Spatially resolved manganese distribution in antler and human bone

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Motivation

The interest in manganese distribution in bone tissue is induced by the findings suggesting it's possible influence on fixation (or incorporation) of calcium into bones.
The experiments intend to fathom the importance of manganese in bone metabolism and the interdependence of manganese and bone health, as in the context of osteoporosis.

Samples

Deer antlers represent a suitable model for bone research – they are easily accessible, and growing faster, are therefore reflective of the environmental changes (e.g. dietary regimen).
In relation to osteoporosis, human bone samples were analyzed – osteoporotic and healthy bone cuts.

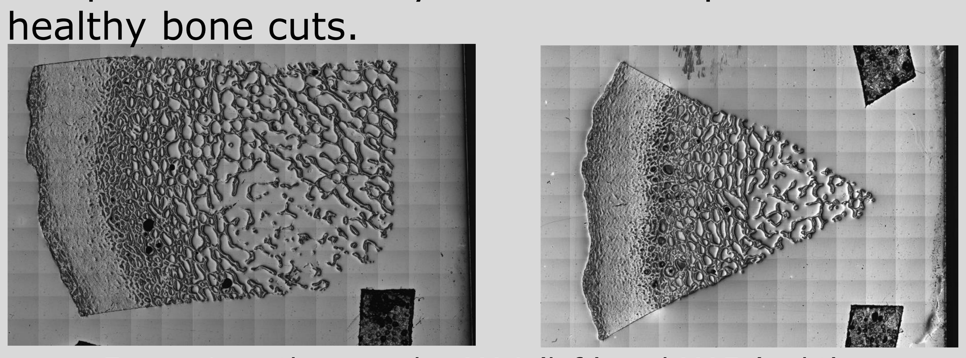

Figure 1: antler samples MA4 (left) and MA5 (right) - cuts embedded in PMMA

Method

The measurements were performed at the FLUO beamline at ANKA (Fig.2).

Characteristics of the setup:

- Primary excitation: 9.2 keV
- Monochromator: W/Si multilayer
- Focusing optics: 2 polycapillary half-lenses (Pb-free at detector)
- Filter: 20 µm Al-foil in front of the detector
- Detector: 50 mm² Vortex
- Digital signal processor: Saturn XIA
- Beam size: 26 µm x 18 µm for antler samples; 27 µm x 18 µm for human bone samples
- Depth resolution; 33.6 µm@Ti-Ka for antler samples; 34.5 µm@Ti-Ka for human bone samples

