WATER QUALITY

New research takes a step towards real-time testing for chemicals of concern

Over 350 000 chemicals and mixtures are registered for commercial use across the globe today. They are used to make a broad range of products that we use in our everyday lives, including pesticides, pharmaceuticals, personal care products, flame retardants, surfactants and various forms of plastic. Traces of these end up in our water, which, as a result, contains a complex cocktail of chemical contaminants and their transformation products. Article by Petro Kotzé.

Though mostly present in low concentrations, the potential negative effects of these potentially hazardous contaminants on both people and the environment are a growing concern. It is now generally accepted that even at low levels, cytotoxic, genotoxic, and carcinogenic substances could damage cells and organisms, with a range of potential impacts including various forms of cancers.

However, micropollutants – generally considered to be chemicals that can pose a risk to lifeforms at microgram per litre or lower concentrations – are not yet commonly monitored in the environment internationally, and existing water quality regulations, including in South Africa, do not currently cover them.

The problem, says Chris Swartz of Chris Swartz Water Utilization Engineers, is that these chemicals mostly occur at very low concentrations, and to test for each of them individually is not only difficult but rarely practically possible. And, he adds, the tests are very expensive. Plus, Swartz points out that by the time you have taken a sample, taken it to the lab for chemical analysis, and received the results, that volume of water already passed through the system, and has done the potential damage.

The challenge is something that South African researchers have been grappling with for some time. And there might be an answer. If we had something that measured the water quality online, in contrast to spot testing and chemical analysis, Swartz says, we could see in real-time on a digital meter what the values are. The first steps towards this aim are now firmly in place. With funding from the Water Research Commission, a group of researchers from the Stellenbosch University Water Institute (SUWI), under the leadership of Swartz in this research project, have developed a framework for future water quality monitoring that takes the mentioned modern-day challenges in mind.

Over and above using real-time sensors, the monitoring develops towards engineered, effect-based biosensors combined with advanced data analysis. Effect-based methods (EBM), also referred to as bioanalytical tools or bioassays, is a different approach to monitoring compared to targeted chemical analyses. Instead of characterising chemicals by their structures, the process aims to characterise them by their potential adverse effects, or key health risks, on whole organisms.

Once developed, it will be the first of its kind for the country and at the forefront of water quality monitoring globally. And, it will be able to detect the presence of contaminants of emerging concern (CECs) that are potentially harmful to human and environmental health at a fraction of the cost of analytical chemistry methods.

The foundation for the work was laid by an earlier project, also led by Swartz.

What lurks in our water?

Among other outputs, this work (**WRC Project No. K5/2369**) resulted in a list of 20 emerging contaminants of concern in reclaimed potable water in southern Africa. The list, Swartz says, contains the most important chemicals that they think should be addressed locally, as a starting point. This list can, in future, be used by the Department of Water and Sanitation to draw up guidelines for water quality for water reuse systems, something that we do not currently have in South Africa (the country relies on World Health Organization and other international guidelines and norms).

The multi-disciplinary work was conducted by researchers from Chris Swartz Water Utilisation Engineers, the CSIR, the University of the Western Cape, Chalmers University of Technology and private consultancy Innovative Research for Water Solutions (INREWASOL). Fieldwork included testing water samples before and after potable water treatment works in large urban areas, including the City of Cape Town.

The list of chemicals includes pharmaceuticals prescribed in the largest volumes in South Africa, country-specific high-risk priority pesticides, and chemicals common to South Africa (like antiretroviral drugs). It also includes chemicals representing each of the groups of CECs. The chemicals on the list are persistent, not removed by conventional water treatment processes and hold the potential for human exposure. Importantly, Swartz says, those included on the list can all currently be detected in water resources.

He says they realised that it is necessary to be able to test for the cumulative effect of this cocktail of chemicals in the water. Swartz explains: "The ideal is to have something in the line, pipe or tank that indicates the presence of CEC, without necessarily telling you what they are. If you can see that there is a loading of unwanted chemicals coming into your system, you can start a more detailed monitoring program to find out what it is that you're working with. Something like this could act as an early warning system."

The study, completed in 2018, recommended that, instead of the traditional chemical testing, a battery of bioassays must rather be employed in a monitoring programme where the reuse of wastewater takes place either intentionally or by accident.

Effect-based monitoring

The use of effect-based methods (EBMs) for water quality screening or monitoring is not new, says Dr Christoff Truter, a researcher at SUWI. The approach has gained a lot of traction

South Africa's water quality regulations do not currently cover micropollutants.

According to South Africa's most recent Blue Drop Audit Report, the quality of the country's drinkable water is getting worse. Nearly half (46%) of all water supply systems do not comply with microbiological standards. These water supply systems pose acute human health risks because drinking water is contaminated by sewage. The report also found that more than two-thirds (67.6%) of all wastewater treatment works are close to failure.

in recent years thanks to research initiatives such as the EU-funded SOLUTIONS and the efforts of the Global Water Research Coalition, amongst others showing the advantages of EBMs. "It entails using certain key pathways that represent the risks that several chemicals can pose providing an indication of risks, but more importantly presence and therefore compromised water quality."

