



## Review article

# Heavy metal, microbial and pesticides residue contaminations are limiting the potential consumption of green leafy vegetables in Ghana: An overview

Patience Atitsogbey<sup>a</sup>, Emmanuel Kyereh<sup>b,c,\*</sup>, Hayford Ofori<sup>b,c</sup>, Paa-Nii T. Johnson<sup>c</sup>, Matilda Steiner-Asiedu<sup>a</sup>

<sup>a</sup> Department of Nutrition and Food Science, University of Ghana, Box LG134, Ghana

<sup>b</sup> Council for Scientific and Industrial Research-Food Research Institute, Box M.20, Accra, Ghana

<sup>c</sup> Department of Agro-processing Technology and Food Biosciences, CSIR-College of Science and Technology, Box M. 20, Accra, Ghana

## ARTICLE INFO

## Keywords:

Green leafy vegetables  
Heavy metal  
Microbial contamination  
Pesticide residues  
Food safety

## ABSTRACT

Green leafy vegetables (such as cocoyam (*Colocasia* spp) leaves, spinach (*Spinach* spp), amaranths (*Amaranthus* spp), roselle leaves (*Hibiscus* spp), and lettuce (*Lactuca* spp)) form a major part of Ghanaian meals providing essential vitamin such as A, B and C and minerals including iron and calcium as well as essential bioactive compounds. However, the practices involved in the production, distribution and handling of these nutrient rich vegetables, by most value chain actors in Ghana, unfortunately pre-dispose them to contamination with pathogens, heavy metals and pesticides residues. These have therefore raised public health concerns regarding the safety and quality of these green leafy vegetables. Understanding the current perspectives of the type of pathogens, heavy metals and pesticide contaminants that are found in leafy vegetables and their health impacts on consumers will go a long way in helping to identify appropriate mitigation measures that could be used to improve the practices involved and thereby help safeguard human health. This review examined reported cases of microbial, heavy metal and pesticides residue contamination of green leafy vegetables in Ghana from 2005 to 2022. Notable pathogenic microorganisms were *Ascaris* eggs and larvae, faecal coliform, *Salmonella* spp., *Staphylococcus aureus*, *Streptococci*, *Clostridium perfringens*, and *Escherichia coli*. In addition, Lead (Pb), Cadmium (Cr), Chromium (Cr), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) have been detected in green leafy vegetables over the years in most Ghanaian cities. Pesticides residues from organochlorine, organophosphorus and synthetic pyrethroid have also been reported. Overall, microbial, heavy metals and pesticide residue contamination of Ghanaian green leafy vegetables on the farms and markets were significant. Hence, mitigation measures to curb the contamination of these vegetables, through the food chain, is urgently required to safeguard public health.

## 1. Introduction

Green leafy vegetables are common diet consumed throughout the world, being essential sources of nutrients, metabolites and antioxidant [1]. The World Health Organization (WHO) emphasized in its 2021 annual report that consumption of safe and quality

\* Corresponding author. Council for Scientific and Industrial Research-Food Research Institute, Box M.20, Accra, Ghana.  
E-mail address: [ekyereh@foodresearchgh.org](mailto:ekyereh@foodresearchgh.org) (E. Kyereh).

<https://doi.org/10.1016/j.heliyon.2023.e15466>

Received 12 September 2022; Received in revised form 4 April 2023; Accepted 10 April 2023

Available online 14 April 2023

2405-8440/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

foods are pre-requisites towards good health [2]. In Ghana, an estimated number of 420,000 foodborne disease cases are reported annually at the health facilities with a death rate of 65,000, costing the Government of Ghana nearly 69 million dollars annually [3]. Fresh leafy vegetables are important parts of healthy diets that can help boost immunity by providing essential nutrients such as vitamins, minerals and fiber [4–7]. Several studies have explained that green leafy vegetables provide protective mechanisms against non-communicable diseases such as cardiovascular diseases, diabetes, overweight and obesity and some cancers [4,6,8–10].

Increased awareness of the health importance of leafy green vegetables in developing countries currently has resulted in their increasing demand and consumption. The increase in demand has spurred the interest in intensive cultivation and full year production [11–14] which has come with its own shortfalls. About 90% of the vegetables consumed in Ghana are mostly grown in limited spaces in urban and peri-urban areas in the cities. The farmers take advantage of the proximity of markets in the cities and the nearness to market centers also help reduce challenges with their postharvest management [15]. Unfortunately, increases in population and urbanization have made water and land for vegetable production in these areas very scarce [16]. Farmers therefore resort to the use of polluted wastewater and fresh poultry manure and intense use of agrochemicals. As a result, there is heavy colonization of pathogen on the leafy vegetables [17–19] high accumulation of heavy metals from old used discarded batteries and metals scraps [20] and agrochemical residues from pesticides and other growth stimulants used for farming [21].

With the rising level of concern about the safety, quality and health risk associated with green leafy vegetables in the Ghanaian farms and markets, a number of studies have been conducted into the microbial, heavy metals and pesticides residues contaminations of leafy green vegetables. This review assessed the current perspectives on heavy metals, microbial, and pesticides residues contamination of fresh leafy green vegetables produced and consumed in Ghana. In terms of scope, our review assesses contributing factors, sources of contamination, levels of concentrations, types of pathogens, the presence of pesticides and heavy metals and their health impacts in the green leafy vegetables produced and sold on the Ghanaian market. This review also examines possible intervention and mitigating programs that might be introduced to reduce the levels of contaminations and therefore improve the overall safety and acceptability of green leafy vegetables. The review has been organized along the following discussion sections; heavy metal residues, microbial and pesticides contaminations.

### 1.1. Heavy metals residue in green leafy vegetables

Heavy metals are a group of metals with relatively high densities, atomic number or weight ranging from 63.5 to 200.6 g mol<sup>-1</sup> like Lead (Pb), Arsenic (As), Mercury (Hg), Cadmium (Cd), Iron (Fe), Zinc (Zn), Chromium (Cr), Copper (Cu), Silver (Ag) and Nickel (Ni) [22–24]. These metals enter the environment through natural and human activities such as mining, car exhausts fumes, industrial discharge and agricultural practices [23–25].

Heavy metals are non-biodegradable and have the potential to bio-accumulate in living organisms including humans and food crops through exposure to low or high concentrations [26]. Their ability to persist in the environment and along the food chain poses serious public health problems [23,24]. The uptake of heavy metals by food crops is however dependent on the acidity of soils on which they are planted, the higher the soil acidity, the more mobile and soluble the heavy metals becomes and increase their potentials to bioaccumulate in food crops and leafy green vegetables such as spinach and lettuce [25,27]. The soils for planting becomes contaminated with heavy metals through the use of pesticides, fertilizers, application of sludge as composts, emissions from incinerators, mining residues, smelting factories and dumping of solid wastes into the environment [28–30].

Recent studies in Tamale Metropolis, Ghana suggested the presence of heavy metals such as Pb, Cd, Cr, Zn, Fe, Cu and Manganese (Mn) in some green leafy vegetables grown within the area [31]. Previous study conducted on 479 vegetables including cabbage and lettuce from farms and major markets in Accra Metropolis from 2013 to 2014 reported that approximately 81% of the vegetables have accumulated heavy metals residues which exceeded the specific release limit (SRL) of the European Union (EU) guideline values (Pb – 0.01 mg/kg; Co – 0.05 mg/kg; , As – 0.002 mg/kg; , Zn – 5 mg/kg, Cr – 1 mg/kg, Mn – 0.55 mg/kg, Cd – 0.005 mg/kg, Fe – 40 mg/kg, Ni – 0.14 mg/kg and Cu 5 mg/kg) [32] with the highest values occurring in lettuce, hence posing a greater health risk to consumers. The heavy metals detected were Pb, Co, As, Zn, Cr, Mn, Cd, Fe, Ni and Cu [30]. In Sekondi-Takoradi Metropolis in the Western region of Ghana, a similar study conducted to determine the presence of heavy metals in indigenous and exotic leafy green vegetables revealed the incidence of Fe, Zn, Hg (Mercury), Cd, Cr and Ni from a major urban farm. Cadmium and Hg were also detected in *Amaranthus spinosus* and *Corchorus olitorius*, which are local delicacies. Although the values were below both local and international daily dietary intake levels, continuous consumption over a period could lead to health hazards [34].

### 1.2. Sources of heavy metals in Ghanaian green leafy vegetables

Green leafy vegetables are able to take up nutrients including heavy metals residues from soils and water and accumulate them in their edible parts [31,35]. Previous studies have shown that vegetables grown on soils polluted with high levels of heavy metals or irrigated with water contaminated with heavy metals accumulate such metals [30,34,36].

More recently, studies in the Western region of Ghana investigated the levels and types of heavy metals in soils from vegetable farms, streams and ground water used to irrigate vegetables. Their results indicated the presence of Hg and Cd in streams with the mean concentration of Hg (0.009 mg/L), and Cd (0.019 mg/L) and about 80% of ground water sources contain heavy metals that exceeds the acceptable limit (Hg 0.006 mg/L and Cd 0.003 mg/L) of FAO [32]. The main sources of heavy metal load in the soils were attributed to household wastes and fertilizer, the mean concentration of the heavy metals in the soils were as follows; Fe (369.06 mgkg<sup>-1</sup>), Hg (0.073mgkg<sup>-1</sup>), Zn (1.71mgkg<sup>-1</sup>) and Cr (0.64mgkg<sup>-1</sup>) [34]. The Goo reservoir which serves as a major source of water for irrigation by vegetable farmers in Navrongo reported high levels of heavy metals such as Cd (1.476 µg/L), Cu (9.387 µg/L), Cr (4.991

$\mu\text{g/L}$ ) and Zn  $6.563 \mu\text{g/L}$ ) detected [36]. The detection levels are clear indication that most leafy vegetables irrigated with water from the Goo reservoir are likely to be contaminated with metals and poses a huge public health challenge in the Navrongo areas.

