DATA ARTICLE

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Health risks due to consumption of pesticides in ready-to-eat vegetables (salads) in Kumasi, Ghana

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Abstract

Background: Pesticide residue levels were determined in ready-to-eat vegetables collected from 16 sites along the food chain; which is, farms, markets, cafeterias and street food vending sites in Kumasi, Ghana. The aim of the study was to determine the concentrations of pesticides residues in two ready-to-eat vegetables and assess the health risks due to consumption of these contaminated vegetables.

Methods: Pesticide residues in ready-to-eat vegetables or salads were extracted by means of the QuEChERS method. Synthetic pyrethroid and organophosphorus pesticides residues in samples were determined using Gas Chromatography with Electron Capture Detector and Pulsed Flame Photometric Detector respectively. Consumption data of ready-to-eat vegetables were obtained from a questionnaire-based dietary survey in the study area. The hazard index and the relative potency factor (RPFs) approaches were used to assess the health risk from chronic cumulative dietary exposure to pesticides.

Results: There were six synthetic pyrethroid residues detected in the ready-to-eat samples at varying concentrations. They were bifenthrin, permethrin, cypermethrin, deltamethrin, lambda-cyhalothrin and fenvalerate. Also, two organophosphates were detected in the samples; which were chlorpyrifos and diazinon. Lambda-cyhalothrin residues was present in all the samples in the study, with the mean concentration of 4.5×10^{-2} mgkg⁻¹. The mean concentration of diazinon in all the samples (0.040 mgkg⁻¹) exceeded the EU MRLs (0.01 mgkg⁻¹), chlopyrifos exceeded its MRL in one sample from the street food vending site and cafeteria each. Deltamethrin, Fenvalerate and Permethrin exceeded their respective MRL in samples from *Asafo* (Street food vending sites) and *Adum* (Cafeteria) and *KNUST* (Farm) respectively. However, the health index of all pesticides residues detected were below the permissible limit. The cumulative intake from the RPF approach for the pesticides were relatively lower than the ADI of the index chemicals.

Conclusion: The concentrations of chlorpyrifos, deltamethrin, fenvalerate, diazinon and permethrin exceeded their respective EU MRLs in some ready-to-eat vegetable samples in the study. The health index and comparison of cumulative intake from RPF with ADI of the index chemicals suggest that pesticide residues in ready-to-eat vegetables could not be considered a major public health problem.

Keywords: Organophosphate, Synthetic pyrethroids, Pesticides, Hazard index, RPF, Vegetables

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Background

Agriculture boosts the economy of the nation as most people depend on it for livelihood (Hossain et al. 2015). In order to meet the increasing demand for food as a result of the increasing population, extensive use of pesticides to kill pests that destroy crops is now popular in most developing countries to increase vield of crops (Akoto et al. 2015; Amoah et al. 2006; Chowdhury et al. 2012). Vegetables are the second major food group consumed after cereals and their products in West Africa (Stadlmayr et al. 2013). Moreover, increased vegetable production is important to Ghana's food security strategy (Akoto et al. 2015). Vegetables consumption promotes good health because of their nutritive components (Verma et al. 2015). But most of the vegetable crops such as chilli (hot) pepper, sweet pepper, tomato and okra are lost on the farm to pests' infestation (Degri and Zainab 2013). As a result, most farmers (over 80%) in Ghana use pesticides and also to protect the crops quality and reduce cost of production.

Reports show that there is an unselective use of pesticides among Ghanaian farmers during vegetable cultivation (Ntow et al., 2006). Furthermore, farmers do not respect pre-harvest interval after using pesticide since consumers demand for vegetables have increased (Darko and Akoto 2008; Ntow et al. 2006). There have been several global attempt to reduce or even eliminate the use of pesticides. However, there is still evidence of their presence in various vegetables as a result of application of doses above the recommended dosage. This is due to the ignorance of most farmers to toxic effects of pesticide overdoses (Horna et al. 2008). Pesticide residues in vegetables have been found to be detrimental to human health particularly when they are freshly consumed (Baig et al. 2009; Chen et al. 2011; Solecki et al. 2005).

Several studies suggest a possible negative correlation between pesticide residues and human and animal health (Berrada et al. 2010; Chowdhury et al. 2012). In animals, there have been adverse effects such as cancer when laboratory animals were exposed to organophosphates and synthetic pyrethroids (Akoto et al. 2015). In humans, diseases such as headaches and nausea are known to be acute symptoms to pesticide exposure (Ali and Tahir 2000; Chowdhury et al. 2012). However, cancer, reproductive defects (Bassil et al. 2007), developmental impairment, immunotoxicity (Berrada et al. 2010), birth defects and endocrine disruption are associated symptoms (Longnecker et al., 1997). According to WHO (2002) pesticide toxicity resulted in about 849,000 death of people globally in 2001 (Hossain et al. 2015). But most of them occurred in the developing countries.

