

An Isotopic Study of Bio-accessible Lead in Wheat, Miswak Toothbrush and Miswak Fruit Using the Continuous On-line Leaching Method with Inductively Coupled Plasma Mass Spectrometry

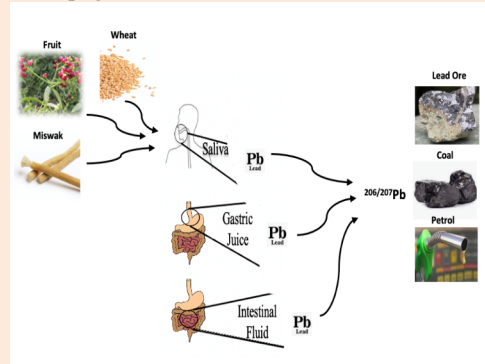
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Received: July 14, 2021; Revised: July 27, 2021; Accepted: July 30, 2021; Available online: July 31, 2021.

DOI: 10.46770/AS.2021.708

ABSTRACT: In this study, sources of Pb are investigated using the $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio measured by inductively coupled plasma quadrupole mass spectrometry in the bio-accessible fractions from wheat, Miswak toothbrush (also used as chewing stick) and Miswak fruit. These samples were obtained for the in vitro batch and on-line leaching methods that mimic the human gastrointestinal tract by sequentially using artificial saliva, gastric juice and intestinal fluid for extraction. The two methods provided similar results according to a Student's *t*-test at the 95% confidence level. Significant ($p < 0.05$) differences in $^{206}\text{Pb}/^{207}\text{Pb}$ were noted at the 95% confidence level in different artificial body fluids. Except for unpolluted Miswak toothbrush and Miswak fruit, which only contained geogenic Pb, the samples contained a mixture of geogenic and anthropogenic Pb, with bio-accessible Pb in saliva being mainly from a geogenic source, whereas bio-accessible Pb in the stomach and intestinal fluids was mainly from anthropogenic sources. Despite the fact that leaded petrol was phased out in Saudi Arabia in 2001, a Miswak toothbrush collected on the side of a busy road after exposure for only a few days and Safeer wheat were still being contaminated with Pb and had an isotopic composition matching that of Pb added to petrol. The $^{208}\text{Pb}/^{206}\text{Pb}$ isotopic ratio for gastric bio-accessible Pb also matched that reported in tetraethyllead. The $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratios of intestinal bio-accessible Pb from Qassim wheat and Safeer wheat point to coal combustion as the source. Overall, these results highlight the need for continued Pb monitoring and the value of examining bio-accessible fractions periodically to identify Pb sources.



INTRODUCTION

Lead contamination of the environment as a result of human activities is a pervasive and serious problem.¹ The wide range of anthropogenic activities involving the release of this metal often makes source identification of Pb necessary in order to plan effective abatement strategies for contaminated sites.¹

Lead is naturally ubiquitous in water, air and soil. However, additional sources of Pb in the atmosphere are caused due to mining activities, burning of coal, smelting, non-ferrous metal refining, waste incineration and combustion of gasoline containing

Pb as anti-knock agent. As a result, Pb accumulates in plants and eventually enters the gastrointestinal tract via ingestion and inhalation, where the bio-accessible portion (released in the gastrointestinal tract) may become bio-available (enter the circulatory system) and cause toxic effects.²⁻⁵

In nature, Pb has four stable isotopes: ^{204}Pb (1%), ^{206}Pb (24%), ^{207}Pb (23%) and ^{208}Pb (52%). The latter three are formed from the radioactive decay of U and Th. Indigenous variation in stable isotope composition can thus be used to identify the Pb sources.⁶⁻⁷ The isotopic composition of Pb in environmental samples can reflect and help discriminate the Pb source.⁸ This fingerprinting

approach is a valuable tool for tracing Pb in the environment.⁹

Over the years, there has been a significant improvement in controlling Pb concentrations in the environment, such as phasing out leaded gasoline in accordance with the ruling of the World Health Organization (WHO).¹⁰ Yet, Pb is still classified as the second most hazardous substance, after only As, as reported by the Agency for Toxic Substances and Disease Registry.¹¹ In Saudi Arabia, despite phasing out leaded gasoline in 2001, Pb continues to accumulate in the environment¹¹ and still exceeds the 0.2-0.3 mg Pb kg⁻¹ food regulation in the region.¹²⁻¹⁶

Lead isotope ratios are commonly measured using thermal ionization mass spectrometry, multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) and inductively coupled plasma quadrupole mass spectrometry (ICP-QMS). Whereas TIMS and MC-ICP-MS are preferred methods for geological dating applications, ICP-QMS is widely used for environmental source studies. In contrast to TIMS and MC-ICP-MS, it is relatively inexpensive and requires minimal sample preparation while allowing sufficiently precise Pb isotope ratio measurements.^{5,17-19}