### 1.3. Levels of heavy metals in Ghanaian green leafy vegetables

A previous study established that 83 cabbage and 113 lettuce samples collected from five farms and five markets in the Accra Metropolis recorded levels of heavy metals exceeding the EU specification of  $0.01 \text{ mgkg}^{-1}$  (As),  $0.01 \text{ mgkg}^{-1}$  (Cd),  $0.05 \text{ mgkg}^{-1}$  (Co),  $0.05 \text{ mgkg}^{-1}$  (Cr),  $0.005 \text{ mgkg}^{-1}$  (Cu),  $2.0 \text{ mgkg}^{-1}$  (Fe),  $0.5 \text{ mgkg}^{-1}$  (Mn),  $0.02 \text{ mgkg}^{-1}$  (Ni),  $0.05 \text{ mgkg}^{-1}$  (Pb) and  $5.0 \text{ mgkg}^{-1}$  (Zn) in leafy green vegetables [30] (Table 1). In their assessment, they reported that most of the irrigated waters were drawn from untreated waste water from residential and commercial areas and streams that receives run off municipal effluent from residential areas. Also, because these farms are situated in the urban areas, other contributing factors such as fumes from heavy traffic emissions were cited as potential contamination points.

A similar study conducted in Sekondi–Takoradi Metropolitan Area in Western Region of Ghana, also reported worrying amounts of heavy metals concentrations of Cd,  $0.027 \text{ mg/kg}$ , Fe,  $36.178 \text{ mg/kg}$  in *Amaranthus spinosus* (Aleefu), Cd,  $0.024 \text{ mg/kg}$  and Fe,  $27.584 \text{ mg/kg}$  in *Corchorus olitorius* (jute leaves), respectively [34]. The mean concentrations of Cd, Fe and Zn recorded for cabbage samples analyzed from the same metropolitan area were  $0.018 \text{ mgkg}^{-1}$ ,  $5.910 \text{ mgkg}^{-1}$  and  $0.079 \text{ mgkg}^{-1}$  and from lettuce,  $0.014 \text{ mgkg}^{-1}$  (Cd),  $16.520 \text{ mgkg}^{-1}$  (Fe) and  $0.075 \text{ mgkg}^{-1}$  (Zn), respectively (Table 1). In both the indigenous and exotic leafy greens, the concentrations for Fe were high compared to Cd and Zn [34]. A comparable study conducted in the Tamale Metropolitan Area, in northern Ghana, reported  $0.04 \text{ mgkg}^{-1}$  (Cd),  $3.23 \text{ mgkg}^{-1}$  (Fe) and  $0.06 \text{ mgkg}^{-1}$  (Zn) for cabbage samples sourced from the main Salaga market in Tamale (Table 2) [31]. Also, higher Cd content, ranging from  $0.5$  to  $4.2 \text{ mgkg}^{-1}$  for cabbage and carrots collected from peri-urban communities compared with those from rural communities in Kumasi, ( $1.6$  to  $1.9 \text{ mgkg}^{-1}$ ) have been reported [38]. Tables 1 and 2 provide detailed summary of the heavy metal concentrations in some selected green leafy vegetables in Ghana.

### 1.4. Health and nutritional implications of heavy metals contamination in green leafy vegetables

Due to the toxic nature of heavy metals, their accumulation in the human body causes serious health problems. Heavy metals bioaccumulate in humans through consumption of contaminated foods which includes green leafy vegetables. The leafy greens absorb the heavy metals such Pb, As, Cd, Cr, Zn, Fe and Cu from soils, water and the environment into their matrix which becomes available to human through the gastrointestinal system. Nickel destroys the nutrients in the leafy green, Fe produces free radicals which damages the DNA and proteins and also causes necrosis and wilting in the leaves. Zinc and Cd decrease plant metabolism and cause oxidative damages while Hg causes physical injury to the leaves and the plants [39]. Consumption of these metals in higher concentration or low concentration, over a long period of time, build ups in the kidney and liver which subsequently leads to kidney, liver, cardiovascular, nervous system and bone diseases [40]. Also, retardation, endocrine disruption, cancers and immunological imbalances are some of the health consequences. These may reduce or prevent the consumption of leafy green vegetables among the populace, which could result in lack of the vital nutrients such as vitamins, minerals and antioxidants in humans with its devastating health problems [31,39, 41–45].

## 2. Microbial contamination of green leafy vegetable

Along the food chain, green leafy vegetables are easily contaminated by pathogenic microbes at multiple stages during production, harvesting, processing, packaging, distribution and handling. These pathogens are attached to the surfaces of the leaves, localized in niches, cracks and crevices. Some become internalized and are largely protected from germicides [46–48]. They are deemed high risk

**Table 1**

Heavy metals detected in some indigenous green leafy vegetables in Sekondi-Takoradi Metropolitan Area.

Green Leafy Vegetable	Area	Heavy Metal Detected	Concentration (mg/kg)	Number of Samples	Summary of findings	References
<i>Amaranthus spinosus</i> (*Aleefu)	Sekondi-Takoradi	Cadmium	0.027	9 duplicates	The mean concentrations were below the safe limit of FAO/WHO	[34]
		Iron	36.178	9 duplicates		
	Metropolis	Mercury	0.0093			
		<i>Corchorus</i>				
		<i>Olitorius</i>				
<i>Olitorius</i> (*Ayoyo)	Sekondi-Takoradi	Zinc	0.202		The metal loads in the vegetable was less than FAO safe limit	[34]
		Cadmium	0.024			
	Metropolis	Iron	27.587			
		Mercury	0.131			
		Cadmium	0.018	9 duplicates		
<i>Brassica oleracea</i> (Cabbage)	Sekondi-Takoradi	Cadmium	0.018	9 duplicates	The mean concentrations were below the safe limit of FAO/WHO	[34]
		Iron	5.910	9 duplicates		
	Metropolis	Mercury	0.001			
		<i>Lactuca sativa</i>				
		<i>(Lettuce)</i>				
<i>Lactuca sativa</i> (Lettuce)	Sekondi-Takoradi	Zinc	0.079		The metal loads in the vegetable was less than FAO safe limit	[34]
		Cadmium	0.014			
	Metropolis	Iron	16.520			
		Mercury	0.002			
		Zinc	0.075			

**Table 2**  
Heavy metals detected in some indigenous green leafy in Accra, Tamale and Navrongo.

Green Leafy Vegetable	Area	Heavy Metal Detected	Concentration (mg/kg)	Number of Samples	Summary of findings	References		
<i>Brassica oleracea</i> (Cabbage)	Tamale Metropolis	Cadmium	0.04	6 samples from 3 vendors	The hazard index of heavy metals load was above 1, signifying non-acceptable level of non-carcinogenic adverse health effect.	[31]		
		Chromium	0.03					
		Manganese	0.01					
		Iron	3.23					
		Zinc	0.06					
<i>Brassica oleracea</i> (Cabbage)	Navrongo (Site B)	Cadmium	0.055 ± 0.08	128 samples	The hazard Index of the metal was above 1, indicating they could be injurious to human health.	[36]		
		Chromium	1.871 ± 0.06					
		<i>Lactuca sativa</i> (Lettuce)	Zinc				0.732 ± 0.15	
		Navrongo (Site B)	Copper				1.489 ± 0.09	
			Cadmium				0.062 ± 0.03	
Chromium	2.063 ± 0.09							
<i>Brassica oleracea</i> (Cabbage)	(Waste water irrigated) Accra (Groundwater Irrigated)	Zinc	0.845 ± 0.27	48 samples each of cabbage, lettuce and ayoyo was used	Even though the hazard indices recorded for all heavy metals indicated that immediate risks currently, continuous consumption of the contaminated vegetables over time can be lethal because these heavy metals accumulate in the body.	[20]		
		Copper	2.751 ± 0.05					
		<i>Brassica oleracea</i> (Cabbage)	Accra (Groundwater Irrigated)				Cadmium	BDL
							Chromium	BDL
		<i>Brassica oleracea</i> (Cabbage)	Accra (Waste water irrigated)				Zinc	9.5 ± 1.4
							Copper	3.3 ± 1.4
		<i>Lactuca sativa</i> (Lettuce)	Accra (Waste water irrigated)				Cobalt	0.4 ± 0.0
							Nickel	1.8 ± 1.0
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Lead	10.5 ± 3.0
							Cadmium	BDL
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Chromium	BDL
							Zinc	9.5 ± 1.5
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Copper	2.6 ± 0.4
							Cobalt	1.0 ± 0.0
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Nickel	1.4 ± 0.1
							Lead	6.7 ± 4.2
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Cadmium	1.1 ± 0.01
							Chromium	1.1 ± 0.0
		<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)				Zinc	10.6 ± 3.5
							Copper	6.3 ± 1.4
<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)	Cobalt	1.1 ± 0.8					
		Nickel	2.8 ± 0.5					
<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)	Lead	10.2 ± 2.5					
		Cadmium	0.50 ± 0.01					
<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)	Chromium	BDL					
		Zinc	9.8 ± 1.0					
<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)	Copper	3.3 ± 2.7					
		Cobalt	1.5 ± 0.0					
<i>Lactuca sativa</i> (Lettuce)	Accra (Groundwater Irrigated)	Nickel	2.8 ± 3.2					
		Lead	8.0 ± 5.2					

\*Local Names.

\*BDL means below detection limit.

produce for microbial contamination because the pathogenic microbes can form biofilms on the leaves [49]. Poor agriculture practices such as planting vegetables on contaminated soils, using untreated poultry manure or manure containing human faecal matter, contaminated water for irrigation, human interactions, and exposure to dusty environment contribute to the microbial loads on the green leafy vegetables [14,47,48]. These poor practices expose the edible parts of the leafy greens to contamination and this contributes significantly to the food safety problems associated with the vegetables because most of these vegetables are consumed either raw or minimally cooked [46,50–53].

