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# Impact of pH and salinity
# on spectroscopic parameters
# of humus

# CONTENT

# FOREWORD

More and more aggressive human activity in unmonitored conditions leads to tremendous changes in the environment. The development of civilization inevitably entails the progressive degradation of the natural environment. Knowledge of the main sources of hazard, as well as their mechanisms, provides a potential possibility of protection against adverse phenomena [5, 47]. Modern human interference in the environment often results in intentional or unintentional disturbance of natural processes occurring in ecosystems, often leading to their degradation. This is often caused by excessive accumulation of heavy metals, xenobiotics, mineral fertilizers and waste substances in the soil, which largely determine the course of humification processes and properties of humus [26]. Human activity exerts a specific impact on the nature and properties of humus occurring in the soil, therefore transformations and properties of humic substances in the areas of increased anthropopressure can reflect the intensity of progressive changes in the ecosystems. Humic substances are one of the key factors in the development of ecosystems. They constitute a major component of humus occurring in small quantities in mineral soils, but due to their properties they play a very important role in the processes occurring in the soil environment. A characteristic feature of humic substances is their high dynamics, which determines the intensity of many processes affecting the circulation of chemical elements in nature. Specific humic-mineral bonding created in ecosystems exposed to anthropopressure might be characterized by distinct properties, which largely modify the circulation of elements in nature. Due to the complexity of processes determining the directions of organic matter transformations in polluted soils, the nature of the resulting products is not sufficiently explored. This generates the need of multidirectional research aiming at explanation of these issues.

It is known that some factors, such as compounds of sulphur or nitrogen, have a destructive influence on the soil, inter alia, by reducing the content of humus [11]. The impact of pollution on humic substances is not limited to changes in their content in soils, but probably affects the composition of humus and properties of humic substances, which results in changes of physical and chemical soil properties.

The main purpose of this study, the results of which are presented in this paper, was to determine the short-term influence of industrial pollution, which leads to soil acidification, alkalization or soil salinity, on spectroscopic properties of humic substances in the soil.

# 1. ECOLOGICAL SIGNIFICANCE OF SOIL HUMIC SUBSTANCES

Soil humus is a natural mixture of different organic substances and mineral-organic compounds in the soil and on its surface, representing different stages of natural decomposition of plant and animal remains [13, 41]. Multiple ecological importance of soil humus results from its specific properties determined by the constitution [1, 13].

## 1.1. STRUCTURE OF HUMIC SUBSTANCES

The main components of soil humus are humic substances: fulvic acids (FA), humic acids (HA) and humins [9, 12, 36]. It is believed that humic substances are a polymolecular and polydisperse mixture of polyelectrolytes, which are produced in the processes of biochemical condensation and polymerization of compounds of plant origin (lignin, cellulose and other saccharides, amino acids, proteins and others) and animal origin (e.g. chitin), as well as products of soil biomass metabolism [13, 36]. The process of incorporating the structural materials into the complex of humic substances is presented schematically in Figure 1.

Humic substances are operationally defined as a mixture of heterogeneous organic substances, from yellow to black in colour, relatively resistant to degradation [1].

Since the composition and properties of humic substances are purely statistical, „fulvic acids", „humic acids" and „humins" should be regarded as a group concept, determining the system of heterogeneous but related organic compounds, differing from each other in the constitution of main radicals, the dispersion degree, condensation, polymerization and other properties [13]. However, one should not overlook the fact that the research material obtained by extraction of humic substances is not the same as the one naturally occurring in the soil. Its properties are largely determined by a procedure of extraction and the type of extraction solvent [19].

According to generally accepted concepts, molecules of humic substances are built of polymeric micelles, whose structure consists of aromatic nuclei, like hydroxyphenols, as well as aliphatic structures, such as carbohydrate, protein and amino acid residues. The following groups play a role of bridges connecting particular structures: $-O-$, $-(CH_2)_n-$, $-NH-$ and $-S-$. Molecules of humic substances have a whole range of functional groups determining their chemical properties: $-COOH$ (carboxylate), $-OH$ (phenolic and alcoholic), $>C=O$ (quinone and ketone), $-NH_2$ (amino and amide) and methoxy groups $-OCH_3$ [12, 13, 18, 19]. Due to the specific character and size of molecules, it is rather difficult to determine the structural model of humic substances. Over the past 50 years, many structural models of humic substances have been proposed, among which Orłow [36] distinguishes two groups: the first one – hypothetical models, the other one – models that base on the results of analyses of humic substances.

In 1948 Dragunow presented a model of structure consisting of di- and trisubstituted aromatic rings, heterocyclic rings containing nitrogen, nitrogen in lateral chains and carbohydrate residues. The model scheme is presented in Figure 2.

In 1951 Kasatoczkin presented a concept according to which a HS molecule contains the so-called nucleus composed of condensed aromatic rings linked with aliphatic chains (in: [19]).
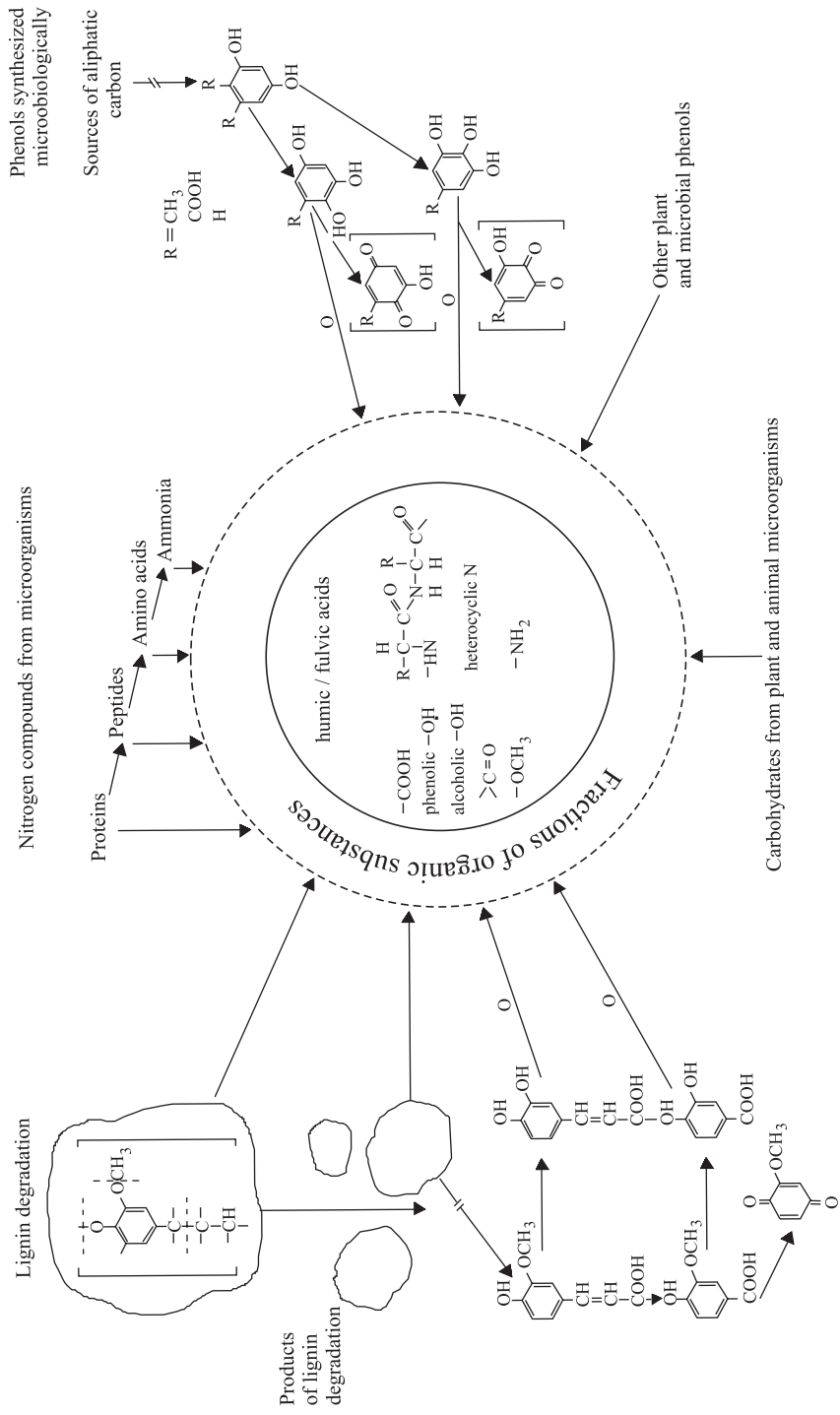
Fig. 1. The process of humic substances formation in ecosystems [16]

Phenols synthesized microobiologically

Sources of aliphatic carbon

R = CH₃
COOH
H

Other plant and microbial phenols

Nitrogen compounds from microorganisms

Ammonia

Amino acids

Peptides

Proteins

Fractions of organic substances

humic / fulvic acids

heterocyclic N

−COOH
phenolic −OH
alcoholic −OH
>C=O
−OCH₃

−NH₂

Carbohydrates from plant and animal microorganisms

Lignin degradation

Products of lignin degradation

7

(1) aromatic rings
(2) nitrogen in cyclic forms
(3) nitrogen in lateral chains
(4) carbohydrates

Fig. 2. Model presenting the structure of humic acids according to Dragunow (in: [36])

According to the model presented by Schnitzer and Khan [44], a HS molecule consists of separate, substituted benzene rings connected into the polymeric system by hydrogen bonds with carboxylate and phenolic groups (Fig. 3).
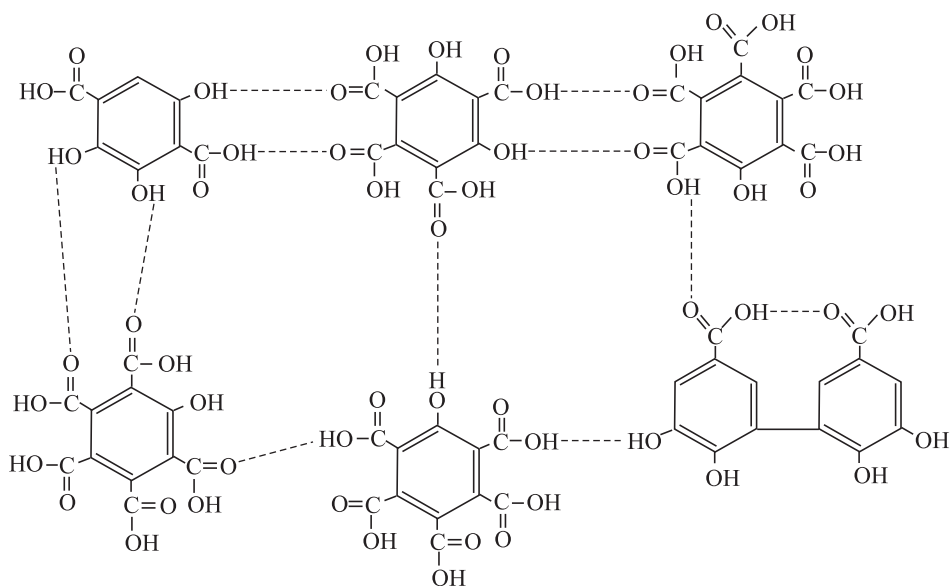


Fig. 3.  Model presenting the structure of fulvic acids according to Schnitzer and Khan [45]

Another attempt at creating a model of HS structure was based on a structure flowchart of humic substances and tended towards a three-segmented structure of molecules: an aromatic nucleus, lateral chains (or bridges) – mainly aliphatic and functional groups.

The structure of humic acids presented by Stevenson in 1982 included the main elements of their structure confirmed by chemical analyses. These are: aromatic ring

systems, free and bound phenolic groups, oxygen bridges, carboxylate groups in the form of substituents on aromatic rings, as well as chains of peptides and carbohydrates.

Introducing new analytical techniques in the analysis of humic substances, such as mass spectroscopy, made it possible to construct a new model of skeleton structure of humic acids. It was found that during pyrolytic decomposition of HAs, several alkyl-aryl compounds are produced, which contain aliphatic substituents covalently bonded with aromatic rings. Figure 4 presents a model of the structure of humic acids based on alkylbenzene, naphthalene and phenanthrene elements. According to authors, this structure is consistent with the properties of humic substances: it can bond both organic (carbohydrates, proteins, fats, biocides) and inorganic components, e.g. clay minerals. Such a structure is flexible – which is in accordance with the results of viscosimetric analysis, and oxidation leads to the production of benzenecarboxylic acids, which are the main product of HA oxidation.
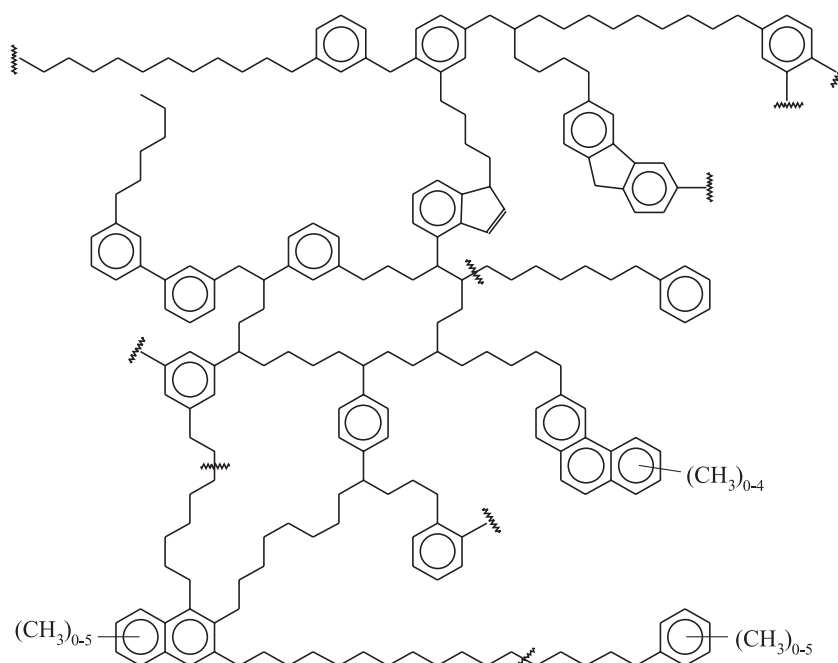


Fig. 4. Model presenting the structure of humic acids according to Schulten, Plage and Schitzer [1991]

The use of analytical methods allowing the examination of a substance in the natural state, without the need of extraction, such as electronic paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR), and particularly NMR CP/MAS (the technique of cross polarization with placing of a sample at the magic angle to the external magnetic field), confirmed the dimerous structure of humic substances (HS). The aromatic part accounts for 21-73% of the molecule of humic compounds. The values of chemical shifts for signals indicate that they can derive from lignin, but before building in the structure of HS they were transformed as a result of oxidation processes.

The aliphatic part of humic substances accounts for 7-36% of the molecule and is probably of microbiological origin [13].

Based on the methods of accelerated sedimentation in ultracentrifuges and images from an electron microscope [2, 16], a theory was developed on the globular shape of HA molecules. A different opinion is represented by Piret (after [44]), who states that humic acids are in the shape of elongated ellipses. The research proved, however, that the shape of molecules of humic acids is determined by concentration, pH and the ionic strength of a solvent [22]. In recent years, the theory of fractals gains some popularity, both in the chemistry of humic substances and in other fields of science [37, 43, 46].

## 1.2. THE SIGNIFICANCE OF HUMIC SUBSTANCES IN THE SOIL

Due to specific structure of humic substances and high diversity of functional groups, they are capable of contributing in the processes of adsorption, ion exchange and complex formation. These properties determine their miscellaneous functions in ecosystems. Humic substances contained in soils determine their biological, chemical and physical properties [12, 13, 20]. The major functions of humic substances in agroecosystems include:

- contribution to the genesis of soils and determination of their properties,
- contribution to the biological cycle of chemical elements,
- regulation of mineral nutrition of embryophytes (higher plants),
- contribution to ion exchange, sorption processes,
- supplying the energy and carbon to microorganisms,
- influence on solubility and migration of many chemical elements,
- regulation of buffer soil properties,
- regulation of oxidation and reduction processes,
- binding and acceleration of degradation of pesticides,
- stimulation of the growth and development of plants,
- inhibition of the development of certain phytopathogens.

**Contribution to the genesis of soils and determination of their properties**

Soil formation and configuration of the profile result from four main processes: inflow, outflow, transportation and transformation. Humic substances participate in all these processes [13]. The presence of humus helps to distinguish the soil from non-soil formations. The content of humus in different genetic soil horizons constitutes an element of soil diagnostics. The surface soil horizons are the richest in humic substances, but the latter occur also in horizons situated beneath the surface. This is a consequence of humic substances migration, in which clay minerals and metal ions often participate, e.g. in podzol soils. The presence of humic substances determines the colour of many soil horizons (humus, cambic, illuvial horizons). Humic substances largely determine a number of physical soil properties, e.g. the structure, temperature, permeability, water capacity, compactness etc. The humus content in soils determines their potential fertility [20].

