



## ORIGINAL PAPER

## LABILE ORGANIC CARBON FRACTIONS AFTER AMENDMENT OF SANDY SOIL WITH MUNICIPAL SEWAGE SLUDGE AND COMPOST\*

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## ABSTRACT

The paper deals with the effect of organic amendments three years after their application on the transformation of organic matter in sandy soil. The labile part of organic carbon has been suggested as a sensitive indicator of changes in soil organic matter. Our study was based on quantification of the hot water-extractable carbon fraction and its optical properties, as well as the easily mineralizable fraction of organic carbon and fulvic acids. The hot water-extractable carbon content was lower in plots amended with sewage sludge than with compost, and it was correlated with easily mineralizable carbon as well as the light fraction of fulvic acids. The analyzed amended soil contained amounts of hot water-extractable carbon that are typical of sandy soils. The optical properties of the carbon fraction soluble in hot water seem to implicate that this dissolved organic carbon in amended plots contained fewer polar functional groups which were hydrophilic. This may suggest the formation of more complex organic compounds with lower affinity to water. The Carbon Management Index values increased after the application of organic amendments as compared to the untreated soil, and were higher for the plots amended with compost. The measurable dynamics of carbon expressed by Lability Index, Carbon Pool Index and Carbon Management Index showed that both compost and sewage sludge increased the lability of organic carbon and, irrespective of some decline in the organic carbon content, an overall balance of carbon is favorable. However, changes in these indices should be monitored over time.

**Keywords:** carbon management index, hot water-extractable carbon, labile organic carbon, organic amendments.

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## INTRODUCTION

The amount of sewage sludge and municipal waste produced in the world is on the increase. Only some of this waste material, rich in organic matter, is applied to agricultural land and urban greenery in order to enhance the physical and chemical soil properties (NYAMANGARA, MZEZEWA 2001, TOBIAŠOVÁ, MIŠKOLCZI 2012, ANGELOVA et al. 2013). It is well known that application of organic amendments produced from municipal waste improves soil properties and resolves the problem of waste disposal (WEBER et al. 2007), but only when these amendments are stabilized and do not contain elevated amounts of potentially dangerous elements, especially heavy metals. Labile fractions of organic matter such as dissolved organic carbon and easily mineralizable carbon compounds can rapidly respond to changes in carbon pools. These labile parts of organic carbon have been suggested as sensitive indicators of soil organic matter changes (SHAHRIARI et al. 2011, SPOHN, GIANI 2011) and important indicators of soil quality (YANGCHUN et al. 2007, STILES et al. 2011).

Humus is the part of organic matter which is generally less labile. Humic substances differ in composition and chemical structure, they are complex and have high molecular weight. The classic fractionation of humus is based on extracting fulvic acids, humic acids and humins, which have different, pH-dependent solubility. The fulvic acid fraction is the least condensed one and has the highest mobility of alkali-soluble fractions, as it consists of organic compounds with low molecular weight. In contrast, humic acids are more condensed and show greater stability (ZAVARZINA et al. 2008).

LOGINOW et al. (1987) and later TIROL-PADRE and LADHA (2004) introduced a method based on the oxidation of organic carbon (OC) by potassium permanganate, which provides qualitative characterization of soil carbon (SHAHRIARI et al. 2011). The method is based on the assumption that the oxidative action of potassium permanganate, under neutral conditions, is comparable to that of enzymes from microorganisms and other soil-borne enzymes. This method has been standardized and modified by BLAIR et al. (1995), and it allows the user to calculate labile and non-labile carbon components, with such tools as the Carbon Management Index (CMI), which compares changes in total and labile carbon as a result of agricultural practice (BLAIR et al. 1995). Also, hot water-extractable carbon (HWC) is a measure of labile OC (HAMKALO, BEDERNICHEK 2014). It correlates strongly with microbial biomass and is therefore thought to be labile in nature (GHANI et al. 2002). HWC is one of the most sensitive indicators among soil biochemical measurements considered to reflect changes in soil organic matter caused by different soil management practices between sites and within an ecosystem. A decrease in HWC may indicate a decline in the content of organic labile nutrients, microbial biomass pool and possibly degradation of soil structure (GHANI et al. 2002). Thus far, dissolved organic matter has not received

enough attention in soil science. Organic matter soluble in water is an important labile fraction as it is the main energy source for soil microorganisms and a primary source of mineralizable N, P, S, in addition to which it influences the availability of ions in soil.

