The Rhine/Danube Monitoring Paradigm: Broader Applications in Sensor Technologies

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Abstract:
The Rhine/Danube paradigm is a robust, highly validated, cybernetic system for water quality monitoring that is poised to be upgraded by technological innovations for sampling, sensing, computer hardware, and data transfer and management protocols. But in addition, the paradigm's infrastructure has to be hardened to withstand terrorist attacks and natural disasters as well as be able to detect, characterize, and respond to the situations themselves. The aim of this paper is to briefly describe some of these technologies and suggest ways that they can be integrated into the paradigm.

Key words: monitoring, biological early warning system (BEWS), biosensors, enzyme inhibition and electrochemistry, lateral-flow device, microspectrophotometry, image and pattern recognition, microarray, polymerase chain reaction (PCR).

Introduction

Advances in the technology of environmental monitoring have been growing steadily over the past decades. Some advances have been used in river monitoring in Europe since the early seventies. Gunatilaka and Diehl (2000) review these developments that began with chemical monitoring but expanded to include biological monitoring systems. Automation became part of monitoring early on where simple physical-chemical parameters would be measured such as pH, O_2 concentration, temperature, turbidity and conductivity, which were measured easily and continuously. As chemical technology became more sophisticated, so did the monitoring that grew to include detecting and measuring micropollutants such as pesticides and industrial pollutants. GC/MS equipment was automated to measure up to 1,000 species of chemicals with increasingly greater sensitivity. But because chemical data alone had little meaning biologically, surveys of in situ plants and animals in the water column and sediment were carried out. Changes in the numbers of organisms within a species or numbers of species gave clues to biologically define the water quality. These were excellent to determine the long-term trends. However, because these surveys were time consuming and on a time scale that did not reflect the immediate situation, enforcement of environmental regulations was difficult. The needed data was too sporadic or lagged to the extent that the polluting event was often missed. As a result quick-responding biological systems were developed, the so-called biological early warning systems (BEWS) comprehensively reviewed by Baldwin and Kramer (1994) and Gunatilaka and Diehl (2000). These systems relied on the organisms' behavioral response to changes in toxicity of the water. BEWS such as the Dynamic *Daphnia* Test (Gunatilaka et al., 2000) and the Musselmonitor® (Kramer and Foekema, 2000) were among ten that played an important role in the monitoring success in Northern Europe (but rarely if ever employed in North America), particularly in the Rhine and Danube River Watersheds. During the Sandoz incident in 1986 when tons of pesticides were released into the Rhine River, a BEWS consisting of a *Daphnia* monitor 500 km downstream from Basel detected toxicity in the water of the, by then, much diluted pesticides, giving an alarm situation. It was this alarm that eventually stimulated governments throughout the world to more closely monitor the waters by mandating a wider application automated monitoring including the newer BEWS protocols.

As terrorism becomes a daily possibility throughout the world, greater attention needs to be given to the safety and security of water resources. In response, world governments have increased spending to anticipate the needs for better protection. For example, the US government has ordered huge budgetary increases in response, requesting $6 billion USD alone to combat bioterrorism for 2003 (Agres, 2002). Improvements in detection of pathogenic agents and toxins will be sought; and this paper will review some of these newer sensor technologies and how they might be employed in detecting weapons of mass destruction (WMD).

The Rhine/Danube Monitoring Paradigm

Thus, developed a complex monitoring system that we call here the Rhine/Danube Monitoring Paradigm (RDMP). This paradigm includes the entire system of monitoring: the sensors and collectors, the identification and measurement of the chemicals, the biological response, the integration of this information from many monitoring stations, the evaluation of this information to decide if there is an alarm situation, the storage of this information for archival purposes and for the possible creation of intelligent systems. The alarm system is complex because of the importance of being able to recognize false alarms on the one hand and detecting pollutants at sufficiently low levels on the other. As this paradigm developed, success in environmental protection followed. After three decades of continuous, automated, on-line monitoring (chemical and biological), this technology has contributed significantly to the dramatic improvement of stream and water quality in this region from alphamesosaprobic to betamesosaprobic. The RDMP was born as globalization of economies began to intensify. As the GAAT and NAFTA continue to enable the expansion of international trade, so too, organizations of dark and devious intent are blossoming. Thus, infrastructure managers will need to expand and intensify environmental (and more specifically water) monitoring particularly aimed at international/cross boundary pollution and terrorist threats.

