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DEPARTMENT OF CHEMISTRY

EVALUATION OF ORGANOPHOSPHATE PESTICIDE RESIDUES IN SELECTED VEGETABLES SOLD IN NAGONGERA TOWN COUNCIL, TORORO DISTRICT, UGANDA.

BY

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A RESEACH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE EDUCATION OF BUSITEMA UNIVERSITY

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DECLARATION

I, **MUKYALA MARIAM,** declare that this research project is my original work and has not been submitted to any other institution for academic credit.

Name: MUKYALA MARIAM

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Date: 31/08/2026

APPROVAL.

This research project has been submitted with the approval of the supervisor.

Supervisors name: DR. KAMOGA OMAR.

Signature: _____

Date: 31/08/2026.

DEDICATION.

This research report is dedicated to my family, especially my father Mr. Waiswa Patrick Nsadhu, friends, and all those who supported me throughout my academic journ

ACKNOWLEDGEMENT.

I would like to express my deepest gratitude to Almighty God for His guidance and protection throughout this study and also to my supervisor Dr. Kamoga Omar for the guidance, encouragement, and constructive criticism provided during the research period. Special thanks to Busitema University Nagongera Campus for providing the resources to conduct this study. I also extend appreciation to the laboratory staff and my lectures. I am grateful to my peers for their constructive feedback and to my family members for their support and unwavering encouragement.

ABSTRACT.

Organophosphate pesticides are widely used in vegetable production to control pests and diseases, but inappropriate application practices may result in pesticide residues remaining on vegetables intended for human consumption. Monitoring of such residues is important for food safety; however, access to advanced analytical techniques for pesticide-residue analysis may be limited in some local laboratory settings. This study therefore evaluated organophosphate pesticide residues in selected vegetables sold in Nagongera Town Council, Tororo District, Uganda, using an ammonium molybdate UV–Visible spectrophotometric approach.

A laboratory-based analytical study was conducted on tomatoes, cabbage and Sukuma wiki collected from selected markets, roadside vendors and shops within Nagongera Town Council. The samples were homogenized and extracted using analytical-grade acetone. The extracts were subsequently subjected to concentrated sulphuric acid digestion to convert phosphorus associated with the extracted organophosphate compounds into inorganic phosphate. Phosphate standard solutions were prepared from potassium dihydrogen phosphate and used for calibration. The digested samples were treated with ammonium molybdate and ascorbic acid and analysed spectrophotometrically at 670nm.

Measurable concentrations of inorganic phosphate derived from organophosphate pesticide residues were detected in all the selected vegetable types. The concentrations varied among the vegetables, with Sukuma wiki recording the highest mean concentration (33.615 mg/kg), followed by cabbage (27.490 mg/kg), while tomatoes recorded the lowest mean concentration (16.685 mg/kg).

The study demonstrates the potential application of UV–Visible spectrophotometry as an accessible screening approach for inorganic phosphate derived from organophosphate pesticide residues in vegetables, particularly where access to advanced chromatographic instrumentation is limited. However, the method does not identify individual organophosphate pesticide compounds and therefore cannot establish the specific pesticides responsible for the detected residues. Further studies using more specific analytical techniques such as gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC) are recommended to identify and quantify individual pesticide compounds. Regular monitoring of pesticide residues and sensitization of farmers on appropriate pesticide-use practices are also recommended to support food safety in the study area.

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LIST OF ABBREVIATIONS.

Abbreviation	Meaning
OPPs	Organophosphate Pesticides
UV-Vis	Ultraviolet-Visible
WHO	World Health Organization
FAO	Food and Agriculture Organization
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography- Mass Spectrometry
KH_2PO_4	Potassium Dihydrogen Phosphate
Mg	milligrams
SDDs	Sustainable Development Goals.
LMICs	Low- and Middle-income Countries
nm	Nanometer
mg/kg	Milligrams per kilogram
GAP	Good Agricultural Practices

CHARPTER ONE

1.0: INTRODUCTION

1.1 Background to Study

Approximately, Africa uses 1.8 million tons of pesticides, of which 153,901.4 tons are used annually in East Africa, where Uganda is located(Ssemugabo et al., 2022b). Pesticide use for food production such as fruits and vegetables has improved food quantity and quality, consequently improving nutrition and international trade. As such, pesticides are considered a necessary tool in the intensification of agriculture to meet the world's food demands(Ssemugabo et al., 2022b).

Vegetables are an essential component of the human diet. Mostly, vegetable crops are cultivated under high pressure for achieving higher production. This involves repeated application of pesticides for the control of various insect-pests. Chemical pesticides have been widely employed for effective controlling of a pest complex of various vegetable crops, but their indiscriminate use may create health hazards due to toxic residues that may persist in amounts above prescribed Maximum Residue Limits (MRL)(Madan et al., 2002)

Pesticides are substances used to control pests in agriculture, forestry, horticulture and on public lands to increase crop yields, improve the appearance of plant products, facilitate the care of open spaces and for public health purposes. The use of pesticides has been increasing worldwide. Global pesticide use is estimated at 6 million tons of active ingredients annually. Forty seven percent of all pesticides are used in Europe, 24% in Asia, 23% in the United States of America (USA) and 5.8% in the rest of the world(Ssemugabo et al., 2022b).

For a specific pesticide applied to a certain food item, there is a tolerance level that, when exceeded, is called 'violative residue'. Commonly, violation takes place when residues that exceed the established tolerance for a specific food item are detected. Tolerances may not be an accurate standard for health-related levels but are at least suitable for the maximum residue limits that have been set for the use of pesticides by law. Furthermore, violation rates do not consider the degree of consumption of various food items and the existing levels of pesticide residues(Region et al., 2020).

Many organophosphates, carbamates, pyrethroids and neonicotinoids, which are all neurotoxic pesticides, are registered for use in Uganda, and use is increasing with increasing consumption

of fruits and vegetables including tomatoes, cabbage, Amaranthus, Sukumawiki and watermelons, to name but a few. Organophosphates and carbamates pesticides are generally not persistent because they degrade when exposed to sunlight, air and soils, but they often have high solubility and volatility and are heavily used in many farming systems(Ssemugabo et al., 2022a).

