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Determination of Metal Concentrations in Sediments of Big Walnut Creek

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Abstract

The freshwater mussel populations in the lower stretch of Big Walnut Creek in central Ohio have been found to vary along the run of the creek with the lowest population of mussels in the middle stretch. One possible cause for the decline is the presence of contaminants in the sediments of the creek. In this study, nine Sites along the creek were examined. At each Site, sediment samples from a pool region and two quadrants from where mussels were also studied (the area of interest) were collected, along with pseudo feces, which is the waste mussels produce as they filter water and sediment to find food. The concentrations of select metals in the sediments and pseudo feces were determined using acid extraction and analysis with microwave plasma-atomic emission spectroscopy. The concentrations of metals were found to vary along the creek, most notably in the cases of lead, zinc, and calcium, and manganese. However, only calcium and zinc were found to be significantly correlated to the mussel populations. The concentration of calcium was found to positively correlate to mussel populations ($r = 0.715$, $p = 0.03$), while zinc was found to negatively correlate to mussel populations ($r = -0.717$, $p = 0.03$). Additionally, the metal levels in the area of interest were compared to those collected in the year 2000, and the overall quality of the sediments improved over the past two decades with only four occasions where a sample exceeds the threshold effect concentration out of 45 total samples.

Acknowledgements

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Introduction

Freshwater mussels are traditionally used in the assessment of overall aquatic health, and freshwater mussel populations have been declining throughout the United States. It is now estimated that over 70% of the mussel species are extinct or endangered [1]. Freshwater mussels are particularly sensitive to their aqueous environments, and understanding the reason for the decline in these bioindicators is critical in developing remediation strategies where necessary. The ability of freshwater mussels to thrive depends on stable environmental conditions with high quality water and contaminant-free sediments. Contamination present in sediments can especially hinder the health of mussels because of the benthic dwelling characteristic of these organisms. Even simple changes in the ecosystem such as water temperature, turbidity, water velocity, and sedimentation levels can all pose a challenge for the survival of a freshwater mussel [2]. Additionally, mussels rely on a symbiotic relationship with fish.

This study examines hindrances, especially the impacts of metals, on mussel populations in the lower stretch of Big Walnut Creek, located in central Ohio; this stretch begins at the Hoover Reservoir. When Hoover Reservoir was built and officially opened in 1958 at N 40° 08’ 01.13”, W 82° 52’ 45.57” on the Big Walnut Creek, a change in the ecological stability occurred, negatively impacting aquatic populations [3]. Further, Big Walnut Creek faced many periods of historical degradation. Although several organisms have experienced rebounds in their populations, mussels have not. When Hoggarth and Grumney evaluated the lower stretch of Big Walnut Creek in 2013, they found that the upper third of the watershed had the most diversity of mussel populations (Sites 3, 8, and 9 in Figure 1), the middle third had the lowest diversity (Sites 11, 12, and 13 in Figure 1), and the lower third contained an intermediate diversity (Sites 16, 18, and 20) [4]. This suggests that some factor is negatively impacting mussels specifically in the middle stretch, and that their population is rebounding further downstream. Grumney and Hoggarth previously ruled out the symbiotic relationship with fish since areas in which fish populations thrive were not necessarily the places where mussel populations do [4]. Thus, one remaining possibility is that anthropogenic inputs introduce toxins in sediments, especially in the middle stretch, that have an adverse effect on mussels living in the benthic zone.

The anthropogenic inputs could come from land use along Big Walnut, or various tributaries that also feed into it. At river mile 27.0 (between Sites 3 and 8), a tributary leads into Big Walnut Creek that collects runoff from the John Glenn Columbus International Airport. Similarly at river mile 15.8 (near Site 11), Rocky Fork Creek, Blacklick Creek, and Alum Creek converge with Big Walnut Creek at the Three Rivers Metro Park. Twenty-five other tributaries also enter Big Walnut Creek in the study area, which are outlined in Table 1. These anthropogenic inputs may be affecting the metal concentrations in the sediments that then negatively impact mussels, especially in the middle stretch of Big Walnut Creek. In this study, the metals from nine Sites along Big Walnut Creek are analyzed in efforts to determine a cause for decreased mussel survival rates.

In addition to sampling from pools at each Site along Big Walnut Creek, pseudo feces and the area (immediately surrounding) where the mussels are collected (the area of interest or AOI) are analyzed for metals. Freshwater mussels filter water and sediments to find food, and the waste they produce, the pseudo feces, is exported into the surrounding substrate. An indicator a freshwater mussel has lived in a certain location for an extended period of time is that a pocket of very fine sediments, or pseudo feces, surround it. These fine sediments have passed through the mussel during filtration, and if toxins are present, they will accumulate in soft tissues over time [5]. If metal buildup is occurring, this will have adverse physiological effects. Thus, this study involves a comparison of metal levels in pools, area of interest, and pseudo feces at each sampling Site.

Previous studies have shown that some metals negatively impact the recovery and resiliency of mussel populations. Bonneris *et al.* found that when freshwater mussels are exposed to high levels of heavy metals, such as cadmium, copper, and zinc, their tissues will absorb these metals and this causes adverse physiological effects [6]. Spann *et al.* emphasized the exponential decline in populations that occurs when mussels are exposed to heavy metal contamination [7]. Phillips *et al.* found that major storm water contaminants, such as zinc, have an adverse affect on the reburial rates of freshwater mussels [8]. Without proper reburial, mussels are more likely become victims of predation. Perceval *et al.* concluded that long-term exposure to some metals specifically leads to a decrease in the overall health of the community as well as a decrease in fecundity [9]. This is especially alarming because if mussels are not able to reproduce, they will be on an expedited track to endangerment. The effects of hindrance in reburial and feeding limit recovery from these conditions [10]. Therefore, this study will examine the levels of a variety of metals including lead, chromium, aluminum, calcium, manganese, potassium, zinc, and nickel in sediments along Big Walnut Creek.

Metals in the sediments of Big Walnut Creek have been studied previously; however, the study was conducted over fifteen years ago and the correlation between mussel populations and metal content was not studied [11]. In the current study, heavy metal contaminants are analyzed using microwave plasma – atomic emission spectroscopy (MP-AES), and the correlation to the decline of mussel communities along Big Walnut Creek are explored. Relationships between the metal concentrations in sediment collected in the year 2000 and the current study are also examined in this study.

Methods

Reagents. Two metal standard solutions containing lead, selenium, cadmium, zinc, manganese, beryllium, calcium, potassium, aluminum, nickel, sodium, and chromium were purchased from Fisher Scientific. A standard soil sample, with certified amounts of select metals, was purchased from the National Institute of Standards and Technology (NIST).

Sediment Sampling and Preparation. Sediment samples were collected from nine locations along Big Walnut Creek (Sites 3, 8, 9, 11, 12, 13, 16, 18, 20, Figure 1) during the summer of 2016 using the USGS method [12]. Sediment samples were taken from the pool and the two quadrants from which mussels were sampled (AOI), as well as pseudo feces. In addition, pseudo feces samples were taken by hand in an effort to not disrupt the organism. A slam bar with a sludge sampler was used to extract samples for the pool and AOI samples. In the time between sample extraction and sample digestion, sediments were stored in sealed plastic bags. The samples were dried, and approximately 50 grams of each sample were sieved, and about 50 grams were used to measure clay and silt percentages using a bouyoucos hydrometer. The sieved samples were then digested using EPA method 3050B [13]. This method requires digestion in a mix of concentrated nitric acid and 30% hydrogen peroxide to isolate the metals from the sediment matrix. Isolation can occur anywhere from four to six hours, depending on the sample. For example, a sample with more organic matter will take longer to digest than a sample with less organic matter. Samples with less sand particulate also respond better to digestion in comparison to samples with more organic matter.

Mussel Sampling. The Ohio Mussel Survey Protocol was used to analyze mussel populations along Big Walnut Creek [14]. A quadrant system outlined the area of interest, and the quadrants directly followed a sink (pool) in the creek. Each quadrant was scanned twice to find an average value of mussel populations for the location. At Site 11, no living mussels were reported, and a pseudo pseudo-feces sample was taken to serve as another sample for the location.

