

Article

Development of Advanced Nanobiosensors and a Portable Monitoring System for Pesticide Detection at the Point of Need

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Abstract

This work presents the development of an automated and portable monitoring system for the point-of-need detection of tebuconazole and lambda-cyhalothrin. The system features nanoparticle/aptamer-modified electrochemical sensors that are integrated into a microfluidic chip based on polydimethylsiloxane (PDMS). More specifically, rapid and selective detection of both pesticides is achieved using target-specific aptamers immobilized on two-dimensional platinum nanoparticle films that serve as expanded nano-gapped electrodes to enhance sensor sensitivity. The effect of the device substrate (i.e., silicon versus flexible substrates) and measurement setup on biosensing performance has also been investigated. The final monitoring system is characterized by high sensitivity and selectivity in the cases of both target analytes and substrates. The system features a limit of detection of 9.85 pM for tebuconazole, which is one of the lowest reported values in the literature; for lambda-cyhalothrin, it is worth noting that the results reported herein represent one of the few studies on an electrochemical aptamer-based sensor for this analyte, featuring a limit of detection of 48.5 pM. The system is also capable of selectively detecting both targets for complex cross-reactive sample matrices consisting of commercially available pesticides. Moreover, its use could be expanded to detect additional pollutants by functionalizing the biosensor surface with appropriate aptamers.

Keywords: nanoparticles; aptamers; triazole; tebuconazole; pyrethroid; lambda-cyhalothrin; aptasensor; microfluidic chip; electrochemical; point of care



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1. Introduction

Fungicides and insecticides are widely used in modern agriculture to control fungal and pest infestations, thereby preventing crop diseases and minimizing yield losses [1].

Among fungicides, triazoles constitute a prominent class of broad-spectrum compounds due to their effectiveness in inhibiting ergosterol biosynthesis, a key component of fungal cell membranes [2]. Although generally considered to be effective, triazoles have been associated with adverse effects on plant physiology and on the hepatic, reproductive, and endocrine systems of various organisms, including humans, and have been implicated in mutagenesis, carcinogenesis, and teratogenesis [3,4]. Insecticides have similarly played a critical role in improving crop productivity; however, their excessive use has led to the accumulation of persistent residues in the environment, particularly organophosphates, prompting regulatory restrictions and remediation efforts. As a result, the use of pyrethroid pesticides has increased substantially [5]. Pyrethroids, classified into type I and type II compounds [6], are widely applied due to their high efficacy, broad applicability, and moderate persistence. Nevertheless, they can exert neurotoxic effects and negatively impact mammalian development and aquatic ecosystems, contributing to increased morbidity, mortality, and ecological disruption [7,8].

Analytical detection of pesticides is still largely based on conventional chromatographic techniques coupled with mass spectrometry or optical detectors. While highly sensitive, these methods are expensive, time-consuming, and require complex instrumentation [9,10]. In contrast, biosensors and chemical sensors integrated with microfluidic platforms offer a promising alternative, enabling rapid, cost-effective, and portable detection while maintaining adequate sensitivity. Despite these advantages, relatively few biosensors have been developed for fungicide detection. Most reported systems rely on metallic nanoparticles or carbon-based materials for colorimetric or electrochemical detection of triazole fungicides [11–14], whereas only a limited number employ selective recognition elements, such as antibodies, enzymes, or molecularly imprinted polymers [15,16]. For pyrethroid insecticides, sensing strategies have mainly focused on optical approaches, including fluorescence-based sensors, photochemical assays, and SERS platforms using nanostructured materials [17–20].

Aptamers have emerged as highly promising recognition elements for pesticide detection due to their strong affinity, selectivity, and chemical stability. Aptamers have been reported for several triazole fungicides, including tebuconazole (TBZ), enabling sensitive detection using colorimetric and electrochemical approaches [21–29]. For example, TBZ has been detected using aptamer–gold nanoparticle systems with reported limits of detection as low as 128 nM and, in more advanced designs, down to 4.13 nM in real water samples [22,28]. Silver nanoparticle-based assays have further improved sensitivity, achieving detection limits of approximately 10 nM [29]. While electrochemical sensors provide fast and cost-effective detection, their application to real-time in-field monitoring remains limited [30]. In contrast, significantly fewer aptamer-based sensing strategies have been reported for pyrethroid insecticides. In particular, only a limited number of studies have addressed lambda-cyhalothrin (CHL) despite its widespread use. Notably, an aptamer-based colorimetric sensor for CHL achieved a detection limit of 0.0186 µg/mL, representing one of the most sensitive methods reported to date [31]. These studies highlight the strong affinity and selectivity of aptamers for small-molecule pesticides while also underscoring the need for more robust, portable, and electrochemical sensing platforms that are capable of real-time monitoring.

NP-based electrochemical aptasensors (nanomaterial-based sensors and incorporating aptamers) have recently gained a great deal of attention in the food industry due to their rapid response, low detection limits and cost efficiency [32–34]. Following our previous work on pesticide detection [35,36], this study discusses the detection of TBZ and CHL via an electrochemical aptasensor/biosensor based on target-specific aptamers and platinum NPs (Pt NPs) that is integrated into a microfluidic chip (MFC) and ultimately in a fully

automated and portable monitoring point-of-need system. It is worth noting that, according to previous studies, the presence of Pt NPs facilitates the immobilization of aptamers and enhances the overall performance of the sensor [36]. In the results reported herein, the use of target-specific aptamers allows for highly selective target detection via an increase in the measured impedance of the biosensors during electrochemical impedance spectroscopy (EIS) Faradaic measurements. The proposed biosensors were initially developed as standalone devices and, in a second step, integrated within a microfluidic system [37–39]. In the first case, the biosensors were calibrated and optimized in static measurements, where TBZ solutions were drop-casted on top of the biosensors' surface. The biosensors were also integrated into a PDMS-based microfluidic system, forming a biosensing MFC, to demonstrate their potential for enhanced sample analysis and integration into a portable and autonomous PoN system. A comparison between "static" and "continuous flow" measurements confirmed that the use of a microfluidic system improves biosensor performance while requiring only minute amounts of analyte solutions. The biosensing MFC demonstrated a detection limit of 9.85 pM for TBZ, which is, to our knowledge, one of the lowest reported values to date, and 48.5 pM (or 0.0225 µg/mL) for CHL, representing one of the few reports on electrochemical detection of CHL using an aptamer-based device and closely matching the performance of the previously reported aptamer-based colorimetric sensor [31]. The biosensing chip is also capable of selectively detecting TBZ and CHL in the case of commercially available products (selective detection for a complex sample matrix consisting of five commercially available cross-interfering products). In addition, the effect of substrate material on sensor performance has been investigated, proving that silicon and flexible polyimide substrates offer similar results. Unlike most optical-based sensors (fluorescence, colorimetric, etc.), a small-footprint and low-power electrochemical device for fungicide/pesticide detection can be easily integrated into portable point-of-need systems. To this end, the biosensing MFC has been successfully integrated into a simple, compact and portable PoN system for remote, autonomous and on-site monitoring of TBZ and CHL spraying events without any loss in biosensing performance.

2. Materials and Methods

2.1. Aptamers and Aptamer Preparation

The T3N CGTACGGAATTCGCTAGCGTGTCATAATGGTC CTCTGGGATCCGAG CTCCACGTG and LCT-1-39 (ACCGACCGTGCTGGACTCT-AGGGGAAGCACGGGCGG GCG) aptamers, used for the detection of tebuconazole (TBZ) and lambda-cyhalothrin (CHL), respectively, were synthesized by Integrated DNA Technologies (IDT) (Leuven, Belgium). As reported in the literature, T3N and LCT-1-39 exhibit high affinity and specificity for TBZ [22] and CHL [31], respectively. The binding affinity of the T3N aptamer has been previously characterized by Isothermal Titration Calorimetry (ITC), which is a well-established technique for determining thermodynamic binding constants. In that study [22], fitting of the ITC binding isotherm using a single-site binding model yielded a dissociation constant (K_d) in the low nanomolar range (approximately 1–5 nM), indicating strong and specific interaction between the aptamer and its target. As far as the LCT-1-39 aptamer is concerned, its K_d value was determined via Microscale Thermophoresis (MST), resulting in a K_d value of 10.27 [31]. Both values fall within the low-nanomolar range, confirming high-affinity aptamer–target interactions and thus showcasing similarly strong binding performance. It is worth noting that the concentration-dependent response as well as the saturation observed in our measurements, as discussed in the Results Section of this paper, are consistent with the high-affinity binding behavior reported for the selected aptamers, supporting their suitability for sensing applications. In addition to their high affinity, the specificity of the aptamers has been experimentally validated in previous studies; selectivity

