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Chapter

In-Situ Contaminant Detection by Portable and Potentially Real-Time Sensing Systems

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Abstract

This chapter aims to provide information on the progress of research into water quality analyses, providing an overview of the state of the art, including novel research achievements, in the detection of water contaminants. After a brief introduction to the main sensing systems' characteristics, the attention will be devoted to two different classes of pollutants: organic and inorganic. Microbiological analyses concerning the monitoring of bacterial load in water and chemical analyses with a special focus on mercury, related to heavy metal pollution, and nitrogen compounds, i.e. nitrate ion and ammonium ion, are discussed. Particular attention will be devoted to all sensing systems that are in principle portable and able to make real-time measurements in situ.

Keywords: water quality, bacteria, heavy metals, nitrogen compounds, silicon photomultiplier, biosensors, electrochemical sensors, conductive polymers, metallic nanoparticles, catalysis

1. Introduction

Water is essential for life and its quality is crucial for human health and environmental sustainability. Regular analysis of water is important to ensure its safety and purity. Microbiological analysis is fundamental for detecting harmful bacteria such as, for example, *Escherichia coli, Legionella,* and *Salmonella*, or dangerous chemical presence, i.e. heavy metals or hydrocarbons, which can cause serious waterborne diseases if present in drinking water [1]. Chemical analysis, on the other hand, helps prevent environmental problems, such as eutrophication due to excessive nitrogen compounds, or human health problems that can arise from contamination by heavy metals such as mercury. For these reasons, a careful analysis of water samples is crucial for human health and environmental protection. The different contamination issues must be investigated separately to determine the best detection strategies. This chapter aims to describe the various sensory detection systems (design and manufacture) of bacteria, mercury (as an example of heavy metal), and nitrogen compounds in water.

Monitoring bacteria in water is essential to ensure public health and prevent waterborne diseases such as cholera, typhoid fever, and other types of waterborne diseases. Contaminated water can lead to serious epidemics, especially in communities with limited access to clean water. In many developing countries, water is still known as "black" [2]. These countries are characterized by high population density and should have good water quality for the population to survive. Bacterial indicators may differ among countries, e.g. total coliforms, fecal coliforms, heterotrophic micro-organisms, *Clostridium perfringens*, etc. [3]. This monitoring allows us to evaluate the effectiveness of disinfection systems already in use within the Water Safety Plans [4, 5] and to test and develop antibiofilm methods to further contribute to improving water quality [6, 7]. Regular monitoring helps to detect bacterial contamination early, enabling timely intervention and treatment to prevent the spread of the disease. Among traditional monitoring methods, such as bacterial culture and membrane filtration, new techniques have been developed such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), advanced methods that sequence DNA from water samples, providing comprehensive data on bacterial communities, and in the case of NGS allowing the identification of known and unknown pathogens.

Innovative methods of bacterial monitoring have been developed using a Silicon PhotoMultiplier (SiPM) [8]. This device is a highly sensitive pixelated photon detector, each pixel being a silicon photodiode operating in avalanche mode [9]. It has a high sensitivity to detect even individual photons, a wide dynamic range for varying light intensities, and a rapid response to light signals [10]. It was also employed for monitoring heavy metals, such as mercury by using engineered bacteria as a sensing element emitting a light signal in its presence, which is then transduced into an electrical signal.

Mercury is one of the most difficult heavy metals to detect and the most dangerous element of the periodic table, excluding the radioactive ones. It exists in three forms with different properties and toxicity: elemental or metallic mercury (Hg^0), inorganic mercury, and organic mercury compounds (Hg^{2+} and Hg^+, respectively). Each one can be converted into other forms through oxidation, reduction, methylation, and demethylation that occur due to the presence of chemical compounds or through the metabolic process of fishes or plants [11].

Long exposure to mercury can cause neurological disturbances, memory problems, skin rash, and kidney abnormalities. In addition, when combined with other chemical elements, such as sulfur or oxygen, it can lead to severe digestive system corrosion, and when mercury is combined with carbon, especially in fresh and seawater, it accumulates in the food chain and, during pregnancy, it can pass through the placenta, causing developmental abnormalities and cerebral palsy to infants [12, 13].

It is worth noting that Hg^{2+} can accumulate in biofilms in pipes of main water and be converted into other toxic forms. Mercury toxicity depends on the form and its water concentration is expected to be very low. The tolerance value is 1 µg/L for water intended for human consumption and contact recreation and thus revealing water contamination can result essential in avoiding risks to human health [14, 15].

Finally, other chemical species must be monitored to provide a meaningful water analysis and we focused our attention on nitrogen compounds. Nitrogen plays a critical role in the structure and function of life-essential biomolecules, in the cycling of elements in nature, and in the provision of nutrients to plants, significantly contributing to the development and maintenance of life on Earth. Plants absorb nitrogen from the soil mainly in inorganic forms: nitrate and ammonium. Specifically, nitrate ions are absorbed by plants through their roots primarily via an active process that

requires energy as it involves specific cell membrane carriers. In contrast, ammonium ions can be absorbed by plants either through an active process or passive diffusion across cell membranes [16, 17]. Under excessive irrigation, plants are unable to absorb all the nitrate and ammonium ions present in the soil, allowing them to percolate through the soil and enter groundwater or surface water bodies. An excessive presence of these nutrients in groundwater or water bodies can lead to eutrophication which can damage aquatic ecosystems [18]. Furthermore, nitrogen can be produced as the result of disinfection of water with chlorine dioxide or monochloramine. An excessive presence of nitrogen compounds (the so-called disinfection by-products) in drinking water is harmful to humans as nitrates convert to nitrites, which bind to hemoglobin, making it incapable of transporting oxygen to tissues in the body.

Several chemical methodologies exist to monitor the concentration of nitrogen compounds and mercury in water, such as flow injection analysis, spectrophotometry, chemiluminescence, capillary electrophoresis, ion chromatography, FTIR spectroscopy, colorimetric pH detection, ion-selective electrodes, and other optical methods [19–24]. Although these techniques are sensitive and specific, they require sophisticated, expensive, time-consuming systems, and the necessity for sampling campaigns.

Despite the fact that the contaminants to be detected are very different (microorganisms, mercury, and nitrogen compounds), a common demand can be inferred: the need for portable systems to perform in-situ measurements that should be precise, simple to be made, and the devices should provide a detection limit at least within the World Health Organization defined limits [25].

The rest of this chapter reviews the three examples of contaminants and the most investigated detection system to infer their concentration in water samples.

2. Microbiological analysis

The main methods for bacteria detection in water samples employ different techniques, such as bacterial cultural growth, immunological, genetic, physical, and chemical methods. Hereafter, all of them are briefly reviewed.

2.1 Cultural methods

One of the most used methods to detect microorganisms' presence in water is the Heterotrophic Plate Count (HCP). It allows for estimating the total aerobic heterotrophic bacteria charge (organisms taking energy from organic carbon) in each sample. The water specimen is collected using a sterile container and spread over a solid culture medium in Petri dishes to provide nutrients for bacterial growth (**Figure 1**). After dish incubation, the formed bacteria colonies are counted in terms of Colony-Forming Units per milliliter of water (CFU/mL) [26]. However, this technique is usually limited to bench-top equipment.

2.2 Enzyme/substrate methods

These methods are based on colorimetric or fluorometric assays concerning specific enzymatic activities. During bacterial growth, the bacterial enzymatic activity allows the fluorophore to be cleaved from the substrate, increasing the fluorescence signal. The sensitivity is strictly related to the time of analysis. Immunological methods are based on the interaction between a specific antibody (Ab) and a specific antigen (Ag). Ab is
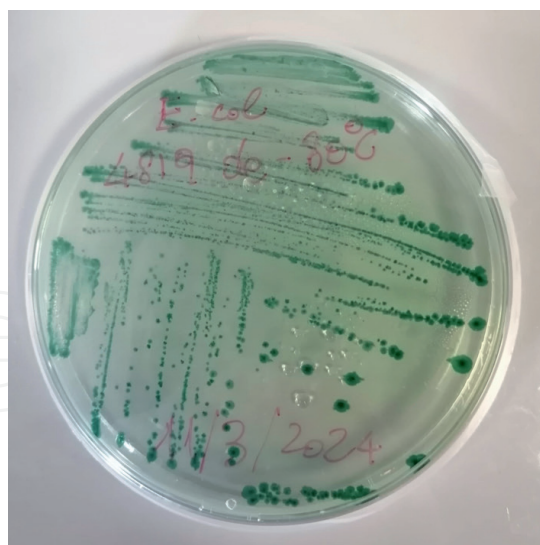
Figure 1.
Escherichia coli *growth on agar medium.*

mounted onto a support system, such as nylon supports, cantilevers, magnetic beads, nanoparticles, graphene quantum dots, electrospun nanofibers, and polymers [27]. The most important immunological techniques are Enzyme-Linked Immunosorbent Assay (ELISA) [28], lateral flow tests (immunochromatographic assays) [29], SPR (Surface Plasmon Resonance) [30], and electrophoresis [31]. However, in most cases, these methods suffer from poor specificity, sometimes returning false-positive results, contributing to issues of low sensitivity [32, 33]. The sensitivities and the specificities of immunological methods depend on the antibody, with a minimum limit of detection (LoD) of 10^5 cells/mL employing ELISA [34]. Finally, the analysis can last from several hours for ELISA to some minutes for lateral flow.

