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## EFFECT OF MUNICIPAL SEWAGE SLUDGE UNDER *SALIX* PLANTATIONS ON DISSOLVED SOIL ORGANIC CARBON POOLS

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**Keywords:** Carbon management index, hot water-extractable carbon, organic amendments, potentially oxidizable carbon.

**Abstract:** Labile fractions of organic matter can rapidly respond to changes in soil and they have been suggested as sensitive indicators of soil organic matter. Two labile fractions of organic carbon in the soils amended with fresh municipal sewage sludge in two rates (equivalent of 60 kg P ha<sup>-1</sup> and 120 kg P ha<sup>-1</sup>) were studied. Soils under studies were overgrown with *Salix* in Germany, Estonia and Poland. In Polish soils application of sewage sludge increased the content of both labile organic carbon fractions (KMnO<sub>4</sub>-C and HWC) for a period of one year. Estonian soils were stable and no distinct changes in labile organic carbon fractions occurred.

### INTRODUCTION

Huge amounts of sewage sludge are produced in every country as a result of wastewater treatment. In Poland the mass of sewage sludge is increasing [4]. Disposal of sewage sludges is an important issue that should be solved without degrading the environment [18]. One of the ways of sewage sludge management is its application on agricultural land under energy crops. However, not enough is known about the effects of such management.

Sewage sludges contain considerable amounts of fresh, uncomplexed organic matter [2] which may be quickly mineralized into CO<sub>2</sub> and other inorganic compounds, or transformed into humus. Labile fractions of organic matter such as dissolved organic carbon and easily oxidizable carbon compounds can rapidly respond to changes in soil and they have been suggested as sensitive indicators of soil organic matter changes [15] and important indicators of soil quality [22].

A method based on the oxidation of organic carbon (OC) by potassium permanganate (KMnO<sub>4</sub>-C) provides qualitative description of soil carbon pool [1]. This fraction of organic carbon is potentially degradable during microbial growth as it quickly oxidizes.

Also, hot water-extractable carbon (HWC) is a measure of labile OC [19]. It strongly correlates with microbial biomass, therefore it is thought to be labile in nature [6]. These two pools of dissolved organic carbon have still not received enough attention in the field of soil science and amendments studies. It is the main energy source for soil microorganisms and a source of N, P, S, and it influences the availability of ions in soil [9, 16].

Labile OC measurements should be used more widely in order to examine soil properties [3, 17], as this part of organic carbon can be potentially easily oxidized to CO<sub>2</sub> and contributes to the diminishing of soil carbon sequestration. Not much is known on the impact of fresh sewage sludge application on labile organic fractions. Does it contribute to an increase of labile organic carbon fraction or non-labile humus fraction? Also, little is known about optical properties of HWC, as such studies are conducted mainly for alkali extracts of humic acids.

The aim of the study is to evaluate and compare two labile fractions of organic carbon in the soil amended with municipal sewage sludge.

## MATERIALS AND METHODS

### *Description of experimental sites*

Soils under studies were overgrown with short-rotation plantations of *Salix*, amended with sewage sludge in Germany, Estonia and Poland. In Germany and in Poland before planting willow plantations cereals were grown for five years and in Estonia the field was a fallow land. In Estonia organic matter of plant material was not incorporated into soils and plants were treated with Roundup preparation. In Poland and Germany regular agricultural activities were performed before willow planting with regular ploughing and harrowing. In Germany the field was located in Schönberg-Holstein in the North of Germany, 3 km from the Baltic Sea and 20 km from the city of Kiel (geographical coordinates: 54°23'50.55" N, 10°23'05.89" E). Precipitation rate during establishing of plantation was 708 mm and average precipitation was 825 mm with annual average temperature 10.8°C. In Estonia the experimental field was situated in Tatra region, Vara commune, with coordinates: 58°30'18" N, 26°55'29" E. Annual precipitation in this region was 620 mm, and at the year of planting 680 mm. An average temperature was 5.2°C. In Poland the field was located in Lisiecice, in Opole Region, Pawłowice commune, with coordinates: 50°15'38" N, 17°52'36" E. Annual precipitation was 725 mm (with average in the region 650 mm), and annual temperature was 8.2°C.

