Energetic Algae ('EnAlgae')

Project no. 215G

Public Output

Biochemical composition and quality assessment of native macroalgae collected along the Flemish coast

Authors
Benny F.G. Pycke, eCOAST
Marco Faasse, eCOAST

Contributors
Sophie Sleuyter, Laboratorium ECCA

Please cite this document as follows:

Pycke B.F.G. & M. Faasse. 2015. Biochemical composition and quality assessment of native macroalgae collected along the Flemish coast, Public Output report of the EnAlgae project, Oostende, December 2015, pp. 30, Available online at www-flanders-maritime-cluster.be.

This document is an output from the Energetic Algae ('EnAlgae') project, which has received European Regional Development Funding through the INTERREG IVB NWE programme. This study was commissioned by Flanders' Maritime Cluster and was financially supported by Flanders and the province of West Flanders.

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Contents

1 PART 1: LITERATURE SURVEY

1.1 Overview of Flemish seaweeds with potential for cultivation

Flemish coast. The Flemish coast consists mainly of relatively flat sandy beaches exposed to wave action and has a tidal range of about 3-5 meters. With a paucity of protected areas (such as bays), the Flemish coast lacks the natural environments that are most favorable for the development of rich seaweed populations. Seaweed growth along the Flemish coast is limited mainly to artificial structures that are either fixed and undergo tidal flows (*e.g.,* groynes, breakwaters, piers, port walls, and ropes) or float (*e.g.,* pontoons, buoys, and hulls of vessels). To a lesser extent seaweeds also occur on locations inland, including the nature reserve "Zwin" (Knokke-Heist), and the artificial lake "Spuikom" (Ostend).

Seaweed biodiversity. Seaweed biodiversity in the Belgian sector of the North Sea is relatively poor compared to the adjacent beaches of the Côte d'Opale, or those in Normandy and Brittany (Coppejans, 1998). The *"Guide to Seaweeds of the North of France and Belgium"* describes 192 macroalgae species of which 78 are known to occur along the Flemish coast (Coppejans, 1998), and about 20 species are harvested for commercial use in France today (Mesnildrey *et al*., 2012). A Dutch desk study from 2009 investigating seaweed species for cultivation in Zeeland described 221 seaweed species in the Scheldt delta area, of which 18 species were found to be most appropriate for cultivation and consumption (Busink *et al.*, 2009).

Seaweed species. The artificial hard substrates along the Flemish coast are often quite densely covered with seaweeds. The green seaweed zone is ubiquitous on hard substrates and consists mainly of sea lettuce (*Ulva* sp.), gutweed (ex-*Enteromorpha* sp., now *Ulva* sp.), and *Blidingia*species. Bladderwrack (*Fucus vesiculosus*) is the principal large brown seaweed along the Flemish coast. As a result of sand scouring, groynes are typically not covered with large brown seaweeds. On higher dams and in less exposed port areas, clear brown seaweed zones can develop with bladderwrack (*Fucus vesiculosus*),toothed wrack (*Fucus serratus*), spiral wrack (*Fucus spiralis*), and rockweed (*Ascophylum nodosum*). In such areas, the two large non-native species Japanese wireweed (*Sargassum muticum*) en wakame (*Undaria pinnatifida*) will also develop. The number of red seaweed species is somewhat limited in Belgium; in the port areas, mainly Laver (*Porphyra* sp.), *Polysiphonia* spp., and *Ceramium* spp. occur and are accompanied by a couple of additional species (MIRA, 2006). A small number of seaweed species native to the Flemish coast currently are harvested and/or cultivated in European countries (**Table 1**). Still, those species that are typically investigated for cultivation in the European Union (including *Laminaria digitata* and *Saccharina latissima*) are not easily found along the Flemish coast because of their preference for permanently submerged, non-turbid locations that are protected from sand scouring.

Environmental factors. Influenced mainly by tides and the turbidity of the water, Flemish seaweeds will grow predominantly in the mediolittoral (periodically submerged) and infralittoral (constantly submerged) zones. Only a limited number of species (including *Blidingia* sp.) occur in the supralittoral zone (*i.e.*, splash zone). Most species of seaweed in Belgium are well adapted to surfacing for several hours per day and many species can lose substantial amounts of moisture (up to 90%) without being damaged. In the Dutch Scheldt delta, seaweeds rarely occur more than 3 m under the low tide level (Busink *et al.*, 2009). This is due to the high turbidity of the water and poor permeation of light as well as the lack of proper substrates below those depths (Busink *et al.*, 2009). In Belgium the largest biodiversity on groynes and breakwaters typically occurs on the Eastern side of the structure because it is typically more protected against sand scouring.

Despite its low natural seaweed biodiversity, the Belgian North Sea can still be a favorable environment to cultivate native or non-native seaweeds because of the favorable physicochemical properties of the water and the increasing economic interest in seaweeds. In France, almost 60.000 tons of seaweed are produced annually (for a turnover of about 2.7 million EUR, or 45 EUR/ton), but only 50 tons originate from seaweed aquaculture; the rest comes from wild seaweeds harvested offshore or onshore, and from wrecked seaweed. Seaweed aquaculture exists in France, but only 2 species are grown, namely the non-native species *Undaria pinnatifida* (wakame) and *Saccharina latissima* (kombu). Six seaweed farms are active in Brittany (4 in North and 2 in South), and one more is located in Vendée. Brittany also has two hatcheries that produce plantlets (Mesnildrey *et al.*, 2012).

Industry-driven research into the biochemical composition of native seaweeds can provide useful information to set up a business case for cultivation or processing of seaweeds in Flanders. The present report focuses on three genera that are native to the Flemish coast, have the largest economic potential (*e.g.*, added value), and could be cultivated in Flanders. These three selected genera of native seaweeds comprise type species listed below (all of which are authorized for human consumption in France) (Burtin, 2003).

1. ***Ulva* species:** The sea lettuce *Ulva lactuca* is a small green alga (up to 30 cm across) with a broad, crumpled frond that is tough, translucent and membranous. The species attaches to rock via a small holdfast. *Ulva lactuca* is found at all levels of the intertidal zone. In very sheltered conditions, plants that have been detached from the substrate can continue to grow, forming extensive floating communities. The plant tolerates brackish conditions and can be found on suitable substrata in estuaries (Mesnildrey *et al.,* 2012).
2. ***Fucus* species:** *Fucus serratus*, the toothed wrack, is a robust, shrubby olive-brown seaweed that grows in high densities low on the seashore. The fronds do not bear air bladders. The whole plant typically grows to approximately 60 cm in length. The fronds have a serrated edge and grow from a short stalk. *F. serratus* is found on hard substrata on the lower shore in areas of coastline sheltered from sand scouring (Mesnildrey *et al.,* 2012). *Fucus vesiculosus* is common on the mid-shore, typically found below *Fucus spiralis* in a zone further up the shore from *Fucus serratus*. This species is found on intertidal rocky shores in a wide range of exposures and can be found in high densities living for approximately 4-5 years. (Mesnildrey *et al.,* 2012). *Fucus spiralis* is an intertidal seaweed that is found on the upper shore below the zone of *Pelvetia canaliculata* and above *Fucus vesiculosus* and *Ascophyllum nodosum*. This species attaches to rocky substrates on sheltered to moderately exposed shores. The alga grows up to 40 cm in length, does not bear air bladders and lives for up to 4 years. The species can tolerate a high level of desiccation. The fronds have a characteristic ridge along the edge of the receptacles (Mesnildrey *et al.,* 2012).
3. ***Porphyra* species:** *Porphyra umbilicalis*, also called purple laver, is a small red alga (up to 20 cm across) with an irregularly shaped, broad frond that is membranous but tough. This species is highly adaptable to conditions on different parts of the rocky shore and is able to withstand prolonged periods of exposure to the air and a greater degree of wave action than most other red algae. This species occurs alone or in dense colonies throughout the intertidal area but is found most frequently at upper levels (Mesnildrey *et al.,* 2012). Conversely, *Porphyra purpurea* often occurs in the lower levels of the intertidal area.

Table 1: *Overview of selected benthic macroalgae (seaweed) species native to the Flemish coast with respective economic descriptors. Selection based on their commonness and commercial interest in Western Europe. ([a]: Mesnildrey et al., 2012; [b]: Atack et al.).*

Species	Applications[b]	Harvested (2010)[b]	Harvesting period[a]	Farming (incl. trials)[b]	Landings (tons/FR/2010)[a,*]
Green algae					
Ulva sp.	AGRI, VEGE	GB, IR, FR, NOR, SP, PO	Year-round	FR	77*
Blidingia sp.	-	-	-	-	-
Brown algae					
Fucus spp.	AGRI, HEAL, CHEM	GB, IR, FR, SP, PO	Year-round	-	2.681
Ascophyllum nodosum	HEAL	GB, IR, FR, NOR, SP	Year-round	-	1.030
Red algae					
Porphyra umbilicalis	VEGE, HEAL	GB, FR, SP	March to August	IR, FR, NOR	53
Porphyra purpurea	-	-	March to August	-	-

AGRI: Agricultural supplies; VEGE: Seaweed vegetable; HEAL; Health & well-being; CHEM: Food processing & chemistry; GB: Great-Britain; IR: Ireland; SP: Spain; PO: Portugal; FR: France; NOR: Norway; - : Not reported.

*: Landings in fresh/wet weight equivalent.

1.2 Composition of Flemish seaweed species

While seaweeds are used in Europe predominantly as a source of thickening and gelling agents for various industrial applications, they also exhibit original and interesting nutritional properties. From a nutritional standpoint, the principal value of seaweeds is their high mineral (iodine, calcium) and soluble dietary fiber contents, the occurrence of vitamin B12 and specific components such as astaxanthin, fucoxanthin, β-carotene, fucosterol, and phlorotannin. As a result, seaweeds are a valuable source of health benefit molecules for food processing and nutraceutical industry (Burtin, 2003).