More studies need to be done on pesticides because of their widespread misapplication and long range atmospheric transport and deposition (Akoto et al. 2013; Darko and Acquah 2008). An increase rate of pesticide use by farmers in Ghana to maximize profitability (Darko 2009). Moreover in Ghana, studies show the presence of pesticides in cereals (Akoto et al. 2013), fish and water sediments (Darko et al. 2008), even in the atmosphere (Hogarh et al. 2014) and in vegetables (Akoto et al. 2015; Botwe et al. 2011; Darko and Akoto 2008). Most of these studies in Ghana, showed the presence of pesticides in vegetables at the market level (Akoto et al. 2015; Bempah et al. 2016). It is therefore, important to assess the health effects on consumers of vegetables (cabbage and lettuce) that are eaten raw in Ghana, especially along the food chain (farm, market, cafeterias and restaurants). This could be evaluated if the levels of pesticide residues in ready-to-eat vegetables (salads) are determined since data on them are scanty. The aims of this study were to determine the concentrations of the pesticide residues in ready-to-eat vegetables (salads) and to assess the health risk they pose to consumers.

Methods

Materials

The ready-to-eat vegetables consisting of fresh cabbages (*Brassica oleracea*) and lettuces (*Lactuca sativa*) were sampled from 16 sites in Kumasi, Ghana. The sample sites consisted of 4 farms, 4 market centres, 4 street food vending points and 4 cafeterias.

The glassware used for extraction of the pesticide residues in the vegetables were thoroughly cleaned with detergent and distilled water. BDH Chemical Ltd., UK, provided the reagents and solvents and they were all of analytical grade. The various pesticide standards were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

Methods

Sampling

Eight hundred grams (800 g) of ready-to-eat lettuce and cabbage each per sampling site were taken from the farms and market centres (Table 1). Also, 800 grams of salad (mixture of cabbage and lettuce) was sampled from each street food vending sites and cafeteria (Table 1) over a 3-weeks period in October 2015.

Vegetables were stored in labeled bags and transported to the laboratory where they were kept refrigerated at 4 °C until analysis.

A semi structured food frequency questionnaire (Additional file 1) was used in an interview schedule involving 406 respondents including farmers, vegetable sellers, consumers, food vendors and restaurateurs.

Table I sumpling areas of ready to cat regetables	Table	1	Sampling	areas of	^f read	y-to-eat vegetables
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Categories of sites	Sampling sites	Global Positioning System (GPS) Readings
Farm sites	Atonsu	6° 39′ 42.23″N, 1° 35′ 12.32″W
	Chirepatre	6° 39′ 23.35″N, 1° 34′ 41.64″W
	KNUST	6° 40' 0.68"N, 1° 34' 54.90"W
	Tanoso	6° 41′ 54.02″N, 1° 40′ 42.23″W
Market centres	Ayigya	6° 41′ 21.77″N, 1° 34′ 21.56″W
	Kejetia	6° 41′ 46.24″N, 1° 37′ 9.15″W
	Kwadaso	6° 41′ 54.67″N, 1° 38′ 57.04″W
	Santasi	6° 39′ 20.34″N, 1° 38′ 16.66″W
Street food	Aboabo	6° 41′ 59.10″N, 1° 35′ 53.95″W
vending sites	Asafo	6° 41′ 18.82″N, 1° 36′ 55.89″W
	Bantama	6° 42' 7.19"N, 1° 36' 59.03"W
	KNUST	6° 40' 37.57"N, 1° 34' 27.37"W
Cafeterias &	Adum	6° 41′ 36.70″N, 1° 37′ 18.62″W
Restaurants	Airport Roundabout	6° 42' 22.50"N, 1° 36' 2.46"W
	Fante New Town	6° 41′ 27.66″N, 1° 36′ 47.94″W
	Suame	6° 42′ 46.27″N, 1° 37′ 44.67″W

Attached to the questionnaire, different quantities of readyto-eat vegetables (5, 6.05, 10, 13, 15, 20, 25, 30 and 50 g) were added in zip bags. Respondents were asked to identify the quantities they consume per serving. This helped to determine the amount of vegetables eaten by the respondents; which was used in determining the consumption rates. Additional information on socio-demographic data and the body weight of the respondents were obtained from the questionnaire.

