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Bioluminescent bioreporter pad biosensor for monitoring water toxicity

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ABSTRACT

Toxicants in water sources are of concern. We developed a tool that is affordable and easy-to-use for monitoring toxicity in water. It is a biosensor composed of disposable bioreporter pads (calcium alginate matrix with immobilized bacteria) and a non-disposable CMOS photodetector. Various parameters to enhance the sensor's signal have been tested, including the effect of alginate and bacterium concentrations. The effect of various toxicants, as well as, environmental samples were tested by evaluating their effect on bacterial luminescence. This is the first step in the creation of a sensitive and simple operative tool that may be used in different environments.

1. Introduction

Worldwide toxicity of freshwater systems is of great concern, as toxicants may enter water reservoirs from the surrounding ground, industrial plants, accidental spillage and more [1,2], therefore, developing tools to test rapidly water putatively contaminated, will enable a quick response in case of a hazard. Although analytical approaches (e.g. gas chromatography (GC), inductively coupled plasma mass spectrometry (ICP-MS) or high-performance liquid chromatography (HPLC)) are very common and widely used to detect toxicants, they exhibit some disadvantages as they are time-consuming (days to weeks to obtain the results) and provide only the detection of a single compound or a group of structurally related compounds at any given time [3–5]. In addition, all these techniques require skilled personnel and expensive equipment. Another disadvantage is that the biological effects of compounds cannot be determined with chemical analysis.

Therefore, a portable, simple to use, cheap and sensitive method of detection is needed to facilitate on-site testing in order to obtain a response on-site. Biosensors may provide the needed type of said technologies. A biosensor is usually described as a self-contained, integrated device, capable of providing selective quantitative or semi-quantitative analytical information and which uses a biological recognition element (biospecific bio-receptor) and a transducer placed in intimate contact via some form of chemical immobilization [6]. Optical transducers are very commonly used in biosensing technology, due to their sensitivity, remote and real-time operation, multiplexing, and immunity to electromagnetic interferences [7]. Furthermore, development and optimization of the complementary metal–oxide–semiconductors (CMOS) technologies has provided the developers of cheap biosensors the possibility to develop real portable, affordable and sensitive devices [8]. Today's availability of highly integrated CMOS circuits on the one hand, and modern miniaturized biotech tools on the other, have had a high potential in the diagnostic field [9]. Different bioreceptors (e.g. antibodies, enzymes and DNA) have been coupled to CMOS technology, but for now still suffer from complicated sample preparation protocols and measurement procedures, thus, making these approaches not yet optimal for end-users. Whole cell microorganisms, on the other hand, are low cost, grow fast, and provide a rich potential in genetic modification varieties enabling the creation of a multitude of sensing entities enabling to selectively target different analytes. They are easy to handle, and are sensitive to a wide variety of environmental stimuli, thus, these engineered bacterial/yeast strains are becoming quite popular as bioreporter entities that are used to date in many applications [10,11]. Such microorganisms were genetically engineered to produce measurable signals (e.g. light) and to respond in a specific manner to particular classes of compounds (e.g. heavy

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metals [12], endocrine destructive compounds (EDCs) [13,14], antibiotics [15], aromatic compounds [16,17]), and more. These microorganisms when exposed to untreated samples, will provide a luminescent response that evaluates the toxicity level of a sample while providing at times an idea of the type of toxicant found therein. Immobilization of these bioreporter bacteria onto CMOS chips, will allow the creation of portable, cheap and sensitive devices. Different immobilization methodologies have been employed in the past (e.g. adsorption, covalent chemistry, antibody functionalized surfaces or agarose gel, and sol–gel films [18–21]) to immobilize whole cells to various surfaces, with either chemical or physical methods, with entrapment, being the preferred method, such as with hydrogels, in particular with calcium alginate [22]. Alginates spontaneously form gels in a single non stressful step process for the bacteria in the presence of divalent ions (e.g. calcium [23], strontium [24]). The high porosity provided by the open lattice structure, hydrated environment provided by the gels, which both enable controlled diffusion, pliability and biocompatibility, makes these alginates an optimal choice for cell entrapment and encapsulation [25]. Whole cell biosensors based on alginate-immobilized bacteria, were, in previous studies of ours, coupled to, either a liquid light guide or a fiber optic, and these have been successfully used to monitor water or air, both in a static system or in a continuous measurement mode [26–28]. In the present study, bioluminescent bacteria were trapped within calcium alginate pads that were placed just above a CMOS sensor surface so as to monitor cell light activity. This is the first prototype of a proposed biosensor “BioPen”, to be a fully integrated disposable system. The CMOS-alginate entrapped cells were validated with a sensitive and conventional bench top commercial luminometer. Being at a lower price, and size, and dispatchable, the Biopen module showed the same sensitivity (10^{-10} mol L^{-1} formaldehyde threshold) and the same dose dependency as with a laboratory-based instrument that is still expensive however, being both commercial and available now.

