

SOIL INGESTION AND LEAD CONCENTRATION IN WILDLIFE SPECIES

by

Erin E. Connor

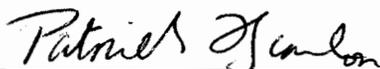
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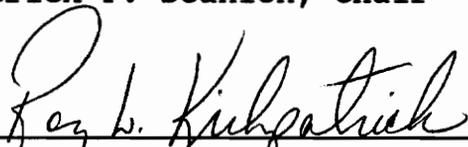
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Committee Chair: Patrick F. Scanlon  
Fisheries and Wildlife Sciences

## ABSTRACT

Problems related to estimating soil ingestion by wildlife species from analysis of feces were examined. Soil ingestion was investigated as a means by which wildlife may be exposed to environmental contaminants, particularly Pb.

Titanium (Ti) and acid-insoluble residue (AIR) tracer methods for estimating soil ingestion were compared. The two methods were not significantly ( $P > 0.05$ ) different when diet consisted of 10% (dry weight, d.w.) soil. When diet contained 5% soil, soil ingestion was more accurately estimated using the Ti method. Digestibility of soil can be ignored in the equation for quantifying soil ingestion from analysis of feces.

Soil ingestion, as percentage dry matter intake (% DMI), by five wildlife species was estimated from analysis of feces or intestinal contents using the Ti tracer method. Soil ingestion by mourning doves (Zenaida macroura) was estimated using AIR analysis of crop contents. Mean ( $\pm$  S.E.) soil ingestion by Canada geese (Branta canadensis) and mallards (Anas platyrhynchos) were 4.92 ( $\pm$  0.60) and 11.73

( $\pm 1.54$ ), respectively. Diets of short-tailed shrews (Blarina brevicauda) consisted of 5.20 ( $\pm 1.87$ )% soil, white-footed mice (Peromyscus leucopus) 16.21 ( $\pm 4.85$ )%, meadow voles (Microtus pennsylvanicus) 2.01 ( $\pm 0.34$ )%, and mourning doves 0.83 ( $\pm 0.41$ )% soil.

Mallards collected from Killarney Lake, northern Idaho were analyzed for free erythrocyte protoporphyrin, fecal, liver, and kidney Pb concentrations, and soil/sediment ingestion. Sediment from the area contained 4485 ppm Pb (d.w.). Protoporphyrin was a poor indicator of Pb contamination at this level of exposure and/or under these conditions. Soil/sediment ingestion by mallards averaged 7.5% DMI. Tissue Pb concentrations indicated mallards were suffering from chronic exposure to low concentrations of Pb.

Sediment collected from Killarney Lake (4485 ppm Pb) was fed to northern bobwhites (Colinus virginianus) at 8% DMI for 21 d. Lead concentrations in blood, liver, and kidneys were determined and compared to control values. Treated and control birds showed no significant decline ( $P > 0.05$ ) in feed intake and body mass did not change by greater than  $\pm 1\%$  over time. Lead in treated birds averaged 7 ppm (d.w.) in liver, 30 ppm (d.w.) in kidneys, and 126 ppb (wet weight, w.w.) in blood. Liver and kidney Pb concentrations of controls were  $< 0.1$  ppm (d.w.) and averaged 630 ppm (w.w.) in blood.

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## INTRODUCTION

Studies have demonstrated that soil ingestion, both voluntary and involuntary, occurs in domestic and wildlife species (Healy, 1967, 1968, 1969; Arthur and Alldredge, 1979; Arthur and Gates, 1988). Ingested soil may serve as a potential source of contaminant uptake. Tracer methods used to estimate soil ingestion by animals from their feces have several limitations. These techniques use substances that are predominant in soil but scarce in plant and animal tissues, such as the elements Al, Si, Ti or acid insoluble residue (AIR), as a tracer for soil. The concentration of tracer in the feces of the animal is measured and then the quantity of soil in the diet is estimated. The parameters of the equation for quantifying soil ingestion are dry matter digestibility of the diet (metabolizability in the case of avian species), the concentration of tracer substance in the soil consumed by the animal, and the concentration of tracer substance in the diet.

Several problems arise in estimating the above values. First, in order to estimate digestibility of the diet of an animal, the contents of the diet must be known. However, the range of diet components is wide among omnivorous species, and diets vary with food availability throughout the year. In addition, digestibility of a diet is

influenced by the stage of maturity of plants, the nutritive ratio of the diet, and the level of nutrition of the animal (Maynard et al., 1979). Furthermore, each feed ingredient in a diet may influence digestibility of the others (Maynard et al., 1979).

Secondly, in order to estimate the concentration of tracer in the soil consumed by an animal, representative soil/sediment samples must be available for analysis from the area where ingestion occurred. In highly mobile species, such as birds, the area of feeding may be large. Over great distances, the concentration of tracer substance in the soil may vary considerably. The concentration of tracer also varies depending upon the particle size of the soil analyzed. Therefore, it is difficult to accurately estimate tracer concentration in the soil ingested by an animal.

Thirdly, it is difficult to estimate the concentration of tracer originating in the diet itself. Acid insoluble residue concentration of plants varies among species and depends upon the stage of development of plants within the same species. Titanium concentration of living tissues is extremely low in comparison to soil concentrations and is generally ignored in the soil ingestion estimation equation.

Fourthly, other factors not accounted for in the soil ingestion equation limit the predictability of the tracer

method. For example, it is not known whether the organic matter present in the ingested soil contributes significant digestible material to the diet, for which an adjustment should be made. Further, in the case of birds that ingest grit, the grinding of grit in the gizzard constantly adds small amounts of tracer to the feces. On the other hand, the gizzard of a bird may act as a sink for ingested soil and in effect, reduce the concentration of tracer found in the feces relative to the level of soil ingested. It should also be noted that the action of grit may increase digestibility of coarse feed by as much as 10% (Titus, 1955, not seen, cited in Sturkie, 1976).

This thesis clarifies some difficulties in estimating avian soil ingestion. The Ti and AIR tracer methods will be compared, digestibility of soil will be tested, and uptake of Pb from contaminated sediments will be measured in wild mallards and laboratory northern bobwhites.

## CHAPTER 1: Evaluating Methods for Quantifying Soil Ingestion by Wildlife Species

### Summary

The Ti and AIR tracer methods for estimating soil ingestion as percentage DMI by northern bobwhites (Colinus virginianus) were compared by feeding birds a known concentration of soil in the diet and analyzing feces using the two methods. The two methods were not significantly ( $P > 0.05$ ) different when the diet consisted of 10% (dry weight) soil. When diets contained 5% soil, soil ingestion was more accurately estimated using the Ti method.

Detection limits of the AIR method were 3.8% for a 0.25 g sample and 2.7% for a 0.5 g sample. Thus, the AIR method would best be applied to samples exceeding 0.5 g (d.w.) in mass and expected to contain high soil concentrations. The Ti method required more sophisticated sample preparation, more technical training, and expensive laboratory equipment. The AIR method was preferred over the Ti method in terms of low cost and fast rate of turnover of results.

Apparent dry matter metabolizability of a soil containing 56% organic matter was estimated at 13%. It was concluded that digestibility contributed by soil ingested by an animal can be ignored in the equation for quantifying

soil ingestion from analysis of feces because the organic matter concentration of soil is generally < 10% and percentage soil in the diet is generally low. Thus, digestible material contributed by the soil fraction of the diet is insignificant compared to digestible material contributed by food materials.

## **Introduction**

### **Tracer Technique for Quantifying Soil Ingestion**

The quantity of soil ingested by an animal can be estimated from the concentration of soil measured in feces. Fecal soil concentration is generally determined in one of two ways. The first method spectrophotometrically measures the concentration of an element in feces that is predominant in soil but not in plant or animal tissues and, thereby, can be used as a tracer for soil. The element most commonly used as a tracer is Ti, although Si and Al also have been used with varying success. The tracer should not be absorbed or metabolized by the animal. Generally, feces and representative soil samples are dried, homogenized, digested in acid, and the concentration of the tracer element is measured spectrographically. Soil intake (S.I.) as percentage of dry matter intake (% DMI) can then be estimated using an equation like the following (from

Fleming, 1986) where Ti was used as a tracer:

Equation 1.1

$$S.I. (\%) = \frac{(1-D) Ti_f}{Ti_s - D \cdot Ti_f} \times 100$$

where:

- D = dry matter digestibility of the diet (%)
- Ti<sub>s</sub> = Ti content of soil (ppm)
- Ti<sub>f</sub> = Ti content of feces (ppm)

### The Use of Ti, Si, and Al as Tracers

There are problems in using both Al and Si as tracers, particularly in studies involving wildlife species. Jones and Handreck (1965) found that when Si is fed to sheep (Ovis aries) as a constituent of pasture plants, recovery of Si in feces is close to 100% when Si content of the diet is 0.94 to 2.84% DMI. However, the proportion of Si excreted in urine increased with decreasing Si content of the diet. Thus, the element is absorbed from the alimentary tract. Overall, Si can be used as a tracer in quantifying soil ingestion. However, collection of both feces and urine must be done. In avian species, this is not a problem. In addition, one must account for the proportion of Si in feces and urine originating from vegetation in the diet as opposed to soil intake. In studies involving wild mammalian species, both conditions are impossible to meet.

Furthermore, a study by Greger and Baier (1983) on humans involving feeding Al at 5 mg/day and 125 mg/day revealed that the element is absorbed from the gastrointestinal tract as shown by elevated Al concentrations in blood sera. However, the total amount of Al ingested was almost equal to that excreted in urine and eliminated in feces. Similar to Jones and Handreck's (1965) study with Si, the proportion of Al eliminated by way of urine was greater in subjects on a low Al diet (26%) than those on a high Al diet (14%). Allen et al. (1986; 1991) demonstrated that Al added to the diet is absorbed and retained by ruminants, although absorption of Al from soil appears to be low. Aluminum may also be accumulated in forage plants under certain conditions (Muchovej et al., 1986), making Al a poor tracer for soil, particularly in wildlife species in which the Al content of the feed cannot easily be determined. The conditions under which Si and Al have value as tracers seem to be sufficiently demanding that their use with wildlife species, both mammalian and avian, is precluded.

Therefore, of the candidate elements, Ti appears to be the best tracer for both mammals and birds (if one is to develop one consistent tracer). However, there are some weaknesses in using Ti as a tracer. First, some Ti in the diet may be absorbed by the animal, although the quantities do not appear to be nearly as substantial as with Al and Si.

Binder et al. (1986) compared the use of Ti, Si, and Al in estimating soil ingestion of children. They found that estimates were 10 times greater when using Ti as a tracer than when using Si or Al. The difference probably was due to the fact that only fecal samples were collected from the children. As a result, the amounts of Si and Al excreted in urine were not accounted for, making it appear as though soil ingestion was much lower in these individuals.

Secondly, Ti concentrations of plant food materials are generally less than 1 ppm and are not incorporated into the soil ingestion estimation equation. However, ignoring the Ti content of plants would not always be appropriate. Titanium concentrations averaging 8.8 ppm were measured in forages of mule deer by Arthur and Alldredge (1979). Forage samples were washed with water in an ultrasound bath prior to analysis and, therefore, were free from surface contamination of soil. Thus, a correction factor for the quantity of Ti coming from the diet itself had to be added to their equation for estimating soil ingestion. Thirdly, the abundance of Ti in soils may vary considerably from place to place. Soils in the U.S. may contain 300 to 15,000 ppm Ti although they average 3000 ppm (Shacklette et al., 1971). This variation can greatly affect estimates in cases where animals, particularly birds, move over large areas within a single feeding period and consume soil from several

different locations, or ingest sediments often carried by water over great distances from various sources. Titanium concentration of soils also varies with particle size. Brebner et al. (1985) found that Ti concentration of topsoil decreases as particle size decreases to 20 to 50  $\mu\text{m}$  but the finest fraction ( $< 20 \mu\text{m}$ ) was richer in Ti than intermediate fractions. Thus, the heterogeneity of Ti distribution in soil fractions limits predictability of soil ingestion from the tracer technique. Despite these weaknesses, Ti measurement is presently the preferred method for estimating low soil concentrations in the diet.

#### **The Use of AIR as a Tracer**

The second approach to estimating fecal soil concentration is to measure percentage AIR of feces. Fecal samples are dry-ashed, leached in concentrated acid, then ignited in a muffle furnace. Soil intake (S.I.) as % DMI is then estimated using the following (from Beyer et al., in review):

Equation 1.2

$$S.I. (\%) = \frac{A_d - A_f + DA_f}{DA_f - A_s + A_d}$$

where:

$A_f$  = AIR (ash/dry weight) in feces

D = dry matter digestibility of feed (%)

$A_d$  = AIR (ash/dry weight) in feed without  
soil

$A_s$  = AIR (ash/dry weight) in soil

The major problems with using AIR to estimate soil ingestion from feces are in estimating AIR concentration of feed and in estimating digestibility of feed. Both can be highly variable in diets of wild species, particularly in omnivores whose diets are extremely unpredictable. Even diets of herbivores vary greatly in AIR content. Mayland et al. (1975) measured AIR concentration of three forage species - squirreltail (Sitanian hystrix), cheatgrass (Bromus tectorum), and bluegrass (Poa secunda) in southern Idaho. Acid insoluble residue concentrations were found to differ greatly among species as well as within the same species throughout the year. The AIR concentration of the three species varied between 1 and 13% from June to November.

