

Heavy Metals in Philippine Rice (*Oryza Sativa*) using Nafion- $[\text{Ru}(\text{bpy})_3]^{2+}$ -Gold Nanoparticles Modified Glassy Carbon Electrodes

S. Palisoc^{1,2}, C. Canquin¹ and M. Natividad^{1,2,*}

¹Condensed Matter Physics Laboratory, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines

²Condensed Matter Research Unit, CENSER, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines

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Abstract: Nafion- $\text{Ru}(\text{bpy})_3^{2+}$ -Gold Nanoparticles (AuNPs) modified glassy carbon electrodes were fabricated and were used in detecting heavy metals in Philippine rice samples by differential pulse voltammetry (DPV). The said electrodes were fabricated via the drop coating technique. The concentrations of the modifiers, $\text{Ru}(\text{bpy})_3^{2+}$ and AuNPs, were varied to determine the best electrode for detecting lead, cadmium, and copper. Based on DPV results, the electrode with 5 mg $\text{Ru}(\text{bpy})_3^{2+}$ and 3 mg AuNPs was chosen as the best electrode. The calibration curves of the best electrode showed strong correlation values of 0.9984 for lead, 0.9905 for cadmium, and 0.9933 for copper. The limit of detection for lead, cadmium, and copper were 10 mg/L, 10 mg/L, and 200 mg/L, respectively. Atomic absorption spectrometry was used as a cross reference to verify the results from DPV. The lead and cadmium content of all the rice samples exceeded the World Health Organization (WHO) limit that the body can take. Some samples contained copper above the WHO limit.

Keywords: Differential pulse voltammetry, heavy metals, Nafion, ruthenium bipyridyl, gold nanoparticles

1. INTRODUCTION

In almost every dining table in the Philippines, whether it is breakfast, lunch, dinner or snack, rice has always been present. Without rice, a Filipino meal will never be complete. Thus, it is important to study the quality of rice that people get from the farm. Soil contamination is one of the main problems that farmers experience when planting crops. The reason is that heavy metals in the soil are absorbed by crops through their roots. This makes the rice that people eat dangerous to their health. Fan et al. [1] conducted a study to determine lead (Pb), cadmium (Cd), arsenic (As), manganese (Mn), and antimony (Sb) concentrations in soil and brown rice samples from three heavy metal mining areas in Hunan Province, central China, and to assess the potential health risks to local inhabitants. It was found in the study that local soil and brown rice were contaminated with Cd, Pb, Sb, and As. The results also showed that potential noncarcinogenic and carcinogenic health risks exist for local inhabitants.

The health hazards of cadmium, lead and copper are alarming. Cadmium is a toxic metal that can affect the stomach which may lead to intense vomiting and diarrhea or in worst case scenario,

death. It can also affect the kidney and bones if the body is exposed to low levels of cadmium for a long time [2]. Lead can affect the central nervous system and can cause the bones and kidneys to malfunction if high levels are absorbed by the body [3]. Copper when consumed in excessive quantities, leads to different neurological disorders, such as Wilson's disease [4].

Due to the reasons mentioned above, detecting heavy metals in Philippine rice is very important. Many analytical methods of detecting trace heavy metals in a sample are available. These include atomic adsorption spectroscopy, inductively coupled plasma mass spectrometry, and inductively coupled plasma atomic emission spectrometry. Although these methods give accurate results, the complicated preparations of samples, procedures and as well as the very expensive cost are a big down side [5]. In comparison with these analytical methods, electrochemical techniques such as anodic stripping voltammetry (ASV) and differential pulse voltammetry (DPV), are more suitable methods to use in detecting trace heavy metals because of their high sensitivity, low cost, and good reproducibility [6].

