Supplementary Materials

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These supplementary figures and tables are a variable hodgepodge of things that didn't fit in the main manuscript, and additional things I thought might be useful. The best way to explore/verify the results and interpretations is to download all the data and code (https://doi.org/10.5281/zenodo.7649824) and just play around with it yourself. Enjoy!

Samples

Calculus samples are a combination of leftovers from a previous aDNA study and newly sampled individuals. In some cases individuals from the previous study were sampled again (if not enough calculus was left over from the previous study) (Table 1).

Selection

Table 1: Table showing which individuals were sampled in this study and which individuals were sampled in the previous study. When both TRUE, the individual was sampled twice.

ID	this study	previous study
MB107	TRUE	TRUE
MB116	FALSE	TRUE
MB117	TRUE	FALSE
MB120	FALSE	TRUE
MB121	TRUE	FALSE
MB131	FALSE	TRUE
MB158	FALSE	TRUE
MB163	FALSE	TRUE
MB18	FALSE	TRUE
MB180	FALSE	TRUE
MB186	TRUE	TRUE
MB204	FALSE	TRUE
MB207	TRUE	TRUE
MB235	FALSE	TRUE
MB251	TRUE	FALSE
MB255	FALSE	TRUE
MB261	TRUE	FALSE
MB266	FALSE	TRUE

ID	this study	previous study
MB287	TRUE	FALSE
MB29	FALSE	TRUE
MB292	TRUE	FALSE
MB298	TRUE	TRUE
MB30	FALSE	TRUE
MB301	TRUE	FALSE
MB301	TRUE	FALSE
MB329	TRUE	FALSE
MB361	FALSE	TRUE
MB365	TRUE	FALSE
MB366	TRUE	TRUE
MB37	FALSE	TRUE
MB392	FALSE	TRUE
MB396	FALSE	TRUE
MB406	TRUE	TRUE
MB407	FALSE	TRUE
MB410	TRUE	FALSE
MB423	TRUE	TRUE
MB425	FALSE	TRUE
MB53	FALSE	TRUE
MB60	FALSE	TRUE
MB65	FALSE	TRUE
MB69	FALSE	TRUE
MB78	FALSE	TRUE
MB9	TRUE	FALSE
MB9	TRUE	FALSE

Demographics

The sample consists of 41 individuals, most of which are middle adult male individuals (Figure 1). Middle adult males were preferentially targeted due to larger calculus deposits (observation) and the sample age and sex distribution is therefore not representative of the population. This was also done to limit potential confounding factors, and because pipe notches, which served as a positive control for tobacco, are predominantly seen in male individuals at the site.

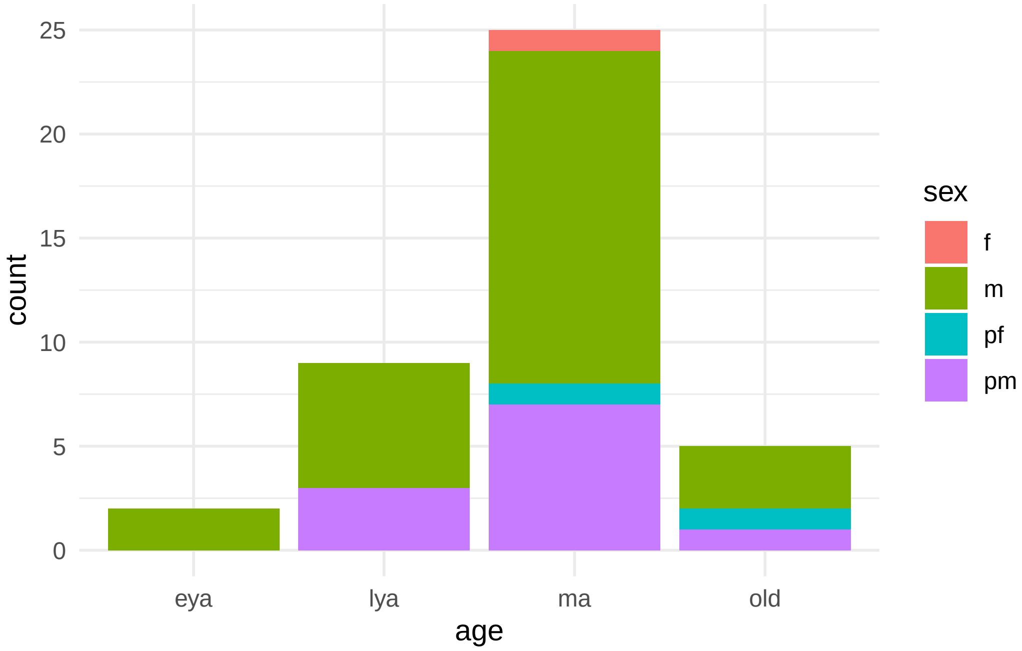


Figure 1: Distribution of age and sex in the sample. f = female; pf = probable female; pm = probable male; m = male; eya = early young adult (18-24 years); lya = late young adult (25-34 years); ma = middle adult (35-49 years); old = old adult (50+ years).

Missing data

An overview of the missing teeth can be found in Figure 2. Missing scores per tooth can be found in Table 2 and Figure 3.

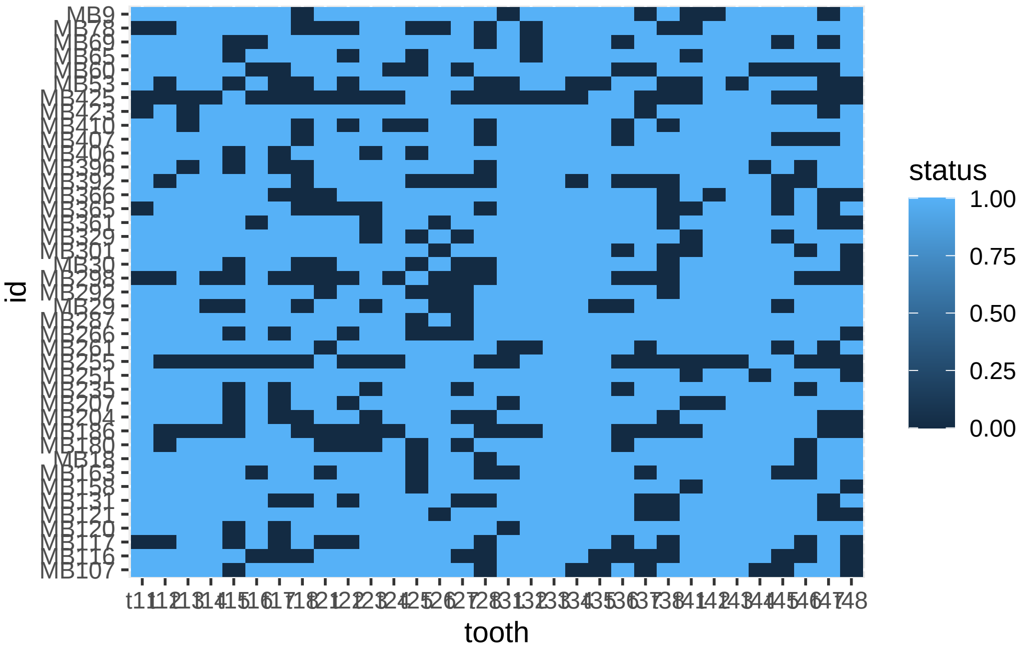


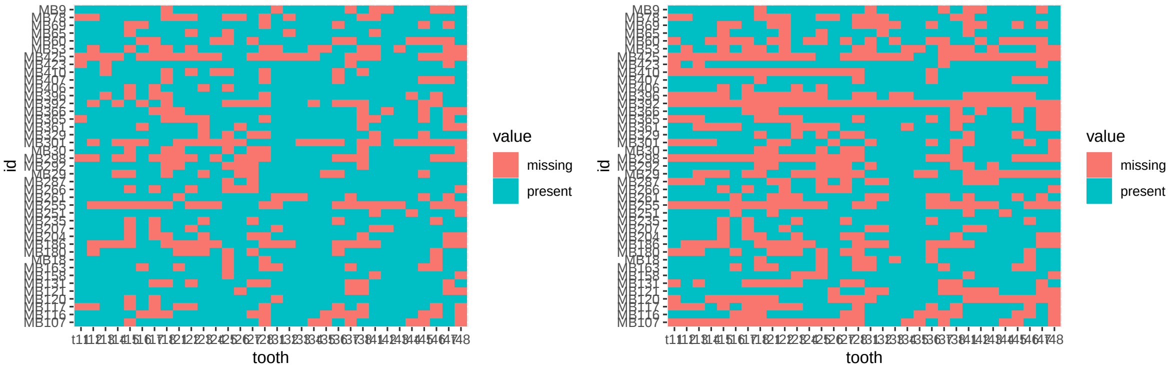
Figure 2: Heatmap of missing teeth per individual in the sample. 1 = present, 2 = missing.

Table 2: Table of missing scores by tooth.

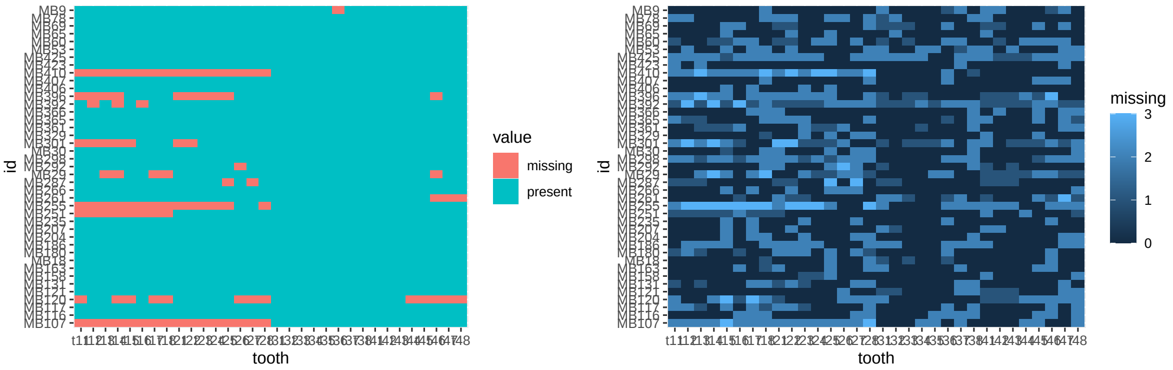
score	t11	t12	t13	t14	t15	t16	t17	t18	t21	t22	t23	t24	t25	t26	t27	t28	t31	t32	t33	t34	t35	t36	t37	t38	t41	t42	t43	t44	t45	t46	t47	t48
caries	6	10	6	7	18	9	16	19	13	15	12	7	16	9	15	21	9	5	3	5	5	14	14	19	13	4	3	5	14	15	18	17
periodontitis	17	15	17	13	21	15	20	25	24	22	20	12	21	12	18	24	19	10	6	6	5	17	15	18	22	13	10	8	16	16	20	20
periapical	7	7	7	9	6	5	6	6	5	5	4	4	5	4	4	4	0	0	0	0	0	1	0	0	0	0	0	1	1	4	2	2

UHPLC analysis

The UHPLC-MS/MS method was validated in a separate study on cadavers received for forensic autopsy and toxicological analysis. Results from dental calculus were validated against compounds detected in whole blood samples from the same individuals (Sørensen et al. 2021). In the original method, samples were washed three times to remove residual substances from the surface of the calculus that originated from oral fluids, and only extract substances from



(a) Caries (b) Periodontitis



(c) Periapical lesions (d) Combined caries, periodontitis, periapical.

Figure 3: Plots

the calculus. In our samples the washes served to remove potential contaminants from the burial environment and post-excavation handling.

Briefly, dental calculus was treated with citric acid and the dissolution extracts were cleaned using weak and strong polymeric cation-exchange sorbents. Samples were washed with 0.5 mL MeOH for 10 seconds. Samples were weighed before and after each wash. The wash solvent was evaporated to a residual volume of 10 µl and added 50µl 30% methanol. Samples were air-dried for 24 hours at room temperature after each wash. Extracts from each wash were analysed by injecting 5 µL into the column on an Exion UHPLC system that consisted of two Exion AD pumps, an Exion AD multiplate autosampler set at 10 ± 2 °C and an Exion AC column oven set at 40 ± 2 °C (Sciex, Ontario, Canada). Separation was performed using a Raptor Biphenyl UHPLC column (2.7 mm, 2.1 mm I.D. × 100 mm) (Restek, Bellefonte, PA). The mass spectrometer was a Sciex QTRAP 6500+ with a TurboIonSpray probe for electrospray ionisation.
The remaining calculus was dissolved using lysing tube beads in 800 μL of 0.5 M citric acid (CA) and 50 μL stable isotope-labelled analogue used as internal standards (SIL-IS) solution for 1 h at ambient temperature with gentle shaking. The suspension was then mixed with 800 μL MeOH and centrifuged at 10,000 × g for 5 mins, and analysed by the same method as the wash extracts. Data analysis was performed using Analyst 1.7 and MultiQuant 3.0.3 (Sciex). Raw quantities of compounds are presented in ng and concentrations as ng / mg.

The samples in the replication batch were processed in the same way, but analysed on different equipment used exclusively for oral samples.

Raw quantities of compounds detected in the dissolved calculus from batches 1 and 2 are presented in Table 4 and Table 5. Since these tables may or may not be legible in PDF format, not to mention that they don't adhere to FAIR principles in this format, the raw data can be downloaded from Zenodo (https://doi.org/10.5281/zenodo.8061483).

Table 3: Target compounds and lower limits of quantitation (LLOQ).