The biochemical composition of seaweeds can differ between species, but it can also change in function of time. This is because seaweeds are exposed to seasonal variations of abiotic factors and different life stages that influence their metabolic responses (photosynthesis and growth rates) and levels of proximate constituents (Khairy & El-Shafay, 2013). As a result, all compositional data obtained from natural resources will only have a relative significance because the time of sampling, sampling locations, and life stage will vary between studies and the plants were grown under uncontrolled conditions. Seaweeds are wild marine plants, and specific laboratory analysis may vary due to naturally occurring fluctuations in the plants. These fluctuations may be due to time of year, tidal flows, weather patterns, and other factors. Seasonal variations in the chemical composition and nutritional value have been reported in common marine seaweeds from different parts of the world. The nutritional properties of seaweeds and their seasonal oscillation are typically poorly known but can be evaluated from the chemical composition. Ecological significance and knowledge of chemical composition of marine seaweed is important for both assessment of nutritional value to marine invertebrate or vertebrate herbivores and for the evaluation of potential sources of protein, carbohydrate and lipid for commercial use, or for possible human consumption (Banerjee *et al*., 2009). In the case of cultivated seaweeds, the nutritional composition and contaminant levels should be determined routinely on a batch level for legal and quality assurance purposes.

The respective chemical compositions described in the literature for the three selected seaweed species (*Ulva* sp., *Fucus* sp., and *Porphyra* sp.) is discussed in Part III of this report alongside the data obtained for the Flemish seaweeds in the frame of this research. Below are discussed the principal constituents that were targeted in the present research.

Overview of valuable constituents

Polysaccharides and dietary fibres. Seaweeds contain large amounts of polysaccharides, notably cell wall structural polysaccharides that are extracted by the hydrocolloid industry: alginate from brown seaweeds, carrageenans and agar from red seaweeds (**Table 2**). Other minor polysaccharides are found in the cell wall: fucoidans (from brown seaweeds), xylans (from certain red and green seaweeds), ulvans in green seaweeds. Seaweeds also contain storage polysaccharides, notably laminarin (*β*-1,3- glucan) in brown seaweeds and floridean starch (amylopectin- like glucan) in red seaweeds. Most of these polysaccharides (agars, carrageenans, ulvans and fucoidans), are not digested by humans or human intestinal bacteria, and therefore can be regarded as dietary fibres. Among polysaccharides, fucoidans were particularly studied as they showed medically-relevant biological activities (including anti-thrombotic, anti-coagulant, anticancer, anti-proliferative, anti-viral, and anti-complementary agent, anti-inflammatory activities). Conversely, xylans and laminarans are completely and rapidly degraded by intestinal bacteria, whereas alginates are only partly degraded and lead to a substantial production of short chain fatty acids (Burtin, 2003).

Proteins and amino acids. The protein content of brown seaweeds is generally low (average: 5-15 % of the dry weight), whereas higher protein contents are recorded in green and red seaweeds (on average 10-30 % of the dry weight). In some red seaweeds, such as *Palmaria palmata* (dulse) and *Porphyra tenera* (nori), proteins can represent up to 35 and 47% of the dry matter, respectively. These levels are comparable to those found in high-protein vegetables such as soybeans (in which proteins represents 35% of the dry mass). The protein levels of *Ulva* spp. are in the range 15-20% of the dry weight. Except for *Undaria pinnatifida*, which contains 11-24 % proteins, other brown seaweeds (*Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Himanthalia elongata*) have relatively low protein contents. (Burtin, 2003).

Tabel 2: *Global production of phycocolloids in 2009. (Bixler & Porse, 2010).*

Type	Production (tonnes)	Worth (million $)	Seaweeds
Agars	9.600	173	Incl. Gelidium, Gracilaria
Carrageenans	50.000	>527	Incl. Chondrus crispus
Alginates	26.500	318	Phaeophyceae

Mineral Fraction. Seaweeds draw from the sea a wealth of mineral elements, macro-elements and trace elements. The mineral fraction of some seaweeds accounts for up to 36% of dry matter. The brown seaweeds have traditionally been used for treating the condition of thyroid goitre. The link between iodine and the thyroid hormones was established soon afterwards. Laminaria sp. and Fucus vesiculosus in particular are known to have a high content of iodine, with the former the main source as it contains 1.500 to 8.000 mg/kg dry weight. Fucus spp. typically contains 500 to 1.000 mg/kg dry weight of iodine. Conversely, Ulva spp. and Porphyra umbilicalis contain substantially lower levels of iodine, one study report levels of 16 mg/kg wet weight and 13 mg/kg wet weight, respectively (MacArtain et al., 2007). Seaweeds are also one of the most important vegetable sources of calcium. Calcium content may be as high as 7% of the dry weight in macroalgae and up to 25 to 34% in the red seaweed Lithothamnium calcareum. Seaweed consumption may thus be useful in the case of expectant mothers, adolescents and elderly at risk of a calcium deficiency. Yet, in April 2015 the Superior Health Council of Belgium has published recommendations to limit the daily use of seaweeds for children and expecting women (SHC, 2015) as a result of the deleterious health effects associated with the excessive consumption of seaweeds, which typically contain elevated levels of non-essential metals. Despite its high mineral contents, the co-occurrence of certain minerals with anionic polysaccharides (alginate, agar or carrageenan) may limit their absorption. For instance, the strong affinity of divalent cations (particularly calcium) for carboxylic polysaccharides (alginates) probably limits their availability. In contrast, the weakness of the linkages between polysaccharides and iodine allows rapid release of this element (Burtin, 2003).

Lipids and fatty acids. Lipids represent only 1-5% of algal dry matter and show an interesting polyunsaturated fatty acid composition particularly regarding omega-3 and omega-6 acids which may play a role in the prevention of cardio-vascular diseases, osteoarthritis diabetes, and many other diseases (Simopoulos, 2002). The green algae show high levels of alpha linolenic acid (ALA, ω3 C18:3), but this omega-3 acid is less potent than the longer chains omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but ALA is still widely used by the food industry as an alternative, more cost-effective, omega-3 fatty acid. Conversely, the red and brown algae are rich in fatty acids with 20 carbon atoms: eicosapentanoïc acid (EPA, ω3 C20:5) and arachidonic acid (AA, ω6 C20:4) (Burtin, 2003).

Besides fatty acids, unsaponifiable fraction of seaweeds was found to contain carotenoids (such as *β*-carotene, lutein and violaxanthin in red and green seaweads, fucoxanthin in brown seaweeds), tocopherols, sterols (such as fucosterol in brown seaweeds), and terpenoids. Lipidic extracts of some edible seaweeds showed antioxidant activity and a synergistic effect with the tocopherol (Burtin, 2003).

Polyphenols (micronutrient). Seaweeds can contain elevated levels of polyphenols with anti-oxidative properties (Keyrouz *et al.*, 2010). Algal polyphenols, also called phlorotannins, differ from terrestrial plant polyphenols. Phlorotannins constitute an extremely heterogeneous group of molecules providing a wide range of potential biological activity. Antioxidants are of interest for the potential health-benefits associated with their ability to capture free radicals (Okawa *et al.*, 2001). Different species can differ in polyphenol content, but how much polyphenols can be extracted from certain species also depends on the availability of optimized extraction procedures (Keyrouz *et al.*, 2010). The method selected for drying seaweeds also has a major impact on the anti-oxidant levels in the product. Elevated temperatures (>50°C) and prolonged drying times are ill-fated (Cox, 2012). Still, for *Himanthalia elongata* drying temperatures of 35-40°C were found more favorable than 25-30°C (presumably because of the shorter drying time at higher temperatures) (Gupta *et al*., 2011). Drying the seaweeds with desiccated air is also beneficial for the drying times as well as the anti-oxidant levels (Djaeni & Sari, 2015). Highest contents are found in brown seaweeds, where phlorotannins range from 5 to 15% of the dried weight. Antioxidant activity of polyphenols extracted from brown and red seaweeds has already been demonstrated by *in vitro* assays (Burtin, 2003).

Carotenoids (micronutrient). Carotenoids are powerful antioxidants. Recent studies have shown the correlation between a diet rich in carotenoids and a lower risk of cardio-vascular disease, cancers (β-carotene, lycopene), as well as opthalmological diseases (lutein, zeaxanthin)(Stahl & Sies, 2005). Brown seaweeds are particularly rich in carotenoids especially in fucoxanthin, β-carotene, violaxanthin. The main carotenoids in the red algae are the β-carotene and α-carotene and their dihydroxylated derivatives: zeaxanthin and lutein. The carotenoid composition of the green algae is similar to that of higher plants: the main carotenoids present are the β-carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin (its metabolite, astaxanthin) and neoxanthin. A lot of studies demonstrated the antioxidant properties of the algal carotenoids and the role they play in preventing pathologies linked to oxidative stress (Burtin, 2003).

Mannitol (micronutrient). Mannitol is a valuable compound with many applications, such as in medicine (as diuretic or for flavoring) and food products (as sweetener or coating agent). It has low glycemic and insulinemic indices, and has a low hygroscopicity. Mannitol is found in almost all plants, including seaweeds from which it can be directly extracted. In China, isolation from seaweed is the most common form of mannitol production. Mannitol is particularly abundant in brown algae, such as *Laminaria* species. Seaweed exudates can contain around 20% of mannitol.

