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# Evaluation of efficiency of technologies for wastewater sludge hygienisation

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## Abstract

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The paper compares the methods of hygienisation of wastewater treatment plants sludges with respect to meet legislative requirements of conditions for using the treated sludge on agricultural land. The paper draws a comparison of the experimental results between the method of hygienisation and stabilisation of the sludge through the autothermic aerobic thermophilic stabilisation (AATS) by pure oxygen and the method of sludge hygienisation by pasteurization. Results of the experiment confirm that sewage sludge treated by both assessed hygienisation technologies meets legislation requirements for application to agricultural soil.

**Keywords:** wastewater treatment plant; sludge; indicator microorganism; microbiological standards; sludge, stabilisation

Wastewater treatment systems typically involve the generation of continuous and high volumes of sludge (SINTON et al. 1998). Proper management of sludge disposal is imperative for effective wastewater treatment. The use of stabilised sewage sludge (biosolids) for various land applications increases and represents a disposal solution that is both economical and environmentally friendly (SALSALI et al. 2006). Sewage sludge acts as the reservoir of several indicator and pathogenic microorganisms (ARTHURSON 2008). Sewage sludge utilization in agricultural production has been gaining increasing interest in recent years (STONE et al. 1998). The use of materials containing large numbers of microorganisms can, under certain conditions, contaminate surface or ground-waters. The risk of groundwater contamination from biosolids may be greatest with pathogenic microorganisms due to their relatively small size and poor adsorption by soil particles (FUKUSHI et al. 2003; HORAN et al. 2004). The disinfection degree, which is influenced by a variety of interacting operational variables and conditions, is however, highly depend-

ent on time and temperature (MAIER et al. 2006). The composition of the most abundant facultative anaerobic bacteria populations (faecal coliforms and enterococci) in sludge can be modified after different treatments. These involve the disposal or reuse of sludge and include: anaerobic digesters, incineration, composting, pasteurization and lime treatments (BONJOCH, BLANCH 2009). Anaerobic digestion is an appropriate technique for the treatment of sludge before final disposal (SAHLSTRÖM et al. 2004). LANG and SMITH 2008 stated that the behaviour of *Escherichia coli* and *Salmonella* under mesophilic conditions appeared to be related to the retention time and to a limited extent, the sample matrix, but there was no evidence of direct thermal inactivation taking place at mesophilic temperatures. Anaerobic digestion was used to stabilise the sewage sludge produced by wastewater treatment plants and, eventually, to eliminate pathogens for more than a century (METCALF, EDDY 2003). The autothermic thermophilic aerobic digestion (ATAD) enhances the quality of the raw sludge that is treated in a biological reactor in the presence of

oxygen (LAPARA, ALLEMAN 1999). Nucleate boiling, which can transfer large amounts of thermal energy at small temperature difference, reveals pasteurization method as to biological sludge (LIU, LEE 2000). Continuously feeding influent wastewater containing diverse bacterial species to a wastewater treatment-activated sludge bioreactor may influence the activated sludge bacterial community temporal dynamics (LEE et al. 2015). The thickened sludge is a very complicated colloidal system, which is composed of organic and inorganic particles. Thickened sewage sludge has the similar rheological properties as activated sewage sludge or digested sewage sludge (TRÁVNÍČEK, JUNGA 2014).

The goal of this paper is to evaluate the experiment with application of autothermic aerobic thermophilic stabilisation (AATS) by pure oxygen (technology OSS – Oxyterm sludge system®), and the method of sludge hygienisation by pasteurization and the study of two different methods for hygienisation of wastewater treatment plant sludges, to compare their efficiency and a level of secondary contamination by indicator microorganisms before their subsequent treatment. Results of this paper confirm that sludge treated by both assessed hygienisation technologies meets legislation requirements for agricultural utilization.