2.3 Genetic methods

Genetic methods are highly specific due to their affinity to specific nucleic acid sequences. The polymerase chain reaction (PCR) [35] is the most used technique employing appropriate short nucleic acid sequences (primers) for DNA sequence amplification relative to bacterial strains. It is characterized by the execution of several steps at different temperatures (see **Figure 2**). The fluorescence phenomenon can be coupled with PCR to monitor bacterial DNA amplification in real time, to obtain quantitative information about the initial amount of DNA contained in the sample under analysis; this technique is known as "quantitative PCR" or "qPCR" [36, 37], which is easy to automate and can be used when pathogens concentration is below the LoD of other assays. Recently, researchers developed an "isothermal technique" to obviate the shortcomings of PCR, which employs in the same way the nucleic acid-based method for bacteria strain identification by amplifying nucleic acids at a fixed temperature [38]. In particular, the loop-mediated isothermal amplification (LAMP) technique has gained popularity due to its specificity, sensitivity, and stability [1, 39]. The disadvantages of these methods are the quality of the sample, which remarkably impacts the sensitivity of the detection, and the presence of inhibitors which create limitations in the analysis of samples. Immuno-PCR (IPCR) exceeds these limits since it combines ELISA with qPCR. Such a combination allows the ELISA to enhance the LoD about 100–1000 times using qPCR to amplify the system signal [40].
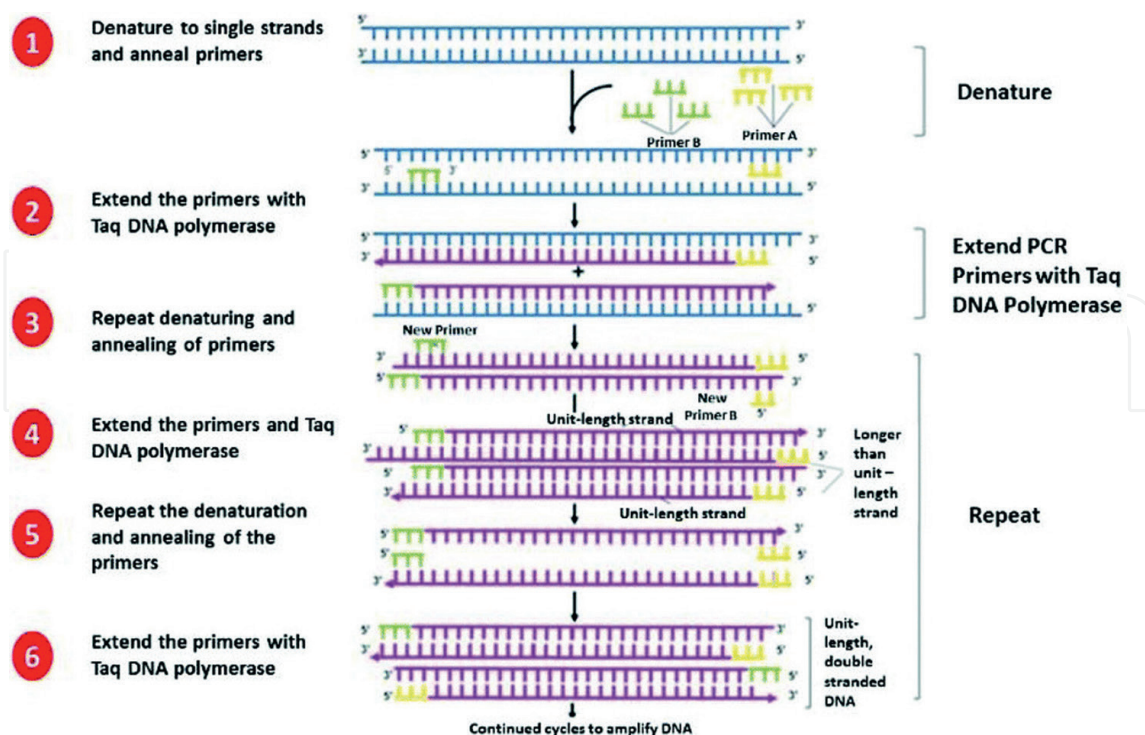
Figure 2.
Scheme representing PCR cycle. Reprinted with permission from Rajapaksha et al. [35].

2.4 Mass spectroscopy methods

Mass spectroscopy techniques are indicated for their accuracy, selectivity, reproducibility, and high Signal-to-Noise Ratios (SNR). Tandem Mass Spectrometry (MS/MS) [41], nanoscale Liquid Chromatography coupled with MS/MS (nano LC-MS/MS) [42], and Matrix-Assisted Laser Desorption-Ionization Time Of Flight mass spectrometry (MALDI-TOF) [43, 44] are the most used techniques for detection and identification of bacterial proteins peptide sequences by comparing the obtained mass spectra to associated databases. The limitation linked to these methods concerns the similarities between organisms and the limited number of spectra in the databases, which can lead to poor discrimination between species or misidentifications [44]. Furthermore, these techniques are not indicated for quantitative analysis.

2.5 Fourier transform infrared (FT-IR), raman spectroscopy, and laser-induced breakdown spectroscopy (LIBS)

Fourier Transform Infrared (FT-IR) is a rapid tool indicated for the investigation of the total composition of all components of the bacterial cells under analysis by using infrared spectroscopy [45], although precise identification with this technique remains to be developed. Raman spectroscopy is currently used for the classification of complex biological samples, due to its fast and non-destructive methodology [46]. Finally, Laser-Induced Breakdown Spectroscopy (LIBS) is a rising spectroscopic method based on atomic emission, in which elemental composition is determined by placing samples in a laser plasma and observing the resulting spectra. For example, LIBS is used to differentiate different types of bacteria and their metabolic state [47]. The main advantages of LIBS-based bacterial detection technology are the speed of analysis (some minutes) and the ability to detect pathogens on all types of surfaces.

However, all the described techniques require the presence of skilled human operators for sample preparation, treatment, processing, and results interpretation.

2.6 Methods based on ATP-luciferin-driven chemiluminescent reaction

Currently, chemiluminescent reaction based on the interaction between adenosine triphosphate (ATP) and luciferin is one of the most sensitive methods for bacterial load detection [48]. It allows obtaining quantitative information about viable cell concentration in a sample [49–51] by employing the internal ATP after appropriate lysis procedures. This reaction is catalyzed by the enzyme luciferase, to obtain luciferyl adenylate that combines with oxygen to produce oxyluciferin and a photon at 560 nm. The reaction is reported in the following [52]:

$$luciferin + ATP + O_2 \underset{luciferase}{\overset{Mg^{2+}}{\rightarrow}} oxyluciferin + AMP + CO_2 + pyrophosphate + hv \quad (1)$$
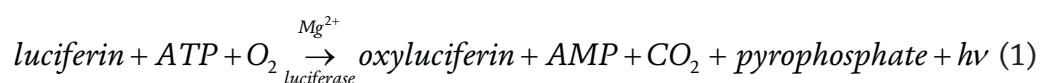
Some commercial readers based on ATP-luciferin-driven reactions reach low limits of detection, such as BioThema ATP Kit SL [53], Luminultra Quench-Gone Aqueous (QGA) Test Kit [54], Hygiena handy type luminometer [55], with LoDs reaching values down to 10^{-12} M, corresponding to about 5×10^2 cells/mL if a mean amount of ATP equal to 1 fg for the single viable cell is considered (see Microbial Equivalent definition [56]).

Hu et al. [57] developed a low-cost and portable device based on the ATP-luciferin-driven chemiluminescence reaction taking place in test tubes for bacterial charge detection, employing a SiPM as a detector. The system reaches a LoD for ATP concentration of 3.6×10^{-11} M, corresponding to about 1.8×10^4 cells/mL.

The described devices also require skilled human operators and long sample treatment times. To overcome such an issue, Santangelo et al. [8] designed and developed a prototype of a low-cost, miniaturizable, real-time, and remote-sensing microfluidic SiPM-based sensor for bacterial ATP detection, which does not require the presence of human operators, allowing to reach a LoD for bacterial charge of about 4×10^6 cells/mL (8×10^{-9} M for ATP).

Recently, Capuano et al. [58] developed an optimized low-cost, miniaturizable, real-time, and remote-sensing SiPM-based sensor based on static ATP-luciferin chemiluminescent reaction measurements (**Figure 3**), allowing the detection of water total bacteria concentration down to about 10^5 cells/mL (2×10^{-10} M for ATP).

It is worth noting that, although the described devices return detailed information about the concentration of the total bacterial load, these systems are not selective,
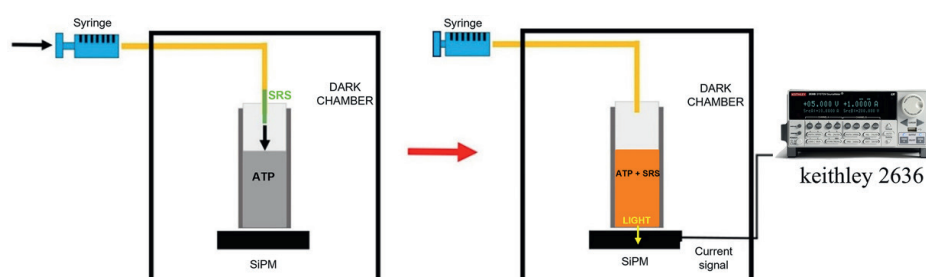

Figure 3.
Schematic layout of the ATP detection system developed by Capuano et al. [58] (reprinting permitted). The chemiluminescent reaction takes place when the reactants contained in the standard reaction solution (SRS) interact with ATP. The light emitted during the reaction is acquired through a SiPM, obtaining a current signal proportional to ATP concentration.

since ATP is contained in similar quantities in all viable cells, and discrimination among different bacterial species is not achieved.

