

VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

BRNO UNIVERSITY OF TECHNOLOGY

FAKULTA CHEMICKÁ
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FACULTY OF CHEMISTRY
INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY

CORRELATION OF THERMOANALYTICAL DATA WITH PRIMARY
PROPERTIES
OF HUMIFIED SUBSTRATA

DIZERTAČNÍ PRÁCE
DOCTORAL THESIS

AUTOR PRÁCE
AUTHOR

Ing. JIŘÍ KISLINGER

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KORELACE TERMOANALYTICKÝCH DAT S PRIMÁRNÍMI CHARAKTERISTIKAMI
HUMIFIKOVANÝCH SUBSTRÁTŮ

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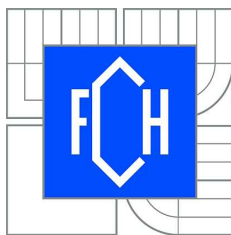
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ABSTRACT

This doctoral thesis is composed as a thermoanalytical study of humified substrata, i.e. humic substances and soils, obtained from several different sources and of various types in order to cover wide range of primary characteristics. It enquires after linear correlations (using Pearson product-moment correlation coefficient) between thermo-oxidative degradation kinetics (observed by means of thermogravimetric analysis (TGA) and expressed as conversion time assessed by non-isothermal isoconversional method) and standard characteristics, such as amount of aromatic and carboxylic carbon present in humic substances determined by nuclear magnetic resonance (NMR) spectroscopy. The main aim is to shed light on the processes of thermo-oxidative degradation of humified pool of natural organic matter and to search for trends between primary and secondary structure of humic substances. The second goal lies within correlating thermogravimetric weight losses of whole soil samples to their microbiological stability measured in laboratory experiments as soil respiration via carbon dioxide evolution method. The purpose consists in assessment of the interrelationship existence and in disclosing what kind of processes occurring during soil respiration can be easily detected by TGA. Results of the work bring more detailed view on thermal analysis of soils and other humified matter. The major benefit of this work is the presentation of emerging applications of thermal analysis for the characterization of soil organic matter and furthermore the contribution to the development of application of thermal analysis techniques in humus science.

ABSTRAKT

Tato dizertační práce je napsána jako termoanalytická studie humifikovaných substrátů, tj. huminových látek a půdních vzorků, získaných z několika různých zdrojů tak, aby byl pokryt co nejširší rozsah primárních charakteristik. Práce hledá lineární korelace (s využitím Pearsonova korelačního koeficientu) mezi kinetikou termo-oxidační degradace (sledovanou pomocí termogravimetrie (TGA) a vyjádřenou konverzním časem stanoveným neizotermickou izokonverzní metodou) a standardními charakteristikami, jako je množství aromatického a karboxylového uhlíku přítomného v huminových látkách stanoveného spektroskopii nukleární magnetické rezonance (NMR). Hlavním cílem této práce je objasnit proces termo-oxidační degradace humifikované části přírodní organické hmoty a hledat závislosti mezi primární a sekundární strukturou huminových látek. Dalším úkolem je nalezení korelace mezi termogravimetrickým úbytkem hmotnosti půdních vzorků a jejich mikrobiální stabilitou měřenou v laboratorních experimentech jakožto půdní respirace (tedy uvolňování oxidu uhličitého). Účelem je zjistit, zdali vzájemné propojení vůbec existuje, a objasnit jaké druhy procesů, které provázejí půdní respiraci, je možné jednoduše odhalit pomocí TGA. Výsledky práce skýtají detailnější pohled na termickou analýzu půdních vzorků a jiných humifikovaných materiálů. Hlavním přínosem této práce je prezentace vyskytujících se aplikací termické analýzy pro charakterizaci půdní organické hmoty a dále také příspěvek k rozvoji aplikací technik termické analýzy v půdní vědě.

KEYWORDS

soil, soil organic matter, soil respiration, humic substances, thermogravimetric analysis, isoconversional methods, correlation

KLÍČOVÁ SLOVA

půda, půdní organická hmota, půdní respirace, huminové látky, termogravimetrická analýza, izokonverzní metody, korelace

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DECLARATION

I declare that the doctoral thesis has been elaborated by myself and that all the quotations from the used literary sources are accurate and complete. The content of the doctoral thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by the supervisor, the dean of the Faculty of Chemistry, BUT and Prof. Dr. habil. Christian Siewert (Hochschule Weihenstephan-Triesdorf).

PROHLÁŠENÍ

Prohlašuji, že jsem disertační práci vypracoval samostatně a že všechny použité literární zdroje jsem správně a úplně citoval. Disertační práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího diplomové práce, děkana FCH VUT a Prof. Dr. habil. Christiana Siewerta (Hochschule Weihenstephan-Triesdorf).

.....
student's signature
podpis studenta

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In this place, I should probably dedicate some words of thanks to certain people. Not an easy task to do... Anyway, thank you, all who deserve it; I'm sure you know who you are. I am endlessly grateful for your unconditional help and support without hidden agenda in such manner that words cannot sufficiently express my innermost feelings. Thanks again!

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1. INTRODUCTION

The Protocols of the Kyoto Convention on Global Changes impose significant reduction of greenhouse gases input to the atmosphere. Such process demands fundamental understanding of carbon (C) stabilization in soils because the amount of organic matter stored in soils represents one of the largest reservoirs of organic carbon on the global scale. Consequently, any change in the size and the turnover rate of soil carbon pools may potentially alter the atmospheric CO₂ concentration and the global climate. The mechanisms for carbon stabilization in soils are still not well understood and the ultimate potential for C stabilization in soils is unknown. Current soil organic matter (SOM) turnover models are not fully process-oriented and thus the simulation of ecosystem response to environmental changes such as management and the changing climate is still difficult. [1]

Both soil fertility and stability are related to the organic matter content of soil. Many functions of SOM are due to its more stabilised fraction, the humified materials, and to the balance between the labile and the stabilised fractions. Changes in SOM content are related to changes in microbial biomass turnover, because they reflect the balance between rates of microbial organic matter accumulation and degradation. However, SOM as a whole responds less quickly to changing soil conditions than does microbial biomass. Therefore, the nature of the organic matter itself, rather than its concentration, may be a more sensitive indicator of the changes in soil quality due to changes in environmental conditions and agricultural management. Chemical and structural characteristics of humic substances are known to be better predictors of the rate of SOM turnover than SOM content itself. This suggests the possibility of using humic substances as indicators of SOM turnover. [2]

A reliable prediction of soil organic matter properties is complicated by the number of different analytical methods to be used. To save costs and time, it is desirable to introduce a method that should simultaneously screen both static and dynamic soil properties. A method of choice is represented by thermal analysis (TA). Thermal analysis techniques have been used for a long time in the study of soils, particularly in clay mineralogy. While there exists a large volume of literature on the theoretical underpinnings of thermal analysis and its application to the analysis of minerals [3–9], the exothermic reactions associated with the thermal oxidation of soil organic matter have received much less attention. The proposition that soil thermograms represent characteristic properties and can be used to characterize soil organic matter quality has existed for decades. However, only recently has the number of tested soils and fractions been large enough to test this proposition and the potential link between thermal and biological soil organic matter stability. [10–14] Due to the increasing demands for rapid and quantitative assessments of soil organic matter quality, thermal analysis techniques are unique means to characterize the complete continuum that comprises soil organic matter. The experimental setup and interpretation of thermal analysis must, however, go beyond the simplified qualitative and quantitative level and therefore requires careful attention to analytical parameters and post-analysis data manipulations.

2. STATE OF THE ART

2.1 The concept of soil

“Soils are the surface mineral and organic formations, always more or less coloured by humus, which constantly manifest themselves as a result of the combined activity of the following agencies; living and dead organisms (plants and animals), parent material, climate and relief.”

V. V. Dokuchaev (1879)

“The soil is teeming with life. It is a world of darkness, of caverns, tunnels and crevices inhabited by a bizarre assortment of living creatures...”

J. A. Wallwork (1975)

“The nation that destroys its soil, destroys itself.”

F. D. Roosevelt (1937)

2.1.1 Basic information

There is a little merit to give a rigorous definition of soil because of the complexity of its make-up, and of the physical, chemical and biological forces that act on it. Nor is it necessary to do so, for soil means different things to different users. [15] Despite, some official definitions have been given. The traditional meaning of soil is that it is the natural medium for the growth of land plants. In 1975 the Soil Conservation Service offered a more inclusive definition in the book *Keys to Soil Taxonomy*: “Soil is the collection of natural bodies on the earth’s surface, in places modified or even made by man of earthy materials, containing living matter and supporting or capable of supporting plants out-of-doors.” [16] The 1999 edition of the same book presented a new definition of soil to take into the account the soils of Antarctica, where the climate is too harsh to support higher plants. It is long and involved definition and is not presented here because the earlier one is adequate enough. [17]

Another given definition says that most soil is a loose mass of fragmented and chemically weathered rock with an admixture of humus, which is partially decomposed organic matter. In the wet areas of humid regions, plant residue may accumulate several feet thick to form a peat soil, but in dry regions, soil organic matter may be low throughout the landscape. Soil is very diverse over the face of the earth, but if properly managed, it serves the mankind well. [18]

Soil is at the interface between the atmosphere and the lithosphere. It also has an interface with bodies of fresh and salt water (the hydrosphere). The soil sustains the growth of many plants and animals, and so forms part of the biosphere. From the agricultural and environmental point of view, soil is very simply defined as a part of natural environment of the Earth forming from regolith of superficial rock and decaying debris of organisms. It is an open

dynamic system with proceeding mass and energy flow. Soil consists of solid, liquid and gaseous phase together with soil organisms, edaphon, their metabolic products and all the organic residues in different state of mineralization. Climate, vegetation, agricultural cultivation, bedrock or land topography are examples of soil-forming factors that define the soil quality and production rate (from centuries to millennia). Soil constitutes the base of almost all land vegetation. It is also very important part of nutrition chain for all mankind. Another definition – more like economical – says that soil is one of primary factors participating in production process. In this case soil represents also all non-renewable natural resources, thus it is not only the soil as defined by the previous definition. The area on Earth covered with soil is called the pedosphere and the science dealing with soil and related topics is pedology. [19]

Soil forms at the interface between the atmosphere and the consolidated or loose deposits of the Earth's crust. Physical and chemical weathering, denudation and redeposition combined with the activities of a succession of colonizing plants and animals moulds a distinctive soil body from the milieu of rock minerals in the parent material. The process of soil formation, called pedogenesis, culminates in the remarkably variable differentiation of the soil material into a series of horizons that constitute the soil profile. The horizons are distinguished, in the field at least, by their visible and tangible properties such as colour, hardness and structural organisation. In Fig. 1 the horizons are denoted as O (organic), A (surface), B (substrate), and C (substratum). In some descriptions it is also possible to find the bedrock or parent material (R) but it is not a part of soil itself. [20]

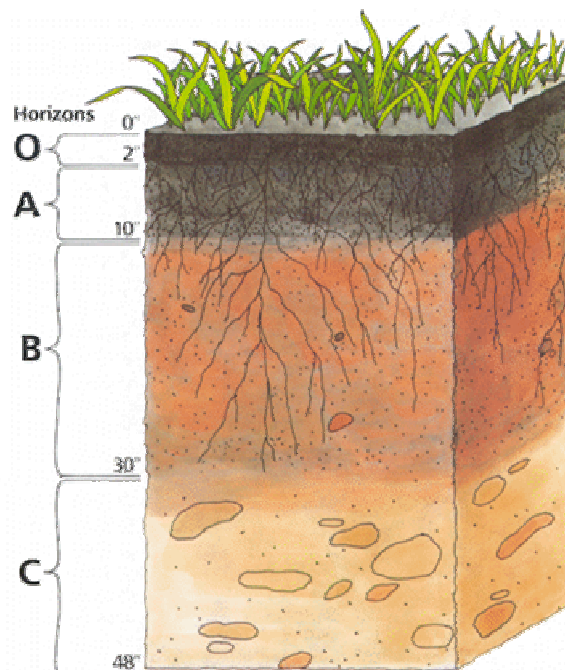


Fig. 1 *Scheme of a soil profile with organic (O), surface (A), substrate (B) and substratum (C) horizon*

The intimate mixing of mineral and organic materials forms a porous fabric that is permeated by water and air. It is a favourable habitat for a variety of plant and animal life (Fig. 2). [15]

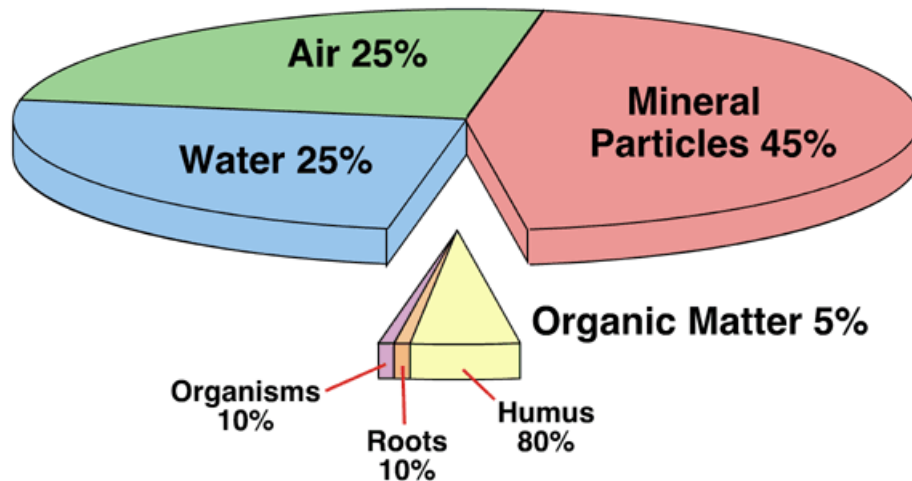


Fig. 2 Average proportions of the main soil components

2.1.2 Mineral composition of soils

Rock fragments and mineral particles in soil vary enormously in size from boulders and stones down to sand grains and very small particles that are beyond the resolving power of an optical microscope ($<0.2 \mu\text{m}$). Particles smaller than $1 \mu\text{m}$ are classed as colloidal. An arbitrary division is made by size-grading soil into material:

- that passes through a sieve with 2 mm diameter holes – the *fine earth*
- that retained on the sieve ($>2 \text{ mm}$) – the *stones* or *gravel*, but smaller than
- fragments $>600 \text{ mm}$, which are called *boulders*.

The distribution of particle sizes of the fine earth determines the soil texture. In practice, such distribution is usually subdivided into several class intervals that define the size limits of the sand, silt and clay fractions. The extent of this subdivision, and the class limit chosen, vary from country to country and even between institutions within countries. The major systems in use are those adopted by the Soil Survey Staff of the United States Department of Agriculture (USDA), the British Standards Institution and the International Union of Soil Sciences. All 3 systems set the upper limit for clay at $2 \mu\text{m}$ diameter but differ in the upper limit chosen for silt and the way in which the sand fraction is subdivided. [15]

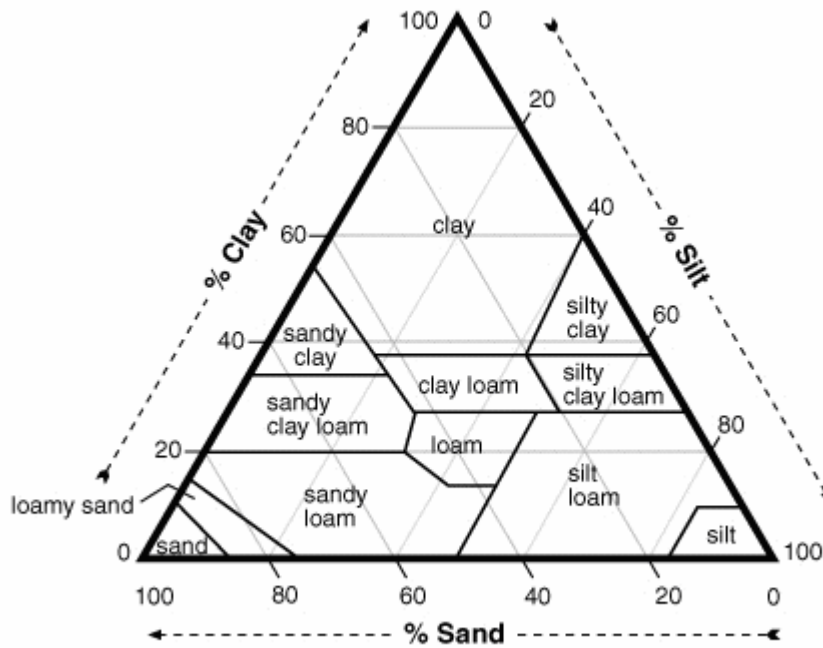


Fig. 3 Composition of 12 major textural classes by USDA [22]

Texture is a property that changes only slowly in time, and is an important determinant of the soil's response to water – its stickiness, mouldability and permeability: its capacity to retain cations, and its rate of adjustment to ambient temperature changes. Texture should not be confused with tilth, of which it is said that a good farmer can recognize it with his boot, but no soil scientist can describe it. Tilth refers to the condition of the surface of ploughed soil prepared for seed sowing: how sticky it is when wet and how hard it sets when dry.

The adsorption of water and solutes by clay fraction particles ($< 2 \mu\text{m}$ size) depends not only on their large specific surface area, but also on the nature of the minerals present. The basic crystal structures consist of sheets of Si in tetrahedral coordination with $\text{O}[\text{SiO}_2]_n$ and sheets of Al in octahedral coordination with $\text{OH}[\text{Al}_2(\text{OH})_6]_n$. Crystal layers are formed by the sharing of O atoms between contiguous silica and alumina sheets, giving rise to minerals with Si/Al mol ratios ≤ 1 (imogolite and the allophanes), the 1:1 layer lattice minerals (kaolinites) and the 2:1 layer lattice minerals (illites, vermiculites and smectites). Isomorphous substitution ($\text{Al}^{3+} \rightarrow \text{Si}^{4+}$ and $\text{Fe}^{2+}, \text{Mg}^{2+} \rightarrow \text{Al}^{3+}$) in the lattice results in an overall net layer charge ranging from < 0.005 to 2 moles of negative charge per unit cell for kaolinite to muscovite, respectively. The formation of inner-sphere complexes between the surface and unhydrated K^+ ions in the interlayer spaces of the micas effectively neutralizes the layer charge and permits large crystals to form. On weathering, K^+ is replaced by cations of higher ionic potential, such as Ca^{2+} and Mg^{2+} and layers of water molecules intrude into the interlayer spaces – the basal spacing of the mineral increases. The Ca^{2+} and Mg^{2+} , which form outer-sphere complexes with the surfaces, are freely exchangeable with other cations in the soil solution. [21]

The edge faces of the clay crystals, especially kaolinite, and the surfaces of iron and aluminium oxides (e.g. goethite and gibbsite) bear variable charges depending on the association or dissociation of protons at exposed O and OH groups, respectively. The surfaces are positively charged at low pH and negatively charged at high pH. The cation and anion charges adsorbed (in mols of charge per unit mass) measure the CEC and AEC respectively of the mineral. CEC ranges from 3 to 150 cmol^+ per kg of the clay minerals and AEC from 30 to 50 cmol^- per kg of the sesquioxides.

Clays, in which the interlayer surfaces are freely accessible to water and solutes are called expanding lattice clays. The total surface area is then the sum of the internal and external areas, and approaches $800 \text{ m}^2 \text{ g}^{-1}$ for a fully dispersed Na-montmorillonite. However, when the crystal lattice layers are strongly bonded to another, as in kaolinite, the surface area comprises only the external surface and may vary from 5 to $100 \text{ m}^2 \text{ g}^{-1}$ depending on the crystal size. [15]

2.1.3 Soil organic matter

Soil organic matter is formed from plant litter, dead roots, animal remains and excreta. It is a repository for the essential elements required by the next generation of organisms. Release of these elements or mineralization depends on the decomposition rate and the demands made by the heterogeneous population of soil organisms.

The living organisms (biomass) vary in size from the macrofauna, vertebrate animals of the burrowing type, to the mesofauna, invertebrates such as mites, spring-tails, insects, earthworms and nematodes, to the microorganisms – broadly subdivided into the prokaryotes and eukaryotes and comprising the bacteria, actinomycetes, fungi, algae and protozoa. The most important of the mesofauna are the earthworms in temperate soils, especially under grassland, and termites in many soils of the tropics and subtropics. Earthworm biomass normally ranges from 0.5 to 1.5 t ha^{-1} , whereas the microbial biomass makes up 2–4 per cent of the total organic C in soil, or $0.5\text{--}2 \text{ t ha}^{-1}$.

Annual rates of litter fall range from $0.1 \text{ t (C) ha}^{-1}$ in alpine and arctic forests to 10 t (C) ha^{-1} in tropical rain forests. Carbon substrates from rhizosphere deposition and the death of roots also make an important contribution that is difficult to quantify. The interaction of microorganisms and mesofauna in decomposing litter leads not only to the release of mineral nutrients but also to the synthesis of complex new organic compounds that are more resistant to attack. This process is called humification. [15]

SOM is a key attribute to soil quality. It affects, directly or indirectly, many physical, chemical and biological properties that control soil productivity and resistance to degradation. Changes in the quantity of SOM and the equilibrium level of SOM depend on the interaction of five factors: climate, landscape, texture, inputs and disturbance. Some of these factors, called SOM capacity factors, can be managed, whereas others cannot. As SOM enters and resides in soil, it is subjected to processes that alter its composition and quantity. Fundamental soil processes, such as humification, aggregation, translocation, erosion, leaching and mineralization are driven by the capacity factors. These capacity factors and soil processes, in turn, largely dictate the management system imposed on soil. [23]

SOM is traditionally subdivided into non-humic and humic substances (HS). Non-humic substances include those with well-defined chemical structure and properties, e.g. carbohydrates, proteins, fats, waxes, dyes, resins, peptides, amino acids and other low molecular organic substances that are easily available to soil microorganisms. The bulk of SOM, however, are HS since they form up to 80 % of it. Besides, HS are also present in waters, peats, sediments, leonardites, lignites and brown-black coals and other natural materials. [24]

Table 1 *Percentage distribution of components in SOM*

Material type		Usual percentage range (by weight)
Non-humic substances	Lipids	1–6
	Carbohydrates	5–25
	Proteins/Peptides/Amino Acids	9–16
	Others	trace
Humic substances		60–80

There are several ways of classification of HS. The most frequent approach divides these substances according to their solubility in acids and alkalis into three basic groups: (1) fulvic acids – soluble under all pH conditions; (2) humic acids – not soluble under acidic conditions ($\text{pH} < 2$) but soluble at basic pH values; and (3) humins – insoluble at any pH value. Despite the fact that HS are under long-term intensive and extensive study and their importance has been acknowledged for over 150 years, they belong to the nature’s least understood materials. Most commonly are described as amorphous, partly aromatic, polyelectrolyte-like materials, their molecular weight being a matter of heated debates. They are responsible for brown or black colour of rivers and lakes. According to recent results, it is suggested that HS are mixtures of plant and microbial carbohydrates, proteins and lipids, together with partially degraded lignins and tannins and also including microbial materials such as melanins. [24]

The chemical structure of humic compounds has proved difficult to elucidate. Scientists have attempted to shed light upon the structure of humic acids for many years. Some post 1970 suggestions for formulae of humic acids were given by Haworth, Stein, Stevenson (Fig. 4) or Schulten and Schnitzer (Fig. 5).

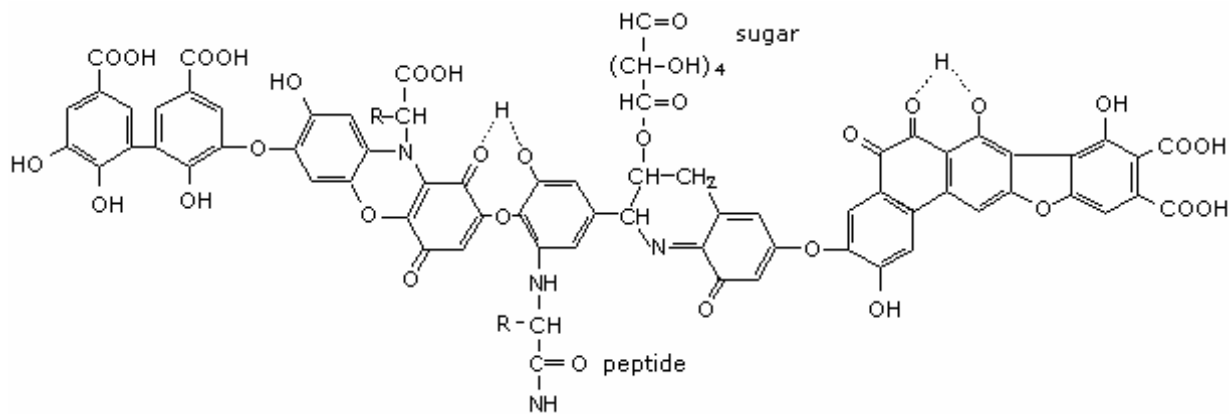


Fig. 4 *The structure of humic acid by Stevenson (1982)*

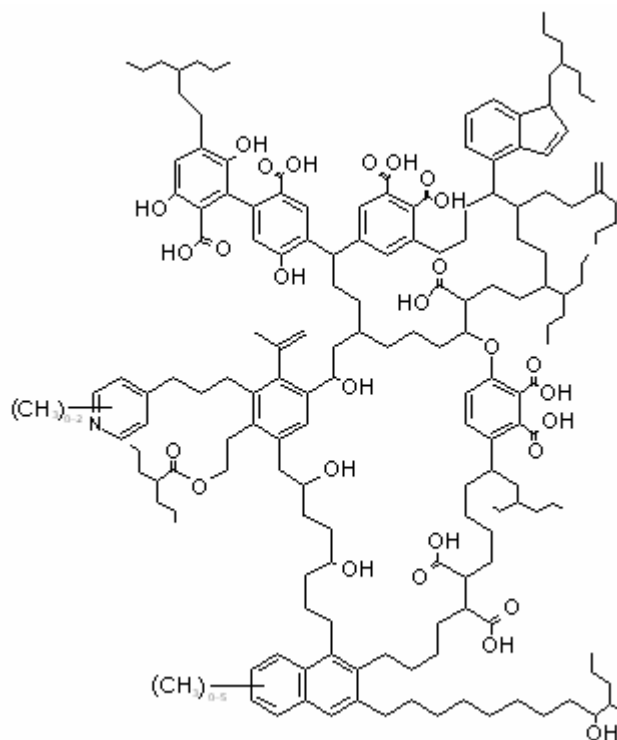


Fig. 5 *Humic acid (by Schulten and Schnitzer), only part of the structure depicted*

Examination of their evidence put forward these traditional structures, showed that there is no strong argument to support any of them. [26] Nevertheless, the most recent HA model structure taking into account the system complexity is presented in Figure 6.

