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Does Circularizing Source-Separated Food Waste Present A Risk To Our Food?

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About a third of the food produced annually is wasted. Food waste recycling can be a way to close the loop and attain a more sustainable food system, however the system must be carefully monitored and managed to avoid the introduction and build-up of contaminants. To study the potential presence of contaminants in food waste, source-separated food waste was collected and screened for five classes of contaminants (physical contaminants, heavy metals, halogenated organic contaminants, pathogens and antibiotic resistance genes) from two separate regulatory environments (voluntary vs mandated food separation). The regulatory environment did not affect the level of contamination, except there was more physical contamination in Maine, where food waste diversion is not mandated. Fifty-seven percent of samples had some form of non-compostable waste. Most of the heavy metals tested were not detected. Copper and zinc were detected in most samples but were always below the most stringent global standards for compost. Some samples had detectable halogenated organics, which is cause for concern because some are known to accumulate in the food chain. Foodborne pathogens were seldom detected and should be killed during treatment, but this could pose a risk to collectors and haulers. Antibiotic resistance genes were detected in most samples. This could jeopardize the utility of antibiotics used to fight infections. More research is needed to determine the fate of antibiotic resistance

genes and halogenated organics during treatment, and the risk of their accumulation in a circular food system.

DEDICATION

To my family: my parents, my brother and my cousins.

Your love and support have always helped me to become better and stronger.

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1 INTRODUCTION

1.1 Background

About a third of the food produced globally goes to waste each year (Gustavsson, Cederberg, Sonesson, Otterdijk, & Meybeck, 2011). This loss is fundamentally unsustainable due to the inherent consumption of water, energy and materials during the production, processing, storage and transport of food that is not productively used. Food waste would best be recycled and reused in a way that minimizes the environmental burden and preferably allows for recovery of part of the resources initially used in its production, processing and transport (Pleissner, 2018). Food loss is defined as the decrease in edible food mass through the production, post-harvest and processing stages of the food system. Food waste is similar to food loss, but it occurs at the retail and consumer stage, and is related to retailers' and consumers' behavior (Gustavsson et al., 2011)

Food loss generated during production includes damaged products left in the field and good products with low or no commercial value that are not harvested. Food processing and manufacturing units produce food loss due to reasons such as damage during transport, spoilage or contamination during storage, and loses during processing. The retail system also generates food waste for reasons such as problems in conserving food products for a long period of time, handling, and lack of cold storage. Food waste generated at the consumer level is due to over-purchasing, bad storage, over-preparation, portioning, and cooking, as well as confusion between terms "best by" and "use by" dates (Girotto, Alibardi, & Cossu, 2015).

Food waste can comprise of edible and inedible waste. The most common food waste management practice is landfilling, which is not beneficial from human health and environmental protection viewpoints due to the production and uncontrolled release of methane, which is a powerful greenhouse gas. The food waste management hierarchy puts reuse as the preferred

option if food is still qualitatively good enough for human consumption (Garcia-Garcia et al., 2017; Pleissner, 2018). Reuse by diverting unwanted or excess food to hungry people achieves the primary goal of the food system: to feed people. If not fit for human consumption, the next best option is to feed animals. There will always be some portion that cannot be guaranteed safe from a biosafety perspective or cannot be economically transported to where it is needed. The next level on the hierarchy is recycling, which is an appropriate option for inedible food scraps. In food scrap recycling, the nutrient and perhaps some of the energy content of the waste material can be recovered through composting or anerobic digestion, which stabilize the material, reduce the pathogen content and produce a product that provides organic matter and nutrients needed to replenish the soil. In the case of anaerobic digestion, energy can also be recovered in the form of methane gas. The focus of this thesis is on food waste recycling- the recovery of nutrients from food waste to be used as fertilizer for further food production. Replenishing the soil with nutrients recovered in the form of food is needed to create a sustainable, circular food system (Garcia-Garcia et al., 2017; Pleissner, 2018).

Recycling organic waste, especially that derived from municipal, agricultural and agro-industrial sources, is recommended as a means of approaching sustainability (Tella et al., 2013). Recycling food waste helps to recycle nutrients back to the soil and to produce more food. This practice contributes to a circular food system with no waste outputs and fewer inputs to food production systems. Anaerobic digestion (AD) and composting are two broadly applicable technologies for organic waste management (Lin, Xu, Ge, & Li, 2018). AD is an anaerobic biological process that converts organic waste into biogas which contains the fuel methane, and a stable digestate containing plant nutrients and organic matter. Composting is an aerobic biological process that decomposes the easily degraded organic components of the waste and produces a soil amendment that slowly releases plant nutrients and improves the water-holding capacity and texture of soil. Both digestate and compost are nutrient-rich mixtures and can be

used as organic amendments to soil to reduce the need for fertilizer addition and improve crop yield (Lin et al., 2018).

In 2017, about 41 million tons of food waste were generated in the US, of which only 6.3% was diverted for composting (U.S. EPA, 2019). Food waste in landfills has the potential to emit the greenhouse gases methane and carbon dioxide (Morone, Koutinas, Gathergood, Arshadi, & Matharu, 2019). Food waste is associated with direct and indirect environmental impacts such as soil erosion, deforestation, water and air pollution, and greenhouse gas emissions. Sustainable food waste management plans and policies are therefore needed (Schanes, Dobernig, & Gözet, 2018). Many states and cities have prioritized food waste management, adopting ambitious programs to eliminate food waste completely. Governments at different levels are coming up with policies and plans to reduce and recycle food waste to avoid sending it to landfill. Such plans and policies may use mandatory or voluntary approaches (Sandson & Leib, 2019). The New England region in the US has been aggressive on the goal of diverting food waste from landfill. Four out of six states in New England have mandatory landfill bans on food waste. Vermont has the nation's most ambitious plan to divert all food waste, including residential, starting in 2020 (Sandson & Leib, 2019). Under the Universal Recycling Law, Vermont has added food waste to the list of materials that cannot be "knowingly" landfilled. The ban started with large generators in 2014 and has progressed to smaller individual generators, and will complete implementation to include residential customers by 2020 (Sandson & Leib, 2019). Maine, another New England state, does not have a mandatory food waste separation and landfill ban. However, subscription services to collect and haul food waste to recycling facilities are available in some parts if the state, and there are several drop-off locations available for residents to divert food waste voluntarily.

Diverting food waste to AD and composting provides a mechanism to recover nutrients and circularize the food production system, but the approach will only be feasible and safe as long as the food waste is uncontaminated with other waste materials and toxicants. Compost products and digestates can be contaminated with potentially toxic elements, pathogens, organic xenobiotics and antibiotic-resistant genes if the input materials are contaminated (Bloem et al., 2017; Bonetta et al., 2014; Fijalkowski, Rorat, Grobelak, & Kacprzak, 2017; Hargreaves, Adl, & Warman, 2008). In order to make a sustainable circular food system, evaluation of policies to encourage food waste diversion and recycling is needed to avoid creation of other unanticipated problems, like increasing the load of heavy metals, pathogens and organic pollutants in our food through application of organic waste residuals to agricultural soils (Cerda et al., 2018; Knapp, Allesch, Müller, & Bockreis, 2017). The policies and plans should be such that they help in generating valuable secondary (recycled) products with contaminant levels below the limits that are deemed safe.

There are points of potential contamination at every stage of the food system, from production to processing, transportation, retail and consumption. Food waste contaminants can include environmental contaminants, food processing contaminants, unapproved adulterants and food additives, household and industrial contaminants from poor source separation, and contaminants that migrate into the food (Nerín, Aznar, & Carrizo, 2016; Rather, Koh, Paek, & Lim, 2017; Stephen R Smith, 2009). Many researchers have reported high heavy metal content, persistent organic pollutants (especially pesticide residues), antibiotic-resistant genes, food-borne pathogens, and other contaminants in different foods around the world. In addition to trace contaminants, food waste may be contaminated with materials associated with food consumption like plastics, toothpicks, papers, paper towels and so on. (Chu, Fan, Wang, & Huang, 2019). Collection and sorting systems greatly influence food waste quality. Inorganic and organic contaminants present in the food waste or introduced during processing determine the impurity

level at the end of these processes (Cerda et al., 2018). When contaminated food enters as feedstock for composting or anaerobic digestion, it increases the probability of contaminating the entire food system, as the end products are used as organic amendments in soil used for the production of more food and can be taken up by the plants (Cerda et al., 2018; R. M. Clarke & Cummins, 2015; Miller, Heringa, Kim, & Jiang, 2013)

It is hard to estimate and evaluate the environmental consequences of waste management initiatives without prior knowledge of the physio-chemical and biological characteristics of the input materials (Götze, Boldrin, Scheutz, & Astrup, 2016). Waste materials are variable over both space and time. Furthermore, sampling is challenging, labor intensive and costly (Götze et al., 2016). This research was conducted to determine the physical, chemical and biological characteristics of source-separated food waste intended as feedstocks for composting and anaerobic digestion in New England. Food waste was screened for different classes of contaminants including heavy metals (Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Selenium (Sn), Titanium (Ti) and Zinc (Zn)), extractable organic halides (EOX), Perfluoroalkyl substances (PFAS), foodborne pathogens (shiga toxin-producing *Escherichia coli* (STEC), non-typhoidal *Salmonella* and *Listeria monocytogenes*) and antibiotic resistance genes (ARGs) (tetracycline resistance (*tet*(M)), beta-lactamase gene (bla_{TEM}) and colistin resistance (*mcr-1*) gene). . This research is first of its kind to monitor all the possible four contaminants in the source -separated food waste.

1.2 Objective

The main objective of this research were:

- To screen for different classes of possible contaminants in food waste meant for recycling (composting and anaerobic digestion).

- To compile literature on contamination along the food system to identify possible pathways for the contaminants to enter the food system.
- To evaluate the effect of regulatory environment (mandatory landfill ban vs voluntary recycling scheme) on the level of contamination in source-separated food waste.
- To assess the effect of source type on levels of contamination.

1.3 Thesis Outline

This research is summarized in five chapters including this introduction (Chapter 1).

Chapter 2 presents a literature review on the studied classes of contaminants, discusses the possible pathways for the entry of those contaminants into our food system, and gives a range of concentration of those contaminants observed worldwide.

Chapter 3 describes the methods used to collect, process and analyze source-separated food waste samples.

Chapter 4 presents the results obtained in the food waste contamination survey, and discussion about how the results compared with the existing data in the literature, as well as implications of the results.

Chapter 5: provides conclusions derived from the analysis of the results and comparison to existing work, and recommendations for future work in this field.

2 LITERATURE REVIEW

Contamination of the food system can come from a variety of sources and practices along the supply chain from production, to processing, to transport and storage, to consumption and source handling of the wastes. The likelihood of contamination depends on the kind of contaminants, the opportunity and likelihood of exposure, and the characteristics of the food itself, which determines whether contaminants will partition into it. The following literature review summarizes the characteristics of heavy metals, organo-halogenated compounds, antibiotic resistance genes and pathogens and compiles reports of their concentrations in food at various stages of the food system.

2.1 Heavy Metals

Heavy metals are natural elements having density greater than 4.5 g/cm^3 (Logan, Henry, Schnoor, Overcash, & McAvoy, 1999). Some heavy metals are essential micronutrients with beneficial impacts on growth as long as their concentrations remain low, however at high concentrations, heavy metals are toxic to plants, animals and human beings (C. Garcia, n.d.; Epstein, Chaney, Henrys, & Locans, 1992).

Heavy metals are widely distributed in water, soil and air. Continuous urban runoff, agricultural runoff, wastewater discharge from cities and industries, and air emissions and subsequent deposition from the combustion of fossil fuels, cement production, mining, extractive metallurgy, and pulp and paper production are some of the sources which increase heavy metal levels in aquatic media (Hossain, Ahmed, & Sarker, 2018; Zuliani et al., 2019).Once heavy metals get into an aquatic environment, they are redistributed throughout the water column, deposited or accumulated in sediments, and are consumed by aquatic biota (Makedonski, Peycheva, & Stancheva, 2017).

Likewise, in the case of fresh produce, once contaminants are introduced into environmental media, they can be absorbed and accumulated in edible and non-edible plant tissues during growth. Plants can take them up through atmospheric foliar deposition (Margenat et al., 2018). Vegetables with expanded leafy surface areas are susceptible to dust and air particle accumulation. Heavy metal uptake and translocation to various plant tissues is dependent on plant species, absorption, retention, plant morphology and physiology (Khan, Malik, Muhammad, Ullah, & Qadir, 2015). Cadmium (Cd) is highly mobile, poorly adsorbed to soil and phytoavailable, and therefore often detected in the aerial parts of plants (Hajeb, Sloth, Shakibazadeh, Mahyudin, & Afsah-Hejri, 2014). Higher bioaccumulation factors (BCFs) have been observed for leafy vegetables than other kinds of plants in many studies suggesting a strong ability to accumulate metals from soil (Dziubanek, Piekut, Rusin, Baranowska, & Hajok, 2015; Lian et al., 2019). Although lead (Pb) has low transfer coefficients and is strongly bound to soil colloids, human lead dietary intake primarily occurs through food originating from plants (Hajeb et al., 2014). Table 2.1 shows the range of heavy metals detected in food obtained in Food and Drug Administration (USFDA) study that included samples from all parts of the country during each season and from supermarkets, grocery stores and fast food restaurants in 2017. Baked cod, pan cooked ground beef, pan cooked liver (beef/calf), and canned tuna were the foods with the highest heavy metal concentrations.

Table 2.1: Range of heavy metals in US food

Metals	Concentration (mg/kg)
Arsenic (As)	0-4.23
Cadmium (Cd)	0-0.477
Chromium (Cr)	0-0.959
Copper (Cu)	0-161
Mercury (Hg)	0-0.062
Nickel (Ni)	0-5.4
Lead (Pb)	0-0.036

Table 2.1 continued

Zinc (Zn)	0-216

(U.S. Food and Drug Administration, 2019)

2.1.1 Sources of Heavy Metals in Food

Sources of heavy metals in the production stage include aerial deposition, irrigation with contaminated water, which includes discharge from wastewater treatment plants, industrial and road runoff into the field, food collected from areas with a high amount of phosphorus fertilizers, and proximity to industrial areas (Dziubanek et al., 2015; Khan et al., 2015; Lian et al., 2019; Margenat et al., 2018; McBride et al., 2014; Zuliani et al., 2019).

Sources of heavy metals in meat include metals introduced in feed, drinking water, and mineral supplements used in excess of recommended limits (Abbas et al., 2019; Hajeb et al., 2014; Hu, Zhang, Chen, Cheng, & Tao, 2018). Higher levels of Pb and Cd that could not be explained by the amounts in feed were seen in a study by Abbas et al. (2019). This demonstrates an exterior source, such as the water used for irrigating crops or forage consumed by the livestock; or the presence of phosphorus-containing minerals supplied as feed ingredients. Milk from industrial areas had higher heavy metal concentrations than milk from non-industrial areas due to environmental factors (Zhou, Zheng, Su, & Wang, 2019).

Heavy metals can also migrate into food from packaging materials (Filippinia et al., 2019). Canned food is especially prone to migration of tin, depending on the food pH, storage time, the temperature of the canned foods, exposure to air of opened canned food, corrosion of the can, and poor lacquering (Filippinia et al., 2019; Ikem & Egiebor, 2005). Packaging materials produced with recycled materials can also contribute to heavy metal contamination and migration (Whitt, Brown, Danes, & Vorst, 2016). Cooking processes like frying, grilling, boiling, etc. and precooking treatments such as the peeling of vegetables can influence heavy metal levels

positively or negatively (Hadayat et al., 2018; Hajeb et al., 2014; Perelló, Martí-Cid, Llobet, & Domingo, 2008).

The range of concentrations of heavy metals measured in different types of food in the world is shown in the Table 2.3. The table captures data at production, processing and retail stages. From the table we can see that heavy metals have been detected in all the stages of food supply and in almost all kinds of food that are commonly consumed. Because we do not have a universal data reporting method, some of the data were reported on a wet weight basis, whereas some were in dry and a few did not specify. Regardless of the reporting weight basis, the highest concentration of Pb (38.8 mg/kg), Cd (3.9 mg/kg) and As (52.48 mg/kg ww) were seen in the samples taken from the retail stage of the food system. Likewise, Hg (6.605 mg/kg ww), Cr (6.55 mg/kg ww), Zn (182 mg/kg ww), Ni (24.65 mg/kg ww) and Cu (224.3 mg/kg ww) were highest in samples taken from the production stage. Tin was highest in canned food. The most contaminated samples were taken from highly contaminated areas like areas impacted by illegal hazardous waste dumping, mining activities, industrial emissions, irrigation with contaminated wastewater or use of metal-based fertilizers and pesticides.

The most stringent regulatory limits for contaminants in compost, and US EPA regulatory limits for land application of biosolids are given in Table 2.2. In the US there are no federally mandated regulatory limits for heavy metals, however many states have developed their own heavy metal limits in compost. We have tried to compare the heavy metal content in food with the stringent compost regulatory limits. When the values were reported in wet weight, we assumed a moisture content of 74%, the average of our food waste samples (see Table 4.2), to convert to a presumed dry weight basis. If wet or dry weight basis was not stated, we assumed they were reported on a dry weight basis and did not adjust the values. The values that are bold in the table exceeded the stringent regulatory limits.

Table 2.2: Regulatory levels of heavy metals in compost

Heavy metals	US EPA CFR 40/503 Sludge Rule (mg/kg dw)	Limits (mg/kg dw)	Country
Mercury (Hg)	17	0.2	Netherland
Lead (Pb)	300	45	Austria and UK
Cadmium (Cd)	39	0.7	Austria and Netherland
Chromium (Cr)	1200	50	Netherland
Copper (Cu)	1500	25	Netherland
Nickel (Ni)	420	10	Netherland
Arsenic (As)	41	13	Canada
Zinc (Zn)	2800	75	Netherland

(Amlinger et al., 2004)

Table 2.3: Range of heavy metals detected in foods at different stages of food supply chain

Country	Food (unit, weight)	Concentration	Author
		Production	
USA	Vegetables (mg/kg, ww)	Pb[1]: 0.0023-2.1, Cd[2]: 0.0021-**0.36**	A
USA	Vegetables (mg/kg, ww)	Pb: <LOD-0.180, Cd: <LOD-0.133	B
USA	Fish (mg/kg, ww)	Hg[3]: 0.005-**6.605**	C
Bangladesh	Fish (mg/kg ww)	As[4]: 0.001-0.002, Cr: 0-0.01, Hg: 0.004-0.007	D
China	Vegetable (mg/kg, ww)	Cd:0.01-**0.66**, Pb: 0.01-1.53, Hg: 0.001-0.043, Zn[5]: 3.14-**58.85**	E
China	Milk (µg/L, ww)	Pb: 0.03-10.46, As: 0.002-**5.01**, Cr[6]: 0.02-5.01, Cd: 0.01-**0.27**	F

Table 2.3 continued

Ghana	Meat (mg/kg, ww)	Cr: nd-0.28, Ni[7]: nd-0.68, Cu[8]: 0.28-**224.3**, Zn: 3.8-**182.2**, As: nd-0.56, Cd: nd-**0.56**, Hg: nd-**0.25**, Pb: nd-0.41	G
India	Chicken and eggs (mg/kg, ww)	Cr: 0.16-1.63, Cu: 0.77-**48.79**, Mn[9]: 0.36-4.67, Ni: 0.13-**2.86**, Pb: 0.01-2, Se[10]: 0.14-1.52, Zn: 6.58-**72.79**	H
Italy	Fresh produce (mg/kg, ww)	As: 0.0005-0.4590, Cd: 0.0005-0**.2150**, Cr: 0.0005-6.5490, Cu: 0.0005-**77.8690**, Hg: 0.0005-**1.4900**, Ni: 0.0005-**24.6520**, Pb: 0.0005-**24.6520**, Sn: 0.0005-8.4450, Ti[13]: 0.0005-7.3440, Zn: 0.0005-**162.5650**	I
Pakistan	Chicken meat (mg/kg, dw)	Cd: 0.016-0.030, Pb: 0.14-0.35, Ni: 0.34-0.59, Zn: 2.72-4.98	J
Philippines	Vegetables (mg/kg)	Cd: 0.05904-**0.69678**, Pb: 0.09680-0.49208	K
Poland	Vegetables (mg/kg, ww)	Pb: <0.0400-3.88, Cd: <0.0600-**1.70**	L

Table 2.3 continued

Spain	Lettuce (mg/kg, ww)	Pb:0.03-0.45, Cd: 0.004-0.04, Ni: 0.04-0.61, Cu: 0.40-0.96, Zn: 1-3.41, Hg: 0.000301-0.00167, As: 0.0000575-0.00230	M
Slovenia	Fish (mg/kg, dw)	As: 0.100-0.775, Cd: 0.0525-0.112, Cr: 0.035-0.316, Pb: nd-0.547, Hg: 0.0762-**5.12**, Zn: 18.6-43.8, Cu: 0.58-6.45, MeHg[11]: 0.071-4.09	N
USA	Canned fish (mg/kg, ww)	Hg: 0.02-**0.74**, Ag: 0-0.20 As: 0-1.72 Cd: 0-0.05 Cr: 0-0.30 Pb: 0-0.03 Mn: 0.01-2.55 Ni: 0-0.78 Co: 0-0.10 Cu: 0.01-**20.5** Sn: 0.04-28.7 Vn: 0-0.31 Zn: 0.14-**97.8**	O
Italy	Canned food, median value (mg/kg)	Sn[12]: 0-0.017	P