Target	LLOQ
6-MAM	0.025
A29	0.050
Absinthin	0.500
Alprazolam	0.025
Amphetamine	0.100
Apigenin	1.000
Benzoylecgonine	0.025
Buprenorphine	0.050
CBD	0.050
CBN	0.050
Caffeine	0.050
Carvone (only MeOH extracts)	5.000

Target	LLOQ
Catechin	0.050
Cathinone	0.100
Chlordiazepoxide	0.050
Chlorprothixene	0.050
Clonazepam	0.025
Clonazepam, 7-amino	0.200
Cocaine	0.025
Codeine	0.050
Cotinine	0.050
Diazepam	0.010
EDDP	0.010
Fentanyl	0.010
Furosemide	0.025
Gabapentin	0.100
Heroin	0.025
Hydrocodone	0.010
Hydromorphone	0.025
Hyoscyamine	0.050
Kaempferol	0.500
Ketamine	0.010
Ketobemidone	0.010
Lamotrigine	0.500
Levamisole	0.025
Lidocaine	0.010
Luteolin	0.100
MDA	0.050
MDE	0.050
MDMA	0.050
Methadone	0.010
Methamphetamine	0.050
Methylphenidate	0.100
Midazolam	0.050
Mirtazepine	0.050
Morphine	0.050
Morphine-3-glu	0.050
Morphine-6-glu	0.050
Nicotine	0.100
Nitrazepam	0.025
Nitrazepam, 7-amino	0.100
Norbuprenorphine	0.100
Norcodeine	0.050

Target	LLOQ
Nordazepam	0.010
Noscapine	0.025
Oxazepam	0.025
Oxycodone	0.025
Oxymorphone	0.050
Papaverine	0.025
Paracetamol	0.200
Pregabalin	0.200
Quercetin (only MeOH extracts)	0.050
Quetiapine	0.050
Salicylic acid	0.500
Sertraline	0.025
THC	0.100
THC-COOH	0.025
THC-COOH-glu	0.010
THC-OH	0.050
THCA-A	0.025
THCV	0.050
THCVA	0.010
Temazepam	0.005
Terbutaline	0.050
Thebaine	0.050
Theophylline	0.010

Table 4: Results from the UHPLC analysis first batch. Quantity of compound in the dissolved calculus, represented in ng and rounded to 3 digits after the decimal.

sample	batch	id	weight	sex	age	completeness	preservation	pipe_notches	caffeine	theophylline	cotinine	nicotine	salicylic	thca-a	cbn	thcva	thc	cbd	cocaine
1	batch1	MB40	1.650	pm	old	partial	fair	1	0.000	0.000	0.000	0.000	0.000	0.169	0.000	0.000	0.000	0.000	0.350
2	batch1	MB249	28.850	m	old	complete	good	2	0.289	0.038	0.844	5.155	16.821	0.628	0.000	0.000	0.000	0.000	0.104
3	batch1	MB496	0.375	m	ma	complete	excellent	6	0.229	0.023	0.000	0.000	20.340	5.000	0.000	0.000	0.000	0.000	0.000
4	batch1	MB224	9.425	m	ma	partial	fair	4	0.171	0.000	0.242	1.641	1.188	0.000	0.000	0.000	0.000	0.000	0.189
5	batch1	MB316	29.200	pm	ma	complete	good	6	0.165	0.024	5.558	35.933	23.631	4.000	0.000	0.000	0.000	0.000	0.000
6	batch1	MB339	42.275	pf	ma	complete	good	3	0.077	0.031	0.000	0.000	2.949	0.044	0.000	0.000	0.000	0.000	0.000
7	batch1	MB227	8.200	f	ma	complete	good	0	0.000	0.000	0.000	0.000	6.080	0.000	0.000	0.000	0.000	0.000	0.000
8	batch1	MB196	8.400	m	ma	complete	good	7	0.000	0.000	0.000	0.000	9.053	0.000	0.000	0.000	0.000	0.000	0.000
9	batch1	MB318	6.575	m	ma	complete	good	2	0.348	0.070	0.000	0.000	12.520	6.000	0.000	0.000	0.000	0.000	0.000
10	batch1	MB216	21.750	m	lya	partial	fair	0	0.257	0.036	0.000	0.000	15.710	8.215	0.000	0.000	0.000	0.000	0.000
11	batch1	MB112	41.900	pm	lya	complete	excellent	8	0.180	0.030	0.181	0.453	35.670	2.000	0.000	0.000	0.000	0.000	0.000

sample	batch	id	weight	sex	age	completeness	preservation	pipe_notch	caffeine	theophylline	cotinine	nicotine	salicylic	thca-a	cbn	thcv	thc	cbd	cocaine
12	batch1	MB9	3.800	m	ma	complete	fair	4	0.101	0.000	0.000	0.109	19.645	0.000	0.000	0.000	0.000	0.000	0.000
13	batch1	MB423	7.050	m	ma	complete	good	7	0.000	0.000	0.000	0.000	9.837	0.000	0.000	0.000	0.000	0.000	0.000
14	batch1	MB107	15.975	m	lya	complete	excellent	3	0.152	0.029	0.282	1.430	17.657	0.000	0.000	0.000	0.000	0.000	0.000
15	batch1	MB251	10.525	m	eya	complete	excellent	2	0.108	0.014	0.000	0.000	6.337	0.000	0.000	0.000	0.000	0.000	0.148
16	batch1	MB301	32.100	pm	ma	complete	excellent	7	0.669	0.035	0.102	0.453	19.978	0.000	0.000	0.000	0.000	0.000	0.000
17	batch1	MB207	20.300	m	lya	complete	excellent	2	0.000	0.000	0.572	2.768	12.488	0.000	0.000	0.000	0.000	0.000	0.000
18	batch1	MB365	25.600	m	ma	complete	good	3	0.270	0.024	0.000	0.000	44.242	0.000	0.000	0.000	0.000	0.000	0.000
19	batch1	MB117	2.875	pm	lya	complete	good	6	0.000	0.000	0.000	0.000	0.742	16.491	2390	0.073	1.242	0.000	0.000
20	batch1	MB131	15.100	m	ma	complete	excellent	6	0.064	0.000	0.079	0.000	7.892	0.000	0.000	0.000	0.000	0.000	0.000
21	batch1	MB204	10.825	pm	ma	complete	good	6	0.410	0.025	0.239	1.599	11.001	0.000	0.000	0.000	0.000	0.000	0.000
22	batch1	MB30	11.675	m	ma	complete	excellent	4	0.000	0.000	0.213	0.913	11.589	0.000	0.000	0.000	0.000	0.000	0.000
23	batch1	MB407	16.300	pm	ma	complete	good	5	0.093	0.013	0.094	0.715	18.172	0.000	0.000	0.000	0.000	0.000	0.000
24	batch1	MB78	29.750	m	old	complete	excellent	6	0.237	0.019	2.855	15.962	20.141	0.000	0.000	0.000	0.000	0.000	0.000
25	batch1	MB163	7.475	m	old	complete	excellent	6	0.392	0.000	0.369	1.023	16.936	0.000	0.000	0.000	0.000	0.000	0.000
26	batch1	MB18	12.500	m	lya	complete	good	5	0.000	0.000	0.000	0.000	26.665	0.000	0.000	0.000	0.000	0.000	0.000
27	batch1	MB53	11.600	m	ma	complete	good	5	0.130	0.000	0.000	0.000	10.394	0.000	0.000	0.000	0.000	0.000	0.000
28	batch1	MB235	30.900	m	ma	complete	excellent	5	0.109	0.020	0.000	0.000	40.479	0.000	0.000	0.000	0.000	0.000	0.000
29	batch1	MB69	22.725	m	ma	partial	good	0	0.130	0.011	0.000	0.000	25.373	0.000	0.000	0.000	0.000	0.000	0.000
30	batch1	MB180	25.450	m	ma	complete	excellent	5	0.000	0.000	0.000	0.000	21.180	0.000	0.000	0.000	0.000	0.000	0.056
31	batch1	MB266	19.850	m	ma	complete	excellent	5	0.248	0.020	0.000	0.000	29.075	0.000	0.000	0.000	0.000	0.000	0.000
32	batch1	MB65	41.775	m	lya	complete	excellent	6	0.731	0.024	1.276	7.772	16.825	0.109	0.000	0.000	0.000	0.000	0.152
33	batch1	MB116	22.275	pm	ma	partial	fair	6	0.144	0.000	0.286	2.130	9.975	5.199	0.000	0.000	0.000	0.000	1.038
34	batch1	MB396	27.200	pm	lya	complete	excellent	6	0.074	0.000	0.054	0.118	25.842	0.000	0.000	0.000	0.000	0.000	0.351
35	batch1	MB255	36.050	pm	ma	partial	good	3	0.174	0.000	0.000	0.000	13.126	0.000	0.000	0.000	0.000	0.000	0.000
36	batch1	MB425	17.400	pf	old	complete	fair	0	0.000	0.000	0.206	0.733	7.852	0.280	0.000	0.000	0.000	0.000	0.000
37	batch1	MB361	30.875	m	ma	complete	excellent	3	0.085	0.021	0.000	0.125	35.325	0.000	0.000	0.000	0.000	0.000	0.000
38	batch1	MB158	30.075	m	ma	fragmentary	fair	6	0.093	0.000	0.230	1.429	21.472	0.000	0.000	0.000	0.000	0.000	0.000
39	batch1	MB29	18.025	m	lya	complete	excellent	1	0.000	0.000	0.000	0.000	6.255	0.000	0.104	0.000	0.000	0.000	0.150
40	batch1	MB120	17.750	m	eya	complete	excellent	3	0.067	0.000	0.098	0.548	18.118	0.000	0.000	0.000	0.000	0.000	0.000
41	batch1	MB60	33.900	pm	ma	complete	good	0	0.208	0.000	0.000	0.000	5.598	0.000	2.946	0.000	0.000	0.476	0.000
42	batch1	MB37	1.200	NA	NA	NA	NA	NA	0.000	0.000	0.000	0.000	2.275	0.000	0.000	0.000	0.000	0.000	0.000

Table 5: Results from the UHPLC analysis second batch. Quantity of compound in calculus after third wash, represented in ng and rounded to 3 digits after the decimal.

batch	sample	id	weight	presence	conc	sex	age	completeness	preservation	pipe_notch	caffeine	theophylline	cotinine	nicotine	salicylic	thca-a	cbn	thcv	thc	cbd	cocaine
batch	12	MB410	NA	NA	NA	pm	old	partial	fair	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch	22	MB298	NA	NA	NA	m	old	complete	good	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

batch	sample	pid	weight	presence	conc	sex	age	complete	response	prescription_n	caffeine	theophylline	cotinine	nicotine	salicyl	thca-a	cbn	thcv	thc	cbd	cocaine
batch3	12	MB406	7.025	1	0.0094093	m	ma	complete	excellent	6	0.066	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch3	12	MB406	7.025	0	0.0000000	m	ma	complete	excellent	6	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch3	12	MB406	7.025	1	1.5145907	m	ma	complete	excellent	6	NA	NA	NA	NA	10.640	NA	NA	NA	NA	NA	NA
batch4	12	MB299	1.250	0	0.0000000	m	ma	partial	fair	4	0.000	0.000	NA	NA	NA	0	0	0	0	0	0
batch4	12	MB299	1.250	1	0.1697436	m	ma	partial	fair	4	NA	NA	0.331	NA	NA	NA	NA	NA	NA	NA	NA
batch4	12	MB299	1.250	1	0.5794872	m	ma	partial	fair	4	NA	NA	NA	1.130	NA	NA	NA	NA	NA	NA	NA
batch4	12	MB299	1.250	1	0.2564103	m	ma	partial	fair	4	NA	NA	NA	NA	0.500	NA	NA	NA	NA	NA	NA
batch5	12	MB329	NA	NA	NA	pm	ma	complete	good	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch6	12	MB398	2.250	1	0.0162105	pf	ma	complete	good	3	0.046	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch6	12	MB398	2.250	0	0.0000000	pf	ma	complete	good	3	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch6	12	MB398	2.250	1	0.1015439	pf	ma	complete	good	3	NA	NA	NA	NA	0.289	NA	NA	NA	NA	NA	NA
batch7	12	MB287	NA	NA	NA	f	ma	complete	good	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch8	12	MB128	1.6850	1	0.0042975	m	ma	complete	good	7	0.094	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch8	12	MB128	1.6850	0	0.0000000	m	ma	complete	good	7	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch8	12	MB128	1.6850	1	0.0335652	m	ma	complete	good	7	NA	NA	0.733	NA	NA	NA	NA	NA	NA	NA	NA
batch8	12	MB128	1.6850	1	0.1803204	m	ma	complete	good	7	NA	NA	NA	3.940	NA	NA	NA	NA	NA	NA	NA
batch8	12	MB128	1.6850	1	0.2810069	m	ma	complete	good	7	NA	NA	NA	NA	6.140	NA	NA	NA	NA	NA	NA
batch9	12	MB368	1.6000	1	0.0068049	m	ma	complete	good	2	0.122	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch9	12	MB368	1.6000	1	0.0003222	m	ma	complete	good	2	NA	0.006	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch9	12	MB368	1.6000	0	0.0000000	m	ma	complete	good	2	NA	NA	0.000	0.000	NA	0	0	0	0	0	0
batch9	12	MB368	1.6000	1	0.5733333	m	ma	complete	good	2	NA	NA	NA	NA	10.320	NA	NA	NA	NA	NA	NA
batch10	12	MB261	NA	NA	NA	m	lya	partial	fair	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch11	12	MB121	NA	NA	NA	pm	lya	complete	excellent	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch12	12	MB9	NA	NA	NA	m	ma	complete	fair	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch13	12	MB427	1.3675	1	0.0045997	m	ma	complete	good	7	0.081	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch13	12	MB427	1.3675	0	0.0000000	m	ma	complete	good	7	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch13	12	MB427	1.3675	1	1.5620934	m	ma	complete	good	7	NA	NA	NA	NA	27.610	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	1	0.0487586	m	lya	complete	excellent	3	0.283	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	1	0.0021552	m	lya	complete	excellent	3	NA	0.013	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	1	0.0873966	m	lya	complete	excellent	3	NA	NA	0.507	NA	NA	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	1	0.3931034	m	lya	complete	excellent	3	NA	NA	NA	2.280	NA	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	1	1.7706897	m	lya	complete	excellent	3	NA	NA	NA	NA	10.270	NA	NA	NA	NA	NA	NA
batch14	12	MB108	5.700	0	0.0000000	m	lya	complete	excellent	3	NA	NA	NA	NA	NA	0	0	0	0	0	0
batch15	12	MB258	7.100	1	0.0159231	m	eya	complete	excellent	2	0.124	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch15	12	MB258	7.100	1	0.0007436	m	eya	complete	excellent	2	NA	0.006	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch15	12	MB258	7.100	0	0.0000000	m	eya	complete	excellent	2	NA	NA	0.000	0.000	NA	0	0	0	0	0	0
batch15	12	MB258	7.100	1	0.4807692	m	eya	complete	excellent	2	NA	NA	NA	NA	3.750	NA	NA	NA	NA	NA	NA
batch16	12	MB301	NA	NA	NA	pm	ma	complete	excellent	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch17	12	MB208	1.7550	1	0.0068841	m	lya	complete	excellent	2	0.128	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch17	12	MB208	1.7550	1	0.0007278	m	lya	complete	excellent	2	NA	0.014	NA	NA	NA	NA	NA	NA	NA	NA	NA