## MATERIAL AND METHODS

The first analysed waste water treatment plant is located in Tetčice, South Moravia region, Czech Republic. The operational capacity is 15,000 (population equivalent) and 2,250 m<sup>3</sup> of waste water a day. Technological conception is low-rate mechanical-biological waste water treatment plant with high efficiency disposal of nitrogen and chemical coagulation of phosphorus. Activated sludge technology consists of couple of oxidation tanks (overall 36.00 m wide, 12.00 m long and 4.80 m high; reaction volume 1,900 m<sup>3</sup>) and pneumatic aeration system. Sludge management system consists of 3 tanks for accumulation and treatment of sludge. Activated sludge is continually pumped from activated sludge process to flotation sludge thickener. Thickened sludge is consequently pumped to the aerated and air-mixed equalization tank. Sludge is pumped (20-hour cycle) to the autothermic aerobic thermophilic stabilisation reactor with middle hydraulic detention time of approximately 21 days. The sludge is warmed-

up with using biologically mediate and controlled oxidation by oxygen and temperature is maintained at hygienisation temperature above 55°C. Second equalization tank is used for homogenization of the hygienised sludge. Capacities in both equalization tanks are maintained in technological parameters which warrant specified duration of heat sludge treatment (min. 20 days).

The second analysed waste water treatment plant is located in Tišnov, South Moravia region, Czech Republic. The operational capacity is 18,000 (population equivalent) and 2,400 m<sup>3</sup> of waste water a day. Technological conception is low-rate mechanical-biological waste water treatment plant. Activated sludge technology consists of couple of mixing tanks (overall 4.80 m wide, 15.00 m long and 3.00 m high; reaction volume 347 m<sup>3</sup>) and aeration system with 6 surface mechanical aerators. Activated sludge is continually pumped to the accumulation tank. Next process is anaerobic fermentation in anaerobic fermenter and pasteurization in pasteurizer. Pasteurization of the sludge proceeds with continual intensive mixing at temperature 70°C for a period of 60 minutes.

Sampling of the sludge is in compliance with requirements ČSN EN ISO 5667-13:2011 (Water quality – Sampling – Part 13: Guidance on sampling of sludges) and ČSN EN ISO 7218:2008 (Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations). Determination of total solids (TS) and water content is according to ČSN EN 12880:2001 (Characterization of sludges – Determination of dry residue and water content) and ČSN EN 15934:2013 (Sludge, treated biowaste, soil and waste – Calculation of dry matter fraction after determination of dry residue or water content). Samples weight was determined with using the analytical balance Adventurer Pro (OHAUS Co., Greifensee, Switzerland) (accuracy 1 mg). Samples were dried in laboratory drier Venticell – (BMT Medical Technology s.r.o., Brno, Czech Republic) (drying process for a period of 12 h at 105 ± 5°C). Share of total solids in percent by weight is given according to equation:

$$w_{dr} = \frac{m_3 - m_1}{m_2 - m_1} \times f \quad (\%) \quad (1)$$

where:  $w_{dr}$  – the concentration of all matters in sample of sludge (the rest after drying) (%);  $m_1$  – the weight of empty crucible (g);  $m_2$  – the weight of crucible with sample (g);  $m_3$  – the weight of crucible with dried sample (g);

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$f$  – the calculation factor ( $f = 100$ ) for expression of result in percent by weight (%)

Determination of loss on ignition is done according to ČSN EN 15169:2007 (Characterization of waste - Determination of loss on ignition in waste, sludge and sediments) and ČSN EN 15935:2013 (Sludge, treated biowaste, soil and waste – Determination of loss on ignition). The sample is annealing to the constant weight at  $550 \pm 25^\circ\text{C}$ . Next, there is determination of sample weight differences before and after ignition. Loss of ignition is given according to equation:

$$w_{loi} = \frac{m_3 - m_4}{m_3 - m_1} \times 100 \quad (\%) \quad (2)$$

where:  $w_{loi}$  – the loss of ignition (%);  $m_1$  – the weight of empty crucible (g);  $m_3$  – the weight of crucible with dried sample (g);  $m_4$  – the weight of crucible after ignition of sample (g)

Determination of indicator microorganisms is performed with the homogenized sludge sample. As the next step, starting suspension and denary thinning along with hygienic security are done. Thinned starting suspension is used for inoculation of sets in Petri dishes with solid selective medium designated for detection of indicator microorganisms. Scale of thinning is chosen with reference to final number of colonies on one Petri dish (range 15 as far as 150 CFU).

