Supplementary material

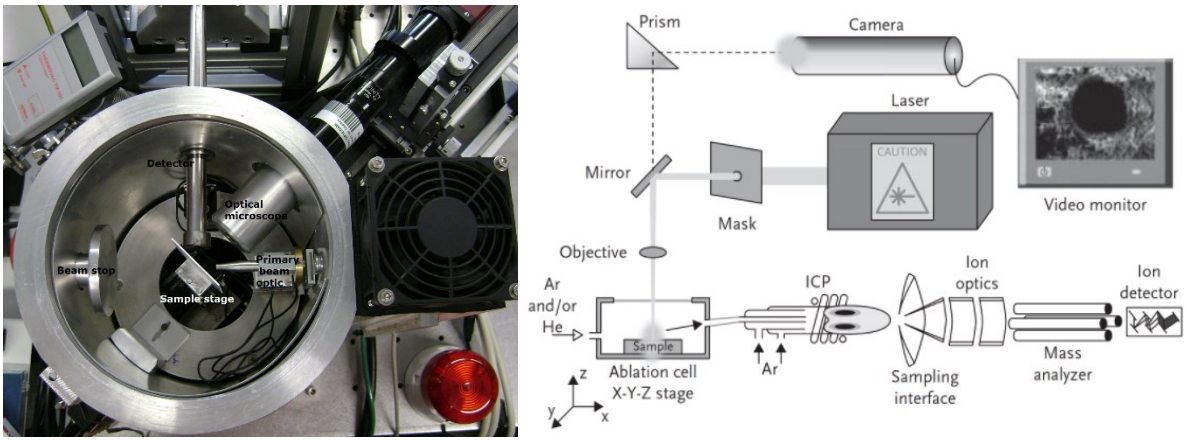

Figure S1. Left: µXRF setup at Atominstitut. Right: Schematic representation of LA-ICP-MS setup (source: https://www.ams.ugent.be/laser-ablation-icp-ms)

ICP-MS (characterization of self-prepared porcine cartilage standard)

The K and S content of the self-made porcine cartilage standard was determined via conventional ICP-MS analysis. After drying the sample powder in a drying cabinet at 65 °C for 60 h, 10 mg sample were weighed in and digested in 1 mL of a 1/1 volumetric mixture of concentrated HNO_3 (65 m%, EMSURE®, Merck, Darmstadt, Germany) and H_2O_2 (30 m%, EMSURE®, Merck, Darmstadt, Germany) at 60°C for 5 h. Three replicate digestions were performed. The obtained solutions were diluted 1/20 v/v proir to the analysis. For signal quantification, external calibration using a certified single element ICP-standard solution containing S (Specpure®, Alfa Aesar, Thermo Fisher Scientific, Kandel, Germany) and K (Certipur®, Merck, Darmstadt, Germany) was applied. An In standard solution (Certipur®, Merck, Darmstadt, Germany), was added to all samples and standards for a final concentration of 10 µg/kg and used as internal standard in order to correct instrumental drifts and variations in sample introduction.

An iCAP Q ICP-MS instrument (Thermo Fisher Scientific, Bremen, Germany) equipped with a quadrupole mass analyzer, a concentric PFA nebulizer, and a Peltier cooled quartz cyclonic spray chamber was used for the analysis. For data acquisition, Qtegra software provided by the manufacturer of the instrument was used. Prior to the measurement, the tune settings of the MS instrumentation were optimized for the maximum ^{115}In signal. Additionally, the oxide ratio was monitored by the $^{140}Ce^{16}O/^{140}Ce$ ratio, which was below 2.0% for the analysis. To minimize the influence of polyatomic interferences, kinetic energy discrimination (KED) mode utilizing a collision cell containing a mixture of helium with 7% hydrogen was used. Detailed information about the used instrumental settings can be found in Table S1.

