

PEAT HUMIC SUBSTANCES AND EARTHWORM BIOHUMUS EXTRACTS FOR AGRICULTURAL APPLICATIONS

Gunārs Bremanis*, Māris Klaviņš**, Oskars Purmalis**, Raitis Ziemelis***, and Solveiga Maļecka*

* State Stende Cereals Breeding Institute, "Dižzemes", p/o Dižstende, Libagu pag., Talsu nov., LV-3258, LATVIA; stende.selekcija@apollo.lv

** Department of Environmental Science, University of Latvia, Raiņa bulv. 19, Rīga, LV-1586, LATVIA; oskars.purmalis@lu.lv

*** Modul House Ltd, Pils iela 28, Ventspils, LATVIA; raitis.ziemelis@modulhouse.lv

Contributed by Māris Klaviņš

Extracts for agricultural use were prepared using cavitation method at 45 °C and at 95 °C from peat and earthworm biohumus. Samples of all studied extracts had similar dry-matter content. Peat humic substances and earthworm biohumus extracts were analysed using UV-Vis, FT-IR, and fluorescence spectrometry. Trace element analysis was performed with atomic absorption spectrometry. Extracts were tested for acidity, conductivity, humic acid concentration, and carboxyl group concentration. Hydrophobicity was assessed using a water-polyethylene glycol two-phase system and calculation of the partition coefficient. Total nitrogen and phosphorus concentration was determined. Concentration of the humic substances slightly changed during the heating process. Significant changes in total phosphorus concentration was observed both in earthworm humus and peat extract. Thermal treatment caused decrease of hydrophobicity and lower concentration of organic matter in extracts, while carboxyl group concentrations in isolated humic substances practically did not change. The calculated ratio from UV-Vis spectra showed significant difference in humification degree of samples.

Key words: humic acid extracts, vermicompost, cavitation, peat.

INTRODUCTION

The agricultural application of humic substances is gaining more and more attention (Mohanty *et al.*, 2002; Makkar *et al.*, 2002). New extraction methods have been elaborated to obtain concentrated extracts of humic substances (Martynenko *et al.*, 2012). Humic acid properties are studied very well, but study of extracted solutions from vermicompost and peat using cavitation are significantly less (Atiyeh *et al.*, 2002). Typically, industrially produced vermicompost and peat have been analysed using simple methods (pH, N, P and metal concentration, humidity, density) and in some cases effects on agricultural crops have been described, but there is gap in knowledge about the structural composition and properties of these products.

The main goal of this study was descriptive analysis of vermicompost and peat extracts using methods commonly applied for study of humic acid properties.

MATERIALS AND METHODS

Vermicompost from "Kvarki" Ltd. was used in the study (composition: 0.7% N, 0.39% P, 60.6 % humidity; pH 7.9).

Transitional mire peat (elemental composition (%): C/H/N/S = 49.87/5.09/1.59/0.72) from a littoral lowland was used for extraction of humic acids.

Cavitation technology was used for preparation of peat and earthworm biohumus extracts (Ziemelis, 2011). Cavitation is the formation and then immediate implosion of cavities in liquid bubbles. This method is based on mixture of organic matter and water treatment with cavitation and formation of an organic dispersion. For better extraction of humic acids, potassium hydroxide solution (1%) was used.

Solvent engineering technique can be used for any organic substances (such as peat, wood, mud, seaweed, straw, grass, worms and bird droppings) to provide a concentrated liquid form of organic humus. The technique utilizes the following equipment (see Fig. 1).