Thermostable coliform bacteria from the tested samples are determined on the surface of selective medium (mFC agar; Merck Corp., USA). Petri dishes with inoculated agar are incubated in temperature-controlled oven at  $44.5 \pm 0.2^\circ\text{C}$  for period of 24 hours. Enterococci are determined from thinned starting suspension with inoculated Petri dishes with m-enterococci selective medium (Slanetz – Bartley agar; Merck Corp., USA). Petri dishes with inoculated agar are incubated in temperature-controlled oven at  $44.5 \pm 0.2^\circ\text{C}$  for period of 24 hours. *Salmonella* spp. bacteria are determined in four consecutive following steps. First step it is selective propagation of bacteria with inoculation of tested samples in pepton solution buffer water (incubation at  $36 \pm 2^\circ\text{C}$  for period of 18 h). After propagation of bacteria, inoculation of two liquid medium is performed. First selective medium contains magnesium chloride and malachite green (ČSN ISO 19250:2011, Water quality – Detection of *Salmonella* spp.). Petri dishes with inoculated liquid medium are incubated in temper-

ature-controlled oven at  $41.5 \pm 1^\circ\text{C}$  for two periods of 24 hours. Second selective medium contains sodium selenite and cysteine (ČSN ISO 19250:2011). Petri dishes with inoculated liquid medium are incubated in temperature-controlled oven at  $36 \pm 2^\circ\text{C}$  for two periods of 24 hours. Each of those cultures is subsequently inoculated in two solid selective mediums – agar with phenolic red and brilliant green (so-called BGA agar by Edel and Kampelmacher) and agar with xylose, lysine and desoxycholate (so-called XLD agar).

After incubation process all of typical bacterial colonies on each plate which contains less than 150 colonies are quantified. Number of indicator microorganisms ( $N$ ) per 1 g of sample is determined in accordance to equation:

$$N = \frac{\sum c}{(n_1 + 0.1 \times n_2) \times d \times V} \quad (\text{CFU}) \quad (3)$$

where:  $\sum c$  – total sum of all colonies counted after identification on chosen plates;  $n_1$  – number of plates applied for calculation out of the first thinning;  $n_2$  – number of plates applied for calculation out of the second thinning;  $d$  – calculation factor of the first thinning;  $V$  – capacity of inoculum (ml);  $N$  – number of indicator microorganisms per 1 g of sample; CFU – colony-forming unit

From the results of number of indicator microorganisms in 10 samples in input (A) and 10 samples in output (B) of technological arrangement the median value is calculated. Level of inactivation is calculated in accordance to equation:

$$\log IR = \log med A - \log med B \quad (4)$$

Concentration of chosen hazardous materials and elements (concretely As, Cd, Cu, Hg, Cr, Ni, Pb, Zn, AOX, PCB) in sludge are important evaluative indicator of sludge for use on agriculture soil. Limits of content and methods for experimental determination of hazardous materials and equipment are given by legislation regulation (Decree of the Ministry of Environment No. 382/2001 Coll. on the conditions for using treated sludge on agricultural land).

Taking of samples for this survey was realized in the years 2008 to 2013. The samples were taken before and after the hygienisation process as well as hygienised and stabilised sludge from dumping site. The evaluated parameters were: total solids, loss of ignition, number of indicator microorganisms and concentration of chosen hazardous materials and elements.

