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DETECTION AND ALLEVIATION OF PESTICIDE RESIDUE IN FOOD AND WATER

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Nutrition and Food Science

by
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August 2016

To The Almighty Lord Jesus: The God of second chance.

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ABSTRACT

Use of pesticide has become part of modern day agricultural practice. Some pesticides can remain in the environment for decades and contaminate surface water that is used for irrigation of produce. Two studies were conducted- first to examine possible pesticide residue in surface water and some fruits, vegetables and cereals in Louisiana; and second was to alleviate possible pesticide residues in the water using zeolite filtration. Samples of 8 foods (tomato, corn, rice, blueberry, cucumber, cabbage, wheat and melon) and 35 surface waters were studied using a QuEChERS extraction method for food samples and an EPA method for the water samples. Gas chromatography-mass spectrometry was used to analyze water and food samples. Alleviation of pesticide residues was attempted for 10 water samples using a natural zeolite filtration. One water sample was filtered through a surfactant (HDTMA-Cl)-modified-zeolite. Eighteen pesticides were detected in the surface water samples and 5 in the food samples. Pesticides detected were below FDA limit but 0.18 ppm cypermethrin found in tomato was 90 % close to the FDA limit (0.2 ppm). Alleviation was achieved in 9 water samples out of 10 samples that were filtered through zeolite. The highest removal of pesticides from water with zeolite was 100 % in bifenthrin in CLC sample, followed by 99.1 % in atrazine in the same sample. Minimum reduction of 10.9 % was in metolachlor in sample BRH. Further reduction of pesticide residues up to 50 % was recorded in the SMZ treatment as the concentrations of 4 out of 8 pesticide residues were reduced. This study suggests the need to intermittently monitor pesticide contamination in our food and water.

CHAPTER 1
INTRODUCTION

The importance of food and water to maintain life cannot be overemphasized. In order to maintain a healthy nutritional diet, food and water must be consumed without contamination from pollution.

Food and water contaminates are an undesirable event at the terrestrial and/or aquatic terrains as it causes ill health and ultimately could lead to death of the affected organisms. Major source of contaminants include sewage, fertilizer and pesticides. Of all the pollutants, the most common are pesticides (Fenik *et al*., 2011). Pesticides are chemicals used in controlling menaces like insect, disease, or weed that are considered impediments to healthy growth of plants; be it horticultural or food crops. Targets of chemical treatments usually include the soil, crop plants, weeds or insects. In farms and gardens, common pesticides used are either insecticides, fungicides or herbicides.

Leaching is a form of environmental pollution which is a phenomenon whereby chemicals drain away from the treated region to non-targeted environments. By this, surface waters have the potential of getting contaminated when irrigation water that has passed over pesticide-treated plants and/or the environment drain or leach into the surface waters (Starner *et al*., 2005). Storms could sometime result in spontaneous flow of contaminated water into surface water (Boithias *et al*., 2014). Another source of pollution is drift that occurs if a pesticide spray misses its targets having been deflected by the wind or resulting from the error of missing the intended, thereby landing on a non-targeted farm area. When the level of the pesticide contamination reaches a critical level in food, ground waters, lakes, rivers or ponds, it becomes an issue that could lead to illness or death in the organisms that depend on such.

Water plays a central role in human life. Asides from the basic routine drinking of water, it is used in irrigation of crops, and serves as home for aquatic lives. Most aquatic organisms are edible to humans and are rich in important food substances like protein, oil and vitamins D and E (Sidhu, 2003); most of which are required for a balanced diet in humans. Omega 3 oil is found in some fishes (Artham *et al*., 2008) and is part of every cell compositions in human body. Zinc is required for healthy skin, muscles and fertility in humans. Oysters, marine fish, and croakers are good source of Zn as they uptake an ample of it from the sea deposits (Chipman *et al*., 1958). Dietary guidelines of most nations worldwide recommend fish along with other seafood for human consumption (WHO, 2003). Crawfish, sometimes also referred to as crayfish, is a good source of low-fat protein, 36-45 % of crude protein, and vitamins A and D, minerals such as calcium, potassium, copper, zinc and iodine (Ibironke *et al*., 2014). Louisiana has the largest crawfish production in the United States of America, accounting for 90% of the total USA production. Its total annual shipments of crawfish between the years 2006 and 2011 had doubled to $195.8 million (The New York Times, 2012). It is an important component of Louisiana economy and that of the United States in general. Given that crawfish have minimal tolerance to pollutants, every trace of pollution in Louisiana waters pose a big threat to her lucrative crawfish industry.

Cereals, fruits and vegetables are among the most commonly grown foods in many parts of the world. In the United States of America, especially in the State of Louisiana, mostly grown in this category includes Wheat (*Triticum aestivum*), Tomato (*Solanum lycopersicum*), Blueberry (*Cynococcus*), Corn (sweet corn- *Zea Mays*), Cucumber (*cucumis sativus*), Cabbage (*Brassica oleracea*), Honeydew (melon- *cucumis melo*) and Rice (*Oryza sativa*). Wheat, rice and corn are cereal grain crops. In year 2013, wheat (713 million tons) is the third largest produced crop

world over following rice (745 million tons) as the second and corn (1,016 million tons) the first. Wheat contains about 8-15% protein and therefore serves as a good source of vegetable protein (Shewry, 2009). It is the main ingredient in many bakeries and fast food menus world-wide. Rice is rich in dietary fiber and some vitamin complexes like nicotinic acid (niacin), riboflavin and thiamin (FAO, 2004). Corn serves as a good source of dietary fiber. Processed sweet corn has been confirmed to have a higher anti-oxidant activity compared to fresh ones. Thermal processing of sweet corn could increase its anti-oxidant activity by 44% (Dewanto, 2002).

Tomato, Cucumber, Cabbage and Honeydew are vegetables; and are generally edible as ingredients in dishes, sauces, salads and stews. Tomato is the most consumed canned vegetable in America; and it is the fourth most consumed fresh vegetable following onions, head lettuce and potatoes as first, second and third most consumed respectively (Canene-Adams *et al.*, 2005). Tomato contains a phytochemical called lycopene which has been found to be associated with decreased risk of prostate cancer and cardiovascular disease (Wilkinson and Chodak, 2003; Cohen, 2002).

Cucumber contains vitamins C and A and therefore is therapeutic as these vitamins are required in the body to fight ailments. Vitamin A is usefuk in enhancing vision in human while vitamin C helps in blood clotting. It is used in skin treatments like cooling, healing and recovery of irritated skin, wrinkles, and sunburn (Akhtar *et al*, 2011). Cucumber contains curbitacin D and 23, 24-dihydrocucurbitacin D which help in prevention of tyrosinase and melanin synthesis (Jian *et al.*, 2005). Cabbage is nutritive as it has glucosinolates, a group of secondary metabolites which convert to isothiocyanates that has an anticarcinogenic potential (Oerlemans *et al.*, 2006; Verkerk and Dekker, 2004; Craig, 1997). According to Gene Lester (1997), honeydew melon is rich in vitamin C, potassium, vitamin B-6 and fibers. Vitamin C acts as an anti-oxidant, that is, a

neutralizer of beneficial free radicals that might in turn be hazardous to our body cells if not neutralized. Our hearts, muscles, blood vessels and nerves need potassium for normal functioning. Vitamin B-6 serves as co-enzyme in the body. Fibers help in digestive system. Blueberries provide vitamins C and E, and also have anthocyanin and polyphenolic antioxidants (Wu *et al*., 2004). They are sources of dietary fiber and manganese (McLeay, 2012). Dietary fiber is good in heart disease prevention and also make the stomach feel full thereby preventing the risk of excess weight resulting from overeating (Slavin, 2013). Manganese is required to help process carbohydrates, proteins and cholesterols in the body (Muhammad *et al*., 2012).

A naturally occurring chemical compound commonly referred to as zeolite could be used to filter out contaminates such as pesticides from water. About 40 zeolites are found commonly in nature as a volcanic mineral, while some 150 others have been artificially synthesized. They are chemically made up of hydrated alumina (AlO_4) and silica (SiO_4) in an interlinked tetrahedron. Elements of zeolite are aluminum, silicon and oxygen. Zeolites are very stable in nature as they do not react with most elements neither do they undergo oxidation. They are hard solid that do not burn nor melt easily. Its melting point is over 1000 ^{0}C (Woodford, 2014). They withstand high pressures and do not dissolve in water or other inorganic solvents. Zeolites have open-frame like structure and special ability to trap molecules inside them. An average pore size of a natural zeolite like clinoptilolite is 0.3 – 2 nm. In its natural state, it is safe to handle but may become unsafe when in fibrous form especially to skin or if inhaled. Among many uses of zeolite is its use is in water softening by binding to the calcium and magnesium in the water thereby replacing them with its own sodium (Woodford, 2014). In Frankston, Australia (Zeolite in Agriculture, 2015), zeolite has been reported to have enhanced carrot yield up to 10%, reduced

leaching, increased fertilizer usage, early ripening, reduced nitrate and improved vitamin levels when compared with same carrot grown without zeolite.

Zeolite could be used for removal of pesticides in farmland soils or waters. Zeolite has a negatively charged surface that allows attraction to cation exchange. They act as molecular sieves by binding to molecules such as ammonium and other active ingredients of pesticides (Lemic *et al.*, 2006). Removal of pesticides (belonging to the chemical families of atrazine, lindane and diazinone) from waste water have been demonstrated using organo-zeolite modified by stearyldimethylbenzylammoniumchloride (SDBAC) (Jovan Lemic *et al*., 2006). Erdem *et al*. (2004) demonstrated the potential of natural zeolites in removal of heavy metal cations from industrial waste water.

Human diets depend on food for their protein, vitamins and minerals in order to maintain a balanced diet. Anything hindering production of these foods is directly or indirectly hindering human well-being. The need for a remedial measure against contaminations of these foods with pesticides can therefore not be overemphasized at this stage. After harvesting produce are washed in water before they are delivered for sale. Zeolite-filtered water could be used to remove pesticides from fruits and vegetable wash water in order to prevent the possible residue of pesticide in produce. This study therefore aims at keeping track of the possible pesticide contamination in fruit and vegetable crops as well as irrigation waters across Louisiana. The effect of zeolite in reducing the possible pesticide residues in surface waters will also be addressed in the course of this research study.

Tracking pesticide residues in water and crops in the state of Louisiana as well as developing technique for removal of pesticides from water using zeolite filtration system will be the two main focus of this study. The specific objectives of this research will therefore include:

1. Detect pesticide residues in selected cereals, fruits, vegetables and water in the State of Louisiana.

2. Develop a zeolite filtration system for removal of pesticides from water.

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CHAPTER 2
LITERATURE REVIEW

2.1 Pesticides

Pesticides can be chemicals that are used for repelling, controlling or killing pests, pesticides could be an herbicide used to control weeds; fungicides against fungi; insecticides (e.g. nematicide, termiticide, repellant, etc.) in combating insects or rodenticides against rodents. The herbicide, insecticide, rodenticide and/or fungicide required depend on the type of agriculture in play. Some pesticides like the organochlorine family are being banned in many nations of the world since 2007 due to their toxicity and persistent nature in the environment (Ulenik *et al*., 2013). Lindane, an organochlorine insecticide is still in use in some parts of the USA as it is kept as a secondary treatment against lice and scabies (WHO, 2005; Engler, 2009).

2.2 Louisiana Agriculture

The income generated from Louisiana agriculture comes majorly from crops up to 60% while the remaining 40 % comes from livestock. Leading food and/or cash crops in Louisiana amongst which sugarcane is the first include rice, soybeans, cotton, and corn. Top vegetable crops are sweet potatoes and tomatoes, while leading fruit crops are peaches, strawberries and melon (Louisiana, 2016a).

2.3 Use of Pesticides in Louisiana

According to Louisiana (2016b), based on the crops grown, the types of pesticides commonly used in Louisiana are as highlighted in Table 1.1. The insecticides are in the families of organophosphate, carbamate, pyrethrins / pyrethroids and organochlorines. Herbicides used are mostly in the families of triazine, phenoxy, chlorophenoxy, organophosphorus and pyridine. Fungicides are benimidazole, dithiocarbamate, organochlorine and phthalimide. The rodenticides

are mostly coumarin family. Amongst these chemical families of pesticides, the most heavily used in the USA is the Triazine; and the two primary triazine herbicides predominant are atrazine and simazine (Walther, 2003).

Table 2.1 Common Pesticides used in Louisiana.

Chemical Family	Pesticide Type
	Insecticide
Organophosphate	Chlorpyrifos, diazinon, malathion, methyl parathion
Carbamate	Aldicarb, carbaryl
Pyrethrins / pyrethroids	Cypermethrin, λ-cyhalothrin, permethrin, bifenthrin, pyrethrin
Organochlorine	Endosulfan, lindane
	Herbicide
Triazine	Atrazine
Phenoxy	2,4 - Dichlorophenoxy acetic acid (2,4-D)
Chlorophenoxy	Dicamba
Organophosphorus	Glyphosate
Pyridine	Triclopyr
	Fungicide
Benzimidazole	Benomyl
Dithiocarbamate	Mancozeb
Organochlorine	Chlorothalonil
Phthalimide	Captan
	Rodenticide
Coumarin	Bromadiolone, brodifacoum

2.4 Insecticides

2.4.1 Organophosphate. Pesticides belonging to this group are very strong insecticides and are being discouraged from being used around residential area due to their adverse effect on humans that get exposed to them (Louisiana, 2016b). It affects the nervous system, and can cause shortness of breath, abnormal salivation, vomiting, headache, dizziness and chest complications including convulsion and paralysis that could cause death. Diazinon for instance, inhibits an enzyme that inactivates the neurotransmitter acetycholine in any organisms exposed to a harmful amount of it (Ecobichon and Joy, 1994; Pesando *et al*., 2003). Chlorpyrifos is widely used in cotton and corn (Williams et al., 1999). The dose of a chemical that becomes lethal in 50% population of experimental animals studied is called acute oral lethal dose fifty, simply put as LD50. The acute dermal LD50 of chlorpyrifos is 202 mg/kg (Gaines, 1969).

2.4.2 Carbamate. This family comprise of insecticides with broad spectrum of activity as they are applied to vegetables, fruits and cereal crops. They are usually applied towards the maturity of crop implying higher risk of exposure is likely being the time growers visit their farm most regularly (Rowayshed *et al*., 2013). Carbamate is used against mites, houseflies among others (El-Saeid, 2003; Randhawa *et al*., 2007). Symptoms resulting from exposure to carbamate are similar to those in organophosphate poisoning. They include headache, dizziness, extreme weakness, twitching or tremor, slow heartbeat, sensation of swelling or tightness in the chest, sweating and nausea. Carbaryl (sevin) is used by tomato growers in Louisiana. The oral acute LD50 for carbaryl is 500 - 850 mg/kg (Kidd and James, 1987).

2.4.3 Pyrethrins / pyrethroids. These insecticide have been used since 1900s (Metcalf, 2000). They are considered not very toxic, recommended for home use, and are usually labeled as low

toxicity pesticides (Bradberry, 2005). They hinder detoxification in insect resulting in its mortality. These are used against cabbage looper and cucumber beetle (Caldwell *et al*., 2013). Bifenthrin is used against termites in gardens and house environment. The oral rat LD50 for bifenthrin is greater than 5,000 mg/kg and is considered relatively non-toxic (Talstar, 2008).

2.4.4 Organochlorines. The pesticides in this group are very toxic. Prolonged exposure could lead to depressed nervous system activity, and seizures. They inhibit chloride flow into an insect's nerve (Coats, 1990). Endosulfan and lindane are toxic to humans and aquatic organisms resulting in acute and chronic symptoms even if exposed to a low level of them (Guerin 2001; UNEP 2009; Zucchini-Pascal *et al*. 2009). Both lindane and endosulfan have been banned but selected few nations still use them (Herna´ndez-Rodrı´guez et al. 2006; Hussain et al. 2007; Rivero et al. 2012). Lindane oral acute LD50 in rats is 88-190 mg/kg (Smith, 1991).

2.5 Herbicides

2.5.1 Triazine. This is one of the oldest weed controlling chemicals dating back to early 1950s. Some are selective while others are non-selective in their herbicidal activities. Selective are targeted against certain weeds while the non-selective is all encompassing. Most commonly used among others in this group include atrazine and metribuzin. Atrazine is used as selective herbicide in sweet corn and sugarcane. They inhibit electron transport in photosynthesis reaction in plants. Exposure to triazine results in eye, skin and respiratory tract irritations (Fishel, 2015). Atrazine is considered moderately toxic with an LD50 of 1300 mg/kg (Bachman and Patterson, 1999).

2.5.2 Phenoxy. 2,4-Dichlorophenoxy acetic acid (2,4-D) is a plant growth hormone in the class of auxins. It is the most commonly used phenoxy herbicides. It came into play in 1946. It is used

in killing broad leaf plants. In monocots like wheat or corn farming, it serves as selective herbicide against broad-leaf weeds by enhancing their uncontrollable growth unto mortality. According to Fraser *et al*., 1984, the LD50 for 2, 4-D is 750 mg/kg.

2.5.3 Chlorophenoxy. Dicamba is a popular chlorophenoxy herbicide. It is a selective herbicide as it targets broadleaf and woody plants considered as weeds. Dicamba is an auxin, and its mode of action in plant is synonymous to that of 2,4-D as they induce overgrowth in an uncontrollably fashion until the weeds die. They are used in farms, gardens and homes. Though dicamba is low in toxicity but inhalation, ingestion or any form of exposure to harmful dose of dicamba may result in vomiting, loss of appetite, diarrhea, shortness of breath, excess saliva (NPIC, 2012). The LD50 for dicamba is 1028 mg/kg (Fraser *et al*., 1984).

2.5.4 Organophosphorus. Glyphosate is a widely used organophosphorus; and most widely used herbicide globally with 11% global herbicide sales (Powels *et al*., 1997). Glyphosate is non-selective and its approach is hindering of synthesis of enzyme needed for normal growth in plants (Kools *et al*., 2005). Being non-selective, glyphosate is used mostly in farms where crop varieties that have resistance to glyphosate are grown. Examples are roundup ready corn and soybean varieties. It is used in farms and homes. It is low in toxicity. Exposure to glyphosate can cause eye, nose, throat or skin irritation, vomiting, diarrhea or excessive saliva. The acute oral LD50 for glyphosate in rat is 5,600 mg/kg (National Library of Medicine, 1992).

2.5.5 Pyridine. Commonly used in this group is trichlopyr. It was first registered in 1979. It is a herbicide popularly applied in rice field and lawns for the control of woody and herbaceous weeds. Pyridine is selective in its herbicidal action and mode of action is synonymous to that of phytohormones like 2,4-D and dicamba whereby inducing an uncontrollable overgrowth in the

unwanted plants. It is corrosive to skin and eye upon contact with harmful dose. The oral LD50 of trichlopyr in rats is 630-729 mg/kg and 2000 -3000 mg/kg depending on the formulated products as they vary (Extoxnet, 1992).

2.6 Fungicides

2.6.1 Benzimidazole. Benomyl is a fungicide in this group. Benzimidazole is known for treating nematode and trematode infections in pet animals like dog and cat. It is used in controlling roundworms, tapeworms, and adult flukes. Its mode of action is by binding to the fungal microtubules and stopping hyphal growth; also binds to spindle microtubules and blocks nuclear division. This is a safe pesticide, as it does not bind to the tubulin of the cells of the animal being treated but rather to the cells of the target parasite. LD50 of benomyl in rats is greater than 10,000 mg/kg and greater than 3,400 mg/kg in rabbits confirming its low risk of acute toxicity (Kidd and James, 1991).

