Online Biomonitoring for integrated smart real-time water management

Online Biomonitoring has been developed and applied in monitoring toxic pulses of large transboundary rivers and at drinking water intakes since the Sandoz accident at the River Rhine in 1986. However, the biomonitors of this 1st generation are often not sensitive enough or fail the requirements for animal welfare, e.g. fishmonitors. During the 2nd generation, the Multispecies Freshwater Biomonitor© (MFB) has been developed and used in many different applications such as monitoring rivers, wastewater treatment plant effluents, drinking water intakes and groundwater in situ. Based on intensive scientific ecotoxicological research with many different invertebrate species the MFB has established large datasets on the behavioural responses of aquatic animals to chemical stressors.
This paper summarizes the main results from different applications of the MFB in practical water management applications. Depending on the wastewater composition regarding toxic compounds the stand-alone time for the MFB has been one to two weeks while monitoring the effluent water before/after a 4th purification step based on active coal or ozonation. The MFB was able to (1) detect process disturbances in the biological purification step, to (2) prove the purification efficiency of a wastewater treatment plant and (3) prove the success of advanced purification steps in WWTPs. Moreover, the MFB was able to detect pollution waves in the rivers Rhine and Meuse as well as Nitrate/Nitrite pollution combined with pharmaceuticals in a groundwater pipe in situ.

1. Introduction

An online biomonitor (or toximeter) operates on real-time basis and consists of a living organism with a sensitive behavioural or physiological response to chemical stressors as "sensor", a quantitative recording unit of this response and a PC unit with specialized software to detect behavioural differences and to interprete and classify them as water quality alarm. Online biomonitors also named continuous or dynamic biotests or biotest automates function as biological early warning systems to rapidly and early detect changes in water quality which provoke responses in organisms (Gerhardt, 1999). The task of an online biomonitor 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.
The development and installation of biomonitors along the river Rhine started soon after the Sandoz accident in 1986, where both the ecosystem health and the drinking water intakes along the Rhine were seriously affected by a pollution accident with longterm effects over several months. Biomonitors using fish, *Daphnia spp.* and mussels as sensors were installed and have been operated for many years. The different types of online biomonitors are described in detail in Gerhardt 1999. However, due to low sensitivity (e.g. mussels), animal welfare concerns (e.g. fish) as well as high maintenance efforts combined with low numbers of accidents, online biomonitors have been closed down in the last years. However, the decrease in pollution accidents might have been due to the presence of online biomonitors which allow for tracking the pollution sources, deliver data for juridical prosecution and the application of the polluters pay principle. The 2nd generation of online biomonitors combined with automated water samplers is less maintenance-intensive and more sensitive and flexible. Moreover, new test species have been established, to allow for higher sensitivity and tailored applications. These online biomonitors still represent a useful part in integrated water quality monitoring as not all chemicals and their degradation products can be recorded simultaneously and in real-time and only organisms can show how the different substances cause synergistic or antagonistic effects as surrogates for the protection of both biodiversity and human health. Therefore, online biomonitors are still necessary in spite of technological advances in chemical monitoring.
In the context of the EU Water Framework Directive online biomonitors can be used in different applications: Operative and overview monitoring sites are checked only every few years by the analysis of biological quality components, e.g. monitoring of macrozoobenthos in a seasonal sample. In between there is no control. This would especially be needed for water bodies of specific concern, e.g. rare and protected species, fish habitats, environmentally protected areas, biodiversity hotspots and in drinking water protection areas. Investigative biomonitoring with online biomonitors is useful where environmental quality standards are repeatedly surpassed, pollution is suspected, success of restoration and improvement measures has to be documented, etc.

Moreover, online biomonitors are useful at point pollution discharges, such as wastewater treatment plants. Here not only the emission but also the immission of potential toxicity has to be monitored continuously, esp. as waste water effluents change in their composition and concentrations of toxicants on daily and seasonal basis. Moreover, small water bodies < 10 km^2 catchment do not fall under the regulations of the EU Water Framework Directive, i.e. there are still no established guidelines how to protect them.
In the following chapters, the Multispecies Freshwater Biomonitor (MFB) as a 2nd generation online biomonitor is presented in its different new applications for real-time biomonitoring of surface water, waste water and groundwater.

