BACTERIOLOGICAL AND PARASITOLOGICAL RISKS ASSOCIATED WITH AGRICULTURAL WASTEWATERS AND SEWAGE SUBJECTED TO BIOLOGICAL TREATMENT

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SUMMARY

The aim of our study was to investigate the bacteriological and parasitological risk associated with the products of aerobic treatment of pig slurry and municipal sewage. We focused on the quality of effluent and on sludge/pig slurry solids from two wastewater treatment plants (pig slurry WWTP-1; municipal wastewater WWTP-2). Efficiency of removal of selected bacteria ranged between 79.8 and 97.9%. No helminth eggs were found in effluents. One sample of sludge (out of 40) contained 1 egg of Ascaris spp. and another 1 one egg of Trichuris spp.

Keywords: helminth eggs, bacteria, wastewater treatment, pig slurry, sewage sludge

INTRODUCTION

Agricultural use of organic waste materials is an option favourable from the economical point of view but there are certain aspects that have to be respected with regard to environmental protection and the necessity to protect humans, animals and plants from undesired infections (Papajová *et al*., 2002).

The requirement for spreading diseases by raw and processed wastes is that the material must become infected with the causative organisms, which must survive treatment or storage, remain capable of causing disease and survive in the material until a human or animal host is encountered. The type of pathogens most commonly found in sewage and sewage sludge depends on the state of health of the population, as well as the presence of hospitals, meat processing plants and abbatoirs in the area (Bruce and Davis, 1983). Sewage sludge may contain a large variety of bacterial and viral pathogens including *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and enteroviruses as well as eggs of parasites such as *Ascaris lumbricoides* and oocysts *Cryptosporidium* spp. and *Giardia* spp. (Straub *et al*., 1993).

With regard to disposal of pig excrements, bacteria such as *Salmonella* spp., *Escherichia coli*, *Mycobacterium* spp., *Enterococcus* spp., *Streptococcus* spp., *Staphylococcus* spp. pose a potential threat to animal and human health. In addition to that protozoa (*Isospora* spp., *Balantidium coli*) and eggs or larvae of enteronematodes (*Ascaris suum*, *Oesophagostomum* spp., *Trichuris suis*) are also found in pig faeces. *Ascaris* eggs and coccidial oocysts are hygienically the most hazardous, primarily for their high resistance in the environment (Novák *et al.*, 1998; Dubinský, Juriš and Moncol, 2000).

MATERIAL AND METHODS

In the first stage of our study, we examined samples from waste-water treatment plant (WWTP-1) treating approx. 500 $m^3.d^{-1}$ of pig slurry and 320 $m^3.d^{-1}$ of village sewage. The solid portion of raw wastewater is separated on vibrating sieves before biological treatment and is stabilised later by simple storage for different period of time before application to soil. Samples for chemical and bacteriological examination were taken in monthly intervals. Parasite eggs and oocysts were determined in individual stages of the treatment (influent, effluent, solid fraction).
In the second stage, we investigated samples from wastewater treatment plant (WWTP-2) treating 1300 $l.s^{-1}$ of municipal wastewater. The biological stage in this plant is aerobic and the sewage sludge produced is subjected to anaerobic and aerobic treatment and dewatering.. Samples for chemical and bacteriological examination were taken in monthly intervals for one year and parasitological examination for the presence of helminth eggs and oocysts was performed twice during this period.

Of chemical parameters determined in the study we include only pH as one of the most important factors affecting survival of micro-organisms. Additional results of chemical examinations were reported elsewhere (Venglovský *et al*., 2005).

Bacteriological examination consisted of determination of plate counts of mesophilic, coliform and faecal coliform bacteria (STN 83 0531-4 and STN-ISO 9308-2) on solid cultivation media (Endo agar, Imuna, Slovakia) and faecal streptococci in the municipal sludges (STN-EN ISO 7899-2) on Slanetz-Bartley agar (Biomark, India).

Parasitological examination of solid samples was carried out by the method of Kazacos (1983). The helminth eggs from liquid samples (influent and effluent) were isolated by a sedimentation-floatation method of Cherepanov (1982), which is a modification of the method of Romanenko (1968) using saturated sacharose solution of specific gravity 1.30.

