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Organic Fertilizers: Public Health Intricacies

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Abstract

Organic fertilizers are an essential source for plant nutrients and a soil conditioner in agriculture. Due to its sources and the composition of the organic inputs as well as the type, functionality and failures of the applied treatment process, the organic fertilizer may contain various amounts of infectious agents and toxic chemicals, especially the antibiotics that can be introduced to the subsequent food chain. A range of human and animal pathogens of bacterial, viral and parasitic origin have been the cause of food-borne epidemics due to unintended contamination from organic fertilizers. The use of antibiotics by humans and in animal feeds will also end up in the organic fertilizers. These antibiotics and other chemicals, depending on the sources of the organics, will enhance the likelihood of occurrence of resistant and multi-resistant strains of microorganisms in society and have been reported to cause ecotoxicological environmental effects and disruption of the ecological balance. Exposure of microorganisms to sublethal concentration of antibiotics in the organic products induces antibiotic resistance. WHO guidelines for the reuse of excreta and other organic matters identify the risk for the exposed groups to the reuse of the excreta and are applicable in the use of organic fertilizers in agriculture.

Keywords: organic fertilizers, food-borne illnesses, pathogens, antibiotics, ecotoxicity, WHO

1. Introduction

The potential health intricacies linked with organic fertilizers relate to their origin, their treatment and human exposure within a system perspective from origin to use, including products like crop type. Since organic fertilizers mainly are "faecal material/manure and urine

from different animals and/or humans, with the addition of plant materials (organic solid wastes), or in special situations waste materials 1 from food or plant processing industries", the origin of the different fractions and their amounts partly defines the risk. Usually the risk is outbalanced by a wide range of benefits that the use of organic fertilizer exerts in agriculture as nutritional fertilizers and for soil conditioning. It has been further implied as more environmental friendly than the inorganic fertilizers [1] and its effect more tender on biotic components of the ecosystem without much shift in the ecological balance [2]. This is partly reflected by organisms like earthworms which may be negatively affected by inorganic fertilizers but promoted by the use of organic fertilizers and also incorporated as decomposers in aerobic composting processes [3, 4].

As this chapter deals with the public health aspects and risks involved, we define the organic materials utilized by its sources and thus relate to the following:

- Human faecal materials (also sludge from domestic treatment plants and from on-site sanitation, e.g. pit latrine emptying).

- Human urine (if separated).

- Animal manure (some risk differences depending on the species of animals/birds).

- Animal urine (often collected/spread separately, but impacted by the animal faeces).

- Other types of organic solid wastes (plant materials, domestic, industrial from organic food/ fodder processing industries).

Additionally, the risk may relate to some storage-specific factors like

- Regrowth of specific bacterial pathogens or opportunistic ones (occurs when the material that, for example should be/are composted, are not well stabilized or broken down. During these circumstances, for example *Escherichia coli*, *Salmonella* sp., *Listeria* sp. and spore formers will regrow in the material if present).

- When the collected/stored/kept organic fractions or mixture thereof (see above) function as a breeding site for flies and mosquitoes that serve as vectors of parasitic diseases.

- Development of spore-forming thermophilic fungi and Actinomycetes in composting processes, where the spores can cause diseases in both immune-competent and immune-compromised individuals upon inhalation. An example of such an organism is *Aspergillus fumigatus*.

Based on source, the risk will vary to a great extent, depending on the health of the animals/ humans that primarily defines the microbial concentration and partly occurrence of antibiotics and chemical components in the organic wastes (from domestic or animal sludge fractions) that may be conveyed to the agricultural sites and crops fertilized. Additional components may apply if organic industrial wastes are utilized. An indirect organic fertilization may occur through irrigation using wastewater effluent, where the nutrient load serves as an advantage. This is widely applied in developing countries [5]. However, this may result in additional inputs of antibiotics, toxic organic and inorganic compounds and pathogens. All these concepts

are further deliberated in this chapter. The possibilities of recycling food-borne pathogens via agricultural crops to the final end consumers of the crops will additionally be discussed. Food-borne pathogens are especially important for animal faecal-based fertilizers used on fruits and vegetables farms meant to supply salads in restaurants. Other dynamics are residual antibiotics which are sometimes locked in the components of the organic fertilizers with attending public health implications to be further enumerated in this chapter.

2. Treatment and risk reduction

The concept of organic fertilization is ever worthwhile, with combined considerations of the public health intricacies that cannot be overemphasized [6]. Several alternative treatment methods can be employed to stabilize the organic fertilizers before use and at the same time reduce the concentrations of potential pathogens, thereby the risks. The efficiency of these will vary based on time, load and different external factors.

	Pathogens	Treatment option or process	Log reduction	Duration (months)	References
1	*Escherichia coli*	Settling ponds	2.2	6	[7, 8]
		Unplanted drying/dewatering beds (for pretreatment)	4.9–5.5	5	[9]
		Composting (window, thermophilic)	4	4	[10]
		pH elevation >9	2	1	[11]
		Anaerobic (mesophilic)	2	0.67	[12]
2	Helminths eggs (WHO, 2006)	Settling ponds	3	4	[13]
		Planted dewatering drying beds (constructed wetlands)	1.5	12	[14]
		Unplanted drying/dewatering beds (for pretreatment)	0.5	0.3–0.6	[15]
		Composting (window, thermophilic)	1.5–2.0	3	[16]
		pH elevation >9	3	6	[17]
		Anaerobic (mesophilic)	0.5	0.5–1.0	[18, 19]
3	Viruses	Settling ponds (enteroviruses)	1.5	3.3	[20]
		Planted dewatering drying beds (constructed wetlands)	99.9% with aluminium	––	[21]
		Unplanted drying/dewatering beds (for pretreatment)	1–3	––	[22]
		pH elevation >9 (Porcine circovirus type 2)	2	1	[23]
		Anaerobic (mesophilic) (Norovirus)	1.1–1.4	0.8	[24]

Table 1. Efficiency of some pathogens' reduction techniques for low-cost sludge treatment strategies.

