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# Quantification of lead in cooking utensils and vegetables using square wave anodic stripping voltammetry

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## Abstract

A simple and sensitive voltammetric method using *in-situ* bismuth film modified glassy carbon electrode (BiFGE) and Nafion-coated bismuth film modified glassy carbon electrode (NC-BiFGE) were used to determine the amount of lead(II) present in locally produced (at Kombolcha, 376 km North of Addis Ababa, Ethiopia) and imported cooking utensils and vegetable samples before and after cooking with the utensils. The voltammetric method was validated using standard spectroscopic method and recovery tests. The amount of lead(II) found in the locally produced utensil ( $6.48 \text{ mg L}^{-1}$ ) was very high compared to the imported utensil ( $0.007 \text{ mg L}^{-1}$ ). Moreover, a 3–5 fold increase in the amount of lead(II) was found when different vegetables were cooked with the local utensil as a result of the leaching out of the lead(II) from the cooking utensil.

Keywords: Food science, Analytical chemistry

## 1. Introduction

Lead, which has been in use since the ancient times, is the second among the priority list of hazardous substances (Ashraf et al., 2017). This toxic metal is being continually introduced into the environment through discharge from various manufacturing industries like storage batteries, printing, painting, pigments, dying

and leaded glass (Kaplan Ince et al., 2017; Shalini et al., 2017). Unlike organic pollutants, it is not at all degradable and contaminates the soils, the water body and agricultural products. Accumulation of lead metal in human bodies and other living organisms can take place through either direct intake or through the food chain system. Several reports have shown that the accumulation of lead metal in humans beyond the permissible limit can cause severe damage to kidney, impair the immune and central nervous systems and also adversely affect the intellectual development of children (Flora et al., 2012; Gidlow, 2004; Muhammad Ekramul Mahmud et al., 2016; Nevin, 2009).

For this reason, there is a growing interest and need, particularly in developing countries to develop a simple, sensitive, cheap and precise method for monitoring the level of the toxic metal in the environment. The standard analytical methods used for metal analysis like atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) are very expensive instruments, require well equipped laboratories and highly trained man power and are not suitable for analysis in the field. On the other hand, electroanalytical methods are cheap, do not require highly skilled personnel and are suitable for routine analysis including applications in fieldwork. Anodic stripping voltammetry (ASV) is the conventional electroanalytical technique for determination of trace metals. For many years mercury electrode as hanging mercury-drop electrode (HMDE) or mercury-film electrode (MFE) was the best electrode material for ASV because of its high cathodic hydrogen overpotential and ability to form amalgams with many heavy metals. However, due to its high toxicity, it is banned in some countries. As a result other electrode materials like bismuth-film electrode (BiFE) (Wang et al., 2000) and antimony-film electrode (SbFE) (Hocevar et al., 2007) are being investigated as alternate electrode materials. The sensitivity of such film electrodes is attributed to the combination of preconcentration step with advanced measurement procedures like square wave voltammetry that generates favorable signal-background ratio (Wang, 2006). Moreover, four to six metals can be measured simultaneously in various matrixes at concentration levels down to  $10^{-10}$  M, utilizing relatively inexpensive and portable instrumentation. Bismuth-film electrode (BiFE) was used in this work because of its high sensitivity, high reproducibility, low background current, low toxicity, better linearity, wide potential window, and ease of preparation (Wang et al., 2000). The influence of surface active materials in different matrices on the current response of such metal film electrode can be reduced and the sensitivity further being improved using Nafion-coated BiFE (Gouveia-Caridade et al., 2006). The method was successfully applied to determine heavy metals in different samples like vegetables (Xu et al., 2008a), wine (Kefala et al., 2004), urine (Kefala et al., 2004), human hair (Kefala, 2003), medicinal plants (Xu et al., 2008b) and milk (Ping et al., 2014). In our recent work (Tesfaw et al., 2016), ASV using BiFE was demonstrated for studying

the adsorption behavior of lead ions onto a natural biopolymer without requiring the commonly used and expensive atomic spectroscopic methods. The application of anodic stripping voltammetry for the determination of lead ion in locally produced cooking utensils and the increased levels of lead in the vegetables while cooking with the lead containing utensils is reported in this work.

