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Calculating and Comparing Bioaccessible Lead Concentrations in Soils from the Garfield Ridge, West Elsdon, and Lakeview Community Areas Using a PBET and a Mehlich-3 Digestion Method

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ABSTRACT Lead exposure through soil poses potential health risks to those in areas with higher soil-Pb levels, specifically urban areas. The soil-Pb concentrations recommended by the EPA are based on measurement of total Pb concentration, yet only a percentage of the total Pb can actually be absorbed into the body. The percentage of Pb in soil that can be absorbed into the blood through ingestion is referred to as bioaccessible Pb and can be a more accurate way of measuring potential dangers of Pb in soils. This study measured the amount of bioaccessible Pb in the community areas of West Elsdon, Garfield Ridge, and Lakeview through comparing a modified physiologically-based extraction test (PBET) and Mehlich-3 digestion method. Both methods of digestion were compared based on potential accuracy, correlation, and procedural efficiency. GLM analysis showed no significant differences in the mean bioaccessible Pb extracted between Lakeview and GR/WE, nor between the PBET method in comparison to the Mehlich-3 method. The GLM analysis did show a significant difference when considering both site and method, where the mean bioaccessible Pb content extracted from the Lakeview site using the PBET method was significantly greater than the mean bioaccessible Pb content extracted from Garfield Ridge/West Elsdon using the Mehlich-3 method (P = 0.003)

INTRODUCTION
Pb occurs in soils naturally, but higher concentrations are often found in urban areas. Much of this is because Pb remains in soils from a variety of sources, including Pb-based paints, automobile exhaust, and certain industrial practices, decades after initial deposition, which creates a buildup of Pb over time (Attanayake et. al., 2014; Bradham et. al., 2017; Misenheimer et. al., 2018; Steele, 2022). Several studies have shown correlations to Pb-related illness and increased child blood Pb levels (BLLs), specifically in urban areas with higher soil Pb concentrations (Surkan et. al., 2007, Bradham et. al., 2014). In Chicago, Black and Hispanic/Latinx communities have been disproportionately impacted by Pb contaminated soils (Steele, 2022). To avoid Pb contamination through soils, the Environmental Protection Agency (EPA) recommends that soils in children’s play-areas maintain a total soil-Pb

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level below 400 ppm (below 1200 ppm for non-play areas).

While measuring total Pb content in soil can give an estimate as to how hazardous a soil is, measuring bioaccessible Pb can help improve the reliability of a soil sample’s hazard level regarding Pb toxicity (Reis et. al., 2014; Wu et. al., 2020). Bioaccessible Pb is reported as a percentage of the total Pb content that is soluble in low pH solutions which mimic the gastric digestion process, and therefore calculates the amount of Pb that could theoretically be absorbed by the body (Bradham et. al., 2017).

Because bioaccessible Pb measures the amount of Pb in soils that can be absorbed into the bloodstream via ingestion, measuring bioaccessible Pb can be used to highlight the danger of Pb in soils more accurately. One way of measuring bioaccessible Pb is through using a gastric digestion, where a soil sample is digested in a solution that mimics properties of stomach acid. These methods, including the physiologically-based extraction test (PBET), produce consistent results, but can be time consuming and produce an excess of Pb solution. Other methods have been used to measure bioaccessible Pb, including the Mehlich-3 extraction, which produces significantly less waste than the PBET, but may not be as accurate (Basta et. al., 2013).

In this study, by measuring and mapping bioaccessible Pb spatial patterns, a more accurate risk assessment of Chicago soils can be determined. Furthermore, through comparing the bioaccessible Pb extraction ability of both the PBET and Mehlich-3 extraction methods, the efficiency, effectiveness, and correlation between the two extraction methods can be determined. We intend to build on the soil profile described by Garcia (2022). Garcia initially hypothesized that the Garfield Ridge and West Elsdon community areas would have higher soil Pb concentrations than those measured in soil samples collected from the Lakeview community area given the demographic differences between the areas (Garfield Ridge and West Elsdon being predominantly Hispanic/Latinx, and Lakeview being predominantly White), but ultimately found that the opposite was the case.