Metal Analysis. Samples and calibration standards from 100 to 9000 ppb were analyzed using Microwave Plasma - Atomic Emission Spectroscopy (MP-AES, Agilent) for several metals, including lead, zinc, chromium, nickel, manganese, calcium, aluminum, and potassium. Additionally, the certified sample from NIST was analyzed. A method blank and calibration check was tested every ten samples to ensure the instrument and quality control criteria. The wavelengths at which each sample is analyzed is described in Table 2. The quality control information is summarized in Appendix A.

Statistical Analysis. Differences in metal concentrations and a comparison to mussel population data were analyzed using SPSS software. To discern if there were statistically significant differences compared to the EPA study in 2000 [10], t-tests were used.

Results

A total of 45 sediment samples were measured across nine different Sites along the lower stretch of Big Walnut Creek. The metal levels were examined for changes along the length of the creek and in comparison to the threshold effect concentration. The consensus based threshold effect concentrations (TEC) are descriptors for sediments that defines a minimum concentration of contaminants that can be present before adverse effects on benthic organisms are observed [15].

Lead Values in Sediment Samples: The lead levels in all sediment samples are in Table 3, and are compared to the TEC for dry weight sediments for lead of 35.8 mg/kg of sample. The average values of the area of interest (AOI) samples shows Sites three, nine, and thirteen above the TEC (see Figure 2). The average AOI lead concentration are statistically different in the Sites sampled, $F (8, 25) = 8.865$, $p < 0.0005$. In the samples taken from pseudo feces (PF), Sites eight, nine, eleven, and thirteen have concentrations of lead higher than the TEC. The average concentrations of lead in the PF are statistically different in the Sites sampled, $F (7, 18) = 7.723$, $p < 0.0005$. The p values for AOI and PF results can be found in Tables 4 and 5.

Chromium Values in Sediment Samples: Chromium levels in sediment collected from Big Walnut Creek are summarized in Table 6**.** The TEC for dry weight sediments for chromium is 43.4 mg/kg of sample. None of the Sites sampled in this study were above the TEC (see Figure 3); however, there is a statistical difference between the AOI levels, $F (8, 25) = 4.534$, $p = 0.002$, and PF concentrations, $F (7, 21) = 2.719$, $p = 0.036$. Sites 11 and 13 were the slightly elevated at the pool concentrations, and there is not a notable trend in the PF and the AOI points. The p values for AOI and PF levels can be found in Tables 7 and 8.

Aluminum Values in Sediment Samples: Aluminum levels in sediment collected from Big Walnut Creek are summarized in Table 9. A TEC value for aluminum in sediments is not reported; however, values can be visualized in Figure 4. There is a statistical difference between the AOI levels throughout the creek, $F (8, 25) = 6.332$, $p <0.0005$, and there is not a statistical difference between the PF concentrations, $F (7, 21) = 0.578$, $p = 0.766$. The p values for AOI and PF results can be found in Tables 10 and 11.

Calcium Values in Sediment Samples: Calcium levels in sediments collected form Big Walnut Creek are summarized in Table 12. A TEC value for calcium in sediments is not reported; however, values can be visualized in Figure 5. There is a statistical difference between the AOI concentrations, $F (8, 23) = 14.101$, $p < 0.0005$ and the PF levels, $F (4, 18) = 16.018$, $p < 0.0005$. The p values for AOI and PF concentrations can be found in Tables 13 and 14.

Manganese Values in Sediment Samples: Manganese levels in sediments collected from Big Walnut Creek are summarized in Table 15. A TEC value for manganese in sediments is not reported; however, values can be visualized in Figure 6. There is a statistical difference between the AOI concentrations, $F (8, 25) = 13.623$, $p < 0.0005$, and there is not a statistical difference between the PF levels, $F (7, 21) = 1.823$, $p = 0.135$. The p values for AOI and PF findings can be found in Tables 16 and 17.

Nickel Values in Sediment Samples: Nickel levels in sediments collected from Big Walnut Creek are summarized in Table 18**.** The TEC value for dry weight of sediments for nickel is 22.7 mg/kg of sample. There is not a statistical difference between the AOI concentrations, $F (8, 25) = 1.672$, $p = 0.155$, and there is a statistical difference between the PF levels, $F (7, 21) = 6.516$, $p < 0.0005$. All samples from Sites 3, and 8, were above the reported TEC values. The samples, excluding PF, were above the TEC at Sites 16 and 20. Only the PF from Site 9 were above the TEC, and the pool data from Site 13 were above the TEC (see Figure 7). The p values for AOI and PF results can be found in Tables 19 and 20.

Potassium Values in Sediment Samples: Potassium levels in sediments collected from Big Walnut Creek are summarized in Table 21. A TEC value for potassium is not reported; however, values can be visualized in Figure 8. There is a statistical different between the AOI concentrations, $F (8, 25) = 15.150$, $p < 0.0005$, and there is not a statistical difference between the PF results, $F (7, 21) = 0.494$, $p = 0.828$. The p values for AOI and PF levels can be found in Tables 22 and 23.

Zinc Values in Sediment Samples: Zinc levels in sediments collected from Big Walnut Creek are summarized in Table 24. The TEC value for dry weight of sediments for zinc is 121 mg/kg of sample (see Figure 9). At Site 11, the PF and pool zinc concentrations are above the TEC, and at Site 13, the pool result is above the reported TEC. There is a statistical difference between the AOI concentrations, $F (8, 25) = 7.824$, $p < 0.0005$, and between the PF levels, $F (7, 21) = 7.122$, $p < 0.0005$. The p values for AOI and PF data can be found in Tables 25 and 26.

Discussion

Spatial Comparison of Metal Concentrations in Big Walnut Creek

One unexpected finding was that Site 3, the most upstream location, had the highest concentrations of most metals. Based on changes in land use moving downstream, metal concentrations were expected to be lowest at Site 3 and increase downstream. These values were expected to increase based on changes in land use. For example, immediately below Hoover Dam, the land use is 46% residential, 22% open space, and 1% industrial. However, below Three Creeks Metro Park (near Site 11), the land use is only 15% residential, and 9% open space while the percent of industrial use increases to 18% [16]. The increased industrial land use would likely increase the amounts of metals rather than decrease them. Thus, these results cannot be explained at the present time.

For most of the metals (aluminum, chromium, calcium, lead, nickel, potassium, zinc) the second highest concentration was observed at Site 8. This is downstream of the John Glenn Columbus International Airport, and the Airport Tributary feeds into Big Walnut Creek in this region. Sediments from the Airport tributary were previously found to be contaminated with metals, including aluminum, chromium, and zinc [10]. Further, there have been other noted spills to the Big Walnut Creek basin, including a spill of heavy metals from Claycraft Brick Plant No. 2 in this region, which may also contribute to elevated metal concentrations at Site 8 [10]. Note that there are more TEC exceedances at Sites 9 and 11, which indicates that this stretch of the creek would be the most toxic to benthic organisms (see Table 10).

Relationship of Metal Levels in Pseudofeces and Area of Interest

The initial hypothesis of the study was that metal levels in pseudo feces (PF) would be higher than in the surrounding sediment (AOI) as mussels filter the sediment while feeding, removing some materials and concentrating metals in their output. This hypothesis is supported by previous studies [17]. However, there is an inconsistent relationship between the changes in metal concentrations in the AOI to the PF. At some Sites, the metal concentration was found to be higher in the AOI than the PF, while at others the opposite was true. This was observed for all the metals in this study. Other factors may influence these inconsistencies, such as water temperature. Mubiana and Blust found that as temperature increased, the uptake of heavy metals, such as lead, increased within soft tissues of the marine bivalve *Mytilus edulis* [18]. This would result in lower amounts of metals in surrounding pseudo feces. However, as temperature decreased, the organism was able to release the metals in their system, which would increase the concentration of metals in PF. Temperature variability along Big Walnut Creek could account for the inconsistent relationship between the changes in metal concentrations in the AOI and PF. The temperature variability may be due to changes in tree cover, which is known to assist in controlling the temperature of the water, and it is important in the homeostatic condition of the mussels.

