

**JIMMA UNIVERSITY**  
**SCHOOL OF GRADUATE STUDIES**  
**DEPARTMENT OF CHEMISTRY**



**M.Sc. THESIS**  
**ON**  
**THE DEVELOPMENT OF ELECTROANALYTICAL METHOD FOR**  
**THE DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC**  
**ACID USING BISMUTH MODIFIED SCREEN PRINTED CARBON**  
**ELECTRODES**

**OCTOBER, 2015**  
**JIMMA ETHIOPIA**

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**BY**

**TEMESGEN TAKELE NIGUSO**

**ADVISOR: TEFAYE REFERA SORETA (PhD)**

**CO-ADVISOR: EPHREM TILAHUN (M.Sc.)**

**A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES  
JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN  
CHEMISTRY (ANALYTICAL)**

**JIMMA, ETHIOPIA**

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**Approved by board of examiners**

**External examiner**

**signature**

**date**

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**Internal examiner**

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**Advisors**

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**Department head**

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## Declaration

I, the undersigned declare that this is my original work and has not been presented for research in any other University.

Temesgen Takele

This M.Sc Thesis has been submitted with our approval as supervisors

Supervisors name

Date

Signature

Tesfaye Refera (PhD)

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Chemistry Department

Jimma University

Ephrem Tilahun (M.Sc)

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Chemistry Department

Jimma University

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## Abbreviations

|          |   |
|----------|---|
| BiFE     | Bismuth film electrode                      |
| CA       | Chronoamperometry                           |
| CME      | Chemically modified electrode               |
| CNT      | Carbon nanotubes                            |
| CPE      | Carbon paste electrode                      |
| CV       | Cyclic voltammetry                          |
| DCP      | Dichlorophenolindophenol                    |
| 2,4-D    | 2,4- Dichlorophenoxy acetic acid            |
| DPV      | Differential pulse voltammetry              |
| EPA      | Environmental protection agency             |
| GCE      | Glassy carbon electrode                     |
| LOD      | Limit of detection                          |
| LOQ      | Limit of quantification                     |
| MB       | Meldola's blue                              |
| MFEs     | Mercury film electrodes                     |
| NAH      | Nicotinamide adenine dinucleotide hydride   |
| PET      | poly 3,4-ethylenedioxythiophene             |
| SPCE     | Screen printed carbon electrode             |
| SAM      | Self-assembled monolayer                    |
| SWV      | Square wave voltammetry                     |
| TTF/TCNQ | Tetrathiafulvalene tetracyanoquinodimethane |

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## **Abstract**

The aim of this work was to develop electroanalytical method for the analysis of 2,4-Dichlorophenoxy acetic acid (2,4-D) through electrode modification. The electrode modification has been done by single potential step electrodeposition of bismuth film on screen-printed carbon electrodes. Under the optimized conditions deposition potential -1050 mV, deposition time 180 s, concentration bath Bi (III) ion 2 mM and pH 11; the nitrated 2,4-D showed dynamic linear concentration range from 11.1 to 74.3  $\mu\text{M}$  with regression coefficient (R) of 0.9988. The limits of detection and quantification were 3.15 and 10.5  $\mu\text{M}$ , respectively. The proposed method offered higher sensitivity compared to bare screen-printed carbon electrode. The modified electrode showed good reproducibility, repeatability, stability and recovery for the analysis of 2,4-D. The developed method was used for electroanalysis of 2,4-D in soil and water samples collected from Sito Kebele, Dedo district Jimma zone.

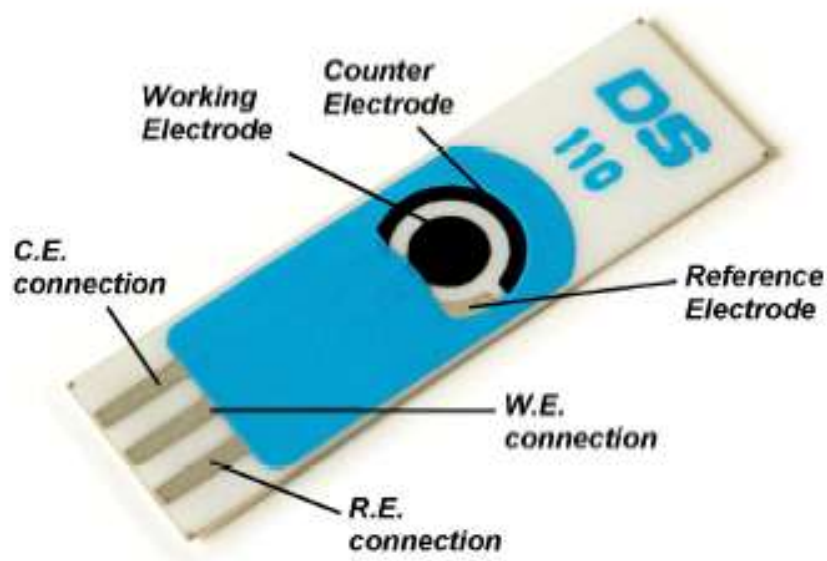
## 1. Introduction

Electroanalysis principally concerned with the relationship between electrical quantities such as current, potential, or charge and chemical parameters like concentration. The measurement of those electrical parameters for analytical purposes has found a wide range of applications, including environmental monitoring, industrial quality control, or biomedical analysis. Electroanalysis has been recognized as a powerful tool for the analysis of heavy metal ions, organic pollutants, and pharmaceutical drugs, pesticides (herbicides, insecticides, and fungicides) and so on. Some electroanalytical methods have been reported for the analysis of herbicide 2,4-D using conventional carbon electrodes and mercury electrodes<sup>1-3</sup>. The herbicide 2,4-D and its derivative exhibits slow electron transfer rate suggesting that the redox process is electrochemically irreversible. The electroinactivity of 2,4-D has been reported under ordinary conditions and the determination of this compound by electrochemical methods is just not straightforward<sup>4</sup>. So, to determine 2,4-D and its residue by electrochemical method the conversion of electroinactive 2,4-D to its electroactive derivative is required and this has been accomplished by labeling it with a nitro group.

Several methods have been reported for determination of the herbicide 2,4-D including high-performance liquid chromatography<sup>5</sup> and gas chromatography coupled with mass spectrometry and spectrophotometry<sup>6</sup>. In comparison with these techniques, electroanalytical methods offer invaluable advantages due to its good sensitivity, reduction in solvent and sample consumption, high-speed analysis, portability as well as simplicity<sup>7</sup>.

With the advancements in electroanalytical science, conventional carbon electrodes and mercury electrodes are cumbersome to use<sup>8</sup>. Because conventional carbon electrodes requires polishing and electrode treatments where as mercury electrodes is inconvenient due to its well-known toxicity. Moreover, in electroanalytical techniques, working electrode might be insensitive to apply in the detection of some chemical substances. So electrode surface modification is needed to improve the sensitivity, selectivity and stability of the electrodes to acquire enhanced response for analytical need<sup>9</sup>. Hence, disposable

screen-printed carbon electrodes (SPCEs) (Fig.1) have proven to replace the above-mentioned electrodes with the surface modification by immobilization of modifying agents on the electrode surface.



**Figure 1.1** Design of a disposable and portable screen printed electrode (with reference, working and auxiliary electrodes on the same substrate)<sup>10</sup>.

Because SPCE avoids contamination and also eliminates problem such as loss of responses due to surface fouling of the electrodes as it is used only once and then discarded<sup>11</sup>.

Therefore, in this study a new electroanalytical method utilizing disposable screen-printed carbon electrode modified with bismuth film was developed for electroanalysis of 2,4-D.

## **2. Literature review**

### **2.1 Electroanalysis**

Electroanalytical methods have received a great deal of attention because of their simplicity, portability, lower detection limit, and inexpensiveness when compared with the other analytical techniques like spectroscopic and chromatographic techniques. Thus, electroanalysis could be a powerful analytical method utilized in pharmaceuticals industry and environmental applications. For instance, heavy metal ions and organic pollutants are considered as one of the major problems in environmental contamination. Hence, electroanalysis can be used as one of the potential analytical method for the analysis of heavy metal ions in different environmental and biological fluids<sup>12-16</sup>, organic pollutants such as hydrazine, phenol<sup>17, 18</sup>, in biomedical applications for the detection of glucose<sup>19</sup>, hormones<sup>20</sup>, cells<sup>21</sup> and tumor markers<sup>22</sup>. The method is used in the detection of extremely low levels of macromolecules like proteins<sup>23</sup> and nucleic acids<sup>24</sup>. In pharmaceutical industry, it is used for the analysis of the level of the constituents of drugs<sup>25, 26</sup>. Moreover, it is a useful tool for analysis of most electroactive organic compounds used for pest and herb control<sup>27-30</sup>.

### **2.2 Electrode surface modification**

Working electrodes might be insensitive to be applied in the detection of some chemical substances in electroanalytical methods. Modification of electrode surface results in improving the properties of the selected working electrodes<sup>9</sup>. Thus, surface modification of electrode encompasses incorporating of a reagent on the electrode surface or into the matrix of the selected electrode. When chemical reagents are incorporated on the electrode surface the resulting electrode is called chemically modified electrode. A chemically modified electrode (CME) is an electrode prepared by surface modification with a thin film of selected conducting materials for the purpose of improving the chemical, electrochemical, optical, and electrical and electron transfer properties of the film in a rational, chemically designed manner<sup>31</sup>. For electroanalytical purposes, a CME can be designed as a powerful sensing device, by deliberate modification of the surface or bulk



matrix material of the electrode with a selected reagent that governs its electrochemical properties<sup>32</sup>. It results from a deliberate immobilization of a modifier onto the electrode surface through chemical reactions, chemisorptions, composite formation or polymer coating. CMEs have different properties from those of the unmodified substrate because the electrode surface is altered by immobilizing molecules in a rational fashion so that the electrode thereafter displays new properties.

## **2.3 Strategies of modifying electrodes**

The strategies of modifying electrodes surface may include adsorption, covalent attachment, bulk modification of carbon composite materials, electrochemical deposition and so on.

### **2.3.1 Adsorption**

The modifying procedures include physisorption or chemisorption, which undergo physical or chemical interaction of the modifier onto the electrode surface respectively.

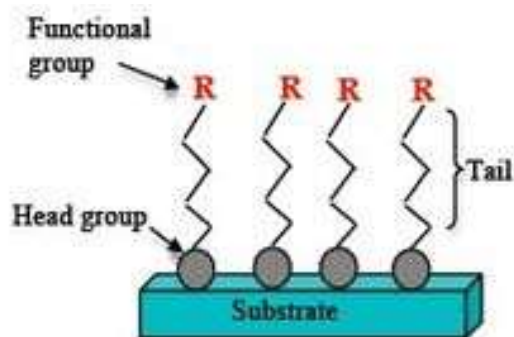
#### **i. Physisorption**

The method utilizes the adsorption of substances on a substrate surface from solution by drop-coating followed by solvent evaporation<sup>31</sup>. For this approach redox mediator such as potassium hexacyanoferrate (II), methyl blue (MB) and dichlorophenolindophenol (DCPIP) have been adsorbed on to glassy carbon electrodes for the electrocatalysis of the oxidation of NADH<sup>33</sup>. And also carbon nanotubes (CNT), which are electrode support material, can be physisorbed onto substrates such as gold<sup>34</sup> or carbon<sup>35</sup>. On the other hand, a polymer modifies the electrodes surfaces by physical adsorption. For example, the functionalized polythiophene such as poly 3, 4-ethylenedioxythiophene (PEDOT) in an enzyme based biosensor. PEDOT served both as an electrocatalytic non-diffusional mediator and as an entrapment matrix for the enzyme<sup>36</sup>.

