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Screening for Heavy Metals in Tea Leaves from Bangladesh Using X-Ray Fluorescence

Abstract

Trace metal contamination is a global health issue. This study evaluated boxed and loose-leaf tea from Bangladesh using a Handheld X-ray Fluorescence (XRF) analyzer for lead and other metals to rapidly screen tea as a potential hot spot of heavy metal exposure. Of the 33 elements measured, several priority pollutants were below the limit of detection (LOD) in all samples, including lead (LOD 2.17 mg/kg) and arsenic (LOD 1.68 mg/kg). Loose-leaf tea samples had higher copper (1.5-fold), zinc (1.3-fold), and manganese (1.8-fold) concentrations compared to boxed tea. Estimated daily intake (EDI) of lead from tea was calculated using three assumed extractability levels, 20%, 50%, and 100%, and assuming the lead concentration was at the LOD. The EDI for lead ranged from 0.008 µg/kg/bw day to 0.041 µg/kg/bw day for adult males and 0.010 µg/kg/bw day to 0.049 µg/kg/bw day for adult females. Based on these tested samples, tea is not likely a primary source of lead exposure in the two sampled areas, Sirajdikhan and Pabna. Future research can evaluate potential processing steps for why some metals were higher in loose-leaf tea compared to boxed tea, including copper, zinc, and manganese. Other potential lead sources could be tested in the tea preparation process, including water sources and dishware used to prepare and consume the tea.

Keywords

Metals, Lead (Pb), Arsenic (As), X-Ray Fluorescence, XRF, Bangladesh

Cover Page Footnote

Amber Wolf graduated magna cum laude from Duke University in May 2020, where she double majored in Global Health and Music with a concentration in piano performance. She will matriculate to the Icahn School of Medicine at Mount Sinai in August 2020. Amber conducted this research as a part of an international research trip to Bangladesh in the summer of 2019, during which she led the sample collection of tea leaves in Bangladesh and sample analysis at the Harvard T.H. Chan School of Public Health. Amber completed this research project as a portion of a summer undergraduate experiential learning activity through Duke University's Global Health Institute. Affiliations of other authors: Aaron J. Specht: Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA Mi-Sun Lee: Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA Maitreyi Mazumdar: Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA; Department of Neurology, Boston Children's Hospital, Boston, MA John F. Obrycki: Department of Neurology, Boston Children's Hospital, Boston, MA ACKNOWLEDGEMENTS: Amber completed this research project as a portion of a summer undergraduate experiential learning activity through Duke University's Global Health Institute. Research supported by the Harvard T.H. Chan NIEHS Center for Environmental Health (P30 ES000002) and Amber's travel supported by R01 ES027825.

Screening for Heavy Metals in Tea Leaves from Bangladesh Using X-Ray Fluorescence

Amber Wolf

Introduction

Trace metal contamination of food and water is a global public health issue.^{1,2,3} In southeast Asia, metal contamination has been reported in several foods, including vegetables, fish, milk, and tea leaves.^{4,5,6} Some of the reported levels were several times above international guidelines. For example, poultry, fish, vegetables, and rice tested in southwestern Dhaka, Bangladesh had lead levels 3- to 10-fold higher than World Health Organization (WHO) standards.⁷

In Bangladesh, possible food contamination with lead is an important public health research question. Spices, such as turmeric,

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¹ Bhowmik AK, Alamdar A, Katsoyiannis I, et al. Mapping human health risks from exposure to trace metal contamination of drinking water sources in Pakistan. *Science of the Total Environment*. 2015;538:306-316.

² Ha NN, Agusa T, Ramu K, et al. Contamination by trace elements at e-waste recycling sites in Bangalore, India. *Chemosphere*. 2009;76(1):9-15.

³ Filippelli G, Anenberg S, Taylor M, van Geen A, Khreis H. New Approaches to Identifying and Reducing the Global Burden of Disease From Pollution. *Geohealth*. 2020;4(4):e2018GH000167.

⁴ Islam GMR, Khan FE, Hoque MM, Jolly YN. Consumption of unsafe food in the adjacent area of Hazaribag tannery campus and Buriganga River embankments of Bangladesh: heavy metal contamination. *Environmental Monitoring and Assessment*. 2014;186(11):7233-7244.

⁵ Akele ML, Abebe DZ, Alemu AK, Assefa AG, Madhusudhan A, de Oliveira RR. Analysis of trace metal concentrations in raw cow's milk from three dairy farms in North Gondar, Ethiopia: chemometric approach. *Environmental Monitoring and Assessment*. 2017;189(10).

⁶ Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal At Spectrom*. 1998;13(6):521-525.

⁷ Islam GMR, Khan FE, Hoque MM, Jolly YN. Consumption of unsafe food in the adjacent area of Hazaribag tannery campus and Buriganga River embankments of Bangladesh: heavy metal contamination. *Environmental Monitoring and Assessment*. 2014;186(11):7233-7244.

are one potential source of lead exposure in Bangladesh^{8,9} and elsewhere.¹⁰ Tea is another potential source of exposure to lead and other metals, such as manganese, cadmium, and chromium.^{11,12,13} A prospective birth cohort study to evaluate metal exposures and birth outcomes in Bangladesh found that maternal tea intake during pregnancy was positively correlated with cord blood lead levels in newborns from two locations, Sirajdikhan and Pabna, which suggests that tea may be an unexpected source of lead exposure in certain areas of Bangladesh.¹⁴ This prospective birth cohort motivated this current study to investigate tea as a potential source of lead exposure.

