Assessment of pesticide residues and nutrient levels in some vegetable samples

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ABSTRACT

The human diet relies heavily on vegetables as a source of nutrients. Vegetable consumption has increased due to changing lifestyles and the need to maintain health. Pesticide residues in vegetables are now a major global concern. Consumers might be at risk for health problems if they consume vegetables that have residual pesticide levels exceeding their individual maximum residue limits (MRL). Thus, to know the extent of residual contamination, there is a need to explore pesticide remainders in vegetables. However, pesticides are harmful to human health and the environment. It is essential to predict the level of pesticides in different vegetables to secure the human health and save the environment. The current research was designed based on four market samples such as: Potol or pointed gourd (*Trichosanthes dioica*), Korola or bitter gourd (*Momordica charantia*), Tomato (*Solanum lycopersicum*), Kacha morich or Green chili (*Capsicum frutescens*) and Phulcopi or cauliflower (*Brassica oleracea var. botrytis*) collected from two districts of Bangladesh to determine the level of residual pesticides.

The vegetables were taken out by the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuECHERS) method, cleaned-up by Primary secondary amine (PSA), $MgSO_4$ and analyzed by gas chromatograph equipped with an electron capture detector (GC-ECD). In the present research work, pesticide residues (alpha-BHC, gamma-BHC, beta-BHC,delta-BHC, heptachlor, aldrin, heptachlor-epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan-I, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, endosulfan-II, endrin aldehyde, 4,4'-DDT, endosulfan sulfate, methoxychlor, and endrin ketone) were analyzed in these vegetable samples using a GC-ECD.

The calibration range of the 20 Organochlorine Pesticides (OCP) pesticides was kept within the range of 5–200 ng/g. The curves followed linear relationships with good correlation coefficient (r^2) values within the rangeof 0.91-0.98. The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 0.00001-0.0013 ng/g and 0.0001-0.0134 ng/g, respectively. Recoveries were found between 70–120% with relative standard deviations up to 10% in the pointed gourd, between 70–119% with relative standard deviations up to 11% in the bitter gourd, between 71–121% with relative standard deviations up to 10% in tomato, between 70–120% with relative standard deviations up to 11% in chili and between 70–117% with relative

standard deviations up to 10% in cauliflower at two different spiking levels for three replicates. Pesticide remainders in vegetable samples collected from the Hatirpool area were found as 4,4'-DDE (9.36-12.91 ng/g), 4,4'-DDD (27.16-27.47 ng/g), 4,4'-DDT (7.77-84.88 ng/g) from Kaptan Bazar area residues were 4,4'-DDD (47.02 ng/g) and 4,4'-DDT (4.03-21.25 ng/g) from Shibpur Bazar area residues were 4,4'-DDT (0.68-6.36 ng/g) and from College gate Bazar, Narsingdi area residues were 4,4'-DDT (3.70-17.28 ng/g). Whereas, alpha-BHC, gamma-BHC, beta- BHC, delta-BHC, heptachlor, aldrin, heptachlor-epoxide, *trans*-chlordane, *cis*- chlordane, endosulfan-I, dieldrin, endrin, endosulfan- II, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone were below detection limit. The moisture and ash contents of samples were 85–96% and 0.38–0.55%, respectively.

Fatty acid compositions were analyzed by Gas chromatography equipped with flame ionization detector (GC-FID) by comparing the retention times of thirteen standardmethyl ester fatty acids. Unsaturated fatty acids were found in the highest amounts compared to saturated fatty acids. The relative percentage of fatty acids ranged from palmitoleic acid (67.73- 100%), Octadecanoic acid (91.43%) and erucic acid (32.26%), respectively. Palmitoleic acid was found in the highest amounts among all unsaturated fatty acids. The protein content was found 0.73, 1.81, 1.02, 0.71 and 2.69% for pointed gourd, bitter gourd, tomato, chili, and cauliflower, respectively. Pointed gourd, bitter gourd, tomato, chili and cauliflower had carbohydrate contents around 2,7,4,8 and 4 g/100 respectively.

The homogenized vegetables were dried by freeze drier and digested with HNO_3 and H_2O_2 before analyzing by an Atomic Absorption Spectrophotometer (AAS) for determination of micronutrients such as Zn, Fe, Mn, Ca, Cd, Cr, Cu, Ni and Pb. The concentrations of micronutrients in vegetables were found in the range of 3.95 ± 1.94 to 519.85 ± 192.50 mg/kg. The levels were to be 26.14-50.03 mg/kg for Zn, 26.16-75.31 mg/kg for Fe, 11.83-34.65 mg/kg for Mn, 187.40-519.85 mg/kg for Ca and 3.95-12.77 mg/kg for Cu. Cadmium and Ni were obtained in two vegetables. Cromium and Pb were observed in bitter gourd and chili, respectively.

1. INTRODUCTION

1.1 Background

Vegetables are plant portions that are eaten as food by people and other animals. It may be the most direct and affordable way to ensure better nutrition for all. Plants can be taken either raw or cooked. Numerous nutritionists advise consumers to eat lots of vegetables. Seed vegetables may have high dry matter content and high starch content. Dietary fibers, flavor contents, vitamins and minerals are secondary compositions. Vegetables contain nitrogen compounds, protein, amino acids, carbohydrates, amines, polysaccharides, lipids, organic acids, phenolic compounds and aroma substances (Belitz et al., 2004).

Vegetables are a crucial part of the diet since they provide nutrients including calcium, iron, protein, vitamins, and other substances (Shoeb et al., 2020**)**. The inclusion of vegetables in human meals has exacerbated their consumption as a part of a daily meal (Ross et al., 2011**)**. Vegetables have low-fat, high nutritional value, high dietary fiber, enriched with minerals and vitamins and possess very high nutritional value (Lin and Ge, 2015; Kostova et al., 2008).

Micronutrients in vegetables have many health benefits for example potassium helps to control healthy blood pressure, cholesterol level and the chance of heart diseases are reduced by dietary fiber content, folate lessens the risk of birth defects, vitamin A helps to maintain health eyes, skin and vitamin C helps to absorb iron and keep teeth healthy (Schreinemachers et al., 2018). The World Health Organization (WHO) recommends that to prevent chronic diseases minimum daily intake of 400 g of fruits and vegetables are needed (WHO/FAO, 2003).

1.2 Classification of Vegetables

Vegetables may be classified by following five different methods (Dhaliwal, 2017). They are

1. Botanical classification
2. Classification based on strength or temperature
3. Classification based on plant part used
4. Classification based on culture
5. Classification based on life cycle

1. Botanical classification: It is a universally accepted method of botanical classification of crop plants and animals, which was first renowned by a taxonomist named Linnaeus. Morphological and cytological similarities and dissimilarities, floral biology, crossability behavior, and place of origin are the major characteristics of this type of classification. This classification includes a group of plants into kingdoms, divisions, sub-divisions, phylum, sub-phylum, class, sub-class, order, family, genera, species, sub-species and varieties. It is subdivided into two classes.

i. Monocotyledoneae: This class includes onions, tulips, lilies and garlic.
ii. Dicotyledoneae: This class involves flowering plants like roses, geraniums, magnolias, and hollyhocks.

2. Hardiness or temperature: This is based on how well plants can withstand frost. Hardy plants can withstand frost. It is mainly in the winter season if they can adapt to the average temperature of 15-18 °C per month. There are two other subgroups that can be added to this categorization. They are:

1. Tolerant /hardy vegetables: these involve cabbage, broccoli, asparagus, peas, brussels sprouts, collard, garlic, chive, knol-khol, kale, leek, onion, parsley and radish.
2. Semi-tolerant/semi-hardy vegetables: these involve potatoes, cauliflower, lettuce, carrots and cabbage.

3. Classification based on plant parts used: Consumer and post-harvest handling are an important classification of vegetables. Vegetables can be categorized as leaves, stems, fruits, pods, flowers, roots, tubers and seeds depending on the part of the plant that is edible.

4. Grouping based on culture: Based on culture, vegetables are classified into different groups. Vegetables are further classified into: leafy vegetables, cucurbit crops, peas and beans (pod vegetables), perennial vegetables and tuber vegetables.

5. Life cycle-based classification: Vegetables can be divided into three categories: biennials, annuals, and perennials. Annual plants make up the majority of vegetable crops. These crops go from seed to harvest in just one growing season. A biennial needs two and perennial vegetables grow for more than two. Examples of annual plants are mustard, lettuce, corn, wheat and watermelon. Biennials include onions, cabbage, radish, carrot and petunias. Perennials involve mango, banana, ginger, tomatoes and coconut.

1.3 Description of some Vegetables

1.3.1 Pointed gourd (*Trichosanthes dioica*)

Trichosanthes dioica is locally known as potol (pointed gourd). It is included in the Cucurbitaceae family. It is a vine and a dioecious plant with heart-shaped leaves. It looks like green with white stripes. It may be small and round in shape or thick and long. Its length is normally 5-15 cm. The plant grows in a moderately warm and humid climate and on sandy loam soil. Pointed gourds have more nutritional value than other cucurbits. It is very rich in vitamins, minerals, protein and fibers (Kumar and Singh, 2012) (Figure 1.1).

Figure 1.1: Pointed gourd (*Trichosanthes dioica*)

1.3.2 Bitter gourd (*Momordica charantia*)

The bitter gourd (*Momordica charantia*) is a green-colored vegetable with white to translucent flesh that tests bitter. It is a part of the Cucurbitaceae family and is known as locally korola. It is a very popular vegetable in Bangladesh and is mostly grown during the summer season throughout the country. It has many nutritional and medicinal values. The edible portion of bitter gourd contains calcium, protein, carbohydrates, iron, phosphorus, vitamin A, and vitamin C, which are crucial for human nutrition (Mila et al., 2015; Singh and Gaikwad, 2012).

Active compounds like triterpenes, steroids and fatty acids like palmitic, stearic, lauric, and linoleic as well as minerals like Cu, Fe, Mg and Zn were identified in bitter gourd (Yuwai et al., 1991). A lot of people utilize it for food and medication and has a long history of use to protect against various diseases like diabetes, psoriasis, and infection (Abascal and Yarnell, 2005) (Figure 1.2).

Figure 1.2: Bitter gourd (*Momordica charantia*)

1.3.3 The Profile of Tomato (*Solanum lycopersycum*)

Tomatoes (*Solanum lycopersycum*) are edible berries and are widely grown in temperate climates across the world. It is available on the market all year round and it is produced mainly from December to March. It is an essential source of vitamins A and C. It prevents cancer by providing

antioxidant agents like lycopene. Regular tomato consumption can help to prevent different diseases like respiratory disorders, joint pain, night blindness, short-sightedness, and other diseases. It is grown in the majority of backyard gardens and fields and it may grow in a variety of soil types and climate in Bangladesh (Hossain and Abdulla, 2015**)**.

A carotenoid ingredient called lycopene gives tomatoes their rich red color, which possesses antioxidant properties, and because of having several conjugated dines, it has two times greater potency than beta-carotene and ten times greater potency than vitamin E. When tomatoes are consumed, lycopene levels are observed at an appreciable level in the human body (Burton-Freeman and Reimers, 2011**)** (Figure 1.3).

Figure 1.3: Tomato (*Solanum lycopersicum*)

1.3.4 Chili (*Capsicum frutescens*)

Green chili (*Capsicum frutescens*) belongs to the Solanaceae family, which is an important spice in Bangladesh. It is grown both in the winter and summer seasons. It is used in the preparation of different salads and curries. In Bangladesh, more than 66,000 hectares of chili crops are grown,

and more than 52,000 metric tons of chili are produced annually. It is a good source of vitamin C and phosphorus (Borgi, 2019) (Figure 1.4).

Figure 1.4: Green chili (*Capsicum frutescens*)

1.3.5 Cauliflower (*Brassica oleracea*)

Cauliflower (*Brassica oleracea*) is a member of the family of Brassicaceae. It is grown mostly during the winter months. It provides calcium, vitamin C, and vitamin A in good amounts. Total cultivation of cauliflower is about 9400 hectares and production of it is about 73,500 metric tons annually in Bangladesh (Shawon et al., 2018**)**. It not only meets the demand for food production but also provides a balanced diet. When it is consumed regularly, it is seen to exert health-promoting effects, for example, a reduction in chronic diseases and various types of cancer (Jahangir et al., 2009; Comhaire, 2014). Cauliflower plants have been found to contain carotenoids, ascorbic acid, phenolics, tocopherols, and well-known antioxidants (De Pascale et al., 2007) (Figure 1.5).

Figure 1.5: Cauliflower (*Brassica oleracea***)**

1.4 Pesticides

Pesticides are chemical substances widely used in agriculture to improve production, boost quality, and prolong the duration of storage of food crops (Chapman, 2002; Atuanya and Onuoha, 2018). Several pests growing with the vegetables cause an overuse of pesticides, which seriously harms the environment. One type of synthetic organic chemical that has found widespread application due to its low cost and great insecticidal effectiveness is pesticides. For example, chlorinated pesticides can bioaccumulate in fatty tissues and biomagnify in the food chain, which can weaken the human immune system. They are also highly neurotoxic (Yu et al., 2021; Abreu-Villaca and Levin, 2017; Grilo et al., 2013).

Pesticides extend the sustainability of crops or agricultural products that have been taken by both adults and kids in the control of diseases. They provide several benefits to society in terms of agricultural productivity, and pesticide use has increased, particularly in developing countries, and has spread to the fastest expanding markets in Asia, Africa, the Eastern Mediterranean and South and Central America (Atuanya and Onuoha., 2018; Adeyemi et al., 2011). Pests are managed using pesticides in agriculture and households. These are very useful components to protect against human diseases transmitted by insects or rodents. The global economy has been expanding

significantly in both industrial and agricultural sectors as a result of high yields following the usage of agrochemicals (Shoeb et al., 2020; Munia et al., 2018).

Pesticides are a serious problem associated with food safety issues. Despite having several benefits, pesticides are highly harmful to humans and are compounds that are both stable and mobilized in the environment. Over use of pesticides results in their presence in food and vegetables. They may enter the human body at a high risk and can lead to poisoning and diseases because of their stability, toxicity, and capacity for bioaccumulation (Fenik et al., 2011; Cairns and Sherma, 1992) (Figure 1.6).

Figure 1.6: Use of pesticides in Bangladesh

1.5 Types of Pesticides

Pesticides are meant to eliminate, discourage, and render hazardous organisms harmless. (Nshimiyimana et al., 2014; WHO, 2010). Pesticides are divided into various categories based on the species they target (insecticides, herbicides, fungicides and rodenticides are only a few examples of the major categories), or according to the chemical processes they perform (organochlorines, organophosphates, carbamates, etc.). Although pesticides have helped agricultural resources, their use poses risks to public health (Anger and Kintz, 2009; Aprea et al, 2002).

Besides their chronic toxicities (long-term exposure) and toxicities (accidental or suicide) may be linked to a variety of human health problems, including infertility, cancer, etc. toxicities (accidental or suicide) (Alavanja et al, 2004). Particularly efficient tools for pulverizing pest control, organochlorine and organophosphorus pesticides have been used mostly in agriculture around the world. These families of insecticides are environmental contaminants because of their great chemical stability and lipid solubility. As a result, these pesticides are frequently found in fish, wildlife, adipose tissue, and breast milk, which are all parts of the food chain that are accessible to both animals and people (Liu and Pleil, 2002; Liberda et al, 2014).

The classification of pesticides based on their chemical nature is divided into five categories. These are organochlorines (DDT, BHC, Chlordane, Heptachlor, DDD, eldrin, dieldrin, aldrin, lindane and endosulfan). Organophosphates (Trichlorofan, malathion, abate, fenthion, dimethoate, dichlorovos), carbamates (Methyl carbofuran, methyl dimethan, thio pebulate, dithiomethan, methyl carbaryl, thio butylate) pyrethroids (Cypermethrin, dimethrin, furethrin, fenvelerate, alphamethrin, tetramethrin) and phenyl amides (Carbanilates, chlororprofan, carbanilates carbetamide, acylanalide butachlor, acylanalide dicryl).

1.6 Organochlorine Pesticides

Due to their low cost and strong insecticidal efficacy, organochlorine pesticides (OCPs) are one outstanding synthetic chlorinated organic chemical that has been utilized all over the world. While the OCPs are highly neurotoxic, have the capacity to bioaccumulate on fatty tissues and biomagnify through the food chain, inhibiting the human immune system in the interim, they would also significantly harm the ecological environment due to their high toxicity and inability to degrade (Yu et al., 2021; Abreu-Villaca and Levin, 2017; Grilo et al., 2013). OCPs have thus been prohibited in developed nations since the mid-1970s. Even after almost 40 years, these chemicals remain dangerous. The detection rates of OCPs in environmental media are low because of their great stability, extended half-life, complex breakdown, and slow metabolism in organisms (Mahugija et al., 2014; Shukla et al., 2006) and food (Li et al., 2018; Zhang et al., 2015). The safety of vegetables has drawn increasing attention due to people's desire for nutritious diet.

OCPs are polychlorinated compounds, which are highly electronegative elements and exhibit the characteristics of high-temperature gasification; as a result, it is required to create sensitive and selective ways to monitor OCPs in vegetables. Aldrin, endrin, dieldrin, chlordane, heptachlor, DDT, toxaphene, endosulfan and hexachlorobenzene are only a few of the OCPs (HCB) (Papadakis et al., 2015). High persistence, low polarity, low aqueous solubility, and high lipid solubility are characteristics of these molecules (lipophilicity). They may pose a health danger to people since they are ecotoxic, non-biodegradable, and able to bioaccumulate and biomagnify in humans through consumption of infected fruits and vegetables (Afful et al., 2010). The Danger Quotient (RQ) approach is used to calculate the potential risk of pesticide exposure to non-target organisms.

The RQ is the difference between the predicted no-effect concentration and the measured environmental concentration (MEC) (PNEC). Using an assessment factor (AF) of 100, the predicted no-effect concentration (PNEC) was calculated by doubling the LC50. The evaluation factor considers the uncertainty in extrapolating from lab toxicity tests for a small number of substances of dietary and non-dietary products to the real environment. The LC_{50} was obtained from Chapman et al (Chapman et al, 2002) and the RQ was from Papadakis et al (Papadakis et al., 2015). Though organochlorine insecticides were used worldwide to control typhus and malaria, these have now outlawed in most developed nations (Aktar et al., 2009). Some organochlorine pesticides are discussed below.

1.7 Different Types of Organochlorine Pesticides and Their Toxicology

1.7.1 DDT

The organochlorine compound dichlorodiphenyltrichloroethane (DDT) was synthesized in 1874, and agricultural applications of DDT began after World War II (Blus, 2011). Dichlorodiphenyldichloroethylene (DDE) is the degradative product of DDT where hydrogen chloride (HCl) is eliminated and dichlorodiphenyldichloroethane (DDD) is formed by the reductive dichlorination process (Cann, 2005). It can affect the nervous system by interfering with nerve impulses. It is environmentally very persistent, highly toxic, and accumulates in fat and water. It is an endocrine disruptor considered to be carcinogenic, mutagenic, and also teratogenic (Hejmej et al., 2011) (Figure 1.7-1.8).

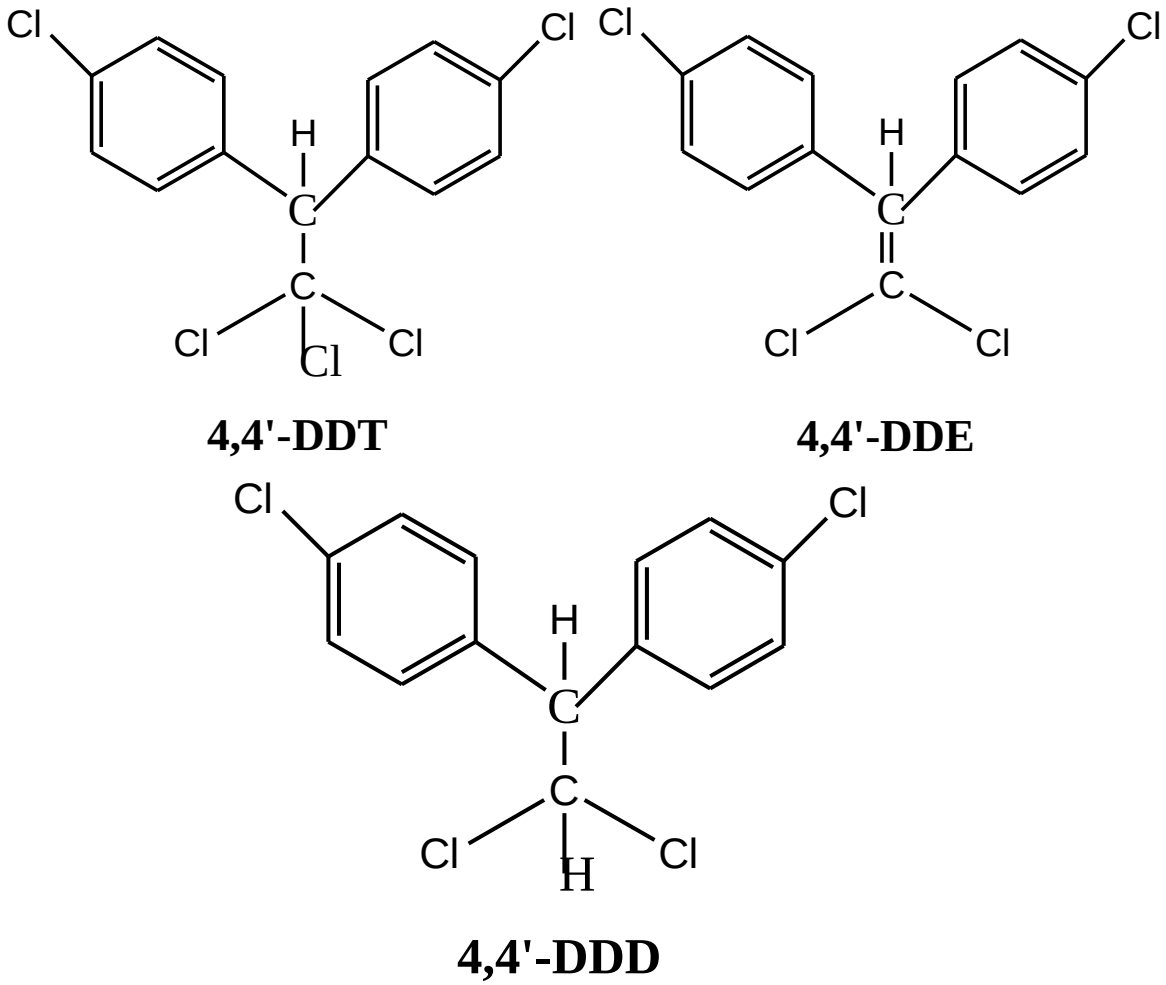


Figure 1.7: Chemical structure of DDT residues

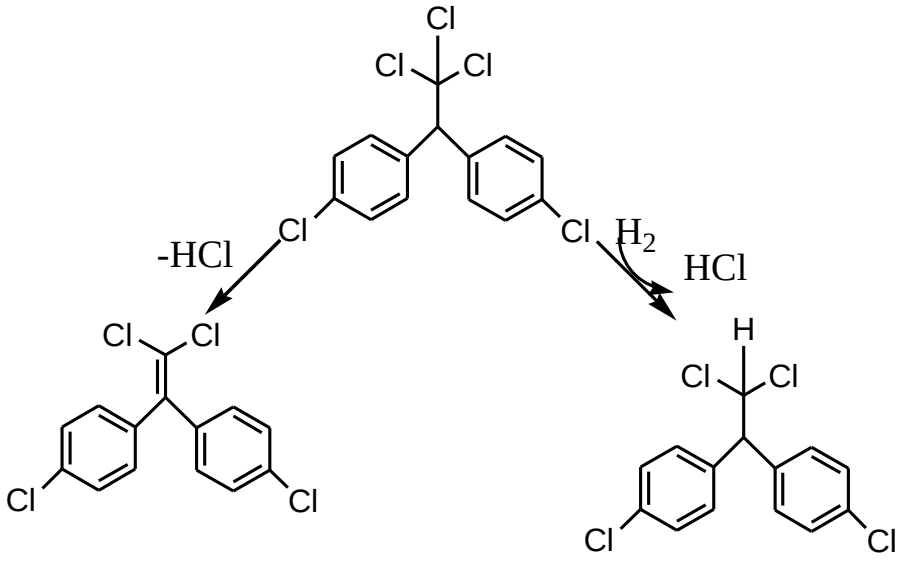


Figure 1.8: Degradation of DDT to DDE (left) and DDD (right)

1.7.2 Aldrine and Dieldrin

Aldrin and dieldrin are chlorinated insecticides used to treat seeds. These are white solid crystals and have been used in various formulations like wettable powders, emulsifiable concentrates, granules, dust, and solutions in hydrocarbon liquids (Figure 1.9).

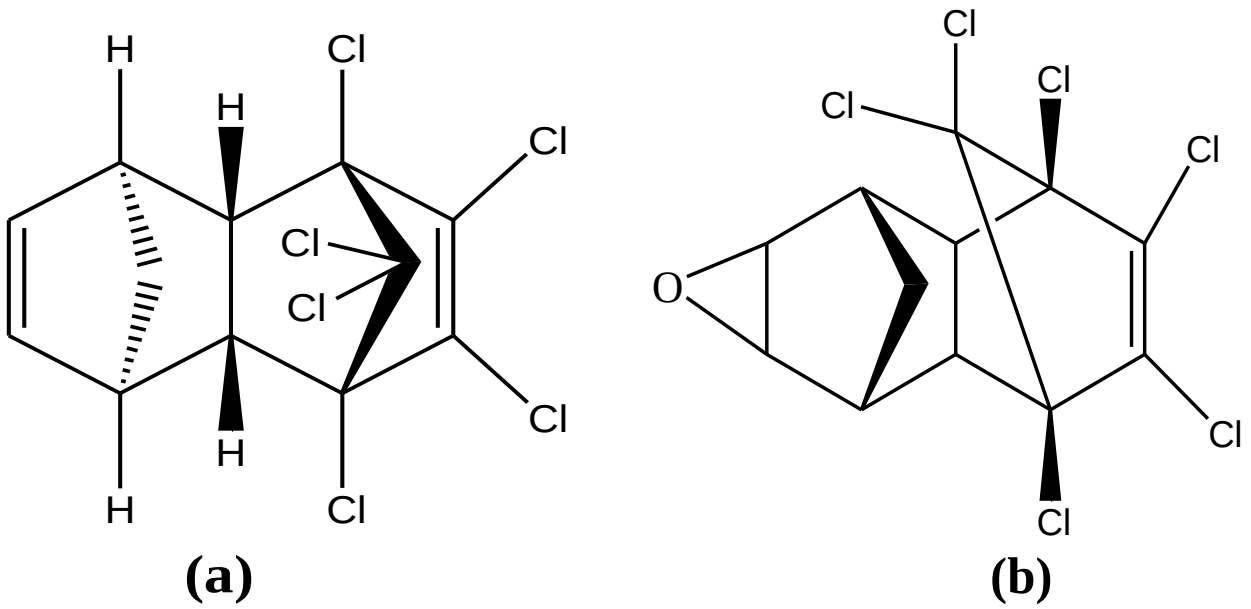


Figure 1.9: Structure of aldrin (a) and dieldrin (b)

1.7.3 Endrin, Endrin aldehyde and Endrin ketone

The toxicology profile of endrin is associated with both endrin ketone and endrin aldehyde. Endrin was used as a pesticide. Endrin ketone and endrin aldehyde are not industrial products, but they are found as impurities or degradation products of endrin. Endrin ketone and endrin aldehyde can be produced from the breakdown of endrin by uncovering to intense light or heat. Exposure to endrin can have a number of negative outcomes, including death and serious central nervous system damage. Headaches, nauseousness, dizziness, vomiting, anxiety, and convulsions are symptoms of endrin poisoning. After being exposed to high levels of endrin, some of these symptoms could last for weeks (PHSE, 1996). Their structures are shown in Figure-1.10.

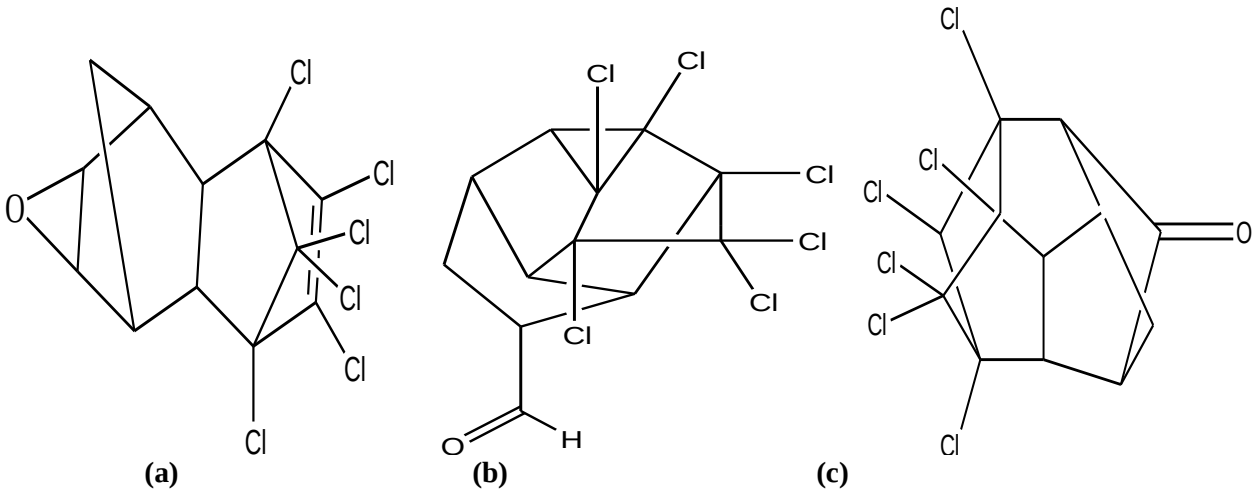
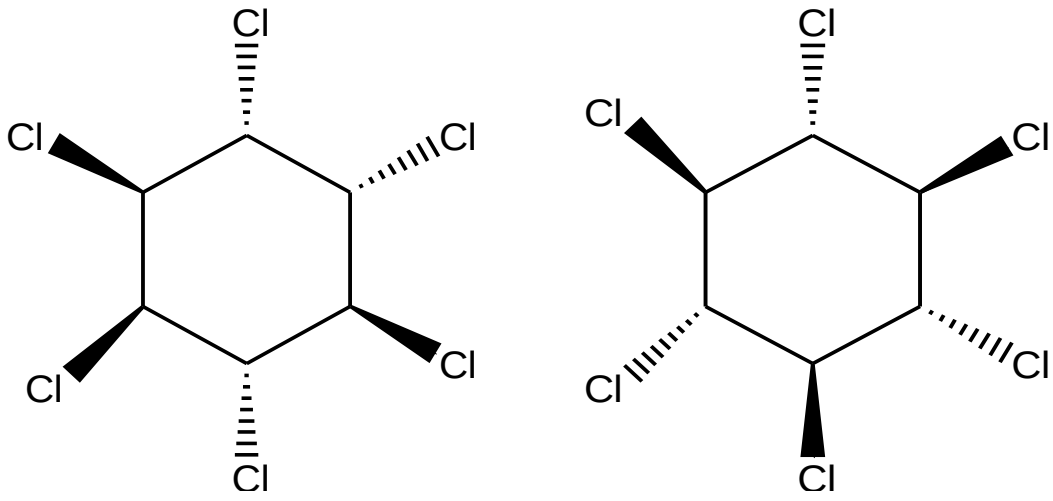


Figure 1.10: Structure of endrin (a), endrin aldehyde (b) and endrin ketone (c)

1.7.4 BHC (α, β, δ) and Lindane (γ-BHC)

The chemical formula of benzene hexachloride (BHC) is $C_6H_6Cl_6$ and it is employed as an organochlorine insecticide. These pesticides have several stereoisomers like α-, β-, γ- and δ – BHC. γ- BHC is known as Lindane or gammaxene. The common toxic effects of γ- BHC are dysfunction of the central nervous system, seizures, ataxia, confusion, etc. (Paul et al., 2013). Workers in the agricultural field were found to have increased lung cancer risk and used Lindane as a pesticide for a long time (National Toxicology Program, 2011). It was also reported that γ-BHC is responsible for inhibiting cell division in human peripheral blood lymphocytes (Krieger, 2001) (Figure 1.11).



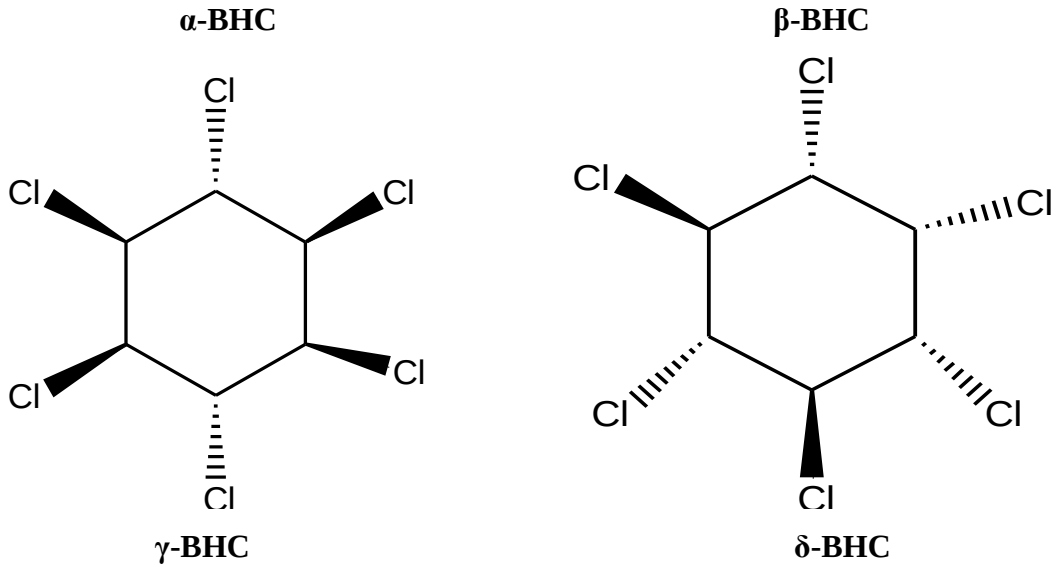
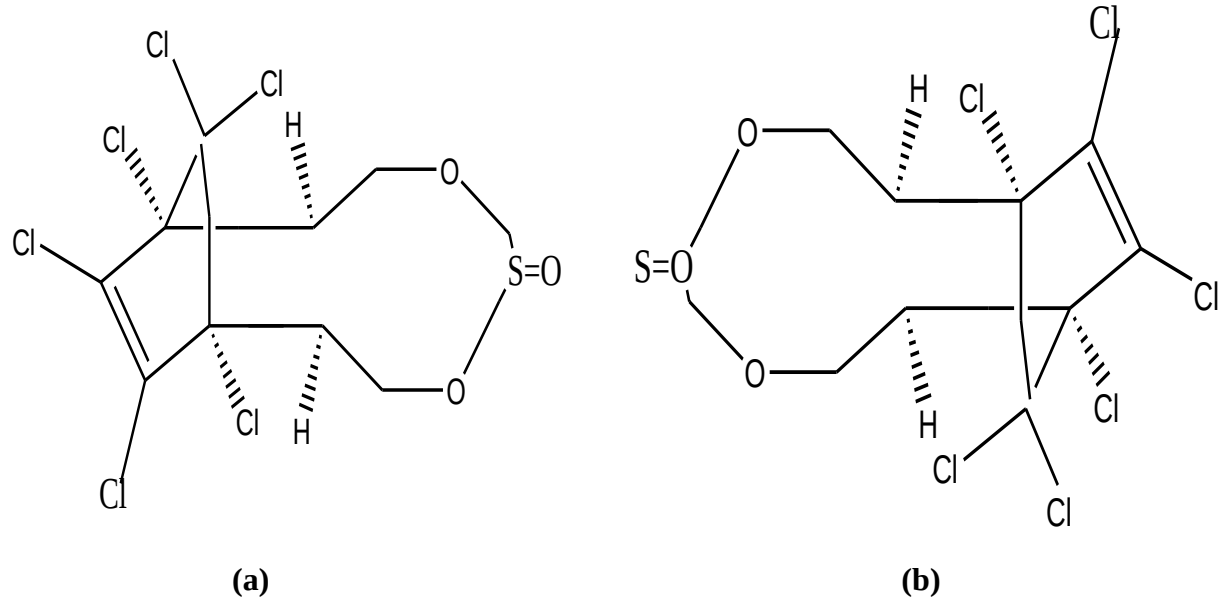


Figure 1.11: Structure of α-BHC, β-BHC,γ-BHC and δ-BHC

1.7.5 Endosulfan (I and II) and Endosulfan Sulfate

Endosulfan ($C_9H_6Cl_6O_3S$) (Figure 1.12-1.13) is a derivative of hexachlorocyclopentadiene and a 7:3 mixture of stereoisomers designated I and II. It is a semivolatile and relatively persistent compound found in soil, sediment, and water, even though it is not directly used in it (Baselt, 2014). It is degraded by soil microorganisms. It has been used worldwide as a pesticide since the 1950s (Berntssen et al., 2017). It is neurotoxic for insects, mammals, and even humans, and its LD_{50} value was found at 30 mg/kg for female rats (Rossi, 2002).



