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TRANSMISSION ELECTRON MICROSCOPY (TEM) AS A TOOL FOR IDENTIFICATION OF COMBUSTION PRODUCTS: APPLICATION TO BLACK LAYERS IN SPELEOTHEMS

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Abstract: The present study deals with the application of High Resolution Transmission Electron Microscopy (HRTEM) to dark layers, occurring in the speleothems of Domica Cave (Slovakia). Chemical pre-treatment was necessary for sample purification and the effective extraction of carbon soot. For purposes of comparison, soot aggregates obtained from laboratory experiments on the combustion of beech wood and collected from a diesel engine also were studied. HRTEM analyses of combustion products permit a distinction to be made between soot aggregates that originated in different combustion processes. The diameter of spherical, primary particles depends on the conditions of combustion, notably temperature. Burning in diesel engines produces soot with relatively small, primary particles (diameter $d_p = 34 \pm 4$ nm). Primary, spherical particles of soot aggregates, obtained from the combustion of beech wood, were larger (diameter $d_p = 42 \pm 5$ nm). The diameters of primary particles of soot separated from Domica flowstones (samples DOM1 and DOM2) were similar to the wood samples ($d_p = 50 \pm 9$ nm). Another type of carbonaceous particle, obtained in the combustion process, had a spherical shape, but the diameter of about 50–500 nm was significantly larger than that of soot. Analyses performed on two samples (DOM S1 and DOM S2) confirmed that the black laminae owed their colour to particles, formed during wood combustion and later retained in the speleothems.

Key words: Transmission electron microscopy, soot aggregates, wood combustion, speleothems, dark layers.

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INTRODUCTION

Optical microscopy and scanning electron microscopy (SEM) are common techniques in mineralogical and structural investigations of geological and archaeological samples. The more complex and advanced transmission electron microscopy (TEM) so far has been rarely used in geological and archaeological research. However, several examples could be given of where it confirmed its usefulness. TEM was used together with other analytical methods in a comparative study of ancient pottery (Mata *et al.*, 2002), to identify copper- and iron-based pigments (Vermilion *et al.*, 2003), to characterize the microstructures of opaque glass mosaics from a mediaeval church in Italy (Roe *et al.*, 2006) and for studies of paintings on a Thracian tomb wall (Glavcheva *et al.*, 2016). The crystallography, the composition and the defects of ancient Egyptian cosmetic powder were examined by TEM (Deeb *et al.*, 2004). High resolution mode (HRTEM) was used in studies on the role of iron in authentic and synthetic samples of Maya blue (a famous blue pigment, composed of palygorskite clay and indigophase; Polette *et al.*, 2002). TEM was used in investigations on the role of goethite dehydration in the process of red pigment production (Pomies *et al.*, 1999a, b) and for the identification of bone heated to low temperatures (Koon *et al.*, 2003). A promising area of application for TEM, important to geology and archaeology, is to products of combustion. In this case, TEM, and especially its high resolution mode (HRTEM), is the only technique able to image and thus to distinguish the different nanostructures (different spatial organizations of the nanometre-sized graphene layers in the nm–μm range).

The black residue that remains after wood combustion is a common material for archaeological and geological investigations. Most published results from these analyses concern carbonaceous microstructure studies, radiocarbon

analyses and stable isotopic analyses in aerosols and clastic deposits (Currie *et al.*, 1997; Masiello *et al.*, 2002; Brodowski *et al.*, 2005). Investigations of soot, treated as laboratory analogues of interstellar carbonaceous matter, were applied for astrophysical purposes, to evaluate the possible implication of soot for the greenhouse effect (Carpentier *et al.*, 2012). The primary reason for these studies was increasing interest in the potentially harmful impacts of nanoparticles on humans and the environment (see review and references in Nowack and Bucheli, 2007). Only a few studies have examined the carbonaceous material from caves. These studies have mostly been for archaeological purposes and to provide information about genesis of the carbon (Petranek and Pouba, 1951; Bennington *et al.*, 1962; Watson, 1966; Hill, 1982; Steelman *et al.*, 2002; Gradziński *et al.*, 2002, 2003, 2007; Chang *et al.*, 2008).

Residues from wood combustion may form a concentrated black layer or may be dispersed in the sediment. In the second case, the identification of the products of burning wood is more difficult. The investigation of the accumulated products of wood combustion in sediments provides an opportunity for determination of their provenance, as a result of human activity or natural phenomena, such as forest or meadow fires. In archaeological and geological investigations, it is also very important to distinguish the products from different sources that can be found commonly in the modern environment (e.g., wood burning in fires, fossil fuel combustion in engines and by industry). The application of credible procedures for the separation and identification of combustion products is necessary.

Dark, almost black layers inside or covering the surfaces of speleothems are relatively frequent in caves worldwide (see references in Gradziński *et al.*, 2003). The black layers could have been formed as a result of burning inside a cave in the following way. During the burning of fires or torches, black smoke with microscopic, solid particles appeared. The particles floated upwards and were deposited on the cool surfaces of the speleothems or the cave walls. The black particles also could have been transported by wind or by water from outside the cave. Sometimes layers of calcite later were formed on the surface, protecting the black layer (laminae) against destruction. Until now, optical and scanning electron microscopy techniques were applied in investigations of such black layers. On the basis of the results obtained, it was concluded that the black layers consist of not entirely carbonized, organic compounds and charcoal particles (Gradziński *et al.*, 2007 and references cited here). It is not complete information, since during the combustion of organic material a number of other solid particles are also formed (Fernandes *et al.*, 2003; Chen *et al.*, 2005; Zupančič *et al*., 2011; Šebela *et al*., 2015). The most common combustion products include soot aggregates, described as C_{soot} in the next part of the paper. C_{soot} has a characteristic structure and can be identified easily, even in extremely low concentrations. C_{soot} particles are small (nanometric in size, 1 nm = 1^{-9} m) and form chain-like aggregates that are irregular in shape. For this reason, C_{soot} aggregates could easily float and stick to the cold surface of a stalagmite or the cave walls. So far, C_{soot} particles were passed over in geoscience investigations as being too small to be easily detected and observed, using optical and scanning electron microscopes. The aim of the present authors is to apply TEM and especially its high resolution mode (HRTEM) in such research (Williams and Carter, 1996). As distinct from scanning electron microscopy, in TEM the electron beam is transmitted by the sample and the signals detected (elastically and inelastically scattered electrons) are collected below the sample. The main advantage of using TEM is the possibility of obtaining high resolution and investigating nanometric particles. Because of their characteristic morphology (described below), C_{soot} particles are easily distinguishable from other solid particles (Pósfai and Molnár, 2000). TEM makes possible the identification of C_{soot} on the basis of the analysis of extremely small amounts of material (mass below 1 nanogram). As carbon soot particles form only during combustion, even such a small amount of material can be sufficient to confirm that the combustion process (natural or caused by people) occurred at a specific place.

