IMPACT OF DIET ON Pb BIOACCESSIBILITY FOR WILDLIFE *IN VITRO* METHODS

By

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ABSTRACT

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Human activities have introduced Pb into the environment, posing a risk not just to humans, but to wildlife as well. Ecological risk assessment is used to assess risk of stressors, such as lead, to wildlife. Currently, risk assessment relies on the assumption that lead exposure results in complete absorption, i.e. 100% relative bioavailability. In many cases this is inaccurate, as bioavailability of lead decreases depending on the media with which it is consumed. Thus, not knowing the bioavailability of metals can lead to overestimation of exposure, impacting risk assessment and making remediation efforts more expensive. Animal models and *in vitro* methods are used to characterize human bioavailability for Pb in soils. The exposure pathway for human health risk assessment is ingestion of soil while fasting, but the pathway for wildlife is exposure through feeding. *In vitro* methods for wildlife should be used for measuring bioavailability of Pb in diet, rather than in soil. The impacts of diet on *in vitro* bioaccessible Pb (i.e. IVBA Pb) have not yet been examined. In this current study, *in vitro* methods that have been found to accurately predict bioavailable Pb for Japanese quail (*Courtnix japonica*) were further tested to see if there would be changes in IVBA Pb in six different diets spiked with lead acetate. Diet impacted IVBA Pb in some in vitro methods, and the effects varied both with method and with diet. Method pH and Ca, fiber, and phytate content were not strongly correlated with changes in Pb solubility, indicating complex interactions between Pb and dietary components. Further research is needed to discern which dietary components impact bioavailability. *In vitro* methods for wildlife should be correlated with animal models to develop predictive methods that can quickly and inexpensively estimate bioavailability of contaminated diets for ecological risk assessment.

ACKNOWLEDGEMENTS

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INTRODUCTION

Anthropogenic activities have led to widespread heavy metal pollution in the environment (Abadin et al., 2007). In order to remediate polluted sites, their risk to wildlife must be assessed. Lead is a common and widespread pollutant that is toxic to humans and wildlife. At least 1,272 of the 1,684 of the 2007 proposed EPA National Priorities List hazardous waste sites are contaminated with lead. Lead has been used in gasoline, pesticides, and paint, and continues to be used in batteries and ammunition (Abadin et al., 2007). The sink for lead in the environment is soil, where it can dissolve in soil water and be taken up by plants. Plants can accumulate Pb at levels toxic to animals before phytotoxicity (Ackah et al., 2014). Lead accumulates in the bones and vital organs, and can cause weakness, incoordination, anemia, anorexia and weight loss, decreased fertility, kidney and liver failure, brain damage, and death (Beyer et al., 2013; Francisco et al., 2003).

Risk to wildlife from heavy metals is determined using ecological risk assessment. Ecological risk assessment is “a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors” (EPA, 1992). The process involves characterizing effects and exposure. Exposure is quantified to determine the dose with known adverse effects. A combination of several factors is used to determine exposure from ingestion of heavy metal-contaminated media, including bioavailability. This is the portion of the ingested metal that is absorbed from the gastrointestinal tract. Relative bioavailability is the amount of Pb absorbed from soil or media divided by the amount of Pb absorbed from a maximally available form, usually lead acetate in water (Drexler and Brattin, 2007). If not otherwise known, relative bioavailability is assumed to be 100%. In many cases this is inaccurate, in part because the bioavailability of heavy metals is decreased by

the media with which it is consumed. The media can provide sorption sites, change the digesta pH, and contain components that could increase or decrease bioavailability (Beyer et al., 2016). Not considering bioavailability can cause overestimation of risk, leading to unnecessary remediation. This has been recognized in human health risk assessment, and the EPA has adjusted the default bioavailability of soil lead from 100% to 60% relative bioavailability (EPA, 2007). Several *in vitro* methods have been developed to rapidly and inexpensively predict bioavailability for individual soils. Those that have been calibrated with robust *in vitro-in vivo* correlations (IVIVC) can be used to better evaluate risk from exposure (Zia et al., 2011).