Low-cost options for pathogen reduction and nutrient recovery from faecal sludge are of special importance to low-income countries. They include settling ponds, planted dewatering drying beds (constructed wetlands), unplanted drying/dewatering beds (for pretreatment), composting (window, thermophilic), pH elevation > 9, anaerobic (mesophilic) and simple storage. They have varying pathogen reduction efficiencies on bacteria, parasitic protozoa and viruses. **Table 1** summarizes the efficiencies of these pathogen reduction techniques with *E. coli* as an example for the bacterial group, helminth's eggs for parasites and some viral examples as stated. The figures serve as examples. Variations can be large based on prevailing local conditions.

Other methods most commonly used in developed countries to treat the sludge include incineration and pasteurization. The former one ensures a total destruction of all pathogenic organisms while the efficiency of the later one depends on time and applied temperature (normally 70°C for at least 1 h). Irradiation with *β*- or *γ*-rays is an approved method in the USA, and it reduces the pathogenic content to a high extend but is not widely used.

2.1. Organic waste stabilization

Organic wastes can be used as soil amendments or organic fertilizers after an effective stabilization and disinfection. Effective stabilization and disinfection of sewage sludge prior to land application are important not only to protect human health. Currently, some of the most commonly used waste stabilization methods are composting (solid state), aerobic digestion (liquid state), anaerobic digestion, lime stabilization [25, 26] and sludge drying. The aerobic and anaerobic methods of waste stabilization are among the most prominent [27]. Furthermore, there have been growing concerns about the survival of pathogenic microorganisms in sewage treatment processes, resulting in the release of antibiotic resistant microbial species to the environment [28, 29]. These are further considered below.

2.2. Composting

Composting is defined as the biological conversion of organic wastes, under controlled conditions, into a hygienic, humus-rich, relatively biostable product that improves land and fertilizes plants [30]. It has the combined effect of pathogen reduction while at the same time stabilizes and converts the organic wastes into product that can be easily handled [31, 32]. The type and concentration of pathogens present in sewage sludge is largely determined by a number of factors including population's state of health, presence of hospitals, abattoirs and factories processing meat [33]. Composting is one of the essential decontamination processes to reduce the load of pathogens in animal wastes. The composting efficiency to ensure inactivation of pathogens depends on allotted time and temperature. Inefficient composting leaves loads of pathogenic bacteria which may be passed on to the end consumers.

Metals such as zinc, copper, cadmium, lead, arsenic, chromium, mercury, vanadium and nickel are usually of great concern [34] when sludge from industrial effluent are used as feed stock for composting both from a health perspective and in the degradation of the productivity of land. Industrial sludge may contain elevated heavy metal concentration which makes them

unsafe for garden use. Despite the fact that copper and zinc are important micronutrients, the possibility of bioaccumulation to phytotoxic or deleterious level for human consumption still makes them a concern.

Zoonoses are among the public health concerns associated with improperly sanitized organic fertilizer. Zoonotic diseases and emerging zoonoses that could be associated with organic fertilizer includes salmonellosis, entrohaemorrhagic *E. coli* (EHEC), anthrax and Newcastle diseases just to mention a few [35]. *Thermoactinomyces vulgaris* is another organism of importance. It produces heat-resistant endospores that can survive high temperature during composting. This organism is the causative agent of "farmer's lung" which is an allergic disease of the respiratory system of agricultural workers. The pathogens present in soil amendments are directly related to the organic waste source. The reduction or removal of pathogens in a compost will depend on the composting temperature and the process used [36]. This implies that improperly carried out composting leaves the organic matter poorly sanitized with the compost becoming a source of recontamination with pathogenic or parasitic organisms [37]. *E. coli*, *Salmonella* sp. and a few others possess advantage for regrowth in compost [38, 39]. Also, due to rich nutrient composition, contaminating *E. coli* grows very rapidly in pre-sanitized organic fertilizers [40–43] that is not properly composted or stabilized. *Salmonella* spp. equally grow in composted sewage sludge if the carbon/nitrogen ratio is >15 and the manure content 0.2 index.

Organisms	Lethal temperature and necessary time
Salmonella spp.	15–20 at 60°C; 1 h at 55°C
Escherichia coli	15–20 at 60°C; 1 h at 55°C
Entamoeba histolytica	68°C; time not given
Taenia saginata	5 min at 71°C
Necator americanus	50 min at 50°C
Shigella spp.	1 h at 55°C
Mycobacterium tuberculosis	20 min at 70°C
Corynebacterium diphtheria	45 min at 55°C; 4 min at 70°C
Ascaris lumbricoides eggs	60 min at 50°C; 7 min 55°C
Viruses	25 min at 70°C

Table 2. Temperature-time relationship required for killing specific pathogens [35, 36, 49].

