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# Diatom-based assessment of the ecological status of the Venta River, Kuldīga

**Ieva Grudzinska**[1*], **Megija Florentīne**[2]

[1] *University of Latvia, Institute of Biology, Jelgavas iela 1, LV-1004 Riga, Latvia*
[2] *Riga 1st Secondary School, Akademijas laukums 1, LV-1050 Riga, Latvia*
[*] Corresponding author: ieva.grudzinska@lu.lv

**Abstract:** Pressure from land use patterns, climate change, and urbanisation on rivers is an important socio-ecological issue that requires management and biomonitoring. The ecological status of the Venta River at Kuldīga was assessed using bioindicators, specifically diatoms, which are widely used in monitoring of streams and rivers worldwide, especially in European countries. Ecological status was defined by calculating Specific Pollution Sensitivity Index (IPS). In addition, diatom diversity was determined in the studied part of the river. A total of six sampling sites were selected along a 10 km stretch of the river in the territory of Kuldīga. The average distance between sampling sites was 1.5 km.

A total of 118 taxa were identified in all samples. The highest species diversity (67 taxa) was observed in sample 2. Individual rarefaction was calculated if exactly 500 diatom valves were counted in all samples. The lowest species diversity was observed in samples 6 (45 taxa) and 6A (17 taxa). Samples 1 and 5 were the most similar in terms of species composition. This is probably because both sites have similar physical characteristics that include sandy beaches with a slow current.

The most abundant diatoms were *Cocconeis placentula* Ehrenberg, *Amphora pediculus* (Kützing) Grunow, *Nitzschia fossilis* (Grunow) Grunow, *Navicula capitatoradiata* Germain ex Gasse, *Navicula antonii* Lange-Bertalot, *Amphora libyca* Ehrenberg, and *Sellaphora nigri* (De Notaris) Wetzel & Ector, which are commonly found in eutrophic waters. Diatom analysis suggests that the Venta might be at risk for eutrophication. For the most abundant diatom taxa, the susceptibility to pollution was assessed as III (medium), but there were also diatoms with IV and V (very sensitive to pollution). This shows that the Venta River in Kuldīga overall has low levels of pollution. All study sites were rated as having 'good' ecological status according to the IPS index (12.4-14.1 IPS).

**Key words:** aquatic pollution, bioindicators, eutrophication, Specific Pollution Sensitivity Index (IPS), diatoms, Latvia

## Introduction

According to the Europe 2020 strategy, one of seven societal challenges Europe must face is the adaptation to climate change and its influence on the aquatic environment. Freshwater resources are essential for human well-being. However, over the last decades Europe's freshwater resources have faced increasing pressure (EEA, 2018). The main drivers of deterioration in water quality are urbanisation, industrialisation, intensive agriculture, and climate change (Alan, 2004; Jury & Vaux, 2005). A cornerstone of EU environmental policy is protecting water resources and ensuring their ecological quality with an aim of ensuring access to good or high quality water for all EU citizens. One of the Water Framework Directive (WFD) targets was to create uniform standards for water policy within the EU and to aim for at least 'good status' for all water bodies in the EU by 2015. In 2018, the European Environment Agency (EEA) reported that only 40% of monitored European water bodies met water quality standards (EEA, 2018). This means that stakeholders (e.g., water resource management organisations and policymakers, nature protection organizations) and researchers must work closely together to reach the WFD aims for 'good status' for all water bodies.

In general, the term "ecological status" is used to describe the combination of biological quality, physicochemical and hydromorphological quality. This combination is generally expressed as an ecological quality ratio (observed/reference), which is into five scale-status classes (high, good, moderate, poor, and bad) (EC, 2003; Masouras et al., 2021). In lotic ecosystems, the most used bioindicators are phytobenthos, benthic invertebrates, fish, macrophytes, as well as phytoplankton (Masouras et al., 2021). Diatoms are the dominant component of phytobenthos and have a fundamental ecological role in aquatic ecosystems. During photosynthesis, inorganic carbon ($CO_2$) is taken up and converted into organic compounds, as well as diatoms participate in phosphorus, nitrogen and silica cycles (Julius and Theriot, 2010; Mann, 1999). Due to their short lifecycle, diatoms respond fast to any natural and anthropogenic disturbance, making them more sensitive to changes in environmental parameters (e.g., inorganic nutrients, organic pollutants, pH, temperature, salinity, and heavy metals) than other biotic groups (Julius and Theriot, 2010; Kelly and Whitton, 1995; Laine et al., 2014; Wu et al., 2016). Therefore, they are good indicators of water quality and land use change (Stevenson, 2014; Stevenson et al., 2010), and as such are the most common indicator organisms in biological assessment in the WFD (Almeida et al., 2014).