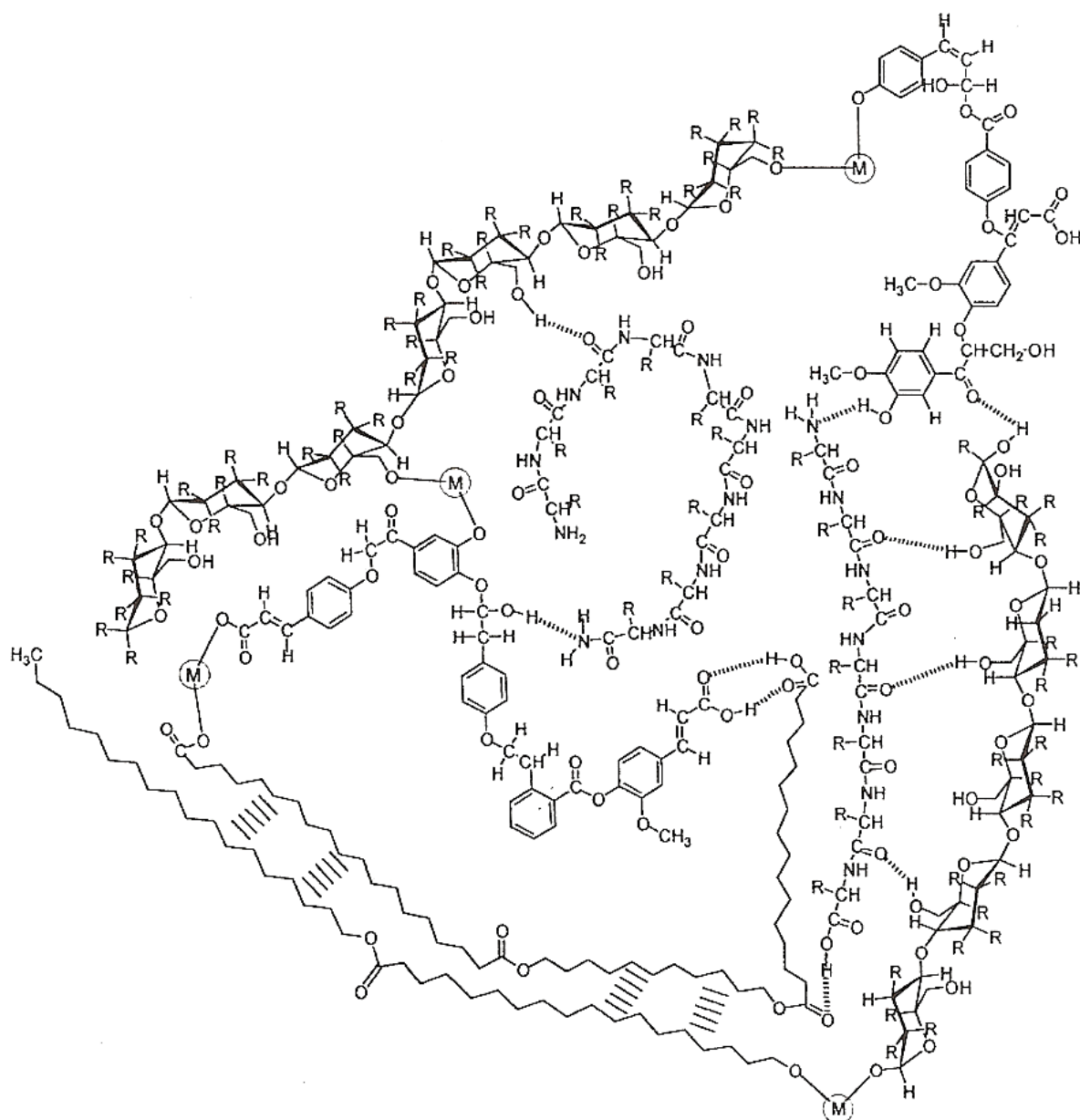


Fig.6 Recent model structure of humic acid according to Simpson et al. (2002). [27]

The common view of humic substances was that they were polydisperse macromolecules created by random polymers. Their basic structural units were derived from well-described biopolymers. [28] The molecular weight of HS was therefore believed to range from 500 Da for some aquatic HS to more than 10^6 Da for soil HA. [25]

The amphiphilic nature of HA and to a lesser content of FA has given rise to the micellar (membrane-like or detergent) model for these compounds. [29] Other studies indicated that the spontaneous aggregation of aqueous HA could be intramolecular (involving a single polymer chain) or intermolecular (involving multiple chains). In the former case, it is thought that humic polymers fold and coil in a manner that creates structured regions that can be linked to knots in a string. The interior of these assemblies is relatively hydrophobic, while the exterior is more hydrophilic. The structure is similar to that of surfactant micelle or better

pseudomicelle, although it is constrained by the intramolecular nature of the arrangement, and the polydispersity of material. [30],[31]

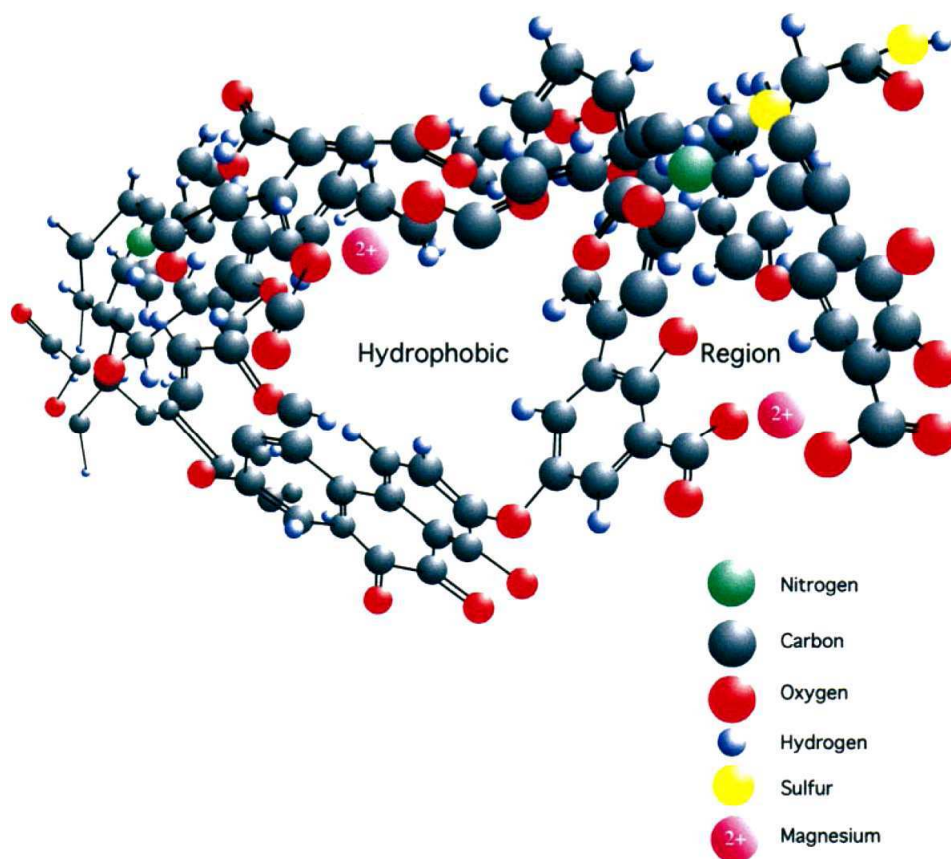


Fig. 7 Portion of the proposed "type" structure of humic acid [32]

The traditional view that HS are polymers is not substantiated by any direct evidence but is assumed only on the basis of laboratory experiments with model molecules and unwarranted results produced by incorrectly applying either analytical procedures or mathematical treatments developed for purified and undisputed biopolymers. Recent results obtained by either low pressure or high performance size exclusion chromatography have shown that the large molecular size currently reported for HS is only superficially apparent and instead they should be considered as self-assembled supramolecular associations of heterogeneous molecules. The conformation of HS in solutions appears to be stabilized mainly by weak dispersive forces such as van der Waals, π - π , or CH- π and hydrogen bonds. These humic associations show only apparent high molecular dimensions, which can be reversibly disrupted by either treating humic solutions with low concentrations of mineral and monocarboxylic acids or slightly varying the mobile phase composition but keeping ionic strength constant. [33] Confirmation of previous results was obtained using NMR technique. As mentioned above, humic materials are complex mixtures of many components, which vary in ratio and structure with sample origin and preparation procedure. While aggregates will likely be "held together" through a complex combination of hydrophobic associations, charge interactions, hydrogen bonds, and metal bridging, results shown in the NMR study suggest that metal ions play a crucial role in aggregate formation and stability. It has been found that humic aggregates consist of molecules with molecular weight up to 2000 Daltons. [34] Later results of Piccolo and Spiteller indicate that the average molecular masses calculated from

electrospray ionization mass spectroscopy (ESI-MS) spectra of terrestrial humic substances appear to be in accordance with those previously measured for aquatic humic matter. The values found for terrestrial humic samples – 600–1500 Da – are from one to three orders of magnitude lower than those which are commonly reported for molecular weight of soil humic substances. These values are in line with the supramolecular model of humic substances by which relatively small and heterogeneous humic molecules self-assemble by multiple non-covalent interactions in only apparently large molecular sizes. [36] Furthermore, findings of Šmejkalová and Piccolo using diffusion ordered nuclear magnetic resonance spectroscopy (DOSY-NMR) showed that carbohydrates had the largest molecular size in FA, whereas alkyl or aromatic components had the largest molecular size in HA. At increasing concentrations, an aggregation into apparently larger associations for all humic samples was indicated. When humic solutions were brought from alkaline to acidic pH (3.6), the results suggested a decrease of aggregation and molecular size. A general comparison of HA and FA molecular sizes was achieved by multivariate statistical analysis. While a larger extent of aggregation and disaggregation was observed for HA than for FA, no aggregation was detected, under similar conditions, for a true macropolymeric standard. Such difference between a polymeric molecule and humic samples, is in line with the supramolecular nature of humic matter. The possible formation of humic micelles was also investigated by both changes of diffusivity in DOSY spectra and shift of ^1H NMR signals. [35]

The value of regular additions of organic matter to the soil has been recognized by growers since prehistoric times. It has a:

- nutritional function; it serves as a source of N, P for plant growth,
- biological function; it profoundly affects the activities of microfloral and microfaunal organisms,
- physical and physico-chemical function; it promotes good soil structure, thereby improving tilth, aeration, and retention of moisture and increasing buffering and exchange capacity of soils.

Humus also plays an indirect role in soil through its effect on the uptake of micronutrients by plants and the performance of herbicides and other agricultural chemicals. It should be emphasized that the importance of any given factor will depend upon such environmental conditions as climate and cropping history. HS are known to significantly affect the behaviour of some pollutants in natural environments, such as trace metal speciation and toxicity, solubilization and adsorption of hydrophobic pollutants, disinfection by-product formation, aqueous photochemistry, mineral growth and dissolution, and redox behaviour in soils. Recent studies have also suggested that HS may be dynamically involved in carbon and electron flow in anaerobic environments, which implies its potential role in pollutant transformation, and, in some cases, detoxification. Furthermore, being an all-natural substance, it is especially attractive for this use, because its own pollution potential is minimal. [25]

2.1.4 Soil respiration

Soil respiration is defined as absorption of oxygen or the discharge of carbon dioxide by microbial activity and includes gas exchange from aerobic and anaerobic metabolism. Soil

respiration results from degradation of biologically accessible organic substances and their mineralization. This biological soil reaction is the summary of many individual activities, which result from the condition of the particular soil and its constituents. Under normal conditions, an ecological balance is achieved in the soil between the organisms and their activities. In these conditions, basic respiration is obtained. If the balance is disturbed (for example by the addition of degradable organic substances), a change in soil respiration will be observed as a result of more intense microbiological growth and mineralization activity. The presence of poisonous substances, such as may be found in wastes, can lead to limitation of soil respiration. The ability of a soil to discharge CO₂ is thus an indication of the biological function of the soil in its entirety, taking into consideration all the constituents of the soil. With appropriate modifications, soil respiration can be employed to carry out further biological determinations, including the biological degradability of substances (such as pesticides, plastics, and fertilizers), toxicity tests to assess contaminated soils, substrate induced respiration rate, microbial biomass etc. [37]

The importance of soil organisms in promoting the turnover of carbon has already been hinted. Aerobic respiration involves the breakdown or dissimilation of complex C molecules, oxygen being consumed and CO₂, water and energy for cellular growth being released. Under such conditions, the respiratory quotient, defined as:

$$RQ = \frac{\text{volume of CO}_2 \text{ released}}{\text{volume of O}_2 \text{ consumed}} \quad (1)$$

is equal to 1. When respiration is anaerobic, however, the RQ rises to infinity because O₂ is no longer consumed but CO₂ continues to be evolved. The respiratory activity of the soil organisms or respiration rate, R , is measured as CO₂ is released (or O₂ consumed) per unit soil volume per unit time. Soil respiration is augmented by the respiration of living plant roots, and, further, the respiration of microorganisms is greatly stimulated by the abundance of carbonaceous material (mucilage, sloughed-off cells and exudates) in the soil immediately around the root. The stimulus to the soil respiration provided by the presence of the crop can as farth as the respiration rates for cropped soil can even triple in winter months and double in summer months in comparison with fallow soil.

An instrument designed to measure respiration rates is called a respirometer. For field measurements, a Rothamsted soil respirometer can be used. In principle, soil moisture content is regulated by controlled watering and drainage; temperature is recorded and the circulating air is monitored for O₂, the deficit being made good by electrolytic generators. Carbon dioxide is absorbed in vessels containing soda lime and measured. Such measurements show that the respiration rate depends on (1) soil conditions, such as organic matter content and quality, O₂ supply and moisture; (2) cultivation and cropping practices; and (3) environmental factors, principally temperature. The effect of temperature on respiration rate is expressed by the equation:

$$R = R_0 Q^{\frac{T}{10}}, \quad (2)$$

where R and R_0 are the respiration rates at temperature T and 0 °C, respectively, and Q is the magnitude of the increase in R for 10 °C rise in temperature, called the $Q-10$ factor, which lies between 2 and 3. Temperature change is the cause of large seasonal fluctuations in soil respiration rate in temperature climates. [15]

Soil respiration is a subject that is of concern not only to ecologists but also to scientists who study atmospheric dynamics and earth system functioning. As an integral part of the ecosystem carbon cycle, soil respiration is related to various components of ecosystem production. Soil respiration is also intimately associated with nutrient processes such as decomposition and mineralization. Moreover, soil respiration plays a critical role in regulating atmospheric CO₂ concentration and climate dynamics in the earth system. Thus, it becomes relevant to the mitigation of climate change and the implementation of international climate treaties in terms of carbon storage and trading. [38]

2.1.5 Environmental aspects and threats

Organic matter levels tend to be reasonably well maintained under natural and semi-natural conditions where the nutrient cycle is unbroken. In circumstances where there is human intervention and there is a need to modify topsoils, as in farming land, the nutrient cycle is likely to be interrupted and amounts of organic matter decline. It is estimated that levels of organic matter can fall by up to 50 percent when arable agriculture replaces natural vegetation, such as grassland or woodland.

Thus in the past 60 years since the introduction of intensive farming there has been a measurable decline in organic matter levels in arable soils. The other assets of organic matter, such as good structure, are not usually provided by chemical fertiliser. Regular ploughing, frequent cultivations and use of some fertilisers have led to a decline in organic matter levels on many places and this has brought about deterioration in soil structure. Granular and crumb topsoil structures have been replaced by coarser structures or even by complete loss of structure. This in turn restricts penetration of rainwater which instead flows over the surface causing soil erosion. Much of the increased soil erosion on farmland in the past 100 years can be linked to declining levels of organic matter.

It is true that natural problems in the soil are relatively rare; much more common are problems where human activity has led to polluted or contaminated soils. Mostly, human pollution of soils can be linked to industries such as mining for metals. Much pollution and contamination occurred during the development of industry from the 1800s onwards, although there are still soils today contaminated from Roman times. In other parts of the world soils can become contaminated when drinking and irrigation waters containing pollutants are pumped up to the surface.

Soils have become polluted and contaminated through the industrial use of land and this has led to incidences of damage to the soil. This in turn can affect the health. To try to limit the effects of pollution, government bodies are set up to encourage and enforce greater awareness of these problems and the clean up of polluted contaminated land. [39]

2.2 Other sources of natural organic matter

Up to now, soil has been mentioned as the major source of natural organic matter. But there are several more humified materials worth mentioning. The most important ones are peats and miscellaneous types of coals. They all play crucial roles in a process called *coalification*.

The so-called coalification or carbonification includes the process of diagenesis, and the metamorphic changes of the coal. The diagenesis of the coals is often referred to as “biochemical coalification”, whereas the metamorphism of coals is called “geochemical coalification”. The coalification begins with peat and ends with highly metamorphic anthracite as demonstrated in Figure 8. Graphite is formed only at pressures and temperatures prevailing during the metamorphism of rock. The diagenesis of rocks should be considered as terminated with the transformation of anthracite to graphite. Inasmuch as carbonification is a very complex process one must use different properties to measure the rank of a coal. The values of the different rank parameters change with the degree of carbonification. [40]

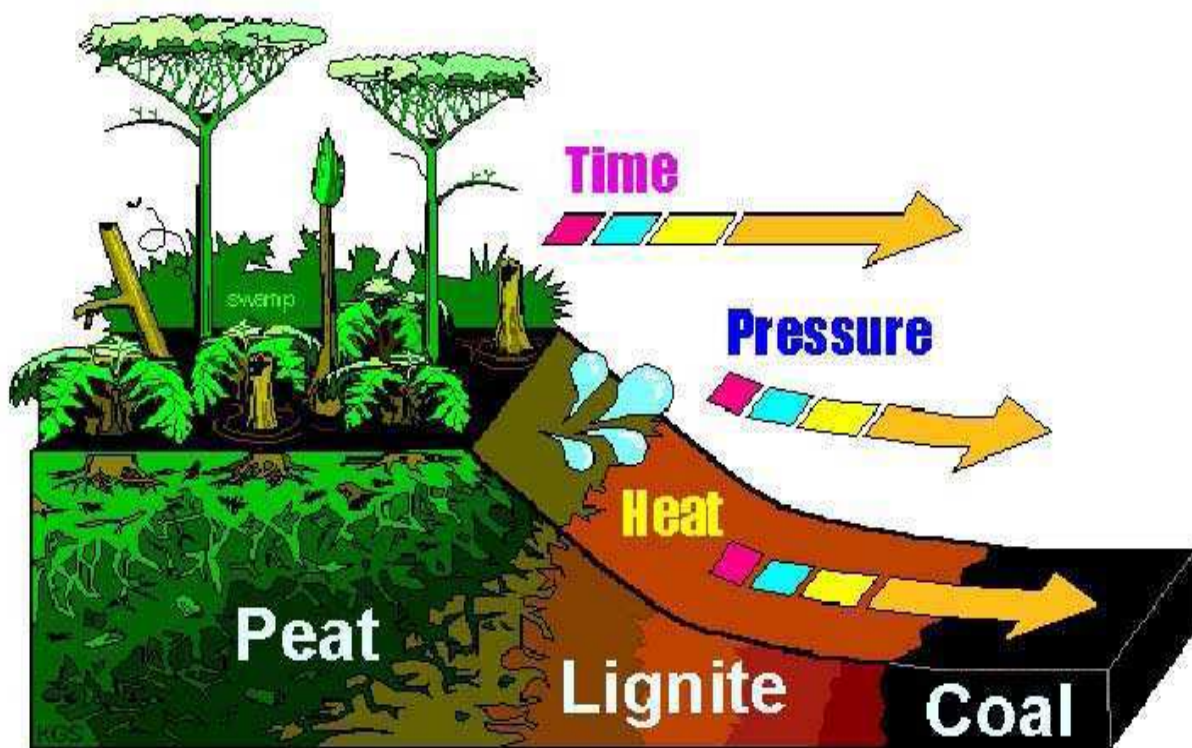


Fig. 8 Schematic of the coalification process

Most coals originate from peats or low moors with plant associations of forests or reeds. Plants usually decompose after their death, i.e. under the influence of oxygen they are converted into gaseous compounds and water. In swamps with a high water table and lack of aeration, however, the plant residues are in a reducing environment, which is conducive to the process of peat formation.

One can differentiate between humic coals and sapropelic coals. Humic coals originate from true peats, which develop through the accumulation of dead plant matter at the site of the peat forming plants. Sapropelic coals, on the other hand, are formed from organic muds, which are

deposited on the floor of poorly aerated quiet-water lakes and ponds. They contain many allochthonous elements. The sapropels do not undergo peatification, but pass through a process of “saprofication”, which is characterized by putrefaction processes under anaerobic conditions. The sapropelic coals are relatively rare. Most humic coals originate from forest peats, and thus mainly from wood and bark substances, leaves and roots of swamp vegetation. In swamps with herbaceous plant associations (e.g. reed marshes), the roots of the sedges and grasses play an important role in the formation of peat. During the process of peat formation, wood, bark, leaves and roots are usually almost completely transformed into humic substances, which are characterized by relatively high oxygen content. These humic substances eventually form vitrinite. Exinite is formed from the chemically resistant and relatively hydrogen-rich plant components such as pollen and spores, leaf epidermis (cuticles), resins, and waxes. These substances are of minor importance in comparison with the humic matter. Besides, relatively carbon-rich macerals (i.e. smallest petrographical unit of coal) occur in coals such as fusinite and micrinite, which are combined under the term inertinite. These components originate from the strong aerobic decomposition of plant residues at the peat surface, with the exception of a part of the fusinite which is derived from fossil charcoal. [41]

Chemically, the parent material of coals consists mainly of cellulose, hemicelluloses and lignins, with minor amounts of proteins, sugars, pentosanes, pectines, tannins and bitumens. The bitumens comprise such substances as fats, oils, waxes, resins, sterins, sporopollenins, cutine and suberine. The inorganic components of the coal originate partially from the plants. Most of them, however, were transported by water or air into the swamps (clay, silt, sand); or they were precipitated syngenetically or epigenetically from solutions in the peat or coal (pyrite, quartz, calcite, siderite, dolomite, etc.).

The principal characteristics of a coal are its thickness, lateral continuity, rank, maceral content and quality. Apart from rank, which is governed by burial and subsequent tectonic history, the remaining properties are determined by factors controlling the mire where the peat originally formed. These factors include type of mire, type(s) of vegetation, growth rate, degree of humification, base-level changes, and rate of clastic sediment input.

About 3 % of the of the earth’s surface is covered by peat, totalling 310 million hectares. During the last years, numerous studies have attempted to understand more fully how peat-producing wetlands or mires are developed and maintained, and in particular how post-depositional factors influence the formation of coals. Peat producing wetlands can be divided into ombrogenous peatlands or mires (owing their origin to rainfall) and topogenous peatlands (owing their origin to a place and its surface/groundwater regime). A great variety of topogenous peats form when water-logging of vegetation is caused by groundwater, but ombrogenous peats are of greater extent but less varied in character. The classification of the two hydrological categories of mire lists a number of widely used terms. Moore has defined a number of these:

- *mire* is now accepted as a general term for peat-forming ecosystems of all types,
- *bog* is generally confined to ombrotrophic peat-forming ecosystems,

- *bog forest* consists of ombrotrophic forested vegetation, usually an upper storey of coniferous trees and a ground layer of sphagnum moss,
- *marsh* is an imprecise term used to denote wetlands characterized by floating vegetation of different kinds including reeds and sedges, but controlled by rheotrophic hydrology,
- *fen* is a rheotrophic ecosystem in which the dry season water table may be below the surface of the peat,
- *swamp* is a rheotrophic in which the dry season water table is almost always above the surface of the sediment; it is an aquatic ecosystem dominated by emergent vegetation,
- *floating swamps* develop around the fringes of lakes and estuaries and extend out over open water; these platforms can be thick and extensive particularly in tropical areas,
- *swamp forest* is a specific type of swamp in which trees are an important constituent, e.g. mangrove swamp. [42]

The resultant characteristics of coals are primarily influenced by the following factors during peat formation: type of deposition, the peat-forming plant communities, the nutrient supply, acidity, bacterial activity, temperature and redox potential. The following equation must balance for a mire formation and peat accumulation:

$$\text{Inflow} + \text{Precipitation} = \text{Outflow} + \text{Evapotranspiration} + \text{Retention} \quad (3)$$

The conditions necessary for peat accumulation are therefore a balance between plant production and organic decay. Both are a function of climate, plant production and organic decay; such decay of plant material within the peat profile is known as humification (as described above). The upper part of the peat profile is subject to fluctuations in the water table and is where humification is most active. The preservation of organic matter requires rapid burial or anoxic conditions, the latter being present in the waterlogged section of the peat profile. In addition, an organic-rich system will become anoxic faster than an organic-poor one as the decay process consumes oxygen. This process is influenced by higher temperatures, decay rates being fastest in hot climates. Rates of humification are also affected by the acidity of the groundwater, as high acidity suppresses microbial activity in the peat. Peat formation can be initiated by:

- terrestrialization, which is the replacement due to the setting up of a body of water (pond, lake, lagoon, interdistributary bay) by a mire;
- paludification, which is the replacement of a dry land by a mire, e.g. due to a rising groundwater table. [43]

One general system of nomenclature divides coal into four major types: anthracite coal, bituminous coal, subbituminous coal and lignite coal. Lignite is the coal that is lowest on the metamorphic scale. It may vary in colour from brown to brown-black and is often considered to be intermediate between peat and the subbituminous coals. Lignite is often distinguished from the subbituminous coals having a lower carbon content and higher moisture content. Lignite may dry out and crumble in air and is certainly liable to spontaneous combustion. It is known to contain high portion of humic acids, usually up to 80 %. [44]

Slight oxidation of lignite deposits produces so-called leonardite, which is even richer in humic acids content (up to 90 %). These materials are traditional sources for humic acids extraction, used also by the International Humic Substances Society. [45]

Because of the challenging ecological conditions of peat wetlands, they are home to many rare and specialised organisms that are found nowhere else. Some environmental organisations and scientists have pointed out that the large-scale removal of peat from bogs in Britain, Ireland and Finland is destroying wildlife habitats. It takes centuries for a peat bog to regenerate. Recent studies indicate that the world's largest peat bog, located in Western Siberia (of the size of France and Germany combined), is thawing for the first time in 11,000 years. As the permafrost melts, it could release billions of tonnes of methane into the atmosphere. The world's peatlands are thought to contain 180 to 455 petagrams of sequestered carbon, and they release into the atmosphere 20 to 45 teragrams of methane annually. The peatlands' contribution to long-term fluctuations in these atmospheric gases has been a matter of considerable debate. [46] Peat has high carbon content and can burn under low moisture conditions. Once ignited by the presence of a heat source (e.g. a wildfire penetrating the subsurface), it smoulders. These smouldering fires can burn undetected for very long periods of time (months, years and even centuries) propagating in a creeping fashion through the underground peat layer. Peat fires are emerging as a global threat with significant economic, social and ecological impacts. Recent burning of peat bogs in Indonesia, with their large and deep growths containing more than 50 billion tons of carbon, has contributed to increases in world carbon dioxide levels. Peat deposits in southeast Asia could be destroyed by 2040. In 1997, it is estimated that peat and forest fires in Indonesia released between 0.81 and 2.57 Gt of carbon; equivalent to 13–40 percent of the amount released by global fossil fuel burning, and greater than the carbon uptake of the world's biosphere. These fires may be responsible for the acceleration in the increase in carbon dioxide levels since 1998. [47],[48]

As mentioned above, profound release of carbon dioxide and methane to the atmosphere is related to peat degradation. Carbon dioxide is known in both scientific and laical community as greenhouse gas with its effects; but other gases should not be forgotten. The contribution to the greenhouse effect by a gas is affected by both the characteristics of the gas and its abundance. For example, on a molecule-for-molecule basis, methane is about eight times stronger greenhouse gas than carbon dioxide [49], but it is present in much smaller concentrations so that its total contribution is smaller. When these gases are ranked by their contribution to the greenhouse effect, the most important are:

- water vapour, which contributes 36–72 %,
- carbon dioxide, which contributes 9–26 %,
- methane, which contributes 4–9 % and
- ozone, which contributes 3–7 %. [50]

2.3 Thermal analysis

2.3.1 Fundamentals

The International Confederation for Thermal Analysis and Calorimetry (ICTAC) defines thermal analysis as a group of techniques in which a property of the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed. The programme may involve heating or cooling at a fixed (or variable) rate of temperature change, or holding the temperature constant, or any sequence of these. Since hardly any measurement is worth doing if the temperature is not controlled, almost all measurements are some type of thermal analysis. The basic theories to be used in thermal analysis are equilibrium and non-equilibrium thermodynamics and kinetics. [51]

TA, in its various guises, is widely employed in both scientific and industrial domains. The ability of these techniques to characterize, quantitatively and qualitatively, a huge variety of materials over a considerable range has been crucial in their acceptance as analytical techniques. The operational simplicity and quickly received results mean that TA is employed in an ever increasing range of applications. [52]

2.3.2 Characteristics

The advantages of TA over other analytical methods can be summarized as follows:

- the sample can be studied over a wide temperature range using various temperature programmes,
- almost any physical form of sample (solid, liquid or gel) can be accommodated using a variety of sample vessels or attachments,
- a small amount of sample is required (cca 0.1–50 mg),
- the atmosphere in the vicinity of the sample can be standardized,
- the time required to complete an experiment ranges from several minutes to several hours,
- TA instruments are reasonably priced.

TA data are indirect and should be collated with results from other measurements (for example NMR, FTIR, X-ray diffractometry and so on) before the molecular processes responsible for the observed behaviour can be elucidated. The recorded data are influenced by experimental parameters, such as the sample dimension and mass, the heating/cooling rate, the nature and composition of the atmosphere in the region of the sample and the thermal and mechanical history of the sample. [52]

Temperature program can be described by linear dependence:

$$T = T_0 + \beta t, \quad (4)$$

where T is temperature at time t , T_0 is the initial measuring temperature and β is the temperature coefficient. When $\beta > 0$, the sample is heated, when $\beta < 0$, one can speak of linear cooling and when $\beta = 0$, the measurement is under isothermal conditions. [53]

2.3.3 Arrangement of TA instruments

The general arrangement of TA apparatus consists of a physical property sensor, a controlled-atmosphere furnace, a temperature programmer and a recording device. Tab. 2 lists the most common forms of TA. Modern TA apparatus is generally interfaced to a computer (work station), which oversees operation of the instrument controlling the temperature range, heating and cooling rate, flow of purge gas and data accumulation and storage. Various types of data analysis are performed by the computer. A trend in modern TA is to use a single work station to operate several instruments simultaneously. [52]

Table 2 *Conventional forms of TA*

Measured property	TA method	Abbreviation
Mass	Thermogravimetric Analysis	TGA
Difference temperature	Differential Thermal Analysis	DTA
Alternating temperature	Alternating Current Calorimetry	ACC
Enthalpy	Differential Scanning Calorimetry	DSC
Length, volume	Dilatometry	
Deformation	Thermomechanical Analysis	TMA
	Dynamic Mechanical Analysis	DMA
Electric current	Thermostimulated Current	TSC
Luminescence	Thermoluminescence	TL

2.3.4 Thermogravimetric analysis (TGA)

Thermogravimetry is the branch of TA which examines the mass change of a sample as a function of temperature in the scanning mode or as a function of time in the isothermal mode. Not all thermal events bring about the change of the mass of the sample (e.g. melting, crystallization or glass transition), but there are some very important exceptions which include desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition. TG is used to characterize the decomposition and thermal stability of materials under a variety of conditions and to examine the kinetics of the physico-chemical processes occurring in the sample. The mass change characteristics of a material are strongly dependent on the experimental conditions employed. Factors such as sample mass, volume and physical form, the shape and the nature of the sample holder, the nature and pressure of the atmosphere in the sample chamber and the scanning rate all have important influences on the characteristics of the recorded TG curve. TG curves are normally plotted with the mass change (Δm) expressed as a percentage on the vertical axis and temperature (T) or time (t) on the horizontal axis. [52]

The most frequent use of TG is in determining the material thermal stability, relative volatility and also process kinetics. Coupling of TG and DTA enables some other characteristics to be measured and assessed, for example temperatures of first and second order phase transitions, namely changes of crystal modification, melting, sublimation, boiling or glass transition. [54]

The size and shape of crucible can appreciably influence the course of the TG curve. If the volatility of a substance is followed under isothermal conditions, the rate of volatilization depends on the shape of the crucible, as this determines the surface area

of the sample. The size of the crucible and the amount of the sample play a major role as they affect the method of heating and also the rate of diffusion of gases liberated from the sample. From this point of view, it appears that the best results are obtained with crucibles in the shape of a small shallow dish permitting homogeneous heating of the sample over a relatively large surface area. It is important to take into account other properties of the investigated substance such as decrepitation or foaming during the heating. If any of these occurs, the crucible described is unsuitable and cylindrical, conical or closed crucible should be used. The shallow crucible is also unsuitable in cases where a gas stream is used, as aerodynamic effects cause complications. Another effect that can influence the course of measurements is the construction of the crucible. The effect of the material is negligible if Pt, Au or Al crucibles are used. Sample properties influence mostly the course of measurements. One of the most important parameters is its quantity which influence the time required for the reaction to take place throughout the whole volume of the sample. Thermogravimetry requires heating and weighing of the sample simultaneously. This means there must be no contact between the sample and the furnace wall. Thus the problem of heating the sample is primarily the one of heat transfer. This is controlled by a number of factors, e.g. the properties, size and nature of the sample, and the enthalpy change of any reactions undergone by the sample. A temperature gradient is formed between the sample and the furnace wall, which introduces errors into measurement of the sample temperature and determination of the range of temperature reactions being investigated. A temperature gradient may also be formed inside the sample. The temperature difference between the furnace wall and the sample is affected primarily by sample properties, the enthalpy change of any reactions it undergoes, and the rate of temperature increase. The atmosphere in the reaction space of the furnace is also very important factor influencing the measurements. If the sample is dried or decomposed in air, the atmosphere in the vicinity of the crucible is continually changing owing to liberation of gaseous products or reaction of the sample with the original atmosphere. The question of the sensitivity of the weighing mechanism and the recording system is closely related to the amount of sample used. The mass of the sample may exert an appreciable influence on the course of the reactions being followed. Often, in order to follow the reaction under required conditions, as low the sample weight as possible is taken. Great attention must always be devoted to long-term stability of the thermobalance, periodic calibration of weight ranges, quality and strength of the record, etc. These factors will introduce errors into the TG measurement mainly in prolonged experiment and at high sensitivities of measurement. [55]

2.3.5 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry observes the enthalpy changes. DSC measures the rate of heat flow and compares its difference between the sample and the reference material. [55] The measurement of sample and reference is carried out in two crucibles. A substance, which doesn't undergo any thermal changes in the temperature range of the measurement, serves as a reference. Most frequently an empty crucible is used. [56]

Based on mechanism of operation, DSC can be classified into two types:

- Heat-Flux DSC and
- Power-Compensated DSC.

