Stream Monitoring using Near-infrared Spectroscopy of Epilithic Material

Jan Persson
Stream Monitoring
using
Near-infrared Spectroscopy of Epilithic Material

av

Jan Persson

Akademisk avhandling

som för avläggande av filosofie licentiatexamen framläggs till offentligt försvar fredagen 1 juni 2007 i KB3A9 (Lilla Hörsalen) KBC-huset, kl 10.00.

Environmental Change Assessment
Department of Ecology and Environmental Science
Umeå University
Umeå 2007
Stream Monitoring using Near-infrared Spectroscopy of Epilithic Material

Abstract

The European Union Water Framework Directive (WFD), with initiatives to manage surface water resources, has increased the need for fast and inexpensive methods for monitoring conditions in streams. The objective of this thesis is to assess the potential of near-infrared spectroscopy (NIRS) of epilithic material to become such method. NIRS, which is a technique that is commonly used in industry for process monitoring and quality control of products, registers the chemical properties of organic material on a molecular level. Epilithic material, i.e. the layer of dead and alive material that covers stone surfaces in streams, is continuously influenced by the stream water that flows over it, and it has the potential to integrate chemical and biological conditions over time. The temporal integration is a significant factor, since conditions in streams can change within hours or days.

The thesis consists of two published papers. In the first paper a new sampler for epilithic material, the Stone Brusher, was described and the performance evaluated. The Stone Brusher is designed to take qualitative or semi-quantitative samples of epilithic material from stones at 7-50 cm water depth. The epilithic material is dislodged from the stone surface with a rotating brush enclosed in a chamber, and the material is drawn up directly into the sample bottle with an air-cylinder. The operator takes a sample quickly and without putting hands into the water. The sampler is made of plastic, stainless steel and aluminium and weighs 3.1 kg. It is designed to meet the demand for standardized sampling for research and environmental monitoring and to improve working conditions for sampling personnel. The equipment allows sampling from surfaces of bedrock and large stones that cannot be lifted from the bottom. Using data of near-infrared spectroscopy and diatom analyses, this new sampler was evaluated in comparison to the toothbrush method, a primitive method which is the current standard in EU. The results indicate that the Stone Brusher reduces sampling variability compared with the toothbrush method.

In the second paper, the Stone Brusher was used to collect epilithic material from 65 sites (42 uncontaminated and 23 contaminated) from streams in the widespread mining area called the Skellefte-district in Västerbotten, northern Sweden, in order to test the hypothesis that impact on the epilithic material caused by emissions from mining and mining-related industries can be detected using NIRS. The epilithic material was filtered onto glass fibre filters, measured by NIRS, and the results were modelled using Principal Component Analysis (PCA). The NIRS approach was evaluated by comparing it with the results of chemical and diatom analyses of the same samples. Based on PCA, the NIRS data distinguished contaminated from uncontaminated sites and performed slightly better than chemical analyses and clearly better than diatom analyses. Of the streams designated a priori as contaminated, 74 % were identified as contaminated, 65 % by chemical analysis and 26 % by diatom analysis. Unlike chemical analyses of water or of epilithic material samples, NIRS data reflect biological impacts in the streams. Given that, and the simplicity of NIRS-analyses, further studies to assess the use of NIRS of epilithic material are justified. NIRS has the potential to become a fast method for screening in regions where large numbers of streams occur to find impacted streams or as a routine method for temporal monitoring in selected streams for early detection of environmental impact, similar to process monitoring in industry.

Key words: Streams, environmental monitoring, Water Framework Directive, water quality, biological impact, epilithic material, near-infrared spectroscopy, diatoms


Signature: Date: 1 June 2007
Stream Monitoring using Near-infrared Spectroscopy of Epilithic Material

Jan Persson
List of papers
This licentiate thesis is based on the following two papers, which are referred to in the text by Roman numerals.


Paper I is reproduced with permission from Hydrobiologia 560, 385-392. © Springer.