Several diatom indices are regularly implemented to evaluate the ecological status of water bodies, these include the Specific Pollution Sensitivity Index (IPS; Coste, 1982), Trophic Diatom Index (TDI; Kelly and Whitton, 1995), Biological Diatom Index (BDI; Coste et al., 2009) and others (Masouras et al., 2021; Stevenson et al., 2010; Tan et al., 2017). For this study, the IPS was chosen to determine the water quality in a river, as it correlates with water quality parameters such as nutrient enrichment, organic pollution, and conductivity (Descy and Coste, 1991). The IPS is one of the most precise diatom-based indices (Tan et al., 2017), as it incorporates the largest data set among all diatom indices, more than 2000 taxa (Descy and Coste, 1991).

The ecological quality of different aquatic ecosystems in the territory of Latvia has been monitored by Latvian Environment, Geology and Meteorology Centre (LEGMC), but benthic diatoms have not been regularly and extensively used as bioindicators. Therefore, this study focuses on a particular part of the Venta River that flows through Kuldīga. The catchment includes residential areas with allotments, industrial areas and agricultural lands. Urbanization and intensive agriculture often have an observable impact on the river's ecology, as increased pollution tends to interfere with physiological processes in living organisms. This often leads to pathologies, which in turn can further reduce quality and expectancy of life at a societal cost. The monitoring of pollution in the Venta is crucial for establishing a baseline and timing of informed and appropriate municipal-level interventions for prevention and mitigation.

The aim of this study was to assess the ecological status of the Venta at Kuldīga using diatoms as bioindicators and to determine diatom diversity in the studied part of the river.

## Study site

The Venta River begins in Užventis, Šiauliai County, Lithuania (168 km), runs through western part of Latvia (178 km) and flows into the Baltic Sea at Ventspils, Latvia. The basin system is ca. 11 800 km$^2$ large, of which ca. 50% is forested. The upper part of the river has a faster current, while downstream of Kuldīga, the river meanders along a calm course. The shores are mostly overgrown and have a few bathing areas. In some places, there are exposed outcrops of reddish sandstone. The largest tributary Abava (Latvia) is 134 km long. The river usually freezes at the end of December, and the ice starts to melt at the beginning of March. The largest settlements along the Venta River are Mažeikiai (population size: 38 819 in 2018) in Lithuania and Kuldīga and Ventspils (population sizes: 10 109 and 33 372 in 2021, respectively) in Latvia. The Venta River flows from a dolomite bed into sandstone, forming the largest waterfall in Latvia – the Venta waterfall (in Latvian: Ventas Rumba) in the town centre of Kuldīga.

The study was carried out along a 10 km stretch of the Venta River in the territory of Kuldīga town (Fig. 1). The average distance between sampling sites was 1.5 km. In total, six sampling sites were selected (Table 1). Fig. 2 illustrates sampling sites along the Venta River.

