Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

„Direct determination of lead in bones using slurry sampling high-resolution continuum source electrothermal atomic absorption spectrometry“

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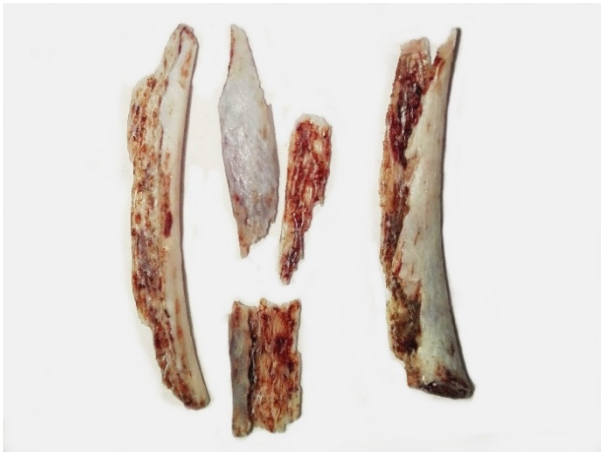
Figure S1 Bone **s**ample preparation and processing: (a) a rib sample with remnants of adherent tissue, (b) sample of the rib after cleaning with a ceramic knife, (c) bone specimens in sterile freeze-drying containers, (d) freeze dried bone sample, (e) a stainless steel vial (1" height x 1/2" diameter) with stainless steel ball pestle (1/4" diameter), (f) resulting sample after 5 min of grinding time, scale given by CZ coin of 23 mm diameter.

a

b

c



d

e

f

Figure S2. Absorbance spectra of PO recorded at different temperatures in the vicinity of the lead line at 217.001 nm

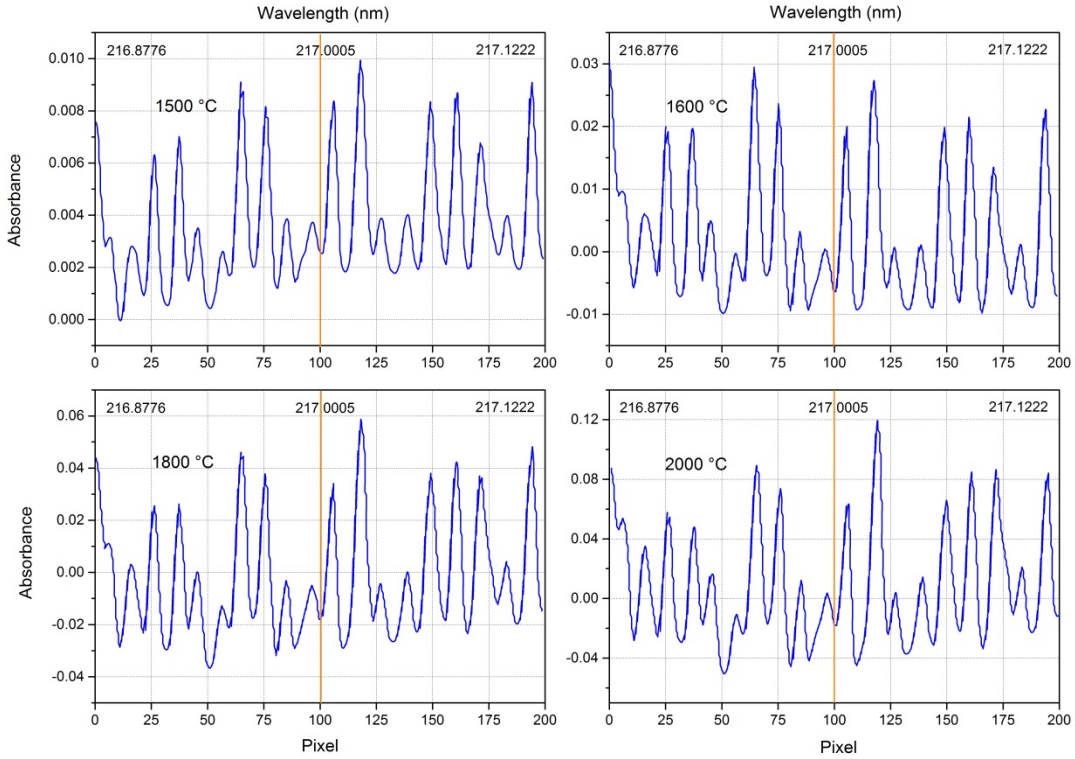


Figure S3. The influence of the amount of hydroxyapatite on the integrated absorbance determined at the Pb line 217.001 nm in the presence of 1 μg Pd + 50 μg citric acid for reagent blank samples without (black line) and with the use of automatic correction with the use of reference PO spectra and a least-square algorithm (red line). Pyrolysis and atomization temperature was 1000 and 2100 °C, respectively.