Environmental Contaminants. Seaweeds grow in natural environments that are rarely pristine and protected from ubiquitous anthropogenic pollutants, such as heavy metals, deleterious organic compounds, and microbial contaminants. As a result, the ingestion of seaweeds may constitute a health risk if they contain elevated levels of such contaminants. France was the first European country to establish a specific regulation concerning the use of seaweeds for human consumption as non–traditional food substances. Currently, 12 macroalgae (6 brown algae, 5 red algae, 2 green algae) and two microalgae are authorized as vegetables and condiments, including the three selected genera (*Ulva*, *Fucus*, and *Porphyra*). These edible seaweeds must meet safety regulations in terms of toxicological and bacteriological criteria (**Table 3**). This regulation, in addition to the potential nutritional properties of seaweeds, allows the food industry to include seaweeds as raw or semi-processed materials in the formulation of seafood products.

In Belgium, non-essential metals (including lead, cadmium, mercury, arsenic, and tin) in seaweeds are regulated via the European and/or Belgian legislation (FOD Volksgezondheid, Veiligheid van de Voedselketen, en Leefmilieu, 2015). The maximum permitted levels for these metals vary per food type as some food products are used more frequently than others and may represent more important exposure routes. Because seaweeds are not commonly consumed by Europeans, they are not catalogued together with the conventional vegetables, but are catalogued under food supplements. Food supplements typically have less stringent limits compared to the conventional Western vegetables because for the average European citizen the former food type represents a more important source of contaminants compared to food supplements. Yet, because vegetarians are more likely to consume more seaweed (compared to the average European citizen), the Superior Council for Health has published recommendations to limit daily seaweed consumption because of the elevated levels of arsenic and other contaminants in seaweed compared to other vegetables (SHC, 2015).

Table 3: *Quality criteria applied to edible seaweeds sold in France. (From: Burtin, 2003; CEVA, 2014).*

Criteria	Limit *(unit)*
Toxic minerals	*(mg/kg dry matter)*
Iodine	2,000
Lead	5.0
Tin	5.0
Arsenic (inorganic)	3.0
Cadmium	0.5
Mercury	0.1
Microorganisms	*(colony-forming units/g)*
Aerobes	100
Anaerobes	100
Fecal coliforms	10
Clostridium perfringens	1

1.3 Applications of Flemish seaweed species

Seaweed can be used for many applications. The uses of seaweeds depend on the species. Historically, seaweeds were used in Europe for heating, in mattresses, for feed, and for human food in times of starvation. Additionally, seaweeds were used to extract soda, hydrocolloids, and iodine, a popular disinfectant in the 19th century. The primary uses may have evolved, but some traditional uses remain and are still observed in some coastal areas, for example the use of seaweed for cattle feed and for soil improvement (Mesnildrey *et al*., 2012).

Some seaweeds can be eaten as a vegetable, while others are used as fertilizers or processed mainly for the production of hydrocolloids, including carrageenan, agar-agar, and alginate. The final products depend on the processes used. The food-processing industry, chemistry and microbiology are the main markets for seaweed in France, with 75 % of the harvested seaweed (domestic production and imports) used for this sector (**Figure 1**). Approximately 25 % of the seaweed is used in the agricultural, health and well-being sector (Mesnildrey *et al*., 2012). Seaweeds are of interest for the mineral content (Ca, P, Mg, I, Na), vitamins (A, B1, B2, C, D, E), polysaccharides, protein content, lipids and polyunsaturated fatty acids, and pigments (phycobilins & carotenoids, e.g. *β*-carotene, astaxanthin, fucoxanthin, and zeaxanthin). Oleic acids are of interest for their antioxidant activities and linolenic acid for its antimicrobial activity (Ferronato *et al*., 2012).

Because most of the French seaweed is harvested on the shores of Brittany, many companies have established in the area. Yet, it should be emphasized that the principal species harvested in France for hydrocolloid industry (namely *Laminaria* sp., *Gelidium* sp., *Chondrus* sp., and *Mastocarpus* sp.) are not among the main wild species occurring along the Flemish coast. Still, *Porphyra* spp., which occur widely in Flanders, are a known source of agar-agar (Mesnildrey *et al*., 2012).

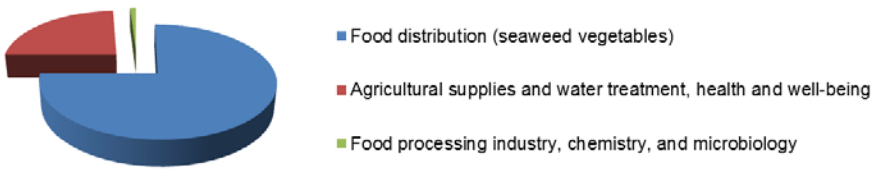

Figure 1: *Main uses of seaweed in France (French production & imports). (Adapted from: Mesnildrey et al., 2012).*

Overview of valuable constituents

Food processing, chemistry, and microbiology. In France, *Laminaria digitata* and *L. hyperborea* are harvested and processed for the production of alginate, which is used in the in the global textile industry, in food-processing industries, in biotechnological applications, and some other industries. Agar-agar is obtained from *Gelidium* spp*., Gracilaria* spp*.* and *Porphyra* spp*.* and is used in the food-processing industry and in microbiology laboratories to prepare culture media. Carrageenans are produced from *Chondrus crispus* and *Mastocarpus stellatus*. Carrageenans are especially used for thickening, gelling and stabilizing in the manufacturing of dairy and meat products (*e.g.,* ham), but carrageenans from imported red algae (*Kappaphycus* and *Eucheuma* from Southeast Asia) are also used in pet food (Mesnildrey *et al*., 2012). Thus, with the exception of *Porphyra* spp., the potential of connecting with opportunities in the hydrocolloid market is limited for Flemish seaweeds because the principal species do not occur along the Flemish coast.

Agriculture & feed. The primary applications of algae in agriculture are as fertilizers and feed additives for cattle for which seaweeds are processed into powders or liquid extracts. Fertilizers are spread on grounds in powder form, as microballs or pulverised in liquid form. The main algae species used are *Ascophyllum nodosum, Fucus* spp*.* and coralline red algae, which favor the growth of plants and resistance against diseases. Indeed, seaweed produces defensive substances in response to aggression by gastropods. The Fucales can also be used as nutritional supplements in animal food for their digestive qualities; they are processed in flours mixed with food (Mesnildrey *et al*., 2012). Seaweeds are also added to feeds for salmon and sea urchin (Ferronato *et al*., 2012). In the animal feed industry, seaweeds are often used as blends of different species depending on the required properties of the product. Based on the species used today in agriculture, agricultural use may be a route that is feasible to market Flemish seaweeds. Yet, it will be economically more favorable to target niche markets with a higher added value, such as the food industry and the health and well-being industry.

Food. Some seaweed species can be eaten like vegetables. In that case, the food processing industry uses the entire alga and uses a range of conservation processes. Algae can be dried or served fresh, frozen, canned or salted, and incorporated into other products such as cheese. Seaweed products are sold as raw materials or are mixed in ready meals (*e.g*., salads or tapenades). Currently, 20 seaweed species (among which *Ulva* spp., *Fucus* spp., and *Porphyra* spp.) are considered edible as raw material or an intermediate product for the food processing industry. These species are often cultivated or gathered on shore by gatherers on foot. Seaweed can be added to many dishes such as tartars, mustard, beer and traditional Asian dishes. However, seaweed is not yet a component in mainstream food habits in Europe (Mesnildrey *et al*., 2012). Most of the seaweeds occurring in Belgium are sold for direct human consumption (with the exception of *F. spiralis* and *F. serratus*).

Health & well-being. The seaweed species used in the cosmetic and pharmaceutical sector are often the same as those used in the food industry. Yet, the number is expanding since research and development are continually finding new applications for additional species. In the cosmetic sector, seaweed is used as a plant extract, crushed (*e.g.,* for peels) or as a coloring agent. The active principles are especially sought and differ according to the species as follows, *e.g.*, *F. vesiculosus* for its beta-glucans, *F. spiralis* for its draining properties, *Ascophyllum nodosum* for its polyphenols and antioxidants. Generally, cosmetic companies process products for large brands. Because marketing holds an important place in the cosmetics industry, seaweed is often used to create an image of natural products with benefits from the sea. There are many outlets for seaweed in the pharmaceutical sector, and researchers continue to discover new virtues of seaweed. Seaweed is recognized for its benefits against high blood pressure and cardiovascular diseases. Several pharmaceuticals utilize active principles from seaweed (Mesnildrey *et al*., 2012).

Overview of species-specific applications

Commercial uses of *Ulva lactuca.* *Ulva lactuca* is available in different forms from companies in countries which include the UK, Ireland, France, Germany, Vietnam, China, Canada, and the United States. It is sold both in fresh and dried form, in flakes, powders, and salad mixes. *Ulva* is a delicate seaweed with a mild flavor. *Ulva lactuca* is sometimes eaten as "green laver", but it is considered inferior to purple laver. It is used as a seasoning by itself and in blends, and can be found in soups and salads. It is a key ingredient in many cosmetic and personal care items such as soap, lotion, toner, lifting cream, eye cream, lip cream, makeup remover, body polish, bath soaks, anti-aging products, shaving lotion, shampoo, conditioner, and serums. It is also a component in gardening and fertilizer products. Leading cosmetic brands which use *Ulva lactuca* as an ingredient in anti-aging products cite that it contains a high concentration of amino acids (proline, glycine, lysine) which are the basic components of proteins, and that it has an ability to stimulate the cells in the connective tissues to synthesize collagen. This in turn can improve the skin's elasticity and reduces lines and wrinkles (Seaweed Industry Association, 2015).

