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HUMIC SUBSTANCES IN THE BALTIC SEDIMENTS*

I. ISOLATION AND PRELIMINARY CHARACTERIZATION OF HUMIC SUBSTANCES

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1. INTRODUCTION

Organic matter plays an important role in physico-chemical and biochemical processes taking place in the bottom sediments of shallow marine basins. The energy released upon decomposition of organic compounds is utilized by a variety of marine organisms to maintain their life activity. Organic substances contained in bottom deposits influence their physical, mechanical and chemical properties, such as humidity, consistency, red-ox potential ec., and determine the direction of diagenetical reactions within the sediments [3].

Naturally occurring organic substances contain functional groups which are capable of binding ions of metals, thereby expressing ion-exchange properties [11]. It is assumed that humic substances, which usually represent a substantial part of the marine organic matter in bottom sediments, play a significant role in the turnover of a number of heavy metals and transition elements in the marine environment.

In order to elucidate the physical and chemical properties of humic substances and characterize their ion-exchange capability, they have to be isolated from the bulk of marine sediment in reasonably pure state. A common isolation procedure consists in the multiple extraction of the sediments with aqueous solutions of sodium or potassium hydroxide of various strengths (usually 0.1—0.5 M) [2—5, 7, 9]. The crude alkaline ex-

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tract containing humic and fulvic acids is then acidified to pH 2—3. The humic acids form an insoluble precipitate, which is separated by filtration or centrifugation from the mother solution containing fulvic acids. Purification of humic acids can be effected by consecutive dissolution — precipitation steps, high-speed centrifugation, dialysis and ion-exchange chromatography [9, 11].

Humic substances are characterized by their elementary composition and presence of functional groups [13, 15, 16], complexing properties [11, 12], isotope composition [9], growth promoting properties [8], etc.

The use of strong hydroxide solutions has been reported to alter the nature of isolated compounds significantly [1]. The alteration can even go further during the subsequent purification steps, e.g. dialysis causes loss of substances with low molecular weight, and ion exchange chromatography leads to retention of some compounds on the resin bed due to adsorption effects.

In this work an attempt has been made to modify the commonly used extraction procedures for humic acids, the aim being to shorten the extraction time and reduce the number of extractions without affecting the overall yield of humic acids. Efforts were made to find a suitable purification method by which substantial losses of the material could be avoided.

2. EXPERIMENTS

2.1. MATERIALS

All experiments were carried out on bottom material from the Bay of Gdańsk (southern Baltic). Samples were taken with a snap mud sampler in the region of Vistula river discharge, about 1 km off the river mouth, from a depth of 7 m. Geographical coordinates of the sampling site were as follows: φ 54° 22.3'N λ = 18° 59.5'E.

Part of the sediment representing the uppermost 10 cm layer was transferred to twist-off top glass jars and brought to the laboratory. Analyses were commenced on fresh material within 20 hours after collection. The moisture content of the sediment was 78.5% and the organic carbon content was 5.45% dry weight.

Preliminary preparation of the sediment sample for the isolation of humic acids was made using a procedure described by Rashid and King [11], with slight modifications. A sample of natural moisture was centrifuged for 20 minutes at 5,000 g and the supernate, which contained negligible amounts of coloured substances, was discarded. The sediment was treated with an equal volume of 0.1 M HCl to decompose carbonates and the mixture was adjusted to pH 2 with 1 M HCl while thoroughly

swirling. After 16 hours the mixture was centrifuged, the supernate was discarded and the residue washed with distilled water and carefully neutralized to pH 7.0 with 0.05 M KOH. The mixture was centrifuged again, and after removing the liquid the sample was homogenized and the moisture determined. The sample was then divided into three sub-samples on which subsequent extraction experiments were performed using different extraction procedures.