2. Materials and methods

2.1. Materials

LB Broth, Miller (10 g. tryptone, 5 g. yeast extract and 10 g. sodium chloride) were purchased from Becton Dickinson (USA). Low viscosity alginic acid sodium salt (A2158), medium viscosity alginic acid sodium salt (A2033), calcium chloride, 96+% A.C.S reagent (C5670), formaldehyde 37% solution (F8775), kanamycin sulfate (K0879), mercury(II) acetate (83352),a hydroxide (A6899) were purchased from Sigma,while ethanol was purchased from Romical.

2.2. Bacterial strain

The *Escherichia coli* strain used in this study, was *E. coli* TV1061, which was obtained from S. Belkin (Hebrew University, Jerusalem, Israel). The strain harbors a plasmid-borne fusion of the different promoters to a reporter gene. The heat-shock *grpE* [26] promoter in the strain *E. coli* TV1061 is sensitive to metabolic changes, such as with cytotoxic substances. This promoter is chromosomally integrated to the *lux*CDABE reporter operon, which has five promotorless structural genes responsible for both the heterodimeric luciferase units (lux A and B) and the synthesis of the luciferase substrate, tetradecanal, by an ATP- and NADPH-dependent multienzyme complex composed of fatty acid reductase, transferase, and synthetase (lux C, D and E) [27]. Strain stocks were stored at −80 °C with 20% (v/v) of glycerol, as a cell cryoprotectant additive [28]. The bioreporter strains from the stock solution were placed on LB-agar plates (NaCl 5 g L^{-1}, yeast extract 5 g L^{-1}, tryptone 10 g L^{-1}, agar 15 g L^{-1}) supplemented with 50 μg mL−1 kanamycin and, after incubation for two days at 37 °C in an incubator (Gerhardt, Germany), were stored at 4 °C for future use in experiments.

2.3. Growth conditions

Bacterial cultivation prior to measurements was performed in 10 mL LB-medium (NaCl 5 g L^{-1}, yeast extract 5 g L^{-1}, tryptone 10 g L^{-1}) [29] supplemented with 50 μg mL^{-1} kanamycin. Cells were grown overnight at 37 °C in a rotary thermo-shaker (MaxQ 4450, ThermoScientific, USA) at 120 rpm in the presence of the antibiotic. Cultures were then diluted to approximately 10^7 cells mL^{-1} and re-grown in 10 mL LB at 30 °C without shaking, until an early exponential phase (OD600 of 0.2), as determined by an Ultrospec 2100 Pro spectrophotometer (Amersham, England).

2.4. Immobilization procedure

The harvested cells were mixed with 4% (w/v) low viscosity sodium alginate solution at a 1:3 (cells:alginate) ratio. With a syringe pump (kdScientific KDS100) connected to a 3 mL syringe (MEDI-PLUS), itself attached to a rubber tube with a 3 mm inner diameter spout, the alginate/bacteria solution was dripped at a flow rate of 10 ml/h into a 0.5 mol L^{-1} $CaCl_2$ solution, spontaneously forming spherical pads of 4 mm diameter, a shape taken thanks to the fact that the $CaCl_2$ was placed in a flat bottomed well. Prior to placing the beads inside an insert, excess of $CaCl_2$ was removed, including drying them for half hour on a clear and dry paper.

2.5. Instrument setup

To monitor bacterial activity, a field-operable biosensor was designed as described in Fig. 1. The sensor (CMOS photodetector-ULS solution kit by Anitoa (Palo Alto, CA)) was placed in a light-tight box, to prevent light interference. To receive and analyze the data, a specific software has been developed by the Anitoa company, which enabled us to monitor the bioluminescent signal, and to handle the data in real-time. Thereupon a CMOS photosensitive surface, a glass well was placed, itself containing calcium alginate pads made of a mixture of both alginate and bacteria. This setup allows emitted light from the stimulated bacteria to reach the CMOS photodetector surface without direct contact between the bacteria and the CMOS photodetector.

Parameters putatively affecting our biosensor performance were tested, such as calcium alginate and bacterial concentrations. All experiments were performed at room temperature.

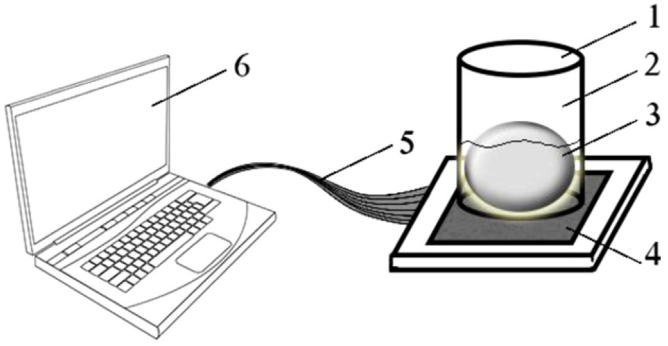

Fig.1. Schematic presentation of the biosensor. where (1) is a flat bottom insert, (2) a liquid sample, (3) a bioreporter pad, (4) a CMOS photodetector, (5) a USB cable and (6) a laptop.

