

Trace level determination of cadmium and lead in coffee (*Coffea*) using gold nanoparticles modified graphene paste electrode

S. Palisoc^{1,2}, J. Leoncini¹ and M. Natividad^{1,2,*}

¹De La Salle University, Condensed Matter Research Laboratory, Physics Department, 2401 Taft Avenue, PH922 Manila, Philippines

²De La Salle University, Condensed Matter Research Unit, CENSER, 2401 Taft Avenue, PH922 Manila, Philippines

*Correspondence: michelle.natividad@dlsu.edu.ph

Abstract. Gold nanoparticles (AuNP) modified graphene paste electrodes (GPE) were fabricated using graphene powder, gold nanoparticles, and mineral oil. The fabricated electrodes were used as working electrode in anodic stripping voltammetry (ASV) for the determination of trace concentrations of cadmium (Cd^{2+}) and lead (Pb^{2+}). The modified GPE was characterized using scanning electron microscopy and cyclic voltammetry. Optimization of the electrode's AuNP content and the ASV parameters was performed. It was determined that the GPE modified with 0.5 mg AuNP obtained the highest anodic current peaks for both Cd^{2+} and Pb^{2+} . The calibration curves obtained using the said electrode showed a linear relationship between heavy metal concentration and peak current and the detection limits were found to be 256 ppb for lead and 267 ppb cadmium. The modified electrode was successful in determining traces of Cd^{2+} and Pb^{2+} in coffee samples. The presence of the heavy metals in the samples were verified using atomic absorption spectroscopy.

Key words: gold nanoparticles, graphene, heavy metals, coffee, voltammetry.

INTRODUCTION

Heavy metals when accumulated in soils and plants pose great risks to human health through direct ingestion or the food chain (soil-plant-human or soil-plant-animal-human) (Wuana & Okieimen, 2011). Any kind of heavy metal is considered to be a contaminant if found where it is unusual or if it has detrimental effects on humans and on the environment. Some metals such iron, cobalt, copper, manganese, molybdenum, and zinc, in trace amounts, are required by the human body (Soetan et al., 2010). They are part of the 'minerals' the 'glow' portion of the food pyramid. Consumed in large amounts, however, they are dangerously toxic and cause serious damage to human health. Heavy metals such as lead, cadmium, mercury, and arsenic have no beneficial effect on the human body. These metals, when accumulated in the human body over time, cause serious damage to the liver and kidneys or even the nervous system (Singh et al., 2011). Furthermore, these metals displace the significant nutritional minerals from their original place and cause to hinder its biological functions (Jaishankar et al., 2014).

Coffee plantations are now adversely affected by environmental pollutants emanating from mining sites near them. Even if the mines are considerably far from the plantations, their effluents are transported by the river systems passing through them. These river systems are the sources of irrigation of the plantations. Through absorption by the root, heavy metals are accumulated in plants (Dalcorsio et al., 2013). These metals are transported via the xylem and distributed to the above-ground sink tissues. However, the absorption and distribution of heavy metals in plants vary depending on the property of the species. A study was conducted to investigate the effects of cadmium (Cd), zinc (Zn) and nickel (Ni) on adult coffee plants (Tezotto et al., 2012). The result showed that after three months, the heavy metals penetrated the soil within the first 50 cm depth. This is the region where the concentration of the coffee plant roots is found. The leaf is not obviously affected but the Zn level in branches are very high. In the same study, it was found that coffee plants are highly tolerant to the metals and high doses of metals transport only little amount to the beans that are what humans are consuming.

Due to the possible adverse effects of heavy metals on human health through consumption of contaminated food and beverages, it is necessary to determine their heavy metal content. One of the methods used for heavy metal detection is anodic stripping voltammetry (ASV) which is a cost-effective method as opposed to spectroscopic methods. In ASV, the choice of the working electrode material is crucial for the success of the detection process. In recent years, modified carbon paste electrodes (MCPE) have been used as the working electrode in voltammetry due to its simplicity, versatility, low cost and easy construction (Almeida & Giannetti, 2002; Vytras et al., 2009; Afkhami et al., 2013; Zaidi, 2013; Kalambat & Srivastava, 2015; Chen et al., 2016; Saleh et al., 2018). Carbon-based nanomaterials such as graphene have been used in the construction of MCPEs due to their excellent conductivity which makes them ideal for sensor applications (Parvin, 2011; Gutierrez et al., 2014; Palisoc et al., 2018a, b). They possess unique physical and chemical properties such as their large surface area, high mechanical strength, and excellent thermal & electrical conductivity (Palisoc et al., 2016; Palisoc et al., 2019). Gold nanoparticles (AuNPs) are used as electrode modifier due to its good conductivity and large surface area. These nanoparticles show excellent sensitivity and selectivity in detecting trace heavy metals (Palisoc et al., 2017a, b).

