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Field-Based Effects Measures

IN SITU ON-LINE TOXICITY BIOMONITORING IN WATER: RECENT DEVELOPMENTS

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Abstract—In situ on-line biomonitoring is an emerging branch of aquatic biomonitoring. On-line biomonitoring systems use behavioral and/or physiological stress responses of caged test organisms exposed in situ either in a bypass system or directly in-stream. Sudden pollution waves are detected by several existing single-species on-line biomonitors, which until now have been placed mostly in streamside laboratories. However, recent achievements have been multispecies biomonitors, mobile biomonitors for direct in-stream use, development of new instruments, new methods for data analysis and alarm generation, biomonitors for use in soil and sediment, and scientific research supporting responses as seen in on-line biomonitors by linking them to other biological and ecological effects. Mobile on-line monitoring platforms containing an array of biomonitors, biosensors, and chemical monitoring equipment might be the future trend, especially in monitoring transboundary rivers at country borders as well as in coastal zones.

Keywords—In situ tests On-line biomonitors Biological early warning systems Toxicity monitoring

INTRODUCTION

Biomonitoring is a truly interdisciplinary applied science based on both ecology and ecotoxicology [1]. Two types of biomonitoring methods that integrate these disciplines exist; trend biomonitoring (using ecological methods and community structure; e.g., diversity indices, similarity indices, species richness, and percentage of Ephemeroptera, Plecoptera, and Trichoptera taxa), and community function (e.g., Functional Feeding Groups) and rapid bioassessment methods (e.g., saproby index, biotic indices, and Biological Monitoring Working Party index). However, other indicator methods might be added depending on the purpose of biomonitoring, such as fish health status, macrophytes, or algae; the latter two methods are invaluable for eutrophication monitoring. All these methods are, unfortunately, not based on ecotoxicology, and because pollution is more often caused by chemical contaminant mixtures than by oxygen depletion, we need to improve current ecotoxicological methods for pollution monitoring. Several applicable methods include in situ on-line biomonitoring with so-called biological early warning systems (BEWS) below toxic effluents or along large rivers, especially at national borders, to detect toxicity spikes and to provide data regarding potential permit violations. Moreover, rapid toxicity tests can be applied directly in situ based on behavioral measurements (e.g., avoidance responses) or on survival, growth, genomics, or other metrics. In situ cage exposures are another appropriate method for long-term exposures. [1].

The development of on-line and off-line in situ biotests for toxicological assessment and biomonitoring of water quality follows the United Nations Agenda 21 (http://www.un.org), which highlights the protection and sustainable management of water as a restricted resource. Biomonitoring, especially on-line toxicity biomonitoring, is an emerging field that is playing an increasing role in the surveillance of environmental quality, often as a complement to chemical monitoring [1–3], and should be integrated in the European Water Framework Directive.

On-line biomonitors frequently use behavior as an endpoint, which provides a visual and, thus, measurable response at the whole-organism level. This method generates fast and sensitive results that can be integrated into many biological functions [4]. The key is to employ invertebrates or younger life stages that tend to be more sensitive to toxicants and respond more rapidly than adult stages or vertebrates, but with the proviso that the effects are nonlethal.

The basic idea of an automated biological sensor for water-quality management was first proposed by Cairns et al. [5]. Automated biomonitors operate on a real-time basis using living organisms as sensors, ideally providing a continuous flow of information regarding water quality. On-line biomonitors, continuous (dynamic) biotests, automated biotests, or BEWS are characterized by three components: a test organism, an automated detection system, and an alarm system. The tasks of on-line biomonitors are to detect pollution waves by recording the ''integrated biological response'' of the test organism to pollution spikes [3].

BACKGROUND

In 1986, a Sandoz chemical storage building in Basel (Switzerland) caught fire, spewing approximately 40 tons of insecticides and 400 kg of atrazine into the Rhine River, resulting in the devastation of a large portion of the river's biocoenosis as well as drinking-water production (40 waterworks had to close for one month) from an already polluted river [6]. Shortly after the accident, an automated biomonitoring system, such as the dynamic *Daphnia* test (a device that electronically measures *Daphnia* swimming behavior) [7], located 500 km down-

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stream detected and reported the event. Samples taken at the time of the alarm showed that the same types of pesticides triggering these alarms were stored in the Sandoz facility. As a result of the speed and accuracy of this single monitoring station detection, many European governments mandated additional monitoring stations along the river. The BEWS grew into a new paradigm of environmental monitoring, especially in large European rivers at country borders.