assays demonstrated low cross-reactivity toward similar analytes, confirming the high binding specificity of the aptamers [22,31]. The aptamers were dissolved in ultrapure water and desalted by centrifugation with Vivaspin 500 spin columns MWCO3K according to manufacturer's instructions. The aptamers were properly folded through the implementation of the following regime and the use of a MS-3000 Orbital Shaker and Incubator (Allsheng, Hangzhou, China): aptamer working solutions were heated at full power to 90 °C, held at this temperature for a further 3 min and then slowly cooled down to 25 °C at 0.5 °C/min while constantly being stirred at 300 rpm. Aptamer immobilization onto the sensor surfaces was achieved with the use of a high-ionic-strength 1 M sodium phosphate buffer pH 8.0, whereas impedimetric measurements were acquired in working buffer (20 mM Tris, 100 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, and pH 7.4) supplemented with 1.0 mM ferri/ferrocyanide redox couple [Fe(CN)₆]^{4+/3+} employed during aptamer selection. To improve on the organization of the immobilized aptamers onto the sensor surface, the latter were backfilled with 6-mercapto-1-hexanol (MCH), which was prepared by diluting a 100 mM ethanolic solution of the chemical to 1 mM in immobilization buffer. All the employed buffers were prepared with the use of ultrapure water [resistivity 18.2 MΩ·cm]. Stock solutions of both pesticides were prepared in acetonitrile, while working solutions were prepared by further diluting the stock solution with working buffer. A detailed list containing all other chemicals, solvents, and pesticides used in this paper can be found in Appendix A.

2.2. Electrode Patterning and Nanoparticle Film Development

Si (100) wafers with a thermal oxide of 300 nm in thickness and 125 µm thick polyimide substrates (purchased from Goodfellow, Cambridge, UK) were used as substrate materials for biosensor development. A two-electrode architecture was employed throughout the experiments; specifically, interdigitated electrodes (IDEs) were patterned across the entire surface of the available substrates using optical lithography and the e-gun deposition of Ti/Au (4/40 nm) (Mantis, Birmingham, United Kingdom, QUAD Series). The Ti/Au IDEs are shown in Figure 1. The finger spacing of IDEs was 10 µm in all fabricated devices. As a next step, a Pt NP layer is deposited over the IDEs using a modified magnetron DC sputtering technique. Sputtering fabrication parameters were as follows: target to substrate distance 20 cm, argon flow 60 sccm, base pressure prior to NP deposition 10^{−5} mbar, base pressure during NP deposition 10^{−3} mbar, sputtering current and voltage 0.1 A and 300 V, respectively, NP deposition duration 2 min, and platinum target pre-sputtering for 1–2 min. The sputtering system allows control over Pt NP diameter (herein 4 nm) as well as of surface coverage. In this case, NP surface coverage was 49%, which is right below the percolation threshold and results in an inter-particle distance of a few nm. The resistance of the NP-based devices had a mean value of 1.05 MΩ, featuring a resistance variation of ~3%.

2.3. Surface Biofunctionalization

The entire process is shown in Scheme 1. For the modification of the sensor surfaces with the target-specific aptamers, the coordination chemistry of organosulfur compounds with metals and especially with noble metals, such as gold and platinum, was effectively utilized [40,41]. Thiol-modified T3N and LCT-1-39 aptamers were drop-casted onto the NP-decorated IDEs at a concentration of 1 µM in immobilization buffer and incubated overnight at room temperature in a humid environment to prevent evaporation of the buffer. Prior to the exposure of the sensors to the backfilling solution, excess aptamers were rinsed off the surfaces with the use of immobilization buffer. Surface backfilling was

achieved by incubating the surfaces with a solution of 1 mM 6-mercapto-1-hexanol (MCH) for 1 h in a humid environment and rinsed off with immobilization buffer.

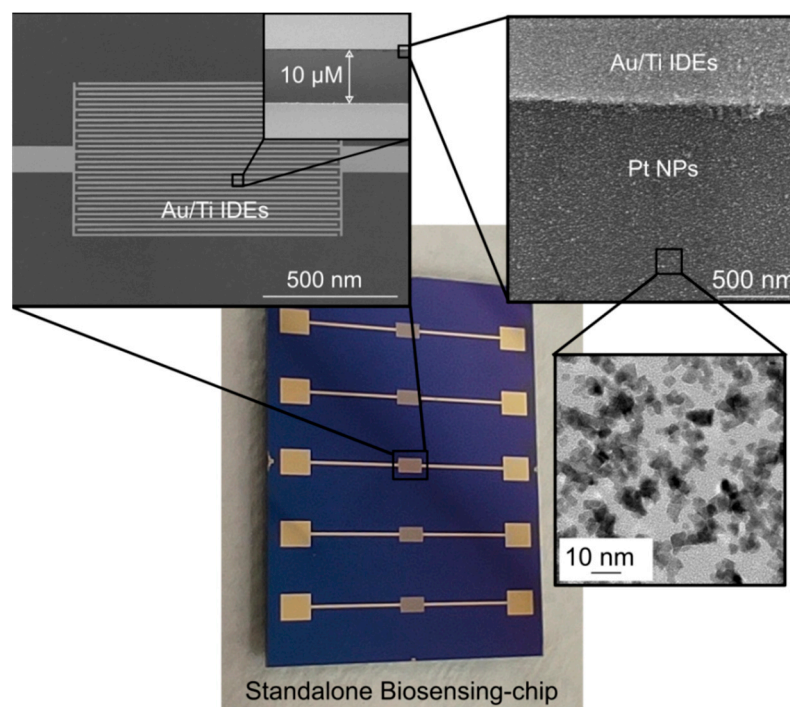
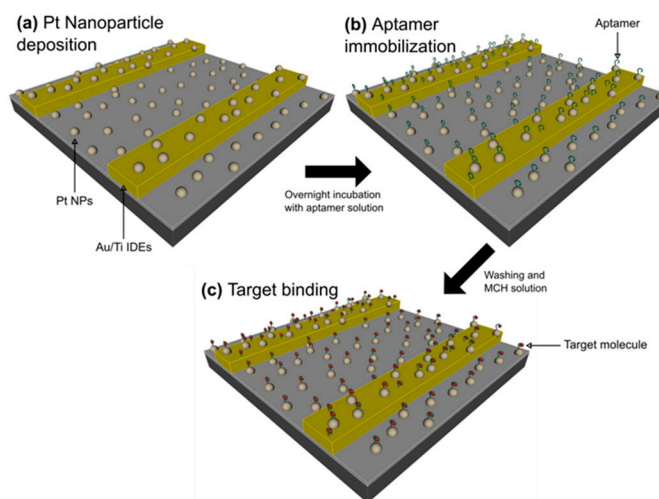


Figure 1. Optical, SEM and TEM images of the device and the platinum nanoparticle layer (Pt NP). SEM images show the Au/Ti interdigitated electrodes (IDEs), the inter-finger spacing of 10 μm and the Pt NPs. TEM images show the Pt NP layer in higher resolution.



Scheme 1. Schematic representation of the various stages of biosensor development from the deposition of the platinum nanoparticle layer (Pt NP) on the sensor's surface to aptamer immobilization and aptamer to target binding (i.e., tebuconazole and lambda-cyhalothrin).

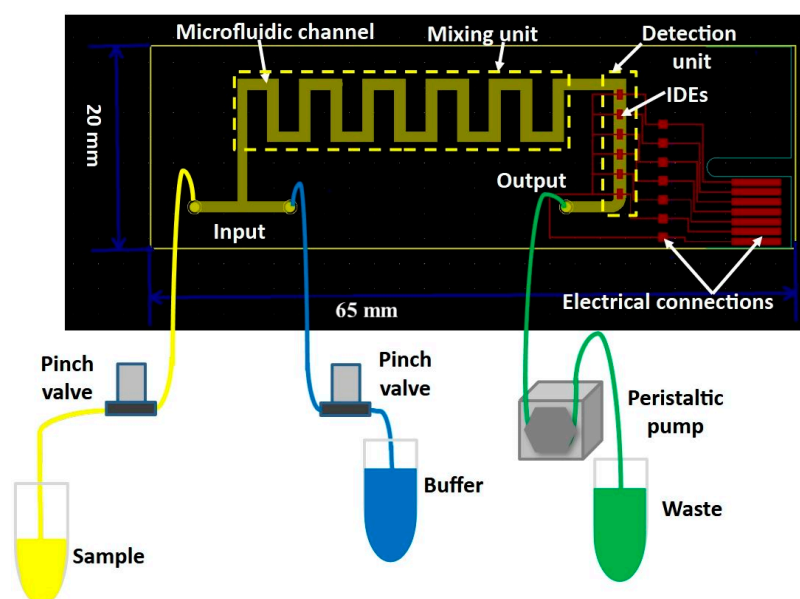
2.4. EIS Measurements

EIS measurements were performed using an Agilent 4284A impedance meter. The measurements were obtained at a frequency range between 100 and 1000 Hz, with a modulation AC voltage of 10 mV and a DC voltage of 0.1 V. For the static measurements (standalone sensor measurements via drop-casting), the biosensors were measured serially using a Karl Suss manual probe station and a two-probe setup. They were fabricated in 15 separate batches, with an average of 18 biosensors per batch. All measurements