2.3.5.1 Heat-Flux DSC

This type (Fig. 9) concerns measurement of the temperature difference between the sample and reference as a function of temperature or time, under controlled temperature conditions. The temperature difference is proportional to the change in the heat flux (energy input per unit time). The thermocouples are attached to the base of the sample and reference holders. A second series of thermocouples measures the temperature of the furnace and of the heat-sensitive plate. The variation in heat flux causes an incremental temperature difference to be measured between the heat-sensitive plate and the furnace. The heat capacity of the heat-sensitive plate as a function of temperature by adiabatic calorimetry during the manufacturing process, allowing an estimation of the enthalpy of transition to be made from the incremental temperature fluctuation. [52]

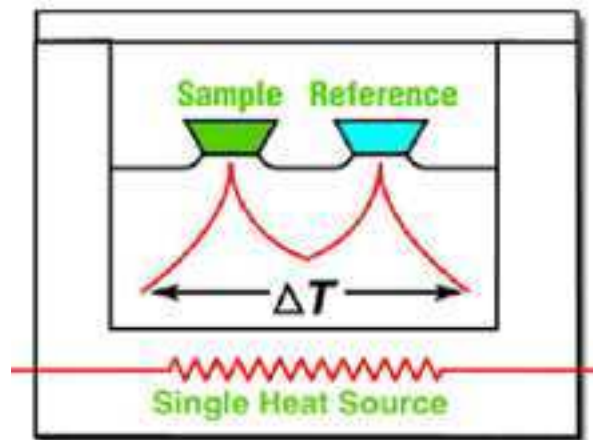


Fig. 9 Block diagram of heat-flux DSC

2.3.5.2 Power-Compensated DSC

This type (Fig. 10) is informally called the true DSC. The sample and reference holders are individually equipped with a resistance sensor, which measures the temperature of the base of the holder, and a resistance heater. If a temperature difference is detected between the sample and reference, due to a phase change in the sample, energy is supplied until the temperature difference is less than a threshold value, typically < 0.01 K. The energy input per unit time is recorded as a function of temperature or time. A simplified consideration of the thermal properties of this configuration shows that the energy input is proportional to the heat capacity of the sample. [52]

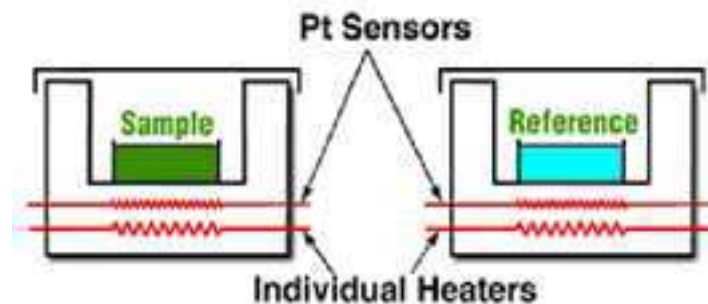


Fig. 10 Block diagram of power-compensated DSC

2.3.5.3 Advantages and drawbacks of DSC

Both types of DSC have their advantages and drawbacks but the final result is the same, they both provide same information.

The benefit of heat-flux DSC is the possibility of measuring larger-volume samples. This type has very high sensitivity and the maximal temperature may reach values over 900 °C. The disadvantage is low heating and cooling rate when using older types of instruments.

Principal advantage of power compensation DSC is no need of calibration, because the heat is obtained directly from electric energy supplied to the sample or reference (calibration is still needed to convert this energy into effective units). High scanning rate can be obtained. The disadvantage of such arrangement is the necessity of extremely high sensitive electronic system and his susceptibility to fluctuating environment to avoid compensating effects, which are not caused by the sample. [57]

2.4 Reaction kinetics

Processes in condensed phase are extensively studied by thermoanalytical methods. Mechanisms of these processes are very often unknown or too complicated to be characterized by a simple kinetic model. They tend to occur in multiple steps that have different rates. To describe their kinetics, the methods based on the single-step approximation are often used, either the model-free or model-fitting ones.

2.4.1 Single-step approximation

It is generally recognized that the rate of the processes in condensed phase is a function of temperature and conversion:

$$\frac{d\alpha}{dt} = \Phi(T, \alpha). \quad (5)$$

The single-step kinetics approximation employs the assumption that the function Φ in Eq. (5) can be expressed as a product of two separable functions independent of each other, the first one, $k(T)$, depending solely on temperature T and the other one, $f(\alpha)$, depending solely on the conversion of the process, α :

$$\Phi(T, \alpha) = k(T)f(\alpha). \quad (6)$$

Combining Eqs (5) and (6), the rate of the complex multi-step condensed-state process can be formally described as

$$\frac{d\alpha}{dt} = k(T)f(\alpha). \quad (7)$$

Equation (7) is mostly called the general rate equation. It resembles a single-step kinetic equation, even though it is a representation of the kinetics of a complex condensed-phase process. The single-step approximation thus resides in substituting in generally complex set of kinetic equations by the sole single-step kinetic equation. This is the first attribute of the single-step approximation. The temperature function in Eq. (7) is mostly considered to be the rate constant and the conversion function is considered to reflect the mechanism of the process. [58] However, this interpretation may be incorrect. Since Eq. (7) is a mathematical formulation of the single-step approximation, the functions $k(T)$ and $f(\alpha)$ represent, in general, just the temperature and conversion components of the kinetic hypersurface. The kinetic hypersurface is a dependence of conversion as a function of time and temperature. [59] With only for few exceptions, the temperature function is expressed by the Arrhenius equation

$$k(T) = A \exp\left[-\frac{E}{RT}\right], \quad (8)$$

where A and E are considered the pre-exponential factor and the activation energy, respectively, T is the absolute temperature and R stands for the gas constant. It has been justified that, since $k(T)$ is not the rate constant and E cannot be mechanistically interpreted in the term of free energy barrier, there is no reason to be confined to the Arrhenius relationship and use of two non-Arrhenius temperature functions was suggested:

$$k(T) = AT^m \quad (9)$$

$$k(T) = Ae^{DT} \quad (10)$$

$$k(T) = \frac{\left(\ln \frac{T_{\infty}}{T_{\infty} - T} \right)^{\frac{1-a}{a}}}{(T_{\infty} - T)^a}, \quad (11)$$

where m , D , T_{∞} and a are parameters. A great advantage of the application of Eqs (9), (10) and (11) is that, in contrary to Eq. (8), the temperature integral can be expressed in a closed form. Unlike the temperature functions, there is a wide range of conversions applied. Practically every function is connected with a certain idea of reaction mechanism. [60],[61]

Thermoanalytical techniques (DSC, TGA) provide an aggregate, overlapped signal of all reaction steps occurring in the sample. For the same process and time-temperature regime, the values of conversions and reaction rates obtained by various thermoanalytical methods may be different. Hence, in the kinetic description of DSC experimental data, analysis of the rate and quantity of heat released/absorbed is done. Analogously, in the kinetic description of TGA data, rate and quantity of mass loss is analysed. [58]

2.4.2 Isoconversional methods

The isoconversional methods may be best known through their most popular representatives, the methods of Friedman; Ozawa, Flynn and Wall; and recently also Šimon. Interestingly, all three methods were conceived by the researchers working on thermal degradation of polymers and since then they have been mostly used in polymer kinetic studies. The isoconversional methods require performing a series of experiments at different temperature programs and yield the values of effective activation energy as a function of conversion. More often than not, the activation energy is found to vary with the extent of conversion. The full potential of the isoconversional methods has been appreciated as the analysis of the energy dependences was brought to the forefront and demonstrated that they can be used for exploring the mechanisms of processes and for predicting kinetics. These two features make a foundation of the isoconversional kinetic analysis or so-called “model-free kinetics”.

The principal idea of the isoconversional method is very simple, there are only two basic assumptions:

- (I) Rates of the processes in the condensed state is generally a function of temperature and conversion, Eqs (5)–(7). The main implication of Eq. (7) is that the conversion function $f(\alpha)$ at a fixed value of α holds for any temperature or temperature regime, i.e. the mechanism of the process is solely a function of conversion, not a function of temperature.
- (II) The activation parameters are obtained from a set of kinetic runs from the dependences of time *vs.* temperature (for isothermal measurements), temperature *vs.* heating rate (for integral and incremental methods with linear heating rate) or from reaction rate *vs.* temperature (for the differential Friedman method). The evaluation is carried out at fixed conversion.

2.4.2.1 Isothermal isoconversional method

For isothermal conditions, the rate constant is a constant. After separation of variables, Eq. (7) can be rearranged into the form:

$$\int_0^{\alpha} \frac{d\alpha}{f(\alpha)} = k \int_0^{t_{\alpha}} dt, \quad (12)$$

where t_α is the time at which the conversion α is reached. If a primitive function of the integral at the left side of Eq. (9) is denoted as F , one can get:

$$F(\alpha) - F(0) = kt_\alpha \Rightarrow t_\alpha = \frac{F(\alpha) - F(0)}{k}. \quad (13)$$

The temperature dependence of the rate constant k is usually expressed by the Arrhenius equation, Eq. (8). Combination of Eqs (8) and (13) gives the expression for the time t :

$$t_\alpha = \frac{1}{A_\alpha \exp[-B_\alpha/T]}, \quad (14)$$

where the activation parameters A_α and B_α are given as

$$A_\alpha = \frac{A}{F(\alpha) - F(0)}. \quad (15)$$

$$B_\alpha = E/R$$

The subscript α at A_α , B_α and t_α designates the values related to the fixed value of conversion. Hence, the parameters A_α and B_α can be obtained from a series of isothermal measurements, even by direct treatment using Eq. (14) or from its logarithmic transformation leading to the linear dependence $\ln t_\alpha = f(1/T)$.

2.4.2.2 Isoconversional methods at linear heating

(I) Integral methods

For non-isothermal conditions, combination of Eqs (7) and (8) gives:

$$\frac{d\alpha}{dt} = kf(\alpha) = A \exp\left[-\frac{E}{RT}\right] f(\alpha). \quad (16)$$

After separation of variables and integration, the latter equation gives the result:

$$\int_0^\alpha \frac{d\alpha}{f(\alpha)} = A \int_0^{t_\alpha} \exp\left[-\frac{E}{RT}\right] dt. \quad (17)$$

Taking into account Eqs (13) and (15) can be rewritten as:

$$1 = A_\alpha \int_0^{t_\alpha} \exp\left[-\frac{B_\alpha}{T}\right] dt. \quad (18)$$

Equation (18) should hold for any temperature regime and enables to calculate the time at which fixed conversion t_α is reached. For the linear heating programme, the furnace temperature can be expressed as

$$T_f = T_0 + \beta t, \quad (19)$$

where T_f is the furnace temperature, T_0 is the starting temperature of the measurement and β stands for the heating rate. It is mostly assumed that the temperature of the sample, T , equals that of the furnace ($T = T_f$). From Eqs (18) and (19) can thus be obtained:

$$\beta = A_\alpha \int_0^{T_\alpha} \exp\left[-\frac{B_\alpha}{T}\right] dT, \quad (20)$$

where T_α is the temperature at which the fixed conversion α is reached. The lower integration limit in Eq. (20) is usually set $T_0 = 0$ K since there is no process observed to occur in the sample at the starting temperature of the experiment. The temperature integral at the right side of Eq. (20) cannot be expressed in a closed form. Using the Doyle and Coats–Redfern approximations for the temperature integral in Eq. (20), the Flynn-Wall-Ozawa (FWO) and Kissinger-Akahira-Sunose (KAS) methods, respectively, can be derived. The kinetic

parameters are usually obtained from a set of isoconversional temperatures for various heating rates, from the linearized transformation of Eq. (20):

$$\ln\left(\frac{\beta}{T_a^a}\right) = f\left(\frac{1}{T_a}\right), \quad (21)$$

where $a = 0$ for FWO method and $a = 2$ for KAS method. From Eq. (20) the non-linear isoconversional method can be derived.

(II) Differential method

For the linear temperature program, from Eq. (16) one can obtain:

$$\left(\frac{d\alpha}{dt}\right)_\alpha = \left(\beta \frac{d\alpha}{dT}\right)_\alpha = [A_k f(\alpha)] \exp\left[-\frac{E}{RT}\right] = A_\alpha \exp\left[-\frac{B_\alpha}{T_a}\right]. \quad (22)$$

Equation (22) is generally used after a logarithmic transformation:

$$\ln\left(\beta \frac{d\alpha}{dT}\right)_\alpha = \ln A_\alpha - \frac{B_\alpha}{T}. \quad (23)$$

From Eq. (23) it follows that the dependence of $\ln(\beta d\alpha/dT) = f(1/T)$ should be linear. The method represented by Eq. (23) is named after Friedman.

(III) Incremental method

Since the Friedman method employs instantaneous rate values, it is very sensitive to experimental noise and tends to be numerically unstable. Using incremental methods can obviate this trouble. An incremental integral isoconversional method has been recently suggested. For the linear heating program, if Eq. (17) is integrated within the conversion increment $\alpha_1 - \alpha_2$, one can arrive at:

$$\beta = \frac{A}{F(\alpha_2) - F(\alpha_1)} \int_{T_1}^{T_2} \exp\left[-\frac{B_\alpha}{T}\right] dT = A_\alpha \int_{T_1}^{T_2} \exp\left[-\frac{B_\alpha}{T}\right] dT. \quad (24)$$

In this case, the definition of the parameter A_α is obvious from Eq. (24). Due to the integration, the influence of experimental noise is eliminated to a great extent. For an infinitely small increment, Eq. (24) degenerates into Eq. (22). If it is set $\alpha_1 = 0$ at $T_1 = 0$, Eq. (24) degenerates into Eq. (20). From Eq. (24), the incremental isoconversional method to account for variation in the activation energy can be derived. [62]

2.4.3 Kinetic predictions – Induction period

Predictions are among the most important practical features of kinetic analysis. They are widely used to evaluate the kinetic behaviour of materials beyond the temperature regions of experimental measurements. For instance, thermal stability can be estimated as the time to reach a certain extent of conversion at a given temperature. Kinetic predictions of this type can be easily accomplished by using the induction period dependence evaluated by an isoconversional method. [63]

Since Eq. (7) is general, it is assumed that it describes also the kinetics of the reactions occurring during the induction period (IP). The existence of these reactions is not detected by the experimental technique used; however they have to take place as a preparatory stage preceding the main process (oxidation, vulcanization, degradation, etc.). For the assessment, the conversion α in Eq. (17) is the conversion of the reactions occurring during IP

corresponding to the end of IP, i.e. to the start of the main process detected by the apparatus, and t_α is the length of IP. Further it is assumed that the conversion α is the same for any temperature. Following the Eq. (18) the temperature dependence of IP can be expressed as:

$$t_i = A \exp(B/T). \quad (25)$$

T_α in Eq. (20) is then the temperature of the end of induction period, i.e. the beginning of the degradation process registered as weight loss (in case of TGA). As Eq. (20) indicates, when increasing the rate of heating, the degradation temperature also increases. [64]

The experimental use of the isoconversional predictive procedure is huge. They can be applied to determine the stability of pharmaceuticals, antioxidant activities of various types of materials, stabilization effects or residual stability and protection factors assessment [65–73] but they have already been successfully employed also in studying HS. [74],[75]

2.5 Thermal analysis of humified substrata

Used in combination with XRD, IR and other chemical analyses, thermal analysis techniques are invaluable tools in mineralogy, geology, pedology, soil chemistry, and physics. They allow the qualitative identification and quantitative analysis of clays and many minerals, and also the identification of the different forms of water in soils, oxidation of all forms of organic matter and inorganic materials, phase transitions, etc. The sensitivity of the DTA–DSC methods makes it possible to detect the presence of minerals at the limit of detection of XRD (e.g. goethite, gibbsite at concentration less than 0.25%, substances with short distance atomic arrangement) in clays and soils. All the techniques used enable the balance of the mineral transformations to be established as a function of different geochemical processes both in a weathering profile and in a topographic sequence. Reasonably precise quantitative analysis is possible of hydroxides and oxyhydroxides of iron and aluminium, as well as of clays and particularly of 1:1 kaolinite and halloysite. Continental sediments containing organic complexes can be studied by controlled oxidizing or non-oxidizing pyrolysis, and the evolved gases are analyzed by FTIR spectroscopy. DSC can quantify enthalpy changes during dehydration, dehydroxylation and other forms of structural analysis of evolved gases by FTIR or mass spectrometry. It is possible to calculate the exact chemical transformations undergone by the sample. Thermal analysis is particularly useful for the study of soil genesis, the study of soils rich in *para*-crystalline compounds (e.g. andosols, histosols) and characterization of the evolution of compounds with short-distance atomic arrangement that cannot be directly analyzed by XRD. In certain cases it is possible to use the thermogravimetric method in specific surfaces of clays (internal and external surfaces) by impregnation of the sample with a monomolecular layer of an organic material (e.g. the EGME method) or of a gas (e.g. the BET method). Instrumental thermal dilatometric methods developed by ceramists can be used instead of manual measurements of contraction–dilatation (coefficient of linear extension). [76]

In the next paragraphs there are several examples of use of thermoanalytical methods, such as thermogravimetry (TG), differential scanning calorimetry (DSC) or differential thermal analysis (DTA) in elucidation of structural and some other, basic features of decayed natural heterogeneous organic matter or materials derived.

Already in 1974 Petrosyan et al. found that complex method of simultaneous evaluation of temperature changes (dT), enthalpy (DTA), weight loss (TG) and the rate of weight loss ($DrTGA$) in combination with other methods gives broader possibilities in studying the mineralogical composition of fine-grained fractions of soils and the physical and chemical properties of humic compounds. As an independent method it permits 1) to give a quantitative evaluation of adsorbed water and interlayer water content, residual humus or non-silicate oxides in fine-grained fractions of soils; and 2) to show the binary composition of humic molecules, the relation of separate atomic clusters and their thermal stability. It was concluded that it is possible to use this method to reveal differences within the same group of humus acids of genetically close types of soils and even of soils from various horizons of the same soil profile, besides its conventional use for structural chemical analysis. Great potential of this tool was seen in the biogeochemistry of humus formation, in the evaluation

of the role of humus compounds in soil formation and of the migration of products of soil formation along the soil profile. [77]

There is a research series of Schaumann on humic matter and TA. The first work focused on glass transitions of purified humic acids. Samples of sandy forest soil were scanned by DSC and the glass transition temperature was 79 °C for air-dried samples and 77 °C for pre-moistened samples. In addition to the glass transition, an exothermic peak around 30 °C was detected in pre-moistened samples. This process corresponds to loss of water of soil organic matter. However, the nature of this process is not yet well understood. This study showed that the macromolecular behaviour of SOM, as was indicated by DSC, reacts to the moisture state of soil organic matter. [78] In another study, hydration of a peat sample was investigated with DSC in terms of glass transition behaviour and the formation of freezable and unfreezable water. Special attention was drawn to the development of these characteristics in the course of hydration and to plasticizing and antiplasticizing effects of water. [79] The study of Hurrass and Schaumann has shown that the glass transition behaviour of SOM in unfractionated soil samples is strongly influenced by time and thermal history. The shift of glass transition-like step transitions to higher temperatures during sample storage below T_g points to an aging process comparable to synthetic polymers. This process is distinctly affected by alterations of the moisture status of the soil samples. A comprehensive understanding of the ageing process of SOM most probably represents an important key to predict the ageing of contaminants in soil. [80]

Gibbs et al. used DTA to obtain thermo-analytical curves over the temperature range 50–1000 °C for 66 soil types from Barbados and nearby areas. Endothermic and exothermic peak areas under these curves were determined and used as the basis of a system derived for identifying soil types. In this system, curves for different soil types were unique with respect to occurrence of endothermic and exothermic peaks and the magnitude of their corresponding peak areas. On re-examination of a selected number of soil samples after 18 months storage, each soil showed the same number of peaks at the same temperature but with altered intensities and in most cases a reduction in peak size, changes consistent with desiccation and spontaneous decomposition. [81] Gibbs also tried to estimate relative fertility of soils using TG in the range 50–1000 °C. The relative thermal activity of each soil sample correlated significantly with CEC and clay-related exchange capacity (correlation coefficients ranged from 0.44 to 0.7). On the basis of TG, intra-specific variations within soil associations were found to be as significant as inter-specific variations with some of the least as well as the most fertile soils. [82]

DSC was used by Leifeld et al. to infer thermal properties of soil organic matter in long-term static fertilization experiment, which has been established in 1902. Four treatments (null – N, change from null to manuring in 1978 – NM, change from manuring to null in 1978 – MN, and permanent manure and mineral fertilization since 1902 – M) were sampled in 2004. Soil organic carbon contents were highest for M, lowest for N and similar for MN and NM. Three heat flow peaks at around 354 °C, 430 °C and 520 °C, which were assigned to as thermally labile and stable SOM and combustion residues from lignin, respectively, characterized all DSC thermograms. DSC peak temperatures were relatively constant among treatments, but peak heights normalized to the organic C content of the soil were significantly different for

labile and stable SOM. Labile C was higher for $M > MN = NM = N$, and stable C decreased in the order $N = NM > MN = M$, showing that agricultural depletion of SOM increases the share of thermally stable C. Lignite-derived C was not affected by management, suggesting a homogeneous deposition across treatments. [83]

DeLapp et al. in their works focused on experimental investigation of thermodynamic properties of natural organic matter (NOM). Whether the fractions of NOM possess the same thermodynamic characteristics as the whole NOM from which they are derived. Advanced thermal characterization techniques were employed to quantify thermal expansion coefficients (α), constant-pressure specific heat capacities (C_p), and thermal transition temperatures (T_i) of several aquatic- and terrestrial-derived NOM. For the first time, glass transition behaviour was reported for a series of NOM fractions derived from the same whole aquatic or terrestrial source, including humic acid-, fulvic acid-, and carbohydrate-based NOM, and terrestrial humin. TMA, DSC, and TMDSC measurements revealed T_i ranging from -87°C for a terrestrial carbohydrate fraction to 62°C for the humin fraction. The NOM generally followed a trend of increasing T_i from carbohydrate to fulvic acid to humic acid to humin, and greater T_i associated with terrestrial fractions relative to aquatic fractions, similar to that expected for macromolecules possessing greater rigidity and larger molecular weight. Many of the NOM samples also possessed evidence of multiple transitions, similar to β and α transitions of synthetic macromolecules. The presence of multiple transitions in fractionated NOM, however, was not necessarily reflected in whole NOM, suggesting other potential influences in the thermal behaviour of the whole NOM relative to fractionated NOM. [84],[85]

The group of Dell'Abate found a possibility to use thermal analysis for quick characterisation of chemical changes in the organic matter of composted materials. Nine samples were taken at progressive stages of maturation from the same trench of a fully automated composting plant. DSC and TG were simultaneously performed in static air conditions on whole ground composts. Progressive stages in the composting process yielded samples whose DSC traces revealed appreciable modifications in thermal patterns that agreed well with quantitative data obtained from TG measurements. The ratio between the mass losses associated with two main exothermic reactions showed a good sensitivity in detecting the changes in the level of stabilization during the composting process. According to these results, thermal methods are valid and comparative in evaluating the level of bio-transformation of materials during a composting process. [86]

Buurman and Aran investigated decay of organic matter from number of podzol profiles on wind-blown sands by incubation and thermal analysis. Decomposition rates obtained by incubation were compared with temperature maxima of thermal degradation. For all investigated horizons, there was a general decrease of the rate of microbial decomposition with a shift of the first thermal oxidation maximum towards higher temperatures. This suggests that thermal characteristics of soil organic matter are related to decomposability by microbes and that they might be used to define organic matter quality in specific cases. [87]

Buurman et al. present in their study that the thermal stability of humic substances depends not only on the counter-ion neutralizing the negative charges of the most acidic functional groups, but also on the organic compounds which may come in contact with humus. In the case of sodium-humates, the thermal stability of humic conformations is increased substantially when organic solvents such as methanol or small organic acids enhance the hydrophobic forces holding the humic molecules together. The intermolecular linking between small humic molecules provided by divalent and trivalent cations such as in Ca- and Al-humates increasingly reduces the effect of organic solvents and the relative enhancement of thermal stability. The results suggest that an additional mechanism of humus stabilization in soil may be hypothesized besides the formation of complexes with di- and tri-valent cations. Humus stabilization against microbial degradation may also occur by a progressive, non-specific hydrophobic aggregation induced by low-molecular-weight organic acids exuded by plant roots. [88]

Rosa et al. made an investigation of the influence of alkaline extraction on the characteristics of humic substances extracted from Brazilian soil samples. Humic substances from seven different soils samples collected in Brazil were extracted using the procedure recommended by the IHSS. Soils, HS and humins were characterized by TG and DTA, which revealed a difference between the content and structural characteristics of organic matter present in HS and humin fractions in relation to their soils. The results indicated that alkaline extraction alters the characteristics of humic substances during the extraction process, underlining the importance of developing methodologies and analytical procedures that allow organic matter in soils to be studied without extracting it. [89]

Simultaneous TG and DTA of different lake aquatic humic-solute fractions were investigated under atmospheric temperature and pressure by Peuravuori et al. The samples were subjected for the destruction of organic matter within the 105–600 °C range at a rate of 5 °C/min. For each different humic-solute fraction, four-to-five partially overlapping thermal degradation stages were obtained from the shapes of the TG curves. A first-order approximation was applied to estimate the energy of activation for different multi-step degradation stages. A powerful reaction took place at 370–500 °C for each acidic humic-solute fraction which indicated the decomposition of a very stable structural nucleus with relatively strong bond energies. The results of TG-DTA demonstrated that the combination of the four diverse humic-solute pieces, isolated/fractionated by the XAD™ technique in strongly acidic conditions, consisted of structural subunits the behaviour of which resembled that of the bulk of humic solutes isolated by the DEAE procedure. Apart from this, the diverse humic-solute fractions do not occur wholly independently in the bulk but are, to some extent, aggregated via different bondings. [90]

Kučerík et al. studied thermo-oxidative behaviour of lignite humic fractions obtained by sequential extraction at different pH values. The aromaticity degree has proven to play a certain role in HS stability/lability. Moreover, higher degree of aliphaticity observed at samples extracted at lower pH did not show dependency on the peak temperature of the first decomposition step associated with decomposition of aliphatic moieties and functional groups. On the other hand, such dependency was observed in higher temperature range where destruction of aromatic structures took part and lower C/H degree of HA samples

shifted maxima to higher temperatures. [91] Further study evaluated changes of onset temperatures. They served as a value of stability of the influence of model organic compounds on HA. Moreover, ratio of mass losses recorded at high and low temperatures (degree of apparent aromaticity) determined the value of affliction. Surprisingly, such parameter reflects more the qualitative than quantitative relationship between biodegradable and humified parts. [92] The mechanisms of processes in condensed phase are very often unknown or too complicated to be characterized by a simple kinetic model. They tend to occur in multiple steps that have different rates. To describe their kinetics, the methods based on the single-step approximation are often used, either model-free or model-fitting ones. Isoconversional methods offer uncomplicated approach to stability evaluation without a deeper insight into the mechanism of the process under study and are especially suitable for kinetic predictions. They were employed several times during the study of humic substances. In [93] the values of induction period of oxidative decomposition of HAs gave information on stability of humic matter at elevated temperatures. These values were believed to correlate with organic matter stability even at lower temperatures. Following work assessed the influence of above mentioned compounds on HA stability using model-free kinetics. Comparison of obtained conversion times brought partial insight into the kinetics and consequently into the role of individual compounds in the thermo-oxidative degradation/stability of secondary HA structure. [94] Finally, Kučerík and Kislínger applied Arrhenius equation as temperature function in study of kinetic parameters of thermo-oxidative degradation of HAs. Their results indicate that some high correlation values between such parameters and elemental analysis may resemble the mechanism of degradation of certain elements *per se*. [95]

Barros recently published a review about the application of calorimetry to study soil properties and its metabolism. Although this research has increased slowly but continuously during the last 30 years, it is true that it has received poor attention. One reason for that could be the complexity of the soil and the difficulties to investigate it from a thermodynamic point of view. In his paper he demonstrates that calorimetry constitutes a very suitable method to face the main topics related to soil quality and activity. Very well known indicators used in soil research can be measured by different thermal and calorimetric methods such as DSC, TG and isothermal calorimetry (ITC). TG and DSC are both temperature scanning methods used for estimation of certain properties of the soil material such as organic matter, ignition temperature, humification index, quartz content and so on, whereas the study of the soil microbial metabolism is conducted under essentially isothermal conditions by ITC. In this review, the contributions of these techniques to different topics in soil research are described and their importance for the environmental concern is discussed in the light of this new era. [96]

A vast and probably one of the most important researches in this field was done by Siewert. Soil samples from various climatic regions and geologies from agriculture and forestry were used to recognize patterns and relationships between SOM components and their biological turnover processes. The results indicate the suitability of thermogravimetry for quantifying those fractions of SOM that are biologically degradable and humifiable. Simultaneously, a close relationship was established between these SOM components and the quantity of bound water. Soils that were used for agriculture showed characteristic deviations.

The incubation experiments indicated different soil respiration dynamics after rewetting air-dried samples. There was a clear dependency on the soil formation and the vegetation in natural ecological systems; however, the dependency was altered by agricultural soil utilization. Links between the results of both methods (thermogravimetry and incubation) were utilized for developing a method of thermogravimetric soil analysis (TGSA). The method makes it possible to measure the regional specifics of the SOM using large-scale uniformities, a differentiation between characteristics of SOM in natural and in utilized soils, as well as an approximation of soil properties (e.g. contents of C, N, clay, and carbonate). Evaluation of TGSA was favoured by low work expenditures. The results give evidence for the conclusiveness of the method. Several possibilities for expanding the current analysis were identified by examination of extreme locations. Restrictions regarding application of the method were limited to hydromorphic soils. [97],[98],[99]

3. AIM OF THE WORK

The number of works presented in previous paragraphs demonstrated the potential of thermal analysis in research of natural organic matter. However, an overwhelming majority of the applications published up to now used thermal analysis only for qualitative and quantitative determination of several parameters overlooking the great potential of thermal analysis in prediction and modelling of processes. Those are generally called “the accelerated tests”. It is obvious that the humified substrata develop in time, their chemistry and biochemistry being kinetic-dependent. Thus, the application of thermal analysis in natural organic matter research should be extended by this possibility.

