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D1.1 Progress report on the design and development of the sustainable nanosensors

Project Coordinator (PC):	Prof. Majlinda VASJARI
	Email: majlinda.vasjari@fshn.edu.al
PC Organization Name:	UT
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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 Tiranes (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.

Table of Contents

Executive Summary

This document consists in a progress report describing the design and development of the sustainable nanosensors achieved by M12 in the context of the SUSNANO project.

The fluorographene chemistry technique is used to synthesize a variety of carbon-based derivatives modified with diverse functional groups such as carboxylic groups (referred to as graphene acid; GA), graphene enriched with nitrogen (referred to as GN3 or NGA), a graphene system incorporating iron (referred to as GA-Fe), a graphene derivative terminated with alkynes, and carbon dots extensively functionalized with carboxylic groups. The innovative nanomaterials are characterized and then investigated as potential platforms for the advancement of innovative electrochemical sensors for antibiotics, HM and pesticides detection.

Graphene derivatives and/or integrated with metallic nanoparticles, metal oxides are used for bulk and surface modification of carbon-based electrodes. These sensors have shown a very good analytical performance in terms of sensitivity and limit of detection especially for pesticides determinations.

A new technique has been experimented to produce a transferable penetrating material on any surface. A laser engraver is used to reduce graphene oxide (GO) and metal simultaneously. These MNPs@rGO electrodes have been tested for the electroanalytical detection of various molecules including heavy metals. These sensors have shown outstanding performance offering opportunities for the development of innovative sensing devices, particularly for flexible electronics and electroanalytical applications.

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

SUMMARY:
The experimentation of new modifiers for the bulk or surface modification of carbon-based electrodes has been the objective of scientific research. Graphene derivatives and/or integrated with metallic nanoparticles, GO, rGO/MeNp (Me-np: Ni, Cu, Ag, Au, Co), Metal Oxides (ZnO, Fe_2O_3) are prepared and are tested with electrochemical techniques (CV) and also by spectrometric techniques SEM, TEM and EDS. The presence of the modifier in the carbon paste considerably improves the shape of the signals, and other analytical performance parameters of the tested sensors to detect antibiotics, pesticides and HM. Glassy carbon electrodes modified with thin films of different derivative carbon modifiers synthesized in the labs of Palacky University (GA, GAN, GCN, GN3, GAFe and Sin500), are tested to detect pesticides. These sensors have shown a very good analytical performance in terms of sensitivity and limit of detection especially for pesticides determinations.
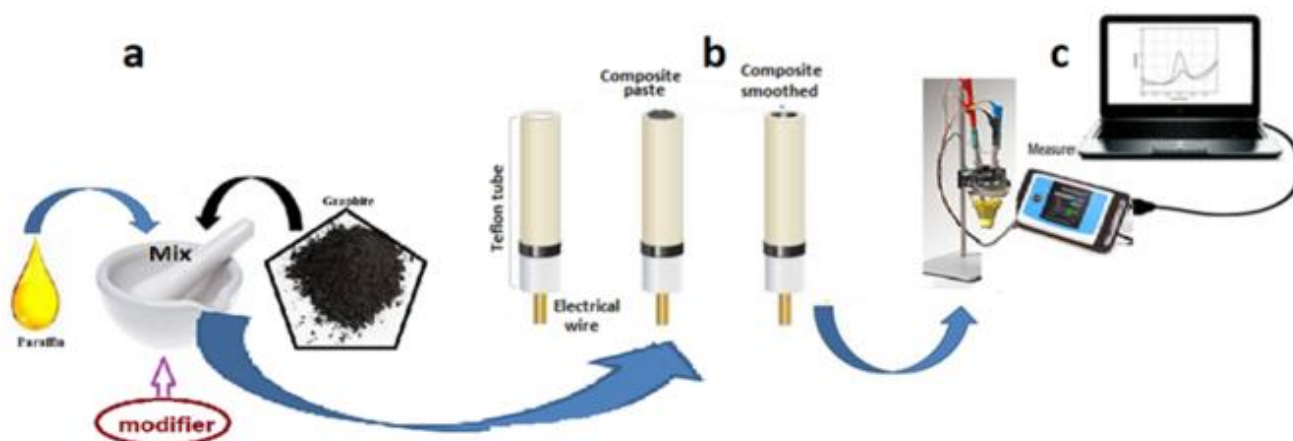
Scheme: a) The graphite powder, paraffin and modifiers were mix gently for several minutes, together until a uniform paste was obtained; b) The carbon paste was packed into the plastic tub containing a copper wire as the external electric contact. A glass surface was used to smooth the surface of the electrode before the measurements; (c) Measurements.

1.1 Preparation of modifiers (carbon derivatives) Graphene Oxide (GO), Graphene oxides doped with Me-np (Me-np: Ni, Cu, Ag, Au, Co), Metal Oxides (ZnO, Fe_2O_3)

Carbon-based modifiers for CPE were prepared in the laboratory based on the hummer method (with a few modifications). Firstly, graphene is oxidized to graphene oxide and then the graphene oxide is doped with different metals. The procedures of each stage are given below:

The procedure for preparation of the Graphen Oxide: In 1.5 g of graphene and 1.5 g of $NaNO_3$ was added 69 ml of sulfuric acid (H_2SO_4 cc). The system was mixed until a homogeneous solution was formed, and then it was kept for 15 minutes at 0^oC (in a container filled with ice and cold water). Then 9 g of $KMnO_4$ was added slowly and the mixture was magnetically stirred for 1 hour in room temperature and later was diluted with 100 ml of distilled water. The solution was heated at 90^oC for one hour in a hotplate and then was diluted with 300 ml distilled water and 10 ml H_2O_2 30%. The solution was maintained for 30 minutes in an ultrasonic bath at 25^oC. 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^oC.

The procedure for preparation of rGO/Me (Me-metal has been: Ni, Cu, Ag, Au). 0.1g GO and 0.3g metal salt ($NiSO_4$, $CuSO_4$, $AgNO_3$, $CoSO_4$), were mixed with 20 ml distillated 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.

The procedure for preparation of rGO-ZnO: Initially, ZnO is prepared. 1.43 g $ZnSO_4$ x $7H_2O$ and 5.29 $Na_3C_6H_9O_9$, were mixed continuously and slowly with 100 mL H_2O. Then 1 g NaOH is added and the system was left in the stirring conditions for 2 hours in room temperature. After that the system was centrifuged for 5 min at 10,000 rpm. The precipitate obtained of ZnO, is washed 3 times with distilled H_2O and leave at 60°C overnight for drying. In the mixture of 0.1 g GO and 0.16 g ZnO (prepared in the lab), 15 mL of distilled water was added and left for 24 h in stirring conditions and room temperature. Then the system was placed in an ultrasound bath for 4 hours and then was centrifuged for 5 minutes at 5000 rpm. The precipitate is dried in a thermostat at 50°C.

* The procedure for preparation of GO- Fe_2O_3. A mixture of 0.1 g GO (previously prepared), and 0.143 g Fe_2O_3 in 15 mL distilled water was kept in room temperature overnight and then in ultrasonic bath for 4 h. Latter on was centrifugated for 5 minutes at 5000 rpm and dried for 1 h në 50°C.

1.2 Preparation of bulk modified electrodes: Carbon paste electrodes modified with modifiers prepared in the lab

Bulk modified electrode (CPE-rGO/Me), was prepared by mixing 1.0 g graphite powder (with particle size 90 - 72 μm), 300 μl paraffin oil and 100mg modifier (rGO/Me, rGO-ZnO, GO- Fe2O3, NTC), for 30 min until a homogeneous paste is obtained. The prepared composite material was kept in a refrigerator at 40C for 24 h. Before measurements the paste was packed into the teflon tube with internal diameter 8 mm and outer diameter of 12 mm containing a copper wire as the external electric contact. The electrode surface was smoothed on a glass surface before immersed in the cell for electrochemical measurement.