There is therefore need to ensure that the mature compost does not contain plant and human pathogens. In composting, the thermophilic temperature is the effective determinant of destroying the pathogen and the efficiency further related to the exposure time. The required time at a given temperature for efficient pathogen inactivation, according to USEPA [44] can be estimated using a time-temperature formula:

$$D = 131700000 / 10^{0.1400t}$$

where D is time in days and t is temperature (°C).

Organisms	US		New Zealand	UK	New South Wales	EU
	Class A	Class B	Class A		Class A	
Escherichia coli	N/A		<100 MPN/g	1000 CFU/g	N/A	0/50 g
Faecal coliforms	<1000 MPN/g	<2,000,000 MPN/g	N/A		<1000 MPN/g	
Salmonella spp.	<3 MPN/4 g total solids		<1/25 g	Nil	0/50 g	<10^3 MPN/ g
Enteric viruses	<1 PFU/4 g		<1 PFU/4 g		<1 PFU/4 g	
Helminth ova	<1/4 g		1/4 g		<1/4 g	

MPCN, most probable cytophatic number; MPN, most probable number; PFU, plaque-forming unit.

Table 3. Standards for maximum concentrations of pathogens in biosolids and composts used as organic fertilizers [49, 52, 53].

In a properly ventilated composting pile, the temperature usually reaches between 55 and 68°C. This temperature level can last for a few days to months depending on the size of the system and the composition of the ingredients [45–47] and is the determinant for the sanitization effectiveness. The average time required for killing specific pathogen is exemplified below (**Table 2**). *Salmonella* spp. and *E. coli* have been known as pathogen indicator bacteria in organic fertilizer, supplemented with soil-transmitted helminths [48] and enteric viruses when a broader spectrum of organisms needs to be assessed. Several national and international standards/guidelines have been established to ensure public health safety when using these organic fertilizers (**Table 3**). Due to high heat resistance of some bacteriophages, they have been suggested as an indicator of properly sterilized compost [35].

2.3. Aerobic digestion

This occurs in engineered ecosystems where biomass consisting of a mixed microbial community and other solids are constantly maintained in a suspension in an aerobic basin supported by mixing [50]. This is usually used in stabilizing sewage and wastewater, producing high-quality treated effluent through the metabolic reactions of the microbial community [51]. The sanitation efficiency of this system depends largely on time, temperature and loading rates [28, 50]. This process still yields poorly stabilized organic matter with a fluid product, having little or no volume reduction and pathogen reduction efficiency is usually low [28].

Moreover, using them as organic fertilizers in an inefficiently sanitized stage can further result in direct microbial contamination of surface water or via runoff from lands amended with such organic waste [28] in addition to their direct exposure effects and effects through crops. Most aerobic sewage sludge treatment plants operate at mesophilic temperatures (30–35°C). Within

this temperature range, the stabilization processes are inefficient in the removal of viruses, bacteria and Parasite's eggs [28].

2.4. Anaerobic digestion

Anaerobic digestion involves the breakdown of complex organic material into simple mono-merics or fraction and production of biogas (bioenergy) in closed system through the activity of anaerobic microorganisms [54]. Anaerobic digestion can be carried out either at mesophilic (30–38°C) or at thermophilic (50–55°C) temperatures. Compared to composting, there is lesser heat generation during anaerobic decomposition, which reduces the sanitizing effect of the process on organic waste [37]. Digesting organics at high temperatures reduces the time required for bacterial inactivation, which eventually results in faster bacterial kill during thermophilic digestion compared to mesophilic [55]. Bacterial spores including *Bacillus cereus* and *Clostridium perfringens* are normally resistant to temperature inactivation at both mesophilic and thermophilic ranges [55–57]. Chauret et al. [58] also noted the resistance of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts to anaerobic sludge digestion. This finding is of importance since *Cryptosporidium* sp. oocysts can persist in soil amended with sludge for at least 30 days [59].

2.5. Lime (alkaline) stabilization

Lime stabilization is a preferred alternative compared to anaerobic and aerobic stabilization processes due to its cost efficiency and enhanced sanitizing effect [25, 60]. It effectively reduces the concentration of pathogens in sludge (**Table 4**), heavy metal availability and enhances its agricultural uses [25]. Free calcium ions resulting from the lime solution form complexes with odorous sulphur species and organic mercaptans; moreover, the high pH precipitates metals from the sludge thereby reducing their solubility and availability. Alkaline stabilization involves the addition of lime slurry in the form of $Ca(OH)_2$ or CaO to the liquid sludge in order to raise its pH to about 12 or higher [60]. Apart from the high pH, the addition of quicklime to the liquid sludge can result in thermophilic temperature (up till 70°C) which inactivates the viruses, bacteria and other microorganisms [61,62]. In a study by Farzadkia and Bazrafshan [25], addition of lime slurry to sewage sludge resulted in a reduction of faecal coliforms with more than 99.99% in stabilized sludge. Arthurson [26] noted that there is a need for further investigation on the potential of alkaline stabilization methods since this process is an effective sewage sludge sanitization method but some contradictory results exist.

Type of treatment	Viruses	Bacteria	Parasite egg
Pasteurization (heat, 30 min at 70°C)	Good	Good	Good
Irradiation (ionizing radiation, 300 rad)	Poor	Good	Good
Lime treatment			
Slacked lime (high pH)	Good	Good	Good
Quick lime (High pH; 80°C)	Good	Good	Good

Type of treatment	Viruses	Bacteria	Parasite egg
Anaerobic digestion			
Mesophilic (30–35°C)	Poor	Poor	Poor
Thermophilic (50–55°C)	Good	Good	Good
Aerobic digestion			
Mesophilic (up to 20°C)	Poor	Poor	Poor
Thermophilic (50–55°C)	Good	Good	Good
Compost (50–60°C)	–	Good	Good

Table 4. Pathogen-reduction performance of the different treatments of sludge [28, 63, 64].