Labile OC measurements should be used more widely in order to examine soil properties (SCHULZ 2004), as this part of organic carbon can be potentially easily oxidized to CO<sub>2</sub> and therefore contributes to the diminishing of soil carbon sequestration. Not enough is known on the impact of different amendments on labile organic fractions. Also, little is known about optical properties of HWC, as such studies are commonly conducted for alkali extracts of humic acids.

The aim of the study was to evaluate and compare amounts and quality of labile fractions of organic carbon in the soil amended with municipal sewage sludge and composted wastes, as well as to quantify carbon indices for both applied amendments.

## MATERIAL AND METHODS

A two-factorial experiment was established in four replications on research fields of the University of Warmia and Mazury in Olsztyn, NE Poland. Municipal sewage sludge (SS) and compost (COM) were applied once on Arenosol. The soil studied contained 90% of sand ( $\phi$  2.0-0.05 mm), 8% of silt ( $\phi$  0.05-0.002 mm) and 2% of clay ( $\phi$  < 0.002 mm) fractions. According to the USDA texture classes and PTG (2009), the soil was classified as sand. The pH<sub>KCl</sub> values of the control soil in the studied layers was 6.74. The soil cation exchange capacity was 8.72 cmol(+) kg<sup>-1</sup> and base saturation amounted to 85.38%. The dominant cation was calcium. The applied organic amendments were mixed with the soil to a depth of 10 cm, after which lawn grasses of different varieties were sown. The doses of the amendments were as follows: 0, 30, 60, 90, 120 and 150 tones of fresh mass per hectare. The compost was produced from selected municipal waste using the technology of DANO biostabilization (in Suwałki, in specially constructed chambers – biostabilizers; the process takes up to 6 days). Organic waste, paper and leftovers were disintegrated and mixed at a fixed temperature, moisture and oxygen content. Then, the mixture was biostabilized and any uncomposted particles (metal, plastic, ceramic, etc.), if present, were removed. The sewage sludge used in the experiment is a product from a municipal treatment plant (for a city with a population of 200,000 people). The sewage sludge applied on the investigated plots was fresh. The main properties of COM and SS applied were as follow:

- compost: dry matter (DM) = 55.85%, organic carbon = 15.3%, pH<sub>KCl</sub> = 7.5, total N = 8.0 g kg<sup>-1</sup>, total P = 3.6 g kg<sup>-1</sup>, total K = 7.7 g kg<sup>-1</sup>, total Ca = 69.0 g kg<sup>-1</sup>;

– sewage sludge: dry matter (DM) = 18.25%, organic carbon = 31.6%,  $\text{pH}_{\text{KCl}} = 7.7$ , total N = 44.4 g kg<sup>-1</sup>, total P = 17.6 g kg<sup>-1</sup>, total K = 10.0 g kg<sup>-1</sup>, total Ca = 44.2 g kg<sup>-1</sup>.

The content of all macroelements in the amendments was calculated on the dry matter basis. The amount of organic carbon introduced into the soil with the applied amendments is given in Table 1.

