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Online monitoring of heavy metal–related toxicity using flow-through and floating microbial fuel cell biosensors

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Abstract Elevated concentrations of heavy metals in water caused by mining activities create significant risks to the environment. Traditional biological methods used to assess heavy metal–related toxicity in aquatic environments are lengthy and labor intensive. Real-time biomonitoring approaches eliminate some of these limitations and provide a more accurate indication of toxicity. This study describes the performance of a flow-through and floating design microbial fuel cell (MFC) biosensors for real-time detection of copper (Cu) and other heavy metal–related toxicity in aquatic environments. Several biomonitoring tests were carried out using Cu and mining effluents as toxicants. The biosensors were able to detect, in real-time, Cu-related toxicity at concentrations as low as 35 - 40 μg L^{-1}, as confirmed by a *Daphnia* assay. A comparison of the floating biosensor's outputs with *Daphnia magna* survival rates showed a linear correlation with a coefficient of determination (R^2) higher than 0.9. In addition, the flow-through biosensor was shown to be able to detect differences in the quality of two mining effluents with different compositions of heavy metals. Finally, the biosensor's real-time field performance was investigated in two aquatic environments in the Sudbury, Ontario region of Canada.

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Introduction

The exponential increase in the demand for heavy metals (Ferguson 1990) has led to an increase in the mining of ore deposits around the world (Northey et al. 2013). During mining, heavy metals are inadvertently released into the natural environment and may persist, sometimes, up to hundreds of years after a mine closure (Peplow 1999). Although some of these metals (e.g., copper) are important for metabolic processes, an increase in their bioavailability due to higher concentrations causes toxicity in many aquatic biota (Waalkes et al. 1992). Subsequently, regulatory limits for heavy metal concentrations in water bodies receiving effluents from industrial processes are enforced and water quality is evaluated through toxicity bioassays of water samples around industrial effluent discharge zones. These tests could either be conducted in vivo (*Daphnia*, microalgae, fish) or in vitro using cultured bacteria or mammalian cells (Farré and Barceló 2003; Reemtsma 2001). For example, in Canada *Daphnia* and rainbow trout, (*Oncorhynchus mykiss*) toxicity tests are regulated under the Metal Mining Effluent Regulations (MMERs).

Despite numerous advantages, toxicity bioassays have several disadvantages, such as tedious logistics related to sampling and transportation, high costs, and lengthy testing times. Several other factors including remoteness of certain effluent discharge zones and seasonal climatic variations may lead to infrequent sampling and non-

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detection of short-term toxic spills. In addition, the environmental relevance of bioassay results may not be accurate because of differences between site and laboratory conditions. Toxicity has been shown to depend on site-specific factors like water chemistry and ambient temperature (Erickson et al. 1996). Recently, inexpensive bioelectrochemical systems, such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), have been shown to be capable of fast detection of heavy metal toxicity using electroactive microorganisms (Adekunle et al. 2019a, b; Yu et al. 2017; Dávila et al. 2011; Di Lorenzo et al. 2014; Kim et al. 2007; Chung et al. 2016). In bioelectrochemical systems, electroactive microorganisms transfer electrons to the anode. In MFCs, these electrons are used at the cathode for an oxygen reduction reaction (Logan et al. 2006), while in MECs the electrons are used for a hydrogen evolution reaction (Rozendal et al. 2007). This electron flow is measurable as current. Heavy metals reduce this flow (electrical current) due to their ability to compete with the anode as an electron acceptor, or due to their toxicity to electroactive microorganisms (Patil et al. 2010). Also, heavy metals can be used as terminal electron acceptors at the cathode (Chung et al. 2016). Notably, the MFC biosensors have been shown to have faster response times when compared to the MEC biosensors (Adekunle et al. 2019a).

In this work, we examine the possibility of continuous online (real-time) copper-related toxicity biomonitoring in aquatic environments using flow-through and floating microbial fuel cell (MFC)–based biosensors. The flow-through biosensor design was initially used for continuous monitoring of toxicity in surface water collected in the proximity of a copper (Cu) mining site and in deionized water amended with Cu. The floating biosensor's performance was verified by comparing its outputs with the results of a *Daphnia* toxicity assay. Finally, the performance of the floating biosensor in two aquatic environments within a watershed receiving mining effluents was evaluated. To our knowledge, this is the first attempt at deploying an MFC-based floating biosensor for Cu-related toxicity biomonitoring in a real aquatic environment.

Materials and methods

Analytical methods and stock solutions

Three types of water were used in the biosensor tests. The first was moderately hard water (MHW) composed of the following (in mg L^{-1}): $CaSO_4$ (60), $MgSO_4$ (122.86), $NaHCO_3$ (96), and KCl (4). Secondly, surface water from Hannah Lake and Junction Creek located near Sudbury, ON, Canada, was used as an environmental reference. A stock solution containing 1 g L^{-1} of Cu was prepared in MHW. The desired Cu concentration in MHW used in the tests was prepared by diluting the Cu stock solution. Also, samples of mining effluents from an iron mine in Northern Quebec containing several metals were collected from the inflow and outflow streams of a mining tailing pond.

The total chemical oxygen demand (tCOD) of the surface waters were measured according to Standard Methods (APHA 1995). Concentration of Cu and other heavy metals was measured by inductive coupled plasma mass spectrophotometry (ICP-MS), as described elsewhere (Beauchemin 2010).

Biosensor design and operation

Flow-through and floating biosensors were used in this study. The flow-through biosensor was used for most of the tests carried out in the laboratory, while the floating biosensor was developed for field deployment. The flow-through biosensor was constructed following the previously described design of a membraneless air-breathing cathode MFC (Grondin et al. 2012). The anode compartment was 100 mL in volume and contained a 10-mm-thick anode made of two layers of 10 × 5 cm carbon felts (SGL Group, Charlotte, NC, USA). The air cathode was made of a 10 × 5 cm E4 electrode containing manganese oxide (Electric Fuel Ltd, Bet Shemesh, Israel). Figure 1 a shows a schematic diagram of the flow-through biosensor.