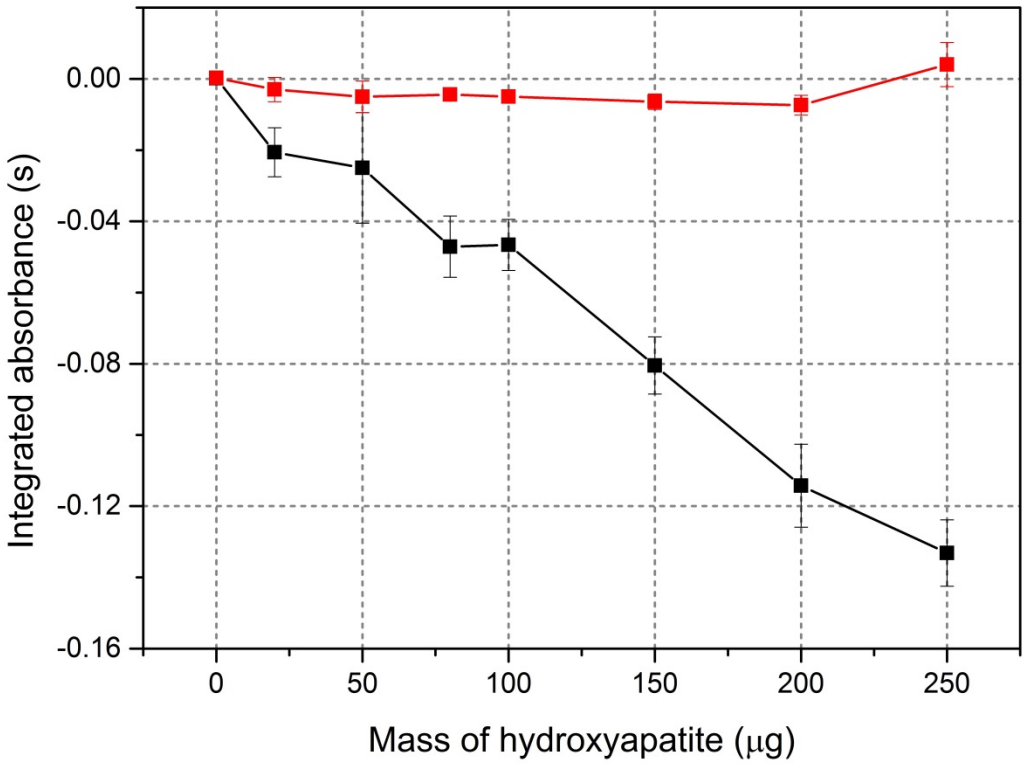


Figure S4a. X-ray diffractogram of NIST SRM® 1486

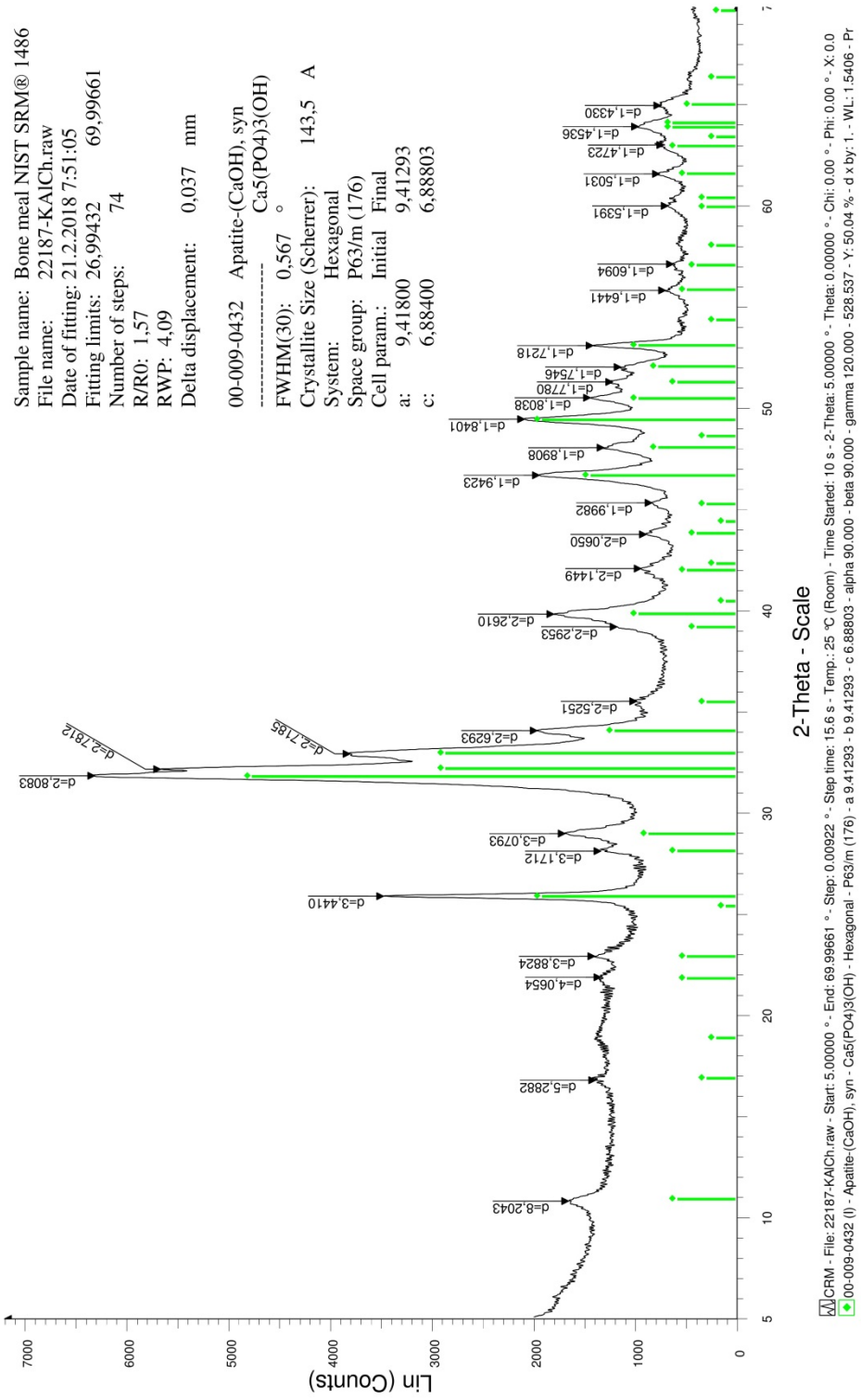