As stated above, the correlation between soil respiration (proportional to the microbial activity) and mass loss in several temperature regions have already been described. However, up to now, there was not observed the attempt to use that knowledge for prediction of soil organic matter respiration. It is clear that in case of success, there can be suggested a novel, rapid and cheap method to model the behaviour of SOM under specific conditions and moreover, also the overall mechanisms of SOM degradation can be suggested. As a result, the notion can be used to improve the models of carbon and other elements flux and accumulation in nature having consequences for example in remediation and carbon sequestration techniques.

Humic substances play a key role in chemistry and biochemistry of soils and other humified substrata. Their chemistry is very complex and the understanding is still limited. As mentioned before, its quality more than quantity is important for stabilization of natural organic matter. However, the definition of that “quality” is still a problem. Recent results [92–94] showed that the information included in kinetics of degradation of humified materials is still not fully appreciated and exploited. Such approach has an advantage, especially in isoconversional kinetics, where the progress and “mechanisms” of degradation can be appreciated step-by-step and thus the role of a specific molecule in a mixture can be elucidated. With this respect, additional correlations are promising since this information is crucial in chemical-based soil organic matter carbon sequestration techniques. Last but not least, the transfer of such knowledge from pure humic substances to soil should bring information on the role of mineral part of soil which is frequently overlooked in this issue.

General objective of this work is to contribute to the development of the application of thermal analysis techniques in humus science, and to present the emerging application of TA for the characterization of soil organic matter. Understanding of the SOM properties and their relationship to results obtained by means of physical-chemical method should contribute to the contemporary problems associated with the gradual decrease of SOM quality and desertification.

4. EXPERIMENTAL PART

4.1 Humic substances

The samples of humic substances (fulvic and humic acids) were provided by RNDr. František Novák, CSc. (Biology Centre of the Academy of Sciences of the Czech Republic). Isolation of samples, NMR spectroscopy and elemental analysis were performed by the sample provider and/or related affiliations.

4.1.1 Isolation of humic and fulvic acids

Humic and fulvic acids were isolated from individual soil horizons of long-term research stands. Soil samples were air-dried and sieved (<2 mm). HA and FA were extracted using 0.1 M NaOH at a solution/soil ratio of 20:1 after soil decalcification by 0.1 M HCl. FA were isolated from the mother liquor after HA precipitation, using the standard method with a XAD 8 resin column. [100] The sodium humates and fulvates were converted into H⁺ form and freeze-dried.

4.1.2 ¹³C NMR spectroscopy

Quantitative ¹³C liquid-state nuclear magnetic resonance (¹³C LS NMR) spectra of HA and FA dissolved in 0.1 M NaOD were recorded employing a Bruker Avance DRX 500 spectrometer operating at 125.75 MHz using following parameters: recycle delay of 5 s, number of scans = 20000, and inverse gated decoupling. The free induction decay was processed with line broadening of 20 Hz. The ¹³C chemical shifts were referenced to tetramethylsilane; quantification was achieved by instrumental integration of NMR peaks. Local minimum at 108 ppm as the border between aromatic and O-alkyl regions was used. The chemical shift regions 0–45, 45–108, 108–160 and 160–220 ppm were assigned to alkyl carbon (C), O-alkyl C, aromatic C, and carboxylic C, respectively. [101] Aromaticity was calculated as the ratio between aromatic and the sum of aromatic and aliphatic (alkyl + O-alkyl) carbons. [102]

4.1.3 Thermal analysis

Thermogravimetric profiles of humic and fulvic acid samples were recorded employing TA Instruments TGA Q 5000 IR device. Approximately 2–3 mg of each sample were exposed to linear heating at 7 different heating rates (0.5; 1; 3; 5; 7; 10; 15 K min⁻¹) from room temperature up to 600°C. Dynamic oxidative atmosphere with air-flow 25 ml min⁻¹ was used. The furnace was temperature-calibrated using Curie point standards (perkalloy, nickel, iron).

4.1.4 Mathematical data processing

The thermogravimetric records showed two or three major steps of weight losses, the first one attributable to loss of water, whereas the others (in Fig. 11 marked within the vertical lines) to degradation of organic matter. The latter steps were used to assess the stability of examined samples and were considered together in order to cover the degradation process and integrate it as one. The beginning as well as the end of the process was determined by the 1st derivative of the TGA curve (DTG), which has the meaning of weight loss rate. The mass losses

obtained at different heating rates were recalculated to conversion intervals (0–100 %). From this interval, several conversions were selected (each 10 % step) and for those the conversion times were calculated out of the conversion temperatures (listed in Appendix I, two randomly selected heating rates). From the non-isothermal measurements, the kinetic parameters of four different temperature functions were assessed. The calculations described here were carried out by the software KINPAR [103] and Origin[®]. The conversion times (listed in Appendices II–V) were calculated using respective equations for 25 °C and 100 °C. Furthermore, DTG values (listed in Appendix VI) calculated for 10 °C temperature regions were used for correlations as well.

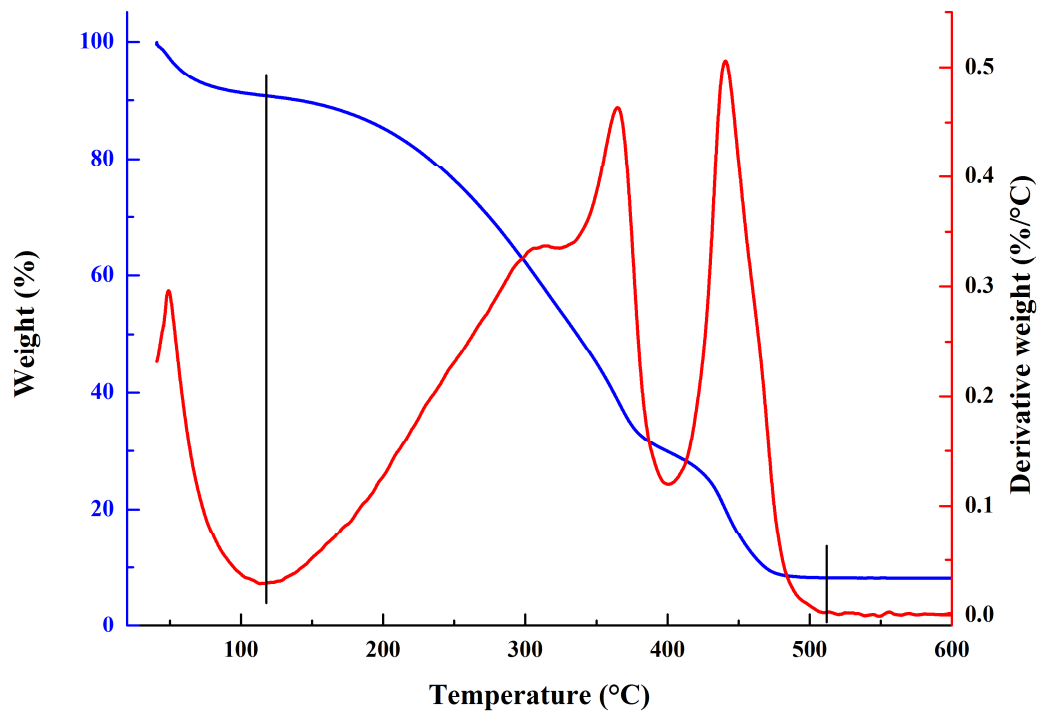


Fig. 11 *Thermogravimetric record of thermo-oxidative degradation of sample A65FK, heating rate 10 °C min⁻¹*

The correlation values between the conversion times or DTG values and values of primary characteristics of HS samples were calculated using the Pearson product-moment correlation coefficient, which is obtained by dividing the covariance of the two variables by the product of their standard deviations. The correlation coefficient R between two random variables X and Y with expected values μ_X and μ_Y and standard deviations σ_X and σ_Y is defined as:

$$R = \frac{\text{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E((X - \mu_X)(Y - \mu_Y))}{\sigma_X \sigma_Y}, \quad (26)$$

where E is the expected value operator and cov means covariance. Since $\mu_X = E(X)$, $\sigma_X^2 = E(X^2) - E^2(X)$ and likewise for Y , we may also write

$$R = \frac{E(XY) - E(X)E(Y)}{\sqrt{E(X^2) - E^2(X)}\sqrt{E(Y^2) - E^2(Y)}}. \quad (27)$$

The correlation is defined only if both of the standard deviations are finite and both of them are nonzero. It is a corollary of the Cauchy-Schwarz inequality that the correlation cannot exceed 1 in absolute value. The correlation is 1 in the case of an increasing linear relationship,

-1 in the case of a decreasing linear relationship, and some value in between in all other cases, indicating the degree of linear dependence between the variables. The closer the coefficient is to either -1 or 1, the stronger the correlation between the variables. If the variables are independent then the correlation is 0, but the converse is not true because the correlation coefficient detects only linear dependencies between two variables. If we suppose the random variable X is uniformly distributed on the interval from -1 to 1, and $Y=X^2$. Then Y is completely determined by X , so that X and Y are dependent, but their correlation is zero; they are uncorrelated. However, in the special case when X and Y are jointly normal, independence is equivalent to uncorrelatedness. A correlation between two variables is diluted in the presence of measurement error around estimates of one or both variables, in which case disattenuation provides a more accurate coefficient. [104] R was computed using Microsoft Excel[®].

4.2 Soil samples

Twelve soil samples were collected in the National Park Berchtesgaden (Germany) nearby Hirschbichl. Six sampling sites comprised alp pastures (the only two sites used for agriculture, i.e. affected by periodic human activity), ravine forests (both deciduous and coniferous) or a spikenard-meadow. Six samples were collected in the National Park Bayerischer Wald, around Zwieslerwaldhaus. The three sampling sites included only coniferous forests (spruce, fir). Four other samples originated from the National Park Hainich, Craula. Two deciduous forests and a moss-marsh area samples were collected. Six samples from four sampling sites were taken in the National Park Harz, nearby Schierke. The soils are of mixed forests and meadow cover. The last sampling location was a Biosphere Reservation Schorfheide-Chorin, nearby Chorin and Brodowin. Six samples from four sampling sites comprised mixed forests, meadow and an alder-tree-moor. The above mentioned locations were selected in order to obtain soils representing a variety of geological composition under native vegetation or extensive land uses (i.e. pastures and meadows). They serve as reference objects in order to detect intrinsic soil features and regulation processes. For an overview of sampling locations, see Figure 12, for more detailed characteristics of the locations, see Table 4 on page 66.



Fig. 12 *Sampling locations in Germany*

4.2.1 Sample collection and analyses

Each soil sampling was carried out in area of at least 30 m diameter. In order to obtain enough soil, approximately 60 cuts down to depth of 0–30 cm using a core-sampling device (Göttinger Bohrstock, Fig. 13) were performed. Where the soil horizons were clearly differentiable, B and/or C horizon samples were collected. Fresh soil samples were air-dried in the thinnest possible layers at a maximum of 25 °C and then sieved (mesh diameter 2 mm). Such treated samples were stored in a cold, dark and moisture-free place for following analyses and incubation experiments. A brief description of the site locations and soil samples is given in Table 5 (page 77).



Fig. 13 *Göttinger Bohrstock*

4.2.1.1 Basic soil analyses

Total organic carbon (C_{org}), nitrogen (N) and sulphur (S) contents were determined by combustion with an elemental analyzer (Elementar Vario EL III). The content of carbonates was detected by repeated elemental analysis before and after treatment of soils with HCl.

Particle size distribution was performed according to DIN/ISO 11277 [105] in order to assess the sand, silt and clay content. The field water retention capacity was determined on ceramic plates at pF 1.8 according to DIN/ISO 11274 [106].

4.2.1.2 Incubation experiment

To establish optimal conditions for microorganisms in the incubation experiments, the air-dried soil samples were re-moistened with distilled water to 80 % of their water retention capacity (pF 1.8). In order to start the experiment immediately after the addition of water, the soils were thoroughly mixed to prevent from gradients of water and soluble organic matter and to reduce variations in the CO₂ release between four replicate measurements. Measurement of CO₂ evolution began immediately after re-moistening. Basal respiration experiments were carried out using the Respicond device (A. Nordgren Innovations AB, Figure 14). Twenty grams of soil were placed in a vessel with a small container including 10 ml of 0.6 M KOH solution. Electrical conductivity of the solution was measured to monitor the CO₂ evolution during varying time intervals. The data collection frequency was continually adjusted during the course of the experiment. Initially, one mean value was recorded every 30 minutes. As the experiment progressed, the data collection frequency was reduced to one mean value every 12 hours per measuring channel. An additional data reduction was performed in the course of the data analysis. Therefore, the data presented here represent only a fraction of what was actually measured. The KOH solution was periodically changed to prevent its saturation during the experiment. The whole incubation experiment lasted for 80 days to detect the effects of both short- and long-term respiration.



Fig. 14 *Schematic and photograph of Respicond device*

4.2.1.3 Thermogravimetric analysis (TGA)

To ensure comparable sample preparation for TGA analyses, the air-dried and sieved samples were additionally conditioned for at least 7 days at 76 % relative humidity, which was maintained using saturated NaCl solution. The TGA was carried out using Mettler Toledo TGA/SDTA 851 device. The temperature program included heating of air-dried soils samples (typically 0.3–1.2 g) in 0.8 ml ceramic pans from 25 °C up to 950 °C with heating rate of 5 °C per minute. During analyses, the sample chamber was purged with an air stream of 76 %

relative humidity (RH valid for 25 °C) and flow rate of 200 ml min⁻¹. TGA data were collected every 4 seconds (i.e. every 0.3 °C temperature increase). Both incubated and non-incubated soil samples were analyzed by TGA. Prior to the TGA, the incubated soils were air-dried again. Derivation results were calculated from mass losses recorded in 10 °C temperature intervals.

4.2.2 Mathematical data processing

In order to describe the interrelations between results of both TGA and incubation experiments, correlation analyses using least square method linear regression were performed with Microsoft Excel[®], Statistica, and SPSS. Presented data are related to coefficients of determination with significant level of at least 95 % probability ($R^2 = 0.6$). The values of determination coefficients are not shown here to quote the closeness of interrelations or explain reasons for its existence. The aim was to use the comparison of correlations to reveal the most interesting time periods during incubation and temperature intervals in thermal analyses as a basic methodological step in the development of future experiments. Since the correlation above 650 °C gave results of low practical relevance and the thermally induced mass losses are very low, the presented data are reported only to this temperature limit.

5. RESULTS AND DISCUSSION

5.1 Humic samples

5.1.1 Thermogravimetric analysis following the isoconversional approach

Figure 15 demonstrates basic thermoanalytical feature, i.e. the same process occurs at higher temperature with increasing heating rate. This phenomenon can be better described using the derivative thermogravimetric curve (Fig. 16). The peak temperatures should correspond to the same stage of degradation, i.e. the degradation process is at the very same stage. E.g. the second major degradation step culminates (peak local maximum) at 395 °C for heating rate 0.5 °C min⁻¹, whereas for 15 °C min⁻¹ the peak temperature rises to 450 °C.

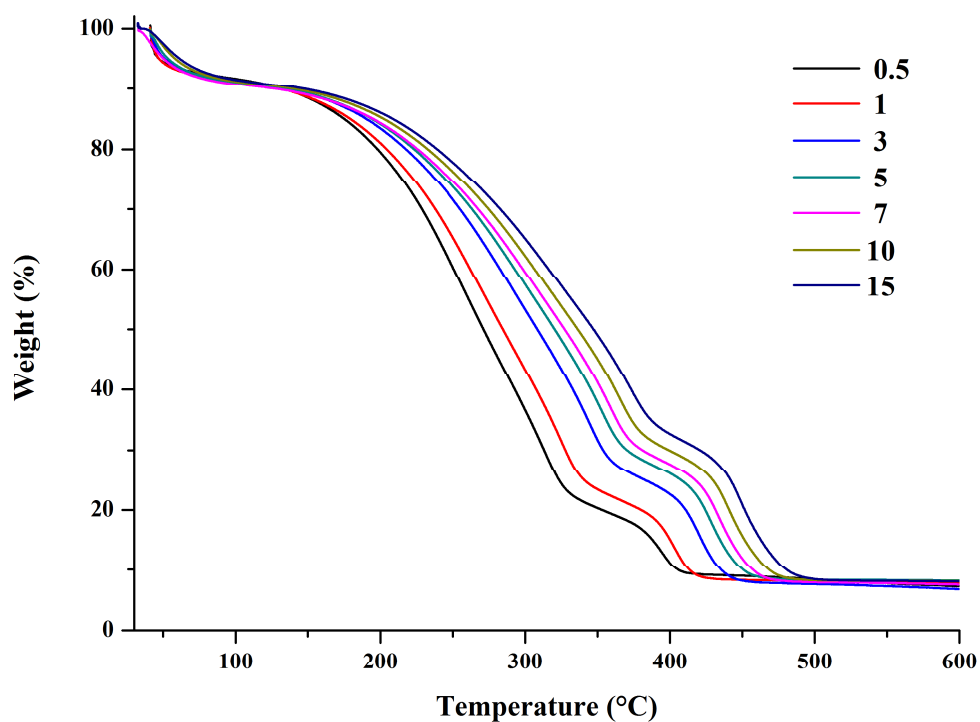


Fig. 15 TGA curves of sample A65FK, different heating rates (in °C min⁻¹)

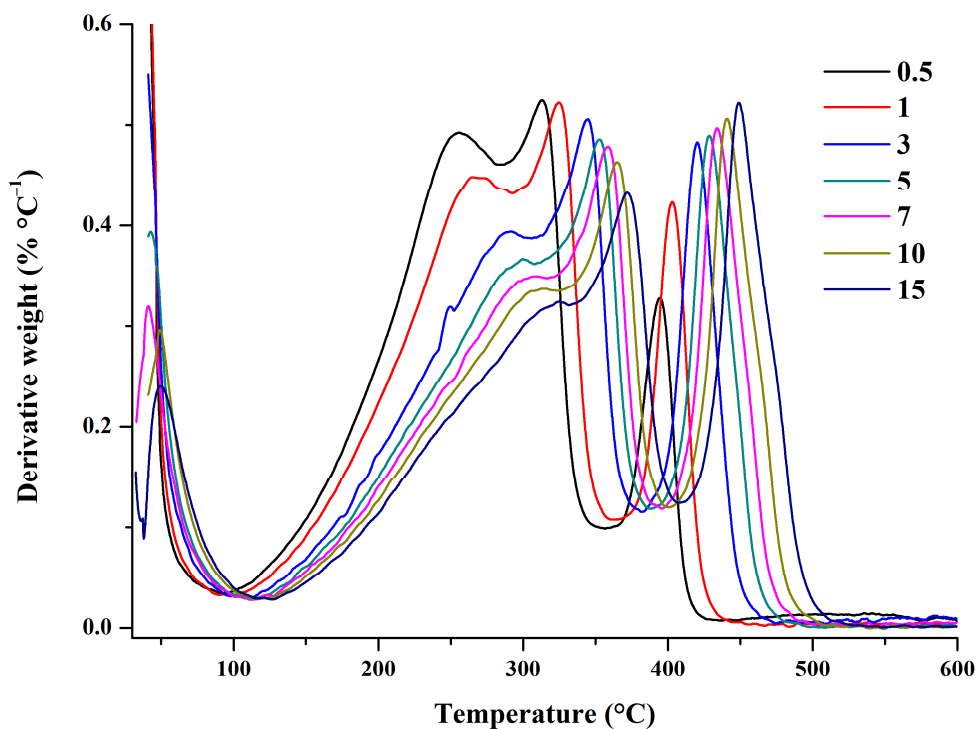


Fig. 16 DTG curves of sample A65FK, different heating rates (in °C min⁻¹)

The above mentioned facts are confirmed in Figure 17, where conversion is plotted against conversion temperature for seven different heating rates for randomly chosen sample. The curves do not cross each other during the whole course of degradation. This is also a condition for isoconversional methods to be used successfully.

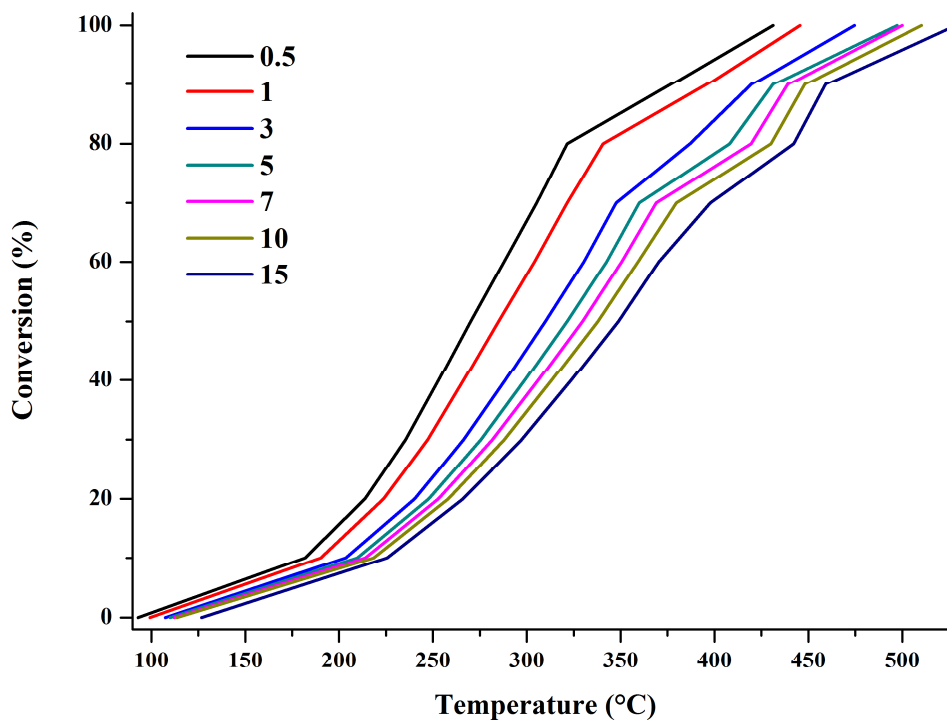


Fig. 17 Conversion curves of sample A65FK, different heating rates (in °C min⁻¹)

The progress of different stages of degradation can be visualized using just the conversion curves at specific heating rate β . But it must be taken into account that application of such approach can be biased by the non-ideal baseline feature. There can also arise some problems in choosing the right heating rate for the comparison among samples. It is generally known, the at lower heating rates some weak processes doesn't have to be necessarily recorded, on the other hand, when using high heating rates, some of the processes may overlap. Moreover, any slight non-ideality in the original TGA analysis can lead to distortion of further results. Anyway, the dependence of conversion temperatures on conversion (Fig. 18) was compared for heating rate $5\text{ }^{\circ}\text{C min}^{-1}$. It is obviously difficult, though possible, to draw conclusions out of this preview. E.g. sample B660FK (light blue line) has the lowest thermo-oxidative stability from 10 to 60 % of conversion, which then rapidly increases and at the end of the degradation process it is the most stable sample of them all, etc.

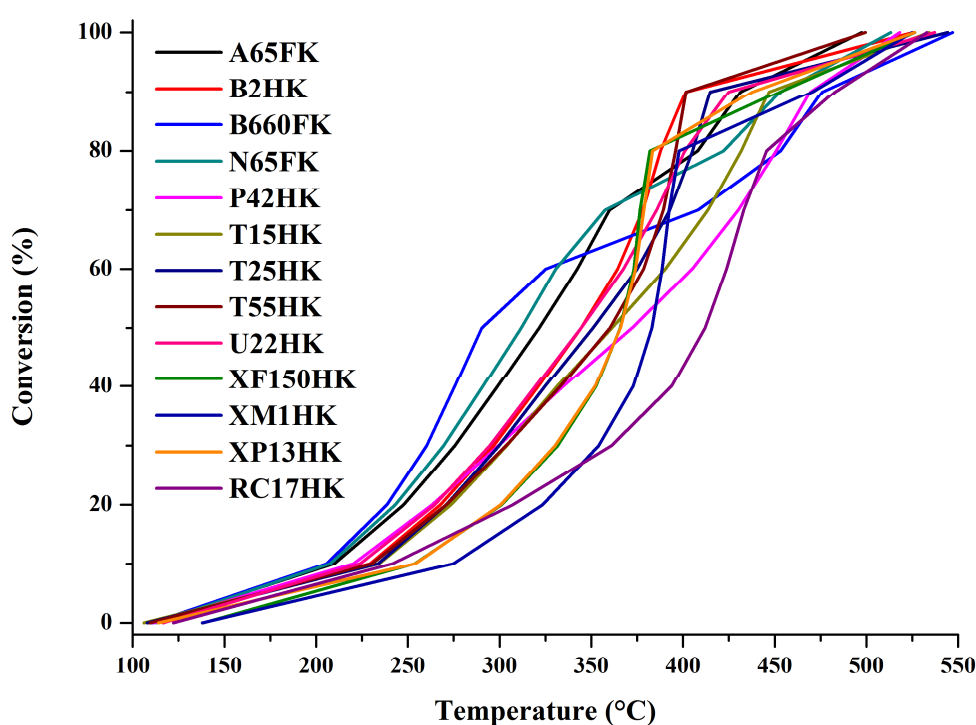


Fig. 18 Comparison of conversion curves of all humic samples, heating rate $5\text{ }^{\circ}\text{C min}^{-1}$

To avoid the problem described in the previous paragraph, it is better to use the isoconversional approach, where at least 3 different heating rates must be used, and therefore, more precise results should be reached.

According to [107], many problems can appear when data obtained at higher temperatures are extrapolated to lower ones. Comparison between conversion times of five randomly selected samples of humic substances samples extrapolated to $25\text{ }^{\circ}\text{C}$ (Fig. 19) and $100\text{ }^{\circ}\text{C}$ (Fig. 20) has proved that these data are proportional and thus any of these temperatures could have been used. For further calculations the temperature $25\text{ }^{\circ}\text{C}$ was adopted. Possible discrepancy emerging from use of different extrapolation temperature is in further correlations of minor importance.

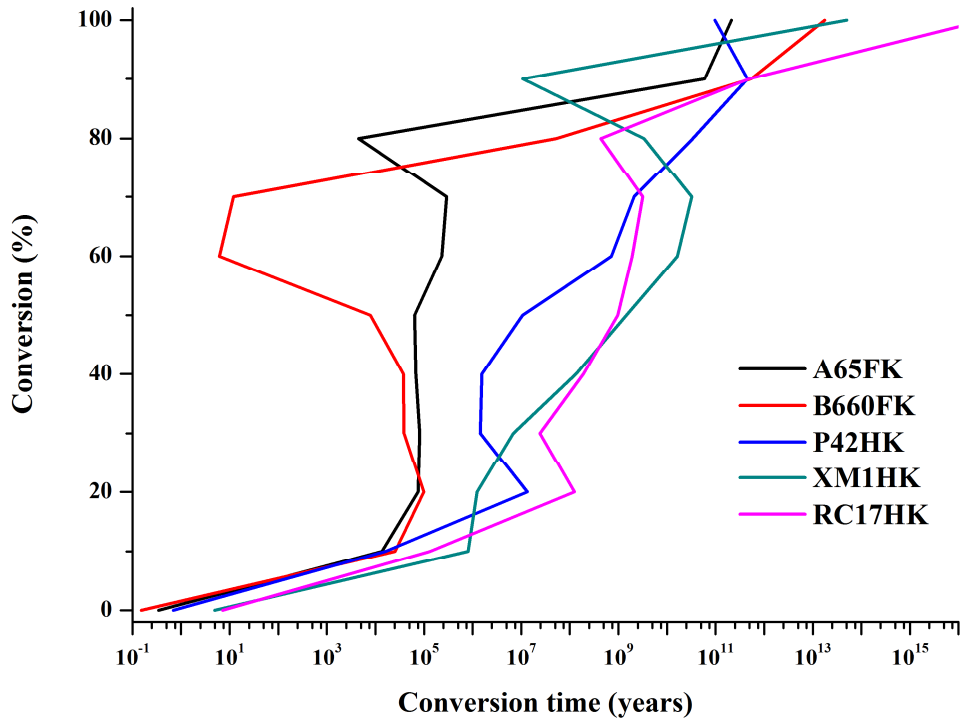


Fig. 19 *Dependence of conversion time on conversion, extrapolation to 25 °C*

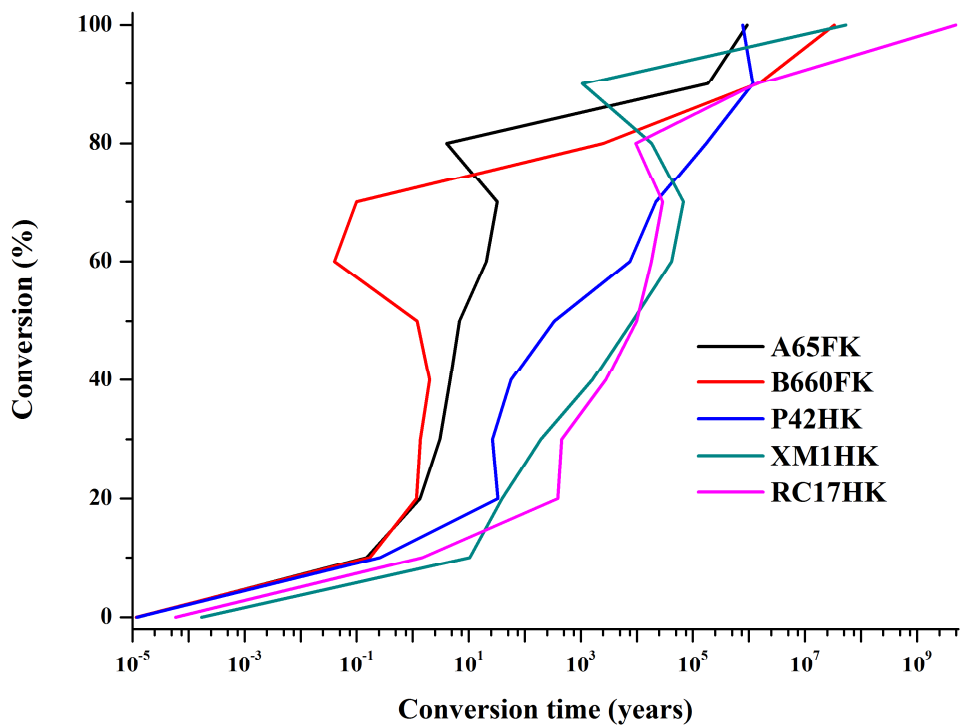


Fig. 20 *Dependence of conversion time on conversion, extrapolation to 100 °C*

In Appendix 1, isoconversional temperatures for two randomly selected heating rates for all humic samples are listed. Out of the isoconversional temperatures (using all previously mentioned heating rates), parameters of respective temperature functions were assessed employing the mathematical apparatus described in chapter 2.4. And finally, using the parameters, isoconversional times were determined. Calculated values of isoconversional times at specific conversions have meaning of stabilities of samples and their corresponding intermediates and reflect mutual differences in kinetics of degradation of measured samples. The aim was to avoid any transformation of experimental data by linearization of functional dependences to obtain reliable values of isoconversional time since such approach leads to deformation of distribution errors, heteroscedasticity and a shift in the position in minima of the sum squares between experimental and calculated values. [59]

5.1.2 Aromaticity degree in humic substances

As mentioned above, one of the traditional ways of classification of humic substances, the widely accepted one, divides them according to their solubility in acids and alkalis into three basic groups: fulvic acids, humic acids and humins. Though this approach may be regarded as rather forced it still resembles the nature and abundance of certain functional groups present in humic molecule. Occurrence and distribution of such groups use to be expressed as humification index. Such feature can be easily illustrated as ratio of present aromatic and aliphatic moieties, i.e. degree of aromaticity. This degree may be visualized as superficial indicator of stability. However, the aromatic C content is not direct sign of sample quality, its origin and nature has to be considered. [25] Still, in some simple cases, direct link between humification degree and the substrate ageing was found. [108] A good agreement between the aromaticity of Leonardite HA measured by NMR and thermal analysis data (thermogravimetry in particular) was already discovered. The statements were based on the extent of weight losses below 653 K in oxidative atmosphere; and the remaining residue at 1223 K under nitrogen atmosphere should indicate previously present aromatic structures. [109] Another study showed that elemental composition and parameters of thermal decomposition can be promising for classification of sedimentary HAs. The results infer that nitrogen content in HAs is inversely proportional to aromaticity of HA molecules and to their heat of combustion. [110] The understanding of fate and behaviour of aliphatic and/or aromatic parts of SOM plays an important role in several concrete environmental implications. Organic matter in sedimentary environments, that lack terrestrial organic matter additions, contains predominantly aliphatic moieties. It is important to consider the role of aliphatic structures in the sorption of nonionic, hydrophobic organic contaminants. Furthermore, aliphatic domains require more attention when considering the uptake, sequestration, and bioavailability of organic contaminants in soil and sedimentary environments simply due to their presence, persistence, and sorptive capability. [111]

The selection of humic substances samples was performed in order to cover the source, where HS are the most abundant and play an important role in nature (soils), having various chemical and physical characteristics suitable for this study. Thus, the primary objective of this part of study is to assess the stability of humic substances of various origin (therefore with different amounts of aromatic and also carboxylic carbon) using four distinct equations as temperature functions. Obtained data were correlated with the chemical composition of humic substances. The linear correlation between obtained isoconversional times and values

of primary properties of HS samples was evaluated using the Pearson product–moment correlation coefficient. The task is to elucidate the influence of aromatic and carboxylic C content on the thermo-oxidative stability of the samples. The discovery of the right approximation could contribute to modeling of C flux in natural systems. It is important to mention that the aim is not to establish any equations enabling calculation of NMR and elemental analysis data from thermogravimetric records but to shed light on the processes of thermo-oxidative degradation of humified pool of natural organic matter; to search for an interrelationships and trends between primary (analytical) and secondary (physical) structure of humic substances; and to search for the suitable temperature function providing data with the highest correlation coefficient.