Using bioavailability in ecological risk assessment would improve understanding of exposure pathways and potentially reduce the cost of remediation efforts. *In vitro* methods could be used to rapidly assess bioavailability to animals, just as is currently done for human health risk assessment. Work done by Beyer et al. found that some *in vitro* methods developed for predicting bioavailability in humans were also predictive for Japanese quail fed diet mixed with Pb-contaminated soil (2016). Two methods were found to accurately predict Pb bioavailability: Ohio State University *In vitro* Gastrointestinal method, gastric phase (OSU IVG GE) and USEPA Method 1340, modified to use pH 2.5 instead of pH 1.5 ((Basta et al., 2007; EPA, 2013).

The exposure pathway for human health risk assessment assumes the worst-case scenario for exposure, which is ingestion of contaminated soil while fasting. However, in ecological risk assessment, the exposure pathway is the most-likely scenario, which is ingestion of contaminated diet or incidental ingestion of soil while feeding. Diet, rather than soil, is the main factor influencing bioavailability for wildlife.

Additional information is needed on how diet impacts the bioavailability of Pb. Previous studies have shown that diet can reduce bioavailability of Pb (Ragan, 1983). Dietary components of plant matter such as phosphates, calcium, phytate, fiber, oxalate, and tannins have all been shown to reduce bioavailability of metals. (Dendougui and Schwedt, 2004; Schroder et al., 2004). It has been reported that diet reduces the absorption of water-soluble Pb primarily due to the presence of phosphate and calcium (Heard and Chamberlain, 1982; Rabinowitz et al., 1980). Rose and Quarterman fed rats a diet contaminated with 200 mg/kg Pb supplemented with either 10g/kg phytate, a plant P storage compound, or 6 g/kg Ca and found reduced Pb accumulation in blood, liver, and bone in each case (1984).

Diet reduces bioaccessibility of Pb in soil when added to soil in *in vitro* methods (Basta et al., 2007; Schroder et al., 2004). However, the impact of diet, and particularly of different diets, on *in vitro* Pb bioaccessibility alone yet to be assessed. The aim of the study was to determine whether diet influenced bioaccessibility of lead in *in vitro* methods and whether a correlation could be found between any components of the diet and bioaccessibility.

MATERIALS AND METHODS

Design of the Study

The bioaccessibility of Pb in 15 diets was measured with the two *in vitro* tests found previously to accurately predict the bioavailability of Pb in Japanese quail: the gastric phase of "Ohio State University *In vitro* Gastrointestinal" method (OSU IVG GE) and USEPA method 1340 at pH 2.5. Additionally, the intestinal phase of OSU IVG was performed (OSU IVG IE), and USEPA method 1340 was conducted at pH 1.5, as this is the standard pH used for the method. NIST 2710a Montana I Soil was used as the quality control soil in the extractions. Total

metal content of the diets was determined by acid digestion with HNO_3 and HCl. Three replicates were tested in the total analysis and two replicates were used in the *in vitro* analyses. Elemental analysis was conducted with an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Varian 720, Varian, Inc.)

Diet Preparation

Six different diets were spiked with lead acetate. The diets were chosen because they are standard diets used in wildlife risk assessment. The surface of one kilogram of diet was sprayed with a solution of Pb acetate and mixed, repeating until the diets were spiked according to the amounts given in Table 1. In total 15 diets were analyzed. A 15 gram subsample of each diet was finely ground until <2mm and homogenous, then oven dried at 36ºC for ten days until dry.

Acid Digestion

9.0 mL of trace-metal-grade nitric acid and 3.0 mL of trace-metal grade hydrochloric acid were added to 0.5±0.0005 gram of diet for 24 hours. 38 mL of water was then added to each sample and the samples shaken to homogenize the solution. Samples were then allowed to settle overnight and 10 mL of solution decanted for analysis.

USEPA Method 1340 (EPA, 2013)

1±0.001gram of diet was placed in a 175 mL high density polyethylene (HDPE) bottle. 100±0.5 mL of gastric solution (0.4 M glycine) that had been preheated to 37°C was added. Samples were then placed on a rotator shaker in a 37°C incubator and rotated at 30±2rpm for 1 hour. Solution pH was checked at 30 minutes and adjusted to 1.5±0.05 with dropwise addition of saturated Na_2CO_3 solution or 50% trace-metal-grade HCl solution. A 10mL aliquot of

suspension was collected with a syringe and filtered (0.45 um) after one hour. The extraction solution pH should be pH 1.5±0.5 at the end of the extraction. All samples were between pH 1.49-1.53 at the end of the extraction.