**Supplying the energy and carbon to microorganisms**

Due to the chemical composition and energy value of humus compounds, these compounds can constitute a source of energy, carbon and nitrogen for soil microorganisms. Cultures of microorganisms on nutrient media, where humic

substances were the only source of carbon and nitrogen, revealed that many bacteria and fungi are capable of using these nutritional sources. Microorganisms that decompose HS also use some chemical elements which constitute the ash for their metabolic processes [31].

**Contribution to ion exchange and sorption processes**

Due to a large specific surface area and functional groups, humic substances are capable of cation-exchange sorption [13, 23]. This capability is several times higher compared with soil mineral elements (Table 1).

Table 1. Cation exchange capacity (CEC) of clay minerals and humic acids [13]

| Type of material | CEC (cmol(+)·kg$^{-1}$ of material) |
|---|---|
| kaolinite | 3-5 |
| illite | 30-40 |
| montmorillonite | 80-150 |
| humic acids | 485-870 |
| fulvic acids | up to 1,400 |

25-95% of the total cation exchange capacity in the surface soil horizons depends on the presence of humic substances [13, 20, 23, 39]. Nearly the total exchange capacity of peat deposits is determined by organic matter. A small amount of organic matter contained in sandy soils is very important for sorption of cations and prevention of their eluviation [29]. Cation exchange capacity is associated with the soil reaction and the extent of humic substances humification. The degree of humification determines the quantity of functional groups (especially carboxylate and phenolic ones), whereas the pH value determines the degree of their ionization (which is related to the capacity of free exchange of hydrogen ions for alkaline ions and the other way around).

**The impact on solubility and migration of chemical elements, as well as on the processes of oxidation and reduction**

Humic substances can bind metal ions into salts or chelates [3, 34]. It is assumed that the stability of complex compounds of humic substances with cations of multivalent metals is the highest at pH of ca. 5 and increases together with a metal valence according to the series:

$$Fe^{3+} > Al^{3+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+} = Ca^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}.$$
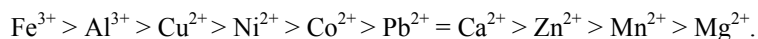
The resulting organic-mineral bonds determine the mobility of metal ions, their relocation in the soil profile and precipitation, and hence they affect the course of the soil-forming process and the development of the soil profile – e.g. the process of podzolization [13, 23]. This is also important for immobilization of toxic metals in the soil environment, which results in at least partial detoxification of the environment [20]. The resulting complexes prevent eluviation of nutrients and facilitate their uptake by plants – e.g. the presence of humic substances enhances the availability of phosphates. The ability of humic substances to form stable, complex compounds with ions of aluminium (toxic to plants) is of major significance in acid soils. The research revealed that in arable soil containing 1% humus the concentration of as little as 10 mg $Al^{3+}·kg^{-1}$ of soil induced a considerable reduction of crops. However, with the 5-6% content of humus, a harmful effect of aluminium ions occurred only at the concentration of 150 mg

$Al^{3+}\cdot kg^{-1}$ of soil [33]. The research also confirms the involvement of humic substances in the reduction of ions of toxic chromium (VI) to chromium (III), and its immobilization in the soil in the form of chromium (III) hydroxide [13]. Mineral--organic compounds are important for the geochemistry of trace elements. Through the influence of organic matter exerted on rocks and minerals, they become dissolved and trace elements are released (Fig. 5).
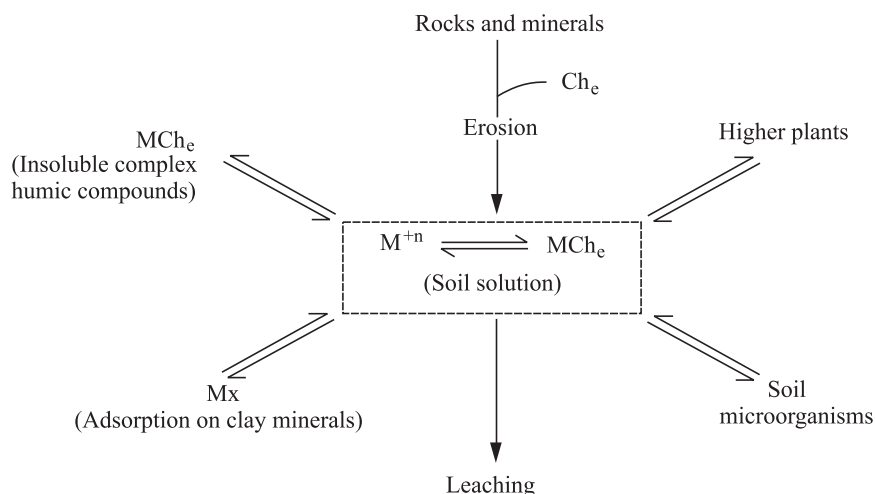


Fig. 5. Contribution of organic matter to the relocation of trace elements in soil [1]

**Regulation of buffer soil properties**

Ion-exchange properties of humic substances are considerably important for the determination of buffer soil properties. Soil organic matter forms a specific type of buffer, which has the capacity to operate within a broad range of pH. A decreased pH value of the soil solution causes a reduction in the degree of dissociation of acid functional (carboxylate and phenolic) groups, which in turn results in binding of free hydrogen ions. Hydrogen ions can also be exchanged for alkaline ions adsorbed on organic matter. In the case of excessive numbers of alkaline ions, a reverse process can occur [13, 39].

**Binding and acceleration of degradation of pesticides**

Humus compounds are also capable of binding the organic molecules of e.g. pesticides to form durable, not toxic complexes [1, 13, 20]. The nature of a bond depends on the type of pesticide and soil conditions. Cationic pesticides can be bound by carboxylate and hydroxyl groups of HS, producing salt-like bonds. S-triazine and aniline herbicides are bound by means of intermolecular interactions (van der Waals forces or hydrogen bonds). The degree of pesticide bonding is correlated with the content of organic matter in soil. Bonds of pesticides with organic matter are more prone to degradation [13].

**Inhibition of the development of certain phytopathogens**

Soil humus compounds are capable of inhibiting the development of certain phytopathogens. Myśków et al. [34] proved that the presence of organic matter reduces the development of phytopathogenic fungi, which are responsible for diseases of cereals through supporting the development of saprophytes antagonistic to these pathogens.

**Biostimulation of the growth and development of plants**

Humic substances exert a direct influence on plants at all stages of their development. HS have a protective effect, similarly to growth stimulators and other biologically active compounds, e.g. vitamins, antibiotics, enzymes, phytohormones. It was found that plants can directly uptake macromolecules of humic substances. Depending on the concentration and the type of humic substances, there were observed stimulating or inhibiting effects on the development of plants. The research revealed that humic substances affect the processes of seed germination and the root system development. They also determine the obtained yields (increasing them to 15% in the conditions of complete NPK fertilization), and they can modify their chemical composition [20, 32].

**Regulation of mineral nutrition of embryophytes (higher plants)**

Due to the contribution of humic substances in the processes of adsorption--desorption of biogenic ions, they constitute a "store" of ions that play a significant role in the mineral nutrition of plants. Humic substances contain on average 4-6% of nitrogen, which is released in the mineralization processes and is made available to plants. Due to a slow rate of the process of mineralization of nitrogen from humic substances, they constitute a reservoir of soil nitrogen. Humic substances are a group of compounds that are relatively resistant to biodegradation, but the research revealed that some bacteria, actinomycetes and fungi are capable of decomposing them. In the processes of biodecomposition, carbon from humic substances is used as energy material. A certain portion of nitrogen and biogenic elements released during these processes can be directly available to higher plants, while the remaining part becomes available only after microorganisms die.

## 2. SOIL POLLUTION

Soil is a receiver of many pollutants. Apart from covering it with concrete, asphalt and tar, chemical contamination gives a cause for concern. The presence of chemical contaminants in the soil is a consequence of, i.a., using the pesticides and excessive amounts of fertilizers, and also atmospheric immission.

Moreover, different substances penetrate into soil in an uncontrolled manner. Soil constitutes a specific sorbent, filter and buffer in relation to environmental pollution. However, the "defence mechanisms" of soils, being subjected to continuous direct and indirect effects of harmful substances, are sometimes interrupted, which results in soil degradation, and pollutants are spread to other elements of the environment and food products [23, 50].

According to Prusinkiewicz (after [13]), degradation of soils is a natural or artificial reduction in their fertility and productivity due to a deterioration in their physical, chemical, microbiological and other properties, important for life and development of plants. And thus, each factor reducing the production capacity of soils is regarded as a degrading factor. The most common types of soil degradation are as follows:
  – soil depletion (impoverishment) of nutrients and disturbance of the ionic equilibrium,
  – acidification or alkalization,
  – excessive loss of humus,
  – desiccation or flooding,
  – salinity,
  – water and aeolian erosion,
  – distortion in the soil structure,
  – mechanical contamination,
  – pollution with phytotoxic compounds,
  – biological contamination [13, 47].

Together with the increasing number of emission sources, classification of pollution into global, regional and local becomes less and less legitimate. Typical sources of local pollution, such as small production plants and service workshops, as well as living and "civilization" human activity, cause contamination of an increasingly global extent. The range of contamination caused by ethyl-gasoline-powered cars, which was once considered to be local and then regional, nowadays covers all continents.

The major sources of soil pollution are as follows: excessively used fertilizers, pesticides, precipitation of harmful substances (including solid – dust and gaseous substances, as well as substances suspended and dissolved in precipitation water), sewage, waste material and transfrontier pollution (e.g. harmful leakage and dusting from unprotected landfill sites, leaking of fuels etc.) [23, 50]. The recorded pollution of soils in Poland is generally not higher than in other regions of Europe. Nevertheless, the soil pollution poses a serious threat to human health due to uncontrolled exploitation of soils, particularly for horticultural crops [23]. Soil impoverishment regarding nutrients, as a form of degradation, basically occurred together with the beginnings of agriculture. During that time, due to the lack of knowledge about transformations taking place in the soil under the influence of cultivation, it was particularly intensive. At present, we can also face this problem in the case of intensive or monoculture cultivation, i.e. when components removed with crops are not replenished with fertilizers [13].

Among factors degrading the natural environment in Poland, soil erosion seems to be the most important one in terms of extent, consequences and duration [27]. Approximately ¼ of the country is threatened with surface, gully and wind erosion [13, 24, 27]. Soil erosion occurring under the influence of nature forces, supported or initiated by different forms of anthropopressure, accelerates the destruction of the surface soil horizon. The soil material, which is eluted by water and runs off with river waters to the sea, accumulates at the foot of hills and mountains or in terrain depressions in the form of deluvia and alluvia. Wind erosion induces deflation of the finest soil particles. This reduces the content of organic matter and fine mineral particles, which alters physical and chemical soil properties [13, 27].

Soil desiccation is a consequence of permanent groundwater table lowering caused by land drainage. Excessive drainage can be associated with inappropriate land-improvement treatments, the increasing consumption of water in industry and municipal services, as well as effects of strip mines. Soil desiccation affects the organic matter. It appears from the research by Prusinkiewicz and Kosakowski (after [42]) that shrinkage of humus colloids, which follows the soil desiccation, facilitates mutual approaching of hydrophilic (water-soluble) groups of humic substances and consequently, hydrogen bonds develop between them. This results in the dominance of hydrophobic groups on the surface of the solid phase, which in turn reduces the soil wettability [42]. Excessive soil moisture and flooding is a frequent phenomenon in mining districts. This can also be associated with malfunctioning of land reclamation and water erosion [13].

Alkaline substances, which induce alkalization of soils, get into the soil environment together with particulates (dust) emitted by cement, limestone and magnesite plants, and also together with dust (urea) and gases (ammonia) emitted by nitrogen plants. Slightly alkaline dust can also be introduced into the atmosphere by power industry (ashes after coal combustion) and metallurgy (metal oxides) [13, 42]. Seasonally in urbanized areas, the soil receives large doses of sodium and calcium salts (applied as agents preventing after-snowfall road slipperiness), which always leads to the strong alkalization of the substrate [4, 15, 42]. According to the research by Badora and Filipek [4, 15], alkalization of soils is connected with winter road treatments by means of a salt mixture, which results in a considerable alkalization of soils adjacent to priority snow-removal routes within the zone of up to 10 m wide. In the regions bordering on soda plants, one can observe progressing alkalization and excessive soil salinity. In the region of Janikowskie Soda Plants, the area of highly saline soils exceeded 100 ha [20]. Alkalization is caused by saturation of the sorption complex with sodium ions, and then, in the presence of $CO_2$, readily soluble sodium carbonate is produced, the hydrolysis of which induces an increase in the soil pH [8, 17]. The concentration of sodium ions in the sorption complex in adjacent soils was up to 6.4 cmol(+)·$kg^{-1}$ soil and was significantly correlated with a low content of exchangeable potassium (0.005-0.024 cmol(+)·$kg^{-1}$ soil) [7]. At first, the effect of alkaline emissions resembles the effect of soil liming. Through neutralization of acidity, they increase the biological soil activity, accelerate the processes of organic matter mineralization and increase the amount of nutrients entering the biological cycle. At the same time, due to the saturation of the sorption complex, mainly by calcium ions, assimilation of other cations (displaced by calcium ions from the sorption complex) is enhanced. The prolonged alkaline emissions have consequences similar to overliming. Due to excessive decomposition of organic matter, the sorption capacity of soil is reduced. Almost all ions disappear from the mineral part of the sorption complex except for

calcium ions [13, 42]. Environmental changes in areas situated within the range of immissions of alkaline nitrogen compounds are determined by one-sided overnutrition of plants with nitrogen compounds and the general impairment of soil environment functioning, rather than changes in the soil reaction [42]. One of the adverse effects of soil alkalization, in particular forest soils, is the extinction of fungi living in symbiosis with many tree species [39].

Acidifying influence of industrial immission on soils is mainly related to the effect of sulphur dioxide and sulphuric(VI) acid [42]. From the analysis of data on the volume of $SO_2$ emission in Poland (Fig. 6), it appears that we are dealing with an upward trend despite numerous investments aiming at its reduction [48].

$SO_2$ mln t·year$^{-1}$
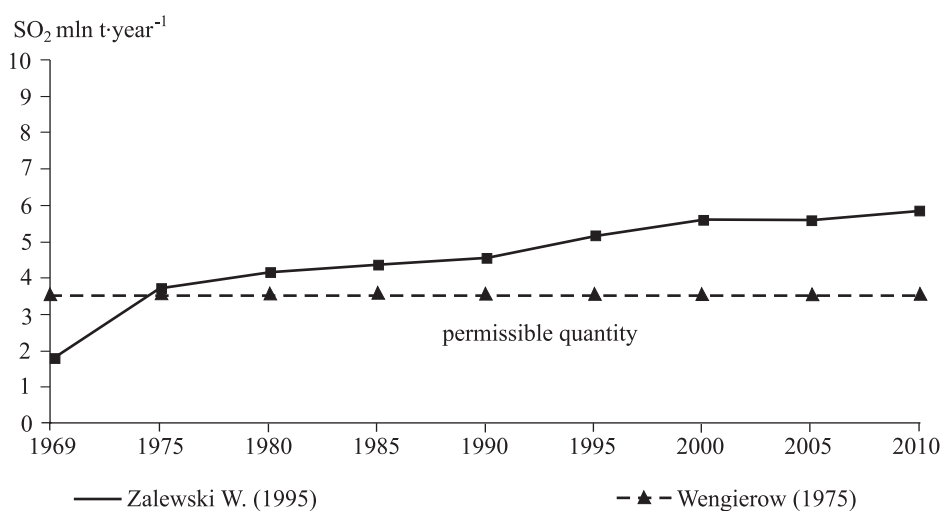


Fig. 6.  To date and forecast emission of $SO_2$ in Poland according to different sources [48]

The significance of nitrogen oxides and nitric(V) acid is secondary but still increasing [42]. Sulphur dioxide and other sulphur compounds emitted into the atmosphere are oxidized to sulphuric(VI) acid. The process of $SO_2$ oxidation (Fig. 7) proceeds through the intermediate stage – formation of sulphurous(IV) acid via contact with water, which is then catalytically oxidized to sulphuric(VI) acid.

If there is no enough water vapour in the air, $SO_2$ remains even up to 30 days and during that time it can be carried over considerable distances from a source of emission [48].