The method of preparation of peat and earthworm biohumus extract contains the following steps:

Peat humus extracts mixture preparation:

- raw material thawed to operating temperature — 856 kW;

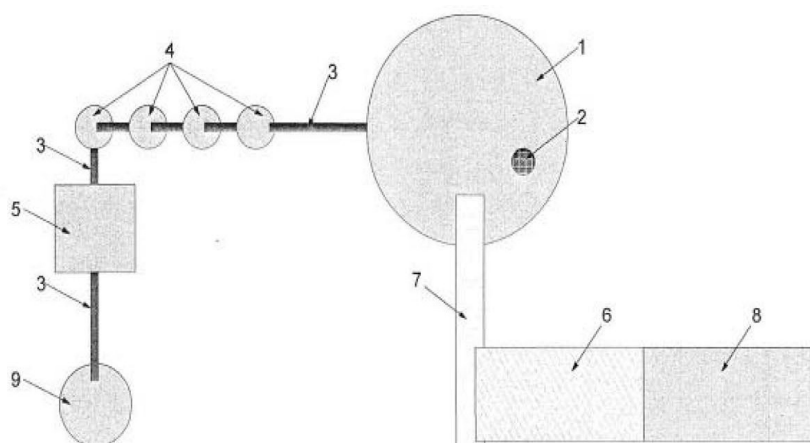


Fig. 1. Applied equipment for preparation of peat and earthworm biohumus extracts: dissolution tank (1), submersible pump (2), substances transfusion tubes or channels (3), (4) filtration system, cavitation device or emulsifier (5), organic matter sifting (6), organic feed device, such as an elevator (7), organic substance grinding device (8), and organic substance concentrate discharge tank (9).

- hardware rinsed with H_2O – 15 kW – $8 \text{ m}^3 \text{ H}_2\text{O}$;
- 4 t sieved peat, 1.5 t sifted peat remains — 10 kW;
- 1.5 t peat is soaked and $1 \text{ m}^3 \text{ H}_2\text{O} + 50 \text{ kg KOH}$ (potassium hydroxide) are added;
- in 1.8 tonne tank is mixed (445 kW) with pumps, then passed through a filter to remove solid mineral particles (sand, etc.) and fed to the cavitation reactor;
- 2 h cavitation (845 kW), then pumped through filters to a storage tank (5 kW). Results in about 1.2 tonnes of subcavitating peat humus organic mixture;
- $0.3 \text{ m}^3 \text{ H}_2\text{O}$ added to storage tank to obtain 1.5 t peat humus extract mixture, which is filled in 20-liter cans for storage.

Earthworm biohumus extract mixture preparation:

- raw material thawed to operating temperature — 850 kW;
- hardware rinsed with H_2O – 15 kW – $8 \text{ m}^3 \text{ H}_2\text{O}$;
- 3 tonnes of earthworm biohumus sifted, 1.2 t sifted earthworm biohumus remains — 10 kW;
- 1.2 t earthworm biohumus soaked in $1 \text{ m}^3 \text{ H}_2\text{O} + 50 \text{ kg KOH}$;
- mixed in 1.8 tonne tank (445 kW) with pumps, then passed through a filter to remove solid mineral particles (sand, etc.) and fed to a cavitation reactor;
- 2-h cavitation (845 kW), then pumped through filters to a storage tank (5 kW). Results in about 0.9 tonnes of subcavitating earthworms biohumus mixture;
- $0.6 \text{ m}^3 \text{ H}_2\text{O}$ added to storage tank to obtain 1.5 t earthworm biohumus extract mixture, which is filled in 20-liter cans for storage.

Boiling of peat humus extracts mixture:

From a storage tank, 750 l peat humus extract mixture (5 kW) is pumped into a boiler, where it is heated with an electrode to 95°C for 8 h (1200 kW). The result is 600 l of boiled peat humus extract mixture, which is filled in 20 l

cans for storage. Aggregate and tank are rinsed with $1 \text{ m}^3 \text{ H}_2\text{O}$.

Boiling of earthworm biohumus extract mixture:

From a storage tank, 350 l earthworm biohumus extract mixture (5 kW) is pumped into a boiler, where it is heated with an electrode to 95°C for 8 h (1200 kW). The result is 300 l of boiled earthworm biohumus extract mixture, which is filled in 20 l cans for storage. Aggregate and tank are rinsed with $1 \text{ m}^3 \text{ H}_2\text{O}$.

