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# Electrochemical DNA biosensor for analysis of wastewater samples

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**Abstract**

The application of a disposable electrochemical DNA biosensor to wastewater samples is reported. The DNA biosensor is assembled by immobilising double-stranded calf thymus DNA on the surface of a disposable, carbon screen-printed electrode (SPE). The oxidation signal of the guanine base, obtained by a square wave voltammetric scan, is used as analytical signal. The presence of compounds with affinity for DNA is measured by their effect on the guanine oxidation. The comparison of the results with a toxicity test based on bioluminescent bacteria has confirmed the applicability of the method to real samples.


*Keywords:* DNA biosensor; Screen-printed electrodes; Wastewater samples; Toxalert® 100

## 1. Introduction

One of the potentially major applications of a DNA electrochemical biosensor [1–3] could be the testing of water, food, soil and plant samples for the presence of pathogenic microorganisms and for the presence of analytes (carcinogens, drugs, mutagenic pollutants, etc.) with binding affinities for DNA. The binding of small molecules to DNA and generally DNA damage has been described through the variation of the electrochemical signal of guanine [1–9]. Intercalation has been observed with planar aromatic molecules, and classical compounds are daunomycin, ethidium bromide, acridine dyes, etc. [10–12]. Alternatively, some positively charged compounds can bind to the DNA via electrostatic interaction with the phosphate backbone [13].

In this paper, we report the DNA biosensor analysis of wastewater samples collected in different Italian towns. The biosensor was developed by immobilizing double-stranded calf thymus DNA at a fixed potential onto the working electrode surface of a screen-printed electrochemical cell. The calf thymus DNA biosensor was then immersed in the sample solution. After 2 min of interaction, a square wave voltammetric scan was carried out to evaluate the oxidation signal of guanine residues.

We found interesting correlations with a commercial toxicity test (Toxalert® 100 obtained from Merck) based on the bioluminescence inhibition of the bacterium *Vibrio fischeri*. The *V. fischeri* bioluminescence test is a standard toxicity test widely recognised in many legislations [14]. The bioluminescence is directly linked to the vitality (the metabolic status) of the bacterial cell. A toxic substance causes changes to the cellular state, i.e. damage to the cell wall, cell membrane, the electron transport chain, enzymes, cytoplasmatic constituents; these changes are rapidly reflected in a decrease in the bioluminescence signal that can be measured with a photomultiplier in a luminometer.

More than 20 aquatic toxicity tests are reported in the 20th edition of the *Standard Methods for the Examination of Water and Wastewater* [15]; however, we chose the Toxalert® 100 assay since bacterial bioluminescent assays are rapid, reproducible and cost-effective tests, and also because many of the other bioassays are not routinely applicable.

The author proposes the electrochemical DNA biosensor as a screening device for the rapid bioanalysis of environmental pollution; this is a different concept from proposing this device as a toxicity test since, in the term toxicity, more complex reactions are involved than the simple binding of a molecule to another molecule (such as the binding of toxicants to the DNA molecules). Nevertheless, in our opinion, each sample that determines a variation in the DNA biosensor response is a sample that could contain possible dangerous pollutants, and thus must be monitored more carefully.

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Electrochemical DNA biosensors are in line with the requirements of in situ screening measurements, since all the equipment needed for the DNA biosensor electrochemical analysis is portable.

## 2. Materials and methods

### 2.1. Apparatus and reagents

Electrochemical measurements were performed with a μAutolab electrochemical analysis system with a GPES 4.5 software package (Ecochemie, Utrecht, Holland), in connection with a VA-Stand 663 (Metrohm, Milano, Italy). The planar, screen-printed electrochemical cell (1.5 cm × 3.0 cm) consists of a graphite working electrode, a graphite counter electrode and a silver pseudoreference electrode. The procedure and reagents to make screen-printed electrodes (SPEs) were reported elsewhere [16]. The graphite screen-printed working electrode surface is 3 mm in diameter. Each electrode is disposable.

The luminometer Toxalert® 100 and all the reagents used to perform cytotoxicity tests were kindly provided by Merck (Darmstadt, Germany).

Sodium acetate, sodium chloride, acetic acid and potassium chloride were purchased from Merck.

Double-stranded calf thymus DNA was obtained from Sigma (Milano, Italy).