Pesticide quantification

Extraction and clean-up of pesticide residues

Extraction and clean-up of pesticide residues was performed by means of the QuEChERS method of Payá et al. (2007) with slight modification. Ten grams of homogenous sample of vegetables (lettuce and cabbage) were weighed into 50 mL centrifuge tube. The mixture was vortexed for 1 min to obtain a uniform mixture after 10 mL of acetonitrile was added to the mixture. A mixture of salt (4 g of magnesium sulphate anhydrous, 1 g of sodium chloride, 1 g of trisodium citrate dehydrate and 0.5 g disodium hydrogencitrate sesquihydrate) was added to the acetonitrile-based mixture. The resultant mixture was vigorously macerated for 1 min and centrifuged at 3000 rpm for 5 min. Aliquots (6 mL) of the extract (organic phase) was transferred into centrifuge tube which contains 150 mg primary secondary amine and 900 mg magnesium sulphate and vortexed for 30 s followed by centrifugation for 5 min at 3000 rpm. A 4 mL aliquot of extract was transferred into a pear shaped flask and 40 μ L of 5% formic acid solution in acetonitrile (ν/ν) was added to adjust the pH of the extracts to 5. The filtrate was concentrated to near dryness on a rotary evaporator (Rotary R-210). One milliliter of ethyl acetate and 20 μ L of ethyl acetate (ν/ν) containing a percentage of glycol solution was used to re-dissolve the concentrated filtrate before transferring the extract into 2 mL vial for quantification by GC with ECD and PFPD.

Determination of pesticides

Separation and quantification of synthetic pyrethroid pesticides were carried out on Varian CP 3800 gas chromatograph with a CombiPAL autosampler equipped with an Electron Capture Detector (ECD, ⁶³Ni), on 30 m + 10 mEZ Guard 0.25 mm internal diameter fused silica capillary column coated with VF-5 ms (0.25-µm film). The initial column oven temperature was 70 °C, held for 2 min and increased to 180 °C at a rate of 25 °C min⁻¹, and then from 180 °C to 300 °C at a rate of 5 °C min⁻¹. Purified nitrogen gas was used as carrier gas at the flow rate of 1.0 mL min⁻¹ and as the make-up gas of 29 mL min⁻¹. The injector and detector temperatures were maintained at 270 °C and 300 °C, respectively. The injection volume was 1.0 µL. Separation and quantification of organophosphate pesticide residues were carried out using Varian CP-3800 gas chromatograph with a CombiPAL autosampler equipped with Pulse Flame Photometric Detector on 30-mm by 0.25-mm internal diameter fused silica capillary column coated with VF-1701 ms (0.25-µm film). The column oven temperature was programmed as: initial temperature 70 °C, then increased to 200 °C at a rate of 25 °Cmin⁻¹, and then increased to 250 °C at a rate of 20 °Cmin⁻¹, keeping the final temperature for 2 min. The carrier gas was nitrogen gas at the flow rate of 2 mLmin⁻¹. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The injector volume into the GC was 2.0 µL.

In order to check interference from the samples, blank analyses were also performed. The retention times of the reference standards were used in detecting the pesticide residues in the extracts. Quantification was achieved by comparing sample peak areas with those of the external reference standards under the same conditions. The sample extracts and reagent blanks were fortified with mixed 0.01 mg/kg of pesticide standards to determine the recovery of the method. The results were calculated with the peak area compared to that of the calibration standards to determine the residue quantitatively. The limits of detection for this method mentioned above were 0.001 mgkg⁻¹, which were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC using the quotient of 3.3 σ and the slope of the calibration curve. The precision of this method ranged from 0.01 mgkg⁻¹ to 1.0 mgkg⁻¹, which was determined by its fortified recoveries for all matrixes ranging from 70 to 120%, its relative standard deviation with range from 3.0 to 12.6% and the correlations were greater than 0.99.

Risk assessment Hazard index

To estimate the risk of non-carcinogenic effects, the estimated daily intakes (EDI) were divided by their corresponding acceptable daily intake (ADI) as shown in Eq. 1. If HI is greater than 1, is an indication of non-carcinogenic risk (Wang et al. 2011).

$$HI = \frac{EDI}{ADI} \tag{1}$$

ADI (mgkg⁻¹ day⁻¹) of bifenthrin, cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin, chlorpyrifos and diazinon (Hossain et al., 2015; Akoto et al., 2015) were obtained from various epidemiological studies and recorded in databases by various health departments. Whiles the average EDI of the various pesticides (mgkg⁻¹d⁻¹) were calculated using Eq. 2.

$$EDI = \frac{Cp \times CRp}{B_w c} \tag{2}$$

Where, CRp is the consumption rate (kgd^{-1}) of pesticides via vegetables, Cp is the mean concentration of pesticide residues $(mgkg^{-1})$ in vegetable samples and B_Wc is the average body weight (kg) of consumers.

