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SUSNANO

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**D1.2 Report on laboratory validation
of sustainable nanosensors**

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SUSNANO's overall aim is to boost the scientific excellence and innovation capacity in sustainable nanosensors for water pollution detection of Universiteti i Tiranës (UT) and its high-quality Twinning partners: Fundació Institut Català de Nanociència i Nanotecnologia, Univerzita Palackého v Olomouci and Intelligentsia Consultants Sàrl. To achieve this aim, SUSNANO will implement a research and innovation strategy over 3 years based upon 5 objectives implemented via 5 corresponding WPs:

Objective 1: Conduct exploratory research on sustainable nanosensors to detect water pollution in Albania

The goal is to develop innovative sustainable nanosensors to detect heavy metals, pesticides and antibiotics. The validated sensors will be used in field tests to provide an environmental assessment of rivers and lakes in Albania.

Objective 2: Transfer knowledge between experienced researchers (ERs) of UT and the Twinning partners

The goal is to organise short term staff exchanges, trainings and seminars for UT's ER's and the Twinning partners' ERs to complement the preparatory research undertaken in Objective 1.

Objective 3: Enhance career prospects of early-stage researchers (ESRs) of UT and the Twinning partners

The goal is to enhance the career prospects of UT's ESRs and the Twinning partners' ESRs by organising short- and medium-term exchanges, training workshops/seminars, summer schools & joint PhD programme.

Objective 4: Improve UT's management and administrative capacity for European R&D programmes

The goal is to improve the skills of UT's Directorate of Scientific Research, Projects and Foreign Relations in proposal preparation, project management and innovation management for European R&D funding programmes.

Objective 5: Raise the research profile of UT and the Twinning partners

The goal is to raise the research profile and scientific reputation of UT and the Twinning partners through a comprehensive range of dissemination, exploitation, communication & outreach activities.

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Executive Summary

This document consists in a report describing the laboratory validation of the sustainable nanosensors in the context of the SUSNANO project.

Composite sensors modified with graphene derivatives and/or integrated with metallic nanoparticles, metal oxides were developed for the quantification of three different types of water pollutants: heavy metals (mercury, lead, cadmium, copper), pesticides (paraquat, imidacloprid, thiamethoxam) and antibiotics (ampicillin, penicillin, azithromycin, tetracycline). Developed materials were evaluated through characterization techniques including for instance: microscopic techniques (HR)TEM, SEM, AFM), spectroscopic methods (XPS, EPR, Raman, FTIR) and electrochemical techniques (CV, pulse methods and EIS). Experimental results have shown that the improvement in the nanosensor's analytical performance, due to the presence of the modifiers, depends on the type of modifier used and the analyte being targeted. The following sections describe in detail the preparation and validation of different electrode platforms in terms of sensitivity, selectivity, working linear range, recovery, relative standard deviation, limit of detection (LoD) and limit of quantification (LoQ).

The fluorographene chemistry technique was used to synthesize a variety of carbon-based derivatives modified with diverse functional groups. Innovative nanomaterials, such as nitrogen-doped Graphene Acid (NGA) and its oxidized form nitrogen-doped graphene acid dots (NGA-D), were used as potential platforms for the development of nanosensors for heavy metals and pesticide detection. These sensors demonstrated high selectivity, sensitivity, recovery, stability and excellent LoD. Modification of screen-printed electrodes via inkjet printing technology using NGA modifier was optimized, achieving sufficient performance for pesticide detection. A paper-based detection system using NGA-D was developed for ultra-low-cost, on-site water quality monitoring.

A graphene-based aptasensor was developed for the detection of antibiotics (ampicillin). The functioning mechanism of the aptasensors enabled maximum selectivity and high sensitivity, as demonstrated in the detection of ampicillin in water samples.

Reduced graphene oxide (rGO)-based electrodes integrated with metallic nanoparticles (MNPs) and bismuth nanorods (BiNRds), as well as inkjet-printed gold (Au-IJP) electrodes, were fabricated using laser engraving and inkjet printing technologies. These sensors were tested for heavy metals determinations. The incorporation of BiNRds into rGO electrodes significantly improved the electrochemical signal while maintaining cost-effectiveness, and environmental sustainability. The printed rGO@BiNRds sensors are particularly promising for environmental applications, including in situ and flow measurements, offering a robust platform for monitoring heavy metals in single and mixed solutions.

The research successfully validated the analytical performance of the developed nanosensors and demonstrated their availability for use in water monitoring.

1 Research Sub-Topic A: Composite electrodes for sustainable nanosensors

Summary

The study aimed to develop and validate composite electrodes capable of detecting heavy metals, pesticides and antibiotics aiming to achieve high analytical performance parameters.

1.1 Laboratory validation for detecting Heavy Metals

1.1.1 Development and validation of nanosensor for detecting Hg^{2+}

Preparation of GO

The preparation of GO was based, on the Hummer method. Initially, in 1.5 g of graphene and 1.5 g of sodium nitrate ($NaNO_3$) 69 mL of concentrated sulfuric acid (H_2SO_4 cc) was added to the 500 ml beaker and the mixture was continuously stirred until a homogeneous solution was achieved. The beaker was then placed in a container filled with ice and cold water and continuously stirred for 15 minutes. Following this, 9 g of potassium permanganate ($KMnO_4$) was slowly added to the mixture, and the beaker was placed in continuous magnetic stirring for 1 hour at room temperature. Afterward, it was transferred to a container with ice and cold water, and 100 mL of distilled water was slowly added to the solution to prevent vigorous reactions with the acid. Then heated in a furnace at a temperature exceeding $90^\circ C$ for an hour. After heating, 300 mL of distilled water and 10 mL of H_2O_2 30% were added to the glass. Then, the glass was placed in an ultrasonic bath for 30 minutes at a temperature of $25^\circ C$. Then the system was centrifuged for 10 minutes at 5000 rpm. The filtrate obtained is washed 3 times with HCl 10% followed by 3 times with distilled water. The precipitate was dried overnight in a thermostat at $40^\circ C$.

Preparation of rGO@Au

0.1g GO and 0.3g metal salt ($HAuCl_4$), were mixed with 20 ml distilled water. The mixture was kept under magnetic stirrer in room temperature for 2 hours and later on, for 4 hours in ultrasonic bath. Then the system was centrifuged for 10 minutes at 5000 rpm and the precipitate was dried in a thermostat at $80^\circ C$ overnight.

Preparation of - rGO@Au/CPE

In a porcelain mortar, 2 g of carbon powder (particle size: 90 μm to 70 μm), 200 mg of pre-synthesized rGO@Au, and 600 μl of paraffin were thoroughly mixed for one hour to achieve a uniform mixture. The resulting paste was refrigerated for 24 hours and then stored in a dry place. Approximately 1 gram of the paste was transferred into the electrode body, connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor rGO@Au/CPE for quantification of Hg^{2+}

Analytical performance of the rGO@Au/CPE for Hg^{2+} determination is studied using different electrochemical techniques: CV, DPV and SWV integrating a preconcentration step. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: rGO@Au /CPE (working), Ag/AgCl/ Cl^- (reference electrode), and Pt (counter electrode).

The optimal SWASV technical parameters are:

- o preconcentration potential -0.1V
- o SWASV scanning -0.1 to 0.8V
- o t (pre-concentration) = 120 s
- o E step = 0.005 V
- o A= 0.06 V
- o Frequency 25 Hz
- o 0.1 M acetate buffer pH= 4.5

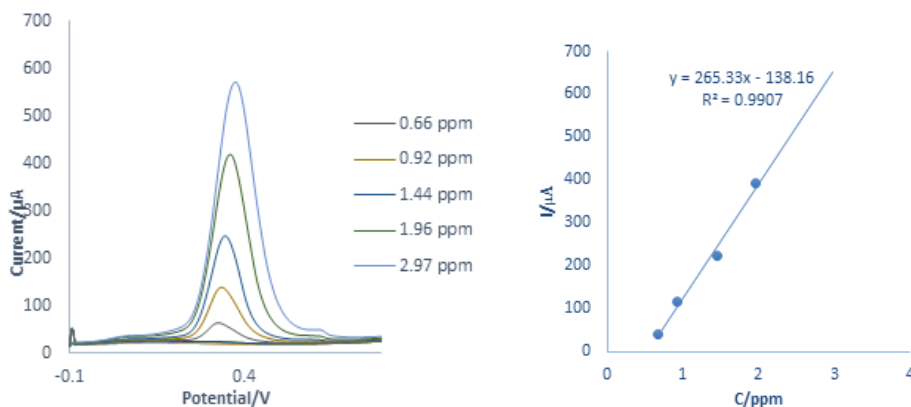


Figure 1: SWAS Voltammograms recorded in different concentrations of Hg²⁺ in 0.1M acetate buffer pH=4.5 using rGO@Au/CPE. Corresponding linear calibration curve.