Table S1. Instrumental parameters ICP-MS analysis

	Thermo iCAP
RF power	1500 W
Plasma gas flow (Ar)	14 l min^{-1}
Nebulizer gas flow (Ar)	0.99 l min^{-1}
Auxiliary gas flow (Ar)	1.2 l min^{-1}
Collision gas flow (He + 7% H_2)	3 ml min^{-1}
Kinetic energy barrier	3 V
Dwell time per isotope	10 ms
Cones	Ni
Measured isotopes	^{34}S, ^{39}K, ^{115}In

Table S2. Potential polyatomic interferences ICP-MS analysis

Isotope	**Interference**
^{24}Mg	$^{12}C_2^+$
^{31}P	$^{14}N^{16}O^1H^+$, $^{15}N^{15}N^1H^+$, $^{15}N^{16}O^+$, $^{14}N^{17}O^+$, $^{13}C^{18}O^+$, $^{12}C^{18}O^1H^+$
^{34}S	$^{15}N^{18}O^1H^+$, $^{16}O^{18}O^+$, $^{17}O_2^+$, $^{16}O^{17}O^1H^+$
^{39}K	$^{38}Ar^1H^+$
^{42}Ca	$^{40}Ar^1H_2^+$
^{50}Cr	$^{34}S^{16}O^+$, $^{36}Ar^{14}N^+$, $^{36}S^{14}N^+$, $^{32}S^{18}O^+$, $^{33}S^{17}O^+$
^{55}Mn	$^{40}Ar^{14}N^1H^+$, $^{39}K^{16}O^+$, $^{40}Ar^{15}N^+$, $^{38}Ar^{17}O^+$, $^{36}Ar^{18}O^1H^+$,$^{38}Ar^{16}O^1H^+$, $^{23}Na^{32}S^+$
^{56}Fe	$^{40}Ar^{16}O^+$, $^{40}Ca^{16}O^+$, $^{40}Ar^{15}N^1H^+$, $^{38}Ar^{18}O^+$, $^{38}Ar^{17}O^1H^+$
^{59}Co	$^{43}Ca^{16}O^+$, $^{42}Ca^{16}O^1H^+$, $^{36}Ar^{23}Na^+$, $^{40}Ar^{18}O^1H^+$
^{64}Zn	$^{32}S^{16}O_2^+$, $^{31}P^{16}O_2{}^1H^+$, $^{48}Ca^{16}O^+$, $^{32}S_2^+$, $^{31}P^{16}O^{17}O^+$, $^{36}Ar^{14}N_2^+$