Researchers such as Saha and Zaman (2012) have revealed that there could be an interactive and/or additive effects upon the exposure of two or more pollutants. This could lead to a combined health index. Because of this, the US EPA (2000) proposed a hazard index method; which has been used to estimate risk posed by a group of pesticides that act by a common mechanism or that are toxicologically similar (Reffstrup et al. 2010). According to Reffstrup et al. (2010) combined hazard index (CHI) is determined using Eq. 3.

$$CHI = \frac{E_1}{A_1} + \frac{E_2}{A_2} + \dots + \frac{E_i}{A_i} = \sum_{i=1}^n \frac{E_i}{A_i}$$
(3)

Where, E_1 , E_2 , E_m and E_i are the estimated daily intakes of each individual pesticide (i) in a mixture of n pesticides in the food sample. Whereas A_1 , A_2 , A_m and A_i are the acceptable daily intakes (ADIs) for each pesticide (US EPA 2000). If the hazard index exceeds 1, it means the overall mixture of pesticide residue has exceeded the maximum acceptable limit and might pose risk to consumers.

Relative potency factors

The study applied relative potency factor (RPF) approach as described in Quijano et al. (2016) to estimate the chronic cumulative exposure to pesticides in the study area. This method used methamidophos and deltamethrin as index compounds. An index compound is a compound with an extensive toxicological database and one of the best studied within the group. Other studies, (Boon et al. 2008; Jensen et al. 2009), used this method to estimate total OP and carbamates exposure in diets. The averages of pesticide concentrations, consumption rate, and body weights were used in the study to calculate the consumer risk of pesticides via readyto-eat vegetables. The averages were used to typify a more practical and useful scenario in real world sample analysis. Consumption rate of individual pesticide via ready-to-eat vegetables was calculated from the results of the survey and the laboratory reports of pesticide residues. The individual estimated daily intakes (iEDI) of pesticide contaminated ready-to-eat vegetables was calculated using Eq. 4.

 $iEDI(mg/meankgbodyweight/day) = C_r \times F_r$ (4)

Where, C_r is concentrations of pesticide residues in ready-to-eat vegetables and F_r is the consumption rate of ready-to-eat vegetables.

Methamidophos and deltamethrin were selected as index compounds for OP (USEPA 2006) and SP (USEPA 2011) respectively in the RPF approach. RPF for OP were derived from literature and considered the benchmark dose at 10% Acetylcholinesterase (AChE) inhibition (BMD_{10}) in the brain of female rats (Jensen et al. 2009; USEPA 2006). Fortunately, the 2 OP were found within the 24 OP compounds that used methamidophos as index compounds that were published by the EPA. For pyrethroids, out of the 15 SP in the common assessment group (CAG) published by US EPA, six were discussed in this study. These published RPFs of SP were determined by dividing the chemical specific BMD_{20} by the BMD_{20} of the index chemical deltamethrin (USEPA 2011) as shown in Additional file 2. Chronic cumulative exposure was expressed as methamidophos- and deltamethrinequivalents by multiplying the iEDI of each mean pesticide value by its adjusted RPF and adding up the different equivalents to one cumulative intake. Where adjusted RPF is a product of the RPF and a safety factor (Food Quality Protection Act "FQPA" in 1996)

LOCATION	Sample ID	Bifen thrin	Chlor pyrifos	Cyperm ethrin	Deltam ethrin	Diazinon	Fenva lerate	Lambda- cyhalothrin	Perme thrin
Knust(farm)	RVS 1	BDL	0.048	BDL	BDL	BDL	BDL	0.010	0.460
Atonso	RVS 2	0.090	0.034	0.070	BDL	BDL	0.004	0.050	BDL
Chipatre	RVS 3	BDL	0.049	BDL	BDL	0.179	0.001	0.020	BDL
Tanoso	RVS 4	0.320	BDL	BDL	BDL	BDL	0.003	0.040	BDL
Ayigya	RVS 5	BDL	BDL	BDL	BDL	BDL	0.001	0.110	BDL
Kejetia	RVS 6	0.007	BDL	0.002	BDL	BDL	BDL	0.020	BDL
Santasi	RVS 7	BDL	0.050	BDL	BDL	BDL	0.002	0.070	BDL
Kwadaso	RVS 8	0.030	BDL	BDL	0.001	BDL	0.011	0.050	0.040
Airport R	RVS 9	BDL	BDL	BDL	0.002	BDL	0.002	0.040	BDL
Fante	RVS 10	0.010	0.050	BDL	0.003	0.128	0.008	0.060	BDL
Adum	RVS 11	BDL	0.040	BDL	BDL	0.066	0.050	0.010	BDL
Suame	RVS 12	BDL	0.089	BDL	0.004	BDL	0.010	0.030	BDL
Aboabo	RVS 13	BDL	0.090	BDL	BDL	BDL	0.006	0.060	BDL
Knust	RVS 14	BDL	BDL	BDL	BDL	BDL	0.005	0.130	0.002
Asafo	RVS 15	BDL	0.038	BDL	0.021	0.184	0.006	0.010	BDL
Bantama	RVS 16	0.080	BDL	BDL	BDL	BDL	BDL	0.004	BDL
MRL		1	0.05	1	0.1	0.01	0.02	0.2	0.05

Table 2 Concentration of pesticide residues in ready-to-eat vegetables (mgkg⁻¹)

MRL = Maximum Residue Limit issued by European Union (EU, 2013)

Bold and Italic numbers exceeded their corresponding MRL

incorporated in many RPF (U.S. Environmental Protection Agency 2002). This is protective of human health for the pesticide cumulative risk assessment since there is not sufficient information to refine the interspecies factor. To assess whether there was a risk, the mean of the cumulative intake of OP and SP were compared to the ADI of the corresponding index compound.