Because many Black and Hispanic/Latinx communities are disproportionately impacted by Pb in soil, it is possible that some areas may have higher percentages of bioaccessible Pb. This would explain the disproportionate impacts of Pb-related health issues present in areas where total Pb may not be as high. Given this, we hypothesized that Lakeview soils would have concentrations in lower bioaccessible Pb percentages than Garfield Ridge and West Elsdon. Furthermore, given the results presented by Basta et. al. (2013) comparing bioaccessible Pb extracted using a Mehlich-3 extraction compared to a standard in-vitro extraction, we also hypothesized that the Mehlich-3 and PBET testing methods would be comparable in Pb extraction abilities.

METHODS

Soil Preparation

All of the soil used for this study had been previously collected. The samples collected from the Lakeview Community Area were collected by former DePaul student Ben Goedert in 2019. The soil samples from West Elsdon and Garfield Ridge were collected by a team of community scientists led by former DePaul student Jennifer Garcia (Garcia, 2022). The West Elsdon and Garfield Ridge Community Areas were combined as one site due to their geographic proximity and similar area in order to compare with the Lakeview community area. For both Lakeview and Garfield Ridge/West Elsdon (GR/WE) five random locations from each census tract were chosen for sample collection using Create Random Points tool in ArcMap (ArcGIS). All of the samples had been processed by being ground and filtered through a #10 sieve to collect any particles < 2.00 mm. The collected particulates were then processed using a ball mill and passed through a #325 sieve (270 µm). For each sample location, two bioaccessible Pb extractions were completed.

PBET

The PBET extraction solution was made as described by Attanayke et. al (2014) and adjusted to pH = 2.5 using trace metal Hydrochloric Acid (HCl). The solution was heated in a hot water bath to ~37ºC and 100 mL of the solution was added to 1.00 g of processed soil. The soil and solution were shaken at 100 rpm in a hot water bath heated to ~37ºC for 1 hour to digest the soil. Once digested, the samples were filtered through a 0.45µm disc filter via vacuum filtration.
Mehlich-3

A Mehlich-3 solution (Mehlich, 1984) was prepared and adjusted to pH = 2.5 using trace metal grade HCl. 10.0 mL of Mehlich-3 was added to 1.00 g of processed soil and shaken for 5 minutes at 100 rpm to digest. The samples were filtered through a 0.45 µm disc filter via vacuum filtration.

All filtered solutions were analyzed on a flame atomic absorption spectrometer (Varian Corporation, Chicago, IL) calibrated with a SpexCertiprep 1000 ppm (2% HNO3) stock solution diluted to a 10 ppm Pb standard solution. To measure Pb concentrations (mg/kg, or ppm) in the solution. Plots for Lakeview and GR/WE were made to compare PBET to Mehlich-3 extracted bioaccessible Pb content in ppm ($P_{ba}$ (ppm)). These concentrations were also used to calculate the amount of bioaccessible Pb extracted from each sample in ppm. Percent bioaccessible Pb ($\%P_{ba}$) for each sample was calculated using total Pb ($P_{T}$ (ppm)) measurements made by Goedert (Lakeview) and Garcia (West Elsdon-Garfield Ridge) (Equation 1).

$$\%P_{ba} = \frac{P_{ba}(ppm)}{P_{T}(ppm)} \times 100\% \quad (1)$$

Average bioaccessible Pb concentration was calculated for each census tract. Spatial patterns of bioaccessible Pb measured by both methods were mapped using ArcGIS. Quality analysis and quality control (QA/QC) was performed by measuring the bioaccessible Pb content in a Standard Reference Material (Montana IIa soil) using each method. Replicates samples were completed for both PBET and Mehlich-3 methods for 14 Lakeview samples and 13 GR/WE samples. The percent bioaccessible Pb extracted for the replicate samples were plotted to determine consistency in methodology. Samples that yielded bioaccessible Pb percent ($P_{ba,\%}$) > 100% were not used in any data analysis (Lakeview PBET Samples 3 and 16).