In Ghana, there is a growing concern about the safety of green leafy vegetables for human consumption. This is because of poor pre- and post-harvest practices which expose these vegetables to microbial contamination [54]. Whilst exotic vegetables are mostly used for cold salads with minimal or no heat applied, the indigenous leafy vegetables are usually used for stews and soups to which moderate or high heat has been applied. Several studies have pointed out consumption of green leafy vegetables with no or minimal heat applied, make them probable vehicles for foodborne diseases [18,55,56].

The possible sources of microbial contamination of leafy vegetables identified in Ghana includes dams and ground water, waste water usage, soil for cultivation and manure used as fertilizer, harvesting vegetables with bare hands, uncleaned transporting vans, un-sanitized sacks used for packaging the vegetables from the farms to market centers, non-potable water used in washing the leafy vegetables at the market centers [18,55,57–62].

Water, an important commodity for farming, is needed by the crops before and after germination through to maturity [57,63]. Scarcity of water in rural, peri-urban and urban areas for farming activities coupled with increased demand, prolonged dry seasons and cost of safe potable water have all contributed to the diversion of using wastewater for irrigation by farmers. Interestingly, some of the

farmers use the unclean water to wash soil-soaked harvested vegetables just before sending them to the marketing centers [64,65], hence leaving prone to contamination. Waste water from residential and industrial drains, streams and rivers which are pumped into shallow wells and used for leafy vegetable irrigation have been also found to be major sources of parasitic and pathogenic microbial contamination of the leafy vegetables which are hazardous to human health [64,65]. For example, about 70% of green leafy vegetable farmers in Accra and Kumasi use wastewater for irrigation [19]. In Cape Coast, 90.5% of spring onions, 76.2% of lettuce and 66.7% of cabbage were found to be contaminated with parasites [60]. Parasitic contaminations detected in leafy vegetables in Navrongo exceeded the WHO/FAO permissible levels [18,36,54,57–60,63,66].

Additionally, the soil also serves as another vehicle for pathogenic microbial contamination. The soil becomes contaminated through the use of untreated or treated poultry and cow droppings that are mixed with the soil intended to serve as manure for the cultivation of the vegetable. Even though these manures are used because they are cheap and readily available, they contain parasitic micro-organisms that are easily transferrable from the soil to the crops [54,60,66].

### 2.1. Types of microorganisms recorded on some Ghanaian green leafy vegetables

Several studies have identified different types of microbial pathogens and parasites that habitually colonize fresh green leafy vegetables consumed in Ghana. These include *Ascaris* eggs and larvae, faecal coliform, *Salmonella* spp., *Staphylococcus aureus* and *Streptococci*, *Strongyloides* spp, *Trichuris trichiura*, and *Entamoeba coli*. Others include *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Shigella*, yeast sp and molds. Also, *Enterobacter* spp., *Enterococci*, *Klebsiella* spp., *Serratia marcescens* were isolated, whereas fungi of

**Table 3**  
Summary of microorganisms detected in exotic green leafy vegetables in Ghana.

Type of vegetable	Microbes detected	Source of Reference-Research title	Summary of findings	Reference
Lettuce, Cabbage, Spring Onions	-Salmonella, enterococci, faecal coliform, molds and yeast -E. coli, Salmonella spp, Bacillus cerus, Shigella spp. -Strongyloides spp, Hookworm, Trichuris trichiura, <i>Ascaris lumbricoides</i> and <i>Entamoeba coli</i> . <i>Escherichia coli</i> , Faecal coliform, Total coliform	-Microbial quality of leafy green vegetables grown or sold in Accra Metropolis, Ghana -Microbial quality of ready-to-eat vegetable salads vended in central business district of Tamale, Ghana -Parasitic profile of fresh vegetables sold in selected markets of the Cape Coast Metropolis of Ghana Irrigation water quality and its impact on the physicochemical and microbiological contamination of vegetables produced from market gardening: a case of the Veia Irrigation Dam, U.E.R., Ghana	-Results shows that sampled leafy green vegetables have poor microbial quality -Leafy vegetable salads sold on the street of Tamale were unwholesome for human consumption -The study showed that most of the leafy vegetables bought from the markets were highly contaminated with intestinal parasites The level of bacteria detected on the vegetables were above the WHO acceptable limits.	[18] [56] [60] [62]
Lettuce, Cabbage, Spring Onions	- Bacteria: <i>Escherichia coli</i> , <i>Enterobacter</i> spp., <i>Klebsiella</i> spp., <i>Salmonella</i> spp., <i>Serratia marcescens</i> , and <i>Staphylococcus</i> Fungi: <i>Aspergillus</i> , <i>Candida</i> , <i>Fusarium</i> , <i>Penicillium</i> , and <i>Rhodotorula</i> - Faecal coliform, <i>Salmonella</i> , <i>E. Coli</i> , helminth eggs -Total coliforms, faecal coliform, <i>Escherichia coli</i>	-Microbiological contamination of some fresh leafy vegetables sold in Cape Coast, Ghana. -Heavy metals and Microbial contaminants of some vegetables irrigated with Goo reservoir water, Navrongo, Ghana. -Presence of pathogenic <i>E. coli</i> in ready-to be-eaten salads from food vendors in the Kumasi Metropolis, Ghana - Coliform contamination of Peri-urban grown vegetables and potential Public Health Risks: evidence from Kumasi, Ghana	-The research revealed that the vegetables are significantly contaminated and have poor microbial quality hence, could be injurious to health -The high microbial contamination levels shown indicated foodborne disease risk should these vegetables be consumed -The presence of <i>E. coli</i> in the salads should be a public health concern The results showed unacceptable levels of microbial contamination on the green leafy vegetables analyzed which could results in public health threats for consumers	[55] [36] [69] [57]
African spinach, African eggplant leaves, roselle leave (kenaf) and jute leaves	-Salmonella, enterococci, faecal coliform, molds and yeast -Bacillus cereus, <i>Clostridium perfringens</i> , <i>Escherichia coli</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Streptococcus</i> spp, <i>Staphylococcus</i> spp, Yeast sp., and total coliform	-Microbial quality of leafy green vegetables grown or sold in Accra Metropolis, Ghana -Potential links between irrigation water microbial quality and fresh vegetables quality in the Upper East Region of Ghana subsistence farming	-Results shows that sampled leafy green vegetables have poor microbial quality -Similar pathogenic microbes were found in both irrigation water and on the leafy vegetables, hence links between the two exist. The presence of harmful microorganisms on the vegetables raises public health issues for consumers	[18] [68]

the genera *Aspergillus*, *Candida*, *Fusarium*, *Penicillium*, and *Rhodotorula* were also found [18,36,55,56,60,66–69]. Table 3 provide detailed summary of typical microorganisms that have been respectively detected on both exotic and indigenous green leafy vegetables grown Ghana.

## 2.2. Levels of microbial contamination recorded on some leafy vegetables in Ghana

Microbial examination conducted on fresh leafy vegetables on some selected farms and at the market places shows various levels of microbial contamination [18]. Green leafy vegetables such as cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), African spinach (*Amaranthus* sp), African eggplant leaves (*Solanum macrocarpon*), roselle leaves (*Hibiscus sabdariffa*), and jute leaves (*Corchorus olitorius*) collected from 50 farms and four major markets in 2018 within Accra, show the following levels of contamination on all vegetables; the mean aerobic bacteria count on all sampled green leafy vegetables ranged from 8.30 to 9.20 log CFU/g. The mean yeast and mold counts were from 4.25 to 5.73 log CFU/g. Mean fecal coliform counts ranged from 4.28 to 5.81 log CFU/g and enterococcus counts from 2.93 to 4.53 log CFU/g [18].

Besides, varied microbial contamination levels were recorded for 30 samples of ready-to-eat fresh green leafy vegetable salads (mostly exotic vegetables) in Tamale, in the Northern region of Ghana. These vegetable salads samples were collected from the central business area in Tamale. They showed the presence of *E. coli* in 96.7% of the salads with average microbial counts of 7.56 log CFU/g, *Bacillus cereus* were present in 93.3% of the salads with counts ranging from 0 to 7.44 log CFU/g, whilst 73.3 and 76.7% of the analyzed salads had *Salmonella* spp. and *Shigella* spp. with the plate counts ranging from 0 to 4.54 log CFU/g and 0 to 5.54 log CFU/g, respectively [56].