5.1.3 Primary properties of humic samples

Chemical composition data are summarized in Table 3; possible sulphur traces in humic substances are included in oxygen content. The content of all kinds of C, H, N, O and ash is expressed as mass percentage. The highest carbon content possessed lignite and oxyhumolite humic acids XM1HK and XP13HK, respectively. Since their sources are low-rank coals, high amount of carbon (mostly conjugated and cyclic) is expected. On the other hand, the lowest C content can be observed at all three samples of fulvic acids, i.e. A65FK, B660FK and N65FK. This is in line with the distribution of oxygen in humic substances, which is connected with carboxylic groups, phenolic and alcoholic OH groups, ketones and chinones. That is the reason of highest content of O in fulvic acids samples, i.e. the most soluble humic substances fraction. Nitrogen content in humic substances is regularly attributed to hydrolysable amino and imino groups [25] and therefore the oldest (in comparison with soil samples) lignite and oxyhumolite samples possess relatively low values. Ash content is directly connected with purification procedure and due to harsh conditions during extraction (extreme pH values and ionic strength) can only hardly be linked with the origin of the samples. The content of both aromatic and carboxylic carbon in the samples of humic substances is coherent with above mentioned elemental character. High amount of conjugated and mainly aromatic carbon is present in brown coal samples, where at the same time low amount of aliphatic chains ending with carboxylic functional groups is expected. And vice versa for the fulvic acids samples. High amount of carboxylic C (responsible for solubility in aqueous media) corresponds to low amount of aromatic C.

Table 3 *Basic description of HS samples and their primary characteristics*

Sample	Locality, substrate	Characterization	HA/FA	aromatic C	carboxylic C	C	H	N	O	ash
				weight %						
A65FK	Ore Mts., Alžbětinka, mountain spruce forest	Soil, Podzol, Bh horizon	FA	43.0	24.4	44.6	3.8	1.3	42.2	8.2
N65FK	Ore Mts., Alžbětinka, mountain spruce forest	Soil, Podzol, Bh horizon	FA	42.9	37.6	44.5	3.9	1.1	42.8	7.8
T15HK	Bohemian Forest, Trojmezí, mountain spruce forest	Soil, Podzol, Ol horizon	HA	23.7	14.4	53.2	5.5	3.0	36.6	1.7
T25HK	Bohemian Forest, Trojmezí, mountain spruce forest	Soil, Podzol, Of horizon	HA	46.5	15.1	50.1	3.3	2.6	41.8	2.2
T55HK	Bohemian Forest, Trojmezí, mountain spruce forest	Soil, Podzol, A horizon	HA	37.0	21.0	48.0	2.4	2.5	44.3	2.8
P42HK	South Bohemia, Chelčice, abandoned field (12 years)	Soil, Cambisol, Ap horizon	HA	33.0	18.0	45.0	3.5	3.9	36.5	11.1
U22HK	South Bohemia, Chelčice, abandoned field (18 years)	Soil, Cambisol, A horizon	HA	45.0	11.2	48.9	3.0	2.9	41.2	4.0
B660FK	Bohemian Forest, Boubín, mountain spruce forest	Soil, Spodo-Dystric Cambisol, B horizon	FA	29.0	41.5	43.6	4.2	1.6	41.0	9.6
B2HK	Bohemian Forest, Boubín, mountain spruce forest	Soil, Spodo-Dystric Cambisol, Of horizon	HA	30.2	17.4	49.5	5.0	2.8	40.0	2.8
RC17HK	Russia, Samara, black soil	Soil, Mollisol, A horizon	HA	33.0	25.9	49.7	3.2	3.1	41.1	2.8
XF150HK	Fluka	Purified from commercial humate	HA	34.6	13.8	51.6	3.4	1.0	39.2	4.8
XM1HK	Oxyhumolite, Bílina	Mine Václav, 1989	HA	60.1	13.0	54.0	3.9	1.5	37.3	3.4
XP13HK	Lignite, Mikulčice	Mine Mír, 2003	HA	21.0	12.7	53.7	4.2	1.3	38.5	2.2

5.1.4 Relationship between isoconversional stability and primary characteristics

The tables in Appendices 1–4 report the calculated isoconversional times for the set of humic and fulvic acids using Arrhenius (Eq. (8)) and three non-Arrhenius equations (Eqs (9)–(11)) as temperature functions. The respective coefficients of mutual linear correlations are graphically illustrated in Figures 21–28.

Figures 21–24 display the level of correlations of thermo-oxidative stability of HS samples with the amount of aromatic and carboxylic carbon using Eqs (8)–(11), each figure for the respective equation. One can see that the trend in correlation coefficients through the whole range of conversion is in Figures 22–24 more or less the same; the only slight difference is the value of correlation level. The highest positive correlation was observed at 90 % of conversion for carboxylic carbon. On the other hand, together with 0 % conversion, these are the only values with positive correlation. As already hinted in [95], values on the edge of conversion interval may give erroneous results, since it may sometimes be difficult to assess the beginning and the end of degradation process. The middle part of conversion range exhibits quite stable correlation level, values are between 0.25 and 0.5. Even though these values doesn't mean any significant correlation, we still may speak about logical explanation, since the presence of higher amount of carboxylic carbon in natural organic matter causes its lability. Carboxylic groups being the ending functional groups present the most suitable degradation sites in oxidative atmosphere at elevated temperature when CO₂ and H₂O is released and the structure starts to collapse. In Figure 21 such manner can be observed only at conversions 10 % and 50–80 %.

When we examine the correlation between thermo-oxidative stability and the amount of aromatic carbon in the samples, no such pattern as in the case of carboxylic C is observed. If we focus just on the mid-conversion as in the previous case in Figures 22–24, between 10 and 80 % of conversion we can see both positive and negative correlation. The highest value is positive and reaches 0.4. This should mean, that high amount of aromatic carbon present in humic substances causes thermo-oxidative stability of the sample. The degradation of organic matter generally proceeds from labile molecules at lower temperatures (aliphatic moieties) to more stable ones (aromatic cores) at higher temperatures. When Eq. (8) as the temperature function is applied (Fig. 21), the correlation pattern changes substantially. Still, very narrow area with relatively high positive correlation (up to 0.5) can be found between 40 and 60 % of conversion. We must take into account that different samples (even with the same degree of aromaticity) may possess very diverse structures containing the aromatic carbon. These exhibit different stability and thus the correlation doesn't exist. This is also in line with recent statements that the aromaticity itself is not a direct indicator of humic substances stability. [90],[92] It seems that during the degradation of humic substances, not only the presence but also distribution of polar groups play an important role. Mutual weak interactions may rather have more stabilizing effect than the chemical nature of present hydrocarbons. The overall stability is thus origin-dependent.

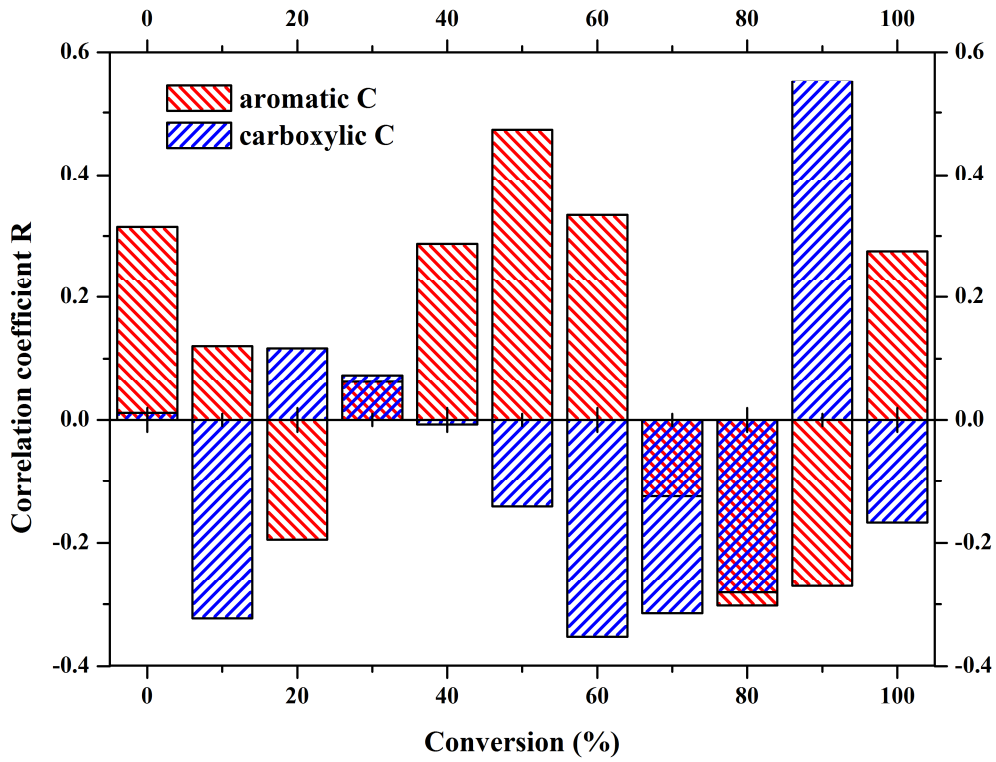


Fig. 21 Correlation of conversion time with aromatic and carboxylic C using Eq. (8)

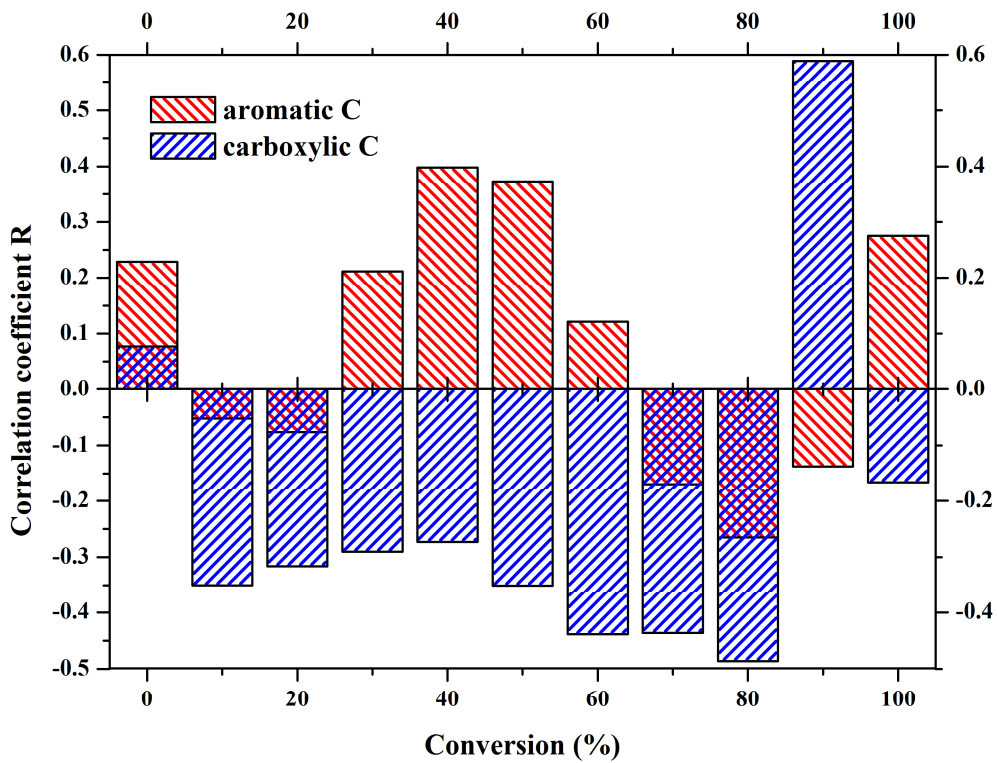


Fig. 22 Correlation of conversion time with aromatic and carboxylic C using Eq. (10)

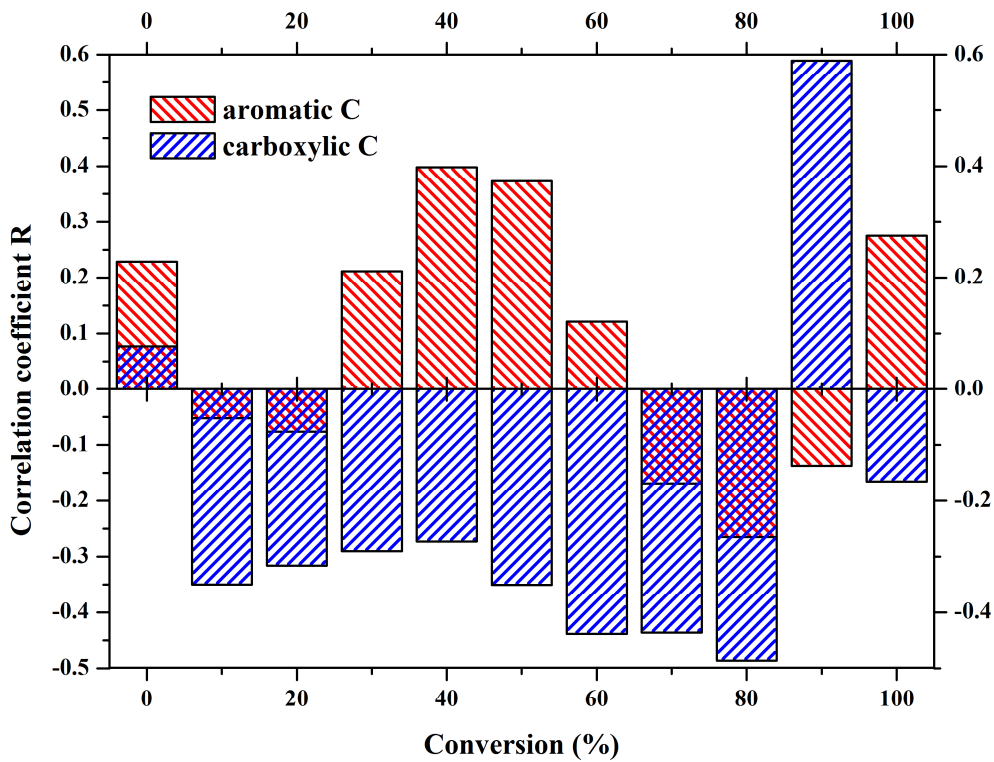


Fig. 23 Correlation of conversion time with aromatic and carboxylic C using Eq. (9)

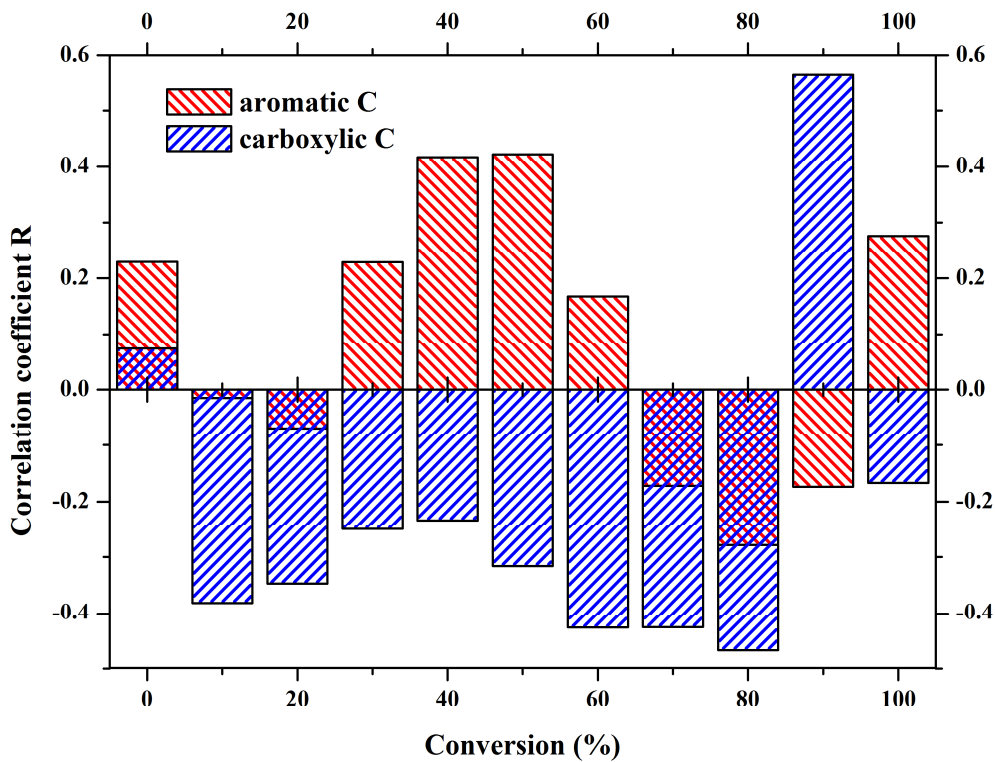


Fig. 24 Correlation of conversion time with aromatic and carboxylic C using Eq. (11)

As previously published in [95], the correlations between thermo-oxidative degradation stability of humic substances expressed as conversion time using Arrhenius equation (Eq. (8)) as temperature function and their elemental composition has already been proved. High positive correlation with the carbon content and a reasonable negative correlation with the oxygen content were found. On the contrary, N content gave substantially lower correlation coefficients. Ratio C/H indicating the aromaticity degree of humic samples did not show any significant correlation. Relatively high value of correlation coefficients provided ratios C/(O+N) and C/O, respectively. As can be seen in Figures 25 and 29, previous conclusions can be confirmed with only a few exceptions. The values of correlation coefficients are substantially lower and do not follow exactly the same trend, mainly concerning the positions of maximal correlations throughout the conversion range. Nevertheless, correlation of isoconversional times with C content gives positive values of correlation, while O gives almost exclusively negative values. The values for N are so small that one can conclude that there is either no significant correlation between correlated parameters or an unexpected experimental error occurred. The highest value (0.6) of linear correlation of kinetics of degradation showed carbon content at 10 % and 60 %. Relatively high correlation was also observed for O content. The highest values were determined for 10 and 80 % of degradation. Within this conversion range the correlation coefficients are noticeably lower but it still indicates the destabilization effect of oxygen on overall humic substances structure stability. Content of N showed a weak influence on the stability of humic substances. The initial increase and later decrease of correlation level can be associated with the stability and degradation of heterocyclic compounds, which are present in humic substances. Additional correlations were also carried out with C/H, C/O and C/(O+N) and some significant results were observed (Fig. 29). In fact, C/O and C/(O+N) reflect the role of the organic carbon content in samples and C/H aromaticity degree of humic substances and they are traditionally used as a distinguishing characteristics of soil, sediment and sedimentary rock. [25] Some relatively interesting values of linear correlations can be identified. At conversions 10 and 60 % the values within 0.6–0.8 (both C/O and C/(O+N)) were reached. In contrast, C/H reached the correlation maximally –0.25 at 10 % of degradation and positive values were even lower, which means no dependence between the parameters.

In this work, also other equations serving as temperature function were used. As in previous case of correlating aromatic and carboxylic carbon, also here all three non-Arrhenius equations show the same patterns, regardless of what is correlated. Figures 26–28 display linear correlation of conversion times and elemental composition of HS and Figs. 30–32 show such correlation with elemental ratios. The values of correlation coefficient are not changing in such dramatic manner through the conversion range as in Figs. 25 and 29 and some clear trends can be identified. E.g. high carbon content is responsible for overall HS structure stability, while vice versa in case of oxygen content. Nitrogen present in humic substances works as a stabilizer at lower conversions (nb. high correlation coefficient, over 0.8, at conversion 20 %) but destabilizes the structure at higher temperatures for reasons mentioned above. Concerning the elemental ratios (Figs. 30–32), the situation is more or less the same as with Arrhenius equation. No correlation of HS stability and C/H can be found. On the other hand, quite high values for other two ratios are reached through relatively wide conversion range (40–80 %).

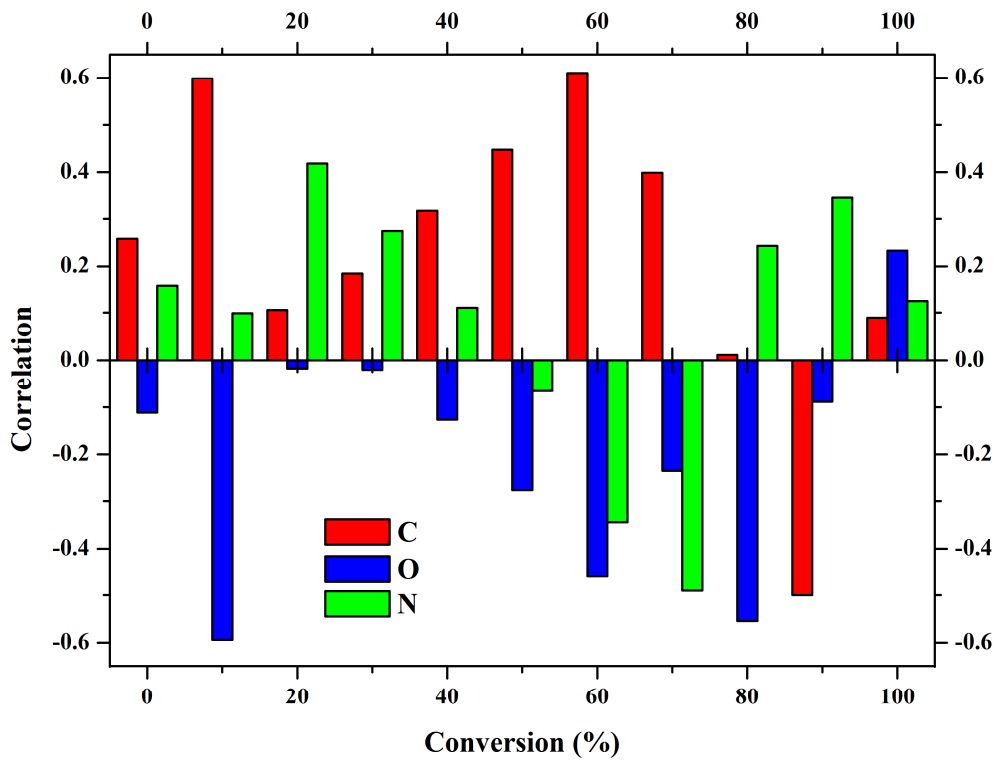


Fig. 25 Correlation of conversion time with elemental composition using Eq. (8)

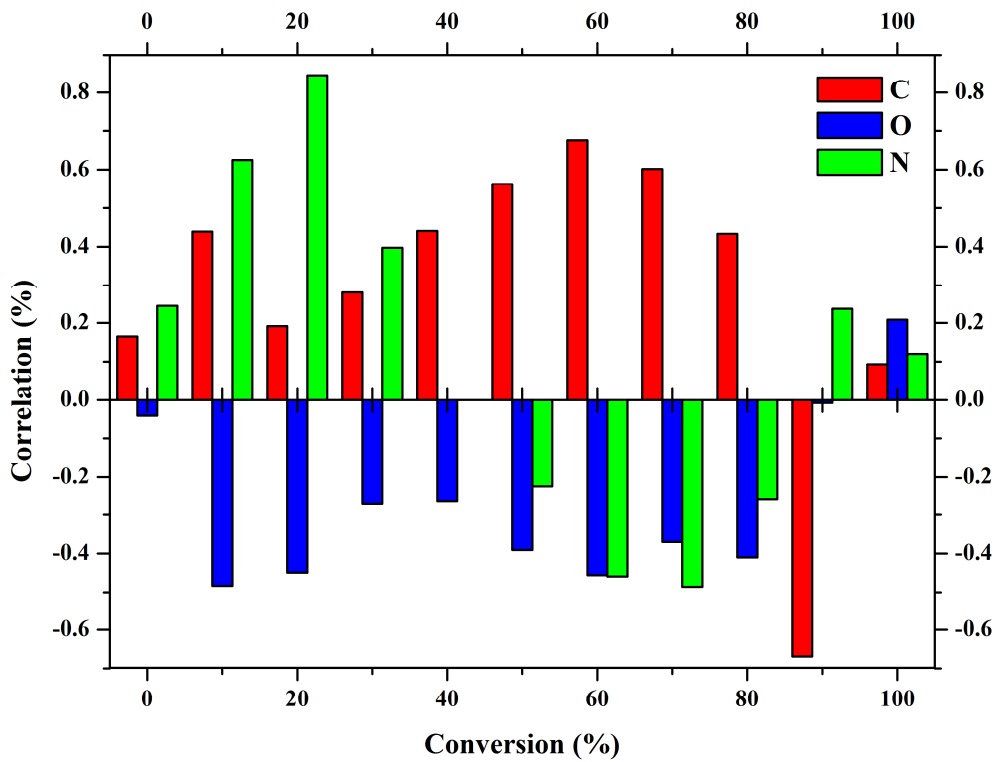


Fig. 26 Correlation of conversion time with elemental composition using Eq. (9)

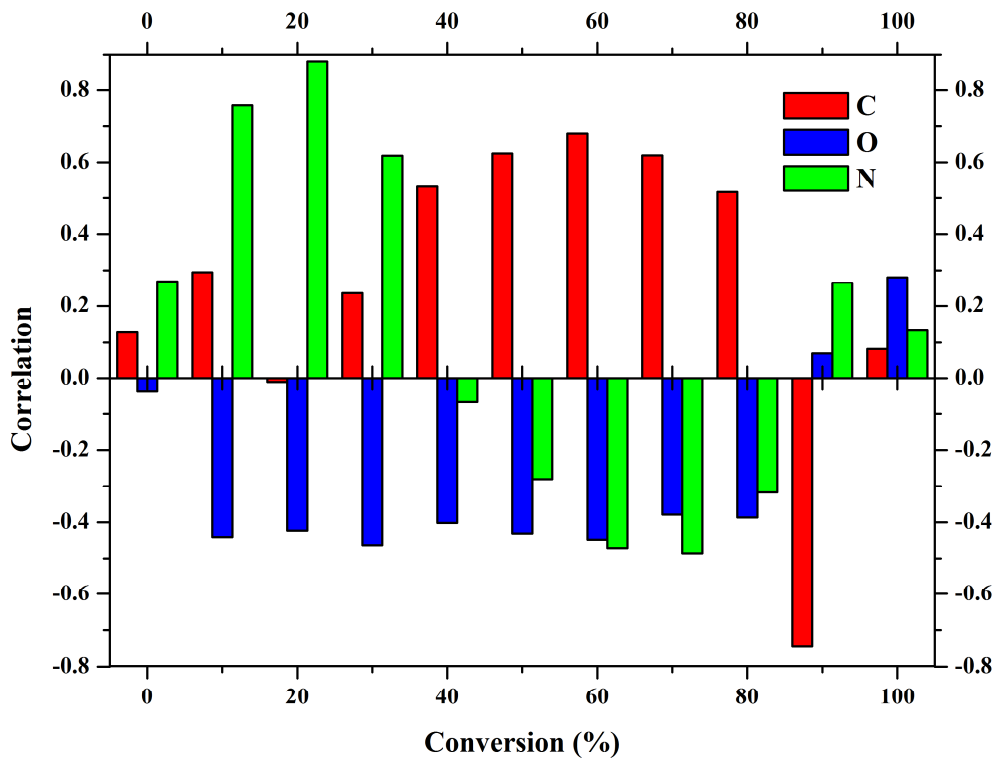


Fig. 27 Correlation of conversion time with elemental composition using Eq. (10)