In the case of birds, deliberate ingestion of grit further complicates the issue of AIR measurement. As grit is degraded by mechanical action of the gizzard, acid insoluble particles may be added to the digesta increasing fecal AIR concentration. This results in an overestimation of soil ingestion by avian species. Furthermore, this approach requires an estimate of AIR concentration of soil,

which varies among different soil types with percentage organic matter and particle size (McGrath et al. 1982). This becomes particularly important when estimating soil ingestion by highly mobile species such as birds or those that may have ingested feeds contaminated with high organic soils or sediments.

### **Estimating Diet Digestibility**

When estimating soil ingestion based on fecal analysis for any tracer element, digestibility of the diet consumed must be considered in order to account for the loss of mass through digestion. In domestic species in which the contents of the diet are known and the location of feeding is within a small area and/or is essentially constant, it is relatively easy to accurately estimate these values. However, diets of many wildlife species are extremely variable because they are opportunistic feeders. Thus, it is difficult to accurately predict what digestibility of the diet will be.

### **Digestibility of Soil**

Neither the Ti or AIR methods take into account digestibility of the organic fraction of ingested soil. In most cases, this is not a problem since mineral soils are generally < 10% organic matter. However, some soils in

wetland areas may easily reach 50% organic matter and, therefore, contribute significant amounts of potentially digestible soil material to the diet. Digestibility of the organic fraction of soil may need to be accounted for in the equation for estimating soil ingestion in many instances, e.g., with high organic soils or with ingestion of sediment.

Overall, it would be useful to determine whether the AIR method or the Ti method is a more accurate and useful technique in estimating soil ingestion by animals. In addition, digestibility of highly organic soil should be investigated in order to determine whether it should be considered as a variable in the equation for calculating soil ingestion from analysis of feces.

**Experiment 1: Comparison of Ti and AIR Methods for  
Estimating Soil Ingested in the Diet From  
Analysis of Feces**

- Objectives:**
- (1) To determine whether Ti or AIR methods give a more accurate estimate of soil ingestion by captive animals.
  - (2) To determine which technique is a more practical approach in studies involving wildlife species.
  - (3) To determine apparent dry matter

metabolizability of a soil for an avian species.

## Methods

Soil high in organic matter concentration (56%) was fed to northern bobwhites to determine the metabolizability of the soil organic fraction and to compare the Ti and AIR methods for estimating soil intake from the analysis of feces. Soil was collected from Knowles Marsh 1, Patuxent Wildlife Research Center, Laurel, MD. Soil was dried at 55°C and passed through a #20 sieve (840 $\mu$ m). Organic matter concentration of the soil was determined by loss of ignition as described by Bear (1964). Acid insoluble residue concentration of the soil was determined using methods of Stafford and McGrath (1986) and Ti concentration was determined by flameless atomic absorption spectrophotometry using a Perkin-Elmer Zeeman 5100 atomic absorption spectrophotometer with an HGA-600 graphite furnace and an AS-60 autosampler following acid digestion. The method detection limits for AIR for 0.25 and 0.5 g (d.w.) samples was calculated using methods recommended by the U.S. Environmental Protection Agency (EPA) (1982), as the standard deviation measured for a series of spiked samples multiplied by the Student's t value for a probability of

0.01. Soil was added to ground poultry ration (Southern States Gamebird Starter Grower Medicated) at 0% (control), 5% and 10% of dry weight. Grit was not added to the diet. Refer to APPENDIX A for the ingredients and analysis of the poultry ration.

Ten subsamples each of the 0%, 5%, and 10% soil added diets were analyzed for AIR and Ti content. The mean AIR and Ti concentrations of the 0% soil added diet were subtracted from the concentrations obtained from the analysis of each subsample of the 5% and 10% soil added diets to correct for AIR and Ti contamination of the diet prior to the addition of soil. The actual percentage soil in the 5% and 10% soil added diets was then calculated as the mean of each subsample's AIR or Ti concentration divided by the respective concentration in the added soil, multiplied by 100.

To determine the accuracy of soil ingestion estimates from feces, 30 northern bobwhites (14 males, 16 females) obtained from Wildlife International, Easton, MD were assigned at random to one of three treatment groups at approximately 18 w of age. All birds were housed individually in hanging wire laying cages (51 cm x 30 cm x 25 cm) and were provided with individual food and water containers. Light was maintained at 13L:11D throughout the study.

Six days prior to the experiment, all birds were placed on the control diet to acclimate them to a ground ration. On d 1 of the study, each treatment group was given the appropriate diet (0%, 5% or 10% soil) ad libitum. On d 4, a low absorbent paper liner (KRAFT brown wrapping paper) was placed under each cage. On d 5, excreta uncontaminated by spilled feed was collected from each tray.

Individual excreta samples were dried at 55°C for 24 h. Each sample was crushed and mixed with a mortar and pestle and divided into two subsamples, one for Ti analysis and one for AIR analysis. The percentage soil ingested was estimated using Equations 1.1 and 1.2 and compared to the concentration of soil measured in the diet. For the comparison of estimated soil ingestion to actual soil intake, it was assumed that any Ti or AIR measured in the feces came from the presence of soil in the diet and not from the diet itself since there was no way of knowing whether the AIR and Ti present in the poultry ration prior to the addition of soil was from soil and dust contamination or the diet itself. Therefore, in Equation 1.2,  $A_d = 0$ . The mean dry matter metabolizability for each diet estimated in this experiment was used as the digestibility estimate in Equations 1.1 and 1.2.

On d 6 of the study, a clean paper liner was placed under each cage. For d 6, 7, and 8, the amount of feed

consumed by each bird was determined by weighing the amount of feed given and subtracting the amount of feed remaining the following day. On d 9, the total amount of excreta in each tray was collected, dried at 55°C, and weighed. Spilled feed was collected, weighed, and the weight subtracted from the total feed intake. The apparent percentage dry matter metabolizability (X) of each diet was estimated using the following equation:

Equation 1.3

$$X = \left(1 - \frac{E}{C}\right) \times 100$$

where:

E = g excreta produced

C = g feed consumed

### **Preparation of Samples for Atomic Absorption**

#### **Spectrophotometry**

Samples were dried at 55°C for at least 24 h. Approximately 0.25 g of sample was weighed using an analytic balance accurate to 0.0001 g into an acid-washed 50 ml PYREX graduated digestion tube. Samples were done in duplicate whenever adequate sample was available. Nitric acid, (4 ml HNO<sub>3</sub> Fisher reagent grade) was added to each sample and allowed to stand at room temperature for at least 24 h. Perchloric acid (2 ml HClO<sub>4</sub> Fisher reagent grade) was added

to each sample (Muchovej et al., 1986). Tubes were heated in a digestion block and digested until samples became colorless and approximately 1 ml of liquid remained (approximately 8 h). Samples were cooled and diluted with deionized water to 12.5 ml. Any further dilutions were made with 0.1 N nitric acid. At least two blanks were included with every 58 samples digested. Standard solutions (FisherChemical Atomic Absorption Reference Standard Solution) were prepared in 0.1 N nitric acid.

### **Statistical Analyses**

Statistical analyses were conducted with Statistix Version 3.5 (Analytical Software, 1991). All comparisons of means were made using a Mann-Whitney U-test. Alpha was set at 0.05 for all statistical inferences.

### **Results**

#### **Comparison of Ti and AIR Methods for Estimating Soil Ingested in the Diet From the Analysis of Feces**

The Knowles Marsh I soil contained 55.6% organic matter, 39.0% AIR, and 693 ppm Ti (Table 1.1). The method detection limit for AIR was found to be 3.80% and 2.71% for a 0.25 and 0.5 g sample, respectively. The estimated

Table 1.1. Characteristics of the Knowles Marsh I soil added to the diet

Characteristic	n	Mean $\pm$ S.E.
Organic Matter, %	3	55.6 $\pm$ 0.96
AIR, %	5	39.0 $\pm$ 1.22
Ti Concentration (ppm)	5	693.3 $\pm$ 41.40

percentage soil of each diet was significantly different ( $P < 0.001$ ) between the two methods at all three dietary soil concentrations. The mean ( $\pm$  S.E.) Ti and AIR concentrations of the 0% soil added diet were 23 ( $\pm$  1) ppm and 0.76 ( $\pm$  0.05)%, respectively. When Ti and AIR concentrations measured in the 0% soil added diet were subtracted from results of the analyses of the 5% and 10% soil added diets, it was found that the AIR method gave the most accurate and consistent results (Table 1.2). The percent error in using the AIR method was  $< 3\%$  whereas percent error using the Ti method was as great as 47%. The coefficients of variation (C.V.) using the two methods were practically equal for the 5% soil added diet. However, the C.V. using the Ti method was over three times the C.V. using the AIR method for the 10% soil added diet. The Ti method consistently overestimated percentage soil added to the diet and the AIR method consistently underestimated percent soil added to the diet.

Estimates of soil ingestion based on fecal analysis of each bird using the Ti method at the 0% and 5% soil added levels were significantly different ( $P < 0.01$ ) from percentage soil actually in the diet as determined by Ti analysis (Table 1.3). Estimates of soil ingestion at the 0%, 5%, and 10% soil added levels were significantly different ( $P < 0.001$ ) from percentage soil measured in the

Table 1.2. The actual percent soil (Mean  $\pm$  S.E.) measured in the 0%, 5%, and 10% soil added diets

Percentage Soil Added to the Diet	N	<u>Ti Method</u>		<u>AIR Method</u>	
		Actual Percentage Soil Measured in the Diet <sup>a</sup>	% Error	Actual Percentage Soil Measured in the Diet <sup>a</sup>	% Error
0	10				
5	10	7.35 $\pm$ 0.49	+47.0	4.93 $\pm$ 0.33	-1.4
10	10	12.49 $\pm$ 0.72	+24.9	9.78 $\pm$ 0.17	-2.2

<sup>a</sup> The mean AIR and Ti concentrations of the 0% soil added diet were subtracted from the AIR and Ti concentrations measured in the subsamples of the 5% and 10% soil added diets to account for AIR and Ti present in the diet prior to the addition of soil.

Table 1.3. Comparison of percentage soil measured in the diet (Mean  $\pm$  S.E.) to estimated percent soil in diet (Mean  $\pm$  S.E. ) from analysis of feces

Percentage Soil Measured in Diet <sup>a</sup>	<u>Ti Method</u>		<u>AIR Method</u>		P-value
	Estimated Percentage Soil in Diet <sup>b</sup> from Feces	P-value	Percentage Soil Measured in Diet <sup>a</sup>	Estimated Percentage Soil in Diet <sup>b</sup> from Feces	
3.33 $\pm$ 0.15	2.05 $\pm$ 0.14	< 0.001	1.99 $\pm$ 0.13	7.27 $\pm$ 0.24	< 0.0001
10.68 $\pm$ 0.49	7.17 $\pm$ 0.88	< 0.010	6.92 $\pm$ 0.33	9.50 $\pm$ 0.51	0.0005
15.82 $\pm$ 0.72	16.34 $\pm$ 1.93	0.678	11.77 $\pm$ 0.17	15.50 $\pm$ 0.78	0.0009

<sup>a</sup> All Ti and AIR present in the diet was assumed to be from soil and not the diet itself.

<sup>b</sup> Based on values obtained from Equations 1.1 or 1.2.

diets using AIR analysis (Table 1.3). However, at the 10% soil concentration, there was no significant difference ( $P > 0.05$ ) between those estimates determined by the AIR method and those obtained using the Ti method. Estimates at the 0% and 5% soil concentrations using both methods were significantly different ( $P < 0.02$ ) from one another. When estimated percentage soil in the diet from the analysis of feces from the 0% soil added diet was subtracted from the 5% and 10% soil added diet estimates, the corrected estimates of soil ingestion became 0%, 5.12%, and 14.29% for the Ti method and 0%, 2.23%, and 8.23% for AIR.

#### Determining Apparent Dry Matter Metabolizability of Soil

Apparent dry matter metabolizability of the 0%, 5%, and 10% soil diets were 56.74, 58.50, and 52.38%, respectively (Table 1.4). Apparent dry matter metabolizability of the soil fraction in the 5% soil diet could not be determined from metabolizability of the 0% and 5% diets because metabolizabilities of the two diets were not significantly different ( $P > 0.05$ ) from one another. However, dry matter metabolizability of the 10% soil diet was significantly different ( $P < 0.05$ ) from the 0% soil diet. Dry matter metabolizability of the soil fraction of the 10% diet ( $M_s$ ) was calculated as 13% (i.e. 13% of 100% soil which in turn was 55.6% organic matter) using Equation 1.4.

Table 1.4. Apparent dry matter metabolizability (Mean  $\pm$  S.E.) of 0%, 5%, and 10% soil added diets in northern bobwhites

Diet	N	Percentage Dry Matter Metabolizability
0%	10	56.74 $\pm$ 0.43 <sup>a</sup>
5%	10	58.50 $\pm$ 1.42 <sup>a</sup>
10%	10	52.38 $\pm$ 0.72 <sup>b</sup>

<sup>a,b</sup> Column means having different superscripts are significantly different ( $P < 0.05$ ) from one another.