Preparation of boiled peat humus and earthworm biohumus mixture:

300 l boiled peat humus organic mixture is added together with 300 l earthworm biohumus mixture in a 1 m^3 tank, and mixed with a submersible pump (5 kW). The result is 600 l of mixture, which is filled in 20 l cans for storage. Aggregate and tank are rinsed with $1 \text{ m}^3 \text{ H}_2\text{O}$.

Boiled earthworm biohumus and the common earthworm biohumus mixture preparation:

300 l of boiled earthworm biohumus organic mixture is added together with 300 l of earthworm biohumus mixture in a 1 m^3 tank and mixed with a submersible pump (5 kW). The result is 600 l of the mixture, which is filled in 20 l cans for storage. Aggregate and tank are rinsed with $1 \text{ m}^3 \text{ H}_2\text{O}$.

Overview of the preparation of peat and earthworm biohumus extracts and used abbreviations are given in Table 1.

Table 1

PREPARATION OF PEAT AND EARTHWORM BIOHUMUS EXTRACTS AND USED ABBREVIATIONS

Sample No.	Description of solutions
B 45	earthworm biohumus extract obtained by cavitation with KOH at 45°C
HS 45	peat extract obtained by cavitation with KOH, at 45°C
B 95	earthworm biohumus extract obtained by cavitation with KOH at 45°C with successive heating at 95°C
HS 95	peat extract obtained by cavitation with KOH at 45°C , with successive heating at 95°C

KOH, potassium hydroxide

Humic acids were extracted from peat and biohumus with 8% KOH. After shaking for 24 h, the alkaline slurry was filtered through glass wool, and the particle-free filtrate was acidified to a pH 1 with addition of 6 M HCl. This solution was allowed to settle and then centrifuged; sediment (which contained HA) was washed with distilled water and freeze-dried.

FT-IR spectra were collected using a Spectrum BX (Perkin Elmer) spectrophotometer in KBr pellets. Transmittance ratios $T_{3400/2900}$ and $T_{3400/1600}$ (transmittance at 3400 characteristic for O-H stretching against transmittance at 2900 cm^{-1} characteristic for aliphatic C-H stretching and transmittance at 3400 against transmittance at 1600 characteristic for aromatic C=C and strongly H-bonded C=O of conjugated ketones) was used to characterise major functional groups of humic substances.

UV/Vis spectra were recorded on a Thermospectronic Helios γ UV (Thermo electron Co) spectrophotometer in a 1-cm quartz cuvette. The ratio E_4/E_6 (Chen *et al.*, 1977): ratio of absorbance at 465 and 665 nm and ratio $E_{2/6}$ (Albrecht *et al.*, 2011): absorbance at 280 and 664 nm were determined for the peat and earthworm biohumus extracts.

An automatic titrator TitroLine Easy (Schott-Geräte GmbH) was used to measure carboxylic group concentration in the peat and earthworm biohumus extracts. The known Caacetate method (Tan, 2005), based on the formation of acetic acid, was used for determination of the total amount of carboxylic groups. HA (20 mg) were weighed into 100 ml Erlenmeyer flask and under N_2 10 ml of the 0.2 N calcium acetate solution were added. Samples were potentiometrically titrated to pH 9.0 with 0.1 N NaOH.

Hydrophobicity of humic substances was characterised by their distribution between water and polyethylene glycol (PEG) phases (PEG 20000, Fluka) (Zavarzina *et al.*, 2002), quantified as the distribution coefficient $K_{\text{PEG/W}}$ (analogous to octanol water distribution coefficient — K_{ow}). The 10 % PEG-10% $(\text{NH}_4)_2\text{SO}_4$ -Extract- H_2O systems were prepared by mixing 2 ml of 30 % PEG solution with 2 ml of solution of ammonium sulphate and 2 ml of peat and earthworm biohumus extracts. The mixtures were shaken for 10 minutes. After complete phase separation, 1 ml were taken from each phase and diluted 10 times in 0.05 M NaHCO_3 . Then the absorbances at 465 nm were measured on a DR/2000 spectrophotometer (Hach Co). The distribution coefficients were calculated as following: $K_{\text{PEGW}} = \text{absorbance at 465 nm of the top (PEG-rich) phase} / \text{absorbance at 465 nm of the bottom phase}$.