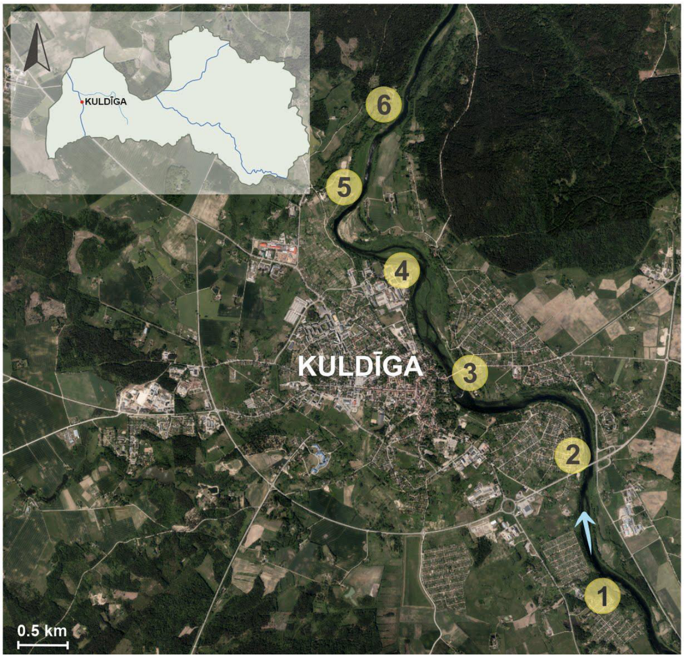

Figure 1. Study area of Venta in Kuldīga, Latvia. Sampling sites are marked with yellow circles. The light blue arrow indicates the direction of flow of the Venta River (LGIA, 2019)

Table 1. Description of sampling sites

| Nr. | Water depth | Coordinates | Description | Potential sources of pollution |
|---|---|---|---|---|
| 1. | 0.5 m | 56°56′55″<br>22°0′40″ | Swimming area on the left bank of the Venta in Ābeļciems, near private houses | Adjacent nearby allotments, agricultural land |
| 2. | 0.5 m | 56°57′42″<br>22°0′05″ | Left bank of the Venta near the bridge, construction works on the opposite bank | Construction works, pollution from road traffic, using sand and salt on the road |
| 3. | 0.2 m | 56°58′09″<br>21°58′47″ | Venta waterfall. The right bank of the Venta | Popular tourist destination, located in the city centre, 400–600 m from construction debris disposal site on the left bank of the Venta |
| 4. | 0.5 m | 56°58′49″<br>21°58′15″ | A swimming area on the left bank of the Venta, with automobile repair shops nearby | Automobile repair shops, industrial area |

| | | | | |
|---|---|---|---|---|
| 5. | 0.7 m | 56°59′17″<br>21°57′35″ | Swimming area on the left bank of the Venta River. Opposite is the water treatment plant | Agricultural land, incompletely treated sewage, residents using the bathing area |
| 6. | 0.5 m | 56°59′40″<br>21°57′57″ | Left bank of the river Venta, near the castle mound | Water from the treatment plant flows into the opposite bank. Here, the tributary Krāčupīte flows past the factory and the allotment gardens |