2.2. EXTRACTION — METHOD A

A 145 g undried sub-sample which contained 31% moisture, was mixed with 300 cm³ 0.5 M KOH solution and left for 16 hours with occasional stirring. The mixture was centrifuged for 20 mins. at 5,000 g, and the residue washed with 100 cm³ 0.5 M KOH and separated by centrifugation. The solutions were combined and the residue was mixed with a second 300 cm³ portion of 0.5 M KOH, then the extraction and washing were repeated as above. The total number of extractions was ten. The solutions from each extraction were further processed to obtain pure preparations of humic acids. Minute mineral particles were removed by centrifugation for 30 minutes at 6,000 g then the extracts were acidified with conc. HCl to pH 2 and left overnight. The precipitates were separated by centrifugation and washed with 50 cm³ distilled water and the liquids saved to retrieve fulvic acids. Purification of humic acids was achieved by consecutive dissolution of the precipitates in 0.5 M KOH, centrifugation and re-precipitation with conc. HCl to pH 2, this having been done four to six times to achieve a satisfactory degree of demineralization of the preparations. The final precipitates were washed with 0.01 M HCl followed by redistilled water, until the washing solution was no longer acidic and weighed after oven-drying at 70°C. The preparations were then analyzed for the total ash content and elementary composition, and IR, VIS and UV absorption spectra were recorded.

The solutions remaining after the isolation of humic acids contained fulvic acids and large quantities of inorganic salts. They were joined and neutralized with 0.05 M KOH to pH 7 and vacuum-evaporated at 70°C in a rota-vapour device to about 100 cm³. The solution was acidified with a few drops of conc. HCl to pH 2 and left overnight. Any precipitate was removed by filtering the solution through a Whatman GF/A glass-fibre filter. To remove salts the solution was applied on the top of a glass column $\varnothing 4 \times 40$ cm filled with Sephadex G — 10 and the column bed was washed with redistilled water. The eluate was collected in several fractions, and each fraction was checked for the presence of chloride ions. The salt-free fractions were combined and evaporated in

a rota-vapour at 70°C to dryness. The residue was then analyzed for the total ash content, elementary composition, and IR, VIS and UV absorption spectra were recorded. The schematic diagram of the isolation of humic and fulvic acids from sediments is shown in Fig. 1 and the course of alkaline extraction in Fig. 2.

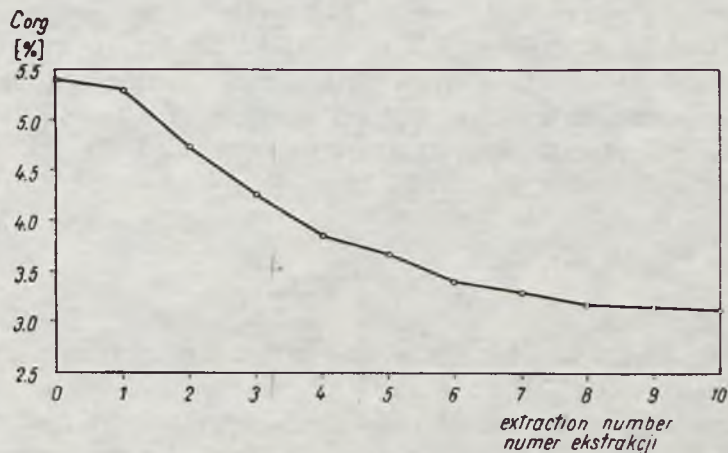


Fig. 2. Changes of organic carbon content in the sediment sample in the course of extraction with 0.5 M KOH (method A)

Ryc. 2. Zmiany w zawartości węgla organicznego w próbce osadów ekstrahowanej przy użyciu 0,5 M (metoda A)

2.3. EXTRACTION — METHOD BI

A portion of the sediment, which had been pre-treated as described in paragraph 2.1. was consecutively extracted with eight 300 cm³ portions of 0.1 solution of EDTA (disodium salt). The extracts were dialysed through a cellophane bag (Kalle Aktiengesellschaft, West Germany) against distilled water, the dialysates were evaporated under reduced pressure to 1/4 of the original volume, and acidified with conc. HCl to pH 2. After 24 hrs. the precipitate of humic acids was centrifuged, washed with diluted HCl and distilled water, and dried. The yield of humic acids for each extraction was determined gravimetrically, and the preparations were thoroughly mixed to obtain a homogeneous composite sample. On this sample the determination of the total ash content and elementary composition was carried out, and absorption spectra in IR, VIS and UV light were taken.

