Abhandlung Band 45/2005 ISSN 0077-6149	Neue Forschungen zum Höhlenbären in Europa	Seite 11-18	Naturhistorische Gesellschaft Nürnberg e.V. Marientorgraben 8, 90402 Nürnberg

Martina Ábelová

Dental cementum of bears (Ursidae) - possibilities of microscopic study

Zusammenfassung:

Die Analyse der Mikrostruktur des Zahnzements gibt uns Informationen zum Individualalter und ebenso zur Jahreszeit des Todes. Die in diesem Artikel untersuchten Zähne stammen aus einem Survey in der Höhle „Za hájovnou" (Javoříčko Karst, Tschechische Republik). Die Funde datieren ins Mittelpleistozän. Für die Untersuchung der Mikrostruktur wurden Dünnschliffe mit einem Polarisationsmikroskop untersucht; dabei wurde eine Kombination von Ätzen, Anfärben und Kathodolumineszenz verwendet. Jede dieser Methoden hat sowohl Vor- als auch Nachteile. Kathodolumineszenz wurde hier erstmals für die Mikrostruktur-Untersuchung von Zähnen verwendet.

Abstract:

Dental cementum microstructure analysis on the bear teeth can tell us about the age of an individual and also about the season of his death. The teeth used in this study come from a survey of cave „Za hájovnou" (Javoříčko Karst CZ). The age of these findings belongs to middle Pleistocene. For the microstructure study, methods of thin sections study in polarisation microscope was used; it is a combination of etching and colouring and cathodoluminescence. Each of these methods have both advantages and disadvantages. Cathodoluminescence was used for the study of dental microstructures for the first time.

Résumé:

L'analyse des structures microscopiques du ciment dentaire livre des informations concernant l'âge individuel et la saison de la mort du sujet. Les dents présentées dans cet article proviennent d'une prospection faite dans la grotte „Za hájovnou" (Karst du Javoříčko, République Tchèque) et datent du Pléistocène moyen. Pour cette analyse, des lamelles ont été étudiées sous microscope à polarisation en utilisant une combinaison de corrosion, coloration et de cathodo-luminescence. Chacune de ces méthodes présente aussi bien des avantages que des inconvénients. La cathodo-luminescence a été utilisée ici pour la première fois dans le cadre de l'étude de dents.

Key words: Javoříčko Karst, Ursidae, dental cementum, thin sections, cathodoluminescence

Introduction

There are numbers of fossil findings of fossil bears at many localities, mainly in cave sediments, in the course of whole Pleistocene. Among these belong the skeleton and teeth of individuals of various ontogenetic stages. It is very important to know the individual age of studied findings for the structure of given community. However, the tooth crown having been worn away, it is impossible to determine the individual age of a given individual. The analysis of teeth wear allow as to assess only the relative age categories (see KURTÉN 1958; STINER 1998). Crucial way to a reliable estimation of individual age is to use the microstructure analysis of dental cementum on the basis of counting cement increments. On the basis of this method, it is possible to determine also the season in which an animal died.
We can often encounter with an application of cement analysis for the age estimation of fossil (DEBELJAK 1966, GUŽVICA 1991) and also of recent bears (CRAIGHEAD et al. 1970, SAUER et al. 1966, STONEBERG & JONKEL 1966), especially in foreign literature. Neither the preparation of samples for this study is a problem. What can help us in the preparation stage, are already verified working techniques which were shown for example by BEASLEY et al. (1992); BURKE (1993); DEBELJAK (1966, 2000). FANCY (1980); MORRIS, (1978); STALLIBRASS (1982) give us an overall view on this working method. Cement analysis is successfully used not only for the bears, but also for many mammals (MATSON 1981). In the scope of our research we compared several methods for study of dental cementum of bears. Observed material comes from a survey of cave „Za hájovnou" (Javoříčko Karst, CZ).

Geological situation of the locality

Cave „Za hájovnou" belongs to the system of Javoříčko caves (North Moravia, Czech Republic). They are components of Javoříčko karst, which geologically belongs to the North part of Konice-Mladeč stretch. Carbonate sedimentation is made of devonian limestones (ŠTELCL et al. 1998). There are two different genetic types of sediments. There are partly fluvial sediments, which come from the time period, when the cave functioned as draft (they come from Matuyama chron). Above this complex, there are terrestrial sediments (mainly from interglacial Holstein). The current explored length of the cave is 80 m, the deepest place reaches 40 m depth. This is the typical bear cave. The age of findings belongs to the middle Pleistocene. Apart from bears there were found *Canis lupus, Panthera spelaea*, *Panthera mosbachensis* ?, *Equus* sp., *Bos/Bison*, *Sus scrofa* and *Rupicapra/Capra*.

