REAL-TIME SENSING AS ALERT SYSTEM FOR SUBSTANCES OF CONCERN

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Need for online water sensors as early warning systems for detection of contamination events

The presence of contaminants and pathogens in the environment is of increasing concern because they can have adverse effects on aquatic lifeforms, and their presence means more and more advanced treatment is required before water can be used for potable purposes. Although background levels of these substances of concern are typically low, they can already have an adverse effect at those levels, e.g. cytotoxicity, genotoxicity, and carcinogenesis (US EPA, 2021). Because of large variations in the occurrence and concentration of these substances, it should be evaluated through routine monitoring approaches. However, identification of such contaminants of emerging concern (CECs) are normally regarded as time- and cost-intensive. To achieve a meaningful monitoring programme for CECs, a sampling and analysis frequency is hence required that cannot be achieved with classical grab sampling. Monitoring programmes for early detection of CECs should therefore rely on online sensor systems. These sensors should produce a warning when elevated concentrations of substances of concern (CEC and pathogens) are present.

The capabilities of available online systems, however, are very different from those of the highly advanced analytical systems available in a laboratory. Online sensors typically measure physical properties of the water, such as electrical conductivity, light absorption, or refractive index (RI), or they measure individual substances through selective methods (e.g. using ion selective electrodes). Commercially available online sensors today are not yet capable of measuring the individual contaminants of concern, and due to the low concentrations of CECs, their direct measurement will probably remain restricted to sophisticated equipment. It will also not be feasible to develop such targeted CEC detectors, as the list of potentially harmful CECs continues to expand with more research results becoming available. This problem is exacerbated by the fact that human behaviour and activities, industrial types, as well climatic conditions all have an effect on the CECs to be found and persisting in a region. The same limitation is valid for emerging pathogens.

Aims and Objectives

With the demonstrated risk of toxicity to humans as well as the ecosystem, there is clearly a need for the establishment of smart sensors that are affordable with sufficient adaptability to be suitable for use in varying conditions. The overall goal of this project was therefore to collate information on relevant technologies, including those that are currently not considered for CEC detection but with potential to expand this field. The specific aims of the project were therefore to:

1. Establish a knowledge basis on sensing systems for smart water quality monitoring.
2. Draw up desired characteristics for sensing technologies for rapid detection of substances of concern and develop appropriate test protocols.
3. Select promising sensing technologies and do bench scale evaluation of these technologies.
4. Draw up a comprehensive evaluation report containing the results of the evaluation, with specific conclusions and recommendations for subsequent implementation at water sources nationally.

Scope

A review of the technologies and systems currently in use or the topics of research programmes were carried out and reported in Chapter 2. The chapter includes a consideration of desired characteristics for online sensors and rapid detection systems. The evaluation work reported here (Chapters 3 and 4) focussed on the potential utility of the CEMS system to detect CECs, specifically to assess the system's sensitivity to the low concentrations at which CECs are typically found in the environment i.e., the CEMS reactors were exposed to a range of CECs, to assess the impact on microbial metabolism and determine the minimum concentrations for measurable effects (Chapter 3). This was followed by exposing of the CEMS reactors to environmental waters containing high levels of CECs.

In Chapter 4, the potential of near-infrared (NIR) spectroscopy combined with multivariate data analysis techniques was investigated to differentiate between pure water samples and water samples spiked with three different CECs (acetaminophen, benzotriazole and caffeine) at different concentrations.

New developments and promising technologies for early-warning systems and effect-based monitoring are discussed in Chapter 5, which then focussed on using the BioTrac system as further development of the CEMS for early warning.

Conclusions

Carbon dioxide evolution measurement system (CEMS)

While experimental data showed that high CEC concentrations inhibit microbial metabolism, CEC concentrations, up to 300 times of what is frequently detected in South African wastewater effluent, had no impact on the metabolic rate of the indigenous biofilms.

This result showed that environmental CEC concentrations are too low to impact microbial metabolism, which underscores the high degree of metabolic redundancy of microbial communities i.e., extensive gene and species pool enable self-regulation to continuously adjust to available nutrients and inhibitors.

NIR Spectroscopy

The research conducted on near-infrared (NIR) spectroscopy combined with multivariate data analysis techniques has provided valuable insights into its potential application as an early warning system for water quality monitoring, particularly in municipal water and wastewater treatment plants, as well as other water reclamation and reuse facilities. The following aspects point out how this technique can be applied effectively:

<u>High Precision Instrumentation:</u> The study demonstrated high instrument precision through repeatability and reproducibility tests. This reliability is crucial for an early warning system as it ensures consistency in detecting contaminants.

<u>Detection of Contaminants:</u> NIR spectroscopy, when combined with multivariate data analysis, showed promise in differentiating between pure water and water samples spiked with various contaminants (CECs) at different concentrations. This ability to detect contaminants is the core function of any early warning system.

<u>Limit of Detection:</u> The research provided valuable insights into the limit of detection for specific contaminants. For example, it determined a limit of detection of 10 mg/L for acetaminophen and even lower limits for

benzotriazole and caffeine. Knowing these limits allows for a more accurate assessment of the severity of contamination events.

Ecological Relevance: While the technique worked well for detecting contaminants at relatively high concentrations, it raised concerns about its ecological relevance in scenarios where dilution occurs, such as confluence of large rivers or during rainfall events. This insight is essential for designing effective monitoring strategies.

Group Monitoring (Cocktail Approach): Given the complexity of water contamination, focusing on individual chemicals may not always be practical. The suggestion to focus on a group of contaminants (cocktail approach) acknowledges the need to consider multiple pollutants simultaneously, which is more representative of real-world contamination scenarios.

Complimentary Early Warning System: NIR spectroscopy combined with multivariate data analysis can serve as a complementary tool within an early warning system. It can aid in rapidly identifying the presence of contaminants or deviations from normal water quality parameters.

Cost-Effective Monitoring: Implementing this technique could lead to more cost-effective monitoring programs in water treatment plants. Traditional methods of monitoring water quality can be labour-intensive and time-consuming. NIR spectroscopy offers a faster and potentially more economical alternative.

Routine Surveillance: This technique could enable routine surveillance of water sources, helping operators and authorities respond quickly to contamination events. Early detection can lead to faster mitigation efforts, reducing the impact of contaminants on public health and the environment.

Future Research: The study highlights the need for further research to refine the technique and expand its applicability. This includes exploring its effectiveness for a wider range of contaminants and under various environmental conditions.

Other real-time sensors

The use of biosensors for the screening of CECs have advanced considerably in the past two decades and includes both targeted (single chemical) and effect-based (combined biological activity) approaches. Effect-based methods, representing key biological modes of action as proxy for health risks, can provide integrated estimates of hazardous chemical burdens. When considering the sheer number of chemicals potentially present in effluent, drinking water or environmental samples, targeted screening through analytical chemistry, or single chemical biosensors is impractical, and effect-based methods are preferred.

Although real-time effect-based biosensors are not currently available, such technology may likely be the future of water quality monitoring. Increased investment and research efforts are needed for the development of effect-based biosensor devices representing key health risks. Biosensor devices utilizing engineered microbes are promising vehicles for effect-based sensing and could enable remote deployment and routine testing at a fraction of the cost of analytical chemistry methods.

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TABLE OF CONTENTS

EXECUTIVE SUMMARY III

ACKNOWLEDGEMENTS VI

LIST OF FIGURES X

LIST OF TABLES XI

ABBREVIATIONS XII

CHAPTER 1: INTRODUCTION 1

1.1 CONTEXTUALISATION 1

1.2 AIMS AND OBJECTIVES 3

1.3 SCOPE 3

CHAPTER 2: LITERATURE REVIEW 4

2.1 INTRODUCTION 4

2.1.1 Water quality monitoring and its significance 4

2.1.2 Limitations of traditional water quality monitoring methods 6

2.1.3 Emergence of online and real-time water quality sensors 7

2.2 ONLINE AND REAL-TIME WATER QUALITY SENSORS 10

2.2.1 Optical Sensors 10

2.2.2 Chemical Sensors 10

2.2.3 Electrochemical Sensors 10

2.2.4 Biosensors and biological monitoring sensors 11

2.3 PRINCIPLES AND OPERATION OF EARLY WARNING SYSTEMS (EWS) 12

2.3.1 Background of EWS for water quality monitoring 12

2.3.2 Principles of EWS for water quality monitoring 12

2.3.3 Operation of EWS for water quality monitoring 13

2.4 BENEFITS AND LIMITATIONS OF EARLY WARNING SYSTEMS 13

2.4.1 Benefits 13

2.4.2 Limitations 14

2.5 CHALLENGES AND FUTURE DIRECTIONS 15

2.6 CONCLUSION 16

CHAPTER 3: CARBON DIOXIDE EVOLUTION MEASUREMENT SYSTEM (CEMS) 18

3.1 INTRODUCTION 18

3.2 METHODOLOGY 18

3.2.1 Sampling and inoculum freezer stocks preparation 18

3.2.2 CEMS reactor inoculum preparation 19
3.2.3 Carbon Dioxide Evolution Measurement System 19
3.2.4 Sample preparation for CEC quantification 22
3.3 INTERIM RESULTS 22
3.3.1 Bench-scale evaluation of CEMS as a sensor for individual CEC detection 22
3.3.2 Bench-scale evaluation of CEMS as sensor for CEC detection in environmental water sources24
3.4 DISCUSSION 26
3.5 INTERIM CONCLUSIONS 27
CHAPTER 4: NEAR-INFRARED SPECTROSCOPY 29
4.1 INTRODUCTION 29
4.2 METHODOLOGY 30
4.2.1 Instrumentation 30
4.2.2 Chemicals of Emerging Concern (CECs) 30
4.2.3 Sample Preparation 31
4.2.4 NIR Instrumentation 31
4.2.5 NIR Spectra Collection 31
4.2.6 Spectral Data Analysis 31
4.3 RESULTS AND DISCUSSION 34
4.3.1 Repeatability and Reproducibility 34
4.3.2 Spectral Analysis of CEC Solutions 36
4.3.3 Wavelength Selection 37
4.3.4 Principal component analysis (PCA) 39
4.3.5 Classification 41
4.4 CONCLUSIONS AND RECOMMENDATIONS 44
CHAPTER 5: NEW DEVELOPMENTS AND PROMISING TECHNOLOGIES 46
5.1 INTRODUCTION 46
5.2 THE NEED FOR NEW APPROACHES TOWARDS EARLY WARNING SYSTEMS FOR CECS 47
5.3 USING THE BIOTRAC SYSTEM AS FURTHER DEVELOPMENT OF THE CEMS FOR EARLY WARNING 49
5.3.1 BioTrac system 49
5.3.2 Monitoring the effect of CECs on microbial communities 50
5.3.3 BioTrac as a warning system 51
5.3.4 Future Developments 51

CHAPTER 6: KNOWLEDGE TRANSFER AND CAPACITY BUILDING 52

6.1 INTRODUCTION 52

6.2 WORKSHOP 1: KNOWLEDGE DISSEMINATION 52

6.3 WORKSHOP 2: FEEDBACK AND FINDINGS 52

6.4 CAPACITY BUILDING 53

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS 54

7.1 CARBON DIOXIDE EVOLUTION MEASUREMENT SYSTEM (CEMS) 54

7.2 NIR SPECTROSCOPY 54

7.3 OTHER REAL-TIME SENSORS 54

CHAPTER 8: WAY FORWARD FOR REAL-TIME WATER SENSING 55

8.1 INTRODUCTION 55

8.2 THE WAY FORWARD FOR REAL-TIME EFFECT-BASED EARLY WARNING SYSTEMS 55

8.3 MICROBIAL BIOSENSORS AS PART OF WATER QUALITY MONITORING FRAMEWORKS AND FUTURE RESEARCH NEEDS 59

REFERENCES 61

APPENDICES 73

APPENDIX A: WORKSHOP 1 73

Appendix A1: Programme 73

Appendix A2: Attendance List 74

APPENDIX B: WORKSHOP 2 75

Appendix B1: Programme 75

Appendix B2: Attendance List 76

LIST OF FIGURES

Figure 3.1: Diagram of CEMS principal 19

Figure 3.2: CEMS setup for CEC/environmental test water exposure. 20

Figure 3.3: Metabolic profile of a pure culture biofilm, expressed as CO_2 production, when exposed to 100 and 1000 ug/L sulfamethoxazole for 2 hours (indicated by the red sections), in that order. 23

Figure 3.4: Metabolic profile of an environmentally relevant mixed community biofilm, expressed as CO_2 production, when grown up on 1 g/L TSB and exposed to filtered river water receiving high loads of CEC contamination for 2 hours (start of exposure indicated by red arrow). 24

Figure 3.5: Metabolic profile of an environmentally relevant mixed community biofilm, expressed as CO_2 production, when grown up on 1 g/L TSB and exposed to filtered wastewater effluent for 2 hours (stat of exposure indicated by red arrow). 25

Figure 4.1: Schematic diagram of aa FT-NIR instrument 30

Figure 4.2: Standard deviation plot of the repeatability and reproducibility tests 36

Figure 4.3: Unprocessed mean spectra of MilliQ, acetaminophen, benzotriazole and caffeine solutions 37

Figure 4.4: Loading plots of the first principal component (PC1) applied to the synchronized averaged data and pre-processed with mean centering for (a) acetaminophen, (b) benzotriazole and (c) caffeine for each experimental day. 38

Figure 4.5: PCA analysis (SG filter, 1st derivative, 2nd order polynomial, 21 points and mean centered pre-processed) of MilliQ (red) and (a) acetaminophen (red) (b) benzotriazole (blue) and (c) caffeine (purple), irrespective of concentration in the 1300-2200 nm range. 40

Figure 4.6: PCA analysis (SG filter, 1st derivative, 2nd order polynomial, 21 points and mean centred pre-processed) of water and caffeine for the high concentration range (10-1000 mg/L). 40

Figure 5.1: Future IoT of EBM biosensor applications 49

Figure 6.1: Participants of the Feedback of Findings workshop held on 17 February 2023 53

LIST OF TABLES

Table 2.1: Key limitations associated with traditional water quality sensors 6

Table 2.2: Water quality parameters grouped as physical, chemical and biological parameters. 8

Table 2.3: Water quality parameters that are measured with online sensing technology (Adapted from Park et al. (2020)) 9

Table 3.1: The effect of environmentally relevant CEC on pure culture biofilm metabolic rate at 100 and 1000 ug/L, as well as the maximum concentrations of the respective CECs in South African wastewater effluent. NOE = No Observed Effect. Adapted from Rogowska et al. (2019) 23

Table 3.2: The total organic carbon, total inorganic carbon and total carbon of 2 environmental samples and 1 g/L TSB growth medium. 25

Table 3.3: CEC quantification in the Plankenbrug river and wastewater effluent using LCMS/MS. 26

Table 4.1: Accuracy classifications on determining the performance of a model 34

Table 4.2: The repeatability and reproducibility of water on the NIR spectrometer 35

Table 4.3: Overall performance measures of the calibration and cross-validation PLS-DA models for acetaminophen evaluated at the different concentration ranges. 41

Table 4.4: Overall performance measures of the calibration and cross-validation PLS-DA models for benzotriazole evaluated at the different concentration ranges. 42

Table 4.5: Overall performance measures of the calibration and cross-validation PLS-DA models for caffeine evaluated at the different concentration ranges. 43

ABBREVIATIONS

ACM	Acetaminophen
BPA	Bisphenol A
BZT	Benzotriazole
CAF	Central Analytical Facility (Stellenbosch University)
CAF	caffeine
CBZ	carbamazepine
CEC	contaminant of emerging concern / chemical of emerging concern
CEMS	Carbon dioxide Evolution Measurement System
CNT	carbon nanotube
COD	chemical oxygen demand
DBP	disinfection by-product
DCF	diclofenac
DO	dissolved oxygen
DWTP	drinking water treatment plant
EBM	effect-based monitoring
EC	Electrical conductivity
EC	European Commission
ECHA	European Chemical Agency
EFR	efavirenz
EQS	Environmental quality standards
EWS	early warning systems
FT-NIR	Fourier transform near-infrared
GC-MS	Gas Chromatography Mass Spectrometry
HPLC-MS	High Pressure Liquid Chromatography Mass Spectrometry
MOA	mode of action
NIR	near-infrared
NOE	no observed effect
ORP	oxidation reduction potential
PCA	principal component analysis
PFOS	perfluorooctane sulfonic acid

RI	refractive index
SPE	solid-phase extraction
SMX	sulfamethoxazole
TDS	total dissolved solids
TIC	total inorganic carbon
TOC	total organic carbon
US EPA	US Environmental Protection Agency
UV	ultraviolet
UV_{254}	ultraviolet absorption at 254 nm

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CHAPTER 1: INTRODUCTION

1.1 Contextualisation

The presence of contaminants and pathogens in the environment is of increasing concern because they can have adverse effects on aquatic lifeforms, and their presence means more and more advanced treatment is required before water can be used, e.g. for drinking water. Although background levels of these substances of concern are typically low, they can already have an adverse effect at those levels, e.g. cytotoxicity, genotoxicity, and carcinogenesis (US EPA, 2021). It is known that these background concentrations fluctuate (as much as between ng/L and µg/L levels in different environmental settings), and the variation can often be followed by periodical grab sampling and analysis. Such variation should be evaluated through routine monitoring approaches. However, identification of such contaminants of emerging concern (CECs) are normally regarded as time- and cost-intensive.

When the water is being used for human consumption, monitoring of the quality requires higher frequency of analysis. Besides the background concentrations, incidents of pollution can lead to a rapid increase in levels of CECs or pathogens. Such incidents can be of a very diverse nature, including agricultural runoff, sewer overflows, industrial discharges, accidental spillage, and intentional contamination. As the concentrations of the contaminants during such events can be orders of magnitude higher than the background concentrations, their effect can be severe. For example, a drinking water treatment facility is designed for a specific removal efficiency. When the influent concentration is much higher than expected, the contaminant can break through the treatment barriers and enter the drinking water distribution system. It should be noted that most, if not all, drinking water treatment plants (DWTPs) also do not regularly screen for the vast number of CECs and pathogens that are not part of SANS 241 water quality standards.

To monitor for pollution events, a sampling and analysis frequency is required that is not achievable with classical grab sampling. Especially in remote areas, the costs would be too high for a sustained sampling campaign. Such event monitoring for early detection should therefore rely on online sensor systems. These sensors should produce a warning when elevated concentrations of substances of concern (CEC and pathogens) are present. The capabilities of available online systems, however, are very different from those of the highly advanced analytical systems available in a laboratory. Online sensors typically measure physical properties of the water, such as electrical conductivity, light absorption, or refractive index (RI), or they measure individual substances through selective methods (e.g. using ion selective electrodes). Commercially available online sensors today are not yet capable of measuring the individual contaminants of concern, and due to the low concentrations of CECs, their direct measurement will probably remain restricted to sophisticated equipment. It will also not be feasible to develop such targeted CEC detectors, as the list of potentially harmful CECs continues to expand with more research results becoming available. This problem is exacerbated by the fact that human behaviour and activities, industrial types, as well climatic conditions all have an effect on the CECs to be found and persisting in a region. The same limitation is valid for emerging pathogens.

What can be achieved using available online sensors is monitoring for events? Besides indicating undesired chemical or microbiological contaminants, the types of events listed above typically display an increased level of a broad range of substances and/or particles. In reality, they do not only cause an increase in the concentration of one or a few substances, but a substantial change of the water matrix, along with various mixture interactions of chemical contaminants. For example, an exceptional contamination event such as an accidental (industrial) spill may only contain a low number of contaminants, or even individual substances at elevated concentrations. On the other hand, an illegal discharge of sewage into a natural stream may contain a number of substances at low concentrations, of which a combination of substances at low concentrations may pose an adverse health effect.

As monitoring for the individual substances or pathogens is not practical, an early warning system should focus on the detection of contamination events using existing chemical- and biological markers to assist with possible classification of the contamination event and if possible, classification of relevant events. This is preferably done using a sensor or combination of sensors with good sensitivity for a broad range of substances, ideally to serve as a proxy for more than one, or classes of, contaminants. A change in the signal of such a sensor (above or below an established threshold value) can be used as an indicator for events, which can then be investigated in more detail once they have been detected.

Much work on the selection of combinations of sensors has been performed for monitoring of drinking water quality (Van den Broeke, 2017). This has shown that generic parameters such as refractive index (RI), electrical conductivity (EC) and total organic carbon (TOC) (e.g. through UV absorbance) are capable of detecting chemical spillage events (M. V. Storey et al., 2011b). Refractive index, being the only truly generic sensor type, covers the broadest range of substances. Additional sensors have been added to perform a preliminary classification of the contamination event (Van den Broeke, 2017).

Existing sensor-based systems for chemical indicators are generally limited to physical-chemical parameters (dissolved oxygen (DO), EC, pH, total dissolved solids (TDS), chemical oxygen demand (COD), etc.) in the test water system that provide information on water quality but does not indicate any association with emerging contaminants that may be present in the test system.

It is impossible to develop CEC-specific online sensor systems for the vast range of organic contaminants that are regularly detected in freshwater systems. It is also not possible to select only a few CECs as surrogates that will give an indication of the total CEC load in water systems. For this reason, it is necessary to draw correlations between existing online sensor systems and priority CECs, drawn up in WRC Project K5/2369 (Swartz et al., 2018), to evaluate whether physico-chemical indicators (DO, EC, pH, TDS, COD, UV254, etc.) as a collective may serve as an alarm system for more in-depth intervention of water quality monitoring such as identification of emerging contaminants and/or emerging pathogens.

The application of real-time, online sensor-based systems in surface waters and/or water treatment facilities may provide alarm systems for CEC monitoring if direct/indirect correlations can be drawn between physico-chemical water quality parameters.

The ‘alarm/threshold’ parameters of the physico-chemical indicators or combination thereof can then be tested in the field to see whether the loads of CECs increase when the combination of physico-chemical indicators increase or decrease. These ‘threshold’ parameters can also be based on the conventional water quality parameters (e.g. SANS241 parameters or acceptable levels for wastewater effluent or river water quality).

The possibility also arises to develop an activation switch such that if these physico-chemical indicator concentrations exceed a certain threshold, an automated sampling device will be turned on that will collect samples for further chemical and/or microbiological testing in the laboratory (off-line).

1.2 Aims and Objectives

The last three decades saw marked increases in recognition of the potential risks associated with CECs, as evidence by the increase in reports in the scientific literature. For instance, a Google scholar search published articles on the theme increased from 4,750 to 11,200 to 24,000 per decade following 1990, 2000 and 2010, respectively. Many of the studies being reported focused on detection in different environments – including development of methods for detection, and studies on the degradation of the chemicals. The vast majority of the techniques reported on involve analytical methods that rely on sophisticated equipment and availability of expensive analytical standards and are often restricted to central analytical facilities with processing times ranging from days to weeks. As a result, the work remained primarily in the research domain with limited uptake for routine monitoring, or utility for early warning of risk.

With the demonstrated risk of toxicity to humans as well as the ecosystem, there is clearly a need for the establishment of smart sensors that are affordable with sufficient adaptability to be suitable for use in varying conditions. The overall goal of this project was therefore to collate information on relevant technologies, including those that are currently not considered for CEC detection but with potential to expand this field. The specific aims of the project were therefore to:

a. Establish a knowledge centre on sensing systems for smart water quality monitoring.
b. Draw up desired characteristics for sensing technologies for rapid detection of substances of concern and develop appropriate test protocols.
c. Select promising sensing technologies and do bench scale evaluation of these technologies.
d. Draw up a comprehensive evaluation report containing the results of the evaluation, with specific conclusions and recommendations for subsequent implementation at water sources nationally.

1.3 Scope

The work reported here (Chapter 3) focussed on the potential utility of the CEMS system to detect CECs, specifically to assess the system's sensitivity to the low concentrations at which CECs are typically found in the environment i.e., the CEMS reactors were exposed to a range of CECs, to assess the impact on microbial metabolism and determine the minimum concentrations for measurable effects. This was followed by exposing of the CEMS reactors to environmental waters containing high levels of CECs.

In Chapter 4, the potential of near-infrared (NIR) spectroscopy combined with multivariate data analysis techniques was investigated to differentiate between pure water samples and water samples spiked with three different CECs (acetaminophen, benzotriazole and caffeine) at different concentrations.

New developments and promising technologies for early-warning systems and effect-based monitoring is discussed in Chapter 5, which then focussed on using the BioTrac system as further development of the CEMS for early warning.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Water is the most precious resource on the planet and essential for sustaining all life on earth. However, one of the biggest problems arid and semi-arid countries, such as South Africa, are facing is the depletion of their water resources and the deterioration of water quality (Adewumi et al., 2010). The quality of life of populations is directly related to the availability of good quality freshwater resources. For this reason, communities that obtain their water from surface water sources have an increasing interest in the potential impact that poor source water quality may have on the provision of safe drinking water.