The sediment, after the Na₂EDTA extractions, was washed four times with 400 cm³ of distilled water and brought in contact with 300 cm³ of

0.2 M KOH for 16 hrs., with occasional stirring. The alkaline extraction was repeated three times and the humic and fulvic acids were isolated using the same procedure as in Method A.

2.4. EXTRACTION — METHOD B2

An aliquot portion of the pre-treated sediment sample was extracted with two 300 cm³ portions of 0.1 M Na₂EDTA for 16 hrs. each, the residues were washed three times with distilled water and the extraction was continued using three 300 cm³ portions of 0.2 M KOH for each extraction. Subsequent separations were performed on the combined alkaline extracts as described in 2.1.2.

3. DISCUSSION

The data characterizing extraction efficiency of humic and fulvic acids by the three different methods are presented in Table 1. The results indicate that all methods tested yield similar quantities of both the acids, and for the sediment sample used in this trial the amounts of humic and fulvic acids were 2.4 to 3.1 and 0.49 to 0.54 percent of dry sediment, respectively. The higher values for both acids were obtained when alkaline extractions were made on the Na₂EDTA pre-treated samples.

Table 1
Tabela 1

Recovery of humic and fulvic acids from sediment samples by different extraction methods

Wydajność kwasów fulwinowych i humusowych uzyskanych z osadu dennego po zastosowaniu ekstrakcji metodą A oraz metodą B 1 i B 2

Extraction method Metoda ekstrakcji	Sample size (dry) Ilość osadu	C org.	Recovery Wydajność				Extraction conditions Warunki ekstrakcji	
			C org.	HA	FA	HA		FA
				KH	KF	KH		KF
g	%	g		%				
A	100	5,45	2,420	0,485	22,4	4,5	0.5M KOH 8x	
B1	100	5,45	0,255	—	2,3	—	Na ₂ EDTA 8x	
			2,715	0,525	24,9	4,8	0.2M KOH 3x	
B2	100	5,45	—	—	—	—	Na ₂ EDTA 2x	
			3,070	0,540	28,1	5,0	0.2M KOH 3x	

Assuming the total organic matter in the sediment to be twice the amount of organic carbon, humic acids constitute about 28% of the organic matter, whereas fulvic acids are much less abundant and amount to some 5% of the total organic matter.

The total ash content in the respective fractions of humic and fulvic acids is shown in Table 2. The results for humic acids vary within a narrow range of 2.2 to 2.8% irrespective of the method of extraction. The only result which fell outside this range was for the B1 (OHA) fraction, which had not been subjected to multiple purification procedure. The ash content in the fulvic acids is much greater and amounts to up to 15.3%, which can be indicative of greater affinity of this toward cations.

Table 2
Tabela 2

Elementary composition of humic and fulvic acids isolated
from Baltic sediments

Skład elementarny kwasów humusowych i fulwinowych
wyodrębnionych z osadów dennych Morza Bałtyckiego

Extraction method (fraction number) Metoda ekstrakcji (nr frakcji)	Ash content Zawar- tość popiołu	Elementary composition *				C/H	C/N
		Skład elementarny					
	%	C%	H%	N%	O + S%		
A (1KH**)	2,2	53,94	6,31	5,74	34,01	8,54	9,39
A (3KH)	2,7	55,39	6,75	6,44	31,42	8,20	8,60
A (5KH)	2,3	55,96	6,26	5,98	31,80	8,93	9,35
A (7KH)	2,8	56,61	6,28	5,88	31,23	9,01	9,62
B 1 (0KH)	5,28	53,63	6,49	5,50	33,98	8,26	9,08
B 1 (1KH)	2,8	55,73	6,41	5,83	32,33	8,60	9,52
B 1 (2KH)	2,4	56,92	6,17	6,02	30,89	9,22	9,41
B 2 (1KH)	2,3	55,17	6,35	5,93	32,55	8,61	9,30
B 2 (2KH)	2,2	56,81	6,14	5,96	31,09	9,26	9,53
A (KF ***)	15,1	47,32	7,77	5,61	39,30	6,09	8,43
B 1 (KF)	13,3	46,59	7,38	5,88	40,15	6,31	7,92
B 2 (KF)	12,9	46,68	7,75	6,13	30,84	6,35	7,61

C, H and N contents are expressed on ash free substance.