Significant levels of microbial contaminations were also found in leafy vegetables from three main markets in Cape Coast; Kotokuraba, Abura, and University of Cape Coast Science markets. Microbial examination was conducted showed the prevalence of intestinal parasites on 90.5% of spring onion, 76.2% of lettuce and 66.7% of cabbage [60]. Mean bacterial counts recorded for cabbage, lettuce and spring onions from the Kotokuraba Market in Cape Coast were  $1.93 \times 10^8$ ,  $1.23 \times 10^8$ , and  $1.17 \times 10^8$  CFU/ml, respectively. From Abura Market also in Cape Coast, the mean bacterial counts recorded were  $9.9 \times 10^7$ ,  $2.8 \times 10^7$ , and  $6.60 \times 10^7$  CFU/ml, respectively, for the same leafy vegetables [55,60]. Microbial loads were detected on kenaf (*Hibiscus cannabinus*), African spinach (*Amaranthus cruentus*) and lettuce (*Lactuca sativa*) from three farms in Tono, Upper East region as well. *Bacillus cereus* load ranged from  $3 \times 10^5$  CFU/g to  $74 \times 10^5$  CFU/g, while *Staphylococcus* spp. load ranged from 0 to  $21 \times 10^5$  CFU/g also *Clostridium perfringens* load ranged from  $37 \times 10^5$  CFU/g to  $80 \times 10^5$  CFU/g. *Escherichia coli* counts ranged from  $4 \times 10^5$  CFU/g to  $80 \times 10^5$  CFU/g while *Salmonella* spp. counts ranged from 0 to  $70 \times 10^5$  CFU/g. For yeast, the counts ranged from 0 to  $75 \times 10^1$  CFU/g [68]. In 2020, microbial analysis on cabbage and lettuce in Navrongo also in the Upper East region revealed microbial contamination levels, for cabbage, the mean microbial loads are; faecal coliform counts ranges from  $2.6 \times 10^7$  CFU/g to  $3.0 \times 10^7$  CFU/g, *E. coli*;  $2.3 \times 10^7$  CFU/g to  $2.8 \times 10^7$  CFU/g; Helminths eggs;  $2.8 \times 10^7$  CFU/g to  $3.1 \times 10^7$  CFU/g and salmonella counts ranges from  $2.1 \times 10^7$  CFU/g to  $2.4 \times 10^7$  CFU/g and for lettuce, the mean microbial loads for faecal coliform ranges from  $2.9 \times 10^7$  CFU/g to  $3.1 \times 10^7$  CFU/g, *E. coli* counts are  $2.3 \times 10^7$  CFU/g to  $2.9 \times 10^7$  CFU/g, the helminths eggs counts ranges from  $2.7 \times 10^7$  CFU/g to  $2.9 \times 10^7$  CFU/g and the plate count for salmonella ranges from  $2.2 \times 10^7$  CFU/g to  $2.3 \times 10^7$  CFU/g [36]. Similarly, analysis conducted on spring onion and cabbage grown and irrigated with water from the Veve Dam in the Upper East Region of Ghana showed results that exceeded the acceptable limits of World Health Organization. Faecal coliform and *E. coli* loads on the spring onions are;  $3.32 \pm 0.07$  and  $3.25 \pm 0.05$  (log CFU/10 g) while that of cabbage are;  $3.50 \pm 0.04$  and  $3.34 \pm 0.08$  (log CFU/10 g) [62].

In Kumasi, microbial examination was conducted on fresh salads sampled from ten suburbs among street food vendors shows the presence of total coliforms and *E. coli*. The mean log CFU/g counts are  $6.35 \pm 0.09$  for total coliforms and  $5.1 \pm 0.1$ , for *E. coli* respectively [69]. A similar study conducted in Kumasi gave mean total coliform/100 ml concentration for spring onions, lettuce and cabbage were  $9.15 \times 10^9$ ,  $4.7 \times 10^7$  and  $8.3 \times 10^7$  respectively. The mean faecal coliform counts for spring onions, lettuce and cabbage were  $1.5 \times 10^8$ ,  $4.15 \times 10^7$  and  $2.15 \times 10^7$ , respectively, also the mean *E. coli* counts for spring onions, lettuce and cabbage were  $1.4 \times 10^8$ ,  $2.2 \times 10^7$  and  $3.2 \times 10^7$  [57]. Also, highly significant levels of faecal coliforms and helminths were isolated on 72 lettuce samples at farm gates in Kumasi [70].

## 2.3. Health and nutritional implications of microbial contamination in different leafy vegetables

However, the presence of harmful microorganisms such as *Ascaris* spp, *Staphylococcus aureus*, *Streptococci*, *Strongyloides* spp, *Trichuris trichiura*, *Bacillus cereus*, *Clostridium perfringens*, *E. coli*, *Shigella*, yeast sp and molds, *Enterobacter* spp., *Salmonella* and *Enterococci*, *Klebsiella* on the leafy green vegetables are risk factors for foodborne disease outbreaks such as cholera, typhoid, diarrhea and dysentery which affects health and nutrition of consumers of such contaminated leafy green vegetables [14,36,46,72–74]. Another microbial hazards to freshly cut ready-to-eat leafy greens by these disease-causing microorganisms is their ability to cause spoilage and odour rendering salads unsafe for human consumption [10,46].

## 3. Pesticide residues in green leafy vegetables

Pesticides application on vegetable farms is an important agricultural practice because it is needed to control or prevent unwanted organisms such as insects, fungi, bacteria and weeds which cause destruction to farm produce [76]. Green leafy vegetables are very susceptible to the destructions caused by these pests and diseases, especially when damaged or have senescing tissue. Hence, farmers use pesticides such as Carbofuran, Lambda-cyhalothrin, Cypermethrin, Diazinon, Fenitrothion, Chlorpyrifos, Endosulfan to prevent

or control the infestation while ensuring quality and boosting production to meet market demands [76,78].

However, the excessive use of these pesticides results in residual effect which affects the wholesomeness of the vegetables [51,79,80]. For instance, 14C-fenpicoxamid (phenyl- and pyridine) metabolism assessed in cabbages grown in open-air environment with 30-day intervals re-treatment, harvested on 7 days after spraying (mature leaves) and 14 days after spraying (immature leaves) shows over 98% of the total residue in cabbage samples was extracted with acetonitrile and aqueous acetonitrile, containing 0.1% phosphoric acid" [81]. Likewise, a study conducted in the USA using spring onions and chive leaves in a five-trial experiment with a re-treatment of cyazofamid for 7 days and zero pre-harvest intervals (PHI) shows cyazofamid residue levels of 0.46, 0.48, 0.54, 0.77 and 1.1 mg/kg but the allowable daily intake of this pesticide is 0.2–0.044 mg/kg. However when the leafy green onions were harvested 35 days after the last application showed negligible cyazofamid residue level [81].

By applying pesticides at various stages of growth of the leafy vegetables, farmers introduce chemicals into the environment and into the vegetables. Also, soil and water bodies used for farming have been found to be other sources that retains agrochemicals and transfer the chemicals into the vegetables [80,83,85].

The soil has high retention capacity for dichlorodiphenyltrichloroethane (DDT) and other organophosphate and organochlorine pesticides and this makes the soil a risk factor for surface, shallow or groundwater pesticides in and around the vegetable farms as its content leaches into the water bodies [80,84,85]. In Ghana, the application of pesticide (insecticide, fungicides, herbicides, bactericides, and rodenticides) in vegetable production has been on the increase over the past years. It is estimated that about 87% of vegetables and fruits farmers in Ghana uses chemical pesticides to control pest and diseases in Ghana [86]. They further reported predominant usage of herbicides in vegetable production in Ghana because of farmers perception of weed control. Further studies have

**Table 4**  
Selected pesticides residues in leafy green vegetables in Accra Metropolis.

Vegetables	Area	Pesticides Detected	Concentration (mg/ kg) $\times 10^{-2}$	Number of Samples	Detection Method	Reference
Cabbage	Accra Metropolis	p,p'-DDE	0.04	267	Quick, easy, cheap, effective, rugged and safe analytical procedure	[91]
		Aldrin	0.01			
		Chlorfenvinphos	<0.01–0.06			
		Fenitrothion	<0.01–0.06			
		Permethrin	<0.01–0.15			
		Endosulfan sulphate	0.05			
		Deltamethrin	<0.01–1.60			
		Fenvalerate	<0.01–0.23			
		Cypermethrin	<0.01–0.27			
		Methoxychlor	<0.01			
Fonofos	0.43					
Diazinon	<0.01–0.15					
Lettuce	Accra Metropolis	$\alpha$ -Endosulfan	0.05	252	Quick, easy, cheap, effective, rugged and safe analytical procedure	[91]
		Beta-endosulfan p,	0.04			
		p'-DDE	<0.01–0.28			
		Diazinon	<0.01–7.88			
		Dimethoate	<0.01–0.09			
		Chlorpyrifos	<0.01–1.27			
		Deltamethrin	<0.01–1.19			
		Fenvalerate	<0.01–0.61			
		Cypermethrin	<0.01–0.19			
		Lambda-cyhalothrin	<0.01–0.92			
Bifenthrin	<0.01–0.39					
Fenitrothion	<0.01–0.03					
Cabbage	Accra Metropolis	$\beta$ -Endosulfan	1.6–2.0	50	multi-residue method based on solid-phase extraction followed by gas chromatography–mass spectrometry	[88]
		Endosulfan sulphate	2.7–3.1			
		p,p-DDT	1.2–1.8			
		p,p-DDE	1.2–1.6			
		p,p-DDD	2.1–2.5			
		$\gamma$ -Chlordane	0.9–1.3			
		Heptachlor	0.9–1.3			
		Lindane	1.3–1.9			
		$\beta$ -HCH	0.8–1.4			
		Endrin	2.2–2.6			
Lettuce	Accra Metropolis	$\beta$ -Endosulfan	0.9–1.3	50	Multi-residue method based on solid-phase extraction followed by gas chromatography–mass spectrometry	[88]
		Endosulfan sulphate	2.0–2.6			
		p,p-DDT	2.8–3.6			
		p,p-DDE	2.7–3.5			
		p,p-DDD	2.6–3.0			
		$\gamma$ -Chlordane	0.6–1.8			
		Heptachlor	0.6–1.8			
		Lindane	1.8–2.4			
		$\beta$ -HCH	1.3–1.9			
		Endrin	1.8–2.4			

shown high levels of contamination of pesticides (DDT) with residue levels of <0.01 to 165.81 mg/kg in soils from leafy green vegetable farms and ground water close to the farms in some parts of Ghana [85].

### 3.1. Common types of pesticide residues found some Ghanaian fresh leafy vegetable

Green leafy vegetables grown in Ghana have been found to contain organochlorine, organophosphorus and synthetic pyrethroid pesticides. Although organochlorine pesticides including methoxychlor, DDT, aldrin, and beta-HCH are banned worldwide, some Ghanaian vegetable farmers continue to use them illegally, hence their presence in some green leafy vegetables [83,85,88–90].

Analysis conducted on about 4,000 leafy green vegetables from major markets in Accra revealed the presence of fenvalerate, fenitrothion, lambda-cyhalothrin, dimethoate, permethrin, deltamethrin, lindane, endosulfan, chlorpyrifos, aldrin, enderin, parathion, heptachlor, phorate and DDT [83,91]. Furthermore, a three year study, from 2010 to 2012, in which 252 samples each of cabbage and lettuce from Accra were analyzed, showed positive results for Deltamethrin, Cypermethrin, Cyfluthrin, Pirimiphos-methyl,  $\alpha$ -Endosulfan, p, p'-DDE, Bifenthrin and Chlorpyrifos which were above the maximum residue limits (MRL) [91].