The calibration curve was repeated three times, and performance parameters such as sensitivity, linear range, limit of detection, and standard addition recovery were calculated. All parameters are summarized in Table 1.

1.1.2 Development and validation of nanosensor for detecting Pb²⁺

Preparation of rGO@Ni

0.1g GO and 0.3g metal salt (NiSO₄), were mixed with 20 ml distilled water. The mixture was kept under magnetic stirrer in room temperature for 2 hours and later on, for 4 hours in ultrasonic bath. Then the system was centrifuged for 10 minutes at 5000 rpm and the precipitate was dried in a thermostat at 80°C overnight.

Preparation of - rGO@Ni/CPE

In a porcelain mortar, 2 g of carbon powder (particle size: 90 μm to 70 μm), 200 mg of pre-synthesized rGO@Ni, and 600 μl of paraffin were thoroughly mixed for one hour to achieve a uniform mixture. The resulting paste was refrigerated for 24 hours and then stored in a dry place. Approximately 1 gram of the paste was transferred into the electrode body, connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor rGO@Ni /CPE for quantification of Pb²⁺

The analytical performance of the rGO@Ni /CPE for Pb²⁺ determination is studied using different electrochemical techniques: CV, DPV, and SWV, which integrate a pre-concentration step. Experimental conditions such as pH, supporting electrolyte, and parameters relating to the technique used are optimized carefully.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: rGO@Ni/CPE (working), Ag/AgCl/Cl⁻ (reference electrode), and Pt (counter electrode).

The optimal SWASV technical parameters are:

- o preconcentration potential -1V
- o SWASV scanning -1 to 0V
- o t (pre-concentration) = 120 s
- o E step = 0.005 V
- o A= 0.06 V
- o Frequency 30 Hz
- o M acetate buffer pH= 4.5

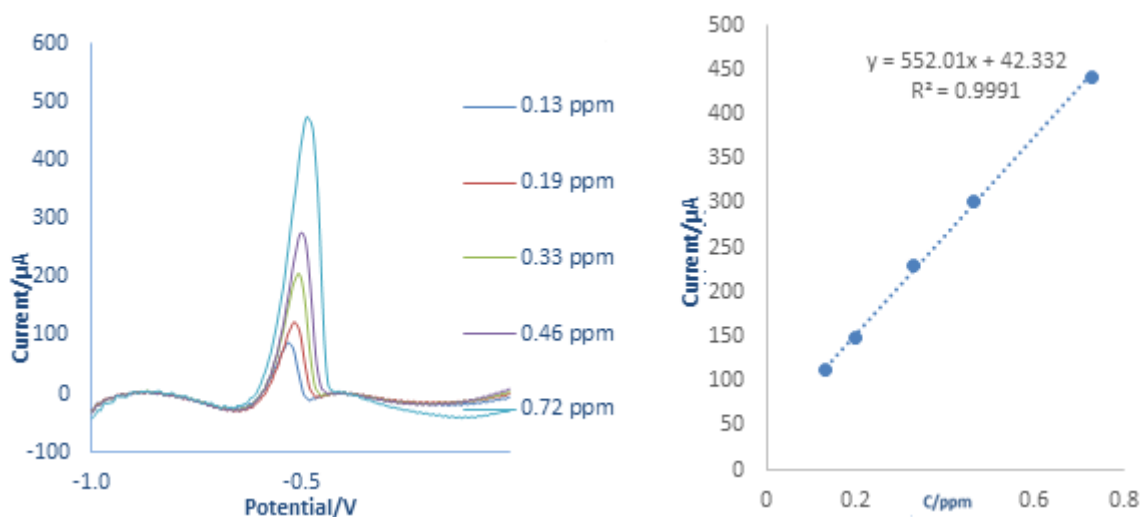


Figure 2: SWAS Voltammogram recorded in different concentrations of Pb²⁺ in 0.1M acetate buffer pH=4.5 using rGO@Ni/CPE. Corresponding calibration plot.

The calibration curve was repeated three times, and performance parameters such as sensitivity, linear range, limit of detection, and standard addition recovery were calculated. All parameters are summarized in Table 1.

1.1.3 Development and validation of nanosensor for detecting Cd²⁺

Preparation of - rGO@Ni /CPE

In a porcelain mortar, 2 g of carbon powder (particle size: 90 µm to 70 µm), 200 mg of pre-synthesized rGO@Ni (explained in 1.1.2), and 600 µl of paraffin were thoroughly mixed for one hour to achieve a uniform mixture. The resulting paste was refrigerated for 24 hours and then stored in a dry place. Approximately 1 gram of the paste was transferred into the electrode body and connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor rGO@Ni /CPE for quantification of Cd²⁺

Analytical performance of the rGO@Ni/CPE for Cd²⁺ determination is studied using different electrochemical techniques: CV, DPV, and SWV, which integrate a pre-concentration step. Experimental conditions such as pH, supporting electrolyte, and parameters relating to the technique used are carefully optimized.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: rGO@Ni/CPE (working), Ag/AgCl/Cl⁻ (reference electrode), and Pt (counter electrode).

The optimal SWASV technical parameters are:

- o preconcentration potential -1.2V
- o SWASV scanning -1.2 to 0V
- o t (pre-concentration) = 120 s
- o E step = 0.005 V
- o A= 0.03 V
- o Frequency 10 Hz
- o 0.1 M acetate buffer pH= 4.5

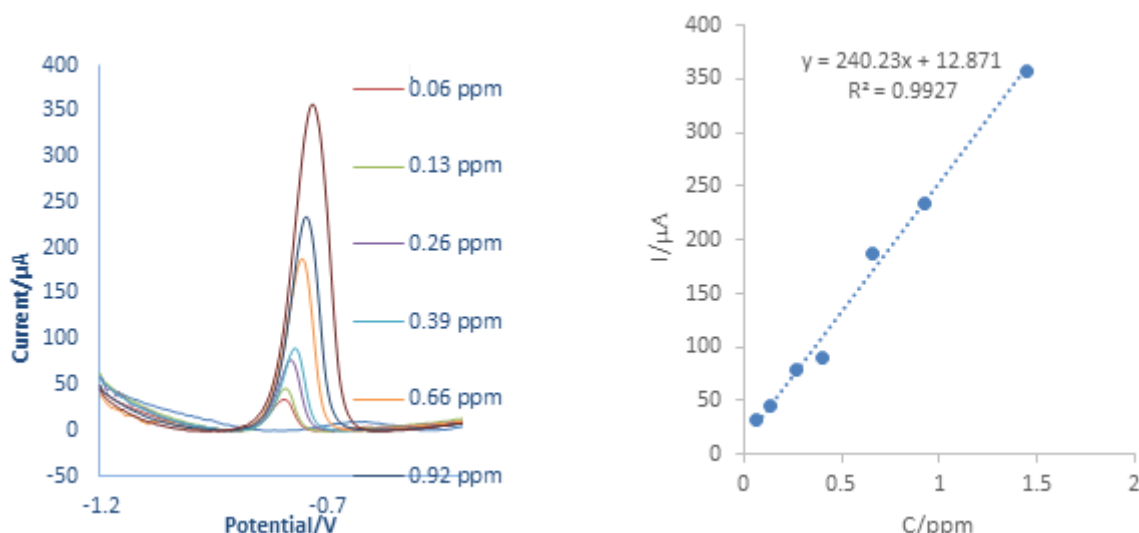


Figure 3: SWAS Voltammogram recorded in different concentrations of Cd²⁺ in 0.1M acetate buffer pH=4.5 using rGO@Ni/CPE. Corresponding calibration plot.