### 2.2. Real sample collection

The Water Supply Companies provided the wastewater samples from a wastewater treatment plant of the towns of Firenze, Parma, Bologna and Roma.

Wastewater treatment plant sampling was performed before (influent) and after (effluent) the treatment. All the plants used biological treatment.

Treatment-plant operators collected influent and effluent samples as 24-h composite samples. Samples were stored in Pyrex borosilicate glass bottles at 4 °C. Before the analysis, the wastewater samples were filtered using single-use syringe filters, pore size 0.45 μm, obtained from Sartorious (Firenze, Italy). To perform the analysis with the Toxalert® 100, the pH of the samples were adjusted to the neutral range; then the samples were ready to be tested. To analyse the samples with the DNA biosensor, concentrated acetate buffer (2 M) solution was added to 5 ml of sample obtaining a final concentration of 0.25 M.

Sample preservation was accomplished by storing bottles at 4 °C immediately after sampling and during the transportation.

### 2.3. DNA biosensor

The electrode surface was pretreated by applying a potential of +1.6 V for 3 min.

The biosensor was developed by immobilising double-stranded calf thymus DNA at fixed potential (+0.5 V vs. Ag screen-printed pseudoreference electrode for 120 s) onto the screen-printed electrode surface. During the immobilisation step, the strip was immersed in acetate buffer solution containing 20 ppm of double-stranded calf thymus DNA. Then a cleaning step was performed by immersion of the biosensor in a clean acetate buffer solution, at open-circuit condition. The incubation step was performed by placing 20 μl of the sample solutions onto the surface of the graphite working electrode. After 2 min the sensor was washed, immersed in acetate buffer and a square wave voltammetric scan was carried out to evaluate the oxidation of guanine residues on the electrode surface. The area of the guanine peak (around +1.0 V vs. Ag screen-printed pseudoreference electrode) was measured. Potentially toxic compounds present in water or wastewater samples were evaluated by changes of the electrochemical signal of guanine. We have estimated the DNA modification with the value of the percentage of response decrease (% R), which is the ratio

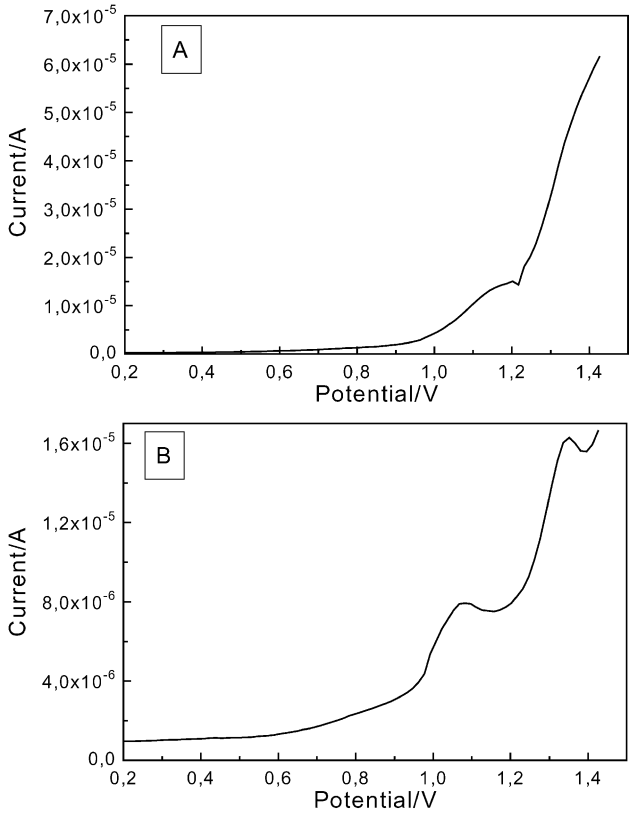


Fig. 1. Oxidation of calf thymus double-stranded DNA immobilised onto the electrode surface without (A) and with (B) the electrode surface conditioning step. The oxidation peak of the guanine base is around +1.0 V vs. Ag pseudo reference SPE. In (B), using conditioned electrode, note, the peak of the adenine base (around +1.3 V vs. Ag pseudo reference SPE). DNA immobilisation: 2 min at +0.5 V vs. Ag pseudo reference SPE; square wave parameters: frequency 200 Hz, step potential 15 mV, amplitude 40 mV.

of the guanine peak area after the interaction with the sample ($GPA_s$), and the guanine peak area after the interaction with the buffer solution ($GPA_b$): % R=[($GPA_s$/$GPA_b$) − 1] × 100.