Experimental field was a randomized block design, and each sludge treatment was repeated five times. Each block consisted of three sludge treatments for one willow clone.

### *Soil classification*

According to World Reference Base for Soil Resources [12] Polish and German soils studied were classified as *Cambisols*. They contain less than 2.5% of OC in topsoil and do not have *mollic* horizon. Their subsurface horizon is *cambic* and their texture is loamy. Estonian soils were classified as *Phaeozems*. They have topsoil rich in organic matter which contains > 2.5% of OC. Organic matter content, colour and pH enable to classify it as *mollic* diagnostic horizon. Their texture is sandy loam. General characteristics of soils of experimental fields are presented in Table 1.

Table 1. General properties of the tested soils

Depth [cm]	LOI	OC	TN	C:N	pH		Texture <sup>a)</sup>
					KCl	H <sub>2</sub> O	
ESTONIA							
0-30	68.5	25.87	2.30	11.25	6.03	6.56	sandy loam
30-80	24.6	8.21	0.80	10.26	7.50	7.41	silty sandy loam
80-150	24.5	0.84	0.11	7.64	7.75	7.65	sandy loam
GERMANY							
0-30	41.4	11.50	1.53	7.52	5.06	6.44	sandy loam
30-80	14.7	4.80	0.73	6.58	6.02	6.17	sandy clay loam
80-150	8.8	2.60	0.28	9.29	6.54	6.50	sandy clay loam
POLAND							
0-30	36.1	12.88	1.14	11.30	4.44	5.40	silt loam
30-80	13.6	5.47	0.62	8.82	5.29	6.14	sandy loam
80-120	10.1	3.79	0.24	15.79	5.71	6.48	sandy loam
120-150	5.4	2.20	0.23	9.57	5.45	6.30	sandy loam

<sup>a)</sup> Texture according to USDA texture classes (USDA – United States Department of Agriculture)

LOI – Loss-on-ignition, TN – total nitrogen

### ***Parameters of sewage sludge***

Estimation of the impact of application of sewage sludge requires consideration of its properties. Sewage sludge by its nature is always a heterogenous mixture of different fractions, such as organic matter originating from wastewater and inorganic precipitates of coagulants.

Chemical composition of sewage sludge is typical for this type of organic amendments. The sewage sludge applied on studied soils contained considerable amounts of organic matter and nutrients (Table 2). Sewage sludge was stabilized with lime which is a normal practice at sewage treatment plants.

Sewage sludge was applied once in two rates calculated on the basis of phosphorus content. Sewage sludge was incorporated and thoroughly mixed by disc harrows two weeks before willow planting. First rate (sewage sludge single rate – SSs) contained 60 kg P per hectare and the second (sewage sludge double rate – SSd) contained 120 kg P per hectare. The amount of organic matter introduced with sewage sludge with the single rate and double rate, respectively were as follows: in Estonia 1.59 and 3.18 t per hectare, in Germany 0.99 and 1.98 t per hectare, in Poland 1.97 and 3.94 t per hectare.

### ***Soil properties***

In spring 2006 and 2007 soil samples were taken from a depth of 0–20 cm, air dried and visible plant remnants were removed by hand picking. At first, the soil samples were ground to pass through a 2.00 mm sieve, and then, for chemical analysis, through 0.25 mm sieve. Loss-on-ignition (LOI) was determined after dry ashing of soil samples

Table 2. General properties of the sewage sludge applied

Parameter	ESTONIA	GERMANY	POLAND
Dry matter [%]	29.79	23.30	43.96
Organic matter [% DM]	52.94	46.58	43.67
pH	7.88	n.d. <sup>a)</sup>	11.05
Total content of macronutrients [g kg <sup>-1</sup> DM]			
N	22.7	23.1	27.5
P	20.0	28.2	13.3
K	3.0	n.d.	n.d.
Ca	55.0	n.d.	78.8
Mg	7.2	n.d.	8.8
Total content of heavy metals [mg kg <sup>-1</sup> DM]			
Cd	2.5	0.9	3.5
Cr	190	24	30
Cu	190	699	130
Hg	0.71·10 <sup>-6</sup>	0.27·10 <sup>-6</sup>	0.85·10 <sup>-6</sup>
Ni	53	15	17
Pb	51	16	65
Zn	720	478	1140

<sup>a)</sup> n.d. – not determined

for 6 hours at a temperature of 550°C. Organic carbon content was measured with a spectrophotometer after oxidation with potassium dichromate [11]. Total nitrogen was determined by Kjeldahl method and soil reaction was determined potentiometrically in water and potassium chloride [21].