2.1.1 Water quality monitoring and its significance

Water quality monitoring is a critical process that involves the regular assessment and analysis of various physical, chemical, biological, and microbiological parameters to determine the health and safety of water resources. The significance of water quality monitoring lies in its ability to safeguard human health, protect ecosystems, support sustainable development, and ensure the availability of clean water for various purposes.

Regular monitoring of water quality is crucial for safeguarding human health. It plays a significant role in identifying potential risks, such as disease-causing pathogens like cholera, typhoid, and dysentery, thereby aiding in the prevention of outbreaks (Altenburger et al., 2019). Additionally, monitoring helps mitigate health hazards associated with exposure to pollutants such as heavy metals, pesticides, and industrial chemicals. However, monitoring chemical substances has become increasingly challenging due to the vast number of chemicals used in our daily lives and commerce, which can find their way into our water sources. It is important to note that current methods of chemical analysis and understanding of chemical toxicity only cover a fraction of the estimated 80 000 chemical compounds in commercial use in the United States, as reported by the EPA (Bradley et al., 2018).

Furthermore, monitoring of water quality is essential for the preservation and maintenance of diverse aquatic ecosystems, encompassing lakes, rivers, wetlands, and coastal areas (Baron et al., 2002). This process plays a crucial role in assessing the impacts of pollution and ensuring the equilibrium of these ecosystems. By monitoring water quality, we can effectively gauge the health of these bodies of water and take necessary measures to sustainably manage these invaluable resources. Furthermore, it promotes the well-being of fish populations and other wildlife, as they depend on clean and healthy water habitats to thrive (Assessment, 2008).

Water quality monitoring plays a vital role in various aspects, including public water supply systems, sustainable agriculture, industrial settings, and recreational areas. One of its primary purposes is to ensure that public water supply systems meet safety standards and maintain the potability of drinking water sources (Rodriguez et al., 2009). By regularly monitoring water quality, potential contaminants and deviations from safety standards can be identified and addressed promptly, safeguarding the health of consumers.

In agriculture, water quality monitoring helps support sustainable practices. It allows for the assessment of salinity levels, nutrient concentrations, and the presence of any contaminants that could potentially affect crop productivity (Pastori et al., 2015). By understanding and managing water quality, farmers can make informed decisions about irrigation and nutrient management, promoting sustainable agricultural practices while minimizing environmental impacts.

Industrial settings and recreational areas also greatly benefit from water quality monitoring. Monitoring helps prevent contamination of water sources by identifying potential pollutants and sources of contamination. This information enables industries to implement appropriate measures to prevent the release of harmful substances and maintain safety standards. In recreational areas, monitoring water quality ensures that the water is safe for activities such as swimming and boating, protecting the health of individuals engaging in water-based recreation (Keiser et al., 2018).

Water quality monitoring encompasses the measurement and analysis of a wide range of physical, chemical, and biological parameters to evaluate the condition and safety of water resources.

Physical parameters, such as temperature, turbidity, and conductivity, offer valuable insights into water clarity, thermal suitability for aquatic life, and the presence of dissolved substances. Monitoring flow rate and water level aids in understanding hydrological patterns and assessing the impacts of human activities on watercourses (Depetris, 2021).

Chemical parameters, including pH, dissolved oxygen (DO), and nutrient levels, play a crucial role in determining the biological health of water bodies. They indicate the presence of pollutants or excessive nutrient loading, which can have detrimental effects on aquatic ecosystems. Chemical monitoring also involves tracking heavy metals and organic pollutants to identify industrial pollution sources and assess potential risks to the ecosystem and human health (Carstea et al., 2016).

Biological parameters involve the study of aquatic macroinvertebrates, algae, and bacteria. These indicators provide valuable insights into the overall health and ecological integrity of water bodies. Changes in the composition and abundance of these organisms can signify pollution or environmental degradation, enabling timely interventions to mitigate potential harms (Holt, 2010).

Water quality monitoring encompasses various techniques to gather data and assess the condition of water resources. Some commonly used monitoring techniques include:

1. **Grab Sampling and Laboratory Analysis**: This traditional method involves manually collecting water samples at specific locations and time intervals. These samples are then transported to a laboratory for analysis using techniques such as titration, colorimetry, spectrophotometry, or chromatography. This approach provides detailed and comprehensive information but may have longer turnaround times for results.
2. **On-site Testing and Portable Devices**: These techniques enable real-time measurements of certain parameters directly in the field. Portable meters or kits are used to measure parameters like pH, conductivity, turbidity, dissolved oxygen, and nutrient levels. They provide quick results and are useful for rapid assessments or on-the-spot measurements.
3. **Remote Sensing and Advanced Monitoring Technologies**: Remote sensing techniques, such as satellite imagery, provide large-scale assessments of water quality parameters. They can monitor

parameters like temperature, turbidity, and algal blooms over wide areas. Sensor networks are deployed in water bodies to continuously monitor parameters like temperature, pH, DO, and conductivity, providing real-time data for analysis and decision-making. Automated water quality samplers are devices that collect water samples at specified intervals, which are later analysed in a laboratory. This method allows for frequent monitoring and analysis of water samples.

In summary, water quality monitoring is an indispensable practice in modern society due to its far-reaching implications. It plays a pivotal role in safeguarding public health, protecting the environment, and ensuring the sustainable utilization of water resources. By employing a range of monitoring techniques, stakeholders can gather vital information to inform decision-making and promote responsible water management practices.

2.1.2 Limitations of traditional water quality monitoring methods

Traditional water quality monitoring techniques have been used for many years to assess the health and safety of water resources. While these methods have provided valuable insights, they are not without limitations. These limitations have prompted researchers and engineers to explore alternative technologies and approaches to overcome these challenges. This section explores the shortcomings of traditional water quality monitoring techniques and highlight the need for innovative approaches to overcome these limitations (Korostynska et al., 2013; Gholizadeh et al., 2016).

The key limitations associated with traditional water quality sensors are discussed in Table 2.1.

Table 2.1: Key limitations associated with traditional water quality sensors.

Limited parameter detection	Most traditional water quality sensors are designed to measure specific parameters such as temperature, pH, dissolved oxygen, turbidity, and conductivity. While these parameters provide essential information about the water's basic characteristics, they may not cover the full spectrum of potential pollutants or contaminants that could be present in a water body. As a result, some pollutants or emerging contaminants may go undetected, leading to an incomplete understanding of water quality.
Lack of real-time monitoring	Many traditional water quality sensors operate on a grab-sample basis, where water samples are collected at specific intervals and then analysed in a laboratory setting. This approach results in delayed data and an inability to monitor water quality in real-time. Real-time data is crucial for responding promptly to water quality issues, especially in scenarios where rapid changes in water conditions can occur.
High maintenance and calibration	Traditional sensors require regular maintenance and calibration to ensure accurate and reliable measurements. The complexity of these maintenance procedures may make them impractical for continuous monitoring in remote or inaccessible locations. Additionally, the costs associated with maintenance and calibration can be significant, especially for large-scale monitoring networks.

Susceptibility to fouling and drift	Water quality sensors deployed in natural water bodies are susceptible to fouling, which occurs when organic matter or mineral deposits accumulate on sensor surfaces. Fouling can lead to measurement inaccuracies and increased maintenance requirements. Moreover, some sensors may experience drift over time, resulting in gradual inaccuracies that require frequent recalibration.
Limited special coverage	Due to cost constraints and the complexity of deploying traditional sensors, it is challenging to achieve extensive spatial coverage in large water bodies. This limitation can lead to a lack of representative data and hinder comprehensive water quality assessment.
Incompatibility with harsh environments	Traditional water quality sensors may not be designed to withstand extreme environmental conditions, such as high or low temperatures, turbulent waters, or harsh chemical environments. These conditions can damage the sensors or affect their accuracy, limiting their applicability in challenging settings.
Lack of versatility	Individual traditional water quality sensors are typically optimised for specific parameters, making them less versatile in handling diverse water quality monitoring needs. Integrating multiple sensors to cover various parameters can be complex and costly.
Cost and Accessibility	Many traditional water quality sensors can be expensive to purchase, install, and maintain. This cost factor may limit their deployment in developing regions or small-scale projects, where affordable and accessible monitoring solutions are crucial for ensuring safe water resources.

In conclusion, while traditional water quality sensors have been valuable tools for decades, they come with inherent limitations that can restrict their effectiveness and applicability in certain scenarios. To address these limitations and enhance water quality monitoring capabilities, advancements in sensor technology, data processing, and integration with emerging technologies are being pursued to provide more comprehensive, accurate, and cost-effective solutions for water quality assessment.

2.1.3 Emergence of online and real-time water quality sensors

The emergence of online and real-time water quality sensors represents a significant advancement in the field of water quality monitoring. Unlike traditional sensors that rely on grab-sample analysis in a laboratory setting, online and real-time sensors provide continuous and instantaneous data, enabling more efficient and effective water quality assessment. These sensors leverage modern technologies and innovative approaches to address the limitations of traditional sensors, revolutionizing the way we monitor and manage water resources.

Water quality monitoring is the foundation on which water quality management is based, and provides information for important decision making, such as describing the water resources and identifying actual and emerging water quality problems, plant management, pollution control and reduction, and limiting the environmental impact of discharges.

The application of online water quality sensors can have various objectives, such as (Capodaglio, 2017):

1. Contaminant source identification
2. Monitoring of discharges into source water
3. Best management practices/protection of water sources
4. Drinking water quality protection
5. Emergency response

These objectives will enable water resource managers to define potential contamination sources in relation to the vulnerability of source water, identify pollution spills, prevent water source deterioration, allow for the appropriate responses to the presence of contamination and allow for drinking water pollution control, risk management and modification of treatment processes.

To achieve these objectives, a monitoring plan is developed, which include the selection of the monitoring site and monitoring variables. Online monitoring instrumentation must be placed at selected, representative locations in the water system network, depending on the requirements of the desired objective. The "ideal" sensor location for the control of contaminants is as close as to the potential contaminant source as possible.

Depending on the monitoring objective and the vulnerability of the water resource, various water quality parameters will be monitored. These parameters are often grouped into three general groups, namely physical, chemical, and biological. The chemical parameters can then be subdivided into organic and inorganic parameters. Table 2.2 summarises the types of water quality parameters.

Table 2.2: Water quality parameters grouped as physical, chemical and biological parameters.

Physical	Chemical		Biological
	Inorganic	Organic	
Turbidity	pH	BOD	Algae
Temperature	Acidity	COD	Viruses
Colour	Alkalinity	TOC	Protozoa
Taste and Odour	Chloride	Hydrocarbons	Pathogens
Total Dissolved Solids (TDS)	Chlorine residuals	UV adsorption	
Electrical Conductivity (EC)	Dissolved Oxygen	VOCs	
	Metals	Pesticides	
	Nutrients	DBPs	
	Cyanide	CECs	

Table 2.3 list water quality parameters that are measured with online sensing technology, adapted from (Park et al., 2020).

The emergence of online and real-time water quality sensors marks a transformative shift in how we approach water quality monitoring. By providing continuous, accurate, and readily accessible data, these sensors contribute significantly to safeguarding water resources, supporting sustainable development, and ensuring the well-being of both ecosystems and human populations. As technology continues to advance, the potential for even more sophisticated and versatile water quality monitoring solutions becomes increasingly promising.

Table 2.3: Water quality parameters that are measured with online sensing technology (Adapted from (Park et al., 2020))

Content	Parameter	Sensor Type
Basic-item monitoring	pH, DO, EC, temperature, ORP, turbidity	In-situ electrodes, colourimetry, conductivity cell, membrane electrode, optical sensor, potentiometric, thermistor, nephelometric.
Organic-compound monitoring	COD	In situ electrochemical sensor
Nutrient monitoring	Nitrite	Using an optical sensor where nitrate concentration is determined from the relationship between UV light absorbance and nitrate concentration in a water sample
Harmful algal blooms (HABs) Monitoring	Nitrate, Ammonium, Phosphate	Wet chemistry sensor where the nutrient concentration is measured based on a colorimetric reaction
	Chl-a	Using satellite images (Chl-a concentration is determined from the empirical relationship between satellite image and Chl-a concentration) In situ optical sensor with wireless data transport network
	Phycocyanin	In situ fluorometric sensor
	Cyanobacteria biomass	Using satellite images (Cyanobacteria biomass concentration is determined from the empirical relationship between satellite image and cyanobacteria biomass)
HABs monitoring using hyperspectral image (HSI)	Chl-a	Chl-a concentration is determined from the empirical relationship between HSI and Chl-a concentration
	Phycocyanin	Phycocyanin concentration is determined from the empirical relationship between HSI and phycocyanin concentration
	Cyanobacteria biomass	Cyanobacteria biomass concentration is determined from the empirical relationship between HSI and cyanobacteria biomass
Physical status for water quantity monitoring	Water level	In situ acoustic sensor where the distance from the surface of the water to bottom is measured from the echoes of the acoustic waves
	Velocity	Velocity sensor

2.2 Online and Real-Time Water Quality Sensors

In recent years, the market for online and real-time water quality sensors has witnessed significant growth due to advancements in sensor technology and the increasing need for continuous monitoring of water resources. These sensors utilize various principles and methods to measure a wide range of water quality parameters accurately and in real-time. It's important to note that many modern online and real-time water quality sensors are designed to be versatile, capable of measuring multiple parameters simultaneously. Additionally, these sensors often come equipped with advanced features like data transmission via wireless networks, integration with IoT platforms, and user-friendly data visualization interfaces (Geetha & Gouthami, 2017). As technology continues to advance, the market for online and real-time water quality sensors is expected to expand further, offering increasingly sophisticated and comprehensive monitoring solutions for safeguarding water resources, and maintaining ecological balance.

There are various types of online and real-time water quality sensors available on the market today (Banna et al., 2014). The types and uses of these sensors are discussed in the section below.

2.2.1 Optical Sensors

Optical online and real-time water quality sensors represent a cutting-edge and rapidly evolving technology in the field of water quality monitoring. Optical sensors employ the interaction of light with water to measure specific parameters. Different water quality parameters absorb, scatter, or emit light in unique ways, allowing these sensors to detect and quantify parameters such as organic matter, turbidity, chlorophyll-a (a proxy for algae), and coloured dissolved organic matter (CDOM). By using optical principles, these sensors can provide multi-parameter measurements without the need for multiple physical sensors.

2.2.2 Chemical Sensors

Chemical sensors work based on chemical reactions that occur between the target analyte in the water and a chemical reagent within the sensor. The reaction results in a measurable change in the sensor's properties, such as colour, conductivity, or optical properties.

The use of chemical sensors is one of the most promising opportunities to carry out inexpensive and real-time monitoring of water quality. Plenty of chemical sensors has been developed for the analysis of heavy metals, organic pollutants, bacteria and their metabolites, etc. (Lvova et al., 2019). Such systems are composed by a number "of semi-selective sensors with overlapping specificities and with differentiated responses towards different analytes of complex samples, accompanied with signal-processing and pattern recognition which enables a rational decision" (Vagin et al., 2016).

2.2.3 Electrochemical Sensors

Electrochemical sensors utilize electrodes to measure the electrical properties of the water. They rely on the interaction between the target analyte and the electrode surface, leading to electrical changes that are proportional to the analyte concentration (Baranwal et al., 2022).

On-line electrochemical sensors can detect and quantify the concentration of a target analyte in real-time. These sensors typically consist of a working electrode, a reference electrode, and a counter electrode, from which an electrochemical reaction between the target analyte and the working electrode generates an electrical signal that can be measured and correlated to the concentration of the analyte in the water sample.

Several on-line electrochemical sensors have been developed to detect CECs in environmental surface waters (Hassan et al., 2021). For example, a biosensor based on a carbon nanotube (CNT) electrode was developed for the detection of triclosan, a common antimicrobial agent, in water samples. The CNT electrode was functionalized with a specific antibody to triclosan, and the binding of triclosan to the antibody caused a change in the electrical properties of the electrode. The biosensor was highly sensitive, with a limit of detection of 2 ng/mL and was able to detect triclosan in real water samples.

Another on-line electrochemical sensor was developed for the detection of sulfamethoxazole, a broad-spectrum antibiotic that are regularly detected in wastewater effluent and surface water samples worldwide. The sensor used a boron-doped diamond electrode and a pulsed amperometric detection technique to quantify the concentration of sulfamethoxazole in real-time. The sensor was highly sensitive, with a limit of detection of 0.1 ng/mL and was able to detect sulfamethoxazole in real water samples.

In addition, an on-line electrochemical sensor was developed for the detection of bisphenol A (BPA) in water samples. The sensor used a gold electrode and an electrochemical impedance spectroscopy technique to quantify the concentration of BPA in real-time. The sensor was highly sensitive, with a limit of detection of 4.4 ng/mL and was able to detect BPA in real water samples.

Overall, on-line electrochemical sensors offer several advantages for the detection of CECs in environmental surface waters, including real-time monitoring, high sensitivity, and low detection limits. However, these sensors may require frequent calibration and maintenance, and their performance may be affected by interfering substances in the water sample. Therefore, further research is needed to develop and optimize on-line electrochemical sensors for specific CECs, and to evaluate their performance more under real-world conditions.

2.2.4 Biosensors and biological monitoring sensors

"Biosensor" and "biological monitoring sensor" are terms often used interchangeably, but they have slightly different meanings.

A biosensor is a device that combines a biological component (such as enzymes, antibodies, or microorganisms) with a physicochemical detector (such as an electrode or an optical sensor) to detect and measure a specific biological or chemical analyte. The biological component of the biosensor interacts with the target analyte, leading to a measurable signal that is converted into a quantitative result by the physicochemical detector (Yang et al., 2016; Zhang et al., 2020). Biosensors do not typically have the analytical capacity to quantifiably determine individual chemical pollutants but rather reports on the whole toxicological footprint of the test site water.

Biological monitoring sensors, on the other hand, refer to a broader category of sensors used for biological monitoring. These sensors can include not only biosensors but also other types of sensors that directly or indirectly measure biological parameters. The task of an online bio-monitor is to detect pollution spikes as quickly as possible by measuring the summation parameter "biological effect", in contrast to chemical online monitoring, which records substance concentrations of single pollutants, which not necessarily result in biological effects. The organism responds to the whole cocktail of toxic substances in the environment and gives a direct answer of ecological relevance, as the organisms are the ultimate protection goal.

In summary, a biosensor specifically refers to a sensor that utilizes a biological component for detecting and quantifying a specific analyte, while biological monitoring sensor is a broader term encompassing sensors used to monitor various biological parameters, which can include biosensors and other types of sensors that measure biological data.

2.3 Principles and Operation of Early Warning Systems (EWS)

2.3.1 Background of EWS for water quality monitoring

EWS are being widely used to detect irregularities in natural/ human induced processes and to inform users about pending threats such as wildland fires, earthquakes, hydro-meteorological hazards, epidemics and food security (UNEP, 2012a). EWS should generate this information in time to prevent or minimize the impact of these threats to human health, the economic system or other vulnerable aspects (Bartrand et al., 2017).

EWS are important because the sooner, and more accurately, short- and long-term risks can be identified, the more likely it is that the impact of adversities or negative trends can be minimized (UNEP, 2012a). The concept of "early" differs from situation to situation. In some cases, this can be hours, whilst in other situations weeks or even years. The two main factors that determine the timescale at which an EWS needs to be effective are:

1. Type of source (e.g. groundwater, shallow groundwater or surface water)
2. Type of threat (e.g. sudden-onset (immediate risk), slow-onset (cumulative change))

The goal of EWS is to provide a fast and accurate means to distinguish between normal variations and contamination events. Ideally, EWS should (Raich, 2013a):

- provide a rapid response.
- include a sufficiently wide range of potential contaminants that can be detected.
- exhibit a significant degree of automation, including automatic sampling.
- allow acquisition, maintenance, and upgrades at an affordable cost.
- require low skill and training.
- identify the source of the contaminant and allow an accurate prediction of the location and concentration downstream of the detection point.
- demonstrate sufficient sensitivity to detect contaminants.
- permit minimal false-positives/false-negatives.
- exhibit robustness and ruggedness to continually operate in a water environment.
- allow remote operation and adjustment.
- function continuously.

Nevertheless, it is not feasible at the moment to deploy an EWS that will accomplish all the requirements mentioned above. However, current EWS could potentially detect pollution events, triggering the deployment of the more costly and labour-intensive conventional sampling and analytical methods, to quantify pollution parameters.

2.3.2 Principles of EWS for water quality monitoring

Early warning systems for monitoring water quality rely on real-time data collection of various parameters. These parameters include temperature, pH, dissolved oxygen, turbidity, conductivity, chemical concentrations (e.g. nutrients, heavy metals, and organic pollutants), and biological indicators (e.g. harmful algal blooms or faecal coliforms). The advantage of real-time monitoring lies in its ability to promptly detect any changes in water quality, allowing for the immediate identification of potential pollution events (Quansah et al., 2010).

To gather data from multiple locations, early warning systems employ strategically placed sensor networks in water bodies. These networks are designed to cover a wide area, including critical points like water intake locations, areas near industrial facilities, and downstream of urban areas (Murray, 2010).

The core of any early warning system is the integration and analysis of the data collected by the sensor network. Utilizing advanced data processing techniques, such as machine learning algorithms and statistical models, the system can identify trends, anomalies, and potential water quality issues (Luo et al., 2022). By combining historical data with real-time information, the system's predictive capabilities are enhanced, enabling the recognition of emerging threats.

To ensure an effective response, early warning systems set specific thresholds and triggers for each monitored parameter. These thresholds indicate critical levels beyond which water quality is considered unacceptable or hazardous. When any parameter exceeds its defined threshold, a trigger is activated, instantly alerting responsible authorities or operators to take immediate action.

2.3.3 Operation of EWS for water quality monitoring

The early warning system operates by continuously collecting data from sensors strategically installed in the water body. These sensors measure various water quality parameters at regular intervals, resulting in a vast amount of data being generated. To ensure seamless access to this data, it is transmitted in real-time to a central database or cloud-based platform through wireless communication networks. This accessibility allows decision-makers and stakeholders to stay informed promptly (Quansah et al., 2010).

The integrated data undergoes analysis using sophisticated algorithms and models to identify patterns, trends, and potential pollution events. This analysis involves comparing current data with historical records and predefined thresholds to determine whether water quality is within acceptable limits (Quansah et al., 2010). Should the early warning system detect any parameter exceeding its threshold, an alert is generated and instantly sent to designated personnel through various communication channels, such as email, SMS, or mobile applications. These alerts provide crucial information about the identified water quality issue and its location, enabling a prompt response. Upon receiving an alert, responsible authorities and stakeholders can take immediate action. They investigate the cause of the water quality problem, initiate necessary mitigation measures, and inform the public about potential risks.

Furthermore, early warning systems facilitate the generation of comprehensive reports on water quality trends and incidents. These reports are instrumental in understanding the effectiveness of response measures and serve as valuable resources for future planning and policy-making (Luo et al., 2022).

2.4 Benefits and Limitations of Early Warning Systems

2.4.1 Benefits

Early warning systems for water quality monitoring offer a range of benefits, contributing significantly to environmental protection, public health, and overall water management. Some of the key advantages include their ability to quickly identify the presence of contaminants, such as pollutants, toxic chemicals, or harmful microorganisms, in water sources. This allows authorities to take prompt action to prevent further contamination or mitigate potential health risks (Raich, 2013b).

By detecting water quality issues early on, these systems help safeguard public health, reducing the risk of waterborne illnesses and protecting vulnerable populations, such as children and the elderly. Moreover, early detection of pollutants can prevent damage to aquatic ecosystems, preserving biodiversity and ensuring the well-being of aquatic life. It enables timely interventions to mitigate the impact of contamination on sensitive species and habitats.

Addressing water quality issues at an early stage is often more cost-effective than dealing with extensive pollution after it has spread. Implementing appropriate measures promptly can significantly reduce the costs associated with remediation efforts. Early warning systems provide real-time or near-real-time data on water quality, ensuring that any changes in water conditions are quickly identified, allowing for immediate responses (Luo et al., 2022; Miller et al., 2023).

These systems are invaluable during natural disasters or industrial accidents that can affect water quality, as they enable authorities to respond rapidly, prevent further contamination, and protect communities from potential hazards. Furthermore, many countries have water quality standards and regulations that industries and municipalities must adhere to. Early warning systems enable continuous monitoring to ensure compliance and avoid penalties for violating environmental regulations.