Material and methods

The teeth of bear are composed, from margin towards the centre, created of cement, enamel and dentine. Cement (cementum) is deposited inside

alveola, on the outer surface of extended dentine part of the tooth. Basically, it is a calcified tissue enclosing the dentine of the tooth stub (Shoshany & Tassy 1996). Enamel (enamelum) constitutes the hardest tissue in a vertebrate body. It is composed of up to 98% inorganic salts (mainly calcium phosphate or hydroxiapatite) and only 2% of organic matter (Roček 2002). Dentine (dentinum) constitutes the main part of the tooth, having its composition similar to a bone (Samuel et al. 2000). The core cavity is located in the centre of the tooth. During the lifetime (of an animal), it contains blood vessels, nerves and lymphatic tissues.

The study of dental cementum from canines was used to cement analysis. The thickness of the cementum increases with age. Dental cementum is composed of „winter" and „summer" layers. They could be compared to the growth rings in trees (Debeljak 2000). For this analysis, a choice of material is very important. We can use each kind of teeth for this analysis. With canines and last molars (C, M^2, M_3), we should add one year when counting the cementum increments, because they erupted nearly a year later than other permanent teeth (Debeljak 1997). Canines could be ideal for cementum analysis; they have the thickest coat of cementum and, furthermore, sex can also be determined from their size. Last Molars are the worst choice, because they often have anomalous and irregularly developed cementum layers (Debeljak 2000).

We used three different methods for the study of microstructures of dental cementum. These methods were compared in order to find out whether they are suitable for different preserved teeth and for other purposes.

1. Thin sections

We made transverse petrographical thin sections from teeth roots. The cut went perpendicularly to the roots of the teeth (Fig. 1). When cement analysis

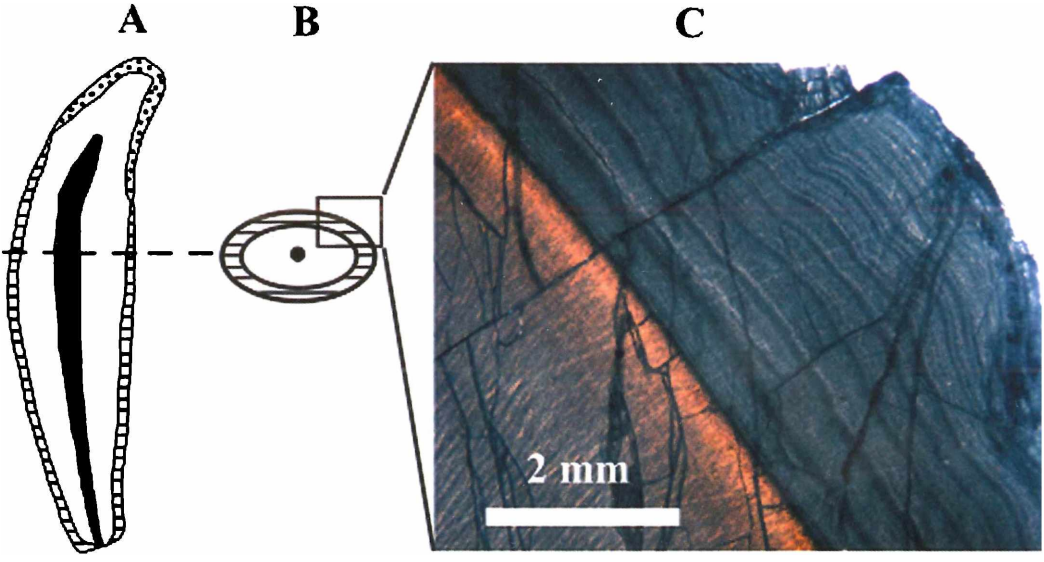

Fig. 1. Sketch of canine in longitudinal cross-section (A), transverse section of the rooth (B) & (C) - transverse thin section of bear canine showing the boundary dentine/cement and annual increments in cementum. Used Nikon 2,5. polarizers. Caption: cement (hatching), dentine (white), pulp cavity (black), enamel (dotted). (Photo: author)

