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Fabrication of an Effective Gold Nanoparticle/Graphene/Nafion® Modified Glassy Carbon Electrode for High Sensitive Detection of Trace Cd^{2+}, Pb^{2+} and Cu^{2+} in Tobacco and Tobacco Products

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The heavy metal contamination in tobacco (*nicotania tabacum*) and tobacco products was investigated in this study. Samples of commercially available cigarettes and traditional Philippine cigars, dried tobacco leaves, tobacco stalk, and soil were tested to confirm their heavy metal content. The World Health Organization (WHO) limits for Cd^{2+}, Pb^{2+}, and Cu^{2+} are 3 ppb, 10 ppb and 2 ppm, respectively. All the cigarette brands and variants in this study are all way above the WHO's toxicity limits for the aforementioned heavy metals. Henceforth, all the cigarette variants in this study can be inferred to be dangerously toxic. The heavy metal detection was done with a glassy carbon electrode modified with gold nanoparticles (AuNP), graphene and Nafion® using the drop coating method. The modified electrodes were optimized by varying the concentration of graphene and AuNP and their effects were subsequently determined by the measurement of their analytical sensitivity, limits of detection, and limits of quantitation. Atomic absorption spectroscopy was performed to validate the concentrations of the heavy metals detected via anodic stripping voltammetry. The statistically insignificant difference between the concentrations detected through anodic stripping voltammetry and atomic absorption spectroscopy shows that the modified electrodes exhibited optimum detection properties. In addition, the transfer factors from soil to tobacco stalk, as well as, soil to tobacco leaf were also computed.

Keywords: Anodic stripping voltammetry, gold nanoparticles, graphene, Nafion®, heavy metals, glassy carbon electrode, tobacco, cigarettes

1. INTRODUCTION

Tobacco use kills over 6 million people every year; and at least 600,000 of which are from secondhand smoke exposure. From this, the World Health Organization stated that the use of tobacco is the leading cause of preventable deaths worldwide [1]. Cigarettes contain nicotine, which is a highly addictive drug and is naturally found in the tobacco plant. Heavy metals such as cadmium, chromium, lead, and nickel are also present in cigarette smoke and are considered to be carcinogenic [2].

By definition, heavy metals have a specific gravity of 5.0 g cm^{-3} and are usually poisonous [3]. Heavy metals can be found almost everywhere, making it inevitable to totally eliminate its use [4]. These heavy metals can cause anemia, encephalopathy, renal dysfunction, and bone structure damage [5]. Thus, there has been an increasing interest in the development of highly sensitive analytical methods for the detection of trace amounts of toxic heavy metals. Electrochemical detection of the heavy metal content in products, such as cigarettes, is important due to the rising number of concerns about their effects to the environment and to society. Anodic stripping voltammetry (ASV) has been widely recognized as a powerful technique for the determination of trace metal ions due to an effective preconcentration step followed by electrochemical stripping measurements of the accumulated analytes. Stripping analysis using a chemically modified electrode has been proven to show significant selectivity toward some metal ions. The choice of the working electrode is crucial for the success of the stripping operation. A mercury based electrode has been mostly used for ASV due to its wide potential window toward negative potential values. However, the defects of mercury, such as its toxicity and difficulties associated with storage and disposal, encumber its use.

Various materials are used in modifying the working electrode for different applications. In metal detection, Nafion® is usually used as a coating material due to its chemical and thermal stability. However, Nafion® is not conductive. Hence, mediators, such as AuNPs and graphene, are needed for electron transport [6-14]. Carbon based nanomaterials such as graphene have been shown to be ideal for sensor applications since they possess unique physical and chemical properties such as their large surface area, high mechanical strength, and excellent thermal & electrical conductivity [15]. The good conductivity and large surface area of AuNPs can improve the response in voltammetric and amperometric methods of electrochemical sensors. According to Wang et al. [16], AuNPs showed excellent sensitivity and selectivity in detecting trace heavy metals.

In this study, electrochemical detection of Cd^{2+}, Pb^{2+}, and Cu^{2+} was accomplished by using glassy carbon electrodes (GCE) that were modified with gold nanoparticle (AuNP), graphene and Nafion® as the working electrode via ASV. The said electrodes were fabricated by depositing AuNP/graphene/Nafion® on to the GCEs using the drop coating method [17]. Concentrations of AuNP and graphene were varied at 1 mg, 2 mg, and 3 mg; and were mixed with 10 wt% Nafion® solution and ethanol. Using ASV, the effects of varying the modifier concentrations on the detection of Pb^{2+}, Cd^{2+}, and Cu^{2+} were analyzed. The optimum modifier concentrations (i.e., the concentration that would produce the highest anodic current peaks) were used in determining the sensitivity, limit of detection, and limit of quantitation of the electrodes through the calibration curves. Commercially available cigarettes were used in the real sample analysis for heavy metal detection by the AuNP/graphene/Nafion® modified GCE.