Human intake of pesticide residues in food commodities can be higher than intake of these substances in water consumption and air inhalation. Therefore, regular monitoring of residue levels in fruits and vegetables is required to protect consumer's health(Mebdoua et al., 2017)

Routine monitoring of OPs residues in vegetables is constrained by lack of analytical infrastructures for example Gas Chromatography- Mass Spectrometry is the reference method but is absent in most district and regional laboratories, including Busitema University Nagongera Campus and Tororo District Health Office. This study proposes a low-cost UV-Vis spectrophotometric method based on molybdenum blue chemistry to screen for total OP residues.

1.2 Statement of the problem

The increased use of pesticides in vegetable farming in Uganda has raised concerns about food safety and public health. Studies have shown that pesticide residues are present in vegetables sold in Ugandan markets, with some exceeding maximum residue limits (MRLS). Additionally, Uganda does not have a comprehensive national monitoring system for pesticide residues in food.

In Nagongera Town Council, most vegetables are sourced from local farms where pesticide use practices are largely unregulated. Consumers may therefore be exposed to harmful levels of organophosphate residues, which are associated with neurological and chronic health effects.

Despite the risks, there is limited data on the levels of organophosphate pesticide residues in the commonly consumed vegetables such as Cabbage, Tomatoes and Sukuma Wiki sold in this region. Advanced analytical techniques such as gas chromatography(GC) and other chromatographic methods can provide specific identification and quantification of individual pesticide compounds. However, access to such techniques is limited by the availability of specialized equipment, laboratory facilities and associated analytical requirements.

Therefore, this study uses a low cost and accessible UV-Visible spectrophotometric method for determination of inorganic phosphates derived from organophosphate pesticide residues extracted from the vegetable samples.

1.3 Objectives of the Study

1.3.1 The general objective

To evaluate organophosphate pesticides residues in the selected vegetables sold in Nagongera Town Council, Tororo District, Uganda.

1.3.2 Specific Objectives

i) To collect tomatoes, cabbages, and Sukuma wiki samples from the market, roadside vendors and shops in Nagongera Town Council.

ii) To determine the concentrations of inorganic phosphate derived from organophosphate pesticide residues in selected vegetables sold in Nagongera Town Council.

iii) To determine the variation in organophosphate-derived phosphate concentrations among the selected vegetables.

iv) To compare the concentration of inorganic phosphates derived from organophosphate pesticide residue among the selected vegetable samples.

1.4 Research Questions

i) What are the concentrations of inorganic phosphates derived from organophosphate pesticide residues in the selected vegetables(Sukuma wiki, Cabbage, and tomatoes) sold in Nagongera Town Council?

ii) Is there a difference in the concentrations of inorganic phosphates derived from organophosphate pesticide residues among the selected vegetables?

iii) Which vegetable contains the highest level of organophosphate pesticide residues?

iv) What is the variation in organophosphate-derived phosphate concentrations among samples of selected vegetables?

1.5 Justification of the Study

The justification for this study stems from several critical factors. Firstly, this study addresses a critical gap in food safety monitoring in Tororo District by developing a method usable at Busitema University, the research creates local capacity for pesticide surveillance without

relying on central laboratories. Findings will inform Tororo District Health office interventions and protect consumers. The work contributes to SDG 3: Good Health and Well-being, and Uganda's National Development Plan III on food safety. It also provides a model for other resource-limited institutions in Uganda.

Secondly, organophosphate pesticides are widely used in Uganda's agricultural sector, posing potential health risks to consumers due to residues in food. Specifically, in Nagongera, Tororo District, the use of these pesticides in vegetable farming is prevalent, yet there is limited data on the levels of residues in commonly consumed vegetables like Cabbage, Tomatoes, and Sukuma wiki. This lack of data hinders efforts to ensure food safety and protect public health. Furthermore, understanding pesticide residue levels is crucial for informing regulatory bodies and guiding farmers on safe

pesticide use and handling practices. Without this study, consumers may be exposed to harmful pesticide levels, and regulatory efforts may remain ineffective.

1.6 Significance of the study

The significance of this study is multifaceted. By assessing organophosphate pesticide residues in vegetables, this research will contribute critical data on food safety, enabling stakeholders to identify potential health risks associated with dietary exposure.

The findings will inform targeted interventions to mitigate risks, protecting consumer health and promoting safer vegetable production practices. Additionally, the study's results will provide valuable insights for regulatory bodies, guiding policy formulation and enforcement on pesticide use and handling. Ultimately, this study has the potential to enhance food safety standards, reduce public health risks, and strengthen Uganda's agricultural sector, and well-being. The study also demonstrates a validated low-cost method that regional universities can adopt for pesticide screening.

1.7 Scope of the Study

1.7.1 Geographical Scope

The study was conducted in Nagongera Town Council, Tororo District, Uganda. Vegetable samples(cabbage, Sukuma wiki, and tomatoes) were obtained from selected selling points within the town council and taken to the laboratory for analysis.

1.7.2 Content Scope

The study focused on the evaluation of organophosphate pesticide residues in selected vegetables sold in Nagongera Town council. The selected vegetables were tomatoes, cabbage and Sukuma wiki.

The study involved extraction of organophosphate pesticide residues from the vegetable samples, followed by acid digestion to convert phosphorus associated with organophosphate pesticide residues into inorganic phosphates. The resulting inorganic phosphate was determined using UV-Visible spectrophotometric method involving colour development with ammonium molybdate and ascorbic acid.

The study focused on determining the concentration of inorganic phosphate derived from organophosphate pesticide residues, comparing the concentrations among selected vegetables and determining the variation among samples of the selected vegetables.

The study did not identify individual organophosphate pesticide compounds, since the analytical method used was based on the determination of inorganic phosphates following digestion.

1.7.3 Time Scope

The study was conducted during the period specified in the approved research schedule. This covered the collection of vegetable samples, laboratory preparation and analysis, data analysis and compilation of the research report.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Organophosphate Pesticides.

Organophosphate pesticides are phosphorus-containing compounds commonly used in agriculture for insect pest control. Their effectiveness is largely associated with their ability to interfere with the normal functioning of the nervous system of target organisms. Many organophosphate pesticides act through inhibition of the enzyme acetylcholinesterase, resulting in disruption of normal cholinergic transmission.

Organophosphate pesticides have been widely used because of their effectiveness against a broad range of agricultural pests. However, their use has raised concerns about possible exposure of agricultural workers and consumers through contaminated food and the environment.

