



Protocol for fresh seaweed sample collection

Version 1.0 – May 2020

This sample collection protocol has been compiled as part of the 'Seaweed Food Safety' pilot project being commissioned by the Ministry of Agriculture within the framework of the Societal Innovation Program ProSeaweed, Nature and Food Quality (LNV), and carried out by Wageningen Food Safety Research (WFSR) in collaboration with the Noordzeeboerderij Foundation (NZB) and seaweed producers in the Netherlands.

Within this pilot project special attention has been paid to the development of a practical sampling protocol aiming at taking samples in a standardized way, and that can then be considered representative regarding a certain time and for a specific location. In addition, during the pilot a significant number of samples are taken according to the developed protocol with the aim of obtaining an impression of the variations in the concentrations of known and unknown potential harmful substances: concentrations of micro- and macronutrients, toxins and contaminants can vary considerably during the growth of seaweed during the season, both between the different seaweed classes (green, brown and red), as well as in the same populations of a specific species, even when situated at short distances from each other.

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The present protocol has been worked out in detail for the green seaweed Sea lettuce (*Ulva sp*) and the brown seaweed Sugar Kelp (*Saccharina Latissima*).

From literature little information is available about the homogeneity of the possible variations in concentrations within one particular seaweed field. This could mean that with any given sample from a particular field, the concentrations found in that sample might not accurately represent the seaweed field as a whole.

Furthermore, no standards are in place yet for representative sample collection of seaweed. A number of EU member states have therefore asked CEN Working Group 454 – which focuses on standardisation projects around algae and algae products – to establish such a standard. The standardisation project for representative sample collection will not be able to start until 2021 at the earliest, and it depends on how much of a priority the development of such a standard is for the EU member states, compared to other standardisations.

The 'Seaweed Food Safety' pilot can be seen as a precursor to such a future European standard, and its focus therefore is to enable reliable conclusions to be drawn about the concentrations of heavy metals, inorganic arsenic and iodine in the production sites of Sea Lettuce (*Ulva sp*) and Sugar Kelp (*Saccharina Latissima*).

The present sampling protocol serves as practical support for the sampling of different classes and types of seaweed, and is a necessary intermediate step in the final sampling strategy, which should lead to representative sample taking in seaweed fields of different magnitudes.

The sample collection protocol has been structured in a generic way so that samples can be analysed not just for the contaminants mentioned above (heavy metals, inorganic arsenic, iodine) but also for other potentially hazardous substances (including dioxins and polycyclic aromatic hydrocarbons (PAHs), mineral oils, natural toxins, pesticides and radioactivity). The protocol also provides instructions for samples that need to be analysed for microbiological hazards.

1 Preparation

Preparations for sample collection need to include specifying the type of site (offshore, inshore, onshore¹) and the species of seaweed that can be collected there. For every producer, the number of samples to be taken from the production site needs to be determined in advance; the total number of samples will depend on the size of the seaweed cultivation field (for offshore/inshore sites) or the number of tanks used for cultivation (for onshore sites).

The accessibility of the sample collection site is also relevant. For offshore or inshore sites, a boat (and skipper) might need to be available. Weather conditions should preferably be calm and stable for offshore and inshore sample collection.

For determining the date/period of possible sample collection it is also advisable to take into account the growing seasons of the relevant seaweeds. Brown algae

such as Sugar Kelp (*Saccharina Latissima*) are winter algae and grow from October-November to May-June. The harvesting season is logically also around this last period, and depends largely on the water temperature. Once the temperature reaches 18 °C or more, Sugar Kelp (*Saccharina Latissima*) barely grows anymore, and lots of other biomass starts to grow on the seaweed. Sea Lettuce (*Ulva sp*) is a summer algae, and can be harvested several times a year. The growing season for cultivated Sea Lettuce (*Ulva sp*) runs from May-June to September-October. However, it may still be harvested in the wild after that time, depending on the decrease in the water temperature. For inshore sample collection the time of day is also relevant: the tide and resulting water level will determine both the accessibility of the seaweed field and the easiness of taking the planned samples.

¹ Offshore: open sea; inshore: estuaries/inland seas; onshore: on land



2 Equipment

Prior to the sampling moment it is important to discuss with the seaweed producer what equipment will be available on site for performing the sample collection.