The desired parameters of preformed biomonitors include full automation, real-time detection and data generation, sensitivity to pollution, rapid response, easy operation, reliable alarm interpretation, minimal false alarms, and minimal costs, maintenance efforts, and training requirements. The organisms chosen range from vertebrates (fish) to arthropods (insects, crustaceans, and daphnids) via oligochaetes to microbes (algae and bacteria). In higher organisms, behavior is the response type that generally is recorded with biomonitors. In microbes, physiological responses, such as bioluminescence, have been chosen as endpoints. Generally, these systems are part of a streamside monitoring station that also includes the ability to detect and measure chemical and physical parameters. Chemical detection often is automated and can include detection of known pollutants expected to be found as a result of accidental industrial spills or agricultural runoff.

BIOMONITORS: STATE OF THE ART

Most bacterial biomonitors are based on recording respiration (bacterial respirometers); however, some use bacterial growth (i.e., measure turbidity or cell density) or bioluminescence via a luminometer [8,9]. Bioelectrodes (bioprobes) are being developed that measure photosynthetic activity of immobilized green algae or cyanobacteria. Bacterial biomonitors are useful for monitoring wastewater treatment processes and effluents. Several problems with these tests include clogging, continuous growth of the bacteria, low sensitivity, and addition of nutrients and media manipulating the test water characteristics (for a detailed review, see Gerhardt [3]).

Algae biomonitors are invaluable for the detection of herbicides in the water. These biomonitors measure cell growth, oxygen production, direct or delayed fluorescence or fluorescence modulation, or sometimes, motility of the flagellate *Euglena gracilis* [8,10]. Most of types of systems work on a semicontinuous basis, with measurement intervals of 30 min. Higher plants, such as *Lemna* sp., also have been used for offline ecotoxicity testing (for a detailed review, see Gerhardt [3]).

Biological early warning systems with animals often use behavioral parameters, because these parameters are among the most sensitive and the first to recognize pollutants. These systems are nondestructive, and measures can be repeated. Bivalves have been used in so-called passive trend biomonitoring; caged bivalves have been exposed to effluents and the bioaccumulation of toxicants measured after a predetermined exposure (Mussel-watch). The BEWS using mussels are based on recording the shell closure, such as the *Dreissena*-Monitor® (Envicontrol, Cologne, Germany) and the Musselmonitor® (Delta Consult, Kapelle, The Netherlands) [11].

Daphnia spp. have been used extensively in ecotoxicology, resulting in copious amounts of literature describing their response to contaminants. The dynamic water flea test was the first BEWS, monitoring the passage of swimming *Daphnia* spp. through infrared light beams [12]. This biomonitor has recently been replaced by video-based technology [13] because of technical errors [3]. The movement of one healthy water flea up and down through many light beams, with the remaining dead water fleas resting on the bottom of the exposure vessel, produced data similar to those of numerous healthy water fleas passing through fewer light beams. Video tracking has rectified this error by monitoring each individual water flea; however, cross-over swimming is still a problem. Water flea tests often use prefiltered test water, which eliminates particle-bound toxicants. The European Water Framework Directive recommends using unfiltered water for testing purposes. Biomonitors that pretreat the water through filtration will not be able to fulfill this demand. The water fleas need to be fed continuously by a stable algae culture, which might represent another uncertainty.

Fish biomonitors also have been among the first biomonitors utilized, especially for those systems based on rheotaxis [14], in which fish are anticipated to exhibit rheotaxis when exposed to pollution waves. The fish then drift downstream to potentially more favorable conditions. This behavior can be seen as an avoidance reaction. This system, especially when used with *Leuciscus idus*, is insensitive and generates ethical concerns because of the small size of the test aquaria [3]. Modern systems are being developed that are based on tracking by video groups of fish, using larger aquaria, and recording swimming speed, turning rate, and swarm formation as test parameters. Moreover, some noncontact bioelectric systems have been developed, recording ventilation rate, cough rate, heart rate, and swimming behavior of different test species [15,16]. Tropical electrical fish exhibit an electrical organ discharge that has been used in biomonitoring systems, but this parameter is too variable for accurate monitoring of pollutants [17].

Smaller running waters are governed predominantly by benthic macroinvertebrates [18]. This organism group has been neglected in on-line biomonitors, apart from use in the Multispecies Freshwater Biomonitor® (MFB; LimCo International, Ibbenbueren, Germany), in which epibenthic and inbenthic insects, crustaceans, and oligochaetes have been tested.

MULTISPECIES MONITORS

Until now, only single-species biomonitors have existed; therefore, many monitoring stations are equipped with a battery of different monitors for different species, such as those for *Daphnia* sp., fish, algae, and mussels. This is an inefficient method when accounting for the costs of installation, maintenance, and manpower efforts, because each monitor needs regular attendance. The aim should be to develop test systems, based on universal recording principles, that are flexible and modular in construction. The MFB is the first animal monitor fulfilling these requirements. The high number of individually recording channels allows a higher number of replicates to be performed within one test species and with a larger variety of simultaneously observed test species. The test unit is flexible in both size and type of construction, and it is adaptable to the space and ecological requirements of many different test species. The recording principle for this system is nonoptical, universal, and based on quadropole-impedance conversion technology [1,16].