3. Heavy metals detection in water: mercury (Hg^2+)

Many techniques have been developed for detecting mercury in water samples providing high-performance quantification. The traditional analysis systems need bulky and costly laboratory instruments, require specialized personnel, and are not suitable for in-situ monitoring.

Chromatographic and spectrometric methods are the most used in mercury analysis and these powerful techniques can be coupled with other monitoring systems. Sanchez-Rodas et al. [59] coupled chromatographic and non-chromatographic separation with Atomic Fluorescence Spectrometry (AFS). The latter is a technique based on the absorption of radiation of characteristic wavelengths by an atomic vapor with subsequent detection of radiationally deactivated states via emission in a direction orthogonal to the excitation source. This method is extremely sensitive and selective in determining environmentally and biomedically important elements such as mercury, arsenic, lead, and cadmium. The separation of the mercury species can occur either by Gas Chromatography (GC) or High-Performance Liquid Chromatography (HPLC). Cold Vapor (CV) is employed with liquid chromatography to convert inorganic Hg to its elemental state. Using liquid chromatography combined with AFS detection (HPLC-CV-AFS) leads to detection limits of 0.2 µg/L for methylmercury (MeHg), 0.07 µg/L for Hg (II), 0.06 µg/L for phenylmercury (PhHg), and 0.12 µg/L for ethylmercury (EtHg) [59]. Gas chromatography has been more widely used than liquid chromatography for Hg detection with AFS. In this case, pyrolysis has been employed as an intermediate step for Hg species oxidation [60]. Detection limits of GC-pyro-AFS have been improved over time reaching 0.13 ng/L for Hg (II) and 0.01 ng/L for MeHg [59].

Other strategies have been developed without chromatographic separation using AFS as a detection process. These include various types of CV-AFS using different chemicals for Hg revealing. In particular, a hydrostatically modified electro-osmotic flow and a newly developed interface have been used for AFS exploitation, detecting Hg(I), MeHg, EtHg, and PhHg in the range of (6.8–16.5 µg/L) [61], while conjunction of flow injection with AFS using a modified flow-through chamber as the interface was employed for fast separation of Hg(II) and MeHg with detection limits of 0.1 and 0.2 µg/mL when applied to water samples [62].

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique with LoD lower than ng/L and great linearity in revealing elements. The requirement of elemental forms leads to coupling versatile separation techniques like HPLC, GC, and Capillary Electrophoresis (CE) with a highly sensitive detector, such as ICP-MS, for ultra-trace elemental speciation analysis [63]. Employing the HPLC-ICP-MS method LoD of 0.07 ng/L for inorganic mercury and 0.02 ng/L for MeHg$^+$ was reported in investigating mercury in seawater samples [64]. Analytical features, such as low detection limits and wide linear calibration ranges, make AFS and ICP-MS great atomic detectors in the speciation and detection process. Compared to ICP techniques, AFS provides additional advantages: low acquisition and running costs, robustness, and ease of operation [59].

In the last decades, many efforts have been made to develop alternative miniaturizable systems to achieve inorganic mercury analysis in water samples. Lots of sensors

based on electrochemical techniques and optical methods employing colorimetry, fluorimetry, and Surface-Enhanced Raman Scattering (SERS) have been designed revealing extremely promising thanks to their high sensitivity and selectivity, fast results, and cost-effectiveness [65, 66].

Electrodes modified with biochemical or chemical compounds raised great attention. The advantages of using them are the large surface area, high electrical conductivity, biocompatibility, heterogeneous electron transfer, and mechanical strength. Many types of electrodes revealing mercury have been designed. Among them, it is worth mentioning the following: polymer-based electrodes which use conductive polymers that contain functional groups binding toward mercury; Ion-Imprinted Polymers (IIP) modified electrodes that have high binding capacity, selectivity, and stability [67]; electrodes based on complexing agents forming complexes with mercury ions [68]; DNA-modified electrodes in which oligonucleotides that contain T-T mismatches can selectively bind mercury with a LoD lower than 0.5 μg/L [69]; finally, nanoelectrodes that show high surface area with higher working electrode surface, adsorption capacity, and catalyzing electron transfer.

Nanomaterials have been widely used in biosensing and various strategies have been developed for mercury detection. Carbon nanomaterials have higher sensitivity, and, among them, carbon nanotubes have raised attention. Specifically, Single-Walled Carbon Nanotubes (SWCNTs) and Multi-Walled Carbon Nanotubes (MWCNTs) have more advantages. Metal nanoparticles show higher response in the field of electroanalysis compared to unmodified electrodes and in some cases, increase the transfer of electrons between electroactive substances and electrodes [70]. Lastly, there is a growing interest in hybrid nanomaterials formed by metallic and carbon structures, such as gold nanoparticles coupled with Carbon Nanotubes (AuNPs/CNTs) [71, 72].

Biosensors have received widespread attention for their relatively low cost, miniaturization, and easy-to-use characteristics. A biosensor is made of two main parts: a biorecognition element that specifically binds to the analyte to be detected. It can be a nucleic acid, antibody, a whole-cell, aptamers, enzymes, or biomimetic receptors; a transducing element that transduces biological response in an electrochemical, optical, magnetic, or acoustic signal [73].

Some drawbacks can occur when using biorecognition elements such as enzymes, biomimetic receptors, or antibodies. Enzymatic-based biosensors are not specific in binding mercury leading to poor selectivity because various heavy metals may cause cross reactivity. In addition, antibody-based biosensors also show some limitations due to reaction conditions, like temperature, pH, and ionic strength affecting antibody activity [74].

Whole cell-based biosensors are based on cells, in most cases microorganisms which can be natural or recombinant [75], that act like biorecognition elements. There are many advantages to using whole-cell biosensors. Firstly, cells can be easily cultivated compared to enzymes making whole cell-based biosensors cheaper than enzyme-based ones. Moreover, microbial sensors are more selective than enzyme ones, thanks to pathways used in microorganisms [76]. Lastly, whole cell-based biosensors usually do not require sample preparation or preconcentration [77] and, compared to antibody-based biosensors, present higher resistance to environmental conditions (e.g. changes in pH, temperature, etc.) [78].

Sciuto et al. [12] developed a miniaturized optical system for inorganic mercury (Hg^{2+}) detection in water, combining engineered *E. coli,* capable of emitting light in the presence of mercury, with a Silicon PhotoMultiplier (SiPM) used as a detector (**Figure 4**). The sensing element used for revealing Hg^{2+} was a strain of *E. coli* modified
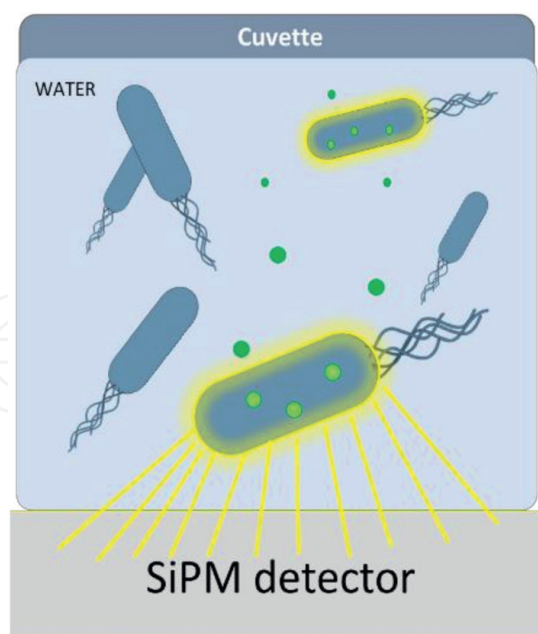
Figure 4.
Design of detecting system. The upper part stands for cuvette with a water sample containing mercury and bacteria emitting light detected by the SiPM (lower part of the figure). Reprinted with permission from Sciuto et al. [12].

with the recombinant plasmid containing a fusion between the gene *merR*, coding for transcription repressor of mercury resistance operon, and genes *luxCDABE*, that encode for bacterial luciferase and its substrate, all regulated by the *PmerT* promoter. Expression of lux genes is induced when Hg^{2+} enters bacteria cells from the water sample and binds to MerR protein, causing the release of its repression on the promoter. This triggers the expression of luciferase and its substrate whose interaction causes 490 nm blue-green light emission that can be detected by SiPM (**Figure 5**).

Bioluminescence intensity was related to a mercury concentration in water samples ranging from 0.25 to 200 µg/L, with a LoD of 0.15 µg/L and providing results in only 20 minutes.
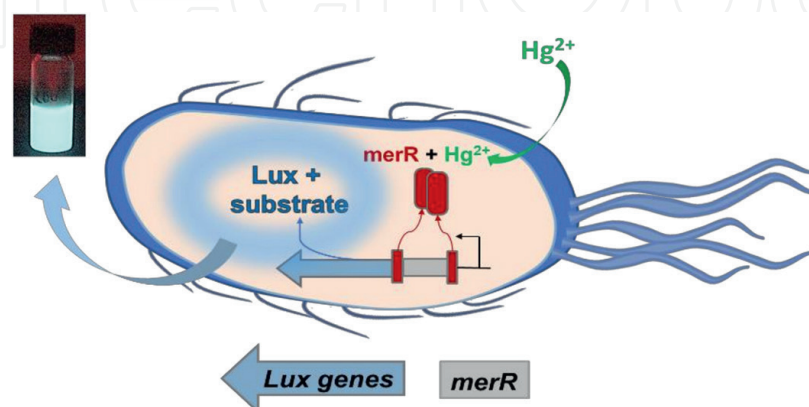

Figure 5.
The mechanism for Lux genes transcription. Mercury binds to protein MerR that provides the de-repression of promoter regulating transcription of luciferase and its substrate. Reprinted with permission from Sciuto et al. [12].