The process of acidification affects the deterioration of physical, chemical and biological properties of soils. As a consequence, ectohumus dominated by fulvic acids develops. It does not have properties of sticking the soil particles together and thus, no lumpy structure develops, and next a sudden deterioration of water-air conditions follows [14]. The effect of acidifying agents is reflected in the fact that the natural buffer capacity of soils and their pH reaction is reduced. The most resistant to progressive acidification are soils rich in calcium carbonate, which is a component of the carbonate buffer and which helps to preserve the pH range within 6.8-7.2. Long-term acidification of soils results in the balanced pH all over the soil profile [13, 37, 42].

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The SO$_2$ oxidation scheme shows:

$2\text{-}3\% \ S_2 \xrightarrow{ax} SO_3 \xrightarrow{H_2O} H_2SO_4 \xrightarrow{H_2O} H^+ + HSO_4^- \xrightarrow{H_2O} 2H^+ + SO_4^{2-}$

$\approx 5 \ M H_2SO_4$

$SO_2$

$97\text{-}98\% \ SO_2 \xrightarrow{H_2O} H_2SO_3 \xrightarrow{H_2O} H^+ + HSO_3^- \xrightarrow{H_2O} 2H^+ + SO_3^{2-}$

$\downarrow Fe^{3+}, Al^{3+}, NH_4^+ \downarrow$

$2H^+ + SO_4^{2-} \qquad 2H^+ + SO_4^{2-}$

$H_2SO_4$
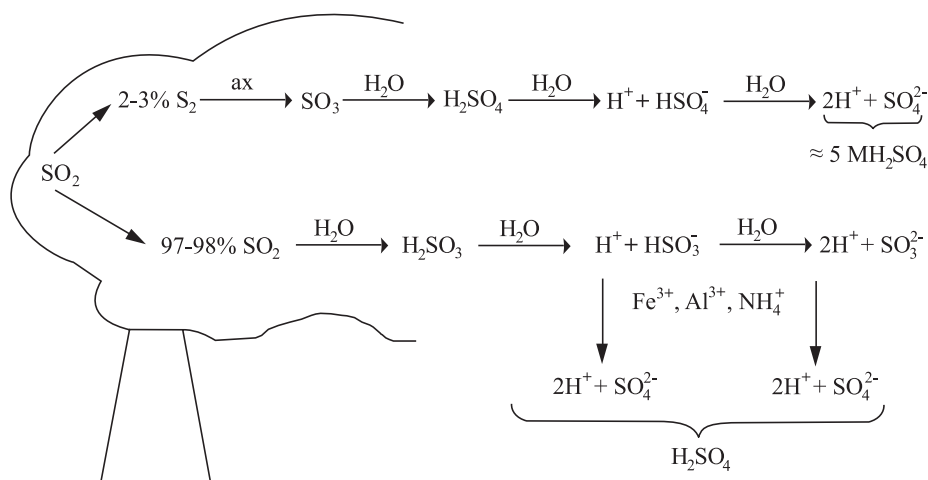
Fig. 7. SO$_2$ oxidation in the atmosphere [48]

In acid soils, usually with the granulometric composition of sand, the amount of mobile aluminium excessively increases, and at the same time the amount of alkaline cations drops. Drozd [11] draw attention to the fact that a low degree of saturation with alkaline cations of the sorption complex in soils beneath the degraded spruce forests and dwarf mountain pine in the Karkonosze Mts could be the cause contributing to dying of forest stands. These soils are very susceptible to unfavourable effects of chemical contamination, and their fertility is quickly reduced. Acid soils are characterized by a low assimilability of nutrients. Trace elements introduced into light acid soils are readily dissolved in soil solutions, therefore they are intensively absorbed by plants and readily migrate to subterranean waters. Phosphates with iron(III) and aluminium ions build insoluble compounds, and consequently they are unavailable for plants. Processes occurring in such soils increase the ecological risk in areas with the emission of sulphur and trace metals [23, 38].

Acidification adversely affects the metabolism of microorganisms and reduces the intensity of mineralization of organic matter in soil. Low pH has an inhibiting effect on many bacterial processes in soil, e.g. elemental nitrogen fixation, nitrification and ammonification [13, 14, 42]. The place of bacteria is taken by fungi, however they do not take over the functions of bacteria. In the case of emission containing the nitric(V) acid into the soil with nitrogen deficiency, the first response of plants could be a positive one. Only a long-term influence of a contamination factor induces adverse effects associated with the nitrogen overdose and disturbances in the ratio between particular nutrients [42].

Acid soils do not create good conditions for the growth and development of cultivated plants, which in turn provides an opportunity for the abundant occurrence of acidophilous weeds that can be regarded as the so-called indicators of strong acidification [14].

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Degradation of chemical soil properties is also associated with the increased content of heavy metals, which mainly come from industrial processes, as well as garbage and technological waste applied in fertilization and soil liming. Most of the trace elements settle on the soil surface in agriculture regions of Europe in relatively similar quantities. This fallout usually exceeds the amount of trace elements carried away with plant crops and leached as a result of subterranean water runoff into a catchment area. This clearly indicates a tendency to accumulation of chemical elements in the surface soil horizons. Certain trace elements, particularly those occurring in anionic forms (boron, fluorine, complex anions) build readily soluble and therefore mobile forms. Cationic forms of trace metals build readily soluble bonding, particularly in acid environment, e.g. in soils polluted with sulphur compounds, which creates an additional risk for the chemical equilibrium of agroecosystems. In the conditions of strongly acid soils, these chemical elements are easily absorbed by plants and more quickly penetrate into subterranean waters [14, 23].

# 3. THE EXPERIMENTAL PART

## 3.1. THE RESEARCH MATERIAL

Samples of 5 types of soils were collected from the Kujawy-Pomerania Region:
− humic gleysol, horizon M, 0-20 cm (Stopka) – No. 1,
− proper mollic gleysols, horizon $A_p$, 0-20 cm (Orłowo) – No. 2,
− proper mollic gleysols, horizon $A_a$, 20-30 cm (Orłowo) – No. 3,
− typical arenosol, horizon A, 0-20 cm (Brzoza) – No. 4,
− typical luvisol, horizon $A_p$, 0-20 cm (Kobylniki) – No. 5,
− typical cambisol, horizon $A_p$, 0-20 cm (Mierzwin) – No. 6.

Before incubation, soil samples were cleaned of plant residues (roots and above-
-ground parts of plants). The collected samples, whose main description is presented in
Tables 2 and 3, constituted the initial material for the incubation experiment.

Table 2. Grain composition of soil samples

| Sample symbol | Content of fractions [%] | | | Granulometric (according to particle size) | |
|---|---|---|---|---|---|
| | sand 1-0.1 mm | silt 0.1-0.02 mm | clay <0.02 mm | groups | subgroups |
| 1 | 61 | 16 | 23 | loam | sandy loam |
| 2 | 53 | 20 | 27 | loam | sandy loam |
| 3 | 55 | 22 | 23 | loam | sandy loam |
| 4 | 91 | 5 | 4 | sand | loose sand |
| 5 | 63 | 25 | 12 | sand | light loamy sand |
| 6 | 68 | 21 | 11 | sand | light loamy sand |

The incubation experiment was conducted in plastic pots of 1 $dm^3$ volume; plastic
tubes were used for aeration of the incubated material (3 pieces per pot). The incubation
was continued for 1 year (from October to September) in conditions of constant
moisture content (at the level of 50% field water capacity), in variable temperature
corresponding to the annual temperature characteristics of the region. Pots were
successively filled with material of all types of soils, according to the following scheme:

| Symbol of experimental variants | Incubated material |
|---|---|
| 1/0 | soil without contamination after one-year incubation (control sample) |
| | soil + acidifying agent |
| 1k1 | + 22.5 $cm^3$ of acidifying solution·1 $dm^{-3}$ of soil |
| 1k2 | + 30.0 $cm^3$ of acidifying solution·1 $dm^{-3}$ of soil |
| 1k3 | + 37.5 $cm^3$ of acidifying solution·1 $dm^{-3}$ of soil |
| | soil + alkalizing agent |
| 1a1 | + 22.5 $cm^3$ of alkalizing solution·1 $dm^{-3}$ of soil |
| 1a2 | + 30.0 $cm^3$ of alkalizing solution·1 $dm^{-3}$ of soil |
| 1a3 | + 37.5 $cm^3$ of alkalizing solution·1 $dm^{-3}$ of soil |
| | soil + salt mixture |
| 1z1 | + 4 g of salt mixture·1 $dm^{-3}$ of soil |
| 1z2 | + 8 g of salt mixture·1 $dm^{-3}$ of soil |
| 1z3 | + 12 g of salt mixture·1 $dm^{-3}$ of soil |

Table 3. Basic characteristics of soil samples

| Sample symbol | C [g·kg⁻¹] | N [g·kg⁻¹] | C:N | pH H₂O | pH KCl | EC [mS·cm⁻¹] | SBM [m²·g⁻¹] | T | S [cmol(+)·kg⁻¹] | H_w | V [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21.5 | 3.0 | 7.16 | 6.79 | 5.91 | 0.27 | 51.54 | 9.7 | 8.9 | 0.8 | 90.3 |
| 2 | 12.2 | 1.2 | 10.16 | 7.87 | 7.75 | 0.26 | 97.61 | 19.9 | 19.9 | 0.0 | 100.0 |
| 3 | 15.1 | 1.6 | 9.43 | 7.72 | 7.46 | 0.31 | 100.76 | 21.1 | 21.0 | 0.1 | 99.6 |
| 4 | 11.4 | 0.8 | 14.25 | 4.94 | 3.92 | 0.08 | 9.29 | 2.7 | 0.0 | 2.7 | 0.0 |
| 5 | 7.4 | 0.7 | 10.57 | 6.54 | 5.61 | 0.13 | 16.91 | 8.1 | 7.4 | 0.7 | 91.4 |
| 6 | 6.5 | 0.7 | 9.28 | 6.12 | 5.25 | 0.27 | 22.48 | 10.1 | 9.2 | 0.9 | 90.9 |

EC – electrolytic conductivity
SBM – specific surface area
T – total exchange capacity
S – cation-exchange capacity
H_w – hydrolytic acidity
V – saturation

0.075 M solution in relation to sulphuric (VI) acid and 0.15 M solution in relation to nitric(V) acid were used for acidification of samples. Alkalization of samples was performed by adding 0.075 M solution in relation to calcium hydroxide and 0.15 M solution in relation to sodium hydroxide. The salinity was obtained by mixing the samples with the mixture of solid salts: calcium and sodium chlorides (in the mole ratio of 1:1).

After incubation, samples from pots were air-dried, sieved through a sieve with a mesh diameter of 1 mm and analysed.

## 3.2. RESEARCH METHODS

### 3.2.1. Organic carbon (TOC)

The content of organic carbon was determined using the Autoanalyzer Primax, produced by the company Skalar B.V. (Breda, the Netherlands), based on the difference between the content of total carbon (TC) and inorganic carbon (carbonates, IC). Determination of TC consists in catalytic combustion (catalyst: cobalt activated by chromium(III)) of a sample in a quartz crucible in a stove, at the temperature of 1200°C and detection of released $CO_2$ in infrared radiation. Determination of IC consists in analysing the amount of $CO_2$ separated by orthophosphoric acid(V) at room temperature from a soil sample (detection of $CO_2$ in infrared). The content of TOC in soil samples, determined by the method of catalytic combustion, is higher than that determined by standard methods: Tiurin (1.4-times) and Alten (1.2-times).

### 3.2.2. Total nitrogen ($N_t$)

Total nitrogen ($N_t$) was determined with Kjeldahl's method. The method consists in the complete thermal decomposition of organic matter in the presence of concentrated sulphuric acid(VI) and transformation of the total nitrogen into the ammonia form bound by sulphate ions(VI). The resulting ammonia nitrogen is determined by the distillation method, where ammonia is bound in a receiver by sulphuric acid(VI) [28].

### 3.2.3. Soil reaction

The potentiometric method was applied to measure the soil reaction. It consists in taking measurements of pH using a pH-meter [type 540GLP produced by the company WTW]. Measurements were taken in a suspension: soil samples with water or 1 M KCl (10 g soil + 25 mL of deionized $H_2O$ or 1 M KCl). Measurements were taken after 24 h after samples were flooded with the appropriate solution [28].

### 3.2.4. Electrolytic conductivity (EC)

Electrolytic conductivity was determined by the conductometric method, with a conductometer CM204 produced by Slandi. Measurements were taken in the suspension of 10 g soil + 20 mL of deionized $H_2O$ [30].

### 3.2.5. Specific surface area

Specific surface area (SSA) of soil samples was determined by the methylene blue adsorption method (MB) [30]. The method consists in spectrophotometric (665 nm) determination of methylene blue, which was not adsorbed by soil samples. Specific surface area was calculated from the formula:

$$SBM = \frac{(a-b)\cdot 10^{-3}}{320\cdot g} \cdot Am \cdot N_A$$

where:

SBM – specific surface area [$m^2 \cdot g^{-1}$],
*a* – the amount of methylene blue used for adsorption [mg],
*b* – the amount of methylene blue left in the solution after sorption [mg],
*g* – analytical soil sample [g],
*Am* – the surface area occupied by one molecule of methylene blue ($130 \cdot 10^{-20}$ $m^2$),
$N_A$ – the Avogadro constant ($6.02 \cdot 10^{23}$),
320 – molar mass of methylene blue.

### 3.2.6. Total exchange capacity (T), hydrolytic acidity (Hw) and total exchangeable bases - base exchange capacity (S), the degree of base saturation (V)

Base exchange capacity (S) was determined by Kappen's method [30]. It consists in displacement of basic cations of 0.1 M HCl from the sorption complex. The excess of acid was titrated with 0.1 M NaOH, and the total of bases (base exchange capacity) was calculated from the relation:

$$S = (50 - a)\cdot 0.1 \cdot 10$$

where:

S – base exchange capacity [$cmol(+)\cdot kg^{-1}$ soil]
50 – the volume of the filtrate used for titration [mL]
a – the volume of NaOH used for titration [mL]

In order to determine the hydrolytic acidity, Kappen's method was applied. It consists in displacement from the sorption complex exchangeable ions $H^+$ and $Al^{3+}$ by the solution of $CH_3COONa$ with pH of 8.2, and in determination of the amount of displaced ions by titration with 0.1 M NaOH. Hydrolytic acidity was calculated from the formula:

$$H_w = a\cdot n\cdot 5\cdot 1.5$$

where:

$H_w$ – exchangeable acidity [$cmol(+)\cdot kg^{-1}$ soil]
a – the volume of NaOH used for titration [mL]
n – molar concentration of NaOH
5 – the conversion factor
1.5 – the empirical factor determined by Kappen

The total sorptive capacity was calculated based on the known value of the base exchange capacity (S) and hydrolytic acidity ($H_w$):

$$T = S + H$$

The degree of base saturation (V) was calculated from the formula:

$$V = \frac{S}{T} \cdot 100\%$$

### 3.2.7. Grain composition

Grain composition was determined by the areometric method according to Prószyński. Based on the granulometric composition, granulometric (grain-size) groups and subgroups of soils were determined according to the Polish Society of Soil Science [30].

### 3.3. PREPARATIONS OF HUMIC ACIDS (HAS)

### 3.3.1. Extraction of humic acids

Extraction of humic acids was carried out with 0.1 M NaOH after decalcification with 0.05 M HCl. The extraction was conducted according to the following scheme:
- decalcification with 0.05 M HCl (1:10 w/v); after 24 h of decalcification, the solution was centrifuged, and the residue was rinsed with the distilled water until neutral reaction,
- the extraction of humic acids; after decalcification, 0.1 M solution of NaOH (1:10 w/v) was poured over the residue, stirred, and after 24 h the extract of humic acids was centrifuged,
- precipitation of humic acids; the alkaline extract of humic acids was acidified with 2 M HCl to pH = 2, and centrifuged after 24 h,
- purification of humic acids with the solution of HCl-HF (5 mL HCl + 5 mL HF + 990 mL $H_2O$), samples were flooded with the purifying mixture and centrifuged after 24 h, rinsed with distilled water until reaction to chlorides disappears, preparations were frozen and lyophilized.

### 3.3.2. Elemental composition

The elemental composition was determined with the autoanalyzer CHN 2400 produced by Perkin-Elmer. The oxygen content (precisely − oxygen and sulphur) was calculated from the difference between the total content of C, H, N and the value of 100%. The results of the analysis are presented in weight and atomic percentage per ash-free substance. Based on the elemental composition, the degree of internal oxidation of humic acids was calculated according to the following formula [51]:

$$\omega = [(2O + 3N) - H]/C$$

where:

C, H, O, N – content of carbon, hydrogen, oxygen and nitrogen in atomic percentage.

