Spectroscopic, Textural and Thermal Characterization Methods of Biostimulants Based on Sodium Humate

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Plant biostimulants, an emerging class of agricultural inputs, are complex products. The reproducibility of their specific action on plant metabolism and plant physiology, which lead to an enhanced nutrient use efficiency, stress tolerance and edible yield quality, is still a challenge. Development of quality insurance systems for plant biostimulants need complex investigation based on adapted analytical, physico-chemical and chemical methods. The objective of this work was to characterize commercial humate biostimulants through different analytical techniques (Fourier transform infrared spectroscopy - FTIR, thermogravimetric analysis- TGA) and to evaluate their textural and chemical (pH, C, N, humic acids, inorganic components) parameters. The first derivative curve from TG analysis showed decomposition of different compounds, classified according to the results obtained by FTIR. The humic substances determined by TGA method was comparable with the results obtained by gravimetric reference method. The inductively coupled plasma-optical emission spectrometry (ICP-OES) technique was applied to determine the inorganic elements either from the production process of humate or from raw materials, as well as for the control of humate in terms of requirements for safety and quality. Their complementary properties obtaining through different analytical techniques provide essential information on the chemical characteristics of the humate plant biostimulant formulations.

Keywords: sodium humate; humic acids, FTIR, TGA, BET, ICP-OES, biostimulant

Plant biostimulants represent a new class of products used as inputs in the plant cultivation technologies, which act on plant biochemistry and physiology [1-3], increasing the water and nutrient uptake and use efficiency, enhancing the tolerance of cultivated plants to biotic and abiotic stress, improving crop quality, mainly due to a higher accumulation of the bioactive compounds into edible yield [4]. Most biostimulants currently used are complex mixtures of (bio)chemicals defined as *a formulated product of biological origin that improves plant productivity as a consequence of the novel, or emergent properties of the complex of constituents, and not as a sole consequence of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds* [5].

The main categories of biostimulants for plants, other than plant beneficial microorganisms [6] are: humic and fulvic acids [7]; protein hydrolysates / peptides and amino acids [8] and other amino derivatives compounds such as glycine-betaine [9]; algae [10, 11] and plants extracts [12]; beneficial elements [13], especially soluble silicon / silicic acid [14] and inorganic compounds as phosphite [15]; chitosan [16] and other biopolymers [17].

Humic substances (HS) are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues, but also from the metabolic activity of soil microbes using these substrates. HS are collections of heterogeneous compounds, originally categorized according to their molecular weights and solubility into humins, humic acids and fulvic acids. Humic substances have been recognized for long as essential contributors to soil fertility, acting on physical, physico-chemical, chemical and biological properties of the soil. Most biostimulant effects of HS refer to the amelioration of root nutrition, via different mechanisms [18].

Clearly, the reproducibility of the biological action of these bioproducts obtained from natural/renewable raw materials depends on their characterization and standardization. In order to achieve such characterization, which is indispensable for defining the regulatory framework for these bioproducts it is necessary to develop fast and accessible methods of analysis, used both in the obtaining process of these bioproducts and to demonstrate conformity with essential requirements of products for safety and quality.

The need for standardization is reflected by the establishment of a new CEN Technical Committe*e Plant Biostimulants and Agricultural Micro-organisms*. This technical committee in the field of plant biostimulants and agricultural micro-organisms comes in support of EC proposal for the elaboration a new regulation which will extend the scope of the previous fertilizers regulation to several new product families, including biostimulants [2].

Different studies [19-21] have shown methodological approaches for the characterization of biostimulants, e.g. those based on brown macro-algae.

Despite the fact that comprehensive information on plant growth and yield studies of biostimulants exist, the chemical composition of these complex mixtures is much more difficult to obtain. The objective of this study was to characterize two commercial humate and its major humic fraction (humic acids) using the chemical, textural, thermic and spectroscopic methods. The humate biostimulants characterization was done considering the corroboration of the information obtained through different analytical techniques FTIR, BET, TGA, TOC/TN_b, ICP-OES. The results of our work can be useful for small and medium enterprises (SME), which are manufacturers of humates, for characterization of their bioproducts.