### **Carbon fractions**

Potentially oxidizable carbon content was estimated by the method described by Blair *et al.* [1]. Soil samples containing 20 mg of OC and 50 ml of 0.0333 mol L<sup>-1</sup> potassium permanganate (KMnO<sub>4</sub>) were used and shaken for 30 minutes on BIOSAN PSU 20 multi-shaker. Then the solutions were filtered through glass filters and a change in KMnO<sub>4</sub> concentration was estimated using 0.05 mol L<sup>-1</sup> oxalic acid (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>). On the basis of the change in KMnO<sub>4</sub> concentration the amount of OC that oxidized was calculated. The amount of C that oxidized is assumed to be the labile carbon (KMnO<sub>4</sub>-C) and the difference between the OC and KMnO<sub>4</sub>-C is termed the non-labile carbon (non-KMnO<sub>4</sub>-C). The Carbon Pool Index (CPI) was calculated based on changes in organic carbon (OC). The Lability Index (LI) was determined based on changes in the proportion of KMnO<sub>4</sub>-C in the samples taken from the plots that were not amended with sewage sludge – control (reference site) and in the samples representing the plots amended with SS. The LI and CPI indices were used to calculate the Carbon Management Index (CMI).

The hot water-extractable C (HWC) was determined on air-dried soil samples (duplicates) according to the method described by Sparling *et al.* [19]. Briefly, 4 g air-dried soil was incubated with 20 ml demineralized water in a capped test-tube at the temperature of 70°C for 18 h. The tubes were shaken by hand at the end of the incubation and then filtered through Whatman ME 25/21 ST 0.45 µm membrane filters (mixed cellulose ester). Soluble C (HWC) was measured on Shimadzu 5000 TOC analyzer.

For HWC extracts optical properties were examined in 10 mm quartz trays using two-channel scanning spectrophotometer Shimadzu UV-1601PC at the range of 254 nm ( $A_{254}$ ), 465 nm ( $A_{465}$ ) and 665 nm ( $A_{665}$ ).

All analyses were performed in duplicate. All results are expressed on an oven-dry soil weight basis (temperature of drying 105°C). Statistical analyses were conducted with Statistica 7.0. Pearson linear correlations were used to assess the relationship between various organic carbon fractions at  $P \leq 0.01$ , for  $n = 30$ .

## RESULTS

The content of organic carbon in the studied soil samples increased after application of sewage sludge in the first year in Germany and Poland. In Estonia, the amount of OC in control and amended plots was similar (Fig. 1). Nevertheless, it should be noted that organic carbon decreased by 20–30% in the second year after application of sewage sludge and the decrease affected also control soil samples.



Fig. 1. Labile and non-labile organic carbon fractions in the soils studied

In Polish soil samples the application of sewage sludge considerably increased the content of both labile organic carbon fractions ( $\text{KMnO}_4\text{-C}$  and HWC) as well as non-labile organic carbon (Fig. 1). However, in the second year (2007) the hot water-extractable carbon and easily oxidizable carbon amounted to the contents reported for control plots. In Estonian soil samples, the amounts of  $\text{KMnO}_4\text{-C}$  in the amended soil samples and control ones were similar in both 2006 and 2007. However, non-labile organic carbon fraction decreased simultaneously with an increase of hot water-extractable carbon (Fig. 1). The HWC in Estonian soil samples, contrary to Polish soils, did not decrease considerably. In German soil samples the changes in easily oxidizable organic carbon varied. In the first year after application of sewage sludge, only plots amended with a single rate had higher contents of oxidizable carbon. In the second year, similarly to Estonian and Polish soils, the quantity of  $\text{KMnO}_4\text{-C}$  decreased. The amount of hot water-extractable carbon increased in 2006 and was considerably lower in 2007.

