

# Microbial contamination of the sand from the wastewater treatment plants

M. SZOSTKOVÁ<sup>1</sup>, T. VÍTEŽ<sup>2</sup>

<sup>1</sup>*Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition,  
Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic*

<sup>2</sup>*Department of Agriculture, Food and Environmental Engineering, Faculty of Agronomy,  
Mendel University in Brno, Brno, Czech Republic*

## Abstract

SZOSTKOVÁ M., VÍTEŽ T., 2010. **Microbial contamination of the sand from the wastewater treatment plants.** Res. Agr. Eng., 56: 147–153.

Primary treatment of domestic wastewater represents an extensive range of physical and chemical activities which directly or indirectly affect functionality of the treatment plant as a whole. The aforementioned effect might be rather significant in many respects. However, an incorrectly designed or operated primary treatment might result in an unnecessary increase of operating costs and, principally, a negative impact on the biological level or sludge treatment and disposal. The subject matter of this contribution comprises contemplations related to functionality of this level, both with respect to its relation to functionality of wastewater treatment plant and the matter of created waste in case of which disposal has become more and more expensive and complicated. The measurement results show that sewage sand from different wastewater treatment plants contains different amount of organic material 1.19–82%. The content of the organic material relates to the content of microorganisms which oscillated in a range of  $1.53 \times 10^4$ – $7.34 \times 10^6$  CFU/g for coliform bacteria including *Escherichia coli*,  $5.57 \times 10^1$ – $4.36 \times 10^4$  CFU/g for enterococci, and  $3.13 \times 10^2$ – $2.19 \times 10^5$  CFU/g for faecal coliform bacteria.

**Keywords:** wastewater treatment; primary treatment; detritus tank; wastewater treatment sand; microbial contamination

A detritus tank proves to have a key role in the process of primary treatment of wastewater. The principal task of a detritus tank is to collect the maximum possible amount of mineral substances from wastewater in a manner ensuring that organic substances will remain present in the uplift and they are to flow to the subsequent treatment level. The structure of a detritus tank is to ensure that solely sand without any organic material will be settling down. However, it is highly complicated to achieve these conditions considering the high level of inflow irregularity, and therefore a high level of concentration of organic material is to be considered to be present in the excavated mate-

rial (SHUVAL, FATTAL 2003). It is essential to return these organic materials in the treatment process. Should a detritus tank fail to function, the mixture of organic material and sand creates a sediment layer that causes major problems in the following stages of wastewater treatment. Sand separated in the wastewater treatment process may, however, contain germs of pathogenic microorganisms that would – in high concentrations – represent a risk pertaining to its subsequent treatment (SCHROEDER, WUERTZ 2003; GERARDI, ZIMMERMAN 2004; BITTON 2005).

**Legislative Requirements:** As regards the legislation, the matter of treatment of sand from waste-

Table 1. Microbiological criteria: use of sludge on agricultural land

Sludge category	Admissible amount of microorganisms (CFU) in 1 g of solids of applied sludge		
	thermotolerant coliform bacteria	enterococci	<i>Salmonella</i> sp.
I.	$< 10^3$	$< 10^3$	negative
II.	$10^3-10^6$	$10^3-10^6$	not determined

Category I – sludge that may be generally used on soils exploited in agriculture in case that the remaining provisions of the Decree are complied with; Category II – sludge that may be used on agricultural land to be used for growing of technical produce and on soils in which vegetables or intensively fertile fruit orchards are not to be planted within at least three years from the time of use of sludge – while complying with the principles of occupational health and safety as well as remaining provisions of the Decree