The data provided by early warning systems is valuable for informed decision-making and long-term water resource planning. Historical data can also aid in identifying trends and patterns related to water quality. Transparent sharing of water quality data through these systems raises public awareness about water issues, leading to greater support for conservation efforts and sustainable water management practices.

Additionally, early warning systems can be integrated into broader smart water management networks, enabling more efficient water distribution, reducing wastage, and optimizing resource usage (Miller et al., 2023). Overall, early warning systems for water quality monitoring play a crucial role in protecting human health, preserving ecosystems, and ensuring the sustainable management of water resources. They are an essential tool for modern water management practices in both urban and rural areas.

2.4.2 Limitations

Early Warning Systems for water quality monitoring offer significant benefits, but they also come with certain limitations and challenges that must be considered (Miller et al., 2023). Implementing and maintaining a robust EWS can be technically complex and expensive, especially in large or remote areas. The costs involved in setting up monitoring equipment, data management systems, and ongoing maintenance can present a significant barrier for some regions or communities.

One of the main challenges with EWS is dealing with the vast amounts of real-time data it generates. Interpreting and analysing this data effectively can be difficult without skilled personnel and advanced data analytics tools, making it hard to extract meaningful insights for informed decision-making (Luo et al., 2022; Miller et al., 2023). Moreover, EWS, particularly automated systems, can produce false alarms or nuisance alerts due to sensor malfunctions, calibration issues, or transient changes in water quality that don't indicate a significant threat. Frequent false alarms can lead to complacency and reduced trust in the system's reliability.

The reliability of the sensors used in EWS is crucial for accurate and timely detection of water quality changes. Regular maintenance and calibration are necessary to ensure their effectiveness as sensors can deteriorate over time, leading to inaccuracies or failures in data collection.

Another concern is that EWS may not provide comprehensive coverage of all water sources in a region, with monitoring stations often concentrated in specific areas. This leaves other water bodies unmonitored, which could lead to undetected contamination events. In remote or less developed regions, unreliable data transmission and internet connectivity can hamper real-time updates and alerts, causing delays in response times. Even with rapid detection, there can be a time lag between identifying a contamination event and implementing a response due to verification, decision-making processes, and logistical constraints, allowing the contamination to spread further (Murray, 2010; UNEP, 2012b).

EWS typically focuses on specific water quality parameters, potentially leaving emerging pollutants or new contaminants outside the monitoring program, thus leaving potential risks undetected. Additionally, EWS may not always be equipped to detect emerging contaminants, such as certain pharmaceuticals, microplastics, or nanomaterials. As these substances gain more attention, EWS may need updates to accommodate their monitoring.

Integrating EWS into existing water management systems and regulatory frameworks can be challenging due to bureaucratic processes, lack of coordination among agencies, and differing priorities.

Despite these limitations, early warning systems remain valuable tools for water quality monitoring. Their effectiveness can be enhanced through ongoing technological advancements, increased capacity-building efforts, and collaboration between stakeholders to address these challenges proactively.

2.5 Challenges and Future Directions

Early warning systems (EWS) and online water quality monitoring have witnessed significant advancements, yet challenges persist in their implementation (M. V. Storey et al., 2011a). One major concern is ensuring the accuracy and reliability of the data collected by monitoring systems. Any inaccuracies could lead to incorrect assessments and ineffective responses. To avoid these inaccuracies regular calibration and maintenance of sensors are necessary. Sensor drift and fouling can also impact the reliability of the data if not addressed promptly.

Establishing efficient and reliable real-time data transmission is critical for timely response and decision-making. Technical issues or communication failures can disrupt the effectiveness of the EWS. This may be especially challenging in South Africa where daily electricity outages can interrupt data transmission over internet networks.

Additionally, the interpretation and integration of vast amounts of data from various sources also present challenges. Developing intelligent algorithms and systems to interpret and integrate data efficiently is essential for quick decision-making.

Furthermore, the implementation and maintenance costs of EWS and online monitoring systems can be a barrier for many regions, particularly in developing countries with limited financial resources and infrastructure.

Finally, while traditional water quality parameters are well-monitored, emerging contaminants like pharmaceuticals and microplastics demand improved detection methods.

Looking ahead, advancements in sensor technology can lead to smaller, more affordable, and more accurate sensors capable of detecting a broader range of parameters, including emerging contaminants. Integration of artificial intelligence and machine learning algorithms can enhance data analysis and prediction capabilities. Autonomous monitoring systems, such as drones and underwater robots, offer the potential for better spatial and temporal coverage of water bodies. Involving the public in water quality monitoring through citizen science initiatives can increase data collection points and promote community engagement. Standardizing data collection, sharing, and reporting protocols can improve interoperability between monitoring systems and encourage collaboration. Integration of remote sensing technologies like satellite imagery can complement ground-based efforts. Stronger policies and regulations related to water quality management can support the adoption and expansion of monitoring systems, fostering better water resource management and environmental protection.

Overall, addressing these challenges and exploring future directions can enhance the effectiveness of early warning systems and online water quality monitoring, contributing to better water resource management and environmental protection.

2.6 Conclusion

Water quality monitoring is crucial for preserving good quality freshwater in arid and semi-arid regions like South Africa, ensuring human health, preserving ecosystems, and supporting various sectors. It involves assessing physical, chemical, biological, and microbiological parameters. Traditional monitoring methods have limitations, but online and real-time sensors offer continuous, accurate data collection, transforming water quality monitoring. These sensors, including optical, chemical, electrochemical, biosensors, and biological monitoring sensors, provide versatile solutions for detecting contaminants and safeguarding water resources.

Advancements in sensor technology have driven the growth of the market for online and real-time water quality sensors. These sensors offer versatile features, such as wireless data transmission and user-friendly interfaces. They enable continuous monitoring, high sensitivity, and the detection of low contaminant concentrations, contributing to timely responses to water quality issues.

Early Warning Systems (EWS) are widely used to detect irregularities in various processes and inform users about impending threats. EWS aim to generate timely information to prevent or minimize the impact of threats on human health, the economy, or other aspects. The effectiveness of EWS depends on their ability to identify risks early, with the concept of "early" varying based on the situation. EWS for water quality monitoring distinguish between normal variations and contamination events rapidly and accurately. Ideal EWS exhibit features like rapid response, automation, affordability, source identification, and minimal false positives/negatives. However, deploying EWS meeting all these requirements can be challenging.

EWS for water quality monitoring rely on real-time data collection of multiple parameters using strategically placed sensors. Advanced data analysis techniques detect trends, anomalies, and potential pollution events. Specific thresholds and triggers define unacceptable water quality levels, and when exceeded, alerts are generated to prompt action. EWS operate by continuously collecting and transmitting data to a central database, enabling rapid response and comprehensive reporting for future planning and policymaking.

In summary, water quality monitoring and EWS are crucial for safeguarding water resources, public health, and ecosystems. Advances in sensor technology and real-time monitoring enhance our ability to address water quality challenges effectively, offering continuous, accurate data for informed decision-making.

CHAPTER 3: CARBON DIOXIDE EVOLUTION MEASUREMENT SYSTEM (CEMS)

3.1 Introduction

The production of carbon dioxide is a universal indicator of respiration and subsequently a parameter of life, often exploited as an indicator of ecosystem health (Stone et al., 2021). The Carbon Dioxide Evolution Measurement System (CEMS) was developed to study whole-biofilm metabolic profiles, using microbiologically produced CO_2 (Kroukamp and Wolfaardt, 2009). In a continuous flow system, inoculated with relevant pure/mixed culture biofilms, the produced CO_2 is trapped and carried with ambient air to an analyser, while the data is logged in real-time. It has previously been used to study biofilm metabolism and metabolic responses to antibiotic treatments (Jackson et al., 2015,2019; Ronan et al., 2016). This led to further development of the CEMS system for use as a real-time water quality monitoring tool, by tracking the metabolic response to environmental pollution and physicochemical parameter fluctuations (Stone et al., 2021), as well as delineating CO_2 production during autotrophic ammonia oxidation, and the effect of increased biomass retention in fixed-film autotrophic nitrification systems, respectively (Ronan, Evan et al., 2021 a,b). The system also found application for *in situ* monitoring of CO_2 sequestration by photoautotrophic biofilms, and interaction between CO_2-consuming autotrophy and CO_2-producing heterotrophy in studies focussing on wastewater valorisation (Ronan, Patrick et al., 2020; 2021a) as well as the impact of acute nitrogen starvation on CO_2 uptake and biomass sloughing (Ronan, Patrick et al., submitted). The rapid understanding of microbial activity at a community level does not require expensive and time consuming genetic, proteomic and metabolic profiling of conventional methods to assess microbial metabolism and health.

The work reported here focussed on the potential utility of the CEMS system to detect CECs, specifically to assess the system's sensitivity to the low concentrations at which CECs are typically found in the environment i.e., the CEMS reactors were exposed to a range of CECs, to assess the impact on microbial metabolism and determine the minimum concentrations for measurable effects. This was followed by exposing of the CEMS reactors to environmental waters containing high levels of CECs.

3.2 Methodology

3.2.1 Sampling and inoculum freezer stocks preparation

For pure culture stocks, a sterile 250 mL conical flask with 50 mL 1 g/L tryptone soy broth (TSB) (Merck, Germany) was inoculated with 500 µL of a pure culture of *Pseudomonas CT07* (Bester et al., 2013) and incubated at 26°C for 24 hours with agitation. Aliquots were made in 1.5 mL Eppendorf tubes in glycerol (40% v/v final concentration) and frozen at -30°C for subsequent experimentation.

For mixed microbial community stocks, a sample was collected from the Plankenbrug river in Stellenbosch, Western Cape (-33.931050;18.889808). Briefly, aqueous, sediment and biofilm samples were collected and combined in a sterile 50 mL Falcon tube and transported on ice to the laboratory within one hour. Aliquots were made in 1.5 mL Eppendorf tubes in glycerol (40% v/v final concentration) and frozen away at -30°C for subsequent experimentation.

River water and wastewater effluent as test water for the CEMS reactors were collected on the day of experimentation. Plankenbrug river water was collected in 5 L containers (cleaned with MeOH and ddH_2O) and transported on ice to the laboratory and stored at 4°C until used. Wastewater effluent was collected in the same manner as the Plankenbrug river water, from a wastewater treatment plant (WWTP) in the Western Cape, South Africa.

3.2.2 CEMS reactor inoculum preparation

To prepare pure culture and mixed community inocula for biofilm studies, 500 µL of the *Pseudomonas CT07* or mixed microbial community freezer stock was added to 50 mL of 1 g/L TSB in a 250 mL conical flask. The flask was incubated at 26°C for 24 hours with agitation. After 24 hours, the culture was passaged to fresh growth medium and grown up at the same conditions until an OD of 0.6 (600 nm). Cell concentration of the inoculum was determined with plate counts on 1 g/L tryptone soy agar (TSA), in triplicate. Either the pure or mixed community culture was used to inoculate the CEMS system for all subsequent experimentation.

3.2.3 Carbon Dioxide Evolution Measurement System

The carbon dioxide evolution measurement system (CEMS) allows for the metabolic profiling of biofilms in real-time, as previously described by Kroukamp and Wolfaardt (2009). The principle relies on the use of a CO_2 analyser to detect CO_2 produced by a biofilm, as a result of metabolism and respiration. Briefly, a biofilm is cultivated in a gas-permeable silicone tubing in a continuous flow system (Figure 3.1). The silicone tubing is encased in an outer Tygon tubing (gas-impermeable) where the CO_2 produced by the biofilm, which diffuses across the silicone membrane, is collected in the annular space and transported to a CO_2 analyser with ambient air sweeper gas.

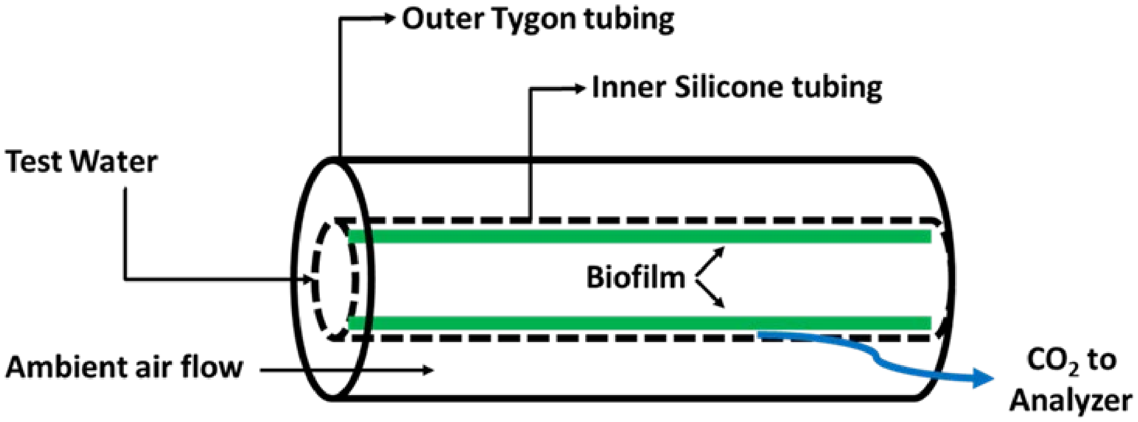

Figure 3.1: Diagram of CEMS principal

3.2.3.1 CEMS reactor setup and growth conditions

The CEMS system was constructed for biofilm cultivation studies to determine the effect that CECs may have on microbial metabolism and subsequently evaluate the use of CEMS as a sensor for water quality changes. The CEMS reactor (Figure 3.2) was set up with 110 cm gas-permeable silicone tubing (1.6 mm inner diameter, 2.4 mm outer diameter) and placed within 100 cm Tygon gas-impermeable tubing (4.8 mm inner diameter, 7.9 mm outer diameter) and sealed at the ends. The silicone tubing is inoculated with a relevant microbial culture and the CO_2 produced as a result of biofilm growth diffuses across the silicone tubing and is transported with ambient air flow to a CO_2 analyser (Aquasignum, Canada). The CEMS reactor was submerged in a water bath (26 ± 1°C) to maintain a constant temperature. Silicone tubing (1.6 mm inner diameter, 3.2 mm outer diameter) was used in a continuous flow system to provide the CEMS reactor with sterile growth medium and to transport the effluent to a waste container. Bubble traps were connected in-line prior to the CEMS reactor, to minimise biofilm disruption due to gas bubbles (He et al., 2011).

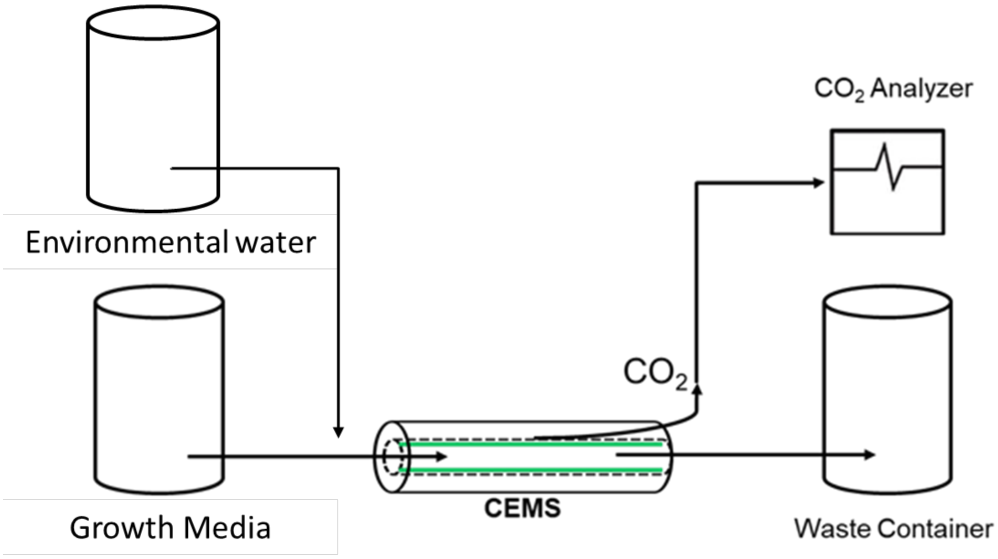

Figure 3.2: CEMS setup for CEC/environmental test water exposure.

The CEMS reactor was sterilised with a 10% v/v bleach solution (4 hours), followed by sterile ddH_2O (12 hours) to rinse residual bleach out of the system. Subsequently, the system was fed with 1 g/L TSB for 2 hours prior to inoculation. For inoculation, the peristaltic pump (Watson Marlow 205S, 12 mL/hr) supplying the growth medium was switched off and the silicone tubes clamped off. The CEMS reactor was inoculated with 200 µL of the passaged culture (OD 0.6, 600 nm) by injecting the silicone tubing with a sterile syringe (25G needle). The pump was left off for 2 hours to allow the inoculum to adhere to the inner surface of the silicone tubing. Once media flow was resumed, the CO_2 analysers were set to log CO_2 concentration every 30 s. Each CEMS reactor was run in parallel with a sterile and negative control, set up in an identical manner.

3.2.3.2 Bench-scale evaluation of CEMS as a CEC sensor

Bench-scale evaluations of CEMS as a CEC sensor followed a phased approach, where in phase one, growth media were spiked with analytical standards of different concentrations of environmentally relevant CECs to assess the effect on biofilm metabolism. The second phase transitioned from the synthetic growth medium to environmental water (river and WWTW effluent) to assess the effect of a cocktail of CECs in more complex water matrices.

For the first phase, the CEMS reactor was prepared and inoculated with the pure culture inoculum as described above, fed with 1 g/L TSB and allowed to reach steady-state conditions (48 hours) as indicated by the CO_2 production, in real time. Additionally, 500 mL of standard growth medium (1 g/L TSB) was prepared and spiked with an individual CEC to a final concentration of 100 µg/L. The flask supplying the CEMS reactor with medium was switched to the CEC containing medium for 2 hours, exposing the biofilm to the respective CEC at standard conditions. Another 48 hours was allowed after switching back to pure growth medium to allow the system to recover to steady-state conditions. Once recovered, the CEMS reactor was exposed to 1000 µg/L CEC in standard growth medium for two hours. The CEMS reactors were exposed to the following environmentally relevant CECs, individually at both 100 and 1000 µg/L: sulfamethoxazole (SMX), carbamazepine (CBZ), caffeine, (CAF), diclofenac (DCF), acetaminophen (ACM), efavirenz (EFR) and benzotriazole (BZT).

3.2.3.3 Bench-scale evaluation of CEMS as a CEC sensor in environmental water sources

The second phase of the bench-scale evaluations required assessment of the CEMS reactor as a water quality sensor when exposed to environmental water sources. Similarly, the CEMS reactor was inoculated with the mixed microbial community as described above, fed with 1 g/L TSB and allowed to reach steady-state conditions (48 hours) as indicated by the CO_2 production, in real time. The collected Plankenbrug river water was filter sterilised (2.7 µm and 0.22 µm, Whatman) and switched with the growth medium for 2 hours, then switched back (Figure 3.2). The biofilm metabolic rate was logged in real-time, to show an immediate effect upon exposure to the environmental test water. The CEMS reactor was exposed to wastewater effluent in the same manner as described above. Both environmental samples, as well as the growth medium was subjected to total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC) analysis to determine the environmental samples' available carbon content compared to the growth medium (Central Analytical Facility (CAF), Stellenbosch University).

3.2.4 Sample preparation for CEC quantification

Solid Phase Extraction (SPE) for High Performance Liquid Chromatography and Mass Spectrometry (HPLC-MS) was performed as described by Petrie et al. (2016a). Briefly, collected samples (100 mL) were spiked with the corresponding internal standards to a final concentration of 50 µg/L (in the final eluted sample) before filtering through a 0.7 µm glass fibre filters. Oasis HLB 3 cc Extraction cartridges were conditioned with 2 mL of methanol (MeOH) followed by 2 mL of ddH_2O at a rate of less than 1 mL/min. The filtered sample was then passed through the cartridge at a rate of less than 5 mL/min. After allowing for complete drying of the cartridge, the sample was eluted from the cartridge with 4 mL of MeOH under gravity and collected in 5 mL glass test tubes. All glassware used during SPE and subsequent HPLC-MS processing procedures were rinsed with MeOH and ddH_20. The eluted samples were dried under nitrogen and reconstituted in 1000 µL of MeOH (100x concentrated). After resuspension by vortexing, 175 µL of the sample was transferred to polypropylene HPLC-MS vials with glass inserts and subjected to HPLC-MS analysis (HPLC; Waters AQUITY) (CAF, Stellenbosch University).

3.3 Interim Results

3.3.1 Bench-scale evaluation of CEMS as a sensor for individual CEC detection

The CEMS reactor was exposed to a range of environmentally relevant CECs, to assess if the administered concentrations influence biofilm metabolism. Exposure to 100 µg/L of the antibiotic SMX (Figure 3.3, first red box), for 2 hours, showed no measurable effect on the metabolic profile of the biofilm as compared to the negative control. Similarly, when exposed to 1000 µg/L SMX (Figure 3.3, second red box), there still was no measurable effect on the biofilm metabolism, expressed as CO_2 production. These concentrations are equivalent to more than 30x and 300x the concentration measured in wastewater effluent, respectively (Table 3.1). Table 3.1 summarises the results obtained from the bench-scale evaluations for individual CEC exposure to the CEMS reactors. No observed effect (NOE) was seen for all CECs tested in this experiment at both 100 and 1000 µg/L concentrations as with exposure to SMX. At 100 µg/L exposure to the CEMS reactors, it is 3-25 times the maximum concentrations measured in South African wastewater effluent, for the six additional CECs investigated. Furthermore, there was also no observable effect on the biofilm metabolic rate at 1000 µg/L.

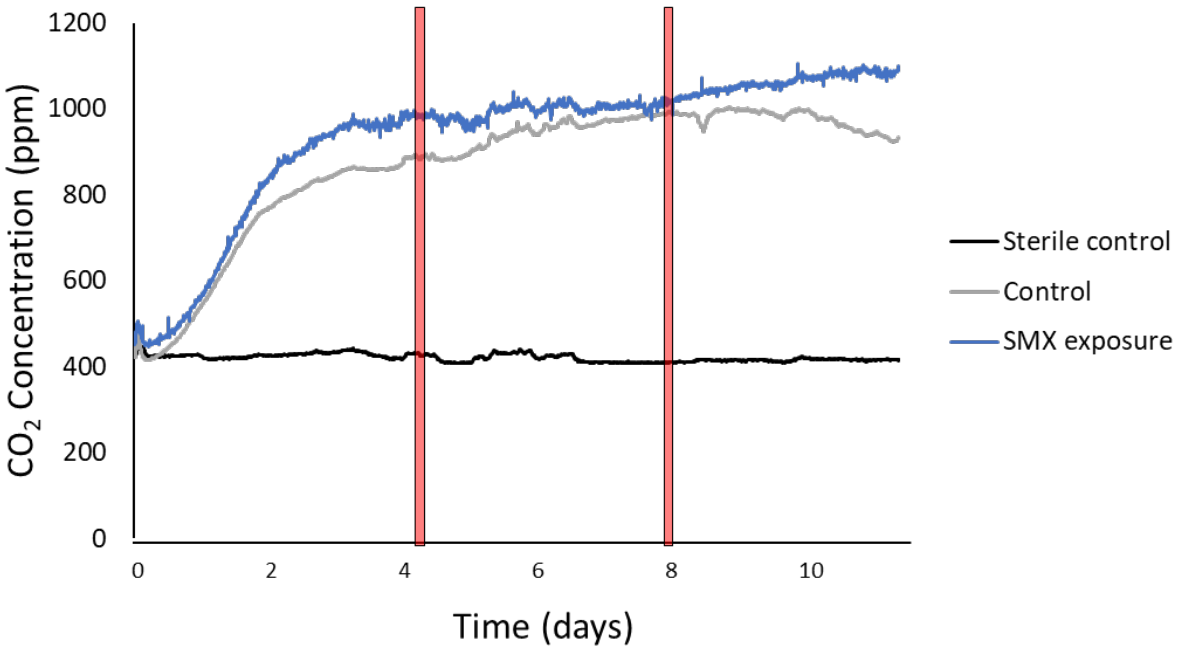

Figure 3.3: Metabolic profile of a pure culture biofilm, expressed as CO$_2$ production, when exposed to 100 and 1000 ug/L sulfamethoxazole for 2 hours (indicated by the red sections), in that order.