(a) **(b)**

Figure 1.12: Structure of (a) endosulfan-I (b) endosulfan-II

Endosulfan can either be oxidized or hydrolyzed to produce endosulfan sulfate and endosulfan diol, which can then be released into the environment. Endosulfan sulfate is also toxic like other environmental contaminants and it is persistent in its parent compound form (Singh and Singh, 2010).

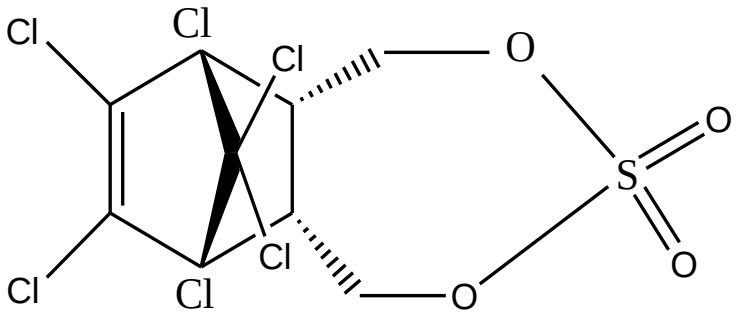


Figure1.13: Structure of endosulfan sulfate

1.7.6 Heptachlor and Heptachlor Epoxide

Heptachlor ($C_{10}H_5Cl_7$) (Figure 1.14) is a synthetic white powdered chemical substance that smells like camphor. Heptachlor epoxide is also a white powdered compound and it is formed when Heptachlor is broken down by bacteria and animals. Heptachlor and Heptachlor epoxide were largely used in agriculture to control insects. These can be dissolved in water (ATSDR, 2000). Humans can absorb the very dangerous chemical heptachlor through their skin, lungs, and digestive system. It is also reported that both heptachlor and heptachlor epoxide are linked with infertility and the improper development of offspring (Kenyanya, 2019).

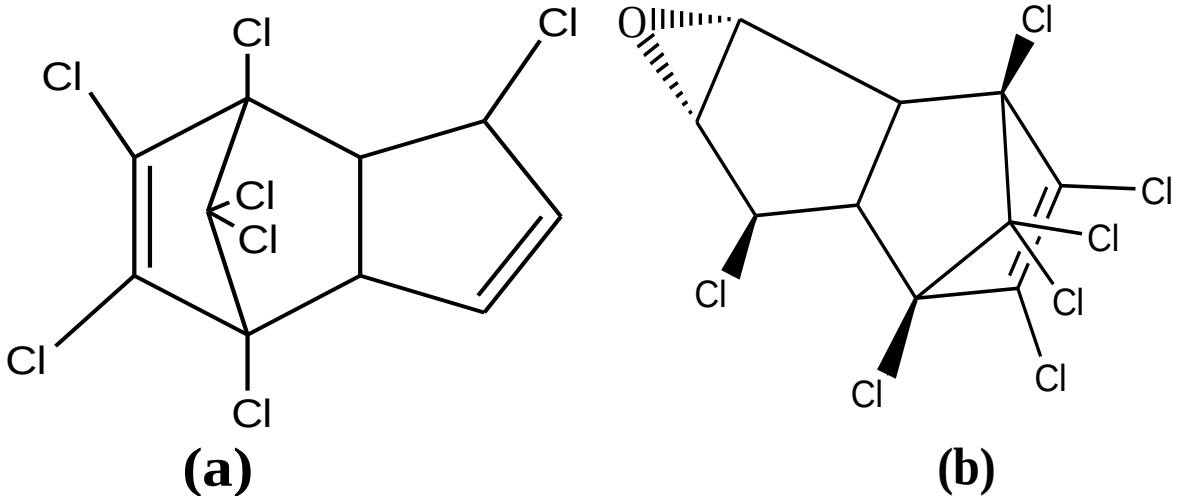


Figure 1.14: Structure of (a) heptachlor and (b) heptachlor epoxide

1.7.7 Chlordane

The chemical formula of chlordane is $C_{10}H_6Cl_8$ (Figure 1.15). It is also called Octachlor and Velsicol 1068. It is an organochlorine substance applied to both food and non-food crops, residential lawns, and gardens as a pesticide against insects. It is a by-product of synthetic rubber manufacturing. Because of its negative effects on health of people and the environment, the United States Environmental Protection Agency (EPA) outlawed the use of chlordane in 1983, without a termiticide, which was used in wooden structures. It is a mixture of over 50 closely related chemicals and variations in the makeup of chlordane can be different as a result of toxicity (Abadin and Goetchius, 1994).

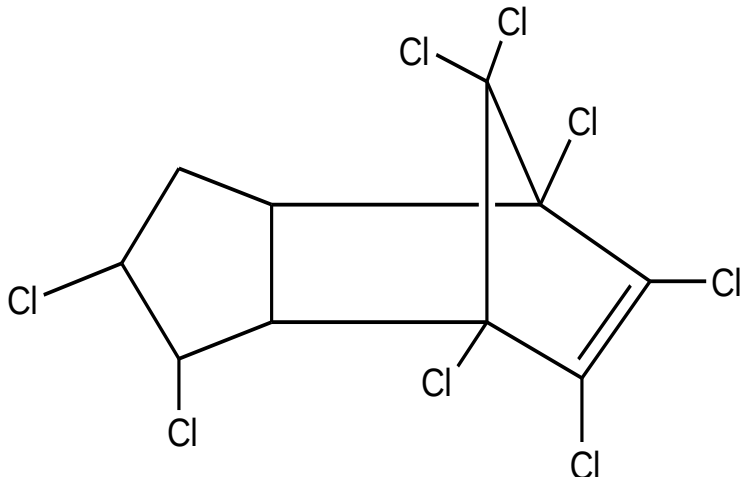


Figure 1.15: Structure of chlordane

1.7.8 Methoxychlor

Methoxychlor ($C_{16}H_{15}Cl_3O_2$) (Figure 1.16), also known as DMDT, Metox, or Margate, was used to control insects and was thought to be the replacement for DDT. It is a chlorinated pesticide. It is white to pale yellow and is insoluble in water. It is detected in water bodies, plants, sediments, biota, and humans. It is persistent and bioaccumulative in the environment. It is harmful to aquatic organisms and animals, and it can be transported far from its production location (Adeyi et al., 2021; Sosan et al., 2020).

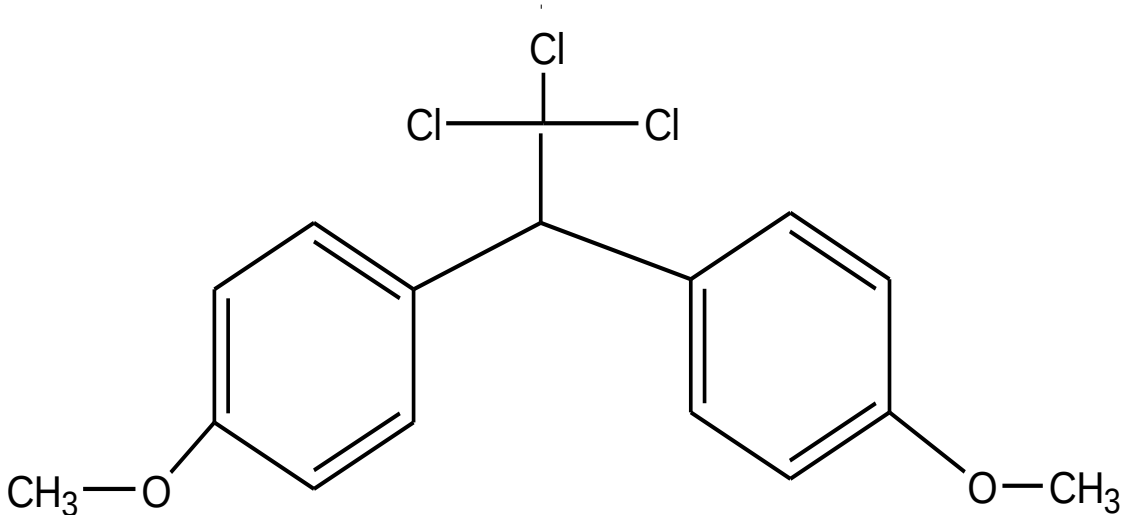


Figure 1.16: Structure of methoxychlor

1.8 Fatty Acid

A fatty acid is an aliphatic carboxylic acid with a long, saturated or unsaturated chain. The majority of naturally occurring fatty acids, which range in number from 4 to 28, have an unbranched chain with an even number of carbon atoms (Moss et al., 1997). Triglycerides or phospholipids are often the sources of fatty acids. The building blocks of cell membranes, an energy source for the body, and a source of energy for muscles, the heart, and other organs are all provided by fatty acids. Fatty acids that are not utilized for energy are changed into triglycerides.

1.9 Fats and Oils from vegetables

Fats are glycerides of long-chain fatty acids. The fat is solid. Chemically, both fats are composed of triglycerides. The majority of the calories from fat are deposited in triglycerides. Fats provide energy. The production of bile acids and other hormones importantly depends on cholesterol. Generally, fats are mostly of animal origin, whereas oils are of vegetative origin. Triglycerides are a component of dietary fats and oils. Oils are typically liquid at normal temperature, while fats are typically solid. The qualities of fatty acids are connected to the features of fats and oils. The melting point decreases with the higher number of double bond and the melting point increases with the higher number of carbon chains (Hayes and Expert, 2010).

1.10 Types of Fatty Acids (Rustan and Dravon, 2001)

(i) Saturated Fatty Acids

(ii) Unsaturated Fatty Acids

(iii) Essential Fatty Acids

(iv) *Trans* Fatty Acids

(v) Free Fatty Acids

1.11 Saturated Fatty Acids

Long chain carboxylic acids with no double bonds, such as saturated fatty acids, typically contain between 12 and 24 carbon atoms. The name and structure of some fatty acids are given in the following Figure 1.17.

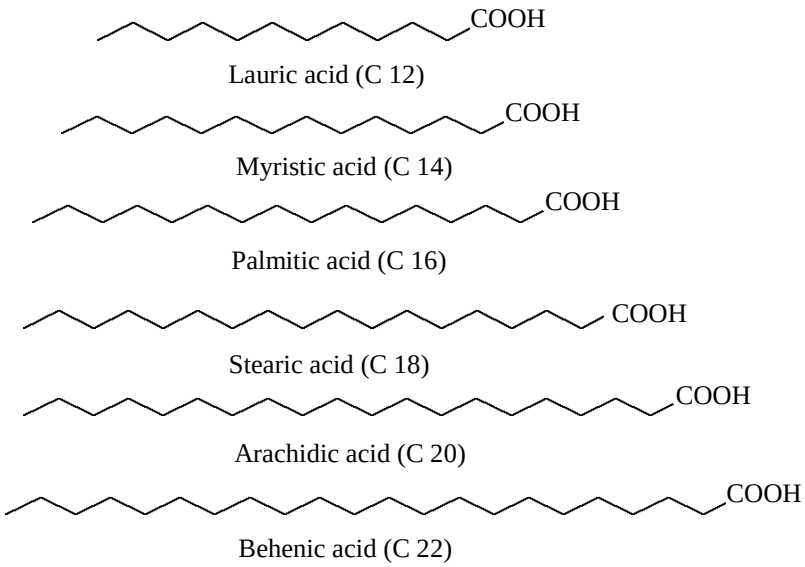


Figure 1.17: Structure of some common saturated fatty acids

1.12 Unsaturated Fatty Acids

Unsaturated fatty acids are those that have one or more carbon-to-carbon double bonds. The most prevalent fatty acid in nature is Eric acid (*cis*-9-octadecenoic acid). A fatty acid is said to as monounsaturated if it has one double bond. Polyunsaturated fatty acids are those that have more than one double bonds.

1.13 Essential Fatty Acids

Humans are unable to manufacture vital fatty acids, thus they must be obtained through diet. Long-chain polyunsaturated fatty acids called essential fatty acids are produced from linolenic and oleic acids. Omega-3 and Omega-6 are the two families of important fatty acids. Because the body can produce a little quantity of omega-9 on its own, it is necessary but not "vital."

1.14 *Trans* Fatty Acids

According to research, for unknown reasons, *trans* fats are more frequently linked to circulatory disorders like atherosclerosis and coronary heart disease than *cis* fats. But it is known that *trans* fats raise LDL cholesterol and lower HDL cholesterol, just like saturated fats (Lupton et al., 2002).

1.15 Heavy Metals

Metallic substances with a relative high density compared to water are referred to as heavy metals. Assuming that toxicity and heaviness are related, heavy metals also include metalloids like arsenic that can cause toxicity at low exposure levels. Environmental contamination by these metals has recently been linked to rising ecological and worldwide public health concerns. Additionally, due to an exponential expansion in their use in a variety of industrial, agricultural, household, and technical applications, human exposure has increased significantly (Fergusson, 1990; Duffus, 2002; Bradl, 2005). Geogenic, industrial, agricultural, pharmaceutical, home effluents, and atmospheric sources are some of the sources of heavy metals in the environment (He et al., 2005).

Even though heavy metals are naturally occurring substances that are present throughout the earth's crust, most environmental pollution and human exposure are caused by anthropogenic activities (He et al., 2005; Shallari et al., 1998). It has also been claimed that natural events like weathering and volcanic eruptions greatly contribute to heavy metal contamination (Nriagu, 1989). According to studies, metals including cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) are necessary nutrients. A lack of these micronutrients can lead to a number of deficiency illnesses (Chang et al., 1996).

Heavy metals are also referred to as trace elements because of their occurrence in diverse environmental matrices at trace amounts (ppb to less than 10 ppm) (Arruti et al., 2010). The necessary heavy metals influence the biochemistry and physiology of plants and animals. They play an important role in various oxidation-reduction reactions. Non-essential metals include those with no known biological uses, such as aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ge), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag)
(Chang et al., 1996).

1.16 Effects of Heavy Metals on Aquatic Life and Human Health

The quality and variety of aquatic life have declined during the industrial period due to pollution. Industries release garbage into waterways that contains heavy metals. These heavy metals build up in various fish organs, which kills the animal. Thus, heavy metal pollution threatens vegetables' primary supply of protein (Kamaruzzaman et al., 2011). Metals alter erythrocyte osmotic resistance through influencing the reticuloendothelial system and hematopoiesis. At various stages of the diseased process, the red and white blood cells are deformed both quantitatively and qualitatively (Perez et al., 2001).

The body can be acquired by heavy metals, resulting in chronic diseases. Metal toxicity is influenced by the absorbed dose, the exposure route, and the length of time. By displacing the original metals from their native binding sites, these heavy metals can connect with protein sites and cause cell dysfunction as a result of their toxicity. The binding of heavy metals to DNA and nuclear proteins can deteriorate biological macromolecules oxidatively (Liu et al., 2010). When certain metals reach particular threshold amounts, they become toxic (Kamaruzzaman et al., 2011). Copper, nickel, zinc, cobalt, and cadmium are the most often discovered heavy metals in wastewater, and they all pose threats to both human health and the environment.

1.17 Sources and Toxicology of Some Heavy Metals

1.17.1 Chromium (Cr)

A naturally occurring metal having oxidation states ranging from chromium (II) to chromium (III), chromium (Cr) is found in the crust of the earth (VI) (EC., 2006). The trivalent [Cr (III)] form of

chromium compounds is the most stable, and chromium released into the environment as a result of the anthropogenic activity is mostly in the hexavalent [Cr (VI)] form (U.S. EPA, 1992) Chromium (Cr (VI)) hexavalent is a harmful industrial contaminant. Inhalation is the main way that people are exposed to chromium, and the lung is the main organ that is affected. Substantial human exposure can take place through the skin and ingestion. (Connett and Wetterhahn, 1983)**.** As a result of exposure to Cr (VI)-containing substances in the workplace and environment, humans have been related to kidney impairment, allergy and asthma attacks, and lung cancer (De Flora et al., 1990).

1.17.2 Lead (Pb)

In trace proportions, lead is a bluish-gray metal that occurs naturally in the earth's crust. Numerous home, agricultural, and industrial uses exist for lead. Currently, it is employed in the manufacture of metal goods, ammunition, and lead-acid batteries (solder and pipes). In the United States in 2004, an estimated 1.52 million metric tons of lead were used for various industrial purposes **(**Gabby, 2006**).** The kidney absorbs the most lead, followed by the liver and the rest of the body's soft tissues, including the heart and brain (Flora, 2006)**.** Lead absorbed by the pregnant mother is readily transferred to the developing fetus. Human evidence corroborates animal findings, linking prenatal exposure to lead with reduced birth weight and preterm delivery (Corpas et al., 1995) and with neurodevelopmental abnormalities in offspring (Huel et al., 1992).

1.17.3 Cadmium (Cd)

Cadmium is accumulated in sedimentary rocks and marine phosphates, which roughly contain about 15 mg/kg (WHO, 1992). Various industrial activities like the production of alloys, pigments, and batteries employ cadmium (Wilson et al., 1988). Ingestion of food or inhalation of cigarette smoke are the two ways to be exposed to cadmium. Symptoms of acute cadmium ingestion include abdominal discomfort, a burning sensation, nausea, vomiting, salivation, cramping in the muscles, vertigo, shock, unconsciousness, and convulsions (Baselt and Cravey, 1982). Norepinephrine, serotonin, and acetylcholine levels are depressed by chronic exposure to cadmium (Singhal et al., 1976). Adenocarcinomas and other prostatic proliferative lesions can also be brought on by systemic or direct exposure. Cadmium levels were discovered to be inversely associated to birth weight and length in cord blood, maternal blood, or maternal urine (Nishijo et al., 2004).

1.17.4 Copper (Cu)

Drinking water containing dissolved copper has a flavor that is harsh and has a light blue or blue-green tint. Due to its adaptability, it has a wide range of commercial uses. Copper is used to create electrical wiring, pipes, valves, fittings, coinage, kitchen utensils, and building materials. Copper compounds are also utilized in the production of azo dyes, fungicides, algicides, insecticides, wood preservatives, lithography, engraving, engraving, and pyrotechnics. Adding copper compounds as a nutrient to fertilizers and animal feeds can promote copper compounds (Landner and Lindestrom, 1999).

In the form of colour and nutrient additions, they are also employed in food. For the purpose of controlling algae, copper sulfate pentahydrate is occasionally added to surface water (Number, 1988). The main way that humans are exposed to copper is through food. A good source of dietary copper include liver and other organ meats, shellfish, nuts, seeds, and whole grains. Copper is an essential nutrient (Russell et al., 2001). Since copper accumulates in the liver, brain, and eyes, it may cause Wilson disease, which is an autosomal recessive disorder (National Research Council, 2000).

1.17.5 Nickel (Ni)

In the first transition series of the periodic table of elements, nickel is the last metallic element, and its most common oxidation states are 0 and 2+ (Cotton et al., 1999). There have been reports that Ni exposure modifies the hemocyte defense system in the mud crab *Scylla serrate* (Vijayavel et al., 2009). Nickel's harmful effects on the immune system in mammals have led to extensive research in this area. Nickel seems to behave more like an ion regulatory toxin in aquatic invertebrates, especially in acute doses (Min et al., 2015).

1.17.6 Iron (Fe)

Minerals include iron. Red blood cells' hemoglobin and muscle cells' myoglobin contain the majority of the body's iron. Transporting oxygen and carbon dioxide need iron. Additionally, it plays other crucial roles in the body. Foods including beef, fish, tofu, beans, spinach, cereal, and other items also contain it. When taken orally in the proper dosages, iron is safe for the majority

of people. However, it may result in negative side effects like nausea, vomiting, diarrhea, and constipation (Tolkien et al., 2015).

1.18 Objective of the Work

Fresh veggies are a valuable source of vitamins, minerals, and other elements, but they can also contain harmful chemicals like pesticides. Plant-based food can become polluted with pesticides under different conditions and at different times before ingestion. Pesticides circulate and migrate to numerous parts of the environment, especially the atmosphere and hydrosphere, regardless of the method of application (Stocka et al., 2016; Biziuk, 2001; Moreno et al., 2006). Pesticides for direct application to fruits and vegetables and pesticides transported by rain or wind to neighboring crops and areas where they are unwanted or harmful (Hajslova and Zrostlikova, 2003; Stocka et al., 2011). Pesticides enter the plant through the root system and travel from the interior to various anatomical areas of the plant (from the root system to stems and leaves). The rate of diffusion is influenced by factors like the characteristics of living things, the chemical composition of soil or pesticides, and atmospheric conditions.

Plants have very few opportunities to excrete waste (the only way is through the leaves transpiring systems). Plants are therefore exposed to a significant amount of insecticides. Fruits and vegetables that have collected pesticides can go through physical, chemical, photochemical, and biochemical transformations that cause irreversible changes and a variety of damage (Róaski et al., 1992). The primary causes of pesticide contamination of fruits and vegetables are agricultural practices and insufficient regulation of their use (Namieśnik and Szefer, 2008; Zhang et al., 2012). It is frequently impossible to completely eradicate pesticides from fruit and vegetable crops. However, their content can be cut down to a level that is essentially health-safe. According to recent studies, pesticides are dangerous for both the environment and human health.

This study's goal was to evaluate the amounts of pesticide residues in Bangladesh's most popular vegetables. The data is also very significant for formulating policies intended to lessen or eliminate threats to human health posed by hazardous pesticide residues in raw and processed agricultural goods. The key criteria used to choose the pesticides was how widely they were used in Bangladesh's vegetable industry. DDT and the majority of organochlorine pesticides have long

been prohibited in Bangladesh. However, ongoing monitoring of these pesticides is necessary due to their long-term persistence in the environment, particularly in agricultural soils, and the potential for entering the food chain through plant uptake (Jallow et al., 2017; Weaver et al., 2012; Florence et al., 2015). As a result, organochlorine pesticide residues were investigate in this study. Therefore, main objectives of the present study were:

a) Identification and quantification of the residual levels of organochlorine pesticides (20 pesticides) in vegetable samples collected from the local market using a gas chromatograph with a detector for electron capture (GC-ECD)

b) Determination of moisture and ash content of vegetable samples

c) Identification and quantification of fatty acid compositions of collected samples utilizing a flame ionization detector fitted gas chromatograph (GC-FID)

d) Analysis of these samples' protein, phenolic, flavonoid, and carbohydrate contents as well as their antioxidant capacity

e) Investigation of micronutrients (Zn, Fe, Mn, Ca, Cd, Cr, Cu, Ni, and Pb) of these vegetable samples by Atomic Absorption Spectrophotometer (AAS)

2. EXPERIMENTAL

2.1 Chemicals, Reagents and Solvents

Analytical grade solvents and reagents used to carry out the whole experiments were purchased from (E, Merck, Germany). Hexane was used for the preparation of the sample and standard solution. Ethyl acetate was used to extract vegetable samples and methanol was used to determine antioxidants, phenolic and flavonoids contents. During research work, 0.5M HCl was used for fatty acid extraction, methanol (99.5% w/w, Sigma Aldrich), 0.5M, 0.1N, and 50% sodium hydroxide, BF_3-methanol complex (E. Merck, Germany), Folin-ciocalteu reagent (freshly prepared), and H_2O_2 were also used.

2.1.2 Preparation of alcoholic NaOH Solution

An alcoholic NaOH solution was prepared for saponification of the oil. NaOH (1.0867g) was taken in a volumetric flask (50 mL) and the volume was adjusted to 50 mL with methanol. The resulted solution was Methanolic NaOH of concentration 0.5 M.

2.1.3 Solid Reagents

Different types of organic and inorganic chemical reagents were used in the laboratory during the research work. A list of them is given below.

- Analytical grade anhydrous magnesium sulfate ($MgSO_4$)
- Sodium chloride (NaCl) (Merck, Germany)
- Sodium sulfate (Na_2SO_4) (Scharlab S.L., 08181 Sentmenat, Spain)
- Na_2CO_3 (anhydrous sodium carbonate)
- Crystalline $AlCl_3$
- Crystalline sodium acetate
- Crystalline sodium phosphate
- Anhydrous ammonium molybdate
- Primary secondary amine (PSA) (SUPELCO, USA)
- Potassium sulfate
- Copper sulfate

2.1.4 Standard Compounds

A mixture of twenty organochlorine pesticides containing 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, BHC (α, β, γ, and δ), heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, methoxychlor, endosulfan (I and II), endosulfan sulfate and chlordane (*cis* and *trans*) was injected into GC ECD as the standard pesticide mixture.

A standard mixture of fatty acids containing caprylic acid, capric acid, myristic acid, lauric acid, palmitic acid, palmitoleic acid, linoleic acid, oleic acid, stearic acid, archidic acid, behenic acid, lignoceric acid solution was purchased from "Sigma-Aldrich, France". This fatty acid mixture was injected in GC-FID as the standard acid mixture.

2.1.5 Glass Apparatus

A list of glass and plastic materials used in research work is given below:

- Round Bottom Flasks (RBF) (10, 50, 100, 250, 500 mL)
- Teflon tubes (50 mL)
- Funnel
- Separatory funnel
- Graduated test tubes and vials
- Test-tube racks
- Graduated pipette (25.0, 10.0, 5.0, 2.0, and 1.0 mL)
- Pasture pipette
- Micro-pipette
- Beaker
- Conical flasks
- Ring stands and clamps
- Condenser
- Volumetric flasks, (250, 100 and 50 mL)
- Measuring cylinder
- Reagent bottles (Pyrex Glass)
- Pear-Shaped flask
- Porcelain crucible
- Spatula
- GC-vial

- Desiccator
- Jip-locked plastic bags etc.

2.1.6 Treatment of Glass and Plastic Equipment

All necessary glass equipment was cleaned with soap in water, followed by water rinses, two rounds of distilled water, and acetone. Before usage, all glassware was stored by covering it with aluminum foil after being heated at 300 °C for around 4 hours.

2.1.7 Analytical Instrument

During the experiment the following equipment and small apparatus were used-

- Gas chromatography with Electron Capture Detector (ECD) (Model GC-2030, Shimadzu, Japan)
- Gas chromatography equipped with Flame Ionization Detector (GC-FID) (Model: GC-2025, Shimadzu, Japan)
- Rotary vacuum evaporator (Heidolph, Germany)
- Carbolite furnace (GSM 11/8 Hope Valley, S336RB, England)
- Analytical balance (FR-200, NDO-450 ND, Japan)
- Centrifuge machine (Hanil Science Industrial Co. Ltd., Model-Combi 514 R or by Heraeus Sepatechm (Labofuge A), with rotation up to 4000 rpm
- Normal kitchen blender (Miyako Chopper, Japan)
- Freeze dryer (LABCONCO, USA)
- Oven (GSM 11/8 Hope valley, S336RB, England)
- Ultrasonicator bath
- Laboratory test sieves
- Atomic Absorption Spectrophotometer (AAS)-(Model- AA-7000. Shimadzu

2.1.8 Furnace and its uses

Different inorganic compounds were activated by heating the crucibles, they were heated above 300 ºC. Again both the moisture content at 105 ºC and the ash content above 700 ºC were determined. All glasswares were heated in a carbolite furnace (GSM 11/8 Hope Valley, S336RB, England) for about 3 hours (Figure 2.1).

Figure 2.1: Carbolite Furnace

2.1.9 Analytical Balance

An analytical balance (FR-200, NDO-450 ND, Japan) was used to take all measureme (Figure 2.2**).**

Figure 2.2: Analytical Balance

2.1.10 Centrifuge Machine

Centrifugation was carried out in a centrifuge machine (Hanil Science Industrial Co. Ltd. Model-Combi 514 R) during the conductance measurement. A picture of a centrifuge machine shown in Figure 2.3.

Figure 2.3: Centrifuge machine

2.1.11 Homogenizer

It was required to make the vegetable samples homogenous after cleaning and chopping. Using a standard kitchen blender, vegetable samples were homogenized and mixed (Figure 2.4).

Figure 2.4: Homogenizing blender

2.1.12 Oven

Different inorganic compounds, like anhydrous sodium sulfate (Na_2SO_4), were activated by heating at temperatures above 100 °C. To properly clean the glass apparatus, it was heated above 50 °C. All glassware was heated in the oven at 50 °C for about 3 hours (Figure 2.5).

Figure 2.5: Oven

2.1.13 Shaking

All shaking was carried out with the help of a vortex machine (Figure 2.6).

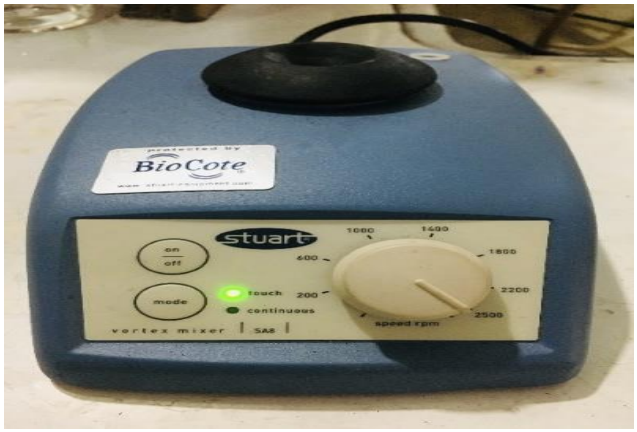


Figure 2.6: Vortex machine

2.1.14 Ultrasonicator Bath

Sonication was used to speed dissolution by breaking intermolecular interactions and to remove dissolved gases from liquid (degassing) (Figure 2.7).

Figure 2.7: Ultrasonicator bath

2.1.15 Freeze Dryer

The samples were dried with a freeze-dryer (Figure 2.8). All freeze-drying was done to remove water and traces of organic solvents. The aqueous samples were first frozen in round-bottomed flasks and finally, the materials were subjected to a freeze-drying operation.

Figure 2.8: Freeze dryer (LABCONCO, USA)

2.1.16 Rotary Vacuum Evaporator

A rotary vacuum evaporator (Heidolph, Germany) was used to evaporate the solvent from the solution under reduced pressure (Figure 2.9). Since many organic compounds are volatile and easily decomposed at high temperatures, evaporation was carried out at 40 ^{o}C under reduced pressure. The temperature of the water bath was not allowed to rise above 40 ^{o}C.

Figure 2.9: Rotary Vacuum Evaporator

2.1.17 Reflux Apparatus

A reflux apparatus (Figure 2.10) was used for the condensation of vapors which then return to the system from which they were vaporized. It is used to produce a reaction at specific temperatures without the loss of solvent. The main purpose of refluxing a solution is to heat a solution in a controlled manner at a constant temperature. All of the refluxes were performed in the apparatus shown below.

Figure 2.10: Reflux apparatus

2.1.18 Filtration

The clean organic solvent extracts were made residual water-free or filtered by adding anhydrous sodium sulfate in a pasture pipette using cotton.

2.2 Preparation of Standard (mixture of organochlorine Pesticides) Solutions

2.2.1 Preparation of Primary Standard Solutions

The primary standard solutions (100 µg/L) were prepared by separately dissolving a standard reference sample (appropriate amount) of twenty standard organochlorine pesticides in n-hexane. The prepared solutions in 100 mL volumetric flasks were labeled, indicating the name of each standard, the concentration, and the date of preparation. The meniscuses of the solutions were marked with permanent black ink and stored in the freezer (-20 °C).

2.2.2 Preparation of Middle and Working Standard Solutions

The primary standard solutions were taken out of the freezer to reach room temperature and checked the meniscus of the layer. These primary standard solutions were diluted as the middle and working standard solutions, respectively. These solutions were labeled, indicating the name of the standard, concentration, and date of preparation. The meniscuses of the solutions were marked with permanent ink and also stored in a freezer (-20 °C) away from the pesticide residue laboratory.

2.2.3 Injection of Standards to GC-ECD

A blank was injected first to check whether the column was clean or not. Afterward, the reference standard solution was injected. The working standard solutions were prepared by serial dilution of the middle standard solution with the solvent. The prepared standard solutions were injected in order to increase their concentration. The peak area was recorded by corresponding to each retention time. The presence of different compositions and pesticides was identified by comparing standard solution chromatograms with sample chromatograms.

2.2.4 Calibration Curves

The working standard solutions were serially diluted and prepared in six different concentrations to obtain standard calibration curves. The calibration curves were linear over the range of the verified concentrations, as shown by the details of the correlation coefficients (r^2) for the linearity. The Codex guidelines recommended an r^2 value of 0.95. The calibration curves were made using Microsoft Excel-2010 software.

2.2.5 Selectivity, Sensitivity and Linearity

Selectivity (or specificity) was assessed by analyzing standard mixtures of pesticides, blank matrices, and blank matrices spiked with the mixture of pesticides simultaneously and by checking their retention times.

The sensitivity of the instruments was assessed by determining the limits of detection (LODs) and limits of quantification (LOQs) for each pesticide in the matrix.

Linearity was also evaluated by constructing calibration curves for each pesticide by injecting a standard mixture to GC-ECD at 5–6 different concentration levels, covering the expected range of pesticides that might be present in the samples.

2.2.6 Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is the smallest concentration from which the presence of an analyte can be deduced, and the limit of quantification (LOQ) is the lowest concentration from which it is possible to quantify the analyte with a reasonable degree of statistical certainty (Bernal, 2014). The limit of detection (LOD) was determined by injecting serially diluted mixtures of standard OCPs solution in GC-ECD. For LOD, the peak area of each standard was considered 3 times higher than the base line noise i.e., signal to noise ratio was 3:1, whereas the limits of quantification (LOQ) were determined with a signal-to-noise ratio of ten.

2.2.7 Identification and quantification of Organochlorine Pesticides (OCPs) by GC-ECD

The reference standard solution was injected into the GC-ECD and, under the same conditions, a cleaned extract of samples was also injected. By comparing the retention times (retention times of standards and unknown known areas to be the same under identical analytical conditions) of the different peaks of the sample with the retention times of the standard compounds, residue present in the samples was identified. The number of unknown analytes in the respective samples was quantified from the standard calibration curve prepared from standard working solutions.

The quantification of the residual amount of OCPs was carried out by using reference standard solutions. The peaks of OCPs were obtained at a specific retention time. By comparing the retention time of the peak present in the sample chromatogram with the chromatogram of 20 standard OCPs, the OCPs present in that particular sample were identified. Quantitative determination was carried out by comparing the peak area of each OCPs in the sample extract with

that of standard solutions. From the calibration curve, the amount of each OCPs present in the sample extract was calculated using the following formula.

$$y = mx + c$$

Where,

y= Peak area

x = Concentration of OCPs solution

m = Slope of calibration curve

c = Intercept in Y- axis

2.2.8 Control

For recovery experiments, control or untreated samples were used, which were previously confirmed to have no pesticide residue. Three control samples were spiked with a known amount of pesticides followed by a respective extraction and clean-up procedure to determine the matrix effect under the analysis method. Reagent blank was done following the same extraction procedure and cleaned up method, using only solvent and reagents (in the absence of sample) to make the analysis rational.

2.2.9 Spiking and Recovery Experiment

The recovery experiments were conducted on uncontaminated control samples by spiking the samples at three replicates at two or three concentration levels. The spiked samples were permitted to equilibrate for 3–4 h before extraction, to allow the pesticide to penetrate the matrix. Then the spiked samples were subsequently processed by following the respective extraction and clean-up procedures. The recovery of each analyte was calculated according to:

$$\textbf{Recovery (\%)} = \frac{\mathbf{C_{spiked\ sample} - C_{unspiked\ sample}}}{\mathbf{C_{added\ standard}}} \text{ X } 100\%$$

2.3 Analysis of Vegetable Samples

2.3.1 Sampling Site

Fresh vegetable samples were collected from the local markets of Hatirpool Bazar, Kaptan Bazar of Dhaka District and Shibpur Bazar, College gate Bazar of Narsingdi District (Figure 2.11). Vegetables were selected according to their availability, accessibility, and demand of consumers in the local market places.