The floating biosensor was 3D printed using a polylactic acid (PLA) filament. It had a dimension of 10 × 10 × 3 cm. The frame was printed with a 25% infill factor to ensure the biosensor's flotation. The slits on the biosensor's bottom allowed for liquid movement through the biosensor. To limit anode exposure to dissolved oxygen in water, a 5-mm-thick layer of synthetic fibers (Scotch-Brite™, 3M Company, St. Paul, MN, USA) was placed between the anode and the bottom wall of the biosensor. The anode in the floating biosensor was 10 mm thick and made of two layers of 10 × 5 cm carbon felt (SGL Group, Charlotte, NC, USA). The air cathode was 10 × 10 cm. Figure 1 b shows a schematic diagram of the floating biosensor.

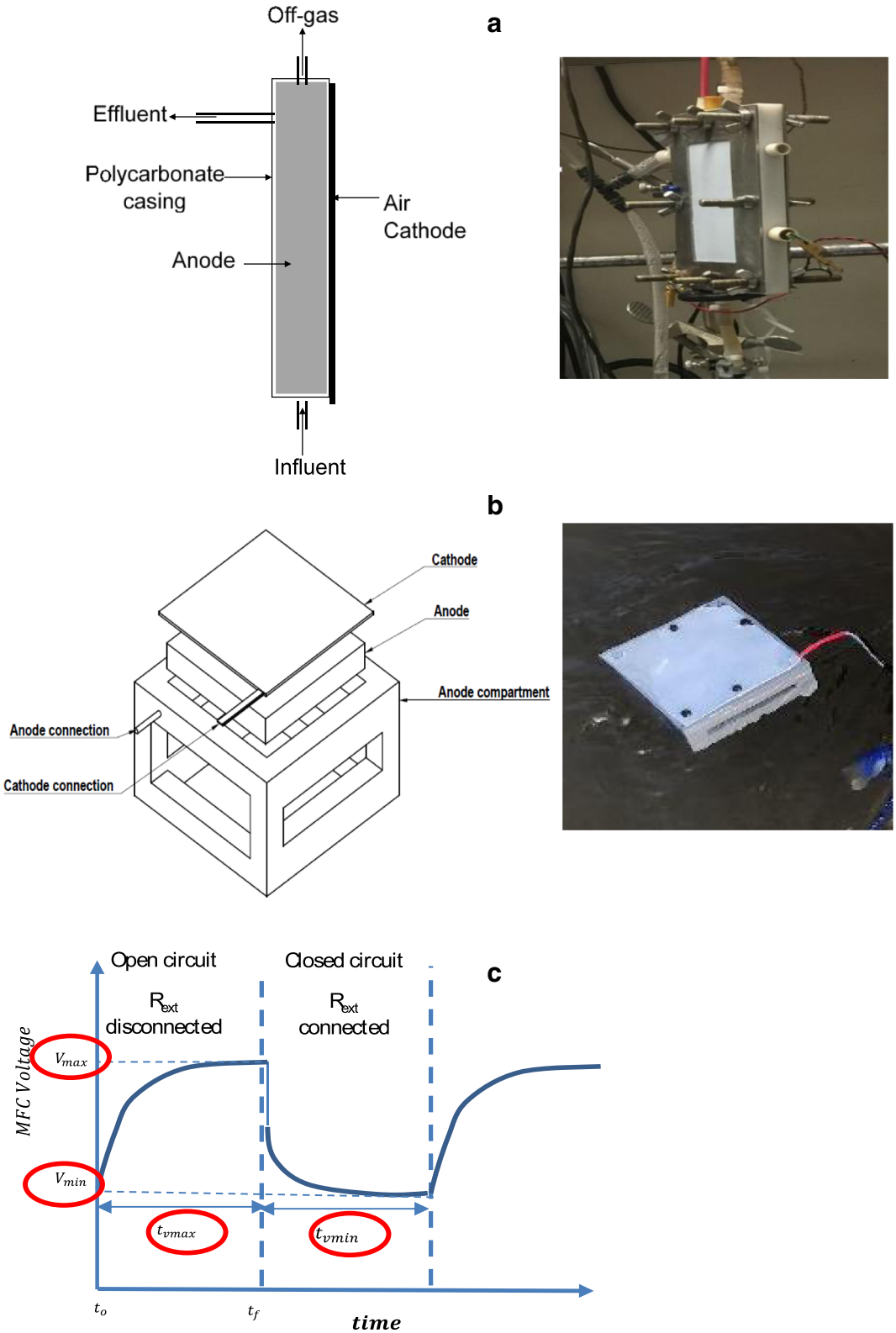

Fig. 1 Schematic diagrams showing the flow-through biosensor design (**a**), floating biosensor design (**b**), and biosensor operation in the external resistance connection/disconnection mode (**c**). Biosensor outputs used for toxicity estimation are shown in circles

A 100-Ω resistor was used for the operation of the flow-through biosensor, while a 12-kΩ resistor was used for the operation of the floating biosensor. The biosensor outputs are measured under steady-state conditions during operation with either connected or disconnected external resistance (periodic connection/disconnection mode of operation). In the proposed algorithm, the measurement cycle starts with biosensor connection to R_{ext}. Thereafter, the biosensor voltage (V_s) is constantly monitored to detect pseudo-steady-state voltage corresponding to V_{min}. Once steady-state voltage is attained, R_{ext} is disconnected and the voltage is monitored again to estimate the open-circuit voltage denoted as V_{max}. For each measurement, the voltage is considered at a steady state once its variation is lower than a predefined small value (ε) for several (e.g., 3–5) consecutive data acquisitions. The times required to achieve V_{min} and V_{max} are recorded as t_{vmin} and t_{vmax}, respectively. Data filtering (e.g., by an exponential filter) is used to reduce noise. Detailed description of the algorithm is provided in our previous study (Adekunle et al. 2019b). Figure 1 c depicts the time diagram of the biosensor's operation and the steady-state outputs (in red circles). The connection/disconnection of the external resistor was achieved using G6S-2F relays (Omron LLS, IL, USA). The control algorithms for the biosensors were implemented in Python (Python https://www.python.org/) on a Raspberry Pi-3 Model B microprocessor. A LabJack U3-LV (LabJack Corp., Lakewood, CO, USA) was used for data acquisition. For the field test, the microprocessor and the data acquisition board were assembled in a water proof case and powered by a 50-W solar panel.