Figure S4b. X-ray diffractogram of rabbit rib bone

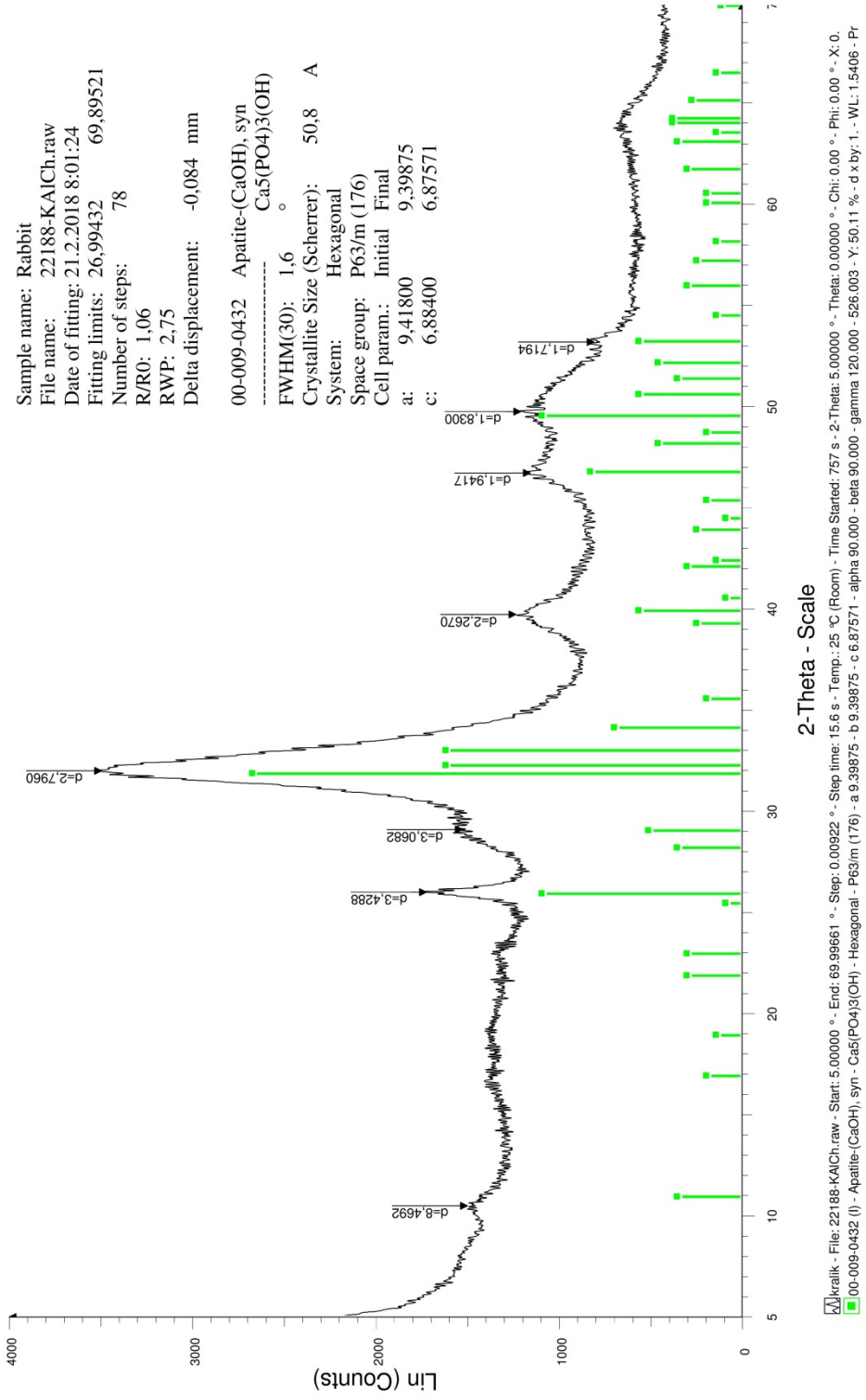


Figure S4c. X-ray diffractogram of a river otter rib bone