2.3.2 Sample Collections

Five different types of fresh vegetables such as pointed gourd, bitter gourd, tomato, green chili, and cauliflower were collected for this research work. All these vegetable samples are the most commercially used for local consumption. After collection, all samples were kept in a chilled box and transported to the laboratory as soon as possible.

The identification details of each sample including common Bangla name, English name, scientific name and family name are shown in Table 2.1. Pictorial (Figure 2.12) views of these vegetables are shown below-

Pointed gourd (*Trichosanthes dioica*)

Bitter gourd (*Momordica charantia*)

Tomato (*Solanum lycopersicum*)

Green chili (*Capsicum frutescens*)

Cauliflower (*Brassica oleracea*)

Figure 2.11: Collected fresh vegetable samples

Figure 2.12: Sample Collection from Narsingdi, Shibpur Market

Table 2.1: Names and nature of different fresh vegetable samples

Local Name	English Name	Scientific Name	Family
Potol	Pointed gourd	*Trichosanthes dioica*	Cucurbitaceae
Korola	Bitter gourd	*Momordica charantia*	Cucurbitaceae
Tomato	Tomato	*Solanum lycopersicum*	Solanaceae
Kacha morich	Green chili	*Capsicum frutescens*	Solanaceae
Phulcopi	Cauliflower	*Brassica oleracea var. Botrytis. Preferred*	Brassicaceae

Table 2.2: List of Collected Vegetable samples

Hatirpool Bazar 25.08.2020	Kaptan Bazar 10.10.2020	Narsingdi (Shibpur Bazar) 07.12.2020	Narsingdi (College gate Bazar) 07.12.2020
PH-1A	PK-2A	PS-3A	PC-4A
PH-1B	PK-2B	PS-3B	PC-4B
PH-1C	PK-2C	PS-3C	PC-4C
KRH-1A	KRK-2A	KRS-3A	KRC-4A
KRH-1B	KRK-2B	KRS-3B	KRC-4B
KRH-1C	KRK-2C	KRS-3C	KRC-4C
TH-1A	TK-2A	TS-3A	TC-4A
TH-1B	TK-2B	TS-3B	TC-4B
TH-1C	TK-2C	TS-3C	TC-4C
KMH-1A	KMK-2A	KMS-3A	KMC-4A
KMH-1B	KMK-2B	KMS-3B	KMC-4B
KMH-1C	KMK-2C	KMS-3C	KMC-4C
CH-1A	CK-2A	CS-3A	CC-4A
CH-1B	CK-2B	CS-3B	CC-4B
CH-1C	CK-2C	CS-3C	CC-4C

Note: P-Pointed gourd; KR-Bitter gourd; T-Tomato; KM- Chili and C- Cauliflower

2.3.3 Sample Storage

The samples were taken into a labeled zip-locked bag and and labeled. In the laboratory, the samples were kept in freezer at -20 ^{0}C until extraction.

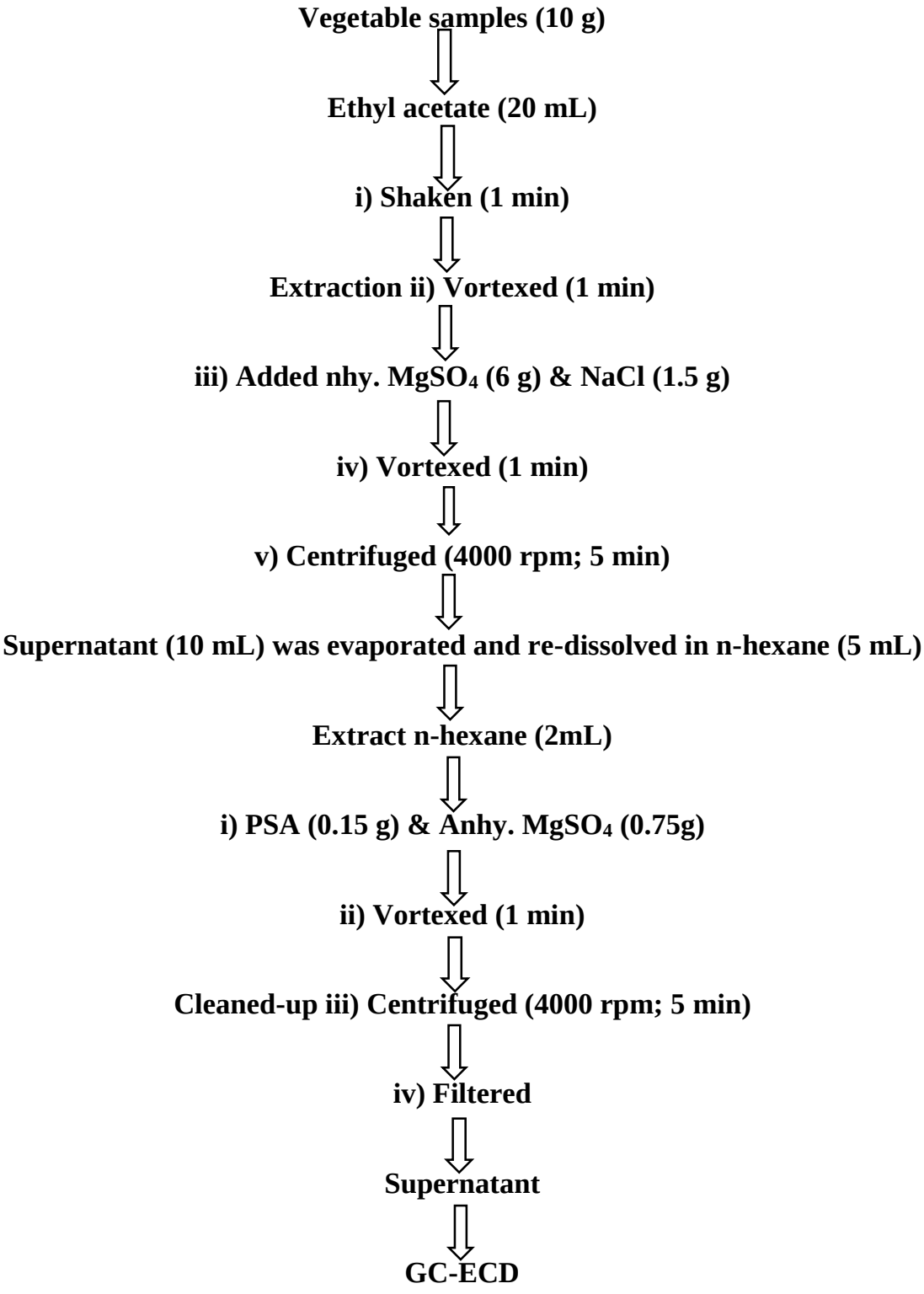
2.3.4 Preservation of Samples

The samples were chopped and thoroughly blended in a normal kitchen blender to obtain a homogeneous sample for weighing and storing in a refrigerator at 0 °C for the estimation of pesticide residues. The same but different amounts of samples were dried and a kitchen blender was used to make powder and stored at room temperature for the determination of fatty acids, total carbohydrates, phenolic content, flavonoid content, antioxidant capacity, and heavy metal analysis.

2.3.5 Extraction and Clean-Up process

In a 50 mL screw cup Teflon tube, 10 g of homogenized vegetable samples were taken, and ethyl acetate (20 mL) was added. The content was shaken for 1 minute in hand and vortexed for 1 minute. Anhydrous $MgSO_4$ (6 g) and NaCl (1.5 g) were added and vortexed for another 1 min and then centrifuged for 5 minutes at 4000 rpm. The supernatant (10 mL) was transferred to a round-bottom flask (100 mL), evaporated to dryness, and reconstituted in n-hexane (5 mL).

The extracted sample (2 mL) was taken in a screw test tube (10 mL), then primary secondary amine (PSA) (0.15 g) and anhydrous $MgSO_4$ (0.75 g) were added to it. The content was vortexed for another 1 min and then centrifuged for 5 minutes at 4000 rpm. The supernatant was passed through a cotton filter and transferred to a clean GC vial and finally analyzed by GC-ECD (Scheme-1).



Scheme 1: Extraction and cleaned-up method of vegetable samples

2.3.6 Analytical Condition of GC-ECD

Gas chromatograph equipped with a Ni Electron Capture Detector (ECD) (Figure 2.13) was used for the determination of pesticide residues in the pointed gourd, bitter gourd, tomato, chili, and cauliflower samples. A non-polar (HP-5 MS) capillary column of 30 m long x 250 m i.d. x 0.25 μm film thickness from Agilent, USA was used to carry out the separation. Nitrogen was used as both a carrier (column flow of 1.92 mL/min.) and makeup gas. The injector and detector temperatures were 200 °C and 300 °C, respectively. All injections were made in split-less mode, and the injection volume was 1μL. The oven temperature was programmed at 150 °C for 2 minutes, then increase at 10 °C per minute to 295 °C for 4 minutes. Identification of residues was achieved by running samples and external reference standards in GC and then comparing the corresponding retention times. All parameters used in GC-ECD (Figure 2.13) is given below:

Parameter	Condition	
Injection volume	1.0 μL	
Injection mode	Splitless mode	
Injector temperature	200 °C	
Carrier gas	N_2	
Column flow	1.0 mL/min	
Make up gas	N_2	
Detector temperature	300 °C	
Column oven temperature program		
Rate, (°C/minute)	Temperature, (°C)	Hold time (minute)
	Initial temp. 150 °C	2 min
5.0 °C	Final temp. 295 °C	4 min
Total program time (minute)	35 min	

Figure 2.13: Gas Chromatograph (SHIMADZU-2030) with electron capture detector

2.4 Moisture Content

Moisture content was determined according to the standard AOAC (2005) method, which is described in the following steps:

(i) The empty porcelain crucible and lid were dried in the oven at 105 ^{o}C and transferred to a desiccator to cool, and the weight of the empty crucible and lid was recorded.

(ii) 2 g of each sample was taken into different crucibles.

(iii) The crucibles containing the samples were placed in the oven and dried for 4 hours at 105 ^{o}C.

(iv) After drying, the dishes with partially covered lids were transferred to the desiccator for cooling. The crucibles with dried samples were reweighed.

The moisture content was calculated by following formula:

$$\textbf{Moisture content (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

2.5 Ash Content

Ash content was determined according to the standard AOAC (2005) method, which is described below:

(i) The empty porcelain crucible and lid were dried in the oven at 105 ^{o}C and transferred to a desiccator to cool, and the weight of the empty crucible was recorded.

(ii) About 2 g of each sample was taken into different crucibles.

(iii) The crucibles with samples were placed in the carbolite furnace and burnt for 4 hours at 700 ^{0}C.

(iv) After drying, the dishes with partially covered lids were transferred to the desiccator for cooling. The crucibles with cooked and samples were reweighed.

The ash content was then calculated using the following formula:

$$\textbf{Ash content (\%)} = \frac{\text{Weight of sample before ashing} - \text{Weight of sample after ashing}}{\text{Weight of sample before ashing}} \times 100$$

2.6 Analysis of Fatty Acids by GC-FID

Extraction Process

Five different freeze-dried samples were extracted with 100 mL n-hexane. The dried extract of pointed gourd (P), bitter gourd (KR), tomato (T), green chili (KM) and cauliflower (C) were collected 50, 40, 20, 100 and 100 mg respectively. Taking these amounts for individual samples into a 100 mL round bottom flask. The flask was vortexed after adding 0.5M of alcoholic sodium hydroxide. Then it was refluxed and evaporated to dryness. 5 mL of water was then added and acidified with dilute HCl (0.5 M). All the materials were transferred into a separating funnel (100 mL) and partitioned with n-hexane. Discarding the lower (aqueous) part, the organic phase was

treated with anhydrous Na_2SO_4 and transferred into a pear-shaped flask and then pear-shaped to dry. A 1.5 mL BF_3 methanol complex was added to the dried material and refluxed in a boiling water bath. It was evaporated to dryness and n-hexane was added to transfer it into a GC vial for analysis by Shimadzu GC-2025 (GC-FID).

Table 2.3: Retention time of standard fatty acids

Standard Fatty Acid	Retention Time (minute)	Chemical type
Octanoic acid	3.45	Saturated
Decanoic acid	6.91	Saturated
Lauric acid	11.36	Saturated
Tetradecanoic acid	17.77	Saturated
Palmitic acid	24.89	Saturated
Palmitoleic acid	25.83	Unsaturated
Octadecanoic acid	32.98	Saturated
Cis-9-oleic acid	33.27	Unsaturated
Linoleic acid	34.48	Unsaturated
Arachidic acid	43.03	Saturated
Linolenic acid	49.96	Unsaturated
Docosanoic acid	51.12	Saturated
Erucic acid	58.71	Unsaturated

A Gas Chromatograph having Flame Ionization Detector (FID) was used for identification and quantification of fatty acids (Figure: 2.14). Separations were performed on WCOT quartz capillary (DB-5) column (30 m in length and 0.25 mm in diameter). The temperature program in the oven was as followed: 120 ^oC for 1 min (hold) then increased by 7 ^oC/min to 280 ^oC and again hold for 6 minute. N_2 was used as carrier gas with a column flow rate of 2 mL/minute. Air and hydrogen gases were used as fuel for FID. The injector temperature was 280 ^oC, and the detector temperature was 290 ^oC. Inject volume: 1 µL.

Figure 2.14: Gas Chromatograph (SHIMADZU-2025) with Flame Ionization Detector (FID)

GC-FID analysis conditions:

Injection volume: 1.0 µL

Injection mode: Splitless/split mode

Spit ratio: 1:80

Injector temperature: 275 ^{o}C

Career gas: N_2

Gas for flame: H_2 and air

Column flow: 1.78 mL/min

Detector temperature: 285 ^{o}C

Program: 28 min

Table 2.4: Table for the condition of analysis in GC-FID

Column oven temperature program Rate, ℃/minute	**Temperature, ℃**	**Hold time (minute)**
-	120.0	1.0
7 ^{o}C	280	6.0

2.7 Analysis of Protein in Vegetable Samples

The protein content was analyzed by the Kjeldhal method which involves three steps:

Sample Preparation

Digestion:

Place 1.0 g of the homogenized and dried sample in a VELP 100 mL micro digestion tube. Fill the tube with 5.0 g of K_2SO_4 and 0.1 g of $CuSO_4$.To begin, combine 5.0 mL of conc. H_2SO_4 (97%) and 1.0 mL of H_2O_2. Insert the digestion tube into the VELP digestion system hole, connect the exhaust system, and turn on the exhaust system first, then the digestion system, gradually increasing the temperature up to 420 ^oC. After completion of digestion, a blue-colored clear solution will appear. After digestion, cool the solution to room temperature.

Distillation:

Check all reagent container connections and water supply to the system, and then turn on the system and wash the distillation system first. Put the digested solution in the VELP distillation tube and then place the tube in position and place the conical flask containing 50 mL of standardized 0.1N H_2SO_4 and methyl orange indicator. Load the desired program and run the system. After completion of distillation, take the conical flask for titration.

2.7.1 Analytical Procedure

Standardization of H_2SO_4:

Take 10 mL of 0.1N Na_2CO_3 with a pipette in a clean conical flask. Pour 0.1N H_2SO_4 into the burette. Add 5-7 drops of methyl orange indicator in the conical flask. Titrate the 0.1N H_2SO_4 against 0.1N Na_2CO_3 to an endpoint of light yellow to orange. Repeat the process at least three times.

(b) Standardization of NaOH

Take 10 mL of standardized 0.1N H_2SO_4 with a pipette in a clean conical flask. Fill the burette with 0.1N NaOH. Add 5-7 drops of methyl orange indicator in the conical flask. Titrate the 0.1N NaOH against 0.1N H_2SO_4 to an endpoint of orange to light yellow. Repeat the process at least three times.

(c) Titration of excess H_2SO_4

After completion of the distillation, the conical flask is taken for titration of excess H_2SO_4 (after capturing NH_3). Fill the burette with 0.1N NaOH and titrate the excess H_2SO_4 against 0.1N NaOH to an endpoint of orange to light yellow. Repeat the process at least three times.

The concentration of protein is

[(mL standard acid x acid normality)-(mL standard base x base normality)] x 1.4007 x 6.25/weight of sample in grams.

The conversion factor for vegetable nitrogen to protein is 6.25.

2.8 Determination of Total Phenolic Content

The total phenolics were determined by the modified folin-ciocalteu method (Wolfe et al., 2003). 1 mL of different methanol extracts of pointed gourd, bitter gourd, tomato, chili, and cauliflower were taken separately in different test tubes, then added 5 mL of folin-ciocalteu's reagent (1: 10 v/v distilled water) and 4 mL of sodium carbonate solution. The solutions were then vortexed for 15 seconds for proper mixing and allowed to stand for 30 min at 40 ^{0}C for color development. After 30 minutes of reaction, absorbance was measured against the blank in a double beam UV/Visible spectrophotometer (UV-1800), at an absorption maximum of 765 nm. Three readings were taken for each experimental sample to get reproducible results. The total phenolic content was determined and expressed as mg gallic acid equivalents per gram of dry extract using the equation obtained from a standard gallic acid calibration curve, $y = 0.0137x + 0.0611$, $r^2 = 0.999$.

2.9 Determination of Total Flavonoid Content

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the extracts (Chang et al., 2002). 5 mL of each extract was individually mixed with 2.5 mL of aluminum trichloride ($AlCl_3$) solution. They were allowed to stand for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 430 nm with a double beam UV-visible spectrophotometer. The total flavonoid content was determined as mg of quercetin equivalent per gram using the equation obtained from a standard quercetin calibration curve $y = 0.0048x + 0.0204$; $r^2 = 0.994$.

2.10 Determination of Total Antioxidant Capacity

The total antioxidant capacity of sample extracts was evaluated by the phosphomolybdenum assay method (Prieto et al., 1999), which is based on the reduction of (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex in acidic conditions. 0.6 mL of each extract was allowed to mix with 6.0 mL of the reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate). This reaction mixture was incubated at 95 C for 90 min. The absorbance was measured at 695 nm using a spectrophotometer against a blank solution. The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from a standard ascorbic acid calibration curve, y = 0.0041x-0.0092, $r^2 = 0.998$.

2.11 Total Carbohydrates Analysis

2.11.1 Preparation of Standard Solution

Ten mg of analytical grade glucose was weighed and put into a 10 mL volumetric flask. After preparing the standard solution, various dilutions were made by pipetting from a known volume of the standard solution. Different concentrations of solutions, such as 0.020, 0.040, 0.050, 0.080, 0.10, 0.150, and 0.20 mg/mL were prepared. For each experiment, 500 µL of standard solution was taken and 50 µL of 80% phenol was added and vortexed for 30 sec. Then, 3 mL of 98% H_2SO_4 was added. A reddish brown colored solution was obtained and the absorbance was measured at 488 nm.

2.11.2 Modified Molisch Test

An aqueous phenol (80%) solution was prepared by mixing phenol (8 g) with water (10 mL). On shaking, the mixture became homogeneous. It was stored in a stoppered bottle and was employed for the test. 500 µL sample was taken in a test tube. Aqueous phenol (80%, 50 µL) was added with it and mixed well by a vortex for 30 sec. Concentrated H_2SO_4 (98%, 3 mL) was added to the solution and it was mixed thoroughly by a vortex for 30 sec. The development of a reddish M-

brown colour indicated the presence of carbohydrates. A UV spectrophotometer was used to measure the absorbance of colored solutions.

2.11.3 Sample Preparation

The sample (1.5 g) was taken in a test tube and 10 mL of water was added to it. Then it was kept at room temperature for 24 hours. Then the extract was filtered. This aqueous extract was diluted. A 20 µL extract was taken in a total of 2000 µL volume of water. Then, for each run, a 500 µL solution was used. Absorbance of the solution was taken at a wavelength of 488 nm. By using the standard calibration curve of D-glucose, the amount of carbohydrate in each sample was determined (Figure 2.15).

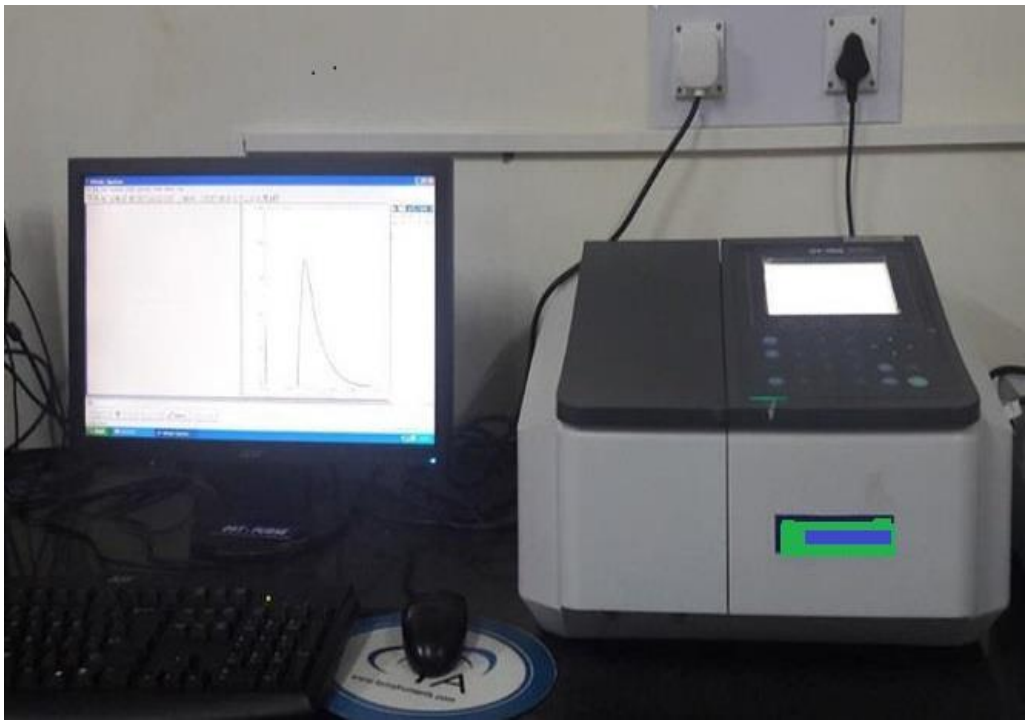


Figure 2.15: UV-Visible spectrophotometer

2.12 Micronutrient Analysis

2.12.1 Sample Preparation and Digestion Procedure for Micronutrient Analysis

The vegetable samples were ground homogenously by the blender. The blended samples were weighed and freeze dried by a freeze dryer. All freeze-drying was done to remove water and traces of organic solvents. The aqueous samples were first frozen in round-bottomed flasks in a freezer below -18 °C and finally the materials were subjected to a freeze-drying operation. The dried samples were ready for further digestion.

Heavy metals Zn, Fe, Mn, Ca, Cd, Cr, Cu, Ni and Pb were analyzed in a flame atomizer based Atomic Absorption Spectrometer using a hollow cathode lamp as a radiation source 5.0 g of dried vegetable powder was placed in a silica crucible and placed in a muffle furnace at 700 ^{0}C for 4 hours. The sample was then cooled down to room temperature and the heating process was repeated three times. The ash was then dissolved by adding concentrated HNO_3 and filling up the volumetric flask with deionized water. Then the sample was prepared to analyze minerals with the Atomic Absorption Spectrophotometer (AAS) (Figure 2.16).

Figure 2.16: Atomic Absorption Spectrophotometer

3. RESULTS AND DISCUSSION

3.1 Analysis of Standards by GC-ECD

The working standard solutions were prepared by serial dilution of a mixture of twenty organochlorine pesticides of alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan-I, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, endosulfan-II, endrin aldehyde, 4,4'-DDT, endosulfan sulfate, methoxychlor and endrin ketone with n-hexane. For the formation of the calibration curves, all the standard solutions were prepared over the concentration range of 5, 10, 25, 50, 100 and 200 ng/g of working standard and injected into the GC-ECD with increasing concentration following the GC-ECD analytical conditions as described in section 2.3.6. Retention time and corresponding peak area were recorded. The standard calibration curve in GC-ECD was constructed by plotting the integrated areas under the peaks against the concentration of standard using Microsoft Office Excel 2016 software. From the calibration curve, the OCPs present in the sample were calculated. The calibration curves are presented in Figures 3.1-3.10. The repeatability of the retention times was less than 15 for 6 injections. The standard solution was left outside of the refrigerator to reach room temperature before being injected into the GC-ECD. Then a definite amount of the solution was withdrawn after checking the meniscus mark and putting a new mark after the withdrawal. The withdrawn solution was transferred into a screw cap test tube with PTFE lining, and a definite volume of n-hexane was added to prepare working standard solutions of a definite concentration.

Table 3.1: The concentration of standard alpha-BHC and gamma-BHC solutions and their relative peak areas

Concentration of alpha-BHC (ng/g)	Peak area	Concentration of gamma-BHC (ng/g)	Peak area
5	5303	5	19428
10	4008	10	15170
25	8591	25	23483
50	14038	50	43319
100	30671	100	99183
200	46198	200	160821

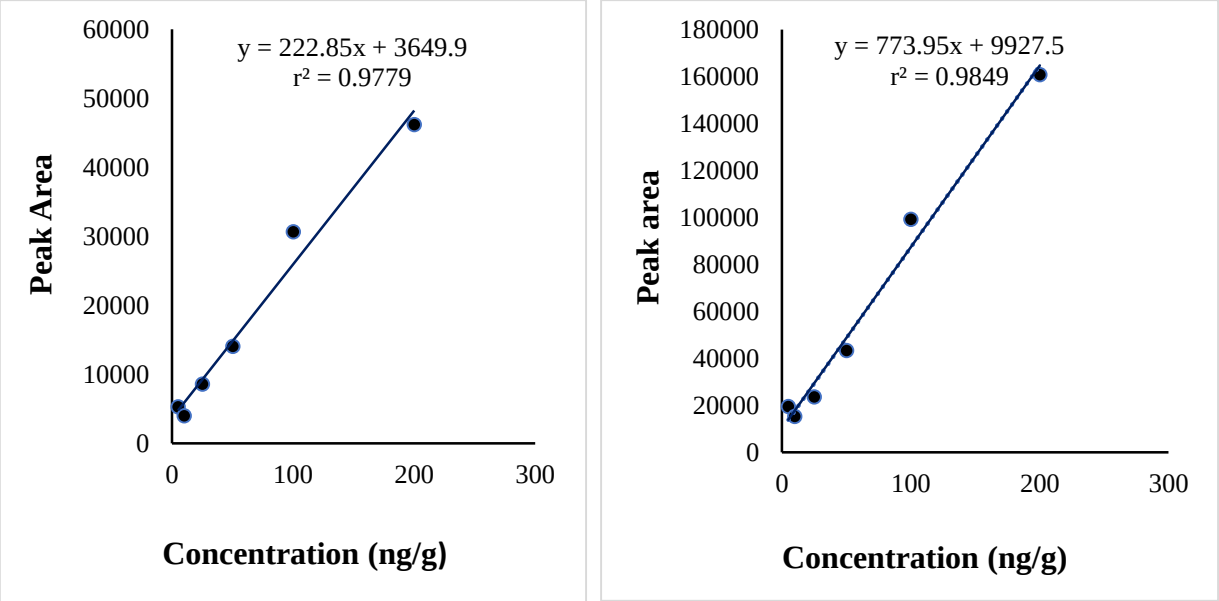


Figure 3.1: Calibration curves of alpha-BHC (left) and gamma-BHC (right)

Table 3.2: The concentration of standard beta-BHC and delta-BHC solutions and their relative peak areas

Concentration of Standard belta-BHC(ng/g)	Peak Area	Concentration of Standard delta-BHC (ng/g)	Peak Area
5	9181	5	30392
10	16759	10	34644
25	163603	25	89529
50	314568	50	191490
100	544497	100	400913
200	738989	200	645707

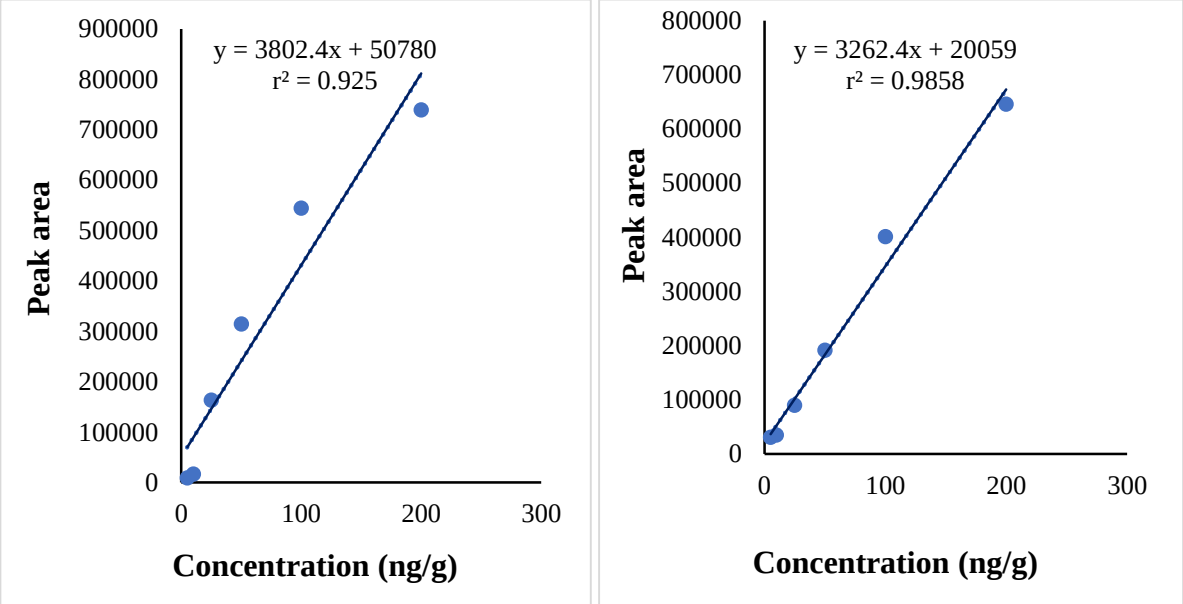


Figure 3.2: Calibration curves of beta-BHC (left) and delta-BHC (right)

Table 3.3: The concentration of standard heptachlor and aldrin solutions and their relative peak areas

Concentration of Standard Heptachlor (ng/g)	Peak Area	Concentration of Standard Aldrin (ng/g)	Peak Area
5	15005	5	27650
10	15648	10	26843
25	23770	25	43193
50	44456	50	82566
100	95326	100	165247
200	149753	200	262147

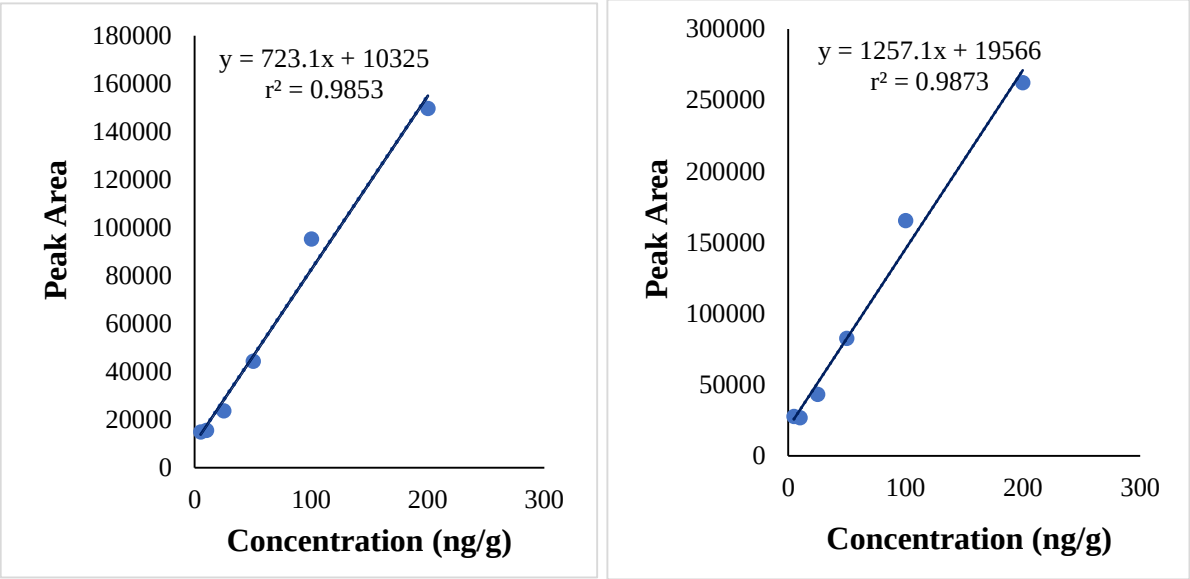


Figure 3.3: Calibration curves of heptachlor (left) and aldrin (right)

Table 3.4: The concentration of standard endrin and 4,4'-DDD solutions and their relative peak areas

Concentration of Standard Endrin (ng/g)	Peak area	Concentration of Standard 4,4'-DDD (ng/g)	Peak area
5	29893	5	81705
10	31038	10	103530
25	157059	25	221890
50	301202	50	396478
100	547928	100	679099
200	787645	200	971374

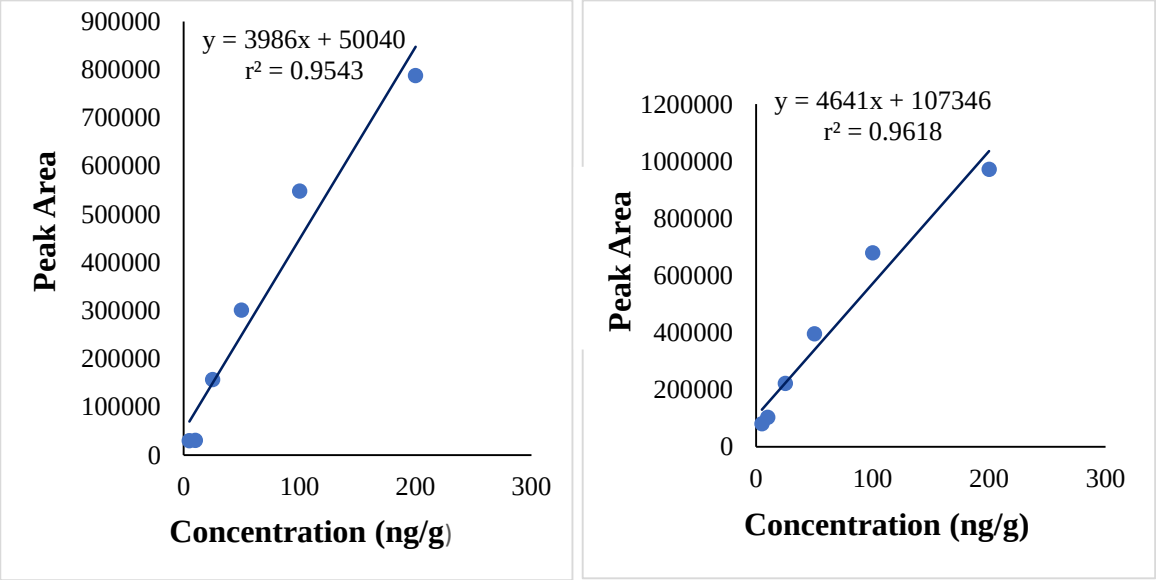


Figure 3.4: Calibration curves of endrin (left) and 4,4'-DDD (right)

Table 3.5: The concentration of standard endosulfan-II and endrin aldehyde solutions and their relative peak areas

Concentration of Endosulfan-ll (ng/g)	Peak area	Concentration of Endrin aldehyde (ng/g)	Peak area
5	51428	5	68803
10	75816	10	90895
25	224094	25	134927
50	400086	50	287269
100	692547	100	528013
200	988872	200	821837