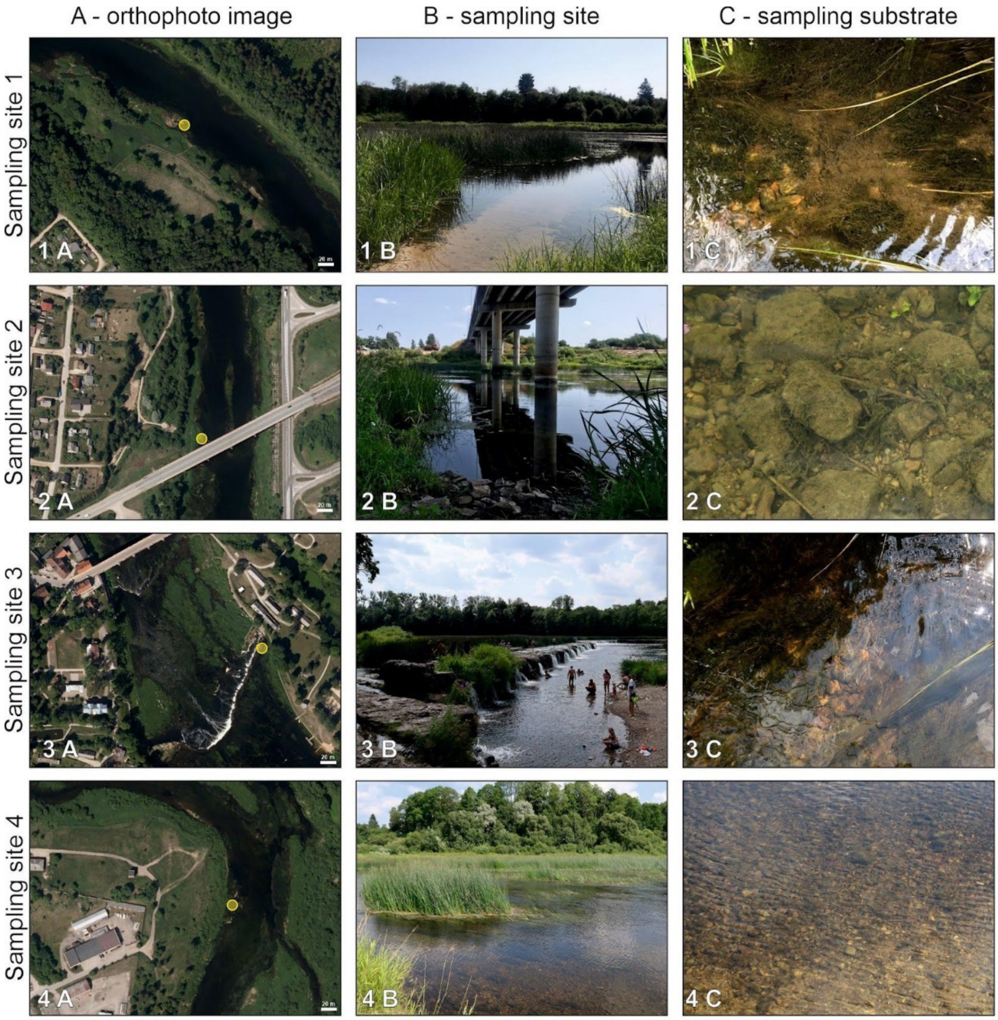

Figure 2a. Sampling sites along the Venta River. A – orthophoto image (LGIA, 2019), sampling site indicated with a yellow circle, B – sampling site, C – sampling substrate

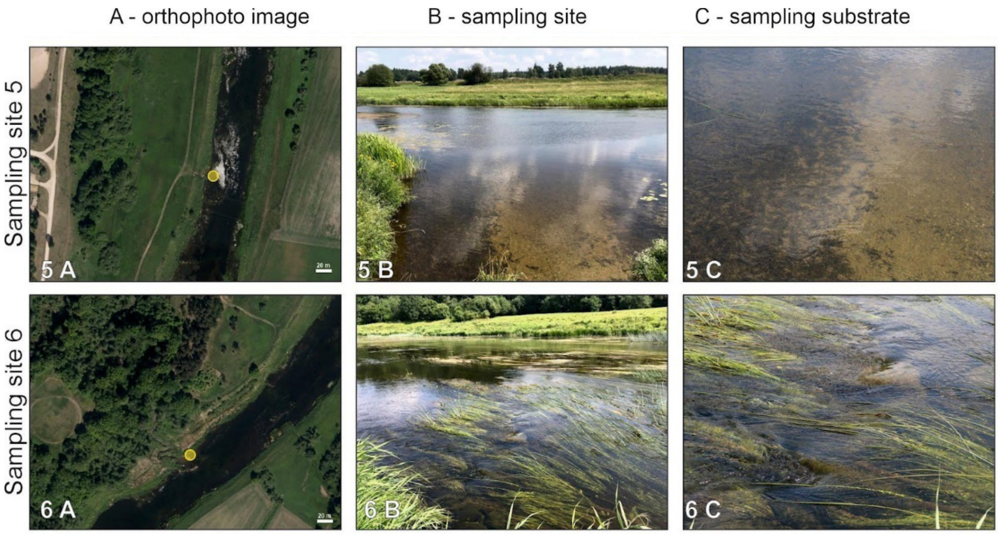

Figure 2b. Sampling sites along the Venta River. A – orthophoto image (LGIA, 2019), sampling site indicated with a yellow circle, B – sampling site, C – sampling substrate

## Material and methods

### Sampling

In total, six sites were selected in the Venta River in Kuldīga, Latvia. The study sites were selected by assessing the potential sources of pollution in the river. The samples were collected on 26th July 2021. Before sampling, the area was visually documented by photo camera, and exact coordinates were marked on the map.