water treatment plants is not thoroughly covered. Considering the valid legislation, principally Act No. 185/2001 Coll. on waste as amended, resp. Decree No. 381/2001 Coll. laying down the Catalogue of Wastes as amended, sand from a detritus tank is – in accordance with the Annex No. 1 to the Decree – included in the Catalogue of Waste under the name of “Waste from detritus tank” and ref. No. 19 08 02 as other waste and such sand is handled in the respective manner. This practice is enforced in spite of the fact that such waste might feature at minimum one of the hazardous properties stipulated in the Annex No. 2 to Act No. 185/2001 Coll. or contain one of the components due to which waste is regarded as hazardous in accordance with the Annex No. 5 to Act No. 185/2001 Coll. The property referred to above is contingent contagiousness. Therefore, we conducted microbiological analyses of sand from wastewater treatment plants. As suggested above, an unambiguous procedure applicable to sand from wastewater treatment plants does not exist and due to this fact the guidelines applicable to handling of wastewater treatment sludge – more specifically Decree No. 382/2001 Coll. on conditions of the use of the treated sludge on agricultural land as amended – were selected to serve as a basis. The aforementioned Decree stipulates the technical conditions applicable to use of sludge on agricultural land as well as limit concentrations of selected hazardous substances in sludge and soil including microbiological criteria (MATĚJŮ 2001). The Decree also stipulates admissible amounts of indicator microorganisms. The scope covers thermotolerant coliform bacteria, enterococci and *Salmonella* sp. (Table 1).

Considering the most common manner of sand removal by the means of disposal, it is necessary to face additional legislative requirements resulting from the valid judicial practice. The respective scope covers principally the conditions applicable to

depositing of sand from a detritus tank in a landfill. They are governed by Decree No. 294/2005 Coll. on the conditions of depositing waste in landfills and its use on the surface of the ground (as amended). The aforementioned Decree expressly stipulates the conditions upon which sand from a detritus tank may be deposited in a landfill. As regards other waste, the Decree specifies three subgroups of landfills (S-OO1, S-OO2, S-OO3); sand from a detritus tank may be deposited in landfills labelled S-OO1 and S-OO2 – on the basis of expressly specified conditions that are stipulated in the Annex No. 4 to Decree No. 294/2005 Coll. Considering the conditions stipulated in the Annex No. 4 to Decree No. 294/2005 Coll., the most significant problem proves to be related to the total content of organic carbon (TOC) which should remain under 5%; in case of exceeding TOC, the problem pertains to dissolved organic carbon (DOC) which is monitored in the aqueous liquor of deposited sand and in case of which the value should not exceed 80 mg/l. The effort focused on prevention of depositing slowly organically decomposable materials in landfills resulted in incorporation of TOC and DOC parameters into our judicial practice and causes numerous problems. The most significant problem pertains to an exact definition of the respective terms as well as selection of a suitable analytical method applicable to determination of TOC and DOC – due to the fact that in practice it is possible to encounter diametrically different and mutually incompatible configurations and contents of the respective values in analyzed samples. The aforementioned fact was not resolved even by the Standard applicable to determination of TOC content [Czech/European Standard ČSN EN 13137 (2002) – Determination of TOC in waste, sludge, and sediments] which stipulates the only proposed method of determination to be incineration of samples in oxygen at high temperatures.

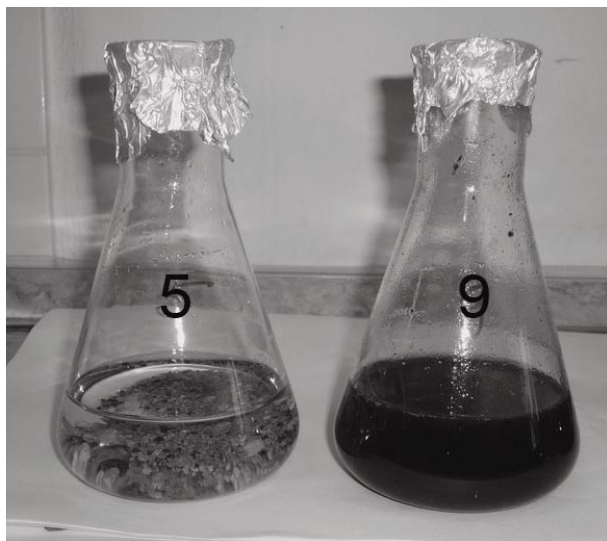


Fig. 1. Samples of sand after 20 min of stirring in distilled water (Photo by M. Szostkova)