Table 3.1: The effect of environmentally relevant CEC on pure culture biofilm metabolic rate at 100 and 1000 ug/L, as well as the maximum concentrations of the respective CECs in South African wastewater effluent. NOE = No Observed Effect. Adapted from Rogowska et al. (2019)

Micropollutant	**Class**	**CEMS response**		**Concentration (µg/L)**	**Reference**
		100 µg/L	**1000 µg/L**	**SA waste water effluent**	
Carbamazepine	Anti-epileptic drug	NOE	NOE	4,61	Deeb et al. (2017)
Sulfamethoxazole	Antibiotic	NOE	NOE	3,25	Oliveira et al. (2015)
Acetaminophen	Painkiller	NOE	NOE	11,73	Petrie et al. (2015)
Benzotriazole	Corrosion inhibitor	NOE	NOE	22,1 - 24,3	Deeb et al. (2017)
Caffeine	Phsyco-active drug	NOE	NOE	11,45	Gros et al. (2017)
Efavirenz	Anti-retroviral	NOE	NOE	34,8	Abafe et al. (2018)
Diclofenac	Non-steroidal anti-inflammatory	NOE	NOE	23,5	Madikizela and Chimuka (2017)

3.3.2 Bench-scale evaluation of CEMS as sensor for CEC detection in environmental water sources

The CEMS reactor was exposed to river water which receives high loads of contamination from surrounding urban discharge, to assess if the concentrations of CECs in environmental samples influence biofilm metabolism. The CEMS reactor showed a pronounced effect when exposed to filtered Plankenbrug river water for 2 hours (Figure 3.4). The metabolic rate of the biofilm, expressed as CO_2 production, showed an immediate effect (within 3 minutes) upon exposure to the river water containing a cocktail of CECs at different concentrations (Table 3.2). CO_2 production was reduced from 910 mg/L to less than 670 mg/L. After exposure to the river water, and upon switching back to the growth media, the CO_2 production steadily increased to normal levels within 24 hours.

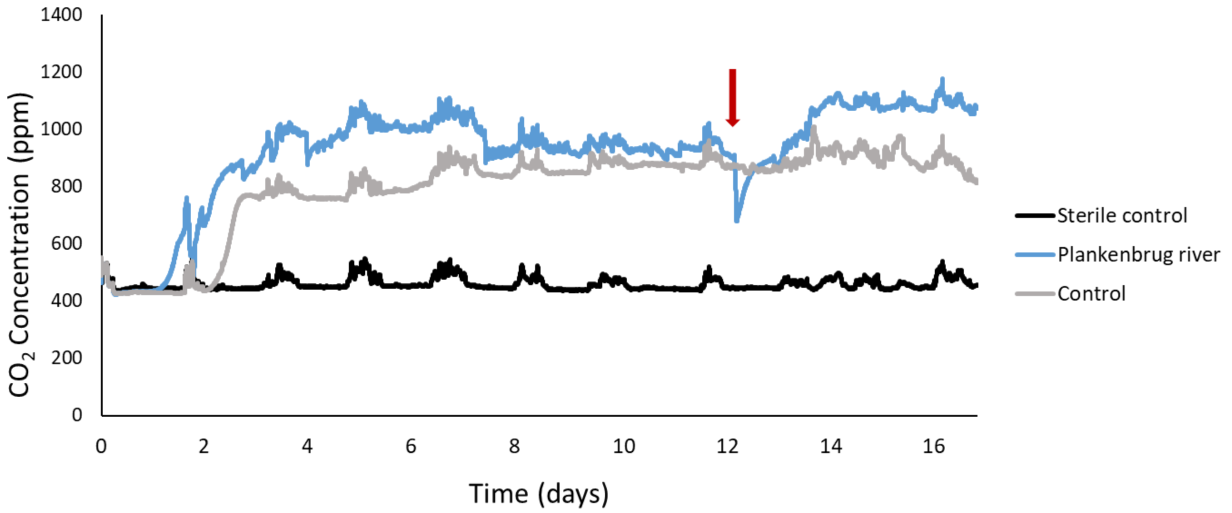

Figure 3.4: Metabolic profile of an environmentally relevant mixed community biofilm, expressed as CO_2 production, when grown up on 1 g/L TSB and exposed to filtered river water receiving high loads of CEC contamination for 2 hours (start of exposure indicated by red arrow).

As with exposure to river water, the CEMS reactor showed a prominent response to exposure to filtered wastewater effluent. CO_2 production was reduced from 1248 mg/L to 903 mg/L at the lowest point within 3 minutes of switching to the wastewater effluent and the reduction in metabolic rate was significantly different from the negative control. The river water and wastewater were analysed for CEC quantification and was found to have high concentrations of CECs, relative to environmental impact (Table 3).

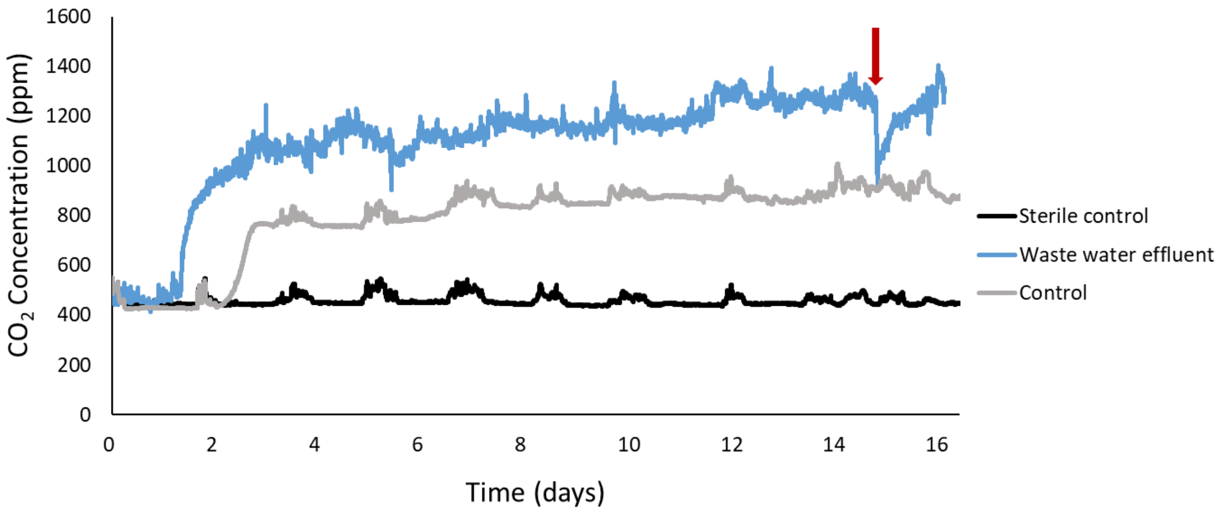

Figure 3.5: Metabolic profile of an environmentally relevant mixed community biofilm, expressed as CO_2 production, when grown up on 1 g/L TSB and exposed to filtered wastewater effluent for 2 hours (stat of exposure indicated by red arrow).

The total carbon content of the growth medium was significantly higher than the two environmental water samples Table 3.2, with total organic carbon constituting more than 99% of the total carbon. In contrast, for the Plankenbrug river and WWTW effluent samples, inorganic carbon constituted about 70% and 66% of the total carbon content present in the samples, respectively.

Table 3.2: The total organic carbon, total inorganic carbon and total carbon of 2 environmental samples and 1 g/L TSB growth medium.

Sample type	TOC (mg/L)	TIC (mg/L)	TC (mg/L)
Plankenbrug river	15.25 ± 0.11	35.11 ± 0.48	50.37 ± 0.44
Wastewater effluent	14.15 ± 0.21	30.97 ± 0.09	45.13 ± 0.21
1 g/L TSB	311.01 ± 3.06	1.77 ± 0.22	312.78 ± 2.85

Table 3.3 summarises the CEC concentrations quantified in the two environmental samples. The concentrations detected is significantly less than the 100 ug/L (100,000 ng) of CECs than was exposed to the CEMS reactors.

Table 3.3: CEC quantification in the Plankenbrug river and wastewater effluent using LCMS/MS.

CEC	Plankenbrug river		Wastewater effluent	
	Concentration (ng/L)	Standard deviation	Concentration (ng/L)	Standard deviation
Benzotriazole	100,00	1,41	78,00	4,24
Methamphetamine	193,00	7,07	4,50	0,71
Acetaminophen	7100,50	118,09	2,50	3,54
1,7-dimethylxanthine	462,00	1,41	17,50	2,12
MDMA	0,00	0,00	1,00	0,00
Caffeine	1101,50	57,28	33,50	2,12
Atrazine	0,00	0,00	6,50	2,12
Naproxen	5,50	3,54	6,50	9,19
Carbamazepine	6,50	0,71	430,00	4,24
Emtricitabine	6096,50	205,77	772,00	24,04
Methaqualone	318,00	4,24	21,50	2,12
Sulfamethoxazole	720,50	30,41	792,00	18,38
Tramadol	46,00	5,66	590,00	19,80
Venlafaxine	20,50	0,71	267,00	12,73
Benzolecgonine	0,50	0,71	3,50	0,71
Trimethoprim	107,50	9,19	12,00	2,83
Diclofenac	38,00	0,00	59,00	0,00
Codeine	54,50	10,61	10,00	0,00
Cocaine	1,00	0,00	0,00	0,00
Efavirenz	1917,50	45,96	3334,00	148,49
Cetirizine	20,50	2,12	374,50	0,71
Tetracycline	0,00	0,00	57,50	2,12

3.4 Discussion

The CEMS reactors, when exposed to individual CECs showed no measurable effect on the CO_2 production of the biofilm. The administered concentrations are much higher than what is frequently detected in South African wastewater effluents for the respective CECs. This demonstrates that even though the selected CECs have inhibitory effects on microbial communities, the concentrations are simply too low to impact microbial metabolism. Additionally, the complexity and diversity of microbial communities enable them to rapidly adapt and shift taxonomic diversity to sudden changes in nutrient availability and toxins (Louca et al., 2018; Trivedi et al., 2020). This highly adaptable nature of biofilms limits the use of the CEMS system to detect CECs at environmental concentrations. Although microbial metabolism has been found to be affected by CECs, such as the antibiotic sulfamethoxazole (SMX), it is only at much higher concentrations than what is detected in the environment. Tucker (2021) found that microbial communities exposed to 646 000 µg/L of SMX had an inhibitory effect on microbial metabolism. This shows the unrealistically high concentrations needed for the detection of a CEC such as SMX, using microbial metabolism.

The CEMS reactors showed a sharp decline in metabolic rate when switched from the synthetic growth medium to river water receiving high levels of pollution form surrounding urban discharge. A similar effect was seen when exposed to WWTW effluent. This prompted measurement of the total organic carbon in the respective media, which suggested that, although the environmental samples had high levels of CECs present, the decrease in CO2 production is not necessarily CEC specific, as the switch from growth medium to river and WWTW effluent results in a significant decrease in nutrient concentration. Upon analysis of the available carbon content of the environmental samples compared to the growth medium, it was found that the river and wastewater effluent had only 14% and 16% organic carbon available for metabolism, respectively. Therefore, the sudden change is metabolic rate upon exposure to environmental waters is most likely due to available nutrient (Oliveira et al., 2015), as the first phase of the bench-scale evaluations showed that environmental concentrations of CECs is too low to impact microbial metabolism. Furthermore, it highlights the gap in development phases of CEC sensors compared to real-world applications, where the complexity and concentrations of CECs in environmental samples are often overlooked.

The low concentrations of CECs quantified in the two environmental samples (Table 3) and the no observed effect of the CECs on microbial metabolism at 100 and 1000 µg/L supports the findings above. Although the concentrations are too low to detect using CEMS, many of the detected CECs were at concentrations that have an environmental impact, especially endocrine disruption in higher organisms (Kumar and Xagoraraki, 2010). Therefore, effect-based monitoring of CECs, using ecotoxicological assays, should rather be considered as opposed to near impossible attempts to detect CECs at the reported low concentrations.

3.5 Interim Conclusions

The Carbon Dioxide Evolution Measurement system was shown to not be indicative of high concentrations of CEC upon exposure, relative to environmental conditions. Concentrations of up to 300 times of what is frequently detected in South African wastewater effluent had no impact on the metabolic rate of the biofilm. During bench-scale evaluations where growth medium was switched with river water that receives high levels of pollution form surrounding urban discharge, there was a significant decline in the metabolic rate of the biofilm. A similar effect was observed with wastewater effluent exposure, reducing metabolism significantly with 3 minutes of exposure. LCMS/MS analysis of the two environmental water sources indicated that both samples had a range of CECs present. Although the switch to environmental test water affected microbial metabolism, it is not necessarily a CEC, or CEC cocktail, specific effect and is likely due to nutrient changes in the media composition. Carbon content analysis confirmed the latter, as the river water and wastewater effluent had only about 14% and 16% of the total carbon available compared to the growth medium.

Although a promising technology in principle, the reality is that there is too much other labile carbon sources in environmental waters and CECs concentrations in the environment is simply too low to be detected without intensive targeted analysis. The results show the real-time capability of CEMS, but also that environmental CEC concentrations are too low to impact overall microbial metabolism. This demonstrates the high degree of metabolic redundancy of microbial communities i.e., the extensive gene and species pool enable self-regulation to continuously adapt to available nutrients and inhibitors. Secondly, it demonstrates the significant challenge to find a substitute for analytical methods to detect CECs in environmental samples at these low concentrations.

Overall, the labour, required infrastructure and associated cost prohibit routine application of analytical methods such as LCMS/MS for real-time sensing of micropollutants. Furthermore, this project showed that the high degree of metabolic redundancy and tolerance to perturbations amongst microbial communities render biofilm metabolism not sufficiently sensitive to be exploited the current CEMS system as an option for real-time sensing of micropollutants. Therefore, the promise shown by the recombinant yeast cell estrogen-screening assay should be investigated as an alternative for incorporating in the CEMS system with the aid of the Internet of Things (Klopper et al., 2020), as an alternative to biofilm metabolism that is currently employed.

CHAPTER 4: NEAR-INFRARED SPECTROSCOPY

4.1 Introduction

The monitoring of chemicals of emerging concern (CECs) in water has been a subject of increasing concern over the past few years. Quantifying these compounds is challenging due to their low concentrations, complex matrices, and wide range of compounds with broad physico-chemical properties. Therefore, monitoring water resources for these chemicals is a complex problem.

The traditional methods applied for the quantification and monitoring of these compounds are advanced detection techniques which include solid phase extraction (SPE) and liquid chromatography paired with mass spectrometry (LC-MS/MS). This is an adaptable and dependable technique for recognizing and measuring contaminations in stream water and wastewater tests (Tripathi et al., 2020). These techniques require sampling regimes and extensive sample preparation which makes them costly and time-consuming. Therefore, there is a need for reliable, fast and low-cost monitoring techniques that can provide a warning of any irregular change in water quality and indicate the necessity for analysis by the standard methods (Tripathi et al., 2020).

Monitoring of a number of single quality parameters of water does not ensure the evaluation of all possible changes in the water molecular system. It is therefore necessary to apply a holistic method, which can monitor the water in real-time, to assess whether it has been in the range of its average probability to change (Kovacs et al., 2015).

Spectroscopy methods such as X-ray, infrared spectroscopy (IR), THz spectroscopy, NIR spectroscopy and others, using light as a probe, proved to be especially valuable tools for water studies and have contributed immensely to elucidation of various aspects of water systems. In general, water-light interaction over the entire electromagnetic spectrum has significantly contributed to a better understanding of water molecular systems.

Water molecules absorb radiation over the entire range of the electromagnetic spectrum. In contrast to mid- and far-infrared, where water strongly absorbs, allowing analysis of only very thin samples, in the NIR part of the spectrum, water absorption is much weaker, therefore offering the possibility of analysing 'thicker' samples and objects rapidly, in a completely non-destructive and non-invasive manner, and with none or little sample preparation. NIR spectroscopy is a vibrational spectroscopy technique in the wavelength range of 750-500 nm (wavenumbers: 13 300-4000 cm^{-1}) (Pasquini, 2003). Using light of the NIR range, it is very easy to acquire spectral data of various aqueous and biological systems in real time without disruption of their state and dynamics. Near infrared spectroscopy thus offers a unique window of opportunities to observe the water molecular network as a scaffold – a matrix of every system of which it is an intrinsic part of – in relation to all other contributing elements and factors shaping the system structure, state and resulting dynamics – without any disruptions (Muncan & Tsenkova, 2019)

Being rapid and non-destructive, NIR spectroscopy is a powerful technique with a wide range of applications, whose horizons have been further expanded by aquaphotomics. Since the establishment of aquaphotomics more than a decade ago, it has grown into a vast and multidisciplinary scientific field, encompassing many research areas. Changes in the absorption spectrum of water are used for quantification of the solutes present in water, even when the solutes do not absorb NIR light at all (Grant et al., 1989; Tsenkova, 2009; Gowen et al., 2015).

4.2 Methodology

4.2.1 Instrumentation

A NIR spectrophotometer has three main components, a light source, a wavelength isolator, and a detector (Ozaki et al., 2020). Fourier transform near-infrared (FT-NIR) spectrophotometers contain an interferometer which produces an interferogram, which is a complex signal that contains all the frequencies that make up the infrared spectrum (Burns & Ciurczak, 2008).

A tungsten halogen lamp produces light that is directed to the first polarizer. The polarised light then passes through a double refracting block, which splits the light into two orthogonally polarised components with a static phase shift. Two double refracting wedges are placed after the refracting block, the first refracting wedge is stationary, while the second refracting wedge constantly moves backwards and forwards. This results in an ongoing phase shift between the light beams. The phase-shifted beams are merged back into one light beam with intensity variation at the second polarizer. The light beam then passes through the sample and is detected by the interferogram detector, producing an interferogram which is converted to a spectrum by Fourier transforms (Burns & Ciurczak, 2008). Figure 4.1 shows a schematic diagram of an FT-NIR instrument.

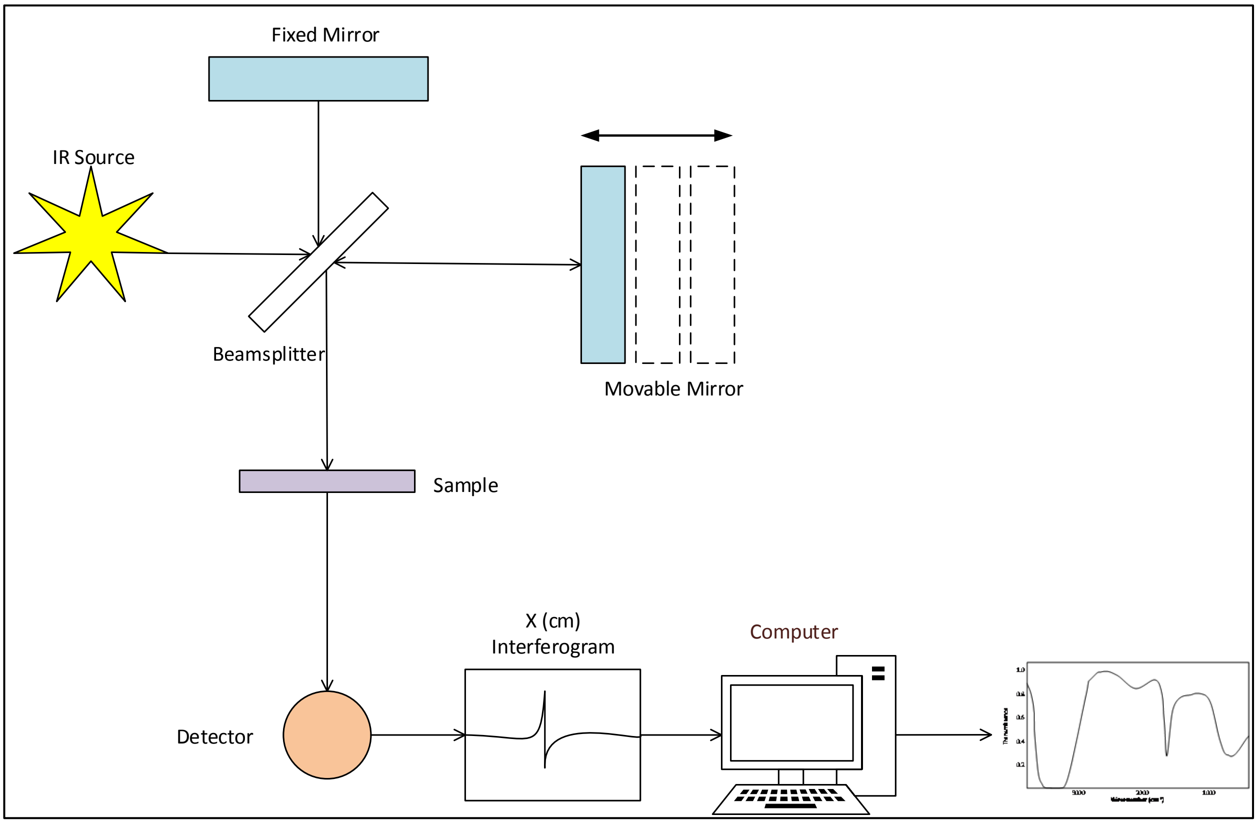

Figure 4.1: Schematic diagram of aa FT-NIR instrument

4.2.2 Chemicals of Emerging Concern (CECs)

For the spiking experiments, three chemicals were selected, namely Acetaminophen (Sigma-Aldrich, CAS No: 103-90-2) Benzotriazole (Sigma-Aldrich, CAS No: 95-14-7) and Caffeine (Sigma-Aldrich, CAS No: 58-08-2). All chemicals were readily available at the Department of Microbiology, Stellenbosch University.

4.2.3 Sample Preparation

The spiked water samples were prepared by formulating working stock solutions of 1000 mg/L (1 g/L) by direct dilution by dissolving 50 mg of the chemical in 50 mL of deionised water from a MilliQ water purification system (Biopak® Polisher, MERCK), which will be referred to only as **water** for the remainder of this report. Stock solutions were mixed using a magnetic stirrer for approximately 15 minutes to ensure that the chemical is completely dissolved and homogeneously mixed with the water. Further dilutions were made by series dilution in water to create a series of the following concentrations: 100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001 mg/L. The dilutions were made with the same water as the stock solution, to ensure that the changes in the absorbance signal were due to the chemicals and not due to a change in the purification of the water.

4.2.4 NIR Instrumentation

All spectra were obtained in the wavelength range of 1000-2500 nm using a Buchi NIRFlex N-500 Fourier transform NIR spectrophotometer (BÜCHI Labortechnik AG, Flawil, Switzerland). The instrument was fitted with a tungsten halogen lamp, a temperature-controlled Indium Gallium Arsenide (InGaAs) detector and a temperature-controlled cuvette holder and was operated using NIRWare software suite. The instrument performed 32 successive scans per sample, at a signal-to-noise ratio of 10 000 and a resolution of 8 cm^{-1} with a data point every 4 cm^{-1} which result in 1501 data points.

4.2.5 NIR Spectra Collection

The experimental work was conducted in six experimental phases. Each sample was scanned at 32°C (±0.1°C) using a quartz cuvette (Hellma Analytics, Germany) with a pathlength of 0.2 mm, filled with 80 µL of the sample. Each sample was scanned three consecutive times and NIR spectra of the samples were collected in an order randomised with respect to chemical and concentration. To monitor any potentially interfering signals, control measurements of water were taken at the beginning, end and after every fifth sample of each experiment. Between each measurement, the cuvette was cleaned thoroughly with acetone followed by the water and subsequently dried under a gentle stream of air. The total number of spectra was 612 (3 chemicals x 3 ranges x 3 concentrations x 6 days x 3 consecutive scans) including pure water samples (6 days x 7-time points x 3 consecutive scans).

4.2.6 Spectral Data Analysis

All data analysis was conducted in MATLAB (The Math Works, Inc. MATLAB. Version 2020a, The Math Works, Inc., 2020. Computer Software) and the PLS_Toolbox [Solo] (Solo 9.0 (R9.0) (2021). Eigenvector Research, Inc., Manson, WA USA 98831; software available at http://www.eigenvector.com) multivariate data analysis software packages.

The effect of scanning over a number of days were eliminated by synchronising the spectra (Kovacs et al., 2015). This was done by subtracting the mean spectra of the deionised water from each spectrum of the same day, then adding the total average spectrum of all deionised water scanned back to all the sample spectra (Kovacs et al., 2015). The triplicate spectra for each sample were averaged prior to pre-processing, exploratory data analysis and model development.

4.2.6.1 Repeatability and Reproducibility

The repeatability of the instrument and reproducibility of the measurement was determined by calculating the standard deviation (SD) and coefficient of variation (CV) for the repeated scans. The CV is expressed as the ratio of the SD over the average of the spectral data (Bazar et al., 2016).