Table 1  
Quantities of organic carbon (kg ha<sup>-1</sup> DM) incorporated into the soil with municipal compost and fresh sewage sludge

Amendment dose (t ha <sup>-1</sup> )	Compost (COM)	Sewage sludge (SS)
30	2558	1734
60	5116	3469
90	7674	5203
120	10 232	6937
150	12 790	8672

Three years after the experiment was set up, 48 composite soil samples were taken to a depth of 0-10 cm, air dried and visible plant remains were removed manually. First, the soil samples were ground to pass through a 2.00 mm sieve, and then, for some chemical analyses, through a 0.25 mm sieve. Loss-on-ignition (LOI) was determined after dry combustion of soil samples for 6 hours at a temperature of 550°C. Total organic carbon (OC) was measured with a spectrophotometer after oxidation with sulfochromic medium (ISO 14235).

The content of potentially oxidizable carbon was estimated by the method described by BLAIR et al. (1995) and TIROL-PADRE, LADHA (2004). Soil samples containing 30 mg of OC were weighed into plastic bottles and 50 ml of 0.0333 M potassium permanganate (KMnO<sub>4</sub>) was added to each bottle, which was then shaken for 30 min on a BIOSAN PSU 20 multi-shaker. Additionally, a blank sample without soil was analyzed. Then the solutions were filtered through glass filters and the change in the KMnO<sub>4</sub> concentration was estimated by titration with 0.05 M oxalic acid (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>). On the basis of the change in the KMnO<sub>4</sub> concentration, the amount of oxidized C was calculated and considered as labile carbon (CL). The difference between OC and CL was assumed to be the non-labile carbon fraction (NCL). The Carbon Pool Index (CPI) was calculated based on changes in OC in amended soils compared to the OC content in the reference samples (untreated plots). Lability of carbon (L) was calculated as a quotient between CL and CNL. The Lability Index (LI) was determined on the basis of changes in the proportion of CL in the samples of non-fertilized soils (a reference site) and in fertilized soils. The LI and CPI indices were used to calculate the Carbon Management Index (CMI), as described by BLAIR et al. (1995).

Hot-water extractable C (HWC) was determined in air-dried soil samples according to the method described by SPARLING et al. (1998). Briefly, 4 g of air-dried soil was incubated with 20 ml demineralised water in a capped test-tube at 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). The HWC in the filtered solution was measured on a Shimadzu 5000 TOC analyzer.

For HWC extracts, optical properties were examined at the range of 254 nm, 465 nm and 665 nm using a two-channel scanning spectrophotometer Shimadzu UV-1601PC and 10 mm quartz trays. The normalized absorbance value – index  $SUVA_{254}$  (Specific Ultra Violet Absorbance) was calculated according to the formula:

$$SUVA_{254} = (2.303 \cdot A_{254}) / (0.01 \cdot HWC) \text{ [m}^{-1} \text{ (mg l}^{-1}\text{)}^{-1}\text{]} \text{ (GŁAŻEWSKI, PARSZUTO 2002),}$$

where: 2.303 – arithmetic factor,

$A_{254}$  – absorbance at 254 nm,

0.01 – optical path length (m),

HWC – content of hot water-extractable carbon (mg l<sup>-1</sup>).

Four soil samples for every dose of the studied organic amendments were analyzed. All analyses were performed in duplicate. All results were expressed on the oven-dry soil weight basis (the 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.

## RESULTS

Our research showed that three years after the application of municipal compost, the content of OC increased but the differences were not statistically significant. Soil total organic carbon varied across the doses of the amendments, with the lowest carbon content in the plot amended with sewage sludge – 16 948 kg ha<sup>-1</sup>. In the case of SS, organic compounds were mineralized and even a decrease in the OC content was observed for the treatments of 30-90 t ha<sup>-1</sup> compared to untreated soil (Table 2).