Benthic diatoms were sampled on rocks, which were chosen as targeted natural substrata, as periphyton samples on aquatic plants can be highly variable (Stevenson et al., 2010). The sampling procedure was carried out in accordance with the guidelines for sampling diatoms on rocks described in Kelly et al., (1998), recommendations in the European Union monitoring programs (WFD: EC, 2000) and the EU Standard for sampling (EN 13946; EU, 2014). In the river, five rocks (samples 1–6) of similar size with an obvious diatom film were chosen. In addition, periphyton from aquatic plants (samples 2A, 3A and 6A) in three sites were sampled in order to compare diatom composition on different substrata. The water depth, at which the stones were found, was measured and marked in a field journal. The rocks or aquatic plants were placed in a bowl and softly brushed with a toothbrush. Each sample was placed in a clean 50 ml centrifuge tube labelled with the name and number of the sampling site and date of the fieldwork campaign.

## Microscope slide preparation

In the laboratory, the samples were centrifuged to remove excess water. The remaining residue was treated following the diatom sample preparation protocol (Battarbee et al., 2001). At first, samples were treated with 10% hydrochloric acid (HCl) to remove $CaCO_3$. After carbonates have reacted in the samples, they were washed three times with distilled water. Then, 35% hydrogen peroxide ($H_2O_2$) was added to remove organic matter. Samples were placed in a water bath where the temperature gradually increased from 50 °C to 85 °C. During the gradual temperature rise, a few drops of $H_2O_2$ were added to the sample until the reaction was complete. The samples were again rinsed three times with distilled water. A drop of the remaining material was put on a cover slip and left to dry overnight at room temperature. The microscope slides were properly labelled and placed on a hot plate. A drop of *Naphrax* was applied on the warm microscope slide, on which the dried cover slip with the sample on it was carefully placed. Since *Naphrax* contains the organic solvent toluene, the microscope slides were placed on the stove in a fume hood and remained there until the toluene evaporated (i.e., the sample stopped bubbling).

## Diatom identification and analysis

The European Standard (EN 14407; EU, 2014) for the WFD was followed for diatom analysis. Diatoms were identified and enumerated to species level (if possible) under the microscopes AmScope SME-F8BH and Leica DM2500 LED by using oil immersion at 1000× magnification. A minimum of 500 diatom valves was counted per slide to estimate relative abundance of taxa. The diatom taxonomy was based on diatom floras (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b; Lange-Bertalot et al., 2017), as well as on the internet sources Diatoms of North America (diatoms.org).

Samples were grouped by applying incremental sum of squares cluster analysis (CONISS) performed on the full percentage sum (transformed by square root function) diatom data with Tilia (Grimm, 2011). The diatom species richness was assessed using rarefaction analysis (Birks and Line, 1992) in the software PAST 4.03 (Hammer et al., 2001). Rarefaction analysis was chosen as it fits well for data in which count sizes vary and, thus, must be normalised ($n = 500$).

## Calculation of Specific Pollution Sensitivity Index

The Specific Pollution Sensitivity Index (*IPS*) is one of the most commonly used diatom indicators, which uses taxonomic composition of assemblages, the relative abundance of taxa in the sample (taxonomic composition) and the environmental preferences and tolerances of taxa (autecological attributes of taxa or taxa traits) (Stevenson et al., 2010; Masouras et al., 2021). Calculations of *IPS* are usually done at the species level. The OMNIDIA software (Lecointe et al., 1993) was used to calculate the IPS indices.

Calculation formula:

$IPS_{(1-5)} = \Sigma\, S \times V \times A \Sigma V \times A$ (Coste, 1982)

where: $A$ – Abundance of the alga
$V$ – species ecological sensitivity index (Versatility)
$S$ – Sensitivity to pollution

Algal abundance ($A$) is calculated as: the number of individuals of a species to the total number of diatoms in the sample. The ecological sensitivity index ($V$) of a species is a number between 1 and 3, where 1 stands for the widest ecological niche while 3 indicates diatoms with the narrowest ecological niche. Sensitivity to pollution ($S$) is characterised by a number between 1 and 5, where 5 denotes the taxa most sensitive to pollution.