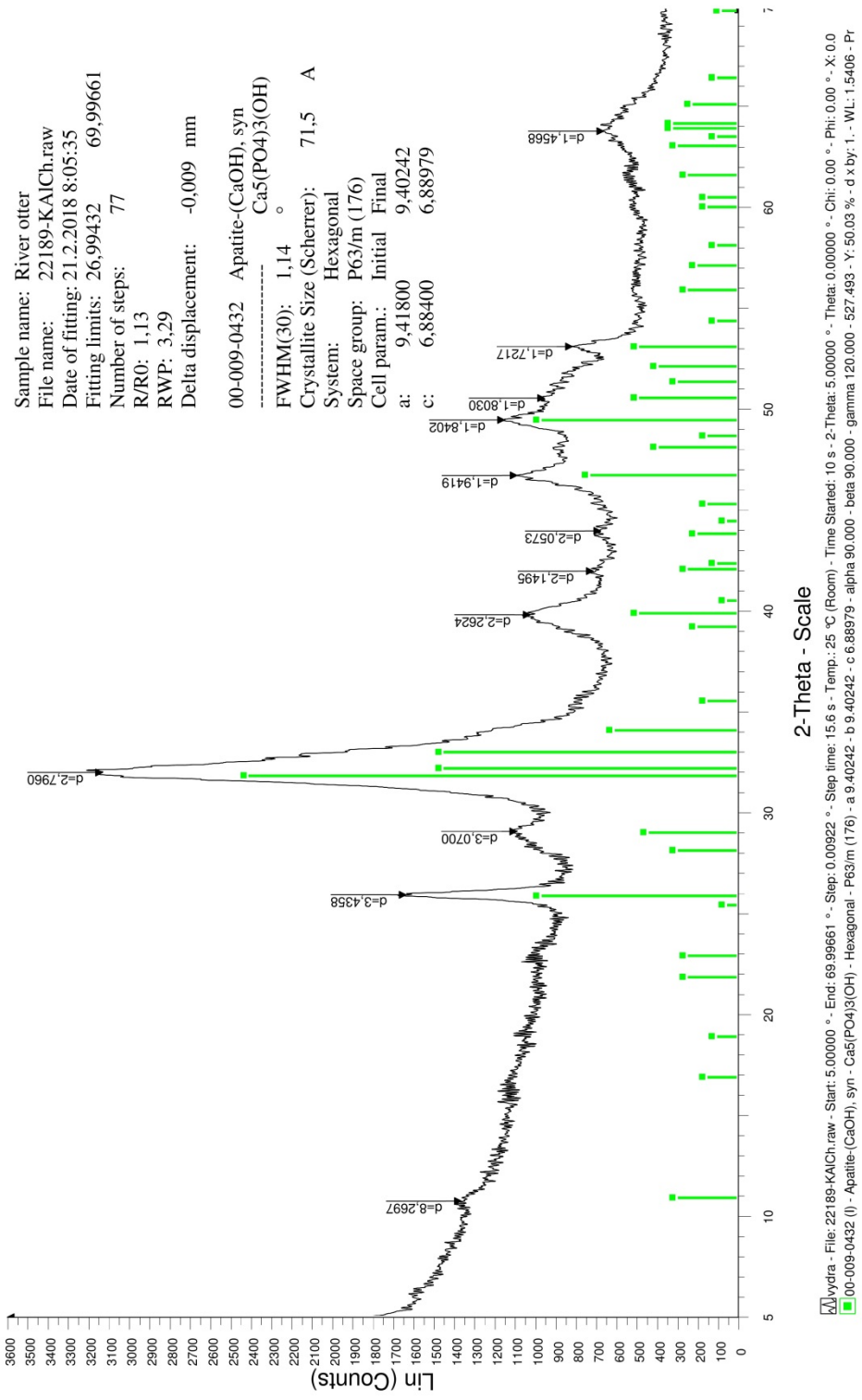


Fig.S5 Effect of grinding time on the particle size distribution of rabbit (red line) and otter (blue line) bones