Table 1. Statistical evaluation of microbiological parameters of sludge samples from waste water treatment plant Tetčice

Statistical parameter	Activated sludge		Hygienized sludge		Stabilized sludge	
	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)
Count measurement	18	18	18	18	18	18
Valid measurement (%)	100	100	100	100	100	100
Average	$3.06 \times 10^5$	$1.90 \times 10^5$	0.00	0.00	$1.72 \times 10^1$	$1.39 \times 10^1$
Geometrical mean	$2.02 \times 10^5$	$1.21 \times 10^5$	–	–	–	–
Harmonic mean	$1.31 \times 10^5$	$8.30 \times 10^4$	–	–	–	–
Median	$1.80 \times 10^5$	$1.05 \times 10^5$	0	0	–	–
Mode	multiple	$4.30 \times 10^4$	0	0	< 10	< 10
Frequency of mode	1	2	18	18	15	15
Sum	5,514,000	3,424,000	0	0	–	–
Minimum	$3.20 \times 10^4$	$2.90 \times 10^4$	0	0	0	0
Maximum	$9.29 \times 10^5$	$8.80 \times 10^5$	0	0	$1.60 \times 10^2$	$1.00 \times 10^2$
Lower quartile	$1.10 \times 10^5$	$5.70 \times 10^4$	0	0	< 10	< 10
Upper quartile	$4.80 \times 10^5$	$2.09 \times 10^5$	0	0	< 10	< 10
The variance	$7.98 \times 10^{10}$	$4.75 \times 10^{10}$	0.00	0.00	–	–
Standard deviation	$2.82 \times 10^5$	$2.18 \times 10^5$	0.00	0.00	–	–
The coefficient of variation	92.22	114.59	0.00	0.00	–	–
Standard error	$6.66 \times 10^4$	$5.14 \times 10^4$	0.00	0.00	8.43	5.12

TS – total solids; TS – total solids; CFU – colony forming units

**RESULTS AND DISCUSSION**

The microbiological parameters of the activated sludge from waste water treatment plant Tetčice incoming into the process of stabilisation and hygieni-

sation are following: average value  $1.90 \times 10^5$  CFU of thermostable coliform bacteria and  $3.06 \times 10^5$  CFU of enterococci per 1 gram of sludge total solids. The maximal given number is  $8.80 \times 10^5$  CFU of thermostable coliform bacteria and  $9.29 \times 10^5$  CFU

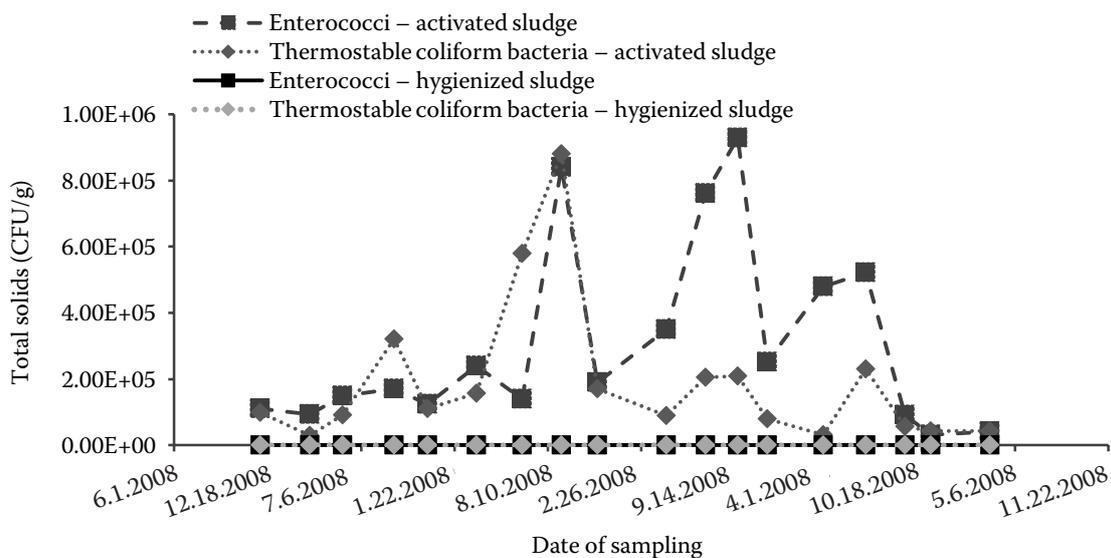


Fig. 1. Evaluation of thermostable coliform bacteria and enterococci in sludge samples from waste water treatment plant Tetčice

