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CHAPTER I

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ESTIMATION OF SANITARY STATUS OF SEWAGE TREATED IN CONSTRUCTED WETLAND SYSTEMS*

Introduction

Domestic sewage is one of the factors causing bacteriological contamination of surface and ground waters. Untreated sewage brings to the waters enormous amounts of microorganisms – bacteria, viruses, fungi, and protozoa, called the allochtonic i.e. introduced flora. Most of the allochtonic flora is typical microflora living in the gastrointestinal tract of humans and higher animals, constituting so-called physiological flora of the organism. It is mainly composed of rods of *Escherichia coli*, enterococci *Enterococcus faecalis* and sporifying clostridia *Clostridium perfringens*, that are excreted together with the faeces (ZAREMBA, BOROWSKI 1997, LIBUDISZ, KOWAL 2000, SMYŁŁA 2005). Untreated domestic sewage may also contain pathogens and potential pathogens, e.g. those causing typhoid fever, paratyphoid fever, bacterial dysentery, campylobacteriosis, tularemia, tuberculosis and cholera (KLUCZEK 1999). According to KLUCZEK (1999), the most frequent pathogenic bacteria occurring in sewage include rods of *Salmonella* and *Mycobacterium tuberculosis*. Other pathogenic bacteria isolated from sewage include *Clostridium*, *Yersinia*, *Brucella*, *Campylobacter*, as well as *Bacillus anthracis*, *Vibrio cholerae*, *Listeria monocytogenes* and enteropathogenic strains of *Escherichia coli* (VENGLOVSKY et al. 1997, OSEK 1999).

Intestinal bacteria are excreted with the faeces in enormous amounts, e.g. 1 gram of human faeces contains on average ca. 1.3×10^7 cells of *E.coli* and 3.0×10^6 cells of *E. faecalis* (SMYŁŁA et al. 2003). Such huge amounts of bacteria in the faeces contribute to the bacterial contamination of waste waters. The species and quantitative composition of microorganisms occurring in sewage are closely related with the health status of inhabitants who produce the sewage (SIMMONS 1997, OLAŃCZUK-NEYMAN 2003). The survival time of pathogenic bacteria outside of the organism of a sick person is usually long enough for the bacteria to constitute a hazard of proliferation of contagious diseases through the water (SIMMONS 1997).

The numbers of bacteria in waste waters are subject to notable variation, but generally depend on the population inhabiting a catchment (GEORGE et al. 2002, OLAŃCZUK-NEYMAN 2003). Therefore, the numbers of bacteria in waste waters are

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greatly varied. Usually the numbers of bacteria from the *coli* group, of faecal origin (thermo-tolerant), in raw sewage vary from 10^6 to 10^8 in 100 cm^3 (GEORGE 2002).

The monitoring and assessment of the level of contamination of waters and waste waters more and more often involve the application of comprehensive analysis of sensory, physicochemical and microbiological indices. Those last ones permit reasonable approximation of the sanitary status of the environment, the time of occurrence and duration of microbiological contamination, the type and source of contamination, and the potential health hazard caused by the presence of pathogens (ŁOMOTOWSKI, SZPINDOR 1999). Current obligatory microbiological tests performed within the scope of assessment of sanitary status of sewage are based primarily on isolating faecal contamination indicating bacteria that constitute permanent natural intestinal microflora of humans and higher animals (KOSAREWICZ et al. 1999).

In microbiological studies on sewage performed to date much less attention has been paid to microscopic fungi. They have mainly been focused on the qualitative composition of those microbial groups, and on the occurrence of pathogenic species in particular. In Poland, studies on the occurrence of pathogenic and potentially pathogenic fungi in sewage and sewage sludge have been conducted by ULFIG (1981, 1986) and by GRABIŃSKA-ŁONIEWSKA et al. (1993). GRABIŃSKA-ŁONIEWSKA et al. (1993) studied the occurrence of yeast and yeast-like organisms in municipal sewage. Those authors demonstrated that typical sludge species included *Geotrichum candidum* and *Trichosporon cutaneum* – fungi potentially pathogenic for humans. Frequently observed in sewage and sewage sludge pathogenic and potentially pathogenic fungi include also dermatophytes causing dermatomycosis, also so-called geophilous dermatophytes (ULFIG 1986). Those fungi participate in processes of sewage treatment, e.g. in the removal of keratin matter, but also are indicator microorganisms in the assessment of degree of contamination with pathogenic microorganisms (ULFIG 1983, KORNIŁŁOWICZ 1993a). Information on other microscopic fungi in sewage and sewage sludge are highly fragmentary (BECKER et al. 1954, COOKE 1970, WOLLETT, HENDRICK 1970, ULFIG et al. 1996). In particular, there is a lack of data on the population sizes of those microbial groups in sewage, and on changes in their numbers in relation to the degree of purification of liquid wastes.