## MATERIAL AND METHODS

Samples of the sand were collected at nine wastewater treatment plants (WWTPs) in the South Moravia Region, Czech Republic (Tetčice, Střelice, Zbraslav, Ořechov, Blansko, Boskovice, Letovice, and Jedovnice). On average, three samples were collected at each WWTP in the time period of September 2008 to March 2009. The collection of samples was based on Czech Standard ČSN ISO No. 10381-6 (1998). On the days of collection the respective samples were transported to a laboratory in sterile sample containers (at a temperature not exceeding 5°C), thus preventing their secondary contamination. The samples were weighed immediately after receipt; the content of total solids and ash-free dry mass were determined and a microbiological analysis was conducted.

The methodology applicable to physical analysis of sand, i.e. determination of the content of total solids and ash-free dry mass is stipulated in Czech Standard ČSN No. 83 0550 (Section 3) (1978). Total solids content and ash-free dry mass in sand samples were determined by use of electric muffle furnace LMH 07/12 which is designed to measure incineration processes, drying, degradation, reheating, thermal treatments etc. Analytical laboratory balances Radwag AS 220/X, were used for precise weighing, readability to 0.0001 g. A well-mixed sample of 10 g was evaporated in a weighed dish and dried to constant weight in an electric muffle furnace at the temperature 103°C to 105°C. The increase in weight over that of the empty dish represents the total solids (%). Af-

ter total solid assessment the dish with sample was put back to electric muffle furnace at 550°C. The difference in weight over that of the dish after total solid assessment represents the ash-free dry mass (%), which expresses the content of organic material in the sand sample.

### The sample preparation for the estimation of microbiological parameters

The suspension was prepared by homogenizing of 20 g sample of sand in 150 ml of sterile solution in a blender for 20 min. After filtration (Whatman No. 1, Merck, Czech Republic) the suspension was used for all following microbiological tests.

### Thermotolerant coliform bacteria

A standard method according to Czech Standard ČSN ISO 4832 (1995) was used for the detection and identification of thermotolerant coliform bacteria in the sand samples. Dilution of suspension was made according to Czech Standard ČSN EN ISO 6887-1 (1999). Petri dishes with m-FC agar (Merck, Germany) were inoculated with 100 µl of the sample and consequently incubated at  $44 \pm 1^\circ\text{C}$  for 18–24 h. Thermotolerant coliform bacteria were indicated by the presence of dark blue colonies on agar.

### Coliform bacteria

A standard method according to Czech Standard ČSN ISO 4832 (1995) was used for the detection of all coliform bacteria in the sand samples. Dilution of suspension was made according to Czech Standard ČSN EN ISO 6887-1 (1999). Petri dishes with ENDO agar (Merck, Germany) were inoculated with 100 µl of the sample and consequently incubated at  $37 \pm 1^\circ\text{C}$  for 24–48 h. Coliform bacteria were indicated by the presence of white or red colonies on agar.

### Enterococci

A standard method according to Czech/European Standard ČSN EN ISO 7899-2 (2001) was used for the detection and identification of intestinal enterococci in the sand samples. Petri dishes with m-Enterococcus selective agar according to Sla-