4. Nitrogen compounds detection in water: nitrate and ammonia ions

4.1 Electrochemical sensors

Electrochemical sensors are composed of three electrodes: the working electrode (WE), the reference electrode (RE), and the counter electrode (CE). They can be manufactured using screen-printing technology, by printing the electrodes on a low-cost solid substrate with reproducible chemical performance (**Figure 6**).

The inks used for printing electrodes determine the properties of the electrochemical cell. The appropriate functionalization of the WE surface plays a key role in the development of sensitive and selective chemical sensors and biosensors. This involves modifying the electrode surface with specific chemical molecules, metal nanoparticles, or biological molecules that have a high affinity for the target analyte. For example, enzymes can be bonded to the electrode to selectively interact with certain chemicals; gold nanoparticles can be used to improve electrical conductivity and sensitivity. The functionalization process increases the specificity and efficiency of the sensor, enabling accurate and reliable detection of various substances in a sample. The miniaturized design allows these electrochemical cells to be portable and suitable for real-time analysis and on-site measurements due to their linear output, low power demand, rapid response, high sensitivity, and capacity to operate at room temperature [79, 80].

The electrochemical sensors undergo an electrochemical reaction on the WE, resulting in changes in current, potential, charge, conductivity, or impedance, that can be measured using different electrochemical techniques. The most used electrochemical techniques are voltammetry, amperometry, potentiometry, and impedance spectroscopy.

In voltammetry, the current as a function of applied potential is measured. By varying the potential at the working electrode, information on redox reactions, kinetics, and thermodynamics of species in solution can be obtained. The most common types of voltammetry include Cyclic Voltammetry (CV), Linear Sweep Voltammetry (LSV), Differential Pulse Voltammetry (DPV), and Square-Wave Voltammetry (SWV).

Amperometry measures current at a constant applied potential. It is often used for the detection and quantification of analytes in solution. The current is directly proportional to the concentration of the electroactive species, allowing for sensitive detection limits. In contrast, chronoamperometry measures current as a function of time at a fixed applied potential. It is often used to study reaction kinetics, electrode processes, and diffusion-controlled processes.
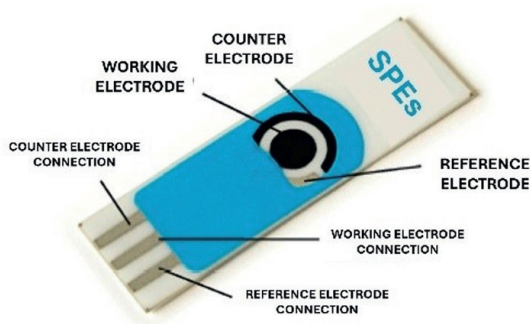

Figure 6.
Scheme of screen-printed electrodes (SPEs).

Potentiometry measures the potential of an electrochemical cell without current flow. It is commonly used in pH and ion-selective electrode measurements. Potentiometric sensors can be selective for specific ions using ion-selective membranes.

Impedance spectroscopy is a technique that measures the electrical impedance of an electrochemical system as a function of frequency. It can provide information on the kinetics of electrode processes, charge transfer resistance, bilayer capacitance, and diffusion processes.

These electrochemical techniques offer valuable insights into the behavior of chemical species at electrode interfaces and are widely used in environmental analysis and biochemistry [81, 82].

4.2 Nitrogen compounds detection in water

To date, various types of electrochemical sensors have been developed to detect NO_3^- [64–66]. Generally, nitrate ion electrochemical sensors are developed using specific nitrate ionophore molecules deposited on the working electrodes, or by exploiting the reduction reaction of nitrate using metal particles. Copper (Cu) is one of the most effective metals to catalyze the electroreduction of NO_3^- due to its high conductivity (5.8×10^7 S/m), which improves charge transfer [83–85], compared to platinum (Pt), silver (Ag), and gold (Au). Researchers showed that Cu deposition, increasing the electroactive surface area of the working electrode, lowers the LoD of electrochemical NO_3^- sensors. For instance, Inam et al. [86] developed a flexible screen-printed amperometric NO_3^- sensor, functionalized with copper nanoclusters deposited via cyclic voltammetry on silver WE. The obtained copper nanocluster morphology and size were uniform all over the Ag surface (**Figure 6a**). The device showed a high capability to detect NO_3^- in water in a dynamic concentration range from 50 to 5000 μM (**Figure 7b**) with a LoD of 0.207 nM using LSV.

Farina et al. [87, 88] developed an electrochemical amperometric NO_3^- sensor, functionalized with Cu micro-flowers electrodeposited via CV on Carbon WE (**Figure 8a**). The flower-like structure of the crystals favors a greater catalytic effect for nitrate reduction since increases the mass transport and the electron transfer
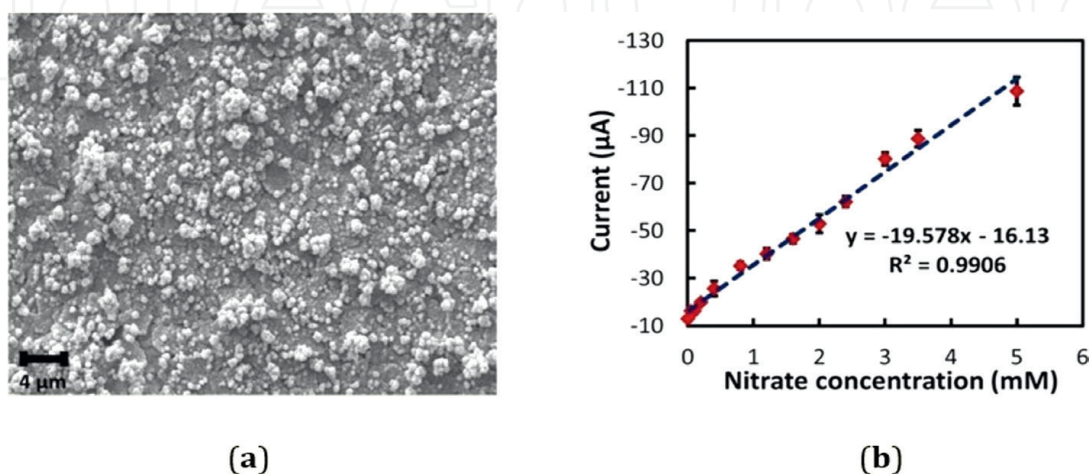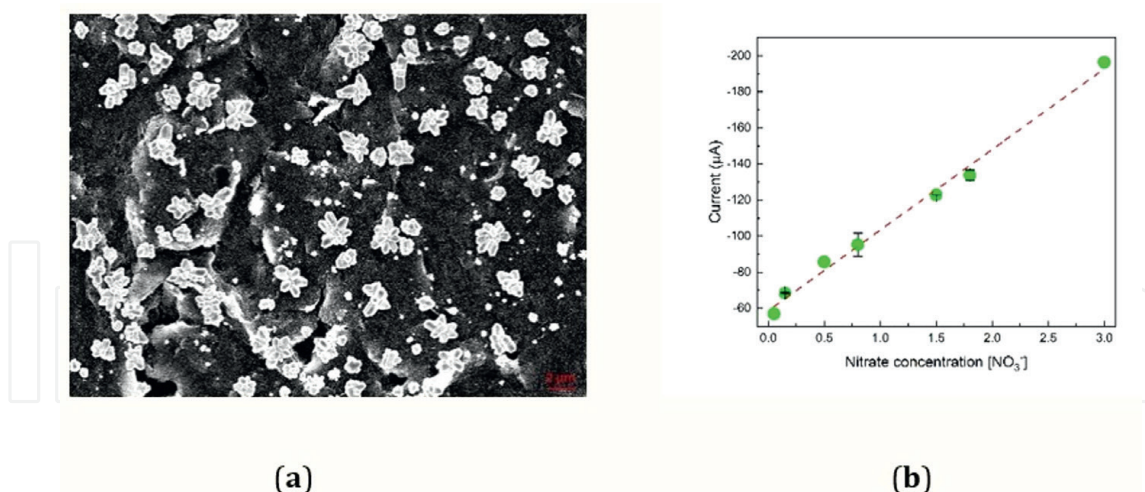

Figure 7.
(a) SEM micrograph of Cu/Ag surface characterized by a uniform deposition of Cu nanoclusters; (b) Calibration curve of the Cu/Ag sensor for NO_3^- detection in water. Reprinted with permission from Inam et al. [86].

Figure 8.
(a) SEM micrograph of Cu/C surface characterized by a Cu micro-flowers deposition; (b) Calibration curve of the Cu/C sensor for NO_3^- detection in water. Reprinted with permission from Farina et al. [87].

process. The obtained sensor showed a LoD of 0.87 μM for NO_3^- in water and a wide dynamic concentration range from 0.05 to 3.00 mM using LSV (**Figure 8b**).

Nitrate detection was investigated by Hyusein et al. [89] using commercial carbon nanofibers (CNF) and single-walled carbon nanotube (SWCNT) electrodes modified with Cu and Pd-Cu in sulfuric acid solution via LSV. The data (**Figure 9b** and **d**) show a higher sensitivity of the reductive currents for the Pd-Cu modified CNF and SWCNT electrodes than for the Cu modified ones. In particular, the electroanalytical response of the Pd-Cu modified SWCNT electrode shows an extended concentration range of non-linear electroanalytical response. Instead, Pd-Cu modified CNF electrode remains linear but in a restricted concentration range. Therefore, the SWCNT modified with Cu and bi-metallic Pd-Cu particles showed a high sensitivity in a larger concentration range of 0.1 to 7.8 mM with a LoD of 52 μM.