The analysis of a sample can be carried out in 8 min.

The supporting electrolyte for the voltammetric experiments and for any step in the biosensor set up was acetate buffer 0.25 M pH 4.7 and KCl 100 mM.

Square wave voltammetry parameters were frequency, 200 Hz; step potential, 15 mV; amplitude, 40 mV; potential range +0.2V ÷ 1.4 V vs. Ag pseudoreference electrode.

### 2.4. Toxalert® 100 procedure

In all the experiments the osmolality of all standard and sample solutions were adjusted to 2% NaCl for optimal reagent performance. To express the toxicity we have used the percentage of inhibition (% I), determined by comparing the response given by a saline control solution to that corresponding to the sample as a function of the incubation time. For all the experiments we used an incubation time of 30 min. The bioluminescence inhibition is determined by:

$$I\% = [(I_{0c} - I_f)/I_{0c}] \times 100$$

where $I_{0c}$ is the corrected value of luminescence intensity of the control test suspension in relative luminescence units (RLU) and $I_f$ is the luminescence intensity of the test sample after the contact time of 30 min in RLU [17].

## 3. Results and discussion

### 3.1. Biosensor development

The biosensor test format involves four main steps: the electrochemical conditioning of the electrode surface, the calf thymus double-stranded DNA immobilisation, the interaction with the sample solution and the electrode surface interrogation. All the measurements were carried out in

