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Pathogens in septage in Vietnam

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ABSTRACT

Septage is widely acknowledged as a major source of infectious pathogens while disposal of septage, and the operation and maintenance of septic tanks, is not regulated in many developing countries. Twenty untreated septage and septage sludge samples were taken from Can Tho City, Vietnam to examine their pathogen content, and indicator micro-organisms. *Escherichia coli* and *Enterococcus* spp. were detected in all samples, regardless of sludge storage time. Phages were detected in 80% of samples. *Salmonella* spp. were detected in 70% of the untreated septage and 60% of septage sludge samples. Concentrations of phages and bacteria tested in septage sludge after many years of tank storage were much higher than the expected levels. Helminth ova were present in 95% of untreated septage samples with an average of 450 ova l^{-1}, and were detected in all septage sludge samples with an average of 16,000 ova l^{-1}. Twelve varieties of helminth ova were identified. More helminth ova varieties in higher concentrations were found in septage than those reported from stool samples. The varieties' frequency ranged from 10% to 50% and *Ascaris lumbricoides* predominated. Results show that pathogens and indicator micro-organisms, especially helminth ova, accumulate in sludge. Thus helminth ova should be considered when septage sludge is treated and used for agriculture. Proper health protection measures must be applied for people handling septage.

1. Introduction

Disposal of septage sludge and operating and maintaining septic tanks are a problem in developing countries like Vietnam. Septage is widely acknowledged as a major source of infectious pathogens like enteric bacteria, viruses, protozoa and helminths. It has been recently reported that 75% of Ho Chi Minh City's septage sludge is discharged directly into the environment (Cuong, 2008). In Nam Dinh City (Northern Vietnam), septage sludge was discharged into fishponds and on fields or wherever the pump truck driver found a place to dump it (Klingel et al., 2001). Harada et al. (2008) showed that 89.6% of septic tanks in Ha Noi have never been desludged and that they underperformed; a situation that is typical of the country as a whole (Bao, 1996). In general, septic tanks in Vietnam are emptied only when blockages occur or odour become unbearable, which arises when the tank is full and untreated septage leaks and contaminates the surface water.

Surveys of septage and strategies for improving faecal sludge management have been undertaken in some provinces in Vietnam (Klingel et al., 2001; Bao, 1996). Those studies focused on physio-chemical aspects of septage and its treatment. Yet the microbiological make-up of untreated septage and septage sludge in Vietnam is not well documented, especially the variety of helminths whose infections are considered a burden in Vietnam (Trang et al., 2007; Dung et al., 2007). Most studies of human helminth infections among people in Vietnam using stool samples have focused on soil-transmitted helminths (STH) like *Ascaris lumbricoides*, *Trichuris* spp. and hookworms (Van der Hoek et al., 2003; Trang et al. 2007; Do et al., 2007; Nguyen et al., 2006). Of all helminth ova likely to be present in wastewater STH are simply known as common intestinal worms and are a public health concern (Scott, 2003). However, many more varieties of helminth eggs exist (Feachem et al., 1983), especially in warm climates with poor sanitary conditions which typify Vietnam. *Taenia* spp., for instance, are responsible for most cases of adult-onset epilepsy in the world since the larval stage of this zoonotic cestode invades the human brain (Gonzalez et al., 2006).

Most septic tanks in Vietnam receive only black water (Viet-Anh et al., 2007; Harada et al., 2008). In terms of nutrient recycling the septage sludge might be fit for agriculture. Traditionally human excreta such as sludge from bucket latrines were used as fertilizer in Northern Vietnam. Today the use of such sludge is decreasing due to the replacement of bucket latrines by septic tanks, in both urban and rural areas (Klingel et al., 2001). In some cases septage sludge is used as an alternative fertilizer in agriculture and aquaculture without any prior treatment. Since most septage sludge remains in septic tanks for a long time, up to 30 years in the case of one survey conducted in Ha Noi (Harada et al., 2008), most pathogens may be eliminated in this long-term stored sludge. However,

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the microbial characteristics of sludge after many years tank storage have not yet been determined.

Maintenance of septic tanks requires septage sludge to be removed on a regular base. Orders to empty septic tanks, either by vacuum track or manually, are made by house owners and they have to pay for the service. Klingel et al. (2001) found that one-third of septic tanks in Nam Dinh City were only ever emptied manually, with workers not taking any health protection measures. In most cases, the tanks are not constructed for regular maintenance and emptying is combined with reconstruction, e.g. opening the floor for access to the tank. Thus workers are exposed to untreated septage and septage sludge in most cases. However, there is little information about the danger of the substrate they are working with and the chance of contracting an infection.

The objective of this study was to examine the pathogenic content and indicator organisms in septage in Can Tho City, Southern Vietnam so as to give an overview of the population's health situation and possible uses of sludge for agriculture and aquaculture, as well as the identification of treatment needs for the removal of pathogens and their safe management. Besides septage sludge, untreated septage was also tested since it is the effluent in full septic tanks. This untreated effluent is often discharged directly to surface water without any treatment.