#### **ii. Chemisorption**

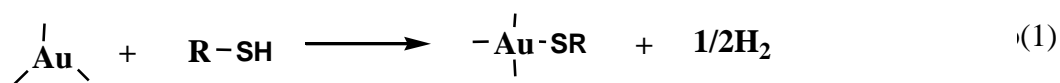
Even though physisorbed systems are very useful for analytical applications, stability is always a matter of concern for such electrodes. Thus, chemisorbed routes provide more

thermally stable CMEs. In this approach, the film is strongly adsorbed, or chemisorbed, onto the surface of the electrode. A good example is self-assembled monolayers (SAMs) of organosulphur compounds with long chain groups on Au in which the thiol group binds tightly to the gold electrode. The well-ordered monolayers formed by alkanethiols (Fig. 2.1) can be used to immobilize proteins close to an electrode surface with a high degree of control over the molecular recognition interface<sup>37</sup>. Because of this ability, SAMs have been widely used for investigations of the interactions of proteins and electrodes and for the fabrication of biosensors.



**Figure 2.1** Representation of a SAM structure

In the case of an alkythiol, chemisorptions (Equation 1) reaction can be written as<sup>38</sup>

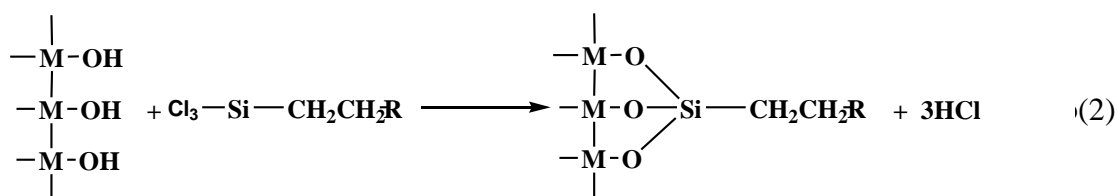


Where, Au represents a gold atom at the electrode surface and R is the alkyl substituent.

### 2.3.2 Covalent attachment of a modifying layer

This strategy involves stronger attachment of the desired chemical component to the substrate surfaces by covalent linking to form a monolayer. Organosilanes, diazonium and cyanouric chloride are the chemical component used in this modification strategy. Carbon surfaces are found to be efficient for covalent modifications due to their alterable functionalities. For example aryl diazonium and acrylacetate-based compounds have all been used for the development of stable compact films in covalent bonding modification

strategy. Attractive features of covalently modified electrode surfaces include their stability under hydrodynamic conditions and greater tolerance to organic solutions, relative to polymeric coatings<sup>39</sup>. This modification strategy can be used for the construction of biosensors that eliminate the need for mediation as it creates intimate contact between the redox active biomolecule and the electrode using covalent attachment, and direct electron transfer<sup>39</sup>. The illustrative example for the covalent bonding approach of modifying electrode involves reaction of a surface hydroxyl with a hydrolytically-unstable Organosilanes represented in equation 2:<sup>38</sup>



Where, M-OH represents a hydroxyl group on an electrode surface and R on the silane is the functional group that is to be attached to the electrode surface.

### 2.3.3 Composite or integration of modifying agents into the electrode

A method that has the chemical modifier mixed with an electrode matrix material. For this method, an electron transfer mediators mixed with carbon particles in a carbon paste electrode has been used. For example carbon paste electrode chemically modified with Tetrathiafulvalene tetracyanoquinodimethane (TTF/TCNQ) for amperometric detection of reduced glutathione<sup>40</sup> can be mentioned as an example.

### 2.3.4 Electrochemical deposition

It is also called redox deposition. This strategy relies on the variation of oxidation (ionic) state, so that film formation will occur often irreversibly when a substance is oxidized or reduced<sup>31</sup>.

## 2.4 Application of surface modification

The surface modification of electrodes have made great success in various areas of research and development, such as electrocatalysis, electronics, biosensors and electroanalysis<sup>41</sup>,

<sup>42</sup>. Surface modifications of electrodes have been employed as powerful tools for concentration determination and surface structure identification<sup>43</sup> <sup>43</sup>. It is broadly used in electroanalysis in improving sensitivity, selectivity and/or stability allowing enhanced response in order to meet analytical needs.

## **2.5 Screen printed electrodes**

Screen printed carbon electrodes (SPCEs) is a disposable electrode type used to perform clinical, environmental or industrial analysis away from a centralized laboratory as this device is easy to use, inexpensive, portable, reproducible, and sensitive electrochemical sensors for the determination of trace levels of pollutants and toxic compounds<sup>44-46</sup>. A disposable screen-printed electrode has several advantages, such as preventing contamination between samples, constant sensitivity and high reproducibility of different printed sensors<sup>47</sup>. Many ink-type substrates have been used for the fabrication disposable electrochemical sensors<sup>48</sup>. The most successful substrates used for the construction of this electrochemical sensor include carbon and the noble metals. Taking into consideration that carbon is inexpensive, requires a very low firing temperature (20-120 °C) and conductive; it is the most widely used substrate for the economic fabrication of disposable electrodes as compared to inks based on noble metals, such as Au, Pt, etc., require a firing temperature of more than 1000 °C.

## **2.6 Bismuth electrode**

Bismuth is an environmentally friendly element, with very low toxicity and the use of this metal as an electrode was introduced in early stage 21<sup>st</sup> century for electroanalytical purposes<sup>49</sup>. Due to its very low toxicity, it was introduced as an alternative to mercury electrodes in electroanalysis and exhibits similar electroanalytical performance to mercury<sup>50</sup>.

However, unlike mercury, bismuth is solid at room temperature. In the field of electroanalytical techniques bismuth electrodes has been widely used in the analysis involving trace metal with electrochemical stripping methods<sup>51</sup>. Due to its high cathodic over-potential window which pushed background limit to more negative potentials,

bismuth is an attractive electrode material for the determination of reducible organic compounds as well<sup>52</sup>.

There are different types of bismuth electrodes used for organic electroanalysis (bismuth-film electrodes, bismuth-bulk electrodes, and bismuth nanoparticles modified electrodes)<sup>52</sup>. Bismuth-film electrodes (BiFEs) are normally fabricated by in-situ or ex-situ electroplating a thin bismuth film on the surface of a conductive support<sup>51</sup>. In an in situ electroplating, bismuth ions are introduced to the sample solution prior to the analytical procedure, and bismuth films are simultaneously plated on the electrode surfaces with the accumulation of analyte. Hence, it is only suitable for the analysis of trace metals by anodic stripping voltammetry, which involves cathodic electrolysis step<sup>53</sup>. In the ex-situ electroplating, a pre-plated BiFEs is employed as the working electrode. This involves pre-plating of the substrate from a separate plating solution containing Bi (III) in acidic media to avoid hydrolysis of the metal cation. BiFEs which are fabricated by the ex-situ method are usually used in organic electroanalysis<sup>52</sup>. The presence of KBr improves the adherence of the bismuth film on the electrode surface. The bismuth plating potential is normally less negative than  $-0.6$  V. Different materials like copper, glassy carbon, carbon paste, and screen-printed carbon ink were used as supports for the generation of BiFEs. The advantages of BiFEs are the possibility for efficient regeneration of the electrode surface by removing the used film but the surface homogeneity of the electrode surface is rather variable and dependent on the plating conditions<sup>53</sup>. As compared to mercury film electrodes (MFEs), BiFEs have lower potential window because of the fact that bismuth is more easily oxidized than mercury on the anodic limits<sup>53</sup>. However, the cathodic limit potential is almost the same as MFEs.

## **2.7 Agrochemicals**

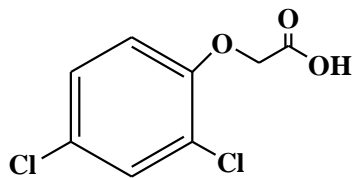
Agrochemicals are chemicals applied to agricultural fields to help in intensifying crop production and reduce the effects of pests and parasites<sup>54</sup>. There are different types of agrochemicals used to control pests and parasites, namely herbicides, insecticides, fungicides, nematocides, molluscicides etc. However, the use of these agrochemicals may have effect on environment as they could be easily washed into the streams or the soil,

eventually contaminating ground water reserves when applied to farmlands. Therefore, human beings are constantly exposed to these chemical species present in the environment. Among these herbicides are the most widely used chemicals in the world, and dangerous to human health and particularly the chlorophenoxy herbicides have received special attention because of their wide distribution and extensive use as plant growth regulators. Chlorophenoxy herbicides such as 2,4-D can be easily transferred to surface and ground water due to their polar nature and relatively good solubility<sup>5,55</sup>.

### **2.7.1 2,4-Dichlorophenoxyacetic acid**

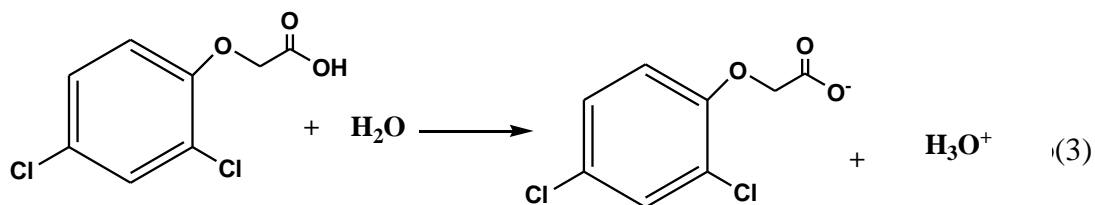
2,4-D (scheme 1.) belongs to a group of chemicals known as a chlorophenoxy compounds that are used widely to control broad leaf weeds and grasses in cultivated crops since the late 1940s. It has been used as a systemic herbicide by causing rapid cell growth in vascular tissue<sup>56</sup>. In addition to the acid form, there are numerous salts and esters derivatives of 2,4-D which vary in their physical and chemical properties and environmental behaviors. These include 2,4-D sodium salt, 2,4-D dimethylamine salt (DMA), 2,4-D 2-butoxyethyl ester (BEE) and so on<sup>57</sup>. When applied to different field and aquatic environments, the initial degradation products of most of these derivatives are the acid forms. The hydrolysis, aqueous photolysis, anaerobic aquatic, aqueous aerobic, aerobic, soil photolysis and field dissipation half-lives of 2,4-D are 39, 13, 312, 15, 66, 393 and 59.3 days respectively. The degradation products of 2,4-D are 2,4-Dichlorophenol (2,4-DCP), succinic acid, chlorohydroquinone, 2,4-Dichlorocatechol, 1,2,4-Benzenetriol, 2,4-D anion, 4-Chlorophen-ol, 2,4-Dichloroanisole (2,4-DCA) and 2,4-Dichloro-5-hydrophenoxyacetic acid.

