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Review article

Hygienic and ecological risks connected with utilization of animal manures and biosolids in agriculture[☆]

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Abstract

In recent years the fate of human and animal pathogen microorganisms as a potential pollutant of the environment has been paid increased attention. Substantial quantities of these compounds and their metabolites are excreted, flushed down the drain, discarded as waste, or left over in animal feedlots. After passing to the sewer, several of these compounds are not adequately eliminated by the methods that are currently used in sewage treatment. Substantial quantities of biosolids and livestock manure end up on agricultural land. Effective sanitation of the environment, particularly of some of its special parts, which can be a source of spreading of diseases, plays an important role in prevention of infectious diseases. In this respect special attention should be paid to the disinfection of infected farm animal excrements. Sanitation of excrements should, on the one hand, ensure effective inhibition of infectious agents and, on the other hand, comply with the requirement of preserving the composition of the manure so it can be used in agricultural production.

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1. Introduction

Livestock manure has been spread on the land for many centuries not only for disposal but also as

fertilizer. Before the advent of inorganic fertilizers, it was the principal source of plant nutrients added to the soil. Manure and other wastes of various agricultural animals often contain high concentrations of human pathogens. Moving manures from region to region represents a seemingly simple solution to the environmental problems of those areas with excess nutrients. However, this approach is fraught with problems based on the scale of the operation, nutrient monitoring and, in some cases, disease risks. The scale of the problem is mostly attributable to the

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volume of liquid slurry and requirements on its storage before it can be applied to the soil. The solid wastes (e.g. farmyard manure) could be beneficially used without problem on the farm or locally. Treatment has a clear role in the overall management package, but only some of the systems emerging are both practicable and effective at the farm level.

Land application of biosolids provides agricultural benefits and presents a cost-effective method of sludge disposal following wastewater treatment. However, reuse of this product presents health concerns that must be addressed and satisfied before land application becomes an accepted practice. Health concerns include pathogen transmission to food or agricultural workers, contamination of ground water or surface water with fecal material from field runoff, and build-up of heavy metals or organic contaminants.

2. Pathogens in animal manures and in biosolids

Manure pathogen levels depend on the source animal, the animal's state of health, and how the manure was stored or treated before use. Non-point (diffuse) sources of contamination by manure are pastured animals, roaming wild animals and leaching or runoff from agricultural areas. Point sources of manure contamination include animal feedlots, animal housing facilities, and manure storage areas, such as lagoons. Special attention should be paid to the pig slurry solids collected in the first stage of manure treatment plants on farms as they are used almost exclusively for agriculture purposes and require proper sanitation (Venglovsky et al., 2005). The point sources may also contribute to diffuse contamination of soil and water by manure (runoff or leaching).

Infected animals shed more than 30 causative agents of public health importance, and many of these are of special importance in faeces and wastewater. The use of faeces and wastewater in agriculture may result in public health risk only if all of the following prerequisites concur:

- (a) if an infective dose of an excreted pathogen reaches the pond or a natural water body, or if the pathogen multiplies in an intermediate host

residing in the pond or in the aquatic environment to form an infective dose;

- (b) if this infective dose reaches a human host through contacts or consumption of the aquacultural products;
- (c) if this host becomes infected;
- (d) if this infection causes disease or further transmission.

A different group of pathogens are present in human wastes and in animal manures (Table 1).

2.1. Bacteria

Many pathogenic bacteria are present in animal manures. The bacterial pathogens most important with regard to human health include, for example, *Salmonella* sp., *E. coli* O157 H7, *Campylobacter jejuni*, *Yersinia enterocolitica* and *C. perfringens*. *Salmonella* are *Enterobacteriaceae* which are widely distributed in the environment and include more than 2000 serotypes. They are one of the most predominant pathogenic bacteria in wastewater and include serotypes that cause typhoid, paratyphoid fever and gastroenteritis. It has been estimated that 0.1% of the population excretes *Salmonella* at any given time. This pathogen produces an endotoxin that causes fever, nausea and diarrhea and may be fatal if not properly treated by antibiotics (Bitton, 1994). Serotypes implicated in food contamination are *S. paratyphi* and *S. typhimurium*. These serotypes can grow readily in contaminated foods and cause food poisoning (Cabada et al., 1995). Shiga-like-toxin-producing *Escherichia coli* strains are major food-borne bacterial pathogens that have been implicated in diarrhea, hemorrhagic colitis, and the hemolytic-uremic syndrome. Because *E. coli* is common in biosolids and has the potential for regrowth (Straub et al., 1993), it is important to assess its survival in biosolids.