Temporal Comparison of Metal Concentrations in Big Walnut Creek

The average metal concentrations found in the current study are compared in Table 27 to those found in sediments from the Ohio EPA study in the year 2000 [11]. Two Sites (11 and 20) align between 2000 and 2016 with respect to river mile, while two others Sites (3 and 8) are within 4.7 and 3.7 river miles of each other, respectively. The amount of Sites that have metal concentrations above the TEC value has decreased from eleven sites in the year 2000 to only four in 2016. Specifically, nickel levels remain a concern, while chromium, lead, and zinc values have declined below the TEC, with the exception of lead at Site 3. However, this Site had a high level of variance (39.1% Relative Standard Deviation) between duplicate samples that suggest additional testing is warranted. Further, the metal concentrations have decreased at all sampling locations relative to the 2000 levels for chromium, lead, manganese, and zinc (with the exception of Site 3), while few changes were observed for aluminum, calcium, and nickel. Thus, it appears that the overall health of Big Walnut Creek with respect to metals in sediments is improving.

Correlations between Metal Concentrations in Sediment and Mussel Population

To examine the relationship between metal in the sediments and mussel population of the lower stretch of Big Walnut Creek, Pearson's correlations were run. There are differences in R values for AOI, PF, and pool metal concentrations when looking at a specific metal. For example, manganese was found to have a moderate negative correlation to manganese concentrations in the AOI and pool (R=-0.46 and -0.43, respectively), but a weak positive correlation in the PF (R=0.15) This suggests that the amount of metal in each type of sediment may be impacting mussels differently. However, there are other possible causes for this as well. The differences in R values among sample types may also be due to variability in measuring AOI duplicates that Pearson's correlation does not take into account. Additionally, there is no Site 3 PF data to include in the analysis of the relationship of mussel populations to metal concentrations, and at Site 11, a pseudo pseudo feces sample was used, which may not be a truly representative sample.

The concentration of calcium in the AOI was found to positively correlate to mussel populations ($r = 0.715$, $p = 0.03$), while zinc in the AOI was found to negatively correlate to mussel populations ($r = -0.717$, $p = 0.03$). Calcium likely has a high positive correlation with mussel populations because it is utilized in the process of growing the shell of the mussel, while the negative correlation to zinc levels is likely due to the negative effects of zinc on mussel reburial rates [7]. It is also interesting to note that the threshold effect concentration was exceeded for zinc in PF only at Site 11, which is where the decrease in mussel population began. This further supports the conclusion that zinc is negatively impacting mussel populations. No other metals were found to have a statistically significant correlation to mussel population; this may be due to the limited number of Sites studied, which limits the statistical power in the analysis.

Correlation between Manganese and Mussel Population

Although not statistically significant, it is interesting to note that manganese levels in AOI sediments had a moderate negative correlation to mussel population ($R= -0.46$). To the best of the author's knowledge, no studies of the relationship between mussels and metals in sediment of freshwater systems have been completed that include manganese; therefore, it is especially noteworthy that this study found a moderate correlation to manganese. However, it is known that manganese negatively impacts mussels. Manganese converts into a bioavailable state when oxygen levels in the water are low. In a simulated laboratory system, Oweson and Henroth found that, under conditions in which manganese is bioavailable, marine invertebrates, including mussels, had a decrease in haemocyte levels as well as an impairment of bactericidal capacity [19]. At higher water temperatures, the oxygen solubility decreases. Thus, under high water temperatures and elevated levels of manganese, mussel health would be negatively affected, which may explain the observed correlation in this study.

Conclusions

This study examined the concentration of select metals (aluminum, calcium, chromium, lead, manganese, nickel, potassium, and zinc) in sediments from the lower stretch of Big Walnut Creek. Metal levels were found to vary along the length of the creek, although no consistent trends emerged. Further, there were no clear trends in the relationship of metals in different sample types. As mussels filter the water to find food and excrete pseudo feces, the metal levels were expected to increase from the surrounding sediments to the pseudo feces based on findings from previous studies, but no clear trends were found in the current study. The quality of the sediments in Big Walnut Creek was found to improve, however, as metal levels decreased in comparison to concentrations determined 16 years earlier.

This study was the first to examine the relationship between metals in sediments of Big Walnut Creek and its mussel population. Mussel populations positively correlate to calcium in sediments, suggesting calcium is needed and may offset toxic metal effects. However, mussel population adversely correlates to zinc and manganese, which may be related to the impacts of zinc on mussel reburial rates and manganese on mussel haemocyte levels and bactericidal capacity.

Future studies should include a more extensive sample collection at each location to account for the heterogeneity that is present in sediment samples, which may limit the data interpretation. It is especially important that pseudo feces (PF) are collected in sufficient quantity at Site 3 since there was not enough to analyze in the current study. Additionally, a different statistical model should be used to examine the results in order to examine the interactions among metals rather than determining the impact of one metal at a time on the mussel population.

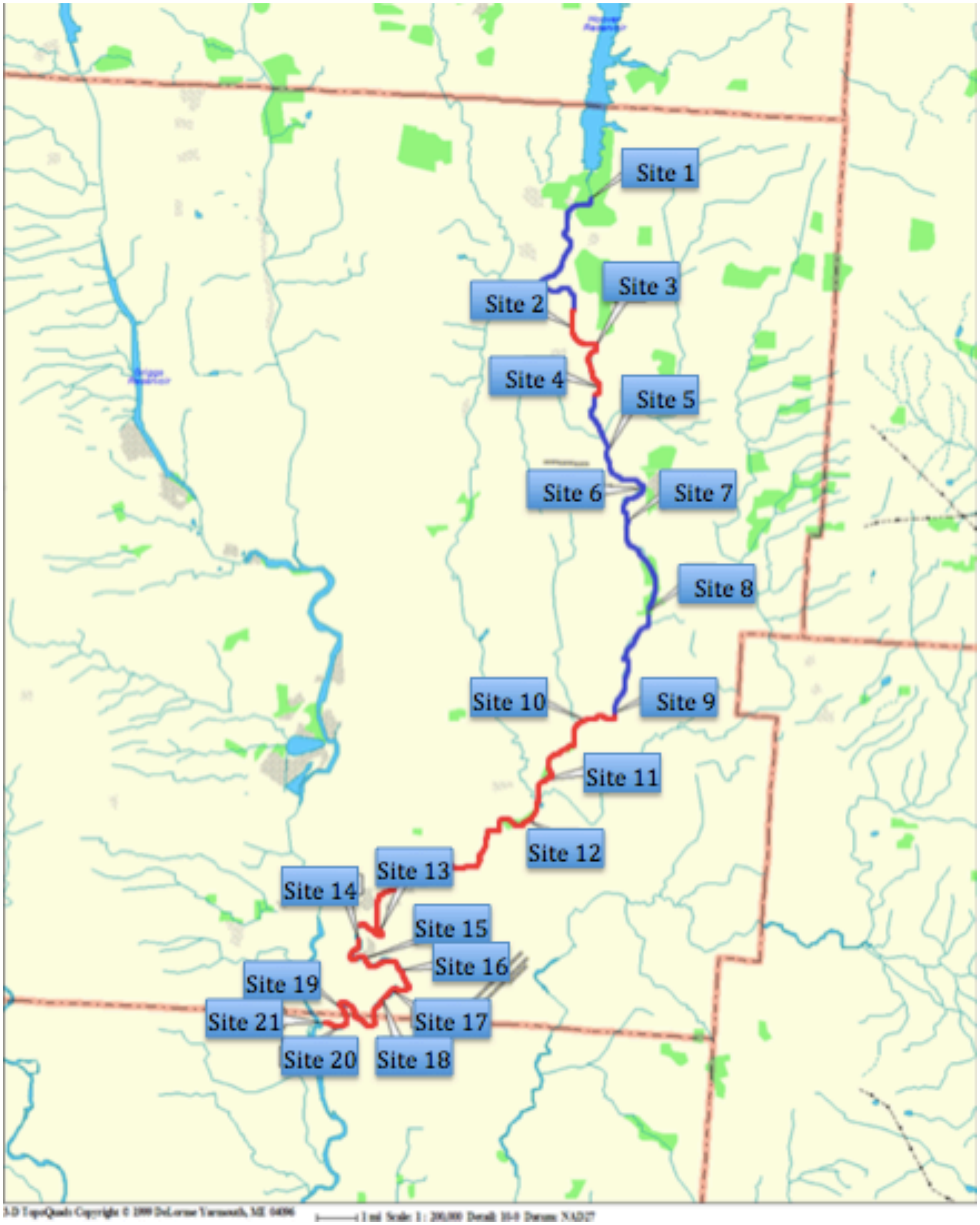

Figure 1. Map of sediment and mussel extraction Sites along lower Big Walnut Creek.