In recent years there has been an increase in the level of ecological awareness of inhabitants of towns and villages in Poland, and therefore, for purposes of protection of the water environment, more and more sewage treatment installations are being constructed, collective ones as well as small household systems. Small household systems are installed mainly is areas that have no connection larger sewage disposal systems. A solution that has been gaining increasing popularity is the constructed wetland system.

In the world constructed wetlands have been in use for more than 30 years, for the treatment of household, industrial, rainfall, and agricultural sewage (SEIDEL 1967, KICKUTH 1977). In Poland, the oldest constructed wetlands have been in operation for over a dozen years (KOWALIK, OBARSKA-PEMPKOWIAK 1998]. In most cases those are single-stage installations, with horizontal (HF-CW “horizontal flow constructed wetland”) or vertical (VF-CW “vertical flow constructed wetland”) flow of waste waters treated, in which reed or willow are employed (HABERL et al.

1995, KOWALIK OBARSKA-PEMPKOWIAK 1998). Recently, however, multi-stage constructed wetlands are built, so-called hybrid systems, composed of two or three HF-CW and VF-CW beds that ensure better conditions for biological purification of waste waters (KOWALIK, OBARSKA-PEMPKOWIAK 1998, LUEDERITZ et al. 2001, OBARSKA-PEMPKOWIAK, GAJEWSKA 2003, ARIAS et al. 2004, GAJEWSKA et al. 2004, TUSZYŃSKA et al. 2004, OBARSKA-PEMPKOWIAK 2005; OBARSKA-PEMPKOWIAK, GAJEWSKA 2005; VYMAZAL 2005).

Constructed wetlands are the object of research in Poland as well as in the world, yet so far there is a shortage of results concerning the microbiological and sanitary status of sewage treated in constructed wetland systems. The objective of the study presented herein is estimation of the sanitary condition of sewage treated in 4 constructed wetlands.

Objects and methods of the study

The study on the sanitary status of treated sewage was conducted at 4 constructed wetlands located within the Lublin Province. A characterisation of the objects under analysis is given below.

Object No. 1. A soil-plant (single stage) vertical flow constructed wetland with common reed *Phragmites australis* Cav. Trin. Ex Steud., with maximum throughput of 60 $m^3 \cdot d^{-1}$, located in Sobieszyn. At present the mean diurnal amount of sewage supplied to the wetland is 24.7 $m^3 \cdot d^{-1}$, and the hydraulic loading rate of sewage on the surface of the bed is on average 0.020 $m^3 \cdot m^{-2} \cdot d^{-1}$. The object has been in operation since 1995 and is located at the Agriculture Schools Complex in Sobieszyn near Kock.

The constructed wetland is made up of a two-chamber preliminary settler (with active volume of 75 m^3), a sewage pumping unit, a distribution well, four parallel beds with reed, with a combined surface area of 1227 m^2, and a collector well. The beds are constructed of several layers of soil, one layer of fabric, and drains. The surface layer, with a depth of 0.2 m, is a humus cover.

Underneath is a layer of loose sand of the same depth, directly overlying a filtering fabric 1.2 mm thick. Beneath the filter fabric there is a 0.3 m layer of dolomite gravel with grain sizes of 16–32 mm. Underneath that there is a layer of drains collecting the effluent, each with a diameter of 100 mm.

The next down and final layer, with a thickness of 0.1 m, is sand, directly overlying a PEHD geomembrane, 1 mm thick, the function of which is to provide total isolation of the bed from the natural soil. The effluent flowing out of the system is a forest-edge ditch that directs the treated sewage to the soil (ŁOSZAK, PODLASZEWSKI 2000).

Object No. 2. A soil-plant (single stage) horizontal flow constructed wetland with willow *Salix viminalis* L., with maximum throughput of 2 $m^3 \cdot d^{-1}$, located in Jastków. At present the mean amount of sewage supplied to the wetland per day is 1.2 $m^3 \cdot d^{-1}$, and the hydraulic loading rate of sewage on the surface of the bed is an average of 0.006 $m^3 \cdot m^{-2} \cdot d^{-1}$. The installation has been in operation since 1994 and its sole function is purification of domestic sewage from an 11-person household. The constructed wetland has a two-chamber preliminary settler (with active capacity of 13.7 m^3) and a soil-plant bed with average depth of 1.1 m and a surface area of

186 m^2. The bed is filled with lose medium-grained sand. The surface layer is a humus cover planted with willow. The bed is isolated from the natural soil with PEHD foil 1 mm thick. The receptacle for treated sewage flowing out of the bed is a pond with surface area of 1190 m^2 (DRUPKA et al.1992).