2. METHODOLOGY

Nafion® solution was purchased from Fuel Cell Earth (Wakefield, MA, USA). Gold nanoparticles, ethanol, 0.3 µm and 0.05 µm of alumina slurry were all sourced from Sigma Aldrich. Graphene powder was purchased from Graphene Supermarket. Laboratory grade heavy metals, cadmium chloride, copper chloride, and lead chloride were procured from Pharmchem (Bahadurgarh, Haryana, India). Stock solutions of heavy metals were prepared with high purity deionized water.

A BOSCH SAE200 electronic balance (BOSCH-Wägesysteme GmbH, Junginen, Germany) was used to measure 1 mg, 2mg, and 3mg of AuNP and graphene, and 0.5844 g of NaCl. A Transferpette®S micropipette was used in coating the solutions onto the surface of the glassy carbon electrodes. All glassware was put in the BANDELIN SONOREX ultrasonicator bath (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) for cleaning. A BST8-stat potentiostat/galvanostat (MTI Corporation, Richmond, CA, USA) was used as the ASV set-up for the electrochemical measurements. Atomic Absorption Spectroscopy was done with the use of AA-6300 Shimadzu Atomic Absorption Spectrophotometer.

Glassy carbon electrodes were polished sequentially with 0.5 µm and 0.3 µm of alumina slurry to obtain a highly lustrous surface. The electrodes were then ultrasonicated in ethanol for five minutes, rinsed with distilled water, and air-dried. The GCEs were wrapped with Teflon tape, with only 1mm exposed from the tip.

Graphene powder and AuNP were both weighed at 1 mg, 2 mg, and 3 mg; and were mixed with a solution of 0.5 ml 10 wt% Nafion® and 4.5 ml ethanol. The mixtures were ultrasonicated for 2 hours in order to obtain a uniformly dispersed solution. The resulting suspension was deposited on the GCEs using the drop coating method. The electrodes were then air dried for at least 2 hours in ambient room temperature.

Solutions containing 10 parts per million (ppm) of $CdCl_2$, $CuCl_{2,}$ and $PbCl_2$ were used in the optimization of the parameters. This was done by adding 1 mg of $CdCl_2$, $CuCl_{2,}$ and $PbCl_2$ to the analyte with 0.5844 g of NaCl (0.1 M NaCl).

A stock solution of 100 ppm of $CdCl_2$, $CuCl_{2,}$ and $PbCl_2$ was used in obtaining the calibration curves for each of the heavy metals in the study. This was done by adding 10 mg of $CdCl_2$, $CuCl_{2,}$ and $PbCl_2$ to a salt solution. The resulting solution was used in obtaining the calibration curve by subsequently adding aliquots of the stock solution to a solution containing 0.1 M of NaCl.

A fabricated electrode was placed in the voltammetry cell together with the saturated calomel electrode (reference electrode) and the platinum coil (counter electrode). A sodium chloride solution was used as the electrolyte in the process. Different concentrations of $CdCl_2$, $PbCl_2$, and $CuCl_2$ were added to determine the figures of merit.

For the optimization of parameters, first, a 10 ppm solution of $CdCl_2$, $PbCl_2$, and $CuCl_2$ was used as an analyte. Second, aliquots of a 100 ppm solution of $CdCl_2$, $PbCl_2$, and $CuCl_2$ were added to NaCl solution and were then used in obtaining the calibration curve. Increments of the aliquot were from 0.09 µL, 1 µL, 9 µL, 25 µL, 200 µL, 400 µL, 600 µL, 800 µL, and 1000 µL for $CdCl_2$ and $PbCl_2$; while aliquots of 1 mL, 5 mL, 6 mL, 7 mL, 8 mL, and 9 mL of $CuCl_2$ were used. Stoichiometry was done to get the roght concentrations of Cd^{2+}, Cu^{2+}, and Pb^{2+} present in the aliquots.

Five brands of commercially available cigarettes and various types of which were used as samples in this study. The samples were labeled as PH1A, PH1B, PH1C, PH1D, PH2A, PH2B, PH2C, PH3, ID, PH4A and PH4B. Two cigar samples, labeled PH CIG1 and PH CIG2, were also used for comparison.

2.1. Plant and Soil Sampling

Samples of dried tobacco leaves, a tobacco stalk, and soil were obtained from Candon, Ilocos Sur. The city of Candon is known to be the largest tobacco (*nicotania tabacum*) source in the Philippines with 1,475 hectares of tobacco farm. The abundant presence of gold and silver mining sites around the region has contributed to the heavy metal content in tobacco [18,19]. All samples were initially stored in dessicators; after which, they were chemically digested in alundum crucibles within 72 hours upon procurement.