For many years, the hanging mercury drop electrode was the most commonly used working electrode in a typical voltammetry experiment. However, this electrode is toxic and has limited applications in analysis. Additionally, the surface area of a drop of mer-

*To whom correspondence should be addressed:
Email: michelle.natividad@dlsu.edu.ph
Phone: +632 5360229

cury is never constant and the applied voltage produces changes in surface tension which makes mercury's drop size change as well [7]. Thus, chemically modified electrodes are now being used as working electrodes in voltammetry instead of mercury based electrodes [8-24]. Chemically modified electrodes are fabricated by coating the electrode surface with a thin film of polymer such as Nafion, incorporated with nanomaterials such as gold nanoparticles (AuNP) and redox active species such Tris (2, 2'-bipyridyl) ruthenium (II) ($\text{Ru}(\text{bpy})_3^{2+}$). The modification can minimize the overpotential required for oxidation and reduction of the metals, increase the electrode's sensitivity and selectivity during the detection of metals, enhance electrochemical properties, and avoid electrode fouling [25].

In this study, glassy carbon electrodes were modified by Nafion, $[\text{Ru}(\text{bpy})_3]^{2+}$ and AuNP. The resulting electrodes were used to detect lead, cadmium and copper in Philippine rice samples.

2. MATERIALS AND METHODS

2.1. Preparation of Nafion[®] - $\text{Ru}(\text{bpy})_3^{2+}$ - AuNPs stock solutions

The coating solutions of Nafion[®] - $\text{Ru}(\text{bpy})_3^{2+}$ - AuNP were prepared by dissolving 1 mg, 2 mg, and 4 mg of the redox modifier $\text{Ru}(\text{bpy})_3^{2+}$ and 1 mg, 2 mg, and 3 mg of AuNPs in 20 mL of reagent grade methanol. With the use of a Transferrpette micropipette, 0.2 ml of 10% Nafion solution was added to the solution. Tris(bipyridyl)ruthenium(II) dichloride and AuNPs were each placed on wax papers and were all weighed using a BOSH SAE200 electronic balance. Lastly, the stock solutions were sonicated using a Bandarex Sonorex for about 2 hours for the $\text{Ru}(\text{bpy})_3^{2+}$ and AuNPs to be completely homogenized.

2.2. Fabrication of the modified glassy carbon electrode (GCE)

The bare glassy carbon electrode was polished using alumina slurry and was washed afterwards with distilled water. The electrode was subsequently ultra-sonicated with the use of BANDELIN SONOREX. The solution in sonicating the electrode consisted of distilled water and ethanol. The sonication process took about 5 minutes.

The drop coating technique was used to fabricate the modified glassy carbon electrode. A Transferrpette[®] micropipette was used to drop the prepared stock solution onto the electrode. The electrode was later on air dried for about 1.5 hours at room temperature before it was used as a working electrode in differential pulse voltammetry.

2.3. Cyclic Voltammetry and Differential Pulse Voltammetry

All measurements for cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were obtained using a BST8-Stat Potentiostat/Galvanostat. An electrochemical cell with a three electrode set up was used in cyclic voltammetry and differential pulse voltammetry. The Nafion[®] - $\text{Ru}(\text{bpy})_3^{2+}$ - AuNPs modified electrode was used as the working electrode, a platinum wire as a counter electrode and a saturated calomel electrode as a reference electrode.

For cyclic voltammetry, the scan rate used was at 0.2 V/s and the potential was set at 1.25 V. All measurements in cyclic voltammetry were scanned 10 times. In differential pulse voltammetry, the

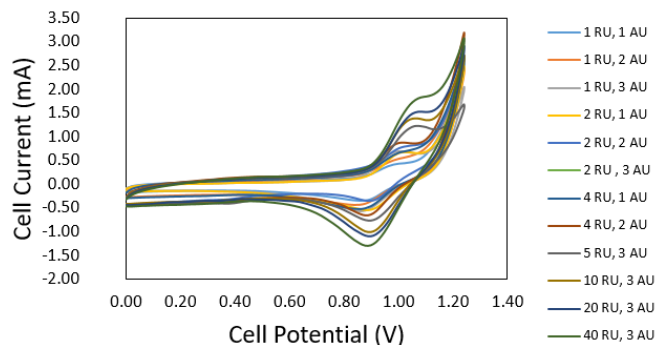


Figure 1. Cyclic Voltammograms of the fabricated electrodes with different modifier concentrations. Supporting electrolyte: 0.1 M NaCl solution; CV parameters: scan rate: scan rate: 0.2 V/s; potential: 1.25 V.

potential was set at -1.0 V, the accumulation time at 150 secs, and the deposition time at 30 secs.