The precision of the instrument and the repeatability of the analytical process were tested by repeating the measurements multiple times under the same conditions. This was done by scanning the same cuvette of water continuously at 40s intervals in the same position. Ten spectra were recorded for this test. The reproducibility of the analytical process was achieved by first scanning and reloading the same water sample ten times in the same position, allowing for a 40s incubation interval between scans. A second reproducibility test was done by refilling the cuvette with new water after each scan. Each newly filled cuvette of water was allowed to incubate for 40s before the first scan was taken to minimise temperature variation. Ten spectra were also recorded for the final test.

4.2.6.2 Pre-processing

A number of pre-processing treatments were applied to the spectra. The purpose of applying pre-processing to the spectra is to remove physical occurrences in the spectra in order to improve the subsequent exploratory, classification and multivariate regression analysis (Rinnan et al., 2009).

Pre-processing techniques that were evaluated to determine which combination would produce the best results included: mean centring (MC) (Agelet & Hurburgh, 2010), standard normal variance (SNV) (Barnes et al., 1989) and Savitzky-Golay (SG) (Savitzky & Golay, 1964) first and second derivates and smoothing.

4.2.6.3 Exploratory Analysis

Principal component analysis (PCA) was applied to the mean centred absorbance spectra to explore the data and identify patterns and to visualise the information present in the spectral data set and to identify outliers (Cowe & McNicol, 1985). PCA was also used to examine the wavelength ranges at which the experiments were most repeatable. For consistency, all PCA models were build using four principal components (PCs) and both the PCA score and loading plots were used to analyse the data.

4.2.6.4 Classification with Partial Least Squares Discriminant Analysis (PLS-DA)

The exploratory data analyses were followed by a multivariate data analysis to determine whether the chemicals could be differentiated from the water, irrespective of concentration and to determine at which concentration no differentiation could be achieved. Each model was tested using cross-validation, where part of the sample set was excluded from the calibration set and the generated model was then validated on the excluded samples (Kovacs et al., 2022). This method of validation was chosen due to the small sample sizes that were available. The cross-validation method allowed for the procedure to be repeated iteratively and thereby ensuring that all samples were included in the validation set at least once. The cross-validation method that were used for analysis was the Venetian Blinds method, where one sample per blind was excluded from the data each time.

Partial Least Squares Discriminant Analysis (PLS-DA) (Wold, 1975) models were developed to differentiate between the water and the spiked CEC water samples. The data in the 1300-2200 nm range were subjected to a range of pre-processing techniques and calibration models were constructed. The maximum number of latent variables was defined according to the minimum value of the root-mean-square error (RMSE) of the cross validation. The spectra of the was designated a dummy index of 0 (zero) while those of the chemicals was designated an index of 1 (one).

The overall performance of the models in, combination with the various pre-processing techniques, was validated by calculating the classification accuracy (Equation (1)), which illustrates the efficacy of the model. A false positive occurs when a negative response (or incorrect class) is classified as a positive response (correct class) and consequently a false negative occurs when a positive response (correct class) is classified as a negative response (incorrect class).

Classification accuracy, sensitivity and specificity will be calculated according to Equations (1) (2) and (3) respectively. The misclassification rate, precision and F1 score was calculated according to Equations (4) (5) (6), respectively. Classification accuracy is used to prove the effectiveness of the overall model. Sensitivity is also known as the true positive response that describes the proportion of true positives that are correctly identified by a diagnostic test (Zhu et al., 2010). Specificity is the proportion of the true negatives correctly identified by a diagnostic test. It suggests how good the test is at identifying normal (negative) condition (Zhu et al., 2010). Finally, the misclassification rate is calculated according to Equation (4) to show how often the classification prediction was incorrect. The F1 score is used to compare the performance of two classifiers.

$$Classification\ Accuracy\ (\%) = \frac{TP + TN}{TP + TN + FP + FN} \times 100 \tag{1}$$

$$Sensitvity\ or\ recall\ (\%) = \frac{TP}{TP + FN} \times 100 \tag{2}$$

$$Specificity\ (\%) = \frac{TN}{TN + FP} \times 100 \tag{3}$$

$$Misclassifcation\ rate\ (\%) = \frac{FP + FN}{TP + TN + FP + FN} \times 100 \tag{4}$$

$$Precision\ (\%) = \frac{TP}{TP + FP} \times 100 \tag{5}$$

$$F1\ score\ (\%) = \frac{2 \times precision \times recall}{precision + recall} \tag{6}$$

Where:

True positives (TP) = positive responses classified as positive responses

True negatives (TN) = negative responses classified as negative responses

False positives (FP) = negative responses classified as positive responses

False negatives (FN) = positive responses classified as negative responses

Table 4.1 shows the most commonly used classification accuracies in measuring the effectiveness of a model (Zhu et al., 2010).

Table 4.1: Accuracy classifications on determining the performance of a model.

Range	Classification
90-100%	Probably overfitted
80-90%	Excellent
70-80%	Good
60-70%	Poor

4.3 Results and Discussion

4.3.1 Repeatability and Reproducibility

The spectral precision of the instrument can be determined by calculating the standard deviation (SD) and coefficient of variation (CV) of spectral data at selected wavelengths, representing relevant peaks to the investigation at hand (Williams, 2013). It was suggested that ideally four wavelengths should be used. Three wavelengths that were chosen to evaluate the average absorbance and standard deviations was as follows: wavelength 1190 nm, 1450 nm, and 1950 nm which are the three of the main absorbance peaks of water in the NIR region (Curcio & Petty, 1951).

Table 4.2 shows the repeatability and reproducibility test results. The low SD values of the repeatability test indicate a low variation between the measurements, indicating a high precision instrument. An increasing trend in the SD value of the spectral data is observed. (Williams, 2013) noted that the SD value of the spectral data tends to increase with increasing wavelength in the repeatability tests.

Table 4.2: The repeatability and reproducibility of water on the NIR spectrometer

		Wavelength		
		1190 nm	**1450 nm**	**1950 nm**
Repeatability	**Average (abs)**	0.044345	0.313135	1.097222
	SD	0.000083	0.000371	0.001644
	CV%	0.19%	0.12%	0.15%
Reproducibility 1	**Average (abs)**	0.041324	0.310538	1.094099
	SD	0.000358	0.000399	0.001705
	CV%	0.87%	0.13%	0.16%
Reproducibility 2	**Average (abs)**	0.041794	0.317181	1.119526
	SD	0.000485	0.001643	0.007060
	CV%	1.16%	0.52%	0.63%

Figure 4.2 shows the SD curves for the three tests. It can be seen that the SD of the two reproducibility tests is higher along the spectrum, because for the first reproducibility test the cuvette was removed from the instrument after each scan and for the second reproducibility test, the water was replaced after each scan. Removal of the cuvette may cause changes in the geometry of the samples, which introduces error (Bazar et al., 2016) and changing the water after each scan introduces temperature variations which then cause fluctuations in the absorbance peak. The shift in the repeatability curve may be due to the temperature change in the sample after being repeatedly scanned. samples with a high moisture content is especially susceptible to changes in the temperature due to the vibrations and rotations of O-H in the water molecule (Maeda et al., 1995). Even so, the repeatability measurements caused less deviation from the average than the reproducibility tests. The results indicated that smaller deviations occurred when the sample was scanned repeatedly at the same position, rather than removing and/or replacing the liquid for each measurement.

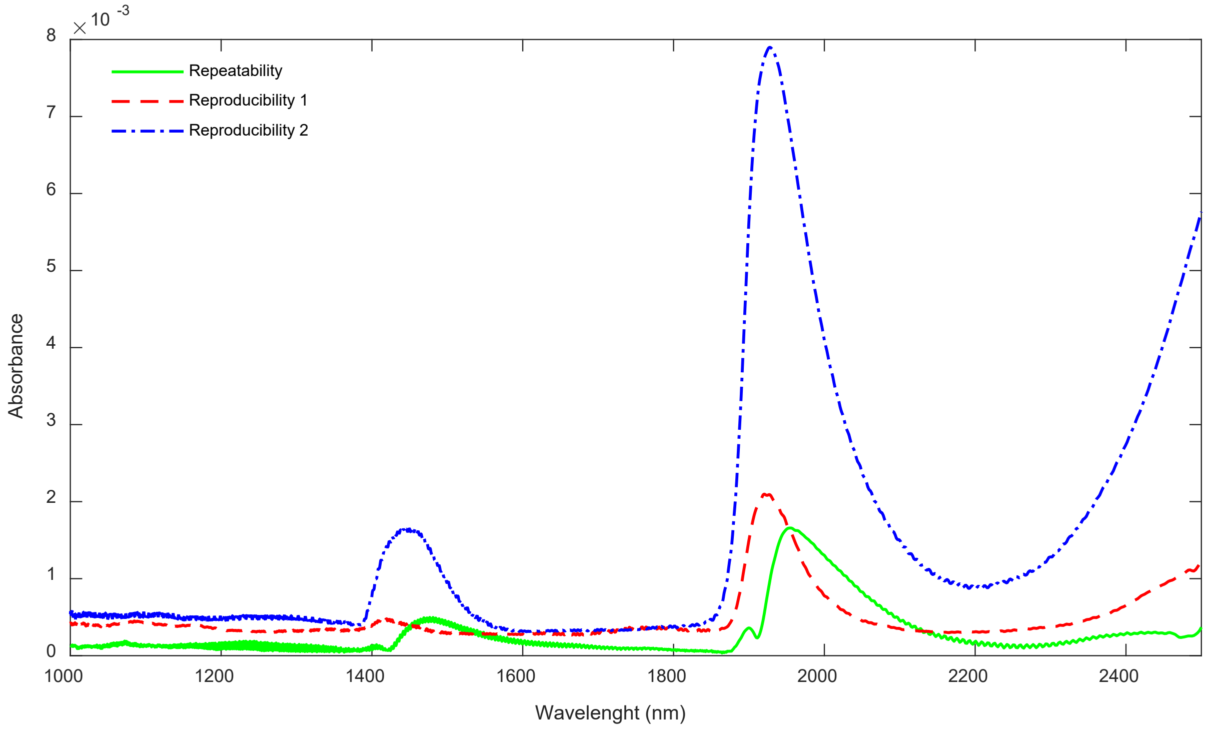

Figure 4.2: Standard deviation plot of the repeatability and reproducibility tests

4.3.2 Spectral Analysis of CEC Solutions

The mean spectra of water and the three chemical solutions are shown in Figure 4.3, which can be used to investigate, determine, and compare chemical properties. However, the spectra of the three chemicals are overlapping and appears to be identical to one another as well as with the water spectra. Due to the strong absorption of water in the 1450 nm and 1950 nm regions, any changes to the molecular structure of water will be very subtle and not visible to the naked eye. For that reason, multivariate analysis is required to extract information hidden within the spectra.

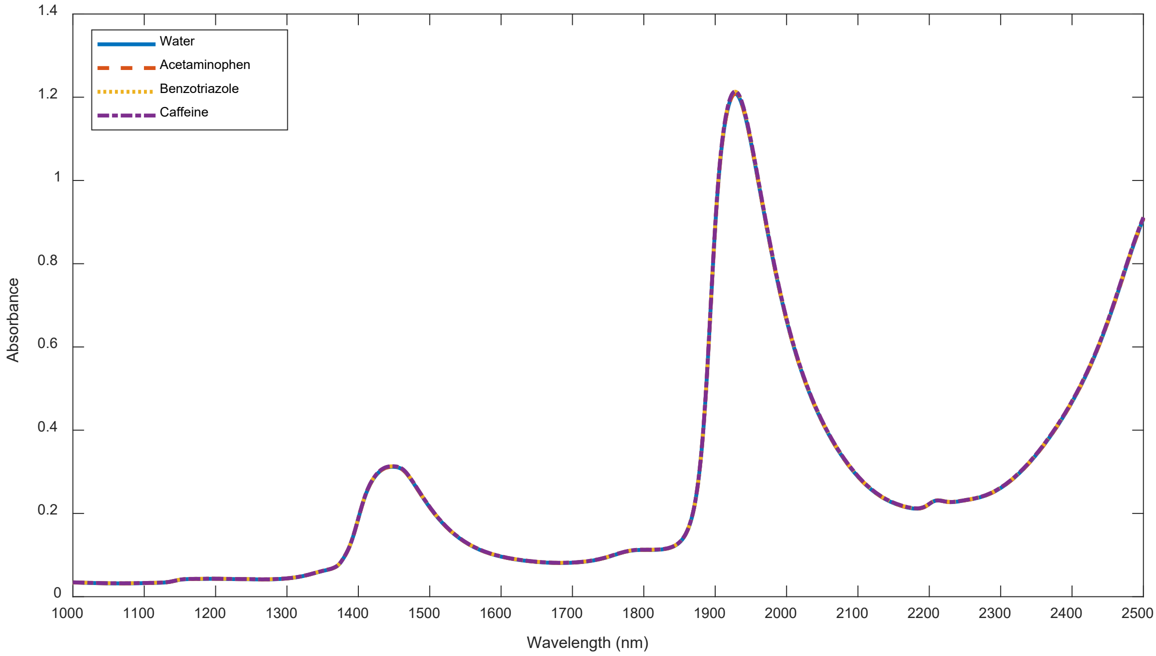

Figure 4.3: Unprocessed mean spectra of MilliQ, acetaminophen, benzotriazole and caffeine solutions

4.3.3 Wavelength Selection

To investigate which wavelength regions would be the most suitable for data modelling, the data were split into different wavelength ranges, from 1000-2500 in steps of 300 nm. Principal Component Analysis (PCA) was applied to the data for each experimental day and wavelength range and the 1st Principal Component (PC) loadings for each day were compared. Figure 4.4 illustrates the loading plots obtained for each wavelength range for the three chemicals, acetaminophen, benzotriazole and caffeine.

It can be observed from the PC1 loadings, that the data from the wavelength region less than 1300 nm is far noisier than that of the wavelength regions from 1300-2500 nm. The noise evident in the spectral edge can be related to the performance of the detector which is generally of lower efficiency at that wavelength region (Gowen et al., 2011). Since very little about the absorption band of water at wavelengths higher than 2200 nm, the higher wavelength range (2200-2500 nm) will also be excluded.

Subsequent analysis was therefore conducted in the wavelength regions 1300-1600 nm and 1600-2200 nm, which had the most striking features.

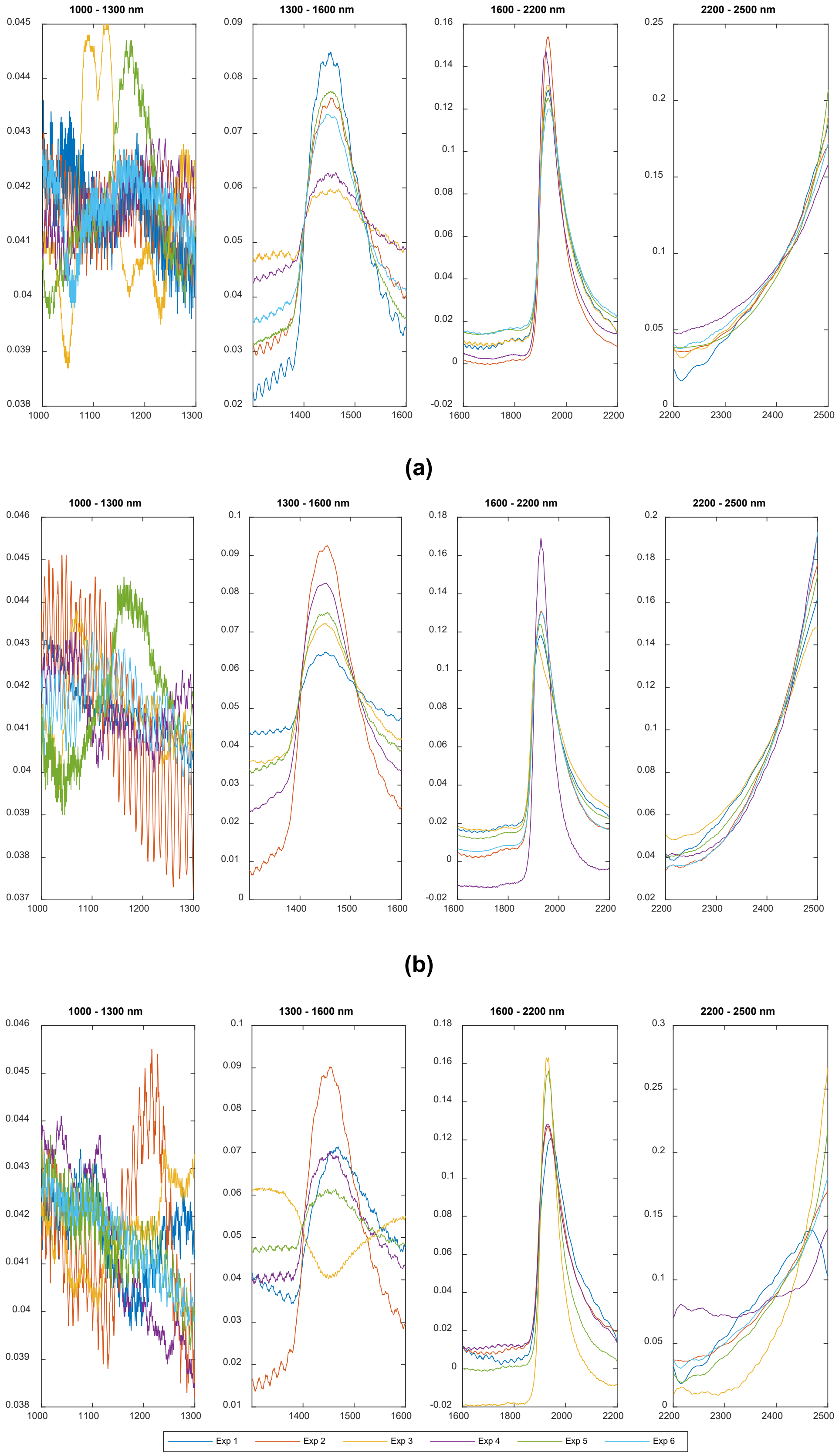

Figure 4.4: Loading plots of the first principal component (PC1) applied to the synchronized averaged data and pre-processed with mean centering for (a) acetaminophen, (b) benzotriazole and (c) caffeine for each experimental day.

4.3.4 Principal component analysis (PCA)

4.3.4.1 Outlier removal

Outliers were investigated by building PCA models on the water data sets and each of the concentration data sets for each chemical. Samples with high leverages were further evaluated to determine whether they are outliers. As outlier removal is an iterative process, this procedure was repeated again after each new model until all outliers were removed. In total, six water samples were removed from the data set. By visual inspection of the original and pre-processed spectra of each of the nine concentrations for each chemical, it could be seen that in some cases there was spectra that deviated from the baseline however, due to the smaller sample sets of the chemicals (6 samples per concentration), the removal of just one sample drastically influenced the model. PCA models for each of the nine concentrations of each chemical was performed to identify any outliers in these data sets. For all three chemicals across all concentrations, no outliers were identified with inspection of the influence plots or PC1 vs PC2 score plots. Subsequently, no outliers were identified and removed from the chemical data sets.

4.3.4.2 PCA of chemicals

PCA was performed on the spectral data of all three chemicals, irrespective of the concentration over the 1300-2200 nm wavelength region. The data was pre-processed with the Savitzky-Golay filter using the 1st derivative, 2nd order polynomial and 21 points, SNV and mean centring. The first two principal components (PC1 & PC2) accounted for 93.5%, 87.9% and 84.1% of the variance for acetaminophen, benzotriazole, and caffeine, respectively. However, visually, separation between the water and the spiked samples occurred in the direction of the third principal component (PC3), as illustrated in Figure 4.5. (Kovacs et al., 2015) noted that the highest variation of spectral data may arise from different factors, such as sample temperature, environment humidity and other perturbations. Therefore, by looking at the PCs with the highest variation may not always be satisfactory and that PCs with lower variance may contain the information required.

Further exploration of the PCA score plots revealed that separation between water and acetaminophen and benzoreiazole could be observed at a maximum concentration of 10 mg/L. Separation between water and caffeine was, however, observed at a maximum concentration of 0.01 mg/L (Figure 4.6). Further classification analysis will attempt to determine at which concentration the chemicals can no longer be distinguished from the water.

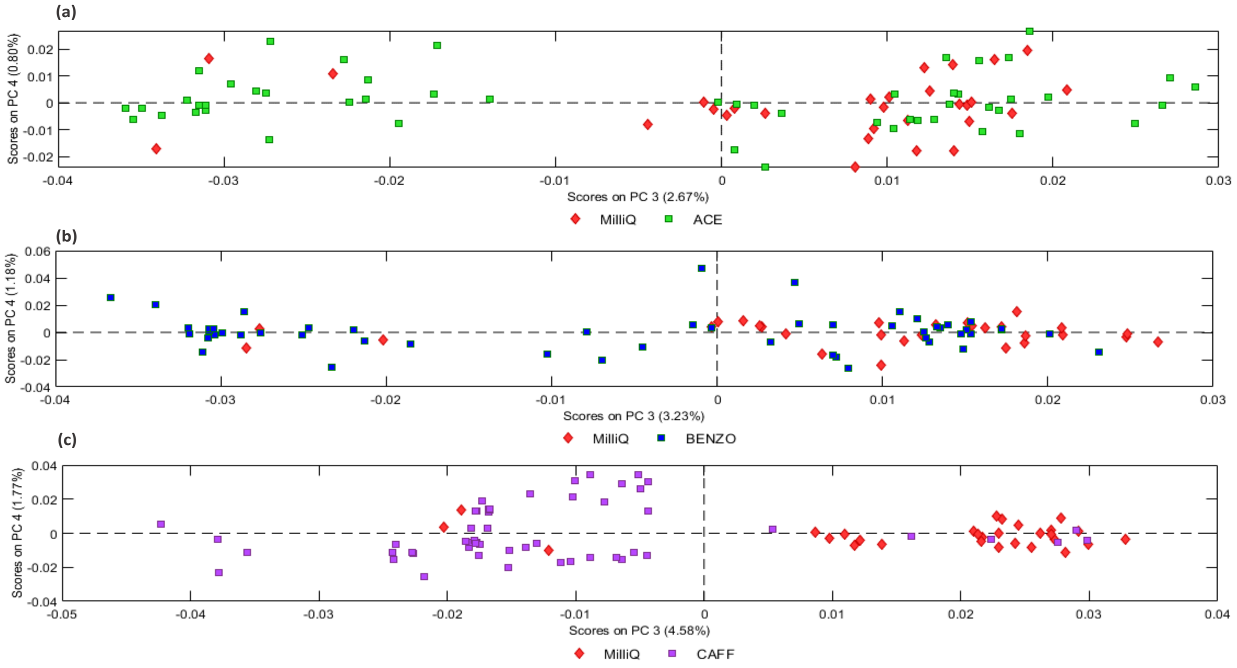

Figure 4.5: PCA analysis (SG filter, 1st derivative, 2nd order polynomial, 21 points and mean centered pre-processed) of MilliQ (red) and (a) acetaminophen (red) (b) benzotriazole (blue) and (c) caffeine (purple), irrespective of concentration in the 1300-2200 nm range.

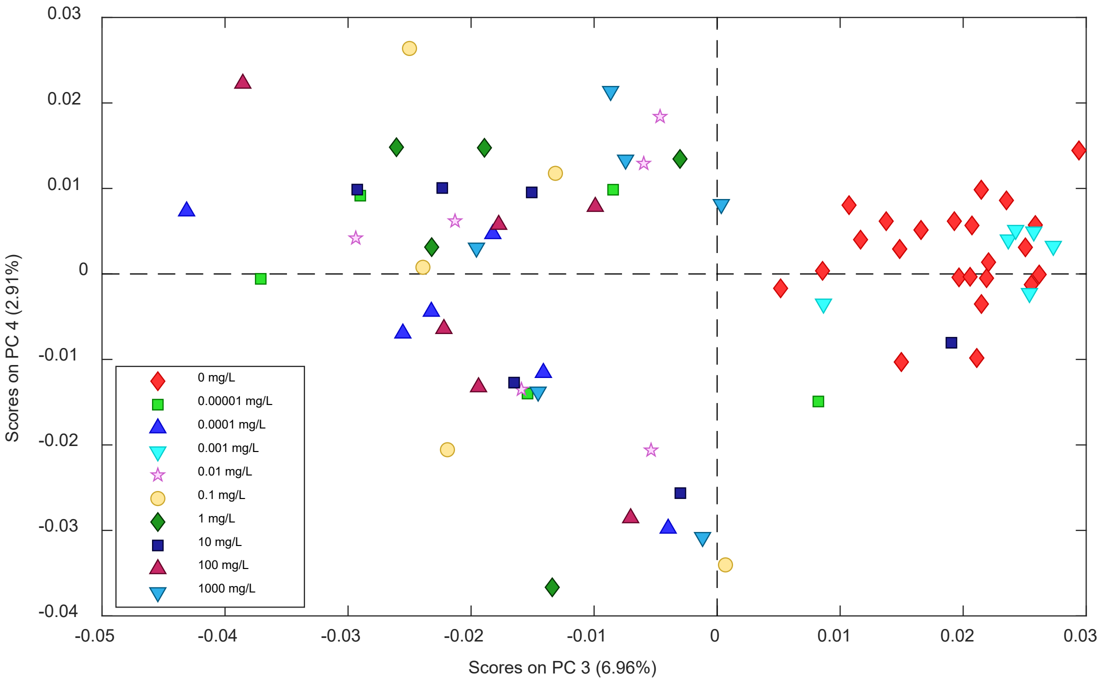

Figure 4.6: PCA analysis (SG filter, 1st derivative, 2nd order polynomial, 21 points and mean centred pre-processed) of water and caffeine for the high concentration range (10-1000 mg/L).

