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CHARACTERISATION OF THE SANITARY STATUS OF DOMESTIC SEWAGE GENERATED IN HOUSEHOLDS IN RURAL AREAS[1]

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Summary. The paper presents a characterisation of the sanitary status of raw domestic sewage from 3 households in rural areas on the province of Lublin (Poland). The sewage studied contained very large amounts of coli group bacteria – from $1.2 \cdot 10^6$ to $110.5 \cdot 10^6$ of bacteria in a 100 cm^3 sample of sewage. The numbers of faecal type *coli* group bacteria were generally several-fold lower – from $0.43 \cdot 10^6$ to $25.65 \cdot 10^6$ bacteria in a 100 cm^3 sample of sewage. The raw sewage under analysis contained also fairly high levels of saprophytic fungi – from 213 to 802 cfu/cm^3 of sewage sample. The numbers of potentially pathogenic fungi were usually slightly higher than in the case of saprophytic fungi – from 402 to 1080 cfu/cm^3 of sewage sample. The results indicate that in rural areas domestic sewage contains very high numbers of microorganisms that, from the sanitary-epidemiological point of view, constitute a significant threat to the health-safety status of the natural environment. This entails the necessity of their elimination in sewage treatment plants before the sewage can be directed to a receiver.

Key words: domestic sewage, sanitary status, *coli* group bacteria, fungi, rural areas

INTRODUCTION

Domestic sewage constitutes one of the primary factors causing bacteriological contamination of surface and ground waters. It is also one of the causes of mycological contamination of ground waters. It contains very high quantities of bacteria, viruses, fungi and protozoa, referred to as the allochthonous flora or introduced flora. Most of those microorganisms form a part of the typical micro-

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biota living in the human and higher animal gastrointestinal tract, forming the so-called physiological microflora of the organism. It includes primarily rods of *Escherichia coli*, *Enterococcus faecalis* and sporulating bacilli *Clostridium perfringens* which are discharged with the faeces [Zaremba and Borowski 1997, Libudisz and Kowal 2000, Smyłła 2005].

In domestic sewage there ay also apear pathogenic and potentially pathogenic organisms that cause bacterial diseases, e.g. typhoid fever, paratyphoid fevers, bacterial dysentery, campylobacteriosis, tularemia, tuberculosis and cholera. Sewage is also the habitat of numerous pathogenic fungi: anthropo- , zoo- and geophilous dermatophytes causing dermatomycosis; pathogenic anascogenic yeasts and moulds causing mycoses of the organs and mycotoxin poisoning. Pathogenic bacteria most often encountered in sewage include rods of *Salmonella* and *Mycobacterium tuberculosis* [Kluczek 1999]. Moreover, in domestic sewage the presence of such pathogenic bacteria as those of the genus *Clostridium*, *Yersinia*, *Brucella*, and *Campylobacter* was found, including species *Clostridium botulinum, Yersinia enterocolitica, Campylobacter jejuni* and *Bacillus anthracis*, *Vibrio cholerae*, *Listeria monocytogenes,* as well as entheropatogenic strains of *Escherichia coli* [Venglovsky *et al*. 1997, Osek 1999, Grabińska-Łoniewska and Siński 2010]. The pathogenic or potentially pathogenic fungi frequently isolated from sewage include also anscogenic yeasts from the genus *Candida, Cryptococcus*, dermatophytes of the genus *Trichophyton* and *Microsporum*, toxicogenic moulds i.e. *Aspergillus flavus* [Cooke 1970, Ulfig 1986, Zamorska 2007].

The objective of the study presented here was characterisation of the sanitary status of raw domestic sewage generated in 3 households in rural areas in the province of Lublin (Poland). Apart from the commonly used sanitary status indicators such as *coli* group and faecal type *coli* group bacteria, also the numbers of saprophytic and potentially pathogenic fungi were determined in the sewage.

MATERIAL AND METHODS

The objects at which the study was conducted are situated in the localities of Jastków (object No. 1), Dąbrowica (object No. 2) and Janów (object No. 3). The sewage from the households of the objects under analysis is purified with the mechanical-biological method in preliminary settlers and in constructed wetland systems.