MOBILE IN SITU PLATFORMS

Biomonitoring methodology also can be carried out by small and, sometimes, remotely powered mobile units. Such mobile platforms consist of an array of biological and chemical sensors and biomonitors. The cost for such a unit can be significantly less than that of the permanent station, increasing the number of monitoring locations in a watershed, especially at remote locations.

Mobile platforms are available at various stages of development. One of the first was the Musselmonitor [11,19], which measured mussel valve movements. Another system is the remotely powered, mobile system [20], which measures clam gape in real time as a response to toxins via a multiprobe. The bluegill ventilatory monitor described by Shedd et al. [21] is a third system that is combined with a multiprobe and mounted on a mobile, remote platform. Both mussel and fish systems are inhibited by the use of a single species, and mussels systems have been found to be less sensitive because of the closure of their valves. The MFB uses a variety of species simultaneously as test organisms, offering a large sensor target and a vast range of sensitivity (what one species may fail to detect, others might), thus broadening the surveillance power. This system also is mobile, and a chemical multiprobe can be added if required [22]. The RUSS Dynamic Profiler (Apprise Technologies, Duluth, MN, USA) is independently powered, communicates with a shore-based computer, and can use a variety of probes, but so far, it lacks a biomonitoring component. Details regarding sensitivity and species-number information for this system are not yet available. Moreover, mobile platforms need to be constructed with self-supporting energy supplies, and automated sample-grab technology must be included to capture samples during an alarm event so that more sophisticated analysis and measurements can be carried out in a laboratory.

NEW DEVELOPMENTS AND APPLICATIONS IN ORGANISMIC BIOMONITORS

Nonoptical systems

The Aquatic Biomonitor™ system described by Shedd et al. [21,23–25] and by van der Schalie et al. [26,27] is a self-contained unit that can be used remotely as an effective means of source-water protection. The toxicity aspect continually measures gill purge, ventilatory, and body movement in the common bluegill (fish). This system suspends the fish in a tank and exposes it to a continuous flow of water. Physical and chemical parameters are measured simultaneously, and the data are transmitted to a central command station. When fish behavior or water-quality parameters change suddenly, samples are grabbed automatically, and an autodialer notifies the station. The Aquatic Biomonitor system has been field tested at Fort Detrick (MD, USA) and the New York City Department of Environmental Protection (NY, USA) for more than two years. Although such alarms are rare, the system has detected toxic events at both sites. An expert system that models fish behavior is now being used to further refine the alarm process.

The MFB, based on quadropole-impedance conversion, is a multispecies, on-line biomonitor for all moving aquatic and terrestrial animal species, recording the behavior of organisms in different media, such as unfiltered water, soil, and sediment. The MFB consists of flow-through test chambers with the individual test organisms, impedance recorder, and PC unit with specific software for data acquisition, analysis, and e-mail alert (Fig. 1). So far, approximately 35 different animal species have been used in the MFB [22], the smallest organism being the nematode *Caenorhabditis elegans* [28]. The measurement unit is the test chamber, a flow-through acrylglass tube with two pairs of stainless-steel electrodes attached to the chamber walls. One pair of electrodes generates a high-frequency alternating current, and the second pair of electrodes measures changes in the impedance, which are produced by a moving animal in the electrical field [3,22]. Vertical and horizontal arrangements of chambers can be used, such as a three-level chamber to cover the three different compartments (sediment, water, and air) [22] or recording vertical movements from phototaxis of zooplankton [29]. Because this measurement principle is nonoptical, the MFB does not affect the normal behavior of the animal because of additional light sources; prefiltering the test water is unnecessary. This system also can be used to monitor animals in soil and sediment. The MFB software analyzes the recorded behavior with a discrete Fast Fourier Transformation, presenting a typical behavioral fingerprint of locomotive and ventilatory behaviors. Alarm generation is based on different mathematical methods using fixed thresholds (moving average method) and dynamic thresholds (jump detector, slope operator, and Double Sigma detector) as well as neurofuzzy approaches.

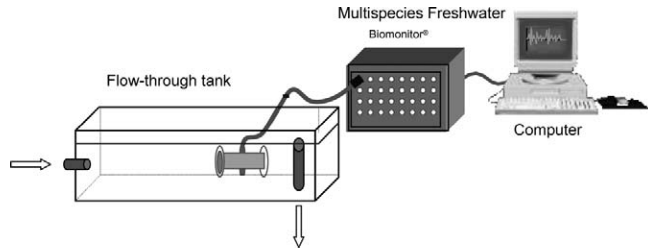

Fig. 1. The Multispecies Freshwater Biomonitor® (MFB) test system (LimCo International, Ibbenbueren, Germany), which consists of the flow-through tank, the test chambers connected to the MFB instrument, and the computer.