USEPA Method 1340 with pH Modification

This modified procedure was performed as described above except the solution pH was adjusted to pH 2.5±0.05 using dropwise addition of saturated Na_2CO_3 solution or 50% trace-metal-grade HCl solution. The extraction solution pH should be pH 2.5±0.5 at the end of the extraction. All samples were between pH 2.44-2.54 at the end of the extraction.

OSU IVG (Basta et al., 2007), Modified for Use with End Over End Rotator

1±0.001gram of diet was placed in a 175 mL HDPE bottle. 150±0.5 mL of gastric solution (0.1 M NaCl and 1% w/w porcine pepsin) that had been preheated to 37ºC was added. Samples were then placed on a rotator shaker in a 37°C incubator and rotated at 30±2 rpm for 1 hour. The pH was adjusted at 30 minutes to 1.8±0.01 using 50% trace-metal-grade HCl solution. A 10mL aliquot of suspension was collected with a syringe and filtered (0.45 um) after one hour. The extraction solution pH should be pH 1.8±0.1 at the end of the extraction. All samples were between pH 1.77-1.84 at the end of the extraction. Then 2mL of saturated Na_2CO_3 solution, 0.525 g porcine bile, and 0.0525 g porcine pancreatin were added. The pH was immediately adjusted to 6.5±0.02 with dropwise addition of saturated Na_2CO_3 solution or 50% trace-metal-grade HCl solution. The sample was rotated for 2 hours, and the pH adjusted at 1 hour. A 10 mL of aliquot of suspension was collected with a syringe and filtered (0.45 um) after two hours. The extraction solution pH should be pH 6.5±0.5 at the end of the extraction. All samples were between pH 6.57-6.70 at the end of the extraction.

Analysis

IVBA Pb was calculated by dividing the Pb concentration measured in the *in vitro* methods by the total diet Pb content measured in the acid digestion. Whether changes in bioavailability for each method were significant was determined using a one sample t-test comparing mean Pb recovery of all diets with spike recovery. Simple regression analysis was used to identify diet components that demonstrated significant relationships with IVBA Pb. Visual MINTEQ v. 3.0 (Gustafsson, 2012) was used to model potential mineral saturation and formation.

RESULTS AND DISCUSSION

Diet Metal Concentrations

Total digest concentrations of Pb, P, and Ca found by acid digestion for the spiked samples are given in Table 2. The actual Pb content of the spiked samples varied from the intended spikes, but recoveries of other elements were in the expected ranges for the diets, so complete digestion of the sample was verified. Reference diets contained undetectable amounts of Pb, while spiked diets contained between 17 mg/kg and 2053 mg/kg. Diets had high amounts of P and Ca, ranging from 1911 mg/kg P in the grass forage diet (15) to 7,982 mg/kg P in the layer diet (11), and from 58 mg/kg Ca in corn (13) to 29,767 mg/kg Ca in the layer diet (11).

In vitro Bioaccessible Pb

Recovered Pb is given in Table 3, and the IVBA Pb calculated as the amount of recovered Pb over the total Pb is given in Table 4. The gastric phase of OSU IVG did not show significant reductions in IVBA (112-87%, mean 100%, $p > 0.25$). USEPA method1340 at pH 1.5

showed significant reductions in IVBA (85%-100%, mean 92%, $p<0.05$). However, the margin of error for the ICP-OES regression was ±15%, so for practical purposes the reduction was not significant. USEPA Method 1340 at pH 2.5 did show significant reduction in bioaccessibility ($p<0.05$), with IVBA ranging between 45%-85%, with an overall mean of 66%. Grass forage (15) and laboratory rodent diet (12) caused the greatest reductions in IVBA (reductions 50% and 42%, respectively), while corn (13) and oats (14) had the least reductions (15% and 16%, respectively). For the game farm maintenance diet, the proportion of Pb recovered increased with increasing spike amounts. IVBA of some diets, like corn, was relatively unchanged between 1340 at pH 1.5 and pH 2.5. Pb in corn was 95% soluble at pH 1.5 and 80% soluble at pH 2.5. Other diets, including grass forage, showed great reductions in IVBA at the higher pH of 2.5. Pb in grass forage was 100% bioaccessible at pH 1.5, while at pH 2.5 only 50% was recovered.

