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Application of a modified method of humic acids extraction as an efficient process in the production of formulations for agricultural purposes

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Due to their properties, humic acids (HA) can interact with macro- and micronutrients and retain plant nutrients in the soil profile. The aim of the presented work was to develop a new integrated 4-step process for the isolation of humic acids from peat with their simultaneous enrichment in macronutrients. The study demonstrated the possibility of replacing traditional solutions used in the extraction of humic acids with solutions that are a source of macronutrients. For the alkaline extraction step, a process assisted with ultrasound was implemented, which allowed to increase the efficiency of humic acids isolation. Optimization of this step has shown that, with the application of ultrasound in the new procedure, it is possible to obtain the humic acids isolation efficiency of more than 60%. The qualitative analysis of the products obtained confirmed the presence of structures characteristic of humic acids, including key functional groups.

Keywords: Humic acids (HA), Peat, Ultrasound-assisted extraction, Fertilizers, Process optimization.

INTRODUCTION

One of the main problems of Polish agriculture is the poor quality of the soils, determined mainly by the type of bedrock. More than 70% of Poland's soils were formed on the basis of clays and till sands of the Pleistocene epoch. More than 40% of them are estimated to be characterised by low agricultural suitability, which is reflected in the low production potential of an average hectare of arable land in Poland, compared to the soils of countries of the European Union[1, 2].

In addition, in some soil profiles, due to global warming, climate change, and agricultural intensification, which causes, among other things, dryness, acid or alkaline reaction, and lack of organic matter in the soil, nutrient uptake by plants can be limited. Humic substances (HS) have begun to be added to soil in different regions of the world to address this problem and increase agricultural yields[3–6]. These complex organic compounds play an important role in the process of forming soil structure. The presence in the soil of humus substances, including humic acids (HA), affects the structure of the soil, increasing its sorption properties, permeability, and water retention capacity, which is one of the factors that determine the efficiency of water uptake by plant root systems, as well as mineral nutrients supplied by fertilisation[7, 8]. In addition, by improving the physical and chemical properties of soil, the diversity of soil microorganisms and soil enzymatic activity is also increased[9]. HA exhibits the ability to stabilise ammonium, which improves the availability of nitrogen in the soil. The application of humic acids indirectly increases the solubilization of phosphorus in the soil, reduces the sorption, and increases the desorption of phosphate ions in the soil, thereby increasing the amount of phosphorus in the soil solution[6, 10].

The process of isolating humic acids is a multistep procedure, which involves the preparation of raw material, alkaline extraction, and separation of humic acids from the extract by lowering the pH. In the alkaline extraction step, in addition to traditional extraction, where mass transfer is intensified by mechanical stirring, ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE) methods are used. This allows a similar amount of humic substances to be obtained from a given mass of raw material in much shorter time[11–15]. The UAE is characterized by high reproducibility, consumption of a small amount of energy, reduction of wastewater, and the possibility of introducing this type of support into an industrial process. During UAE, processes such as fragmentation, erosion, ultrasonic capillary effect, sonoporation, local shear stress, or detexturation occur simultaneously or sequentially. These occur due to the propagation of ultrasonic waves in the extraction medium, resulting in the formation of cavitation bubbles in the medium. The implosion of such bubbles causes exfoliation of the sample surface, erosion, and disintegration, as well as inducing macroturbulence and micromixing. All of these effects increase the reactivity of the medium and mass transfer due to reduced particle size and facilitated solvent access[16–17].

HS, available from many fertilizer manufacturers or specialized supply companies, comes in many different forms (granules, powder, liquid, and flakes), that is vary due to the application technique, i.e., soil application, foliar application, seed treatment, root dipping, drip irrigation, and application along with mineral fertilisers. The combined use of HA and mineral fertilizers alleviates the problems associated with continuous cropping systems. With HA, the number of basic macronutrients (nitrogen, phosphorus, and potassium) and organic matter in the soil available to plants increases, resulting in an increased uptake of macronutrients by plants. Various HS salts, such as potassium and calcium humate, are used as fertilizer additives to increase soil fertility. Increasing the level of natural HS, such as potassium humate, reduces the need for commercial fertilizers as it improves soil fertility[18].

This study aimed to implement an integrated procedure for the isolation of humic acids with their simultaneous enrichment with macronutrients. Furthermore, to intensify the isolation process, the alkaline extraction step was assisted by the use of ultrasound, and statistical analysis was implemented to evaluate the effect of the process parameters of that part of the procedure on

the efficiency of the HA isolation. On the basis of the attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR) for the selected samples, a qualitative analysis of HA was performed. The developed procedure of humic acid isolation allowed for the obtainment of HS with an increase in nitrogen, phosphorus, and potassium content, eliminating the necessity to combine HS products with mineral fertilisers, which is the primary method of application of humic acids in agriculture.

MATERIALS AND METHODS

Raw material and chemicals

In the presented study, peat was used as a source of humic acids. The raw material was taken from a peat bog located in the northern part of Poland. On the basis of its structure analysis, it was classified as pseudofibrus peat. To prepare it for the extraction process, the raw material was air-dried and ground, using an analytical mill, and passed through a sieve with a mesh diameter of 2 mm. The peat thus prepared was stored in a tightly closed polypropylene container.

For the isolation of humic acids, chemicals have been used that were carriers of fertilizer macronutrients. For this purpose, KOH, $NH_3 \cdot H_2O$, H_3PO_4 and HNO_3 solutions were used. Their concentrations depended on the type of process variant and on the alkaline extraction step conditions described by the experimental matrix. The solutions were prepared with the use of deionised water (conductivity <1 $\mu S \cdot cm^{-1}$) and analytical grade reagents: solid potassium hydroxide, ammonia (25 %mass), phosphoric acid (85 %mass), and nitric acid (65 %mass), which were purchased from POCH (Avantor Performance Material, Gliwice, Poland).

Procedure of humic acids isolation

For the implementation of the integrated process of extraction of humic acids and enrichment with fertilisers macronutrients (nitrogen, phosphorus, and potassium), four variants of the humic acids isolation procedure were considered. They differed in the type of acid or base solution that was introduced into the system at a certain stage of the process (Table 1).