In this study, a novel gold nanoparticle modified graphene paste electrode (AuNP/GPE) was fabricated, optimized, and used to detect heavy metals in coffee (*Coffea*) via anodic stripping voltammetry.

MATERIALS AND METHODS

Glassware and equipment

A BOSCHE SAE200 electronic balance (BOSCH-Wagesysteme GmbH, Hungingen, Germany) was used in measuring the amounts of graphene and AuNPs. All glassware was cleaned using a BANDELIN SONOREX ultrasonic bath (BANDELIN electronic GmbH & Co. KG, Berlin, Germany). A BST8 potentiostat/galvanostat was used in the cyclic voltammetry and anodic stripping voltammetry analyses. A JEOL 5300 scanning electron microscope was used to characterize the morphology of the fabricated electrodes.

Chemicals, reagents, and real samples

Graphene nanopowder (multilayer graphene; average flake thickness: 60 nm) was purchased from Graphene Supermarket (Calverton, NY, USA). Gold nanopowder (< 100 nm particle size), sodium chloride, lead chloride, cadmium chloride, copper chloride, mineral oil, and nitric acid were purchased from Sigma, Aldrich (Sigma-Aldrich Pte Ltd, Singapore). Organic Arabica coffee beans, coffee leaves, stems and soil in which the coffee was grown were used for real sample analysis.

Fabrication of bare and AuNP-modified GPEs

The graphene paste mixture was prepared by mixing 0.80 μL of mineral oil, 0.21 g of graphene powder and varying amounts of AuNP for 30 min using agate mortar and pestle. The resulting mixture was packed in a Teflon syringe with a diameter of 6mm. The tip of the electrode was polished with wet filter paper and a copper wire was attached to it with silver paste for the ohmic contact.

Mineral Oil and AuNP amount optimization

The amount of AuNP was varied and the amount that yields the highest anodic current peak was determined. The amount of AuNP are the following: 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 1 mg, 1.5 mg, and 2.0 mg. The amount of graphene and mineral oil were held constant at 0.21 g and 0.80 μL , respectively. The optimized electrode was used to simultaneously detect trace amounts of cadmium and lead.

Cyclic voltammetry and Anodic stripping voltammetry

Cyclic voltammetry (CV) and ASV measurements were done using a BST8 potentiostat/galvanostat. The working electrode, which is the fabricated AuNP/GPE, was placed together with the silver/silver chloride reference electrode and platinum counter electrode in the voltammetric cell. Sodium chloride of mass 0.5844 g was dissolved in 100 mL of deionized water to make the electrolyte solution.

Real Sample Analysis

Chopped coffee leaves and stalks, and ground coffee beans were dried in a furnace. One gram of each sample was weighed and carbonized on a hot plate and then transferred to the furnace with a temperature of 500 $^{\circ}\text{C}$ to dry ash the sample. The ashes were dissolved in nitric acid and placed back on the hot plate for the acid to evaporate. The sample was then transferred to an electrolyte solution for ASV and atomic absorption spectroscopy (AAS) analyses to simultaneously detect Pb^{2+} and Cd^{2+} .

Atomic Absorption Spectrometry

The presence of cadmium and lead in the real samples was verified by flame atomic absorption spectroscopy (AAS) using a Shimadzu (Japan) AA-6300 atomic absorption spectrophotometer. Standard solutions of lead and cadmium were used to obtain the calibration curve. Three trials were done in the AAS analysis of the real samples.