2.2. Acid Digestion and Real Sample Analysis

The samples were weighed at 2.5 g each and placed inside the furnace at 450 °C for at least 6 hours, or until a white ash was obtained. Two mL of nitric acid was then added to the samples and was evaporated to dryness. After allowing to evaporate, the samples were put back in the furnace at 450 °C for 30 minutes. One mL of hydrochloric acid was then added to each sample and was diluted to 100 mL with deionized water. Sodium chloride of mass 0.5844 g was added to the resulting analyte to obtain a 0.1M of NaCl solution [20].

Prior to weighing, non-soil particles were removed using a pair of laboratory tweezers. One gram of soil was put in a beaker and 10 mL of nitric acid was then added to the sample. The crucible containing the sample was heated until the solution was completely clear and solid traces of the soil sample were no longer visible. The solution was then diluted to 100 mL with deionized water and 0.5844 g of sodium chloride was added to the resulting analyte to obtain a 0.1M NaCl solution [21].

Anodic stripping voltammetry was used to determine the heavy metal content of the samples in the study. The optimized parameters were applied to determine the concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} present in every sample. The concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} were computed using the calibration curve equation for each of the heavy metals.

To validate the concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} in the samples, atomic absorption spectroscopy (AAS) was performed. Stock solutions of the same concentrations used in the ASV calibration curves were first prepared, and from which AAS calibration curves were obtained. However, AAS has a detection limit of 150 ppb, limiting the concentrations that will be used for comparison. The resulting AAS calibration curve equations were used to compute for the concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} present in the solutions.

3. RESULTS AND DISCUSSION

The determination of the best modified electrode was done by varying the concentrations of AuNP and graphene in the coating solution. The concentration of graphene was set at 1 mg, 2 mg, and 3 mg per 5 ml Nafion®. The concentration of AuNP was varied at 1mg, 2mg, and 3mg for every concentration of graphene. The resulting electrodes were used to simultaneously detect constant amounts (10 ppm each) of Cd^{2+} and Pb^{2+}, and sequentially for Cu^{2+} via anodic stripping voltammetry with the use of the following optimum parameters: the scan rate was set to 100 mV/s, initial potential of -0.95 V, deposition time at 60 seconds, and rest period at 30 seconds.

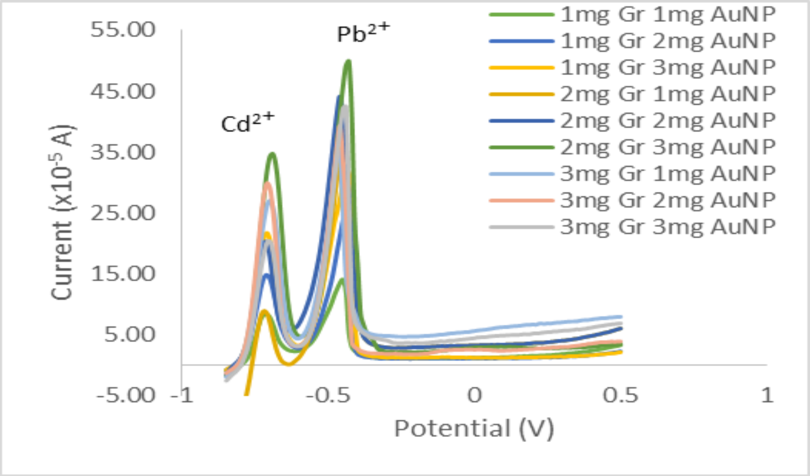

Figure 1. ASV curves of Pb^{2+} and Cd^{2+} for varying amounts of electrode modifiers, AuNP and graphene. Supporting electrolyte: 0.1M NaCl solution, 10ppm Pb(II), 10ppm Cd(II). ASV parameters: scan rate = 100 mV/s, initial potential = -0.95 V, deposition time = 60 s, and rest period = 30 seconds.