The $IPS_{(1-5)}$ index calculated above is used to calculate the final $IPS$. This is used to determine the ecological status of a water body.

$IPS_{(\text{final})} = (IPS_{(1-5)} \times 4.75) - 3.75$

Table 2. IPS ecological quality indicators (Kokorite et al., 2018)

| IPS Specific Pollution Sensitivity Index | | | | |
|---|---|---|---|---|
| High | Good | Moderate | Poor | Bad |
| > 15.5 | 15.5–12.0 | 12.0–9.5 | 9.5–6.9 | < 6.9 |

## Results and discussion

### Benthic diatom assemblages in Venta

A total of 118 diatom taxa belonging to 47 genera were found in nine samples (six from rocks, Samples 1–6, and three, Samples 2A, 3A and 6A, from macrophytes) from the Venta River. Only diatom taxa accounting for ≥ 2% of the diatom abundances are presented in the diatom diagram of Venta (Fig. 3).

The most abundant diatoms were *Cocconeis placentula* Ehrenberg (mean = 18.9%; max = 52.8%), *Amphora pediculus* (Kützing) Grunow (mean = 15.9%; max = 30%), *Nitzschia fossilis* (Grunow) Grunow (mean = 4.5%; max = 13%), *Navicula capitatoradiata* Germain ex Gasse (mean = 3.9%; max = 8.1%), *Navicula antonii* Lange-Bertalot (mean = 2.6%; max = 6.9%), *Amphora libyca* Ehrenberg (mean = 3%; max = 6.4%), and *Sellaphora nigri* (De Notaris) Wetzel & Ector (mean = 2.5%, max = 5.1%), which are commonly found in eutrophic waters. In significant quantities indifferent taxa, such as *Achnanthidium minutissimum* (Kützing) Czarnecki (mean = 2.5%, max = 6.1%), *Navicula cryptotenella* Lange-Bertalot (mean = 6%; max = 10.3%) in all samples and *Ulnaria ulna* (Nitzsch) Compère (max = 8.6%) in sample 4. Diatom data suggests that the Venta River might be at risk of eutrophication.

The majority are eutrophic diatoms 49–72% (median 60%), the lowest number of eutrophic diatoms is in sample 2 (49%), but the highest (72%) in sample 3, while in samples 6 and 6A were observed the highest abundance of hypereutrophic taxa (4–5%). The highest species diversity (rarefaction *n* = 500) was observed in sample 2 (67 taxa), while the lowest species diversity was observed in samples 6 (45 species) and 6A (17 taxa). The most similar samples in terms of species are samples 1 and 5 (Fig. 3). This is probably because both sites have similar physical characteristics that include sandy beaches with a slow current (Fig. 2 – 1B and 5B).

