Trace Metal Biomonitoring and Assessment in Groundwater, Soil and Pearl Millet (Pennisetum glaucum) Plants in Industrial and Urban Areas in Tehama by Using Square-Wave Voltammetry

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Abstract: Different experimental approaches have been suggested in the last few decades to determine metal species in complex matrices of unknown composition which are underground water, soil and Pennisetum glaucum (Pearl millet) plant samples, which used mainly for forage production as indicators of environmental pollution. The methods are mainly focused on the determination of single species or groups of species. The more recent developments in trace elements speciation are reviewed focusing on methods for labile and free metal determination. Electrochemical procedures with low detection limit as anodic stripping voltammetry (ASV) and the competing ligand exchange with adsorption cathodic stripping voltammetry (AdCSV) have been widely employed in metal distribution studies. Other electrochemical methods such as square-wave voltammetric (SWV) seem to be promising to evaluate the free metal concentration at the low levels of environmental samples at industrial city (Hodeidah) and historically interesting village in Yemen (Zabid) during the summer of 2008. The application of square-wave voltammetry (SWV) for the determination of six elements viz. Cd (II), Pb (II), Cu (II), Co (II), Ni (II) and Cr (VI) in environmental samples has been examined and optimized. The cathodic and anodic types of the SWV technique were examined for the detection of these metal ions. It was found that the square-wave anodic stripping voltammetry is the conventional technique for the determination of Cd (II), Pb (II) and Cu (II), but square-wave adsorptive cathodic stripping voltammetric method is used for the determination of Co (II), Ni (II) and Cr (VI). Various experimental parameters, which influence the response of the mercury film electrode to these metal ions, were optimized. The results of physicochemical parameters showed that the temperature values varied from 19°C to 25°C and pH values from 7.35 to 8.93 and the transparency from 40cm to 220cm. The detection limits of these metal ions were 0.0275, 0.3917, 0.0449, 0.0425, 0.1893 and 0.2792 µg/kg for Cd (II), Pb (II), Cu (II), Co (II), Ni (II) and Cr (VI), respectively; with very good accuracy (standard deviation is below 2%). Interference from coexisting ions was successfully investigated. A comparison of analytical data for analyzing real samples was carried out between the SWV method and the graphite furnace atomic absorption spectrophotometric (GFAAS) method. By the standard addition method, the relative standard deviation (RSD) were 0.14433 - 4.58762% and relative error (RE) 0.2037 - 6.938% values. Statistically, from one way analysis of variance, the highest significant changes in all heavy metal contents versus location differences (P < 0.0001) were calculated using SWV and lowest significance changes (P < 0.01 and P < 0.05) were recorded in Cu, Co and Cr using GFAAS method, which in turn confirm the sensitivity of SWV method. The great advantage of SWV is the simplicity, selectivity, sensitivity and shortening analysis time over the GFAAS method.

Keywords: Stripping voltammetry · Cd · Pb · Cu · Ni · Co · Cr · water · Soil · Pennisetum glaucum plant

INTRODUCTION

A number of methods based on different concepts and experimental procedures have been proposed and widely used to this aim. The ideal speciation technique should be able to quantify the amount of free and bound metal and also to provide some insight on dynamic changes in behavior of the species, which seems to play
an important role on the bioavailability and consequently on toxicity of metals [1-2]. Spectroscopic techniques, as graphite furnace atomic absorption spectroscopy (GF-AAS) and inductively coupled plasma mass spectroscopy (ICP-MS), but also neutron activation analysis (NAA) have very low detection limits and have been widely used for trace metal analysis. However, these methods can be used for speciation analysis only by coupling them to separation procedures [3].

In contrast, the electrochemical techniques are well suited to directly assess particular metal species, so that they have been and still are, widely used also for in situ application [4]. Some electrochemical methods make possible the determination of metal species and of the free metal ion concentration, which is one of the foremost goals in metal speciation analysis. This particular species is of fundamental interest for determining the quality of environmental water, as assumed in some successful toxicity models, such as the Biotic Ligand Model, BLIM [5] and the Free Ion Activity Model FIAAM [6]. They are based on the concept that the toxicity is mainly due to the free metal ion permeability through the biological membranes. Although recent studies showed the relevant toxicological role of hydrophobic neutral complexes, at least for copper (II) [7] and a few other toxic and essential metals [8], the free metal ion is still widely recognized as the most important species permeating through biological membranes. Its determination in environmental samples is difficult because of the very low concentrations and of the presence of interferences. There is a limited number of methods with the required selectivity and detection limit. Potentiometry with ion selective electrodes (ISE) is in principle the ideal method for this evaluation, because it does not disturb the equilibria and the sample composition during the analytical procedure [9]. Potentiometry is often used as a reference method for assessing other speciation techniques [10].