Biosensing tests

Experiments were carried out in 5 phases. As proposed in our previous work (Adekunle et al. 2019b), toxicity was estimated using online measurements of V_{max} (open-circuit voltage), V_{min} (voltage during biosensor connection to an electrical load), and t_{vmin} (time required to achieve stable V_{min} value). In general, the biosensor outputs of V_{max} and V_{min} decrease with increasing toxicity, while the t_{vmin} value increases.

The initial biosensing tests were conducted in the laboratory with the flow-through sensor (biosensor design used in phases 1–3) to investigate various Copper (Cu) exposure scenarios. In phase 1, collected surface water samples were spiked with a 1 g L^{-1} $CuSO_4$ stock solution to achieve Cu concentrations ranging from 15 to 774 μg L^{-1} and fed to the sensor. In phase 2, MWH containing different levels of Cu, ranging from 0 to 160 μg L^{-1}, was continuously fed to the flow-through sensor. In phase 3, the flow-through sensor was fed with mining effluent containing several heavy metals. This effluent was collected at an iron mine in Northern Quebec. In preparation for field tests, the suitability and response of the floating biosensor to Cu spikes was examined in phase 4. This phase involved assessing the biosensor's response to the presence of Cu and comparing it to the results obtained with the *Daphnia* toxicity assay. Finally, in phase 5, a field biomonitoring trial was conducted using floating biosensors deployed at two locations near Sudbury, ON, Canada. These locations were within a watershed that receives effluents from a Cu and nickel mine. One of these locations, referred to as Junction Creek (GPS 46° 27′ 59.8″ N 81° 02′ 12.1″ W), spans about 25 km and directly receives mining effluent discharges (Weber et al. 2008). The second location (Hannah Lake; GPS 46° 26′ 44.6″ N 81° 02′ 01.5″ W) spans approximately 27.7 ha and is classified as mesotrophic. Hannah Lake has 6 known fish species and does not receive mining effluents.

Daphnia toxicity assay

The *Daphnia* toxicity assay was carried out to compare the biosensor response with a standardized toxicity test. In this test, concurrent exposures of the biosensor and *Daphnia magna* were conducted in a modified 48-h lethality test. The exposure vessel for the toxicity test consisted of a 9-L plexiglass tank that was lightly aerated for the duration of the test. Four exposure cups, each consisting of 4″ PVC pipe with a mesh bottom, were suspended in water and each cup held 10 *D. magna* (total $n = 40$). The biosensor was also placed in the tank so that both the biosensor and *D. magna* were exposed to the same concentration of Cu. Exposures were conducted by initiating tests with the lowest concentration of Cu (9.375 μg L^{-1}) for 48 h, after which both the biosensor and *D. magna* were removed. The biosensor was placed into clean water and *D. magna* were counted for survival. This was repeated four times with increasing concentrations of Cu (Table 1). The maximum Cu exposure concentration was determined when < 50% of *D. magna* survived.

In addition, a reference toxicity test with Cu was also conducted with *D. magna* to ensure LC50s calculated in

Table 1 Concentrations of copper used in tests comparing the biosensor response to the Daphnia assay

Cu concentration (μg L^{-1})	
Biosensor and Daphnia test	Reference Daphnia test
9.38	3.1
18.8	6.3
28.1	12.5
37.5	25.0
n/a	50.0

the biosensor comparison test were similar. For both tests (biosensor and reference toxicity), a modification to the standardized test was incorporated to include 5-day-old *D. magna*. The reason for this modification was to ensure a minimum size for *D. magna*, which would prevent their escape from the mesh bottom cups into the larger tank. The use of 5-day-old species allowed us to accurately determine lethality in each cup. In addition, a comparative toxicity test with 24-h-old *D. magna* was also conducted to ensure that sensitivities were similar between ages. The reference toxicity tests were conducted in accordance with the Environment and Climate Change Canada's biological test method (EPS1/RM/11). In brief, ten 5-day-old and ten 24-h-old *D. magna* were placed into 150 mL of MHW for 48 h. Experimental design consisted of 4 replicates per treatment and 5 concentrations of Cu plus control (Table 1). Survival was determined after 48 h.

Results and discussion

Biosensor response to changes in copper concentration

To investigate the mechanism of Cu ion interference with MFC current, the flow-through biosensor's response to spikes of Cu was examined. The biosensor was fed with water collected at Hannah Lake (near Sudbury, ON, Canada). This water had a COD content of 48 mg L^{-1}; i.e., a small amount of organic materials was available for microbial metabolic activities. Water was recirculated through the biosensor at a flow rate of 1 L day^{-1}. Cu concentration spikes were achieved by adding a predetermined amount of a $CuSO_4$ stock solution to the lake water. The Cu concentration was measured at least 24 h after each spike and was regarded as the steady-state Cu concentration provided to the biosensor.

The biosensor outputs changed in response to spikes in Cu concentrations of up to 800 μg L^{-1}. In particular, the biosensor outputs related to the closed-circuit operation (t_{vmin} and V_{min}) showed significant linear correlation with the measured Cu concentrations (Fig. 2). V_{min} values decreased and t_{vmin} increased after each spike in Cu concentration. However, the biosensor recovered after each spike, suggesting the effect of several factors, including partial removal of Cu by the biosensor (Tao et al. 2011), adaptation of electroactive microbial populations to the presence of Cu, and sufficiently high flow rate of the feed, which limited the accumulation of Cu in the anodic biofilm (Shen et al. 2013; Pham et al. 2008). Indeed, the appearance of new electroactive microbial populations after exposure to dissolved metals was demonstrated in our previous study (Adekunle et al. 2019b). Also, the presence of organic matter in surface water improves the biosensor resilience (Adekunle et al. 2019a). A significant reduction of dissolved Cu concentration (based on the comparison of initial spike concentration and steady-state Cu concentration), totaling up to 1.76 mg L^{-1} of Cu removed in the biosensor, was observed in a spike test, where a Cu concentration of 2.45 mg L^{-1} was measured immediately after spiking with 1 g L^{-1} $CuSO_4$ stock solution. A Cu concentration of 0.7 mg L^{-1} was measured after 2 days. This suggests that dissolved Cu ions were used as terminal electron acceptors in the biosensor, resulting in their reduction to insoluble Cu salts or elemental Cu at the cathode.

