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Validation of Modified Electrode Surfaces as Sensitive Analytical Tools in Soil Analysis; Detection of Total Concentration of Zn (II), Cd(II), Pb(II), Cu(II)/Cu (I) along with Handling Complicated Sample Matrices

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Abstract: Heavy metals constitute pollutants particularly common in air, water, and soil. They are present in both urban and rural environments, on land and in marine ecosystems, causing serious environmental problems since they do not degrade easily, remain almost unchanged for long periods, and bioaccumulate. Detection, though especially quantification, is a systematic process as monitoring of their levels needs to be carried out at regular intervals since there is often seasonal variation. The need for rapid and low-cost determination of metals is therefore considerable. In the present study absorption spectrometry along with a bismuth film plated electrode surface applying stripping voltammetry, a sensitive voltammetric method is being compared and analytically validated. Atomic absorption spectroscopy (AAS) represents a well-established analytical technique while anodic stripping voltammetry is being criticized for its applicability in complicated sample matrices such as soil samples. This sample-handling challenge is being investigated in the present study. The results show that both methods AAS and ASV were satisfactorily correlated and showed that the concentration of metals is lower than the limit values, but with increasing trend. Therefore, continuous monitoring of metal levels in the urban complex of the city is necessary and a matter of great importance. Regarding Cd, the detection limits of Cd are lower compared to those of AAS when using the graphite furnace, while an overestimation was noted when using flame-AAS in comparison with those found with SWASV. The SWASV method has the advantage of being cheaper and faster, enabling the simultaneous determination of heavy elements across the range of concentrations that these elements can occur in Mediterranean soils. Additionally, a dsDNA biosensor is suggested for the discrimination of Cu(I) along with Cu(II) based upon the oxidation peak of guanine and adenine residues can be applied in the redox speciation analysis of copper in soil which represents an issue of great importance.

Keywords: heavy metals; anodic stripping voltammetry; bismuth film electrode; atomic absorption spectroscopy; speciation analysis; urban and agricultural soils; natural and spiked samples

1. Introduction

Heavy metals constitute an environmental health risk that humanity has been concerned about for the last decades [1,2]. Several of them in small quantities (trace amounts), such as copper (Cu) and zinc (Zn), are essential for the smooth and safe production process of growth and reproduction of living beings [3]. However, once they reach hazardous concentrations, they pose a risk both to the environment they are present in as well as to the plant or animal organisms [4]. Meanwhile, a few metals such as cadmium (Cd) and lead (Pb) have no beneficial effect on living organisms, even when present in small quantities. Probably the greatest problem is that heavy metals have the potential to remain unchanged for long periods, as they have long half-lives [5]. They also bio-accumulate, in both the abiotic environment and in living organisms, causing numerous diseases, such as neurological disorders, cancer, and even death [6]. Their presence is widespread on land and sea, as well as in the air, water, and soil. It is well known that soils are subject to

long-term pollution, and therefore the ongoing recording and monitoring of their concentrations is necessary [7]. The sources of their origin are numerous, with two major classes dominating: geochemical and anthropogenic. Minerals and rocks in soils constitute the main source of their origin, as they are decomposed by physical (air, rain, ice), chemical (oxidation, hydrolysis), and other factors. However, human activities, industrial processes, heating fuel use, exhaust gases from wheeled vehicles, etc. generate heavy metal pollution in the mainly urban environment [8,9]. In agricultural soils, the indiscriminate use of fertilizers and plant protection products along with biological treatment sludge often contain heavy metals in small or large quantities.

Soil contains multiple heavy metals, such as Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, A^{g+}, and As^{3+}/As^{5+}. The concentrations of Cu^{2+} and Zn^{2}, in the soil, are remarkably higher compared to those of Pb^{2+} and Cd^{2+} [10,11] Interferences can affect the electroanalytical determination of Pb^{2+} and Cd^{2+} , due to complex and alloy formation [12].