2.2. Parasites

At the present, helminthic pathogens play a minor role in industrialised countries. Many helminths are, however, endemic in most tropical, less industrialised countries, and have, via waste-fed aquaculture, various potential transmission patterns. *Ascaris* (roundworms),

Table 1
Pathogens in human wastes and in animal manures (Filip et al., 1988)

Pathogen	Disease
<i>Virus</i>	
Enterovirus	Gastroenteritis
Rotavirus	Gastroenteritis
Parvovirus	Gastroenteritis
Adenovirus	Respiratory infections
Hepatitis A virus	Viral hepatitis
Polio virus	Poliomyelitis
Coxsackie virus	Meningitis
<i>Bacteria</i>	
<i>Salmonella</i> (1700 serotypes)	Salmonellosis
<i>Shigellae</i>	Shigellosis
<i>Mycobacterium tuberculosis</i>	Tuberculosis
<i>Vibrio cholerae</i>	Cholera
<i>Escherichia coli</i>	Gastroenteritis
<i>Yersinia enterocolica</i>	Gastroenteritis
<i>Clostridium perfringens</i>	Gastroenteritis, gangrene
<i>Clostridium botulinum</i>	Botulism
<i>Listeria monocytogenes</i>	Encephalitis
<i>Campylobacter jejuni</i>	Gastroenteritis
<i>Fungi</i>	
<i>Candida</i> sp	Mycoses (skin and systemic)
<i>Tricosporon cutaneum</i>	Skin mycosis
<i>Aspergillus fumigatus</i>	Lung mycosis
<i>Trichophyton</i> sp.	Skin mycosis
<i>Epidermophyton</i> sp.	Skin mycosis
<i>Microsporium</i> sp.	Skin mycosis
<i>Protozoa</i>	
<i>Entamoeba</i> sp.	Amoebic dysentery
<i>Giardia lamblia</i>	Giardiasis
<i>Balantidium coli</i> (rare)	Dysentery
<i>Cryptosporidium parvum</i>	Diarrhoeal illness
<i>Acanthamoeba</i> (rare)	Meningoencephalitis
<i>Helminths</i>	
<i>Ascaris lumbricoides</i>	Human large round worm
<i>Ancylostoma</i> sp.	Hookworm
<i>Necator americanus</i>	Common hookworm of man
<i>Enterobius vermicularis</i>	Human pinworm
<i>Strongyloides stercoralis</i>	Small roundworm
<i>Trichuris trichiura</i>	Human whipworm
<i>Taenia solium</i>	Human tapeworm
<i>Hymenolepis nana</i>	Dwarf tapeworm

the most common helminthic pathogen in developing countries, may be carried passively by fish and other aquacultural products although its major foci are soils and waste-fertilised crops. The three main transmission routes for *Ascaris* are: transmission

from faeces-contaminated surfaces and materials, transmission to workers in fields that have been fertilised with faeces or sewage and transmission by consumption of vegetables fertilised with faeces or sewage (Feachem et al., 1983; Varadyova et al., 2001).

2.3. Viruses

More than 140 enteric viruses can be transmitted by biosolids. The calciviruses, adenoviruses, hepatitis A and E viruses, astroviruses, and rotaviruses are of particular concern. It is notable, however, that zoonotic viruses of humans continue to be discovered or appear to re-emerge as important human pathogens. Recent among these are the SARS virus (a coronavirus), monkeypox virus (a poxvirus), and hepatitis E virus. A quantitative risk-assessment model is available to assess the risk of infection from exposure to this pathogen (Haas et al., 2000).

3. Survival of pathogens

Pathogens may persist in liquid manure for a long time depending on storage conditions, type of slurry, storage temperature and pathogen type. They will be inactivated after exposure to the environment but may survive long enough to be of public and/or animal health concern.

The main factors influencing die-off are temperature, dryness and UV-light. Experimentally, reduction in temperature and increase in solids content has been shown to enhance pathogen survival (Feachem et al., 1983). Previous studies on the fate of *E. coli* O157:H7 in bovine feces revealed that the pathogen survived for 42 to 49 days at 37 °C, for 49 to 56 days at 22 °C, and for 63 to 70 days at 5 °C (Wang et al., 1996). Another study of *E. coli* O157:H7 in manure heaps revealed that the pathogen could survive for up to 47 days, 4 months, and 21 months in bovine manure and aerated and non aerated ovine manure, respectively (Kudva et al., 1998). Furthermore ammonia (NH₃) is known to be bactericidal. Therefore, bacteria die-off is enhanced at pH ≥ 8.5–9 due to an increase in the NH₃/NH₄ ratio. According to Strauch (1987) 90% of *Salmonella* reduction is associated with pH decrease in the substrate. The

decrease of the pH value during storage is influenced by the natural bacterial flora producing fatty acids which have toxic effects upon *Salmonella*. The latter, in contrast to natural bacterial flora, are not able to secure nutrients and this probably causes their die-off (Strauch, 1987). Niewolak (1994) stated that microorganisms (*Escherichia*, *Salmonella*) that reach the soil by means of application of contaminated pig slurry can penetrate to the depth of 160–180 cm. Henry et al. (1995) isolated *Salmonella* from pasture 2 months, and from soil 8 months, after the application of contaminated pig slurry. This is the reason why it is necessary to ensure proper sanitation of contaminated slurry.