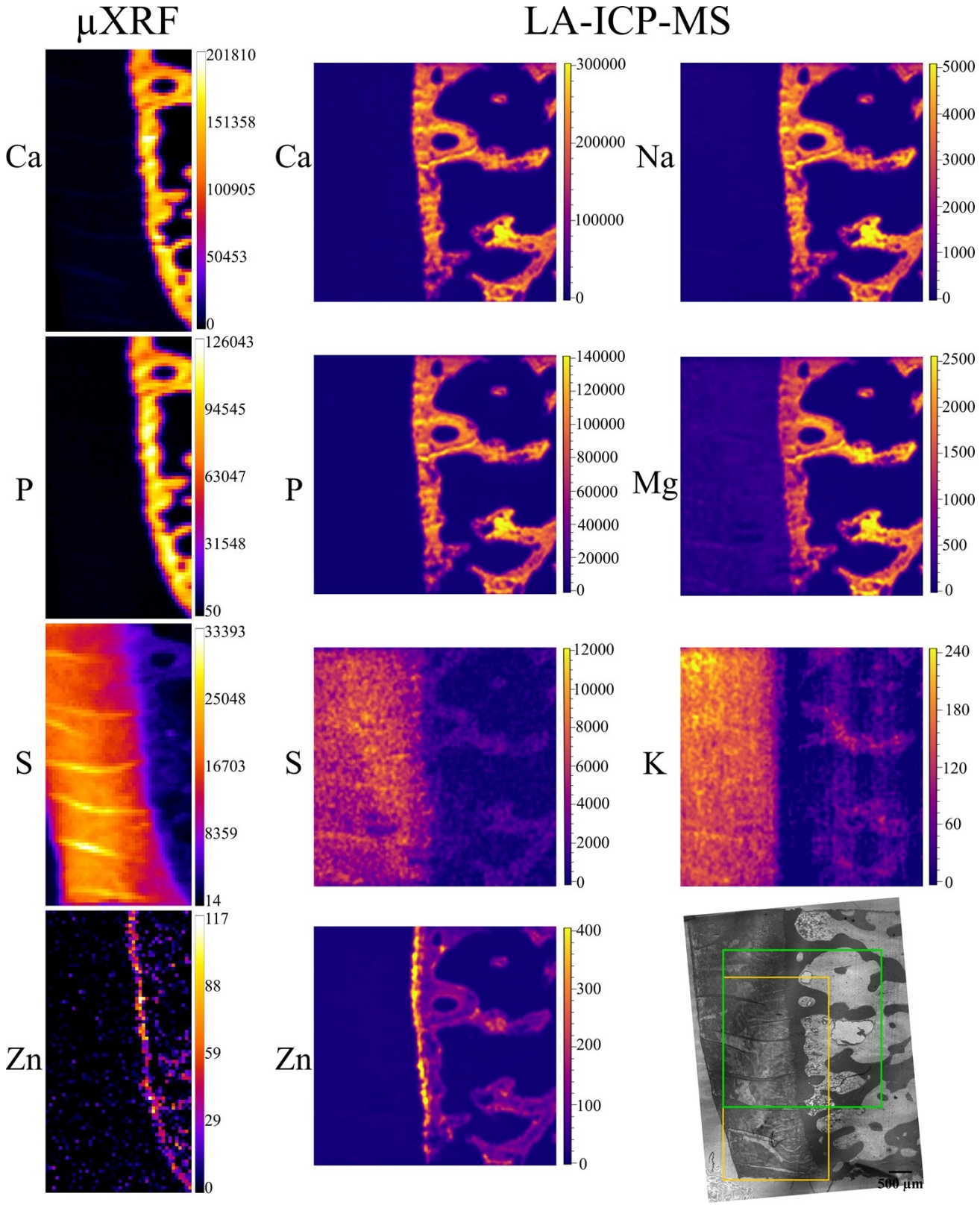

Figure S2. Original scans on 15 µm slice: elemental maps obtained by µXRF and LA-ICP-MS. The micrograph (transmitted light microscopy) in the low right corner with the scanned regions (µXRF – yellow, LA-ICP-MS – green rectangle). The units for both µXRF and LA-ICP-MS maps are µg/g. Please note, that S µXRF map is subject to fitting artifact within mineralized tissue (no S actually detected), detailed explanation is provided in the manuscript.