Voltammetry, including a preconcentration step have been widely applied for metal ion determination in environmental waters, because of their sensitivity and low detection limits. Many efforts have been spent to model the data produced by these methods, because in some way they disturb the metal–ligand equilibria, at least near the electrode, where the metal is consumed. Thus the problem is about the relation between the signal and the metal species which actually contribute to the current. For example with anodic stripping voltammetry (ASV), the current is due to the free metal ion at the surface of the electrode, either that moving from the bulk of the solution by diffusion and that produced by the dissociation of the labile complexes in the diffusion layer. This is a dynamic concept related to the residence time of the species in the diffusion layer and on their kinetical lability [11-12].

Complexes are defined as inert if they do not contribute to the overall current and only the free metal reduction occurs and as labile if they are characterized by high rates of association/dissociation. Criteria to assess the kinetic lability have been proposed. Since the measured current may depend also on the presence of heterogeneous ligands and organic matter which can be adsorbed at the electrode surface [13], ASV is mainly used to evaluate the complexing capacity of natural ligands by titration with the metal ion of concern [14].

Sensitive and stable monitoring of heavy metals in seawater using screen-printed electrodes (SPE) is presented. The analytical performance of SPEs coupled with square wave anodic stripping voltammetry (SWASV) for the simultaneous determination of Pb and Cd in seawater samples, in the low μg L⁻¹ range, is evaluated. The stripping response for the heavy metals following 2min deposition was linear over the concentration range examined (10–2000 μg L⁻¹) with detection limits of 1.8 and 2.9 μg L⁻¹ for Pb and Cd, respectively. The accuracy of the method was validated by analyzing metal contents in different spiked seawater samples and comparing these results to those obtained with the well-established anodic stripping voltammetry using the hanging mercury drop electrode. Moreover, a certified reference material was also used and the results obtained were satisfactory [15].

The rechargeable mercury film silver based electrode Hg (Ag) FE applied for determination of Cr (VI) traces using catalytic adsorptive stripping voltammetry (CADSV) will be presented. The film electrode is characterized by its very good surface reproducibility (not less than 2%) and long-term stability (1500–2000 measurement cycles). The mechanical refreshing of mercury film is realized in the specially constructed device, in a time shorter than 1–2s [16]. However, mercury film electrodes (MFEs) are more suitable than the HMDEs for field applications and online measurements by virtue of their robustness, mechanical stability and sample maintenance [17-18]. Mercury-film electrodes, when combined with square-wave adsorptive stripping voltammetry (SWA dSV), can lead to a suitable trace analysis technique that is fast and sensitive tolerant to dissolve oxygen [19].

It is estimated that only 50% of dairy farms in the dry land regions of Victoria have suitable dairy effluent systems and of these only 25% are managed effectively. Furthermore, most farmers apply effluent to less than 10% of their available land, often to the same area each year.
Despite being seen by many farmers as an undesirable waste, dairy effluent contains relatively large amounts of agronomically useful nutrients especially nitrogen (N) and potassium (K). Studies in New Zealand [20] indicate substantial dry matter (DM) yield increases are possible when effluent is applied to perennial pasture throughout the year. It should however be noted that the climatic conditions under which these trials were conducted are different to those in Southern Victoria where effluent application in winter is likely to lead to nutrient run off or leaching. Given this limitation, there is potential to investigate the use of effluent on summer active forages. Previous work [21] highlighted (DM) yield increase when effluent was applied to turnips.

The introduction of heavy metals and metalloids to the food chain via various anthropogenic sources poses a serious threat to human health. In particular, the husbandry and/or processing practices adopted during the growth and handling of foods represent a major source of contamination by inorganic and organic substances. Owing to the growing concern in this area, many national and international regulatory bodies on food quality have lowered the maximum permissible levels of toxic metals in human food. As an example, the maximum tolerable daily intake set by FAO/WHO Joint Expert Committee on Food Additives for cadmium and lead for all sources (including air, food and water) are 1.0 - 1.2 μg/kg and 3.5 - 4.0 μg/kg body mass, respectively. Consequently, the control of the concentrations of trace metals in foods and possible minimization of food pollution by improving husbandry and food processing practices has become a significant aspect in achieving high food quality. As food is one of the main sources of toxic metals, it constitutes a significant pathway through which toxic metals are ingested by human and animals. Therefore, the on-going monitoring of these substances in foods and beverages with a high degree of accuracy, reliability and sensitivity at trace and ultra-trace concentrations is necessary for the protection of human health [22].