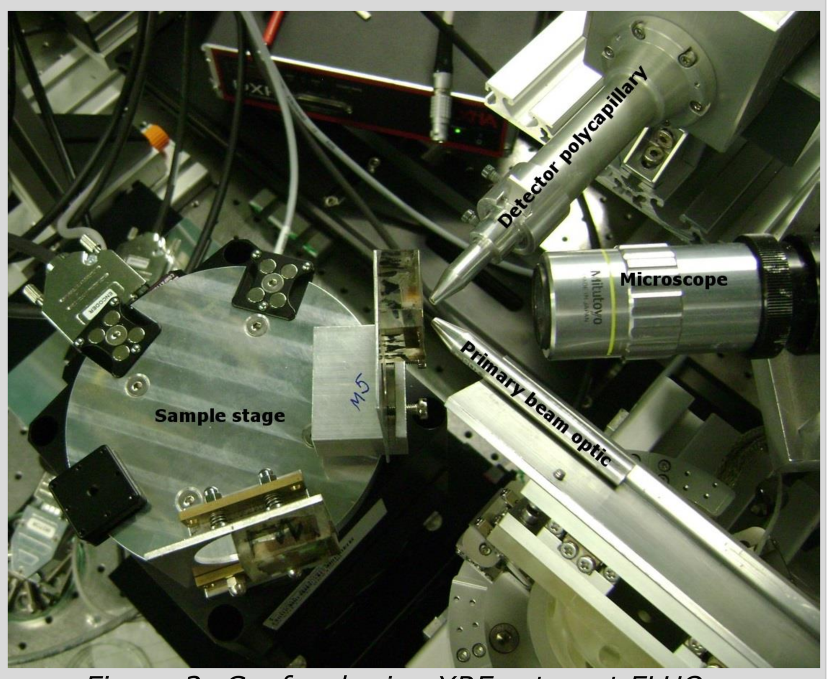

Figure 2: Confocal microXRF setup at FLUO beamline, ANKA

Results and data interpretation

1. Deer antlers

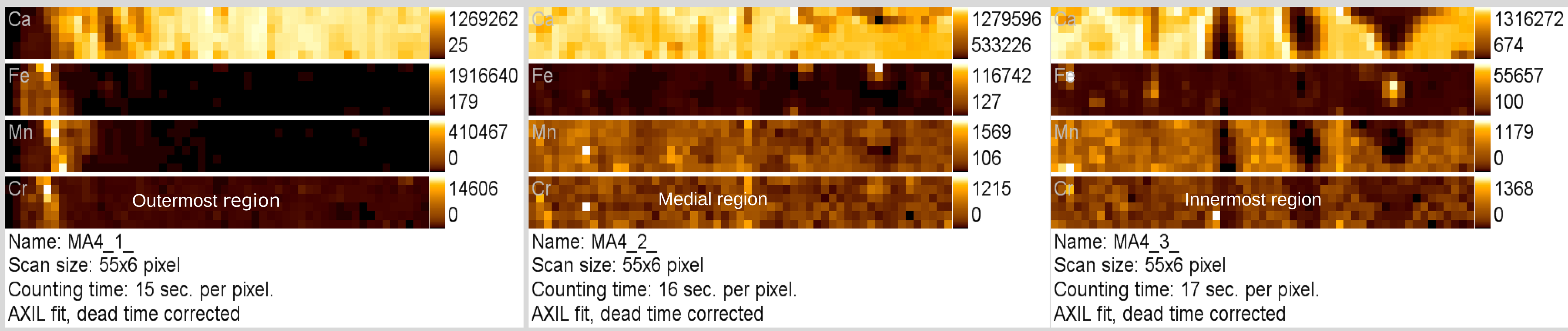

Figure 3: Elemental maps of sample MA4

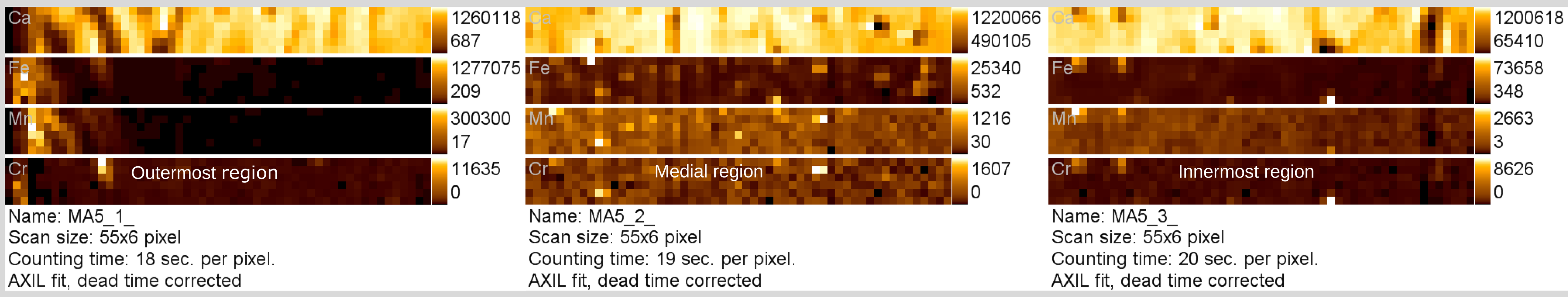

Figure 4: Elemental maps of sample MA5

Figure 5: Mn-Ca ratios of sample MA4 (order of the areas the same as on Fig.3)

Figure 6: Mn-Ca ratios of sample MA5 (order of the areas the same as on Fig.4)

Two antler samples were measured, MA4 and MA5 – cuts obtained from the same animal, but taken from different parts of antlers – MA4 being positioned closer to the base, MA5 – closer to the crown of antler.
The scanning was performed in the direction from outside of antler to inside, the area determined for scan was divided into three subregions of the same size – outermost region of antler, comprising low-mineralized structures, like skin and periost; medial region – mostly cortical bone and innermost region, which includes transition zone between cortical and trabecular bone.
The data analysis was performed using QXAS-AXIL software package. The elemental maps for following elements were produced: Ca, Fe, Mn, Cr (Fig.3-4). Though Fe and Cr in certain concentrations are considered as natural constituents of bone along with Mn, metal contamination arising from sample preparation need to be taken care of.
Therefore further data treatment using the software of X-ray Lab was applied, which includes exclusion of metal contamination (through setting a threshold depending on Cr countrate values) and getting the normalized (to 100mA and 1s) countrate ratios of Mn/(Ca+Mn), which allows for better overview of Mn distribution in the calcified bone matrix (Fig.5-6). The higher values for ratios in the outermost regions of the both samples attest the fact that Mn is needed in the sites of bone undergoing mineralization.