The calibration curve was repeated three times, and performance parameters such as sensitivity, linear range, limit of detection, and standard addition recovery were calculated. All parameters are summarized in Table 1.

Table 1. Summary of analytical performance parameters of nanosensors for quantification of HM

Sensor	Analyte	Method	Sensitiv. µA /ppm	R ²	Linear range ppm	LOD ppm	LOQ ppm	Recovery %	C _{Heavy metal Recovery} (ppm)
rGO@Ni/CPE	Cd ²⁺	SWASV	240.23	0.9927	0.066-1.44	0.017	0.058	81-119	0.066-0.39
rGO@Ni/CPE	Pb ²⁺	SWASV	552.01	0.9991	0.13-0.72	0.12	0.39	98-102	0.13-0.33
rGO@Au/CPE	Hg ²⁺	SWASV	265.33	0.9907	0.66- 1.96	0.31	1.02	94-103	1.44- 1.96

1.2 Laboratory validation for detecting Pesticides

1.2.1 Development and validation of nanosensor for detecting Paraquat

Preparation of - rGO@Ni/CPE

In a porcelain mortar, 2 g of carbon powder (particle size: 90 μm to 70 μm), 200 mg of pre-synthesized rGO@Ni (explained in 1.1.2), and 600 μl of paraffin were thoroughly mixed for one hour to achieve a uniform mixture. The resulting paste was refrigerated for 24 hours and then stored in a dry place. Approximately 1 gram of the paste was transferred into the electrode body, connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor rGO@Ni/CPE for quantification of Paraquat

The analytical performance of the rGO@Ni/CPE for Paraquat determination was evaluated through various electrochemical techniques, including cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). Optimization of the operating conditions, supporting electrolyte, as well as the pH value, was conducted to enhance the accuracy of the results. The best results were achieved with DPV using a three-electrode setup consisting of: rGO@Ni/CPE (working electrode), Ag/AgCl/Cl⁻ (reference electrode) and Pt (counter electrode).

Optimal DPV technical parameters:

- $t_{\text{equilibr}}=10\text{s}$
- $E_{\text{step}}=0.01\text{V}$
- $E_{\text{pulse}}=0.05\text{V}$
- $t_{\text{pulse}}=0.05\text{s}$
- Scan rate: 50mV/s
- Potential range: -0.3V ; -1.4V.

The calibration curve was repeated three times ($n=3$) to evaluate performance parameters such as sensitivity, linear range, limit of detection and addition recovery. All parameters are summarized in Table 2.

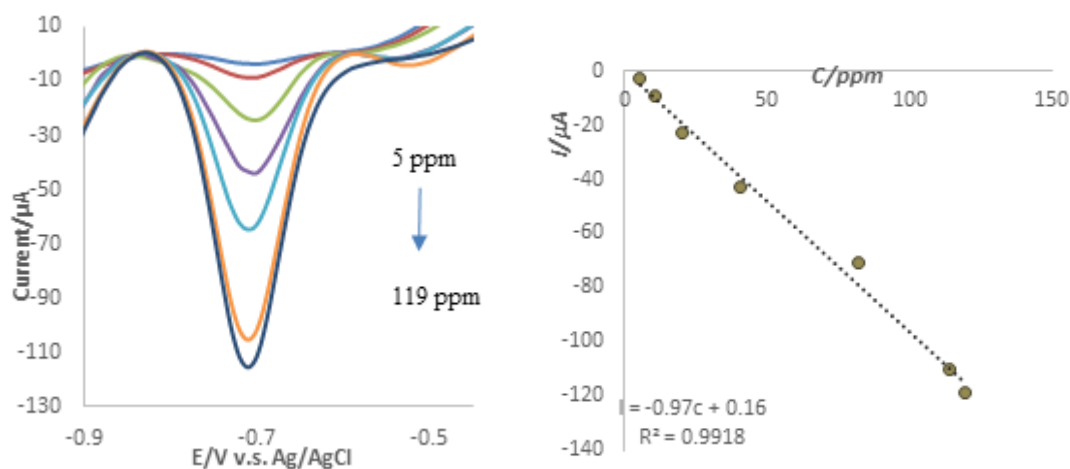


Fig.4 DPVs responses recorded in different concentrations of paraquat. All measurements were performed in phosphate buffer saline (PBS), pH 6.76. Corresponding calibration graph.

1.2.2 Development and validation of nanosensor for detecting Imidacloprid

Preparation of rGO@Ni (explained in 1.1.2)

Preparation of - rGO@Ni/CPE (explained in 1.2.1)

Analytical performance parameter of nanosensor CPE/rGO-Ni for quantification of Imidacloprid

The analytical performance of the rGO@Ni/CPE for imidacloprid determination was evaluated using various electrochemical techniques: cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The optimization of the experimental conditions such as supporting electrolyte, pH value, voltammetry techniques parameters, are performed and carefully followed. The best results were achieved with DPV using a three-electrode system: rGO@Ni/CPE (working electrode), Ag/AgCl/Cl⁻ (reference electrode) and Pt (counter electrode).

The optimal DPV technical parameters are:

- $t_{\text{equilibr}}=10\text{s}$
- $E_{\text{step}}=0.01\text{V}$
- $E_{\text{pulse}}=0.05\text{V}$
- $t_{\text{pulse}}=0.05\text{s}$
- Scan rate 50mV/s
- Potential range: -0.3V ; -1.4V.

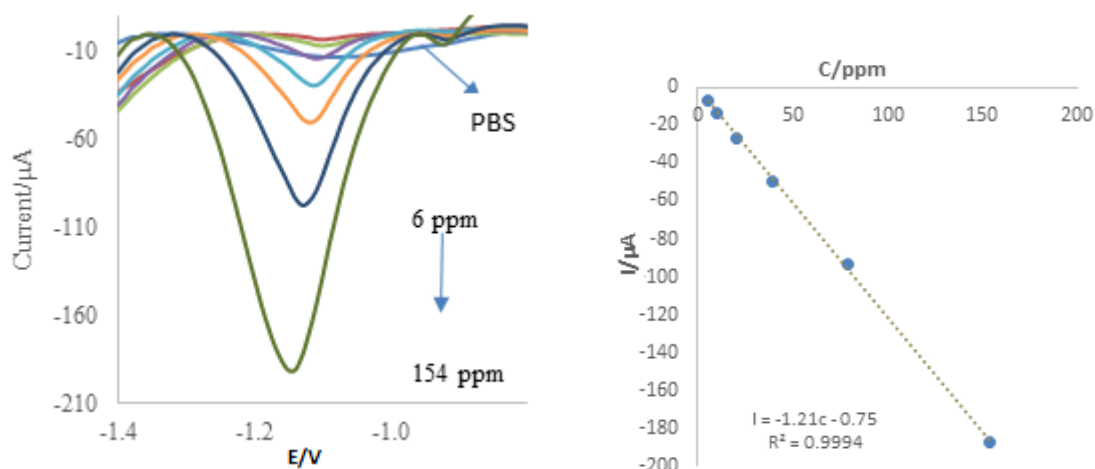


Fig.5 DPVs responses recorded in different concentrations of imidacloprid. All measurements were performed in phosphate buffer saline (PBS), pH 6.76. Corresponding calibration graph.

The calibration curve underwent three repetitions ($n=3$) and analytical performance parameters in terms of sensitivity, linear range, limit of detection, limit of quantification and addition recovery were subsequently calculated. A summary of all parameters can be found in Table 2.