were performed in a custom-made electrochemical cell of 80 μL capacity whereby the concentration of TBZ/CHL was increasing with a step of 1 order of magnitude, starting from 1 pM up to 10 μM . Prior to each measurement the target analyte was drop-casted into the cell and remained for 30 min in order to ensure a completed incubation process, which is translated to proper binding between aptamers and target analytes and maximum saturation of the biosensing surface with analyte molecules (i.e., TBZ, CHL, etc.). As a next step, the target analyte was washed using buffer, and finally the cell was filled with the same amount (80 mL) of buffer solution and measured once more. For the integrated biosensing chip's measurements, all of the aforementioned steps have been repeated by introducing successively all of the required solutions into the microfluidic channels. After the 30 min incubation process, EIS measurements were performed. It is worth noting that no air or air bubbles were introduced inside the microfluidic channels during the biosensors' characterization, ensuring the complete and continuous wetting of their surface. In addition, all of the reported biosensor calibration results featuring the microfluidic system, discussed in Sections 3.1 and 3.2, have been realized under static conditions, i.e., with zero flow-rate.

2.5. Development of the Microfluidic Chip and the Standalone Environmental-Monitoring PoN System

Details on the development and fabrication of the microfluidic chip as well as its integration with the hybrid nanoparticle/aptamer sensor can be found in Appendix B. The PoN system consists primarily of the microfluidic chip, the chip holder, and various auxiliary components, including the peristaltic pump, the two pinch valves, appropriate tubing, and the interface board, which are essential parts of both the hydraulic circuit and the biosensor measurement system. The microfluidic chip serves as the central component of the microfluidic PoN system and, together with the holder, is linked to the other components, as illustrated in Scheme 2. Simultaneously, the electrical interfaces transmit the measurement data to the electronic sensor reading circuit. Description of the autonomous monitoring PoN-system can be found in Appendix C.



Scheme 2. The key components of the microfluidic system. The microfluidic system has been designed with separate “sample” and “buffer” inlets in case sample and buffer mixing is needed.

3. Results

3.1. Biosensor Characterization and Integration in a Biosensing Chip

The NP/aptamer biosensors have been tested and optimized towards TBZ and CHL detection via EIS measurements. Measurements have been performed by drop-casting the test solutions on standalone biosensors inside a custom-made electrochemical cell, as described in Section 2.4, as well as in an integrated MFC. The rationale behind the development of standalone biosensors lies in their simpler and faster development, their facile use and their easy measurement/characterization, which does not require the MFC architecture (even in the case of commercially available PoN systems). As mentioned in the Materials and Methods Section, devices with an NP surface coverage of 49% have been used for sensor development; this resulted in a measured device mean resistance of 1.05 MΩ. This resistance value range has been shown to lead to optimal device performance for aptamer-based biosensors, as reported previously [35,36]. In the results reported herein, 20 standalone biosensors and 40 biosensors incorporated in the integrated MFC have been tested, demonstrating a measured resistance variation of ~3% due to slight NP surface coverage differences that can be attributed to the DC sputtering technique.

In a previous study, this group reported increased sensitivity for Pt NP-based biosensors targeting similar small-molecule analytes (i.e., for ACTM detection) when compared to devices without any NP modification [35,36]. Figure 2a,b show the Bode plots for standalone biosensors in frequency ranges between 100 and 1000 Hz and 100 and 105 Hz, respectively, and for a TBZ concentration range from 0.940 nM to 10 μM; similar results have been obtained during the integrated MFC characterization for both TBZ and CHL. The total impedance Z was calculated using the following formula:

$$Z = \sqrt{R^2 + X^2} \quad (1)$$

where R is the real part of impedance, while X refers to the imaginary part. Note that each curve represents a different TBZ concentration as well as the pure buffer solution. As can be seen for a frequency range close to 100 Hz (Figure 2b), each curve can be clearly distinguished. Specifically, concentrations from 10 μM down to 0.940 nM show different impedance values at 100 Hz. Fitting between the experimental data and a proposed equivalent circuit model can be found in Appendix D.

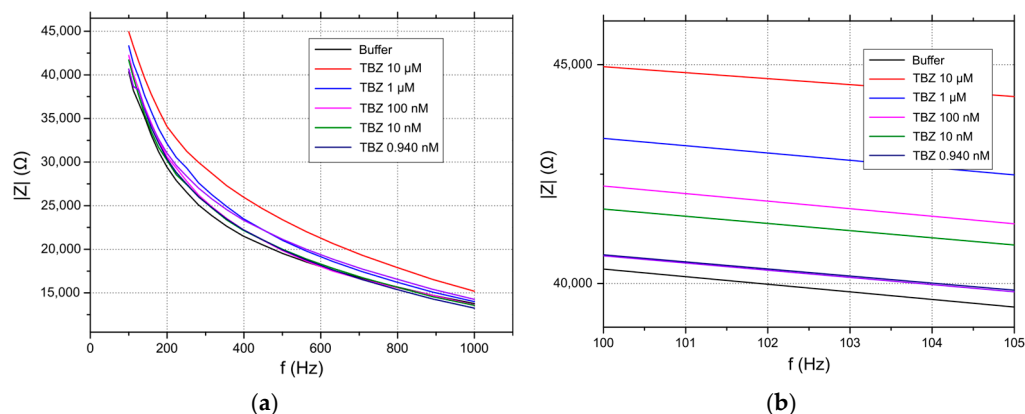


Figure 2. Bode plots showing the EIS response of standalone biosensors for different TBZ concentrations and for frequency ranges of (a) 100–1000 Hz and (b) 100–105 Hz. Similar results, albeit with higher sensor response, have been obtained for the integrated biosensing chip.

Following the successful demonstration of the biosensors' potential for TBZ detection via drop-casting experiments, the sensors have been integrated into a biosensing chip with a respective microfluidic channel, as described in Appendix B. To further assess and evaluate

the overall characterization results of both standalone devices and of those integrated into the biosensing chip, a calibration curve for standalone biosensors as well as for biosensors integrated into the MFC was plotted (Figure 3). $\Delta Z/Z_b$ is the difference between the impedance value of each concentration at 100 Hz and the impedance value of the buffer solution (or tap water in the case of commercially available pesticides) divided by the latter; as can be clearly seen, $\Delta Z/Z_b$ is decreasing as the TBZ concentration decreases. For all the characterization results that follow, the LoD of the biosensors has been calculated as the mean value of the response of all the biosensors tested in that concentration; error bars correspond to the standard deviation of the mean value. We note that 20 biosensors were used for each target analyte and for the control experiments, i.e., TBZ, CHL, difenoconazole (DFZ), hexaconazole (HXZ), deltamethrin (DMT), and cypermethrin (CMT). Regarding the characterization of standalone biosensors in the custom-made electrochemical cell, the LoD of the standalone biosensors has been found to be 0.940 nM. The LoD has been investigated by introducing incrementally increasing TBZ concentrations in the electrochemical cell (increment step: 10 pM) for TBZ concentrations in the range between 800 pM and 10 nM. The resolution of the biosensor for TBZ has been found to be 78 pM (increment step: 1 pM). It is remarked that the biosensors' response remains constant for TBZ concentrations $>10 \mu\text{M}$ (tested in the range between 10 and 300 μM). Moreover, the linear correlation between impedance and TBZ concentration can be attributed to the hindering of the redox process between ferri-ferro species and the electrode/NP surface after TBZ capture in the aptamer layer.