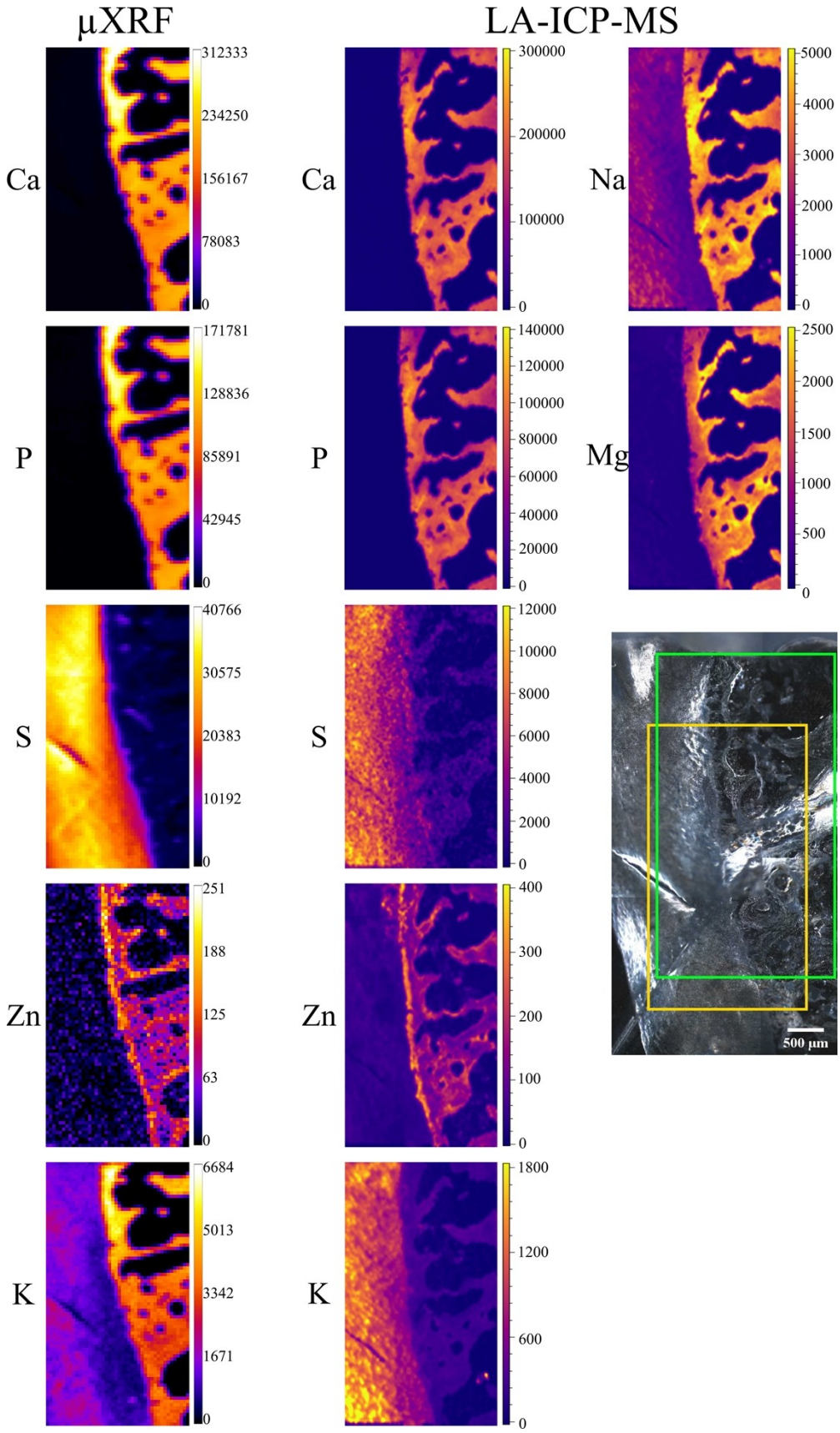

Figure S3. Original scans on 41 µm slice: elemental maps obtained by µXRF and LA-ICP-MS. The micrograph in the low right corner with the scanned regions (µXRF – yellow, LA-ICP-MS – green rectangle). The units for both µXRF and LA-ICP-MS maps are µg/g. Please note, that K µXRF map is subject to fitting artifact within mineralized issue (no K actually detected), detailed explanation is provided in the manuscript.

Analysis of µXRF maps with regard to elemental content within specific histological areas

The analysis was performed in ImageJ using the original µXRF scans (as shown in Fig. S2-S3). The mask for the calcification area was created on Ca map, tidemark mask – on Zn map, and cartilage mask on S map – see Fig. S4. The selection was done subjectively, as to include most of the area of interest, while minimizing the overlap with the adjacent tissue. The calcification zone includes bone and mineralized cartilage.