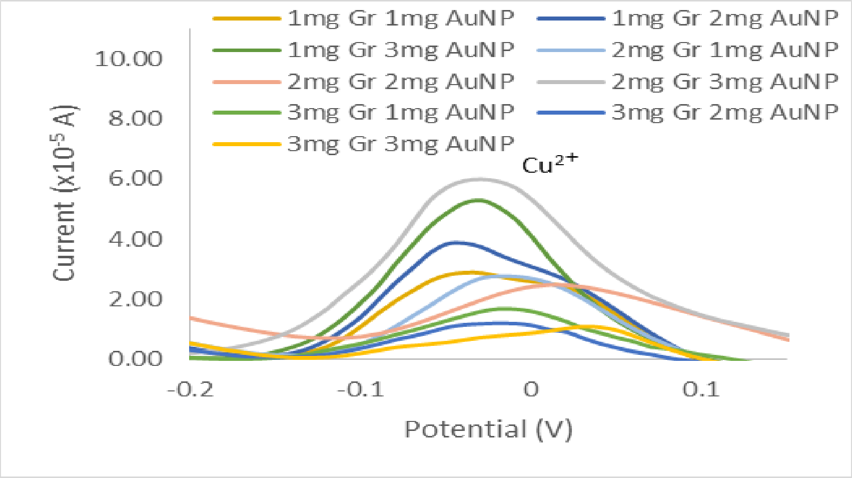

Figure 2. ASV curves of Pb^{2+} and Cd^{2+} for varying amounts of electrode modifiers, AuNP and graphene. Supporting electrolyte: 0.1M NaCl solution, 10ppm Pb(II), 10ppm Cd(II). ASV parameters: scan rate = 100 mV/s, initial potential = -0.95 V, deposition time = 60 s, and rest period = 30 seconds.

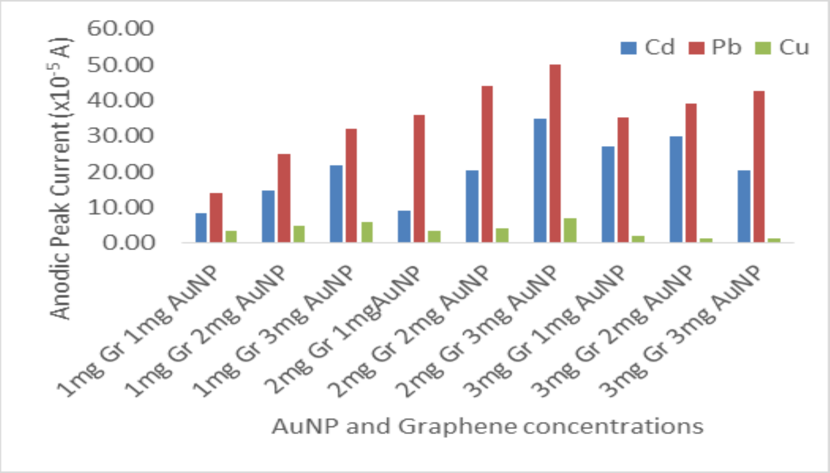

Figure 3. Comparison of anodic current peaks for varying AuNP and Graphene for the detection of Pb^{2+}, Cd^{2+}, and Cu^{2+}.

Figures 1 and 2 show the ASV curves obtained from the electrochemical measurements. The shift in the peak potentials of Pb^{2+}, Cd^{2+} and Cu^{2+} indicates that the deposition and stripping of the heavy metals to/from the electrode surface are affected by the amounts of Au and Gr on the modified electrode. An increasing trend can be observed from the anodic current peaks for 1 mg and 2 mg graphene as the concentration of AuNP is increased (Fig. 3). Since the electrode fabricated with 2 mg graphene and 3 mg AuNP exhibited the highest anodic current peaks for Cd^{2+}, Pb^{2+}, and Cu^{2+}, it was considered to be the concentration that will give the optimum measurements for the heavy metals in the study.

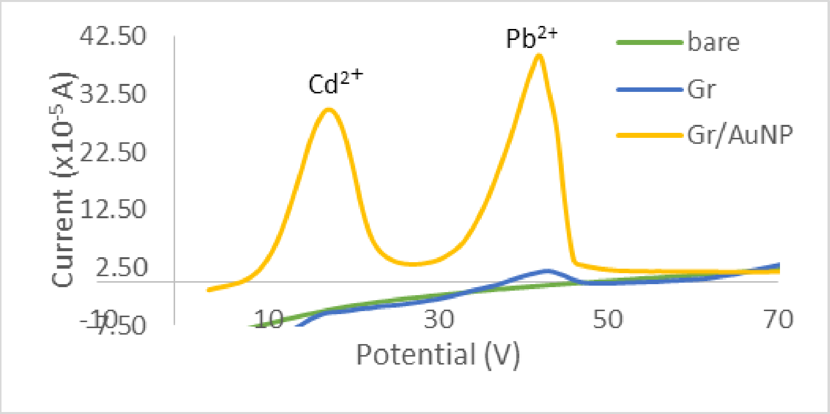

Figure 4. Comparison of ASV curves of Pb^{2+} and Cd^{2+} using a bare GCE (green) , graphene/Nafion modified GCE (blue) and graphene/AuNP/Nafion modified GCE (yellow). Supporting electrolyte: 0.1M NaCl solution, 10ppm Pb(II), 10ppm Cd(II). ASV parameters: scan rate = 100 mV/s, initial potential = -0.95 V, deposition time = 60 s, and rest period = 30 seconds.