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Experimental part

Materials and methods

Chemicals and biostimulant formulations

The humic acids standard was purchased from Sigma-Aldrich, sodium hydroxide from Scharlau and hydrochloric acid from Merck. The Certipur ICP-element standard solution IV (Merck) and the Phosphor ICP standard (Merck), both with concentrations of 1000 mg.L^{-1} were used for prepared standard solutions. The digestions of sodium humate samples were done using HNO_3 65% and HF 48% purchased from Scharlau. Doubly distilled water was used to prepare the standard solutions. All reagents used in the experiments were of analytical grade. The commercial biostimulant formulation evaluated in this study was liquid (LH) and solid humate (SH).

Reference method

Humic substances determined by TGA method which is described below is based on the gravimetric method as a reference method. The alkaline extraction, separation, and determination of humic acids were performed as described in this reference [22].

Fourier transform infrared spectroscopy

The Fourier Transform Infrared Spectroscopy method, FTIR, was used both by transmission technique, in KBr pellet for humic and fulvic acids and humate samples. Spectrum recordings were done on an Perkin Elmer FTIR Spectrum GX apparatus, from 4000 cm^{-1} to 400 cm^{-1} accumulating 32 spectra, at a resolution of 4 cm^{-1}.

Textural characterization

Nitrogen physisorption was used to characterize and compare the textural properties of a humic acid standard and a commercial humate. The textural characteristics of the two samples were evaluated from nitrogen adsorption/desorption isotherms recorded at the liquid nitrogen temperature using a Quantachrome Nova 2200e equipment. The standard Brunauer-Emmett-Teller (BET) equation was applied to calculate the specific surface area of the analyzed samples. The total pore volume was estimated from the amount of gas adsorbed at a relative pressure (p/po) value close to unity. The pore size distribution was determined from the adsorption branch of the isotherm using Barrett-Joyner-Halenda (BJH) model. The t-plot method was used to estimate the external surface of the humic materials [23]. Prior to adsorption measurements, amounts of 90-100 mg of each sample were vacuum - degassed at room temperature for 24 h. The experimental data processing was performed using Nova Win version 11.03 software.

Thermogravimetric analysis

Analysis of the humate biostimulants and the humic acids standard was carried out using a TGA/SDTA 851 thermogravimetric analyser (Mettler Toledo). The samples and reference material were heated from room temperature to 900°C in alumina crucibles at a heating rate of 20°C min^{-1} in air at a flow rate of 50 mL min^{-1}. The thermal weight loss characteristics were observed as thermograms and quantitative evaluations were carried out on the curves using STARe evaluation software as derivative thermograms.

Chemical analysis

The *p*H of the formulation was measured using 10 % aqueous solution of the original product.

The elemental compositions of the samples were determined by inductively coupled plasma-optical emission spectroscopy (Optima 2100 DV ICP-OES System) instrument. Humate samples were digested using Multiwave 3000 (Anton Paar) model microwave digestion system. The RF power used was 1300 watts, plasma flow was 15 L/min, Auxiliary gas flow was kept at 0.2 L/min, Nebulizer Flow was kept at 0.8 L/min, and Pump Flow Rate was kept at 1.5 L/min. Plasma view was in the axial/radial mode. Approximately 0.2 g of sample was weighed and transferred into a pressure-resistant PTFE (polytetrafluoroethylene) vessel, and the mixture of acids (65 % HNO_3 + 48 % HF, 10:2 mL) was added. The procedure mentioned above was following by the complexation stage with boric acid (H_3BO_3). Boric acid is added following digestion to complex F^- in solution. The reaction mixture was subjected to an evaporation procedure in order to remove the acids after the final digestion. The ICP-OES measurements were performed for the diluted solutions. The standard solutions for calibration curves were made from reference standards Certipur ICP-element standard solution IV of 1000 mg/L. Determination of Cd, Co, Cr, Cu, Ni, Pb was achieved by constructing a multipoint standard curve covering the range of analyte concentrations in samples (2-100 µg.L^{-1}). Analytical determination of selenium it was done on a calibration curve in the range 50- 300 µg.L^{-1}. Phosphorus analysis was performed on a calibrated curve containing standards with concentrations of 0.3-1 mg.L^{-1}.