1.2.3 Development and validation of nanosensor for detecting Thiamethoxam

Preparation of rGO@Ni (explained in 1.1.2)

Preparation of - rGO@Ni/CPE (explained in 1.2.1)

Analytical performance parameter of nanosensor - rGO@Ni/CPE for quantification of Thiamethoxam

Electrochemical measurements and analytical performance for detecting thiamethoxam were conducted using the following electrochemical methods: cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). Furthermore, the operating conditions for CV and SWV techniques, supporting electrolyte, as well as the pH value, were optimized. The results obtained through DPV indicated successful detection using a three-electrode system consisting of: - rGO@Ni/CPE as the working electrode, Ag/AgCl/Cl⁻ as the reference electrode and Pt as the counter electrode.

The optimal DPV technical parameters are:

- $t_{\text{equilibr}}=10\text{s}$
- $E_{\text{step}} = 0.01\text{V}$
- $E_{\text{pulse}}= 0.05\text{V}$
- $t_{\text{pulse}}= 0.05\text{s}$
- Scan rate 50mV/s
- Potential range : -0.8V ; -1.4V.

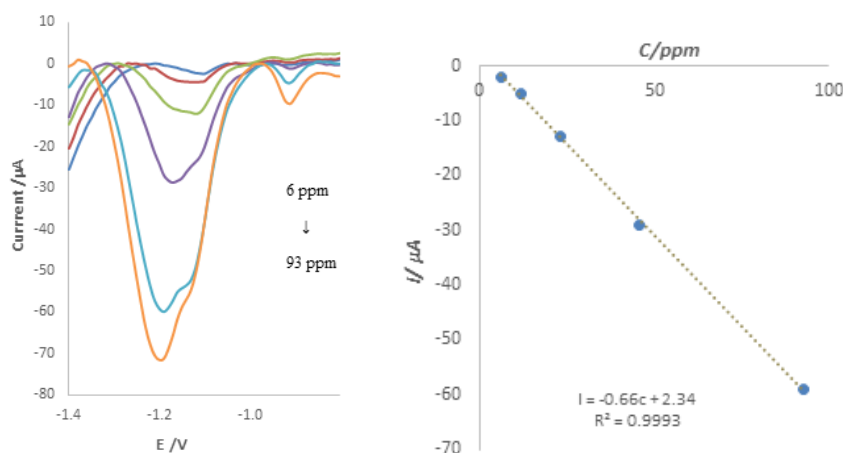


Fig. 6 DPVs responses to increasing concentration of thiamethoxam. All measurements were performed in phosphate buffer saline (PBS), pH 6.76 with CPE/rGO-Ni . Scan rate 50mV/s, $E_{\text{puls}} 0.05\text{V}$, $t_{\text{pulse}}= 0.05\text{s}$. Corresponding calibration graph

The calibration curve was conducted three times ($n=3$) to assess performance parameters including sensitivity, linear range, limit of detection, and addition recovery. A summary of these parameters is provided in Table 2.



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Table 2. Summary of analytical performance parameters of nanosensors for quantification of pesticides

Sensor	Pesticide	Method	Sensit. ($\mu\text{A}/\text{ppm}$)	Linear range (ppm)	LoD (ppm)	LoQ (ppm)	Relative Standard Deviation	R ²	Recovery %	C _{pesticide} , recovery ppm
rGO@Ni/CPE	PQ	DPV	0.97	5-119	0.44	1.46	4.8%	0.9918	89- 117	11-21
rGO@Ni/CPE	IMI	DPV	1.21	6-154)	1.31	4.39	1.1%	0.9994	91 - 106	6-21
rGO@Ni/CPE	THMX	DPV	0.66	6-93)	3.96	13.21	7.5%	0.9993	92- 108	12- 6

1.3 Laboratory validation for detecting Antibiotics

1.3.1 Development and validation of nanosensor for detecting Penicillin

Preparation of GO-Cu/CPE

Mix 0.1 g of graphene oxide (GO) previously prepared (explained in 1.1.1) with 0.63 g of $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ and 15 ml of distilled water, for 24 h at RTP. Then, for 4 h, the mixture is placed in an ultrasonic bath for more efficient mixing. For 5 min, it is then placed in a centrifuge at 5000 rpm and dried in a thermostat for 1 h at 50°C .

Then, 1 g of carbon powder (particle size: 90 μm to 70 μm) is homogenized in a mortar with 0.1 g of GO-Cu and 300 μL of paraffin for 1 hour and placed in a refrigerator (4°C) for 24 hours. Approximately 1 gram of the paste was transferred into the electrode body connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor GO-Cu/CPE for quantification of Penicillin (PEN)

Analytical performance of the GO-Cu/CPE for PEN determination is studied using different electrochemical techniques: CV, DPV and SWV. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Differential Pulse Voltammetry (DPV $t_{eq}=10\text{s}$, $E=0-1.8\text{ V}$, scan rate 0.05 V/s) using three electrode system: GO-Cu/CPE (working), Ag/AgCl/ Cl^- (reference electrode) and Pt (counter electrode).

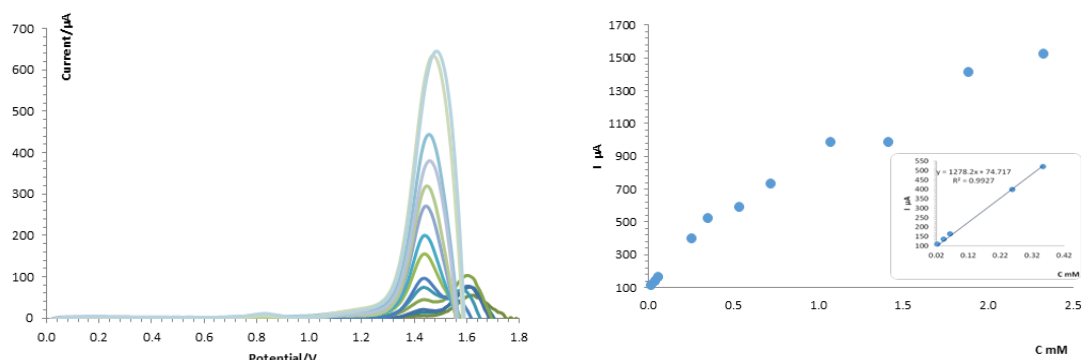


Fig. 7 DPVs recorded in different concentrations of penicillin (0.01-2.3 mM) in 0.1M acetate buffer pH=5 using GO-Cu/CPE. $t_{eq}=10\text{s}$, $E=0-1.8\text{ V}$, scan rate 0.05 V/s ; Corresponding calibration graph

The calibration curve was conducted three times ($n=3$) to assess performance parameters including sensitivity, linear range, limit of detection, and addition recovery. A summary of these parameters is provided in Table 3.

1.3.2 Development and validation of nanosensor for detecting Azithromycin

Preparation of ZnO/CPE

1.43 g of $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ was mixed lightly with 5.29 g of $\text{Na}_3\text{C}_6\text{H}_9\text{O}_9$ and 100 mL of H_2O . Then, 1 g of NaOH is added and after 2 hours the mixture was transferred to a plastic tube and centrifugated for 5 min, 10000 rpm. The precipitate was washed several (3) times with distilled H_2O and leave it at 60°C for 12 hours.

2 g of carbon powder is homogenized in a mortar with 0.2 g of pre-synthesized ZnO and 600 μL of paraffin for 1 hour and stored in a refrigerator (4°C) for 24 hours, then was transferred into the electrode body, connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor ZnO/CPE for quantification of Azithromycin (AZI)

Analytical performance of the ZnO/CPE for AZI determination is studied using different electrochemical techniques: CV, DPV and SWV. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Differential Pulse Voltammetry (DPV $t_{\text{eq}} = 0$ s, $E_{\text{beg}} = 0$ V, $E_{\text{end}} = 1.8$ V, $E_{\text{step}} = 5$ mV, $\text{Ampit} = 100$ mV, frequency = 5 Hz) using three electrode system: ZnO/CPE (working), Ag/AgCl/ Cl^- (reference electrode) and Pt (counter electrode).