Equation 1.4

$$M_s = \frac{M_0 - (0.90 \times M_{10})}{0.10}$$

where:

$M_0$  = % metabolizability of the 0% soil diet

$M_{10}$  = % metabolizability of the 10% soil diet

Thus, metabolizability of the organic fraction of the soil was as great as 23.4% (assuming the inorganic fraction of the soil was not metabolizable).

## Discussion

### Comparison of Ti and AIR Methods for Estimating Soil Ingested in the Diet From the Analysis of Feces

Based on results of the diet analysis using Ti and AIR methods, it appeared that AIR was the preferred method. Standard errors of the means of the 0%, 5%, and 10% soil added diets were consistently lower using the AIR method and values were closer to the actual percentage soil in the diet. In estimating dietary soil concentration from fecal analysis however, it was found that when the diet was 10% soil, the results obtained by the two methods were not significantly different. Yet when the soil concentration of the diet was 5%, the Ti method was more accurate.

A diet that contains < 5% soil probably is not unusual

and does not pose much risk to an animal except in cases where contamination of the soil is extremely high. Diets containing 5 - 10% soil may pose a risk to animals in highly contaminated areas. Soil ingestion at these levels would best be estimated using the Ti method. At high levels of soil ingestion, it appears that the AIR and Ti methods estimate soil ingestion equally well. The fact that the concentration of soil added to the diet could be estimated accurately by AIR analysis of the diet but that the AIR method could not accurately estimate percentage soil in the diet from analysis of feces at low concentrations of soil intake (but consistently underestimated percentage soil in the diet [corrected values]), suggests that AIR is absorbed at low concentrations by animals. The greatest effect is seen when soil ingestion is low.

Thus, it appears that AIR analysis can be used in place of Ti analysis only in cases where the sample mass to be analyzed is at least 0.5 g, AIR concentration of the diet without soil can be estimated, and the quantity of soil ingested is expected to be high (> 10%). The Ti method should be used in all other cases. The AIR method has many advantages over the Ti method including very simple, low cost sample preparation and analysis, little technical training required to analyze samples, quick turn-over of results, limited use of reagents and glassware, and a lesser

chance of sample contamination. In addition, both Ti and AIR methods depend upon estimating Ti or AIR concentration of the soil in the area where the animal was feeding. As previously mentioned, the location of feeding is impossible to pinpoint in many wildlife species, particularly birds. Therefore, getting an exact value for Ti or AIR concentration of the soil where ingestion occurred is impossible. Finally, Ti concentration of soils can range from 300 to 15,000 ppm (Shacklette et al., 1971) whereas AIR concentration of most soils is probably between 90 and 100%. Therefore, the chances of an estimate for Ti concentration of the soil in the location of feeding being unrepresentative are greater than that for the concentration of AIR in the soil being incorrect. On the other hand, the AIR method necessitates estimating the concentration of AIR coming from the diet whereas the Ti method does not require an estimate of Ti concentration of the diet in most cases. In this experiment, the diet without soil added contained a moderate concentration of Ti, but the Ti likely came from dust or low level soil contamination and not the food itself. Acid insoluble residue, on the other hand, could have come from dust or soil contamination or the food itself since plant materials often contain AIR.

In conclusion, the method of choice depends upon several factors including the species of interest and its

diet, the required accuracy of the soil ingestion estimate, and the equipment and personnel available to conduct the analysis.

#### Determining Apparent Dry Matter Metabolizability of Soil

It is extremely difficult to determine the true metabolizability of a diet consisting only of soil for an avian species. Sibbald (1977) found that simple diet mixtures had true metabolizable energy (TME) values similar to those calculated from TME values of the component parts. That is, TME values of feedingstuffs are additive. Thus, this principle was used to determine the percentage dry matter metabolizability of soil alone based on the metabolizability of diets composed of 0%, 5%, and 10% soil. Unfortunately, the metabolizability of the 0% and 5% soil diets were not different statistically so that the metabolizability contributed by the soil fraction could not be determined. The difference between the 0% and 10% soil diets suggested that the soil component was 13% metabolizable.

The purpose of knowing the metabolizability of the soil fraction is to determine whether it needs to be considered in the soil ingestion estimation equation. The equation is based on the assumption that the soil fraction of the diet is indigestible. The study showed that even if soil is

consumed by an animal at 5% DMI and the soil is as great as 56% organic matter, it does not significantly change the metabolizability of the diet. If a diet contains at least 10% soil of which 56% is organic matter, the metabolizability value of the diet will change significantly. As for whether or not this will change the soil ingestion estimate, suppose an animal's diet is 59% digestible, the Ti concentration of the soil in the area is 1000 ppm, and the Ti concentration of the feces is 50 ppm. The estimated soil ingestion would be 2.11%. If the actual digestibility of the diet were 52% as opposed to 59% (the same difference found between the 5% and 10% soil diets in this study; Table 1.4), then keeping all other values constant, the estimate of soil ingestion would be 2.46%. A difference of this magnitude is of minor concern. Under these conditions, the diet digestibility estimate would have to be over- or underestimated by 20% in order to result in a 1% change in the soil ingestion estimate. In conclusion, the digestible dry matter contributed by the soil in the diet need not be accounted for in the soil ingestion estimation equation.

## CHAPTER 2: A Survey of Soil Ingestion by Select Wildlife Species

### Summary

Soil ingestion (% DMI) by six wildlife species was estimated from analysis of feces or intestinal contents using the Ti and AIR tracer methods. The mean ( $\pm$  S.E.) soil ingestion by Canada geese (Branta canadensis) and mallards (Anas platyrhynchos) from the VPI&SU duck pond were 4.92 ( $\pm$  0.60) and 11.73 ( $\pm$  1.54), respectively. Diets of short-tailed shrews (Blarina brevicauda) consisted of 5.20 ( $\pm$  1.87)% soil, white-footed mice (Peromyscus leucopus) 16.21 ( $\pm$  4.85)%, meadow voles (Microtus pennsylvanicus) 2.01 ( $\pm$  0.34)%, and mourning doves (Zenaida macroura) 0.83 ( $\pm$  0.41)% soil.

### Introduction

In order to determine whether wild animals acquire significant amounts of environmental contaminants from ingesting soil, the quantity of soil ingested by various wildlife species must be investigated. Studies of domestic species and a few wildlife species have shown that soil intake can be quite high (Table 2.1) and may contribute to

Table 2.1. Soil ingestion by various species as determined by Ti analysis

SPECIES	SOIL INGESTION	SOURCE
Dairy cow	0.52 - 1.75 kg/day <sup>a</sup>	Healy (1968) Fries et al. (1982a)
	0.25 - 2.41% DMI <sup>b</sup>	
	0.52 - 0.81% DMI <sup>c</sup>	
	1.38 - 2.43% DMI <sup>d</sup>	
	1.56 - 3.77% DMI <sup>e</sup>	
Beef cow	0.1 - 1.5 kg/day	Mayland et al. (1975) Kirby and Stuth (1980)
	0.28 - 0.84 kg/day <sup>f</sup>	
Swine ( <u>Sus scrofa domesticus</u> )	1.2 - 5.7% DMI <sup>g</sup>	Fries et al. (1982b)
	3.3 - 8.0% DMI <sup>h</sup>	
Sheep ( <u>Ovis aries</u> )	0.01 - 0.20 kg/day	Field and Purves (1964) Healy (1967)
	0.4 kg/day	
Mule deer ( <u>Odocoileus hemionus</u> )	18.3 g/day <sup>i</sup>	Arthur and Alldredge (1979)
	29.6 g/day <sup>j</sup>	
	7.7 g/day <sup>k</sup>	
	8.8 g/day <sup>l</sup>	
Pronghorn antelope ( <u>Antilocapra americana</u> )	5.4% DMI	Arthur and Gates (1988)
Black-tailed jackrabbit ( <u>Lepus californicus</u> )	6.3% DMI	Arthur and Gates (1988)
Hispid cotton rat ( <u>Sigmodon hispidus</u> )	2.8% DMI	Garten (1980)

<sup>a</sup> 0.75 - 2 cows/acre

<sup>b</sup> non-lactating, access to unpaved lots with no vegetation

<sup>c</sup> non-lactating, confined to concrete

<sup>d</sup> non-lactating, on pasture and supplemental feed

<sup>e</sup> non-lactating, access to unpaved lots with sparse vegetation

<sup>f</sup> determined by AIR analysis

g on lots with bare soil  
h on grass pasture  
i winter  
j spring  
k summer  
l fall

the uptake of such contaminants as plutonium (Arthur and Alldredge, 1979; Blincoe et al., 1981), DDT (Healy, 1968, Harrison et al., 1970), polybrominated biphenyls (Fries et al., 1982), Cd and chlorinated hydrocarbons (Hansen, 1981), polychlorinated biphenyls (Fries, 1982), dioxin (Umbreit et al., 1987), metals (Thornton and Abrahams, 1983; Russell et al., 1985; Fleming, 1986; Neufeld, 1987; Hegstrom and West, 1989), and organophosphates (Driver, 1991).

Soil ingestion by animals may be deliberate or involuntary. Domestic cattle (Bos taurus) appear to consume soil deliberately in order to obtain micro-minerals (Healy, 1969; Maynard et al., 1979). Likewise, wild ungulates (particularly in arid regions) use mineral licks to obtain elements such as Na (Cowan and Brink, 1949; Kreulen and Jager, 1984). Many avian species ingest grit as well as a source of minerals, particularly Ca, and as an aid in the grinding of food particles in the gizzard.

Many factors are involved in involuntary uptake of soil. The rate of soil ingestion among domestic species is affected by stocking density and condition of pastures (Healy, 1967, 1968, 1969) including soil moisture, soil texture, forage availability, and plant morphology. In areas where vegetative cover is sparse or well-grazed (i.e. probably short), soil ingestion rates are increased. Soils are also deposited on vegetation by wind in dry climates and

by water during periods of heavy rainfall. Soil may be consumed with roots of vegetation during foraging. Animals that consume earthworms ingest soil that is on the surface of the worm as well as the soil present in its gastrointestinal tract. Grooming is yet another source of soil intake by animals. Thus, soil ingestion may be quite substantial in many species and serve as a potential pathway for contaminant uptake.

### **Experiment 1: Estimating Soil Ingestion by Wildlife Species**

**Objective:** To estimate soil ingestion by select wildlife species.

#### **Methods**

Fresh feces was collected from mallards in June and July 1991 and Canada geese in September 1991 feeding at the VPI&SU duck pond and individual samples were frozen until Ti analysis could be done. Soil samples for Ti analysis were collected also from the surface 5 cm of soil surrounding the pond. In addition, 20 meadow voles, 20 short-tailed shrews, and 20 white-footed mice were snap-trapped on the VPI&SU campus between the last week of May and the third week of November. The majority of the short-tailed shrews were

captured in September, the white-footed mice in November, and the meadow voles in September and October, 1991. All meadow voles and white-footed mice were captured at the Southgate and Center woods locations, respectively. Short-tailed shrews were captured at both locations. Soil samples for Ti analysis were collected from the surface 5 cm of soil throughout the trap site. Small mammals were frozen whole until lower intestinal fecal pellets of each could be removed for Ti analysis. Complete digestive tracts were obtained also from hunter-harvested mourning doves from Floyd County, VA. Crop contents of each mourning dove were removed for AIR analysis. (Fecal contents were too small to conduct AIR analysis).

Estimates of diet dry matter digestibility of each survey species except mourning doves (Table 2.2) were based on values obtained from the literature. A value of 0.85 was used for short-tailed shrews based on findings of Buckner (1964) (not seen; cited by Grodzinski and Wunder, 1975). Buckner (1964) reported an assimilation coefficient of 0.80 for short-tailed shrews. Coefficients of assimilation (metabolizability) are generally about 5% lower than coefficients of digestibility in insectivorous and carnivorous small mammals due to the loss of energy as urine (Grodzinski and Wunder, 1975). Estimates of digestibility based on energy may be slightly greater than those based on

Table 2.2. Estimated diet dry matter digestibilities of survey species based on values obtained from the literature

Species	Diet Digestibility
Short-tailed shrew ( <u>Blarina brevicauda</u> )	0.85
White-footed mouse ( <u>Peromyscus leucopus</u> )	0.78
Meadow vole ( <u>Microtus pennsylvanicus</u> )	0.69
Mallard ( <u>Anas platyrhynchos</u> )	0.21
Canada goose ( <u>Branta canadensis</u> )	0.25

dry weight. However, Servello et al. (1983) and MacPherson et al. (1984) reported percentage digestible dry matter values and percentage digestible energy values for several diets fed to meadow voles and the two differed by only 0.1 to 4.0%. Thus, the percentage digestible energy values reported in Grodzinski and Wunder (1975) and Johnson and Groepper (1970) were used as estimates of percentage dry matter digestibility for small mammals in the present study. Digestibility was estimated at 0.78 for white-footed mice based on assimilation efficiencies of 0.77 and 0.87 (Johnson and Groepper, 1970), and a digestibility coefficient of 0.71 (Maxell, 1973; not seen; cited by Grodzinski and Wunder, 1975) reported for deer mice (Peromyscus maniculatus). MacPherson et al. (1984) found a mean dry matter digestibility of 0.69 for meadow voles from March to January. This value was nearly identical to the mean of digestibility coefficients reviewed by Grodzinski and Wunder (1975). Thus, a value of 0.69 was used for the present study. A value of 0.25 was used for Canada geese based on a study by Buchsbaum et al. (1986) in which Canada geese were fed a diet of marsh grass (Spartina alterniflora). A value of 0.21 was used for mallards based on metabolizable energy values reported by Muztar et al. (1977) and converted to apparent metabolizable energy coefficients (MEC) by Karasov (1990). Metabolizable energy coefficient values ranging

from 0.15 to 0.32 were reported for mallards fed diets of alfalfa (Medicago sativa), a green algae (Cladophora), duckweed (Lemna minor), watermilfoil, pondweed, and wild celery (Vallisneria americana). The mean value of reported MEC's for mallards was 0.24. Dry matter digestibility can be estimated (on average) by subtracting 0.03 from the MEC for herbage and fruit diets (Karasov, 1990).