In object No. 1 the sewage is purified in a single-stage horizontal flow constructed wetland with willow *Salix viminalis* L., with maximum throughput of 2 $m^3 \cdot d^{-1}$. In objects No. 2 and 3 sewage is treated in hybrid horizontal and vertical flow constructed wetlands with willow *Salix viminalis* L. and common reed *Phragmites australis* Cav. Trin. Ex Steud., with maximum throughput values of 0.6 and 0.45 $m^3 \cdot d^{-1}$, respectively. The constructed wetland systems in question and their efficiency of removal of bacteria and fungi have been described in detail in a paper by Jóźwiakowski *et al*. [2009].

Samples of sewage for microbiological analyses within the scope of this study were taken from the first chamber of the preliminary settlers of the objects studied, in accordance with the relevant standards (PN-74/C-04620/00, PN-EN 25667-2: 1999), in February, May, August and November, 2009. Characterisation of the physical and chemical parameters of the raw sewage is given in Table 1.

Table 1. Mean concentrations of pollutions in raw sewage generated in households in rural areas in the province of Lublin in 2009

Parameter	Object No. 1	Object No. 2	Object No. 3
Temp. of sewage, ^{o}C	17.4 (±4.7)	16.1 (±4.4)	16.0 (±3.3)
pH	7.07 (±0.11)	7.66 (±0.33)	7.45 (±0.25)
O_2, mg·dm^{-3}	0.30 (±0.09)	0.44 (±0.13)	0.42 (±0.11)
TSS, mg·dm^{-3}	191 (±20.8)	293 (±70.3)	319 (±320)
BOD_5, mg O_2·dm^{-3}	291 (±97.3)	273 (±102)	238 (±86)
COD, mg O_2·dm^{-3}	523 (±138)	578 (±210)	455 (±138)
N-NH_4, mg·dm^{-3}	60.4 (±21.2)	116.4 (±20.7)	55.6 (±24.2)
N-NO_3, mg·dm^{-3}	1.53 (±0.56)	1.63 (±0.82)	1.40 (±1.08)
N-NO_2, mg·dm^{-3}	0.184 (±0.057)	0.239 (±0.088)	0.150 (±0.023)
$N_{tot.}$, mg·dm^{-3}	76.8 (±21.0)	155 (±19.2)	75 (±26.0)
P_{tot}, mg·dm^{-3}	32.0 (±9.9)	44.1 (±6.9)	24.9 (±6.2)
K, mg·dm^{-3}	193 (±48.8)	247 (±52.6)	135 (±56.4)

The presence of *coli* group bacteria in the samples was determined with the fermentation method, and the presence of faecal type *coli* group bacteria following the current standards PN-75-C-04615/05 and PN-77-C-04615/07.

Coli group bacteria are Gram-negative rods that do not produce spores, grow in relatively anaerobic conditions and ferment lactose, producing acid and gas, during 24–48 hours at temperature of 35–37ºC. They belong to the genus *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* within the family *Enterobacteriaceae*. Faecal type *coli* group acteria occurring in sewage are mainly *Escherichia coli* that are present in large amounts in human faeces, having the capacity of fermenting lactose, with the generation of acid and gas, at temperature of 44ºC during 24–48 hours. Determination of *coli* group bacteria with the fermentation method was performed through the inoculation of decimal dilutions of samples (dilution in Ringer fluid – PN-ISO 9308-1) in the binary system onto the Ejkman liquid medium (lactose, bromocresol purple) in test tubes with Dürham tubes, followed by incubation at 37 and 44ºC. The results were read after 24 and 48 days of culturing. The results were accepted as positive when total change of colouring of the substrate (from violet to yellow) and the evolution of gas were observed. Doubtful results (small amount of gas with no or weak acidification) were subjected to a verification procedure, i.e. inoculation of the Endo medium, and to supplementary analysis through inoculation for repeated fermentation, preparation of dyed specimen with the Gram method, and the test for cytochrome oxidase. The final results were read from Tables pro-

vided in the standards, and given in the form of the most probable number (MPN) of *coli* group bacteria in 100 cm^3 of sample, and the *coli* titre, i.e. the smallest volume of analysed sample in which the presence of *coli* group bacteria can still be detected.