To assess and identify the Pb exposure pathways, Pb isotope ratios can be measured in blood samples. For example, the Pb isotope ratios in children's blood were reported to match those in soil and house dust, suggesting their ingestion as the source of Pb in blood.²⁰ Similarly, the Pb isotopic composition in children's blood in China corresponded to that found in the ash of coal combustion and particulate matter. This suggests that the inhalation of these air particulates from coal ash, contaminated during coal combustion, was a significant contributor to Pb in their blood.²¹ In another case, the Pb isotope ratios in children's blood agreed with those found in the vegetables, wheat, drinking water, and air particulates from the local areas, suggesting that dietary and inhalation pathways were the most likely sources for these children's Pb in blood.²²

An alternative to measuring bio-available Pb isotope ratios is to determine Pb isotope ratios in the bio-accessible fraction, which circumvents the above difficulties. Given that the bio-accessible fraction²⁴ is equal to the bio-available fraction in the worst-case scenario, Pb isotope ratios may thus be measured in the bio-accessible fraction of environmental samples to realistically estimate the main Pb contamination sources.

The continuous on-line leaching method, where gastrointestinal reagents are sequentially pumped through a mini-column of sample while the effluent is continuously monitored by ICP-QMS, allows bio-accessibility measurements in a fraction of the time required for batch methods because continuous exposure of the sample to fresh reagents shifts the dissolution equilibrium to the right.²⁴ In addition, it provides real-time information that can help discriminate different sources.²⁴ It revealed two sources of Pb being released in gastric juice from corn bran, i.e., two peaks in the

temporal profile, where one peak showed a Pb isotopic composition matching that of tetraethyllead (TEL) previously added to gasoline as the anti-knock agent.²⁵

Given the problematic Pb levels in Saudi Arabia, in addition to Pb up to 100% bio-accessible from wheat,¹⁵ Miswak natural toothbrush (also used as a chewing stick)²⁶ and Miswak fruit,²⁶ source apportionment would be useful. For instance, if most of the Pb is anthropogenic, actions can then be taken to reduce contamination. The aim of this research was to investigate the isotopic composition of bio-accessible Pb from these samples. The results obtained by a conventional batch method to measure bio-accessibility were compared to those obtained by the on-line leaching method previously developed.²⁴ The resulting Pb isotopic composition was then compared to that reported in the literature to identify the most probable source(s) of Pb in wheat, Miswak toothbrush and Miswak fruit from Saudi Arabia. To the best knowledge of the authors, Pb source apportionment has never been conducted on such samples.

EXPERIMENTAL

Instrumentation. Lead isotope ratio measurement was performed using a Varian 820MS ICP-QMS (Varian Inc., Mulgrave, Victoria, Australia), with Bruker Quantum software. The instrument was equipped with a Burgener Mira Mist nebulizer (Burgener Research, Mississauga, ON, Canada) inserted into a Scott double-pass spray chamber (SCP Science, Baie d'Urfé, QC, Canada) and a collision-reaction interface (CRI). To maintain CeO⁺/Ce⁺<2% and Ba⁺⁺/Ba⁺<2%, the instrument was tuned daily using 5 µg L⁻¹ Be, Mg, Co, In, Ce, Pb and Ba in 2% (v/v) HNO₃. The operating conditions are summarized in Table 1. Data acquisition was performed in the steady-state mode with 10 s integration for the batch method and in time-resolved mode for the on-line leaching method, with three points per peak, one scan per replicate, a dwell time of 80 ms and 0.025 a.m.u. spacing.

Samples. Saudi Arabia is located in Western Asia and is geographically the largest state in the Middle East. Wheat samples in this study were purchased from grocery stores in the Western province of Saudi Arabia. As shown in Fig. 1, they originated from

Table 1. ICP-QMS (Varian 820MS) Operating Conditions

Parameter	Setting
Ar plasma gas flow rate	18.0 L min ⁻¹
Ar auxiliary gas flow rate	1.80 L min ⁻¹
Ar nebulizer flow rate	1.05 L min ⁻¹
Sample uptake rate	0.8 mL min ⁻¹
Sampling position	6.2 mm
Ar sheath gas flow rate	0.06 L min ⁻¹
Radio-frequency Power	1.40 kW
CRI skimmer H ₂ gas flow rate	85 mL min ⁻¹
Monitored signals	²⁰⁶ Pb ⁺ , ²⁰⁷ Pb ⁺ , ²⁰⁸ Pb ⁺