batch	sample	pid	weight	presence	conc	sex	age	completeness	preservation	[illegible]	caffeine	theophylline	cotinine	nicotine	salicyl	thca-a	cbn	thcv	thc	cbd	cocaine
batch17	2	[illegible]	[illegible]	1	0.1315364	m	lya	complete	excellent	2	NA	NA	2.440	NA	NA	NA	NA	NA	NA	NA	NA
batch17	2	[illegible]	[illegible]	1	0.5800539	m	lya	complete	excellent	2	NA	NA	NA	10.760	NA	NA	NA	NA	NA	NA	NA
batch17	2	[illegible]	[illegible]	1	0.2954178	m	lya	complete	excellent	2	NA	NA	NA	NA	5.480	NA	NA	NA	NA	NA	NA
batch17	2	[illegible]	[illegible]	0	0.0000000	m	lya	complete	excellent	2	NA	NA	NA	NA	NA	0	0	0	0	0	0
batch18	2	MB365	NA	NA	NA	m	ma	complete	good	3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch19	2	MB117	NA	NA	NA	pm	lya	complete	good	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch20	2	[illegible]	[illegible]	1	0.0074923	m	ma	complete	excellent	6	0.049	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch20	2	[illegible]	[illegible]	0	0.0000000	m	ma	complete	excellent	6	NA	0.000	NA	0.000	NA	0	0	0	0	0	0
batch20	2	[illegible]	[illegible]	1	0.0122462	m	ma	complete	excellent	6	NA	NA	0.080	NA	NA	NA	NA	NA	NA	NA	NA
batch20	2	[illegible]	[illegible]	1	0.9246154	m	ma	complete	excellent	6	NA	NA	NA	NA	6.010	NA	NA	NA	NA	NA	NA
batch21	2	[illegible]	[illegible]	1	0.0213637	pm	ma	complete	good	6	0.086	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch21	2	[illegible]	[illegible]	0	0.0000000	pm	ma	complete	good	6	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch21	2	[illegible]	[illegible]	1	0.0516770	pm	ma	complete	good	6	NA	NA	0.208	NA	NA	NA	NA	NA	NA	NA	NA
batch21	2	[illegible]	[illegible]	1	0.1907578	pm	ma	complete	good	6	NA	NA	NA	0.768	NA	NA	NA	NA	NA	NA	NA
batch21	2	[illegible]	[illegible]	1	2.5515528	pm	ma	complete	good	6	NA	NA	NA	NA	10.270	NA	NA	NA	NA	NA	NA
batch22	2	[illegible]	[illegible]	0	0.0000000	m	ma	complete	excellent	4	0.000	0.000	NA	NA	NA	0	0	0	0	0	0
batch22	2	[illegible]	[illegible]	1	0.0244848	m	ma	complete	excellent	4	NA	NA	0.141	NA	NA	NA	NA	NA	NA	NA	NA
batch22	2	[illegible]	[illegible]	1	0.0822165	m	ma	complete	excellent	4	NA	NA	NA	0.475	NA	NA	NA	NA	NA	NA	NA
batch22	2	[illegible]	[illegible]	1	1.7367965	m	ma	complete	excellent	4	NA	NA	NA	NA	10.030	NA	NA	NA	NA	NA	NA
batch23	2	[illegible]	[illegible]	1	0.0239059	pm	ma	complete	good	5	0.051	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch23	2	[illegible]	[illegible]	0	0.0000000	pm	ma	complete	good	5	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch23	2	[illegible]	[illegible]	1	0.0258353	pm	ma	complete	good	5	NA	NA	0.055	NA	NA	NA	NA	NA	NA	NA	NA
batch23	2	[illegible]	[illegible]	1	0.1200471	pm	ma	complete	good	5	NA	NA	NA	0.255	NA	NA	NA	NA	NA	NA	NA
batch23	2	[illegible]	[illegible]	1	1.6611765	pm	ma	complete	good	5	NA	NA	NA	NA	3.530	NA	NA	NA	NA	NA	NA
batch24	2	[illegible]	[illegible]	1	0.0079243	m	old	complete	excellent	6	0.063	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch24	2	[illegible]	[illegible]	0	0.0000000	m	old	complete	excellent	6	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch24	2	[illegible]	[illegible]	1	0.0144732	m	old	complete	excellent	6	NA	NA	0.115	NA	NA	NA	NA	NA	NA	NA	NA
batch24	2	[illegible]	[illegible]	1	0.0580315	m	old	complete	excellent	6	NA	NA	NA	0.460	NA	NA	NA	NA	NA	NA	NA
batch24	2	[illegible]	[illegible]	1	1.9167192	m	old	complete	excellent	6	NA	NA	NA	NA	15.190	NA	NA	NA	NA	NA	NA
batch25	2	[illegible]	[illegible]	1	0.0242783	m	old	complete	excellent	6	0.070	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch25	2	[illegible]	[illegible]	0	0.0000000	m	old	complete	excellent	6	NA	0.000	NA	0.000	NA	0	0	0	0	0	0
batch25	2	[illegible]	[illegible]	1	0.0115478	m	old	complete	excellent	6	NA	NA	0.033	NA	NA	NA	NA	NA	NA	NA	NA
batch25	2	[illegible]	[illegible]	1	3.1165217	m	old	complete	excellent	6	NA	NA	NA	NA	8.960	NA	NA	NA	NA	NA	NA
batch26	2	[illegible]	[illegible]	0	0.0000000	m	lya	complete	good	5	0.000	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch26	2	[illegible]	[illegible]	1	2.5000000	m	lya	complete	good	5	NA	NA	NA	NA	17.750	NA	NA	NA	NA	NA	NA
batch27	2	[illegible]	[illegible]	1	0.0202752	m	ma	complete	good	5	0.110	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch27	2	[illegible]	[illegible]	0	0.0000000	m	ma	complete	good	5	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch27	2	[illegible]	[illegible]	1	1.6366972	m	ma	complete	good	5	NA	NA	NA	NA	8.920	NA	NA	NA	NA	NA	NA
batch28	2	[illegible]	[illegible]	1	0.0053046	m	ma	complete	excellent	5	0.049	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch28	2	[illegible]	[illegible]	0	0.0000000	m	ma	complete	excellent	5	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0

batch	sample	pid	weight	presence	conc	sex	age	completeness	preservation	[illegible]	caffeine	theophylline	cotinine	nicotine	salicyl	thca-a	cbn	thcv	thc	cbd	cocaine
batch2	28	MB932	25.75	1	0.9045822	m	ma	complete	excellent	5	NA	NA	NA	NA	8.390	NA	NA	NA	NA	NA	NA
batch2	29	MB69	9.75	1	0.0191254	m	ma	partial	good	0	0.133	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	29	MB69	9.75	0	0.0000000	m	ma	partial	good	0	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch2	29	MB69	9.75	1	1.9039427	m	ma	partial	good	0	NA	NA	NA	NA	13.280	NA	NA	NA	NA	NA	NA
batch2	30	MB168	30.75	0	0.0000000	m	ma	complete	excellent	5	0.000	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch2	30	MB168	30.75	1	1.5043137	m	ma	complete	excellent	5	NA	NA	NA	NA	9.590	NA	NA	NA	NA	NA	NA
batch2	31	MB216	16.100	1	0.0151863	m	ma	complete	excellent	5	0.244	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	31	MB216	16.100	1	0.0007267	m	ma	complete	excellent	5	NA	0.012	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	31	MB216	16.100	0	0.0000000	m	ma	complete	excellent	5	NA	NA	0.000	0.000	NA	0	0	0	0	0	0
batch2	31	MB216	16.100	1	2.2453416	m	ma	complete	excellent	5	NA	NA	NA	NA	36.150	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	1	0.0300144	m	lya	complete	excellent	6	0.834	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	1	0.0003273	m	lya	complete	excellent	6	NA	0.009	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	1	0.0413669	m	lya	complete	excellent	6	NA	NA	1.150	NA	NA	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	1	0.2356115	m	lya	complete	excellent	6	NA	NA	NA	6.550	NA	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	1	0.3978417	m	lya	complete	excellent	6	NA	NA	NA	NA	11.060	NA	NA	NA	NA	NA	NA
batch2	32	MB65	27.800	0	0.0000000	m	lya	complete	excellent	6	NA	NA	NA	NA	NA	0	0	0	0	0	0
batch2	33	MB16	NA	NA	NA	pm	ma	partial	fair	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	34	MB396	NA	NA	NA	pm	lya	complete	excellent	6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	35	MB245	5.550	1	0.0112593	pm	ma	partial	good	3	0.046	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	35	MB245	5.550	0	0.0000000	pm	ma	partial	good	3	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch2	35	MB245	5.550	1	0.3530864	pm	ma	partial	good	3	NA	NA	NA	NA	1.430	NA	NA	NA	NA	NA	NA
batch2	36	MB483	2.325	0	0.0000000	pf	old	complete	fair	0	0.000	0.000	NA	NA	NA	0	0	0	0	0	0
batch2	36	MB483	2.325	1	0.0073153	pf	old	complete	fair	0	NA	NA	0.061	NA	NA	NA	NA	NA	NA	NA	NA
batch2	36	MB483	2.325	1	0.0225826	pf	old	complete	fair	0	NA	NA	NA	0.188	NA	NA	NA	NA	NA	NA	NA
batch2	36	MB483	2.325	1	0.6786787	pf	old	complete	fair	0	NA	NA	NA	NA	5.650	NA	NA	NA	NA	NA	NA
batch2	37	MB316	4.1525	1	0.0055697	m	ma	complete	excellent	3	0.081	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	37	MB316	4.1525	0	0.0000000	m	ma	complete	excellent	3	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch2	37	MB316	4.1525	1	0.0043580	m	ma	complete	excellent	3	NA	NA	0.063	NA	NA	NA	NA	NA	NA	NA	NA
batch2	37	MB316	4.1525	1	0.0061274	m	ma	complete	excellent	3	NA	NA	NA	0.089	NA	NA	NA	NA	NA	NA	NA
batch2	37	MB316	4.1525	1	1.6351119	m	ma	complete	excellent	3	NA	NA	NA	NA	23.750	NA	NA	NA	NA	NA	NA
batch2	38	MB175	5.800	1	0.0463600	m	ma	fragmentary	fair	6	0.348	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	38	MB175	5.800	0	0.0000000	m	ma	fragmentary	fair	6	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch2	38	MB175	5.800	1	0.0055867	m	ma	fragmentary	fair	6	NA	NA	0.042	NA	NA	NA	NA	NA	NA	NA	NA
batch2	38	MB175	5.800	1	0.0200267	m	ma	fragmentary	fair	6	NA	NA	NA	0.150	NA	NA	NA	NA	NA	NA	NA
batch2	38	MB175	5.800	1	0.5320000	m	ma	fragmentary	fair	6	NA	NA	NA	NA	3.990	NA	NA	NA	NA	NA	NA
batch2	39	MB21	9.075	0	0.0000000	m	lya	complete	excellent	1	0.000	0.000	0.000	0.000	0.000	0	0	0	0	0	0
batch2	40	MB92	15.50	1	0.0130366	m	eya	complete	excellent	3	0.124	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	40	MB92	15.50	0	0.0000000	m	eya	complete	excellent	3	NA	0.000	NA	NA	NA	0	0	0	0	0	0
batch2	40	MB92	15.50	1	0.0329005	m	eya	complete	excellent	3	NA	NA	0.314	NA	NA	NA	NA	NA	NA	NA	NA
batch2	40	MB92	15.50	1	0.1602094	m	eya	complete	excellent	3	NA	NA	NA	1.530	NA	NA	NA	NA	NA	NA	NA

batch	sample	id	weight	presence	conc	sex	age	completeness	preservation	position	caffeine	theophylline	cotinine	nicotine	salicylic	thca-a	cbn	thcv	thc	cbd	cocaine
batch2	40	MB19	25.50	1	1.0743455	m	ya	complete	excellent	3	NA	NA	NA	NA	10.260	NA	NA	NA	NA	NA	NA
batch2	41	MB60	14.175	1	0.0052063	pm	ma	complete	good	0	0.074	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
batch2	41	MB60	14.175	0	0.0000000	pm	ma	complete	good	0	NA	0.000	0.000	0.000	NA	0	0	0	0	0	0
batch2	41	MB60	14.175	1	0.2913580	pm	ma	complete	good	0	NA	NA	NA	NA	4.130	NA	NA	NA	NA	NA	NA
batch2	42	MB37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Authentication

No modern synthetic drugs were detected in any of the samples.