Paper II is reproduced with permission from Environmental Science & Technology 41, 2874-2880. © American Chemical Society.
Abstract
The European Union Water Framework Directive (WFD), with initiatives to manage surface water resources, has increased the need for fast and inexpensive methods for monitoring conditions in streams. The objective of this thesis is to assess the potential of near-infrared spectroscopy (NIRS) of epilithic material to become such method. NIRS, which is a technique that is commonly used in industry for process monitoring and quality control of products, registers the chemical properties of organic material on a molecular level. Epilithic material, i.e. the layer of dead and alive material that covers stone surfaces in streams, is continuously influenced by the stream water that flows over it, and it has the potential to integrate chemical and biological conditions over time. The temporal integration is a significant factor, since conditions in streams can change within hours or days.

The thesis consists of two published papers. In the first paper a new sampler for epilithic material, the Stone Brusher, was described and the performance evaluated. The Stone Brusher is designed to take qualitative or semi-quantitative samples of epilithic material from stones at 7-50 cm water depth. The epilithic material is dislodged from the stone surface with a rotating brush enclosed in a chamber, and the material is drawn up directly into the sample bottle with an air-cylinder. The operator takes a sample quickly and without putting hands into the water. The sampler is made of plastic, stainless steel and aluminium and weighs 3.1 kg. It is designed to meet the demand for standardized sampling for research and environmental monitoring and to improve working conditions for sampling personnel. The equipment allows sampling from surfaces of bedrock and large stones that cannot be lifted from the bottom. Using data of near-infrared spectroscopy and diatom analyses, this new sampler was evaluated in comparison to the toothbrush method, a primitive method which is the current standard in EU. The results indicate that the Stone Brusher reduces sampling variability compared with the toothbrush method.

In the second paper, the Stone Brusher was used to collect epilithic material from 65 sites (42 uncontaminated and 23 contaminated) from streams in the widespread mining area called the Skellefte-district in Västerbotten, northern Sweden, in order to test the hypothesis that impact on the epilithic material caused by emissions from mining and mining-related industries can be detected using NIRS. The epilithic material was filtered onto glass fibre filters, measured by NIRS, and the results were modelled using Principal Component Analysis (PCA). The NIRS approach was evaluated by comparing it with the results of chemical and diatom analyses of the same samples. Based on PCA, the NIRS data distinguished contaminated from uncontaminated sites and performed slightly better than chemical analyses and clearly better than diatom analyses. Of the streams designated a priori as contaminated, 74 % were identified as contaminated by NIRS, 65 % by chemical analysis and 26 % by diatom analysis. Unlike chemical analyses of water or of epilithic material samples, NIRS data reflect biological impacts in the streams. Given that, and the simplicity of NIRS-analyses, further studies to assess the use of NIRS of epilithic material are justified. NIRS has the potential to become a fast method for screening in regions where large numbers of streams occur to find impacted streams or as a routine method for temporal monitoring in selected streams for early detection of environmental impact, similar to process monitoring in industry.
Introduction

The EU Water Framework Directive (WFD) was adopted by the European Parliament and the Council (Directive 2000/60/EC) in 2000. The main objectives are to manage Europe’s freshwater resources by restoring polluted waters and ensure that unpolluted waters are maintained in a good state. According to the WFD, all surface waters should have a ‘good status’ by 2015, and as a consequence, the demands for action and control have increased. To achieve the goals of the WFD, monitoring programs are required, the results of which are reported to the European Environment Agency (EEA) in Copenhagen. In Sweden, the national monitoring program is coordinated by the Swedish Environmental Protection Agency, the central environmental authority under the Swedish Government. The monitoring is administrated by the Water Authorities, the County Administration Boards and other regional actors.

Considering freshwater issues, the US Environmental Protection Agency (EPA) was ahead of the EU since it established the ‘Clean water act’ already in 1972. Australia, Japan and Brazil also have freshwater monitoring programs; in other areas of the world extensive water monitoring occurs only sparsely, but will likely commence following increased awareness of the need to manage surface water resources.

Freshwaters are exposed to different kinds of anthropogenic impact, such as emissions of nutrients from agriculture and forestry, acid emissions from fossil fuel combustion, and toxic chemical compounds and elements from activities such as mining and metal production. At present, lake and stream monitoring is performed using biological parameters, such as fish, macro-invertebrates, macrophytes and algae, and with physical and chemical parameters such as pH, TOC, DOC, metals, conductivity, alkalinity, water colour, nutrients and oxygen saturation (Hauer and Lamberti 1996). Although all these methods individually provide information about chemical and ecological conditions, a battery of analyses is usually required to get a comprehensive picture of the status of the streams. Many of the methods currently used require experts and are time consuming and expensive. Simple, inexpensive and standardized methods are desirable for better comparison between studies and also between countries (Aloi 1990), and my thesis aims at developing such a method for stream monitoring based on near-infrared spectroscopy (NIRS) of epilithic material. Although this approach has a potential for monitoring a variety of environmental parameters, I focus here on the impact from mining and mining-related industries.

