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Analysis of Honey Bee Hive Products as a Model for Monitoring Pesticide Usage in Agroecosystems

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Abstract
Global food and nutritional security majorly rely on honey bees for pollination. Furthermore, honey bees (*Apis mellifera*), are considered as reliable biological indicators of environmental contamination because they pick up chemical pollutants in the air or in flowers as they search for food. As a result, the honey bee colony environment acts as a reservoir for a diversity of resources of floral origin and therefore analyzing hive products is more cost effective compared to monitoring individual crops. Effective methods for monitoring agrochemicals contamination in the environment can therefore be achieved by continuous analysis of honey bee products. We investigated pesticide residues in honey and pollen collected from honey bee hives in various agro-ecological zones across Kenya over a period of two years (September 2013 to August 2015) to determine the circulating organic chemical pollutants in the environment. A total of 36 pesticide residues were detected belonging to three chemical classes; insecticides (>50%) fungicides (27%) and herbicides (20%) with majority of the pesticides detected in pollen compared to honey. Although herbicides appeared to be the least prevalent, they were detected at the highest concentrations of up to 356 ppb in honey compared to insecticides which were detected at fairly low concentrations (0.1 to 53 ppb). Our findings highlight the need to create greater awareness of the ecological consequences of wide scale use of agro-chemicals in agriculture.
Keywords: Pesticide residues, honey bees (*Apis mellifera*), honey and pollen

1. Introduction

Agrochemicals are crucial to modern agriculture as they protect crops from pests and disease invasion thereby boosting crop productivity that is much needed to meet the world food demands (József, 2013; Aktar, 2009). Globally, millions of tons of pesticides are applied annually, but only a small fraction (<1%) effectively reaches the target organisms, and the remainder is deposited either in the soil, atmosphere or water, contaminating the environment and non-target organisms (József, 2013; Horrigan, 2002). In Africa, pesticides use represents less than 5% of the total amount of pesticides used worldwide but many developing countries have large stockpiles of obsolete pesticides, usually scattered over various sites (PAN UK, 2007, World Bank, 2013). These pesticides are in deplorable state and are hazardous to both human and environmental health (Dinham, 2003; World Bank, 2013).

Moreover, the rapid increase in human population in African countries requires more food supply putting a strain on agricultural land available for crop production (Naidoo, 2010; Williamson, 2008). In Kenya and other African nations, most farmers are risk-averse and having small farm sizes strive to maximize their output by using fertilizers and other pesticides (Aduol, 2005). A recent survey carried out in Kenya showed that farmers used a dose above the recommended levels in their effort to reduce pest damage (Gitonga *et al., 2*010). These farmers are accustomed to pesticide use in response to any signs of crop damage and most have little or no knowledge on alternative pest-management approaches (Dinham, 2003; Lekei, 2014). As a result, the tendency to rely on pesticides use to enhance agricultural output in Africa is on the rise. This dependency on pesticides threatens food safety, causes health risks and environmental problems, and deepens the inequality between rich and poor African farmers (Ngowi, 2007; Williamson, 2008; Oesterlund, 2014). Additionally, most farmers have limited knowledge on pesticides and their widespread use of these pesticides in agriculture results in inappropriate use (Kimani, 1995; Lekei, 2014). The subsequent slow degradation of some of these pesticides unfavorably affects the whole ecosystem by entering into the food chain and polluting the air, soil and water (Asenso-Okyere, 2011; Lekei, 2014). As a consequence, methods for monitoring pesticide residues circulating in African agro-ecosystems are required to prevent their eventual toxicity to human health and the potential hazard to the conservation of the ecological equilibrium. Conversely, analysis of trace pesticides in the environment across a large spatial area requires laborious and expensive sample effort which is a major hurdle for most African countries.