2.4. Preparation of Real Samples

Philippine rice samples were bought from the market and some were obtained from the farm. Before the rice samples were tested, they underwent dry-ashing and acid digestion. With the use of a Thermolyne 48000 Furnace, the rice samples were dry-ashed at 600°C until they were completely decomposed. It took about 12 hours for the rice samples to be completely decomposed. After the rice samples were dry-ashed, the completely decomposed samples were acid digested. Hydrochloric acid (3-5 mL) was added to the decomposed samples which were then heated in a hot plate until the acid evaporated. After evaporation, the samples were added to an electrolyte solution which was later on used for the detection of lead, cadmium and copper by differential pulse voltammetry.

3. RESULTS AND DISCUSSION

3.1. Cyclic Voltammetry Characterization

The cyclic voltammograms of the fabricated electrodes with different amounts of $\text{Ru}(\text{bpy})_3^{2+}$ and AuNPs are shown in Figure 1. As can be observed from the graph, the anodic peak current increased with increasing concentrations of $\text{Ru}(\text{bpy})_3^{2+}$ and AuNPs. This indicates that the conductivity of the electrodes increased as the concentrations of the modifiers increased.

The reversibility or the ratio of the anodic and cathodic peak currents of the modified electrodes was determined from the cyclic voltammograms (Figure 2). The ratios of the anodic peak current to cathodic peak current of all the electrodes are greater than 1 indicating that the anodic peak currents are greater than the cathodic peak currents. The electrode with 1 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 1 mg AuNPs is the most reversible since its reversibility is closest to 1.

In every cyclic voltammetry run, the electrode was scanned 10 times in order to determine its stability. The percentage relative standard deviation (%RSD) of anodic peak currents for the 10 scans are shown in Figure 3. It is evident from the figure that the most stable or the electrode with the lowest %RSD is the one which was modified with 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs. A low %RSD indicates that the multiple scans of the electrode are relatively consistent with each other which signifies better stability of

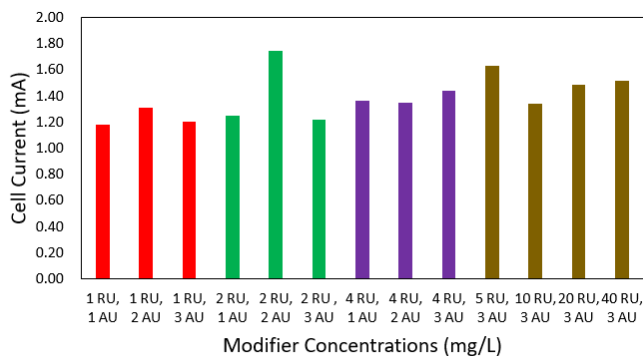


Figure 2. Reversibility of the fabricated electrodes with different modifier concentrations.

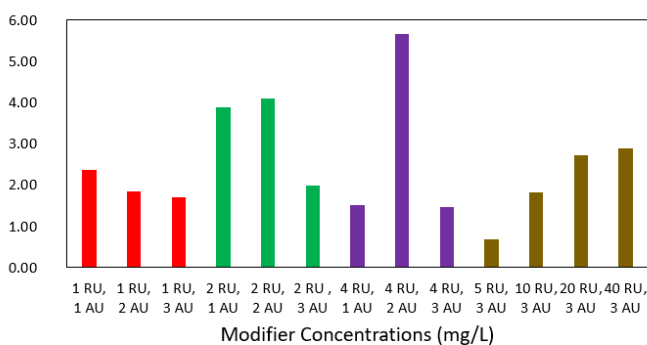


Figure 3. Percentage relative standard deviation of the modified electrodes.

the modified electrode and allows for more precise and more reproducible results [23]. A high %RSD indicates loss of $[\text{Ru}(\text{bpy})_3]^{2+}$ with respect to time.