Continuous biomonitoring of copper-related toxicity

In Canada, the regulatory limit set for Cu in aquatic environments is 5 μg L^{-1}, although this is usually adjusted based on site-specific factors which affect toxicity, such as water chemistry (MacDonald et al. 2002). Therefore, in this test, the response of the biosensor to Cu concentrations in a range of 35 to 160 μg L^{-1} was examined. The tested Cu concentrations were achieved by continuous dilution of a syringe pump fed Cu stock solution with MHW fed with a peristaltic pump. The total flow rate of the resulting Cu-containing stream was 288 mL day^{-1}. The influent Cu concentration was estimated using the flow rates of the Cu stock solution and MHW. Cu concentration in the effluent of the biosensor, for each influent concentration used, was also measured while the

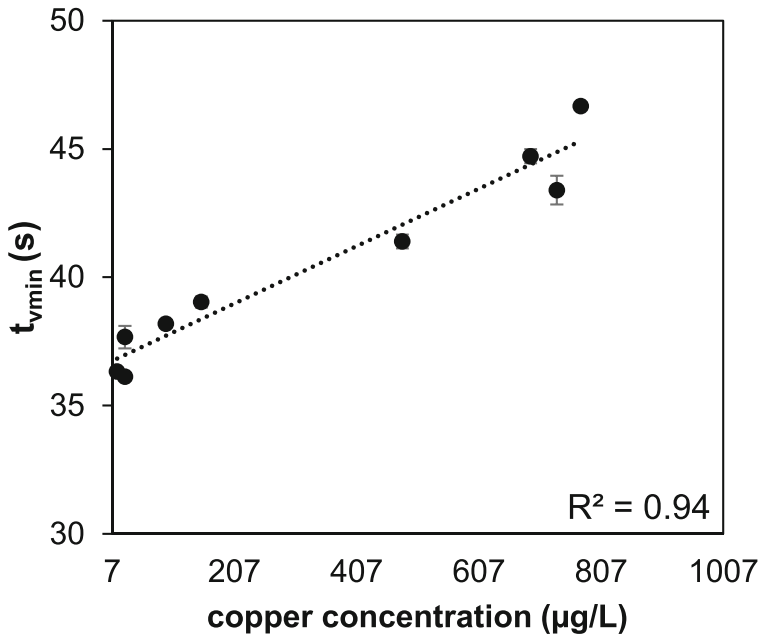

Fig. 2 Detection of copper concentration spikes in surface water by biosensor showing linear correlation between the biosensor output (t_{vmin}) and copper concentration

biosensor's output was averaged over a day before each change in concentration.

Overall, the biosensor was able to continuously detect changes in the influent Cu concentration. The open-circuit voltage (V_{max}) and the closed-circuit voltage (V_{min}) of the biosensor reduced with increasing Cu concentration. This strongly suggests that the Cu^{2+} was still toxic at the low concentrations tested, thereby reducing the measurable microbial electroactivity. As expected, in the biosensor's effluent, the measured Cu concentrations were lower than the estimated influent concentration (data not shown). The reduction in the measured Cu concentration again suggests that one major bioelectrochemical interference pathway is related to the electrochemical reduction of Cu^{2+}, as suggested in several other studies (Tao et al. 2011; Heijne et al. 2010). In fact, blue-colored deposits (color of Cu compounds) were seen on the cathode when the biosensor was opened at the end of the experiment (picture not shown). Overall, Cu toxicity had a greater effect on V_{min} than on V_{max}, as can be seen from the ratio of the two parameters (V_{max}/V_{min}). This can be expected since V_{min} values directly reflect the rate of electron transfer to the anode, as well as bacterial activity, processes which are severely affected by the presence of electron acceptors such as heavy metals and Cu. The time to achieve the pseudo-steady-state values (t_{vmin}) also increased with an increase in Cu concentration, generally indicating reduced performance of the electroactive bacteria.

The toxic effect of Cu on microorganisms (Zhu and Logan 2014) and the bioaccumulation of Cu in the anodic biofilm can be attributed to the observed slow recovery of the biosensor when the Cu concentration was reduced from an initial high concentration. It should be noted that although bioaccumulation can be reduced at higher flow rates (as seen in the spike test), increasing flow rates reduce the biosensor's sensitivity and performance, due to a reduction in retention time and exposure of the anodic biofilm to increased levels of dissolved oxygen. The slow recovery of the biosensor likely skewed the determination of coefficient R^2 values obtained from a linear correlation of the influent Cu concentration and the biosensor outputs (Fig. 3). Subsequently, it can be concluded that an MFC-based biosensor is most suitable for detecting short-term spikes in the concentration of Cu and other known toxicants, while long-term exposure of the biosensor to elevated levels of heavy metals could result in the adaptation of electroactive bacteria to heavy metal toxicity. Nevertheless, a significant deviation from the established concentration of heavy metals might be detected by the sensor; i.e., it can be used as an alarm to indicate abrupt changes in water quality. It is important to note that the operation of the biosensor on MHW with no added organic matter implies that the major electroactive microorganisms in the biosensor could be lithotrophic.