Isolation of the humic acids from peat based on the procedure described by Swift[19] and recommended by the International Humic Substances Society (IHSS) with some modifications. Generally, the procedure was divided into four steps. The aim of the first of it was to decalcify the peat with the use of acid solutions. In a 1 liter polypropylene bottle, 15 grams of peat were placed and acidified to a pH equal to 2 by adding 1 M nitric or phosphoric acid (depending on the process variant). Subsequently, 0.1 M acid solution was added to the mixture, in such an amount that the ratio of acid solution to raw material mass was equal to 10:1. The bottle with acidified peat was shaken for 1 hour at room temperature, with the use of a water bath shaker. After the decalcification part, the peat was separated by sedimentation and solution decantation. In the second step of the proposed process, the peat was neutralised to pH = 7 by adding, depending on the variant, 1 M KOH or $NH_3 \cdot H_2O$.

The third part of the HA isolation procedure focused on the alkaline extraction of humic acids from decalcified and neutralized peat. It was the essential step for the whole process because it allowed for the transfer of humic acid from the solid raw material to the liquid phase (extract). The ratio of peat mass to extractant volume was equal to 1:15. For this step ultrasound-assisted extraction (UAE) was implemented. It allowed for increasing the efficiency of the isolation of humic acids, which was proved in our previous study[20]. In this part, the influence of various values of UAE conditions, such as temperature, ultrasound efficiency, extraction time, and extractant concentration, on the efficiency of HA isolation was examined. Next, based on the analysis of the results, for the variant that resulted in the highest efficiency for the tested points, the process modelling, using fractional factorial design (FrFD), was implemented.

After the extraction, the phases were separated by centrifugation (15 min with 2000 rpm). For this process, the MPW-352 centrifuge was used. Moreover, after this step, the extract was vacuum-filtered with the use of a hard qualitative paper laboratory filter. The additional vacuum filtration step allowed the separation of solid elements that did not separate after the centrifugation process.

The last step of the described method was associated with the precipitation of the humic acids fraction from the extract by its acidification to a pH of approximately 2 with the use of 1 M HNO_3 or H_3PO_4 solution. After the acid addition, the extract was stored at 5 °C for 12 hours. The precipitated HA fraction was then isolated by filtration with the use of quantitative filter papers and dried to the mass constant.

The efficiency of HA isolation was calculated using Equation (1), where HA^{daf} represents the amount of humic acids isolated from peat, calculated for the dry ash-free state, %mass, m_1, m_2 and m_3 means the mass of the HA fraction after its drying, g, the mass of ash after burning of the humic acids, g, and the mass of the peat, g, respectively. M is the moisture content, %mass, and A is the ash content, %mass. The results were related to the mass of raw material in the dry ash-free state. Because of this, the moisture and ash content of the analysed samples was determined by drying them at 105 °C and then burning them at 650 °C to a constant mass.

$$HA^{daf} = \frac{10\,000 \cdot (m_1 - m_2)}{m_3 \cdot (100 - M - A)} \quad (1)$$

Table 1. Summary of the process variants to obtain humic acids enriched in fertilizer macronutrients

Variant designation	Type of solution used			
	Raw material decalcification (step A)	pH correction (step B)	Alkaline extraction (step C)	Extract acidification (step D)
I	H_3PO_4	KOH	$NH_3 \cdot H_2O$	HNO_3
II	HNO_3	KOH	$NH_3 \cdot H_2O$	H_3PO_4
III	H_3PO_4	$NH_3 \cdot H_2O$	KOH	HNO_3
IV	HNO_3	$NH_3 \cdot H_2O$	KOH	H_3PO_4

Macronutrients determination

To describe the influence of the chemicals used in the HA isolation procedure on the content of nitrogen, phosphorus, and potassium compounds in the humic acids obtained, the analysis of these macronutrients was performed in selected HA and peat samples.

The nitrogen compounds content was determined by the modified Kjeldahl method, which is recommended for organic samples[21]. The method consists of sample mineralization, resulting in the conversion of all nitrogen forms to ammonia. The ammonia is then distilled from the alkaline solution and absorbed in the known volume of the standard solution of H_2SO_4. The excess acid was titrated using a standard solution of NaOH, in the presence of an indicator. The nitrogen content may be calculated using Equation (2), where: N is the nitrogen content in all forms, %mass, V_1 and V_2 represent the volume of standard solution of 0.05 M H_2SO_4 and the volume of standard solution of 0.1 M NaOH, respectively, mol · dm^{-3}, and m is the mass of the sample, which was used in the analysis, g.

$$N = \frac{0.14 \cdot (V_1 - V_2)}{m} \tag{2}$$

The content of phosphorus was analyzed using a gravimetric method based on precipitation of phosphates in the form of magnesium-ammonia phosphate with the use of a magnesium mixture. The precipitate was then roasted at 800 °C to magnesium pyrophosphate and burnt at 1050 °C to a constant mass[22]. The phosphorus content in the samples analysed was determined using Equation (3), where P_2O_5 is the phosphorus content calculated in P_2O_5, %mass, m_1 and m_2 are the mass of the magnesium pyrophosphate ash after burning and the mass of the sample, respectively, g.

$$P_2O_5 = \frac{63.8 \cdot m_1}{m_2} \tag{3}$$

The measurement of the potassium content, after mineralization and dilution of the samples, was done using a flame photometer. The results were specified based on the calibration curve, determined in ppm, and converted to % of the mass of K_2O according to Equation (4), where K is the potassium concentration in the tested samples, ppm.

$$K_2O = 1.205 \cdot 0.01 \cdot K \tag{4}$$

Design of experiment

Based on the results of macronutrients content and, all the above, the efficiency of HA isolation for four considered variants, the best integrated procedure of humic acids isolation and their enriched with nitrogen, phosphorus, and potassium was chosen. The design of the experiment focused on the third step of the procedure related to the alkaline extraction of HA from the peat. The four crucial parameters for that step: alkaline extractant concentration, ultrasound intensity, extraction time, and temperature were examined. The influence of the parameters tested on the efficiency of HA isolation for peat was tested with the use of a fractional factorial design (FrFD), and each independent variable was determined in the experimental space by coded at three levels, which is presented in Table 2.