The chemical formula of 2,4-D is  $C_8H_6Cl_2O_3$ . It is white to brownish crystalline solid. The solubility of 2,4-D in water is pH dependent. Its solubility in water at 25°C in pH 5, 7 and 9 are 29.9, 44.6 and 43.1 g L<sup>-1</sup> respectively<sup>58</sup>. It is soluble in organic solvent and it has pKa value of 2.64.



**Scheme 1.** Structure of 2, 4-Dichlorophenoxy acetic acid

The acid dissociation reaction equation for 2,4-D is represented in equation 3



### 2.7.2 Toxicity of 2,4-D

Due to its widespread distribution and ability to drift off-site, 2,4-D is potentially toxic to humans and may cause health effects<sup>59</sup>. 2,4-D is considered as being moderately toxic based on environmental protection agency (EPA) toxicity classification. It produces serious eye and skin irritation among agricultural workers. Prolonged exposure of 2,4-D through breathing can cause coughing, dizziness, burning and temporal loss of muscle coordination to human beings. It was also reported that exposure with 2,4-D can also bring blood, liver, and kidney toxicity. Furthermore, it is one of the few herbicides to cause nervous system damage, which include inflamed nerve endings, lack of coordination, stiffness in the arms and legs, inability to walk, fatigue, stupor, coma, and death<sup>60</sup>.

## 2.8 Electroanalysis of agrochemicals

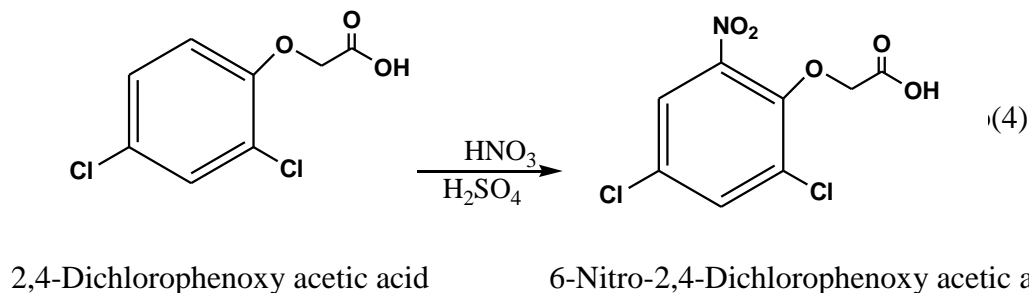
Agrochemicals such as herbicides and insecticides are essential to help in intensifying crop production. Because of their increasing use in agricultural field, the residue and metabolites of these agrochemicals can contaminate the environment<sup>61</sup>. For this reason, the monitoring of agrochemicals and their metabolites is important to ensure environmental safety. Therefore, there is a wide range of studies concerned with electroanalytical methods for

monitoring of these chemical substances in environmental samples. For example, organo-chlorine<sup>62-64</sup>, organophosphate<sup>65-67</sup>, triazine<sup>68-71</sup> and carbamates<sup>72, 73</sup>.

### 2.8.1 Electroanalysis of 2, 4- D

It was reported that 2,4-D is electroinactive under ordinary conditions<sup>4</sup>. Thus, the determination of this compound by electrochemical methods is just not straightforward. In order to determine 2,4-D and its residue by electrochemical method the conversion of electroinactive 2,4-D to its electroactive derivative is required and this is accomplished by labeling it with a nitro group. The nitration reaction product of 2,4-D is 6 - nitro - 2,4-D<sup>4</sup>.

The chemical equation for the nitration of 2,4-D is represented in equation 4:



In many literature the electroanalysis of 2,4-D and its derivative exhibits slow electron transfer rate which indicated that electrochemically irreversible process<sup>74</sup>. The expected redox peak potential for 2,4-D was approximately between -300 mV to -700 mV versus Ag/AgCl reference electrode<sup>3</sup>. The electrochemical behavior of 2,4-D was investigated using electrocatalytic reduction at palladium-modified glassy carbon electrodes<sup>1</sup>, a glassy carbon electrode modified with cobalt chloride (III) protoporphyrin IX<sup>2</sup>, graphite-polyurethane composite electrode and square wave voltammetry (SWV)<sup>3</sup>. And also a disposable immunomagnetic electrochemical sensor based on a perfluorosulfonate-coated screen-printed electrode for the determination of 2,4-D<sup>75</sup>, and screen-printed electrochemical immunosensors<sup>76</sup> were employed.

The above mentioned electrodes such as mercury electrode, glassy carbon electrodes, and carbon paste electrodes, which were used for the electroanalysis of 2,4-D herbicides, have the following limitations. For example, mercury electrodes are toxic, glassy carbon



electrodes requires polishing of the electrode surface and pretreatment which may leads to contamination and loss of response due to surface fouling of the electrode and carbon paste electrode might have the tendency of the organic binder to go in to solutions containing fractions of organic solvents resulting in undesired response<sup>8</sup>.

Therefore, these limitations of the conventional electrodes such as mercury electrodes, glassy carbon electrodes and carbon paste electrodes could be solved by the replacement of these electrodes with screen printed carbon electrode modified with bismuth film for electroanalysis of 2,4-D.

## **2.9 Statement of the problem**

A number of research units are concerned with development of finding simple and reliable means for the determination of herbicides and their residues in environment due to their frequent usage and environmental pollution problem. The residues and metabolites of these herbicides can easily be washed into the streams or the soil, and eventually contaminating ground water reserves. Although efforts have been made in detecting herbicides residues and metabolites by using different techniques, the development of other optional analytical methods with low cost, comparable precision, accuracy, sensitivity and selectivity are required for the determination of herbicides residues and their metabolites. Hence, this study was focused on the development of new analytical method for the determination/analysis of 2,4-D using bismuth film modified SPCEs.

## **2.10 Hypothesis**

Bismuth film modified SPCE could be used for electroanalysis of 2,4-D because of the modification strategy in increasing the surface area of the electrode and also expanding the potential window in negative working ranges needed for the electroanalysis of this herbicide. Bismuth film modified electrodes can have attractive properties such as high sensitivity, wide negative potential range, and insensitivity to dissolved oxygen. Therefore the modification of electrodes with this modifying agent could enhance the sensitivity for 2,4-D electroanalysis.

## **2.11 Objectives of the Study**

### **2.11.1 General objective**

- To develop electrochemical method for the electroanalysis of 2,4-D based on bismuth film modified SPCE.

### **2.11.2 Specific objectives**

- ✓ To modify screen-printed carbon electrode with bismuth film and investigate the electrochemical response for 2,4-D
- ✓ To optimize experimental parameters for electroanalysis of 2,4-D using the modified electrode
- ✓ To apply the bismuth film modified screen-printed electrodes for the Electroanalysis of real samples

## **2.12 Significance of the study**

Herbicides such as 2,4-D are extensively used to control unwanted plants in the agricultural field. The residue and metabolites of these herbicides can enter in to the surrounding water by leaching, run off and wind and can contaminate the water system. Hence, the herbicide can significantly affect the health of human beings as well as animals. Therefore, the development of a new, simple, cost effective and reliable analytical method for the analysis of this herbicide would help to determine the level of contamination so that appropriate measure could be taken the concerned bodies to prevent the side-effects of these pollutants.

### **3. Materials and methods**

#### **3.1 Chemicals**

Bismuth nitrate ( $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ , 98%, M & B laboratory reagent), boric acid ( $\text{H}_3\text{BO}_3$ , 99.5%, Techno Pharmachem), 2,4-Dichlorophenoxyacetic acid ( $\text{C}_8\text{H}_6\text{O}_3\text{Cl}_2$ , 97%, Aldrich), sodium perchlorate ( $\text{NaClO}_4$ , 98%, Sigma), potassium nitrate ( $\text{KNO}_3$ , 99 -100.5%, Riedel de Haen), dichloromethane ( $\text{CH}_2\text{Cl}_2$ , BDH ), hydrochloric acid ( $\text{HCl}$ , 37%, Riedel de Haen), potassium hexacyanoferrate (97%, Labmark Chemicals) sodium hydroxide ( $\text{NaOH}$ , 96%, LeSOL laboratory reagent), potassium chloride ( $\text{KCl}$ , 99%, Finkem), phosphoric acid ( $\text{H}_3\text{PO}_4$ , 85%, Riedel de Haen), glacial acetic acid ( $\text{CH}_3\text{COOH}$ , 100%, BDH ), and distilled water were used.

#### **3.2 Instruments**

Screen-printed carbon electrodes (Dropsens 110) connected to Epsilon (BASi) electroanalyzer using Dropsens connector (DRP-DSC70506) was used for electrochemical measurements. The BASi Epsilon was operated using Basic Epsilon EC-Version 1.40.70 software. ATC pH meter (353) was used to measure pH.

#### **3.3 Methods**

##### **3.3.1 Procedure for nitration of 2,4-D**

Nitrated 2,4-D was synthesized (appendix I) using the procedure reported by Maleki et al <sup>4</sup>. 2 g of 2,4-D was dissolved in a solution containing 2 mL dichloromethane 5 mL concentrated sulfuric acid and the resulting solution was kept in ice bath at 0-5 °C. While the temperature of the solution was maintained at 0-5 °C, 1.5 mL concentrated nitric acid was added drop wise over 30 min to the above solution. A yellow paste was formed in the vessel. The reaction mixture was then transferred into 30 mL 10% aqueous  $\text{Na}_2\text{SO}_4$  and the separated organic phase was washed with 20 mL 10% aqueous  $\text{Na}_2\text{SO}_4$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$  using desiccator and the solvent was evaporated. The dried yellow solid was weighed and, the yield of nitrated 2,4-D was calculated. Finally, its melting point was measured.

### **3.3.2 Procedure for preparation of solution**

A total of 3.72 mM stock standard solution of nitrated 2,4-D was prepared in 25 mL volumetric flask, by dissolving 25 mg of the nitrated 2,4-D in a Britton Robinson (BR) buffer of pH 11. BR buffer solution, which contains 40 mM phosphoric acid, 40 mM acetic acid and 40 mM boric acid, was prepared by dissolving 2.7 mL H<sub>3</sub>PO<sub>4</sub>, 2.3 mL CH<sub>3</sub>COOH and 2.474 g H<sub>3</sub>BO<sub>3</sub> in small amount of distilled water and diluting it up to the mark in 1 L volumetric flask. The pH was adjusted to the desired level using 1 M NaOH solution. Working solution was prepared by taking appropriate volume from the stock solution and diluting it with a buffer of pH 11.

### **3.3.3 Electrochemical Measurements**

The cyclic voltammetry and amperometric experiments were carried out using a Basic Epsilon EC-Version 1.40.70 voltammetric analyzer (Bio-analytical Systems, USA), controlled with basic Epsilon software. SPCE (Dropsens 101) was used with carbon as the working electrode and carbon counter electrode & silver as pseudo reference electrode. All potentials were reported with respect to this reference electrode. A magnetic stirrer (BASi C3 Cell stand) was used for stirring solution during amperometric measurements.