Table 2.3 continued

Spain	Cooking effects (mg/kg)	Before Cooking: As: 0.050-2.086, Cd: Nd-0.007, Hg: Nd-**0.355**, Pb: nd- 0.084 after cooking: As: 0.092-3.281, Cd: Nd-0.012, Hg: Nd- **0.421**, Pb: Nd-0.060	Q
		Retail	
USA	Fish (mg/kg, ww)	As: 0.23-3.3, Cd: 0.00013-0.02, Cr: 0.03-0.34, Pb: 0.04-0.34, Hg: 0.01-**0.65**	R
USA	Vegetables (mg/kg)	Cu: 0.6-**30**, Pb: 4.1-**27**, Zn: 1.7-**65**, Cr: <0.1, Cd: <0.1, Ni: <0.04	S
USA	Vegetables (mg/kg, ww)	As: 0.00124-0.020, Cd: 0.00062-0.057, Pb: 0.0005-0.065, Ni: 0.005-0.217, Cu: 0.127-2.654, Zn: 1.125-3.880	T
USA	Produce (mg/kg, ww)	Pb: <LOD-0.057, Cd: <LOD-0.051	U

Table 2.3 continued

Bulgaria	Fish (fillet mean concentration mg/kg, ww)	As: 0.38-1.1, Cd: <0.010-0.015, Pb: <0.06-0.08, Hg: 0.05-0.16, Zn: 5.2-11, Cu: 0.34-1.4	V
China	Vegetables (mg/kg, ww)	Cd: <LOD-**1.9900**, Pb: <LOD-3.0500, As: <LOD-0.5200, Hg: <LOD-**0.4860**, Cr: <LOD-0.8300	W
China	Foodstuffs derived from animals (mg/kg, ww)	Pb: 0.035-0.055, Cd: 0.0004-**0.352**, Hg: nd-0.037, As: 0.0036-1.8	X
Italy	Seafood (mg/kg, ww)	As: 5.35-**52.48**, Cd: <0.01-0.14, Pb: <0.001-0.21, Hg: 0.04-**0.84**	Y
Pakistan	Vegetables (mg/kg)	Cr0.8-5.2, Ni: 1.1-7, Pb: 7.3-38.8, Cd: 0.11-**3.9**, Cu: 0.1-3.9, Zn: 2.9-27.5	Z
Portugal	Different food (mg/kg, ww)	As: 0.003-**16.70**, Cd: <LOD-**0.30810**, Pb: 0.00371-0.19218,	A1
Romania	Pork (mg/kg)	Pb: 0.35-1.06, Cd: 0.04-0.24, Cu: 0.65-1.55, Zn: 23.1-55.9	A2

Table 2.3 continued

Taiwan	Livestock meat (mg/kg)	As: <0.002-0.075, Cd: <0.002-0.103, Pb: <0.002-0.321	A3
Turkey	Fish (mean concentration) (mg/kg, ww)	Cd: 0.010-**1.122**, Pb: 0.019-0.822, Hg: 0.0074-**1.75**, Cu: 0.234-1.890	A4

1: Lead, 2: Cadmium, 3: Mercury, 4: Arsenic, 5: Zinc, 6: Chromium, 7: Nickel, 8: Copper, 9: Manganese, 10: Selenium, 11: Methylmercury, 12: Tin and 13: Titanium

A= (McBride et al., 2014), B= (Kohrman & Chamberlain, 2014), C= (USEPA, 2009), D= (Hossain et al., 2018), E= (Lian et al., 2019), F= (Zhou et al., 2019), G= (Bortey-sam et al., 2015), H= (Girihttps & Singh, 2019), I= (Esposito et al., 2018), J= (Abbas et al., 2019), K= (Palisoc, Natividad, Jesus, & Carlos, 2018), L= (Dziubanek et al., 2015), M= (Margenat et al., 2018), N= (Zuliani et al., 2019), O= (De Mello Lazarini, Milani, Yamashita, Saron, & Morgano, 2019), P= (Filippinia et al., 2019), Q= (Perelló et al., 2008), R= (Burger & Gochfeld, 2005), S= (F. Mehari, Greene, L. Duncan, & Olawale Fakayode, 2015), T= (Hadayat et al., 2018), U= (Kohrman & Chamberlain, 2014), V= (Makedonski et al., 2017), W= (Liang et al., 2018), X= (Wu et al., 2016), Y= (Traina et al., 2019), Z= (Khan et al., 2015), A1= (Ventura et al., 2018), A2= (Hoha, Costăchescu, Leahu, & Păsărin, 2014), A3= (Chen, Lin, Kao, & Shih, 2013) and A4= (Keskin et al., 2007).

2.2 Halogenated Compounds

Organohalogenated contaminants are usually synthetic organic chemicals with one or more halogens (chlorine, bromine, iodine and fluorine) substituted for hydrogens in the molecule. They are highly persistent in the environment, are mostly lipophilic and often bioaccumulation potential. Many halogenated compounds are classified as persistent organic pollutants or POPs. POPs are resistant to environmental degradation (chemical, biological and photolytic) and therefore have a long half-life (Alharbi, Basheer, Khattab, & Ali, 2018; Jones & Voogt, 1999). POPs tend to be hydrophobic and partition strongly to the solid matrix (organic matter) in the aquatic and soil environment. Also, they tend to be lipophilic and partition into lipids in organisms, which slows their metabolism in organisms, resulting in accumulation in food chain (Jones & Voogt, 1999). Many are volatile or semi-volatile, and migrate from soils, vegetation, and aquatic bodies into the atmosphere. Volatilization enables them to travel long distances and deposit far from the source (Jones & Voogt, 1999). Examples of POPs include pesticides,

industrial chemicals, and by-products of industrial processes that are semi-volatile and toxic, and many are halogenated. Organochlorine pesticides (OCPs), Polybrominated diphenyl ethers (PBDEs), Hexabromocyclododecane (HBCD), and organophosphate esters (OPEs) are some of the classes of organic halides that are persistent and ubiquitously present in the environment (Pedro et al., 2018; Schecter, Colacino, et al., 2010; Schecter, Haffner, et al., 2010; Wang & Kannan, 2018). POPs consist of many chemicals associated with endocrine disruption and other toxic impacts, and some are known or suspected carcinogens (Jones & Voogt, 1999).

Poly and per fluorinated alkyl substances (PFASs) are a family of molecules consisting of linear or branched carbon chains and can be fully or partially fluorinated. Fluoroalkyl moieties have high thermal, chemical and biochemical stability due to the larger size of the fluorine atom compared to hydrogen, and the strength of the carbon-fluorine bond (Ghisi, Vamerali, & Manzetti, 2019). They have the unique property of repelling both water and oil, so they have become popular in paper coatings and packaging; as surface protection products used on carpet and clothing to resist stains and water; as nonstick coatings on cookware; as industrial surfactants; and in the manufacture of fire-resistant foams (Fair et al., 2019; Schecter, Colacino, et al., 2010). PFASs have affinity to serum albumin and fatty acid binding protein and some show a bioaccumulation potential (Ahrens & Bundschuh, 2014; Haukas, Berger, Hop, Gullikesen, & Gabrielsen, 2007).

More than 90% of organochlorine compound exposure occurs through food, mainly of animal origin (Ábalos et al., 2019; Fair et al., 2018; Ferrante et al., 2017; Schecter, Colacino, et al., 2010). One study on fish has revealed higher PBDE (0.075-4806 ng/g ww) in US fish samples than in European (<LOD-353 ng/g ww) and Asian countries (0.03-1726 ng/g ww) (Eljarrat & Barceló, 2018; Schecter, Haffner, et al., 2010). PFASs are also present in fish sampled in the US: perfluorooctanesulfonic acid (PFOS) in US fish ranged between 4.8-147 ng/g ww, and

perfluorooctanoic acid (PFOA) ranged between <0.2- <36 ng/g ww between 1980-2005 (D'Hollander, Voogt, Coen, & Bervoets, 2010). These data show that fish in the US are highly contaminated with POPs and fish is an important protein source in the food system.

In the 2017 pesticide monitoring program, the USFDA analyzed 6,069 foods consumed by humans (1,799 domestic and 4,270 imported foods). They found that 96.2% of domestic food and 89.6% of imported food samples were compliant with federal standards. No pesticides were detected in 52.5% and 50% of the domestic and imported samples respectively (U.S. Food and Drug Administration, 2017). By food group, 1.6% of the grain products (N=252), 1.8% of fruits (N=387), 9.4% of vegetables (N=563) and 2.7% of the other foods (N=147) had pesticide residues above regulatory limits in domestic food; and 14.1% of grain samples (N=601), 0.5% of fish samples (N=217), 7.9% of fruits (N=1198), 12.5% of vegetables (N=1819) and 8.2% of other foods (N=429) were above the limits among the imported food samples (U.S. Food and Drug Administration, 2017). The list of foods with concentrations above the regulatory limit for four persistent pesticides is shown in Table 2.4.

Table 2.4: Foods containing pesticide residues above regulatory limits

Pesticide	Food
Dieldrin	Squash
DDT	Cilantro, lettuce loose leaf
Endosulfan	Snow peas, leaf and steam vegetables dried or paste, squash
Malathion	Berries dried, hawthorn, cilantro, leaf and steam vegetables

(U.S. Food and Drug Administration (2017)

2.2.1 Sources of POPs in Food

Sources of POPs contamination during the production of food crops include wastewater use for irrigation, atmospheric deposition, runoff from contaminated sites to the fields, urban activities, pesticides and fertilizers (Batt, Wathen, Lazorchak, Olsen, & Kincaid, 2017; Blocksom

et al., 2010; Fair et al., 2019; Nerín et al., 2016; Rather et al., 2017). Other sources of OCs and polychlorinated biphenyl (PCB) contamination can be due to proximity to toxic waste sources and atmospheric deposition (Olatunji, 2019; Witczak & Abdel-gawad, 2012).

For livestock, grazing on contaminated soil and grass, proximity to chemical production areas, and local exposure routes such as paints, sealants and coatings used in the structures in which the animals are housed, are some of the routes of exposure in addition to contaminated feed and water (Ferrante et al., 2017; Pajurek, Pietron, Maszewski, Mikolajczyk, & Piskorska-pliszczynska, 2019; Weber et al., 2018; Zennegg, 2018).

Sources of PFASs in plants include irrigation with contaminated water, application of polluted sewage sludges or industrial wastes to soil, atmospheric deposition from PFASs emission sources like firefighting training locations and airports (Ghisi et al., 2019). Absorption of PFASs by plants depends on chain lengths, functional groups, plant species, abundance and characteristics of soil organic matter. It is also seen that shorter chain PFASs accumulate in leaves and fruits whereas longer chain compounds tend to accumulate in roots (Ghisi et al., 2019; Scher et al., 2018). Perfluorobutyrate (PFBA), Perfluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA) and PFOA are the major substances detected in water and produce samples (Scher et al., 2018). However there are relatively few studies on this subject and more research is necessary to fully understand the uptake mechanisms (Scher et al., 2018).

Packaging and processing practices are shown to be the possible sources of POPs in food (Jogsten et al., 2009; Schecter, Colacino, et al., 2010; Schecter, Haffner, et al., 2010; Wang & Kannan, 2018). More than 6000 chemicals can be used as food contact materials in the US and European Union (EU). Migration of chemicals can occur from packaging materials into food (Nerín et al., 2016). There is always a chance that harmful, non-intentionally added substances (NIAS) may be produced and to migrate from recycled packages (Geueke, Wagner, & Muncke,

2014). Also, deterioration of packaging speeds up when stored under direct sunlight, which likely increases the rate of migration of contaminants into food (Rather et al., 2017).

In a lab experiment examining 15 kinds of food packaging materials, PFOAs were detected in all the samples. The rate of mass transfer into food was high. Significant PFOA migration occurred after only two hours and equilibrium was reached after the 24-hour time point (Xu, Noonan, & Begley, 2013). Also, in another experiment conducted on 407 samples of food packaging materials collected from five regions of the US, 33% had detectable fluorine concentrations ranging from 16 to 800 nmol of F/cm^2 (Schaider et al., 2017). Fluorine was more commonly detected in grease-proof products, namely food contact papers, than in products holding liquids or non-food-contact surfaces (Schaider et al., 2017). For many samples, there was signal for unknown polyfluorinated compounds, suggesting the presence of organofluorine compounds in those samples. These examples show the potential for migration of PFAS from food packaging materials lined with PFAS. As PFAS compounds are linked with serious health effects, monitoring and finding a better packaging system is an immediate need.

Cooking processes can increase or decrease the concentration of POPs, with inconsistent results among studies, and no underlying mechanisms identified (Jogsten et al., 2009; Moon, Kim, & Oh, 2019). PCB can be formed from the reactions of polycyclic aromatic hydrocarbons (PAHs) with metallic components in ingredients or cookware under certain high temperature conditions (Moon et al., 2019). Thus, cooking processes can also become a source of POPs in food.

Halogenated compounds are found in different foods at production, processing and retail stages as shown in Table 2.5. They have been detected almost in all kinds of food consumed worldwide. Most of the halogenated compounds are toxic at low concentrations and many bioaccumulate, so detection at even low levels is potentially dangerous for human health. As per

Table 2.5, PCB (857 ng/g ww), PBDE (311 ng/g ww) and DDT(294 ng/g ww) levels were highest in fish collected from US rivers (production stage). Similarly, PFOS (66.3 ng/g ww) was also detected at high concentrations in the fish collected from South Carolina (USA). PFOA (8 ng/g ww) was highest in the fish collected from Sava river basin which touches six European countries' territories. All these fish samples were taken from areas with high industrial activity, areas with chemical industries, high pesticide application rates and discharge from wastewater treatment plants. These sources might be discharging halogenated compounds into the rivers where they bioaccumulated in the fish.

Table 2.5: Concentrations of POPs detected in food worldwide

Country	Food	Concentration	Author
		Production Stage	
USA	Fish (ng/g, ww)	ΣPCB[1]: nd-857, ΣPBDE[2]: nd-311, ΣDDT[3]: nd-294, ΣChlordane: nd- 311	A
USA	Fish (mean ng/g, ww)	ΣPCBs: 57.23, ΣPBDE: 1.9, ΣOCPs[4]: 22.63, ΣPOPs[5]: 81.76	B
USA	Fish (ng/g, ww) (Range of mean)	Chordane: 3.69-23.77, Dieldrin: 5.47-18.32, PBDEs: 4.43-45.66, PCBs: 7.41-123.12	C
USA	Fish (ng/g, ww)	Total PFAS[6]: 6.20-85.4, Total PFSA[7]: 2.79-72.1, Total PFCA[8]: 3.40-23, PFOS[9]: 2.53-66.3	D

Table 2.5 continued

USA	Fresh Produce (ng/g, ww)	PFBA[10]: ND[21]-33, PFOA[11]: ND-0.26, PFOS: ND-0.38	E
Egypt	Cattle (ng/g,lw)	ΣHCHs[12]: 2-1827, HCB[13]: 0.4-105, Σdrins: 2-470, ΣCHLs[14]: 0.5-277, ΣDDTs: 1-308, ΣOCPs: ND-2827	F
France	Hake (ng/g, dw.)	BDE-47: 0.09-65, CB-153: 2.7-2154	G
India	Rice (ng/g, dw	DDT: ND-110, HCH: 2-215	H
Italy	Goat Milk (ng/g, ww)	Σ6PCB: nd-4.02, Σ20PCB: nd-7.34, HCB: nd-0.22, ΣDDT: nd-0.20	I
Poland	Produce (range of mean ng/g, ww.)	OCP: 21.57-190.63, DDT: 0.52-16.74, Σ7PCBs: 0.12-3.71	J
Sava River Basin	Fish (ng/g, ww)	PBDEs: 0.65-11.5, PFOA: <MLOQ-8, PFOS: <MLOQ[21]-17	K
South Africa	Produce (ng/g, ww)	DDTs: 38.9-66.1, PCBs: 90.9-234	L
		PROCESSING	
Korea	Seafood and different cooking methods (ng/g, ww)	PCB: 0.01-20.6	M
Spain	Raw, cooked and packaged food (ng/g, ww)	PFHxS[15]: <0.001-<0.250, PFOS: <0.001-0.330, PFHxA[16]: <0.001-0.118, PFOA: <0.063-<0.600	N

Table 2.5 continued

		Retail Stage	
USA	Meat, fish, dairy, cheese and vegetables (ng/g, ww)	PCBs: ND-5.87, PFASs: ND-1.8, HCHs: ND-0.62, DDTs: ND-18.94, Dieldrin: ND-2.30	O
USA	(ng/g, ww)	HBCD: ND-0.593, PBDEs: ND-1.486	P
Cameroon	dried foods (ng/g)	Aldrin: 1.2-464.6, Dieldrin: 1.2-60.4, Endrin: 1.2- 33.7, Heptachlor: 1.2- 123.6, Malathion: 0.0073-5526.9, o,p'-DDT: 1.3-15.6, p,p'-DDD[17]: 1.2-24.1, p,p'-DDE: 1.3-27.6, p,p'-DDT: 3.3-146.6, α-Endosulfan: 1.2-41.5, β-endosulfan: 1.7-1.7 and β-HCH: 1.2-137.1 mg/kg	Q
Canada	Composite food Samples from TDS (ng/g, ww)	PFOA: <0.5-3.6, PFNA[18]: <1 and 4.5 ng/g and PFOS: <0.6-2.7 ng/g	R
Portugal	Duplicate Diet (ng/g ww)	PBDEs: <LOD-0.23, HBCDDs[19]: <LOD-1.2, PCBs: <LOD-0.95, HCHs: 0.0093-0.16, HCB: <LOD-0.062, CHLs: <LOD-1 and DDTs: 0.11-0.73	S

1: Polychlorinated Biphenyls, 2: Polybrominated Diphenyl Ethers, 3: Dichloro Diphenyl Trichloroethane, 4: organochlorine pesticide, 5: Persistent Organic Pollutant, 6: Per and polyfluoroalkyl substances, 7: Perfluorinated sulfonates, 8: Perfluorinated carboxylic acid, 9: Perfluorooctane sulfonate, 10: Perfluorobutanoic acid, 11: Perfluorooctanoic acid, 12: Hexachlorocyclohexane, 13: Hexachlorobenzene, 14: Chlordanes, 15:

Perfluorohexanesulfonate, 16: Perfluorohexanoic acid, 17: Dichlorodiphenyldichloroethane, 18: Perfluorononanoate, 19: Hexabromocyclododecane, 21: Non-detection and 22: methods limit of quantification

A= (Batt et al., 2017), B= (Fair et al., 2018), C= (Blocksom et al., 2010)B, D= (Fair et al., 2019), E= (Scher et al., 2018), F= (Mahmoud et al., 2016), G= (Bodiguel et al., n.d.), H= (Babu et al., 2002), I= (Ferrante et al., 2017), J= (Witczak & Abdel-gawad, 2012), K= (Ábalos et al., 2019), L= (Olatunji, 2019), M= (Moon et al., 2019), N= (Jogsten et al., 2009), O= (Schecter, Colacino, et al., 2010), P= (Schecter, Haffner, et al., 2010), Q= (Galani et al., 2018), R= (Ronson, Ao, & Abeka, 2007) and S= (Coelho et al., 2016)

2.3 Antibiotic Resistance Genes (ARGs)

ARGs are genes that confer antibiotic resistance. They may be encoded in the genome or on mobile genetic elements. They can be acquired by mutation, uptake from the environment (transformation), direct transfer from another organisms (conjugation), or transfer by viral infection (transduction) (Jose L. Martinez, Baquero, & Anderson, 2007). Antibiotics are used for disease treatment and prevention, but also for non-medicinal purposes, such as feed proficiency enhancers and growth promoters (Bengtsson-Palme Johan, 2017; Van, Yidana, Smooker, & Coloe, 2019). After administration to humans or animals, antibiotics are partially metabolized and a sizeable fraction (30-90%) is passed into urine and feces (Lee et al., 2017). Because wastewater treatment systems and animal waste handling methods have variable removal efficiencies, between 60% and 90% of the antibiotics may eventually be returned to the environment (R. Clarke, Healy, Fenton, & Cummins, 2018; Pan & Chu, 2017; S. R. Smith, 2009). ARGs may also be spread or introduced to the environment through activities such as spreading of biosolids or contaminated compost on land or the use of reclaimed water for irrigation (R. M. Clarke & Cummins, 2015; Lau, Engelen, Gordon, Renaud, & Topp, 2017).