RESULTS AND DISCUSSION

Optimization of electrode modifier

The determination of the best-modified electrode was done by varying the amounts of AuNP while the graphene powder (0.21 g) and mineral oil (0.80 μL) were held constant. Eight electrodes were prepared with different amounts of AuNP from 0.1 mg to 2.0 mg. The resulting electrodes were then used to detect 10 ppm each of Cd^{2+} and Pb^{2+} . The bar graph in Fig. 1 shows that 0.5 mg AuNP attained the highest peak current for both Cd^{2+} and Pb^{2+} . Therefore, this was considered as the optimum AuNP content in this study.

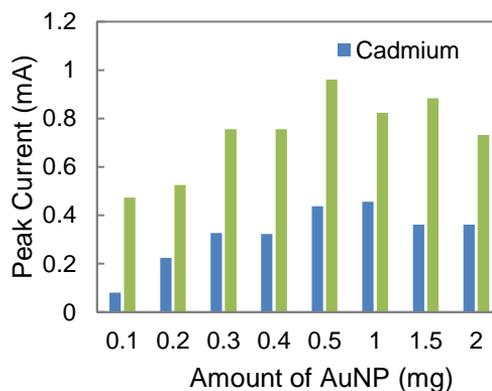


Figure 1. Comparison of peak currents of Cd^{2+} and Pb^{2+} using AuNP/GPE with varying AuNP content.

Characterization of the fabricated electrodes

Cyclic voltammetry was used to characterize the electrochemical response of the fabricated electrodes. Fig. 2 shows the cyclic voltammograms of the bare GPE for 10 scans. The graph shows that the bare electrode is conducting but there are no peaks. In Fig. 3, the cyclic voltammograms obtained from the AuNP-modified GPE show that an anodic peak is present but it is not reversible. This indicates that the AuNP modified GPE is more conductive as compared to the bare GPE.

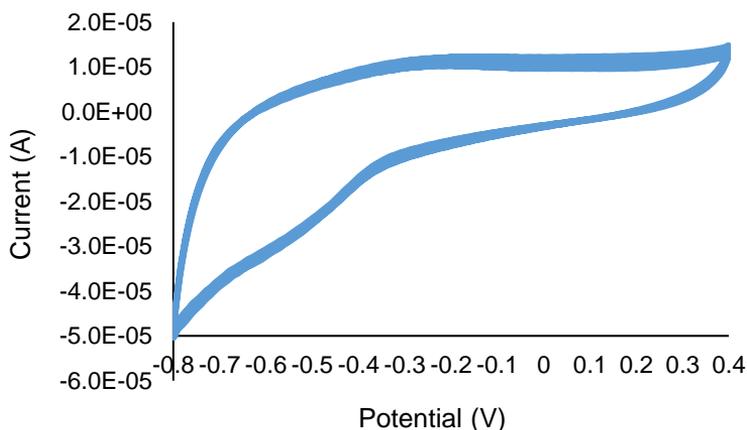


Figure 2. Cyclic voltammograms of the bare GPE for 10 scans.

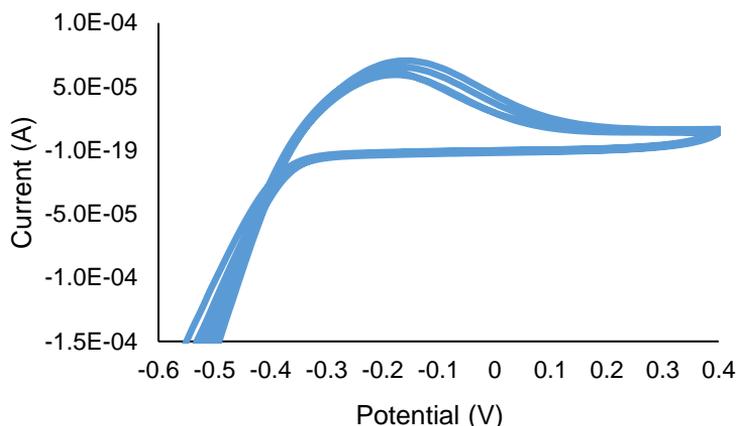


Figure 3. Cyclic voltammograms of the AuNP-modified GPE for 10 scans.

Fig. 4 shows the SEM image of the bare GPE at 20,000x magnification. The flakes reveal the presence of graphene in the electrode. Fig. 5 shows the SEM image of the AuNP-modified GPE. The granular microstructures represent the gold nanoparticles that adhered to the graphene surfaces. The gold nanoparticles can be seen as white cloudy spherical shaped particles based on the SEM image.

