

Soil and Groundwater Fecal Contamination as a Result of Sewage Sludge Land Application

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Received: June 23, 2006

Accepted: March 20, 2007

Abstract

The release of sludge-born bacteria and their further subsurface transport was studied. The migration of bacteria was investigated in column experiments which were carried out under the conditions corresponding to naturally occurring extreme rainfall. Coal fly ash as well as coarse and medium grained sand, whose properties are similar to the soils present in degraded areas, were used as column beds. Sewage sludge was applied on the top of column beds in the quantity corresponding to the best land-reclamation practice. *Clostridium perfringens* and fecal coliforms were used as bio-tracers of fecal pollution. The obtained results showed the dynamic of bacterial cells' leaching from the sludge matrix, and the dynamic of their infiltration through the column beds to the effluents. The bacterial breakthrough curves obtained for the fly ash and for the sandy media differ significantly, reflecting the differences in transport processes and in the survival of bacterial cells. It has been found that the fly ash layer, whose thickness equals 0.80 m, can be regarded as an effective filter, which limits bacterial migration. When sludge is applied to the sandy soils, about 0.02% of the initial number of sludge bacteria can migrate downwards the sandy layer and can cause contamination of potentially shallow aquifers.

Keywords: sewage sludge, soil remediation, fecal microorganisms, bacterial migration

Introduction

Sewage sludge generated at wastewater treatment plants should be disposed of safely and economically. Land application is a popular option for its disposal and seems to be the most convenient and the least expensive. The potential bacterial contamination of soil and groundwater limits, however, the land application of sewage sludge. In the US, a significant link between waterborne disease outbreaks and the consumption of contaminated groundwater, particularly from private noncommunity wells, has been found [1-3]. When aquifer protection is taken into account, the understanding

of bacterial cells' migration in porous media becomes of great importance.

The transport of bacteria in soils, in the majority of studies, was analyzed using influent solutions with suspended cells applied to the soil surfaces, and the experiments were carried out mostly in saturated conditions [4-9, and others]. The data obtained in those experiments are only partly applicable to the migration of sludge-borne bacterial cells. Microorganisms present in sewage sludge, must first be realized from the sludge matrix, before they infiltrate through the soil profile, and only in a few studies was this problem analyzed [10-12].

In the present paper, the bacteriological aspects of sewage sludge land disposal on sandy soils and on coal fly ash are discussed. The samples of sandy soils and of coal fly ash were obtained from the local degraded areas.

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The lack of organic matter and biogenic compounds, and consequently the lack of vegetative protection cause the wind and the rain erosion of those soils. Sewage sludge is recommended as a means of recycling nutrients and organic matter, and enhancing soil quality. It seems to be a valuable material for the reconditioning of degraded areas [13, 14]. The migration of sludge-borne pathogens through soils into groundwater has become, however, a major concern to environmentalists and the general public [15].

In the present study, the effect of cyclic rain infiltration and of drainage events on the sludge-born bacteria's leaching, and on migration was analyzed. In general, straining, adsorption and inactivation are the main processes controlling bacterial transport in porous media. In unsaturated soils, bacteria travel with mobile water [16] and their cells can interact with the air or solid phase, which results in temporary or permanent immobilization. In such conditions bacteria can also be entrapped in stagnant pore water between gas bubbles. During rain events, connectivity between mobile and immobile water increases, allowing bacteria to migrate with the advancing wetting front.

The assumption of the study was to conduct the experiments in the worst possible conditions, which ensured the maximum efficiency of bacteria leaching from sewage sludge, and the advanced bacterial migration down the column. Thus, the laboratory tests were carried out in the atmospheric conditions corresponding to the extreme rainfall reported in the region of northern Poland whose precipitation height equals 100 mm per day [17]. In such conditions the flow rate of infiltrating rain water is the main factor controlling the bacterial transport in the unsaturated media, with the negligible meaning of bacterial cells' interactions with media interfaces [4, 18]. The leaching of bacteria from the sewage sludge and bacterial vertical migration was analyzed using *Clostridium perfringens* and fecal coliforms (FC), bio-tracers commonly present in sewage sludge [19-21], and compared with the transport of sludge-born NO_3^- ion. In the column experiments, nitrate nitrogen is an accepted pollution tracer [22, 23].