Statistical Analysis

The data were compared between sites, where one site was West Elsdon and Garfield Ridge (combined as a single site) and the other was Lakeview. The Pb data from each site were tested for normality in R (RStudio Team). None of the data sets were normally distributed and using the fitistrplus, logspline, and MASS data sets in R, we found the data for both extraction methods for both community areas was best fit to a gamma distribution. To compare bioaccessible Pb concentration (in ppm) and the percent bioaccessible Pb extracted by site and extraction method, we used generalized linear models (GLM) so that the comparison would not be influenced based on differences in sample size. Of the data points for the PBET distribution, two samples yielded bioaccessible Pb levels greater than 100% (Lakeview PBET samples 3 and 16), and therefore the data for the GLMs was analyzed without those data values present. The GLMs were also used to compare amount of bioaccessible Pb extracted (in both % and mg/kg) between sample locations for the PBET and Mehlich-3.

Mapping

The individual sample locations were mapped using ArcGIS. The average bioaccessible Pb content was taken for each census tract for the Lakeview and GR/WE community areas for both methods.

RESULTS

Analysis of the maps for each site showed differences in the bioaccessible Pb content extracted by each method. For the Lakeview community area, the maximum average bioaccessible Pb content found was in census tract 63301 (279.4 ppm) for the PBET method (Figure 1), and in census tract 61500 (90.4 ppm) for the Mehlich-3 method (Figure 2). Similarly, for the GR/WE community area, the maximum average bioaccessible Pb content was found in census tract 5610 (27.1 ppm) for the PBET and census tract 6204 (27.4 ppm) for Mehlich-3. In Lakeview, the minimum average bioaccessible Pb content was in census tract 5602 for both methods and was 7.7 ppm for the PBET and 3.5 ppm for the Mehlich-3 method (data not shown).
In Lakeview soils, bioaccessible Pb concentrations extracted using the PBET ranged from 1.00 ppm to 538.92 ppm with an average of 53 ppm (average bioaccessible Pb percent of 18%). The Mehlich-3 extraction of Lakeview soils yielded a range of bioaccessible Pb content of 0.70 ppm to 241.13 ppm with an average of 36 ppm (10.8%). In GR/WE soils, PBET bioaccessible Pb concentrations ranged from 6.97 ppm to 72.0 ppm, with an average of 19.47 ppm (13%). Mehlich-3 extracted soils from GR/WE ranged from 1.6 ppm to 126.17 ppm, with an average of 19.71 ppm (14%). The average, mean, maximum, and minimum bioaccessible Pb extracted by method from each site is shown in Table 1.

Results of the GLM showed no significant difference in the mean bioaccessible Pb content extracted between sites in both ppm (P = .1163) and percentage (P = .49). The results of GLM showed that there may be a significant difference between the mean bioaccessible Pb extracted by PBET compared to the Mehlich-3 extraction in both ppm (P = 0.0479) and percentage (P = 0.019). This, however, may be due to the much more significant difference between the mean...
bioaccessible Pb extracted when both site and method are considered. When considering both site and method, the GLM showed a significant difference between the amount of bioaccessible Pb extracted from Lakeview site using the PBET method in comparison to the GR/WE site using the Mehlich-3 method in both ppm ($P = 0.003$) and percentage ($P = 0.000239$). Results of the GLM analysis are shown in tables 2 and 3. The mean bioaccessible Pb extracted considering site and method is represented graphically for both bioaccessible Pb in ppm and percentage in Figures 5 and 6.