3.2. Determination of Best Modified Electrode for Lead and Cadmium by Differential Pulse Voltammetry

The determination of the best modified electrode was done by varying the concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$ and AuNPs. The concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$ was varied at 1 mg, 2 mg, and 4 mg and for every concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$, the AuNPs concentration was varied at 1 mg, 2 mg, 3 mg. The fabricated electrodes were then used in detecting 10 mg/L of Cd^{2+} and Pb^{2+} . Figure 4 shows the differential pulse voltammograms of the electrodes with different modifier concentrations. As can be observed in Figure 5, the peak currents increased linearly with increasing AuNPs concentration. Since the peaks constantly increased, additional concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$ were tested until optimization was achieved. The electrode optimized at 4 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs for cadmium. However, the electrode optimized at 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs for lead. Since the peak current still rose at 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs for lead, 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs was the chosen as the best electrode.

3.3. Determination of Best Modified Electrode for Copper by Differential Pulse Voltammetry

Since it was proven already from the previous section that

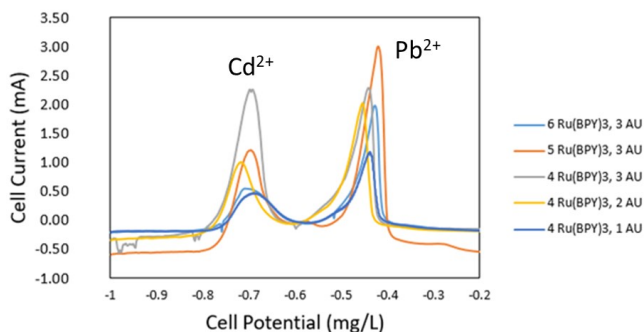
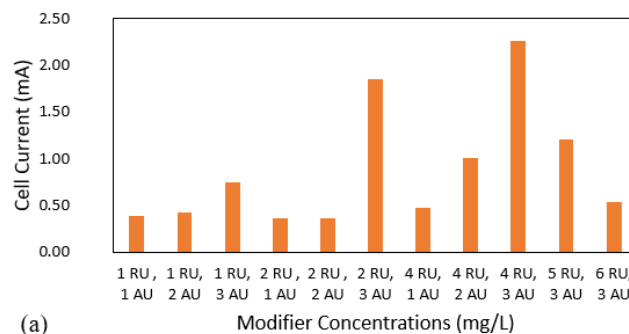
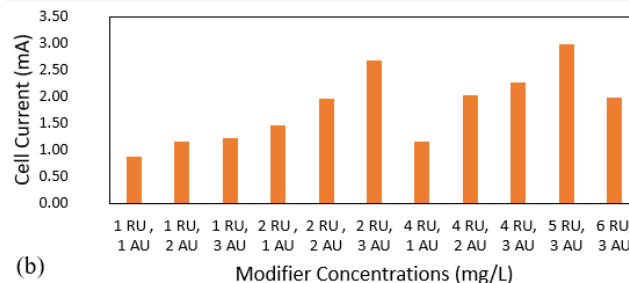


Figure 4. DPV curves of the fabricated electrodes with different modifier concentrations. Supporting electrolyte: 0.1 M NaCl solution, 10 mg/L Cd, 10mg/L Pb; DPV parameters: potential = -1.0 V; accumulation time = 150 s; deposition time = 30 s.



(a)



(b)

Figure 5. (a) Peak current comparison for (a) cadmium and (b) lead using electrodes with varying concentrations of $[\text{Ru}(\text{bpy})_3]^{2+}$ and AuNPs.

AuNPs improve the anodic peak currents by increasing it linearly, only the concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$ was varied for the determination of the best modified electrode for copper. For every 3 mg of AuNPs, the concentration of $[\text{Ru}(\text{bpy})_3]^{2+}$ was varied at 1 mg, 2 mg, 4 mg, 5 mg, and 6 mg. The fabricated electrodes were later on used in detecting 10 mg/L of Cu^{2+} .

Figure 6 shows that the Cu peak currents increased linearly until it optimized at 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs. Thus, the electrode modified with 5 mg $[\text{Ru}(\text{bpy})_3]^{2+}$ and 3 mg AuNPs was considered to be the best electrode for copper. It can be seen in the voltammograms for copper detection, that there were stripping peaks produced at 0.1 V–0.2 V. These peaks are caused by the oxidation of Cu^{2+} [23].