Over time, a range of techniques has been developed for detecting heavy metal ions (HMIs) in soil samples, each with its own set of advantages and limitations, including inductively coupled plasma mass spectrometry (ICP-MS) [13,14], inductively coupled plasma optical emission spectrometry (ICP-OES) [15], flame atomic absorption spectrophotometry (FAAS) [16,17] and atomic absorption spectroscopy (AAS) [18–20]. These methods vary in their focus, sometimes emphasizing parameters and other times the choice of analysis method. While spectrometric methods like AAS and ICP-MS offer exceptional accuracy and sensitivity, they come with drawbacks such as high cost, time intensity, and the inability to conduct on-site measurements. Thus, selecting the appropriate technique depends on factors like required precision, sensitivity, cost considerations, and the feasibility of on-site analysis.

Although these techniques offer high sensitivity and selectivity, they have several notable limitations. On the other hand, electrochemical techniques like voltammetry, offer distinct advantages including lower cost, ease of use, rapid analysis, simplicity portability, suitability for field monitoring of environmental samples, as well as high sensitivity and selectivity [21,22].

Voltammetry stands out among other electrochemical techniques, as the leading electrochemical method for its exceptional sensitivity, making it capable of in situ simultaneous determination and measurement of heavy metals in different soil samples [21]. This method includes several types, including cyclic voltammetry (CV) [23], square wave voltammetry (SWV) [24], linear scan voltammetry (LSV), and differential pulse voltammetry (DPV) [25]. The discrimination between these techniques arise from the different way of the applied potential during stripping procedure [26]. It has been reported that the simultaneous electrochemical determination of Cd^{2+}, Pb^{2+}, and Zn^{2+} with a glassy carbon (GCE) electrode can be achieved using anodic stripping differential pulse voltammetry (DP-ASV) [27].

Additionally, DPV and SWV exhibit better detection sensitivity compared to the other techniques. In addition, electroanalysis offers promising analytical methodologies for the sensitive Pb determination It is worth mentioning that ~~ESA~~ electroanalysis can also be easily combined with inexpensive and user-friendly instruments.

The square wave anodic stripping voltammetry (SWASV) as an electroanalytical technique for the detection of Zn^{2+}, Cd^{2+}, Cu^{2+}, and Pb^{2+}, has been considered as a promising analytical tool arising from its improved analytical characteristics in the determination of heavy metals of considerable concern. On the other hand, certain ions introduce interferences in the determination of heavy metals [12]. In this method, an electrode is used which can be modified for a specific purpose of use.

During the last years chemically modified electrodes offer remarkable and advantageous prospects in chemical analysis. Chemical modification involves carbon materials along with bioelements such as enzymes, deoxyribonucleic acid etc

Due to its characteristics, bismuth is chosen in this experiment, to be used as a film on electrodes, such as glassy carbon electrodes (GCEs), which, in turn, find applications in various sample analyses (environmental, biological, etc.). Carbon electrodes mainly glassy carbon and carbon paste offer an important platform for the development of modified electrode surfaces based on bismuth film which restores the merits of mercury film excluding its toxicity. The unique character of bismuth film electrode allows its growing importance in its application in the chemical analysis [28]

The purpose of this paper is to present a simple and easy-to-apply analytical method for the preparation of a glassy carbon electrode modified with bismuth film that can be used for the sensitive and selective simultaneous voltammetric determination of the total concentration of cadmium, lead, zinc, and copper in soil samples using square wave voltammetry. Moreoverin this work a electrochemical biosensor is being suggested in the differentiation in the behavior of Cu(I) as well as Cu(II) with double-stranded DNA which results in a sensitive and selective analytical tool for redox copper speciation in soil.

2. Materials and Methods

2.1. Handling of Samples

The soil samples included in the study fell into three different groups: natural soils derived from agricultural soils that have been intensively cultivated for years [7], soils from the urban fabric of crowded cities[29,30] and soils that have been artificially or laboratory contaminated [31] in order to achieve high concentrations of metals, particularly cadmium, considerably higher than usual in soils [1,2]. The soil samples, both from rural and urban areas, were contaminated (spiked) by adding appropriate volumes of metal nitrate solutions. The solutions were mixed with the soil samples by spraying and mechanical agitation for 15 minutes. They were then transferred to black plastic bags and kept there to incubate for a month. Aeration was applied in order to maintain moisture content at 50% level.