Zawartość C, H, N podano w % przeliczonych dla substancji pozbawionych popiołu.

* O + S values were obtained by subtraction of the sum of C + H + N from 100%.

Wartość O + S wyliczono z różnicy: 100% - (%C + %H + %N).

** Humic acids

Kwasy humusowe

*** Fulvic acids

Kwasy fulwinowe

The course of alkaline extraction of the sediment in method A is shown in Fig. 2. Numbers on the ordinate show the total organic carbon left in the sediment after consecutive extraction steps. The overall decrease of C org. in the sediment after ten extractions was 43%.

It should be pointed out that a little higher recovery of humic and fulvic acids was obtained when Na₂EDTA treatment was applied prior to alkaline extraction, even though fewer extractions with more diluted KOH solutions were carried out. The effectiveness of Na₂EDTA alone in solubilizing humic acids in the sediment proved to be negligible in the first three extractions and appreciable quantities of humic substances began to appear starting from the fourth extract. The rate of extraction was slow though, the overall yield of eight Na₂EDTA extractions having been but 250 mg per 100 g of dry sediment.

The enhancing effect of Na₂EDTA on the alkaline extraction of humic acids can presumably be attributed to complexing properties of ethylen diamine tetraacetic acid molecules toward positively charged ions of metals. Humic matter is known to form insoluble compounds with multivalent cations, and adsorb on mineral particles [10, 12, 16]. In the presence of Na₂EDTA exchange of ligands takes place and multivalent cations complexed with EDTA are no longer capable of rendering the humic matter in insoluble form. If adsorption of humic acids on clay particles is effected via cations loosely bound to aluminosilicate structures [16] by complexing these cations with Na₂EDTA the bonds are broken and humic anions are made available for alkaline extraction.

The results of elementary analysis of humic and fulvic acids are given in Table 2. They are expressed on an ash-free substance basis. The results indicate that the carbon content of consecutive humic acids fractions obtained in method A increases gradually from 53.94% to 56.61%. At the same time the hydrogen content decreases in the same order, whereas the values for nitrogen are within the range of 5.74 to 6.44%, the variation being irregular. The C/H ratio, which is regarded as indicative of the degree of condensation of humic acids [2], increases with the increase of the carbon content. Such results may speak in favour of the belief that during the consecutive alkaline extractions humic acids of increasingly condensed molecules pass into the solution.

The elementary composition of humic acids extracted using the three different methods is much the same, which can be regarded as evidence that no substantial alteration of the nature of extracted substances has occurred. In comparison with humic acids fulvic acids contain higher proportions of hydrogen and a lower carbon content, which results in great differences in the C/H ratio for both acids.

Absorption spectra, in visual and ultraviolet light, of selected humic

and fulvic acid fractions are shown in Figs. 3 and 4. Spectra of humic acids bear the same character for all the fractions, and indicate two deflections on the absorption curves, one at 405 nm and the other at 665 nm, with continual increase of absorbance towards a shorter wavelength region. On absorption spectra of fulvic acids no such deflection