Another aim of this study is to provide proof that the dark (almost black) coloured layers within the speleothems studied are dyed by compounds formed during the combustion of wood, which in combination with the analysis of their distribution within the cave can be confirmation that black layers are the result of wood combustion in particular caves. Efforts were focused on several aspects: (1) the characterization of carbon soot (C_{soot}) particles formed during wood combustion in conditions similar to the burning of bonfires and torches, (2) the development of a method of extraction and purification of carbon soot (C_{soot}) from speleothems layers, (3) the characterization of solid carbonaceous particles from speleothem layers and (4) the comparison of soot aggregates separated from speleothems and from a modern diesel engine.

CARBON SOOT (C_{SOOT}) FORMATION AND MORPHOLOGY

Soot is the product of the incomplete combustion of organic materials, very common in the environment. It consists of almost only carbon atoms (as in diamond, graphite, coal, carbon nanotubes, fullerenes and others). Soot is characterised by a specific structure at nanometric scale. It is worth emphasizing that up to a few dozen years ago, soot was presented as an example of amorphous and completely disordered material. This untrue opinion can still be found in numerous publications. Misunderstandings concerning these materials also are connected with the terminology used. Soot, carbon black and black carbon describe similar fractions of carbon materials and sometimes are used interchangeably. The most frequently used term “soot” is reserved for the product, formed during the combustion of substances, including a considerable amount of carbon, without sufficient access of the oxygen. It concerns mainly natural situations, such as fires in forests or meadows, as well as human activity connected with wood, coal or oil/gas combustion in stoves, furnaces and car engines. All of the phenomena described coexist with the formation of smaller or larger amount of black smoke and emissions of fine solid carbonaceous particles to the atmosphere. The particles are

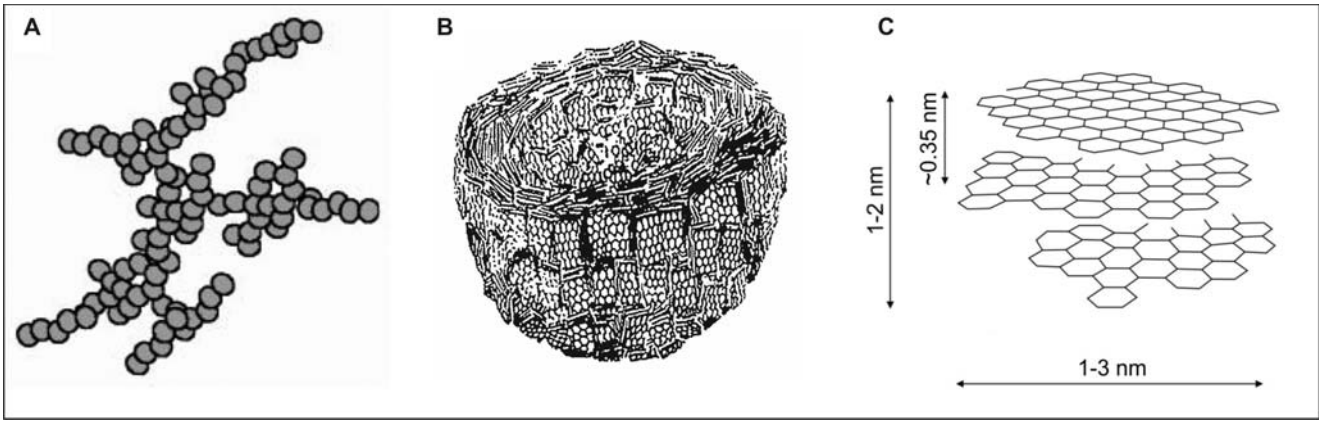

Fig. 1. Multiscale organization of C_{soot} particles (scheme, not drawn to scale). **A.** A soot aggregate composed of spherical particles. **B.** Spherical primary particle internal structure (Heidenreich *et al*., 1968). **C**. Basic unit of spherical soot particle – ordered domain composed of small, parallel carbon layers.

diverse in size (from a few nanometres to a few hundreds of micrometres), structure, morphology and chemical composition. All of them include a significant amount of carbon, but additionally small amounts of other elements can be present.

A special type of industrial carbon material is "carbon black" (CB; Hess and Herd, 1993). CB finds applications in the production of pigments, varnishes and inks, as a filler in rubber compounds (tires) and as an additive to plastics. CB also is formed as a result of the combustion of organic matter. The main difference with soot is that CB is obtained under controlled conditions (pressure, temperature, precursors). As a result, a highly homogenous black powder is obtained, consisting of fine, solid particles with the characteristic soot morphology (Fig. 1A). CB aggregates consist of from a few dozen to a few thousand spherical, primary particles. The diameter of each primary particle is about a few to a few dozen nanometres. The number of primary particles and their diameters and connections depend on manufacturing parameters (mainly temperature, pressure and duration of the process; Kuhner and Voll, 1993). Soot, formed as a result of combustion in uncontrolled (natural) conditions (e.g., fires, bonfires), is a mixture of diverse types of particle (e.g., tar balls, charcoal), but usually a considerable part of them is particles with a morphology characteristic for CB, in the present paper described as C_{soot}. In some areas of science (research connected with climate changes, if the fraction of carbon obtained as a result of combustion processes is evaluated), for substances consisting mainly of carbon, the term black carbon (BC) is used (Bond *et al.*, 2013). BC, in this sense, consists of carbon particles emitted to the atmosphere. Those dark particles in the atmosphere (which can later fall and accumulate on the ground or in water reservoirs), absorb sunlight and contribute to increases in temperature. The opposite of them is organic carbon (OC), which consists of particles scattering the light (Bond *et al.*, 2013).

C_{soot} particles form during the incomplete combustion of organic materials (Donnet *et al*., 1993; Bockhorn, 1994). Under ideal conditions, CO_2 and H_2O are the main products of burning reactions. If the amount of available oxygen is not sufficient for complete oxidation, C_{soot} particles are formed. Because wood combustion is a violent and heterogeneous process, there are always some volumes, in which the amount of oxygen is not sufficient. Then a condensation nucleus of merely a few atoms of carbon occurs and around them spherical, primary particles with a diameter about 10–100 nm are formed, consisting of merely a few million carbon atoms. Next the spherical, primary particles join, forming three-dimensional, complex aggregates. The smallest, indivisible unit of soot particles is an aggregate, schematically presented in Fig. 1A. Aggregates consist of joined, solid spherules (primary particles, Fig. 1B), which form long, branched chains. Typically, aggregates have an irregular shape. Sometimes aggregates are more compact and close, but even then empty space occupies a significant part of them. Pores are present within and among the aggregates. As a result, their exterior surface is very large in comparison to their mass (even more than 1000 m^2/g). Spherical, primary particles with a diameter of 10–50 nm are characterized by an onion-like nanostructure and comprise smaller, amorphous and ordered domains. The diameter of spherical, primary particles is controlled by the conditions of burning, mainly the burning temperature. The amorphous domains, situated mainly in the central part of the particle, are disordered mixtures of polycyclic, aromatic hydrocarbons (PAH) and other organic and inorganic components. Ordered domains (Fig. 1C) in the exterior part are arranged concentrically, parallel to the particle perimeter. The distance between the parallel carbon layers is constant and approximately equals 0.335 nm, similar to pure graphite (Franklin, 1951; Rouzaud *et al.*, 2004).