4.3.5 Classification

PLS-DA models for all three chemicals were constructed to determine if classification of the chemicals could be achieved. For each chemical, models were constructed first for the entire concentration range and then for the high (10-1000 mg/L), medium (0.01-1 mg/L) and low (0.00001-0.001 mg/L) concentration ranges. Each time the data was subjected to a range of pre-processing techniques to determine which technique or combination thereof will produce the best calibration models. Models with an overall performance of between 70-90% were considered good models.

Table 4.3 shows the overall performance measures for the models constructed for the acetaminophen data. Poor classification was observed for the combined concentration set, with classification accuracies of less than 70%. However, for the high and medium concentration ranges, good classification of the ACE samples was achieved, but less so for the lowest concentration range. Two pre-processing technique combinations proved to produce good models for this data set: the combination of (1) mean centring and Savitzky-Golay (2nd derivative, 2nd order polynomial and 21 points) and (2) mean centring, SNV and Savitzky-Golay (1st derivative, 2nd order polynomial and 21 points).

Table 4.3: Overall performance measures of the calibration and cross-validation PLS-DA models for acetaminophen evaluated at the different concentration ranges.

	CONCENTRATION: ALL (0.00001-1000 mg/L)				**CONCENTRATION: HIGH (10-1000 mg/L)**			
		Calibration		**CV**		**Calibration**		**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	**5**	**67.8%**	**32.2%**	**63.3%**	4	90.7%	9.3%	83.3%
MC + SNV	6	66.7%	33.3%	62.2%	3	87.0%	13.0%	87.0%
MC + SGd1 (21)	3	65.6%	34.4%	58.9%	3	90.7%	9.3%	88.9%
MC + SGd2 (21)	3	65.6%	34.4%	65.4%	4	88.9%	11.1%	88.9%
MC + SNV + SGd1(21)	3	63.3%	36.7%	58.9%	**2**	**92.6%**	**7.4%**	**88.9%**
MC + SNV + SGd2(21)	4	63.3%	36.7%	65.6%	5	92.6%	7.4%	85.2%
	CONCENTRATION: MEDIUM (0.01-1 mg/L)				**CONCENTRATION: LOW (0.00001-0.001 mg/L)**			
		Calibration		**CV**		**Calibration**		**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	2	61.1%	38.9%	63.0%	4	64.8%	35.2%	61.1%
MC + SNV	3	70.4%	29.6%	50.0%	4	68.5%	31.5%	57.4%
MC + SGd1 (21)	4	74.1%	25.9%	63.0%	**3**	**66.7%**	**33.3%**	**68.5%**
MC + SGd2 (21)	5	75.9%	24.1%	64.8%	4	72.2%	27.8%	55.6%
MC + SNV + SGd1(21)	**5**	**77.8%**	**22.2%**	**59.3%**	4	61.1%	38.9%	51.9%
MC + SNV + SGd2(21)	3	74.1%	25.9%	63.0%	4	68.5%	31.5%	46.3%

(%CA) Classification Accuracy; (%MC) Misclassification Accuracy; (CV) Cross-Validation; (MC) Mean centring; (SNV) Standard normal variance; [SGd1(21) Savitzky-Golay (1st derivative, 2nd order polynomial, 21 points)]; [SGd2(21) Savitzky-Golay (2nd derivative, 2nd order polynomial, 21 points)]

Table 4.4 shows the overall performance measures for the models constructed for the benzotriazole data. The models performed well for the high and medium concentration ranges. Classification accuracies of 77.8% of the high concentration range indicates good model performance. Even higher classification accuracies were achieved in the medium concentration range at 81.5%. Subsequent analysis of the score plots of the medium concentration range showed classification only to a maximum of 0.1 mg/L. These results coincide with the study done by (Béen et al., 2020) that also found that benzotriazole could be distinguished from the water samples at concentrations higher than 0.1 mg/L (100 µg/L). Good classification accuracies are seen in the low concentration range as well; however, the low cross-validation accuracies suggests that the model is overfitting the data.

Table 4.4: Overall performance measures of the calibration and cross-validation PLS-DA models for benzotriazole evaluated at the different concentration ranges.

	CONCENTRATION: ALL (0.00001-1000 mg/L)				**CONCENTRATION: HIGH (10-1000 mg/L)**			
	Calibration			**CV**	**Calibration**			**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	3	56.7%	43.3%	57.8%	4	77.8%	22.2%	77.8%
MC + SNV	3	64.4%	35.6%	61.1%	2	77.8%	22.2%	81.5%
MC + SGd1 (21)	2	64.4%	35.6%	60.0%	2	77.8%	22.2%	77.8%
MC + SGd2 (21)	2	70.0%	30.0%	62.2%	2	77.8%	22.2%	77.8%
MC + SNV + SGd1(21)	3	67.8%	32.2%	65.6%	3	77.8%	22.2%	74.1%
MC + SNV + SGd2(21)	2	67.8%	32.2%	65.6%	3	79.6%	20.4%	66.7%
	CONCENTRATION: MEDIUM (0.01-1 mg/L)				**CONCENTRATION: LOW (0.00001-0.001 mg/L)**			
	Calibration			**CV**	**Calibration**			**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	3	72.2%	27.8%	64.8%	3	64.8%	35.2%	57.4%
MC + SNV	4	83.3%	16.7%	72.2%	3	64.8%	35.2%	57.4%
MC + SGd1 (21)	4	81.5%	18.5%	79.6%	3	75.9%	24.1%	64.8%
MC + SGd2 (21)	2	83.3%	16.7%	72.2%	4	75.9%	24.1%	64.8%
MC + SNV + SGd1(21)	3	81.5%	18.5%	77.8%	4	72.2%	27.8%	50.0%
MC + SNV + SGd2(21)	2	83.3%	16.7%	79.6%	2	74.1%	25.9%	66.7%

(%CA) Classification Accuracy; (%MC) Misclassification Accuracy; (CV) Cross-Validation; (MC) Mean centring; (SNV) Standard normal variance; [SGd1(21) Savitzky-Golay (1st derivative, 2nd order polynomial, 21 points)]; [SGd2(21) Savitzky-Golay (2nd derivative, 2nd order polynomial, 21 points)]

Table 4.5 shows the overall performance measures for the models constructed for the caffeine data. The PLS-DA models showed good classification accuracies across the entire wavelength range, and therefore corroborating the results from the PCA analysis of caffeine. Models for the medium and low concentration ranges showed satisfactory classification, however, the models were prone to over-fitting the data, leading to over optimistic classification accuracies. The results indicate that NIR spectroscopy combined with chemometrics could accurately distinguish between caffeine and water samples at all concentrations ranges (0.00001-1000 mg/L). Further analysis of the score plots, however, revealed that classification could only be achieved to a maximum concentration of 0.01 mg/L.

Table 4.5: Overall performance measures of the calibration and cross-validation PLS-DA models for caffeine evaluated at the different concentration ranges.

	CONCENTRATION: ALL (0.00001-1000 mg/L)				**CONCENTRATION: HIGH (10-1000 mg/L)**			
		Calibration		**CV**		**Calibration**		**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	6	88.9%	11.1%	87.8%	6	90.7%	9.3%	90.7%
MC + SNV	4	90.0%	10.0%	90.0%	2	85.2%	14.8%	77.8%
MC + SGd1 (21)	3	88.9%	11.1%	88.9%	3	92.6%	7.4%	90.7%
MC + SGd2 (21)	3	83.3%	16.7%	83.3%	2	94.4%	5.6%	90.7%
MC + SNV + SGd1(21)	3	88.9%	11.1%	88.9%	3	92.6%	7.4%	90.7%
MC + SNV + SGd2(21)	4	88.9%	11.1%	86.7%	3	90.7%	9.3%	90.7%
	CONCENTRATION: MEDIUM (0.01-1 mg/L)				**CONCENTRATION: LOW (0.00001-0.001 mg/L)**			
		Calibration		**CV**		**Calibration**		**CV**
Pre-processing	**Number of LVs**	**%CA**	**%MC**	**%CA**	**Number of LVs**	**%CA**	**%MC**	**%CA**
MC	3	72.2%	27.8%	64.8%	3	66.7%	33.3%	63.0%
MC + SNV	4	92.6%	7.4%	88.9%	4	85.2%	14.8%	77.8%
MC + SGd1 (21)	3	90.7%	9.3%	83.3%	4	81.5%	18.5%	77.8%
MC + SGd2 (21)	4	87.0%	13.0%	87.0%	3	79.6%	20.4%	75.9%
MC + SNV + SGd1(21)	3	92.6%	7.4%	88.9%	2	81.5%	18.5%	75.9%
MC + SNV + SGd2(21)	3	87.0%	13.0%	87.0%	2	81.5%	18.5%	75.9%

(%CA) Classification Accuracy; (%MC) Misclassification Accuracy; (CV) Cross-Validation; (MC) Mean centring; (SNV) Standard normal variance; [SGd1(21) Savitzky-Golay (1st derivative, 2nd order polynomial, 21 points)]; [SGd2(21) Savitzky-Golay (2nd derivative, 2nd order polynomial, 21 points)]

4.4 Conclusions and recommendations

The research conducted on near-infrared (NIR) spectroscopy combined with multivariate data analysis techniques has provided valuable insights into its potential application as an early warning system for water quality monitoring, particularly in municipal water and wastewater treatment plants, as well as other water reclamation and reuse facilities. The following points elaborate on how this technique can be applied effectively:

High Precision Instrumentation: The study demonstrated high instrument precision through repeatability and reproducibility tests. This reliability is crucial for an early warning system as it ensures consistency in detecting contaminants.

Detection of Contaminants: NIR spectroscopy, when combined with multivariate data analysis, showed promise in differentiating between pure water and water samples spiked with various contaminants (CECs) at different concentrations. This ability to detect contaminants is the core function of any early warning system.

Limit of Detection: The research provided valuable insights into the limit of detection for specific contaminants. For example, it determined a limit of detection of 10 mg/L for acetaminophen and even lower limits for benzotriazole and caffeine. Knowing these limits allows for a more accurate assessment of the severity of contamination events.

Ecological Relevance: While the technique worked well for detecting contaminants at relatively high concentrations, it raised concerns about its ecological relevance in scenarios where dilution occurs, such as confluence of large rivers or during rainfall events. This insight is essential for designing effective monitoring strategies.

Group Monitoring (Cocktail Approach): Given the complexity of water contamination, focusing on individual chemicals may not always be practical. The suggestion to focus on a group of contaminants (cocktail approach) acknowledges the need to consider multiple pollutants simultaneously, which is more representative of real-world contamination scenarios.

Complimentary Early Warning System: NIR spectroscopy combined with multivariate data analysis can serve as a complementary tool within an early warning system. It can aid in rapidly identifying the presence of contaminants or deviations from normal water quality parameters.

Cost-Effective Monitoring: Implementing this technique could lead to more cost-effective monitoring programs in water treatment plants. Traditional methods of monitoring water quality can be labor-intensive and time-consuming. NIR spectroscopy offers a faster and potentially more economical alternative.

Routine Surveillance: This technique could enable routine surveillance of water sources, helping operators and authorities respond quickly to contamination events. Early detection can lead to faster mitigation efforts, reducing the impact of contaminants on public health and the environment.

Future Research: The study highlights the need for further research to refine the technique and expand its applicability. This includes exploring its effectiveness for a wider range of contaminants and under various environmental conditions.

In conclusion, NIR spectroscopy combined with multivariate data analysis has demonstrated promise as an early warning system for water quality monitoring. While it may not be suitable for all contaminants at low concentrations, it can still play a valuable role in safeguarding water supplies by complementing existing monitoring methods. Further research and development in this field are essential to enhance its effectiveness and broaden its utility in ensuring the safety of water resources.

CHAPTER 5: NEW DEVELOPMENTS AND PROMISING TECHNOLOGIES

5.1 Introduction

Chemicals of emerging concern (CECs) are a group of chemicals that are increasingly being detected in the environment and are of growing concern due to their potential impacts on human and environmental health. Such CECs can therefore be classified as either (1) being detected in high frequencies in environmental waters, but with unknown/limited toxicological risk, or (2) as chemicals that are not being monitored frequently in environmental waters whilst having a high potential to be present in a defined surface water setting and having known toxicological risk to humans and aquatic life. Examples of such CEC types include pharmaceuticals, personal care products, pesticides, and industrial by-products.

Pharmaceuticals, including prescription and over-the-counter drugs, are designed to have biological activity in humans or animals. When these drugs are excreted or disposed of improperly, they can enter the environment and have the potential to affect aquatic organisms, such as fish and amphibians, and even human health. Personal care products, such as cosmetics, soaps, and detergents, contain a variety of chemicals that can also enter the environment and have potential impacts on aquatic organisms. Pesticides are used in agricultural and residential settings to control pests but can also enter the environment and have negative impacts on non-target organisms. Industrial by-products, including chemicals used in manufacturing, can also find their way into the environment, and pose risks to human and environmental health.

Due to the vast number of CECs that can be introduced into freshwaters from various anthropogenic sources, it is imperative to consider CECs more seriously due to their potential negative impacts on human and environmental health. Many of these chemicals are designed to have biological activity, which means they can have unintended impacts on non-target organisms in the environment, ranging from short term lethal toxicity to long term lethal or sublethal toxicity on a population level and across generations.

In addition, CECs are often present in low concentrations in the environment, which can make them difficult to detect and monitor. This means that they may not be regulated or managed in the same way as other pollutants, such as metals or nutrients. For this reason, routine surveillance of CECs should be considered more seriously in water quality management programs to develop early warning systems (EWS) that allows for timely intervention to minimize CEC release and distribution in freshwater resources. Such an EWS consist of a set of procedures and protocols designed to identify and quantify the presence of CECs in water systems, from which such systems should include a combination of automated sensors and manual sampling and analysis techniques that can create a CEC profile within a defined setting in real-time or near real-time.

The importance of EWS for CECs is underscored by the fact that many of these contaminants are difficult to detect using traditional water quality monitoring techniques. This is because they are often present in low concentrations or have complex chemical structures that make them difficult to quantify. EWS for CECs can overcome these limitations by using advanced technologies that can detect contaminants at very low concentrations or identify unique chemical fingerprints that are associated with specific CECs.

5.2 The need for new approaches towards early warning systems for CECs

Water quality is a critical concern for both human health and environmental sustainability. While traditional water quality monitoring methods are proven to provide sensitive and high-resolution profiling of water quality parameters, they still rely on manual sampling and laboratory analysis, which are time-consuming, costly, and often provide delayed results. However, the emergence of online sensing technologies and machine learning algorithms has the potential to revolutionize water quality evaluation by enabling real-time monitoring and analysis of water quality parameters such as temperature, pH, dissolved oxygen, turbidity, and nutrient levels. These technologies can be applied in various water settings, including wastewater treatment works, rivers, estuaries, groundwater, and urban water distribution systems, to provide accurate and timely information for decision-making and effective management of water resources.

Although most of the commercially available on-line sensing technologies are useful to evaluate physico-chemical properties of water matrices, there is a need to develop a system that can allow for routine surveillance of CECs that can pose detrimental health effects on the short- and long term for both ecological- and human health. Intervention for the development and commercial rolling-out of real-time sensing technologies are thus needed to provide an early warning system for water quality management and maintenance.

5.2.1.1 Challenges in conventional CEC detection for ecological risk characterisation and early warning

Routine surveillance for CECs during environmental water quality assessments is a complex and challenging task. There are several challenges that need to be addressed, including:

- High costs to perform routine analysis. Conventional CEC detection, mainly using LC-MS or GC-MS, require a great deal of sample clean-up and pre-concentration to account for sample matrix effects and the low concentrations in which CECs are sometimes found in the environment.
- Identifying the diverse range of organic pollutants found in a defined setting. CECs come from various natural and anthropogenic sources, including pharmaceuticals, personal care products, pesticides, and industrial by-products, and have a high degree of potential mixture interactions. This means that the effects of these chemicals on human and environmental health can be compounded, making it difficult to isolate the effects of individual contaminants. For example, areas with high agricultural activity may have a higher prevalence of pesticides and fertilizers in their water sources, while urban areas may have higher concentrations of personal care products and pharmaceuticals due to higher population density and wastewater discharge. This diversity of contaminants and their sources adds complexity to the surveillance and management of CECs.
- Limitations in timely risk characterization practices for early warning intervention. Risk characterization is a complex process that involves identifying and evaluating potential risks associated with exposure to a contaminant, including dose-response relationships and exposure pathways. However, many CECs have limited toxicological data, making it challenging to accurately assess their potential risks. In addition, the effects of long-term exposure to low levels of CECs are not well understood, further complicating risk characterization.

- Application of on-line sensing technologies in certain areas and/or the unavailability of analytical infrastructure and expertise to perform routine surveillance. Moreover, the diverse range of CECs means that different detection methods may be required for different contaminants, making the development of a universal early warning system challenging.

In conclusion, routine surveillance of CECs during environmental water quality assessments is a complex and challenging task but has shown promise to be improved with on-line technologies and machine learning that shows promise to enable a faster response in CEC risk management. However, various challenges should still be overcome that requires a multi-disciplinary approach involving toxicology, chemistry, environmental engineering, and data analytics, among others.

5.2.1.2 Challenges and limitations towards defined CEC on-line and real-time sensing

On-line sensing technologies have the potential to revolutionize routine surface water quality monitoring for CECs. These technologies provide real-time or near real-time data on water quality parameters, allowing for prompt action to be taken to protect human and environmental health. However, there are several challenges to developing and implementing on-line sensing technologies for specific CECs during routine surface water quality monitoring.

One of the main challenges is the diversity of CECs that may be present in surface water sources. Different CECs have different chemical properties, and some may be present in very low concentrations, making them difficult to detect using traditional on-line sensing technologies. Therefore, a wide range of sensors and detection methods may be required to detect different CECs, and these sensors must be sensitive enough to detect low levels of contaminants. Moreover, the complexity of CEC parent and transformation products limits the selection criteria for the suite of CECs that need to be prioritised. For example, many pharmaceutical and pesticide transformation products are known to present a much higher stability in water and solid matrices whilst having a higher physiological potency and toxicity than their parent counterparts. This can be even more prevalent in settings where wastewater treatment effluent is being discharged due to controlled biotic- and abiotic factors within these settings which can accommodate the conditions for CEC transformation into higher-risk pollutants. Such challenges in selecting the most appropriate surrogate CEC to define risk in a defined setting would thus need to be expanded to the target CECs transformation products, of which their current profiling in freshwater ecosystems is limited.

Another challenge is the complexity of the water matrix in surface water sources. Water from surface sources can contain a diverse range of organic and inorganic compounds, as well as particles and microorganisms, which can interfere with the detection of specific CECs. This means that on-line sensing technologies for specific CECs need to be able to distinguish between the target contaminants and other compounds in the water matrix. Additionally, the development and implementation of on-line sensing technologies require significant resources and infrastructure. The sensors themselves must be developed and validated, and a system for autonomous data gathering and visualisation must be established. Furthermore, the implementation of on-line sensing technologies requires a significant shift in water quality monitoring practices. For these reasons, changes to regulatory frameworks are required along with the adoption of alternative monitoring protocols that can serve as a first-tier profiling mechanism to identify CEC contamination risk.

5.2.1.3 The need for effect-based sensing technologies to account for complex water matrices.

Effect-based monitoring (EBM) is an innovative approach to water quality monitoring that uses biological assays to assess health risks associated with chemical pollutants in effluents or environmental water sources. Unlike traditional methods that rely on individual chemical identification, EBM can provide a more comprehensive and integrated view of the effects of chemical pollution on humans and wildlife (including aquatic ecosystems). By measuring the responses of living organisms such as fish or algae to water samples, or biomarkers representing key EBM can identify the presence of contaminants that may not be detectable by conventional methods. This can help to identify potential risks to aquatic life and human health, even in cases where individual chemical concentrations are below regulatory limits. EBM can also be used to identify emerging contaminants or unknown pollutants, which may not be covered by existing regulations. By providing a more holistic view of the health status of a river system, EBM can inform more effective management strategies and enable better protection of aquatic ecosystems and public health.

Figure 5.1 demonstrates the future IoT of EBM biosensor applications.

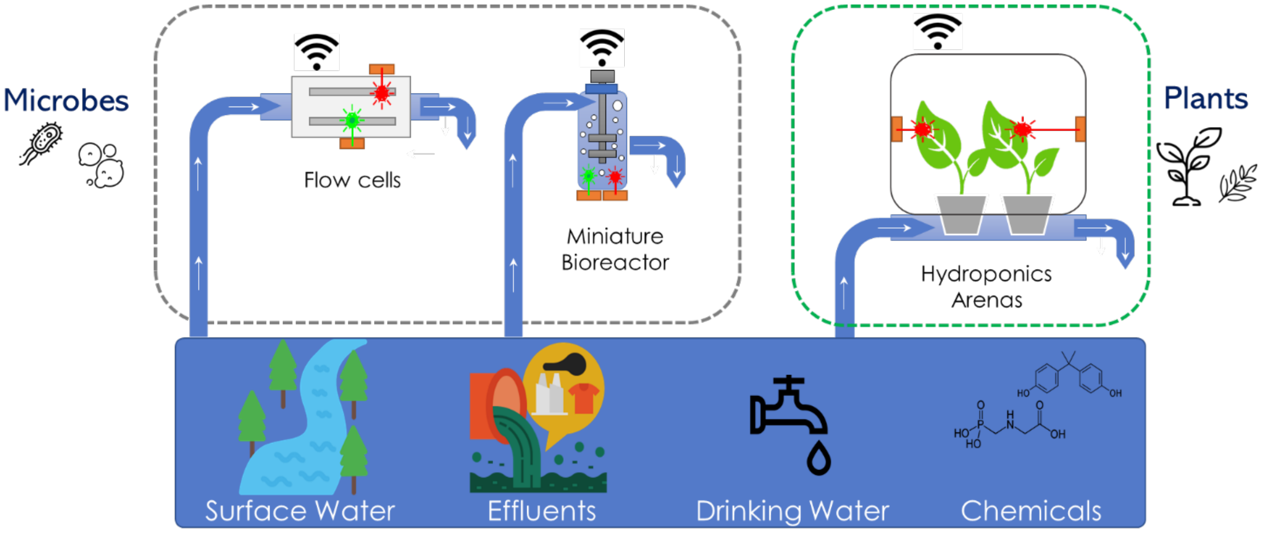

Figure 5.1: Future IoT of EBM biosensor applications

5.3 Using the BioTrac system as further development of the CEMS for early warning

5.3.1 BioTrac system

The maintenance of adequate water quality is of critical importance to various industries. Microbial fouling of water, surfaces or products is a universal problem that affects different sectors, including water purification and wastewater treatment, manufacturing, as well as food production and processing plants. The ability to monitor microbial fouling, both in terms of physical fouling of surfaces as well as metabolic activity, in a continuous, non-destructive and real-time manner could greatly benefit these sectors by providing a warning of deviation from optimal operational parameters. The BioTrac system achieves the monitoring of these microbial fouling parameters through the implementation of various IoT sensors and controllers, and is partly based on the technology and processes developed in the Wolfaardt research group and published in peer-reviewed scientific literature over the past decade (Bott et al., 1997; A. Dumitrache et al., 2013, 2015; A. D. Dumitrache et al., 2013; Eberl et al., 2017; Jackson et al., 2015, 2019; Klopper et al., 2019, 2020, 2023; Kroukamp & Wolfaardt, 2009; E. Ronan et al., 2021; P. Ronan et al., 2020; Stone et al., 2021; Stone, Kroukamp, Korber, et al., 2016; Stone, Kroukamp, McKelvie, et al., 2016; Stone, Kroukamp, Moes, et al., 2016).