In Kumasi, organochlorine residues of methoxychlor and beta-hexachlorocyclohexane (beta-HCH), heptachlor, aldrin,  $\alpha$ -endosulfan, dieldrin, endrin and p,p'-DDT were isolated from leafy green vegetables collected from four farms [85]. Also, in another study conducted in Kumasi (Ayigya, kejetia, Asafo, Adum, Bantama, Suame and Aboabo), on ready-to-eat leafy green vegetable salads revealed the presence of synthetic pyrethroid and organophosphorus pesticides residues [92]. Specifically, the synthetic pyrethroid pesticide residues detected included bifenthrin, permethrin, cypermethrin, deltamethrin, lambda-cyhalothrin and fenvalerate, whilst the organophosphates identified were chlorpyrifos and diazinon [92]. Similarly, in 2005, a study conducted on total of 180 spring onions, cabbage and lettuce samples from three cities namely, Kumasi, Tamale and Accra revealed Chlorpyrifos (Dursban) on 78% of the lettuce, lindane (Gamalin 20) on 31%, endosulfan (Thiodan) on 36%, lambda-cyhalothrin (Karate) on 11%, and dichloro-diphenyl-trichloroethane on 33% [93]. These detected levels mostly exceeded the maximum pesticide residue levels for consumption in vegetables by FAO/WHO [81,93]. Tables 4 and 5 provide detailed summaries of commonly detected pesticides and their concentration levels in leafy green vegetables from some selected locations in Ghana.

### 3.2. Levels of pesticide residues recorded on some Ghanaian leafy vegetables

The Maximum Residue Levels is “the highest level of pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly”. The amount of residues found in all foods must be safe for consumers and must be as low as possible stated in mg/kg” [94]. The European commission’s MRL for organochlorine pesticide residues in or on leafy green vegetables such as cabbage

**Table 5**  
Pesticides residues detected in fresh leafy green vegetables in Kumasi and Accra Metropolis.

Vegetables	Area	Pesticides Detected	Concentration ( $\mu\text{g}/\text{kg}$ )	Number of Samples	Detection Method	Reference
Cabbage	Ayigya, Kumasi	$\beta$ -HCH	0.20 $\pm$ 0.01	1 kg	Varian CP-3800 gas chromatograph equipped with an electron capture detector (ECD) and VF-5ms capillary column (30 m $\times$ 0.25mm $\times$ 0.25 $\mu\text{m}$ ) was used	[85]
		$\gamma$ -HCH	1.40 $\pm$ 0.41			
		$\delta$ -HCH p,p'-DDT	1.50 $\pm$ 1.81			
		Endosulfan sulphate	5.67 $\pm$ 0.44			
		Methoxychlor	28.47 $\pm$ 2.68*			
Lettuce	Ayigya, Kumasi	$\gamma$ -HCH	9.54 $\pm$ 0.02	1 kg	Varian CP-3800 gas chromatograph equipped with an electron capture detector (ECD) and VF-5ms capillary column (30 m $\times$ 0.25mm $\times$ 0.25 $\mu\text{m}$ ) was used	[85]
		Heptachlor	2.29 $\pm$ 0.14			
		Aldrin	22.66 $\pm$ 0.15*			
		$\alpha$ -Endosulfan	0.60 $\pm$ 0.28			
		Dieldrin	1.49 $\pm$ 0.14			
		Endrin p,p'-DDT	1.69 $\pm$ 0.42			
		Methoxychlor	9.34 $\pm$ 0.22			
Salads (Lettuce and cabbage mixture)	Kumasi, Ghana	Synthetic Pyrethroids	0.007–0.320	800 g each from 16 sites	Quick, easy, cheap, effective, rugged and safe analytical procedure and GC (Electron Capture Detector and Pulsed Flame Photometric Detector)	[92]
		Bifenthrin	0.002–0.070			
		Cypermethrin	0.001–0.021			
		Deltamethrin	0.001–0.050			
		Fenvalerate	0.004–0.130			
		Lambda-cyhalothrin	0.002–0.460			
		Permethrin				
Salads (Lettuce and cabbage mixture)	Kumasi, Ghana	<b>Organophosphorus</b>	0.034–0.090	800 g each from 16 sites	Quick, easy, cheap, effective, rugged and safe analytical procedure and GC (Electron Capture Detector and Pulsed Flame Photometric Detector)	[92]
		Chlorpyrifos	0.066–0.184			
		Diazinon				
Lettuce	Accra	Deltamethrin	0.50	252	modified quick, easy, cheap, effective, rugged and safe analytical procedure	[91]
		Cypermethrin	2.00			
		Cyfluthrin	1.00			
		Pirimiphos-methyl	1.00			
		$\alpha$ -Endosulfan	0.50			



and lettuce is 10 µg/kg or 0.01 mg/kg and the acceptable daily intake (ADI) is 3 mg/kg bw [85]. However, research has shown that the levels of methoxychlor detected in some leafy vegetables in Kumasi, Ghana (Table 5) were about 20 times higher ( $184.10 \pm 12.11$ ) than the EU's MRL of 10 µg/kg [85].

A three-year study carried out to determine the presence of pesticides residues in vegetables in major markets in Accra (Table 4) shows that 1,128 leafy vegetable samples exceeded the maximum residue limits of 0.005 mg/kg for organochlorine, 0.01 mg/kg for organophosphorus and 0.01 mg/kg for synthetic pyrethroids as established by EU/WHO [81,91]. Another study in which 400 vegetables including cabbage and lettuce were investigated for the presence of chlorinated pesticide residues (Table 4), results shows that 20% of the samples were above the maximum residue levels [88].

### 3.3. Health and nutritional implications of pesticide residues contamination

The interplay between health and nutrition is intrinsically linked in that consumption of healthy diets, which includes green leafy vegetables due to their high content of vitamins and minerals such as vitamins A, B and C, Ca, Fe, folic acids, dietary fiber, protein and important bioactive compounds leads to good health [95]. Previous studies have shown that non-consumption of these vitamins and mineral rich leafy green vegetables leads to micronutrient deficiencies among the populace [95–97].

Consequently, pesticides residues on leafy green vegetables have been found to affect the nutritional quality of the vegetables and their consumption having several fatal outcomes for consumers [98]. Evidence has shown that consumption of organochlorines, organophosphorus and synthetic pyrethroids in any amount in the leafy vegetables bio-accumulate in the human body and have been associated with disease conditions such as cancer, headache, reproductive health problems, hormone disruption, nervousness, dizziness, confusion, vomiting, convulsion and decreased intelligence [7,51,98,100,101].

Also, chemicals such as chlorine used in treating slightly chopped ready-to-eat fresh leafy vegetable before packaging in order to prolong shelf lives, leaves undesirable impacts as it produces by-products such as chloroforms, chloramines and haloacetic acids which can be fatal to humans hence, raises several public health concerns [10,51,84].

## 4. Interventions to reduce the microbial, heavy metals and pesticides residues in green leafy vegetables

Green leafy vegetables have become a very important part of diets globally, but yet are plagued with the challenge of contamination from microbial, heavy metals and pesticide residues. It has therefore become imperative to devise reliable methods that could help reduce the contamination load in order to make the vegetables safe for human consumption [102–104]. According to the US Environmental protection agency, a method is deemed significant if the treatment results in microbial reduction of 2 log or more. Also, any methods which lowers the residue levels of pesticides below the maximum residue level of 0.1 to 1 ppm is considered effective [105].

### 4.1. Microbial interventions

In decontaminating fresh lettuce, a combined treatment of ozone (9 mg/L) and lactic acid (2.5 ml/L) for 10 min effectively reduced *E. coli* 1.6–2.9 log CFU/g and mesophilic bacteria 1.5–3.5 log CFU/g on the lettuce [106]. Furthermore, washing the leafy vegetables with 1.2 g/L of acidified sodium chlorite (ASC) was shown to removed *E. coli*, *L. monocytogenes*, and *Salmonella* spp. up to 99.9% from the vegetables [105]. They also recommended modern technologies such as Ultrasound, pulsed light (PL) treatment, ultraviolet (UV) light which destroys microorganisms through generation of photoproducts in the DNA of exposed microorganism, High hydrostatic pressure (HHP) technique, Gamma-rays, X-rays radiation which effectively decontaminate leafy vegetables and make them safe for consumption [105]. Likewise, the effectiveness of PL and a HEN (hydrogen peroxide, EDTA and Nisin) was tested in the reduction of *E. coli* on spinach. Intense treatment for 15 s using pulse light alone resulted in the reduction of 2.7 log CFU/g of *E. coli* O157:H7 while the combination of PL and HEN sanitizer yielded a higher result [107]. The combination PL dose (15.75 J/cm<sup>2</sup>) followed by 2 min immersion in HEN whereas in HEN-PL treatment yielded a 4.6 logs CFU/g reduction of *E. Coli* on the fresh spinach [107]. However, EDTA application must be done carefully as long-term exposure to higher concentrations by human dentin can result in removal of phosphorus from the teeth [108].

### 4.2. Heavy metal interventions

In another study, a combination of microbubbles and two oxidizing sanitizers; sodium hypochlorite (NaOCl, 40 and 80 mg/L) and acidic electrolyte water (AEO, 20 and 40 mg/L) used to wash leafy green vegetables for 5 min resulted in the effective reduction of *Salmonella Typhimurium* and *E. coli* on sweet basil and Thai mint with 2–3 log CFU/g (99.2–99.8%) [109].