Figure 4 shows the comparison of the ASV curves of Pb^{2+} and Cd^{2+} using a bare GCE, graphene/Nafion modified GCE and the best graphene/AuNP/Nafion modified GCE. It can be observed from the voltammograms that the detection of Pb^{2+} and Cd^{2+} is greatly enhanced by modifying the GCE by graphene and AuNP. This can be attributed to the increase in the electrical conductivity of the electrode due to the addition of AuNP. Since AuNP is a nanoelectrocatalyst, the heavy metals in the solution underwent faster redox reaction thereby accelerating the electron transfer. The combined high surface-to-volume ratio of graphene and the high conductivity of AuNP made the electrode more sensitive in detecting the heavy metals.

3.1. Calibration Curves

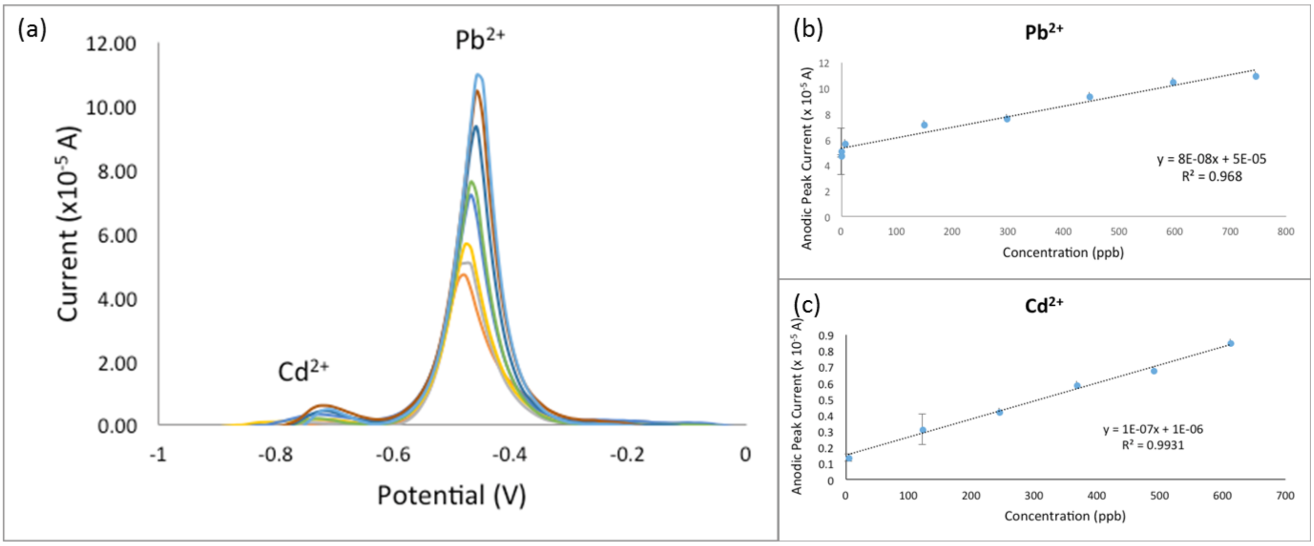

Figure 5. (a) ASV curves recorded in solutions with increasing Pb^{2+} and Cd^{2+} concentrations and the corresponding calibration curves for (b) Pb^{2+} and (c) Cd^{2+}. Supporting electrolyte: 0.1M NaCl solution. ASV parameters: scan rate = 100 mV/s, initial potential = -0.95 V, deposition time = 60 s, and rest period = 30 seconds.

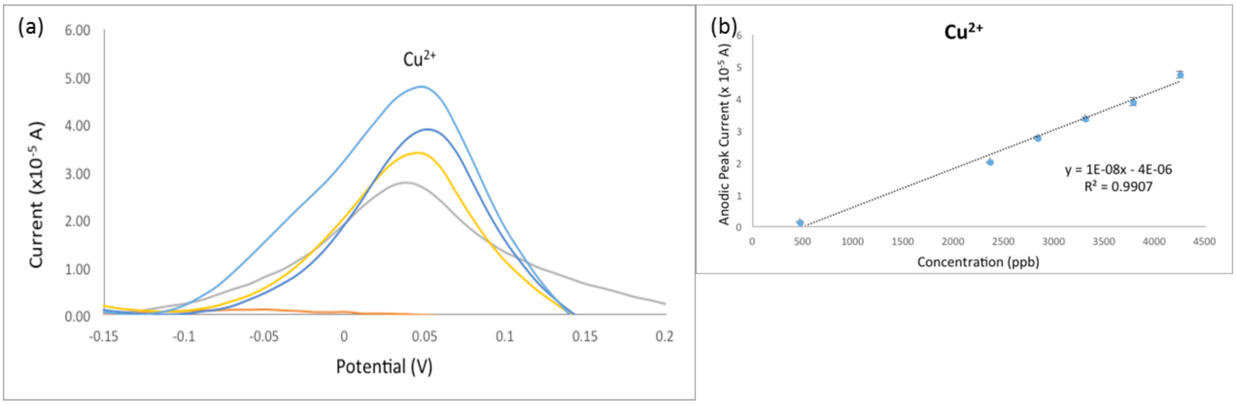

Figure 6. (a) ASV curves recorded in solutions with increasing Cu^{2+} concentrations and the corresponding calibration curves for (b) Cu^{2+}. Supporting electrolyte: 0.1M NaCl solution. ASV parameters: scan rate = 100 mV/s, initial potential = -0.95 V, deposition time = 60 s, and rest period = 30 seconds.