Microbial biosensors have been developed for voltammetric determination of various substances. This method describes the development of a new biosorption based microbial biosensor for determination of Cu^{2+}. The developed biosensor is based on carbon paste electrode consisting of whole cells of Cricinella sp. Cu^{2+} was preconcentrated on the electrode surface at open circuit and then cathodically detected with the reduction of Cu^{2+}. The voltammetric responses were evaluated with respect to percentage cell loading in the carbon paste, preconcentration time, pH of preconcentration solution, scan rate and interferences. The optimum response was realized by biosensor constructed using 5 mg of dry cell weight per 100 mg of carbon paste in pH 5.5 preconcentration solution. Under the optimum experimental conditions, the developed microbial biosensor exhibited an excellent current response to Cu^{2+} over a linear range from 5.0 x 10^{-7} to 1.0 x 10^{-3} M (r^2 = 0.9938) with a detection limit of 5.4 x 10^{-6} M (S/N = 3). The microbial biosensor had good sensitivity and reproducibility (R.S.D. 4.3%, n = 6). Finally, the applicability of the proposed microbial biosensor to voltammetric determination of Cu^{2+} in real sample was also demonstrated and validated with atomic absorption spectrophotometric (AAS) method [23].

The aim of this work is to evaluate the performance of the square-wave stripping voltammetric procedures for the determination of several heavy metals in underground water, soil (0-50 cm), Peniseum plant samples at control environmental location and industrial location.

Experimental Instrumentation

Atomic Absorption Spectrometer Zeenit 700 (Analytikjenaag): The Zeenit700 Atomic Absorption Spectrometer (AAS) is ideal for high performance, low cost, routine elemental analysis. Since the Zeenit700 is a simple to use elemental analysis instrument, it meets a wide range of applications across many analytical sectors. It finds a home supplying analytical advantages in the Chemical, Environmental, Mining, Metallurgical, Pharmaceutical, Energy, Agricultural, Manufacturing and Utilities industries. Convenient automatically aligned 5-lamp turret, each with its own independent power supply. Software Version used for procedures in this manual: WinAAS Version 3.15 is an exceptionally well-designed Windows® based software package that allows you to multi-task within the Window's environment. It is easy to operate, as it provides a working background already familiar to virtually all users. This program is designed to automatically generate calibration curves based on the standard solutions analyzed and operate within an MS Excel format, thereby minimizing statistical & graphical analysis time.

EG&G Princeton Applied Research 273A Potentiostat /Galvanostat: For the voltammetric measurements, an EG&G PAR Model 273A potentiostat with 250/270 research electrochemistry software version 4.0, manufactured by Princeton Applied Research Corporation (NJ) was used with a glassy carbon-working electrode
(K0066, EG&G, 4mm diameter). Stirring was performed with a Teflon coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechnik, Germany). Silver/silver chloride (saturated KCl) was used as a reference electrode and a platinum wire as an auxiliary electrode. A Pyrex glass cell was used for the measurements with the MFE; either this cell or a Teflon cell was used with the MFE. All the pH measurements were made with an Orion Model 601 digital pH meter.

**Chemicals:** Nitric, perchloric and phosphoric acids, sodium monohydrogen phosphate, sodium dihydrogen phosphate, ammonium chloride, ammonium hydroxide, sodium nitrate and borax (Merck) were used. The supporting electrolyte required for the determination of Pb(II), Cd(II) and Cu(II) was a mixture of 0.01M H₂PO₄ and 0.01M HNO₃ acids. For Ni (II) and Co (II) determinations, 0.02M ammonium buffer (NH₄HCl) with 2x10⁻⁵ M dimethylglyoxime (DMG) (Analar Grade) was used [24]. However, 0.01M NaNO₂ and 0.001M mixtures of mono- and dihydrogen phosphate buffer were prepared for the selective detection of Cr (VI) as recommended by Ghandour et al. [25]. Bidistilled water was used for the preparation of all solutions. The optimal conditions and the accuracy of the concentrations determined in this study were checked by the measurements of the certified reference material, Whole Milk Powder 8435 (WMP8435), from the National Institute of Standard and Technology in Canada. The mercury plating solution was 2x10⁻⁷ M Hg (NO₃)₂·H₂O in 0.1M KNO₃ and 0.01M HNO₃ as carrier solution.

**Electrode Preparation:** The plating was carried out by adding the Hg(II) solution in 0.1M KNO₃ and 0.01M HNO₃ while the electrode potential was held at −1.0V; usually, 1 min was a sufficient period to ensure a satisfactory mercury coating of the glassy carbon electrode surface. After each experiment, the mercury film was cleaned by wiping the electrode with a wet tissue followed by plating a new mercury film for performing a new experiment.

**Samples Collection and Treatment:** Samples were collected from two locations at Hodeidah and Zabid, Yemen. Sampling locations are shown in location map (Fig.1): (1) 45° 4’ 6.08” N, 43° 5’ 55.7” E; 40 mASL at Hodeidah and location (2) 12° 14’ 12.63” N, 19° 43’ 18.47” E; 106 mASL at Zabid. Many studies have already been published on the preparation of samples for wet chemical analysis. Two main techniques have been used: alkaline fusion and acidic decomposition or extraction. Alkaline fusion [26-27] induces high blank levels and high detection limits. Due to the small amounts of particulate matter, acidic methods are more suitable. There are many ways to proceed with acids. In addition, various parameters, such as the method of heating, working pressures and acids, have a role to play in the digestion efficiency. The use of low quantities of acid allows small volumes of final solution to be handled and the detection limits to be improved [28]. The principal acids used for particle digestion are HNO₃, HCl, HClO₄, HF and H₂SO₄ [29-32]. However, the use of HF leads to long, dangerous and cumbersome schemes and it is not recommended for routine analysis [33]. Thus, in environmental analytical

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**Fig. 1:** Sampling locations map
chemistry, acid leaching has become a common procedure as an alternative to total digestion. Underground water sample (S₁), two top soil samples S₂ (at the surface) and S₃ (50 cm depth) and *Pennisetum* samples were divided to three subsamples (leaves S₄, stems S₅, and roots S₆).

