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INTEGRATION OF SMFC-BASED BIOSENSOR IN CONSTRUCTED WETLAND FOR REAL TIME MONITORING AND BIOREMEDIATION OF HEAVY METALS CONTAMINATED WATERS

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ABSTRACT. Water contaminated with heavy metals is one of the most serious environmental problems in the world. Their timely detection and removal are of key importance for human health and ecosystems. The possibility of simultaneous real-time monitoring and bioremediation of waters contaminated with heavy metals has been established. Constructed wetlands with integrated sediment microbial fuel cells have been proposed for this purpose. Water contaminated with heavy metals - copper, chromium, nickel and zinc - was examined using them. All studied water samples showed a sudden voltage peak (in the range from 150 to 200 mV) when the contaminant entered the cell and a gradual decrease in voltage corresponding to the decreasing of the contaminant's concentration. Chromium ions were removed the fastest – for 28 hours. The soil played the role of a protective layer for the electroactive microorganisms in the anode area, adsorbing the heavy metals in its upper layer and ensuring the efficient operation of the system. The studies showed an increase in the concentration of heavy metals in the surface soil layer between 5 and 35 mg/kg.

Key words: constructed wetlands, sediment microbial fuel cells, real time monitoring, heavy metals

Introduction

With the rapid progress of global urbanisation and industrialisation, heavy metals are continuously entering the environment. Their accumulation in water, soil, and sediments poses a potential hazard to natural ecosystems, including plants, human health, and other organisms (Rongwei et al., 2024). Naturally occurring heavy metals or metalloids such as zinc, copper, lead, nickel, chromium, cadmium and arsenic are ubiquitous in all compartments of surface systems due to their release during weathering of primary rocks. Their subsequent involvement in various biogeochemical processes, including secondary sorption processes in soils, formation of dissolved or colloidal systems transported through soil and surface water, and uptake by plant roots followed by delocalization in leaves, may favour further transfers of heavy metals along the trophic chain. It is well known that many human activities of the last decades, such as those related to mining and processing of ore and metallurgy have led to many introduction pathways of heavy metals in the environment, thus contributing to an increase in the content of heavy metals in surface waters and soils (Walaszeka et al., 2018).

Mining operations lead to increased discharges of various types of pollutants, including heavy metals, which can threaten the quality of receiving water bodies (Etteieb et al., 2021). Active and historical mining activities release toxic metals into water, soil, and other environments, leading to ecosystem and human health concerns. Zinc, copper, lead, nickel, and chromium are among the most common metals present in high concentrations in these types of polluted waters (Yang et al., 2023). Cadmium is a toxic element that is easily taken up by plants and thus can enter food chains (Rusyn and Gomora-Hernandes, 2024) and bioaccumulate.

The removal of pollutants from wastewater is considered a major challenge in the field of water pollution. Removal of heavy metals in urban wastewater has been studied and many methods have been established to treat wastewater containing heavy metals, such as membrane separation, chemical precipitation and electrolysis. However, the application of these technologies may raise additional concerns, such as the large amount of sludge produced, high energy consumption, and complex maintenance (Mengzi et al., 2020). Among the numerous techniques available for the removal of pollutants, including heavy metals, constructed wetlands are effective in treating wastewater containing heavy metals. Constructed wetlands are considered a promising alternative technology due to their low energy consumption, environmental and economic benefits (Arliyani et al., 2024). The main mechanisms of constructed wetlands that are capable of treating wastewater containing heavy metals include physical retention, filler adsorption and sedimentation, plant adsorption, and microbial biosorption (Khan et al., 2009). Plants are essential in purifying waters of heavy metals in constructed wetlands through phytoremediation, phytostabilisation and phytoextraction processes (Nabuyanda et al., 2022). Also, for the effective removal of heavy metals through constructed wetlands, constructional features, the type of substrate, the course of the water flow and other factors are essential (Saeed et al., 2021).