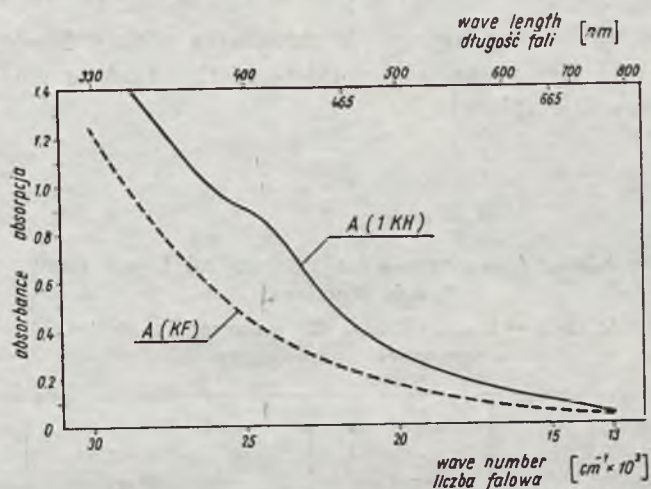


Fig. 3. Absorption spectra of humic (KH) and fulvic (KF) acids in visible light
Ryc. 3. Widmo absorpcyjne kwasów humusowych (KH) i kwasów fulwinowych (KF) w zakresie widzialnym

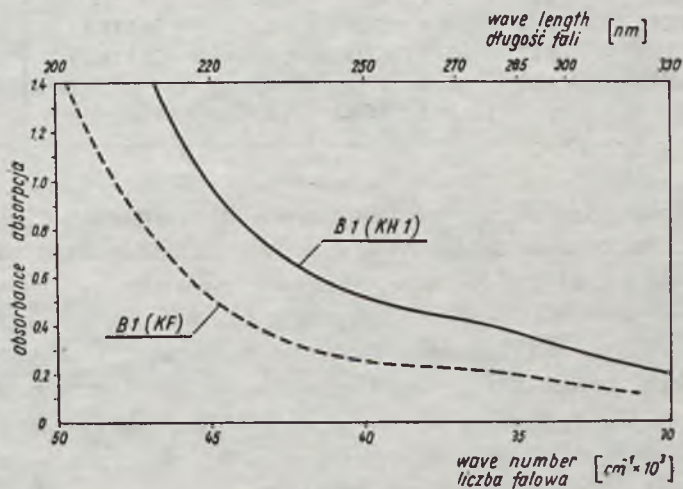


Fig. 4. Absorption spectra of humic (KH) and fulvic (KF) acids in ultraviolet light
Ryc. 4. Widmo absorpcyjne kwasów humusowych (KH) i kwasów fulwinowych (KF) w zakresie nadczerwonym

tions can be seen in visible light, and absorbance values are generally much lower than for humic acid solutions of the same concentration.

Spectra in visible light provide some information about the molecular structure of humic acids. Skopintsev [17] has shown that the absorbance ratio: $K = \frac{A_{465 \text{ nm}}}{A_{665 \text{ nm}}}$ depends on the degree of condensation of humic acid molecules, this being higher for structures with a lower degree of condensation, and vice versa. The validity of this finding was confirmed by Nissenbaum and Kaplan [9].

Table 3
Tabela 3

$K = A_{465 \text{ nm}} : A_{665 \text{ nm}}$ values for different fulvic and humic acid fractions

Wartości $K = E_{465 \text{ nm}} : E_{665 \text{ nm}}$ dla poszczególnych frakcji substancji humusowych

Extraction method (fraction number) Metoda ekstrakcji	K	Extraction method (fraction number) Metoda ekstrakcji	K	Extraction method (fraction number) Metoda ekstrakcji	K
A (1KH)	4.8	B 1 (1KH)	4.7	A (KF)	5.8
A (3KH)	4.65	B 1 (2KH)	4.3	B 1 (KF)	6.1
A (5KH)	4.55	B 2 (1KH)	4.6	B 2 (KF)	5.95
A (7KH)	4.25	B 2 (2KH)	4.35		
		B 1 (OKH)	4.75		

The K values for humic acids obtained by consecutive alkaline extractions of the bottom sediment are shown in Table 3. It can be seen from these data that the K values decrease with the increasing number of extractions, which indicate the growing contribution of more condensed molecules in each subsequent alkaline extract. The same general conclusion can be drawn from the results of elementary analyses performed on the respective fractions of humic acids (Table 2).