Samples were replicated to verify results from the initial analysis. Of the 41 samples initially analysed, 29 samples were replicated.

Only caffeine, theophylline, nicotine, cotinine, and salicylic acid were found in the replicated samples.

Most plots show a large increase in extracted mass of a compound between the calculus wash extracts (wash 1-3) and the dissolved calculus (calc). Most samples containing theophylline and caffeine had the largest quantity of the compound extracted from the first wash, then decreasing in washes 2 and 3. There is an increase between wash 3 and the dissolved calculus in all samples. The patterns are consistent across batches 1 and 2. The pattern we expect to see in a sample is a reduction in the quantity from wash 1 to wash 3, and then another spike in the final extraction from the dissolved calculus, which means the compound is actually 'ancient' or authentic. The compounds that are completely absent in all three washes and present in high quantities in the final extraction may also be suggestive of lab contamination. This has not been thoroughly tested and is only based on what we expect to see. Therefore, the interpretation of these graphs is itself up for interpretation.

Quantity vs. sample weight

There is no clear relationship between the sample weight and the amount of compound detected, except for salicylic acid, where the amount of extracted compound increases with increasing sample weight. In batch 2 there is also a slight positive trend for caffeine, nicotine, and cotinine. Nicotine and cotinine display the same relative relationship between samples. Where the nicotine quantity is high compared to other samples, the cotinine quantity will be similarly high (Figure 6 and Figure 6).

The positive correlation between the weight of the calculus sample and recovered quantities of the compounds suggests sample weight may affect the ability to detect compounds; although, we were able to detect compounds in samples as small as 2 mg (Figure 6 and Figure 7).

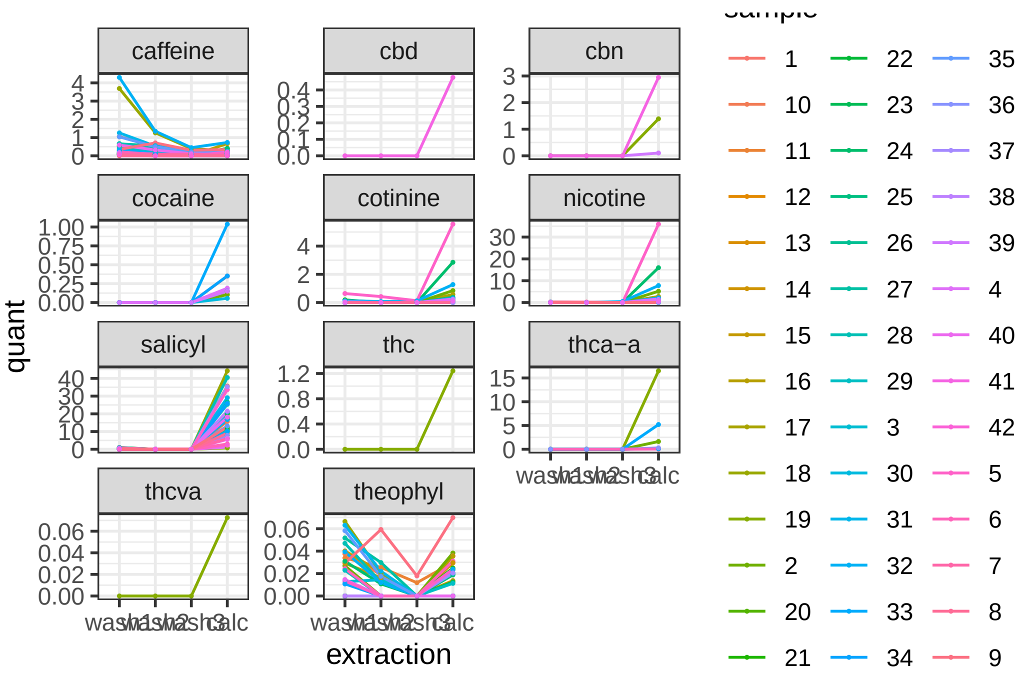


Figure 4: Plot of extracted quantities of each compound across the three washes and calculus extraction in batch 1. Each line represents an individual.

Distribution of compounds detected in the samples

The replication showed that caffeine, theophylline, cotinine, nicotine, and salicylic acid could be consistently detected in the samples, although theophylline detection decreased between batches 1 and 2. CBD, CBN, cocaine, and THCA-A was not detected at all in the second batch.

Detection and preservation

To see if preservation of the skeletal remains had any effect on the detection of compounds, absolute quantities of compounds were compared to the various levels of preservation.

Distribution of state-of-preservation in batches 1 and 2 to make sure the number of skeletons are not affecting the relationships shown above. Given our sample contains a smaller number of individuals with fair preservation, this may bias our interpretations (Figure 12).

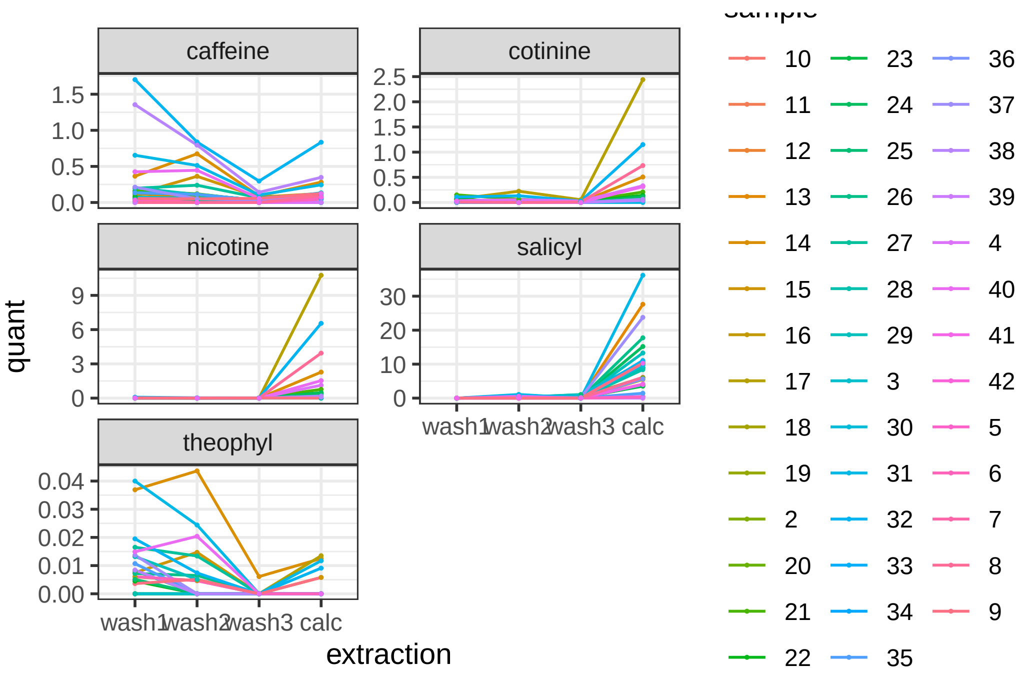


Figure 5: Plot of extracted quantities of each compound across the three washes and calculus extraction in batch 2. Each line represents an individual.

Detection of tobacco

Given that pipe notches are present in the majority of individuals, the presence of pipe notch(es) in an individual and concurrent detection of nicotine and/or cotinine is used as a rough indicator of the accuracy of the method.

We found no correlation between the number of pipe notches and the concentration of nicotine and cotinine, suggesting that our ability to detect tobacco consumption in dental calculus does not necessarily rely on targeting frequent smokers; here, we consider individuals with multiple pipe notches as likely to have been heavy smokers.

No apparent correlation between the number of pipe notches and the concentration of nicotine or cotinine (Figure 13).

The presence of pipe notch(es) in an individual and concurrent detection of nicotine and/or cotinine is used as a crude indicator of the accuracy of the method. When combining the results of both batches, the method was able to detect some form of tobacco in 21 of 35 individuals with a pipe notch (60.0%). When also considering correct the absence of a tobacco alkaloid together with the absence of a pipe notch, the accuracy of the method is 63.2%. Accuracy in the old adult age category is 75.0%.

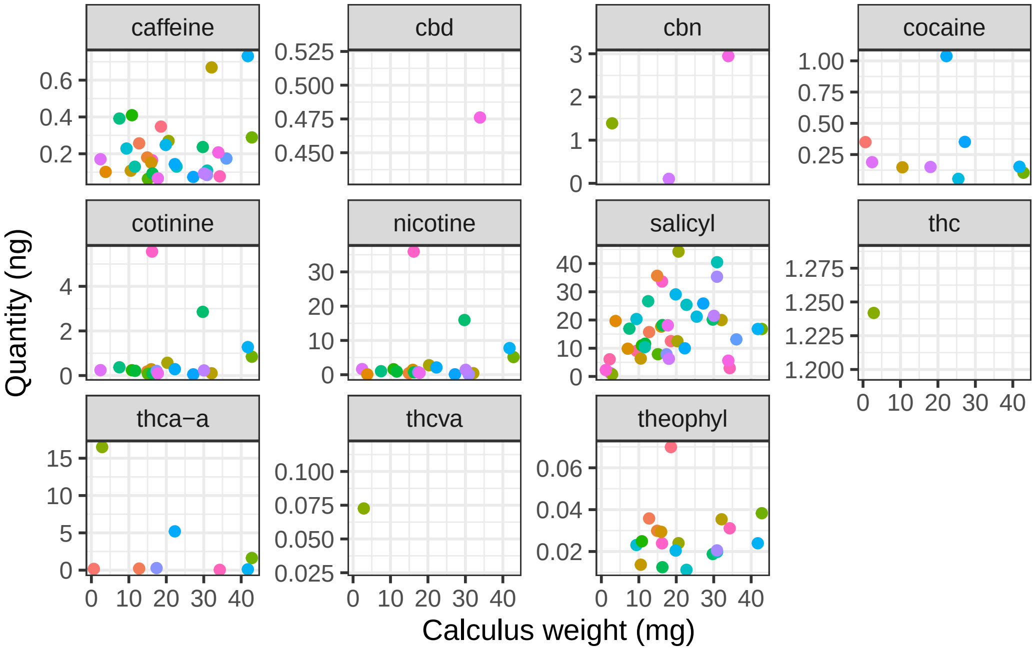


Figure 6: Quantity of a compound (ng) found in a sample plotted against the weight of the calculus sample. Results from batch 1.

In the replicated samples only, tobacco detection was successful in 14 out of 36 pipe smokers (38.9%) Including individuals with absence of a pipe notch and concurrent absence of compounds as a correct identification, gives an overall accuracy of 39.0%.

One individual—an old adult, probable female—was positive for both nicotine and cotinine, and had no signs of a pipe notch.

Dental analysis

Pipe notches were identified by wear on the mesial and distal sides of the crowns between to teeth, resulting from the practice of clenching a pipe between adjacent and isomeric teeth, and which differs from the occlusal wear that occurs through mastication. Wear occurring between adjacent and isomeric teeth were counted as a single pipe notch.

Some of the teeth were missing because they have been sent elsewhere for DNA sampling. These teeth were considered present when determining antemortem loss ratios, and absent when scoring caries, periodontitis, and calculus.

An overview of available teeth can be seen in Figure 14.

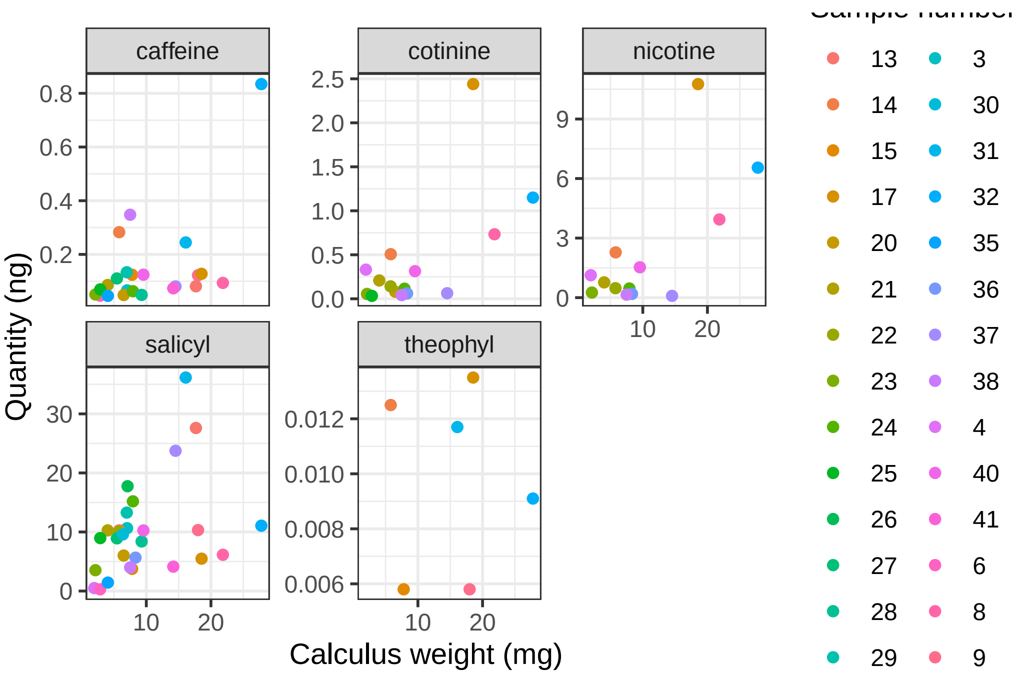


Figure 7: Quantity of a compound (ng) found in a sample plotted against the weight of the calculus sample. Results from batch 2.