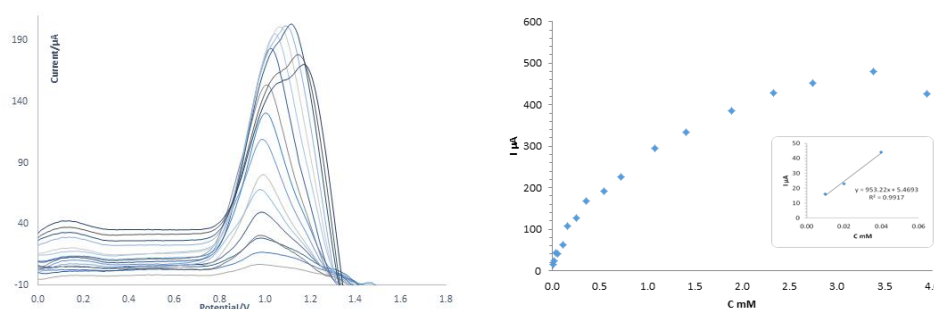


Fig. 8 DPVs recorded in different concentrations of azithromycin (0.01-3.9 mM) in 0.1M acetate buffer pH=5 using ZnO/CPE $t_{\text{eq}}=10\text{s}$, $E = 0-1.8$ V, scan rate 0.05 V/s; Corresponding calibration graph

The calibration curve was conducted three times ($n=3$) to assess performance parameters including sensitivity, linear range, limit of detection, and addition recovery. A summary of these parameters is provided in Table 3.

1.3.3 Development and validation of nanosensor for detecting Tetracycline

Preparation of ZnO(x2)/CPE

2 g of carbon powder is homogenized in a mortar with 0.4 g of pre-synthesized ZnO and 600 μL of paraffin for 1 hour and stored in a refrigerator (4°C) for 24 hours, then was transferred into the electrode body, connected with a conductive wire for electrical contact.

Analytical performance parameter of nanosensor ZnO(x2)/CPE for quantification of tetracycline (TET)

Analytical performance of the ZnO(x2)/CPE for TET determination is studied using different electrochemical techniques: CV, DPV and SWV. Optimization of experimental conditions such as

pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by SWV ($t_{eq} = 60$ s, $E_{beg} = 0.5$ V, $E_{end} = 1.5$ V, $E_{step} = 5$ mV, Amplitude= 50 mV, frequency=100Hz) using three electrode system: ZnO(x2)/CPE (working), Ag/AgCl/Cl⁻ (reference electrode) and Pt (counter electrode).

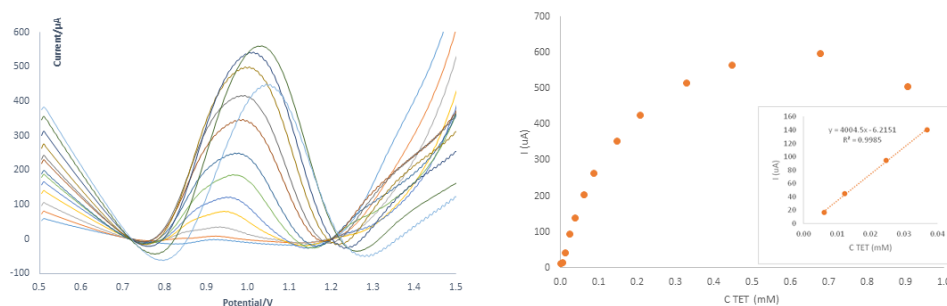


Fig. 9 SWVs recorded in different concentrations of tetracycline (6 μ M-2 mM) in 0.1M acetate buffer pH=5 using CPE/ZnO(x2) t_{eq} =60s, E= 0.5-1.5 V, E step = 5 mV, Ampit.= 50 mV, Frequency= 100 Hz; Corresponding calibration graph

The calibration curve was conducted three times ($n=3$) to assess performance parameters including sensitivity, linear range, limit of detection, and addition recovery. A summary of these parameters is provided in Table 3.

Table 3. Summary of analytical performance parameters of nanosensors for quantification of antibiotics

Sensor	Tecniq.	Anal.	Sensit. (μ A/ppm)	R ²	Linear range (ppm)	LOD (ppm)	LOQ (ppm)	Relative Standard Deviation	Recovery. (%)	Conc. of rec. (ppm)
CPE/GO-Cu	DPV	Pen	3.6	0.993	4-125	4.1	9.5	6.4%	99-120	18-124
CPE/ZnO	DPV	Azi	1.3	0.992	7-30	7	22	5.8%	92-110	8-30
CPE/ZnO(x2)	SWV	Tet	8.3	0.9985	3-18	4	12	2.3%	78-91	4-16

2 Research Sub-Topic B: Paper-based substrates with graphene derivatives for sustainable nanosensors

2.1 Laboratory validation for detecting Heavy Metals

Summary

The study aimed to develop and validate graphene-based nanosensors for detecting and adsorbing heavy metals, specifically cadmium (Cd^{2+}) and lead (Pb^{2+}). The goal was to ensure high selectivity, sensitivity, reusability, and eco-friendliness of the materials.

Materials and Methods

1. Material Design:

- Developed nitrogen-doped graphene derivatives (NG), which were further oxidized and size-reduced to produce nitrogen-doped graphene acid dots (NGA-D).
- NGA-D incorporated carboxyl groups and nitrogen dopants, forming multidentate coordination sites with strong affinity for heavy metals.

2. Validation Parameters:

- Adsorption Capacity: Measured maximum sorption capabilities for Pb^{2+} and Cd^{2+} .
- Selectivity: Assessed performance in the presence of competing ions (e.g., Ca^{2+} , Mg^{2+}).
- Sensitivity: Determined the limits of detection (LoD) for heavy metals.
- Reusability: Evaluated material regeneration through acidic washing and its effect on adsorption efficacy.

3. Testing Conditions:

- Simulated and real water samples, including tap, mineral, and river water, were used.
- Competitive adsorption experiments with mixed metal ions were conducted to test selectivity.

Key Findings

1. Adsorption Performance:

- NGA achieved adsorption capacities of 872 mg/g for Pb^{2+} and 448 mg/g for Cd^{2+} under optimized conditions (100 ppm feed, pH 3.5).
- The capacity was retained even in competitive environments, highlighting selectivity for heavy metals over common water ions like Ca^{2+} and Mg^{2+} .

2. Detection Sensitivity:

- The NGA-D nanosensor exhibited fluorescence quenching upon binding Pb^{2+} and Cd^{2+} , with LoD values of 0.1 nM (Pb^{2+}) and 0.2 nM (Cd^{2+}).
- Sensitivity was consistent across various water matrices, outperforming many state-of-the-art fluorometric and potentiometric sensors.

3. Reusability:

- The material retained over 90% of its adsorption capacity for six cycles of regeneration using acidic washing and NaOH treatment.

4. Practical Applications:

- A paper-based detection system using NGA-D was developed for ultra-low-cost on-site water quality monitoring.
- The system detected Pb^{2+} and Cd^{2+} concentrations as low as 0.5 ppb and 9 ppb, respectively, meeting regulatory standards for drinking water.

Results Overview

Parameter	Pb^{2+}	Cd^{2+}
Adsorption Capacity	872 mg/g	448 mg/g
Limit of Detection	0.1 nM	0.2 nM
Reusability (6 cycles)	>90% retention	-
Competitive Selectivity	High	Moderate

Conclusions

The study successfully validated the performance of NGA-D as a dual-function material for water decontamination and heavy metal detection. Its unique properties include:

- **High adsorption capacity** for selective removal of Pb^{2+} and Cd^{2+} .
- **Unprecedented sensitivity** with LoD values surpassing state-of-the-art technologies.
- **Eco-friendliness** and cost-effectiveness via reusability and paper-based sensor applications.

The materials are promising for real-world applications in environmental monitoring and remediation, with potential for scalability and adaptation to detect other contaminants. Additionally, all results are freely accessible online (<https://doi.org/10.1002/sml.202201003>).

2.2 Laboratory validation for detecting Pesticides

Objective

To develop and validate a graphene-based electrochemical sensor for detecting pesticides, particularly the neonicotinoid imidacloprid, in real-world samples. The sensor was designed to exhibit high sensitivity, selectivity, and usability in point-of-care applications.