Object No. 3. A multi-stage soil-plant constructed wetland with both vertical and horizontal flow, with willow *Salix viminalis* L. and common reed *Phragmites australis* Cav. Trin. Ex Steud., with throughput of 0.6 $m^3 \cdot d^{-1}$, located in Dabrowica. The installation purifies domestic sewage from a 6-person household, and has been in operation since September2006. The first element of the system is a three-chamber settler with active capacity of 4.6 m^3. The second element is an arrangement of two parallel systems of soil-plant beds: system I – first bed with horizontal flow and willow (A) and second bed with vertical flow and common reed (B), system II – first bed with vertical flow and common reed (C) and second bed with horizontal flow and willow (D). All beds (A, B, C, D) have the same surface area of 24 m^2. Average hydraulic loading rate of each bed system is 0.006 $m^3 \cdot m^{-2} \cdot d^{-1}$. The beds with willow (A, D) have a depth of 1.0 m, while the beds with reed (B, C) – 0.8 m. Inclination of the bed bottoms is 3% in the direction of sewage outflow. The beds are filled with crushed stone and medium-grained sand. The beds are isolated from the natural soil by means of PEHD foil 1 mm thick. The receptacle for treated sewage is a mid-field ditch (JÓŹWIAKOWSKI et al. 2006).

Object No. 4. A multi-stage soil-plant constructed wetland with vertical and horizontal flow, with common reed *Phragmites australis* Cav. Trin. Ex Steud. and willow *Salix viminalis* L., with maximum throughput of 0.45 $m^3 \cdot d^{-1}$, located in Janów near Garbów. The constructed wetland purifies domestic sewage from a 3-person household. The object was built at the turn of 2007 and 2008. The first element is a two-chamber settler with active volume of ca. 8.4 m^3. The second element is a system of two beds: 1 – with vertical flow, with common reed *Phragmites australis* Cav. Trin. Ex Steud. (surface area of 18 m^2 and depth of 0.8 m), 2 – with horizontal flow, with willow *Salix viminalis* L (surface area of 30 m^2 and depth of 1.2 m). The beds are filled with crushed stone and loose medium-grained sand. They are isolated from the natural soil by means of PEHD hydro-insulating geomembrane with thickness of 1 mm. Treated sewage is deposited to the ground by means of filtering drainage planted with *Miscanthus giganteus* [JÓŹWIAKOWSKI, GORAL 2007].

Samples of sewage for microbiological analyses were taken from the above objects from the particular stages of purification, as follows: from the preliminary settler – raw sewage, from the tank after the settler – sewage after mechanical purification, from the tank after biological treatment – biologically treated sewage. The samples for analyses were taken in conformance with the relevant standards (PN-74/C-04620/00, PN-EN 25667-2: 1999), in February, May, August and November of 2008. In the samples the numbers of *coli* group bacteria were determined with the fermentation method, and the numbers of faecal bacteria from the *coli* group in compliance with the current standards PN-75-C-04615/05 and PN-77-C-04615/07.

Bacteria from the *coli* group are Gram-negative rods that do not form spores, grow under relatively anaerobic conditions, and ferment lactose, producing acid and gas, within 24-48 hours at temperature of 35-37ºC. They are classified in the genera

Escherichia, *Citrobacter* and *Enterobacter* within the family *Bacteriaceae*. Faecal-type coli bacteria occurring in sewage include *Escherichia coli* that settle in human faeces and have the capability of fermenting lactose, producing acid and gas, within 24-48 hours at temperature of 44^0C. Determination of bacteria from the *coli* group with the fermentation method was done by inoculation of decimal dilutions of samples (dilution in Ringer fluid – PN-ISO 9308-1) in a binary system into Ejkman liquid medium (lactose, bromocresol purple) in test tubes with Dürham tubes, followed by incubation at 37ºC and 44ºC. The results were read after 24 and 48 days of culturing. Results were accepted as positive when the medium changed colour completely (from purple to yellow) and gas was produced. Doubtful results (small amount of gas at no or weak acidification) were verified by inoculation into Endo medium, and subjected to complementary testing by inoculation for repeated fermentation, making coloured preparation with the Gram method, and performing the cytochrome oxidase test. Final results were read from Tables included in the standards and given in the form of the most probable number (MPN) of bacteria from the *coli* group in 100 cm^3 of sample, and as the *coli* form count, i.e. the smallest volume of tested sample in which *coli* group bacteria can still be observed.