One gram of each sample from S₁, S₄, and S₆, was stirred in bidistilled water for 1 h and then filtered. The filtrate of S₁ and S₆ were transferred into 100-mL measuring flask and diluted to the required volume (100mL) by bidistilled water. The second filtrate sample S₄ and S₅ are mixed with 5mL concentrated nitric acid and evaporated to dryness. The residue was dissolved in the bidistilled water and transferred into a 100-mL measuring flask and diluted to the required volume with bidistilled water.

Also, one gram of each *Pennisetum* plant samples S₁, S₄, and S₆ after dryness and grinding were dissolved in 5mL concentrated nitric acid and evaporated to dryness. The residue was dissolved in bidistilled water and transferred to 100mL measuring flask and completed to volume with bidistilled water. All glass ware and polyethylene bottles were soaked in 2M nitric acid for at least 1 week, washed three times with bidistilled water and finally soaked in 0.1M hydrochloric acid until being ready for use.

**Procedures:** The peak current obtained in SW voltammetry is dependent on various instrumental parameters such as SW amplitude, SW frequency and scan increment. These parameters are interrelated and affect the response, but here only the general trends will be examined. It was found that these parameters had little effect on the peak potential. The results of this optimization study show that the set of square-wave amplitude of 25 mV, frequency of 60 Hz and scan increment of 2mV are best suited for routine determination of these metals. Since the scan increment together with the frequency defines the effective scan rate, an increase of either the frequency or the scan increment results in an increase in the effective scan rate. By maintaining both the frequency and the scan increment at 60 Hz and 2 mV, respectively, the effective scan rate is 120mV/s.

Cd (II), Pb (II) and Cu (II) were determined in the same cell. The cell contains a 5mL sample, in presence of 0.01M H₃PO₄, 0.01M HNO₃, and diluted to 10mL using bidistilled water. The deposition potential was controlled at -0.80, -0.63 and -0.30V vs Ag/AgCl electrode for Cd (II), Pb (II) and Cu (II), respectively. The deposition time for each sample was studied and it was found that 60s for Cd (II) and 50s for Pb (II) and Cu (II) are suitable. After the deposition step and a further 15s (equilibrium time), the voltammogram was recorded in the anodic direction. Different concentrations from the metal ions were added to the cell using an automatic pipette while keeping the deposition time constant. The concentrations of Cd (II), Pb (II) and Cu (II) were calculated in the sample using the standard addition method.

In the case of Ni (II) and Co (II) quantization using SWACSV, a mixture of 0.02M ammonium buffer (NH₄Cl + HCl); pH=9 was used as a supporting electrolyte and 2x10⁻³ M DMG was used as a complexing agent. The accumulation potentials were -0.70 and -0.85V for Ni (II) and Co (II) for 30 and 20s preconcentration time, respectively.

To detect Cr (VI) in the samples under consideration, 0.01M sodium nitrate and 0.01M of mixed mono- and disodium hydrogen phosphate buffer were used in the presence of 5mL sample and the volume was diluted to 10mL into the voltammetric cell. The preconcentration potential was adjusted at +0.1V for 30s and the potential was terminated at -0.5 V.

**RESULTS AND DISCUSSION**

**Optimization of the Solution Conditions:** Square-wave anodic stripping voltammetry is a suitable method for the determination of Cd (II), Pb (II) and Cu (II). The metal deposits into the electrode as amalgam for a period of time by electrolysis phenomenon. The effects of supporting electrolyte, pH, deposition potential and deposition time were investigated as shown in Table (1).

\[ \text{M}^{2+} + 2e + \text{Hg} \rightarrow \text{M} \quad \text{(deposition step)} \]

The dissolved amalgam is then reoxidized back to the following equation:

\[ \text{M} \quad \text{Hg} \rightarrow \text{M}^{2+} + 2e + \text{Hg} \quad \text{(stripping step)} \]

While, Ni (II), Co (II) and Cr (VI) have been determined using square-wave adsorptive cathodic stripping voltammetry (SWACSV). This method involves complexation of both metal ions with the ligands, e.g., DMG and adsorbing the resulting complex onto the mercury surface. The preconcentration involves interfacial accumulation of an adsorption layer of the nickel and cobalt bis(dimethylglyoximate) at the mercury film surface. The adsorbed metal complex is electrochemically removed by scanning the electrode potential, in a
Table 1: Anodic square-wave voltammetric (ASWW) conditions for the determination of Cu, Pb and Cd in water, soil and plant samples