The determinations of TC (Total Carbon), TIC (Total Inorganic Carbon), TOC (Total Organic Carbon) were made using Multi N/C 2100 Analytic Jena AG-Germany. Standardized methods were performed as described in this reference [24, 25]. After dilution, the samples were analyzed by difference method. The TOC is obtained by calculating the difference between TC and TIC. The carbon and nitrogen content of the samples were calculated using a previously determined calibration function.

Results and discussions

Fourier transform infrared spectroscopy

The FTIR spectra of humic and fulvic acids have basically the same types of absorption bands, and their spectra differs mainly by their relative intensity of this bands. For this reason all the spectra have been normalized and their baseline, corrected. The main recorded bands for humic acids, compared to those reported in the numerous literature studies [26- 29], figure 1, are: a broad band at 3600 cm^{-1} to 3200 cm^{-1} corresponding to the H-OH bond from alcohols, phenols and organic acids, as well as intermolecular and/or intramolecular hydrogen bonds, and also to N-H groups; two bands at 2923 cm^{-1} and at 2852 cm^{-1} corresponding to the stretch vibration of the -CH_2 group in the alkyl structures; a broad band at 1800 cm^{-1} to 1500 cm^{-1}, with a shoulder at 1622 cm^{-1} corresponding mainly to the C = C aromatic linkages and/or to the C = O group of Amide (I), ketone or quinone.

Also, a second shoulder is recorded at 1571 cm^{-1} to 1557 cm^{-1} characteristic to the C = O group of the Amide (II); a less intense band between 1459 cm^{-1} and 1377 cm^{-1} corresponding to several chemical groups such as: -CH_3, -OH phenolic, COO- and/or ortho-disubstituted aromatic ring; a light band at 1272 cm^{-1} produced by Amide (III) and/or an ether as well as a broad band between 1240 cm^{-1} and 1140 cm^{-1} and between 1100 cm^{-1} and 950 cm^{-1}, with sharp peaks centered at 1191 cm^{-1}, at 1096 cm^{-1}, at 1032 cm^{-1} and at 1009cm^{-1}, which generally characterize aromatic ethers, but also carbohydrates or silicates, which also absorb in these spectral zones. These characteristic absorption bands confirm the presence of strongly oxidized

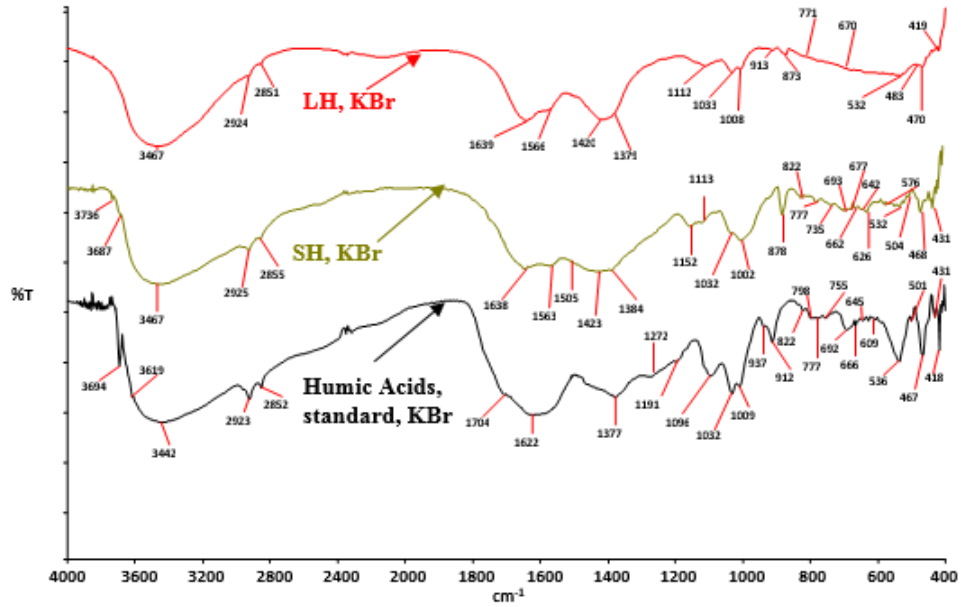

Fig.1. The FTIR spectra of liquid humate (LH); solid humate (SH) and humic acids standard in KBr

