Provisional research protocol

PFAS in aquatic vegetation (hekkelspecie)

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In the context of increasing awareness and concern about the spread of persistent contaminants in both aquatic and terrestrial environments, it is important to gain systematic insight into the presence and distribution of per and polyfluoroalkyl substances (PFAS) in watercourses and related environmental compartments. PFAS are a group of human made substances known for their chemical stability and water and grease repellent properties, while at the same time being persistent, bioaccumulative, and potentially toxic to humans and the environment. Because of these characteristics, there is growing global attention on measuring PFAS in various environmental matrices, including water, soil and biota (where biota refers to plants as well as animals, and animal derived products such as eggs).

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1. Background, objectives and principles

Aquatic plants form a particularly relevant matrix for monitoring PFAS, because their roots, leaves and other surfaces are in direct contact with both the sediment and the water, which are the two largest PFAS reservoirs in the aquatic environment. Plants may therefore play a role in the potential transfer of PFAS to the wider environment beyond the aquatic, through management activities such as ditch cleaning ('schouwen' or 'hekkelen').

Ditch cleaning is a common practice in Dutch water management in which aquatic and riparian flora is periodically removed from ditches and other watercourses to maintain flow and prevent channels from becoming overgrown or silted up. The vegetation and sediments removed during this process (also referred to as hekkelspecie) are, in practice, often deposited on the bank — typically within 25 metres of the watercourse — for temporary storage prior to disposal or reuse. . In some cases the organic material is reused, for example as fertilizer or soil improver, on agricultural land.

Due to the known presence of PFAS in the sediment of various watercourses, concerns have arisen that the removal and processing of the vegetation may also transfer PFAS from the aquatic environment to the terrestrial environment. When hekkelspecie is deposited on the bank or spread elsewhere, there is a potential that PFAS will enter the soil, with possible consequences for local soil quality, groundwater and food safety. The possibility that these substances may, through human activity, be transported further away from their original source underscores the importance of accurate sampling and analysis of aquatic plants during management activities.

At present, there is no standardized or widely accepted approach for sampling and analyzing aquatic plants as an environmental matrix. Recent practical experience and consultations with involved parties show that different laboratories report highly variable results for comparable samples. These differences are likely due to variations in sample preparation, differences in analytical methods, and a lack of uniformity in the selection and handling of plant material. As a result, the measured concentrations are difficult to compare, leading to uncertainty in the interpretation of the results.

To obtain reliable and reproducible data that can serve as a basis for well founded decision making, there is a need for a standardized, substantiated, and practically, feasible, cost-effective procedure for sampling, handling, and analyzing aquatic plants in the context of PFAS research.

This protocol aims to meet that need. It provides a structured description of how fieldwork and laboratory analyses should be carried out for the plants that will end up in future hekkelspecie. Specific attention is given to the selection of sampling locations, the type of plant material, the methods of sampling and storage, and the processing required to obtain analytical results suitable for policy evaluation. The protocol has been developed using a combination of literature research, interviews with field experts, existing analytical methods, and insights from previous pilot projects. The goal is to establish a uniform procedure that yields reliable, verifiable and comparable data, thereby contributing to an ecologically responsible handling of hekkelspecie in PFAS impacted environments.

2. Fieldwork

2.1 Selection of sampling points and plant material

Background

Hekkelen is generally carried out once or twice a year using specialized equipment such as rake buckets (*hekkelbak*), mowing baskets, or hydraulic arms. These are operated from the bank up to approximately halfway across the width of the watercourse or, typically in larger watercourses, from a boat toward the bank. (The method of sampling is, therefore similar to the standard procedures used for sampling sediments and water. The biomass released during this process, known as hekkelspecie, is typically deposited on the bank within 25 meters of the watercourse.

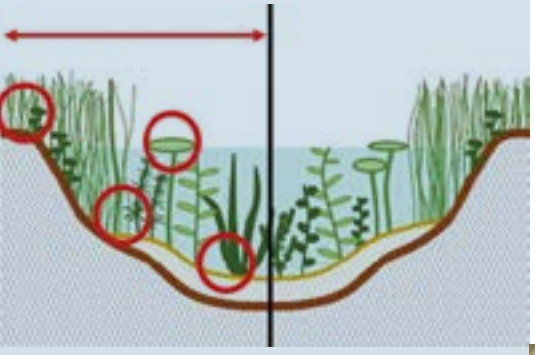
Depending on the region and the degree of contamination, the permitted period during which aquatic plants may remain on the bank varies. In Friesland, this period is at least two days, whereas in the case of PFAS contamination a maximum of five working days applies. The minimum period is intended to give aquatic animals the opportunity to return to the water.

Monsterselectie

The sampling should reflect the hekkel process as closely as possible to minimise the additional effort required. To obtain a representative plant sample of the hekkelspecie, four subsamples should be collected along a transect positioned perpendicular to the watercourse running from the bank to the midpoint of the watercourse. Along each transect, plant material should be collected at four locations, consisting of the various plant species present (riparian plants, aquatic plants on the slope and the bottom, and floating plants), as well as algae. The collection of sediment is avoided as much as possible. The subsamples should then be combined into a single composite sample for each transect.