Both the naturally contaminated and spiked soils were transferred to the Laboratory of Soil Science of the Aristotle University of Thessaloniki in order to be subjected to soil analyses, after having been air-dried and passed through a 2mm diameter sieve. The methodology as described by Dimirkou et al. [32] and Golia and Diakoloukas [33] was then followed.

Nutrients concentration levels were measured according to the related literature [32,33]. Soil texture and soil-water mixture measurements in a ratio of 1:2.5 was used to determine the pH value according to Vougioukos' method [34]. For the Electrical Conductivity (EC) measurements a soil-water saturation paste was used in an equal ratio, i.e. 1:1. Total nitrogen was measured by Kjehldal method and that of assimilable phosphorus by the Olsen method. Concentration of exchangeable potassium was evaluated by applying Flame Photometer (Sherwood 410) after extraction with 0.1M CH_3COONH_4 solution at pH = 7 Organic matter content of soils samples as well as their mixtures was obtained by the Walkley-Black method [35].

Total metal concentrations were determined after extraction using Aqua Regia ($HCl:HNO_3$, 3:1) [36]Atomic absorption spectrometer equipped with flame (Shimatzu 6300) or graphite furnace (Shimatzu 7000) was used. Certified reference material CRM 141R was analysed. Certified concentrations for Cu, Zn, Cd, and Pb were 0.29, 0.68, 0.019, and 0.218 μg L-1, respectively, while the accuracy for metal analysis ranged from 7.8-10.9%.

For the voltammetric measurements, the digested samples were further diluted with deionized water (1:5). Voltammetric Measurements were realized using a Palm Sens Model 1 potentiostat/galvanostat from Echo Chemie in the Netherlands where heavy metal determinations were performed. The electrochemical cell used in this experiment included a glassy carbon working electrode (GCE), an Ag/AgCl reference electrode (RE) saturated with 3 mol L^{-1} KCl, and a platinum wire counter electrode (CE). The electrodes were used to determine heavy metals and more specifically cadmium, lead, and zinc~~.~~ A Sartorius balance and a Consort C830 pH meter were used for relevant measurements. A magnetic stirring bar 8x3 mm, PTFE (HEINZ HERENZ HAMBURG) was used.

Solvents, acids, bases and standard solutions were all of pro analysis grade The supporting electrolyte, which was used with the voltammetric techniques, was 0.1 mol L^{-1} acetate buffer (pH=

4.5), prepared from CH_3COOH and NaOH (CH_3COOH/CH_3COONa) (ACS reagent, Darmstadt, Germany) were provided by Merck. Standard solutions of bismuth, cadmium (Cd), lead (Pb), and zinc (Zn) were provided by Sigma-Aldrich (Darmstadt, Germany). Hydrogen peroxide (30 volume %) was pro analysis, Merck. Double-stranded (ds) calf thymus DNA (D-1501, highly polymerized) was purchased from Sigma. For the study of the electrochemical behavior of dsDNA at the electrode surface, the stock solutions (0.14 g L^{-1}) were prepared in 10 mM Tris–HCl and 1 mM EDTA at pH 8.0. All aqueous solutions were prepared using deionized water. The experiment aimed to determine heavy metals such as Cd, Pb, and Zn in soil samples by using Bi as the substrate of the GCE electrode. The soil solutions were diluted 1:5 in 25 ml volumetric flasks.

Voltammetric procedure aims to determine cadmium, lead, and zinc and this was carried out using standard solutions of known mass concentration of these three metals, using the technique of square wave voltammetry and applying *in situ* plating:

- plating: acetate buffer+ Bi+ H_2O_2; deposition potential -0,3 V; duration 60s
- preconcentration: deposition potential -1,4 V; duration 60s; equilibration time 10s
- conditioning: potential 0,3V; duration 30s

First, the glassy carbon electrode being rinsed with deionized water, and then its surface was polished with 0.1 mm and 0.05 mm alumina powder since the use of a polishing cloth is required to produce a mirror-like surface. . 0.1 mol L^{-1} acetate buffer (CH_3COOH/CH_3COONa), pH 4.7, along with square wave voltammetry (SWV) were applied Electrode surface was modified by a bismuth film electrode . The potential was recorded in the range from −1,400 to +0,0 V, with a potential of 5 mV, pulse potential of 15 mV, and a frequency of 10 Hz Magnetic stirring was used as needed.