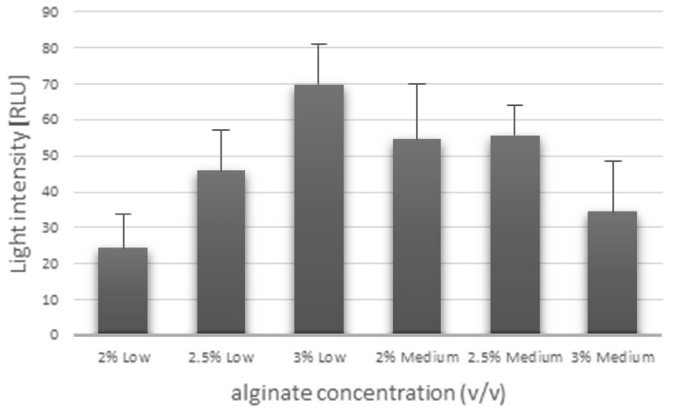

Fig. 2. Effect of calcium alginate concentration on *E. coli* TV1061 responses to 2% (v/v) ethanol.

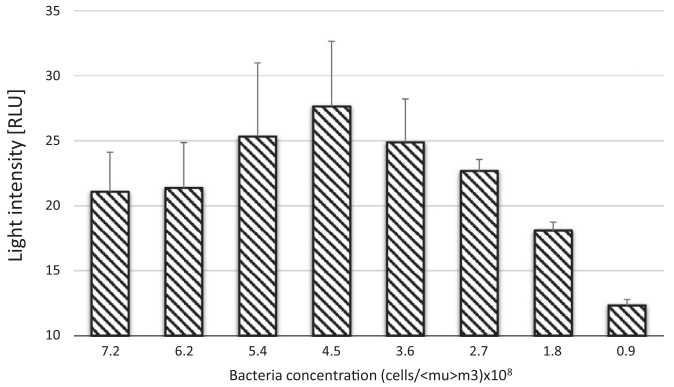

Fig. 3. Effect of the bacterial (7.2, 6.2, 5.4, 4.5, 3.6, 2.7, 1.8 and 0.9 $\times 10^8$ (cells/μm^3 calcium alginate)) concentrations on *E. coli* TV1061 strain responses to the 2% (v/v) ethanol.

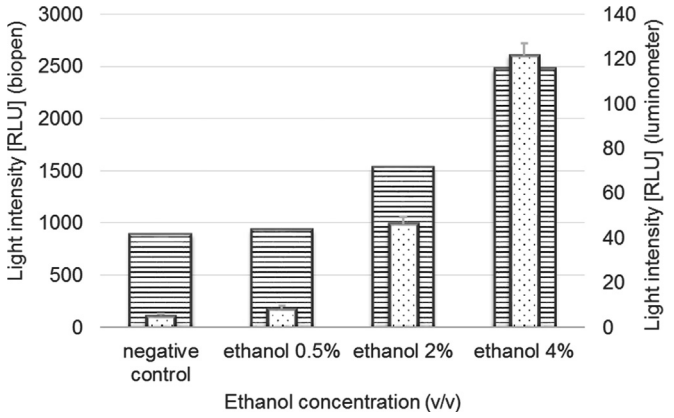

Fig. 4. Exposure of bacteria (TV1016 strain, 4.5×10^8 cells/μm^3) to different concentrations of ethanol. Right *y* axe is a measurement for commercial luminometer (dots) and left is for the CMOS biosensor (lines).

2.6. Pad optimization

2.6.1. Alginate concentration

The effect of low and medium viscosity calcium alginate concentration on bacterial response (*E. coli* TV1061 strain was used as the model microorganism) using three different concentrations (2, 2.5 and 3% (w/v)) was tested. After immobilization and placement of the pads in the glass holder, cells were then exposed to our toxicant model 2% (v/v) ethanol.

2.6.2. Bacterial concentration

The effect of cell density on sensor efficacy was tested by immobilization of eight different bacterial concentrations (7.2, 6.2, 5.4, 4.5, 3.6, 2.7, 1.8 and 0.9×10^8 cells/μm^3 calcium alginate) with the optimal 3% (w/v) calcium alginate beads, and exposed to a 2% (v/v) ethanol solution. Bacteria were concentrated by centrifugation of refreshed cells (E. coli TV1061 strain), and thereafter, pipetting out supernatant.

2.7. Exposure to different toxins

The effects of different pollutants at different concentrations (mercury (10^{-4}, 10^{-6}, 10^{-8}, 10^{-10} and 10^{-12} mol L^{-1}), formaldehyde (10^{-4}, 10^{-6}, 10^{-8}, 10^{-10} and 10^{-12} mol L^{-1}) and ammonium hydroxide (10^{-4}, 10^{-6}, 10^{-8}, 10^{-10} and 10^{-12} mo L^{-1})) were evaluated by placing 20 μL of each toxicant solution to pad-immobilized bacteria and measurement of the triggered cells' response was made with the prototype CMOS sensor integrated device.