CPE (unmodified), CPE/Au-np, CPE/rGO, CPE/Cu@rGO, CPE/Ag@rGO, CPE/Ni@rGO, CPE/Au@rGO, CPE/ZnO, CPE/ZnO/GO, CPE/GO/Fe2O3

1.2.1 Electrochemical characterization of composite using CV in the presence of redox couple Fe(+3)/Fe(+2)

Electrochemical characterizations of the studied sensors were performed using cyclic voltammetry technique (CV). In the electrochemical cell containing 15 ml of acetat buffer pH=5.5, the background current is recorded firstly in the buffer, and after the addition of redox couple Fe(+3)/Fe(+2). The same procedure in room temperature, was used for electrochemical characterizations of the modified CPE. In fig 1 are shown the electrochemical behaviour of unmodified CPE (A) and composite electrodes (B, C, D, E, F).
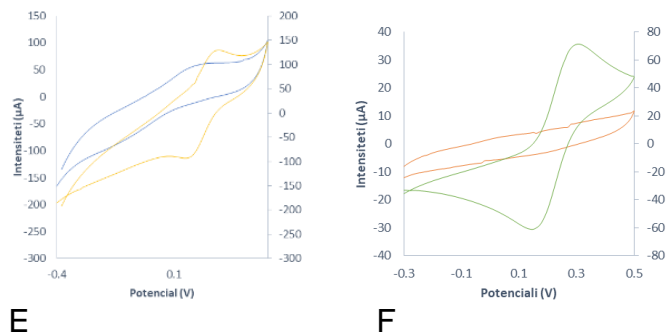

A   B   C   D

E   F
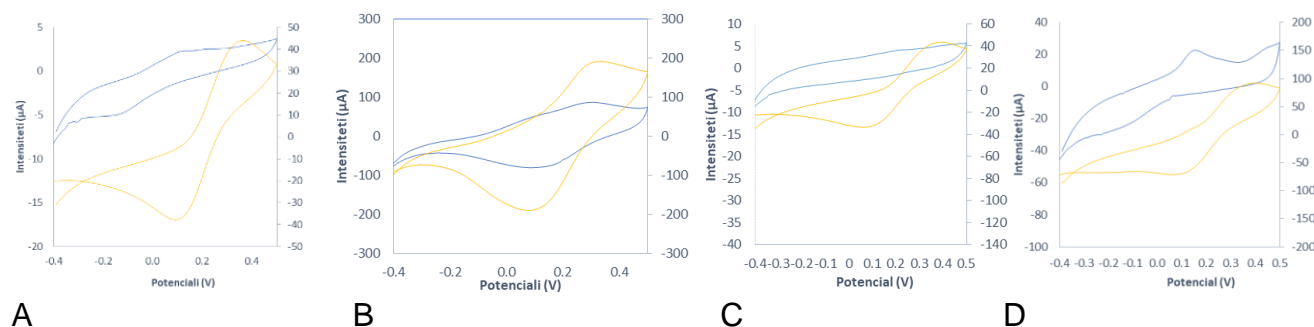
Fig.1 Cyclic voltamogrames recorded in the acetate buffer (pH=5.5) and after addition of the redox couple (Fe2+/Fe3+ 0.31 mM) using: A) CPE; B) CPE/rGO; C) CPE/Ni@rGO; D) CPE/Cu@rGO; E) CPE/Ag@rGO; F) CPE/Au@rGO.

1.2.2 Characterization of surface morphologies and chemical compositions by SEM, EDS, TEM and FTIR techniques

The modified CPE with rGO/Me was characterized by using several spectroscopic techniques. The presence of modifiers in the composite was further investigated using SEM, TEM and EDS analyses to evidence the efficiency of modifier in electrochemical behaviour (Fig 2 and Fig 3).
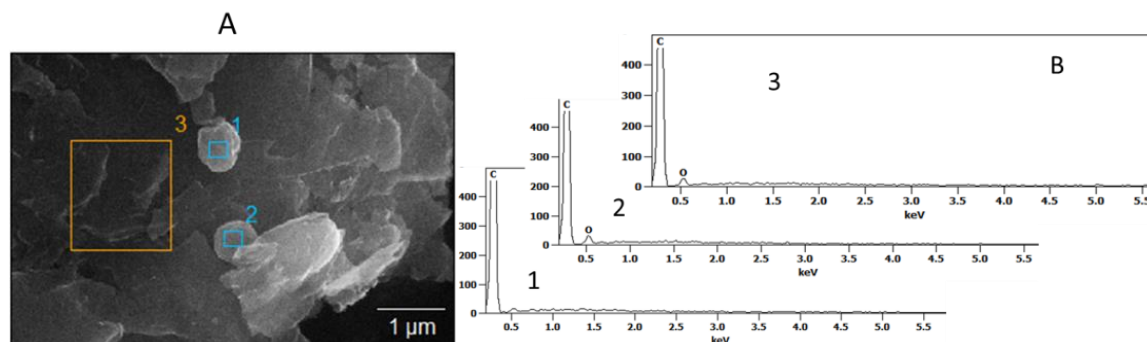

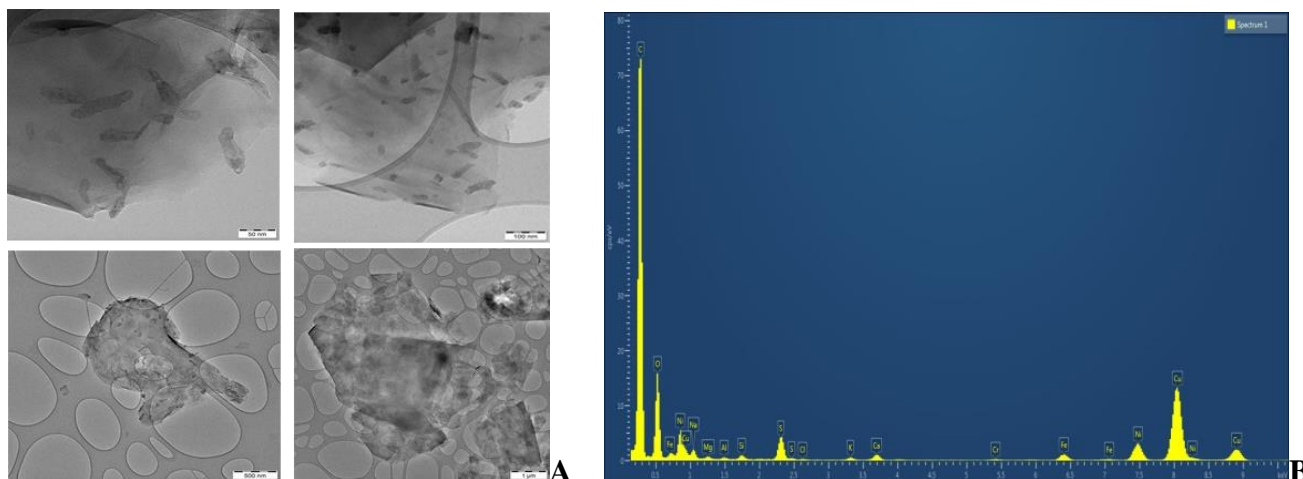Fig 2. (A) SEM image ofCPE/rGO/Ni; (B) EDS of CPE/rGO/Ni corresponding to 1, 2 and 3 respectively.

Fig.3 (A) TEM images in different magnifications and (B) EDS of CPE/rGO/Ni

1.3 Preparation of surface modified electrodes: GCE modified with thin films of nanomaterials (graphene derivatives, metal nanoparticles, hybrid structures:GO/Me-np)

Glassy carbon electrodes were modified with thin films of different derivative carbon modifiers synthesized in the labs of Palacky University: GA, GAN, GCN, GN3, GAFe and Sin500.

Modification of GCE:
The surface of GCE is cleaned as usually with Al_2O_3 powder (5μm), in wet conditions, rinsed with alcohol and dried. A drop of modifier (10μl) was dropped onto the surface and left to dry at room temperature for 30-40 minutes.

1.3.1 Electrochemical characterization of modified GCE using CV in the presence of redox couple Fe(+3)/Fe(+2)

Electrochemical characterizations of the modified GCE were performed by cyclic voltammetry technique (CV), recorded using GCE and modified GCE (fig 4).
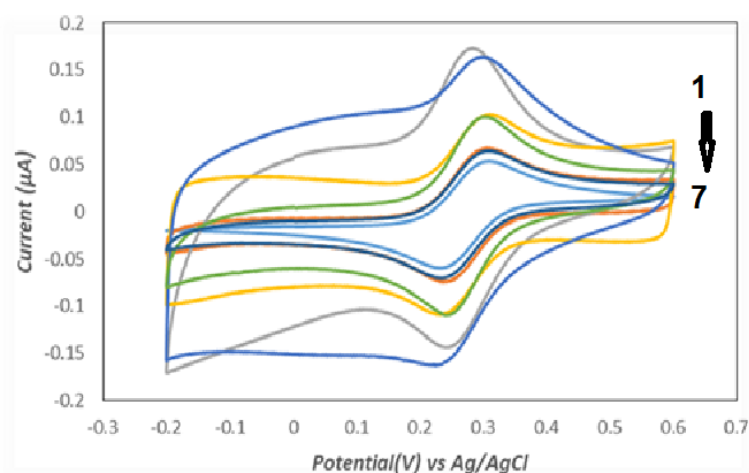

Fig.4 CVs recorded in the presence of redox couple Fe+3/Fe+2, using: GA/GCE (1), GAN/GCE (2), GCN/GCE (3), GAFe/GCE (4), GN3/GCE (5), SiN500/GCE (6) and bare GCE (7)

It is shown that the modified electrodes have better electrochemical behaviour than the unmodified one and especially GCE modified with GAN and GA.