Antibiotics in the environment exert a selective pressure on microbes which leads to the emergence and amplification of ARGs and antibiotic resistant bacteria (ARBs) (Ben et al., 2019). Trace concentrations of antibiotics are enough to trigger resistance development mechanisms (S. R. Smith, 2009). ARGs can be disseminated among microorganisms including pathogens through horizontal gene transfer (HGT), which is the movement of genetic materials between cells (Lau et al., 2017). Presently the rate of development of new antibiotics is constant but ARBs and ARGs

are increasing. Serious concerns arise when ARGs are transferred to human pathogens which make antibiotics ineffective in their treatment. The CDC estimates that in the United States, antibiotic resistant infections affect a minimum of two million people annually resulting in 23,000 deaths(Pepper, Brooks, & Gerba, 2018).

More antibiotics are currently used in the animal production sector than in the human health sector. Antibiotics are also used to protect plants from diseases, although the amount is much less than in animal rearing (Bengtsson-Palme Johan, 2017; Hudson et al., 2017) . In the USA in 2015, antibiotics used to treat infections in the human healthcare sector was about 60% of the amount of antibiotics used on food-producing animals, and 70% of medically important antibiotics were also sold for use in animals (Caniça, Manageiro, Abriouel, Moran-Gilad, & Franz, 2019).

Colistin is the last resort antibiotic used to treat human infections caused by clinically resistant gram-negative bacteria such as carbapenem- resistant Enterobacteriaceae. This means that colistin is a last-line treatment option against multidrug resistant gram negative Enterobacteriaceae. In 2015, a bacterium with plasmid-mediated colistin resistance conferred by the *mcr-1* gene was isolated from animals, raw meats and patients in China. Since then, additional varieties of colistin resistance genes, namely *mcr-2*, *mcr-3*, and *mcr-4* have been isolated worldwide (Garch, Jong, Bertrand, Hocquet, & Sauget, 2018). This is putting the human healthcare system at risk.

2.3.1 Sources of ARGs in Food

There are several reports of the association between the use of antibiotics in food-producing animals and antibiotic resistance in bacteria isolated from humans (Jose Luis Martinez, 2009). The use of antibiotics in livestock is associated with the emergence of antibiotic resistance in food-borne pathogens and livestock bacteria (Zwe et al., 2018). Multidrug resistant *Salmonella*,

Escherichia coli (E. coli), Campylobacter and other foodborne pathogens and opportunistic pathogens have been isolated from food -producing animals and fresh produce at different stages of the food system in recent years (Bosilevac, Guerini, Kalchayanand, & Koohmaraie, 2009; Del Collo et al., 2017; Holvoet, Sampers, Callens, & Dewulf, 2013; Karumathil, Yin, Kollanoor-johny, & Venkitanarayanan, 2016; S. Liu & Kilonzo-nthenge, 2017; Schwaiger, Helmke, Hölzel, & Bauer, 2011; Sivagami, Vignesh, Srinivasan, Divyapriya, & Nambi, 2018; Sjölund-Karlsson et al., 2013; Zwe et al., 2018). These microbes were resistant to azithromycin, tetracycline, nalidixic acid, amikacin, ciprofloxacin, trimethoprim-sulfamethoxazole and cephalosporin (Bosilevac et al., 2009; Sjölund-Karlsson et al., 2013). Antibiotic-resistance genes such as ceftriaxone-, aminoglycoside-, beta-lactam-, chloramphenicol-, sulfamethoxazole-, tetracycline-, and trimethoprim-resistance genes have all been detected in *Salmonella* (Iwamoto et al., 2017; Sjölund-Karlsson et al., 2013).

In the processing environment, contaminated surfaces can be a source for the transfer of ARGs along the food chain, as shown in a Malaysian market study (Hudson et al., 2017). Bacteria on contaminated surfaces take up genetic materials and become resistant. At a new chicken farm antibiotic-resistance gene were detected in litter samples after the arrival of the flock, but not before. The operators denied using antibiotics, which indicates either the amplification of resistance genes already in the environment or introduction with the broiler chicks as the carrier from their previous environment (Brooks, McLaughlin, Adeli, & Miles, 2016). This shows that ARGs can spread resistance in the inter-connected environment.

Techniques to kill or inactivate microbial populations such as the use of preservatives, temperature, or salt may be used during processing. These methods create stress in the microbes leading to the inactivation of many. However the same processes can also stimulate the transfer of

ARGs among microbes exposed to prolonged exposure to such stresses (Perez-Rodriguez & Taban, 2019).

Cross-contamination is likely to occur during transport of food and in the processing environment. Antibiotic-resistant *Salmonella* have been isolated from the environment where animals are held prior to slaughter. These lairage areas can then act as a contamination source, passing resistant organisms to subsequent groups of animals on their way to slaughter (Hudson et al., 2017).

Tetracycline-resistant genes are frequently detected in food and foodborne bacteria (Sharma et al., 2019; Xiong, Sun, Shi, & Yan, 2019). In their studies, there was a difference between the ARGs detected in land-based agriculture and aquatic food products, probably due to differences in microbial communities, and environmental structure. Upon analysis of swine manure, it was found that the same ARGs that dominated in swine manure were most commonly detected in fresh produce (*tet*(M), *aadA* and *qacE*) (Xiong et al., 2019). This indicates contaminated swine manure could be a route for dissemination of ARGs in fresh produce.

Misuse of antibiotics for rapid growth and disease prevention has triggered multi drug resistance in foodborne pathogens (Sharma et al., 2019). Colistin is widely used as growth promoter in animals (Ghafur et al., 2019; Monte et al., 2017). Colistin-resistance genes are spreading widely throughout the environment. *Mcr-1* and *mcr-2* have been detected in swine cecae, pork carcasses, chicken meat and mutton in the Belgium, Brazil and India respectively (Garcia-graells et al., 2018; Ghafur et al., 2019; Meinersmann, Ladely, Plumblee, Cook, & Thacker, 2017; Monte et al., 2017). In contrast, 1000 STEC isolates collected from 2006 through 2014 from livestock, wildlife, produce, soil and water samples from a major food-producing region of California, and that were screened for *mcr-1* and *mcr-2* genes tested negative (Mavrici, Yambao, Lee, Quiñones, & He, 2017). In the US colistin has never been used in animals whereas

colistin has been widely used as growth promoter in India, China and Brazil (Sun et al., 2017). Because of this, the detection of colistin resistance genes is higher in those countries as compared to the US.

The prevalence of antimicrobial resistant organisms has been shown to decline along the food chain in tandem with the increase in food hygiene (Hudson et al., 2017). Most supermarkets only carry washed vegetables maintained in cool conditions throughout the supply chain, which might cause lower resistance rates than in farm samples (Schwaiger et al., 2011). Use of chloramphenicol in food animals was banned in Europe in 1994. On comparing isolates obtained in 1996/1997 and 2004/2005, the resistance rates were lower in the isolates from 2004/2005. This is likely due to the decrease in the usage of chloramphenicol (Schwaiger et al., 2011).

The literature review on pathogens in food indicates that they are not very common or abundant in food. This means that ARGs are more likely to reside in non-pathogenic microbes than pathogenic microbes in the food chain supply. It is not easy to track the survival rates of the antibiotic-resistant population, but there is a high likelihood that once they get into the food system, they will stay there, grow and they will be detectable in raw food or ready to eat food (Perez-Rodriguez & Taban, 2019).

Table 2.6 shows the prevalence of ARGs in different food, especially in pathogens isolated from food, collected from different countries at production, processing and retail stage of the food system. The available data shows that researchers are primarily interested in how many foodborne pathogen isolates have ARGs or are resistant to antibiotics as opposed to looking for ARGs directly from food, which would provide information on the background abundance of these genes. On comparing beta-lactam resistance genes between the food system stages, at most bla_{TEM} was detected in 57% of the isolates in processing stage and bla_{CMY} was present in 92% of the examined isolates in retail stage. Furthermore, many of the isolates obtained from the food are

resistant to more than one antibiotic or have more than one resistance gene i.e. multi drug resistance (MDR).

Table 2.6: Occurrence of ARGs in food at different stages of food supply chain

Country	Food	ARGs
		Production Stage
USA[A]	Milk	41 *Campylobacter spp*. (38 *C.jejuni*. 2 *C. coli* and 1 *C. coli*) was subjected to 9 common antimicrobial testing. 26/38 (68.4%) *C. jejuni* were resistant to tetracycline, 5/38 (13.2%) was resistant to both ciprofloxacin and nalidixic acid. 12 *C.jejuni* isolates were susceptible to all 9 antimicrobials testing. *C. lari* was resistant to ciprofloxacin and nalidixic acid. *C.coli* was resistant to all the tested 9 antimicrobial substances.
USA[B]	Fruits and vegetables	None of the samples yielded any positive samples for colistin resistant gene
Germany [C]	Farm (299) and supermarket (702)	number of samples from farm had showed more resistance than the supermarket samples
Nigeria [D]	Food animals	Measured in *E. coli* isolates: bla_{TEM}: 54/211, bla_{CMY}: 126/211, bla_{CTX}: 6/211, bla_{OXA}: 2/211, bla_{SHV}: 0
Spain [E]	Fresh produce (ARGs screened in the phage extract	75% of the tested samples showed upto 9 ARGs in the lettuce and 82% soil samples showed upto 6 ARGs. Cucumber (69% upto 4 ARGs and 27% upto 3 ARGs respectively). The most abundant group was $bla_{CTX\text{-}M\text{-}9}$, bla_{TEM} and $bla_{VIM.}$
		Processing Stage
USA[F]	*Salmonella* isolates from slaughterhouse and processing plants	Ceftriaxone resistance *Salmonella* detected in 509 (26.2%) of 1940 *Salmonella* isolates from chicken, 167(9%) of 1862 isolates from ground turkey and 21 (13.5%) of 155 isolates from ground beef.
USA[G]	Ground beef processing	0.6% MDR *Salmonella* isolates resisted from 2 to 10 tested antibiotics including tetracycline
USA and Canada[H]	Slaughterhouse and retail *Salmonella* isolate along with human	*Tet*(A): 45/56, *tet*(B): 8/56, *tet*(C): 8/56, *tet*(D): 7/56, *tet(R)*: 50/56, bla_{TEM}: 32/56, bla_{CMY}: 30/56, bla_{PSE}: 36/56.
USA[I]	Ground beef samples	*Tet*(M): 64/75, Relative Abundance: 10^{-5}-1 (values not accurate, extracted from graph)
		Tet(B): 10/75, Relative Abundance: $<10^{-5}$->10^{-4}(values not accurate, extracted from graph)
		Tet(A): 29/75, Relative Abundance: $>10^{-4}$-<1(values not accurate, extracted from graph)
Canada [K]	26 *E. coli* obtained from commercial ground beef	Polymerase Chain Reaction (PCR) confirmation: *tet*(A)-1/26, *tet*(B)- 9/26, *tet*(C)- 5/26, bla_{CMY}-5/26, and bla_{TEM}- 11/26

Table 2.6 continued

India [L]	Processing and retail shop (the shops acted both of these phase)	Measured in total *Salmonella* isolates:*Tet*(A): 70/70, *tet*(B): 0/70, *tet*(G): 0/70, b*la*$_{TEM}$: 17/67, *bla*$_{PSE}$-1:1/67, *bla*$_{CMY}$: 1/67
		Retail Stage
USA and Canada [M]	Retail meat, ceca and food animals	USA
		bla$_{CMY}$: retail: 57/77, food animals: 138/140 isolates
		bla$_{TEM-1}$: retail meat: 8/77, food animals: 15/140
		Canada
		bla$_{CMY}$: retail meat: 48/52, food animals: 28/42,
		bla$_{TEM-1}$: retail meat: 1/52, food animals: 2/42
Belgium [N]	*Salmonella* from different food	32/398 in 2012, 18/296 in 2013, 38/294 in 2014 and 17/427 in 2015 were found to be colistin resistant. Total: 105/1415
		mcr-1: 2/105
		mcr-2: 1/105
		Mcr-1 and *mcr-2* was found in pork carcasses in 2012. The other one was the poultry samples
Brazil [O]	Chicken	*Mcr-1:*8/41
China [P]	Rectal from pig slaughterhouse and retail meat	Colistin resistant E. coli isolates
		Slaughter: 166/804 (21%)
		Retail Meat: 78/523, (15%)
		First report of plasmid mediated colistin resistance mechanism in animals
India [Q]	*E. coli* from raw meat, vegetables from shops and households	Colistin resistant organisms: vegetables: 23/63, fish samples: 11/21, poultry samples: 12/19, mutton: 3/4 and fruits: 2/3
		PCR screening showed that 3/71 *E. coli* harbored *mcr-1* gene (1 mutton and 2 poultry meat samples)

A= (Del Collo et al., 2017), B= (Mavrici et al., 2017), C= (Schwaiger et al., 2011), D= (Adenipekun et al., 2019), E= (Larrañaga et al., 2018), F= (Iwamoto et al., 2017), G= (Bosilevac et al., 2009), H= (Glenn et al., 2013), I= (Vikram et al., 2018), J= (Folster et al., 2012), K= (Aslam, Diarra, Service, & Rempel, 2009), L= (Sharma et al., 2019), M= (Sjölund-Karlsson et al., 2013), N= (Garcia-graells et al., 2018), O= (Monte et al., 2017), P= (Y.-Y. Liu et al., 2016) and Q= (Ghafur et al., 2019).

2.4 Pathogens

Foodborne illness can be acquired through ingestion of foodborne pathogens, or ingestion of toxins produced by toxigenic pathogens in food products (Bintsis, 2017). *Salmonella* and pathogenic E. coli are the top foodborne pathogens. These pathogens produce more infections in Asia and Africa than elsewhere (Fegan & Jenson, 2018). The US Center for Disease Control

(CDC) estimates that each year one in six Americans (i.e. 48 million) suffers from foodborne illness (Hoagland, Ximenes, Ku, & Ladisch, 2018). Once a food source has become contaminated, outbreaks occur rapidly, infecting many people (Hoagland et al., 2018). There were 839 documented food-related outbreaks in 2017 in the US, resulting in 14,471 reported cases of illness, 822 hospitalizations and 21 deaths (https://wwwn.cdc.gov/norsdashboard/). *Norovirus*, *Salmonella*, *Campylobacte*r, *Bacillus*, and *E. coli* (pathogenic) are some of the food borne pathogen responsible for those outbreaks, illnesses, hospitalizations and deaths. In 2017, *norovirus* infections caused more outbreaks, illnesses and hospitalizations than the other pathogens. However, *Salmonella* was responsible for a greater number of deaths than other pathogens. The types of food responsible for causing illnesses, hospitalizations and deaths were found to be meat, poultry, dairy, fruits, vegetables, seafood, grains, and nuts. Although the literature shows a low prevalence of food-borne pathogens in our commonly consumed food, the data in Table 2.7 shows that the consequences of exposure through food can be severe, and a sizeable number of people are affected annually.

Table 2.7: Outbreak data due to foodborne pathogens in US in 2017

Pathogens	Outbreaks	Illness	Hospitalized	Death
Bacillus	25	704	56	2
Campylobacter	27	770	117	1
Clostridium	57	1480	64	3
E. coli	27	770	117	1
Norovirus	318	6389	54	4
Listeria	7	28	27	3
Shigella	4	54	10	0
Salmonella	125	3228	528	9
Staphylococcus	22	559	56	2
Streptococcus	1	62	0	0
Vibrio	20	91	5	0

(NORS, CDC, 2019)

2.4.1 Sources of Pathogens in Food

Sources of pathogen contamination of fresh produce at the farm level include livestock and human movement, land-application of raw manure, irrigation water and water, immature compost application, contaminated soil, and runoff from compost and manure stockpiles on the farm (Bilung, Chai, Tahar, Ted, & Apun, 2018; Ceuppens et al., 2014; Ssemanda et al., 2018). Possible sources of contamination at dairy farms are fecal contamination, contaminated crops or feedstock, contaminated housing and water (Del Collo et al., 2017; Mcauley, Mcmillan, Moore, Fegan, & Fox, 2014). Produce leaves that touch the ground are more prone to pathogenic contamination than plants whose leaves have not (Reddy, Wang, Adams, & Feng, 2016). Water distribution systems such as surface furrow and drip irrigation system pose less risk than sprinkler systems because the latter irrigation water comes in contact with the edible portion of the plants (Alegbeleye, Singleton, & Sant'Ana, 2018).

Processing steps are often found to be more susceptible to contamination than production steps (Heredia et al., 2016; Ilic, Odomeru, & LeJeune, 2008; Johnston et al., 2005; Perez-Arnedo & Gonzalez-Fandos, 2019). Environmental samples (soil, feces, water), poorly sanitized food contact surfaces (conveyor belt, knives, slices etc.) and poorly sanitized non-food contact surfaces (walls, drains, floors etc.), unhygienic design of plants, unregulated traffic patterns, non-sanitized worker's hands, transport trailers and crates are some of the sources of contamination (Heredia et al., 2016; Johnston et al., 2006; Li et al., 2017; Muhterem-Uyar et al., 2015; Perez-Arnedo & Gonzalez-Fandos, 2019). High contamination in meat processing plants (probably due to cross contamination from animal carcasses) and cutting and packaging rooms was reported to be due to unhygienic design of bleeding, plucking and evisceration equipment (Muhterem-Uyar et al., 2015; Perez-Arnedo & Gonzalez-Fandos, 2019) . Cross contamination of foodborne pathogens can occur during transportation or while animals are waiting in lairage before slaughter. When

transport vehicles are contaminated with foodborne pathogens and are then used to transport other food, it promotes cross contamination (Carrasco, Morales-rueda, & García-gimeno, 2012; Larsen et al., n.d.). Biofilms (thin slime layers of bacteria) are the major vehicle for microbial food contamination (Ripolles-avila, Hascoët, Martínez-suárez, Capita, & Rodríguez-jerez, 2019). A large variety of strains were isolated from vegetables collected from various processing industries in the Republic of Ireland, implying that soil is more likely the source of contamination than the processing staff or equipment (Leong, Alvarez-ordóñez, & Jordan, 2014).

At the retail stage, observed food contamination may originate at the retail site or from previous stages in the food supply (production and processing) as shown in a study by Dickins et al., (2016). Shelf life, packaging materials and style, rodents and refrigeration systems are some of the factors which need to be taken into consideration for prevention of further contamination (Sharma et al., 2019; Trimoulinard et al., 2017). Various field management techniques, poor regulatory guidance, emphasis on minimal application of antibiotics and interest in the organic processes could be some of the possible reasons for the high prevalence of pathogens in locally grown produce collected from a farmer's market in West Virginia (Li et al., 2017). The other identified contamination source can be the manipulation activities such as inappropriate disinfection process done in the food items (open and unpacked food) (Panel HanaVojkovská et al., 2017). When food with foodborne pathogens is prepared for consumption in a kitchen, surfaces and implements can transfer pathogens from one food to another, causing cross contamination (Mol, Akay, & Guney, 2018; Redmond & Griffith, 2003).

Various studies have demonstrated that fresh produce has a low incidence of foodborne pathogens as shown in Table 2.8 (Cheruiyot, Parveen, Hashem, & Bowers, 2016; Denis, Zhang, Leroux, Trudel, & Bietlot, 2016; Mukherjee, Speh, Jones, Buesing, & Diez-gonzalez, 2006; Reddy et al., 2016; Seow, Ágoston, Phua, & Yuk, 2012). This can be attributed to stringent

regulation and enforcement of food and produce safety protocols as shown in study by Luchansky et al.,(2017) where *L. monocytogenes* decreased with time following the change in industrial practice behavior and regulatory practice. Another reason for the low prevalence can be due to improved knowledge of the biology and ecology of *L. monocytogenes* since the first recognized food-borne outbreaks in the early 1980s (Cheruiyot et al., 2016). Table 2.8 shows the incidence of foodborne pathogens in different foods from around the world. The highest incidence of *Salmonella* (16.03%) and *Campylobacter* (82%) occurred at the retail stage. The most positive *L. monocytogenes* (up to 26.19%) was detected at the processing stage. Likewise, pathogenic *E. coli* (33%) was most prevalent in the samples taken from the production stage. Some of the studies yielded no- or very low incidences of food borne pathogens. Even a low incidence of pathogens can be problematic when the food is eaten raw, however if the food is cooked to high enough temperature, most pathogens will be killed or rendered harmless. Poorly sanitized food contact and non-food contact surfaces, unhygienic design of the processing plants and cross contamination were the possible reason behind the high prevalence of *L. monocytogenes*. Lack of use of antimicrobials in the post-harvest control process in organic fresh produce was suspected to contribute to the higher prevalence of *Salmonella* than in conventional supermarket samples. Contamination at the brooder house or in the post slaughter stages were suspected to be the possible reason for high prevalence of *Campylobacter* in chicken.