The numbers of fungi were determined with the plate dilution method, using the Martin medium (saprotrophic fungi) and the Sabouraud medium (fungi potentially pathogenic for humans and animals). Saprotrophic fungi were cultured at 25ºC, while potentially pathogenic fungi - at 30ºC. Colonies grown were counted and the results were given in $cfu \cdot 1cm^{-3}$ of sample. In all cases 3 parallel replications were made.

The efficiency of elimination of bacteria and fungi was estimated on the basis of their mean population values in the input and output sewage from the particular elements of the constructed wetlands analysed in 2008. The obtained results of populations of *coli* group bacteria were compared with the values given in the REGULATION OF THE MINISTER FOR THE ENVIRONMENT [2004] which introduces five classes of water purity in Poland. In the microbiological aspect, that division is based on the numbers of coli group bacteria in 100 cm^3 of water and faecal type *coli* group bacteria in 100 cm^3 of water (Tab.1).

Table 1

Limit values of microbiological indices of purity of surface waters according to the REGULATION OF THE MINISTER FOR THE ENVIRONMENT (2004)

Microbiological indices	Limit values in water purity classes I-V				
	I	II	III	IV	V
Number of faecal type *coli* group bacteria in 100 ml	20	200	2 000	20 000	>20 000
Number of *coli* group bacteria in 100 ml	50	500	5 000	50 000	>50 000

Effects of removal of *coli* group and faecal type *coli* group bacteria

The results of determinations concerning the populations of *coli* group and faecal type *coli* group bacteria in the sewage from the constructed wetlands under analysis, at the particular stages of purification, are presented in Tables 2 and 3.

Raw sewage contained very large numbers of bacteria of the type of *Escherichia coli* – the mean MPN value varied from $8.3 \cdot 10^6$ bacteria in 100 cm^3 of the sample from the constructed wetland in Sobieszyn to $4.2 \cdot 10^7$ bacteria in 100 cm^3 of the sewage sample from the constructed wetland in Jastków. The values for faecal type *E. coli* were generally several-fold lower – mean MPN value varied from $2.1 \cdot 10^6$ bacteria in 100 cm^3 in the sample of sewage from the constructed wetland in Sobieszyn to $8.2 \cdot 10^6$ bacteria in 100 $cm^3$of sewage sample from the system in Dąbrowica.

Table 2

Numbers of *coli* group bacteria (MPN) in 100 ml of sewage
from the constructed wetlands in 2008

Object No. 1 – Sobieszyn				
Kind of sewage	II	V	VIII	XI
Raw sewage	$700 \cdot 10^3$	$24000 \cdot 10^3$	$2400 \cdot 10^3$	$6200 \cdot 10^3$
Treated sewage	$24 \cdot 10^3$	$24 \cdot 10^3$	$62 \cdot 10^3$	$2.4 \cdot 10^3$
Object No. 2 – Jastków				
Kind of sewage	II	V	VIII	XI
Raw sewage	$70000 \cdot 10^3$	$70000 \cdot 10^3$	$2400 \cdot 10^3$	$24000 \cdot 10^3$
Sewage after settler	$7000 \cdot 10^3$	$1300 \cdot 10^3$	$2400 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after willow bed	$7 \cdot 10^3$	$70 \cdot 10^3$	$240 \cdot 10^3$	$6.2 \cdot 10^3$
Object No. 3 – Dąbrowica				
System I				
Kind of sewage	II	V	VIII	XI
Raw sewage	$7000 \cdot 10^3$	$24000 \cdot 10^3$	$24000 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after settler	$7000 \cdot 10^3$	$700 \cdot 10^3$	$24000 \cdot 10^3$	$24000 \cdot 10^3$
Sewage after bed A	$7000 \cdot 10^3$	$130 \cdot 10^3$	$6200 \cdot 10^3$	$2400 \cdot 10^3$
Sewage after bed B	$0.7 \cdot 10^3$	$70 \cdot 10^3$	$23 \cdot 10^3$	$1.3 \cdot 10^3$
System II				
Raw sewage	$7000 \cdot 10^3$	$2400 \cdot 10^3$	$24000 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after settler	$7000 \cdot 10^3$	$700 \cdot 10^3$	$24000 \cdot 10^3$	$24000 \cdot 10^3$
Sewage after bed C	$24 \cdot 10^3$	$240 \cdot 10^3$	$23 \cdot 10^3$	$130 \cdot 10^3$
Sewage afer bed D	$2.40 \cdot 10^3$	$2.40 \cdot 10^3$	$2.30 \cdot 10^3$	$0.62 \cdot 10^3$
Object No. 4 – Janów				
Kind of sewage	II	V	VIII	XI
Raw sewage	n.sew.	$24 \cdot 10^3$	$24000 \cdot 10^3$	$2300 \cdot 10^3$
Sewage after reed bed	n.sew.	$2.4 \cdot 10^3$	$62 \cdot 10^3$	$62 \cdot 10^3$
Sewage after willow bed	n.sew.	$0.62 \cdot 10^3$	$0.24 \cdot 10^3$	$23 \cdot 10^3$