Commercial uses of *Fucus vesiculosus.* *F. vesiculosus* is included as an ingredient in over 100 types of products from Italy, UK, Ireland, France, Australia, the United States, and Canada. Some of these are very expensive personal care and cosmetics products from leading sellers and brands. Product types include anti-aging products, firming and slenderizing gels, muds, and creams, anti-cellulite products, eye gels, and toners. There are facial and body masques, exfoliating lotions and soaps, scrubs, cleansers, and cleansing muds. For bath and shower, one can purchase bath soaks, bubble baths, shower gels, body wash, shampoos, and conditioners. Additional skin treatments include night creams, day creams, hand creams, massage oils, moisturizing lotions, and sunscreens. For health, *F. vesiculosus* is used as food and in diet supplements for humans, pets, horses, and other animals, including iodine supplements. It can be found in different semi-processed forms such as flakes and powders for gardens and crops, and is also in many fertilizers (Seaweed Industry Association, 2015).

Primary chemical constituents of *F. vesiculosus* include mucilage, alginate, mannitol, *β*-carotene, zeaxanthin, iodine and iodine salts, bromine, potassium, and many other minerals, volatile oils, as well as polysaccharides. When used in hot seawater baths or steamed the plants are said to release certain substances that promote good skin, lower blood pressure and ease arthritic and rheumatic pains. *F. vesiculosus* has been shown to help women with abnormal menstrual cycling patterns and menstrual-related disease histories. A popular use of *F. vesiculosus* in herbal medicine is as a source of iodine (it was the original source of iodine, discovered in 1811), an essential nutrient for the thyroid gland; it can be used in the treatment of underactive thyroid glands (hypothyroidism) and goitre, a swelling of the thyroid gland related to iodine deficiency (Seaweed Industry Association, 2015).

Its ability as a thyroid stimulant may also help counter obesity by increasing metabolic rate. *F. vesiculosus* is reported to activate the flow of lymph, which reduces swelling and fluid retention. Even though iodine is present in seaweeds in relatively low quantities, it appears that its effects are reinforced and synergized by the presence of other substances (sugars, amino acids, mineral salts) so as to form a complex, the active function of which is to demolish deposits of hypodermic lipids. Preparations including iodine have been used in beauty treatments of cellulite thanks to the degenerative effect of the iodine on the cells in subcutaneous fatty tissue. *F. vesiculosus* is also recognized for its softening, emollient and invigorating effects, and therefore it can be put to good use also in the preparation of cosmetics for skin which is dry, faded and aging. *F. vesiculosus* is commonly used as a food in Japan, though less so in Europe and North America. It can be stored dried, and make a nutritious tea, and added to soups and stews in flakes or powder form for flavor (Seaweed Industry Association, 2015).

Commercial uses of *Porphyra umbilicalis.* *Porphyra umbilicalis* is rich in protein, vitamins A, C, E, and B, and trace minerals, and also rich in omega-3 polyunsaturated fatty acids (EPA and DHA). It contains special compounds named "mycosporine-like amino acids" (MAAs) which are valuable in certain types of personal care products. Industry markets the favorable properties associated with compounds from *Porphyra umbilicalis* by claiming that it:

- acts as a natural bio-protector against UV-A induced damage
- prevents the formation of "sun burn cells" and premature photo-aging
- protects cell structures, especially membrane lipids and DNA from damage by UV-induced radicals
- helps in the re-equalization of lipid deficient skins
- increases epidermal hydration
- protects against Trans Epidermal Water Loss
- improves intercellular cohesion
- reinforces the skin barrier function
- restores cell membrane structure after irritant damage
- has oxygenating properties that helps revitalize stressed and fatigued skin
- is able to diminish the appearance of fine lines and wrinkles

As a result, it is used in many different product applications, such as:

- daily UV protective skin care, sun care,
- anti-photo-aging care
- cares for dry, reactive and sensitive skins
- lip care
- aftersun care

Specific products identified so far from France, Ireland, Italy, Spain, United States utilizing *Porphyra umbilicalis* include regenerating face creams and anti-aging facial creams, facial masks, and aftershave balm. For food, it is sold in flaked and whole leaf form, as a nori substitute, as laver, and is used as an ingredient in several snack mixes and condiments. It is also used as a pet nutrition supplement (Seaweed Industry Association, 2015).

1.4 Methods for seaweed drying

Historically, French coastal populations gathered wrecked seaweed after storms for soil improvement. Men collected algae from the sea even in winter with large rakes, and women carried algae with litter at the edge of shores. Then, the algae were spread on dunes to be dried for year-round preservation. Until 1978, seaweed was dried on dunes, a procedure which was then replaced by industrial processes, but small-scale producers in Europe still dry small batches of seaweeds manually on drying racks. Currently, different techniques are used to process seaweeds depending on their final use (*e.g.,* for direct human consumption, as condiment, or for use in feed) and on whether the algae are to be used fresh or dried. Still, most seaweed is dried before being processed (Mesnildrey *et al*., 2012), because wet seaweed can only be stored wet for about 24h before the plants start to decay.

Seaweeds contain a large amount (75-85%) of water and 15-25% of organic components and minerals. Optimal storage conditions for fresh seaweeds is between 15-17°C in the dark and storage temps below 12.5°C can cause chilling injury (Ferronato *et al*., 2012). Sometimes the algae are cleaned with clean sea water of fresh water and they are dried in a greenhouse or the sun for 24-48h depending on the species. Because of this elevated water content and the natural resilience of some species to losing water, drying some seaweeds (*e.g., Fucus* spp.) on an industrial scale may require a relatively energy-intensive process compared to other species (*e.g., Porphyra* spp. or *Ulva* spp.).

A number of industry and academic professionals with an expertise in seaweed cultivation (*n* = 5 and 2, respectively) were contacted to inquire what protocols are typically used to dry seaweeds on a commercial (semi-)industrial scale. Our questions focused on the temperature and the methodology, and were not limited to the targeted market (human or animal consumption, or other). The ultimate goal of the survey was (*i*) to get insights into current best practices and (*ii*) to steer the experimental setup, so the results would be relevant to the seaweed industry. The results of the survey are discussed in Part 3 of the report.

1.5 Available pilots for drying seaweeds

Nearly 90 companies process or sell products made using seaweed in France. Among these companies, more than 40 companies using seaweed are active in the health and well-being sectors. Almost a quarter of these companies operate within the agricultural supply and water treatment sectors. One quarter of the companies provide seaweed as vegetables for human consumption. A total of 7 companies use algae to produce hydrocolloids. The enterprises using edible seaweed for human consumption are small or medium in size. These newer types of industry have been developed in the last few years because of the increasing demand from European consumers. In France, all edible algae are harvested manually (Mesnildrey *et al*., 2012).

The proximity to the harvesting areas is the main factor defining the factory location for the processing industry. In France, the production of alginate and cattle feed requires vast quantities of raw seaweed. Seaweed cannot be transported profitably to other regions by road because of the large number of trucks required to move the vast volume of material. The added transportation costs would make the seaweed product uncompetitive compared to the dried products imported from abroad. For this reason, the main industries working with raw seaweed (such as Cargill's alginate and carrageenan production plants in Lannilis, Brittany and Baupte, Normandy, respectively) are located near the main areas producing the seaweed. In addition to using local raw materials, the seaweed processing industries also import dried seaweed when the local resource is out of season. The French seaweed industry imports consist of fresh and/or dried seaweed from Chile (4.478 tons) and the Philippines (3.660 tons) (Mesnildrey *et al*., 2012).

In Belgium, the seaweed producing industry is non-existent. Yet, there are many sites where entrepreneurs and companies can optimize seaweed processing protocols. Seaweed drying can be optimized under laboratory conditions at the **Flemish universities** or in **commercial laboratories** (such as Laboratory ECCA in Merelbeke), or on a semi-industrial scale at *e.g.* the **ILVO Food Pilot** (Melle) for spray drying, lyophilizing, or air drying. On larger scale, many companies located in Flanders possess drying installations for agricultural products, wood products, or sludge. A conceivable issue with these (semi-)industrial sized facilities is getting access to the facilities without perturbing the main activities of the company, but some companies rent out their drying installations and provide consulting services. It is recommended to test seaweed drying in facilities certified to process products for human or animal consumption (to minimize complications with post-drying analysis of the seaweeds). Still, the process parameters can be determined in any full-scale dryer. Though the drying process can be developed in any location, ultimately, commercial seaweed drying should be performed close to the landing site, which in all likelihood will be a Flemish harbor.
A small selection of examples of companies from different industry sectors where drying may be tested and optimized is listed below for illustration purposes:

Eco Treasures nv is a company located in Lokeren (East Flanders). The company aims to extract high quality products from natural primary and secondary resources in a prudent, sustainable, and economical way. Eco Treasures has a pilot dryer for short feasibility tests on pilot scale (50 L). The pilot dryer is ideal for exploratory tests on the drying of natural feedstock or intermediates, and is available to third parties for drying tests. The company is a member of the Flanders Innovation Hub for Sustainable Chemistry. (www.ecotreasures.be)

Bioagrico bvba is a company located in Beert (Pepingen, Flemish Brabant) that harvests yew (*Taxus baccata*) clippings in the yards of about 20.000 customers for the pharmaceutical industry and the production of cervical and breast cancer medication. Yew clippings are ground in a rotating drum drier, a process that is similar to the drying process for seaweeds. The seasonal nature of the harvesting of the yew clippings makes this company an interesting potential partner for small-scale tests for industrial seaweed drying. (www.bioagrico.be)

Parmatam nv is a company located in Zwevegem (West Flanders) offering their expertise in drying of wood and wood drying equipment. The company's focus on providing wood drying services to customers makes them a potentially interesting party to contact for drying of larger batches of seaweed in their drying rooms that can be used for several weeks. The company also provides consulting services and distributes specialized drying equipment. (parmatamhoutdrogers.be/)