Pollinators, particularly honey bees (*Apis mellifera*), are considered as reliable biological indicators (Giorgio, 2003; Porrini, 2002) because they reveal the chemical contaminants in the environment which they intercept in the air or in flowers as they search for food (Wallowork-Barber, 1992; Fernandez, 2002). Since honey bees have great mobility to forage vast areas, they can be monitored cheaply for chemical pollutants by analyzing their hive products. Honey bees are also well known to be highly susceptible to most chemicals and are typically used as representatives of non-target beneficial insects by environmental agencies worldwide to measure toxicity of pesticides during registration process (Desneux, 2007; Stoner, 2013; Solecki, 2006). Exposure of honey bees to

pesticides either leads to their sudden death, if the chemical is highly toxic or the doses are high or sub-lethal effects which may compromise their immunity or foraging behavior and the return of the contaminated food to the hive exposes the whole colony. The presence of these contaminants in honey bee food can then be detected using appropriate analytical methods (Fernandez, 2002; Kevan 1999; Porrini *et al.* 2000). As a result, continuous analysis of honey bees and their products can be employed as a method for monitoring fluctuations in pesticide exposure and their levels in the environment at any given time. So far, information on the levels of pesticide residues in hive products from Africa its environs are scanty.

Herein, pesticide residue analysis of honey bees and their products collected from different agro-ecological zones in Kenya over a two year period (September 2013-August 2015) was performed as a first step to monitor the extent of environmental contamination in Africa and also provide some insights on chemical residues currently circulating in this country that could affect beneficial arthropods, the environment and the community at large. A multi-residue analytical approach was employed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the acquired data was used to generate a map on distribution of various pesticides that are currently present in the surveyed regions across Kenya.

1.1 Ethical consideration

Prior to undertaking the study in each of the selected agro-ecological zones, informed consents were obtained from the owners of the honey bee colonies after explaining to them the background and the objectives of the study. The participants in this study were mainly small holder farmers working individually or as part of a beekeepers association group.

2.0. Materials and methods

2.1 Selection of study sites

Study sites were selected to reflect the major agro-ecological zones responsible for over 80% of food production in Kenya, see Figure 1. The major food crops present in these regions were maize, beans, and various vegetable and fruit crops with the exception of one site in Kiambu which consisted of a mixture of large scale farming (horticultural, coffee, french beans and pineapple farms) and small scale farming containing maize and beans. In each of these agro-ecological zones, eight apiaries spread apart (>10 km from each other) were randomly selected. The choice of the apiaries from each of these sites was based on the number and the strength of active colonies present.