In the last ten years, significant progress has been made in the research and industrial application of constructed wetlands - microbial fuel cells (CW-MFC) (Miworunyuie et al., 2025). The main advantages of this technology are low cost for construction, operation and maintenance, low energy consumption, environmentally friendly technology with the potential for highly efficient cogeneration of energy (Rusyn and Gomora-Hernandes, 2024). However, the application of CW-MFC for engineering needs requires additional studies on the various synergistic mechanisms of pollutant degradation, the rapid and correct reading of operational indicators, and the stable operation of the system during long-term operation under various environmental factors (Xie et al., 2024).

Currently, most chemical testing for wastewater quality monitoring is performed off-site and causes long-term delays for wastewater treatment plants (Hang et al., 2020). As a consequence, such methods are not suitable for real-time monitoring, thus limiting rapid response to pollution events

(Corbella et al., 2019). Online sensors are gaining critical importance for monitoring the quality of treated wastewater because they can provide online early warning systems for timely precautions and solutions (Hang et al., 2020). Bioelectrochemical systems (BES) are emerging as a strong competitor to traditional cumbersome water monitoring methodologies (Lei et al., 2021). Scientific publications show that there is a growing popularity of BES experience as a biosensor for organic pollutants, COD and BOD, dissolved oxygen, heavy metals and others (Na et al., 2019, Olias et al., 2020). Electrochemical biosensors have some advantages over conventional sensors, such as: use of microorganisms as a sensitive element and a very sharp response to toxic substances. Also, they have a simpler and cheaper construction. Therefore, these devices are suitable for online detection of pollutants in wastewater (Naik and Jujjavarapu, 2020; Rui et al., 2022).

Since these sensors provide electrical signals based on the performance of anodic electroactive microorganisms, they have major drawback. Toxic contaminants would inhibit microorganisms and even cause irreparable damage, making it impossible to trace subsequent contamination. This makes it unrealistic to use MFC-based biosensors for in situ monitoring of heavy metal pollution in aquatic environments (Schneider et al., 2016).

Sensors based on sediment microbial fuel cells (SMFC) avoid the inhibition of electroactive microorganisms. The main advantages of the sensor include:

- instead of using the inhibition of electroactive bacteria in the sediment, it captures heavy metal pollutants released into the water, reducing them on the cathode and raising the voltage.
- the sensor uses natural electroactive bacteria living in the sediment, generating a base voltage, therefore the inoculation process can be avoided.
- the sensor components are mounted in an integrated system; thus, monitoring is convenient and starts with placing the sensor in a wet area (Liu et al, 2020).

In this context, the present study aimed to evaluate the feasibility of integrating a sediment microbial fuel cell into a constructed wetland and investigate the effectiveness of simultaneous bioremediation and real-time monitoring of waters contaminated with heavy metals – copper, chromium, nickel and zinc. This work is believed to provide additional, valuable results for the potential sensing application of an SMFC-based biosensor in a constructed wetland.

Materials and methods

Soil Sampling

For the purpose of the study, river sediment was taken from Ivanyanska river, Bulgaria (42°42'27.1"N 23°11'46.4"E), from a depth of 20-30 cm. After taking the river sediment, the larger pieces were removed by sieving through 2 mm. In order to carry out physicochemical analyses, part of the sieved soil was air-dried. Soil pH was measured by pH-meter HANNA HI-9021 determined at 1:2.5 (soil: water). Soil electrical conductivity (EC) was measured by WTW LF 197-S determined at 1:5 (soil: water). The main soil physicochemical properties were as follows: pH 6.68, EC 154.45 µS/cm. The soil was classified as sandy clay loam, the Kjeldahl-N content was 0.21% and the dissolved organic carbon - 66.14 mg/kg. The amount of copper measured was 59.5 mg/kg, chrome – 20.2 mg/kg, nickel – 13.0 mg/kg and zinc – 105 mg/kg. The soil thus prepared was then used to fill constructed wetlands with integrated sediment microbial fuel cells (Figure 1). The concentration of heavy metals was determined by preliminary extraction and ICP-OES.

Description of the laboratory installation

A scheme of the laboratory installation of a constructed wetland with integrated sediment microbial fuel cell is shown in Figure 1.

