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Evaluating the efficacy of protocols for genomic DNA isolation from periphyton microbial communities used for water quality monitoring

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INTRODUCTION

The Water Framework Directive (2000/60/EC) requires Member States to ensure **good water quality in their water bodies** focusing on both chemical and ecological quality. Biodiversity analysis on 356 Wallon water bodies are therefore performed on a regular basis (every 2 or 6 years).

Thanks to the **Green Deal**, Europe aims to put **biodiversity** on the **path to recovery by 2030** with implication for society, nature and climate (see *Figure 1*). Tools and methods are thus necessary to quantify and qualify the water bodies.

The aim of the **AquaBioSens** project (FEDER project included in the Smart Water Management @ccros Scales project led by the University of Liège) is to develop a **bioindicator** for water quality monitoring by using **genomic analysis of periphyton** (see *Figure 1*). Periphyton is an aquatic biofilm that adheres to substrates and when under influence of environmental factors, changes occur in its community composition. This bioindicator will **complement** already existing **biodiversity indices** such as the diatom benthic index.

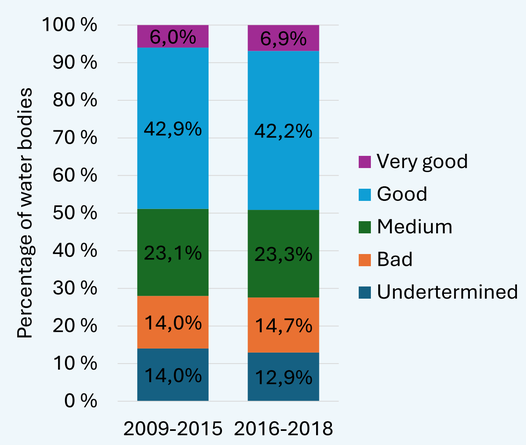

Figure 1: Global biodiversity indices of Walloon water bodies (SPW)

MATERIAL & METHODS

The first stage is to evaluate the efficacy of eleven **DNA extraction kits** for periphyton DNA isolation according to two aspects: total **DNA quantity and quality** recovery and **recovery of targeted species groups** (bacteria, microalgae and protozoa).

① The **sampling of the periphyton** was conducted as described by Pawlowski et al. (2020). Stones covered with periphyton were collected from three rivers in Liège. The periphyton was then scraped off using a toothbrush. The three samples were subsequently pooled, homogenized, aliquoted and stored at -80°C.

② Eleven **DNA extraction kits** designed for soil, plant and animal tissues, biofilm and water, and microbial samples have been assessed. Three **cell lysis technologies** using beads have been applied: FastPrep-24™ 5G, Vibro-Broyeur MM 400 and the Vortex-Genie 2.

③ The analysis of the DNA extracts was carried out using the Quantus™ to assess **DNA quantity**, by analyzing the **length of the extracted DNA** using the fragment analyzer system (Agilent), by barcoding **sequencing 16S** (V1-V3) and **18S** (V9) genes (Illumina).

RESULTS

Data from 5 kits out of 11 are presented here (see *Figure 2* legend).

There is no statistically (t-test, alpha < 0.05) significant differences among the five DNA extraction kits regarding the **amount of DNA collected**.

The **fragment length** of the extracted DNA was analyzed (GIGA department, University of Liège). The **MicroB-VG** produced the longest DNA fragments (38065) compared to the other DNA extraction protocols (8684.3 ± 4155.1 bp).

Figure 2 presents the results of 18S and 16S sequencing (Simpson's α-diversity value > 0.875 for all samples), focusing on classes and orders with a relative abundance > 3%. For **18S**, the **MicroB-VG** DNA extraction protocol appears to extract a more diverse range of classes, including green algae (***Chlorophyceae***). Diatoms (***Bacillariophyceae***) were extracted by all protocols, but their abundance is < 3%. For **16S**, at the phylum level, all protocols were able to extract ***Cyanobacteria*** (6.8 ± 1.6%), but at the order level, only three protocols, including **MicroB-VG**, successfully extracted *Nostocales* (the only order within *Cyanobacteria* with an abundance > 3%).