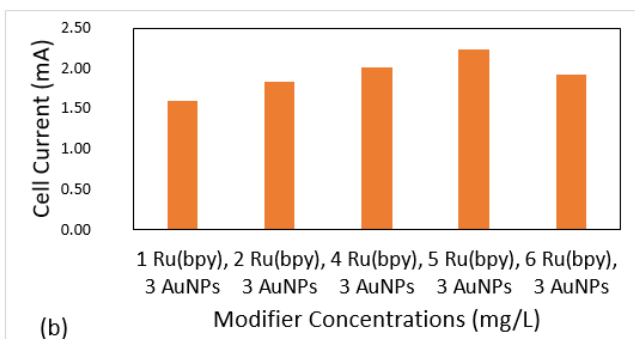
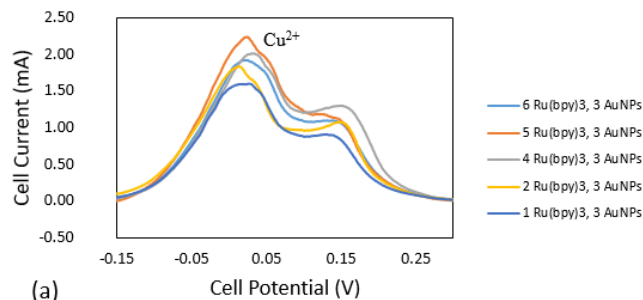


Figure 6. (a) DPV curves for Cu²⁺ using electrodes with varying concentrations of [Ru(bpy)₃]²⁺ and constant AuNPs concentration. Supporting electrolyte: 0.1 M NaCl solution, 10 mg/L Cu; DPV parameters: potential: -1.0 V; accumulation time: 150 s; deposition time: 30 s. (b) Peak current comparison for copper using electrodes with varying concentrations of [Ru(bpy)₃]²⁺ and constant 3 mg AuNPs concentration.

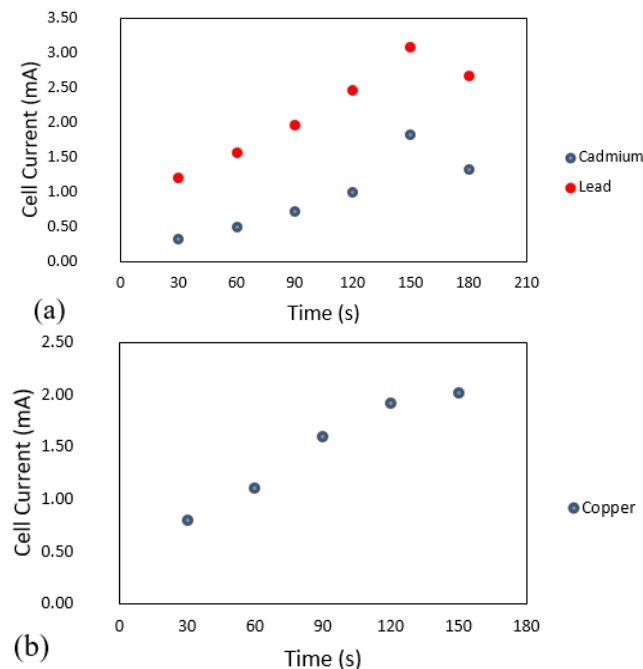


Figure 7. Peak currents of 10 µg/L each of (a) Cd²⁺, Pb²⁺ and (b) Cu²⁺ for different accumulation time.