n.sew. – no sewage

For comparison, in raw sewage at the sewage treatment plant in Częstochowa 10^6 faecal *coli* bacteria were found in 100 cm^3 of sewage sample (SMYŁŁA et al. 2003), while in sewage at the treatment plant in Gdynia as much as $1.8 \cdot 10^{20} \cdot 100\ cm^{-3}$ faecal type *coli* bacteria were noted, and in Gdańsk – $9.3 \cdot 10^{18} \cdot 100\ cm^{-3}$ (SZUMILAS et al. 2001). Whereas, the numbers of bacteria of *Escherichia coli* type in sewage supplied to household sewage treatment installations with filtration drainage located in the communes of Lubraniec and Nakło varied from $2.51 \cdot 10^7$ to $7.39 \cdot 10^7$ $cfu \cdot cm^{-3}$ (BUDZIŃSKA et al. 2007), which – converted to values per 100 cm^3 - gives from $2.51 \cdot 10^9$ to $7.39 \cdot 10^9$ bacteria. Comparatively, those were populations from 100 to 1000-fold greater than those in the raw sewage supplied to the constructed wetlands in Jastków and Sobieszyn, respectively.

Table 3

Numbers of faecal type *coli* group bacteria (MPN) in 100 ml of sewage from the constructed wetlands in 2008

Object No. 1 – Sobieszyn				
Kind of sewage	II	V	VIII	XI
Raw sewage	$240 \cdot 10^3$	$6200 \cdot 10^3$	$1300 \cdot 10^3$	$620 \cdot 10^3$
Treated sewage	$7.0 \cdot 10^3$	$2.4 \cdot 10^3$	$23 \cdot 10^3$	$0.62 \cdot 10^3$
Object No. 2 – Jastków				
Kind of sewage	II	V	VIII	XI
Raw sewage	$24000 \cdot 10^3$	$2400 \cdot 10^3$	$620 \cdot 10^3$	$2400 \cdot 10^3$
Sewage after settler	$70000 \cdot 10^3$	$620 \cdot 10^3$	$1300 \cdot 10^3$	$2400 \cdot 10^3$
Sewage after willow bed	$0.21 \cdot 10^3$	$24 \cdot 10^3$	$62 \cdot 10^3$	$1.3 \cdot 10^3$
Object No. 3 – Dąbrowica				
System I				
Kind of sewage	II	V	VIII	XI
Raw sewage	$2400 \cdot 10^3$	$240 \cdot 10^3$	$24000 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after settler	$620 \cdot 10^3$	$130 \cdot 10^3$	$6200 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after bed A	$24 \cdot 10^3$	$130 \cdot 10^3$	$1620 \cdot 10^3$	$2400 \cdot 10^3$
Sewage after bed B	$0.24 \cdot 10^3$	$21 \cdot 10^3$	$23 \cdot 10^3$	$0.24 \cdot 10^3$
System II				
Raw sewage	$2400 \cdot 10^3$	$240 \cdot 10^3$	$24000 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after settler	$620 \cdot 10^3$	$130 \cdot 10^3$	$6200 \cdot 10^3$	$6200 \cdot 10^3$
Sewage after bed C	$6.2 \cdot 10^3$	$240 \cdot 10^3$	$6.2 \cdot 10^3$	$50 \cdot 10^3$
Sewage after bed D	$1.3 \cdot 10^3$	$0.24 \cdot 10^3$	$0.62 \cdot 10^3$	$0.13 \cdot 10^3$
Object No. 4 – Janów				
Kind of sewage	II	V	VIII	XI
Raw sewage	n.sew.	$62 \cdot 10^3$	$6200 \cdot 10^3$	$620 \cdot 10^3$
Sewage after reed bed	n.sew.	$0.24 \cdot 10^3$	$2.4 \cdot 10^3$	$6.2 \cdot 10^3$
Sewage after willow bed	n.sew.	$0.062 \cdot 10^3$	$0.24 \cdot 10^3$	$0.62 \cdot 10^3$

n.sew. – no sewage

During the mechanical purification of sewage in the settlers of the constructed wetlands under analysis a low efficiency of removal of bacteria of *E. coli*. and *E. coli* of faecal type was observed. It was only at the biological stage that clear effluents were obtained, with mean values of *coli* form count at MPN of $1.93 \cdot 10^3$ – $80.8 \cdot 10^3$ cells in 100 cm^3 of analysed sample. The mean values of faecal *coli* form count varied within the range of MPN $0.31 \cdot 10^3$ – $21.9 \cdot 10^3$ cells in 100 cm^3 of analysed sample. The results obtained are lower by 1-2 orders of magnitude than data given in the literature.