Outline of principles and of the Dynamic *Daphnia* Test and Musselmonitor®

The well-designed biomonitor responds to an environmental stress rapidly (at least earlier than it would in humans), that the response needs to be an easily and unambiguously identified effect, and that the effect has to be nonlethal (Butterworth, 1995). Other features of a successful biomonitor are that it is cost effective, reliable, and robust. Two of the more successful BEWS that have these attributes are the Dynamic *Daphnia* Test (Gunatilaka et al., 2000) and Musselmonitor® (Kramer and Foekema, 2000). Both tests, fully described in Butterworth et al. (2000), measure behavioral changes in response to environmental stress to *Daphnia* (or the common water flea) and the zebra mussel, respectively. In the Dynamic *Daphnia* Test, swimming behavior is measured as the animal swims through IR beams in a column of water that is continuously replenished by river water. Each pass the animal makes through a light beam is recorded and the rate of passes is proportional to pollutant present in the water. In the Musselmonitor®, shell opening of the animal is measured in response to pollutants it detects in the water. The higher the concentration of pollutant present, the higher the rate of shell closing is detected electrically and recorded. Both tests measure sublethal levels of pollutants, continuously, automatically where data are transmitted by telephone lines to a central computer. There, ‘alarm’ software in addition to archiving the monitoring data, will make and broadcast decisions on alarm events.

Newer biosensor technologies

The above biological systems are very useful possessing all the features for success, but they are not without problems. For instance, although they will be useful in the foreseeable future, they require continual maintenance and careful quality control of the test animals. However, there are things that they cannot test for: they cannot detect the presence of specific pathogens or toxins or complex mixtures of pollutants or toxins. Furthermore the entire RDMP system as it stands has to rely on GC/MS to identify all candidate compounds. Advances in biosensor technology may lead to solutions to these obstacles. For example, new, remote-acting technologies as such as (1.) screen-printed, disposable biosensors, which utilize enzymatic systems to detect and identify specific pollutants; (2.) lateral-flow devices with electrochemical detectors will be able detect and measure specific microbial pathogens; (3.) miniaturized, cost-effective, optical sensors and biosensors employing microspectrophotometers of plastic, optical fibers will be able to detect, identify and measure specific pollutants; and (4.) systems of image and pattern recognition algorithms will be able to identify and measure cellular pathogens or (5.) decipher binding patterns on microarrays of binding/receptor proteins that could be able to identify critical, complex mixtures of pollutants or toxins; or specific DNA sequences to identify groups of organisms or patterns of sequences, or (6.) gene replication (polymerase chair reaction or PCR) technology to amplify unknown sequences in order to compare with known reference sequences.

1. Screen-printed biosensors (Cowell, et al. 2000) are in stages of development where compounds such as those capable of detecting and measuring the amounts of organophosphate-based pesticides (OPP) in drinking water and foods. The biosensor measures acetylcholinesterase inhibition by OPP where the electron shuttle of the conversion of thiocholine RSH to RSSR the oxidized form that sends a current in an underlying screen-printed carbon electrode. The sensitivity is down to levels of 10^{-5} M in river water with high speed and specificity. It is not inconceivable that such technology could be adapted for continuous monitoring where the sensors would be automatically purged or have a mechanism to introduce fresh sensor cassettes into the monitoring chamber. Applications of this technology in response to a terrorist attack of neurotoxins and nerve gas are obvious. Distinction of other pesticides and specially-weaponized neurotoxins might require knowing an inhibitor of the appropriate key enzyme in the neurotransmitter path. In addition to being highly specific, and fast, the devices can be miniaturized and mass-produced at low cost.