To estimate the AIR content of the diet (without soil) of mourning doves, seeds of smartweed (Polygonum spp.) and Jimson weed (Datura stramonium) were collected, rinsed in deionized water and analyzed for AIR content. Soil samples from the area where doves were shot were not collected because the doves were harvested by hunters and location of feeding was not known. Acid insoluble residue content of the soil was estimated at 99%.

Soil ingestion by each species except mourning doves was estimated using Equation 2.1.

Equation 2.1

$$S.I. (\%) = \frac{(1-D) Ti_f}{Ti_s - D \cdot Ti_f} \times 100$$

where:

- D = dry matter digestibility of the diet (%)
- Ti<sub>s</sub> = Ti content of soil (ppm)
- Ti<sub>f</sub> = Ti content of feces (ppm)

## Results

Mean Ti concentrations in soils from each sampling area ranged from 334 to 1417 ppm (Table 2.3). Soil ingestion by each species examined ranged from 2% to slightly over 16% among small mammals and from nearly 1% to 12% in avian species (Table 2.4). All estimates for small mammals were based on analysis of intestinal contents. A few small mammals did not have fecal material present in the intestines which reduced the samples size from 20 in all species collected.

Acid insoluble residue content of seeds representing the diet of mourning doves was below the limit of detection for AIR. Twenty-two mourning doves were collected for AIR analysis of crop contents. However, only 15 crops contained adequate sample volume for analysis. Soil ingestion by mourning doves was estimated by assuming ash content of soil from the collection area was 99% and diet without soil was 2% AIR. Eleven of the 15 crop samples analyzed resulted in values below the limit of detection. For these samples, a value of one half the detection limit was assigned. Two percent (0.02) was subtracted from each value obtained for AIR concentration of crop contents to account for the AIR contributed by the diet itself. In cases where AIR concentration was less than 2%, a value of zero was

Table 2.3. Mean Ti concentration (ppm) in soils from each sampling area

Location	N	Mean	S.E.
Center Woods	8	333.95	22.87
Southgate	5	1042.52	92.19
Duck Pond	10	1417.49	63.40
Killarney Lake, ID	3	662.77	194.40

Table 2.4. Soil ingestion (% DMI) by select wildlife species calculated from fecal or intestinal content samples

Species	N	Soil Ingestion (Mean $\pm$ S.E.)
<u>Ti Method</u>		
Short-tailed shrew ( <u>Blarina brevicauda</u> )	17	5.20 $\pm$ 1.87
White-footed mouse ( <u>Peromyscus leucopus</u> )	17	16.21 $\pm$ 4.85
Meadow vole ( <u>Microtus pennsylvanicus</u> )	19	2.01 $\pm$ 0.34
Mallard ( <u>Anas platyrhynchos</u> )	30	11.73 $\pm$ 1.54
Canada goose ( <u>Branta canadensis</u> )	20	4.92 $\pm$ 0.60
<u>AIR Method</u>		
Mourning dove ( <u>Zenaida macroura</u> )	15	0.83 $\pm$ 0.41 <sup>a</sup>

<sup>a</sup> based on crop contents

assigned. The remaining value was divided by 0.99 and multiplied by 100 to give percentage soil ingested.

## **Discussion**

There was considerable variation in soil ingestion by the three small mammal species surveyed. Meadow voles, a semi-fossorial species exhibited the lowest soil ingestion and the lowest standard error at  $2.01 \pm 0.34\%$  DMI. The diet of meadow voles consists of green vegetation (grasses, clover, plantain), tubers, seed heads, flowers, and leaves of grasses (Whitaker, 1980). A diet consisting of low-growing vegetation and tubers together with a semi-fossorial lifestyle would suggest a high likelihood of soil ingestion. However, this was not shown by the present study. The estimate based on the present study, however, was nearly identical to the estimate made by Beyer et al. (in review) of 2.4% based on AIR analysis.

Diets of short-tailed shrews and white-footed mice are much more diverse compared to the diet of meadow voles as illustrated by larger standard errors in soil intake for these two species. The diet of white-footed mice consists of nuts, seeds, fruits, beetles, caterpillars, and other insects (Whitaker, 1980) and would not appear to be conducive to soil ingestion. It is possible, however, that

these food items contain surface contamination of soil. The results of the present study showed that white-footed mice ingested the highest amount of soil of all species surveyed. Beyer et al. (in review) estimated soil ingestion at < 2% DMI based on AIR analysis of nine pooled samples of four mice each. Diet digestibility was estimated at 65% as opposed to 78% used in this study. If 78% had been used in their study, soil intake would have been estimated at an even lower amount. The best explanation for the extremely large estimate in this study is that the Ti concentration of soil sampled in the trapping area (i.e. Center woods, 334 ppm Ti) was not representative of soil ingested by the animals captured. If a higher Ti concentration such as 1000 ppm, a value similar to that seen at the two other locations sampled on the VPI&SU campus, had been used in the soil ingestion equation, the estimated soil ingestion would have been  $4.05 \pm 1.05\%$  DMI, a result more consistent with expected concentrations.

Estimated soil ingestion by short-tailed shrews was also variable as would be expected based on the diet of this species. Shrews consume earthworms, snails, centipedes, beetles and other invertebrates, subterranean fungus (Endogone), and occasionally mice and smaller shrews (Whitaker, 1980). Earthworms likely supply the majority of dietary soil. In addition, this species lives underground

and excavates its burrows with its snout and forefeet. Therefore, it was expected that soil ingestion would be high. Estimated soil intake (% DMI) in the present study ( $5.20 \pm 1.87$ ) was based on animals obtained from two locations, one being Center woods, the area where soil Ti may have been underestimated. Estimated soil ingestion based on only the 10 animals obtained from the Southgate location was  $1.71 \pm 0.64\%$  DMI, a value slightly lower than the estimate based on animals from both sampling sites combined. If a value of 1000 ppm Ti were used for the Center woods soil and all animals were included, the mean soil ingestion would become  $2.48 \pm 0.73\%$  DMI. In any case, the soil ingestion by this species was not as high as might be expected based on its burrowing behavior. Beyer et al. (in review) found similar results with groundhogs (Marmota monax) and prairie dogs (Cynomys ludovicianus) (both burrowing species) which had a mean soil ingestion of  $< 3\%$  soil.

Beyer et al. (in review) estimated soil ingestion by mallards at approximately  $4\%$  DMI using AIR analysis. Mallards from the VPI&SU duck pond on average ingested a greater amount of soil. Canada geese ingested slightly less soil than those studied by Beyer et al. (in review) using AIR analysis. Beyer et al. (in review) estimated soil ingestion at  $8.2\%$  DMI whereas this study found soil

ingestion to be  $4.92 \pm 0.60\%$  DMI. Diets of mallards and Canada geese are similar, consisting primarily of plant materials, seeds, and possibly aquatic invertebrates. Thus, one would expect soil ingestion by the two species to be similar. However, feeding styles of the two species differ in that Canada geese are typically ground feeders whereas mallards are dabblers, which may predispose them to slightly higher soil ingestion due to intake of sediments suspended in water during feeding.

Finally, soil ingestion by mourning doves was estimated at  $< 1\%$  DMI based on AIR analysis. Considering that mourning doves are strictly seed eaters and birds were collected in early September when seeds are still primarily on plants, this estimate seems reasonable. The majority of AIR in the crops of mourning doves probably resulted from the intake of grit.

Results of the present survey clearly show that wildlife species ingest soil and that any pollutants contained in soils are thus consumed. Potential harm that these pollutants may cause depends upon several factors including the concentration of the pollutant in the soil and the bioavailability of the pollutant once ingested. Chapter 3 will attempt to examine a few of these factors.

## **CHAPTER 3: A Case Study: Lead Uptake in Waterfowl of the Lower Coeur d'Alene River and Lateral Lakes of Northern Idaho**

### **Summary**

In Experiment 1, mallards were collected by shooting from Killarney Lake, northern Idaho, an area of extensive Pb contamination, and analyzed for blood free erythrocyte protoporphyrin (PP), fecal, liver, and kidney Pb concentrations, and soil/sediment ingestion. Sediment collected in the area was found to contain 4485 ppm Pb. At least 86% of mallards sampled had elevated ( $\geq 10$  ppm dry weight) liver and kidney concentrations of Pb and at least 21% had concentrations indicating acute Pb exposure ( $\geq 24$  ppm dry weight). Protoporphyrin was found to be a poor indicator of Pb contamination at this level of exposure and/or under these conditions. Soil/sediment ingestion by mallards averaged 7.5% DMI. Fecal Pb concentrations averaged 130 ppm but were not significantly ( $P < 0.05$ ) correlated to soil/sediment ingestion. It was concluded from the study that mallards from the area were suffering from chronic exposure to low concentrations of Pb, likely from ingestion of Pb contaminated sediments.

In Experiment 2, sediment collected from Killarney Lake

was added to the diet of captive northern bobwhites at 8% DMI for a period of 21 d. The concentration of Pb in blood, liver, and kidneys of each bird was determined and compared to control values. Treated birds showed no significant decline ( $P > 0.05$ ) in feed intake or body mass over time but 90% exhibited blood Pb concentrations consistent with clinical Pb poisoning and all birds had elevated liver and kidney Pb concentrations. It was shown that tissue Pb accumulation can occur from the ingestion of Pb contaminated sediments in the diet.

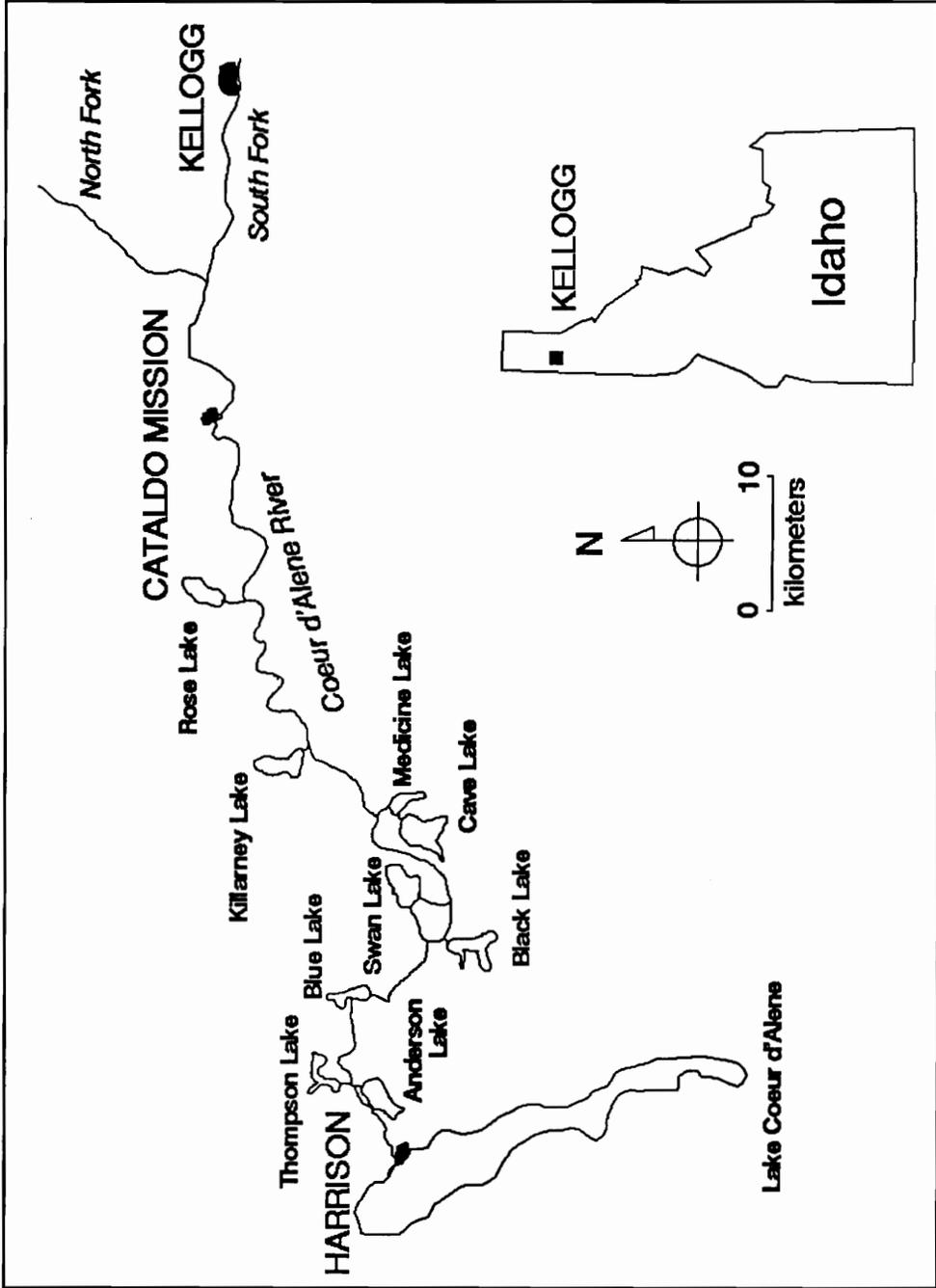
## **Introduction**

### **Lower Coeur D'Alene River Valley, Northern Idaho**

The lower Coeur d'Alene River Valley consists in part of the Coeur d'Alene River and a number of lateral lakes and seasonally flooded marshes (Figure 3.1). These waterbodies serve as excellent habitat for a variety of wildlife species. Mining of Pb, Ag, Au and other metals has been practiced in the Coeur d'Alene River Valley (Figure 3.2) since the 1880's. The area is one of the world's major producers of Ag, Pb, and Zn. The industry flourished until 1981 when several plants and smelters including the Pb smelter, phosphoric acid/fertilizer plant, electrolytic Zn plant, Cd and sulfuric acid plants at the Bunker Hill