Labile organic matter pools were studied, taking into consideration the following principal indicators: the content of HWC and the part of organic carbon that is easily oxidized with KMnO<sub>4</sub>. The content of HWC in control plots was the lowest (0.361 t ha<sup>-1</sup> ± 0.187), and increased by 22-23% after the addition of compost and sewage sludge. The highest content of this dissolved carbon was recorded in the plots amended with the dose 90 t ha<sup>-1</sup>. In compost-amended and sewage sludge-amended plots, it amounted to 0.441 and

Table 2

Organic carbon fractions studied ( $n = 48$ ). The values are arithmetic means from all experimental plots

Treatment*		LOI	OC	HWC	CL
		(kg ha <sup>-1</sup> )			
Reference	MN	34 302	17 945	361	626
	SD	2 627	2 103	18	176
30 / COM	MN	40 728	18 365	436	818
	SD	2 015	1 675	44	104
60 / COM	MN	40 980	19 562	404	807
	SD	2 414	698	38	142
90 / COM	MN	39 528	19 582	441	1132
	SD	4 705	2 294	41	142
120 / COM	MN	38 326	18 586	411	760
	SD	2 713	469	58	93
150 / COM	MN	43 628	20 457	432	926
	SD	3 031	1 345	34	56
COM	MN	39 582	19 083	414	845
	SD	3 134	941	30	171
30 / SS	MN	35 114	17 488	382	895
	SD	2 594	2 458	99	264
60 / SS	MN	35 901	17 412	398	867
	SD	2 947	2 104	93	148
90 / SS	MN	38 797	16 948	446	995
	SD	1 873	642	110	180
120 / SS	MN	39 111	20 993	376	798
	SD	1 721	636	36	87
150 / SS	MN	40 280	18 324	386	892
	SD	1 001	856	29	48
SS	MN	37 840	18 233	398	890
	SD	2 218	1 621	28	71

\* COM and SS doses in tons of fresh matter per hectare, MN – mean, SD – standard deviation, LOI – loss-on-ignition, OC – organic carbon, CL – labile organic carbon oxidized with  $\text{KMnO}_4$ , HWC – hot-water extractable carbon

0.446 t ha<sup>-1</sup>, respectively. However, when the share of HWC in OC is taken into consideration, fluctuations were observed in some amended plots (Figure 1).

The content of CL in amended soil plots ranged from 0.376 to 0.446 t ha<sup>-1</sup>, and was higher than in the control plots (0.361 t ha<sup>-1</sup>). Similarly to the results