In Polish and German soil samples significant positive correlations between HWC and OC ( $r = 0.929$  in Polish soils and  $r = 0.649$  in German soil samples), as well as HWC and  $\text{KMnO}_4\text{-C}$  ( $r = 0.901$  in Polish soils and  $r = 0.540$  in German soil samples) were stated (Fig. 2A, 2B). For Estonian soil samples the correlation was significant only in the case of HWC and  $\text{KMnO}_4\text{-C}$ .

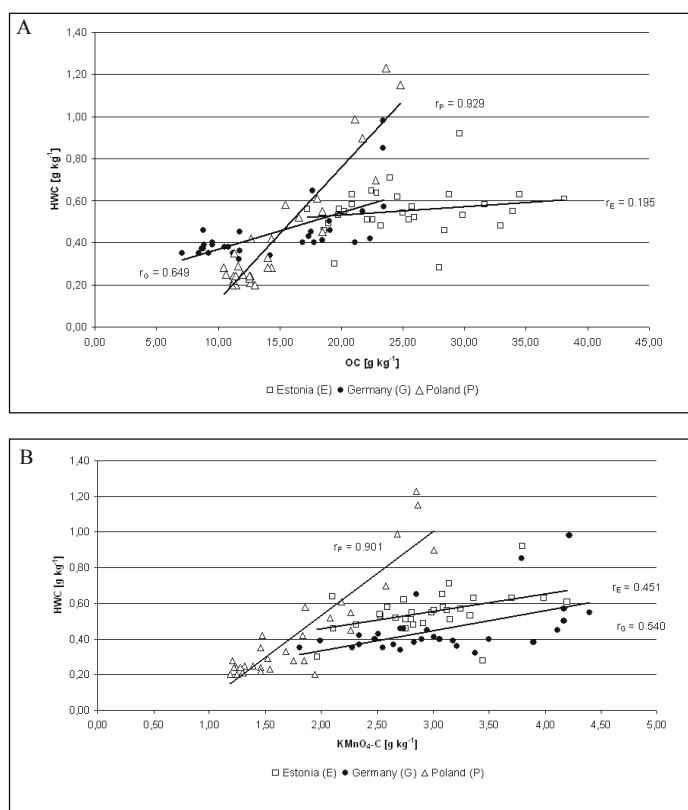


Fig. 2. Dependence of hot water-extractable carbon on total organic carbon (A) and easily oxidizable carbon (B)

Spectral analysis revealed that the values of absorbance  $A_{254}$  measured in HWC extracts in Polish soil samples in 2006 were the lowest. In control soil samples these values were half lower than in the soils amended with sewage sludge. However, in 2007 the values of  $A_{254}$  in Polish soils were similar in each sampled soil. In Estonian soils, the values of  $A_{254}$  were considerably higher and similar in 2006 and 2007. In German soil samples, similarly to Estonian, the soils amended with sewage sludge seem to be more parallel to the control plots and have similar values of  $A_{254}$  at different HWC content (Table 3).

The ratio of the absorbance at 465 nm and 665 nm was the lowest in Estonian and Polish soil samples in 2006. In 2007 in Polish soils it remained the same. However, in Estonian soils it was considerably higher (Table 3). Contrary to Estonian soil samples, in German soils, E4/6 ratio was the highest in 2006 and the lowest in 2007 (Table 3).

The lability of organic carbon (L) and lability index (LI) in 2006 was higher in control plots than in the soils amended with sewage sludge (Table 4). However, in 2007 the values of L and LI were higher in the amended soils as compared to reference. The carbon pool index (CPI) of Estonian soil samples was lower in amended soil than in the reference. In Polish soils the dependence was inverse. The highest values of CPI were noted in amended plots in 2006 as well as in 2007. In German soil samples the CPI value increased in the first year after sewage sludge application but in the second year it was lower than in reference samples. Nevertheless, each application of sewage sludge increased the carbon management index (CMI) as compared to the reference soil. In German and Estonian soils the increase was higher in 2007, whereas in Polish soils a considerable increase of CMI value was noted in 2006. In 2007 it was still high, but lower than in the first year after application of sewage sludge.