As can be seen in Figure 3, the integrated biosensing chip has proven to outperform the standalone sensors; this is to be expected since microfluidic systems offer increased sensitivity and stability due to the small amounts of required analytes as well as being well isolated by the environment [37]. In this case, the integrated biosensing chip was able to detect TBZ concentrations as low as 9.85 pM, showing good selectivity, as can be seen in Figure 3a. The LoD has been investigated by introducing incrementally increasing TBZ concentrations (increment step: 0.05 pM) for TBZ concentrations in the ranges between 1 pM and 20 pM and 90 and 110 pM. The resolution of the biosensor for TBZ has been found to be 8.1 pM (increment step: 0.1 pM). The results obtained during the integrated biosensing system's characterization indicate response values of $\sim 1.22\%$ for 9.830 pM of TBZ and $\sim 21.7\%$ in the case of 10 μM . Since the integrated MFC offers improved sensing performance, the detection of CHL was then performed solely via the microfluidic system and is shown in Figure 3b; in this case the biosensors feature a LoD of 48.5 pM (response value of $\sim 0.96\%$). The LoD has been investigated by introducing incrementally increasing CHL concentrations (increment step: 0.05 pM) for CHL concentrations in the range between 40 pM and 120 pM. The resolution of the biosensor for CHL has been found to be 15.4 pM (increment step: 0.1 pM). Linear fitting has been performed for results reported in Figure 3 for silicon-based sensors and across the entire measurement range via OriginLab. Linearity indicators such as Adjusted R-square (R^2) and Residual Sum of Squares (RSS) varied within 0.62–0.78 and 12.47–60.56, respectively, pointing to poor linearity, as expected for binding-based sensors, such as the one considered here. However, within the intermediate concentration range (10^{-5} – $10^{-1} \mu\text{M}$), the response exhibits a strong linear dependence on the logarithm of the concentration. Linear regression of the response versus $\log_{10}$ of concentration (response = $a + b \log_{10}(C)$) resulted in statistically significant slopes ($p < 0.01$) and high coefficients of determination ($R^2 \approx 0.94$ and 0.97) across the datasets, indicating a well-defined analytical working range. At higher concentrations ($>10^{-1} \mu\text{M}$), the response deviates from linearity and reaches a plateau, consistent with saturation of binding sites on the sensor surface. Linear behavior with the log of concentration is often recorded in aptamer-based electrochemical sensors, where rapid increase in sensor

response for low concentrations is often the case, followed by saturation as the concentration increases [42]. Linear regression of the sensor response from 10^{-5} μM to 10^{-1} μM versus $\log_{10}(\text{concentration})$ resulted in the following equations for TBZ and CHL of $\Delta Z/Z_b = 1.51 \log_{10}(C_{\text{TBZ}}) + 8.72$ and $\Delta Z/Z_b = 1.62 \log_{10}(C_{\text{CHL}}) + 8.03$, respectively, indicating a well-defined log-linear response prior to saturation. For concentrations in the range between 10 and 300 μM , the biosensors' response remains close to the one reported for 10 μM for both TBZ and CHL; this indicates that the concentration of 10 μM is the threshold above which the biosensors' response is saturated, resulting in a constant response recorded via the EIS measurements. As can be seen in Figure 3, the selectivity of the device was also studied inside the MFC by testing the biosensors using cross-interfering analytes, i.e., TBZ, CHL, DFZ, HXZ, DMT, and CMT; DFZ/HXZ and DMT/CMT belong in the triazole and pyrethroid family of plant-protective products, respectively. What is more, the signal-to-noise ratio (SNR) of the device has been calculated for biosensors integrated into the MFC. Signal (μ) has been calculated as the mean value of the impedance at 100 Hz for a TBZ concentration of 9.85 pM: $\mu_{\text{TBZ}} = 40,671 \Omega$. Noise (σ) has been calculated as the standard deviation of the impedance at 100 Hz for exposure to buffer solution: $\sigma_{\text{TBZ}} = 157 \Omega$ ($\text{CV}_{\text{TBZ}} = 0.39\%$); this results in $\text{SNR}_{\text{linear}} = \mu_{\text{TBZ}}/\sigma_{\text{TBZ}} = 259.05$ and $\text{SNR}_{\text{dB}} = 20 \log_{10}(\mu_{\text{TBZ}}/\sigma_{\text{TBZ}}) = 48.3 \text{ dB}$. In the case of CHL and for a concentration of 48.5 pM ($\mu_{\text{CHL}} = 40,562 \Omega$, $\sigma_{\text{CHL}} = 142 \Omega$, $\text{CV}_{\text{CHL}} = 0.35\%$), $\text{SNR}_{\text{linear}} = 285.6$ and $\text{SNR}_{\text{dB}} = 49.1 \text{ dB}$.

The impedance response of the electrochemical biosensor originates from interfacial changes occurring at the NP-modified active surface of the sensor upon target binding. In the absence of a target analyte, the Pt NP layer facilitates efficient electron transfer and features a large surface area and increased surface roughness, serving as a high-density immobilization center for aptamers. Upon binding of TBZ or CHL to the immobilized aptamer, steric hindrance, aptamer conformational rearrangement, and local charge redistribution occur at the interface, leading to partial blocking of the electrode surface and increased charge-transfer resistance. The increase in device impedance (measured at 100 Hz) therefore reflects changes in interfacial electron transfer kinetics. The observed concentration-dependent decrease in charge transfer is consistent with a binding-controlled sensing mechanism and confirms successful molecular recognition at the biosensor's surface. On the other hand, the overall stability of the aptamer-modified sensing interface is ensured by both the immobilization strategy and the intrinsic binding properties of the aptamer. In the present work, the aptamers were thiol-functionalized at one end, enabling strong and stable attachment to the platinum nanoparticles through thiol-metal interactions. Such interactions are widely employed in electrochemical biosensors due to their high chemical stability and resistance to desorption. In addition, as discussed above, the selected aptamers exhibit high binding affinity and specificity toward their respective target analytes, contributing to stable and reproducible target recognition at the sensor surface. The robustness of the sensing interface is further supported experimentally by the reproducible impedance responses obtained during measurements and by the saturation behavior observed at higher analyte concentrations, indicating that the aptamer layer remains intact and functional throughout the detection process. It should be noted that the current versions of the biosensor and the integrated point-of-need (PoN) system discussed in Section 3.2 are designed for single-use operation. Nevertheless, the platform could be readily adapted for sensor regeneration and reuse in future implementations by incorporating additional microfluidic channels or reservoirs for the delivery of regeneration and washing solutions, as discussed in Section 2.3. These aspects will be the subject of future investigation.

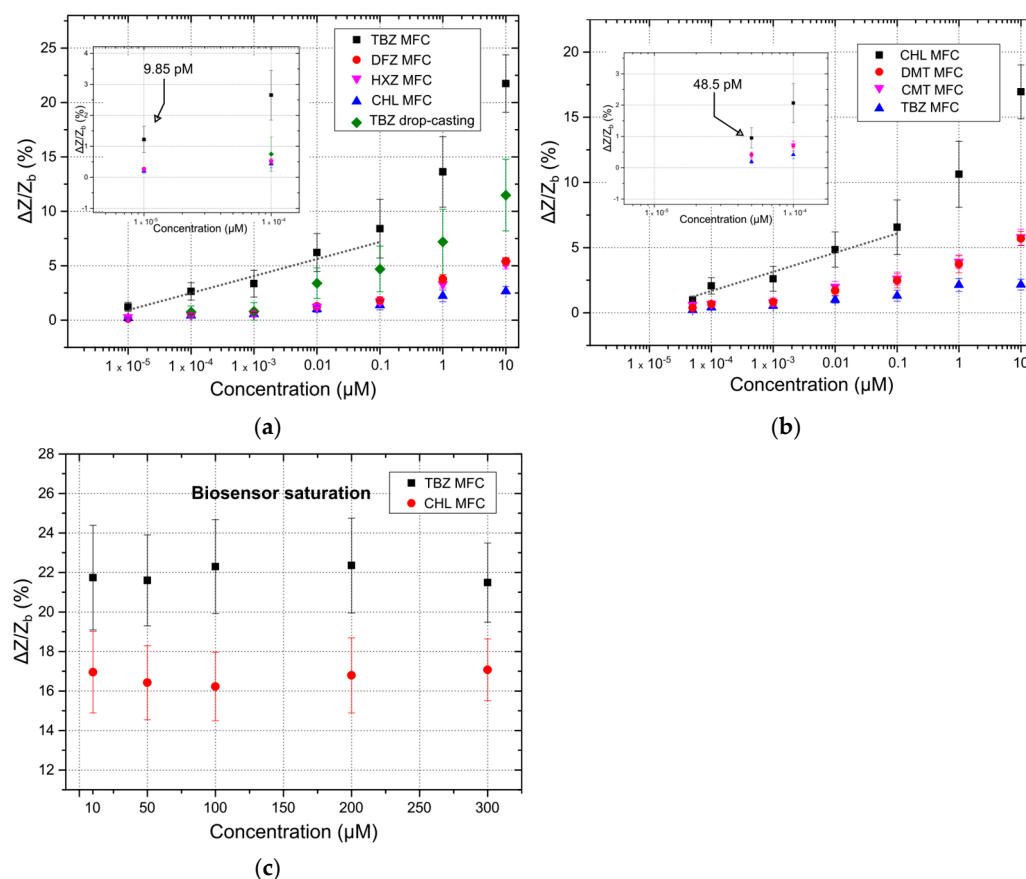


Figure 3. Calibration curve for biosensors integrated into the microfluidic chip (MFC) functionalized with (a) tebuconazole (TBZ)- and (b) lambda-cyhalothrin (CHL)-specific aptamers. (a) Also incorporates results of standalone sensor calibration (TBZ drop-casting) for comparison. Error bars correspond to the standard deviation after the measurement of 20 distinctive biosensors for each target analyte (i.e., TBZ and CHL), while dashed lines correspond to the linear fitting over $\log_{10}$ (concentration). Selectivity of the biosensors has been studied using cross-reactive targets, i.e., difenconazole (DFZ)/hexaconazole (HXZ) and deltamethrin (DMT)/cypermethrin (CMT) for TBZ and CHL biosensors, respectively. Inset figures show in more detail the biosensors' response for concentrations close to their limit of detection (LoD). (c) Biosensor saturation data over the concentration range of 10–300 μM .