is used, it is possible to apply also longitudinal thin sections. The cut runs parallelly with axes of the teeth. When making thin sections, it is necessary to keep in mind, that dentin is hard, but also very brittle. For that reason, it is needful to pour the teeth to a synthetic resin before cutting. We have to cover the thin section with protective glass, because even small per cent of air humidity may cause cracking and therefore devalue thin sections.
On these thin sections we observed „winter" and „summer" increments in cement layer under the polarisation microscope. We used the ocular with 2.5, 4, 10 a 20 a 40x magnification. We recorded the final picture with digital camera Olympus or with camera installed onto microscope. When we used the camera, we transferred observed picture directly to a computer.

2. Etching and colouring (After Debeljak 1996)

We cut the roots of the teeth transversely. Ground sections are etched with 10-20% orthophosphoric acid (H_3PO_4) for 5-10 minutes and rinsed with water afterwards. Then, they were stained with approx. 0,5% aqueous

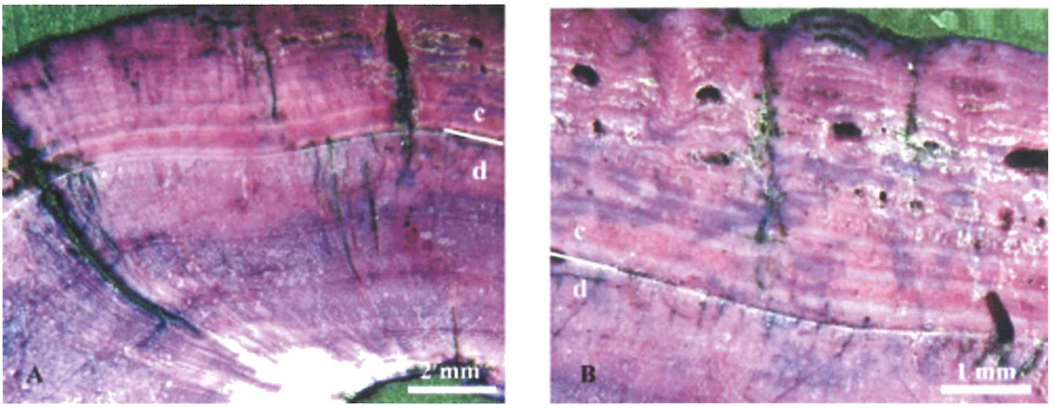

Fig. 2. Dental cementum and dentine in the bear. Etched, stained sections. Individual age estimation: about 16 years. (Photo: author).

solution of gentian violet (hexamethyl-*p*-rosaniline chloride or crystal violet). Gentian violet is applied to root section with a paintbrush and rinsed with tap water after few seconds. The intense violet colour disappears if the specimen is immersed in the acid for the second time. This preparation is studied under binocular. Sharp contrast zones mark the transition from „summer" to „winter" increments (Fig. 2).

3. Cathodoluminescence

This term is to represent emissions of visible light caused by impact of accelerated electrons onto a rock or a mineral. Intensity of emitted light depends mainly on concentration of the activators. Activators are atoms whose presence in the structure of studied material causes the luminescence. Here, for example, belong atoms of manganese and elements of rare soils (other elements, mainly divalent iron, inhibit the luminescence).