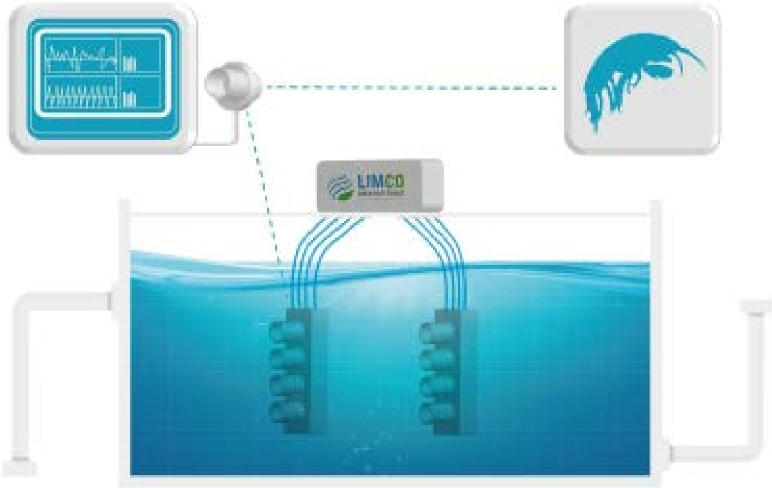

Figure 1: *Schematic drawing of the components of the Multispecies Freshwater Biomonitor, MFB*
Top: PC-unit and sensor chamber (left), sensor animal (right);
Bottom: flow-through installation with 2 times 4 sensor chambers connected to the MFB-instrument and exposed in the current.

2. Methods

The Multispecies Freshwater Biomonitor© (MFB) (**Fig. 1**) uses cylindrical flow-through test chambers with net-carrying srew-lids on both ends. The nets have different mesh size depending on the size of the test species. Also chamber size can vary in order to fulfill the ecological and space requirements for the species to be used. The smallest animal size of ca. 1 mm (e.g. copepods) has been applied in a flow-through test chamber block (Microimpedance Sensor System©, MSS) (Gerhardt, 2018), whereas the largest animals of ca. 10 cm size have been applied in chambers of 40 cm lengths and 20 cm in diameter. Moreover, rows of chambers can be arranged to measure e.g. drift, swimming speed, burrowing behavior of soil or sediment organisms or phototaxis (Gerhardt et al. 2006). These few examples show the wide range of potential applications of the MFB for recording aquatic, soil and sediment quality.
The recording principle is based on the non-optic quadrupole impedance conversion technology: two pairs of stainless-steel electrode plates are placed at the inner walls of the acrylglass chambers (Gerhardt et al. 1994). Whereas one electrode pair generates a high frequency alternating current over the test chamber filled with medium (water, sediment), a second non-current carrying pair of electrodes senses the changes in this electrical field caused by the movements of a test organisms placed in the chamber (Gerhardt, 1999, Gerhardt et al. 1994). The signals are processed in real-time using a discrete fast Fourier Transformation (FFT) with Hamming function, which leads to a histogram of signal frequencies which can be allocated to different types of behavior, such as gill ventilation, locomotion and inactivity. Alarm algorithms consist of ARIMA-models, Double Sigma and slope detectors, allowing for sensitive warnings in case of pollution changes in the water.

3. Results

River monitoring

In situ online biomonitoring with the Multispecies Freshwater Biomonitor, MFB, was performed in streamside powered monitoring stations at the rivers Meuse (NL), Aller (GER) and Rhine (F/GER) using Gammarus pulex as sensor species. G. pulex responded to pollution pulses in the Meuse river and during longterm monitoring in the Rhine river to several chemical irregularities. In the river Aller, no biological alarms were recorded during the test period, which corresponded to the chemical analyses (Gerhardt et al. 2007).