Table 1. Description of sampling Sites and inputs by river mile along Big Walnut Creek.

River Mile	Element
1.5	**Site 20**
3.6	**Site 18**
5.1	**Site 16**
5.3	Unidentified Outfall - 4IJ00014001
8.0	Lockbourne Ditch – 4IJ00006001
8.1	**Site 13**
9.7	Hamilton Meadows Waste Water Treatment - 4IZ00021
9.8	S&S Aggregates – 4IJ00023002
10.1	S&S Aggregates – 4IJ00023001
11.2	Unnamed tributary to Obetz
11.5	? Sandpit – 4IJ00079001
12.7	Unnamed tributary with two outfalls – R.M. 0.01 4IZ00050 and at R.M. 3.09 4IE0000401
13.2	**Site 12**
14.6	Unnamed tributary to Groveport – R.M. 1.95 4IZ00030 WWTP
15.32	Alum Creek Enters Big Walnut Creek
15.33	Blacklick Creek Enters Big Walnut Creek
15.8	Three Rivers Metro Park
15.9	**Site 11**
17.9	Mason Run to Whitehall – R.M. 4.0 4I000001 (Department of Defense)
19.0	Unnamed tributary with no outfalls
19.7	Unnamed tributary with no outfalls
20.1	**Site 9**
20.2	Unnamed tributary with no outfalls
22.0	Unnamed tributary with no outfalls
22.8	Unnamed tributary with no outfalls
23.3	**Site 8**
23.8	Unnamed tributary with no outfalls
24.2	Unnamed tributary with outfalls at R.M. 1.08 4IC00006002 & R.M. 1.15 4IC00006001
26.0	Unnamed tributary with no outfalls
26.4	4IN00039001
26.5	Unnamed tributary with no outfalls
26.7	Unnamed tributary with no outfalls
27.3	Unnamed tributary with no outfalls to Columbus International Airport
27.0	Immediately downstream of Columbus International Airport
28.3	Rocky Fork to Gahanna no outfalls identified
29.7	McKenna Creek
30.7	**Site 3**
32.6	Hoover Dam

Table 2. Optimized wavelengths for MP-AES analysis for highest sensitivity to the various elements.

Element	Wavelength (nm)
Zinc	472.215
Nickel	341.476
Chromium	425.433
Lead	405.781
Maganese	259.372
Calcium	393.152
Aluminum	396.152
Potassium	766.491

Table 3. Lead values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Lead							
Site Number	**PF**	**AOI**	**Pool**	**TEC**	**Stdev (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		59.68	23.83	35.80		27.11	0.23
8	41.29	29.82		35.80	9.73	9.16	
9	63.80	59.90	35.76	35.80	12.30	0.01	0.11
11	62.36	22.65	30.31	35.80	0.11	1.50	0.16
12	37.77	33.21	27.05	35.80	29.25	1.24	0.16
13	48.29	70.25	84.24	35.80	0.15	32.68	1.60
16	21.75	27.62	29.85	35.80	5.25	7.64	0.10
18	26.24	22.42	31.15	35.80	0.25	2.08	0.22
20	19.05	19.71	64.36	35.80	4.69	5.05	0.01

Table 4. Listing on p values from ANOVA analysis of lead concentrations in AOI at sampling Sites along Big Wanlut Creek.

Lead AOI - Games and Howell (Homogeneity, p<0.0005 & Robust p<0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.399	1.000	0.53	0.464	0.999	0.338	0.246	0.399
AOI Site 8			0.026	0.624	0.983	0.33	1.000	0.581	0.398
AOI Site 9				p < 0.0005	p < 0.005	0.985	0.014	p < 0.0005	0.002
AOI Site 11					p < 0.005	0.221	0.814	0.999	0.814
AOI Site 12						0.362	0.718	0.001	0.043
AOI Site 13							0.276	0.216	0.189
AOI Site 16								0.766	0.543
AOI Site 18									0.92
AOI Site 20									

Table 5. Listing on p values from ANOVA analysis of lead concentrations in PF at sampling Sites along Big Wanlut Creek.

Lead PF: Games and Howell (Homogeneity, p<0.0005 & Robust p<0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.121	0.071	1	0.617	0.073	0.175	0.047
PF Site 9			1	0.579	0.301	0.012	0.03	0.011
PF Site 11				0.56	0.001	0.002	0.002	0.001
PF Site 12					0.969	0.836	0.943	0.745
PF Site 13						0.007	0.001	0.004
PF Site 16							0.56	0.969
PF Site 18								0.187
PF Site 20								

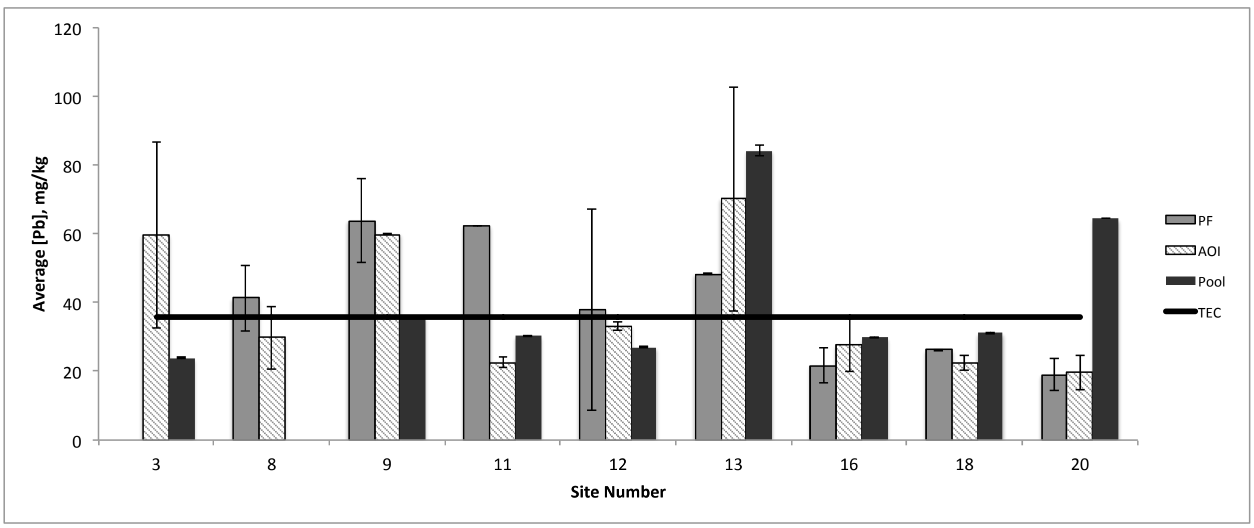

Figure 2. Average lead values for each location along lower Big Walnut Creek.

Table 6. Chromium values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Chromium							
Site Number	**PF**	**AOI**	**Pool**	**TEC**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		12.17	12.24	43.40		0.39	0.02
8	13.37	10.09		43.40	2.07	3.26	
9	10.83	14.20	11.79	43.40	2.13	0.02	0.03
11	13.57	10.01	18.91	43.40	0.00	3.87	0.18
12	10.87	11.78	9.29	43.40	5.10	1.73	0.01
13	8.89	11.29	19.00	43.40	0.02	0.86	0.26
16	7.56	7.77	12.70	43.40	1.79	0.06	0.02
18	11.34	9.71	16.94	43.40	0.04	1.97	0.04
20	8.60	7.53	15.34	43.40	1.71	1.51	0.02

Table 7. Listing on p values from ANOVA analysis of chromium concentrations in AOI at sampling Sites along Big Wanlut Creek.

Chromium AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.792	0.007	0.863	0.999	0.479	0.002	0.31	0.027
AOI Site 8			0.304	1	0.945	0.982	0.886	1	0.714
AOI Site 9				0.405	0.246	0.025	0.002	0.075	0.012
AOI Site 11					0.963	0.989	0.952	1	0.832
AOI Site 12						0.998	0.089	0.626	0.049
AOI Site 13							0.009	0.695	0.038
AOI Site 16								0.765	0.934
AOI Site 18									0.521
AOI Site 20									

Table 8. Listing on p values from ANOVA analysis of chromium concentrations in PF at sampling Sites along Big Wanlut Creek.