In reducing heavy metals contamination on leafy green vegetables, a study suggested grafting which truncates the heavy metal uptake by the roots of the leafy vegetables into the leaves. Similarly, in a study between grafted and non-grafted shoots of vegetables, the results revealed lower concentrations of Sr, Mn, Cr, Ti, Pb, Ni and Cd in the grafted leafy green vegetables compared to the non-grafted vegetables [39,110]. Transgenic planting is a form of genetic engineering technique which enhances the phytoremediation capability of plants in reducing hazardous heavy metals in the environment. These transgenic plants prevent accumulation of heavy metals such as Hg and Cd by truncating root uptakes, hence accumulation in the leaves are prevented [39,111,112]. Previous studies have successfully explored phytoremediation technique in reducing the levels of heavy metals in the soil, where higher plants were used to absorb both essential and non-essential metals including Cd [114–117]. Transgenic planting and phytoremediation techniques can be adapted in Ghana to reduce heavy metals in leafy vegetables.

### 4.3. Pesticides interventions

For reduction of pesticide residue concentration in leafy vegetables, various methods have been suggested. For instance, findings showed the reduction of methamidophos and dimethoate on spinach and cabbage using a strong acidic electrolysed oxidizing (AC) water and alkaline electrolysed (AK) water. Mixing a strong acidic electrolysed oxidizing (AC) water with either methamidophos or dimethoate reduced the initial concentrations by more than 99% [118]. Furthermore, NaOH solution (pH 11.24) or strong alkaline electrolysed (AK) water resulted in a 98% of methamidophos in 10 min. Using AC water to wash three times every 3 min, followed by an AK water washing for 3 min with shaking (AC-333, AK-3) increased the reducing effect significantly [118].

Another method which has been reported to be efficient and effective in the removal or reduction of pesticides residues on leafy vegetables is the ultrasound aided extraction (UAE). Ultrasound cleaning technique is a faster method which penetrates all the crevices of the vegetable. This method has aided the extraction of different pesticides including organophosphate, carbamates, triazoles, and pyrethroids from six vegetable matrices [119,120]. Also, a recent study investigated the effect of ozone, lactic acid and combination treatments on the control of pesticide contaminants of fresh vegetables and reported that combined treatment of vegetables with lactic acid and ozone effectively remove 26–97% chlorpyrifos and 62–100% of spiked  $\lambda$ -cyhalothrin from various vegetables [106]. The difference between normal washing with water and the combine treatment of ozone and lactic of the vegetables to remove chlorpyrifos was statistically significant at  $p < 0.05$  [106].

Soaking Chinese-kale for 15 min in 0.1 M acetic acid has been proven to remove 43 and 90% of methomyl, carbaryl, and pesticides from the vegetable [105,122]. The study suggested that high redox potential (617 mV) and the acidity of the acetic acid (pH 3.74) solution could be the reason for the carbaryl degradation [105,122]. Electrolyzed water also known as electrolyzed oxidizing water (EOW) and electrolyzed reducing water (ERW) have shown effectiveness in removing pesticide residues from spinach. The EW due to its strong oxidizing capacity has been used to effectively remove pesticides from fresh vegetables [105]. While the EOW on the other hand was effective in removing pesticides such as diazinon, phosmet, and cyprodinil on fresh spinach [124].

## 5. Recommendations for producers, retailers and consumers

With the evidence provided to us from the published articles on the microbial, heavy metals and pesticides residue contamination of leafy green vegetables in Ghana, we recommend that;

- The Ministry of Food and Agriculture (MOFA), and the government should strengthen legislation, and oversight responsibility on food crop production in Ghana. That is, they must increase education on the health hazards associated with the utilization of untreated poultry or cow dung as manure usage, the usage of untreated waste water from drains and sewages for irrigation purposes, water from unprotected and polluted well and reservoirs used on the farms to avoid microbial contamination.
- Awareness must be increased among retailers on the health risk linked with the use of untreated water for sprinkling on fresh leafy vegetables in the market places. Also, agricultural centered NGOs must include sensitization of farmers and the public on the sources of heavy metals in the leafy greens and the health risks associated with heavy metals consumption through leafy green vegetables.
- Farmers should be educated on the dangers associated with the excessive application of permissible agrochemicals and the avoidance of the usage of banned agrochemicals for vegetable production, how the chemicals can bio accumulate in their body leading to diseases and death; and how their actions are putting public health at risk.
- Public education in churches and mosques, local opinion leaders, dramas on television and radios to drum home the health problems associated with the use of unsafe water, and unsafe farm and cooking practices must be initiated and sustained. Knowledge must be shared during the public education on the safe methods of washing fresh leafy vegetables and cooking time of the vegetables to render the harmful microbes ineffective or completely eliminate. Also, awareness must be created on the available methods of decontaminating the green leafy vegetables to remove or reduce pesticides and heavy metals to improve quality and safety.
- Proper labelling of package leafy vegetables indicating type of chemicals and quantity used for production and preservation should be stated. Compulsory quarterly medical examination for leafy green vegetable farmers so as to ascertain the level of chemical bio accumulation and worm infestation in order to minimize and or prevent cross contamination of the fresh leafy green vegetables through handling on the farms should be instituted.
- Regular testing of water and soil on the farms to ascertain level of microbial, heavy metals and pesticide residue contamination should be carried out by the relevant agencies. More microbial and pesticide residue contamination research covering many varieties of indigenous Ghanaian green leafy vegetables is recommended.

## 6. Conclusion

Available data show high levels of microbial contaminations of both exotic and few indigenous green leafy vegetables analyzed from some Ghanaian market especially in Accra, Kumasi, Tamale and Navrongo. Heavy metals such as Pb, Cd, As, Fe, Zn, Hg and Cu were as well found on these leafy vegetables. Although the application of pesticides is necessary for increase food production which is important in preventing food insecurity, data available has shown excessive usage of the pesticides which leaves residues that exceeds the maximum residue level permitted by European Union Commission/World Health Organization in the analyzed vegetables samples. However, there is the need for public education on safe use of pesticides coupled with their health implication. Education on safe

vegetable production and consumption coupled with the health risks associated with not adhering to guidelines should be incorporated in Ghana's educational curriculum at all levels. However, it is imperative to consider simple detection methods at the farm level to help farmers with rapid analysis of green leafy vegetables after harvest.

#### Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

#### Data availability statement

Data included in article/supp. material/referenced in article.

#### Declaration of competing interest

The authors declare no conflict of interest.

#### References

- [1] P.R. Shakya, N.M. Khwaounjoo, Heavy metal contamination in green leafy vegetables collected from different market sites of Kathmandu and their associated health risks, *Sci. World J.* 11 (11) (2013) 37–42.
- [2] O. World Health, WHO urges governments to promote healthy food in public facilities, *Pridobljeno* 19 (1) (2021) 2022.
- [3] P.F. Ababio, P.J.F.C. Lovatt, A review on food safety and food hygiene studies in Ghana, *J. Food Contr.* 47 (2015) 92–97.
- [4] D.F. Maffei, N.F. de Arruda Silveira, and M.d.P.L.M.J.F.C. Catanazi, Microbiological quality of organic and conventional vegetables sold in Brazil, *Food Control* 29 (1) (2013) 226–230.
- [5] R.S. Bhutada, R. Rathi, D.J.I.J.o.R.i.P.S. Dasar, Immunity boosting diet during Covid 19, *Int. J. Res. Pharm. Sci.* (1) (2020) 11.
- [6] K. Adak, Indian vegetables boost immunity and truncate obesity: a review, *Int. Res. J. Adv. Sci. Hub* 2 (2020) 48–52.
- [7] S. Elgueta, et al., Pesticide Residues in Leafy Vegetables and Human Health Risk Assessment in North Central Agricultural Areas of Chile, 2017, pp. 105–112, 10(2).
- [8] G.-Y. Tang, et al., Effects of vegetables on cardiovascular diseases and related mechanisms, *Nutrients* 9 (8) (2017) 857.
- [9] E.M. Alissa, G.A. Ferns, Nutrition, Dietary fruits and vegetables and cardiovascular diseases risk, *J. Crit. Rev. Food Sci.* 57 (9) (2017) 1950–1962.
- [10] R.K. Saini, E.Y. Ko, Y.-S. Keum, Minimally processed ready-to-eat baby-leaf vegetables, *Prod. Proc. Stor. Microb. Saf. Nutr. Poten.* 33 (6) (2017) 644–663.
- [11] E.O. Gido, et al., Consumer acceptance of leafy African indigenous vegetables: comparison between rural and urban dwellers, *Int. J. Veg. Sci.* 23 (4) (2017) 346–361.
- [12] K. Abass, J.K. Ganle, K.J.G. Afriyie, 'The germs are not harmful': health risk perceptions among consumers of peri-urban grown vegetables in Kumasi, Ghana, *J GeoJournal* 82 (6) (2017) 1213–1227.
- [13] P.B. Sant'Anna, et al., Microbiological safety of ready-to-eat minimally processed vegetables in Brazil: an overview, *J. Sci. Food Agric.* 100 (13) (2020) 4664–4670.
- [14] G.I. Balali, et al., Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world, *Int. J. Microb.* 2020 (2020).
- [15] B. Keraita, et al., Harnessing farmers' knowledge and perceptions for health-risk reduction in wastewater-irrigated agriculture, in: P. Drechsel, et al. (Eds.), *Wastewater irrigation and health: Assessing and mitigating risk in low-income countries*, Int. Water Manag. Inst., 2010, pp. 337–354.
- [16] K. Afriyie, K. Abass, J.A.A.J.I.J.o.U.S.D. Adomako, Urbanisation of the rural landscape: assessing the effects in peri-urban Kumasi, *Int. J. Urban Sustain. Dev.* 6 (1) (2014) 1–19.
- [17] C. Samuel Jerry, et al., Microbial contamination in vegetables at the farm gate due to irrigation with wastewater in the tamale metropolis of Northern Ghana, *J. Environ. Protect.* (2013) 2013.
- [18] J.K. Quansah, et al., Microbial quality of leafy green vegetables grown or sold in Accra metropolis, Ghana, *Food Control* 86 (2018) 302–309.
- [19] B. Keraita, et al., Reducing microbial contamination on wastewater-irrigated lettuce by cessation of irrigation before harvesting, *Trop. Med. Int. Health* 12 (2007) 8–14.
- [20] I. Lente, et al., Heavy metal pollution of vegetable crops irrigated with wastewater 41 heavy metal pollution of vegetable crops irrigated with wastewater in Accra, Ghana, *West Afr. J. Appl. Ecol.* 22 (1) (2014) 41–58.
- [21] J. Mariyono, A. Kuntariningsih, T. Kompas, Pesticide use in Indonesian vegetable farming and its determinants, *Manag. Environ. Quality Int. J.* 29 (2018) 305–323.
- [22] S.M. Shadman, et al., Aptamer-based electrochemical biosensors, in: *Electrochemical Biosensors*, Elsevier, 2019, pp. 213–251.
- [23] M. Yadav, R. Gupta, R.K. Sharma, Green and sustainable pathways for wastewater purification, in: *Advances in Water Purification Techniques*, Elsevier, 2019, pp. 355–383.
- [24] D. Santos, et al., Zebrafish Early Life Stages for Toxicological Screening: Insights from Molecular and Biochemical Markers, in: *Advances in Molecular Toxicology*, Elsevier, 2018, pp. 151–179.
- [25] S. Martin, W. Griswold, in: *Human Health Effects of Heavy Metals*. Environmental Science and Technology Briefs for Citizens, vol. 15, 2009, pp. 1–6.
- [26] A. Medynska-Juraszek, et al., The effects of rabbit-manure-derived biochar Co-application with compost on the availability and heavy metal uptake by green leafy vegetables, *Agronomy* 12 (10) (2022) 2552.
- [27] G. Pandey, S. Madhuri, Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veter. Fish. Sci.* 2 (2) (2014) 17–23.
- [28] R. Singh, et al., Heavy metals and living systems: an overview, *Indian J. Pharmacol.* 43 (3) (2011) 246.
- [29] C.M. Hussain, R. Kecili, *Modern Environmental Analysis Techniques for Pollutants*, Elsevier, 2019.
- [30] A. Donkor, et al., Evaluation of trace metals in vegetables sampled from farm and market sites of Accra Metropolis, Ghana, *Int. J. Environ. Stud.* 74 (2) (2017) 315–324.
- [31] S.T. Ametepey, et al., Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis, Ghana, *Int. J. Flow Control* 5 (1) (2018) 1–8.
- [32] European Food Safety Authority, Technical Guide on Metals and Alloys Used in Food Contact Materials and Articles. 2nd Edition. European Committee for Food Contact Materials and Articles (Partial Agreement)(CD-P-MCA) 21 March-29 April, 2022, 2022.
- [33] A.O. Affum, et al., Quality assessment and potential health risk of heavy metals in leafy and non-leafy vegetables irrigated with groundwater and municipal-waste-dominated stream in the Western Region, Ghana, *J Heliyon* 6 (12) (2020), e05829.
- [34] B. Ghasemidehkordi, et al., Arsenic and boron levels in irrigation water, soil, and green leafy vegetables, *Int. J. Veg. Sci.* 24 (2) (2018) 115–121.
- [35] A. Theophilus Atio, W.J.J.o.A. Conrad Atogi-Akwoa, E.R. International, Heavy metal and microbial contaminants of some vegetables irrigated with Goo reservoir water, Navrongo, Ghana, *J. Agr. Ecol. Res. Int.* (2020) 11–24.