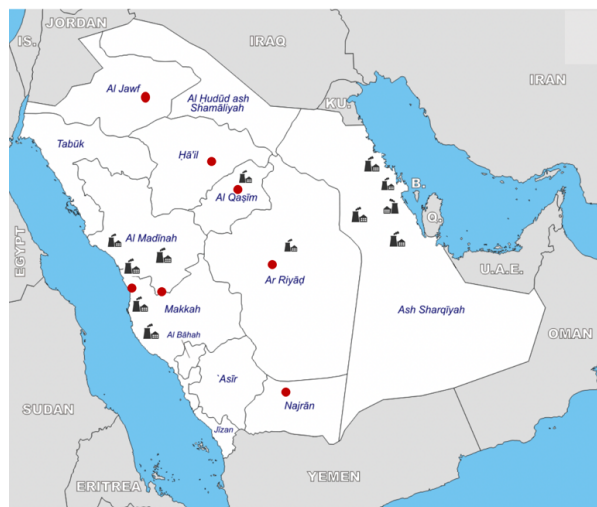


Fig. 1 Map of Saudi Arabia with sample sources in red. Industry signs indicate sites of major industries.²⁷

different regions: Northern (Dubai), Western (Nakleeh), South-Western (Najran) and Central Arabia (Noqrah, Qassim and Safer). All-natural toothbrush and Miswak's fruits were collected from one Miswak tree (*Salvadora persica*) in Al Lith city, located on the coast of the Red Sea (Western province).

The Miswak toothbrushes were 30-cm long, with a 1-cm diameter. Unpolluted Miswak toothbrushes and Miswak fruits were collected from one Miswak tree and placed directly in separate polyethylene bags upon collection, whereas polluted Miswak toothbrushes were sampled from the same Miswak tree but handled normally and transported for 180 km to Jeddah city to be sold in an open market on the roadside of a busy street. After a few days, one Miswak toothbrush was placed in a polyethylene bag for later analysis. Then, these samples were ground by hand to a fine powder with a porcelain pestle and mortar and kept in polyethylene bags at 4°C until analysis.

Reagents. Artificial saliva was prepared by mixing 6.8 g of KH_2PO_4 (ACS grade; Fisher Scientific, Ottawa, ON, Canada) and 77 mL of 0.2 mol L^{-1} NaOH (ACS grade; BioShop, Burlington, ON, Canada). After adjusting the pH to 6.5 with 0.2 mol L^{-1} NaOH, the sample was diluted to 1 L with doubly deionized water (DDW) (Arium Pro UV/DI, Sartorius Stedim Biotech, Göttingen, Germany).

For preparation of gastric juice (pH. 1.2), 2.0 g of NaCl (ACS grade; BioShop), 3.2 g of pepsin (Sigma-Aldrich, Oakville, ON, Canada) and 7.0 mL of sub-boiled HCl (ACS grade; Fisher Scientific) were combined, then diluted to 1 L with DDW.

Intestinal fluid was prepared by mixing 6.8 g of KH_2PO_4 , 10 g of pancreatin (Sigma-Aldrich, St. Louis, MO, USA) and 77 mL of 0.2 mol L^{-1} NaOH, then diluting to 1 L with DDW after adjusting the pH to 6.8 with 0.2 mol L^{-1} NaOH.

All acids were purified using a DST-1000 sub-boiling distillation system (Saville, Minnetonka, MN, USA).

Batch method. Approximately 1 g of sample was placed in a 50 mL falcon tube with artificial saliva. The test tube was then placed in a shaker for 10 min at 37°C to mimic human body temperature. Then, the tube was centrifuged for 15 min at 4100 rpm and 3°C. The supernatant was decanted, filtered, and collected in a clean bottle (high-density polyethylene). The same procedure was repeated with artificial gastric juice and finally intestinal juice, each shaken for 2 h.

On-line leaching method. An amount of 0.4 g of ground sample was rolled in glass wool and then inserted into a mini-column (5-cm long polytetrafluoroethylene tube with 5/16-in outer diameter and 1/4-in inner diameter). A clean glass wool plug was placed at each end of the mini-column to trap the sample, maximize the flow and minimize clogging. The ICP-QMS instrument's peristaltic pump controlled the flow rate of the artificial body fluids that were sequentially pumped through the mini-column to the nebulizer. A thermostatically controlled water bath (Haake, Berlin, Germany) maintained the mini-column and gastro-intestinal reagents at 37°C.

Data processing. For the batch method, the Pb isotope ratios were readily obtained by dividing the average steady-state signal of $^{206}\text{Pb}^+$ by that of $^{207}\text{Pb}^+$. For the transient signal resulting from the on-line method, the point-by-point signal intensity of $^{206}\text{Pb}^+$ was plotted versus $^{207}\text{Pb}^+$ using Microsoft Office Excel 2016. This yielded a straight line whose slope was the $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio. This approach was reported to provide more accurate ratios than a ratio of peak areas or the average point-by-point ratio.²⁸ The same approach was used to obtain the $^{208}\text{Pb}/^{206}\text{Pb}$ isotope ratio to confirm TEL as a source.