2.2 Use of Organophosphate Pesticides in Agriculture

Global pesticide use is estimated at 6 million tons of active ingredients annually. Forty seven percent of all pesticides are used in Europe, 24% in Asia, 23% in the United States of America (USA) and 5.8% in the rest of the world. Herbicides, insecticides and fungicides are the most used pesticides worldwide. Insecticides are more common in low-and middle-income countries (LMICs), whereas herbicides and fungicides are more heavily used in high income countries (HICs). While Africa contributes 2% to 4% of the global pesticide consumption, their use has increased by 26.1% in recent years. Approximately, Africa uses 1.8 million tons of pesticides, of which 153,901.4 tons are used annually in East Africa, where Uganda is located(Ssemugabo et al., 2022b).

In vegetable production, pesticides may be applied at different stages of crop growth to control insect pests and diseases. However, the benefits of pesticide application depend on appropriate use, including adherence to recommended application rates and preharvest intervals.

2.3 Sources of Pesticide Residues in Vegetables.

Pesticide residues may remain in vegetables following the application of pesticides during cultivation. The existing study identifies several practices that may contribute to increased residues, including excessive pesticide application, failure to observe recommended

withdrawal or pre- harvest periods, repeated spraying, and poor agricultural practices. Farmers who do not follow recommended mixing concentrations and preharvest intervals may contribute to increased pesticide residues remaining on vegetables when they are harvest and marketed. Since vegetables are commonly consumed as part of the human diet, contaminated vegetables can provide a route of dietary exposure to pesticide residues.

The amount of residue remaining on vegetables may therefore depend on pesticide-use practices, the type of crop, environmental conditions and the period between pesticide application and harvesting.

2.4 Health Impacts of Organophosphate Pesticides.

Pesticides are known to be a public health issue and have been reported to cause toxic effects on humans, ranging from acute effects such as dizziness, headaches, rashes, and nausea to chronic effects such as cancer, neurotoxicity, genotoxicity, reproductive disorders, and endocrine system dysfunctions. Both acute and chronic diseases can result from their exposure. The risks were usually related to toxicity and quantity of the pesticide used, the approach of accomplishment, amount and frequency of contact with pesticide and the person that is exposed during application(Chaikasem & Roi-et, 2020).

Among various pesticide classes, organophosphorus pesticide (OPPs) group is the most widely used class of agricultural pesticides. In recent years, many studies have proved OPPs to be mutagenic, carcinogenic, cytotoxic, genotoxic, teratogenic and immunotoxic. Some of the pesticides belonging to OPPs group are also known for their interferences in reproduction especially in males. There are reports on the testicular toxic effects of parathion (OPP) in males and mouse and chromosomal aberrations in peripheral lymphocytes of mouse. Few studies reported that the exposure of OPPs had posed a direct potential risk to human health by inhibiting acetyl cholinesterase and modifying cholinergic signaling. OPPs have tendency to bind to the enzyme acetyl cholinesterase and to disrupt nerve functioning which further result in paralysis and death(Sharma et al., 2010).

Dietary exposure is therefore an important consideration when evaluating pesticide residues in vegetables. Monitoring residues in vegetables can provide information that contribute to food-safety assessment and public-health protection.

2.5 OPP Residues in Vegetables.

In Uganda, the volume of pesticides used has increased from 338t in the 1960s to 18,928.16t in 2019(Ssemugabo et al., 2022a). Many farmers do not follow recommended mixing concentrations on label instructions and pre-harvest intervals. Such improper pesticide use practices may result in higher levels of pesticide residues in fruits and vegetables that leave the farm to the final consumer. While they are important sources of minerals, vitamins, and other healthful nutrients, consumption of fruits and vegetables contaminated with

pesticide can be a route of exposure to hazardous chemicals(Ssemugabo et al., 2022a)

Vegetables differ in their ability to retain pesticide residues. Leafy vegetables may have extensive exposed surfaces that can come into contact with pesticide sprays. Consequently, differences in residue levels may occur between leafy vegetables and vegetables with different surface characteristics.

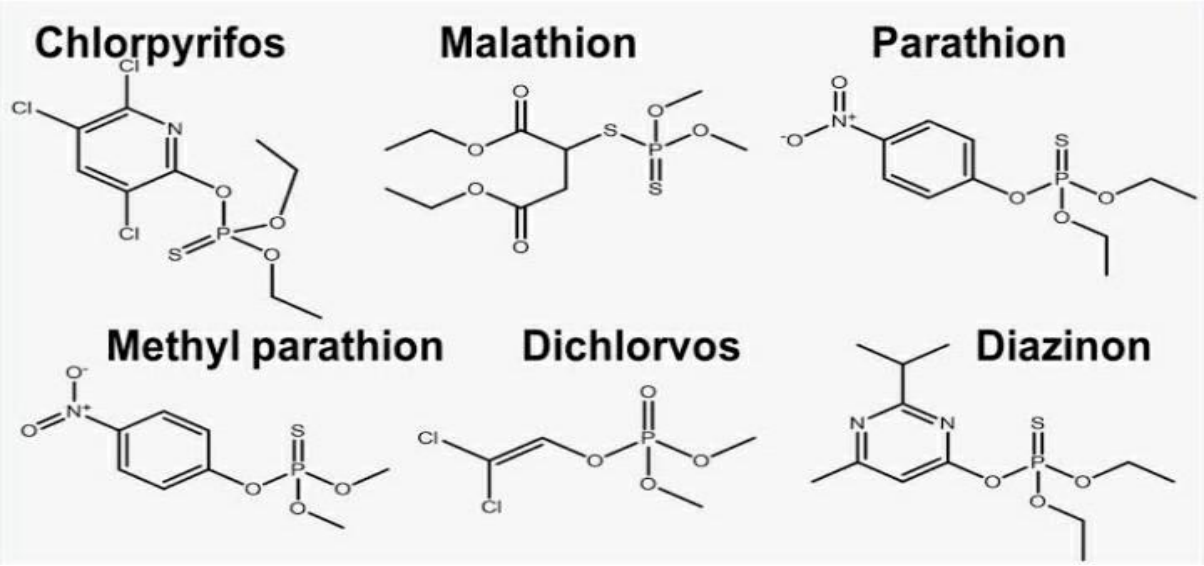
The study specifically selected Sukuma wiki, cabbage and tomatoes for investigation because they are commonly consumed vegetables sold within Nagongera Town Council.