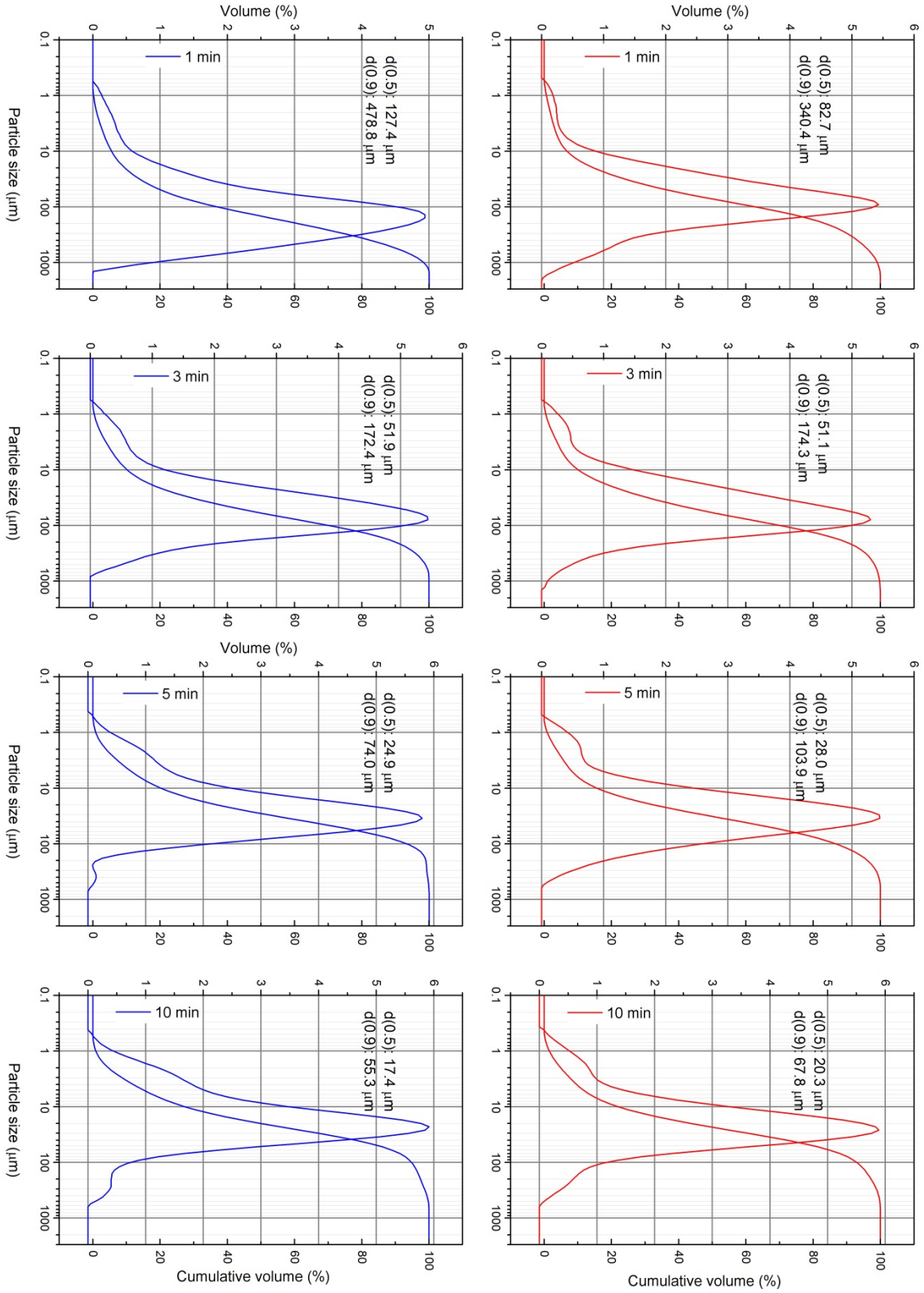
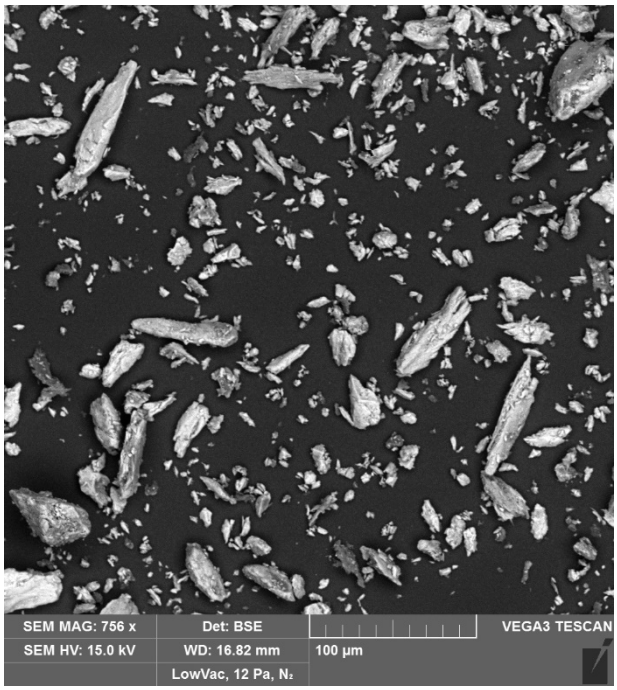
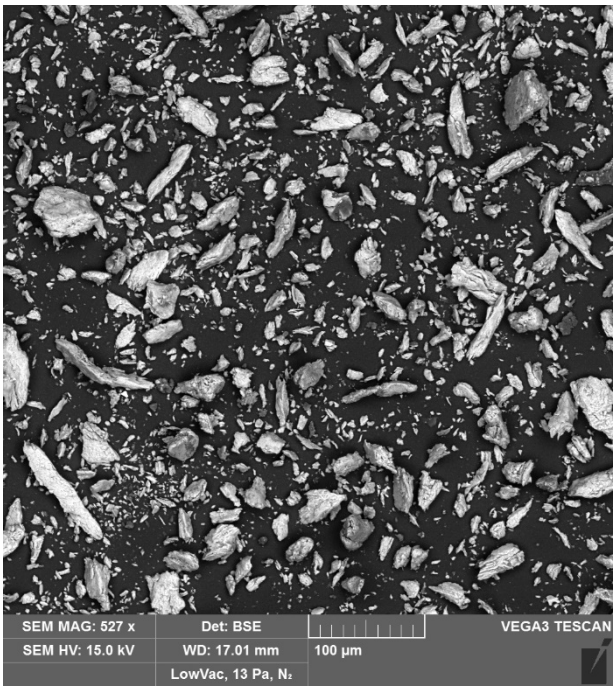


Fig.S6 SEM images of (A) rabbit and (B) river otter bone after 3 min of grinding



A



B

Figure S7. The effect of pyrolysis (A) and atomization (B) temperature on the integrated absorbance of 200 pg Pb in aqueous standard without (○) and with 1 μg Pd + 50 μg citric acid (●) chemical modifier.