To compare the results from the on-line and batch methods, an F test was first performed at the 95% confidence level to determine if there was a significant difference in variance. The appropriate Student's *t*-test was then performed at the 95% confidence level to establish if there was a significant difference between the results. To compare leaching of the three gastro-intestinal reagents, analysis of variance (ANOVA) was conducted: a significant difference ($p < 0.05$) between the Pb isotope ratios by the two methods for a given leaching reagent indicates that there is at least one source significantly different from the others. Tukey Pairwise analysis was then conducted to reveal which Pb source in the body fluids was significantly different.

RESULTS AND DISCUSSION

$^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio in bio-accessible fraction from wheat. Fingerprinting based on the $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio is commonly used to distinguish anthropogenic Pb input from that of natural origin in environmental samples.²⁹ The ratios measured in the bio-accessible fraction from the wheat samples in each artificial

Table 2. $^{206}\text{Pb}/^{207}\text{Pb}$ Ratios \pm Standard Deviation (% relative standard deviation (% RSD)) Measured in Saliva, Gastric Juice and Intestinal Fluid Leachates from Wheat Samples by On-line Continuous Leaching and Batch Methods, with Student's *t*-test at the 95% Confidence level (*n*=3-5)

Region	Leaching reagent	Method	Pb ratio (\pm SD) (RSD)	Student's <i>t</i>	
				Found	Table
Dubai	Saliva	Batch	1.14 \pm 0.02(2)	1.54	3.18
		On-line	1.12 \pm 0.01 (1)		
	Gastric juice	Batch	1.08 \pm 0.10 (9)	0.66	2.36
		On-line	1.05 \pm 0.03 (3)		
	Intestinal fluid	Batch	1.11 \pm 0.03 (3)	2.43	2.45
		On-line	1.06 \pm 0.03 (3)		
Nakleeh	Saliva	Batch	1.14 \pm 0.03 (3)	0.23	2.45
		On-line	1.15 \pm 0.02 (2)		
	Gastric juice	Batch	1.06 \pm 0.08 (7)	0.63	2.36
		On-line	1.03 \pm 0.07 (7)		
	Intestinal fluid	Batch	1.07 \pm 0.08 (7)	0.49	2.44
		On-line	1.05 \pm 0.04 (4)		
Qassim	Saliva	Batch	1.08 \pm 0.06 (5)	0.74	2.45
		On-line	1.06 \pm 0.02 (2)		
	Gastric juice	Batch	1.19 \pm 0.11 (9)	0.38	2.36
		On-line	1.17 \pm 0.03 (3)		
	Intestinal fluid	Batch	1.17 \pm 0.20 (17)	0.29	2.77
		On-line	1.16 \pm 0.02 (2)		
Najran	Saliva	Batch	1.14 \pm 0.03 (3)	1.25	2.45
		On-line	1.15 \pm 0.03 (3)		
	Gastric juice	Batch	1.05 \pm 0.05 (5)	0.07	2.31
		On-line	1.05 \pm 0.03 (3)		
	Intestinal fluid	Batch	1.08 \pm 0.04 (4)	1.52	2.36
		On-line	1.04 \pm 0.04 (4)		
Noqrah	Saliva	Batch	1.08 \pm 0.30 (27)	0.98	2.57
		On-line	1.02 \pm 0.07 (7)		
	Gastric juice	Batch	1.03 \pm 0.08 (8)	0.08	2.45
		On-line	1.03 \pm 0.04 (4)		
	Intestinal fluid	Batch	1.09 \pm 0.05 (5)	2.26	2.36
		On-line	1.01 \pm 0.06 (6)		
Safeer	Saliva	Batch	1.12 \pm 0.02 (2)	0.25	2.45
		On-line	1.13 \pm 0.05 (4)		
	Gastric juice	Batch	1.20 \pm 0.09 (8)	0.04	2.57
		On-line	1.21 \pm 0.02 (2)		
	Intestinal fluid	Batch	1.16 \pm 0.02 (2)	0.48	2.77
		On-line	1.17 \pm 0.02 (2)		

leaching reagent by the conventional batch method were compared to those with the on-line leaching method, as listed in Table 2. The results are in agreement based on the Student's *t*-test

at the 95% confidence level, confirming similar results by the two methods. However, the precision, expressed as relative standard deviation (RSD), for the ratios by the on-line method was in general better than by the batch method. Indeed, the 2-7% range with the on-line method is clearly narrower than the 2-27% range with the batch method.

The Pb released from the wheat samples obtained from Dubai, Nakleeh, Najran and Safeer has an isotopic composition that falls within the 1.12-1.15 range previously reported for geogenic Pb, i.e., indigenous Pb ores in Saudi Arabia.³⁰ The $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio in saliva was found to be significantly different from those in gastric juice and intestinal fluid ($p < 0.05$) at the 95% confidence level.