## 2. Experimental

### 2.1. Reagents

All chemicals used were of analytical grade. Nitric acid,  $\text{HNO}_3$ , 69% (Sigma-Aldrich, USA), hydrochloric acid,  $\text{HCl}$ , 36% (Fisher Scientific, UK), perchloric acid,  $\text{HClO}_4$ , 70% (Fluka, Switzerland) and 30% hydrogen peroxide,  $\text{H}_2\text{O}_2$ , (BDH, England) were used for sample digestion.  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  (Riedel-de Haen) and  $\text{Pb}(\text{NO}_3)_2$  (Wagtech International Ltd. UK), were used for preparation of bismuth modified electrode and for the preparation of the standard solutions, respectively. Sodium acetate,  $\text{CH}_3\text{COONa}$  (BDH Chemicals Ltd. Poole, England) and acetic acid,  $\text{CH}_3\text{COOH}$  (Merck Darmstadt, Germany) were used to prepare the supporting electrolyte (0.1 M acetate buffer, pH 4.6). Nafion solutions (1%) were prepared with absolute ethanol from a stock solution of 5% Nafion (Sigma Aldrich) for the preparation of Nafion modified electrode.  $\text{Cr}(\text{NO}_3)_3$ ,  $\text{Cd}(\text{NO}_3)_2$ ,  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$ ,  $\text{Ni}(\text{NO}_3)_2$  and  $\text{Al}(\text{NO}_3)_3$  (BDH Chemicals Ltd Poole, England) and  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (Riedel-de Haen) were used for interference study. Deionized water was used to prepare all standard and sample solutions throughout the experiment.

### 2.2. Sample collection and preparation

Locally produced and imported cooking utensils were purchased from Kombolcha, 376 km away from Addis Ababa and from a market (made in India) in Addis Ababa, Ethiopia, respectively (Fig. 1). The utensils were washed with deionized water thoroughly and dried. In order to get good representative samples of the whole utensils, several pieces from the different parts of the utensils were taken by drilling using High Speed Steel Twist Drills (Osborn-Mushet Tools Ltd. made in the U.K.). The drilled pieces of samples were mixed and then digested in aqua regia. Fresh vegetable samples were also purchased from a local supermarket. The



**Fig. 1.** Pictures of locally produced (left) and imported cooking utensils (right).

collected vegetable samples were divided into three. The first part of vegetable samples were dried at 60 °C for 12 h, powdered and digested. The sample was directly analyzed and the lead ion was determined using anodic stripping technique as a control. The second and third parts of vegetable samples were cooked in locally produced and imported utensils for two hours, respectively. The cooked vegetable samples were dried at 60 °C for 12 h, powdered and digested and then its lead content was determined by similar method. The leaching of lead ions from the utensil to the food were verified by comparing the results obtained from the cooked and uncooked samples.

### 2.3. Digestion of cooking utensil samples

Cooking utensils were digested according to reported procedures (Ehi-Eromosele et al., 2012). Briefly, one gram of a well-homogenized sample from each material was weighed into a flat-bottom Pyrex flask and 12 mL of freshly prepared aquaregia (3 mL HNO<sub>3</sub> + 9 mL HCl) were added. The flasks were heated for 2 h on a hot plate between 100–150 °C. The mixtures were allowed to cool and then filtered through a Whatman No. 42 filter paper into a 50 mL standard volumetric flask. The filtrates were diluted to 50 mL with 0.1 M acetate buffer (pH 4.6) solution.

### 2.4. Digestion of tomato, cabbage and red onion samples

Similarly vegetable samples were digested according to reported procedures (Viqarun et al., 2005), where about 0.4 g of the dried and homogenized vegetable samples were first weighed in a flat-bottom Pyrex flask. The samples were mixed with 2.0 mL of conc. HNO<sub>3</sub> and 0.5 mL of conc. HClO<sub>4</sub> and were initially heated at 80–100 °C and then at 100–150 °C till dissolution of the samples were completed. After dissolution, the samples were cooled to 70 °C and 0.5 mL of 30% H<sub>2</sub>O<sub>2</sub> was added to remove any organic residues. The mixtures were then heated to dryness. About 2.0 mL of deionized water and 0.1 mL HNO<sub>3</sub> were added to the dried mixtures and heated at 100–150 °C for 2 h. After repeated additions of deionized water the mixtures were heated to dryness in order to expel the last traces of H<sub>2</sub>O<sub>2</sub>. Finally, whitish residues were obtained and dissolved in 10.0 mL of 0.1 M acetate buffer solution for electroanalytical measurements.