2. Lateral-flow devices (LFD) containing belts of antibodies embedded in nitrocellulose strips in combination with proprietary, screen-printed carbon electrodes (SPCE) can detect at very low concentrations cells having catalase activity including pathogenic bacteria in weapons of mass destruction (WMD) such as anthrax or simply sewage bacteria that force beach closings (Cowell et al., 1994). Here a cassette containing an LFD charged with antibodies specific to candidate bacteria where each antibody band lies directly over a particular SPCE circuit. The sample containing an unknown mixture of suspended bacteria is introduced to the LFD, it flows through the matrix to an absorption pad, and specific cells bind to the specific antibody belts. An aliquot of peroxide solution is introduced to the cassette, and the resulting electrochemical reaction of peroxide in the presence of bacterial catalase is detected and recorded in each of the underlying SPCEs. In addition to the high specificity, there is, because of the enzymatic amplification, a sensitivity of 10 cells per 200 uL in 10 min. Weaponized anthrax is in the form of spores, and because spores do not have catalase activity, an additional 60 min would be required for germination. Again, it is not

inconceivable that such technology could be adapted to continuous monitoring either by purging the cassette or more likely, by automatically inserting of a fresh cassette. This system will be rather inexpensive, the cost and size of the cassette would be equivalent to a home pregnancy test.

3. Plastic, fiber optics in microspectrophotometers are able to detect specific pollutants (Scully et al. 2000; Wong et al., 2001) at relatively high sensitivity, specificity, and speed. In particular, evanescent wave (EW) immunosensors are made from an optical fiber where parts of the cladding are removed and replaced with a biological sensing agent (for example, fluorescently-labelled antibody). An interrogative light is directed within the fiber, interacts with the biological sensing layer, which in turn re-emits the EW at a longer wave length that is captured by the detector. Examples in the literature show atrazine in water at a detection limit of 2nM or 0.1 ug/L and parathion to 0.3 ug/L. Adaptability to automation would require recharging the biological sensing layer or a method of introducing new EW units. Creating EW units sensitive to weapons of mass destruction such as ricin would depend on the type of antibodies used. The devices can be miniaturized and mass-produced.

4. Image recognition of cellular pathogens such as *Giardia* or *Cryptosporidia* can be identified and measured using a patented continuous microbiotal recognition method of Butterworth and Das (2000). Das and Butterworth (2000) have shown that using simple phase microscopy and proprietary, sealed microchambers coupled with particular software, these organisms can be continuously and automatically identified and enumerated. The specificity, speed, and potential for automation using nonpathogenic organisms are being convincingly established. Because *Giardia* and *Cryptosporidia* are seen mainly in cyst form, each positively identified cyst would be purged into an incubation chamber to test the cyst's viability. The speed of identification of cysts will be in real time, the cost would be less than $5,000 USD. Flow cytometry coupled with immuno-specific stains can also identify these organisms, as well as any other pathogenic cells. However, this technology is more costly by an order of magnitude and it is unlikely in the near future that it would capable of automated monitoring.

5. Protein microarray technology may be a successful approach to understanding the, as yet unpredictable, effects of complex mixtures. Recent work from our laboratory (McGowen, et al., 2000) indicates that complex mixtures of pollutants can have unpredictable results depending on the concentrations of three separate toxic chemicals. In some cases the effect was additive, others subtractive, others had no effect regardless of how high or low the concentrations were made, and still other combinations were synergistic at extremely low concentrations. Microarrays of binding proteins including antibodies or receptor proteins (specific for each toxin) in conjunction with stains specific for each binding protein could identify toxin mixtures rapidly, with high specificity and speed (MacBeath and Scheiber, 2000; Zhu et al., 2001; see Lee et al. (2002 for nanoarray technology that might have greater specificity that microarrays but with different detection requirements). Such arrays have particular staining patterns, a matrix of colored dots that would be recognized by appropriate pattern recognition software (see part 4) above) in an array reader. Arrays of binding proteins for specific toxins could give staining patterns that could be compared with specific toxicity patterns such as those we have described above to develop a database to help identify unexpected toxic cocktails. Designers of WMD might try to make toxic, multicomponent cocktails, where any component could go undetected or raise no concern. But as a particular mixture such a cocktail could have high toxicity. Microarrays might be able to help in their detection. Specifically-designed arrays or information from the database might be able to identify