Chromium PF: Games and Howell (Homogeneity, p < 0.005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.354	0.561	0.395	0.239	0.165	0.382	0.228
PF Site 9			0.267	1	0.498	0.108	0.996	0.545
PF Site 11				0.858	0.004	0.003	0.02	0.035
PF Site 12					0.956	0.649	1	0.942
PF Site 13						0.115	0.004	0.999
PF Site 16							0.011	0.572
PF Site 18								0.165
PF Site 20								

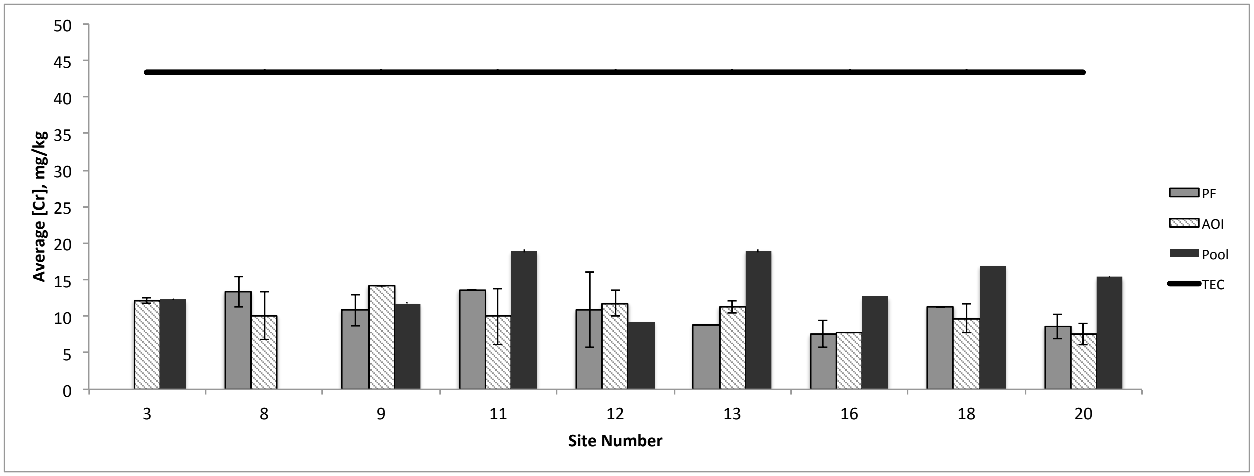

Figure 3. Average chromium values for each location along Big Walnut Creek.

Table 9. Aluminum values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Aluminum						
Site Number	**PF**	**AOI**	**Pool**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		4626.96	5814.09		48.26	27.22
8	5082.47	3879.54		879.93	1169.66	
9	3601.36	4547.19	4275.94	824.35	3.99	11.79
11	4415.10	2896.37	5475.18	26.56	924.81	106.42
12	3850.81	3411.27	3507.55	1522.87	583.66	10.86
13	2919.06	3517.39	4486.10	5.50	561.56	76.67
16	2756.06	2703.85	4538.97	643.29	166.53	13.97
18	4375.57	3473.00	5131.00	20.45	43.67	10.49
20	3080.45	2651.61	4313.44	625.28	509.88	3.71

Table 10. Listing on p values from ANOVA analysis of aluminum concentrations in AOI at sampling Sites along Big Wanlut Creek.

Aluminum AOI - Games and Howell (Homogeneity, p<0.0005 & Robust p<0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.789	0.164	0.127	0.098	0.107	0.016	p < 0.0005	0.016
AOI Site 8			0.853	0.779	0.986	0.996	0.638	0.983	0.461
AOI Site 9				0.144	0.119	0.131	0.019	p < 0.0005	0.019
AOI Site 11					0.936	0.866	1	0.81	0.999
AOI Site 12						1	0.708	1	0.4
AOI Site 13							0.534	1.000	0.279
AOI Site 16								0.282	0.984
AOI Site 18									0.176
AOI Site 20									

Table 11. Listing on p values from ANOVA analysis of auluminum concentrations in PF at sampling Sites along Big Wanlut Creek.

Aluminum PF: Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.997	0.816	0.991	1	1	0.826	1
PF Site 9			0.44	1	0.571	0.288	0.463	0.894
PF Site 11				0.965	0.015	0.007	0.921	0.08
PF Site 12					0.782	0.569	0.971	0.914
PF Site 13						0.452	0.001	0.994
PF Site 16							0.007	0.669
PF Site 18								0.084
PF Site 20								

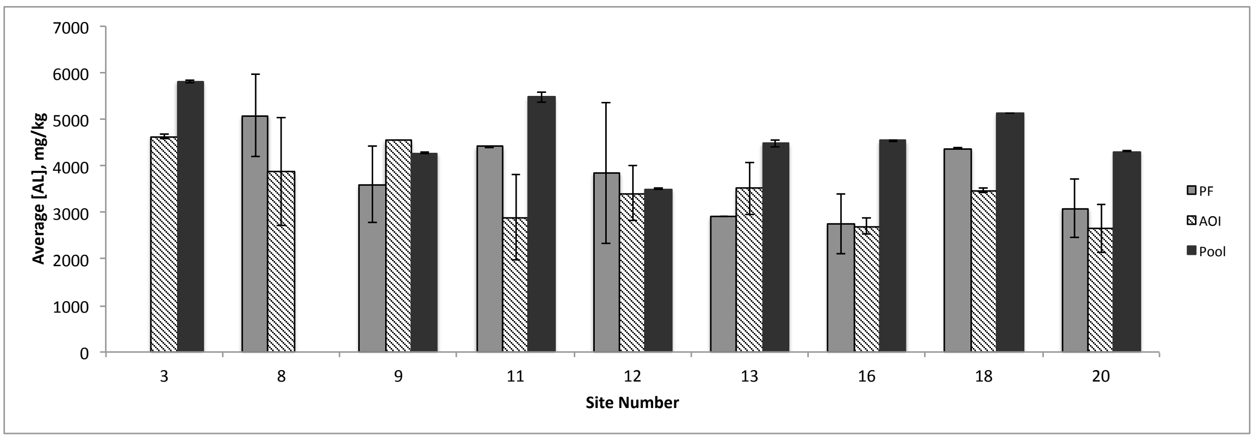

Figure 4. Average aluminum values for each location along Big Walnut Creek.

Table 12. Calcium values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Calcium						
Site Number	**PF**	**AOI**	**Pool**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		21572.33	20376.81		16109.21	72.14
8	31824.97	37877.76		5265.77	260.32	
9	35921.03	40598.72		6145.09	137.97	
11		66466.93			26111.72	
12	73503.89	116907.13	21739.40	15822.30	0.00	120.11
13		63966.73			4268.93	
16	1378.12	1352.01	2269.91	321.47	83.09	33.47
18	58321.15	66373.58	71297.57	76.08	10096.80	184.74
20	12280.43	1326.15	2157.21	14875.86	255.12	127.43

Table 13. Listing on p values from ANOVA analysis of calcium concentrations in AOI at sampling Sites along Big Wanlut Creek.

Calcium AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.453	0.345	0.145	0.053	0.043	1	0.023	1
AOI Site 8			0.056	0.397	0.141	0.004	0.026	0.042	0.012
AOI Site 9				0.469	0.159	0.006	0.018	0.055	0.007
AOI Site 11					0.752	1.000	0.132	1	0.18
AOI Site 12						0.499	0.065	0.586	0.082
AOI Site 13							p < 0.0005	0.999	p < 0.0005
AOI Site 16								0.0003	0.599
AOI Site 18									0.009
AOI Site 20									

Table 14. Listing on p values from ANOVA analysis of calcium concentrations in PF at sampling Sites along Big Wanlut Creek.