- [38] E. Mensah, et al., Influence of human activities and land use on heavy metal concentrations in irrigated vegetables in Ghana and their health implications. *Appropriate Technologies for Environmental Protection in the Developing World*, in: Selected Papers from ERTEP 2007, July 17–19 2007, 2009, pp. 9–14. Ghana, Africa.
- [39] M. Edelstein, M.J.S.H. Ben-Hur, Heavy metals and metalloids: sources, risks and strategies to reduce their accumulation in horticultural crops, *Sci. Hortic.* 234 (2018) 431–444.
- [40] K.O. Omeje, et al., Quantification of heavy metals and pesticide residues in widely consumed Nigerian food crops using atomic absorption spectroscopy (AAS) and gas chromatography (GC), *Toxins* 13 (12) (2021) 870.
- [41] M. Li, et al., Total and bioaccessible heavy metals in cabbage from major producing cities in Southwest China: health risk assessment and cytotoxicity, *RSC Adv.* 11 (20) (2021) 12306–12314.
- [42] T. Eliku, S.J.E.S. Leta, P. Research, Heavy metals bioconcentration from soil to vegetables and appraisal of health risk in Koka and Wonji farms, Ethiopia, *Environ. Sci. Pollut. Control Ser.* 24 (12) (2017) 11807–11815.
- [43] L. Huang, et al., Cadmium uptake from soil and transport by leafy vegetables: a meta-analysis, *Environ. Pollut.* 264 (2020) 114677.
- [44] G.N. Nambafu, Extent of heavy metals contamination in leafy vegetables among peri-urban farmers, *Asi. J. Res. Botany* (2020) 38–46.
- [45] H.R. Gebeyehu, L.D. Bayissa, Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia, *PLoS One* 15 (1) (2020), e0227883.
- [46] L. Mogren, et al., The hurdle approach—A holistic concept for controlling food safety risks associated with pathogenic bacterial contamination of leafy green vegetables. A review, *Front. Microb.* 9 (2018) 1965.
- [47] M.N. Losio, et al., Microbiological survey of raw and ready-to-eat leafy green vegetables marketed in Italy, *Int. J. Food Microbiol.* 210 (2015) 88–91.
- [48] S.A. Mir, et al., Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens, *Food Control* 85 (2018) 235–244.
- [49] S. Sela, S. Manulis-Sasson, What else can we do to mitigate contamination of fresh produce by foodborne pathogens? *Microb. Biotechnol.* 8 (1) (2015) 29.
- [50] B. Machado-Moreira, et al., Microbial contamination of fresh produce: what, where, and how? *Compr. Rev. Food Sci. Food Saf.* 18 (6) (2019) 1727–1750.
- [51] G.A. Santarelli, et al., Assessment of pesticide residues and microbial contamination in raw leafy green vegetables marketed in Italy, *Food Control* 85 (2018) 350–358.
- [52] B.V. Jagannathan, P.P. Vijayakumar, The need for prevention-based food safety programs for fresh produce, *Food Protect. Trends* 39 (6) (2019) 572–579.
- [53] M.I. Gil, et al., Pre-And Postharvest Preventive Measures And Intervention Strategies To Control Microbial Food Safety Hazards Of Fresh Leafy Vegetables, 2015, pp. 453–468, 55(4).
- [54] J.K. Quansah, et al., Pre-and post-harvest Practices of urban leafy green vegetable Farmers in Accra, Ghana and their Association with microbial Quality of vegetables produced, *Agriculture* 10 (1) (2020) 18.
- [55] L. Yafetto, et al., Microbiological contamination of some fresh leafy vegetables sold in Cape Coast, Ghana, *Ghana J. Sci.* 60 (2) (2019) 11–23.
- [56] G. Abakari, S.J. Cobbina, E. Yeleliere, Microbial quality of ready-to-eat vegetable salads vended in the central business district of Tamale, Ghana, *Int. J. Flow Control* (1) (2018) 5.
- [57] K. Abass, J.K. Ganle, E. Adaborna, Coliform contamination of peri-urban grown vegetables and potential public health risks: evidence from Kumasi, Ghana, *J. Community Health* 41 (2) (2016) 392–397.
- [58] P. Antwi-Agyei, et al., A farm to fork risk assessment for the use of wastewater in agriculture in Accra, Ghana, *J PLoS one* 10 (11) (2015), e0142346.
- [59] K. Abass, A.F.S. Owusu, R.M. Gyasi, Market vegetable hygiene practices and health risk perceptions of vegetable sellers in urban Ghana, *Int. J. Environ. Health Res.* 29 (2) (2019) 221–236.
- [60] K. Dankwa, et al., Parasitic profile of fresh vegetables sold in selected markets of the Cape Coast Metropolis in Ghana, *Ann. Res. Rev. Biol.* (2018) 1–7.
- [61] P.K. Amisah-Reynolds, et al., Parasitic contamination in ready-to-eat salads in the Accra metropolis, Ghana, *South Asi. J. Paras.* (2019) 1–11.
- [62] N.B. Doui, et al., Irrigation water quality and its impact on the physicochemical and microbiological contamination of vegetables produced from market gardening: a case of the Veia Irrigation Dam, UER, Ghana, *J. Water* (2021).
- [63] D. Acheampong, et al., Assessing the effectiveness and impact of agricultural water management interventions: the case of small reservoirs in northern Ghana, *J. Agr. Water Manag.* 209 (2018) 163–170.
- [64] N. Thi Van Ha, et al., Bacterial contamination of raw vegetables, vegetable-related water and river water in Ho Chi Minh City, Vietnam, *Water Sci. Technol.* 58 (12) (2008) 2403–2411.
- [65] P. Amoah, et al., Irrigated urban vegetable production in Ghana: microbiological contamination in farms and markets and associated consumer risk groups, *J. Water Health* 5 (3) (2007) 455–466.
- [66] I.D. Amoah, et al., Contribution of wastewater irrigation to soil transmitted helminths infection among vegetable farmers in Kumasi, Ghana, *J. PLoS Neg. Trop. Dis.* 10 (12) (2016), e0005161.
- [67] F.J. Yakubu, et al., Microbial analysis of leafy vegetables in iceless cooling facility. *International Journal of Environment, Agri. Biotechn.* 3 (1) (2018) 239054.
- [68] L. Adetunde, et al., Potential links between irrigation water microbiological quality and fresh vegetables quality in Upper East region of Ghana subsistence farming, *J. Ann. Res. Rev. Biol.* (2015) 347–354.
- [69] A. Abubakari, et al., Presence of pathogenic *E. coli* in ready-to-be-eaten salad food from vendors in the Kumasi Metropolis, Ghana, *Afr. J. Microbiol. Res.* 9 (21) (2015) 1440–1445.
- [70] P. Amoah, P. Drechsel, R.C. Abaidoo, Irrigated urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination, *Irrigat. Drain.* 54 (S1) (2005) S49–S61.
- [72] J. Fung, et al., Microbiological quality of urban-vended salad and its association with gastrointestinal diseases in Kumasi, Ghana. *International Journal of Food Safety, Nutr. Pub. Health* 4 (2–4) (2011) 152–166.
- [73] J.A. Finger, et al., Overview of foodborne disease outbreaks in Brazil from 2000 to 2018, *Foods* 8 (10) (2019) 434.
- [74] K.O. Duedu, et al., A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-air markets in Accra, Ghana, *BMC Res. Notes* 7 (1) (2014) 1–6.
- [76] M. Shammil, et al., Pesticide exposures towards health and environmental hazard in Bangladesh: a case study on farmers' perception, *J. Saudi Soci. Agri. Sci.* 19 (2) (2020) 161–173.
- [78] B.T. Mengistie, A.P.J. Mol, P. Oosterveer, Pesticide use practices among smallholder vegetable farmers in Ethiopian Central Rift Valley, *Environ. Dev. Sustain.* 19 (1) (2017) 301–324.
- [79] J.J. Lu, B. Science, Trending of pesticide residues and consumer's health risk, *Int. J. Chem. Biomol. Sci.* 1 (3) (2015) 85–89.
- [80] L. Wu, et al., Seasonal variation and exposure risk assessment of pesticide residues in vegetables from Xinjiang Uygur Autonomous Region of China during 2010–2014, *J. Food Compos. Anal.* 58 (2017) 1–9.
- [81] W.H.O. FAO, Pesticide Residues in Food 2018-Report 2018-Joint FAO/WHO Meeting on Pesticide Residues, 2018.
- [83] A. Donkor, et al., Pesticide residues in fruits and vegetables in Ghana: a review, *Environ. Sci. Pollut. Res. Int.* 23 (19) (2016) 18966–18987.
- [84] L.A. Thompson, et al., Organochlorine pesticide contamination of foods in Africa: incidence and public health significance, *J. Vet. Med. Sci.* (2017) 16–214.
- [85] V.K. Bolor, et al., Human risk assessment of organochlorine pesticide residues in vegetables from Kumasi, Ghana, *J. Chem.* 2018 (2018) 1–11.
- [86] B. Dinham, Growing vegetables in developing countries for local urban populations and export markets: problems confronting small-scale producers, *Pest Manag. Sci.* 59 (5) (2003) 575–582.
- [88] C.K. Bempah, et al., Dietary exposure to chlorinated pesticide residues in fruits and vegetables from Ghanaian markets, *J. Food Comp.* 46 (2016) 103–113.
- [89] G. Blankson, et al., Contamination levels of organophosphorus and synthetic pyrethroid pesticides in vegetables marketed in Accra, Ghana, *Food Control* 68 (2016) 174–180.
- [90] O. Akoto, et al., Estimation of human health risk associated with the consumption of pesticide-contaminated vegetables from Kumasi, Ghana, *Environ. Monit. Assess.* 187 (5) (2015) 244.