According to the FrFD for the conditions presented, the creation of an experimental matrix consisting of 27 points was required. Moreover, for the improvement of the quality of statistical analysis, and reducing the noise, the three extra points, which were characteristic of the central point of the experimental space, were added to the plan. Due to that, it was possible to increase the degree of freedom in the analysis of variance (ANOVA).

ATR/FTIR analysis

The attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FTIR) spectra were recorded using a Bruker Vertex 70 spectrometer for identification of the functional groups characteristic of the HA samples. They were mixed with KBr (approximately 2 mg of sample for 200 mg of KBr) and prepared as pellets. Spectra were collected in the 400–4000 cm^{-1} region at room temperature with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Peat characteristics

To evaluate peat as a raw material for the HA isolation process, moisture, ash, and macronutrient analyses were performed. The results are shown in Table 3. The evaluated parameters made it possible to determine the efficiency of HA isolation in terms of dry ash-free peat. Based on the determinations of the macronutrients of fertilizer in peat, it was possible to determine whether the nitrogen, phosphorus, and potassium compounds

Table 2. Independent variables values and codes for fractional factorial design levels

Independent variable	Unit	Designation	Values for coded levels		
			–1	0	1
Extractant concentration	mol · dm^{-3}	X_1	0.1	0.3	0.5
Ultrasound intensity	W · cm^{-2}	X_2	0.2	0.3	0.4
Extraction time	min	X_3	45	90	135
Temperature	°C	X_4	30	45	60

Table 3. Results of the physicochemical analyzes of peat, which was used as a raw material in the procedure of humic acids isolation

Number of analysis	Moisture content [%mass]	Ash content [%mass]	Fertilizers macronutrients content		
			Nitrogen [%mass]	Phosphorus [%mass P_2O_5]	Potassium [%mass K_2O]
I	72.94	11.30	2.08	0.55	0.11
II	77.68	12.40	1.35	0.45	0.13
III	70.90	11.98	1.53	0.43	0.23
Arithmetic mean	73.84	11.89	1.65	0.48	0.14

contained in the isolated humic acids came primarily from the raw material or were introduced as a result of the application of appropriate acid and alkaline solutions. Each of the mentioned analyses was performed in triplicate, and the arithmetic mean was taken as a final result.

Physicochemical studies of peat can also provide general information on its structure and degree of decomposition. The average moisture content for the analyzed material was 73.84 %mass and was relatively high in relation to the mass of the raw material. However, the result for this parameter was lower according to the average moisture content of the peat, which, according to Fuchsman, is between 80 and 90 %mass[23]. The lower moisture content may indicate an increase in mineral content. This is confirmed by analysis of the ash content. Compared to the average result for that analysis (11.89 %mass.) with the data from the literature, it can be concluded that the ash content for the evaluated peat is slightly above the standard results, which range from 3 to 10 %mass[24].

Based on analyses of micronutrients content, it is possible to assess the structure and degree of decomposition of the studied peat. According to the literature, the average nitrogen content of peat is in the range of 1 to 2 %mass, and increases for samples with a higher degree of decomposition[25]. Jonczak points out that for peats from the northern part of Poland, these values may be higher, up to 3 %mass[26]. When the average total nitrogen content in the analysed raw material (1.65 %mass) with the data from the literature, it can be concluded that the studied peat was characterised by an average degree of decomposition. This is also confirmed by the results for total phosphorus (0.48 %mass of P_2O_5). Paleckiene et al. showed that the phosphorus content in peat is negatively correlated with its degree of decomposition. This study showed that for pseudofibrous peat with an average degree of decomposition, which are defined on the von Post scale as H5, the total phosphorus content was 0.42 %mass of P_2O_5. The result for the total potassium content of the evaluated peat (0.14 %mass of K_2O) was slightly higher compared to the literature, where the range of values for pseudofibrous, middle decomposed peat, was from 0.01 to 0.12 %mass of K_2O. This difference may be due to the depth of sampling[27].

Assessment of process variants

To determine the best variant of the integrated process of isolating humic acids from peat, with their simultaneous enrichment in macronutrients, two criteria were taken into account, which were evaluated in terms of the order in which acids and alkaline solutions were implemented into the process, as well as in relation to the conditions of the ultrasound-assisted alkaline extraction step of the procedure. The first of the evaluated criterium was the average content of total nitrogen, phosphorus, and potassium in the isolated humic acids samples. Table 4 presents the results of the average macronutrients content of the humic acids extracted according to the experimental points evaluated. The nitrogen, phosphorus, and potassium content was higher for all experimental points, compared to the content of macroelements in the peat. This means that application of solutions that are a source of macronutrients leads to an increase in their content in isolated humic acids. On the basis of the comparison of results for the various variants, it can be concluded that higher amounts of macronutrients were obtained when the carrier solution was implemented in the ultrasound-assisted alkaline extraction and precipitation steps, which are strictly related to the isolation of the humic fraction from the raw material, while the other two steps of the developed technology are related to the preparation (decalcification) of the raw material and have little impact on the level of enrichment of the final product in macronutrients. Therefore, the highest potassium content was observed under the same conditions of the alkaline extraction step for variants III and IV, for which alkaline extraction was performed with the use of KOH solutions. Similarly, the highest total nitrogen content was obtained for variants I and II, that is, when ammonia solutions were used in step three of the procedure. However, comparing these variants, a slightly higher total nitrogen content was observed for variant I, due to the use of the HNO_3 solution for the precipitation of humic acids from alkaline extract. And the highest phosphorus content was in humic acids samples isolated according to procedure IV, which was related to the use of the H_3PO_4 solution in the precipitation step.