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Table 2. Concentration (CFU/g total solids) of chosen hazardous materials and elements in sludge samples from waste water treatment plant Tetčice

Type of hazardous material or element	Average value	Maximal value	Limit value
Arsenic	2.93	7.4	30
Cadmium	1.23	2.3	5
Chromium	41.69	65	200
Copper	178.65	257	500
Mercury	1.08	2.2	4
Nckel	33.59	53	100
Lead	59.89	92	200
Zinc	1,288.00	1,652	2,500
AOX	146.55	293	500
PCB (sum of 6 congeners)	0.28	0.36	0.6

of enterococci per 1 g of sludge total solids. The statistical evaluation of other microbiological parameters of sludge samples from waste water treatment Tetčice is given in Table 1 and Fig. 1.

In terms of all performed laboratory tests, thermostable coliform bacteria, enterococci or *Salmonella* spp. bacteria were not detected in hygienised sludge. Application of autothermic aerobic thermophilic stabilisation and hygienisation of sludge caused 100% dispatch of monitored indicator microorganisms. Level of inactivation is calculated (IR) 5 log for enterococci as well as thermostable coliform bacteria. The results of indicator microorganism determination in sludge treated by autothermic aerobic thermophilic stabilisation and hygienisation process (AATS) confirm the results of ZWIEFELHOFER (1985) and BENEŠOVÁ (2004). Those authors stated that AATS process ensures perfect stabilisation and hygienisation of sludge,

which means total dispatch of *Salmonella* spp. bacteria and reduction of the number of enterococci and thermostable coliform bacteria on values of  $< 10^3$  CFU per 1 gram of sludge total solids.

The number of thermostable coliform bacteria and enterococci  $< 10$  (CFU/g of TS) was determined in stabilised sludge from dumping site (period of deposition  $> 10$  days). No *Salmonella* spp. bacteria were detected in either of the obtained sludge samples. Only one of obtained samples revealed enterococci as  $1.60 \times 10^2$  (CFU/g of TS) and coliform bacteria as  $1.00 \times 10^2$  (CFU/g of TS). These values represent recontamination of sludge in all likelihood caused by incorrect sampling. Despite this fact, stabilised sludge from dumping site meets all of the legislation microbiological criteria (category I) for application to agricultural soil.

The results of chemical analyses of sludge from waste water treatment Tetčice confirm that neither of the monitored indicators for sludge evaluation exceeds legislation limits of application to agricultural soil. Concentrations of chosen hazardous materials and elements in sludge samples are presented in Table 2.

The agrochemical characteristics of analysed sludge samples from waste water treatment plant Tetčice are following: average value of nitrogen content is 4.16% of total solids, phosphorus 2.55% of dry matter, kalium 0.54% of dry matter, calcium 3.54% of total solids and magnesium 0.79% of total solids. Agrochemical composition of sludge for agricultural soil application is a rich source of majority of mineral elements necessary for plant growth and suitable alternative for industrial fertilizers supply. The agrochemical characteristics are presented in Fig. 2.