2.8. Environmental samples tested

Tests with environmental samples were performed using water from putatively polluted locations in the Amazon and Lachish rivers (Israel, Lat. 31 °48′56.68″ N Lon. 34 °38′35.66″ E) using our pad biosensor integrated system, and the toxicants therin were partially analyzed by chemical composition analysis (Fig. 7). Collected water has been stored at 4 °C to prevent quality loss as much as possible. These samples are the same as previously published [26,27] so as to enable a comparative measurement of sensitivity.

2.9. Data analysis

The bioluminescence signal of the bacterial response to spiked toxicants and environmental samples, was expressed as the induction factor, calculated using the following formula: Induction factor=Bi/BC, where Bi is the value of the bioluminescence signal for the tested toxin and BC is the value for the control.

3. Results

3.1. Comparison of the prototype device to commercial methodology

To determine the capability of the proposed new system (CMOS-alginate-bioreporter based integrated biosensor), in comparison to that of a commercial luminometer, several ethanol concentrations (0.5%, 2% and 4% (w/v)) were tested. All conditions were kept otherwise the same (Fig. 4), and both systems showed both the same dose dependency and sensitivity.

3.2. Optimization steps

3.2.1. Alginate concentration effect on induction performance

The effect of alginate concentrations (2%, 2.5% and 3% (w/v) at low viscosity (250 cps) and medium viscosity (> 2000 cps)) used for immobilizing bioluminescent bioreporter bacteria was evaluated in the sensor performance by exposing the cells to 2% (v/v) ethanol (Fig. 2). In general, increasing alginate concentration, enabled the greater induction of cell response, while the highest bacterial response was obtained with 3% (w/v), low viscosity alginate solution.

3.2.2. Bacterial concentration effect on induction performance

We determined the effect various bacterial concentrations (7.2, 6.2, 5.4, 4.5, 3.6, 2.7, 1.8 and 0.9×10^8 (cells/μm^3 calcium alginate))