China and Indonesia were major providers of coal to Saudi Arabia during the last three decades of the 20th century, which had a $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio in the 1.15-1.18 range.³¹⁻³² The Pb released by intestinal fluid from the wheat from Qassim and Safeer has an isotopic composition that falls within this range, indicating that coal burning may be responsible for this more radiogenic Pb. In each case, the isotopic ratio is significantly different from that in saliva ($p < 0.05$) at the 95% confidence level. There is also evidence of Pb from leaded gasoline ($^{206}\text{Pb}/^{207}\text{Pb}$ of 1.21)³³ in the gastric juice leachate from Safeer wheat, indicating that the soil contamination from decades of leaded petrol use in that region is still reflected in wheat.

The lower isotope ratios in gastric juice and intestinal fluid leachates of wheat from Dubai, Nakleeh and Najran compared to geogenic Pb, is likely a result of industrial Pb emissions, e.g., from copper smelters.³⁴ The different geographical origins of these samples (North, West and Southern-West, respectively) suggest atmospheric transport of anthropogenic Pb. Storms are well known to occur in those regions, which carry large amounts of particulate matter over long distances.³⁵

The similarity or difference between sources of Pb are also evident when plotting the temporal leaching profile of Pb versus that of Cd, which was recently used to help visualize different sources of these elements in Miswak.¹⁶ For example, Fig. 2 shows very different correlations between the temporal profiles of Pb and Cd for two wheat samples (from Noqrah and Safeer) that contained similar concentrations of Pb (2000 \pm 520 $\mu\text{g kg}^{-1}$ and 2000 \pm 400 $\mu\text{g kg}^{-1}$ respectively).¹⁵ The less bio-accessible Pb in Noqrah wheat¹⁵ seems to be from a single source, supporting the similar $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio in saliva, gastric juice and intestinal fluid. In contrast, three different sources are clearly indicated by the three different correlations between Pb and Cd for Safeer wheat (Fig. 2), in agreement with the significantly different isotopic ratios in each of the three gastro-intestinal reagents.

$^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio in bio-accessible fraction from Miswak toothbrush and Miswak fruit. Table 3 shows the $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratios obtained in the saliva, gastric juice and

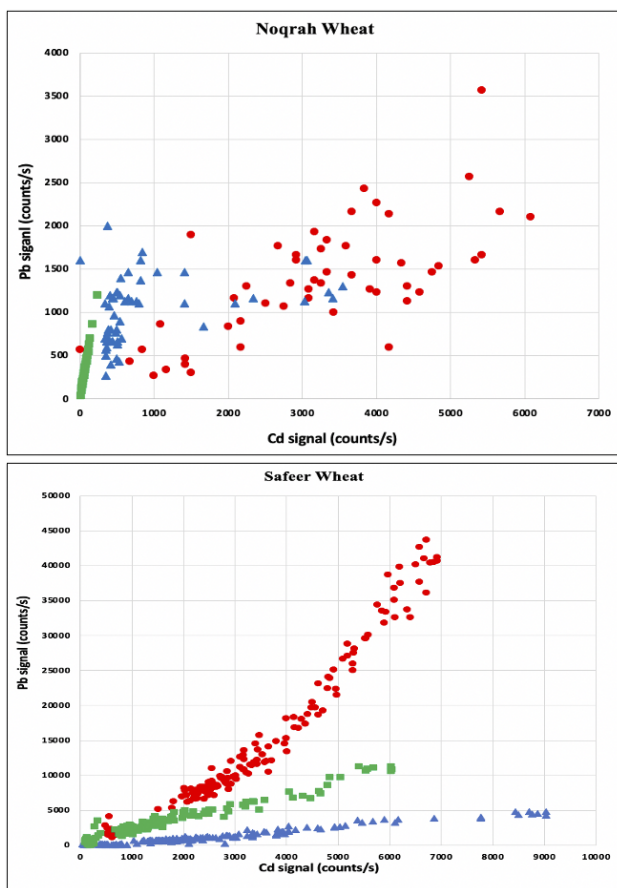


Fig. 2 Correlation between the leaching profiles of Pb and Cd with artificial saliva (blue triangles), gastric juice (red circles) and intestinal fluid (green squares) in Noqrah wheat (top) and Safer wheat (bottom).