Alltech Flanders bvba, part of the Alltech Group, *i.e.* an Irish-American producer of natural feed additives, has constructed a drying plant in Klerken (West Flanders) to start operating in 2015. The plant is equipped with drum barrels that are also capable of processing 90.000-100.000 tons of wet material to generate about 9.000 tons of dried products on an annual basis (about 125-130 tons of additives per day). (www.dvo.be)

AVEVE Agro Riemst nv is an AVEVE Group production site in Limburg where corn is dried on an industrial scale (batches of tens of tons). (www.aveve.be)

Farms. Some farms such as the horse farm of Thierry de Pas in Pas de Bois-Guilbert (France) have small scale (9 kW) drying installations for the processing of wheat, hay, and other agricultural products on site. Small farms such as this one may represent an easily accessible option for using or renting the drying equipment. (www.biogas-e.be)

Aquafin nv has four installations operational in Flanders for the drying of sewage sludge. The plants are located in the wastewater treatment plants of Deurne, Houthalen, Leuven, and Bruges which have been operational since 1998, 2001, 2003, and 2001, respectively. Aquafin dries 99,96% of the sludge they produce at these locations, where the material (about 90% dry matter) is pelleted prior to being incinerated. In 2012, Aquafin produced 32.381 tons of dried product of which 37.9% was incinerated in the Bruges incineration plant. Drying installations used for drying materials not intended for human or animal consumption, such as these sludge dryers, are not recommended because of the inherent risk of contamination of the seaweeds with chemical and microbial contaminants. (www.aquafin.be)

2 PART 2: BIOCHEMICAL COMPOSITION OF NATIVE FLEMISH SEAWEEDS

This part of the report describes the seaweed sampling campaign and the results of the chemical analysis of three wet seaweed samples.

2.1 Seaweed species selection

During the sampling campaign, seaweed species were chosen to have in our selection native Flemish seaweeds representing the **different Divisions** (*Chlorophyta*/green seaweeds, *Rhodophyta*/red seaweeds, and *Phaeophyta*/brown seaweeds). Possible **uses of seaweeds** were also taken into consideration, so that at least one species of significance for direct human consumption and at least one of significance for its biochemical constituents was included.

Seaweed species were only considered for sampling when they were native to the Belgian part of the North Sea because only **native species** are eligible for mariculture. Non-native species can be cultivated, but only in closed systems on land that do not affect the natural biodiversity (Regulation (EC) No 708/2007 and its amendment Regulation (EU) No 304/2011). As an additional parameter for selection, the seaweeds had to occur in **reasonable amounts** during the sampling period (i.e., July-August). Preference was also given to species that are currently consumed directly or used in consumer products. During the selection process the reference guide by Coppejans (1998) was used for selecting and identifying species occurring in the different littoral zones along the Belgian coast.

Given the criteria listed above, the final selection of seaweed species comprised *Ulva* sp. (EN: sea lettuce), *Fucus vesiculosus* (EN: bladderwrack), and *Porphyra umbilicalis* (EN: laver). The related brown and red seaweed species *Fucus spiralis* and *Porphyra purpurea* were also considered as alternative species should our first choice not be available at the selected sampling sites. Species frequently used or considered for commercial mariculture such as *Laminaria digitata* and *Saccharina latissima* (a.k.a. *L. saccharina*) (Mesnildrey et al., 2012) were not considered because they are rare or nonexistent in Belgium and occur below the intertidal zone (*i.e.*, the infralittoral zone), and thus not easily accessible at the selected sampling sites.

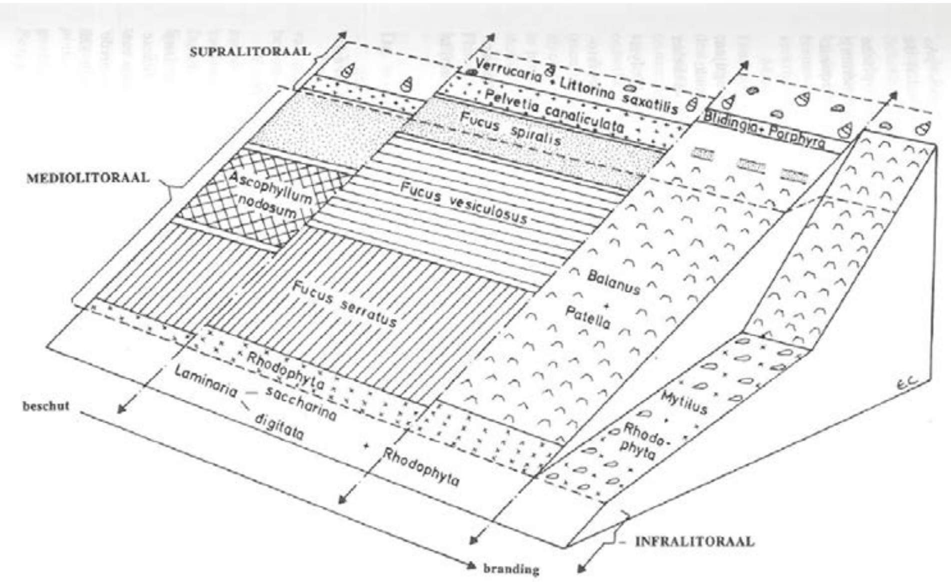

Figure 2: *Schematic representation of the seaweed zonation along the North Sea coast near Boulogne-sur-Mer. From: Coppejans, 1998.*

2.2 Sampling site selection

Seaweed growth along the Belgian coast is associated mainly to the occurrence of artificial surfaces, such as groynes, breakwaters, quay walls, piers, and ropes. At such sites, seaweeds will also attach to natural surfaces, such as the shells of oysters and mussels. Seaweed biodiversity at a given site depends on the littoral zone and the extent of exposure to wave action and sand scour. As a result of these and other factors such as water turbidity, seaweeds occur in horizontal bands or zones (**Figure 2**).

Groynes (NL: strandhoofden) were not retained as sampling location because many of them along the Belgian coast are covered with sand (*e.g.*, at Heist) or because they are not sufficiently submerged in the intertidal zone (*e.g.*, at Koksijde). Breakwaters (NL: golfbrekers of havendammen) that are located in the top and middle part of the intertidal zone are exposed to erosion due to suspended sand, which is negatively associated with seaweed growth. Dikes typically do not reach the middle of the intertidal zone and also suffer erosion from suspended sediments. Poles and piers have a relatively small surface area and are also exposed to erosion from suspended sediments. Seaweed growth on dikes, poles, and piers is typically limited to green seaweeds, *Chlorophyta* (such as the Ulvaceaen *Blidingia* spp.). On breakwaters and the walls of ports mostly *Fucaceae* are found (Engledow *et al*., 2001, Volckaert *et al*., 2004). From unpublished research performed by eCOAST, it is known that green, brown, and red seaweeds thrive and are numerous in some inland locations, including the Spuikom at Ostend.

For seaweed sampling we have chosen the Western breakwater of the port of Zeebrugge, the Eastern bank of the inner harbor of the port of Ostend, and submerged ropes and pontoons in the Spuikom (Ostend) (**Figure 3**). Our surveys of candidates along the Belgian coast sites in the week of July 13, 2015 showed that green, brown, and red seaweeds (among which our candidate species *Ulva* spp., *Fucus* spp., and *Porphyra* spp.) grow abundantly at these three selected locations.

2.3 Seaweed sampling

In the week of July 13 2015 several candidate sites along the Flemish coast were inspected for the occurrence of our candidate species, and the three sampling sites were selected. On July 20 2015, two sites (Port of Zeebrugge and Port of Oostende) were visited around low tide, namely between 8h30 and 10h00 and between 11h30 and 13h00 respectively. On the same day, the third site, the Spuikom at Ostend, was also visited between 14h30 and 15h15 to sample seaweeds growing close to the pontoon of the local business, *De Oesterput*, with the assistance of the business-owner, Jacky Puystens. The exact sampling locations are shown in **Figure 3**.

Table 4: *Overview of the samples submitted for analysis with their respective sampling locations.*

Sample #	Analysis of wet samples	Analysis after drying
1	*Ulva* sp. (Port of Ostend)	*Fucus* sp. (Port of Ostend)
2	*Fucus* sp. (Port of Zeebrugge)	*Fucus* sp. (Port of Ostend)
3	*Porphyra umbilicalis* (Port of Ostend)	

As mentioned above, three seaweed species were selected to cover a range of potential uses and taxonomic groups of seaweeds occurring along the Flemish coast. Additional species were collected at the different sampling locations as back-up samples, but ultimately only three species from two locations were submitted for analysis (**Table 4**). *Ulva* sp. sampled in the Spuikom was not retained for analysis because the plants were partly in a state of senescence and overgrown with micro- and macroscopic organisms (**Figure 4**). All samples were cleared of biological contaminants and senescent parts by removing those parts and rinsing with fresh seawater. The samples were collected in 6 L polypropylene freezer bags, stored at 4°C using ice packs in cool boxes, and transported to the laboratory on the day of sampling, where they were stored at -20°C until analysis. **Figures 5 and 6** provide a view of the seaweed zonation at the ports of Zeebrugge (exposed to wave action) and Ostend (protected from wave action).