Ingram et al. [30] presents a toxicity early warning system (TEW) for pulp and paper effluent. A local mill in Thunder Bay (ON, Canada), in collaboration with Lakehead University, is engaged in the development of an industrial biomonitoring system to prevent the possibility of toxic events. This system, referred to as the TEW, is currently being constructed to monitor continuously, in real time, the toxicity of pulp mill effluent to aquatic organisms before the effluent enters the environment. The TEW uses the behavioral response of rainbow trout (*Oncorhynchus mykiss*), an ecologically sensitive species, as an indicator of potential toxicity. By monitoring the behavior of captive trout exposed to a geometric series of pulp mill effluent, a behavioral ''response-to-toxicity-concentration'' library can be developed. Specific behavioral responses, such as cough frequency, whole-body movement, ventilatory frequency, and ventilatory depth, can be used in correlation with tested toxicity levels to monitor pulp mill effluent as it travels through the outflow pipes of the mill. The recording principle is based on a Wheatstone bridge circuit, which requires the implementation of two tanks. This system compares and measures the resistance of an unknown current (passes through the tank containing a fish) against a known current (passes through the tank without fish) [31]. The results are displayed as the degree of difference between the two currents via a pulse frequency.

Both tanks are part of a flow-through system in which untreated river water flows at a steady velocity through both tanks, later replaced by a specific concentration of effluent. A current in the microvolt range is passed through both tanks, initiating at a stainless-steel electrode positioned at the head

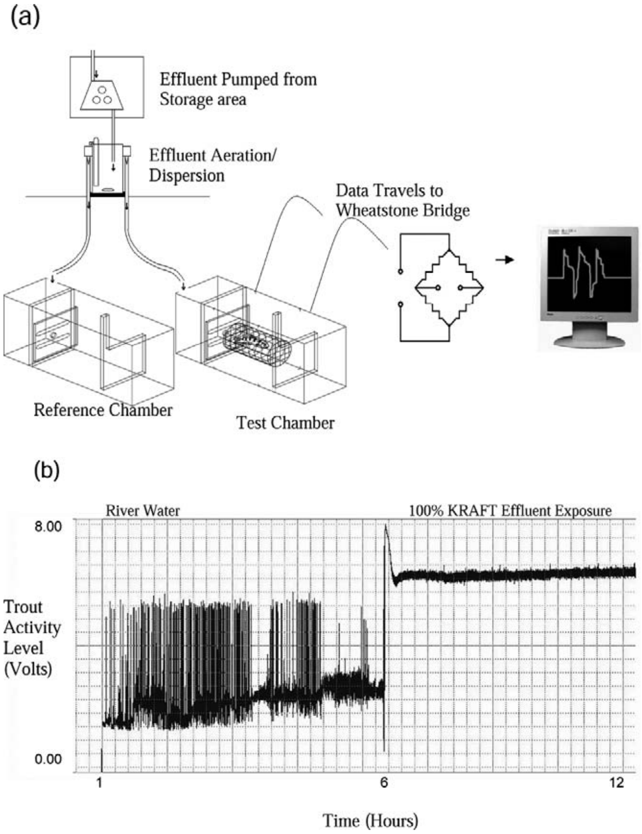

Fig. 2. (**a**) Scheme of the Toxicity Early Warning System (TEW) schematic, displaying inflow from effluent storage area to Wheatstone circuitry system (Lakehead University, Thunder Bay, ON, Canada). Two tanks are used for every trout. The Wheatstone bridge measures the degree or scale of difference between the electrical currents passing through these two tanks and displays the results as a waveform on a computer screen. (**b**) TEW: Exposure to 100% effluent. The TEW used rainbow trout (*Oncorhynchus mykiss*) during its testing. Rainbow trout were exposed to a 100% dilution of effluent from a local pulp and paper mill, the results of which show fish behavior before effluent exposure (6 h) and fish behavior to effluent exposure (6 h).

of the tank and exiting through an electrode positioned at the foot of the tank. In the fish test tank, the fish will swim, breathe, and move. This movement inhibits the flow of the current as it passes from one electrode to the other. The frequency and/or scale of the fish movement will be reflected in the amount of energy expended by the current as it passes through the tank. This energy expenditure will be compared to the current passing through the reference tank, and the degree of difference between these two currents, recorded in real time, will be amplified and electronically displayed on a computer monitor (Fig. 2).