humic acids, which is the basic structure of fulvic acids, which are in very good concordance with the literature [30-32]. The humates samples were analyzed both by transmission technique, in KBr pellet, and by attenuated total reflectance, in order to find out which spectral technique provides more information for their characterization. More informations have been obtained by transmission technique, and the main recorded absorption bands, in concordance with literature [33-37]. As a general feature, spectrum has a high absorption intensity for the three types of bands, namely: at 3400 cm^{-1}, at 1650 cm^{-1} and at 1034 cm^{-1}. The first corresponds to the hydrogen bond, H-OH, and/or the intermolecular and/or intramolecular OH bond, of OH ... OH type, and/or the NH group, the second occurs mainly in benzoic compounds and the third band mainly belongs to the C-O-C etheric bond.

Textural characterization

The N_2 adsorption/desorption isotherms of humic acid and sodium humate (not shown here) are quite similar representing a combination of II and IV type isotherms according to IUPAC classification and indicate the mesoporous character of the two samples [38].

The textural parameters characterizing the porous structure and pore size distributions of the analyzed samples are presented in table 1 and figure 2.

Table 1

TEXTURAL PARAMETERS OF HUMIC ACIDS STANDARD AND SODIUM HUMATE SAMPLE (SH)

Sample	S_{BET} (m^2/g)	S_{ext} (m^2/g)	V_t (cc/g)	V_M (cc/g)	D_p (nm)
Humic acids	2.59	2.59	0.009	0.009	3.54
Sodium humate	1.45	1.45	0.003	0.003	4.29

S_{BET}: BET specific surface area; S_{ext}: t-plot external surface area; V_t: volume of gas adsorbed at p/p_o ~1.0 ; V_M: BJH mesopores volume; D_p: BJH mesopores diameter.

As can be seen in table 1, the both samples have low specific surface areas. The surface area and total pore volume of the sodium humate are smaller (1.45 m^2/g, 0.003 cc/g)) as compared to humic acid (2.59 m^2/g, 0.009 cc/g). Generally, the surface areas of humic materials can vary in a large range in connection with their particular structure, composition, particle size, etc. [39, 41]. Our data suggest that the investigated samples may have low chelating capacity with metal ions and low interaction with different

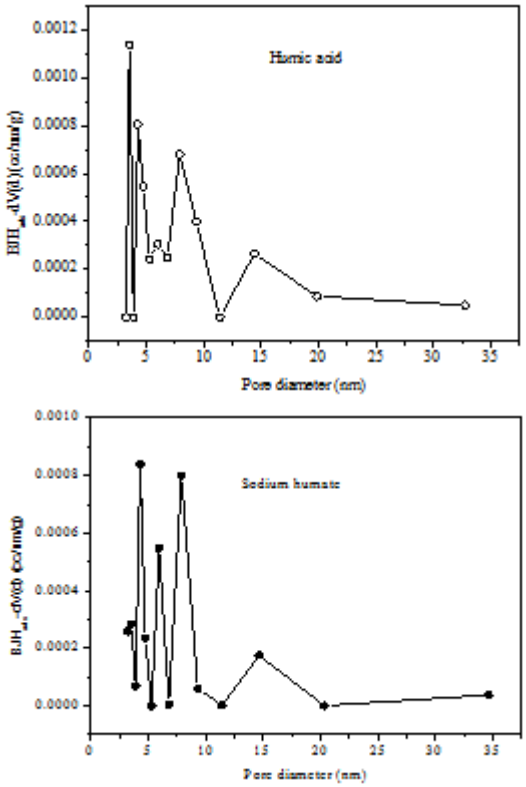

Fig.2.Pore size distributions of humic acids standard and sodium humate (SH)

organic compounds, which is due to their small specific surface areas. The pore size distributions in figure 2, evidence the mesoporosity of the humic acid and sodium humate investigated in this study. As can be seen in figure 2, humic acid has a quite narrow mesopores size distribution with a maximum situated at 3.54 nm. Moreover, a significant proportion of larger mesopores of 5-20 nm is observed in humic acid.