Calcium PF: Games and Howell (Homogeneity, p < 0.005 & Robust = 0.001)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.179	X	0.002	X	0.894	X	0.776
PF Site 9			X	0.03	X	0.066	X	0.104
PF Site 11				X	X	X	X	X
PF Site 12					X	0.019	X	0.016
PF Site 13						X	X	X
PF Site 16							X	0.797
PF Site 18								X
PF Site 20								

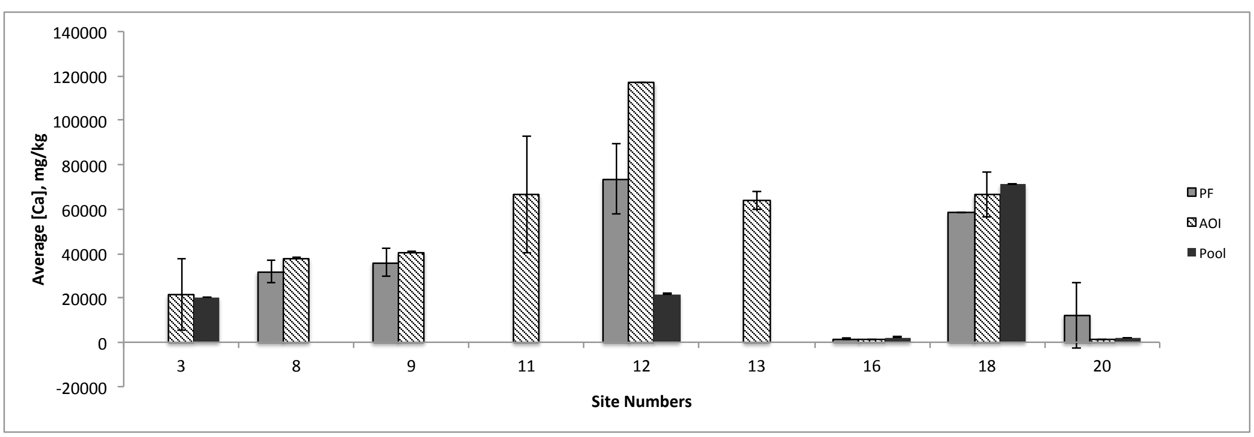

Figure 5. Average calcium values for each location along Big Walnut Creek.

Table 15. Manganese values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Manganese						
Site Number	**PF**	**AOI**	**Pool**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		536.73	328.00		115.91	1.29
8	484.92	190.94		105.80	72.94	
9	340.53	381.68	250.21	43.38	2.17	0.72
11	295.34	250.22	228.47	0.26	57.32	2.37
12	484.36	327.07	186.87	165.32	43.58	0.87
13	523.37	336.12	316.45	0.82	42.92	7.13
16	220.56	270.28	218.91	44.60	79.51	0.11
18	415.33	350.19	321.22	0.86	0.12	0.24
20	264.49	264.95	389.67	32.84	55.51	4.90

Table 16. Listing on p values from ANOVA analysis of manganese concentrations in AOI at sampling Sites along Big Wanlut Creek.

Manganese AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.017	0.269	0.04	0.121	0.138	0.052	0.167	0.05
AOI Site 8			0.051	0.795	0.109	0.087	0.685	0.089	0.601
AOI Site 9				0.073	0.307	0.42	0.243	0.003	0.092
AOI Site 11					0.341	0.249	1	0.165	1
AOI Site 12						1	0.806	0.96	0.519
AOI Site 13							0.695	0.999	0.386
AOI Site 16								0.508	1
AOI Site 18									0.226
AOI Site 20									

Table 17. Listing on p values from ANOVA analysis of manganese concentrations in PF at sampling Sites along Big Wanlut Creek.

Manganese PF: Games and Howell (Homogeneity, p <0.0005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.999	1	0.711	0.35	0.989	0.868	1
PF Site 9			0.402	0.55	0.012	0.034	0.138	0.136
PF Site 11				0.339	p < 0.0005	0.148	p < 0.0005	0.479
PF Site 12					0.996	0.159	0.941	0.248
PF Site 13						0.003	p < 0.0005	0.002
PF Site 16							0.011	0.576
PF Site 18								0.009
PF Site 20								

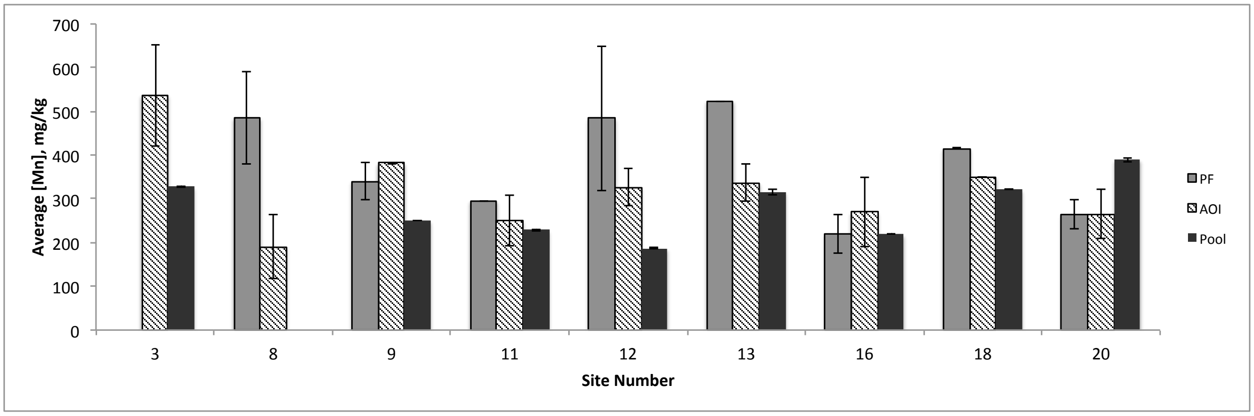

Figure 6. Average manganese values for each location along Big Walnut Creek.

Table 18. Nickel values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Nickel							
Site Number	**PF**	**AOI**	**Pool**	**TEC**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		35.02	37.90	22.70		7.47	0.01
8	108.78	28.55		22.70	20.65	8.07	
9	33.89	16.16	22.37	22.70	15.87	0.81	0.10
11	24.17	11.19		22.70	0.32	4.52	
12	14.50	10.89	42.65	22.70	2.86	5.73	0.11
13	7.26	14.54	41.88	22.70	0.04	0.62	1.84
16	10.14	45.34	47.86	22.70	10.89	39.87	0.07
18	17.38	15.63	31.35	22.70	0.02	1.34	0.14
20	8.32	60.71	147.00	22.70	7.05	67.03	0.08

Table 19. Listing on p values from ANOVA analysis of nickel concentrations in AOI at sampling Sites along Big Wanlut Creek.

Nickel AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust, p = 0.005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.848	0.052	0.013	0.012	0.045	0.997	0.049	0.997
AOI Site 8			0.196	0.065	0.064	0.148	0.96	0.177	0.984
AOI Site 9				0.406	0.545	0.526	0.698	0.996	0.902
AOI Site 11					1	0.693	0.583	0.491	0.85
AOI Site 12						0.797	0.579	0.527	0.847
AOI Site 13							0.658	0.693	0.886
AOI Site 16								0.685	1
AOI Site 18									0.897
AOI Site 20									

Table 20. Listing on p values from ANOVA analysis of nickel concentrations in PF at sampling Sites along Big Wanlut Creek.