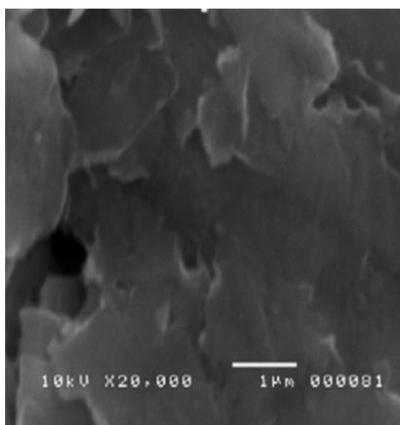


Figure 4. SEM image of bare GPE.

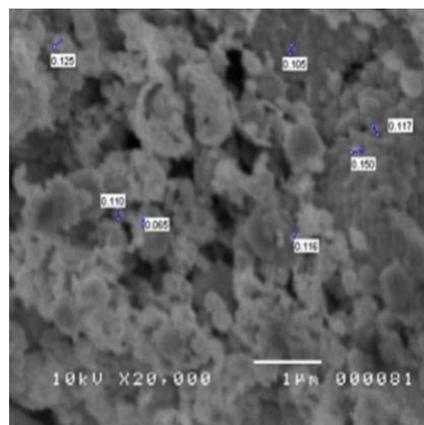


Figure 5. SEM image of AuNP-GPE.

Optimization of ASV Parameters

To obtain the highest peak current for the optimized electrode, different ASV parameters such as accumulation time, accumulation potential, deposition time, deposition potential and scan rate were optimized. The optimization of ASV parameters used the best-modified electrode to detect 10 ppm each of Cd^{2+} and Pb^{2+} in the electrolyte solution. Results were obtained by observing the relationship between the parameters and the peak current.

Accumulation Time

The accumulation time was set from 30 seconds to 180 seconds with increments of 30 seconds. A time with less than 30 s increment e.g. 15 s would be inefficient whereas a time increment greater than 30 e.g. 60 s would result in bigger gaps of data points and may affect the smoothness of the voltammogram. Fig. 6 shows that 60 s as the optimum accumulation time.

Deposition Time

To determine the optimum deposition time, the accumulation potential was set to -0.80V and accumulation time to 60s and were held constant. The deposition time was varied from 30s to 150s with 30s interval. From Fig. 7, it can be observed that the peak current is highest at 90s.

Deposition Potential

The deposition potential was varied from -0.8V to 1.0V. It can be seen from the bar graph in Fig. 8 that the optimum deposition potential is 0.8V for Cd and -0.85V for Pb.

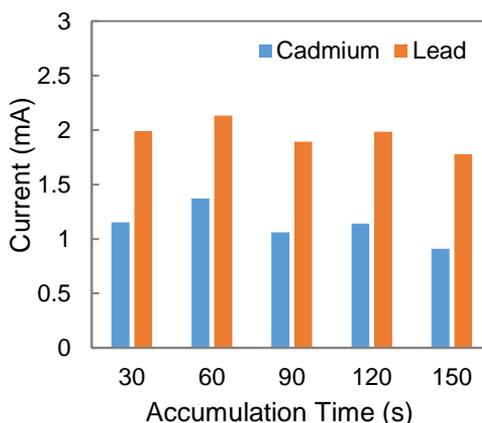


Figure 6. Optimization of accumulation time.

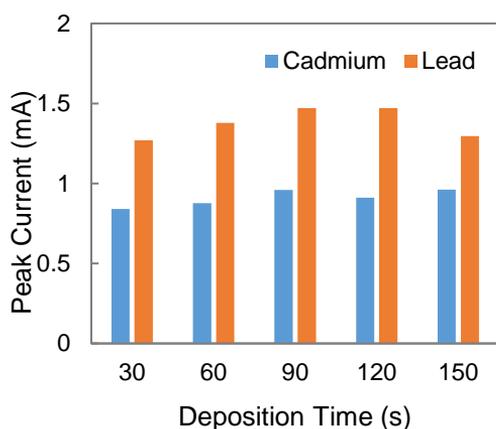


Figure 7. Optimization of deposition time.