METHODS AND MATERIALS

Transmission electron microscopy for C_{soot} investigations

C_{soot} particles are too small to be analyzed and imaged by light microscopy. The resolution of modern scanning electron microscopes is sufficient for such a task. This is confirmed by the result presented in Fig. 2, where a charac-

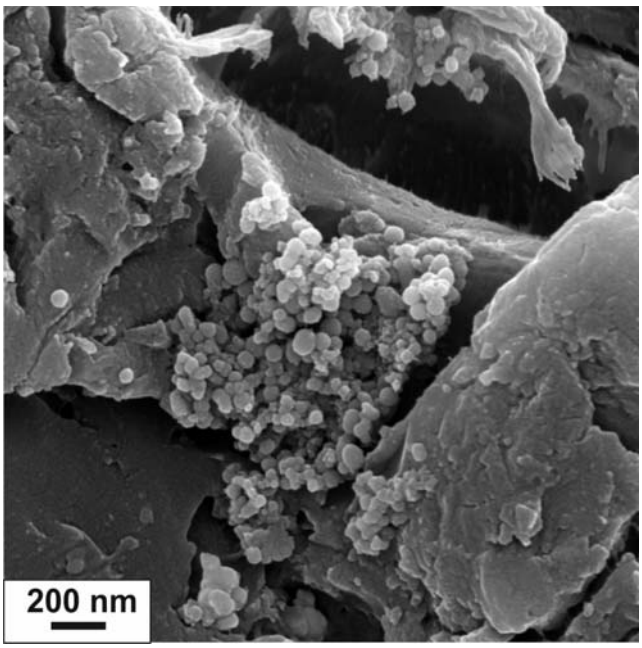

Fig. 2. SEM image of the C_{soot} aggregate in flowstone sample collected in Domica Cave (Slovakia).

teristic soot aggregate, including spherical, primary particles with a diameter about 50 nm, is visible. Nevertheless, detailed investigations of C_{soot} with scanning electron microscopy are not effective and particle identification is not completely credible with that technique. Usually for identification of particles observed by means of a scanning electron microscope, energy dispersive X-ray spectroscopy (EDS) is additionally applied, which make possible determination of the chemical composition (Williams and Carter, 2009). In the case of C_{soot} , EDS is useless, because its spatial resolution is too small. The signal detected is collected from a volume with a diameter equal to about 500 nm, which is too large for the investigation of soot aggregates. The second reason is that carbon (as a light element, Z = 6) is not credibly detected by EDS.

There are two main advantages in the application of transmission electron microscopy (TEM) for investigations of samples, formed as a result of the combustion of organic materials, such as wood. Firstly, it makes possible investigation not only of the morphology (imaging of the exterior shape of the particle), but also its internal structure (imaging of the arrangement of carbon layers inside the particles). The arrangement of carbon layers is characteristic for particles obtained from combustion processes and it directly confirms that these particles were formed in such a way. Secondly, the analysis of chemical composition using of EDS spectroscopy in transmission microscope is more precise (spatial resolution is about a few nanometres, characteristic X-ray emission is less absorbed in thin samples) and for that reason, more credible.

The primary probe applied in TEM is high-energy electrons (accelerated to a few hundreds of keV). The distance that can be resolved on TEM images depends on their wavelength and for modern instruments lies in the range from 50 to 100 pm. Owing to Coulomb forces, electrons interact strongly with matter and various signals can be obtained at the same place and at the same time, which is an important advantage of TEM. Elastically scattered electrons are used for imaging and diffraction, inelastically scattered electrons for electron energy loss spectrometry (EELS) and X-ray emission for energy dispersive X-ray spectroscopy (EDS). The last two are used for chemical analysis (Williams and Carter, 1996; Spence, 2003). Because only electrons transmitted through samples are analysed, the samples have to be very thin (<200 nm). For C_{soot} that condition is met automatically, because the diameter of primary particles is less than 100 nm. C_{soot} consists of small, curved and strongly defected carbon layers (one-atom-thick graphitic layers). The distance between them is equal to about 0.335 nm (typical for graphite). If they are parallel to the optical axis of the microscope, they are visible on TEM images as lines (Oberlin, 1989). Layers arranged not parallel to the optical axis are transparent. In the case of C_{soot}, layers parallel to optical axis are at the same time approximately parallel to each other and to the exterior surface of the primary particles. Such an onion-like arrangement of carbon layers occurs only for C_{soot} particles and it is connected with the process of their formation during combustion.

Analytical procedures

Speleothems samples pre-treatment

Two methods of specimen preparation were tested. First, a small amount of sample was scratched using a sharp tool (scalpel) and dispersed in ethanol, using an ultrasound. The principal disadvantages of this treatment were that the resulting specimen consisted of fragments of the sample, not sufficiently crushed to be observed effectively by TEM; well prepared samples for TEM observation should be less than 200 nm thick. Moreover, the specimen obtained included mainly mineral particles and even during very careful observation carbonaceous particles could not be seen between others. For these reasons, the speleothem fragments were chemically treated. The samples were crushed first and next ground in an agate mortar. Then they were dissolved in 2% HCl (3 × 24 h) to remove carbonates and finally during 1 hour in 40% HF to remove mineral grains. Finally, the samples were cleaned repeatedly (about 10 times) in distilled water. As a result, an intense, dark solution was obtained. One drop of that solution was diluted in ethanol and dispersed for 3 minutes by ultrasound. Finally, one drop of almost transparent liquid, containing uniformly dispersed nanoparticles of the tested material, was put on a TEM grid, coated with a lacey carbon film. After ethanol evaporation, the sample was ready for TEM observations.

TEM Procedure

TEM imaging was performed on a Philips Tecnai F20 (point resolution of 0.24 nm), operated at 200 kV accelerating voltage. High resolution TEM (HRTEM) images were recorded under optimal focus conditions.

For TEM characterization, particles were deposited on the perforated carbon film, the thickness of which was approximately 50 nm. The presence of the carbon film prevented good-quality HRTEM images from being obtained

and caused false results in the EDS analysis. For this reason, only unsupported particles, located over the holes in the carbon support, were analyzed. Spectroscopic studies were performed simultaneously by means of the same microscope. The chemical composition permits the clear distinction of carbonaceous and mineral particles. The spatial resolution of a chemical analysis is limited by the degree of focus of the electron beam and is equal to 2 nm. SEM images in In-Lens mode were obtained using a Zeiss Supra 35 field emission SEM.