Most studies involving elemental analysis of tea leaves use inductively coupled plasma spectroscopic methods (ICP-AES, ICP-MS)^{15,16,17} or flame and graphite furnace atomic absorption spectrometry (GF-AAS).¹⁸ Two previous studies of tea samples

⁸ Forsyth JE, Weaver KL, Maher K, et al. Sources of Blood Lead Exposure in Rural Bangladesh. *Environmental Science & Technology*. 2019;53(19):11429-11436.

⁹ Gleason K, Shine JP, Shobnam N, et al. Contaminated turmeric is a potential source of lead exposure for children in rural Bangladesh. *Journal of environmental and public health*. 2014;2014:730636.

¹⁰ Hore P, Alex-Oni K, Sedlar S, Nagin D. A Spoonful of Lead: A 10-Year Look at Spices as a Potential Source of Lead Exposure. *Journal of Public Health Management and Practice*. 2019;25:S63-S70.

¹¹ Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal At Spectrom*. 1998;13(6):521-525.

¹² Brzezicha-Cirocka J, Grembecka M, Szefer P. Monitoring of essential and heavy metals in green tea from different geographical origins. *Environmental Monitoring and Assessment*. 2016;188(3):11.

¹³ Moreda-Pineiro A, Fisher A, Hill SJ. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *J Food Compos Anal*. 2003;16(2):195-211.

¹⁴ Mi-Sun Lee K-DE, Mostofa Golam, Quazi Quamruzzaman, Molly L. Kile, Maitreyi Mazumdar, David C. Christiani. Umbilical Cord Blood Metals and Birth Size in Bangladesh Children *Submitted manuscript*. 2019.

¹⁵ Moreda-Pineiro A, Fisher A, Hill SJ. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *J Food Compos Anal*. 2003;16(2):195-211.

¹⁶ Han WY, Zhao FJ, Shi YZ, Ma LF, Ruan JY. Scale and causes of lead contamination in Chinese tea. *Environmental Pollution*. 2006;139(1):125-132.

¹⁷ Zhang J, Yang RD, Chen R, Peng YS, Wen XF, Gao L. Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China. *International journal of environmental research and public health*. 2018;15(1):22.

¹⁸ Rashid MH, Fardous Z, Chowdhury MAZ, et al. Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: an evaluation of six digestion methods. *Chem Cent J*. 2016;10:13.

from Bangladesh reported lead concentrations of 0.44¹⁹ and 0.34²⁰ mg/kg. Of the two previous studies of tea leaves from Bangladesh, one study used GF-AAS²¹ and the other used ICP-MS.²² These techniques are destructive of samples, expensive, and time consuming. X-ray fluorescence (XRF) is a non-destructive analytical technique used for qualitative and quantitative analysis with minimal sample preparation requirements.²³ The technique measures fluorescent X-rays to identify each element present in the sample from the element's unique emission energies.²⁴ Handheld XRF units provide a portable method for testing material compositions that allows for rapid screening of heavy metal contamination and identification of potential hot spots with elevated metal concentrations. Two disadvantages of the handheld XRF device are (1) the limit of detection (LOD) is higher than ICP and AAS, and (2) XRF analyzes total, not bioavailable, elemental content. However, Palmer et al. (2009) report that while the LODs of the Handheld XRF are orders of magnitude higher than other methods such as ICP, the analyzer is more than sufficient for detecting acute and long-term toxic levels of certain elements, including As, Cd, Hg, and Pb.²⁵

The current study uses a handheld XRF analyzer to test if brands of tea in Bangladesh contain lead or other metals at concentrations that could cause detrimental health outcomes. The XRF was selected as a rapid screening tool to test for high levels of heavy metals in tea leaves, similar to how this device is routinely used in home lead exposure screening assessments.

¹⁹ Ibid.

²⁰ Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal At Spectrom.* 1998;13(6):521-525.

²¹ Rashid MH, Fardous Z, Chowdhury MAZ, et al. Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: an evaluation of six digestion methods. *Chem Cent J.* 2016;10:13.

²² Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal At Spectrom.* 1998;13(6):521-525.

²³ Palmer PK, Jacobs R, Baker P, Ferguson K, Webber S. Use of Field-Portable XRF Analyzers for Rapid Screening of Toxic Elements in FDA-Regulated Products. *Journal of Agricultural and Food Chemistry.* 2009;7(7): 2605-2613.

²⁴ Scientific T. XRF Technology. <https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental-isotope-analysis-learning-center/elemental-analysis-information/xrf-technology.html>.

²⁵ Palmer PK, Jacobs R, Baker P, Ferguson K, Webber S. Use of Field-Portable XRF Analyzers for Rapid Screening of Toxic Elements in FDA-Regulated Products. *Journal of Agricultural and Food Chemistry.* 2009;7(7): 2605-2613.

Materials and Methods

Sample Collection

Ten brands of tea, including bagged and loose-leaf, were purchased from markets in Sirajdikhan and Pabna in Bangladesh during July 2019. These two locations were selected because they are the primary recruitment sites for the birth cohort study that prompted this investigation of tea leaves. Six brands of bagged tea were purchased at one market in Sirajdikhan and four brands of loose-leaf tea were purchased at a market in Pabna, the locations of which are represented in **Figure 1**. All samples were collected using a market basket sampling technique, which mimics how families would buy and consume tea in their daily lives. As samples were chosen randomly, the type of tea (loose-leaf vs. bagged) was not controlled for at either location. This market basket sampling method is similar to that of the Food and Drug Administration's (FDA) Total Diet Study, an ongoing study that buys samples of food at retail outlets throughout the U.S. to calculate the U.S. population's estimated annual dietary intakes of certain contaminants and nutrients.²⁶