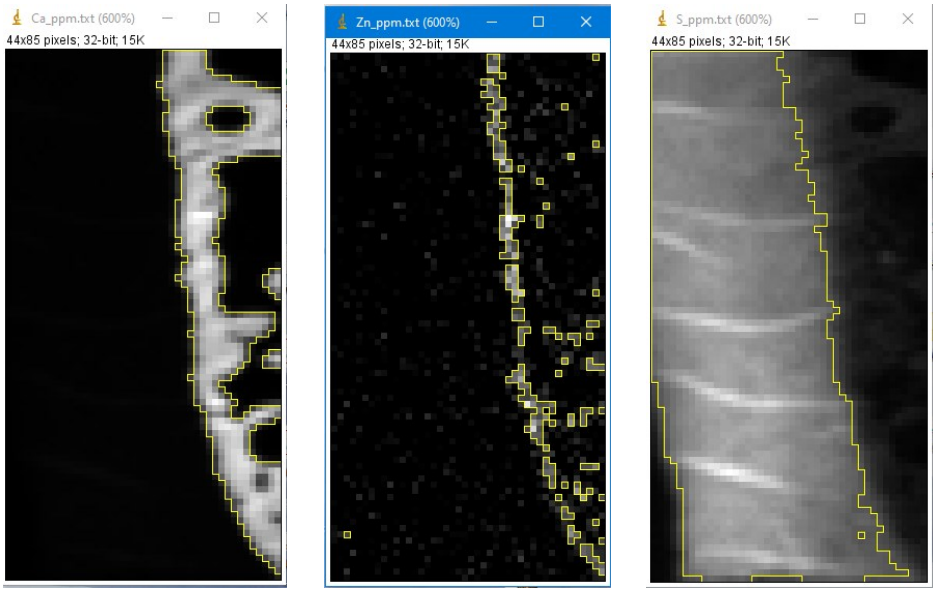

Figure S4. 15µm slice: left – µXRF Ca map with the "calcification zone" mask applied on it; middle – µXRF Zn map with the "tidemark" mask; right – µXRF S map with the "cartilage" mask

The masks (yellow line) were applied to the elemental maps of interest. The mean value, standard deviation for the area within the mask as well as minimum and maximum values for the 15 µm sample are given in Table S3.

Table S3. 15µm slice – comparison of elemental concentrations (µg/g) obtained by µXRF in calcification area, tidemark and cartilage areas (as shown in Fig. S4)

15 µm	**Calcification area** – 20% Ca signal (XRF), 747 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
Ca	122655.5 ± 36378.9	40366.2 – 201810.4
P	80263.2 ± 22738.3	14149 – 126043.2
Zn	16 ± 21	0 – 117.3
	Tidemark – 5% Zn signal (XRF), 181 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
Zn	48.3 ± 17.3	29.2 – 117.3
Ca	103549 ± 40001	79.9 – 201591.4
P	65510 ± 23769	274.2 – 110238.7
	Cartilage (hyaline) – 62% S signal (XRF), 2331 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
S	17518.5 ± 3663.6	10879.7 – 33392.7
Ca	2540.6 ± 4051.3	725.9 – 102477
P	1174 ± 2358.6	240.9 – 39284.6
Zn	1.3 ± 4.4	0 – 106.5

Similar procedure was performed for µXRF elemental maps of 41 µm slice (Fig. S5). As the tidemark zone is less pronounced, we could not separate this histological area based on Zn; a mask "Zn zone" includes pixels with high Zn values in mineralized cartilage (including the tidemark) and in bone. The results are presented in Table S4.