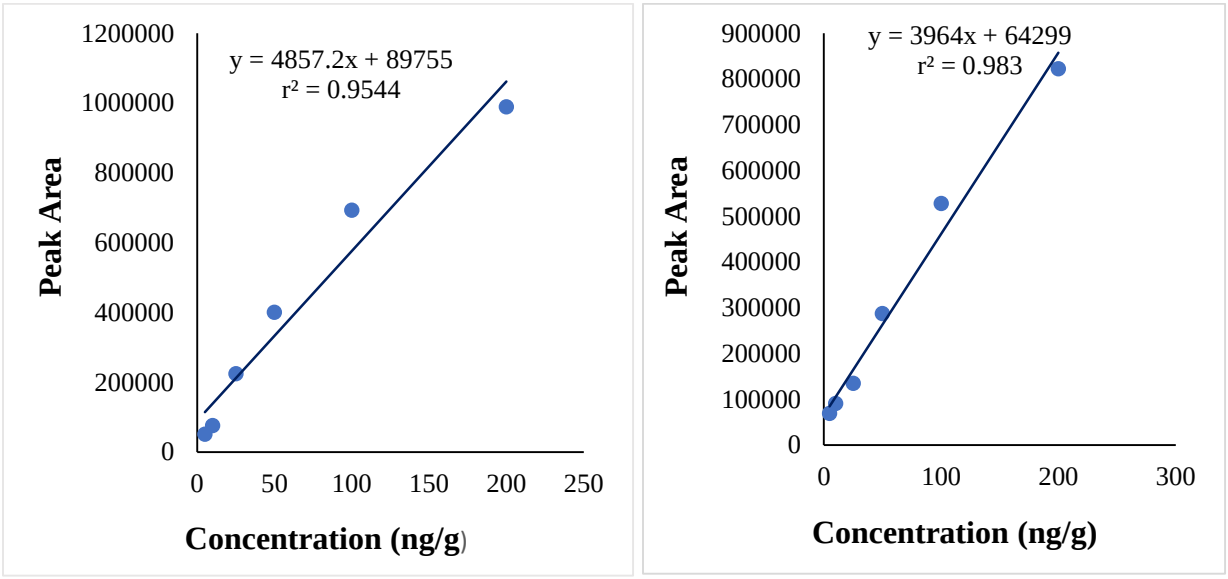


Figure 3.5: Calibration curves of endosulfan-II (left) and endrin aldehyde (right)

Table 3.6: The concentration of standard 4,4'-DDT and endosulfan sulfate solutions and their relative peak areas

Concentration of 4,4-DDT (ng/g)	Peak area	Concentration of Endosulfan sulfate (ng/g)	Peak area
5	7027	5	40129
10	9712	10	60115
25	40889	25	162556
50	77432	50	302109
100	137226	100	525668
200	229706	200	782737

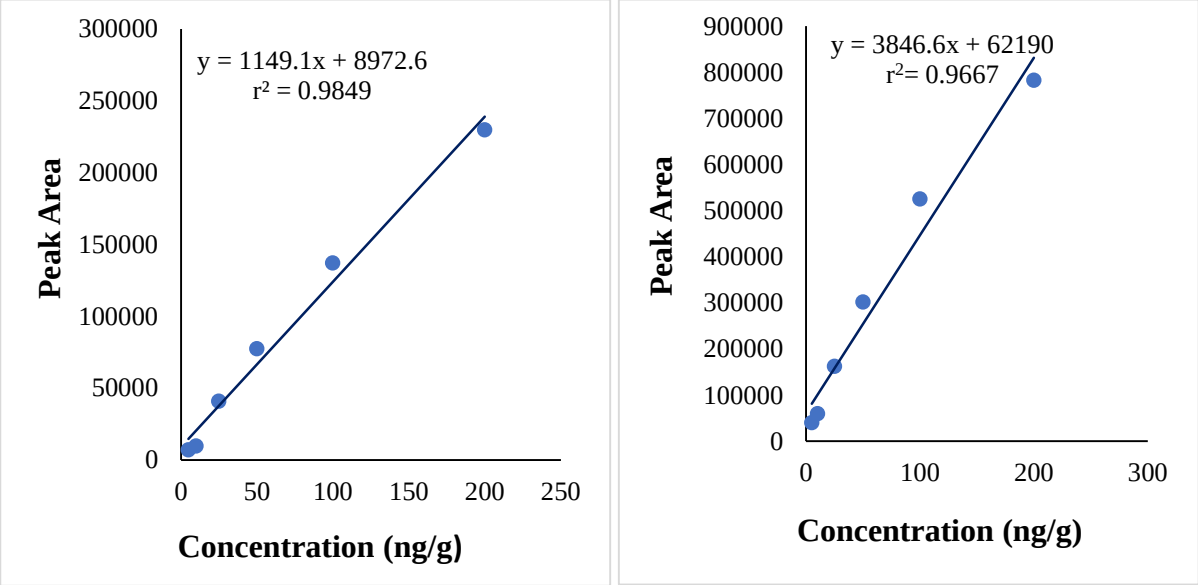
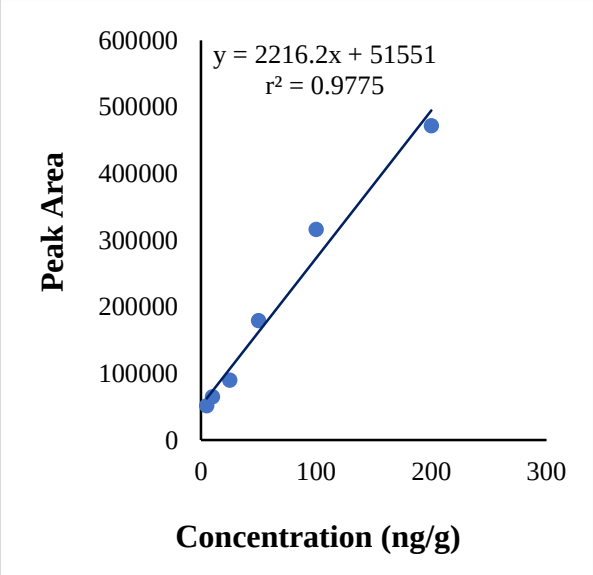


Figure 3.6: Calibration curves of 4,4'-DDT (left) and endosulfan sulfate (right)

Table 3.7: The concentration of standard methoxychlor and endrin ketone solutions and their relative peak areas

Concentration of methoxychlor (ng/g)	Peak area	Concentration of endrin ketone (ng/g)	Peak area
5	51581	5	32165
10	64888	10	50050
25	89742	25	154719
50	179303	50	298959
100	316190	100	520927
200	471907	200	771106



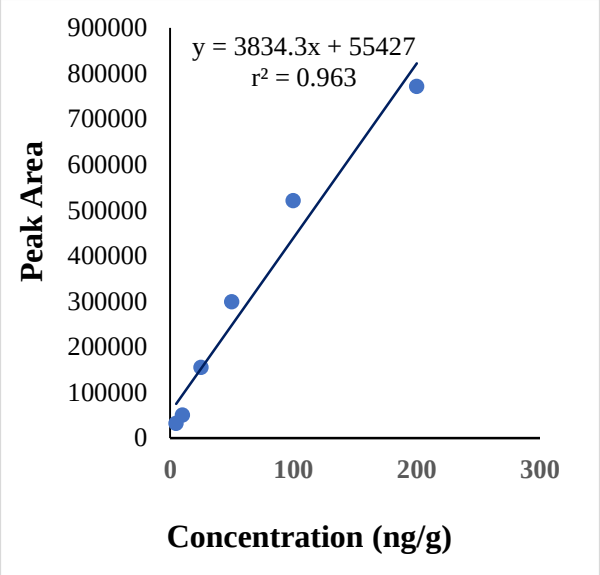


Figure 3.7: Calibration curves of methoxychlor (left) and endrin ketone (right)

3.2 Limit of Detection (LOD) and Limit of Quantification (LOQ) (Method Validation)

To make standard calibration curves of twenty organochlorine pesticides containing 4,4'-DDT, 4,4'-DDE, 4,4'-DDD, BHC (α, β, γ, and δ), heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, methoxychlor, endosulfan (I and II), endosulfan sulfate and chlordane (*cis* and *trans*) were serially diluted to obtain 6 different concentrations. The linearity of the method was well demonstrated with an r^2 (regression coefficient) having a value in the range of 0.919-0.987 (Table: 3.8), which is higher than the value recommended by the Codex guideline (r^2 = 0.95). To elucidate the sensitivity of the experiment, the limit of detection (LOD) and the limit of quantification (LOQ) were determined. The LOD of twenty pesticides was found to be ranging from 0.00001 to 0.0013 ng/g and the LOQ was found to be ranging from 0.00003 to 0.0134 ng/g.

Table 3.8: Name, Linear Regression Equation, and Linearity (r^2) of Different Pesticides

PESTICIDE	Retention time	Linear Regression Equation	Correlation Coefficients(r^2)	LOD (ng/g)	LOQ (ng/g)
Alpha-BHC	14.24	Y 222.85x+3649.9	0.977	0.0013	0.0134
Gamma-BHC	15.82	Y =773.95x+9927.5	0.984	0.0004	0.0034
Beta-BHC	16.19	Y =3802.4x+50780	0.925	0.00001	0.00003
Delta-BHC	17.45	Y =3262.4x+20059	0.985	0.0002	0.0018
Hepachlor	17.69	Y =723.1x+10325	0.985	0.0004	0.0044
Aldrin	19.05	Y =1257.1x+19566	0.987	0.0009	0.0088
Heptachlor epoxide	21.41	Y =5279.1x+94649	0.956	0.00004	0.0004
Trans-Chlordane	22.17	Y =5130.3x+75159	0.949	0.00003	0.0003
Cis-Chlordane	22.75	Y =5869.1x+105562	0.951	0.00001	0.0001
Endosulfan I	22.97	Y =4492.9x+199183	0.947	0.00002	0.0002
4,4′-DDE	23.43	Y=6612.7x+131546	0.958	0.0001	0.0007
Dieldrin	24.06	Y=4336.7x+309793	0.919	0.0002	0.0016
Endrin	25.25	Y = 3986x+50040	0.954	0.0001	0.0008
4,4′-DDD	25.67	Y =4641x+107346	0.961	0.0001	0.0006
Endosulfan II	26.07	Y =4857.2x+89755	0.954	0.00002	0.0002
Endrin aldehyde	26.89	Y =3964x+64299	0.983	0.0005	0.0046
4,4′-DDT	27.39	Y =1149.1x+8972.6	0.984	0.00102	0.01020
Endosulfan Sulfate	28.46	Y =3846.6x+62190	0.966	0.00002	0.0002
Methoxychlor	29.52	Y =2216.2x+51551	0.977	0.0011	0.0107
Endrin Ketone	30.54	Y = 3834.3x+55427	0.963	0.00004	0.0004

3.3 Linearity

For the construction of the calibration curve, the solution of the mixture of 20 OCP standards of alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan-I, 4,4′-DDE, dieldrin, endrin, 4,4′-DDD, endosulfan-II, endrin aldehyde, 4,4′-DDT, endosulfan sulfate, methoxychlor and endrin ketone was prepared with n-hexane over a concentration range of 5–200 ng/g. The calibration curve was prepared by plotting the concentration of the calibration standards on the X-axis and the corresponding peak area on the Y-axis. The calibration curve was obtained for the mixture of 20 OCP standards using the Microsoft Office Excel 2016 program, which was linear with a correlation coefficient, of $r^2 \leq 0.99$. The residual concentrations of r^2 ranged from 0.919–0.987 in the spiked and market samples.

3.4 Recovery of the Standard Pesticides from Control Vegetable Samples

The extraction efficiency was assessed by doing recovery experiments before analyzing pointed gourd, bitter gourd, tomato, chili, and cauliflower samples. The recovery experiments were performed in three replicates at two fortified concentrations. The recovery experiment was carried out using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEchERS) method as described in Methodology (Scheme-1). The sample extract was analyzed by GC-ECD following the same analytical conditions as the standard. Retention time and corresponding peak area were recorded. The recovery was calculated using the standard calibration curve. The result is shown in the Tables 3.9.1-3.9.5**.** The percent recovery of pesticide from the pointed gourd, bitter gourd, tomato, chili and cauliflower samples revealed that the extraction method was acceptable. Results of recoveries indicate that the QuEchERS method would be a good alternative than the traditional solvent-solvent extraction method because the former is very effective for pre-concentration and clean-up of environmental samples.

Table 3.9.1: Data of the Recovery experiment from pointed gourd

Pesticides	Spiking Level (ng/g)	Mean Recovery (%)	RSD (%)
alpha-BHC	25	84.11	3.13
	50	83.81	8.82
gamma-BHC	5	97.72	4.28
	10	120.11	5.00
beta-BHC	5	95.47	2.82
	10	73.91	4.24
delta-BHC	5	101.22	2.66
	10	86.36	9.12
heptachlor	5	70.02	2.57
	10	73.08	1.17
aldrin	100	70.69	8.00
	200	74.59	3.59
heptachlor epoxide	5	96.06	4.30
	10	80.87	8.03
trans-chlordane	10	115.77	3.72
	25	104.48	10.23
cis-chlordane	10	94.00	6.94
	25	91.96	7.07
endosulfan-I	25	103.96	3.88
	50	89.86	1.57
4,4'-DDE	5	91.66	3.68
	10	94.11	1.92
dieldrin	25	72.41	4.28
	50	82.16	0.48
endrin	5	82.83	5.59
	10	78.63	6.63
4,4'-DDD	5	72.92	0.24
	10	103.06	2.42
endosulfan-II	5	82.49	0.52
	10	94.47	2.56
4,4'-DDT	10	80.01	5.43
	25	73.43	3.96
endosulfan sulfate	5	86.08	7.15
	10	79.57	3.52
methoxychlor	5	105.23	3.93
	10	119.71	5.20
endrin ketone	5	76.24	5.01
	10	85.24	4.10

Table 3.9.2: Data of the Recovery experiment from Bitter gourd

Pesticides	Spiking Level (ng/g)	Mean Recovery (%)	RSD (%)
gamma-BHC	25	116.86	9.69
	50	99.87	6.53
beta-BHC	5	100.97	0.13
	10	76.81	6.22
delta-BHC	5	92.76	0.08
	10	103.89	2.96
heptachlor	5	117.74	5.42
	10	90.04	6.15
aldrin	50	86.07	5.15
	100	113.02	6.58
heptachlor epoxide	5	99.34	6.69
	10	79.91	2.94
trans-chlordane	10	73.06	10.94
	25	70.04	9.93
cis-chlordane	5	96.63	3.52
	10	81.64	0.61
endosulfan-I	25	73.36	7.12
	50	93.46	0.50
4,4'-DDE	5	73.93	7.04
	10	80.58	2.20
dieldrin	25	91.51	0.54
	50	71.23	0.94
endrin	5	89.04	0.83
	25	76.73	0.57
4,4'-DDD	5	88.52	11.94
	10	101.38	11.43
endosulfan-II	5	86.63	3.11
	10	70.61	4.35
4,4'-DDT	5	78.97	7.21
	10	116.85	6.69
endosulfan sulfate	5	72.86	7.84
	10	101.10	2.54
methoxychlor	5	119.35	5.40
	10	94.41	3.01
endrin ketone	5	117.96	6.28
	10	87.51	0.43

Table 3.9.3: Data of the Recovery experiment from Tomato

Pesticides	Spiking Level (ng/g)	Mean Recovery (%)	RSD (%)
alpha-BHC	5	90.02	2.51
	10	114.67	9.94
gamma-BHC	5	80.62	2.07
	10	82.25	0.74
beta-BHC	5	107.36	6.37
	10	107.14	7.09
delta-BHC	5	82.79	3.55
	10	76.79	5.89
heptachlor	10	81.49	4.89
	50	74.03	9.60
aldrin	50	115.08	10.70
	100	93.05	8.60
heptachlor epoxide	5	99.67	1.57
	10	91.29	3.73
trans-chlordane	10	91.37	6.15
	25	71.17	7.78
cis-chlordane	5	102.37	8.15
	10	92.90	5.19
endosulfan-I	25	106.77	5.53
	50	87.55	6.70
4,4'-DDE	5	79.43	7.24
	10	93.02	10.42
dieldrin	25	93.84	5.38
	50	91.21	6.53
endrin	5	88.34	5.54
	25	79.57	4.02
4,4'-DDD	5	97.24	9.70
	10	71.51	5.25
endosulfan-II	5	90.28	7.67
	10	72.55	3.92
4,4'-DDT	5	117.79	6.09
	10	104.79	7.32
endosulfan sulfate	5	83.07	8.89
	10	81.88	0.58
methoxychlor	5	121.34	7.04
	10	100.76	9.36
endrin ketone	5	107.25	8.95
	10	108.96	4.00

Table 3.9.4: Data of the Recovery experiment from Chili

Pesticides	Spiking Level (ng/g)	Mean Recovery (%)	RSD (%)
alpha-BHC	5	97.42	7.30
	10	116.26	7.80
gamma-BHC	5	70.51	7.68
	10	76.01	8.63
beta-BHC	5	116.86	4.46
	10	86.47	7.76
delta-BHC	5	108.24	5.63
	10	94.50	2.12
heptachlor	5	111.80	10.47
	10	90.38	7.07
aldrin	100	95.11	10.11
	200	73.92	7.68
heptachlor epoxide	5	112.80	9.45
	10	70.21	10.23
trans-chlordane	10	73.59	8.66
	25	109.13	7.14
cis-chlordane	5	69.70	1.37
	10	98.21	8.65
endosulfan-I	25	93.48	2.05
	50	94.46	10.18
4,4'-DDE	5	89.44	2.65
	10	90.28	3.19
dieldrin	5	118.94	6.13
	10	75.19	8.27
endrin	5	113.13	8.92
	10	98.34	0.91
4,4'-DDD	5	85.23	1.16
	10	72.61	1.58
endosulfan-II	5	75.73	6.58
	10	105.44	1.80
4,4'-DDT	25	120.32	3.33
	50	99.57	1.20
endosulfan sulfate	5	89.11	5.51
	10	94.07	7.27
methoxychlor	5	76.59	11.83
	25	102.09	7.47
endrin ketone	5	80.62	8.21
	10	94.33	4.29

Table 3.9.5: Data of the Recovery experiment from Cauliflower

Pesticides	Spiking Level (ng/g)	Mean Recovery (%)	RSD (%)
alpha-BHC	5	101.94	1.89
	10	115.97	6.31
gamma-BHC	5	84.16	4.40
	10	71.31	8.49
beta-BHC	25	75.11	2.20
	50	96.31	3.74
delta-BHC	5	105.52	7.54
	10	104.12	1.63
heptachlor	5	107.15	6.63
	10	104.29	6.20
aldrin	25	107.65	10.14
	50	115.53	1.09
heptachlor epoxide	5	73.00	3.67
	10	75.20	2.66
trans-chlordane	10	76.15	1.74
	25	104.11	6.22
cis-chlordane	5	73.55	10.10
	10	93.74	1.51
endosulfan-I	25	79.48	4.98
	50	101.49	3.80
4,4'-DDE	5	101.70	4.72
	10	91.99	6.97
dieldrin	25	94.35	7.75
	50	81.56	6.92
endrin	5	75.31	6.59
	25	75.70	7.98
4,4'-DDD	5	81.56	4.83
	10	98.69	1.31
endosulfan-II	5	98.39	5.82
	10	89.35	8.65
4,4'-DDT	5	74.14	5.88
	10	117.32	6.13
endosulfan sulfate	5	70.35	3.49
	10	109.91	8.06
methoxychlor	5	117.20	7.81
	10	97.99	5.91
endrin ketone	5	92.54	2.81
	10	80.80	4.24

3.5 Accuracy and Precision

Accuracy is expressed as a percentage of the recovery. Precision is expressed by the relative standard deviation (RSD). The table (3.9.1-3.9.5) shows the results regarding the accuracy and precision of the method. These accuracy and precision values were consistent with the ranges listed in the Codex guidelines. Accuracy values were varied from 70-120% with relative standard deviations (precision) up to 10% in pointed gourd, 70–119% with relative standard deviation up to 11% in bitter gourd, 71-121% with relative standard deviation up to 10% in tomato, 70–120% with relative standard deviation up to 11% in chili, 70–117% with relative standard deviation up to 10% in cauliflower which is acceptable in analytical purpose. For analyzing pesticide residues at ng/g level, accuracy and recovery of 70-120% considered acceptable (Bempah et al., 2011).

3.6 Specificity

The unnecessary compounds interfering with the analytes were examined by comparing the chromatograms of the standard, blank sample, and fortified samples. There were no interference peaks at the retention times of alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan-I, 4,4'-DDE, dieldrin, endrin, 4,4'-DDD, endosulfan-II, endrin aldehyde, 4,4'-DDT, endosulfan sulfate, methoxychlor and endrin ketone.

3.7 Determination of Organochlorine Pesticides in Vegetable Samples

The extraction of the samples was carried out by QuEChERS Method. After extraction and clean-up of the sample, the residual amount of OCPs was determined by using GC-ECD. Identification of the organochlorine pesticides was done by comparing the retention times of the corresponding certified standards. The identified OCPs and their concentrations (ng/g) are shown in Tables 3.10-3.13.

Table 3.10: Concentration (ng/g) of organochlorine pesticides in fresh vegetable samples collected from Hatirpool bazar, Dhaka

Organochlorine Pesticides	Concentration of organochlorine pesticides (ng/g)				
	Pointed gourd	Bitter gourd	Tomato	Chili	Cauliflower
alpha-BHC	-	-	-	-	-
gamma-BHC	-	-	-	-	-
beta-BHC	-	-	-	-	-
delta-BHC	-	-	-	-	-
heptachlor	-	-	-	-	-
aldrin	-	-	-	-	-
heptachlor epoxide	-	-	-	-	-
trans-Chlordane	-	-	-	-	-
cis-Chlordane	-	-	-	-	-
endosulfan-I	-	-	-	-	-
4,4'-DDE	12.91	-	-	9.36	-
dieldrin	-	-	-	-	-
endirn	-	-	-	-	-
4,4'-DDD	27.47	-	-	27.16	-
endosulfan-II	-	-	-	-	-
endrin aldehyde		-	-	-	-
4,4'-DDT	7.77	62.09	-	84.88	-
endosulfan sulfate	-	-	-	-	-
methoxychlor		-	-	-	
endrin ketone	-	-	-	-	-

Note: (-) indicates below detection limit

Five varieties of vegetables were collected from Hatirpool Bazar, Dhaka for the analysis of twenty organochlorine pesticides (OCPs). Three different OCPs were identified and quantified in the pointed gourd. 4,4'-DDT was quantified in bitter gourd and three OCPs were quantified in chili. No OCPs was detected both in tomato and cauliflower. From Table 3.10, it was found that alpha-BHC, gamma-BHC, beta- BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-

chlordane, *cis*-chlordane, endosulfan-I, dieldrin, endrin, endosulfan-II, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone were below the detection limit. In pointed gourd, 4,4'-DDE (12.91 ng/g), 4,4´-DDD (27.47ng/g) and 4,4´-DDT (7.77 ng/g) were found where the concentration of 4,4´-DDD was the highest and that of 4,4´-DDT was the lowest. The presence of 4,4´-DDT (62.09 ng/g) was found in bitter gourd. In chili, 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT have been found as 9, 27 and 84 ng/g, respectively.

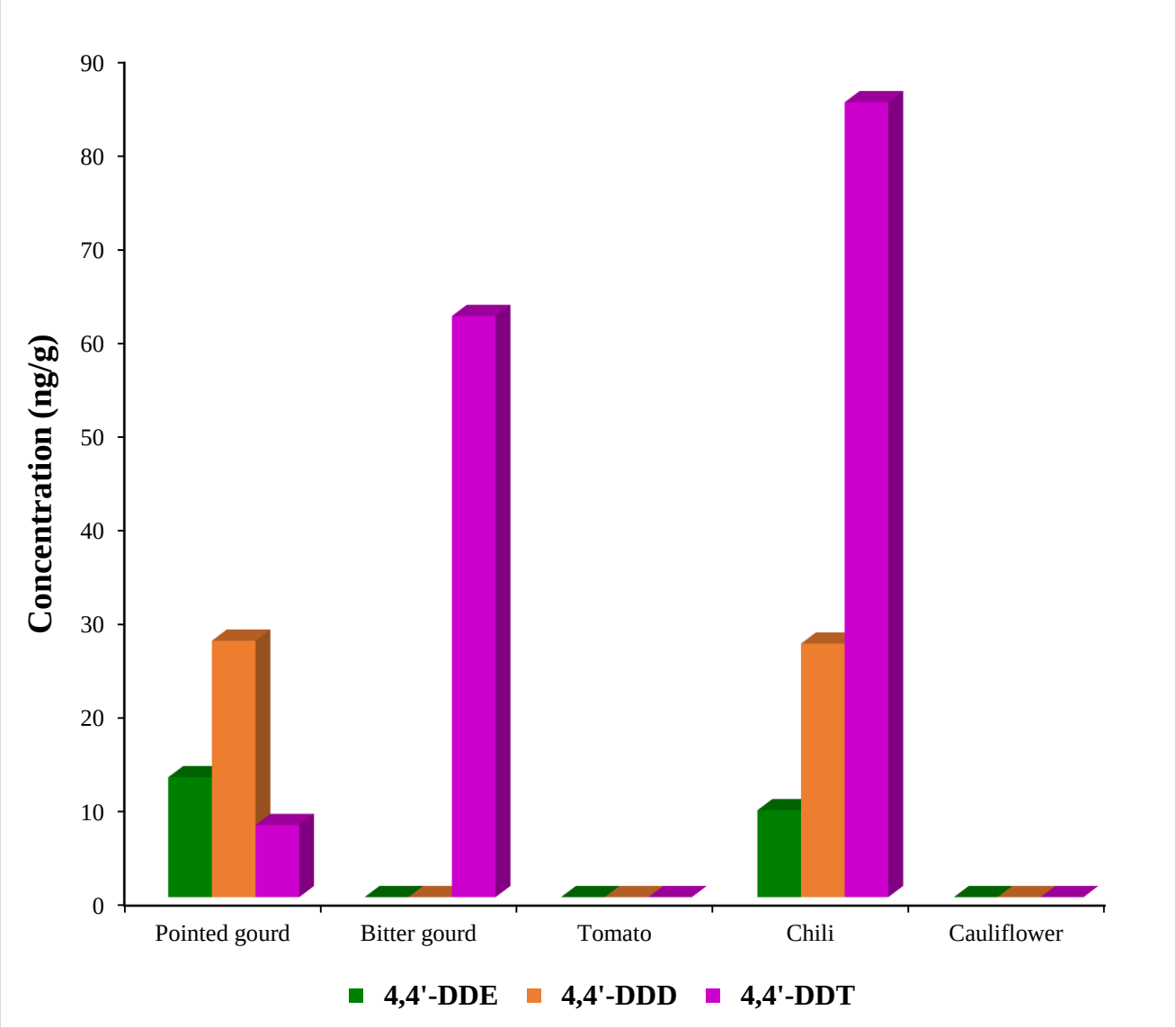


Figure 3.8: Concentration (ng/g) of organochlorine pesticides in samples collected from Hatirpool Bazar, Dhaka

Table 3.11: Concentration (ng/g) of organochlorine pesticides in fresh vegetable samples collected from Kaptan Bazar, Dhaka

Organochlorine Pesticides	Concentration of organochlorine pesticides (ng/g)				
	Pointed gourd	Bitter gourd	Tomato	Chili	Cauliflower
alpha-BHC	-	-	-	-	-
gamma-BHC	-	-	-	-	-
beta-BHC	-	-	-	-	-
delta-BHC	-	-	-	-	-
heptachlor	-	-	-	-	-
aldrin	-	-	-	-	-
heptachlor epoxide	-	-		-	-
trans- chlordane	-	-	-	-	-
cis-chlordane	-	-	-	-	-
endosulfan-I	-	-	-	-	-
4,4'-DDE	-	-	-	-	-
dieldrin	-	-	-	-	-
endirn	-	-	-	-	-
4,4'-DDD	-	-	-	47.02	-
endosulfan-II	-	-	-	-	-
endrin aldehyde	-	-	-	-	-
4,4'-DDT	6.52	4.03	4.87	21.25	6.12
endosulfan sulfate	-	-	-	-	-
methoxychlor	-	-	-	-	-
endrin ketone	-		-	-	-

Note: (-) indicates below detection limit-

Pointed gourd, bitter gourd, tomato, chili, and cauliflower samples were purchased from the local market of Kaptan Bazar, Dhaka. 4,4'-DDT was identified and quantified in all the samples. It was

found as 6.52, 4.03, 4.87, 21.25 and 6.12 ng/g in pointed gourd, bitter gourd, tomato, chili and cauliflower, respectively. However, 4,4'-DDD (47.02 ng/g) also was present in chili.

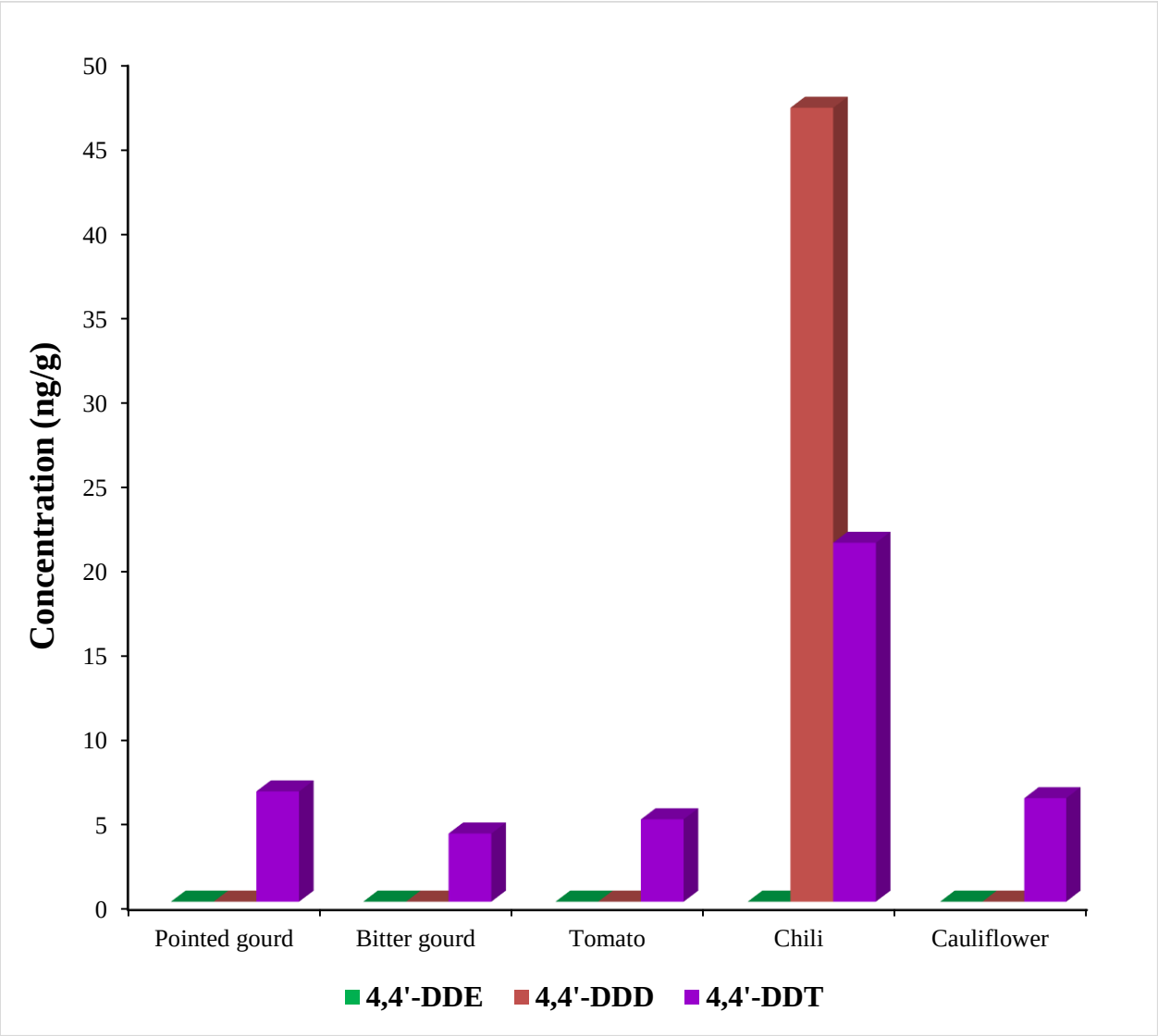


Figure 3.9: Concentration (ng/g) of organochlorine pesticides in samples collected from Kaptan Bazar, Dhaka

Table-3.12: Concentration (ng/g) of organochlorine pesticides in fresh vegetable samples collected from Shibpur Bazar, Narsingdi

Organochlorine Pesticides	Concentration of organochlorine pesticides (ng/g)				
	Pointed gourd	Bitter gourd	Tomato	Chili	Cauliflower
alpha-BHC	-	-	-	-	-
gamma-BHC	-	-	-	-	-
beta-BHC	-	-	-	-	-
delta -BHC	-	-	-	-	-
hepachlor	-	-	-	-	-
aldrin	-	-		-	-
heptachlor epoxide	-	-	-	-	-
trans- chlordane	-	-	-	-	-
cis-chlordane	-	-	-	-	-
endosulfan-I		-	-	-	-
4,4'-DDE	-	-	-	-	-
dieldrin	-	-	-	-	-
endirn	-	-	-	-	-
4,4'-DDD	-	-	-	-	-
endosulfan- II	-	-	-	-	-
endrin aldehyde	-	-	-	-	-
4,4'-DDT	2.68	3.85	6.36	3.77	0.68
endosulfan sulfate	-	-	-	-	-
methoxychlor	-	-	-	-	-
endrin ketone	-	-	-	-	-

Note: (-) indicates below detection limit

Vegetables were collected from Shibpur Bazar, Narsingdi for analyzing twenty organochlorine pesticides (OCPs). Alpha-BHC, gamma-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan-I, 4,4´-DDE, dieldrin, endrin, 4,4´-DDD, endosulfan-II, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone were all below the detection limit in all samples. Only 4,4´-DDT was detected and quantified.

Tomato was contaminated with the highest level of 4,4´-DDT among all vegetables and it was 6.36 ng/g. Pointed gourd, bitter gourd, chili and cauliflower contained 2.68, 3.85, 3.77 and 0.68 ng/g, respectively. It is obvious that cauliflower was the least contaminated vegetable.

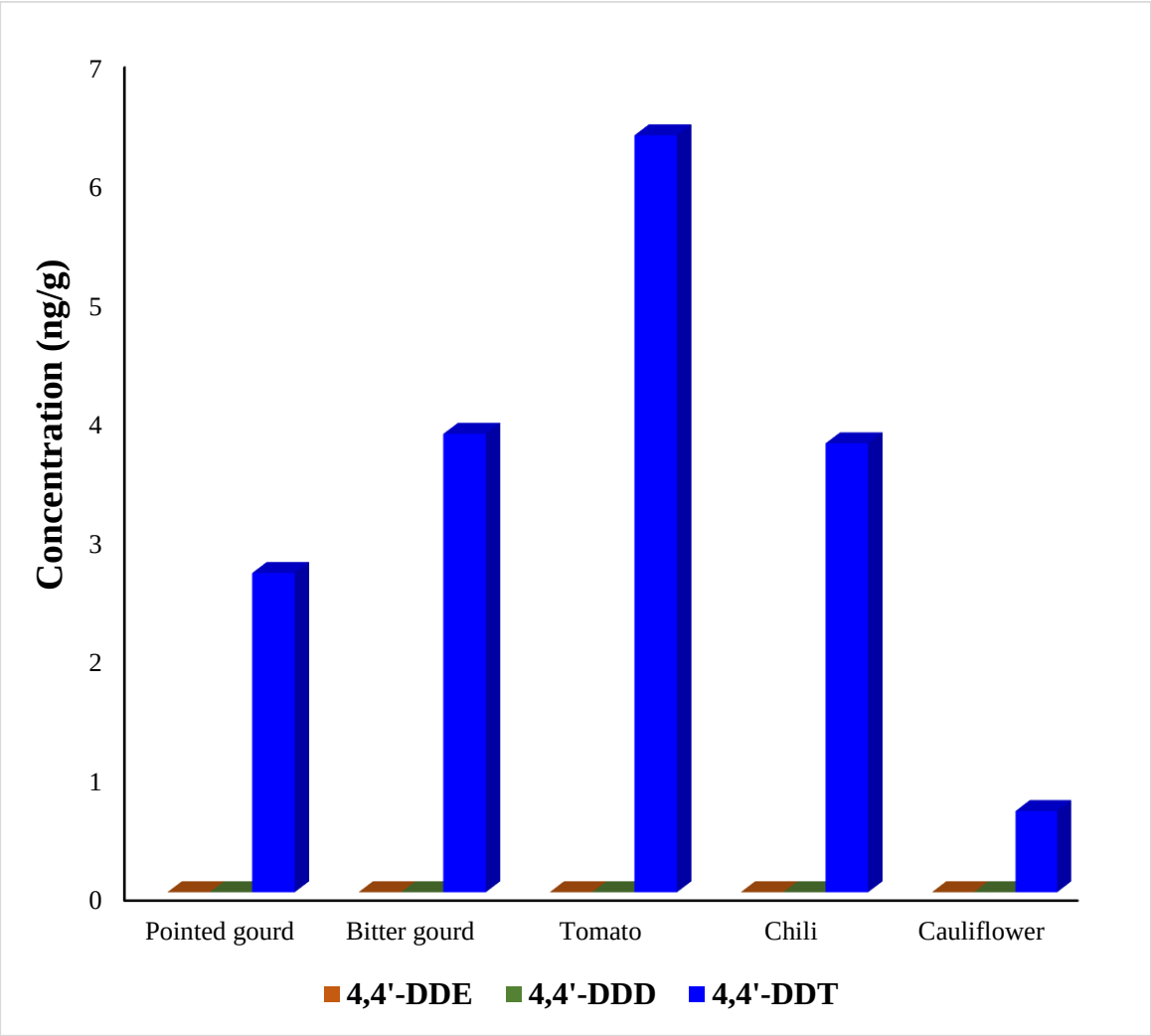


Figure 3.10: Concentration (ng/g) of organochlorine pesticides in samples collected from Shibpur Bazar, Narsingdi

Table 3.13: Concentration (ng/g) of organochlorine pesticides in fresh vegetable samples collected from College gate Bazar, Narsingdi

Organochlorine Pesticides	Concentration of organochlorine pesticides (ng/g)				
	Pointed gourd	Bitter gourd	Tomato	Chili	Cauliflower
alpha -BHC	-	-	-	-	-
gamma-BHC	-	-	-	-	-
beta-BHC	-	-	-	-	-
delta- BHC	-	-	-	-	-
heptachlor	-	-	-	-	-
aldrin	-	-	-	-	-
heptachlor epoxide	-	-	-	-	-
trans-chlordane	-	-	-	-	-
cis-chlordane	-	-	-	-	-
endosulfan-I	-	-	-	-	-
4,4'-DDE	-	-	-	-	-
dieldrin	-	-	-	-	-
endirn	-	-	-	-	-
4,4'-DDD	-	-	-	-	-
endosulfan-II	-	-	-	-	-
endrin aldehyde	-	-	-	-	-
4,4'-DDT	9.09	8.20	8.27	17.28	3.70
endosulfan sulfate	-	-	-	-	-
methoxychlor	-	-	-	-	-
endrin ketone	-	-	-	-	-

Note: (-) indicates below detection limit

Pointed gourd, bitter gourd, tomato, chili, and cauliflower were purchased from the local market of College gate, Narsingdi. Only one organochlorine pesticide (4,4´-DDT) was identified and quantified in the samples. From Table 3.13 it was found that the contamination level of 4,4´-DDT was the highest in chili (17.28 ng/g) and it was almost the same (8-9 ng/g) in pointed gourd, bitter gourd and tomato. The lowest level of contamination was found in cauliflower with 3.70 ng/g.