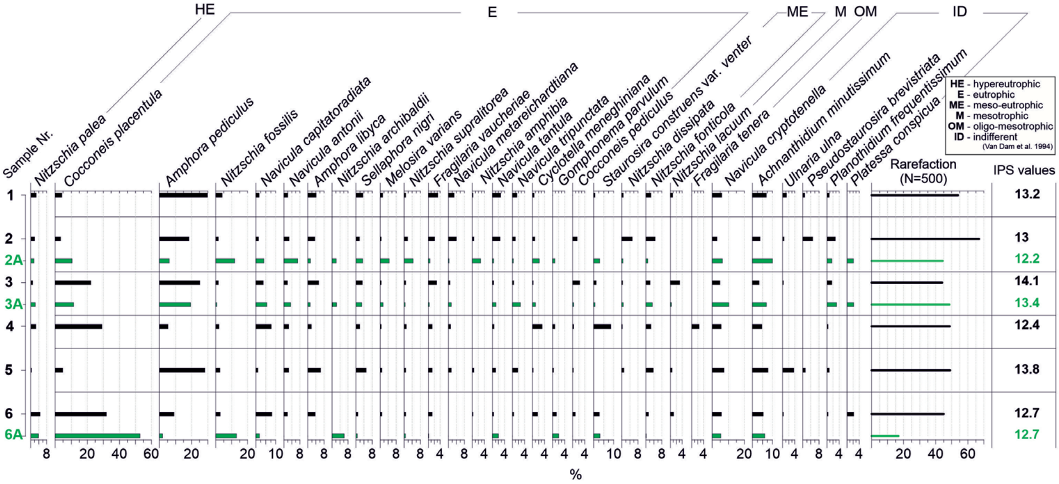

Figure 3. Diagram of selected diatoms. Only diatoms › 2% at least in one sample are presented. Diatoms are grouped according to the trophic state where they are found most often (Van Dam et al., 1994). 'A' indicates samples taken from macrophytes

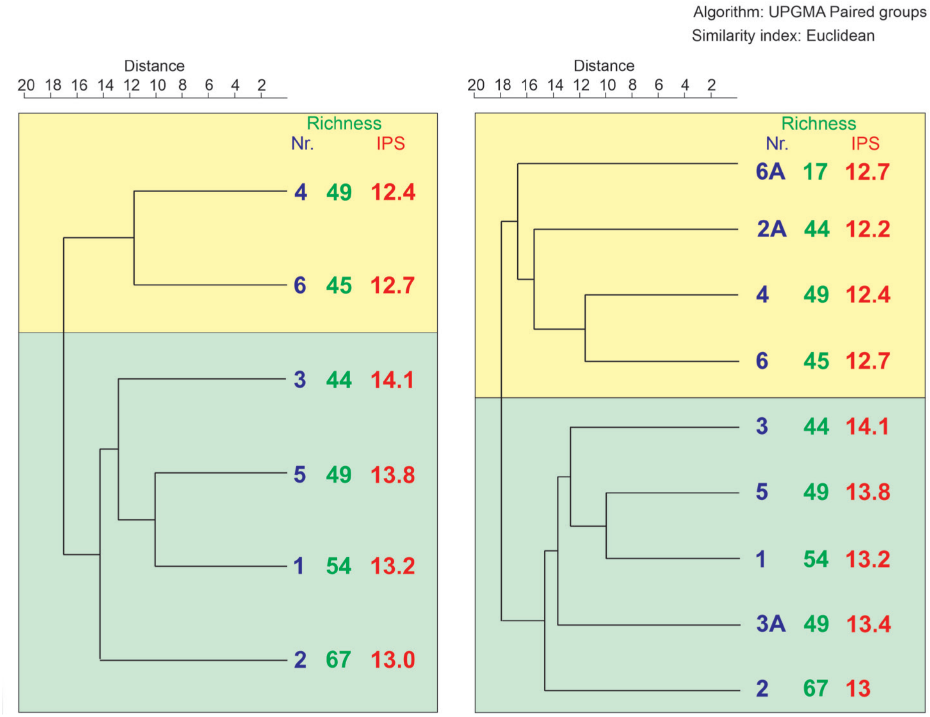

Figure 4. Cluster plots from clustering analysis (CONISS). On the left side, diagram shows clusters of samples scraped from rocks, and the diagram on the right side shows the clusters of the most similar samples taken from different substrates (rocks and macrophytes)

## Water quality assessment in the Venta River

The precision and accuracy of performance of the IPS partly depends on degree of overlap between the taxa list integrated in the IPS and those that occur in the analysed samples (Tan et al., 2017). More than 95% of all diatom taxa identified in the Venta River samples were found in the IPS list.

Overall, all samples met the 'good' ecological status according to the IPS values (Fig. 3 and 4, Table 2) in the part of Venta River flowing through Kuldīga town. The lowest value from samples scraped from rocks was calculated for sample 4 (12.4 IPS), while the highest IPS value had sample 3 (14.1 IPS). If samples brushed from macrophytes are considered, then the lowest IPS value was calculated to sample 2A (12.2 IPS). IPS values tend to be lower for samples 2A and 3A, which biofilms were taken from submerged macrophytes, it might be explained by epiphytic diatom ability to uptake additional nutrients through the plant. Therefore, it is important to choose a targeted substrate (if possible) at the beginning of field work (Stevenson et al., 2010). As mentioned before, in this study the targeted substrate is submerged medium size rocks.