<table>
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<th>Lakeview</th>
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Table 1. Data summary for PBET and Mehlich-3 extractions performed on soils from Lakeview and soils from Garfield Ridge/West Elsdon.

| Estimate | Std. Error | $z$ | Pr($>|z|$) | Estimate | Std. Error | $z$ | Pr($>|z|$) |
|----------|------------|-----|-----------|----------|------------|-----|-----------|
| Intercept | 0.035813   | 0.004489 | 7.977 | 1.50E-15 | Intercept | 0.072383 | 0.006482 | 11.168 | <2e-16  |
| Site      | -0.00796   | 0.005065 | -1.57  | 0.1163   | Method    | 0.006564 | 0.009591 | 0.684  | 0.493743 |
| Method    | 0.015525   | 0.007847 | 1.979  | 0.0479   | Site      | 0.020122 | 0.008543 | 2.355  | 0.018501 |
| Site:Method | -0.02475 | 0.008341 | -2.97  | 0.003    | Site:Method | -0.04263 | 0.011604 | -3.674 | 0.000239 |

Table 2. GLM p-values for comparing bioaccessible soil-Pb concentration (ppm) between sites and extraction methods.

Table 3. GLM p-values for comparing bioaccessible soil-Pb percent between sites and extraction methods.

Figure 5. Mean bioaccessible (BA) Pb content extracted in ppm by each method from Lakeview and Garfield Ridge/West Elsdon soils with standard error.

Figure 6. Mean bioaccessible (BA) Pb percent extracted by each method from Lakeview and Garfield Ridge/West Elsdon soils with standard error.
To check for any correlation between methods at each site, a plot comparing the bioaccessible Pb concentrations (ppm) obtained via the PBET, and the Mehlich-3 methods was made for each site (Figures 7 and 8). A line of best fit was also calculated along with an R² value to determine correlation between the PBET and Mehlich-3 methods for each sample location.

To check for consistency within each method, a plot of the replicate samples was also made for Lakeview and GR/WE (Figure 9). These plots showed significant correlation between the PBET replicates (R² = 0.9743). For the Mehlich-3 extraction, there was less correlation between the amount of bioaccessible Pb extracted using the same method on the same site (R² = 0.7005) (data not shown).

**Figure 7.** Plot comparing the bioaccessible Pb content obtained using the PBET method and the Mehlich-3 digestion method for Lakeview soils. Line of best fit was determined to be \( \hat{y} = 0.3665x + 16.417 \) with an overall (R² = 0.4178).

**Figure 8.** Plot comparing the bioaccessible Pb content obtained using the PBET method and the Mehlich-3 digestion method for Garfield Ridge/West Elsdon soils. Line of best fit was determined to be \( \hat{y} = 1.608x - 3.3979 \) (R² = 0.5359).

**DISCUSSION**

When considering both site and method in the GLM, the negative estimate value showed that the mean bioaccessible Pb content extracted using the Mehlich-3 method in GR/WE was significantly lower than the mean bioaccessible Pb content extracted using the PBET method in Lakeview (P = .0003). This was also seen when comparing mean bioaccessible Pb percentages, the Mehlich-3 method in GR/WE was significantly less than that found using the PBET method in Lakeview (P = .000239). This would indicate that the method which extracts higher Pb content from soils is dependent on the soil’s origin.

From the GLMs comparing the two sites alone, there was no significant difference found between mean bioaccessible Pb content from Lakeview
and GR/WE when measured in both ppm (P = 0.1163) and in percentage (P = .4937). In comparing methods, the GLM shows that there might be a significant difference in the mean bioaccessible Pb extracted between methods, but this seems to be misleading. The GLM indicated that in comparing method alone, the PBET extraction method yields a statistically significantly higher mean bioaccessible Pb content (in ppm) comparison to the Mehlich-3 method (P = 0.047). However, because this value is so close to the 95% confidence interval threshold, and because Lakeview showed much higher mean bioaccessible Pb content compared to GR/WE only when being analyzed with the PBET method, it is likely that the Lakeview soils extracted using the PBET method skewed the overall mean comparison of the two sites. For the GLM comparing the mean bioaccessible Pb percentages, the PBET method showed a significantly higher mean bioaccessible Pb percentage compared to the Mehlich-3 method (P = .0185). Again, this is likely be skewed by the mean bioaccessible Pb percentage extracted from Lakeview soils using the PBET method. Because the PBET method only yielded a higher mean bioaccessible Pb content in the Lakeview area, it is not possible to determine any significant differences between the mean bioaccessible Pb yield for each method.