The calibration curves were obtained by varying the analyte concentrations from 67 parts per trillion (ppt) to 745 parts per billion (ppb) for Pb^{2+}, from 5 ppb to 613 ppb for Cd^{2+}, and from 472 ppb to 4253 ppb for Cu^{2+}. The resulting concentrations were then plotted against the corresponding current peaks to obtain the calibration curve for each metal (Figs. 5 and 6). The shift in the peak potential for Cu is due to the change in pH of the analyte solution. The Pearson correlation coefficients (R^2) for all calibration curves are close to 1, indicating that there is a strong linear relationship between the reduction current and heavy metal concentration [22,23].

3.2. Real Sample Analysis

Using the best electrode, i.e, 2 mg graphene and 3mg AuNP, and the optimum parameters, the concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} present in the samples were determined by substituting the values of the resulting anodic current peaks as the y-values in the calibration curve equations.

Cadmium, lead, and copper were detected via AAS to verify the ASV results. The concentrations of Cd^{2+}, Pb^{2+}, and Cu^{2+} were determined by substituting the value of the obtained absorbance values as the y-values in the resulting calibration curve equations. The statistically insignificant difference in the concentrations detected via ASV and AAS indicates that ASV is a reliable method for heavy metal detection.

Table 1. Comparison between Cd^{2+} concentrations detected via ASV and AAS

Sample	CADMIUM				
	ASV (ppb)	AAS (ppb)	%error	%diff	SD (ppb)
PH 1A	89.20	109.50	18.54%	20.43%	14.35
PH 1B	56.95	62.50	8.88%	9.29%	3.92
PH 1C	36.60	37.00	1.08%	1.09%	0.28
PH 1D	82.40	91.50	9.95%	10.47%	6.43
PH 2A	87.61	90.40	3.09%	3.13%	1.97
PH 2B	77.70	84.50	8.05%	8.38%	4.81
PH 2C	33.90	38.67	12.33%	13.14%	3.37
PH 3	73.43	86.50	15.11%	16.34%	9.24
ID	101.37	132.00	23.21%	26.25%	21.66
PH 4A	72.50	84.00	13.69%	14.70%	8.13
PH 4B	62.76	79.36	20.91%	23.36%	11.74
PH CIG 1	89.11	94.38	5.59%	5.75%	3.73
PH CIG 2	71.67	69.85	2.61%	2.57%	1.29
LEAVES	93.13	97.37	4.35%	4.45%	3.00
STALK	90.10	94.48	4.63%	4.74%	3.10
SOIL	58.33	67.49	13.57%	14.56%	6.48

From Table 1, cigarette sample 1D had the highest Cd^{2+} content with 101.37 ppb. Sample PH2C had the lowest Cd^{2+} content with 33.90 ppb. From Table 2, cigarette sample PH3 had the highest Pb^{2+} content with 712.50 ppb. Sample PH1B had the lowest Pb^{2+} content with 462.25 ppb.

From Table 3, cigarette sample PH1D had the highest Cu^{2+} content with 8847.00 ppb. Sample PH1B had the lowest Cu^{2+} content with 485.20 ppb. Although PH1B appears to have the least Pb^{2+} and Cu^{2+} contamination, it is still way above the WHO toxicity limit of 10 ppb and 2 ppm, respectively [24, 25]. The World Health Organization (WHO) limit for Cd^{2+} is 3 ppb [26]. The cigarette brands had Cd^{2+} content from 33.90 ppb to 101.37 ppb, the cigars had Cd^{2+} contents of 89.11ppb and 71.67 ppb. The heavy metal content of the commercial cigarettes and cigars in this study were all way above the WHO toxicity limit. Therefore, they can be inferred to be dangerously toxic. The leaves and stalks of the tobaco plant, as well as the soil, from Candon, Ilocos Sur, Philippines were also analyzed for their heavy metal contamination. They were also found to have Cd^{2+}, Pb^{2+} and Cu^{2+} contamination way above the WHO toxicity limit. This can be attributed to the contamination from the two river systems, namely, the Abra and the Cagayan River systems, which are the sources of the irrigation water for the tobacco farms in Candon. These two river systems traverse several silver and mining sites which dispose of their copper, lead and cadmium wastes indiscriminately into the said river systems.