<table>
<thead>
<tr>
<th>Metal Ions</th>
<th>Supporting electrolyte (M)</th>
<th>pH</th>
<th>Deposition potential (V)</th>
<th>Final potential (V)</th>
<th>Deposition time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd(II)</td>
<td>0.01 M HNO₃ + 0.01 M H₂PO₄</td>
<td>2.3</td>
<td>-0.89</td>
<td>-0.61</td>
<td>60</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>0.01 M HNO₃ + 0.01 M H₂PO₄</td>
<td>2.1</td>
<td>-0.65</td>
<td>-0.26</td>
<td>30</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>0.01 M HNO₃ + 0.01 M H₂PO₄</td>
<td>2.4</td>
<td>-0.30</td>
<td>+0.12</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2: Adsorptive cathodic square-wave voltammetric (SWA-CSV) conditions for the determination of Co, Ni and Cr in water and plants samples

<table>
<thead>
<tr>
<th>Metal Ions</th>
<th>Supporting electrolyte (M)</th>
<th>pH</th>
<th>Accumulation potential (V)</th>
<th>Final potential (V)</th>
<th>Preconcentration time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(II)</td>
<td>0.02 M (H₂SO₄-H₂O + 2x10⁻⁴ M DMGl)</td>
<td>9.0</td>
<td>-0.70</td>
<td>-1.2</td>
<td>30</td>
</tr>
<tr>
<td>Co(II)</td>
<td>0.01 M (H₂SO₄-H₂O + 2x10⁻⁴ M DMGl)</td>
<td>9.2</td>
<td>-0.85</td>
<td>-1.35</td>
<td>10</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>0.001 M Na₂HPO₄ - NaH₂PO₄ + 0.01 M NaNO₃</td>
<td>7.2</td>
<td>+0.10</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

The optimal operating parameters such as supporting electrolyte, pH, preconcentration time and accumulation potential are tabulated in Table 2.

For Cr (VI) determination, peak height was attained with a maximum value in the presence of 0.01 M of monosodium hydrogen phosphate buffer and 0.01 M NaNO₃ with pH = 7.2. It was observed that a sharp cathodic peak is located at -0.15 V. This peak is due to the adsorption of the reduced form; Cr (III) phosphate hydrolyzed species obtained from the reduction of Cr (VI) to Cr (III) which coats the surface of the electrode [34]. The influence of accumulation potential and preconcentration time on the peak current has been examined and the optimal conditions have been set up for the detection of Cr (VI) as shown in Table 2. Figures 2 and 3 represent ASW and SWA-CSV voltammograms obtained for Cd, Pb, or Cu and Ni, Co or Cr, in soil (S2) sample, respectively. Some heavy metals percentages analyzed using two methods (SWCS and AAS) in underground water, soil and Pennisetum glaucum plant in two different locations (Hodeidah and Zabid) as shown in Fig. 4.

The use of computer-controlled equipment for a flexible choice of parameters makes it possible to eliminate the background current. The procedure of background current correction is very simple. The blank correction leads, in some cases, to a significant increase in the determination of sensitivity. Thus, for the determination of Ni and Co in underground water, soil and Pennisetum glaucum plant samples, the background correction helps to eliminate the Ni signal corresponding to Ni contamination of the supporting electrolyte and to increase the sensitivity of Co determination [35]. The great advantage of square wave voltammetry (SWV) is the rejection of all currents that correspond to slow electrode processes. In voltammetric trace analysis, oxygen reduction often produces unacceptably large and unstable residual currents. However, with SWV the oxygen reduction current is almost completely rejected [36].

Analytical Limits (Limit of Detection and Determination): The detection (XN) and determination (XB) limits have been established as the concentration expressed in nanograms of the element per gram of sample, giving a current reading statistically different from that of the blank and they have been calculated by dividing 3 and 10 times the standard deviation of the current readings of the blank by the slope of the analytical curve, respectively.

\[ X_N = t_p \times sc0 \times \sqrt{\frac{1}{n} + \frac{1}{m} + \sum_{i=1}^{k} \frac{x_m^2}{(X_i - x_m)^2}} \]

\[ sc0 = \frac{sy0}{\Gamma(x_m)} \quad \text{co} = 0.0044/\text{Slop} \]

\[ X_B = 3 \times t_{p2} \times sc0 \times \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(3X_N - x_m)^2}{\sum_{i=1}^{k} (X_i - x_m)^2}} \]

Based on blank replicates: \( X_N = (3 \times SD_{nl} \times co) / 0.0044 \) and \( XB = (10 \times SD_{nl} \times co) / 0.0044 \)