### **3.3.4 Electrode preparation**

Screen-printed carbon electrodes were first thoroughly rinsed with distilled water and electrochemically conditioned by potential scanning from 0 to -1100 mV in 1 M KCl for at least five complete scans at 100 mVs<sup>-1</sup>. During which the high background current due to carbon oxidation was minimized. Then, the CV of bare screen-printed electrodes in the presence of potassium hexacyanoferrate was measured from -200 mV to 400 mV to determine the area of the electrodes. The electrodes were dried and immediately used following the cleaning and conditioning steps.

### **3.3.5 Preparation of bismuth film modified SPCE**

Bismuth film modified screen-printed carbon electrodes were prepared by potentiostatic deposition of bismuth on the SPCEs from the bismuth deposition bath (2 mM Bi

(NO<sub>3</sub>)<sub>3</sub>.5H<sub>2</sub>O in 1 M HCl). To prepare bismuth film-modified electrode, bismuth was electrochemically deposited from 2 mM Bi (NO<sub>3</sub>)<sub>3</sub>.5H<sub>2</sub>O by applying constant deposition potential of -1050 mV for 180 s. The modified SPCEs were rinsed with distilled water and immediately used for electrochemical studies of nitrated 2,4-D.

### **3.3.6 Modifying strategies of the electrodes by using bismuth film**

Parameters for the pre-plating of the screen-printed carbon electrode by bismuth using cyclic voltammetry and potential step technique were optimized based on electrochemical response for the analyte at the electrodes. Plating solution containing Bi (III) in acidic media was used to avoid hydrolysis of the metal cation in the presence of KBr to improve the adherence of the bismuth film on the electrode surface<sup>52</sup>. Parameters such as initial concentration of the deposition bath, the deposition potential and deposition time were optimized for electrodeposition of bismuth film. The optimum conditions were selected based on response to the analyte electrochemical signal.

#### **i. Optimization of deposition time**

As reported by Tufa et al.<sup>77</sup>, bismuth film was deposited on the SPCE from 2 mM Bi (III) deposition bath at a step potential of -1200 mV. The effect of deposition time was studied by varying the time applied to the applied potential for 60 , 120 , 180 , 240 , 300 and 360 s. Using the prepared Bi-film electrodes, the current response for 0.372 mM nitrated 2,4-D was recorded. The appropriate deposition time was selected based the response for the nitrated 2,4-D.

#### **ii. Optimization of deposition potential**

The Bi film deposition potential was optimized within the potential range from -950 mV to -1250 mV as dictated by the CV of 2 mM Bi(NO<sub>3</sub>)<sub>3</sub> at the SPCE. After deposition of Bi film at selected potentials within this range for 180 s, the modified SPCE was used to measure the reduction peak for 0.372 mM of the nitrated 2,4-D . The deposition potential that gave the highest analytical signal was selected.

### **iii. Optimization of bath concentration**

Bismuth deposition bath concentrations that range from 0.5 mM to 3 mM was tested to optimized bath concentration at -1050 mV deposition potential for 180 s. The reduction peak current for the nitrated 2,4-D was at the Bi film modified electrodes obtained from different deposition baths was compared to selected the appropriate deposition bath concentration.

### **iv. Optimization of pH**

For pH optimization, the amperometry of 0.372 mM nitrated 2,4-D was run at Bi film modified SPCE (obtained from deposition bath of 2 mM, at -1050 mV for 180 s), using the optimized deposition potential, deposition time and concentration. The resulting current signal of the nitrated 2,4-D were plotted against the pH of the buffer solution.

### **v. Procedure for calibration curve**

Three SPCEs were modified by bismuth film using the above-optimized condition. The amperometry at applied potential -550 mV of nitrated 2,4-D whose concentration ranging from 11.1 to 74.3  $\mu\text{M}$  were run on the same day under similar conditions using these modified electrodes. The average current signal of the analyte from the modified electrodes were plotted against the concentration of nitrated 2,4-D and the calibration curve was plotted.

Three bare SPCE were taken and the chronoamperometry of nitrated 2,4-D at applied potential of -550 mV for concentration ranging from 22.3 to 59.5  $\mu\text{M}$  were run. The average current signal of the analyte from the three different electrodes were plotted against the concentration of nitrated 2,4-D.

### **vi. Procedure for reproducibility and repeatability study**

The reproducibility of the bismuth film modified SPCEs was examined by measuring the same concentration 37.2  $\mu\text{M}$  of nitrated 2,4-D on three modified electrodes in three consecutive days with triplicate measurement in each day. The relative standard deviation

(RSD) of the measured current signal was calculated to determine reproducibility of the modification strategy among the electrodes.

For the repeatability study, the bismuth-modified electrode was prepared under the optimum conditions. Nine successive voltammetric measurements of 37.2  $\mu\text{M}$  nitrated 2,4-D were made on the same day for a modified electrode. The RSD of the measurements was used to estimate the repeatability of the measurement.

#### **vii. Procedure for stability study**

Three SPCE were modified with bismuth film under the optimized conditions on the same day. The amperometry of 37.2  $\mu\text{M}$  nitrated 2,4-D was measured on the first day, seventh day and fourteenth day. In each day, six measurements were made at each electrode. The average current signals of the six measurements in the first day was compared to that of the fourteenth day to determine the stability of the modification procedure developed.

#### **3.3.7 Procedure for sample collection**

Soil and water samples were collected from Dedo district, Sito Kebele around Jimma zone, Ethiopia (**appendix II**). Its location in terms of UTM is 799083N-846083N and 244535E-302787E. Six soil samples from one farmland were randomly collected following standard sampling procedure. The soil samples were mixed and finally one composite soil sample was obtained (**appendix III**). In addition, water sample was collected from Dase River in Sito Kebele about 200 m from the farmland.

#### **3.3.8 Procedure for the extraction of 2,4-D from water and soil samples**

##### **i. Extraction of 2,4-D from soil samples**

One gram of air dried soil sample (**appendix IV**) was dissolved in 100 mL of water and the pH of solution was adjusted to  $\sim 9$  with 1 M NaOH solution. Organic materials that could be present in the sample were separated using the procedure reported by Maleki et al.<sup>4</sup>.

Ten millimeter of the soil solution was transferred to a separating funnel. Then the pH of the aqueous solution was adjusted approximately to 1 with 1 M HCl. The separation was

performed with 5 mL dichloromethane. The acid solution was extracted with dichloromethane. In the final step the dichloromethane phase (expected to contain 2,4-D) was back extracted with 10 mL alkaline aqueous solution (pH~9) and the aqueous solution obtained was nitrated applying procedures reported by Maleki et al.<sup>4</sup> and analyzed by the proposed electrochemical method.

**ii. Extraction of 2,4-D from water samples**

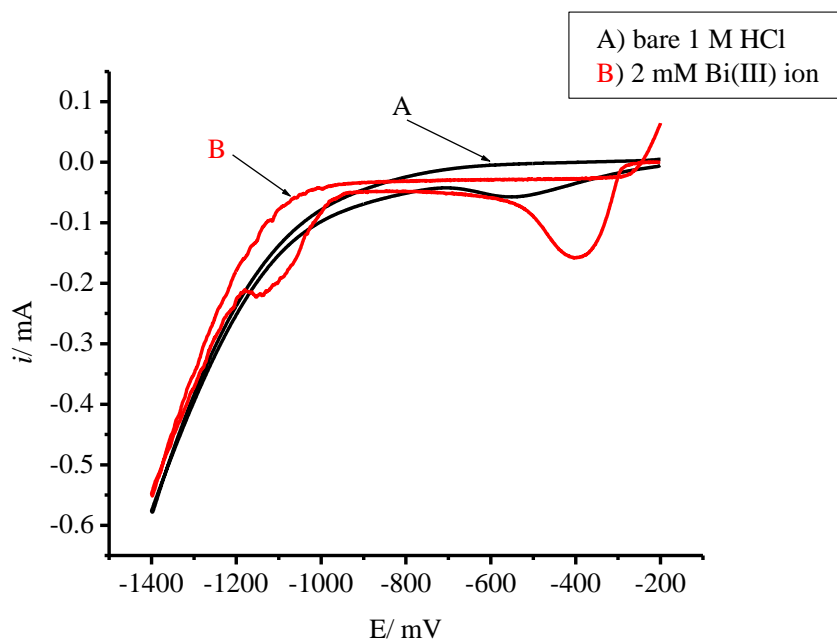
For the analysis of 2,4-D from water samples the procedures reported by Maleki et al.<sup>4</sup> were used. 10 mL of water sample was transferred to a separating funnel. The pH of solution was adjusted to approximately 1 with HCl (1 M) and the separation was performed with 5 mL of dichloromethane. The organic phase was back extracted with 10 mL alkaline solution (pH ~ 9 adjusted with 1 M NaOH). The aqueous solution obtained was nitrated applying procedures reported by Maleki et al.<sup>4</sup>. Finally it was analyzed by the proposed electrochemical method.



## 4. Results and discussion

### 4.1 Cyclic voltammetry of Bismuth (III) ion

The cyclic voltammetry of Bare SPCE in electrolyte (1 M HCl) and in 2 mM  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  was as shown (Fig. 4.1). From the cyclic voltammogram the potential which are more negative than -950 mV can reduces the  $\text{Bi}^{3+}$  ion in to its metallic form.

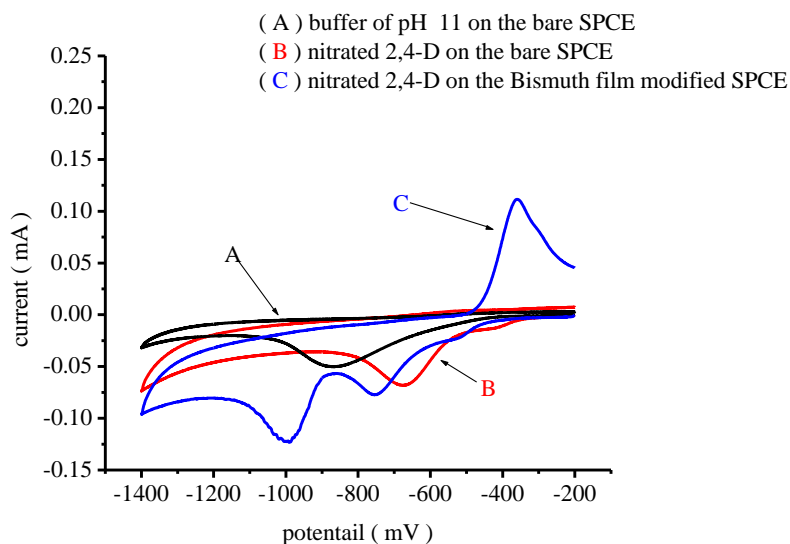


**Figure 4.1** Cyclic voltammogram of (A) bare 1 M HCl and (B) 2 mM Bi (III) ion.

So, Bi film was electrochemically deposited on the SPCE from 2 mM  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  by applying constant deposition potential of -1050 mV for 180 s.

### 4.2 Voltammetric behavior of nitrated 2,4-D at bismuth film modified SPCE

The cyclic voltammogram of nitrated 2,4-D at the bare, and bismuth film-modified SPCEs based on the above mentioned procedure (section 3.3.5) were depicted in (Fig. 4.2). It is evident that the voltammogram of bismuth film modified SPCEs showed an increment in signal for the analyte than that of the bare SPCE within the studied potential window.