2.6 Regulatory Framework.

Pesticide residue monitoring is the only tool to control the quantity of pesticides on food. For the past few decades, regulatory authorities in many countries have been setting up monitoring systems for agricultural products and the environment. The surveillance focuses on the proper use of pesticides in terms of authorization and registration (application rates and pre-harvested intervals), and on compliance with maximum residue limits (MRLs). Pesticide residue monitoring is also recognized as a significant aspect of initiatives to reduce potential hazards to human health. MRLs encourage food safety by restricting the concentration of a residue permitted on a commodity, and by limiting the type of commodity on which it is allowed.

The establishment of MRLs is based on good agricultural practices (GAP) data on food derived from commodities. MRLs are not toxicological limits, but they must be toxicologically acceptable. Exceeded MRLs are strong indicators of violations of GAP(Chen et al., 2011).

2.7 Structures of organophosphates



2.8 Spectrophotometric Determination of Phosphates.

Spectrophotometric determination of phosphates is based on the formation of phosphomolybdenum blue complex under acidic conditions. The intensity of the blue color formed is related to the concentration in solution. In the present study, this principle was applied after the organophosphate-containing extracts had undergone acid digestion. The digestion converted organically bound phosphorus into inorganic phosphates, which could then participate in the colour-development reaction.

The resulting-colored solution was measured using a UV-Visible spectrometer. The study specifies the use of a 6705 UV-Visible spectrometer at 670 nm

2.9 Acetone Extraction of Organophosphate Residues.

Extraction is an important stage in pesticide-residue analysis because the compounds of interest must first be separated from the vegetable matrix. This methodology used analytical-grade acetone to extract organophosphate residues from homogenized vegetable samples.

Acetone was selected because of its ability to dissolve many organic compounds and facilitate extraction from vegetable tissues. Following extraction, the sample extracts were subjected to further treatment before spectrophotometric determination.

2.10 Sulphuric Acid Digestion and Conversion to Inorganic Phosphate

The conversion of organophosphate-associated phosphorus into inorganic phosphate is a central part of the analytical procedure used in this study.

According to this study, concentrated sulphuric acid was used to digest the extracted material and convert organically bound phosphorus into inorganic phosphate ions suitable for subsequent spectrophotometric determination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials and apparatus.

The apparatus used during the study included an analytical balance for accurate measurement of vegetable samples and reagents, a laboratory blender for homogenization of the vegetable samples, and a water bath for controlled heating during sample preparation. Other apparatus included a fume hood, filter papers, stainless-steel knives, zipper bags, a vortex mixer, and a refrigerator for temporary storage of prepared samples. A 6705 UV–Visible spectrophotometer was used for the spectrophotometric determination of phosphate at 670 nm. Measuring cylinders of 10 mL and 100 mL capacities, filter funnels, beakers of 100 mL, 250 mL and 1000 mL capacities, 1 cm path-length cuvettes, glass rods, test tubes and 250 mL conical flasks were also used during the laboratory procedures.

3.2 Reagents.

All chemicals used in the study were of analytical grade. The reagents included potassium dihydrogen phosphate, ascorbic acid, ammonium molybdate, concentrated sulphuric acid, distilled water, acetone and anhydrous sodium sulphate. Potassium dihydrogen phosphate was used in the preparation of phosphate standard solutions, while ammonium molybdate and ascorbic acid were used for colour development during spectrophotometric determination.

chopped into small pieces using a stainless-steel knife and then kept in tight zipper bags at 4°C before analysis to prevent the disappearance or degradation of pesticide residues till further analysis. Each chopped vegetable sample was appropriately mixed for homogeneity using a laboratory blender to obtain a uniform matrix. Then, the homogenized samples were stored at -20^0C in the laboratory refrigerator.

3.3 PREPARATION OF STOCK SOLUTIONS

All reagents used in the study were of analytical grade. The reagents and standard solutions were prepared using distilled or deionized water as appropriate. The preparation of the phosphate stock solution, working standards, ammonium molybdate solution, ascorbic acid solution and diluted sulphuric acid was carried out as described below.

3.3.1 Preparation of Potassium dihydrogen phosphate Stock solution.

A primary phosphate stock solution was prepared using analytical-grade potassium dihydrogen phosphate (KH_2PO_4). 0.219 g of dried potassium dihydrogen phosphate crystals was accurately weighed using an analytical balance and transferred into a clean beaker. The crystals were dissolved in 1000 mL of deionized water to prepare a primary phosphate standard solution with a concentration of 50 mg/L. The prepared stock solution was thoroughly mixed to ensure uniform distribution of the dissolved phosphate and was subsequently used for preparation of the working phosphate standards.

Working phosphate standard solutions were prepared from the primary potassium dihydrogen phosphate stock solution by serial dilution. Appropriate volumes of the stock solution were transferred into separate volumetric containers and diluted to 100 mL with distilled water to obtain phosphate concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 mg/L. The prepared standards covered the expected concentration range of phosphate in the vegetable samples and were subsequently used to establish the calibration relationship between phosphate concentration and spectrophotometric absorbance.

The preparation of the working standards was as follows:

Table 3.4: dilutions of the stock solutions

Volume of stock solution (ml)	Diluted to 100ml using distilled water(ml)	Concentration of the diluted stock solution(mg/l)
0	100	0
1	100	0.5
2	100	1
3	100	1.5
4	100	2

3.3.2 Preparation of Ammonium Molybdate Solution

Ammonium molybdate solution was prepared by accurately weighing 5 g of solid ammonium molybdate crystals using an analytical balance. The crystals were dissolved in 50 mL of distilled water, and the resulting solution was thoroughly mixed to obtain a homogeneous reagent (10% solution of ammonium molybdate solution) for the colour-development stage of

phosphate determination. In the subsequent analytical procedure, the prepared ammonium molybdate reagent was used for formation of the coloured phosphomolybdenum complex.

3.3.3 Preparation of Ascorbic Acid Solution

A fresh ascorbic acid solution was prepared for use as the reducing agent during colour development. 2.5 g of solid ascorbic acid was accurately weighed using an analytical balance and transferred into a clean container. The crystals were dissolved in 50 mL of distilled water and the solution was thoroughly mixed to obtain a homogeneous 5% ascorbic acid solution. The freshly prepared solution was subsequently used during the development of the blue phosphomolybdenum complex.