### 2.5. Apparatus

Square wave anodic stripping voltammetry (SWASV) measurements were performed using CH Instruments Electrochemical Analyzer (CHI840C). A three-electrode system consisting of bismuth-film modified glassy carbon (GC-BiFE) and Nafion-coated bismuth-film modified glassy carbon electrodes (GC-NCBiFE) working electrodes, Ag/AgCl/KCl (saturated) reference electrode, and a platinum

wire counter electrode were employed. All electrochemical experiments were carried out in one-compartment voltammetric cell at ambient temperature. Magnetic stirrer together with magnetic bar was used, whenever necessary. AAS and ICP-MS measurements were carried out using Analytik jena (ZEE nit 700p) and 6100 Perkin Elmer ICP-MS instruments, respectively.

## 2.6. Preparation of the modified electrodes

The glassy carbon electrode (GCE) was polished with a 0.05  $\mu\text{m}$  aluminum oxide powder on a polishing cloth, rinsed with deionized water and dried. Nafion solution (1%) and dimethylformamide (DMF) (5  $\mu\text{L}$  drop each) were sequentially placed on the surface of the glassy carbon electrode and were left to dry at room temperature. The electrochemical deposition of bismuth and the lead ions on the bare GCE and the Nafion-coated GCE were done by *in situ* potentiostatic deposition method using the three-electrode system from 0.1 M acetate buffer solution (pH 4.6) containing 1  $\text{mg L}^{-1}$  of bismuth(III) ions at a deposition potential of  $-1.2$  V for 240 s.

## 2.7. Stripping analysis of lead

Following the deposition of both the bismuth modifier and the lead metal, square wave anodic stripping voltammograms were recorded by scanning the potential from  $-1.2$  to  $+0.2$  V with a frequency of 15 Hz, potential step of 4 mV, and amplitude of 25 mV. Prior to each measurement, the electrode was cleaned by holding the potential of working electrode at  $+0.3$  V for 30 s, while stirring the solution. The experiments were carried out at room temperature.

## 2.8. Standard addition method

After the current response of the sample solution was measured, known concentrations of lead ions were subsequently added. The current responses were then measured for every addition. The unknown concentrations of lead ions in the samples were calculated using Eq. (1).

$$C_x = \frac{i_x C_s}{i_{x+s} - i_x} \quad (1)$$

where  $C_x$  and  $C_s$  are the concentration of the analyte in the sample and concentration of the standard added, respectively,  $i_x$  and  $i_{x+s}$  are the current responses of the solutions containing  $C_x$  and after addition  $C_s$ . The slopes of the straight lines obtained when the current response of the solutions were plotted against the added concentrations were compared to that of the calibration curve to confirm the absence of matrix effect in the electroanalytical determination.

## 2.9. Statistical analysis

Statistical analysis was performed using SigmaPlot (version 11.0 Software, San Jose, CA, USA) for calculating the  $p$ -values. The analysis considers the 95% confidence interval for the confidence level  $\alpha = 0.05$ .