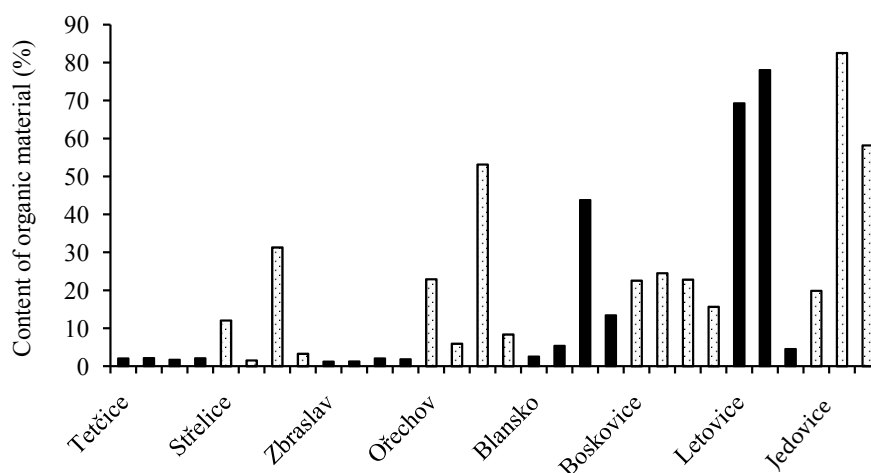


Fig. 2. Content of organic material in the samples of sand from various WWTPs (Wastewater treatment plants)

netz and Bartley (Merck, Germany) were inoculated with 100  $\mu$ l of the sample. Dishes with samples were incubated in an inverted position at  $37 \pm 1^\circ\text{C}$  for 4 hours and consequently at  $44 \pm 0.5^\circ\text{C}$  for 20 to 44 h. Enterococcus was indicated by the presence of pink to maroon colonies on agar.

## RESULTS

Samples of wastewater treatment sand collected at various WWTPs showed visually different amounts of organic material content. Fig. 1 documents the aforementioned differences using samples collected at WWTP Zbraslav (left) and WWTP Ořechov (right). Sample No. 5 (Zbraslav) is clear, it contains 95% of total solids and its content of organic material is only 1.2%. Quite on the contrary, Sample

No. 9 (Ořechov) is conspicuously turbid, it contains only 68% of total solids and its content of organic material was determined to reach 23% (Fig. 2).

All the collected samples were also subject to a microbiological analysis focused on indicator groups of microorganisms that are commonly determined in wastewater treatment sludge prior to its contingent use on agricultural land. The scope covers coliform bacteria including *Escherichia coli*, enterococci, and faecal coliform bacteria (Fig. 3).

The charts in Fig 3a, b, c contain lines that mark limit values for Category I (solid line) and Category II (dashed line) of the sewage sludge. Values of sand contamination are shown in Table 2.

The measured data were graphically compared (Fig 4a, b, c) in order to evaluate the dependence of organic material content on contamination of samples by indicator groups of microorganisms.

Table 2. The values of the microbial contamination of the waste sand from various waste water treatment plants

Sand sample	Coliform bacteria including <i>E. coli</i> $\times 10^4$ CFU/g of total solids			Enterococci $\times 10^3$ CFU/g of total solids			Faecal coliform bacteria $\times 10^3$ CFU/g of total solids		
	min	max	arithmetic mean	min	max	arithmetic mean	min	max	arithmetic mean
Tetčice	1.69	7.08	4.47	–	–	–	0.31	0.31	0.31
Střelice	2.08	425.00	113.42	0.06	26.35	8.07	3.55	118.61	61.08
Zbraslav	2.09	34.50	18.63	1.29	10.18	4.98	13.80	18.66	16.23
Ořechov	24.00	606.00	275.30	3.43	33.88	15.93	141.73	219.28	180.51
Blansko	24.10	127.00	43.20	0.09	4.64	1.25	0.85	7.28	4.78
Boskovice	13.70	734.00	36.49	2.18	43.62	19.00	8.70	130.19	57.28
Letovice	1.53	24.60	12.01	1.56	33.65	21.99	0.80	27.72	10.82
Jedovice	3.20	3.92	3.56	15.67	36.54	22.62	3.82	3.92	3.87