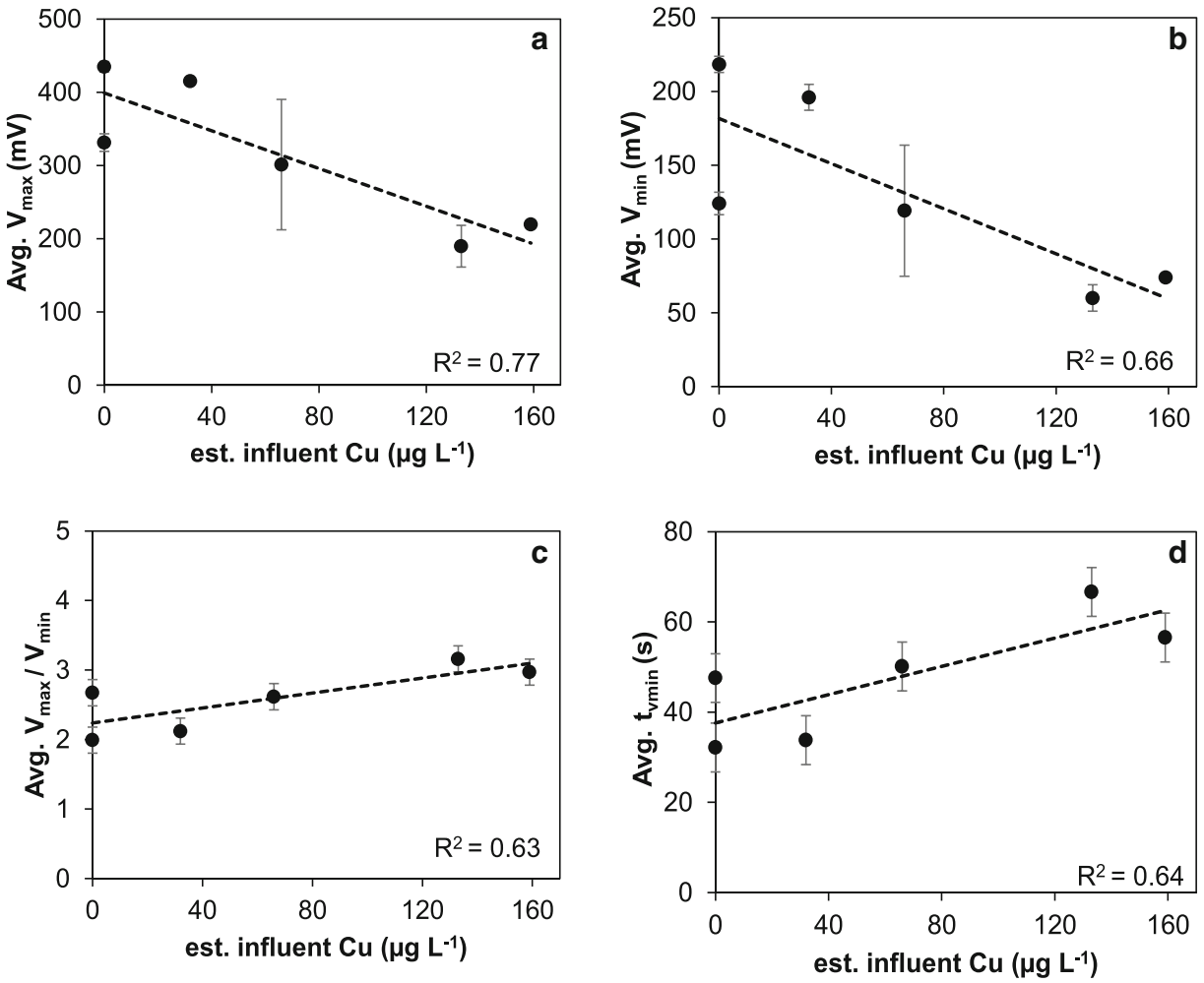

Fig. 3 Dependence of the biosensor outputs V_{max} (**a**), V_{min} (**b**), V_{max}/V_{min} (**c**), and t_{vmin} (**d**) on the influent copper concentrations in moderately hard water during continuous monitoring tests

Biosensor response to mining effluents

As mentioned earlier, mining effluents contain multiple heavy metals. Also, aquatic environments can receive contaminants originating from different sources. For example, the watershed in Sudbury, ON, typically receives effluents from mining, urban run-off, a sewage treatment plant, an industrial waste treatment plant, etc. Junction Creek is an example of a natural water body in this watershed that directly receives all of these effluents (Weber et al. 2008; Dubé et al. 2006). Therefore, it was important to investigate the biosensor's response to mining effluents containing multiple heavy metals. In order to evaluate such responses, effluents were collected from the inflow and outflow streams of a mining tailing pond at an iron mine. The collected effluent samples were fed into the flow-through biosensor at a flow rate of 1 L day^{-1}, corresponding to an HRT of 6.9 h. This flow rate was higher than in the previous test and was selected to facilitate washout of any accumulated solids and to reduce bioaccumulation in the biosensor as heavy metals' concentrations were high. MHW was used as a baseline for comparing biosensor responses.

Table 2 shows the composition of metals in each type of mining water used in this test. As shown in Fig. 4, the biosensor responded to each change in the influent stream composition. The start of each feed type is denoted with arrows. It can be inferred that the biosensor's response reflected either the presence and/or relative concentration of heavy metals in the

Table 2 Concentrations of heavy metals in mining effluents collected from a tailing pond inflow and outflow. The collected water was used for the biosensor test in phase 3

Metals	Inflow (μg L^{-1})	Outflow (μg L^{-1})
Zinc	42	< 7.0
Vanadium	3.5	< 2.0
Lead	0.89	< 0.50
Manganese	250	12
Iron	14000	440
Copper	3.3	< 1.0
Aluminum	840	62

influent stream. The inflow with the highest concentration of metals led to a reduction of the biosensor's output (V_{max}, V_{min}) and increased the closed-circuit time (t_{vmin}). On day 2, when the outflow water containing lower concentration of heavy metals was introduced, the biosensor's outputs were immediately increased. The highest V_{max} and V_{min} values and lowest t_{vmin} were recorded when MHW solution was fed to the biosensor. These results confirm that the biosensor is sensitive to a wide range of heavy metals in mining effluents. Also, this test demonstrates that the biosensor can be conveniently used for detecting sudden changes in toxicity, for example by setting thresholds for changes in the biosensor outputs that trigger an alarm message. In other words, the biosensor can be proposed as a real-time early warning device for toxicity monitoring.