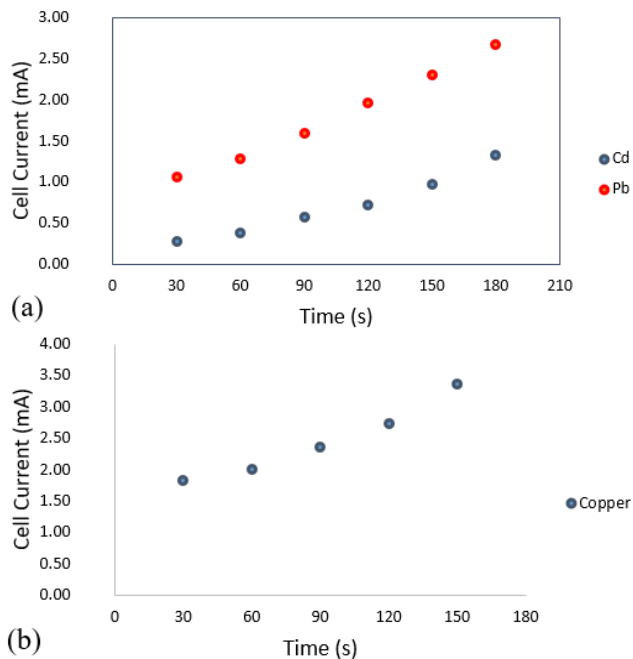


Figure 8. Peak currents of 10 µg/L each of (a) Cd²⁺, Pb²⁺ and (b) Cu²⁺ for different deposition time.

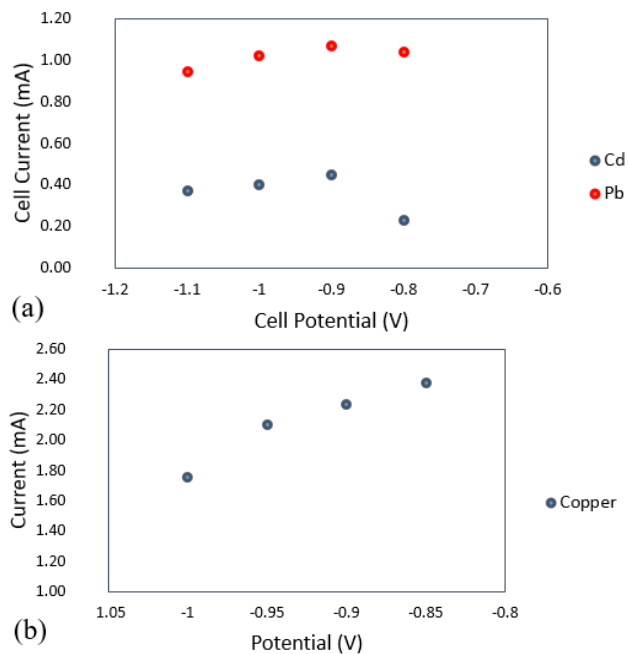


Figure 9. Peak currents of 10 µg/L each of (a) Cd²⁺, Pb²⁺ and (b) Cu²⁺ for different deposition potentials.

3.4. Optimization of DPV parameters

In order to get the highest anodic peak current of the best electrode, the accumulation time, deposition time, and deposition potential were varied. To determine the optimized results for the accumulation time, deposition time, and deposition potential, 10