1.3.2 Characterization of modified GCE using electrochemical impedance spectroscopy

From the voltammograms in fig 5 it can be seen that the presence of the modifying film GAN, GA have better affect in accelerating the transfer of electrons on the GCE surface.
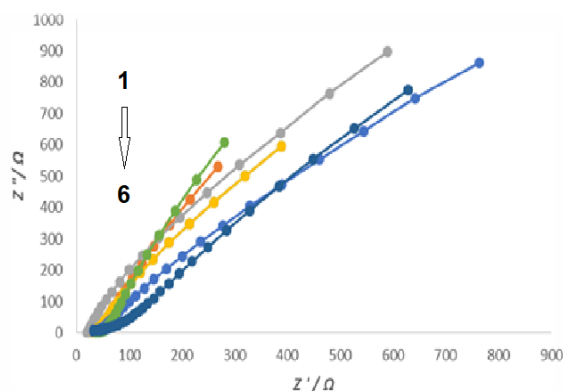.

Fig.5 Voltamograms recorded in 0.1M KCl + 5.0mM $[Fe(CN)_6]^{3-/4-}$ using GAN/EKQ (1), GA/EKQ (2), GCN/EKQ (3), GAFe/EKQ (4), GN3/EKQ (5), SiN500/EKQ (6)

1.4 Electrochemical behaviour (analytical performance), of new developed sensors in the presence of

1.4.1 Antibiotics (penicillin, azythromycine)

1.4.1.1 Electrochemical behaviour of bulk modified CPE in the presence of penicillin

Electrochemical behaviour of developed sensors CPE/rGO, CPE/Ag@rGO and CPE/Cu@rGO are tested in the presence of penicillin in acetate buffer pH=5 using Cyclic voltammetry and DPV. Better analytical performances are obtained using the CPE modified with Cu@rGO. N fig 6 it is shown the DPV voltammograms recorded in different concentrations of penicillin (0.01mM to 2.76mM). The calibration graph is derived from voltammograms and the linear range and sensitivity is calculated (1.28mA/mM)
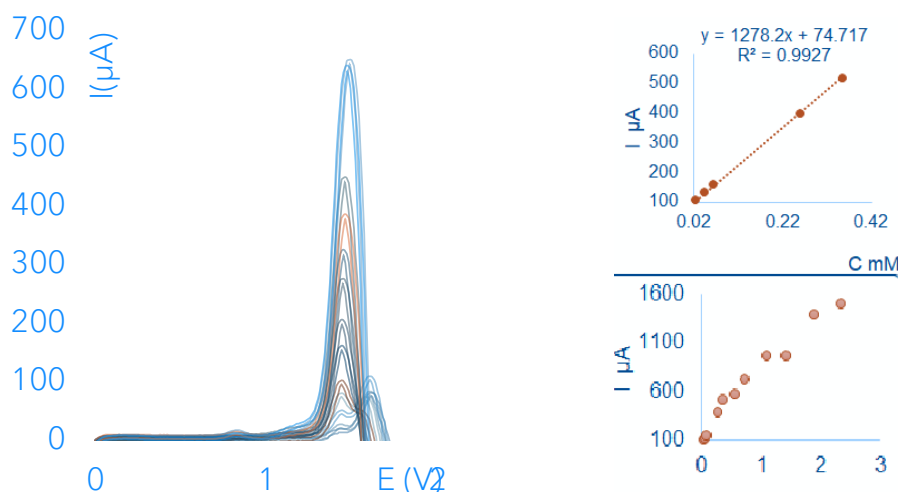

Fig 6. DPV and calibration graph in acetate buffer pH 5, using Cu@rGO (Concentration of PEN from 0.01mM to 2.76mM; t_{eq} =10s, E_{begin}= 0V, E_{final}=1.8V, E_{step}=0.01V, E_{puls}=0.05V, t_{puls}=0.05s, Scan rate =0.05V/s

1.4.1.2 Electrochemical behaviour of bulk modified CPE in the presence of azytromicine

Electrochemical behavior of developed sensors CPE/rGO, CPE/Ag@rGO and CPE/Cu@rGO , CPE/Ni@rGO and CPE/ZnO@rGO and CPE/Fe$_2$O$_3$@rGO are tested in the presence of azytromicine in acatat buffer pH=5 using CV , DPV and SWV. Better analytical performance are obtained using the sensor modified with Cu@rGO and ZnO@rGO (fig 7 a, b, c)
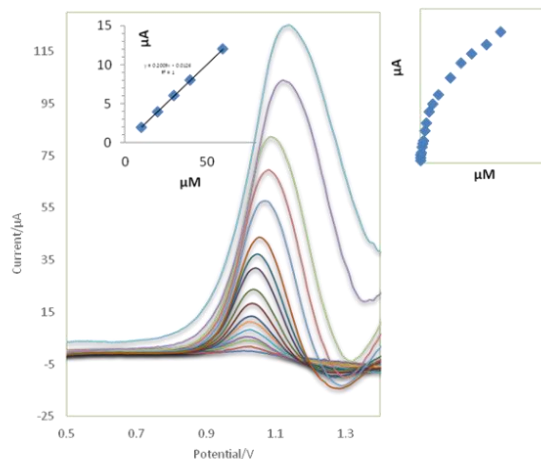
Fig 7 (a) DPV and calibration graph using CPE/Cu@rGO, recorded in acetate buffer solution pH=5; t_{eq}=10 s, scan rate=0.05v/s; Concentrations of azytromicine 0.01-0.6 mM.
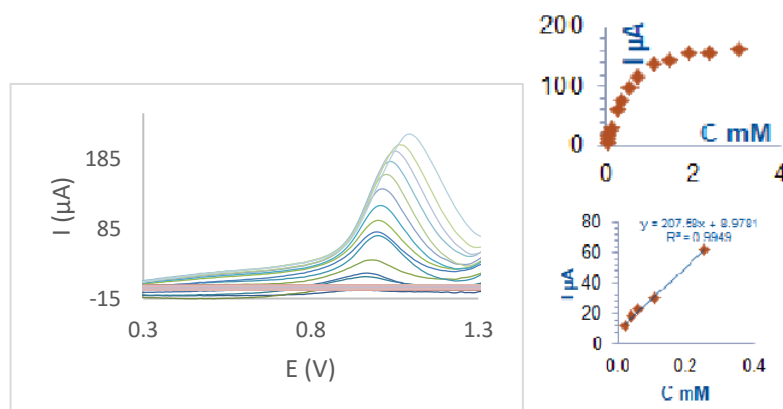
Fig.7 (b)..DPV and calibration graph using CPE/ZnO@rGO, recorded in acetate buffer solution pH=5; E=1-1.8 V, t_{eq}=10 s, scan rate=0.05v/s; Concentrations of azytromicine 0.01- 3.76 mM.

Fig 7 (c) .S WV and calibration graph using CPE/ZnO@rGO, recorded in acetate buffer solution pH=5; t_{eq}= 10s; amplitude= 0.05V; v= 5Hz; Concentrations of azytromicine 0.01- 3.76 mM.
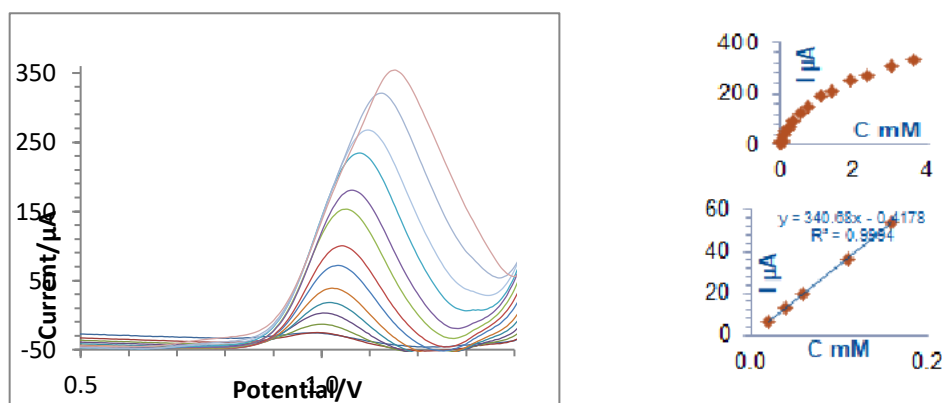
Calibration graphs are derived from the voltammograms and the linear range and sensitivity are calculated and are presented in tab 1. The sensitivity is higher performing with SWV.