### 3.3.3. Infrared spectrometry (IR)

Infrared spectra of humic acids were prepared with the use of an infrared spectrometer with Fourier transformation, Perkin-Elmer BX, within the range of 400--4000 $cm^{-1}$, for tablets of 3 mg HA in 800 mg KBr. The differences in the structure of humic acid molecules (occurring under the influence of pollution) were determined based on the infrared spectra, i.e. their width, intensity, the presence or the absence of particular absorption bands.

### 3.3.4. Ultraviolet and visible spectrometry (UV-Vis)

Visible spectra were prepared for 0.02% solutions of humic acids in 0.1 M NaOH, using a spectrometer Perkin Elmer Lambda 20. Recording of ultraviolet spectra was performed after dilution of the initial solution with 0.1 M NaOH in the ratio of 1:5.

Based on the determined absorbance values at the wavelengths of 280 ($A_{280}$), 400 ($A_{400}$), 465 ($A_{465}$), 600 ($A_{600}$) and 665 nm ($A_{665}$), values of the coefficients were calculated:

$A_{2/4}$ – the ratio of absorbance values at the wavelengths of 280 and 465 nm,
$A_{2/6}$ – the ratio of absorbance values at the wavelengths of 280 and 665 nm,
$A_{4/6}$ – the ratio of absorbance values at the wavelengths of 465 and 665 nm,
$\Delta logK = logA_{400} - logA_{600}$ [25].

### 3.3.5. Susceptibility of humic acids to chemical oxidation

Measurements of visible spectra of humic acids can be applied in the evaluation of their susceptibility to oxidation. Exposure of humic acids to hydrogen peroxide is a relatively simple method of determining their susceptibility to chemical oxidation.

For that purpose, 0.02% solutions of HAs in 0.1 M NaOH were prepared, which were treated with 3% solution of $H_2O_2$ in the volumetric proportion 1:1. Measurements of the absorbance were taken for stock solutions and after 24 h of oxidation at the wavelengths of 465 and 665 nm. For these wavelengths, a drop in the absorbance values was calculated for humate solutions after the reaction, and it was expressed in the percentage of the initial absorbance value of the reference solution:

$$\Delta A^{ox} = [(A_0 - A^{ox})/A_0] \cdot 100\%$$

where:

$A_0$ – absorbance of the solution before oxidation
$A^{ox}$ – absorbance of the solution after oxidation,

and a drop (in %) in a value of the parameter $A_{4/6}$:
$$DA^{ox}_{4/6} = [(A^{ox}_{4/6} - A_{4/6})/A_{4/6}] \cdot 100\%$$

The analysis of variance performed on the results of the analyses, for the experiments with 2 and 3 factors, with replications or without replications, was made with the statistical software ANALWAR developed and made available by Prof. Dr. hab. Franciszek Rudnicki (Professor, Ph.D.) and Dr. Karol Kotwica (Ph.D.) from the Department of Plant Cultivation and Biometrics.

# 4. THE RESULTS

## 4.1. SOIL SAMPLES

### 4.1.1. The main properties of the incubated soil samples

In order to assess the impact of simulated pollution on the properties of soil samples, measurements of pH were taken for the soil suspension in water and in 1 M solution of KCl ($pH_{H2O}$, $pH_{KCl}$), as well as measurements of conductivity (EC). The results are presented in Tables 4 and 5.

For the variants: acidification and alkalization, the pH reaction of soil samples was the physicochemical parameter that characterized the process of their contamination. For the samples of mollic gleysols (2 and 3), alkalization and acidification in the applied doses did not cause any statistically significant changes in pH values, measured both in water and in 1 M KCl. The biggest changes of pH in the variant with acidification were recorded for arenosol samples (4) – with naturally acid reaction (Table 4). The difference in the value of $pH_{H2O}$ between the control sample and the sample acidified with the maximum dose amounted to ca. 1.5. Alkalization changed the pH reaction of luvisol to the largest extent; the initial pH value was 6.5 – for the third dose of the alkalizing factor, the $pH_{H2O}$ value of the sample was 7.23. Both acidification and alkalization of soil samples caused a minor increase in the conductivity (EC) values. Statistical analysis of the obtained results, which was carried out with the analysis of variance for experiments with 2 factors, revealed that different pH values of samples were significant in all variants only with reference to the soil types. The dose of pollutants affects the pH reaction of the studied soils in a statistically significantly different way, depending on the type of pollutants (Table 6), which was a consequence of the soil buffer capacity.

In the variant of representative (model) soil salinity, the value of conductivity (EC) was accepted as a parameter confirming the increasing salinity of the material. For samples of all types of soils, the conductivity value increased 10 times already after the first dose of salt was applied, on average above 3.5 mS·cm$^{-1}$. The second dose increased the conductivity value on average up to ca. 6 mS·cm$^{-1}$, whereas the third dose – over 9 mS·cm$^{-1}$. Salinity of samples did not induce any significant changes in the soil reaction (Table 5).

Statistical analysis on the obtained results revealed the significance of differences between EC values for soil types (luvisol, arenosol and humic gleysol), as well as for different doses of salt (Table 5).

Table 4. Physicochemical parameters of incubated soil samples

| Sample symbol | EC [mS·cm⁻¹] | pH_H2O | pH_KCl |
|---|---|---|---|
| 1/0 | 0.59 | 6.36 | 5.55 |
| 1k1 | 0.66 | 6.21 | 5.49 |
| 1k2 | 0.63 | 6.12 | 5.46 |
| 1k3 | 1.09 | 5.67 | 5.06 |
| 1a1 | 0.44 | 6.52 | 5.68 |
| 1a2 | 0.52 | 6.61 | 5.94 |
| 1a3 | 0.51 | 6.83 | 6.04 |
| 1z1 | 4.07 | 6.19 | 6.30 |
| 1z2 | 6.63 | 5.81 | 5.82 |
| 1z3 | 10.83 | 5.77 | 5.47 |
| 2/0 | 0.38 | 7.97 | 7.84 |
| 2k1 | 0.53 | 7.85 | 7.84 |
| 2k2 | 0.73 | 7.94 | 7.80 |
| 2k3 | 1.04 | 7.92 | 7.81 |
| 2a1 | 0.42 | 8.01 | 7.86 |
| 2a2 | 0.41 | 8.22 | 7.55 |
| 2a3 | 0.41 | 8.20 | 7.58 |
| 2z1 | 4.02 | 7.66 | 7.67 |
| 2z2 | 6.13 | 7.37 | 7.60 |
| 2z3 | 9.89 | 7.29 | 7.27 |

| Sample symbol | EC [mS·cm⁻¹] | pH_H2O | pH_KCl |
|---|---|---|---|
| 3/0 | 0.42 | 7.81 | 7.64 |
| 3k1 | 0.76 | 7.72 | 7.45 |
| 3k2 | 0.87 | 7.70 | 7.54 |
| 3k3 | 1.10 | 7.70 | 7.60 |
| 3a1 | 0.45 | 7.81 | 7.66 |
| 3a2 | 0.49 | 7.92 | 7.63 |
| 3a3 | 0.55 | 7.89 | 7.69 |
| 3z1 | 3.83 | 7.47 | 7.34 |
| 3z2 | 6.30 | 7.37 | 7.25 |
| 3z3 | 9.11 | 7.13 | 7.17 |
| 4/0 | 0.22 | 4.77 | 4.34 |
| 4k1 | 0.42 | 3.92 | 3.68 |
| 4k2 | 0.54 | 3.51 | 3.36 |
| 4k3 | 0.56 | 3.43 | 3.35 |
| 4a1 | 0.17 | 5.04 | 3.83 |
| 4a2 | 0.13 | 5.40 | 3.96 |
| 4a3 | 0.30 | 5.64 | 4.31 |
| 4z1 | 2.69 | 3.93 | 3.93 |
| 4z2 | 5.16 | 3.71 | 3.94 |
| 4z3 | 9.04 | 3.37 | 3.95 |

| Sample symbol | EC [mS·cm⁻¹] | pH_H2O | pH_KCl |
|---|---|---|---|
| 5/0 | 0.21 | 5.95 | 5.35 |
| 5k1 | 0.44 | 5.55 | 4.83 |
| 5k2 | 0.56 | 5.53 | 4.77 |
| 5k3 | 0.78 | 5.11 | 4.53 |
| 5a1 | 0.24 | 6.59 | 6.31 |
| 5a2 | 0.26 | 7.23 | 6.77 |
| 5a3 | 0.28 | 7.23 | 7.20 |
| 5z1 | 3.10 | 5.65 | 5.53 |
| 5z2 | 5.92 | 5.86 | 5.60 |
| 5z3 | 7.77 | 5.84 | 5.78 |
| 6/0 | 0.28 | 5.80 | 5.11 |
| 6k1 | 0.55 | 5.46 | 4.70 |
| 6k2 | 0.67 | 5.22 | 4.52 |
| 6k3 | 0.77 | 4.93 | 4.32 |
| 6a1 | 0.30 | 6.12 | 5.27 |
| 6a2 | 0.32 | 6.13 | 5.47 |
| 6a3 | 0.38 | 6.14 | 5.33 |
| 6z1 | 3.62 | 5.74 | 5.44 |
| 6z2 | 6.90 | 5.98 | 5.82 |
| 6z3 | 8.79 | 5.93 | 5.92 |

Table 5. Mean values of physicochemical parameters of incubated soil samples

| | | Acidification (k) | | | | | Alkalization (a) | | | | | Salinity (z) | | |
| | | Mean values | | | | | Mean values | | | | | Mean values | | |
| Soil type | Dose | $pH_{H_2O}$ | $pH_{KCl}$ | EC | Soil type | Dose | $pH_{H_2O}$ | $pH_{KCl}$ | EC | Soil type | Dose | $pH_{H_2O}$ | $pH_{KCl}$ | EC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 6.09 | 5.39 | 0.743 | 1 | | 6.58 | 5.80 | 0.515 | 1 | | 6.03 | 5.79 | 5.530 |
| 2 | | 7.92 | 7.82 | 0.670 | 2 | | 8.10 | 7.71 | 0.405 | 2 | | 7.57 | 7.60 | 5.105 |
| 3 | | 7.61 | 7.56 | 0.788 | 3 | | 7.86 | 7.66 | 0.478 | 3 | | 7.45 | 7.35 | 4.915 |
| 4 | | 3.91 | 3.68 | 0.435 | 4 | | 5.21 | 4.11 | 0.205 | 4 | | 3.95 | 4.04 | 4.278 |
| 5 | | 5.54 | 4.87 | 0.498 | 5 | | 6.75 | 6.41 | 0.248 | 5 | | 5.83 | 5.57 | 4.250 |
| 6 | | 5.35 | 4.66 | 0.567 | 6 | | 6.05 | 5.30 | 0.320 | 6 | | 5.86 | 5.57 | 4.898 |
| LSD | | 0.606 | 0.464 | 0.202 | LSD | | 0.505 | 0.817 | 0.103 | LSD | | 0.568 | 0.679 | 1.182 |
| | 0 | 6.44 | 5.97 | 0.350 | | 0 | 6.44 | 5.97 | 0.350 | | 0 | 6.44 | 5.97 | 0.350 |
| | 1 | 6.04 | 5.66 | 0.560 | | 1 | 6.68 | 6.10 | 0.337 | | 1 | 6.11 | 6.04 | 3.555 |
| | 2 | 6.00 | 5.57 | 0.667 | | 2 | 6.92 | 6.22 | 0.355 | | 2 | 6.02 | 6.01 | 6.173 |
| | 3 | 5.80 | 5.44 | 0.890 | | 3 | 7.00 | 6.36 | 0.405 | | 3 | 5.89 | 5.92 | 9.238 |
| | LSD | ns | 0.336 | 0.146 | | LSD | 0.366 | ns | ns | | LSD | ns | ns | 0.856 |

EC: electrolytic conductivity [$mS \cdot cm^{-1}$]
ns – non-significant differences

Table 6. Elemental composition of humic acids isolated from samples of humic gleysol

| Sample symbol | Weight % | | | | Atomic % | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | N | O | C | H | N | O |
| 1\0 | 47.42 | 4.41 | 3.61 | 44.56 | 34.65 | 38.67 | 2.26 | 24.42 |
| 1k1 | 49.37 | 3.84 | 3.46 | 43.32 | 37.72 | 35.19 | 2.27 | 24.82 |
| 1k2 | 51.02 | 3.99 | 3.47 | 41.53 | 38.36 | 35.99 | 2.23 | 23.42 |
| 1k3 | 51.66 | 4.29 | 3.45 | 40.60 | 37.84 | 37.69 | 2.17 | 22.30 |
| 1a1 | 49.82 | 4.31 | 3.78 | 42.09 | 36.53 | 37.96 | 2.37 | 23.14 |
| 1a2 | 49.37 | 3.93 | 3.42 | 43.28 | 37.44 | 35.72 | 2.23 | 24.62 |
| 1a3 | 47.15 | 4.30 | 3.53 | 45.02 | 34.80 | 38.05 | 2.23 | 24.92 |
| 1z1 | 51.96 | 3.91 | 3.59 | 40.54 | 39.25 | 35.45 | 2.33 | 22.97 |
| 1z2 | 48.17 | 3.58 | 3.60 | 44.65 | 37.74 | 33.61 | 2.42 | 26.23 |
| 1z3 | 55.78 | 4.87 | 4.70 | 47.32 | 38.10 | 39.91 | 2.75 | 24.24 |

## 4.2. PREPARATIONS OF HUMIC ACIDS

### 4.2.1. Elemental composition

Elemental composition is one of the main properties of organic compounds, based on which one can identify them and infer about their structure. The results of the analysis on the elemental composition of humic acids (HAs) are presented in Tables 6-17.

Table 7. Elemental composition of humic acids isolated from samples of mollic gleysols (horizon Ap)

| Sample symbol | Weight % | | | | Atomic % | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | N | O | C | H | N | O |
| 2/0 | 52.36 | 3.59 | 3.47 | 40.58 | 40.64 | 33.43 | 2.31 | 23.62 |
| 2k1 | 49.85 | 3.99 | 3.53 | 42.63 | 37.55 | 36.08 | 2.28 | 24.09 |
| 2k2 | 48.77 | 3.76 | 3.50 | 43.96 | 37.54 | 34.77 | 2.31 | 25.38 |
| 2k3 | 50.36 | 5.10 | 3.97 | 40.57 | 34.65 | 42.08 | 2.34 | 20.93 |
| 2a1 | 50.41 | 3.91 | 3.99 | 41.68 | 38.17 | 35.56 | 2.59 | 23.67 |
| 2a2 | 51.36 | 3.87 | 3.47 | 41.29 | 38.97 | 35.28 | 2.26 | 23.49 |
| 2a3 | 50.59 | 3.72 | 3.59 | 42.10 | 38.94 | 34.39 | 2.37 | 24.30 |
| 2z1 | 47.42 | 4.16 | 3.52 | 44.91 | 35.39 | 37.22 | 2.25 | 25.14 |
| 2z2 | 50.30 | 3.67 | 3.27 | 42.76 | 38.93 | 34.08 | 2.17 | 24.82 |
| 2z3 | 52.54 | 3.85 | 4.23 | 44.57 | 38.68 | 34.05 | 2.67 | 24.61 |

Table 8. Elemental composition of humic acids isolated from samples of mollic gleysols (horizon Aa)

| Sample symbol | Weight % | | | | Atomic % | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | N | O | C | H | N | O |
| 3/0 | 49.51 | 4.51 | 4.06 | 41.93 | 35.75 | 39.04 | 2.51 | 22.71 |
| 3k1 | 49.40 | 4.31 | 3.77 | 42.52 | 36.25 | 37.97 | 2.37 | 23.40 |
| 3k2 | 49.58 | 4.37 | 4.13 | 41.92 | 36.20 | 38.26 | 2.58 | 22.95 |
| 3k3 | 49.67 | 3.88 | 3.91 | 42.53 | 37.76 | 35.44 | 2.55 | 24.25 |
| 3a1 | 52.54 | 4.02 | 3.79 | 39.64 | 39.27 | 36.07 | 2.43 | 22.22 |
| 3a2 | 47.98 | 4.27 | 3.66 | 44.10 | 35.45 | 37.81 | 2.31 | 24.43 |
| 3a3 | 51.52 | 4.24 | 3.89 | 40.35 | 37.88 | 37.42 | 2.45 | 22.25 |
| 3z1 | 48.12 | 4.07 | 3.87 | 43.94 | 36.12 | 36.65 | 2.49 | 24.74 |
| 3z2 | 50.40 | 3.90 | 3.87 | 41.83 | 38.21 | 35.49 | 2.51 | 23.79 |
| 3z3 | 50.17 | 3.95 | 3.88 | 41.99 | 37.89 | 35.81 | 2.51 | 23.78 |