In a study at the sewage treatment plant in Gdańsk, OLAŃCZUK-NEYMAN (2003) found, at the outlet, populations of faecal type *coli* bacteria at the level of 10^4 – $10^5 \cdot 100$ cm^{-3}, and at Dębogóra, $2.4 \cdot 10^4$ – $2.5 \cdot 10^5$ in 100 cm^3. In a study at the sewage treatment plant in Częstochowa, populations of faecal *coli* bacteria at the outlet were of the order of 10^3 – $10^4 \cdot 100$ cm^{-3} (SMYŁŁA et al. 2003). Whereas, the numbers of *Escherichia coli* bacteria in sewage on the outlet of the household sewage treatment systems with filtration drainage in the communes of Lubraniec and Nakło was from $8.03 \cdot 10^1$ to $9.07 \cdot 10^1$ $cfu \cdot cm^{-3}$ (BUDZIŃSKA et al. 2007), which corresponds to $8.03 \cdot 10^3$ – $9.07 \cdot 10^3$ bacteria in 100 cm^3. These results are similar to those obtained for the constructed wetland systems under analysis.

In the opinion of KOSAREWICZ et al. (1999), after mechanical and biological purification of sewage the number of *coli bacillus* rods usually varies from 1000 to 100 000 in 1 dm^3. Application of additional purification processes permits further reduction of the content of those microorganisms.

Populations of *coli* group and faecal type *coli* group bacteria obtained in the multi-stage constructed wetland systems (objects No. 3 and 4) most often corresponded to water purity classes II, III or IV. Treated sewage with those purity classes can be used for agricultural needs, e.g. for the watering of gardens or lawns. In the single-stage constructed wetlands (objects No. 1 and 2) the mean numbers of bacteria from the *coli* group and faecal type *coli* qualify the treated sewage under analysis in water purity classes IV or V. The highest numbers of bacteria of the type of *E. coli* and faecal *E. coli* were noted at object No. 2 (constructed wetland in Jastków), in operation since 1994.

Based on the bacteriological analyses performed it was found that the small constructed wetlands under study are characterised by very good efficiency of removal of bacteria of faecal type *coli* group and those of the *coli* group (99.60 – 99.99%). The best efficiency of removal of *coli* group and faecal type *coli* group bacteria (above 99.91%) was obtained in the multi-stage systems in Janów - object No. 4, and in Dąbrowica – object No. 3 (system 2), and the worst in the single-stage reed system in Sobieszyn – object No. 1 (under 99.66%). SZUMILAS et al. (2001) report that modern sewage treatment systems are capable of eliminating more than 99.999% of *coil* group bacteria through biological purification. According to TALARKO (2003), the efficiency of elimination of *coli* form bacteria in soil-plant filters amounts to approximately 99%, while BERGIER et al. (2002) maintain that constructed wetlands with horizontal flow are characterised by faecal bacteria removal rates at the level of 98.8%.

Effects of removal of saprotrophic and potentially pathogenic fungi

Wastewaters and surface waters are the habitat of numerous fungi. In surface waters there occur typically aquatic fungi, primarily zoosporic, as well as yeasts (KORNIŁŁOWICZ 1991, KORNIŁŁOWICZ, SZEMBER 1991, DYNOWSKA 1995, CZECZUGA et al. 2002, KIZIEWICZ 2004a,b). Next to those, depending on the degree of pollution with allochtonic organic matter, in surface waters there occur, frequently in large numbers, so-called non-aquatic fungi, most often of soil or sewage origin (PARK 1972, KORNIŁŁOWICZ 1993a,b, 1994a,b, DYNOWSKA 1995).

Sewage fungi are characterised by notable diversity of taxonomic and physiological groups related with plant and animal organisms and with soil, including phytopathogenic species as well as those pathogenic for humans and animals (BECKER et al. 1954, COOKE 1970, WOLLETT, HENDRICK 1970, ULFIG and KORCZ 1983, GRABIŃSKA-ŁONIEWSKA 1993, ULFIG et al. 1996). Therefore, determinations of fungi at sewage treatment installations are significant not only from the general biological but also from the sanitary point of view (ULFIG 1986). TOMLINSON and WILLIAMS (1975), as well as KORNIŁŁOWICZ (1993) and ULFIG (1993), are of the opinion that certain fungi play an important role in processes of sewage purification, and are also used as bioindicators of pollution of surface waters.

The results of determinations concerning the populations of saprotrophic and potentially pathogenic fungi in the sewage from the constructed wetlands under analysis are presented in Tables 4 and 5.