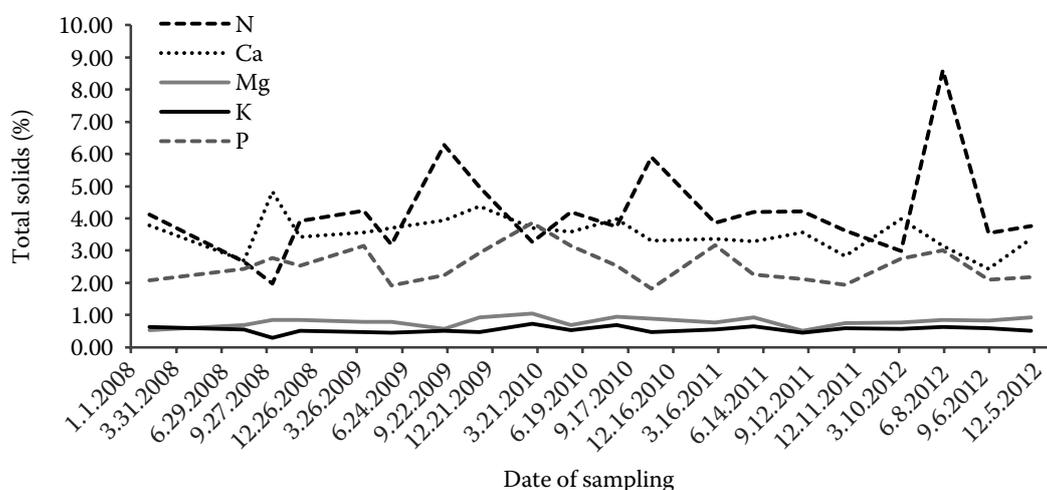


Fig. 2. Agrochemical characteristics of sludge samples from waste water treatment plant Tetčice

Table 3. Statistical evaluation of microbiological parameters of sludge samples from waste water treatment plant Tišnov

Statistical parameter	Non-treated sludge		Hygienized sludge		Stabilized sludge	
	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)	enterococci (CFU/g TS)	thermostable coliform bacteria (CFU/g TS)
Count measurement	18	18	18	18	18	18
Valid measurement (%)	100	100	100	100	100	100
Average	$8.15 \times 10^5$	$1.77 \times 10^7$	$2.81 \times 10^2$	$1.68 \times 10^2$	$2.47 \times 10^3$	$8.25 \times 10^2$
Geometrical mean	$5.45 \times 10^5$	$9.94 \times 10^5$	–	–	$8.07 \times 10^2$	$5.57 \times 10^2$
Harmonic mean	$3.71 \times 10^5$	$3.07 \times 10^5$	–	–	$4.55 \times 10^2$	$3.83 \times 10^2$
Median	$5.49 \times 10^5$	$4.75 \times 10^5$	$3.20 \times 10^2$	$1.05 \times 10^2$	$7.25 \times 10^2$	$5.20 \times 10^2$
Mode	$1.90 \times 10^6$	multiple	multiple	0	$4.00 \times 10^2$	multiple
Frequency of mode	2	1	2	3	2	2
Sum	14,673,000	318,101,000	5,050	3,025	44,410	14,843
Minimum	$1.40 \times 10^5$	$8.70 \times 10^4$	0	0	$9.00 \times 10^1$	$1.20 \times 10^2$
Maximum	$1.92 \times 10^6$	$1.76 \times 10^8$	$5.20 \times 10^2$	$5.50 \times 10^2$	$2.80 \times 10^4$	$2.55 \times 10^3$
Lower quartile	$2.37 \times 10^5$	$1.82 \times 10^5$	$1.20 \times 10^2$	$2.00 \times 10^1$	$4.00 \times 10^2$	$2.60 \times 10^2$
Upper quartile	$1.43 \times 10^6$	$2.51 \times 10^6$	$4.20 \times 10^2$	$3.00 \times 10^2$	$1.27 \times 10^3$	$1.20 \times 10^3$
Variance	$4.67 \times 10^{11}$	$2.27 \times 10^{15}$	$3.23 \times 10^4$	$2.94 \times 10^4$	$4.14 \times 10^7$	$5.69 \times 10^5$
Standard deviation	$6.84 \times 10^5$	$4.76 \times 10^7$	$1.80 \times 10^2$	$1.71 \times 10^2$	$6.43 \times 10^3$	$7.54 \times 10^2$
Coefficient of variation	83.87	269.47	64.01	102.02	260.66	91.50
Standard error	$1.61 \times 10^5$	$1.12 \times 10^7$	$4.20 \times 10^1$	$4.00 \times 10^1$	$1.52 \times 10^3$	$1.78 \times 10^2$

TS – total solids; CFU – colony forming units

The microbiological parameters of activated sludge from waste water treatment plant Tišnov incoming into process of stabilisation and hygienisation are following: average value  $1.77 \times 10^7$  CFU of thermostable coliform bacteria and  $8.15 \times 10^5$  CFU of enterococci per 1 g of sludge total solids.