For sampling the different plant types, the following procedure should be applied:

- Aquatic plants are usually raked during hekkelen, causing the entire plant to be pulled out including the roots. These roots should therefore be included in the



Sampling pattern within the transect

sampling for hekkelspecie. During sampling, aquatic plants can also be loosened from the bottom using a rake so that root structures are included as well.

- Floating plants (such as duckweed) end up in the hekkelspecie in their entirety and can be collected using a dip net.
- Riparian plants (such as reed) are generally mowed or partially pulled loose with the rake bucket during hekkelen. Roots typically do not enter the hekkelspecie in this process, meaning the root zone of these plants should be excluded from sampling.

In research on the concentrations of PFAS in vegetation, the recommended standard approach is not to differentiate between plant species. Four representative subsamples are collected per transect, including as many different plants, plant parts, and species as possible. These subsamples are then combined into a single composite sample. This approach aligns with actual hekkelen practice, in which hekkelspecie is usually treated and processed as a homogeneous mixture. As a result, botanical expertise is not required, and a representative picture is obtained of PFAS concentrations in the total plant material released during maintenance activities. However, during sampling it must be ensured that the composite sample is representative of the vegetation present. For example, it should be avoided that certain plant species or plant parts are overly represented in the composite sample relative to their actual presence in the field.

Sampling period

For the most representative and comparable results, the research must take place within a defined period, as PFAS concentrations can vary depending on the growth stage of the plants. Ideally, sampling should be conducted after the longest day, from late May onwards (week 22), when PFAS concentrations in plants are expected to be at their highest. This is related to reduced nutrient uptake by the plant during this phase and the transition towards flowering and seed formation.

2.2 Collecting, packaging and handling samples

Regardless of the specific research objectives, both the actual hekkelen process and the sampling must be carried out using PFAS free materials and under strict hygienic conditions to minimise cross-contamination. This includes, but is not limited to:

- No use of clothing or gloves with a fluoropolymer coating or PFAS treatment. According to current best practice, latex gloves appear to be suitable, provided that they are uncoated. Nitrile disposable gloves can also be used, as long as they are PFAS-free with no fluorine coating. Silicone gloves are generally reusable and are less commonly used during sampling due to the potential for cross-contamination.
- Do not use waders, rainwear, or water repellent clothing or footwear that has been treated with a fluoropolymer coating, as these often contain PFAS. The use of LDPE or PVC clothing is allowed.
- Do not use cosmetics, moisturizers, hand creams, sunscreens, insect repellents, or similar products that may contain PFAS. Use only products that are entirely composed of natural ingredients. If the use of such products is still necessary for personal protection reasons, ensure that direct contact with the sample material is avoided, for example by wearing gloves and a lab coat or similar protective clothing.
- All materials that come into contact with the sample material during sampling, storage, transport and laboratory analysis must be PFAS free. This includes, but is not limited to:
    - » Sampling equipment (such as scoops or grabbers)
    - » Sample containers, including lids and closures
    - » Cutting mats or other surfaces on which plant material is processed
- Avoid the use of plastics that contain fluoropolymers, such as:
    - » Polytetrafluoroethylene (PTFE or Teflon)
    - » Polyvinylidene fluoride (PVDF)
    - » Other fluoropolymer containing materials or coatings

These precautionary measures are essential to minimise the potential for contamination of the samples and to reduce the uncertainty of the analytical results. For additional information on PFAS sampling and analytical methods, reference is made to the PFAS sampling guideline (Handreiking PFAS bemonsteren), prepared by Arcadis. Although these guidelines were specifically developed for soil and water samples, many of the guidelines and recommendations are also relevant for the sampling of vegetation.

For each sampling location, one container should be filled with the collected plant material, including any roots and adhering soil or sediment that may be present. The material should be drained or shaken for a few seconds to remove excess water prior to it being placed in the container. The sample must not be rinsed or thoroughly shaken off, because this is also not done in practice during mowing or mechanical removal for hekkelen. A minimum quantity of 10 grams should be collected for each sample, to ensure there is sufficient for laboratory analysis Ideally, 15 grams should be collected. A photograph of each sample should be taken before packaging to document its condition and composition.

Per analyse wordt na homogenisatie in het laboratorium doorgaans 5 gram materiaal ingezet. Daarom is voor elk monster een minimale hoeveelheid van 10 gram vereist, zodat er voldoende beschikbaar is voor de initiële analyse én eventuele heranalyses. Bij voorkeur wordt 15 gram verzameld, zodat optimaal kan worden voldaan aan de huidige laboratoriumbehoeften. Van elk monster wordt vóór het verpakken een foto genomen, om de staat en samenstelling van het monster te documenteren.

