Hoption Cann, S. A., Morley, D. R., & van Netten, J. P. (2000). Elemental and radioactive analysis of commercially available seaweed. *Science of the Total Environment, 255*(1–3), 169–175.

Westby, T., Duignan, G., Smyth, T., & Cadogan, A. (2016). Method validation and determination of total iodine in seaweed bathwater. *Botanica Marina, 59*(4), 241–249.

WHO (2004). Iodine status worldwide WHO global database on iodine deficiency. http://apps.who.int/iris/bitstream/10665/43010/1/9241592001.pdf. Accessed 29 Sept 2016.

WHO (2007). Assessment of the iodine deficiency disorders and monitoring their elimination. A guide for programme managers. http://apps.who.int/iris/bitstream/10665/43781/1/9789241595827_eng.pdf. Accessed 19 Oct 2016.

Zheng, J., Takata, H., Tagami, K., Aono, T., Fujita, K., & Uchida, S. (2012). Rapid determination of total iodine in Japanese coastal seawater using SF-ICP-MS. *Microchemical Journal, 100*, 42–47.

Zimmermann, M. B. (2008). Iodine requirements and the risks and benefits of correcting iodine deficiency in populations. *Journal of Trace Elements in Medicine and Biology, 22*, 81–92.