The maximal given number is  $1.76 \times 10^8$  CFU of thermostable coliform bacteria and  $1.92 \times 10^6$  CFU of enterococci per 1 g of sludge total solids. The statistical evaluation of other microbiological parameters of sludge samples from waste water treatment Tišnov is given in Table 3 and Fig. 3.

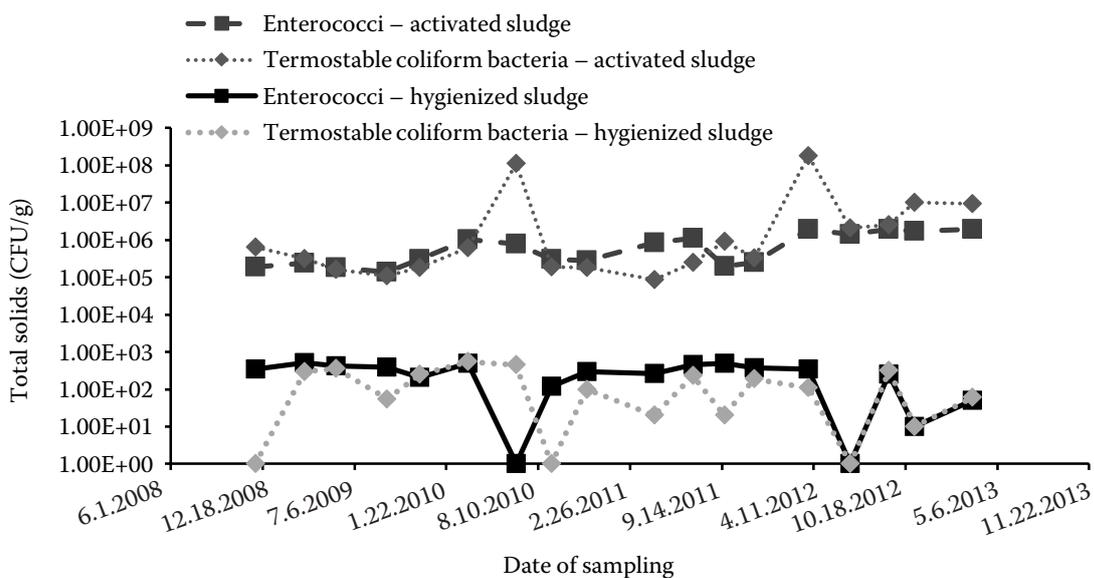


Fig. 3. Evaluation of thermostable coliform bacteria and enterococci in sludge samples from waste water treatment plant Tišnov

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Table 4. Concentration (CFU/g TS) of chosen hazardous materials and elements in sludge samples from waste water treatment plant Tišnov

Type of hazardous material or element	Average value	Maximal value	Limit value
Arsenic	1.48	5.0	30
Cadmium	0.93	1.8	5
Chromium	37.21	62.0	200
Copper	179.00	257	500
Mercury	1.85	3.4	4
Nickel	28.55	41.0	100
Lead	53.22	72.0	200
Zinc	1,169.65	1,443	2,500
AOX	371.00	391	500
PCB (sum of 6 congeners)	0.06	0.20	0.6

The following microbiological parameters of hygienised (pasteurized) sludge from waste water treatment plant Tišnov were determined: average value  $1.68 \times 10^2$  CFU of thermostable coliform bacteria and  $2.81 \times 10^2$  CFU of enterococci per 1 g of hygienised sludge total solids. The maximal given number is  $5.50 \times 10^2$  CFU of thermostable coliform bacteria and  $5.20 \times 10^2$  CFU of enterococci per 1 g of hygienised sludge total solids. In terms of all performed laboratory tests, no thermostable coliform bacteria, enterococci or *Salmonella* spp. bacteria were detected in hygienised sludge. Average rate of dispatch monitored indicator microorganisms defined from measurements come to 99.95% of thermostable coliform bacteria and 99.91 % of enterococci. The results of indicator microorganism determination in sludge treated by pasteurization (temperature 70°C for a period of

60 min) stabilisation and hygienisation process confirm the results of BENEŠOVÁ (2004) and STRAUCH (1998). Those authors stated that pasteurization process ensures a large degree of devitalization of pathogenic microorganisms.