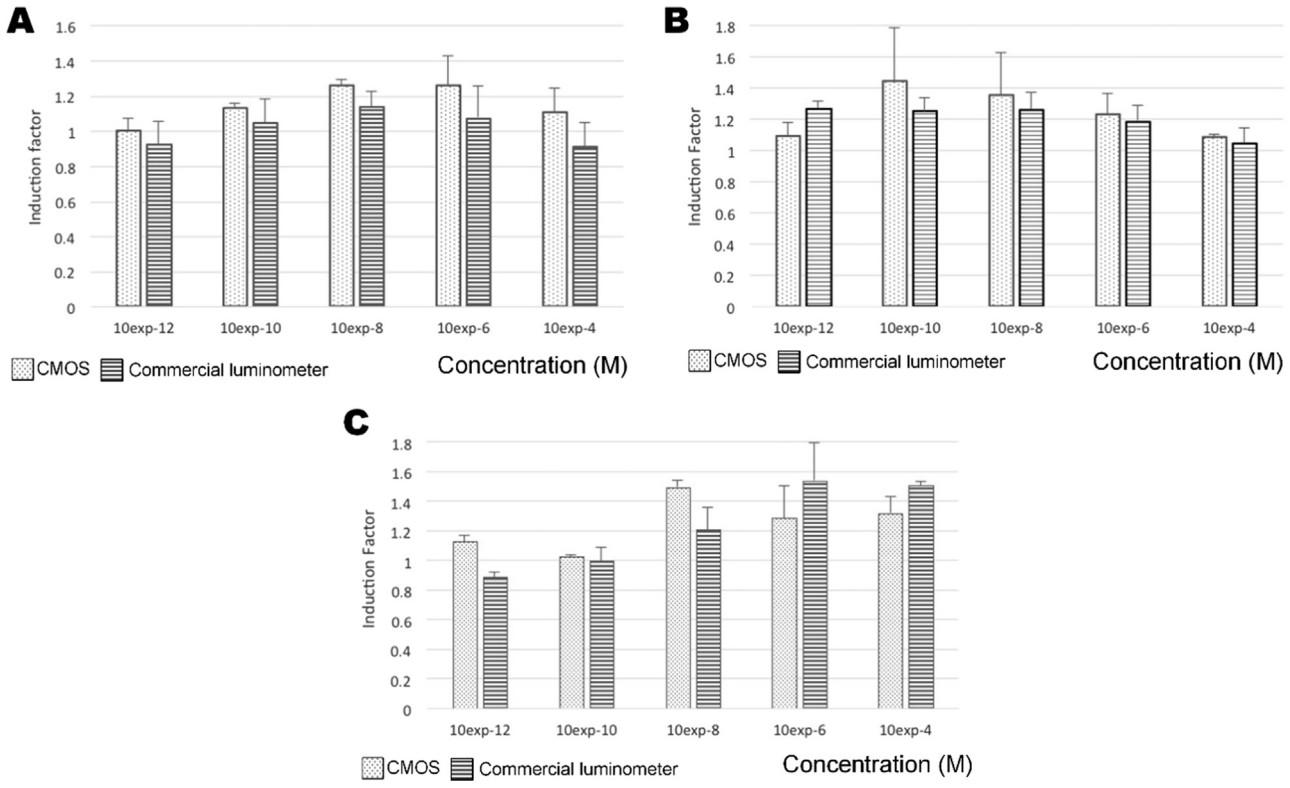

Fig. 5. Effect of exposing bacteria (TV1016 strain) to the different concentrations of (A) mercury, (B) ammonium hydroxide and (C) Formaldehyde.

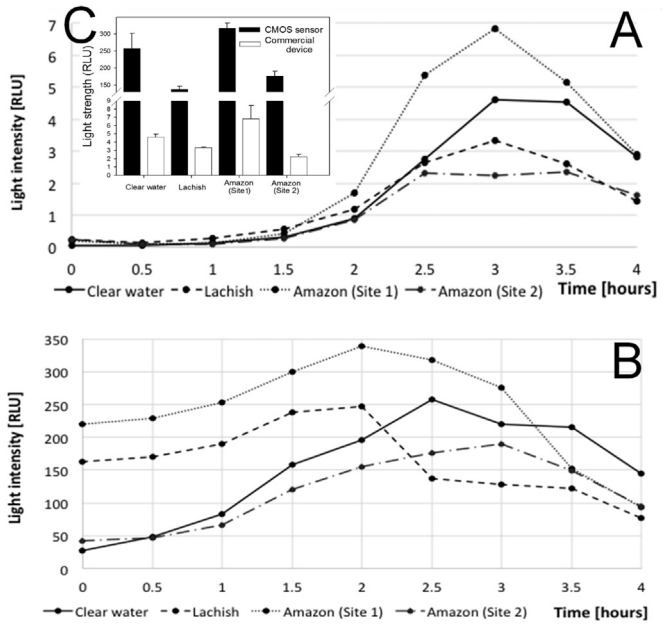

Fig. 6. Exposure of bioreporter bacteria *E. coli* TV1061 to different environmental samples. Bacteria were immobilized in calcium alginate matrices and measured with (A) commercial luminometer, (B) CMOS based biosensor. While (C) is the maximum luminescent values for both used technologies.

have on signal response while residing in calcium alginate matrices (Fig. 3). Immobilized bacteria were exposed to a model 2% (v/v) ethanol toxicant to induce a glow response. Increasing cell density provided higher light response, with the optimum/maximum response obtained from a 4.5×10^8 concentration (cells/μm^3 calcium alginate), whilst a further increase in cell concentration, had the detrimental effect in decreasing their overall light response (Fig. 2).

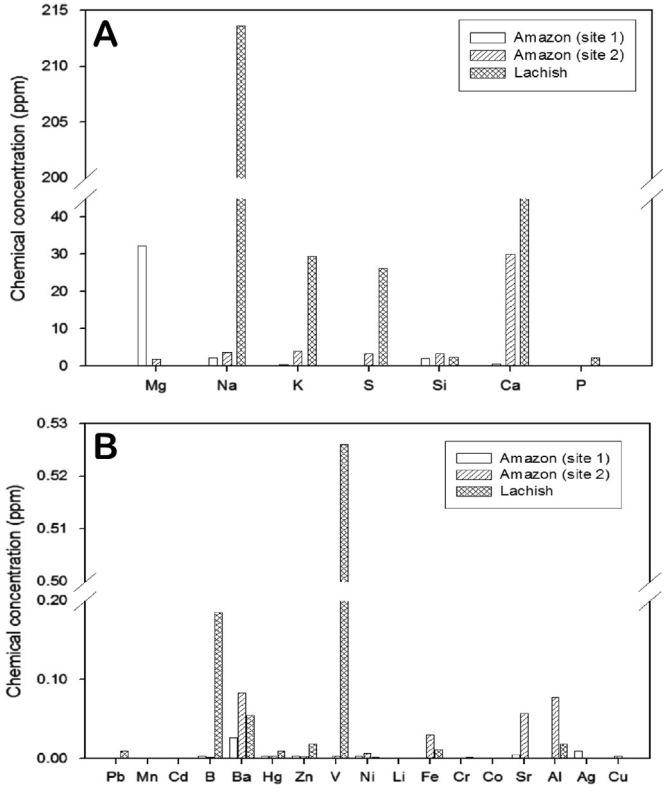

Fig. 7. Analytical results of Amazonia and Lachish water. All chemicals concentrations are in ppm units.