BioTrac continuously analyses fluctuations of microbial parameters, due to changing environmental conditions, to establish the level of biofouling in a water source, system or process and can thus inform system operations and maintenance requirements in real-time. An example of the system output is illustrated in Figure 5.2 (adapted from Klopper et al. 2020).

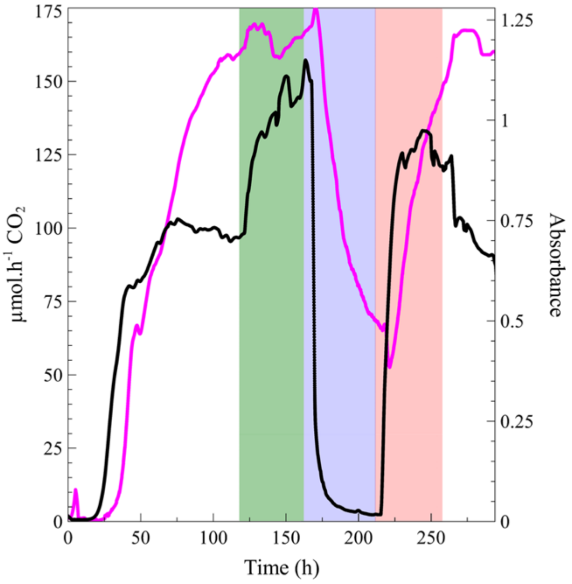

Figure 5.2: Metabolic activity (measured as CO_2 production rate, black line) and physical attachment (biomass, magenta line) of a *Pseudomonas aeruginosa* biofilm cultivated under flow during changing nutrient concentrations. White section (0 to 120h), biofilm development under low nutrient conditions. Green section (120 to 165h), exposure of established biofilm to high nutrient concentration. Purple section (165h to 215h), removal of nutrients and exposure of the biofilm to buffer only. Pink section (215 to 265h), addition of medium concentration of nutrients. White section (265h onwards), re-introduction of low nutrient concentration.

5.3.2 Monitoring the effect of CECs on microbial communities

Conventional approaches to the detection and quantification of CECs are generally not feasible for anything other than small/academic studies, due to various limitations. As previously discussed, LC-MS or GC-MS require specialised equipment, technical staff and require a great deal of sample processing prior to analysis. These methods have the capability to detect and quantify the concentrations of CECs but cannot inform the user with information on the potential biological effects of these compounds. In fact, the ultra-low concentrations of CECs detected are often below the concentrations where biological effects have been observed. Observable effects of CECs on microbiological communities are often only evident in the microgram to milligram per millilitre range, which is substantially higher than concentrations found in unpolluted water sources. As previously mentioned (Section 3), although the use of native microbial communities to monitor the effects of CECs is promising in principle, the stark reality is that CECs concentrations found in the environment are too low. This, coupled with the availability of more labile carbon sources in natural environments may dampen the effects of CECs on native microbial communities and limit their biodegradation.

The need to artificially concentrate CECs to levels where a biological effect can be observed further hinders the scalability of types of analyses. However, the potential to use microbial biofilms, which represent lower trophic levels, may facilitate CEC monitoring if these compounds bio-accumulate in biofilm biomass over time, leading to *in-situ* concentration and the potential of eliciting a biological effect.

5.3.3 BioTrac as a warning system

The ability of BioTrac to monitor the response of sessile microbial communities in real-time may facilitate its use as a warning system for the intentional or unintentional release of industrial effluent into water bodies. Further expansion of the system can leverage the real-time monitoring ability of BioTrac for a type of wastewater forensics, by coupling a warning signal to initiation of water collection by in-line auto samplers for subsequent analysis by conventional and advanced laboratory-based systems (e.g. LC-MS). This may allow better monitoring of water sources and limiting the impact of pollution events. Figure 5.3 illustrates the typical response of microbial communities to the influx of foreign compounds as detected by BioTrac.

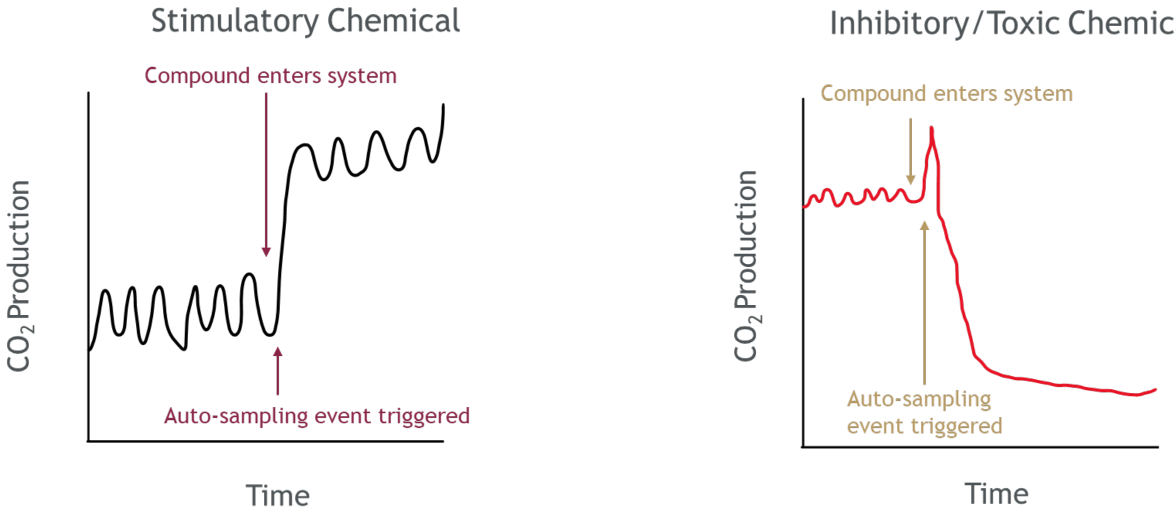

Figure 5.3: Stimulation or inhibition of microbial respiration (CO_2 production), as detected in real-time by BioSpec can trigger auto-sampling of the water source for subsequent laboratory analyses.

5.3.4 Future Developments

The expansion of the current BioTrac system into effects-based monitoring (EBM) and waste-water forensics (WWF) is currently underway. The integration of existing effects-based screening tools (yeast estrogen screen, etc.) with the BioTrac platform may allow for enhanced monitoring of specific effects. Additionally, the expansion of the BioTrac system to allow for monitoring and water sampling in the event of a warning signal, can expand the system to better understand the timing, origins and risks of accidental pollution or intentional dumping events.

CHAPTER 6: KNOWLEDGE TRANSFER AND CAPACITY BUILDING

6.1 Introduction

Knowledge dissemination to the water sector took place by means of two workshops that were presented during the execution of the project, viz. a first virtual workshop that was presented in August 2021, and a second workshop that was presented in Durbanville in February 2023. The outcomes of the workshops are described briefly below, with more details of the workshops provided in Appendices A and B of the report.

6.2 Workshop 1: Knowledge dissemination

A virtual inception and knowledge dissemination workshop was held on **19 August 2021** to introduce the topic of real-time sensing as alert system for substances of concern. Invitations were sent to all relevant stakeholders and role players in the water and wastewater industry. The workshop was held on Teams and a total number of 69 persons attended the workshop/webinar.

The workshop has as its objective presentations on the scope, aims and methodology of the project. A general discussion and Q&A session was then held to obtain the inputs of the stakeholders on the proposed aims and methodology of the project. No specific contributions were made, therefore indicating that no significant changes will be made to the methodology, and the project can therefore continue with the aims and methodology as contained in the original proposal.

The workshop programme and attendance list can be found in Appendices A1 and A2 of this report.

6.3 Workshop 2: Feedback and Findings

A workshop was held on **17 February 2023** at the Durbanville Hills Winery during which feedback on the findings of the project was provided to stakeholders, end-users, suppliers, researchers and other role-players with interest in the field, and discussions was held on available sensors and end-user experiences with sensor systems. A group photo of the workshop attendees is shown in Figure 6.1.

The workshop was divided in two parts. The morning session consisted of presentations on the project scope, aims, methodology and findings, as well as presentations on the need for early warning systems and the way forward with effect base monitoring. A key speaker, Dr Bill Harding, also presented on why Sensors makes sense. In the afternoon a discussion session was held on research, development, experiences, trends and needs for smart systems on real-time sensors and detection systems for pollutants in water sources.

The workshop programme and attendance list can be found in Appendices B1 and B2 of this report.

Figure 6.1: Participants of the Feedback of Findings workshop held on 17 February 2023

6.4 Capacity building

Mr JHL Bröcker registered for his PhD in microbiology (Stellenbosch University, department of Microbiology) in January 2021 and is expected to hand in his dissertation in December 2023, in time for March 2024 graduation at Stellenbosch University. As part of his PhD, a chapter focusses on the occurrence and persistence of chemical of emerging concern (CEC) in aquatic environments. The work he did as part of this project involved the use of the CEMS for CEC detection.

Ms C Lourens registered for her Master's in Chemical Engineering (Stellenbosch University, Department of Process Engineering) in January 2020 and handed in her final dissertation in January 2023. As part of the project, her master's project investigated the potential of near-infrared spectroscopy for the detection of low-level CECs in environmental waters. The results and findings of her studies are included as a chapter in this project report (Chapter 4).

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 Carbon dioxide evolution measurement system (CEMS)

While experimental data showed that high CEC concentrations inhibit microbial metabolism as indicated in the Progress Report, CEC concentrations, up to 300 times of what is frequently detected in South African wastewater effluent had no impact on the metabolic rate of the indigenous biofilms.

This result showed that environmental CEC concentrations are too low to impact microbial metabolism, which underscores the high degree of metabolic redundancy of microbial communities i.e., extensive gene and species pool enable self-regulation to continuously adjust to available nutrients and inhibitors.

7.2 NIR Spectroscopy

NIR spectroscopy combined with multivariate data analysis has shown promising potential for detecting contaminants of emerging concern at mg/L and even high µg/L levels. However, implementation of the method as a (near)-real time early warning system for detecting CECs at environmental concentrations (at ng/L levels) is not yet a practical possibility.

Further relevant research should certainly be pursued, since the application of this technique as a complimentary early warning system for municipal water and wastewater treatment plants (as well as other water reclamation and reuse plants), could enable easier and more cost-effective monitoring programmes at such plants.

7.3 Other real-time sensors

The use of biosensors for the screening of CECs have advanced considerably in the past two decades and includes both targeted (single chemical) and effect-based (combined biological activity) approaches. Effect-based methods, representing key biological modes of action as proxy for health risks, can provide integrated estimates of hazardous chemical burdens. When considering the sheer number of chemicals potentially present in effluent, drinking water or environmental samples, targeted screening through analytical chemistry, or single chemical biosensors is impractical, and effect-based methods are preferred.

Although real-time effect-based biosensors are not currently available, such technology may likely be the future of water quality monitoring. Increased investment and research efforts are needed for the development of effect-based biosensor devices representing key health risks. Biosensor devices utilizing engineered microbes are promising vehicles for effect-based sensing and could enable remote deployment and routine testing at a fraction of the cost of analytical chemistry methods.

CHAPTER 8: WAY FORWARD FOR REAL-TIME WATER SENSING

8.1 Introduction

The number of chemicals classified as hazardous is increasing annually, with the European Chemical Agency (ECHA) already regulating more than 4000 chemicals in 2023 and the US Environmental Protection Agency (USEPA) having more than 85000 listed chemicals on the Toxic Substances Control Act (TSCA). As a result, the comprehensive monitoring of water quality in relation to micropollutant and other hazardous substance burdens is becoming more of a challenge. Analytical chemistry is becoming impractical due to the sheer amount of potentially hazardous substances present and the associated human resource and economic cost requirements for the analyses. As an alternative, biological assays can be applied to screen for the risks of specific adverse health or adverse ecological effects associated with chemicals present in a particular sample providing an integrated estimate of the hazardous chemical burden (Brack et al., 2019). In fact, effect-based methods (EBMs) featuring biological assays are advocated as the norm for the testing of complex environmental samples such as effluents, surface, or groundwater for potentially hazardous chemicals (including chemicals of emerging concern) (Brack et al., 2019; Kruger et al., 2022; Neale et al., 2022; Wernersson et al., 2015).

Effect-based methods include laboratory *in vitro* or *in vivo* bioassays representing individual- or a collection of chemical modes of action (MOAs) or toxicity parameters. Such biological assays have been applied for decades in chemical hazard assessments or for the testing of environmental samples (Laroche et al., 1970). However, the majority of assays being proposed as part of EBM frameworks in the developed world require highly trained personnel and are costly to perform (e.g. mammalian cell reporter gene assays) (Brack et al., 2019; Robitaille et al., 2022; Wernersson et al., 2015).

Microbes including bacteria, fungi and microalgae have been genetically engineered to sense potential health risks to humans or other organisms associated with chemical exposure (Ma et al., 2022). Such microbial biosensors can represent specific molecular initiating- or key events within known adverse outcome pathways (Ankley et al., 2010), hence enabling the identification of hazard classes of chemicals (e.g. endocrine disruptors, carcinogens, neurotoxins, etc.) and constituting EBMs. Microbes have been applied as part of remotely deployed or static inline real-time toxicity testing digital devices (Wlodkowic & Karpinski, 2021). Although devices with the capacity to sense adverse biological activity associated with chemical exposure in real-time and not simply toxicity, is yet to be developed, engineered microbes appears to be promising candidates for the purpose (Ma et al., 2022).

8.2 The way forward for real-time effect-based early warning systems

8.2.1.1 EBM Testing Frameworks of relevance to CECs

The European Commission (EC) Water Framework Directive (WFD, 2000/60/EC) and Groundwater Directive (GWD, 2006/118/EC) function to protect surface waters and groundwater in the European Union. The guidelines feature both ecological and chemical status criteria to assess the quality of water resources. As part of the chemical status assessment, the EC WFD currently include a list of 45 priority substances with environmental quality standards (EQS) described for each compound, representing guideline limits for compliance purposes (Directive 2013/39/EU – ANNEX1). The existing priority substances list consists predominantly of banned legacy chemicals, classified as persistent organic pollutants (POPs), including organochlorine insecticides p,p'DDT, aldrin and endosulfan, industrial chemicals such as the brominated flame retardant HBCDD, perfluorooctane sulfonic acid (PFOS), and a number of polycyclic aromatic hydrocarbons (PAHs). Selected non-banned substances are also listed as priority including the herbicides atrazine and simazine. Surprisingly, no pharmaceuticals are currently classified as priority by the EC WFD.

In October 2022 proposed changes to the EC WFD and GWD were published which included the addition of 23 compounds to the priority substances list. The revised list include a number of pharmaceuticals, additional fluorinated compounds (apart from PFOS), and other well-known CECs such as the steroid hormone estradiol, the plasticiser bisphenol-A, the herbicide glyphosate, and the biocide triclosan. Notably, the amended WFD would require member states to apply effect-based methods (EBM) to screen for estrogenicity but no other health hazard categories. The move to include EBM is not a surprise seeing that the need for such an approach to monitoring has been advocated for nearly a decade by leaders in the field of environmental toxicology (Wernersson et al., 2015). In fact, the EC-funded SOLUTIONS project unequivocally demonstrated the need for EBM to monitor complex samples such as effluents, groundwater, surface water and even drinking water (Brack et al., 2019). Nonetheless, although the amendment to the water protection directives includes EBM, environmental quality standard (EQS) values to function as compliance guidelines will not be required yet. The EC proposes the amendment of the WFD to “require Member States to carry out effect-based monitoring to assess the presence of estrogenic hormones in water bodies, in view of possible future setting of effect-based trigger values”. This implies that EBM efforts will simply be data collection and further exploration of the need for EBM for monitoring in future. The hesitancy to enforce the use of EBMs and EQS as part of regulation, and in particular as proposed for the revised WC WFD, has been criticised due to the obvious advantages of EBMs for monitoring (Backhaus, 2023). The inclusion of only a single mechanism of action as part of EBM, i.e., estrogenicity, is surprising considering the reality of other potential adverse risks associated with chemical exposure.

The Global Water Research Coalition (GWRC) is one of the leaders in characterizing EBM frameworks for the monitoring of drinking water, effluents and environmental waters with the end goal of promoting EBM as part of regulation. The GWRC have published a number of documents as part of the "Effect Based Monitoring in Water Safety Planning" programme including fact sheets for water operators and legislators (GWRC, 2023b, 2023a; P. Neale et al., 2020; P. A. Neale et al., 2022). The recent publications represent a significant step forward for the promotion and characterization of EBM as part of future regulation and policies related to water quality. The prescribed endpoints suggested by the GWRC are estrogen receptor (ER – estrogenicity), aryl hydrocarbon receptor (AhR) activation and oxidative stress (OS) for recycled water and ER, AhR, oxidative stress and genotoxicity for drinking water. These endpoints constitute chemical modes of action and collectively represent adverse health risks (Figure 8.1). The South African Water Research Commission (WRC) was a founding member of the GWRC in 2002 and remains one of the core 12 global entities in the alliance. The WRC is therefore involved with the GWRC EBM characterization and implementation efforts and funds local projects related to EBM performed in collaboration with the GWRC such as the current C2020/2021-00348 project entitled "Implementation of effect-based methods for water quality assessment". The funding of EBM research is of critical value to build capacity and increase awareness of the value of the approach for water quality monitoring in South Africa.

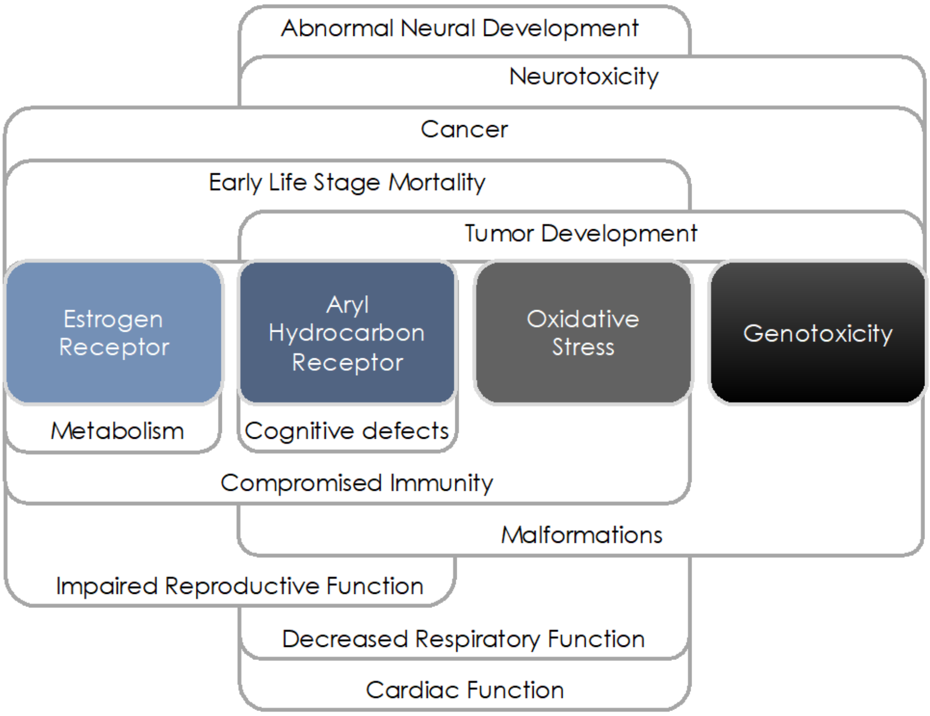

Figure 8.1. Key "effects" representing chemical modes of action potentially leading to adverse health risks identified by the Global Water Research Coalition to be included in effect-based method testing frameworks.

The only governmental authority presently enforcing the use of EBM as part of regulation related to water quality is the State of California, USA. In particular, the State Water Board (SWB) Recycled Water Policy (effective on April 8, 2019) specifies the screening for ER and AhR activation in recycled water. Evidently California has taken the lead globally in terms of EBM-based monitoring and have produced detailed guidelines specifying sample collection, processing and testing through EBMs to aid water operators (WateReuse, 2020).

Although the California SWB is presently the only authority requiring effect-based screening, and the European Commission possibly in the near future should the amendment to the WFD be accepted, other territories will most likely follow suite in the coming years due to economic and practical advantages of EBM compared to analytical chemistry approaches, and growing water security concerns. However, the *in vitro* assays applied as part of EBM-based monitoring are expensive and require skilled personnel to perform. Conventional assays are furthermore not compatible with IoT devices and real-time applications. Engineered microbes represent a promising alternative to conventional cell based assays for effect-based testing because such organisms can report chemical MOAs relevant to humans and other vertebrates such as the effects prescribed by the GWRC (Figure 8.2). The characteristics of microbes such as ease of cultivation, viability after lyophilization, and low cost of production are further advantages over mammalian cell or whole organism assays, in support of application in deployable digital devices for effect-based monitoring.

Engineered microbes can produce signals detectable by digital sensors in response to chemical exposure. The four broad categories of reporting technologies compatible with engineered microbes include enzymatic reactions, fluorescence, luminescence and amperometric (Figure 8.2). Such reporters can be coupled to molecular sensing cascades and provide an indication of the presence of certain classes of chemicals. Digital sensors can be used to detect the activation of a reporter and therefore the presence of groups of chemicals that could potentially lead to adverse health effects (Figure 8.2).

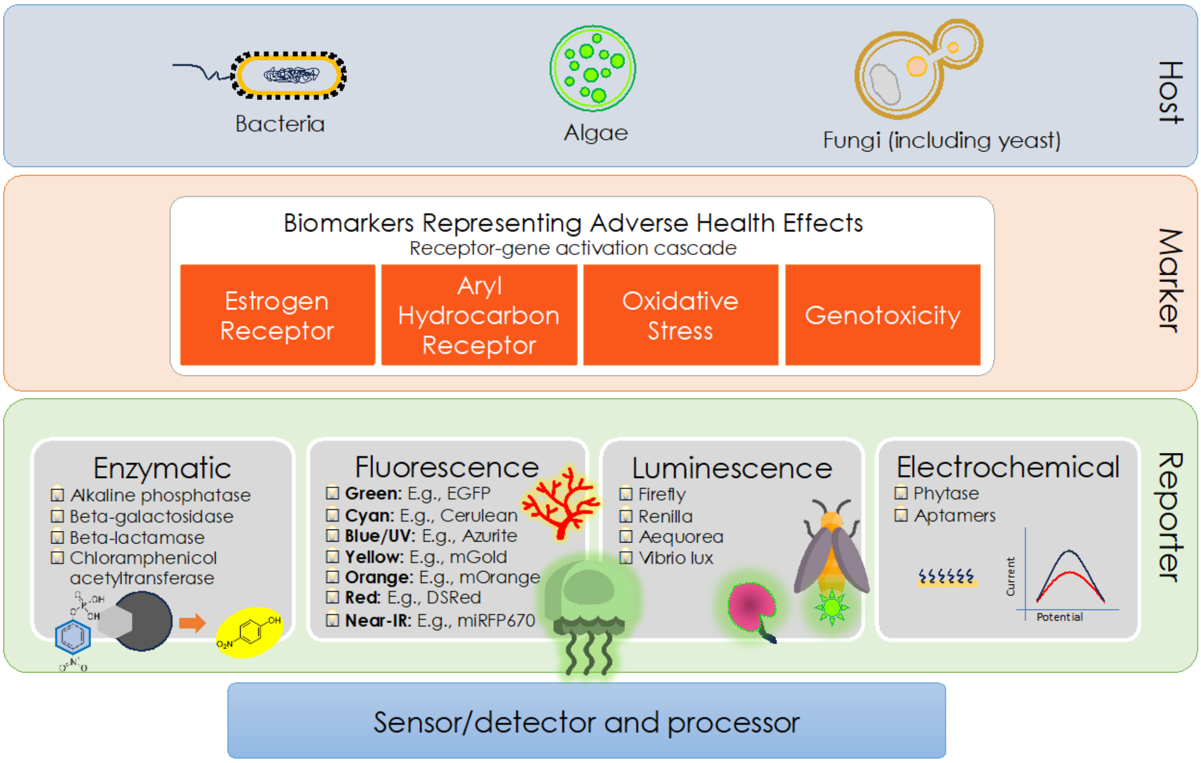

Figure 8.2. Application of engineered microbes for effect-based monitoring of water quality as part of digital sensor devices. The biomarkers included are based on the testing framework proposed by the Global Water Research Coalition (GWRC) for drinking water and effluents.

