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as the autochthonous bio-sensor: research and application as a pre-alarm system

Real time recording of the quality of surface water through *Unio elongatulus* as the autochthonous biosensor: research and application as a pre-alarm system

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Introduction

Correct management of the drinking water sector makes it necessary to constantly improve the quality of water at the same time as containing production costs, in compliance with the current legislation concerning the protection of waters (legislative decree no. 152/99). The most profitable way to monitor the quality of water, both as a source of supply and as the supplied product, envisages supervision, at the same time and, where possible, prior, to chemical-physical monitoring, by means of biological tests and/or bio-sensors, capable of quickly identifying any changes in water resources and avoiding undesirable effects on living systems and, in particular, on man.

With this in mind, a biological pre-alarm system (Mosselmonitor® , Delta Consult – Holland) was installed, in October 1996, at the monitoring station on the river Po, belonging to A.A.M. Torino SpA to monitor water entering the water purification plants. This system uses eight bi-valve molluscs belonging to the *Unio elongatulus* species, autochthonous of the river Po.

Unio elongatulus is one of the long life-cycle biological sensors, and provides measurable responses that can be attributed to effects of acute toxicity. Under “normal” conditions the valves are open for breathing and feeding, while, in the presence of stimuli that are perceived as hostile, they can close for a long time (this event was recorded by the instrument as alarm C, *closed* condition) or increase the opening and closing frequency (alarm A, *hyper-activity*). As a further alternative, the organisms can decrease the volumes of water filtered per time unit (alarm D, *slowing down*). The positions of the valves are recorded via an electro-magnetic induction system and the electronic sensor consists in two coils stuck onto the valves; these elements are not “invasive” for the molluscs because they are not inserted inside them and do not cause them additional stress.

The aims of the current research are as follows:

- to assess the sensitivity of the system to the pollutants concerned, in other words to substances to which the considered water resource, i.e. the river Po, are vulnerable;
- to design a system that combines good sensitivity with a minimum emission of false alarms.

The correct management of the instrument, i.e. its appropriate maintenance and the necessary planning of the method to be used in analysing the data obtained, is of particular importance in constant monitoring.

Toxicity tests

The concentrations of the investigated pollutants that were tested were selected on the basis of CMAs (Maximum Permitted Concentration) established by the regulations in force for waters destined for human consumption (DPR 236/88). An average of four concentrations were prepared for each pollutant, administered in increasing order in the water subjected to monitoring, in order to verify, during a suitable period of time (usually 24 hours), if and when alarms occur (LOEC: lowest observed effect concentration), and their type and duration. The results obtained are given in the following table.

Pollutant	CMA	LOEC	Type of alarm generated
Cadmium			
Copper			
Mercury			
Zinc			
Nickel			
Lead			
Aluminium			
Ammonia			
Nitrate			
Nitrite			
Laurilsulphate			
Terbumeton			

(*) as the sum of anti-parasites and similar products

The influence of environmental and operating conditions on monitoring

It was shown that the behaviour of the bi-valves depends to some extent on certain environmental parameters, such as the amount of dissolved oxygen and the temperature of the water; minor influence seems to be exercised by turbidity, the speed of flow of the water and mechanical stimuli (noises, vibrations). Moreover, it was also discovered that the activity of the molluscs follows a circadian rhythm.

The importance played by dissolved oxygen in maintaining an environment that is favourable for the life of the bio-sensors was observed during fluctuations following natural events (such as, for

example, the considerable increase in the turbidity of water as a result of meteorological phenomena), but it was also verified through scheduled surveys. Observations under conditions of hypoxia were carried out in June 1997 and May 1998, and verification tests were carried out in August, October and November 1998, from March to June 1999 and from January to March 2000. When the increase in turbidity is added to the hypoxia, *Unio elongatulus* tends to close its valves and therefore, to present a clear isolation reaction. Only rarely were type A alarms found, in such conditions, and they were, however, short; (2-4 hours), type D alarms were more frequent, attributable to temporary attempts to “test” environmental conditions: recordings of type C alarms, were, however, predominant, lasting as long as 15 hours a day, especially during the summer. As far as the speed of flow of the water is concerned, the reduction in flow can generate short alarms (lasting for less than 2 hours) in certain situations.