AMTL

Ratios of antemortem lost teeth per present teeth at the site. Calculated per individual (Table 6), tooth class (Table 7), and tooth type (Table 8)

Table 6: AMTL ratio per individual.

id	n_teeth	count	ratio
MB107	27	3	0.1111111
MB116	27	5	0.1851852
MB117	23	2	0.0869565
MB120	29	0	0.0000000
MB121	29	2	0.0689655
MB131	26	2	0.0769231
MB158	29	0	0.0000000
MB163	31	5	0.1612903
MB18	32	1	0.0312500
MB180	26	2	0.0769231
MB186	25	11	0.4400000

id	n_teeth	count	ratio
MB204	26	3	0.1153846
MB207	28	2	0.0714286
MB235	32	4	0.1250000
MB251	31	0	0.0000000
MB255	18	7	0.3888889
MB261	26	0	0.0000000
MB266	30	3	0.1000000
MB287	32	0	0.0000000
MB29	26	3	0.1153846
MB292	30	1	0.0333333
MB298	31	17	0.5483871
MB30	27	1	0.0370370
MB301	30	3	0.1000000
MB329	30	1	0.0333333
MB361	29	2	0.0689655
MB365	28	6	0.2142857
MB366	25	1	0.0400000
MB392	27	5	0.1851852
MB396	27	0	0.0000000
MB406	30	0	0.0000000
MB407	30	2	0.0666667
MB410	26	2	0.0769231
MB423	28	0	0.0000000
MB425	25	17	0.6800000
MB53	27	7	0.2592593
MB60	31	8	0.2580645
MB65	30	1	0.0333333
MB69	30	3	0.1000000
MB78	28	5	0.1785714
MB9	28	0	0.0000000

Table 7: AMTL ratio per tooth class.

class	n_teeth	count	ratio
canine	151	7	0.0463576
incisor	283	27	0.0954064
molar	414	80	0.1932367
premolar	302	23	0.0761589

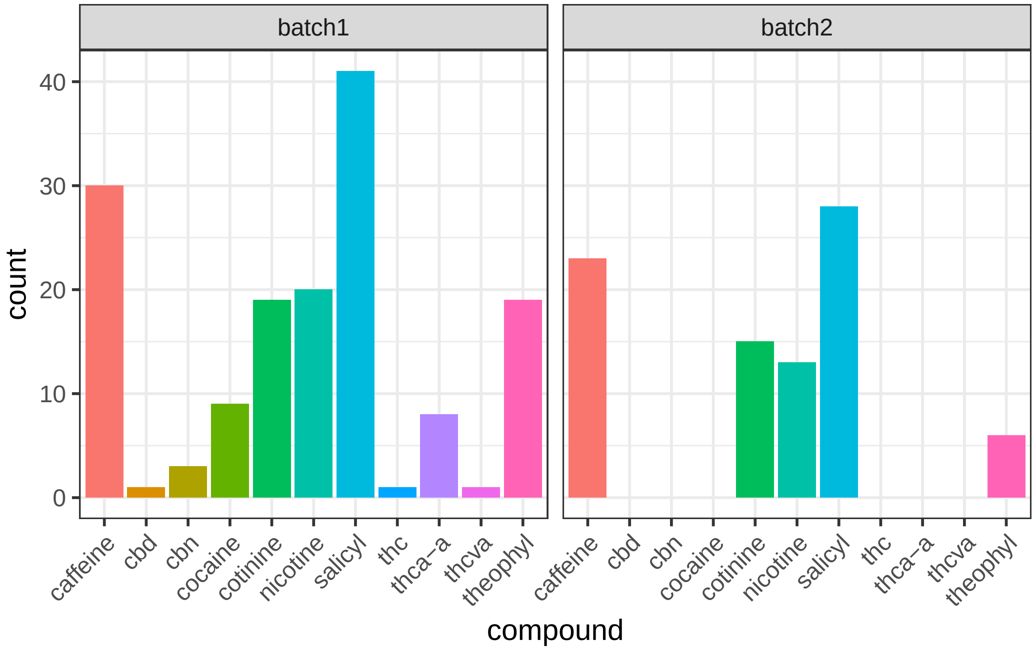


Figure 8: Number of individuals in which each compound was detected between batch 1 and 2.

Table 8: AMTL ratio per tooth type.

type	n_teeth	count	ratio
c	151	7	0.0463576
i1	140	15	0.1071429
i2	143	12	0.0839161
m1	160	39	0.2437500
m2	150	31	0.2066667
m3	104	10	0.0961538
pm1	155	9	0.0580645
pm2	147	14	0.0952381

Caries

Caries were scored as the location on each individual tooth. Multiple locations on a single tooth were separated with `;`. The size of caries was also recorded, but not used in further analysis. Large caries that cover multiple surfaces with an unknown origin were recorded as ‘crown’.

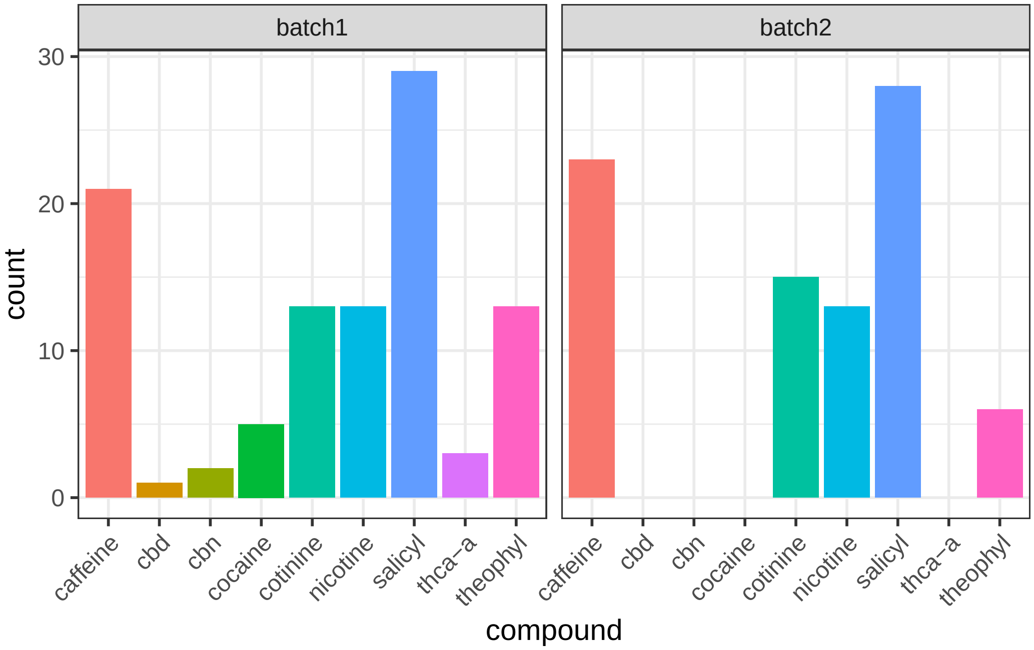


Figure 9: Number of individuals in which each compound was detected between batch 1 and 2. Only showing replicated individuals.

code	surface
mes	mesial surface
dis	distal surface
lin	lingual surface
buc	buccal surface (including labial surface)
occ	occlusal surface (including incisal surface)
crown	caries covers 2+ surfaces
none	No caries visible on surface
NA	Not observable/tooth missing

In the 950 that were examined, 167 teeth had caries (17.6%). This frequency has very little meaning, and was further broken down into a ratio for each individual and each tooth class (Table 10 and Figure 15). As expected, the molars have a higher frequency of caries than the other teeth.

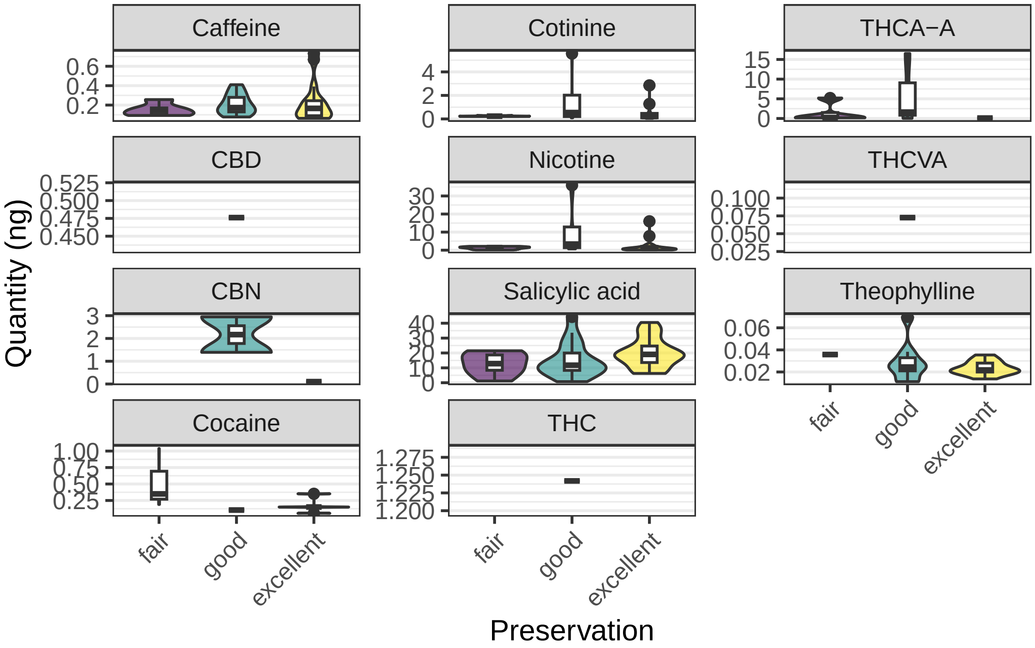


Figure 10: Plot of relationship between the absolute quantity of a detected compound (ng) and the overall skeletal preservation of the individuals in which the compound was detected. Showing results for batch 1.

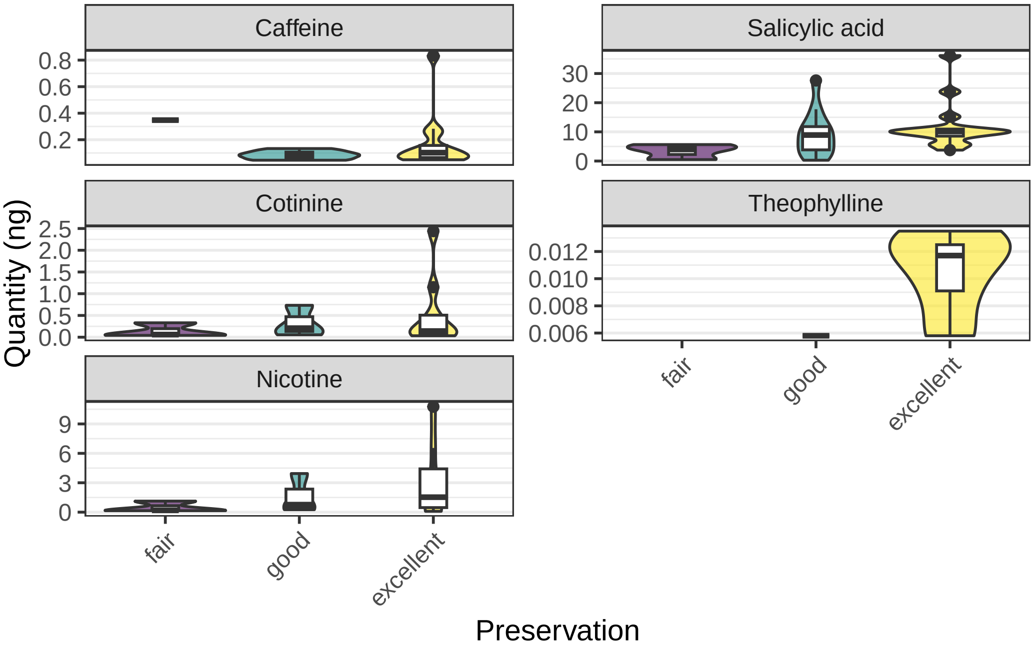


Figure 11: Plot of relationship between the absolute quantity of a detected compound (ng) and the overall skeletal preservation of the individuals in which the compound was detected. Showing results for batch 2.

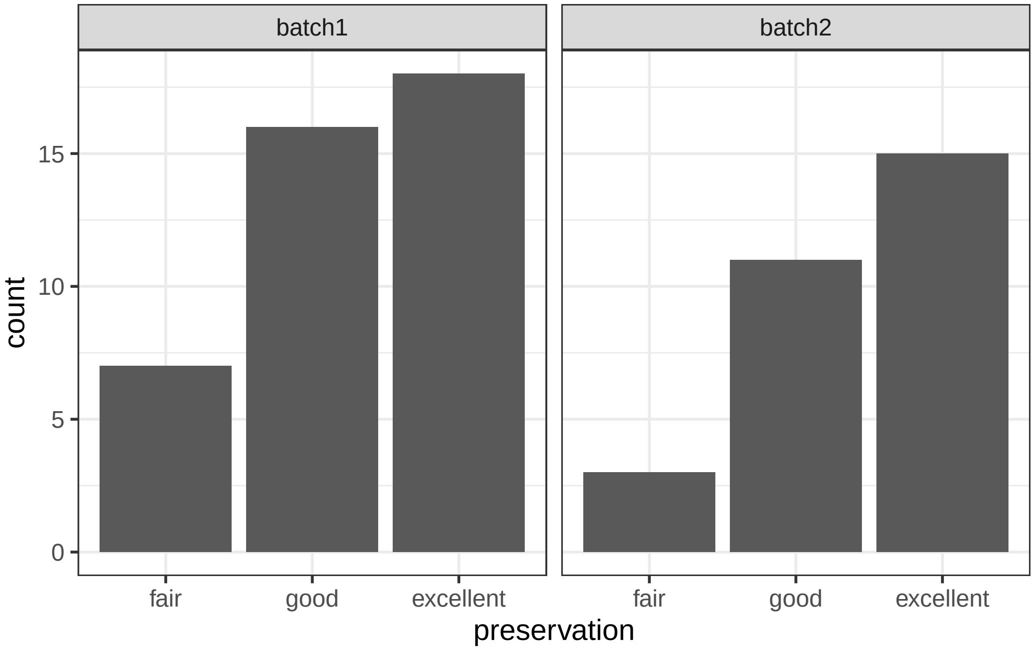


Figure 12: Plot of the number of skeletons in each state of preservation separated by batch.