The sodium humate sample is characterized by a multimodal pore size distribution in the *mezzo* region evidenced by the highest maximum centered at 4.29 nm followed by the other two quite intense maxima situated at 7 nm and 9 nm, respectively.

As compared to humic acid, the presence of larger mesopores (10-20 nm) is also observed in sodium humate sample. It can be noted that the larger mesopores in sodium humate might result from the widening of the original mesopores during the activation process of the humic acid [40].

Sample/standard	Determination	Results, %
Humic acids standard	Mass loss between 25-200 ^{0}C	2.0
	Mass loss between 200-385 ^{0}C	15.4
	Mass loss between 385-900 ^{0}C	50.3
	Residue at 900 ^{0}C	32.3
Solide humate	Mass loss between 25-280 ^{0}C	12.7
	Mass loss between 280-615 ^{0}C	10.0
	Mass loss between 615-900 ^{0}C	21.7
	Residue at 900 ^{0}C	55.6
Liquid humate	Mass loss between 25-150 ^{0}C	86.1
	Mass loss between 150-550 ^{0}C	1.5
	Mass loss between 550-900 ^{0}C	4.4
	Residue at 900 ^{0}C	8.0

Table 2
TGA RESULTS OF HUMIC ACID STANDARD AND HUMATE SAMPLES

Thermogravimetric analysis

Thermogravimetric (TG) and the corresponding differential thermogravimetric (DTG) curves for humic acids reference standard and humate samples are shown in figure 3. In the DTG curves can be observed three regions with peaks between 25-150 ^{0}C, 150-385^{0}C, 385-900 ^{0}C for humic acids standard and 25-280 ^{0}C, 280-615 ^{0}C, 615-900 ^{0}C for SH (table 2).

The DTG curve of humic acids standard presents a small peak around 100 ^{0}C and two exothermic peaks at about 340 ^{0}C and at about 470^{0}C. The DTG curves of the solid humate sample exhibit a peak at 104 ^{0}C, small exothermic peaks at about 320^{0}C and at about 460 ^{0}C and a strong exothermic peak at 708 ^{0}C. Whereas the decomposition reactions of humic substances are not well separated the assignments of the peaks of DTG curves was performed considering the results obtained from FTIR corroborated with experiments previously reported [42].

The first step is attributed to the elimination of moisture for humic acids (HAs) and fulvic acids (FAs), the second step is due to decarboxylation and dehydration and the third step with peaks in 400-780 ^{0}C can be attributed to the decomposition of condensed aromatic nucleus. The thermostable organo-mineral compounds from FAs are decomposed at higher temperature [42].

Humic substances determined by TGA method was based on the mass losses of the humic acid standard and humate samples and knowing the humic acids content of the standard determined by the reference method. Humic substances of the formulations determined by TGA method were 8.0 % (LH) and 69.0 % (SH) the differences from those obtained by the reference method may be due to the presence of other substances like fulvic acids. The HS were evaluated with this quick, cheap method, which does not require a complex preparation of the sample, the results obtained being comparable to those determined by the reference method (gravimetric).

Chemical analysis

The compositions of liquid humate (LH) and solid humate (SH) determined by gravimetric and TOC/TN_b methods are summarized in table 3.

The gravimetric dry matter (DM) content of the formulation was 12.1 (LH) and 91.4 % (SH). Carbon total (TC) concentration of LH and SH samples obtained by TOC/TN_b method was 3.34% and 22.6 %, while N concentration was 0.08% and 0.6% with the resultant C/N ratios of 41.8 to 37.7 (Table 3). Similar observations have been reported by other authors [43].