For the purpose of the study, four constructed wetlands with integrated sediment microbial fuel cells were prepared. The constructed wetland was a plastic container with a volume of 10 dm^3. A graphite electrode with an area of 120 cm^2 was placed at the bottom of the vessel. The vessel was filled with river sediment with a volume of 8 dm^3. The cell was then filled with 2 dm^3 pure water and a second 120 cm^2 graphite electrode was placed in the surface layer of the water. The wetland was then planted with Typha angustifolia. The physicochemical characteristics of water were pH 7.48, Eh 264 mV and EC 48 µS/cm measured by Vernier LabQuest Mini.

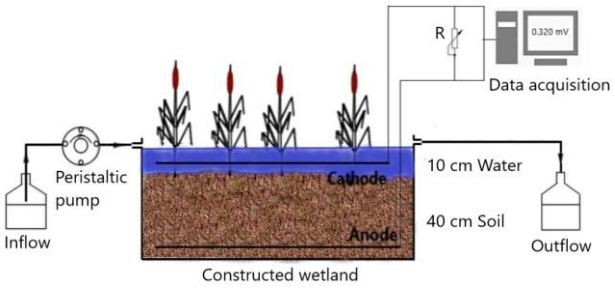


Fig. 1. Technological scheme of the laboratory installation

The heavy metal solutions pass through the cathode zone with surface flow, where heavy metal ions play the role of an additional electron acceptor and increase the voltage of the cell by changing the electrochemical parameters (Liu et al., 2020). Initially, clean water was supplied to the cells until a constant base voltage was reached. After that, the heavy metal solutions were supplied to the individual cells with a hydraulic retention time of seven days, and the change in cell voltage was continuously recorded. The concentration of heavy metals was measured periodically according to the dynamics of the voltage.

Heavy metals pollution

For the purposes of the study, four synthetic solutions containing heavy metals were prepared. The first one containing 25 mg/L Cu ($CuSO_4.5H_2O$), the second 25 mg/L Cr (K_2CrO_7), the third 25 mg/L Ni ($NiSO_4.7H_2O$) and the last 25 mg/L Zn ($ZnSO_4.7H_2O$).

Analytical methods

To determine the response of voltage signals to heavy metals shock, the biosensors` voltage was measured and recorded with Vernier LabQuest Mini and Logger Lite software.

The pH and EC parameters were measured at certain points of the laboratory installation. Conductivity and pH were measured using a WTW Multi 3510 IDS and corresponding electrodes. In order to determine the adsorption of heavy metals from the soil, after the end of the experiments, their concentration in the soil was studied by preliminary extraction and ICP-OES. The concentration of heavy metals (Cu, Cr, Ni and Zn) in water was determined by ICP-OES.

Results and discussion

To establish the applicability of the constructed wetlands with integrated sediment microbial fuel cells for real-time monitoring and bioremediation of copper pollution, clean water in surface flow was fed into the first of the cells for 72 hours. As can be seen in Figure 2, the cell maintains a stable baseline voltage in the range of 338-340 mV throughout.

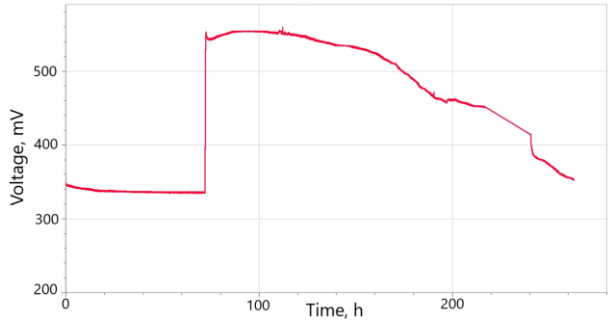


Fig. 2. The voltage dynamics of constructed wetland under copper pollution

At the 72nd hour, the supply of a copper solution with a concentration of 25 mg/L began. As can be seen from the figure, when the solution starts to flow, the voltage increases in leaps and bounds to 550 mV. It is this peak in voltage that can be used in practice to determine the presence of copper contamination. In the following hours, a gradual lowering of the voltage is observed, which corresponds to a decrease in the concentration of copper in the outgoing water. At the 200th hour, a copper concentration of 15.34 mg/L was determined by ICP, and at the 260th hour it was below the sensitivity of the method (0.05 mg/L). The voltage decreases proportionally with the concentration of copper, and it is 458 mV and 462 mV at the 200th and 260th hour, respectively. The graph obtained shows that, in addition to the presence of copper pollution, such a biosensor can also give us information about the degree of treatment of the pollutant in the constructed wetland.