The list of required equipment will vary depending on the type of production site and the specific location. [Appendix 1](#) provides a checklist for the various types of equipment.

3 Sample collection

3.1 Sample quantities required

The quantity of fresh sample material taken should be at least 500 grams per sample, dried to the extent that it is no longer dripping. This quantity will ensure that an analysis can be made of a sufficiently representative and homogenous sample. Where possible, a (manual) centrifuge should be used to dry the seaweed. If that is not possible, take into account how much sample weight will be lost. Estimated weight loss after centrifuging is 25% for Sugar Kelp (*Saccharina Latissima*) and 50% for Sea Lettuce (*Ulva sp.*). The sample collection quantity for Sugar Kelp (*Saccharina Latissima*) should therefore be at least 750 grams, and for Sea Lettuce (*Ulva sp.*) at least 1000 grams.

3.2 Measures for preventing contamination

Contamination of the samples must always be avoided. The following general measures should be observed:

- Wash hands before taking samples
- Wear strong plastic or neoprene gloves when taking samples
- Avoid touching your head (face/hair) or other body parts while wearing the gloves
- Avoid toilet breaks while taking samples²

3.3 General procedure

- Use the sample collection administration form to draw a schematic overview of the site (on land, include the length, width and depth of the tanks; for inshore/offshore locations include the dimensions and position of the field), and number the sample taking positions ([Appendix 2](#))
- Before taking any samples, check that the equipment (e.g. landing net, cool box and centrifuge) is clean. Only use clean equipment
- Disinfect the equipment to be used
- Apply the general hygiene measures (see 3.2)
- Take the seaweed sample
- Collect at least the minimum quantity of seaweed

- required for the analysis (500 grams). Take account of the moisture contained in the seaweed (take at least 750g of wet Sugar Kelp, or up to 1kg of wet Sea Lettuce)
- Try to dry the sample as much as possible (using a (hand) centrifuge if possible):
 - Place the seaweed in a centrifuge bag, and place that in the centrifuge. If no automatic centrifuge is available, use a manual one
 - Centrifuge the seaweed until no more water is released
- Weigh the seaweed; the minimal amount should be 500 grams sample material
- Place the seaweed in a plastic bag with an ID number
- Enter the following details on the sample collection administration form ([Appendix 2](#)):
 - Company name
 - Sample collection site
 - Sample collection date
 - Sample weight
 - Type/species of seaweed
 - number of the plastic bag
- Write down the details above on the plastic sample bag itself, using a waterproof marker. The sequence numbers of the samples taken can also be included
- Seal the plastic bag using waterproof tape
- Place the plastic bag in a cool box
- Rinse any equipment used, such as the centrifuge, using fresh water or seawater before it is used again (this applies to every new sample taken); if no water is available, a wash bottle with a nozzle containing clean water should be brought to the site
- Using a clean towel or cloth and/or water, clean everything that has been in contact with the sample material before any subsequent samples are taken. Use fresh cleaning materials to clean between each sample

Register the details above on the sample collection administration form ([Appendix 2](#)). Take photos of any important details related to the site and/or deviations from the protocol, and record them using the reporting template ([Appendix 3](#)).

² Where this does happen, consider: the need to keep a sufficient distance from the sampling collection site, flow direction, and washing hands again



During the Seaweed Food Safety pilot taking photos of the sample collection process was recommended to help transfer knowledge and information. For this at least 1 photo of the site, 1 of the seaweed, and 1 of the sample collection method were taken. An example of a completed reporting template is provided in [Appendix 4](#).

3.4 Sample collection methods for specific species

Sample collection methods can vary depending on what purpose the samples are taken for. This protocol describes the process of collecting samples of seaweed to analyse it for the presence of potential hazards with human consumption as destination. The sample collection process described in this protocol is therefore focused on the edible components of the seaweed.