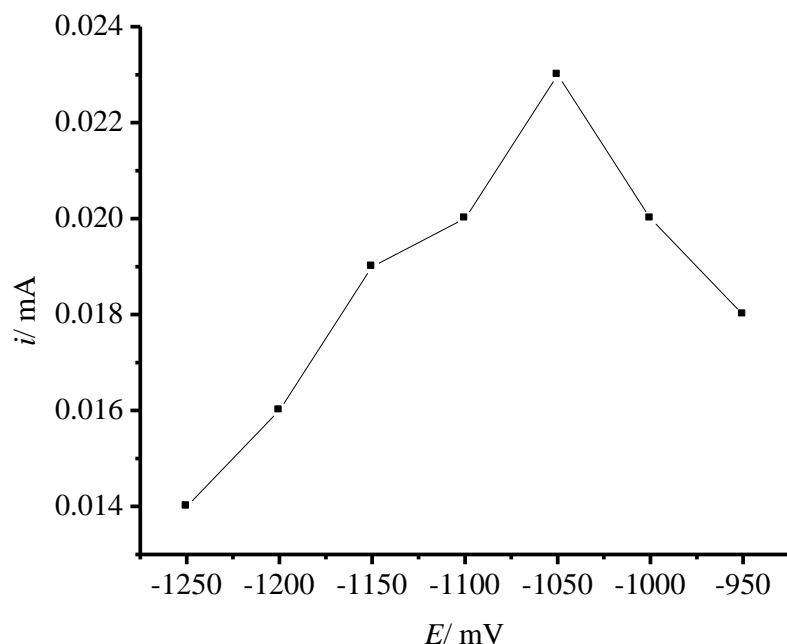
**Figure 4.2** Cyclic voltammogram of (A) buffer of pH 11 on bare SPCE, (B) nitrated 2,4-D on- bare SPCE, and (C) nitrated 2,4-D on the bismuth modified SPCE.

Furthermore, the cyclic voltammogram revealed that the potentials, which are more negative than -300 mV, can reduce nitrated 2,4-D on the bare SPCE. However, when the SPCE is modified with Bismuth, the reduction potential of nitrated 2,4-D slightly shifted to potentials that are more negative and the reduction starts -350 mV since bismuth electrode have high over potential for the reduction of  $\text{H}_3\text{O}^+$  to  $\text{H}_2$  similar to mercury electrodes<sup>50</sup>.

### 4.3 Optimization of parameters for the determination 2,4-D

#### 4.3.1 Effect of deposition potential

For the study of the deposition potential of bismuth film on the SPCE, the cyclic voltammetry of the plating solution containing 2 mM Bi (III) in 1 M HCl depicted in (Fig. 4.1). Then the effect of deposition potential for the determination of the nitrated 2,4-D was studied in the potential range of -950 to -1250 mV as it was shown in (Fig. 4.3).

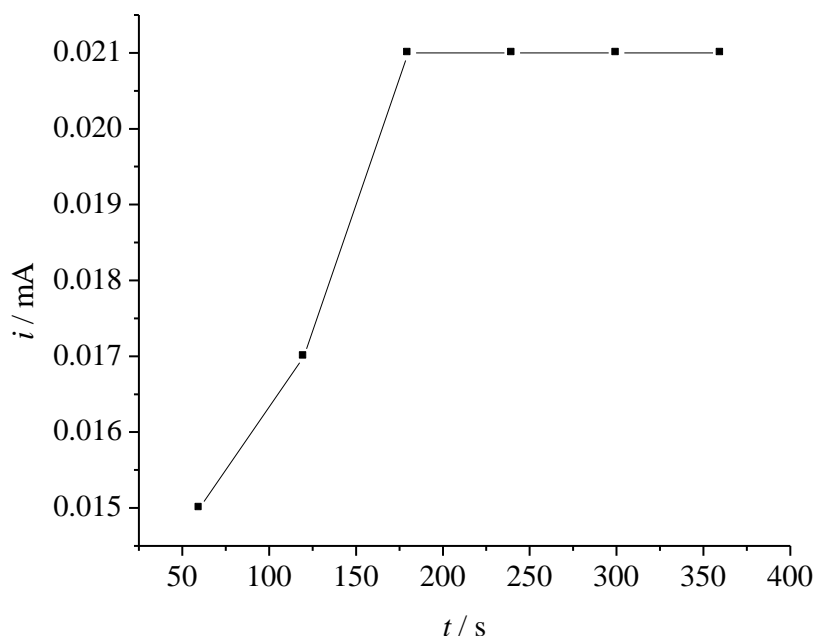


**Figure 4.3** Effect of deposition potential upon voltammetric response for 0.372  $\mu\text{M}$  nitrated 2,4-D. Experimental conditions: 2 mM Bi (III) and deposition time 180 s.

The result obtained in (Fig. 4.3) suggests that deposition potential of -1050 mV gives the best analytical signal and the BiFE obtained under these conditions was selected for the electroanalysis of nitrated 2,4-D as this potential offered the highest signal for the analyte. This result is in line with the effect of the choice of deposition potential which was investigated by Kokkinos et al.<sup>78</sup> to have best quality of bismuth film. The background hydrogen evolution that occurs at a more negative potential has to be avoided.

#### 4.3.2 Effect of deposition time

The bismuth film deposition time is one of the decisive parameters which is used to control the thickness of the bismuth film<sup>79</sup>. The effect of the bismuth plating time was studied in the range 60–360 s as shown in (Fig. 4.4).

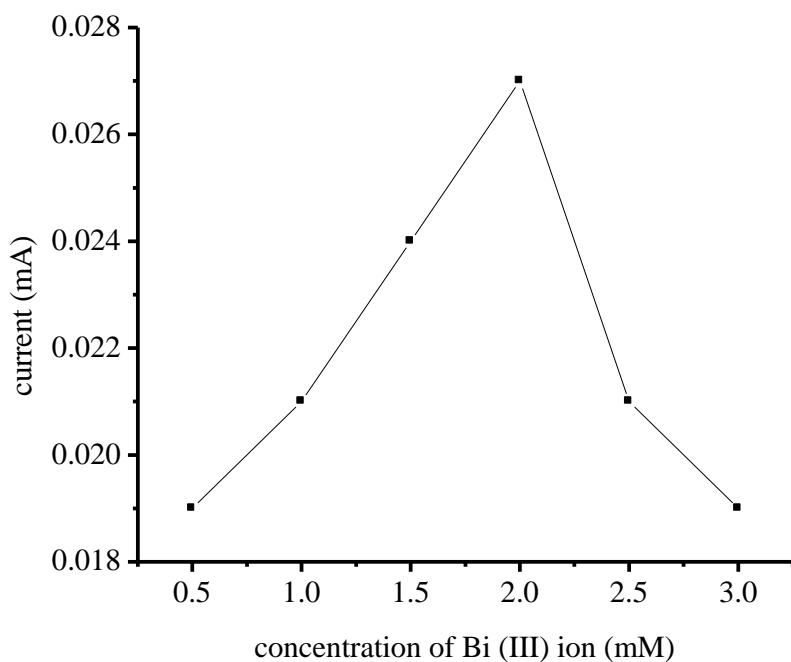


**Figure 4.4** Effect of deposition time upon voltammetric response for 0.372  $\mu\text{M}$  nitrated 2,4-D. Experimental condition: 2 mM Bi (III) and deposition potential -1050 mV.

The peak current the nitrated 2,4-D goes on increasing with increasing bismuth deposition time up to 180 s and remained constant beyond this deposition time which suggested that bismuth deposition time of 180 s was found to offer the highest sensitivity for the determination of nitrated 2,4-D. The increment of the peak current of nitrated 2,4-D with increase in time is due to the increase in thickness of the film with increase deposition time. However, further increasing deposition time resulted in depletion of  $\text{Bi}^{3+}$  at electrode-electrolyte interface<sup>77, 79</sup>.

### 4.3.3 Effect of bath concentration

The other important parameter which influences the thickness of bismuth film is the bismuth ion concentration on the peak current of the nitrated 2,4-D. The effect of concentration bath of bismuth ion was examined in the range of 0.5 to 3 mM as shown in (Fig. 4.5) for 0.372  $\mu\text{M}$  nitrated 2,4-D solution in buffer at pH 11.

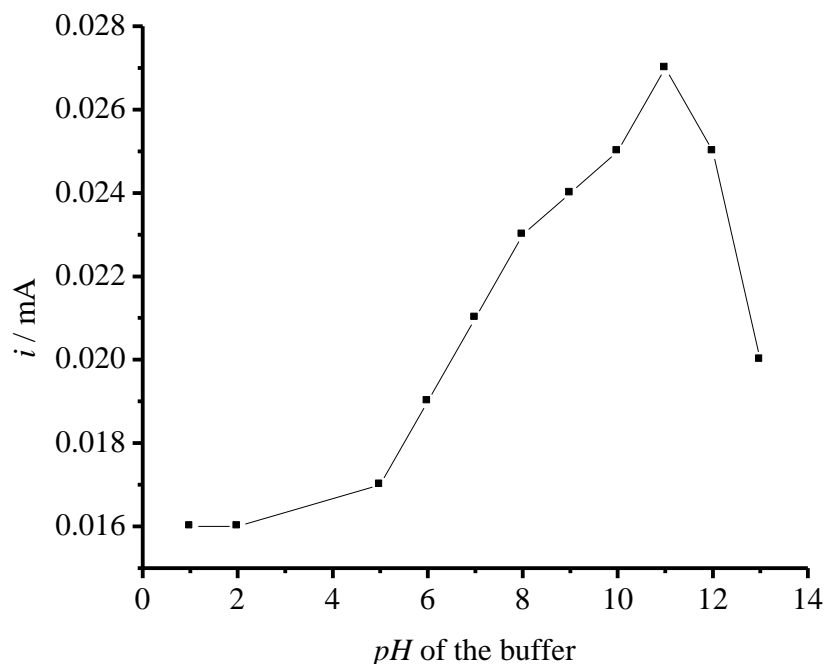


**Figure 4.5** Effect of bath concentration upon voltammetric response for 0.372  $\mu\text{M}$  nitrated 2,4-D. experimental condition: deposition potential -1050 mV and deposition time 180 s.

The result indicated in (Fig. 4.5) showed that the peak current of the nitrated 2,4-D started increasing up to 2 mM Bi (III) ion and started decreasing for concentration beyond 2 mM Bi (III) ion. The increased in peak current for the increase in Bi (III) ion concentration up to 2 mM is attributed to the increase in the bismuth film thickness<sup>80</sup>. However, when the concentration of Bi (III) ion becomes higher, peaks decreased with increasing bismuth film thickness this is due to cracking of the film at higher concentration. Hence, this result is in agreement with result reported for the optimization of bath concentration<sup>77, 79</sup>.

#### 4.3.4 Effect of pH of the supporting electrolyte

The pH of the electrolyte was also an important parameter that could influence the response of the electrode in the electroanalysis of 2,4-D. The effect of the pH of the supporting electrolyte on the electrode response was studied in the range 1–13 in BR buffer. The results obtained are given in (Fig. 4.6) that indicates the optimum response is at pH 11.

