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OUTLINE OF THE HISTORY OF STUDIES ON THE EFFECT OF HUMIC COMPOUNDS ON ALGAE

Contents: 1. Introduction, 2. Review of papers, 3. General discussion; Streszczenie; References

1. INTRODUCTION

Humic substances form a wide class of acidic compounds derived from polymerized, oxidized polyphenols and quinones containing nitrogen in the molecule, either in the ring or in the side chain. All of them are more or less dark in colour and produce hydrophilic colloidal systems. They very greatly as regards molecule size and magnitude of colloidal particles, as well as solubility in water, bases, acids and organic solvents, the latter being the result of not only the physico-chemical dissimilarities between individual compounds, but also the effect of different chemical composition, particularly quantitative differences in the functional groups of the molecule.

Algologists have long been aware of the presence of humic substances in natural waters, but none took serious interest in the matter. They were thought to be rather accessory indicators of water quality than true ecological factors.

2. REVIEW OF PAPERS

Uspenski [22] should be considered to be the first author who fully appreciated the function of humic compounds in the environment and life of algae. He carried out extensive studies on the dependence of the fresh water algae population composition on the contents of humic substances and experimented on their influence on the growth of individual species in pure cultures; the experiments were performed mainly on the genus Volvox, one particularly difficult to cultivate. He reached the following conclusion: iron turned out to be one of the major factors effecting fresh water algae, its content and chemical state determining the quantitative and qualitative composition of the algae population. Simultaneously, Uspenski stated that the biological value of iron in water depended to a great extent on the content of humic substances, thus he attributed them a high ecological rank. According to his findings, humic compounds, by binding iron, made it inaccessible to algae, thus: when there was a deficiency of iron, humic compounds hindered the algal vegetation and when it was in excess — facilitated it. As particular species differed in iron demand, humic compounds modified the quantitative relation of the algal population. Uspenski presented the results of his studies and the ensuing ecological ideas in an extensive treatise entitled *Eisen als Factor für die Verbreitung niederer Wasserpflanzen* [22].

Uspenski's hypothesis was contradicted by Pringsheim (1934), who published *Untersuchungen zu Uspenskis Eisenhypothese der Algenverbreitung*. In his paper, he strongly objected to Uspenski's methods of determining iron utilizable by algae. Due to this criticism, Uspenski's papers were almost forgotten and only a short remark on his opinions is to be found in a text-book on algae physiology edited by Levin [11], which also contained Pringsheim's criticism of the chemical methods applied by him (see Wiesner's article in the above-mentioned text-book, p. 270). Reffering to this remark Wiesner recalled a paper by Gran (1933) suggesting that the fluctuations in phytoplankton populations and density observed in the Gulf of Maine were caused by periodical changes in the contents of both — iron and humic substances.

Lhotský [12] in his paper cited, among others, an article by Lwoff and Lederer (1935) in which the authors attempted to elucidate the mode of action of humic compounds on the growth and proliferation of algae. Unfortunately, this paper was unobtainable.

Pringsheim, who successfully applied aqueous soil extracts to algae cultivation tried to find the components of the soil extracts responsible for the effects observed. The results of these experiments, reported under the characteristic title *Das Rätsel der Erdabkochung* [17], indicated that the soil extracts contained a certain growth factor important for algae (reproduction). From the author's point of view, this was an organic compound resistant to treatment by various substances, thermostable, but degradable by hydrogen peroxide. This growth factor could be replaced by an "artificial humus" made of caramel. Pringsheim noticed a slight analogy between iron and the alleged growth factor, but he did not give any suggestions on the matter, and concluded the humic compounds to be inadequate as growth agents.

Not being convinced of Pringsheim's opinions I conducted several experiments concerning the effect of natural humic acids, obtained from peat by standard methods, on crude cultures of various green algae, Flagellatae, Sarcodina and Infusoriae. The addition of humic acid to the culture medium either stimulated or inhibited the cell reproduction, depending on the humic acid concentration and species examined. Similar observations were made for chemotaxis of Euglena and Rhizopoda. Amebozoa appeared to be particularly sensitive to higher humic acid concentration and demonstrated early symptoms of necrobiosis. On the basis of these and similar experiments [3] it was possible to assume that colloidal solutions of humic acids acted mainly as physico-chemical agents during contact between the colloid and the plant or animal protoplasm.