polluted Miswak toothbrush and Miswak fruit. Again, there is good agreement between the batch and the on-line leaching methods according to a Student's *t*-test at the 95% confidence level. The precision of ratios with the on-line method, 1-5 % RSD, is again in general better than the 1-18% RSD with the batch method. For unpolluted Miswak toothbrush and Miswak fruit, the three ratios are similar to those measured in Saudi Arabia's western Pb ores (1.12-1.15).³⁰ Moreover, $^{206}\text{Pb}/^{207}\text{Pb}$ values for unpolluted Miswak toothbrush and Miswak fruit are not statistically different from each other, which is commensurate with their being collected from the same tree. However, the significantly higher $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio for polluted Miswak toothbrush in the gastric phase indicates an anthropogenic source of Pb that corresponds to leaded petrol (with a $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio of 1.21).³³ Although at least two different sources of Pb were indicated by the correlations between the Pb and Cd temporal profiles in each leaching reagent,¹⁶ these results were unexpected, given that leaded gasoline was discontinued in 2001. However, they support reports of leaded gasoline being mixed with unleaded gasoline.³⁶⁻³⁷ In any case, exposing a Miswak toothbrush to emissions from vehicular traffic for only a few days was clearly sufficient for it to become contaminated with a different source of Pb.

Table 3. $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio \pm standard deviation (% RSD) measured in saliva, gastric juice and intestinal fluid leachates from natural toothbrush samples by on-line continuous leaching and the batch method, with Student's *t*-test at the 95% confidence level ($n=3-5$)

Miswak Samples	Reagent	Method	Pb ratio (\pm SD) (RSD)	Student's <i>t</i>	
				Found	Table
Unpolluted	Saliva	Batch	1.15 \pm 0.02 (2)	0.22	2.45
		On-line	1.15 \pm 0.02 (2)		
	Gastric juice	Batch	1.11 \pm 0.08 (7)	0.40	2.36
		On-line	1.12 \pm 0.02 (2)		
	Intestinal fluid	Batch	1.15 \pm 0.04 (4)	0.09	2.57
		On-line	1.15 \pm 0.06 (5)		
Polluted	Saliva	Batch	1.16 \pm 0.03 (3)	0.37	2.57
		On-line	1.15 \pm 0.02 (2)		
	Gastric juice	Batch	1.21 \pm 0.22 (18)	0.04	2.31
		On-line	1.22 \pm 0.04 (3)		
	Intestinal fluid	Batch	1.14 \pm 0.08 (7)	0.06	2.45
		On-line	1.14 \pm 0.02 (2)		
Fruit	Saliva	Batch	1.14 \pm 0.02 (2)	0.46	2.77
		On-line	1.14 \pm 0.01 (1)		
	Gastric juice	Batch	1.13 \pm 0.01 (1)	1.26	2.77
		On-line	1.12 \pm 0.02 (2)		
	Intestinal fluid	Batch	1.11 \pm 0.01 (1)	1.21	2.57
		On-line	1.12 \pm 0.02 (2)		

Table 4. $^{208}\text{Pb}/^{206}\text{Pb}$ ratio \pm standard deviation (% RSD) measured in gastric juice leachates from Safer wheat and polluted Miswak samples by on-line continuous leaching and batch methods, with Student's *t*-test at the 95% confidence level ($n=3-5$).

Sample	Method	Pb ratio (\pm SD) (RSD)	Student's <i>t</i>	
			Found	Table
Safer wheat	Batch	2.05 \pm 0.07 (3)	0.59	3.18
	On-line	2.07 \pm 0.04 (2)		
Polluted Miswak	Batch	2.04 \pm 0.12 (6)	0.52	2.31
	On-line	2.06 \pm 0.04 (2)		
TEL ³⁸⁻⁴⁰		2.05		

Confirmation of TEL as a source of Pb in wheat and polluted Miswak toothbrush. To confirm that TEL was a source of Pb in Safer wheat and polluted Miswak toothbrush, and rule out any potential spectroscopic interference that might have spuriously increased $^{206}\text{Pb}/^{207}\text{Pb}$, the $^{208}\text{Pb}/^{206}\text{Pb}$ isotopic ratio, was also measured in the gastric juice fraction. The results are summarized in Table 4 and clearly confirm TEL as a source of Pb. Again, there was no significant difference between the ratios obtained by the batch and on-line methods according to a Student's *t*-test at the 95% confidence level. The on-line method again provided more precise ratios than the batch method. Given that this Pb source appeared on Miswak toothbrush after only a few days of exposure on the side of a busy road, it also indicates the continued use of TEL in gasoline.

CONCLUSIONS

For the first time, the $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio was successfully used as a fingerprinting tool on the bio-accessible fractions from wheat, Miswak toothbrush and Miswak fruit samples from Saudi Arabia. In addition to geogenic Pb, anthropogenic Pb from leaded petrol, coal combustion and other industrial sources were identified in bio-accessible Pb. Anthropogenic Pb thus remains a significant pollutant, which may pose a potential risk to human health. The on-line leaching method affords visual confirmation of different Pb sources through the significantly different correlations that then result between the temporal profiles of Pb and Cd. Future Pb monitoring programs could use this approach to assess whether Pb mitigation strategies are being effective.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Saudi Arabian Government and the Natural Sciences and Engineering Research Council of Canada (RGPNM 39487-2018).