Results and Discussion

Pesticide residues

The concentration of pesticide residues in the samples are shown in Table 2.

The study shows that all samples analyzed were contaminated with either one or more pesticides. Only 2 organophosphorus pesticides residues were detected out of the 13 considered in the samples (Table 2). Furthermore, 6 out of 9 synthetic pyrethroid pesticides were detected in the samples (Table 2). Among the detected pesticide residues in the samples, only lambdacyhalothrin having varying concentrations ranging from 0.004 to 0.130 $mgkg^{-1}$ were detected in all the 16 samples. Lambda-cyhalothrin is used to control pests such as a cabbage worms, lettuce flea beetles, leaf miners and aphids. Nine out of the 16 samples had concentrations ranging from 0.034 to 0.090 mgkg⁻¹ of chlorpyrifos in them, with concentrations in two samples (*RVS* 12 = 0.089 and *RVS* $13 = 0.090 \text{ mgkg}^{-1}$) exceeding its maximum residue limit of 0.05 mgkg⁻¹ (EU 2013). Moreover, the concentration of diazinon ranging from 0.066 to 0.184 mgkg⁻¹ exceeded their maximum residue limit of 0.01 mgkg⁻¹ (EU 2013). Diazinon residues were identified in 4 of the 16 samples. However, varying concentrations of synthetic pyrethroid pesticides (ranging from 0.007 to 0.320 mgkg⁻¹ for bifenthrin, 0.002 to 0.070 mgkg⁻¹ for cypermethrin and 0.001 to 0.021 mgkg⁻¹ for deltamethrin) detected in the samples were within their corresponding maxresiduelimit. Fenvalerate and permethrin imum detected in samples RVS 11 and RVS 1 had their concentrations above their maximum residue limits respectively. Seven samples had at least a pesticide concentration which was greater than their respective maximum residuelimits.

Residues of cypermethrin were detected in 2 of the 16 samples at a mean concentration of 0.005 $mgkg^{-1}$ which was lower than concentration of cypermethrin in cabbage and lettuce as 0.071 $mgkg^{-1}$ and 0.079 $mgkg^{-1}$ respectively in a study conducted by Yuan et al. (2014). Their study also recorded a higher mean concentration of chlorpyrifos in cabbage and lettuce as 0.09 $mgkg^{-1}$ and 0.024 $mgkg^{-1}$ respectively.

Two samples *RVS 1* (0.460 mgkg⁻¹) for permethrin and *RVS 3* (0.179 mgkg⁻¹) for diazinon from farm sites in *KNUST* and *Chirapatre* exceeded their respective MRL. The other samples (*RVS 12* and *13* for chlorpyrifos, *RVS 15* for deltamethrin, *RVS 11* for fenvalerate and RVS 10, 11, and 15 for diazinon) that exceeded their MRL were either from cafeteria or street food vending sites. This differs from expectation as samples from farm sites were expected to have higher pesticide residue levels since pesticides application occurs there. According to Pérez et al. (2016) processes such as blanching, cooking, frying, peeling and washing reduces pesticide residue levels in fruits and vegetables. Pesticide residues that are loosely attached to the surface were observed to be reduced by washing while peeling removes even those that have penetrated the cuticles of the fruits or vegetables. Contrary, washing of vegetables could increase the pesticide residues sampled from cafeterias and street food vending sites in this study. This is because earlier research revealed that people along the vegetable chain wash several vegetables in the same water in a container without changing these waters; this could re-contaminate these vegetables (Amoah et al. 2008). Moreover, most of these pesticides such as lambda-cyhalothrin are not made only as agricultural insecticides for food and non-food crops but they are used indoors and outdoors for homes, hospitals, and other buildings (World Health Organization 1990).