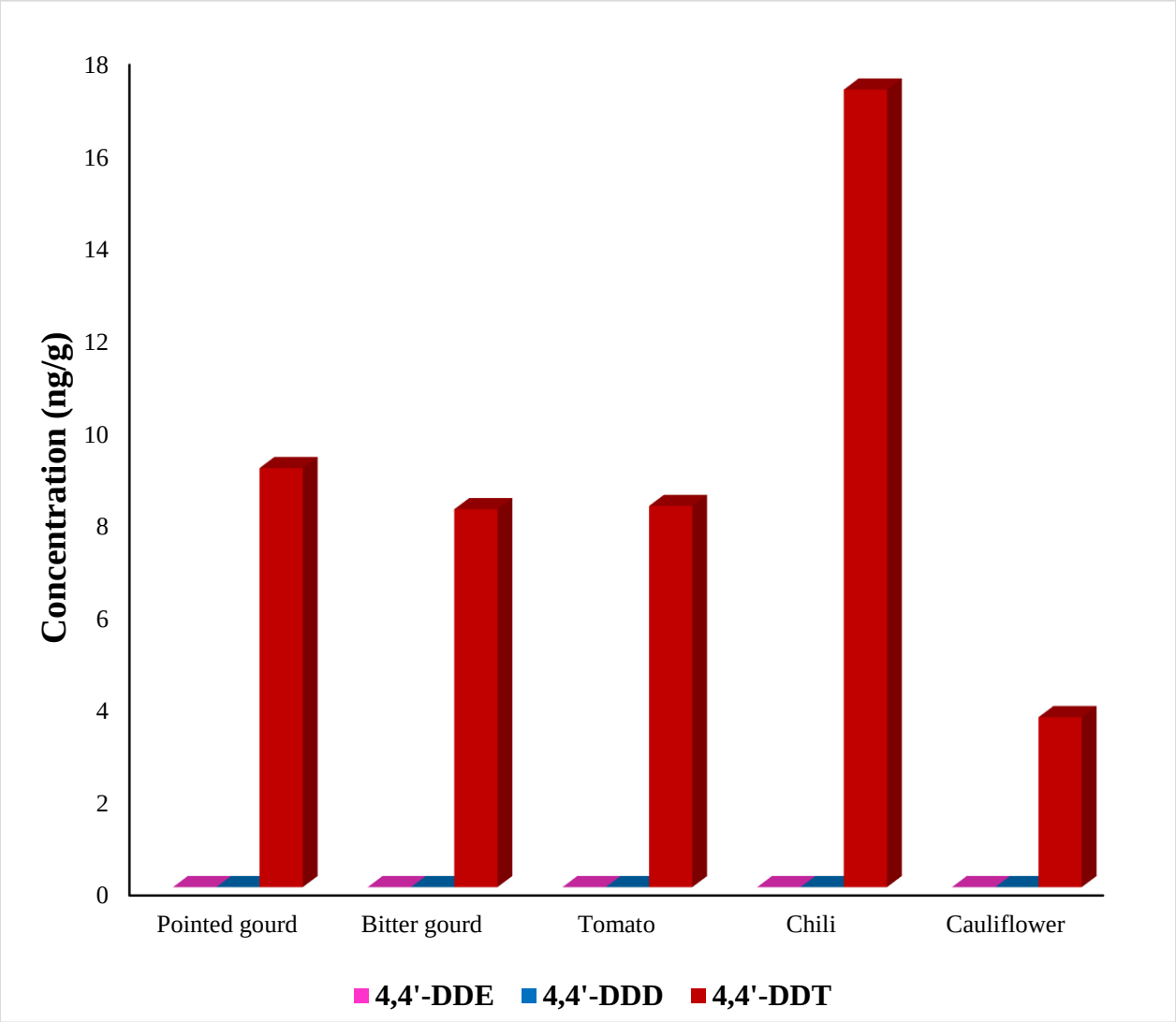


Figure 3.11: Concentration (ng/g) of organochlorine pesticides in samples collected from College gate Bazar, Narsingdi

The investigation was carried out to determine the concentration and distribution of organochlorine pesticides in five fresh vegetable samples. For quantification of pesticides from these vegetable

samples, twenty OCPs reference standards were used. Peaks of the pesticides were obtained at different retention times. The retention time of the peak present in the sample chromatogram was compared with the chromatogram of the standard solution. Solutions of different concentrations were made from the certified standard. Using Microsoft Excel-2016 software, calibration curves for these pesticides were made. From the calibration curve, the number of pesticides present in the sample was calculated and tabulated in Tables 3.10-3.13.

The studies were conducted by collecting 60 samples of a pointed gourd (potol), bitter gourd (korola), tomato, chili, and cauliflower from local markets in Bangladesh. The samples collected from Hatirpool Bazar and Kaptan Bazar, Dhaka, were also found to contain relatively more residual pesticides than the samples collected from Shibpur Bazar and College gate Bazar, Narsingdi. This indicates that vegetables sold in these two markets in Dhaka are more contaminated by pesticides than in the other two markets in Narsingdi. Among the twenty pesticides, 4,4'-DDT was found in the highest concentration in all the samples collected from different markets.

3.8 Amount of Moisture in Vegetable samples

Moisture content for vegetables is an important parameter used to measure how much water is present in vegetables, which plays a vital role in processing, storage, transportation, and perishability. This measurement is very important economically, especially for food processing industries. The highest amount of moisture content among five different vegetables was found in a pointed gourd (potol) with a value of 25.76 ± 3.16 %. All other vegetables like chili (kacha morich), tomato (tomato), bitter gourd (korola), and cauliflower (phulcopi) contain a moisture content of around (19-23) %. Reduced water content can control the growth of microorganisms and reduce deterioration processes. The moisture content result is shown in the Table 3.14.

Table 3.14: Moisture content of the vegetable sample (g/100g)

Sample	(a,b,c)	Amount of moisture (g/100g)	Standard deviation	Amount of moisture (g/100g)
T	a	24.40	0.60	23.72 ± 0.60
	b	23.50		
	c	23.25		
P	a	23.51	3.16	25.76 ± 3.16
	b	24.40		
	c	29.37		
KM	a	21.82	1.97	22.95 ± 1.97
	b	21.81		
	c	25.23		
KR	a	23.35	0.14	23.26 ± 0.14
	b	23.34		
	c	23.10		
C	a	22.34	4.18	19.92 ± 4.18
	b	22.34		
	c	15.09		

T- Tomato, P- Pointed gourd, KM- Chili, KR- Bitter gourd and C- Cauliflower

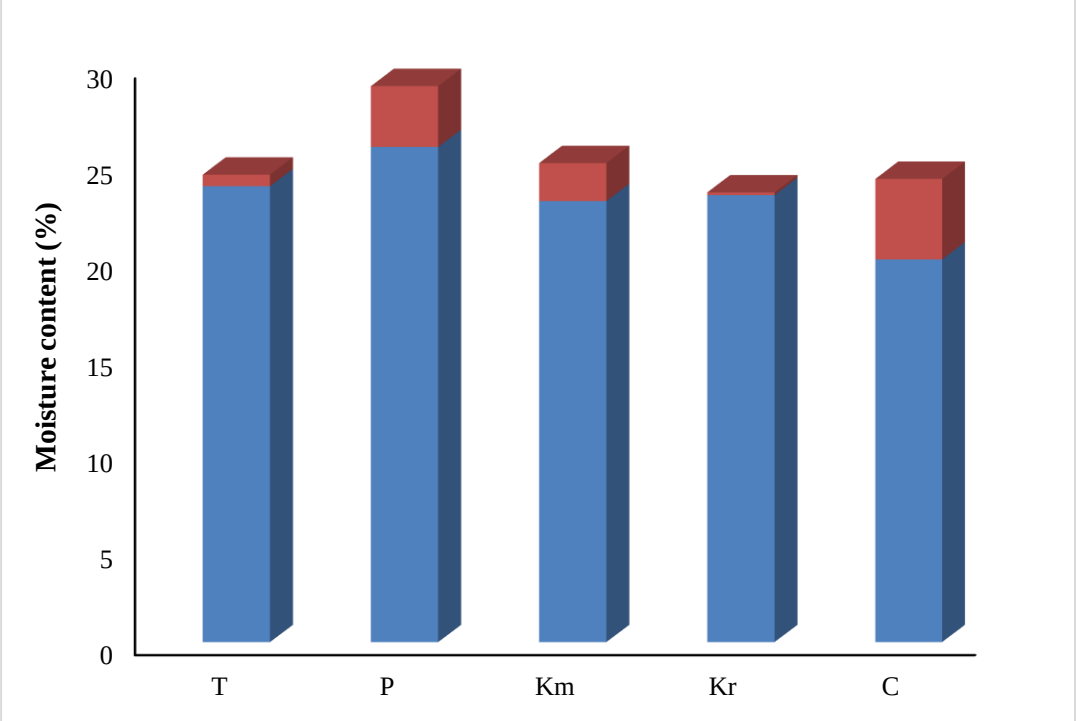


Figure 3.12: Moisture content in vegetable samples

3.9 Amount of Ash in Vegetable samples

The ash content of vegetables indicates the inorganic residue remaining after complete oxidation of organic components present in the sample. It is the measure of minerals or inorganic components like Na, K, Ca, Fe and Ni. It is used for proximate analysis using high temperatures (Afify et al., 2017)**.** The ash content of pointed gourd (P), bitter gourd (KR), tomato (T), chili (KM) and cauliflower (C) ranged from 0.38 to 0.50%. The ash content result is shown in the Table 3.15.

Table 3.15: Ash content of vegetable samples (g/100g)

Sample	(a,b,c)	Amount of ash (g/100g)	Standard deviation	Amount of ash (g/100g)
P	a	0.3931	0.0037	0.39 ± 0.0037
	b	0.3990		
	c	0.3922		
KR	a	0.3901	0.09945	0.39 ± 0.09945
	b	0.4895		
	c	0.2906		
T	a	0.4453	0.145812	0.44 ± 0.1458
	b	0.2897		
	c	0.5811		
KM	a	0.04963	0.403717	0.50 ± 0.4037
	b	0.63477		
	c	0.82404		
C	a	0.3359	0.126939	0.38 ± 0.1269
	b	0.5278		
	c	0.2879		

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

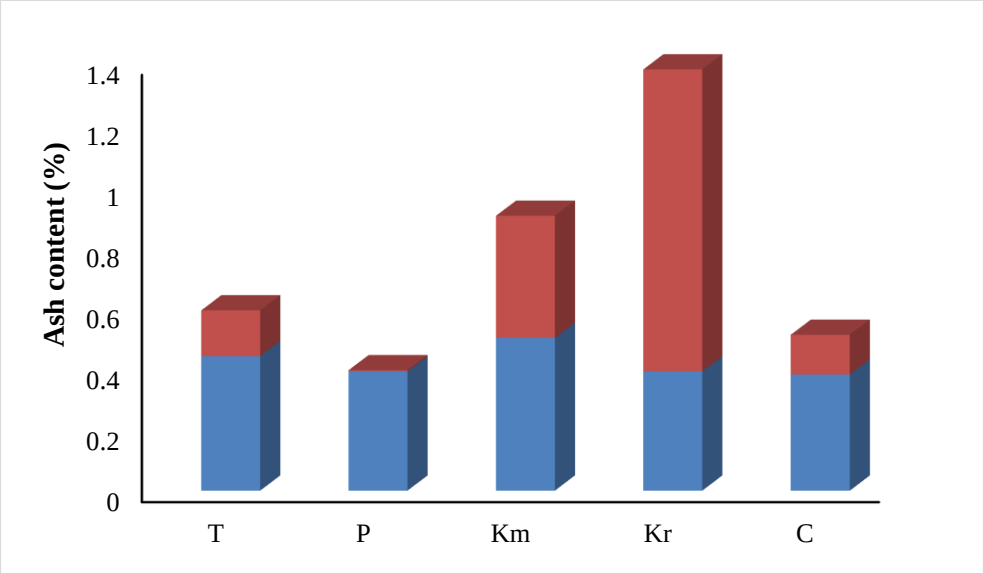


Figure 3.13: Ash content in vegetable samples

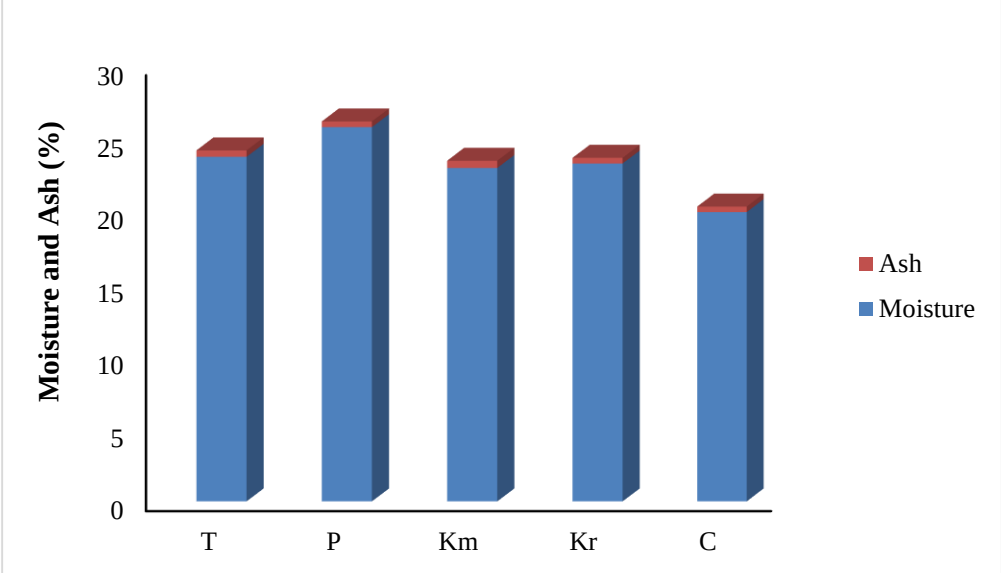


Figure 3.14: Moisture and Ash content in vegetable Samples

3.10 Analysis of Fatty Acids

Fatty acids in vegetable samples were identified with the retention time of the standard of fatty acids in the GC chromatogram. The mixture of standard fatty acids (1µL) gave a chromatogram. A sample (1µL) was injected into the injector of the GC at the same condition. Relative percentage of different fatty acids = (Area of the fatty acid X 100)/ Total area

Relative percentage of different fatty acids = (Area of the fatty acid X 100)/ Total area

Table 3.16: Retention time of standard fatty acids

Standard Fatty Acid	RetentionTime (minute)	Chemical type
Octanoic acid	3.45	Saturated
Decanoic acid	6.91	Saturated
Lauric acid	11.36	Saturated
Tetradecanoic acid	17.77	Saturated
Palmitic acid	24.89	Saturated
Palmitoleic acid	25.83	Unsaturated
Octadecanoic acud	32.98	Saturated
cis-9-oleic acid	33.27	Unsaturated
Linoleic acid	34.48	Unsaturated
Arachidic acid	43.03	Saturated
Linolenic acid	49.96	Unsaturated
Docosanoic acid	51.12	Saturated
Erucic acid	58.71	Unsaturated

Table 3.17: Fatty acids found in the hexane extract of different vegetables

Sample Name	Palmitoleic Acid	Octadecanoic Acid	Erucic Acid
HKR	67.73%	N.D	32.26%
HT	9.36%	91.43%	N.D
HKM	100%	N.D	N.D

Note: (N.D): Indicates Not Detected

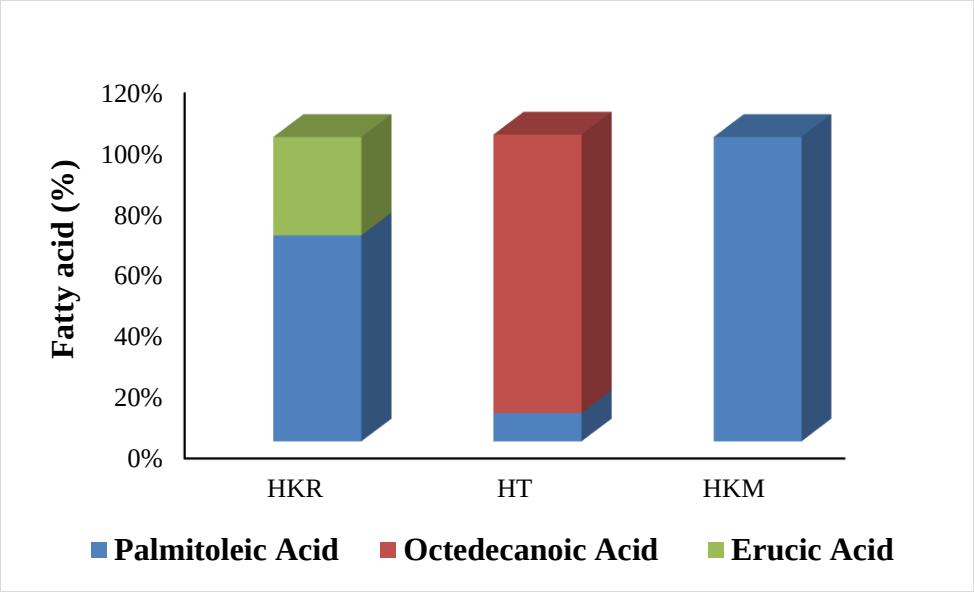


Figure 3.15: Percent of fatty acids found in the hexane extract of different vegetables

The hexane extracts of vegetables were analyzes by GC-FID. Three fatty acids (palmitoleic acid, octadecanoic acid, and erucic acid) were identified in three different samples, such as bitter gourd, tomato, and chili. In chili, only palmitoleic acid was identified. In tomato, palmitoleic acid and octadecanoic acid were identified. In bitter gourd, palmitoleic acid and erucic acid were identified.

3.11 Analysis of Protein in Vegetable Samples

The strength of sulfuric acid was 0.1144 N, and the volume of acid was 25.00 mL. The strength of sodium hydroxide was 0.102 N.

Table 3.18: Determination of Protein in vegetable samples

Sample ID	Required Volume of Sodium Hydroxide(mL)	Sample Weight(g)	(Protein %)
P	27.20	1.0214	0.73
KR	26.00	1.0047	1.81
T	26.80	1.0874	1.02
KM	27.20	1.0624	0.71
C	25.00	1.0076	2.69

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

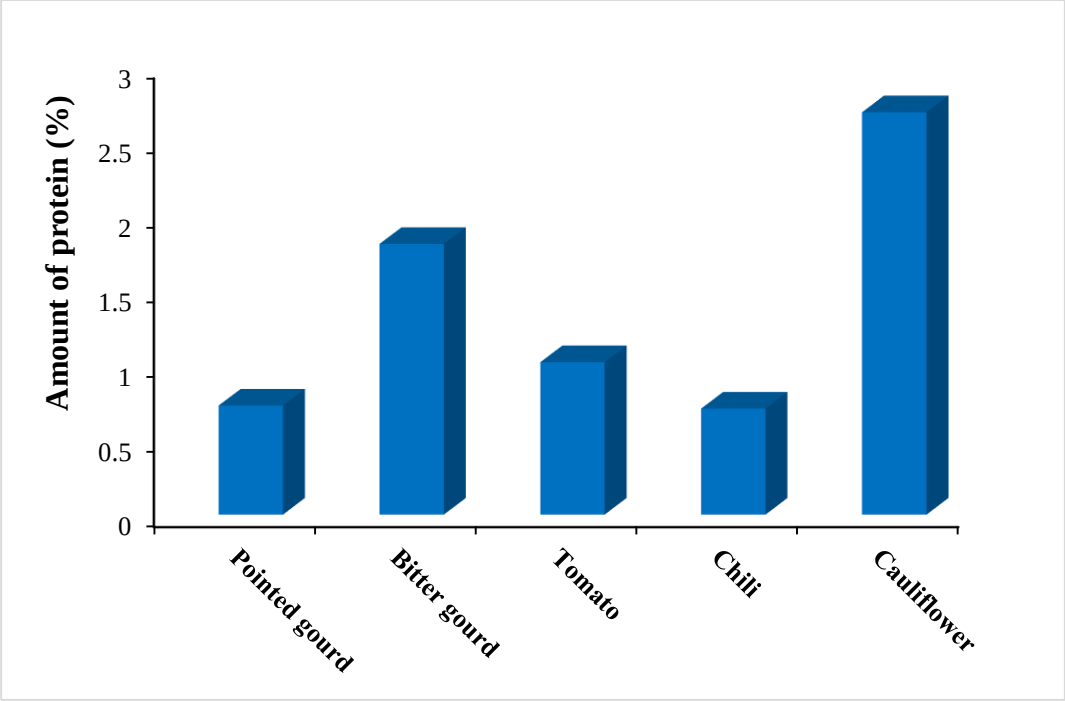


Figure 3.16: Protein content in different vegetable samples

The highest amount of protein was found in cauliflower with 2.69% and the lowest amount was found in chili with 0.71%. The amount of protein in a bitter gourd was 1.81% and in tomato was 1.02% of dry extract.

3.12 Total Phenolic content

The methanol extract of chili was found to contain the maximum amount of total phenolic content with 33.86 ± 4.34 mg gallic acid equivalent per gram (GAE/g) of dry extract, and it was followed by cauliflower with 32 mg GAE/g. The lowest amount of phenolic content among five different vegetables was found in tomatoes. The phenolic content of bitter gourd was found to be 14 mg GAE/g of dry extract.

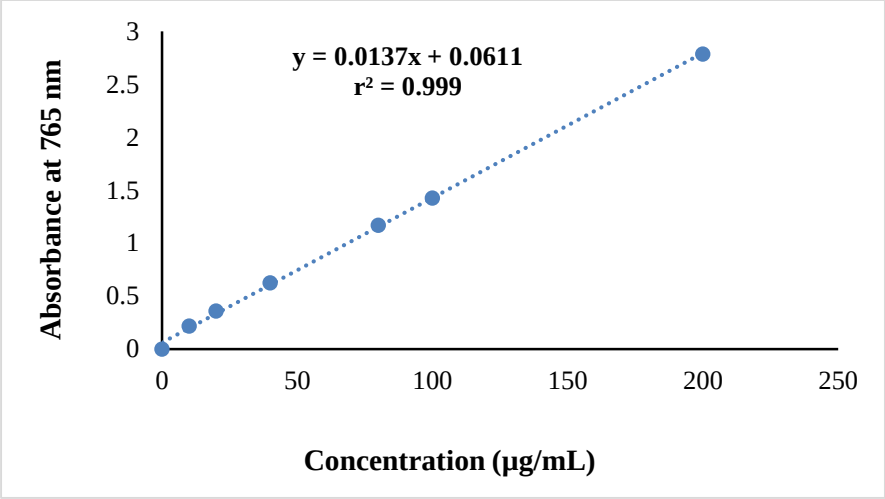


Figure 3.17: Calibration curve of Gallic acid

Table 3.19: Total phenolic content of different extracts

Different Extracts	Total Phenolic Content (mg GAE/g)
MP	6.71 ± 4.28
MKR	14.45 ± 4.22
MT	5.98 ± 4.34
MKM	33.86 ± 4.34
MC	32.0 ± 4.20

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

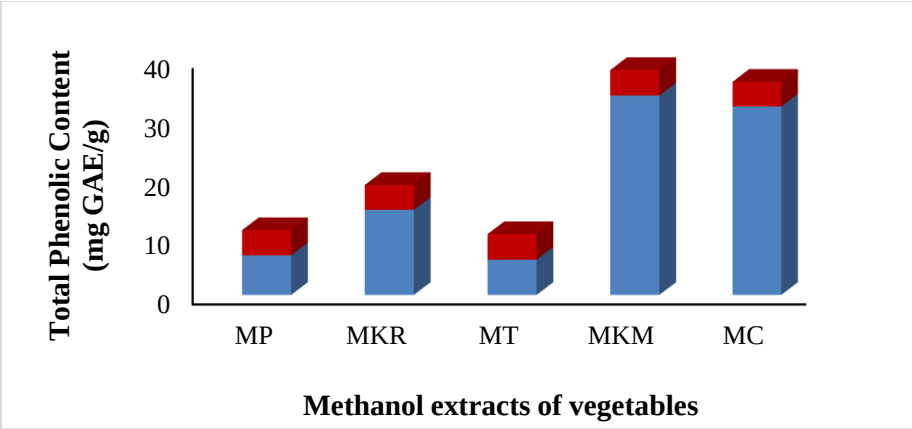


Figure 3.18: Total phenolic content of methanol extracts of different vegetables

3.13 Total Flavonoid Content

The highest amount of flavonoid content was found in bitter gourd with 37.64 ± 3.72 mg quercetin equivalent per gram (QE/g) of dry extract, whereas the lowest amount was found in cauliflower (MC) with 6 mg QE/g of dry extract. The flavonoid content of pointed gourd (MP), tomato (MT), and chili (MKM) was almost the same.

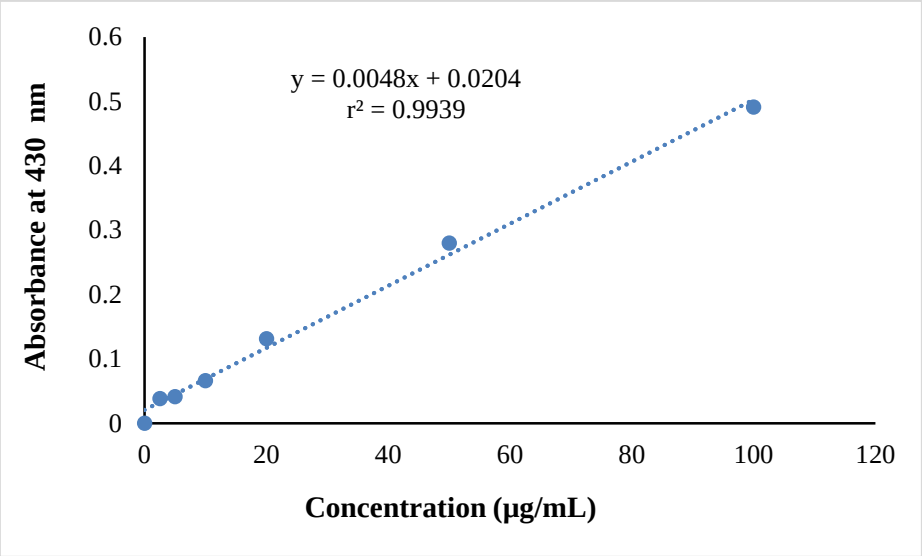


Figure 3.19: Calibration curve of Quercetin

Table 3.20: Total flavonoid content of different extracts of vegetables

Different Extracts	Total Flavonoid Content (mg QE/g)
MP	8.68 ± 2.64
MKR	37.64 ± 3.72
MT	9.52 ± 2.72
MKM	7.22 ± 4.27
MC	6.18 ± 2.45

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

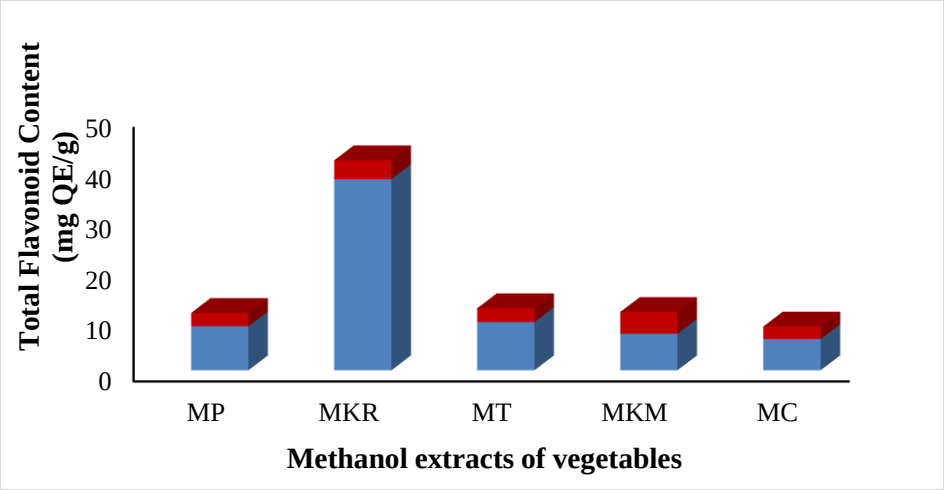


Figure 3.20: Total flavonoid content of methanol extracts of different vegetables

3.14 Total Antioxidant Capacity

Among five different vegetable samples, bitter gourd and chili showed the highest antioxidant capacity with a value of 53 mg ascorbic acid equivalent per gram (AAE/g) of dry extract. Cauliflower showed lower antioxidant capacity (29.07 ± 2.73 mg AAE/g) than chili and bitter gourd. The lowest amount of antioxidant capacity was found in a pointed gourd with 17 mg AAE/g of dry extract. The antioxidant capacity of tomato was found at 22 mg AAE/g of dry extract.

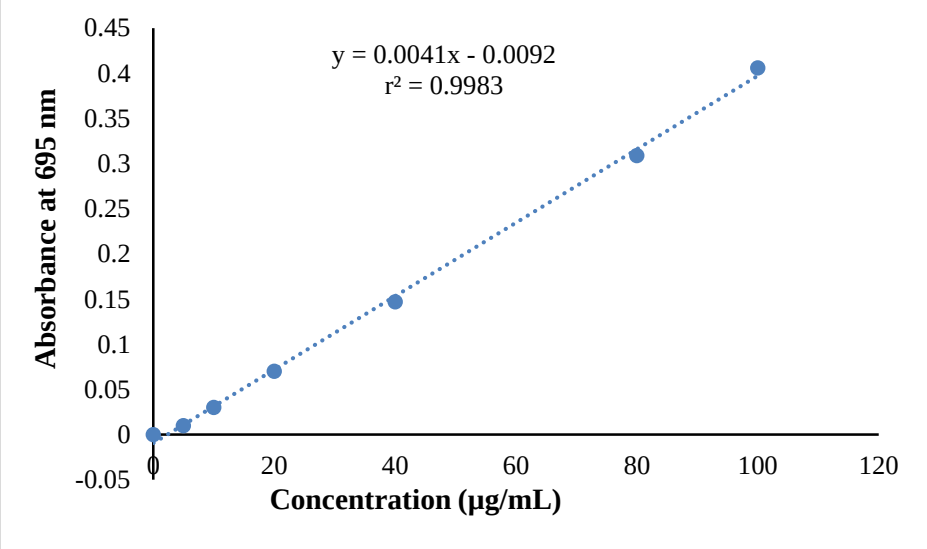


Figure 3.21: Calibration curve of Ascorbic acid

Table 3.21: Total antioxidant capacity of different extracts of vegetables

Different Extracts	Total antioxidant capacity (mg AAE/g)
MP	17.36 ± 3.04
MKR	53.46 ± 3.00
MT	22.48± 2.87
MKM	53.95 ± 3.14
MC	29.07 ± 2.73

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

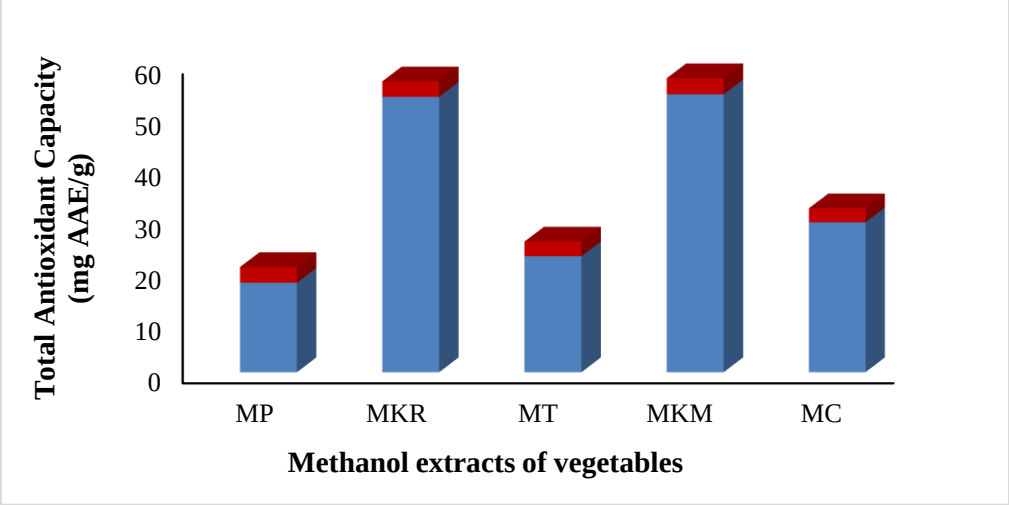


Figure 3.22: Total antioxidant capacity of methanol extracts of different vegetables

3.15 Analysis of Total Carbohydrates

Five samples were analyzed to determine total carbohydrates. Almost the same amount is present in both samples. The highest amount of carbohydrates among five different vegetables collected from different markets of Dhaka city was found in chili (kacha morich) with 8.4 ± 4.56 g/100g. It was followed by bitter gourd (korola) with 7.13 ± 4.21 g/100g. However, the lowest amount of carbohydrates was found in pointed gourd (potol) with a value of 2.28 ± 2.12 g/100g.