2. Material and methods

Samples were taken between September and November 2008 from septic tanks from 20 single-family dwellings as they were being emptied by a pump truck. Two samples were taken from each tank: one at a depth of 10 cm (untreated septage), the other from the centre (septage sludge) when the tanker had extracted half the contents. All septic tanks were full at sampling. The emptying intervals ranged from 1 to 20 years. Septic tanks with two compartments and a storage volume of 1–2 m^3 predominated (16 out of 20 tanks). The number of users of septic tank per household ranged from two to ten with an average of five. No readymade inspection hatches existed in the surveyed tanks. Workers had to damage house floors as the septic tanks were located in the basements.

Samples arrived at the laboratory within 1 h and were stored at 4 °C before analysis. Samples were processed within 24 h of sampling with the exception of helminth ova. Analyses included pH, dry matter (DM) and somatic coliphages (SC), male-specific bacteriophages (MSB), *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., and helminth ova. MSB and somatic coliphages were counted by the single-agar-layer technique as described in ISO 10705-1 and ISO 10705-2. *E. coli* was counted on Chromocult®Coliform Agar (Merck) after 24 h incubation at 36 ± 1 °C. *Enterococcus faecalis* was enumerated on Enterococcus Selective Agar according to Slanetz and Bartley (Merck) after 48 h of incubation at 36 ± 1 °C. *Salmonella* spp. were counted via the most probable number (MPN) method in Rappaport–Vassiliadis broth (48-h incubation at 36 ± 1 °C) and Hektoen agar (24-h incubation at 36 ± 1 °C). Helminth ova were determined by using WHO guidelines (Ayres and Mara, 1996). Ova with incomplete walls and empty ova were not counted. Only fertile specimens of *A. lumbricoides* ova were counted.

3. Results and discussion

3.1. Characteristics of untreated septage samples

Untreated septage was low in dry matter (average DM = 0.24%). The pH values ranged from 7.3 to 7.5. Occurrence and levels of indicators and pathogens studied in untreated septage are reported in Table 1. Helminth ova detected were those of *A. lumbricoides*, *Enterobius vermicularis*, *Hymenolepis diminuta*, *Hymenolepis nana*, *Taenia* spp., *Capillaria philippinensis* and hookworm. Their frequency varied from 20 to 40% of samples. The average concentration for each species ranged from 6 to 190 no.l^{-1} (data not shown).

When the septic tank is full, untreated septage often flows directly to, and contaminates, the surface water. The average concentration of *E. coli* in the untreated septage does not meet the Vietnamese Domestic Wastewater Discharge Standards (TCVN 6772:2000), as it only allows for levels of total coliform in the range of 1000 to 10,000 MPN/100 ml. In our study we only measured *E. coli* and found levels above the limit in all samples (Table 1).

As the studied tanks were filled with sludge, the hydraulic retention time of the wastewater was short. Helminth ova in the overflow do not sediment fast enough to be captured in the sludge accumulated at the tank's base, and the ova risk ending up in the surface water, which is used by many people every day. Thus increasing the frequency of emptying means the risk of transmitting helminths to the surface water is reduced. While residents must obtain a construction permit for the design of a septic tank before houses are built, there are neither regulations nor legislation for their emptying. Thus septic tanks should be built in such a way as to be easily accessed for maintenance, e.g. removable access covers should be installed for easy inspection of sludge level and emptying. Besides raising awareness in the general population, there must be changes to the legal and policy frameworks if septic tanks are to perform optimally and decrease the risk of contamination to surface water.

3.2. Characteristics of septage sludge samples

Septage sludge had an average dry matter of 5.4%. The pH varied from 6.7 to 7.4. *E. coli*, *Enterococcus* spp., and helminth eggs were detected in all samples tested (Table 2). Phages and bacteria found in septage sludge were in accord with the concentration found by Lepeuple et al. (2004). Yet the percentage of phage-positive samples differed spatially depending on the health situation of different human populations. Lucena et al. (2003) for instance reported that MSB were detected in all septage sludge samples in Buenos Aires (Argentina), while Calci et al. (1998) found that only 58% of samples collected in Southern Rhode Island (USA) were MSB positive.

That helminth ova were detected in 100% of septage sludges tested is supported by a study of Bao (1996) in Northern Vietnam, although the concentration range observed in the presented study is wider. There was no correlation found between helminth ova concentration and retention time of tank sludge (data not shown). From approximately 300 m^3 of septage sludge discharged daily into the environment in Ho

Table 1
Concentration of micro-organisms tested in untreated septage samples ($n = 20$; average dry matter = 0.24%).

Organism tested	Unit	Range	Mean	Median	SD	% positive
Somatic coliphage	pfu ml^{-1}	ND–1.9×10^5	1800	150	25,000	80
Male-specific bacteriophage	pfu ml^{-1}	ND–1000	520	600	380	80
E. coli	CFU ml^{-1}	2,000–3.5×10^5	68,000	11,000	34,000	100
Salmonella spp.	MPN/100 ml	ND–7000	1300	310	270	70
Enterococcus spp.	CFU ml^{-1}	640–24,000	8500	5300	4900	100
Helminth ova	no.l^{-1}	ND–1200	450	240	440	95

ND = not detected.