Materials and Methods

1. Material Design:

- Developed a novel graphene derivative, **Nitrogen-doped Graphene Acid (NGA)**, optimized for modification of screen-printed electrodes via inkjet printing technology.

2. Validation Parameters:

- **Sensitivity:** Determined the detection limit using electrochemical square wave voltammetry (SWV).
- **Selectivity:** Evaluated against structurally similar pesticides and other compounds relevant to water pollution.
- **Real-Sample Efficacy:** Assessed performance in spiked tap water and river water samples.
- **Stability:** Tested storage stability over two weeks under standard conditions.

3. Testing Conditions:

- Utilized disposable **screen-printed carbon electrodes (SPCEs)** as the sensor platform.
- Electrochemical detection relied on signal amplification in the presence of imidacloprid.

Key Findings

1. Sensitivity:

- Achieved a detection limit (LoD) of **4.4 μM** , suitable for monitoring imidacloprid in industrial effluents where permissible pesticide levels are typically set at or above this threshold.

2. Selectivity:

- The sensor demonstrated high selectivity for neonicotinoids, with no interference observed from halogenated pesticides, such as hexachlorobenzene.

3. Real-Sample Performance:

- **Recovery Rates:**
 - Tap water: **In progress** (experiments ongoing).
 - Lake water: **In progress** (experiments ongoing).

4. Stability:

- Retained functionality and accuracy for at least **two weeks** under standard storage conditions.



Results Overview

Parameter	Result
Detection Limit (LoD)	4.4 μ M
Selectivity	Specific to neonicotinoids (medium-high)
Recovery Rates	Tap water: In progress; Lake water: In progress

2.3 Laboratory validation for detecting Antibiotics

Objective

To develop and validate a graphene-based aptasensor capable of detecting antibiotics, particularly ampicillin, in diverse and complex samples. The device aimed for high sensitivity, selectivity, and usability in point-of-care applications.

Materials and Methods

1. Material Design:

- Developed a novel graphene derivative (GA-NH-YN) with alkyne functional groups through fluorographene chemistry.
- Covalent conjugation of DNA aptamers specific to ampicillin using copper-catalysed azide-alkyne cycloaddition (CuAAC) "click" chemistry.

2. Validation Parameters:

- **Sensitivity:** Detection limit determined through electrochemical signals.
- **Selectivity:** Tested against structurally similar antibiotics and other compounds.
- **Real-Sample Efficacy:** Evaluated in tap water, saliva, and milk spiked with ampicillin.
- **Stability:** Assessed storage stability over four weeks.

3. Testing Conditions:

- Utilized disposable screen-printed carbon electrodes (SPCEs) as the sensor platform.
- Electrochemical detection based on signal changes induced by aptamer conformational shifts upon binding with ampicillin.

Key Findings

1. Sensitivity:

- Achieved a detection limit of 1.36 nM (approximately 0.5 µg/L), surpassing regulatory thresholds for antibiotic residues in milk (4 µg/L in Europe).

2. Selectivity:

- The aptasensor demonstrated high specificity for ampicillin with negligible responses to other antibiotics, including penicillin G, amoxicillin, azithromycin, and tetracycline.

3. Real-Sample Performance:

- Recovery rates in complex samples:
 - Tap water: 104%
 - Saliva: 90%
 - Milk: 88%
- Highlights robustness in detecting antibiotics amidst potential interfering species like proteins and lipids.

4. Stability:

- The aptasensor retained functionality for at least four weeks, with a slight performance improvement attributed to aptamer conformational adjustments.

5. Point-of-Care Usability:

- The portable setup with a micropotentiostat connected to a smartphone showed minimal deviations in results, enabling practical field applications.

Results Overview

Parameter	Result
Detection Limit (LoD)	1.36 nM
Selectivity	High (specific to ampicillin)
Recovery Rates	Tap water: 104%; Saliva: 90%; Milk: 88%
Stability	Retained functionality for 4 weeks

Conclusions

The validation confirmed the high efficacy of the aptasensor for detecting ampicillin in real-world and complex samples. Key attributes include:

- **High Sensitivity:** LoD far below regulatory requirements.
- **Robust Selectivity:** Exceptional discrimination against structurally similar compounds.
- **Real-Sample Utility:** Effective in challenging matrices like milk and saliva.
- **Cost-Effectiveness:** Disposable carbon electrode base and simple preparation protocol enhance accessibility for widespread use.

The aptasensor's versatility offers a promising platform for adapting detection mechanisms to other antibiotics or analytes, enabling rapid and reliable monitoring in healthcare and environmental applications. Additionally, all results are freely accessible online (<https://doi.org/10.1002/sml.202207216>).

3 Research Sub-Topic C: Printing technologies for sustainable nanosensors

3.1 Laboratory validation for detecting Heavy Metals

Summary

This report details the laboratory validation of various electrode systems for detecting heavy metals, focusing on the optimization of analytical parameters and the evaluation of performance. We developed and tested reduced graphene oxide (rGO)-based electrodes with integrated metallic nanoparticles (MNPs) and bismuth nanorods (BiNRds), alongside inkjet-printed gold (Au-IJP) electrodes. The incorporation of BiNRds into rGO electrodes represents a significant advancement due to their cost-effectiveness, environmental friendliness, and superior resistance to interference compared to traditional mercury-based electrodes.

The study evaluated the performance of -rGO@Au, -rGO@BiNRds, and Au-IJP electrodes for detecting Pb^{2+} , Cd^{2+} , and Cu^{2+} ions. Each electrode demonstrated distinct strengths: the Au-IJP electrodes showed high sensitivity and a wider linear range compared to -rGO@Au, while -rGO@BiNRds provided the broadest linear range and was the most effective for simultaneous multi-metal detection. The results indicate that -rGO@BiNRds electrodes are especially promising for environmental applications, offering a robust platform for heavy metal monitoring in single and mixed solutions. Additionally, the inkjet-printed gold electrodes (Au-IJP) offer a scalable and reproducible alternative for heavy metal detection with excellent performance for Pb^{2+} ions.

Objective

The primary objective of this work was to validate the laboratory performance of multiple electrode systems, including nanocomposite-based rGO electrodes and inkjet-printed gold electrodes, for detecting heavy metals. The optimization of analytical parameters, sensitivity, linear range, and the ability to detect multiple analytes simultaneously were evaluated.

3.1.1 Preparation of rGO@Au printed electrode

1.23 g of GO (1%) was diluted in 5.2 ml of 50 mM of a gold salt solution prepared in distilled water; then the dispersion was stirred for 5 min and filtered onto a PVDF membrane. After the filtration process, the film was allowed to dry at a temperature of 37 °C for 20 minutes. The dried film was aligned according to the pattern design and treated with a GRBL laser. The laser assistance facilitates the creation of a system consisting of three electrodes, with both the reference and auxiliary electrodes being made from the identical material as the working electrode, in this case - rGo@Au. The silver electrical contacts were screen printed onto PET (polyethylene terephthalate). The electrodes provided by laser reduction are severed and moved onto the PET substrate using a hydraulic press.

Analytical performance parameter of rGO@Au printed electrode for quantification of Pb^{2+}

Analytical performance of rGO@Au printed electrode for Pb^{2+} determination is studied using different electrochemical techniques: CV, DPV and SWV integrating a preconcentration step. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: working, reference electrode and counter electrode made from rGO@Au material.