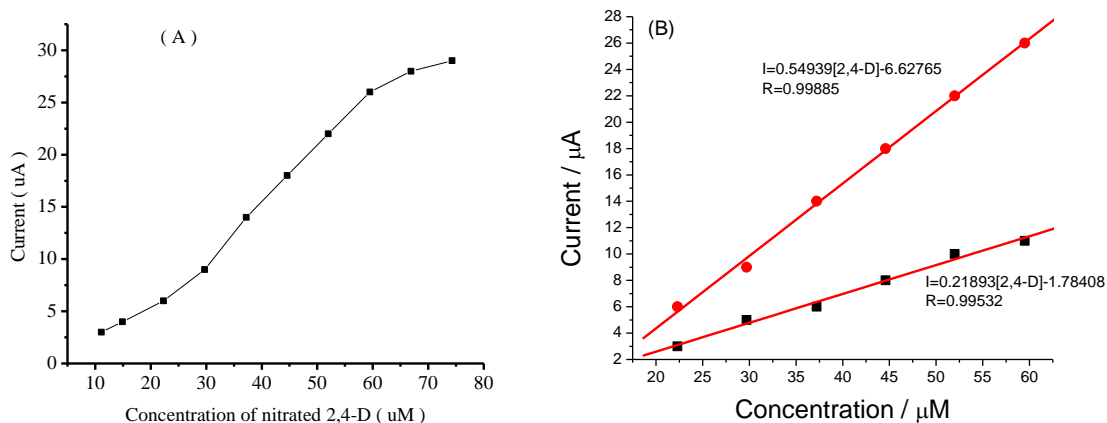


**Figure 4.6** Effect of pH of the supporting electrolyte upon voltammetric response for solution containing 0.372  $\mu\text{M}$  nitrated 2,4-D. Experimental conditions: 2 mM Bi (III), deposition time 180 s and deposition potential -1050 mV.

This may be due to the existence of the anionic form of the target analyte in the alkaline environments. On the other hand, for the  $\text{pH} < 6$  reduction peak may overlaps with the hydrogen evolution signal so that best analytical signal could not be observed in this pH range. Therefore, buffer of pH 11 has been taken as the optimum pH for the determination of 2,4-D throughout the experiments, which is in close agreement with previous reports<sup>4</sup>.

#### 4.4 Calibration curve

The calibration curve was established using the optimum parameters described for the determinations of nitrated 2,4-D with triplicate measurements. It exhibits dynamic linear range from 11.1 to 74.3  $\mu\text{M}$  of nitrated 2,4-D (Fig. 4.7A). The plot of the reduction peak current versus the concentration nitrated 2,4-D was linear with regression coefficient (R) 0.9989 in the concentration range from 22.3 to 59.5  $\mu\text{M}$  (Fig. 4.7B).



**Figure 4.7** A) The dynamic calibration curve for nitrated 2,4-D at the bismuth film modified SPCE (B) Comparison of the bare and bismuth film modified SPCE in a selected concentration range.

The limit of detection (LOD) which is three times the standard deviation of the blank divided by the slope of the curve and limit of quantification (LOQ), which refers to ten times the standard deviation of the blank divided by the slope of the curve, were calculated to be 3.15 and 10.5  $\mu\text{M}$ , respectively. These findings thus clearly confirm the reliability of the developed electroanalytical method for determination of the target analyte in the environmental samples.

#### 4.5 Repeatability and reproducibility

The repeatability and reproducibility BiFEs were determined by successive voltammetric measurements using 37.2  $\mu\text{M}$  nitrated 2,4-D in BR buffer solution at pH 11. In the repeatability study, nine successive voltammetric measurements on the same day were made under the same experimental condition, and the RSD was 5.84%. This suggested that the proposed BiFEs have good repeatability.

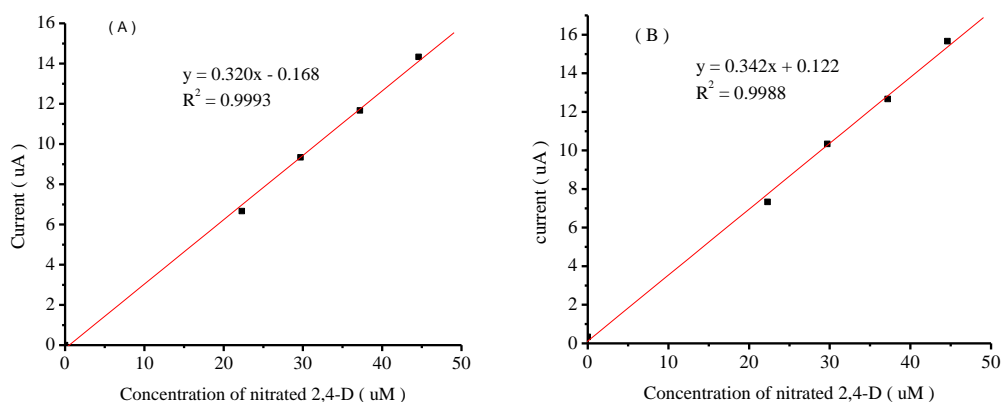
The reproducibility of the BiFE was also examined by measuring 37.2  $\mu\text{M}$  of nitrated 2,4-D on three different days with triplicate measurement. The RSD obtained were 4.66% for three independently prepared modified electrodes. This confirmed that the experimental results for the proposed BiFEs were reproducible.

## 4.6 Stability of the BiFE

The stability of the bismuth film modified SPCE after keeping it for two weeks was studied by amperometric measurements of 37.2  $\mu\text{M}$  nitrated 2,4-D. The peak current for the nitrated 2,4-D obtained after keeping the modified electrodes at room temperature for two weeks has maintained 95.7% of the initial current response. This result indicated that there was insignificant peak current change lower than 5%, showing that the BiFE had good stability.

## 4.7 Analysis of 2,4-D in real sample using Bismuth modified SPCE

To only validate the applicability of the developed method, water and soil samples were used for analysis of 2,4-D by standard addition method. The results were presented in (Fig. 4.8).



**Figure 4.8** Calibration curve for standard addition of 2,4-D of (A) water sample and (B) soil sample.

From the results obtained for the analysis of 2,4-D in the soil sample, the level of 2,4-D could be detected but it was below quantification limit. Whereas in water samples, the level of 2,4-D could not be detected using the proposed method.



#### 4.8 Recoveries of 2,4-D from soil and water samples

For the recovery studies, 10 mL of each of the nitrated water and soil samples were transferred into electrochemical cell. The recovery was performed by spiking three different concentrations of nitrated 2,4-D (22.3, 29.7, and 37.2  $\mu\text{M}$ ) to each sample, and the resultant current peak of chronoamperometry was obtained using the proposed BiFEs.

**Table 4.1** Recoveries of 2,4-D from real samples (n = 3)

| sample | Added $\mu\text{M}$ | Found $\mu\text{M}$ | Recovery% | RDS% |
|--------|---------------------|---------------------|-----------|------|
| water  | 22.3                | 22.4                | 100.4     | 8.66 |
|        | 29.7                | 31.8                | 107.1     | 5.97 |
|        | 37.2                | 34.9                | 93.8      | 5.09 |
| soil   | 22.3                | 23.1                | 103.6     | 7.53 |
|        | 29.7                | 31.8                | 107.1     | 5.41 |
|        | 37.2                | 37.7                | 101.3     | 4.56 |

The recovery obtained for 2,4-D in the real samples using the bismuth film modified SPCE ranges from 93.8 to 107.1% as illustrated in (Table 4.1) for water and soil samples. These results may suggest that the matrices of the real samples do not have significant effects on the determination of the 2,4-D from real water and soil samples.

#### 4.9 Comparison of the current developed method with the reported other electroanalytical methods

The developed electroanalytical method for the determination of 2,4-D herbicide in water and soil samples by using bismuth film modified SPCE was comparable with the values reported in literature as shown in (Table 4.2). The proposed method has shown moderate performance.

**Table 4.2** Comparison of the new developed method in the present work and other methods described in the literature for the determination of 2,4-D

| Electrodes   | Techniques | LOD                  | Reference |
|--|------------|----------------------|-----------|
| Protoporphyrin IX cobalt(III) chloride<br>modified GCE | CA         | 0.98 $\mu\text{M}$   | 2         |
| Silica-gel modified carbon paste electrode             | CA         | 400 $\text{mM}$      | 81        |
| Mercury electrode                                      | ADS        | 0.226 $\mu\text{M}$  | 82        |
| Static mercury electrode                               | DPV        | 0.0226 $\mu\text{M}$ | 4         |
| Graphite-polyurethane                                  | SWV        | 0.0796 $\mu\text{M}$ | 3         |
| Bismuth film modified SPCE                             | CA         | 3.15 $\mu\text{M}$   | This work |

## **5. Conclusion and recommendation**

### **5.1 Conclusion**

In this study, new electroanalytical method has been developed for the analysis of 2,4-D by the electrodeposition of bismuth film on the surface of screen printed carbon electrodes. The developed method has showed dynamic linear concentration range from 11.1 to 74.3  $\mu\text{M}$  of the nitrated 2,4-D with regression coefficient of (R)0.9988. The limits of detection and quantification were 3.15 and 10.5  $\mu\text{M}$  respectively. The method has exhibited very good electroanalytical properties such as high sensitivity, low LOD and wide linear dynamic range for the determination of 2,4-D. The method has advantageous due to its inexpensiveness, simplicity, fast time analysis and lesser toxicity.

### **5.2 Recommendation**

Based on the findings of this study, the sensitivity and the analytical performance of the developed method for the determination of 2,4-D was successfully addressed. However, the selectivity of the method was not studied due to the lack of standard chemicals required for the interference study. So, to determine the level of 2,4-D herbicide in environmental samples other researchers can apply the developed method for further investigation.

## References

1. Casella, I. G.; Contursi, M., Electrocatalytic reduction of chlorophenoxy acids at palladium-modified glassy carbon electrodes. *Electrochim. Acta* **2007**,*52*, 7028-7034.
2. Chaiyasith, S.; Tangkuaram, T.; Chaiyasith, P., Electrocatalytical of chlorophenoxy-carboxylic acids at a protoporphyrin IX cobalt (III) chloride modified glassy carbon electrode. *J. Electroanal. Chem.* **2005**,*581*, 104-110.
3. Ramos de Andrade, F.; Alves de Toledo, R.; Manoel Pedro Vaz, C., Electroanalytical methodology for the direct determination of 2, 4-dichlorophenoxyacetic acid in soil samples using a graphite-polyurethane electrode. *Int. J. Electrochem.* **2014**
4. Maleki, N.; Safavi, A.; Hasanpour, F.; Shahbaazi, H., Determination of traces of 2, 4-dichlorophenoxy acetic acid in environmental Samples. *Sci. Iran.* **2008**,*15*, 430-434.
5. Jankowska, A.; Biesaga, M.; Drzewicz, P.; Trojanowicz, M.; Pyrzyn'ska, K., Chromatographic separation of chlorophenoxy acid herbicides and their radiolytic degradation products in water samples. *Water Res.* **2004**,*38*, 3259-3264.
6. Kundu, S.; Pal, A.; Dikshit, A. K., UV induced degradation of herbicide 2, 4-D: kinetics, mechanism and effect of various conditions on the degradation. *Sep. Purif. Technol.* **2005**,*44*, 121-129.
7. Wang, J., *Analytical electrochemistry*. John Wiley & Sons: 2006.
8. Uslu, B.; Ozkan, S. A., Solid electrodes in electroanalytical chemistry: present applications and prospects for high throughput screening of drug compounds. *Comb. Chem. High Throughput Screening* **2007**,*10*, 495-513.
9. Stradiotto, N. R.; Yamanaka, H.; Zandoni, M. V. B., Electrochemical sensors: a powerful tool in analytical chemistry. *J. Braz. Chem. Soc.* **2003**,*14*, 159-173.
10. [http://www.dropsens.com/en/screen\\_printed\\_electrodes\\_pag.html](http://www.dropsens.com/en/screen_printed_electrodes_pag.html) accessed date october 19.