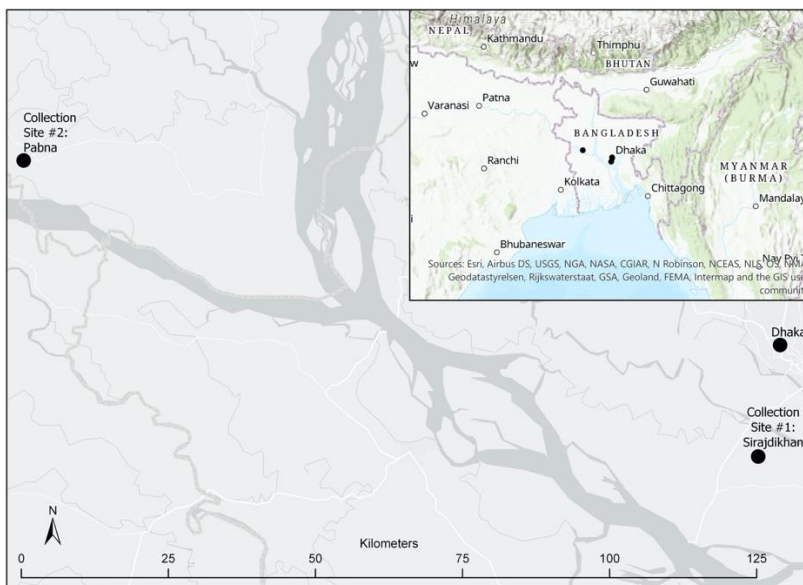


Figure 1. General map of Bangladesh showing the location of the capital city, Dhaka, and the two tea sampling sites, Pabna and Sirajdikhan.

²⁶ Administration FaD. Total Diet Study Design.

<https://www.fda.gov/food/total-diet-study/total-diet-study-design>. Published 2018.

The 10 tea samples were analyzed at the Harvard T.H. Chan School of Public Health Trace Metals Laboratory using a Niton XL3t GOLDD+ handheld XRF unit (Thermo Fisher Scientific, Billerica, MA). The XRF unit was calibrated prior to use, both internally by the instrument when it was turned on and while testing against a known sample or calibration disc. Prior to measuring the tea samples, standard calibration curves were calculated for specific modes of measurement dependent on the medium and composition of samples being measured. The XRF does not yet have a measurement mode specifically for tea leaves. In this study, the measurement mode was selected to be soil as this was the closest mode available to plant samples, as recommended by Palmer et al. (2009).²⁷

Two subsamples of each brand of tea were analyzed for a total of 20 XRF read events. Tea bags were placed directly on the XRF instrument platform, and loose-leaf tea was poured onto a blank sheet of paper prior to analysis. The blank sheet of paper was tested to ensure that there was no contamination with any metals. The average read time for each subsample was 150 seconds. The LOD for a given element is reported as twice the standard deviation and was reported on a scale of parts per million. The XRF provided measurements on 33 elements.

Exposure Calculations

The estimated daily intake (EDI) was calculated for lead using three assumed extractability levels, 20%, 50%, and 100%. The 20% solubility estimate was based on previous research conducted by Dalipi *et al.* (2018) classifying lead as poorly extractable from tea samples.²⁸ The completely soluble scenario (100%) was included as a worst-case exposure estimate. The half soluble scenario (50%) was included as a middle exposure value. The estimated daily intake (EDI) of lead was calculated using the LOD, and the average daily amount of tea consumed by an individual in Bangladesh, as illustrated in **Equation 1**.

$$EDI = FIR * C * E$$

Equation 1.

In this equation, FIR was the food ingestion rate (kg/person/day), C was the arithmetic average metal concentration

²⁷ Palmer P, Jacobs R, Baker P, Ferguson K, Webber S. Use of Field-Portable XRF Analyzers for Rapid Screening of Toxic Elements in FDA-Regulated Products. *Journal of Agricultural and Food Chemistry*. 2009;7(7): 2605-2613.

²⁸ Dalipi R, Borgese L, Tsuji K, Bontempi E, Depero LE. Elemental analysis of teas, herbs and their infusions by means of total reflection X-ray fluorescence. *J Food Compos Anal*. 2018;67:128-134.

in the sample (mg/kg), and E was the extractability of the metal when the tea was prepared by steeping. The estimated FIR was calculated by using an assumed consumption of 61.9 thousand tonnes²⁹ and a total population of 152.8 million people,³⁰ for a total consumption of 0.4051 kg of tea per person per year. When calculating tea consumption on a per-day basis, this was equivalent to 1.1 g per person per day. Body weight is an additional parameter needed to estimate metal exposure. Using values from Waid et al. (2017) dataset, the average weight of 58.4 kg and 48.9 kg was used to estimate adult male and female weight, respectively.³¹

Standards for Comparison

The government of Bangladesh currently does not have national limits for metal contaminants in food. Shaheen *et al.* (2016) used the maximum allowable concentration (MAC) set by FAO/WHO³² in their study of heavy metals in fruits and vegetables in Bangladesh. However, the FAO/WHO did not set specified values for tea leaves.³³ The WHO's evaluation of food additives and contaminants, which was conducted specifically for lead, estimated that a chronic dietary exposure of 1.3 µg of Pb/kg bodyweight/day corresponded to a 1 mmHg increase in systolic blood pressure.³⁴ As a result, the daily intake values for lead were compared to these blood pressure endpoints for adults.