At this point, it should be noted that, to our knowledge, such a low detection limit of TBZ has not previously been reported in the literature. In addition, the LoD that was estimated herein for CHL is substantially close to one of the best performing sensors in the literature for an aptamer-based colorimetric device.

As a following step, the entire process of biosensor and MFC development has been repeated for polyimide substrates using 15 distinctive biosensors for each target analyte. As can be seen in Figure 4, the incorporation of flexible polyimide substrates does not affect the performance of the integrated MFC, which is similar to the one reported for TBZ and CHL detection on silicon substrates. This can be attributed to the fact that critical parameters such as surface roughness, dielectric constant, etc., are quite similar for SiO_2 and polyimide, allowing the self-assembly of the Pt NP and aptamer layers in a similar manner. In order to assess whether signals corresponding to different concentrations are statistically distinguishable, despite relative standard deviations of approximately 20–30%, we performed one-way analysis of variance (ANOVA) using OriginLab for each of the datasets reported in Figure 4a,b. As can be seen in Table 1 across all sensor response-data, ANOVA revealed a highly significant effect of concentration on signal response ($p < 0.001$ in all cases). The

corresponding F-statistic values ranged from 239 to 252, with effect sizes (n^2) of approximately 0.92 in all cases, indicating that over 90% of the total variance in signal intensity can be attributed to concentration differences rather than experimental variability (e.g., noise, etc.). More specifically, large n^2 values demonstrate that between-concentration differences substantially exceed within-concentration variability, even for low-concentration (close to the limit of detection), where relative standard deviations are highest, highlighting that the separation between the data-points remains statistically robust. Flexible substrates such as polyimide (kapton) have lately found use in the development of sensors that are mainly intended for wearable applications. As a result, small-footprint biosensing systems such as the one discussed in the context of this paper could be integrated into a wearable device that could be used, e.g., for the safety of farmers by detecting environmental pollutants or in plant-wearable sensors intended for food safety, precision agriculture or smart farming [43]. What is more, microfluidic systems technology is moving towards the incorporation of flexible, polymeric substrates such as polyimide [44]. Additionally, there are many biomarkers that need to be detected in the sub-nM regime that cannot be accessed via wearable devices; in such cases, polyimide-based aptasensors/biosensors have been used as implantable devices for assessing and quantifying such biomarkers [45,46]; as a result, the fact that the proposed technology for developing the integrated biosensing chip is directly transferable to polyimide substrates is of added value.

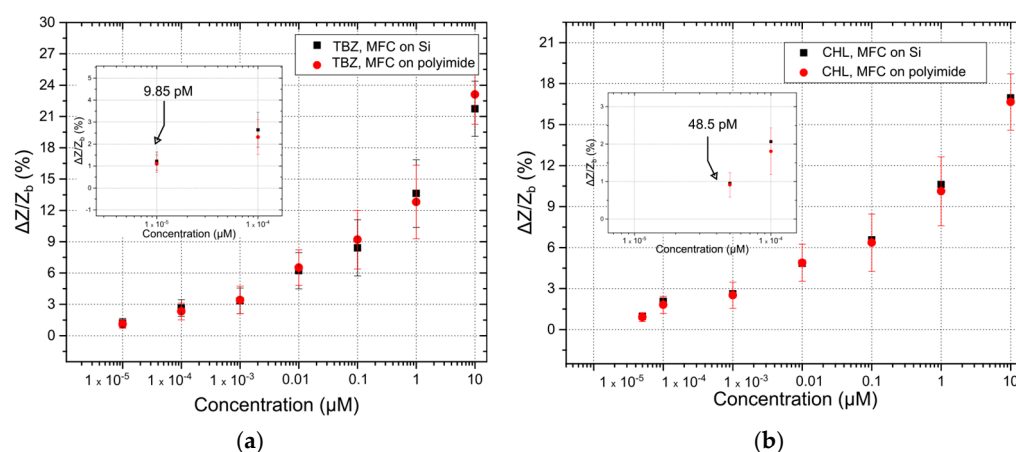


Figure 4. Effect of substrate material on sensor response. Calibration curves for (a) silicon- and (b) polyimide-based MFCs. The biosensors were functionalized with tebuconazole (TBZ)- and lambda-cyhalothrin (CHL)-specific aptamers. Error bars correspond to the standard deviation after the measurement of 15 distinctive biosensors developed on polyimide substrates and 20 biosensors on silicon substrates. Inset figures show in more detail the biosensors' response for concentrations close to their limit of detection (LoD).

Table 1. ANOVA results for results reported in Figure 4.

ANOVA 2			
	<i>p</i> -Value	F-Value	n^2
Silicon, TBZ detection	<0.0001	252.29	0.92
Polyimide, TBZ detection	<0.0001	244.85	0.92
Silicon, CHL detection	<0.0001	248.54	0.92
Polyimide, CHL detection	<0.0001	239.42	0.92

Finally, experiments with commercially available pesticides have also been realized (Figure 5). More specifically, tap water has been used to prepare solutions of Flint Max 75 WG (Bayer AG, Leverkusen, Germany), Score 25 EC (Syngenta, Basel, Switzerland), Profil

20 SG (Nisso Chemical Europe GmbH, Düsseldorf, Germany), Pointer 10 CS (SIPCAM SpA, Milan, Italy), and DECIS 25 EC (Bayer AG, Leverkusen, Germany), which are commercially available fungicides/insecticides for TBZ, difenoconazole (DFZ), ACTM, CHL, and deltamethrin (DMT), respectively. Solutions have been prepared in their recommended concentrations (TBZ, DFZ, CHL, DMT and ACTM concentrations of 326 μ M, 935 μ M, 55 μ M, 24,7 μ M and 225 μ M, respectively, as discussed in Appendix A). Since it is envisioned that the biosensing chip will ultimately be used in the field and specifically in a greenhouse environment for actual crop monitoring, the results reported in Figure 5 correspond to the biosensor's response in a complex sample matrix (a solution containing all five products in equal parts) so as to assess the biosensor's capacity to detect TBZ or CHL in the presence of similar cross-interfering substances (DFZ and DMT) or pesticides of a different group (ACTM). Twenty biosensors functionalized with TBZ- and CHL-specific aptamers (i.e., T3N and LCT-1-39) were tested using a mixture containing all of the aforementioned commercially available products. It is evident that the biosensing MFC is able to successfully detect each TBZ- or CHL-based pesticide (Flint Max 75 WG and Pointer 10 CS), with a response that is close to the "threshold" response of the biosensor (concentrations equal or higher to 10 μ M). One-way ANOVA has also been performed using OriginLab. The ANOVA for sensors functionalized with the TBZ aptamer returned an F-statistic value of 137.11 (much larger than the critical F-value of 2.5) and p -value < 0.001 , signaling a highly significant difference in the mean response across all five target analytes. Dunnett's post hoc test for selectivity has also been performed to compare the biosensors' response to Flint Max to each of the four remaining target analytes; in this case the minimum significant difference (MSD) required for verifying good selectivity was 2.54. By subtracting the biosensors' response to control target analytes from the response to Flint Max, it can be inferred that, for TBZ-biosensors, the MSD is no less than 16.8 (signaling strong selectivity). In the case of biosensors functionalized with the CHL aptamer, an F-statistic value of 113.3 and p -value < 0.05 were obtained, while MSD was 2.24; these results confirm once more strong selectivity in the case of CHL.

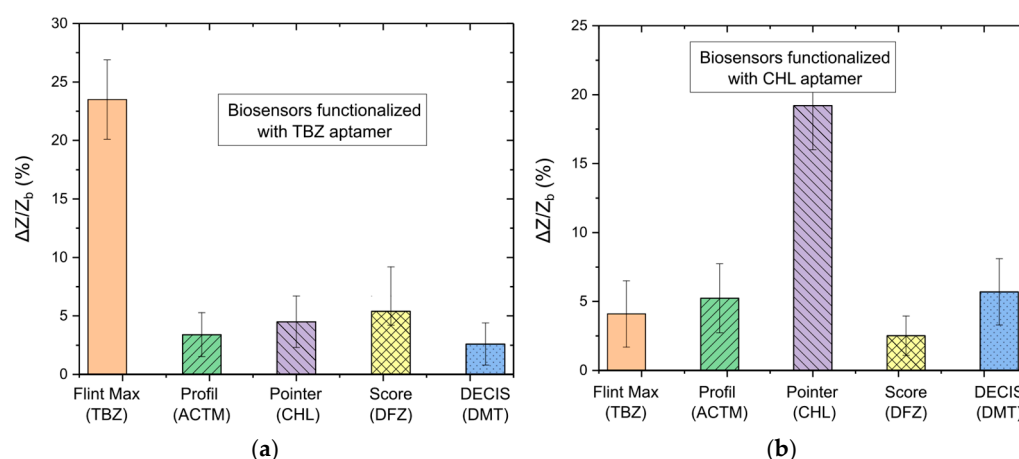


Figure 5. Aptamer/NP-based biosensors integrated into a microfluidic chip for the detection of commercially available pesticides based on tebuconazole (TBZ), lambda-cyhalothrin (CHL), difenoconazole (DFZ), acetamiprid (ACTM) and deltamethrin (DMT). (a) Response and selectivity study for TBZ-specific biosensors for a mixture containing all five pesticides. (b) Response and selectivity study for CHL-specific biosensors for a mixture containing all five pesticides/fungicides. Error bars correspond to the standard deviation after the measurement of 10 distinctive biosensors for each of the three pesticides.