Bioaccessibility was greatly reduced for the intestinal phase of OSU IVG, with values ranging from -13%-45%, with an overall mean of 14% ($P<0.05$). The Pb values of Diet 7 were near the detection limit of the instrument, which caused variability in the data and the -13% recovery. Blank spike recoveries for OSU IVG IE were 2%, as Pb is largely insoluble at pH 6.5. The game farm maintenance chow (7-10) and layer chow (12) diets had completely precipitated Pb. Other diets had soluble Pb remaining, up to 45% in the case of grass forage (15), indicating that some diets had components which held Pb in solution. Chelating agents, such as organic acids from diets, may be responsible for increased bioaccessibility of Pb in these diets.

Interestingly, grass forage had the least reduction in Pb bioaccessibility for OSU IVG IE, and game farm maintenance chow had the greatest Pb reductions, reversed from 1340 at pH 2.5. Pb in the laboratory rodent diet was the most bioaccessible in 1340 at pH 2.5, and was nearly insoluble in OSU IVG IE (13% Pb recovered). Corn and oats behaved similarly: both had limited

reductions in 1340 at pH 2.5 (80% and 76% soluble, respectively), and had high recoveries in OSU IVG IE (both 35%). For the game farm maintenance diet, Pb was completely insolubilized in OSU IVG IE for all spike amounts.

Bioaccessibility of the OSU IVG IE and 2.5 methods was compared with both diet total and soluble P and Ca for each method. Total P content was found to be strongly correlated ($P<0.0005$) with bioaccessible Pb for the OSU IVG IE method (Figure 1). A strong correlation between total P and OSU IVG IE, with a coefficient of determination (r^2) of 0.98, suggests that P impacts the bioaccessibility of Pb for that method. However, no relationship was found between soluble P or %bioaccessible P and IVBA Pb for OSU IVG IE. Additionally, no relationship was found between IVBA Pb of 1340 at pH 2.5 and total, soluble, and % bioaccessible P, and correlations between IVBA Pb with total, soluble, or % bioaccessible Ca were not seen for either method.

Dietary components that have been previously found to impact the bioavailability of Pb and other metals include phytate and fiber (Rose and Quarterman, 1987; Dendougui and Schwedt, 2004). Estimated phytate values for the diets were compared to bioaccessible Pb for Diets 12-15 (Table 6). No correlation was found, suggesting that in the *in vitro* tests other forms of P present in the diets may also be contributing to bioaccessibility (Figure 7). Similarly, no correlation was found when comparing estimated protein and fiber content of all diets to bioaccessible Pb. Only estimates were used for fiber, phytate, and protein values; true values may produce better correlations. A distinction was not made between soluble and insoluble fiber, the forming having a higher binding capacity for Pb (Ou et al., 1999). Diets are variable in their composition, and what may drive bioavailability reductions in one diet may not in another.

Phytate can reduce the bioavailability of metals, and is broken down by phytase. Phytase is naturally present in seeds and is active between pH 2 and pH 6 (Naves et al., 2012). Commercial feed may have phytase added to aid in P absorption. Diet 11, Layena chicken feed, contained added phytase. Phytase activity may occur in methods that are within this active pH range, potentially impacting Pb binding and correlations between phytate and IVBA Pb.

Mineral Saturation Modeling

A comprehensive elemental analysis of diets was conducted to determine if there were correlations between dietary components and bioavailability. Geochemical modeling of *in vitro* solution chemistry by MINTEQ was used to determine possible oversaturation of minerals. MINTEQ modeling showed that that for 1340 at pH 1.5 and OSU IVG GE all Pb containing minerals were undersaturated and therefore soluble, which was supported by the results of these *in vitro*s. Part of this can be explained by pH: Pb is soluble at low pH, and becomes increasingly insoluble as pH rises. MINTEQ data suggests all Pb minerals were also undersaturated for 1340 at pH 2.5. This was supported by the blank spike recoveries for 1340 at pH 2.5, which averaged 90%. Given pH alone, most diet Pb should have been soluble. However, bioaccessibility ranged between 45-85%, suggesting that dietary components reduced bioaccessibility.