2.6.2 Dithiocarbamate. Mancozeb is a dithiocarbamate chemical used as fungicide in tomato against early and late blights, anthracnose, leaf mould, grey leaf spot and phoma rot; lettuce against downy mildew, anthracnose, and septoria leaf spot (Primefacts 223, 2006). The LD50 for mancozeb in rats is 4,500-11,200 mg/kg. Dermal LD50 in rabbit (when applied to its skin) is 5,000-15,000 mg/kg (EPA, 1987), meaning that it is a mild skin irritant.

2.6.3 Organochlorine. Is used as fungicide in peanut, potato and tomato farms. It is used on lawns and golf courses. Its mode of action is by hindering enzymatic reactions in fungi leading to their deaths (Ronald, 1973). Chlorathalonil is toxic and could cause eye irritation, and kidney damage. The oral LD50 is greater than 10,000 mg/kg in rats (Kidd and James, 1991; US National Library of Medicine, 1995).

2.6.4 Phthalimide. Captan is a phthalimide fungicide used in controlling fungi diseases in fruits and vegetables. It is less toxic and therefore can be used in the field and at homes. However, a prolonged high dose could be cytotoxic. It is non-toxic to birds but toxic to fish (Kidd *et al*., 1991). Oral LD50 of captan in rat is 8400-15,000 mg/kg confirming its low acute toxicity (Chemical Information System, 1988).

2.7 Rodenticide

2.7.1 Coumarin. Is an anticoagulant in its rodenticidal action. It inhibits enzyme, and vitamin K epoxide reductase. This results to death due to a decrease in vitamin K in the blood system causing inability of the rodent's blood to clot. Brodifacoum, a registered coumarin, has been in use since 1970s (British Crop Protection Council, 2000). According to the World Health Organisation (1995), one of the most abundant brown rats – *R. norvegicus* has an oral LD50 of brodifacoum as 0.26 mg/kg and its half-life in soil is 157 days.

2.8 Pesticides in Food and Water

Pesticides used in field crops belong to either organic or inorganic group. Organophosphorus, organochlorine and organonitrogen pesticides are groups of pesticides under which most organic pesticides used in field crops belong. Among these, the most widely used in the USA and especially in Louisiana agriculture is organonitrogen that includes triazine family where herbicide atrazine belongs (Walther, 2003). Contrary to expectation of pesticides to control menaces like weed, fungi, bugs, and rodents, and disappear from the plants, its produce and environment without any trace of harmful residues left behind, there are cases where it is either applied at an overdose rate or added by erosion or storm resulting in such pesticide remaining in food or water as residues (Fenik *et al*, 2011).

2.9 Detection of Pesticides in Food and Water

Analytical methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used in the past but were not confirmatory in their output results considering matrix interference, probably due to the type of detectors (electron capture-ECD, flame photometric – FPD, and nitrogen-phosphorus-NPD) used in the GC (Schachterle *et al.*, 1996). However, mass spectrometry (MS) has succeeded in this aspect even though MS full scan sometimes fails to provide adequate sensitivity in real samples in selected ion monitoring (SIM) mode giving room to false positives due to reduced qualitative data (Arrebola *et al*., 1999). The modern use of the combination of gas chromatography and mass spectrometry (GC-MS) has solved the problem of shortfalls inherent in the singular use of GC and/or MS. The combination of GC-MS provides analysis of trace amounts of pesticide residues in diverse samples ranging from biological fluids (Vidal *et al*., 1998; Frias *et al*., 2001; Uroz *et al*., 2001), waters (Pablo-Espada et al., 1999; Vidal et al., 2000) or fruits and vegetables (Arrebola *et al*., 2001; Gamon *et al*., 2001).

Walther (2003) reported the presence of triazines particularly atrazine and simazine in Upper Terrebonne basin of Louisiana surface waters.

2.10 Removal of Pesticides in Water

The use of clinoptilolite - a natural zeolite, in removing organic contamination (pesticides) from surface waters was reported. The success of this method was said to be a function of pH, initial concentrations of humic acid and ammonia, temperature and contact duration (Mergeta *et al*., 2013). Removal of ammonia and humic acid was best with zeolite at the

pH value close to waters' natural pH (Moussavia *et al*., 2011). Removal of Fe and Mn ions from underground water samples using natural and modified zeolite confirmed 22-90% and 61-100 % success for natural zeolite (Inglezakis *et al*., 2002). Lemic *et al*. (2006) detected atrazine in ground water and with SDBAC SMZ were able to remove atrazine from the water (Lemic *et al*., 2007).

2.11 Development of Zeolite for Removal of Pesticides in Water

Zeolite could be tailored according to the type of contaminants to which it will be subjected by modifying its surface properties. Modification of a zeolite enhances its adsorption capacity, that is, its ability to remove contaminants from water. Surfactants, organic molecules with high functionality in filtration capacity could be cationic in its polarity, with certain level of CEC (cation exchange capacity) depending on the type of surfactant used. The compound targeted to be isolated in a pesticide residue determines the kind of surfactant that will be developed to modify the zeolite. Removal of atrazine, lindane, diazinone group of pesticides will require stearyldimethylbenzylammoniumchloride (SDBAC 80%, 19% propan-2-ol, 1% water) surfactant modified zeolite (Lemic *et al*., 2006, Roxana Apreutesei *et al.,* 2008). Removal of 4-chlorophenol according to Haggerty and Bowman (1994) and Apreutesei *et al.,* (2008), requires hexadecyltrimethylammonium bromide or chloride (HDTMA-Br 57.6 ml/32 g zeolite); Removal of chromate and perchloroethylene from distilled water and waters from Elizabeth City and Oak Ridge, TN respectively (Bowman, 2005).

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CHAPTER 3
DETECTION OF PESTICIDES IN FOOD AND WATER

3.1 Introduction

Pesticides are very useful tools in agriculture, for instance, removal of weeds, insects and infections/diseases controls are the main reasons why the use of pesticides will likely continued to be used by farmers. Apart from the risk involved in exceeding recommended application rates, persistence of some pesticides in the environment heightens the need for regular monitoring of food and water. Detection of pesticides in food and water is advantageous in terms of economic measures. The inherent loss in case of consumption of contaminated food and/or water in terms of health hazards when it comes to cost of treatment, irreparable loss, etc., is much greater compared to the cost of efforts in the detection process.

Many detection methods for pesticides used in the past could be summarized under three headings such as multiresidue methods (MRMs), single residue methods (SRMs) and semiquantitative and qualitative methods (US Congress, 1988). However, none of these combines the best features in an analytical condition such as: high recovery rate (>85%); accuracy; high sample throughput (say 20 samples in half an hour); efficiency (in terms of use of solvent, labware needed, and bench space); adaptability (single person with little training can handle); rugged (having allowance to cleanup fatty acids and other organic acids commonly found in foods); safe (in that solvent- acetonitrile is dispensed through an auto-dispenser minimizing risk of spillage/contact); mobile lab (with chopper, balance and centrifuge, the bench space is ready for use) (Lehotay *et al*., 2005). MRM can detect residue and also is applicable to monitoring multiple residues as the name implies, but unable to identify the pesticide residue. SRM may be less sensitive than MRM in terms of detection of pesticides and

also confined to monitoring single residues, but it does a better job monitoring some pesticides that are very hazardous. SRM is less efficient in monitoring multi residues as it requires much glassware, evaporative apparatus, chromatography and detectors, etc., whereas MRM uses the same apparatus to run multiple residues. Semiqualitative and quantitative methods will not provide the qualitative and quantitative information required in details. A semiqualitative method provides the range of pesticide found while quantitative will only indicate whether the detected amount is over or within the tolerance threshold. Following the need for a new method, QuEChERS (quick, easy, cheap, effective, rugged and safe), is an analytical method that is recent and closest to fulfilling the required analytical conditions used in the detection of pesticides in matrix samples due to its high recoveries, accuracy, high thoroughput etc. (Braganca *et al*., 2012; Wilkowska and Bizuik, 2011) is used in the detection of pesticides in food especially fruits and vegetables (Salvia *et al*., 2012). QuEChERS is therefore the extraction methodology used for the food samples during this study.

This study was conducted to determine if pesticide residues are in food and water collected from different locations in Louisiana.

3.2 Materials and Methods

3.2.1 Food and Water Sample Storage and Preparation. Surface water and food samples were collected at different locations in Louisiana. These were sourced from the pool of samples being routinely submitted to the Pesticide Laboratory of the Agricultural Chemistry department, Louisiana State University through the Louisiana State Department of Agriculture and Forestry (LDAF). Food samples as shown in Figure 3.1, A through 3.1, H include wheat, tomato, blueberry, corn, cucumber, cabbage, honeydew and rice. The food samples were obtained form

different parishes in Louisiana. As outlined in Table 3.1, there were 6 tomato samples obtained-2 from Amite and one each from Boyce, OakGrove, Epps and Coushatta. Sweet corn consists of 3 samples, each from Alexandria, Winsboro and Dixie. One melon came from Breau Bridge; and 2 blueberries with 1 each from Franklinton and Ringgold. Two wheat samples were both from Deridder. Cucumber, cabbage and rice contained 1 sample each and were from Pollock, Lafayette and Eunice respectively. All the food samples were received in June 2015 except rice that was delivered in August 2015. All the 35 waters were received in May 2015.

Table 3.1 Food samples

Food	Amount	Source
Tomato	6	Amite(2), Boyce, OakGrove, Epps, Coushatta
Corn	3	Alexandria, Winsboro, Dixie
Melon	1	Breau Bridge
Blueberries	2	Franklinton, Ringgold
Cucumber	1	Pollock
Cabbage	1	Lafayette
Wheat	2	Deridder (2)
Rice	1	Eunice

Water samples and their sources were as listed in Table 3.2. Each sample was labeled after its source by abbreviating the name of the source. For instance, sample BPH was obtained from Bayou Pierre, Hwy 1 S of Powha; sample CRH from Cane River, Hwy 1, 1 mile N. gal; CLC from Chatlin Lake Canal, Hwy 457 T2N etc. All water samples were stored at 4 ^{0}C and

food samples stored at -20 ^{0}C until each was analyzed. Each food sample was retrieved from the freezer and kept overnight in the cooler (4 ^{0}C) to allow for thawing. Few samples were blended completely, for instance, tomato, melon, cucumber, corn, and cabbage. Food samples like wheat, rice and blueberry were shuffled before selection for grinding. The grains were ground whole. Juicy samples like tomato, blueberry, corn, cucumber, cabbage, and honeydew were blended into puree (Fig. 3.2, A) using Robot Coupe (Fig. 3.2, C). Rice and wheat, being grains was each blended into powder (Fig. 3.2, B) using a Majic blender (Fig. 3.2, D). Each prepared sample was labeled separately, poured into glass quart jars, and stored at -20 ^{0}C until ready to analyze.

Table 3.2 Water Samples

Water	Source
BPH	Bayou Pierre, Hwy 1 S of Powha (*WM-S-A-01)
CRH	Cane River, Hwy 1, 1 mile N. Gal (WM-S-A-02)
CLC	Chatlin Lake Canal, Hwy 457 T2N (WM-S-A-04)
CDG	Coulee Des Grues, hwy 115-SW (WM-S-A-05)
BCH	Big Creek Hwy 80 at Holly Ridge (WM-S-M-03)
LTC	Little Turkey Creek, Hwy 128 T1 (WM-S-M-05)
LBT	Lake Bruin T12N R12E S29 (WM-S-M-06)
TRH2	Tensas River Hwy 15 at Clayon (WM-S-M-07)
CBS	Cross Bayou-S of Hwy 84 T7N R8E (WM-S-M-08)
BTI	Bayou Teche I-10 at Breaux Brid (WM-S-O-06)
BPI	Bayou Portage I-10 at Henderson (WM-S-O-07)
BDP	Bayou Du Portage Hwy 679 T10S R (WM-S-O-08)

(Table 3.2 Continued)

Water	Source
LCH	Lasalle Coulee Hwy 182 at Cade (WM-S-O-09)
VRH	Vermillon River Hwy 14 at Abbev (WM-S-O-10)
BTH	Bayou Tech Hwy 87 at Olivier (WM-S-O-11)
BGT	Bayou Grosse Tete at Frisco Hwy (WM-S-B-01)
BGT2	Bayou Grosse Tete at I-10 at GR (WM-S-B-04)
BTH2	Bayou Tigre Hwy 404 T11S-RSE (WM-S-B-05)
BRH2	Blind River Hwy 61 T11S-RSE (WM-S-B-06)
HRH	Houston River Hwy 27, 2 MI N.O (WM-S-C-02)
BDC	Bayou De Cannes, Hwy 98 2 MI, W (WM-S-C-03)
BPH	Bayou Plaquemine Hwy 98 4 MI (WM-S-C-04)
EBL	East Bayou Lacassine ½ Mile W (WM-S-C-05)
MRH	Mermentau River Hwy 90 at Merme (WM-S-C-06)
BLH	Bayou Lacassine Hwy 14 T11S R5 (WM-S-C-07)
BSM	Bayou Serpent at Manuel Road (WM-S-C-09)
BBH	Black Bayou, Hwy 530 2 MI. E. of Foley AL 36535 (WM-S-S-01)
BPH2	Bayou Pierre, Hwy 530 2 MI. E. of Foley AL 36535 (WM-S-S-03)
BRH	Boeuf River, Hwy 2 T2 IN R8E S25 Eunice LA (WM-S-M-01)
BMH	Bayou Macon, Hwy 134 Poverty POI Eunice, LA (WM-S-M-02)
TRH	Tensas River Hwy 80 at Tendal, Eunice, LA (WM-S-M-04)
BQD	Bayou Queue De Turtue Hwy 13 T Metairie (WM-S-C-08)
GBH	Grand Bayou Hwy 70 T12S-R13E Washgton (WM-S-B-07)

(Table 3.2 Continued)

Water	Source
BLR	Bayou Lafourche at Raceland T1 Port Barre (WM-S-B-08)
BTG	Bayou Terrebonne at Gray T16S-Port Barre (WM-S-B-09)

*WM = Water monitoring.

Figure 3.1 (A) tomato, (B) blueberry, (C) corn, (D) cucumber, (E) cabbage, (F) honeydew. (G) wheat and (H) rice.

Figure 3.2 (A) Purees of tomato, blueberry, corn, cucumber, cabbage and honeydew. (B) Powders of wheat and rice. (C) Robot Coupe. (D) Majic blender.

3.2.2 Pesticide Residue Extraction in Fresh Surface Water. Sodium sulfate was poured to almost fill a large ceramic filter funnel (1 liter size) with a rubber stopper on the stem. This was attached to the top of a 2-liter filter flask. The flask was attached to a vacuum source and rinsed with pet ether three times. The sodium sulfate was dried by spreading on aluminum foil under a hood. Dried sodium sulfate was packaged in a clean dry container, labeled and dated.

Water sample was allowed to warm up to room temperature having been stored in a cooler at 4 ^{0}C. Into a graduated cylinder, 500 ml of surface water samples were measured and

transferred to a 1-liter separatory funnel. With a 100 ml graduated cylinder, 75 ml of methylene chloride was measured and added to the surface water sample. The surface water samples were vented to prevent breakage of glass due to pressure. The surface water samples were capped and shaken for 1.5 minutes, with occasional release of pressure every 15-20 seconds. A large funnel was prepared for each sample by plugging the stem with a small amount of rinsed glass wool in the bowl and filling approximately a quarter full with sodium sulfate. The bottom layer was drained from the separatory funnel through the prepared funnel into a 400 ml beaker. As carried out earlier, an addition of methylene chloride, subsequent shaking / pressure release every 15-20 sec, and draining the bottom layer from the separatory funnel were repeated two more times with the bottom layer drained. The drained sample was placed in a 400 ml beaker in a water bath at 40-50 ^{0}C, and was evaporated to about 1 ml volume. With a pipette, 2-3 ml of hexane was measured and added into the evaporated sample, and returned to the water bath for further evaporation until about 1 ml volume remained. Hexane was added until 12 ml volume was reached. A small funnel was prepared with a small amount of glass wool added in order to plug its drain. A funnel was placed on a 15 ml graduated test tube in a rack. Sodium sulfate was poured into the funnel bowl until almost the one-third full mark. The sample was transferred from the beaker into the 15ml tube by pouring through the small prepared funnel and a clean stopper cork was placed on the tube containing the sample.

The sample tube was placed in a water bath that was set to 35 ^{0}C. While the samples were in the water bath, nitrogen was blowing on individual samples through a vent connected to a Pasteur pipette into each sample tube. With occasional adjustment of the nitrogen vent, sample tubes were left in the water bath until the sample volume was concentrated to slightly below 1 ml. Hexane was added to make the final sample volume to the 1 ml mark using a Pasteur pipette

and a dropper. Surface water samples, positive and negative controls were prepared in vials for GC-MS (gas chromatography-mass spectrometry) analysis.

3.2.3 Pesticide Residue Extraction in Food Samples. The extraction technique used was the quick, effective, cheap, easy and safe (QuEChERS) method. The stages involved in QuEChERS used in the extraction of food samples toward the detection of pesticide in this study comprised of sample homogenization (blending for vegetables and fruits, or grinding for grains), weighing, spiking (addition of standard solution for measuring extraction efficiency and quantitation of analytes), addition of extraction solvent, buffering and drying (addition of extraction salt), separation of organic layer from the sample (centrifuge), Clean-up (with dispersive Solid Phase Extraction (dSPE) which contains carbon black (carbon 12- C12 and graphitized carbon black-GCB), primary secondary amine (PSA) and magnesium sulphate to remove matrix compounds like chlorophyll, proteins, fats), separation of supernatant from dSPE junk (centrifuge), sample vial preparation for GC-MS analysis. Magnessium sulphate as a salt enhances the ionic strength of the extraction solution that increases the amount of analytes suspended by the sorbents, that is, C12 and GCB. Unwanted dirts and debris like chlorophyll are removed by the PSA. The extraction solvent acetonitrile (ACN) eventually extracts the suspended analytes from the sorbents and also separates the organic layer that contains the analytes at the upper layer away from the lower layer which contains the unwanted debris from the matrix sample. The analytes are concentrated by placing the tube in a water bath at 35 ^{0}C with nitrogen gas blowing through a vent in the analyte solution.