Methodology

The column experiments were conducted in four series: one lasted 30 days – series A, and three lasted 7 days – series B, C, and D. Cylindrical plexiglas columns of to-

tal length of 1.0 m and with the internal diameter of 5.4 cm were used in all the series. The construction of the columns enabled their disassembling and collection of soil samples from the estimated levels (Fig. 1). Coarse grained sand (CGS), medium grained sand (MGS), and coal fly ash (FA) were used as column beds. The basic features of the analyzed filter media are summarized in Table 1.

Air-dried CGS, MGS and FA were sieved (<1mm) and, to eliminate the impact of their autochthonous microflora on the obtained results, autoclaved prior to every experiment. The columns were filled to the height of 0.8 m. To achieve high packing density, the columns were completely saturated from the bottom up with filter-sterilized ($\phi = 0.45 \mu\text{m}$) deionized water (pH from 6.5 to 6.6). Once saturation was reached, the water content of each column was determined by weighing. Next, the columns were allowed to drain until the stable, minimum moisture content was achieved. The porosity of the filter beds was calculated, and was equal to 38.18 ± 0.6 , 41.07 ± 0.32 and 72.99 ± 0.59 for CGS, MGS and FA, respectively.

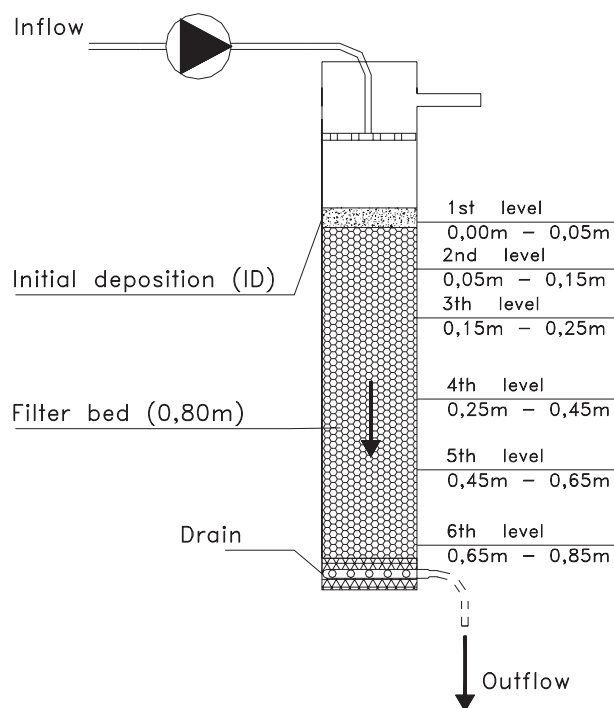


Fig. 1. Scheme of the experimental column.

Table 1. Selected properties of the filter media used in the column experiments.

Filter media	Grain size d_{50} [mm]	Uniformity coefficient d_{60} / d_{10}	Silt fraction [%]	Clay fraction [%]	Organic matter [%]	N_{NO_3} [g kg ⁻¹ dm]	pH
CGS	0.70	3.0	0.00	0.00	0.20 ± 0.01	< 0.001	6.2
MGS	0.25	3.2	1.33	0.00	0.39 ± 0.02	0.001	5.9
FA ^a	0.10	5.0	25.61	1.21	1.31 ± 0.11	0.020	8.4

^a av. concentration of heavy metals in FA [mg kg⁻¹ dm]: Cr-89.0; Zn-120.2; Cd-2.4; Cu-80.6; Ni-3.7; Pb-39.0