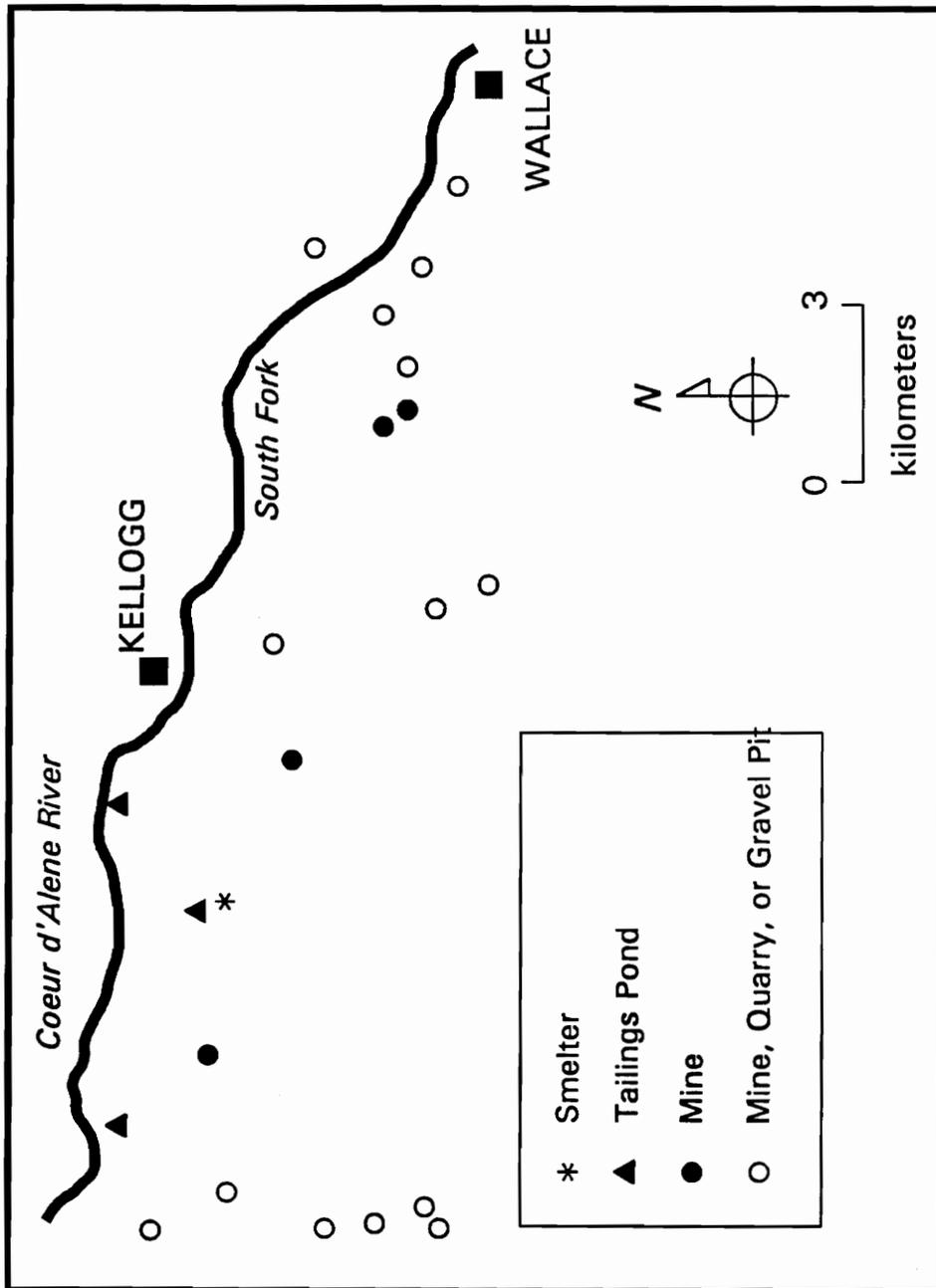
**Figure 3.1** Coeur d'Alene River and its lateral lakes





**Figure 3.2 Sources of heavy metal contamination near Kellogg, Idaho**





Complex at Kellogg were closed (Krieger, 1990). Mining activities still continue today, but at a reduced rate and smelting activities have been discontinued. As a result of present and past activities, large quantities of mine wastes containing Pb, Cd, Zn, and other metals have been discharged into the South Fork of the Coeur d'Alene River. Neufeld (1987) reported that Pb appeared to be the most damaging element to wildlife in the area.

In 1968, settling ponds and higher smelting stacks were constructed to reduce the quantity of wastes discharged into the river. However, over 10,000 acres of wetlands in the Coeur d'Alene River system remain contaminated (Neufeld, 1987). In the early 1980's, a 54 km<sup>2</sup> area around the Bunker Hill Mine and Complex was designated an Environmental Protection Agency (EPA) Superfund Clean-up Site as a result of heavy metal contamination from mine wastes (Casteel et al., 1991). Efforts are still underway to assess and reduce the risks of heavy metal contamination to wildlife in the area.

Kemmerer et al. (1923) reported that the Coeur d'Alene River was so laden with mine and mill tailings that they could be "traced far out into the clear waters of the lake [Coeur d'Alene Lake]." In a 1964 waste disposal study, it was estimated an average of 2000 metric tons of mine slimes were discharged into the South Fork of the Coeur d'Alene

River each day (Johnson et al., 1975). The slimes likely contained heavy metals and contributed large quantities of metals to the river and surrounding lakes. In May 1976, Pb moving downstream in the river was measured by the EPA at 125 kg/day, but dropped to 9 kg/day in August 1986, a period of low mining activity (Neufeld, 1987). Neufeld's report does not indicate how or what form of Pb was measured in the river. Despite the extensive contamination of the valley bottom, discharging of heavy metals is still allowed with EPA National Pollution Discharge Elimination System (NPDES) Permits. In 1986, average daily discharges from eight mining corporations totalled approximately 5000 kg of Pb which were released under NPDES Permits into the South Fork of the river (Neufeld, 1987). Total Pb per year may have reached over 12,500 kg based on maximum daily discharge permits. Emissions of Pb from the main smelter in the Coeur d'Alene valley averaged 8.3 to 11.7 metric tons each month from 1955 to 1973 (Burrows et al., 1981).

The impacts of mining activities in the Coeur d'Alene Valley have been well documented. In 1968, the only organisms present in the main stem of the Coeur d'Alene River below the mining district were Chironomids (midgefly larvae) and there were no macrobenthic species in the South Fork of the river based on surveys by Savage (1970) and Savage and Rabe (1973) (not seen, cited by Johnson et al.,

1975). Reports of Pb poisoning in dogs, horses, and children in the Coeur d'Alene Valley were made in the 1970's. A study of 178 children living within one mile of the smelter complex at Kellogg was conducted by the Idaho Department of Health and Welfare in 1974 and revealed 98% had blood Pb > 400 ppb wet weight (w.w.) (subclinical Pb poisoning) and 2% had blood Pb > 800 ppb (clinical Pb poisoning) (Johnson et al., 1975). The major source of the Pb was thought to be inhalation of airborne Pb originating from the smelter complex and exposure to contaminated soil and dust in and around their homes. The conclusions of Johnson et al. (1975) are supported by findings of Krieger (1990) who found yard soils and house dusts at the Bunker Hill Superfund Site contained 2500 to 30,000 ppm and 1800 to 36,000 ppm Pb, respectively.

Blus et al. (1987) measured concentrations of Pb in mink (Mustela vison), muskrats (Ondatra zibethicus) and small mammals downstream of the smelter complex from 1981 to 1983. They found maximum liver concentrations of Pb were greater than concentrations known to cause morbidity and death in laboratory animals. They concluded that small mammal populations in the area likely had declined due to Pb toxicity and "associated secondary effects" on food supply and cover.

Waterfowl mortality due to Pb poisoning has been

recorded in the Lower Coeur d'Alene River since 1924 (Chupp and Dalke, 1964). A number of studies have been conducted since that time attempting to assess the impacts of heavy metal contamination in the Coeur d'Alene River and adjacent marshes on waterfowl populations. Chupp and Dalke (1964) collected information on waterfowl mortality by reviewing literature and interviewing state and federal conservation agency personnel. Three emergent aquatic plant samples (species not reported by authors) taken in the lower 16 km of the Coeur d'Alene Valley and 12 soil samples taken from the top 15 cm were collected and analyzed for Pb concentration. Soil and plants were found to contain 150 to 9600 ppm (d.w.) and 1500 to 3700 ppm (d.w.) Pb, respectively. It is difficult to determine from the report, however, whether the Pb from plants was actually in the plants or adhered to plant surfaces. Jones and Clement (1972) found that only a small proportion of Pb in soil/sediment is available to plant roots and that the majority of Pb that is absorbed remains in the roots. In addition, Chupp and Dalke (1964) examined 13 waterfowl of various species for Pb poisoning. Livers of five tundra swans (Cygnus columbianus) contained 18 to 37 ppm (w.w.) Pb and two tibia/fibula samples contained 40 to 50 ppm (w.w.) Pb. Scanlon (1982) provided data on wet weight versus dry weight of mallard tissues to aid in the comparison of

reports of contaminant concentrations of tissues based on wet weights and dry weights. His study revealed the dry weight of kidney was 23%, liver was 33%, and bone 82%. Thus Chupp and Dalke's findings convert to 55 to 112 ppm (d.w.) in livers and 49 to 61 ppm (d.w.) in bone. A concentration of 10 ppm (d.w.) in liver is considered a "background level" (Scanlon et al., 1980) and 24 to 80 ppm (d.w.) in liver generally indicates recent, probably debilitating, Pb exposure (Longcore et al., 1974). Neufeld (1987) states that a concentration of 1 ppm Pb (w.w.; 3 ppm d.w. converted) indicates that exposure of some form has occurred and a concentration of 10 ppm (w.w.; 30 ppm d.w. converted) or greater indicates Pb toxicity and is generally accompanied by outward signs of Pb poisoning. Longcore et al. (1974) reviewed the literature for Pb concentrations in livers of Pb poisoned waterfowl and concluded that Pb concentrations indicative of lead poisoning are "arbitrary." Therefore, the final diagnosis of Pb poisoning should be based on tissue concentrations of Pb as well as other physiological and histopathological signs of Pb exposure. Chupp and Dalke (1964) concluded that the primary cause of waterfowl mortality in the Coeur d'Alene area was contamination by mine wastes. Waterfowl probably were consuming contaminated sediments with vegetation or directly as grit.

In an Idaho Department of Fish and Game Report, Neufeld (1987) reported that the likelihood of swan mortality in the Coeur d'Alene River area depends upon water levels and the deposition of new sediments on vegetation that swans eat, during winter floods. The ingestion of roots and tubers by swans could also provide uptake of contaminated sediments. The water levels in the Coeur d'Alene River system are subject to extreme seasonal fluctuations depending on snow melt and discharges from the Washington Water Power Company's Post Falls dam. The water level of the lateral lakes of the Coeur d'Alene River may vary seasonally by several meters. He also suggested contaminated sediments were used by swans as grit which contributed to their Pb exposure. However, based on the minute particle size observed in the sediments collected in the present study, it is unlikely that waterfowl use the sediments as a source of grit.