Sugar Kelp (*Saccharina Latissima*)

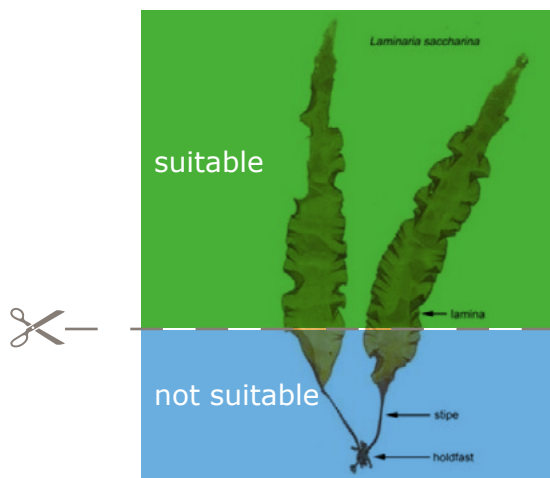
Type: brown algae

Harvested once a year

The lower section (holdfast and stipe) are generally not consumed. These sections are therefore not included in samples.

Cut the leaf off the seaweed, around 5cm from the stem. Take the leaf as a sample.

Note: at the end of the growing season, seeds can set in this type of seaweed ([Appendix 4](#)). These strands of seaweed should not be included in the sample, as the composition of such a leaf can be significantly different to the rest of the seaweed.



(Photo from Reith et al, 2005)

Weight loss after centrifuging: around 25%

Sea Lettuce (*Ulva sp*)

Type: green algae

Harvested several times a year

Sea Lettuce is edible in its entirety, so all of it can be included in a sample (photo left).

Towards the end of the growing season, Sea Lettuce can start to take on a translucent and plastic-like appearance. This makes it unsuitable for consumption. This material is not suitable for consumption.

Any translucent, plastic-like plant material should be removed (photo right).



Fresh seaweed, suitable for sample collection and consumption



Seaweed, not suitable for sample collection and consumption

(photo left from Groenendijk et al, 2006, right Christine M. Young)

Weight loss after centrifuging: around 50%





3.5 Sample collection methods for specific sites

Besides the differences between the types of algae themselves (see 3.4) there are also differences between the sites where they seaweed is cultivated and harvested. For several locations the sample collection process is described:

- Onshore (on land)
- Inshore (estuaries and inland seas)
- Offshore (open sea)

3.5.1 Onshore sample collection

Seaweed cultivated on land is grown in tanks filled with seawater. These are semi-closed systems connected to the sea through a seawater pipe.

Seaweed cultivated on land is grown in tanks filled with seawater. These are semi-closed systems connected to the sea through a seawater pipe.

- Systematic: taking a sample from every tank in use (assuming that each tank contains the same type of seaweed, and that the seaweed is at the same stage of growth)
- Random: taking a random selection of samples drawn from all the tanks
- Risk-factor based: sample collection based on indications of an increased risk of certain hazards

The sample collection should be carried out as follows:

- Take the seaweed sample in accordance with the sample collection process described in [section 3.3](#);
- Scoop the seaweed out of the tank using a landing net (note: this can also be done by hand if gloves are worn)
- If necessary, the seaweed can be temporarily stored in a clean plastic container (of around 5-10 litres)
- Proceed in accordance with the instructions in [section 3.3](#)

3.5.2 Inshore sample collection

There are several inshore sites in the Netherlands where cultivated and wild seaweed can be harvested:

- the coast of the Wadden Islands
- the Eastern Scheldt

One of the features of inshore sites is tidal variation, as these waters are directly connected to the sea. Sample collection at these sites needs to take accessibility into account. Different issues also need to be considered for samples taken close to the coast and those taken further out.

For sample collection close to the coast, the following measures need to be taken:

- If possible, do not venture out onto the mud flats alone, due to the risk of quicksand, getting stuck, incoming tides, etcetera. Bring a mobile phone with you for emergencies, and have an emergency number at hand
- Take precautionary measures and be aware of incoming tides, any quicksand, etcetera
- Check the tide at the site for the time that sample collection is scheduled to take place. Taking samples at low tide is preferred, so that the sampling sites are readily accessible in fishing waders or chest waders. Seaweed is also located much closer to the sea floor at low tide, which makes sample collection significantly easier
- When taking samples, try to minimise disturbances to the sea floor, as this generally contains higher levels of chemical contaminants and swirling sand in the water can cause those contaminants to end up on the seaweed
- Try to get the samples into the sample bags with as little sand as possible



Further away from the coast (where sites are difficult to access on foot) the following measures should be taken:

- Ensure that a (motor) boat is available to provide access to the seaweed field. The boat should have a shallow draft so it can travel across the seaweed field without damaging the seaweed. If possible, use only paddles to move across the top of the seaweed and between the lines
- Ensure that equipment is available to pull the seaweed lines up and to cut the seaweed from the lines

The sample collection should be carried out as follows:

- Bring one or more numbered/labelled containers for temporary storage, corresponding to however many samples are to be taken. These should be able to hold around 1 kg of seaweed. Other storage equipment – preferably plastic, such as a cool box – can also be used
- Try to take samples in a way that is similar to how the seaweed is normally harvested
- Take the seaweed sample in accordance with the sample collection process described in [section 3.3](#)
- Shake off the water (avoiding contamination with sand etcetera), and place the samples in the temporary storage containers
- Place clear sticky tape across the labels, so that the information is still legible if it gets wet;
- Proceed in accordance with the instructions in [section 3.3](#)

Back on land:

- Carry out the remaining steps from [section 3.3](#) that could not be performed on the water
- If samples need to be (re)packed from temporary containers into sample bags, ensure this is performed on a clean surface. Clean the surface in between sample transfers (using for example seawater)

If the samples are to be (re)packed from temporary containers, make sure there is sufficient ground coverage (such as plastic bags) in compliance with general and specific hygiene measures ([section 3.2](#)). If possible, transfer the samples indoors on a clean stainless steel table.

3.5.3 Offshore sample collection

There is currently one offshore site in the Netherlands, with seaweed being cultivated in the North Sea. The following conditions and equipment are recommended for offshore sample collection:

- A reasonably calm sea. Ideally the sample collection should take place on a day with stable weather conditions and not much wind
- Thermal clothing (depending on the temperatures at sea)
- Windproof and waterproof rain suits and boots
- A life jacket or survival suit
- One or more divers to grab the lines and harvest the samples

Depending on the type of boat used for the sample collection, some additional safety rules may apply. These should be discussed in advance.

Offshore sample collection generally regularly involves harvesting a complete seaweed line, rather than cutting seaweed strands from a line as would be the case with inshore sample collection. The line can be stored on the boat in a drum or cool box (with some seawater, to keep it fresh) before being further processed back on land.

Preparation:

Given the conditions out at sea, it is advisable to complete some of the protocol requirements before setting off on the boat. This includes completing some of the details on the bags and sample forms.

- Bring some numbered/labelled drums, cool boxes or spare sample bags (corresponding to the number of samples to be taken) big enough to hold an entire seaweed line
- Place clear sticky tape across the labels, so that the information is still legible if it gets wet

Sample collection:

- Take the seaweed sample in accordance with the sample collection process described in [section 3.3](#)
- Keep the samples fully separated (it might be useful to have drums with differently coloured lids)
- Note any important details on the sample collection form or mark the map to show where the samples were taken

Back on land:

- Carry out the remaining steps from [section 3.3](#) that could not be done on the water
- If samples need to be (re)packed from temporary containers into sample bags, ensure this is done on a clean surface. Clean the surface in between sample transfers (using for example seawater)





4 Transporting samples

The samples should be transported to the analysis site (WFSR in Wageningen) at no more than 7° C. They should be delivered to WFSR in Wageningen within 24 hours, or to one of the other WUR sites: Wageningen Marine Research in Yerseke, IJmuiden or Den Helder.

If the samples need microbiological analysis, a temperature data logger needs to be included with the package during transportation.

If same-day delivery to one of the sites mentioned above is not possible, the seaweed will need temporary refrigerated storage elsewhere (see section 5).

5 Temporary storage of samples

If samples cannot be delivered to WFSR within the maximum window of 24 hours, they will need to be temporarily stored elsewhere.