The second criterium, which was taken into account for choosing the best variant of the integrated humic acids isolation procedure with their enrichment in macronutrients, was the influence of the alkaline extraction conditions on the efficiency of the isolation of humic acids from decalcified peat. For increasing the extraction efficiency, compared with traditional procedure, where the mass transfer is intensified by shaking, the ultrasound-assisted process was implemented. The results of this part of the procedure evaluation are presented in

Table 4. Results of the macronutrients content in humic acids for the four evaluated variants of their isolation

Variant	Conditions of the alkaline extraction step				Total macronutrients content		
	Temperature [°C]	Extractant concentration [mol · dm^{-3}]	Ultrasound intensity [W · cm^{-2}]	Time [min]	nitrogen [%mass]	phosphates [%mass of P_2O_5]	Potassium [% mass of K_2O]
I	30	0.1	0.2	45	3.11	0.71	2.44
	45	0.3	0.3	90	6.02	1.02	3.24
	60	0.5	0.4	135	8.55	1.04	4.82
II	30	0.1	0.2	45	3.02	5.74	1.92
	45	0.3	0.3	90	5.59	6.23	2.86
	60	0.5	0.4	135	7.23	7.55	5.04
III	30	0.1	0.2	45	2.74	0.93	4.30
	45	0.3	0.3	90	3.68	2.23	7.72
	60	0.5	0.4	135	4.13	3.37	11.21
IV	30	0.1	0.2	45	2.26	7.31	5.15
	45	0.3	0.3	90	2.85	7.79	8.06
	60	0.5	0.4	135	3.96	9.18	10.39

Table 5. The efficiencies obtained in HA isolation suggested that the increase in the values of the parameters of the ultrasound-assisted alkaline extraction step, such as temperature, ultrasound intensity, time, and extractant concentration, have a positive effect on the amount of isolated humic acids and cause an increase in the efficiency of the procedure. When comparing the efficiency obtained depending on the order of the introduction of the solution, that is, for different variants of the procedure for isolating humic acids enriched in macronutrients, it can be concluded that the highest amount of HA can be isolated from the peat for variant III, where the peat is decalcified with the use of H_3PO_4 solutions, neutralized to pH=7 using 1 M $NH_3 \cdot H_2O$, ultrasound-assisted alkaline extraction was carried out with the use of KOH solutions and the humic acid fraction was precipitated by addition of 1 M HNO_3.

Based on the results obtained for the four variants of the humic acids isolation procedure, for further study, which included a detailed evaluation of the influence of the parameters of the ultrasound-assisted alkaline extraction step on the isolation of humic acid from peat, variant III was selected. This choice was based mainly on the results of the efficiency of humic acids extraction because the assessment of the micronutrients content in isolated humic acids did not allow to clear define the procedure variant, which would lead to obtaining the humic acids with the highest content of three macronutrient simultaneously. However, humic acids isolated according to the procedure described in variant III, in addition to the highest efficiency of the ultrasound-assisted alkaline extraction step (more than 60%), were characterized by the highest potassium content, as well as the high amount of total nitrogen, which was related to the use in variant III of the procedure KOH and HNO_3 solutions at the ultrasound-assisted alkaline extraction and humic acids precipitation steps, respectively.

Efficiency of humic acids isolation

A detailed assessment of the efficiency of humic acids isolation was focused on the evaluation of the ultrasound-assisted alkaline extraction of humic acids, which was performed on the basis of the statistical analysis of the influence of the extraction conditions on the efficiency of humic acids isolation. For this purpose, an experimental matrix was created for four independent variables. It is based on the fractional factorial design (FrFD). According to that design plan, the experimental matrix that takes into account four process parameters, whose values were coded at three levels, should consist of 27 experimental points. In the presented study, the matrix has been extended by 3 additional points, defined by the centre of the experimental space evaluated. The matrix created was presented as Table 6. It consists of 30 experimental points, where the values of the independent variables were presented in coded form, that are compatible with the information presented in Table 2. For each point, an actual result of the efficiency of humic acids extraction from peat was given. In addition, in Table 6, the efficiencies of the humic acids obtained that were predicted based on the polynomial equation were also defined. The addition of three experiments to the built matrix allows to an increase in the total number of degrees of freedom (df) in the analysis of variance (ANOVA), thus, it allowed the increase in the precision of the analysis. Moreover, the analysis of the central point in triplicate caused a noise reduction that may be defined as a random result and could cause a disruption in the statistical evaluation.

Statistical analysis

To describe the influence of independent parameters of ultrasound-assisted alkaline extraction on the efficiency of humic acids isolation in the integrated process of HA obtaining with their enrichment with macronutrients, statistical analysis was implemented. It was made on the basis of the results for experimental points defined by the design matrix. The main element of the evaluation of the process was to determine the effect estimates of the parameters of the ultrasound-assisted alkaline extraction step on the response. It was presented in Table 7. Based on the p-value (p) and factors of the Fischer test (F), it was possible to determine the significance of the linear, quadratic, and interaction effects of the process parameters on the response. In the analysis, an effect was assumed to be statistically significant when the p-value for it was less than 0.05.

Based on the results presented in Table 7 it can be concluded that the linear effects of extractant concentration (X_1), ultrasound intensity (X_2), extraction time (X_3), and temperature (X_4) were statistically significant. Moreover, all were higher than 0, which means that they have a positive effect on the efficiency of the HA isolation in the evaluated process. Furthermore, the quadratic effect of time (X_3^2), as well as temperature (X_4^2), was also characterised by a p value lower than 0.05, but the first has a negative value, which means that the increase in the time of the ultrasound-assisted

Table 5. Results of the efficiency of the ultrasound-assisted alkaline extraction step for the four variants evaluated

Variant	Conditions of the alkaline extraction step				Efficiency of humic acids isolation [mass%]
	Temperature [°C]	Extractant concentration [mol · dm^{-3}]	Ultrasound intensity [W · cm^{-2}]	Time [min]	
I	30	0.1	0.2	45	7.02
	45	0.3	0.3	90	46.09
	60	0.5	0.4	135	32.67
II	30	0.1	0.2	45	6.23
	45	0.3	0.3	90	44.65
	60	0.5	0.4	135	31.28
III	30	0.1	0.2	45	9.23
	45	0.3	0.3	90	33.11
	60	0.5	0.4	135	60.28
IV	30	0.1	0.2	45	8.74
	45	0.3	0.3	90	30.23
	60	0.5	0.4	135	55.38