Optical systems

Kang et al. [32] presents a BEWS based on fish using the Japanese medaka (*Oryzias latipes*) to monitor mixtures of toxic chemicals in water supplies (Fig. 3). Several tests were conducted to evaluate the detection ability of the medaka sensor for single substances (e.g., potassium cyanide [KCN], phenol, and pesticides) as well as for chemical mixtures. The test system is comprised of a test chamber with a continuous-flow system (400 ml/min), in which the water temperature was kept at 22 ± 1°C (mean ± standard deviation throughout). Medaka (four to six months posthatching; total length, 3.2 ± 0.25 cm; weight, 0.3 ± 0.06 g) were exposed to nominal concentrations

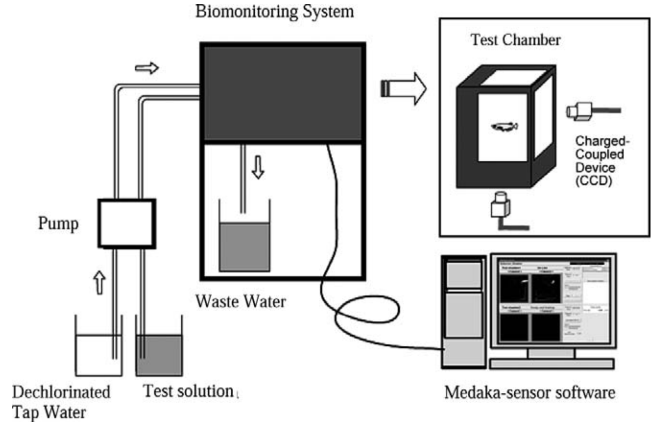

Fig. 3. Scheme of the Medaka biomonitor system (Seiko Electric, Hakata-ku, Fukuoka, Japan).

of single chemicals and a mixture of chemicals. The effects of the test chemicals on the swimming behavior of medaka were observed for 1 h. For the first time, three-dimensional data from two cameras, exposed in front and at the side of the aquarium, were analyzed to detect abnormal behaviors, including rapid transfer, staying just below the water-surface layer of the aquarium, and death. At the start of the test, only dechlorinated water was run through the test chamber for 30 min, and then the test solution was released into the test chamber for 60 min. In addition, medaka were exposed to three mixtures of KCN and phenol and a mixture of three pesticides (fenitrothion, benthiocarb, and diazinon). Also, the mixture toxicity test results were compared with the results of the individual toxicity tests for each chemical. Results of this study are summarized in Table 1. It was concluded that the detection time was faster and more accurate for mixed chemical exposure. Data were analyzed by fractal analysis [33].

Leem et al. [34] used image-processing systems to study the effect of a pesticide on *Daphnia* behavior. Four movement patterns frequently observed before and after the treatment were selected, quantified, and compared. Quantitative investigations on the variables of the movement tracks suggested the usefulness of response behavior as a monitoring tool for environmental assessment. This *Daphnia* biomonitor could be used for evaluation of toxicity reduction in groundwater remediation through application of a series of preconcentrated groundwaters [35].

CAPMON (CapMon, ALS, Elleaer 8, Herlev, Denmark) is a computer-aided system that monitors heart rate and movements in mussels and crustaceans. The sensors being attached to the shell or carapace of the test animal [36].

The light-emitting response of bacteria already has been used in the Microtox™ Toxicity Test (Strategic Diagnostics, Newark, NJ, USA) and in the Lumi-CELL ER bioassay, which is based on transgene bacteria that are especially sensitive to endocrine-disrupting chemicals [37]. However, these are off-line biotests rather than on-line biomonitors, because they do not run continuously and need to replace the organisms after each measurement. Appels [38] stressed that a microbial toxicity assay (Microtox) opens possibilities for application in wastewater and cooling water as well as for a screening tool of toxicity problems. Anako et al. [39] described two separate strains of luminescent bacteria: Both bioluminescence as well as oxygen uptake were effective early warning indicators for the protection of activated sludge systems.

Table 1. Time until detection of abnormal behavior during the 60-min exposure of fish in the medaka sensor system[a]

		Time until detection during the 60-min exposure test by behavior index		
Test chemicals	Nominal concn. (mg/L)	Staying surface[b]	Rapid transfer	Death
KCN	0.5	—	—	—
	1	34 (1)	—	—
Phenol	6.25	—	—	—
	12.5	—	36 (1)	—
KCN + phenol	0.5 + 6.25	—	—	—
	0.5 + 12.5	—	33.3 ± 10.1 (3)	—
	1 + 6.25	—	34.5 ± 7.6 (4)	—
	1 + 12.5	—	28.5 ± 5.1 (4)	50 (1)
MEP	10	—	—	—
Benthiocarb	10	—	—	—
Diazinon	10	—	—	—
MEP	20	53 ± 6.1 (3)	54 (1)	
Benthiocarb	20	39.5 ± 0.7 (2)	—	
Diazinon	20	—	—	—
MEP + benthiocarb	10 + 10	35.5 ± 10.2 (4)	37 ± 9.9 (2)	—
Benthiocarb + diazinon	10 + 10	45 ± 0 (2)	40 (1)	—
Diazinon + MEP	10 + 10	38.3 ± 7.2 (3)	36 (1)	—

[a] KCN = potassium cyanide; MEP = fenitrothion. Numbers in parentheses represent medaka that showed abnormal behavior, with the abnormality detected in the medaka sensor system during 60 min of exposure. Each test was conducted four times using different medaka.
[b] Staying surface refers to the behavior of staying just below the water surface.