Materials studied

Sample from diesel engine

The sample from a diesel engine ("diesel soot") was obtained directly from the tail pipe of a diesel engine. A few milligrams of the samples were dispersed in ethanol in two successive dilutions and ultrasound was used for dispersion of primary aggregates without secondary aggregates. A drop of suspension was deposited onto TEM grids, coated with a lacey carbon film. Such a procedure is a commonly used technique for soot sample preparation (e.g., Vander Wal *et al.*, 2007).

Samples from wood combustion

During the first stage of research, soot samples were collected during wood combustion under laboratory conditions. In order to obtain comparative material, an experiment on the burning of dry beech wood was carried out. TEM grids coated with a lacey carbon film were placed about 20–30 cm above the fire. Particles were collected, when the fire burned intensively. Particles stuck to the carbon film. Such samples could be tested in the transmission electron microscope.

Speleothems samples

Black layers in the flowstone collected in Domica Cave (Slovakia) were selected for the present study. Domica Cave is located in the Slovak Karst, near the Slovak-Hungarian border and is the Slovakian part of an extensive cave system with a total length of approximately 25 km. The Hungarian part of this system is named Baradla. Domica Cave is more than 5,000 m long (Droppa, 1970). The cave was settled by prehistoric humans in the Upper Palaeolithic and mainly during the Neolithic (Bárta, 1965; Lichardus, 1968, 1974; Soják, 2008). Samples were collected from the Hall of Courage (Seň odvahy) and the Virgin Passage (Panenská Chodba). The black-coloured layers in speleothems (samples DOM S1 and DOM S2) had been an object of earlier studies (see Gradziński *et al.*, 2007) in order to estimate the age of the prehistoric human activity in caves. As the black layers occur only in some speleothem' zones, this indicates that they were dyed periodically. The authors assumed that black layers gained their colour as a result of the burning of bonfires and torches by prehistoric men (Gradziński *et al.*, 2007). Optical microscopy and SEM investigations revealed that the black layers include carbonaceous particles connected with combustion processes, but there was no unambiguous proof that such particles were formed during wood combustion inside the caves.

RESULTS

Characterization of particles in the sample collected from a diesel engine

A typical diesel soot aggregate, collected from a diesel engine, is presented in Fig. 3A. It shows a branching structure and consists of a few hundred primary particles, which are very similar in size (diameter $d_p = 34 \pm 4$ nm). The boundaries between primary particles are not distinct. Numerous nuclei are visible inside the primary particles; this confirms that the soot was formed in a fast, vigorous process. As is typical for soot, the primary particles show concentrically stacked carbon layers (Fig. 3B), but the exterior, ordered shell is thin, only a few (up to 10) nanometres.

Characterization of particles in the sample collected during wood combustion

A typical soot aggregate formed during wood (beech) combustion is presented in Fig. 4A. An aggregate is stuck on the carbon film covering the microscopy grid. EDS analysis showed that the aggregate consists of almost pure carbon, with a small, additional amount of O, and vestigial amounts of Si and K. There are about 100 spherical, primary particles, very similar in size (diameter $d_p = 42 \pm 5$ nm). On the HRTEM image (Fig. 4B), the characteristic nanostructure is visible with a distinct exterior part. That part is well ordered, with carbon layers concentrically arranged, parallel to each other and to the exterior surface of the primary soot particle. The authors must emphasize that all of the other aggregates looked similar. They usually included a few dozen to a maximum of 200 regular (spherical), primary particles. This is consistent with earlier observations of the particulate emissions from wood combustion in old stoves, whereas soot aggregates formed in diesel engines include up to few thousand primary particles, which are significantly smaller ($d_p = 24 \pm 7$ nm) and more irregular in shape (Clague *et al.*, 1999; Kocbach 2006).

In addition to C_{soot} carbonaceous particles were also observed (Fig. 5). They were similar in chemical composition. All of them include mainly C and O; the amount of oxygen was higher than for C_{soot}, EDS analysis detected trace amounts of Si and K. In contrast to C_{soot} such particles did not form aggregates. Moreover, they had a different morphology (size and shape) and nanostructure (carbon layers arrangement). Some of them were irregular in shape (Fig. 5A). Irregular particles had different sizes, between a few nanometres and a few micrometers. Carbon layers visible on high-resolution TEM images showed an anisotropic arrangement (Fig. 5B). The other type of carbonaceous particle had a spherical shape and a diameter of about 50–500 nm (Fig. 5C). Even under high magnification, carbon layers are not visible (Fig. 5D), which is characteristic for C_{soot} and charcoal. In earlier papers, such amorphous particles were named tar balls (Posfai *et al.*, 2004). Amorphous-like micro- or nanoparticles were also observed in the nascent soot, obtained under laboratory conditions, especially at lower temperatures (Alfe, 2009; Rouzaud *et al.*, 2015). The lack of any microstructure clearly means that tar balls are a distinct particle type, rather than just individual soot spherules.