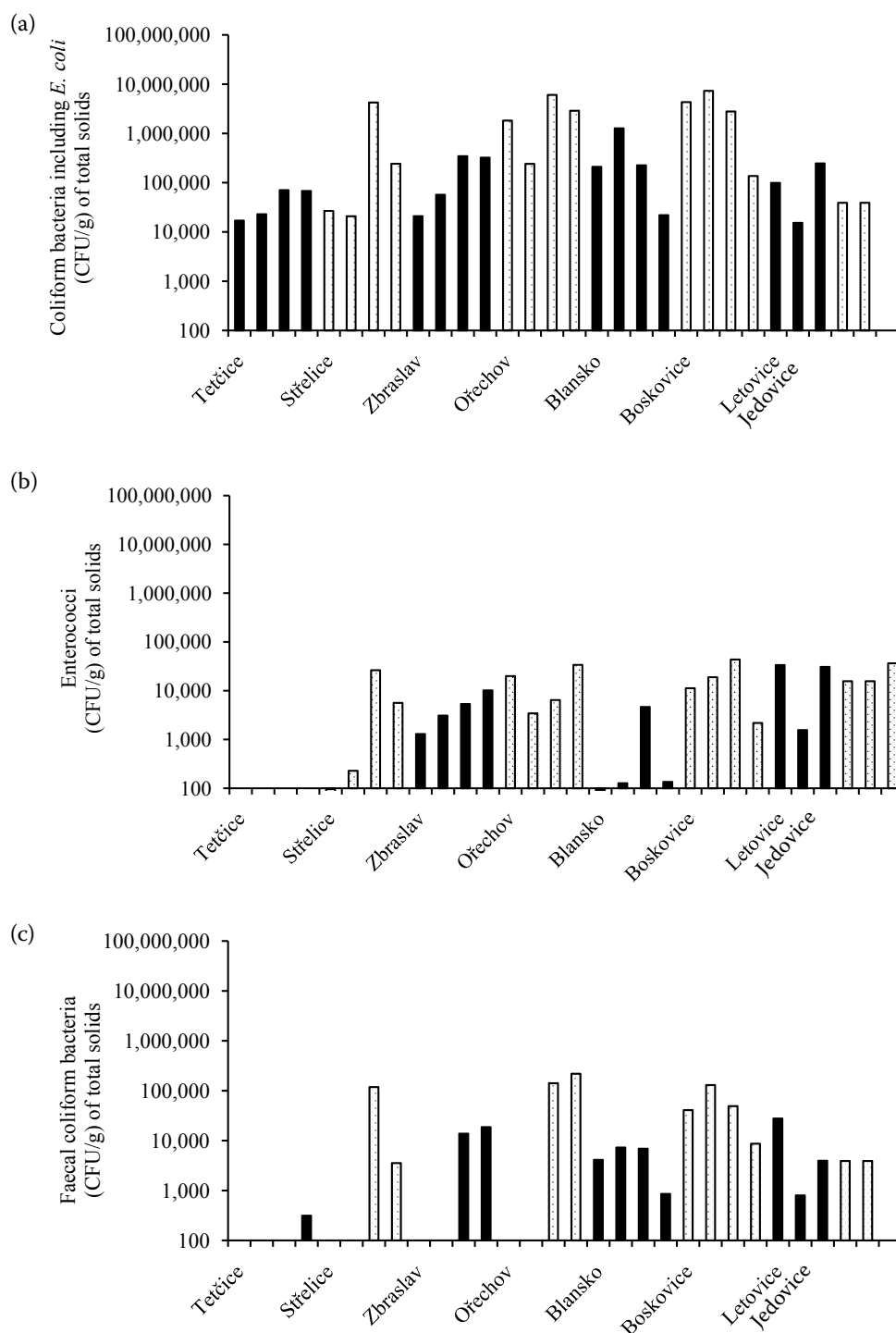


Fig. 3. Coliform bacteria including *E. coli* (a), enterococci (b), faecal coliform bacteria (c) in samples of sand from various WWTPs (Wastewater treatment plants)

## DISCUSSION

The presented results document microbial contamination of samples of sand from nine wastewater treatment plants located in the South Moravia Region. Samples collected gradually – in the course of 4 months – sometimes proved to feature similar parameters (e.g.