3.3. Testing known chemicals in prepared solutions

After optimizing both alginate and bacterial concentration, the biosensor was exposed to three different model pollutants

(formaldehyde, ammonium hydroxide and mercury) (Fig. 5). No visible effect was observed with the exposure of the chosen bioreporter bacteria to mercury (Fig. 5A) with both the commercial luminometer and the CMOS sensor; indeed, the bacterial luminescence values were similar with and without addition of the said chemical. Minor induction effects were observed with the addition of ammonium hydroxide (Fig. 5B). Both of the aforementioned tested approaches showed the same dose dependency and behavior at different toxicant concentrations. Of the three tested chemicals, formaldehyde has shown the highest induction factor values (Fig. 5C). In the latter case, sensitivity of the commercial instrument was one fold higher than that of the CMOS-based device. It is possible that the photomultiplier found within the luminometer is more sensitive, albeit much more expensive than the CMOS chip used in the miniaturized integrated system.

3.4. Environmental testing

For each of the examined samples from the three environmental sites, calcium alginate immobilized bacteria responded differently. Lachish water inhibited the reporter strain in both of the used approaches (Fig. 6). On the other hand, bioluminescent bacteria responded differently with the two different water samples from the Amazon sites. In this case, site 1 samples, induced bioluminescent responses in the *E. coli* TV1061 strain, while site 2 sample inhibited cell output signals (Fig. 6). Inducing and Inhibition effects of the water samples from the Amazon and Lachish rivers, suggested the presence of pollutants in the tested water (Fig. 6). Both of the used technologies, allowed a clear observation of the putative toxicity. To find out the possible toxicants found within the toxic environmental samples (Lachish and Amazonia rivers), and suspected because of the strong positive signals observed, their respective chemical composition was tested with an optical emission spectrometer (ICP). This approach allowed for the qualitative determination of metal-based compounds in the samples. Both samples that either induced or inhibited the bioluminescence response in the bioreporter bacteria, were found to be contaminated with many different heavy metals (Fig. 7), thus suggesting a possible source for the responses found herein. Obviously, organic pollutants may also contribute to these effects, but these have not been determined in this study.

4. Discussion

Simplicity, low price and portability are the main issues in developing dispatchable environmental water toxicity sensors and in the present research we tried to come up with a reasonable integrated device offering a combination of these stringent facets. Indeed, this work is a logical outcome of the previously developed fiber optic and liquid light guide biosensors, that enabled real-time monitoring of air and water toxicity through the use of a waveguide [26,29]. In spite of past innovations a more miniaturized and portable monitoring system has largely eluded us due to a lack of compact and cheap opto-electronic photodetectors and instrumentation. In this paper, we strive to solve this problem by introducing ultra low-light CMOS bio-imager sensors that are sensitive, highly integrated and of low cost, so as to enable a cheap portable bioluminescent-sensor based toxicity monitoring instrument (Fig. 1). Similar to the previous setups, calcium alginate pads were stored in a refrigerator and then when needed were easily placed (with a one-click action) at the biosensor end-face (CMOS sensor). It is our pill-like concept: removal of a test pad from a stored closed foil compartment as found in common medication storage compartments. The analyte toxicity will then be tested by simple dipping of the biosensor into the tested sample in either a solution or a suspension. Thus, simplicity of the measurement processes, real portability, price of the device and possibility to choose pads of interest containing a plethora of bacterial strains in pill-like containers are making this approach useful for onsite toxicology measurements. Further developments of the system could be seen as an array of pads, each containing a different bacterial strain, thus simultaneously testing different types of materials, providing a fingerprint, which will provide a more complete analysis in a single step.

4.1. Optimization procedures

We first sought to determine the capability of the CMOS based biosensor to detect chemicals in water samples via bioreporter luminescence production. Sensitivity of such measurements were compared to that of a commercially available device (bench top luminometer). The luminometer was found to exhibit higher sensitivity as reported elsewhere too [30], however, it is nevertheless much more expensive and not amenable for field work, and thus can only be operated in a laboratory environment. Both, the luminometer and the CMOS sensor-based device described herein, have shown similar bacterial luminescence trends, however, eventhough the luminometer shows results with a better resolution, the CMOS-based device is by far more portable, affordable and easy to operate. Furthermore, it did show overall the same sensitivity despite being of a different price league. It's important to mention that our model 0.5% (v/v) ethanol toxicant was the lowest measurable signal in both cases. This could be due to the fast ethanol evaporation rates, before enough could diffuse into the calcium alginate matrix and produce some damage effects on the immobilized cells or simply that lower ethanol concentrations do not have toxic effects on these bacteria, which is more likely.