3.3.4 Preparation of diluted sulphuric acid for the phosphate standards

Diluted sulphuric acid was prepared from concentrated sulphuric acid for use during phosphate-standard preparation and colour development. 50 mL of distilled water was first placed in a clean 100 mL beaker. 5.6 mL of concentrated sulphuric acid was then added slowly to the water while gently stirring. The mixture was allowed to cool, after which distilled water was added to the required volume and the resulting solution was thoroughly mixed. The prepared diluted sulphuric acid was subsequently used in the preparation and treatment of the phosphate standards.

3.3.5 Preparation of Reagent Blank for Phosphate Standards

A reagent blank was prepared using the same procedure as that used for the phosphate standards, except that potassium dihydrogen phosphate was excluded. 10 mL of distilled water was transferred into a clean test tube, followed by 0.5 mL of diluted sulphuric acid, 2 mL of ammonium molybdate solution and 3 drops of ascorbic acid solution. The mixture was thoroughly mixed and allowed to stand for 15 minutes to allow colour development. The resulting blank was used to zero the spectrophotometer before measurement of the phosphate standards.

3.3.6 Preparation of Vegetable-Sample Reagent Blank

A separate blank was prepared to account for any contribution from the extraction and digestion reagents. The complete extraction and digestion procedure was performed without adding vegetable material. 50 mL of analytical-grade acetone was placed in an empty 250ml conical flask and subjected to the same extraction procedure used for the vegetable samples. Followed by 25 mL of concentrated sulphuric acid. The mixture was heated in a water bath at 60–80°C

for 60 minutes and subsequently allowed to cool. The resulting mixture was diluted with distilled water to the mark in a 250ml conical flask. 10 ml of the resultant mixture was transferred into a test tube. For colour development, 2 mL of ammonium molybdate solution was added to 10 mL of the prepared solution, followed by 3 drops of ascorbic acid solution. The mixture was thoroughly mixed and allowed to stand for 15 minutes before spectrophotometric analysis.

3.4 Methods of Sample Preparation and Sample Collection

3.4.1 Study Area

The study was conducted in Nagongera Town Council, Tororo District, Uganda. Vegetable samples were collected from selected markets, roadside vendors and shops within the town council. Laboratory analysis was conducted in the Chemistry Laboratory at Busitema University. The study focused on tomatoes, cabbage and Sukuma wiki because these vegetables are commonly consumed and were available from the selected selling points.

3.4.2 Sample Collection

Approximately 30 vegetable samples, comprising tomatoes, cabbage and Sukuma wiki, were collected from selected markets, roadside vendors and shops within Nagongera Town Council, Tororo District. The samples consisted of approximately 10 samples of each vegetable type. Each sample was placed in an individually labelled polythene bag, identified using an appropriate sample number, and transported to the Busitema University Chemistry Laboratory under cool conditions for further preparation and analysis.

3.4.3 Sample Preparation

Upon arrival at the laboratory, each vegetable sample was washed with distilled water to remove surface dirt and then allowed to air-dry at room temperature. The edible portions were chopped into small pieces using a stainless-steel knife and placed in tightly sealed zipper bags. The samples were stored at 4°C before analysis to minimize degradation or loss of pesticide residues. Each sample was subsequently homogenized using a laboratory blender to obtain a uniform matrix and stored in a laboratory refrigerator at −20°C until analysis.

3.4.4 Extraction of Organophosphate Pesticide Residues

For each vegetable sample, 20.0 g of the homogenized sample was accurately weighed into a 250 mL conical flask. 50 mL of analytical-grade acetone was added as the extraction solvent.

The flask was sealed and shaken vigorously for 30 minutes using a vortex machine to facilitate extraction of pesticide residues. The mixture was then allowed to stand for approximately 10 minutes to facilitate phase separation before being filtered through filter paper into a clean 250 mL conical flask. 5 g of anhydrous sodium sulphate was added to the filtrate to remove residual moisture.

3.4.5 Acid Digestion

Following extraction, 25 mL of concentrated sulphuric acid was slowly added to the extracted sample. The mixture was heated in a water bath maintained at 60–80°C for 60 minutes. This digestion step converted the phosphorus associated with the extracted organophosphate compounds into inorganic phosphate ions. The mixture was then allowed to cool, transferred into a 250 mL volumetric flask, and diluted to the mark with distilled water. The resulting solution contained inorganic phosphate derived from the organophosphate pesticide residues.

3.4.6 Colour Development

For colour development, 10 mL of the hydrolysed sample was transferred into a clean test tube. 2 mL of ammonium molybdate solution was added, followed by 3 drops of freshly prepared ascorbic acid solution as the reducing agent. The mixture was thoroughly mixed and allowed to stand for 15 minutes to allow development of the blue phosphomolybdenum colour.

3.4.7 Spectrophotometric Analysis

The 6705 UV–Visible spectrophotometer was switched on and allowed to warm up for approximately 20 minutes to stabilize the lamp and detector. The instrument was set to absorbance mode at a wavelength of 670 nm, and clean 1 cm path-length cuvettes were used. The phosphate standards were first analyzed after zeroing the instrument with the appropriate reagent blank. The absorbance of each standard was measured and recorded at 670 nm, and a calibration curve of absorbance against phosphate concentration was prepared. The vegetable samples were subsequently analyzed using the same procedure after zeroing the instrument with the vegetable-sample blank.

3.5 Data Analysis.

The data obtained from the spectrophotometric analysis were entered and processed using Microsoft Excel. The absorbance readings obtained from the phosphate standard solutions were used to construct a five-point external calibration curve by plotting absorbance against phosphate concentration. Linear regression analysis was used to establish the relationship

between absorbance and phosphate concentration. The resulting calibration relationship was then used to determine the phosphate concentrations corresponding to the absorbance readings obtained from the vegetable samples.

The concentrations obtained from the three replicate analyses of each vegetable type were summarized using descriptive statistics. The arithmetic mean was determined for each vegetable type to provide a representative concentration of inorganic phosphate derived from organophosphate pesticide residues. The mean concentrations for tomatoes, cabbage and Sukuma wiki were subsequently compared to assess the variation in residue levels among the selected vegetables.

The results were presented in tables and graphical form to facilitate clear interpretation and comparison of the concentrations obtained from the different vegetable samples.