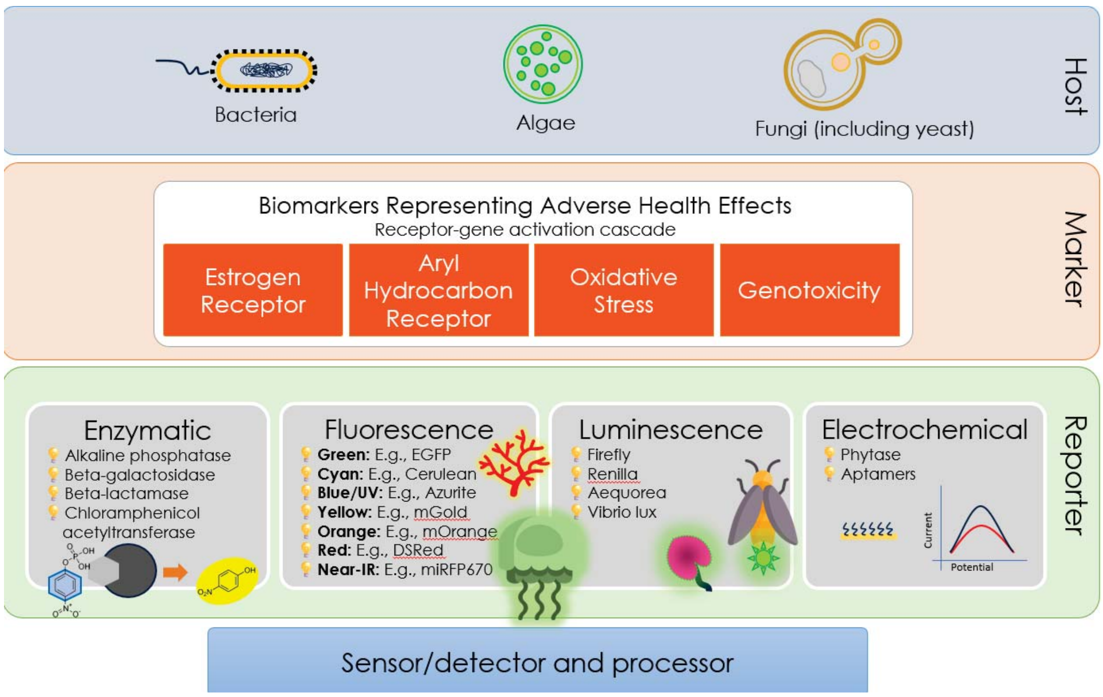
Towards this purpose, researchers are continuously looking for surrogate parameters for the CECs, Swartz explains. The classic example is estrogenicity, says Truter, who did his PhD in zoology and focused on the use of biological assays to screen South African water bodies for the risk of endocrine disruption. There are more than 300 chemicals that are known to be estrogenic based on the US EPA Endocrine Disruptor Screening Program, he says. These chemicals can bind to a human estrogen receptor, or those of other vertebrates including aquatic species, potentially inducing a cascade of effects. One of the best-characterised estrogenic effects is male fish that becomes feminised, he explains. If estrogenicity is picked up in water, it can be interpreted as an indication that some of the estrogenic chemicals are present.

Another good example of an indicator is sucralose, a component found in artificial sweeteners, Swartz says. Since it is man-made, once you pick up sucralose in a river, you know that there has been some human discharge, like sewage or urine into that source. And, it means there are going to be a lot of other potential pharmaceutical products and hormones present too.

This also applies to pathogens (disease-causing microorganisms) with ***E. coli*** being a good example of a widely used indicator. The pathogen is an indicator that there's sewage present in the water, Swartz says. Another is caffeine, which is also included on the list of 20 chemicals identified in the earlier-mentioned project. Caffeine is an indicator of the presence of a certain group of chemicals possibly in the water, Swartz says.

This knowledge of indicator compounds or pathogens can be applied to identify and develop biosensors functioning as EBMs. A yeast cell, for example, can be engineered to sense estrogenicity, and be applied as part of a digital interface to form a biosensor. The yeast cell can be seen to represent human exposure, Truter explains. When such cells are exposed to a water sample potentially containing thousands of chemicals, and it shows a measurable response, it will indicate that there's a risk, he says.

"Engineered microbes can predict whether a particular chemical or mixture of chemicals within a water sample, or other matrix, would actually trigger a biological effect in a human or other vertebrates such as fish or amphibians." In comparison to expensive, individual chemical analysis, Truter says "these effect-based methods are geared to screen samples containing complex mixtures of chemicals." The purpose of EBMs is to show the presence of chemicals, which indicate risk, and more broadly also overall water quality.