Wastewater monitoring

The first application ever of an online biomonitor in wastewater monitoring was performed from 2012–2016 in a WWTP in Switzerland with the MFB and eight gammarids as test organisms. Regularly high values of Ammonium (> 2 mg/l) in the effluent caused serious mortality of the gammarids in the MFB and simultaneously impairment of the biological cleaning step (**Fig. 2**). A water sample taken during the event of gammarid dying revealed toxic substances in the wastewater (carbamates > 10 mg/l). In this case study the MFB was able to clarify the reasons of low performance of the microbiological purification process and lead to the responsible causes, which could be handled thereafter (Gerhardt, 2019; Bühler et al. 2014). In order to evaluate the potential toxic effect of the treated wastewater on the river biocoenosis a comparison was performed using two MFB systems, one spiked with treated effluent and one spiked with river water. Whereas the gammarids rapidly died within one week in the wastewater they survived with only sporadic deaths in the river water. This shows, that the effluent of this WWTP is still toxic and needs further optimization in the purification processes (Gerhardt, 2019). In another WWTP a pilot trial was performed using two MFB systems, one placed before and one after the advanced active coal step. Differences in behavior and mortality of the gammarids showed that during

Figure 2: *Longterm signals of eight gammarids in the MFB exposed to treated wastewater effluent (red curves). On top of each graph the status bar shows normal behaviour (green) and inactivity (red). Five animals died during the exposure.*

the active coal step toxicity could be reduced so that mortality of the gammarids decreased (Gerhardt, 2019).

Groundwater monitoring

Two stygal species, Niphargopsis casparyi (Pratz, 1866) and Proasellus slavus (Remy, 1948), were introduced as novel biomonitoring candidates for groundwater applications. Copper sensitivity of the stygal and two surface species, Gammarus fossarum and Daphnia magna, was recorded in acute and chronic toxicity tests and in multiple copper pulse experiments. Regarding mortality, copper toxicity tests showed low impact of acute copper pulses onto the two stygal species N. casparyi and P. slavus, but high impact on the surface water species. Chronic exposure led to increased mortality in G. fossarum and P. slavus, but not on N. casparyi (Grimm & Gerhardt 2018).

During a 2-month long experiment, the applicability of N. casparyi in water quality online biomonitoring was established in a drinking water utility and its performance as biomonitor species compared to G. fossarum and to another biomonitor using daphnids. The experiments revealed pronounced, useful, and reproducible behavioural responses towards copper for G. fossarum, N. casparyi, D. magna, but not for P. slavus, making them promising candidates for online biomonitoring (Grimm & Gerhardt 2018).

The effects of pig manure applied as fertilizer on an agricultural field with crop was studied in situ during several simulated rain events. The effects on groundwater crustaceans were monitored continuously using the Multispecies Freshwater Biomonitor© (MFB) adapted to groundwater conditions and in situ application using the amphipods Gammarus fossarum (Koch, 1835) and Niphargopsis casparyi (Pratz, 1866) as indicator species. Pig manure was applied on the test field, once spiked with sulfamethoxazole. Three rainfall events of different intensity and duration were simulated directly after the fertilization and the responses of the animals in the online biomonitor were quantitatively recorded for 24h to seven days (**Fig. 3**). G. fossarum responded within 24 h with decreasing spontaneous locomotor activity to the components in the pig manure and elevated nitrate levels which were washed out from the surrounding soils. N. casparyi responded in two experiments of seven days' duration with decreasing activity to elevated nitrate levels in the 1st experiment and in the 2nd experiment to increased nitrite and sulfamethoxazole levels showing decreased activity and 62.5% mortality. The 3 field experiments showed (1) successful operation of the online biomonitor MFB in situ, (2) G. fossarum sensitivity to nitrate, (3) groundwater crustaceans sensitivity to nitrate, nitrite and sulfamethoxazole. We recommend to use the MFB with groundwater crustaceans (= groundwater biomonitor) for long term in situ biomonitoring of groundwater ecosystem quality (Gerhardt et al. 2020).

4. Conclusions

The Multispecies Freshwater Biomonitor (MFB) has successfully been used during the past 25 years in laboratory ecotoxicty testing with different aquatic invertebrates exposed to both inorganic and organic substances and complex effluents.

Figure 3: *The experimental agricultural field in Germany, showing the sprinklers simulating rainfall events in the back as well as solar panel for powering the instruments, such as the autosampler and the Multispecies Freshwater Biomonitor, MFB, and the battery storage station (from left to right)*

The MFB has proven to be able to detect process disturbances, to show the purification success of advanced waste water treatment steps and to monitor effluent water quality both at the emission and the immission sides.
Moreover, the MFB has proven to be applicable as stand-alone monitor operated by solar power in situ for groundwater biomonitoring of agricultural chemicals using both surface and groundwater amphipods as sensor species.

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