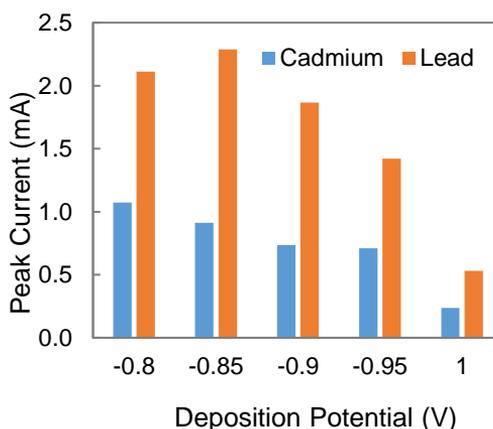


Figure 8. Bar graphs of optimization of deposition potential.

Calibration Curve

The calibration curves of the best electrode were obtained by varying the concentration of cadmium and lead from 750 ppb to 1,000 ppb. Figs 9 to 11 show the voltammograms and the obtained calibration curves for Cd^{2+} and Pb^{2+} . It can be seen from the figure that the peak current increased as the heavy metal concentration

increased which means that more heavy metals accumulate on the surface of the electrode. The Pearson correlation coefficient (R^2) for both heavy metals are close to 1 indicating that there is a strong linear relationship between the peak current and heavy metal concentration.

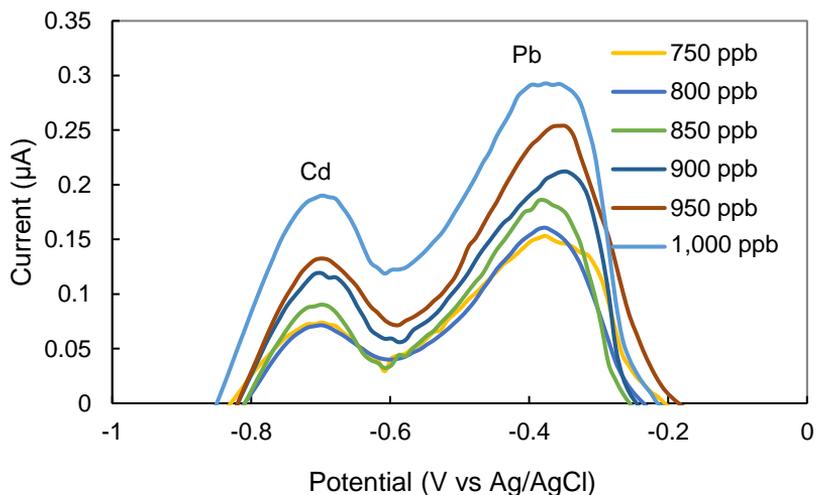


Figure 9. Anodic stripping voltammograms for various concentrations of Pb and Cd.

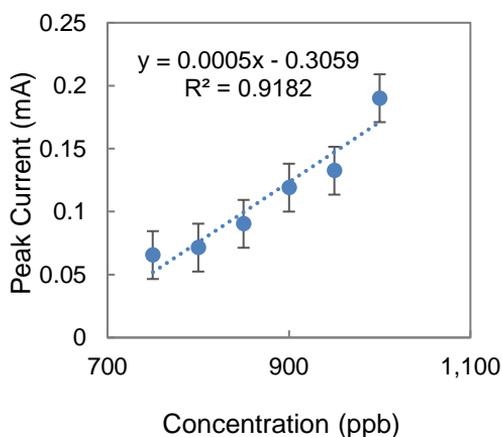


Figure 10. Calibration curve for cadmium.

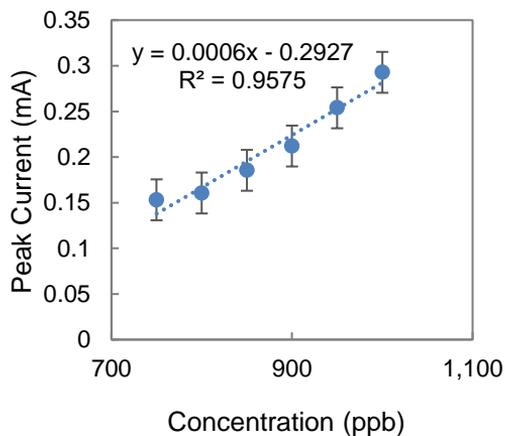


Figure 11. Calibration curve for lead.