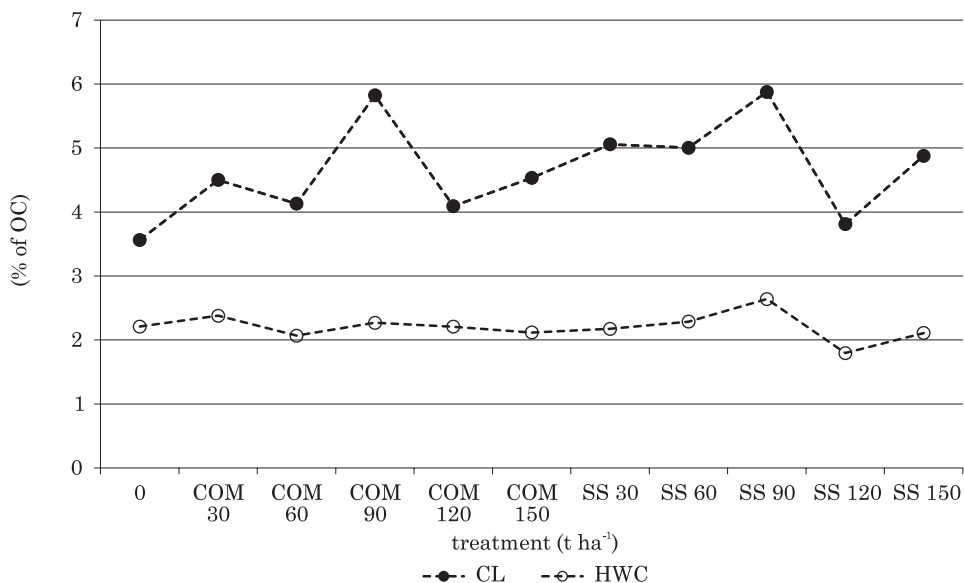


Fig. 1. Share of the analyzed carbon fractions in OC with regard to treatments

on HWC, the amount of CL varied within the type of amendment – either increasing or decreasing – irrespective of the dose of an amendment. It should also be noted that for the dose of 90 t of amendment per hectare, CL reached the highest values: 1.132 and 0.995 t ha<sup>-1</sup> for plots amended with compost and sewage sludge, respectively. This labile carbon increased by 30-87% in the plots treated with COM and by 32-62% after the amendment with SS (Table 3). Nonetheless, as presented in Figure 1, its share in OC is generally higher in the samples amended with sewage sludge than in the ones amended with compost. These two organic carbon pools (CL and HWC) increased after the addition of organic amendments. However, this dependence was not statistically significant (Figure 2) according to Pearson's correlation tests ( $p = 0.05$ ).

Some of the optical properties of the HWC fraction are shown in Table 4. The values of  $A_{254}$  in control, SS and COM plots were low. However, the distinction between min. and max. values was remarkable. The highest discrepancy was reported for control plots (0.551) and the lowest one (0.062) for the plots amended with SS (Table 3). The dependences were not statistically significant (Pearson's correlation test;  $p = 0.05$ ). Plots amended with SS seem to be more parallel to the control plots and have similar values of  $A_{254}$  at different HWC content (Figure 3a).

Carbon indices of the soils

Treatment	<i>n</i>	CL	CNL	OC	L	CPI	LI	CMI
		(g kg <sup>-1</sup> )						
Reference	8	0.40	11.07	11.47	0.036	1.000	1.000	100.0
30 / COM	4	0.53	11.26	11.79	0.047	1.027	1.279	131.4
60 / COM	4	0.52	12.20	12.72	0.043	1.109	1.179	130.8
90 / COM	4	0.74	12.11	12.85	0.061	1.121	1.685	188.8
120 / COM	4	0.52	12.07	12.59	0.043	1.097	1.173	128.6
150 / COM	4	0.64	13.47	14.11	0.047	1.230	1.302	160.0
Mean/COM	20	0.59	12.22	12.81	0.048	1.117	1.324	147.9
30 / SS	4	0.57	10.62	11.19	0.054	0.975	1.480	144.3
60 / SS	4	0.56	10.71	11.27	0.052	0.982	1.439	141.4
90 / SS	4	0.65	10.46	11.11	0.062	0.969	1.711	165.7
120 / SS	4	0.53	13.28	13.81	0.040	1.204	1.085	130.6
150 / SS	4	0.59	11.62	12.21	0.051	1.065	1.404	149.5
Mean/SS	20	0.58	11.34	11.92	0.052	1.039	1.424	146.3

*n* – number of samples, CL, CNL and OC are mean values,  $L = CL / CNL$ ,  $CPI = OC_{\text{sample}} / OC_{\text{reference}}$ ,  $LI = L_{\text{sample}} / L_{\text{reference}}$ ,  $CMI = CPI \times LI \times 100$