Lhotský [12] carried out extensive studies on the growth of algae populations and their uptake of biogenic salts in the presence of extracts of brown coal. The experiments were performed on pure cultures. The most sensitive of the 21 species investigated proved to be Chlorogonium elongatum and Ch. euchlorum, as opposed to Chlorella vulgaris and Ankistrodesmus falcatus which were resistant. The optimal concentration for Chlorogonium profliferation ranged from 10 to 100 ppm depending on the coal applied. Higher concentrations were toxic. The stimulatory effect was particularly significant in the adaptation and final growth phases of the culture.

Brown coal extracts protected the algae against the toxic effect of excess cations of heavy metals in the culture medium efface the harmful effects of too high or too low pH, hence the best stimulatory results were observed at unsuitable pH values. Higher stimulation was noticed when there was a deficiency of phosphate ions in culture media than in the case of optimum contents of this element.

Aqueous extracts from brown coal proved to be better than extracts from soily humus substrates, while polyphenols afforded considerably lower stimulation, and often inhibited or even prevented the growth of algae. The author was of the opinion that humic acids constituted the biologically active components of brown coal extracts and that they acted by means of their colloidal properties or by affecting the metabolism.

Květ' [7] conducted comparative studies on the population growth rate (mainly Chlorogonium euchlorum) of algae exposed to aqueous extracts from raised bog, peat, peatmoss and plants of the genus Sphagnum from pure cultures. Using photocolorimetric measurements of the algae culture density he found similar stimulatory effectiveness of all the extracts applied, at concentrations of about 10^{-3}, these being expressed as the amount of dry mater dissolved in the extract. It should be noted that the extracts were prepared at temperatures of over 60°C and subsequently sterilized at high temperatures. Under such conditions, oxidation and polymerization of polyphenols present in the plant extracts was inevitable.

Kyć [8] attempted to explain the mechanism of the action of humic compounds on algae. He carried out investigations on the influence of sodium humate, natural — from compost and model — from caramel, on cell reproduction and dry weight accumulation in pure cultures of Scenedesmus quadricauda. The experiments were carried out in a photo-thermostat with Uspenski's medium, on which the species developed very well. The author found that both the natural and the model sodium humates increased the content of dry weight in the culture. The increase was noticeable over an extended period of cultivation while the cell division was stimulated only at the beginning of the culture development and particularly at low humate concentrations (1 - 10 ppm of dry weight), with an optimum of 100 ppm for dry weight stimulation. Apart from this, cell division was noticeable at low light intensity (300 lx) and diminished at higher — 5000 lx. The best conditions for stimulation were as follows: deficiency of iron, excess of copper and inappropriate pH. The stimulation effect ensued from neither the absorption of light by humate, which was proved in experiments based on filtration of light through a humate layer, nor from the occurrence of some growth substances — which was ruled out by the application of model compound. The contents of microelements in natural humate only supplemented the stimulatory function, as was illustrated by tests with humate ash and model humate. Eventually the author came to the conclusion that biological activity of humate consisted in its sorptive and ion exchange properties.

Jurajda [6] conducted a comparative study of the effect of sodium humate on the growth of two green algae: Scenedesmus quadricauda and Gonium pectorale. Fourteen-day cultures on Pringsheim medium in a photothermostat indicated the susceptibility of Scenedesmus to iron deficiency and predilection of Gonium for calcium. Despite this, the optimum pH of the medium for Scenedesmus was higher than that for Gonium. Sodium humate in amounts of 100 ppm (calculated per dry weight unit) stimulated cell division as well as the increase of dry matter production in Scenedesmus cultures. The effect was particularly conspicuous where there was an iron deficiency and inappropriate pH in the medium. Gonium cultures reacted negatively to exposure to sodium humate, generally inhibiting cultures growth, especially at higher pH. These results suggested that the mechanism of humate biological activity consisted in regulation of the utilization of mineral components of the medium. The conclusions seemed highly justified, considering data on the influence of humic substances on plant physiology and the information on this topic contained in chemical-agricultural papers, see the monography by Trojanowski [21]. As far as the results of the relation of stimulation or inhibition rates to the pH of the medium are concerned, they conformed with those of Lhotský [12] and Kyć [8], but they were considerably harder to interpret. The phenomenon of the pH function could not be explained by simple change in accessibility of individual mineral components of the medium for algae. Rypáček's report [18] on the occurrence of plasmolysis in cells of highly organized plants and in the algae Nitella in the presence of humic compounds requires very careful inspection. According to this author humic acids delay plasmolysis as well as de-plasmolysis, which would indicate the depletion of the permeability of protoplasm, this depending on the pH of the extraneous solution. This subject will be reconsidered in the general discussion.