3.2.3.1 Tomato. Six tomato samples labeled 3x, 5x, 7x, 10x, 11x, and 14x were extracted towards possible pesticide residue detection. On a weighing balance, 10 g of tomato puree of

each of the 6 varieties was measured into 50 ml plastic centrifuge tube. A reagent blank sample, which was the negative control, was prepared by pipetting 10 ml of milliQ water into a 50 ml centrifuge tube. Immediately after weighing, spike samples, which were the positive controls, were sorted out, labeled separately and spikes added to each of them accordingly. There was a low and a medium spike each for variety 3x, a medium and a high spike each for variety 5x, a medium spike for 7x, a low spike for 10x and a low spike for 14x. All spiked samples were vortexed and allowed to wait for 30 minutes. With an auto dispenser, 10 ml of solvent (acetonitrile) was added to each of the samples. Samples were shaken using elbow and shoulder than the wrists, and once again vortexed. One pack of an extraction salt of QuEChERS containing 1200 mg magnesium sulphate ($MgSO_4$), 400 mg primary and secondary amine (PSA), 400 mg carbon 18 (C18) and 400 mg graphitized carbon black (GCB) was added to each sample. $MgSO_4$ helps enhance the ionic strength of the extraction solution thereby increasing the attraction of analytes towards sorbents C18 and GCB. PSA helps to remove matrix junks like the chlorophyll. Elbows and shoulders were once again used to shake the salted samples, after which they were centrifuged for 10 minutes at 3500 rpm for 15 minutes. Coming out of the centrifuge, separate layers were formed distinctly, and the upper layer (acetonitrile extract) was carefully pipetted into 15 ml centrifuge tube containing dSPE 150 $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg GCB. These were vortexed and centrifuged for 1 minute at 3500 rpm. Sample vials were prepared for GC-MS. A total of 28 sample vials comprising of 1 solvent (acetonitrile), 3 ACN standards, 4 matrix standards, 3 solvent standards, 1 reagent blank, 3x, low spike 3x, medium spike 3x, 5x, 5x duplicate, medium spike 5x, high spike 5x, 7x, 7x duplicate, 7x medium spike, 14x, 14x duplicate, 14x low spike, 10x, 10x low spike, and 11x were prepared and ran. All samples were loaded into the GC/MS analyzer and the machine operated as earlier outlined.

Details of preparation of standard samples, matrix samples, and solvent samples are as outlined in appendix 1. Sequences of the runs were as outlined in appendix 2.

3.2.3.2 Corn. Whole grains from 5 cobs of corn from each sample were scraped into the blender. As shown in the sequence in appendix 3, three corn samples 6x, 8x and 13x were prepared as explained in section 3.2.3.1 for tomato. A total of fourteen samples were prepared from the 3 samples including the reagent blank and solvent standard, making a total of 4 samples from each of the varieties. 6x matrix standard, 6x spike, 6x, 6x duplicate were from variety 6x. Four samples from variety 8x were 8x matrix standard, 8x spike, 8x, and 8x duplicate. The four from 13x were 13x matrix standard, 13x spike, 13x, and 13 duplicate. All these 14 samples were loaded into the GC-MS in 3 replicates.

3.2.3.3 Blueberry. About 50 pieces of blueberry fruits were ground for each sample. Extraction of two samples of blueberries namely 4x and 12x was done as stated for tomato in section 3.2.3.1. Ten samples with reagent blank inclusive (4x, 4x duplicate, 4x low spike, 4x medium spike, 12x, 12x duplicate, 12x low spike, 12x medium spike and 12x high spike) were prepared and ran in the chromatography for both samples.

3.2.3.4 Cucumber. Four cucumbers were sliced and blended. The same extraction process carried out for tomato in section 3.2.3.1 was done for cucumber sample 9x. Four standards were prepared – 2 matrix standards and 2 solvent standards. In addition to the standards, 5 samples were prepared as 9x, 9x duplicate, 9x low spike, 9x medium spike and 9x high spike. Samples were ran in the GC-MS as duplicate in replication.

3.2.3.5 Melon (honeydew). One whole melon of sample 15x was extracted as done for tomato (section 3.2.3.1). The matrix standards prepared were two as well as 2 solvent standards. Other samples prepared and ran along with the standards includes 15x, 15x duplicate, 15x low spike, and 15x high spike. Samples were replicated twice as loaded and ran in the GC-MS.

3.2.3.6 Cabbage. Sample extraction as carried out on tomato (section 3.2.3.1) was repeated for 1 whole piece of cabbage. Two solvent standards, 2 matrix standards, 16x, 16 duplicate, 16 low spike and 16x medium spike were the samples prepared and run for cabbage.

3.2.3.7 Wheat. About 100 g of whole grains of wheat was measured into the majic blender for each of samples 1x and 2x**.** The two wheat samples 1x and 2x were extracted as outlined for tomato in section 3.2.3.1. Seventeen samples were prepared for both varieties. Reagent blank, two matrix standards for each of the varieties, 2 solvent standards for each of the samples making 8 standards followed by 1x, 1x duplicate, 1x low spike, 1x high spike, 2x, 2x duplicate, 2x low spike, and 2x medium spike. The 16 samples were replicated twice during run in the GC-MS machine.

3.2.3.8 Rice. About 100 g of rice sample 17x was measured and blended into powder. Rice sample was extracted in the same way as done for tomato (section 3.2.3.1). Reagent blank, two solvent standards, two matrix standards, 17x, 17 duplicate, 17x low spike, 17x medium spike and 17x high spike were samples prepared followed by replicating twice during machine run.

3.2.4 Gas Chromatography-Mass Spectrometry. Both gas chromatography and mass spectrometry combined in one as GC-MS were from Agilent company. The GC component was Agilent 6890 while the MS was Agilent 5975 quadrupole. The series autosampler 6890 series

was used to inject sample extracts and standards into the GC-MS. The column was a Restek 35 MS-GC column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness. For the instrument control and quantitative data analysis, software was required and the software used was Agilent ChemStation. Injection volume was 2 µl with pulsed spitless at 20 psi pressure pulse for 0.74 minutes. Injector temperature was 250 ^{0}C and a transfer line temperature of 280 ^{0}C. Helium gas was the carrier mobile phase with a constant flow at 1.5 ml/min. The temperature of the system was programmed with an initial temperature set to 120 ^{0}C and held for 2 min after which it was elevated to 340 ^{0}C at 30 ^{0}C/min rate prior to the final hold of 2 minutes. The total run based on these settings was 12.33 minutes. The mode at which the MS was operated was electron impact ionization (EI) with MS ion source at 230 ^{0}C and the quadrupole at 150 ^{0}C. Electron nultiplier was set at 200 V above the calibration standard PFTBA (Perfluorotributylamine) autotuned setting. Selected ion monitoring (SIM) mode was used for screening and quantitative analysis of targeted pesticides. The initial identification of a pesticide in the sample was based on the detection of its characteristic ion peaks and their relative abundances as well as the comparison of its retention time with those observed in the analytical standard. The particular retention times and quantitation ions for the SIM mode analysis of the pesticides is as shown in Table 3.3. Full-scan (50-450m/z) MS analyses were conducted to confirm the pesticide's detection by comparison to mass spectral libraries from both commercial sources and internally generated spectra. This comparison was automated using the NIST (National Institute of Standards and Technology) AMDIS (Automated Mass spectral Deconvolution and Identification System) software. Retention time confirmation against the analytical standard in full-scan MS mode was also required for confirmation. Pesticides with multiple peaks are summed for quantification.

3.2.5 Trends of Some Pesticide Residues in the Surface Water Samples. From the database of the pesticide lab of agricultural chemistry dept. of LSU, data for the recent past 4 years (2012-2015) from the results of analysis of pesticide residues in some surface waters was accessed. Water samples collected each year was done in summer in the month of May. In order to show the trends of either an increase or reduction in the levels of pesticide residues detected from the same sources year-in year-out, selected water samples studied included BPH, CLC, BBH, BRH, BCH, TRH2, BPI and CDG.

Table 3.3 Retention time and quantitation ion for target compounds and their degradation products involved in this study

Compound	t_R (min)	Q_{ion} (m/z)	Compound	t_R (min)	Q_{ion} (m/z)
Carbofuran deg.	4.08	164	Metribuzin	7.18	198
Eptam	4.24	128	Malathion	7.20	173
Etridiazole	5.04	183	Metolachlor	7.22	162
Trifluralin	5.60	306	Chlorpyrifos	7.26	197
Molinate (Ordram)	5.57	126	MB45950fm	7.29	420
Captan deg.	5.67	79	Fipronil	7.35	367
Tefluthrin	6.17	177	Pendameth	7.50	252
Thimet	6.17	75	Bromacil	7.50	207
DesEthylAtrazine	6.24	172	Cyanazine	7.57	225
DesIsopropylAtz	6.28	173	MB46136fm	7.80	383
Prometone	6.34	225	Captan	7.80	79
Terbufos	6.40	231	Propicon1	8.56	259

(Table 3.3 continued)

Compound	t_R (min)	Q_{ion} (m/z)	Compound	t_R (min)	Q_{ion} (m/z)
Diazinon	6.40	137	Bifenthrin	8.57	181
Tebupirimiphos	6.42	261	Propicon2	8.59	259
Atrazine	6.50	200	Norflurazon	8.76	303
Clomazone	6.53	125	λ-cyhalot1	8.91	181
Carbofuran	6.65	164	λ-cyhalot	8.99	197
MB46513,Fip. met.	6.76	388	Hexazinone	9.01	171
Acetochlor	6.87	223	Cyfluthrin 1	9.69	206
Dimethenamid	6.91	154	Cypermet1	9.88	181
Terbacil	6.93	161	Cyfluthrin 3	9.76	206
Alachlor	6.95	188	Cypermet2	9.95	181
Prometryn	7.05	241	Esfenvalera1	10.36	167
Propanil	7.10	161	Esfenvalerate	10.45	167
Metalaxyl	7.10	249	Azoxystrobin	11.51	344
Methyl Parathion	7.16	263	Carbaryl	5.84	144
Acephate	5.62	136	Methamido	4.45	141
Endosulfan I	8.18	237	Endosulfan II	8.79	195
Endosulfan SO_4	9.08	272	Permethrin I	9.53	163
Permethrin 2	9.57	163			

λ = lambda; DesIsopropylAtz = desethylatrazine; MB46136fm = MB46136, Fip. met.; MB45950 = MB45950, Fip. met. Pendameth = Pendamethalin; Propicon2 = Propiconazole 2; Propicon1 = Propiconazole 1; λ-cyhalot1 = Lambda-cyhalothrin 1; λ-cyhalot = Lambda-cyhalothrin; Cypermethrin 1 = Cypermet1; Cypermethrin 2 = Cypermet2; Esfenvalerate 1 = Esfenvalera1; Methamido = Methamidiphos.

3.3 Statistical Analysis

Six calcualtions were required in computing the results obtained in the chromatographic analysis of both the water and food samples. These calculations are as outlined as follows:

1. Calculate the on column conc. of the sample:

This equaltion 1 solves for “x” (x will be the same unit as the standard conc.)

Std. conc. µg/ml/std. area = on column conc. “x” µg/ml/sample area

(The assumption of this formula is that the injection vol. of the std. & sample are the same value: e.g. 10 µl).

2. On column concentration “x” is calculated in terms of the amount of sample it actually signifies:

On column conc. “x” (µg/ml)/sample wt (g) X vol of extract solvent(ml) X final vol (ml)/original vol (ml) = amount reported found in the sample (ppm or µg/g)

3. The spiking rate was calculated:

The spiking rate does not depend on the volume of extraction neither on any dilutions nor concentrations.

Vol of std added (ml) X std con (µg/ml) /wt of sample (g) = spiking rate (ppm)

4. The efficiency of the methodology was confirmed through the value of the spike recovery:

Amount reported in the sample (ppm) /spiking rate (ppm) X 100 = % Recovery

5. The on column concentration expected from a spike was calculated and it provides a clue as

per where matrix standard is needed to be in order to use it to calculate the recovery rate.

Volume of standard added (ml) x standard conc. (µg/ml) / volume of extraction solvent (ml) x original volume (ml) / final volume (ml) = spike conc. on column (µg/ml).

6. The amount of sample represented in the liquid injected onto column was calculated. This

represents the amount that could be be written on the worksheet as final dilution. This also stands for the sample aount that gets to the detector. Note that the more sample to the detector, the lower the limit of detection and the dirtier the injection will be:

weight of sample (g) / vol. of extraction solvent (ml) x original vol. (ml) / final vol. (ml) = sample amount to the detector (g/ml)

This makes a factor out of equation 2 above (on column concentration "x" adjustment for the amount of sample it signifies):

"x" (µg/ml) / sample amount to the detector (g/ml) = amount reported found in the sample in ppm (µg/g).

3.4 Results

3.4.1 Pesticide in Surface Waters. Chromatographic analysis of the 35 water samples analyzed indicated that different pesticides were found in them. As outlined in Tables 3.4a through 3.4e, the total number of pesticides that were detected across the 35 surface waters was 17. Since there is no threshold set for pesticide residues in surface water, the closest way to interpret the possible impact of the pesticide levels detected in this study is to compare them with the threshold set for

potable waters by the EPA (United States Environmental Protection Agency). However, the EPA has thresholds published for selected pesticides like atrazine, glyphosate and 2,4-D. The limits are for atrazine 0.003 ppm, 0.07 ppm for 2,4-D and 0.7 ppm for glyphosate. In comparison to these standards, 4 waters (0.00648 ppm in CLC, 0.0062 ppm in BRH, 0.00624 ppm in BCH, and 0.01188 ppm in CBS) gone above the atrazine limit.

Table 3.4a Pesticide residues detected in surface waters (ppm).

	BPH	CRH	CLC	CDG	BBH	BPH2	BRH	Stdev
Atrazine	0.0002	0.00033	0.00648	0.00068	0.00178	0.00052	0.0062	0.003
AM PA	0.35	0.35	0.35	0.35	ND	ND	0.35	0.00
Glyphosate	0.35	0.35	0.35	0.35	ND	ND	0.35	0.00
Quinclorac	ND	0.0002	ND	ND	ND	ND	0.0043	0.003
Desethatz	ND	ND	0.00074	ND	ND	ND	0.00062	8.5E-05
Metolachlor	ND	ND	0.00108	0.00084	0.00116	0.00042	0.0172	0.007
Fluometuron	ND	ND	ND	ND	ND	0.00075	0.0014	4.6E-04
Diuron	ND	ND	ND	ND	ND	0.0002	ND	ND
Acetochlor	ND	ND	ND	ND	ND	ND	ND	ND
Clomazone	ND	ND	ND	ND	ND	ND	0.0024	ND
Metribuzin	ND	ND	ND	ND	ND	ND	0.00034	ND
Trifluralin	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	ND	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND
Bromacil	ND	ND	ND	ND	ND	ND	ND	ND

(Table 3.4a continued)

	BPH	CRH	CLC	CDG	BBH	BPH2	BRH	Stdev
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND
Acifluorfen	ND	ND	ND	ND	ND	ND	0.00022	ND

Each water sample was labeled as abbreviation of the name of its source as outlined in Table 3.2; ND = Not detected. Desethatz = Desethylatrazine.

Table 3.4b Pesticide residues detected in surface waters (ppm).

	TRH	LTC	LBT	TRH2	CBS	BTI	BPI	Stdev
Atrazine	0.00274	0.00246	0.0006	0.00038	0.01188	0.00038	0.00072	0.004
AM PA	ND	0.35	0.35	0.35	0.35	0.35	0.35	6.1E-17
Glyphosate	0.35	0.35	0.35	0.35	0.35	0.35	0.35	6.0E-17
Quinclorac	ND	ND	ND	ND	ND	ND	ND	ND
Desethatz	ND	ND	ND	ND	0.00122	ND	0.0002	7.2E-04
Metolachlor	0.01204	0.00106	0.00036	0.0034	0.00396	0.00049	0.00074	0.004
Fluometuron	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	0.0023	ND	0.00106	ND	ND	ND	8.8E-04
Acetochlor	ND	ND	ND	0.00022	ND	ND	ND	ND
Clomazone	ND	ND	ND	ND	ND	0.0002	ND	ND
Metribuzin	0.00086	ND	ND	0.0003	ND	ND	0.00028	3.3E-04
Trifluralin	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	ND	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND
Bromacil	ND	ND	ND	ND	ND	ND	ND	ND
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND

(Table 3.4b continued)

	TRH	LTC	LBT	TRH2	CBS	BTI	BPI	Stdev
Acifluorfen	ND	ND	ND	ND	ND	ND	ND	ND

Each water sample was labeled as abbreviation of the name of its source as outlined in Table 3.2; ND = Not detected. Desethatz = Desethylatrazine.

Table 3.4c Pesticide residues detected in surface waters (ppm).

	VRH	BTH	BGT	BGT2	BTH2	BRH2	GBH	Stdev
Atrazine	0.00026	ND	ND	ND	ND	0.00032	ND	4.3E-05
AM PA	0.35	0.35	ND	ND	ND	ND	ND	0.00
Glyphosate	0.35	0.35	ND	ND	ND	ND	ND	0.00
Quinclorac	ND	ND	ND	ND	ND	ND	ND	ND
Desethatz	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	0.0002	ND	ND	ND	0.00038	ND	ND	1.3E-04
Fluometuron	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	ND	ND	ND	0.00037	0.00054	ND	1.2E-04
Acetochlor	ND	ND	ND	ND	ND	ND	ND	ND
Clomazone	ND	ND	ND	ND	ND	ND	ND	ND
Metribuzin	0.0002	0.00068	0.00238	0.00054	0.00024	0.00023	0.0017	8.5E-04
Trifluralin	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	0.0002	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND
Bromacil	ND	ND	ND	ND	ND	ND	ND	ND
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND

(Table 3.4c continued)

	VRH	BTH	BGT	BGT2	BTH2	BRH2	GBH	Stdev
Acifluorfen	ND	ND	ND	ND	ND	ND	ND	ND

Each water sample was labeled as abbreviation of the name of its source as outlined in Table 3.2; ND = Not detected. Desethatz = Desethylatrazine.

Table 3.4d Pesticide residues detected in surface waters (ppm).

	HRH	BDC	BPH	EBL	MRH	BLH	BQD	Stdev
Atrazine	ND	ND	ND	ND	ND	ND	ND	ND
AM PA	ND	ND	ND	ND	ND	ND	ND	ND
Glyphosate	ND	ND	ND	ND	ND	ND	ND	ND
Quinclorac	ND	0.00058	0.0003	0.00142	0.0004	ND	0.0002	4.9E-04
Desethatz	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	0.00032	ND	ND	ND	0.00182	1.1E-03
Fluometuron	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	0.00029	ND	0.0002	ND	ND	ND	6.4E-05
Acetochlor	ND	ND	ND	ND	ND	ND	ND	ND
Clomazone	ND	0.0003	0.00032	0.00058	0.00022	0.0002	0.0002	1.5E-04
Metribuzin	ND	ND	ND	ND	ND	ND	0.00036	ND
Trifluralin	ND	ND	ND	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	0.0003	ND	ND	ND	ND
Dicamba	ND	ND	ND	ND	ND	ND	ND	ND
Bromacil	ND	0.00042	ND	ND	ND	ND	ND	ND

(Table 3.4d continued)

	HRH	BDC	BPH	EBL	MRH	BLH	BQD	Stdev
2,4-D	0.00036	ND	ND	ND	ND	ND	ND	ND
Acifluorfen	ND	ND	ND	ND	ND	ND	ND	ND

Each water sample was labeled as abbreviation of the name of its source as outlined in Table 3.2; ND = Not detected. Desethatz = Desethylatrazine.

Table 3.4e Pesticide residues detected in surface waters (ppm).