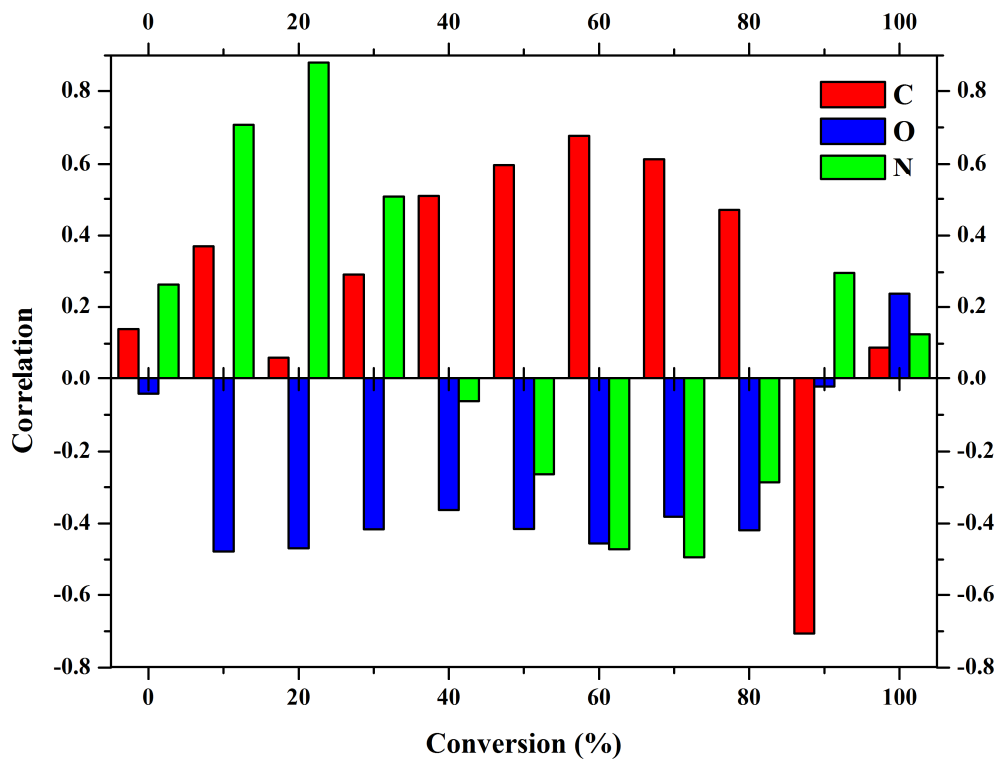


Fig. 28 Correlation of conversion time with elemental composition using Eq. (11)

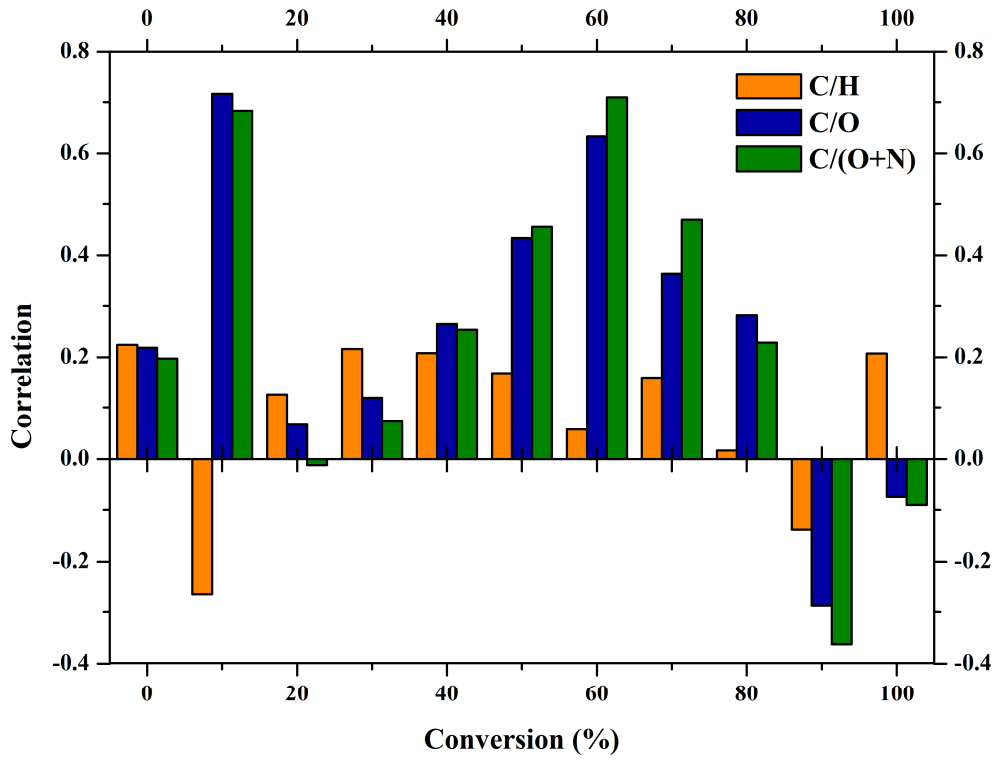


Fig. 29 Correlation of conversion time with elemental ratios using Eq. (8)

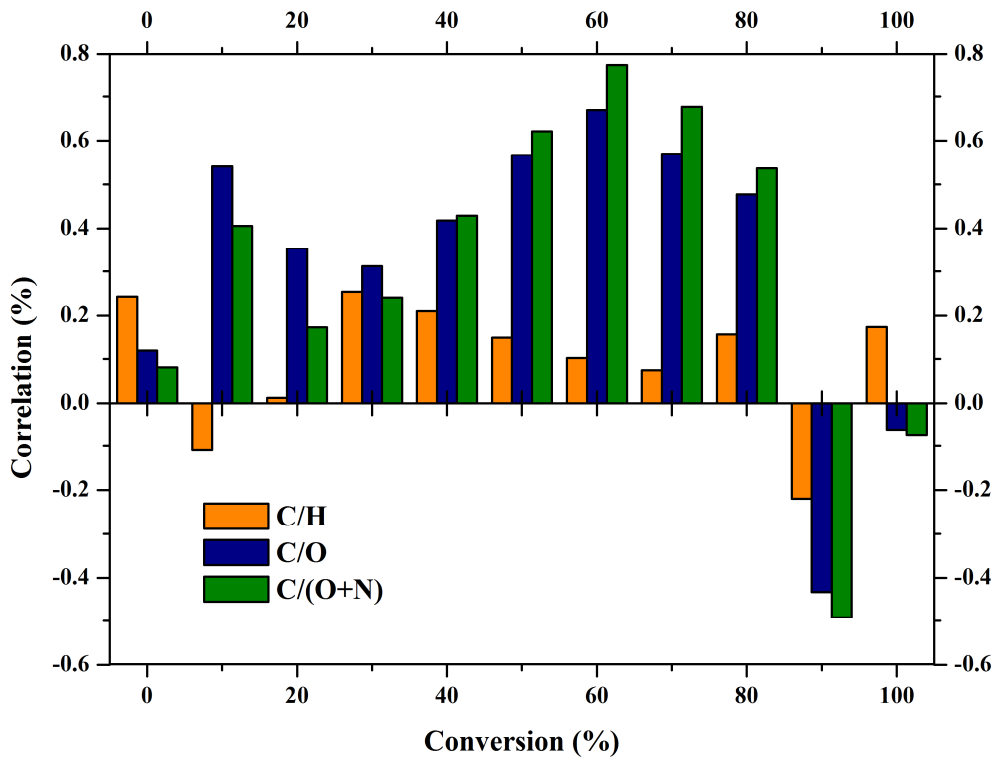


Fig. 30 Correlation of conversion time with elemental ratios using Eq. (9)

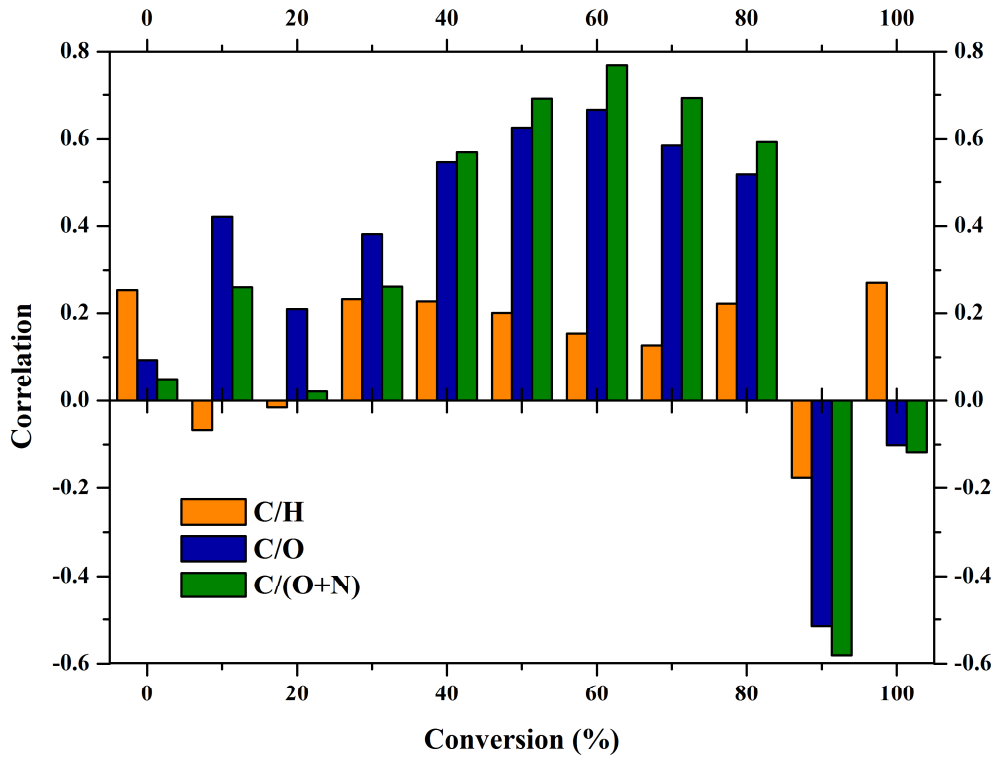


Fig. 31 Correlation of conversion time with elemental ratios using Eq. (10)

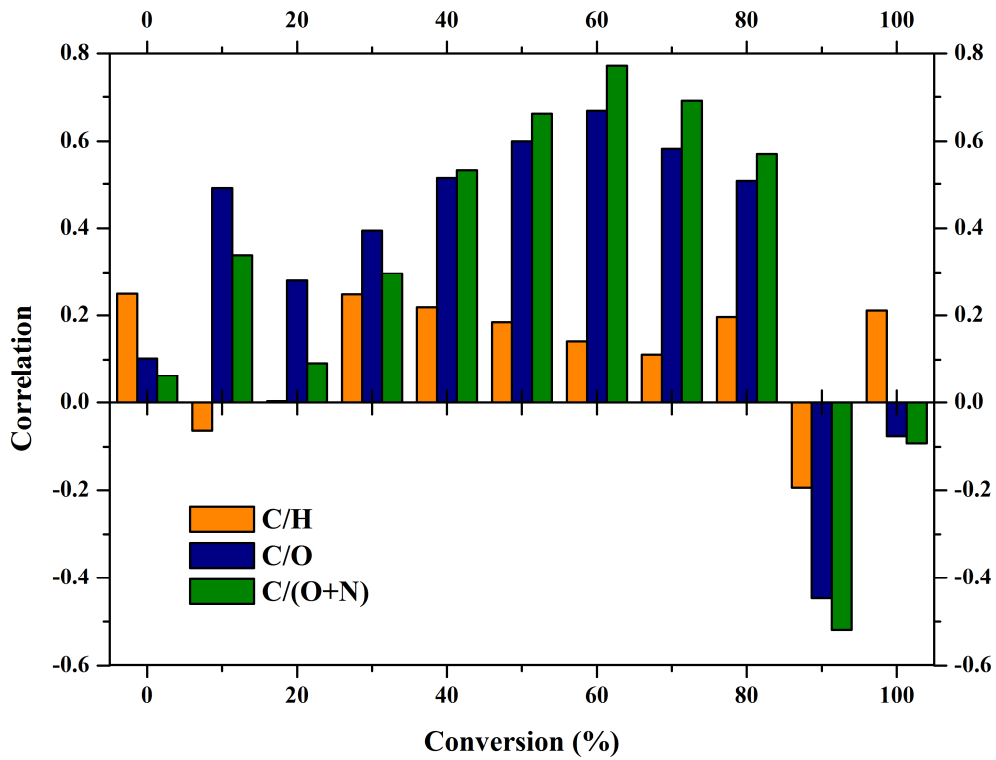


Fig. 32 Correlation of conversion time with elemental ratios using Eq. (11)

5.1.5 Relationship between TGA weight losses and primary characteristics

As discussed above, the mathematical apparatus of isoconversional methods applied to thermo-oxidative degradation of humic substances didn't bring any significant correlations with primary properties of HS. Therefore, other approach with zero mathematical modification was used for the same purpose. Thermogravimetric weight losses in 10 °C intervals (employing heating rate 5 °C min⁻¹) were determined and then correlated to HS properties. This method has been successfully applied by Siewert when studying whole soil samples and allows rapid estimation of amounts of organic C, total N, clay and carbonates. [98] Although this method is suitable exclusively for soil substrates, the effort was driven to find out, if there exists any correlation between TGA weight losses in narrow 10 °C intervals and primary characteristics. Values of weight losses in the specific temperature intervals can be found in Appendix VI. Figure 33 displays the level of correlation between the TGA weight losses and amount of aromatic and carboxylic C in HS samples. As can be seen, the highest positive and negative correlation for aromatic C reaches 0.4 (400 °C) and -0.4 (450 °C), respectively. That means there is no specific temperature, at which the aromatic moieties would degrade. Higher correlation could be reached using multiple temperature regions, since the aromatic cores are cleaved according to their type and degree of condensation during wider temperature scale. On the other hand the correlation with carboxylic carbon brings some interesting remarks. The correlation coefficient reaches quite high values, up to 0.85 at low temperatures to 280 °C. That means that carboxylic functional groups are responsible for initial lability. At higher temperatures (350 °C) the correlation reaches negative values down to -0.7. The stability at this temperature region may be caused by the fact that all labile moieties are already decomposed thanks to the first stage and at the same time, the temperature is not high enough for the rest of the organic matter to degrade.

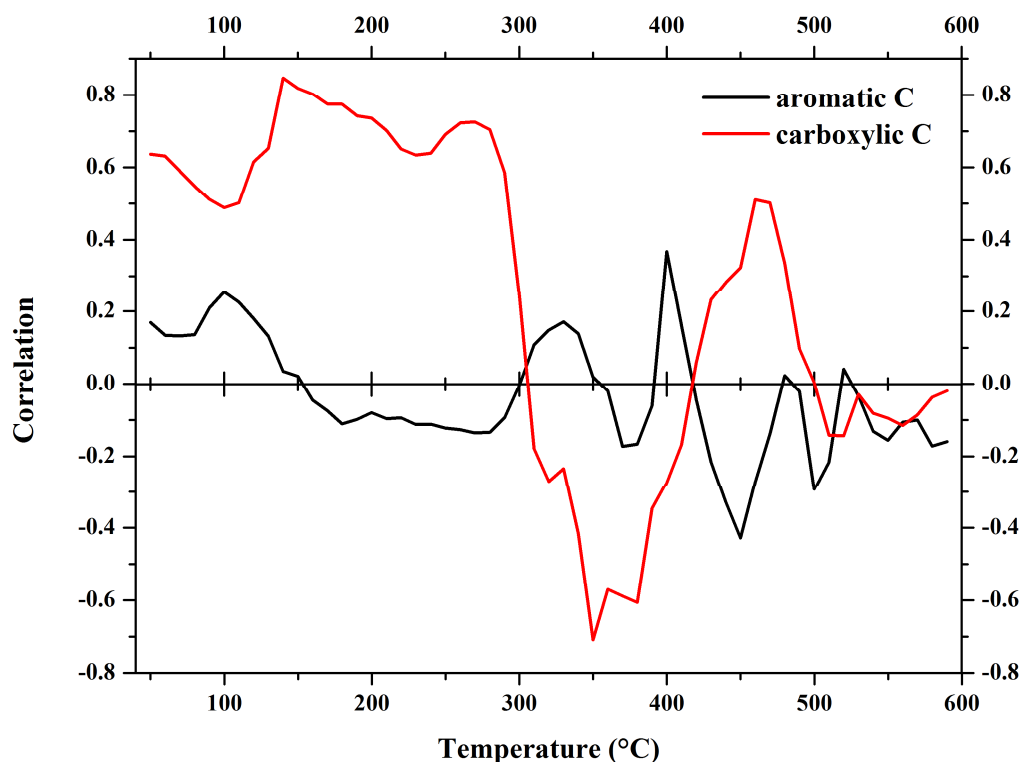


Fig. 33 Correlation of TGA weight losses with aromatic and carboxylic C

Figure 34 shows correlations between thermogravimetric weight losses and elemental composition of HS. From the course of the curves one could generalize that high amount of carbon initially stabilizes humic matter. On the other hand, high amount of oxygen is responsible for decomposition at lower temperatures, which is in line with previous findings about carboxylic C, where major part of the overall oxygen content is stored. The increasing values of N correlation curve in the course of increasing temperature hint on initial stability of N-containing groups, whereas at temperatures above 500 °C the stability decreases thanks to degradation mainly of heterocyclic and other nitrogen functional groups.

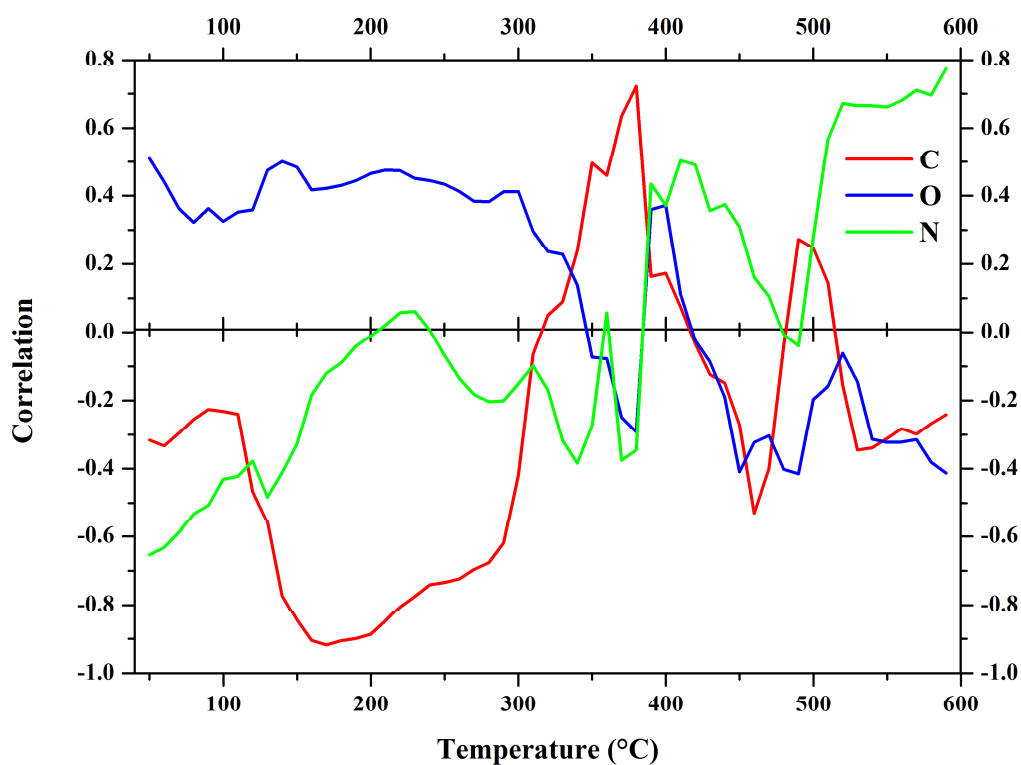


Fig. 34 Correlation of TGA weight losses with elemental composition

The pattern of all three curves in Figure 35 probably doesn't reflect any trend during thermo-oxidative degradation concerning the elemental ratios of humic substances. They are highly influenced by carbon content (cf. red correlation curve in Fig. 34). Despite some high correlation coefficients, drawing conclusion out of these values is not recommended. Moreover, the correlation at the end of the temperature interval in all cases may be untrustworthy, since the end of the degradation process is at most of the samples already ended by approx. 500 °C and the weight losses (if any) can be ascribed to non-idealistic baseline feature. This leads to irregular data distribution and/or zero Y variance.

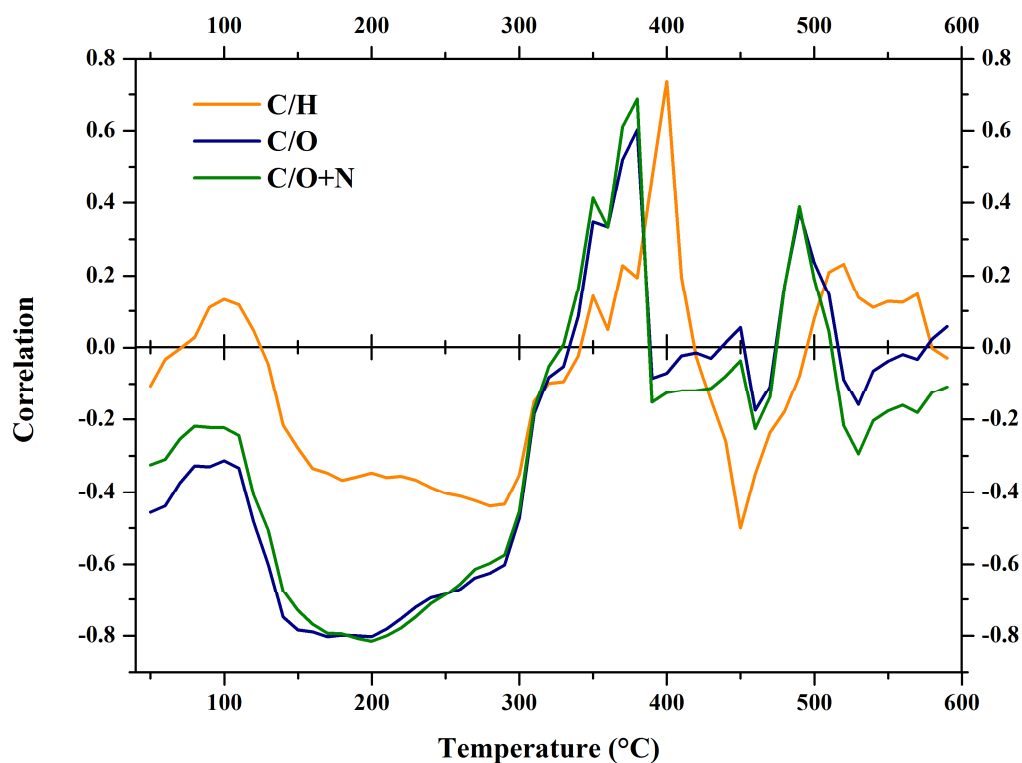


Fig. 35 Correlation of TGA weight losses with elemental ratios

As mentioned above, the thermo-oxidative stability (expressed as conversion time) must logically increase with increasing conversion. As can be seen in Figures 19 and 20, this assumption is not fulfilled. The possible explanation of such behaviour can consist in the fact that some of the attributes of single-step approximation is not complied. The plausible kinetic description, which should provide unvarying values of adjustable parameters both for temperature and conversion functions over the whole range of experimental data may be unsuitable. The single-step kinetics approximation enables a mathematical description of the kinetics of solid-state reactions. The correct mathematical description should recover the values of conversion and the rate of the reaction under study for a given couple of time and temperature. In general, the temperature and conversion functions represent the temperature and conversion components of the kinetic hypersurface so that the adjustable parameters occurring in the both functions have no obvious physical meaning. They may vary with the range of experimental data so that the extrapolation of the results measured too far outside the experimental range cannot be considered trustworthy. The impossibility of far-reaching extrapolation is a weakness of the single-step approximation. [58]

5.2 Soil samples

5.2.1 Chemical, biological, and thermal characteristics

Soil samples taken from extensive and natural land uses represented the main soil types under natural vegetation or low intensity (grazing) agriculture. More detailed characteristics of soil sampling sites can be found in Table 4. These soils showed a wide range of physical and chemical characteristics (Table 5, see page 77). Clay content ranged from 0 to 41 %, water content at pF 1.8 from 110 to 740 g kg⁻¹, organic carbon content from 8.4 to 272 g kg⁻¹, etc.

Table 4 *Description of soil sampling locations*

National Park	Description	GPS coordinates		alt.
		N	E	m
BERCHTESGADEN Hirschbichl	Low altitude alp pasture	47° 33' 35.9"	12° 47' 56.4"	1115
	Medium altitude alp pasture	47° 33' 26.6"	12° 48' 30.5"	1327
	Ravine forest	47° 33' 26.3"	12° 47' 46.6"	1055
	Acidic meadow with spikenard	47° 33' 44.1"	12° 48' 01.3"	1077
	Orchid–Beech forest	47° 33' 41.0"	12° 47' 55.1"	1034
	Erosion valley	47° 33' 24.0"	12° 47' 52.6"	1179
BAVARIAN FOREST Zwieslerwaldhaus	Spruce–Fir–Beech forest	49° 05' 50.3"	13° 13' 52.3"	740
	Spruce–Fir–Beech primeval forest	49° 05' 46.7"	13° 15' 00.5"	790
	Spruce–Fir–Beech forest	49° 06' 09.9"	13° 16' 33.2"	1200
HAINICH Craula	Wood Clubrush–Beech forest	51° 04' 08.1"	10° 26' 58.5"	480
	Orchid–Beech forest (Shallow founded Rendzina)	51° 03' 41.3"	10° 24' 43.0"	350
	Moss–Marsh area	51° 05' 16.4"	10° 25' 45.3"	470
HARZ Schierke	Wood Clubrush–Beech forest	51° 50' 48.5"	10° 39' 31.1"	430
	Spruce–Alder forest	51° 49' 34.2"	10° 38' 04.8"	635
	Three-lobed liverwort–Spruce forest	51° 47' 31.1"	10° 38' 14.7"	1025
	Reedgrass meadow	51° 47' 56.5"	10° 37' 12.3"	1230
SCHORFHEIDE- CHORIN Chorin/Brodowin	Red Beech forest	52° 53' 30.5"	13° 53' 31.1"	120
	Pine–Red Beech–Oak forest	52° 52' 59.3"	13° 53' 54.6"	115
	Pine–Red Beech forest	52° 53' 01.7"	13° 55' 30.1"	95
	Herbaceous layer border Alder carr / Beech forest	52° 53' 22.3"	13° 55' 25.2"	90
	Semi-arid meadow (Drumlin)	52° 54' 47.1"	13° 58' 55.5"	120

Figure 36 shows the mean values of cumulative evolved CO₂ from all 33 soil samples incubated (nb. incubation = respiration) under optimal moisture conditions (80 % of pF 1.8) and constant temperature (25 °C). It reveals the highest increase in the respiration rate at the beginning of respiration experiments with a decrease during incubation time.

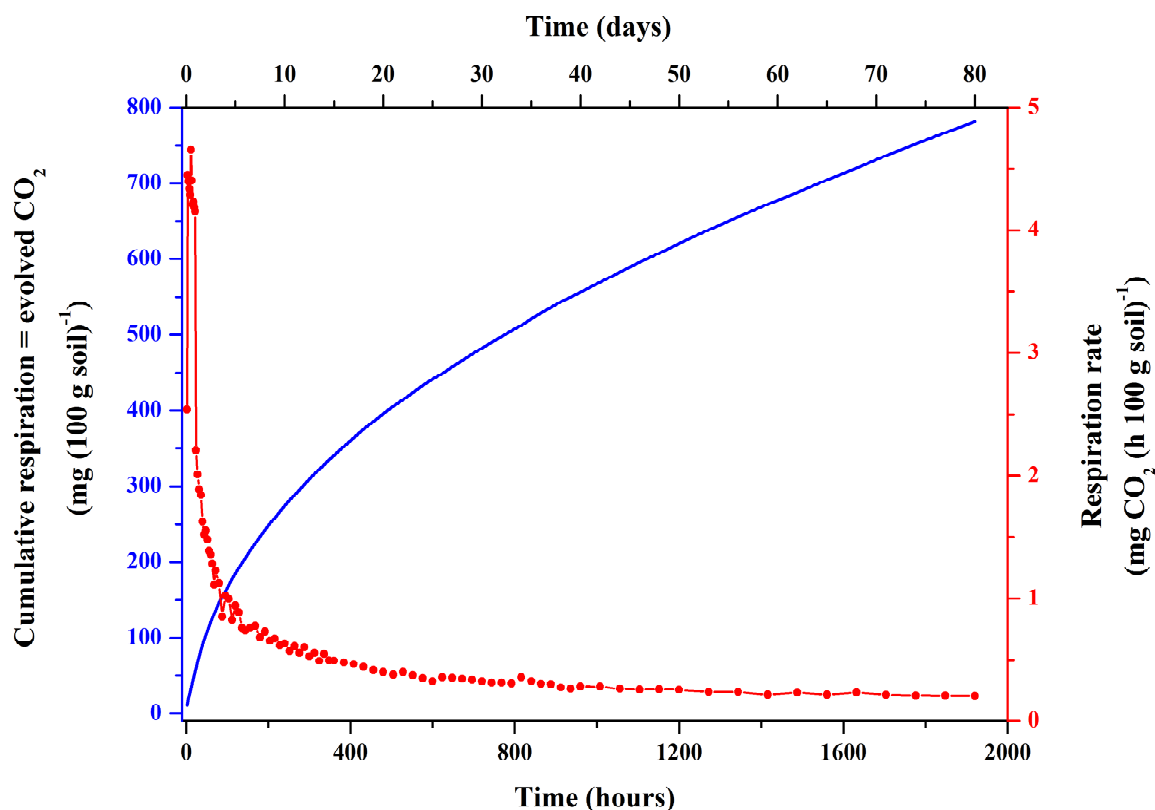


Fig. 36 *Averaged curve of soil cumulative incubation and its derivative (respiration rate)*

An averaged thermogravimetric profile (TGA) of all non-incubated samples and its derivation (DTG) are reported in Figure 37. The processes occurring in soils during thermo-oxidative degradation have been described in scientific literature several times. [107][113][114] From this preview, the DTG curve provides a good resolution of the stages occurring during thermo-oxidative degradation. The temperature interval from room temperature to temperatures around 110 °C was attributed to the moisture evaporation, whereas higher temperature areas were ascribed to degradation (oxidation/combustion) of soil organic (second DTG peak, up to cca. 600 °C) and inorganic (third DTG peak, mainly carbonates, around 800° C) matter.