The μMac-ToxScreen™ (Checklight, Qiryat-Tivon, Israel) [40,41] is mentioned here as an innovative, automated, on-line water-quality monitoring system that uses luminescent bacteria as biosensors to detect μg/L concentrations of toxic organic and inorganic chemical pollutants in surface water or groundwater as well as in raw and treated drinking water (Fig. 4). The ToxScreen bioassay uses a renewable suspension of luminescent bacteria. When the bacteria are automatically mixed with a water sample, their light production, which is tied directly to cell respiration and other critical metabolic pathways, is decreased in proportion to the toxicity in the sample. The analytical part of the instrument is an automatic analyzer that uses a patented technology, called Loop Flow Analysis, that is widely used in a variety of on-line chemical analyzers for water quality [42] stand-alone use, surface water, groundwater [43], and seawater [44]. At 14-d intervals, the instrument is resupplied with a fresh inventory of liquid assay buffers and a freshly hydrated suspension of the freeze-dried luminescent bacteria. Automatic safeguards have been engineered into the system to assure reagent and data quality as well as appropriate instrument functioning. The instrument also is equipped with autocalibration features to assure reliable instrument performance; microprocessor-based system controls provide data storage, data downloading, real-time communication with a remote computer, and user-adjustable alarm levels. To allow the automatization of the bioluminescence-based bioassay, the standard Loop Flow Reactor [45] was modified to include a photomultiplier tube with internal counting to enable real-time evaluation of the changes in bacterial luminescence.

In Table 2, the concentration at which 50% inhibition is

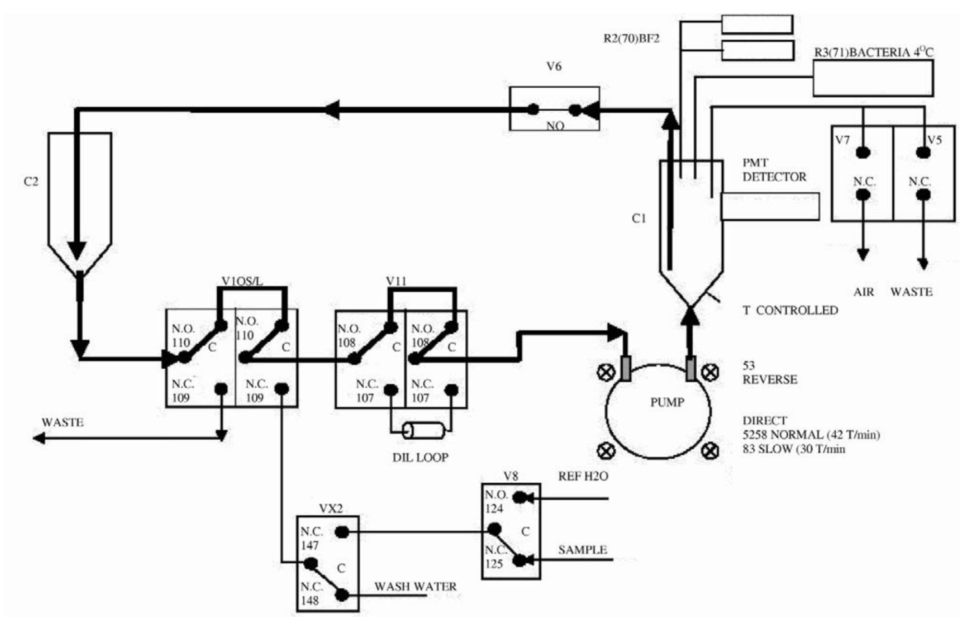

Fig. 4. Scheme of the ToxScreen system (CheckLight, Qiryat-Tiv'on, Israel).