The numbers of fungi were determined with the dilution plate method using the Martin medium (saprotrophic fungi) [Martin 1950] and the Sabouraud medium (fungi potentially pathogenic for humans and animals) [Dvořak and Otčenašek 1969]. Saprotrophic fungi were cultured at 25ºC, while potentially pathogenic fungi at 30ºC. The colonies grown were counted, and the results were given in cfu/cm^3 of sample. Three parallel replications were always made.

RESULTS AND DISCUSSION

Coli group and faecal type *coli* group bacteria

The sewage studied contained very large amounts of *coli* group bacteria – the mean value of the MPN varied from $1.235 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage from object No. 3 to $110.5 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage from object No. 1 (Tab. 2). The numbers of faecal type *coli* group bacteria were usually several-fold smaller – mean value of MPN varied from $0.427 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage from object No. 3 to $25.65 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage from object No. 2 (Tab. 3). Dominant in that group was *Escherichia coli*, a species that, next to strains which are harmless commen

Table 2. Numbers of *coli* group bacteria in MPN /100 cm^3 (incubation at 37ºC) in domestic sewage produced in households in rural areas

Object number	Time of analyses				
	II	V	VIII	XI	Mean value
1	$13 \cdot 10^6$	$2.4 \cdot 10^6$	$24 \cdot 10^6$	$62 \cdot 10^6$	$25.35 \cdot 10^6$
2	$70 \cdot 10^6$	$62 \cdot 10^6$	$240 \cdot 10^6$	$70 \cdot 10^6$	$110.5 \cdot 10^6$
3	$1.3 \cdot 10^6$	$0.62 \cdot 10^6$	$2.4 \cdot 10^6$	$0.62 \cdot 10^6$	$1.235 \cdot 10^6$

Table 3. Numbers of faecal type *coli* group bacteria in MPN /100 cm^3 (incubation at 44ºC) in domestic sewage produced in households in rural areas

Object number	Time of analyses				
	II	V	VIII	XI	Mean value
1	$2.4 \cdot 10^6$	$0.62 \cdot 10^6$	$2.3 \cdot 10^6$	$24 \cdot 10^6$	$7.33 \cdot 10^6$
2	$24 \cdot 10^6$	$6.2 \cdot 10^6$	$2.4 \cdot 10^6$	$70 \cdot 10^6$	$25.65 \cdot 10^6$
3	$0.24 \cdot 10^6$	$0.23 \cdot 10^6$	$0.62 \cdot 10^6$	$0.62 \cdot 10^6$	$0.427 \cdot 10^6$

sals, may include pathogenic strains causing food poisoning as well as certain serious extraintestinal disorders such as inflammation of the urinary tract and meningitis. Although the primary source of infections with pathogenic strains of

E. coli is food, especially of animal origin, the bacteria have also been isolated from sewage sludge [Sahlström *et al.* 2004], which indicates the possibility of their occurrence in sewage.

For comparison, in raw sewage in the sewage treatment plant in Częstochowa 10^6 faecal type *coli* group bacteria were found in 100 cm^3 sample of sewage [Smyłła *et al*. 2003], while in sewage from the treatment plant in Gdynia as much as $1.8 \cdot 10^{20}$ faecal type *coli* group bacteria was determined in 100 cm^3 sewage sample, and in Gdańsk – $9.3 \cdot 10^{18}$/100 cm^3 [Szumilas *et al*. 2001]. Whereas, the numbers of *Escherichia coli* bacteria in sewage flowing into household sewage treatment facilities with filtration drainage in the communes of Lubraniec and Nakło were from $2.51 \cdot 10^9$ to $7.39 \cdot 10^9$ in 100 cm^3 samples of sewage [Budzińska *et al*. 2007]. Comparatively, those were values from 100 to 1000-fold greater than in the raw domestic sewage from the objects studied.

In turn, in Florence (Italy), sewage supplied to a hybrid constructed wetland (HF-VF) contained from $4 \cdot 10^5$ to $3 \cdot 10^7$ of *coli* group bacteria and faecal type *coli* group bacteria [Masi *et al*. 2004]. Similar numbers of faecal type *coli* group bacteria ($1.3 \cdot 10^5$ to $3 \cdot 10^6$) were found in municipal sewage treated in a constructed wetland in Arizona (USA) [Karpiscak *et al*. 1996].

