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

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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 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. In southeast Asia, metal contamination has been reported in several foods, including vegetables, fish, milk, and tea leaves. 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.

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

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are one potential source of lead exposure in Bangladesh\textsuperscript{8,9} and elsewhere.\textsuperscript{10} Tea is another potential source of exposure to lead and other metals, such as manganese, cadmium, and chromium.\textsuperscript{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.\textsuperscript{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),\textsuperscript{15,16,17} or flame and graphite furnace atomic absorption spectrometry (GF-AAS).\textsuperscript{18} Two previous studies of tea samples
from Bangladesh reported lead concentrations of 0.44\textsuperscript{19} and 0.34\textsuperscript{20} mg/kg. Of the two previous studies of tea leaves from Bangladesh, one study used GF-AAS\textsuperscript{21} and the other used ICP-MS.\textsuperscript{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.\textsuperscript{23} The technique measures fluorescent X-rays to identify each element present in the sample from the element’s unique emission energies.\textsuperscript{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.\textsuperscript{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.

\textsuperscript{19} Ibid.


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.  

![Figure 1](https://www.fda.gov/food/total-diet-study/total-diet-study-design). Published 2018.

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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).  

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. 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.

\[
\text{EDI} = \text{FIR} \times \text{C} \times \text{E}
\]

**Equation 1.**

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

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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 tonnes\textsuperscript{29} and a total population of 152.8 million people\textsuperscript{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.\textsuperscript{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/WHO\textsuperscript{32} in their study of heavy metals in fruits and vegetables in Bangladesh. However, the FAO/WHO did not set specified values for tea leaves.\textsuperscript{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.\textsuperscript{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).\textsuperscript{35} After computing weighted standard errors of the mean,

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


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


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

Pollutants. 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.\(^a\)

<table>
<thead>
<tr>
<th>Element</th>
<th>Boxed tea, Sirajdikhan (n=6)</th>
<th>Loose-leaf tea, Pabna (n=4)</th>
<th>LOD(^b)</th>
<th>P-value(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Max</td>
<td>Mean ± SE</td>
<td>Max</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------</td>
<td>-----------------------------</td>
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<tr>
<td>---------------</td>
<td>-------------------------------</td>
<td>-----------------------------</td>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td><strong>EPA Priority Pollutants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antimony</td>
<td>LOD ± 0.95</td>
<td>LOD</td>
<td>LOD ± 1.4</td>
<td>LOD</td>
</tr>
<tr>
<td>Arsenic</td>
<td>LOD ± 0.13</td>
<td>LOD</td>
<td>LOD ± 0.19</td>
<td>LOD</td>
</tr>
<tr>
<td>Cadmium</td>
<td>LOD ± 0.74</td>
<td>LOD</td>
<td>LOD ± 1.1</td>
<td>LOD</td>
</tr>
<tr>
<td>Chromium(^c)</td>
<td>8.8 ± 3.9</td>
<td>9.2</td>
<td>9.6 ± 3.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Copper</td>
<td>23 ± 1.7</td>
<td>34</td>
<td>35 ± 2.7</td>
<td>57</td>
</tr>
<tr>
<td>Lead</td>
<td>LOD ± 0.22</td>
<td>LOD</td>
<td>LOD ± 0.32</td>
<td>LOD</td>
</tr>
<tr>
<td>Mercury</td>
<td>LOD ± 0.93</td>
<td>LOD</td>
<td>LOD ± 1.42</td>
<td>LOD</td>
</tr>
<tr>
<td>Nickel</td>
<td>LOD ± 4.0</td>
<td>LOD</td>
<td>LOD ± 6.25</td>
<td>LOD</td>
</tr>
<tr>
<td>Selenium</td>
<td>LOD ± 0.13</td>
<td>LOD</td>
<td>LOD ± 0.18</td>
<td>LOD</td>
</tr>
<tr>
<td>Silver</td>
<td>LOD ± 0.29</td>
<td>LOD</td>
<td>LOD ± 0.41</td>
<td>LOD</td>
</tr>
<tr>
<td>Zinc</td>
<td>34 ± 0.79</td>
<td>97</td>
<td>45 ± 1.19</td>
<td>56</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Element</th>
<th>Boxed tea, Sirajdikhan (n=6)</th>
<th>Loose-leaf tea, Pabna (n=4)</th>
<th>LOD(^b)</th>
<th>P-value(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Max</td>
<td>Mean ± SE</td>
<td>Max</td>
</tr>
<tr>
<td>Calcium</td>
<td>111,657 ± 5,639</td>
<td>120,268</td>
<td>109,820 ± 6,452</td>
<td>112,632</td>
</tr>
<tr>
<td>Iron</td>
<td>268 ± 17</td>
<td>456</td>
<td>410 ± 32</td>
<td>642</td>
</tr>
<tr>
<td>Manganese(^c)</td>
<td>470 ± 40</td>
<td>1,216</td>
<td>866 ± 72</td>
<td>1,174</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>10.8 ± 0.08</td>
<td>11.8</td>
<td>11.3 ± 0.12</td>
<td>12.2</td>
</tr>
<tr>
<td>Potassium</td>
<td>12,479 ± 1,634</td>
<td>14,432</td>
<td>13,808 ± 2,030</td>
<td>14,597</td>
</tr>
<tr>
<td>Rubidium</td>
<td>62.8 ± 0.14</td>
<td>72.1</td>
<td>81.7 ± 0.25</td>
<td>100.6</td>
</tr>
<tr>
<td>Strontium</td>
<td>20.9 ± 0.05</td>
<td>54.2</td>
<td>27.4 ± 0.08</td>
<td>45.7</td>
</tr>
<tr>
<td>Sulfur</td>
<td>819 ± 1,606</td>
<td>1008</td>
<td>941 ± 1,878</td>
<td>960</td>
</tr>
<tr>
<td>Titanium</td>
<td>207 ± 24</td>
<td>244</td>
<td>189 ± 28</td>
<td>198</td>
</tr>
<tr>
<td>Tungsten(^c)</td>
<td>25.9 ± 9.2</td>
<td>41.2</td>
<td>34.9 ± 13.5</td>
<td>69.9</td>
</tr>
<tr>
<td>Zirconium</td>
<td>8.7 ± 0.05</td>
<td>10.0</td>
<td>9.1 ± 0.08</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Other Metals above LOD

Other metals below LOD

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\(^a\)Source: EPA Priority Pollutant list [40 CFR Part 423, Appendix A].

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

\(^c\)Some values were imputed as the subsample specific LOD as discussed in the methods section.

\(^d\)Calculated 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 $142 \pm 94$ mg/kg greater in the loose-leaf tea (1.5-fold greater, $p=0.012$). Manganese was $397 \pm 215$ mg/kg greater in the loose-leaf tea (1.8-fold greater, $p=0.005$). Molybdenum was $0.48 \pm 0.36$ mg/kg greater in the loose-leaf tea (1.04-fold greater, $p=0.017$). Rubidium was $18.9 \pm 0.7$ mg/kg greater in the loose-leaf tea (1.3-fold greater, $p<0.0001$). Strontium was $6.6 \pm 0.2$ mg/kg greater in the loose-leaf tea (1.3-fold greater, $p<0.0001$). Finally, zirconium was $0.36 \pm 0.22$ 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 µg/kg bw/day. For adult females, the EDI ranged from 0.010 to 0.049 µg/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. When considered as a single exposure, lead from tea would not reach the 1.3 µg/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.
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 µg/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 0.44\textsuperscript{39} and 0.34\textsuperscript{40} 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


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

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