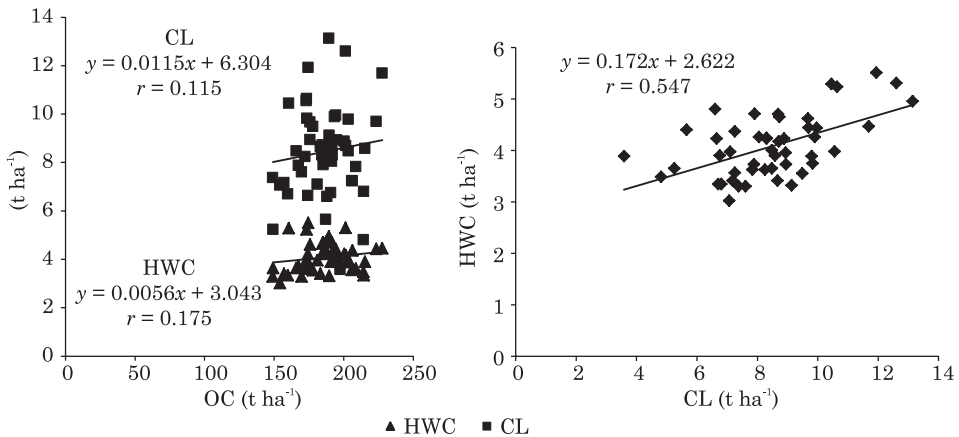


Fig. 2. Relationships between the organic carbon fractions studied

## DISCUSSION

Generally, soil organic compounds play a key role in agriculture and affect soil quality as they influence physical, chemical and biological soil properties. The principal indicator of the amount of organic substances is



Table 4

## Optical properties of HWC solutions

Treatment		$A_{254}$	$SUVA_{254}$	$A_{465}/A_{665}$
Reference	min-max	0.797 - 1.348	4.908 - 6.050	5.024 - 13.789
	mean	1.119	5.303	8.853
Sewage sludge	min-max	1.070 - 1.132	4.624 - 5.139	7.564 - 10.664
	mean	1.098	4.956	8.773
Compost	min-max	1.233 - 1.313	4.776 - 5.137	7.927 - 9.784
	mean	1.271	5.005	8.847

$A_{254}$  – absorbance at 254 nm,  $SUVA_{254}$  – normalized absorbance value at the range of 254 nm,  $A_{465}/A_{665}$  – quotient of absorbance at 465 nm and 665 nm

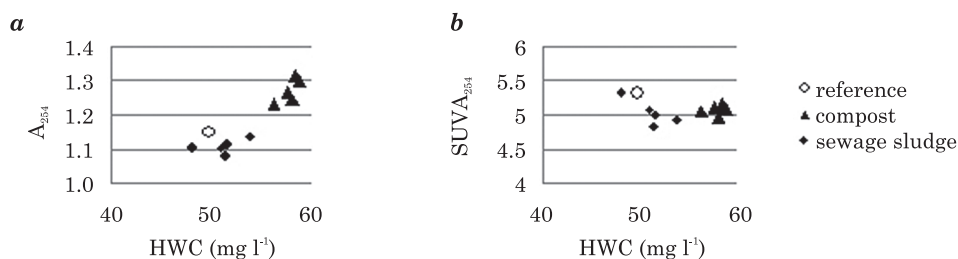


Fig. 3. Relationships between the HWC content and  $A_{254}$  (a),  $SUVA_{254}$  (b) (mean values for treatments)

organic carbon. Soil organic carbon sequestration is influenced by many factors (including climate, type of soil, type of amendments added into the soil), which affect the rate of decomposition of organic carbon and which can increase the content of soil organic carbon (KWIATKOWSKA-MALINA 2015, ZHU et al. 2015). Our research showed that three years after the application of sewage sludge, organic carbon incorporated into the soil was substantially mineralized. GASCÓ et al. (2004) stated that SS is a source of fulvic-like substances, which are easily mineralized within a year. In light soils occurring in Poland, due to climatic conditions, mineralization of organic matter introduced into the soil is observed (KALEMBASA, BECHER 2009). This explains the decrease in OC after the application of sewage sludge into the studied soil. However, in the case of compost, the OC after three years was still higher than in control plots (untreated soil), suggesting that compost contains stable organic compounds which are not readily mineralized. Thus, compost is a better (stable) organic amendment in comparison to sewage sludge. Short-term changes in soil organic carbon may be detected by the changes in labile organic fractions, such as CL (easily oxidizable carbon) and HWC (microbial biomass carbon) (ZHU et al. 2015). HWC and CL can be seen as indicators of soil quality because they are sensitive to changes in soil management practice (HAYNES, 2005). These two organic carbon pools increased after the addition of organic amendments, although the lack of correlation between HWC and

OC or between CL and OC suggests that the SOC content is not the prime indicator of the amount of labile C pools. This conclusion is consistent with earlier statements of HAYNES (2005) that labile organic matter fractions can vary remarkably.