Biosensor response comparison to Daphnia assay

The *Daphnia magna* lethality assay is a standardized toxicity test that is used regularly in determining

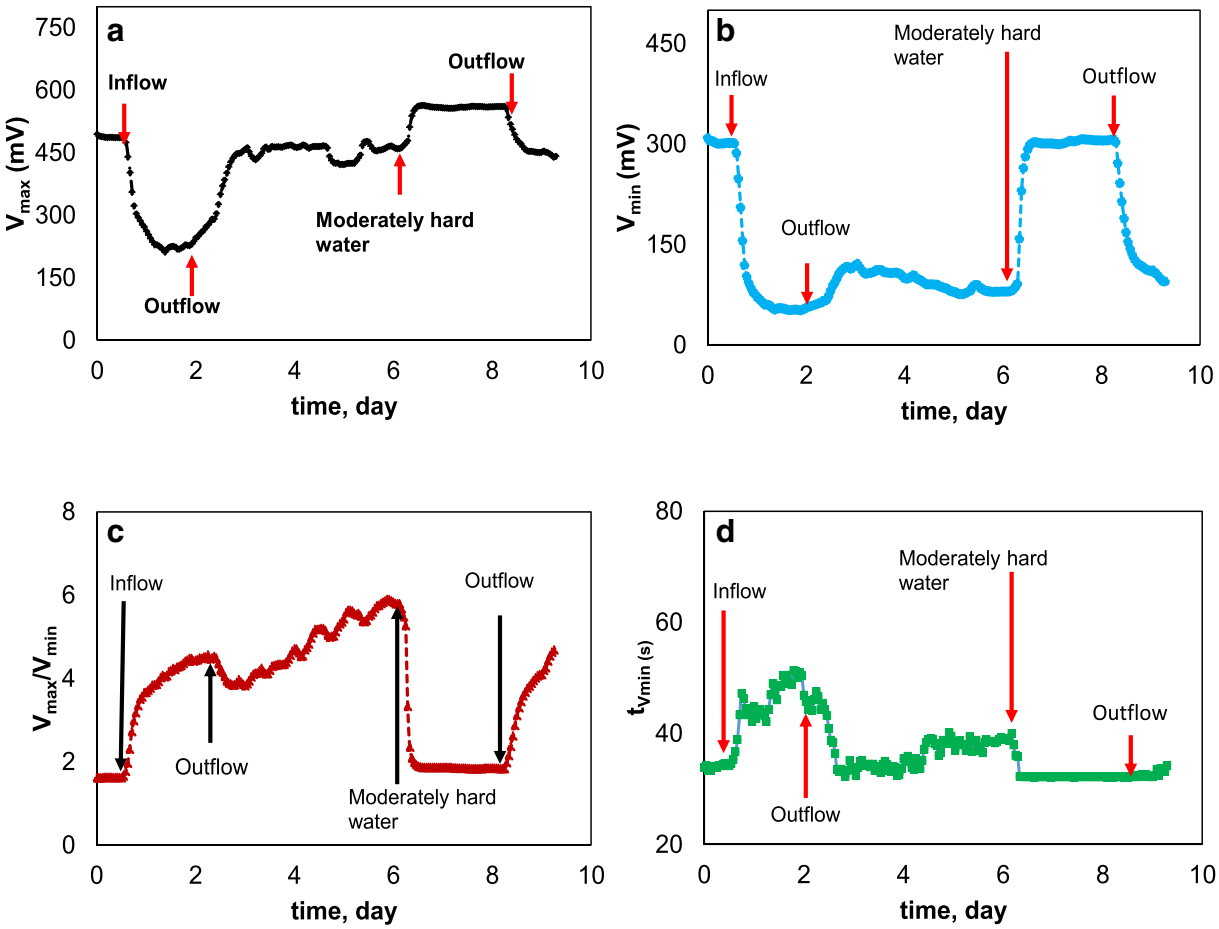

Fig. 4 Detection of heavy metal–related toxicity in mining effluents by the biosensor. Changes in V_{max} (**a**), V_{min} (**b**), V_{max}/V_{min} ratio (**c**), and t_{Vmin} (**d**) as a function of time. Arrows indicate changes in water type

toxicity in aquatic environments and is a regulated test under the Metal Mine Effluent Regulations (SOR/2002-222) to determine compliance (OECD 1984; ASTM 1988; Atienzar et al. 2001). Therefore, a comparison of the floating biosensor response to the results of a Daphnia assay was carried out to validate the results obtained from the biosensing tests. The floating biosensor design (Fig. 1 b) was used for the comparison test because it presents practical advantages for field deployment.

In the Daphnia toxicity tests, survival of *D. magna* decreased with increasing concentrations of Cu in all tests conducted (Fig. 5a). Both reference toxicity tests conducted with < 24-h-old and 5-day-old neonates resulted in similar LC50s (Table 3), indicating the use of 5-day-old *D. magna* does not reduce sensitivity to Cu. The LC50 obtained from the biosensor test was similar to the reference toxicity tests. This would confirm that the responses observed in the biosensor test reflect those from the standardized reference toxicity test procedure. The biosensor on the other hand had an increase in the average V_{max}/V_{min} values, relating to the rate of reduction in the biosensor voltages, as seen in previous tests (Fig. 5b). Therefore, it can be suggested that both the *Daphnia* assay and the biosensor detected Cu-related toxicity in the MHW simultaneously. Figure 5 c compares the results of both the biosensor monitoring and the *Daphnia* assay, and it can be seen that the correlation between them is linear (R^2 = 0.92). In addition, the results from this comparison test also suggest that the floating biosensor design is capable of detecting changes in aquatic toxicity. It also verifies that the biosensor responses observed in phase 1–3 tests are related to the toxicity of Cu. Further confirmation was obtained with periodic spikes of Cu (concentration not determined) over 20 days and as can be seen in Fig. 6, the biosensor was able to detect spikes as seen in V_{max} and V_{min} outputs. It is important to state that transport-related diffusion limitations (He et al. 2005) due to passive water flow may affect the floating biosensor's response time and sensitivity (Jiang et al. 2015). However, the aforementioned adverse effect was reduced in this test due to the addition of a sparger in the tank. It is expected that water currents in aquatic environments may help to reduce such transport limitations in the floating biosensor design, although the risk of overexposure to toxicants and dissolved oxygen may also occur. Accordingly, a field trial of the floating biosensor design is necessary.