The variations of inorganic elements listed in table 4, showing a relationship between high residue contents of the formulations and high concentrations of sodium attributed to the production process of the humate samples.

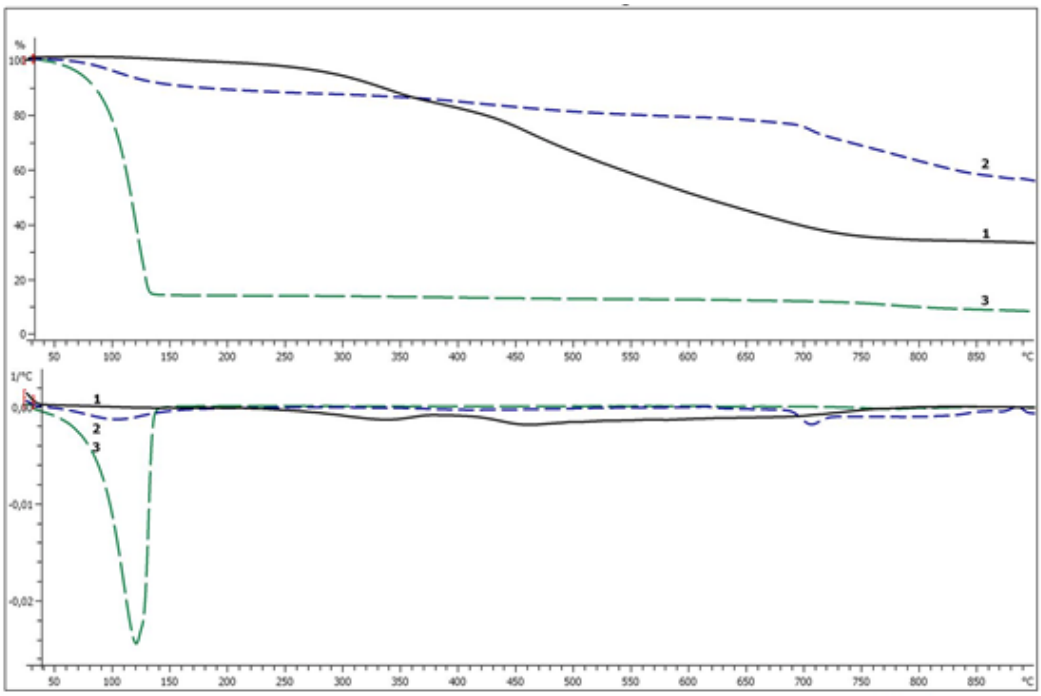

Fig. 3. The TG curves of 1- humic acids standard, 2- solid (SH) and 3-liquid humate (LH)

Formulation		Liquid Humate	Solid humate
pH		11.7	12.1
Dry matter, %		12.08	91.38
TIC, %		0.24	1.8
TC, %		3.34	22.6
TOC, %		3.10	20.8
TN, %		0.08	0.6
C:N ratio		41.8	37.7
Reference method	Humic acids, %	6.49	65.9
TGA	Humic substances, %	8.0	69.0