In the next step, some basic optimization procedures were made by testing the effect of alginate entrapment concentration and viscosity on light coupling efficiency. Bacteria immobilized in low viscosity alginates, enabled an increase in light response with the increase in alginate concentration (from 2 to 3% (w/v)). On the other hand, bioreporter bacteria immobilized in the >2000 cps alginate (medium viscosity) showed a decrease in their luminescence output signals with increase in the alginate concentration (3% w/v), due to lower diffusion rate which allows for a more gradual and altogether lower response. The immobilization of cells in calcium alginate has been widely studied and used in many applications [31–35] including our previous bacterial alginate-based biosensors for air and water toxicity measurements [26,27]. It has been reported that a lower diffusion rate of ethanol in alginate, was due to a decrease in the number and increase in length of the pores, rather than a decrease in the pore diameter [36]. Thus, porosity must be carefully optimized in order for the device to function as a biosensor without limiting the diffusion of the water pollutants inside the immobilization matrix. Additional studies have shown the direct effect of alginate concentrations on molecule diffusion rates [37,38]. The optimum alginate concentration in the present application was found to be 3% (w/v) with 250 cps viscosity rate and will be adopted in future experiments.

We then studied the effect of the concentration of entrapped cells found within a pad on the activities of the sensor (Fig. 4). Bioreporter cell density is one of the crucial parameters in device development, since changes in bacterial cell concentrations are expected to yield two opposite effects: a larger number of cells will increase the luminescent signal output to be measured, but at the same time it will lead to negative effects such as shading, quenching, issues in thermodynamic parameters such as toxicant diffusion reduction and oxygen limitations, as well as lowered

metabolite exchange with the environment [27]. Indeed, increase in the cell concentration induced increasing light responses only at the lower (between 0.9×10^8 and 4.5×10^8 cells mL^{-1}) tested bacterial densities. At the higher bacterial concentrations (between 4.5×10^8 and 7.2×10^8 cells mL^{-1}), lower photon detection efficiencies were obtained (Fig. 4). Many studies reported decreased diffusion rates of chemicals into alginates with increased loadings of microorganisms [39–41], explaining the weaker bacterial response from lower ethanol diffusion rates into the matrix. Reduced oxygen diffusion to the deeper bacterial layers in the alginate matrix may also be a reason for the lower output signal. At higher cell populations, less oxygen will be overall available to each individual cell and diffusion rate may be decreased and this fact may explain the direct effect this will have on the metabolic activity of the bacterial cell, notably that of bioluminescence generation [34]. The bacterial concentration producing highest bacterial responses was adopted and used in the next experiments.

4.2. Response of the biosensor to various water contaminating pollutants

For further validation of our CMOS system, the *E. coli* TV1061 bioreporter strain, that is sensitive to cytotoxic stresses, was also exposed to several common and environmental chemicals at different spiked concentrations, and in parallel validated with a commercial luminometer. Calcium alginate immobilized cells were exposed to different concentrations of mercury (Fig. 5A), which is a common pollutant in many lakes, rivers and oceans [42,43] including in China were it was found in six major shallow freshwater lakes [44]. Similar to previous studies [27], bacteria exposed to mercury demonstrate lower sensitivity in a strain such as *E. coli* TV1061, suggesting a non-cytotoxic mode of damage. Indeed, emphasis on genotoxicity of mercuric compounds has been reported in both *in vitro* and *in vivo* model systems [45,46] as well as in bacterial mutagenicity assays [47]. As expected, both methodologies showed similar response patterns. Heavy metal sensitive strains (up to 2.5 mg L^{-1}) may be used in the future for more specific and sensitive mercury measurements, but this was not the purpose of our paper to study bioreporter selectivities. In a way we may see this as a negative control and when part of a panel of different bioreporter strains then it would be part of what would make an identification fingerprint. Another set of fresh immobilized bacteria were then exposed to ammonium hydroxide, which is one of the other highly toxic chemicals for human beings and wildlife, as it is produced in both fertilizer and chemical factories. The dissolution of this chemical into running and drinking water may cause severe health problems such as skin, throat, and lung cancer as well as permanent blindness [48]. As expected, bacterial responses were higher than for mercury, as ammonium hydroxide is cytotoxic to bacteria. Ammonium hydroxide also has genotoxicity as it has been reported in mammalian recombinant fluorescent tests [49]. In this particular case, and unexplained to us, the CMOS device showed a higher sensitivity to this particular chemical (i.e. 1×10^{-10} mol L^{-1}) than when measured with the commercial luminometer using microplate-alginate pad well biosensors (Fig. 5B). Finally, cells were also exposed to different concentrations of formaldehyde (Fig. 5C). Formaldehyde (HCHO) is one of industry's most important and widely used chemical and millions of people in the United States, and worldwide, are exposed to it at the workplace, whilst environmental exposures may even be more common [50]. From all the aforementioned tested chemicals, the strongest inducing effect on the *E. coli* TV1061 strain was with formaldehyde (in both measuring technologies), confirming its cytotoxicity to the said bioreporter (Fig. 5C) and exhibiting measured low concentrations (i.e. 1×10^{-8} mol L^{-1}) with the CMOS based biosensor, that are comparable to other published approaches [51–53]. Interestingly, our biosensor showed a ten times more sensitive response (i.e. 1×10^{-8} mol L^{-1}) than the commercial device (i.e. 1×10^{-6} mol L^{-1}), despite consisting of a lower sensitivity to the photodetector. The increased sensitivity may originate from the possibility of placing the pads directly onto the CMOS endface enabling a greater amount of collected photons. To summarize, for all tested chemicals, the CMOS based biosensor had a similar or better sensitivity than the commercial luminometer. These results suggest that our biosensor may therefore be used for on-site measurements.