Statistical Analysis

Summary statistics for each metal were calculated by tea type (boxed tea and loose-leaf tea). Weighted means and weighted standard errors of the mean were first calculated by tea type and then by sampling location following Bevington and Robinson (2003).³⁵ After computing weighted standard errors of the mean,

²⁹ Chang K. *World tea production and trade: current and future development. Table 3. World tea consumption.*: FAO Intergovernmental Group on Tea;2015.

³⁰ Population Estimates and Projections. In: Data TWB, Catalog, eds2019.

³¹ Waid JL, Bogard JR, Thilste SH, Gabrysha S. Estimates of average energy requirements in Bangladesh: Adult Male Equivalent values for use in analyzing household consumption and expenditure surveys. *Data in Brief*. 2017;14:101-106.

³² Codex Alimentarius. In: Food and Agriculture Organization/World Health Organization; Amended 2015.

³³ Shaheen N, Irfan NM, Khan IN, Islam S, Islam MS, Ahmed MK. Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh. *Chemosphere*. 2016;152:431-438.

³⁴ Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2011. <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511#>.

³⁵ Bevington PR, Robinson DK. *Data Reduction and Error Analysis for the Physical Sciences*. 3 ed. New York, NY: McGraw-Hill Higher Education.

the weighted standard deviation for samples collected from each location was calculated by multiplying the weighted standard error of the mean by the square root of the location sample size [Sirajdikhan (n=6), Pabna (n=4)].

These calculations were applied to metal concentrations based on combinations of reported and below limit of detection values. Of the 33 metals, 10 metals had values above LOD for all 20 subsamples. These 10 metals had weighted means and weighted standard errors calculated as described above. Twenty metals had values below LOD for all 20 subsamples. These 20 metals had an average reported as “<LOD” and the weighted standard deviation was calculated as described above. Three metals (tungsten, manganese, and chromium) had a mixture of reported and below LOD values across the 20 subsamples. For these three metals, the <LOD values were imputed as twice the subsample-specific reported standard deviation, which is equal to the LOD for that subsample. Two imputations occurred for manganese, 19 for chromium, and seven for tungsten.

If the instrument measured value for lead was below the limit of detection, the calculated detection limit of 2.17 mg of lead per kg of tea was utilized to estimate an upper limit for potential lead exposure as part of a theoretical risk assessment. When a measurement is below the limit of detection, it cannot be assumed that the concentration of the contaminant is zero, therefore using the limit of detection is one option available for estimating exposure.³⁶

Weighted mean and standard error calculations were conducted in Microsoft Excel (v. 2016). Two mean t-tests were conducted using Stata software (v. 16, Stata Corp., College Station, Texas) to determine if any metal concentrations were significantly different based on collection site. These tests were only conducted when the group mean for each sample location was calculated and not listed as LOD. Stata code used to calculate the p-values for t-tests is included in **Supplemental Table 1**. For interested readers, the dataset used in this study is provided as a supplemental data file. No ethical approval was required because research did not involve human subjects.

RESULTS

The metal concentrations across the two sites are listed in **Tables 1 and 2**. Metals in **Table 1** are part of the EPA’s Priority Pollutant List while metals in **Table 2** are not EPA Priority

³⁶ Pollutants. NRCUCoRAoHA. *Science and Judgment in Risk Assessment*. Washington (DC): National Academies Press (US); 1994.

Pollutants.³⁷ The tea samples were primarily composed of calcium, potassium, sulfur, manganese, and iron. Approximately 10-11% of the tea samples by weight were calcium and were 1.2-1.4% potassium. These metals are all essential plant nutrients. At both sampling locations, many of the Priority Pollutants were reported below their respective limits of detection (LODs), including antimony, arsenic, cadmium, lead, mercury, nickel, selenium, and silver.

Table 1. Mean, standard error, and limits of detection (LOD) for metals in tea samples.^a

Element	Boxed tea, Sirajdikhan (n=6)		Loose-leaf tea, Pabna (n=4)		LOD ^b	P- value ^d
	Mean ± SE	Max	Mean ± SE	Max		
-----Concentration (mg/kg)-----						
EPA Priority Pollutants						
Antimony	LOD ± 0.95	LOD	LOD ± 1.4	LOD	4.54	-
Arsenic	LOD ± 0.13	LOD	LOD ± 0.19	LOD	1.68	-
Cadmium	LOD ± 0.74	LOD	LOD ± 1.1	LOD	4.01	-
Chromium ^c	8.8 ± 3.9	9.2	9.6 ± 3.7	10.7	8.55	0.878
Copper	23 ± 1.7	34	35 ± 2.7	57	6.20	0.013
Lead	LOD ± 0.22	LOD	LOD ± 0.32	LOD	2.17	-
Mercury	LOD ± 0.93	LOD	LOD ± 1.42	LOD	4.55	-
Nickel	LOD ± 4.0	LOD	LOD ± 6.25	LOD	9.42	-
Selenium	LOD ± 0.13	LOD	LOD ± 0.18	LOD	1.65	-
Silver	LOD ± 0.29	LOD	LOD ± 0.41	LOD	2.49	-
Zinc	34 ± 0.79	97	45 ± 1.19	56	4.22	0.012

³⁷ Priority Pollutant List. In: US Environmental Protection Agency; 2014.