these mixtures. It is not inconceivable that such technology could be adapted continuous monitoring systems. Microarrays of DNA work on the same principle and have the potential to identify pathogenic organisms. DNA arrays at present appear to have little use because of the length of preparation times required to obtain results, but there are commercially available products being produced that are minimizing these times. Array readers are relatively expensive; and organism viability detection is not yet possible.

6. Polymerase chain reaction (PCR) technology is fast becoming a way to identify organisms by their DNA. Large numbers of copies, made of an organism's DNA from a tiny sample, can be identified via base sequence analysis. The development of the technology is allowing such identifications to become more rapid. However the equipment is still costly and requires fairly skilled technicians to perform. Automation of the technology is far off and cannot yet determine whether the organisms are alive.

The first four technologies have the greatest chance of being incorporated into a RDMP system because they are closer to being automated. The latter two technologies at least for the present could serve well in an adjunct capacity.

Secure water supply systems and crisis management

Globally significant investments have been made continuously in the public sector over many years for safe and reliable water supplies and infrastructure. But global threats to public health and safety have forced infrastructure managers to rethink preparedness for terrorist attacks as well as natural disasters. Against this background, there is a clear need for water supply systems to be 'life-line' systems which are resilient against disasters of all kinds, and there is an urgent need for development of well-organized crisis-management systems.

In the last two decades on-line monitoring equipment has been improved to measure very accurately simple as well as complex parameters such as herbicides, pesticides and a vast array of other toxic substances. These are examples of real-life applications. If well maintained with frequent supervision, these instruments have proved to be reliable and give continuous information for a relatively long time as shown by the groundwater management system developed for the city of Vienna (Gunatilaka and Dreher, 2001; Dreher and Gunatilaka, 1998, 2001). Now we have to use the technology developed to address recent problems such as bioterrorism.

Integration of and also the development of redundancy in electronic instrumentation and control systems is a critical approach. New computer systems having internationally open architecture are downsized to operate in real time, are highly reliable and networked (Middleton and Goodwin, 1990). Ohto (1998) described present developments with the current status of instrumentation, control and automation by the concept of '3C - Fusion' (already in progress in Japan) where fusion of controls, computing and communication has to be accelerated (a current problem in Europe). This information would be used in ultra-high speed, digital communication systems deployed with control systems to utilize advanced mathematical models, fuzzy logic controls (De Silva, 1995), also neuro- and knowledge-based models integrated into a single system for broad-based management and control of large water distribution systems (Olsson and Piani, 1992; Åström, K.J. and Wittenmark, 1990; Hafner, 1994)

It is hoped that WMD will be detected by biosensors before humans are affected. But unfortunately, disease outbreaks such as those caused by *Cryptosporidia* or anthrax often may be the first indication of a terrorist attack, and resource managers and homeland defence personnel will have to know the source and location of pathogen or toxin release. This will require database development for area topography, maps, pipelines, plant facilities, equipment management and maintenance including automatic reading of meters. The use of optical fibre, cable networks used among other purposes, remote supervising control of facilities (monitoring, supervising, security, information and transmission) will also be required. In addition broader usage of systems analysis, expert systems, fuzzy logic and artificial neural network control will be part of a successful management approach.

New technologies in the Rhine/Danube Monitoring Paradigm

Although the above examples are not meant to be exhaustive, they at least point to where automated water monitoring can go in the next decade. All the methods have the potential to be incorporated into the RDMP: to increase the accuracy, speed, and specificity at the same time to introduce new monitoring concepts, replace bulky and high maintenance equipment and to reduce costs. The current threats to the infrastructure have induced governments to significantly expand funding for research, development and implementation leading the authors to believe that, together with the zeal and determination of scientists and managers, the improvements such as those enumerated above will occur within the decade.

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