Dry matter was determined after drying at 105 °C. Loss on ignition (LOI) method was applied to estimate organic matter content in solutions. LOI_{550} was determined by ashing samples at 550 °C.

Metal concentrations were measured by graphite furnace atomic absorption spectrometry (GFAAS) (Perkin-Elmer AAnalyst 200) with background correction. Na and K con-

centrations were measured using a flame photometer PAZ-3 with air-propane flame.

RESULTS

Spectral methods were used to describe vermicompost and peat extract properties. UV-Vis spectra showed higher amount of ketones for peat extracts (Fig. 2).

Fluorescence shows that aromaticity is higher for extracts from vermicompost and thermally treated samples (Fig. 3).

The results on calculated absorbance ratios E_4/E_6 , $E_{2/6}$ and pH, EVS, and dry matter content of peat and earthworm biohumus extracts are given in Table 2. Calculated transmittance ratios $T_{3400/2900}$ and $T_{3400/1600}$ of peat and earthworm biohumus extracts are shown in Table 3.

Figure 4 shows Fourier transformed infra-red spectra of peat and earthworm biohumus extracts.

Other indices of organic matter properties are given in Table 4. The HA/FA ratios indicate that the vermicompost extracts contain higher concentration of fulvic acids than those of peat and that these are increased by heating. Concentration of carboxyl groups in vermicompost extracts was higher than in that of peat. Analysis of total nitrogen and

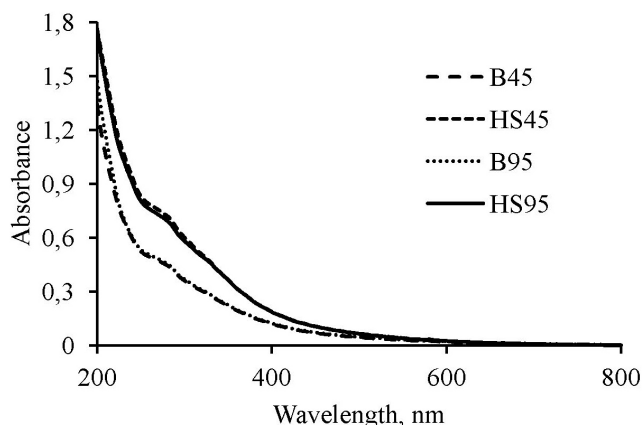


Fig. 2. UV-Vis spectra of peat and earthworm biohumus extracts.

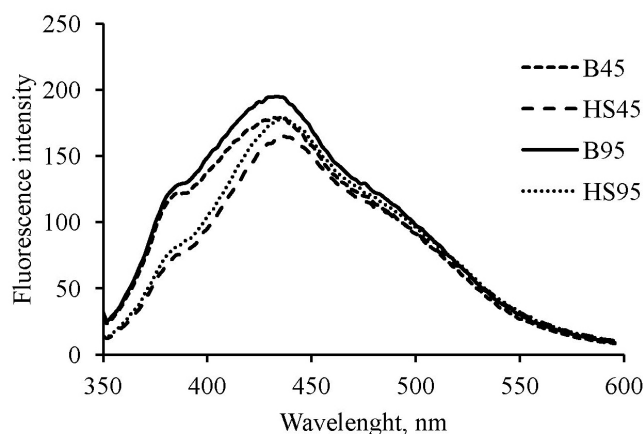


Fig. 3. Fluorescence spectra of peat and earthworm biohumus extracts.

Table 2

CALCULATED ABSORBANCE RATIOS E_4/E_6 , $E_{2/6}$ AND pH, EVS, DRY MATTER, % TO PEAT AND EARTHWORM BIOHUMUS EXTRACTS

	2011		2012		2011			2012		
	E_4/E_6	$E_{2/6}$	E_4/E_6	$E_{2/6}$	pH	EVS, $\mu\text{S}/\text{cm}$	dry matter, %	pH	EVS, $\mu\text{S}/\text{cm}$	dry matter, %
B 45	2.86	15.33	5.45	40.54	7.35	1448	0.19	10.80	1396	0.17
HS 45	3.90	23.95	6.57	51.57	7.23	962	0.21	10.35	1554	0.20
B 95	3.73	26.45	4.85	35.00	7.01	1156	0.17	11.07	1395	0.17
HS 95	4.47	28.05	6.50	49.64	7.58	1073	0.20	11.51	1916	0.20

For abbreviations see Table 1.