In general, lambda-cyhalothrin was prevalent in the all the samples analyzed, followed by fenvalerate, chlorpyrifos, bifenthrin, deltamethrin, diazinon, permethrin and cypermethrin. However, in terms of concentration in samples, the highest was diazinon followed by lambda-cyhalothrin, chlorpyrifos, bifenthrin, permethrin and fenvalerate; indicating higher concentration of OP than SP in samples. Permethrin residues were identified in 3 of the 16 samples with a mean concentration of 0.031 mgkg⁻¹ whiles fenvalerate was detected in 12 of the samples at a mean concentration of 0.007 mgkg ⁻¹. Bifenthrin was detected in 6 of the samples at a mean concentration of 0.034 mgkg⁻¹. The range of concentrations of bifenthrin reported in the current study were higher than the study of González-Rodríguez et al. (2008), who reported a range of bifenthrin concentration in lettuce as 0.02 to 0.05 mgkg⁻¹ in Spain. For the synthetic pyrethroid, lambda-cyhalothrin recorded the highest concentration whereas the lowest concentration was deltamethrin. Moreover, diazinon recorded the highest concentration with chlorpyrifos being the lowest for the organophosphates. All the pesticides, except diazinon, recorded mean concentrations lower than their maximum residuelimits. The results indicate the use of pesticides by farmers and their concentrations suggesting farmers' practices towards the safe use of pesticides.. Besides, pesticide application and pest infestation would influence the amount of residues found in them. The coexistence of many of these vegetables grown together on the same farms will create the right conditions for different pests attacks; which will lead to the application of several pesticides.

Consumption trends of ready-to-eat vegetables

In total, 404 stakeholders of different groups among the ready-to-eat vegetables farm to fork chain responded to the study making a response rate of 99.5%. Non-participation was because of interview refusals (0.5%). From the interview schedule, majority of the people (56%) who patronize ready-to eat vegetables in the study area were within 16 to 30 years' age group and 42% above 30 years. The consumption frequencies revealed that females (51.23%) patronized ready-to-eat vegetables more than males (48.77%). A similar survey conducted in the study area (Fung et al. 2011) was different as males patronize more ready-toeat vegetables. This might be as a result of the variation in respondents. Respondents in this study included all the various people along the vegetable food chain (farmers, vegetable sellers, food vendors, buyers (consumers) and restauranteurs). Whereas only buyers (consumers) of salads at the cafeterias and street vending sites were interviewed in the study conducted by Fung et al. (2011). However, a non-parametric Levene's test showed a homogeneity of variance (p > 0.05) between the gender; indicating no significant statistical difference between males and females that consume ready-to-eat vegetables in Kumasi (detailed in Additional file 2).

Consumers of ready-to-eat vegetables in this study consumes averagely 18.60 g per meal, and 355 consumers (87.44%) claim they consume these salads once a day if they do eat them. Twelve respondents (2.96%) consumed ready-to-eat vegetables thrice a day while 9.60% consumed twice a day. Moreover, the study revealed that over a quarter (29.80%) of the consumers consume ready-to-eat-vegetables once a week while 67 (16.50%) twice a week. Fifty-three respondents (13.05%) consume ready-to-eat vegetables once every 2 weeks, 48 (11.83%) once every month and 11 (2.71%) every day. The mean consumption rate per day was recorded as 23.5 gd⁻¹, while the average body weight of consumers in the study area was 68.13 kg.

Based on these concentrations and the consumption trends of salads, the average estimated daily intake were determined and subsequently the health risk assessment. Several methods such as the point of departure index (PODI), and the margin of exposure (MOE) could be used to evaluate the risk; however, the study used hazard index (HI) and the RPF approach, although they all have their limitations. The RPF approach, for example, can only be used if the effects of the individual substances are dose-additive (Boon et al. 2008).

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Pesticide	Mean (mgkg ⁻¹)	EU MRL (mgkg ⁻¹)	ADI (mgkg ⁻¹ day ⁻¹)	EDI (mgkg ⁻¹ day ⁻¹)	HI	HR
Bifenthrin	0.034	1.00	0.02	1.17×10^{-5}	5.86×10^{-4}	No
Chlorpyrifos	0.031	0.05	0.01	1.07×10^{-5}	1.07×10^{-3}	No
Cypermethrin	0.005	1.00	0.05	1.73×10^{-6}	3.45×10^{-5}	No
Deltamethrin	0.002	0.10	0.01	6.90×10^{-7}	6.90×10^{-5}	No
Diazinon	0.040	0.01	0.01	1.38×10^{-5}	1.38×10^{-3}	No
Fenvalerate	0.007	0.02	0.02	2.42×10^{-6}	1.21×10^{-4}	No
Lambda-cyhalothrin	0.045	0.20	0.01	1.55×10^{-5}	1.55×10^{-3}	No
Permethrin	0.031	0.05	0.05	1.07×10^{-5}	2.14×10^{-4}	No

Table 3 The mean concentration of pesticides, their EU MRL, EDI, ADI and health risk estimation through the consumption of ready-to-eat vegetables or salad samples (n = 16)

ADI of pesticides (Osei Akoto et al., 2015; Hossain et al., 2015)

EU MRL of pesticides (EU, 2013)