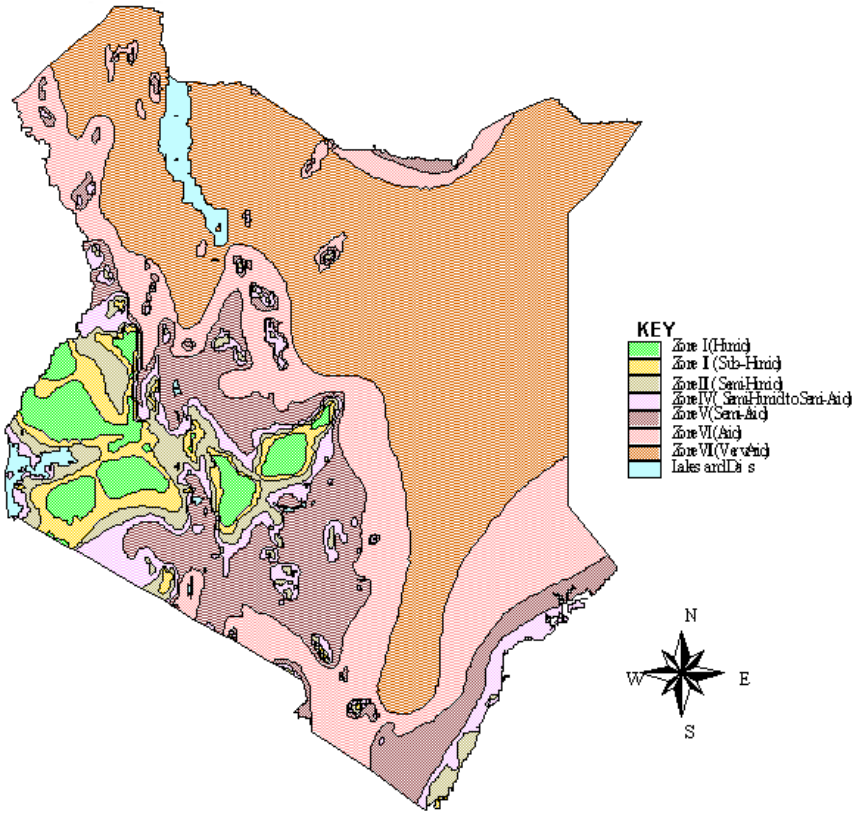

Figure 1: Map of Kenya highlighting study sites from different agro-ecological zones (Macharia, 2004)

2.2 Sample collection and storage

Sample collection was performed over a period of 2 years (September 2013 to August 2015). From the eight selected apiaries, five colonies were randomly selected for sample collection. Samples (honey and pollen) were collected from the apiaries in each of the sites provided in Figure 1 except for Isiolo, Laikipia, and Nairobi where samples were collected from only two apiaries per site due to limited number of available apiaries. Samples collection was conducted during different seasons, Nov-Dec (short rains), Feb-March (start of long rains) and July-Aug (dry season). A total of 261 honey samples and 322 pollen samples were collected during the study period. All samples were immediately stored in either liquid nitrogen or cooler boxes and transported to the laboratory where they were stored at -80^{O}C until further analysis.

2.3 Chemicals and reagents

All pesticide standards were of high purity (>90%) and were obtained from Sigma-Aldrich (Chemie GmbH, Germany) and Dr Ehrenstorfer GmbH (Augsburg, Germany). These standards were stored according to manufacturer's recommendations until use. Pesticide stock solutions were prepared in acetonitrile at 1µg/mL and stored in amber screw-capped glass vials at -20^{o}C until analysis.

2.4 Sample preparation

Samples of the same matrix from each apiary, which constituted of 5 colonies, were pooled and prepared following the QuEChERS method (Anastassiades et al., 2013). Briefly, from each pooled sample matrix, 5g of honey or 3g of pollen were weighed into a 50ml falcon tube and 10ml of water added and the mixture was homogenized. Acetonitrile (10ml) plus a mixture of QuEChERS salts were added. The samples were vortexed for 1 min and centrifuged at 4,200 rpm for 5 min. For quality control monitoring, a blank sample was spiked with a mixture of pesticides of interest at limit of quantification (0.1 µg kg^{-1}) and was processed with a neat blank matrix (non-spiked blank) along with the other samples of each batch of each sample matrix.

Clean-up procedure was performed by taking 1.0 ml of aliquot into 2 ml eppendorf tube and cleaned using dispersive solid phase extraction, packed with 150 mg $MgSO_4$, 50 mg PSA. Pollen samples were additionally cleaned using graphitized carbon to remove excess pigment. The cleaned extracts were centrifuged and filtered through hydrophilic PTFE 0.2 µm. The final extract was diluted at 1:1 (v/v) with water before transferring into auto-sampler vial for LC-MS/MS.

2.5 LC-MS/MS instrumentation

Analysis was performed using an ultra high performance liquid chromatography (UHPLC) Agilent 1290 series coupled to a 6490 model triple quadrupole mass spectrometer (Agilent technologies) with an ifunnel JetStream electrospray source operating in the positive ion mode. Nitrogen was used both as a nebuliser and as the collision gas. Data acquisition and processing was performed using Mass Hunter Data Acquisition; Qualitative and Quantitative analysis software (Agilent Technologies, Palo Alto, CA, v.B.06 and v.B.07).