Epilithic material, i.e., the layer of organic and inorganic material that covers stone surfaces in streams, has a great potential as an alternative approach for stream monitoring. Epilithic material is composed of material deposited from the water, as well as algae, bacteria, fungi and other benthic organisms. The submerged epilithic material is continuously exposed to and influenced by the stream water that flows over it. The epilithic material reflects both the physical and chemical conditions in the water and as such integrates chemical and ecological variability over time. Temporal integration is a significant factor, since conditions in streams can change within hours or days (Davies et al. 1992; Laudon and Bishop 2002) and traditional monitoring must for economic reasons rely on one or a few samples each year.

Near-infrared spectroscopy (NIRS) is a non-destructive technique that is commonly used in industry for control of product quality and process monitoring, for example in the
food and the pharmaceutical industries (Givens et al. 1997; Reich 2005; Scarff et al. 2006). The technique is based on absorption of near-infrared radiation by polar bonds, such as C-H, N-H and O-H, and thereby describes the molecular composition of the organic material (Siesler et al. 2002). NIRS has also been used to a limited extent in ecological and limnological studies (Malley et al. 1993; Foley et al. 1998; Dåbakk et al. 2000; Korsman et al. 2001), but has still not found wide application in environmental monitoring. NIRS analyses are rapid and require limited sample preparation and the instrumentation is relatively inexpensive.

The hypothesis examined in this thesis is that impact on the epilithic material, either directly or indirectly, caused by the emissions from mining and metal-related industries can be detected using NIRS, and furthermore, that the NIRS-technique can become a fast and inexpensive complement to frequent water sampling and methods that require involvement of specialists, such as most biological analyses. Near-infrared spectroscopy performed on epilithic samples can be used as a screening method to detect environmental impacts, which then can be further assessed by specialized methods.

The most common use of NIRS is to calibrate the NIR-spectra against some measured constituent of the analyzed material, e.g., protein or water content of products in food industry (Siesler et al. 2002), or against properties such as lake-water pH or TOC in environmental contexts, and then use these calibrations to infer the parameter of interest in unknown samples based on their NIR spectra (Korsman et al. 1992; Malley and Williams 1997; Dåbakk et al. 2000). This approach utilizes the simplicity, robustness and cost efficiency of NIRS, but it also requires analysis and establishment of a transfer function (model) of some constituent that carries the wanted information. Calibration against a particular constituent also risks utilizing only a fraction of the total environmental pressure that is reflected in the analyzed material. The approach used in this thesis is to register the natural variation in uncontaminated forest streams using NIRS and establish a model based on these sites against which unknown samples are compared. This utilizes more fully the power of NIRS to identify systematic variation and impact in the organic chemical composition of the sample, and is advantageous in comparison to modelling against only one selected chemical element in order to discover environmental pressure on biota by diffuse and complex environmental contamination.

Samples of epilithic material from 65 stream sites in Västerbotten, northern Sweden, were used for the study, see list of sites in Paper II, Table 1. The streams comprised both forest streams without any direct contamination source and streams affected by mining-related activities. To evaluate the performance of NIRS, the NIRS method was compared with chemical and diatom analyses, made on the same samples of epilithic material. Heavy metal analyses and in particular analyses of stable lead isotopes \(^{206}\text{Pb}/^{207}\text{Pb}\) ratios indicate influence from mining-related industries since lead is present in most sulphide ores, and the isotopic signature of the ores and the common bedrock is different. Lead is composed of the stable isotopes \(^{206}\text{Pb}, ^{207}\text{Pb}, ^{206}\text{Pb}\) and \(^{204}\text{Pb}\). The ratio \(^{206}\text{Pb}/^{207}\text{Pb}\) is a recognized tool to detect lead origin. The natural geogenic \(^{206}\text{Pb}/^{207}\text{Pb}\) ratio in bedrock and uncontaminated soils in Sweden is 1.5 ± 0.2 SD (Brännvall et al. 2001), while the ratio of the lead ore in the study area in northern Sweden is 1.02-1.12 (Billström 1996), i.e., it has a much lower ratio than the common geogenic lead. Given the large difference in the isotope ratio, a small influence from mining can be detected. Diatoms are unicellular algae that are very common in streams and are sensitive to water
quality including mining-related elements (Medley and Clements 1998; Niyogi et al. 2002). Diatoms are one of the main components of the epilithic material. To evaluate data of NIRS, chemistry, and diatom analyses, Principal Component Analysis (PCA) was used, as well as more conventional methods for chemistry and diatoms.