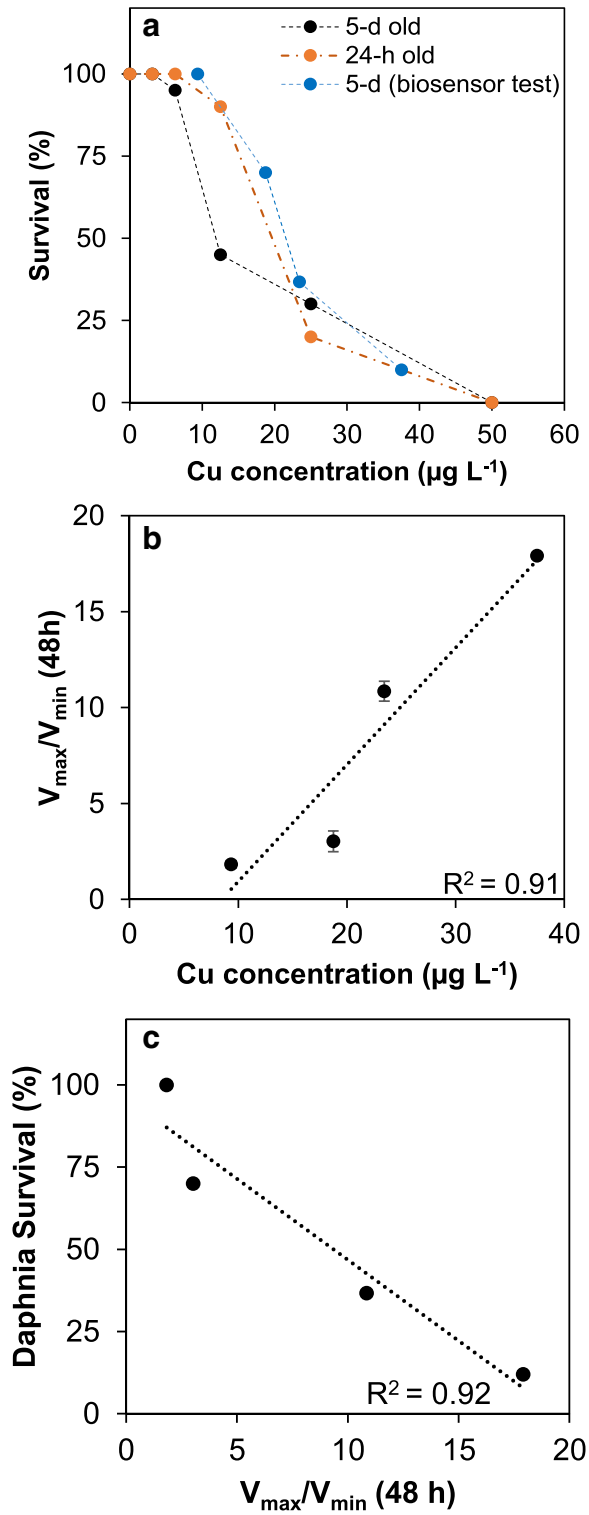

Fig. 5 Detection of copper-induced toxicity. Daphnia magna survival rates in the biosensor and reference test (**a**), biosensor (V_{max}/V_{min}) output (**b**), a comparison of the biosensor to Daphnia assay outputs (**c**)

Table 3 The LC50's calculated in the *Daphnia* toxicity assays

Test	LC50 (μg L^{-1} $CuSO_4$)
Daphnia assay 24 h	24.4
Daphnia assay 5 days	21.3
Biosensor test	23.5

Biosensor field trial

Following the confirmation of the biosensor's continuous monitoring capability in the floating design configuration, a field test was carried out in June 2018. The floating biosensor was deployed and attached to a buoy at two locations in Sudbury, ON. Analysis of field results from both locations shows that the biosensors were able to detect differences in the quality of two aquatic environments (Fig. 7). A sharp decline in the open-circuit voltage (V_{max}) of the biosensor, from approximately 310 mV to 22 mV, can be seen when the sensor was placed in Junction Creek, which receives multiple effluents and is expected to be more toxic than the other location. On the other hand, in Hannah Lake, the open-circuit voltage of the biosensor averaged approximately 250 mV, although the signal was not as stable as in Junction Creek. It is hypothesized that the signal fluctuations reflected the biosensor's exposure to wind and waves on the lake. Regardless, the difference between the aquatic environments can be seen within 2 h