To overcome the problems encountered in conditions of hypoxia, it was considered appropriate to introduce an aeration system in the monitoring tank, an expedient that was used from November 1998. This turned out to be useful both during pollution simulation tests (i.e. tests where the toxic substances concerned are added to the measuring chamber), and during temporary increases to turbidity, while it did not have any particular influence on normal monitoring conditions, at the most, avoiding the appearance of false alarms.

Optimisation of acquisition parameters

Temperature must be taken into consideration when calibrating the method to be used in acquiring data as this has a strong influence on the behaviour of the tested molluscs. In fact, in spite of the fact that daily fluctuations in temperature do not usually exceed the interval of two degrees and therefore do not cause anomalies in the behaviour of the bio-sensors, seasonal variations have shown the need to identify threshold-values and therefore, in order to avoid false alarms, acquisition parameters should be modified to correspond with these.

For alarm C, for example, three temperature intervals were identified that are capable of changing the number of bio-sensors necessary for signalling the alarm: during summer months ($T=20-24^{\circ}\text{C}$) the longer photo-period favours greater filtering activity. Therefore the simultaneous closure of four out of eight specimens is sufficiently significant. Vice versa, during the winter ($T=<10^{\circ}\text{C}$), all vital functions slow down, therefore, in order to avoid an excessive number of false alarms, the sensitivity of the system must be reduced, increasing the number of closed sensors to six out of eight in order to cause the alarm. During intermediary seasons ($T=10-20^{\circ}\text{C}$) the optimal number of closed testers is five out of eight.

Type D and A alarms are less prone to seasonal variations.

A detailed analysis of the data and the subsequent changes made to the method of acquiring results has led to a considerable reduction in the signalling of undesired alarms. Between the end of 1996 and the beginning of 1997 an over-sensitive recording protocol resulted in 324 hours of alarms in just four months, most of which were unjustified: this was due to the application of *standard* acquisition parameters, suggested by the supplier, that had been suitable in similar situations in which, however, another bio-sensor had been used, *Dreissena polymorpha*; this species is the tester most commonly used to bio-monitor fresh surface waters, but it is not autochthonous of Italian fresh waters. The experience gained from this study of the behaviour of *Unio elongatulus* and on how the system works has made it possible to reduce the total number of unjustified alarms, during 1998-99, to less than 6%, using the toxicity tests as a reference.

Monitoring the quality of the water of the river Po

The suitability of the Mosselmonitor® as a pre-alarm system has been verified in actual circumstances during two episodes of pollution of the river Po: during the first episode (September 1998) the presence of oily patches on the river was recorded and the instrument registered a C alarm, lasting five hours, and a D alarm, lasting six hours. In January 1999, on the other hand, surfactants were encountered in the river waters and, during the same days, a C alarm, lasting fifteen hours, was registered.

Conclusions

In the light of the results obtained, the constant use of the bio-monitoring system with *Unio elongatulus* was shown to be advantageous, both in terms of the characteristics of the instrumentation and those of the bio-sensor used.

The characteristics of this instrumentation, in fact, are that it operates constantly, it has a high degree of automation and requires minimum maintenance: it is also extremely versatile because it can be calibrated at various levels of sensitivity.

Unio elongatulus is an organism that has many of the characteristics of the “ideal bio-sensor”, because it is autochthonous of internal Italian waters, it is easy to find and to handle and does not need special care. Moreover, it has demonstrated a suitable sensitivity to the use in question both in pollution simulations and under normal monitoring conditions. The large amount of data acquired has also made it possible to considerably increase knowledge about the ecology of this mollusc. Further research developments can be identified in the simulation of contamination to water resources due to mixed toxic flows with a typical composition, in order to assess any synergetic or additive effects, and also in the application of the instrument in conditions of strong biological

pollution. In fact, it would be extremely interesting to investigate the responses of bio-sensors not only in the presence of xeno-biotic substances but also in bio-toxicity situations, such as, for example, the strong eutrophication of a surface water body, in order to evaluate not only the quality of the water but the quality of the considered habitat, with particular reference to slow-flowing water.