Table 6. Experimental matrix with actual and predicted results for four independent variables of the ultrasound-assisted alkaline extraction step in the humic acids isolation procedure

Run	Independent variables				Efficiency of HA isolation [%]	
	Extractant Concentration (X_1)	Ultrasound intensity (X_2)	Time (X_3)	Temperature (X_4)	Actual	Predicted
	$mol \cdot dm^{-3}$	$W \cdot cm^{-2}$	min	°C		
1	–1	–1	–1	–1	9.23	8.52
2	–1	–1	0	1	39.23	40.78
3	–1	–1	1	0	22.16	23.88
4	–1	0	–1	1	35.76	32.69
5	–1	0	0	0	23.86	26.35
6	–1	0	1	–1	22.19	21.76
7	–1	1	–1	0	17.03	18.26
8	–1	1	0	–1	24.68	24.23
9	–1	1	1	1	46.52	45.93
10	0	–1	–1	1	37.13	37.90
11	0	–1	0	0	30.88	31.56
12	0	–1	1	–1	26.98	26.97
13	0	0	–1	0	24.57	23.47
14	0	0	0	–1	29.18	29.44
15	0	0	1	1	54.06	51.14
16	0	1	–1	–1	20.55	21.36
17	0	1	0	1	52.28	53.61
18	0	1	1	0	36.61	36.71
19	1	–1	–1	0	29.86	28.68
20	1	–1	0	–1	36.13	34.65
21	1	–1	1	1	55.43	56.34
22	1	0	–1	–1	24.66	26.56
23	1	0	0	1	58.16	58.82
24	1	0	1	0	40.81	41.92
25	1	1	–1	1	49.39	50.73
26	1	1	0	0	46.01	44.39
27	1	1	1	–1	39.71	39.80
28 (C)	0	0	0	0	33.11	34.10
29 (C)	0	0	0	0	35.67	34.10
30 (C)	0	0	0	0	36.93	34.10

Table 7. Effects estimates of the independent variables of the ultrasound-assisted alkaline extraction on the efficiency of humic acids isolation

Variable	Effect	Standard error	p-value	F-Value	Remarks
X_1	15.50	0.92	0.0003	285.34	Significant
X_1^2	–0.87	1.50	0.6203	0.34	Not Significant
X_2	5.08	0.92	0.0311	30.69	Significant
X_2^2	–1.21	1.50	0.5053	0.65	Not Significant
X_3	10.70	0.92	0.0073	135.95	Significant
X_3^2	–10.25	1.50	0.0207	46.88	Significant
X_4	21.63	0.92	0.0017	555.54	Significant
X_4^2	12.61	1.50	0.0138	70.86	Significant
$X_1 \cdot X_2$	–0.73	1.16	0.5944	0.40	Not Significant
$X_1 \cdot X_3$	–0.74	1.16	0.5909	0.40	Not Significant
$X_1 \cdot X_4$	–0.19	1.16	0.8870	0.03	Not Significant
$X_2 \cdot X_3$	1.21	1.16	0.4077	1.08	Not Significant
$X_2 \cdot X_4$	0.82	1.16	0.5543	0.50	Not Significant
$X_3 \cdot X_4$	–0.30	1.16	0.8200	0.07	Not Significant

alkaline extraction step in the developed procedure can cause a decrease in the efficiency of HA isolation from peat. This is most likely due to the degradation of humic acids, treated with long-term sonication, into products with lower molecular mass.

The detailed effect of changes in the values of the independent variables of the ultrasound-assisted alkaline extraction process on the isolation efficiency of humic acids is presented graphically, using response surface plots, which are summarized in Figure 1. Their shape is in line with the determined effect estimates and showed the positive effect of linear increase of independent variables values on the response. Furthermore, the plot that describes the isolation efficiency as a function of time and ultrasound intensity (Figure 1d) indicates the negative value of the quadratic effect of time, which is related to flattening of this graph for the higher values of independent variables.

The statistical assessment of the ultrasound-assisted alkaline extraction step of the humic acid isolation procedure with their simultaneous enrichment in nitrogen, phosphorus, and potassium compounds allowed the