Fulvic acids show higher K values than humic acids which may be associated with lower molecular weight of the former [9].

Both humic and fulvic acids comprise a variety of chemical compounds with different molecular sizes and elementary composition [10, 15] which fact makes them a difficult object of study. Infrared spectrometry

provides useful means of elucidating the functional composition of such complex substances. In examining IR spectra of humic and fulvic acids attention is given to the following wave number regions: 3200 cm^{-1} — stretching vibrations of —OH and —NH_2 bonds, 1720 cm^{-1} stretching vibrations of >C=O in esters and aliphatic acids, 1650 cm^{-1} — stretching vibrations of peptide bonds in proteins and >C=C< in aromatic rings 1530 cm^{-1} — amide II bonds in proteins, 1240 cm^{-1} — usually accompanied with 1530 cm^{-1} absorption band, 1200 cm^{-1} — usually

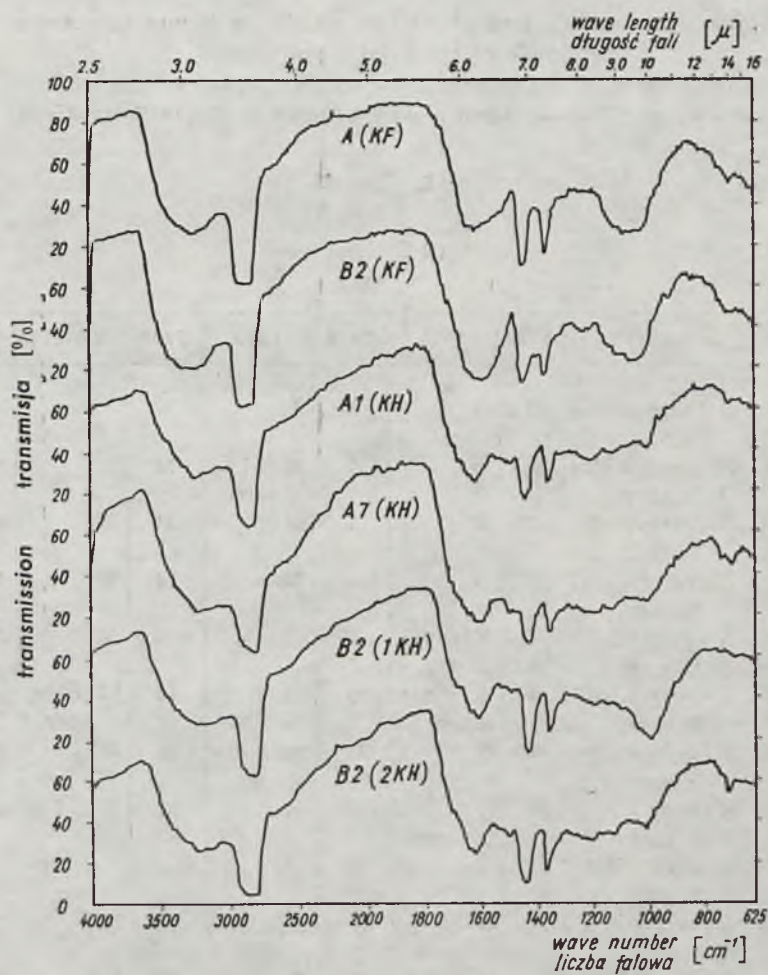


Fig. 5. Infrared transmission spectra of separate fractions of humic (KH) and fulvic (KF) acids

Ryc. 5. Widmo absorpcyjne poszczególnych frakcji kwasów humusowych (KH) i kwasów fulwinowych (KF) w zakresie podczerwonym

accompanied with a carbonyl band at 1720 cm^{-1} , $1200 - 1100\text{ cm}^{-1}$ — cyclic ethers, 1050 cm^{-1} — a band characteristic for $>C=O$ in polysaccharides, $1000 - 700\text{ cm}^{-1}$ — a band characteristic for silicoorganic compounds.