With this said, there were still differences seen between the two methods. Analysis comparing the two extraction methods using the maps generated for the Lakeview community area (Figures 1 and 2) showed that the census tracts containing the maximum and minimum average bioaccessible Pb content were in different census tracts based on extraction method. Similarly, in the maps generated for GR/WE, the maximum average bioaccessible Pb content is located in different census tracts depending on the extraction method, but the minimum average bioaccessible Pb content was in the same census tract (Figures 3 and 4). The differences in bioaccessible Pb distribution shown on maps comparing methods indicate that the two extraction methods do not provide similar or even proportional bioaccessible Pb extraction results.

Discrepancies between the extraction methods are further supported by the plot comparing the bioaccessible Pb content extracted between the PBET and Mehlich-3 methods (Figures 5 and 6). Low correlation was found at both sites comparing the two methods, which would indicate that the two methods do yield different bioaccessible Pb concentrations in ppm. Both plots show fairly low $R^2$ values (both less than 0.6), indicating that the overall correlation between the two methods for a given location is also fairly low. In comparison, research done by Basta. et. al. (2013) comparing an in-vitro bioaccessible Pb extraction to a Mehlich-3 extraction yielded an $R^2$ value of 0.938 at the same pH. The weaker correlation found in this experiment comparing the PBET and Mehlich-3 methods would further support the idea that the PBET and Mehlich-3 extraction methods were not comparable in bioaccessible Pb extraction ability.

While there is evidence to show that the PBET and Mehlich-3 extraction methods perform differently, we cannot be certain as to which method provides more accurate and precise results, and thus it is unclear as to which method is better for bioaccessible Pb analysis. There is currently no EPA bioaccessible Pb standard, so while both extraction methods were performed on the Montana II 2711a total Pb standard soil, there is no bioaccessible Pb value reported for this sample. One study by Hamel et. al. (1999) showed that the Montana I 2710 soil, a similar standard sample, has a bioaccessible Pb content of 62 (± 1)%, which is higher than the observed Pb extracted using the Mehlich-3 method (42.6%) and lower than the Pb extracted using the PBET (73.8%). A NIST publication by Mackey et. al. (2010) showed that “acid-extractable” Pb (noted as a generalized method of testing for bioaccessibility of metals in soils) for the Montana II 2711a standard soil was 93%, which is higher than both the PBET and Mehlich-3 yielded in this experiment for the same standard. These studies alone show a large range, which makes it difficult to determine what an accurate level of bioaccessible Pb is present in the standard sample tested. In either case, the Mehlich-3 extracted Pb from the standard sample was lower than the reported Pb extracted for both studies,
which could indicate that the PBET is more accurate.

Precision within method was tested by generating plots comparing replicate samples for each extraction method (Figure 7). This plot showed that there was a stronger correlation in replicate PBET samples ($R^2 = 0.9743$) compared to replicate Mehlich-3 samples ($R^2 = 0.7005$). This would indicate that the Mehlich-3 extraction of bioaccessible Pb is much more variable than the PBET extracted bioaccessible Pb, and thus the PBET samples would be more precise.