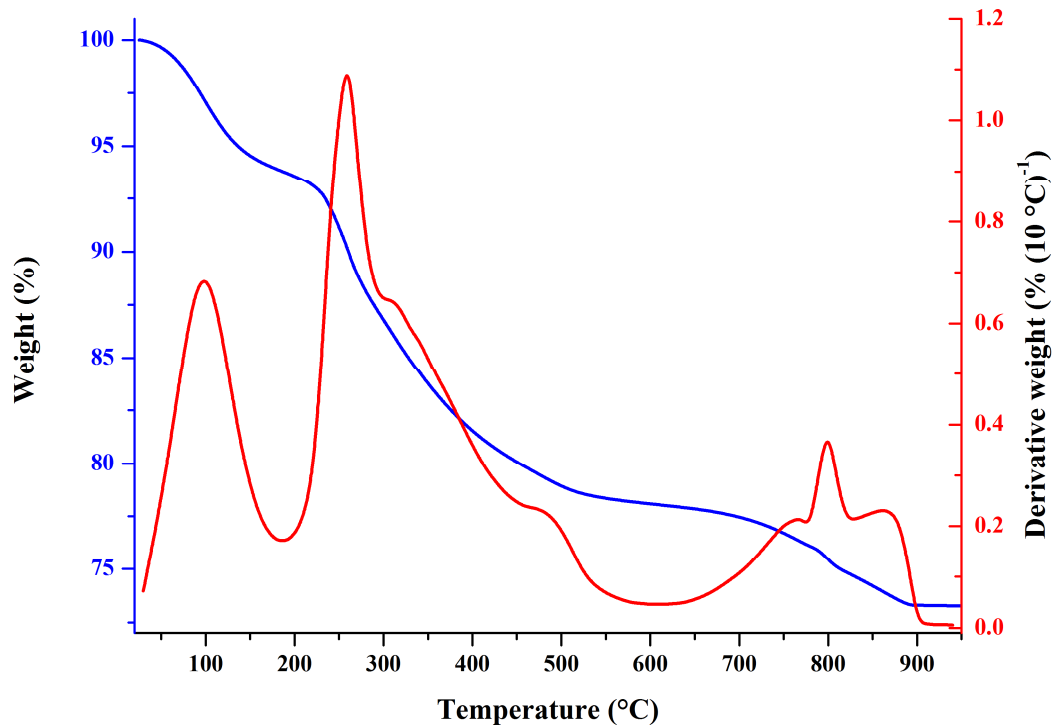


Fig. 37 *Averaged thermogravimetric and derivative thermogravimetric record of non-incubated soil samples*

In previous studies the mass loss recorded in pre-dried soils (at 105 °C), from 110 °C to 550 °C was used for the determination of SOM content. [77],[115] Figure 38 shows a randomly chosen example of mass losses from analyzed soils samples in relation to cumulative respired CO₂ in soil incubation experiments after seven days. It demonstrates a statistically significant but on the other hand weak dependence between these two parameters.

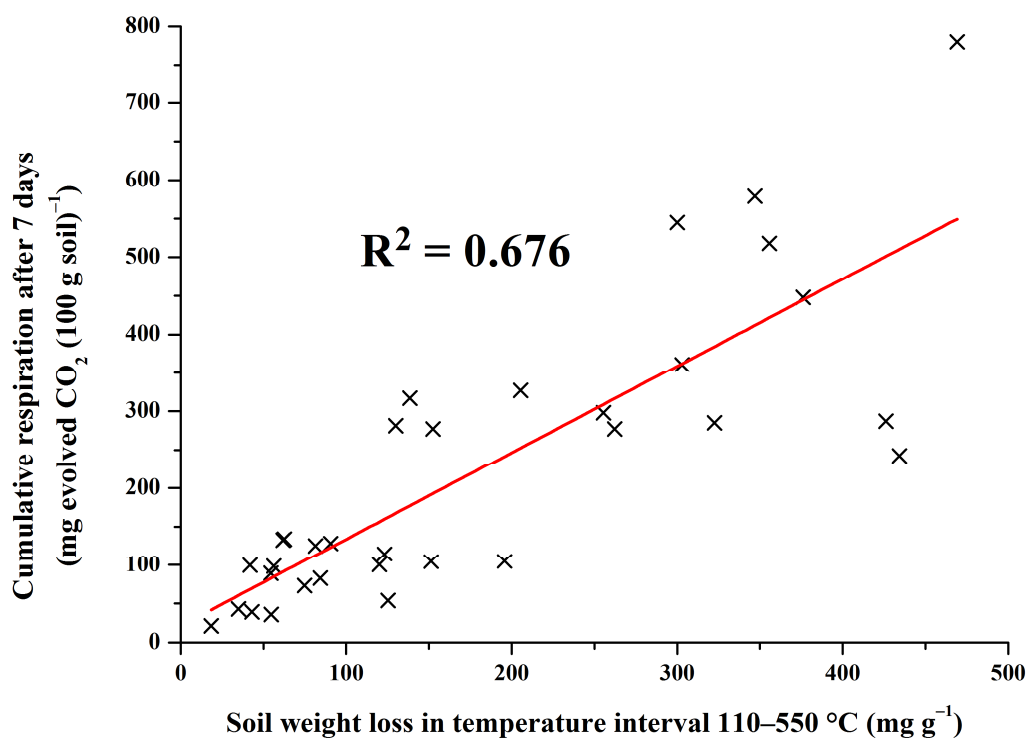


Fig. 38 *Dependence of total CO₂ amount evolved after 7 days of incubation on thermally induced mass losses in temperature interval 110–550 °C*

5.2.2 Time/temperature relationship between TGA and laboratory incubation

As shown previously by Siewert in [97], the mass loss of soil at any temperature interval (obtained from TGA) can be positively correlated with data from soil respiration at any time of incubation. Results of such calculations are depicted in Figure 39. It shows changing values of correlation coefficients (R^2) between TGA and cumulative soil respiration with time of incubation for two selected temperature intervals in TGA. The highest values ($R^2 = 0.67$) are reached after approximately two days of incubation following a sharp increase during the first hours of incubation. With increasing time, the dependence between results of both methods becomes lower and nearly constant at around $R^2 = 0.5$. Both selected temperature intervals in TGA give similar results. It can be concluded from this data that continuous incubation could bring more information only in case of very long time periods of incubation exceeding the measuring period reported here.

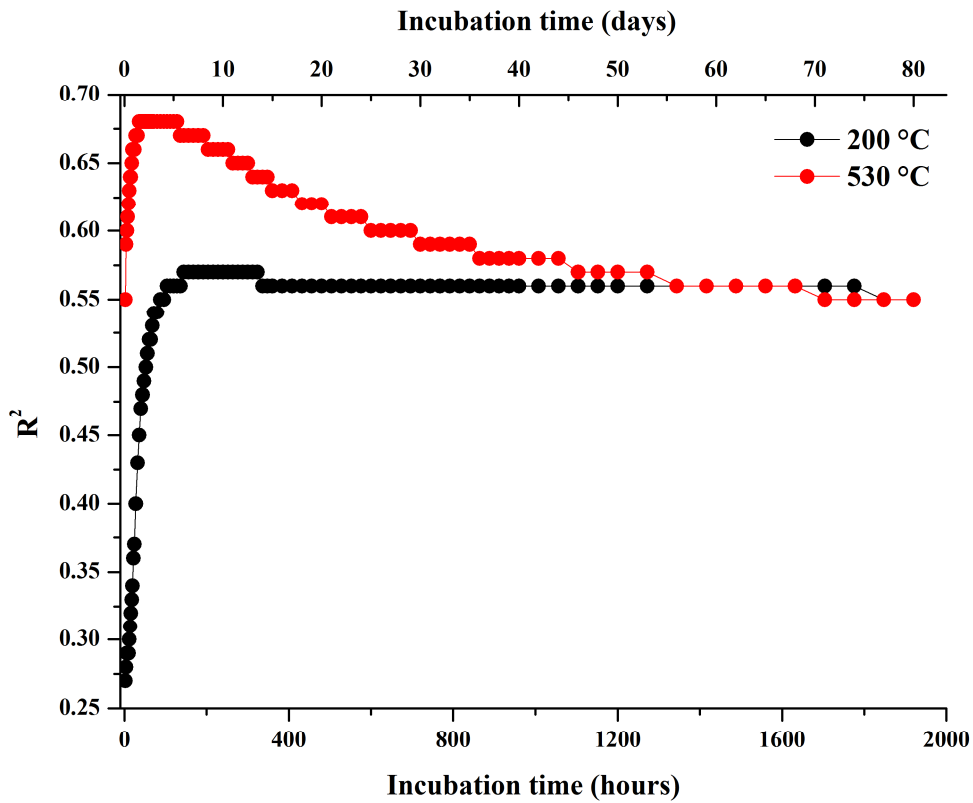


Fig. 39 Changing levels of determination coefficients with time of incubation for two temperatures in TGA

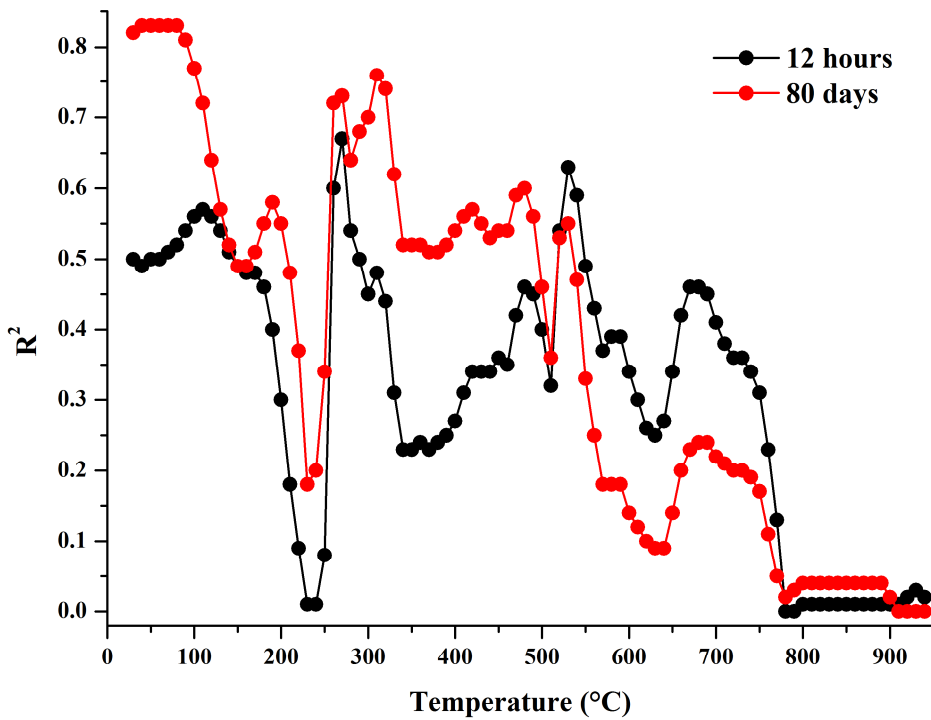


Fig. 40 Changing levels of determination coefficients with increasing temperature in TGA for two periods in incubation experiment

Figure 40 summarizes correlation coefficients of two selected incubation times with TGA results over the whole temperature range from 30 °C to 950 °C. Despite very different results, long-term incubation (red dots) provides closer correlation with TGA mass losses except in the temperature area around 400 °C. The highest coefficients of determination were found mainly below 120 °C and at around 300 °C, i.e. in regions of water evaporation and degradation of organic matter.

These results confirm that the temperature area from room temperature up to 650 °C should be considered. The main characteristics of dependencies between both methods can be obtained after short incubation time. The absolute level of determination coefficients are not of the same importance. Low coefficients, e.g. between thermal mass losses at around 220 °C with soil microbial respiration can be caused by the lack of dependencies or by methodical artifacts or other disturbances. High coefficients should be validated with extended sample sets and analyzed also with other statistical methods before their potential use and interpretations.

For this purpose, exemplary data reported in Figures 39 and 40 were combined and the extended information value can be illustrated in 3D charts. Figs. 41 and 42 show an example of such concept. In these figures, coefficients of determination are depicted in colour scale as the third dimension (Z-axis). The scale is differentiated according to the significance of the correlation, i.e. the darker colour, the closer correlation. First, the colour changes with 0.05 step up to 0.8 (of coefficient of determination) and then with 0.01 step. The Z-axis represents the mutual interrelations between CO₂ evolution in incubation experiments with re-moistened soils and thermally induced mass losses of air-dried soils in TGA.

The highest determination coefficients with R^2 around 0.85 can be found for thermally induced mass losses at the temperature interval between 30 °C and 110 °C and the CO₂ evolution rate after one day of incubation (brown and black areas in Figure 41). The second area with statistical significance but lower correlation coefficients can be seen between mass losses in the temperature interval from 260 °C to 320 °C and incubation after approximately one day. Other significant correlations were also found for different incubation time periods at temperatures above 450 °C. Figure 42 demonstrates similar data but using the cumulative CO₂ evolution. It implies that the correlation in the late stage of incubation is interlinked with the correlation in the first stage of incubation. However, it is difficult to recognize to which extend this correlation was caused by intrinsic soil properties or if it is a consequence of earlier stage of respiration, or both cases.

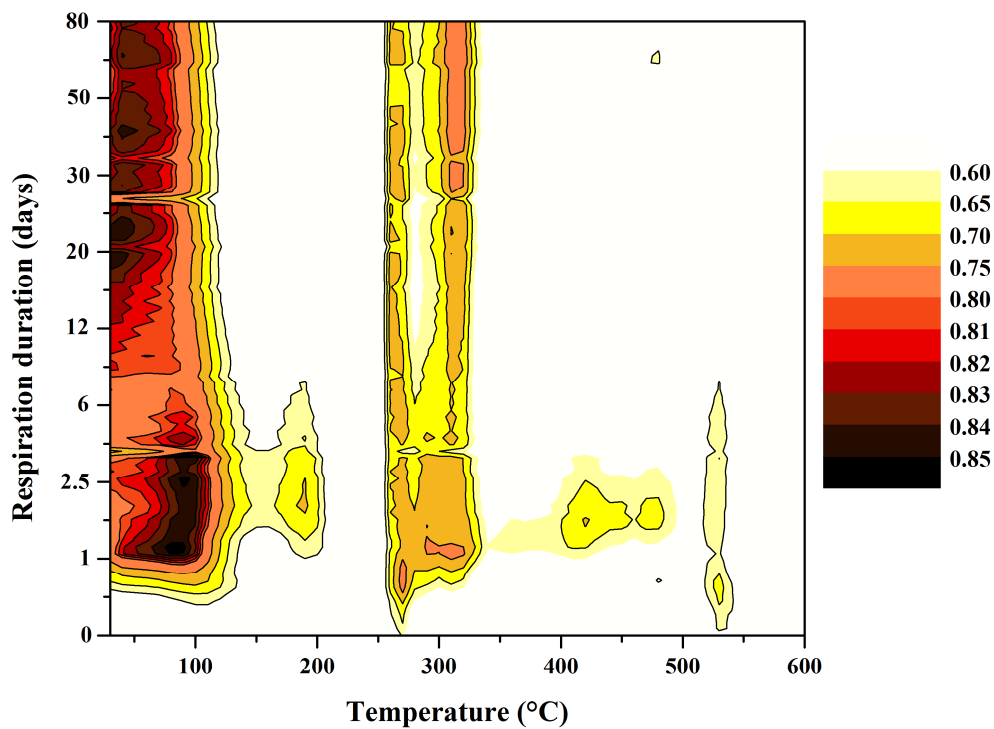


Fig. 41 *Matrix of determination coefficients representing dependence between the rate of CO₂ evolution and TGA mass losses*

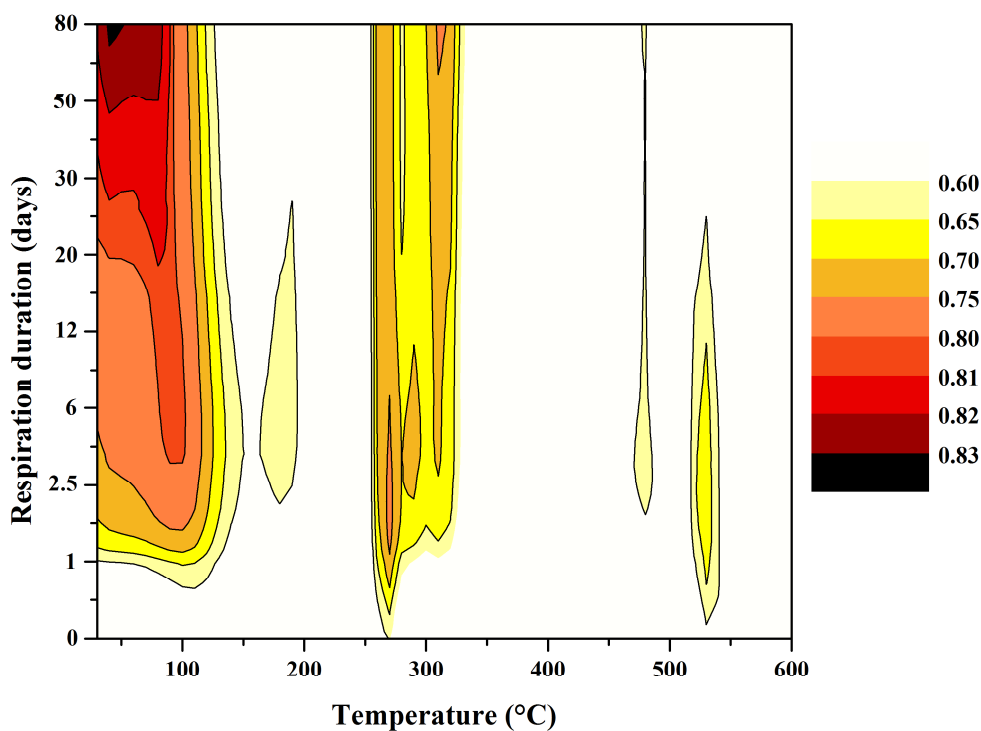


Fig. 42 *Matrix of determination coefficients representing dependence between the cumulative CO₂ evolution and TGA mass losses*

In order to understand the meaning of interrelations between mass loss at lower temperatures in TGA and soil respiration, it is necessary to understand processes occurring during soil heating and induced mass losses. The mass loss of air-dried samples at lower temperatures up to approximately 150 °C is mainly connected with free and bound water evaporation. In soils, this water loss correlates with clay content. [98] Few publications discuss the hygroscopicity of fresh plant materials; more of them report humic substances and other components of soils as a source of the water detected in thermal analyses. [116],[117] Mass losses at higher temperatures are usually attributed to the degradation of soil organic matter components of different thermal stability. Around 550 °C the onset of degradation of carbonates is usually reported as the main reason of mass loss. [98] From this point of view, it is surprising to find the closest correlations of soil respiration in re-moistened soils with TGA mass losses occurring in the area of water evaporation in air-dried soils. The steep increase of these correlations immediately after the beginning of the incubation is unexpected too, but already reported for different sample sets. [97]

The influence of water in air-dried soils on biological process in re-moistened soils could be associated with many factors. The kinetics of wetting and complete hydration of soil organic and inorganic matter has been demonstrated (including wetting and swelling) to take up to three weeks [117] and thus the kinetics of hydration are likely to play a role in the increase of correlation coefficient with time. It implies several questions. Could the interaction of water with soil organic matter be considered as regulation factor of biological processes in soils, and what is the influence of swelling, solvation, dissociation, diffusion and other phenomena on those processes? These questions reflect the lack of information regarding the unique soil properties associated with its long-term genesis, implying the complex and often poorly understood mechanisms between chemical, physical, and biological properties in soils.

High correlation of mass losses at temperatures above 230 °C with biological activity after few hours of incubation was expected since in this temperature interval the organic matter, used by microorganisms during the soil respiration, is decomposed. [97] However, the reason for relatively low levels of the coefficients of determination (i.e. $R^2 < 0.50$) was not clear.

If one plots the dependence of cumulatively evolved CO₂ after 5 days of incubation against thermally induced mass loss at 300 °C (area of significant correlation in Fig. 41, $R^2 = 0.77$), Figure 43 is obtained. From the pattern of the data points it can be easily concluded that linear fitting is not the most suitable one. In this case, power dependence yields much higher determination coefficient ($R^2 = 0.95$). Such trend cannot be easily explained and is not followed in each combination in dependence: incubation time vs. temperature region.

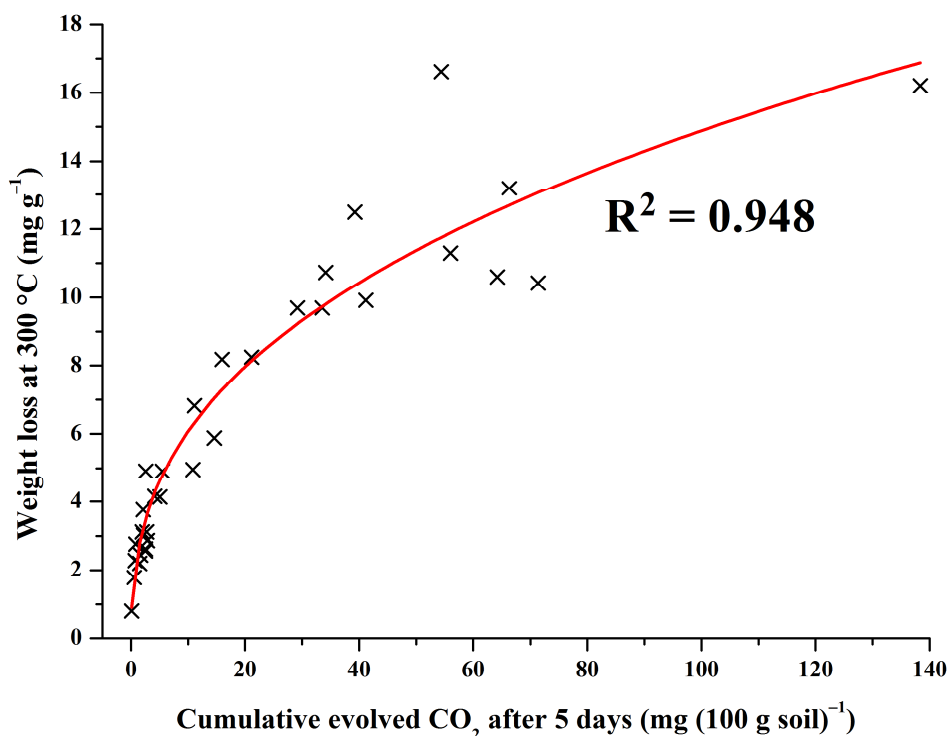


Fig. 43 Power fit of TGA weight loss at 300 °C depending on cumulative evolved CO₂ after 5 days of incubation

Such and even higher coefficients can also be obtained using multiple regressions, hidden, non-trivial dependencies and TGA data from two or more temperature areas at the same time. These promising evaluations are not shown because for this type of correlation and further conclusions, higher number of samples (to cover wider range of respiration behaviour and mass losses) and additional experiments to discover the nature of these correlations is needed.

5.2.3 Effect of incubation on thermally induced mass loss

One can obtain more information about the relevant biological processes by comparing TGA of samples incubated for 80 days and non-incubated ones as shown in Figure 44. The subtracted difference between averaged curves obtained from all samples is displayed in the bottom part of the figure with separate scale to reveal changes in DTG induced by microbiological decay. This curve illustrates an increase of mass during respiration experiments evaporating and/or degrading at around 110 °C and 225 °C. On the contrary, after incubation, there was a relative decrease of mass loss at temperature >240 °C, with a huge peak between 250 °C and 290 °C. It is thought that the effect of rewetting and drying is small as most of the changes occur when the soil is initially dried. These results confirm similar trends found previously in other soil samples. [118] The reason of such phenomenon is not clear. However, the changes in mass losses at around 100 °C comparing the incubated to non-incubated samples suggest the interrelation of water binding to biological processes in soils, e.g. by accumulation of hygroscopic substances or changing chemical composition by growing microorganisms, redistribution of soil aggregates, and changes in organic complexes. The hypothesis is that one of the possible explanations of the relative increase in mass loss of incubated samples than in the non-incubated ones at temperatures around 200 °C could be

attributed to the growth of microorganism population with lower thermal stability, i.e. higher content of labile biopolymers such as proteins, polysaccharides and other biomolecules present as the building blocks of microorganism bodies. From the temperature 250 °C onward, there is a decrease in TGA weight losses in incubated samples (Fig. 44, blue line, negative values). Thermally induced mass losses in this temperature interval in incubated samples coincide with the mineralization of organic matter by microorganisms and transformation of labile molecules into heat and CO₂. However, the confirmation of these statements is beyond the scope of this work.

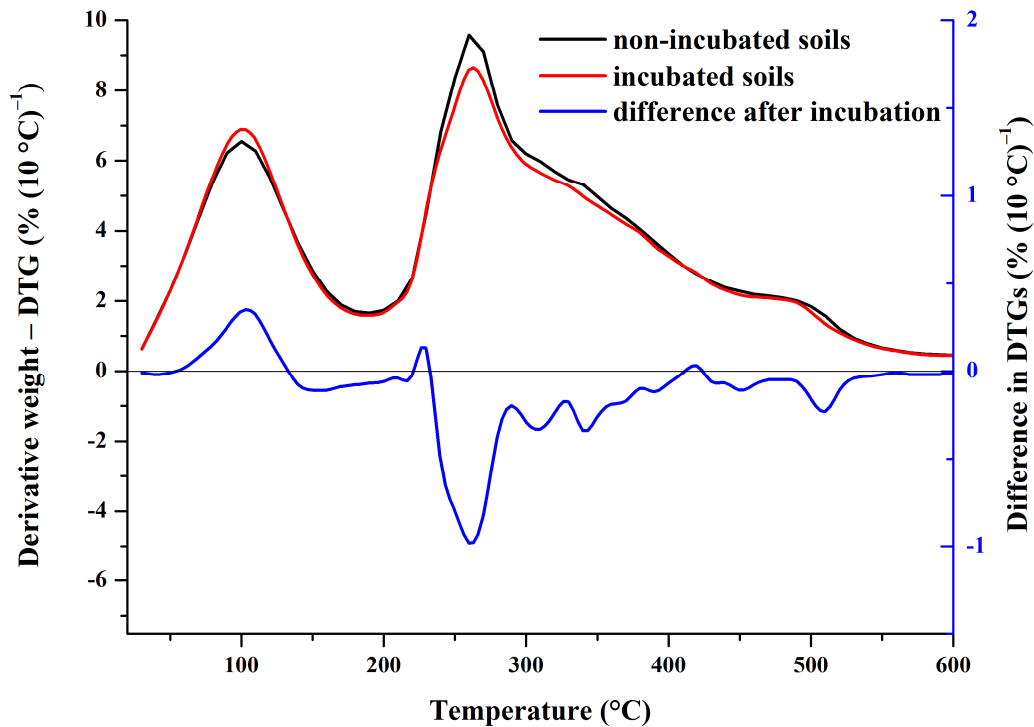


Fig. 44 *Averaged derivative TGA profiles of all samples before and after incubation experiment and their difference*

5.2.4 Determination of soil organic carbon (SOC) using TGA?

Using previously published experiences [98], the content of organic carbon of investigated soil samples determined by elemental analyses was compared with carbon content determined by TGA. The results are reported in Figure 45. According to Siewert [98], the combination of results obtained from mass losses in several temperature intervals is recommended to determine the soil organic carbon. In the present study, despite the use of a very narrow temperature interval of mass losses between 340 °C and 350 °C, a close correlation with results of elemental analysis was confirmed as one of the temperature intervals with closest correlations to total carbon content determined by elemental analyses. In addition, the figure shows a possibility to determine carbon content with TGA in a wider concentration range in comparison with recommendations reported previously. This makes this area highly interesting for further investigations. If very small mass losses in narrow temperature intervals reflect the whole carbon content with high accuracy, there should also be a connection to biological transformation processes in soils. Since such determination works only for

relatively non-disturbed soils (but not for mineral substrates containing organic carbon and soils under extreme conditions, e.g. desert soils with heavy surface changes caused by wind or permafrost soils with intensive cryoturbation), it makes the TGA an interesting tool to search for intrinsic soil regulation processes that should be combined with other methods to detect the reasons of found correlations.

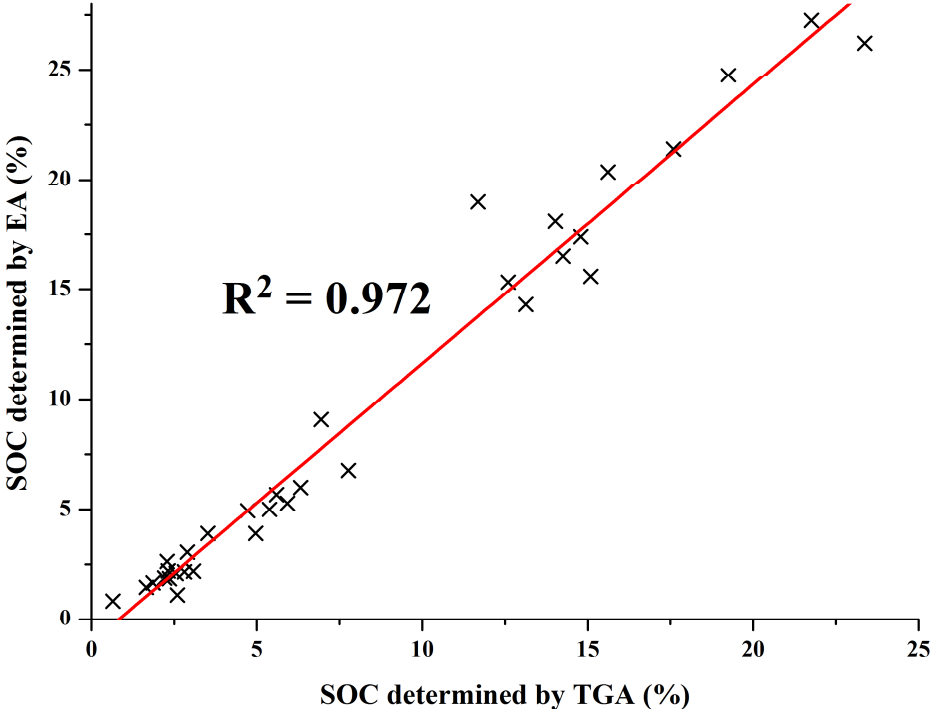


Fig. 45 Correlation between soil organic carbon content determined by elemental (EA) and thermogravimetric analysis (TGA)

Table 5 *Brief description and basic characteristics of studied soils*

Location	Land use	Coordinates	Altitude	# sites	# samples	pF 1.8	organic C	N	Sand	Silt	Clay
			m			g kg ⁻¹			%		
Berchtesgaden	pastures, forests (all types)	N 47° 33' E 12° 48'	1050–1330	6	12	260–890	11.3–272.5	0.13–1.39	15–52	38–51	0–34
Bayerischer Wald	coniferous forests	N 49° 05' E 13° 15'	740–1200	3	6	260–520	50.1–214.0	0.22–1.19	49–56	32–37	0–15
Hainich	deciduous forests, marsh (moss)	N 51° 04' E 10° 25'	350–480	3	4	360–460	18.6–90.8	0.17–0.43	2–7	55–71	27–43
Harz	mixed forests, meadows	N 51° 48' E 10° 38'	430–1230	4	6	230–740	39.1–262.1	0.17–1.37	21–74	20–54	0–24
Schorfheide-Chorin	mixed forests, meadows	N 52° 53' E 13° 55'	90–120	5	6	110–720	8.4–165.0	0.05–0.95	63–90	6–27	0–10

6. CONCLUSION

Determined isoconversional times of humic substances samples were correlated to their primary characteristics, such as the content of aromatic and carboxylic carbon or other elemental features. During the calculations, four distinct (Arrhenius and 3 non-Arrhenius) equations were used as temperature functions. As a measure of mutual interrelationship, Pearson product-moment correlation coefficient was employed. The effort to correlate above mentioned parameters linearly resulted in little success concerning the correlation values. On the other hand, in case of e.g. carboxylic carbon or oxygen content, undisputable interrelations were found and described. The non-Arrhenius temperature function gave substantially higher correlation values; using the Arrhenius function, previously published findings were confirmed. In order to perform the least possible mathematical modifications of raw thermoanalytical data to avoid possible accumulation of errors, values of derivative thermogravimetric analyses were also correlated to primary properties of humic substances. Unlike in soil samples, where this approach leads to perfect agreement between elemental and thermogravimetric analysis, humic samples neither show high correlation coefficients nor was any trend of correlation during degradation observed.