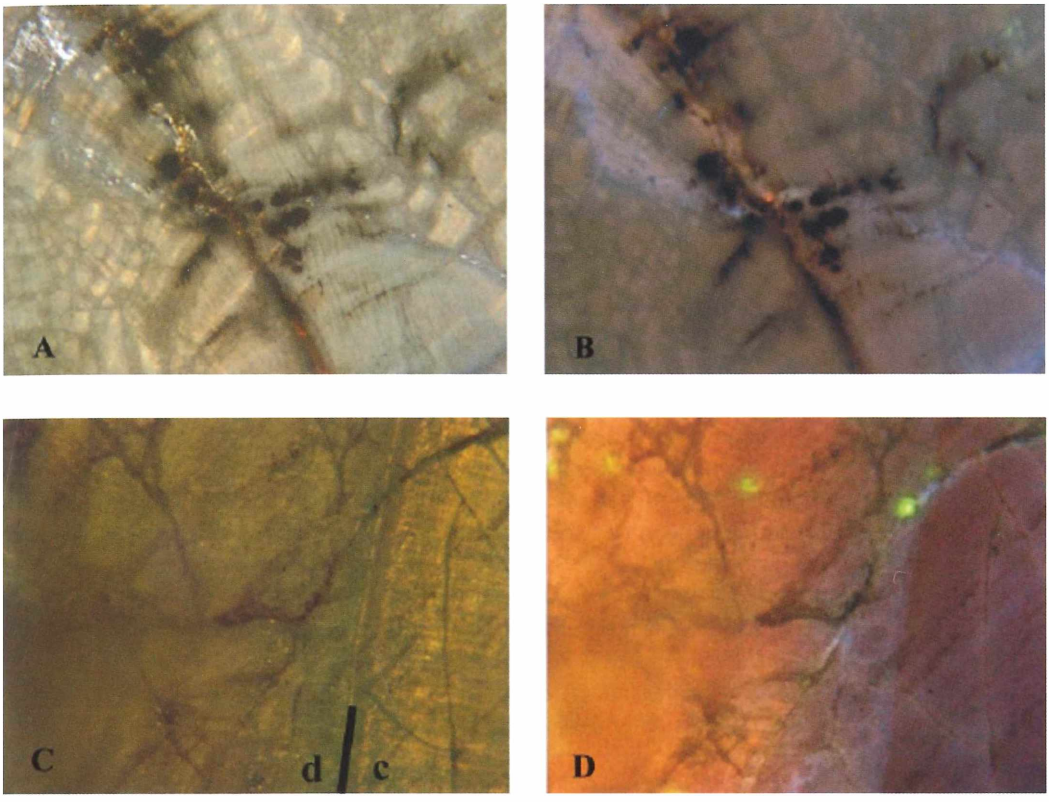

Fig. 3. Bear canine observed in polarisation microscope (A, C) and with the use of cathodoluminescence (B, D). d/c is dentin/cement boundary.

For this method we made uncovered polished thin sections from the teeth roots. The cut crossed perpendicularly the roots of the teeth similarly like in the previous methods. We then covered this thin section with carbon. Subsequently was the surface of the sample exhibited to the flow of accelerated electrons in the vacuum. This caused emitting of visible light. This light we can not only observe, but even photograph or subject to spectral analysis (LEICHMANN & JELÍNEK 2005). This method hasn´t been used for such study up to the present.

Results and interpretations

Three different methods were used for the purpose of distinguishing annual layers of cement on bear canines. This increment is possible to utilize to determine the death season of an animal and estimates an animal age. Bright („summer“) and dark („winter“) increments together create one year. „Summer“ and „winter“ increments differ in colour and structure. This structure is obvious thanks to the distinct organisation of collagen fibres and cell content, which is influenced by a relative percentage of mineral and organic fraction (CARLSON 1991, HILLSON 1986).
Among the advantages which follow from using of shown methods belong: amount of bear teeth in quaternary sediments, we do not need the whole

preserved tooth, examined teeth could be fossil and also recent, we do not need a very large magnification when analysing increments; using of these methods is relatively cheap and fast, and what is most important, these methods are possible to apply also on other species of mammals.
Observation of preparations with gentian violet under the microscope is the cheapest and easiest from all used methods. This method is suitable when the preparation of the thin section is complicated by poorly preserved material of teeth. Teeth can be perished, but it is common, that even on the first sight solid teeth, later show to be fragile or are negatively influenced by sedimentary environment. In this case, „erase" of incremental layers occurs, and they become invisible for observation under the microscope.
Observing of thin sections under polarisation microscope have benefits in contrast to the previous method. It is possible to distinguish the colour of the increments and we can study it under large magnification.
Observation under classical microscope brings us information mainly about colour, index of fracture and other optical properties about the sample. On the other hand, observation with application of cathodoluminescence brings us information about the presence, spacing and about other features of activators. With combination of these two types of observation, it is possible to document the structures which originated in individual period of animal life. In some cases, it is possible that luminescence is not observable as a result of weathering, efflorescence, blurring or admixing of foreign material (Fig. 3 B). In other cases (Fig. 3 D) luminescence is not present in the part of a sample or only in minimum (it is weakly preserved in cement in our case) or only so-called „shortly lived luminescence" can occur. (in our case in dentine). In the case of „shortly lived luminescence" the zonality (increments) is visible, but because of a very short emission - lasting only a few seconds, the luminescence can not be properly photographically documented.
All these methods have smaller disadvantages. Cementum analysis belongs to the destructive methods. When we want to make thin sections or preparation we have to cut the teeth. But under the careful manipulation it is possible to glue the pieces of the tooth together. Material of the teeth is hard but fragile. We have to count with this fact when preparing the thin sections. Furthermore, the older the individual is, the denser and finer will the increments be. This fact could make their counting more difficult.