Depending on the type of analysis required, different temperature requirements apply,

- Samples for microbiological analysis need to be stored above 0° C.
- For longer term storage (> 24 hours) samples for all types of analysis other than microbiological can be stored between -80° C and -20° C

6 Appendices

Appendix 1 Sample collection equipment

Appendix 2 Sample collection administration form

Appendix 3 Reporting template

Appendix 4 Example of sample collection reporting

This research has been funded by the Ministry of Agriculture, Nature and Food Quality (LNV) en NVWA-BuRO. The budget of LNV is financed from the Multi-year Mission-Driven Innovation Program (MMIP) Seaweed for Food and Feed/proseaweed, which has been acting in the Top sectors Agri & Food under the framework of the Kennis- en Innovatie Agenda Landbouw, Water en Voedsel

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Foto credits

Reith, J. H., E. P. Deurwaarder, K. Hemmes, A. P. W. M. Curvers, P. Kamermans, W. Brandenburg and G. Zeeman (2005). Bio-offshore : Grootschalige teelt van zeewieren in combinatie met offshore windparken in de Noordzee. ECN-C--05-008. Petten, Energieonderzoek Centrum Nederland.

Groenendijk, F. C., P. Bikker, R. Blaauw, W. A. Brandenburg, S. W. K. van den Burg, P. F. H. Harmsen, R. G. Jak, P. Kamermans, M. M. van Krimpen, H. Prins, M. Stuiver, A. K. van der Werf, M. M. Scholl and J. Wald (2016). North-Sea-Weed-Chain: Sustainable seaweed from the North Sea; an exploration of the value chain, IMARES.

Young, C.M.



Appendix 1 Sample collection equipment

Item	Required?	On site	Who
Clipboard with printout of sample collection protocol and sample collection administration form			
Plastic sleeve for documents			
Waterproof marker (2x)			
Landing net			
Grass scissors/cutter			
Gloves (preferably neoprene, at least 2 pairs)			
Strong shoes/boots			
Waterproof clothing			
Towel(s)			
Optional: long-sleeve gloves			
Roll of paper towels or cloth towels to clean equipment between samples (1 piece per sample)			
Wash bottle with clean water (quantity depends on number of samples)			
Weighing scales			
(Manual) Centrifuge			
Plastic sample bags			
Plastic tub (around 10 litres)			
Tape (preferably yellow waterproof tape)			
Cool box + freezer blocks (max 4 kg per cool box)			
Smartphone (with camera and location tracking)			

Additional items for inshore sample collection

Item	Required?	On site	Who
Wading boots and fish or chest wader(s)			
Motor boat (with shallow draft) and skipper			
Paddles			
Equipment for bringing lines up (such as a hook)			
Rubbish bags (max 10 litres)			
Numbered/marked drums for storing samples (1 per sample)			
Transparent tape (to stick over the labels)			

Additional items for offshore sample collection

Item	Required?	On site	Who
Thermal clothing			
Windproof and waterproof rain suit			
Life jacket / survival suit			
One or more divers to remove the seaweed from the lines			
Numbered/marked drums for storing samples/lines (1 per sample)			



Appendix 2 Sample collection administration form (part 1)

Name company

Address

Sample location

Sampling date / time

Sampling provided by

Class / Species of seaweed

Sample number / Sample ID

Additional information (e.g. weight)

1

2

3

4

5

6

7

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11

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14

15

Other remarks



Sample collection administration form (part 2)

Sketch of site (map of sample locations)

Sketch of cultivation on line locations (number, length, depth)



Appendix 3 Reporting template

Sample collection from:

Date:

Site	Seaweed	Method
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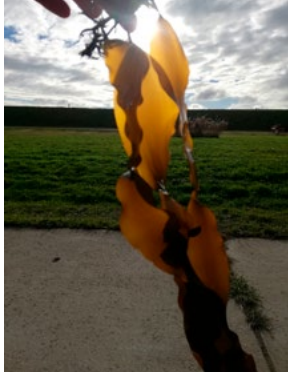

Important details about the site / deviations from protocol:



Appendix 4 Example of sample collection reporting

Reporting on samples taken on land

Date: 1 May 2020

Site	Seaweed	Method
		

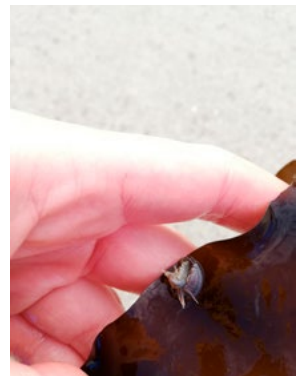
Important details about the site / deviations from protocol:



Cutting of Sugar Kelp (*Saccharina Latissima*)



Seaweeds with seeds not to be used as samples



Shellfish/crustaceans present in the seaweed



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