Where,

- \( t_p \) = Student factor for significance level \( p \) (one-tailed), \( t_s = t(1-p) \)
- \( t_{p2} \) = Student factor for significance level \( p \) (two-tailed), \( t_{p2} = t((1-p)/2) \)
- \( p \) = Significance level 0.00 -1.00, \( t = t \) distribution function (Student distribution)
- \( n \) = number of standard, \( m \) = number of replicates per standard
- \( x_m \) = mean concentration \( x_m = (\sum X_i) / n \), \( x_i \) = entered calibration concentration (known value)
- \( sy0(1) \) = residual variance of calibration function including outliers
- \( sy0(2) \) = residual variance of calibration function without outliers
- \( \Gamma(x) \) = slope of calibration function for \( x \)
- \( SD_{nl} \) = standard deviation of measured values of IDL sample.
Fig. 2: ASWV voltammograms of Cd, Pb and Cu in soil sample (S2) at location (1), without digestion at 60s deposition times: (a) Sample of Cd, Pb and Cd metal ions; (b) 8.00, 9.63, 5.42, and 6.82 ppb of Cu, Pb and Cd metal ions; (c) 14.55, 18.00, 10.20, and 9.2 ppb of Cu, Pb and Cd metal ions; (d) 21.23, 36.23, 15.53, and 13.25 ppb of Cu, Pb and Cd metal ions; and (e) 27.29, 45.95, 29.59, and 19.12 ppb of Cu, Pb and Cd metal ions.

Fig. 3: SWA4CS voltammograms of Cr, Ni, and Co in soil sample (S2) at location (1), with digestion at 30s deposition times: (a) Sample of Cr, Ni, and Co metal ions; (b) 1.63, 0.81, and 25 ppb of Cr, Ni and Co metal ions; (c) 5.26, 2.36, and 50 ppb of Cr, Ni and Co metal ions; and (d) 9.4, 3.78, and 75 ppb of Cr, Ni and Co metal ions.
Fig. 4: Heavy metals percentages analyzed using two methods (SWCS and AAS) in water, soil and plant in two different locations (Hodeida and Zabid, Yemen).

Fig. 5: Calibration plot (i_p/c) of Pb (II) obtained by standard addition method at E_s=-0.63V, t_s=30s and pulse height=25mV for (a) the element present alone in the solution and (b) the element mixed with 0.1 mg/L of the other five investigated elements.
Table 3: Determination of Co, Pd, Cu, Cd, Ni and Cr(III) detected in soil, water and plant samples (n=6)

<table>
<thead>
<tr>
<th>Location</th>
<th>Co (μg/kg)</th>
<th>Pd (μg/kg)</th>
<th>Cu (μg/kg)</th>
<th>Ni (μg/kg)</th>
<th>Cd (μg/kg)</th>
<th>Cr (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>0.494±0.0040</td>
<td>0.430±0.023</td>
<td>9.73±0.02</td>
<td>9.34±0.05</td>
<td>16.71±0.53</td>
<td>15.66±0.52</td>
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<tr>
<td>Location 2</td>
<td>0.77±0.05</td>
<td>0.65±0.06</td>
<td>9.26±0.05</td>
<td>9.34±0.05</td>
<td>16.71±0.53</td>
<td>15.66±0.52</td>
</tr>
<tr>
<td>Location 3</td>
<td>0.81±0.01</td>
<td>0.93±0.08</td>
<td>25.93±0.93</td>
<td>34.73±1.93</td>
<td>12.34±0.13</td>
<td>12.34±0.13</td>
</tr>
<tr>
<td>Location 4</td>
<td>0.79±0.04</td>
<td>0.38±0.04</td>
<td>35.94±1.43</td>
<td>15.14±0.41</td>
<td>12.34±0.13</td>
<td>12.34±0.13</td>
</tr>
<tr>
<td>Location 5</td>
<td>0.70±0.10</td>
<td>0.95±0.04</td>
<td>35.94±1.43</td>
<td>15.14±0.41</td>
<td>12.34±0.13</td>
<td>12.34±0.13</td>
</tr>
<tr>
<td>Location 6</td>
<td>0.14±0.00</td>
<td>0.20±0.01</td>
<td>0.83±0.03</td>
<td>0.91±0.07</td>
<td>1.18±0.01</td>
<td>1.48±0.01</td>
</tr>
<tr>
<td>Location 7</td>
<td>0.17±0.05</td>
<td>0.15±0.05</td>
<td>11.74±0.01</td>
<td>11.04±0.02</td>
<td>1.24±0.01</td>
<td>1.24±0.01</td>
</tr>
<tr>
<td>Location 8</td>
<td>0.99±0.02</td>
<td>0.79±0.03</td>
<td>27.09±0.02</td>
<td>25.93±0.93</td>
<td>12.34±0.13</td>
<td>12.34±0.13</td>
</tr>
</tbody>
</table>

Location 1 (n=6)