In this study, higher values of MPN of *coli* group and faecal type *coli* group bacteria were noted in object No. 2 compared to objects Nos. 1 and 3. Object No. 2 received sewage with the highest load of contaminants of faecal origin. In the analysis of the physicochemical composition of the raw sewage from that object noteworthy is the high concentration of biogens and COD, with mean concentration of total nitrogen amounting to 155 $mg \cdot dm^{-3}$, total phosphorus to 44.1 $mg \cdot dm^{-3}$, and the level of COD being 578 mg $O_2 \cdot dm^{-3}$ (Tab. 1). These are values far in excess of levels defined as characteristic of domestic-household sewage [Heidrich *et al*. 2008]. Grabińska-Łoniewska and Siński [2010] report that in waters rich in nutrients and with elevated temperature (above 20ºC) there is a possibility of intensive multiplication of *coli* group bacteria, including the pathogenic strains of *Escherichia coli*. In the summer season the temperature of the studied sewage reached 22.8ºC (object No. 1), 21.6ºC (object No. 2) and 20.6ºC (object No. 3).

Saprotrophic and potentially pathogenic fungi

The raw sewage analysed contained also fairly large amounts of saprotrophic fungi – their average numbers varied from 213 cfu/cm^3 of sewage sample from object No. 3 to 802 cfu/cm^3 of sewage sample from object No. 2 (Tab. 4). The numbers of potentially pathogenic fungi were usually slightly higher, with average numbers in the raw sewage varying from 402 cfu/cm^3 for objects No. 1 and 3 to 1080 cfu/cm^3 in the sewage sample from oject No. 2 (Tab. 5).

It can be assumed that the isolated mycobiota comprised so-called geophilic fungi, i.e. relatively aquatic, also known as non-aquatic [Park 1972]. They are fungi of land origin that may grow in an aquatic environment under conditions

Table 4. Numbers of saprotrophic fungi (cfu · cm^{-3}) in domestic sewage generated in households in rural areas

Object number	Time of analyses				
	II	V	VIII	XI	Mean value
1	126.7 (±32.14)	546.7 (±61.10)	140.0 (±26.45)	353.3 (±50.33)	292
2	153.3 (±25.16)	690.0 (±10.00)	1063.3 (±97.12)	1300.0 (±26.45)	802
3	70.0 (±16.45)	210.0 (±10.00)	376.7 (±25.16)	196.7 (±15.27)	213

Table 5. Numbers f potentially pathogenic fungi (cfu · cm^{-3}) in domestic sewage generated in households in rural areas

Object number	Time of analyses				
	II	V	VIII	XI	Mean value
1	126.7 (±32.14)	510.0 (±60.82)	400.0 (±20.00)	570.0 (±26.45)	402
2	286.7 (±20.82)	800.0 (±73.20)	1633.3 (±15.27)	1600.0 (±10.00)	1080
3	50.0 (± 0)	263.3 (±51.31)	1092.0 (±35.35)	203.3 (±30.55)	402

of its contamination with organic matter and biogens, as it happens in sewage [Cooke 1970]. This suggestion is supported by the results of our earlier research [Korniłłowicz 1995] that indicate the absence, among fungi isolated with the dilution plate method, of typically aquatic species, i.e. zoospore fungi of the aquatic *Hyphomycetes* [Batko 1975].

The numbers of saprotrophic fungi determined in the objects studied are similar to those of saprotrophic fungi isolated from raw domestic sewage in Ohio (USA) by Cooke [1970]. That author, using a similar method for the isolation of the fungi (dilution plate method, Martin medium), isolated from 120 to 900 cfu of mould fungi from 1 cm^3 of the sewage. Apart from typically saprotrophic fungal species, Cooke [1970] demonstrated the presence of numerous species potentially pathogenic and pathogenic to humans.