WWTP Tetčice) while the values determined at other WWTPs featured major fluctuations. For example, at WWTP Střelice the difference between individual samples represented 160 times higher amounts of coliform bacteria and 470 times higher amounts of enterococci (Fig 3a, b). The respective results reflect the technological equipment at individual WWTPs,

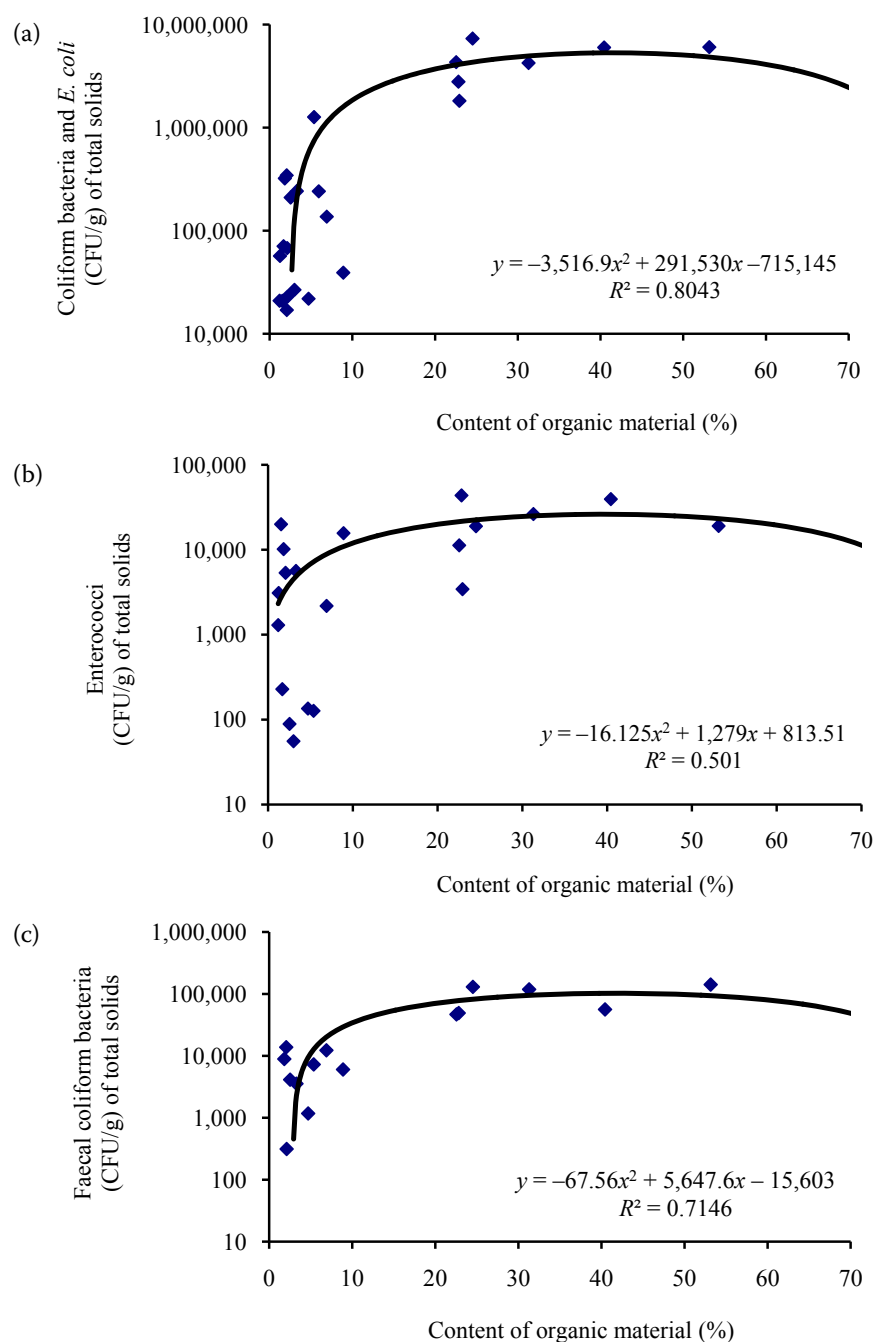


Fig. 4. Relation between contamination of samples by coliform bacteria (a), enterococci (b), faecal coliform bacteria (c) and the content of organic material

rainfall prior to collection of samples as well as numerous other factors that might affect the quality of inflowing wastewater, thus affecting its microbial contamination as well. The chart in Fig. 3a shows a rather high level of contamination of samples by coliform bacteria. A third of the samples do not comply with the microbiological criteria applicable to use for Category II sludge on agricultural land (Table 1). However, as regards the presence of enterococci and

faecal coliform bacteria, all the analyzed samples of wastewater treatment sand prove to comply with the above-mentioned criteria (Figs 3b, c).