<table>
<thead>
<tr>
<th>By using (SPCE) method</th>
<th>By using (AECA) method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anodic stripping voltammetry</td>
<td>Cathodic stripping voltammetry</td>
</tr>
</tbody>
</table>

Table 4: Significance changes in heavy metals in two locations and in underground water, soil and Permian clay samples using one way analysis of variance (n=6) versus location differences

<table>
<thead>
<tr>
<th>Samples</th>
<th>Co (μg/kg)</th>
<th>Pd (μg/kg)</th>
<th>Cr (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (G)</td>
<td>(5.3±0.4)*</td>
<td>(1.7±0.1)*</td>
<td>(1.6±0.7)*</td>
</tr>
<tr>
<td>E1 (1)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E2 (2)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E3 (3)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E4 (4)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E5 (5)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E6 (6)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E7 (7)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
<tr>
<td>E8 (8)</td>
<td>(1.6±0.7)*</td>
<td>(5.6±0.7)*</td>
<td>(2.5±0.5)*</td>
</tr>
</tbody>
</table>

Table 5: Relative standard deviation (RSD %) and relative error (RE %) values of various concentration levels of the investigated elements under the optimal conditions

<table>
<thead>
<tr>
<th>Element</th>
<th>Taked (μg/kg)</th>
<th>Found (μg/kg)</th>
<th>RSD (%)</th>
<th>RE (%)</th>
<th>Spike</th>
<th>Intersect</th>
<th>LDR (μg/kg)</th>
<th>C(3x)</th>
<th>RSD</th>
<th>Linear Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>1.00</td>
<td>0.993±0.0040</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
<td>0.0071±1</td>
<td>1.0±0.00</td>
<td>0.8±0.00</td>
<td>0.1±0.00</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1.00</td>
<td>1.049±0.0107</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
<td>0.0071±1</td>
<td>1.0±0.00</td>
<td>0.8±0.00</td>
<td>0.1±0.00</td>
</tr>
<tr>
<td>Ni(III)</td>
<td>1.00</td>
<td>0.959±0.0069</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
<td>0.0071±1</td>
<td>1.0±0.00</td>
<td>0.8±0.00</td>
<td>0.1±0.00</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>1.00</td>
<td>1.057±0.0069</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
<td>0.0071±1</td>
<td>1.0±0.00</td>
<td>0.8±0.00</td>
<td>0.1±0.00</td>
</tr>
<tr>
<td>Pd(IV)</td>
<td>1.00</td>
<td>1.049±0.0107</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
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<td>0.1±0.00</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>1.00</td>
<td>0.959±0.0069</td>
<td>0.43±0.03</td>
<td>0.13±0.01</td>
<td>12.99±0.04</td>
<td>19.93±0.05</td>
<td>0.0071±1</td>
<td>1.0±0.00</td>
<td>0.8±0.00</td>
<td>0.1±0.00</td>
</tr>
</tbody>
</table>

By using (SPCE) method (n=6)

By using (AECA) method (n=6)

Data of concentration in two locations:

S1: Underwater, water; S2: soil without digestion (at the surface); S3: soil with digestion (at the surface); S4: soil with digestion (50cm depth) and S5: leaves, S6: stems and roots, S7: Permian clay samples.

Data from graphite furnace atomic absorption spectrophotometry.

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From these values and taking into account the dilution and sample size, the detection (XL) and determination (XC) limits in nanograms per gram of sample have been calculated [37].

Table 3 represents the results of the determination of six elements in water (S), soil (S1, S2, S3, and S4) and *Pennisetum glaucum* plant samples (S5, S6, and S7) with SWV and GFAAS. For both methods, the standard deviation ranged from 0.0001% to 1.59% for all metals. The concentrations of trace elements in all samples under investigation have been calculated using the standard addition method. This method was chosen because the matrix effects were less influenced by this procedure. By drawing the concentration of the metal added against the peak current, the concentration of the metal in the sample can be obtained as can be seen in Figures 2 and 3. The GFAAS method has been applied for the determination of the six metals (Cd, Pb, Cu, Ni, Co and Cr) to validate the stripping voltammetric procedure, the accuracy was evaluated by carrying out recovery assays at parts per billion levels (n=6). Since no certified reference sample was available (Table 5). Precision of the method has been estimated from the standard deviation, relative error (%) and the correlation coefficient for six replicate analyses of sample solutions and it provides values greater than 0.0986, 0.2037 and 0.99 for all metals, respectively.

**Test of Linearity of Calibration Plots:** The linearity between ip and concentration C was tested for the investigated elements by the standard addition method under the optimal conditions (Fig. 5). Satisfactory linearity was obtained over the concentration range generally found in the underground water for all investigated elements. The formation of intermetallic compounds between the investigated metal ions may cause an error in their determination. This aspect was investigated by comparing the slopes of the ip/concentration plots for each metal present in the solution separately and mixed with 0.1 mg/L of the other investigated metals. Slope value (a) of the calibration plot of the pure element solution and slope value (b) of the plot of the element mixed with the other five investigated elements are not significantly different (Table 5). This indicated that the intermetallic compound formation between the investigated elements in the mercury film at glassy carbon electrode surface under the optimal conditions of the proposed analysis scheme is small and can be neglected.