4.3. Environmental monitoring

Two different environmental water sources were tested. The first was water from Lachish River, which flows in Ashdod, Israel and has plagued the city's residents for years. Rainwater carrying large amounts of contaminations from road surfaces is drawn off into the sea without treatment and both the coastal strip and Lachish river are now polluted [54]. Fig. 6 shows the inhibition effect of the samples on cell activity. Bacterial strain luminescence, in both measuring technologies, were inhibited suggesting not only the presence of toxic compounds in the water, but also it's cytotoxicity. Indeed, the chemical composition analysis of the Lachish water, shows the presence of many different toxicants that have known cytotoxic effects on the bioreporter bacteria used in this study (Fig. 7). For example, cytotoxic effects of lead [55], zinc [56], vanadium [57], sulfur [58], aluminum [59] and the oxidative effect of chromium [60] are well studied, and support our results. The second source of water samples was collected from the Amazon River (French Guiana), downstream of lands rich with gold mines from two different sites. Rivers in many South American countries are contaminated with mercury as a result of ruthless, illegal gold extraction [61–63]. Indeed, ICP results show the presence of both gold and mercury in the tested samples (Fig. 7). In this case, bioreporter bacteria responded differently to the water samples from two tested Amazon sites. Samples from site 1 induced and from site 2 inhibited bioreporter cells in both devices (Fig. 6). ICP tests (Fig. 7) show that the chemical composition of the two Amazon samples is very different. The site 2 sample includes much higher concentrations of heavy metals, in some cases, even higher levels than those found in the industry polluted Lachish river. This could be a possible reason for the observed cell response as follows (Fig. 6). Site 1 samples induced luminescence suggesting a certain degree of cytotoxicity of these Amazon water samples. For almost all tested chemicals, the toxicant values were much higher in the Lachish river, than that of the Amazon site 1 samples (Fig. 7), suggesting a higher contamination degree of the water. Several studies are being conducted to assess the genotoxicity of water bodies receiving industrial effluents [64,65]. Similar to Amazon site 2 samples, these water samples consisted of many toxicants (heavy metals) at lower concentration, with clear toxic effects. Thus, the presence of these metals (e.g. Sr, Ba, Al) in site 2 samples and absent in site 1 (Fig. 7), is reflected in differences in the cells' response (Fig. 6). Chemical composition of the tested environmental [27] water correlates well to the bacterial response, where the Amazon water site 1 samples seem to induce the bioreporter strain used, while the site 2 and Lachish river samples seem to inhibit bacterial luminescence. In both used measurement devices (CMOS and luminometer) a similar response pattern for all tested environmental samples was observed. Furthermore, these results were correlated to the previously published data (Table 1).

In all the three aforementioned cases, our CMOS based biosensor showed its capability to respond to the presence of a selection of chemicals tested at the same rate and sensitivity as the commercial instrument, the only limitation being the selected

Table 1
Effect of the different environmental samples on the bioreporter bacteria as measured by either the CMOS-based biosensor device or the luminometer.

Sample	CMOS	Liquid light guide	Commercial luminometer
Amazon (site 1)	Induction effect [a]	Induction effect [b]	Induction effect [a,b]
Amazon (site 2)	Inhibition effect [a]	–	Inhibition effect [a,b]
Lachish	Inhibition effect [a]	Inhibition effect [b]	Inhibition effect [a,b]
Sea of Galilee	–	No visible effect [b]	No visible effect [b]

[a] This paper.
[b] [27].

bioreporter. Thus, with similar sensitivity and with many additional advantages (e.g. price, simplicity and portability) our biosensor may be more attractive to the endpoint users.

5. Conclusion

A portable biosensor for water quality monitoring was developed, tested and compared to a commercially available device. The biosensor is built from two parts, a non-disposable one (CMOS photodetector) and a one-time replaceable (bioluminescence bacteria immobilized into calcium alginate pads) component. After optimization steps (e.g. determination effect of the alginate concentration, viscosity and bacteria density on sensor responses), both the CMOS and luminometer-based devices were exposed to spiked common environmental toxicants, where the CMOS biosensor exhibited higher sensitivity. Thereafter, environmental samples from three different sources were tested, with the CMOS based technology showing similar response sensitivity and kinetic patterns and ratios as that of the commercial device. Thus, in addition to its sensitivity, the developed biosensor is more user-friendly, portable, has a lower detection limit and requires smaller amounts of sample volume. These advantages make this application attractive for use in many ground-based applications, as well as, including water-quality analysis in underdeveloped regions of the world.

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