Water quality and ecological status determined by IPS indices of diatom samples collected in 2021 indicate good water quality and correspond to data collected by the Latvian Environment, Geology and Meteorology Centre (LEGMC) in 2018 and 2019 (Table 3). The latter data provide a total biological assessment of two closest monitoring stations to the study site and show 'good' ecological quality. While total physicochemical assessment indicates 'poor' water quality in two upstream monitoring stations in 2019 due to elevated total nitrogen (TN; 4.3–4.5 mg/l), other chemical indicators show 'high' or 'good' water quality. Observed elevated TN might be caused by increased runoff from agriculture lands (Kaliff, 2002). Data from 2020 (LEGMC, 2021) from two other monitoring stations along the Venta River upstream from Kuldīga also show elevated TN (3.45–3.72 mg/l), indicating 'moderate' ecological status. In addition, biological assessment from one of the stations (representing 2020) also shows 'good' ecological status in the Venta River, as the ones close to Kuldīga (representing 2018 and 2019).

Table 3. Monitoring results (LEGMC, 2020 and 2019): the numbers for biological quality elements, hydromorphological and total ecological quality indicate ecological quality class (1 – high, 2 – good, 3 – moderate, 4 – poor, 5 – bad), and numbers for chemical parameters represent yearly average concentrations

| **Monitoring station** | **Venta** | | |
|---|---|---|---|
| | **0.5 km upstream of Kuldīga** | **1.0 km downstream of Kuldīga** | **Upstream of Ēdas** |
| **Year** | **2019** | **2018** | **2019** |
| Benthic invertebrates | 2 | 1 | 1 |
| Macrophytes | 2 | N | 3 |
| Phytobenthos | | 2 | |
| Total biological assessment | 2 | 2 | 3 |
| $O_2$, mg/l | 11.4 | 11.4 | 11.2 |
| $BOD_5$, mg $O_2$/l | 1.4 | 1.07 | 1.4 |
| N-$NH_4$, mg/l | 0.03 | 0.06 | 0.05 |
| Total N, mg/l | 4.5 | 1.9 | 4.3 |
| Total P, mg/l | 0.055 | 0.051 | 0.058 |
| Cu, µg/l | 1.6 | 1.1 | 1.5 |
| Zn, µg/l | 1.1 | 1.1 | 1.0 |
| Total physicochemical assessment | 4 | 2 | 4 |
| Summary ecological quality | 3 | 2 | 3 |
| Hydromorphological modifications | 2 | 2 | 3 |

Colour chart of quality criteria:

| | | | | |
|---|---|---|---|---|
| High | Good | Moderate | Poor | Bad |

Rivers usually experience high variability in discharge, water chemistry, temperature, and light availability (Stevenson et al., 2010) throughout the vegetative period. Therefore, one-time measurements of environmental parameters might not provide a precise characterisation of physical and chemical conditions of the river or stream (Stevenson et al., 2010). Thus, phytobenthos (e.g., diatom-based) autecological indices are particularly valuable in water quality assessments, as a one-time assay of diatom taxa composition and abundance in lotic ecosystems provides a better characterisation of environmental conditions (Stevenson et al., 2010, Stevenson, 2014).

## Conclusions

Calculating autecological indices based on species composition and abundance of benthic diatoms is an effective approach to determine ecological status of aquatic ecosystems including rivers and streams.

Diatom analysis suggests that the Venta River might be at risk of eutrophication. This could be mitigated by controlling the use of fertilizers on the surrounding agricultural lands. Pollution might also enter the river from neighbouring allotments. To reduce the risk of pollution, it should be checked whether all allotments with summer houses are connected to the urban sewerage network.

Calculated IPS index values show that the Venta River in Kuldīga overall has low levels of pollution. All study sites were rated as having ‘good’ ecological status on the IPS index. The highest diatom diversity was observed in Sample 2 (67 taxa), which was taken from the rocks, and the lowest diversity was in the sample taken from the water plants (17 taxa) – sample 6A.

## Acknowledgements

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