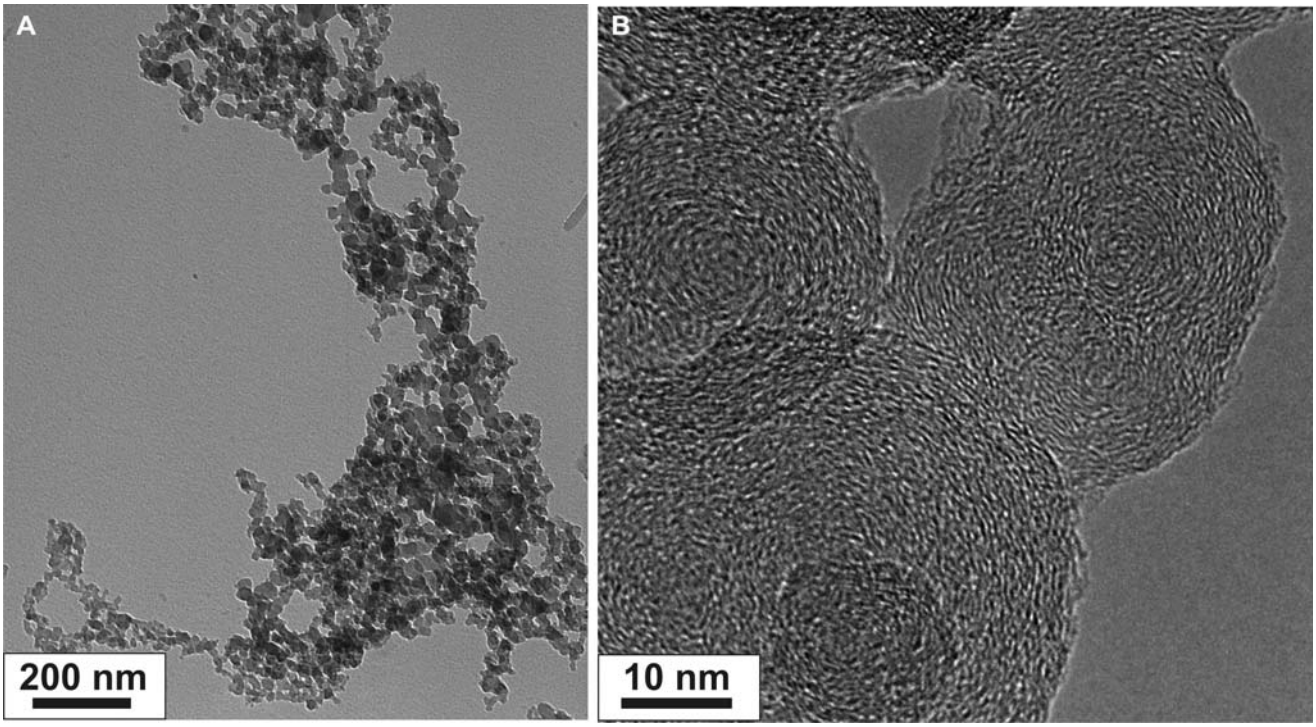

Fig. 3. Typical soot sample collected from diesel engine. **A.** TEM image of typical diesel soot aggregate showing branching structure. **B.** HRTEM image of diesel soot primary particles in A showing concentrically stacked carbon layers.

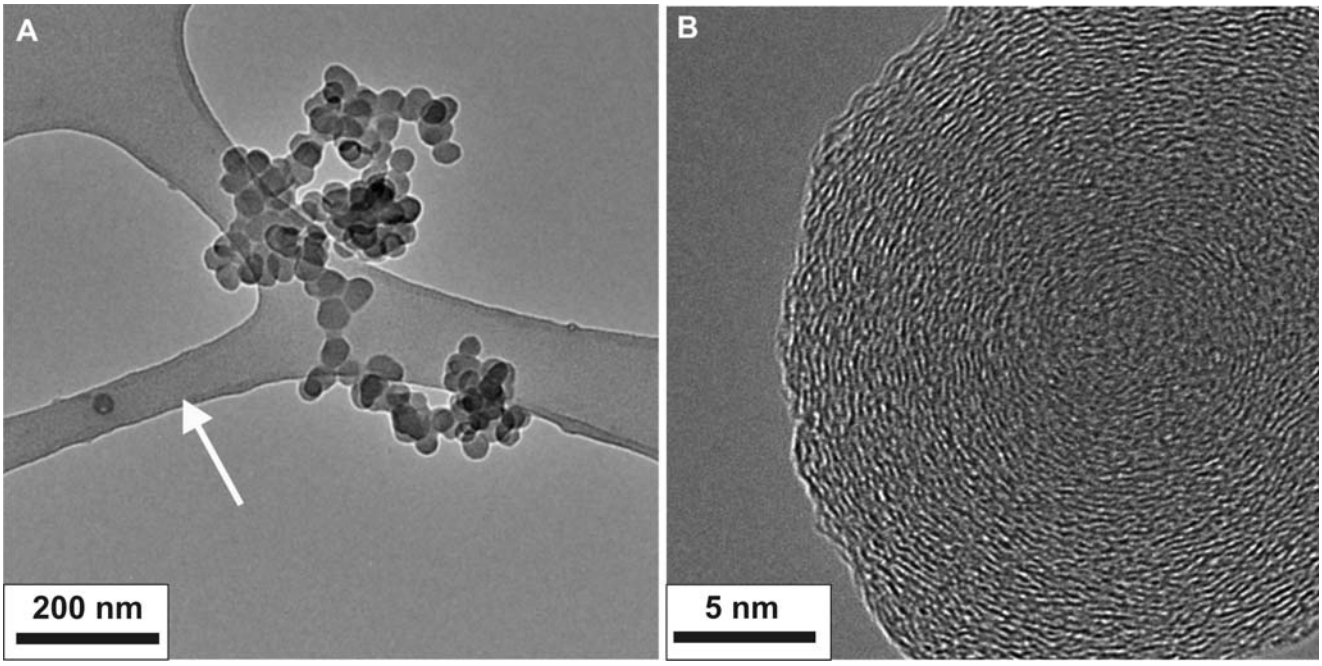

Fig. 4. TEM image of typical soot aggregates formed during wood combustion. **A.** Aggregate stuck to the carbon film covering microscopy grid (arrow). **B.** HRTEM image of spherical primary particle with concentric nanostructure visible.

Characterization of particles in speleothem samples scratched and dispersed in ethanol (without chemical treatment)

During observations of samples prepared this way, only few soot aggregates were noticed. Because such particles are very common in the air, in the opinion of the authors, such an amount of particles is not sufficient to prove that C_{soot} particles were present in speleothem samples; they could have been absorbed onto the grid surface during sample preparation. In the sample investigated, other organic particles were very common. They were different in size,