The stabilized sewage sludge, collected from the local wastewater treatment plant 'Wschód' in Gdańsk, was a mixture of primary and secondary sludge which has been previously stored in piles for three months. The dosage of the sludge in each experiment was equal to 23 g (dm) of sewage sludge per column. This value was equivalent to 100 Mg of sewage sludge per hectare. To simulate conditions of land reclamation, sewage sludge was first mixed with coarse grained sand (CGS) in ratio 1:3 (m/m) and then placed as a layer (thickness about 5 cm) on the top of each column bed. In the paper, the sewage sludge/sand mixture is called the initial deposition (ID). The mass of the initial deposition applied to each column was equal to 81.05 ± 0.50 g (dm). Basic properties of the ID are shown in Table 2.

The column experiments were conducted under atmospheric conditions corresponding to extreme rainfall (100 mm per day). In the study, filter-sterilized, deionized water (pH from 6.5 to 6.6) was used as rainwater. Each column was equipped with a sprinkler that homogeneously applied water to the top surface. The volume of water applied to each column was equal to 0.230 dm^3 per day, supplied in a period of 12 h followed by 12 h drainage.

In the pilot-run series A, sorption abilities of the selected filter media (CGS, MGS, FA) were determined. Three experiments have been carried out for 30 days, each one in the separate column, packed with different filter medium: coarse grained sand was in the first column (A1), medium grained sand in the second (A2), and coal fly ash in the third (A3). The data obtained in pilot series A showed that the concentration of sludge-borne NO_3^- ions and bio-tracers reached the highest level during the first seven days of the experiments, regardless of the filter medium type. Thus, that period of time was established as sufficiently long for series B, C, and D. Each of the short series (B, C, D) was conducted in four columns simultaneously. The four columns operated under the same flow and filter medium conditions. CGS, MGS, and FA were used in series B, C, and D, respectively.

The column effluents were collected into sterile bottles during the experiments and the samples were taken once a day for the determination of *Clostridium perfringens* and fecal coliforms presence. Additionally, pH, and nitrate nitrogen content were measured. At the end of each filtration period, the columns were disassembled and samples of the initial deposition (ID_{end}) as well as filter media were taken from 6 levels as it is shown in Fig. 1.

The measurements were made according to standards methods [24-28]. Solid and liquid samples were homogenized prior to each analysis. The membrane filtration

method (MF) was used for determination of *Clostridium perfringens* and fecal coliforms. Nitrate ion concentration was analyzed using spectrometric method with sulfosalicylic acid. The values of pH were estimated electrometrically, in solid samples in the 1:5 (volume fraction) suspension of soil in water. The grain-size sieve analysis of the initial deposition (ID) and of the filter medium (CGS, MGS, FA) samples was also conducted.

Results and Discussion

The sludge-borne fecal contamination of the filter beds and the effluents was assessed using indicator microorganisms. The presence of bio-tracers in the column profiles and in the column effluents indicated transport abilities of examined media. The column experiments were conducted in the conditions of extreme rainfall and the bacterial transport was analyzed in sand (CGS, MGS) and fly ash (FA) media. On the basis of the granulometric analysis coarse and medium grained sand qualified as medium permeable, while fly ash as less permeable. The daily volume of the filter-sterilized deionized water applied to each column was equal to 0.230 dm^3 . The total volume of effluents was close to the total amount of the input water for each column (water recovery was over 95%).

