

# Characterization of Natural Organic Colloids Derived from Sewage Sludge

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## Abstract

Fractionation of sewage sludge colloids has been achieved using the split-flow-thin (SPLITT) fractionation technique. It reveals that the majority of organic carbon as well as heavy metals is carried by particles smaller than 1  $\mu\text{m}$ . Optimization of volumetric flowrates and application of full-feed depletion mode of SPLITT fractionation allows concentrating the smallest colloids. Additionally, HPLC and  $^{13}\text{C}$  NMR analyses has been performed to characterize the organic colloids. The  $^{13}\text{C}$  NMR spectrum confirms that smaller fraction contained elevated contents of aliphatic, aromatic and carboxylic groups characteristic of humic compounds. HPLC analysis separates two fractions of substances and compares obtained chromatograms with separation of the humic standard.

**Keywords:** heavy metal speciation, sewage sludge, SPLITT fractionation

## Introduction

The number of wastewater treatment plants (WWTP) is increasing constantly due to restrictions in water policies. Nowadays, wastewater treatment plants are recognized as point sources of heavy metal pollution into river systems. One of the major problems of the WWTP is sewage sludge utilization. This type of valuable waste can often contain various contaminations that exclude them as a compost material. The presence of heavy metals in compost causes a significant environmental hazard, and is one of most difficult contamination problems to solve.

Several research studies investigating the quality of sewage sludge and composts show significant differences of metals content [1-4]. Their concentration depends on waste type, seasonality and other meteorological conditions. On the other hand, the toxicity of metal is related to its dominant form in the environment. Operational speciation is applied to characterize the toxicity [5-10].

Commonly used selective sequential extraction (SSE) based on the Tessier method or the BCR procedure gives the information on chemical speciation of heavy metals and the toxicity of sewage sludge. However, physical speciation is also important from the point of view of sewage purification.

The aim of the present study was characterization of physical speciation of selected heavy metals in sewage sludge. The split-flow-thin fractionation (SPLITT) system has been applied to separate different fractions of colloids derived from deactivated sewage sludge.

SPLITT was developed by Giddings in the early 1980's [11] and has become a member of the field-flow fractionation (FFF) family. Separation in SPLITT is based on the same principles as other FFF methods [12], but it has some unique properties. The fractionation channel consists of two inlets and two outlets (Fig. 1). Separation is based on a combined action of the non-uniform flow velocity profile of a liquid through the channel and transverse gravitational field applied perpendicularly to this carrier.

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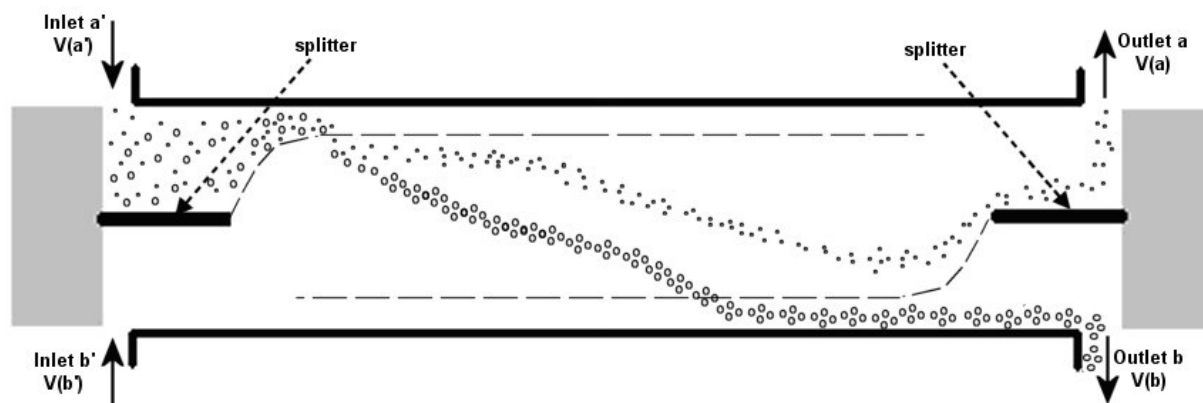


Fig. 1. Cross-section of SPLITT channel and illustration of the separation process.