Figures of Merit

The limit of detection (LOD) of the fabricated AuNP-GPE was found to be 267 ppb for cadmium and 256 ppb for lead while the limit of quantitation (LOQ) is 810 ppb for cadmium and 776 ppb for lead. The performance comparison of the fabricated electrode with previous works Table 1.

Table 1. Performance comparison of the fabricated electrode with other works

Electrode	Modifier	LOD	Method	Reference
ITO	[Ru(NH ₃) ₆] ³⁺ /Nafion	Pb & Cd – 500 ppb	ASV	Palisoc et al., 2015
GCE	AuNP/[Ru(NH ₃) ₆] ³⁺ /Nafion	Pb – 45 ppb Cd – 200 ppb	ASV	Palisoc et al., 2017a
GCE	Chitosan/carbon nanotubes	Pb – 600 ppb Cd – 800 ppb	SWASV	Wu et al., 2017
CPE	AgNP	Pb – 111 ppb Cd – 183 ppb	ASV	Palisoc et al., 2018b
GPE	AuNP	Pb – 256 ppb Cd – 267 ppb	ASV	this work

ITO – Indium Tin Oxide; GCE – Glassy Carbon Electrode; CPE – Carbon Paste Electrode.

Real Sample Analysis

A total of twenty (20) samples from five (5) different farms were obtained. The selection of the samples was composed of soil, stalks, leaves, and beans (raw). Each sample underwent dry ashing and acid digestion and was subjected to ASV and AAS analyses. The ASV results of the 20 samples are summarized in Table 2. The corresponding bar graph is shown in Fig. 12. It can be seen from Table 2 and Fig. 12 that Cd²⁺ was detected in all the samples while Pb²⁺ was not detected in some samples. The concentration of Pb²⁺ in said samples was probably below the detection limit of the electrode. Table 3 and the corresponding bar graph in Fig. 13 show the results obtained from AAS. It can be observed that Cd²⁺ was present in all the samples verifying the ASV result. Lead was also not detected in some samples. The discrepancies in the ASV and AAS results can be attributed to the different LOD and sensitivities of the two methods.

Table 2. Results of Real Sample Analysis via ASV

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Soil					
Cd (in ppb)	1978.33	1953.33	630	686.67	1,400
Pb (in ppb)	ND	ND	607.43	ND	910
Stalk					
Cd (in ppb)	970	711.67	785	815.83	1,400
Pb (in ppb)	ND	633.71	741.43	ND	910
Leaves					
Cd (in ppb)	711.67	715	800	785	719.83
Pb (in ppb)	ND	690	ND	738.57	ND
Beans					
Cd (in ppb)	677	691.66	810	708.33	675.83
Pb (in ppb)	ND	ND	ND	ND	ND

ND – not detected.

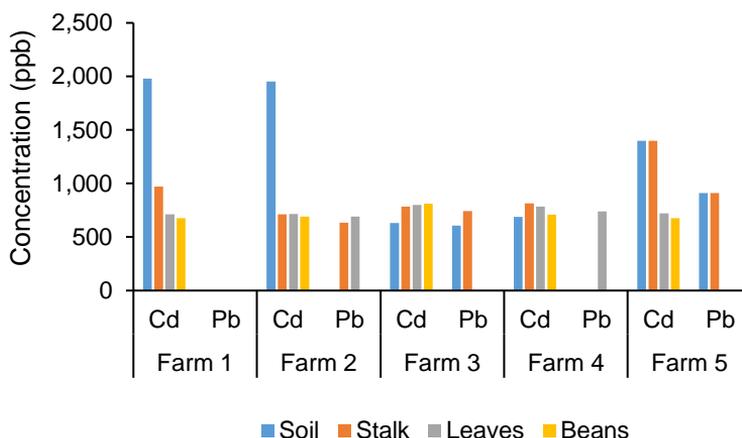


Figure 12. Comparison of Cd and Pb concentrations in the real samples measured using ASV.