Table 3

CALCULATED TRANSMITTANCE RATIOS $T_{3400/2900}$ AND $T_{3400/1600}$ TO PEAT AND EARTHWORM BIOHUMUS EXTRACTS

	2011		2012	
	$T_{3400/2900}$	$T_{3400/1600}$	$T_{3400/2900}$	$T_{3400/1600}$
B 45	0.985	1.009	0.887	1.034
HS 45	0.992	1.058	0.889	1.052
B 95	0.979	1.015	0.859	1.010
HS 95	0.982	1.048	0.917	1.070

For abbreviations see Table 1.

Table 4

RESULTS OF PEAT AND EARTHWORM BIOHUMUS EXTRACT COMPOSITION ANALYSIS

Sample No	LOI ₅₅₀	TOC, mg/l	HA conc., mg/l	HA/FA ratio	COOH ⁻ mmol/g	K _{PEG/W}
2011						
B 45	60.73	318	1465	2.41	3.0	18.76
HS 45	43.23	692	1316	7.14	5.0	8.85
B 95	57.51	348	869	1.80	4.2	9.40
HS 95	54.63	602	1196	5.58	5.3	8.40
2012						
B 45	41.35	521	792	3.63	3.1	15.65
HS 45	38.63	595	960	3.32	3.6	11.00
B 95	47.38	525	781	3.44	2.7	6.75
HS 95	39.77	578	1020	2.38	3.5	9.17

TOC, total organic carbon; HA, humic acid; FA, fulvic acid. For other abbreviations see Table 1.

phosphorus showed that total nitrogen in peat extract was two times less than in vermicompost. Also, vermicompost had significantly higher concentration of NO_2^- and NH_4^+ . Metal concentrations in peat and earthworm biohumus extracts are shown in Table 5.

DISCUSSION

The use of peat substrates and vermicompost in agriculture is well known. They positively influence productivity of crops and enhance soil quality. Vermicompost contains major and minor nutrients in plant-available forms, enzymes, vitamins and plant growth hormones (Borah *et al.*, 2007). Peat contains substances such as proteins and humic acids (Jun-zhang *et al.*, 2004). Recently, there have been numer-

Table 5

ANALYSIS OF METAL CONCENTRATIONS IN PEAT AND EARTHWORM BIOHUMUS EXTRACTS

Sample	Mg mg/l	K mg/l	Ca mg/l	Fe mg/l	Mn mg/l	Cu mg/l	Zn mg/l	As mg/l
2011								
B 45	31	320	98	4.8	0.29	0.040	0.13	0.007
HS 45	14	303	59	5.7	0.04	0.050	0.14	0.008
B 95	18	296	73	5.8	0.25	0.530	0.36	0.005
HS 95	15	326	76	6.3	0.02	0.040	0.81	0.005
2012								
B 45	3.4	471	23	5.1	0.11	0.040	0.18	0.009
HS 45	0.3	595	11	3.9	0.02	0.017	0.36	0.015
B 95	3.5	459	24	5.6	0.13	0.054	0.23	0.008
HS 95	0.3	568	11	3.7	0.01	0.012	0.40	0.009

For abbreviations see Table 1.