This behavior can be traced back to the morphological composition of the electrodes. Specifically, the Pd-Cu modified CNF electrode shows individual well-separated Pd particles where the deposition of Cu resulted in nucleation and growth of well-separated Cu particles with larger sizes (**Figure 9a**). Instead, Pd-Cu modified SWCNT electrode shows a dense population of overlapping Pd particles that decreased the C surface available for the nucleation and growth of Cu. Therefore, the Cu deposition formed micrometric crystals in the defects of the C structure (**Figure 9b**). Probably, the contact between Cu and Pd in areas populated by Pd NPs led to a sensitive and stable electroanalytical signal for nitrate reduction.

Conducting polymers are suitable for the detection of ammonium ions in water due to their electrical conductivity, ion exchange capacity, high sensitivity, and selectivity toward specific ions such as ammonia (NH_4^+). They can be easily functionalized or modified to increase their affinity toward target ions, improving sensor performance in terms of detection limits and response time. When NH_4^+ reaches a conductive polymer-based sensor, it can undergo ion exchange reactions with the polymer, resulting in changes in the electrical conductivity of the material that can be correlated with the analyte concentration. Their compatibility with microfabrication techniques also allows the development of miniaturized sensors for real-time
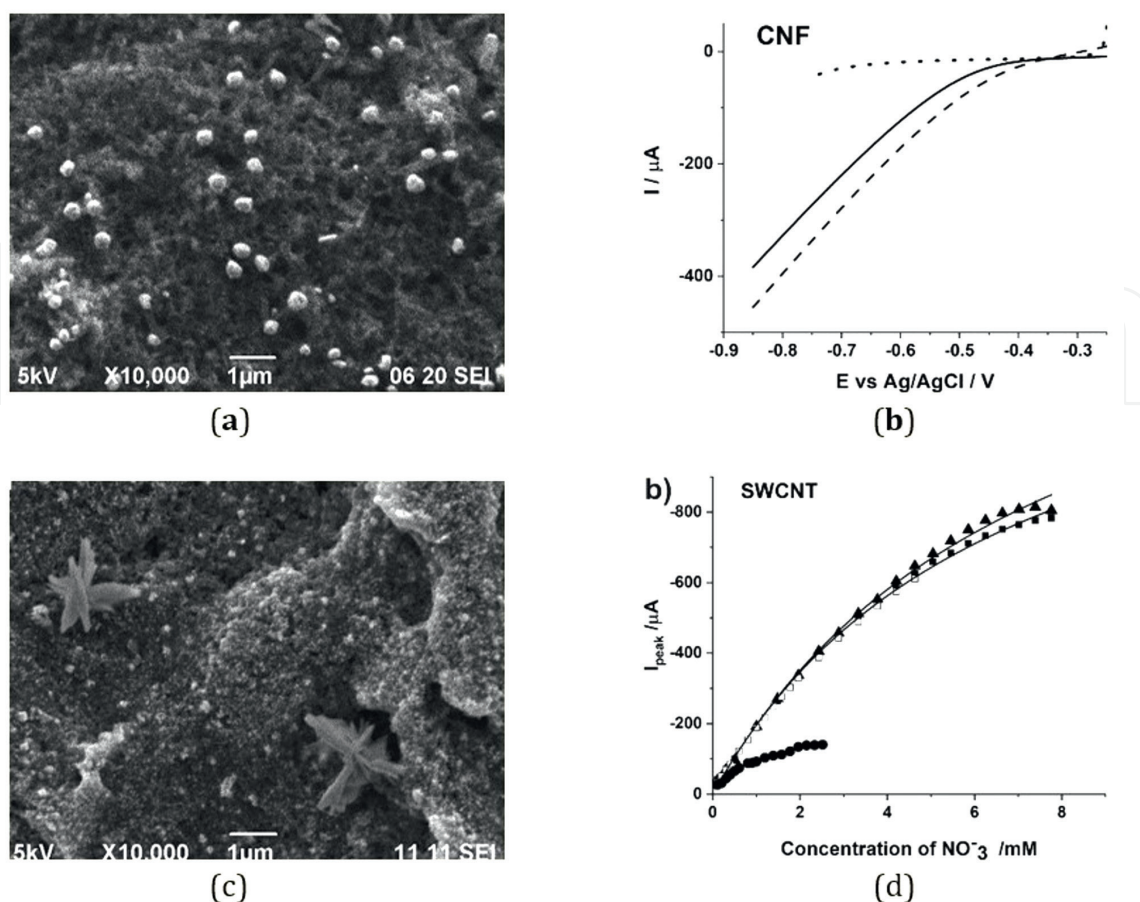
Figure 9.
(a) SEM micrograph of Pd-Cu/CNF surface; (b) Calibration curve of Pd-Cu/CNF sensor for NO$_3^-$ detection in water; (c) SEM micrograph surface of Pd-Cu/SWCNT; (d) Calibration curve of Pd-Cu/SWCNT sensor for NO$_3^-$ detection in water. Reprinted with permission from Hyusein et al. [89].

monitoring applications. A key aspect of the conductive polymers' behavior is doping, which involves the introduction of dopant molecules or ions into the polymer matrix to increase its electrical conductivity. Dopants for conducting or semiconducting polymers include inorganic and organic acids. Doping can significantly alter the electronic structure of the polymer, leading to an increase in the concentration and mobility of charge carriers [90].

Furthermore, the conductive properties of polymers can be improved by metal nanoparticles. When conducting polymers interact with metallic nanoparticles, such as gold or silver nanoparticles, their conductivity can be further enhanced due to the synergistic effects between the two materials. The presence of metal nanoparticles provides additional pathways for charge transport, which can lead to a significant increase in conductivity compared to pristine conducting polymers.

Farina et al. [90] developed two detection electrodes for NH$_4^+$ detection. The first uses PANI electro-polymerized (PANIep) via CV on commercial screen-printed carbon electrodes and the second uses commercial PANI screen-printed carbon electrodes, both modified with electrodeposited Au NPs. Functionalizing the PANI with Au NPs enhanced the conductivity and performance of the system. The sensing mechanism of the devices is based on the deprotonation reaction of PANI, the oxidation of NH$_4^+$, and the subsequent reduction and oxidation of PANI. The results show
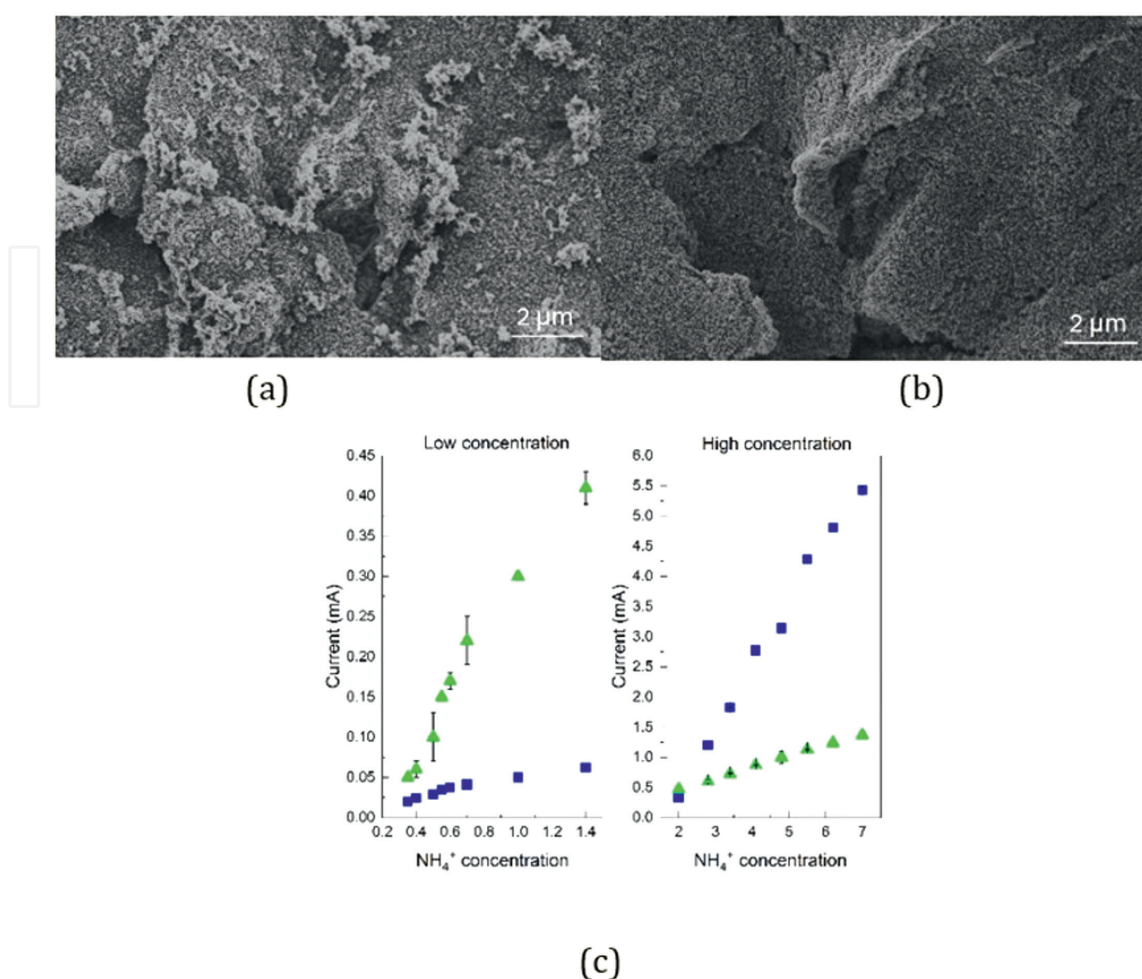
Figure 10.
(a) SEM micrograph of Au/PANIep/C surface; (b) SEM micrograph surface of Au/PANI/C; (c) Calibration curve of Au/PANIep/C (green triangles) and Au/PANI/C (blue squares) sensor for NH_4^+ detection in water. Reprinted with permission from Farina et al. [90].

that the Au/PANI/C electrode performs better for high NH_4^+ concentrations (0.34 μM LoD) and worse for low NH_4^+ concentrations (0.01 μM LoD) than the Au/PANIep/C WE, which shows an opposite trend (0.03 μM LoD for low NH_4^+ concentration and 0.07 μM LoD for high NH_4^+ concentration) (**Figure 10c**). This phenomenon can be explained by considering the distribution of polyaniline on the carbon electrode, which can significantly influence the sensitivity and dynamic range of sensors for amperometric detection of ammonium ions in water (**Figure 10a** and **b**).