SPLITT has an ability to separate relatively large quantities of sample (milligrams or even grams) in a reasonable amount of time. Fractionation can be performed in full-feed depletion mode (FFDSF) [13]. In this mode only one inlet with sample flow is used. Such an operational mode avoids the dilution of the sample, but the mechanism of separation remains the same as in conventional mode with two inlets.

The strategy to remove particles equal or bigger than a specific diameter ( $d_c$ ) is usually applied. The cut-off diameter is related to the velocity of transport region by a simple equation:

$$V(t) = \frac{bL G d_c^2 |\Delta\rho|}{18\eta_o} \quad (1)$$

...where:

$V(t)$  is the velocity of transport region,  
 $b$  and  $L$  are dimensions of the SPLITT channel,  
 $G$  is the gravitational constant,  
 $\Delta\rho$  is the difference in density between the particle and the carrier,  
 $\eta$  is carrier viscosity.

$V(t)$  is equal to the difference between velocity in upper inlet  $V(a')$  and upper outlet  $V(a)$ . In theory, there are infinite numbers of pairs of  $V(a)$  and  $V(a')$  for given  $V(t)$ . In practice however, the appropriate ratio of  $V(a')$  to  $V(a)$  has to be optimized to obtain the appropriate resolution and throughput of the sample.

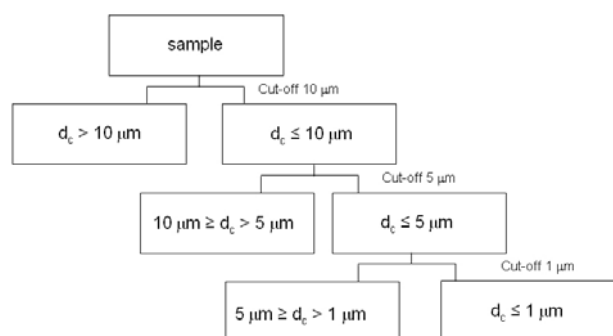


Fig. 2. Fractionation scheme.

Table 1. Optimized values of flow rates for desired cut-off.

cut-off [ $\mu\text{m}$ ]	$V(t)$ [mL/min]	$V(a')$ [mL/min]
10	6.17	8.72
5	3.66	5.89
1	0.15	2.83

## Experimental Procedures

Samples of sewage sludge were taken from the Municipal Treatment Plant in Toruń in the period of autumn 2008 to the spring 2009. The samples were shaken with deionized water (30 g of sample with 200 mL of water) by 30 minutes. After this, supernatant was taken to obtain fractions of particles below 10  $\mu\text{m}$ . The sedimentation process has been repeated three times to purify the fraction from bigger particles. The sedimentation time was calculated according to a simplified Mason-Weaver equation. The obtained fraction has been fractionated by the SPLITT system (SF-1000 STD, PostNova Analytics, USA) in FFDSF mode. (The fractionation scheme is given in Fig. 2.) Flowrates for particular cut-off values ( $d_c$ ) have been optimized before separation using silica particles with strictly defined sizes. The optimal values of flows are presented in Table 1. Three fractions were separated from sewage sludge.

The application of small flowrate on outlet  $V(a)$  for fractionation of smaller particles gives an additional goal – these particles were concentrated from bulk solution.

Purity of fractions have been examined by microscopic observation of samples with fluorescence stereomicroscope equipped with a CCD camera (SZX16, Olympus, Japan). Additionally, the smallest fractions have been analyzed by a laser light scattering detector (Zetasizer Nano, Malvern, UK) due to the fact that it was very uncertain to measure the sizes of particles equal to or smaller than 1  $\mu\text{m}$  by optic microscope.

The collected fractions were characterized by various analytical methods. Total organic content (TOC) was measured by a TOC analyzer (TOC 5000, Shimadzu, Japan).