Table 2. Mean, standard error, and limits of detection (LOD) for metals that are not EPA Priority Pollutants

Element	Boxed tea, Sirajdikhan (n=6)		Loose-leaf tea, Pabna (n=4)		LOD ^b	P- value ^d
	Mean ± SE	Max	Mean ± SE	Max		
-----Concentration (mg/kg)-----						
Other Metals above LOD						
Calcium	111,657 ± 5,639	120,268	109,820 ± 6,452	112,632	328	0.84
Iron	268 ± 17	456	410 ± 32	642	20.82	0.012
Manganese ^c	470 ± 40	1,216	866 ± 72	1,174	31.91	0.005
Molybdenum	10.8 ± 0.08	11.8	11.3 ± 0.12	12.2	1.33	0.017
Potassium	12,479 ± 1,634	14,432	13,808 ± 2,030	14,597	180	0.627
Rubidium	62.8 ± 0.14	72.1	81.7 ± 0.25	100.6	1.83	<0.0001
Strontium	20.9 ± 0.05	54.2	27.4 ± 0.08	45.7	1.07	<0.0001
Sulfur	819 ± 1,606	1008	941 ± 1,878	960	176	0.962
Titanium	207 ± 24	244	189 ± 28	198	81.50	0.644
Tungsten ^c	25.9 ± 9.2	41.2	34.9 ± 13.5	69.9	14.53	0.601
Zirconium	8.7 ± 0.05	10.0	9.1 ± 0.08	10.0	1.06	0.009
Other metals below LOD						
Barium	LOD ± 10.5	LOD	LOD ± 15.6	LOD	15.16	-
Cesium	LOD ± 0.58	LOD	LOD ± 0.87	LOD	3.57	-
Cobalt	LOD ± 9.0	LOD	LOD ± 14.8	LOD	14.36	-
Gold	LOD ± 0.34	LOD	LOD ± 0.48	LOD	2.68	-
Palladium	LOD ± 0.41	LOD	LOD ± 0.61	LOD	2.99	-
Scandium	LOD ± 199	LOD	LOD ± 277	LOD	61.70	-
Tellurium	LOD ± 4.2	LOD	LOD ± 6.2	LOD	9.59	-
Thorium	LOD ± 0.15	LOD	LOD ± 0.21	LOD	1.75	-
Tin	LOD ± 0.38	LOD	LOD ± 0.54	LOD	2.85	-
Uranium	LOD ± 0.56	LOD	LOD ± 0.93	LOD	3.56	-
Vanadium	LOD ± 4.0	LOD	LOD ± 4.7	LOD	8.81	-

^aSource: EPA Priority Pollutant list [40 CFR Part 423, Appendix A].

^bCalculated as the average error reported by the XRF over all 20 reads (10 samples *2 replicates per sample).

^cSome values were imputed as the subsample specific LOD as discussed in the methods section.

^dCalculated using mean and standard deviation assuming unequal variances in Stata v. 16. See Supplemental Table 1 for the code used.

Both copper and zinc were higher in the loose-leaf teas collected from Pabna than the boxed teas from Sirajdikhan. The loose-leaf tea had 11.9 ± 8.1 mg/kg (95% confidence interval) more copper than the boxed teas ($p=0.013$). Similarly, the loose-leaf tea had 5.3 ± 3.6 mg/kg more zinc than the boxed teas ($p=0.012$). When expressed as a fold difference by dividing the mean concentrations from the loose-leaf teas by the concentration from the boxed teas, the loose-leaf tea had approximately 1.5-fold more copper and 1.3-fold more zinc.

For the non-priority pollutant metals, a similar trend was observed, with higher metal concentrations in loose-leaf teas compared to boxed teas, shown in **Table 2**. Iron was 142 ± 94 mg/kg greater in the loose-leaf tea (1.5-fold greater, $p=0.012$). Manganese was 397 ± 215 mg/kg greater in the loose-leaf tea (1.8-fold greater, $p=0.005$). Molybdenum was 0.48 ± 0.36 mg/kg greater in the loose-leaf tea (1.04-fold greater, $p=0.017$). Rubidium was 18.9 ± 0.7 mg/kg greater in the loose-leaf tea (1.3-fold greater, $p<0.0001$). Strontium was 6.6 ± 0.2 mg/kg greater in the loose-leaf tea (1.3-fold greater, $p<0.0001$). Finally, zirconium was 0.36 ± 0.22 mg/kg greater in the loose-leaf tea (1.05-fold greater, $p=0.009$).

Manganese, chromium, and tungsten presented a mixture of below LOD and reported metal concentrations. Only two subsamples (from the same site) of tea had manganese reported as below LOD while the remaining samples ranged from 550 to 1330 mg/kg. For chromium, all samples were reported below LOD except for a single sample reporting 11.6 mg/kg.

The EDI for adult males ranged from 0.008 to 0.041 $\mu\text{g}/\text{kg}$ bw/day. For adult females, the EDI ranged from 0.010 to 0.049 $\mu\text{g}/\text{kg}$ bw/day. According to the WHO's evaluation of food additives and contaminants, a chronic dietary exposure of 1.3 μg of lead/kg bw/day corresponded to an estimated increase in systolic blood pressure of 1 mmHg.³⁸ When considered as a single exposure, lead from tea would not reach the 1.3 $\mu\text{g}/\text{kg}$ bw/day threshold if a handheld XRF analyzer indicated the sample was below the limit of detection, and the sample was assumed to have a lead concentration at the limit of detection, as illustrated by **Figure 2**.