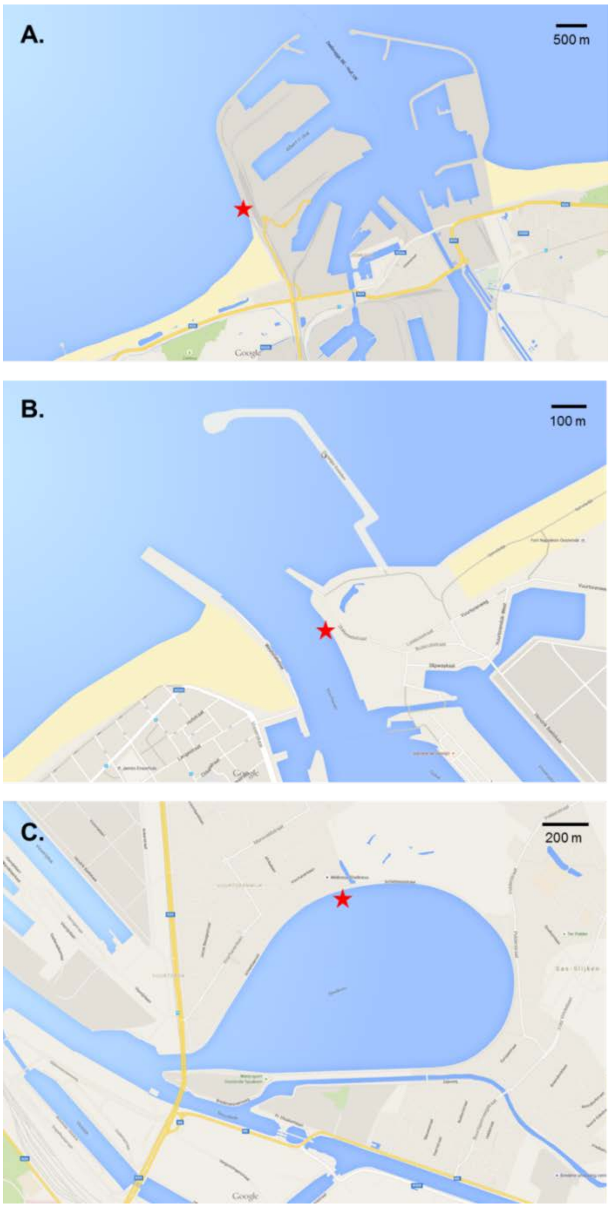

Figure 3: *Overview of the precise locations that were visited for seaweed sampling. A: Port of Zeebrugge, B: Port of Ostend, and C: Spuikom.*

Figure 4: *Macroscopic appearance of Ulva sp. sampled at the Spuikom on July 20, 2015.*

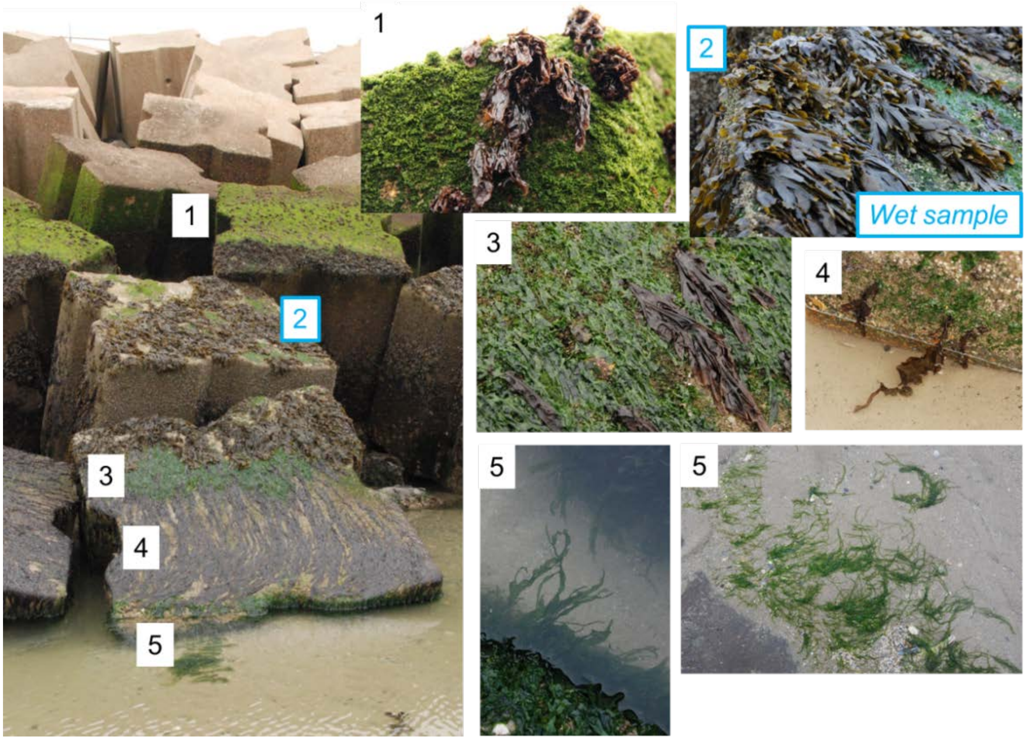

Figure 5: *Zonation of seaweed species at the Zeebrugge sampling location, where Fucus sp. (2) was sampled for analysis. (1) Blidingia sp. & Porphyra umbilicalis; (4) Porphyra purpurea; and (5) Ulva sp. & ex-Enteromorpha sp.*

Figure 6: *Zonation of seaweed species at the Ostend sampling location, where Porphyra umbilicalis (1), Fucus sp. (3), and Ulva sp. (4) were sampled for analysis.*

2.4 Seaweed chemical composition

The chemical composition of three seaweed species were determined by Laboratory ECCA (Merelbeke) and the results are provided in **Table 5** and **Table 6**.

Ulva sp. Our survey of the literature showed that dry matter content of *Ulva* sp. can vary substantially across the world ranging from 5.96% in Iran up to 56.1% in New Zealand (Tabarsa *et al*., 2012; Dean *et al*., 2013), so the cost associated with drying the plants may vary substantially depending on site-specific parameters, such as location. Also, the relative fractions of ash, protein, carbohydrates, and lipids of seaweeds can vary between studies and locations, so the business model of cultivating seaweeds in a given location has to be evaluated case-by-case. Still, the biochemical composition of *Ulva* sp. from Ostend was largely similar to the composition reported by a French producer near Brest in Brittany (**Table 6**). Compared to studies with *Ulva* sp. collected in Iran, Tunisia, Brazil, and New Zealand, *Ulva* sp. analyzed here had relatively elevated mineral, protein, and lipid contents, but a relatively low carbohydrate content (Tabarsa *et al*., 2012; Yiach et al., 2011; de Pádua *et al*., 2004; Dean *et al*., 2013). The total phosphorous content was in the same range as *Ulva* sp. from New Zealand (400 mg/kg compared to 268-333 mg/kg), but the sulfur content was rather low compared to *Ulva* sp. from New Zealand (3.500 mg/kg versus 10.990-15.070 mg/kg) (Dean *et al*., 2013). The levels of As, Pb, Cd, and Hg in *Ulva* sp. from Ostend (0.86 mg/kg, 0,47 mg/kg, <0,050 mg/kg, and <0.050 mg/kg, respectively) were also lower or similar compared to the levels found in New Zealand (1,9-4,6 mg/kg, 2,0-3,2 mg/kg, 0,03-0,05 mg/kg, and <0,01-0,01 mg/kg, respectively) (Dean *et al*., 2013) and the levels found in commercially-distributed *Ulva rigida* (**Table 6**). Still, all concentrations of non-essential (heavy) metals were well below the European limits for food supplements (Regulation (EC) No. 1881/2006), namely for lead & cadmium: 3,0 mg/kg fresh weight, and for mercury: 0,10 mg/kg fresh weight. Fresh *Ulva* sp. also does not exceed the maximum arsenic level of 1 mg/kg of product regulated through the Royal Decree (KB, 2014), though it would exceed the maximum arsenic level if it were sold as dry food supplement (**Table 6**).

Porphyra umbilicalis. Unlike *Ulva* sp. where the relative biochemical composition differed somewhat from the plants from France, *P. umbilicalis* from Flanders overlaps with the French ranges for ash, protein, and carbohydrate content, with a notable deviation for lipid content (4,1% *vs*. 1-2%)(**Table 6**). Nori, which is prepared from closely related to the seaweeds (*P. tenera* and *P. yeeziensis*), typically contains 85,0% of moisture, 5,81% of proteins 5,11% of carbohydrates, and 0,28% of lipids. Hence, the chemical composition of *P. umbilicalis* from Flanders (**Table 5**) is also very similar to the nutritional composition reported for Nori (wikipedia.org, 2015). In *P. umbilicalis* from Ostend iodine was not detected (<591 mg/kg DW), whereas *Porphyra* sp. from China and South-Korea were found to contain between 2407-3108 mg/kg DW of iodine (Hwang *et al*., 2013). *P. umbilicalis* in Flanders contains relatively low levels of the heavy metals (**Table 6**) compared to *Porphyra* sp. sampled in China and South-Korea (arsenic: 19,5 mg/kg DW *vs.* 32,0-43,8 mg/kg DW; mercury: <0,30 mg/kg DW *vs.*<0,1-<0,1 mg/kg DW; lead: 0,53 mg/kg DW *vs.*0,26-1,57 mg/kg DW, and cadmium: <0,30 mg/kg DW *vs.*1,6-3,4 mg/kg DW) (Hwang *et al*., 2013). The heavy metal levels are also in the same order of magnitude as commercially-distributed *P. umbilicalis* (**Table 6**). The levels of all heavy metals in fresh *Porphyra* sp. were well below the European limits for food supplements (Regulation (EC) No. 1881/2006), but the arsenic levels exceeded the Belgian limit of 1 mg/kg of product in food supplements on both the fresh and dry weight basis (KB, 2014) (**Table 5 & 6**).