After collection, the samples of vegetation should be immediately packaged in airtight, UV-resistant, PFAS-free containers. This packaging protects the sample from degradation or chemical changes caused by exposure to sunlight during storage and transport to the laboratory. If UV resistant material is not available, the sample should be stored in a cool, dark place and transported to the laboratory as soon as possible. For small quantities (approximately 15 grams), it is recommended that sludge jars or similar small PFAS-free containers are used. The use of standard plastic bags is discouraged unless it can be demonstrated that they do not contain or release PFAS.

Regardless of the type of packaging, samples must always be stored in a dark and cool environment immediately after sampling. Preferably, the samples should be delivered to the laboratory within a few hours.

3. Laboratory analyses

The analyses should be carried out in accordance with the guideline Implementing regulation- 2022/1428- EN- EUR-Lex. This is the European regulation on sampling and analytical methods for monitoring PFAS in certain foodstuffs. However, various aspects relating to the analysis of vegetation are not specifically addressed in this regulation and are therefore discussed below.

Sampling method

The collected plant samples should be analyzed for the presence of PFAS using advanced laboratory techniques. Liquid chromatography coupled with tandem mass spectrometry (LC MS/MS) should be used for this purpose, an analytical method known for its high sensitivity and precision in detecting PFAS in complex matrices such as biotic material.

Pretreatment: drying or not drying

There are two approaches regarding the condition of the plant sample prior to analysis:

1. Some laboratories choose to dry the plants first, after which the dried material is ground and analyzed.
2. Other laboratories analyze the wet sample as collected in the field.

Both methods have advantages and disadvantages. Drying plants prior to analysis has the advantage that results can be expressed per gram of dry weight, which allows for more accurate comparison with reference values from toxicological or ecological studies and comparison between the results of testing of vegetation carried out for research or consultancy purposes. In addition, after cutting, the shredded material always remains on the waterside for some time, where it drains and dries naturally to some extent before further processing.

The disadvantage of drying the plant material prior to analysis is that volatile or less stable PFAS compoundsmay be lost . Analyzing wet material avoids this risk but introduces greater variability due to the fluctuating moisture content of plants.

For this protocol, analyses will be carried out on wet, unevenly drained material as collected in the field. This choice aligns best with mowing practice, because in this way the material is examined in the same condition in which it was collected. It also minimizes the risk of losing volatile PFAS compounds. At the same time, sampling wet material provides a more realistic representation of what actually accumulates on the waterside in the field.

Pretreatment: grinding and homogenization

It is essential that the samples are ground and homogenized in the laboratory prior to analysis. This means that all plant parts, roots, and any adhering water, silt, or soil are ground together into one homogeneous sample. In this way, a representative overall picture is obtained of the PFAS concentrations in the total vegetation debris, as is accumulated during actual ditch maintenance. Moreover, this ensures that the ratio between plant material and silt corresponds to real world conditions.

Compounds to be analyzed

Many commercial laboratories currently (as of mid 2025) use a standard PFAS analytical suite designed for soil, groundwater or firefighting water. These suites are often limited to a selection of 24* to 30** more common PFAS compounds, such as PFOS, PFOA, GenX and the substances listed on the PFAS advisory list. Other s exist , for example, tests for PFOS and PFOA only.

For research involving plant material, it is important that the analytical suite includes the short chain substances, such as perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluorobutanesulfonic acid (PFBS). These compounds are more mobile and more readily taken up by roots, which means they can accumulate to relatively high concentrations in aquatic and riparian plants. These compounds are part of the aforementioned PFAS advisory list.

*Concawe_PFAS_EQS_reflections_article_publication.pdf

**PFAS advieslijst 2019: https://iplo.nl/publish/pages/224246/1907012-pfas_-_advieslijst_tbv_tijdelijk_handelingskader_v4.pdf

For this reason, it is generally not necessary to explicitly request an extended PFAS package to ensure inclusion of these short chain forms. At the same time, it should be noted that the selection of commonly analyzed PFAS compounds is not well documented and follows no clear prioritization method. As a result, this list is subject to change, and will be dependent on developments in PFAS research, the availability of new information relating to specific PFAS, and the ability of commercial laboratories to develop accredited methods of analysis. It is therefore, good practice to verify in advance whether the selected suiteincludes the relevant short chain forms.

If required, in addition to the standard PFAS analysis, extractable organofluorine (EOF) analysis can also be undertaken. EOF analysis can be valuable because it detects unknown or non specifically measured organofluorine compounds that are not individually analyzed in a regular PFAS determination. This provides a more complete picture of the total load of fluorine containing substances within a sample.

In the context of recent EU developments, trifluoroacetic acid (TFA), a degradation product of certain PFAS, is also being added to the priority list of PFAS in surface water. However, experience with EOF analyses and the inclusion of TFA is still variable, which can make interpretation of results challenging. Completing EOF analysis can be useful, but the practical application and results must be interpreted with care.

Want to know more?

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