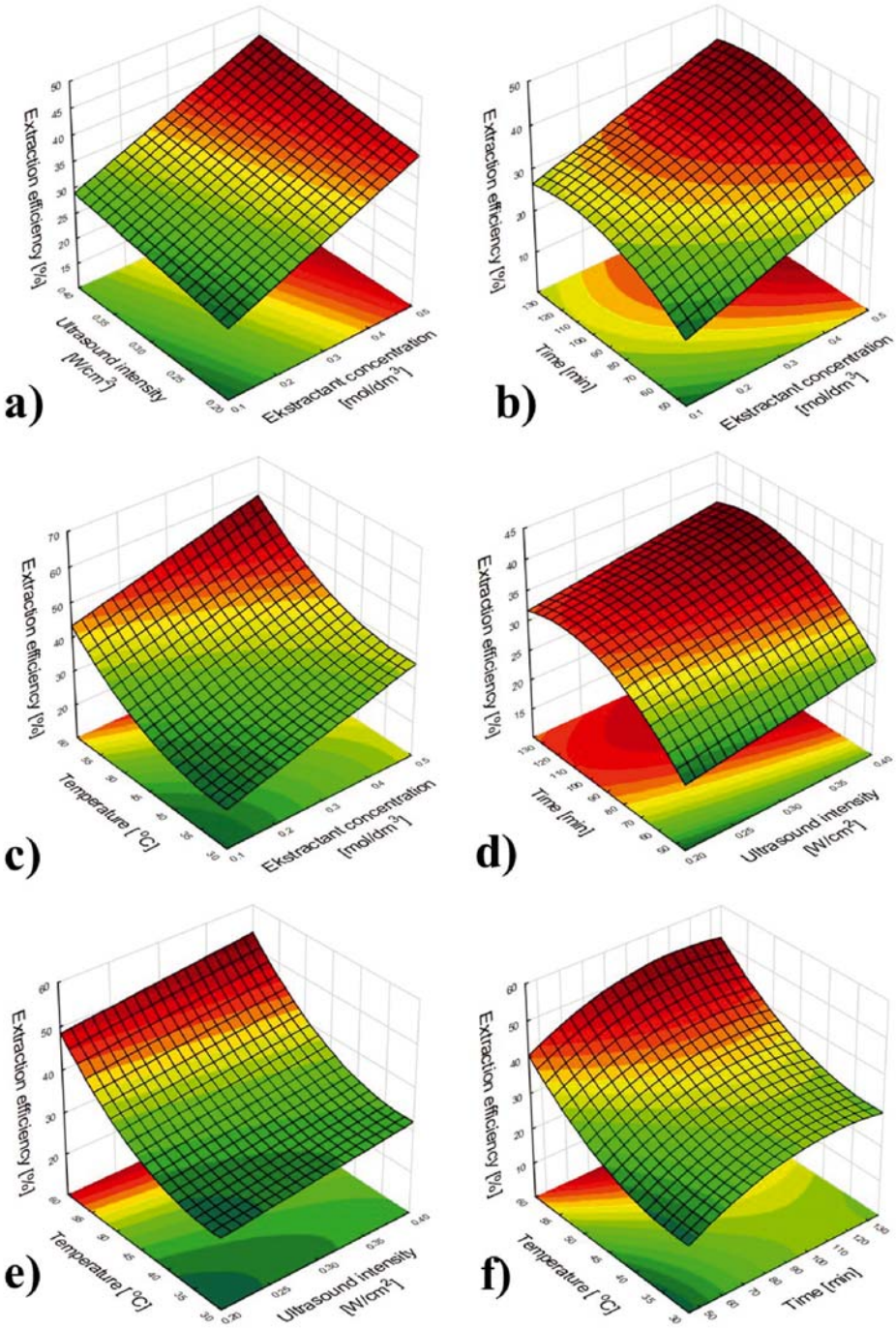


Figure 1. Response surface plots of the influence of ultrasound-assisted alkaline extraction parameters on the efficiency of humic acids isolation from peat

creation of the polynomial equation (5), which describes the efficiency of the isolation of HA as a function of the extraction parameters. It was related to the coded values of independent variables. Due to that, the coefficients for individual elements, which were statistically significant, were equal to half of their effect estimates.

$$Y = 34.10 + 7.75 \cdot X_1 + 2.54 \cdot X_2 + 5.34 \cdot X_3 - 5.28 \cdot X_3^2 + 10.81 \cdot X_4 + 6.16 \cdot X_4^2 \quad (5)$$

Analysis of variance (ANOVA) showed that the lack of fit for the created polynomial equation was characterised by the p-value equalling 0.6621 which means that the equation was statistically significant. The coefficient of determination (R^2) for a polynomial model was 0.9885. This indicates that 98.85% of the variations of the evaluated process may be explained by the model presented as Equation (5). The high R^2 value is also confirmed by the high fit of the actual results to the predicted based on the model, which is presented in Figure 2.

Qualitative analysis of humic acids

For the assessment of the influence of ultrasound on the quality of the obtained product, HA extracted by both alkaline extraction conducted by mechanical shaking (S) and ultrasound-assisted extraction (UAE) were subjected to ATR/FTIR analysis. Extractions were conducted for different values of independent variables of alkaline extraction step (extractant concentration, time, temperature, and ultrasound intensity for the UAE process). Samples extracted from raw material after its decalcification with H_3PO_4 and neutralisation with the use of 1 M $NH_3 \cdot H_2O$ solutions were evaluated. Humic acids were extracted with KOH solution and 1 M HNO_3

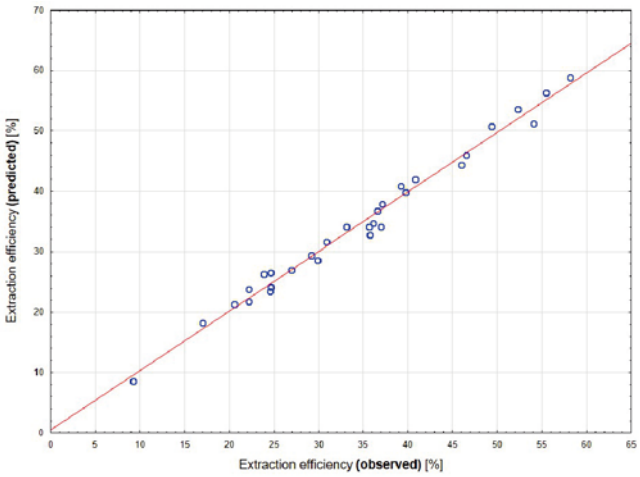


Figure 2. Predicted vs actual results for the efficiency of humic acids isolation

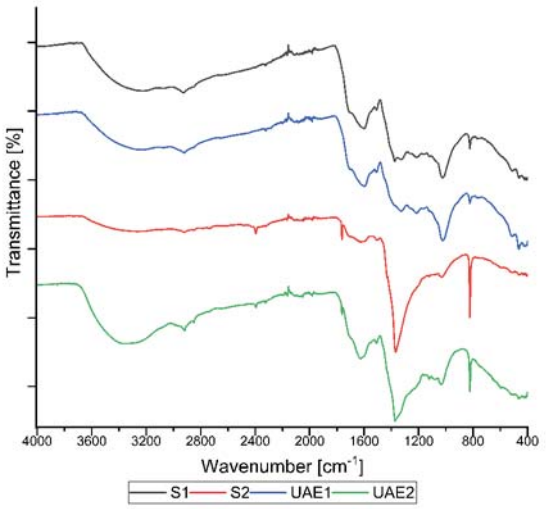


Figure 3. ATR/FTIR spectra for the analyzed humic acids

was used in the extract acidification step. The samples were labelled with symbols that refer to the method of conducting the alkaline extraction (S for shaking, or UAE for the ultrasound-assisted process). Table 8 summarizes the parameters of the extraction method.