Table 3.22: Absorbance of standard D-glucose solution at different concentration

Concentration (µg/mL)	Absorbance at 488 nm
00	0.00
20	0.163
40	0.239
50	0.26
80	0.412
100	0.559
150	0.849
200	1.162

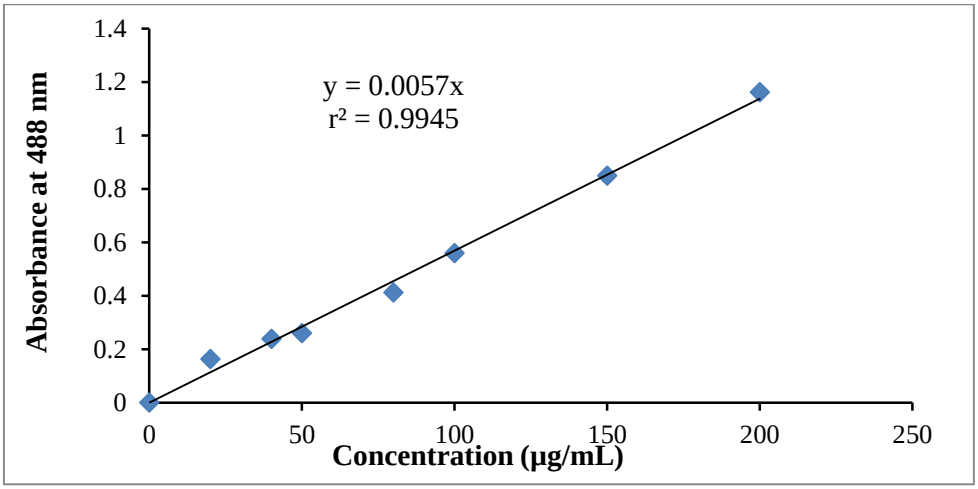


Figure 3.23: Calibration curve for standard D-glucose

Table 3.23: Total carbohydrates in vegetable samples

Samples	Amount of carbohydrates (g/100)
P	2.28 ± 2.12
KR	7.13 ± 4.21
T	4.25 ± 3.33
KM	8.4 ± 4.56
C	4.7 ± 2.26

P- Pointed gourd, KR- Bitter gourd, T- Tomato, KM- Chili and C- Cauliflower

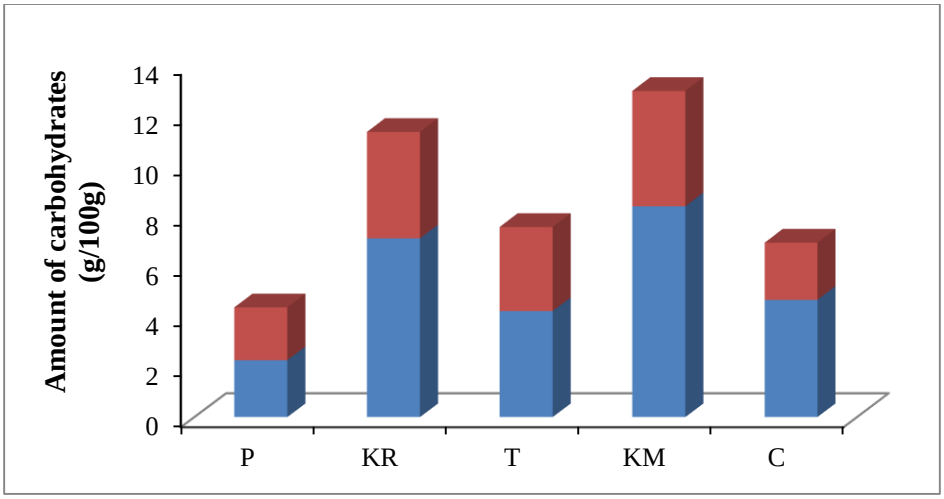


Figure 3.24: Total carbohydrates in vegetable samples

3.16 Micronutrient Analysis in Fresh Vegetable Samples

3.16.1 Calibration curves

Heavy metals such as Zn, Fe, Mn, Ca, Cd, Cr, Cu, Ni and Pb were found in vegetable samples and measured in mg/kg using an Atomic Absorption Spectrophotometer (AAS). The calibration curves for heavy metals were prepared using Microsoft Excel-2016 software.

3.16.2 Absorbance of Different Concentration of Standard Micronutrient

Table 3.24: Absorbance of standard Zn solutions of different concentrations

Concentration of Zn (ppm)	Absorbance
00	0.0004
0.1	0.0504
0.2	0.0984
0.6	0.2845
0.8	0.3852

Element: Zn (r = 0.9999)

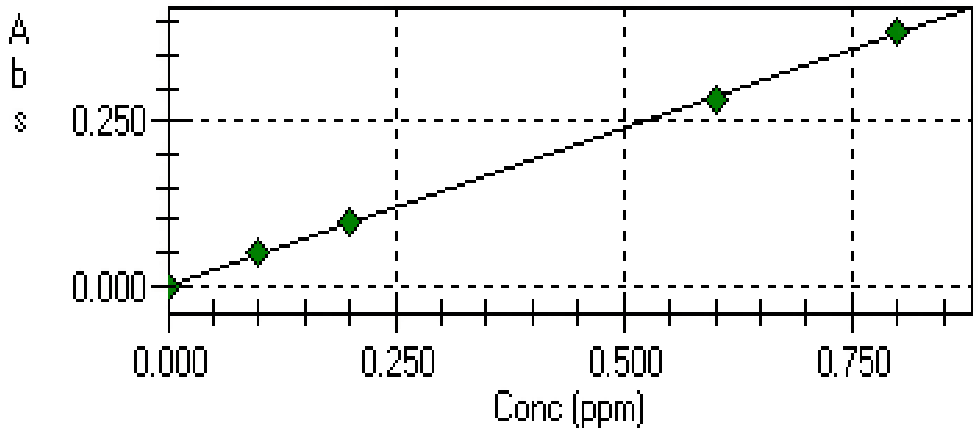


Figure 3.25: Calibration curve of standard Zn solutions

Table 3.25: Absorbance of standard Fe solutions of different concentrations

Concentration of Fe (ppm)	Absorbance
0	0.0008
1	0.1120
2	0.2087
4	0.4038

Element: Fe (r = 0.9996)

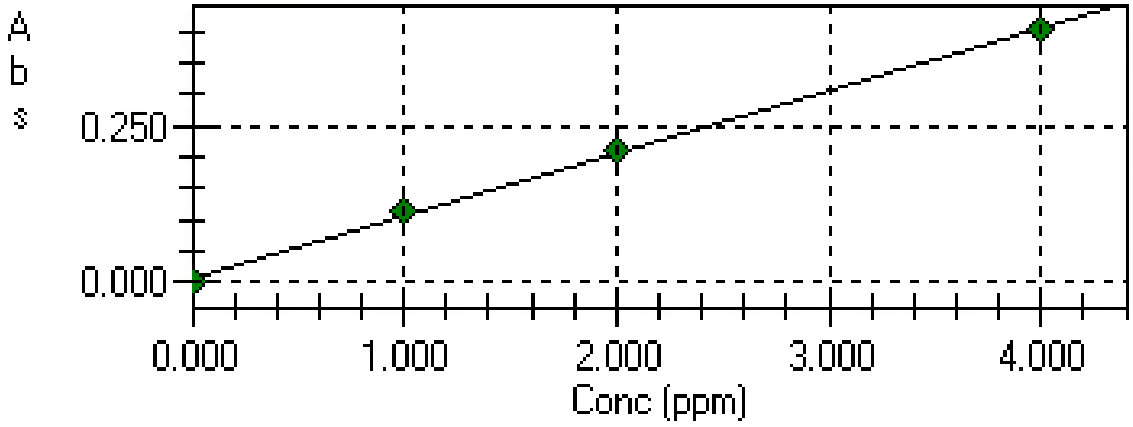


Figure 3.26: Calibration curve of standard Fe solutions

Table 3.26: Absorbance of standard Mn solutions of different concentrations

Concentration of Mn (ppm)	Absorbance
0	0.0004
1	0.2440
2	0.4975

Element: Mn (r = 0.9999)

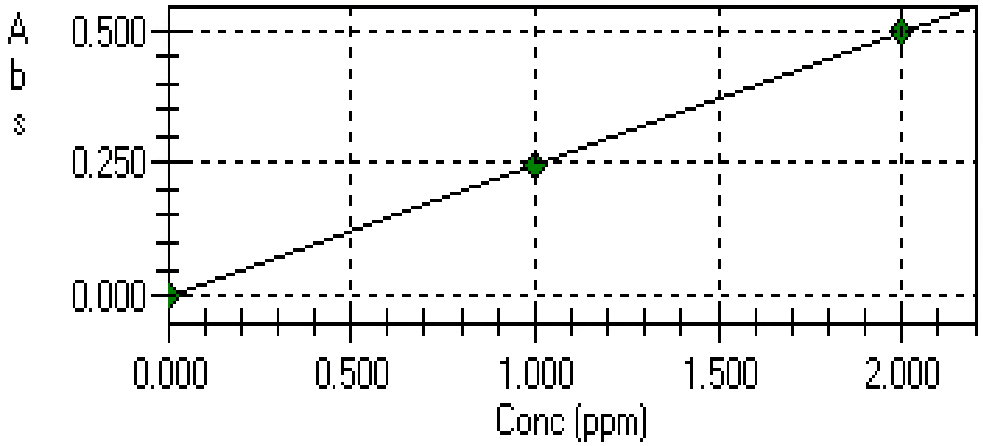


Figure 3.27: Calibration curve of standard Mn solutions

Table 3.27: Absorbance of standard Ca solutions of different concentrations

Concentration of Ca (ppm)	Absorbance
00	0.0001
0.5	0.0092
1	0.0171
2	0.0373
4	0.0713
6	0.1093

Element: Ca (r = 0.9998

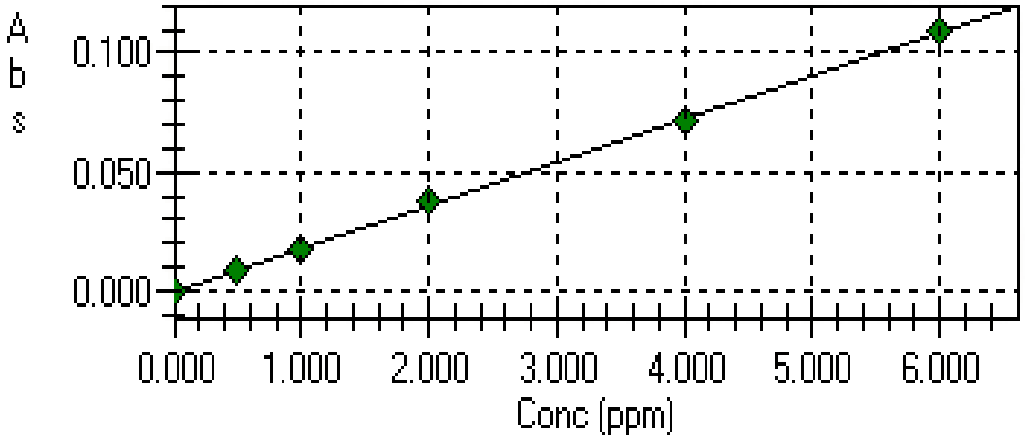


Figure 3.28: Calibration curve of standard Ca solutions

Table 3.28: Absorbance of standard Cd solutions of different concentrations

Concentration of Cd (ppm)	Absorbance
00	00
0.1	0.0462
0.2	0.1034
0.8	0.4234

Element: Cd (r = 0.9999)

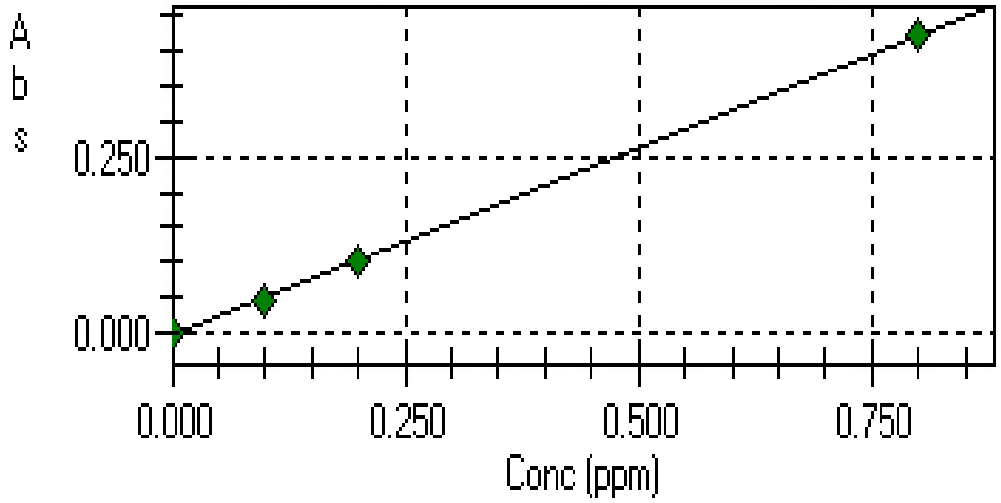


Figure 3.29: Calibration curve of standard Cd solutions

Table 3.29: Absorbance of standard Cr solutions of different concentrations

Concentration of Cr (ppm)	Absorbance
00	0.0003
0.25	0.0096
0.5	0.0190
1	0.0384

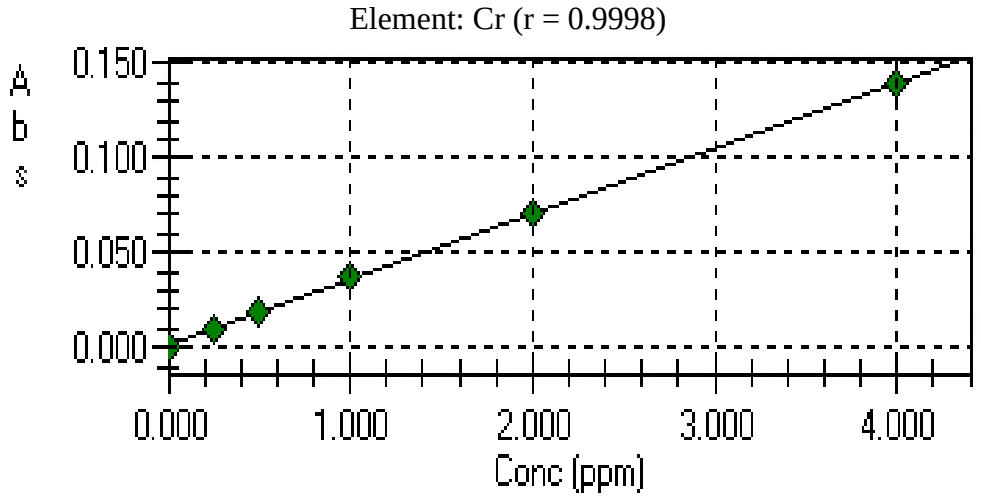


Figure 3.30: Calibration curve of standard Cr solutions

Table 3.30: Absorbance of standard Cu solutions of different concentrations

Concentration of Cu (ppm)	Absorbance
00	0.0013
0.5	0.0776
2	0.3140
4	0.6024

Element: Cu (r = 0.9998)

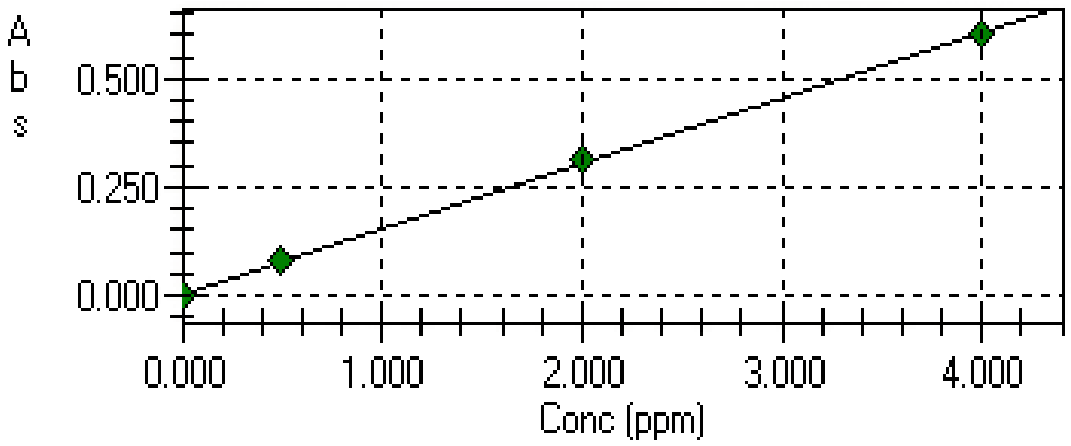


Figure 3.31: Calibration curve of standard Cu solutions

Table 3.31: Absorbance of standard Ni solution of different concentrations

Concentration of Ni (ppm)	Absorbance
0	0.0007
1	0.1163
2	0.2285

Element: Ni (r = 0.9999)

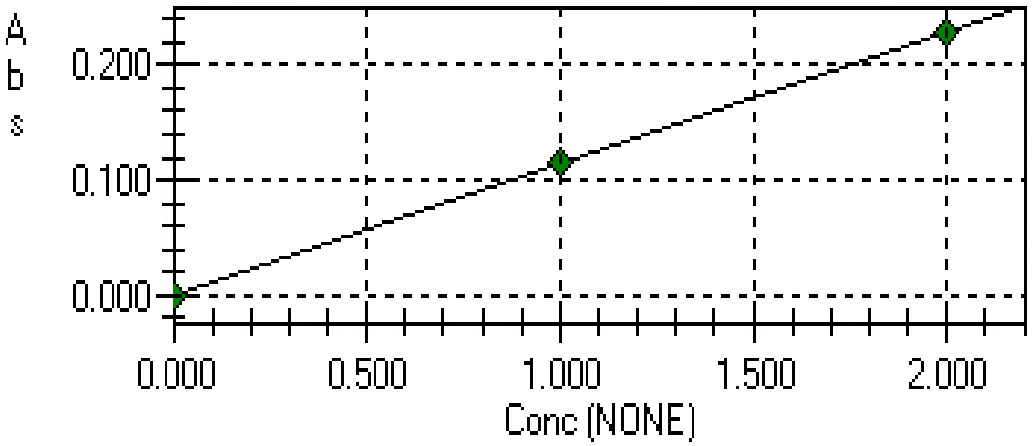


Figure 3.32: Calibration curve of standard Ni solutions

Table 3.32: Absorbance of standard Pb solutions of different concentrations

Concentration of Pb (ppm)	Absorbance
00	0.0003
0.5	0.0108
1	0.0226
2	0.0445

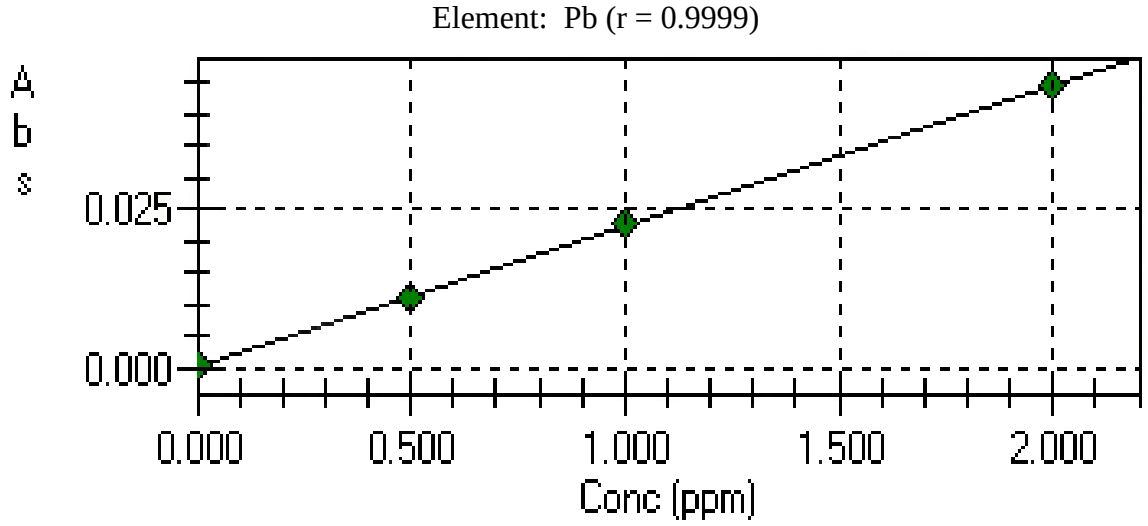


Figure 3.33: Calibration curve of standard Pb solutions

3.16.3 Quantification of Micronutrients

The study of heavy metal analysis in vegetables is important concerning human consumption of vegetables. Several studies showed that heavy metal contamination may considerably vary among the different species of vegetables. For heavy metal analysis, samples were freeze-dried, digested, and finally analyzed by AAS.

Almost all the heavy metals were found in all the samples at different concentrations. The concentration of heavy metals in vegetables is given in the Table.3.33.

Table 3.33: Concentration of Micronutrients (mg/kg) in the Fresh Vegetable Samples

Sample Name	Concentration of Micronutrients (mg/kg)								
	Zn	**Fe**	**Mn**	**Ca**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**
P1a	42.70	22.84	12.81	196.62	<0.1	<0.1	8.58	6.42	<0.5
P1b	43.84	49.82	12.26	700.48	<0.1	<0.1	1.86	<0.1	<0.5
P1c	35.41	19.67	12.59	369.86	<0.1	<0.1	1.45	<0.1	<0.5
P	40.6 5 ± 4.57	30.78 ± 16.57	12.55 ± 0.28	422.32 ± 255.99			3.96 ± 4.00		
KR1a	50.67	71.53	36.46	62.59	<0.1	0.16	7.40	<0.1	<0.5
KR1b	21.85	62.56	22.05	422.05	<0.1	<0.1	3.11	<0.1	<0.5
KR1c	35.94	91.85	45.43	352.44	<0.1	<0.1	6.51	4.00	<0.5
KR	36.15 ± 14.41	75.31 ± 15.00	34.65 ± 11.79	279.03 ± 190.64			5.67 ± 2.26		
T1a	46.39	17.40	19.37	336.35	0.17	<0.1	5.94	0.30	<0.5
T1b	54.91	40.17	19.28	720.41	<0.1	<0.1	3.84	<0.1	<0.5
T1c	48.79	20.91	17.12	502.79	<0.1	<0.1	2.06	<0.1	<0.5
T	50.03 ± 4.39	26.16 ± 12.25	18.59 ± 1.27	519.85 ± 192.50			3.95 ± 1.94		
KM1a	22.79	40.63	12.49	120.89	0.30	<0.1	2.51	<0.1	<0.5
KM1b	23.72	60.29	12.75	385.45	<0.1	<0.1	6.25	<0.1	0.5
KM1c	31.92	19.95	10.77	55.87	0.11	<0.1	6.11	<0.1	<0.5
KM	26.14 ± 5.02	40.29 ± 20.17	12.00 ± 1.07	187.40 ± 174.57			4.96 ± 2.12		
C1a	31.87	54.78	11.75	493.03	<0.1	<0.1	12.51	<0.1	<0.5
C1b	24.98	88.93	11.99	288.77	<0.1	<0.1	9.96	<0.1	<0.5
C1c	28.67	65.24	11.76	472.52	<0.1	<0.1	15.83	<0.1	<0.5
C	28.51 ± 3.45	69.65 ± 17.49	11.83 ± 0.13	418.11 ± 112.48			12.77 ± 2.94		

Micronutrient concentrations in vegetable samples ranged from 3.95±1.94 to 519.85±192.50 mg/kg. According to the table, the average concentration of calcium was the highest and it was

followed by zinc, iron, manganese and copper. The decreasing trend for average accumulated micronutrient concentrations in the vegetable samples is as follows: Tomato (618.58 mg/kg) > Cauliflower (540.87 mg/kg) > Pointed gourd (510.26 mg/kg) > Bitter gourd (430.81 mg/kg) > Chili (270.79 mg/kg).

3.16.4 Zinc Content

Zinc is discovered as zinc sulfide in the earth's crust. Zinc is beneficial in many cases and is less toxic than Cr, Cd and Pb, but excess consumption of zinc is harmful to humans. An overdose of zinc decreases the HDL cholesterol level and increases the risk of heart disease (Yilmaz, 2009). Ali & Al-Qahtani was reported that the concentration of Zn in vegetables was found between 3.56 and 4.59 mg/kg as recommended by the international standard (Ali & Al-Qahtani, 2012). According to WHO, generally, the set limit for Zn is 5 mg/kg (World Health Organization, 1994). Moreover, Elbagermi et al measured the heavy metal intake based on the consumption of vegetables. When the intake of Zn is 0.8 mg/day, the mean level of consumption is 8.15 mg/kg (Elbagermi et al., 2012**).** Three homogenized samples of each variety were used for the determination of heavy metals. The results have been expressed as both individual and mean with standard deviation **(**Table 3.33**)**. In the present study, the concentration of Zn was found to be highest in tomatoes (54.91 mg/kg), whereas the lowest amount was in the bitter gourd (21.85 mg/kg). Zn content averages 40.65 ± 4.57 mg/kg in pointed gourd, 36.15 ± 14.41 mg/kg in bitter gourd, 50.03 ± 4.39 mg/kg in tomato, 26.14 ± 5.02 mg/kg in chili and 28.51 ± 3.45 mg/kg in cauliflower.

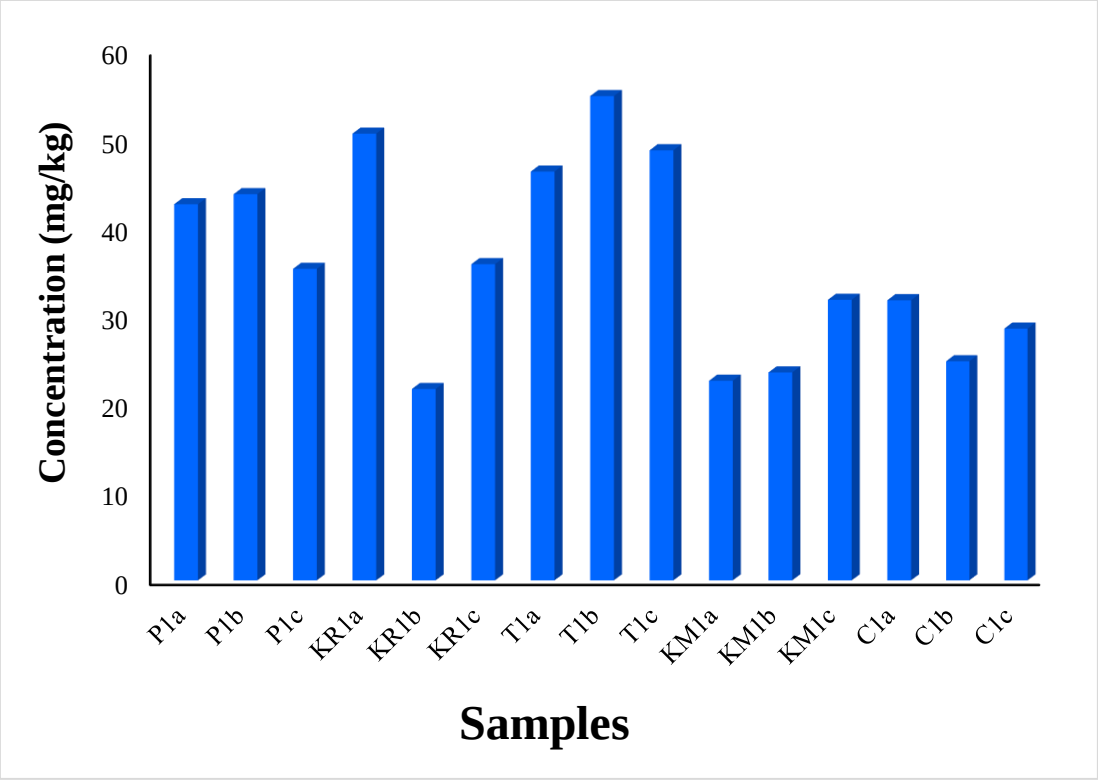


Figure 3.34: Concentration (mg/kg) of Zinc in vegetable samples

3.16.5 Iron Content

Iron is an important nutrient that plays a vital role in different functions in the human body. Iron deficiency causes a variety of health problems, including anemia, immune system dysfunction, and decreased reproductive capacity. However, an overdose of iron can affect you negatively too. Excess iron may have various harmful effects on the human body, like dizziness, vomiting, nausea, diarrhea, liver damage, cardiovascular disease, and metabolic functions. Bukva et al reported that the recommended daily intake of Fe may vary based on age and gender for example, 8 mg for males, 18 mg for women, 11 mg for adolescents, 10 mg for children, and 0.27 mg for infants (Bukva et al., 2019). Chiroma et al reported that the maximum permissible limit of iron in the vegetable samples is 425 µg/g (Chiroma et al., 2014). In the present study, the highest concentration of Fe was found in a bitter gourd with a value of approximately 91 mg/kg, which does not exceed the permissible limit. It was followed by cauliflower, with a value of around 89

mg/kg. The mean concentration of iron in a pointed gourd, bitter gourd, tomato, chili, and cauliflower was found to be 30.78 ±16.57, 75.31 ± 15.00, 26.16 ± 12.25, 40.29 ± 20.17, and 69.65 ± 17.49 mg/kg, respectively.

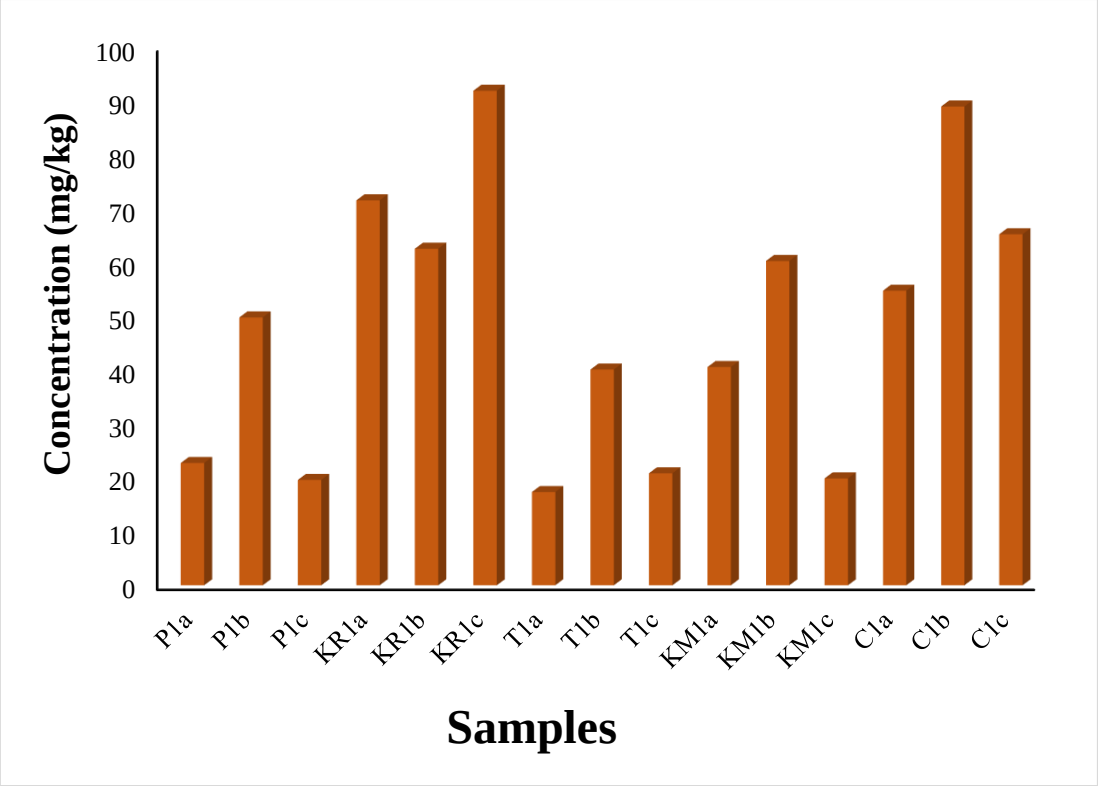


Figure 3.35: Concentration (mg/kg) of Iron in vegetable samples

3.16.6 Manganese Content

Manganese is a mineral element that has both nutritional and toxic effects. It plays a vital role as a constituent of multiple enzymes in different physiologic processes and it is an activator of other enzymes (Nielsen, 1999). Ugulu's et al reported that Mn is an important element for plant growth and is necessary for a small amount. It also aids in the enhancement of various biochemical activities, such as the antioxidant enzyme superoxide dismutase (Ugulu, 2015). WHO and FAO jointly recommended the permissible limit for manganese be 500 mg/kg in vegetables (FAO/WHO, 2001).

The present study reveals that the manganese content in different vegetables ranged from 10.77 to 45.43 mg/kg, which is lower than the permissible limit for manganese. The highest Mn content was found in bitter gourd and the lowest was in chili. The mean concentrations of the highest and lowest are 34.65 ± 11.79 and 12.00 ± 1.07 mg/kg, respectively. The concentration of Mn were found to be 12.55 ± 0.28 mg/kg in pointed gourd, 18.59 ± 1.27 mg/kg in tomato and 11.83 ± 0.13 mg/kg in cauliflower. Since the concentration of Mn content in all vegetables has been observed under the permissible limit, these vegetables will be helpful for people in their metabolic function.

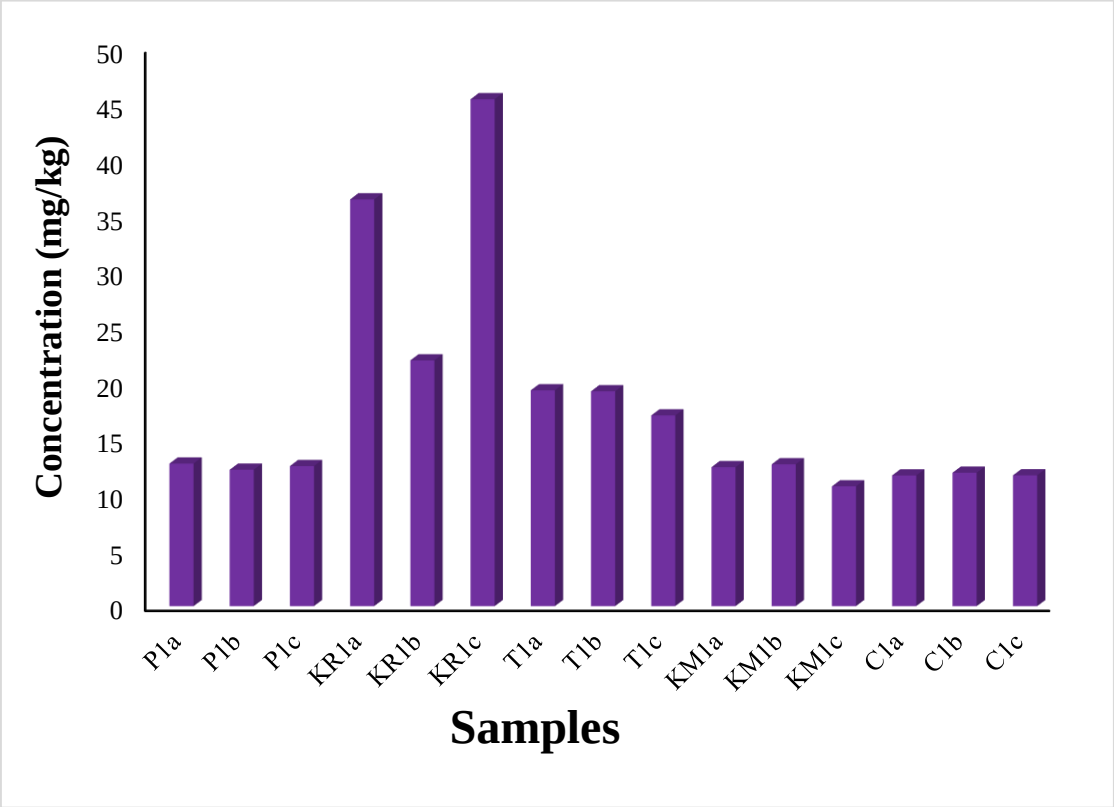


Figure 3.36: Concentration (mg/kg) of Mn in vegetable samples

3.16.7 Calcium Content

Calcium is an element that acts as a mineral and is often linked with healthy bones and teeth. It is a very important element for blood clotting, building up muscles, and regulating normal heart rhythms and functions of nerves. The bones contain almost 99% of the body's calcium, and the rest 1% is stored in blood, tissues, and muscles. The main sources of calcium are milk, cheese, dairy foods, green leafy vegetables, etc. Overdosage of calcium supplements, for example, more

than 1500 mg/day, may result in a variety of side effects such as stomach pain, diarrhea, and other gastrointestinal effects. If there is no nutritional disorder and there is enough vitamin D in the human body, an intake of calcium of even below 300 mg or above 1000 mg a day is harmful (Reid et al., 2015). The FAO and WHO jointly recommended even lower intakes of calcium for adults of 400-500 mg/day in 1974 (World Health Organisation, 1974). The study reveals that the calcium content in various vegetables collected from different markets ranged from 55.87 to 720.41 mg/kg. The highest concentration of Ca was found in tomatoes and it was followed by pointed gourds with 700 mg/kg. Most vegetables contain a good amount of calcium, which means eating vegetables every day can fulfill our body's calcium demand, and as a natural mineral, calcium does not have any side effects. The mean concentrations of Ca in pointed gourd, bitter gourd, tomato, chili, and cauliflower were estimated as 422.32 ± 255, 279.03 ± 190, 519.85 ± 192, 187.40 ± 174, and 418.11 ± 112 mg/kg, respectively.