The results of soil experiments confirmed the existence of close interrelationship between soil biological respiration estimated via CO₂ evolution in incubation experiments and thermal stability detected by mass losses using TGA. The combination of incubation experiments with thermal and simple elemental analyses can open the way to new methodical approaches. Narrow temperature intervals are useful for carbon content determination and imply a connection to carbon regulation processes. The possibility to monitor changes in soil composition induced by microbial activity confirms the attractiveness of TGA to identify soil components that change during biological processes and should be analyzed with other methods. Obtained results hint at the importance of water-binding processes and should be verified as a key factor for transformation processes in carbon cycling. Therefore, hydration of air-dried samples has to be taken into account during sampling and sample preparation for soil incubation and TGA experiments to get comparable results about intrinsic properties of soils as a product of interrelated biological and non-biological processes.

Summarizing all results, the combination of TGA with other analytical techniques and methods and approaches should promote better understanding of humic substances degradation behaviour and of soil intrinsic properties and distinguish them from other mineral substrates containing carbon upon long-term formation processes.

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8. LIST OF ABBREVIATIONS

ACC	alternating current calorimetry
AEC	anion exchange capacity
BET	Brunauer-Emmett-Teller theory (for the physical adsorption of gas molecules on a solid surface)
CEC	cation exchange capacity
DEAE	diethylaminoethyl (cellulose)
DMA	dynamic mechanical analysis
DOSY-NMR	diffusion ordered nuclear magnetic resonance spectroscopy
DrTGA	derivative thermogravimetric analysis
DSC	differential scanning calorimetry
DTA	differential thermal analysis
DTG	derivative thermogravimetric analysis
EGME	ethylene glycol monomethyl ether (2-methoxyethanol)
ESI-MS	electrospray ionization mass spectroscopy
FA	fulvic acid
FTIR	Fourier transform infra red (spectroscopy)
HA	humic acid
HS	humic substances
ICTAC	International Confederation for Thermal Analysis and Calorimetry
IHSS	International Humic Substances Society
ITC	isothermal calorimetry
NaOD	deuterated sodium hydroxide
NMR	nuclear magnetic resonance
NOM	natural organic matter
NPK	nitrogen-phosphorus-kalium (potassium)
SOC	soil organic carbon
SOM	soil organic matter
TA	thermal analysis
T _g	glass transition temperature
TG	thermogravimetry
TGA	thermogravimetric analysis
TGSA	thermogravimetric soil analysis
TL	thermoluminescence
TMA	thermomechanical analysis
TMDSC	temperature-modulated differential scanning calorimetry
TSC	thermostimulated current
USDA	United States Department of Agriculture
XAD	adsorbent resin
XRD	X-ray diffraction

9. LIST OF PUBLICATIONS AND ACTIVITIES

Impacted publications:

1. KISLINGER, J., Kučerík, J., Demyan, M.S., Siewert, C.: Does thermal analysis reflect biological properties of soils? Submitted to *European Journal of Soil Science*. 2010
2. Kučerík, J., KISLINGER, J., Majzlík, P., Pekař, M.: Correlation of humic substances chemical properties and their thermo-oxidative degradation kinetics. Part 1. Arrhenius equation as the temperature function. *Journal of Thermal Analysis and Calorimetry*. 2009, vol. 98, no. 1, p. 207–214. ISSN: 1388-6150.
3. Válková, D.; KISLINGER, J., Pekař, M., Kučerík, J.: The kinetics of thermo-oxidative humic acids degradation studied by isoconversional methods. *Journal of Thermal Analysis and Calorimetry*. 2007, vol. 89, no. 3, p. 957–964. ISSN: 1388-6150.
4. Kučerík, J.; Kamenářová, D.; Válková, D.; Pekař, M.; KISLINGER, J.: The role of various compounds in humic acids stability studied by TG and DTA. *Journal of Thermal Analysis and Calorimetry*. 2006, vol. 84, no. 3, p. 715–720. ISSN: 1388-6150.

Given speeches:

5. Jahrestagung der Deutschen Bodenkundlichen Gesellschaft 2009, Bonn: KISLINGER, J.: Thermal and biological stability of soil organic matter. In: *Böden - eine endliche Ressource*. Bonn, 2009.
6. 4th Meeting on Chemistry and Life 2008, Brno: KISLINGER, J., Novák, F., Kučerík, J.: Role of Aromaticity in Humic Substances Degradation Kinetics Using Non-Arrhenius Temperature Functions. *Chemické listy*, 2008, vol. 102, no. S, p. 1086–1088. ISSN: 1213-7103.

Conference contributions:

7. Kislinger, J., Kučerík, J., Siewert, C.: Does thermal stability of soil organic matter resemble its microbiological properties? In *Advances in Natural Organic Matter and Humic Substances Research 2008-2010 (Vol. 2), Proceedings Book of the Communications presented to the 15th Meeting of the International Humic Substances Society, Tenerife - Canary Islands*. Sevilla & Puerto de la Cruz, Spain: International Humic Substances Society, Institutional Repository of Consejo Superior de Investigaciones Científicas (CSIC), 2010. p. 299-303.

8. Kislinger, J., Novák, F., Kučerík, J.: Role of Aromaticity Degree in the Stability of Humic Substances. In *Proceedings of the 14th Meeting of International Humic Substances Society: From molecular understanding to innovative applications of humic substances*. Leninskíe Gory 1–3, Moscow, Russia: Department of Chemistry, Lomonosov Moscow State University, 2008. p. 253–256. ISBN: 80-248-0786-6.
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10. Kislinger, J., Kučerík, J.: Relationship between soil respiration and kinetic parameters of thermo-oxidative degradation. In *Livro de resumos do VII EBSH*. Florianopolis, Brazilie: Brazilian humic substances society, 2007. p. 5.
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12. Čechlovská, H., Kislinger, J., Pekař, M., Kučerík, J.: Kinetika degradace frakcí huminových kyselin ve vztahu k jejich biologické dostupnosti. In *Sborník příspěvků*. Brno: Masarykova univerzita, 2007. p. 25–26. ISBN: 978-80-210-4235-3.
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17. Válková, D., Kučerík, J., Pekař, M., Kislinger, J.: Application of isoconversional methods for evaluation of kinetics of thermo-oxidative humic acids degradation studied by DSC. In *Termanal 2005*. Bratislava: Slovenská chemická spoločnosť, 2005. p. 106–107. ISBN: 80-227-2285-5.

Trainings and courses:

1. Workshop Landnutzung Sibirien (FH Weihenstephan, Freising, 2008)
2. 'Interpretation of IR vibration spectra' course (Institute of Chemical Technology Prague, 2008)
3. International Summer School – Natural Organic Matter: A source for environment and nanotechnologies (University of Naples, 2007)
4. NETZSCH Workshop – thermal analysis (Brno, 2006)
5. Summer School of Thermal Analysis and Calorimetry (Slovak University of Technology in Bratislava, 2004 and 2005)
6. METTLER TOLEDO Workshop – thermal analysis (Brno, 2004 and 2008)

10. LIST OF APPENDICES

- APPENDIX I: Conversion temperatures (in °C) of humic samples for two heating rates (1 °C min⁻¹ in the upper line and 7 °C min⁻¹ in the lower line)
- APPENDIX II: Conversion times (in years) of humic samples using Eq. (8), extrapolated to 25 °C
- APPENDIX III: Conversion times (in years) of humic samples using Eq. (9), extrapolated to 25 °C
- APPENDIX IV: Conversion times (in years) of humic samples using Eq. (10), extrapolated to 25 °C
- APPENDIX V: Conversion times (in years) of humic samples using Eq. (11), extrapolated to 25 °C
- APPENDIX VI: DTG values (mg weight loss per 1 g sample) of humic samples in 10 °C intervals

APPENDIX I – PART I

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
A65FK	99.24	190.12	223.74	247.13	266.33	285.02	304.07	321.56	340.70	396.01	445.57
	112.20	213.85	252.81	281.70	306.09	329.61	350.55	368.93	419.52	439.13	499.99
B2HK	92.62	208.05	241.74	265.81	285.98	306.19	325.72	340.81	350.89	360.54	496.35
	111.42	233.59	273.09	303.32	328.47	352.63	372.82	386.59	396.51	410.39	532.70
B660FK	95.75	187.07	215.95	233.86	246.67	257.95	271.06	320.66	405.01	434.02	499.02
	112.33	209.59	243.71	265.94	281.81	297.85	342.66	423.41	462.56	485.37	563.77
N65FK	99.57	188.08	219.55	241.57	259.32	276.23	293.58	308.69	354.71	409.47	470.32
	116.28	211.80	248.54	275.91	298.37	320.02	339.34	378.07	433.98	462.68	533.69
P42HK	100.81	201.85	240.13	270.99	300.95	334.19	365.42	390.52	411.05	429.45	475.41
	115.77	225.69	269.50	306.96	343.43	382.05	415.77	441.97	462.62	481.29	537.41
T15HK	97.10	214.79	248.92	273.74	295.89	320.84	347.97	371.14	389.05	404.04	506.21
	118.81	239.06	279.15	310.95	339.65	370.34	399.87	423.56	441.36	456.53	543.52
T25HK	98.15	214.01	246.45	270.42	291.12	313.17	335.94	354.20	366.67	375.41	516.80
	108.40	238.37	276.46	306.57	332.94	359.78	383.99	401.85	414.22	424.25	549.45

APPENDIX I – PART II

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
T55HK	102.36	209.33	244.79	272.11	297.40	322.51	341.79	353.45	360.13	365.15	485.06
	115.76	235.00	276.60	310.21	341.02	368.44	386.71	397.51	403.98	410.14	509.09
U22HK	103.60	204.14	239.81	264.92	285.64	306.77	328.56	347.25	362.90	383.90	508.50
	117.25	227.65	269.36	300.98	327.01	352.67	376.13	394.45	410.08	436.92	545.65
XF150HK	118.53	228.30	269.86	297.17	317.41	331.57	340.79	346.56	350.30	397.17	485.81
	143.90	260.16	308.88	340.33	361.10	373.65	377.88	383.12	389.85	462.39	535.43
XM1HK	123.44	249.57	291.00	319.33	338.59	349.96	356.51	360.55	363.93	417.18	484.09
	143.14	280.76	330.76	362.09	380.88	391.03	396.82	400.65	407.18	478.50	534.19
XP13HK	99.93	229.13	268.96	295.62	316.93	332.13	341.41	346.86	350.93	373.19	483.74
	119.10	260.00	307.90	338.18	359.83	373.31	380.97	385.10	390.62	443.96	541.84
RC17HK	113.19	220.64	277.97	326.07	356.40	373.28	383.84	392.09	401.19	437.89	492.70
	126.26	246.52	313.57	368.60	402.24	420.66	432.20	441.98	455.95	490.45	555.90

APPENDIX II

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
A65FK	3.45E-01	1.36E+04	7.61E+04	8.18E+04	6.80E+04	6.38E+04	2.33E+05	2.93E+05	4.49E+03	5.95E+10	2.14E+11
B2HK	8.68E-02	1.35E+05	3.68E+05	2.05E+05	1.75E+05	3.26E+05	2.45E+06	3.22E+07	1.50E+08	9.27E+06	2.51E+16
B660FK	1.49E-01	2.52E+04	9.86E+04	3.88E+04	3.76E+04	7.84E+03	6.12E+00	1.19E+01	5.21E+07	5.42E+11	1.76E+13
N65FK	1.89E-01	9.01E+03	1.51E+05	4.12E+04	3.57E+04	4.89E+04	9.93E+04	1.82E+02	2.30E+03	2.83E+10	3.82E+11
P42HK	6.94E-01	1.60E+04	1.34E+07	1.44E+06	1.55E+06	1.06E+07	7.09E+08	2.11E+09	3.41E+10	4.50E+11	9.71E+10
T15HK	3.27E-02	9.55E+05	2.74E+07	8.98E+05	4.62E+05	6.55E+05	4.02E+06	5.16E+07	4.29E+08	1.94E+09	1.22E+16
T25HK	4.21E-01	6.04E+04	9.45E+05	5.89E+05	3.72E+05	5.53E+05	4.60E+06	1.09E+08	4.75E+08	1.90E+09	1.22E+24
T55HK	2.56E-01	4.11E+04	2.87E+06	3.29E+05	4.09E+05	2.72E+06	5.29E+07	4.15E+08	2.19E+09	7.55E+08	8.21E+22
U22HK	1.43E-01	1.66E+04	7.46E+05	6.05E+05	6.12E+05	5.81E+05	3.08E+06	4.03E+07	2.48E+08	2.15E+07	1.09E+18
XF150HK	2.18E-01	4.57E+04	1.62E+05	5.56E+05	4.86E+06	7.16E+07	2.02E+09	2.32E+11	9.90E+09	7.09E+05	5.38E+17
XM1HK	4.87E+00	8.08E+05	1.22E+06	6.88E+06	1.34E+08	1.47E+09	1.64E+10	3.22E+10	3.40E+09	1.07E+07	5.06E+13
XP13HK	3.00E-02	3.11E+04	8.67E+04	4.29E+05	6.79E+06	1.61E+08	9.77E+09	7.83E+10	2.03E+10	8.73E+05	7.90E+13
RC17HK	7.13E+00	1.33E+05	1.25E+08	2.40E+07	1.92E+08	9.60E+08	1.92E+09	3.19E+09	4.37E+08	4.91E+11	3.43E+16

APPENDIX III

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
A65FK	2.48E-01	2.59E+02	4.97E+02	3.60E+02	2.32E+02	1.83E+02	3.10E+02	2.91E+02	1.34E+01	3.45E+05	3.07E+05
B2HK	6.38E-02	9.91E+02	1.01E+03	5.05E+02	3.61E+02	4.25E+02	1.12E+03	5.31E+03	1.31E+04	1.87E+03	1.90E+08
B660FK	1.66E-01	4.46E+02	4.27E+02	2.48E+02	1.91E+02	5.58E+01	2.77E-01	3.04E-01	3.27E+03	6.50E+05	2.23E+06
N65FK	6.22E-02	1.87E+02	3.82E+02	2.18E+02	1.66E+02	1.26E+02	2.06E+02	2.57E+00	7.88E+00	8.91E+04	3.64E+05
P42HK	4.85E-01	1.66E+03	3.51E+03	2.30E+03	1.34E+03	2.58E+03	1.08E+04	4.53E+04	1.62E+05	5.50E+05	1.08E+05
T15HK	5.38E-03	4.52E+03	4.12E+03	1.13E+03	5.53E+02	4.72E+02	1.25E+03	4.62E+03	1.45E+04	3.17E+04	1.59E+08
T25HK	2.75E-01	2.06E+03	2.34E+03	9.41E+02	5.51E+02	5.51E+02	1.66E+03	6.92E+03	2.44E+04	2.59E+04	3.40E+14
T55HK	2.24E-01	7.67E+02	1.00E+03	6.93E+02	4.63E+02	1.26E+03	7.07E+03	3.10E+04	6.82E+04	3.81E+04	6.40E+12
U22HK	8.76E-02	1.67E+03	2.34E+03	1.11E+03	6.84E+02	5.41E+02	1.24E+03	5.29E+03	1.60E+04	2.30E+03	2.52E+08
XF150HK	6.13E-02	3.25E+02	5.10E+02	7.26E+02	2.68E+03	1.25E+04	1.73E+05	6.14E+05	2.68E+05	2.29E+02	2.88E+07
XM1HK	1.65E+00	1.90E+03	1.32E+03	3.62E+03	1.66E+04	7.26E+04	3.47E+05	4.13E+05	7.75E+04	1.05E+03	4.51E+06
XP13HK	1.36E-02	2.25E+02	2.53E+02	5.33E+02	2.58E+03	2.09E+04	2.99E+05	1.05E+06	3.56E+05	2.31E+02	4.94E+06
RC17HK	3.47E+00	2.18E+03	2.98E+03	5.58E+03	1.48E+04	2.81E+04	5.50E+04	5.68E+04	1.13E+04	4.68E+05	2.16E+08

APPENDIX IV

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
A65FK	1.00E-01	1.22E+01	1.45E+01	8.33E+00	5.03E+00	3.60E+00	4.93E+00	4.30E+00	4.09E-01	2.79E+02	1.63E+02
B2HK	3.03E-02	2.66E+01	2.06E+01	9.34E+00	5.86E+00	5.46E+00	1.05E+01	2.70E+01	4.46E+01	1.13E+01	7.58E+03
B660FK	6.72E-02	1.89E+01	1.28E+01	7.49E+00	5.50E+00	1.95E+00	4.36E-02	3.25E-02	1.13E+01	2.95E+02	4.37E+02
N65FK	2.97E-02	1.03E+01	1.15E+01	6.59E+00	4.30E+00	3.11E+00	3.91E+00	1.68E-01	2.55E-01	9.85E+01	1.80E+02
P42HK	1.80E-01	4.68E+01	5.75E+01	2.79E+01	1.59E+01	1.81E+01	3.72E+01	8.20E+01	1.82E+02	3.42E+02	8.56E+01
T15HK	8.63E-03	7.96E+01	5.00E+01	1.70E+01	7.99E+00	5.68E+00	9.17E+00	1.94E+01	3.57E+01	5.25E+01	6.75E+03
T25HK	1.07E-01	4.63E+01	3.33E+01	1.43E+01	7.98E+00	6.54E+00	1.19E+01	3.03E+01	5.73E+01	6.46E+01	4.50E+07
T55HK	8.57E-02	2.32E+01	1.93E+01	1.05E+01	7.32E+00	1.18E+01	3.24E+01	7.72E+01	1.26E+02	8.08E+01	7.22E+06
U22HK	3.88E-02	4.25E+01	3.75E+01	1.62E+01	9.07E+00	7.19E+00	1.12E+01	2.63E+01	4.62E+01	1.21E+01	8.37E+03
XF150HK	2.60E-02	1.13E+01	1.02E+01	1.18E+01	2.35E+01	6.60E+01	3.21E+02	7.62E+02	4.34E+02	2.59E+00	3.13E+03
XM1HK	4.29E-01	3.41E+01	1.71E+01	2.50E+01	6.46E+01	1.63E+02	4.19E+02	4.77E+02	1.63E+02	5.25E+00	8.81E+02
XP13HK	7.47E-03	7.34E+00	5.80E+00	7.97E+00	2.03E+01	7.02E+01	3.90E+02	1.00E+03	4.64E+02	2.37E+00	7.33E+02
RC17HK	8.73E-01	4.41E+01	2.89E+01	3.10E+01	4.47E+01	6.82E+01	9.03E+01	8.99E+01	2.91E+01	2.44E+02	7.72E+03

APPENDIX V

	Conversion (%)										
	0	10	20	30	40	50	60	70	80	90	100
A65FK	1.11E-01	2.22E+01	3.13E+01	1.89E+01	1.17E+01	8.62E+00	1.32E+01	1.21E+01	8.40E-01	2.09E+03	1.48E+03
B2HK	3.22E-02	5.57E+01	4.98E+01	2.32E+01	1.49E+01	1.49E+01	3.39E+01	1.06E+02	1.99E+02	4.26E+01	2.02E+05
B660FK	7.33E-02	3.50E+01	2.64E+01	1.59E+01	1.19E+01	3.98E+00	6.02E-02	4.19E-02	4.95E+01	2.80E+03	6.38E+03
N65FK	3.21E-02	1.84E+01	2.39E+01	1.43E+01	9.57E+00	7.12E+00	9.73E+00	2.95E-01	4.85E-01	6.67E+02	1.89E+03
P42HK	2.02E-01	1.00E+02	1.54E+02	8.10E+01	4.98E+01	6.67E+01	1.77E+02	4.99E+02	1.42E+03	3.32E+03	7.85E+02
T15HK	9.02E-03	1.89E+02	1.37E+02	4.68E+01	2.21E+01	1.65E+01	3.17E+01	8.35E+01	1.84E+02	3.11E+02	1.94E+05
T25HK	1.18E-01	1.05E+02	8.60E+01	3.78E+01	2.16E+01	1.88E+01	4.07E+01	1.30E+02	2.89E+02	3.47E+02	1.01E+10
T55HK	9.45E-02	4.87E+01	4.69E+01	2.73E+01	2.01E+01	3.82E+01	1.31E+02	3.78E+02	6.91E+02	4.25E+02	7.32E+08
U22HK	4.19E-02	9.06E+01	9.50E+01	4.25E+01	2.44E+01	2.04E+01	3.69E+01	1.06E+02	2.20E+02	5.08E+01	2.37E+05
XF150HK	2.87E-02	2.48E+01	2.65E+01	3.52E+01	8.39E+01	2.92E+02	1.87E+03	5.23E+03	2.79E+03	8.80E+00	6.58E+04
XM1HK	5.21E-01	9.08E+01	5.14E+01	8.99E+01	2.91E+02	8.94E+02	2.76E+03	3.27E+03	9.64E+02	2.08E+01	1.40E+04
XP13HK	7.77E-03	1.52E+01	1.38E+01	2.19E+01	6.90E+01	3.06E+02	2.29E+03	7.03E+03	2.96E+03	6.97E+00	1.11E+04
RC17HK	1.05E+00	1.03E+02	8.56E+01	1.16E+02	2.04E+02	3.61E+02	5.28E+02	5.48E+02	1.52E+02	2.20E+03	2.12E+05

APPENDIX VI – PART I

	A65FK	B2HK	B660FK	N65FK	P42HK	T15HK	T25HK	T55HK	U22HK	XF150HK	XM1HK	XP13HK	RC17HK
50	30.75	20.10	26.24	30.60	17.19	18.90	23.64	22.42	17.57	23.54	23.79	24.55	28.49
60	15.06	9.30	13.39	15.53	8.77	7.86	10.26	10.55	8.65	13.15	11.66	12.02	15.48
70	9.11	5.11	7.84	9.15	5.01	3.83	5.09	6.04	4.25	8.09	7.18	6.84	9.89
80	5.97	3.07	4.87	5.87	3.07	2.08	2.80	3.79	2.32	5.25	4.78	4.24	6.97
90	4.31	1.81	3.25	4.12	1.75	1.09	1.77	2.72	1.53	3.66	3.51	2.67	5.27
100	3.37	1.13	2.32	3.31	1.49	0.76	1.23	1.99	1.21	2.72	2.82	1.87	4.37
110	2.91	1.35	2.18	2.83	1.31	0.91	1.08	1.86	1.44	2.39	2.42	1.72	3.74
120	3.12	1.57	2.52	3.20	2.11	1.18	1.32	2.03	1.81	2.47	2.28	1.92	3.48
130	3.87	2.19	3.30	3.67	2.16	1.68	1.71	2.35	2.73	3.10	2.34	2.26	3.56
140	4.70	2.71	4.65	4.91	3.05	2.45	2.47	2.96	3.22	3.35	2.51	2.69	3.93
150	6.26	3.62	6.19	6.34	4.16	3.41	3.16	3.82	4.65	3.94	2.98	3.32	4.45
160	7.57	4.73	8.00	7.84	5.89	4.53	4.07	4.71	5.97	4.56	3.26	3.87	5.06
170	9.02	6.25	10.12	9.57	7.39	5.46	5.23	5.85	7.60	5.20	3.53	4.62	5.61
180	10.59	7.78	12.42	11.61	8.74	6.86	6.54	7.15	9.15	5.86	3.86	5.55	6.49
190	13.02	9.62	14.95	14.06	10.59	8.64	8.26	8.95	11.31	6.45	4.32	6.31	7.46
200	14.85	11.79	17.88	16.77	12.35	10.39	10.65	10.94	13.47	7.24	5.18	7.18	8.60
210	17.47	14.61	20.73	19.25	13.87	13.35	13.38	13.41	15.46	8.19	5.83	8.03	9.71
220	19.81	17.48	23.50	21.97	15.70	16.22	16.57	15.78	18.10	9.17	6.62	9.12	10.68
230	22.04	19.96	27.43	24.27	17.60	19.18	19.73	18.07	20.15	10.64	7.57	10.36	11.57
240	24.40	22.31	31.98	26.46	18.22	21.45	22.39	19.90	21.87	12.02	8.88	11.68	11.99
250	26.29	23.90	38.27	29.83	19.15	23.16	24.23	21.59	23.35	13.48	10.00	13.18	12.65
260	29.00	25.89	46.62	32.76	20.06	24.34	25.54	23.35	25.30	15.03	11.42	14.72	12.90
270	31.77	28.19	55.29	35.95	21.01	26.11	27.84	24.70	27.72	16.79	12.75	17.00	13.06
280	34.10	31.23	57.59	38.57	21.93	28.04	30.15	26.08	30.11	18.50	14.24	19.47	13.30
290	35.48	33.78	49.12	39.81	22.24	30.93	32.93	27.58	33.90	20.76	16.18	22.27	13.39
300	36.64	36.49	32.51	40.02	22.96	33.04	35.35	28.83	35.78	23.38	17.93	25.34	13.72
310	36.44	37.58	16.55	40.56	22.49	34.15	36.61	29.49	36.96	26.14	20.09	28.29	13.96
320	37.13	38.01	11.90	43.07	22.67	33.91	36.53	29.65	36.89	29.32	22.68	31.36	14.51

APPENDIX VI – PART II

	A65FK	B2HK	B660FK	N65FK	P42HK	T15HK	T25HK	T55HK	U22HK	XF150HK	XM1HK	XP13HK	RC17HK
330	38.41	38.98	10.54	48.72	21.57	33.05	35.49	29.92	36.51	33.89	25.56	34.81	15.21
340	42.60	39.91	9.77	40.94	21.20	31.93	32.80	30.82	37.40	39.23	28.58	39.57	16.20
350	47.54	43.24	9.53	17.13	21.12	30.69	34.77	33.48	37.74	49.18	33.15	47.91	17.82
360	41.28	50.06	9.28	11.38	20.92	30.25	36.82	38.22	40.38	6.78	41.26	65.71	19.73
370	20.97	62.82	9.38	10.17	21.34	30.71	40.32	48.17	45.56	146.62	57.32	112.47	22.48
380	13.16	87.91	9.65	10.00	22.80	32.31	47.18	67.61	52.83	136.23	99.48	183.04	26.38
390	11.91	93.78	10.01	10.97	24.23	35.01	57.62	113.93	67.81	13.75	20.12	36.65	32.84
400	13.50	30.96	10.56	13.29	26.20	38.27	76.19	128.46	57.37	8.21	61.66	7.61	43.57
410	19.46	6.47	11.57	16.47	28.76	43.52	93.28	12.71	44.05	8.04	8.81	6.20	58.34
420	36.99	5.63	13.21	19.87	31.81	49.51	43.96	5.20	25.49	8.74	9.54	5.84	77.84
430	48.09	6.40	16.25	24.68	35.75	56.28	5.55	9.62	10.54	10.01	9.55	5.92	90.21
440	35.44	6.90	21.64	28.85	38.33	61.68	3.07	12.04	7.62	11.63	7.90	6.74	68.09
450	20.19	7.17	28.83	28.75	41.03	52.54	3.83	9.09	8.41	13.75	7.37	8.94	23.52
460	6.52	7.54	35.04	26.17	42.59	14.59	5.33	5.89	9.49	14.96	10.46	12.69	15.18
470	2.11	8.72	37.91	24.90	39.94	6.58	6.02	3.91	9.88	15.50	19.31	15.21	25.19
480	0.66	10.63	35.66	18.59	28.32	8.19	4.89	2.62	10.34	16.45	34.15	17.67	32.07
490	0.42	13.21	28.27	6.15	14.67	8.96	3.56	2.33	10.94	19.59	32.11	17.91	33.37
500	0.08	9.56	11.64	1.53	8.33	7.65	3.17	1.78	10.63	13.31	7.94	11.06	32.50
510	0.12	3.14	4.55	0.40	5.14	3.63	3.47	1.59	8.24	3.54	2.14	3.83	10.51
520	0.12	1.19	2.95	0.30	3.89	1.67	4.21	1.67	4.09	0.68	0.96	0.93	1.94
530	0.13	1.07	1.65	0.39	4.02	0.76	2.62	1.62	1.08	0.38	0.46	0.26	0.60
540	0.06	0.84	0.62	0.16	4.12	0.35	0.55	1.27	0.63	0.24	0.30	0.22	0.45
550	0.20	0.66	0.39	0.14	3.67	0.50	0.23	1.35	0.45	0.16	0.33	0.27	0.48
560	0.08	1.06	0.35	0.22	3.79	0.52	0.29	1.48	0.50	0.20	0.56	0.11	0.51
570	0.23	0.68	0.42	0.20	2.84	0.58	0.29	1.20	0.48	0.19	0.51	0.08	0.64
580	0.26	0.98	0.46	0.23	2.69	0.73	0.22	1.01	0.11	0.22	0.56	0.05	0.77
590	0.28	0.91	0.63	0.26	2.38	0.94	0.25	0.91	0.41	0.12	0.70	0.07	0.98