2.2. dsDNA Modified Electrode

dsDNA modified electrode preparation was realized applying pre-treatment at +1.7 V for 1 min at bare electrode surface while dsDNA was subsequently immobilized at electrode surface for 5 min at +0.5 V. Measuremens were recorded in 0.2 M acetate buffer solution scanning from +0.1 V with a scan rate of 50 $mV \cdot s^{-1}$.

Results were statistically processed applying SPSS package (IBM SPSS Statistics 26) and Microsoft Office Excel. Every experiment was performed for five times. Fisher's least significant difference (LSD) test at p = 0.05 was performed.

3. Results and Discussion

3.1. Physico-Chemical Features of Soil Samples

The following Table 1 presents the physico-chemical properties of the soil samples.

Table 1. Values of the physico-chemical parameters of the soil samples (n=30).

	pH (1:1)	EC (µS cm^{-1})	OM (%)	Clay (%)	Sand (%)	$CaCO_3$ (%)
Minimum Value	7.12	1224	0.55	11	21	8.65
Maximum Value	8.82	4431	3.43	56	67	19.34
Mean Value	7.49	2205	1.97	24	44	11.33
Relative Standard Deviation	0.55	11.13	0.88	2.30	6.43	1.33
Kurtosis Coefficient	1.339	-0.385	-0.543	-0.008	-0.082	-0.760
Skewness Coefficient	0.690	0.643	-0.213	1.0088	-0.944	0.331

The soil samples selected for the present study are alkaline, as in cases both in rural and mainly in urban areas, i.e. urban and peri-urban areas, the soils are alkaline [30,37]. The electrical conductivity and organic matter values suggest common values found in agricultural and urban soils investigated [38]. The highest values of electrical conductivity have been observed on soils located close to the sea, mainly around the ports in the cities where urban soils were collected, while in agricultural soils it is mostly the salts that contribute to the increase in EC values that are found in the fertilizers used [29,33]. Soils also exhibit great variability concerning the values of clay and sand. Mónok, et al. [8] studied and compared the physico-chemical properties of urban soils versus cultivated soils, concluding that the properties of urban soils show considerable similarities between them, often following a known and recurring pattern.

In the following Table 2 the nutrients along the toxic metals concentrations are presented.

Table 2. Values of nutrients and heavy metals concentrations in the soil samples (n=30).

	N	**P**	**K**	**Cu**	**Zn**	**Pb**	**Cd**
	(%)			(mg kg^{-1})			
Minimum Value	0.1	7.81	45.12	17.44	28.13	4.54	0.17
10th-perc[a]	0.12	10.41	57.61	19.45	33.14	9.15	0.24
50th-perc[b]	0.15	14.66	66.87	26.78	47.31	28.55	0.76
Average	0.19	16.62	71.77	29.88	49.02	29.93	0.88
90th-perc[c]	0.2	17.67	88.89	32.45	52.07	39.72	0.91
Maximum Value	0.22	22.77	97.73	34.76	69.11	43.54	1.05
EU Limits[d]				140	300	300	3

[a] 10th percentile; [b] 50th percentile; [c] 90th percentile; [d] 86/278/EEC Directive [39].

Lu et al. [37] investigated the consequences of pH fluctuations, mainly caused by endogenous redox reactions, as well as by soil buffering capacity, on Cd(II) speciation in rice paddy soils when flooding and drying cycles occur. Lue et al. [40] also investigated the potential factors, such as soil pH, the extent of urban green space, along with climatic conditions, that may determine and ultimately mitigate the accumulation of toxic heavy metals in urban soils. Researchers Skorbiłowicz et al. [41] studied the concentrations of potentially toxic elements observed in soils around the perimeter of high traffic roads, on the bialystok-budzisko route in Northeastern Poland, where significantly high concentrations of Pb and Cd are present. The soil samples of the study which were naturally contaminated, were found to have concentrations lower than the maximum permissible limits established by the European Union [39]. The metal values were much higher in the spiked soils, as these concentrations are hardly found in the real life, even in mines, and would be a significant risk to people's health [30,42,43]. However, the presented concentrations were selected to meet the requirements of the investigation into the limitations of metals' quantitative determination using certain analytical techniques.