	BMH	BCH	BDP	LCH	BLR	BTG	BSM	Stdev
Atrazine	0.0018	0.00624	ND	0.00054	ND	ND	ND	0.003
AM PA	0.35	0.35	0.35	0.35	ND	ND	ND	0.00
Glyphosate	0.35	0.35	0.35	0.35	ND	ND	ND	0.00
Quinclorac	ND	ND	ND	ND	ND	ND	0.00054	ND
Desethatz	0.00058	0.00054	ND	ND	ND	ND	ND	2.8E-05
Metolachlor	0.0006	0.0039	ND	ND	0.00034	ND	ND	2.0E-03
Fluometuron	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	ND	ND	ND	0.00056	ND	0.00075	ND	1.3E-04
Acetochlor	ND	0.00028	ND	ND	ND	ND	ND	ND
Clomazone	ND	ND	ND	ND	ND	ND	0.00042	ND
Metribuzin	0.00266	0.00036	0.00172	0.00036	0.0006	ND	ND	1.0E-03
Trifluralin	ND	ND	0.00028	ND	ND	ND	ND	ND
Triclopyr	ND	ND	ND	ND	ND	ND	0.00028	ND
Dicamba	ND	ND	ND	ND	0.00104	ND	ND	ND
Bromacil	ND	ND	ND	ND	ND	0.00034	ND	ND

(Table 3.4e continued)

	BMH	BCH	BDP	LCH	BLR	BTG	BSM	Stdev
2,4-D	ND	ND	ND	ND	ND	ND	ND	ND
Acifluorfen	ND	ND	ND	ND	ND	ND	ND	ND

Each water sample was labeled as abbreviation of the name of its source as outlined in Table 3.2; ND = Not detected. Desethatz = Desethylatrazine.

As outlined in Table 3.4, the lowest among the 4 samples that over the threshold was sample BRH and was 107 % higher than the EPA limit for potable waters. The highest above threshold sample was recorded in CBS at 296 % above the limit.

Table 3.5 Percentage of Atrazine above Limit (comparing surface water with EPA potable water limit).

Sample	Surface water Atrazine Detected	Potable water EPA Limit	Difference	% above limit
CLC	0.0065	0.003	0.00348	116
BRH	0.0062	0.003	0.00320	107
BCH	0.0062	0.003	0.00324	108
CBS	0.0119	0.003	0.00890	296

3.4.2 Pesticide in Food. Tomato, melon and rice were found with pesticides as shown in Table 3.5. Corn, blueberry, cucumber and cabbage showed no pesticide residues. Out of the 6 varieties of tomatoes analyzed, 3 of them – samples 2, 4 and 6 respectively showed presence of sevin

(carbaryl), cypermethrin and cyfluthrin. Concentration of the carbaryl found was 0.110 ppm, while that of cypermethrin and cyfluthrin were 0.180 and 0.110 ppm respectively. The FDA tolerance threshold in tomatoes was 5.000, 0.200 and 0.200 ppm for carbaryl, cypermethrin and cyfluthrin respectively.

Azoxystrobin was found in melon at the level of 0.057 ppm. The FDA tolerance rate was 0.300 ppm in melon. The rice variety contained 0.031 ppm propiconazole and 0.027 ppm azoxystrobin. Tolerance rate in rice as provided by the FDA was 7 ppm for propiconazole and 5 ppm for azoxystrobin.

Table 3.6 Pesticides found in food.

Food sample	Sample number	Sample name	Pesticide detected	Amount (ppm)	Tolerance (ppm)
Tomato	1	3x	None	-	-
Tomato	2	5x	Sevin	0.110	5.000
Tomato	3	7x	None	-	-
Tomato	4	10x	Cypermethrin	0.180	0.200
Tomato	5	11x	None	-	-
Tomato	6	14x	Cyfluthrin	0.110	0.200
Corn	1	6x	None	-	-
Corn	2	8x	None	-	-
Corn	3	13x	None	-	-
Blueberry	1	4x	None	-	-
Blueberry	2	12x	None	-	-
Cucumber	1	9x	None	-	-

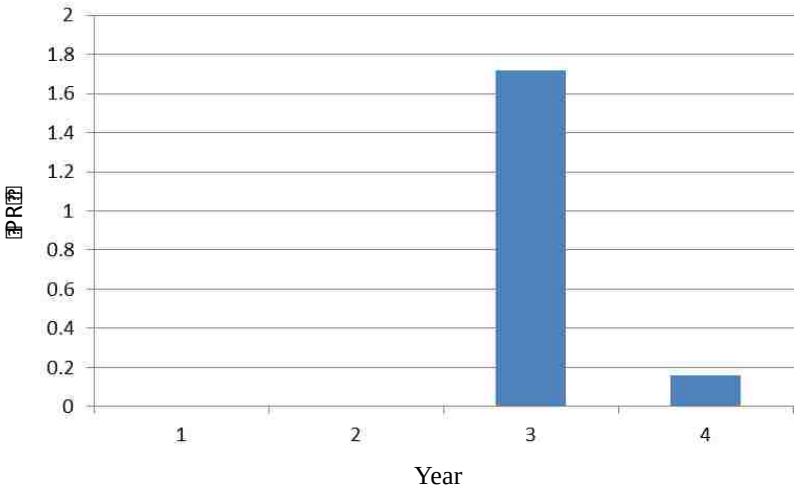
(Table 3.6 continued)

Food sample	Sample number	Sample name	Pesticide detected	Amount (ppm)	Tolerance (ppm)
Melon (Honeydew)	1	15x	Azoxystrobin	0.057	0.300
Cabbage	1	16x	None	-	-
Wheat	1	1x	None	-	-
Wheat	2	2x	None	-	-
Rice	1	17x	Propiconazole	0.031	7.000
Rice	1	17x	Azoxystrobin	0.027	5.000

3.4.3 Trends of Some Pesticide Residues in the Surface Water Samples. Data gathered for the recent past 4 years (2012-2015) from the results of analysis of pesticide residues in some surface waters was accessed. Data from selected water samples BPH, CLC, BBH, BRH, BCH, TRH2, BPI and CDG were plotted into graphs (Figures 3.3a through 3.3l) in order to show the trends of either an increase or reduction in the levels of pesticide residues detected from the same source year-in year-out. Figure 3.3a showed a decline in atrazine level. Year 2012 and 2013 data showed no atrazine was detected in sample BPH but comparing year 2014 and 2015 revealed a fall in atrazine level from 1.72 ppb in 2014 to 0.16 ppb in 2015.

Figure 3.2b showed a steady increase in atrazine level in the sample CLC as record confirmed 0.4 ppb in 2012, 2.26 ppb in 2013, 4.62 ppb in 2014 and 6.48 ppb in 2015. In sample BBH, atrazine content also is on the increase (Figure 3.3c) starting at 0.2 ppb in 2012, 0.66 ppb in 2-13, ND (no detection) in 2014 and finally 1.78 in 2015. In Figure 3.3d, atrazine level in

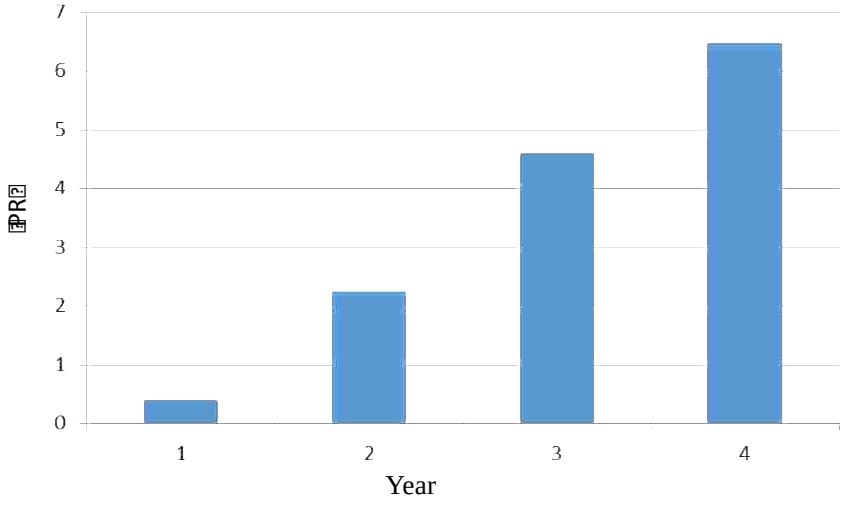
2012 in BRH sample was at 2.36 ppb; while there was ND in both 2013 and 2014, year 2015 experienced an increase to 6.2 ppb.



Legend
Year 1 = 2012
Year 2 = 2013
Year 3 = 2014
Year 4 = 2015
PR = pesticide residue

BPH-ATRAZINE PR	Level (ppb)
2012	0
2013	0
2014	1.72
2015	0.16

Figure 3.3a Atrazine in BPH sample from 2012 through 2015.



Legend
Year 1 = 2012
Year 2 = 2013
Year 3 = 2014
Year 4 = 2015
PR = pesticide residue

CLC-ATRAZINE PR	Level (ppb)
2012	0.4
2013	2.26
2014	4.62
2015	6.48

Figure 3.3b Atrazine in CLC sample from 2012 through 2015.

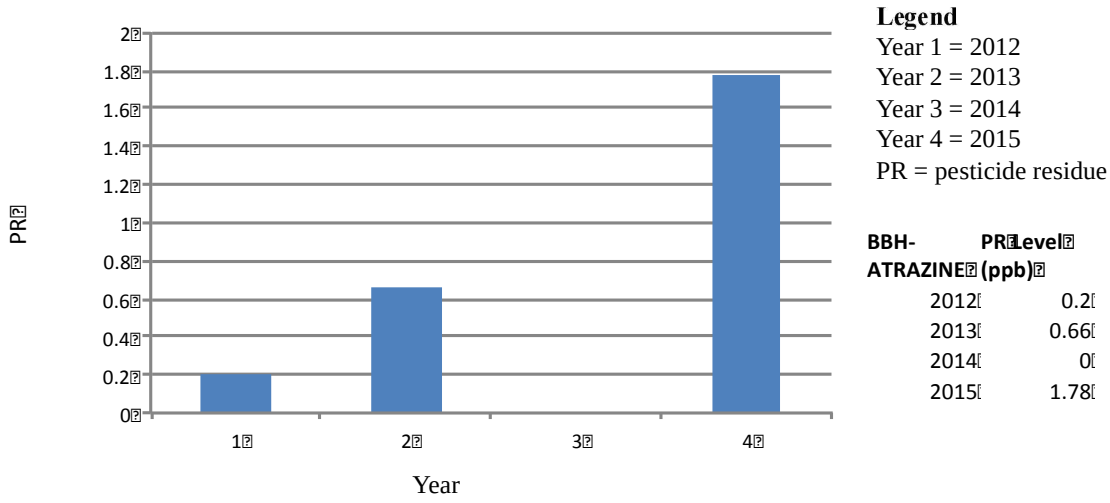


Figure 3.3c Atrazine in BBH sample from 2012 through 2015.

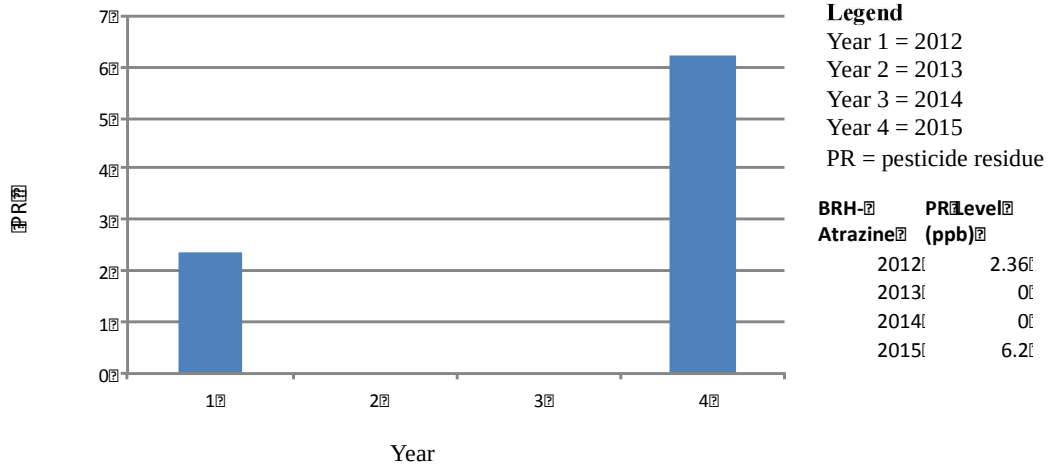


Figure 3.3d Atrazine in BRH sample from 2012 through 2015.

A fluctuation was observed in the atrazine levels in sample BCH (Figure 3.3e) as the level was 6.24 in 2013, dropped to 1.38 in 2014 and finally back to 6.24 in 2015. No detection of atrazine in 2012. In sample TRH2 (Figure 3.3f), there was no detection in 2013 and 2014; but the 2012 atrazine level was 1.28 and a decrease to 0.38 in 2015 was observed. Another fluctuation

as seen in Figure 3.3g, was in atrazine level that was 1.16 in 2012, dropped to 0.7 in 2013, increased to 2.32 in 2014 and dropped back to 0.72 in 2015.

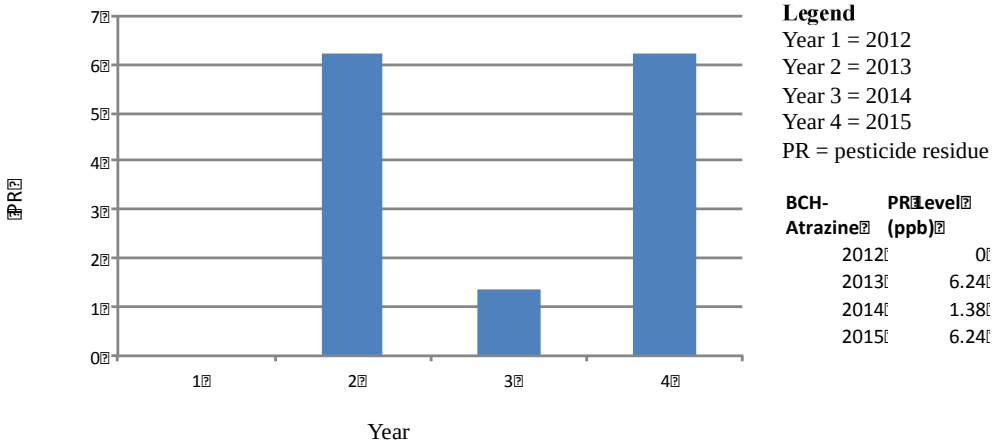


Figure 3.3e Atrazine in BCH sample from 2012 through 2015.

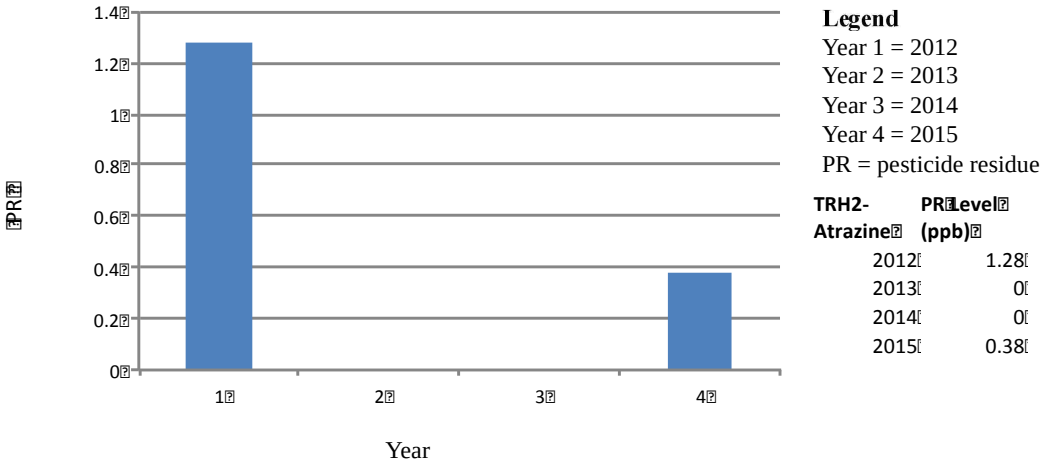


Figure 3.3f Atrazine in TRH2 sample from 2012 through 2015.

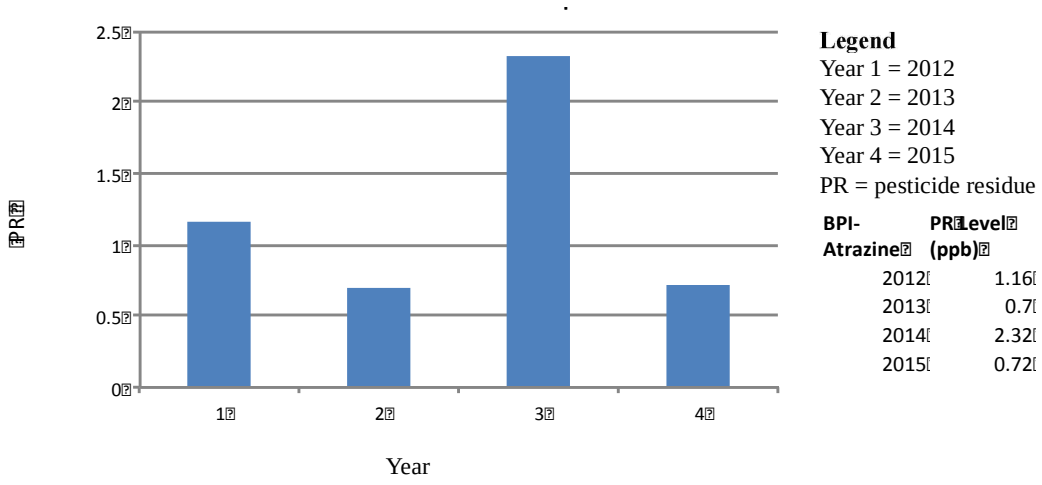


Figure 3.3g Atrazine in BPI sample from 2012 through 2015.

Metolachlor in sample BPH (Figure 3.3h) was not detected in 2012 and 2013, but it level was 1.72 in 2014 and a drop to 0.16 in 2015. In the sample CDG, there was no detection as recorded for metolachlor levels in 2013 and 2014 (Figure 3.3i); but incidentally there was an equal level of 0.84 recorded in 2012 as repeated in 2015.

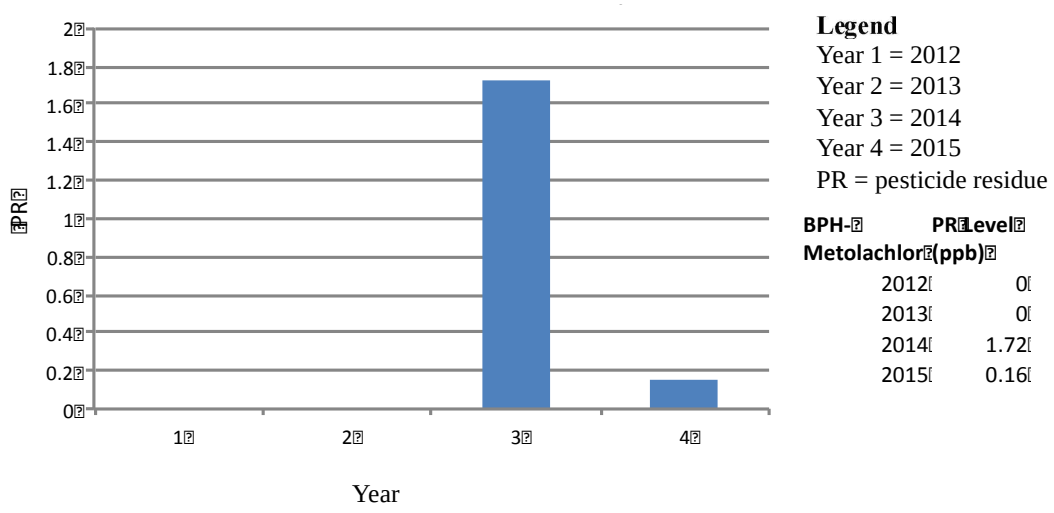


Figure 3.3h Metolachlor in BPH sample from 2012 through 2015.