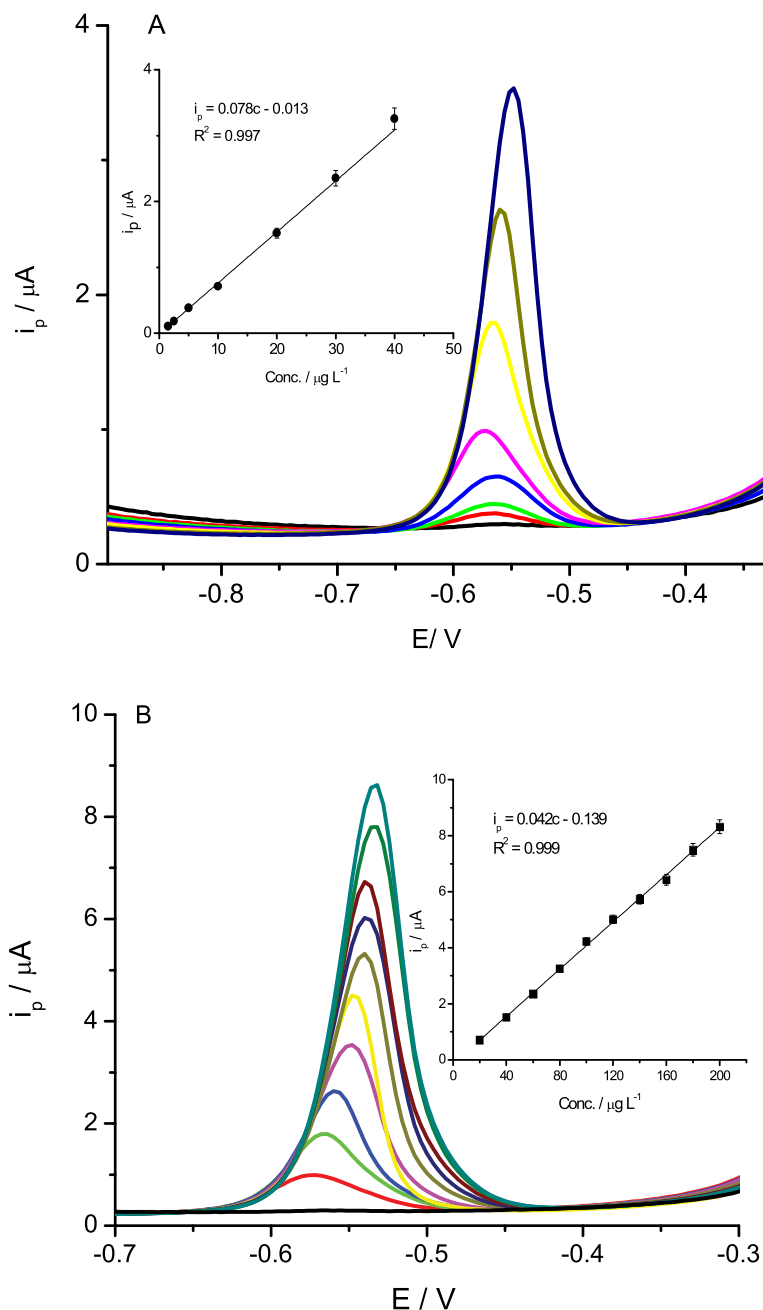
## 2.10. Interference studies

A known amount of potentially interfering metal ions was spiked to an aliquot of 10 mL of digested sample containing  $100 \mu\text{g L}^{-1}$  of lead ion to investigate their effect on the detection of lead ions according to reported procedure (Zhang et al., 2017). The amounts of interfering ion concentrations spiked were 10 and 100-fold of that of lead ion concentration. The anodic stripping currents of lead ion in the absence and presence of the interfering metal ions were compared.

## 3. Results and discussion

Square wave anodic stripping voltammetry (SWASV) using bismuth-film modified glassy carbon (GC-BiFE) and Nafion-coated bismuth-film modified glassy carbon electrodes (GC-NCBiFE) were used to quantify lead ions in vegetables, imported and locally produced cooking utensils. The anodic stripping currents were found to be linear as a function of the concentrations of lead ions in the ranges of  $20\text{--}200 \mu\text{g L}^{-1}$  and  $1.5\text{--}40 \mu\text{g L}^{-1}$  for GC-BiFE and GC-NCBiFE, respectively. A linear equation of  $i_p = 0.078c - 0.013$  with a correlation coefficient of 0.997 was obtained for GC-NCBiFE as shown in Fig. 2A, which is better in detecting lower concentration of lead ions compared to that of GC-BiFE ( $i_p = 0.042c - 0.139$ ,  $R^2 = 0.999$ ) (Fig. 2B). The sensitivity for lead ions at GC-NCBiFE was found to be two times higher than that of GC-BiFE. The sensitivity for lead ions at GC-NCBiFE was found to be two times higher than that of GC-BiFE. This result can be ascribed to the cation ion-exchange ability of Nafion film. The negatively charged sulfonate groups in the Nafion film could facilitate the non-faradaic preconcentration of metal ions (Xu et al., 2008a). Similar significant improvement in sensitivity was reported in the literature using differential pulse anodic stripping voltammetry analysis (Xu et al., 2008b). The calibration curve of GC-BiFE was only employed for the determination of lead ion up to  $20 \mu\text{g L}^{-1}$  but for further lower concentration, GC-NCBiFE was used due to its higher sensitivity.