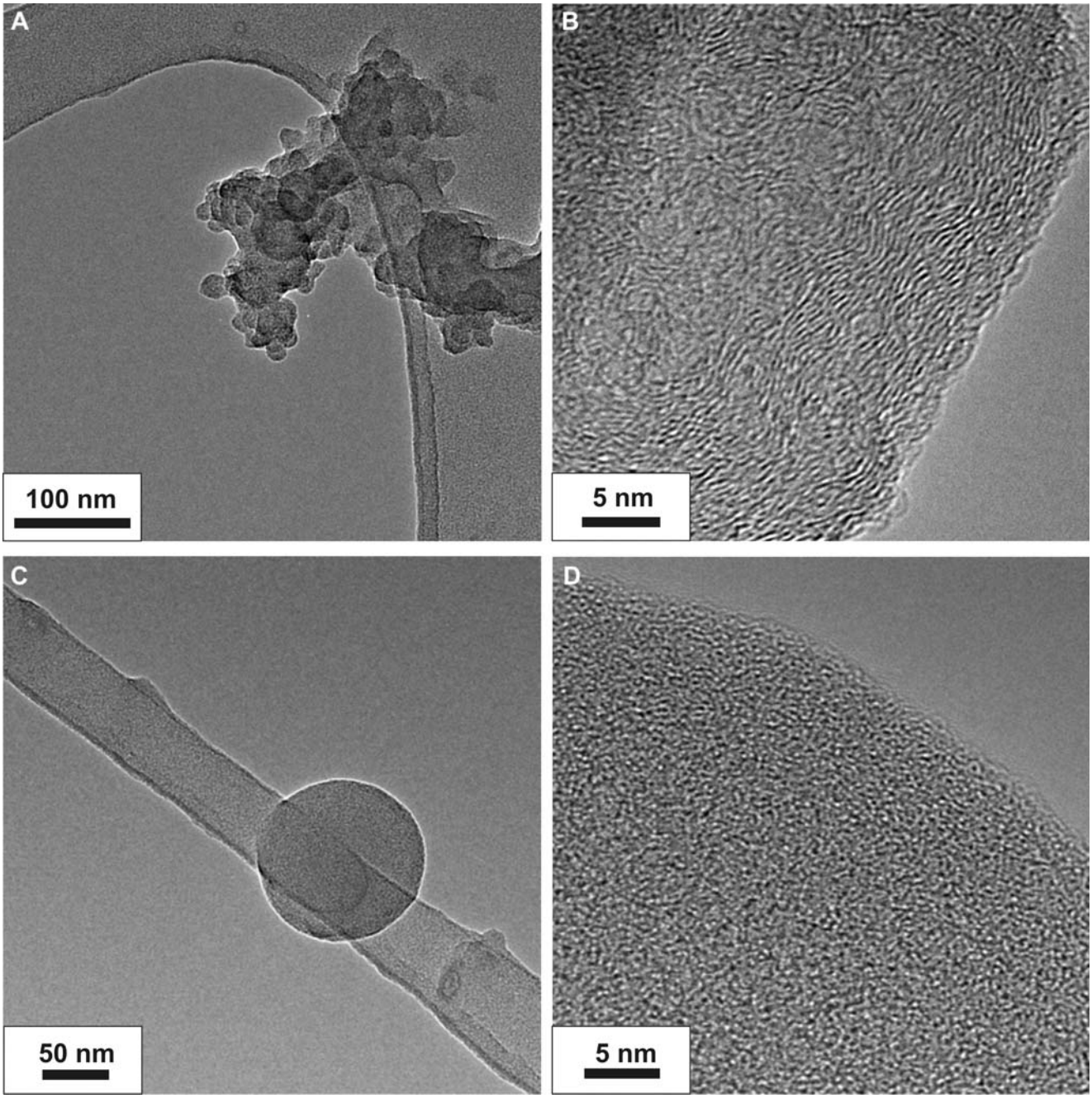

Fig. 5. Carbonaceous particles formed during wood combustion. **A.** TEM image of irregular carbonaceous particle. **B.** HRTEM image of particle presented in Fig. 4A with carbon layers not forming visible concentric nanostructure. **C.** TEM image of spherical tar ball particle. **D.** HRTEM image of particle presented in Fig. 4C with amorphous nanostructure visible.

some of them relatively large, more than 1000 nm (Fig. 6). Such particles are similar to a drop of liquid and contain mainly C and O, as was confirmed by EDS analysis. These organic particles were probably hydrated on arrival onto the speleothem. Such particles had been observed by other researchers. They are the most abundant particle type in the smoke from biomass-burning fires (Posfai, 2004) and experimental fires of pinewood ignited in caves (Rouzaud *et al*., 2015).

Characterization of particles in chemically treated speleothem samples

In samples with HCl treatment, soot aggregates were common, but still they were not a predominant component. Observations were additionally hampered as carbonaceous particles were be stuck together with other mineral particles (Fig. 7). Fortunately, because of their characteristic morphology, C_{soot} particles could be identified unambiguously. The observed particles bear a close similarity to C_{soot} particles, collected during wood combustion.

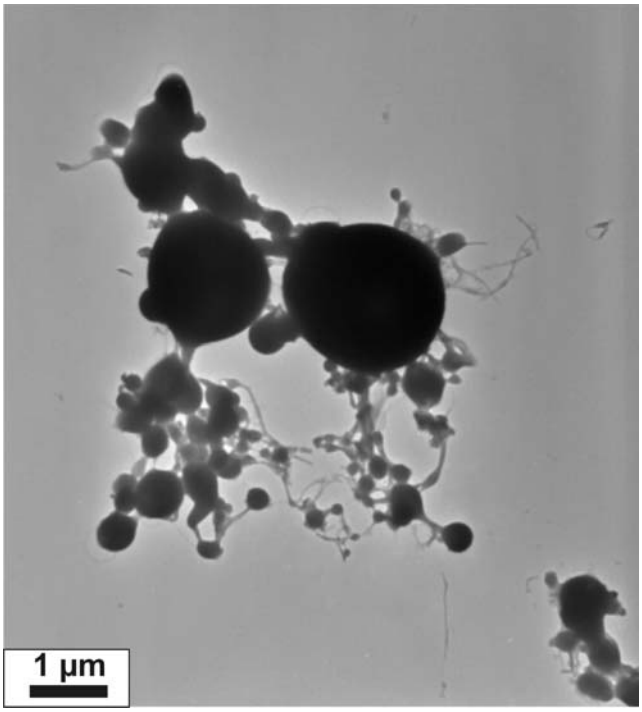

Fig. 6. Organic particles separated from flowstone sample DOM S2.

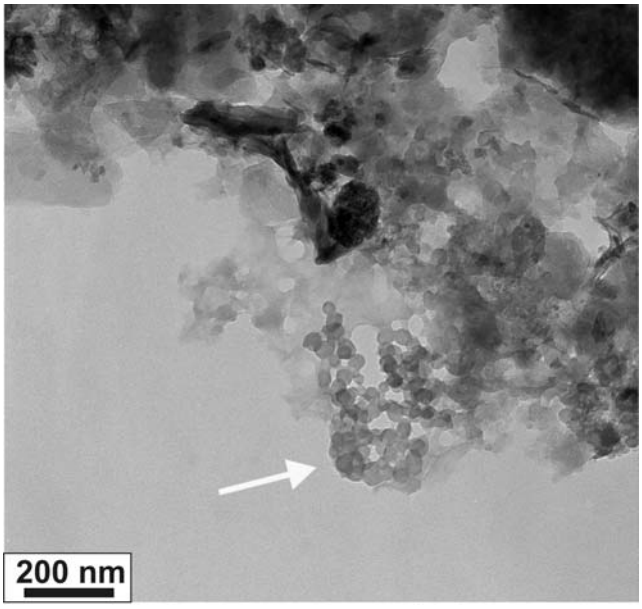

Fig. 7. TEM image of sample DOM S1 after dissolving in 2% HCl. Soot aggregate indicated by arrow.

After HF treatment, the observations were more unambiguous. Fig. 8A presents a sample after HF treatment. A comparable amount of soot aggregates and other carbonaceous particles, as well as a small amount of mineral particles, are visible. Aggregates consist of spherical, primary particles, similar in size (Fig. 8B). Primary particles are distinct, which indicates that aggregation occurred after complete formation of the primary particles. The particles are relatively large (here $d_p = 50 \pm 9$ nm, obtained for N = 67 diameter measurements of primary particles). In Fig. 8B, a concentric nanostructure is clearly visible, which undoubtedly confirms that observed particles were formed during the combustion of organic material.