Figure 3 illustrates the ATR/FTIR spectra of the analyzed samples. From them, the presence of groups characteristic of the HA fractions can be inferred. However, because of the complex molecular structure of the fractions evaluated, the vibrations of individual groups may have been blocked; in addition, some of the peaks may have overlapped or shifted. The band in the range of wave numbers 3000–3600 cm^{-1} corresponds to stretching vibrations of O-H groups[28]. Small peaks at 2920 and 2850 cm^{-1} are associated with the stretching of C-H bonds of the methylene (-CH_2-) and methyl (-CH_3) groups, respectively, characteristic of aliphatic chains[29]. The presence of these structures is also evidenced by the C-H deformation vibration peak at approximately 1400 cm^{-1} [30]. Signals in the 1500–1800 cm^{-1} range correspond to the C=O stretching in carboxyl, carbonyl or amide groups[31]. Vibrations in this range of wave numbers may also involve C=C stretching within aromatic structures[32]. The group of peaks seen in the range of 900–1200 cm^{-1} is characteristic of C-O stretching , which may indicate the presence of polysaccharide structures, ester groups, and alcohols[33]. Signals in the 700–900 cm^{-1} range indicate deformations of the C-H within aromatic structures [29]. A group of peaks that appear below 700 cm^{-1} is associated with the presence of mineral impurities[30].

CONCLUSIONS

The application of humic acids in agriculture improves soil structure and has a positive effect on the availability of macro and micronutrients for plants. In this study, the integrated procedure for the isolation of humic acids from peat with their simultaneous enrichment with nitrogen, phosphorus, and potassium was proposed. To increase the efficiency of HA isolation, the alkaline extraction step was assisted by the use of ultrasound. The proposed procedure allows the production of the specialised humic product for agriculture purposes, which may be an alternative to traditional humic formulations that are mixed with mineral fertilizers. The results of the research showed the possibility of replacing the HCl and NaOH solutions, which are recommended by IHSS, with acids and alkaline solutions, which are sources of macronutrients. It has been shown that the content of nitrogen, phosphorus, and potassium depends mainly on the type of solutions used in the alkaline extraction and precipitation steps of humic acids. Evaluation of the four variants of the HA isolation procedure tested showed that the highest efficiency of product isolation was obtained, when for the peat preparation step H_3PO_4 and $NH_3 \cdot H_2O$ solutions, and for the ultrasound-assisted alkaline extraction and precipitation of humic acids KOH and HNO_3 solutions were used, respectively. Statistical analysis of ultrasound-assisted alkaline extraction for that variant showed in most cases a positive effect of independent parameters on the efficiency of HA isolation. However, the extension of the time of that step may cause a decrease in process efficiency, which was stated on the basis of the negative quadratic effect of that parameter. The quality analysis of humic acids, with the use of ATR/FTIR spectroscopy, showed the presence in the isolated samples of structures that are characteristic of HA, especially carboxyl and carbonyl groups, that are crucial for the interaction of humic acids with macro and microelements.

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Table 8. Summary of parameters for obtaining humic acid samples subjected to ATR/FTIR analysis

Sample Identification	Ultrasound intensity [W·cm^{-2}]	Type and concentration of extractant	Extraction time [min]	Temperature [°C]
1 S	N/A	0.1 M KOH	45	30
1 UAE	0,2			
2 S	N/A	0.5 M KOH	135	60
2 UAE	0,4			

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LITERATURE CITED

1. Krasowicz, S., Oleszek, W., Horabik, J., Debicki, R. Jankowiak, J., Stuczynski, T. & Jadczyszyn, J. (2011). Racjonalne gospodarowanie środowiskiem glebowym Polski. *Pol. J. Agron.* 7, 43–58.

2. Skłodowski, P. & Bielska, A. (2009). Właściwości i urodzajność gleb Polski - podstawą kształtowania relacji rolno-środowiskowych. *Water Environ. Rural Areas.* 9(4), 203–14.

3. Liu, M., Wang, C., Wang, F. & Xie, Y. (2019). Vermicompost and humic fertilizer improve coastal saline soil by regulating soil aggregates and the bacterial community. *Arch. Agron. Soil Sci.* 65(3), 281–293. DOI: 10.1080/03650340.2018.1498083.

4. Olk, D.C., Bloom, P.R., Perdue, E.M., McKnight, D.M., Chen, Y., Farenhorst, A., Senesi, N., Chin, Y.P., Schmitt-Kopplin, P., Hertkorn, N. & Harir, M. (2019). Environmental and Agricultural Relevance of Humic Fractions Extracted by Alkali from Soils and Natural Waters. *J. Environ. Qual.* 48(2), 217–232. DOI: 10.2134/JEQ2019.02.0041.

5. Šimanský, V., Juriga, M., Jonczak, J., Uzarowicz, Ł. & Stępień, W. (2019). How relationships between soil organic matter parameters and soil structure characteristics are affected by the long-term fertilization of a sandy soil. *Geoderma.* 342, 75–84. DOI: 10.1016/J.GEODERMA.2019.02.020.

6. Bhatt, P. & Singh, V.K. (2022). Effect of humic acid on soil properties and crop production – A review. *Indian J. Agric. Sci.* 92(12), 1423–1430. DOI: 10.56093/ijas.v92i12.124948.

7. Deepamala, M., Pooja, M., Sucheta, S. & Alok, K. (2017). Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of Pisum sativum. *Appl. Soil Ecol.* 110, 97–108. DOI: 10.1016/j.apsoil.2016.10.008.

8. Knyazev, D.A., Fokin, A.D. & Knyazev, V.D. (2002). The role of humic substances in formation of ion-conductive soil structures. *Eurasian Soil Sci.* 35(2), 132–8.

9. Lumactud, R.A., Gorim, L.Y. & Thilakarathna, M.S. (2022). Impacts of humic-based products on the microbial community structure and functions toward sustainable agriculture. *Front. Sustain. Food Syst.* 6. DOI: 10.3389/FSUFS.2022.977121/BIBTEX.