3. Potential human pathogens in organic fertilizers from faecal materials and implications

Pathogen-free organic fertilizer can be developed and microbiological safety assured for the reuse of sludge and manure. Various factors affecting the survival of pathogens in composting include time, temperature, pH, aerobic/anaerobic, biological activity, UV or irradiation, moisture, combination and chemical effects (e.g. ammonia). These factors are considered with regard to some pathogens discussed in Sections 3.1 and 3.2.

The inherent pathogens in an organic fertilizer depend on the animal source of the faecal materials used. When considering heat-dependent anoxic degradation of product for manure from dairy cattle, studies have shown the rate of kill of *E. coli* O157:H7 at 55°C to be 3 logs per 30 min and 4 logs per 100 min [70]. **Table 5** show the heat-based inactivation of some pathogens with values of decimal reduction time (D) at test temperature (T) Thermal death times for *Salmonella* to achieve reduction of 9 log has been reported to be 40 min at 55°C. Some pathogens can survive for longer periods of time in compost especially when they are located on the surface part of the compost pile where the heat effects may be inefficient. They also survive better in mesophilic composting (<45°C) than at elevated temperature. Moisture availability in biowaste compost (denoted by water activity, a_w) is also an important determinant for the survival time of many pathogens.

	Pathogens	T (°C)	D_T (s)	References
Protozoan parasites	Soil-transmitted helminths (STHs)	20–30°C	Several	[69]
Bacteria	*Campylobacter* sp.	55.4–61.2	89–10.3	[70]
	Escherichia coli	55–70	1281.6–1.86	[71]
	Escherichia coli O157:H7	55	1500	[72]
	Listeria sp.	55–70	3370.14–7.56	[73]
	Salmonella sp.	55–70	3370.14–7.56	[74]

	Pathogens	T (°C)	D_T (s)	References
		55	720	[75]
Virus	Hepatitis A	80	73.2–733.2	[76]
		90	13.2–180	
	Rotavirus	~20°C	20–100	
	Coliphage	51	1860	[75]
Fungi	*Botrytis cinerea*	40–48	1800–36	[77]
	Monilinia fructigena	39–45	1302–150	[77]
	Monascus ruber	70	2238–4379	[78]

Table 5. Heat inactivation: values of decimal reduction time (*D*) at test temperature (*T*) (Adapted from Romdhana [68]).

3.1. Soil-transmitted helminths

Both human and animal waste and wastewater may contain different soil-transmitted helminths (STHs). These are among the most resistant microorganisms and will develop in soils or poorly treated biosolids from the non-invasive stages that are excreted to an infective one. Thus, when poorly handled, soil and crops get contaminated with eggs or larvae of STHs, which in turn will be transmitted orally through crops or due to accidental ingestion (e.g. *Ascaris* sp.) or penetrate bare skin (hookworms). Due to their resistance to environmental stress, helminth parasite eggs are widely used as hygiene indicators. STHs are resistant to sublethal composting temperatures and they require longer time at alkaline pH (months at pH 9–10, but much more rapid at pH 11–12) to effect appreciable die-off. A report by Jensen and Vrsle [65] showed that it would take a period of 117 days to achieve 99% die-off of an *Ascaris suum* eggs when placed on human excreta with pH levels between 9.4 and 11.6. When temperatures of above 50°C are reached, a rapid die-off occurs. Thus, a properly composted night soil with crop residues can destroy the parasitic infective stages efficiently.

When assessing the effectiveness of composting, *A. suum* eggs from pigs may be utilized as a model for the survival of human parasitic roundworm, *A. lumbricoides* [66,67].

3.2. Zoonotic organisms in waste dung as components of organic fertilizer

Chicken litters and pig dungs are rich in nutrients and are valuable animal wastes as organic fertilizer. However, chicken litter exemplify one organic fertilizer that may contain important human pathogens like *Salmonella* sp., *Campylobacter jejuni* and *Listeria monocytogenes*. If not properly sanitized these pathogens can easily get deposited on crop/plants, with transmission to consumers with, for example, fruits and vegetables [79,80]. Several human pathogens have been reported in organic fertilizer and may be conveyed to human, while other may function as animal or plant pathogens [81]. *L. monocytogenes* is a typical example of a pathogen easily conveyed via food crop.
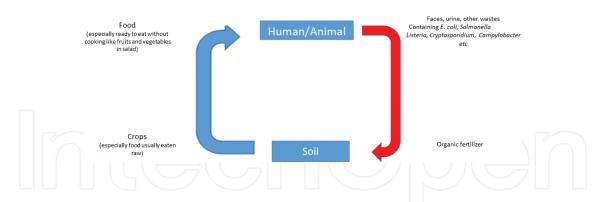
Figure 1. The cycling of potential pathogens in faecal materials for making organic fertilizer.
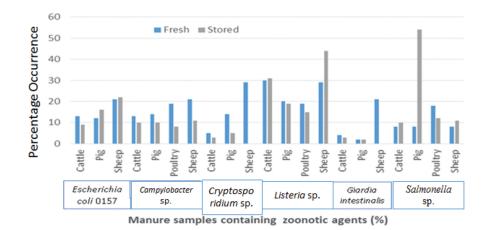

Figure 2. Prevalence and concentration of zoonotic pathogens observed in British livestock manure (modified based on Ref. [84]).