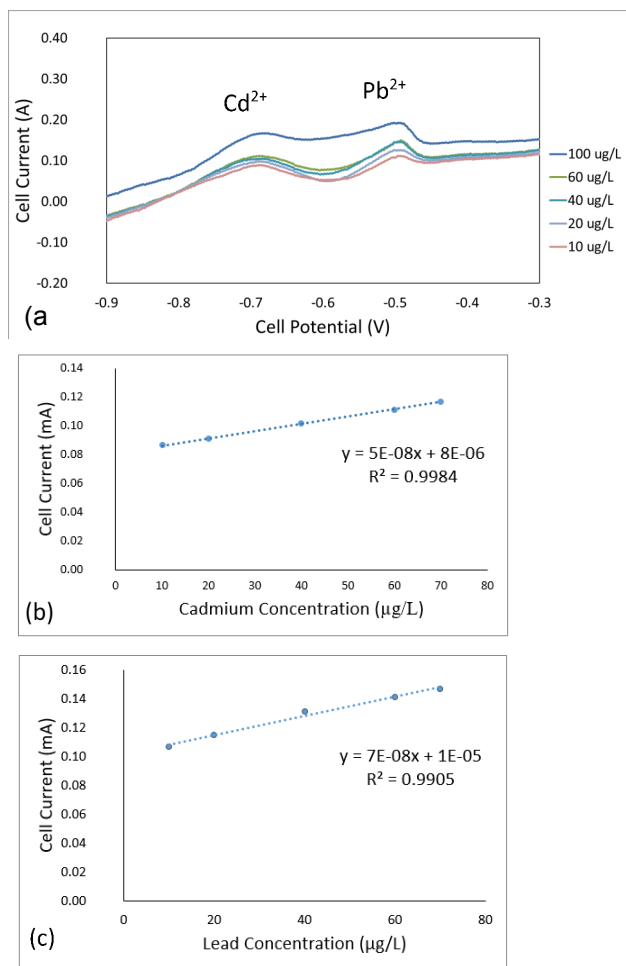


Figure 10. (a) DPV curves recorded in solutions with increasing Cd²⁺ and Pb²⁺ concentrations. Supporting electrolyte: 0.1 M NaCl solution, 10 mg/L Cu; DPV parameters: potential: -1.0 V; accumulation time: 150 s; deposition time: 30 s. Calibration curves for (b) cadmium and (c) lead.

µg/L each of Cd²⁺, Pb²⁺ and Cu²⁺ were used.

The accumulation time was varied at 30 s, 60 s, 90 s, 120 s, 150 s and 180 s. The deposition time was set constant at 30 s while the deposition potential was set constant at -1.0 V. In Figure 7, it is observed that the peaks for both cadmium and lead optimized at 150 seconds.

The deposition time was also varied at 30 s, 60 s, 90 s, 120 s, 150 s and 180 s. The accumulation time was set constant at 30 seconds while the deposition potential was set at 1.0 V. It can be seen in Figure 8 that the peak currents were constantly and linearly increasing. The deposition time of 30 s was chosen for time efficiency.

The deposition potential was varied at -0.8 V, -0.9 V, -1.0 V, and -1.1 V. The accumulation time and deposition time were both set at constant 30 seconds. As seen in Figure 9, the optimized deposition potential is -0.9 V for Cd²⁺ and Pb²⁺ and -0.85 V for Cu²⁺.

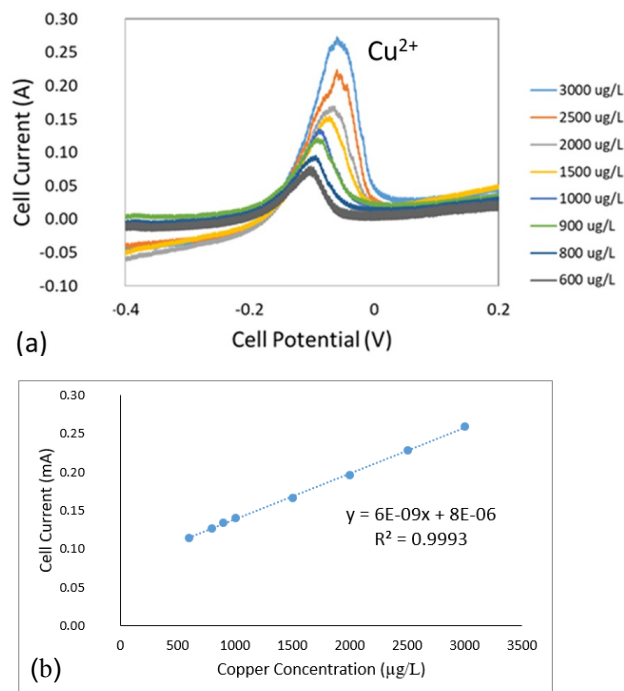


Figure 11. (a) DPV curves recorded in solutions with increasing Cu²⁺ concentrations. Supporting electrolyte: 0.1 M NaCl solution, 10 mg/L Cu; DPV parameters: potential: -1.0 V; accumulation time: 150 s; deposition time: 30 s. (b) Calibration curve for copper.

3.5. Calibration Curves for Lead and Cadmium

The calibration curves for lead and cadmium were obtained by varying the concentrations of lead and cadmium at 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, and 100 mg/L using the best modified electrode as working electrode (Figure 10). Increasing the concentration of Pb²⁺ and Cd²⁺ resulted to a higher anodic peak current. This can be attributed to the increase in the amounts of the heavy metals that were deposited onto the surface of the electrodes when their concentration was increased.