CHAPTER FOUR:

4.0 RESULTS AND DISCUSSION

4.1 Results.

4.1.1Calibration curve results.

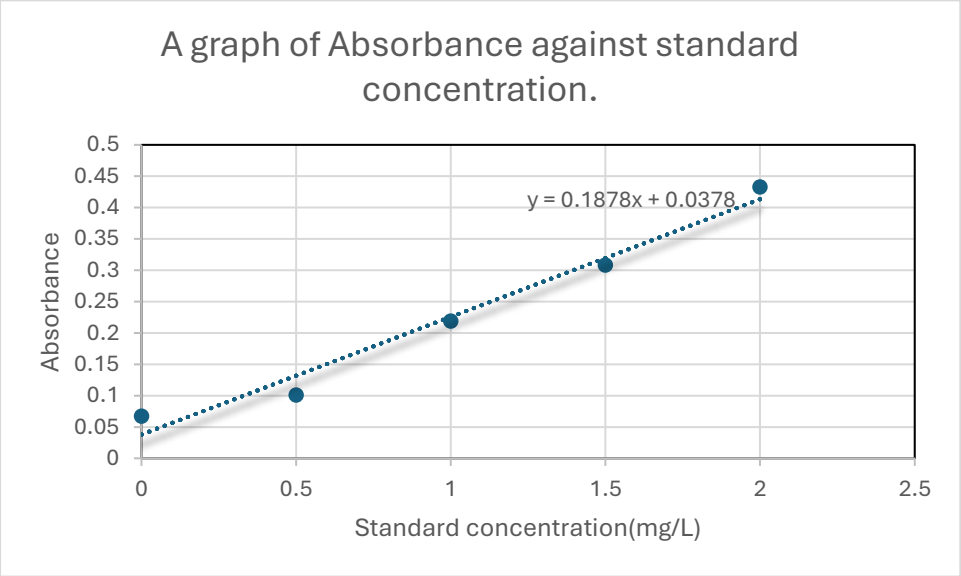


Figure 4.1 Calibration curve obtained using the reference standards of potassium dihydrogen phosphate

The calibration curve was prepared from the phosphate standard solutions by plotting absorbance against phosphate concentration. Linear regression was used to establish the relationship between absorbance and phosphate concentration. The resulting regression equation was subsequently used to determine the phosphate concentration corresponding to the absorbance measured for each vegetable sample. The calculated sample concentrations were then used in determining the concentration of inorganic phosphate derived from organophosphate pesticide residues in the vegetable samples.

4.1.2 Concentrations of inorganic phosphate derived from organophosphate pesticide residues in selected vegetables

Three replicate analyses were carried out for each of the selected vegetables: tomatoes, cabbage and Sukuma wiki. The concentrations obtained from the analyses are presented in Table

Table 4.1 concentration of inorganic phosphates derived from organophosphate pesticide residues in selected vegetable samples

Vegetable Sample	Test Sample 1 (mg/kg)	Test Sample2(mg/kg)	Test Sample 3(mg/kg)	Mean concentration (mg/kg)
Tomato	16.710	17.115	16.230	16.685
Cabbage	27.015	28.050	27.405	27.490
Sukuma wiki	33.000	34.200	33.645	33.615

The results indicate that the concentration of inorganic phosphate derived from organophosphate pesticide residues varied among the three vegetable types. Sukuma wiki recorded the highest mean concentration of 33.615 mg/kg, followed by cabbage with a mean concentration of 27.490 mg/kg, while tomatoes recorded the lowest mean concentration of 16.685 mg/kg.

The order of the mean concentrations was therefore:

Sukuma wiki > Cabbage > Tomato.

4.1.3 Variation among replicate measurements

There was some variation among the three replicate measurements for each vegetable. The tomato samples recorded concentrations ranging from 16.230 to 17.115 mg/kg. Cabbage concentrations ranged from 27.015 to 28.050 mg/kg, while Sukuma wiki concentrations ranged from 33.000 to 34.200 mg/kg.

The relatively small differences between replicate measurements indicate that the measurements for each vegetable were reasonably consistent under the analytical conditions used.

4.1.4 Comparative concentration of the selected vegetables

Table 4.2 mean concentrations of inorganic phosphates in the selected vegetables

Vegetable Sample	Mean concentration of inorganic phosphates(mg/kg)
Tomato	16.685
Cabbage	27.490

Sukuma wiki	33.615

The mean concentrations obtained for the three vegetables show that Sukuma wiki had the highest concentration, followed by cabbage and then tomatoes.

Sukuma wiki had a mean concentration of 33.615 mg/kg, which was 6.125 mg/kg higher than cabbage and 16.930 mg/kg higher than tomatoes. Cabbage also recorded a mean concentration that was 10.805 mg/kg higher than tomatoes.

These differences demonstrate that the concentration of inorganic phosphate derived from organophosphate pesticide residues was not the same among the selected vegetable types.

Presentation of results using a bar graph

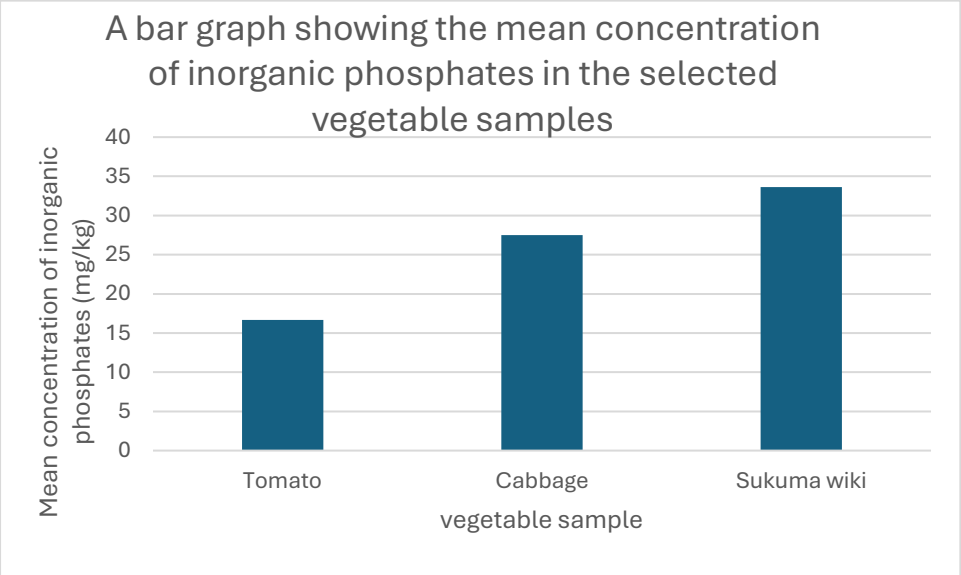


Figure 4.2 A bar graph showing the mean concentration of inorganic phosphates in the selected vegetable sample