Table 3
CHEMICAL CHARACTERISTICS OF DIFFERENT FORMULATIONS

Sample	Element	ICP-OES Wavelength (nm)	Concentration	Unit
Solid humate	Na	589.592	8.76	% (w/w)
	Mg	285.213	0.238	% (w/w)
	Ca	422.673	1.07	% (w/w)
	K	766.490	0.58	% (w/w)
	Al	396.153	1.51	% (w/w)
	Fe	238.204	1.14	% (w/w)
	Zn	213.857	0.027	% (w/w)
	Mn	257.610	72.8	mg/kg
	As	228.812	0.518	mg/kg
	Cd	228.802	< 1.3*	mg/kg
	Co	228.616	< 0.8*	mg/kg
	Cr	267.716	< 0.9*	mg/kg
	Cu	327.393	< 0.9*	mg/kg
	Ni	231.604	< 2.1*	mg/kg
	Pb	220.353	7.69	mg/kg
	Se	196.026	37.2	mg/kg
	P	213.617	< 1.1*	mg/kg
Liquid humate	Na	589.592	1.04	% (w/w)
	Mg	285.213	0.027	% (w/w)
	Ca	422.673	0.089	% (w/w)
	K	766.490	0.011	mg/kg
	Al	396.153	0.090	% (w/w)
	Fe	238.204	0.043	% (w/w)
	Zn	213.857	4.29	mg/kg
	Mn	257.610	6.99	mg/kg
	As	228.812	0.377	mg/kg
	Cd	228.802	< 1.3*	mg/kg
	Co	228.616	< 0.8*	mg/kg
	Cr	267.716	< 0.9*	mg/kg
	Cu	327.393	< 0.9*	mg/kg
	Ni	231.604	< 2.1*	mg/kg
	Pb	220.353	4.47	mg/kg
	Se	196.026	30.1	mg/kg
	P	213.617	< 1.1*	mg/kg

Table 4
MEASURED CONCENTRATIONS OF METALS FROM SODIUM HUMATE MATERIALS BY ICP-OES

*The limit of quantitation (LOQ) for determination of metals by (ICP-OES)

The concentrations of Cd, Cr, Pb, Ni can be assimilated to the raw materials being consistent with Annex 3 - Initial analysis for consideration by the CEN/TC *Plant Biostimulants and Agricultural MicroOrganisms* where is indicated for cadmium, Cr VI and lead a content of max. 3 mg/kg dry matter, 2 mg/kg dry matter and 1020 mg/kg dry matter respectively [2].

Conclusions

Commercial humate, were characterized by various chemical, textural and instrumental methods. The results presented show that through the applied analytical techniques (FTIR, BET, TGA, TOC/TN_b, ICP-OES, gravimetry) the characterization the sodium humate samples was done based on their complementary chemical properties. The identification of major compounds was done by FTIR. As a general characterization of FTIR spectra for each of the three studied samples, one humic acids standard from Sigma Aldrich, and two commercial humates products, there have been observed three spectral zones: at about 3400 cm^{-1} for hydrogen bond, and/or H-OH, and/or the NH group, at about 1650 cm^{-1} mainly for unsaturated compounds, and at about 1034 cm^{-1} mainly for the C-O-C etheric bond. The humic acids are characterized especially

by presence of C=O bound from COO^- groups at about 1704 cm^{-1} and of OH bound from COOH groups at about 912 cm^{-1}. The sodium humates are characterized by presence of C=O bound from Amide I and/or aromatic C=C skeletal at about 1638 cm^{-1} and of N-H deformation and C=N stretching of Amide II band at about 1563 cm^{-1}.

Nitrogen physisorption data have evidenced the mesoporous structure of the humic acid and sodium humate. Taking into account the small specific surface areas, a limited number of organic compounds can be adsorbed on the investigated materials. Both humic acid and sodium humate have quite narrow mesopores size distributions. The larger mesopores size of sodium humate (4.29 nm) as compared to humic acid (3.54 nm) can be connected to the used activation process.

The quantitative information about organic and inorganic fractions from humate was obtained by TGA analysis. Humic substances of the formulations determined by TGA method were 8.0 % (LH) and 69.0 % (SH) the differences (1.5% for LH and 3.1% for SH) from those obtained by the reference method may be due to the presence of other substances like fulvic acids. The HS were evaluated with this quick, cheap method, which does not require a complex preparation of the sample.

Useful information for biostimulant producers results from the N content of the humate samples which in our case is much lower than the value reported by Schnitzer [44] of 3.2%, for *ideal* humic acid soil makes simultaneous sources of inorganic N indispensable to prevent N deficiency in crops treated with these biostimulants.

The ICP-OES technique complete the information about the inorganic fraction thus sodium was assigned to the production process of humate biostimulants and Mg, Se, As, Cd, Co, Cr, Cu, Pb, Ni and P were attributed to the humate raw materials.

The applied methods can be useful for humates caracterization necessary for the production of biostimulants with reproducible biological activity.

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