Table 3. Optical properties of HWC solution

Treatment	HWC	$A_{254}$	$E_{4/6}$	HWC	$A_{254}$	$E_{4/6}$
	2006			2007		
ESTONIA						
	mg L <sup>-1</sup>			mg L <sup>-1</sup>		
Control	113.755	0.733	1.91	108.806	0.631	7.67
SSs	111.435	0.666	1.90	107.763	0.598	5.25
SSd	108.844	0.712	2.62	133.435	0.712	5.20
GERMANY						
Control	103.230	0.594	10.00	71.867	0.543	1.32
SSs	109.388	0.765	6.50	73.269	0.554	1.32
SSd	100.333	0.620	7.00	81.502	0.563	1.33
POLAND						
Control	73.856	0.055	2.00	43.824	0.046	2.00
SSs	154.050	0.091	3.00	48.790	0.048	3.00
SSd	155.720	0.091	2.00	52.178	0.045	2.00

Table 4. Organic carbon pool indices of soils studied

Soils	n <sup>a)</sup>	L <sup>b)</sup>	CPI <sup>c)</sup>	LI <sup>d)</sup>	CMI <sup>e)</sup>	L	CPI	LI	CMI
		2006				2007			
ESTONIA									
Control (reference)	5	0.130	1.000	1.000	100.00	0.162	1.000	1.000	100.00
SSs	5	0.125	0.990	0.964	95.46	0.177	0.948	1.095	103.83
SSd	5	0.132	0.973	1.017	98.92	0.176	0.927	1.086	100.67
GERMANY									
Control (reference)	5	0.216	1.000	1.000	100.00	0.217	1.000	1.000	100.00
SSs	5	0.199	1.167	0.921	107.44	0.311	0.869	1.433	124.43
SSd	5	0.194	1.052	0.898	94.54	0.276	0.925	1.269	117.39
POLAND									
Control (reference)	5	0.142	1.000	1.000	100.00	0.124	1.000	1.000	100.00
SSs	5	0.141	1.488	0.993	147.67	0.153	1.059	1.231	130.38
SSd	5	0.139	1.654	0.978	161.83	0.124	1.049	0.997	104.62

<sup>a)</sup> n – number of samples; <sup>b)</sup> L =  $\text{KMnO}_4\text{-C} / \text{non-KMnO}_4\text{-C}$ ; <sup>c)</sup> CPI =  $\text{OC}_{\text{sample}} / \text{OC}_{\text{reference}}$ ;

<sup>d)</sup> LI =  $L_{\text{sample}} / L_{\text{reference}}$ ; <sup>e)</sup> CMI =  $\text{CPI} \times \text{LI} \times 100$

Table 5. ANOVA analysis – F values for organic carbon (OC), hot water-extractable carbon (HWC) and easily oxidizable carbon ( $\text{KMnO}_4\text{-C}$ )

	GERMANY			POLAND			ESTONIA		
	OC	HWC	$\text{KMnO}_4\text{-C}$	OC	HWC	$\text{KMnO}_4\text{-C}$	OC	HWC	$\text{KMnO}_4\text{-C}$
	$\text{g kg}^{-1}$								
Time (A)	138.684*	8.139*	3.606	54.378*	39.170*	55.760*	20.097*	0.452	2.988
Dose (B)	1.598	0.056	0.548	12.760*	4.897*	12.724*	3.436*	0.535	0.385
A×B	0.177	0.261	0.259	9.385*	4.448*	7.180*	1.604	3.586*	0.565

\*significant at  $p \leq 0.05$

## DISCUSSION

Sludge, more recently being called biosolids, helps the plant growth and provides a source of organic matter for soil. In general, many farmers are aware that the so-called biosolids can reduce costs of fertilizer and generally improve their soils [10]. It is limited by the content of heavy metals and amounts of nutrients [14] which may cause eutrophication. Therefore, testing of the sludge must be performed regularly. Species of *Salix* generally grow well on sandy and loamy soils [20, 13], which were examined in the study.

The study showed that the changes in organic carbon content (decrease of OC) include both soils amended with sewage sludge and control ones. It is assumed that sewage sludge is a primary source of fulvic-like substances that are easily mineralized



within a year [5]. Moreover, the changes are also due to heterogeneity and variability of organic carbon in space and time.