The average number of thermostable coliform bacteria  $8.25 \times 10^2$  (CFU/g, d.m.) and enterococci  $2.47 \times 10^3$  (CFU/g, d.m.) was determined in stabilised sludge from the dumping site (period of deposition >10 days). No *Salmonella* spp. bacteria were detected in either of the obtained sludge samples. These values represent recontamination of sludge. Recontamination can be caused by expansion of survived microorganism or in a way of storage – contamination from the surrounding environment. Despite this fact, stabilised sludge from the dumping site meets the legislation microbiological criteria (category II) for application to agricultural soil destined only for technical crop production (non-food production).

The results of chemical analyses of sludge from waste water treatment Tišnov confirm that neither of the monitored indicators for sludge evaluation exceeds legislation limits for application to agricultural soil. Concentrations of chosen hazardous materials and elements in sludge samples are presented in Table 4.

The agrochemical characteristics of analysed sludge samples from waste water treatment plant Tišnov are following: average value of nitrogen content is 3.81% of dry matter, phosphorus 2.59% of dry matter, kalium 0.40 % of dry matter, calcium 3.62% of dry matter and magnesium 0.62% of dry matter. The agrochemical characteristics are presented in Fig. 4.

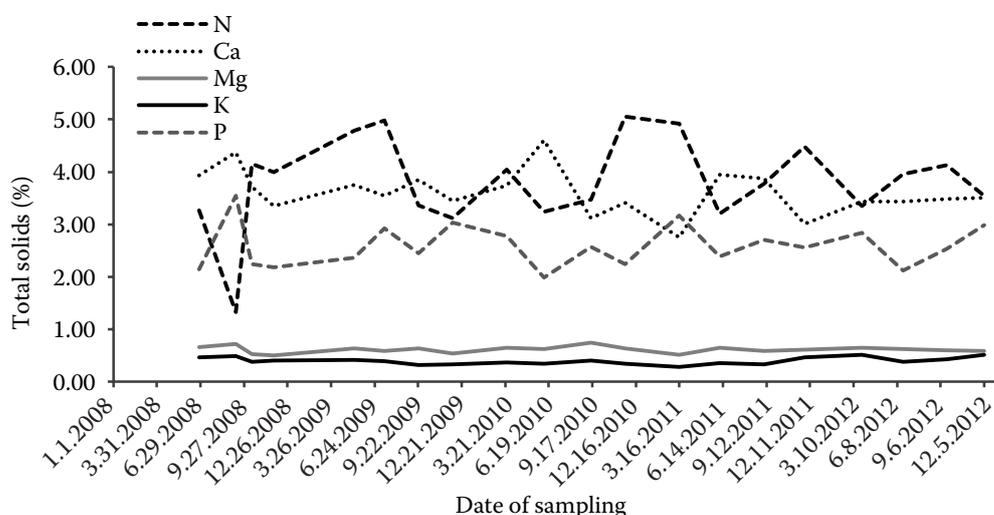


Fig. 4. Agrochemical characteristics of sludge samples from waste water treatment plant Tišnov

## CONCLUSION

Sludge from waste water treatment plants can be defined as concentrated mixture of mineral and organic materials removed from sewerage water by waste water treatment process. Sludge is characterized by large variability of physical, chemical and microbial properties. These properties depend on origin and way of waste water and sludge treatment. Results of this experimental study confirm that sewage sludge treated by both assessed hygienisation technologies meets legislation requirements for application to agricultural soil (technology of pasteurization – sludge category I and technology of autothermic aerobic thermophilic stabilisation and hygienisation process – category II).

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