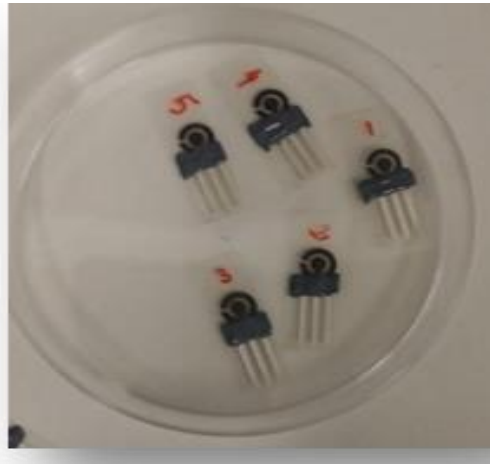


Fig. 10 Photo of rGO@Au printed electrodes

The optimal SWASV technical parameters are:

- preconcentration potential -0.8 V
- SWASV scanning -0.8 V to 0 V
- $t = 240$ s
- E step = 0.005 V
- $A = 10$ mV
- $f = 25$ Hz
- 0.1 M acetate buffer pH= 4.5

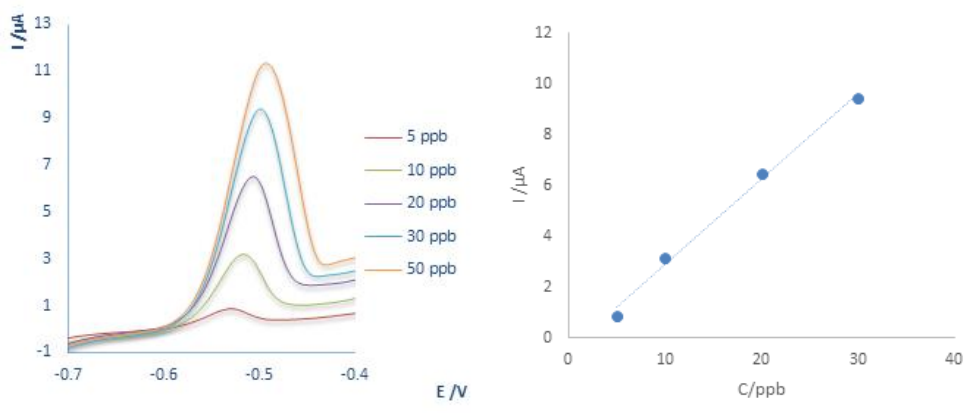


Fig. 11 SWAS Voltammograms recorded in different concentrations of Pb^{2+} in 0.1M acetate buffer pH=4.5 using rGO@Au under optimized condition; Corresponding calibration plot between Current (μA) and Concentration (ppb)

Table 4: Analytical performance parameters of rGO@Au printed nanosensor

Sensor	Analyte	Method	Sensitivity $\mu A / ppb$	R^2	Linear range ppb
-rGO@Au	Pb^{2+}	SWASV	0.3367	0.9941	5- 30

3.1.2 Preparation of Gold inject printed electrodes (Au-IJP), for Pb²⁺ quantification

The sensors were printed using the EPSON XP15000 printer and Gold Nano™ AuNP ink for the working and auxiliary electrodes, while Silver Nano™ AgNP was used for printing the reference electrode. The sensors are printed on a Mitsubishi Paper Mills substrate according to a previously optimized procedure.



Fig. 12 Photo of Au-IJP sensor

Analytical performance parameter of Au-IJP printed electrode for quantification of Pb²⁺

Analytical performance of Au-IJP printed electrode for Pb²⁺ determination is studied using the following electrochemical techniques: CV, DPV and SWV integrating a preconcentration step. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: working & reference electrode made from Au and counter electrode made from Ag.

The optimal SWASV technical parameters are:

- preconcentration potential -0.5 V
- SWASV scanning -0.5 V to 0.3 V
- $t = 180$ s
- E step = 0.005 V
- $A = 50$ mV
- $f = 25$ Hz
- 0.1 M acetate buffer pH= 4.5

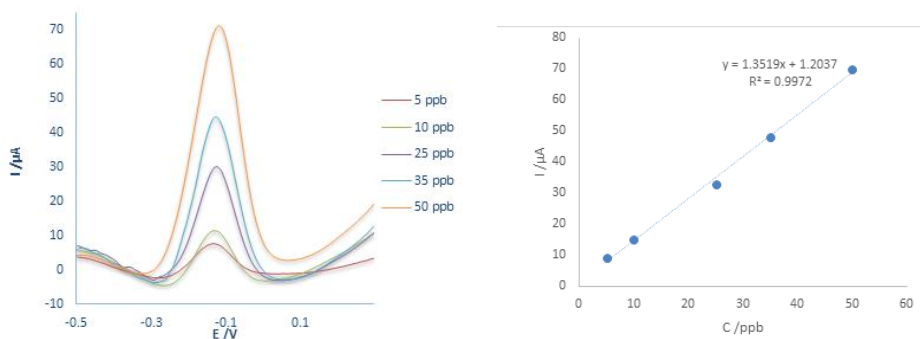


Fig.13: SWAS Voltammogram recorded in different concentrations of Pb²⁺ in 0.1M acetate buffer pH=4.5 using Au-Ijp under optimized condition. Corresponding calibration plot between Current (μA) and Concentration (ppb)

Table 5: Analytical performance parameters for Au-IjP sensor

Sensor	Analyte	Method	Sensitivity $\mu\text{A} / \text{ppb}$	R^2	Linear range ppb
Au- IJP	Pb^{2+}	SWASV	1.3519	0.9972	4 - 50

3.1.3 Preparation of rGO@BiNRds printed electrode for Pb^{2+} , Cd^{2+} , and Cu^{2+} detection

Preparation of rGO@BiNRds is done following similar procedure as is explained in 3.1.1 and instead of Au salt a Bi material was used.

Analytical performance parameter of rGO@BiNRds printed electrode for quantification of Pb^{2+} , Cd^{2+} , and Cu^{2+}

Analytical performance of rGO@BiNRds printed sensors for HM (Pb^{2+} , Cd^{2+} , Cu^{2+}) determination is studied using the following electrochemical techniques: CV, DPV and SWV integrating a preconcentration step. Optimization of experimental conditions such as pH, supporting electrolyte and also parameters relating to the technique used are followed carefully.

The best results are obtained by Square Waved Anodic Stripping Voltammetry using three electrode systems: working, reference electrode and counter electrode made from rGO@BiNRds material.

The optimal SWASV technical parameters for HM quantification are:

- preconcentration potential -1.2 V
- SWASV scanning -1.2 V to 0 V
- $t = 500\text{ s}$
- $E\text{ step} = 0.005\text{ V}$
- $A = 50\text{ mV}$
- $f = 1\text{ Hz}$
- 0.1 M acetate buffer $\text{pH} = 4.5$

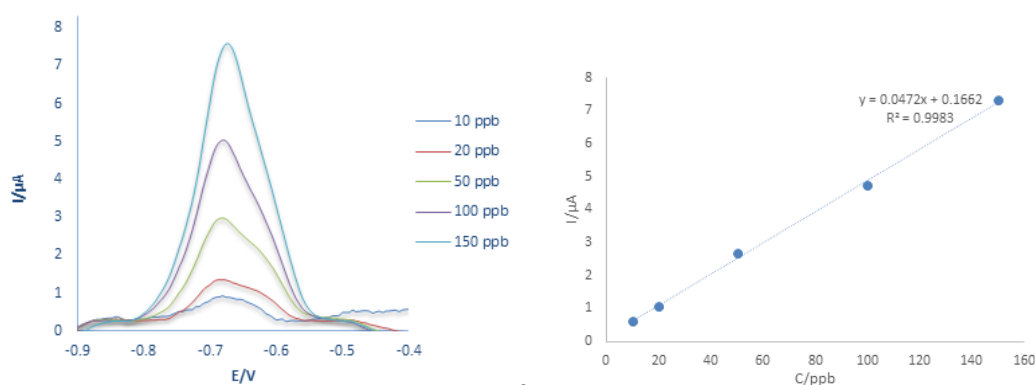


Fig.14 SWAS Voltammogram recorded in different concentrations of Pb^{2+} in 0.1M acetate buffer $\text{pH} = 4.5$ using rGO@BiNRds under optimized condition Corresponding calibration plot between Current (μA) and Concentration (ppb)

The calibration curve was repeated three times (n=3) to evaluate performance parameters such as sensitivity, linear range, limit of detection and addition recovery. All parameters are summarized in Table 6.

