DETERMINATION OF THE ELEMENT DISTRIBUTION IN SAUROPOD LONG BONES BY MICRO-XRF

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ABSTRACT
The distribution of elements in sauropod humeri and femora was determined by micro-X-ray-fluorescence analysis (µ-XRF). Element distribution maps enable correlations between bone architecture and spatial distribution of the chemical elements. The investigations reveal no significant macroscopic gradients in the element distribution across the fossil bone cross-section. More detailed investigations of areas in the periosteal and the endosteal region of the bones were performed by area scans and are visualized by element distribution maps.

KEYWORDS
X-ray fluorescence, sauropod dinosaur, bone, diagenesis, Tendaguru

INTRODUCTION
Their extraordinary size leaves sauropod dinosaurs among the biologically most interesting vertebrates [1]. Thus, sauropod biology is currently studied by an interdisciplinary team of researchers in the DFG research unit “Biology of the Sauropod Dinosaurs: The Evolution of Gigantism” [2]. Recent estimates based on photogrammetric measurements in actual skeletons [3] or on scientific reconstructions [4] place common sauropods consistently in the 15t to 50t category. Sauropods are tetrapods, a body similar to proboscideans (elephants) is combined with a very small head on a very long neck and a long tail.

The main emphasis of our studies in the framework of the larger interdisciplinary Research Unit on Sauropod Biology is on studying the architecture of the fossilised sauropod bones. We aim at contributing to a solution of the problem whether the hierarchical structure of sauropod bones was adapted to their extraordinary mass. Due to the very long time that has passed since the sauropods lived (their diversity and range peak was in the Late Jurassic and they went extinct at the end of the Cretaceous, 65 million years ago) sauropod bones today are fossilised. During the burial and the fossilisation of the sauropod bones, their organic parts degenerated and the bone histology, bone porosity, protein content, the crystallinity of the bone apatite, carbonate content, and their content of chemical species in general changed [5]. A number of studies have been concerned with the relative stabilities of various elements with respect to diagenetic changes [6,7] since the elements present in the fossil bone may also allow for conclusions e.g. regarding the dietary habits of ancient populations and extinct animals.

The sauropod bones excavated from the faunas of the African Tendaguru Beds of Late Jurassic age are surprisingly little altered at the histological level, thus e.g. growth pattern of Barosaurus brancai and Brachiosaurus africanus could be determined [1]. The microstructure and element distribution in fossilized sauropod bones from the Tendaguru Beds however has scarcely been
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studied. Romer et al. [8] determined the U and Pb distribution in the bones aiming at age estimates, but found the Pb content of the bones severely influenced by diagenetic processes. Tütken et al. [9] performed electron micro probe (EMPA) analyses on fossils from sympatric dinosaur fossils. The EMPA studies however are not sensitive to trace elements such as Sr, which is among the most resistant elements to diagenetic changes [6]. Our group earlier performed non-destructive investigations of the element distribution in the Tendaguru sauropod bones using proton induced X-ray emission (PIXE) [10], the gauge volume used in those measurement however was 2mm x 2mm, thus the elements observed cannot be allocated to specific regions of the fossilized bones. The aim of the study presented here was to determine the spatial variation of elements in the fossilized bones and to distinguish between elements mainly present in pore filling minerals and elements mainly present in the former hydroxyapatite.

EXPERIMENTAL DETAILS

Sample selection: We chose samples from a growth series of humeri and femora of Brachiosaurus brancai and Barosaurus africanus, which were excavated during an expedition between 1909 and 1913 that are housed at the Naturkundemuseum of the Humboldt-Universität, Berlin, Germany [1, 11-13].

Specimen preparation: Sample of the sauropod bones were extracted by a core-drilling technique. The cores were taken in the narrowest part of the diaphysis of both fossilized humeri and femora of Brachiosaurus brancai and Barosaurus africanus [1]. Specimens were extracted from these cores by slicing them along their longitudinal axis. Both halves of the cylinder thus contain a whole cross-section of the bones, from the trabecular bone inside the medullar cavity up to the laminar bone at the surface. The plane surface of the half cylinder was ground on SiC, polished with diamond paste and cleaned in ethanol before the µ-XRF measurements.

Micro-XRF Measurements: Measurements of the elemental distribution in sauropod bones have been performed using the micro-focus setup at the Atomic Institute, TU Wien, Vienna, Austria [14]. The µ-XRF spectrometer consists of a vacuum chamber comprising a manual adjustment unit for the X-ray optics and a motor driven, software controlled sample stage for performing spatially resolved µ-XRF scans. Within this set of experiments the point focus of a 2kW Mo diffraction tube operated at 40kV and 30mA was used as excitation source. The primary X-ray beam was focussed by a monolithic polycapillary to a focal spot of 60µm. Recording of the fluorescence photons was accomplished by a silicon drift detector with an active area of 10mm². The angle between incident beam and sample as well as sample and detector is fixed to 45° each. To optically control the measurement position, the system is equipped with a CCD camera coupled to an optical microscope. Peak deconvolution and subtraction of the radiation background for each measured point was performed using the AXIL software from the QXAS package in automatic mode (batch mode).

RESULTS AND DISCUSSION

The µ-XRF experiments revealed that the elements P, Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Pb, U, Sr, Y and Mo are present in the sauropod bones (fig.1). These elements have previously also identified in the PIXE measurements [10] and in complementary EDX analyses [15] in the scanning electron microscope. µ-XRF line-scans across the bone cross-sections were performed
and two areas (fig. 2) were investigated in detail in a sauropod bone (specimen number Ba A1, *Barosaurus africanus*, right humerus, 99 cm long, thus an adult animal according to [1]).