Table 2. Comparison between Pb^{2+} concentrations detected via ASV and AAS

Sample	LEAD				
	ASV (ppb)	AAS (ppb)	%error	%diff	SD (ppb)
PH 1A	488.25	480.00	1.72%	1.70%	5.83
PH 1B	462.25	528.33	12.51%	13.34%	46.73
PH 1C	582.13	586.33	0.72%	0.72%	2.98
PH 1D	594.38	598.33	0.66%	0.66%	2.80
PH 2A	575.51	630.00	8.65%	9.04%	38.53
PH 2B	622.98	616.67	1.02%	1.02%	4.46
PH 2C	617.94	653.33	5.42%	5.57%	25.03
PH 3	712.50	737.50	3.39%	3.45%	17.68
ID	620.63	710.00	12.59%	13.43%	63.19
PH 4A	615.00	674.32	8.80%	9.20%	41.95
PH 4B	620.48	649.84	4.52%	4.62%	20.76
PH CIG 1	498.76	539.56	7.56%	7.86%	28.85
PH CIG 2	617.12	692.81	10.93%	11.56%	53.52
LEAVES	618.55	674.42	8.28%	8.64%	39.50
STALK	622.55	692.58	10.11%	10.65%	49.52
SOIL	570.24	601.37	5.18%	5.31%	22.01

Table 3. Comparison between Cu^{2+} concentrations detected via ASV and AAS

Sample	COPPER				
	ASV (ppb)	AAS (ppb)	%error	%diff	SD (ppb)
PH 1A	8265.00	8377.78	1.35%	1.36%	79.75
PH 1B	485.20	496.67	2.31%	2.34%	8.11

PH 1C	2643.00	3544.44	25.43%	29.14%	637.42
PH 1D	8847.00	9149.00	3.30%	3.36%	213.55
PH 2A	7376.00	8931.00	17.41%	19.07%	1099.55
PH 2B	1078.30	1243.44	13.28%	14.23%	116.77
PH 2C	1449.00	1879.00	22.88%	25.84%	304.06
PH 3	555.10	551.00	0.74%	0.74%	2.90
ID	4124.84	3822.22	7.92%	7.62%	213.98
PH 4A	1559.00	1620.81	3.81%	3.89%	43.71
PH 4B	3407.56	3628.90	6.10%	6.29%	156.51
PH CIG 1	877.12	1405.44	37.59%	46.29%	373.58
PH CIG 2	1945.97	1993.87	2.40%	2.43%	33.87
LEAVES	1636.26	2001.26	18.24%	20.07%	258.09
STALK	578.17	1011.98	42.87%	54.56%	306.75
SOIL	55796.20	59845.61	6.77%	7.00%	2863.37

3.3. Transfer Factor

A relative measure of the transfer of the trace element from the soil to the plant is expressed by the transfer factor (TF). The transfer factor is an indication of the plant species ability or tendency to uptake a certain element from the soil [27].

Soil to plant metal transfer was computed as transfer factor. It was calculated by using the equation [28]:

$$TF = \frac{C_{plant}}{C_{soil}} \qquad \text{(Eq. 3.1)}$$

where, C_{Plant} is the concentration of heavy metals in plants and C_{Soil} is the concentration of heavy metals in soil.

Table 4. Transfer factor for plant and soil concentrations

	C soil (ppb)	C stalk (ppb)	C leaves (ppb)	Transfer Factor (soil to stalk)	Transfer Factor (soil to leaves)
Cd	58.33	90.10	93.13	1.54	1.60
Pb	570.24	622.55	618.55	1.09	1.08
Cu	55796.20	578.17	1636.26	0.01	0.03

There was a significant transfer from soil to stalk and from soil to leaves, in the case of cadmium and lead (Table 4). A transfer factor greater than 1 can be explained by the absorption of heavy metals of both the stalk and the leaves from other sources like the irrigation water and air pollution from gas exhaust emitted by motorized vehicles since the tobacco fields are near public roads. The computed low transfer factor from copper may be explained by the possibility that there was really minimal copper content in the soil or the tobacco leaves and stalk do not absorb copper efficiently.

3.4. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) is the smallest concentration at which the targeted metals in an analyte can be quantitated with a linear response, while the limit of quantitation (LOQ) is defined to be the smallest concentration of the targeted metals in an analyte that can be detected with no guarantee about the bias or precision of the assay. Experimental LOD is the lowest analyte concentration detected experimentally. It was determined by decreasing the analyte concentration in the electrolyte solution until it cannot be detected anymore.

$$LOD = \frac{3.3\sigma}{m} \qquad \text{(Eq. 3.2)}$$

$$LOQ = \frac{10\sigma}{\mathrm{m}} \qquad \text{(Eq. 3.3)}$$

where σ refers to the standard deviation of the blank signal and S is the slope of the calibration curve [29, 30].