REFERENCES

1. B. Eldering, G. Amund, H. Kunzendorf, and E. J. Krogstad, *Appl. Geochem.*, 2002, **17**, 493-502. [https://doi.org/10.1016/S0883-2927\(01\)00119-6](https://doi.org/10.1016/S0883-2927(01)00119-6)
2. H. M. Xu, J. J. Cao, K. F. Ho, H. Ding, Y. M. Han, G. H. Wang, J. C. Chow, J. G. Watson, S. D. Khol, J. Qiang, and W. T. Li, *Atmos. Environ.*, 2012, **46**, 217-224. <https://doi.org/10.1016/j.atmosenv.2011.09.078>
3. L. S. J. Tsuji, B. C. Wainman, I. D. Martin, C. Sutherland, J. P. Weber, P. Dumas, and E. Nieboer, *Sci. Total Environ.*, 2008, **393**, 291-298. <https://doi.org/10.1016/j.scitotenv.2008.01.022>
4. X. Y. Bi, Z. G. Li, S. X. Wang, L. Zhang, R. Xu, J. L. Liu, H. M. Yang and M. Z. Guo, *Environ. Sci. Technol.*, 2017, **51**, 13502-13508. <https://doi.org/10.1021/acs.est.7b04119>
5. H. Cheng and Y. Hu, *Environ. Pollut.*, 2010, **158**, 1134-1146. <https://doi.org/10.1016/j.envpol.2009.12.028>
6. E. Schnyder, M. Štok, Z. Kosonen, M. Skudnik, D. Mazej, Z. Jeran, and L. Thöni, *Ecol. Indi.*, 2018, **95**, 250-259. <https://doi.org/10.1016/J.ECOLIND.2018.06.072>
7. L. J. Binkowski, W. Meissner, M. Trzeciak, K. Izevbekhai, and J. Barker, *Chemosphere*, 2016, **164**, 436-442. <https://doi.org/10.1016/j.chemosphere.2016.08.120>
8. W. H. Shetaya, E. R. Marzouk, E. F. Mohamed, M. Elkassas, E. H. Bailey, and S. D. Young, *Sci. Total Environ.*, 2018, **618**, 460-468. <https://doi.org/10.1016/j.scitotenv.2017.11.040>
9. M. Tarzia, B. de Vivo, R. Somma, R. A. Ayuso, R. A. R. McGill, and R.R. Parrish, *GEEA.*, 2002, **2**, 45-56. <https://doi.org/10.1144/1467-787302-006>
10. WHO. Initiating an alliance for action: Healthy environments for children. Geneva: World Health Organization, 2002. https://apps.who.int/iris/bitstream/handle/10665/67382/WHO_SDE_PHE_02.06.pdf?sequence=1
11. H. M. Aburas, M. A. Zytoon, and M. I. Abdulsalam, *Clean Soil, Air, Water*, 2001, **39**, 711-719. <https://doi.org/10.1002/clen.201000510>
12. G. E. El-Desoky, M. A. M. Aboul-Soud, Z. A. Al-Othman, M. Habila, and J. P. Giesy, *Environ. Geochem. Health*, 2014, **36**, 583-593. <https://doi.org/10.1007/s10653-013-9582-3>
13. S. Mahboob, H. F. A. Albalawi, F. Almisned, S. Al-quraishy, and Z. Amhad., *B. Environ. Contam. Toxicol.*, 2014, **92**, 61-66. <https://doi.org/10.1007/s00128-013-1139-8>
14. H. Mohamed, P. I. Haris, and E. i. Brima, *Int. J. Environ. Res. Public Health*, 2017, **14**, 1575. <https://doi.org/10.3390/ijerph14121575>
15. R. A. Althobiti and D. Beauchemin, *J. Anal. At. Spectrom.*, 2018, **33**, 642-648. <https://doi.org/10.1039/C8JA00047F>
16. R. A. Althobiti and D. Beauchemin, *J. Anal. At. Spectrom.*, 2021, **36**, 535-539. <https://doi.org/10.1039/D0JA00479K>
17. R. Larcher, G. Nicolini and P. Pangrazzi, *J. Agric. Food Chem.*, 2003, **51**, 5956-5961. <https://doi.org/10.1021/jf021064r>
18. E. Margui, M. Iglesias, I. Queralt and M. Hidalgo, *Sci. Total Environ.*, 2006, **367**, 988-998. <https://doi.org/10.1016/j.scitotenv.2006.03.036>
19. C. J. Lord, *J. Anal. At. Spectrom.*, 1994, **9**, 599-603. <https://doi.org/10.1039/JA9940900599>
20. Y. Yaffe, C.P. Flessel, J. J. Wesolowski, A. del Rosario, G. N. Guirguis, V. Matias, J. W. Gramlich, W. R. Kelly, T. E. Degarmo, and G. C. Coleman, *Arch. Environ. Health*, 1983, **38**, 237-245.
21. F. Liang, G. L. Zhang, M. G. Tan, C. H. Yan, X. L. Li, Y. Li, Y. M. Zhang, and Z. C. Shan, *Environ. Sci. Technol.*, 2010, **44**, 4760-4765. <https://doi.org/10.1021/es9039665>
22. S. Z. Cao, X. L. Duan, X. G. Zhao, B. B. Wang, J. Ma, D. L. Fan, C. Y. Sun, B. He, F. S. Wei, and G. B. Jiang, *Environ. Int.*, 2014, **73**, 158-166. <https://doi.org/10.1016/j.envpol.2014.12.035>
23. L. Nijhawan, M. Janodia, B. Muddukrishna, K. Bhat, K. Bairy, N. Udupa and P. Musmade, *J. Adv. Pharmaceut. Technol. Res.*, 2013, **3**, 134-140. <https://doi.org/10.4103/2231-4040.116779>
24. M. Chu and D. Beauchemin, *J. Anal. At. Spectrom.*, 2004, **19**, 1213-1216. <https://doi.org/10.1039/b403215b>
25. M. Chu and D. Beauchemin, *Can. J. Anal. Sci. Spectrosc.*, 2005, **50**, 289-296. https://doi.org/10.17104/0017-1417_2010_5_424
26. R. A. Althobiti, and D. Beauchemin, *J. Anal. At. Spectrom.*, 2021, **36**, 622-629. <https://doi.org/10.1039/D0JA00447B>
27. <https://www.globalsecurity.org/jhtml/jframe.html#https://www.globalsecurity.org/military/world/gulf/images/map-saudi-industry.jpg||Saudi%20Arabia%20Industry%20Map>, Accessed March 10, 2021.