Boulter et al. [82] reported that *Salmonella* sp. was observed among several other Gram negative bacterial potential pathogens in green compost for organic fertilizer. Even some Gram positive bacteria may occur, for example *Bacillus cereus* which is associated mainly with food poisoning and as a cause of serious and potentially fatal non-gastrointestinal-tract infections [83]. This resembles contamination of the farmland through poorly formulated organic fertilizers that circulates egested pathogens from human or animal back to them or another is pictorial as a cycle (**Figure 1**). Pathogens from wastes like *E. coli, Salmonella* sp., *Listeria* sp., *Cryptosporidium* sp. and *Campylobacter* sp. among others are usually conveyed to the farmland through poorly composted organic fertilizers or through contaminated irrigation water. **Figure 2** illustrates the occurrence of a number of different zoonotic pathogens found in manure [84].

Treated wastewater effluents contain nutrients (nitrogen, phosphorus and potassium), inorganic matter (dissolved minerals) and other chemicals which can complement the enrichment of the farmland in enhancing plants' growth. Enhanced concentrations of different excreted pathogens may also occur in wastewater being used for irrigation. Most of these pathogens are of known aetiologies of various infection (exemplified in **Table 6**). This is likely more prevalent in developing countries where wastewater for irrigation is not pretreated and

disease prevalence may be higher. Intestinal nematodes released with the irrigated water are of special concern. The risk becomes higher in a farmland in which organic fertilizer is already in use, as it enriches the environment for the pathogens to thrive.

Table 6 gives the summary of potential human pathogens found in wastewater effluent and sewage sludge as components that are used for organic fertilizers. Some of these pathogens have been reported in zoonotic infection as discussed hereafter.

Pathogens		Potential disease (s) /Symptoms
Gram positive bacteria	*Staphylococcus* sp.	Osteomyelitis, furuncles, carbuncles, impetigo, wound infections, food poisoning
	Streptococcus sp.	Skin infection, otitis media, respiratory infection
	Clostridium perfringens	Gas gangrene, gastroenteritis (food poisoning)
	Clostridium botulinum	Botulism
	Bacillus anthracis	Anthrax
Z-N positive bacteria	*Mycobacterium* spp.	Leprosy, tuberculosis
Gram negative bacteria	*Salmonella* spp.	Gastroenteritis, typhoid fever
	Shigella spp.	Bacillary dysentery
	Escherichia coli (enteropathogenic strains)	Gastroenteritis
	Pseudomonas aeruginosa	Otitis externa, skin and wound infections (opportunistic pathogen)
	Yersinia enterocolitica	Gastroenteritis
	Campylobacter jejuni	Campylobacteriosis: diarrhoea, fever, nausea, vomiting, abdominal pain, headache
	Listeria monocytogenes	Listeriosis
	Vibrio cholera	Cholera
	V. parahaemolyticus	Acute gastroenteritis
Viruses	*Coronavirus HKU1, Klassevirus* and *Cosavirus*	Severe acute respiratory, gastrointestinal and hepatic illnesses
	Norovirus	Gastroenteritis
	Enteric viruses including *Adenovirus (AdV), hepatitis A virus (HAV) and Rotavirus (RV)*	Enteric infection
	Polioviruses	Poliomyelitis
Parasites	Soil-transmitted helminths (*Ascaris*	**Helminthiases**

Pathogens	Potential disease (s) /Symptoms
lumbricoides, whipworm and hookworm)	
Giardia sp.	Giardiasis
Cyclospora sp.	Cyclosporiasis
Cryptosporidium sp.	Cryptosporidiosis

Table 6. Potential human pathogens identified in municipal wastewater and sewage sludge being used for fertilizing farmland [26].

3.3. Pathogens from organic fertilizers into crops and vegetables

Pathogen can be passed on to crop plants through direct contact, through deposition on the surface or in splash contamination. Human pathogens may also get internalized in plants from fertilizers in the soil, where the probability for internalization may be increased by mechanical damage. The pathogens may migrate within the plants' tissues. **Figure 3** gives a pictorial illustration of the process of internalization of pathogens from organic fertilizers into crops and vegetables. Pathogens in the organic fertilizers are deposited on the surface of the crops and/or vegetables. The pathogens in subsurface parts of the crops are difficult to be removed or disinfected. The potential for enteric pathogens to be absorbed by roots has been considered [85].

Enteric pathogens may further enter plant tissues through both natural apertures (stomata, lateral junctions of roots) and damaged (wounds, cut surfaces) tissue (**Figure 3**). Researchers [86–88] have demonstrated the internalization of *E. coli* from soil into hypocotyl of spinach, lettuce and cabbage using different bioluminescent labels.

Regrowth contribute to high concentration of pathogens. Either *E. coli* O157:H7 or *Salmonella* may Multiply, get internalized into the tissues of raddish [89–91] and mung bean [87]. Surface
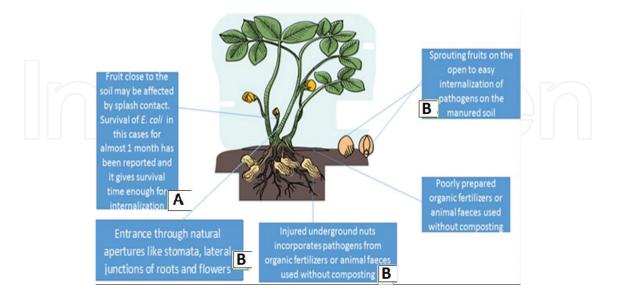

Figure 3. Pathogens' deposition (A) and internalization (B) in crop on an organically fertilized farm.

sterilization will then have little effect as the pathogens are already within the tissues. Similar experiment involving *Salmonella* and alfalfa seeds was demonstrated by Gandhi et al. [92] in which the bacterium penetrated into the hypocotyls.