The µ-XRF line-scan (fig. 3) reveals that P, Ca, Mn, Fe, Sr, Pb and U do not show macroscopic gradients across the bone cross-section. Pb, Sr and U appear to be homogeneously distributed along the line from the periosteal to the endosteal part of the bone. In contrast Fe, Mn, Ca and P show strong local inhomogeneities, with a strong correlation for Fe and Mn and a weaker correlation between Ca and P. The absence of macroscopic gradients in the element distribution indicates that equilibrium may have been reached, thus the driving force for interdiffusion between the elements in the bone and those in its burial environment strongly decreased.

**Fig. 1:** Sum spectrum of the µ-XRF, scanned area 900 x 900 µm (10 x 10 px), measurement time 40s/px

**Fig. 2:** Photo of the polished core drilled out of the cortex of the *Barosaurus africanus* humerus A1. The bone surface is to the left and the endosteal region filled by cancellous bone is to the right. The red line marks line of the scan. The width of the field of view is 4 mm.

**Fig. 1:** Line scan across the cross-section of cortex of the *Barosaurus africanus* bone A1. Scanned distance 37.5mm; step size 500µm; measuring time 40s/point.
Fig. 4: Elemental map obtained from a μ-XRF scan of cancellous bone in the endosteal region of *Barosaurus africanus* humerus A1. Bone trabeculae are intersected at the margin of the map, the center is occupied by diagenetic minerals replacing soft tissue. Scanned area 900x900µm; step size 100µm; measuring time 40s/point. Length of the scale bar corresponds to 200µm.

Fig. 5: Elemental map obtained from a μ-XRF scan in the cortex of *Barosaurus africanus* humerus A1. The bone tissue is primary bone of the laminar fibro-lamellar type. Scanned area 900x900µm; step size 100µm; measuring time 40s/point. Length of the scale bar corresponds to 200µm.
Fig. 6: Micro-XRF of a region of the cortex of *Brachiosaurus brancai* XV. The bone tissue is primary bone of the laminar fibro-lamellar type with interspersed secondary osteons. A diagenetic crack runs across the upper left of the map. Length of the femur: 219 cm. Scanned area 900x900µm; step size 100µm; measuring time 40s/point. Length of the scale bar corresponds to 200µm.

The elemental maps obtained from the area scans provide a more detailed picture of the element distribution in the fossilized bone (fig. 4-6). In the endosteal region (fig. 4) of the bone A1 minerals have filled the cavities between the trabeculae. Previous microscopy investigations and X-ray diffraction \[10, 15\] have shown that calcite is the predominant pore-filling mineral. The µ-XRF elemental map shows these regions to be calcium rich. The calcite appears to be enriched in Pb (see right lower corner of the elemental map). The remains of the hydroxyapatite (X-ray diffraction experiments indicated that it changed to fluorapatite \[10, 15\]) around the calcite inclusion contain P, which presumably remained from the original hydroxyapatite, Sr, U, Fe and Mn. The distribution of Sr and U appear similar. In the periosteal region (fig. 5) pore-filling minerals have invaded the vascular cannals. Apparently the predominant element of the pore-filling minerals appears to be calcite. A comparison of the Mn and the Fe distributions reveals a very strong similarity, probably indicating iron oxides to be present in the former vascular cannals. Again the Sr and the U distributions are well correlated. They are also similar to the P distribution, thus presumably Sr and U are mainly present in the apatite remains. Pb is very inhomogeneously distributed, but shows high fluorescence intensities in the area where both, Sr and the U counts reach their maximum. Since Pb is a decay product of U, this indicates in agreement with \[8\] that Pb has been incorporated into the fossilised bones due to diagenetic processes.

Fig. 6 shows a region of a *Brachiosaurus brancai* bone that contains a presumably diagenetically induced crack in a region with primary fibro-lamellar bone and interspersed secondary osteons. The crack appears to be filled by a mineral with high calcium content. Similarly to the *Barosaurus africanus* fossil bone A1 (fig. 6) higher Sr and U intensities are mainly found in former hydroxyapatite and again appear to be correlated. However, the inhomogeneity in their distribution reveals that they have not been diagenetically averaged within the samples, which is in good agreement with \[7\] who state that their analytical results corroborate that Sr is not
affected by diagenesis. The P distribution in this bone appears also similar to the one of Sr and U. Here Pb is mainly present in the crack-filling mineral. Compared to the *Barosaurus africanus* fossil bone, fig. 4 and fig. 5, the elemental distribution of Fe and Mn in the *Brachiosaurus brancai* fossil bone is more homogeneous in fig. 6 and less well correlated.

CONCLUSIONS

µ-XRF investigations performed on sauropod long bones revealed no macroscopic gradients in the element distribution across the bone cross-section. On a microscopic level inhomogeneities in the chemical composition of the bones are present due to interdiffusion between the bone and its surroundings during the approximately 150 million years burial. The cavities in the bone, e.g. between the trabeculae and the vascular cannals, appear to be filled by minerals, predominantly calcite. These minerals contain various elements beside calcium, e.g. Fe, Mn and Pb. The Pb is a decay product of the U, but, the dissimilarity between the Pb distribution within the fossilized bones shows that it also has been incorporated into the bones as a result of diagenetic processes. Sr and U are mainly encountered in the former bone apatite and their distribution in the microstructure of the fossils appears well correlated.

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REFERENCES

[15] Pyzalla, A., Zaruba, Ch., et al., unpublished results