Nickel PF: Games and Howell (Homogeneity, p <0.0005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.235	0.102	0.052	0.033	0.037	0.064	0.034
PF Site 9			0.782	0.298	0.148	0.211	0.403	0.151
PF Site 11				0.02	0.019	0.269	0.053	0.073
PF Site 12					0.052	0.96	0.43	0.584
PF Site 13						0.993	p < 0.0005	1
PF Site 16							0.731	1
PF Site 18								0.279
PF Site 20								

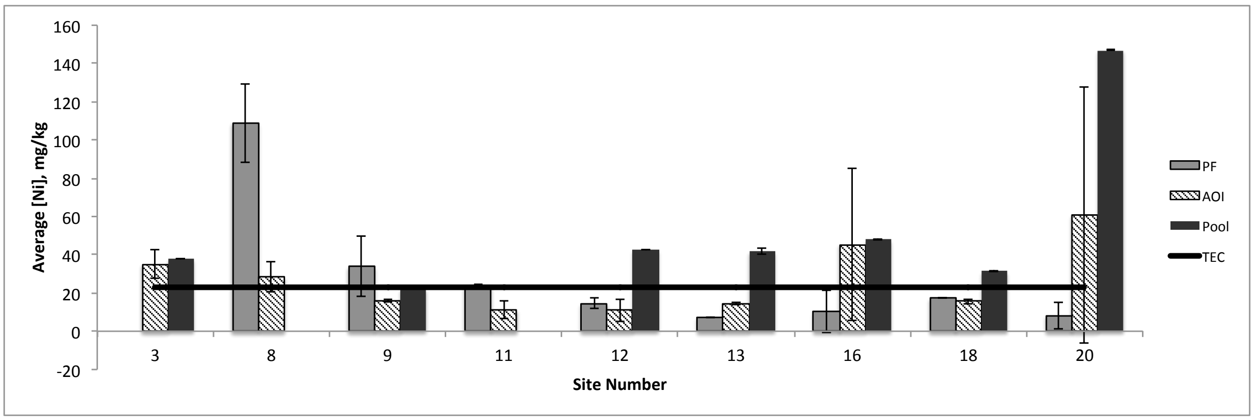

Figure 7. Average nickel values for each location along Big Walnut Creek.

Table 21. Potassium values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Potassium						
Site Number	**PF**	**AOI**	**Pool**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		1180.24	1307.72		165.62	2.50
8	1131.49	860.13		403.33	236.27	
9	771.49	1099.84	895.20	182.02	5.93	0.82
11	770.13	641.10	1489.92	2.29	216.16	1.87
12	797.81	858.43	513.36	235.11	107.67	0.20
13	823.12	751.95	1069.57	0.02	170.41	5.96
16	473.41	529.33	902.74	91.13	123.39	0.15
18	859.34	630.17	1139.46	2.30	119.58	0.33
20	572.76	415.52	794.30	108.66	38.94	128.52

Table 22. Listing on p values from ANOVA analysis of potassium concentrations in AOI at sampling Sites along Big Wanlut Creek.

Potassium AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.313	0.917	0.039	0.097	0.053	0.005	0.01	0.008
AOI Site 8			0.452	0.745	1	0.982	0.252	0.545	0.118
AOI Site 9				0.09	0.077	0.099	0.01	0.016	p < 0.0005
AOI Site 11					0.514	0.973	0.95	1	0.435
AOI Site 12						0.898	0.034	0.138	0.008
AOI Site 13							0.351	0.850	0.101
AOI Site 16								0.85	0.548
AOI Site 18									0.126
AOI Site 20									

Table 23. Listing on p values from ANOVA analysis of potassium concentrations in PF at sampling Sites along Big Wanlut Creek.

Potassium PF: Games and Howell (Homogeneity, p <0.0005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		1	1	0.999	0.995	0.988	0.984	1
PF Site 9			1	1	0.99	0.144	0.897	0.431
PF Site 11				1	0.049	0.025	0.003	0.122
PF Site 12					1	0.228	0.994	0.509
PF Site 13						0.016	0.072	0.066
PF Site 16							0.012	0.684
PF Site 18								0.046
PF Site 20								

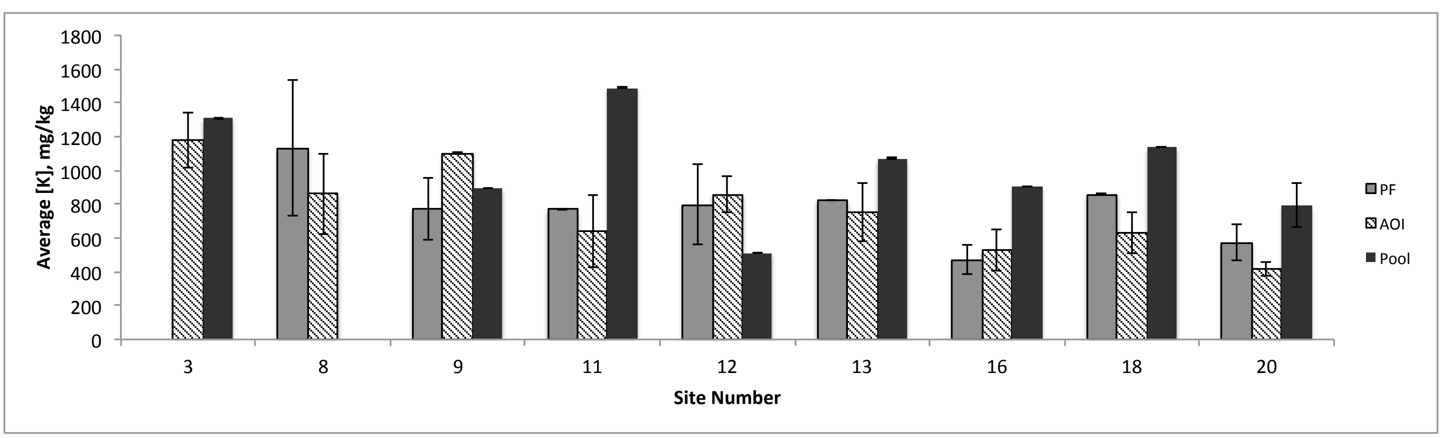

Figure 8. Average potassium values for each location along Big Walnut Creek.

Table 24. Zinc values in mg/kg and standard deviations (STDEV) in PF, AOI, and pool samples compared to the threshold effect concentration (TEC).

Zinc							
Site Number	**PF**	**AOI**	**Pool**	**TEC**	**STDEV (PF)**	**STDEV (AOI)**	**STDEV (Pool)**
3		109.45	84.96	121		29.85	0.28
8	119.33	84.05		121	11.04	32.41	
9	118.78	122.46	83.79	121	21.10	0.47	0.15
11	190.20	57.19	138.26	121	0.29	11.62	1.42
12	68.59	64.63	74.18	121	34.26	8.66	0.00
13	48.98	75.90	136.73	121	0.03	8.25	1.83
16	60.99	63.39	98.34	121	2.89	3.60	0.06
18	75.62	70.68	106.47	121	0.14	16.33	0.01
20	60.32	61.87	78.06	121	4.21	1.77	0.60

Table 25. Listing on p values from ANOVA analysis of zinc concentrations in AOI at sampling Sites along Big Wanlut Creek.

Zinc AOI - Games and Howell (Homogeneity, p < 0.0005 & Robust p < 0.0005)									
	AOI Site 3	**AOI Site 8**	**AOI Site 9**	**AOI Site 11**	**AOI Site 12**	**AOI Site 13**	**AOI Site 16**	**AOI Site 18**	**AOI Site 20**
AOI Site 3		0.86	0.947	0.132	0.203	0.385	0.195	0.31	0.182
AOI Site 8			0.342	0.643	0.845	0.998	0.793	0.971	0.745
AOI Site 9				0.006	0.003	0.005	p < 0.0005	0.03	p < 0.0005
AOI Site 11					0.912	0.193	0.904	0.757	0.965
AOI Site 12						0.45	1	0.99	0.991
AOI Site 13							0.199	0.996	0.152
AOI Site 16								0.949	0.978
AOI Site 18									0.879
AOI Site 20									

Table 26. Listing on p values from ANOVA analysis of zinc concentrations in PF at sampling Sites along Big Wanlut Creek..