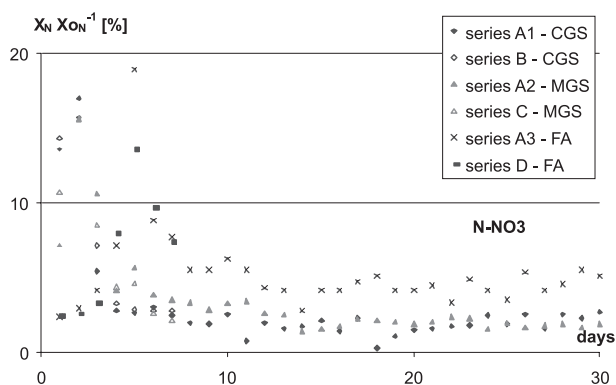
To simulate the practice of land reclamation, the sewage sludge was mixed with the sandy soil and applied to each column as the initial deposition (ID). The average load of nitrate nitrogen (X_{O_N}) introduced with the initial deposition to each column was equal to $6.4 \pm 0.1 \text{ mg N}_{\text{NO}_3}$ per column, whereas the initial number of indicator bacteria (X_0) per column varied from 1.9×10^6 to 10.5×10^6 CFU and from 1.3×10^5 to 5.0×10^5 CFU for *Clostridium perfringens* of fecal coliforms, respectively.

In the analyzed unsaturated flow conditions, the transport of sludge-borne *Clostridium perfringens* and fecal coliforms was compared with the transport of sludge-borne NO_3^- ions. Both the chemical-tracer and the bio-tracers were released and removed from the initial deposition by water percolating through that layer and transported downwards on the column beds to the effluents.

The dynamic of the NO_3^- ions leaching from the initial deposition was determined as a ratio of the nitrate nitrogen mass present in the total volume of daily effluents (X_N) to its initial mass (X_{O_N}) introduced to the column (Fig. 2). The relative breakthrough curve ($X_N X_{\text{O}_N}^{-1}$) started to increase from the first day and reached the highest level on the second day of the experiments for the coarse (series A1

Table 2. Characteristics of the initial deposition (ID).

Water content	Organic matter	N_{NO_3}	Fecal coliform	<i>Cl.perfringens</i>	pH
	%	g kg^{-1} (dm)	CFU g^{-1} (dm)		
20.4 ± 0.4	3.5 ± 0.1	0.08 ± 0.01	$3\ 180 \pm 500$	$78\ 500 \pm 12\ 263$	6.9 ± 0.1



(CGS), A2 - C (MGS), and A3 - D (FA) were equal to 0.97, 0.95, and 0.99 respectively.

Fig. 2. Relative ratio of NO_3^- mass present in the total volume of daily effluents (X_N) to its initial mass (X_{0N}) introduced to the column as a function of time.

and B) and the medium grained sand (series A2 and C), then decreased gradually with time. For the fly ash (series A3 and D), the maximum concentrations of NO_3^- ions in the column effluents were observed on the fifth day (Fig. 2). The longer retardation time, recorded for FA, was connected with the high moisture content in that filter bed. The capillary water content was about four times higher than the amount of water applied daily to the columns. Regardless of the filter material, however, the maximum concentration of nitrate nitrogen in the column effluents was comparable for each series and varied from 4.0 to 5.5 $\text{mg N}_{\text{NO}_3} \text{dm}^{-3}$. Sufficient repeatability and no significant difference in the transport conditions of the chemical-tracer between corresponding experiments of the short series B, C, D and the long series A (respectively A1, A2, A3) were confirmed by the consistence of the obtained data. The correlation coefficients calculated for the corresponding series: A1 – B (CGS), A2 – C (MGS), and A3 – D (FA) were equal to 0.97, 0.95, and 0.99, respectively.

The dynamic of the indicator bacteria leaching from the initial deposition, and their transport through the column beds to the effluents was also determined as a relative ratio of the number of bacterial cells present in the total volume of daily effluents (X_t) to the initial number of bacteria introduced to the column (X_0) as a function of time (Fig. 3). Bacterial transport ($X_t X_0^{-1}$) was calculated for all the series: for series A (A1, A2, A3) lasting 30 days, and for series B, C, and D lasting 7 days. Bacterial retention in the analyzed media was estimated only for 30-day series A (A1, A2, A3) and determined as a dimensionless ratio of $X_t X_0^{-1}$ in function of column's depth (Fig. 3). X_t was defined as a number of indicator bacteria present in the total mass of the filter bed at the analyzed bed's level (Fig. 1).