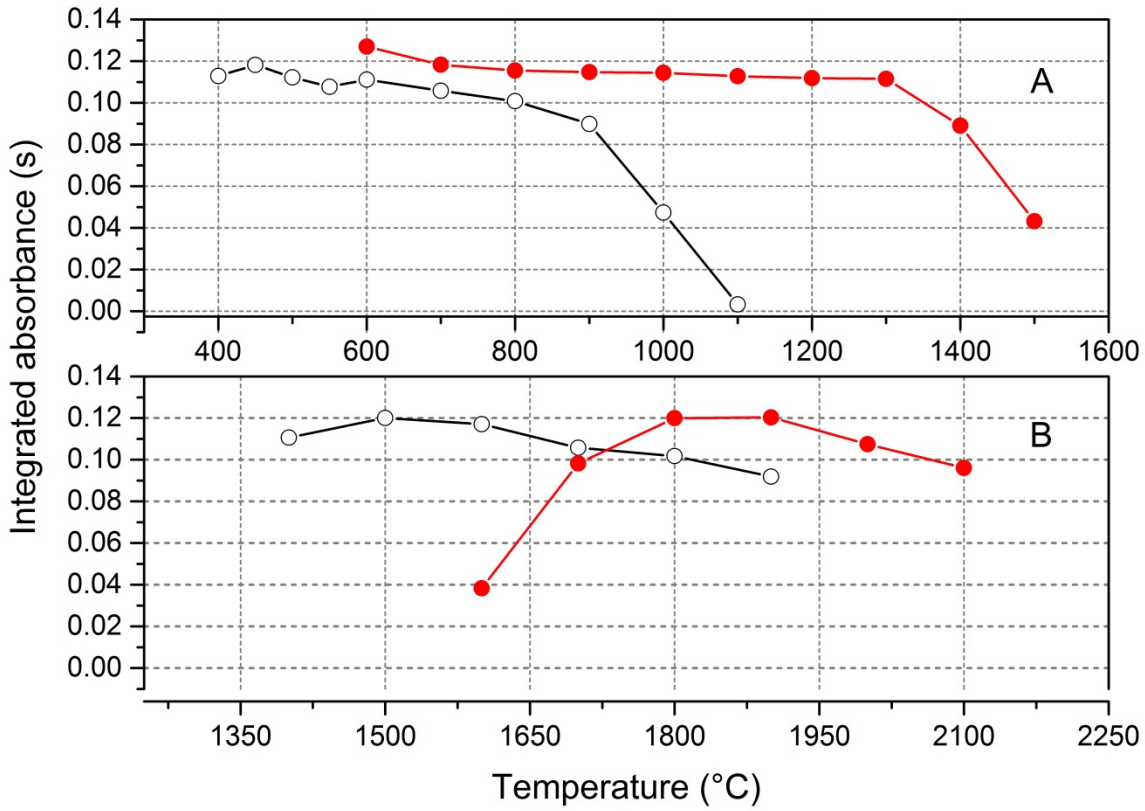


Table S1. Design and experimental results[a] for analysis of Pb in NIST SRM 1486 Bone Meal

Exp.	Replic.	Glycerol (%)	HNO_3 (%)	Sonication (min)	Particles (μm)	Tp (°C)	Ta (°C)	Modifier (μL)	Integrated absorbance
2	1	20.0	0.0	2.0	54	900	2100	10	0.0409
11 (C)	1	10.0	2.5	6.0	160	1150	1800	6	0.1297
23	3	0.0	0.0	2.0	315	1400	2100	2	0.0040
26	3	20.0	5.0	2.0	315	900	1500	2	0.1476
13	2	20.0	0.0	2.0	54	900	2100	10	0.0367
16	2	0.0	0.0	10.0	315	900	1500	10	0.0158
17	2	20.0	0.0	10.0	54	1400	1500	2	0.0040
22 (C)	2	10.0	2.5	6.0	160	1150	1800	6	0.1340
1	1	0.0	0.0	2.0	315	1400	2100	2	0.0055
12	2	0.0	0.0	2.0	315	1400	2100	2	0.0040
28	3	20.0	0.0	10.0	54	1400	1500	2	0.0030
32 (C)	3	10.0	2.5	6.0	160	1150	1800	6	0.1316
20 (C)	2	10.0	2.5	6.0	160	1150	1800	6	0.1325
8	1	20.0	5.0	10.0	315	1400	2100	10	0.0584
33 (C)	3	10.0	2.5	6.0	160	1150	1800	6	0.1203
14	2	0.0	5.0	2.0	54	1400	1500	10	0.0305
19	2	20.0	5.0	10.0	315	1400	2100	10	0.0466
30	3	20.0	5.0	10.0	315	1400	2100	10	0.0472
27	3	0.0	0.0	10.0	315	900	1500	10	0.0457
4	1	20.0	5.0	2.0	315	900	1500	2	0.1303
21 (C)	2	10.0	2.5	6.0	160	1150	1800	6	0.1065
9 (C)	1	10.0	2.5	6.0	160	1150	1800	6	0.1191
25	3	0.0	5.0	2.0	54	1400	1500	10	0.0225
31 (C)	3	10.0	2.5	6.0	160	1150	1800	6	0.1237
6	1	20.0	0.0	10.0	54	1400	1500	2	0.0017
3	1	0.0	5.0	2.0	54	1400	1500	10	0.0300
7	1	0.0	5.0	10.0	54	900	2100	2	0.1288
15	2	20.0	5.0	2.0	315	900	1500	2	0.1233
29	3	0.0	5.0	10.0	54	900	2100	2	0.1262
24	3	20.0	0.0	2.0	54	900	2100	10	0.0400
18	2	0.0	5.0	10.0	54	900	2100	2	0.1217
5	1	0.0	0.0	10.0	315	900	1500	10	0.0261
10 (C)	1	10.0	2.5	6.0	160	1150	1800	6	0.1271