The next experiment aimed to establish the application possibilities of the constructed wetlands with integrated sediment microbial fuel cells for real-time monitoring and bioremediation of chromium pollution. The first experiment showed that after the rapid stabilisation of the cell, the baseline voltage when supplying pure water did not change for up to three days. The time for supplying pure water in subsequent experiments was reduced in order to optimise the studies.

The second cell was supplied with clean water for 52 hours. As can be seen from Figure 3, during these hours the cell maintains a stable base voltage in the range of 377-379 mV.

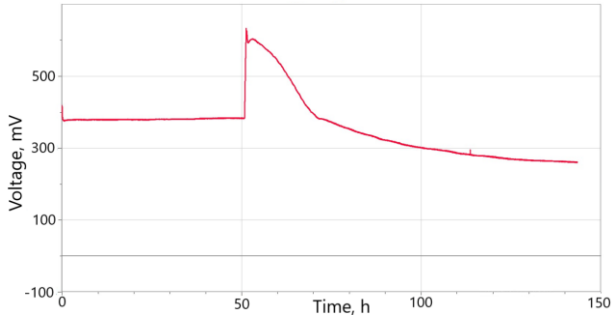


Fig. 3. The voltage dynamics of constructed wetland under chromium pollution

At the 52nd hour, the supply of chromium solution with a concentration of 25 mg/L began. As can be seen from the figure, when the solution starts to flow, the voltage rises instantly to 575 mV, and this peak in the voltage serves to determine the beginning of chromium contamination. In the following hours, a rapid decrease in voltage is observed, which, in addition to the ongoing biochemical and physicochemical processes of chromium removal in the cell, may also be due to its easy reduction to Cr(III) and the precipitation of the latter as hydroxide (Tandukar et al., 2009; Li, 2024). At the 63rd hour, a chromium concentration of 13.84 mg/L was determined by ICP, and at the 80th hour it was below the sensitivity of the method (0.05 mg/l). The voltage decreases proportionally with the concentration of chromium, and it is 500 mV and 343 mV at the 63rd and 80th hours, respectively. The voltage continues to decrease as it reaches a new baseline around 270 mV.

The third cell was used to establish the application of constructed wetlands with integrated sediment microbial fuel cells for real-time monitoring and bioremediation of nickel pollution. With a surface run of water, the supply of clean water to the third cell began for 22 hours. Figure 4 shows that during these hours the cell maintains a stable base voltage of 300 mV.

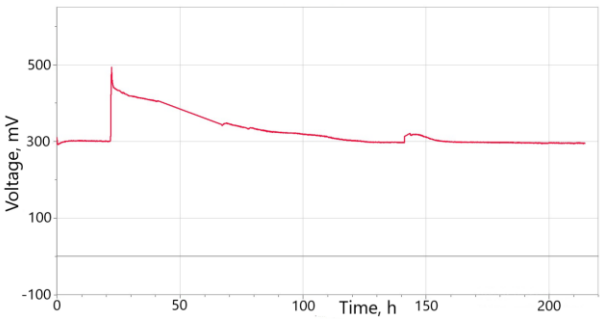


Fig. 4. The voltage dynamics of constructed wetland under nickel pollution

At the 22nd hour when starting to supply a nickel solution with a surface flow of the water, with a concentration of 25 mg/L a voltage peak is produced, reaching instantaneously to 497 mV and marking the moment of occurrence of nickel contamination. In the following hours, a gradual decrease of the voltage is observed, and at the 130th hour it reaches the base for the cell of 300 mV. At the 70th hour, the concentration of nickel was determined by ICP-OES to be 6.91 mg/L, and at the 300th hour it was 0.45 mg/L. By the end of the experiment (215th hour), the cell's baseline voltage did not change (300 mV), indicating that after the removal of the contaminant, the cell continued to work effectively as a biosensor for real-time nickel monitoring and that further contamination should be reflected as a new voltage peak.