Résumé

Dans plusieurs localités notamment dans des sédiments caverneux se trouve un grande nombre de débris fossiles des ours parmi lesquelles on compte des squelettes et des dents des individus de différentes phases ontogénétiques. Pour la structure d' une communauté donnée il est très important de connaître l'âge individuel des trouvailles étudiées. Néanmoins la couronne d'une dent une fois usée, il n'est pas possible de déterminer précisement l'âge d'un individu donné. L'analyse de l'usure des dents ne nous permet établir

que relativement des trances d'âge. Ce qui est important pour évaluer avec certitude l'âge individuel c'est utilser l'analyse des microstructures du cément et en examiner les accroissements. En vertu de cette méthode il est possible de déterminer même la saison dans laquelle un individu étudiée est mort.
Pour étudier cettes microstructure on a utilisé et on a comparé les méthodes de l'observation des échantillons dans un microscope polarisant, la combinaison de l'attaque et de la teinture et la méthode d' luminescence cathodique. Chaque méthode a ses avantages et ses inconvénients. L'iluminescence cathodique a été utilisée pour la première fois pour l'étude des microstructures du cément.
Les dents analisées des ours qui ont été utilisé pour l'étude, proviennent de l'exploration de la grotte Za Hájovnou (Javoříčský kras, République Tchèque). C'est une grotte typique pour les ours. L'âge des trouvailles date du pléistocèn moyen - interglaciaire Holstein.

Resumé

In vielen Fundstellen, vor allem in Höhlensedimenten, haben sich im Laufe des gesamten Pleistozäns fossile Bärenreste abgelagert. Unter diese Funde gehören Skelette und Zähne von Individuen verschiedener ontogenetischer Stadien.
Für die Struktur der überlieferten Gemeinschaft ist es sehr wichtig, das Individualalter der untersuchten Funde zu kennen. Mit dem Abkauungsgrad der Zahnkrone kann man nicht genau das Alter des jeweiligen Individuums feststellen. Die Analyse der Abnutzung der Zähne erlaubt uns nur eine relative Bestimmung der Alters-Kategorie.
Ein wichtiger Weg zu einer zuverlässigen Schätzung des Alters ist die Benutzung der Analyse der Mikrostruktur des Zahnes aufgrund des Zuwachses des Zements. Aufgrund dieser Methode kann man auch die Jahreszeit des Todes des erforschten Objektes bestimmen. Für die Untersuchung der Mikrostruktur an Schliffen hat man Vergleichsmethoden mit einem Polarisationsmikroskop genutzt, sowie eine Kombination aus Ätzung und Färbung, und die Methode der Kathodolumineszenz.
Jede von diesen Methoden hat ihre Vor- und Nachteile. Die analysierten Bärenzähne stammen aus der Höhle **Za hájovnou** (Javoříčský kras, Tschechische Republik). Es ist eine typische Bärenhöhle. Das Alter der Funde gehört zum mittleren Pleistozän, dem Holstein-Interglazial.

References

Beasley, M. J., Brown, W. A. B., Legge, A. J. (1992): Incremental banding in dental cementum: methods of preparation of teeth from archeological sites and for modern comparative specimens.- International Journal of Osteoarchaeology, 2, 37-50, Chichester.

Burke, A. M. (1993): Observation of incremental growth structures in dental cementum using the scanning electron microscope.- Archaeozoologia, 5 (2), 41-54, Grenoble.

Carlson, S. J. (1991): Vertebrate Dental Structures.- In: Carter, J. G. (ed.): Skeletal Biomineralization: Patterns, Process and Evolutionary Trends.- 531-556, New York (Van Nostrand Reinhold).