Tab. 2 Performance parameter of CPE/ZnO@rGO in the presence of azytromicine

| Technique | Parameter of performance | | |
	Linear range (µM)	Sensitivity (µA/mM)	R^2
DPV	19.96-255.57	208	0.9949
	255.57 – 723	113	0.9959
SWV	0-158.31	**336**	0.9990
	19.96-158.31	**341**	0.9994

1.4.2 Heavy metals (Pb, Hg)

1.4.2.1 Electrochemical behaviour of bulk modified CPE in the presence of Pb

Modified CPE (CPE/rGO, CPE/Ag@rGO, CPE/Ni@rGO, CPE/Au@rGO), were tested in the presence of Pb. Cyclic voltammograms were recorded after the Pb additions and it was found that the peak of oxidation and reduction of Pb increases with the increase of it's concentration. The better performances obtained with CPE/Ni@rGO and CPE/Au@rGO are shown below. The cyclic voltammogram recorded using CPE/Au@rGO and CPE/Ni@rGO and respective calibration graph and presented in fig.8 (A and B) and fig 9 (A and B)

A   B

Fig 8 A) Cyclic voltammograms recorded using the CPE/Au@rGO in acetate buffer solution pH=3; at different concentrations of Pb^{+2}; scan rate 100 mv/s. B) Corresponding calibration graph.

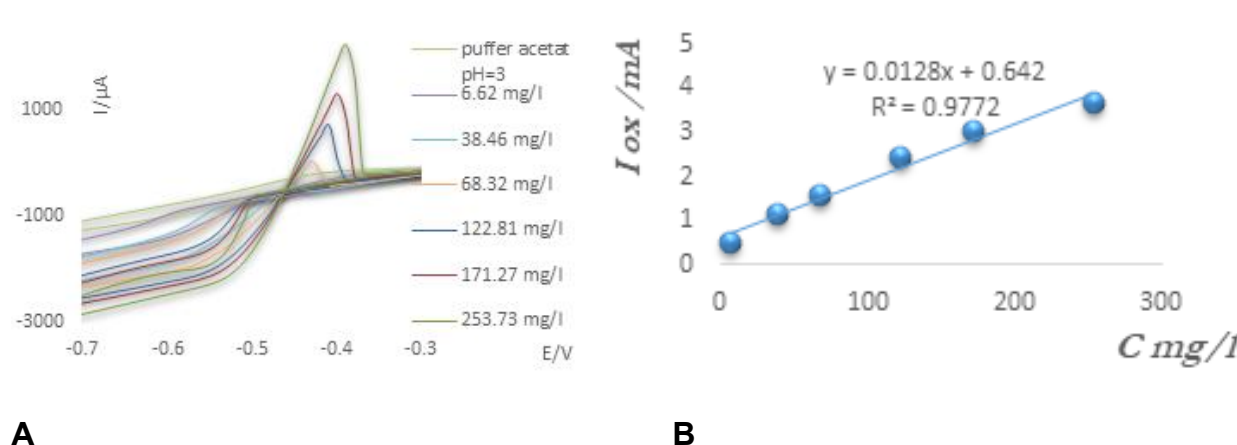A   B

Fig 9 A)Cyclic voltammograms recorded using the CPE/Ni@rGO in acetate buffer solution pH=3; at different concentrations of Pb^{+2}; scan rate 100 mv/s. B) Corresponding calibration graph.
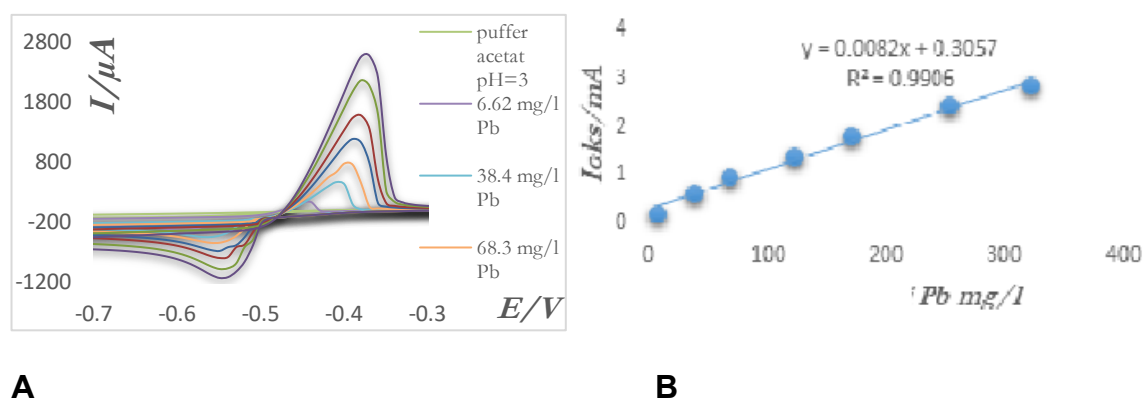
In Tab 2 are summarised the performance parameter of experimented sensors.

Table 2: Performance parameter of the studied composite sensors in the presence of Pb^{+2}; buffer acetate pH=3

Sensor	Modifier	Analyte	Technique	Analytical performance parameters		
				snsitivity mA/(mg/l)	R^2	Linear range mg/l
CPE	rGO	Pb^{2+}	CV	0.007	0.9953	6.62 - 253.73
CPE	Ni@rGO	Pb^{2+}	CV	0.0082	0.9967	6.62 - 253.73
CPE	Au@rGO	Pb^{2+}	CV	0.0128	0.9972	38.46 - 253.73

The effect of pH is studied using CPE modified with Ni@rGO in the presence of Pb and the results are given in the table 3.

Table 3 Comparison of analytical performance of CPE/Ni@rGO using acetate buffer pH=3, pH=5.5 for Pb quantification

Analyte	Technique	Acetate buffer (pH)	Analytical performance parameters		
			Sensitivity mA/(mg/L)	R2	Linear range mg/l
Pb^{2+}	CV	5.5	0.0073	0.9953	6.62 - 253.7
Pb^{2+}	CV	3	0.0087	0.9918	6.62– 253.7

1.4.2.2 Electrochemical behaviour of bulk modified CPE in the presence of Hg

Modified CPE (CPE/rGO, CPE/Ag@rGO, CPE/Ni@rGO, CPE/Au@rGO), were tested in the presence of Hg by using Cyclic Voltammetry technique. CVs were recorded after each Hg additions. Measurements are performed in different pH: acetate buffer in pH=3 and pH=5.5 and in PBS pH=7. (Tab 4).

Tab.4 Comparison of analytical performance of CPE/Au@rGO using acetate buffer pH=3 ; pH=5.5 and PBS pH=7 for Hg quantification

Sensor	Analyte	Technique	Electrolyte (pH)	Analytical performance parameters		
				Sensitivity mA/(mg/l)	R2	Linear range mg/l
CPE/Au@rGO	Hg^{2+}	CV	7	0.0157	0.9959	13.15 - 85.36
CPE/Au@rGO	Hg^{2+}	CV	5.5	0.0112	0.9939	6.62 -85.36
CPE/Au@rGO	Hg^{2+}	CV	3	0.0049	0.9944	38.46 -127.91

1.4.3 Pesticides (paraquat, imidacloprid)

1.4.3.1 Electrochemical behaviour of bulk modified CPE in the presence of imidacloprid

Modified CPE (CPE/Ni@rGO, CPE/Cu@rGO, CPE/Ag@rGO, CPE/Au@rGO), were tested in the presence of imidacloprid using Cyclic Voltammetry technique. The proportional dependence of the signal as a function of the imidacloprid concentration is observed only in the case of the CPE/Ni@rGO sensor (fig 10).