3.4. Fate of pathogens in consumers of the plants products

Pathogens associated with plant products can be conveyed to consumers through crops mainly eaten raw from contaminated organic fertilizers. Surface washing will reduce surface-associated pathogens [48, 93]. Fruit- and vegetable-related outbreaks have been reported globally, affecting from a few infected person to causing major epidemics [94–96]. One recent outbreak was in 2011, affecting several countries in Europe involving ingestion of *E. coli* O157 from fruits and vegetables [94]. About 46 million food-related cases with 400,000 hospitalization and 3000 deaths were summarized by Scallan et al. [95,96]. The increasing numbers of immunocompromised individuals globally will enhance the effects of pathogens from contaminated fruits and vegetables. The risk to public health exists, and it is imperative for each country to remodel the agriculture extension to address this challenge.

The original source before food contamination differs. Some pathogens like norovirus and *Salmonella* sp. serotype *Typhi* are sustained in human reservoirs, but several others are sustained in animal reservoir. Surface contamination and/or internalization of pathogens in fruits and vegetable may not be the major pathway for contamination of food supply. However, the outbreaks via this channel may have high public health significance [97, 98]. An estimated, 131 produce-related food-borne outbreaks were reported in the USA between 1996 and 2010. A large *E. coli* O157:H7 outbreak of food-related illness involving vegetable occurred in 1996 in Japan in which >11,000 individuals were reported severely ill. Several deaths occurred among young school children [98].

In England, 60 outbreaks of food-related illnesses from fruit- and vegetable-related infections were reported during 7 years, beginning from 1992. Contamination with human pathogens on farms can be attributed not only to faeces from human, and manure from farm and wild animal but also to poor environmental waste handling [99]. A report of an *E. coli* outbreak confirmed the contributions of water, manure from cattle dung and wild pig faeces, etc. towards contamination on spinach [100]. The same strains of pathogens found in spinach fields have also been found internalized in the spinach. This informed the initiation of safety plan against *E. coli* O157 infection through pathogens in vegetables [101].

When fruits harbour internalized pathogens, they pose an enhanced risk especially when used for sprouted seeds and unpasteurized fruit juices [102–104]. This is more important for internalized fruits as surface contaminant are usually steam washed away in processing companies.

3.5. Exposure pathway and health risks when reusing contaminated organic materials as agricultural fertilizers

Consumption of crops, including fodder crops, serves as the most common transmission pathway to chemical and pathogens from biosolids used as fertilizer. Investigations have also

been performed related to contamination of crops used for medicinal products and supplements [105]. The direct exposure of agricultural workers is also significant and relates to different transmission routes, as well as the frequency and duration of exposure. Farmworker exposure has been examined [106, 107], including the impact on family members [108]. Direct exposure relates to the level of manual work and mechanization. The risk further relates to the type of fertilizer, from human and animal urine to untreated or treated wastewater, manure or human excreta. A special situation is when stored organic fractions or mixture thereof function as breeding site for fly/mosquito vectors of parasitic disease or attract vermin's that can act as carriers of pathogens. This is for example considered in the USEPA guidelines [109].

In addition to microbiological contaminants, organic fertilizers may, especially when sludge constitute parts of the input material, contain metals and other chemicals that may affect the receiving soils as well as be of relevance for occupational exposures. To appropriately assess human risk from chemicals found in biosolids, the form of the chemicals, and their fate, transport and bioavailability needs to be known, for example, arsenic, lead, mercury, antibiotics.

Jerkins et al. [110] reported two studies that were suggestive that compost workers were affected by fungi. One cross-sectional study in Germany reported a significant increase in symptoms from lungs and airways as well as dermal effects and related these to increased exposure to fungi and Actinomycetes. The other was a prospective study in multiple US cities where significant increases in eye and skin irritation occurred and fungal colonization was documented but no serological evidence of other infections was reported. Indirect evidences were presented by Harrison and Oakes [111] that reported 39 incidence of illness among neighbours to biosolids application sites. The evidences were however not appropriately backed up.

The infection risks have been estimated using quantitative microbial risk assessment (QMRA) when urine or human faeces are used for garden fertilization [112]. A study in South Africa reported enhanced infection risks of *Salmonella* sp. and *Ascaris* sp. associated with spinach or carrots fertilized with human excreta [113]. An assessment of the health risk associated with daily consumption of vegetables (lettuce, 11.5 g) fertilized with compost was done by Watanabe et al. [114]. If the concentration of pathogenic virus in compost, for example is 10^{-1}–10^2 PFU/g of lettuce, the risk would still be higher than the WHO tolerable annual infection risks.