Wang et al. [91] developed an electrochemical biosensor for monitoring ammoniacal nitrogen in aquaculture water. A SPEC/AuNPs/PMB modified electrode was prepared by electrodeposition and electro-polymerization using Au NPs and methylene blue. The electrochemical behavior of reduced nicotinamide adenine dinucleotide (NADH) on the surface of the modified electrode was studied by CV. An α-Ketoglutarate substrate and glutamate dehydrogenase were coated on the modified electrode to form a functional enzyme membrane (**Figure 11a**). The ammonia nitrogen in the water sample could be calculated indirectly by measuring the consumption of NADH in the reaction (**Figure 11b**). The biosensor exhibited a linear range from 0.65 to 300 μM with a detection limit of 0.65 μM NH_4^+.
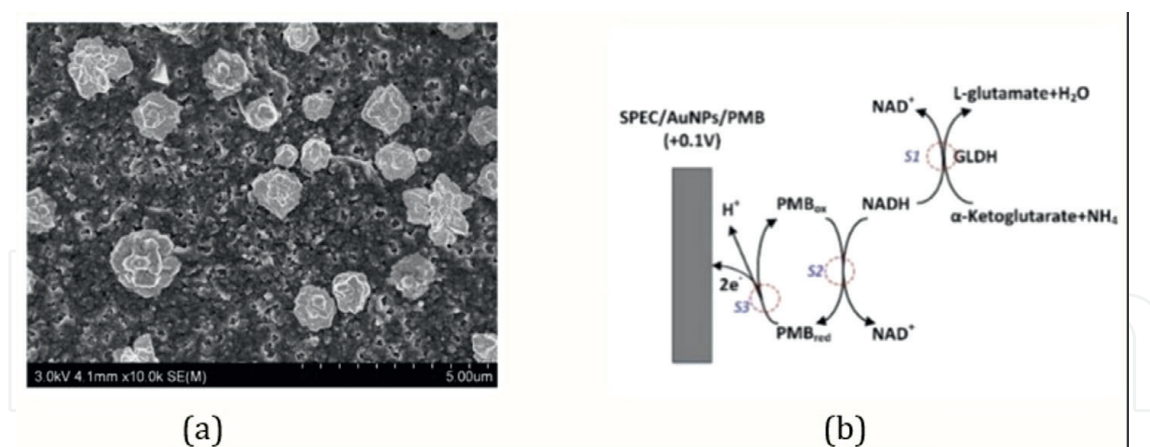
Figure 11.
(a) SEM micrograph of modified electrode surface; (b) Schematic illustration of NH_4^+ detection. Reprinted with permission from Wang et al. [91].

5. Conclusions

In conclusion, ensuring the quality of water through regular microbiological and chemical analyses is essential for safeguarding human health and promoting environmental sustainability. Detecting harmful bacteria like, for example, *E. coli*, *Legionella*, and *Salmonella* prevents serious waterborne diseases while monitoring nitrogen compounds and heavy metals, such as mercury, helps prevent environmental degradation and health issues. The development of advanced techniques, including electrochemical sensors, and optical methodologies using SiPM, enhances our ability to detect and respond to contaminants promptly and accurately. By integrating these innovative methods into Water Safety Plans, we can achieve more effective monitoring, ensuring safe and clean water for all uses and protecting public health and ecosystems.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Caruso G, Coniglio MA, Laganà P, Fasciana T, Arcoleo G, Arrigo I, et al. Validation of a loop-mediated isothermal amplification-based kit for the detection of legionella pneumophila in environmental samples according to ISO/TS 12869:2012. Microorganisms. 2024;**12**:961. DOI: 10.3390/microorganisms12050961

[2] Afshan N, Kazmi M, Khan SA, Jafri N. Bacteriological survey of drinking water in Karachi. In: Proceedings of the IEEE 11th International Bhurban Conference on Applied Sciences and Technology (IBCAST). Islamabad, Pakistan. New York City, United States: Institute of Electrical and Electronics Engineers (IEEE); 14-18 January 2014. p. 92-94. DOI: 10.1109/IBCAST.2014.6778129

[3] Lugo JL, Lugo ER, De La Puente M. A systematic review of microorganisms as indicators of recreational water quality in natural and drinking water systems. Journal of Water and Health. 2021;**19**(1):20-28. DOI: 10.2166/WH.2020.179

[4] Coniglio MA, Ferrante M, Yassin MH. Preventing health care associated Legionellosis: Results after 3 years of continuous disinfection of hot water with Monochloramine and an effective water safety plan. International Journal of Environmental Research and Public Health. 2018;**15**:1594. DOI: 10.3390/ijerph15081594

[5] Sciuto EL, Laganà P, Filice S, Scalese S, Libertino S, Corso D, et al. Environmental management of legionella in domestic water systems: Consolidated and innovative approaches for disinfection methods and risk assessment. Microorganisms. 2021;**9**:577. DOI: 10.3390/microorganisms9030577

[6] Sciuto EL, Filice S, Coniglio MA, Faro G, Gradon L, Galati C, et al. Antimicrobials s-PBC coatings for innovative multifunctional water filters. Molecules. 2020;**25**:5196. DOI: 10.3390/molecules25215196

[7] Filice S, Sciuto EL, Scalese S, Faro G, Libertino S, Corso D, et al. Innovative Antibiofilm smart surface against legionella for water systems. Microorganisms. 2022;**10**(5):870. DOI: 10.3390/microorganisms10050870

[8] Santangelo MF, Libertino S, Turner APF, Filippini D, Mak WC. Integrating printed microfluidics with silicon photomultipliers for miniaturised and highly sensitive ATP bioluminescence detection. Biosensors & Bioelectronics. 2018;**99**:464-470. DOI: 10.1016/j.bios.2017.07.055

[9] Pagano R, Corso D, Lombardo S, Valvo G, Sanfilippo D, Fallica G, et al. Dark current in silicon photomultiplier pixels: Data and model. IEEE Transactions on Electron Devices. 2012;**59**(9):2410. DOI: 10.1109/TED.2012.2205689

[10] Pagano R, Valvo G, Sanfilippo D, Libertino S, Corso D, Fallica PG, et al. Silicon photomultiplier device architecture with dark current improved to the ultimate physical limit. Applied Physics Letters. 2013;**102**(18):183502. DOI: 10.1063/1.4804192

[11] Sciuto EL, Coniglio MA, Corso D, van der Meer JR, Acerbi F, Gola A, et al. Biosensors in monitoring water quality and safety: An example of a miniaturizable whole-cell based sensor for hg2+ optical detection in water. Water. 2019;**11**:1986. DOI: 10.3390/w11101986

[12] Sciuto EL, Corso D, Libertino S, van der Meer JR, Faro G, Coniglio MA. A miniaturized microbe-silicon-chip based on bioluminescent engineered *Escherichia coli* for the evaluation of water quality and safety. International Journal of Environmental Research and Public Health. 2021;**18**(14):7580. DOI: 10.3390/ijerph18147580

[13] Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, et al. Methylmercury exposure and health e ects in humans: A worldwide concern. Ambio. 2007;**36**:3-11. DOI: 10.1579/0044-7447 (2007)36[3,MEAHEI]2.0.CO;2

[14] Lepak JM, Shayler HA, Kraft CE, Knuth BA. Mercury contamination in sport fish in the Northeastern United States: Considerations for future data collection. Bioscience. 2009;**59**:174-181. DOI: 10.1525/bio.2009.59.2.10

[15] Water Quality. Ambient Water Quality Guidelines for Mercury Overview Report—First Update; Ministry of Environment: Victoria. Canada: BC; 1981

[16] Forde BG, Clarkson DT. Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives, J.A. Callow. Advances in Botanical Research. 1999;**30**:1-90. DOI: 10.1016/S0065-2296(08)60226-8

[17] Bryan NS, van Grinsven H. Chapter three - the role of nitrate in human health. Donald L. Sparks. Advances in Agronomy. 2013;**119**:153-182. DOI: 10.1016/B978-0-12-407247-3.00003-2

[18] Edwards TM, Puglis HJ, Kent DB, López Durán J, Bradshaw LM, Farag AM. Ammonia and aquatic ecosystems – A review of global sources, biogeochemical cycling, and effects on fish. Science of The Total Environment. 2024;**907**:167911. DOI: 10.1016/j.scitotenv.2023.167911

[19] Li D, Xu X, Li Z, Wang T, Wang C. Detection methods of ammonia nitrogen in water: A review. Trends in Analytical Chemistry. 2020;**127**:115890. DOI: 10.1016/j.trac.2020.115890