Hydrobiologists and even algologists habitually, although incorrectly, include Cyanophyceae among the algae. This is illustrated in the classic handbook on the physiology of algae edited by Levin [11], the authors of which are fully aware of the mistake from the point of view of the plant systematics (as stated in the introduction), and yet the book describes the Cyanophyceae physiology in detail. I shall therefore follow their example and focus my attention on this species.

An employee of the Botanical Gardens of Wrocław, Sławiński, in search of a practical means of counteracting Cyanophyceae in aquaria, one which would be harmless to higher plants and fish living in aquaria, tested certain humic substances derived from peat, produced under the supervision of Prof. Niklewski of Elbląg. Basing on the results presented in my paper [3] Sławiński tried to stop the development of Cyanophyceae by the application of a substantial concentration of humus, but not harmful to other organisms. The experiments proved successful and were published in "Wiadomości Botaniczne" in 1958 [20], the method now being used in the Botanical Gardens of Wrocław.

Having in view the scientific explanation of the fact Skinder [19] conducted a number of investigations on the effect of sodium humate from peat and compost on pure cultures of Cyanophyceae Oscillatoria sancta, the species frequently encountered in aquaria. Oscillatoria sancta were cultivated in a photothermostat on Ghu No. 10 medium modified by Gerloff [1], with various doses of calcium, magnesium and iron ions and different pH values. The author compared the effectiveness of humates from peat and compost with that afforded by sodium versenate. It was found that both humates, similar to versenate, strongly inhibited the proliferation of Oscillatoria, and only humate of peat showed slight stimulatory activity at very low concentration — 10 ppm. An increase in the calcium content, and to a certain extent also iron and magnesium, reduced or even broke up the inhibitory effect of humate. As humate however, did not counteract the harmful effect of excess calcium, the inhibition of algal growth caused by humate could not be explained by impeding calcium uptake. Humate, does, in fact obstruct the calcium consumption by Cyanophyceae and even extracts the element from the cell, which was proved by measuring the calcium content in the medium prior to and on completion of the experiment. Similar to the results obtained by Lhotský, Květ and Kyć, the author found the maximum biological activity of humate in unfavourable pH conditions of the medium. Thus the mechanism of humate behaviour should be related to the possibility of binding ions, as well as to the direct effect on plasm.

In a paper on the influence of chelating agents on Cyanophyceae Lange [10] mentioned that fulvic acids were valuable to Cyanophyceae, if applied in appropriate concentrations, this in respect of their chelating properties. Fulvic acids revealed stronger biological activity as compared to chelating products obtained by the chemical industry. Unfortunately, the previous article by Lange [9], concerning fulvic acids, was not available.

Up to the present, algology lacks papers in which the biological activity of individual compounds or fractions of humic substances are compared.

3. GENERAL DISCUSSION

The results of algological studies on humic substances should be confronted with the data from agrochemical and physiological investigations concerning this problem in relation to higher plants. I shall not discuss the extensive literature on the subject and confine myself to four synthetic sources, i.e. the monography by Trojanowski *The transformation of organic substances in soil* [21], two articles by Gumiński [4, 5] and one by Lisiak [13].

None the less, the conclusions arising from the agrochemical and physiological studies carried out on plants and yeast, should be compared with the results of algological observations.

Chemical investigations of humic substances revealed that besides colloid sorptive properties, these compounds possess even chelating properties in relation to multivalent cations, and also indirectly phosphates. The binding strength of humic acids differs in relation to particular ions and greatly depends on the pH. Individual compounds (fulvic, humic and hymatomelanic acids) and their fractions bind particular cations with different strength. On the other hand, the ability of algae cells to extract the cations bound in humic colloids varies according to the species and the physiological conditions of the organism. Generally, the algae utilize well iron and badly — or not at all — calcium and copper. Experiments with radioactive tracers led to the conclusion that macromolecular compounds (humic and hymatomelanic acids) do not penetrate the plasmolemma, while compounds of relatively low molecular weight (e.g. fulvic acid) penetrate the cells and are then distributed in the tissues. Rypáček [18] observed the effect of fulvic acids on the movement of some protoplasmatic granulation in the alga Nitella.