Several Pb containing minerals could have theoretically formed in OSU IVG IE, and all had similar saturation indices (Table 5). Most of these minerals contained P. Chloropyromorphite ($Pb_5(PO_4)_3Cl$) was the mineral that was most likely to precipitate for all diets. The minerals and their saturation indices for the diets were relatively similar, despite there being large differences in bioavailability between the diets (-13%-45%). Again, this suggests that other components besides diet metals were responsible for reducing bioaccessibility

There are several issues that need to be addressed before *in vitro* methods can be used to estimate Pb exposure for wildlife. One limitation of this study was the use of diets spiked with lead acetate, a very soluble form of Pb. In actuality, wildlife would be exposed to Pb through diets contaminated by plant uptake and assimilation of Pb and through contaminated soil, which will have various forms of Pb with different properties. Another potential limitation of using an *in vitro* method for diets with high Ca and P is that these metals are absorbed competitively with Pb in the small intestine. This impact cannot be observed in an *in vitro* as only bioaccessible Pb is measured, i.e. the amount that is available for absorption, not the amount that will actually be absorbed. These elements have higher concentrations in diets than in soil. Diets sampled contained up to 4x more Ca and 13x more P than the median values for US soils (Smith et al., 2013). The quantity of food consumed will be much greater than the amount of contaminated soil incidentally ingested with feeding. Even if diet is contaminated, Pb is not easily accumulated by plants and is phytotoxic, so concentrations in aboveground tissue may be relatively low, generally <100 ppm (Liu et al., 2008; Malar et al., 2014; Chatterjee et al., 2006; Lamb et al., 2010). All of these factors result in high amounts P and Ca to compete with Pb absorption. This may impact the accuracy of an *in vitro* method.

Some diets were difficult to analyze using these methods. A few samples clogged the filters and took more time to extract. If several samples in a batch are difficult to extract and each takes a few minutes to filter, then the measured bioaccessible Pb could change as reaction time increases. The intestinal phase of OSU IVG had to be diluted immediately as many samples precipitated within a day. The low Pb recoveries of this method combined with the inability to analyze undiluted samples caused relative standard deviations to be high in some diets with very low Pb. These factors limit this method's usefulness in evaluating diets.

A method for processing and spiking diets needs to be standardized. Samples were spiked unevenly and most had Pb concentrations that were lower than intended. Diets had different particle sizes, which may have contributed to the poor spiking. Some samples were dusty, and the dust may have absorbed more Pb and then settled to the bottom of the container. Additionally, nonhomogeneous samples that are spiked before processing like the whole oats and cracked corn have layers that are exposed to Pb and layers that are not. Samples such as these may have different interactions and form different Pb minerals during aging than the same samples that were spiked after being ground and homogenized.

Continuing work on this study includes measuring the phytate content of diets to compare to bioaccessibility. Additionally, a meat-based diet will be analyzed. Higher protein diets may interact differently with methods that include pepsin and other proteases, like OSU IVG. Ultimately, in order to be useful *in vitro* methods must be predictive. Bioaccessibility tests need to be calibrated with *in vivo – in vitro* correlation (IVIVC) regressions with animal models.

CONCLUSIONS

Bioaccessibility varied greatly among methods and diets. The greatest reductions were seen in OSU IVG IE, with an average 14% recovery, and reductions were also seen in 1340 at pH 2.5, with an average 66% recovery. No significant reductions were seen with 1340 at pH 1.5 and OSU IVG GE. Some diets decreased Pb bioaccessibility in 1340 at pH 2.5, while some diets increased Pb bioaccessibility in OSU IVG IE. These experiments show that diet does impact *in vitro* bioaccessibility of Pb, and that the effect depends both upon the method and the diet. While total P content of diet appeared to be involved in reducing bioavailability, not all of the impacts

could be explained by P alone. Interactions of dietary components and metals are complex, and no strong relationships were found between a single component and bioaccessibility.