The HWC values achieved were also compared to the ranges of HWC for soils regarding their supply with SOM suggested by SCHULZ (1997). The analyzed soils contained medium (0.25-0.30 g kg<sup>-1</sup>) amounts of HWC, which are recommended for sandy and loamy soils. Nevertheless, it should be noted that lower amounts were reported for the plots amended with SS and higher for COM (Table 2). This indicates that composts provide more substrate for microorganisms.

Our study showed that HWC is correlated with CL (Figure 2). MUNGAI, MOTAVALLI (2006) stated that soil containing more CL had a more favorable biological activity and contained more substrates for microbial populations, and would release more CO<sub>2</sub>. CONTEH et al. (1999) show that CL was significantly related to the soil microbial biomass carbon and that this KMnO<sub>4</sub>-oxidizable carbon is mainly comprised of soil polysaccharides and some unidentified aromatic compounds.

Qualitative description of dissolved organic carbon in soils is still a poorly recognized issue. Optical properties of this fraction in the soil samples amended with COM and SS may suggest qualitative changes in the fraction of dissolved organic carbon. Absorbance at 254 nm ( $A_{254}$ ) depends on the condensation of the aromatic core of the molecule (ANDERSEN et al. 2000). The value of  $A_{254}$  on compost-amended plots is higher and indicates that this fraction of SOM is more aromatic. However, it seems that this value is more closely related to the content of HWC. Therefore, we measured the normalized absorbance called the SUVA<sub>254</sub> index. It is assumed that the higher the SUVA<sub>254</sub>, the higher the number of polar functional groups in the molecule. This index is lower in the amended plots than in the control ones (Table 3). By the values of this parameter (Figure 3b) it can be stated that the amount of polar functional groups decreases after the addition of amendments. This may indicate the formation of more complex organic compounds with lower affinity to water after the application of amendments. Generally, a reduction in the SUVA value in mineral soils was observed by other authors (KOTHAWALA et al. 2012).

The ratio of the absorbance at 465 nm and 665 nm ( $A_{465}/A_{665}$ ) is considered to be inversely related to the molecular size of humus substances. This ratio was slightly higher in the control plot samples than in the amended plots (Table 3). It suggests that the degree of humification of organic matter of the soil sampled from the analyzed plots is low. However, it should also be stated that three years after the application of the amendments, the optical properties of the amended plots and control plots became similar.

The application of organic amendments was beneficial regarding CL. This labile carbon increased by 30-87% in the plots treated with COM and by

32-62% after the amendment with SS (Table 4). It also had an effect on L (lability of carbon), the values of which were always higher for the amended plots than for the control ones. Greater L as well as LI were reported for the plots amended with SS than for those amended with COM. The Carbon Pool Index increased in the plots treated with COM (Table 4). Carbon compounds contained in SS must have been rapidly mineralized as the CPI values declined. This had some influence on the CMI. The CMI values increased after the application of organic amendments, but were higher for the plots fertilized with COM. Taking into account the pools of CL, CNL and OC, a more definite picture of the soil carbon is obtained. The CMI values provide data of the changes of the C pool between the treatments and - when monitored over time - they provide relative data of the dynamics of carbon (KALISZ et al. 2012).

## CONCLUSIONS

1. Municipal sewage sludge and compost produced from municipal waste used as organic amendments in green areas improved the dynamics of OC.

2. Three years after the application of COM, the content of SOM and OC was higher compared to untreated control soil.

3. The content of CL, which is thought to be a substrate for microbial activity, and the HWC content increased after the application of both amendments.

4. The content of HWC after the addition of organic amendments increased but the amounts determined were not higher than ones determined in typical sandy soils. The HWC fraction contributed to the formation of less hydrophilic organic substances.

5. The measurable dynamics of carbon expressed by LI, CPI and CMI values showed that both COM and SS increased the lability of OC and, irrespective of some decline of OC, the overall balance of C is favorable.

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