3.2. Voltammetric Determination

3.2.1. Bismuth Film-Modified Electrode

Since the year 2000) bismuth film modified electrode , unceasingly has been attracting increasing attention in electroanalysis. In this context, bismuth film electrodes (Bi-FEs) have become an attractive new subject of electro-analytical research as a potential replacement for mercury electrodes.. Bismuth is known as “green metal” and represents a competitive alternative to mercury which is known as a highly toxic one. Sensors based on bismuth film modification procedures offer remarkable enhancement of sensitivity, selectivity, measurement flexibility; analytical figures of merit enabling them to become very competitive.

Bi-film electrodes (BFEs) are prepared by depositing a thin layer of bismuth onto a suitable substrate material instead of using mercury. The primary advantage of BFEs lies in their eco-friendliness, as bismuth and its salts are minimally toxic. Furthermore, BFEs exhibit analytical characteristics in voltammetric analysis that are roughly equivalent to those of mercury film electrodes (MFEs). This similarity is attributed to bismuth's ability to form "fused alloys" with heavy metals, akin to the amalgams formed by mercury [44].

The electrode modification process with bismuth can be accomplished through two distinct methods:

- Plating of bismuth film in a different supporting electrolyte solution of that of measurement (ex situ)
- Plating of bismuth film in the same supporting electrolyte solution with that of measurement (in situ) [45].

Their primary limitation lies in their restricted anodic range, which hinders their utility in detecting or accumulating species at higher positive potentials. Overall, it can be concluded that BiFEs show great promise as analytical instruments and indeed have the potential to replace Hg-film electrodes (MFEs) [44].

Numerous publications indicate that the detection of heavy metals with BiFE electrode is attributed to Bi's capability to create a " fused alloy" with specific heavy metals, such as lead cadmium and zinc [46]. The modification process for detecting lead on a Bi film electrode typically entails electroplating BiFE onto the electrode surface [45].

For the determination of heavy metals (Zn, Cd, Pb, Cu) we used square wave anodic stripping voltammetry at a bismuth film-modified glassy carbon electrode. The process of accumulation (the electrolysis) is realized across electrochemical reduction of a marked substance by invariable potential, joint from simultaneous mixing solution. This method thanks to high tenderness, and low limits of detection, finds use in the analysis of many natural samples as well as industrial.

Electrode surface is bismuth film plated in parallel with the determined metal ions. During the preconcentration step in anodic stripping voltammetry, from a solution containing added Bi(III) ions, or ex-situ, where bismuth film can be formed in an external plating solution.

Measurement followed potentiostatic accumulation and equilibrium period.

During ex-situ plating, increasing copper concentration was followed by a significant decrease in the bismuth signal. Nearby the electrode surface competition for surface sites is taking place, between the deposited copper and bismuth following a significant overlap in bismuth and copper signals

After bismuth in situ plating~~,~~. the electrode surface sensitivity was remarkably improved giving rise to higher Cd, Pb, and Zn peaks while between bismuth and copper the separation was not favored, since they are competing for the available electrode surface sites. In our previous work [47,48] which involved the simultaneous determination of Zn, Cd, Pb, and Cu using BiFE in samples of biological and environmental concern, the problem of simultaneous determination of Cu along with Zn, Cd, and Pb was studied either by addition of Ga (III) or by addition of H_2O_2. In the case of the addition of Ga(III), the linear range for copper was more limited than the corresponding range obtained after the addition of H_2O_2.

The addition of H_2O_2 favored the simultaneous determination due to the reason that:

a) causes a noticeable shift of the redissolution peak of copper to more positive potential values (at +0.212 V) than expected (at -0.100 V) redissolution improvement

b) eliminates competition between Bi(III) and Cu(II) for free electrode surface sites (interference minimization).

c) ensures an excellent improved correlation of copper concentration with its peak current (r >0.99)

d) achieves lower detection limits Cu(II)

e) gives satisfactory repeatability for consecutive measurements (N=10),

f) guarantees excellent recovery performance (96-108%)

For the reasons given above, in situ, modification of the GCE electrode surface was most preferable compared to the ex situ process for the simultaneous determination of Cd, Pb, and Zn because gives more reliable results

Applying the procedure of in situ plating the limits of detection were 0.70 mg/L for Zn, 0.14 mg/L for Cd, 0.03 mg/L for Pb and 0.38 mg/L for Cu (measured values in the digest) and the relative standard deviations were 8.60% for Zn, 4.75% for Cd and 5.68% for Pb and 5.38% for Pb at the 2.0 mg/L level (n=5). A voltammogram of the simultaneous determination is shown in Figure 1.