Table 9.  Elemental composition of humic acids isolated from arenosol samples

| Sample | Weight % | | | | Atomic % | | | |
|--------|----|----|----|----|----|----|----|----|
| symbol | C | H | N | O | C | H | N | O |
| 4/0 | 51.89 | 4.79 | 3.57 | 39.74 | 36.47 | 40.43 | 2.15 | 20.95 |
| 4k1 | 51.38 | 4.84 | 3.57 | 40.20 | 36.01 | 40.71 | 2.15 | 21.13 |
| 4k2 | 51.16 | 4.70 | 3.52 | 40.63 | 36.28 | 39.96 | 2.14 | 21.61 |
| 4k3 | 51.36 | 4.85 | 3.73 | 40.06 | 35.97 | 40.75 | 2.24 | 21.04 |
| 4a1 | 52.63 | 4.84 | 3.71 | 38.82 | 36.79 | 40.64 | 2.22 | 20.35 |
| 4a2 | 53.92 | 4.95 | 3.66 | 37.47 | 37.31 | 41.07 | 2.17 | 19.45 |
| 4a3 | 49.69 | 4.67 | 3.75 | 41.89 | 35.40 | 39.93 | 2.29 | 22.38 |
| 4z1 | 50.28 | 4.87 | 3.81 | 41.04 | 35.23 | 40.92 | 2.29 | 21.56 |
| 4z2 | 51.96 | 4.57 | 3.54 | 39.93 | 37.18 | 39.22 | 2.17 | 21.43 |
| 4z3 | 53.19 | 4.73 | 3.74 | 38.34 | 37.47 | 40.01 | 2.26 | 20.26 |

Table 10. Elemental composition of humic acids isolated from samples of luvisol

| Sample | Weight % | | | | Atomic % | | | |
|--------|----|----|----|----|----|----|----|----|
| symbol | C | H | N | O | C | H | N | O |
| 5 | 48.49 | 5.20 | 4.79 | 41.52 | 33.18 | 42.70 | 2.81 | 21.32 |
| 5/0 | 46.41 | 5.47 | 4.43 | 43.69 | 31.24 | 44.15 | 2.56 | 22.06 |
| 5k1 | 44.71 | 5.30 | 4.61 | 45.38 | 30.57 | 43.46 | 2.70 | 23.27 |
| 5k2 | 43.80 | 4.99 | 3.75 | 41.69 | 31.71 | 43.32 | 2.33 | 22.64 |
| 5k3 | 49.33 | 5.26 | 4.91 | 40.51 | 33.56 | 42.91 | 2.86 | 20.67 |
| 5a1 | 49.82 | 5.42 | 4.77 | 39.98 | 33.46 | 43.66 | 2.75 | 20.14 |
| 5a2 | 45.44 | 5.43 | 4.56 | 44.57 | 30.72 | 44.03 | 2.64 | 22.60 |
| 5a3 | 46.59 | 5.24 | 4.65 | 43.52 | 31.88 | 43.05 | 2.73 | 22.34 |
| 5z1 | 48.96 | 5.23 | 4.88 | 40.93 | 33.40 | 42.81 | 2.85 | 20.94 |
| 5z2 | 46.02 | 5.50 | 4.56 | 43.92 | 30.92 | 44.33 | 2.62 | 22.13 |
| 5z3 | 43.04 | 4.78 | 3.51 | 39.43 | 32.43 | 43.02 | 2.27 | 22.29 |

Table 11. Elemental composition of humic acids isolated from samples of cambisol

| Sample | Weight % | | | | Atomic % | | | |
|--------|----|----|----|----|----|----|----|----|
| symbol | C | H | N | O | C | H | N | O |
| 6/0 | 48.16 | 5.24 | 4.77 | 41.83 | 32.87 | 42.92 | 2.79 | 21.42 |
| 6k1 | 49.54 | 5.13 | 4.41 | 42.02 | 33.81 | 42.03 | 2.58 | 21.51 |
| 6k2 | 47.51 | 5.49 | 4.52 | 45.27 | 31.42 | 43.54 | 2.56 | 22.46 |
| 6k3 | 49.41 | 5.25 | 4.95 | 40.39 | 33.62 | 42.88 | 2.89 | 20.61 |
| 6a1 | 46.47 | 5.51 | 4.27 | 43.75 | 31.18 | 44.36 | 2.45 | 22.01 |
| 6a2 | 46.26 | 5.17 | 4.56 | 44.01 | 31.86 | 42.71 | 2.69 | 22.73 |
| 6a3 | 48.45 | 5.20 | 4.77 | 41.58 | 33.15 | 42.71 | 2.80 | 21.34 |
| 6z1 | 49.73 | 5.15 | 4.98 | 40.14 | 34.09 | 42.34 | 2.93 | 20.64 |
| 6z2 | 45.46 | 5.12 | 4.55 | 44.88 | 31.48 | 42.52 | 2.70 | 23.31 |
| 6z3 | 49.51 | 6.62 | 4.99 | 39.88 | 32.75 | 44.64 | 2.83 | 19.78 |

Table 12. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from samples of humic gleysol

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 1\0 | 1.12 | 0.065 | 0.70 | 0.63 | 0.489 | 1.14 |
| 1k1 | 0.93 | 0.060 | 0.66 | 0.71 | 0.563 | 1.16 |
| 1k2 | 0.94 | 0.058 | 0.61 | 0.65 | 0.457 | 1.13 |
| 1k3 | 1.00 | 0.057 | 0.59 | 0.59 | 0.354 | 1.10 |
| 1a1 | 1.04 | 0.065 | 0.63 | 0.61 | 0.423 | 1.12 |
| 1a2 | 0.95 | 0.059 | 0.66 | 0.69 | 0.539 | 1.16 |
| 1a3 | 1.09 | 0.064 | 0.72 | 0.65 | 0.531 | 1.15 |
| 1z1 | 0.90 | 0.059 | 0.59 | 0.65 | 0.445 | 1.13 |
| 1z2 | 0.89 | 0.064 | 0.70 | 0.78 | 0.692 | 1.21 |
| 1z3 | 0.92 | 0.072 | 0.64 | 0.69 | 0.573 | 1.17 |

Table 13. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from samples of mollic gleysols (horizon Ap)

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 2/0 | 0.82 | 0.057 | 0.58 | 0.71 | 0.510 | 1.15 |
| 2k1 | 0.96 | 0.061 | 0.64 | 0.67 | 0.504 | 1.14 |
| 2k2 | 0.93 | 0.062 | 0.68 | 0.73 | 0.610 | 1.18 |
| 2k3 | 1.21 | 0.068 | 0.60 | 0.50 | 0.196 | 1.05 |
| 2a1 | 0.93 | 0.068 | 0.62 | 0.67 | 0.512 | 1.15 |
| 2a2 | 0.91 | 0.058 | 0.60 | 0.67 | 0.474 | 1.13 |
| 2a3 | 0.88 | 0.061 | 0.62 | 0.71 | 0.547 | 1.16 |
| 2z1 | 1.05 | 0.064 | 0.71 | 0.68 | 0.560 | 1.16 |
| 2z2 | 0.88 | 0.056 | 0.64 | 0.73 | 0.567 | 1.17 |
| 2z3 | 0.88 | 0.069 | 0.64 | 0.72 | 0.559 | 1.18 |

Table 14. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from samples of mollic gleysols (horizon Ap)

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 3/0 | 1.09 | 0.070 | 0.64 | 0.58 | 0.389 | 1.11 |
| 3k1 | 1.05 | 0.065 | 0.65 | 0.62 | 0.440 | 1.12 |
| 3k2 | 1.06 | 0.071 | 0.63 | 0.60 | 0.425 | 1.12 |
| 3k3 | 0.94 | 0.067 | 0.64 | 0.68 | 0.548 | 1.16 |
| 3a1 | 0.92 | 0.062 | 0.57 | 0.62 | 0.399 | 1.11 |
| 3a2 | 1.07 | 0.065 | 0.69 | 0.65 | 0.508 | 1.15 |
| 3a3 | 0.99 | 0.065 | 0.59 | 0.59 | 0.381 | 1.11 |
| 3z1 | 1.01 | 0.069 | 0.68 | 0.68 | 0.562 | 1.16 |
| 3z2 | 0.93 | 0.066 | 0.62 | 0.67 | 0.514 | 1.15 |
| 3z3 | 0.95 | 0.066 | 0.63 | 0.66 | 0.509 | 1.15 |

Table 15. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from arenosol samples

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 4/0 | 1.11 | 0.059 | 0.57 | 0.52 | 0.217 | 1.06 |
| 4k1 | 1.13 | 0.060 | 0.59 | 0.52 | 0.222 | 1.06 |
| 4k2 | 1.10 | 0.059 | 0.60 | 0.54 | 0.267 | 1.07 |
| 4k3 | 1.13 | 0.062 | 0.59 | 0.52 | 0.224 | 1.06 |
| 4a1 | 1.10 | 0.060 | 0.55 | 0.50 | 0.183 | 1.05 |
| 4a2 | 1.10 | 0.058 | 0.52 | 0.47 | 0.116 | 1.03 |
| 4a3 | 1.13 | 0.065 | 0.63 | 0.56 | 0.330 | 1.09 |
| 4z1 | 1.16 | 0.065 | 0.61 | 0.53 | 0.258 | 1.07 |
| 4z2 | 1.06 | 0.058 | 0.58 | 0.55 | 0.273 | 1.07 |
| 4z3 | 1.07 | 0.060 | 0.54 | 0.51 | 0.194 | 1.05 |

Table 16. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from samples of luvisol

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 5/0 | 1.41 | 0.082 | 0.71 | 0.50 | 0.245 | 1.07 |
| 5k1 | 1.42 | 0.088 | 0.76 | 0.54 | 0.366 | 1.10 |
| 5k2 | 1.37 | 0.074 | 0.71 | 0.52 | 0.283 | 1.08 |
| 5k3 | 1.28 | 0.085 | 0.62 | 0.48 | 0.209 | 1.06 |
| 5a1 | 1.30 | 0.082 | 0.60 | 0.46 | 0.145 | 1.04 |
| 5a2 | 1.43 | 0.086 | 0.74 | 0.51 | 0.296 | 1.08 |
| 5a3 | 1.35 | 0.086 | 0.70 | 0.52 | 0.307 | 1.08 |
| 5z1 | 1.28 | 0.085 | 0.63 | 0.49 | 0.228 | 1.06 |
| 5z2 | 1.43 | 0.085 | 0.72 | 0.50 | 0.252 | 1.07 |
| 5z3 | 1.33 | 0.070 | 0.69 | 0.52 | 0.257 | 1.07 |

Table 17. Values of atomic ratios and the degree of internal oxidation in molecules of humic acids isolated from samples of cambisol

| Sample symbol | H/C | N/C | O/C | O/H | ω | CQ |
|---|---|---|---|---|---|---|
| 6/0 | 1.31 | 0.085 | 0.65 | 0.50 | 0.252 | 1.07 |
| 6k1 | 1.24 | 0.076 | 0.64 | 0.51 | 0.262 | 1.07 |
| 6k2 | 1.39 | 0.081 | 0.71 | 0.52 | 0.288 | 1.08 |
| 6k3 | 1.28 | 0.086 | 0.61 | 0.48 | 0.208 | 1.05 |
| 6a1 | 1.42 | 0.079 | 0.71 | 0.50 | 0.226 | 1.06 |
| 6a2 | 1.34 | 0.084 | 0.71 | 0.53 | 0.340 | 1.09 |
| 6a3 | 1.29 | 0.084 | 0.64 | 0.50 | 0.252 | 1.07 |
| 6z1 | 1.24 | 0.086 | 0.61 | 0.49 | 0.227 | 1.06 |
| 6z2 | 1.35 | 0.086 | 0.74 | 0.55 | 0.387 | 1.11 |
| 6z3 | 1.36 | 0.086 | 0.60 | 0.44 | 0.104 | 1.03 |

Among preparations of humic acids isolated from the control soil samples, HAs from mollic gleysols (2) were characterized by the highest content of carbon (expressed in atomic percentage) – 40.64%, whereas the lowest content of ca. 32% was recorded for samples of HAs extracted from luvisol and cambisol (5, 6). In the preparations obtained after incubation with pollutants, the content of carbon in humic acids extracted

from humic gleysol (1) was higher, and the content of hydrogen was lower compared with initial samples.

The content of nitrogen (in atomic percentage) in initial samples varied from 2.26 (1) to 2.79% (6).

Among the control samples of HAs, humic acids isolated from cambisol (6/0) were characterised by the highest content of hydrogen, whereas HAs from mollic gleysols (2/0) had the lowest content. Incubation with pollutants induced an increase in the content of hydrogen in HAs isolated from mollic gleysols (2) and luvisol (5). However, a decrease in the content of hydrogen was recorded for HAs isolated from humic gleysol (1) and mollic gleysols (3).

Among the preparations isolated from pure soils, HAs from mollic gleysols were characterized by the highest content of oxygen (ca. 24%), whereas preparation 4/0 (ca. 20%) extracted from arenosol had the lowest content. An increase in the oxygen content during incubation was observed for samples of mollic gleysols (2.3).

Based on the statistical analysis of the elemental composition applying the analysis of variance for experiments with 2 factors, humic acids were divided into two groups (I: humic acids isolated from mollic gleysols and humic gleysol, and II: humic acids from luvisol, arenosol and cambisol) – Tables 18-20.

Humic acids from luvisol were characterized by the widest range of the H:C ratio, whereas HAs from mollic gleysols (2 and 3) - by the narrowest one. Incubation of soil samples caused a reduction in average H:C values for HAs isolated from humic gleysol. However, H:C values increased for HAs extracted from mollic gleysols (2). Similar dynamics of changes was observed for atomic ratios N:C and O:C. The highest value of the O:H ratio was recorded for samples of humic acids no. 2 and 3 (extracted from mollic gleysols), the lowest one for HA samples no. 4 and 5 (isolated from arenosol and luvisol, respectively). During the incubation of the soil material, the average value of the O:H ratio increased in acids of humic gleysol, whereas it dropped for HAs from mollic gleysols.

The highest value of the internal oxidation number was recorded for HAs isolated from mollic gleysols ($\omega > 0.4$) and humic gleysol. Other humic acids were characterized by lower values of the internal oxidation number – ca. 0.2. The differences between these two groups of HA preparations were statistically confirmed (Tables 18-20).

Also the parameter CQ had a higher value for HAs from mollic gleysol and humic gleysol (1, 2, 3) – on average ca. 1.4, whereas for humic acids from less rich soils – ca. 1.1. Statistical analysis revealed significant differences between the values of the parameter CQ for HAs isolated from mollic gleysols (2 and 3) and humic gleysol (1) versus other HAs (Tables 18-20).

Statistical analysis performed on the results of the elemental composition of HAs revealed that significant differences in the elemental composition reflect the typological heterogeneity of the research material, and incubation with pollutants did not induce statistically significant changes.