The anodic stripping responses of lead ions in acetate buffer solutions were determined and compared with that of inductively coupled plasma-mass spectroscopy (ICP-MS) in order to validate the anodic stripping method. Acetate buffer solutions were prepared using de-ionized water and tap water separately to analyze the lead ions. The amounts of lead ions in acetate buffer were found to be  $6.22 \mu\text{g L}^{-1}$  in de-ionized water and  $15.96 \mu\text{g L}^{-1}$  in tap water which were comparable with the results found in ICP-MS method  $6.62 \mu\text{g L}^{-1}$  and  $16.09 \mu\text{g}$



**Fig. 2.** Square wave anodic stripping voltammograms as a function of the concentration of lead ions (A) 1.5–40  $\mu\text{g L}^{-1}$  at GC-NCBiFE and (B) 20–200  $\mu\text{g L}^{-1}$  at GC-BiFE in 0.1 M acetate buffer at pH 4.6. Inset: corresponding calibration curves.

$\text{L}^{-1}$ , respectively, ( $p > 0.05$ ). The  $p$ -value showed that there were no significant differences between the two methods. Hence, the simple, cheap and sensitive electroanalytical method provides reliable results for detection of lead ions in environmental samples.

Moreover, the analysis of metal ions for locally produced and imported cooking utensils were performed using AAS and the results of the analysis were given in Table 1. It is noted that aluminum and iron are the main components in both utensils. The basic differences between the two utensils are the amounts of copper, nickel, chromium, zinc, and lead which show relatively higher amounts in locally produced utensil than that of the imported one. Since locally produced utensils were prepared from wasted materials such as pieces of lead water pipes, old used utensils and galvanized metal car bodies [personal interview with producers], that could be the cause for larger concentration of the metal ions in locally produced utensils.

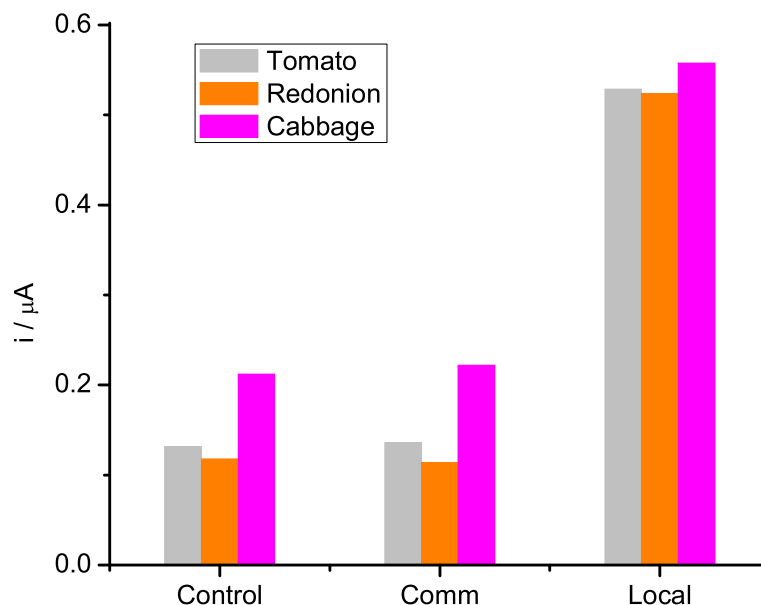
The amount of lead ions in imported cooking utensils was found to be  $7.00 \times 10^{-3}$  mg L<sup>-1</sup> using anodic stripping voltammetry. On the other hand, the amount of lead ions in locally produced utensils was found to be 6.48 mg L<sup>-1</sup> using the anodic stripping method, which is comparable with the amount, obtained using atomic absorption measurement (6.45 mg L<sup>-1</sup>). The amount of lead ions found in locally produced cooking utensil was 922 times higher than that of the commercial utensil and hence, this very high content of lead ions in the locally produced cooking utensils poses a serious health risk and immediate actions needs to be taken not to use the utensil.