Another factors such as concentrations of solids and seasons could influence the survival of bacteria. Larsen and Munch (1986) found that *S. typhimurium* survived in pig and cattle slurry stored at 8 °C for more than 10 weeks with only a small decrease in numbers (from 10^6 to 10^4 /ml). *Y. enterocolitica* was eradicated

by week 6 while *Staphylococcus aureus* was reduced from 10^6 to 10^2 /ml in 9 weeks. In the solid fraction of pig slurry, *S. typhimurium* survived for 26 days in summer and 85 days in winter (Placha et al., 2001), and coliforms were reduced by 90% in 35 and 233 days during the summer and winter time, respectively.

The protozoan parasites often associated with biosolids include *Giardia* and *Cryptosporidium* spp. However, little research has been conducted on the survival of these parasites in biosolids-amended soil. One report documented increased inactivation of *Cryptosporidium parvum* as temperature increased from 35 to 50 °C and water potential decreased (Jenkins et al., 1999). Little is known about the viability of these parasites following land application of biosolids, and research in this area should be encouraged. Helminthes are perhaps the most persistent of enteric pathogens. *Ascaris* eggs survive several years in soils, although very dry or very wet soils decrease survival (Straub et al., 1993; Varadyova et

Table 2
Animal waste treatment processes and pathogen reduction (Strauch, 1987)

Treatment process physical	Pathogen reduction (\log_{10}) with all types of treatment reduction depends on respective microorganism	Main factors affecting the reduction
<i>Heat/Thermal Processes</i>		
Mesophilic	Typically, 1–2	Temperature, contact time, pH, etc.
Thermophilic	Typically, >4	Temperature, contact time, pH, etc.
Freezing	Variable	Waste composition and conditions, temperature, etc.
Drying or desiccation	Typically >4 at <1% moisture; Typically <1 at >5% moisture	Contact time, pH, etc.
Gamma irradiation	Typically >3	Dose, waste, etc.
<i>Chemical</i>		
High pH (>11)	Inactivation at high pH, e.g., alkaline/lime stabilization; >3–4	Contact time, pH, etc.
Low pH (<2 to <5)	Inactivation at low pH; acidification: typically, <2	Contact time, pH, etc.
Ammonia	Inactivation at higher pH where NH_3 predominates	Contact time, pH, other waste constituents
<i>Biological Processes</i>		
Aerobic, mesophilic	Typically 1–2	Solids separation, contact time, reactor design, temp.
Aerobic, thermophilic (composting)	Typically >4	Solids separation, contact time, reactor design, mixing methods, temperature
Anaerobic, mesophilic	Typically 1–2	Contact time, reactor design, solids separation, temperature
Anaerobic, thermophilic	Typically >4	Contact time, reactor design, solids separation, temperature
Silage treatment, mesophilic	Variable	Ensiling conditions
Land application	Highly variable and largely unknown; potentially high	Site-specific factors: temperature, precipitation, vadose zone, loading, sunlight, soil constitution, etc.

al., 2001). Pathogen reductions in animal manures by manure treatment and management processes are summarized in Table 2.

4. Techniques for reducing pathogens

In order to eliminate these risk factors different technologies are used, aerobic thermophilic stabilization with or without a subsequent anaerobic process (see also in this issue the review by Juteau), lime disinfection of sludge by $\text{Ca}(\text{OH})_2$ or CaO , composting of sludge, and other (Plachy, 1995; Novak, 1994). Besides lime the effect of some natural materials such as zeolite, bentonit was investigated (Sasakova et al., 2004; Varadyova et al., 2003). Aerobic stabilization of sewage sludges is a biological oxidation process of organic sludges which makes use of the anabolic and catabolic activity of aerobic microorganisms capable of producing heat. With an accurately run process (adequate aeration and sludge dry residue) the temperature can rise up to the thermophilic range (45–70 °C) in which pathogens are killed and the sludge becomes stabilized, odour free and suitable for agricultural use as a high-quality organic fertilizer (Smith et al., 1975; Dubinsky et al., 2000).