[20] Zhang J-Z, Fischer CJ. A simplified resorcinol method for direct spectrophotometric determination of nitrate in seawater. Marine Chemistry. 2006;**99**(1-4):220-226. DOI: 10.1016/j.marchem.2005.09.008

[21] Goldshleger N, Grinberg A, Harpaz S, Shulzinger A, Abramovich A. Real-time advanced spectroscopic monitoring of ammonia concentration in water. Aquacultural Engineering. 2018;**83**:103-108. DOI: 10.1016/j.aquaeng.2018.10.002

[22] Zhu Y, Yuan D, Huang Y, Ma J, Feng S, Lin K. A modified method for on-line determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell and spectrophotometric detection. Marine Chemistry. 2014;**162**:114-121. DOI: 10.1016/j.marchem.2014.03.011

[23] Amali AJ, Awwad NH, Rana RK, Patra D. Nanoparticle assembled microcapsules for application as pH and ammonia sensor. Analytica Chimica Acta. 2011;**708**:75-83. DOI: 10.1016/j.aca.2011.10.001

[24] Siribunbandal P, Kim Y-H, Osotchan T, Zhu Z, Jaisutti R. Quantitative colorimetric detection of dissolved ammonia using Polydiacetylene sensors enabled by machine learning classifiers. ACS Omega. 2022;7:18714-18721. DOI: 10.1021/acsomega.2c01419

[25] Guidelines for Drinking-Water Quality: Incorporating First Addendum

To Third Edition. Recommendations. World Health Organization. Geneva, Switzerland: Water, Sanitation and Health Team; 2006:1

[26] Understanding Heterotrophic Plate Count (HPC) in Process Water: A Key to Quality Assurance. United States: Sure-BioChem Laboratories, LLC; 2023. Available from: https://www.surebiochem.com/blog/understanding-heterotrophic-plate-count-hpc-in-process-water-a-key-to-quality-assurance#:~:text=HPC%20is%20a%20broad%20measure,grow%20and%20form%20visible%20colonies [Accessed: May 27, 2024]

[27] Castillo-Henríquez L, Brenes-Acuña M, Castro-Rojas A, Cordero-Salmerón R, Lopretti-Correa M, Vega-Baudrit JR. Biosensors for the detection of bacterial and viral clinical pathogens. Sens. 2020;**20**(23):6926. DOI: 10.3390/s20236926

[28] Alhajj M, Zubair M, Farhana A. Enzyme Linked Immunosorbent Assay. 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555922/

[29] Bergua JF, Hu L, Fuentes-Chust C, Álvarez-Diduk R, Hassan AHA, Parolo C, et al. Lateral flow device for water fecal pollution assessment: From troubleshooting of its microfluidics using bioluminescence to colorimetric monitoring of generic *Escherichia coli*. Lab on a Chip. 2021;**21**:2417. DOI: 10.1039/D1LC00090J

[30] Pandiaraj S, Muthuramamoorthy M, Alanazi N, Alodhayb AN. Enhanced sensitivity of SPR-based biosensor for waterborne pathogen monitoring: A numerical analysis. Plasmonics. 2024:1-9. DOI: 10.1007/s11468-023-02183-3

[31] Chu Z, Chen J, Zhang J, Xie Q, Zhang F, Wang Q. Detection of *Escherichia coli* by capillary electrophoresis assisted by large volume sample stacking and nicking endonuclease signal amplification. Journal of Chromatography A. 2023;**1706**:464275. DOI: 10.1016/j.chroma.2023.464275

[32] Dincer C, Bruch R, Costa-Rama E, Fernández-Abedul MT, Merkoçi A, Manz A, et al. Disposable sensors in diagnostics, food, and environmental monitoring. Advanced Materials. 2019;**31**(30):1806739. DOI: 10.1002/adma.201806739

[33] Ramírez-Castillo F, Loera-Muro A, Jacques M, Garneau P, Avelar-González F, Harel J, et al. Waterborne pathogens: Detection methods and challenges. Pathogens. 2015;**4**:307-334. DOI: 10.3390/pathogens4020307

[34] Galikowska E, Kunikowska D, Tokarska-Pietrzak E, Dziadziuszko H, Loś JM, Golec P, et al. Specific detection of salmonella enterica and *Escherichia coli* strains by using ELISA with bacteriophages as recognition agents. European Journal of Clinical Microbiology & Infectious Diseases. 2011;**30**(9):1067, 73. DOI: 10.1007/s10096-011-1193-2

[35] Rajapaksha P, Elbourne A, Gangadoo S, Brown R, Cozzolino D, Chapman J. A review of methods for the detection of pathogenic microorganisms. The Analyst. 2019;**144**:396-411. DOI: 10.1039/C8AN01488D

[36] Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5′----3′ exonuclease activity of Thermus aquaticus DNA polymerase. National Academy of Sciences of the United States of America. 1991;**88**(16):7276-7280. DOI: 10.1073/pnas.88.16.7276

[37] Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology. 1992;**10**:413-417. DOI: 10.1038/nbt0492-413

[38] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research. 2000;**28**:63e. DOI: 10.1093/nar/28.12.e63

[39] Alonso-Padilla J, Cortés-Serra N, Pinazo MJ, Bottazzi ME, Abril M, Barreira F, et al. Strategies to enhance access to diagnosis and treatment for Chagas disease patients in Latin America. Expert Review of Anti-infective Therapy. 2019;**17**:145-157. DOI: 10.1080/14787210.2019.1577731

[40] Adler M, Wacker R, Niemeyer CM. Sensitivity by combination: Immuno-PCR and related technologies. The Analyst. 2008;**133**(6):702-718. DOI: 10.1039/b718587c

[41] Fang P, Yu S, Ma X, Hou L, Li T, Gao K, et al. Applications of tandem mass spectrometry (MS/MS) in antimicrobial peptides field: Current state and new applications. Heliyon. 2024;**10**(7):e28484. DOI: 10.1016/j.heliyon.2024.e28484

[42] Tracz DM, McCorrister SJ, Chong PM, Lee DM, Corbett CR, Westmacott GR. A simple shotgun proteomics method for rapid bacterial identification. Journal of Microbiological Methods. 2013;**94**(1):54-57. DOI: 10.1016/j.mimet.2013.04.008

[43] Ashfaq MY, Da'na DA, Al-Ghouti MA. Application of MALDI-TOF MS for identification of environmental bacteria: A review. Journal of Environmental Management. 2022;**305**:114359, ISSN 0301-4797. DOI: 10.1016/j.jenvman.2021.114359

[44] Rychert J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. Journal of Infectiology and Epidemiology. 2019;**2**(4):1-5. DOI: 10.29245/2689-9981/2019/4.1142

[45] Helm D, Labischinski H, Schallehn G, Naumann D. Classification and identification of bacteria by Fourier-transform infrared spectroscopy. The Journal of General Microbiology. 1991;**137**(1):69-79. DOI: 10.1099/00221287-137-1-69

[46] Rodriguez L, Zhang Z, Wang D. Recent advances of Raman spectroscopy for the analysis of bacteria. Analytical Science Advances. 2023;**4**(3-4):81-95. DOI: 10.1002/ansa.202200066

[47] Multari R.A, Cremers DA, Dupre JA, Gustafson JE. Detection of biological contaminants on foods and food surfaces using laser-induced breakdown spectroscopy (LIBS). Journal of Agricultural and Food Chemistry. 2013;61(36):8687-8694. DOI: 10.1021/jf4029317.

[48] Liu S, Zhao J, Guo Y, Ma X, Sun C, Cai M, et al. Application of ATP-based bioluminescence technology in bacterial detection: A review. The Analyst. 2023;**148**:3452. DOI: 10.1039/d3an00576c

[49] Brolin SE, Wettermark G. Bioluminescence Analysis. New York, NY, USA: VCH; 1992

[50] Ahn JM, Kim BC, Gu MB. Characterization of gltA:luxCDABE fusion in *Escherichia coli* as a toxicity biosensor. Biotechnology and Bioprocess Engineering. 2006;**11**:516-521. DOI: 10.1007/BF02932076

[51] Morciano G, Sarti AC, Marchi S, Missiroli S, Falzoni S, Raffaghello L, et al. Use of luciferase probes to measure ATP in living cells and animals. Nature Protocols. 2017;**12**:1542-1562. DOI: 10.1038/nprot.2017.052

[52] Tanii T, Goto T, Iida T, Koh-Masahara M, Ohdomari I. Fabrication of adenosine triphosphate-molecule recognition chip by means of bioluminous enzyme luciferase. Japanese Journal of Applied Physics. 2001;**40**:1135-1137. DOI: 10.1143/JJAP.40.L1135

[53] Biothema Luminescent Assays. ATP Kit SL. Available from: https://biothema.com/shop/kits/atp-kit-sl/ [Accessed: June 03, 2024]

[54] Luminultra Microbial Monitoring. 2nd Generation ATP Testing. Available from: https://www.luminultra.com/tech/2nd-generation-atp/ [Accessed: June 03, 2024]

[55] Hygiena. Welcome to Smarter ATP Hygiene Monitoring. Available from: https://www.hygiena.com/documents/64121/implementationguide-for-ensure-touch.pdf [Accessed: June 03, 2024]

[56] Luminultra Test Kit Instructions. Available from: https://biotechnologysolutions.com/wp-content/uploads/2016/11/LuminUltra-Test-Kit-Instructions-QGA.pdf [Accessed: June 03, 2024]

[57] Hu Z, Zhang D, Lin H, Ni H, Li H, Guan Y, et al. Low-cost portable bioluminescence detector based on silicon photomultiplier for on-site colony detection. Analytica Chimica Acta. 2021;**1185**:339080. DOI: 10.1016/j.aca.2021.339080