CRAIGHEAD, J. J., CRAIGHEAD, F. C., MCCUTCHEN, H. E. (1970): Age determination of grizzly bears from fourth premolar tooth sections.- Journal of Wildlife Management, 34, 353-363, Washington.

DEBELJAK, I. (1966): A simple preparation technique of cave bear teeth for age determination by cementum increments.- Revue de Paléobiologie, 15 (1), 105-108, Genève.

DEBELJAK, I. (1997): Ontogenetic development of dentition in the cave bear.- Geologija, 39 (1996), 13-77, Ljubljana.

DEBELJAK, I. (2000): Dental cementum in the cave bear; comparision of different preparation techniques.- Geološki zbornik, 15, 25-40, Ljubljana.

FANCY, S. G. (1980): Preparation of Mammalia for the age determination by cementum layers: a review.- Wildlife Society Bulletin, 8, 242-248, Washington.

GUŽVICA, G. (1991): Metoda procjene životne dobi spiljskog medvjeda (Ursus spelaeus) na temelju slojeva u zubnom cementu.- Geološki vjesnik, 44, 9-13, Zagreb.

HILLSON, S. (1986): Teeth.- Cambridge Manuals in Archeology, xix+376, Cambridge (University Press).

KURTÉN, B. (1958): Life and death of the Pleistocene cave bear. A study in paleoecology.- Acta Zoologica Fennica, 90, 1-48, Helsinki.

LEICHMANN, J., JELÍNEK, F. (2005): Katódová luminiscence. Čtení zápisu z dob vzniku horniny.- Vesmír, 84, 143-147, Praha.

MATSON, G. M. (1981): Workbook for cementum analysis.- Matson's Laboratory, Milltown, Montana, 30.

MORRIS, P. (1978): The Use of Teeth for Estimating the Age of Wild Mammals.- In: BUTLER, P. M., JOYSEY, K. A. (eds.): Development, Function and Evolution of Teeth.- 483-494, London (Academic Press).

ROČEK, Z. (2002): Historie obratlovců. Evoluce, fylogeneze, systém.- Academia, 512, Praha.

SAMUEL, O., RORZA, V., ČINČUROVÁ, E., FORDINÁL, K., GALLE, A., HOLEC, P., CHLUPÁČ, I., MICHALÍK, J., ONDREJIČKOVÁ, A., OŽVOLDOVÁ, L., PAPŠOVÁ, J., PEVNÝ, J., PIPÍK, R., REHÁKOVÁ, D., ŠTORCH, P., TUBA, L'., VAČOVÁ, M., ZÁGORŠEK, K., ŽÍT, J. (2000): Geologický slovník. Zoopaleontológia.- Vydavatel'stvo Dionýza Štúra, 527, Bratislava.

SAUER, P. R., FREE, S., BROWNE, S. (1966): Age determination in black bears from canine tooth sections.- New York Fish and Game Journal, 13 (2), 125-139, New York.

SHOSHANY, P., TASSY, P. (eds.) (1996): The Proboscidea. Evolution and Palaeoecology of Elephants and Their Relatives.- 472, New York (Oxford University).

STALLIBRASS, S. (1982): The use of cement layers for absolute ageing of mammalian teeth. A selective review of the literature, with suggestions for studies and alternative applications.- In: WILSON, B., GRIGSON, C., PAYNE, S. (eds.): Ageing and Sexing Animal Bones from Archeological Sites.- BAR British Series, 109, 109-126, Oxford.

STINER, M. C. (1998): Mortality analysis of Pleistocene bears and its paleoanthropological relevance.- Journal of Human Evolution, 34, 303-326, London.

STONEBERG, R. P., JONKEL, C. J. (1966): Age determination of black bears by cementum layers.- Journal of Wildlife Management, 30 (2), 411-414, Washington.

ŠTELCL, J., ZIMÁK, J., NAVRÁTIL, O., SLÁDEK, P., SAS, D. (1998): Geologické faktory a mikroklima speleoterapeutické léčebny v Javoříčských jeskyních.- Scripta Facultatis Scientiarum Naturalium Universitatis Masarykianae Brunensis.- Masarykova univerzita, 25 (1), 47-58, Brno.

Address of author:

Martina Ábelová, Institute of Geological Sciences, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; abelova.m@mail.muni.cz