3.2. Evaluation of the Standalone Monitoring PoN System for Pesticide Detection

As a final step, the silicon biosensing MFC has been integrated into the portable and autonomous environmental-monitoring PoN system, as described in detail in Appendix C. For the results reported in Figure 6, 18 biosensing chips have been used in the PoN system with TBZ and CHL laboratory solutions, while 18 biosensing chips have been used for the commercial pesticide experiments. The PoN characterization process has been performed as described in Section 2.4 for the integrated biosensing chip, albeit the entire process was fully automated, with all the steps controlled by the on-board electronics of the PoN system (pumping and introducing of the necessary solutions in the microfluidic channels, incubation, performing EIS measurements, sensor readout, post-processing of sensor data, etc.).

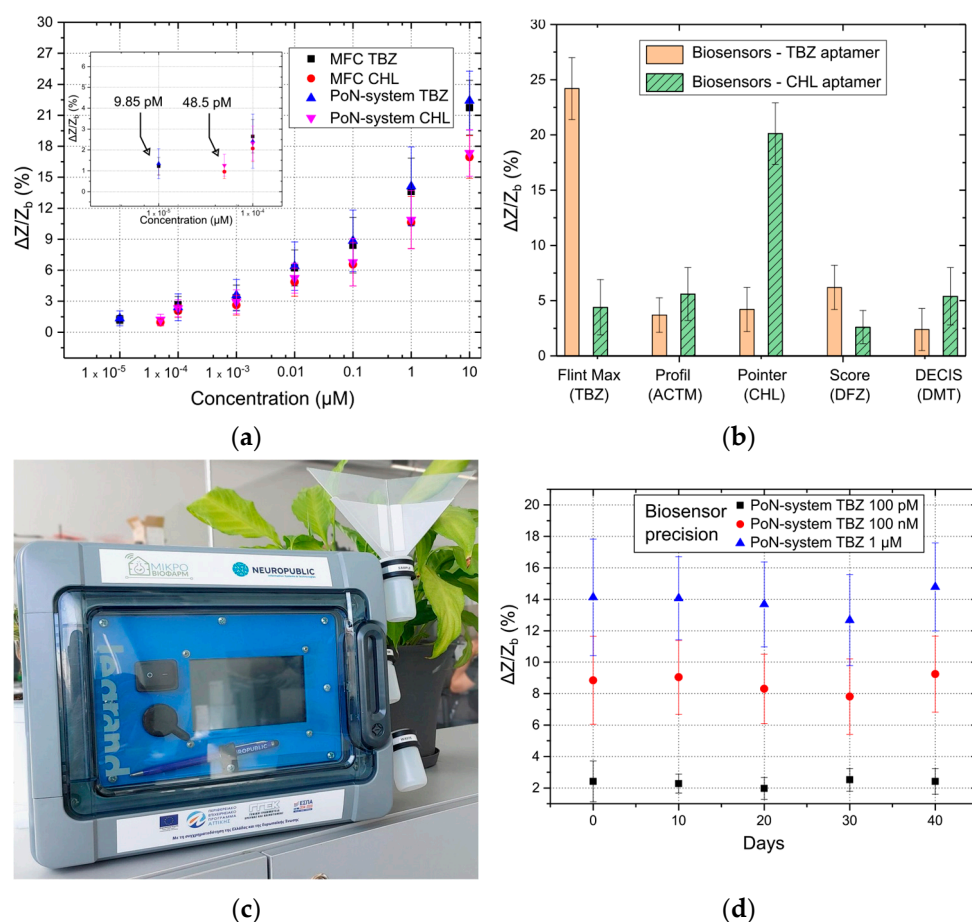


Figure 6. (a) Calibration curve of the autonomous PoN system for tebuconazole (TBZ) and (b) lambda-cyhalothrin (CHL) solutions and comparison with the MFC measurements. (b) Response and selectivity study for the PoN system for a mixture containing all five commercially available pesticides based on tebuconazole (TBZ), lambda-cyhalothrin (CHL), difenoconazole (DFZ), acetamiprid (ACTM) and deltamethrin (DMT). (c) Optical image of the portable and autonomous environmental-monitoring PoN system, with a PMMA artificial leaf for sample collection. (d) Long-term stability and precision of the PoN system over 40 days at fixed TBZ concentrations of 100 pM, 100 nM, and 1 μM .

The successful transfer and automated operation of the biosensing platform, the microfluidic system and the on-board electronics for sensor readout within the portable system were demonstrated by its analytical performance in selectively detecting TBZ and CHL, both in standard solutions as well as in commercial fungicide/pesticide solutions. To be more specific, as can be seen in Figure 6a, the PoN system achieves similar LoDs to

the ones reported for the integrated MFC for both target analytes (i.e., 9.85 pM for TBZ and 48.5 pM for CHL). The PoN system is envisioned as a component of an advanced smart-farming framework (<https://www.gaiasense.gr/en/>, accessed on 1 December 2025) primarily designed for the real-time detection of pesticide spraying events in open-field and greenhouse environments rather than to perform post hoc residue analysis of harvested crops. In contrast to conventional residue analysis approaches that focus on post-harvest testing of food samples, the present platform aims to enable early, fast and on-site identification of pesticide application events, thereby supporting regulatory compliance, worker safety, dosage optimization and the prevention of inappropriate pesticide and potential economic and agricultural losses. While the current study focuses on direct detection of sprayed formulations using an “artificial leaf” as a collection interface, future developments will extend the platform toward the analysis of food-related matrices, real water samples (ground river or lake water) and comparison with established analytical techniques, such as HPLC–MS, to further validate its performance under real-world conditions. To that end and for the detection of commercial pesticides, a complex matrix containing all five commercially available products in equal parts has been introduced via spraying the solution close to the artificial leaf that can be seen in Figure 6c (details can be found in Appendix A). Following the spraying event, the artificial leaf collected part of the sprayed solution inside a plastic container. It is worth mentioning that the MFC requires only small amounts of the test solution for its operation (160 μ L of solution is required in order to fully fill the MFC from end to end, while a quantity of around 20–30 μ L suffices to fully cover the sensing array). The PoN system showed good sensitivity and reliability, while it is also able to detect Flint Max and Pointer solutions in concentrations lower than the ones recommended by their respective manufacturers; in this case the PoN system has been able to successfully detect Flint Max and Pointer solutions in concentrations as low as 12.5 pM and 56.3 pM, respectively. We also note that the electrochemical biosensors discussed herein have been able to successfully perform sample analysis in the context of the PoN system up to two and a half months after their fabrication. The long-term stability and precision of the biosensor were evaluated over 40 days at fixed TBZ concentrations of 100 pM, 100 nM, and 1 μ M. It is worth noting that 25 distinctive biosensors (developed on Si substrates and in five independent sensor batches) have been used with the PoN system. The results of this study can be seen in Figure 6d. Five biosensors have been used for each individual characterization experiment, while error bars correspond to the standard deviation of the response mean value. The coefficient of variation (CV) ranged from approximately 30 to 40% at 100 pM, 25 to 32% at 100 nM, and 18 to 26% at 1 μ M, indicating improved signal stability at higher analyte concentrations and acceptable reproducibility over prolonged storage.