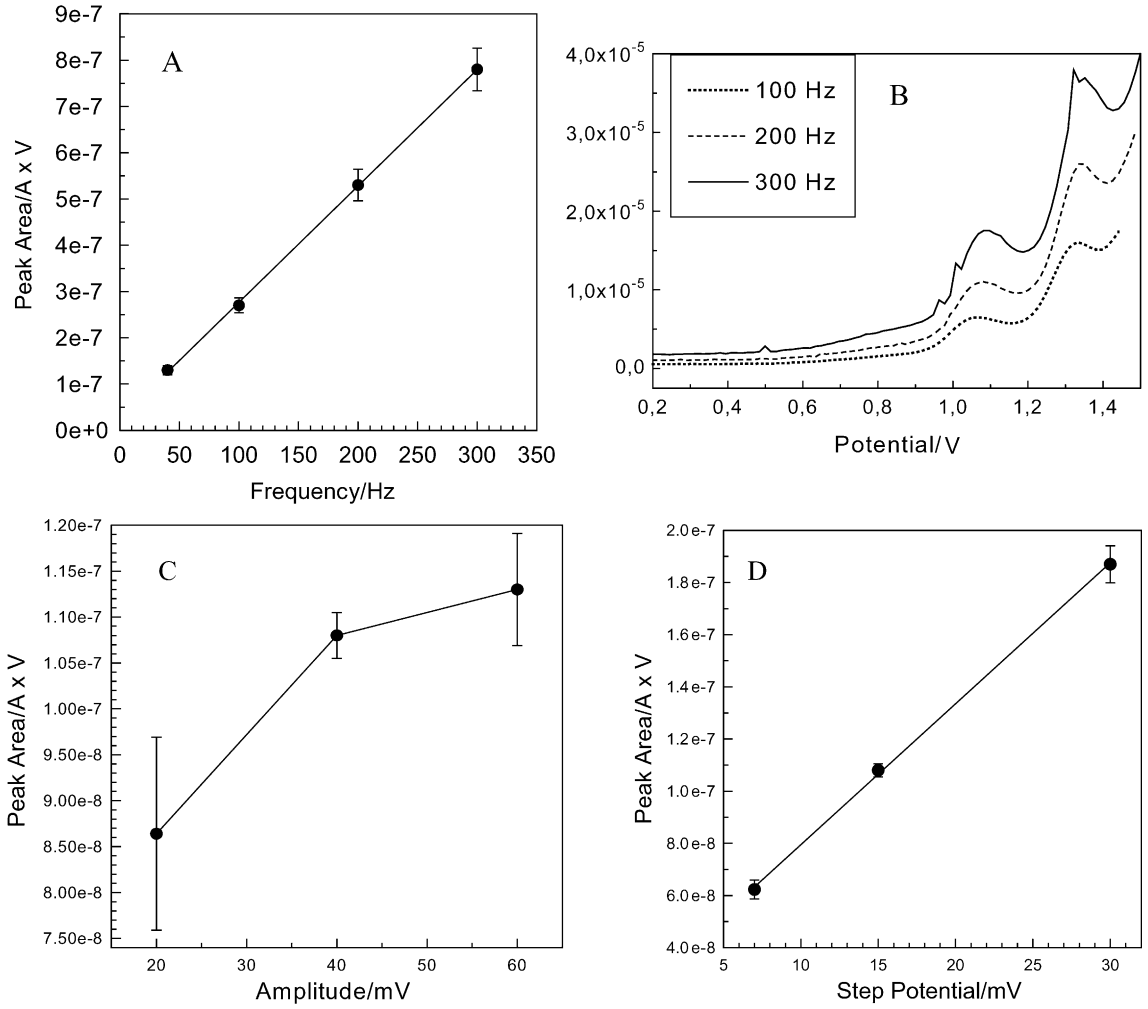


Fig. 2. Effect of the square wave parameters on the guanine oxidation peak. Electrode conditioning +1.6 V vs. Ag pseudo reference SPE, for 3 min: DNA immobilisation: 2 min at +0.5 V vs. Ag pseudo reference SPE.

acetate buffer since this is the best medium for performing this kind of measurement [1–3].

As reported in the literature [18,19], the carbon working surface needs to be conditioned before the immobilisation of the DNA. The application of high potentials in acidic media (acetate buffer pH 4.7) seems to increase the hydrophilic properties of the electrode surface through the introduction of oxygenated functionalities [20] accomplished with an oxidative cleaning [20]. To pretreat the electrode surface we applied a fixed potential of +1.6 V vs. Ag pseudoreference SPE for 3 min, thus increasing the resolution of the analytical signal as reported in Fig. 1.

The DNA immobilisation procedure as well as the interaction step with the sample solution was already optimised [2].

Square wave voltammetry was the method used to detect the oxidation peak of guanine. As already reported in Ref. [21], square wave parameters showed a great influence on the biosensor analytical signal. The DNA signal increased linearly with the frequency up to 300 Hz (Fig. 2A). However, an increase in the electrical noise (Fig. 2B) was also observed for frequency values higher than 200 Hz; thus this value was used as the optimal value. The analytical signal was dependent on the amplitude (Fig. 2C) even if this parameter seems to be less important than the frequency. The peak area increased linearly with the step potential in the range 7–30 mV, but for step potentials higher than 20 mV we observed a worse resolution of the signal (Fig. 2D). Thus, the optimal values were found to be: frequency, 200 Hz; amplitude, 40 mV; step potential, 15 mV.

In our previous papers [1,2,24], the biosensor was tested with many different chemical compounds with relevance in terms of environmental pollution. Compounds such as aromatic amines, endocrine disruptors, surfactants and pesticides were analysed at different concentrations. Fig. 3 voltammogram A, shows the effect of 2-anthramine on the guanine peak. This compound is electroactive and a peak due to the oxidation of the substance was observed. The 2-anthramine decreased the guanine peak even at low concentration, as expected, since it is a well-known mutagen and carcinogen [22]. In addition, bisphenol A is electroactive and a well-shaped peak was reported in Fig. 3 voltammogram C, however, from our experimental results, this compound, an endocrine disruptor, [23], had no effect on the DNA biosensor.

The effect of some of these standard compounds on the Toxalert® 100 test was also studied [24] in order to understand the different sensitivities of the two tests towards different chemical substances. Considering that the Toxalert® 100 test involves the metabolic activity of the bacterial cells instead of a simple interaction between two molecules, as in the DNA biosensor test, an interesting correlation between the two methods was found, even if some molecules did not inhibit the bioluminescence of V. fisheri (aromatic amines) or had no effect on DNA biosensor response (nonylphenol or bisphenol A)