Table 2.8: Occurrence of foodborne pathogens in food from different countries at different stages of the food supply chain

Country	Food	Foodborne Pathogen data (Value, %)	Author
		Production Stage	
USA	Milk	Filter: *Salmonella*: 61/254, *Listeria spp.*: 47/254, *L. monocytogenes*: 14/254 and *E. coli*: 216/254 BTM: *Salmonella*: 11/234, *Listeria spp.*: 6/234, *L. monocytogenes*: 4/234 and *E. coli*: 77/234	A
USA	Milk	Filter: *Campylobacter*: 69/231 BTM: *Campylobacter*: 27/234	B
USA	leafy green samples	*Salmonella*: 15/369 and *E. coli*: 2/369	C
USA	Fruits and vegetables	*Salmonella* and *E. coli* not detected in food out of 2029 fruits and vegetables	D
USA	Produce mostly eaten raw	*Salmonella*: 3/398 (only in cantaloupe) *L. monocytogenes* and Pathogenic *E. coli*: not detected in food	E
Australia	Milk	raw milk: *STEC*: 1/15, *Salmonella*: 1/15 and *Campylobacter* and *Listeria spp.*: not detected in food Milk filter: *Listeria*: 1/9, *Salmonella*: 2/9 and *Campylobacter* and *STEC*: not detected in food	F
Malaysia	Vegetables	*Listeria spp.*: 9/206 and *L. monocytogenes*: not detected in food	G
		Processing Stage	

Table 2.8 continued

USA	18 beef processing industries	*Salmonella*: 172/4136	H
USA	Ground beef	*Salmonella*: 30/370	I
USA	Spinach	*E. coli*: 0/1356, *Shigella*: 0/1311, *Salmonella*: 1/404 (before processing) *Salmonella*: 4/907 (after processing), *L. monocytogenes*: 3/409 and *Listeria spp*. 5/409	J
Brazil	Cheese products	*L. monocytogenes*: 3/16	K
Ireland	Food processing facilities	*L. monocytogenes*: 23/432	L
Six European Countries	Food processing industries	*L. monocytogenes*: Meat: 22/84 and Dairy: 40/1362	M
		Retail Stage	
USA	Meat	*Salmonella*: 25/825, *Campylobacter*: 159/719 and *E. coli*: 179/825	N
USA	Retail meat	*Campylobacter*:3194/24566	O
USA	Chicken	*Campylobacter*: 59/72	P
USA	Vegetables	*Salmonella*: 2/414, *L. monocytogenes*: 1/414 and *E. Coli*: 1/414	Q
USA	Fresh produce	*Salmonella*: 34/212, *Listeria spp*.: 8/212 and *L. monocytogenes*: 4/212	R
USA	Fresh produce	*Salmonella:* 456/111598 (PCR positive), 146/456 isolates from PCR positive samples	S
USA	RTE	*L. monocytogenes*: 116/27389	T
Canada	Fruits and vegetables	*Salmonella*: 10/29391, *L. monocytogenes*: 16/4575, *Campylobacter*: 0/8866 and *E. coli*: 0/23805	U

Table 2.8 continued

China	RTE	*L. monocytogenes*: 57/3974, *Salmonella*: 28/4035, *S. Aureus*: 32/4047 and diarrheagenic *E. coli*: 40/3774	V
Czech Republic	General food	*Salmonella*: 1/339 and *L. monocytogenes*: 17/339	W
India	Meat	*Salmonella*: 28/188	X
India	Meat	*Salmonella*: 16/480, *L. monocytogenes*: 14/480 and *E. coli*: 3/480	Y
Reunion Island, Africa	Sausages	*Salmonella*: 24/203, *Campylobacter*: 3/203, *Listeria spp*.: 61/203 and *L. monocytogenes*: 12/203	Z
Singapore	Vegetables and fruits	*Salmonella*: 0/125	A1
Wales, UK	RTE	*L. monocytogenes*: 58/15228, *Salmonella*: 1/15228, *Campylobacter*: 0/2061	A2

A= (Sonnier et al., 2018), B= (Del Collo et al., 2017), C= (Marine et al., 2015), D= (Mukherjee et al., 2006), E= (Johnston et al., 2005), F= (Mcauley et al., 2014), G= (Bilung et al., 2018), H= (Bosilevac et al., 2009), I = (Vikram et al., 2018), J= (Ilic et al., 2008), K= (Oxaran et al., 2017), L= (Leong et al., 2014), M= (Muhterem-Uyar et al., 2015), N= (C. Zhao et al., 2001), O= (S. Zhao et al., 2010), P= (Dickins et al., 2002), P= (Cheruiyot et al., 2016), R= (Li et al., 2017), S= (Reddy et al., 2016), T= (Luchansky et al., 2017), U= (Denis et al., 2016), V= (Yang et al., 2016), W= (Panel HanaVojkovská et al., 2017), X= (Sharma et al., 2019), Y= (Mritunjay & Kumar, 2017), Z= (Trimoulinard et al., 2017), A1= (Seow et al., 2012) and A2= (Meldrum et al., 2005)

2.5 Observation and Discussion

The above review presents the different possible sources of contaminants that can enter our food system. We have tried to compile contaminant data from the US and other countries at the production, processing, and retail stages. Some contamination exists in all stages of the food system. Each stage has unique contamination sources and mechanisms. Due to differences in methods and protocols among the studies, it is not possible to make sweeping conclusions about which countries or steps in the food chain produce more contamination across the board. The

protocols used in the source studies were different even within the same country. Also, countries differ in the social, economic and legal status which, further complicates comparison. However, we did observe that contaminants are present in food at all stages, and in all countries. The wide concentration ranges observed are due to variability in the production environments, handling procedures, processing, packaging and storage among the foods tested and emphasize the complexity of the system and the difficulty in establishing simple fixes. But some general observations were possible:

1. For halogenated organics, the production stage produced the highest level of contamination for all the investigated halogenated compounds i.e. PCBs, PBDEs, DDT, PFOA and PFOS. Some may also be introduced in packaging and from food contact papers.
2. Heavy metals were also largely introduced at the production stage, although processing and packaging were also significant sources of some metals
3. Pathogens were largely introduced during the processing stage for meat products, but production for vegetables. Handling at retail and consumer sites can also introduce pathogens.
4. Antibiotic resistance genes are mostly introduced during animal rearing as a result of non-therapeutic antibiotic use, and may contaminate meat during processing

From our review, we found that as the food moves away from production stage in the food system, it gets more difficult to assess the source of contamination. When food reaches the retail stage, it may already have been already contaminated at a previous stage of the supply chain, or it could become contaminated at the store. Policies and plans that regulate and monitor the level of contaminants at all stages of the food system have to be put in place to identify

sources. In recent times, the level of contaminants has decreased especially for foodborne pathogens. This can be attributed to effective enforcement of strict regulations.

To summarize, we saw that contaminated soil, contaminated irrigation water, and the application of contaminated compost, manure, or other amendments are the contributing sources for heavy metals, halogenated compounds, ARGs and pathogens at the production stage. Proximity to emission sourced and aerial deposition are additional sources of contamination for heavy metals and halogenated compounds. Antibiotic usage as a growth promoter on animals triggers HGT in bacteria which ultimately leads to proliferation of ARGs in the environment. In the processing environment, food packaging materials and cooking processes are the prominent sources of chemical contamination. Cross contamination, unhygienic practices, contamination of food contact and non-contact surfaces are the reason for the biological contamination. At the retail stage, it becomes harder to accurately identify the sources of contamination. Many countries, especially developed countries, have strict regulations on the use of appropriate packaging materials, appropriate storage temperatures and expiry date labels. These measures have helped reduce, but not eliminate the risks associated with all four contaminant types. Maintaining a hygienic environment in all stages of the food system is essential to minimize biological contamination.

Regardless of the source along the food system, land application of treated food waste will re-introduce remaining contaminants that can be taken up by plants and contribute to their amplification in the food system. As we saw from the literature review, heavy metals, halogenated compounds, ARGs, and foodborne pathogens are prevalent in food, and will end up in the food waste. Their concentration may increase during food waste collection and processing. Heavy metals are recalcitrant materials and they do not degrade with biological treatment (Stephen R Smith, 2009). On the other hand, composting at optimum temperature (>65˚C) limits

the likelihood for human pathogen survival (Gurtler, Doyle, Erickson, Jiang, & Millner, 2018). The fates of halogenated compounds and antibiotic resistance genes in food waste treatment systems are not well understood (R. M. Clarke & Cummins, 2015; J. Zhang et al., 2016).

Contaminated compost/digestate is one of the contributing sources for all classes of investigated contaminants. Food waste acts as a feedstock for anaerobic digestion and composting. Food waste varies widely over time and regionally, furthermore processing practices, such as co-digestion or co-composting with biosolids, can add to the contamination. The heterogeneous nature of food waste and co-digesting and co-composting it with other organics can amplify the risk of entry of contaminants into food system. We argue that routine monitoring of feedstocks that go into such systems is essential. Repeated land application of processed food waste can result in accumulation of contaminants if they are present in the feedstocks. Safe recycling practice such as careful source separation of food waste can help to generate high quality inputs resulting in desirable, and marketable, end products.

3 METHODOLOGY

3.1 Sampling Sites and Sample Collection

Food waste samples were collected from Massachusetts (MA), Vermont (VT) and Maine (ME) in 2018 and 2019. Samples were collected from six different source types i.e. grocery stores, hospitals, retirement communities, restaurants, residential pick-up and drop-off locations, and schools. Two scoops of half-gallon capacity were used to collect samples, however in some cases there was insufficient material to get two full scoops. During field operations, care was taken to avoid cross-contamination. Separate scoops and buckets were used for each type of source. Ice packs were placed in the buckets beneath the sample bags. The buckets were lined with trash bags and samples were poured into the trash bags.

3.2 Sample Pretreatment

In the lab, the following procedure was adopted to avoid cross-contamination between samples. All equipment and surfaces that touched the samples were:

1. Cleaned with 10% bleach.
2. Sprayed or wiped with 70% ethanol
3. Rinsed with deionized water (DI)

Trays, the industrial-grade food processor (Robot-Coupe R602), and any equipment like spoons, scissors or shears that contacted the waste were stainless steel and were always treated as above before processing any samples and in between samples. Food was poured onto sanitized trays. Any non-food items, including papers, were removed. Only food waste was further processed. One of the reasons was that the Robot-Coupe was not able to blend papers. The separated contaminants were inventoried, then weighed, and photos were taken for records. Food waste were then placed in a -20˚C freezer and were processed the next day. Food was blended to

a fine consistency and transferred to two quarter gallon zip-loc bags (S C Johnson & Sons, USA), one whirl-pak bag and one sterile 50 ml centrifuge tube for further lab testing.

3.3 Solids Analytical Methods

3.3.1 Heavy Metals

One of the quarter-gallon zip lock bags of processed food waste was sent to the Maine Soil Testing Lab at the University of Maine for heavy metal and compost test. The compost test includes conductivity, Carbon, Nitrogen, C: N, pH, Phosphorus, Potassium, Magnesium, Calcium, Boron, Iron, Manganese and total solid tests. Heavy metals (Cd, Cr, Ni, Pb, Sn, and Ti) were analyzed by acid digestion using the EPA 3051 method and determined by ICP-OES(EPA, 2007). The detection limit for heavy metals by this method was 2mg/kg d.w.

3.3.2 Extractable Organic Halides (EOX)

The whirl-pak bag was sent to Maine Environmental Laboratory (Yarmouth, ME) for Extractable Organic Halide (EOX) testing. The EOX test is a modification of the AOX (adsorbable organic halide) test, which is used to estimate the total organically bound halogen (chlorine, bromine, and iodine but not fluorine) in liquid samples (Goi, Tubaro, & Dolcetti, 2006). Therefore, EOX is used to determine organohalogens in solids (Pöykiö, Nurmesniemi, & Kivilinna, 2008). Halogenated organic contaminants are considered among the most dangerous organic pollutants due to their persistence and ability to bioaccumulate. Measurement of EOX gives a good estimation of the level of harmful organic halides in waste meant for recycling.

EOX was determined using the EPA 9023 method that employs pyrolysis/microcoulometry to determine halogenated compounds in solids (EPA, 1996). This method does not measure individual components but measures the halogenated compounds as a whole in the samples. The detection limit was 5 mg/kg as Cl^- (by wet weight).

3.3.3 PFAS

Four samples from each regulatory environment that were comparatively fresher than the other food waste samples were selected for PFAS analysis. PFAS analysis was carried out by Eurofins, Test America (West Sacramento, California) using EPA method 537 modified (EPA, 2020). Samples were shipped in the bottles provided by the laboratory, on ice, following chain of custody protocols. To avoid matrix effects on the results, the method was modified to use 1 g of food waste rather than 5 g of solid matrix as in the standard protocol. The samples were tested for 17 different PFASs compounds. They are Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnA), PErfluorododecanoic acid (PFDoA), Perfluorotridecanoic acid (PFTriA), Perfluorotetradecanoic acid (PFTeA), Perfluorobutanesulfonic acid (PFBS), Perfluoroheptanesulfonic acid (PFHpS), Perfluorooctanesulfonic acid (PFOS), Perfluorodecanesulfonic acid (PFDS) and Perfluorooctanesulfonamide (FOSA).

In brief, aliquots of solid samples were fortified with extracted internal standards (EIS), that is, carbon-13 labeled analogs, oxygen -18 labeled analogs, or denatured analogs of the compounds of interest. The fortified aliquots were extracted with a potassium hydroxide (KOH)/methanol solution using an orbital shaker for 3 hours followed by sonication for 12 hours. After centrifuging and filtration, the extracts were then subjected to a solid- phase extraction cleanup, with the PFAS eluted from the cartridge using an ammonium hydroxide/methanol solution. Internal standard is added after diluting the extracts to create a basic methanol/water solution. The extracts were then analyzed by high performance liquid chromatography coupled with mass spectrometry (HPLC/MS/MS) using a C18 column and solvent gradient program. EIS

are used as internal standards to calculate the concentration of target analytes (PFAS) present in the sample. This helps in correcting any analytical bias encountered especially with complex environmental samples. The compounds that did not have an identically labeled analog were quantified using a closely related labeled analog as the EIS.

3.3.4 Deoxyribonucleic Acid (DNA) Extraction

Genomic DNA was extracted from two roughly 0.25 g aliquots from each food sample using the Qiagen Soil DNA Extraction Kit (Qiagen, MD, USA) following their protocol. The two eluted DNA extracts were mixed at the end for downstream processing. This gave us a total volume of 200 µl. The extracted DNA samples were stored at -80 ˚C prior to analysis. DNA concentrations were quantified with a Qubit fluorometer (Invitrogen, CA, USA). All DNA samples were diluted to 5 ng/ µl with nuclease-free water (Thermo-Fisher, Waltham, MA) for qPCR. Some of the DNA samples were concentrated to 5 ng/µl by adding 1/10 volume of 3M Na-Acetate pH 5.2 and 2.5 volumes of ice-cold 100% ethanol to DNA sample, mixing by inverting the tube several times and storing -20˚C for 1 hour for DNA precipitation. The precipitated DNA was recovered by centrifuging at full speed for 20 minutes. The pellet was dried by pipetting off the supernatant and air drying for 15 minutes. DNA free water was added to the tube containing the DNA pellet, and the tube was vortexed. The concentration was determined using the qubit assay, and samples were diluted with nuclease free water as needed.

3.3.5 qPCR Standards

Standards for antibiotic resistance genes *tet*(M) and bla_{TEM} were developed in the lab. Influent and activated sludge samples were obtained from the Orono wastewater treatment plant. DNA was extracted using the Qiagen Soil DNA Extraction Kit as described above, and quantified with a Qubit fluorometer (Invitrogen, CA, USA). DNA was diluted to 50 ng/µl with nuclease-free water (Thermo-Fisher, Waltham, MA).

Polymerase chain reaction (PCR) is a technique to amplify a target DNA template. PCR was performed to develop standards using the primer sets (Table 3.1) and PCR conditions (Table 3.2) listed below. PCR protocols were run for 30 cycles. PCR components in 25 µl consisted of 2X GoTaq ® Green Master Mix (Promega, Fitchburg, WI), 25 pmol of each primer for *tet*(M) and 5 pmole of each primer for bla_{TEM}, 1 µl of DNA and nuclease-free water (Thermo-Fisher, Waltham, MA). To verify the correct target was amplified, 5 µl of fresh PCR product was mixed with 1 µl of 6X loading buffer (NEB, Ipswich, MA) and loaded into 2% agarose gels for electrophoresis at ~130 mV for 45 min. A 100-base pair (bp) ladder (NEB, Ipswich, MA) was run alongside the samples to assess the amplicon length in base pairs. The fresh PCR product with the right size band was quantified with a Qubit fluorometer (Invitrogen, Carlsbad, CA). The amplified fragments were then cloned as described in 3.3.6

E. coli NCTC 13846 DNA was used as the standard for the colistin-resistance gene (*mcr-1*) (Microbiologics, USA). Following their protocol for kwik stik pack, the culture was streak plated onto Luria Bertani (LB) agar plates (25 g LB powder, 15 g agar1L deionized water, autoclaved) and incubated at 37˚C overnight. White colonies were picked with a sterile loop and transferred to LB broth (25 g LB powder and 1L water, autoclaved) the next day and incubated at 37˚C overnight. DNA was extracted from the culture and the concentration was determined. This DNA was subjected to PCR with the *mcr-1* primer set using the method described in Table 3.1 and Table 3.2 for 30 cycles and the fresh PCR product was run in 2% agarose gel as described above. The right size band from the agarose gel was cleaned with QIAquick Gel Extraction Kit (Qiagen, MD, USA) following the manufacturer's protocol. The product was Sanger sequenced (University of Maine, ME, USA) to confirm the presence of the *mcr-1* gene.

Salmonella enterica (ATCC® BAA-1045), *Listeria monocytogenes* (ATCC® 19115) and *E. coli* (ATCC® BAA-184) were kindly provided by Dr. J. Perry (School of Food and

Agriculture, University of Maine). DNA was extracted from stationary phase cultures grown in LB broth using the Qiagen Soil DNA Extraction Kit (Qiagen, MD, USA) for use as standards in our qPCR.

Concentration for the genomic or plasmid DNA was quantified using Qubit fluorometer. The total number of copies of the target gene in plasmid or genomic DNA was calculated using the equation below:

$$\text{Copy}/\mu\text{l} = \frac{DNA\ x\ 6.022{*}10^{23} molecules/mole}{\left(N\ x\ 660\frac{g}{mole}\right) x\ 1{*}10^{9} ng/g}$$ where

DNA: Concentration of DNA (ng/ µl)

N: Length of plasmid or chromosome DNA (bp)

3.3.6 Cloning

The quantified and identity-confirmed fresh PCR products were ligated into the TOPO-TA vector (Invitrogen, Carlsbad, CA) at a vector-insert ratio of 1:2. This was transformed into TOP10 competent cells (Invitrogen, Carlsbad, CA) following the One Shot® chemical transformation protocol. Transformed bacteria were streaked onto LB plates with 50 µg/ml ampicillin (Thermo Fisher Scientific, Waltham, MA) that were previously spread with 40 mg/ml X-gal (Invitrogen, Carlsbad, CA). The plates were incubated at 37˚C overnight. White colonies were picked according to the blue (plasmid without insert) and white (plasmid with insert) screening procedure.

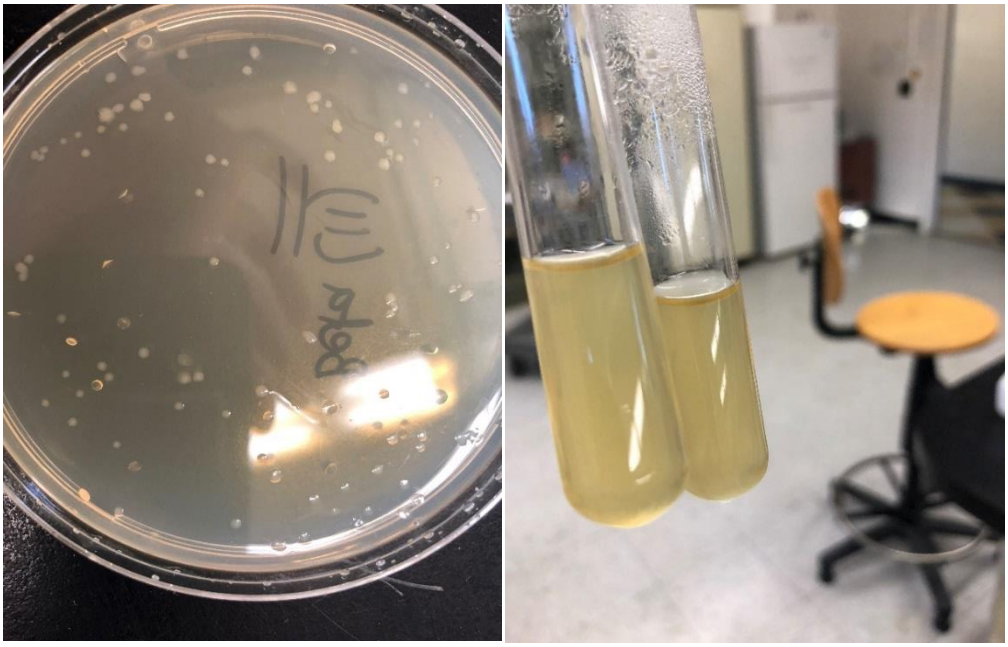


Figure 3.1: From left a) white colonies grown on LB agar plate b) LB broth showing fully grown *E. coli* after being shaken for 13 hours

White colonies were picked and inoculated in 5 ml LB broth with 50 µg/ml ampicillin. The broth was shaken for 8 hours at 37˚C. PCR was again performed following the same protocols as before (3.3.5) with 1µl of this fully-grown bacterial media. Amplicons were electrophoresed in 2% agarose gels to verify the presence of the insert. The cultures that were confirmed as having plasmid with insert were inoculated into new tubes with 5 ml LB broth with 50 µg/ml ampicillin and shaken at 37 ˚C overnight. The next day, plasmids were extracted from the overnight cultures using Plasmid mini prep kits (Invitrogen, Carlsbad, CA). Purified plasmid DNA was sent for Sanger sequencing to confirm the presence of the target gene. This confirmed plasmid DNA was used as the standard for qPCR.