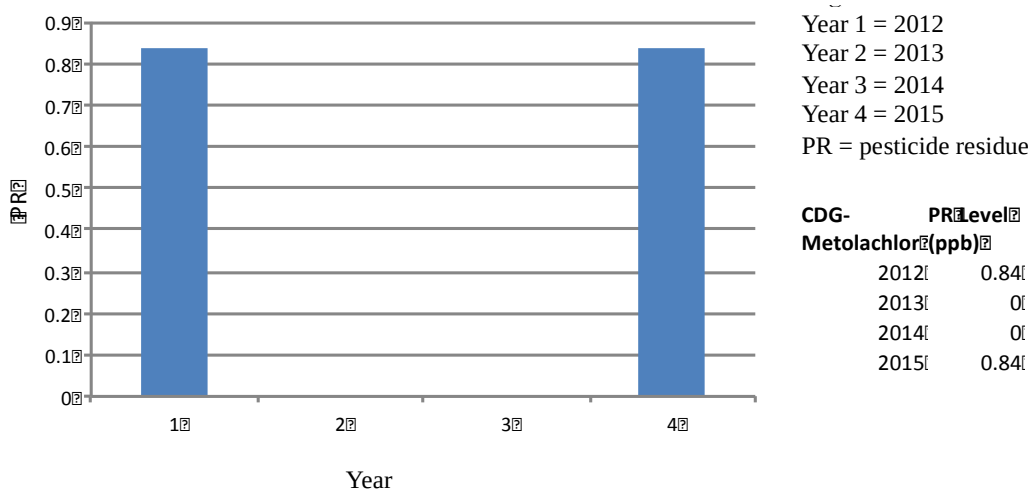


Figure 3.3i Metolachlor in CDG sample from 2012 through 2015.

In Figure 3.3j, metolachlor level in 2012 sample of BBH was 0.2 and an increase in 2015 to 1.16; there was no detection in consecutive years 2013 and 2014. Metolachlor also fluctuated greatly in sample BRH (Figure 3.3k) was at 3.26 level in 2012, increased to 40 in 2013, went down to 4.56 in 2014 and rose to 17.2 in 2015.

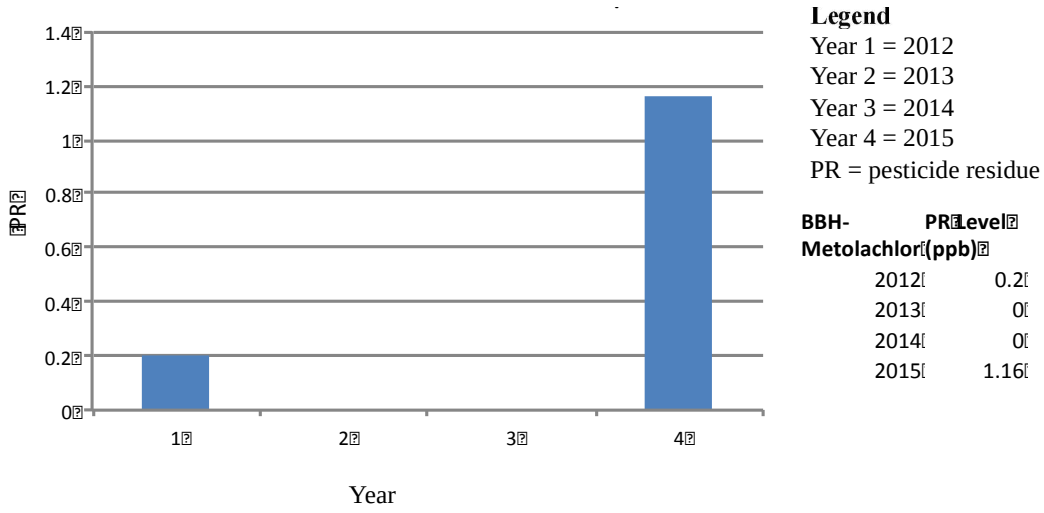


Figure 3.3j Metolachlor in BBH sample from 2012 through 2015.

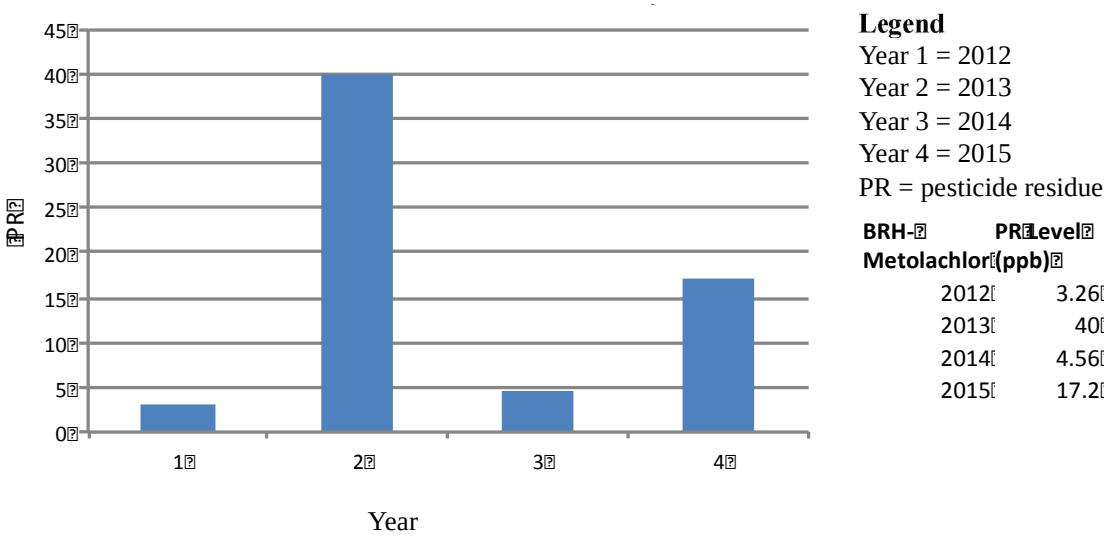


Figure 3.3k Metolachlor in BRH sample from 2012 through 2015.

The last Figure 3.3l shows some mild fluctuations as clomazone was 3.96 ppb in 2012, dropped to 2.48 in 2013, drpped further to 1.96 in 2014 but on the increase in 2015 as it went up to 2.4 ppb.

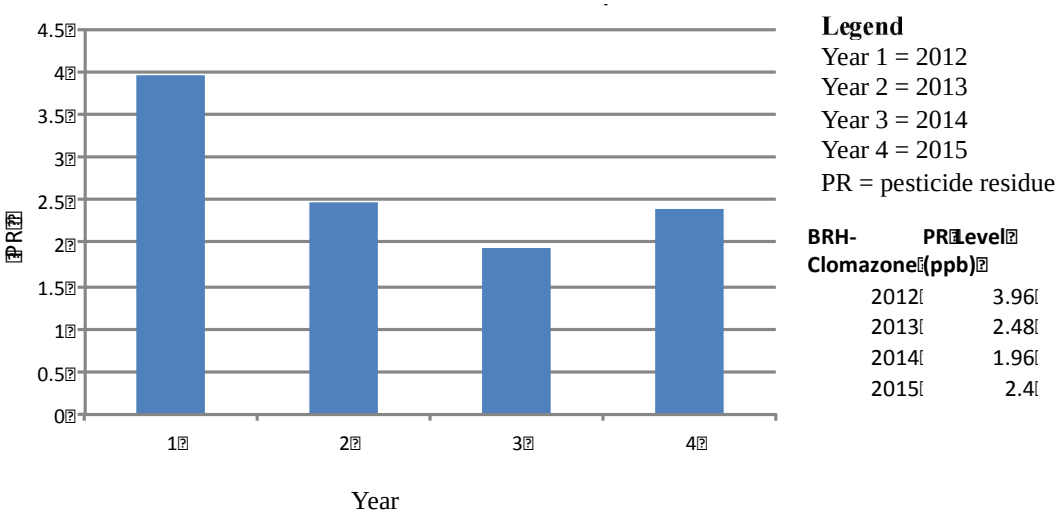


Figure 3.3l Clomazone in BRH sample from 2012 through 2015.

3.5 Discussion

Results obtained in this study are similar to reports of earlier works conducted in this field as some of the pesticides detected in foods and waters studied have been detected by some authors. Walther (2003) detected 0.0375 ppm atrazine in Iberville water district surface water in Upper Terrebonne Basin of Louisiana. This was 1150 % above tolerance limit of 0.003 ppm. This is far above the range value obtained in this study for high concentrations of atrazine. This is about 4 times more than the highest value of 296% obtained in sample CBS in this study. Atrazine in ground water was reported by Lemic *et al* (2006). Atrazine was found in ground water in the United Kingdom to have exceeded potable water limit (0.0001 ppm) in more than 10% of the analyzed samples (Comber, 1999).

The 3 pesticides detected in tomato namely carbaryl, cypermethrin (Ahmed *et al*., 2015; Alamgir *et al*., 2013) and cyfluthrin (Dikshit *et al*., 2003) are insecticides used in its cultivation. Sevin is used to control cutworm, stinkbugs and thrips; Cypermethrin is used to control hornworm; and Cyfluthrin is used against thrips, leafminer and stinkbug (Masabni, 2015). Cypermethrin level of 0.180 ppm detected in tomato is very close to its 0.200 ppm ceiling level as set by the FDA. However, cypermethrin is acid-labile as it degrades with increasing level of acidity. According to Lin *et al* (2005), cypermethrin level in tomato decreases by 30 % within 12 days at 5 ^{0}C in tomato paste pH of 4.3. The degradative product of cypermethrin is 3-Phenoxybenzaldehyde whose health effect is yet unknown but an *in-vitro* study carried out by Lin *et al* (2005) confirmed some endocrine activity associated with cypermethrin breakdown. This may explain part of the reasons why the exposure of tomato consumers to this insecticide may, or may not remain, less of a risk. Azoxystrobin and propiconazole are fungicides.

Azoxystrobin detected in melon and rice in this study is used in melon to control gummy stem blight (Stevenson *et al*., 2004), and in rice to control sheath blight (Groth, 2005). Propiconazole serves the same purpose of controlling sheath blight in rice farming (Jones *et al*., 1987).

While pesticides found in the foods products were below tolerance limits as set by the EPA, those levels detected in surface waters were above the tolerance for atrazine in 4 samples. Since the amount of pesticide residue in water is a function of its usage (Lemic *et al.,* 2006), in addition to our results, the ground water samples and produce from those 4 locations should be monitored for atrazine after which the respective authorities and the users of atrazine in the regions could be advised to take caution.

Trends observed in the atrazine, metolachlor and clomazone in those water samples could be reliably considered since the samplings were done at the same month of May from 2012 through 2015. Since the agricultural activities that characterize this period of the year did not change, it will not be much of a factor but rather some other factors like weather anomalies resulting to storm and erosion may be part of the reasons for such fluctuations.

3.6 References

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CHAPTER 4
ALLEVIATION OF PESTICIDES

4.1 Introduction

Alleviation of pesticide residues in surface water is a step towards water purification as it describes the removal or reduction of pesticides in water. Considering the multi-purpose use of water in food production, from irrigation of crops to postharvest cleaning of farm produce the use of clean water is desired. Detrimental effect of pesticides on human health is one reason why we need to have a method for keeping our food and water pesticide-free. Carson (1958) asserted how lack of caution in pesticide handling could make an environment vulnerable and desolate, as some pesticides, once applied, take ages to degrade thereby constituting an impediment to life and subsequent agricultural practice in such environment. The persistent nature of pesticides in our environments, demands a method of getting rid of unwanted pesticide residues in the soil, waters and atmospheric air around us.

Some of the past efforts made in removing pesticide residues in water include the use of clay (Li *et al.*, 2003; Lemic *et al* 2006), activated carbon (Boussahel *et al.*, 2000; Ogata *et al* 2011) and ozonization (Boussahel *et al.*, 2000). Use of clay is limited by its adsorption capacity due to its shrink-swell behavior and zeolites are free of such flaws (Tarasevich and Polyakov, 1995). Saturation of carbon filters resulting in cost of replacement; and a decrease in the efficiency of activated carbon with increased organic contaminants are limitations in the use of activated carbon (Welte *et al.*, 1996). Formation of byproducts like peroxides, ozonides, organobromine and bromate are associated with the use of ozonization (Welte *et al*., 1996).

A natural zeolite like clipnotilolite is high in its cation exchange capacity due to its net negative charge on the outer surface. When a natural zeolite is further fortified with an overall positive charge on its surface by modification with surfactant(s), its affinity for cation changes to anion and it entraps negatively charged organic ions. These unique attributes of a zeolite are both utilized in this study as we seek to alleviate pesticide residues in surface waters across Louisiana.

4.2 Materials and Methods

4.2.1 Water Filtration through Natural Zeolite- Clinoptilolite. Ten samples -BPH, CLC, CDG, BBH, BRH, BCH, LBT, TRH, BPI, and BDC, were selected from the original pool of 35 samples of surface water studied for detection of pesticide residues as reported in Chapter 3 of this dissertation. The criterion used in selecting those 10 samples was the water samples that had the most pesticide residues based on the results obtained in Chapter 3 of this dissertation.

As shown in Figure 4.1, A, the water filtration system used to filter surface water samples from top to bottom contained 20 g each of gravel, sand and Zeolite. A funnel was placed on the topmost column and filtration was initiated. The filtrate was collected into a 1 liter amber color bottle as shown in Figure 4.1, B. For each water sample, a total of 1000 ml was filtered per 20 g of zeolite after which the filtration system was dismantled, cleaned by hot wash in soap, rinsed in running potable water thrice and allowed to dry before re-assembled and re-used. Fresh zeolite was used for each sample.

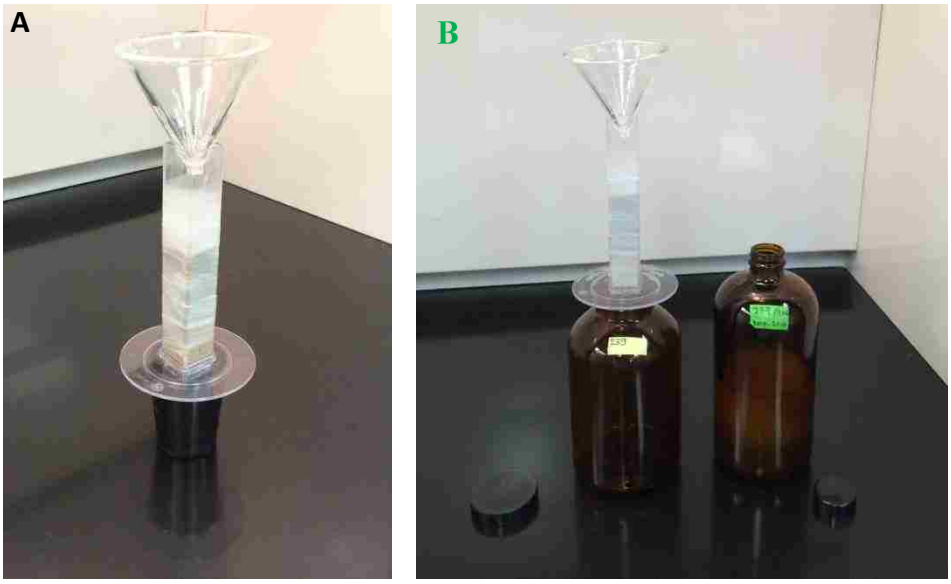

Figure 4.1 (A) Water filtration system (B) Filtration of surface water through natural zeolite and HDTMA-Cl SMZ.

4.2.2 Preparation of HDTMA-Cl (Hexadecyltrimethylammonium chloride)-Surface-Modified Zeolite. As described by Bowman (2005), 0.056 M surfactant –HDTMA-Cl was prepared to treat the natural zeolite used in the earlier section 4.2.1. With a weighing balance, 1.43 g HDTMA-Cl was measured into a 125 ml beaker containing 70 ml of milliQ water. With a gentle swirl until all surfactant dissolved, solution was poured into 100 ml graduated cylinder and milliQ water added up to 80.5 ml final volume. With a weighing balance, 20 g of natural zeolite was measured and dispersed in the 80.5 ml of 0.056 M surfactant for 2 hours. The supernatant was drained away after 2 hours and the surface-modified zeolite (SMZ) was spread out on a clean aluminum foil to air dry overnight.

4.2.3 Water Filtration through HDTMA-Cl (Hexadecyltrimethylammonium chloride)-Surface-Modified Zeolite. The water sample BRH was selected based on the same criterion (highest volume of pesticide residue content) as used in the earlier section 4.2.1. The filtration

system for SMZ consisted of 3 columns in layers. The upper layer was empty followed by a middle layer of natural zeolite and base layer of column of HDTMA-Cl-SMZ.

4.2.4 Pesticide Residue Extractions in both Zeolite-filtered, and SMZ-filtered Waters. As listed in Table 4.1, ten zeolite-filtered water samples were extracted for pesticide residues. The same extraction method used in pesticide residue extraction in fresh surface water in Section 3.2.2 of Chapter 3 was repeated for both sets of samples- 10 zeolite-filtered samples and 1 SMZ-filtered sample. In each case, the same volume of 1000 ml of water was run through the natural zeolite and the SMZ. Sample vials for the GC-MS were prepared and analysis ran.

4.3 Statistical Analysis

Six calcualtions were required in computing the results obtained in the analysis of the water samples as computed for food and water samples analyzed in Chapter 3 with equations outlined in section 3.3. Statistical analytical system (SAS) was employed to run paired student t-test in order to compare the concentration of pesticide residues in the water samples before and after zeolite treatments. The alpha value was set at $P = 0.05$. That is, when the calculated P-value is less than 0.05 then a statistical difference can be declared; at this stage we say we fail to accept the null hypothesis H_o (the null hypothesis says there is no significant difference between the pesticide residue concentrations before and after the zeolite treatment while the converse describes the alternative hypothesis H_a that will in this case adviocates that the pesticide levels before and after zeolite treatments are statistically different). Statistical significance at $P< 0.05$ implies that there is 95 out of 100 chances of repeating the sampling and chromatographic analysis of the water sample of arriving at the same concentration rate of the pesticide residues

detected and reported. This also means that the chance that our detected rates were due to error was 5 in 100.

4.4 Results

4.4.1 Role of Natural Zeolite in Pesticide Alleviation. Reduction in pesticide residues was observed in 9 zeolite-filtered surface waters out of the 10 samples analyzed (Table 4.1). Low standard deviation of the pesticide residue value showed that the recorded values agree meaning there were not disparities amongst the first and second readings.