Raw sewage from the constructed wetlands under analysis contained fairly large amounts of saprotrophic fungi – their average populations varied from 553 cfu·1cm^{-3} of analysed sample of sewage from the system in Sobieszyn (object No. 1) to 2292 cfu·1cm^{-3} of sewage sample from the constructed wetland in Dąbrowica (object No. 3).

In turn, the numbers of potentially pathogenic fungi were usually slightly higher – their mean populations in the raw sewage varied from 705 cfu·1cm^{-3} (object No. 1) to 2808 cfu·1cm^{-3} (object No. 3).

The analysed constructed wetland systems were fairly efficient in the reduction of populations of saprophytic and potentially pathogenic fungi, so their numbers in purified sewage were low. The lowest numbers of saprophytic fungi were noted in sewage on the outlet of the constructed wetland in Dąbrowica (object No. 3) and in Janów (object No. 4) – at 7.0 and 6.0 cfu·1cm^{-3} of analysed sample, respectively.

The fungal populations observed were similar to the number of those microbial groups determined by GRABIŃSKA-ŁONIEWSKA et al. (2007) in mains water after the process of purification. The authors quoted, in samples of river water after the process of purification, found more than 2 cfu after conversion per 1 cm^3 of water (1506 cfu·dm^{-3}). Our own study shows that also the numbers of potentially pathogenic fungi were the lowest in objects No. 3 and 4, at 12.0 and 8.0 cfu·1cm^{-3} of analysed sample, respectively. The highest numbers of saprotrophic fungi – 81 cfu·cm^{-3} of analysed sample – were recorded in treated sewage on the outlet of the constructed wetland in Sobieszyn (object No. 1), and largest populations of potentially pathogenic fungi – 881 cfu·1cm^{-3} of analysed sample – in sewage flowing out from the constructed wetland installation in Jastków (object No. 2).

The research results obtained indicate that the highest efficiency of removal of saprotrophic and potentially pathogenic fungi (above 99.28%) was recorded in the multi-stage constructed wetlands in Janów (object No. 4) and in Dąbrowica (object No. 3 - system I). The lower efficiency of removing fungal groups observed in objects No. 1 and 2 is due to the long period of operation of those systems.

Table 4

Numbers of saprotrophic fungi ($cfu \cdot cm^{-3}$) in sewage from the constructed wetlands in 2008

Object No. 1 - Sobieszyn				
Kind of sewage	II	V	VIII	XI
Raw sewage	183.3 (±57.7)	1466.7 (±75.1)	336.7 (±35.1)	226.7 (±41.6)
Treated sewage	4.7 (±2.0)	4.7 (±0.5)	253.3 (±50.3)	60.7 (±2.1)
Object No. 2 - Jastków				
Kind of sewage	II	V	VIII	XI
Raw sewage	180.0 (±26.5)	2266.7 (±305.5)	500.0 (±100.0)	250.0 (±50.0)
Sewage after settler	106.7 (±11.5)	1766.7 (±51.6)	290.0 (±78.1)	133.3 (±37.8)
Sewage after willow bed	6.0 (±2.6)	29.0 (±2.6)	5.0 (±1.7)	203.3 (±25.1)
Object No. 3 - Dąbrowica				
System I				
Kind of sewage	II	V	VIII	XI
Raw sewage	2533.3 (±351.2)	3200.0 (±500.0)	2666.7 (±321.4)	766.7 (±57.7)
Sewage after settler	1200.0 (±173.2)	933.3 (±115.4)	676.7 (±35.1)	126.7 (±20.8)
Sewage after bed A	146.7 (±20.0)	11.3 (±3.0)	50.3 (±2.8)	386.7 (±31.1)
Sewage after bed B	3.7 (±0.5)	15.0 (±2.6)	6.0 (±2.0)	4.0 (±1.0)
System II				
Raw sewage	2533.3 (±351.2)	3200.0 (±500.0)	2666.7 (±321.4)	766.7 (±57.7)
Sewage after settler	1200.0 (±173.2)	933.3 (±115.4)	676.7 (±35.1)	126.7 (±20.8)
Sewage after bed C	9.7 (±3.6)	63.0 (±10.5)	6.0 (±2.0)	4.0 (±1.0)
Sewage after bed D	8.7 (±5.7)	122.0 (±10.5)	8.3 (±1.0)	72.0 (±4.0)
Object No. 4 - Janów				
Kind of sewage	II	V	VIII	XI
Raw sewage	n.sew.	140.0 (±17.3)	1633.3 (±115.4)	720.0 (±30.0)
Sewage after reed bed	n.sew.	3.3 (±1.1)	11.7 (±2.1)	79.3 (±4.5)
Sewage after willow bed	n.sew.	1.3 (±0.5)	8.0 (±2.0)	9.3 (±1.1)

n.sew. – no sewage

Table 5

Numbers of potentially pathogenic fungi (cfu·cm^{-3}) in sewage from the constructed wetlands in 2008