Fucus sp. The dry matter content and biochemical composition of *Fucus* sp. from Flanders is largely similar to *Fucus vesiculosus* from Canada (Kim, 2012) and Brittany, with only slightly higher protein and lipid contents (**Table 6**). The sulfur, phosphorous, and mannitol contents for Flemish *Fucus* sp. (4,3% DM, 0,16% DM, and 7,6% DM) are largely similar to the levels reported for *F. vesiculosus* from Brittany (1,5% DM, 0,35% DM, and 7,5% DM). Iodine was not detected in Flemish *Fucus* sp. (<540 mg/kg DM). These these findings are consistent with the reports for *F. vesiculosus* from Brittany and Estonia where iodine levels of respectively 500 mg/kg DM and 130-160 mg/kg DM were reported (Agrimer, 2015; Truus *et al*., 2001). The mercury level found in Flemish *Fucus* sp. (<0,27 mg/kg DM) was consistent with the one reported for *F. vesiculosus* from Estonia (0,0063-0,0073 mg/kd DM), but the arsenic levels in Flemish seaweeds were an order of magnitude lower (24,3 mg/kg DM compared to 327 mg/kg DM) (Truus *et al*., 2001) (**Table 6**). The levels of heavy metals were well below the European limits for food supplements (Regulation (EC) No. 1881/2006), but the arsenic levels exceed the Belgian limit of 1mg/kg of product in food supplements on both the fresh and dry weight basis (KB, 2014) (**Table 5 & 6**).

Table 5: *Chemical composition of three fresh seaweed species sampled along the Flemish coast. Fractions and concentrations are shown as wet, unprocessed material. For components that were not detected, the Method Detection Limits are reported.*

Parameter	Unit	*Ulva* sp.	*Porphyra umbilicalis*	*Fucus* sp.
		Port of Ostend	Port of Ostend	Port of Zeebrugge
Biochemical composition				
Moisture	%	88,4	83,1	81,5
Ash	%	3,43	3,45	5,50
Protein	%	3,9	5,0	2,2
Total fat	%	0,4	0,7	0,9
Total carbohydrates	%	3,9	7,8	9,8
Fiber	%	2,0	NR	7,2
Cell content	%	0,4	1,1	0,8
TOC	% DM	32	36	33
TIC	%C/DM	0,207	0,122	0,0170
Highvalue components				
Mannitol	% (m/m)	<0,100	<0,100	1,40
Polyphenols	mg/kg	<0,30	<0,30	<0,30
Elemental composition				
Sulfur (S)	mg/kg	3.500	4.900	7.900
Total phosphorus	mg/kg	400	920	300
Iodine (I)	mg/kg	<100	<100	<100
Arsenic (As)	mg/kg	0,86	3,3	4,5
Lead (Pb)	mg/kg	0,47	0,089	0,082
Cadmium (Cd)	mg/kg	<0,050	<0,050	0,10
Tin (excl. Sn-oxides)	mg/kg	<4,0	<4,0	<4,0
Mercury (Hg)	mg/kg	<0,050	<0,050	<0,050
Energetic value				
kcal	kcal/100g	34,9	57,2	56,6
kjoule	kjoule/100g	148	242	240

***Table 6.** Biochemical compositions of the wet seaweeds expressed as relative fractions of dry matter compared to equivalent data from a seaweed producer in France (Agrimer). The relative fractions were calculated using the percentages of dry matter, which itself are derived from the moisture contents.*

Parameter	Unit	*Ulva* sp.	*Ulva* sp.	*Porphyra umbilicalis*	*Porphyra* sp.	*Fucus* sp.	*Fucus vesiculosus*
		Port of Ostend	Brittany, France [a]	Port of Ostend	Brittany, France [a]	Port of Zeebrugge	Brittany, France [a]
Dry matter (DM)	*%*	*11,6*	*20-30*	*16,9*	*10-35*	*18,5*	*15-30*
Ash	% DM	29,6	17-35	20,4	15-35	29,7	15-30
Protein	% DM	33,6	7-30	29,6	15-47	11,9	5-8
Total carbohydrates	% DM	33,6	41-62	46,2	30-55	53,0	45-70
Total lipids	% DM	3,4	1-3	4,1	1-2	4,9	1-4
Parameter	Unit	*Ulva* sp.	*Ulva rigida*	*Porphyra umbilicalis*	*Porphyra umilicalis*	*Fucus* sp.	*Fucus* sp.
		Port of Ostend	OCS [b]	Port of Ostend	OCS [b]	Port of Zeebrugge	Estonia [c]
Arsenic	mg/kg DM	7,41	6.41-7.06	19,5	28.9-49.5	24,3	327
Lead	mg/kg DM	4,05	1.00-1.05	0,53	<0.008-0.270	0,44	NR
Cadmium	mg/kg DM	<0,43	0.031-0.033	<0,30	0.253-3.10	<0,54	NR
Tin (excl. Sn-oxides)	mg/kg DM	<34,5	NR	<23,6	NR	<21,6	NR
Mercury	mg/kg DM	<0,43	0.018-0.019	<0,30	0.008-0.032	<0,27	0,0063-0,0073

OCS: Origin not specified, NR: Not reported, Concentrations in grey with a "<"-sign indicate the compounds were not detected above the method detection limits.

[a]: Agrimer, 2015; [b]: Besada *et al*., 2009; [c]: Truus *et al*., 2001

3 PART 3: INFLUENCE OF DRYING ON SEAWEED COMPOSITION

This part of the report describes the techniques and possibilities for drying seaweeds on an industrial scale, and reports the results of the seaweed drying experiment.

3.1 Survey regarding industrial seaweed drying

A number of industry and academic professionals (*n* = 5 and 2, respectively) with an expertise in seaweed cultivation or seaweed processing were contacted to inquire what approaches are typically used to dry seaweeds on a commercial (semi-)industrial scale. Our questions focused on the temperature and the methodology, and were not limited to the targeted market (human or animal consumption, or other). The ultimate goal of the survey was (i) to get insights into current best practices and (ii) to steer our experimental setup for seaweed drying, so the findings of our drying experiment would be relevant to the seaweed industry.

Our inquiries showed that seaweed producers are reluctant to disclose the exact protocol used to process their harvests. Producers mentioned that optimization of the drying protocol is a lengthy process and details regarding the procedure are considered a trade secret. For the same reasons the names of our sources are not mentioned in the present report. Most of the contacted experts did disclose that seaweeds should be dried as quickly as possible and at the lowest temperature possible. Different companies use different methods and temperatures, and even within companies individual parameters (such as the temperature) can vary depending on the ultimate use of the seaweeds. For example, seaweeds used for human consumption will be dried differently (*i.e.*, at lower temperatures) from those used in animal feeds.

Seaweeds can be dried directly by blowing hot air over a conveyor belt or through a rotating drum, or dried indirectly by heating the rotating drums externally. One producer also reported using centrifugation as a pre-treatment for a reduction of about 10% of the wet weight. Exact temperatures used to dry seaweeds were never disclosed by producers and may vary depending on the producer. The scientific literature often recommends using temperatures below 50°C to prevent blackening of the seaweeds and degradation of antioxidants. This is consistent with the results of our interviews, where industry contacts mention using temperatures <60°C (and the lower the better) or temperatures between 22-37°C with good ventilation. In the Ph.D. dissertation of S. Cox (Dublin Institute of Technology) published in 2012, the researchers reported comparing different temperatures for seaweed drying and concluded that drying at 40°C for 2 hours was optimal for phytochemical content because the seaweed was shown to increase its antioxidant content (presumably in response to the drying). Longer drying times were also used depending on the ultimate use of the seaweeds. In the same dissertation it is also mentioned that atmospheric drying is the most common preservation method in Ireland for seaweed and that little effort is made to optimize the process in order to capitalize on the nutritional content. Hence, this report suggests that additional scientific research on industrial drying could bolster the nutritional value of dried seaweeds.

During our survey, other relevant factors were also mentioned, namely that some producers rinse seaweeds prior to drying them since the salt may affect steel components and that freeze drying, using dried air, or pressing could be alternative ways of drying seaweeds.

3.2 Drying of Flemish seaweed

On July 20 2015, a large batch of *Fucus* sp. (in excess of 2 kg) was sampled in the Port of Ostend (**Figure 3 and 6**). In order to determine the effect of seaweed drying on their chemical composition, we set out to dry one aliquot (about 1 kg) at a temperature below 50°C (*e.g.*, 40°C using dry air and air flowing over spread out seaweeds) and another aliquot using lyophilization (*i.e.*, freeze drying). Lyophilization would allow seaweed drying without changing the chemical composition as a result of thermal degradation of thermolabile components. Using thermal drying will always result in an altered composition, either due to the response of the plant to the drying or due to the heat-induced transformation of some of its constituents.

Drying and analysis of seaweeds was performed at the same facility, namely the commercial Laboratory ECCA at Merelbeke, Belgium. This laboratory is ISO-certified and is experienced in the processing and analysis of food, feed, and environmental samples.

One aliquot of *Fucus* sp. was dried in a laboratory freeze dryer, another at 50°C using a laboratory oven. The latter sample was dried despite our explicit instructions to the subcontractor not to process the samples until a procedure was provided and not to dry at temperatures of 50°C or higher. After a couple of days, laboratory staff observed blackening of the seaweed as well as what was reported to be mild mold growth (not confirmed using culturing). The supposed mold growth could have been the formation of salt crystals, a process known to occur during seaweed drying. The blackening of *Fucus* sp. at a temperature of 50°C was consistent with previous reports in literature which state that seaweeds should be dried at temperatures below 50°C to avoid darkening. **Figure 7** shows the discoloration of *Fucus* sp. dried at 50°C compared to the freeze-dried aliquot that retained a greener appearance.