4.2 Discussion of Results

The study evaluated organophosphate pesticide residues in selected vegetables sold in Nagongera Town Council, Tororo District, Uganda. The selected vegetables were tomatoes, cabbage and Sukuma wiki. The analytical procedure involved extraction of organophosphate pesticide residues using acetone, followed by sulphuric acid digestion to convert the phosphorus associated with the extracted organophosphate compounds into inorganic phosphate. The resulting inorganic phosphate was subsequently determined using the ammonium molybdate UV–Visible spectrophotometric method. Therefore, the concentrations

reported in this study represent inorganic phosphate derived from organophosphate pesticide residues rather than concentrations of individual organophosphate pesticide compounds.

The results demonstrated that measurable concentrations of inorganic phosphate derived from organophosphate pesticide residues were obtained from all the vegetable types investigated. The mean concentration was highest in Sukuma wiki at 33.615 mg/kg, followed by cabbage at 27.490 mg/kg, while tomatoes recorded the lowest mean concentration of 16.685 mg/kg. This variation indicates that the levels of organophosphate-derived phosphorus were not uniform among the selected vegetable types. The observed differences may be associated with differences in vegetable morphology, pesticide exposure, agricultural practices, environmental conditions and the period between pesticide application and harvesting.

Sukuma wiki recorded the highest mean concentration among the vegetables analyzed. The relatively high concentration observed in Sukuma wiki may be associated with its leafy structure and extensive exposed surface area. The leaves provide surfaces on which pesticide sprays may be deposited and retained during agricultural application. The literature reviewed in this study similarly indicates that leafy vegetables may retain relatively higher amounts of pesticide residues because of their surface characteristics. This may partly explain the higher concentration observed in Sukuma wiki in comparison with tomatoes. However, vegetable morphology alone cannot be considered the sole explanation because pesticide residue levels are also influenced by the type and amount of pesticide applied, frequency of application, environmental conditions and compliance with recommended pre-harvest intervals.

Cabbage recorded the second-highest mean concentration of 27.490 mg/kg. The relatively high concentration in cabbage may also be associated with its leafy nature, which provides considerable surface area for pesticide deposition. Cabbage is frequently cultivated under conditions where pesticides may be applied to control insect pests and other crop diseases. The literature reviewed in the study indicates that repeated or inappropriate pesticide application can increase the likelihood of residues remaining on harvested vegetables. In addition, failure to observe recommended pre-harvest intervals may allow vegetables to be harvested before sufficient degradation or removal of pesticide residues has occurred. However, the present study did not collect information on the specific pesticides used by the farmers supplying the vegetables, their application rates, frequency of application or the exact pre-harvest intervals. Therefore, these factors can only be considered possible explanations for the observed differences rather than confirmed causes.

Tomatoes recorded the lowest mean concentration of 16.685 mg/kg among the three vegetable types. The comparatively lower concentration may be associated with differences in the physical characteristics of tomatoes compared with the leafy vegetables. Tomatoes are fruiting vegetables and have a relatively smooth outer surface, which may result in different pesticide deposition and retention characteristics from those of leafy vegetables. Nevertheless, the detection of measurable concentrations in tomatoes indicates that the absence of a leafy structure does not prevent the occurrence of pesticide-derived residues. Residues may arise from direct pesticide application, environmental exposure and other agricultural practices.

The differences observed among the three vegetables are consistent with the broader concern regarding pesticide residues in vegetables. The literature reviewed in this study indicates that pesticide use is widespread in vegetable production and that residues may remain on food commodities when pesticides are improperly applied or when recommended pre-harvest intervals are not followed.

The present findings are also important in the context of vegetable production and food safety in Uganda. The literature review identified organophosphate pesticides among the pesticide groups used in Uganda and noted their application to commonly consumed vegetables, including tomatoes, cabbage and Sukuma wiki. The detection of organophosphate-derived inorganic phosphate in all three vegetable types therefore provides local evidence that pesticide-related residues can be detected in vegetables sold within Nagongera Town Council. This is particularly relevant because the study area has limited local data on pesticide residues in commonly consumed vegetables.

The replicate measurements obtained for each vegetable type showed relatively close values within the respective samples. This indicates reasonable consistency in the analytical measurements obtained under the conditions of the study. Small differences among replicate measurements may occur because of variations during sample homogenization, extraction, digestion, colour development and spectrophotometric measurement. Variation may also arise from differences in the distribution of pesticide residues within the vegetable material. Since vegetable samples are biological matrices, complete uniformity of pesticide residues throughout an individual sample cannot always be assumed.

The observed differences among the vegetables should therefore be interpreted with consideration of the sampling design. The study used samples collected from selected markets, roadside vendors and shops within Nagongera Town Council. The samples represented

vegetables available at the selected collection points during the study period. Consequently, the results provide information about the sampled vegetables and sampling period rather than establishing the residue levels of all tomatoes, cabbage or Sukuma wiki produced in the wider Tororo District. The study itself recognizes that its findings are specific to Nagongera Town Council and the sampling period.

An important consideration when interpreting the results is the analytical limitation of the method used. The ammonium molybdate spectrophotometric procedure measures phosphate after the organophosphate residues have undergone acid digestion. Consequently, the method provides an indication of the total inorganic phosphate derived from the phosphorus-containing residues but does not distinguish among individual organophosphate pesticide compounds. This limitation is important because different organophosphate pesticides may contribute to the measured phosphorus, and the method cannot establish which specific compound was responsible for the detected concentration. The literature and methodology of the study therefore support the use of more specific analytical techniques, such as GC-MS or HPLC, where identification and quantification of individual pesticide compounds are required.

The results should also be interpreted cautiously with respect to Maximum Residue Limits (MRLs). MRLs are established for specific pesticide active ingredients and specific food commodities. Since the analytical method used in this study did not identify individual organophosphate compounds, the measured inorganic phosphate-derived concentrations should not be directly interpreted as concentrations of a particular pesticide or directly compared with an MRL for a specific pesticide. Instead, the results provide evidence of measurable organophosphate-derived phosphorus in the sampled vegetables. This distinction is important for maintaining scientific accuracy in the interpretation of the findings.