The content of selected heavy metals was analyzed by flame-AAS (AAAnalyst 800, Perkin Elmer, USA). 50 mL of each fraction was filtered through a 0.2  $\mu\text{m}$  syringe membrane. The filter was then demounted and a nylon membrane with colloids was digested in aqua regia, dried and dissolved in 1% (v/v)  $\text{HNO}_3$  to obtain clear solution. Additionally,  $^{13}\text{C}$  NMR spectroscopy and HPLC separation have been used on the smallest ( $<1 \mu\text{m}$ ) fractions. The sample for NMR was dried at  $60^\circ\text{C}$  and spectra were registered at 75.5 MHz. Filtered (0.22  $\mu\text{m}$ ) solution was analyzed by HPLC (Shimadzu LC-10ATVP, Kyoto Japan) with gradient elution ( $\text{H}_2\text{O}/\text{ACN}$ ) to observe changes in different sampling periods and compared with the technical standard of humic acids (Sigma – Aldrich). 20  $\mu\text{L}$  of sample was injected into the HPLC system working at a flowrate of 0.7 mL/min. The conditions were adopted from literature [14-16] and optimized during the investigations.

## Results and Discussion

All fractions separated by SPLITT technique were characterized by a TOC analyzer. The smallest fraction derived from sewage sludge was richest in organic carbon. Colloids smaller than 1  $\mu\text{m}$  contain almost twenty times more organic carbon than other fractions (Table 2) because of the presence of humic and other organic substances. Concentration of different carbon forms in the rest of fractions were similar.

The characteristics of sewage sludge in terms of organic content was investigated in different sampling periods.

Table 2. TOC content in specific fractions.

Fractions	TC [mg/dm <sup>3</sup> ]	IC [mg/dm <sup>3</sup> ]	TOC [mg/dm <sup>3</sup> ]
$d_c > 10 \mu\text{m}$	106.00	81.07	24.93
$10 \mu\text{m} > d_c > 5 \mu\text{m}$	101.4	74.4	27
$5 \mu\text{m} > d_c > 1 \mu\text{m}$	103.8	78.7	25.1
$d_c < 1 \mu\text{m}$	472.9	63.57	409.33

Table 3. TOC content in smallest fractions for samples taken in different periods.

Date	TC [mg/dm <sup>3</sup> ]	IC [mg/dm <sup>3</sup> ]	TOC [mg/dm <sup>3</sup> ]
18 XI 2008	472.9	63.57	409.33
10 XII 2008	741.7	62.42	679.28
3 II 2009	268.8	78.96	189.84

Table 4. Physical speciation of heavy metals in sewage sludge.

Fractions	Ni [mg/dm <sup>3</sup> ]	Cr [mg/dm <sup>3</sup> ]	Cd [mg/dm <sup>3</sup> ]	Pb [mg/dm <sup>3</sup> ]
$10 \mu\text{m} > d_c > 5 \mu\text{m}$	0.4	0.13	0.04	0.07
$5 \mu\text{m} > d_c > 1 \mu\text{m}$	0.5	0.07	0.06	0.05
$d_c < 1 \mu\text{m}$	1.4	0.98	0.67	0.26