Table 3.1: List of the primers used in qPCR for ARGs and pathogens

Name	Primers	Sequence (5'-3')	Amplicon size(bp)	Author
Tetracycline Resistance Gene	*tet*(M) (F)	ACAGAAAGCTTATTATATAAC	171	(Aminov, Garrigues-Jeanjean, & Mackie, 2001)
	tet(M) (R)	TGGCGTGTCTATGATGTTCAC		

Table 3.1 continued

Beta-Lactamase Resistant Gene	*bla*$_{TEM}$ (F)	GCKGCCAACTTACTTCTGACAACG	28	(Xi et al., 2009)
	bla$_{TEM}$ (R)	CTTTATCCGCCTCCATCCAGTCTA		
Colistin Resistant Gene	*mcr-1* (F)	GGGCCTGCGTATTTTAAGCG	183	(Hembach et al., 2017)
	mcr-1 (R)	CATAGGCATTGCTGTGCGTC		
Salmonella	*InvA* (F)	TCGTCATTCCATTACCTACC	118	(Hoorfar, Ahrens, & Radstrom, 2000)
	InvA (R)	AAACGTTGAAAAACTGAGGA		
L. monocytogenes	*hlyA* (F)	TGCAAGTCCTAAGACGCCA	112	(Barbau-piednoir, Botteldoorn, Yde, Mahillon, & Roosens, 2013)
	hlyA (R)	CACTGCATCTCCGTGGTATACTAA		
STEC E. coli	*stx-1* (F)	GTCACAGTAACAAACCGTAACA	95	(Fukushima et al., 2010)
	stx-1 (R)	TCGTTGACTACTTCTTATCTGGA		
16s rRNA	1369	CGGTGAATACGTTCYCGG	143	(Suzuki, Taylor, & DeLong, 2000)
	1492	GGWTACCTTGTTACGACTT		

Table 3.2: qPCR primer conditions and working program

Name	Primer Concentration	Program
Tet(M)	0.4 µM	95˚C 5 mins, 95˚C 15 sec, 55˚C 30 sec and 72˚C 30 sec
Bla$_{TEM}$	0.2 µM	95˚C 15 mins, 95˚C 15 sec, 61˚C 30 sec and 72˚C 30 sec
Mcr-1	0.2 µM	95˚C 10 mins, 95˚C 15 sec, 60˚C 30 sec and 72˚C 30 sec
InvA	0.4 µM	95˚C 15 mins, 95˚C 15 sec, 55˚C 20 sec and 72˚C 30 sec
hlyA	0.5 µM	95˚C 10 mins, 95˚C 15 sec, 60˚C 1min and 72˚C 1 min
Stx-1	0.25 µM	95˚C 10 mins, 95˚C 15 sec, 55˚C 30 sec and 72˚C 30 sec
16S rRNA	0.4 µM	95˚C 10 mins, 95˚C 15 mins, 55˚C for 30 sec and 72˚C 30 sec

3.3.7 Quantitative Polymerase Chain Reaction (qPCR)

qPCR is a technique that allows the real time monitoring in the increase of double stranded DNA template during PCR reactions. Standards were made by diluting the standard DNA (a copy number determined as described above) with nuclease-free water to make standards from 10^7 to 10^3 copies per µL. All the qPCR assays were run using a BioRad CFX96 thermocycler (Bio-Rad Technologies, Hercules, CA) in a total volume of 10µl. qPCR assay consists of 5µl SsoAdv Universal SYBR Green Supermix (Bio-Rad Technologies, Hercules, CA), 1 µl of each primer, 1 µl of DNA (5ng) and 2 µl nuclease-free water. qPCR protocols for each of the targets and their primers information along with primer concentration are given in the Table 3.1 and Table 3.2. All the qPCR protocols were run for 40 cycles.

Standard curves were established by plotting the number of cycles to reach the fluorescence threshold against copy number ($10^7$to 10^3) for all the gene except 16S rRNA ($10^9$to 10^4). The threshold limit was manually set at 60 relative fluorescence unit (RFU) for all the genes except for *L. monocytogenes* which was set at 70 RFU. This threshold was free of background noise and was on the logarithmic phase of DNA amplification. Samples possessing a signal above this value were assessed as positive and were quantified from the standard curve. In some cases, a sample did not reach the signal threshold within the allowable number of amplification cycles (40). In these cases, any sample that had a peak at the right temperature in the melting curve and had the right sized band when run on a 2% agarose gel was scored as positive but below the limit of quantification. A subset of these were Sanger sequenced to confirm the positive score.

Efficiency ranged from 93% to 101%. The assays were run in triplicate along with non-template control (NTC). After the standard curve development, samples were run in batches along with standards, spikes (standards+ samples) and NTC. Control DNA spiked into food DNA samples were run to ensure that the qPCR program quantifies accurately in the sample matrix.

The 16S rRNA gene is found in all bacteria. Thus, quantification of 16S rRNA was used to quantify the total bacterial population. 16S rRNA values were used to calculate the relative abundance of ARGs (fractions of microbes with the gene) in our study.

3.3.8 Microbial Community Analysis

Eighteen fresh samples were picked for Illumina sequencing of amplicons of the V4 region of the small subunit ribosomal RNA (rRNA) for microbial community analysis. DNA extracts were normalized to 5 ng/µl. 30 µl of DNA was transferred into a sterile tube for each sample, and they were placed on ice and shipped overnight to MR DNA (Shallowater, Texas, US) for Illumina sequencing. Universal bacterial primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) were used. Library preparation and sequence determination using paired- end Illumina MiSeq 2 x 250 reads was performed by MR DNA. In brief, 16S rRNA gene V4 region was subjected to 30 cycles using the HotStar Taq Plus Master Mix Kit (Qiagen, USA) under the conditions: 95˚C for 5, 30cycles of 95 ˚C for 30 seconds, 53 ˚C for 40 seconds and 72 ˚C for 1 minutes and elongation at 72 ˚C for 10 minutes. Amplicons were checked on 2% agarose gels to confirm the right sized band and relative intensity of bands. Samples were multiplexed using unique dual indices and were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads and the DNA library was prepared. Sequencing was performed on a MiSeq following manufacturer's guidelines. FASTQ files were used for further data analysis using Divisive Amplicon Denoising Algorithm (DADA2) R package. DADA2 was used for filtering, trimming, dereplicating, inference, merging pair-end reads and chimera identification and removal. Phyloseq another R package was used for visualizing the results. The poor-quality reads were removed. Forward and reverse reads were trimmed off at 240 and 200 bp respectively since quality towards the end drops off. Following the dereplication and inference

steps, the forward and reverse reads that overlapped were merged to create full, denoised paired reads. Subsequently, chimera were identified and removed by the consensus method. The obtained clean sequence variants were assigned taxonomy using a Bayesian classifier method on the manually curated Silva training set Fasta files (Callahan et al., 2016). Sequences identified as chloroplasts and mitochondria were removed. The resulting abundance and taxonomic classification were analyzed and plotted using phyloseq after rarefication. The results are presented in abbreviation form R1 to R18. Their original names are presented in appendix D.

3.4 Data Analysis

The descriptive statistics were performed on Excel 2016 (Microsoft Corp., USA). Other statistical analysis was performed using R open source version 3.6.3. Box plot was plotted in R using package Tidyverse. As data sets did not fit normal distribution, nonparametric analysis Wilcox rank sum test was carried out in R. Statistical significance was defined at 95% confidence intervals, $P < 0.05$. Microbial community analysis was carried out in R using DADA2 and Phyloseq packages.

4 RESULTS

4.1 Physical Contamination

Physical contamination is documented for 72 samples whereas chemical and biological contamination results are documented for 71 samples. On our field visit to a school during one of the trips, the compost bin contained only trash even though the bin was designated for food waste collection. Due to absence of food waste we were not able to document chemical and biological contamination results for that sample. It is not a common practice for hospitals in Maine to participate in source-separated food waste collection (Horton, Nadeau, Flynn, Patterson, & Kleisinger, 2019), therefore, only five hospital samples were collected in the state. Complete description of number of samples collected from each source types that were processed for physical, chemical and biological analysis are shown in Table 4.1.

Table 4.1: Total number of samples tested from each source types for each type of analysis

Source Types	Physical Contamination	Chemical Contamination	Biological Contamination
Grocery	14	14	14
Hospital	10	10	10
Residential	12	12	12
Restaurant	12	12	12
Retirement	12	12	12
School	12	11[1]	11[1]
Total	72	71	71

1: One of the school sample from Maine comprised of physical contaminants only without food waste. Thus, no chemical and biological tests were carried out on that sample. Equal numbers of samples were taken from each regulatory environment (regulated (MA/VT) and unregulated (ME) for each sample type.

Comparisons are made with the available regulatory limits for compost, where available, or biosolids, although our samples are feedstocks for composting or digestion. General physicochemical characteristics of the samples are presented in Table 4.2. The average C:N ratio was 20.31 but these ratios were highly variable. The average pH was 4.58. The nutrient content also varied significantly among samples. Food wastes usually have low C: N ratios (Bong et al., 2018). Usually pH in the range of 3.65 to 7.17 is reported for food waste (Bong et al., 2018). C: N and pH are crucial parameters for maintaining optimum operation in AD and composting (Bong et al., 2018; Cerda et al., 2018). Usually a C: N of 20-30:1 is recommended in both systems (C. Zhang, Su, Baeyens, & Tan, 2014; Zhu, 2007). The N content in food waste varies depending primarily on the protein content of the food (Bong et al., 2018). Food waste nutrient content is highly variable due to variation in collection type, food waste sources, time (festival, long holidays etc.), seasons, geographical locations and many other factors. Our result also shows variation in the nutrient content. They were collected at different times and from different locations. The food waste contents of the samples collected in this research were also different with few samples having only one kind of food whereas other samples had a combination of different types.

Table 4.2: Results of the compost test

Parameters	Range	Average ($\bar{X}$) n=71	S.D.	C.V.(%)
Conductivity (mmhos/cm)	1.90-12.10	7.02	2.04	29
Carbon (%)	29.40-66.90	47.86	5.48	11
Nitrogen (%)	0.53-9.08	2.81	1.31	47
C: N	5.52-81.70	20.31	10.46	52
pH	3.8-6.3	4.58	0.54	12
Phosphorus (%)	0.08-0.74	0.27	0.12	44
Potassium (%)	0.22-3.3	1.04	0.74	71
Magnesium (%)	0.03-0.26	0.11	0.05	45
Calcium (%)	0.04-16	2.11	3.99	189

Table 4.2 continued

Boron (ppm)	0.05-29	8.30	6.59	79
Iron (ppm)	13.10-546	71.19	80.77	113
Manganese (ppm)	-0.54-45	13.95	9.17	66
Total Solid (%)	5.90-79.20	26.23	11.94	46

As stated in the Methods section (3.2), our Robot- Coupe was not able to grind the compostable non-food materials. They were considered contaminants and removed prior to chemical and biological analysis. The wet weight of the contaminants was taken, and the percent contamination was calculated as follows:

$$\%\ Contaminants = \frac{CW}{W} * 100\%$$

Where: CW= Wet weight of contaminants

W= Wet weight of food waste + contaminants

Grocery stores in Maine (not regulated) did not separate their food waste from its packaging although they did segregate food (with or without packaging) from other kinds of waste. The facility that accepted grocery food waste from Maine has a de-packaging system to separate the food waste from packaging and other physical contaminants.

Eighty two percent of the samples had some form of non-food materials which included materials like plastics, napkins, coffee filters, wrapping papers, wood, plastic gloves and fruit stickers. regardless of the regulatory environment. Fifty-seven percent had non-compostable materials like plastic containers, candles, gloves and fruit stickers. Except for the one outlier (school, ME), the non-food materials accounted for up to 39% of the mass of the sample waste. More than 76% of the samples had less than 10% non-food waste by mass. A Wilcox rank sum test was conducted to see there was significant impact of regulatory environment on the mass of non-food contaminants in the samples. Grocery samples from Maine (non-regulated) were

removed because they all went to a facility with a de-packager, so separation from packaging was not required. The median mass of contaminants in food samples from Maine was significantly higher ($p = 0.05$) than that in the regulated states, indicating that waste generators in the regulated states were more efficient at source separation. Box plot is shown in Figure 4.1.

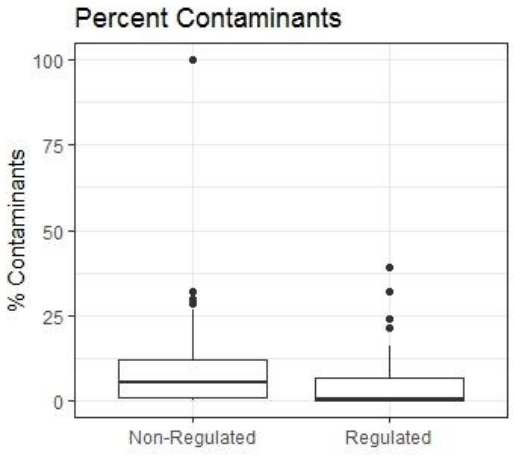


Figure 4.1: Percent by mass of contaminants in food waste samples from regulated and non-regulated states

The lower and upper bounds of the box are the 25th and 75th percentiles, the bold line is the median and the "whiskers" show the range. The data is presented for N= 35 in non-regulated state and N=36 in regulated state

The visible contaminants like paper towels, plastics, toothpicks and papers with prints may contain small amounts of heavy metals. Plastics may contain Cd, Cr and Pb (Chu et al., 2019; Stephen R Smith, 2009). Most oil based plastics are non-biodegradable (Jouhara et al., 2017). Additionally, plastic, glass and metals may pose an occupational risk to waste handlers (Stephen R Smith, 2009). Therefore, good sorting and processing are required to prevent their introduction into the compost. The presence of non-biodegradable materials can degrade the quality of compost produced.

4.2 Chemical Contamination

4.2.1 Heavy Metals

Out of the eight heavy metals analyzed, six (Cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), titanium (Ti) and tin (Sn)) were below the detection limit (2mg/kg) in all samples. Zinc (Zn) and copper (Cu) were the only ones that came out in measurable quantity. The average concentration of Cu in our study was 4.4 (range 0.251-13.8) mg/kg, and the average concentration of zinc was 21.19 (range 4.94-71.1) mg/kg. There were no significant differences between the copper concentrations in regulated vs unregulated samples. The median concentration in hospital food waste was lower than in residential or retirement community samples. The median of residential samples was higher than restaurants and schools as shown in Table 4.3.

Table 4.3: P-value as given by Wilcox Rank Sum test for Cu by source types

Source Types	Hospital	Grocery	Residential	Retirement	Restaurant	School
Hospital		0.138	**0.006**	**0.035**	0.123	0.197
Grocery	0.138		0.145	0.820	0.560	0.244
Residential	**0.006**	0.145		0.068	**0.033**	**0.003**
Retirement	**0.035**	0.820	0.068		0.154	0.196
Restaurant	0.123	0.560	**0.033**	0.154		0.880
School	0.197	0.244	**0.003**	0.196	0.880	

The highlighted values show significant differences at 95% confidence level between the row and column source types.

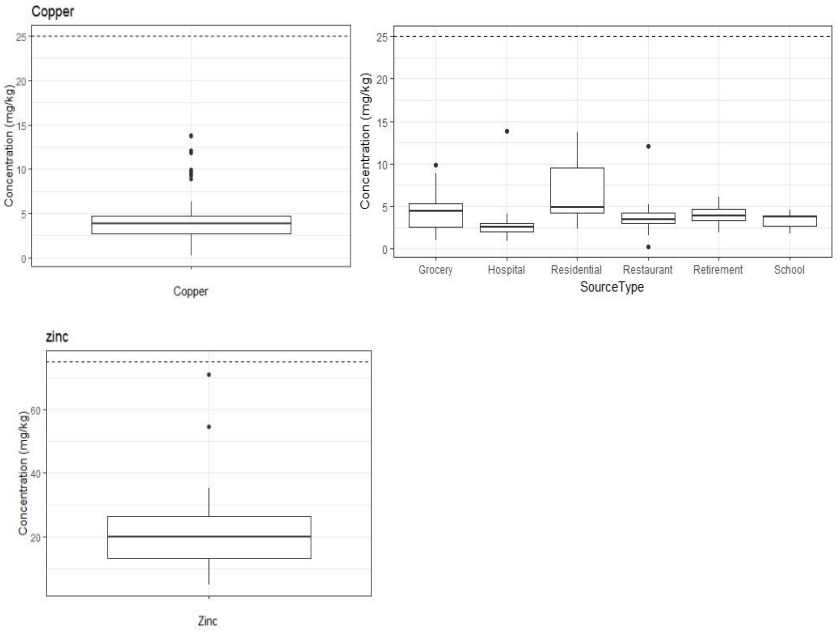


Figure 4.2: Copper and Zinc levels in food samples combined and by source type

Dotted lines show the most stringent regulatory limits imposed for compost. Cu: 25 mg/kg and Zn: 75 mg/kg (BOOM, Netherland). Box plot showing from left to right, a) Copper concentration n = 71 b) Copper concentration by source types n=10 for hospital, n=11 for schools, n =12 for residential, restaurant, retirement and n=14 for grocery c) Zinc concentration n = 71.

Source-separated household wastes have been reported to have smaller amounts of heavy metals than mechanically separated wastes (Amlinger et al., 2004; Logan et al., 1999; Richard T.L., 1992; Sharma V.K., Canditelli M., Fortuna F., 1997). Veeken & Hamelers (2002) compared the heavy metal contents in source-separated biodegradable waste with its background value in the constituent materials. They found that source-separated household biowastes (biological origin indoor and outdoor waste) were not contaminated from other sources. The concentration of heavy metals in our samples are similar to what has been reported in the literature for food (Burger & Gochfeld, 2005; Esposito et al., 2018; F. Mehari et al., 2015; Hadayat et al., 2018; Khan et al., 2015). The heavy metal content in the compost produced from source-separated feedstocks is the natural background concentration of feedstocks (Stephen R Smith, 2009). One of the reasons for low levels of heavy metals in our samples can be due to analysis of source-

separated food waste as mentioned in above studies. Also, we had removed non-food waste materials before processing the food waste. This might have affected the range of heavy metal levels in our food waste samples.

Heavy metals are recalcitrant and do not degrade in food waste treatment systems (Kupper, Bürge, Bachmann, Güsewell, & Mayer, 2014) such as composting or anaerobic digestion (Lin et al., 2018). The concentration of heavy metals may increase due to mineralization of the organic fractions but mobility decreases due to the formation of` organic-matter metal complexes due to the oxidation and microbial immobilization (Farrell & Jones, 2009; García, Hernández, & Costa, 1990). Logan, Henry, Schnoor, Overcash, & McAvoy, (1999) showed that the bioavailability of trace elements in compost derived from MSW (source-separated, or non-source-separated) was lower or similar to than in biosolids (Logan et al., 1999). With comparatively low metal concentration in our study and evaluating the existing literature we conclude there is relatively little risk associated with heavy metals with repeated application of treated source-separated food waste.

4.2.2 Extractable Organic Halides (EOX)

POPs are of persisting nature and have a long half-life. They are problematic because they have a tendency to accumulate in organisms (R. M. Clarke & Cummins, 2015). The majority of POPs are organohalogens, organic compounds containing some carbon-halogen (chlorine, bromine, fluorine) bonds. Some organohalogens are highly toxic, some are harmless, and some degradable, whereas some are highly persistent (Schowanek et al., 2004). EOX/AOX provides a bulk measure of the total sum of organohalogens except fluorinated compounds. Some of the compounds included in EOX are PCB, polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDFs), DDTs and many other compounds. (Rizzardini & Goi, 2014).