2. Human bone samples

The link between osteoporosis and lack of Mn was hypothesized on the base of animal model.
To prove the assumption human bone samples were analyzed. Osteoporotic samples were obtained from male patients with multiple vertebral fractures and low bone mineral density, 47-57 years old; cause of osteoporosis was unknown. As reference, healthy bone tissue samples, non-osteoporotic from male patients (middle aged) were also measured.
Fig.7-8 demonstrate the results obtained for osteoporotic sample MN1 (areas B and D).
Fig.9-10 show the results of measurements of healthy bone sample MN4 (areas B and D).
Homogeneous distribution and higher Mn content can be observed in healthy bone tissue, with about 10-fold difference in Mn-Ca ratios compared to osteoporotic sample.

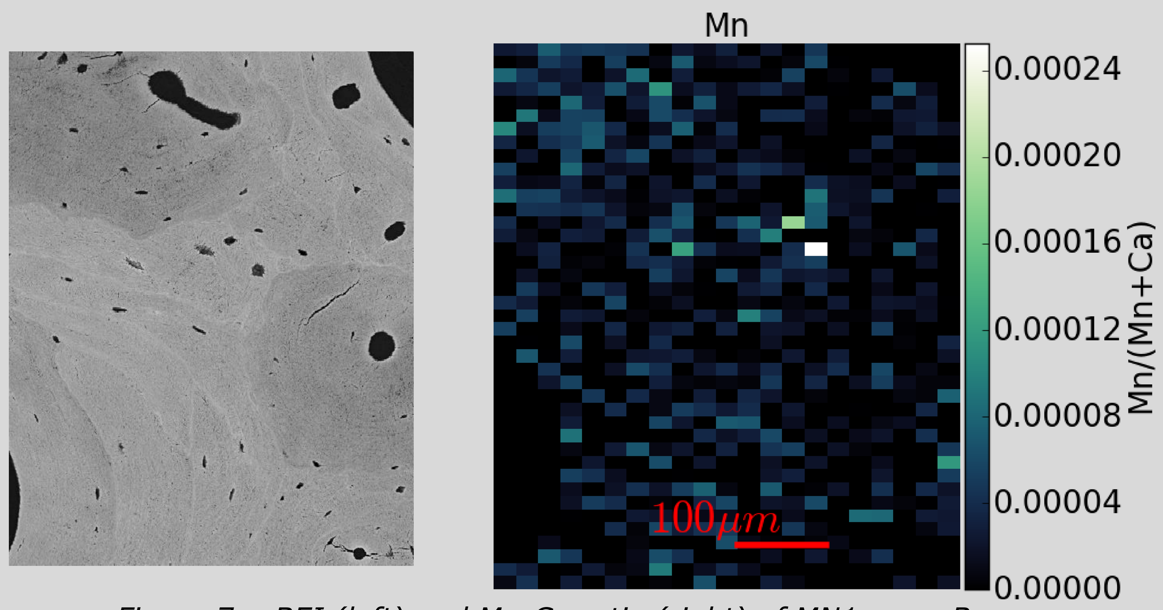

Figure 7: qBEI (left) and Mn-Ca ratio (right) of MN1, area B

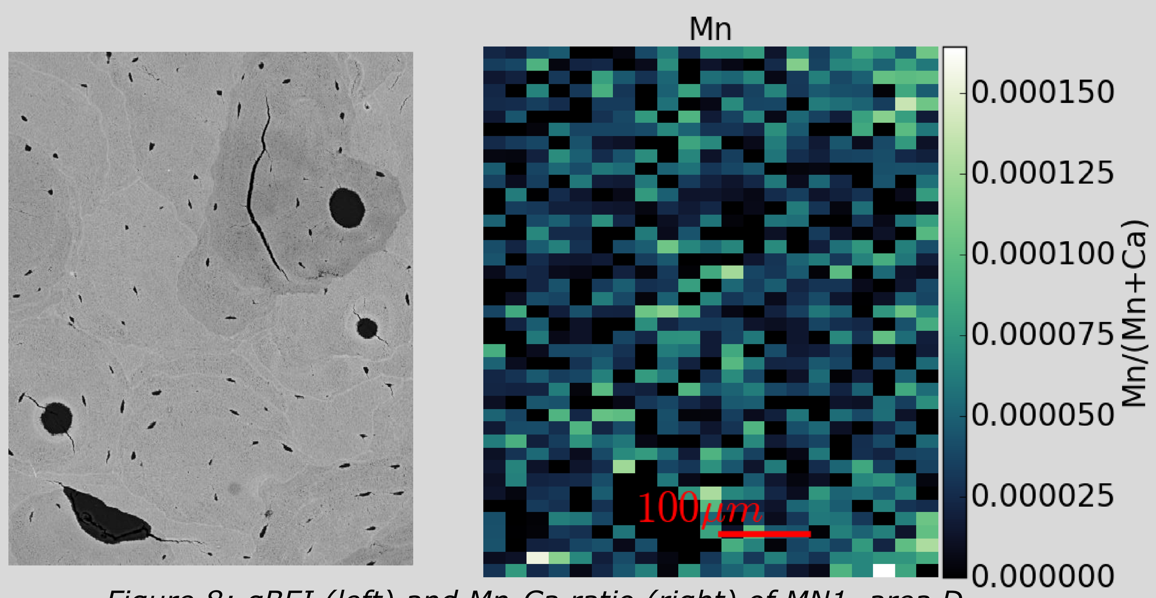

Figure 8: qBEI (left) and Mn-Ca ratio (right) of MN1, area D

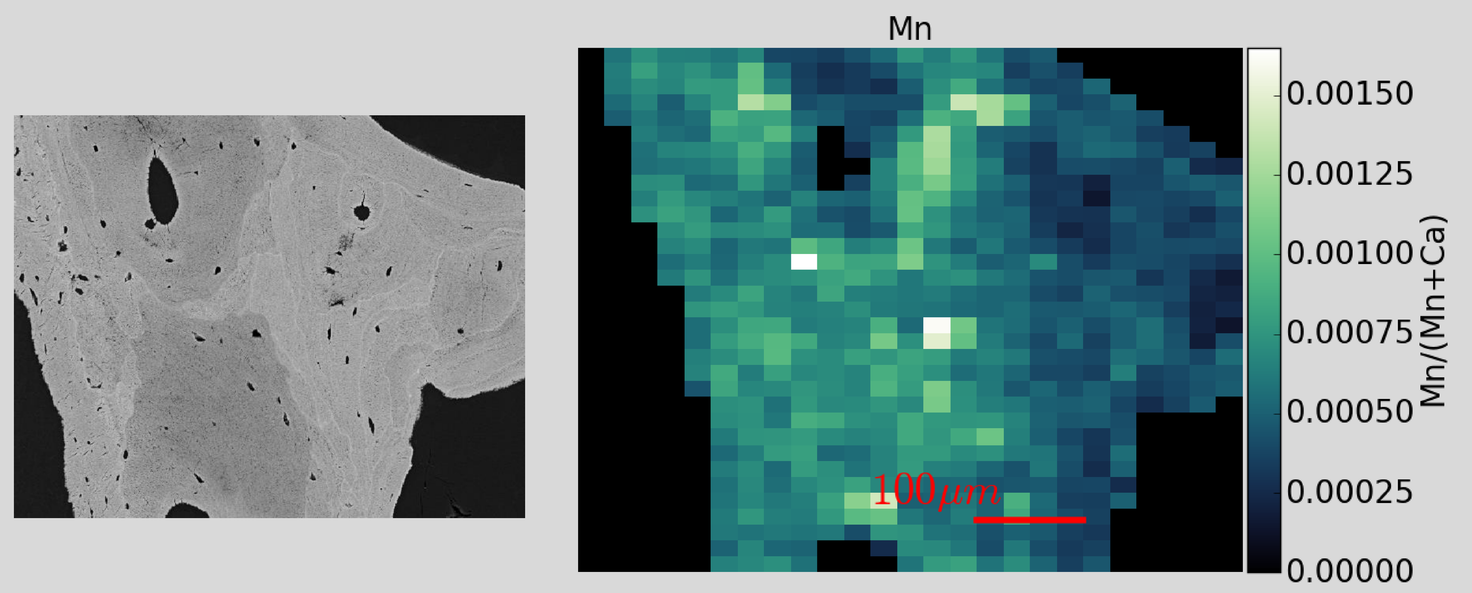

Figure 9: qBEI (left) and Mn-Ca ratio (right) of MN4, area B

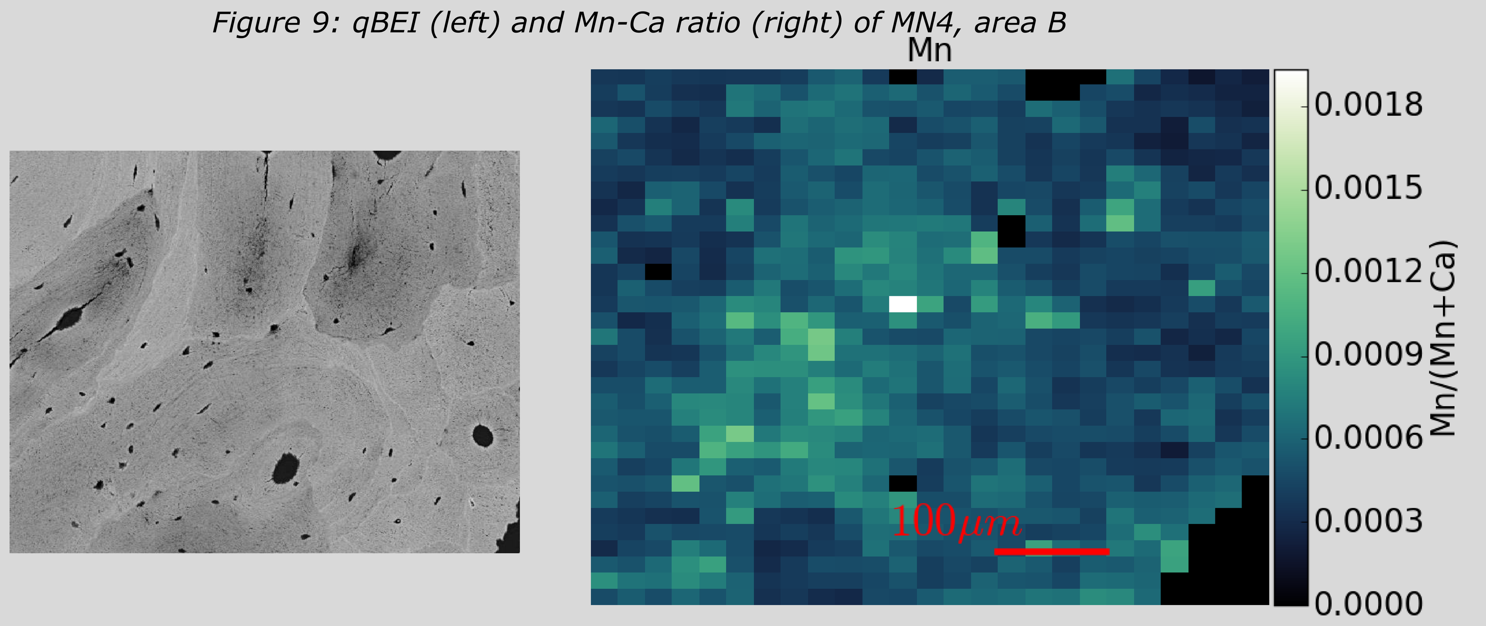

Figure 10: qBEI (left) and Mn-Ca ratio (right) of MN4, area D

Conclusions and Outlook

1. Deer antlers present a suitable model for bone metabolism research
2. Investigation of antlers cuts taken from different animals, kept e.g. under various dietary regimen, might provide us with deeper understanding of bone biology.
3. Preliminary studies of human osteoporotic and osteoporotic samples show differences in Mn distributions.
4. Enlarging sample pool of human bone cuts might lead to more firm conclusions regarding the role of Mn in pathogenic pathways in osteoporosis.

References

[1] B. Pemmer et al., Bone, 57 (2013), 184 - 193.
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