The chromatographic separation was performed on a Rapid Resolution reverse phase column-C18 1.8 µm, 2.1 x 150 mm column (Agilent Technologies). A gradient elution at a flow rate of 0.4 mL/min was used with water and acetonitrile each containing 5 mM ammonium formate in 0.1% formic acid as mobile phase A and B respectively.

2.6 Sample Analysis

A multi-residue approach, using LC-MS/MS for screening, was adapted to search for chemical contaminants against 102 pesticides that were chosen based on the information obtained from the farmers and local agrochemical stores. Data analysis was carried out by monitoring two transition ions where possible for each targeted analyte as per LC-MS/MS criteria for residue analysis provided in SANCO document (SANCO, 2013). The most dominant transition ion was used for quantification whereas the second most intense ion was used as a qualifier for confirmation purposes. To generate calibration curves used for quantification, matrix-matched calibration standards were prepared at seven calibration levels covering 0.05, 0.1, 1, 10, 25 and 50 parts per billion (ppb), including the zero point in blank extracts of the respective matrices. The resulting calibration curves were used to determine the method's limit of quantification (LOQ) and limits of detection (LOD). The LOQ was set as the minimum concentration that could be quantified with acceptable accuracy and precision.

3.0 Results and Discussion

Pesticide residues in pollen and honey (or its concentrate, nectar) are likely to account for most of the chemical contaminant exposures to honey bees and may represent most of the potential risks concerns since bees rely on honey and pollen to meet majority of their nutritional requirements. Results from this study indicate that among the two hive products, pollen contained approximately 90% of the pesticide residues detected while 50% were detected in honey. A total of 36 pesticide residues were detected of which 5 were only found in honey, 18 in pollen,

and 13 in both matrices as shown in Table 1. Previous studies that investigated chemical residues in hive products have also reported a similar trend leading to some researchers concluding that pollen is the most contaminated hive product (Mullin et al., 2010). A recent study conducted in Kenya evaluated two hive products (bees wax and pollen) and found only four pesticides (1-naphthol, chlorothalonil, chlorpyrifos and fluvalinate) at very low concentrations below 50 ppb (Muli et al., 2014). In that study, the highest concentration was found in 1-naphthol (119 ppb), a metabolite for carbayl and naphthalene, but it was only detected in one of the 15 sites investigated. From the current study, only two (chlorpyrifos and chlorothalonil) of the four pesticides were detected, see Table 1. The other two were possibly not detected due to the fact that the site where 1-naphthol was previously detected was not visited in the current study whereas the highly lipophilic nature of fluvalinate is incompatible with the analytical approach employed in this study. Regarding the big difference between the total numbers of residues detected from the previous study (4) and current study (36), one plausible explanation could be due to the fact that more sites were visited (45 sites) compared to the 15 sites that were visited previously, and perhaps the seasonal variations between the two study periods.

Figure 2 represents a summary of the prevalence (%) of all the various chemical classes detected in Kenya while Figure 3 illustrates how these chemical residues are distributed across the country. Overall, it appears that insecticides are the most prevalent pesticides (>50%, including neonicotinoids and acaricides) followed by fungicides (27%) and herbicides (20%), see Figure 2, implying that pests are the major threat to most agricultural crops in the study areas investigated whereas herbicides are the least frequently used.