³⁸ Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2011. <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511#>

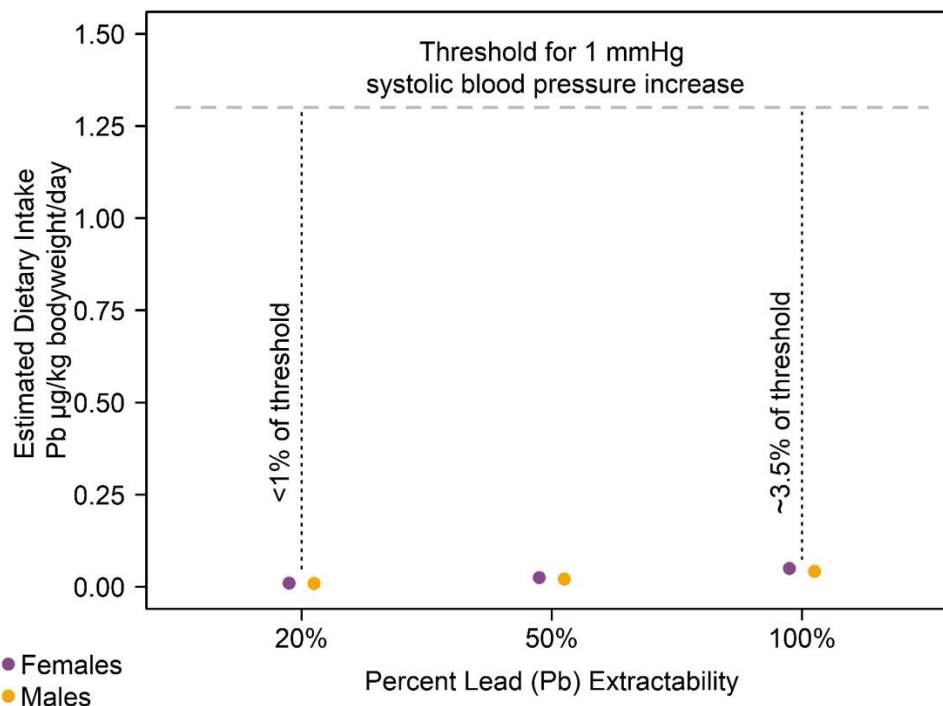


Figure 2. Estimated dietary intake of Pb μg per kg of body weight per day for tea samples containing the limit of detection value 2.17 mg Pb per kg of tea at three extractability levels for females (purple circles) and males (orange circles). Horizontal line represents the 1.3 $\mu\text{g}/\text{kg}$ bw/day estimate for 1 mmHg increase in systolic blood pressure.

Discussion

The results from the samples tested in the current study suggest that tea is not likely a primary source of lead exposure in Sirajdikhan and Pabna. The concentrations found in this study, all below a LOD of 2.17 mg/kg, are similar to the reported lead concentrations of 0.44³⁹ and 0.34⁴⁰ mg/kg found in tea leaves in previous studies. Many metal concentrations were found to be below the limit of detection, particularly for the EPA Priority Pollutants. While it cannot be assumed that there are no health effects at levels below the LOD for a handheld XRF, this study found that the tea samples do not appear to be hot spots of Priority Pollutant metal concentration in the sampling locations. The use of

³⁹ Rashid MH, Fardous Z, Chowdhury MAZ, et al. Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: an evaluation of six digestion methods. *Chem Cent J*. 2016;10:13.

⁴⁰ Marcos A, Fisher A, Rea G, Hill SJ. Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *J Anal At Spectrom*. 1998;13(6):521-525.

the handheld XRF as a screening tool for hot spots of metal contamination is consistent with that of McComb et al. (2014).⁴¹

The trend across all t-tests for metals was that the loose-leaf tea had higher metal concentrations than the boxed tea, including for copper, zinc, and manganese. These differences could reflect greater opportunities for metals to get into the tea due to increased handling during processing, air deposition, or the containers in which the bulk loose-leaf samples were kept before sale. Alternatively, the higher metal levels in the loose-leaf tea samples could have been due to differences in plant species. The difference between loose leaf and bagged tea samples provides an intriguing research question for future study. The variability across tested brands could make estimating metal contamination hazards more difficult due to greater inter-sample (inter-brand) variation. Some metals, such as manganese, had values reported as above and below the limit of detection across boxed and loose-leaf tea. Additional teas could be sampled to test if this variation occurs across a wider number of teas.

One of the limitations of this study is the small sample size and limited geographic area. Due to the fact that a total of 10 tea brands were sampled from two different locations in Bangladesh, the results of this study may not be representative of the rest of the country. Further research should conduct a broader analysis and sample tea from numerous other areas in Bangladesh, including both urban and rural areas. Another limitation of this study involves the estimation of metal intake. Solubility testing and additional surveys to estimate tea consumption in adults can be conducted to more accurately predict metal intake.

Conclusions

A handheld XRF analyzer was used to screen for metal contamination in commonly-consumed brands of tea from Bangladesh. There were no hot spots of lead contamination found in the tested samples of tea leaves. Across all of the 33 metals measured by the XRF, several metals tended to have higher concentrations in the loose-leaf samples compared to the boxed samples, including copper (1.5-fold higher), zinc (1.3-fold higher), and manganese (1.8-fold higher). Future research of trace metal contamination of tea leaves should include solubility testing and additional surveys to estimate tea consumption across age groups to more accurately predict metal intake values. Tea samples could

⁴¹ McComb JQ, Rogers C, Han FX, Tchounwou PB. Rapid Screening of Heavy Metals and Trace Elements in Environmental Samples Using Portable X-Ray Fluorescence Spectrometer, A Comparative Study. *Water Air and Soil Pollution*. 2014;225(12).

be collected at each phase of the processing, distribution, and sale of tea to determine if metal levels were changing from their original conditions. Other studies can also evaluate potential exposures due to other metals in tea, contextualize this with potential metal exposure from other foods, and validate these estimates by collecting extracted tea samples from individual homes. Given the number of samples below the instrument LOD, further measurements for these low levels of metal contamination and potential exposure will require detailed analytical techniques, such as more powerful XRF units or digestion techniques. Nevertheless, the handheld XRF can be used as a tool to locate hot spots of metal contamination in tea leaves.