Table 4.1 Effect of Zeolite treatment on pesticide residue in Surface water

Sample	pH	Pesticide Residue	(ppb)			
			Before	After		
				1st	2nd	Mean±sd n
BPH	7.7	Atrazine	0.2			
		Metolachlor	0.16	0.1	0.14	0.12±0.03 2
CLC	7.7	Atrazine	6.48	0.06	0.06	0.06±0.00 2
		*Desethatz	0.74	0.54	0.56	0.55±0.01 2
		Metolachlor	1.08			
		Bifenthrin	0.02		0	1
CDG	7.2	Atrazine	0.68			
		Metolachlor	0.84	0.72	0.74	0.73±0.01 2
BBH	7.2	Atrazine	1.78	1.34	1.18	1.26±0.11 2
		Metolachlor	1.16	0.92	1.1	1.01±0.13 2
		Acetochlor	0.06			
		Azoxystrobin	0.02			
BRH	7.3	Atrazine	6.2	0.86	0.42	0.64±0.31 2
		Clomazone	2.4		1.54	1

(Table 4.1 continued)

Sample	pH	Pesticide Residue	(ppb)				
			Before	After			
				1st	2nd	Mean±sd	n
		Desethatz	0.62		0.38		1
		Metribuzin	0.34		0.17		1
		Metolachlor	17.2		15.32		1
		Propanil	0.08	0.04	0.02	0.03±0.01	2
		Metalaxyl	0.08		0.06		1
		Dimethenamid	0.16		0.12		1
LBT	7.7	Desethatz	0.22	0.18	0.16	0.17±0.01	2
		Atrazine	0.6				
		Metolachlor	0.36				
		Glyphosate	ND				
		AMPA	ND				
BCH	7.1	Atrazine	6.24	2.7	0.1	1.4±1.84	2
		Desethatz	0.54				
		Acetochlor	0.28				
		Metribuzin	0.36				
		Metolachlor	3.9				
		Clomazone	0.04				
TRH2	7.2	Atrazine	0.38	0.12	0.2	0.16±0.06	2
		Desethatz	0.26		0.06		1
		Metribuzin	0.30				
		Metolachlor	3.40				
		Clomazone	0.18				
		Azoxystrobin	0.06		0.02		1
BPI	7.2	Atrazine	0.72		0.22		1
		Desethatz	0.2				
		Metribuzin	0.28				
		Metolachlor	0.74				
		Metalaxyl	0.12		0.1		1
		Clomazone	0.04				

(Table 4.1 continued)

Sample	pH		Pesticide Residue (ppb)				
			Before	After			
				1st	2nd	Mean±sd	n
		Azoxystobin	0.06		0.04		1
BDC	6.8	Clomazone	0.3				
		Bromacil	0.42				
		Metalaxyl	0.04				
		Metolachlor	0.06				
		Propiconazole	0.12				

*Desethatz = Desethylatrazine.

As explained and shown in Table 4.2 that is outlined on the next page, reduction in pesticide residue levels ranged from the minimum of 10.9 % to a maximum of 100 %. Minimum reduction was recorded in metolachlor in sample BRH, while the maximum was in bifenthrin in sample CLC. A high reduction rate of 99.1% was found in atrazine in the same sample CLC; next to this high atrazine found in CLC was recorded in BRH at level 89.7 % making it the third highest reduction recorded in this study. Atrazine was also alleviated in sample BRH up to 89.7 %. Most high rates of reduction following zeolite filtration were found in atrazine at the rate of 77.6 % in sample BCH; 57.9 % in sample TRH2; and 69.4% in sample BPI. Alleviations recorded above average also included 50 % metribuzin and 62.5 % propanil both in sample BRH; and 66.7 % azoxystrobin in sample TRH2.

Table 4.2 Percentage reduction of pesticide residues in zeolite-filtered surface water.

Sample	Original	Reduced	Pesticide	Before	After	Alleviation (%)
BPH	2	1	Metolachlor	0.16	0.12	25.0
CLC	4	3	Atrazine	6.48	0.06	99.1
			Desethylatz	0.74	0.55	25.7
			Bifenthrin	0.02	0.00	100.0
CDG	2	1	Metolachlor	0.84	0.73	13.1
BBH	4	2	Atrazine	1.78	1.26	29.2
			Metolachlor	1.16	0.13	12.9
BRH	8	8	Atrazine	6.20	0.31	89.7
			Clomazone	2.40	1.54	35.8
			Desethylatz	0.62	0.38	38.7
			Metribuzin	0.34	0.17	50.0
			Metolachlor	17.20	15.32	10.9
			Propanil	0.08	0.03	62.5
			Metalaxyl	0.08	0.06	25.0
			Dimethnamid	0.16	0.12	25.0
LBT	5	1	Desethylatz	0.22	0.17	22.7
BCH	6	1	Atrazine	6.24	1.40	77.6
TRH2	6	3	Atrazine	0.38	0.16	57.9
			Desethylatz	0.26	0.06	76.9
			Azoxystrobin	0.06	0.02	66.7
BPI	7	3	Atrazine	0.72	0.22	69.4
			Metalaxyl	0.12	0.10	16.7
			Azoxystrobin	0.06	0.04	33.3
BDC	5	0	ND	ND	ND	ND

In comparing means of the pesticide residue found before and after filtering water through natural zeolite a paired student t-test was conducted using SAS software. From the SAS outputs shown in Table 4.3 at $P_{critical} = 0.05$, the difference between the atrazine levels before and after filtration of water sample CLC through natural zeolite was highly significant ($P_{calculated} = 0.0001$). Statistical difference ($P_{calc} = 0.03$) was also found between the desethylatrazine levels before and after zeolite treatment in the same water sample CLC. The difference between the levels of atrazine in sample BRH before and after zeolite treatment was also significant ($P_{calc} = 0.03$). No significant difference was found the before and after treatment with zeolite for the pesticide levels in metholachlor in samples BPH, CDG, BBH and LBT. Similarly, the difference found between the pesticide residue concentrations of atrazine before and after zeolite treatment in samples BBH, BCH and TRH2 were not statistically different from each other. There was no statistical difference between propanil levels before and after zeolite zeolite treatment.

Table 4.3 Paired t-test comparison of pesticide residue means before and after zeolite treatment.

Sample	PR	N	Mean	SD	SE	Min	Max	df	t value	Pr > |t|	Sig.
BPH	Metolachlor	2	0.04	0.03	0.02	0.02	0.06	1	2.00	0.30	NS
CLC	Atrazine	2	6.42	0.00	0.00	6.42	6.42	1	Infty	.0001	***
	Desethatz	2	0.19	0.01	0.01	0.18	0.20	1	19.00	0.03	*
CDG	Metolachlor	2	0.11	0.01	0.01	0.10	0.12	1	11.00	0.06	NS
BBH	Atrazine	2	0.52	0.11	0.08	0.44	0.60	1	6.50	0.10	NS
	Metolachlor	2	0.15	0.13	0.09	0.06	0.24	1	1.67	0.34	NS

(Table 4.3 continued)

Sample	PR	N	Mean	SD	SE	Min	Max	df	t value	Pr > |t|	Sig.
BRH	Atrazine	2	5.56	0.31	0.22	5.34	5.78	1	25.27	0.03	*
	Propanil	2	0.05	0.01	0.01	0.04	0.06	1	5.00	0.13	NS
LBT	Desethatz	2	0.05	0.01	0.01	0.04	0.06	1	5.00	0.13	NS
BCH	Atrazine	2	4.84	1.84	1.30	3.54	6.14	1	3.72	0.17	NS
TRH2	Atrazine	2	0.22	0.06	0.04	0.18	0.26	1	5.50	0.12	NS

Sig. = Significance; NS = no significant difference found among the pesticide residue levels recorded before and after treatment with natural zeolite clinoptilolite; * & *** = significant difference and highly suignificant difference respectively, found among the pesticide residue levels recorded before and after treatment with natural zeolite clinoptilolite; SD = standard deviation; SE = standard error; PR = pesticide residue; df = degree of freedom; Pr>t = calculated P value by SAS; Alpha = 0.05 (critical P value).

4.4.2 Role of Surfactant-Modified-Zeolite (SMZ) in Pesticide Alleviation. As summarized in Table 4.4, following SMZ treatment of sample BRH, 6 pesticides were detected out of 8. Propanil and dimethenamid were the undetected by the GC-MS as it were after SMZ treatment. Low standard deviation confirms lack of disparity between the 1st and 2nd data collected during the chromatographic analysis.

Table 4.4 Effect of surfactant-modified-zeolite (SMZ) on pesticide residue in surface water

Sample		Pesticide Residue (ppb)					
		Before	After				
			1st	2nd	Mean	n	Stdev
BRH	Atrazine	6.2	0.34	0.28	0.31±0.04	2	0.04243
	Clomazone	2.4	1.12	0.84	0.98±0.20	2	0.19799

(Table 4.4 continued)

Sample		Pesticide Residue (ppb)						
		Before	After					
			1st	2nd	Mean	n	Stdev
	Desethylatrazine	0.62	0.5	0.34	0.42±0.11	2	0.11314
	Metribuzin	0.34	0.24	0.22	0.23±0.01	2	0.01414
	Metolachlor	17.2	10.16	7.82	8.99±1.66	2	1.65463
	Propanil	0.08	ND	ND			
	Metalaxyl	0.08	0.04	0.04	0.04±0.00	2	0.00000
	Dimethenamid	0.16	ND	ND			

Further reduction of pesticide residues was recorded (Table 4.5) in the sample BRH that was filtered through the surfactant-modified-zeolite (SMZ). A 50 % reduction was observed as 4 out of the 8 residues found were reduced following filtration through SMZ. The 4 compounds that were further reduced compared filtration through natural zeolite included atrazine @ 95 % compared to 89.7 % reduction with natural zeolite (NZ); 59.2 % clomazone compared with 35.8% with NZ; 47.7 % metolachlor compared with 10.9 % with NZ and 50 % metalaxyl compared with 25 % with NZ.

Table 4.5 Percentage reduction of the pesticide residue in surface water filtered through surfactant modified zeolite (SMZ)

Sample		Before Zeolite	After Zeolite	After SMZ	Zeolite % reduction	SMZ % reduction
BRH	Atrazine	6.2	0.64	0.31	89.7	95.0
	Clomazone	2.4	1.54	0.98	35.8	59.2
	DesethylAtrazine	0.62	0.38	0.42	38.7	32.3

(Table 4.5 continued)

Sample	Before Zeolite	After Zeolite	After SMZ	Zeolite % reduction	SMZ % reduction
Metribuzin	0.34	0.17	0.23	50.0	32.4
Metolachlor	17.2	15.32	8.99	10.9	47.7
Metalaxyl	0.08	0.06	0.04	25.0	50.0

As outlined in Table 4.6, paired t-test comparison of pesticide residue means before and after SMZ treatment was conducted. A very significant difference (P_{calc} = 0.003) was found in atrazine between the pesticide level recorded before and after the SMZ treatment of sample BRH. A highly significant difference (P_{calc} < 0.0001) was similarly found in metolaxyl levels before and after SMZ treatment. In pesticide levels recorded for clomazone, desethylatrazine, metribuzin and metolachlor, there was no statistical difference found among them.

Table 4.6 Paired t-test comparison of pesticide residue means before and after SMZ treatment.

Sample	PR	N	Mean	SD	SE	Min	Max	df	t value	Pr > |t|	*Sig.
BRH	Atrazine	2	5.89	0.04	0.03	5.86	5.92	1	196.33	0.003	**
	Clomazone	2	1.42	0.20	0.14	1.28	1.56	1	10.14	0.06	NS
	Desethatz	2	0.20	0.11	0.08	0.12	0.28	1	2.50	0.24	NS
	Metribuzin	2	0.11	0.01	0.01	0.10	0.12	1	11.0	0.06	NS
	Metolachlor	2	8.21	1.66	1.17	7.04	9.38	1	7.02	0.09	NS
	Metolaxyl	2	0.04	0.00	0.00	0.04	0.04	1	infty	<.0001	***

*Sig. = Significance; NS = no significant difference found among the pesticide residue levels recorded before and after treatment with Hexa decyl trimethyl chloride surfactant-modified-zeolite clinoptilolite; ** = very significant difference found between the pesticide residue levels recorded before and after treatment with HDTM-Cl SMZ; SD = standard deviation; SE =

standard error; PR = pesticide residue; df = degree of freedom; Pr>t = calculated P value by SAS; Alpha = 0.05 (critical P value).

Further paired t-test comparison of pesticide levels was conducted between the levels recorded after treatment with natural zeolite and the levels recorded after treatment with surfactant-modified-zeolite. The outcome of this as outlined in Table 4.7 showed a statistical difference in metolaxyl, and the difference observed was highly significant ($P_{calc} < 0.0001$). No statistical difference was observed in atrazine, clomazone, desethyatrazine, metribuzin and metolachlor. However, negative mean value and t value computed for desethylatrazine and metribuzin showed a negative trend because the levels recorded after filtration through the SMZ was higher than the levels after filtration through the natural zeolite. As outlined in Table 4.5, after filtration through natural zeolite desethyatrazine level was reduced from original 0.62 ppb to 0.38 ppb compared to 0.42 ppb at which it was found after filtration through SMZ. After filtration through zeolite, metribuzin level was reduced from 0.34 to 0.17 compared to 0.23 ppb recorded after filtration through SMZ.

Table 4.7 Paired t-test comparison of levels of PR of zeolite-treated and SMZ-treated sample.

Sample	PR	N	Mean (ppb)	SD	SE	Min	Max	df	t value	Pr > |t|	Sig.
BRH	Atrazine	2	0.33	0.04	0.03	0.30	0.36	1	11.0	0.06	NS
	Clomazone	2	0.56	0.20	0.14	0.42	0.70	1	4.00	0.16	NS
	Desethatz	2	-0.04	0.11	0.08	-0.12	0.04	1	-0.50	0.71	NS
	Metribuzin	2	-0.06	0.01	0.01	-0.07	-0.05	1	-6.00	0.11	NS

(Table 4.7 continued)

Sample	PR	N	Mean (ppb)	SD	SE	Min	Max	df	t value	Pr > |t|	Sig.
	Metolachlor	2	6.33	1.66	1.17	5.16	7.50	1	5.41	0.12	NS
	Metolaxyl	2	0.02	0.00	0.00	0.02	0.02	1	infty	<.0001	***

Sig. = Significance; NS = no significant difference found among the pesticide residue levels recorded between zeolite treated and SMZ treated sample BRH; *** = highly suignificant difference found among the pesticide residue levels recorded between zeolite treated and SMZ treated sample BRH; SD = standard deviation; SE = standard error; PR = pesticide residue; df = degree of freedom; Pr>t = calculated P value by SAS; Alpha = 0.05 (critical P value).

4.5 Discussion

As obtained in this study, Smedt *et al*., (2015) reported adsorption of metolaxyl using zeolite. Alleviation of atrazine recorded in this study is similar to reports of Lemic *et al* 2006 and 2007, even though they used SDBAC as their surfactant to modify the zeolite and we used HDTMA-Cl as modifying surfactant. Further reduction of atrazine, clomazone, metolachlor and metalaxyl after filtration through SMZ conforms to the theoretical principle of effect of exchanging CEC property of clinoptilolite with an anion exchange capacity (AEC), thereby enhancing its ability to retain negatively charged organic ions that ordinarily would have escaped.

Differences recorded in the pH of the surface water samples may have impacted on the cation exchange capacity of the zeolite. This finding is in agreement with Mergeta *et al*. (2013) where they confirmed that the success of clinoptilolite in removing organic contaminations is a function of pH, initial concentrations of humic acid and ammonia, temperature and contact duration. Pesticide residues were alleviated in all the samples whose pH ranged between 7.1

through 7.7, while the only sample where no pesticide residue was found had pH 6.8. Similar to the assertion of Mergeta *et al.* (2013) that the optimum temperature at which zeolite could reduce organic contaminants in water is about room temperature which was the reason while sample waters were always allowed to acclimatize to room temperature after retrievied from the cold storage; findings in this study may also imply that water samples need to be above neutral pH in order for the zeolite to work at its optimum as suggested by Moussavia *et al.* (2011) that sample water needs to be about the pH of natural water for the detection of residues to be at its best.

. Atrazine results reported in Chapter 3 detection study showed its alarming concentration increase in the surface waters of 4 locations in Louisiana. Its adsorption by the natural zeolite (clinoptilolite) and SMZ in this section serves as a potential remedy to the concentrations of this herbicide in the waters.

Propanil and dimethenamid were not detected in the water samples and this could be due to low concentration as GC-MS does not detect trace levels. It could also be that they have been totally removed from the sample during the SMZ treatment.

As opposed to the expected event that enhanced reduction be observed when filtered through SMZ, a reversed trend observed in desethylatrazine and metribuzin may imply that they have greater affinity a natural zeolite than for modified modified zeolite by hexadecyltrimethyl ammonium chloride surfactant.

Great affinity of clinoptilolite zeolite for ammonium ion (Mumpton, 1999) is a proof that any trace amount of $NH4^+$ in any of the 11samples studied in this section must have been reduced. However, lack of measurement of $NH4^+$ limited us from any information regarding this aspect. Part of future work would be to examine water for metal contaminants like arsenic

(Sulivan *et al*., 1998); chromate (Bowman, 2003); Fe and Mn (Inglezakis *et al*., 2010); Cd and Pb (Curkovic *et al*., 1997) and the cation $NH4^{+}$.

This study in general serves as a reminder of the need to regularly monitor the pesticide residue in our foods and waters.

4.6 References

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CHAPTER 5
CONCLUSIONS

Agriculture is as important to humans as life as it holds the food baskets. Pesticides on their own are as important as agriculture as they both go hand in hand. However, as important as pesticides are, their uses must be under control in order to maintain a healthy environment.

In this study, all pesticides found in food samples of tomato, blueberry, corn, cucumber, cabbage, melon, wheat and rice were below EPA tolerance. Insecticide cypermethrin detected in tomato was 90 % close to the tolerance level. However, acid-lability of cypermethrin may remain an advantage to consumers of tomato as it will always degrade with increase in acidity level of tomato fruit or puree.

High atrazine level in the surface waters in certain locations of Louisiana will need to be looked into as further study is conducted on those areas. Inclusion of ground waters in those areas will be advised in order to know how much infiltration of surface water contamination is going into ground waters.

Use of natural zeolite cllinoptilolite as demonstrated in this study will go a long way in curtailing excess pesticide in surface waters that might be used in irrigation and other purposes. Surfactant modified zeolite showed to have more affinity for organic contaminants. Through the use of SMZ, zeolite can be tailored towards any organic contaminant of interest per time.

Weather anomalies resulting in wild winds, storms, heavy rainfalls, erosion and flood may be the main reason why fluctuations are so rampant in the 3 pesticide residue levels observed in some water samples across a consecutive period of 4 years. A balance data of a

longer period say 10 years will be required in a future surveillance study in order to create a more reliable feel of the real situation of things as per trends in the pesticide residue levels.

In conclusion, this study is a reminder of the need to regularly quantitate the pesticide residue in our food and water and also develop methods of removing pesticide residues in them.

APPENDIX 1
LIST OF REAGENTS, APPARATUS, CHEMICAL STANDARDS AND SPIKES RECOVERIES

Reagents: Hexane, pesticide grade, Fisher #H300-4; methylene chloride, pesticide grade, Fisher #D142-4; sodium sulfate, ACS certified; granular, 10-60 mesh, Fisher #S415-1; petroleum ether, pesticide grade, Fisher #P480-4; MilliQ water 18.2 mega-ohm; acetonitrile- HPLC grade JTBaker 9017-03; acetonitrile- optima grade Fisher A996-4; acetone- pesticide grade, Fischer A40-4; RESTEK Cat #2622 QuEChERS 1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18 and 400 mg GCB (for extraction); RESTEK Q-sep QuEchERS dSPE Cat #26219 containing 150 $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg GCB, 2 ml pack (graphitized carbon black).