Table 2. Inhibitory concentration leading to 50% light decay (IC50) and lowest detectable level (LDL) values (mg/L) obtained for common toxicants using ToxScreen-II and Microtox Tests™ (in parentheses; CheckLight, Qiryat-Tiv'on, Israel)[a]

Toxicant	IC50 (mg/L)	LDL (mg/L)
Tested in Pro-Metal Buffer		
Arsenic (V)	0.15 (1.5)	0.017 (NF)[a]
Cadmium	0.04 (41.4, 106)	0.024 (20)
Lead	0.15 (11 ,0.6)	0.06 (0.6)
Selenium	1.2 (NF)	0.3 (33)
Copper	0.11 (8)	0.01 (0.8)
Tested in Pro-Organic Buffer		
2,4,5-T	0.03 (52.2, 158)	0.01 (NF)
2,4,-Dinitrophenol	0.09 (10.6)	0.025 (NF)
Arsenic (III)	1.7 (NF)	0.75 (7.8)
DDT	0.07 (7, 13.8)	0.006 (NF)
Potassium cyanide	0.45 (8.4)	0.1 (0.73)
2,4-D	0.03 (5.7, 100)	0.01 (NF)
Flouroacetate	0.5 (NF)	0.05 (50)
Trinitrotolouene	0.4 (NF)	0.03 (19.78)

[a] Microtox median effective concentration and LDL in parentheses are from the Strategic Diagnostics website (http://www.sdix.com). NF = not found in the literature; 2,4-D = 2,4-dichlorophenoxy-ethanoic acid; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

reached for the spiked chemicals were defined and graphically calculated as the inhibitory concentration (mg/L) at which luminescence is inhibited by 50% as compared to the clean-water control sample. The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percentage inhibition significantly greater than that in the negative control. An inhibition was significantly greater than that in the negative control if the average inhibition plus or minus the standard deviation did not include zero. Data generated with the ToxScreen toxicity test were compared to data generated with the Microtox luminescence toxicity test as reported in the literature [46–49].

NEW APPROACHES TO DATA ANALYSIS

On-line data represent repeated measurements of the same organisms to detect significant changes in organism performance during exposure; hence, data are time-dependent. Time-series analysis is the most appropriate statistical approach to smooth and describe a data series and to predict future values as well as detect deviations or trigger alarms. Mostly autoregressive or moving average models are applied either alone or in combination to time-series data. Alarm detection can be done using either fixed or dynamic thresholds. The first biomonitors used fixed, predefined thresholds, generating an alarm if the threshold was surpassed. However, dynamic alarm thresholds consider long-term drift and varying data variability (cases in which false alarms might be raised). Examples of dynamic alarm algorithms, as described by Blohm and Lechelt [50], are the Double Sigma detector, the Adaptive Slope-Adjusted Hinkley detector, and the Slope detector. The Adaptive Slope-Adjusted Hinkley detector has been implemented in several existing biomonitors, and the combination of the three methods recently has been programmed and tested in the MFB, making the Double Sigma detector the most reliable detector and the easiest system to operate (A. Gerhardt, unpublished data). Recent developments in artificial intelligence and expert systems are being implemented and tested in several biomonitors, such as the Aquatic Biomonitor for fish by van der Schalie et al. [27] and the MFB.

The TEW system by Ingram et al. [30] uses the coefficient of variance to determine the degree to which trout behavior may vary from untreated river-water exposure periods to that of effluent exposure periods. An analysis of variance will generate the mean, variance, and standard deviation for the degree of trout activity, and all significant behavioral responses will be flagged and stored in a toxicity library.

The video-based fish monitor by Kang et al. [32,33] analyzed data using fractal dimension recorded every 5 min. The results of the fractal dimension analysis for the three-dimensional data from exposure tests showed no significant differences, and no abnormalities in the water were detected within a 1-h time period. Therefore, fractal dimension analysis might not be useful for the medaka test system.

MONITORS FOR SOIL AND SEDIMENT

Toxicity testing and on-line biomonitoring should consider that many aquatic benthic species need both sediment and water for their development, either at different life stages during their life cycle (e.g., insects and crustaceans) or simultaneously (e.g., oligochaetes).

Recording behavior without affecting the organism survival in the soil and sediment can be accomplished only through nonoptical methods because sediment and soil organisms often are negatively phototactic and artificial light sources might alter their behavior. Organisms hidden in the soil cannot be recorded quantitatively using video-based technology without affecting the animals (e.g., by labeling). Therefore, a new, two-compartment chamber filled with sediment (bottom) and water (top) was designed for the MFB to record the behavior of chironomid larvae. Behavior showing chironomid locomotion through the water and abdominal pumping (undulations) in the sediment was recorded. Chironomids frequently move from one compartment to the other, but the signals in each compartment were clearly separated (Fig. 5). The development of this system offers new applications for ecologically relevant sediment and soil toxicology and biomonitoring with the MFB.

A new apparatus based on radiolabeled earthworms and x-ray tomography moving within a three-dimensional space in a soil core has been developed [51]. Unfortunately, this system only provides behavioral snapshots and requires soil core scanning, which makes the system expensive.