Using such utensils with high lead content for cooking purposes will have a detrimental effect to health since the lead ions could easily leach out from the utensils. To confirm the leaching out of lead ions from such utensils during cooking, first the amount of lead ions present in tomato, cabbage and red onion samples cooked and uncooked were analyzed. The leaching of lead ions from the utensil to the food were determined by taking the difference of the amounts of lead ion obtained from the cooked and the uncooked samples.

**Table 1.** Comparison of metal components determined by AAS for both locally produced and imported utensils.

Metals	Locally produced (mg L <sup>-1</sup> )	RSD (%)	Imported (mg L <sup>-1</sup> )	RSD (%)
Al	9888	1.90	9024	6.94
Ca	<1.00	2.10	1.89	0.30
Cd	<1.00	0.60	<1.00	1.93
Cr	8.91	0.50	<1.00	6.00
Cu	137	2.30	<1.00	0.31
Fe	962	1.02	917	2.97
Ni	11.87	1.05	1.62	0.35
Pb	6.48	1.30	<1.00	2.30
Zn	7.39	0.62	<1.00	2.50





**Fig. 3.** Anodic stripping peak currents for lead content in different vegetables cooked using imported (comm) and locally produced (local) utensils in comparison with that of the the control.

As shown in Fig. 3 the anodic stripping peak currents for lead ions in the vegetables cooked with the locally produced utensils were significantly higher than the corresponding anodic stripping peak currents for lead ions in the vegetables cooked with imported utensils. Furthermore, the anodic stripping peak currents in the vegetables after being cooked in commercial utensil were nearly the same as that of the anodic stripping peak currents in the vegetables themselves before cooking (used as a control).

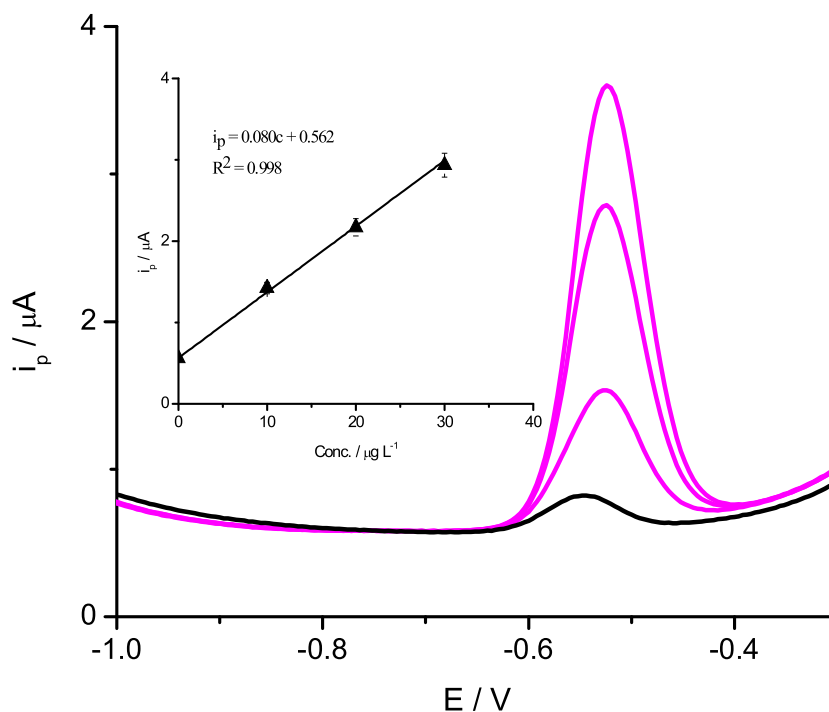
Hence, using the electroanalytical method, the amounts of lead ions in tomato, red onion and cabbage cooked using the locally produced utensils were found to be 1.62, 1.55 and 2.12 mg kg<sup>-1</sup>, respectively. These values are significantly higher than the lead content found in the vegetables before being cooked (0.31, 0.38 and 0.66 mg kg<sup>-1</sup>) (control) with  $p < 0.001$ , as shown in Table 2. On the other hand, the lead contents found in the vegetables after being cooked with imported utensils are nearly comparable to the value found in the control ( $p > 0.05$ ). The results clearly indicates that there was a leaching out of lead ions from the locally produced utensils while cooking the vegetables. The lead contents observed in this study were beyond the permissible level of 0.1 mg kg<sup>-1</sup> (Corguinha et al., 2015) and calls for immediate interventions.