RESULTS

The results of bacteriological and parasitological analysis of samples from WWTP-1 are presented in Tab. 1 and 2. Mean plate counts of mesophilic bacteria ranged between $9.8x10^6$ and $9.2x10^8$ $CFU.ml^{-1}$, of coliform bacteria between $1.0x10^5$ and $8.9x10^8$ $CFU.ml^{-1}$ and of faecal coliform bacteria between $1.0x10^5$ and $8.3x10^7$ $CFU.ml^{-1}$. The numbers of selected groups of bacteria (mesophilic, coliform, faecal coliform) in the influent and effluent from WWTP-1 showed that mean efficiency of removal reached 95.7% for mesophilic bacteria, 86.5% for coliforms and 91.4% for faecal coliform bacteria. This resulted from the decrease in plate counts by approximately two orders of magnitude in most samplings and corresponded to the technology used. Better efficiency was reached in the warmer period (May – October) as the flocculation of activated sludge may be supported by higher temperatures. The plate counts in the solid fraction ($9.4x10^4$ – $3.8.10^9$ $CFU.ml^{-1}$) indicate high population of this substrate with the bacteria of interest. Parasitological examination showed that no helminth eggs were found in effluents from both plants despite their presence in the influent.

Bacteriological examination of samples from WWTP-2 provided different results (Tab. 3). The plate counts of investigated bacteria in the influent were lower due to considerable dilution. Mean plate counts of mesophilic bacteria ranged between $1.4x10^4$ and $4.5x10^5$ $CFU.ml^{-1}$, of coliform bacteria between $6.5x10^4$ and $3.3x10^6$ $CFU.ml^{-1}$ and of faecal coliform bacteria between

$4.1x10^4$ and $7.4x10^5$ CFU.ml^{-1}. The mean efficiency of removal reached 97.9% for mesophilic bacteria, 96.6% for coliforms and 79.8% for faecal coliform bacteria.

None of the samples (n = 10) of influent, effluent and activated sludge allowed us to recover helminth eggs. One of the factors may be the considerable dilution of human faeces in these waste-waters. Out of 40 samples of sludge only two samples were positive, one contained one egg of *Ascaris* spp. and the other one egg of *Trichuris* spp.

DISCUSSION

The epidemiological risk arising from the use of excrements and sludges is related to presence of pathogens in these substrates and their potential survival sometimes for a remarkable period of time. The subsequent direct and indirect transmission of zoonotic agents to farm animals is generally regarded as the most relevant risk factor of agricultural utilization of untreated or insufficiently treated sludge and wastes of animal origin (Juriš *et al*., 2000). Effluents from WWTP are discharged into surface waters where they increase the counts of coliform and faecal coliform bacteria and faecal streptococci. Evaluation of quality of surface waters in Slovakia shows that particularly due to microbiological parameters many rivers belong to lower quality classes (SAŽP, 2004).

The organisms used to monitor the effectiveness of sanitation treatment of organic wastes were *E. coli*, faecal streptococci and *Salmonella* spp. According to Hays *et al*. (1977) considerable number of bacteria and viruses entering the WWTP is devitalised by the treatment but endoparasite developmental stages may remain viable. With regard to their relatively high specific weight they tend to sediment and concentrate in the solid fraction together with undissolved substances and in this manner may be returned into the environment (U. S. Environmental Protection Agency, 1992). While the pathogenic viruses and bacteria may survive in the environment for hours or days, protozoan cysts remain viable for months and eggs of helminths even for years (Sobsey and Shields, 1987) because of the presence of stabilising proteins, lipids and chitin in the wall of nematode eggs (Bruňanská, 1989; Eckert, 2000). In their fully-developed, second-stage larval form, eggs of *Ascaris* spp. are highly resistant and have been frequently used as indicator organisms for water and sewage treatment processes. They may also be a good indicator for the effectiveness of composting to reduce parasites (Mara and Cairncross, 1999).

Our results showed that the efficiency of removal of selected groups of bacteria in both treatment plants corresponded to the technology used. In general, the plate counts decreased by one to two orders of magnitude. Better results were reached in the summer season which can be associated with better flocculation of the activated sludge and therefore also higher entrapment of bacteria in the sludge flocks.