Table 18. Mean values of the elemental composition of humic acids preparations (acidification variant)

| Soil type | Dose | Mean values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | O | H:C | N:C | O:C | O:H | ω | CQ |
| 1 | | 37.14 | 36.89 | 2.23 | 23.74 | 1.00 | 0.060 | 0.64 | 0.65 | 0.466 | 1.13 |
| 2 | | 37.60 | 36.59 | 2.31 | 23.51 | 0.98 | 0.062 | 0.63 | 0.65 | 0.455 | 1.13 |
| 3 | | 36.49 | 37.68 | 2.50 | 23.33 | 1.04 | 0.068 | 0.64 | 0.62 | 0.450 | 1.13 |
| 4 | | 36.18 | 40.36 | 2.17 | 21.18 | 1.12 | 0.060 | 0.59 | 0.53 | 0.232 | 1.06 |
| 5 | | 31.80 | 43.46 | 2.61 | 22.16 | 1.37 | 0.082 | 0.70 | 0.51 | 0.276 | 1.08 |
| 6 | | 32.93 | 42.84 | 2.71 | 21.50 | 1.31 | 0.082 | 0.65 | 0.50 | 0.252 | 1.07 |
| LSD | | 3.609 | 4.54 | 0.262 | 2.161 | 0.223 | 0.010 | 0.084 | 0.116 | 0.198 | 0.062 |
| | 0 | 35.29 | 39.76 | 2.43 | 22.53 | 1.14 | 0.070 | 0.64 | 0.57 | 0.350 | 1.10 |
| | 1 | 35.32 | 39.19 | 2.39 | 23.04 | 1.12 | 0.068 | 0.66 | 0.60 | 0.393 | 1.11 |
| | 2 | 35.25 | 39.31 | 2.36 | 23.08 | 1.13 | 0.067 | 0.66 | 0.59 | 0.388 | 1.11 |
| | 3 | 35.56 | 40.29 | 2.51 | 21.63 | 1.14 | 0.071 | 0.61 | 0.54 | 0.290 | 1.08 |
| LSD | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Table 19. Mean values of the elemental composition of humic acids preparations (alkalization variant)

| Soil type | Dose | Mean values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | O | H:C | N:C | O:C | O:H | ω | CQ |
| 1 | | 35.86 | 37.60 | 2.27 | 24.28 | 1.05 | 0.063 | 0.68 | 0.65 | 0.495 | 1.14 |
| 2 | | 39.18 | 34.67 | 2.38 | 23.77 | 0.89 | 0.061 | 0.61 | 0.69 | 0.511 | 1.15 |
| 3 | | 37.09 | 37.59 | 2.43 | 22.90 | 1.02 | 0.065 | 0.62 | 0.61 | 0.419 | 1.12 |
| 4 | | 36.49 | 40.52 | 2.21 | 20.78 | 1.11 | 0.060 | 0.57 | 0.51 | 0.211 | 1.06 |
| 5 | | 31.82 | 43.72 | 2.67 | 21.79 | 1.37 | 0.084 | 0.69 | 0.50 | 0.241 | 1.07 |
| 6 | | 32.27 | 43.18 | 2.68 | 22.13 | 1.34 | 0.083 | 0.68 | 0.51 | 0.267 | 1.07 |
| LSD | | 3.011 | 2.226 | 0.251 | 2.129 | 0.146 | 0.008 | 0.104 | 0.065 | 0.130 | 0.039 |
| | 0 | 35.27 | 39.77 | 2.43 | 22.53 | 1.14 | 0.070 | 0.64 | 0.57 | 0.350 | 1.10 |
| | 1 | 35.90 | 39.71 | 2.47 | 21.92 | 1.12 | 0.069 | 0.61 | 0.56 | 0.315 | 1.09 |
| | 2 | 35.29 | 39.44 | 2.38 | 23.05 | 1.14 | 0.068 | 0.65 | 0.59 | 0.375 | 1.11 |
| | 3 | 35.34 | 39.26 | 2.49 | 22.92 | 1.12 | 0.071 | 0.65 | 0.59 | 0.391 | 1.11 |
| LSD | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Table 20. Mean values of the elemental composition of humic acids preparations (salinity variant)

| Soil type | Dose | Mean values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | O | H:C | N:C | O:C | O:H | ω | CQ |
| 1 | | 37.44 | 35.66 | 2.44 | 24.47 | 0.96 | 0.065 | 0.66 | 0.69 | 0.550 | 1.16 |
| 2 | | 38.41 | 34.70 | 2.35 | 24.55 | 0.91 | 0.062 | 0.64 | 0.71 | 0.559 | 1.16 |
| 3 | | 37.00 | 36.75 | 2.51 | 23.76 | 1.00 | 0.068 | 0.64 | 0.65 | 0.493 | 1.14 |
| 4 | | 36.59 | 40.15 | 2.28 | 21.05 | 1.10 | 0.060 | 0.58 | 0.53 | 0.235 | 1.06 |
| 5 | | 32.00 | 43.58 | 2.58 | 21.86 | 1.36 | 0.080 | 0.69 | 0.50 | 0.248 | 1.07 |
| 6 | | 32.80 | 43.11 | 2.81 | 21.29 | 1.32 | 0.086 | 0.65 | 0.50 | 0.243 | 1.07 |
| LSD | | 3.664 | 3.196 | 0.390 | 2.111 | 0.185 | 0.012 | 0.108 | 0.079 | 0.157 | 0.048 |
| | 0 | 35.27 | 39.77 | 2.43 | 22.53 | 1.14 | 0.070 | 0.64 | 0.57 | 0.352 | 1.10 |
| | 1 | 35.58 | 39.23 | 2.52 | 22.67 | 1.11 | 0.071 | 0.64 | 0.59 | 0.380 | 1.11 |
| | 2 | 35.74 | 38.21 | 2.43 | 23.62 | 1.09 | 0.069 | 0.67 | 0.63 | 0.447 | 1.13 |
| | 3 | 36.22 | 38.74 | 2.55 | 22.49 | 1.09 | 0.071 | 0.62 | 0.59 | 0.373 | 1.11 |
| LSD | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

ns – non-significant differences

### 4.2.2. Spectra in infrared radiation

Spectra of humic acids in infrared radiation were characterized by the occurrence of numerous absorption bands within the range of wave numbers 1000-4000 cm$^{-1}$, which is associated with their complex structure. Figures 8-13 present infrared spectra of humic acids isolated from the control soil samples.
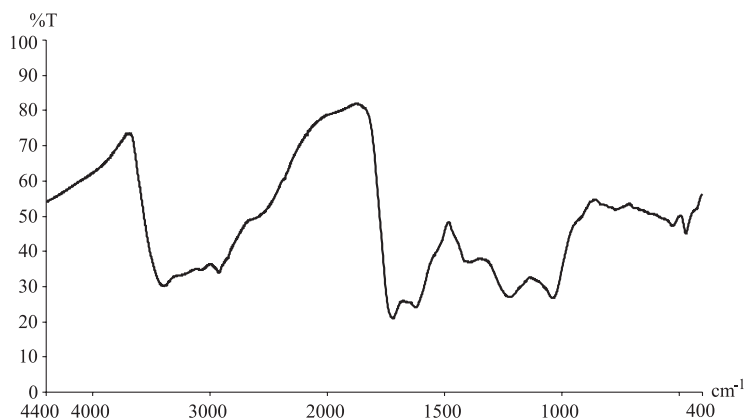


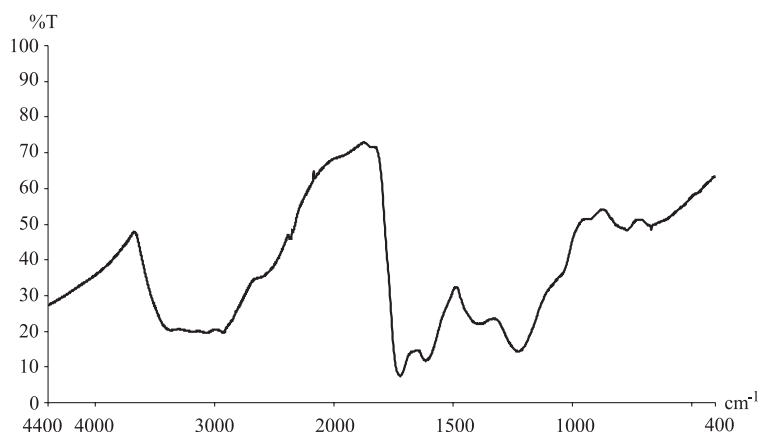Fig. 8. Infrared spectrum of HAs isolated from humic gleysol (1/0)



Fig. 9.  Infrared spectrum of HAs isolated from mollic gleysols (2/0)

Within the range of 3100-3300 cm$^{-1}$, an absorption band was identified, which results from stretching vibrations of hydroxyl groups of alcohols, phenols and carboxylic acids. It is a complex band that also consists of stretching vibrations in hydrogen bonds. A doublet at the wave number of 2860 cm$^{-1}$ together with a complex band within the range of 2920-2960 cm$^{-1}$ corresponds to stretching vibrations of methyl and methylene groups. Within the range of 1600-1660 cm$^{-1}$, a complex band occurred, which corresponds to stretching vibrations C=O, deformation vibrations NH (in primary amides) and stretching vibrations C=C in aromatic rings. Additionally, the presence of aromatic rings (in spectra of humic acids isolated from soils: luvisol (5), arenosol (4) and cambisol (6) was reflected in the occurrence of an absorption band at the wave

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number of 1510 cm$^{-1}$, near the vibrations of deformation bands NH (1540 cm$^{-1}$). At the wave numbers of 1440-1460 cm$^{-1}$, there were bands coming from deformation vibrations of methyl and methylene groups. Spectra of the studied preparations revealed also the presence of ester bonds, carbonyl and hydroxyl groups – an absorption band within the range of 1400-1420 cm$^{-1}$. There was also a clear band at the wave number of 1380 cm$^{-1}$, which is characteristic of carboxylate salts. A specific narrowing and sharpening of this band indicates, however, that the band was associated with the presence of complexed carboxylate groups, and not salt-type bonds. The analysis of the range 1030-1080 cm$^{-1}$ was hindered due to the fact that bands originated from polysaccharide structures were superimposed over the bands from clay minerals.

The process of the soil material incubation with the addition of pollution (regardless of the type of pollutant) did not induce any visible changes in the obtained HAs, therefore HA spectra isolated from samples after incubation were not included. One should pay attention, however, to a high difficulty in the analysis of spectra of this type, as well as to significant differences in the soil ash content of the studied preparations, which significantly affected the quality of the infrared spectra and possibilities of their interpretation.
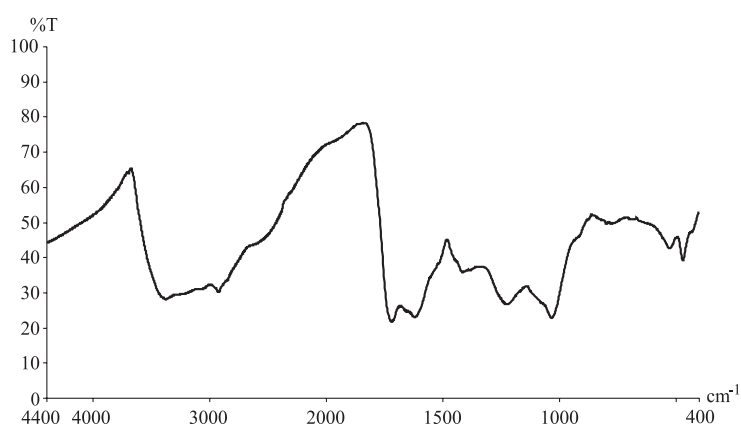
Fig. 10. Infrared spectrum of HAs isolated from mollic gleysols (3/0)
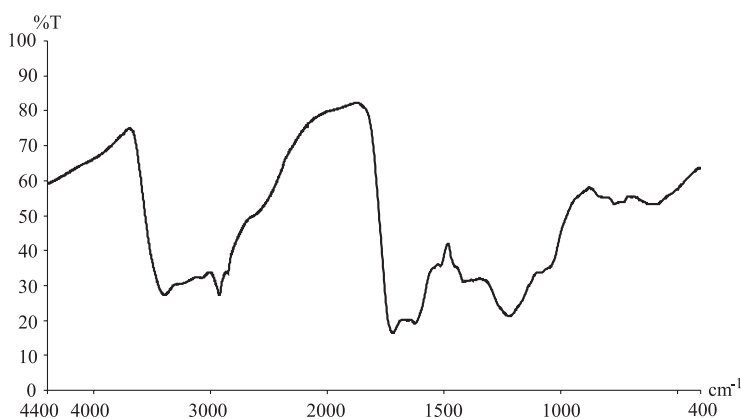
Fig. 11. Infrared spectrum of HAs isolated from arenosol (4/0)
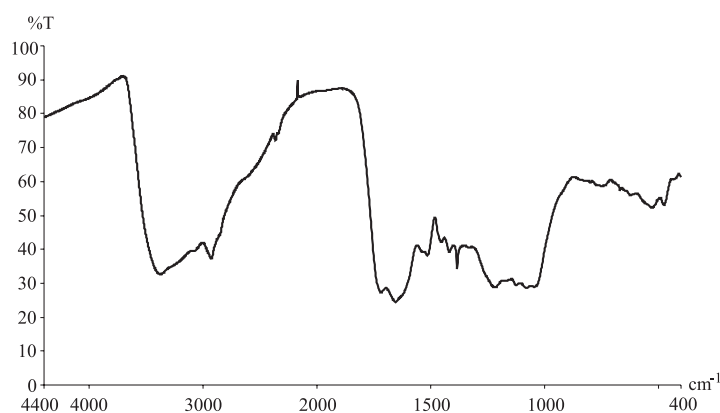
Fig. 12. Infrared spectrum of HAs isolated from luvisol (5/0)
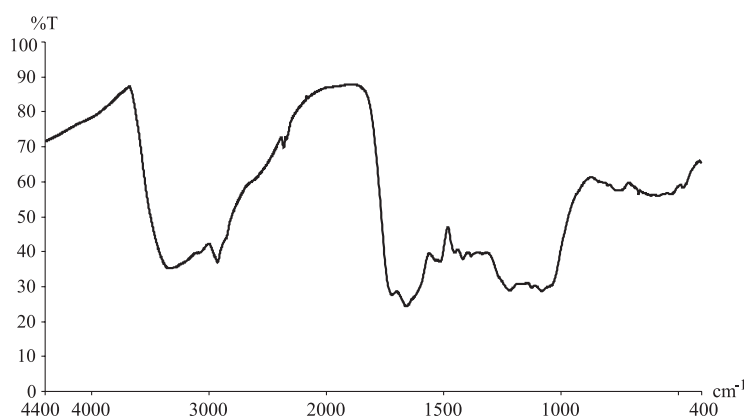


Fig. 13. Infrared spectrum of HAs isolated from cambisol (6/0)

### 4.2.3. Spectrometric parameters

Determination of spectrometric parameters allows the inference about the progress of humification processes and the structure of humic acids. The results of spectrometric analysis within the range of visible and ultraviolet spectra are presented in Tables 21-26 and in Figures 14-15.

The value of the parameter $A_{2/4}$, which determines the amount of substances in the initial stage of decomposition for humic acids isolated from control samples No. 1, 2, 3 was similar and amounted to ca. 4.8; the values of the parameter $A_{2/4}$ for HAs of the other soils were higher (5.44-6.12).

The quotient of the absorbance values at the wavelengths of 280 and 665 nm ($A_{2/6}$), which corresponds to the ratio of lignin to humified material contribution, was the highest for HAs coming from luvisol and cambisol, and the lowest for preparations of HAs isolated from mollic gleysol.

The value of the quotient $A_{4/6}$ (the absorbance quotient at the wavelengths of 465 and 665 nm) that illustrates the degree of condensation of aromatic structures in molecules of humic acids ranged from ca. 3.7 (for HAs extracted from mollic gleysol) to ca. 4.7 (for HAs isolated from samples of cambisol and luvisol). The value of the

parameter $A_{4/6}$ changed considerably only in the case of acidification of humic gleysol samples. Humic acids isolated from acidified samples of this type of soil were characterized by a greater condensation of aromatic structures (increased values of the quotient $A_{4/6}$).

The value of the parameter $\Delta logK$ varied depending on the type of soil. Humic acids isolated from luvisol and cambisol were characterized by the highest values of $\Delta logK$ (ca. 0.70). The lowest values of this parameter were recorded for HA preparations obtained from mollic gleysol (ca. 0.53).