A   B   C   D
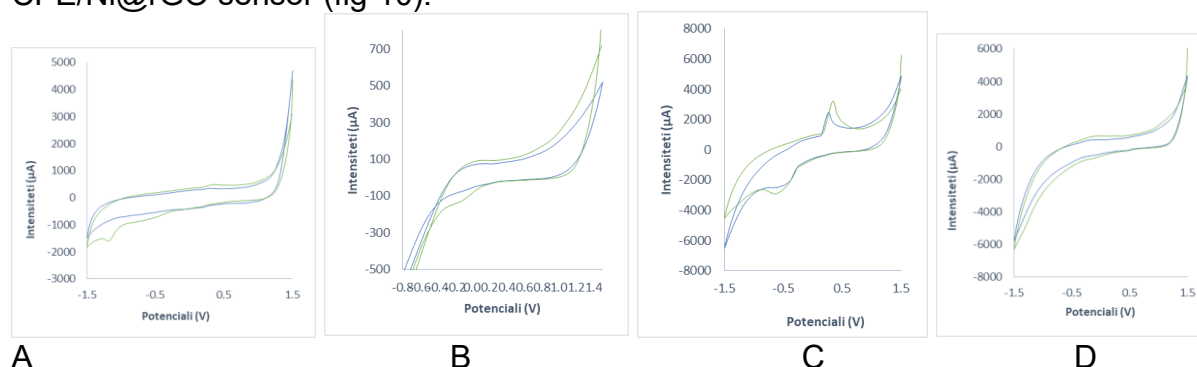
Fig.10 Cyclic voltammogram recorded in the PBS (pH=7) and after addition of 191 ppm imidacloprid, using: A) CPE/Ni@rGO; B) CPE/Cu@rGO; C)CPE/Ag@rGO; D)CPE/Au@rGO.

This sensor is tested applying other electrochemical techniques SWV and DPV. Calibration graphs derived from respective voltammograms are shown in fig 11 and the calculated performances parameters are summarized in tab.5.
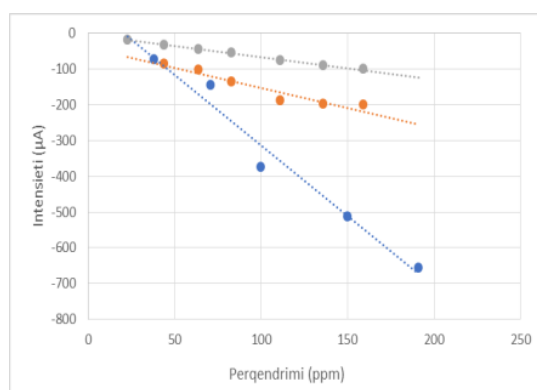
Fig 11 Calibration graph using CPE/Ni@rGO, for determination of imidacloprid using different electrochemical techniques: CV (blu line), SWV (orange line) and DPV (grey line).

Table 5. Performance parameter of CPE/Ni@rGO using different electrochemical techniques. Additions of imidacloprid from 0.07 mM to 1.13mM, in acetate buffer pH=5.5.
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Volt. technique	Linear Range (ppm)	Sens. (µA/ppm)	R^2	Reduction potential
CV	38-191	3.95	0.9719	-1.17 V
DPV	23-159	0.54	0.9936	-1.04 V
SWV	44-111	1.58	0.979	-1.16 V

1.4.3.2 Electrochemical behaviour of modified GCE in the presence of Paraquat

Modified GCE: GA/GCE, SiN500/GCE, GAFe/GCE, GCN/GCE, GAN/GCE, and GN3/GCE are tested in the presence of paraquat using Cyclic Voltammetry technique (fig. 12).
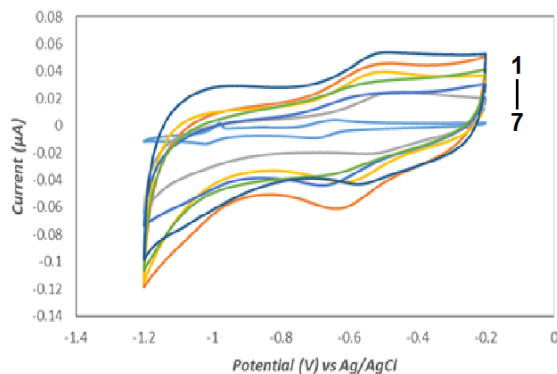
Fig.12 Voltamogrames recorded in 01M PBS (pH=7.04) and 0.1mM paraquat using GA/GCE (1) ,SiN500/GCE (2), GAFe/GCE (3), GCN/GCE (4), GAN/GCE (5),GN3/GCE (6), BARE /GCE (7)

Voltammogram recorded with bare GCE have 2 red/ox peaks of paraquat. In the case of modified GCE only the 1st reduction peak is observed. The high capacitance of modifier film overlaps the second one. The first peak is with interest because quantitatively represents the concentration of the paraquat in the solution.

Quantification of paraquat using modified GCE with thin film of GAFe, is tested after optimization of experimental conditions such as pH, frequency.

Several tests are done using GCE/GAFe and GCE/GN3 towards two other pesticides imidacloprid and thiamethoxan.

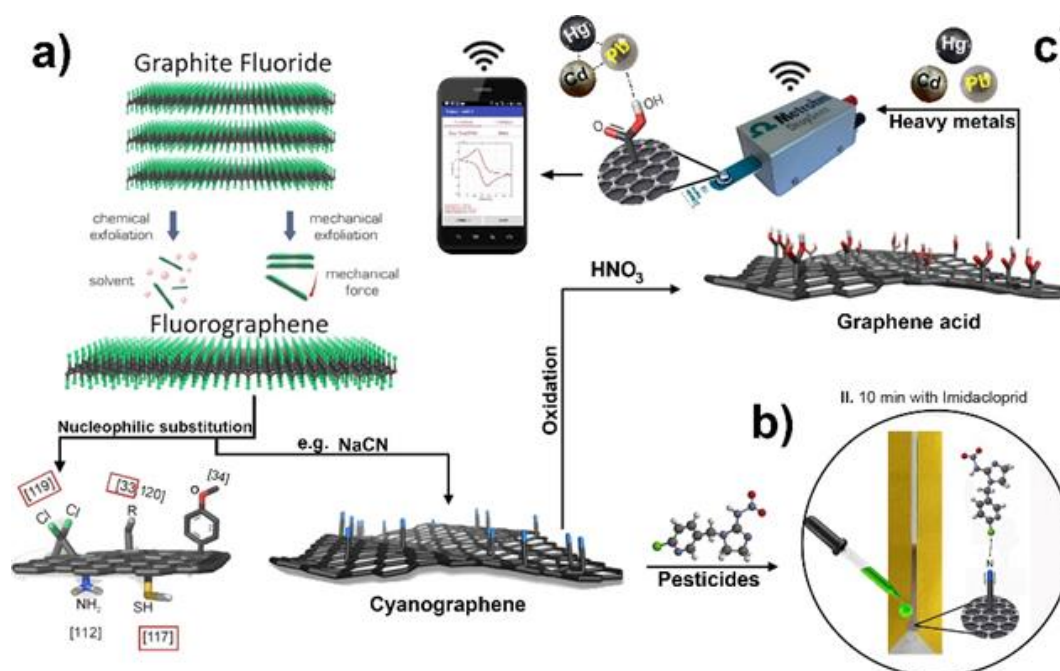## 2 Research Sub-Topic B: Paper-based substrates with graphene derivatives for sustainable nanosensors

SUMMARY:
By employing the techniques of fluorographene chemistry, we have successfully prepared a variety of carbon-based derivatives (including graphene derivatives and carbon dots) that have been modified with diverse functional groups. These tailored derivatives were then investigated as potential platforms for the advancement of innovative electrochemical sensors (see Scheme 1). This collection encompasses, among others, graphene functionalized with carboxylic groups (referred to as graphene acid; GA), graphene enriched with nitrogen (referred to as GN3 or NGA), a graphene system incorporating iron (referred to as GA-Fe), a graphene derivative terminated with alkynes, and carbon dots extensively functionalized with carboxylic groups.

Scheme 1 a) Graphene derivatives prepared via fluorographene chemistry b) Sensor modified with cyanographene as a potential candidate for pesticide detection. c) Schematic illustration of possible electrochemical detection of heavy metals using graphene acid functionalized SPCE.

A comprehensive characterization of all synthesized carbon-based materials was conducted using cutting-edge experimental techniques, including for instance HRTEM, TEM, SEM/FIB, AFM, XPS, and Raman spectroscopy. Additionally, computational chemistry played a vital role in elucidating diverse binding mechanisms, such as those involving antibiotics, and in explaining the quenching of photoluminescence by Hg^{2+} ions.

The research centred around carbon-based systems prepared through fluorographene chemistry has been disseminated through journals like Small (Wiley) and Advanced Optical Materials (ACS). Furthermore, these findings are slated for presentation at prominent international conferences, including nanoBalkan 2023 and The European Conference on Computational and Theoretical Chemistry.