Specimen examples of IR spectra taken for selected fractions of humic and fulvic acids are shown in Fig. 5, and the more important absorption bands are tabulated in Table 4. Interpretation of the spectra is compli-

Table 4

Tabela 4

More important infrared absorption bands for humic substances isolated from Baltic sediments

Ważniejsze maksima absorpcji w podczerwonym zakresie widma dla substancji humusowych z osadów dennych Morza Bałtyckiego

Extraction method (fraction number) Metoda ekstrakcji	IR newoil max (cm^{-1})							
	IR nujol max (cm^{-1})							
	3200	1720	1650	1530	1240	1260	1200-1100	1030
A (1KH)	S (very wide) b. szer.	S	S	M	M	W		M
A (3KH)	S (very wide) b. szer.	S	S	S	M (wide) szer.	M		M
A (5KH)	S (very wide) b. szer.	S	S	S	M	M	W	M
A (7KH)	S (very wide) b. szer.	S	S	S	M	M	W	M
B 1 (1KH)	S (very wide) b. szer.	S	S (wide) szer.	M		S	M	S (wide) szer.
B 1 (2KH)	S (very wide) b. szer.	S	S (wide) szer.	S	M	M	M (wide) szer.	M (wide) szer.
B 2 (1KH)	S (very wide) b. szer.	S	S	W	M (wide) szer.	M	M	S
B 2 (2KH)	S (very wide) b. szer.	S	S (wide) szer.	M	M	M		W
A (KF)	S (very wide) b. szer.	M	S	S		S (wide) szer.	S (wide) szer.	
B 1 (KF)	S (very wide) b. szer.	M	S	S	S (wide) szer.	M	M	S (wide) b. szer.
B 2 (KF)	S (very wide) b. szer.	M	S	S	S (wide) szer.	M	M	S (wide) szer.

S — Strong
silne

M — medium
średnie

W — weak
słabe

cated by the presence of strong and wide absorption bands. It can be seen, however, that the spectra of fulvic acid fractions are all alike, with all the aforementioned bands being well visualized. The more pronounced absorption peaks occur at 1050^{-1} and within a range of $1200 - 1100 \text{ cm}^{-1}$, which were not observed in the humic acid spectra. There are also some additional peaks in humic acid spectra, at 1240 cm^{-1} and 1200 cm^{-1} which are not seen in the spectra of fulvic acids. Humic acids do not seem to undergo alteration in the course of alkaline extraction significant enough to be revealed by the IR spectra.

4. CONCLUSIONS

A comparison of different extraction methods for isolating humic substances from marine sediments indicates that treatment of the sediments with 0.1 M solution of Na_2EDTA prior to alkaline extraction with 0.2 M KOH solution results in faster dissolution rate and greater yield of humic substances compared with methods that make use of hydroxide solutions alone. The results of elementary analysis and IR, VIS and UV spectra show no substantial differences in the physical and chemical properties of the humic substances isolated by both the methods. The effectiveness of Na_2EDTA in enhancing solubilization of organic matter contained in sediments may be due to complexation of heavy metal ions that form insoluble compounds with humic substances. Another factor that may contribute is the action of Na_2EDTA on organo-mineral aggregates, which brings the organic component to solution.

An advantage of the pre-treatment of sediment samples with Na_2EDTA solution is that by using more dilute hydroxide extractants and shortening the extraction procedure organic compounds are less exposed to the adverse environment, thereby yielding products which resemble more closely their original structure.

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SUBSTANCJE HUMUSOWE
W OSADACH DENNYCH MORZA BAŁTYCKIEGO
I. WYODRĘBNIENIE I WSTĘPNA CHARAKTERYSTYKA
SUBSTANCJI HUMUSOWYCH

Streszczenie

Substancje organiczne zawarte w osadach dennych mórz odgrywają ważną rolę w fizykochemicznych i biochemicznych procesach zachodzących w tych osadach. Największą frakcją substancji organicznych osadów dennych stanowią substancje humusowe. Własności substancji humusowych poznane zostały w małym stopniu; wiadomo jednak, że w skład frakcji humusowej zawartej w morskich osadach dennych wchodzi związek znacznie różniący się ciężarem cząsteczkowym, posiadające szereg grup funkcyjnych, takich jak hydroksylowe, karbonylowe, karboksylowe, aminowe, ugrupowania aromatyczne i heterocykliczne. Porównanie wyników badań nad substancjami humusowymi, które zostały uzyskane przez różnych badaczy, sprawia trudności ze względu na stosowanie różnych metod izolowania tych związków z osadów oraz możliwości zmian w naturze wyodrębnionych substancji.