4. Conclusions

In this work, a hybrid nanoparticle/aptamer-based biosensing chip was developed and optimized, and it was ultimately integrated into a portable monitoring point-of-need (PoN) system. The integrated biosensing microfluidic chip is based on the development of an impedimetric electrochemical biosensor for the detection of tebuconazole (TBZ) and lambda-cyhalothrin (CHL). The microfluidic chip, fabricated using PDMS, significantly enhanced the biosensors’ performance and enabled their integration in the final PoN system. To be more specific, biosensors combined with the microfluidic chip showed good selectivity and achieved a limit of detection of 9.85 pM for TBZ, which, to the best of the authors’ knowledge, is one of the lowest reported values in the literature. For CHL, the biosensing chip achieved a detection limit of 48.5 pM (0.024 μ g/mL), outperforming most optical-based sensors for cyhalothrin detection and closely matching one of the best reported

colorimetric aptasensors (0.0186 µg/mL). In addition, the impact of the device substrate on the performance of the integrated chip was investigated, demonstrating that both silicon as well as flexible polyimide substrates are equally suitable for its development. The highly sensitive biosensing microfluidic chip has also been capable of selectively detecting TBZ and CHL in the case of a complex sample matrix consisting of five commercially available pesticides, highlighting the potential of this platform for reliable and accurate pesticide monitoring in the field.

As a final step, the biosensing chip was successfully integrated into a compact, robust, automated and standalone environmental-monitoring PoN system, with the ability to perform point-of-care analysis and assessment of TBZ and CHL, without any loss in biosensing performance. The PoN system incorporates electronic and electromechanical components along with sensor readout electronics to perform automated measurements by collecting sprayed solutions. Future work will focus on the development of algorithms for post-processing of sensor data within a web-based or cloud-enabled architecture; the design of dedicated hardware for the collection of samples from soil, plant leaves, crop surfaces, and environmental waters; the development of appropriate protocols for sample handling and processing using the PoN system; and the optimization of the biofunctionalization strategy toward sensor regeneration and reuse. Particular emphasis will be placed on the establishment of standardized procedures for sample preparation and analysis, enabling the extension of the PoN platform toward complex matrices, including food-related and environmental water samples. In this context, future studies will also include spike-and-recovery experiments to assess analytical performance in realistic aqueous matrices. Furthermore, since electrochemical detection via Faradaic EIS measurements combined with the proposed NP-aptamer-based biosensor constitutes a modular biosensing platform, the PoN system could be extended in the future to enable the detection of additional and varying environmental pollutants by modifying the aptamer layer accordingly.

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Conflicts of Interest: Authors Stergios Dimou-Sakellariou, Nikos Kalatzis and Eleftherios A. Petrakis were employed by the company NEUROPUBLIC S.A. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Chemicals and Buffer Solutions

All chemicals, solvents and pesticides (Pestanal grade) were purchased from Sigma Aldrich (St. Louis, MO, USA). Commercially available products containing additional pesticide-enhancing compounds or even a second pesticide on top of their main active ingredient, TBZ, CHL, DFZ, DMT and ACTM, were employed to assess sensor performance in the presence of possible interferents and in conditions that resemble those anticipated in open crops or greenhouses. To that end, tap water was employed to make up the pesticide working solutions instead of ultrapure water. These formulations were Flint Max 75 WG (50% *w/w* tebuconazole), Score 25 EC (25% difenoconazole, DFZ), Pointer 10 CS (9.5% CHL), DECIS 25 EC (2.5 *w/v* deltamethrin, DMT) and Profil 20 SG (20% Acetamiprid), and they were purchased from an agricultural supply store (Kentro Fytoprostatias AE, Aliartos, Boeotia). An acetamiprid (ACTM)-containing product was also included in the assessment of the cross-reactivity of the developed sensors since ACTM is a widely employed pesticide that belongs to the neonicotinoid family of plant-protective chemicals. Stock solutions for all five products were prepared at the same concentrations as the ones recommended by the manufacturers for use in crops, i.e., 0.20 mg/mL, 0.001 mL/mL, 0.25 mg/mL, 0.5 10–3 mL/mL and 0.25 mg/mL in tap water for Flint Max 75 WG, Score 25 EC, Pointer 10 CS, DECIS 25 EC and Profil 20 SG, respectively. The concentrations of TBZ, DFZ, CHL, DMT and ACTM in the prepared solutions were thus 0.10 mg/mL, 0.38 mg/mL, 0.025 mg/mL, 1.25 10–2 mg/mL and 0.05 mg/mL and hence equal to 326 µM, 935 µM, 55 µM, 24.7 µM and 225 µM, respectively.

Appendix B

Development of an Integrated Biosensing Microfluidic Chip

An integrated chip with a small footprint (20 × 65 mm) encompassing the biosensors as well as microfluidic functions was developed. For the fabrication of the microfluidic channel, polydimethylsiloxane (PDMS) SYLGARD 184 silicone elastomer (10:1), purchased from DowCorning GmbH, was used. The sealing of the PDMS channel opening was done by a piezo-adhesive polyolefin film (StarSeal), purchased from Starlab International GmbH (Hamburg, Germany), whereas a commercially available 5 mm thick polymethyl methacrylate (PMMA) was utilized for the fabrication of the chip holder. Finally, a peristaltic pump was utilized for the circulation of the solutions.

For the fabrication of the integrated biosensing chip, the following steps were followed. As a first step, the biosensors were developed on top of silicon or polyimide substrates, as described in Section 2.1 of the paper. Then, in order to enhance the bonding strength between the PDMS channel and the substrate, a plasma treatment step was performed. More specifically, different conditions were tested in two plasma reactors, a custom-made inductively coupled plasma reactor (ICP) plasma reactor using oxygen as gas and an atmospheric plasma activation reactor using air (Covance multi-purpose plasma system (Femto Science Inc., Korea). The first type of reactor is mainly used for etching and micro-nanotexturing of polymers, whereas the second type of reactor is for activation and cleaning of surfaces and can be used in mild and isotropic conditions. The conditions used in the custom-made ICP are power: 250–1000 W, pressure: ~10 mTorr, duration: 30–60 s, no bias voltage, and O₂ flow: 100 sccm. In the ICP reactor, the milder conditions (250 W power) resulted in better bonding of the PDMS channel on the SiO₂ substrate. In the second type of reactor, the conditions used were even milder (power: 100 W, duration: 60 s, and air flow: 50 sccm), which, in combination with the higher pressure (50 mTorr), resulted in the optimum result regarding the bonding of the PDMS channel and the SiO₂ substrate with no defects.

After the above steps, the deposition of the aptamers is performed through the opening that exists in the detection unit of the PDMS channel, as described in Section 2.2 of the paper. Finally, the opening is sealed with polyolefin film, and the integrated biosensing chip is placed inside a chip holder, which has the necessary microfluidic fittings. The peristaltic pump is used to enable the circulation of the solutions.

Appendix C

Development of a Standalone Environmental-Monitoring PoN System

To facilitate the detection of both pesticides, an electronic system has been developed to enable the readout of impedance changes of electrochemical biosensors. Each biosensor within the array generates an analog signal, which is received by the analog interface unit, the Analog Front End (AFE). This signal is then converted from analog to digital by the Analog/Digital Converter (ADC) unit before being processed and transmitted by the CPU. Additionally, the electromechanical operations required for seamless data collection, physical sampling, and system maintenance are executed by the Electromechanical Unit (EMU), operating under the control of the CPU. A brief overview of the key components involved in the readout electronics of the sensor array is provided below.

The AFE comprises controlled 1V reference voltage sources, powered by local voltage regulators, along with resistors in series, proportional to the resistance of each biosensor to maximize the dynamic measurement range. The voltage supply from the AFE is regulated by the CPU and is positioned as close as possible to the sensor array to minimize interference from other electronic circuits. Interconnection between the multiple boards within the system is achieved using specialized connector arrays. The circuits are powered by local voltage regulators, while the ADC circuits utilize high-precision local reference power supplies to optimize noise reduction. To ensure accurate acquisition of the analog signals from the sensor array, multiple high-precision current sources were employed. The entire analog section was implemented on a printed circuit board (PCB), with all electronic components mounted on a single side. For the development of the digital component of the LoC system, the following elements were implemented: (a) integration of the CPU, (b) internal power supplies to support system operation, (c) Input/Output (I/O) units, and (d) the EMU, which enables the connection of external actuators, facilitating the energy supply required for sampling. A Raspberry Pi Zero 2 W serves as the CPU, managing system operations. Additionally, status indicators, i.e., lights and speakers, have been incorporated to provide real-time feedback on the operational state of the system. Similar to the analog section, the entire digital section was implemented on a PCB, with all electronic components mounted on a single side. The integration of the analog and digital components within the LoC system was achieved using specified connectors in a “Board-on-Board” configuration.

The impedance measurement follows a structured procedure. Once the microfluidic system is supplied with the test solution, individual biosensors are sequentially selected and measured serially, consistent with our previous protocol, using a three-input multiplexer. This multiplexer is implemented with latching relays to prevent interference from excitation currents during measurement. The AFE includes a precision sine wave generator operating at 100 Hz with a symmetrical waveform. The generated signal is subsequently processed by a low-noise amplifier equipped with a high-stability DC coupling circuit, ensuring the input signal maintains the amplitude values of 10 mV (RMS) and 100 mV (DC). To minimize indirect systematic errors, the generated signal passes through an isolator, ensuring zero output impedance before being applied to the sample. A high-resolution transconductance amplifier then measures the current flowing through the selected sensor and transmits it to a peak detector. The output is subsequently fed into the input of the ADC for further

processing. All analog units are powered by a linear power supply with a symmetrical ± 6 V output.