The hot water-extractable pool of C (HWC) can be used as one of the soil quality indicators in soil-plant ecosystems [7]. HWC is one of the most sensitive indicators reflecting the changes in the soil organic matter. Another sensitive indicator is easily oxidizable pool of OC – the  $\text{KMnO}_4\text{-C}$ . The results indicate that the sewage sludge, in the rates applied, did not contribute to distinct changes in the content of HWC and  $\text{KMnO}_4\text{-C}$  comparing fertilized plots to the control ones. In German and Polish soils, which were cultivated for years, these two pools of organic carbon increased only in the first year after amendments, whereas in the second, they were similar to unamended plots. However, in Estonian soils, which were not cultivated but fallowed, these two pools of carbon were similar in control plots and amended ones. The analysis of ANOVA (Table 5) revealed that only in Poland the dose of sewage sludge as well as time of “incubation” of sewage sludge in the soil were significant in relation to organic carbon, hot water-extractable carbon and easily oxidizable carbon pools. The obtained results confirmed previous statements that the changes in dissolved organic carbon pools are more dependent on the previous land use and soil type and less on the rate of amendment applied [8].

Spectral analysis of HWC solutions proved that Estonian soils contained similar amounts of aromatic organic compounds in control plots and after application of SS in 2006 and 2007 ( $A_{254}$  remained unchanged). However, the state of humification of organic compounds and size of organic molecules changed, which was stated on the basis of E4/6. In 2007 these soils contained organic compounds with smaller molecules and the state of humification was lower. In German soils the transformation was opposite. Dissolved organic matter after application of sewage sludge in 2007 contained more humified organic compounds with more complex molecules. Different transformation took place in Polish soils. Their state of humification remained stable and the amount of aromatic compounds was higher only in the first year after application of sewage sludge. Although similar rates of sewage sludge were applied on soils in three different countries under *Salix* plantations, dissolved organic carbon showed differences. This leads us to the statement that the changes occurring in DOC after application of SS depend on the previous use of land and soil type, and are not strongly influenced by the sewage sludge application.

Carbon Pool Index (CPI) represents changes in the content of total organic carbon, whereas the Lability Index (LI) informs about the changes in labile part of carbon between control soil samples (reference) and soil samples fertilized with sewage sludge. These two indices are used to calculate Carbon Management Index (CMI). The CMI value is a measure of rate of change in soil carbon. When a new agricultural practice is introduced, as it is for sewage sludge application, the CMI indicates if such practices cause a decline or rehabilitation of the soil system [1]. The application of sewage sludge contributed to an increase of Carbon Pool Index (CPI) in most investigated cases. However, as reported in the literature, sewage sludge is a source of fulvic-like substances which are mineralized within a year. The study showed that the application of sewage sludge contributed to a better management of carbon.

Generally it can be stated that the rates of sewage sludge applied did not cause undesirable changes in soils. In this respect SS may be applied in order to improve soil properties. However, long-term effects of those applications should be monitored.

Estonian soils were stable in this respect and no distinct changes in labile organic carbon fractions occurred. These soils, opposed to Polish and German soils, were not cultivated for years before the establishment of short-rotation plantations.

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#### WPLYW OSADÓW ŚCIEKOWYCH NA PLANTACJACH *SALIX* NA ZAWARTOŚĆ WĘGLA ROZPUSZCZONEGO W GLEBIE

Labilne frakcje materii organicznej mogą być wyrazem zmian zachodzących w glebie i stanowią one wskaźniki jakości materii organicznej. Badano dwie frakcje labilnego węgla organicznego w glebach nawożonych osadem ściekowym w dwóch dawkach (będące równoważnikami 60 kg P ha<sup>-1</sup> i 120 kg P ha<sup>-1</sup>). Badane gleby były porośnięte gatunkami wierzy krzewiastej w Niemczech, Estonii i Polsce. W badanych glebach w Polsce zastosowanie osadów ściekowych spowodowało wzrost obu analizowanych frakcji węgla (KMnO<sub>4</sub>-C i HWC). Natomiast gleby estońskie były stabilne i nie stwierdzono zmian w labilnych frakcjach węgla organicznego.