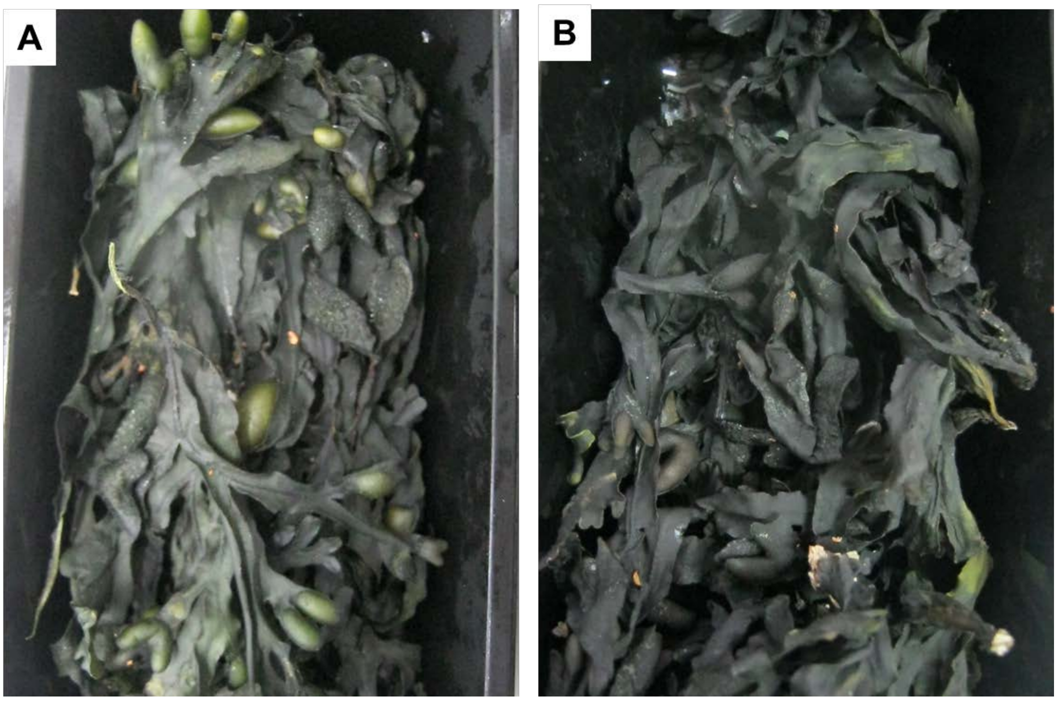

Figure 7: *Fucus sp. collected in trays after the aliquots were lyophilized (A) and heat-dried at 50°C in an oven (B).*

On August 11, 2015, eCOAST scientists returned to the sampling location in the Port of Ostend to collect additional material for replacing the aliquot that was lost because of the drying at 50°C and to complete the comparison between heat-dried and lyophilized *Fucus* sp. Yet, the *Fucus* sp. population at the sampling location was in a state of decay. The available biomass and seaweed density of *Fucus* sp. had decreased substantially compared to July 20, 2015. The quality of the remaining seaweeds was too low to permit a comparison between heat-dried and lyophilized samples. The population of *Fucus* sp. was much darker in overall appearance (**Figure 8A**) and occurred in various stages of senescence and degradation (**Figure 8B and 8C**). Because of the poor overall state of the *Fucus* sp. population in the port of Ostend, it was decided not to perform the planned analyses on heat-dried seaweeds as the decaying plants would not be able to provide a reliable measure of its composition.

Figure 8: *State of the Fucus sp. population on August 11, 2015 in the port of Ostend. (A) General appearance of the site, close-ups of Fucus sp. plants on location (B) and in the eCOAST mobile laboratory (C).*

3.3 Comparative analysis of fresh and dried seaweed

To estimate the impact of seaweed drying, the chemical composition of fresh *Fucus* sp. from the Port of Zeebrugge was compared to the composition of dried *Fucus* sp. from the Port of Ostend. First, *Fucus* sp. from the Ostend was lyophilized in a laboratory-scale device to 8.0% moisture content. Then, the chemical compositions of fresh and dried *Fucus* sp. were estimated assuming a moisture content of 0% (**Table 7**). It should be noted that in this work *Fucus* sp. plants are compared from two different locations (Zeebrugge *vs*. Ostend) and two different exposures to wave action (exposed vs. protected). Yet, both locations have the same orientation (*i.e*., West-southwest) and the same general environment (*i.e*., adjacent to the harbor of the Belgian North Sea).

Using this comparison of fresh and dried *Fucus* sp. (**Table 7**), it was determined that the global biochemical composition is about the same for both locations and thus that lyophilizing does not affect the estimated biochemical compositions substantially.[1] Iodine was not detected in fresh *Fucus* sp., but was detected at 280 mg/kg DM in dried plants. Among the elemental components, most consituents were in the same order of magnitude with the exception of sulfur, which varied substantially from 42.660 mg/kg DM in fresh *Fucus* sp. to 5.870 mg/kg DM in dried plants. The reason for this strong decrease in sulfur content after drying is presently unknown. Finally, the energetic value of *Fucus* sp. decreased somewhat after drying and this decrease is associated with a relative decrease in carbohydrate and lipid content and an increase in ash and protein content. As for the fresh seaweeds, all concentrations of non-essential (heavy) metals in dried *Fucus* sp. were well below the European limits for food supplements (Regulation (EC) No. 1881/2006), namely 3,0 mg/kg fresh weight for lead & cadmium, and 0,10 mg/kg fresh weight for mercury. Conversely, dried *Fucus* sp. exceeded the maximum arsenic level of 1 mg/kg of product regulated through the Royal Decree (KB, 2014) (**Table 7**).

[1] *Note*: Variations of 10-20% are common for analytical analyses on independent samples.

Table 7: *The chemical compositions of fresh and dried (lyophilized) Flemish Fucus sp. compared to the estimated dry matter compositions of fresh and dried Fucus sp. assuming a theoretical moisture content of 0%.*

Parameter	**Species / sampling location**			
	Fucus sp. / Port of Zeebrugge		*Fucus sp. /* Port of Ostend	
	Fresh	Estimated/DM-based	Lyophilized	Estimated/DM-based
Biochemical composition				
Moisture	81,5%	Theoretically, zero	8,0%	Theoretically, zero
Ash	5,5%	29,7% DM	32,42%	35,2%
Protein	2,2%	11,9% DM	12,5%	13,6%
Total fat	0,9%	4,9% DM	3,5%	3,8%
Total carbohydrates	9,8%	52,9% DM	43,5%	47,3%
Fiber	7,2%	38,9% DM	37,0%	40,2%
TOC	33% DM	NA	35% DM	35% DM
TIC	0,017% C/DM	NA	0,0200% C/DM	0,0200% C/DM
Highvalue components				
Mannitol	1,4% (w/w)	NA	NR	NA
Polyfenolen	<0,30 mg/kg	NA	NR	NA
Elemental composition				
Sulfur (S)	7.900 mg/kg	42.660 mg/kg DM	5.400 mg/kg	5.870 mg/kg DM
Total phosphorous	300 mg/kg	1.620 mg/kg DM	2.100 mg/kg	2.283 mg/kg DM
Iodine (I)	<100 mg/kg	<540 mg/kg DM	280 mg/kg DM	280 mg/kg DM
Arsenic (As)	4,5 mg/kg	24,3 mg/kg DM	17 mg/kg	18,5 mg/kg DM
Lead (Pb)	0,082 mg/kg	0,443 mg/kg DM	0,21 mg/kg	0,23 mg/kg DM
Cadmium (Cd)	0,1 mg/kg	0,54 mg/kg DM	0,22 mg/kg	0,24 mg/kg DM
Tin (Sn)(excl. Sn-oxides)	<4,0 mg/kg	<21,6 mg/kg DM	<4,0 mg/kg	<4,3 mg/kg DM
Mercury (Hg)	<0,050 mg/kg	<0,27 mg/kg DM	<0,050 mg/kg	<0,054 mg/kg DM
Energetic value				
kcal	56,6 kcal/100g	305,6 kcal/100g DM	256 kcal/100g	278 kcal/100g DM
kjoule	240 kcal/100g	1.296 kjoule/100g DM	1.080 kjoule/100g	1.174 kjoule/100g DM

Conclusions

The present study showed that:

- native seaweed biodiversity along the Belgian coast is less rich compared to neighboring countries
- some of the most abundant species (in terms of biomass and frequency of occurrence) have existing markets, with *Ulva* sp., *Porphyra* sp., *Fucus* sp. and among the most abundant and commercially-important native species
- there are many potential locations to perform pilot tests for seaweed drying on a pilot or (semi-)industrial scale in Flanders
- the biochemical composition of fresh *Ulva* sp., *Porphyra umbilicalis*, *Fucus* sp. from Flanders is relatively similar to the same species harvested in Brittany, France
- the levels of non-essential metals (mercury, cadmium, and lead) in fresh *Ulva* sp., *Porphyra umbilicalis*, *Fucus* sp. from Flanders meets the European maximum contaminant levels for food supplements, but fresh *Porphyra umbilicalis* and *Fucus* sp. from Flanders exceeds the maximum permitted levels for arsenic in food supplements (limit of 1 mg/kg of product)
- drying *Fucus* sp. using lyophilization does substantially alter the global biochemical composition the dried *Fucus* plants still meet the maximum contaminant levels for mercury, cadmium, and lead, but they exceed the maximum allowed levels of arsenic with one order of magnitude

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This study was commissioned by Flanders' Maritime Cluster and financially supported by Flanders and the province of West Flanders.