As for samples collected during wood combustion in laboratory conditions, spherical amorphous carbonaceous particles were observed also in speleothem samples after chemical treatment. Figure 9 shows soot aggregate and two

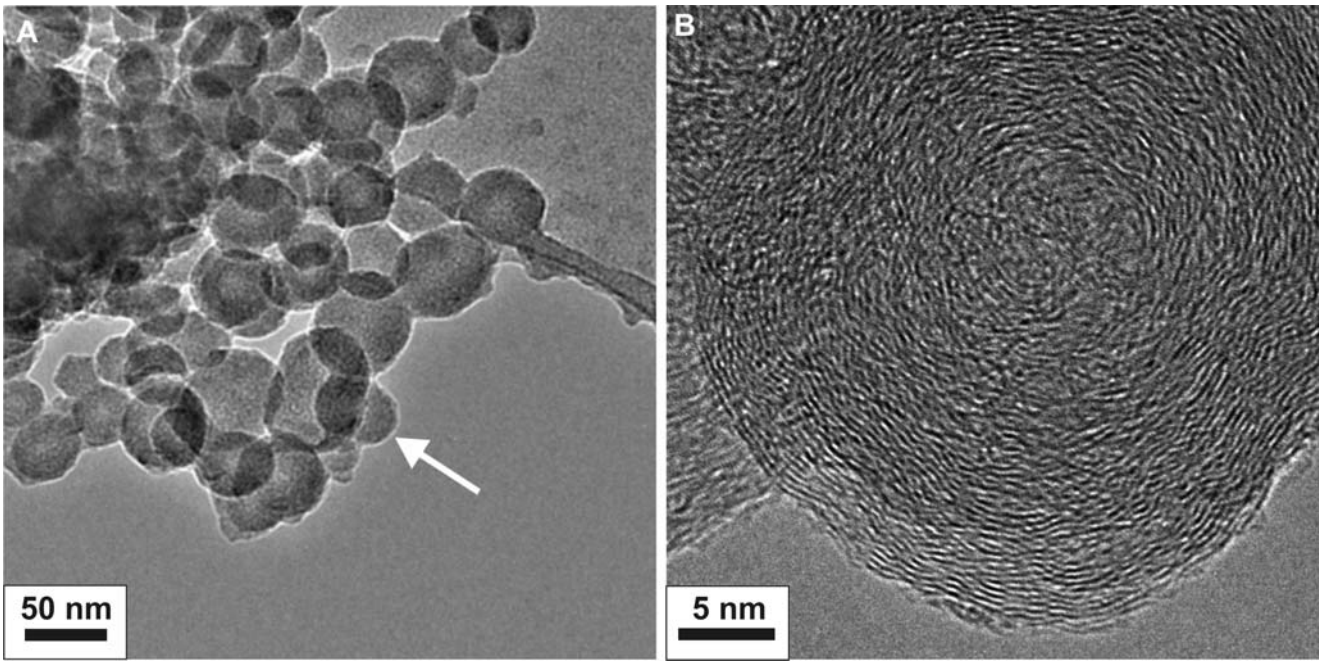

Fig. 8. TEM image of sample DOM S1 after dissolving in 2% HCl and HF. **A.** TEM image, soot aggregate indicated by arrow. **B.** HRTEM image of spherical primary particle indicated by arrow in Fig. 8A with concentric structure of carbon layers visible.

spherical carbonaceous particles (tar balls), about 130 and 150 nm in diameter. The tar balls contained mainly C and O (the oxygen content was higher than for C_{soot}); additionally small amounts of Cl (a residue after HCl treatment), K and Ca were present.

In speleothem samples after chemical treatment, particles with mineral inclusions also were very common (Fig. 10). Their chemical compositions were diverse. The particle in Fig. 10A contains C, O, F, Si, S, Cl, K, Ca (EDS analysis for all aggregate), whereas in Fig. 10B in area indicated by the black arrow (soot aggregate) C, O, and a small amount of K predominate; in the area indicated by the white arrow (mineral particle), C, O, Si, S, Cl, K, Ca, and a small amount of Fe predominate.

DISCUSSION

The aim of this work was to develop a sensitive method for the identification of wood burning products. It is important for proof of human activity in caves in prehistoric times, even if retained traces are not sufficient for other methods. Using TEM equipped with EDS spectrometry, the traces of hearth or torch usage, retained in the speleothems, can be investigated. The obtained results showed that especially the detection of C_{soot} particles, easily recognized from the characteristic morphology and nanostructure, makes analytical TEM a powerful tool in archaeological and geological research and unambiguously confirm that fires or other combustion process took place. Even extremely small amounts of material (<1 ng) can be enough for detection. The same methods can be used for the identification of extremely small quantities of burning traces and studies in sediments and in aerosol samples.

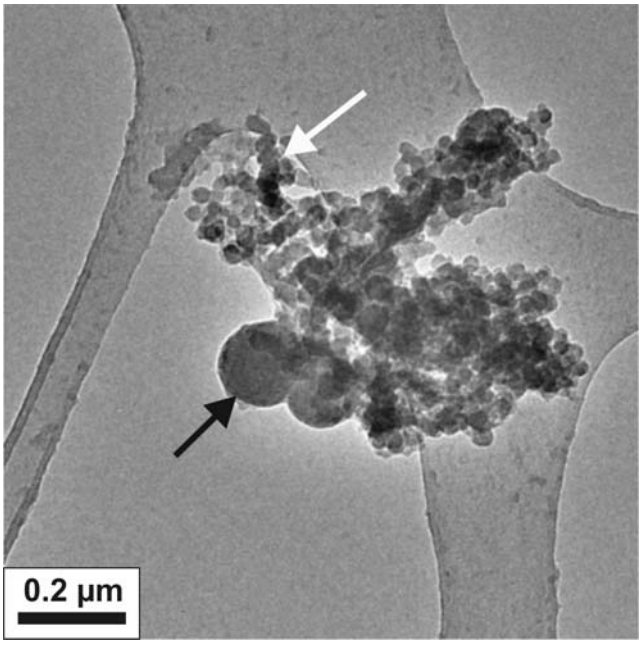

Fig. 9. TEM image of sample DOM S1 after dissolving in 2% HCl and HF. Soot aggregate indicated by white arrow; black arrow indicates two spherical carbonaceous particles.

Analysis performed for two samples (DOM S1 and DOM S2) confirmed that black layers are dyed by particles, formed during wood combustion and later retained in speleothems. Chemical treatment was necessary for effective extraction and purification of carbon soot (C_{soot}) and the proper preparation of specimens for microscopy. High concentrations of C_{soot} particles, as well as localization of the places where speleothem samples were collected,