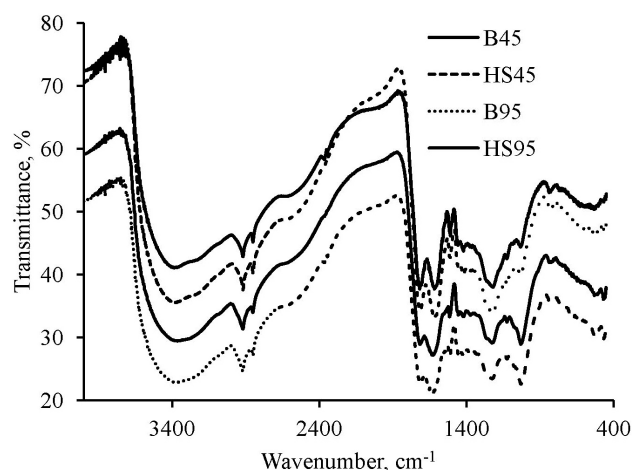


Fig. 4. Fourier transform infra-red spectra of peat and earthworm biohumus extracts.

ous trials on the use of humic substances as fertiliser (Klavins *et al.*, 2004; Peña-Méndez *et al.*, 2005). Humic substances caused significant variation in the numbers of bacterial heterotrophs and autotrophic nitrifiers in the soil. Studies suggest that the mechanism through which humic acids affect both plant and soil microbes may chiefly involve enhancement of cell membrane permeability to nutrients (Valdrighi *et al.*, 1996). Physiological effects of humic substances (HS) on some aspects of plant growth and me-

tabolism depend on the source of HAs, concentration and molecular weight humic fraction (Nardi *et al.*, 2002).

Comparison of vermicompost and peat extracts in potassium humate properties is important to understand their differences, variability and potential effects to crops. Spectral characterisation methods were selected to study vermicompost and peat extract properties. The shape of UV-Vis spectra (Fig. 2) revealed a monotonic decrease with increasing wavelength. Presence of a shoulder between 240 and 290 nm is characteristic of aromatic or unsaturated compounds (conjugation of quinones and ketones) ($C=C$, $C=O$, $N=N$) (Chin *et al.*, 1994). Absorption as well as shoulders of UV-Vis spectra of extracts indicate higher amounts of ketones in peat extract and higher humic acid concentration (Table 4) in peat extract.

Analysed fluorescence spectra of potassium humates shows similarity in origin of bulk material. All spectra showed a sharp peak at 450 nm and shoulder at 480 nm (Fig. 3). The emission fluorescence and spectra region 440–480 nm is influenced by polycondensation between carbonyl group-containing substances and amines, or phenolic structures (Chen *et al.*, 2002).

Fluorescence describes aromaticity of extracts, which is higher for extracts from vermicompost and thermally treated samples. These results correspond to ratios E_4/E_6 and $E_{2/6}$, which are inversely related to aromaticity of the material and relation between humified and non-humified material (Albrecht *et al.*, 2011; Chen *et al.*, 1977) (Table 2). The fluorescence spectra shoulder at 485–510 nm shows degradation products of lignite, which is typical for soil organic material. For example, phenolic components are formed from plant lignin during biological degradation and are an essential part of soil humic material (Maciak and Harms, 1986). Fluorescence spectra as well as ratios E_4/E_6 and $E_{2/6}$ show significant differences between the extracts and the effects of extraction conditions due to the used peat and biohumus and their structural changes during extraction procedure using cavitation. Extracts differ in respect to quantities of aliphatic structures and condensed aromatic structures (Table 3), and in the amount of humified material. Thermal treatment during extraction causes loss of aliphatic compounds and hydrophobicity and also loss of organic matter (Table 4). It could be suggested that hydrolysis and oxidation of organic matter occurs during extraction and storage.

The FT-IR spectrum of the extracts obtained in this experiment were similar. A broad, intense band was present at 3500–3200 cm^{-1} , corresponding to OH stretching (carboxylic, phenolic and alcoholic) associated with inter- and intra-molecular hydrogen bonding. (Fig. 4) The bands at 2936 and 2850 cm^{-1} are associated with symmetric and asymmetric aliphatic CH stretching, while the band at 1750 cm^{-1} can be assigned to $C=O$ stretching of aldehydes or ketones. The band at 1649 cm^{-1} is also due to $C=O$ stretching, but of quinones and amides. Bands characteristic of $C=C$ and $C=N$ stretching of aromatic structures and amides and

CN deformation are present at 1560 and 1466 cm^{-1} . The bands at 1266 and 1166 cm^{-1} can be attributed to CO stretching, OH deformation of carboxylic acids and CO stretching of phenols and esters. The band observed at 1044 cm^{-1} can be assigned to CO stretching of ester groups and the remains of polysaccharides (García *et al.*, 2012).