8.2.1.2 Existing genetically engineered microbial biosensors

To date various genetically modified microbes have been developed for the screening of environmental chemicals including CECs (Reviewed by Ma et al., 2022). The host organisms currently applied for chemical sensing include bacteria, fungi (including yeast) and algae (Figure 8.2). For example, David Wood and colleagues developed *E. coli* strains with the capacity to sense for estrogen receptor, androgen receptor, thyroid receptor and peroxisome proliferator-activated receptor gamma activity respectively (Gierach et al., 2012; Li et al., 2011; Skretas et al., 2007). The microbes applied by Wood et al. features intern circuits to sense chemical MOAs relevant to humans seeing that vertebrate transcription factors cannot be functionally expressed in procaryotes due to differences in cellular machinery compared with eukaryotes. A further example of recombinant bacteria with the capacity to screen for adverse health effects is an *E. coli* strain with the capacity to detect DNA damage (Chen et al., 2021). Recombinant yeast is a more common host than bacteria to represent vertebrate pathways (Reviewed by Wahid et al., 2023). Pham et al. (2012) developed the EstraMonitor featuring recombinant yeast containing an amperometric reporter activated by estrogenic substances. Further examples include yeast with the capacity to sense AhR agonists (Leskinen et al., 2008), oxidative stress (Sharma et al., 2015), and thyroid receptor binding (Shi et al., 2012). Deployable handheld devices to screen for estrogenicity using recombinant yeast has also been developed (Lobsiger et al., 2019). Microalgae has mostly been applied for the sensing herbicides such as diuron, or metals, but not to date to sense for MOA included in the GWRC framework such as estrogenicity (Reviewed by Ma et al., 2022). The field of algae synthetic biology has however advanced in the past decade and some examples include the MoClo golden gate cloning toolkit made available by the Chlamydomonas Resource Centre (University of

Minnesota) which includes parts for various fluorescent proteins, promoters, terminators, selectable markers among others (Crozet et al., 2018).

The potential application of engineered microbes as biosensors extend beyond the previously mentioned endpoints identified by the GWRC (i.e., ER, AhR, OS and mutagenicity), and for example microbes have been produced to detect the presence of hazardous metals including arsenic, cadmium and mercury among others (Ali et al., 2021; Ma et al., 2022). A further promising application of engineered microbe biosensors is in the medical diagnostics setting for the diagnosis of infections, clinical abnormalities (Amrofell et al., 2020; Chang et al., 2021), and most recently cancer tumours (Cooper et al., 2023).

8.3 Microbial biosensors as part of water quality monitoring frameworks and future research needs

Commercially available real-time inline sensors providing data comparable to that generated using bioassays are limited to the AlgaeOnlineAnalyzer, PhycoSense, AlgaeGuard, AlgaeToximeter II, Daphnia Toximeter II, ToxProtect II and Fish Toximeter II (BBE Moldaenke, DE), the benthic invertebrate based SensaGaurd (REMONDIS Aqua, DE), and the MosselMonitor (AquaDect, NL) (Bownik and Wlodkowic, 2021; Kizgin et al., 2023; Moldaenke et al., 2019). These products can provide toxicity data and can effectively function as early warning systems for compromised water quality, but does not test for CECs or other hazardous chemicals per se, with some exceptions such as selected herbicides (Kizgin et al., 2023). Nonetheless, sophisticated instruments such as the AlgaeToximeter II are likely the future of water quality monitoring, especially with the aid of artificial intelligence applications to improve data capture and interpretation. The real-time algae sensors produced by BBE Moldaenke are the only microbe-based technology commercially available, but does not utilize engineered microbes, although it can be expected in future.

Wastewater treatment works are major sources of pharmaceuticals and other CECs in the environment. Although pharmaceuticals are not routinely monitored by the public sector in surface and groundwater in South Africa, the loads of these substances in water resources have likely increased considerably in the recent decades due to WWTP inadequacies (Herbig, 2019). Failing infrastructure, limited capacity and mismanagement are some of the causes of inefficient wastewater treatment in South Africa, as is evident in the DWS Green Drop Reports. A further significant source of sewage pollution and therefore CECs in South African waters is runoff from informal settlements (Archer et al., 2023; Gqomfa et al., 2022; Morole et al., 2022). Not surprisingly, the data of the DWS National Microbial Monitoring Programme (NMMP) provides evidence of the increases in *E. coli* and faecal coliform numbers and hence sewage pollution of surface water in the past two decades, which in turn creates challenges for drinking water operators extracting water from polluted sources. In fact, the presence of pathogens originating from sewage in drinking water is a reality in many parts of South Africa (Luvhimbi et al., 2022) as is evident in the DWS Blue Drop Reports. The skills required and costs involved with advanced water quality assessment such as liquid chromatography mass spectrometry (LCMS) or bioassay testing are likely the two major confounding factors limiting the application of these methods for routine monitoring in developing countries such as South Africa. For example, SANS241, regulating drinking water quality, does not include EBM and the most recent draft standards only a limited number of CECs. Nonetheless, the reality remains that the quality of surface, groundwater and drinking water in South Africa is deteriorating and increased efforts are needed for pollution surveillance to protect public health and ecosystems. Cost effective technology for monitoring hazardous chemicals could be the solution needed for water quality surveillance related to hazardous chemical pollution.

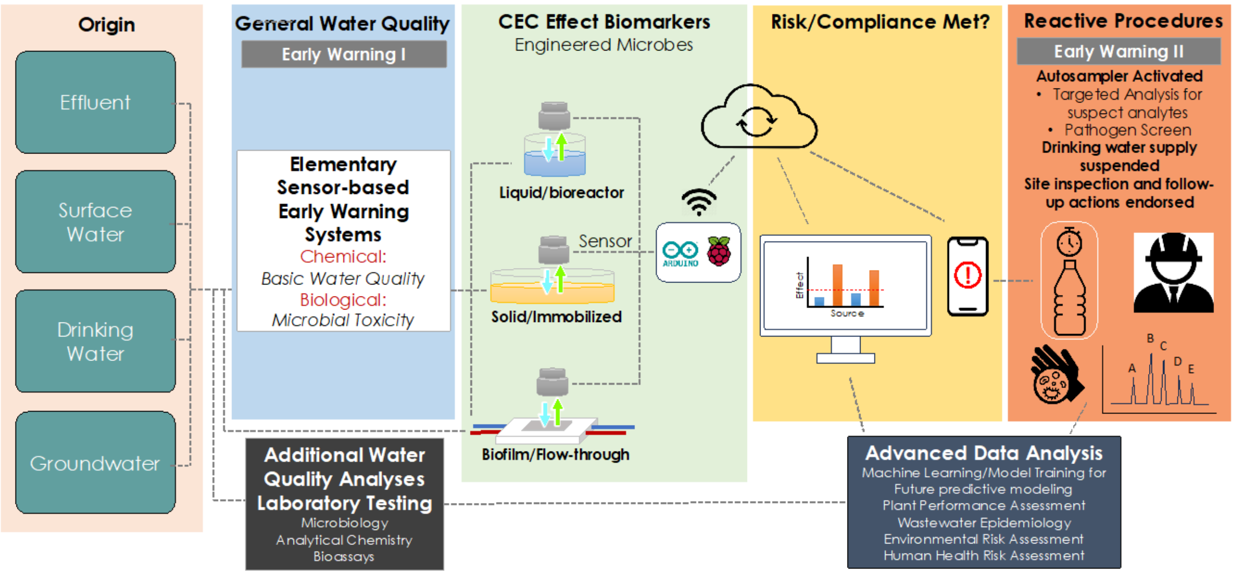

Figure 8.3. Framework for future water quality monitoring utilizing real-time sensors, engineered microbe effect-based methods, and IoT devices.

Real-time effect-based sensors to screen for key chemical modes of action (such as the endpoints prescribed in the GWRC EBM framework) are yet to be developed but may be a reality in coming years. Future research efforts should include the development of digital devices harnessing the advantages of engineered microbes for effect-based monitoring. Such devices can form part of chemical pollution surveillance programmes to monitor surface water, groundwater, effluents, recycled water and drinking water (Figure 8.3). Automated devices that can replace costly laboratory tests for water quality monitoring, will contribute to water security, inform and aid conservation efforts related to aquatic organisms, and preserve human health. Water quality frameworks could feature two phases of early warning based on defined thresholds utilizing (1) basic water quality parameters assessed using conventional sensors and (2) effect-based screening using engineered microbe biosensor IoT devices (Figure 8.3). Continuous data capture can be applied for machine learning to provide site-specific personalized profiles to predict health hazards and non-compliance to EBM guideline limits based on water quality parameters apart from effect-based testing.

To our knowledge, only a single research group in South Africa is currently developing genetically engineered microbes to be applied as part of biosensor platforms. There is a need for future investment directed at biosensor development in the local context because such technology falls within the fourth industrial revolution sphere and has the potential to revolutionize water quality monitoring. The application of microbial biosensors for medical diagnostics is a further incentive for the promotion of increased research efforts in the local setting.

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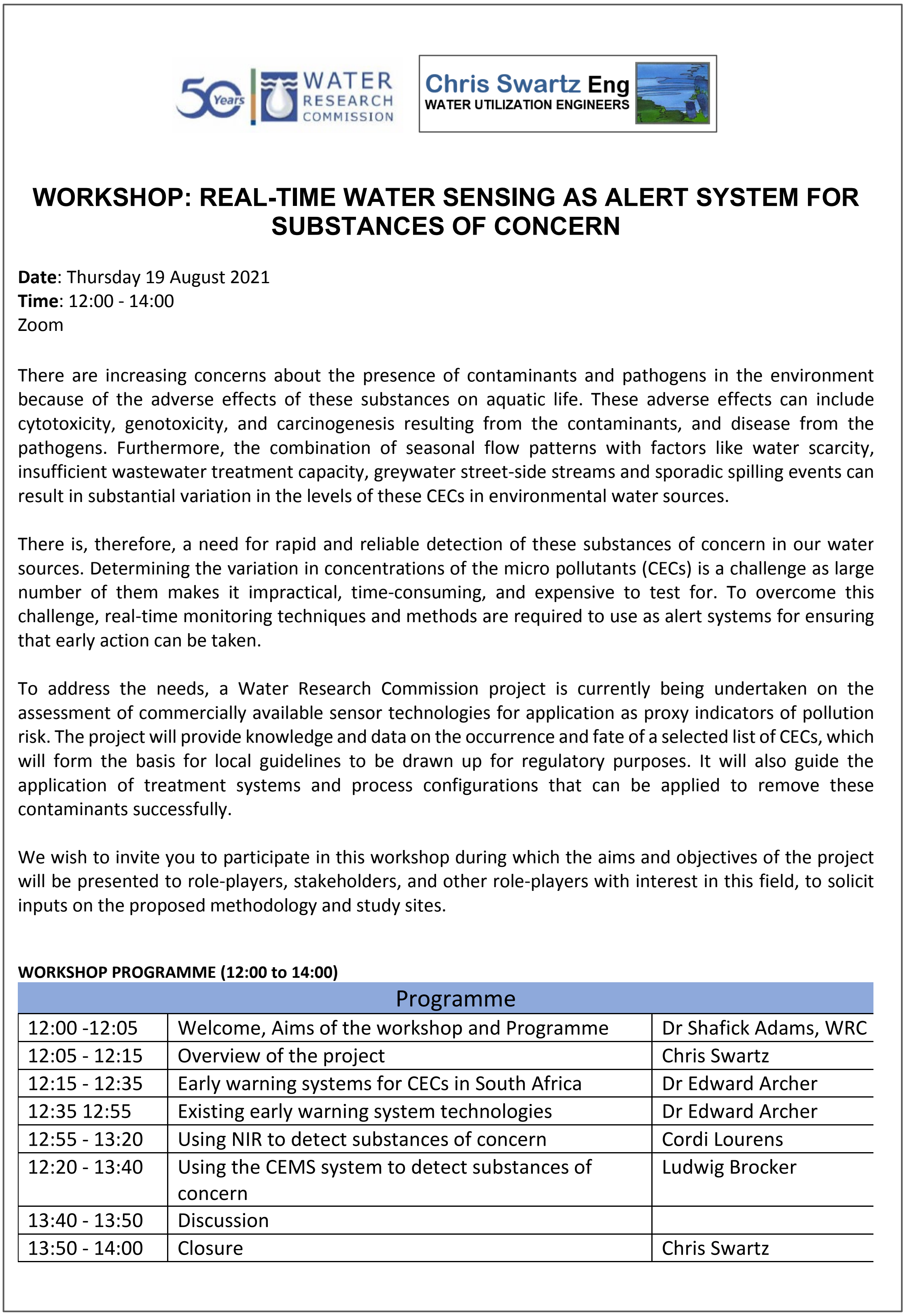
APPENDICES

Appendix A: Workshop 1

Appendix A1: Programme



50 Years WATER RESEARCH COMMISSION

Chris Swartz Eng
WATER UTILIZATION ENGINEERS

WORKSHOP: REAL-TIME WATER SENSING AS ALERT SYSTEM FOR SUBSTANCES OF CONCERN

Date: Thursday 19 August 2021
Time: 12:00 - 14:00
Zoom

There are increasing concerns about the presence of contaminants and pathogens in the environment because of the adverse effects of these substances on aquatic life. These adverse effects can include cytotoxicity, genotoxicity, and carcinogenesis resulting from the contaminants, and disease from the pathogens. Furthermore, the combination of seasonal flow patterns with factors like water scarcity, insufficient wastewater treatment capacity, greywater street-side streams and sporadic spilling events can result in substantial variation in the levels of these CECs in environmental water sources.

There is, therefore, a need for rapid and reliable detection of these substances of concern in our water sources. Determining the variation in concentrations of the micro pollutants (CECs) is a challenge as large number of them makes it impractical, time-consuming, and expensive to test for. To overcome this challenge, real-time monitoring techniques and methods are required to use as alert systems for ensuring that early action can be taken.

To address the needs, a Water Research Commission project is currently being undertaken on the assessment of commercially available sensor technologies for application as proxy indicators of pollution risk. The project will provide knowledge and data on the occurrence and fate of a selected list of CECs, which will form the basis for local guidelines to be drawn up for regulatory purposes. It will also guide the application of treatment systems and process configurations that can be applied to remove these contaminants successfully.

We wish to invite you to participate in this workshop during which the aims and objectives of the project will be presented to role-players, stakeholders, and other role-players with interest in this field, to solicit inputs on the proposed methodology and study sites.

WORKSHOP PROGRAMME (12:00 to 14:00)

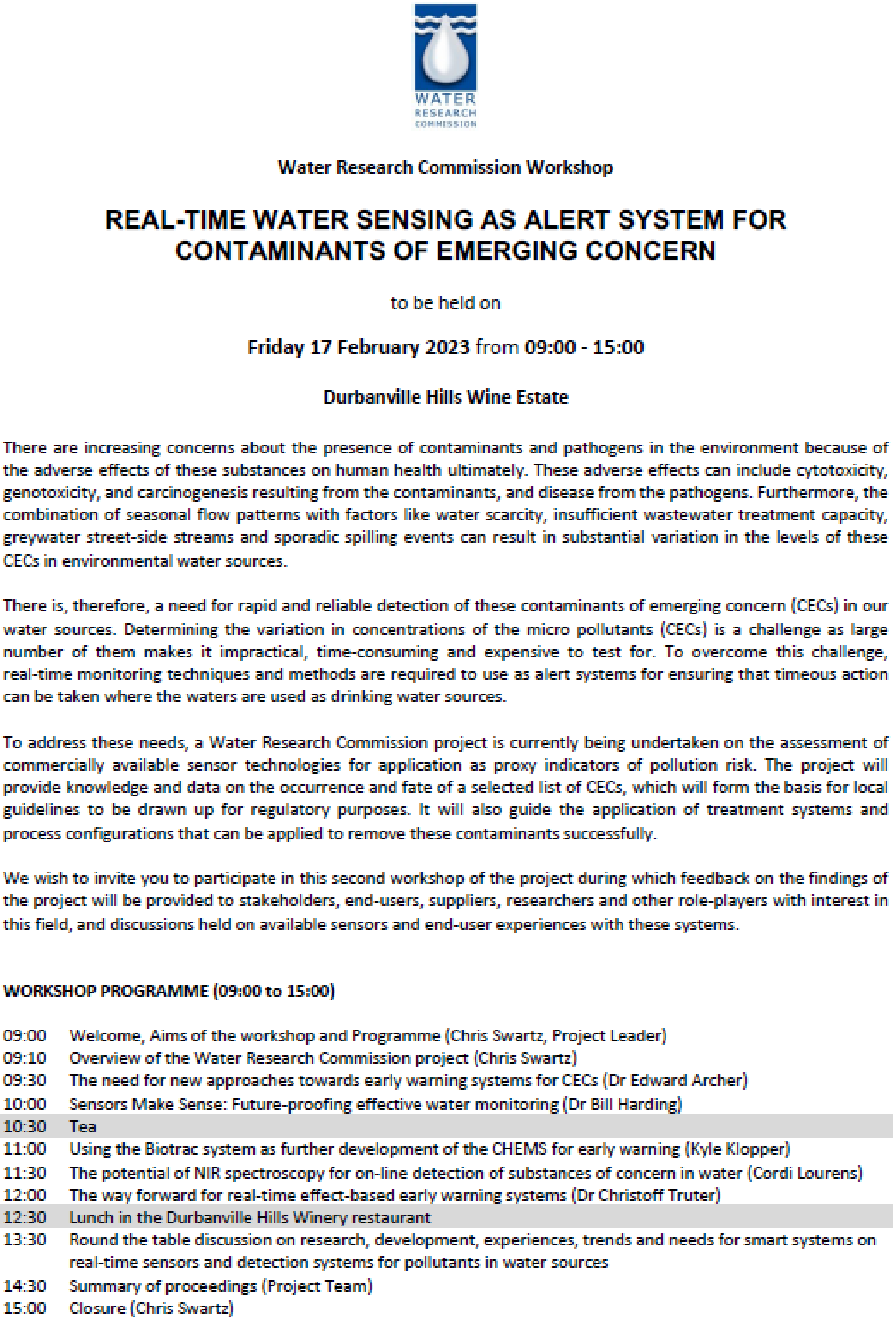
Programme		
12:00 -12:05	Welcome, Aims of the workshop and Programme	Dr Shafick Adams, WRC
12:05 - 12:15	Overview of the project	Chris Swartz
12:15 - 12:35	Early warning systems for CECs in South Africa	Dr Edward Archer
12:35 12:55	Existing early warning system technologies	Dr Edward Archer
12:55 - 13:20	Using NIR to detect substances of concern	Cordi Lourens
12:20 - 13:40	Using the CEMS system to detect substances of concern	Ludwig Brocker
13:40 - 13:50	Discussion	
13:50 - 14:00	Closure	Chris Swartz

Appendix A2: Attendance List

Surname and Name	Surname and Name
Adams, Shafick	Mzimela, Amanda
Aderemi, Banjo Ayoade	Ndou, Innocent
Archer, Edward	Ntshangase, Senamile
Bachoo, Santosh	Paepae, Thulane
Botha, Claudia	Parak, Omar
Bröcker, Ludwig	Phafuli, Lialuma
Bux, Faizal	Ramaliba, Gumani
Charles, Kenny	Razanamahandry, Lovasoa Christine
Daniel, Lawrence	Rebelo, Alanna
de Souza, Philip	Reddick, Jane
Dlamini, Sanele	Rhoda-Baderoen, Firdous
Fennemore, Chris	Robbertse, Jean-Jacques
Genthe, Bettina	Sehume, Onalenma
Hadi, Mahdi	Seihleko, Jethrone
Hendricks, Rahzia	Sekgoele, Thato
Hodgson, Kim	Sekgota, Khethobole
Hoosain, Nirseen	Shete, Sebolelo
Jonginamba, Unathi	Sibiya, Muzi
Kanini, Dephney	Surujlal-Naicker, Swastika
Khumalo, Nqubeko	Swartz, Chris
Kloppers, Wilna	Thamae, Seeng
Kotze, Hennie	Topkin, James
Kumunda, Coster	Tshithukhe, Getrude
Liefferink, Mariette	Tuffuor, Benedict
Lourens, Cordi	Ubomba-Jaswa, Eunice
Manzungu, Henry	van Blerk, Nico
Marais, Savia	Voges, Melanie
Menge, Jürgen	Volschenk, Mercia
Mkhonza, Mdu	Walingo, Noloyiso
Moodie, Elzanne	Walingo, Tom
Mothibi, Tsholofelo	Wolfaardt, Gideon
Muller, Nikite	Zulu, Zama
Muthraparsad, Namisha	Zuma, Lungi

Appendix B: Workshop 2

Appendix B1: Programme



WATER RESEARCH COMMISSION

Water Research Commission Workshop

REAL-TIME WATER SENSING AS ALERT SYSTEM FOR CONTAMINANTS OF EMERGING CONCERN

to be held on

Friday 17 February 2023 from **09:00 - 15:00**

Durbanville Hills Wine Estate

There are increasing concerns about the presence of contaminants and pathogens in the environment because of the adverse effects of these substances on human health ultimately. These adverse effects can include cytotoxicity, genotoxicity, and carcinogenesis resulting from the contaminants, and disease from the pathogens. Furthermore, the combination of seasonal flow patterns with factors like water scarcity, insufficient wastewater treatment capacity, greywater street-side streams and sporadic spilling events can result in substantial variation in the levels of these CECs in environmental water sources.

There is, therefore, a need for rapid and reliable detection of these contaminants of emerging concern (CECs) in our water sources. Determining the variation in concentrations of the micro pollutants (CECs) is a challenge as large number of them makes it impractical, time-consuming and expensive to test for. To overcome this challenge, real-time monitoring techniques and methods are required to use as alert systems for ensuring that timeous action can be taken where the waters are used as drinking water sources.

To address these needs, a Water Research Commission project is currently being undertaken on the assessment of commercially available sensor technologies for application as proxy indicators of pollution risk. The project will provide knowledge and data on the occurrence and fate of a selected list of CECs, which will form the basis for local guidelines to be drawn up for regulatory purposes. It will also guide the application of treatment systems and process configurations that can be applied to remove these contaminants successfully.

We wish to invite you to participate in this second workshop of the project during which feedback on the findings of the project will be provided to stakeholders, end-users, suppliers, researchers and other role-players with interest in this field, and discussions held on available sensors and end-user experiences with these systems.

WORKSHOP PROGRAMME (09:00 to 15:00)

09:00	Welcome, Aims of the workshop and Programme (Chris Swartz, Project Leader)
09:10	Overview of the Water Research Commission project (Chris Swartz)
09:30	The need for new approaches towards early warning systems for CECs (Dr Edward Archer)
10:00	Sensors Make Sense: Future-proofing effective water monitoring (Dr Bill Harding)
10:30	Tea
11:00	Using the Biotrac system as further development of the CHEMS for early warning (Kyle Klopper)
11:30	The potential of NIR spectroscopy for on-line detection of substances of concern in water (Cordi Lourens)
12:00	The way forward for real-time effect-based early warning systems (Dr Christoff Truter)
12:30	Lunch in the Durbanville Hills Winery restaurant
13:30	Round the table discussion on research, development, experiences, trends and needs for smart systems on real-time sensors and detection systems for pollutants in water sources
14:30	Summary of proceedings (Project Team)
15:00	Closure (Chris Swartz)

Appendix B2: Attendance List

NAME	SURNAME	AFFILIATION
Chris	Swartz	CSWUE
Cordi	Lourens	CSWUE
Dr Christoff	Truter	Stellenbosch University
Kyle	Klopper	Stellenbosch University
Dr Swastika	Surujlal-Naicker	City of Cape Town
Hennie	Kotze	City of Cape Town
Louis	Koen	Zutari
Willem	Naudé	Veolia
Dr Kirsty	Carden	Future Water UCT
Dr Bill	Harding	DHE Consulting
Linda	Potgieter	DHE Consulting
André	Kowalewski	Drakenstein Municipality
Dr Jac	Wilsenach	Virtual Consulting
Neil	van Kooten	Integral Labs
Dr Elanna	Bester	Stellenbosch University
Dr Tom	Murry	Stellenbosch University
Danelle	Botha	Stellenbosch University
Bettina	Genthe	Private Consultant
Ruchelle	Schneuwly	UCT
Dr Edward	Archer	Macquarie University, Sydney, Australia
James	Topkin	ERWAT
Jürgen	Menge	Innovative Research for Water Solutions
Prof Gideon	Wolfaardt	SUWI
Dr Marelize	Botes	SUWI