The presence of fungi in sewage constitutes a serious sanitary problem. Among them there may be those causing superficial mycoses, representing dermatophytes, and those causing mycoses of the organs (anascogenic yeats i.e. *Candida albicans)* and toxin-producing fungi that produce carcinogenic, mutagenic or teratogenic mycotoxins. Highly dangerous are so-called opportunistic pathogens, i.e. saprotrophic fungi that attack organisms with hypoimmunity [Ulfig 1986, Grabińska-Łoniewska and Siński 2010]. Due to the threat they pose to human and animal health, those fungi should be eliminated from wastewaters before their introduction in water reservoirs. Another argument for the necessity of maximum reduction of propagation units of geophilic fungi in wastewaters is their high resistance to disinfection with chlorine, greater than that of bacteria. This allows those microorganisms to survive the process of groundwater treatment prior to its introduction in the water supply system [Grabińska-Łoniewska and Siński 2010].

Our study indicates that the raw domestic sewage analysed varied in the degree of mycological contamination. The highest numbers of colony forming units, as in the case of *coli* group bacteria, were recorded at the successive times of analyses (and on average) in object No. 2 (Dąbrowica). That effect should be attributed to higher concentrations of nutrients – total nitrogen, $N\text{-}NH_4$ and total phosphorus – in the sewage in that object (Tab. 1).

Our earlier studies indicate that environments characterised by accumulation of nitrogen and phosphorus compounds are also notable for their greater numbers of fungi compared to environments with lower levels of nitrogen and phosphorus. That effect was observed in bird nests fouled with bird faeces (high concentrations of nitrogen and phosphorus) compared to nests of bird species that do not defecate in their nests [Korniłłowicz-Kowalska *et al*. 2010], and in soils richer in those components compared to soils with low levels of fertility [Korniłłowicz 1983, Korniłłowicz-Kowalska *et al*. 2003].

CONCLUSIONS

1. The domestic sewage under study contained very large numbers of *coli* group bacteria – mean value of MPN varied from $1.2 \cdot 10^6$ to $110.5 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage.

2. The numbers of faecal type *coli* group bacteria were usually several-fold lower – mean value of MPN varied from $0.43 \cdot 10^6$ to $25.65 \cdot 10^6$ bacteria in 100 cm^3 sample of sewage.

3. The raw sewage analysed contained also fairly high numbers of saprotrophic fungi – their average numbers varied from 213 to 802 cfu/cm^3 of sewage sample under analysis.

4. The numbers of potentially pathogenic fungi were usually slightly higher than those of saprotrophic fungi – their average numbers varied from 402 to 1080 cfu/cm^3 of analysed sewage sample.

5. The results indicate that the domestic sewage contains very high numbers of microorganisms, therefore their purification is necessary before they can be directed to a receiver.

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CHARAKTERYSTYKA STANU SANITARNEGO ŚCIEKÓW BYTOWYCH POWSTAJĄCYCH W GOSPODARSTWACH DOMOWYCH NA TERENACH WIEJSKICH

Streszczenie. W pracy przedstawiono charakterystykę stanu sanitarnego surowych ścieków bytowych powstających w 3 gospodarstwach domowych na terenach wiejskich w woj. lubelskim (Polska). Badane ścieki bytowe zawierały bardzo duże ilości bakterii z grupy *coli* – od $1{,}2 \cdot 10^6$ do $110{,}5 \cdot 10^6$ bakterii w 100 cm^3 w próbie ścieków. Liczebności bakterii grupy *coli* typu kałowego były na ogół kilkakrotnie mniejsze – od $0{,}43 \cdot 10^6$ do $25{,}65 \cdot 10^6$ bakterii w 100 cm^3 w próbie ścieków. Analizowane ścieki surowe zawierały również dość dużo grzybów saprotroficznych – od 213 do 802 jtk /cm^3 badanej próby ścieków. Liczebności grzybów potencjalnie chorobotwórczych były na ogół nieznacznie większe niż w przypadku grzybów saprotroficznych, od 402 do 1080 jtk/cm^3 badanej próby ścieków. Uzyskane wyniki wskazują, że ścieki bytowe na terenach wiejskich zawierają bardzo duże ilości drobnoustrojów, które z sanitarno-epidemiologicznego punktu widzenia stanowią znaczne zagrożenie dla stanu czystości środowiska przyrodniczego. Wiąże się z tym konieczność ich usuwania w oczyszczalniach ścieków przed odprowadzeniem do odbiornika.

Słowa kluczowe: ścieki bytowe, stan sanitarny, bakterie grupy coli, grzyby, tereny rolnicze