Table 10: Table of caries ratios per individual per tooth class.

id	class	n_teeth	count	ratio
MB107	canine	4	2	0.5000000
MB107	incisor	8	1	0.1250000
MB107	molar	9	0	0.0000000
MB107	premolar	3	0	0.0000000
MB116	canine	4	1	0.2500000
MB116	incisor	8	0	0.0000000
MB116	molar	2	0	0.0000000
MB116	premolar	6	1	0.1666667
MB117	canine	4	0	0.0000000
MB117	incisor	4	0	0.0000000
MB117	molar	6	3	0.5000000
MB117	premolar	7	0	0.0000000
MB120	canine	4	0	0.0000000
MB120	incisor	7	0	0.0000000
MB120	molar	11	0	0.0000000
MB120	premolar	7	0	0.0000000
MB121	canine	4	0	0.0000000

id	class	n_teeth	count	ratio
MB121	incisor	8	0	0.0000000
MB121	molar	7	4	0.5714286
MB121	premolar	8	0	0.0000000
MB131	canine	4	1	0.2500000
MB131	incisor	7	0	0.0000000
MB131	molar	5	2	0.4000000
MB131	premolar	8	0	0.0000000
MB158	canine	4	0	0.0000000
MB158	incisor	7	0	0.0000000
MB158	molar	11	2	0.1818182
MB158	premolar	7	1	0.1428571
MB163	canine	4	0	0.0000000
MB163	incisor	6	0	0.0000000
MB163	molar	8	4	0.5000000
MB163	premolar	6	0	0.0000000
MB18	canine	4	0	0.0000000
MB18	incisor	8	0	0.0000000
MB18	molar	10	2	0.2000000
MB18	premolar	7	0	0.0000000
MB180	canine	3	1	0.3333333
MB180	incisor	5	0	0.0000000
MB180	molar	9	4	0.4444444
MB180	premolar	7	1	0.1428571
MB186	canine	2	0	0.0000000
MB186	incisor	2	0	0.0000000
MB186	molar	5	1	0.2000000
MB186	premolar	5	1	0.2000000
MB204	canine	3	0	0.0000000
MB204	incisor	8	0	0.0000000
MB204	molar	5	1	0.2000000
MB204	premolar	7	0	0.0000000
MB207	canine	4	0	0.0000000
MB207	incisor	4	0	0.0000000
MB207	molar	11	0	0.0000000
MB207	premolar	7	0	0.0000000
MB235	canine	3	2	0.6666667
MB235	incisor	8	0	0.0000000
MB235	molar	8	4	0.5000000
MB235	premolar	7	6	0.8571429
MB251	canine	4	0	0.0000000
MB251	incisor	7	0	0.0000000

id	class	n_teeth	count	ratio
MB251	molar	11	0	0.0000000
MB251	premolar	7	0	0.0000000
MB255	canine	1	0	0.0000000
MB255	incisor	3	2	0.6666667
MB255	molar	2	2	1.0000000
MB255	premolar	5	1	0.2000000
MB261	canine	3	0	0.0000000
MB261	incisor	5	0	0.0000000
MB261	molar	10	4	0.4000000
MB261	premolar	7	2	0.2857143
MB266	canine	4	0	0.0000000
MB266	incisor	7	0	0.0000000
MB266	molar	8	2	0.2500000
MB266	premolar	6	0	0.0000000
MB287	canine	4	0	0.0000000
MB287	incisor	8	0	0.0000000
MB287	molar	11	4	0.3636364
MB287	premolar	7	0	0.0000000
MB29	canine	3	1	0.3333333
MB29	incisor	8	8	1.0000000
MB29	molar	8	4	0.5000000
MB29	premolar	4	0	0.0000000
MB292	canine	4	0	0.0000000
MB292	incisor	7	0	0.0000000
MB292	molar	8	4	0.5000000
MB292	premolar	7	0	0.0000000
MB298	canine	4	0	0.0000000
MB298	incisor	4	0	0.0000000
MB298	molar	1	0	0.0000000
MB298	premolar	5	0	0.0000000
MB30	canine	4	0	0.0000000
MB30	incisor	7	0	0.0000000
MB30	molar	7	1	0.1428571
MB30	premolar	6	2	0.3333333
MB301	canine	1	0	0.0000000
MB301	incisor	4	1	0.2500000
MB301	molar	5	5	1.0000000
MB301	premolar	0	0	NaN
MB329	canine	3	0	0.0000000
MB329	incisor	7	0	0.0000000
MB329	molar	10	1	0.1000000

id	class	n_teeth	count	ratio
MB329	premolar	6	0	0.0000000
MB361	canine	3	0	0.0000000
MB361	incisor	8	1	0.1250000
MB361	molar	7	0	0.0000000
MB361	premolar	8	3	0.3750000
MB365	canine	3	0	0.0000000
MB365	incisor	4	0	0.0000000
MB365	molar	8	0	0.0000000
MB365	premolar	7	0	0.0000000
MB366	canine	4	0	0.0000000
MB366	incisor	6	0	0.0000000
MB366	molar	7	2	0.2857143
MB366	premolar	7	0	0.0000000
MB392	canine	4	2	0.5000000
MB392	incisor	7	3	0.4285714
MB392	molar	3	3	1.0000000
MB392	premolar	4	3	0.7500000
MB396	canine	3	0	0.0000000
MB396	incisor	8	0	0.0000000
MB396	molar	8	2	0.2500000
MB396	premolar	6	1	0.1666667
MB406	canine	3	0	0.0000000
MB406	incisor	8	0	0.0000000
MB406	molar	11	3	0.2727273
MB406	premolar	6	0	0.0000000
MB407	canine	4	1	0.2500000
MB407	incisor	8	1	0.1250000
MB407	molar	7	3	0.4285714
MB407	premolar	7	0	0.0000000
MB410	canine	3	0	0.0000000
MB410	incisor	7	0	0.0000000
MB410	molar	8	7	0.8750000
MB410	premolar	6	1	0.1666667
MB423	canine	3	0	0.0000000
MB423	incisor	7	0	0.0000000
MB423	molar	10	1	0.1000000
MB423	premolar	8	0	0.0000000
MB425	canine	1	0	0.0000000
MB425	incisor	1	0	0.0000000
MB425	molar	2	1	0.5000000
MB425	premolar	4	2	0.5000000

id	class	n_teeth	count	ratio
MB53	canine	3	0	0.0000000
MB53	incisor	4	0	0.0000000
MB53	molar	6	4	0.6666667
MB53	premolar	5	1	0.2000000
MB60	canine	4	0	0.0000000
MB60	incisor	8	0	0.0000000
MB60	molar	5	1	0.2000000
MB60	premolar	4	2	0.5000000
MB65	canine	4	4	1.0000000
MB65	incisor	5	2	0.4000000
MB65	molar	12	8	0.6666667
MB65	premolar	6	3	0.5000000
MB69	canine	4	2	0.5000000
MB69	incisor	8	0	0.0000000
MB69	molar	8	5	0.6250000
MB69	premolar	6	1	0.1666667
MB78	canine	4	0	0.0000000
MB78	incisor	2	0	0.0000000
MB78	molar	8	3	0.3750000
MB78	premolar	7	2	0.2857143
MB9	canine	4	0	0.0000000
MB9	incisor	5	0	0.0000000
MB9	molar	8	0	0.0000000
MB9	premolar	8	0	0.0000000

Periodontitis

Periodontitis was scored qualitatively on a scale from 0-3 as the amount of horizontal bone loss from the CEJ to the alveolar bone, accounting for ca. 2mm of gingival thickness. The distribution of scores in the pooled sample dentitions can be seen in Figure 17.

Calculus

Calculus was scored on each tooth surface (interproximal surfaces were given a single score) on a scale of 0-3, representing absence of calculus (0) to heavy deposit (3). Distribution of individual calculus indices within the sample, separated by quadrant shows that the lower anterior quadrant had the largest deposits (Figure 18).

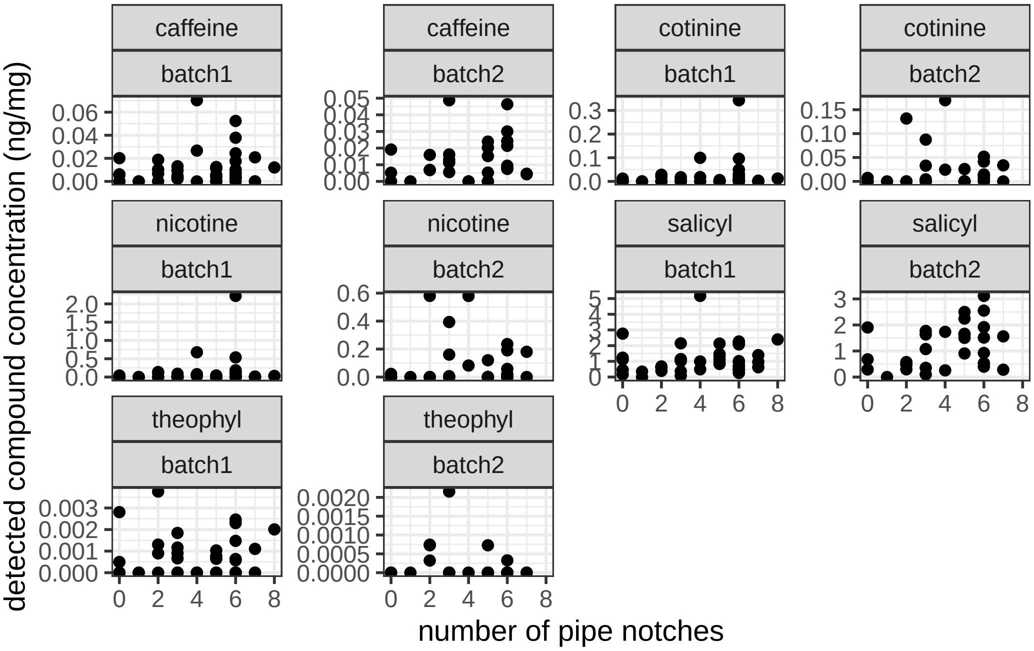


Figure 13: Plot of relationships between the total number of pipe notches in an individual and the concentration of detected compounds. The only relevant comparisons in this case are nicotine and cotinine. The others are just included because I couldn't be bothered filtering them out.

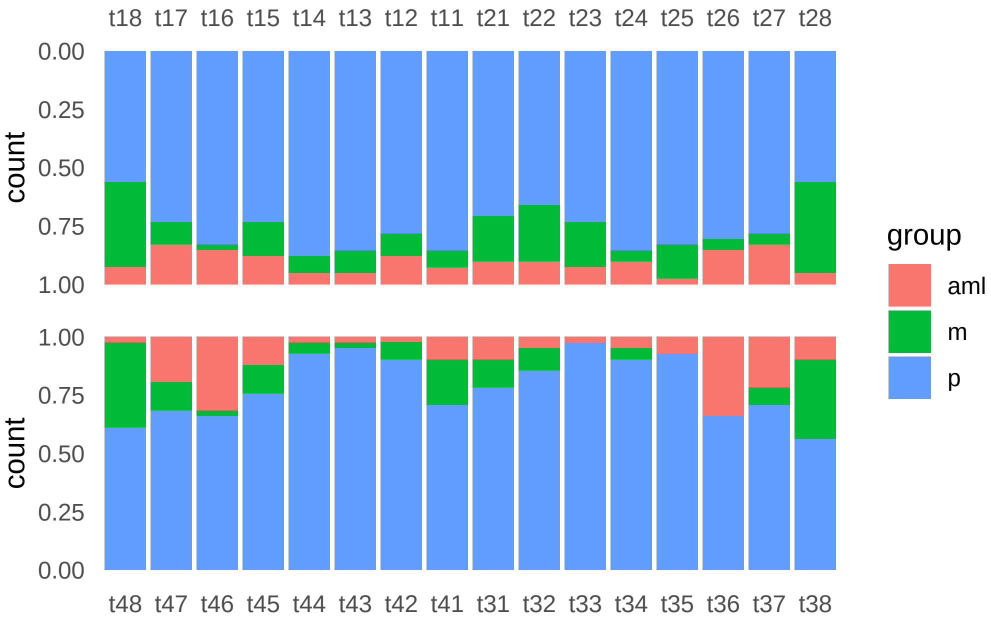


Figure 14: Overview of the dental inventory of the sample. Teeth removed for DNA analysis considered 'present'.