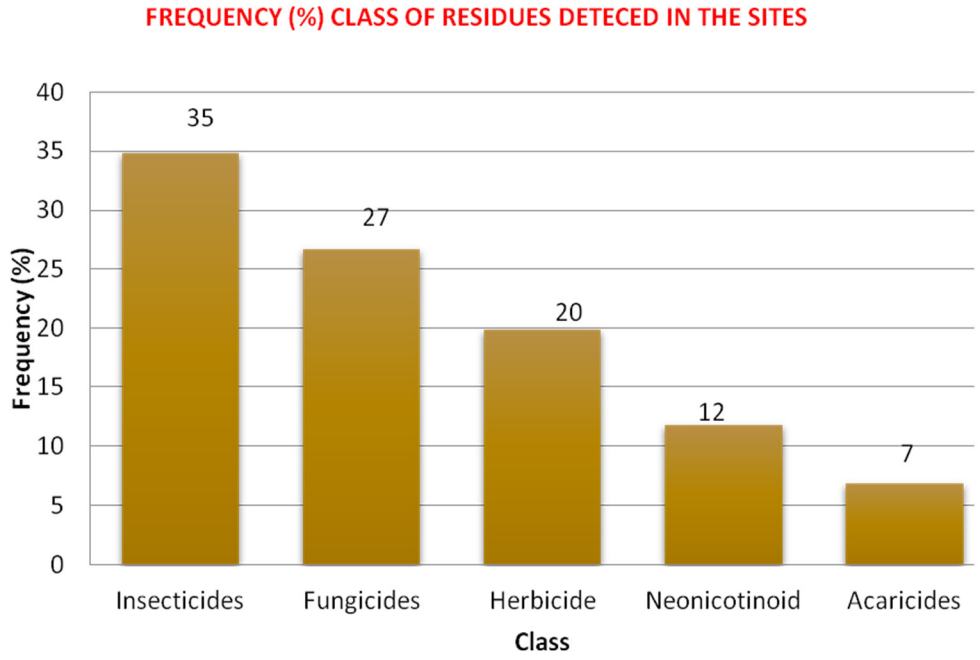

Figure 2: Representative example showing prevalence of different pesticide classes used by Kenyan farmers

Table 1: Concentration of detected pesticides in Kenya and effect on honey bees

Pesticide Residue	Category	Mean Conc. in honey (ppb)	Mean Conc. in pollen (ppb)
Acetamiprid	Insecticide	0.5	N/D
Aldicarb	Insecticide	1.19	27.1
Atrazine	Herbicide	356.7	23.5
Azoxystrobin	Fungicide	30.8	N/D
Bupirimate	Fungicide	N/D	0.47
Carbaryl	Insecticide	0.31	0.4
Carbendazim	Fungicide	0.47	4.64
Chlorpyriphos	Insecticide	0.52	19.5
Chlorpyrofos methyl	Insecticide	N/D	4.14
Clofentazine	Insecticide	N/D	0.83
Chlorothalonil	Fungicide	N/D	0.71
Cymiazole	Insecticide	0.19	0.33
Cymoxanil	Fungicide	1.03	0.14
Cyproconazol	Fungicide	7.04	0.46
Diazinon	Insecticide	1.14	4.12
Dichlorvos	Insecticide	0.6	1.47
Diflubenzuron	Insecticide	N/D	0.73
Dimethoate	Insecticide	1.19	N/D
Epoxyconazole	Fungicide	N/D	0.36
Etofeprox	Insecticide	N/D	3.23
Febuconazole	Fungicide	N/D	0.17
Fenazaquin	Insecticide	N/D	2.55
Flutriafol	Fungicide	0.81	N/D
Hexaconazole	Fungicide	N/D	1.56
Hexythiazox	Insecticide	N/D	0.94
Imidacloprid	Insecticide	0.42	2.19
Malathion	Insecticide	N/D	52.9
Metalaxyl	Fungicide	N/D	0.67
Metribuzin	Herbicide	52.6	3.55
Oxamyl	Insecticide	0.96	5.54
pirimiphos methyl	Insecticide	N/D	2.95
Propamocarb	Fungicide	N/D	0.58
Propiconazole	Fungicide	N/D	0.5
Pyraclostrobin	Fungicide	N/D	0.71
Thiamethoxam	Insecticide	N/D	29.4
Triadimefon	Fungicide	0.89	N/D