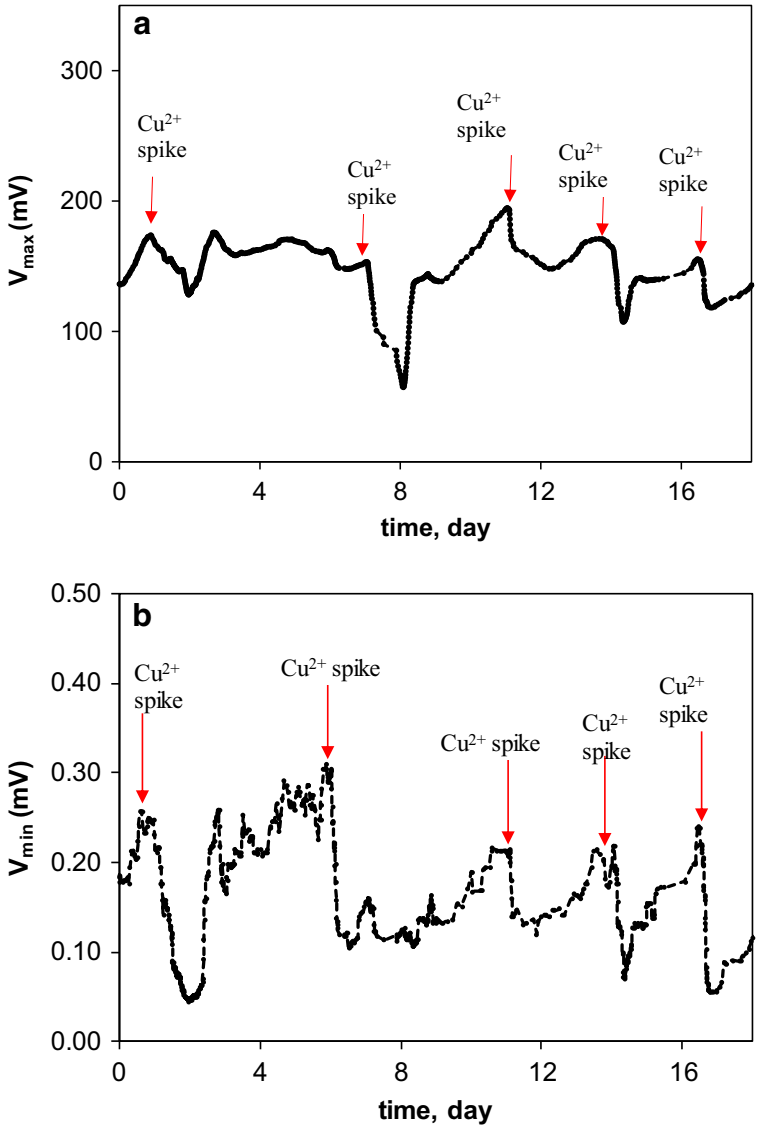

Fig. 6 Continuous detection of copper concentration spikes by the floating biosensor. V_{max} (**a**) and V_{min} (**b**) biosensor outputs are shown during Cu spikes marked by arrows

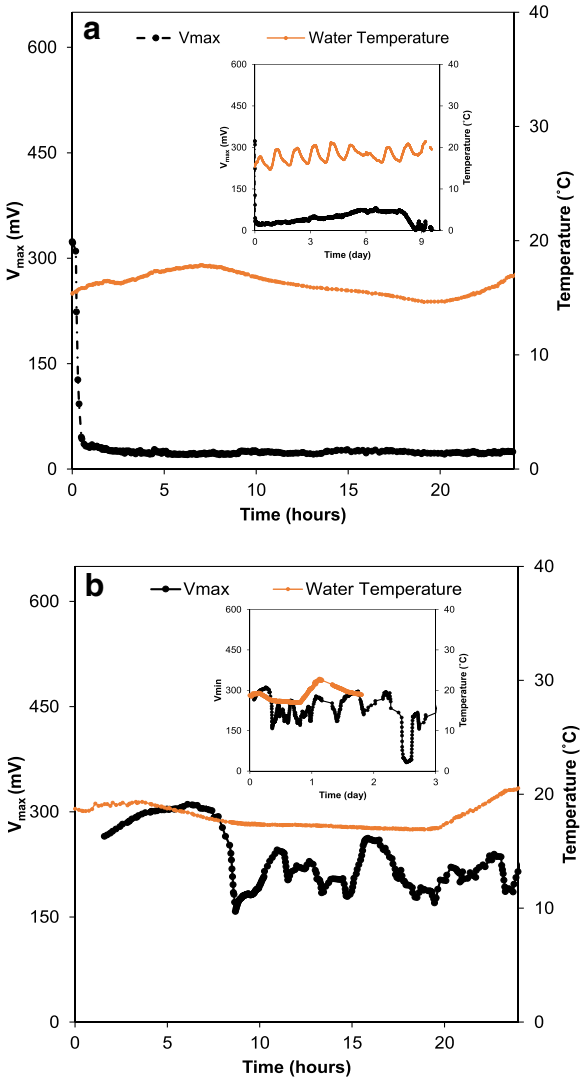

Fig. 7 V_{max} biosensor outputs recorded at two locations near Sudbury, ON. Biosensor outputs at Junction Creek (**a**), and at Hannah Lake (**b**) are recorded over a 24-h period. The insets show the biosensor outputs and water temperature recorded over 10 days for Junction Creek and 3 days for Hannah Lake

of placing the biosensors. Furthermore, after approximately 10 days of operation, the voltage of the biosensor at Junction Creek was reversed (Fig. 7a inset), indicating significant inhibition of the microbial electroactivity due to high concentrations of multiple heavy metals. Bacterial activity in MFCs can be limited by low carbon source concentration (Oh and Logan 2007), but in this case, it can be suggested that the reversal was due to the heavy metals' toxicity (Zhu and Logan 2014; Adekunle et al. 2019a). Unfortunately, data collection continued for only 3 days at Hannah Lake due to the loss of the biosensor, likely due to the presence of wildlife. Figure 7 b inset shows the performance of the biosensor before the signal was lost. Although temperature was continuously monitored and recorded, no observable impact of temperature fluctuations on biosensor performance was noticed. Overall, the field trial showed that the biosensor can be successfully used for continuous toxicity monitoring, although the sensor design needs to be improved.

Conclusion

This work evaluated the possibility of online continuous monitoring of heavy metal–related toxicity in aquatic environments using flow-through and floating MFC-based biosensors. The results of this work show that such biosensors can provide timely detection of toxicity changes. A comparison of the floating biosensor response with results of a *Daphnia* assay shows a linear correlation (R^2 = 0.9) between the two methods, thus confirming that the floating biosensor can be used to determine a toxicity response in surface waters. Finally, our study describes results of a field test involving two floating biosensors. Overall, the study successfully demonstrates the concept of an online biosensor for monitoring toxicity of aquatic environments in remote locations. The proposed MFC-based biosensor is inexpensive, simple to operate, and robust. It should be added that the biosensor response can be caused by several metals, which are either inhibiting activity of electroactive bacteria or competing with the anode as an electron acceptor. Therefore, quantitative estimation of a particular dissolved metal concentration (e.g., Cu) can be biased. Biosensor application as an online tool for qualitative (toxicity event alarm) or semi-qualitative assessment of toxicity can be envisioned.

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