The concentrations of *Clostridium perfringens* and fecal coliform in the effluents were directly related to the type of filter bed. For CGS (series A1 and B) the peak of the bacteria breakthrough curves appeared on the second and third days. The maximal number of the indicator bac-

teria cells varied from 183 to 630 CFU for *Clostridium perfringens* and from 8 to 20 CFU for FC in 100 ml of the effluent. The indicator bacteria concentrations obtained in series A1 (CGS) were higher than the corresponding values in series B. Bacterial migration could be accelerated by larger pore spaces, which may occur in coarse soils [4, 11, 29].

In the medium grained sand effluents (series A2 and C), the indicator bacteria reached the highest level on the fourth day of the experiments with the maximum number of *Clostridium perfringens* ranging from 104 to 240 CFU in 100 ml. The corresponding concentrations of FC did not exceed 5 CFU in 100 ml of effluent.

In the case of fly ash (series A3 and D) the number of the colony forming indicator bacteria present in the column effluents was close or equal to zero.

The laboratory experiments indicated that *Clostridium perfringens* and fecal coliforms were leached from the initial deposition layer and transported throughout the filter beds to the effluents. In the sandy media, the retardation time of the indicator bacteria was similar to the retardation time of the chemical tracer (N_{NO_3}) only for CGS. For MGS such similarity was observed between the retardation time of the bacteria cells and of the less mobile nitrogen compounds as organic and ammonium nitrogen [30]. The bacterial movement comparable to the movement of chemical compounds was obtained in several column and field experiments [22, 31, 32]. In the current study, however, no correlation between the bacteria cells and the nitrate nitrogen movement in the FA beds was found. The indicator bacteria distribution in the fly ash was limited. Only a few cells in colony forming state were recovered from the effluents.

The obtained data suggested that the indicator bacteria were mainly leached from the initial deposition at the beginning of each experiment; however, factors controlling their effective extraction from such a complex substrate as sewage sludge (initial deposition) have not yet been fully recognized [12]. It should also be considered that in conditions of extensive rainfall, the area of air-water interfaces is reduced, thus even sludge bacteria attached to the solid particles can be mobilized in porous media [8, 16, 33].

At the end of series A (A1, A2, A3) the cumulative number of the bacterial cells present in the total volume of effluents ΣX_t was calculated (Table 3). The ratio of ΣX_t to the initial number of bacterial cells introduced to the columns in the initial deposition (X_0) was low, and varied from 0.008 to 0.012% for CGS, from 0.002 to 0.004% for MGS, whereas for FA, it was even less than 0.001%. It suggests that bacteria were retained in the initial deposition or in the column filter media, or were present in the column effluents but in a non-culturable state. The bacteria released from the sewage sludge by the percolating water were transported through the beds to the effluents, although the cell interactions with the filter media interfaces could limit those processes.

The bacterial retention ($X_t X_0^{-1}$) in analyzed media was estimated only for a 30-day series A (A1, A2, A3),

Table 3. Cumulative number of bacteria cells present in total volume of effluents calculated for series A.

Total number of bacteria	Kind of filter bed		
	CGS (A1)	MGS (A2)	FA (A3)
<i>Cl.perfringens</i>	< 3 000	< 800	< 100
Fecal coliform	< 140	< 20	< 10

(av. total volume of effluents obtained in series A was equal to 6.75 dm³)

and determined as a function of column depth (Fig. 3). The presence of the indicator bacteria in the filter beds was directly related to the experimental level and the number of bacteria decreased with depth. The bacterial cells were mostly kept in the initial deposition level (0.0 – 0.1m) where ratio $X_t X_o^{-1}$ varied from 40% to 43% for *Clostridium perfringens* and from 31% to 35% for fecal coliforms. However, the retention ($X_t X_o^{-1}$) of *Clostridium perfringens* in the ID_{end} layer was higher than that of fecal coliforms. It is difficult to say if it was related to a lower facility of *Clostridium perfringens* extraction from the sludge matrix by the simulated extreme rainfall or to the lower FC resistance to environmental stress than spore-forming *Clostridium perfringens* showed.