The same pattern was observed when the distribution of these pesticides across the country was evaluated as shown in Figure 3. This figure illustrates the distribution of pesticides in the two hive matrices (honey and pollen) from different agro-ecological zones in Kenya. It was noted that the concentration and the number of pesticides detected increased with altitude and agricultural intensification. When the various insecticides detected were further examined, organophosphates (31%) and carbamates (33%) appeared to be the most commonly used throughout Kenya as illustrated in Figure 4. This could be attributed to their slow degradation whereas the 1% of synthetic pyrethroids detected could be attributed to their fast degradation from the environment. This observation is in line with studies done by Gambarcorta et al, 2005, which showed that after spraying, pesticides degrade by first order kinetics resulting in a decrease in their residue levels over time. Unfortunately, in most developing countries, farmers' knowledge about pesticides and available alternatives is still remarkably limited and short-term cost considerations still remain an important factor in poor farmers' choices of pesticides. Cheap pesticides that usually have long degradation periods and present a high risk to users, the public or the environment, often continue to be used in place of less hazardous but more expensive alternatives with shorter degradation time.

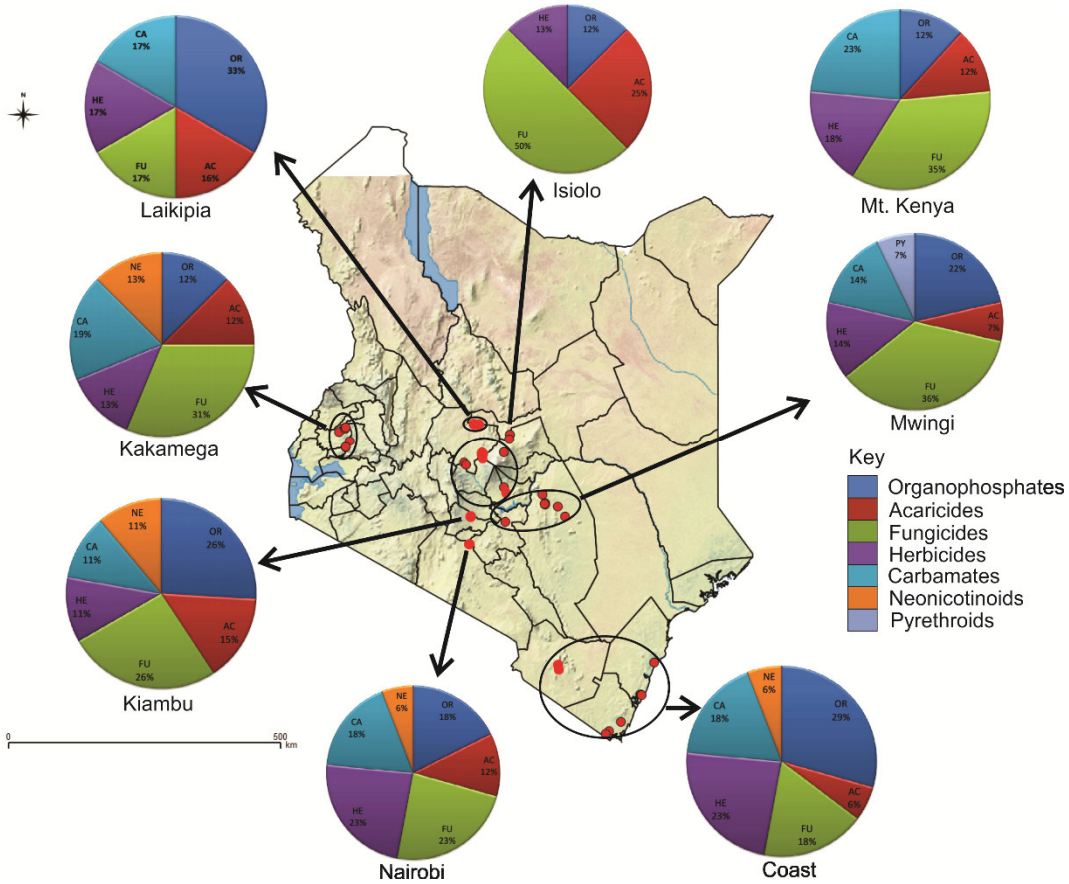

Figure 3: Pesticide distribution in various agro-ecological zones in Kenya

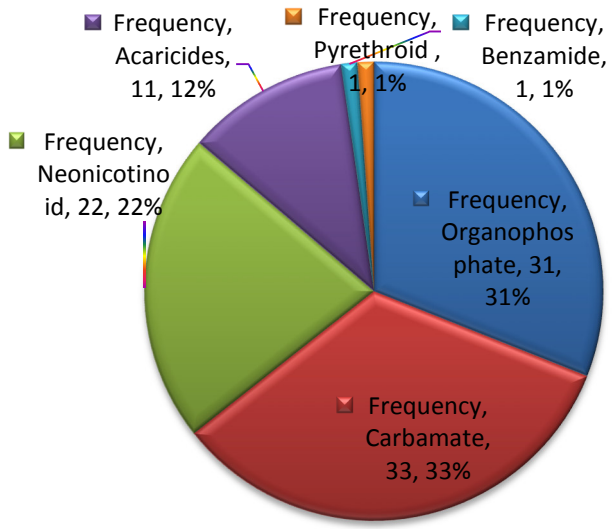

Figure 4: Distribution of different insecticides used in Kenya

Although herbicides appeared to be the least prevalent compared to the other chemical classes, the highest concentration detected from all pesticides originated from atrazine (356 ppb) as shown in Table 1, which is a broad spectrum herbicide used for weed control in maize, sugarcane and pineapple farms in the surveyed regions. Herbicides are generally known to be non-toxic to bees and therefore the levels detected would unlikely pose any hazardous risk to them. Unfortunately no maximum residue limits (MRL) have been set for this chemical in honey or other apicultural products hence its impact to human health requires further investigation. On the contrary, insecticides which seemed to be the most prevalent were detected at the lowest concentrations ranging from 0.1 to 53 ppb, see Table 1. This chemical class is known to be highly toxic to honey bees. Of particular interest are the