Table 3. Results from Real Sample Analysis via AAS

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Soil					
Cd (in ppb)	142	111.67	111.83	107	113.33
Pb (in ppb)	792.5	337.5	1975	292.5	350
Stalk					
Cd (in ppb)	108.67	105.5	105.83	109.33	107.5
Pb (in ppb)	100	6.25	ND	227.5	55
Leaves					
Cd (in ppb)	110	116.33	110.33	113.67	110.67
Pb (in ppb)	82.5	147.5	47.5	92.5	17.5
Beans					
Cd (in ppb)	115.83	105.5	152.83	104.83	107.83
Pb (in ppb)	35	2.5	35	ND	17.5

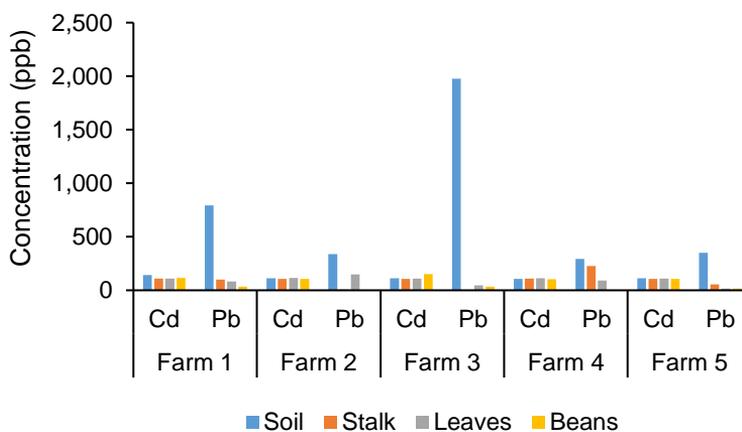


Figure 13. Comparison of Cd and Pb concentrations in the real samples measured using ASV.

The maximum level of heavy metal intake according to the World Health Organization is 10 ppb for lead and 3 ppb for cadmium (WHO, 2008). Based on the results from both AAS and ASV, it can be concluded that most of the samples are toxic. It is probable that the metals that were detected in the samples came from the mine tailings from the mining operations that have accumulated through the soil and nearby waters.

CONCLUSIONS

Gold nanoparticles modified graphene paste electrodes were fabricated and were used for the simultaneous detection of lead and cadmium via anodic stripping voltammetry. The amount of gold nanoparticles was varied while the amounts of graphene and mineral oil were kept constant. The AuNP content that obtained the highest peak current for both Cd^{2+} and Pb^{2+} was the 0.5 mg. Thus, this was chosen as the best-modified electrode. Anodic stripping voltammetry parameters were optimized to improve the performance of the best modified electrode. Under optimum conditions, the calibration curves obtained for both Cd^{2+} and Pb^{2+} showed a linear relationship between the peak current and heavy metal concentration and the limit of detection is 256 ppb for lead and 267 ppb cadmium. The AuNP-GPE was successful in detecting trace metals in coffee and soil samples. The presence of Cd^{2+} and Pb^{2+} in the real samples was verified by AAS analysis.

REFERENCES

- Afkhami, A., Soltani-Felehgari, F. & Madrakian, T. 2013. Gold nanoparticles modified carbon paste electrode as an efficient electrochemical sensor for rapid and sensitive determination of cefixime in urine and pharmaceutical samples. *Electrochimica Acta* **103**, 125–133.
- Almeida, C.M.V.B. & Giannetti, B.F. 2002. A new and practical carbon paste electrode for insoluble and ground samples. *Electrochemistry Communications* **4**, 985–988.
- Chen, G., Hao, X., Li, B.L. & Li, N.B. 2016. Anodic stripping voltammetric measurement of trace cadmium at antimony film modified sodium montmorillonite doped carbon paste electrode. *Sensors and Actuators B: Chemical* **237**, 570–574.
- Dalcorso, G., Manara, A. & Furini, A. 2013. An overview of heavy metal challenge in plants: from roots to shoots. *Metallomics* **5**, 1117–1132.
- Gutierrez, F., Comba, F., Gasnier, A., Gutierrez, A., Galicia, L., Parrado, C, Rubianes, M. & Rivas, G. 2014. Graphene Paste Electrode: Analytical Applications for the Quantification of Dopamine, Phenolic Compounds and Ethanol. *Electroanalysis* **26**, 1–9.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. & Beeregowda, K. 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology* **7**, 60–72.
- Kalambat, P., Biradar, M., Karna, S. & Srivastava, A. 2015. Adsorptive stripping differential pulse voltammetry determination of rivastigimine at graphene nanosheet-gold nanoparticle/carbon paste electrode. *Journal of Electroanalytical Chemistry* **757**, 150–158.
- Palisoc, S., Bentulan, J.M. & Natividad, M. 2019. Determination of trace heavy metals in canned food using Graphene/AuNPs/[$\text{Ru}(\text{NH}_3)_6$] $^{3+}$ /Nafion modified glassy carbon electrodes. *Journal of Food Measurement and Characterization* **13**, 169–176.
- Palisoc, S., Estioko, L.C. & Natividad, M. 2018a. Voltammetric determination of lead and cadmium in vegetables by graphene paste electrode modified with activated carbon from coconut husk. *Materials Research Express* **5**, 1–10.