In the current study, the cells of the indicator bacteria, released from the initial deposition, were mainly retained in the upper part of the columns, within the first 0.10 m beneath the ID_{end} layer. In the sandy soils (series A1 and A2) fecal coliforms did not penetrate, in culturable state, to a depth greater than 0.25 m beneath the ID_{end} layer; in the fly ash (series A3) they were not present even on the first level (0.10 m beneath the ID_{end}). In the case of *Clostridium perfringens*, the bacterial cells were isolated from all the column levels, in a very low number – $X_t X_o^{-1} < 0.5\%$ (< 350 CFU g⁻¹ dm). Compared to the spore-forming *Clostridium perfringens*, vegetative fecal coliforms were more sensitive to the FA environment. It is probable

that the high value of fly ash's pH and the high concentration of heavy metals (see Table 1) limited survival of fecal coliform. Additionally, in the FA media, the presence of 25% of silt and 1% of clay fraction promote bio-tracers' retention. Stated in the experiments, limited bacterial transport and the retention of bacterial cells in the upper part of the soil corresponds to the data presented in literature [29, 34].

The obtained data indicate that features of filter media and bacteria properties were the main factors influencing bacteria distribution in the analyzed conditions. Similar conclusions have been formulated in the works of Smith et al. [4], Schafer et al. [35], Shelton et al. [12], Harvey et al. [36], Abu-Ashour et al. [37], and others.

Gannon et al. [5, 6] and Troxler et al. [38] found out that viable bacterial cells were relatively strongly attached to solid particles, whereas dead and injured cells might be extracted more easily. Shelton's et al. [12] studies of the releasing rate of manure-born coliform bacteria and their transport in stony soil reported a strong correlation between FC concentration in effluents and effluents' turbidity. The data obtained in the current experiments also suggests that bacteria cells were extracted from the initial deposition layer not only as free cells but also as cells attached to small organic particles of sewage sludge. The higher concentration of organic matter in the first 0.10 m of the column beds beneath the ID layer, compared to the background samples (data not shown, [39]), proves the rightness of that assumption. Thus, future research is required to determine how bacterial retention in soils is affected by the presence of sludge-born organic particles [12].

At the end of series A, a cumulative number of the indicator bacteria cells present in the total amount of the column effluents (ΣX_t) as well as in the column beds (ΣX_t) was calculated. The cell balance was then determined as a ratio of bacterial recovery ($X_{rec} = \Sigma X_t + \Sigma X_t$) to X_o . Since the cumulative bacterial number present in the column effluents (ΣX_t) was negligibly low compared to ΣX_r , the bacterial recovery ($X_{rec} X_o^{-1}$) was nearly equal to bacterial retention ($X_t X_o^{-1}$). The low recovery of bacterial cells