[58] Capuano GE, Corso D, Farina R, Pezzotti Escobar G, Screpis GA, Coniglio MA, et al. Miniaturizable Chemiluminescence system for ATP detection in water. Sensors. 2024;**24**:3921. DOI: 10.3390/s24123921

[59] Sanchez-Rodas D, Corns WT, Chen B, Stockwell PB. Atomic fluorescence spectrometry: A suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury. Journal of Analytical Atomic Spectrometry. 2010;**25**:933-946. DOI: 10.1039/b917755h

[60] Cabanero AI, Carvahlo C, Madrid Y, Batoreu C, Camara C. Quantification and speciation of mercury and selenium in fish samples of high consumption in Spain and Portugal. Biological Trace Element Research. 2005;**103**(1):17-36. DOI: 10.1385/BTER:103:1:017

[61] Yan XP, Yin XB, Jiang DQ, He XW. Speciation of mercury by hydrostatically modified electroosmotic flow capillary electrophoresis coupled with volatile species generation atomic fluorescence spectrometry. Analytical Chemistry. 2003;**75**(7):1726-1732. DOI: 10.1021/ac026272x

[62] Wang DD, Li F, Yan XP. On-line hyphenation of flow injection, miniaturized capillary electrophoresis and atomic fluorescence spectrometry for high-throughput speciation analysis. Journal of Chromatography A. 2006;**1117**(2):246-249. DOI: 10.1016/j.chroma.2006.04.023

[63] Popp M, Hann S, Koellensperger G. Environmental application of elemental speciation analysis based on liquid or gas chromatography hyphenated to inductively coupled plasma mass spectrometry—A review. Analytica Chimica Acta. 2010;**668**:114-129. DOI: 10.1016/j.aca.2010.04.036

[64] Cairns WRL, Ranaldo M, Hennebelle R, Turetta C, Capodaglio G,

Ferrari CF, et al. Speciation analysis of mercury in seawater from the lagoon of Venice by on-line pre-concentration HPLC-ICP-MS. Analytica Chimica Acta. 2008;**622**:62-69. DOI: 10.1016/j. aca.2008.05.048

[65] Pujol L, Evrard D, Groenen-Serrano K, Freyssinier M, Ru en-Cizsak A, Gros P. Electrochemical sensors and devices for heavy metals assay in water: The French groups' contribution. Frontiers in Chemistry. 2014;**2**:19. DOI: 10.3389/ fchem.2014.00019

[66] Guerrini L, Rodriguez-Loureiro I, Correa-Duarte MA, Lee YH, Ling XY, García de Abajo FJ, et al. Chemical speciation of heavy metals by surface-enhanced Raman scattering spectroscopy: Identification and quantification of inorganic- and methyl-mercury in water. Nanoscale. 2014;**6**:8368-8375. DOI: 10.1039/ c4nr01464b

[67] Yu J, Zhang YY, Li H, Wan QJ, Li YW, Yang NJ. Electrochemical properties and sensing applications of nanocarbons: A comparative study. Carbon. 2018;**129**:301-309. DOI: 10.1016/j.carbon.2017.11.092

[68] Wang F, Wei X, Wang C, Zhang S, Ye B. Langmuir-Blodgett film of p-tert-butylthiacalix[4]arene modified glassy carbon electrode as voltammetric sensor for the determination of Hg(II). Talanta. 2012;**80**:1198-1204. DOI: 10.1016/j. talanta.2009.09.008

[69] Chen C, Du Y, Li J, Yang X, Wang E. Fabrication of a sensor chip containing Au and Ag electrodes and its application for sensitive Hg (II) determination using chronocoulometry. Analytica Chimica Acta. 2012;**738**:45-50. DOI: 10.1016/j. aca.2012.06.019

[70] Hezard T, Fajerwerg K, Evrard D, Colliere V, Behra P, Gros P. Gold nanoparticles electrodeposited on glassy carbon using cyclic voltammetry: Application to Hg(II) trace analysis. Journal of Electroanalytical Chemistry. 2012;**664**:46-52. DOI: 10.1016/j. jelechem.2011.10.014

[71] Pavithra KG, Sundar Rajan P, Kumar P. Senthil, Rangasamy Gayathri, mercury sources, contaminations, mercury cycle, detection and treatment techniques: A review. Chemosphere. 2023;**312**(1):137314. DOI: 10.1016/j. chemosphere.2022.137314

[72] Martin-Yerga D, Gonzalez-Garcia MB, Costa-Garcia A. Electrochemical determination of mercury: A review. Talanta. 2013;**116**:1091-1104. DOI: 10.1016/j. talanta.2013.07.056

[73] Preedy VR, Patel VB. Biosensors and Environmental Health. Boca Raton, FL, USA: CRC Press; 2012. DOI: 10.1201/ b12775

[74] Dzantiev BB, Zherdev AV. Antibody-Based Biosensors. In Portable Biosensing of Food Toxicants and Environmental Pollutants. Boca Raton, FL, USA: CRC Press; 2013. pp. 161-196. DOI: 10.1201/ b15589-7

[75] Gu MB, Mitchell RJ, Kim BC. Whole-cell-based biosensors for environmental biomonitoring and application. Advances in Biochemical Engineering/ Biotechnology. 2004;**87**:269-305. DOI: 10.1007/b13533

[76] Gui Q, Lawson T, Shan S, Yan L, Liu Y. The application of whole cell-based biosensors for use in environmental analysis and in medical diagnostics. Sensors. 2017;**17**:1623. DOI: 10.3390/s17071623

[77] Hakkila K, Green T, Leskinen P, Ivask A, Marks R, Virta M. Detection of bioavailable heavy metals in EILA Tox-Oregon samples using whole-cell luminescent bacterial sensors in suspension or immobilized onto fibre-optic tips. Journal of Applied Toxicology. 2004;**24**(5):333-342. DOI: 10.1002/jat.1020

[78] Gilchrist KH, Giovangrandi L, Whittington RH, Kovacs GT. Sensitivity of cell-based biosensors to environmental variables. Biosensors & Bioelectronics. 2005;**20**:1397-1406. DOI: 10.1016/j.bios.2004.06.007

[79] Ferrari AGM, Rowley-Neale SJ, Banks CE. Screen-printed electrodes: Transitioning the laboratory in-to-the field. Talanta. 2021;**3**:100032. DOI: 10.1016/j.talo.2021.100032

[80] Hanrahan G, Patil DG, Wang J. Electrochemical sensors for environmental monitoring: Design, development and applications. Journal of Environmental Monitoring. 2004;**6**:657-664. DOI: 10.1039/b403975k

[81] Heejeong R, Dorian T, Yuankai H, Baikun L, Yu L. Electrochemical sensors for nitrogen species: A review. Sensors and Actuators Reports. 2020;**2**:100022. DOI: 10.1016/j.snr.2020.100022

[82] Ronkainen NJ, Halsall HB, Heineman WR. Electrochemical biosensors. Chemical Society Reviews. 2010;**39**:1747-1763. DOI: 10.1039/b714449k

[83] Li Y, Sun J, Bian C, Tong J, Xia S. Micro electrochemical sensor with copper nanoclusters for nitrate determination in freshwaters. Micro & Nano Letters. 2012;**7**(12):1197-1201. DOI: 10.1049/mnl.2012.0533

[84] Bagheri H, Hajian A, Rezaei M, Shir-zadmehr A. Composite of Cu metal nanoparticles multiwall carbon nanotubes-reduced graphene oxide as a novel and high performance platform of the electrochemical sensor for simultaneous determination of nitrite and nitrate. Journal of Hazardous Materials. 2017;**324**:762-772. DOI: 10.1016/j.jhazmat.2016.11.055

[85] Filimonov EV, Shcherbakov AI. Catalytic effect of copper ions on nitrate reduction. Protection of Metals. 2004;**40**(3):280-285. DOI: 10.1023/B:PROM.0000028923.12984.8c

[86] Inam AKMS, Costa Angeli MA, Shkodra B, Douaki A, Avancini E, Magagnin L, et al. Flexible screen-printed electrochemical sensors functionalized with electrodeposited copper for nitrate detection in water. ACS Omega. 2021;**6**(49):33523-33532. DOI: 10.1021/acsomega.1c04296

[87] Farina R; Libertino S. Nitrates Electrocatalytic Detection in Water by Copper Micro-Flowers, Italian Patent Requested (Italian MIMIT) in Date 11/03/2024, File n. 102024000005344

[88] Farina R, D'Arrigo G, Alberti A, Scalese S, Capuano GE, Corso D, et al. Copper micro-flowers for electrocatalytic sensing of nitrate ions in water. Sensors. 2024;**24**(14):4501. DOI: 10.3390/s24144501

[89] Hyusein C, Tsakova V. Nitrate detection at Pd-Cu-modified carbon screen printed electrodes. Journal of Electroanalytical Chemistry. 2023;**930**:117172. DOI: 10.1016/j.jelechem.2023.117172

[90] Farina R, Scalese S, Corso D, Capuano GE, Screpis GA, Coniglio MA, et al. Chronoamperometric ammonium

ions detection in water via conductive polymers and gold nanoparticles. Molecules. 2024;**29**(13):3028. DOI: 10.3390/molecules29133028

[91] Wang C, Wang T, Li Z, Xu X, Zhang X, Li D. An electrochemical enzyme biosensor for ammonium detection in aquaculture using screen-printed electrode modified by gold nanoparticle/Polymethylene blue. Biosensors. 2021;**11**(9):335. DOI: 10.3390/bios11090335