The new sampler (PAPER I)

Sampling of epilithic material in streams is usually made with rather unsophisticated techniques, and the EU standard for sampling of benthic algae is primitive. The current procedure recommended by the European Committee for Standardization is as follows: In moderate and fast flowing streams with pebbles, cobbles and boulders the sampling personnel pick up a cobble, place the cobble in a tray and brush the upper surface with a clean toothbrush or scrape it with a knife and collect the epilithic material in a sample jar (CEN 2003).

There are several reasons for improving the sampling procedure for submerged epilithic material in streams and lakes. First, during the brushing and rinsing procedures the epilithic material from the upper surface of a stone that has been picked up can be mixed with debris of unknown age from the lower side of the stone, i.e., the risk of contamination is high. Second, it can be uncomfortable and unhealthy to put hands into cold or polluted water, and thirdly, in some streams with extensive rock face and big boulders it is very difficult or impossible to find stones that can be picked up and carried to the shore.

The sampler, developed for stream monitoring and presented in this thesis, has a total weight of 3.1 kg and the length is 120 cm. It consists of a brush chamber with a brush connected to a stainless steel rod running inside an aluminum pipe to the top, where there is a rotating knob to twist the brush, which gives a brushed area of 28 cm². The chamber is connected to a sample bottle (150 ml) by plastic tubing. The sample bottle is further connected to an air cylinder which creates an under-pressure to draw 125 ml ± 25 ml of water and epilithic slurry from the brush chamber up into the sample bottle, which is easily attached and exchanged (PAPER I, Figure 1). Stones must be at a depth of 7-50 cm and after the sampling point in the stream has been selected, it takes less than a minute to take a sample for a trained person. In streams the ideal sampling site for use of this equipment is a moderate to fast flowing stream (0.2-1.5 m s⁻¹) with many submerged stones.

The sampler has been tested in different environments, from steep, clear headwater streams in the mountains to slowly flowing, silty streams in the lowland region, in summer as well as in winter. The sampler, which is called the Stone Brusher, was first designed in 1997 and since then, some five thousand samples have been taken from ~130 streams.

The sampler was tested in two studies: i) a comparison with the toothbrush method made in an oligotrophic lake in northern Sweden using near-infrared spectroscopy and ii) diatoms sampled from 46 streams in central Sweden. In the lake along a 100-m straight boulder-ridge shore, 34 stones were sampled with tooth brushes and 34 stones were sampled with the Stone Brusher. To reduce systematic sampling variability along the shore, the two methods were alternated every other sampling until totally 68 equally-sized stones were sampled. Principal Component Analysis (PCA) was used to evaluate
the spectroscopy data. Average, standard deviation and kurtosis were calculated for all six significant principal components. The standard deviation in the Stone Brusher sample group is slightly lower in the first and most important component compared to the toothbrush method, and the kurtosis values of the first three components are more positive when the Stone Brusher has been used compared to the toothbrush method. Kurtosis characterizes the relative peakedness or flatness of a distribution curve compared with the normal distribution curve. Positive kurtosis indicates a relatively peaked distribution and negative kurtosis indicates a relatively flat distribution. This means that if the Stone Brusher is used, fewer samples have to be taken to achieve statistically reliable results.

The stream diatom study was an attempt at an analytical quality control study. 10 sub-samples (diatom slides) from one sample, 10 samples from one big flat stone, 10 stones from the same stream and finally samples from each of 46 streams were assessed with PCA. The within-sample variability was smaller than the between-surface variability, which was smaller than the between-stone variability, which was smaller than the between-stream variability. That is an expected result and supports that the new sampler works satisfactory in streams.