Most of the food waste samples we tested were below the detection limit (5 mg/kg). Ten out of seventy-one samples had measurable values. The range of EOX was 5 - 89.7 mg/kg ww (below detection -191 mg/kg dw), however only one sample was above 12 mg/kg. Currently, we are unable to state why the concentration of one of the food samples was so high. These concentrations were converted to dry weight and samples with detectable EOX are presented in Table 4.4. They are found to be low when compared with the adsorbable organic halide (similar to EOX but used for liquid samples) (AOX) limit of 500 mg/kg dw for land application of sewage sludge used in several European countries (Mininni, Blanch, Lucena, & Berselli, 2015). For statistical analysis, samples with EOX below the detection limit of 5 mg/kg ww were assigned a value of 2.5 mg/kg ww, which produced an average EOX concentration of 4.41 mg/kg ww (17.5 mg/kg dw). Wilcox rank sum test result shows that there was no difference between regulated and non-regulated samples.

Table 4.4: Detected EOX concentration

Sample	Regulation	EOX (mg/kg ww)	T.S.	EOX (mg/kg dw)
Hospital_2	Non-Regulated	6	0.37	16.22
Residential_5	Non-Regulated	10.9	0.34	32.06
Restaurant_2	Non-Regulated	89.7	0.47	190.85
Retirement_2	Non-Regulated	10.5	0.305	34.43
Retirement_3	Non-Regulated	7.3	0.33	22.12
Grocery_5	Regulated	5.2	0.06	86.67
Restaurant_3	Regulated	9.3	0.79	11.77
Retirement_2	Regulated	11.3	0.41	27.56
Retirement_5	Regulated	5.7	0.091	62.64
School_1	Regulated	5	0.31	16.13

Many organo-halogenated compounds are detected in food. More than 90% of human organochlorine exposure occurs through food (Ábalos et al., 2019; Fair et al., 2018; Ferrante et al., 2017; Schecter, Colacino, et al., 2010). EOX average values of 9.03 mg/kg dw and 9.79

mg/kg dw have been reported for sludges from domestic wastewater and agro-industrial waste treatment plants respectively (Rizzardini & Goi, 2014). Our average EOX values are higher than the above values on a dry weight basis. In another study by Lei, Raninger, Run-dong, & Yan-ji, (2008) AOX was detected in the range between 120-170 mg/kg d.w. in household organic wastes and 83-110 mg/kg dw. in compost derived from them. The EOX value reported in our study are well below regulatory limits but two of the detected EOX values (Table 4.4) are above the values listed in the aforementioned studies. Also, the removal of non-food waste especially packaging materials and plastics can lower the detection of EOX and PFAS in our study. This can be also be taken in another way: if we remove all the possible non-food waste materials or at least non-compostable materials, we can have lower contamination in our food waste and thereby increase the quality of the end products of recovery systems.

4.2.3 Perfluoroalkyl Substances (PFASs)

PFASs are fluorinated organic compounds that are lipophobic, hydrophobic but attracted towards protein. Some are highly toxic and some have bioaccumulation potential (Ahrens & Bundschuh, 2014; Haukas et al., 2007). Eight samples were selected for PFAS analysis. Two analytes were detected in our samples as shown in Table 4.5.

Table 4.5: PFAS compounds detected in the food waste samples

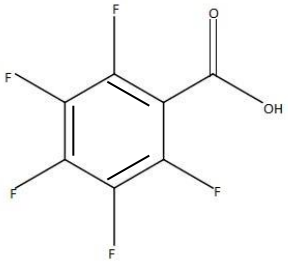
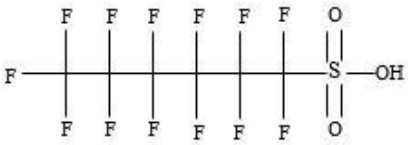
PFBA	PFHxS
	
Short chain compounds (4-C) Toxicity is equivalent to PFOA (Eun et al., 2020).	Long chain (6-C) Bioaccumulation potential (Ghisi et al., 2019).

Table 4.5 continued

It was detected in two food waste samples:	It was detected in one sample:
School food waste: 0.27 µg/kg ww (1.27 µg/kg dw) and Grocery food waste: 0.11 µg/kg ww (0.87 µg/kg dw)	School food waste: 0.15 µg/kg ww (0.7 µg/kg dw)

Fourteen different PFAS compounds were screened in eight samples (four from each regulatory environment) where only two PFAS compounds were detected in two samples.

Both of these samples were from Maine. In a market basket study conducted in Texas, only one sample (fish) contained detectable PFHxS (0.07 µg/kg ww) which is lower than our value (Schecter, Colacino, et al., 2010). In US municipal organic waste compost, PFHxS was found in between 0.17-0.25 µg/kg which is lower than our value (Choi, Lazcano, Youse, Trim, & Lee, 2019).

PFBA, which was detected in two of our samples, is one of the current substitutes for PFOA and PFOS. In a study on PFAS concentrations in eggs conducted in China, PFBA was the second most frequently detected compound, ranging between 1.75 to 110 µg/kg (Su et al., 2017). The low end of this range is an order of magnitude higher than the concentration in our samples. In US municipal organic waste compost, PFBA was detected at levels of 0.15-12.04 ng/g .Our samples are within this range (Choi et al., 2019).

The most widely used PFAS compounds in the past were PFOA and PFOS. These were not detected in any of the samples. Schecter, Colacino, et al. (2010) did not detect PFOS at high concentration in their market basket study, in contrast to past studies, likely due to the voluntary phase out of PFOS and PFOA. They did, however, detect PFOA in their food samples, which might have originated in the packaging materials. This suggests that since the voluntary phase-out and substitution by shorter chain PFAS, PFOA and PFOS are not as widely found in food as they were in the past when they were in wider use (Ghisi et al., 2019; Schecter, Colacino, et al., 2010).

Schaider et al., (2017) had shown the presence of PFAS in food packaging materials found in the US. As PFAS are widely used in food packaging materials (FCMs,) compost derived from them also shows higher PFAS value than the compost that does not have FCMs. Choi et al. (2019) found perfluoroalkyl acid (PFAA) loading in compost facilities that accepted FCMs were higher than in facilities that did not accept FCMs. Thus, food packaging may be a source of PFAS in food waste. Having removed the packaging materials before processing our food samples, we might have lowered the detection of PFAS in our study.

Amlinger et al., (2004) found that most organochlorine compound concentrations are higher in their feedstocks than in finished compost. Thermophilic temperature seems to be effective in removing volatile compounds while microbial reactions are effective at removing labile compounds. The maturation phase in composting can immobilize some recalcitrant POPs. POPs become bound to organic matter, at least in the short term, reducing their bioavailability (Farrell & Jones, 2009). S. R. Smith (2009) has pointed out the efficacy of aerobic processes in removing organic contaminants over anaerobic processes due to the greater range of metabolic pathways available under aerobic conditions. In contrast, dehalogenation is a possible biodegradation process to remove halogenated compounds under anaerobic conditions. However the poor bioavailability of some halogenated compounds can limit their susceptibility to dehalogenation (Stasinakis, 2012). Although defluorination is thermodynamically possible and could produce sufficient energy to support microbial growth, research has shown fluorinated compounds to be stable. The lack of defluorination and biodegradation of these compounds is probably due to the strength of C-F bond resulting very slow reaction kinetics (Stasinakis, 2012).

Environmental occurrence of many organic contaminants is low but they are still toxic at these levels (R. M. Clarke & Cummins, 2015). Food waste recycling is encouraged, and many countries and states in the US have mandatory landfill ban policies (Sandson & Leib, 2019).

Repeated application of treated wastes with even trace levels of bio-accumulative contaminants theoretically leads to accumulation of contaminants in soil that might adversely impact plant growth or enter into the food system (R. M. Clarke & Cummins, 2015). The emerging substitute short chain PFASs are relatively new and very little is known about them. At this point it is necessary to conduct more research regarding the short chain PFASs their environmental occurrence, toxicity and bioaccumulation properties. There is a lack of available information about the impacts and fate of PFAS, therefore it is impossible to judge whether the values we observed are safe.

One of the reasons for low detection of EOX and PFAS can be due to removal of non-food waste components. In a real scenario in a treatment system, papers, coffee filters and other non-food waste except the non-compostable waste are mixed with food waste. In some cases, the presence of small sized non-compostable food waste like fruit stickers, glass and plastic pieces make it difficult to separate completely. Separation of such physical contaminants in our study can influence the EOX and PFAS levels as some of those physical contaminants can act as sources of these contaminants.

4.3 Biological Contaminants

4.3.1 Antibiotic Resistance Genes (ARGs)

Three ARGs were screened in our study. They are a tetracycline resistance gene (*tet*(M)*)*, a beta-lactam resistance gene (bla_{TEM}) and a colistin resistance gene (*mcr-1*). *Mcr-1* confers resistance to polymyxin E, which is considered the last resort drug to combat multidrug-resistant pathogens. Plasmid-borne *mcr-1*-mediated resistance has recently spread throughout the world (Caniça et al., 2019). No *mcr-1* was detected in any of our samples, which was similar to the results of a study by Mavrici et al., (2017) who screened 1000 *E. coli* isolates obtained from wildlife, produce and environmental samples for *mcr-1,* and all came out negative in their study.

Bla$_{TEM}$ confers resistance to beta-lactams which include penicillin and its derivatives, cephalosporins and carbapenems (Rood & Li, 2017). *Bla*$_{TEM}$ was detected in 69 out of 71 samples making a prevalence of 97% in this study, although six of these detections were below the limit of quantification. *Bla*$_{TEM}$ is widely detected. For example, in a study by Thai et al. (2012), it was found in 90% of the *Salmonella* isolates obtained from raw retail beef (Thai et al., 2012). The absolute abundance of *bla*$_{TEM}$ in our samples was from non-detection to 6.66 x 10^{9} copies per gram (dry weight) of food waste with an average of 6.81 x 10^{8} copies/gdw. Its relative abundance, meaning the number of genes per microbe (measured as small subunit rRNA genes), ranged from non-detection to 1.03 with an average of 2.69 x 10^{-2} as shown in Figure 4.3). There was no significant difference between sample regulatory environment or source types as shown by Wilcox sum rank test at 95% confidence interval.

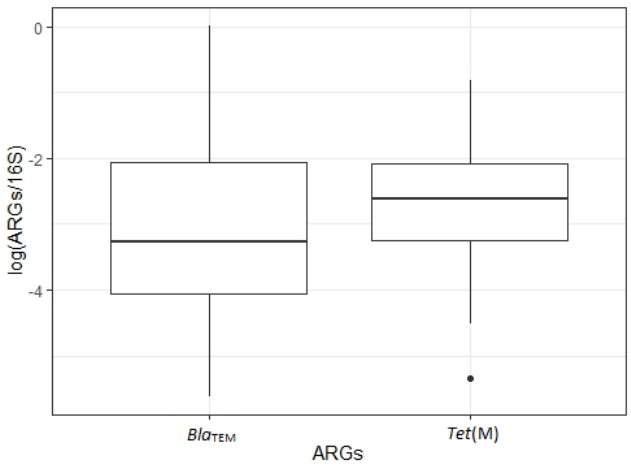


Figure 4.3: Log relative abundance of *tet*(M) and *bla*$_{TEM}$ genes

The data were log transformed prior to make the box and whisker plots. In the figure N=71.

Tetracycline resistance genes are present abundantly in food and foodborne bacteria. *Tet*(M) has a wide range of host genera (>42) which includes both Gram positive and Gram negative and aerobic as well as anaerobic bacteria (Lee et al., 2017). Sixty-eight (96%) of our

samples were positive for *tet(*M). However, 11 were below our limit of quantification. The relative abundance of *tet*(M) was from non-detection to 1.53×10^{-1} copies/gdw with an average of 9×10^{-3} copies/gdw. The mean relative abundance of *tet*(M) in greenhouse soil was found to be 1.96×10^{-3} and in agricultural soil in China was found to be 1.64×10^{-3} (Zeng, Sun, & Zhu, 2019). *Tet*(M) was detected in all the analyzed 51 soil samples from the agricultural field.

The absolute abundance of *tet*(M) ranged between non-detection to 1.53×10^{10} with an average of 6.79×10^{8}. This value is less than reported by Liao et al., (2019) for food waste samples in China which was around 2×10^{9} copies/gdw. In their study on the fate of ARGs in composting, they found that tetracycline and macrolide resistance genes were the most prevalent in the initial days of food waste composting, but their relative abundance decreased at the later stages of treatment. Wilcox rank sum test showed no significant difference between regulatory environments and source types other than between hospital and grocery with median high in hospital.

There is an absence of unequivocal agreement on the survival of ARGs during treatment, with some systems favoring the degradation of ARGs (as observed in the Liao et al., (2019) study) whereas some show increases in ARGs (J. Zhang et al., 2016). Wide ranges of detection of ARGs and uncertainty about the fate of ARGs during treatment makes it difficult to assess their impact on the sustainability of food waste recycling efforts. The detection of ARGs in food animals and fresh produce, however, increases the risk that they will be present in food waste acting as feedstocks to composting and AD operations, and subsequently in organic amendments to the soil. This could lead to proliferation of ARGs in the environment and increase the probability of acquisition of resistance to antibiotics by human pathogens.

4.3.2 Pathogens

All samples were subjected to qPCR for three foodborne pathogens as described in the methodology section 3.3.7. The screened foodborne pathogens were *L. monocytogenes* (due to its ability to grown even in low temperature <4˚C), non-typhoidal *Salmonella* (highest number of deaths in 2017) and STEC (common foodborne pathogen). STEC was not detected in any of our samples, although we were able to detect and accurately quantify control DNA spiked into food sample DNA extracts. Two samples produced a low signal for *Salmonella* and were confirmed to be positive for *Salmonella* by electrophoresis and Sanger sequencing. The copy numbers were not quantified, however, because they were below our limit of quantification. The two samples that were positive for *Salmonella* were a grocery food waste sample from ME and a residential sample from MA. This gives a total of 2.81% positive samples in our study. Similarly, 8 samples were detected positive for *L. monocytogenes.* Although five of these were below the quantification limit, they were confirmed positive for *L. monocytogenes* as described above. Two came from hospital samples, one was from a residential sample and five were from grocery samples.

Miller, Heringa, Kim, & Jiang (2013) showed no detection of *E. coli* (pathogenic), *Salmonella and L. monocytogenes* in organic compost whereas Sundberg et al. (2011) showed detection of pathogens in both source-separated feedstocks for compost and finished compost (Miller et al., 2013; Sundberg et al., 2011). Similarly, the occurrence of foodborne pathogens in food varies in the literature ranging from non-detection to >10% detection from around the world (Bilung et al., 2018; Denis et al., 2016; Ilic et al., 2008; Mcauley et al., 2014; Mukherjee et al., 2006; Panel HanaVojkovská et al., 2017; Sonnier et al., 2018; Yang et al., 2016; C. Zhao et al., 2001). This illustrates the episodic nature of contamination with pathogens and the inherent variability of food waste contamination. We seldom detected foodborne pathogens in our study which can be killed at temperature >65 ˚C.

4.3.3 Next Generation Sequencing

Eighteen samples were subjected to next generation sequencing of the V4 region of 16S rRNA gene to determine the microbial community composition and screen for genera containing known foodborne pathogens. This was undertaken to see if there were potential pathogens that did not belong to the groups we screened by qPCR, which has the advantage of a low detection limit and the ability to discriminate between strains. We obtained 1960 sequence variants after quality control, chimera removal, excluding chloroplasts and mitochondria which most likely came from the food, and rarefication steps. By far, the most abundant phyla were Proteobacteria, and Firmicutes (Figure 4.4), with some samples showing high abundances of Bacteroidetes and Actinobacteria. This result is similar to the phyla reported to be in compost (Cerda et al., 2018). *Bacillus*, *Clostridium*, *Escherichia/Shigella*, *Proteus*, *Staphylococcus*, *Vibrio* and *Yersinia* are some of the genera that are associated with foodborne bacteria (Bintsis, 2017) and that were detected in our samples. A similar result was reported for foods obtained from market in India (Keisam, Tuikhar, Ahmed, & Jeyaram, 2019).

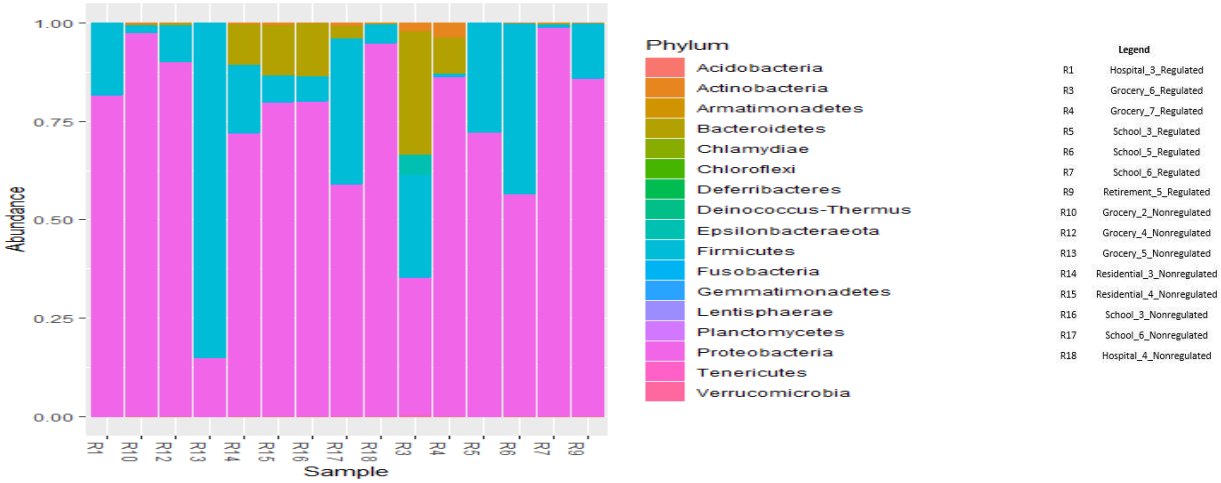


Figure 4.4: Relative abundance by phylum

Three samples were eliminated due to low bacterial abundance.

All of the detected pathogenic genera except *Vibrio* were present in sample grocery_6 from regulated state. *Vibrio* was present only in grocery_5 from non-regulated state (0.027%). *Yersinia* was present in all the samples except for grocery_7 from regulated state with highest abundance in grocery_2 from non-regulated state (15.06%). Residential_4 from non-regulated state had the highest abundance of *Proteus* (4.13%) and *Staphylococcus* (0.22%) among the 15 samples. The taxonomic table did not distinguish between *Escherichia* and *Shigella*. *Escherichia/Shigella* was present in three samples grocery_6 (regulated) (0.02%), grocery_7 (regulated) (0.03) and hospital_4 (non-regulated) (0.64%). Residential_3 (non-regulated) and grocery_6 (regulated) had the highest abundance of *Bacillus* and *Clostridium* respectively. These reported genera have both non-pathogenic strains and pathogenic strains. Thus, this method only gives idea list of potential pathogens that might be present in our food waste.

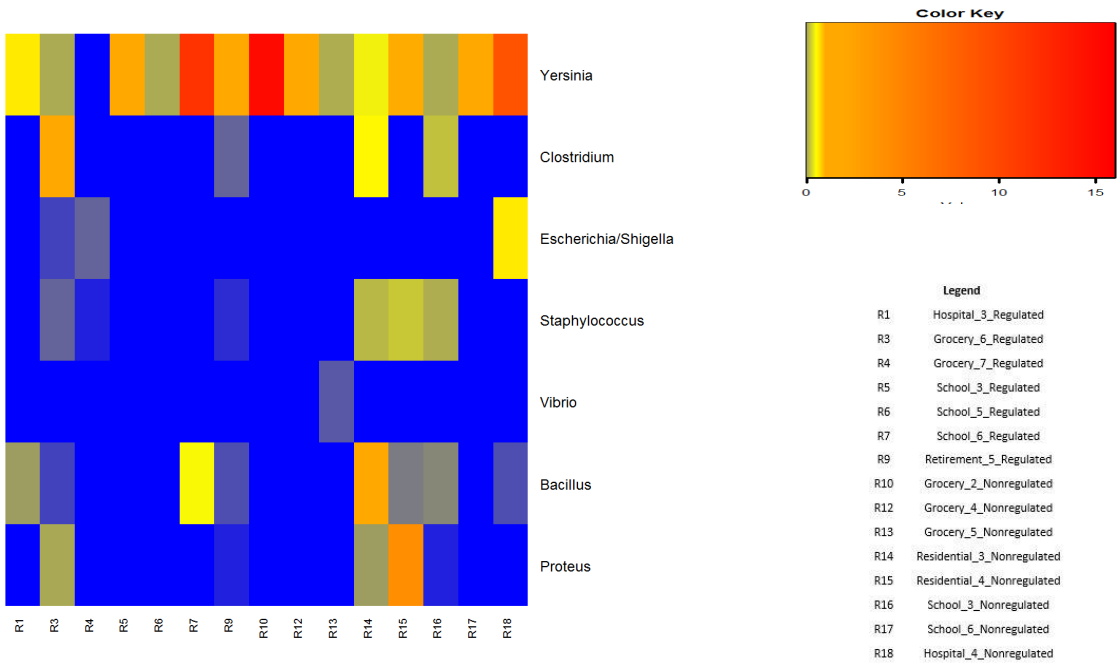


Figure 4.5: Heat map showing foodborne pathogens present in 15 food waste

Abundance from 0-0.05% is shown by blue gradient, 0.05% -1% by yellow gradient and any values above 1% is shown by red gradient.