Table 21. Spectrometric parameters of humic acids isolated from samples of humic gleysol

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta logK$ |
|---|---|---|---|---|
| 1/0 | 4.93 | 20.62 | 4.18 | 0.604 |
| 1k1 | 4.56 | 18.98 | 4.16 | 0.585 |
| 1k2 | 5.87 | 26.79 | 4.57 | 0.611 |
| 1k3 | 5.05 | 22.96 | 4.55 | 0.616 |
| 1a1 | 4.87 | 20.06 | 4.12 | 0.593 |
| 1a2 | 5.41 | 27.93 | 5.17 | 0.648 |
| 1a3 | 4.74 | 19.66 | 4.14 | 0.585 |
| 1z1 | 4.46 | 18.52 | 4.15 | 0.582 |
| 1z2 | 5.07 | 20.82 | 4.10 | 0.588 |
| 1z3 | 4.71 | 21.34 | 4.53 | 0.611 |

Table 22. Spectrometric parameters of humic acids isolated from samples of mollic gleysols (horizon Ap)

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta logK$ |
|---|---|---|---|---|
| 2/0 | 4.70 | 17.42 | 3.71 | 0.526 |
| 2k1 | 4.30 | 16.09 | 3.74 | 0.542 |
| 2k2 | 4.40 | 15.74 | 3.58 | 0.528 |
| 2k3 | 5.56 | 22.19 | 3.99 | 0.618 |
| 2a1 | 4.32 | 16.18 | 3.75 | 0.555 |
| 2a2 | 4.37 | 16.63 | 3.80 | 0.539 |
| 2a3 | 3.52 | 13.35 | 3.80 | 0.539 |
| 2z1 | 4.34 | 15.94 | 3.67 | 0.548 |
| 2z2 | 4.61 | 19.36 | 4.20 | 0.577 |
| 2z3 | 4.55 | 17.10 | 3.76 | 0.557 |

Table 23. Spectrometric parameters of humic acids isolated from samples of mollic gleysols (horizon Ap)

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta logK$ |
|---|---|---|---|---|
| 3/0 | 4.72 | 17.55 | 3.72 | 0.552 |
| 3k1 | 4.46 | 16.41 | 3.68 | 0.550 |
| 3k2 | 4.74 | 17.87 | 3.77 | 0.567 |
| 3k3 | 4.43 | 16.37 | 3.69 | 0.565 |
| 3a1 | 4.40 | 16.43 | 3.73 | 0.537 |
| 3a2 | 4.65 | 16.98 | 3.65 | 0.561 |
| 3a3 | 5.01 | 18.90 | 3.77 | 0.561 |
| 3z1 | 4.97 | 19.62 | 3.94 | 0.577 |
| 3z2 | 4.66 | 17.47 | 3.75 | 0.559 |
| 3z3 | 4.64 | 17.05 | 3.67 | 0.555 |

Table 24. Spectrometric parameters of humic acids isolated from arenosol samples

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta\log K$ |
|---|---|---|---|---|
| 4/0 | 5.44 | 22.87 | 4.21 | 0.606 |
| 4k1 | 5.28 | 21.46 | 4.06 | 0.604 |
| 4k2 | 5.07 | 22.10 | 4.36 | 0.615 |
| 4k3 | 5.47 | 23.02 | 4.21 | 0.611 |
| 4a1 | 5.24 | 22.71 | 4.33 | 0.614 |
| 4a2 | 5.23 | 23.26 | 4.45 | 0.614 |
| 4a3 | 5.32 | 21.74 | 4.09 | 0.611 |
| 4z1 | 6.73 | 28.57 | 4.24 | 0.619 |
| 4z2 | 4.89 | 20.87 | 4.26 | 0.604 |
| 4z3 | 5.20 | 21.61 | 4.16 | 0.609 |

Table 25. Spectrometric parameters of humic acids isolated from luvisol samples

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta\log K$ |
|---|---|---|---|---|
| 5/0 | 6.12 | 29.45 | 4.81 | 0.712 |
| 5k1 | 5.52 | 24.42 | 4.43 | 0.670 |
| 5k2 | 5.31 | 23.95 | 4.51 | 0.670 |
| 5k3 | 7.76 | 38.82 | 5.00 | 0.691 |
| 5a1 | 6.36 | 32.04 | 5.04 | 0.714 |
| 5a2 | 5.55 | 25.32 | 4.56 | 0.676 |
| 5a3 | 5.53 | 24.73 | 4.47 | 0.667 |
| 5z1 | 5.83 | 28.25 | 4.84 | 0.687 |
| 5z2 | 8.01 | 36.79 | 4.60 | 0.690 |
| 5z3 | 5.81 | 25.61 | 4.41 | 0.684 |

Table 26. Spectrometric parameters of humic acids isolated from samples of cambisol

| Sample symbol | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta\log K$ |
|---|---|---|---|---|
| 6/0 | 5.88 | 27.83 | 4.73 | 0.684 |
| 6k1 | 6.18 | 28.76 | 4.66 | 0.712 |
| 6k2 | 6.72 | 30.41 | 4.52 | 0.684 |
| 6k3 | 5.77 | 28.29 | 4.91 | 0.696 |
| 6a1 | 6.17 | 30.41 | 4.93 | 0.709 |
| 6a2 | 5.28 | 24.61 | 4.66 | 0.705 |
| 6a3 | 5.75 | 28.56 | 4.96 | 0.700 |
| 6z1 | 6.15 | 30.09 | 4.90 | 0.699 |
| 6z2 | 6.46 | 29.77 | 4.61 | 0.685 |
| 6z3 | 5.96 | 26.65 | 4.47 | 0.659 |

Statistical analysis of the results revealed no effects of the type of pollutants or their dose on the spectrometric properties of humic acids. The determined differences in the values of spectrometric parameters were not statistically significant, and directions of their changes basically did not depend on a dose of a pollutant – Table 27.
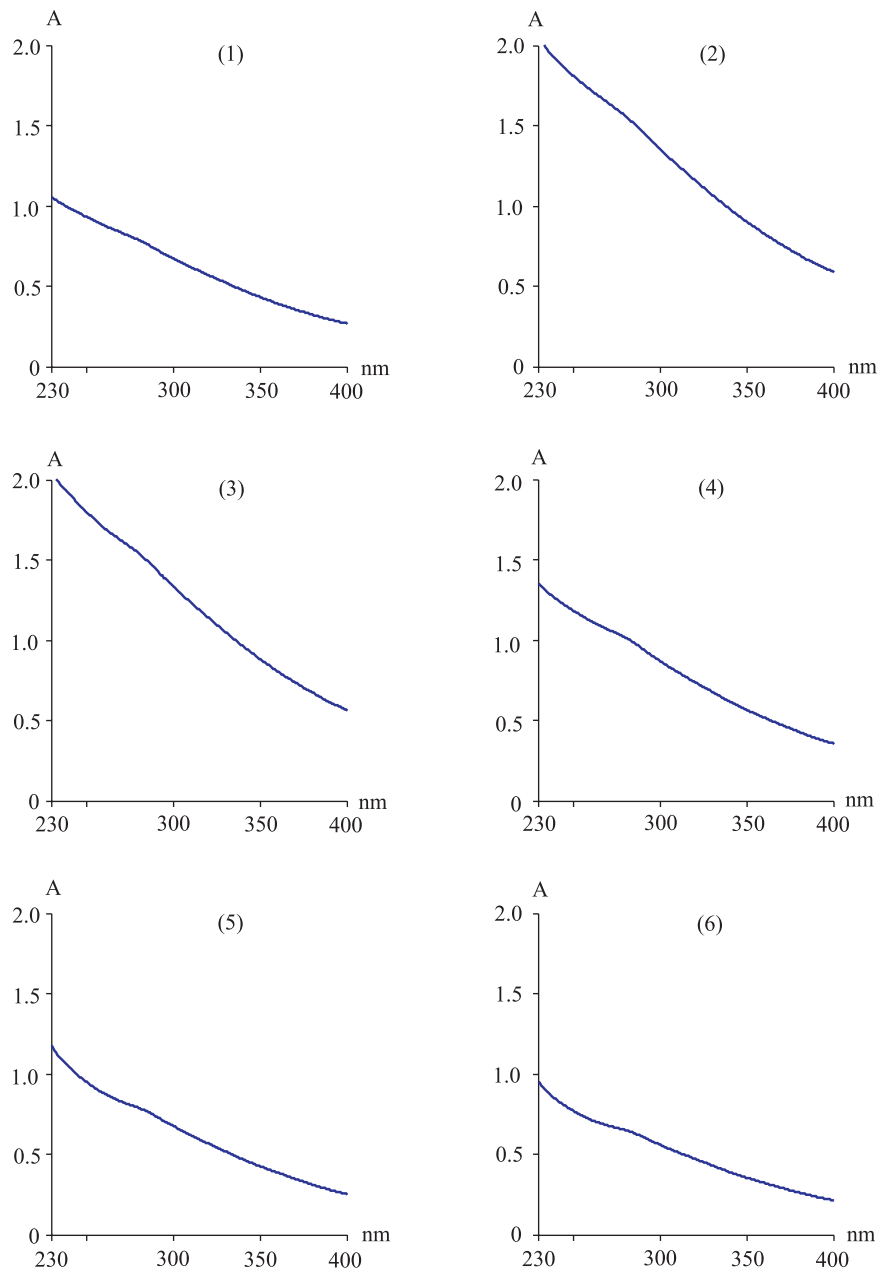
Fig. 14. UV spectra of humic acids isolated from control samples of humic gleysol (1), mollic gleysol (2, 3), arenosol (4), luvisol (5) and cambisol (6)
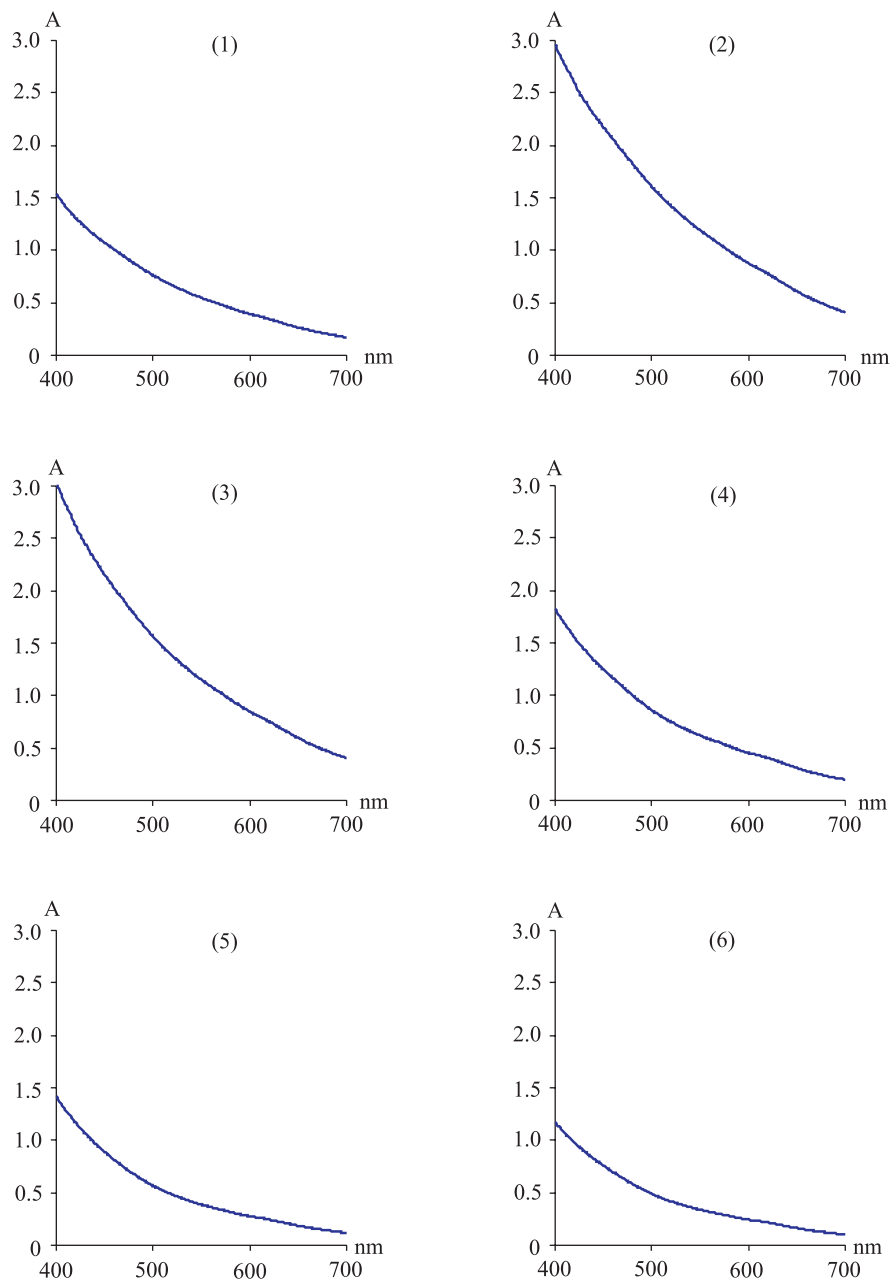
Fig. 15. VIS spectra of humic acids isolated from control samples of humic gleysol (1), mollic gleysol (2, 3), arenosol (4), luvisol (5) and cambisol (6)

Table 27. Spectrometric parameters of humic acids – mean values

**Acidification (k)**

| Soil type | Dose | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta lgK$ |
|---|---|---|---|---|---|
| 1 | | 5.10 | 22.34 | 4.37 | 0.604 |
| 2 | | 4.74 | 17.86 | 3.78 | 0.553 |
| 3 | | 5.59 | 17.05 | 3.72 | 0.558 |
| 4 | | 5.32 | 22.36 | 4.21 | 0.609 |
| 5 | | 6.13 | 29.16 | 4.69 | 0.686 |
| 6 | | 6.14 | 28.82 | 4.71 | 0.694 |
| LSD | | 1.282 | 7.409 | 0.353 | 0.047 |
| | 0 | 5.30 | 22.62 | 4.23 | 0.614 |
| | 1 | 5.05 | 21.02 | 4.12 | 0.611 |
| | 2 | 5.35 | 22.81 | 4.22 | 0.613 |
| | 3 | 5.64 | 25.28 | 4.39 | 0.633 |
| | LSD | ns | ns | ns | ns |

**Alkalization (a)**

| Soil type | Dose | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta lgK$ |
|---|---|---|---|---|---|
| 1 | | 4.99 | 22.07 | 4.40 | 0.608 |
| 2 | | 4.23 | 15.90 | 3.77 | 0.540 |
| 3 | | 4.70 | 17.47 | 3.72 | 0.553 |
| 4 | | 5.31 | 22.65 | 4.27 | 0.611 |
| 5 | | 5.89 | 27.89 | 4.72 | 0.692 |
| 6 | | 5.77 | 27.85 | 4.82 | 0.700 |
| LSD | | 0.794 | 6.022 | 0.559 | 0.041 |
| | 0 | 5.30 | 22.62 | 4.23 | 0.614 |
| | 1 | 5.23 | 22.97 | 4.32 | 0.620 |
| | 2 | 5.08 | 22.46 | 4.38 | 0.624 |
| | 3 | 4.99 | 21.16 | 4.21 | 0.610 |
| | LSD | ns | ns | ns | ns |

**Salinity (z)**

| Soil type | Dose | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta lgK$ |
|---|---|---|---|---|---|
| 1 | | 4.79 | 20.33 | 4.24 | 0.956 |
| 2 | | 4.55 | 17.46 | 3.84 | 0.552 |
| 3 | | 4.75 | 17.92 | 3.77 | 0.561 |
| 4 | | 5.57 | 23.48 | 4.22 | 0.609 |
| 5 | | 6.44 | 30.03 | 4.67 | 0.693 |
| 6 | | 6.13 | 28.59 | 4.68 | 0.682 |
| LSD | | 1.346 | 6.081 | 0.427 | 0.036 |
| | 0 | 5.30 | 22.62 | 4.23 | 0.614 |
| | 1 | 5.41 | 22.50 | 4.29 | 0.619 |
| | 2 | 5.62 | 24.18 | 4.25 | 0.617 |
| | 3 | 5.15 | 21.56 | 4.17 | 0.612 |
| | LSD | ns | ns | ns | ns |

ns – non-significant differences

### 4.2.4. Susceptibility of humic acids preparations to oxidation

Susceptibility of humic acids to oxidation, expressed as a decrease in absorbance at the wavelengths of 465 and 665 nm, bears some important information related to the chemical activity of relevant fractions of humic acids. The results of analyses are presented in Tables 28-33.

Humic acids of mollic gleysols were characterized by the highest resistance to oxidation (2 and 3). A decrease in the absorbance values at the wavelength of 465 nm ($\Delta A_{465}$) amounted on average to 30%, whereas at the wavelength of 665 nm ($\Delta A_{665}$) – to 45%. Absorbance values for HAs of the other soils decreased to over 50% and 60%, respectively. A decrease in the absorbance value was not determined by a pollutant. In all cases, the process of oxidation was mostly related to "mature" humic acids. Based on the difference in the absorbance values at the wavelengths of 665 and 465 nm, it was found that the difference in the susceptibility to oxidation between "mature" and "young" fractions of humic acids was on average ca. 10 percentage points and was not determined by the type of soil and the type of added contaminants. Therefore, the values of the parameter $A_{4/6}$ calculated for the solutions of these humic acids were reduced after 24 hours of oxidation.

Susceptibility to oxidation of humic acids is associated with their origin (the type of soil from which they were isolated), which was confirmed by the statistical analysis of the results. The differences resulting from the presence of contamination in the incubated material were not statistically significant (Table 34).