The near-infrared spectroscopy method (PAPER II)
In regions with many streams, traditional surface-water monitoring programs become expensive. It is desirable therefore to develop methods for fast and simple screening for two reasons. First, to define what the reference condition (the normal undisturbed condition) is in order to select a minimum number of streams with reference conditions for future routine monitoring. Secondly, to identify stream sites where environmental conditions deviate from reference conditions due to human impact and concentrate more of the available expertise resources on these objects by doing full water chemistry and biological assessment. The basic hypothesis of this thesis is that NIRS of epilithic material can become such a screening method. The results of the study presented in Paper II, which were aimed at assessing if emissions from mines and metal-producing facilities could be detected with NIRS, are encouraging.

The basic approach used in this study, to compare NIRS data of unknown samples against NIRS data from reference streams, utilizes the power of NIRS to register systematic deviation in the chemical composition of the organic material of the epilithic samples. The first task was to build a data-set from streams with reference conditions, i.e. in this case streams without any known impact from mines or related industries. The development of a NIRS model that represents the variation of the spectra of reference streams followed established methods (Filho et al. 2004). The first step was a PCA analysis to select a sub-set of representative samples from these reference sites including low, median and high score values on each of the significant principal components to cover all variation in the reference data-set. This sub-set was used to make a model (calibration data-set) for the reference sites (uncontaminated sites). The second step was then to compare all the other samples to that calibration data-set. For PAPER II, a model for contaminated sites was also developed using the same approach.

It was clear that NIRS data could be used to distinguish contaminated epilithic material in mining streams from uncontaminated epilithic material in reference sites. To
discriminate between contaminated and uncontaminated sites we used the deviation between each sample and the principal component model for contaminated and uncontaminated samples, respectively. The deviation between a single sample and the models was visualized by Cooman-plots. Of the 23 sites from contaminated streams, 17 sites (74%) were classified by the PC-model using NIRS-data as contaminated (PAPER II, Figure 1), a result also supported by the chemistry data. In the majority of cases (75%), the NIRS model classified the same sites as contaminated as the chemistry model did. The PC-model based on chemistry data from uncontaminated sites classified 16 sites as significantly different from the model of uncontaminated sites, of which 15 sites (65%) were sites in streams that receive water from mining industries (PAPER II, Figure 2). These were in most cases also the sites that had low $^{206}\text{Pb}/^{207}\text{Pb}$ ratios, i.e. a clear signal of emissions from ore processing (PAPER II, Figure 3).

The internal relationship between the NIRS-based predictions of the sites sampled downstream from the emissions source in the polluted stream Vormbäcken illustrates the performance of the NIRS methodology. In the PCA the sites in Vormbäcken plot according to the expected degree of mining impact, where the sites closest to the emission source have scores furthest from the model of uncontaminated sites and most of the samples downstream of the tailing dam appear sequentially in the plot. The two sites in Vormbäcken that are situated most distant from the contamination source were classified as uncontaminated sites by NIRS (PAPER II, Figure 1). Moreover, the chemistry and diatom data could not distinguish them from uncontaminated sites. This result is not surprising as those sites are situated downstream of an 8 km² lake which probably acts as a sink for pollutants.

Of the 23 stream sites originally designated as mining-related sites, the NIRS-based model classified six as uncontaminated. Besides the two mentioned above, plus another site in the middle of Vormbäcken, two are located near abandoned mines and one is downstream of a 50-year-old test-mining area, i.e., these are sites with a minimal impact from mining (PAPER II, Figure 1).

The separation of the contaminated sites from the PC modelled class of uncontaminated sites is larger for the NIRS results compared to chemistry data. The NIRS-based model not only identified the same samples as the chemical-based model, but the resolution was significantly better. The separation of contaminated samples from uncontaminated was four times larger for the NIRS-based model than the chemical-based model (PAPER II, see x-scale in Figure 1 and 2). The most plausible interpretation of this is that relatively small variations in chemical contents are magnified by effects on the biota and manifested in changed composition of both the living and dead organic material that NIRS registers. This observation further indicates that NIRS actually reveals the impact from metals on the stream ecosystem while chemistry data merely reflect variability in metal concentrations without any information on biological effects. As a result, NIRS is probably a more sensitive tool than chemical analyses to detect mining impact and monitor environmental conditions in streams. This is a desirable property of monitoring methods since early detection of a problem allows early remedial action and lower costs (Allan et al. 2006). Considering NIRS sensitivity and the simplicity of NIRS-analyses, further studies to assess the use of NIRS are justified. NIRS has the potential to become a cost-effective method for screening in regions with large numbers of streams and as a routine method for temporal monitoring in selected streams for early warning of
environmental impact, in a similar manner as NIRS is used for monitoring of industrial processes.