The greater variation in Mehlich-3 extraction of bioaccessible Pb could be indicative of the Mehlich-3 extraction being less effective for correctly predicting a soil’s bioaccessible Pb concentration in comparison to the PBET method. It is also possible that the equipment used was not the most capable of measuring the bioaccessible Pb extracted from the Mehlich-3 method, which could result in the lack of strong correlation between the two methods. In the Mehlich-3 extraction method described by Basta et al. (2013), the samples were analyzed using ICP-OES Method USEPA Method 6010C, which utilizes inductively coupled plasma-atomic emission spectrometry (EPA, 2000). In this experiment, all samples were analyzed using flame absorption spectrometry on a Varian FLAA. The FLAA was calibrated using a 10 ppm Pb standard solution, and thus was calibrated to measure Pb solution concentrations near or less than 10 ppm. This was, for the most part, the case for the PBET samples, which were far more dilute than the Mehlich-3 samples. Because the FLAA was not properly calibrated to read higher Pb concentrations, which were more frequently seen in the Mehlich-3 extraction due to the lower solution volume, it is possible that the Pb levels reported were not the most accurate. Considering this, it is possible that the calibrated range of the FLAA may not have been suitable for reading the Mehlich-3 extracted samples, resulting in inaccurate readings.

Furthermore, throughout using the FLAA for this experiment, there were several occasions where samples had to be run multiple times due to improper set up, as the readings would show negative values of Pb present in the sample. While this was caught when the samples read as containing negative Pb samples, it is possible that some samples were simply read as lower values than were actually present if they contained a high enough Pb concentration. This could have also been an issue for the students testing for total Pb prior to this experiment, which in turn would skew the results of the bioaccessible Pb percentages obtained.

Other differences between the PBET and Mehlich-3 method could be due to general errors in methodology. Due to time constraints, many of the PBET samples were digested in a hot water bath, and then were moved to refrigeration or freezing before filtration. This extra time of soil in solution could have allowed more Pb to be extracted from the soil compared to other samples that were filtered immediately. Similarly, many of the Mehlich-3 samples were digested and then left refrigerated before filtering, which again could alter the amount of Pb extracted.

When comparing the PBET and Mehlich-3 methods as practices, the PBET took more time and materials to produce results. For 1.00 g of processed soil, 100 mL of solution was needed for digestion. Since little solution is needed for analysis (< 5 mL), the excess Pb solution becomes a waste issue. Furthermore, each PBET sample had to be heated and shaken for 1 hour, making the entire process more time consuming. For the Mehlich-3 extraction, only 10 mL was needed for every 1.00 g of soil, which resulted in far less Pb waste solution. The Mehlich-3 extraction also only required 5 minutes of being shaken, which cut down the extraction time significantly.

Because both PBET and Mehlich-3 extraction methods do work to extract some amount of bioaccessible Pb, it is possible that the methods can extract similar amounts of bioaccessible Pb with proper technique and equipment. This could include re-processing and re-analyzing the Lakeview soils to remove any larger bits of soil debris that would usually be filtered out prior to pulverization. This could also include re-analyzing some of the samples on an FLAA calibrated to read higher concentrations of Pb in
solution, or by simply re-analyzing the samples on an FLAA that is set up fully properly. This could also include re-analyzing some of the samples on an FLAA calibrated to read higher concentrations of Pb in solution, or by simply re-analyzing the samples on an FLAA that is set up fully properly, which could allow more concentrated Pb extractant solutions (>10 ppm) to be analyzed more accurately than would be possible on a FLAA calibrated using a 10 ppm solution. Furthermore, preparing and utilizing one or several standard bioaccessible Pb standard solutions to be analyzed alongside the samples would be useful in determining a baseline for accuracy for each method. This would also help us determine how accurate the FLAA is when reading a high concentration Pb solution even when calibrated with a lower concentration Pb solution. Because there was not a known standard used for this experiment, we could not easily determine the accuracy of each method outside of their respective correlation. By adjusting both the extraction process and the analysis technique, as well as having a known bioaccessible Pb standard for comparison, it is possible that the Mehlich-3 extraction method could perform similarly to the PBET method.

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