Results of parasitological analysis showed that eggs of several helminths were present in the influent to WWTP-1 while all effluent samples were negative. Examination of the solid fraction indicated that considerable number of them passed to the solid fraction and therefore this substrate requires further processing before application to agricultural land. This indicates that there is a need for additional treatment of this material especially because it is almost exclusively used for agricultural purposes. Composting may be recommended as a most suitable way of treatment as it inactivates most of the agents, provided that temperatures above 55^{o}C are maintained for sufficient period of time. However, some authors reported that resistant organisms such as *Clostridium perfringens*, *C. botulinum* and the cysts and eggs of protozoan and helminth parasites may survive. There is also a danger that *E. coli* and *Salmonella* spp. may grow in the final

compost if the process has been inefficient and the organic matter remains poorly stabilised. According to Day and Shaw (2000) the temperature of 55°C is sufficient to devitalise *Ascaris lumbricoides* in 60 min and *Entamoeba histolytica* cysts in 1 sec. Thermophilic stabilisation (48.5°C) was sufficient to destroy eggs of *A. suum* in the study by Plachá *et al.* (2002). Burge (1983) observed that 10-fold reduction in *Ascaris* spp. ova was reached within 1.3 min at 60°C. All the above data indicate that composting at temperatures above 55°C for at least 3 days should be sufficient to eliminate or at least minimise the problem.

The samples of influent and effluent from WWTP-2 were negative at helminthological examinations. Of the 40 samples of sludge from this plant only two were positive. It is evident that the current sludge treatment cannot guarantee devitalisation of helminth stages and for this reason liming of sludge and its storage at pH higher than 12 for at least 3 months is recommended before its application to the soil.

ACKNOWLEDGEMENT

The study was supported by the Projects VEGA 1/3485/06, VEGA 2/7190/27 and VEGA 2007.

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Table 1. Plate counts of selected groups of bacteria in the influent and effluent and the solid fraction from WWTP-1

	Influent (CFU.ml^{-1})	**Effluent (CFU.ml^{-1})**	**Efficiency (%)**	**Solid fraction (CFU.kg^{-1})**
Mesophilic bacteria	$9.8x10^6$–$9.2x10^8$	$5.2x10^4$–$1.2x10^7$	95.7	$3.1x10^7$–$3.8x10^9$
Coliform bacteria	$1.0x10^5$–$8.9x10^8$	$2.3x10^3$–$4.1x10^6$	86.5	$3.5x10^5$–$1.6x10^8$
Faecal coliform bact	$1.0x10^5$–$8.3x10^7$	$4.3x10^3$–$6.3x10^5$	91.4	$9.4x10^4$–$2.4x10^6$

Table 2. Parasitological examination of samples from WWTP-1

	Influent (eggs.1000 ml^{-1})	**Effluent (eggs.1000 ml^{-1})**	**Solid fraction (eggs.100g^{-1})**
A. suum	28–29	0	12–35
***Oesophagostomum* spp.**	5–19	0	2–6
***Trichuris* spp.**	1–3	0	1–2
***Hymenolepis* spp.**	0–5	0	0
***Isospora* spp.**	0 – 9*	0*	0*
***Eimeria* spp.**	6 – 34*	0*	2 – 12*

* – oocysts

Table 3. Plate counts of selected groups of bacteria in the influent, effluent and sludge from WWTP-2

	Influent (CFU.ml^{-1})	**Effluent (CFU.ml^{-1})**	**Efficiency (%)**	**Sludge (CFU.ml^{-1})**
Mesophilic bacteria	$1.4x10^4$–$4.5x10^5$	$1.0x10^3$–$5.7x10^4$	97.9	$1.5x10^6$–$8.9x10^7$
Coliform bacteria	$6.5x10^4$–$3.3x10^6$	$1.0x10^2$–$4.4x10^3$	96.6	$2.2.10^6$–$1.3x10^8$
Faecal coliform bacteria	$4.1x10^4$–$7.4x10^5$	$1.1x10^3$–$2.8x10^5$	79.8	$8.6.10^5$–$9.4x10^7$