Peat extracts have more aldehydes, ketones, amides and carboxylic groups, but less esters and remains of polysaccharides. This is due to the humification processes for peat and vermicompost. Lower amount of phenols in 2011 vermicompost extracts indicates that the bulk material has been significantly more decomposed, in relation to the calculated ratios E_4/E_6 and $E_{2/6}$.

Transmittance ratio $T_{3400/2900}$ shows differences of extracts (Table 3) to O-H stretching at 3400 cm^{-1} against C-H stretching at 2900 cm^{-1} characterising hydroxyl- and phenol hydroxyl group and aliphatic compound relationships. Differences between extracts are negligible, but significant differences occur between years due to bulk material composition.

As it is seen from HA/FA ratio (Table 4), vermicompost extracts contains higher concentration of fulvic acids than that of peat, which was increased by heating. Concentration of carboxyl groups in vermicompost extract was higher than in peat extracts. Total nitrogen in peat extract was two times less than in vermicompost (Table 4). Also vermicompost had significantly higher concentration of NO_2^- and NH_4^+ .

Potassium humates extracted from peat and earthworm biohumus with cavitation showed relatively high similarity with respect to their spectroscopic characteristics. Differences between extract properties may vary depending on thermal treatment and properties of vermicompost and peat. Differences were found in amounts of aromatic, aliphatic and phenolic structures in extracted material.

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KŪDRAS UN SLIEKU BIOHUMUSA IZVILKUMU SALĪDZINĀJUMS

Izmantojot kavitācijas metodi, iegūti lauksaimniecībā izmantojami kūdras un slieku biohumusa humīnvielu izvilkumi. Izvilkumi gatavoti divas reizes — 2011. un 2012. gada pavasarī. Kūdra ņemta Kurzemes piejūras zemienē, slieku biohumuss — no Talsu novada Valdgales pagasta sliekkopības uzņēmuma SIA „Kvarki”. No katra substrāta ņemts ieguves procesa 45 °C temperatūrā iegūts un 95 °C temperatūrā karsēts paraugs. Visiem pētītajiem izvilkumiem bija līdzīgs sausnes saturs. Izvilkumi tika analizēti izmantojot ultravioletās-redzamās gaismas (UV-Vis), infrasarkanā (FT-IR) un fluorescences spektrometriju, bet multielementu analīze veikta ar atomu absorbcijas spektrometriju. Šķīdumiem noteikts skābums (pH), elektrovadītspēja, sausnes saturs, humusvielu koncentrācija, noteikta karboksilgrupu koncentrācija un, izmantojot ūdens-polietilēnglikola divfāzu sistēmu, noteikts šķīdumu hidroforums, izteikts kā sadalījuma koeficients. Noteiktas slāpekļa un fosfora kopējās koncentrācijas. Visu paraugu humusvielu saturs, kā jau dabas produktā, ir nedaudz svārstīgs dažādās gatavošanas reizēs, tāpat nedaudz mainās karsējot. No slieku biohumusa iegūtajos 2012. gada izvilkumos kopējais slāpeklis bija ievērojami lielāks nekā 2011. gadā, kas, acīmredzot, izskaidrojams ar atšķirībām to barības bāzē. Kopējā fosfora būtiskas atšķirības starp gatavošanas reizēm konstatētas gan slieku humusa, gan arī kūdras izvilkumiem. Karsētajiem paraugiem samazinās hidroforums, kūdras izvilkumiem nedaudz, bet slieku biohumusa izvilkumiem vairāk kā divas reizes, taču karboksilgrupu daudzums izolētās humīnvielās praktiski nav mainījies. UV spektru analīze parāda ievērojamas atšķirības paraugu humificētā sastāva ziņā.