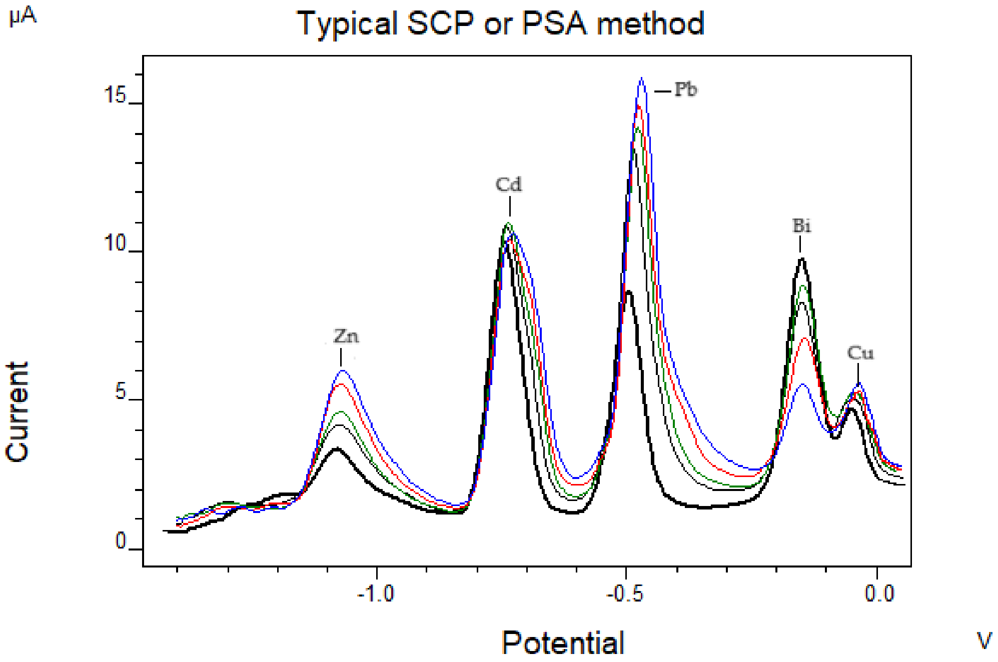
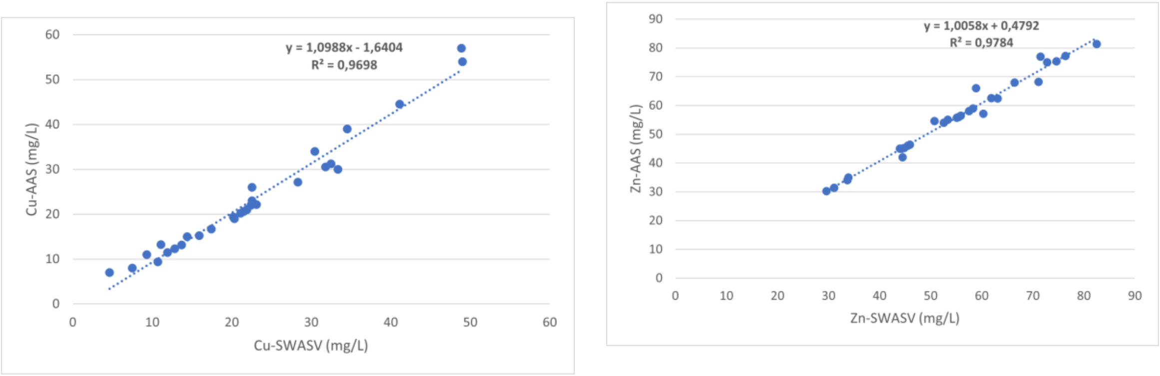