Object No. 1 - Sobieszyn				
Kind of sewage	II	V	VIII	XI
Raw sewage	230.0 (±60.8)	1966.7 (±251.6)	406.7 (±66.5)	216.7 (±28.8)
Treated sewage	1.7 (±0.5)	6.7 (±1.5)	27.0 (±4.3)	116.7 (±15.2)
Object No. 2 - Jastków				
Kind of sewage	II	V	VIII	XI
Raw sewage	130.0 (±10.0)	3233.3 (±251.6)	800.0 (±173.2)	323.3 (±3.8)
Sewage after settler	83.3 (±15.2)	2666.7 (±155.7)	340.0 (±36.1)	280.0 (±6.4)
Sewage after willow bed	7.0 (±1.0)	3333.3 (±161.7)	11.7 (±5.6)	173.3 (±11.5)
Object No. 3 - Dąbrowica				
System I				
Kind of sewage	II	V	VIII	XI
Raw sewage	3833.3 (±208.1)	3600.0 (±316.5)	2933.3 (±450.9)	866.7 (±52.7)
Sewage after settler	2333.3 (±305.5)	933.3 (±321.4)	976.7 (±35.1)	160.0 (±20.0)
Sewage after bed A	206.7 (±66.5)	12.0 (±3.0)	190.0 (±17.3)	413.3 (±37.8)
Sewage after bed B	12.0 (±5.2)	21.0 (±6.2)	4.0 (±1.0)	9.0 (±1.0)
System II				
Raw sewage	3833.3 (±208.1)	3600.0 (±316.5)	2933.3 (±450.9)	866.7 (±52.7)
Sewage after settler	2333.3 (±305.5)	933.3 (±321.4)	976.7 (±35.1)	160.0 (±20.0)
Sewage after bed C	18.3 (±3.7)	123.0 (±11.3)	9.0 (±1.7)	77.0 (±3.6)
Sewage after bed D	13.0 (±2.6)	148.3 (±6.6)	15.0 (±0.6)	6.0 (±1.0)
Object No. 4 - Janów				
Kind of sewage	II	V	VIII	XI
Raw sewage	n.sew.	203.3 (±5.7)	2233.3 (±387.6)	1766.7 (±251.6)
Sewage after reed bed	n.sew.	4.0 (±1.7)	19.7 (±1.5)	70.0 (±4.0)
Sewage after willow bed	n.sew.	3.3 (±5.7)	9.0 (±2.6)	11.0 (±1.7)

n.sew. – no sewage

Summary

Even though at present most sewage ends up in sewage treatment plants, it does not solve the problem of bacterial contamination of surface waters. Classical sewage treatment installations that do not perform specific disinfection reduce the numbers of faecal bacteria by 1–3 orders of magnitude (GEORGE et al. 2002). As the level of contamination of raw sewage supplied to treatment plants is very high, faecal bacteria are also drained off, in enormous amounts, with treated sewage to the environment (GEORGE et al. 2002). Even highly efficient purification of sewage, with removal of biogenic substances, nitrogen and phosphorus, does not ensure simultaneous effective elimination of microorganisms (OLAŃCZUK-NEYMAN 2003), as the efficiency of reduction of bacterial populations in the course of sewage purification depends to a large extent on the numbers of bacteria in raw sewage. SZUMILAS et al. (2001) found 99.999% reduction of the numbers of faecal type coli group bacteria after sewage treatment, yet in spite of such an efficient operation of the sewage treatment plant in question, with the initial pollution at the level of 10^{18}- 10^{20}, the numbers of those bacteria in treated sewage directed to the environment were still huge.

The research results presented here indicate that multi-stage constructed wetland systems ensure highly efficient – above 99% - elimination of bacteria and fungi, while constructed wetland systems with a single soil bed (operated for more than a dozen years) eliminate bacterial and mycological contaminations to a lesser degree.

To protect the aquatic environment from degradation it is necessary to employ more and more efficient technologies of sewage treatment and to conduct microbiological monitoring of the solutions applied. In recent years, certain European countries have been introducing at least a partial disinfection of sewage flowing out of purification plants. In Germany disinfection is applied to sewage going to recreational areas, France disinfects sewage dumped in protected areas, such as bathing zones and areas of mollusc growing, while in Spain disinfection is applied with relation to sewage used for irrigation of arable fields, orchards, sports fields, and gardens (SMYŁŁA et al. 2003).

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