11. Hayat, A.; Marty, J. L., Disposable screen printed electrochemical sensors: Tools for environmental monitoring. *Sensors* **2014**,*14*, 10432-10453.
12. Achary, G.; Kumaraswamy, M.; Viswanatha, R.; Nayaka, Y. A., An organically modified exfoliated graphite electrode for the voltammetric determination of lead ions in contaminated water samples. . *Russ. J. Electrochem.* **2015**,*51*, 679-685.
13. Choi, J.-Y.; Seo, K.; Cho, S.-R.; Oh, J.-R.; Kahng, S.-H.; Park, J., Screen-printed anodic stripping voltammetric sensor containing HgO for heavy metal analysis. *Anal. Chim. Acta* **2001**,*443*, 241-247.
14. Niu, P. F.-S., C.; Gich, M.; Ayora, C.; Roig, A., , Electroanalytical assessment of heavy metals in waters with bismuth nanoparticle-porous carbon paste electrodes. *Electrochim. Acta* **2015**,*165*, 155-161.
15. Rusinek, C. A.; Bange, A.; Papautsky, I.; Heineman, W. R., Cloud point extraction for electroanalysis: Anodic stripping voltammetry of cadmium. *Anal. Chem.* **2015**,*87*, 6133-6140.
16. Simm, A. O.; Banks, C. E.; Compton, R. G., The electrochemical detection of arsenic (III) at a silver electrode. *Electroanalysis* **2005**,*17*, 1727-1733.
17. Sakthinathan, S.; Chen, S.-M., Graphene supported nanocomposite for electrochemical detection of pollutant materials: A short review. *Int. J. Electrochem.* **2015**,*10*, 6527-6536.
18. Honeychurch, K. C.; Hart, J. P., Voltammetric behavior of p-nitrophenol and its trace determination in human urine by liquid chromatography with a dual reductive mode electrochemical detection system. *Electroanalysis* **2007**,*19*, 2176-2184.
19. Kumar, A. S.; Zen, J.-M., Electrochemical investigation of glucose sensor fabricated at copper-plated screen-printed carbon electrodes. *Electroanalysis* **2002**,*14*, 671.
20. La Marca, A.; Stabile, G.; Artenisio, A. C.; Volpe, A., Serum anti-mullerian hormone throughout the human menstrual cycle. *Hum. Reprod.* **2006**,*21*, 3103-3107.

21. Geetha, S.; Rao, C. R.; Vijayan, M.; Trivedi, D., Biosensing and drug delivery by polypyrrole. *Anal. Chim. Acta* **2006**,*568*, 119-125.
22. Wang, X.; Yu, J.; Sreekumar, A.; Varambally, S.; Shen, R.; Giacherio, D.; Mehra, R.; Montie, J. E.; Pienta, K. J.; Sanda, M. G., Autoantibody signatures in prostate cancer. *N. Engl. J. Med.* **2005**,*353*, 1224-1235.
23. Zhang, S.; Ma, H.; Yan, L.; Cao, W.; Yan, T.; Wei, Q.; Du, B., Copper-doped titanium dioxide nanoparticles as dual-functional labels for fabrication of electrochemical immunosensors. *Biosens. Bioelectron.* **2014**,*59*, 335-341.
24. Schlegel, M. K.; Peritz, A. E.; Kittigowittana, K.; Zhang, L.; Meggers, E., Duplex formation of the simplified nucleic acid GNA. *ChemBioChem* **2007**,*8*, 927-932.
25. Li, C.; Zhan, G.; Yang, Q.; Lu, J., Electrochemical investigation of acetaminophen with a carbon nano-tube composite film electrode. *Bull. Korean Chem. Soc.* **2006**,*27*, 1854-1860.
26. Wang, Y.; Rui, Y.; Li, F.; Li, M., Electrodeposition of nickel hexacyanoferrate/layered double hydroxide hybrid film on the gold electrode and its application in the electroanalysis of ascorbic acid. *Electrochim. Acta* **2014**,*117*, 398-404.
27. Garrido, E. M.; Delerue-Matos, C.; Lima, J.; Brett, A. O., Electrochemical methods in pesticides control. *Anal. Lett.* **2004**,*37*, 1755-1791.
28. Hance, R., Polarography of herbicides-A preliminary survey. *Pestic. Sci.* **1970**,*1*, 112-113.
29. De Figueiredo-Filho, L. C. S.; dos Santos, V. B.; Janegitz, B. C.; Guerreiro, T. B.; Fatibello-Filho, O.; Faria, R. C.; Marcolino-Junior, L. H., Differential pulse voltammetric determination of paraquat using a bismuth-film electrode. *Electroanalysis* **2010**,*22*, 1260-1266.
30. Terry, L. A.; White, S. F.; Tigwell, L. J., The application of biosensors to fresh produce and the wider food industry. *J. Agric. Food Chem.* **2005**,*53*, 1309-1316.
31. Durst, R., Chemically modified electrodes: Recommended terminology and definitions (IUPAC recommendations 1997). *Pure Appl. Chem.* **1997**,*69*, 1317-1324.

32. Ciucu, A. A., Chemically modified electrodes in biosensing. *J. Biosens. Bioelectron* **2014**,*5*, 1-10.
33. Prieto-Simon, B.; Fabregas, E., Comparative study of electron mediators used in the electrochemical oxidation of NADH. *Biosens. Bioelectron.* **2004**,*19*, 1131-1138.
34. Guan, J.-G.; Miao, Y.-Q.; Chen, J.-R., Prussian blue modified amperometric FIA biosensor: one-step immunoassay for  $\alpha$ -fetoprotein. *Biosens. Bioelectron.* **2004**,*19*, 789-794.
35. Gong, K.; Dong, Y.; Xiong, S.; Chen, Y.; Mao, L., Novel electrochemical method for sensitive determination of homocysteine with carbon nanotube-based electrodes. *Biosens. Bioelectron.* **2004**,*20*, 253-259.
36. Gerard, M.; Chaubey, A.; Malhotra, B., Application of conducting polymers to biosensors. *Biosens. Bioelectron.* **2002**,*17*, 345-359.
37. Gooding, J. J.; Hibbert, D. B., The application of alkanethiol self-assembled monolayers to enzyme electrodes. *TrAC, Trends Anal. Chem.* **1999**,*18*, 525-533.
38. Foss Jr, C. A.; Martin, C. *Chemically modified electrodes*; DTIC document: 1994.
39. Roddick, A. D., Development and application of electroanalytical techniques for biological matrices. **1995**.
40. Calvo-Marzal, P.; Chumbimuni-Torres, K. Y.; Hoehr, N. F.; de Oliveira Neto, G.; Kubota, L. T., Determination of reduced glutathione using an amperometric carbon paste electrode chemically modified with TTF-TCNQ. *Sensors and Actuators B: Chemical* **2004**,*100*, 333-340.
41. Kalcher, K.; Kauffmann, J.-M.; Wang, J.; Å vancara, I.; Vytras, K.; Neuhold, C.; Yang, Z., Sensors based on carbon paste in electrochemical analysis: a review with particular emphasis on the period 1990-1993. *Electroanalysis* **1995**,*7*, 5-22.
42. Kutner, W.; Wang, J.; L'her, M.; Buck, R. P., Analytical aspects of chemically modified electrodes: classification, critical evaluation and recommendations (IUPAC Recommendations 1998). *Pure Appl. Chem.* **1998**,*70*, 1301-1318.

43. Miu, M.; Angelescu, A.; Kleps, I.; Simion, M., Electrochemical sensors for heavy metals detection in liquid media. *Int. J. Environ. Anal. Chem.* **2005**,*85*, 675-679.
44. Barton, J.; Garcia, M. B. G.; Santos, D. H.; Fanjul-Bolado, P.; Ribotti, A.; McCaul, M.; Diamond, D.; Magni, P., Screen-printed electrodes for environmental monitoring of heavy metal ions: a review. *Microchim. Acta* **2015**, 1-15.
45. Chekin, F.; Gorton, L.; Tapsobea, I., Direct and mediated electrochemistry of peroxidase and its electrocatalysis on a variety of screen-printed carbon electrodes: amperometric hydrogen peroxide and phenols biosensor. *Anal. Bioanal. Chem.* **2015**,*407*, 439-446.
46. Cinti, S.; Arduini, F.; Carbone, M.; Sansone, L.; Cacciotti, I.; Moscone, D.; Palleschi, G., Screen-Printed Electrodes Modified with Carbon Nanomaterials: A Comparison among Carbon Black, Carbon Nanotubes and Graphene. *Electroanalysis* **2015**,*27*, 2230-2238.
47. Radi, A.-E., Voltammetric behaviour of esomeprazole at screen printed carbon electrode and its determination in capsule dosage form. *Eurasian J. Anal. Chem.* **2015**,*9*.
48. Ahmed, M. U.; Hossain, M. M.; Safavieh, M.; Wong, Y. L.; Rahman, I. A.; Zourob, M.; Tamiya, E., Toward the development of smart and low cost point-of-care biosensors based on screen printed electrodes. *Crit. Rev. Biotechnol.* **2015**, 1-11.
49. Asadpour-Zeynali, K.; Najafi-Marandi, P., Bismuth modified disposable pencil-lead electrode for simultaneous determination of 2-nitrophenol and 4-nitrophenol by net analyte signal standard addition method. *Electroanalysis* **2011**,*23*, 2241-2247.
50. Sopha, H.; Hocevar, S. B.; Pihlar, B.; Ogorevc, B., Bismuth film electrode for stripping voltammetric measurement of sildenafil citrate. *Electrochim. Acta* **2012**,*60*, 274-277.
51. Wang, J., Stripping analysis at bismuth electrodes: a review. *Electroanalysis* **2005**,*17*, 1341-1346.