Exposure assessment is a critical part of ecological risk assessment. Considering total diet Pb, rather than bioavailable Pb, often overestimates exposure and therefore risk. Given the large number of Pb contaminated sites, ecological risk assessments should be site specific and accurate to prevent unnecessary remediation from overly conservative assumptions. A better understanding of how diet impacts Pb bioavailability along with the use of *in vitro* Pb bioaccessibility methods could provide more accurate information about potential exposure, reducing remediation costs while ensuring protection for wildlife. Further research is needed to determine if bioaccessible Pb can predict bioavailable Pb in ecological receptors consuming these diets.

TABLES

Table 1. Reference and Spiked Diets.

	Diet	Spike
1	Purina® Game Bird Maintenance Chow	reference
2	Purina® Layena Chicken Feed	reference
3	laboratory rodent	reference
4	large mammal (corn)	reference
5	large mammal (oats)	reference
6	grass forage (timothy, orchard)	reference
7	Purina® Game Bird Maintenance Chow	Pb acetate (20ppm)
8	Purina® Game Bird Maintenance Chow	Pb acetate (100 ppm)
9	Purina® Game Bird Maintenance Chow	Pb acetate (500 ppm)
10	Purina® Game Bird Maintenance Chow	Pb acetate (2500 ppm)
11	Purina® Layena Chicken Feed	Pb acetate (100 ppm)
12	laboratory rodent	Pb acetate (100 ppm)
13	large mammal (corn)	Pb acetate (100 ppm)
14	large mammal (oats)	Pb acetate (100 ppm)
15	grass forage (timothy, orchard)	Pb acetate (100 ppm)

Table 2. Elemental Content and Standard Deviation of Pb, P, and Ca in Spiked Diets.

	Pb	P	Ca
Diet	**mg/kg dry weight** (RSD)		
7	**17** (2.9%)	**6947** (0.6%)	**11147** (16%)
8	**85** (2.7%)	**6855** (1.5%)	**9894** (1.8%)
9	**320** (2.3%)	**7011** (1.1%)	**10470** (1.3%)
10	**2053** (1.2%)	**6868** (1.7%)	**9724** (2.9%)
11	**77** (1.7%)	**7511** (0.7%)	**28687** (2.2%)
12	**87** (4.3%)	**6160** (0.4%)	**8422** (0.4%)
13	**91** (1.8%)	**3014** (2.3%)	**88** (4.9%)
14	**90** (10.4%)	**3286** (3.6%)	**667** (6.4%)
15	**150** (2.9%)	**1767** (1.9%)	**6043** (2.6%)

Table 3. Recovered Pb and Relative Standard Deviation for USEPA Method 1340 at pH 2.5 and pH 1.5, and OSU IVG GE and IE.

Method		USEPA 1340 2.5	USEPA 1340 1.5	OSU IVG GE	OSU IVG IE
Solution pH		2.5	1.5	1.8	6.5
Diet	Total Pb (mg/kg)	**Recovered Pb mg/kg** (RSD)			
7	17	**7.6** (23%)	**14** (11%)	**15** (4.4%)	**0.5** (-19%)
8	85	**60** (2.6%)	**73** (3.3%)	**88** (2.9%)	**3.2** (87%)
9	320	**257** (0.5%)	**292** (3.3%)	**332** (0.9%)	**13** (0.2%)
10	2053	**1749** (0.5%)	**1965** (3.8%)	**2296** (0.9%)	**77** (2.5%)
11	76	**47** (0.1%)	**68** (3%)	**67** (2.5%)	**7.5** (26%)
12	87	**42** (1.6%)	**78** (3.8%)	**83** (3.5%)	**33** (6.6%)
13	91	**73** (0.1%)	**86** (4.9%)	**99** (0%)	**42** (12%)
14	90	**68** (4.8%)	**83** (2.1%)	**93** (0.6%)	**40** (12%)
15	150	**75** (2.6%)	**150** (1.1%)	**153** (2.9%)	**75** (8.5%)

Table 4. Comparison of Bioaccessible Pb (Pb recovered/total Pb) Determined by USEPA Method 1340 at pH 2.5 and pH 1.5, and OSU IVG GE and IE.