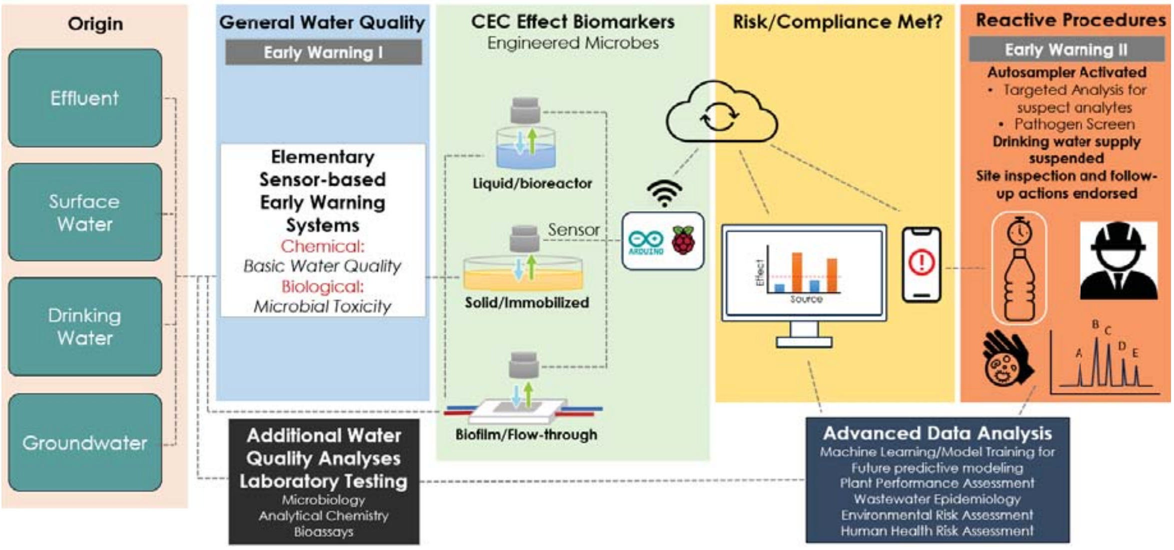


Application of engineered microbes for effect-based monitoring of water quality as part of digital sensor devices. The biomarkers included are based on the testing framework proposed by the Global Water Research Coalition (GWRC) for drinking water and effluents.

The use of engineered microbes representing important biomarkers, was key to the framework for future water quality monitoring suggested by the researchers. The project's final report (**WRC Report No. 3103/1/23**) was just published in November 2023.

A framework for future water quality monitoring

The illustration below suggests a largely automated system for water quality testing.



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

The first step of the system involves the testing of the water source with commercially available sensors that can measure basic water quality parameters like ph, electrical conductivity and total dissolved solids, as well as microbial toxicity. The information is relayed to a software platform that can interpret the data to assess the degree of risk, serving as an early warning step.

The next testing step involves engineered microbes that will act as CEC effect biosensors. This is the tricky one, Truter says, though he adds that synthetic biology is "an exciting space in science" that is evolving fast. "You can, for example, literally put a human gene inside a yeast cell or a bacterial cell with relatively little effort."

In the suggested framework there are three potential ways in which the bioengineered cells can be exposed to the water body. The first one is in a liquid bioreactor format, which is yeast, bacterial or algal cells in a liquid form. The next option is in a solid form, like an agar plate. We have our hopes on the third option, the biofilm format, Truter says. In the illustrated framework, in a very simplified way, this includes a food source (the red line) to feed the biofilm, and the water source being monitored (blue line), running in a continuous flow over the engineered microbes.

The engineered microbes representing key adverse effects (like DNA damage) will be coupled to sensors that detect if something activates the reporters, which can be, for example, in the form of a colour change.

The data from the biosensors and the physical sensors is then interpreted with the help of machine learning to allow risk prediction in real-time. Wastewater treatment works operators or catchment management agencies can then simply receive an interpreted message that flags a problem as it occurs, with an indication of the necessary action to take.

The future of water quality testing

The project's final report points out that although real-time effect-based biosensors are not currently available, such technology may likely be the future of water quality monitoring. They state that increased investment and research efforts are still needed for the development of effect-based biosensor devices representing key health risks. These technologies will enable remote deployment and routine testing at a fraction of the cost of analytical chemistry methods. The research team recommends that 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.

The biosensor development research is exciting because it's still an open canvas, Truter says, in reference particularly to the fields of synthetic biology integrated with data science.

The work will continue in the currently EU-funded MAR2PROTECT project (https://mar2protect.eu/).

However, Swartz adds that there should also be a start towards regulation of these chemicals and their compounds or, making sure that they don't land in the water in the first place.

To download the report, *Real-time sensing as alert system for substances of concern* (**WRC report no. 3103/1/23**), visit: https://bit.ly/3TBbfkm