Apparatus: Separatory funnels, glass, 1-liter with PTFE stopcock, Kimble #29048F-1000; 500 ml graduated cylinder, Kimble #20024D-500; 100 ml graduated cylinder, Kimble #20024D-100; 400 ml beaker, Kimble #14000-400; 100 mm glass funnel, Corning #6140-100; 35 mm glass funnel, Kimble #28950-35; 1 ml volumetric pipette, fisher #13-650B; 15 ml graduated conical centrifuge tube, Corning #8080A-15; corks, size 6; VWR #23420-184; large ceramic filter funnel Fisher #10-356H; 2000 ml filter flask, Kimble #27060-2000; glass wool, Fisher #11-390; water bath, Fisher #15-461-20; aluminum foil, Fisher #01-213-18; Pasteur pipettes, 5 ¾", Fischer #13-678-20A; 13 mm PVDF filters, 0.2 um with tip, Fischer #09-910-2; 1 cc syringes, disposable, Fisher #14-823-2F; disposable polypropylene centrifuge tubes, 15 ml, with plastic screw cap, Fisher #05-538-53D; UPLC autosampler caps, crimp silver aluminum PTFE / silicone / PTPE septum, 11 mm Agilent 5183-4499; Vial inserts, 150 ul with plastic spring, waters WAT094171; solvent dispenser for dispensing 15 ml of extraction solvent; Analytical balance- Mettler PG 802-S; Micro-centrifuge, model 5418, Eppendorf 22620304; Allegra 6 Centrifuge, Beckman-

Coutler; Multi-tube vortexer – set for 50 ml tubes, Fisher 02-215-452; Vortex mixer, Fisher #12-815-18; Nitrogen gas evaporator, organomation Associates, Inc., N-EVAP 112 with OA-SYS heating system.

Solutions: Pesticide stock solution; intermediate working standard; Working standards; Matrix matched standards.

Wheat and Rice samples **1/25/2016**
1X, 2X AND 17X(rice)

SOLVENT STANDARDS
PUT in 1425ml of Acetonitrile in autosampler vial
ADD the ul of each standard below
CAP and VORTEX

A	**Vol used ul**	**Stock Conc ug/ml**	**Solvent ul**	**Final vov ul**	**A CONC ug/ml**
10AGCMS	75	8.00	1425	1500	0.40

B	**Vol used ul OF A**	**A CONC ug/ml**	**Solvent ul**	**Final vov ul**	**Final Conc ug/ml**
LOW LEVEL	300	0.40	900	1200	0.10

95% **MATRIX STANDARDS USING FILTERED MATRIX**

PUT in 1425ml of FILTERED SAMPLE in autosampler vial
ADD the ul of each standard below

CAP and VORTEX 140STD/1500TOTAL 95% MTX 0.907 0.05

FILTERED

A	Vol used ul	Stock Conc ug/ml	MATRIX ul	Final vov ul	A CONC ug/ml
10AGCMS	75	8.00	1425	1500	0.40

21STD/1200TOTAL >95% MTX

B	Vol used ul OF A	A CONC ug/ml	FILTERED MATRIX ul	Final vov ul	Final Conc ug/ml
LOW LEVEL	300	0.40	900	1200	0.10

REPEAT THE MATRIX STANDARD SET A AND B THREE TIMES

- ONCE FOR 1X, ONCE FOR 2X AND ONCE FOR 17X

SHOULD END UP WITH 8 VIALS OF STANDARDS

SOLV A	0.40	SOLVENT (ACETONITRILE)
SOLV B	0.10	SOLVENT (ACETONITRILE)
1X A	0.40	9X MTX CUCUMBER
1X B	0.10	9X MTX CUCUMBER
2X A	0.40	15 MTX MELON
2X B	0.10	15 MTX MELON
17X A	0.40	16 MTX CABBAGE
17X B	0.10	16 MTX CABBAGE

RICE AND WHEAT SPIKES 1/25/2016

LOW SPIKE RATE 10x EU RATE Acetonitrile Sample

	Conc ug/ml	Used ul	Tot vol ml	Weight g	On Column	RATE
10A GC/MS MIX	8.000	**125**	10	5.00	0.100	0.2
			10.125		0.099 ACTUAL	

MED SPIKE RATE

Acetonitrile Sample

	Conc ug/ml	Used ul	Tot vol ml	Weight g	On Column	RATE
10A GC/MS MIX	8.000	**500**	10	5.00	0.400	0.8
			10.5		0.380952 ACTUAL	

HIGH SPIKE RATE

Acetonitrile Sample

	Conc ug/ml	Used ul	Tot vol ml	Weight g	On Column	RATE
10A GC/MS MIX	8.000	**1250**	10	5.00	1.000	1.6
			11.25		0.889 ACTUAL	

TOMATO SPIKES	% Recovery 3X Tomato	% Recovery 3X Tomato	% Recovery 5X Tomato	% Recovery 5X Tomato
(Spike Rate ppm)	0.10 ug/ml	0.41 ug/ml	0.41 ug/ml	1.00 ug/ml

Cucumber, Cabbage, Melon **1/13/2016**

9X, 15X AND 16X

SOLVENT STANDARDS

PUT in 1390ml of Acetonitrile in autosampler vial
ADD the ul of each standard below
CAP and VORTEX

A	**Vol used**	**Stock Conc**	**Solvent**	**Final vov**	**A CONC**

	ul	ug/ml	ul	ul	ug/ml
Tomato	35	17.03	1390	1500	0.40
10AGCMS	75	8.00	1390	1500	0.40
Permethrin	30	20.00	1390	1500	0.40

B	**Vol used ul OF A**	**A CONC ug/ml**	**Solvent ul**	**Final vov ul**	**Final Conc ug/ml**
LOW LEVEL	300	0.40	900	1200	0.10

91% **MATRIX STANDARDS USING FILTERED MATRIX**

PUT in 1390ml of FILTERED SAMPLE in autosampler vial
ADD the ul of each standard below
CAP and VORTEX

140STD/1500TOTAL 91% MTX 0.907

A	**Vol used ul**	**Stock Conc ug/ml**	**FILTERED MATRIX ul**	**Final vov ul**	**A CONC ug/ml**
Tomato	35	17.03	1390	1500	0.40
10AGCMS	75	8.00	1390	1500	0.40
Permethrin	30	20.00	1390	1500	0.40

21STD/1200TOTAL 98% MTX 0.9825

B	**Vol used ul OF A**	**A CONC ug/ml**	**FILTERED MATRIX ul**	**Final vov ul**	**Final Conc ug/ml**
LOW LEVEL	300	0.40	900	1200	0.10

REPEAT THE MATRIX STANDARD SET A AND B THREE TIMES
- ONCE FOR 9X, ONCE FOR 15X AND ONCE FOR 16X

SHOULD END UP WITH 8 VIALS OF STANDARDS

SOLV A	0.40	SOLVENT (ACETONITRILE)
SOLV B	0.10	SOLVENT (ACETONITRILE)
9X A	0.40	9X MTX CUCUMBER
9X B	0.10	9X MTX CUCUMBER
15X A	0.40	15 MTX MELON
15X B	0.10	15 MTX MELON
16X A	0.40	16 MTX CABBAGE
16X B	0.10	16 MTX CABBAGE

BLUEBERRY SAMPLES 12/29/2015

4X AND 12X

SOLVENT STANDARDS

PUT in 1390ml of Acetonitrile in autosampler vial
ADD the ul of each standard below
CAP and VORTEX

	Vol used ul	Stock Conc ug/ml	Solvent ul	Final vov ul	Final Conc ug/ml
Sevin	35	17.03	1390	1500	0.40
10AGCMS	75	8.00	1390	1500	0.40

	Vol used ul	Stock Conc ug/ml	Solvent ul	Final vov ul	Final Conc ug/ml
LOW LEVEL	300	0.40	900	1200	0.10

93% MATRIX STANDARDS USING FILTERED MATRIX

PUT in 1390ml of FILTERED SAMPLE in autosampler vial
ADD the ul of each standard below
CAP and VORTEX

	Vol used ul	Stock Conc ug/ml	FILTERED MATRIX ul	Final vov ul	Final Conc ug/ml
Sevin	35	17.03	1390	1500	0.40
10AGCMS	75	8.00	1390	1500	0.40

	Vol used ul	Stock Conc ug/ml	FILTERED MATRIX ul	Final vov ul	Final Conc ug/ml
LOW LEVEL	300	0.40	900	1200	0.10

REPEAT THE MATRIX STANDARD TWICE - ONCE FOR 4X AND ONCE FOR 12X

SHOULD END UP WITH 6 VIALS OF STANDARDS

0.40	SOLVENT (ACETONITRILE)
0.10	SOLVENT (ACETONITRILE)
0.40	4X MTX
0.10	4X MTX
0.40	12 MTX
0.10	12 MTX

CUCUMBER ---9X, CABBAGE----16X, MELON-----15X

LOW SPIKE RATE 10x EU RATE

	Conc ug/ml	Used ul
Tomato Standard		
Chlorothalonil 03/15 Ace	17.065	**60**
Acephate 08/14 Ace	17.013	60
Methamidophos 9/03 Ace	16.990	60
Endosulfan I 01/00 Hex	16.982	60

Endosulfan II 01/00 Hex	16.973	60
Endosulfan SO4 01/00 Hex	17.002	60
10A GC/MS MIX	8.000	**125**
PERMETHRIN	20.000	50
		235

MED SPIKE RATE

Tomato Standard	**Conc ug/ml**	**Used ul**
Chlorothalonil 03/15 Ace	17.065	**240**
Acephate 08/14 Ace	17.013	240
Methamidophos 9/03 Ace	16.990	240
Endosulfan I 01/00 Hex	16.982	240
Endosulfan II 01/00 Hex	16.973	240
Endosulfan SO4 01/00 Hex	17.002	240
10A GC/MS MIX	8.000	**500**
PERMETHRIN	20.000	200
		940

HIGH SPIKE RATE

Tomato Standard	**Conc ug/ml**	**Used ul**
Chlorothalonil 03/15 Ace	17.065	**600**
Acephate 08/14 Ace	17.013	600
Methamidophos 9/03 Ace	16.990	600
Endosulfan I 01/00 Hex	16.982	600
Endosulfan II 01/00 Hex	16.973	600
Endosulfan SO4 01/00 Hex	17.002	600
10A GC/MS MIX	8.000	**1250**
PERMETHRIN	20.000	500

CORN 11/20/2015

LOW SPIKE RATE 10x EU RATE

Conc	**Used**	Acetonitrile **Tot vol**	Sample **Weight**	**Spike Rate & On**
	85			

	ug/ml	ul	ml	g	Column
10A GC/MS MIX	8.000	**125**	10	10.00	0.100

MED SPIKE RATE	Conc ug/ml	Used ul	Acetonitrile Tot vol ml	Sample Weight g	Spike Rate & On Column
10A GC/MS MIX	8.000	**500**	10	10.00	0.400

HIGH SPIKE RATE	Conc ug/ml	Used ul	Acetonitrile Tot vol ml	Sample Weight g	Spike Rate & On Column
10A GC/MS MIX	8.000	**1250**	10	10.00	1.000

SEVIN (Carbaryl)

10/30/2015

STOCK SOLUTION #1502B R 4/15 E 11/19 EPA REPOSITORY

g	PURITY	VOL	CONVERSION	ug/ml (ppm)
0.01386	0.996	100	1000000	138.05
	99.60%	EToAc	10 TO 6TH	

2 ppm Level in Solvent

ml	Stock	Final Vol	Final Conc
0.087	138.04	6	2.00
87ul	ug/ml	ml	ug/ml (ppm)
		EToAc	

MATRIX STANDARDS USING MATRIX WITHOUT SEVIN PRESENT

ul	ug/ml (ppm)	ul of MTX	Total ul	Final conc	
100	2.00	400	500	0.40	ug/ml (ppm)
100	0.40	300	400	0.10	ug/ml (ppm)

SPIKING LEVEL STANDARD

11/5/2015

ml	Stock	Final Vol	Final Conc
0.74	138.05	6	17.03

11/5/2015

LOW SPIKE RATE 10x EU RATE

Tomato Standard	Conc ug/ml	Used ul	Acetonitrile Tot vol ml	Sample Weight g	Spike Rate & On Column
Chlorothalonil 03/15 Ace	17.065	**60**	10	10.00	0.102
Acephate 08/14 Ace	17.013	60	10	10.00	0.102
Methamidophos 9/03 Ace	16.990	60	10	10.00	0.102
Endosulfan I 01/00 Hex	16.982	60	10	10.00	0.102
Endosulfan II 01/00 Hex	16.973	60	10	10.00	0.102
Endosulfan SO4 01/00 Hex	17.002	60	10	10.00	0.102
10A GC/MS MIX	8.000	**125**	10	10.00	0.100
Sevin 10/15	17.026	60	10	10.00	0.102

MED SPIKE RATE

Tomato Standard	Conc ug/ml	Used ul	Acetonitrile Tot vol ml	Sample Weight g	Spike Rate & On Column
Chlorothalonil 03/15 Ace	17.065	**240**	10	10.00	0.410
Acephate 08/14 Ace	17.013	240	10	10.00	0.408
Methamidophos 9/03 Ace	16.990	240	10	10.00	0.408

Endosulfan I 01/00 Hex	16.982	240	10	10.00	0.408
Endosulfan II 01/00 Hex	16.973	240	10	10.00	0.407
Endosulfan SO4 01/00 Hex	17.002	240	10	10.00	0.408
10A GC/MS MIX	8.000	**500**	10	10.00	0.400
Sevin 10/15	17.026	240	10	10.00	0.409

2350

LOW SPIKE RATE 10x EU RATE

	Conc	Used	Acetonitrile Tot vol
Tomato Standard	**ug/ml**	**ul**	**ml**
Chlorothalonil 03/15 Ace	17.065	**60**	10
Acephate 08/14 Ace	17.013	60	10
Methamidophos 9/03 Ace	16.990	60	10
Endosulfan I 01/00 Hex	16.982	60	10
Endosulfan II 01/00 Hex	16.973	60	10
Endosulfan SO4 01/00 Hex	17.002	60	10
10A GC/MS MIX	8.000	**125**	10

MED SPIKE RATE

	Conc	Used	Acetonitrile Tot vol
Tomato Standard	**ug/ml**	**ul**	**ml**
Chlorothalonil 03/15 Ace	17.065	**240**	10
Acephate 08/14 Ace	17.013	240	10
Methamidophos 9/03 Ace	16.990	240	10
Endosulfan I 01/00 Hex	16.982	240	10
Endosulfan II 01/00 Hex	16.973	240	10
Endosulfan SO4 01/00 Hex	17.002	240	10
10A GC/MS MIX	8.000	**500**	10

HIGH SPIKE RATE

	Conc	Used	Acetonitrile Tot vol
Tomato Standard	**ug/ml**	**ul**	**ml**
Chlorothalonil 03/15 Ace	17.065	**600**	10
Acephate 08/14 Ace	17.013	600	10
Methamidophos 9/03 Ace	16.990	600	10
Endosulfan I 01/00 Hex	16.982	600	10
Endosulfan II 01/00 Hex	16.973	600	10
Endosulfan SO4 01/00 Hex	17.002	600	10
10A GC/MS MIX	8.000	**1250**	10

TOMATO STANDARD FOR FOOD SAFETY SAMPLES

10/28/2015

MIXED STANDARD	**Stock ug/ml**	**Used ml**	**Tot vol ml**	**Final conc ug/ml**	**Target ug/ml**
Chlorothalonil 03/15 Ace	255.97	0.400	6	17.06	17
Acephate 08/14 Ace	416.64	0.245	6	17.00	17
Methamidophos 9/03 Ace	57.27	1.780	6	16.99	17
Endosulfan I 01/00 Hex	115	0.886	6	16.98	17
Endosulfan II 01/00 Hex	268	0.380	6	16.97	17
Endosulfan SO4 01/00 Hex	46.2	2.208	6	17.00	17
		5.899			
		0.101	added Acetone		

Injection test

Tomato Mix	**17 MIX ug/ml**	**Used ul**	**Tot vol ul**	**Final conc ug/ml**

Chlorothalonil 03/15 Ace	17.065	176	1500	2.00	2
Acephate 08/14 Ace	17.013	176	1500	2.00	2
Methamidophos 9/03 Ace	16.990	176	1500	1.99	2
Endosulfan I 01/00 Hex	16.982	176	1500	1.99	2
Endosulfan II 01/00 Hex	16.973	176	1500	1.99	2
Endosulfan SO4 01/00 Hex	17.002	176	1500	1.99	2

1324 Acetone
1324 Acetonitrile

10/29/2015

Solvent Standard @ 0.41 Acetonitrile

	17 MIX ug/ml	**Used ul**	**Tot vol ul**	**Final conc ug/ml**
Chlorothalonil 03/15 Ace	17.065	33	1363	0.413
Acephate 08/14 Ace	17.013	33	1363	0.412
Methamidophos 9/03 Ace	16.990	33	1363	0.411
Endosulfan I 01/00 Hex	16.982	33	1363	0.411
Endosulfan II 01/00 Hex	16.973	33	1363	0.411
Endosulfan SO4 01/00 Hex	17.002	33	1363	0.412

Solvent Standard @ 0.10 Acetonitrile

	0.41 MIX ug/ml	**Used ul**	**Tot vol ul**	**Final conc ug/ml**
Chlorothalonil 03/15 Ace	0.413	300	1200	0.103
Acephate 08/14 Ace	0.412	300	1200	0.103
Methamidophos 9/03 Ace	0.411	300	1200	0.103
Endosulfan I 01/00 Hex	0.411	300	1200	0.103
Endosulfan II 01/00 Hex	0.411	300	1200	0.103
Endosulfan SO4 01/00 Hex	0.412	300	1200	0.103

MATRIX Standard @ 0.41 **3X TOMATO**

	17 MIX	**Used**	**Tot vol**	**Final conc**

	ug/ml	ul	ul	ug/ml
Chlorothalonil 03/15 Ace	17.065	33	1363	0.413
Acephate 08/14 Ace	17.013	33	1363	0.412
Methamidophos 9/03 Ace	16.990	33	1363	0.411
Endosulfan I 01/00 Hex	16.982	33	1363	0.411
Endosulfan II 01/00 Hex	16.973	33	1363	0.411
Endosulfan SO4 01/00 Hex	17.002	33	1363	0.412

3X

MATRIX Standard @ 0.10

TOMATO

	0.41 MIX ug/ml	Used ul	Tot vol ul	Final conc ug/ml
Chlorothalonil 03/15 Ace	0.413	300	1200	0.103
Acephate 08/14 Ace	0.412	300	1200	0.103
Methamidophos 9/03 Ace	0.411	300	1200	0.103
Endosulfan I 01/00 Hex	0.411	300	1200	0.103
Endosulfan II 01/00 Hex	0.411	300	1200	0.103
Endosulfan SO4 01/00 Hex	0.412	300	1200	0.103

Carbofuran deg	110	105	113	97
Eptam	140	108	108	94
Etridiazole	100	108	103	102
Trifluralin	100	100	108	113
Molinate	130	100	100	98
Captan deg	150	115	95	95
Tefluthrin	100	98	85	92
Thimet	100	96	113	106
Desethylatrazine	100	98	103	98
Desisopropylatrazine	100	86	88	88
Prometone	100	96	105	100
Diazinon	100	88	103	98