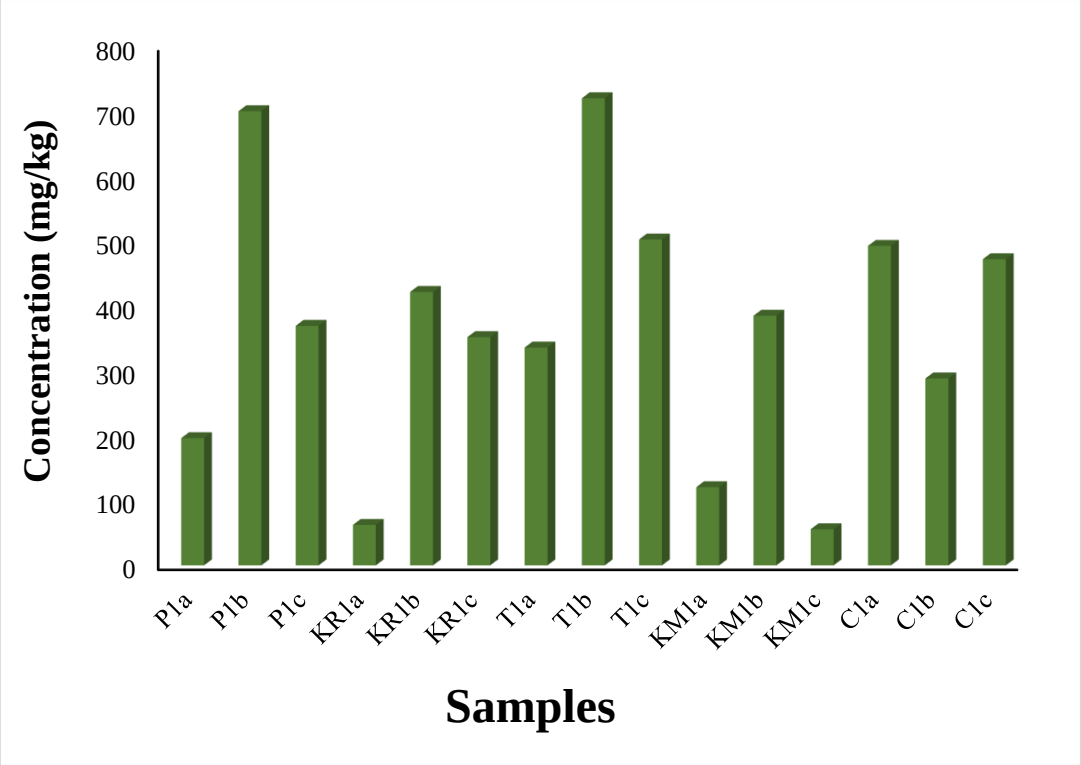


Figure 3.37 : Concentration (mg/kg) of Calcium in vegetable samples

3.16.8 Cadmium Content

Cadmium is considered an environmental pollutant. The source of Cd in the human body is food consumption. Severe toxic symptoms resulting from Cd ingestion are reported between 10 and 326 mg. Fatal ingestions of Cd, producing shock and acute renal failure, occur from ingestions exceeding 350 mg/g. The maximum acceptable level of Cd is 0.05 mg/kg as recommended by the European Community legislation (EC, 2001). Heavy metals are absorbed by vegetables from the soil as well as from surface deposits (Naser et al., 2009). The permissible limit of Cd is 0.2 mg/kg of fresh weight for leafy vegetables and 0.1 mg/kg for stem and root vegetables, set by the Commission of the European Communities (CEC) and World Health Organization (WHO) (Jamali et al., 2007). In this study, the concentration of Cd was found to be less than 0.1 in all samples except tomato with 0.17 mg/kg and chili (0.1-0.3 mg/kg).

3.16.9 Chromium Content

Chromium (Cr) is present in the earth's crust with oxidation states ranging from chromium (II) to chromium (VI). Roussel reported that the acceptable daily intake (ADI) of Cr in elderly people in the USA is 25–37 mg. However, in Sweden, Switzerland, and Finland, the ADI is 50 mg or lower (Roussel et al., 2007). The concentration of Cr in the present study is less than 0.1 mg/kg in all studied vegetables without the exception of bitter gourd with a value of 0.16 mg/kg, but its mean value should be lower. The presence of Cr in the diet has an active involvement in lipid and glucose metabolism. Cr deficiency can impair progress and denature lipid, protein, and glucose metabolism. However, extreme intake of Cr may cause acute pulmonary disorders and damage potential organs like the liver, lungs, and kidney.

The concentration of chromium increases in the environment due to wastewater releases containing chromium, mainly from metallurgical, refractory, and chemical industries. Non-occupational exposure occurs by eating food and drinking water that is contaminated by chromium, whereas occupational exposure is by the respiratory system. Several regulatory and non-regulatory agencies have classified hexavalent chromium [Cr (VI)] as a human carcinogen, which is a toxic industrial pollutant. Adverse health effects induced by Cr (VI) have also been reported in humans. Oxidative damage is considered to be the underlying cause of the genotoxic effects, including chromosomal abnormalities and DNA strand breaks.

3.16.10 Copper Content

Copper is an essential part of several enzymes and it is necessary for the synthesis of hemoglobin, but excess intake of copper can alter liver and kidney function. It was reported that the acceptable limit of copper for human consumption is 10 mg/kg (Amos-Tautua and Onigbinde, 2014). Johnson reported that the usual range of copper in vegetables and fruits is between 0.1 and 0.2 mg/kg (Johnson, 1997). The decreasing trends were seen for the mean concentration of Cu of the selected vegetables were as: cauliflower (12.77 ± 3 mg/kg) > bitter gourd (5.67 ± 3 mg/kg) > chili (4.96 ± 3 mg/kg) > pointed gourd (3.96 ± 4 mg/kg) > tomato (3.95 ± 2 mg/kg). It was reported that the acceptable concentration of copper content is 4.0 mg/100 g dry weight by FAO/WHO in vegetables (Chove et al., 2006). According to FAO/WHO, the mean concentration of copper content exceeds the acceptable limit found in cauliflower and bitter gourd. In the other three vegetables, the values are under the permissible limit.

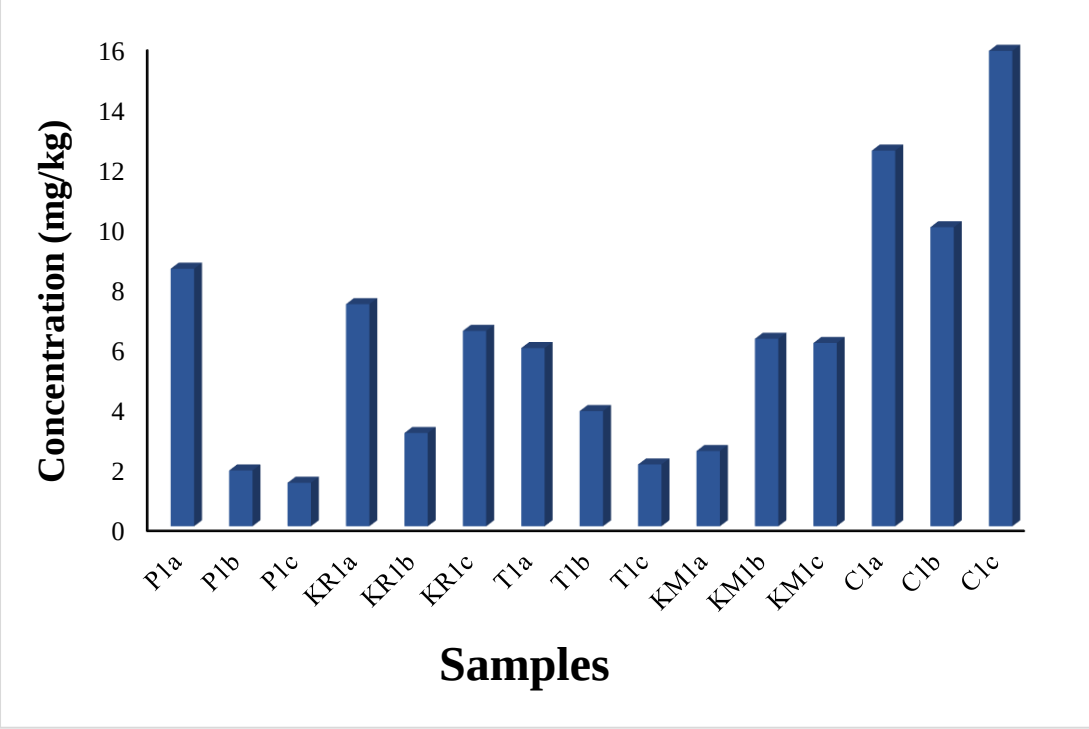


Figure 3.38: Concentration (mg/kg) of Copper in vegetable samples

3.16.11 Nickel Content

The major source of Ni for the human body is food and uptake from natural sources, as well as food processing. It is also an environmental pollutant. An increased incidence of cancer of the lung and nasal cavity caused by a high intake of Ni has also been reported in workers in Ni smelters. Scancar et al reported that the average recommended dietary daily intake is approximately 0.1–0.3 mg of Ni per day and a person living in an urban area usually inhales approximately 0.0004–0.0008 mg of Ni per day (Scancar et al., 2013). According to the Prevention of Food Adulteration Act (PFA), the maximum acceptable limit for Ni is 5 mg/kg (Naser et al., 2009). Yusuf et al. note that since heavy metals are absorbed by vegetables from the soil, the presence of these elements in fertilizers contributes to their being absorbed by various vegetables and results in metal pollution (Yusuf et al., 2003). The concentration of Ni in these studies reveals less than 0.1 mg/kg for most of the vegetables. However, the Ni content of three different vegetables, such as pointed gourd, bitter gourd, and tomato, varies; for example, 6.42 mg/kg for pointed gourd, 4 mg/kg for bitter gourd, and 0.30 mg/kg for tomato, but their mean value should be lower.

3.16.12 Lead (Pb) content

Lead is a significant environmental pollutant. Lead poisoning causes neurotoxicity and cellular inactivation and binds to gastrointestinal enzymes and the renal system. Anthropogenic activities like industry, agriculture, and urban lifestyle enhance the Pb content in vegetables through water and soil (Alegria et al., 1991). Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults. It was reported by FAO/WHO that the maximum permissible concentration of Pb is 0.5 mg/100 g dry weight (Chove et al., 2006). The study describes that the concentration of Pb is less than 0.5 mg/kg in all vegetable samples, which indicates that the vegetables are safe and under the permissible limit reported by FAO.

4. CONCLUSION

Bangladesh is an agro-based country where the majority of the population are directly or indirectly involved in the agricultural sector. Many of the early pesticides, such as DDT and other organochlorine compounds, are often highly toxic, very persistent or pose a threat to the environment. At present, pesticides are very carefully researched compounds to ensure their effectiveness against target organisms, are easily degradable, safe for the environment, and can be used without hazards to the operators or consumers. Pesticide residues in food and crops are a direct result of pesticide application to agricultural fields. There is an urgent need to establish a reliable level of pesticide residues for various agricultural products available on the market.

This study includes the assessment of organochlorine pesticides residue in the vegetable samples. The concentration of 20 OCPs ranged 0.68-84.88 ng/g. It was found that 4,4′-DDT was predominant in all the samples from different market. Alpha-BHC, gamma-BHC, beta- BHC, delta-BHC, heptachlor, aldrin, heptachlor-epoxide, *trans*-chlordane, *cis*- chlordane, endosulfan-I, dieldrin, endrin, endosulfan- II, endrin aldehyde, endosulfan sulphate, methoxychlor and endrin ketone were below detection limit in all the samples and other pesticides were present in moderate level. 4,4′-DDE, 4,4′-DDD and 4,4′-DDT were found in pointed gourd and chili from Hatirpool Bazar. 4,4′-DDT was also found in bitter gourd from Hatirpool Bazar. 4,4′-DDT was found in all five types of vegetables collected from Kaptan Bazar, Shibpur Bazar and College gate Bazar. 4,4′-DDD was also found in chili collected from Kaptan Bazar. Fatty acid composition was analyzed by GC-FID comparing retention time with 13 standards. Palmitoleic acid, octadecanoic acid and erucic acid were found among tomato, chili and bitter gourd.

Determination of Protein by Kjeldhal method was analyzed in five different vegetable samples. The highest protein content (2.69%) was found in cauliflower and the lowest protein content (0.71%) was found in chili. In pointed gourd, bitter gourd and tomato, the protein content was determined as 0.73%, 1.81% and, 1.02%, respectively. The methanol extract of chili contained the maximum amount of phenolic content with 33.86 ± 4.34 mg gallic acid equivalent per gram (GAE/g) of dry extract, and it was followed by cauliflower with 32 mg GAE/g. However, the highest amount of flavonoid content was found in bitter gourd with 37.64 ± 3.72 mg quercetin equivalent per gram (QE/g) of dry extract. Bitter gourd, and chili showed the highest antioxidant

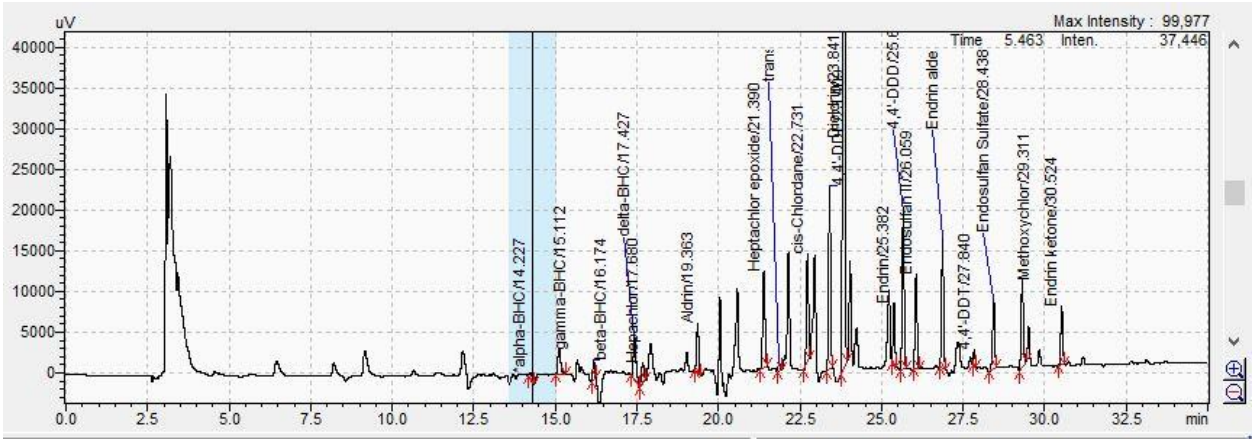
capacity with a value of 53 mg ascorbic acid equivalent per gram (AAE/g) of dry extract compared to pointed gourd, tomato and cauliflower. Total carbohydrate among five different vegetable samples was the highest in chili with 8.4±4.56 g/100g.

Analysis of micronutrients was also carried out. Nine heavy metals (Zn, Fe, Mn, Ca, Cd, Cr, Cu, Ni and Pb) in the vegetables were analyzed by AAS. Zinc was found in pointed gourd (40.65 ppm), bitter gourd (36.15 ppm), tomato (50.03 ppm), chili (26.14 ppm) and cauliflower (28.51ppm). Nickel was found in three samples: pointed gourd (6.42 ppm), bitter gourd (4.00 ppm) and tomato (0.30 ppm). Iron is naturally present in all vegetables. So all the vegetables contained iron in varying concentrations ranged 26.16-75.31 ppm. Though it is very important for the body but excessive presence of Fe can be harmful for health. Manganese was found in the highest concentration in bitter gourd (34.65 ppm) and the lowest in cauliflower (11.83 ppm). Calcium content was present in all vegetables and the values are 422.32 ppm for pointed gourd, 279.03 ppm for bitter gourd, 519.85 ppm for tomato, 187.40 ppm for chili and 418.11 ppm for cauliflower. Copper was found in five vegetables between 3.95 to 12.77 ppm.

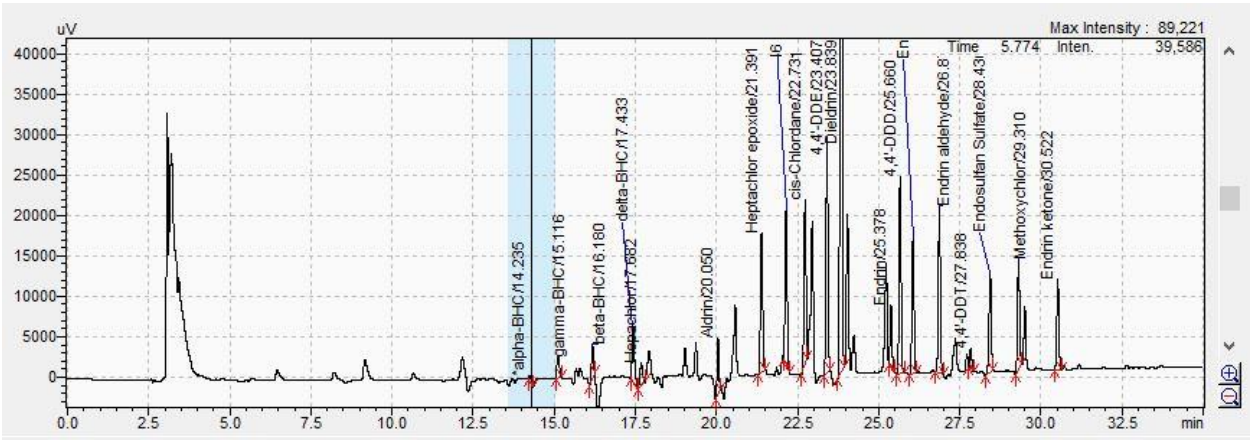
The highest number of pesticides were present in samples collected from Hatirpool Bazar, Dhaka and the lowest number of pesticides were present in samples collected from College gate Bazar, Narsingdi. The more pesticides were identified in samples from Dhaka (Hatirpool and Kaptan Bazar) than the samples from Narsingdi (Shibpur and College gate Bazar).

As farmers are applying pesticides, they need proper awareness and knowledge so that they will not misuse the pesticides. Accumulation of pesticides in human body via consumption of foods can create several health problems. Some important steps such as use of natural pesticides or bio-pesticides, properly harvesting and washing of vegetables, encouraging organic farming, creating awareness among farmers and implementation of pesticides related laws properly may reduce the contamination of pesticides. It can be recommended that before cooking vegetables should be washed several times with water and heat should be used properly so that pesticides can easily be degraded before entering human body.

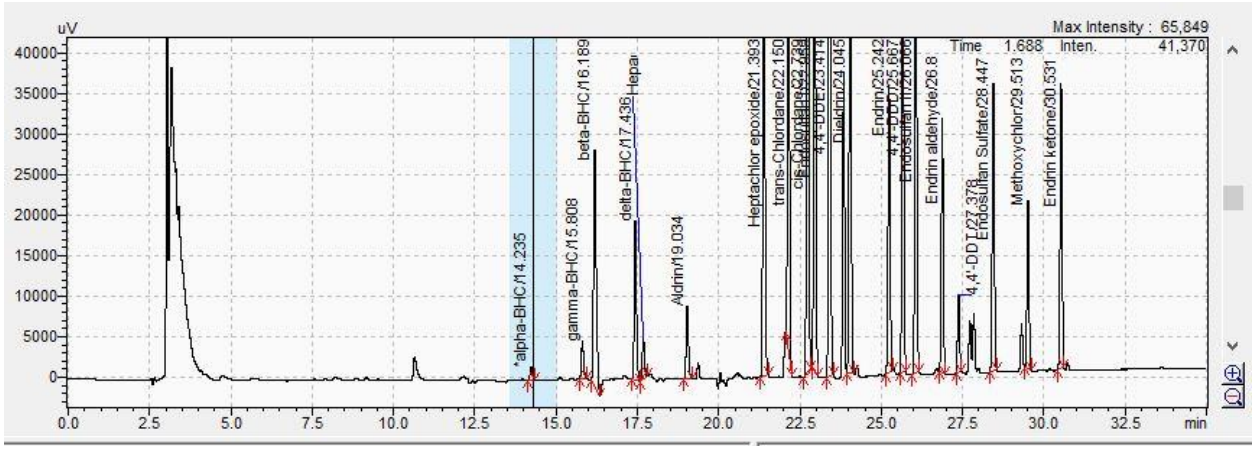
Chromatogram 1: Chromatogram of blank



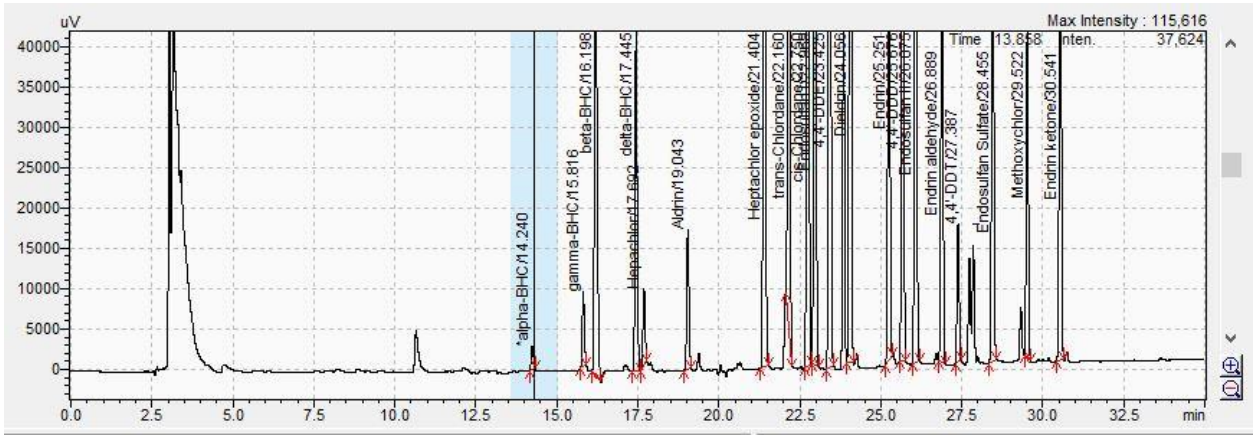
Chromatogram 2: Chromatogram of standard pesticides (5 ng/g)



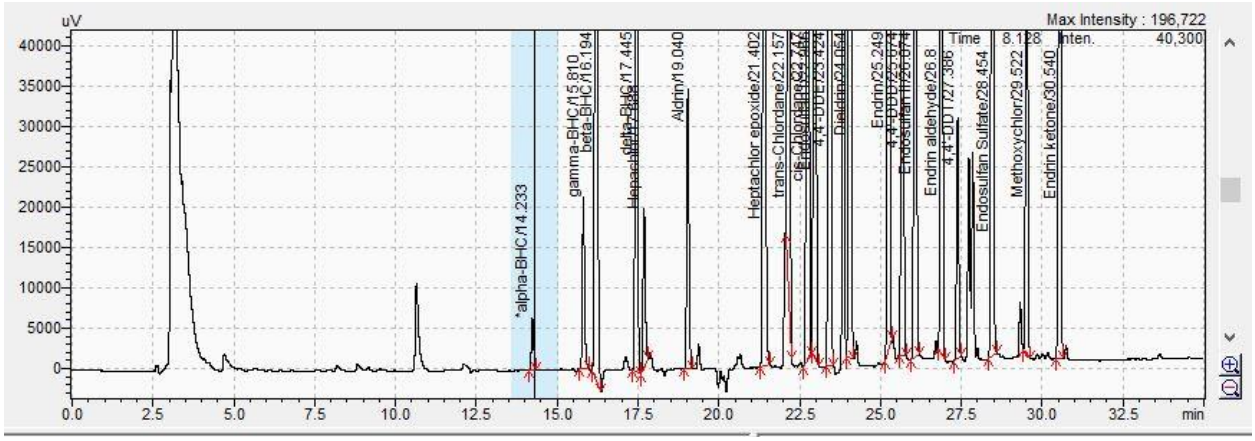
Chromatogram 3: Chromatogram of standard pesticides (10 ng/g)



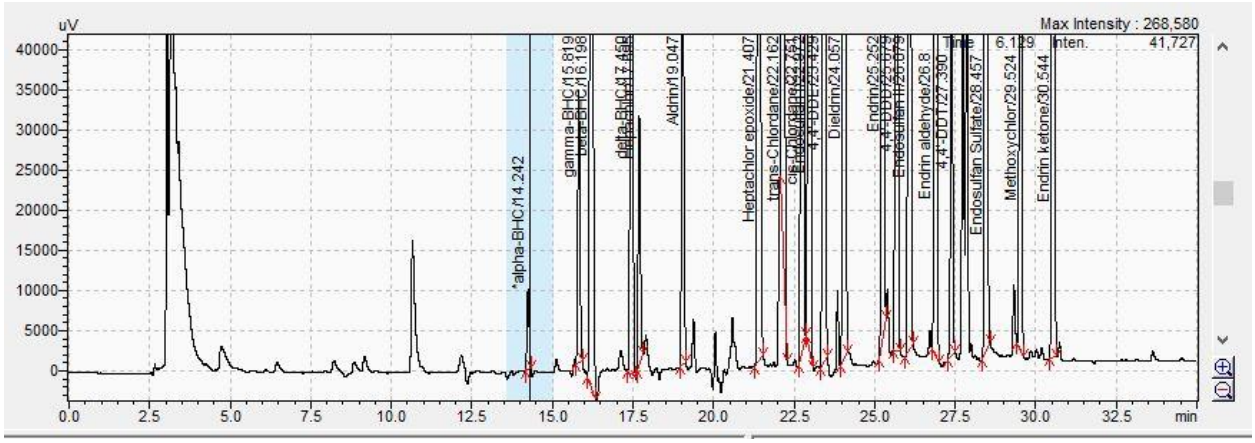
Chromatogram 4: Chromatogram of standard pesticides (25 ng/g)



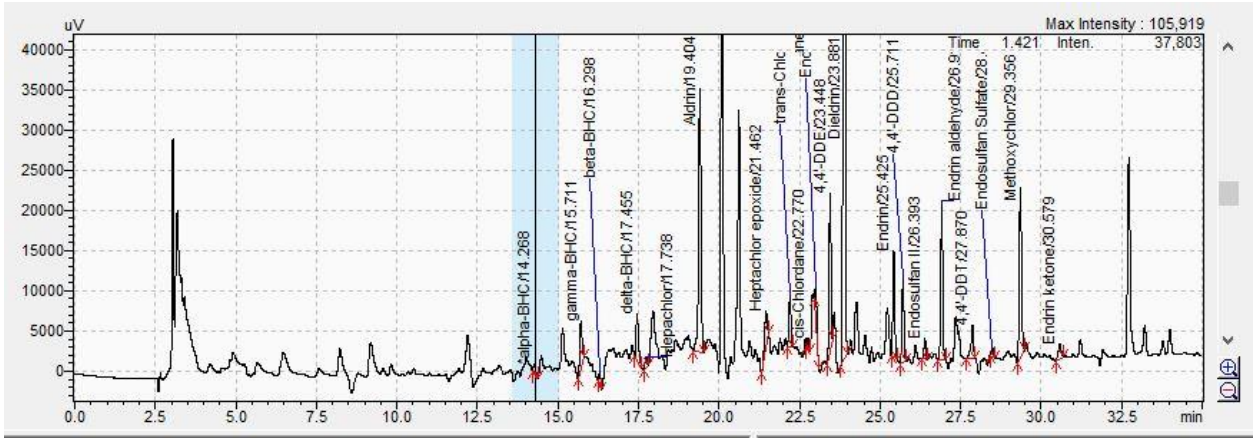
Chromatogram 5: Chromatogram of standard pesticides (50 ng/g)



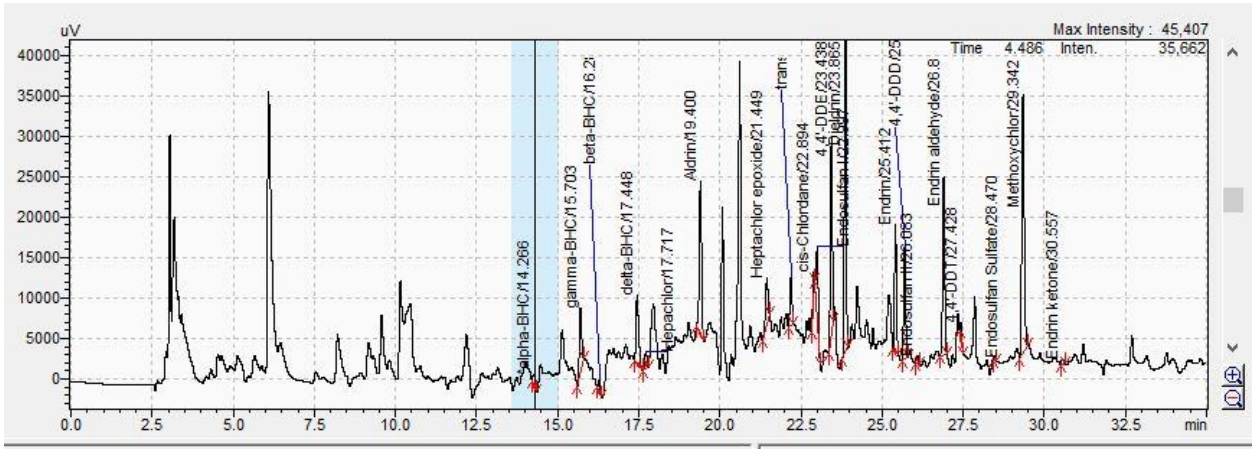
Chromatogram 6: Chromatogram of standard pesticides (100 ng/g)



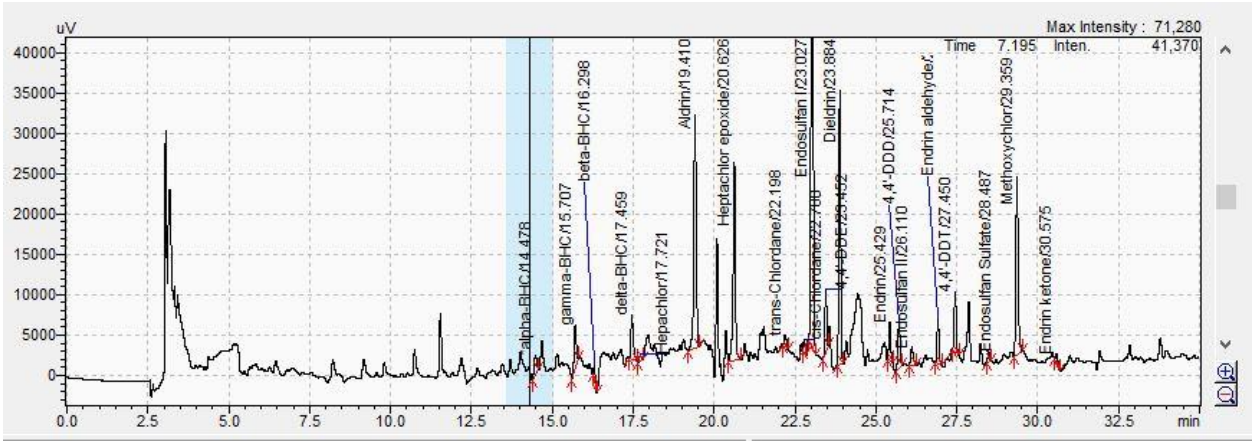
Chromatogram 7: Chromatogram of standard pesticides (200 ng/g)



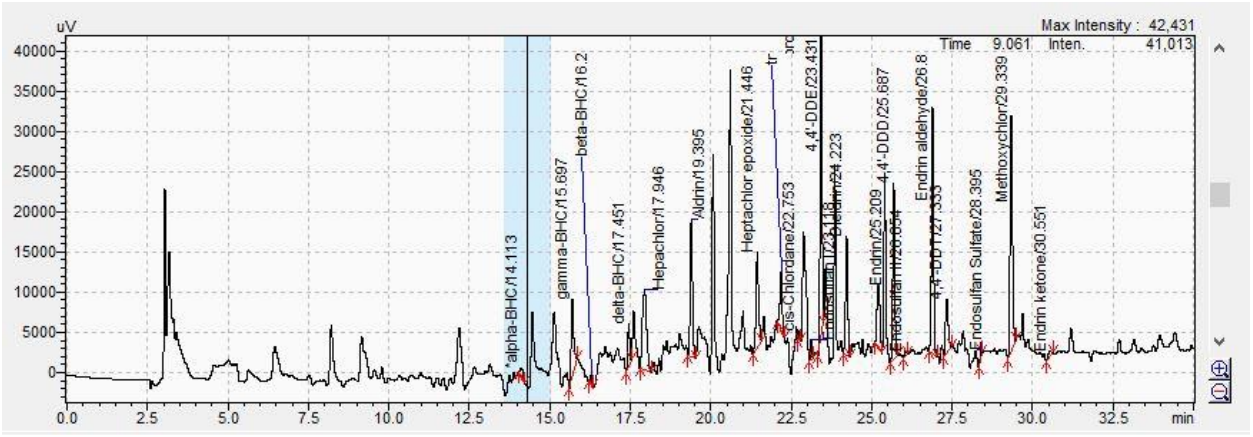
Chromatogram 8: Chromatogram of spiked pointed gourd



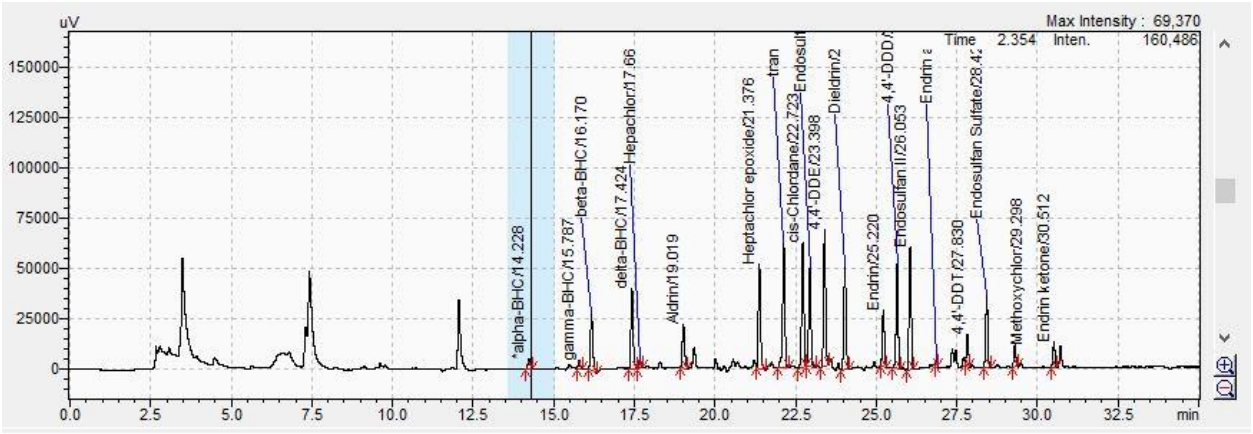
Chromatogram 9: Chromatogram of spiked bitter gourd



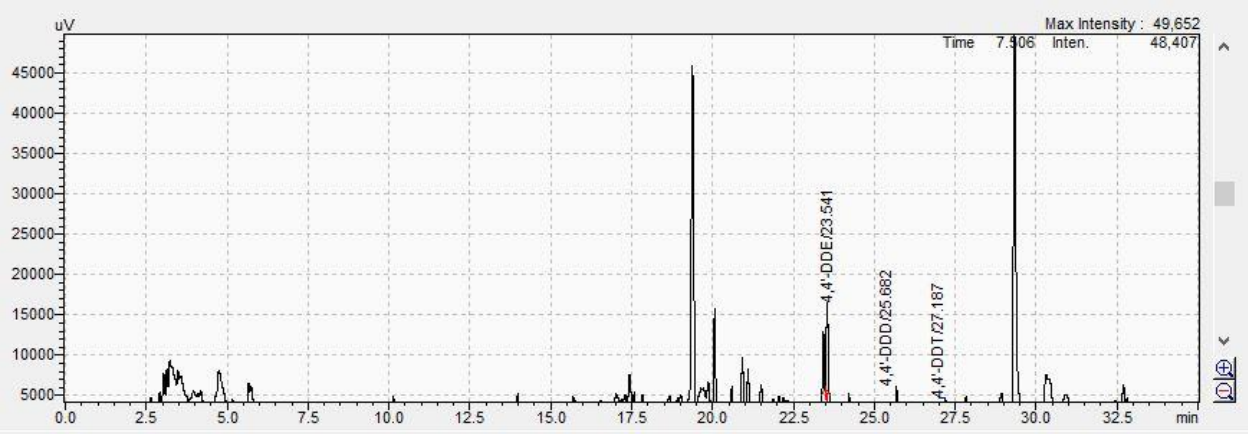
Chromatogram 10: Chromatogram of spiked tomato