The percentage of the content of organic material represents good indicators of microbial loading of samples of sand (Fig. 2). The charts in Fig. 4 represent a generalization of the mathematical relation between the content of organic material in the samples and their microbial contamination.

### References

- BITTON G., 2005. Wastewater Microbiology. 3<sup>rd</sup> Ed. Gainesville, USA, Wiley-Interscience: 746.
- ČSN 83 0550 (Section 3), 1978. Fyzikálně chemický rozbor kalů (Physico-chemical analysis of sludge). Prague, Czech Office for Standards, Methrology and Testing.
- ČSN ISO 4832, 1995. Mikrobiologie potravin a krmiv – Horizontální metoda stanovení počtu koliformních bakterií – Technika počítání kolonií (Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique). Prague, Czech Office for Standards, Methrology and Testing.
- ČSN ISO 10381-6, 1998. Kvalita půdy – Odběr vzorků – Část 6: Pokyny pro odběr, manipulaci a uchovávání půdních vzorků určených pro studium aerobních mikrobiálních procesů v laboratoři (Soil Quality – Sampling – Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory). Prague, Czech Office for Standards, Methrology and Testing.
- ČSN EN ISO 6887-1, 1999. Mikrobiologie potravin a krmiv – Úprava analytických vzorků, příprava výchozí suspenze a desetinasobných ředění – Část 1: Všeobecné pokyny pro přípravu výchozí suspenze a desetinasobných ředění (Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the initial suspension and decimal dilutions). Prague, Czech Office for Standards, Methrology and Testing.
- ČSN EN ISO 7899-2, 2001. Jakost vod – Stanovení intestinálních enterokoků – Část 2: Metoda membránových filtrů (Water quality – Determination of intestinal enterococci – Part 2: Membrane filtration method). Prague, Czech Office for Standards, Methrology and Testing.
- ČSN EN 13137, 2002. Charakterizace odpadů – Stanovení celkového organického uhlíku (TOC) v odpadech, kalech a sedimentech (Characterization of waste – Determination of total organic carbon (TOC) in waste, sludges and sediments). Prague, Czech Office for Standards, Methrology and Testing.
- GERARDI M.H., ZIMMERMAN M.C., 2004. Wastewater Pathogens. Gainesville, USA, Wiley-Interscience: 179.
- MATĚJŮ L., 2001. Stanovení indikátorových mikroorganismů pro mikrobiologická kritéria pro použití kalů na zemědělské půdě ve smyslu vyhlášky č. 382/2001 Sb., o podmínkách použití upravených kalů na zemědělské půdě (Determination of indicator organisms for the microbiological criteria for the use of sludge on agricultural land as defined in the Decree No. 382/2001 Coll. Concerning the application of treated sludge on agricultural land). Prague, Státní zdravotní ústav v Praze.
- SHUVAL H., FATTAL B., 2003. Control of pathogenic microorganisms in wastewater recycling and reuse in agriculture. In: MARA D., HORAN M.J (eds), Handbook of Water and Wastewater Microbiology. London, Academic Press: 241–262.
- SCHROEDER E., WUERTZ S., 2003. Bacteria. In: MARA D., HORAN M.J (eds), Handbook of Water and Wastewater Microbiology. London, Academic Press: 57–68.

Received for publication June 4, 2009

Accepted after corrections July, 20, 2010

---

#### Corresponding author:

Mgr. MONIKA SZOSTKOVÁ, Ph.D., Mendel University in Brno, Faculty of Agronomy,  
Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Zemědělská 1, Brno, Czech Republic  
phone: + 420 545 133 323, e-mail: szostkov@mendelu.cz

---