28. V. N. Epov, S. Berail, M. Jimenez-Moreno, V. Perrot, C. Pecheyran, D. Amouroux, and O. F. X. Donard, *Anal. Chem.*, 2010, **82**, 5652-5662. <https://doi.org/10.1021/ac100648f>
 29. R. M. Ellam., *Sci. Total Environ.*, 2010, **408**, 3490-3492. <https://doi.org/10.1016/j.scitotenv.2010.03.037>
 30. I. A. Al-Saleh, C. Fellows, T. Delves, and A. Taylor, *Ann. Clin. Biochem.*, 1993, **30**, 142-145. <https://doi.org/10.1177/000456329303000205>
 31. M. Díaz-Somoano, M. E. Kylander, M. A. Lopez-Anton, I. S. U. Arez-Ruiz, M. R. Martinez-Tarazon, M. Ferrat, B. Kober, and D. J. Weiss, *Environ. Sci. Technol.*, 2009, **43**, 1078-1085. <https://doi.org/10.1021/es801818r>
 32. <https://www.abarico.it/03-22218/ZFrpl.html>, Accessed February 27, 2021.
 33. J. S. Stacey, B. R. Doe, R. J. Roberts, M. H. Delevaux, and J. W. Gramlich, *Contrib. Mineral. Petrol.*, 1980, **74**, 175-188. <https://doi.org/10.1007/BF01132003>
 34. A. Bollhofer and K. J. Rosman. *Geochim. Cosmochim. Acta*, 2001, **65**, 1727-1740. [https://doi.org/10.1016/S0016-7037\(00\)00630-X](https://doi.org/10.1016/S0016-7037(00)00630-X)
 35. S. Kumar, S. G. Aggarwal, J. Malherbe, J. P. G. Barre, S. Berail, P. K. Gupta, and O. F. X. Donard, *Atmos. Environ.*, 2016, **132**, 179-187. <https://doi.org/10.1016/j.atmosenv.2016.03.002>
 36. <https://www.al-jazirah.com/2004/20040104/ec10.htm>, Accessed April 26, 2021.
 37. <https://www.al-jazirah.com/2001/20010210/rv5.htm>, Accessed April 26, 2021.
 38. A. M. Scheuhammer and D. M. Templeton, *Ecotoxicol.*, 1998, **7**, 37-42. <https://doi.org/10.1023/A:1008855617453>
 39. A. M. Scheuhammer, D. E. Bond, N. M. Burgess, and J. Rodrigue, *Environ. Toxicol. Chem.*, 2003, **22**, 2585-2591. <https://doi.org/10.1897/02-361>
 40. M. M. Larsen, J. S. Blusztajn, O. Andersen, and I. Dahllöf, *J. Environ. Monit.*, 2012, **14**, 2893-2901. <https://doi.org/10.1039/c2em30579h>
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