Standard addition method was used to investigate the matrix effect during the electroanalytical determination of the lead content in various vegetables. Fig. 4 shows a typical square wave anodic stripping voltammograms found for cabbage samples with subsequent additions of 10 μg L<sup>-1</sup> of lead ions together with

**Table 2.** Amount of lead ions found in tomato, red onion, and cabbage before cooking (control) and after being cooked in locally produced (local) and imported (comm.) utensils.

sample	Concentration lead ion ( $\text{mg kg}^{-1}$ )			<i>p</i> -value (control vs comm.)	<i>p</i> -value (control vs local)
	Control	comm.	local		
Tomato	$0.31 \pm 0.01$	$0.34 \pm 0.01$	$1.62 \pm 0.01$	0.264	<0.001
Red onion	$0.38 \pm 0.01$	$0.32 \pm 0.01$	$1.55 \pm 0.02$	0.102	<0.001
Cabbage	$0.66 \pm 0.01$	$0.61 \pm 0.01$	$2.12 \pm 0.01$	0.264	<0.001

corresponding standard addition plot ( $i_p = 0.080c + 0.562$ ,  $R^2 = 0.998$ ). All the other vegetable samples were also analyzed with similar procedures and the equations obtained from standard addition plots were  $i_p = 0.078c + 0.518$ ,  $R^2 = 0.996$  and  $i_p = 0.079c + 0.553$ ,  $R^2 = 0.998$  for tomato and red onion, respectively. The sensitivities of the plots for the cabbage, red onion and tomato were nearly the same as that of the calibration plot ( $0.078 \mu\text{A L mg}^{-1}$ ). Hence, the various sample matrixes did not affect the electroanalytical determination.



**Fig. 4.** Typical anodic stripping voltammograms of lead ions found in the cabbage sample with successive standard additions of  $10 \mu\text{g L}^{-1}$  of lead ions in 0.1 M acetate buffer at pH 4.6. Inset: corresponding standard addition plot.