10. Ampong, K., Thilakaranthna, M.S. & Gorim, L.Y., (2022). Understanding the Role of Humic Acids on Crop Performance and Soil Health. *Front. Agron.* 10, 4. DOI: 10.3389/fagro.2022.848621.

11. Mecozzi, M., Amici, M., Pietrantonio, E. & Romanelli, G. (2002). An ultrasound assisted extraction of the available humic substance from marine sediments. *Ultrason. Sonochem.* 9(1), 11–18. DOI: 10.1016/S1350-4177(01)00098-0.

12. Raposo, J.C., Villanueva, U., Olivares, M., & Madariaga, J.M. (2016). Determination of humic substances in sediments by focused ultrasound extraction and ultraviolet visible spectroscopy. *Microchem. J.* 128, 26–33. DOI: 10.1016/J.MICROC.2016.04.004.

14. Moreda-Piñeiro, A., Bermejo-Barrera, A. & Bermejo-Barrera, P. New trends involving the use of ultrasound energy for the extraction of humic substances from marine sediments. *Anal. Chim. Acta.* 524, 97–107. DOI: 10.1016/j.aca.2004.03.096.

15. Javed, S., Kohli, K. & Ali, M. (2013). Microwave-Assisted Extraction of Fulvic Acid from a Solid Dosage Form: A Statistical Approach. *J. Pharm. Innov.,* 8(3), 175–186. DOI: 10.1007/s12247-013-9157-y.

16. Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S. & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason Sonochem.* 34, 540–560, DOI: 10.1016/j.ultsonch.2016.06.035.

17. Cravotto, G., Mariatti, F., Gunjevic, V., Secondo, M., Villa, M., Parolin, J. & Cavaglià, G. (2018). Pilot scale cavitational reactors and other enabling technologies to design the industrial recovery of polyphenols from agro-food by-products, a technical and economical overview. *Foods.* 7(9). DOI: 10.3390/foods7090130.

18. Karčauskienė, D., Repšienė, R., Ambrazaitienė, D., Mockevičienė, I., Šiaudinis, G. & Skuodienė, R. (2019). A complex assessment of mineral fertilizers with humic substances in an agroecosystem of acid soil. *Zemdirbyste* 106(4), 307–14. DOI: 10.13080/Z-A.2019.106.039.

19. Swift, R.S. (1996). Organic matter characterization. In D. L. Sparks et al. (eds) *Methods of soil analysis. Part 3. Chemical Methods* (pp. 1018-1020). Madison, WI: Soil Science Society of America.

20. Nieweś, D., Huculak-Mączka, M., Braun-Giwerska, M., Marecka, K., Tyc, A., Biegun, M., Hoffmann, K. & Hoffmann J. (2022). Ultrasound-Assisted Extraction of Humic Substances from Peat: Assessment of Process Efficiency and Products' Quality. *Molecules* 27(11), 3413. DOI: 10.3390/molecules27113413.

21. Standard Association of Poland. (1993). Polish standard: Fertilizers – determination of total nitrogen by distillation method. PN-C-87085.

22. Standard Association of Poland. (1988). Polish standard: Fertilizers –test methods for phosphate content. PN-C-87015.

23. Fuchsman, C.H. (1980). *Peat industrial chemistry and technology.* London, United Kingdom: Academic Press Inc.

24. Huat, B.K., Prasad, A., Asadi, A. & Kazemian, S. (2014). *Geotechnics of organic soils and peat.* Leiden, Nederland: CRC Press.

25. Dettmann, U., Kraft, N.N., Rech, R., Heidkamp, A. & Tiemeyer, B. (2021) Analysis of peat soil organic carbon, total nitrogen, soil water content and basal respiration: Is there a 'best' drying temperature? *Geoderma* 403, 115231. DOI: 10.1016/j.geoderma.2021.115231.

26. Jonczak, J. (2013). Effect of peat samples drying on measured content of carbon and nitrogen fractions. *Soil Sci. Ann.* 64(4), 130–134. DOI: 10.2478/ssa-2013-0020.

27. Paleckiene, R., Navikaite, R. & Slinksiene R. (2021). Peat as a raw material for plant nutrients and humic substances. *Sustainability* 13, 6354. DOI: 10.3390/su13116354.

28. Asing, J. Wong, N. & Seng, L. (2009). Optimization of extraction method and characterization of humic acid derived from coals and composts (Pengoptimuman kaedah pengekstrakan dan pencirian asid humik daripada arang batu dan kompos). *J. Trop. Agric. Food Sci.* 37(2), 211–223.

29. Doskočil, L., Burdíková-Szewieczková, J., Enev, V., Kalina, L. & Wasserbauer, J. (2018). Spectral characterization and comparison of humic acids isolated from some European lignites. *Fuel* 213, 123–132, DOI: 10.1016/J.FUEL.2017.10.114.

30. Chang, R.R., Mylotte, R., Hayes, M.H.B., Mclnerney, R. & Tzou, Y.M. (2014). A comparison of the compositional differences between humic fractions isolated by the IHSS and exhaustive extraction procedures. *Naturwissenschaften,* 101(3), 197–209. DOI: 10.1007/S00114-013-1140-4.

31. Fernandes, A.N. Giovanela, M., Esteves, V.I. & Sierra, M.M.S. (2010). Elemental and spectral properties of peat and soil samples and their respective humic substances. *J. Mol. Struct.* 971(1–3), 33–38. DOI: 10.1016/J.MOLSTRUC.2010.02.069.

32. Garcia, D., Cegarra, J., Roig, A. & Abad, M. (1994). Effects of the extraction temperature on the characteristics of a humic fertilizer obtained from lignite. *Bioresour. Technol.* 47(2), 103–106. DOI: 10.1016/0960-8524(94)90106-6.

33. Wali, A., Ben Salah, I., Zerrouki, M., Choukchou-Braham, A., Kamoun, Y. & Ksibi, M. (2019). A novel humic acid extraction procedure from Tunisian lignite. Euro. *Mediterr. J. Environ. Integr.* 4(1), 1–9. DOI: 10.1007/S41207-019-0115-Z/FIGURES/5.