Zinc PF: Games and Howell (Homogeneity, p <0.0005 & Robust p < 0.0005)								
	PF Site 8	**PF Site 9**	**PF Site 11**	**PF Site 12**	**PF Site 13**	**PF Site 16**	**PF Site 18**	**PF Site 20**
PF Site 8		0.623	0.008	0.993	0.475	0.821	0.999	0.806
PF Site 9			0.023	0.212	0.024	0.039	0.09	0.035
PF Site 11				0.02	0.02	p < 0.0005	p < 0.0005	p < 0.0005
PF Site 12					0.819	0.998	0.998	0.996
PF Site 13						0.013	0.004	0.044
PF Site 16							0.007	1
PF Site 18								0.019
PF Site 20								

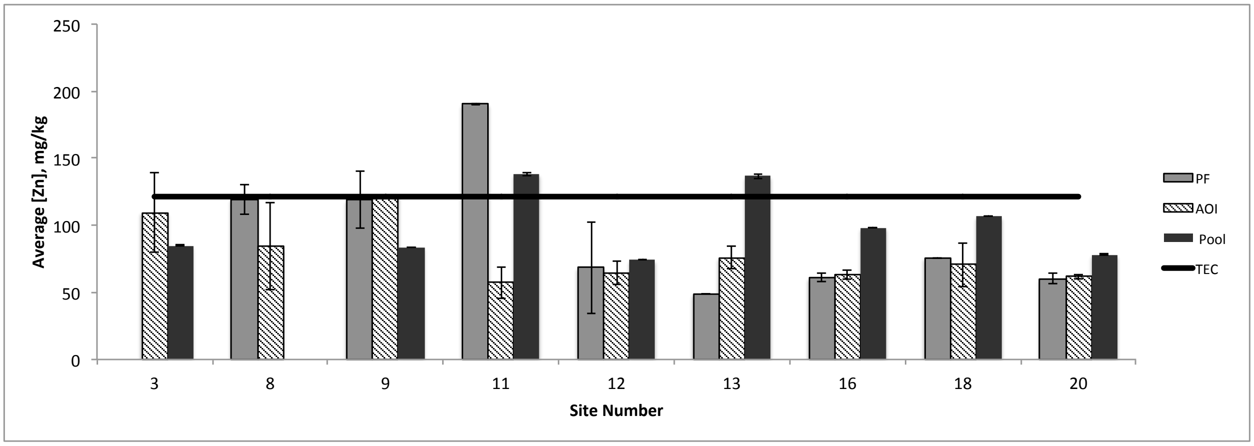

Figure 9. Average zinc values for each location along Big Walnut Creek.

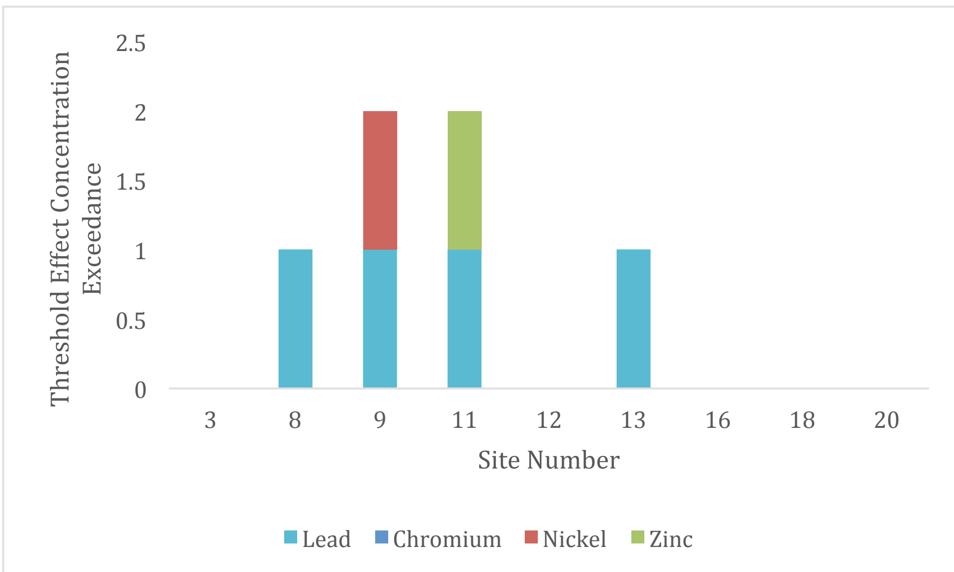

Figure 10: Visually representing the Sites, and metals when applicable, of which exceed the TEC when reported.

Table 27. Temporal Comparison of Metal Concentrations in Big Walnut Creek. The 2016 values are those in sediment collected within the sampling cell. All metal concentration are reported in mg/kg of dry weight. Underlined values indicated concentrations below the method detection limit. Shaded boxes exceed the consensus-based threshold effect concentration (TEC) published in MacDonald, 2000 [15].

River Mile (Site No.)	**Year**	**TEC***	**1.7 (20)**	**15.8 (11)**	**27 (8)**	**34.9 (3)**
Landmark (Location)			US 23 (Lockbourn)	Williams R. (SE Columbus)	Airport tributary (Reynoldsburg)	SR 161 (NE Columbus)
Aluminum	2000		46400	29600	21700	25000
	2016		2650*	2900*	3880*	4630
Calcium	2000		34700	20700	17900	3890
	2016		21600	18900*	66500	1330*
Chromium	2000	43.4	45	25	24.3	25.9
	2016		7.5	10	10.1	12.2
Lead	2000	35.8	49	82	35.8	25.9
	2016		19.7	22.6	29.8	59.7*
Manganese	2000		593	362	357	2290
	2016		265	250	191*	537*
Nickel	2000	22.7	43	33	26.8	25.9
	2016		60.7*	11.2	28.5	35
Potassium	2000		12100	8580	6390	6480
	2016		416	641*	860*	1180
Zinc	2000	121	230	378	147	84.9
	2016		61.9	57.2	84*	109

*Indicates a large difference between two sampling cells. Site 3: the calcium difference between cells is 22,800 mg/kg, lead difference is 38.3 mg/kg, and the manganese difference is 164 mg/kg. Site 8: aluminum difference between cells is 1650 mg/kg, manganese difference is 103 mg/kg, potassium difference is 330 mg/kg, and the zinc difference is 45.8 mg/kg. Site 11: aluminum difference between cells is 1,300 mg/kg, calcium difference is 36,900 mg/kg, and the potassium difference is 305 mg/kg. Site 20: the aluminum difference between cells is 361 mg/kg, and the nickel difference between cells is 95.7 mg/kg

Table 28. Pearson's Correlation R values for sediment values compared to average mussel population at each Site. **Bold** values indicate a large correlation, underlined values indicate a moderate correlation, and unmarked values indicate a small correlation. Calcium and zinc had p values less than 0.05.

Metal	**AOI**	**PF**	**Pool**
Aluminum	**-0.64**	-0.13	-0.35
Calcium	**0.72**	-0.28	-0.01
Chromium	-0.25	-0.04	0.42
Lead	-0.30	0.03	0.18
Manganese	-0.46	0.15	-0.43
Nickel	-0.50	-0.60	-0.26
Potassium	-0.58	-0.18	-0.09
Zinc	**-0.72**	-0.05	**0.57**

Works Cited

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APPENDIX A – QUALITY CONTROL RESULTS

.Method blanks and calibration standards were used to determine the detection limit of the instrument for each metal, which are listed in Table A1. The detection limits were all below metals values calculated for sediment samples. No metal contamination was observed in any blank.

The percent recovery from calibration check standards are also shown in Table A1. A standard reference material (SRM) of soil from NIST was used to characterize the extraction of metals. Percent recoveries for the SRM are in Table A2. Because some of the percent recoveries for metals in both the calibration check standards and SRM are outside acceptable levels, it is possible that the concentrations of metals are underestimated in the current study..

Table A1. Results from Calibration Check Standards and Listing of Limit of Detection (LOD) and Limit of Qualification (LOQ) for Each Metal in ppb.

Element	LOD	LOQ	Calibration Check Solution: Theoretical (ppb)	Percent Recovery
Aluminum	0.18	0.59	1744	95.30%
Calcium	0.09	0.29	8720	95.90%
Chromium	0.01	0.27	175	96.50%
Lead	2.60	8.66	600	106.50%
Maganese	12.31	41.03	120	130%
Nickel	0.30	1.01	175	168%
Potassium	5.07	16.91	3488	99.10%
Zinc	1.31	4.37	180	122.30%

Table 2A. Percent Recoveries from NIST Soil Standard 2587.

Element	mg/kg Calculated	Standard Deviation	mg/kg Actual	Percent Recovery
Aluminum	9136	13.90	58600 ± 1700	15.59%
Calcium	1926	1.49	9270 ± 200	20.77%
Chromium	28.59	0.00	92 ± 11	31.08%
Lead	2840	10.73	3242 ± 57	87.60%
Maganese	378.9	1.04	651 ± 23	58.21%
Nickel	19.52	0.17	36	54.23%
Potassium	927.2	3.63	15830 ± 550	5.86%
Zinc	202.6	0.67	335.8 ± 7.6	60.33%