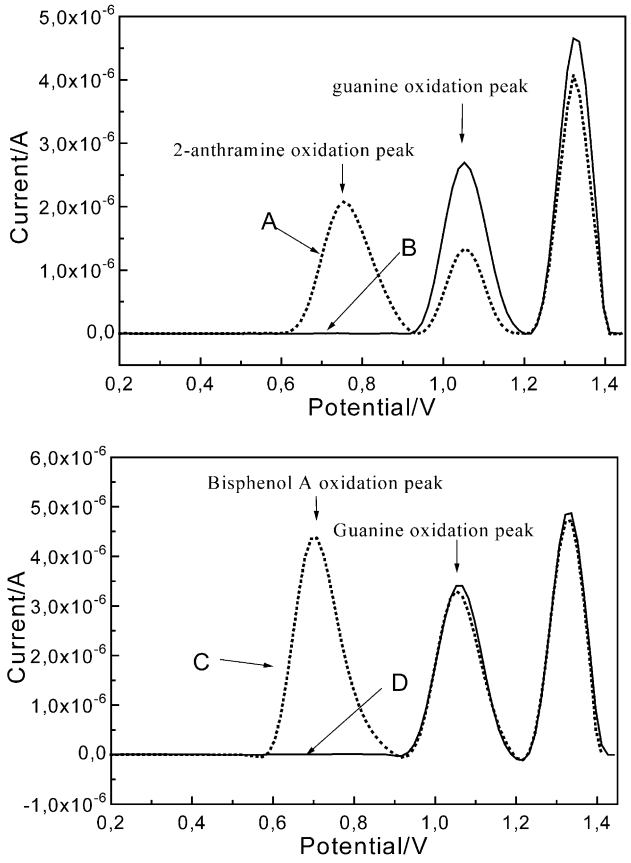


Fig. 3. Analysis of standard solution of 2-anthramine (0.1 ppm) and bisphenol A (5 ppm) with the double-stranded DNA biosensor. Oxidation signal of the DNA biosensor after the interaction with 2-anthramine (0.1 ppm) (A) and bisphenol A (5 ppm) (C), respectively. (B) and (D) DNA biosensor oxidation peaks (guanine ca. +1.0 V and adenine ca. +1.3 V), without interaction with the analyte solutions. Electrode conditioning: 1.6 V vs. Ag pseudo reference SPE, for 3 min: DNA immobilisation: 2 min at 0.5 V vs. Ag pseudo reference SPE. Interaction with the analyte 2 min open-circuit condition. Square wave parameters: frequency 200 Hz, step potential 15 mV, amplitude 40 mV. On the original signals, a baseline correction was performed.

### 3.2. Analysis of wastewater samples

Fig. 4 shows the results of the samples collected in treatment plants of different Italian towns, which receive mainly urban wastes as in the case of the samples collected in Firenze, Bologna and Roma, but also industrial wastes as in the case of Parma samples. For each town, influent and effluent samples were collected and analysed. The samples were tested with both the DNA biosensor and the Toxalert® 100.

For the samples from Firenze, Bologna and Roma, the two tests showed the same trend. Generally higher levels of luminescence inhibition on the influent samples in comparison to the effluent samples were observed with the Toxalert® 100 and the same trend was observed, in terms of the decrease in guanine peak area, with the DNA biosensor.