neonicotinoids, in this case, acetamiprid, imidacloprid and thiamethoxam, which were detected at various concentrations in this study. Specifically thiamethoxam, a broad spectrum systemic insecticide used in control of sucking insects in flowers, vegetables, leaf miner in coffee and for maize and beans seed treatment, was detected at 29 ppb as depicted in Table 1. This level could be hazardous to both humans and honey bees since the MRL acceptable for this chemical in apicultural products that would not pose a health concern is almost 3-fold lower (10ppb) than the concentration reported in this study. This pesticide is also known to be highly toxic to honey bees with an oral lethal dose that would kill 50% of the test population (LD_{50}) at 0.005 µg/bee (US EPA). A closer look at the data obtained from all sites revealed that only one site, Kiambu, contained levels above the set MRL for this compound whereas the other regions where this pesticide was detected contained very low levels (data not shown). This was not suprising considering that Kiambu region was the only site where both small scale and large scale farming of horticultural, french beans, pineapple and coffee farming was practiced and most of the farmers from this region indicated that they use actara or thiamethoxam in their farms to control pests in their coffee and horticultural farms. In addition, the wet season sampling in this region coincided with periods of heavy pesticide spraying and application.

5. Conclusion

A total of 36 pesticides were detected across the four agro-ecological zones in Kenya belonging to a wide array of chemical classes with insecticides as the most predominantly (>50%) used pesticides followed by fungicides whereas herbicides were the least frequently used in the study areas. Although herbicides appeared to be the least prevalent, they were detected at the highest concentrations of up to 356 ppb in honey compared to insecticides which were detected at fairly low concentrations (0.1 to 53 ppb). Our findings highlight the need to create greater awareness of the ecological consequences of wide scale use of agro-chemicals in agriculture. Further investigations are needed to determine the effect of the detected pesticides on Africa's agro-ecosystem, consumers and honey bee health over time and their potential synergic effects.

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