SCIENTIFIC RESEARCH: LINK WITH OTHER BIOLOGICAL PARAMETERS

Biomonitors use either behavioral or physiological responses as an indicator of stress [1]. However, are these behavioral responses relevant for other biological processes (e.g., reproduction, abundance, and predation), and can these responses be linked to suborganismal biomarkers? These important research topics are necessary for calibrating behavioral responses and biomonitors in their ecological relevance and their weight of evidence for water-, soil-, and sediment-quality biomonitoring.

The MFB, for example, has been used to detect pollution by recording behavior, survival, and bioaccumulation under exposure to acid and acid mine drainage [52–54], single metals [4,55], and unfiltered effluents from wastewater treatment plants [56–59] in laboratory as well as in situ (in-stream) with link to ecosystem structure. The MFB also has been used in combination with morphological or biochemical biomarkers using different insect, crustacean, and fish species. The MFB has been applied in marine aquaculture [60] as well as for

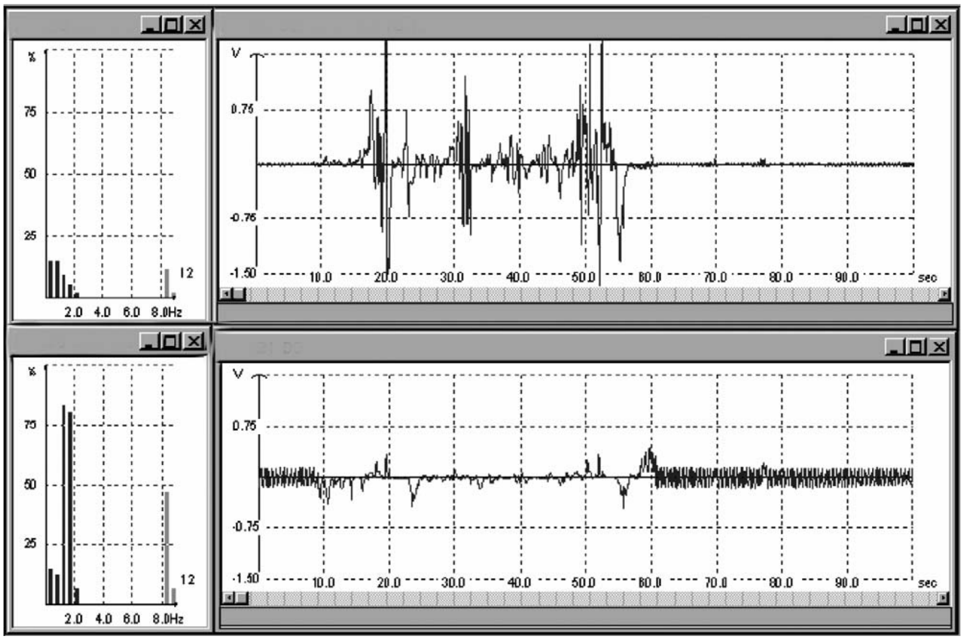

Fig. 5. Behavior of the instar *Chironomus riparius* larva in a novel two-compartment test chamber for the Multispecies Freshwater Biomonitor® (MFB; LimCo International, Ibbenbueren, Germany): The upper compartment contains water, and the lower compartment is filled with sediment. Upper right: Signals of locomotion (V) over time (s); upper left: histogram of the signal frequencies (Fast Fourier Transformation [FFT]); lower right: signals of ventilation (pumping with the abdomen; V) over time (s) in the sediment; lower left: corresponding FFT with a typical signal frequency for ventilation of 1.5 to 2 Hz. Note the movement of the larva from one compartment to the other at approximately 15 and 55 s.

long-term monitoring of polluted surface water from the River Rhine with *Gammarus pulex* and *Daphnia magna* [58].

CONCLUSION

In summary, the development of new biomonitoring instruments is ongoing and should focus on the following topics: Optimization of alarm algorithms, use of indigenous test species for ecologically based biomonitoring purposes (e.g., protection of aquatic ecosystems), and development of self-supporting multimetric platforms that can be used directly in situ (in-stream) in remote areas. These platforms should consist of biomonitors and pollutant-specific biosensors. Biomonitoring systems should also include soil and sediment toxicity assessment and monitoring using basic ecotoxicological research with on-line biomonitors to establish causal links over several biological organization levels, especially linking alarms to effects on the population and ecosystem levels.

To establish on-line biomonitoring worldwide in water-quality control, the legislative basis for these tests needs to be established in many countries. In addition, test systems have to be developed, compared, selected, and standardized, and the costs for investment and maintenance need to be reduced. For example, the International Organization for Standardization is considering a standard for on-line chemical/physical sensors. Similar standardization protocols should be adopted for the BEWS as well.

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