Disclosure Statement

The authors have no conflicts of interest to declare.

Works Cited

- "Total Diet Study Design." 2018, <https://www.fda.gov/food/total-diet-study/total-diet-study-design>.
- "Lead in Food, Foodwares, and Dietary Supplements." 2020, <https://www.fda.gov/food/metals-and-your-food/lead-food-foodwares-and-dietary-supplements>.
- Akele, M. L., D. Z. Abebe, A. K. Alemu, A. G. Assefa, A. Madhusudhan, and R. R. de Oliveira. "Analysis of Trace Metal Concentrations in Raw Cow's Milk from Three Dairy Farms in North Gondar, Ethiopia: Chemometric Approach." *Environmental Monitoring and Assessment* 189, no. 10 (Oct 2017): 499. <https://doi.org/10.1007/s10661-017-6203-0>. <Go to ISI>://WOS:000411166100017.
- Bevington, Philip R., and D. Keith Robinson. *Data Reduction and Error Analysis for the Physical Sciences*. 3 ed. New York, NY: McGraw-Hill Higher Education.
- Bhowmik, Avit Kumar, Ambreen Alamdar, Ioannis Katsoyiannis, Heqing Shen, Nadeem Ali, Syeda Maria Ali, Habib Bokhari, Ralf B. Schaefer, and Syed Ali Musstjab Akber Shah Eqani. "Mapping Human Health Risks from Exposure to Trace Metal Contamination of Drinking Water Sources in Pakistan." *Science of the Total Environment* 538 (Dec 15 2015): 306-16. <https://doi.org/10.1016/j.scitotenv.2015.08.069>. <Go to ISI>://WOS:000363348900031.
- Brzezicha-Cirocka, J., M. Grembecka, and P. Szefer. "Monitoring of Essential and Heavy Metals in Green Tea from Different Geographical Origins." [In English]. Article. *Environmental Monitoring and Assessment* 188, no. 3 (Mar 2016): 11 183. <https://doi.org/10.1007/s10661-016-5157-y>. <Go to ISI>://WOS:000371067600035.
- Chang, Kaison. *World Tea Production and Trade: Current and Future Development. Table 3. World Tea Consumption*. FAO Intergovernmental Group on Tea (2015). <http://www.fao.org/3/a-i4480e.pdf>.

"Codex Alimentarius." Food and Agriculture Organization/World Health Organization, Amended 2015.

<http://www.fao.org/fao-who-codexalimentarius/en/>.

Dalipi, R., L. Borgese, K. Tsuji, E. Bontempi, and L. E. Depero. "Elemental Analysis of Teas, Herbs and Their Infusions by Means of Total Reflection X-Ray Fluorescence." [In English]. Article. *Journal of Food Composition and Analysis* 67 (Apr 2018): 128-34.

<https://doi.org/10.1016/j.jfca.2018.01.010>. <Go to ISI>://WOS:000427213100016.

<https://www.sciencedirect.com/science/article/pii/S0889157518300103?via%3Dihub>.

Filippelli, G., S. Anenberg, M. Taylor, A. van Geen, and H. Khreis. "New Approaches to Identifying and Reducing the Global Burden of Disease from Pollution." *Geohealth* 4, no. 4 (Apr 2020): e2018GH000167.

<https://doi.org/10.1029/2018GH000167>.

<https://www.ncbi.nlm.nih.gov/pubmed/32226911>.

Forsyth, Jenna E., Karrie L. Weaver, Kate Maher, M. Saiful Islam, Rubhana Raqib, Mahbubur Rahman, Scott Fendorf, and Stephen P. Luby. "Sources of Blood Lead Exposure in Rural Bangladesh." *Environmental Science & Technology* 53, no. 19 (2019/10/01 2019): 11429-36.

<https://doi.org/10.1021/acs.est.9b00744>.

<https://doi.org/10.1021/acs.est.9b00744>.

Gleason, Kelsey, James P. Shine, Nadia Shobnam, Lisa B. Rokoff, Hafiza Sultana Suchanda, Md Omar Sharif Ibne Hasan, Golam Mostofa, *et al.* "Contaminated Turmeric Is a Potential Source of Lead Exposure for Children in Rural Bangladesh." [In English]. ; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't. *Journal of environmental and public health* 2014 (2014 (Epub 2014 Aug 2014): 730636. <https://doi.org/10.1155/2014/730636>.

Ha, Nguyen Ngoc, Tetsuro Agusa, Karri Ramu, Nguyen Phuc Cam Tu, Satoko Murata, Keshav A. Bulbule, Peethmbaram Parthasarathy, *et al.* "Contamination by Trace Elements at E-Waste Recycling Sites in Bangalore, India." *Chemosphere* 76, no. 1 (Jun 2009): 9-15.

<https://doi.org/10.1016/j.chemosphere.2009.02.056>. <Go to ISI>://WOS:000266897200002.