Foodborne pathogens like *Salmonella*, *Campylobacter* and *E. coli* (pathogenic) have caused multiple outbreaks, illnesses, hospitalizations and deaths (CDC, 2017, (Fegan & Jenson, 2018). Although, the identification was not carried up to species level, genus level identification also gives insight regarding the potential for occurrence of foodborne pathogens. Depending on the operational parameters like temperature, aeration rate, moisture content, C: N and nutrient contents in treatment systems microbes may either be inactivated or persist in compost or digestate (Gurtler et al., 2018; Sahlström, 2003). Some foodborne pathogens like *Campylobacter* can be thermotolerant as well (Heredia & García, 2018). The use of improperly treated organic waste can be a potential source of foodborne pathogens in our food supply chain (Miller et al., 2013; Sahlström, 2003). Another issue in the detection of such diverse genera of foodborne pathogens in samples that also contain ARGs is the possibility of acquisition of ARGs by pathogens. Foodborne pathogens can be naturally antibiotic resistant or can acquire ARGs through horizontal gene transfer during composting or anaerobic digestion (Ezzariai et al., 2018; Liao et al., 2019). Thus, our composting or anaerobic digestion can enable the exchange of ARGs among the bacteria. With conflicting reports on the survival of pathogens and ARGs in food waste treatment systems, the likelihood of repeated land application of treated food waste residuals serving as an entry point of ARGs and pathogens to the food system remains unknown. The potential for this issue requires more research.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Source-separated food waste was collected from MA, VT, where food waste separation is mandated and from ME, where participation in food waste recycling is voluntary. The samples were screened for physical contaminants, heavy metals, halogenated compounds, ARGs and pathogens. Our initial hypothesis was that the mandate to divert food waste could result in a more contaminated waste stream due to lower intrinsic motivation to participate and even the possibility of resentment at the requirement. Our results did not support this hypothesis. The only statistically significant difference in contamination among the regulatory environment was a higher mass of non-food waste stream in the samples from Maine (voluntary participation). This result could point to effective communication strategies in the roll-out of the food waste diversion efforts in MA and VT.

Visible plastic contamination was found in 57% of the samples. this included materials like coffee cups, lids, packaging, fruit stickers and plastic gloves. Physical contamination, by mass, was higher in Maine than in regulated states. One of the possible ways to reduce the physical contamination would be educating people about the consequences of including non-compostable food waste in the food waste stream. People should be encouraged to do source separation work properly.

The qPCR results showed a high prevalence of ARGs and sporadic presence of foodborne pathogens. *Tet*(M) and bla_{TEM} were present in 96% and 97% of samples respectively. Fortunately, the last-resort colistin resistance gene m*cr-1* was not detected in any of our samples. *Salmonella* was present in two samples and *L. monocytogenes* was present in eight samples. STEC was not detected in any of the sample. Next generation sequencing showed the presence of several genera that contain foodborne pathogens. They are: *Bacillus*, *Staphylococcus*, *Vibrio*,

Yersinia, *Escherichia/Shigella*, *Clostridium* and *Proteus*. The abundance and prevalence of ARGs in samples that also contain pathogens poses a risk of transfer into pathogens, which could result in difficulty treating infections. This is of serious concern as it would reduce the effectiveness of human and veterinary antibiotics against those pathogens over time.

Heavy metal contamination seems to be less problematic in source-separated food waste. All the heavy metals except for Zn and Cu were under the detection limit. All the heavy metals were under the most stringent regulatory limits for compost except the detection limit for Cd using the USEPA method (2 mg/kg) was higher than the more stringent regulatory limit used in Netherland for compost application on organic farms for Cd (0.7 mg/kg). Cd was still under regulatory limits used in the United States and Canada.

There was occasional detection of EOX in this study. PFAS was also detected in two out of eight samples being screened. The concentration of PFAS and most of the EOX values were within the range and low as compared to their values in sludge and compost (Choi et al., 2019; Lei et al., 2008; Rizzardini & Goi, 2014), however we did have some EOX and PFHxS values in excess of levels observed in sludge and organic waste. Although on the surface this might look like low detection, they may be detrimental even in very small concentrations and have the potential to accumulate over time. Very little is known about PFBA and PFHxS which were the PFAS compounds detected in our study. Hence, it is hard to determine what level of detection can be deemed safe.

We had removed all the non-food waste materials before carrying out physical and chemical contamination analysis. This might have caused a lower detection of some contaminants especially halogenated compounds and heavy metals. Separation of non-food waste components helps reduce trace chemical contamination issue. One of the ways to achieve better quality in the end products is removal of non-food materials before the treatment.

The contaminants' survival and transfer into the food system is determined by their fate in the treatment system it is subjected to, and uptake by the plants where the residuals are applied (Bloem et al., 2017). The fate of ARGs and trace organics like PFAS and EOX constituents during treatment is still an area of active research with different studies showing conflicting results (Choi et al., 2019; Liao et al., 2019) . Other than PFOS and PFOA, there is not much available information about PFAS compounds in various matrices. When compost or digestate containing trace contaminants is added as an organic amendment repeatedly, the concentration of contaminants is amplified over time. This poses a threat to the food system. This research makes a case for the need to conduct more research on the nature and fate of ARGs in the feedstocks, treatment systems and final products. Also, this research finds an immediate need to determine the safe level of PFAS, especially the new PFAS substitutes, in compost and anaerobic digestor feedstocks and final products.

5.2 Recommendations

One of the avenues to reduce and divert food waste can be an organic waste ban policy hence diverting food waste out of landfill. Different reuse/recycling opportunities can be promoted to achieve this goal. In our study, we saw that food waste quality was similar regardless of regulatory environment. Initially, we hypothesized that voluntary participation would achieve better quality as participants are more interested and invested in the outcome, and likely more informed about where the materials are going. But this was not the case. In contrast, if there were any differences, the samples from regulated states were actually "cleaner" – this could be due to outreach campaigns and efforts by waste managers to improve the quality of the collected materials. Thus, there seems to be no indication that mandating food waste diversion from landfill causes food waste quality reduction as long as regulation is accompanied by strong outreach

efforts to ensure the quality of the collected food waste. Landfill bans can help to increase the reuse/recycling rates and minimize the food waste.

After the introduction of the organic waste ban in Massachusetts, there was an increase in the amount of food diversion to both organic waste processors and food rescue organizations (Sandson & Leib, 2019). At the same time, there was a positive economic impact due to the generation of over 900 jobs and over $5 million in state and local tax revenue in 2016. If we analyze from an environmental protection viewpoint, the policy helped to reduce hauling food waste to landfill, conserved landfill space, and reduced greenhouse gas generation by avoiding the production and release of methane from landfill. Thus, enforcement of organic waste ban policy can be helpful in reducing food waste and reusing/recycling food waste while creating positive economic and environment impacts.

We collected food waste from the consumer level. Some of the physical contaminants that we encountered in our study were packaging materials, coffee cups, plastic straws, gloves and coffee lids. Also, in our results we saw that non-regulated samples had more physical contamination than regulated samples. Physical contaminants are potential sources of PFASs and EOX in food waste. We also saw that two of the EOX values were similar to values found in sewage sludge. This indicates that there are existing pathways of contamination of food wastes with organohalogen compounds that could pose a threat. Still, the better the source separation job at generator level, the better the quality of food waste, which results in better compost or digestate. Generators must be provided with education and outreach related to food insecurity, food waste prevention, and clear guidance on what should be composted and what should not. They should be motivated, encouraged and incentivized to participate in source separation of food waste.

Once food waste reach recycling facilities, physical contaminants can be hard to separate from the food waste. Some recycling facilities can remove some physical contaminants like packaging materials; however, this requires a large investment in equipment and its operation. However, even after removal of physical contaminants, chemical and biological risk still persists. As we saw, food waste contains chemical and biological contaminants, some of which can even persist in the end products. The recycling operators should play an important role in ensuring the source separation is carried out diligently. They can include provisions such as limiting the contaminant load in the food waste or financially penalizing generators who don't adhere to proper source separation. If legislators restrict the level of contaminants in the food waste, this can encourage the recycling operators to, in turn, invest more in ensuring appropriate source separation at the generator level (Sandson & Leib, 2019).

We saw some pathogen contamination in the food waste. Some of the safe practices for handling waste in recycling facilities to avoid of contracting biological contaminants can include use of gloves, and masks, and processing waste soon after arrival on site. At the consumer and recycling operator levels, one of the ways to lower the risk of transfer of ARGs to pathogens can be accomplished by limiting growth of pathogens by minimizing the time in trash bin or recycling facilities at room temperature. Food waste can be put at low temperature which restricts the growth of most foodborne pathogens. However this approach is unlikely to be feasible, so restrictions on non-therapeutic use of antibiotics would be a more practical method of reducing the overall burden of ARGs in the environment and our food system, and continued attention should be given to reduce the transmission of foodborne pathogens through the food production, processing and retail stages.

For now, to mitigate the unknown associated with halogenated compounds and ARGs, separation of non- food waste, especially food packaging materials, antibiotics, personal care

products, plastics, and disinfectants should be encouraged. However, as seen from the literature review, food waste contains ARGs and halogenated compounds, pointing to the need to find ways to reduce contamination in the food system. Antibiotics should only be used for therapeutic use and should not be used as a growth promoter in animals. This can limit the proliferation of ARGs in the environment and food. We have identified the sources of heavy metals, halogenated compounds, ARGs and pathogens in our literature review. Those sources need to be monitored closely and carefully. If there are any existing regulations on such sources, they should be strictly enforced and monitored. There seems to be inadequacy of information in identifying sources accurately, which needs further research and study especially at retail levels.

5.3 Limitations of our Dataset

While processing food waste samples, we separated the non-food waste materials like plastics, paper, coffee filters, packaging materials, plastic straws etc. In practice, only non-compostable waste is separated by pre-processing most wastes, and not completely efficiently. Sometimes, non-compostable waste like small glass particles and plastics can also get into food waste and separating them can be difficult or impossible. The degree to which physical contaminants contribute to the chemical and biological contamination of the waste has been overlooked in our study due to technical limitation of the processing machine in use. Hence our data could have looked slightly different had we included those components in the analysis. Also, we only collected 36 food waste samples from regulated states and 35 food waste samples from non-regulated state for chemical and biological analyses. With limited statistical power, we could have missed smaller effects of regulation on the contamination of waste materials. Also, we collected samples directly from the generators bins to see the effects of source type, rather than at processing facilities where sampling would have integrated wastes from all sources. Thus, the analysis from individual bins do not reflect the overall quality of the waste generated in a

particular state or regulatory environment and doesn't account for differences in generation rates. Since the source type did not yield significant differences, waste samples in future work should be taken from processing facilities, perhaps after the initial processing steps, to better integrate and represent overall food waste from the particular state.

5.4 Future Research

As a next step, samples from large food waste processing facilities could be evaluated. This should better quantify the level of contaminants in the food waste in different states or regulatory environments. There is lack of knowledge hence lack of consensus, on the fate of ARGs and halogenated compounds during treatment, thus paired sampling of inputs and outputs of treatment should be evaluated. The current presence of contaminants that can accumulate in food and food waste indicates there are current pathways of contamination that should be examined more closely to develop procedural and policy mechanisms to reduce the risk of contamination. Greater clarity in these areas will contribute to the creation of a more sustainable system where food waste residuals can be returned to the land safely.

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APPENDIX A- PHYSIO-CHEMICAL CHARACTERISTICS OF FOOD WASTE

Table A.1: General Compost Characteristics

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Hospital	1	Regulated	6.68	4.3	28.5	13.63	2.8	58.1	4.73	0.21	0.51	0.05	0.25	3.85	83.6
Hospital	2	Regulated	11.1	5.1	25.3	1.31	5.9	56.9	4.08	0.4	0.75	0.11	0.4	6.71	34.7
Hospital	3	Regulated	8.47	4.4	26.6	0.00	8.1	50.6	4.81	0.34	0.82	0.09	0.19	3.45	32.7
Hospital	4	Regulated	32	4.5	32.9	31.76	3.6	54.2	2.04	0.13	0.24	0.12	0.13	3.04	35.7
Hospital	5	Regulated	13.9	4.3	35.9	0.00	6.9	46.3	2.68	0.23	0.4	0.06	0.09	1.43	31
Hospital	6	Non-Regulated	-0.5	5.5	29.3	0.00	9.5	56.2	6.28	0.51	0.5	0.04	0.28	1.21	26.9
Hospital	7	Non-Regulated	4.7	4.3	37.3	1.27	9.6	48.4	3.2	0.26	0.72	0.05	0.09	1.04	27.7
Hospital	8	Non-Regulated	4.57	4.5	26	0.00	7.3	49.8	3.5	0.29	0.79	0.05	0.12	3.11	28.3
Hospital	9	Non-Regulated	5.84	4.6	20.1	2.06	8	48.6	3.52	0.22	1.24	0.13	0.22	4.42	121

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Hospital	10	Non-Regulated	10.7	4.1	23	0.00	7.9	50.4	3.59	0.31	0.65	0.08	0.08	2.01	44
Grocery	1	Regulated	14	4	10.5	0.00	4.4	45.5	1.44	0.2	1.06	0.09	0.2	12.6	220
Grocery	2	Regulated	6.14	4.2	36.6	0.04	8.5	49.9	1.82	0.15	0.62	0.05	0.04	9.89	38.3
Grocery	3	Regulated	38.1	4.4	10.8	2.11	8.1	43	2.1	0.31	2.59	0.22	0.7	18.1	125
Grocery	4	Regulated	6.75	4.3	28.7	0.00	5.5	48.2	2.3	0.16	0.51	0.04	0.06	4.44	40.8
Grocery	5	Regulated	19.5	4.4	5.9	0.00	6.1	45	2.76	0.31	2.98	0.21	0.37	14.5	88.5
Grocery	6	Regulated	29.2	3.8	8.1	0.11	6.1	47.4	2.74	0.32	2.86	0.24	0.72	29	188
Grocery	7	Regulated	19.5	4.5	10.6	0.15	7.3	45	4.36	0.56	3.3	0.26	0.51	26.1	64.4
Grocery	8	Non-Regulated	2.38	4.2	41.4	2.70	5.5	60.3	2.44	0.25	0.36	0.04	0.15	2.06	47
Grocery	9	Non-Regulated	9.28	4.2	25.4	8.06	3.2	43.3	0.53	0.08	0.73	0.06	0.12	4.05	15.5
Grocery	10	Non-Regulated	29.8	4.2	9	0.86	4.8	46.4	2.42	0.34	2.13	0.2	0.53	19.5	285
Grocery	11	Non-Regulated	15.8	4.1	10.4	7.71	6.1	55.1	2.13	0.24	1.5	0.12	0.32	10.5	52.4

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Grocery	12	Non-Regulated	10.8	4	30.3	10.95	9.4	48.8	3.98	0.18	0.8	0.08	0.36	4.57	28.5
Grocery	13	Non-Regulated	7.47	4.2	12.7	0.90	4.6	49	2	0.23	1.2	0.11	0.3	16.4	28.1
Grocery	14	Non-Regulated	12.5	4.3	28.1	8.94	5.4	46.8	1.89	0.2	0.65	0.07	0.19	5.41	51.8
Residential	1	Non-Regulated	23.6	6.3	20	22.88	4.7	45.4	1.95	0.18	1.61	0.2	5.6	20.5	64.2
Residential	2	Non-Regulated	11.1	4.4	13.9	1.35	5.8	46.3	1.72	0.19	2.2	0.12	2.3	13.1	40
Residential	3	Non-Regulated	28.1	4.5	26.7	15.13	5.4	49.4	2.66	0.21	0.86	0.11	0.84	5.99	56.3
Residential	4	Non-Regulated	8.63	4.2	27.6	2.90	5.7	49.4	4.97	0.32	0.88	0.07	0.15	3.26	36.4
Residential	5	Non-Regulated	18.9	3.8	26.2	0.08	9	52.9	2.21	0.23	0.83	0.1	0.39	8.2	83.2
Residential	6	Non-Regulated	14.7	5.6	30.5	6.90	6.3	49.1	1.92	0.65	0.88	0.14	2.7	26.5	48.4
Residential	7	Non-Regulated	26.8	4.8	10.2	29.71	5.7	46.9	2.24	0.2	1.49	0.16	0.74	9.86	83.5

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Residential	8	Non-Regulated	20.1	5.4	29.1	6.82	7.2	43.7	1.91	0.17	1.44	0.14	9.6	12.6	26.4
Residential	9	Non-Regulated	15.5	4.5	27.9	19.93	8.4	50.4	3.73	0.27	0.81	0.1	0.7	4.02	51.1
Residential	10	Non-Regulated	12.4	5.2	19.4	28.29	10.2	48.5	2.91	0.4	1.52	0.11	3.1	9.68	104
Residential	11	Non-Regulated	9.41	4.1	34	6.55	6.9	47.7	1.97	0.26	0.5	0.05	1	4.24	28.3
Residential	12	Non-Regulated	9.68	5	28.6	6.90	8.6	43.9	2.12	0.17	0.96	0.1	3.5	6.68	38.7
Retirement	1	Regulated	6.33	4.4	48.7	4.75	1.9	66.9	1.65	0.12	0.52	0.04	0.08	2.3	48.2
Retirement	2	Regulated	6.04	4.1	40.9	0.70	5.3	52.5	2.51	0.24	0.49	0.06	0.12	1.81	37.4
Retirement	3	Regulated	9.97	4.2	22.1	1.93	5	49.8	1.61	0.23	1.19	0.11	0.24	9.08	42.5
Retirement	4	Regulated	4.89	4.5	18	0.00	7.1	50.1	9.08	0.36	0.75	0.06	0.14	2.87	34.3
Retirement	5	Regulated	31.8	3.9	9.1	0.00	7.2	42.4	2.52	0.35	3.16	0.21	0.84	20.6	89.8

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Retirement	6	Regulated	32	4.4	10.6	0.00	5.4	43	2.76	0.33	2.41	0.22	0.67	19.9	49.2
Retirement	7	Non-Regulated	27.1	4.4	16.4	5.07	5.8	49.5	2.64	0.24	0.91	0.09	0.23	7.32	51.2
Retirement	8	Non-Regulated	12.1	5.1	30.5	13.64	10.3	40.6	3.21	0.74	0.77	0.13	7.4	10.2	184
Retirement	9	Non-Regulated	9.38	4.5	33.2	7.01	9	51.3	5.86	0.3	0.56	0.06	0.08	1.13	77.6
Retirement	10	Non-Regulated	8.43	4.3	24.7	1.07	6.5	46.7	2.87	0.22	0.75	0.07	0.13	5.07	63.4
Retirement	11	Non-Regulated	14.8	4.3	21.4	9.99	6.1	48.8	2.07	0.34	0.72	0.09	0.52	5.55	36.5
Retirement	12	Non-Regulated	11.5	4.4	22.2	0.60	6.2	48.4	3.19	0.31	1.1	0.07	0.27	4.19	37.7
Restaurant	1	Regulated	8.57	4.5	22	7.09	12.1	54.5	2.82	0.37	0.96	0.07	1	4.44	56.2
Restaurant	2	Regulated	18.7	6.3	27.9	21.41	7.3	29.4	1.1	0.12	0.81	0.18	16	8	22.4
Restaurant	3	Regulated	2.06	5.4	79.2	3.90	4.5	46.9	0.87	0.12	0.22	0.03	0.04	0.05	13.1

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Restaurant	4	Regulated	9.1	5.7	34.5	4.74	7.4	48.9	3.01	0.22	0.5	0.09	11	17.1	293
Restaurant	5	Regulated	14.6	4.2	20.9	0.00	8.4	44.1	1.88	0.21	1.27	0.1	0.08	6.4	26.9
Restaurant	6	Regulated	10.6	5.2	32.7	39.01	10.1	45.5	2.75	0.23	0.73	0.11	12	9.61	49
Restaurant	7	Non-Regulated	5.93	5.2	44	2.20	7.6	42.3	1.57	0.15	0.22	0.1	10	2.1	34.7
Restaurant	8	Non-Regulated	45	4.9	47	31.99	9.9	49.9	2.26	0.14	0.37	0.05	3.9	4.8	89.6
Restaurant	9	Non-Regulated	9.19	5.2	38.6	17.77	10.8	38.9	2.02	0.16	0.69	0.13	13	14.9	53
Restaurant	10	Non-Regulated	17.4	5.1	25.9	0.66	5.6	49.6	2.11	0.14	0.82	0.1	0.85	7.29	40.9
Restaurant	11	Non-Regulated	14.3	4.1	11.6	0.44	8.4	40.8	2.4	0.3	1.7	0.11	0.42	11.9	57.6
Restaurant	12	Non-Regulated	5.78	5.2	39.8	5.94	7.3	35.6	1.97	0.16	0.5	0.17	15	10.3	18.7
School	1	Regulated	8.52	4.3	31.3	0.00	9.8	52.6	4.42	0.43	0.62	0.09	0.17	2.28	53.5