Method		USEPA 1340 2.5	USEPA 1340 1.5	OSU IVG GE	OSU IVG IE
Solution pH		2.5	1.5	1.8	6.5
Diet	Diet Pb (mg/kg)				
7	17	45%	85%	87%	-13%[a]
8	85	71%	86%	103%	2%
9	320	80%	91%	104%	2%
10	2053	85%	96%	112%	2%
11	77	61%	89%	88%	3%
12	87	48%	90%	95%	13%
13	91	80%	95%	109%	35%
14	90	76%	92%	103%	35%
15	150	50%	100%	102%	45%
Blank Spike Recovery (150 ppm)		90%	88%	100%	2%
Mean Pb bioaccessibility		66%	92%	100%	14%

[a] Pb content near detection limit of instrument, which caused variability in the data and the negative recovery

Table 5. Theoretical Saturation Indices for Pb Minerals in OSU IVG IE (pH 6.5) Using Visual MINTEQ.

		Saturation Index (log IAP-log Ks)								
		Diet								
Mineral	Formula	9	10	11	12	13	15	17	18	19
Chloropyromorphite	$Pb_5(PO_4)_3Cl$	10.3	12.4	13.7	16.8	9.3	8.6	16.1	9.5	13.0
Plumbgummite	$PbAl_3(PO_4)_2(OH)_5 \cdot H_2O$	7.4	8.0	7.8	8.8	-8.9	-12.1	8.8	-12.6	-9.2
Tsumebite	$Pb_2Cu(PO_4)(SO_4)(OH)$	-2.9	-2.1	-1.5	-.3	3.5	3.1	-1.4	3.0	4.7
Hydroxylpyromorphite	$Pb_5(PO_4)_3OH$	0.3	2.4	3.8	4.1	3.1	2.9	6.1	3.9	7.1
	$Pb_3(PO_4)_2(s)$	0.2	1.4	2.3	4.1	0.3	0.1	3.7	0.6	2.6
	$Pb_2(OH)_3Cl(s)$	-6.4	-5.6	-5.0	-3.8	0.4	0.4	-1.6	0.9	2.0
	$PbMoO_4(s)$	-0.4	0.2	0.3	0.9	-2.1	-2.7	-8.1	-3.3	-1.9

Table 6. Estimated Phytate Values for Diets 12-15.

Diet	Total P (mg/kg)	Phytate P (%)	Phytate (mg/kg)
12	6160	28% [a]	2464
13	3013	57% [b]	1730
14	3286	70% [c]	2300
15	1766.8	10% [d]	177

[a] Laboratory rodent diet 5001, Lab Diet
[b]Tahir et al., 2012.
[c]Ashton and Williams, 1958.
[d]Alkarawi and Zotz, 2014.