- [91] P.O. Fosu, et al., Surveillance of pesticide residues in fruits and vegetables from Accra Metropolis markets, Ghana, 2010–2012: a case study in sub-Saharan Africa, *Environ. Sci. Poll. Res.* 24 (20) (2017) 17187–17205.
- [92] S. Akomea-Frempong, et al., Health risks due to consumption of pesticides in ready-to-eat vegetables (salads) in Kumasi, Ghana, *Int. J. Food Control* 4 (1) (2017) 1–11.
- [93] P. Amoah, et al., Pesticide and pathogen contamination of vegetables in Ghana's urban markets, *Arch. Environ. Contam. Toxicol.* 50 (2006) 1–6.
- [94] C.E. Handford, et al., A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards, *Integrated Environ. Assess. Manag.* 11 (4) (2015) 525–536.
- [95] H. Natesh, L. Abbey, S.J.H.L.J. Asiedu, An overview of nutritional and antinutritional factors in green leafy vegetables, *Horticult. Int. J.* 1 (2) (2017) 11.
- [96] N. Petry, et al., Risk factors for anaemia among Ghanaian women and children vary by population group and climate zone, *Matern. Child Nutr.* (2020), e13076.
- [97] E. Gogo, et al., Postharvest UV-C treatment for extending shelf life and improving nutritional quality of African indigenous leafy vegetables, *Postharvest Biol. Technol.* 129 (2017) 107–117.
- [98] A.O. Adeleye, M.B. Sosan, J.A.O.J.H. Oyekunle, Dietary exposure assessment of organochlorine pesticides in two commonly grown leafy vegetables in South-western Nigeria, *J. Heliyon* 5 (6) (2019), e01895.
- [100] J. Lemos, et al., Risk assessment of exposure to pesticides through dietary intake of vegetables typical of the Mediterranean diet in the Basque Country, *J. Food Comp.* 49 (2016) 35–41.
- [101] D.W. Park, et al., Pesticide residues in leafy vegetables, stalk and stem vegetables from South Korea: a long-term study on safety and health risk assessment, *Food Addit. Contam.* 33 (1) (2016) 105–118.
- [102] T. Eliku, S.J.B.o.e.c. Leta, and toxicology, Assessment of heavy metal contamination in vegetables grown using paper mill wastewater in Wonji Gefersa, Ethiopia, *Bull. Environ. Contam. Toxicol.* 97 (5) (2016) 714–720.
- [103] O. Otitaju, et al., Heavy metal contamination of green leafy vegetable Gardens in Itam Road Construction site in Uyo, Nigeria, *Res. J. Environ. Earth Sci.* 4 (4) (2012) 371–375.
- [104] N. Gupta, et al., Evaluating heavy metals contamination in soil and vegetables in the region of North India: levels, transfer and potential human health risk analysis, *Environ. Toxicol. Pharmacol.* 82 (2021) 103563.
- [105] T. Bhilwadikar, et al., Decontamination of microorganisms and pesticides from fresh fruits and vegetables: a comprehensive review from common household processes to modern techniques, *J. Compreh. Rev. Food Sci. Food Saf.* 18 (4) (2019) 1003–1038.
- [106] S. Pounraj, et al., Effect of ozone, lactic acid and combination treatments on the control of microbial and pesticide contaminants of fresh vegetables, *J. Sci. Food Agric.* 101 (8) (2021) 3422–3428.
- [107] S. Mukhopadhyay, et al., Effects of pulsed light and sanitizer wash combination on inactivation of *Escherichia coli* O157: H7, microbial loads and apparent quality of spinach leaves, *Food Microbiol.* 82 (2019) 127–134.
- [108] A. Serper, S. Çalt, The demineralizing effects of EDTA at different concentrations and pH, *J. Endod.* 28 (7) (2002) 501–502.
- [109] P. Klintham, et al., Combination of microbubbles with oxidizing sanitizers to eliminate *Escherichia coli* and *Salmonella Typhimurium* on Thai leafy vegetables, *Food Control* 77 (2017) 260–269.
- [110] R.M. Balal, et al., Kinnow Mandarin plants grafted on tetraploid rootstocks are more tolerant to Cr-toxicity than those grafted on its diploids one, *J. Environ. Exper. Botany* 140 (2017) 8–18.
- [111] A.G. Assunção, et al., Arabidopsis thaliana transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency, *J. Proceed. Nat. Acad. Sci.* 107 (22) (2010) 10296–10301.
- [112] C.S. Seth, A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up, *Bot. Rev.* 78 (1) (2012) 32–62.
- [114] J. Chen, et al., Short-term enhancement effect of nitrogen addition on microbial degradation and plant uptake of polybrominated diphenyl ethers (PBDEs) in contaminated mangrove soil, *J. Hazard Mater.* 300 (2015) 84–92.
- [115] S. Di Gregorio, et al., Phytomediated biostimulation of the autochthonous bacterial community for the acceleration of the depletion of polycyclic aromatic hydrocarbons in contaminated sediments, *BioMed Res. Int.* (2014) 2014.
- [116] S.H. Elias, et al., Water hyacinth bioremediation for ceramic industry wastewater treatment-application of rhizofiltration system, *Sains Malays.* 43 (9) (2014) 1397–1403.
- [117] J. Mesa, et al., Moving closer towards restoration of contaminated estuaries: bioaugmentation with autochthonous rhizobacteria improves metal rhizoaccumulation in native *Spartina maritima*, *J. Hazard Mater.* 300 (2015) 263–271.
- [118] C.S. Lin, et al., Evaluation of electrolysed water as an agent for reducing methamidophos and dimethoate concentrations in vegetables, *Int. J. Food Sci. Technol.* 41 (9) (2006) 1099–1104.
- [119] E.O. Dos Santos, et al., Sand as a solid support in ultrasound-assisted MSPD: a simple, green and low-cost method for multiresidue pesticide determination in fruits and vegetables, *J. Food Chem.* 297 (2019) 124926.
- [120] S.R. Azam, et al., Efficacy of ultrasound treatment in the removal of pesticide residues from fresh vegetables: a review, *J. Trend. Food Sci.* 97 (2020) 417–432.
- [122] P. Klinthom, A. Halee, S. Methawiwat, The effectiveness of household chemicals in residue removal of methomyl and carbaryl pesticides on Chinese-kale, *Agri. Nat. Res.* 42 (5) (2008) 136–143.
- [124] H. Qi, Q. Huang, Y.-C.J.F.c. Hung, Effectiveness of electrolyzed oxidizing water treatment in removing pesticide residues and its effect on produce quality, *Food Chem.* 239 (2018) 561–568.