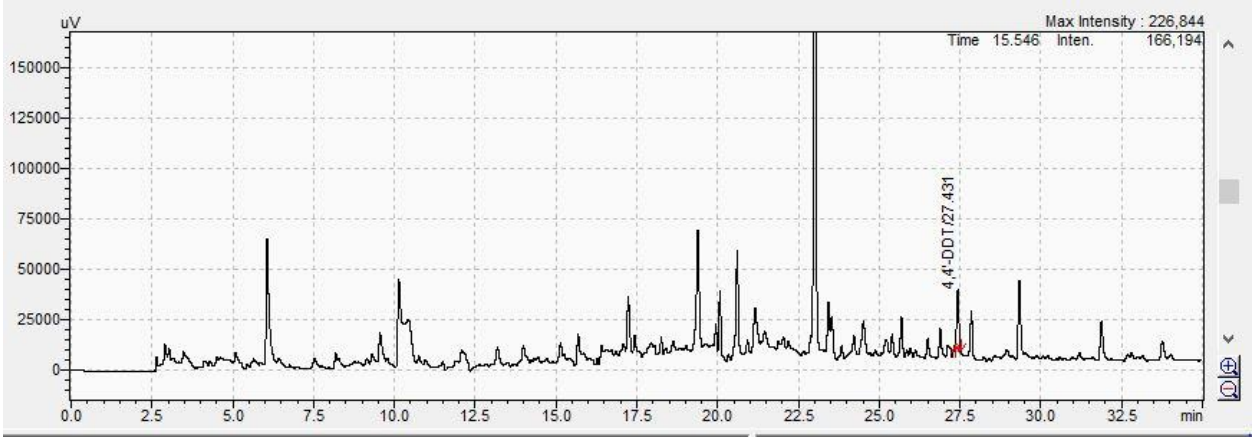
Chromatogram 11: Chromatogram of spiked chili



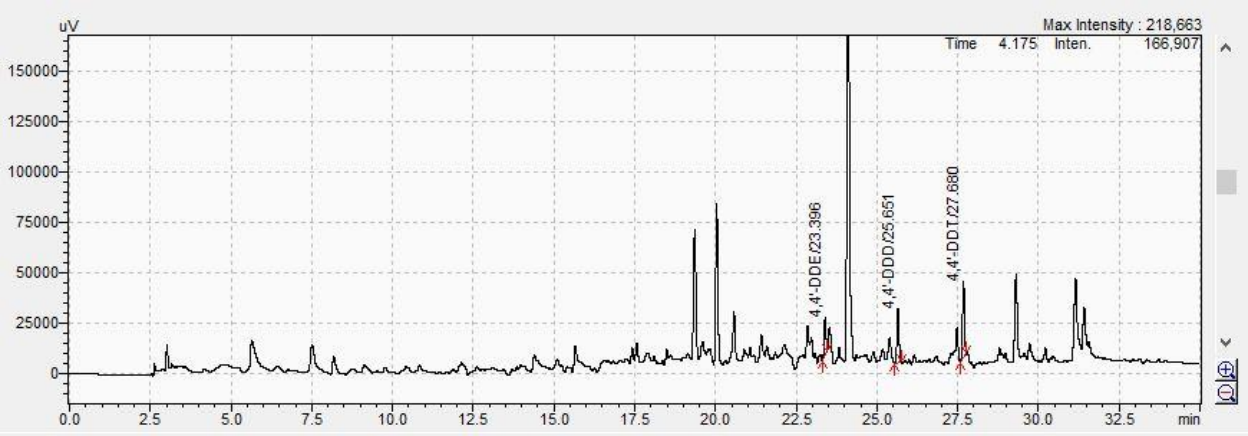
Chromatogram 12: Chromatogram of spiked cauliflower



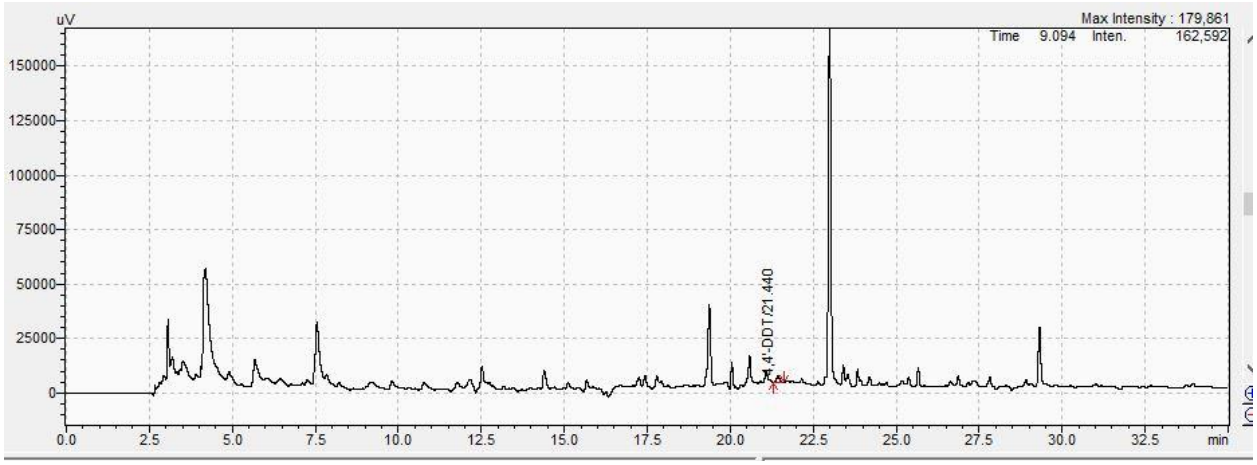
Chromatogram 13: Chromatogram of pointed gourd from Hatirpool Bazar



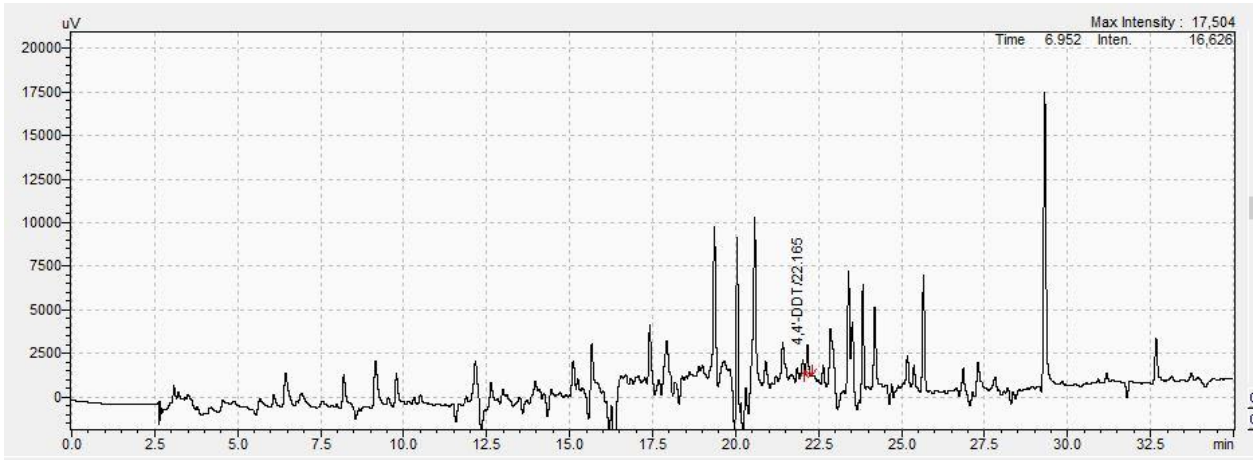
Chromatogram 14: Chromatogram of bitter gourd from Hatirpool Bazar



Chromatogram 15: Chromatogram of chili from Hatirpool Bazar

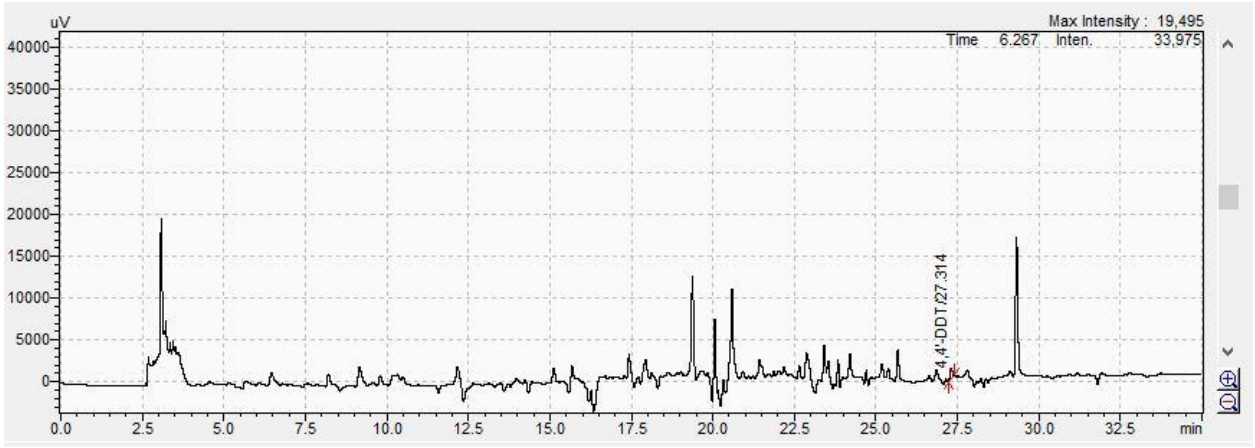


Chromatogram 16: Chromatogram of pointed gourd from Kaptan Bazar



Chromatogram 17: Chromatogram of bitter gourd from Kaptan Bazar

Chromatogram 18: Chromatogram of tomato from Kaptan Bazar



Chromatogram 19: Chromatogram of bitter gourd from Shibpur Bazar

Chromatogram 20: Chromatogram of chili from College gate Bazar

5. REFERENCES

Abascal, K., and Yarnell, E. (2005). Using bitter melon to treat diabetes. *Journal of Alternative and Complementary Therapies*, *11*(4), 179-184.

Abadin, H. G., and Goetchius, P. F. (1994). Agency for Toxic Substances and Disease Registry.

Abreu-Villaca, Y., and Levin, E. D. (2017). Developmental neurotoxicity of succeeding generations of insecticides. *Journal of Environment International*, *99*, 55-77.

Adeyemi, D., Anyakora, C., Ukpo, G., Adedayo, A., and Darko, G. (2011). Evaluation of the levels of organochlorine pesticide residues in water samples of Lagos Lagoon using solid phase extraction method. *Journal of Environmental Chemistry and Ecotoxicology*, *3*(6), 160-166.

Adeyi, A. A., Babalola, B., and Akpotu, S. O. (2021). Occurrence, distribution, and risk of organochlorine pesticides in food and greenness assessment of method. *Journal of Environmental Science and Pollution Research*, *28*(25), 33433-33444.

Afful, S., Anim, A. K., and Serfor-Armah, Y. (2010). Spectrum of organochlorine pesticide residues in fish samples from the Densu Basin. *Research Journal of Environmental and Earth Sciences*, *2*(3), 133-138.

Afify, A. S., Abdalla, A. A., Elsayed, A., Gamuhay, B., Abu-Khadra, A. S., Hassan, M., and Mohamed, A. (2017). Survey on the moisture and ash contents in agricultural commodities in Al-Rass Governorate, Saudi Arabia in 2017. *Assiut Journal of Agricultural Sciences*, *48*(6), 55-62.

Aktar, M. W., Sengupta, D., and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Journal of Interdisciplinary Toxicology*, *2*(1), 1-12.

Alavanja, M. C. R.., Hoppin, J. A., and Kamel, F. (2004). Health effects of chronic pesticide exposure: Cancer and neurotoxicity. *Annual Review of Public Health*, 25, 155-197.

Alegria, A., Barbera, R., Boluda, R., Errecalde, F., Farré, R., and Lagarda, M. J. (1991). Environmental cadmium, lead and nickel contamination: possible relationship between soil and vegetable content. *Fresenius' journal of analytical chemistry*, *339*(9), 654-657.

Ali, M. H. H., and Al-Qahtani, K. M. (2012). Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. *The Egyptian Journal of Aquatic Research,* 38 (1): 31–37.

Amos-Tautua, B. M. W., and Onigbinde, A. O. (2014). Estimation of iron, copper and zinc in some vegetables commonly consumed in Amassoma, Niger Delta, Nigeria. *Pakistan Journal of Nutrition.* 13(12), 742-745.

Anger, J., Kintz, P., Rennes, U. De, and Cedex, R. (2009). Difficultés analytiques de la caractérisation des pesticides dans le sang, Analytical Difficulties in the Characterization of Pesticides in Blood. *Journal of Annales de Toxicologie Analytique,* 21 (3), 131-141.

AOAC (2005) Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of Antiandrogenic_and_estrogenic_compounds_effect_on_development_and_function_of_male_reproductive_system.pdf

Aprea, C., Colosio, C., Mammone, T., Minoia, C., and Maroni, M. (2002). Biological monitoring of pesticide exposure: a review of analytical methods. *Journal of Chromatography B*, *769*(2), 191-219.

Arruti, A., Fernández-Olmo, I., and Irabien, Á. (2010). Evaluation of the contribution of local sources to trace metals levels in urban $PM_{2.5}$ and PM_{10} in the Cantabria region (Northern Spain). *Journal of environmental monitoring*, 12(7), 1451-1458.

ATSDR, T. (2000). ATSDR (Agency for toxic substances and disease registry). *Prepared by clement international corp., under contract*, *205*, 88-0608.

Atuanya, E. I., and Onuoha, T. (2018). Level of organochlorine pesticide residues in selected consumable vegetables commonly sold in Benin city markets. *Journal of Applied Sciences and Environmental Management*, 22(10), 1625-1630.

Baselt, R. (2014). Encyclopedia of Toxicology. *Journal of Analytical Toxicology, 38(7), 464–464.* https://doi.org/10.1093/jat/bku065

Baselt, R. C., and Cravey, R. H. (1982). *Disposition of toxic drugs and chemicals in man* (v. 8). Davis, CA: Biomedical publications.

Belitz, H. D., Grosch, W., and Schieberle, P. (2004). Vegetables and vegetable products. *In Food Chemistry*, 772-805.

Bempah, C. K., Buah-Kwofie, A., Denutsui, D., Asomaning, J., and Tutu, A. O. (2011). Monitoring of pesticide residues in fruits and vegetables and related health risk assessment in Kumasi Metropolis, Ghana. *Research Journal of Environmental and Earth Sciences*, *3*(6), 761-771.

Bernal, E. (2014). Limit of Detection and Limit of Quantification, Determination in Gas Chromatography. Advances in Gas Chromatography, 57-81.

Berntssen, M. H., Maage, A., and Lundebye, A. K. (2017). Chemical contamination of finfish with organic pollutants and metals. In *Chemical Contaminants and Residues in Food* 517-551. Woodhead Publishing.

Biziuk, M. (2001). Pestycydy, Występowanie, Oznaczanie i Unieszkodliwiani, WNT, Warszawa.

Blus, L. J. (2011). 12 DDT, DDD and DDE in Birds. Environmental Contaminants in Biota. 425-444.

Borgi, L. (2019). Inclusion of phosphorus in the nutrition facts label. *Clinical Journal of the American Society of Nephrology*, *14*(1), 139-140.

Bradl, H. (2005). Heavy Metals in the Environment: origin, interaction and remediation. Academic press.

Bukva, M., Kapo, D., Huseinbašić, N., Gojak-Salimović, S., and Huremović, J. (2019). Iron content in fruits, vegetables, herbs and spices samples marketed in Sarajevo, Bosnia and Herzegovina. *Kemija u industriji: Časopis kemičara i kemijskih inženjera Hrvatske*, *68*(7-8), 281-287.

Burton-Freeman, B., and Reimers, K. (2011). Tomato consumption and health: Emerging benefits. *American Journal of Lifestyle Medicine*, 5(2), 182-191.

Cairns, T., and Sherma, J. (1992). Emerging strategies for pesticide analysis. vol. 1. CRC press.

Cann, M. (2005). Environmental Chemistry. Macmillan.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, *10*(3), 178-182.

Chang, L. W., Magos, L., and Suzuki, T. (1996). *Toxicology of metals*. Boca Raton, FL: CRC. 9-28.

Chapman, P. M. (2002). Integrating toxicology and ecology: putting the “eco” into ecotoxicology. Marine pollution bulletin, 44(1), 7-15.

Chiroma, T. M., Ebewele, R. O., and Hymore, F. K. (2014). Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. *International refereed journal of engineering and science*, *3*(2), 01-09.

Chove, B. E., Ballegu, W. R., and Chove, L. M. (2006). Copper and Lead levels in two popular leafy vegetables grown around Morogoro Municipality, Tanzania. *Tanzania Journal of Health Research*, 8(1), 37-40.

Comhaire, F. (2014). Nutriceutical approach to the metabolic syndrome. *Journal of Endocrinology and Metabolic Syndrome*, *3*(3), 1-4.

Connett, P. H., and Wetterhahn, K. E. (1983). Metabolism of the carcinogen chromate by cellular constituents. In *Inorganic elements in biochemistry* (pp. 93-124). Springer, Berlin, Heidelberg.

Corpas, I., Gaspar, I., Martínez, S., Codesal, J., Candelas, S., and Antonio, M. T. (1995). Testicular alterations in rats due to gestational and early lactational administration of lead. *Reproductive Toxicology*, *9*(3), 307-313.

Cotton, F. A., Wilkinson, G., Murillo, C. A., and Bochmann, M. (1999). *Advanced inorganic chemistry*. John Wiley and Sons, Inc.

De Flora, S., Bagnasco, M., Serra, D., and Zanacchi, P. (1990). Genotoxicity of chromium compounds. A review. *Mutation Research/Reviews in Genetic Toxicology*, *238*(2), 99-172.

De Pascale, S., Maggio, A., Pernice, R., Fogliano, V., and Barbieri, G. (2007). Sulphur fertilization may improve the nutritional value of Brassica rapa L. subsp. sylvestris. *European Journal of Agronomy*, *26*(4), 418-424.

Dhaliwal, M. S. (2017). Classification of Vegetable Crops.

Duffus, J. H. (2002). Heavy metals a meaningless term? (IUPAC Technical Report). *Journal of Pure and applied chemistry*, *74*(5), 793-807.

Elbagermi, M. A., Edwards, H. G. M., and Alajtal, A. I. (2012). Monitoring of heavy metal content in fruits and vegetables collected from production and market sites in the Misurata area of Libya. *International Scholarly Research Notices*, *2012*, 1-5. doi:10.5402/2012/827645.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of European Commission*, *364*, 5-2.

FAO/WHO Codex Alimentarius Commission. (2001). Food additives and contaminants. Joint FAO. *WHO Food Standards Programme, ALINORM*, *1*, 1-289.

Fenik, J., Tankiewicz, M., and Biziuk, M. (2011). Properties and determination of pesticides in fruits and vegetables. *Journal of TrAC Trends in Analytical Chemistry*, *30*(6), 814-826.

Fergusson, J. E. (1990). The heavy elements: chemistry, environmental impact and health effects. Pergamon, Oxford.

Flora, S. J., Flora, G., and Saxena, G. (2006). Environmental occurrence, health effects and management of lead poisoning. In *Lead*, 158-228. Elsevier Science BV.

Florence, C., Philippe, L., and Magalie, L. J. (2015). Organochlorine (chlordecone) uptake by root vegetables. *Chemosphere*, 118, 96-102.

Gabby, P. N. (2006). Lead: in mineral commodity summaries. *Reston, VA: US, Geological Survey* Food and Agriculture Organisation of the United Nations, World Health Organisation. Calcium. In: World Health Orga-nisation, ed.Handbook on Human Nutritional Requirements.Geneva: World Health Organisation, 1974, 49–52.

Grilo, T. F., Cardoso, P. G., Pato, P., Duarte, A. C., and Pardal, M. A. (2013). Organochlorine accumulation on a highly consumed bivalve (Scrobicularia plana) and its main implications for human health. *Journal of Science of the Total Environment*, *461*, 188-197.

Hajšlová, J., and Zrostlıkova, J. (2003). Matrix effects in (ultra) trace analysis of pesticide residues in food and biotic matrices. *Journal of Chromatography A*, 1000(1-2), 181-197.

Hayes, K. C., and Expert Panel. (2010). Fatty Acid Expert Roundtable: Key Statements about Fatty Acids. *Journal of the American College of Nutrition*, *29*(sup3), 285S-288S.

He, Z. L., Yang, X. E., and Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *Journal of Trace elements in Medicine and Biology*, *19*(2-3), 125-140.

Hejmej, A., Kotula-Balak, M., and Bilińska, B. (2011). Antiandrogenic and estrogenic compounds: Effect on Development and Function of Male Reproductive System. *Steroids-Clinical Aspect.* 51-82.

Hossain, M. M., and Abdulla, F. (2015). On the production behaviors and forecasting the tomatoes production in Bangladesh. *Journal of Agricultural Economics and Development*, 4(5), 66-74.

Huel, G., Tubert, P., Frery, N., Moreau, T., and Dreyfus, J. (1992). Joint effect of gestational age and maternal lead exposure on psychomotor development of the child at six years. *Neurotoxicology*, *13*(1), 249-254. https://www.researchgate.net/publication/312583829.

Jahangir, M., Kim, H. K., Choi, Y. H., and Verpoorte, R. (2009). Health-affecting compounds in Brassicaceae. *Journal of Comprehensive Reviews in Food Science and Food Safety*, *8*(2), 31-43.

Jallow, M. F., Awadh, D. G., Albaho, M. S., Devi, V. Y., and Ahmad, N. (2017). Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. *International journal of Environmental Research and Public Health*, *14*(8), 833.

Jamali, M. K., Kazi, T. G., Arain, M. B., Afridi, H. I., Jalbani, N., and Memon, A. R. (2007). Heavy metal contents of vegetables grown in soil, irrigated with mixtures of wastewater and sewage sludge in Pakistan, using ultrasonic-assisted pseudo-digestion. *Journal of Agronomy and Crop Science*, *193*(3), 218-228.

Johnson, M. A. (1997). Encyclopaedia of Food Technology and Nutrition (R. Macrae., RK Robinson., MJ Sadler, eds). Academic Press, London, 1237-1243.

Kamaruzzaman, B. Y., Rina, Z., John, B. A., and Jalal, K. C. A. (2011). Heavy metal accumulation in commercially important fishes of south west Malaysian coast. *Research Journal of Environmental Sciences*, 5(6), 595-602.

Kenyanya, T. O. (2019). *Dietery risk assessment for exposure to pesticide residues in exotic vegetables sold in Nakuru town, Kenya* (Doctoral dissertation, Egerton University).

Kostova, D., Kanazirska, V., and Kamburova, M. (2008). A comparative analysis of different vegetable crops for content of manganese and molybdenum. *Agronomy Research*, *6*(2), 477-488.

Krieger, R. (2001). Handbook of pesticide toxicology: principles and agents. Academic press.

Kumar, S., and Singh, B. D. (2012). 5 Pointed Gourd: Botany and Horticulture. *Horticultural Reviews*, 39(1), 203-238.

Landner, L., and Lindestrom, L. (1999). Copper in society and in the environment. Vasteras, Swedish. *Environmental Research Group (MFG), (SCDA S-721 88)*.

Li, J., Li, H., Zhang, W. J., Wang, Y. B., Su, Q., and Wu, L. (2018). Hollow fiber–stir bar sorptive extraction and gas chromatography–mass spectrometry for determination of organochlorine pesticide residues in environmental and food matrices. *Journal of Food Analytical Methods*, *11*(3), 883-891.

Liberda, E. N., Tsuji, L. J., Martin, I. D., Cote, S., Ayotte, P., Dewailly, E., and Nieboer, E. (2014). Plasma concentrations of persistent organic pollutants in the Cree of northern Quebec, Canada: results from the multi-community environment-and-health study. *Journal of Science of the Total Environment*. *470-471*, 818-828.

Lin, C., and Ge, X. L. (2015). Effects of Zinc on the Growth of Several Vegetables. In *Advanced Materials Research*, 1092, 613-616. Trans Tech Publications Ltd.

Liu, P., Wang, C. N., Song, X. Y., and Wu, Y. N. (2010). Dietary intake of lead and cadmium by children and adults–result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method. *International Journal of Hygiene and Environmental Health*, *213*(6), 450-457.

Liu, S., and Pleil, J. D. (2002). Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography–mass spectrometry analysis. *Journal of Chromatography B*, *769*(1), 155-167.

Lupton, J. R., Brooks, J., Butte, N. F., Caballero, B., Flatt, J. P., and Fried, S. K. (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. *National Academy Press: Washington, DC, USA*, *5*, 589-768.

Mahugija, J. A. M., Henkelmann, B., and Schramm, K. W. (2014). Levels, compositions and distributions of organochlorine pesticide residues in soil 5–14 years after clean-up of former storage sites in Tanzania. *Chemosphere*, *117*, 330-337.

Mila, F. A., Rahman, M. S., Nahar, N., Debnath, D., and Shahjada, M. K. H. (2015). Profitability of bitter gourd production in some areas of Narsingdi District. *Journal of Sylhet Agricultural University*, 2, 143-147.

Min, E. Y., Cha, Y. J., and Kang, J. C. (2015). Effects of waterborne nickel on the physiological and immunological parameters of the Pacific abalone Haliotis discus hannai during thermal stress. *Journal of Environmental Science and Pollution Research*, *22*(17), 13546-13555.

Moreno, J. F., Liébanas, F. A., Frenich, A. G., and Vidal, J. M. (2006). Evaluation of different sample treatments for determining pesticide residues in fat vegetable matrices like avocado by low-pressure gas chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1111(1), 97-105.

Moss, G. P., Smith, P. A. S., Tavernier, D., Burrows, H., and Stohner, J. (1997). IUPAC compendium of chemical terminology. *Pure and applied chemistry*, *67*, 1307-1375.

Munia, Z. S., Shoeb, M., Mamun, M. I. R., and Nahar, N. (2018). Dissipation pattern of quinalphos in cauliflower, tomato and bean samples. *Journal of Consumer Protection and Food Safety*, *13*(1), 63-67.

Namieśnik, J., and Szefer, P. (2008). Preparing samples for analysis-the key to analytical success. *Journal of Ecological Chemistry and Engineering S*, 15(2), 167-244.

Naser, H. M., Shil, N. C., Mahmud, N. U., Rashid, M. H., and Hossain, K. M. (2009). Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. *Bangladesh Journal of Agricultural Research*, *34*(4), 545-554.

National Toxicology Program. (2011). Lindane, hexachlorocyclohexane (technical grade), and other hexachlorocyclohexane isomers. *Report on carcinogens: carcinogen profiles*, *12*, 256-258.
National Research Council. (2000). Copper in drinking water.

Nielsen, F. H. (1999). Ultratrace minerals. *Modern Nutrition in Helath and Disease*, 284-303

Nishijo, M., Tawara, K., Honda, R., Nakagawa, H., Tanebe, K., and Saito, S. (2004). Relationship between newborn size and mother's blood cadmium levels, Toyama, Japan. *Archives of Environmental Health: An International Journal*, *59*(1), 22-25.

Nriagu, J. O. (1989). A global assessment of natural sources of atmospheric trace metals. *Nature*, *338*(6210), 47-49.

Nshimiyimana, F. X., El Abidi, A., Fekhaoui, M., Benbakhta, B., Barakate, N., Hami, H., and Soulaymani, A. (2014). Analysis method for pesticide residues in biological matrices: gas chromatography-mass spectrometry. *Journal of Life Sciences*, *8*(6), 489-495.

Number, N. S. (1988). 60. Drinking Water Treatment Chemicals-Health Effects. *Ann. Arbor, Michigan, 9881*.

Papadakis, E. N., Vryzas, Z., Kotopoulou, A., Kintzikoglou, K., Makris, K. C., and Papadopoulou-Mourkidou, E. (2015). A pesticide monitoring survey in rivers and lakes of northern Greece and its human and ecotoxicological risk assessment. *Journal of Ecotoxicology and Environmental Safety*, *116*, 1-9.

Paul, R., Talukdar, A., Bhattacharya, R., and Santra, G. (2013). γ-Benzene hexachloride poisoning leading to acute hepatorenal decompensation. *Case Reports*, bcr2013009851. doi:10.1136/bcr-2013-009851.

Perez, C. B., Boia, C., Pombo, L. and Rebelo, E. (2001). Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electro-thermal atomic absorption spectrometry. *Food chem*, *75*(1), 93-100.

PHSE (Public Health Statement Endrin) (1996). Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry. www.atsdr.cdc.gov/

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, *269*(2), 337-341.

Reid, I. R., Bristow, S. M., and Bolland, M. J. (2015). Calcium supplements: benefits and risks. *Journal of internal medicine*, *278*(4), 354-368.

Ross, A. C., Taylo, C. L., Yaktine, A. L., and Valle, H. B. D. (2011). Dietary Reference Intakes for Calcium and Vitamin D. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, National Academies Press, Washington (DC).

Rossi, L. A. (2002). *Reregistration eligibility decision for endosulfan*. EPA 738-R-02-013. Reregistration Report. US Environmental Protection Agency, Washington, DC.

Roussel, A. M., Andriollo-Sanchez, M., Ferry, M., Bryden, N. A., and Anderson, R. A. (2007). Food chromium content, dietary chromium intake and related biological variables in French free-living elderly. *British journal of nutrition*, *98*(2), 326-331.

Rózanski, L. (1992). Przemiany pestycydów w organizmach zywych iw srodowisku. *PWRiL, Warszawa*.

Russell, R., Beard, J. L., Cousins, R. J., Dunn, J. T., Ferland, G., Hambidge, K., and Yates, A. A. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *A Report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board Institute of Medicine.*

Rustan, A. C., and Drevon, C. A. (2001). Fatty acids: structures and properties. *e LS*.

Ščančar, J., Zuliani, T., and Milačič, R. (2013). Study of nickel content in Ni-rich food products in Slovenia. *Journal of food composition and analysis*, *32*(1), 83-89. doi:10.1016/j.jfca.2013.06.011.

Schreinemachers, P., Simmons, E. B., and Wopereis, M. C.S. (2018). Tapping the economic and nutritional power of vegetables. *Global Food Security*, 16, 36–45. doi.org/10.1016/j.gfs.2017.09.005.

Shallari, S., Schwartz, C., Hasko, A., and Morel, J. L. (1998). Heavy metals in soils and plants of serpentine and industrial sites of Albania. *Journal of Science of the total environment*, *209*(2-3), 133-142.

Shawon, T. R., Rahman, M., Islam, A. K. M. A., Islam, M. R., Saha, D. C., Kamruzzaman, M. A Comparative Financial Evaluation of Selected Vegetables Production in Bangladesh, https://bsmrau.edu.bd/seminar/wp-content/uploads/sites/318/2018/04/Seminar-Paper-Shawon.pdf

Shoeb, M., Sultana, S., Jahan, N., Nahar, N., and Farha, W. (2020). Phytochemical screenings, physicochemical properties and effects of pesticides on vegetables. *Asian Journal of Pharmacogn*, 4(2), 24-41.

Shukla, G., Kumar, A., Bhanti, M., Joseph, P. E., and Taneja, A. (2006). Organochlorine pesticide contamination of ground water in the city of Hyderabad. *Journal of Environment International*, *32*(2), 244-247. doi:10.1016/j.envint.2005.08.027.

Singh, N. S., and Singh, D. K. (2011). Biodegradation of endosulfan and endosulfan sulfate by Achromobacter xylosoxidans strain C8B in broth medium. *Biodegradation*, *22*(5), 845-857.

Singh, S., and Gaikwad, K. K. (2012). Studies on the development and storage stability of bitter gourd lemon function RTS beverage. *International Journal of Processing and Post Harvest Technology*, *3*(2), 306-310.

Singhal, R. L., Merali, Z., and Hrdina, P. D. (1976). January). Aspects of the biochemical toxicology of cadmium. In *Federation proceedings* (v. 35(1), 75-80.

Sosan, M. B., Adeleye, A. O., Oyekunle, J. A. O., Udah, O., Oloruntunbi, P. M., Daramola, M. O., and Saka, W. T. (2020). Dietary risk assessment of organochlorine pesticide residues in maize-based complementary breakfast food products in Nigeria. *Heliyon*, *6*(12), e05803.

Stocka, J., Biziuk, M., and Namieśnik, J. (2016). Analysis of pesticide residue in fruits and vegetables using analytical protocol based on application of the QuEChERS technique and GC-ECD system. *International Journal of Global Environmental Issues*, *15*(1-2), 136-150.

Stocka, J., Tankiewicz, M., Biziuk, M., and Namieśnik, J. (2011). Green aspects of techniques for the determination of currently used pesticides in environmental samples. *International Journal of Molecular Sciences*, 12(11), 7785-7805.

U.S. EPA (1992) Integrated risk information system (IRIS). Environmental Criteria and Assessment Office, United States Environmental Protection Agency, Cincinnati, OH.

Ugulu, I. (2015). Determination of heavy metal accumulation in plant samples by spectrometric techniques in Turkey. *Applied Spectroscopy Reviews*, *50*(2), 113-151.

Vijayavel, K., Gopalakrishnan, S., Thiagarajan, R., and Thilagam, H. (2009). Immunotoxic effects of nickel in the mud crab Scylla serrata. *Journal of Fish and Shellfish Immunology*, *26*(1), 133-139.

Weaver, T. B., Ghadiri, H., Hulugalle, N. R., and Harden, S. (2012). Organochlorine pesticides in soil under irrigated cotton farming systems in Vertisols of the Namoi Valley, north-western New South Wales, Australia. *Chemosphere*, *88*(3), 336-343.

Wilson, D. N. (1988). Cadmium-market trends and influences. In *Cadmium 87. Proceedings of the 6th International Cadmium Conference, London, Cadmium Association* (v.9, p. 16).

Wolfe, K., Wu, X., and Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of agricultural and food chemistry*, *51*(3), 609-614.

World Health Organization. (1992). IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP: report of the twenty-second session, Vienna, 9-13 March 1992.

World Health Organization. (1994). Quality directive of potable water. *WHO*, *197*.

World Health Organization. (2010). International code of conduct on the distribution and use of pesticides: guidelines on pesticide advertising. In *International code of conduct on the distribution and use of pesticides: guidelines on pesticide advertising*.

Yilmaz, F. (2009). The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (Anguilla anguilla, Mugil cephalus and Oreochromis niloticus) inhabiting Koycegiz Lake-Mugla (Turkey). *Turkish Journal of Science and Technology*, *4*(1).

Yu, L., Guo, G., Zhao, J., Zhao, L., Xia, A., He, X., Xing, C., Dong, L and Wang, F. (2021). Determination of Organochlorine Pesticides in Green Leafy Vegetable Samples via Fe3O4 Magnetic Nanoparticles Modified QuEChERS Integrated to Dispersive Liquid-Liquid Microextraction Coupled with Gas Chromatography-Mass Spectrometry. *Journal of Analytical Methods in Chemistry*, *2021*.

Yusuf, A. A., Arowolo, T. A., and Bamgbose, O. (2003). Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Journal of Food and Chemical Toxicology*, 41(3), 375-378.

Yuwai, K. E., Rao, K. S., Kaluwin, C., Jones, G. P., and Rivett, D. E. (1991). Chemical composition of Momordica charantia L. fruits. *Journal of Agricultural and Food Chemistry*, *39*(10), 1762-1763.

Zhang, A., Luo, W., Sun, J., Xiao, H., and Liu, W. (2015). Distribution and uptake pathways of organochlorine pesticides in greenhouse and conventional vegetables. *Journal of Science of the Total Environment*, *505*, 1142-1147.

Zhang, L., Liu, S., Cui, X., Pan, C., Zhang, A., and Chen, F. (2012). A review of sample preparation methods for the pesticide residue analysis in foods. *Open Chemistry*, 10(3), 900-925.