It appears that from 1984 to 1986, Pb shot ingestion was also important in Pb poisoning of ducks of the Coeur d'Alene River system. Neufeld (1987) reported that in 1984, 240 duck gizzards were collected from hunter-harvested ducks from the Coeur d'Alene River Wildlife Management Area to determine the incidence of Pb shot ingestion. The study was repeated for the 1985 and 1986 hunting seasons. The proportion of gizzards containing Pb shot were 29.2, 24.6,

and 6.0% in 1984, 1985, and 1986, respectively. Neufeld (1987) further reported that ducks with Pb shot in their gizzards had liver Pb concentrations averaging 9 ppm (range 2 - 43). Those without Pb shot but in a lead-contaminated environment had mean Pb concentrations ranging from 2 to 4 ppm. Neufeld (1987) did not indicate whether analysis was done on a wet or dry weight basis but given the style in the text, these values probably were wet weights. It is possible that elevated liver Pb may have resulted from previous Pb shot ingestion, but that the pellets were no longer present in the gizzard. Scanlon et al. (1980) compared the liver Pb concentrations in hunter harvested waterfowl species from Maryland with and without ingested Pb shot present in their gizzards and found that 16.2% of waterfowl with no shot in the gizzard had liver Pb concentrations indicative of recent Pb dosing, probably from shot ingestion. However, Neufeld (1987) reported that in an area in Idaho where sediments were not contaminated, ducks without ingested Pb shot had liver Pb concentrations averaging 0.7 ppm (range 0.39 - 3.10). Neufeld concluded that contaminated sediments likely were responsible for the elevated liver Pb concentrations in ducks without Pb shot.

Casteel et al. (1991) examined liver Pb concentrations of hunter-killed ducks collected in 1987 from the Coeur d'Alene River Valley and compared concentrations in ducks

with and without lead shot in their gizzards to evaluate Pb contribution from mining waste versus that from Pb shot. Ducks that had ingested Pb shot had a mean ( $\pm$  S.E.) liver Pb concentration of  $15.18 \pm 2.51$  ppm (w.w.; 46 ppm d.w. converted;  $n = 23$ ) and those without ingested Pb shot had a mean liver lead concentration of  $4.21 \pm 0.47$  ppm (w.w.; 12.76 ppm d.w.;  $n = 85$ ). Three sediment samples collected from a lake within their test site had a mean Pb concentration of 4520 ppm (w.w.; moisture content of sediment not given). Of a total of 108 ducks examined, 23 (21.3%) had ingested Pb shot, indicating a serious Pb shot problem. The authors concluded that steel shot designation of the area in 1986 had not yet impacted the amount of Pb shot ingested by ducks in the area and that although Pb concentrations in sediments were quite high, Pb shot was a greater threat to ducks than mining waste.

#### **Bioavailability of Pb in Soil/Sediments**

Many factors influence the availability of Pb in soils/sediments to plants and animals. Plant roots absorb Pb in the ionic form from soils/sediments (Inyang, 1982). In most cases, Pb ions are adsorbed to clay particles, phosphates, carbonates, hydroxides, and organic matter in soils/sediments making them unavailable for plant uptake, although, soil/sediment conditions such as low pH, organic

matter, phosphate concentrations, and hydration increase the solubility of Pb and its availability to plants (Zimdahl and Koepe, 1979; Inyang, 1982). The concentration of Pb in soil/sediment that can be affected by the nature of the soil/sediment, also affects its availability to plants. For example, Maxfield et al. (1974) found that Pb content in sedimentary layers of the Coeur d'Alene River delta (where the Coeur d'Alene River empties into Coeur d'Alene Lake) follows the order clay > organic matter > silt > sand. In addition, very little aerosol-deposited Pb is absorbed through plant leaves, and Pb absorbed through the roots is not readily translocated to "edible" parts of most plants, depending on the species. Yet, roots may be edible in some species. Little (1973) found that heavy metals deposited on leaves by particulate matter tend to remain as surface deposits rather than being incorporated into the plant tissue. Thus, plants themselves are not likely a significant source of Pb. However, the deposition of Pb on plant surfaces as dust and soil/sediment may be a potential hazard to animals consuming them, depending on the plants' habitats and/or the part of the plant consumed (Zimdahl and Koepe, 1979).

Factors that influence availability of Pb to plants such as adsorption to soil/sediment components and Pb concentration, affect availability of Pb to animals

ingesting soil/sediment. In addition, many dietary factors influence uptake of Pb ions that are available for absorption. For instance, a diet low in Fe, P or Ca or high in Vitamin D tends to increase Pb absorption and toxicity (World Health Organization, 1977). Furthermore, Pb toxicity appears to be alleviated by diets high in protein and Ca. Both decrease absorption of Pb from the digestive tract and reduce Pb storage (Sanderson and Bellrose, 1986). In general, it appears that only 10 to 20% of Pb ingested is absorbed (Coburn, 1951; World Health Organization, 1977).

Freeman et al. (1992) fed Sprague-Dawley rats diets containing 0.2, 0.5, 2, and 5% dietary soil containing mine waste from Butte, Montana for 30 d to determine the bioavailability of Pb present in soil. Soil contained 810 or 3908 ppm Pb. Another group of rats was fed Pb acetate at 1, 10, 25, 100, and 250  $\mu\text{g}$  Pb/g feed to serve as standards. Liver, blood and femurs were analyzed for Pb concentration. It was found that Pb as lead acetate trihydrate ( $(\text{CH}_3\text{CO}_2)_2\text{Pb}\cdot 3\text{H}_2\text{O}$ ) was absorbed more readily by the rats than Pb from soil. Physical signs, body weight, and feed consumption of both groups of treated rats were similar to controls. Mean relative percentage bioavailability values of Pb in soil (defined as the ratio of Pb uptake from that dose of test soil to Pb uptake of the same dose of Pb acetate treatment) were estimated at 20% based on blood Pb

data, 9% based on bone, and 8% based on liver data.

### **Diagnostic Techniques for Pb Poisoning**

A number of methods have been employed to evaluate Pb exposure in avian species. Diagnoses are based on pathological changes and demonstration of elevated Pb concentrations in various body tissues. For studies involving live specimens or birds exposed to low concentrations of Pb which often do not exhibit clinical signs, hematological characteristics have been used to detect Pb exposure. These characteristics include packed cell volume, percentage hemoglobin, blood Pb concentration, delta-aminolevulinic acid dehydratase ( $\delta$ -ALAD) activity, and free erythrocyte PP concentration. Several studies (Mautino and Bell, 1987; Scheuhammer, 1987, 1989; Pain, 1989) have attempted to evaluate these techniques to determine which is the best method for measuring Pb intoxication in avian species.

### **Clinical Signs of Pb Toxicosis**

The external signs of Pb poisoning include weakness and progressive paralysis of the wing, neck, and leg muscles, regurgitation of yellowish crop fluid, and watery green feces (Scott, 1972; Birkhead and Perrins, 1986; Sanderson and Bellrose, 1986; Friend, 1987; Davidson and Nettles,

1988). The vent is often stained green due to the presence of excessive bile in the droppings (Davidson and Nettles, 1988). Birds suffering from prolonged Pb exposure generally have reduced levels or lack subcutaneous and visceral fat (Friend, 1987). Internal lesions may include a brittle, dark gizzard, a distended proventriculus, atrophy of the breast muscles, and impaction of the proventriculus and/or esophagus with food (Birkhead and Perrins, 1986; Sanderson and Bellrose, 1986; Friend, 1987; Davidson and Nettles, 1988). Food becomes impacted as Pb inhibits the normal rhythmic contractions of the alimentary tract. It is believed that Pb acts directly on smooth muscle or associated nerve plexi of the digestive system (Eisler, 1988). Lead also has a number of sublethal effects. It impairs the synthesis of hemoglobin, resulting in anemia, and causes demyelination and axonal degeneration of nerves, resulting in damage to the central nervous system and incoordination (World Health Organization, 1977). Lead also accumulates in kidneys at the proximal convoluted tubules causing interstitial fibrosis and edema (Eisler, 1988). Histological changes in kidney cells such as the presence of internuclear inclusion bodies can be used in diagnosing Pb poisoning (Eisler, 1988). Demonstration of elevated Pb concentrations in blood, liver, kidney, bones, or feathers is another approach to diagnosing Pb poisoning.

### Hematological Characteristics

Lead impairs normal heme biosynthesis and creates a number of changes in the blood which have been used as secondary biological indicators of Pb intoxication. Lead changes the activity of enzymes and production and excretion of intermediates involved in the heme biosynthetic pathway and causes depressed hemoglobin concentration in cases of severe Pb poisoning (Singhal and Thomas, 1980). Hemoglobin concentration can easily be measured in wild animals. Changes in packed cell volume have also been measured; however, Mautino and Bell (1987) and Pain (1989) demonstrated no significant relationship between packed cell volume and blood Pb concentration. Thus, packed cell volume is not a reliable indicator of Pb exposure.

The concentration of free erythrocyte PP, an intermediate in the heme biosynthetic pathway, is commonly used as an index of Pb exposure. Protoporphyrin fluoresces under ultraviolet light and can be rapidly and inexpensively measured in erythrocytes using a spectrofluorometer (Singhal and Thomas, 1980). Protoporphyrin concentration was found by Pain (1989) to be significantly correlated with blood Pb concentration ( $r = 0.683$ ,  $P < 0.001$ ,  $n = 213$ ). The relationship was not significant, however, when blood Pb concentrations of  $> 100 \mu\text{l/dL}$  were excluded from the regression analysis. Mautino and Bell (1987) found similar

results. Thus, the technique should only be used for birds with blood Pb concentrations  $> 100 \mu\text{g/dL}$ . Pain (1989) also suggested that PP should only be used on birds exposed to Pb more than five days previously due to the time it takes for PP concentrations to accumulate in the blood (Roscoe et al., 1979). Furthermore, a study by Scheuhammer (1989) revealed that at least 9.5% of free-ranging mallards with blood Pb  $> 80 \mu\text{g/dL}$  did not exhibit elevated PP. This technique is reliable, but has limited applications.

The activity of  $\delta$ -ALAD, the enzyme which converts aminolevulinic acid to porphobilinogen in heme synthesis is directly inhibited by Pb. The activity of the enzyme, like PP, can be measured relatively inexpensively, rapidly and reproducibly using spectrophotometry. Pain (1989) found  $\delta$ -ALAD activity was depressed within 24 h of Pb exposure. The log  $\delta$ -ALAD activity has been shown to be negatively correlated with blood Pb concentration ( $r = -0.790$  to  $-0.914$ ) (Mautino and Bell, 1987; Scheuhammer, 1987; Pain, 1989). However,  $\delta$ -ALAD activity of the blood is negligible when blood Pb is in excess of  $100 \mu\text{g/dL}$  (Mautino and Bell, 1987; Pain, 1989) and consequently is most useful in cases of subclinical Pb exposure.

The ideal index of Pb exposure appears to be the  $\delta$ -ALAD activity ratio which is the ratio of fully restored enzyme activity to that measured without removing any inhibitory

influence of Pb. This technique has been used by Scheuhammer (1987, 1989) and Pain (1989) to predict Pb intoxication in birds. The log  $\delta$ -ALAD activity ratio is more highly correlated to blood Pb than any other technique ( $r > -0.95$ ) (Scheuhammer, 1987, 1989; Pain, 1989). With this method, Scheuhammer (1987) found dietary concentrations as low as 5 ppm Pb can be detected consistently. In addition, direct comparisons can be made among different species as well as among individuals of the same species since the ratio does not depend upon the absolute level of enzyme activity.

**Experiment 1: Determining Pb Exposure in Mallards of the Coeur d'Alene River System**

- Objectives:**
- (1) To determine the concentrations of Pb in tissues of mallards occupying the lateral lakes of the Coeur d'Alene River system
  - (2) To estimate soil/sediment ingestion by mallards occupying the lateral lakes of the Coeur d'Alene River system

**Methods**

Twenty-nine mallards were collected at Killarney Lake

by shooting using steel shot in mid-August, 1991. It was assumed that these mallards were residents because it was too early for migrants to be arriving in the area. Upon shooting, blood was collected immediately via cardiac puncture using a heparinized syringe, transferred to a heparinized vacutainer tube, and held on ice. Carcasses were held on ice until the liver, kidneys, and entire gastrointestinal tract could be removed. Organs were frozen in liquid nitrogen until Pb analysis could be done. The age (juvenile or adult) of each duck was determined by feather characteristics and the degree of development/regression of the bursa of Fabricius (Larson and Taber, 1980). The sex of each duck was determined also upon dissection and recorded.

Blood was refrigerated for approximately 48 h after collection before measuring PP concentration on an AVIV<sup>®</sup> protoporphyrin hematofluorometer modified for avian blood as described by Blus et al. (1991). Kidneys and liver from each duck were analyzed for Pb concentration by the EPA Environmental Research Laboratory in Corvallis, OR using inductively coupled plasma (ICP) atomic emission spectrometry.