As can be observed from the figures, the Pearson correlation coefficient (R^2) was very close to 1. This means that there is a strong linear relationship between the toxic element concentration and anodic peak current.

3.6. Calibration Curve for Copper

The calibration curves for copper were obtained by varying the concentrations of copper at 600 mg/L, 800 mg/L, 900 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L and 3000 mg/L using the best modified electrode for copper (Figure 11). It can be seen from the figure that the higher the Cu concentration, the higher anodic peak current. This is due to the increase in the amount of Cu deposited onto the surface of the electrode when its concentration was increased.

3.7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LODs and LOQs were calculated and the results are shown in Table 1. As can be observed that the difference between the

experimental LOD and theoretical LOD is not that far. This means that the electrode was sensitive in detecting trace amounts of lead, cadmium, and copper.

3.8. Real Sampling

The results of the real sampling obtained from DPV and AAS are shown in Table 2. As can be observed from the table, cadmium, lead and copper were all detected in the samples. Cadmium and lead concentrations were lower than the copper concentrations obtained. Black rice had the highest content of cadmium at 83.11 mg/L, Brown Rice had the highest content of lead at 302.5 mg/L, and Sinandomeng rice had the highest content of copper at 3,666.67 mg/L. Based on world health organization (WHO), the limit of lead that the body can take is about 50 mg/L, the limit of cadmium that the body can take is about 5 mg/L and the limit of copper that the body can take is about 2000 mg/L. Thus, the concentrations of all real samples exceed the limit for lead and cadmium while only two samples exceeded the limit for copper. Tin was also detected in two rice samples however the concentration of Tin was not determined because the electrode was not calibrated for Tin.

Heavy metals in Philippine rice were detected because of the abundant presence of gold and silver mining sites around the country. Gold and silver are only byproducts of copper and lead mining. Only 0.02% of silver and 2% of gold are extracted from the mining sites and the rest are washed down the river systems which leads to soil contamination. One way of transferring the heavy metals from the mining sites to the rice fields is through the river systems. The whole Region 1 of the Philippines has 29 river systems with some more mountain streams that triples its size during the rainy season. Rivers are the main source of water for irrigation systems in Region 1. Thus, it is possible that the heavy metals in the rice samples are coming from the mining sites around Ilocos Sur.

Table 1. Limit of Detection and Limit of Quatitation

Heavy Metals	Theoretical LOD (µg/L)	Experimental LOD (µg/L)	LOQ (µg/L)
Cd	19.97	10.00	7.26
Pb	7.42	10.00	22.50
Cu	148.78	200.00	374.03

Table 2. Cadmium, Lead and Copper concentrations in the real samples as detected by DPV and AAS.

Rice Sample	Cadmium (µg/L)		Lead (µg/L)		Copper (µg/L)	
	DPV	AAS	DPV	AAS	DPV	AAS
Organic 1	78.22	60.50	53.50	55.00	888.33	512.00
Organic 2	63.78	51.50	-	-	436.67	517.00
Jasmine 1	74.56	34.50	91.88	168.90	778.33	281.00
Jasmine 2	68.11	46.50	52.00	181.53	883.33	422.00
Sinandomeng	78.89	45.00	192.50	110.00	3,666.67	1,392.00
Black rice	83.11	51.50	238.00	259.94	1,041.67	1,170
Brown rice	80.22	37.00	302.50	271.75	516.67	278
Palay	64.11	48.50	-	-	503.33	497

4. CONCLUSION

Nafion-[Ru(bpy)₃]²⁺-AuNPs modified glassy carbon electrodes were used in detecting heavy metals in Philippine rice samples by differential pulse voltammetry. The best electrode chosen in detecting lead, cadmium, and copper was modified with 5 mg [Ru(bpy)₃]²⁺ and 3 mg AuNPs. The best electrode was used to obtain DPV calibration curves which yielded strong correlation values at 0.9984 for lead, 0.9905 for cadmium, and 0.9993 for copper. The limit of detection of the electrode for lead, cadmium, and copper were 10.00 mg/L, 10.00 mg/L, and 200.00 mg/L, respectively. The results of DPV were verified using AAS. The modified electrodes were successful in detecting heavy metals in rice samples.

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