Composting is one of several methods for treating biosolids to create a marketable end product that is easy to handle, store, and use. Several authors including Niewolak and Szelagiewicz (1997), Novak (1994) and Plachy (1995) have reported that composting results in significant decreases in pathogenic bacteria, fungi and helminth eggs and high-quality organic manure with a considerable portion of humic substances. Two of the physical factors for reduction are heating and cavitation. It is difficult to examine the impact of only one physical factor, such as temperature, on inhibition of pathogens. At 52 °C, complete inactivation of helminth eggs requires approximately 20 days. Inactivation with thermophilic alkaline processes and composting of biosolids requires approximately 3 to 5 days. Inactivation will also be affected by other factors such as ammonia, organic constituents, dissolved solids, and hydroxide anions. Biological processing has been effective in the digesting, composting, and storage of biosolids. In these processes, there is mechanical or autothermal heating. Biocidal inactivation has been observed in

lagoon storage. An increase in solids from 4% to 24% resulted in a 5-fold increase in parasite and bacteria die-off and a 25-fold increase in virus die-off (Bowman et al., 2003).

The main disadvantage of using chemical disinfectants is the negative effect of these agents upon the living environment, their price and the demand on the technology of application. An exception can be found in those substances used to disinfect sludges which do not load the environment, are accessible and even capable of improving the dunging quality of sludges (Burton and Turner, 2003). All these criteria are met by liming of sludges. For this purpose lime in the form of lime hydroxide or calcium lime is used. The latter, if added to sludges, reacts with water and produces $\text{Ca}(\text{OH})_2$ and heat (exothermic reaction). The heat acquired and the alkalic pH are the decisive disinfecting factors. However, liming is not appropriate for limestone soils. Since isolated and closed reactors have to be used (Strauch and De Bertoldi, 1985), the technology is more demanding and thus less favourable. Lime hydrate is often used as a flocculant in sludge dehydration and depending on the dose used and the properties of sludges pH grows to alkalic. Even dispersion is a requirement on lime hydrate application (Sattar et al., 1976).

Plachy (1995) investigated that a laboratory aerobic stabilization of the primary sewage sludge within a thermophilic temperatural range (max. stabilization temperature 52.5–62.5 °C) exhibited a 100% lethal effect on eggs of the model nematodes *Toxocara canis* and *Ascaris suum*. Under a temperature reaching the mesophilic range (36.9–38.3 °C), aerobic stabilization reduced the infectivity of *T. canis* eggs to the values

Table 3
Summary of microbial reduction during sludge treatment (Ward et al., 1984)

Treatment	Reduction*		
	Bacteria	Viruses	Parasites
Anaerobic digestion ^a	1–2	1	0
Aerobic digestion	1–2	1	0
Composting	2–3	2–3	2–3
Air drying ^b	2–3	1–3	1–3
Lime stabilization	2–3	3	0

*Scale: 0=<10% reduction; 1=99% reduction; 2=99.9% reduction; 3=99.99% reduction.

^a Mesophilic temperatures (27–37 °C) assumed.

^b Effects depend on moisture levels.

of 1–6% and the viability of *A. suum* nonembryonated eggs remained at 35%.

A summary of pathogen reduction by the various sludge treatment processes is listed in Table 3.

5. Conclusion

Legislation in industrialised countries requires acceptable procedures for the disposal and utilization of sludges. One of these procedures, land application, offers many advantages, some of which are the disposal of cumbersome material, supply of valuable nutrients to agricultural crops, and improvement of soil properties. However, there is public concern about the agricultural use of sludge. Agricultural sludge reuse is acceptable and accepted only if the sanitary quality of the sludge is sufficiently guaranteed and the public concern can be limited. States or multinational organizations have implemented or are implementing regulations which are based on our present knowledge. Regarding pathogens, the regulations for use of sludges for agricultural purposes are based on three principles: (i) a requirement for treatment, including thermal treatment, to reduce the amount of pathogens; (ii) validation of the treatment; and (iii) assurance of the microbiological quality of the sludges. Several countries have developed legislation concerning the use of residual sludge. In France, the decree of January 8th, 1998 specifies the technical regulations governing the spreading of sludge on agricultural land. In the United States, for class A sludge the pathogens (*Salmonella*, enteroviruses and viable nematodes) must be reduced to below detectable levels and for class B (use with restriction) pathogenic bacteria and viruses are adequately reduced in density as demonstrated by a fecal coliform density in the treated sludge of 2×10^6 MPN (Most Probable Number)/g total solids sewage sludge (dry weight basis). Viable helminth ova are not necessarily reduced in a class B sludge (Gantzer et al., 2001).

Thermophilic aerobic stabilization of sewage sludges has been used in Switzerland, Germany, England, less in the USA to prevent transmission of *Salmonella* spp. from the sludges applied to agricultural land. In the first stage sludges are disinfected by a thermophilic anaerobic fermentation, in the second stage stabilization takes place in mesophilic anaerobic

fermentors. To achieve a thermophilic temperatural range of aerobic fermentation, sludges have to be concentrated. The most common way of sludge thickening is gravitation.

The environmental benefits of better manure management will only be fully realized if there is a broad uptake of such techniques in the key areas. It is possible that larger livestock units could accommodate such technology with relatively little support but for smaller farms, it is unlikely to be practical and the option of centralized facilities may need to be considered.

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