Application of graphene derivatives in determination of pesticides
Collaborating with ESR from Albania, our research has revealed the promise of graphene derivatives, including GA, GN3, GCN, and GA-Fe, as viable platforms for detecting the pesticide

paraquat (its structure is illustrated in Figure 1a). Specifically, we have observed that the reduction peak of paraquat can be discerned within the cathodic region at around –0.5 V, as illustrated in Figure 1b. Given that the utilization of diverse graphene-based materials has the capacity to influence the reduction potential of paraquat, it becomes evident that there exists significant potential to construct a multiplex system suitable for detecting various pesticides, such as imidacloprid, thiamethoxam, and others.
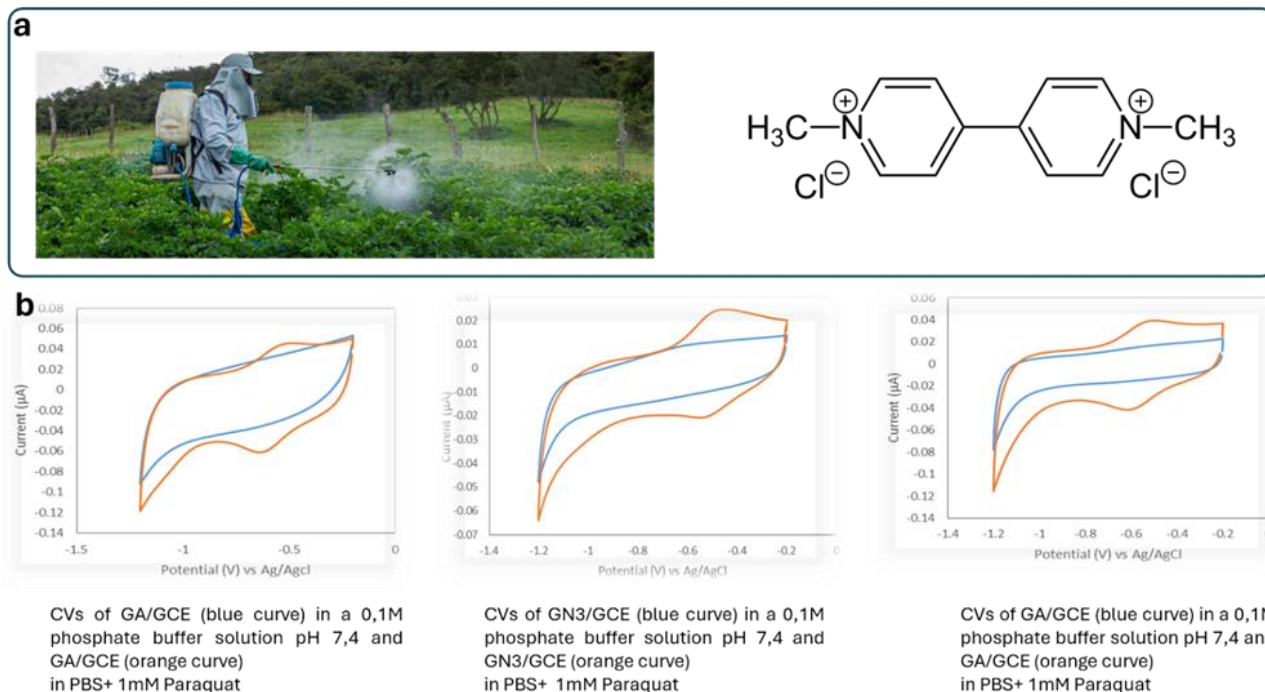

CVs of GA/GCE (blue curve) in a 0,1M phosphate buffer solution pH 7,4 and GA/GCE (orange curve) in PBS+ 1mM Paraquat

CVs of GN3/GCE (blue curve) in a 0,1M phosphate buffer solution pH 7,4 and GN3/GCE (orange curve) in PBS+ 1mM Paraquat

CVs of GA/GCE (blue curve) in a 0,1M phosphate buffer solution pH 7,4 and GA/GCE (orange curve) in PBS+ 1mM Paraquat

Figure 1. a) Chemical structure of paraquat molecule. b) cyclic voltammetry response of GCE modified with GA, GN3 and GCN in 0.1 M PBS buffer solution containing 1 mM paraquat.

Aptasensor suitable for the determination of antibiotic Ampicillin
Employing the principles of fluorographene chemistry, our investigation has unveiled an innovative graphene derivative featuring alkyne termination (referred to as GA-NH-YN, depicted in Figure 2). This derivative has exhibited a remarkable propensity for facile conjugation with biorecognition units through the application of click chemistry. We have effectively demonstrated the straightforward CuAAC conjugation with a DNA aptamer modified with a redox probe, enabling selective binding to the ampicillin antibiotic.

The resulting electrochemical aptasensor, as illustrated in our findings, has demonstrated exceptional selectivity and sensitivity towards ampicillin, even in intricate samples like tap water, saliva, and milk. GA-NH-YN, the developed graphene derivative, offers a ready-to-use platform for click-chemistry-mediated linkage with commercially available biorecognition units modified with azide. This versatility serves as a good start for the creation of a wide array of biosensors.

Moreover, GA-NH-YN has the added advantage of enabling the production of flexible printed electrodes on disposable substrates, facilitating the creation of cost-effective, point-of-care electrochemical sensors operable through mobile devices. Notably, these attributes coalesce with its remarkable selectivity, even in the presence of structurally different antibiotics, and an extended shelf-life.
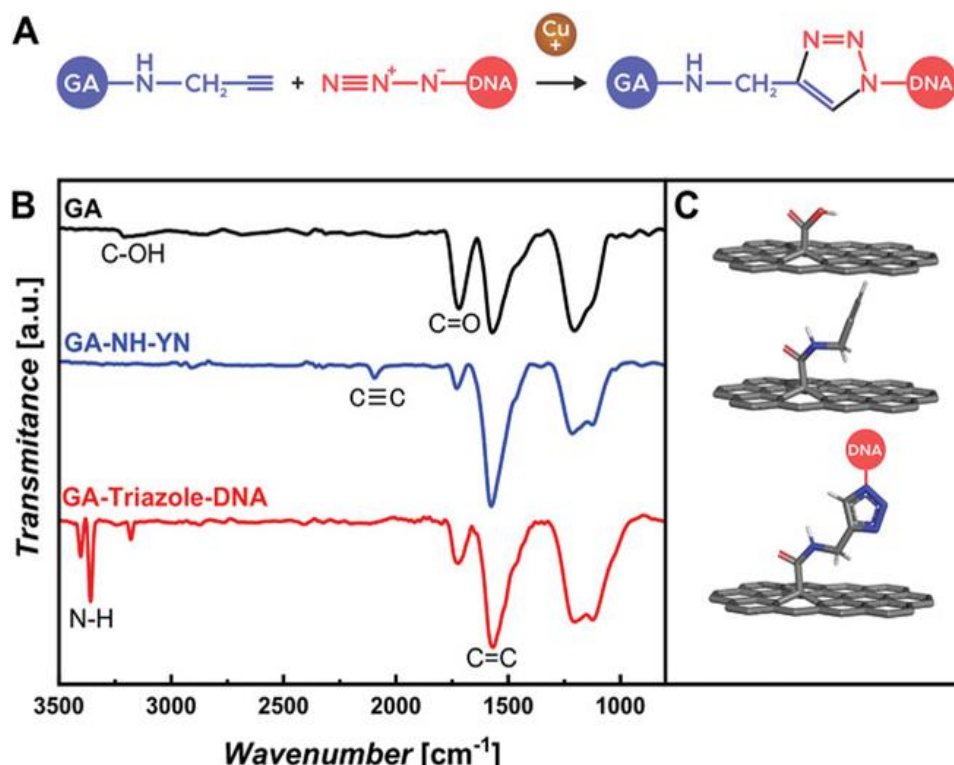
Figure 2. a) Illustration of the click chemistry interaction between GA-NH-YN and a DNA aptamer containing an azide group. b) FTIR spectra, and c) Proposed configurations of the initial nanomaterial (graphene acid – GA), subsequent conjugation with propargylamine (GA-NH-YN), and the click chemistry reaction with the aptamer (GA-Triazole-DNA).

Ultrasensitive and ultra selective photoluminescence determination of inorganic mercury
This study presents the utilization of carboxyl-enriched surface carbon dots (CDs) for the detection of Hg^{2+} in natural water. Notably, the photoluminescence (PL) lifetime of these CDs exhibits a dependence on the concentration of Hg^{2+} (as depicted in Figure 3). Through precise PL lifetime measurements, the determination of exceedingly low levels of Hg^{2+} in water was achieved. Of particular significance is the resistance of this approach to influences from other physical parameters, including pH, ionic strength, and variations in excitation source intensity.