Despite this limitation, the findings demonstrate the potential usefulness of the UV–Visible spectrophotometric approach as a relatively accessible screening method in laboratories where advanced chromatographic equipment is unavailable. The study was partly motivated by limited access to advanced analytical instrumentation such as GC-MS, and the literature review identified the use of UV–Visible spectrophotometry as a lower-cost approach for screening phosphate derived from organophosphate residues. The method therefore provides a practical preliminary approach for generating information on pesticide-related phosphorus in vegetable samples, although confirmatory analysis using more specific techniques would be required to identify individual pesticide compounds.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study evaluated organophosphate pesticide residues in selected vegetables sold in Nagongera Town Council, Tororo District, Uganda, using an acetone extraction procedure followed by sulphuric acid digestion and ammonium molybdate UV–Visible spectrophotometry. The analytical procedure converted phosphorus associated with the extracted organophosphate compounds into inorganic phosphate, which was subsequently determined spectrophotometrically.

The findings demonstrated measurable concentrations of inorganic phosphate derived from organophosphate pesticide residues in all the selected vegetable types. Sukuma wiki recorded the highest mean concentration of 33.615 mg/kg, followed by cabbage with 27.490 mg/kg, while tomatoes recorded the lowest mean concentration of 16.685 mg/kg. The results therefore demonstrated variation in the concentrations detected among the three vegetable types.

The higher concentrations observed in Sukuma wiki and cabbage may be associated with their leafy characteristics and relatively large exposed surfaces, which may facilitate deposition and retention of pesticide residues. However, differences in pesticide application practices, environmental conditions and the interval between pesticide application and harvesting may also contribute to the observed variation.

The study further demonstrated that UV–Visible spectrophotometry can provide a relatively accessible approach for screening inorganic phosphate derived from organophosphate pesticide residues in vegetable samples. Nevertheless, the method used in this study does not identify individual organophosphate pesticide compounds. Consequently, the measured concentrations cannot be attributed to specific pesticides or directly compared with pesticide-specific Maximum Residue Limits.

Overall, the study provides evidence of measurable organophosphate-derived inorganic phosphate in tomatoes, cabbage and Sukuma wiki sold in Nagongera Town Council and highlights the importance of continued monitoring and appropriate management of pesticide use in vegetable production.

5.2 Recommendations

Based on the findings of the study, the following recommendations are made:

1. Regular monitoring of pesticide residues in vegetables should be encouraged by relevant agricultural and public-health authorities to improve food-safety surveillance.

2. Farmers should be sensitized on proper pesticide application practices, including observing recommended application rates and pre-harvest intervals, to minimize pesticide residues on harvested vegetables.

3. Consumers should be encouraged to follow appropriate food-handling practices, including thorough washing of vegetables before consumption, as part of general measures for reducing potential dietary exposure to surface contaminants.

4. Local agricultural extension officers should strengthen farmer education on the safe handling, storage and application of pesticides.

5. Further studies should be conducted using more specific analytical techniques such as GC-MS or HPLC to identify and quantify individual organophosphate pesticide compounds in the vegetables.

6. Laboratories and research institutions should strengthen analytical capacity for pesticide-residue monitoring by improving access to appropriate analytical equipment and technical expertise.

5.3 Areas for Further Research

Further research should be undertaken to provide more comprehensive information on organophosphate pesticide residues in vegetables within Nagongera Town Council and the wider Tororo District. Future studies should consider the following areas:

1. Identification and quantification of individual organophosphate pesticides: Advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC) should be employed to identify and quantify individual pesticide compounds present in the vegetable samples.

2. Assessment of pesticide residues in a larger sample population: Future studies should involve a larger number of vegetable samples collected from a greater number of markets, farms and vendors and across different seasons to improve the representativeness of the findings.

3. Investigation of agricultural practices: Further studies should examine the types of pesticides used by farmers, application rates, frequency of application, mixing practices and adherence to recommended pre-harvest intervals in order to establish factors contributing to pesticide residues in vegetables.

4. Assessment of pesticide residues at different stages of the food chain: Research could investigate pesticide residues at farm level, during transportation, at wholesale and retail markets, and at household level to determine how residue levels change from production to consumption.

5. Evaluation of consumer exposure and potential health risks: Further studies should assess dietary exposure to individual pesticide residues through consumption of contaminated vegetables and evaluate the potential risks associated with long-term exposure.

6. Comparison of analytical methods: Further research could compare the UV–Visible spectrophotometric screening approach used in this study with established chromatographic methods to determine its accuracy, sensitivity and suitability for preliminary pesticide-residue screening.

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APPENDICES

Appendix (i)

Table7.1: Sample Collection Record Sheet

Sample Code	Vegetable Type	Collection Site	Date for Collection
T	Tomato	Nagongera market and shops	1st/05/2026
C	Cabbage	Nagongera roadside market	3rd/05/2026
S	Sukuma wiki	Nagongera market	2nd/05/2026

Appendix (ii)

Table 7.2: Absorbance of phosphate standard solutions

Standard Concentration(mg/L)	Absorbance at 670nm
0.0	0.067
0.5	0.101
1.0	0.219
1.5	0.308
2.0	0.433

Table 7.3: Results for tomato cuvette samples

Sample	Absorbance	Concentration of the inorganic phosphates in the cuvette sample (mg/L)
First tomato cuvette sample	0.247	1.114

Second tomato cuvette sample	0.252	1.141
Third tomato cuvette sample	0.241	1.082

Table7.4: Results for cabbage cuvette samples Cabbage

Sample	Absorbance	Concentration of the inorganic phosphates in the cuvette sample (mg/L)
First cabbage cuvette sample	0.376	1.801
Second cabbage cuvette sample	0.389	1.870
Third cabbage cuvette sample	0.381	1.827

Table 7.5: Results for Sukuma wiki cuvette sample

Sample	Absorbance	Concentration of the inorganic phosphates in the cuvette sample (mg/L)
First Sukuma wiki cuvette sample	0.451	2.200
Second Sukuma wiki cuvette sample	0.466	2.280
Third Sukuma wiki cuvette sample	0.459	2.243