- Han, W. Y., F. J. Zhao, Y. Z. Shi, L. F. Ma, and J. Y. Ruan. "Scale and Causes of Lead Contamination in Chinese Tea." [In English]. Article. *Environmental Pollution* 139, no. 1 (Jan 2006): 125-32.
<https://doi.org/10.1016/j.envpol.2005.04.025>. <Go to ISI>://WOS:000234069100014.
- Hore, Paromita, Kolapo Alex-Oni, Slavenka Sedlar, and Deborah Nagin. "A Spoonful of Lead: A 10-Year Look at Spices as a Potential Source of Lead Exposure." *Journal of Public Health Management and Practice* 25 (2019): S63-S70.
<https://doi.org/10.1097/phh.0000000000000876>.
https://journals.lww.com/jphmp/Fulltext/2019/01001/A_Spoonful_of_Lead_A_10_Year_Look_at_Spices_as_a.11.aspx.
- Islam, G. M. Rabiul, Faysal Elahi Khan, Md Mozammel Hoque, and Yeasmin Nahar Jolly. "Consumption of Unsafe Food in the Adjacent Area of Hazaribag Tannery Campus and Buriganga River Embankments of Bangladesh: Heavy Metal Contamination." *Environmental Monitoring and Assessment* 186, no. 11 (Nov 2014): 7233-44.
<https://doi.org/10.1007/s10661-014-3923-2>. <Go to ISI>://WOS:000343051400019.
- Marcos, A., A. Fisher, G. Rea, and S. J. Hill. "Preliminary Study Using Trace Element Concentrations and a Chemometrics Approach to Determine the Geographical Origin of Tea." [In English]. Article. *Journal of Analytical Atomic Spectrometry* 13, no. 6 (Jun 1998): 521-25.
<https://doi.org/10.1039/a708658j>. <Go to ISI>://WOS:000074276200004.
- McComb, Jacqueline Q., Christian Rogers, Fengxiang X. Han, and Paul B. Tchounwou. "Rapid Screening of Heavy Metals and Trace Elements in Environmental Samples Using Portable X-Ray Fluorescence Spectrometer, a Comparative Study." *Water Air and Soil Pollution* 225, no. 12 (Dec 2014): 2169. <https://doi.org/10.1007/s11270-014-2169-5>. <Go to ISI>://WOS:000345320200003.
- Mi-Sun Lee, Ki-Do Eum, Mostofa Golam, Quazi Quamruzzaman, Molly L. Kile, Maitreyi Mazumdar, David C. Christiani. "Umbilical Cord Blood Metals and Birth Size in Bangladesh Children ". *Submitted manuscript* (2019).

- Moreda-Pineiro, A., A. Fisher, and S. J. Hill. "The Classification of Tea According to Region of Origin Using Pattern Recognition Techniques and Trace Metal Data." [In English]. Article. *Journal of Food Composition and Analysis* 16, no. 2 (Apr 2003): 195-211. [https://doi.org/10.1016/s0889-1575\(02\)00163-1](https://doi.org/10.1016/s0889-1575(02)00163-1). <Go to ISI>://WOS:000182673500010.
- Organization, World Health. "Evaluations of the Joint Fao/Who Expert Committee on Food Additives (Jecfa)." 2011. <https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511#>.
- Palmer, Peter, Richard Jacobs, Peter Baker, Kelly Ferguson, and Siri Webber. "Use of Field-Portable Xrf Analyzers for Rapid Screening of Toxic Elements in Fda-Regulated Products." *Journal of Agricultural and Food Chemistry* 7, no. 7 (2009): 2605-13. <https://doi.org/10.1021/jf803285h>.
- Pollutants., National Research Council (US) Committee on Risk Assessment of Hazardous Air. *Science and Judgment in Risk Assessment*. Washington (DC): National Academies Press (US), 1994. doi:10.17226/2125. <https://www.ncbi.nlm.nih.gov/books/NBK208248/>.
- "Population Estimates and Projections." edited by The World Bank Data and Catalog, 2019. <https://datacatalog.worldbank.org/dataset/population-estimates-and-projections>.
- "Priority Pollutant List." US Environmental Protection Agency, 2014. <https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf>.
- Rashid, M. H., Z. Fardous, M. A. Z. Chowdhury, M. K. Alam, M. L. Bari, M. Moniruzzaman, and S. H. Gan. "Determination of Heavy Metals in the Soils of Tea Plantations and in Fresh and Processed Tea Leaves: An Evaluation of Six Digestion Methods." [In English]. Article. *Chemistry Central Journal* 10 (Feb 2016): 13 7. <https://doi.org/10.1186/s13065-016-0154-3>. <Go to ISI>://WOS:000370316700001.
- "XRF Technology." <https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental->

isotope-analysis-learning-center/elemental-analysis-information/xrf-technology.html.

Shaheen, Nazma, Nafis Md Irfan, Ishrat Nourin Khan, Saiful Islam, Md Saiful Islam, and Md Kawser Ahmed. "Presence of Heavy Metals in Fruits and Vegetables: Health Risk Implications in Bangladesh." *Chemosphere* 152 (Jun 2016): 431-38.
<https://doi.org/10.1016/j.chemosphere.2016.02.060>. <Go to ISI>://WOS:000374616600053.

Waid, Jillian L., Jessica R. Bogard, Shakuntala H. Thilste, and Sabine Gabryscha. "Estimates of Average Energy Requirements in Bangladesh: Adult Male Equivalent Values for Use in Analyzing Household Consumption and Expenditure Surveys." *Data in Brief* 14 (2017): 101-06.
<https://doi.org/10.1016/j.dib.2017.07.022>.

Zhang, J., R. D. Yang, R. Chen, Y. S. Peng, X. F. Wen, and L. Gao. "Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China." [In English]. Article. *International Journal of Environmental Research and Public Health* 15, no. 1 (Jan 2018): 22-33.
<https://doi.org/10.3390/ijerph15010133>. <Go to ISI>://WOS:000424121200132.