Table 5. Limit of detection and limit of quantitation

	Experimental LOD (ppb)	LOD (ppb)	%error	%diff	SD	LOQ (ppb)
Cd	5.519	0.123	43.966	1.913	2.698	0.372
Pb	0.067	0.014	3.657	1.293	0.026	0.044
Cu	472.666	209.041	1.261	0.773	131.812	633.458

The fabricated electrode in this study is deemed significantly capable of detecting cadmium and lead way below the WHO limit of toxicity for both metals (Table 5). ASV using the modified electrode fabricated in this study, therefore, is an efficient and cost effective method in detecting heavy metal toxicity as opposed to the significantly expensive methods such as AAS. Although the fabricated electrode can detect copper, it is not efficient enough to detect copper content within the WHO limit of toxicity.

3.5. Analytical Sensitivity

Analytical sensitivity was defined by Mandel and Stiehler to be the precision in sensitivity. To compute for the analytical sensitivity,the following equation is used:

$$\gamma = \frac{m}{S_s} \quad \text{(Eq. 3.4)}$$

where γ is the analytical sensitivity, m is the slope of the calibration curve, and S_s is the standard deviation of the measurements obtained [23].

As seen in Table 6, the analytical sensitivity of the electrode increases as the concentration of heavy metals increases. The table shows consistently that the greater the concentration, the greater is the sensitivity which is in agreement with the results of a study by Ambruster and Pry [29].

Table 6. Analytical Sensitivity

	Concentration (ppb)	SD (A)	Slope (A/ppb)	Analytical Sensitivity (1/ppb)
Cd	5.52	2.2E-07	1.00E-07	2.21
	122.64	2.9E-07	1.00E-07	2.87
	245.29	3.6E-07	1.00E-07	3.64
	367.93	5.5E-07	1.00E-07	5.53
	490.58	1.3E-06	1.00E-07	12.71
	613.22	2.4E-06	1.00E-07	23.79
Pb	0.07	7.7E-06	8.00E-08	96.35
	0.75	8.2E-06	8.00E-08	103.01
	6.71	9.5E-06	8.00E-08	118.35
	149.01	1.3E-05	8.00E-08	158.01
	298.02	1.8E-05	8.00E-08	224.31
	447.03	1.9E-05	8.00E-08	236.44
	596.05	1.8E-05	8.00E-08	223.59
	745.06	3.6E-05	8.00E-08	447.70
Cu	472.67	1.3E-08	1.00E-08	1.32
	1890.67	4.6E-07	1.00E-08	46.23
	2836.00	8.7E-07	1.00E-08	87.39
	3308.67	9.6E-07	1.00E-08	95.53
	3781.33	1.2E-06	1.00E-08	117.91
	4254.00	1.4E-06	1.00E-08	137.64

3.6. Calibration Sensitivity

Calibration sensitivity simply refers to the slope of the calibration curve. This figure of merit does not account for precision of individual measurements.

Table 7. Calibration Sensitivity

	A/ppb	
	AAS	ASV
Cd	0.0002	1.00E-07
Pb	6.00E-05	8.00E-08
Cu	9.00E-05	1.00E-08

The calibration sensitivities, in terms of A/ppb, for the targeted metals are shown in Table 7. If the two methods are equally precise, the one with steeper calibration curve or the one with higher slope is more sensitive [23]. This indicates that AAS is a more sensitive technique than ASV.

4. CONCLUSIONS

Cadmium, lead and copper were found to be present in popular and commercially available cigarettes in the Philippines which are manufactured in the Philippines and Indonesia. For cadmium, they range in value from 33.90 ppb to 101.37 ppb. For lead contamination, the values range from 462.25 ppb to 712.50 ppb. Copper content ranged from 485.20 ppb to 8847.00 ppb. These are all way above the WHO toxicity limits of 3 ppb, 10 ppb, and 2 ppm for Pb^{2+}, Cu^{2+} and Cd^{2+}, respectively.

The drop-coating technique was used to successfully modify the glassy carbon electrodes. The detection of Cd^{2+}, Pb^{2+} and Cu^{2+} were enhanced by modifying the substrate (GCE) with AuNP/graphene/Nafion®. Of the nine concentrations used for substrate modification, 2 mg graphene and 3 mg AuNP exhibited the highest anodic current peaks for lead, cadmium, and copper, and was therefore chosen as the electrode for metal detection in this study. Under optimum conditions, the electrode exhibited linearity from 67 ppt to 670 ppb for lead, 5.5 ppb to 613 ppb for cadmium, and 473 ppb to 4254 ppb for copper. Although AAS gives better sensitivity, the other figures of merit, viz. limit of detection (LOD) and limit of quantitation (LOQ), do not have significant difference using AAS and ASV. In terms of cost effectiveness and selectivity, ASV was proven to be an effective method for heavy metal detection when compared to AAS. Hence, taking all the figures of merit in to consideration, ASV has more merits for the detection of lead, cadmium, and copper using 2mg graphene/3mg AuNP/Nafion® modified glassy carbon electrode fabricated in this study.

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