The growth effects of humic acids observed in yeast are comparative to those of tannins and gibberellins.

The germination of ligt-sensitive seeds induced by light could be provoked equally well by gibberellins or sodium humate and humate completely or at least partly inhibited the diminished germination of photophobic seeds due to the effect of light. Humate also proved to stimulate germination of various seeds in oxygen deficiency.

Tannins and products of polymerization and oxydation of polyphenols influence plant physiology in the same way as humic compounds and — although they do not contain nitrogen in the molecule — are used as model humic compounds. Similar growth effects to those of natural and model humic compounds, as well as gibberellins were observed in media with controlled concentration of hydrogen ions (in the given medium or mineral solution).

Studies on the processes of plasmolysis and deplasmolysis showed a fairly complicated correlation between the effect of humic compounds, potassium, calcium and hydrogen ions on the permeability of protoplasm.

Humic compounds distinctly influence the activity of many enzymes. The astonishing similarities in the effects of humic compounds, tannins, gibberellins and hydrogen ions are quite easily explained if the following facts are taken into account: that plasmolemma contains a number of enzymes regulating the cell growth and active ion transportation, and that the activity of these enzymes depends strongly on the structure of the plasmolemma.

In this light, the results of physiological experiments in algology can be arranged in order and apparent discrepances (e.g. growth stimulation for some and inhibition for other species or results dependable on pH) become comprehensible. Individual species of algae and their particular cells require various amounts of ions depending on their physiological state, their uptake also differing. For this reason the phase of culture development and the incubation material should be treated with special care in experiments. Humic compounds bind cations with variable strength according to the number and type of functional groups, hence the facilitation or obstruction of the ion uptake by algae. This concerns particularly iron, phosphorus and calcium. Uspenski's conclusions regarding the relation of iron and humic compounds to algae, proved to be true, despite the errors made in the chemical analysis.

The indirect influence of humic acids on the ion uptake by some plants, plays a more important role in aquatic environments than in soil, as a strong inorganic sorptive complex is present in the latter.

The situation becomes more complex by the interaction of humic compounds and hydrogen ions which affect not only the solubility of salt in water and the strength of coordination bond with humic substances, but also the structure of plasmolemma and activity of constituent enzymes.

Thus the results of physiological-algological studies on the influence of humic compounds indicated that the compounds in question affect the medium as well as the plasmolemma structure and the activity of its enzymes. As regards fulvic acids they penetrate the protoplasm, thus their impact reaches further. It is very likely that the accumulation of volutin caused by humic compounds in Scenedesmus, observed by Prát [15], is closely related to this fact. However, very little is known of the biological effect of individual, less defined humic compounds on algae due to the lack of suitable comparative experiments. The effects exerted on algae by aqueous extracts of various brown coals result from the presence of some humic compounds, as justly presumed by Lhotský [12]. This fact was also confirmed in experiments with certain higher plants.

As regards the extracts of Sphagnum plants, the subject of studies by Květ [7], extracts of various peatmoss plants tested on higher plants pointed to the formation of substances resembling humic compounds during treatment with high temperature. So, not surprisingly, in Květ's experiments, the effects of peat and peatmoss extracts caused similar effects to those of humic compounds.

The final conclusion of these considerations is that humic substances are biologically active in relation to algae, as well as to higher plants, and that this activity does not result from any additive substances accompanying these compounds.

Quite different questions arise from the interaction of humic compounds with pesticides occurring in water, but as the matter requires careful consideration we shall not deal with it in this paper.

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ZARYS HISTORII BADAŃ NAD WPŁYWEM ZWIĄZKÓW PRÓCHNICZNYCH NA GLONY

Streszczenie

Przedstawiono wyniki prac ekologicznych i fizjologicznych, które stopniowo ujawniały mechanizm działania związków próchnicznych na glony. Rozróżniono wpływ pośredni poprzez wiązanie kationów wielowartościowych oraz wpływ bezpośredni na

protoplazmę (głównie plazmalemmę), a co za tym idzie na metabolizm. Wskazano na znaczne zróżnicowanie związków próchnicznych, w szczególności pod względem występowania grup funkcyjnych i wielkości cząstek koloidalnych, a także drobin, i na związane z tym różnice w efektywności fizjologicznej, zwłaszcza na tle wahań kwasowości wody. Omówiono zagadnienie różnej reakcji poszczególnych gatunków glonów na związki próchniczne.

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