[a] Results are presented as integrated absorbances evaluated as the means of 3 consecutive analytical runs.

Tp and Ta: pyrolysis and atomization temperature, respectively.

Table S2. Determination of Ca, P, Na and Mg in NIST SRM 1486 Bone Meal, rabbit and otter ribs by EDX

	Mass fraction (%)		
Constituent element	SRM	Rabbit	River otter
Ca	25.82 ± 0.66	25.02 ± 0.64	29.08 ± 0.83
P	12.26 ± 0.41	10.17 ± 0.34	9.97 ± 0.38
Na	0.49 ± 0.06	0.54 ± 0.06	0.74 ± 0.07
Mg	0.42 ± 0.05	0.46 ± 0.05	0.27 ± 0.04

Certified[a] or information[b] mass fraction values (Dry-Mass Basis) for SRM 1486: Ca[a]: 26.58 ± 0.24 %, Mg[a]: 0.466± 0.017 %, P[a]: 12.30 ± 0.19 %, Na[b]: 0.5 %

Table S3. Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	DF	Adj SS	Adj MS	*F*-value	*p*-value[a]
Model	8	0.089235	0.011154	172.63	0.000
Linear	4	0.053645	0.013411	207.56	0.000
x_1 (Glycerol)	1	0.000589	0.000589	9.12	0.006
x_2 (HNO_3)	1	0.025722	0.025722	398.09	0.000
x_3 (Tp)	1	0.021943	0.021943	339.61	0.000
x_4 (Modifier)	1	0.005391	0.005391	83.43	0.000
Square	1	0.035127	0.035127	543.64	0.000
x_1 (Glycerol) × x_1 (Glycerol)	1	0.035127	0.035127	543.64	0.000
2-Way Interaction[b]	3	0.000463	0.000154	2.39	0.094
x_1 (Glycerol) × x_2 (HNO_3)	1	0.000196	0.000196	3.03	0.095
x_1 (Glycerol) × x_3 (Tp)	1	0.000004	0.000004	0.06	0.804
x_1 (Glycerol) × x_4 (Modifier)	1	0.000263	0.000263	4.08	0.055
Error	24	0.001551	0.000065		
Total	32	0.090786			

Model Summary: R^2 = 98.3 %; R^2(adj) = 97.7 %; R^2(pred) = 96.8 %, where: R^2 is the percentage of variation in the response that is explained by the model, R^2(adj) is the percentage of the variation in the response that is explained by the model, adjusted for the number of predictors in the model relative to the number of observations, R^2(pred) provides a measure of how well the regression model predicts the response outside the range of data sample used for the regression.

[a] Parameter is statistically insignificant at significance level 0.05 when its *p*-value is above 0.05

[b] The following terms cannot be estimated and were removed: $x_2 \times x_2$ ($HNO_3 \times HNO_3$); $x_3 \times x_3$ (Tp × Tp); $x_4 \times x_4$ (Modifier × Modifier); $x_2 \times x_3$ (HNO_3 × Tp); $x_2 \times x_4$ (HNO_3 × Modifier); $x_3 \times x_4$ (Tp × Modifier).