52. Lezi, N.; Vyskocil, V.; Economou, A.; Barek, J., Electroanalysis of organic compounds at bismuth electrodes: a short review. *Sensing in electroanalysis* **2012**,*7*, 71-78.
53. March, G.; Nguyen, T. D.; Piro, B., Modified electrodes used for electrochemical detection of metal ions in environmental analysis. *Biosensors* **2015**,*5*, 241-275.
54. Organization, W. H., Public health impact of pesticides used in agriculture. **1990**.
55. Cserhati, T.; Forgacs, E., Phenoxyacetic acids: separation and quantitative determination. *J. Chromatogr. B: Biomed. Sci. Appl.* **1998**,*717*, 157-178.
56. Scientist, S. F. E., Use Information and air monitoring recommendations for the pesticide active ingredient 2,4-Dichlorophenoxy acetic acid dimethylamine salt 2013.
57. EPA, Registration Eligibility Decision for 2,4-D. US Environmental Protection Agency, Office of Pesticide programs Number EPA 738-R-05-002. *Number EPA 738-R-05-002* 2005.
58. Gervais, J.; Luukinen, B.; Buhl, K.; Stone, D., 2, 4-D technical fact sheet; national pesticide information center, Oregon State University Extension Services. 2008.
59. Boivin, A.; Amellal, S.; Schiavon, M.; Van Genuchten, M. T., 2, 4-Dichlorophenoxyacetic acid (2, 4-D) sorption and degradation dynamics in three agricultural soils. *Environmental Pollution* **2005**,*138*, 92-99.
60. Pesticides, B., 2, 4-D Chemical WATCH Factsheet. 2004.
61. Lavy, T., Safety and health in the use of agrochemicals: A Guide. *J. Environ. Qual.* **1992**,*21*, 291-291.
62. Carrai, P.; Nucci, L.; Pergola, F., Polarographic behaviour of alachlor application to analytical determination. *Anal. Lett.* **1992**,*25*, 163-172.
63. Fayyad, M.; Alawi, M.; Issa, I., Differential pulse polarographic determination of organochlorine pesticides. *Anal. Lett.* **1989**,*22*, 1939-1959.

64. Garcia, A. R.; Barrio, A. R.; Carrazon, J. P.; Daez, L. P., Polarographic study of organochlorine pesticides in micellar solutions. *Anal. Chim. Acta* **1991**,*246*, 293-300.
65. Sreedhar, N.; Reddy, P. R. K.; Reddy, G. S.; Reddy, S., Differential pulse polarographic determination of dicrotophos, crotoxypfos and chlorfenvinphos in grains and soils. *Talanta* **1997**,*44*, 1859-1863.
66. Bronstad, J. O.; Friestad, H., Method for determination of glyphosate residues in natural waters based on polarography of the N-nitroso derivative. *Analyst* **1976**,*101*, 820-824.
67. Subbalakshamma, M.; Reddy, S. J., Electrochemical reduction behavior and analysis of some organophosphorous pesticides. *Electroanalysis* **1994**,*6*, 521-526.
68. Ignjatovic, L. M. M., D. A.; Veselinovic, D. S.; Besic, B. R, Polarographic behavior and determination of some S-triazine herbicides. *Electroanalysis* **1993**,*5*, 529-533.
69. Benadikova, H. K., R., Adsorptive stripping voltammetry of some triazine-and nitro group-containing pesticides. *Anal. Lett.* **1984**,*17*, 1519-1531.
70. Lippolis, M.; Concialini, V., Differential pulse polarographic determination of the herbicides atrazine, prometryne and simazine. *Talanta* **1988**,*35*, 235-236.
71. Skopalova, J.; Kotouk • ek, M., Polarographic behaviour of some s-triazine herbicides and their determination by adsorptive stripping voltammetry at the hanging mercury drop electrode. *Fresenius' J. Anal. Chem.* **1995**,*351*, 650-655.
72. Benson, W. R.; Gajan, R. J., Nitrosation of 1-naphthyl N-methylcarbamate and related compounds as it was followed by oscillopolarography. *J. Org. Chem.* **1966**,*31*, 2498-2502.
73. Nouws, H.; Delerue-Matos, C.; Lima, J.; Garrido, E. M.; Vincke, P.; Maes, N., Electroanalytical study of the pesticide asulam. *Int. J. Environ. Anal. Chem.* **2002**,*82*, 69-76.
74. Fontmorin, J.-M.; Fourcade, F.; Geneste, F.; Soutrel, I.; Floner, D.; Amrane, A., Direct electrochemical oxidation of a pesticide, 2, 4-dichlorophenoxyacetic

- acid, at the surface of a graphite felt electrode: Biodegradability improvement. *C. R. Chim.* **2015**,*18*, 32-38.
75. Dequaire, M.; Degrand, C.; Limoges, B., An immunomagnetic electrochemical sensor based on a perfluorosulfonate-coated screen-printed electrode for the determination of 2, 4-dichlorophenoxyacetic acid. *Anal. Chem.* **1999**,*71*, 2571-2577.
76. Kalab, T.; Skladal, P., Disposable multichannel immunosensors for 2, 4-dichlorophenoxyacetic acid using acetylcholinesterase as an enzyme label. *Electroanalysis* **1997**,*9*, 293-297.
77. Tufa, L. T.; Siraj, K.; Soreta, T. R., Electrochemical determination of lead using bismuth modified glassy carbon electrode. *Russ. J. Electrochem.* **2013**,*49*, 59-66.
78. Kokkinos, C.; Economou, A.; Raptis, I.; Efstathiou, C. E., Lithographically fabricated disposable bismuth-film electrodes for the trace determination of Pb (II) and Cd (II) by anodic stripping voltammetry. *Electrochim. Acta* **2008**,*53*, 5294-5299.
79. Baron-Jaimez, J. A.; Marulanda-Arevalo, J. L.; Barba-Ortega, J. J., Electrodes friendly with the environment for detect heavy metal. *Dyna* **2014**,*81*, 122-128.
80. Arduini, F.; Calvo, J. Q.; Palleschi, G.; Moscone, D.; Amine, A., Bismuth-modified electrodes for lead detection. *TrAC, Trends Anal. Chem.* **2010**,*29*, 1295-1304.
81. Prado, A. G.; Barcelos, H. T.; Moura, A. O.; Nunes, A. R.; Gil, E. S., Dichlorophenoxyacetic acid anchored on silica-gel modified carbon paste for the determination of pesticide 2, 4-D. *Int. J. Electrochem. Sci.* **2012**,*7*, 8929-8939.
82. Maleki, N.; Safavi, A.; Shahbaazi, H., Electrochemical determination of 2, 4-D at a mercury electrode. *Anal. Chim. Acta* **2005**,*530*, 69-74.

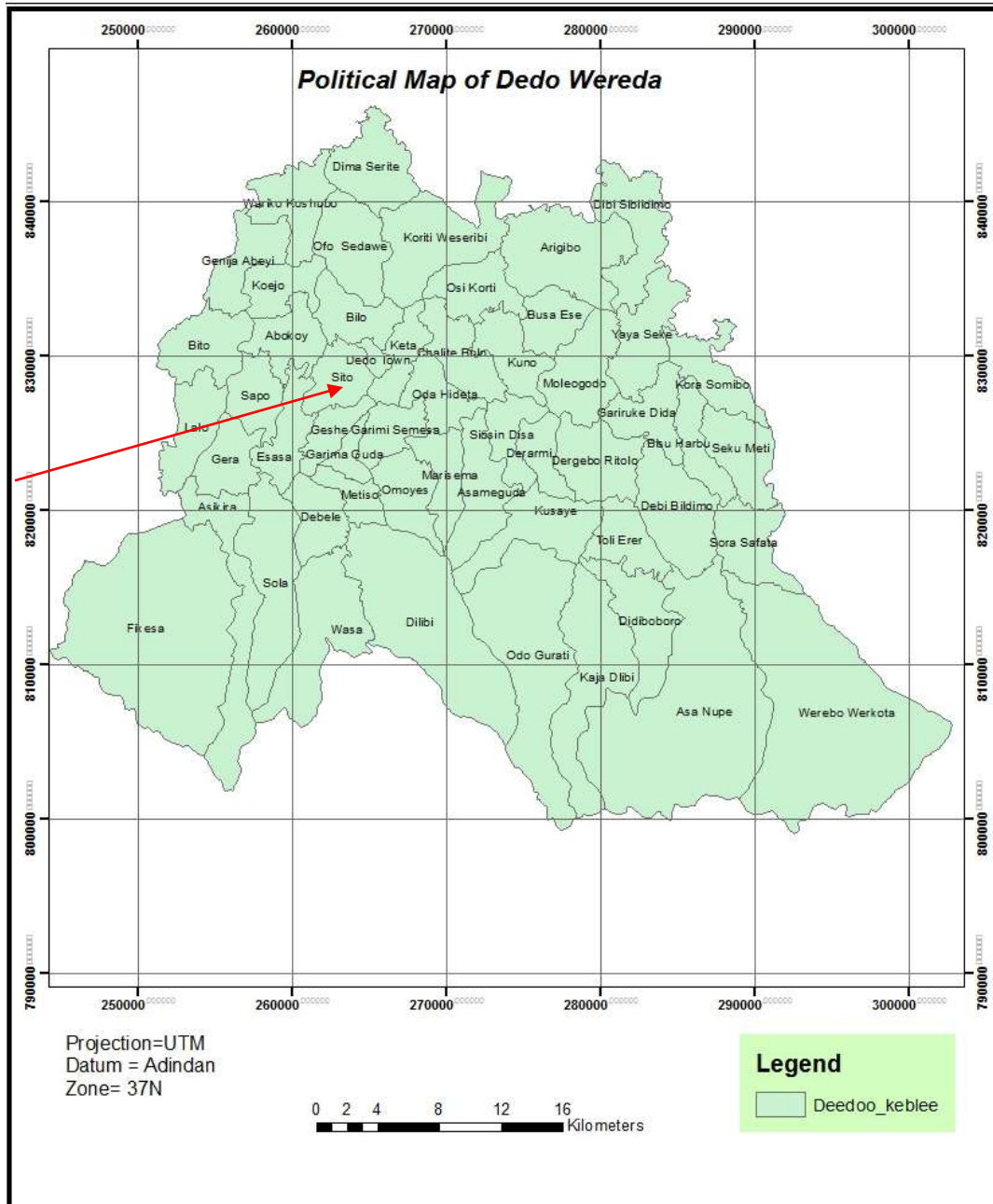
## Appendix I

Synthesis of nitrated 2,4-D



## Appendix II

Map of Dedo district, Jimma zone



## Appendix III

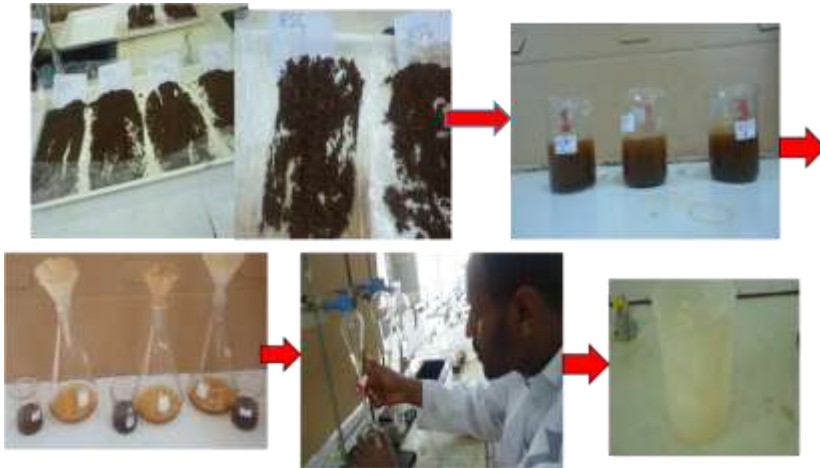
### Water and Soil samples collection



## Appendix IV

Extraction of 2, 4 -D from water and soil samples

soil samples



Water sample