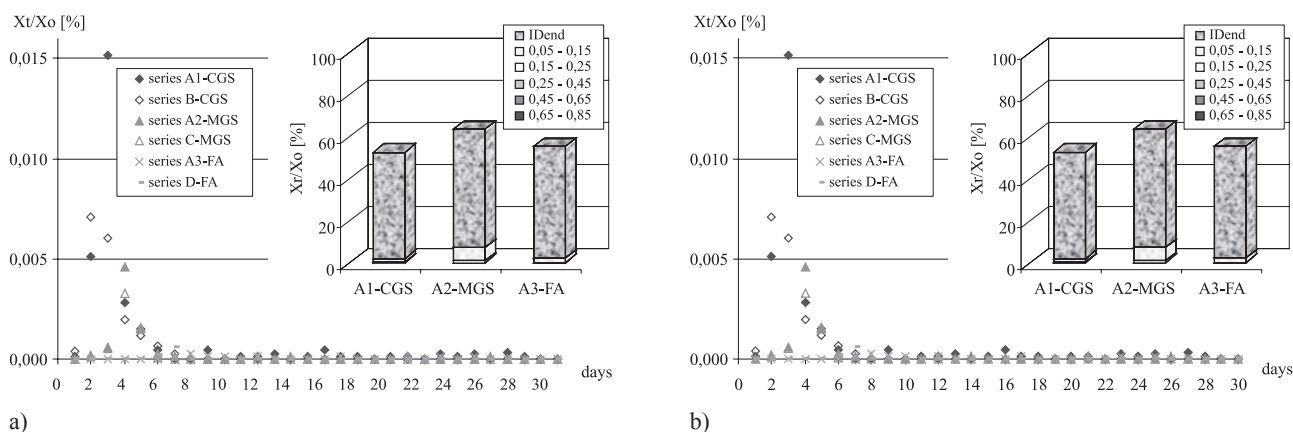


Fig. 3. The dynamic of bacterial transport ($X_t X_o^{-1}$) and bacterial retention ($X_r X_o^{-1}$) in column experiments: a) *Clostridium perfringens*, b) fecal coliforms.

($X_{rec} \cdot X_o^{-1}$), less than 65% for *Clostridium perfringens* and less than 50% for fecal coliforms, indicated that the examined bacteria died during the laboratory tests or were likely to persist mostly in a non-culturable state [40, 41]. Similar low recovery results were obtained by Gannon et al. [5, 6] and Troxler et al. [38].

Conclusions

The indicator microorganisms introduced to the columns with sewage sludge were kept mainly in the initial deposition layer (sewage sludge), whereas cells released from that layer were retained mainly by the first 0.1m of the column beds. The column effluents contained less than 0.02% of the initial number of bacteria. However, the presence of viable cells suggests that in the condition of extensive rainfall, a land application of sewage sludge, especially on sandy soils, can cause the contamination of shallow aquifers. The fly ash, which contains silt and clay fraction, creates a more effective filter, although the bacterial transport could in this case be additionally influenced by the limited survival of bacterial cells, which depends on ashes' pH and high concentration of heavy metals.

The low bacteria recovery (less than 65% of their initial number) may be related not only to the bacterial die-off but also to the presence of cells in a non-culturable state. Thus, the current study raises the questions of using a traditional, cultivating method to determine microorganisms' presence in soil and water samples.

The knowledge of microorganisms' release from sewage sludge seems to be essential for the analysis of the correlation between sewage sludge land application and sewage-borne bacteria transport in soils. The continuation of the presented work is needed to assess the role of non-culturable bacterial cells in soil and groundwater environment.

Appendix

CGS – coarse grained sand,
 dm – dry mass,
 FA – fly ash,
 FC – fecal coliforms,
 ID – mixture of sewage sludge and CGS prepared in ratio 1:3 (m/m),
 ID_{end} – properties of sewage sludge/CGS mixture at the end of each filtration,
 m – mass,
 MGS – medium grained sand,
 MF – membrane filtration,
 X_N – mass of chemical tracer (NO₃⁻) present in total volume of daily effluents,
 X_o – mass of chemical tracer (NO₃⁻) introduced to the column with initial deposition (ID);
 X_o – number of indicator bacteria present in total mass of freshly prepared initial deposition,

X_t – number of indicator bacteria present in the total volume of daily column effluents,
 X_r – number of indicator bacteria present in total mass of filter bed at analyzed column's level,
 ΣX_t – cumulative number of bacterial cells isolated from the total volume of column effluents,
 ΣX_r – cumulative number of bacteria cells present in column beds' subsamples,
 X_{rec} – bacterial recovery ($X_{rec} = \Sigma X_t + \Sigma X_r$)

Acknowledgements

This work was carried out as part of a research project supported by the Committee for Scientific Research 7 T09D 044 21.

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