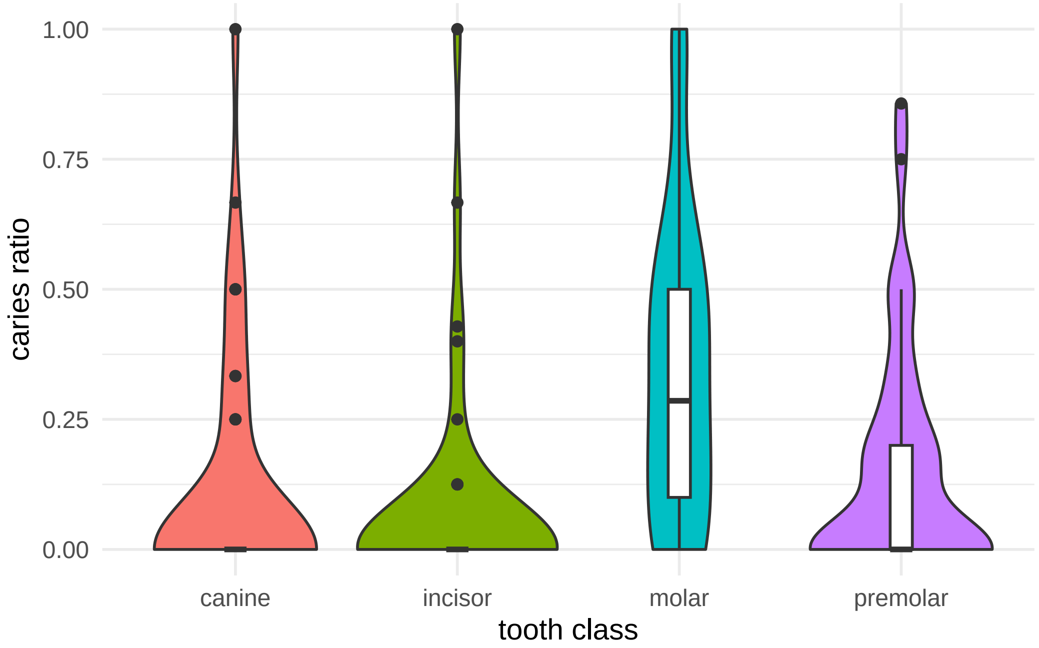


Figure 15: Plot of caries ratios calculated per individual per tooth class.

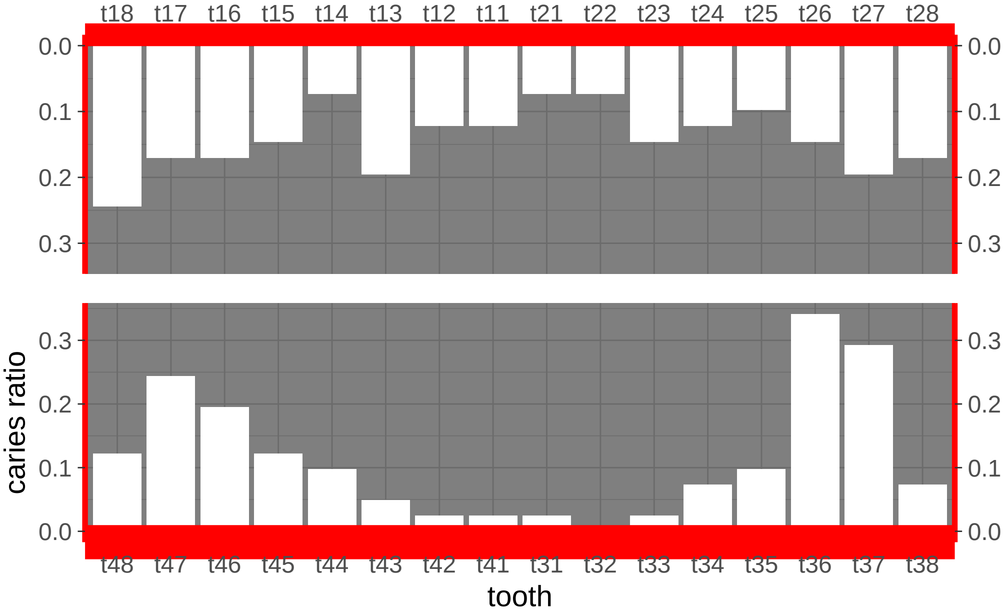


Figure 16: Plot of caries rate per tooth in pooled sample from all individuals. Teeth reordered along the x-axis to match position in the mouth (yes, the plot is supposed to resemble a mouth).

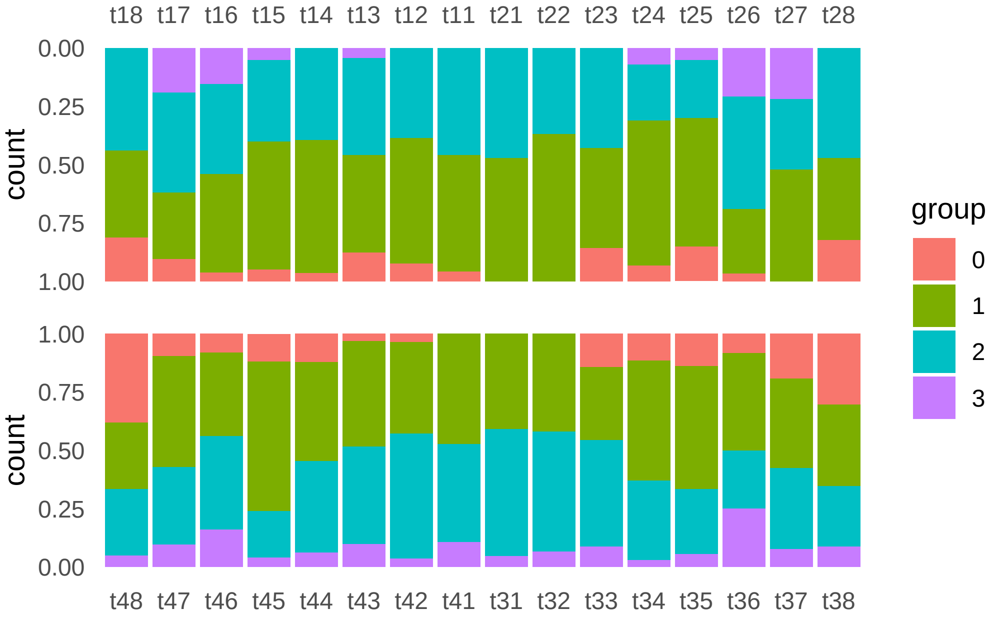


Figure 17: Distribution of periodontitis scores in each tooth (FDI notation) in the pooled sample.

No apparent influence of lower anterior calculus index on the presence/absence of a compound (or vice versa) (Figure 19).

Pathological conditions

Pathological conditions and lesions that occur frequently in the population were included in the analysis. Data were dichotomised to presence/absence to allow statistical analysis. A conservative approach was taken, so when in doubt, absence of a disease was assumed. Osteoarthritis was considered present in cases where eburnation was visible on one or more joint surfaces. Vertebral osteophytosis is identified by marginal lipping and/or osteophyte formation on the margin of the superior and inferior surfaces of the vertebral body. Cribra orbitalia was diagnosed based on the presence of pitting on the superior surface of the orbit. No distinction was made between active or healing lesions. Degenerative disc disease, or spondylosis, is identified as a large diffuse depression of the superior and/or inferior surfaces of the vertebral body (Rogers 2000). Schmorl's nodes are identified as any cortical depressions on the surface of the vertebral body. A note was made whether the lesion perforated the vertebral margin, but both perforating and non-perforating lesions were recorded as present.

Data on chronic maxillary sinusitis from Casna et al. (2021) were included in this study to assess the relationship between upper respiratory diseases with environmental factors (i.e. to-

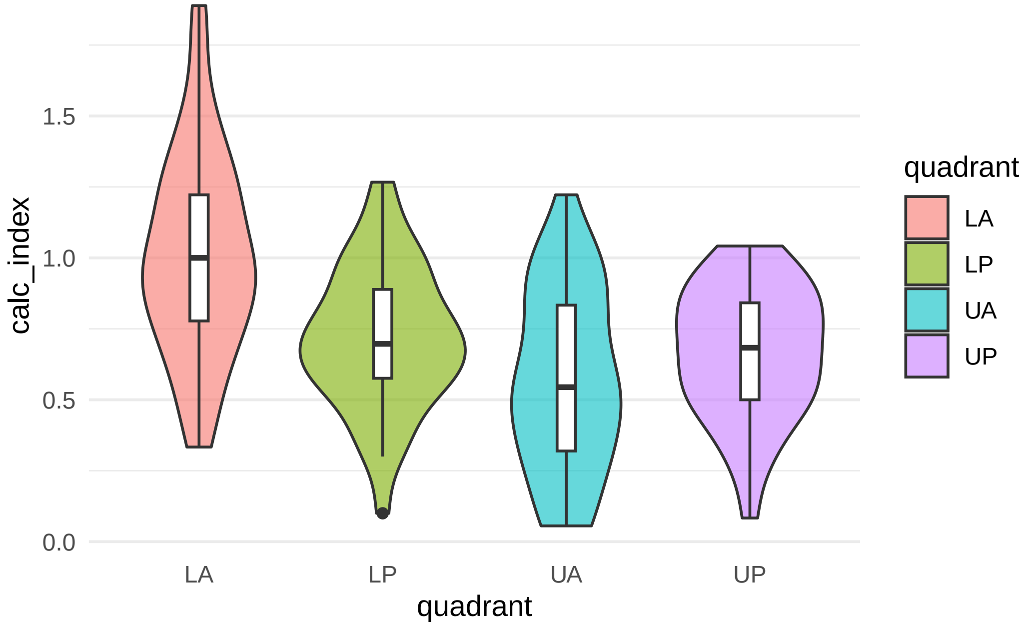


Figure 18: Calculus index per quadrant. LA = lower anterior, LP = lower posterior, UA = upper anterior, UP = upper posterior.

bacco smoke, caffeine consumption). Chronic maxillary sinusitis (CMS) is the inflammation of the lower paranasal sinuses, air-filled pockets located in the skull that defend the organism against inhaled particulate matter and pathogens. This occurs through the production of mucus carried by small hairs toward an opening situated on the superior part of the sinus, where pathogens are drained (Slavin et al. 2005). Without drainage, mucus begins to accumulate in the sinuses, providing an ideal environment for bacterial growth and thereby contributing to inflammation of the mucous membranes and subsequently of the bone surfaces (Jang et al. 2002). Lesions associated with CMS as defined by Boocock, Roberts, and Manchester (1995) were recorded for each individual and classified as “pitting”, “spicule-type bone formation”, “remodeled spicules”, or “white pitted bone”. CMS was scored as absent when the sinus presented smooth surfaces with little or no associated pitting. To facilitate inspection, fragmented sinuses were cleaned using a dry tooth-brush and water where necessary. If the sinuses were not observable with the naked eye, they were examined with a flexible medical endoscope (Pentax, model: FNL-10RBS, ø=4mm; view angle=30°) inserted through minor breaks naturally occurring on the inferior nasal conchae and palatine bone, where the bone tissue is thinner.

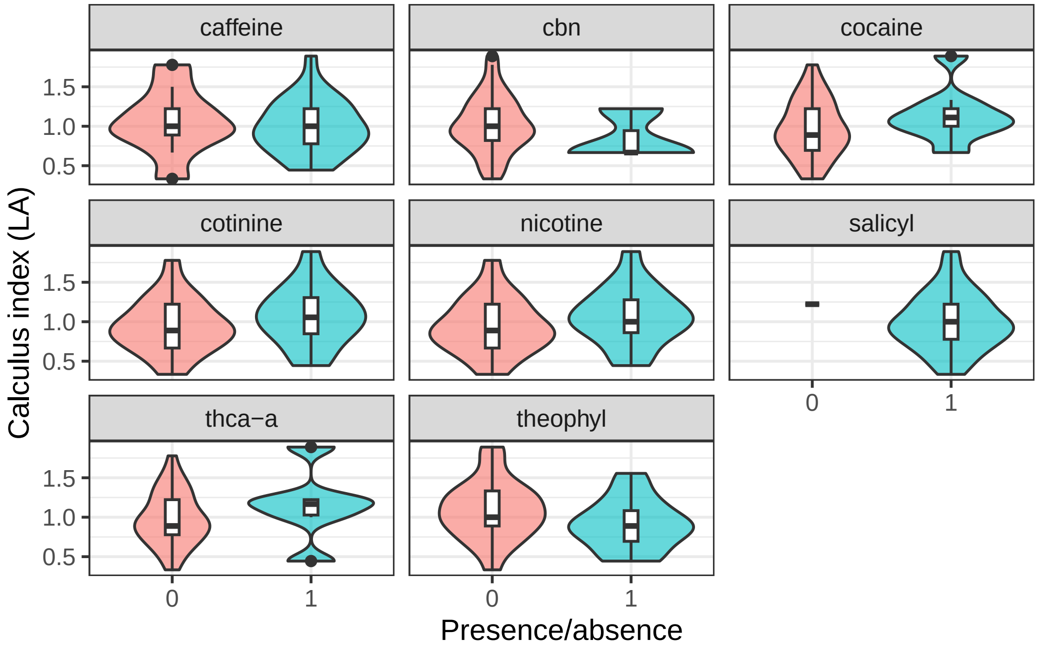


Figure 19: Relationship between the presence (1) or abesence (0) of a compound and the calculus index of the lower anterior quadrant of an individual.

Statistical analysis

Point-biserial correlation

Point-biserial (Pearson) correlation was conducted on compound concentrations, calculus index, caries ratio, and binary variables (Figure 20). This is done to see if any correlations exist prior to discretisation of continuous variables. Irrelevant correlations (anything not between two continuous or a continuous and binary variable) are removed from the plot.