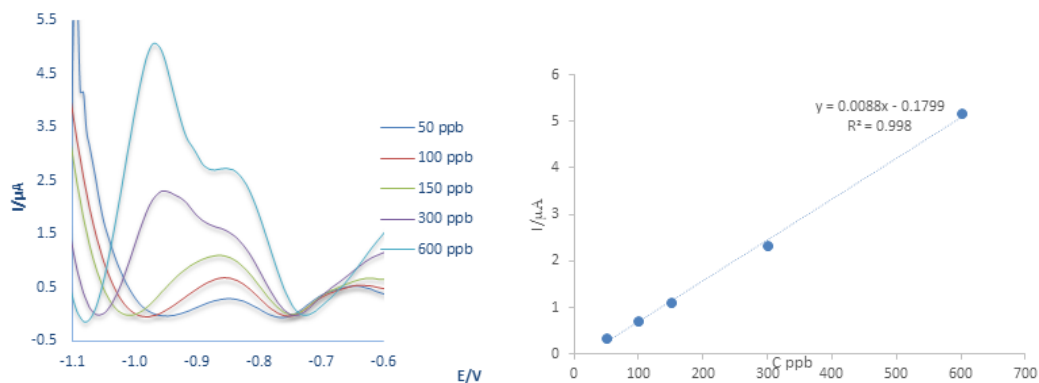


Fig.15 SWAS Voltammograms recorded in different concentrations of Cd^{2+} in 0.1M buffer pH=4.5 using rGO@BiNRds under optimized condition. Corresponding calibration plot between Current (μA) and Concentration (ppb)

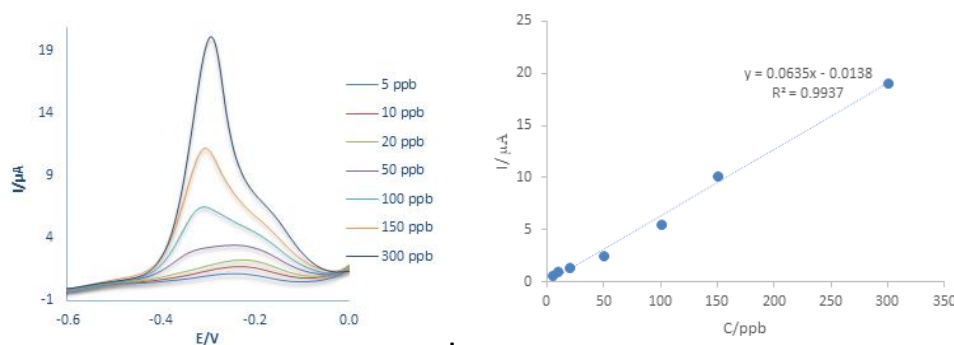


Fig.16 SWAS Voltammograms recorded in different concentrations of Cu^{2+} in 0.1 M acetate buffer pH=4.5 using rGO@BiNRds under optimized condition. Corresponding calibration plot between Current (μA) and Concentration (ppb)

Table 6: Summary of analytical performance parameters of rGO@BiNRds for quantification of HM

Sensor	Analyte	Method	Sensitivity $\mu A / ppb$	R^2	Linear range ppb	LOD ppb	LOQ ppb	Rel. Stand. Dev.%	Recovery %
rGO@BiNRds	Pb^{2+}	SWASV	0.0472	0.9983	10- 150	1.1	3.6	2.78	95-106
rGO@BiNRds	Cd^{2+}	SWASV	0.0088	0.9982	50 - 600	11.1	37.1	8.4	95-118
rGO@BiNRds	Cu^{2+}	SWASV	0.0635	0.9937	5 - 150	2.0	6.7	2.4	87-110



Conclusion

The incorporation of BiNRds into rGO electrodes provided significant benefits, including a broader linear range and higher selectivity for heavy metal ions in complex matrices, making rGO@BiNRds the most suitable for real-world environmental monitoring. Inkjet-printed gold electrodes (Au-IJP) emerged as a scalable and sensitive alternative, particularly for Pb²⁺ detection. Future work will include validation with real environmental samples, testing additional analytes, and further optimization to improve multi-metal detection accuracy.

4 Summary of the results for the best nanosensors

4.1 Heavy Metals

Table 7: Summary of the results for the best nanosensors for detecting heavy metals

Heavy metal	Linear Range Achieved	Limit of Detection Achieved	Recovery Achieved	Relative Standard Deviation Achieved
Pb ²⁺	10- 150 ppb	1.1 ppb	95-106%	2.8%
Pb ²⁺	130-720 ppb	120 ppb	98-102%	2.0%
Pb ²⁺	n/a	0.02 ppb	n/a	n/a
Cd ²⁺	50 – 600 ppb	11ppb	95-118%	8.4%
Cd ²⁺	66-1440 ppb	17ppb	81-119%	4.2%
Cd ²⁺	n/a	0.02 ppb	n/a	n/a
Cu ²⁺	5-150 ppb	2.0 ppb	87-110%	2.4%
Hg ²⁺	660-1960 ppb	310 ppb	94-103%	3.3%

Carbon paste sensors modified with rGO@Me are characterized by good sensitivity, linear working range, good correlation, but with a detection limit higher than the maximum allowed levels defined in world standards (table 1). Thanks to the advantages of easily preparation and usage, they can be applied in cases of monitoring environments contaminated with studied metals (Pb, Cd, Hg).

During this project, carbon derivatives with very good absorption properties for metals were synthesized. Especially nitrogen-doped graphene acid dots (NGA-D) exhibited high selectivity and sensitivity with LoD values of 0.02 ppb for Pb²⁺ and Cd²⁺ much lower than the levels appointed by international standards. These sensors can be used to monitor the environmental state of surface water.

Experimentation of printed sensors based on laser engraver technology have resulted in analytical parameters suitable for use in surface water monitoring (table 6), because they are also characterized by LOD lower than the maximum allowed limit.

4.2 Pesticides

Table 8: Summary of the results for the best nanosensors for detecting pesticides

Pesticide	Linear Range Achieved	Limit of Detection Achieved	Recovery Achieved	Relative Standard Deviation Achieved
Paraquat	5-119 ppm	0.44 ppm	89-117%	4.8%
Imidacloprid	6-154 ppm	1.3 ppm	91-106%	1.1%
Imidacloprid	1.3–102 ppm	1.1 ppm	n/a	< 10%
Thiamethoxam	6-93 ppm	3.69 ppm	92-108%	7.5%

Modified carbon paste sensors are characterized by good sensitivity, linear working range corresponds to ppm, good correlation of linear regression, and good recovery of standard additions (table 2). Those sensors are characterized by detection limits higher than the maximum allowed levels defined in world standards. The good recovery values enable the monitoring of polluted surface waters.

Screen-printed carbon electrodes (SPCEs) modified with Nitrogen-doped Graphene Acid (NGA), are studied and optimized for imidacloprid determination. This sensor resulted with very good

analytical performance characterized by low limit of detection 1 ppm (4.4 μ M), medium to high selectivity and good recovery in tap water. These sensors enable water monitoring.

4.3 Antibiotics

Table 9: Summary of the results for the best nanosensors for detecting antibiotics

Antibiotic	Linear Range Achieved	Limit of Detection Achieved	Recovery Achieved	Relative Standard Deviation Achieved
Ampicillin	10 nM–10 mM	0.48 ppb	104%	n/a
Penicillin	4-125 ppm	4.1 ppm	99-120%	6.4%
Azithromycin	7-30 ppm	7.0 ppm	92-110%	5.8%
Tetracycline	3-18 ppm	4.0 ppm	78-91%	2.3%

Modified carbon paste sensors using GO, GO-Cu and ZnO as modifiers, have resulted with good analytical performance parameters in terms of sensitivity, linear range and recovery. The sensors are characterized by LOD (4-7 ppm), higher than other analytical techniques (tab 3). Consequently, they cannot be used to monitor water with low pollution levels.

Graphene-based aptasensor is developed for ampicillin detection, even in complex samples. The sensor has resulted with very good selectivity, very low LOD of 1.36 nM (approximately 0.5 μ g/L), surpassing regulatory thresholds for antibiotic residues in milk (4 μ g/L in Europe).