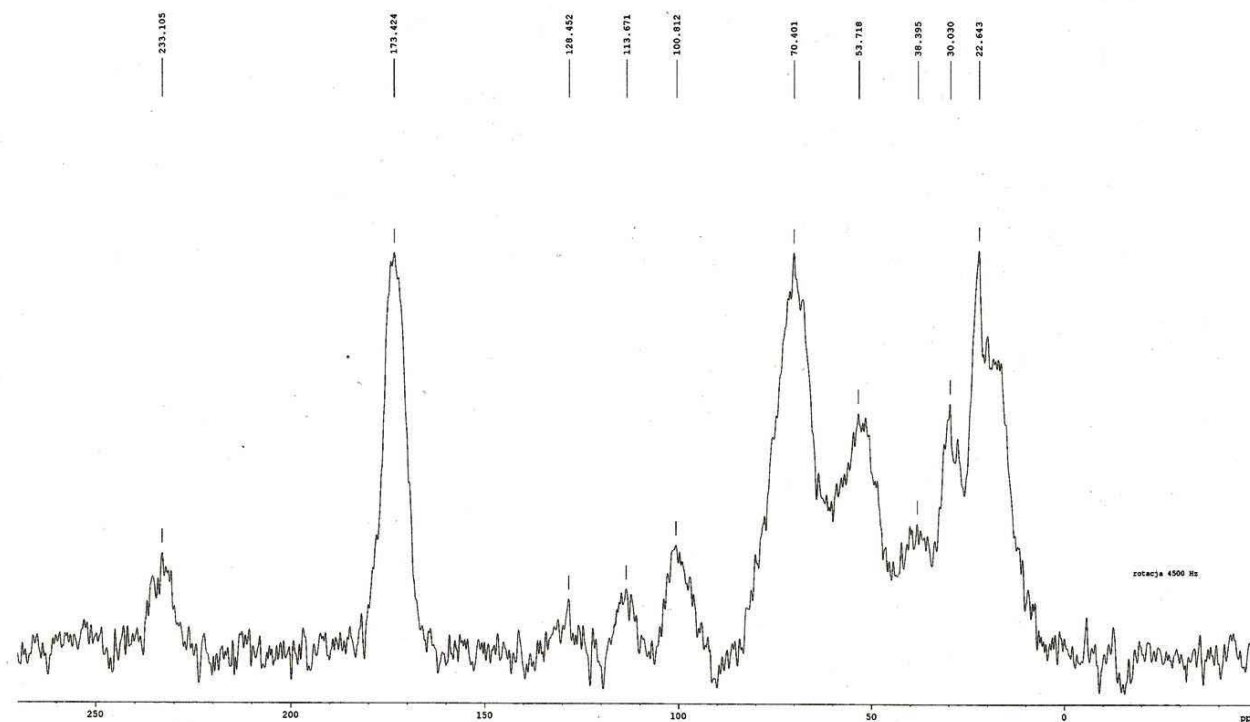


Fig. 3.  $^{13}\text{C}$  NMR spectrum of sewage sludge fraction:  $d_c < 1 \mu\text{m}$ .

TOC content was varied in samples taken in winter season between November and February (Table 3). Variability of TOC was rather high and shows the influence of weather conditions on treatment efficiency and sewage sludge composition. In contrast, the content of inorganic carbon remains almost at the same level in all samples.

Discussion on seasonality of organic content in colloidal fractions need more detailed investigations. Samples should cover all seasons and have to be taken in similar operational conditions of WWTP. Present results show that the content of TOC is obviously related to efficiency of the purification processes in WWTP.

AAS analysis revealed that more than 60% of the total amount of nickel and lead and almost 90% of chromium and cadmium has been carried by the smallest fraction of sewage (Table 4). The relation between concentrations of heavy metals and TOC shows that the majority of heavy metals is bonded with organic matter. In this sense, one can extrapolate present results of physical speciation to qualitative analysis of chemical speciation. Chromium and cadmium occur in sewage sludge as complexes with organic matter, while nickel and lead also exist in other chemical forms.

The results from Table 4 indicate the potential way to remove heavy metals from sewage sludge by physical separation of colloids. This method can be much easier and cheaper than others applied to sewage sludge based on bacteria activity [17, 18], other organisms [19] or natural sorbents [10, 20, 21].

$^{13}\text{C}$  NMR spectrum confirm that fractions with colloids smaller than  $1\ \mu\text{m}$  contain elevated amounts of aliphatic, aromatic and carboxylic groups characteristic of humic compounds (Fig. 3).