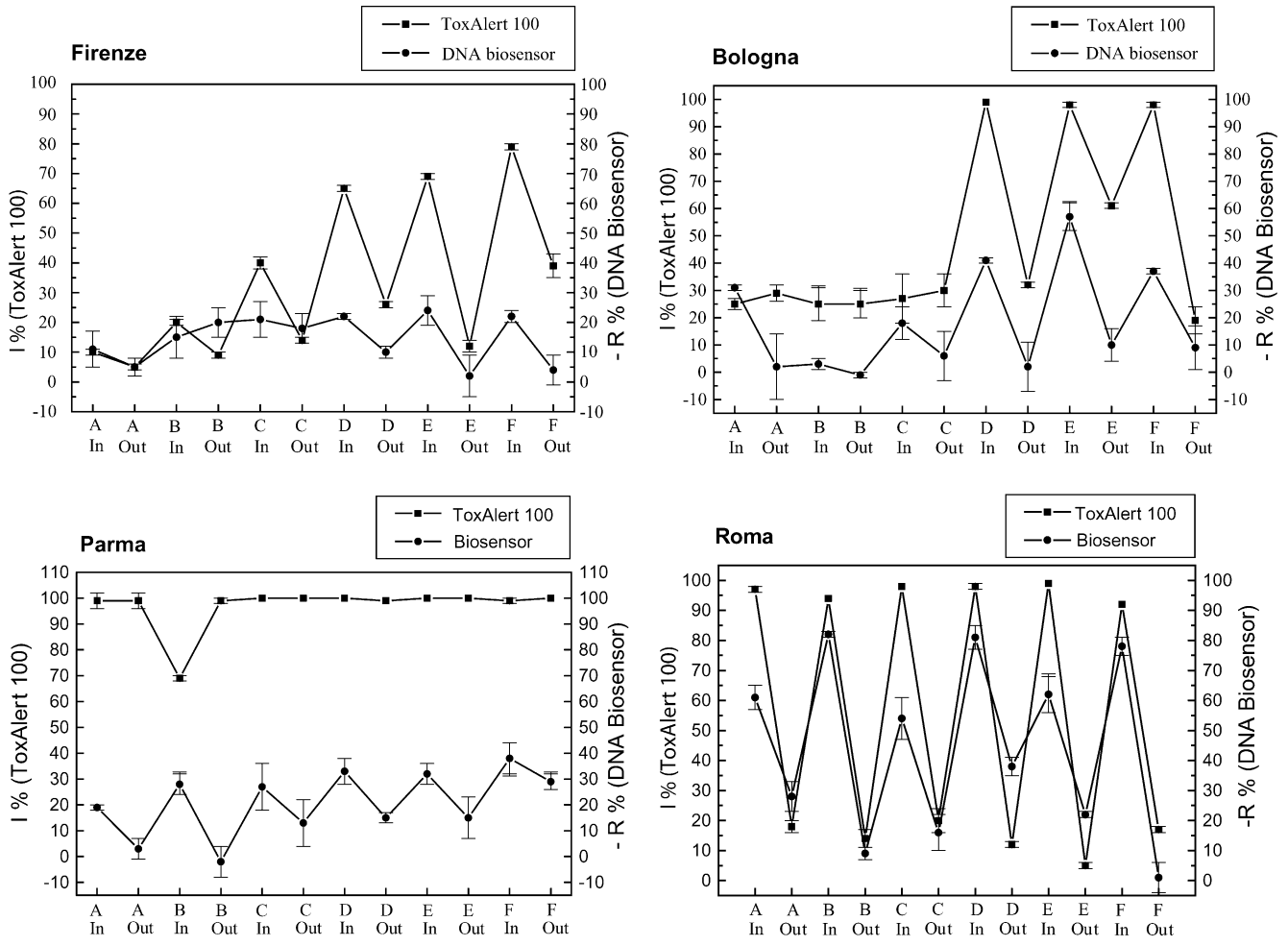


Fig. 4. DNA biosensor and Toxalert® 100 response on influent (In) and effluent (Out) wastewater samples from wastewater treatment plants of different Italian towns (Firenze, Bologna, Parma, Roma).

A completely different trend was observed on the samples of Parma; for these samples a high toxicity level (% I = 100) was observed with the Toxalert® 100 test, on both the influent and effluent samples. On the contrary, the DNA biosensor, which was able to discriminate between the influent and effluent samples, indicated a low presence of molecules with binding affinity for the DNA.

In cases like this one the classical chemical analysis could be very helpful, since it can individuate the molecules or the class of molecules responsible for such high values of bacteria inhibition, but with a very low affinity for the DNA, and thus indicates if there is a false positive (Toxalert 100) or a false negative (DNA Biosensor) response.

## 4. Conclusion

The results of the real sample analysis show a promising correlation between the two tests. The DNA biosensor response indicates just the binding of one or more molecules in the samples with the DNA molecule. The Toxalert response is a more complex response since the metabolic activity of the bacterial cell is involved.

The measurement time with the DNA biosensor is less than 10 min, the instrumentation needed is portable, and this means that it could be very useful for in-field screening analysis or when many samples have to be analysed.

In our opinion, this test is not exhaustive in defining the "toxicity" of a sample, or better, the presence of compounds toxic to human health in a water sample; however, it is important to specify that the real toxicity of a water sample has to be defined by many toxicity tests simultaneously, since the biological component (from DNA to animal, passing through bacteria, fish, algae) of the different tests [15] shows a different sensitivity towards the different compounds.

Nevertheless the DNA biosensor could be a very useful test, integrated in a panel of tests, since it can give rapid and easy to evaluate information on the presence of compounds with affinity to the DNA. Moreover, this test is one of the most competitive in terms of analysis cost and time, with the possibility of developing a very end-user-friendly format,

according to the requirements of a screening test for in field measurements.

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