Table 28. Spectrometric properties of humic acids extracted from humic gleysol after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 1/0 | 1.167 | 0.652 | 0.272 | 0.130 | 44.1 | 52.2 | 5.01 | 19.9 |
| 1k1 | 1.436 | 0.784 | 0.342 | 0.161 | 45.4 | 53.0 | 4.88 | 17.3 |
| 1k2 | 1.884 | 0.927 | 0.409 | 0.163 | 50.8 | 60.2 | 5.69 | 24.5 |
| 1k3 | 1.358 | 0.679 | 0.292 | 0.109 | 50.0 | 62.5 | 6.22 | 36.7 |
| 1a1 | 1.421 | 0.773 | 0.336 | 0.150 | 45.6 | 55.4 | 5.15 | 25.0 |
| 1a2 | 1.292 | 0.658 | 0.251 | 0.097 | 49.1 | 61.6 | 6.81 | 31.7 |
| 1a3 | 1.331 | 0.773 | 0.319 | 0.149 | 41.9 | 53.4 | 5.20 | 25.6 |
| 1z1 | 1.513 | 0.960 | 0.361 | 0.189 | 36.5 | 47.5 | 5.08 | 22.4 |
| 1z2 | 1.273 | 0.717 | 0.307 | 0.150 | 43.7 | 51.2 | 4.77 | 16.3 |
| 1z3 | 1.589 | 0.809 | 0.344 | 0.140 | 49.1 | 59.3 | 5.79 | 27.8 |

Table 29. Spectrometric properties of humic acids extracted from mollic gleysol (horizon Ap) after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 2/0 | 2.009 | 1.316 | 0.533 | 0.279 | 34.5 | 47.6 | 4.71 | 27.0 |
| 2k1 | 1.837 | 1.303 | 0.482 | 0.285 | 29.1 | 40.8 | 4.57 | 22.2 |
| 2k2 | 1.677 | 1.129 | 0.460 | 0.260 | 32.7 | 43.4 | 4.34 | 21.2 |
| 2k3 | 1.002 | 0.440 | 0.251 | 0.100 | 56.1 | 60.2 | 4.40 | 10.3 |
| 2a1 | 1.720 | 1.162 | 0.458 | 0.255 | 32.4 | 44.4 | 4.56 | 21.6 |
| 2a2 | 1.973 | 1.241 | 0.516 | 0.272 | 37.1 | 47.3 | 4.57 | 20.3 |
| 2a3 | 1.769 | 1.114 | 0.475 | 0.243 | 37.0 | 48.9 | 4.58 | 20.5 |
| 2z1 | 1.556 | 1.106 | 0.412 | 0.211 | 28.9 | 48.9 | 5.24 | 42.8 |
| 2z2 | 1.926 | 1.132 | 0.449 | 0.213 | 41.2 | 52.5 | 5.31 | 26.4 |
| 2z3 | 1.639 | 1.108 | 0.435 | 0.246 | 32.4 | 43.5 | 4.51 | 19.9 |

Table 30. Spectrometric properties of humic acids extracted from mollic gleysol (horizon Ap) after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 3/0 | 1.304 | 0.797 | 0.347 | 0.181 | 38.9 | 47.9 | 4.41 | 18.5 |
| 3k1 | 1.413 | 0.936 | 0.377 | 0.224 | 33.8 | 40.5 | 4.17 | 13.3 |
| 3k2 | 1.308 | 0.971 | 0.342 | 0.277 | 25.8 | 19.1 | 3.5 | 14.1 |
| 3k3 | 1.410 | 0.941 | 0.362 | 0.208 | 33.2 | 42.7 | 4.54 | 23.0 |
| 3a1 | 1.856 | 1.206 | 0.487 | 0.277 | 35.0 | 43.1 | 4.35 | 16.6 |
| 3a2 | 1.269 | 0.788 | 0.342 | 0.122 | 37.9 | 64.3 | 6.45 | 21.9 |
| 3a3 | 1.768 | 0.936 | 0.465 | 0.216 | 47.0 | 53.7 | 4.34 | 15.1 |
| 3z1 | 1.472 | 1.162 | 0.371 | 0.307 | 21.1 | 17.3 | 3.78 | 21.3 |
| 3z2 | 1.392 | 1.141 | 0.351 | 0.290 | 18.0 | 17.2 | 3.93 | 23.5 |
| 3z3 | 1.836 | 1.051 | 0.483 | 0.227 | 42.7 | 53.0 | 4.64 | 26.4 |

Table 31. Spectrometric properties of humic acids extracted from arenosol after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 4/0 | 1.330 | 0.574 | 0.318 | 0.115 | 56.8 | 63.7 | 4.98 | 18.3 |
| 4k1 | 1.203 | 0.531 | 0.291 | 0.107 | 55.9 | 63.4 | 4.98 | 22.7 |
| 4k2 | 1.075 | 0.526 | 0.246 | 0.096 | 51.1 | 61.1 | 5.49 | 25.9 |
| 4k3 | 1.203 | 0.515 | 0.289 | 0.103 | 57.2 | 64.5 | 5.01 | 19.0 |
| 4a1 | 1.241 | 0.549 | 0.284 | 0.110 | 55.7 | 61.4 | 5.01 | 15.7 |
| 4a2 | 1.206 | 0.551 | 0.278 | 0.106 | 54.3 | 62.1 | 5.21 | 17.1 |
| 4a3 | 1.080 | 0.529 | 0.263 | 0.113 | 51.0 | 56.9 | 4.68 | 14.4 |
| 4z1 | 1.030 | 0.450 | 0.246 | 0.094 | 56.3 | 61.8 | 4.78 | 12.7 |
| 4z2 | 1.316 | 0.615 | 0.309 | 0.123 | 53.3 | 60.1 | 5.00 | 17.4 |
| 4z3 | 1.339 | 0.617 | 0.314 | 0.120 | 54.0 | 62.0 | 5.16 | 24.0 |

Table 32. Spectrometric properties of humic acids extracted from luvisol after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 5/0 | 0.784 | 0.334 | 0.172 | 0.073 | 57.4 | 57.7 | 4.59 | 16.2 |
| 5k1 | 0.620 | 0.292 | 0.135 | 0.053 | 52.9 | 60.5 | 5.46 | 23.3 |
| 5k2 | 0.777 | 0.365 | 0.170 | 0.073 | 53.0 | 57.3 | 5.04 | 11.8 |
| 5k3 | 0.847 | 0.363 | 0.170 | 0.064 | 57.1 | 62.5 | 5.71 | 14.2 |
| 5a1 | 0.735 | 0.288 | 0.143 | 0.042 | 60.9 | 70.6 | 6.84 | 35.7 |
| 5a2 | 0.653 | 0.312 | 0.141 | 0.056 | 52.2 | 60.6 | 5.62 | 23.2 |
| 5a3 | 0.755 | 0.341 | 0.172 | 0.070 | 54.9 | 59.3 | 4.86 | 8.7 |
| 5z1 | 0.690 | 0.312 | 0.139 | 0.049 | 54.8 | 64.8 | 6.36 | 31.4 |
| 5z2 | 0.680 | 0.282 | 0.154 | 0.059 | 58.6 | 61.9 | 4.81 | 4.6 |
| 5z3 | 0.581 | 0.266 | 0.133 | 0.054 | 54.3 | 59.2 | 4.89 | 10.9 |

Table 33. Spectrometric properties of humic acids extracted from brown soil after their oxidation

| Sample symbol | $A_{465}$ | $A^{ox}_{465}$ | $A_{665}$ | $A^{ox}_{665}$ | $\Delta A^{ox}_{465}$ [%] | $\Delta A^{ox}_{665}$ [%] | $A^{ox}_{4/6}$ | $\Delta A^{ox}_{4/6}$ [%] |
|---|---|---|---|---|---|---|---|---|
| 6/0 | 0.797 | 0.411 | 0.169 | 0.078 | 48.5 | 53.6 | 5.24 | 10.8 |
| 6k1 | 0.507 | 0.265 | 0.119 | 0.057 | 47.8 | 52.1 | 4.63 | 5.8 |
| 6k2 | 0.624 | 0.296 | 0.138 | 0.060 | 52.6 | 56.8 | 4.96 | 9.7 |
| 6k3 | 0.839 | 0.355 | 0.168 | 0.061 | 57.6 | 63.9 | 5.86 | 19.3 |
| 6a1 | 0.706 | 0.340 | 0.143 | 0.063 | 51.9 | 55.8 | 5.38 | 9.1 |
| 6a2 | 0.585 | 0.276 | 0.126 | 0.054 | 52.9 | 57.7 | 5.16 | 10.7 |
| 6a3 | 0.694 | 0.366 | 0.140 | 0.074 | 47.2 | 47.2 | 4.96 | 4.0 |
| 6z1 | 0.758 | 0.359 | 0.154 | 0.062 | 52.6 | 59.9 | 5.84 | 19.2 |
| 6z2 | 0.668 | 0.340 | 0.142 | 0.068 | 49.1 | 52.2 | 5.02 | 8.9 |
| 6z3 | 0.714 | 0.383 | 0.158 | 0.084 | 46.4 | 47.3 | 4.59 | 2.7 |

Table 34. Spectrometric properties of humic acids after their oxidation – mean values

**Acidification (k)**

| Soil type | Dose | Mean values | | | |
|---|---|---|---|---|---|
| | | $\Delta A_{465}$ | $\Delta A_{665}$ | $A^{ox}_{4/6}$ | $\Delta A_{4/6}$ |
| 1 | | 47.58 | 56.96 | 5.45 | 24.60 |
| 2 | | 38.10 | 48.00 | 4.51 | 20.18 |
| 3 | | 32.93 | 41.55 | 4.36 | 17.23 |
| 4 | | 55.25 | 63.18 | 5.12 | 21.48 |
| 5 | | 55.10 | 59.50 | 5.20 | 16.38 |
| 6 | | 51.63 | 57.10 | 5.25 | 11.40 |
| LSD | | 12.43 | 9.712 | 0.827 | ns |
| | 0 | 46.70 | 53.78 | 4.82 | 18.45 |
| | 1 | 44.15 | 52.05 | 4.83 | 17.43 |
| | 2 | 44.33 | 52.32 | 4.97 | 17.86 |
| | 3 | 51.87 | 59.38 | 5.29 | 20.42 |
| | LSD | ns | ns | ns | ns |

**Alkalization (a)**

| Soil type | Dose | Mean values | | | |
|---|---|---|---|---|---|
| | | $\Delta A_{465}$ | $\Delta A_{665}$ | $A^{ox}_{4/6}$ | $\Delta A_{4/6}$ |
| 1 | | 45.18 | 55.65 | 5.54 | 25.55 |
| 2 | | 35.25 | 47.05 | 4.61 | 22.35 |
| 3 | | 39.70 | 49.75 | 4.39 | 18.03 |
| 4 | | 54.45 | 61.03 | 4.97 | 16.38 |
| 5 | | 56.35 | 62.05 | 5.48 | 20.95 |
| 6 | | 50.13 | 56.10 | 5.24 | 8.65 |
| LSD | | 8.445 | 9.320 | 1.215 | 12.11 |
| | 0 | 46.70 | 53.78 | 4.82 | 18.45 |
| | 1 | 46.92 | 55.13 | 5.22 | 20.62 |
| | 2 | 47.25 | 57.27 | 5.30 | 20.82 |
| | 3 | 46.50 | 54.90 | 4.80 | 14.72 |
| | LSD | ns | ns | ns | ns |

**Salinity (z)**

| Soil type | Dose | Mean values | | | |
|---|---|---|---|---|---|
| | | $\Delta A_{465}$ | $\Delta A_{665}$ | $A^{ox}_{4/6}$ | $\Delta A_{4/6}$ |
| 1 | | 43.35 | 52.55 | 5.16 | 21.63 |
| 2 | | 34.25 | 48.13 | 4.94 | 29.03 |
| 3 | | 30.20 | 38.85 | 4.62 | 22.43 |
| 4 | | 55.10 | 61.90 | 4.98 | 18.10 |
| 5 | | 56.28 | 60.90 | 5.16 | 15.78 |
| 6 | | 49.15 | 53.25 | 5.31 | 10.40 |
| LSD | | 14.11 | 16.13 | ns | 16.04 |
| | 0 | 46.70 | 53.78 | 4.82 | 18.45 |
| | 1 | 41.72 | 51.70 | 5.29 | 24.67 |
| | 2 | 43.98 | 50.85 | 4.92 | 16.18 |
| | 3 | 46.48 | 54.05 | 5.08 | 18.63 |
| | LSD | ns | ns | ns | ns |

ns – non-significant differences

# 5. DISCUSSION OF THE RESULTS

## 5.1. SOIL PROPERTIES

Introduction of pollutants into soil will affect the balance of the soil environment. However, the expression level of disturbance of this balance depends mainly on the type of soil.

The processes of acidification of soils with the initial alkaline reaction (mollic gleysol) did not change their reaction, which resulted from a high buffering capacity in the acid environment, as well as high values of saturation with bases of the sorption complex. Similar results were obtained by Pokojska [40] for forest soils. The author reported that the soil horizons which have a considerable amount of bases in the sorption complex are more resistant to acidification. However, the same doses of an acidification mixture introduced into arenosol of a low base saturation level caused interruption in the buffer protection (due to a poor buffering capacity in this respect) and a change in the reaction. Drozd [10] reports that a low degree of soil saturation with alkaline cations in the Karkonosze Mts enhances the degradation of these areas exposed to, inter alia, immission of sulphur oxides (acidification). When soils are exposed to alkaline pollution, soils with a low content of organic carbon turned out to be the most sensitive. However, the response to alkalization was similar for all types of soils and induced a discrete increase in the values of conductivity above $3.5$ mS$\cdot$cm$^{-1}$ already with the first dose. The value of $2$ mS$\cdot$cm$^{-1}$ is generally accepted as permissible, whereas higher values of conductivity are defined as harmful [21]. It appears from the research that protection of soils against salinity is markedly less efficient compared with protection against acidification or alkalization.

## 5.2. PROPERTIES OF HUMIC ACIDS

An absorption band appeared irregularly only in infrared spectra of humic acids at the wave number of $1377$-$1380$ cm$^{-1}$, the specific narrowing and sharpening of which could imply complex formation processes with the contribution of a free electron pair from the oxygen atom of the carboxylate group. This is also confirmed by the results of analyses of the elemental composition of humic acids [35]. Significant differences in the elemental composition were caused by initial diversification of the study material, and not by incubation with simulated pollutants. Humic acids isolated from different types of soils were characterized by a typical content of carbon, hydrogen, nitrogen and oxygen [6, 36]. Due to subtle changes in the elemental composition, values of derived atomic ratios did not reveal any significant changes. The value of the H:C ratio amounted to $0.82$-$1.41$ and was within the range quoted by van Krevelen, which corresponds to aromatic ring systems coupled with the aliphatic chain containing 10 carbon atoms [49].

The subsequent basic analyses of humic acids – spectroscopic analysis within the range of visible and ultraviolet spectra – did not contribute any new information on changes in the properties of HAs during the soil incubation process with some pollutants added. Values of the $A_{2/4}$ ratio ranged from $3.52$ to $7.76$ and were typical of soil humic acids [36]. Values of the parameter $\Delta$logK for all humic acids are at the borderline between type A (acids of a high humification degree) and type B [25]. The lack of significant changes in the values of spectroscopic parameters of HAs during the

incubation of the soil material proves a slow rate of the humification process. This can be associated with a high degree of humic acid humification in the initial matter. Values of the correlation coefficients for spectroscopic parameters and the elemental composition are in accordance with the literature data [25]. A negative value was confirmed for the coefficient of correlation between the parameter $A_{4/6}$ and the carbon content in molecules of humic acids (-0.61), as well as between $A_{4/6}$ and the O:H ratio value (-0.63), and also a positive value for the correlation between $A_{4/6}$ and the H:C ratio value (0.66).

Changes in the absorbance values at the wavelengths of 465 and 665 nm are typical of soil humic acids and are not related to incubation with pollutants but to the research material origin. Humic acids isolated from soils of hydrogenic origin (humic gleysol and mollic gleysol) were characterized by greater susceptibility to chemical oxidation. Regardless of the type of soil, oxidation was more intensive with fractions of "old" humic acids. Susceptibility to chemical oxidation clearly correlates with the elemental composition and spectroscopic properties of humic acids (Table 35).

Table 35. Correlation coefficients of spectroscopic parameters after oxidation of humic acids together with their elemental composition and spectroscopic properties ($\alpha = 0.05$)

|  | C | H | N | O | H:C | N:C | O:C | O:H | $A_{2/4}$ | $A_{2/6}$ | $A_{4/6}$ | $\Delta logK$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\Delta A_{465}^{ox}$ | -0.53 | 0.71 | – | -0.66 | 0.64 | – | – | -0.73 | 0.68 | 0.73 | 0.72 | 0.73 |
| $\Delta A_{665}^{ox}$ | – | 0.56 | – | -0.56 | 0.48 | – | – | -0.60 | 0.54 | 0.61 | 0.66 | 0.61 |
| $A_{4/6}^{ox}$ | – | – | – | – | – | – | – | – | – | 0.55 | 0.76 | 0.54 |

In the spectra of humic acids made in infrared radiation, no changes were recorded, which could result from the presence of pollution in the soil material. Only in some cases a band was recorded which was probably associated with coordinate (dipolar) bonds in the complex system with iron ions(III).

## 6. CONCLUSIONS

1. Short-term exposure of soils to chemical stress (acidification, alkalization, salinity) does not cause significant changes in the structure and spectroscopic properties of humic acids, which mainly depend on the type of soil.
2. Humic acids of hydrogenic soils: humic gleysol and mollic gleysol, compared with humic acids of arenosol, luvisol and cambisol, are characterized by specific properties.
3. Susceptibility of humic acids to oxidation, measured by a change in spectrometric properties within ultraviolet and visible radiation, decreases along with the increased molecular weight of humic acids and increases together with the increased presence of aliphatic structures in these acids.

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