W pracy przedstawiono metodę szybkiego wyodrębniania substancji humusowych z osadów i oczyszczania uzyskanych substancji w warunkach zachowawczych.

Metoda polega na wstępnej obróbce osadu 0,1 M roztworem wersenianu dwusodowego (Na_2EDTA), a następnie ekstrakcji osadu 0,2 M roztworem wodorotlenku potasowego (KOH). Taki sposób izolacji pozwala zwiększyć wydajność ekstrakcji oraz ograniczyć ilość destrukcyjnych ekstrakcji alkalicznych

W wypadku stosowania wyłącznie ekstrakcji alkalicznych konieczna jest dziesięciokrotna ekstrakcja 0,5 M roztworem KOH, co sprawdzono oznaczając zawartość węgla organicznego (c_{org}) w osadzie po kolejnych ekstrakcjach. Uzyskane substancje humusowe rozdzielano na kwasy humusowe i kwasy fulwinowe wykorzystując różnicę rozpuszczalności tych związków w środowisku kwaśnym (pH 2). Kwasy humusowe oczyszczano drogą wirowania, a kwasy fulwinowe — na kolumnie wypełnionej Sephadexem G-10.

W celu scharakteryzowania uzyskanych w trakcie kolejnych ekstrakcji frakcji kwasów humusowych i kwasów fulwinowych wykonano widma absorpcyjne wodnych roztworów tych kwasów w świetle widzialnym i nadfiolecie oraz widma w podczerwieni. Wykonano również analizę składu elementarnego poszczególnych frakcji, oznaczając zawartość węgla, wodoru i azotu.

Widmo absorpcyjne w świetle widzialnym otrzymane dla roztworów kwasów humusowych wykazuje dwa punkty przegięcia: przy długości fali 405 nm i 665 nm. Natomiast widmo kwasów fulwinowych ma charakter monotoniczny w tym zakresie. Widmo absorpcyjne w nadfiolecie dla kwasów humusowych i fulwinowych

posiada punkt przegięcia przy około 280 nm. Widma absorpcyjne w świetle widzialnym i nadfiolecie wykazują wzrost absorpcji wraz ze zmniejszaniem długości fali.

Znacznie bardziej interesujące są widma absorpcyjne w podczerwieni. Pomimo utrudnionej, ze względu na bardzo silne i bardzo szerokie pasma, interpretacji we wszystkich analizowanych frakcjach wykazano istnienie pasm absorpcji wywołanych obecnością grup hydroksylowych, karbonylowych, karboksylowych, aminowych, a także wiązań peptydowych ugrupowań aromatycznych i policukrów.

Analiza elementarna wykazała, że kwasy humusowe zawierają 53,94—56,61% węgla, 6,14—6,75% wodoru oraz 5,74—6,44% azotu. Skład elementarny kwasów fulwinowych jest nieco inny. Zawartość węgla wynosi 46,68—47,32%, zawartość azotu 5,61—6,13%, a zawartość wodoru 7,35—7,77%. Nie stwierdzono zależności pomiędzy charakterem widm w podczerwieni a składem elementarnym badanych związków.

Wzrost wydajności ekstrakcji po wstępnym użyciu wersenianu dwusodowego wytłumaczono kompleksowaniem jonów metali przez silny ligand, jakim jest wersenian dwusodowy. Nie bez znaczenia może być również wpływ wersenianu na strukturę minerałów ilastych, które są głównym czynnikiem adsorbującym substancje humusowe z wody morskiej.

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