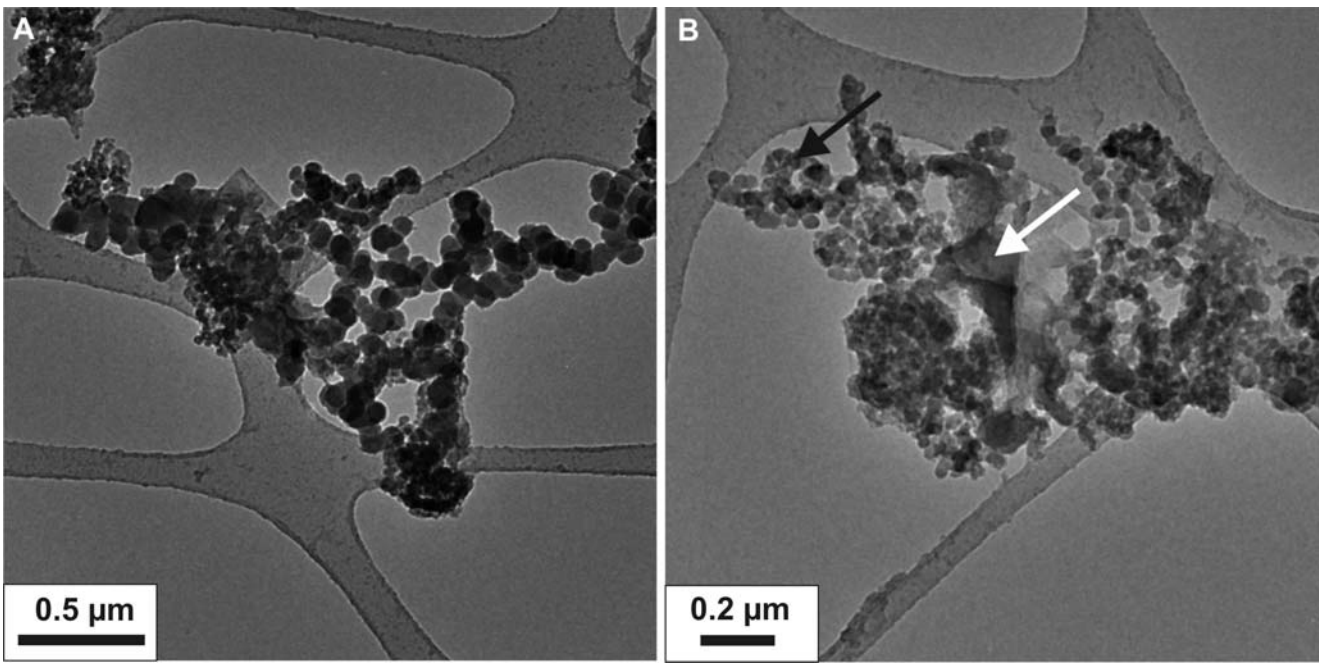

Fig. 10. TEM image of sample DOM S1 after dissolving in 2% HCl and HF. **A.** Soot particles with mineral inclusions. **B.** Soot aggregates (black arrow) and mineral particles (white arrow).

strongly contradict the hypothesis that they could come from the outside. Thus, the presence of carbonaceous particles supports the hypothesis of human activity in the cave, when the black layers were formed.

The diameter of primary, spherical particles depends mostly on the burning temperature. Soot aggregates that originated by high-temperature fuel combustion in diesel engines consist of relatively small, spherical particles with a mean diameter of 24 ± 7 nm (Griffin and Goldberg, 1979; Clague *et al*., 1999; Kocbach *et al.*, 2006). Spherical particles that originated by wood burning in fireplace were significantly larger, with a mean diameter of 41.6 ± 5.4 nm. A similar value (50 ± 9 nm) was obtained for particles, separated from the speleothems studied.

Soot structure is a fingerprint of its conditions of formation. Part of the information about soot formation can be revealed using the method described by the authors. It is worth noting that additional information on the soot structure and formation conditions can be obtained using other methods. HRTEM image analysis (such as the layer length, the interlayer spacing, etc.) could provide quantitative data (Rouzaud and Clinard, 2002). A precise technique for the study of chemical composition, perfectly suited to the study of nanometric particles, is Electron Energy Loss Spectroscopy (EELS; Williams and Carter, 2009). A very promising tool for carbonaceous materials is Raman microspectroscopy, sensitive not only to crystal structure, but also to the molecular structures of disordered carbons. In the case of archaeological research, especially promising is the fact that it allows estimation of the maximum temperature of the fire, where wood was burnt (Rouzaud *et al.*, 2015).

Generally, TEM observation of C_{soot} nanoparticles is not sufficient to prove human activity (making bonfires, using torches) in prehistoric times in a particular cave, but strongly supports such a hypothesis. The carbon particles in caves can originate from two other sources: they could flow with water from the ground above the cave or they could be blown through the entrance of the cave. In contradiction to most other carbon particles, the first solution is improbable in the case of C_{soot}. C_{soot} particles can form only during combustion and then, because of their very special morphology (small mass and diameter of primary particles, fractal-like shape of aggregates), they immediately rise up and do not remain on the ground. C_{soot} particles can return to the Earth's surface only with rain, but then they are extremely diluted (and cannot form black layers). Char particles can be larger (even few micrometers) and remain close to the place of the fire. Such particles can be rinsed off the ground and arrive in the cave. Fortunately, transmission electron microscopy makes it possible to distinguish these two types of carbon particles. Second sources can be eliminated, if the place of sample collection was carefully selected. Even during a powerful fire, the pressure of the air does not increase significantly. It is rather unexpected that a huge amount of smoky air will be forced into the cave. Far from the entrance, the concentration of C_{soot} particles should be low. The traces of that event should be visible evenly on all walls of the cave. Only specific conditions might allow higher concentration of allogenic soot particles inside the passages of a cave. High ventilation of relatively small caves at the time of fires in the vicinity of the cave or located in industrial areas may introduce enough smog for covering a cave's walls with a black layer. Another example is touristic caves with very large numbers of visitors like the caves in South Korea, described in several papers (Jeong *et al.*, 2003; Chang *et al.*, 2008). The main source of the black colour of the walls and the speleothems is high traffic and pollution taken by visitors to the caves. The black-coloured layer on the surfaces of speleothems is important for management, because it reduces the value of tourist caves. Detailed studies of black layers covering speleothems were conducted in the caves of South Korea (Jeong *et al.*, 2003; Chang *et al.*, 2008). On the basis of the detailed chemical and microscopic studies including TEM, the authors found that the main source of the black colour is the products of fuel combustion in car engines.

CONCLUSIONS

On the basis of the sizes of primary particles, characteristics of soot aggregates and chemical composition, the authors concluded that the black layers inside flowstones of Domica Cave originated from wood burning in conditions similar to those of a fireplace. The distribution of the black layers in the cave and the quantity of soot support the hypothesis of a process of combustion inside the cave. Together with other archaeological discoveries (Gradziński *et al.*, 2007), this indicates the existence of human activity in the cave.

HRTEM in combination with EDS has confirmed its usefulness to archaeological research. The methods presented permit the study of the structural diversity of soot particles and their chemical composition to obtain information about the combustion process and to help to determine the source of combusted material. Carbonaceous products allow the recognition of the traces of human occupation and especially that of prehistoric humans (Rouzaud *et al*., 2015). By using TEM, it is possible to identify the forms of disordered carbon, characterized by different nanostructures (e.g., porous, lamellar, fibrous, onion-like) and determine the combustion conditions, in which they were formed. In particular, an advantage of TEM is the possibility of carrying out analyses of extremely small amounts of material.

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