FIGURES

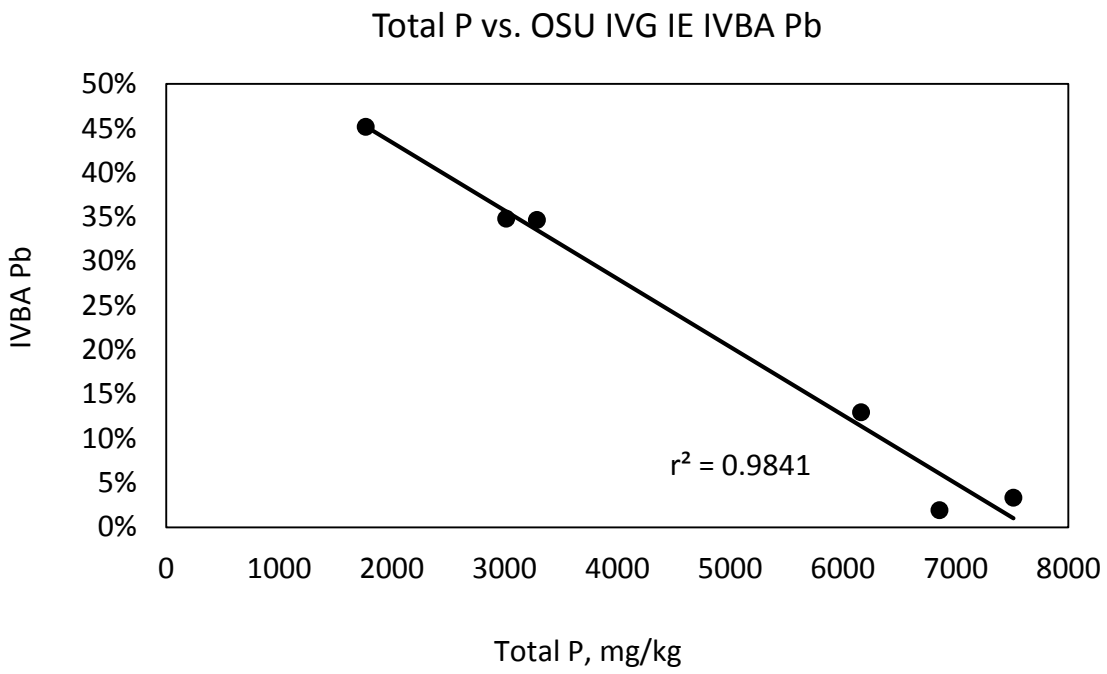

Figure 1. Comparison of Total P and OSU IVG IE Bioaccessible Pb.

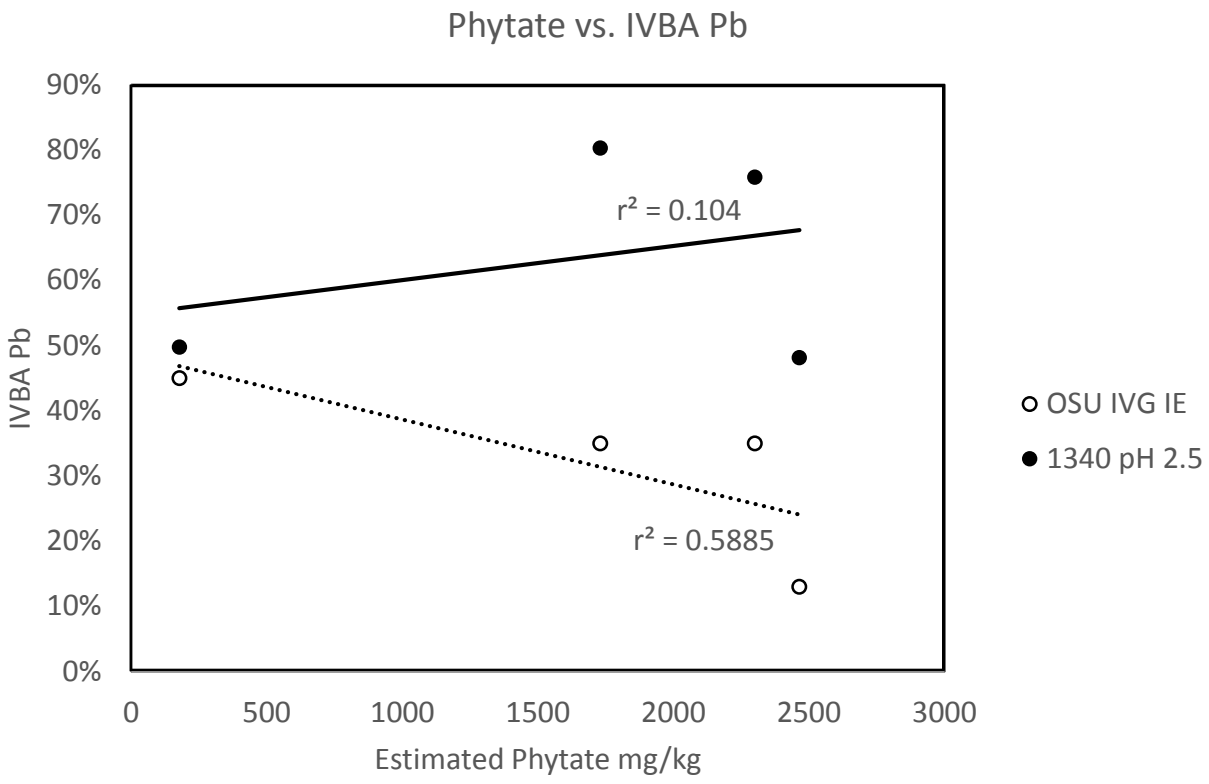

Figure 2. Comparison of Estimated Phytate and IVBA Pb for OSU IVG IE and USEPA 1340 at pH 2.5.

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