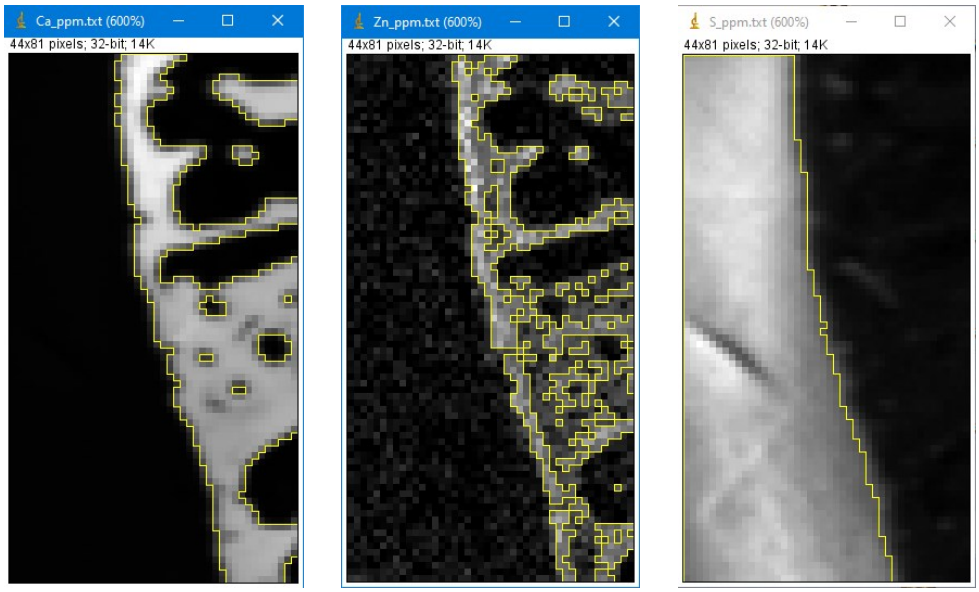

Figure S5. 41µm slice: left – µXRF Ca map with the "calcification zone" mask applied on it; middle – µXRF Zn map with the "Zn zone" mask; right – µXRF S map with the "cartilage" mask

Table S4. 41µm slice – comparison of elemental concentrations (µg/g) obtained by µXRF in calcification area, Zn zone and cartilage areas (as shown in Fig. S5)

41 µm	**Calcification area** – 29% Ca signal (XRF), 1044 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
Ca	189091.4 ± 50386.5	74829.4 – 312333.3
P	100227.8 ± 27150.7	31444.7 – 171781.4
Zn	93.2 ± 38.4	2.9 –250.6
	Zn zone – 16% Zn signal (XRF), 574 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
Zn	120.1 ± 28.9	85.6 – 250.6
Ca	192916 ± 52340.8	3554.7 – 312333.3
P	100955 ± 28672.6	1285.4 – 171781.4
	Cartilage (hyaline) – 52% S signal (XRF), 1839 pixels	
	$\bar{x} \pm s$, *in µg/g*	*Range, in µg/g*
S	27351.8 ± 5879.6	13590 – 40766.2
Ca	4680.8 ± 11184.7	1101.8 – 146076.3
P	2007 ± 6653.8	0 – 82902
Zn	14.2 ± 16	0 – 151.1
K	1330.7 ± 460.1	400.3 – 3872.4

One will notice dissimilarities between the µXRF values in Tables 2-3 (original paper) and the ones provided below. There are several reasons for these differences: 1) different approach to the division into mineralized and cartilage zones; 2) more pixels in the original map (and, therefore, more data points for estimations in Tables S3-S4), compared to the segments of the map shown in Fig .2-3, which are the base for the Tables 2-3; 3) no interpolation applied for plotting the original µXRF maps.

Estimation of LOD and LOQ for LA-ICP-MS and LOD for µXRF

The limits of detection and limits of quantification for LA-ICP-MS were calculated based on the measurements of blank sample (no analyte) as follows:
LOD = MEAN (Blank) + 3 * SD (Blank)

LOQ = MEAN (Blank) + 10 * SD (Blank)

The detection limits for µXRF were calculated using formula below and values obtained after fitting the summary spectra resulting from scanning the pelletized IAEA H-5 (Animal Bone) standard reference material:

$$LOD = \frac{3\sqrt{N_B}}{N_N} \times c$$

,

where N_N and N_B are the peak net area and peak background area of the element of interest, respectively, and c – the certified concentration of this element. The obtained LOD values were normalized to 1000 s.

Results for both methods are included in the Table S5.

Table S5. Summary for LA-ICP-MS and µXRF

LA-ICP-MS							
	23Na	**24Mg**	**31P**	**34S**	**39K**	**42Ca**	**64Zn**
LOD (µg/g)	7.4	0.12	68	1520	135	884	0.17
LOQ (µg/g)	25	0.38	228	5065	451	2947	0.55
µXRF							
			P	**S**	**K**	**Ca**	**Zn**
LOD_{1000} (µg/g)			186	-	525	131	26

Since sulphur is not present in IAEA H-5 standard reference material, and therefore no certified value is available for it, µXRF limits of detection for sulphur could not be estimated.