Nanosensors based on CD photoluminescence lifetime have demonstrated both remarkable sensitivity in detecting Hg^{2+} at sub-parts per billion (ppb) levels, with a limit of detection (LOD) of 0.7 ppb, and remarkable selectivity toward Hg^{2+}. To delve into the specificity of CD-PL-lifetime-based nanosensors, we employed light-induced electron paramagnetic resonance (LEPR) spectroscopy. This elucidated the interaction of the photoexcited state of CDs with various heavy metal ions, such as Hg^{2+}, Cd^{2+}, and Pb^{2+}, revealing a pronounced electronic perturbation upon Hg^{2+} binding.

Complementing the experimental findings, computational analysis utilizing molecular dynamics (MD) simulations and time-dependent density functional theory (TD-DFT) calculations confirmed the observed outcomes. The unique sensitivity of CD photoluminescence to Hg^{2+} ions stems from their distinctive electronic structure, specifically involving a lower-lying charge-transfer state connected to a relativistically stabilized $6s$ orbital in Hg^{2+}.
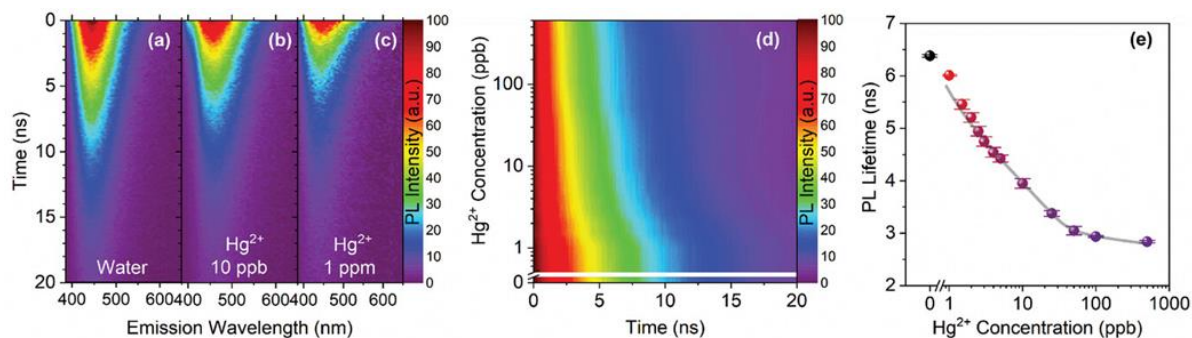
Figure 3. Time-resolved Hg^{2+} concentration-dependent PL emission of CDs. Time-resolved PL emission colour maps of CDs in a) pure water, b) Hg^{2+} solution, 10 ppb, c) Hg^{2+} solution, 1 ppm. d) Colour map of normalized time-resolved PL intensity at the PL emission maximum (λ_{em} = 445 nm) versus Hg^{2+} concentration. e) Extracted PL lifetimes of CDs plotted as a function of Hg^{2+} concentration (experimental data are given as symbols, whereas the line is the fit by Equation 1; error bars represent the standard deviation from three measurements).

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

Summary:

By employing the techniques of fluorographene chemistry, we have successfully prepared a variety of carbon-based derivatives (including graphene derivatives and carbon dots) that have been modified with diverse functional groups. These tailored derivatives were then investigated as potential platforms for the advancement of innovative electrochemical sensors (see Scheme 1). This collection encompasses, among others, graphene functionalized with carboxylic groups (referred to as graphene acid; GA), graphene enriched with nitrogen (referred to as GN3 or NGA), a graphene system incorporating iron (referred to as GA-Fe), a graphene derivative terminated with alkynes, and carbon dots extensively functionalized with carboxylic groups.

We have dedicated our efforts to develop techniques for producing nano-thin layers of reduced graphene oxide (rGO) and other nanocomposites. We have successfully utilized a laser engraver to reduce and pattern graphene oxide (GO), resulting in a conductive nanomaterial that can be transferred onto any surface. Recently, we have achieved the instantaneous laser-induced co-reduction of GO and metal cations, enabling the creation of highly exfoliated conductive rGO nanosheets integrated with metallic nanoparticles (MNPs) in a single procedure. These MNPs@rGO electrodes have undergone extensive testing for electroanalytical detection of various molecules including heavy metals. Additionally, we have demonstrated specific detection abilities for significant biomarkers using Au@rGO electrodes by easily attaching thiolated bioreceptors. These biosensors have shown exceptional performance with heightened sensitivity, offering opportunities for the development of innovative sensing devices, especially for flexible electronics and electroanalytical applications.

Fabrication of Laser-Assisted MNPs@rGO electrodes.

Working solutions containing 30 mM gold/Au(III), 15 mM silver/Ag(I), and 30 mM platinum/Pt(II) salts were prepared in MilliQ water and used promptly. To create the M^{n+}@GO film, 0.4 mL of a 10 mg mL-1 GO stock solution was mixed with 5 mL of the respective metal salt solution. The resulting dispersion was stirred for 5 minutes and then filtered onto a polyvinylidene fluoride (PVDF) membrane with a pore size of 0.1 μm and a diameter of 47 mm.

The filtered material was allowed to dry at room temperature for 20 minutes. Once dry, the film was aligned according to the desired pattern and subjected to treatment with a RayJet CO_2 laser engraver using a 7.4 cm focusing lens in the engraving mode. The laser parameters used were a power of 2.10 W and a speed of 1.50 m s-1. A pattern consisting of 17 key-lock-like working electrodes (WE) with a diameter of 3 mm, designed using Corel Draw software, was employed for the laser scribing. See Scheme 1
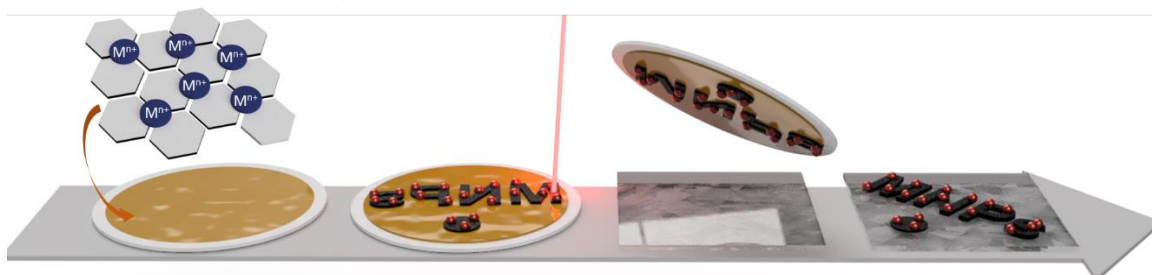
Scheme 1. rGO@MNPs preparation by laser scribing and transferring.

As a control, a GO film was prepared using the same procedure but without the addition of metal sources. For the control, 0.4 mL of a 10 mg mL-1 GO stock solution diluted in 5 mL of MilliQ water was filtered.

In order to explore cost-effective alternatives to CO2 engravers, we have successfully adapted the same methodology for implementation in a diode laser engraver. This device presents a significantly lower price point, amounting to just 5% of the cost of a CO_2 engraver. By incorporating this technology into the University of Tirana, our aim is to enable in-house electrode manufacturing. The diode laser engraver has showcased remarkable advancements in both power and engraving speed, yielding highly satisfactory outcomes See Picture 1. Although the resolution of this equipment falls short of being optimal, the results obtained are akin to those achieved with the renowned RayJet system. Currently, our ongoing efforts involve comprehensive characterization of the electrodes through a variety of techniques including electron microscopy, electrochemical methodologies, and X-ray photoelectron spectroscopy (XPS).

Picture 1 Photographs showing the Rayjet CO_2 laser engraver, the adapted system using a CNC diode laser engraver with an open source software and a picture of an electrode made with this machine.

Heavy metal detection with the MNPs@rGO electrodes.

To evaluate the performance of the MNPs@rGO electrodes for heavy metal detection, we conducted experiments focusing on lead (Pb) as a model analyte. The electrochemical detection parameters were optimized using square wave anodic stripping voltammetry (SWASV), including frequency, potential amplitude, and deposition time.