Contents of the colon (large intestine distal to the cecae) were removed from each duck and analyzed for Ti and Pb concentration. (Colon contents will be referred to as feces for remainder of this discussion). Samples of

sediment (Pywell muck; U.S. Department of Agriculture, Soil Conservation Service, 1981) were collected at random from three locations at the study site for Ti and Pb analysis. Five samples from each location were dried at 55°C, passed through a #20 sieve (840µm), and analyzed for each element. The concentration of soil/sediment in diets of ducks was estimated from feces using the equation from Fleming (1986):

Equation 3.1

$$S.I. (\%) = \frac{(1-D) Ti_f}{Ti_s - D \cdot Ti_f} \times 100$$

where:

D = digestibility of the diet (%)

Ti<sub>s</sub> = Ti content of sediment (ppm)

Ti<sub>f</sub> = Ti content of feces (ppm)

Entire gastrointestinal tracts of each mallard collected at Killarney Lake were X-rayed by the Veterinary Teaching Hospital at VPI&SU for evidence of shot presence. All gastrointestinal tracts found to show shot were dissected to determine if shot was present from the shooting done to collect them. Presence/absence of entry holes for pellets was noted where relevant. Recovered shot were tested with a magnet to determine whether the shot were steel or Pb.

## Statistical Analyses

Statistical analyses were conducted using the Statistical Analysis System (SAS Institute Inc., 1987). A two-way ANOVA with interactions was used to analyze the effect of sex and/or age on PP, liver, kidney, and fecal Pb concentrations, and soil/sediment ingestion. Tukey's Studentized Range (HSD) test was used for follow-up analysis to detect differences between groups. Correlations were made using Statistix Version 3.5 (Analytical Software, 1991). Alpha was set at 0.05 for all statistical inferences.

## Results

A two-way ANOVA showed there was no interaction and Tukey's Studentized Range test showed no significant difference between sex or age for PP, liver, kidney, and fecal Pb concentrations, or soil/sediment ingestion. Therefore results from all mallards analyzed were combined (Table 3.1). The sediment samples collected at Killarney Lake contained  $660 \pm 194$  ppm Ti and  $4500 \pm 184$  ppm Pb which is quite similar to mean ( $\pm$  S.E.) values reported by Krieger (1990) for Killarney Lake of  $4522 \pm 683$  ppm.

Protoporphyrin concentration in the blood could be

Table 3.1 Summary of tissue and fecal analyses<sup>a</sup> of mallards collected from Killarney Lake, Idaho

Parameter	N	Mean $\pm$ S.E.	Range
Liver Pb (ppm)	28	18.71 $\pm$ 2.54	2.91 - 64.14
Kidney Pb (ppm)	28	23.83 $\pm$ 2.84	4.92 - 64.28
Fecal Pb (ppm)	28	129.75 $\pm$ 38.48	<0.01 - 792.07
Soil ingestion (% DMI)	28	7.50 $\pm$ 2.91	0.60 - 73.64
Protoporphyrin ( $\mu$ g/dL)	20	30 $\pm$ 3	9 - 71

<sup>a</sup> Dry matter basis

determined for only 20 of the 29 mallards collected because of a malfunction of the hematofluorometer. There was a significant correlation (Spearman's correlation coefficient,  $r_s = 0.660$ ,  $p < 0.01$ ,  $n = 28$ ) between liver Pb and kidney Pb concentrations. However, no significant correlation existed between liver Pb concentration and PP concentration ( $r_s = 0.182$ ,  $P > 0.05$ ,  $n = 20$ ), soil/sediment ingestion ( $r_s = 0.059$ ,  $P > 0.05$ ,  $n = 27$ ), or fecal Pb concentration ( $r_s = -0.107$ ,  $P > 0.05$ ,  $n = 27$ ). There was also no significant correlation between PP concentration and kidney Pb concentration ( $r_s = 0.232$ ,  $P > 0.05$ ,  $n = 20$ ), soil/sediment ingestion ( $r_s = -0.205$ ,  $P > 0.05$ ,  $n = 19$ ), or fecal Pb concentration ( $r_s = 0.199$ ,  $P > 0.05$ ,  $n = 19$ ). Kidney Pb concentration was not significantly correlated with soil/sediment ingestion ( $r_s = 0.008$ ,  $P > 0.05$ ,  $n = 27$ ) or fecal Pb concentration ( $r_s = 0.235$ ,  $P > 0.05$ ,  $n = 27$ ). Lastly, there was no significant correlation between soil/sediment ingestion and fecal Pb concentration ( $r_s = 0.370$ ,  $P > 0.05$ ,  $n = 28$ ), but the correlation between fecal Ti and fecal Pb was almost significant at  $\alpha = 0.05$  ( $n = 28$ ,  $r_s = 0.370$ ,  $r_{crit} = 0.374$ ).

One Pb shot and metal fragments (composition unknown) were recovered from the gizzard of one mallard examined for shot. The mallard exhibited the highest PP concentration (71  $\mu\text{g/dL}$ ) of the 20 birds examined and moderate to high

concentrations of kidney Pb (36 ppm) and fecal Pb (517 ppm). A second mallard had one steel shot present in its gizzard.

## **Discussion**

Scanlon et al. (1980) suggested 10 ppm Pb (d.w.) in liver as a "background level" for Pb exposure in mallards. Bagley and Locke (1967) found that "background levels" of liver Pb in 11 species of waterfowl with no known Pb exposure averaged 0.5 to 2 ppm (w.w.; 2 to 5 ppm d.w. converted). Longcore et al. (1974) found that liver or kidney Pb concentrations of 6 to 20 ppm (w.w.; 24 to 80 ppm d.w. converted) were indicative of acute Pb exposure. Based on Scanlon, Bagley and Locke, and Longcore's values, 86 to 100% of the mallards sampled at Killarney Lake had liver Pb concentrations above the "background levels" for the studies cited and 21% had concentrations > 24 ppm (d.w.), suggesting acute Pb exposure. Kidney Pb concentrations indicated 93 to 100% were above "background levels" and 36% had concentrations > 24 ppm (d.w.). Lead concentrations of livers were quite similar to values reported by Krieger (1990) for Pb in livers of hunter harvested mallards from Killarney Lake. Krieger found mean ( $\pm$  S.E.) liver Pb at  $6 \pm 3$  ppm (w.w; 17 ppm d.w. converted; n = 15) in the 1986 hunting season and  $4 \pm 1$  ppm (w.w; 12 ppm d.w. converted; n

= 23) in mallards without Pb shot present and  $6 \pm 1$  ppm (w.w.; 19 ppm d.w. converted; n = 23) including ducks with Pb shot present in the 1987 hunting season.

Protoporphyrin was elevated ( $> 40 \mu\text{g/dL}$ ) in only 25% of the 20 mallards examined. Protoporphyrin concentration was not significantly ( $P < 0.05$ ) correlated with liver or kidney Pb concentrations. There was, however, a significant ( $P < 0.05$ ) relationship between liver and kidney Pb concentrations, which suggests that PP was not a reliable indicator of Pb uptake at this level and/or of exposure. The PP concentration probably depends more on recent Pb intake than liver and kidney Pb concentrations. Mautino and Bell (1987) and Pain (1989) found that increases in PP concentration only became significant when blood Pb was  $> 100 \mu\text{g/dL}$ . Thus, the birds in the present study may have been exposed to Pb in the recent past (as shown by tissue residues), yet blood Pb concentrations were no longer elevated enough to induce PP accumulation. Although Pb concentrations in liver and kidneys were well above normal concentrations, none of the mallards exhibited overt signs of Pb poisoning upon dissection. However, the mallards did lack subcutaneous fat which may have been a consequence of recent reproduction and molting. This suggests that the mallards were accumulating Pb in soft tissues from long-term, low-level Pb uptake. The concentrations were not high

enough to induce severe Pb poisoning, but may have been causing subclinical effects such as poor weight gain and fat storage and decreased resistance to disease.

Soil/sediment ingestion and fecal Pb concentrations were not significantly correlated to tissue concentrations of Pb. This is not surprising since both the sediment and fecal Pb concentrations only apply to a restricted period of time, whereas liver and kidney concentrations reflect Pb exposure over longer intervals. It cannot be proven from this study whether the tissue residues of Pb observed were a result of soil/sediment ingestion. However, based on a study by Coburn et al. (1951) in which mallards absorbed 20 to 57% of the Pb fed as an aqueous solution of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] and the current study in which mallards on average ingested approximately 7% DMI soil/sediment containing 4485 ppm Pb, mallards in the Killarney Lake area absorbed 63 - 128 μg Pb/g diet consumed and excreted 107 - 198 μg Pb/g feces produced (digestibility of diet = 21%). This agrees with the mean Pb concentration measured in the feces of Killarney Lake mallards (130 ± 39 ppm Pb). In addition, the correlation between the Ti and Pb concentrations of the feces was almost significant at alpha = 0.05 (n = 28, r<sub>s</sub> = 0.370, r<sub>crit</sub> = 0.374), suggesting the Pb in the feces came from ingestion of contaminated soil/sediment. The fact that Pb shot was present in only

one out of the 29 mallards collected in the Killarney Lake area, yet Pb concentrations in tissues were elevated in 80% of the mallards, further suggests that the Pb contamination resulted from the ingestion of contaminated soil/sediments. It is possible (but not probable), however, that the other 28 mallards had ingested Pb shot in the recent past but had already eliminated the shot or the shot was completely eroded by the action of the gizzard and could not be detected visually.

Lead concentrations of duck livers and kidneys from the present study were similar to concentrations reported by Krieger (1990) for ducks collected from the Coeur d'Alene basin in 1986. However, Krieger (1990) did not indicate in his report the location from which the ducks were obtained or the species of ducks that were analyzed. Krieger (1990) found duck livers had Pb concentrations of  $5 \pm 1$  ppm (w.w.; 16 ppm d.w. converted, n = 54) and duck kidneys contained  $6 \pm 1$  ppm Pb (w.w.; 26 ppm d.w. converted; n = 53). Thus, it can be seen that the problem of Pb contamination in the Coeur d'Alene River Valley continues and additional measures must be taken in order to reduce the exposure of wildlife in the area to contaminated sediments.

**Experiment 2: Toxicity of Killarney Lake Sediments to  
Northern Bobwhites**

**Objective:** To determine whether ingestion of sediment from the Coeur d'Alene River system by northern bobwhites at a concentration considered "normal" for that species in the wild is capable of producing elevated tissue concentrations of Pb.

**Methods**

Sediment collected at Killarney Lake, ID was dried at 55°C, sieved through a #20 sieve (840µm) and added to ground poultry ration (Southern States Gamebird Starter Grower Medicated) at 8% dry weight and mixed in an electric mixer for a minimum of five minutes. Fifteen male northern bobwhites approximately 17 w of age were assigned to one of two treatment groups (eight treated, seven controls). The first group was fed ground poultry ration ad libitum and served as controls. The second group received the ground poultry ration spiked with 8% sediment ad libitum. Birds were housed individually in hanging wire rodent cages (20 cm x 18 cm x 18 cm) and provided with fresh water each day ad libitum. Birds were acclimated to the hanging cages and a ground ration for 14 d prior to the study. Food intake by

each bird was measured each day of the study. Birds were fed the experimental diets for 21 d.

Blood (400 - 1000  $\mu$ l) was collected from each bird via the jugular vein using a heparinized syringe the final day of the study. Body weight of each bird was measured on d 0, 7, 14, and 21 of the study. On d 21, all birds were sacrificed via cervical dislocation. The liver and kidney of each bird was removed, freeze dried and analyzed for Pb concentration via HGA atomic absorption spectrophotometry. Blood samples were diluted five times with 0.1 N HNO<sub>3</sub> and analyzed for Pb concentration via ICP spectrometry.

### **Statistical Analyses**

Statistical analyses were conducted using the Statistical Analysis System (SAS Institute Inc., 1987). An ANOVA with repeated measures was used to analyze the effect of time and/or treatment on feed intake and percent change in body mass. Statistix Version 3.5 (Analytical Software, 1991) was used to conduct a Mann Whitney U-test to analyze the effect of treatment on blood Pb. Alpha was set at 0.05 for all statistical inferences.

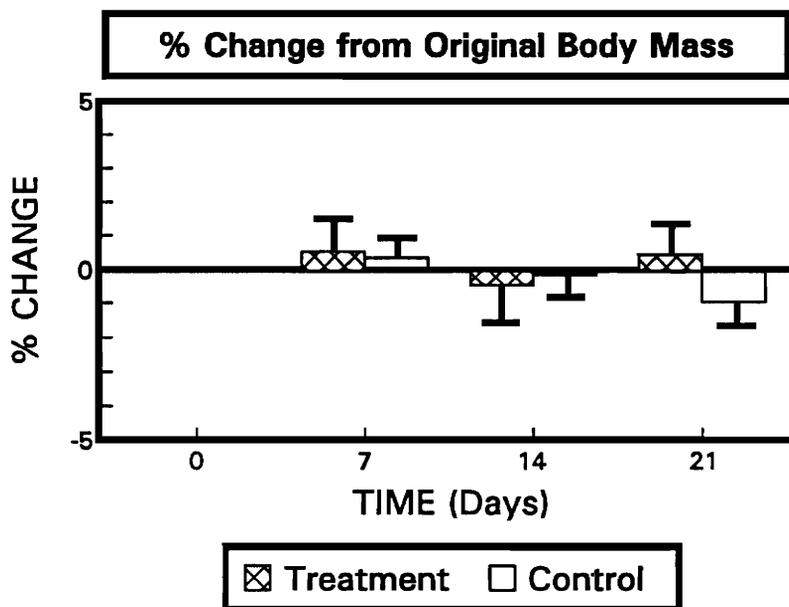
## Results

There was no time x treatment interaction ( $F_{2,12} = 2.6583$ ,  $P = 0.1107$ ) for percent change from the original body mass over time (Figure 3.3), but there was a significant time effect ( $F_{2,12} = 4.0246$ ,  $P = 0.0460$ ). Overall, the mean percent change in body mass of males in both the treatment and control groups did not vary by more than  $\pm 1\%$ . There was no significant difference in percent change in body mass between treated birds and controls on d 7 ( $F_{1,13} = 0.03$ ,  $P = 0.8707$ ), d 14 ( $F_{1,13} = 0.05$ ,  $P = 0.8191$ ) or d 21 ( $F_{1,13} = 1.65$ ,  $P = 0.2213$ ) of the study.