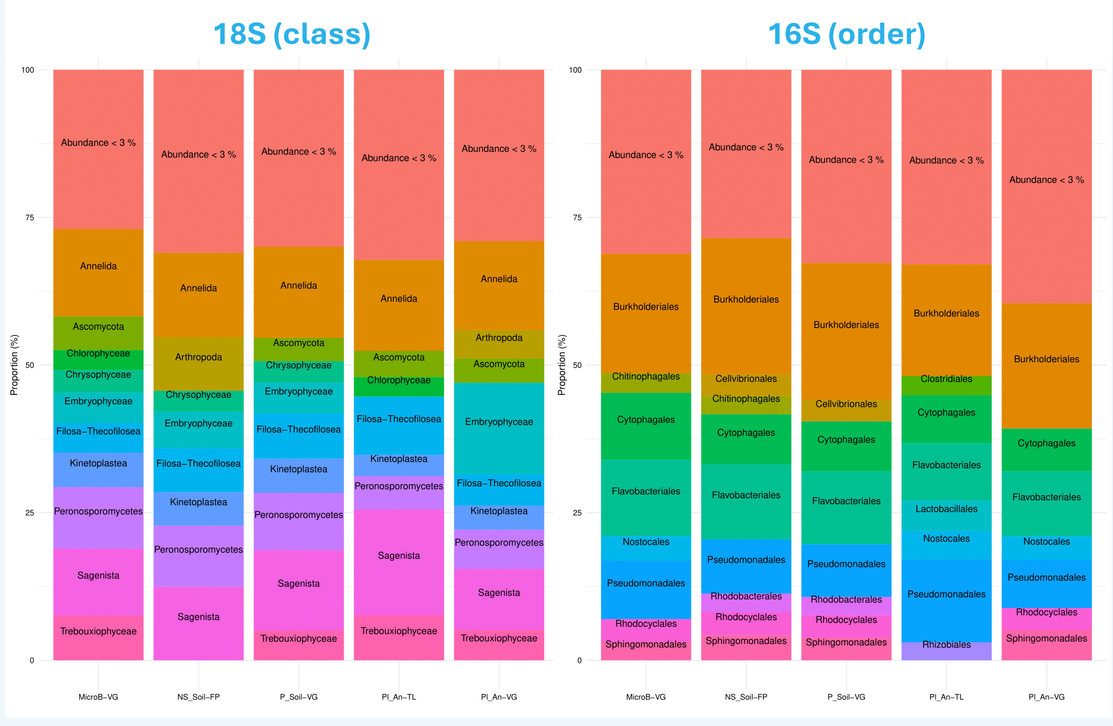

Figure 2: 18S and 16S sequencing (Illumina) results (PR^2 v5.0.0 and Emu v3.4.5 databases) for the 5 investigated DNA extraction kits: MicroB, NucleoMag® DNA microbiome (Macherey-Nagel) ; NS_Soil, NucleoSpin® Soil (Macherey-Nagel) ; P_Soil, DNeasy® PowerSoil Pro (Qiagen) ; Pl_An, FastDNA™ Spin kit for Plant and Animal tissues (MP Biomedicals) and the 3 cell lysis techniques: VG, Vortex-Genie 2 ; FP, FastPrep-24 5G ; TL, Vibro-Broyeux MM 400 (tissue lyser).

CONCLUSION & DISCUSSION

In the first part of the AquaBioSens project, eleven DNA extraction protocols were evaluated based on their efficacy in three key areas: extracting sufficient quantities of DNA, ensuring the necessary quality for subsequent applications, and enabling the identification of a diverse range of taxa.

Combining these three criteria, the **NucleoMag® DNA Microbiome** kit used in conjunction with the Vortex-Genie 2 appears to be the most relevant DNA extraction protocol: with the intention of comparing the future AquaBioSens index to existing and proven indices (i.e., IBD), it is essential to extract diatoms and, more broadly, photosynthetic organisms (i.e. *Cyanobacteria*, *Chlorophyta*, *Stramenopiles*). The chosen DNA extraction kit will be applied to analyze periphyton composition in Walloon rivers using long read sequencing, with the aim of developing the genomic bio-indicator of water quality.

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