The whole system is enclosed in a waterproof electrical panel ($340 \times 282 \times 141$ mm) made of IP65-rated plastic material (Figure A1a). Three HDPE bottles (i.e., sample, buffer, and waste) of 30 mL are positioned on the right side of the panel; the HDPE bottles have been used for delivering test solutions on the biosensor surface during the evaluation stage of the lab-on-a-chip system discussed in Section 3.2 of the paper. The system also incorporates a digital SHT85 sensor (Sensirion, Gerlingen, Germany) to measure the temperature and relative humidity of the operating environment of biosensors. Local operation and communication with the system are facilitated through an integrated 5" LCD HDMI touch screen for Raspberry Pi (800×480 resolution). The touchscreen interface enables access to system functions and data, as well as the configuration settings for optimal use. The software services and the graphical user interface (GUI) enable visualization of biosensor data and support remote system management for possible use in greenhouse settings after integration with smart farming systems (i.e., gaiasense; <https://www.gaiasense.gr/en/>, accessed on 1 December 2025). In this context, a superhydrophobic PMMA surface ($100 \times 100 \times 1$ mm), shaped in the form of a “leaf”, is also located at the top right of the panel to facilitate sample collection after spraying (used for the commercial sample measurements discussed in Section 3.2 of the paper).

The LoC system operates on the Raspbian OS, a Debian-based distribution optimized for Raspberry Pi hardware. Raspbian includes essential software tools and libraries used for remote control and automation, with built-in support for the Python (version 3.13) programming language. The core algorithm for the functionality of the LoC system is implemented in Python and manages communications, data acquisition, processing, storage, and user interface, among others. Each biosensor is locally controlled by Python-based code executed on the Raspberry Pi. The Raspberry Pi transmits sensor readings via HTTPS using a web service (REST API) hosted on a cloud server. Remote control is also possible through this web service, allowing users to start and stop data recording. The web service, developed using the FastAPI library (<https://fastapi.tiangolo.com/>, accessed on 1 December 2025), is served by an ASGI server (Uvicorn; <https://www.uvicorn.org/>, accessed on 1 December 2025) and follows the OpenAPI standard, with documentation provided through the Swagger platform (<https://swagger.io/docs/specification/2-0/what-is-swagger/>, accessed on 1 December 2025).



Figure A1. Overview of the standalone LoC system. (a) The complete system housed in a waterproof electrical enclosure as well as the LCD display interface for real-time monitoring; and (b) the integrated system components.

All collected measurements are stored in a PostgreSQL relational database, with optional support for the PostGIS extension. The measurement management platform is deployed and managed using virtualization technologies through the open-source software platform Docker. The GUI, which enables user interaction with the LoC system functions, is hosted on an Apache server. It utilizes various web technologies, including PHP, JavaScript, HTML, and Bootstrap. Measurement data are presented in the form of time-series diagrams for clear visualization, as shown in Figure A2.

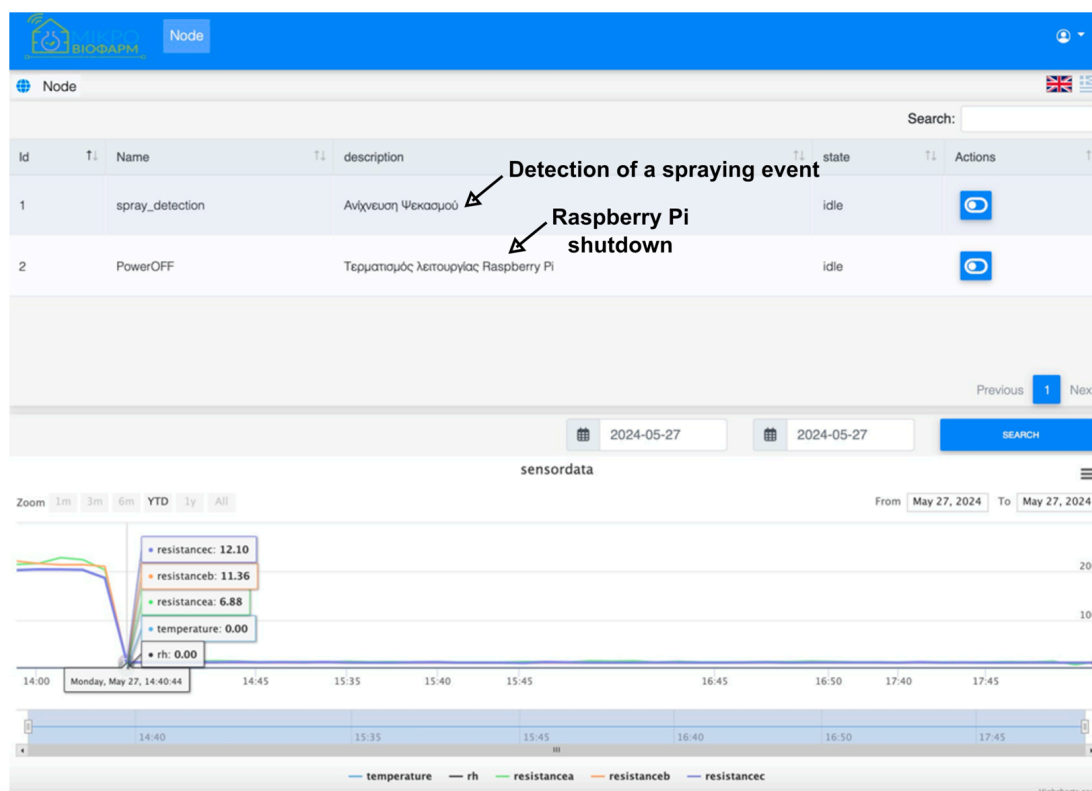


Figure A2. Time-series diagrams of measurements recorded after sampling and detection.

Appendix D

Equivalent Circuit of the Device and Fitting with Experimental Data

An equivalent circuit of the buffer/TBZ electrolyte solution and the sensor's architecture has been tested in order to further understand the experimental results. The equivalent circuit consists of a resistor (R1) and a capacitor (C) connected in parallel, which are the charge transfer resistance and the double-layer capacitance, respectively. This, in turn, is connected in series with another resistor (R2), which is the active electrolyte resistance, as shown in Figure A3a. Importing such an equivalent circuit in LTspice allowed us to reproduce the Bode plots presented in Figure 2 of the paper by adjusting the R1, R2 and C component values in order to achieve the optimal fitting with the experimental data. Figure A3b shows the fitting of the theoretically extracted curves with the experimental ones for all different TBZ concentrations and for a frequency range between 100 and 105 Hz. The electrolyte resistance R2 is constant and equal to ~1 kΩ. Moreover, an adequate fitting is achieved by mainly altering the resistive component (R1) of the circuit rather than the capacitive component (C). Specifically, R1 varied between 2 and 10 kΩ with increasing TBZ concentration, whereas C varied only between 3 and 4 nF at a frequency of 100 Hz.

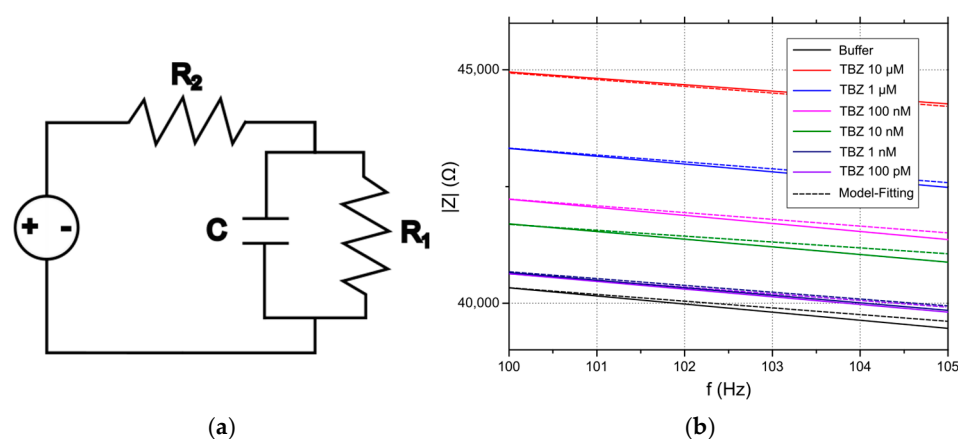


Figure A3. (a) Equivalent circuit of the buffer/TBZ electrolyte solution and the sensor's electrodes. (b) Fitting of the proposed model to the experimental values (Bode plots for varying TBZ concentrations) for a frequency range of 100–105 Hz. Dashed lines represent model fitting for each experimental curve.

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