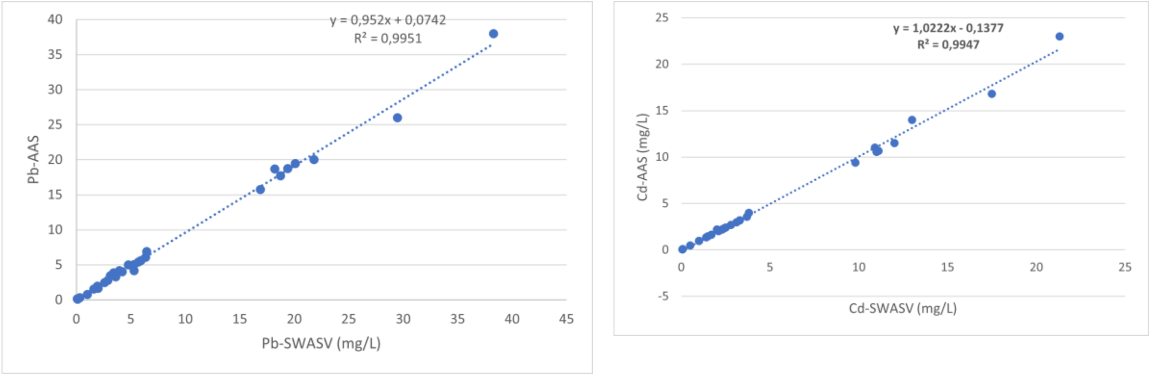
Figure 1. Voltammogram of simultaneous determination of Cu, Zn, Pb, and Cd: 50uL 250 uL, -1.4V for 120s, scanning from -1.4 V (Eend) +0.00 V (Ebegin), 0.12 % v/v H2O2, 450 μL Bi, buffer CH3COOH/CH3COONa (pH=4.7), 10 Hz (SW frequency), 0.005 V (Estep), 0.015 V (Epulse).

The method's accreditation was conducted using the CRM 141R standard soil. For Zn, Cd, Cu and Pb the MQL values were 0.91, 0.88, 1.1, and 0.88 mg kg^{-1}, respectively, while the accuracy for metal analysis ranged from 8.2-10.4 %. In the case of dsDNA-modified electrode Cu concentration was found to be 0.97 mg kg^{-1} with an RSD value of 9.9%. While regarding Cu(II)/Cu(I) redox speciation in the same certified standard soil CRM 141R (spiked with Cu(I)) a recovery of 91,7 % Cu(I) was obtained with an RSD value of 8.7%

Moreover, the analytical method was validated by applying atomic absorption spectrometry Results achieved are shown in Figure 2 proved to be in good agreement (ANOVA, Student's test, 95 % confidence level). This indicates that the method is unbiased and proves its validity and versatility.



(**a**) (**b**)



(c) (d)

Figure 2. This is a figure. Schemes follow the same formatting. Comparison of AAS and SWASV with Bismuth film modified electrode: (a) regarding Cu; (b) regarding Zn; (c) regarding Pb; and (d) regarding Cd.

3.2.2. dsDNA Modified Electrode

According to our previous work [49,50] is possible the redox speciation of copper, based on the interaction of dsDNA along with Cu (I) and Cu (II).

Interaction of Cu (II) with dsDNA resulted to the increase of the characteristic oxidation peak of guanine and the decrease of adenine oxidation peak. The interaction phenomenon proved to be time-dependent.

In the case of Cu (I), dsDNA is electrostatically adsorbed at the electrode surface through while their interaction resulted to a peak at 1.37 V which started to appear with increasing concentrations of Cu(I.-The proposed (bio)sensor can be evaluated for copper speciation in soil samples. In Figure 3 is shown the comparison of AAS and dsDNA modified electrode (based on guanine oxidation peak) regarding Cu(II).