Terbufos	110	98	100	100
Tebupirimphos	100	98	90	97
Atrazine	100	90	98	92
Clomazone	110	100	108	99
Carbofuran	150	128	130	119
MB 46513, Fipronil	130	90	123	96
Acetochlor	100	115	93	107
Dimethamid	130	98	110	101
Terbacil	120	100	103	97
Alachlor	120	98	105	97
Prometryn	90	100	93	108
Propanil	60	50	53	64
Metalaxyl	120	100	108	104
Methyl Parathion	90	96	85	107
Metribuzin	110	108	105	113
Malathion	100	105	103	111
Metolachlor	110	98	103	100
Chlorpyrifos	40	35	38	51
MB4590, Fipronil	110	100	108	110
Fipronil	110	103	108	107
Bromacil	40	45	45	70
Pendamethalin	100	103	95	115
Cyanazine	100	103	103	105
MB 46136 Fipronil	100	105	110	109
Captan	20	65	53	104
Propiconazole	90	92	92	100
Bifenthrin	80	70	68	79
Norflurazon	50	53	53	72
Lambda-cyhalothrin	85	92	92	106
Hexazinone	110	103	105	107
Baythroid	75	84	86	107
Cypermethrin	95	80	83	97
Esfenvalerate	75	78	86	102
Azoxystrobin	70	90	90	118
Methamidophos	113	76	96	112
Acephate	84	83	105	165
Chlorothalonil	0	0	0	0

Endosulfan 1	81	71	91	93
Endosulfan 2	96	81	108	119
Endosulfan Sulfate	88	93	96	91

TOMATO SPIKES (Spike Rate ppm)	% Recovery 7X Tomato 0.40 ug/ml	% Recovery 10X Tomato 0.10 ug/ml	% Recovery 14X Tomato 0.10 ug/ml
Carbofuran deg	98	110	210
Eptam	95	100	160
Etridiazole	88	100	140
Trifluralin	98	90	110
Molinate	93	110	130
Captan deg	103	140	140
Tefluthrin	90	110	120
Thimet	93	100	110
Desethylatrazine	93	100	110
Desisopropylatrazine	80	90	90
Prometone	93	90	120
Diazinon	95	100	100
Terbufos	95	90	110
Tebupirimphos	93	100	110
Atrazine	88	90	110
Clomazone	90	100	110
Carbofuran	100	110	130
MB 46513, Fipronil	95	100	120
Acetochlor	103	110	130
Dimethamid	95	100	120
Terbacil	103	100	120
Alachlor	93	100	110
Prometryn	90	100	110
Propanil	53	50	60
Metalaxyl	98	110	120
Methyl Parathion	93	100	90
Metribuzin	100	110	120
Malathion	100	100	110
Metolachlor	98	100	120

Chlorpyrifos	45	50	50
MB4590, Fipronil	103	100	110
Fipronil	100	100	110
Bromacil	50	40	50
Pendamethalin	95	90	130
Cyanazine	93	100	120
MB 46136 Fipronil	120	120	100
Captan	0	0	0
Propiconazole	89	95	105
Bifenthrin	75	70	80
Norflurazon	58	50	60
Lambda-cyhalothrin	92	120	120
Hexazinone	98	100	110
Baythroid	88	65	+
Cypermethrin	84	+	105
Esfenvalerate	94	75	100
Azoxystrobin	95	80	80
Methamidophos	83	98	113
Acephate	75	123	74
Chlorothalonil	0	0	0
Endosulfan 1	75	76	84
Endosulfan 2	88	107	68
Endosulfan Sulfate	78	93	88
Carbaryl (Sevin)	70	73	73

CABBAGE SPIKES	% Recovery 16X Cabbage	% Recovery 16X Cabbage
(Spike Rate ppm)	0.1	0.4
Carbofuran deg	94%	87%
Eptam	86%	109%
Etridiazole	77%	139%
Trifluralin	80%	117%
Molinate	81%	107%
Captan deg	86%	107%
Tefluthrin	79%	77%

Thimet	84%	107%
Desethylatrazine	77%	90%
Desisopropylatrazine	61%	87%
Prometone	71%	85%
Diazinon	76%	79%
Terbufos	74%	104%
Tebupirimphos	81%	107%
Atrazine	67%	68%
Clomazone	86%	112%
Carbofuran	89%	126%
MB 46513, Fipronil	79%	104%
Acetochlor	93%	90%
Dimethamid	93%	117%
Terbacil	74%	112%
Alachlor	78%	93%
Prometryn	69%	74%
Propanil	38%	52%
Metalaxyl	NA	NA
Methyl Parathion	NA	82%
Metribuzin	77%	96%
Malathion	111%	96%
Metolachlor	75%	90%
Chlorpyrifos	34%	36%
MB4590, Fipronil	80%	101%
Fipronil	76%	96%
Bromacil	75%	98%
Pendamethalin	44%	46%
Cyanazine	99%	96%
MB 46136 Fipronil	121%	107%
Captan	NA	NA
Propiconazole	70%	87%
Bifenthrin	59%	66%
Norflurazon	39%	66%
Lambda-cyhalothrin	66%	82%
Hexazinone	87%	115%
Baythroid	65%	89%
Cypermethrin	61%	93%
Esfenvalerate	65%	97%
Azoxystrobin	84%	123%

Methamidophos	0	77
Acephate	0	0
Chlorothalonil	0	0
Endosulfan 1	88	80
Endosulfan 2	82	88
Endosulfan Sulfate	118	103

Permethrin	54	62

CORN SPIKES (Spike Rate ppm)	% Recovery 6X CORN 0.10 ug/ml	% Recovery 8X CORN 0.40 ug/ml	% Recovery 13X CORN 1.00 ug/ml
Carbofuran deg	80	103	85
Eptam	120	113	80
Etridiazole	180	115	60
Trifluralin	110	103	68
Molinate	120	105	83
Captan deg	0	0	0
Tefluthrin	110	103	81
Thimet	110	98	66
Desethylatrazine	90	123	87
Desisopropylatrazine	70	120	69
Prometone	80	195	84
Diazinon	140	90	60
Terbufos	100	98	71
Tebupirimphos	100	100	75
Atrazine	80	98	72
Clomazone	110	105	69
Carbofuran	140	108	54
MB 46513, Fipronil	80	218	90
Acetochlor	90	110	65
Dimethamid	120	108	84
Terbacil	100	98	80?
Alachlor	90	85	69
Prometryn	100	60	43
Propanil	100	103	72

Metalaxyl	120	113	75
Methyl Parathion	100	115	74
Metribuzin	100	108	80
Malathion	100	110	78
Metolachlor	90	108	95
Chlorpyrifos	90	105	52
MB4590, Fipronil	90	123	255 INTERFERENCE
Fipronil	110	118	79
Bromacil	120	69	61
Pendamethalin	80	90	69
Cyanazine	100	145	132?
MB 46136 Fipronil	100	115	78
Captan	0	0	0
Propiconazole	105	109	76
Bifenthrin	90	103	72
Norflurazon	100	115	74
Lambda-cyhalothrin	110	110	80
Hexazinone	90	115	77
Baythroid	90	109	73
Cypermethrin	95	96	78
Esfenvalerate	100	112	71
Azoxystrobin	90	125	79

BLUEBERRY (Spike Rate ppm)	% Recovery 4X Blueberry 0.10 ug/ml	% Recovery 4X Blueberry 0.40ug/ml	% Recovery 12X Blueberry 0.10 ug/ml	% Recovery 12X Blueberry 0.40ug/ml	% Recovery 12X Blueberry 1.00ug/ml
Carbofuran deg	90	95	58	68	65
Eptam	80	93	69	68	74
Etridiazole	80	88	85	65	81
Trifluralin	70	83	77	70	80
Molinate	100	95	84	73	71
Captan deg	150	63	95	62	64
Tefluthrin	90	80	75	60	66
Thimet	70	75	70	65	70
Desethylatrazine	80	75	84	65	70
Desisopropylatrazine	60	65	55	60	59
Prometone	60	78	64	78	71
Diazinon	80	83	67	75	73

Terbufos	80	75	66	60	67
Tebupirimphos	80	85	71	75	66
Atrazine	60	68	61	63	62
Clomazone	80	90	68	78	71
Carbofuran	100	103	84	70	80
MB 46513, Fipronil	80	100	78	65	62
Acetochlor	100	95	86	98	81
Dimethamid	80	88	75	75	71
Terbacil	80	83	71	73	70
Alachlor	80	85	74	70	68
Prometryn	70	73	74	55	59
Propanil	40	43	40	35	40
Metalaxyl	90	90	77	75	70
Methyl Parathion	90	80	48	63	66
Metribuzin	90	103	77	78	75
Malathion	80	90	75	73	68
Metolachlor	80	88	69	73	70
Chlorpyrifos	40	35	27	38	35
MB4590, Fipronil	80	98	71	88	73
Fipronil	80	100	70	83	77
Bromacil	90	90	75	75	68
Pendamethalin	30	43	35	35	40
Cyanazine	80	88	70	82	72
MB 46136 Fipronil	80	100	85	118	81
Captan	130	63	280	93	95
Propiconazole	65	74	57	68	62
Bifenthrin	60	63	54	63	57
Norflurazon	40	48	37	48	48
Lambda-cyhalothrin	95	86	58	85	71
Hexazinone	70	90	71	80	73
Baythroid	60	83	65	78	71
Cypermethrin	90	78	74	79	74
Esfenvalerate	65	74	58	68	66
Azoxystrobin	70	78	75	78	77
Carbaryl (Sevin)	80	86	50	52	50

CUCUMBER SPIKES	% Recovery 9X Cucumber	% Recovery 9X Cucumber	% Recovery 9X Cucumber
(Spike Rate ppm)	0.1	0.4	1

Carbofuran deg	97%	115%	117%
Eptam	105%	104%	141%
Etridiazole	102%	107%	170%
Trifluralin	106%	150%	194%
Molinate	107%	96%	138%
Captan deg	126%	139%	154%
Tefluthrin	104%	68%	102%
Thimet	98%	128%	162%
Desethylatrazine	103%	74%	126%
Desisopropylatrazine	92%	79%	120%
Prometone	108%	123%	159%
Diazinon	111%	85%	117%
Terbufos	105%	126%	174%
Tebupirimphos	96%	109%	151%
Atrazine	97%	77%	112%
Clomazone	108%	128%	170%
Carbofuran	124%	104%	175%
MB 46513, Fipronil	107%	156%	204%
Acetochlor	99%	115%	151%
Dimethamid	121%	142%	170%
Terbacil	117%	93%	159%
Alachlor	101%	150%	158%
Prometryn	109%	71%	104%
Propanil	52%	49%	100%
Metalaxyl	NA	NA	NA
Methyl Parathion	101%	NA	131%
Metribuzin	108%	96%	123%
Malathion	124%	112%	121%
Metolachlor	111%	90%	136%
Chlorpyrifos	52%	44%	93%
MB4590, Fipronil	116%	112%	181%
Fipronil	103%	109%	181%
Bromacil	106%	109%	156%
Pendamethalin	51%	77%	119%
Cyanazine	112%	123%	125%
MB 46136 Fipronil	113%	139%	138%
Captan	114%	82%	122%
Propiconazole	97%	92%	127%
Bifenthrin	78%	74%	107%

Norflurazon	62%	60%	107%
Lambda-cyhalothrin	101%	98%	116%
Hexazinone	104%	93%	130%
Baythroid	90%	85%	115%
Cypermethrin	98%	77%	112%
Esfenvalerate	79%	79%	114%
Azoxystrobin	100%	98%	146%
Methamidophos	0	85	165
Acephate	0	0	0
Chlorothalonil	0	0	38
Endosulfan 1	69	69	81
Endosulfan 2	60	110	89
Endosulfan Sulfate	99	91	103
Permethrin	78	69	77

MELON SPIKES	% Recovery 15X Melon	% Recovery 15X Melon
(Spike Rate ppm)	0.1	0.81
Carbofuran deg	83%	73%
Eptam	81%	111%
Etridiazole	80%	126%
Trifluralin	91%	115%
Molinate	92%	102%
Captan deg	95%	112%
Tefluthrin	83%	90%
Thimet	82%	104%
Desethylatrazine	81%	101%
Desisopropylatrazine	69%	94%
Prometone	85%	100%
Diazinon	83%	90%
Terbufos	81%	106%
Tebupirimphos	81%	106%
Atrazine	77%	91%
Clomazone	83%	111%
Carbofuran	93%	170%
MB 46513, Fipronil	94%	102%
Acetochlor	91%	104%

Dimethamid	88%	104%
Terbacil	90%	117%
Alachlor	98%	98%
Prometryn	83%	98%
Propanil	39%	59%
Metalaxyl	NA	NA
Methyl Parathion	NA	104%
Metribuzin	92%	106%
Malathion	87%	102%
Metolachlor	87%	102%
Chlorpyrifos	37%	46%
MB4590, Fipronil	90%	102%
Fipronil	88%	104%
Bromacil	85%	105%
Pendamethalin	37%	53%
Cyanazine	81%	105%
MB 46136 Fipronil	88%	98%
Captan	75%	93%
Propiconazole	76%	94%
Bifenthrin	61%	69%
Norflurazon	46%	65%
Lambda-cyhalothrin	87%	91%
Hexazinone	88%	107%
Baythroid	75%	88%
Cypermethrin	78%	78%
Esfenvalerate	68%	83%
Azoxystrobin	72%	88%
Methamidophos	0	101
Acephate	0	0
Chlorothalonil	0	28
Endosulfan 1	70	89
Endosulfan 2	92	89
Endosulfan Sulfate	104	128
Permethrin	60	77

MELON SPIKES	% Recovery 15X Melon	% Recovery 15X Melon

(Spike Rate ppm)	0.1	0.81
Carbofuran deg	83%	73%
Eptam	81%	111%
Etridiazole	80%	126%
Trifluralin	91%	115%
Molinate	92%	102%
Captan deg	95%	112%
Tefluthrin	83%	90%
Thimet	82%	104%
Desethylatrazine	81%	101%
Desisopropylatrazine	69%	94%
Prometone	85%	100%
Diazinon	83%	90%
Terbufos	81%	106%
Tebupirimphos	81%	106%
Atrazine	77%	91%
Clomazone	83%	111%
Carbofuran	93%	170%
MB 46513, Fipronil	94%	102%
Acetochlor	91%	104%
Dimethamid	88%	104%
Terbacil	90%	117%
Alachlor	98%	98%
Prometryn	83%	98%
Propanil	39%	59%
Metalaxyl	NA	NA
Methyl Parathion	NA	104%
Metribuzin	92%	106%
Malathion	87%	102%
Metolachlor	87%	102%
Chlorpyrifos	37%	46%
MB4590, Fipronil	90%	102%
Fipronil	88%	104%
Bromacil	85%	105%
Pendamethalin	37%	53%
Cyanazine	81%	105%
MB 46136 Fipronil	88%	98%
Captan	75%	93%
Propiconazole	76%	94%

Bifenthrin	61%	69%
Norflurazon	46%	65%
Lambda-cyhalothrin	87%	91%
Hexazinone	88%	107%
Baythroid	75%	88%
Cypermethrin	78%	78%
Esfenvalerate	68%	83%
Azoxystrobin	72%	88%
Methamidophos	0	101
Acephate	0	0
Chlorothalonil	0	28
Endosulfan 1	70	89
Endosulfan 2	92	89
Endosulfan Sulfate	104	128
Permethrin	60	77

WATER SPIKES	% Recovery 245 Water	% Recovery MQ WATER
(Spike Rate ppm)	0.4	0.4 NOT ADDED
Carbofuran deg	85%	0%
Eptam	108%	0%
Etridiazole	118%	0%
Trifluralin	140%	0%
Molinate	105%	0%
Captan deg	93%	0%
Tefluthrin	105%	0%
Thimet	123%	0%
Desethylatrazine	+SAMPLE	0%
Desisopropylatrazine	83%	0%
Prometone	115%	0%
Diazinon	110%	0%
Terbufos	125%	0%
Tebupirimphos	128%	0%
Atrazine	+SAMPLE	0%
Clomazone	+SAMPLE	0%

Carbofuran	240%	0%
MB 46513, Fipronil	93%	0%
Acetochlor	+SAMPLE	0%
Dimethamid	118%	0%
Terbacil	143%	0%
Alachlor	108%	0%
Prometryn	150%	0%
Propanil	118%	0%
Metalaxyl	73%	0%
Methyl Parathion	220%	0%
Metribuzin	+SAMPLE	0%
Malathion	198%	0%
Metolachlor	+SAMPLE	0%
Chlorpyrifos	128%	0%
MB4590, Fipronil	123%	0%
Fipronil	195%	0%
Bromacil	133%	0%
Pendamethalin	138%	0%
Cyanazine	140%	0%
MB 46136 Fipronil	115%	0%
Captan	145%	0%
Propiconazole	113%	0%
Bifenthrin	88%	0%
Norflurazon	113%	0%
Lambda-cyhalothrin	105%	0%
Hexazinone	115%	0%
Baythroid	125%	0%
Cypermethrin	113%	0%
Esfenvalerate	110%	0%
Azoxystrobin	123%	0%

APPENDIX 2
SEQUENCE

Example of Tomato sequence ran in GC-MS in this study:

1. Sample 1……tomato acetonitrile
2. Sample 2…..tomato std ACN 2ppm
3. sample 3…..tomato std ACN 0.41ppm
4. Sample 4…..tomato std CAN 0.10 ppm
5. Sample 5…tomato MTX 0.41 ppm
6. Sample 6….tomato MTX 0.10 ppm
7. Sample 10 ….tomato RB
8. Sample 11….tomato 3x
9. Sample 12…. 3x tomato Lo spike
10. Sample 13 …….3x tomato Med spike
11. Sample 14……5x tomato
12. Sample 15…..5x tomato Dupl
13. Sample 16…..5x tomato Med spike
14. Sample 17…..5x tomato Hi spike
15. Sample 7……10A 10AGCMS 0.41 solvent
16. Sample 8….10AGCMS 0.10 solvent
17. Sample 9….10GCMS MTX 0.40 ppm

VITA

Olubode James Adeniyi is Nigerian. He completed his bachelor's degree in chemistry in 1990 from Ondo State University, Ado-Ekiti, Nigeria. He worked from 1990 through 1995 as a research assistant in the plant tissue culture laboratory of biotechnology unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria before starting his master's degree in agronomy (crop science) at the University of Ibadan, Nigeria, where he completed in 1998 and graduated in 2000. He continued working in the IITA plant tissue culture laboratory as lab manager when he completed a master of philosophy degree in agronomy with specialty in plant cell and tissue culture from the same University of Ibadan in 2005. He worked for IITA till 2009 when he came to the Louisiana State University for his doctoral degree program.

'Bode has served as resource person in some biotechnology training courses in the area of plant cell and tissue culture. He has presented posters and papers in workshops and symposiums on plant tissue culture techniques; and attended a short-term training in the University of Naples, Portici, Italy on plant regeneration and transformation using agrobacterium *tumefaciens*. He has published some journal articles on micropropagation and post-flask techniques of cassava and yams. He is a member of International Society for Tropical Root Crops- Africa Branch (ISTRC-AB), American Society of Plant Biologists (ASPB), Institute of Food Technologists (IFT) and American Chemical Society (ACS).