Further investigations on soluble organic matter in sewage sludge were done by HPLC on hydrophobic C18 column (Lichrosorb RP18, Merck, Germany) and revealed that samples contain two fractions: hydrophilic and hydrophobic. The mobile phase was pure water in the first minutes of analysis. The peak obtained between 1 and 3 minutes of analysis (Fig. 4) therefore indicates the appearance of polar and (mostly preferred) macromolecular substances. The interaction of such molecules with solid phase is rather weak. The character of interactions in column was changed with an increase of organic solvent. After 7 min-

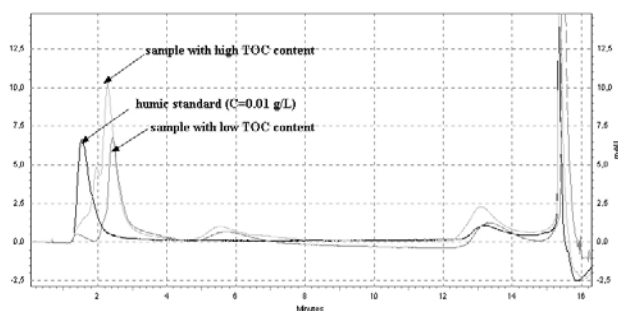


Fig. 4. Chromatograms of fractions with  $d_c < 1\ \mu\text{m}$  and humic standard.

utes the volumic ratio between water and acetonitrile was 1:1, and the proportion is decreasing to more hydrophilic conditions. The second peak therefore contains the non-polar substances. Additionally, one can find a simple correlation between TOC content (Table 3) and peak height on chromatograms. The composition of fractions was found to be very labile even after filtration through a  $0.22\ \mu\text{m}$  filter. Samples were stored in a refrigerator in dark bottles, but periodical HPLC analysis shows significant changes in the shape and positions of peaks.

The comparison between investigated materials and technical standards of humic substances diluted to  $0.01\ \text{g/L}$  show the shift between retention time of the first peak. It can indicate the appearance of different organic substances apart from humic matter in sewage sludge or other inorganic substances. The rest of peaks appears at the same retention time. The differences in composition of substances in sewage sludge and humic standard were obtained for polar fractions.

## Conclusions and Future Outlook

Fractionation of non-uniform colloids from sewage sludge was performed successfully using SPLITT. Application of full-feed depletion mode in SPLITT fractionation avoided the dilution of samples. Moreover, optimization of flowrates allows concentrating the fraction of particles with diameter below  $1\ \mu\text{m}$  to small volume. Analysis of fractions revealed that the majority of heavy metals and organic matter is carried by colloids smaller than  $1\ \mu\text{m}$ . The conclusion is similar to results obtained in previous studies of chemical speciation [22-23], but additionally indicates the potential ways to reduce heavy metals in sewage sludge materials. It can be done by isolating the smallest colloidal fractions by simple sedimentation and producing sewage compost of better quality.

Future investigation will focus on the composition of organics in sewage sludge samples and the characterization of heavy metals complexes with organic matter. Other packing materials for HPLC columns will be used. There is also a need for more sophisticated detection of particle size than the use of an optical microscope. Application of laser light scattering detectors should give quantitative results of particle size distributions of collected fractions. It also provides a fast and reliable way to optimize the fractionation in SPLITT technique.

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## References

1. WOELKI G., FRIEDRICH S., HANSCHMANN G., SALZER R. HPLC fractionation and structural dynamics of humic acids. *Fresenius' J. Anal. Chem.* **5**, 548, 1997.