The latter cell was used to establish the application of constructed wetlands with integrated sediment microbial fuel cells for real-time monitoring and bioremediation of zinc water pollution. A clean water supply through the cell, with a surface flow, was started for 18 hours (Figure 5).

The graph on Figure 5 shows that the cell maintains a stable base voltage of 643 mV during these hours. At the 18th hour, when starting to supply a zinc solution with a concentration of 25 mg/L, a voltage peak of up to 801 mV is obtained and marks the moment of occurrence of zinc contamination.

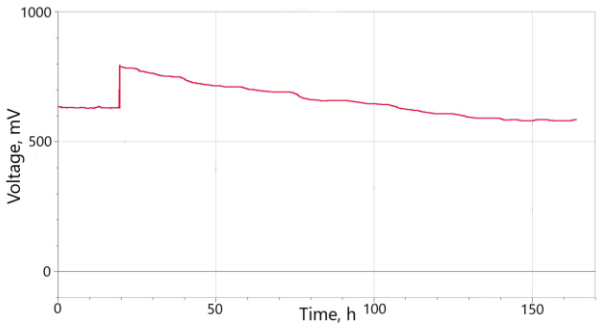


Fig. 5. The voltage dynamics of constructed wetland under zinc pollution

In the following hours, a very smooth decrease in voltage is observed, at the 50th hour it is 713 mV, at the 100th hour it is 658 mV and then it falls below the initial cell voltage, reaching a new baseline for the cell voltage around 600 mV. At the 50th hour, the concentration of zinc determined by ICP was 18.95 mg/L, at the 100th hour it was 8.06 mg/L, and at the end of the experiment the concentration of zinc in the water leaving the installation was 2,62 mg/L.

Adsorption of metals - contaminants by the soil is one of the most serious advantages of soil (sedimentary) microbial fuel cells - based biosensors. In this way, they can be used repeatedly without affecting the electroactive microflora, which is essential for energy generation in the sedimentary microbial fuel cell. Therefore, a study was conducted on the adsorption of heavy metals in the different layers of the soil as the solution passed through the cell. The results are presented in Table 1.

Table 1. *Distribution of pollutants in the different soil layers*

Soil layer	Cu, mg/kg	Cr, mg/kg	Ni, mg/kg	Zn, mg/kg
0-20 cm	69.8	25.7	25.4	140
20-40 cm	60.5	20.5	13.7	108
Initial soil	59.5	20.2	13.0	105

It can be seen from Table 1 that the concentration of heavy metals is significantly higher in the first twenty centimetres of the depth of the layer. In the bottom layer, the concentration of heavy metals remained close to that measured in the initial soil. This indicates that even under shock fouling, the electroactive microorganisms inhabiting the anodic region remain protected from toxic shock, allowing the biosensor to be used long-term under repeated heavy metal shock fouling.

Conclusion

Timely detection and treatment of water contaminated with heavy metals is important for the protection of the environment in the industrial world. Methods offering real-time monitoring and effective treatment should be inexpensive for widespread application. Our proposed development offers simultaneous bioremediation of heavy metal-contaminated waters in a constructed wetland and real-time monitoring through naturally generated voltage from an integrated sediment microbial fuel cell. The results showed an immediate response to contamination with copper, chromium, nickel and zinc in concentrations of 25 mg/l, as well as their ions effective removal. When heavy metal ions were introduced into the system, a sharp jump in voltage between 150 and 200 mV was observed. Chromium ions were removed through the constructed wetland in the shortest time - 28 hours. At the beginning and end of each experiment, the voltage had stable baseline values, indicating the possibility of using this type of biosensors in case of repeated contamination. The top soil layer adsorbs a large part of the heavy metals, preventing them from reaching the electroactive microorganisms in the anode area and compromising the performance of the system. An increase in the concentration of heavy metals in the surface layer of the soil between 5 and 35 mg/kg was observed. Initial research has shown a good opportunity for widespread application of constructed wetlands with integrated sediment microbial fuel cells as systems for real-time heavy metals pollution monitoring and bioremediation of contaminated waters.

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