**Precision, Accuracy and Detection Limits:** Precision and accuracy were determined as relative standard deviation (RSD) and relative error (RE), respectively, by analyzing the same reference standard solution three times for various concentration levels. RSD and RE for three concentration levels of each element are shown in Table 5. The detection limits (LD) of the investigated metals, defined as the metal concentration yielding an analytical peak equal to the minimum detectable one, can be calculated [38]. The given detection limits of the elements under investigation revealed that the proposed scheme of analysis under the optimal conditions is very sensitive and very useful for ultratrace determination of elements.

SWV offers a great advantage in that an experiment is performed much faster than using the normal and differential pulse techniques. Whereas the latter technique typically runs at scan rates of 1–10 mV/s, SWV enables the use of scan rates up to 1 V/s, or more, allowing the determination time to be cut substantially. The comparison between the determination time for SWV and GFAAS shows that the time needed for simultaneous determination of three metals (i.e., Cd, Pb and Cu) by SWV is similar to the time needed for the determination of two elements by GFAAS.

The tolerance for various foreign ions was studied for the determination of 5 x 10⁻⁴ M of each metal ion (individually). It was found that 200-fold molar ratio of Mn(II), Mg(II), Fe(III) and Al(III), 100-fold ratio of Ta(V), W(V), Bi(III) and Fe(II) and 50-fold ratio of Ag(I) and UO₂(II), could be tolerated. However, surfactants, such as Triton X-100 (0.2 mg/L) and tetramethylammonium bromide (10⁻⁴ M), can lower the peak current of each metal alone significantly.

Average percentages of heavy metals were monitored in ground water, soil and millet plant (Fig. 4). In Hodeida, which considered as industrial area, Ni (II) average percentage was the highest in water (46-47%) and Cu (II) (26%) in comparison with other elements, while, Pb (II) average percentage was the highest in soil and millet plant (52-54%), followed by Ni (19-20%). On other hand, in Zabid which considered as urban area, Ni (II) highest percentages (28-29%) and Cr (VI) highest percentages (34-35%) were in ground water. Other wise, Pb (II) highest percentage (52%), Ni (II) (18%) and Cu (II) (11-12%) were recorded in soil and plants.

In conclusion, the differences in Pb(II) average percentages between the two locations was 10%, which could be attributed to extensive domestic and industrial waste release and extensive use of septic tanks and potential waste seepage into ground water reservoir.
Statistical analysis of changes in heavy metals contents in groundwater, soil and millet plants versus location differences (Table 4). From one-way analysis of variance, generally, (SWCS) methods revealed its sensitivity in analysis of heavy metals in different environmental samples in comparison with (AAS) method. The (SWCS) method gave the highest significant changes in heavy metals content in environmental material were attributed to location differences. Where, high F values were estimated and P<0.0001, even at fairly low (650.29) to low (56.37) F values.

In contracts, AAS method gave variable statistical approach. The highest significant changes in all environmental samples were estimated in Pb (II) and Ni (II) due to location differences. Where, F ranged between minimum of 26.5 in millet root to 6.9E+08 in soil at 50 cm depth and P<0.0001. While, fairly low significance changes in Cd (II) were estimated. Where, F value varied from minimum of 228.36 in water to maximum of 7.6E+04 in roots and P<0.0001 in response to location differences. Other heavy metal such as Cu (II) gave low significant changes due to location differences. Where, F =5.42; P<0.05 in digested soil samples at surface in response to location differences. While, lowest F values was estimated in Co (II) (F= 8.88; P<0.05) in millet stems and in Cr (VI) (F=12.35; P<0.01 and F=22.35; P<0.05) in millet stems and digested soil at 50 cm depth, respectively. It can be concluded that great importance of SWCS and it’s liability in heavy metal analysis in different environmental components.

CONCLUSION

From our investigation, it has been observed that SWV is a very sensitive and rapid analytical method. It can be expected that SWV will be very sensitive due to its high scan rate in all cases where the reacting species is accumulated by adsorption on the electrode surface. This mode includes elements forming insoluble compounds with mercury film on the mercury film surface as well as compounds adsorbed on the mercury surface such as Ni and Co. On the other hand, the use of acidified samples (5mL of nitric acid is enough) is necessary to allow an accurate and sensitive determination in water, soil and Pennisetum glaucum plant samples. Thus, the use of nitric acid increases the metal generation efficiency from solid particles and sample pretreatment is kept to a minimum. The short analysis time in SWV makes this method very attractive for routine determination of the described elements in water, soil Pennisetum glaucum plant samples.

REFERENCES