- Palisoc, S., Lee, E., Natividad, M. & Racines, L. 2018b. Silver Nanoparticle Modified Graphene Paste Electrode for the Electrochemical Detection of Lead, Cadmium and Copper. *International Journal of Electrochemical Science* **13**, 8854–8866.
- Palisoc, S., Causing, A.M. & Natividad, M. 2017a. Gold nanoparticle/hexaammineruthenium/Nafion® modified glassy carbon electrodes for trace heavy metal detection in commercial hair dyes. *Analytical Methods* **9**, 4240–4246.
- Palisoc, S., Valeza, N. & Natividad, M. 2017b. Fabrication of an effective gold nanoparticle/graphene/Nafion® modified glassy carbon electrode for high sensitive detection of trace Cd²⁺, Pb²⁺ and Cu²⁺ in tobacco and tobacco products. *International Journal of Electrochemical Science* **12**, 3859–3872.
- Palisoc, S., Natividad, M., Calde, D.M. & Rosopa, E.R. 2016. Trace Determination of Lead and Cadmium using Graphene/[Ru(NH₃)₆]³⁺/Nafion Modified Glassy Carbon Electrodes. *Journal of New Materials for Electrochemical Systems* **19**, 223–228.
- Palisoc, S., Natividad, M., Martinez, N., Ramos, R. & Kaw, K. 2015. Fabrication and electrochemical study of [Ru(NH₃)₆]³⁺/Nafion modified electrodes for the determination of trace amounts of Pb²⁺, Cd²⁺, and Zn²⁺ via anodic stripping voltammetry. *e-Polymers* **16**, 117–123.
- Parvin, M.H. 2011. Graphene paste electrode for detection of chlorpromazine. *Electrochemistry Communications* **13**, 366–369.
- Saleh, T., AlAqad, K. & Rahim, A. 2018. Electrochemical sensor for the determination of ketoconazole based on gold nanoparticles modified carbon paste electrode. *Journal of Molecular Liquids* **256**, 39–48.
- Singh, R., Gautam, N., Mishra, A. & Gupta, R. 2011. Heavy metals and living systems: An overview. *Indian Journal of Pharmacology* **43**, 246–253.
- Soetan, K., Olaiya, C. & Oyewole, O. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science* **4**, 200–222.
- Tezotto, T., Favarin, J.L., Azevedo, R.A., Alleoni, L.R.F. & Mazzafera, P. 2012. Coffee is highly tolerant to cadmium, nickel and zinc: Plant and soil nutritional status, metal distribution and bean yield. *Field Crops Research* **125**, 25–34.
- Vytras, K., Svancara, I. & Metalika, R. 2009. Carbon paste electrodes in electroanalytical chemistry. *Journal of the Serbian Chemical Society* **74**, 1021–1033.
- WHO. 2008. *Guidelines for drinking-water quality*. 3rd edition incorporating 1st and 2nd addenda. Vol. **1**. Recommendations. Geneva, World Health Organization, pp. 392–394.
- Wu, K., Lo, H., Wang, J., Yu, S. & Yan, B. 2017. Electrochemical detection of heavy metal pollutant using crosslinked chitosan/carbon nanotubes thin film electrodes. *Materials Express* **7**, 15–24.
- Wuana, R. & Okieimen, F. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology* **2011**, 1–20.
- Zaidi, S.A. 2013. Graphene: A comprehensive review on its utilization in carbon paste electrodes for improved sensor performance. *International Journal of Electrochemical Science* **8**, 11337–11355.