**Table 3.** Recovery results for the determination of lead ions in the vegetable samples.

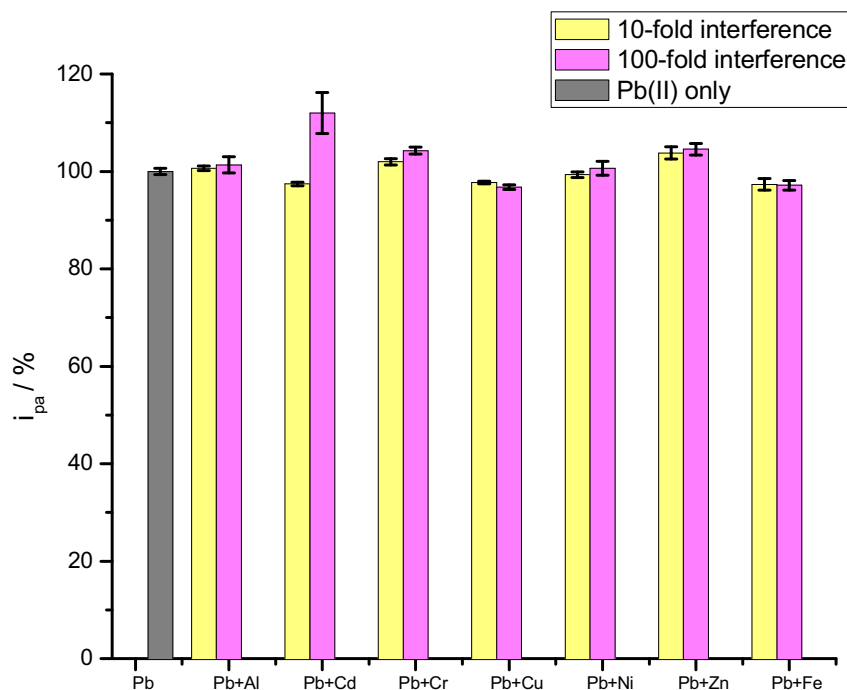
Matrix	Pb(II) added (mg kg <sup>-1</sup> )	Found (mg kg <sup>-1</sup> )			Recovery (%)		
		local	control	Comm.	local	control	Comm.
Tomato	–	1.62 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	–	–	–
	2.50	3.98 ± 0.03	3.04 ± 0.02	2.90 ± 0.02	94.8 ± 0.5	109.7 ± 0.7	102.3 ± 0.1
	5.00	6.99 ± 0.02	5.80 ± 0.05	5.78 ± 0.03	107.6 ± 0.6	110.1 ± 0.1	108.9 ± 0.4
	7.50	9.43 ± 0.03	8.03 ± 0.03	7.78 ± 0.02	104.2 ± 0.8	103.1 ± 0.8	99.2 ± 0.5
Cabbage	–	2.12 ± 0.01	0.66 ± 0.01	0.61 ± 0.01	–	–	–
	2.50	4.37 ± 0.03	3.33 ± 0.03	3.33 ± 0.02	90.1 ± 0.2	107.9 ± 1.0	108.8 ± 0.6
	5.00	6.98 ± 0.02	6.02 ± 0.02	5.78 ± 0.02	97.2 ± 0.2	107.7 ± 0.8	103.5 ± 0.5
	7.50	9.27 ± 0.02	8.74 ± 0.03	9.13 ± 0.02	95.4 ± 0.1	108.0 ± 0.9	113.7 ± 0.7
Red onion	–	1.55 ± 0.01	0.38 ± 0.01	0.32 ± 0.02	–	–	–
	2.50	4.49 ± 0.03	2.99 ± 0.03	2.95 ± 0.03	117.5 ± 0.5	104.5 ± 0.4	105.5 ± 0.2
	5.00	6.81 ± 0.03	6.16 ± 0.02	5.80 ± 0.02	105.2 ± 0.3	115.8 ± 0.8	109.8 ± 0.3
	7.50	9.37 ± 0.02	8.12 ± 0.02	8.35 ± 0.02	104.3 ± 0.1	103.2 ± 0.1	107.2 ± 0.5

To further confirm the validity of the electroanalytical method used for the determination of the lead content in various vegetables, recovery tests were performed using standard addition method. The recovery results were summarized in Table 3 and the values were within the acceptable recovery ranges. This further confirms that the complex matrixes in the vegetable samples do not interfere during the electroanalytical determination of lead ions.

Interference studies were made by adding 10 and 100-fold excess amounts of potentially interfering metal ions to digested utensil samples containing 100 µg L<sup>-1</sup> of lead ion. The percent signal changes found for both 10 and 100-fold concentrations were less than 5% for Al(III), Cr(III), Cu(II), Ni(II), Zn(II) and Fe (III) ions which indicate that the interferences of the metal ions for the determination of the lead ion is insignificant (see Fig. 5). However, addition of 100-fold Cd(II) ion concentration positively interfere the electrochemical response. Fortunately, the amount of Cd(II) ion in the local produced utensil sample was found to be less than 1 mg L<sup>-1</sup> as shown in Table 1, which could have no effect in the analysis.

#### 4. Conclusion

In conclusion square wave anodic stripping voltammetric technique using BiFGE and NC-BiFGE for determination of lead(II) have been successfully utilized for the analysis of different real samples. The method has many advantages like low cost,



**Fig. 5.** Interference percentage of the SWASV responses for the analysis of Pb(II) from cooking utensil samples using BiFE in the presence of only Pb(II) ions and Pb(II) ions combined with other interfering ions.

easy handling and good sensitivity and can be used for routine analysis of lead(II) in different real samples. Further concerted effort is needed to develop this novel electrochemical method for a low cost, efficient, reliable and convenient method for on-site monitoring of lead(II) in different environmental samples. It is noted that the locally produced utensil contains extremely high content of lead(II) which also easily leaches out while cooking foods. Hence, it is recommended that an immediate action to be taken by the concerned authority to ban the use for such utensils of high lead content for household purposes.

## Declarations

### Author contribution statement

Belete Tesfaw: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Solomon Mehretie, Shimelis Admassie: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

## Competing interest statement

The authors declare no conflict of interest.

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## Additional information

No additional information is available for this paper.

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