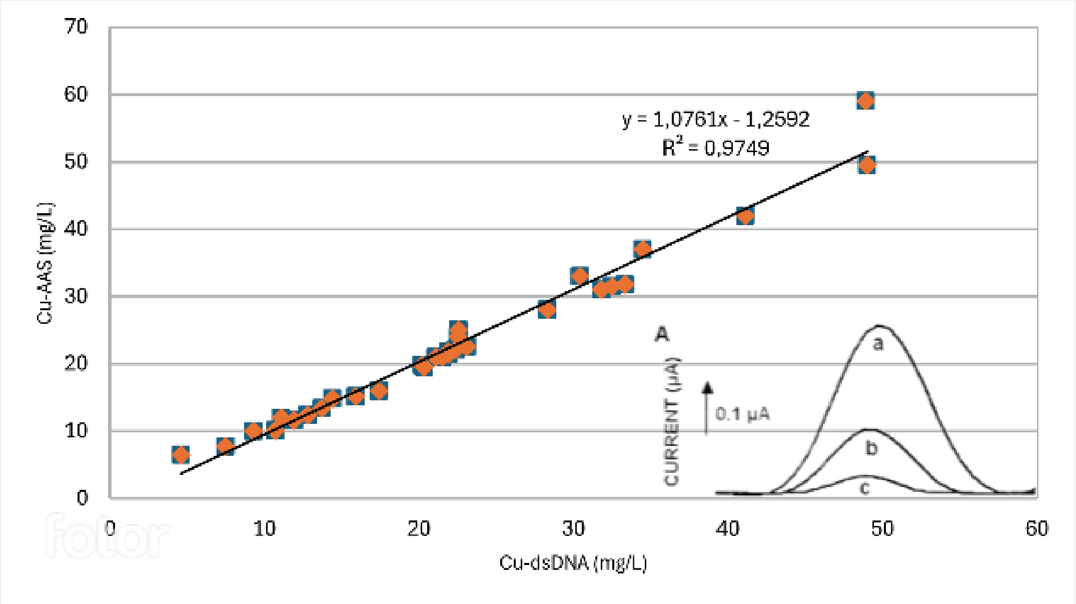


Figure 3. Comparison of AAS and SWASV with dsDNA modified electrode (based on guanine oxidation peak) regarding Cu(II).

4. Conclusions

Heavy metals constitute pollutants particularly common in air, water, and soil. They are present in both urban and rural environments, on land and in marine ecosystems, causing serious environmental problems since they do not degrade easily, remain almost unchanged for long periods, and bioaccumulate. Detection, though especially quantification, is a systematic process as monitoring of their levels needs to be carried out at regular intervals since there is often seasonal variation. The need for rapid and low-cost determination of total metal concentration as well as speciation of copper is therefore considerable. In the present work, the quantitative determination of Cu, Cd, Pb, and Zn was carried out in extracts of soil samples obtained from the urban area of Thessaloniki. Metals were extracted using an Aqua Regia mixture and subsequently determined using two analytical techniques: atomic absorption spectroscopy (AAS), using a flame or graphite furnace fixture, and square wave anodic stripping voltammetry (SWASV), using a bismuth thin film electrode. Regarding the quantification of Cu, Cd, Pb, and Zn the values determined by both methods are in satisfactory agreement among them. Regarding Cd, it was apparent that the detection limits of Cd were lower with AAS than with SWASV when using the graphite furnace, while an overestimation was noted when using flame-AAS in comparison with those found with SWASV. The SWASV method has an advantage as it is cheaper and faster, enabling the simultaneous determination of all four elements across the range of concentrations that these elements can occur in Mediterranean soils. Moreover, a dsDNa-modified electrode surface is proposed as a useful analytical tool for copper speciation, as a future prospect, under an alternative sample handling which could allow the simultaneous presence of Cu(II) along with Cu(I).

Author Contributions: Conceptualization, S.G. and E.E.G.; methodology, S.G and E.E.G.; software, V.K. and S.G.P.; validation, S.G. and E.E.G.; formal analysis, V.K., S.G.P., S.G. and E.E.G.; investigation, S.G. and E.E.G resources, S.G. and E.E.G; data curation V.K., S.G.P., S.G. and E.E.G.; writing—original draft preparation, V.K., S.G.P., S.G. and E.E.G.; writing—review and editing, V.K., S.G.P., S.G. and E.E.G.; visualization, V.K., S.G.P., S.G. and E.E.G.; supervision, S.G. and E.E.G.;.; project administration, S.G. and E.E.G.;.; funding acquisition, S.G. and E.E.G.; All authors have read and agreed to the published version of the manuscript.

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