**Suggestions for further research**

The new sampler, described in Paper I, is a substantial improvement on previous methods. However, as discussed in the paper, the sampler could easily be modified to take quantitative samples.

In Paper II the focus was on the possibility of detecting impact from mining and the results were encouraging, despite the fact that pollution levels of heavy metals were low in the studied streams. Of course, further studies are needed, with a larger data-set and preferably with a larger metal contamination gradient. I argue that the epilithic material is a time-integrating material, unlike water samples that only provide a momentary picture. This hypothesis is supported by an unpublished test I made by repeated sampling in four streams over a period of a month, and a study in two streams with three samplings over one year (Dåbakk 1999). A longer study of temporal changes with more sampling dates of the epilithic material should be performed.

The diatom study reported in Paper II did not show any strong correlation between diatoms and mining impact. This should be investigated more thoroughly, since there are reports in the literature about large effects of mining on diatom assemblages (Medley and Clements 1998; Niyogi et al. 2002). However, in most of those studies the pollution load is significantly larger than in the streams in Västerbotten. Furthermore, the streams in my study that drain the largest pollution sources, such as Brubäcken and Vormbäcken, are extensively limed.

Dåbakk et al. (2000) assessed relationships between NIR spectra of seston collected on glass fibre filters and the following lake water parameters: TOC, water colour, pH and total phosphorus (TP). There were good relationships between NIRS and TOC, water colour and pH, and less strong with TP. Although seston and epilithic material are not the same, it suggests that NIRS can record subtle changes in the properties of the organic material in aquatic environments. It is very likely that NIRS of epilithic material could be used for monitoring stream water acidity, total organic carbon (TOC), and trophic conditions.

Finally, since the interest for diatom analysis as a biological monitoring method is increasing, a comment on this issue is warranted. In my Master thesis (Persson 1998), diatom assemblages in epilithic material and water chemistry data from 65 streams from Dalarna were analysed, and weighted averaging (WA) was used to assess the relationship between diatoms and the water chemistry variables pH, TOC, tot-N, and TP. WA indicated a strong relationship between the diatom assemblages and pH (apparent $R^2 = 0.70$, jackknifed $R^2 = 0.63$). WA indicated also that the diatom assemblage was related to total organic carbon (TOC). No relationships between diatom communities and concentrations of nitrogen and phosphorus were observed in that study; however, the N and P gradient was very short, and in addition to that, the environmental data points were few. In palaeolimnology, modern statistical methods have been developed to infer environmental conditions from sub-fossil diatoms in lake sediments, and these methods are commonly applied (Birks 1998). In stream monitoring, a more traditional approach using indicator species is pre-dominant, but it seems reasonable to believe, as suggested
by my master thesis, that methods from palaeolimnological research could be transferred to stream monitoring as well, and be beneficial for environmental monitoring in running waters.

Acknowledgements
Thanks to Georange in Malå which financed the majority of my work, and my supervisors, Ingemar Renberg who is full of ideas and always had time to answer my questions, and Mats Nilsson for decisive advice. Thanks to Tom Korsman and all my colleagues at the Department of Ecology and Environmental Science, which were helpful and inspired me to work. Hans Hansson, Luleå, was the skilful engineer behind the technical solutions of ‘The Stone Brusher’. Steve Brooks, Julieta Massaferro, Ben Williamson, Malcolm Grant from the Natural History Museum of London, sampled many of the stream sites with me. It was Steve who started it all when he offered collaboration in the summer of 2002, in an EU-funded project lead by Georange in Malå. Birgitta Olsson, Swedish University of Agricultural Sciences (SLU) made all the chemistry analyses.

Tack
Till min familj som ofta hejat på, stundtals tvivlat, skickat matpaket, plockat upp och lämnat av mig på buss- och järnvägsstationer under tider på dygnet när ingen annan vill vara ute.

Literature Cited