Polychoric correlation

Before analysing the sample with a polychoric correlation (Table 11 and Figure 21), the calculus index and caries ratio for each individual was converted to an ordinal variable by using quartiles, providing a score from 0–4.

```
       0%       25%       50%       75%      100% 
0.0000000 0.0800000 0.1428571 0.2500000 0.6296296 
```

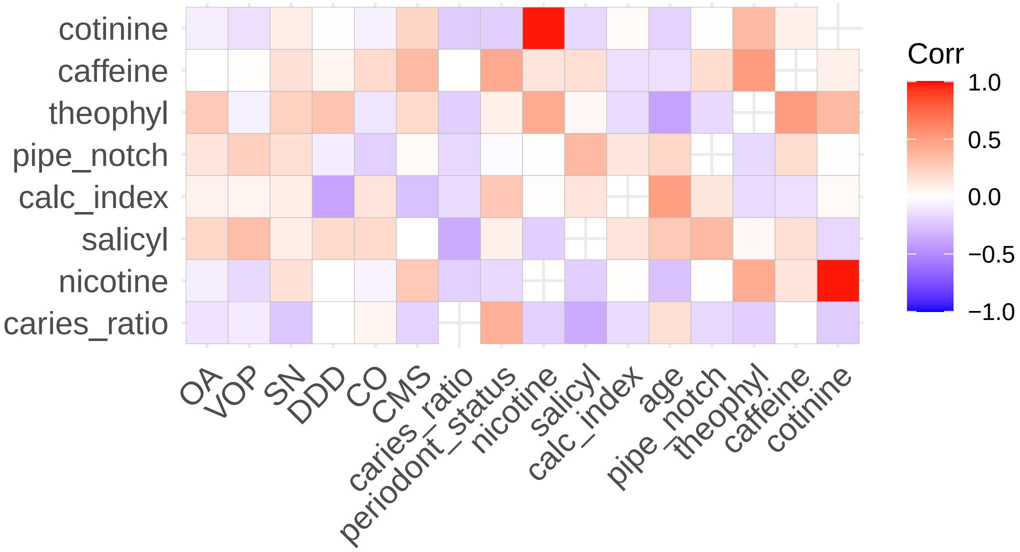


Figure 20: Pearson correlation plot.

```
       0%       25%       50%       75%      100% 
0.2528736 0.5833333 0.7246377 0.8666667 1.0833333 
```

Table 11: Table of polychoric correlations (rho).

	OA	VOP	SN	DDD	CO	CMS	caries_ratio	periodont_status	nicotine	calc_index	age	theophyl	caffeine	cotinine
OA	1.000	0.684	0.365	0.470	-0.146	-0.006	-0.010	0.271	0.001	-0.082	0.389	0.272	0.002	0.292
VOP	0.684	1.000	0.031	0.192	0.364	0.225	-0.008	0.487	-0.151	-0.007	0.387	-0.053	0.345	0.227
SN	0.365	0.031	1.000	-0.083	-0.366	0.254	-0.306	0.067	0.530	0.008	-0.138	0.314	0.005	0.559
DDD	0.470	0.192	-0.083	1.000	-0.069	0.099	0.028	0.033	-0.040	-0.416	-0.019	0.193	-0.160	-0.148
CO	-0.146	0.364	-0.366	-0.069	1.000	0.139	0.011	0.323	0.060	0.057	-0.007	-0.062	0.090	-0.005
CMS	-0.006	0.225	0.254	0.099	0.139	1.000	-0.370	0.104	0.499	-0.237	-0.100	0.080	0.530	0.427
caries_ratio	-0.010	-0.008	-0.306	0.028	0.011	-0.370	1.000	0.523	-0.230	-0.001	0.360	-0.442	-0.131	-0.269

	OA	VOP	SN	DDD	CO	CMS	caries_ratio	periodont_status	nicotine	calc_index	age	theophyl	caffeine	cotinine
periodont_status	0.271	0.487	0.067	0.033	0.323	0.104	0.523	1.000	0.163	0.168	0.407	-0.038	0.494	0.204
nicotine	0.001	-0.151	0.530	-0.040	0.060	0.499	-0.230	0.163	1.000	0.153	-0.025	0.069	-0.039	0.847
calc_index	-0.082	-0.007	0.008	-0.416	0.057	-0.237	-0.001	0.168	0.153	1.000	0.237	-0.150	0.030	0.211
age	0.389	0.387	-0.138	-0.019	-0.007	-0.100	0.360	0.407	-0.025	0.237	1.000	-0.450	0.044	0.147
theophyl	0.272	-0.053	0.314	0.193	-0.062	0.080	-0.442	-0.038	0.069	-0.150	-0.450	1.000	0.322	-0.021
caffeine	0.002	0.345	0.005	-0.160	0.090	0.530	-0.131	0.494	-0.039	0.030	0.044	0.322	1.000	0.024
cotinine	0.292	0.227	0.559	-0.148	-0.005	0.427	-0.269	0.204	0.847	0.211	0.147	-0.021	0.024	1.000

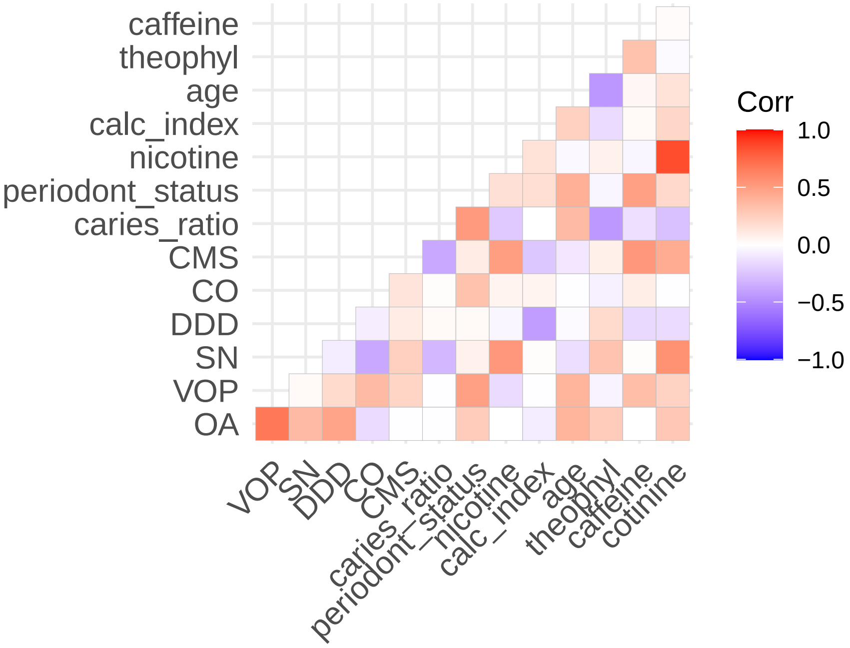


Figure 21: Heatmap of polychoric correlations (rho).

The sample bias caused by targeting larger calculus deposits may also affect the detection of compounds, as caffeinated drinks are often acidic, and may cause a lower calculus formation. We only found a weak negative correlation between caffeine concentration and dental calculus index (Figure 20), but since we targeted individuals with calculus, this may inadvertently have been controlled for during sample collection.

Data dictionary

All raw data are available for download from Zenodo (https://zenodo.org/record/8061483).

metadata.csv

variable	description
id	unique identifier for the individual
sample	sample number for the UHPLC-MS/MS analysis
element	which tooth (FDI notation) was sampled for UHPLC-MS/MS analysis
KZ_element	which tooth (FDI notation) was sampled for aDNA in original study
replicated	Whether the sample was included in the replication batch (TRUE/FALSE)
batch1_weight	weight (mg) of sample in batch 1
batch2_weight	weight (mg) of sample in batch 2

lloq.tsv

variable	description
compound	name of target compound
lloq	Lower limit of quantitation

uhplc-results(_batch2).csv

variable	description
sample	UHPLC-MS/MS sample number
weight	weight of calculus sample in ng before washes
weight_wash<1..3>	weight of calculus sample in ng following each wash
weight_avg	mean of weights

variable	description
_wash<1..3>	Extracted quantity of compound in ng from washes
_calc	Extracted quantity of compound in ng from calculus

dental-inv.csv

Dental inventory

variable	description
id	unique identifier for the individual
t11..t48	status of tooth (FDI notation for variable name)

Dental inventory key:

- p = present
- m = missing (for unknown reason - likely postmortem loss)
- aml = ante-mortem loss
- dna = previously removed for DNA sampling

caries.csv

Caries lesions location

variable	description
id	unique identifier for the individual
t11..t48	location of caries lesion(s)

Caries location key:

- none = no caries present
- mes = mesial
- dis = distal
- lin = lingual
- buc = buccal (and labial)
- occ = occlusal
- crown = crown (large caries lesion covering multiple surfaces)
- root = root
- blank = tooth not present

periodontitis.csv

variable	description
id	unique identifier for the individual
t11..t48	periodontitis score (0-3)

Periodontitis score:

- 0 = none
- 1 = slight
- 2 = moderate
- 3 = severe
- blank = tooth not present

periapical.csv

variable	description
id	unique identifier for the individual
t11..t48	location of periapical lesion

- none = no lesion
- bucc = buccal
- lin = lingual
- perf = perforated alveolar bone
- blank = not scoreable

calculus_full.csv

variable	description
id	unique identifier for the individual
t11_bucc..t48_ip	calculus deposit size (0-3) per tooth surface (bucc = buccal; lin = lingual; ip = interproximal)

- 0 = no calculus

- 1 = slight calculus
- 2 = moderate calculus
- 3 = heavy calculus

path-conditions.csv

Pathological conditions

- OA = osteoarthritis
- IVDD = intervertebral disc disease
- TB = tuberculosis
- DISH = diffuse idiopathic skeletal hyperostosis
- VOP = vertebral osteophytosis
- SN = schmorl's nodes
- DDD = degenerative disc disease
- PNBF = periosteal new bone formation
- OD = osteochondritis dissecans
- CF = cribra femora
- CO = cribra orbitalia

sinusitis.csv

variable	description
id	Individual ID
CMS	Presence (YES) or absence (NO) of chronic maxillary sinusitis
IPR	Presence (YES) or absence (NO) of periosteal reaction on visceral surface of ribs

path-conditions.csv

variable	description
id	Individual ID
OA	Presence/absence of lesions related to osteoarthritis
IVDD	Presence/absence of lesions related to inter-vertebral disc disease
TB	Presence/absence of lesions related to tuberculosis
Mastoiditis	Presence/absence of lesions related to mastoiditis

variable	description
DISH	Presence/absence of lesions related to diffuse idiopathic skeletal hyperostosis
VOP	Presence/absence of lesions related to vertebral osteophytosis
SN	Presence/absence of lesions related to Schmorl's node(s)
DDD	Presence/absence of lesions related to degenerative disc disease
PNBF	Presence/absence of periosteal new bone formation
OD	Presence/absence of lesions related to osteochondritis dissecans
CF	Presence/absence of lesions related to cribra femora
CO	Presence/absence of lesions related to cribra orbitalia

Session information

This report was generated on 2023-10-27 using the following computational environment and dependencies:

```
R version 4.3.1 (2023-06-16)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Pop!_OS 22.04 LTS

Matrix products: default
BLAS:   /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.10.0
LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0

attached base packages:
[1] stats     graphics  grDevices datasets  utils     methods   base

other attached packages:
 [1] mb11CalculusPilot_0.1.0 patchwork_1.1.2         psych_2.2.9
 [4] purrr_1.0.1             glue_1.6.2              stringr_1.5.0
 [7] ggcorrplot_0.1.4        ggplot2_3.4.4           tibble_3.2.1
[10] tidyr_1.3.0             dplyr_1.1.3             readr_2.1.4
[13] here_1.0.1

loaded via a namespace (and not attached):
```

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References

Boocock, P, C. A. Roberts, and K Manchester. 1995. "Maxillary Sinusitis in Medieval Chichester, England." *American Journal of Physical Anthropology* 98 (4): 483–95. https://doi.org/10.1002/ajpa.1330980408.

Casna, Maia, Carla L. Burrell, Rachel Schats, Menno L. P. Hoogland, and Sarah A. Schrader. 2021. "Urbanization and Respiratory Stress in the Northern Low Countries: A Comparative Study of Chronic Maxillary Sinusitis in Two Early Modern Sites from the Netherlands (AD 1626–1866)." *International Journal of Osteoarchaeology* 31 (5): 891–901. https://doi.org/10.1002/oa.3006.

Jang, Y.j., T.w. Koo, S.y. Chung, and S.g. Park. 2002. "Bone Involvement in Chronic Rhinosinusitis Assessed by 99mTc-MDP Bone SPECT." *Clinical Otolaryngology & Allied Sciences* 27 (3): 156–61. https://doi.org/10.1046/j.1365-2273.2002.00553.x.

Rogers, Juliet. 2000. "The Palaeopathology of Joint Disease." In *Human Osteology : In Archaeology and Forensic Science.*, edited by Margaret Cox and Simon Mays, 1st ed, 163–82. Cambridge: Cambridge University Press. https://login.ezproxy.leidenuniv.nl:2443/login?URL=https://search.ebscohost.com/login.aspx?direct=true&db=e000xww&AN=40641&site=ehost-live.

Slavin, Raymond G., Sheldon L. Spector, I. Leonard Bernstein, Raymond G. Slavin, Michael A. Kaliner, David W. Kennedy, Frank S. Virant, et al. 2005. "The Diagnosis and Management of Sinusitis: A Practice Parameter Update." *Journal of Allergy and Clinical Immunology*, The Diagnosis and Management of Sinusitis: A Practice Parameter Update, 116 (December): S13–47. https://doi.org/10.1016/j.jaci.2005.09.048.

Sørensen, Lambert K., Jørgen B. Hasselstrøm, Line S. Larsen, and Dorthe A. Bindslev. 2021. "Entrapment of Drugs in Dental Calculus – Detection Validation Based on Test Results from Post-Mortem Investigations." *Forensic Science International* 319 (February): 110647. https://doi.org/10.1016/j.forsciint.2020.110647.