For mean daily feed intake during d 0 - 4, d 5 - 9, d 10 - 14, and d 15 - 19 (Figure 3.4), there was no time x treatment interaction ( $F_{3,11} = 1.7685$ ,  $P = 0.2111$ ) and no time effect ( $F_{3,11} = 0.1238$ ,  $P = 0.9441$ ). There was also no significant difference between treated birds and controls in mean daily food intake for d 0 - 4 ( $F_{1,13} = 0.23$ ,  $P = 0.6429$ ), d 5 - 9 ( $F_{1,13} = 0.04$ ,  $P = 0.8505$ ), d 10 - 14 ( $F_{1,13} = 0.24$ ,  $P = 0.6329$ ), or d 15 - 19 ( $F_{1,13} = 0.10$ ,  $P = 0.7604$ ).

There was a significant difference ( $P < 0.01$ ) between blood Pb of controls and treated birds taken d 21 of the study (Table 3.2). The analysis of d 21 blood revealed controls had a mean ( $\pm$  S.E.) blood Pb concentration of 630 ( $\pm 37$ ) ppb and ranged from 500 to 750 ppb. Treated birds

**Figure 3.3 Percentage change from original body mass over time**



**Figure 3.4 Mean daily feed intake d 0 - 4, d 5 - 9, d 10 - 14, and d 15 - 19**

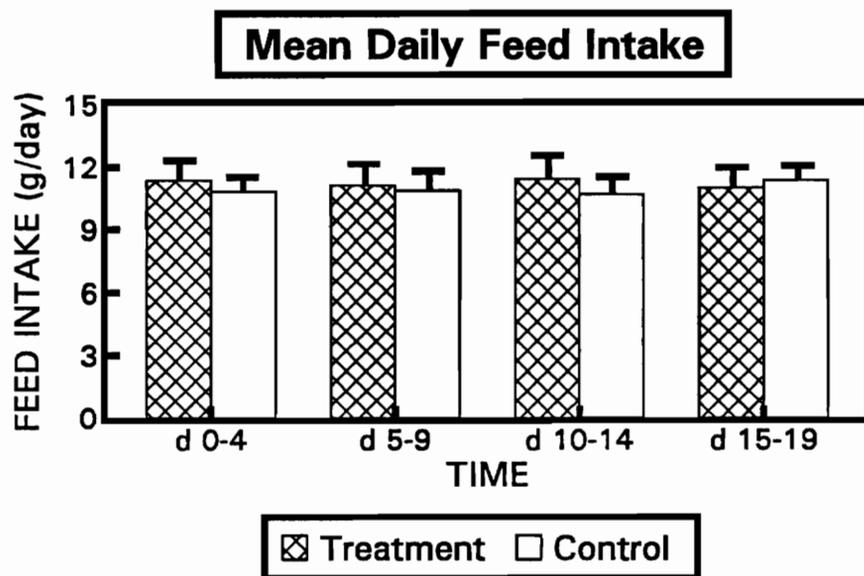


Table 3.2 Influence of Killarney Lake sediment ingestion by northern bobwhites on Pb concentrations<sup>a</sup> (Mean  $\pm$  S.E.) in liver, kidneys, and blood

Parameter	Sediment, 8% DMI	Control
N	8	7
Liver	7.11 $\pm$ 0.81	< 0.10
Kidney	30.33 $\pm$ 6.95 <sup>b</sup>	< 0.10
Blood	1.26 $\pm$ 0.14 <sup>b</sup>	0.63 $\pm$ 0.04 <sup>c,d</sup>

<sup>a</sup> ppm dry weight for liver and kidneys and ppm wet weight for blood

<sup>b,c</sup> Means having different superscripts are significantly (P < 0.05) different from one another.

<sup>d</sup> Based on seven samples (one sample could not be analyzed).

exhibited blood Pb concentrations ranging from 650 to 1750 ppb and the mean ( $\pm$  S.E.) concentration was 1257 ( $\pm$  137) ppb. All of the birds had blood Pb concentrations greater than 150 ppb which is considered indicative of Pb exposure in mallards (Scheuhammer, 1989). Of the seven treated birds, six exhibited blood Pb concentrations  $>$  800 ppb indicative of clinical Pb poisoning in mallards (Scheuhammer, 1989).

Liver and kidney Pb concentrations of all controls were below the detection limit of the ICP ( $<$  100 ppb). However, all treated birds had Pb present in the liver and kidneys. Liver Pb ranged from 5 to 11 ppm (d.w.) and had a mean value ( $\pm$  S.E.) of 7 ( $\pm$  1) ppm (d.w.). Mean ( $\pm$  S.E.) kidney Pb of treated birds was 30 ( $\pm$  7) ppm (d.w.) and ranged from 17 to 48 ppm.

## **Discussion**

The results of this study indicate that northern bobwhites are capable of accumulating tissue residues of Pb as a result of ingesting Pb contaminated sediment in the diet. However, the bobwhites in this study did not exhibit any overt indications of Pb toxicity upon dissection and neither body mass nor feed intake of birds were affected. Blood Pb concentrations were above normal among control

birds but liver and kidney residues could not be detected. This suggests that some form of Pb exposure occurred recently, but the source of this Pb is not known. Blood Pb concentrations of treated birds were significantly greater ( $P < 0.001$ ) than blood Pb concentrations of controls.

The mean liver Pb concentration of treated birds was not great enough to be considered above "background levels" in wild populations. Yet, the mean kidney Pb concentration was great enough to indicate debilitating exposure to Pb. In addition, blood Pb concentrations of treated birds were well above that which is considered Pb poisoned. Dieter and Finley (1978) found that mallards dosed with one Pb shot showed no mortality or significant changes in body weight, blood Pb concentrations of only 1 ppm, and liver Pb concentrations of 2 ppm (w.w.; 7 ppm d.w. converted). Their findings were nearly identical to those in the present study. Dieter and Finley (1978), however, found that at 1 ppm Pb in blood,  $\delta$ -ALAD enzyme activity in blood was reduced by 75% and by 35 to 50% in the brain and liver. An inhibition of  $\delta$ -ALAD of this magnitude could affect heme production and, therefore, cellular processes dependent on heme, including electron transport and ATP generation and cytochrome P-450 activity and hepatic detoxification (Dieter and Finley, 1978). The authors concluded that enzyme inhibition in cells of critical organs such as the brain,

could lead to reduced survival due to impaired motor, visual, and auditory responses. Based on this and the results of the present study, it is difficult to assess the degree to which treated birds were suffering from Pb exposure, but clearly Pb was accumulated to significant concentrations in the tissues of bobwhites consuming a diet of 8% sediment from Killarney Lake, Idaho. The birds in the present study were on a well balanced, high protein diet (26%) which may have reduced the toxic effects of Pb. It is likely that animals on a less nutritious diet would have exhibited the effects of Pb to a greater degree.

## GENERAL DISCUSSION

Soil/sediment ingestion clearly occurs in wildlife as well as domestic species. Ingestion of soil/sediment in highly contaminated areas, such as the heavily mined Coeur d'Alene River Valley of northern Idaho, may be a primary route by which animals are exposed to contaminants in these areas. In order to determine the risks associated with soil/sediment ingestion in a particular species, the amount of soil/sediment ingested by that species must be quantified. Soil/sediment ingestion can be relatively accurately estimated from analysis of feces using the AIR or Ti tracer methods. Either method may be used equally well depending primarily on the quantity of sample to be analyzed and the expected soil concentration in the diet. Soil ingestion expected to be < 10% DMI would best be estimated using the Ti method. In order to calculate the percent soil in the diet, one must be able to estimate the quantity of tracer substance in the soil from the location where the animal fed, digestibility of the animal's diet, and the concentration of tracer in the food consumed by the animal. (The latter generally only applies to the use of the AIR tracer method because plant and animal tissues normally have very low concentrations of Ti). Problems arise in estimating these values, particularly in wildlife species.

Much additional work is needed in the area of estimating soil ingestion by wildlife species because very little information exists in this area and verification of the findings of the present and similar studies is needed.

Waterfowl mortality due to Pb poisoning, particularly among tundra swans, has been well documented in the heavily mined Coeur d'Alene River Valley of northern Idaho (Chupp and Dalke, 1964; Raigini et al., 1977; Neufeld, 1987; Krieger, 1990; Blus et al., 1991). It has been suggested that Pb poisoning resulted, in some cases, from Pb shot ingestion but more often as a result of exposure to Pb from mining activities. Lead is abundant in the sediments of the river and its lateral lakes where waterfowl feed, and it may be ingested directly as grit, or more likely, while feeding on sediment-laden submergent aquatic vegetation, roots, and tubers. Sediments are also ingested inadvertently as waterfowl disturb sediments in shallow waters while in search of food. Plants in the area have also accumulated high concentrations of Pb, particularly in their roots (Krieger, 1990), which are then consumed by waterfowl.

The quantity of soil/sediments ingested by waterfowl depends upon the feeding habits of that particular species. From this study, it was found that mallards, on average, consume soil/sediment at almost 8% DMI. The mallards did not appear to have ingested Pb shot, yet had mean tissue Pb

concentrations well above normal levels, although none exhibited overt signs of Pb toxicity. The study further showed that northern bobwhites fed a diet for 21 d containing 8% dry weight sediment from Killarney Lake had elevated tissue concentrations of Pb which indicates that Pb can be absorbed from the ingestion of sediments from Killarney Lake at the concentration ingested by mallards from the Killarney Lake area.

The findings of the current study suggest that mallards of the Coeur d'Alene River Valley are at less of a risk of Pb poisoning than tundra swans. Based on the feeding habits of tundra swans and the number that have died from acute Pb poisoning in the Coeur d'Alene River Valley, it appears that tundra swans are more likely to ingest high concentrations of sediments than other species of waterfowl. Swans generally consume stems, leaves, roots, and tubers of aquatic vegetation where sediment may be abundant on plant surfaces. They also dig in sediments in order to expose food items, resulting in a cloud of debris through which they must then feed. Further research is needed to estimate soil/sediment ingestion by tundra swans in the Coeur d'Alene River Valley.

Overall, the present study revealed that the concentration of Pb in the sediments of the Coeur d'Alene River Valley from mining activities persist at

concentrations measured in the area in the mid-1970's by Maxfield et al. (1974). The soil/sediments are a threat to waterfowl that consume the soil/sediment as grit and feed on aquatic vegetation that is coated with contaminated sediments during seasonal high water periods. Additional measures must be taken in the area to reduce the quantities of Pb released into the environment by the mining industry currently in operation. Soil ingestion must be considered as a route by which animals are exposed to environmental contaminants in future risk assessment studies.

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**APPENDIX A Analysis and Ingredients of Southern States  
Gamebird Starter and Grower Medicated Feed**

Southern States Cooperative, Inc.  
Richmond, Virginia 23260

ACTIVE DRUG INGREDIENT

Bacitracin MD . . . . . 50 g/ton  
(from Bacitracin Methylene Disalicylate)

GUARANTEED ANALYSIS

Crude Protein . . . . . (min) 26.00%  
Crude Fat . . . . . (min) 4.00%  
Crude Fiber . . . . . (max) 4.00%

INGREDIENTS

Grain Products, Plant Protein Products, Processed Grain By-Products, Animal Protein Products, Dried Hemicellulose Extract, Animal Fat & Hydrolyzed Vegetable Oil, Vitamin A Palmitate (with improved stability), D-Activated Animal Sterol, DL-Methionine, Riboflavin Supplement, Niacinamide, Calcium Pantothenate, Vitamin B12 Supplement, Choline Chloride, Biotin, Thiamine Mononitrate, Pyridoxine Hydrochloride, Vitamin E Supplement, Menadione Sodium Bisulfite Complex (Source of Vitamin K Activity), Folic Acid, Ethoxyquin (A Preservative), Ground Limestone, Dicalcium Phosphate, Iodized Salt, Traces of: Manganous Oxide, Zinc Oxide, Iron Sulfate, Cobalt Carbonate, Copper Oxide, Calcium Iodate, Sodium Selenite.

Code: 11216-GB/B-BRP  
4660 Pellets

## VITA

Erin Elaine Connor was born August 28, 1968 in Baltimore, Maryland. She was graduated from Eleanor Roosevelt High School, Greenbelt, Maryland in 1986 and received her B.S. degree in Animal Science from the University of Maryland, College Park, Maryland in 1989. In January 1990, she became a candidate for the Master of Science degree in Fisheries and Wildlife Sciences (Wildlife Science) at Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

A handwritten signature in black ink that reads "Erin E Connor". The signature is written in a cursive style with a large initial "E" and "C".