4. Residual antibiotics (AB) and antibiotic-resistance genes (ARGs) in organic fertilizers

Veterinary drugs are introduced into the environment through a number of routes like direct applications as in aquaculture, application of manure and/or slurry to agricultural fields and through disposal of wastes during the production processes. An investigation also indicate a link between the proximity of swine farms exposed to these antibiotics through contact with animal feed and development of antibiotic resistance in bacteria among small wild animal accessing into barns and feed storage areas [115]. The presence of drugs and their metabolites

in the environment have frequently been reported. For instance, low levels (<1 µg/L) of antibiotic residues have been detected in surface water samples in both Germany and the USA collected from sites considered susceptible to contamination [116]. The residual antibiotics in organic fertilizers using animal manure from large-scale livestock farms (mainly including slurry and dung from pigs, cows and chicken) have been investigated with their presence confirmed [117].

The residual antibiotics found in organic fertilizers may emanate from administration to humans either as prophylaxis or for therapeutic purposes. They are also being used as components of animal feeds to promote growth, to treat or prevent diseases of farm animals and sometimes against diseases in plants [118–126]. Hence, tetracycline concentration, for example, in liquid organic fertilizer could be as high as 20 mg kg^{-1} [127]. So, the use of manures as organic fertilizers on farmland containing antibiotics is fast becoming a serious environmental issue of concern [128]. Up to 200,000 tons of antibiotics are both used per annum by humans and administered to farm animals [129]. About 70% are consumed as growth promoters [131, 131], irrespective of the 1998 EU embargo [132, 133]. Massive utilization of antibiotics in veterinary practices remains in China, Russia, Europe and the USA [134, 135] where the largest producer and user of antibiotic is China. Tens of thousands tons of penicillin and tetracycline derivatives were produced in early 2000s [136]. The prescription in China is/ was equally over double the amount in the Americas [137].

Therefore, antibiotics that end up in manure or fertilizers might have come from any of the following:

a. Feed additives (especially in fish farming)

b. Human and veterinary drugs

c. Effluents from pharmaceutical industries [138, 139]

Pharmaceuticals are excreted to the environment through the excreta (either mainly through the urine or through the faeces) from humans or animals in a semi-digested active form or as derivatives and end up in wastewater or biosolid. Some of them may be retained in the final organic fertilizers (biosolids), as well as in wastewater and reach surface water and sediments [127, 140, 141]. All kinds of manures, wastewater sludge and excreta from human are vehicles for carrying residual antibiotics in the environment [142–146]. Zhang et al. [146, 147] reported that residual antibiotics were highest in pig manure, followed by chicken manure and cow manure in that order, but this is mainly a reflection of the local situation. The concentration is, as expected, higher from large-scale agriculture farm than from subsistence farm. Rainfall will naturally add to the run-off of these from agricultural land to surface and groundwater. It also enhances the potential for distribution to other biomes with ecotoxic effects. They also get lodged in the soil organisms like earthworms, soil arthropods, fungi and bacteria.

Like internalized pathogens, the potential high uptake of antibiotics by vegetables fertilized with biosolids globally is of enhanced public health concern [148, 149].

Future attention is needed in the issue of bioaccumulation in vegetable with residual antibiotic because

a.   Vegetable rapidly take up harmful substance(s) during short growth cycle. The acute and long-term cytotoxicity effects on the consumers are unclear.

b.   Vegetables, either leafy or root, are often consumed raw. Thermal effect in cooking may affect advantageous sublimation of some harmful compounds, but this is not guaranteed and

c.   They are stored for short-term and consumed fresh, bringing about timely delivery of residual bioaccumulated antibiotics and other pollutants.

Therefore, they bring about any of the following environmental impacts:

a.   Emergence of bacterial resistance through long-time exposure to sublethal concentration of the residual antibiotics, genetic variation resulting from innate adaptive drives of the bacteria and also provide a pseudo-biofilm environment for exchange of antibiotic-resistant genes (ARGs) [150–152]. It is an established fact that exposure to low-level or sublethal or sub-minimum inhibitory concentration (sub-MIC) of antibiotic drug has effects on the bacterial physiology and its genetic or phenotypic variability, and the potentials of antibiotics to function as signalling molecules. All these factors contribute to prompt emergence and spread of antibiotic-resistant bacteria among humans and animals.

   Laboratory-based methods have been developed to determine the effect of exposing bacteria to sublethal concentrations (sub-MIC) of antibiotics. This has affirmed the implication of the antibiotics in environment, including those in organic fertilizers, on the emergence of antibiotic resistance. These kinds of research also encompass the *in vitro* pharmaco dynamic models, concentration and exposure time of susceptible bacteria to selected conventional antibiotics before the emergence of resistance. The concentration variations to be employed for such studies will be informed by the concentration of the extracted antibiotics in the organic fertilizers.

b.   As it is a generally accepted fact that all drugs, including antibiotics have their side effect. It is only advantageous if taken to remove a more serious infection. Continuous exposure of farmers to residual antibiotics in dust [127] from soil fertilized with organic fertilizer exposes them to risk associated with accumulative effect of the gradual exposure.

c.   Ecotoxic effects on other biotic components of the environment.

5. Guidelines for reuse of human and animal waste products as organic fertilizers

The WHO operational monitoring guidelines for the reuse of wastewater, excreta and greywater to fertilize crop strictly advocate certain validation requirements, operation monitoring parameter and technical measures, and verification monitoring are as stated in **Table 7** for safe reuse of waste. WHO guidelines [48] exemplify the die-off efficiency with a temperature of 50°C for at least 1 week before compost or ecohumus is considered safe for

reuse. If this temperature is not achieved, a longer composting/storage time has been advocated by WHO. One to two years of storage is recommended for systems that generate ecohumus for proper removal of bacterial pathogens and appreciable reduction of viral and parasitic protozoa. WHO [48] identified the risk on the exposed groups to the reuse of the excreta and wastewater, and recommended health protective measures. The guidelines also include standards for chemical in fish and vegetables. According to the guideline, ≤1 helminth's eggs (arithmetic mean number) per litre or per gram total solid applies for excreta to be used on edible products and organic fertilizers to which agriculture workers would be exposed. The guidelines also contain threshold values for bacterial pathogens (based on $\leq 10^4$–$\leq 10^5$ CFU *E. coli* per 100 mL or g total solid) and for trematode eggs (absent) in aquaculture.