2. SCANCAR J., MILACIC R., STRAZAR M., BURICA O. Total metal concentrations and partitioning of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge. *Sci. Total Environ.* **250**, (1-3), 9, **2000**.
3. KANDPAL G., RAM B., SRIVASTAVA P. C., SINGH S. K. Effect of metal spiking on different chemical pools and chemically extractable fractions of heavy metals in sewage sludge. *J. Hazard. Mater.* **106**, (2-3), 133, **2004**.
4. HANAY Ö., HASAR H., KOCER N., ASLAN S. Evaluation for Agricultural Usage with Speciation of Heavy Metals in a Municipal Sewage Sludge. *Bull. Environ. Contam. Toxicol.* **1**, 42, **2008**.
5. WALTER I., CUEVAS G. Chemical fractionation of heavy metals in a soil amended with repeated sewage sludge application. *Sci. Total Environ.* **226**, (2-3), 113, **1999**.
6. LASHEEN M. R., AMMAR N. S. Assessment of metals speciation in sewage sludge and stabilized sludge from different Wastewater Treatment Plants, Greater Cairo, Egypt. *J. Hazard. Mater.* **164**, (2-3), 740, **2009**.
7. PÉREZ-CID B., LAVILLA I., BENDICHO C. Comparison between conventional and ultrasound accelerated Tessier sequential extraction schemes for metal fractionation in sewage sludge. *Fresenius J. Anal. Chem.* **363**, 667, **1999**.
8. SHRIVASTAVA S. K., BANERJEE D. K. Speciation of metals in sewage sludge and sludge-amended soils. *Water Air Soil Poll.* **1-4**, 219, **2004**.
9. WONG J. W. C., SELVAM A. Speciation of heavy metals during co-composting of sewage sludge with lime. *Chemosphere* **6**, 980, **2006**.
10. SPRYNSKY M., KOSOBUECKI P., KOWALKOWSKI T., BUSZEWSKI B. Influence of clinoptilolite rock on chemical speciation of selected heavy metals in sewage sludge. *J. Hazard. Mater.* **2**, 310, **2007**.
11. GIDDINGS J. C. A system based on split-flow lateral-transport thin (SPLITT). Separation cells for rapid and continuous particle fractionation. *Sep. Sci. Technol.* **20**, 749, **1985**.
12. KOWALKOWSKI T., CANTADO C., DONDI F., BUSZEWSKI B. Field-flow fractionation: theory, techniques, applications and the challenges, *Crit. Rev. Anal. Chem.* **36**, 1, **2006**.
13. CONTADO C., DONDI F., BECKETT R., GIDDINGS J. C. Separation of particulate environmental samples by SPLITT fractionation using different operating modes. *Anal. Chim. Acta.* **345**, (1-3), 99, **1997**.
14. DEBSKA B., DRAG M., BANACH-SZOTT M. Molecular size distribution and hydrophilic and hydrophobic properties of humic acids isolated from forest soil. *Soil Water Res.* **2**, 45, **2007**.
15. DEBSKA B., GONET I. Share of hydrophilic and hydrophobic fractions in humic acids formed as a result of post-harvest residue decomposition. *Pol. J. Soil Sci.* **1**, 57, **2007**.
16. PREUßE G., FRIEDRICH S., SALZER R. Retention behavior of humic substances in reversed phase HPLC. *Fresenius' J. Anal. Chem.* **2/3**, 268, **2000**.
17. LOMBARDI A. T., GARCIA O., MENEZES W. A. N. The Effects of Bacterial Leaching on Metal Partitioning in Sewage Sludge. *World J. Microbiol. Biotechnol.* **22**, (10), 1013, **2006**.
18. XIANG L., CHAN L. C., WONG J. W. C. Removal of heavy metals from anaerobically digested sewage sludge by isolated indigenous iron-oxidizing bacteria. *Chemosphere* **41**, (1-2), 283, **2000**.
19. SHANABLEH A., GINIGE P. Acidic bioleaching of heavy metals from sewage sludge. *J. Mater. Cycles Waste Manag.* **2**, (1), 43, **2000**.
20. KOSOBUECKI P., KRUK M., BUSZEWSKI B. Immobilization of selected heavy metals in sewage sludge by natural zeolites, *Biores. Technol.* **99**, 5972, **2008**.
21. ZORPAS A. A., VASSILIS I., LOIZIDOU M., GRIGOROPOULOU H. Particle size effects on uptake of heavy metals from sewage sludge compost using natural zeolite clinoptilolite. *J. Coll. Inter. Sc.* **250**, (1), 1, **2002**.
22. KOWALKOWSKI T., BUSZEWSKI B. Sorption and Migration of Selected Heavy Metals in Different Soil Matrices, *Pol. J. Environ. Stud.* **11**, (2), 135, **2002**.
23. KOWALKOWSKI T., BUSZEWSKI B. Soil reclamation by municipal sewage sludge compost: heavy metals transport study. *J. Environ. Sci. Health A.* **44**, 522, **2009**.