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
School	2	Regulated	9.22	4	22.1	15.74	5.8	47.3	1.92	0.21	1.04	0.08	0.34	13.4	95.2
School	3	Regulated	30.1	4.3	17.7	0.27	5.1	46.5	2.27	0.18	0.71	0.13	0.15	6.86	37.1
School	4	Regulated	17.1	4.3	32.7	23.93	7.8	48.3	2.81	0.33	0.82	0.08	0.27	5.97	30.9
School	5	Regulated	6.87	4.4	22.6	10.26	7.2	48.2	2.37	0.28	1.71	0.12	0.26	6.67	39.6
School	6	Regulated	7.5	5.5	32.3	0.00	8.4	36	1.93	0.23	1.43	0.21	13	8.63	38
School	7	Non-Regulated	12.1	4.6	28.3	0.87	7.6	47.6	3.2	0.36	0.62	0.09	0.32	1.31	44.8
School	8	Non-Regulated	8.98	4.2	15	2.52	5.6	44.4	3.19	0.29	1.14	0.1	0.73	12.2	24.9
School	9	Non-Regulated	15.3	4.5	25.4	0.00	10.9	48.7	3.56	0.27	0.78	0.07	0.13	5.4	546
School	10	Non-Regulated	6.74	4.8	44	6.28	8.7	48.9	3.44	0.37	0.4	0.06	0.48	2.56	32.5
School	11	Non-Regulated	15.1	4.8	21.3	0.00	8.1	46	3.73	0.2	0.84	0.12	2.6	5.27	206

Source	S. N.	Regulation	Mn(ppm)	pH	Total solid (%)	Contaminants (%)	Conductivity (mmhos/cm)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Boron (ppm)	Iron (ppm)
Mean			14	4.58	26.2	6.757	7.02	47.9	2.806	0.267	1.04	0.11	2.106	8.303	71.2
Standard Deviation			9.17	0.54	11.9	9.361	2.04	5.48	1.31	0.118	0.70	0.05	3.99	6.59	80.8
Maximum			45	6.3	79.2	39.01	12.1	66.9	9.08	0.74	3.30	0.26	16	29	546
Minimum			-0.5	3.8	5.9	0	1.9	29.4	0.53	0.08	0.22	0.03	0.04	0.05	13.1

APPENDIX B- CONTAMINANTS PRESENT IN FOOD WASTE

Table B. 1: Contaminants Present in Food Waste

Source Types	SN	Regulation	Cu (ppm)	Zn (ppm)	EOX (mg/kg)	*tet*(M)	Bla_{TEM}	*Mcr-1*	*STEC*	*L. monocytogenes*	*Salmonella*
Hospital	1	Regulated	1.54	26	<5	0.002	0.000285	(-)	(-)	(-)	(-)
Hospital	2	Regulated	4.1	28.1	<5	3E-05	9.28E-06	(-)	(-)	(-)	(-)
Hospital	3	Regulated	2.46	22.9	<5	<LOD	2.17E-05	(-)	(-)	(-)	(-)
Hospital	4	Regulated	13.8	8.15	<5	0.071	0.321834	(-)	(-)	(+)	(-)
Hospital	5	Regulated	2.72	14.5	<5	0.008	0.022198	(-)	(-)	(-)	(-)
Hospital	6	Non-Regulated	0.94	34.5	<5	0.014	0	(-)	(-)	(-)	(-)
Hospital	7	Non-Regulated	3.03	24.1	6	6E-04	0	(-)	(-)	(-)	(-)
Hospital	8	Non-Regulated	1.96	15	<5	0.054	0.005327	(-)	(-)	(-)	(-)
Hospital	9	Non-Regulated	2.8	13.9	<5	0.011	0.054765	(-)	(-)	(-)	(-)
Hospital	10	Non-Regulated	2.5	19.7	<5	0.153	1.026049	(-)	(-)	(+)	(-)
Grocery	1	Regulated	4.35	20.6	<5	<LOD	0.000414	(-)	(-)	(-)	(-)
Grocery	2	Regulated	4.56	10.6	<5	2E-04	<LOD	(-)	(-)	(-)	(-)
Grocery	3	Regulated	3.31	24.4	<5	7E-05	<LOD	(-)	(-)	(-)	(-)
Grocery	4	Regulated	1.76	9.22	<5	<LOD	<LOD	(-)	(-)	(+)	(-)
Grocery	5	Regulated	9.89	23.1	5.2	<LOD	<LOD	(-)	(-)	(+)	(-)

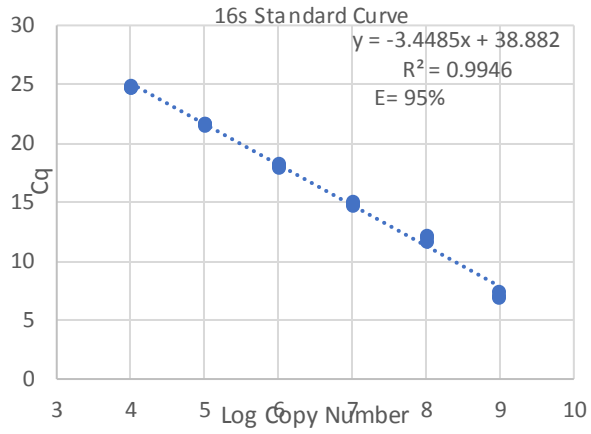
Source Types	SN	Regulation	Cu (ppm)	Zn (ppm)	EOX (mg/kg)	*tet*(M)	Bla_{TEM}	*Mcr-1*	*STEC*	*L. monocytogenes*	*Salmonella*
Grocery	6	Regulated	8.91	71.1	<5	0.064	0.000667	(-)	(-)	(+)	(-)
Grocery	7	Regulated	4.73	32.9	<5	0.019	0.047148	(-)	(-)	(+)	(-)
Grocery	8	Non-Regulated	2.49	13.9	<5	0.007	0.001458	(-)	(-)	(+)	(+)
Grocery	9	Non-Regulated	2.07	4.94	<5	0	8.36E-05	(-)	(-)	(-)	(-)
Grocery	10	Non-Regulated	6.31	23.7	<5	<LOD	<LOD	(-)	(-)	(-)	(-)
Grocery	11	Non-Regulated	5.06	18.6	<5	0	2.6E-05	(-)	(-)	(-)	(-)
Grocery	12	Non-Regulated	1.08	10.3	<5	0.003	0.000186	(-)	(-)	(-)	(-)
Grocery	13	Non-Regulated	5.4	13.4	<5	<LOD	0.001102	(-)	(-)	(-)	(-)
Grocery	14	Non-Regulated	2.76	12.6	<5	0.006	0.026233	(-)	(-)	(-)	(-)
Residential	1	Non-Regulated	9.41	27	<5	0.002	5.19E-06	(-)	(-)	(-)	(-)
Residential	2	Non-Regulated	4.58	18.3	<5	0.001	6.58E-05	(-)	(-)	(-)	(-)
Residential	3	Non-Regulated	9.75	14.7	<5	0.001	0.00033	(-)	(-)	(-)	(-)
Residential	4	Non-Regulated	3.06	20	<5	5E-04	<LOD	(-)	(-)	(-)	(-)
Residential	5	Non-Regulated	13.7	22.7	<5	0.002	0.010995	(-)	(-)	(-)	(-)
Residential	6	Non-Regulated	11.8	28.9	<5	0.003	0.003982	(-)	(-)	(-)	(+)
Residential	7	Non-Regulated	9.3	34.4	<5	8E-04	0.000426	(-)	(-)	(+)	(-)
Residential	8	Non-Regulated	4.3	12.8	<5	1E-04	0.000535	(-)	(-)	(-)	(-)
Residential	9	Non-Regulated	5.26	24.2	<5	0.002	4.44E-05	(-)	(-)	(-)	(-)

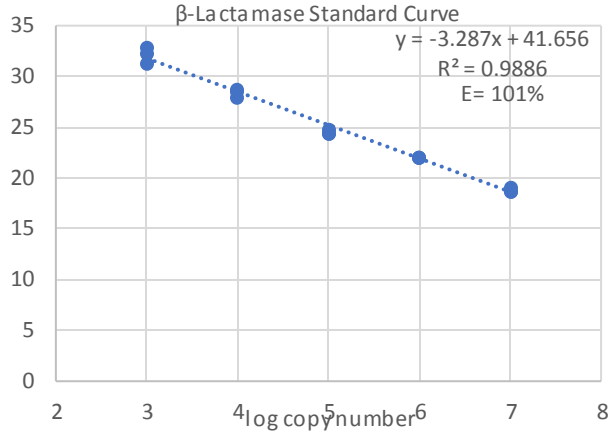
Source Types	SN	Regulation	Cu (ppm)	Zn (ppm)	EOX (mg/kg)	*tet*(M)	*Bla*$_{TEM}$	*Mcr-1*	*STEC*	*L. monocytogenes*	*Salmonella*
Residential	10	Non-Regulated	4.59	34.1	10.9	3E-04	0.002604	(-)	(-)	(-)	(-)
Residential	11	Non-Regulated	2.32	13.1	<5	2E-04	0.001743	(-)	(-)	(-)	(-)
Residential	12	Non-Regulated	4.13	13.7	<5	7E-04	8.85E-05	(-)	(-)	(-)	(-)
Retirement	1	Regulated	3.78	16.3	<5	0.018	0.000141	(-)	(-)	(-)	(-)
Retirement	2	Regulated	1.91	11	11.3	1E-04	4.85E-05	(-)	(-)	(-)	(-)
Retirement	3	Regulated	2.61	13.2	<5	1E-04	2.41E-06	(-)	(-)	(-)	(-)
Retirement	4	Regulated	3.41	15.1	<5	<LOD	0.000123	(-)	(-)	(-)	(-)
Retirement	5	Regulated	3.58	20.3	5.7	6E-04	0.001314	(-)	(-)	(-)	(-)
Retirement	6	Regulated	4.71	35.3	<5	0.013	0.048545	(-)	(-)	(-)	(-)
Retirement	7	Non-Regulated	6.13	24.7	<5	0.004	0.001258	(-)	(-)	(-)	(-)
Retirement	8	Non-Regulated	3.13	29.2	10.5	5E-05	4.51E-05	(-)	(-)	(-)	(-)
Retirement	9	Non-Regulated	4.72	34.4	7.3	0.002	0.004988	(-)	(-)	(-)	(-)
Retirement	10	Non-Regulated	4.57	12.5	<5	<LOD	0.015344	(-)	(-)	(-)	(-)
Retirement	11	Non-Regulated	4.1	17.2	<5	0.003	0.057308	(-)	(-)	(-)	(-)
Retirement	12	Non-Regulated	4.8	22.5	<5	0.006	0.014482	(-)	(-)	(-)	(-)
Restaurant	1	Regulated	3.1	19.8	<5	0.032	3.27E-05	(-)	(-)	(-)	(-)
Restaurant	2	Regulated	5.2	5.74	<5	0	0.000177	(-)	(-)	(-)	(-)
Restaurant	3	Regulated	0.251	10.5	9.3	4E-04	7.19E-05	(-)	(-)	(-)	(-)

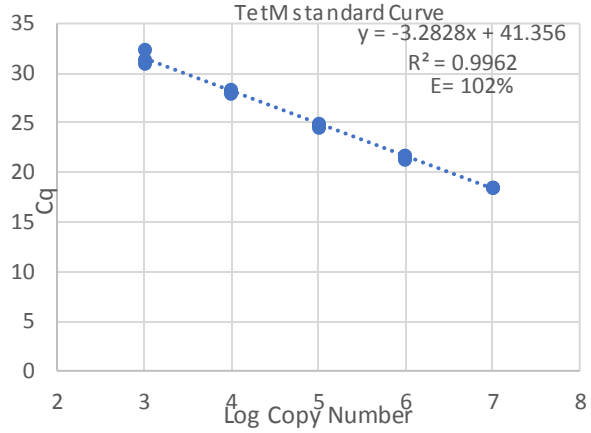
Source Types	SN	Regulation	Cu (ppm)	Zn (ppm)	EOX (mg/kg)	*tet*(M)	*Bla*$_{TEM}$	*Mcr-1*	*STEC*	*L. monocytogenes*	*Salmonella*
Restaurant	4	Regulated	3.94	35.1	<5	0.004	0.000375	(-)	(-)	(-)	(-)
Restaurant	5	Regulated	2.89	29	<5	<LOD	6.53E-05	(-)	(-)	(-)	(-)
Restaurant	6	Regulated	3.04	23.1	<5	4E-05	0.000107	(-)	(-)	(-)	(-)
Restaurant	7	Non-Regulated	1.56	10	<5	0.032	0.000525	(-)	(-)	(-)	(-)
Restaurant	8	Non-Regulated	4.85	22.6	89.7	7E-04	1.99E-05	(-)	(-)	(-)	(-)
Restaurant	9	Non-Regulated	3.85	20	<5	0.003	0.006606	(-)	(-)	(-)	(-)
Restaurant	10	Non-Regulated	12.1	10.1	<5	0.019	0.046761	(-)	(-)	(-)	(-)
Restaurant	11	Non-Regulated	4.06	26	<5	0.008	0.026758	(-)	(-)	(-)	(-)
Restaurant	12	Non-Regulated	3.09	9.28	<5	0.003	0.011229	(-)	(-)	(-)	(-)
School	1	Regulated	3.25	33.2	5	<LOD	0.000212	(-)	(-)	(-)	(-)
School	2	Regulated	3.75	54.7	<5	0.002	0.000408	(-)	(-)	(-)	(-)
School	3	Regulated	4.07	13.4	<5	0.003	1.69E-05	(-)	(-)	(-)	(-)
School	4	Regulated	3.28	23.7	<5	0.045	0.117934	(-)	(-)	(-)	(-)
School	5	Regulated	4.27	20.7	<5	3E-04	0.001831	(-)	(-)	(-)	(-)
School	6	Regulated	2.22	9.67	<5	8E-04	0.003444	(-)	(-)	(-)	(-)
School	7	Non-Regulated	3.78	28.9	<5	0.001	0.016381	(-)	(-)	(-)	(-)
School	8	Non-Regulated	3.83	17.6	<5	0.016	0.004192	(-)	(-)	(-)	(-)
School	9	Non-Regulated	4.54	29.9	<5	5E-06	1.44E-05	(-)	(-)	(-)	(-)

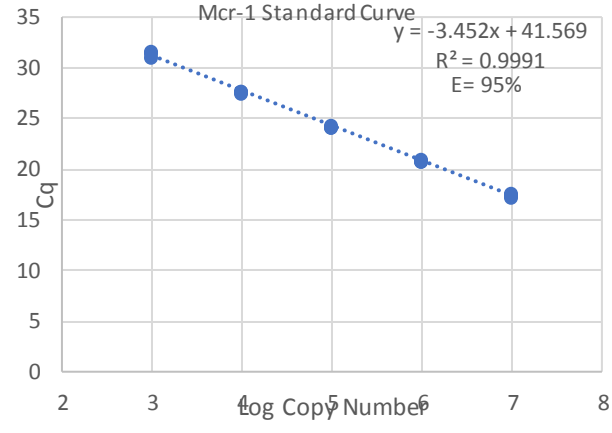
Source Types	SN	Regulation	Cu (ppm)	Zn (ppm)	EOX (mg/kg)	*tet*(M)	Bla_{TEM}	*Mcr-1*	*STEC*	*L. monocytogenes*	*Salmonella*
School	10	Non-Regulated	1.77	27.8	<5	0.002	0.000338	(-)	(-)	(-)	(-)
School	11	Non-Regulated	2.21	11.7	<5	<LOD	0.000226	(-)	(-)	(-)	(-)
Mean			4.410	21.187		0.009	0.027				
Standard Deviation			2.838	10.867		0.023	0.127				
Maximum			13.800	71.100		0.153	1.026				
Minimum			0.251	4.940		0.000	0.000				

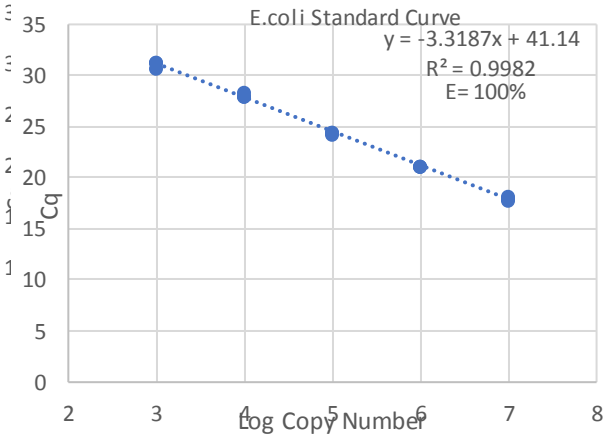
APPENDIX C- STANDARD CURVES

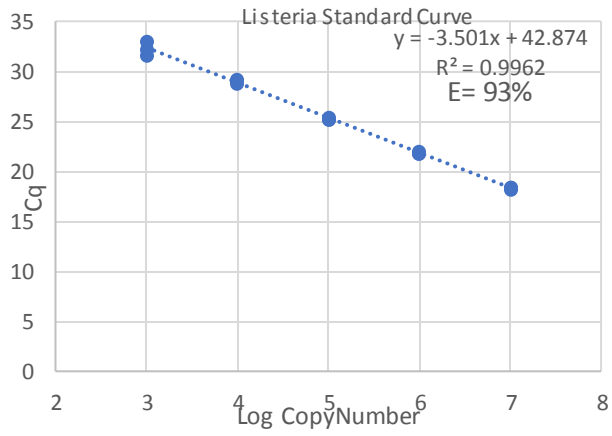












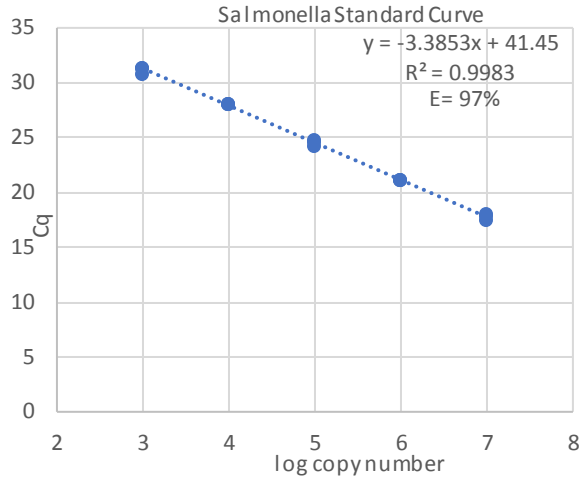


Figure C.1: Standard curves develop for quantification of target DNA in the food DNA sample, from left to right in sequence a) 16S rRNA gene b) *Bla*$_{TEM}$ resistance gene c) *tet*(M) resistance gene d) *mcr-1* resistance gene e) STEC f) *L. monocytogenes* g)*Salmonella*

APPENDIX D- ACRONYMS USED IN MICROBIAL COMMUNITY ANALYSIS

Table D.1: Acronyms used for the samples in microbial community analysis

Names	Source Type	S.N.	Regulation
R1	Hospital	3	Regulated
R2	Grocery	5	Regulated
R3	Grocery	6	Regulated
R4	Grocery	7	Regulated
R5	School	3	Regulated
R6	School	5	Regulated
R7	School	6	Regulated
R8	Retirement	6	Regulated
R9	Retirement	5	Regulated
R10	Grocery	2	Non-Regulated
R11	Grocery	3	Non-Regulated
R12	Grocery	4	Non-Regulated
R13	Grocery	5	Non-Regulated
R14	Residential	3	Non-Regulated
R15	Residential	4	Non-Regulated
R16	School	3	Non-Regulated
R17	School	6	Non-Regulated
R18	Hospital	4	Non-Regulated

BIOGRAPHY OF THE AUTHOR

Astha Thakali was born in Himalayan kingdom Mustang, Nepal in 1992. She moved to Pokhara for her further education and graduated high school from Gandaki Higher Boarding School in 2010. She was always motivated to develop her place and carry out lots of developmental activities in rural Himalayan places. Her interest made her to join Institute of Engineering in Tribhuvan University and pursue a career in Civil Engineering. After graduation, she started to work in the remote earthquake affected areas of Nepal. She was involved in planning, designing and constructing small scale infrastructure works. She realized the importance to bring developmental works in harmony with environmental protection. After working for one year, Astha started her master's in civil engineering with an emphasis in Environmental Engineering at the University of Maine, where she improved her research capabilities. Astha is a candidate for Master of Science degree in Civil Engineering from the University of Maine in May 2020.