Figure 2a illustrates the response of the rGO@MNPs and rGO electrodes to a solution containing 2.5 ppm Pb2+ in 0.1 M acetate buffer at pH 4.5. The blue curve represents the Au@rGO electrode, while the red curve represents the -rGO electrode. Notably, the electrodes containing gold nanoparticles (Au@rGO) exhibited a more resolved peak and higher signal intensity compared to the control (-rGO). These results demonstrate the enhanced electroanalytical performance achieved through the integration of metallic nanoparticles with rGO nanosheets.
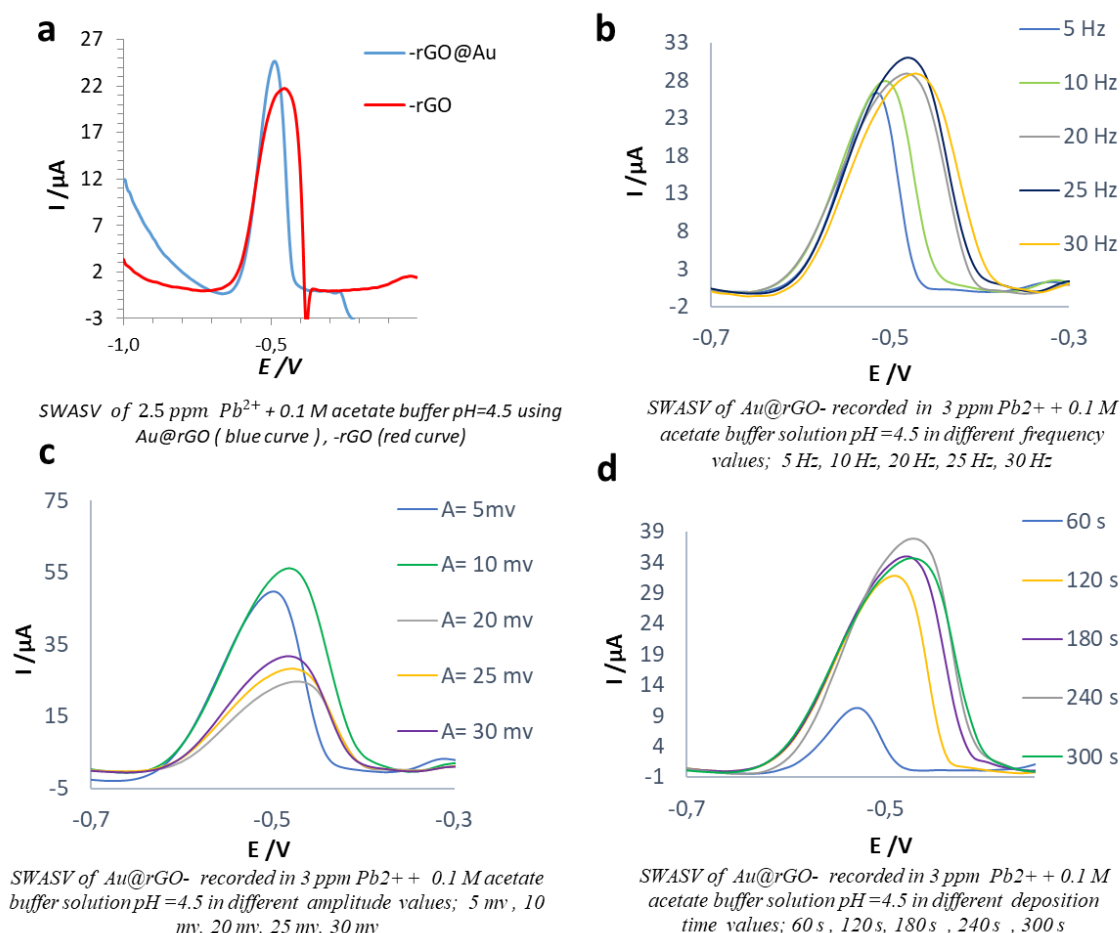
a

SWASV of 2.5 ppm Pb^{2+} + 0.1 M acetate buffer pH=4.5 using Au@rGO (blue curve) , -rGO (red curve)

b

SWASV of Au@rGO- recorded in 3 ppm Pb2+ + 0.1 M acetate buffer solution pH=4.5 in different frequency values; 5 Hz, 10 Hz, 20 Hz, 25 Hz, 30 Hz

c

SWASV of Au@rGO- recorded in 3 ppm Pb2+ + 0.1 M acetate buffer solution pH=4.5 in different amplitude values; 5 mv , 10 mv, 20 mv, 25 mv, 30 mv

d

SWASV of Au@rGO- recorded in 3 ppm Pb2+ + 0.1 M acetate buffer solution pH=4.5 in different deposition time values; 60 s , 120 s, 180 s , 240 s , 300 s

Figure 2. Optimization parameters for Pb^{2+} detection using SWASV.

To further optimize the detection parameters, we varied each parameter in subsequent SWASV experiments to obtain the best signal response. Figure 2b, 2c, and 2d present the effects of varying deposition time, frequency, and potential amplitude, respectively. Through optimization, we determined that using acetate buffer at pH 4.5, the optimum deposition time was 240 seconds, frequency was 25 Hz, and potential amplitude was 10 mV. These optimized parameters significantly improved the sensitivity of the electrodes, enabling the detection of Pb at parts per billion (ppb) levels.

The enhanced performance of the MNPs@rGO electrodes allowed us to extend the linear range of detection from 5 to 30 ppb, as shown in Figure 3. The graph demonstrates a linear relationship between the concentration of Pb and the corresponding electrochemical response, validating the suitability of the MNPs@rGO electrodes for accurate and precise heavy metal detection.
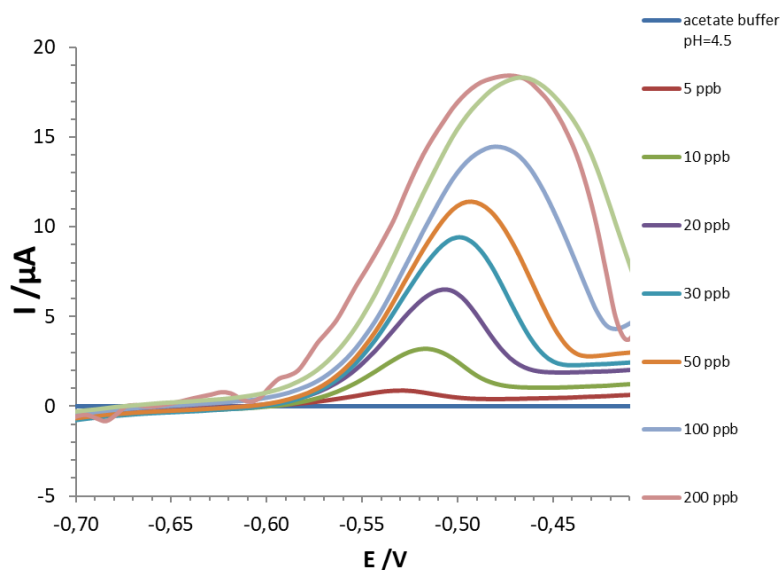
Figure 3. SWASV recorded at different concentration of Pb^{2+} in acetate buffer pH=4.5 using Au@rGO electrodes.

Based on the results obtained with Pb^{2+}, our future steps involve creating calibration curves under the same conditions for other heavy metals such as cadmium (Cd) and mercury (Hg) using the rGO@MNPs electrodes. Additionally, we plan to explore graphene nanocomposites incorporating other metal nanoparticles such as bismuth, silver, platinum, and nickel. By constructing an array of electrodes with different graphene nanocomposites, we aim to resolve peaks resulting from statistical treatments of heavy metal mixtures, ultimately providing precise concentration measurements for each analyte.

To facilitate this process and improve the overall detection capabilities, machine learning methods will be employed to train the system. Gathering a substantial amount of data in a systematic manner will be crucial throughout the project to ensure accurate and reliable detection using the MNPs@rGO electrodes.

It is important to note that the results presented here are a result of collaborative efforts between ICN2 and UT as part of the WP3 program. During the fabrication and characterization of the system, two students from UT stayed at ICN2, contributing to the successful implementation of this research endeavour.

Functionalization of receptors for antibiotics and pesticides detection

ICN2 is supporting UT and UPO by manufacturing similar electrodes for functionalization with aptamers, DNA, etc., to detect antibiotics and pesticides. Antibodies and thiolated aptamers can be easily immobilized on the surface of rGO@AuNPs electrodes, providing an advantage for biosensor fabrication. Successful immobilization of antibodies and ssDNA models has been achieved, and capacitive measurements of different analytes have shown promising results. In the following months, specific aptamers for several antibiotics and pesticides will be tested, and capacitance measurements will be replaced by SWASV for easier measurement.