Hazard index

Hazard indices (HI) for all residues detected are shown in Table 3. Lambda-cyhalothrin was recorded the highest with respect to hazard indices obtained, and the lowest was recorded for diazinon, chlorpyrifos, bifenthrin, permethrin, fenvalerate, deltamethrin and cypermethrin followed in the decreasing order. Again, the order of the HI's were the same in terms of the pesticide residue concentrations detected in the samples except diazinon which was the highest followed by lambda-cyhalothrin. This suggest that HI are almost directly proportional to the pesticide residue concentrations detected in the samples. Whereas for prevalence of pesticide residues, the HI's were mixed-up and followed no particular order. The HI of permethrin, diazinon, chlorpyrifos, fenvalerate and deltamethrin (Table 3) were less than 1 and therefore indicate no health risk. However, these pesticides could accumulate in fatty tissues of consumers and exert chronic health effect since some individual sample concentrations (RVS 1 for permethrin, RVS 3, 10, 11, and 15 for diazinon, RVS 12 and 13 for chlorpyrifos, RVS 11 for fenvalerate and RVS 15 for deltamethrin) exceeded their respective MRL.

The HI of deltamethrin in eggplant (3.1×10^{-3}) and tomato (2.8×10^{-5}) as well as permethrin in eggplant (4.7×10^{-5}) and tomato (4.1×10^{-3}) in the same study area (Akoto et al., 2015) were similar to that determined in this study. In addition, chlorpyrifos in eggplant

Table 4 The combined health risk of various pesticides inready-to-eat vegetables or salads

Pesticide	Ready-to-eat vegetables or salads
Organophosphate	2.45×10^{-3}
Synthetic Pyrethroid	2.57×10^{-3}
Total	5.02×10^{-3}

 (7.7×10^{-3}) , okro (1.2×10^{-3}) and tomatoes (4.1×10^{-3}) were similar to HI of chlorpyrifos in this study. Indicating a moderate approach in pesticide application by vegetable farmers, since the HI were similar and below permissible limit. The hazard indices of all pesticide residues were below one, hence posed no health risk to consumers.

The combined health risks for organophosphates was 2.45×10^{-3} (Table 4). This indicates that consumption of vegetables in the study area pose no significant health risk to consumers as far as organophosphates are concerned. Likewise, synthetic pyrethroids recorded a combined health risk effect of 2.57×10^{-3} , suggesting that people who patronize vegetables may not experience adverse health impacts in their lifetime. The synthetic pyrethroids recorded the highest combined health risk to consumers relatively to organophosphate.

The total non-carcinogenic effects from the consumption of these ready-to eat vegetables is the sum of the combined health risk of organophosphates and synthetic pyrethroids detected in the samples. However, the total non-carcinogenic effects was 5.02×10^{-3} , which is shown in Table 4.

Relative potency factors

Long-term exposure of ready-to-eat vegetable or salad consumers to pesticide residues in Kumasi were low for OP and SP. The pesticides that most contributed to the mean chronic exposure were: chlorpyrifos for OP and lambda-cyhalothrin for SP. Chlorpyrifos contributed most to chronic exposure for OP when the RPF approach was used unlike diazinon when HI was employed. Also, SP contributed most (96.25%) to the chronic cumulative intake/exposure of pesticides detected in the samples. To assess whether there was a risk of exposure, the chronic cumulative intake per each pesticide group, was compared with its

Pesticide	iEDI (mgkg ⁻¹ day ⁻¹)	Adjusted RPF ^a	Concentration expressed in equivalents of index compounds	ADI of Index Compounds
Chlorpyrifos	1.07×10^{-5}	0.12 ^b	1.28×10^{-6}	
Diazinon	1.38×10^{-5}	0.02 ^b	2.76×10^{-7}	
Organophosphate			1.56 × 10 ^{-6 c}	0.004
Bifenthrin	1.17×10^{-5}	1.01	1.18×10^{-5}	
Cypermethrin	1.73×10^{-6}	0.19	3.28×10^{-7}	
Deltamethrin	6.90×10^{-7}	1.00	6.90×10^{-7}	
Fenvalerate	1.21×10^{-4}	0.36	8.69×10^{-7}	
Lambda-cyhalothrin	1.55×10^{-5}	1.63	2.53×10^{-5}	
Permethrin	1.07×10^{-5}	0.09	9.62×10^{-7}	
Synthetic Pyrethroids			4.00×10^{-5} c	0.01

Table 5 Chronic cumulative exposure to OP and SP through ready-to-eat vegetables in Kumasi, Ghana

^aAdjusted RPF = FQPA 10X factors of index compound (USEPA 2006, 2011) \times Oral RPF

^bMethamidophos as index compound and the rest had Deltemethrin as index compound