Control measures	Validation requirements	Operation monitoring parameter and technical measures	Verification monitoring
Fertilizer handling	Reduce direct contact with insufficiently treated material and environmental contamination	Wearing gloves Washing of hands and equipment used	Informed farmers using excreta special equipment available
Fertilized field	Time needed for pathogen die-off under different climatic conditions and withholding time between waste application and crop harvest to ensure minimal contamination	Working excreta into the ground, information and signs avoiding overfertilization	Analyse plants' contamination
Fertilized crop-produce restriction	Survey of product consumers to identify species always eaten after thorough cooking	Harvesting and transport practices	Testing of excreta/greywater to ensure that it meets WHO microbial reduction targets
	Analysis of marketability of different species/crops Economic viability of growing products not for human consumption. Harvesting, transport and trade consumption Contamination of hands, kitchen utensils, food	Withholding time between fertilization and harvest Types of crops grown in excreta use areas crops cooked before eating	Proper preparation and cooking of food products Domestic and food hygiene Hand washing

Table 7. Validation requirements, operation mornitoring parameter and technical measures, and verification monitoring for reuse in fertilization (adapted from WHO [48]).

6. Conclusions and research gaps

The WHO guidelines Vol 2 (Wastewater Use in Agriculture) and Vol 4 (Excreta and Greywater Use in Agriculture) [48, 153] form an evidence base and referral point for risk management

strategies and risk mitigation. As such they are applicable for the planning and implementation of health aspects, especially related to pathogens, of use schemes for organic fertilizers, whether defined as biosolids, faecal sludge, manure, urine or different mixtures of these and with plant materials. The guidelines are building on microbial risk assessment (MRA) with identification and characterization of hazards, exposure assessment and risk characterization and management that can be applied with different levels of sophistication. This can be part of a scenario or model approach or built into a management approach. With modifications but with its different components it formed the base for "Human Health Risk Assessments of Pathogens in Land-applied Biosolids" [154] in the USA, with a model and scenario-based approach. It further forms a base for the simplified risk management approaches within the WHO sanitation safety plans (SSPs) [155].

For organic fertilizers in agriculture, the major differences in the hazard identification and characterization are locally specific, partly driven by the sources of the organic fertilizers used and partly reflecting the regional and socio-economic situations. In this context, the risk may partly be regarded higher in transient and developing global economies. It further relates to the treatment and application barriers, where regulations and enforcement against most often will be more stringent in developed regions and economies [156–158].

The WHO guidelines are further framed around a risk-reduction strategy accounting for a multiple risk barrier approach, which embrace both technical and handling barriers. This is applied to ensure a reduced exposure risk, which in relation to the application of biosolid, faecal sludge or manure etc. should reduce the risks in relation to both the crop and soil, to agricultural workers, communities or due to secondary run-off and impact. The technical reduction barriers here naturally play a fundamental role where different treatment methods have different efficiency. In the USA, a pathogen equivalency committee [159] should be able to assess new methods to ensure a high level of safety. Safety is also ensured in the way that the application is made in the agricultural fields, the crop selection and the impact of environmental factors (e.g. sunlight, temperature etc) on pathogen die-off. Again, large differences occur locally, seasonally and between different economic regions and social strata.

Even if the different risks and the level of risk can be identified, the epidemiological evidences are still poor for different types of organic fertilizers and especially if we should value this transmission route in relation to others. This further relates to different global regions and socio-economic conditions. The study outcomes from specified investigations in the USA, in EU or in Australia, for example, cannot be directly transferred to the conditions and situations on other continents and vice versa.

Low-cost treatment and handling approaches applicable for developing regions need further attention, where seasonal variations also need to be further accounted for.

The evidence base related to microbial die-off under different field conditions need to be substantially broadened and performed studies so far systematized in relation to effect.

The relationship between animal waste, water and environmental quality and human health have been addressed from a zoonotic livestock perspective, including management practices,

exposure interventions and risk analysis but need much further attention related to organic fertilizers [160].

Crop contamination is documented but the relative impact between pre-harvest contamination by organic fertilizers and irrigation water on the one hand and post-harvest handling and storage contamination on the other needs to be further addressed. The specific situation with the potential impact of internalization and uptake of pathogens as compared to deposition on outer surfaces need much more attention and documentation, before long-term handling and management practices can be issued and related to modes of application.

Also, the specific situation, partly addressed in this chapter with uptake of antibiotics (and other organic contaminants) as well as the impact of use of these in livestock and among humans and the further fate in agricultural fields need to be addressed. Linked to this is also the large problem complex with the occurrence, transmission and impact of antibiotic-resistant bacteria especially, but also including other antimicrobial drugs.

At the current stage, the authors believe and conclude that the benefits with human- and animal-based organic fertilizers in the field far outmaster the potential negative impacts. However, we also firmly believe that a broadened evidence base and application of this in a risk-management perspective and framework will further enhance the positive benefits and counteract negative impact.

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