^cChronic cumulative intake/exposure

corresponding ADI. OP and SP chronic cumulative intakes did not exceed the ADI values of the corresponding index compounds (Table 5). According to the results, cumulative exposure to the 22 pesticides included in the study is not so much a problem to people consuming ready-to-eat vegetables that contain pesticide residues of AChE inhibiting compounds with the same mode of action. The study present low values of chronic cumulative exposure relatively to the chronic cumulative exposure in fruits and vegetables in Valencia, Spain (Quijano et al. 2016). The low chronic cumulative exposure values may be to the pesticide residues in only one commodity (ready-to-eat vegetables or salads) as compared to the 19 commodities comprising of fruits and vegetables in their study. The current study used methamidophos as index compound for OP like the Danish study of pesticides in total diets unlike acephate in fruits and vegetables in Valencia (Quijano et al. 2016). The present study used the mean values conversely to the lower and upper bound in the Valencia study and the 50, 90, and 99 percentile in the Dutch study (Boon et al. 2008). The use of different methodologies can lead to differences in the estimated exposures and evidence the need to harmonize the methodologies used in risk assessment. The results indicated that the exposure is higher when HI are used than when BMD-derived RPF are used. Although they were all relatively, lower as compared to their respective ADI.

It is always important to include scientific uncertainties in pesticide risk assessment since they influence the results. One of the uncertainties that was adhere to was the survey period, which was 3 weeks in this study. According to EFSA (2012) more than 2 days are recommended to estimate long-term exposure. However, the study did not include uncertainty factor such as processing factors (washing and peeling) that can influence the calculated exposure; resulting into a relatively lower estimated exposure. Incorporating these uncertainties into pesticide risk assessment would not have influence the results much considering that exposure levels were very low. Additionally, pesticide risk assessments are more appropriate in total diet exposure, nevertheless, the study focused on intakes of pesticide residues via ready-to-eat vegetables or salads.

Conclusion

The study shows that pesticide residues are present in all ready-to-eat vegetable or salad samples analyzed. At least one synthetic pyrethroid pesticide was detected in each sample analyzed whiles organophosphate pesticides were found in more than half of the samples. Fenvalerate and permethrin exceeded their maximum residue limits in one sample each. Likewise, chlorpyrifos exceeded its maximum residue limit in two samples and diazinon in the four samples. The cumulative nature of pesticides in humans makes their presence in ready-to-eat vegetables or salads problematic. Although chlorpyrifos, diazinon fenvalerate and permethrin exceeded their maximum residuelimits, they pose no adverse health effect to consumers per the health risk assessments. The combined health index of the various pesticide groups revealed no significant health risk for dietary ingestion of organophosphates and synthetic pyrethroid pesticide residues in ready-to-eat vegetables or salads. Therefore, no long-term consumer risk is expected. To guarantee food safety, continuous monitoring is recommended for pesticide residues in vegetables especially those eaten raw.

Appendix 1

Pesticide concentrations in the different ready-to-vegetables (mgkg⁻¹) presented as the mean \pm standard deviation (n = 16) with the range in parenthesis

Pesticide		Salad Samples	LOQ	LOD
Synthtic Pyrethroids	Bifenthrin	0.034 ± 0.088 (0.007 - 0.320)	0.003	0.0010
	Cypermethrin	0.005 ± 0.018 (0.002 - 0.070)		0.0010
	Deltamethrin	0.002 ± 0.006 (0.001 - 0.021)		0.0010
	Fenvalerate	0.007 ± 0.014 (0.001 - 0.050)		0.0030
	Lambda- cyhalothrin	0.045 ± 0.059 (0.004 - 0.130)		0.0008
	Permethrin	0.031 ± 0.111 (0.002 - 0.460)		0.0020
	Allethrin	BDL		0.0005
	Cyfluthrin	BDL		0.0003
	Fenpropathrin	BDL		0.0005
Organophosphorus	Chlorpyrifos	0.031 ± 0.043 (0.034 - 0.090)	0.003	0.0008
	Diazinon	0.040 ± 0.077 (0.066 - 0.184)		0.0008
	Chlorfenvinphos	BDL		0.0005
	Dimethoate	BDL		0.0010
	Ethoprophos	BDL		0.0010
	Fenitrothion	BDL		0.0008
	Fonofos	BDL		0.0005
	Malathion	BDL		0.0010
	Methamidophos	BDL		0.0010
	Parathion	BDL		0.0005
	Phorate	BDL		0.0010
	Pirimiphos-methyl	BDL		0.0005
	Profenofos	BDL		0.0010

Additional files

Additional file 1: Food Frequency Questionnaire. (DOCX 17 kb) Additional file 2: Relative potency factors (RPF) used in the cumulative assessment. (DOCX 19 kb)

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Availability of data and materials

I have submitted my raw data (Excel form) as part of Additional file 1 for any references.

Authors' contributions

SAF and IWO designed the study. SAF collected survey and analytical data, and drafted manuscript. IWO, EDJO and GD made substantial contributions to analysis and interpretation of data. GD reviewed manuscript for intellectual content and contributed substantially to the writing of manuscript. All authors read and approved the final manuscript.

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Competing interests

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