HNF-54680
Revision 0

Calendar Year 2012 Assessment of Porcupines and Woody Vegetation in the 100 Areas of the Hanford Site

Prepared for the U.S. Department of Energy
Assistant Secretary for Environmental Management

Contractor for the U.S. Department of Energy
under Contract DE-AC06-09RL14728

P.O. Box 650
Richland, Washington 99352

Approved for Public Release
Further Dissemination Unlimited

Printed in the United States of America

The cover photo is courtesy of Cole Lindsey.

HNF-54680
Revision 0

Calendar Year 2012 Assessment Report for Porcupines and Woody Vegetation in the 100 Areas of the Hanford Site

C. Lindsey and B. Tiller
Mission Support Alliance

Date Published
March 2013

Prepared for the U.S. Department of Energy
Assistant Secretary for Environmental Management

Contractor for the U.S. Department of Energy
under Contract DE-AC06-09RL14728

P.O. Box 650
Richland, Washington 99352

APPROVED
By Janis D. Aardal at 11:03 am, Mar 13, 2013

Release Approval Date

Contents

Figures

Tables

1.0 Introduction

Woody vegetation occurring along the shorelines of the Columbia River has long been identified as a potential pathway for transport of Hanford Site contaminants from the groundwater into the surface environment (Patton et al. 2002; Tiller et al. 1995; Tiller and Poston 2000). Porcupines are relatively common residents along the Columbia River and feed on the bark, leaves, and cambium (Table 1) of woody plants (Fitzner and Gray 1990; Tenneson and Oring 1985). As such, porcupines may be good sentinel indicators of localized contamination from Hanford Site releases because of their relatively limited home range (5-25 hectares [12-62 acres]) and long lifespan (15 years). However, little to no contaminant data exists for porcupines residing on or near the Hanford Site. As such, a small-scale sampling effort was initiated during 2012 to obtain co-located samples of tree bark/cambium tissue and porcupine tissue concentrations of Strontium-90 (Sr^{90}) and trace metals from the riparian zone of the Columbia River near the 100-Areas of the Hanford Site.

The *Aquatic and Riparian Receptor Impact Information for the 100-NR-2 Groundwater Operable Unit* (DOE/RL-2006-26) recorded the maximum concentration of Strontium-90 (Sr^{90}) in riparian mammal tissue at 13.4 pCi/g (fresh wt.), with maximum concentrations in mulberry (*Morus alba*) recorded at 1.6 pCi/g (fresh wt.). While these findings indicated relatively low risk to riparian mammals from exposure to Hanford Site releases, the results suggested the biological concentration factor (BCF) of $Sr^{90}$from vegetation to mammals may have exceeded 8 in this area. Additionally, a separate sampling event, performed in this same area recorded mulberry cambium (bark) Sr^{90} concentrations of 107 and 226 pCi/g (dry wt.) (unpublished data PNNL 2009). This data, combined with a BCF of 8, could result in tissue levels two orders of magnitude higher than documented in DOE/RL-2006-26. Strontium-90, like other radionuclides, releases energy as it decays. This energy can result in deleterious effects to biota at high enough doses (Beyer and Meador 2011). Also, several metals such as lead and cadmium are considered contaminants of concern on the Hanford Site, and could also be present in shoreline vegetation and subsequently ingested by herbivorous mammals such as deer, beaver (*Castor Canadensis*), porcupine (*Erethizon dorsatum*), or mice.

The primary goal of this effort was to determine Sr^{90} and trace metal levels in woody vegetation and a mammalian consumer (such as porcupines) of the woody vegetation, and to examine whether porcupines would be a good indicators of regional environmental contamination within the riparian zone of the Columbia River near the 100 Areas of the Hanford Site. Another goal was to determine whether porcupine quills (modified hairs) could be used as a suitable non-lethal indicator of Sr^{90} and/or trace metal levels in porcupines. This effort also provided results that may be useful for ongoing Hanford Site environmental monitoring, remedial investigations, risk assessments, and/or injury assessments that need to consider “onsite measures” of biological uptake or vegetation-to-mammal Sr^{90} BCFs.

Previously, the Great Basin pocket mouse (*Perognathus parvus*) was identified as the terrestrial/riparian sentinel herbivorous mammalian receptor in the River Corridor Baseline Risk Assessment (DOE/RL-2007-21, Rev.0), with a 100% diet of mixed vegetation. In this prior study, vegetation samples were composited to determine whether there was a correlation between vegetation contaminant levels and the levels in the soils and in higher tropic level receptors such as mammalian herbivores The pocket mouse feeding on mixed vegetation was used to represent all of the organisms in the riparian mammal guild, including porcupines. However, it is possible that the exposure scenario of a pocket mouse feeding on mixed vegetation may not adequately define the scenario of a porcupine feeding on the bark and cambium of woody riparian vegetation growing along the 100 Area shorelines of the Columbia River. A comparison of the two species is shown in Table 1.

Table 1. Comparison of Selected Life History Attributes of Great Basin Pocket Mice and Porcupines

Attribute	Great Basin Pocket Mice	Porcupines
Body mass	0.01 - 0.03 kg	5-15 kg
Lifespan	Most live < 1 yr, maximum 2-3 yr	10-15 yr
Reproduction	Gestation 3-4 wks, 2-8 young per litter, 2-3 litters per year	Gestation 17-18 wks, 1 young per litter, 1 litter per year
Diet	Granivore; diet is mostly seeds, some green grasses; seasonal diet may include 20-25% Insects	Strict herbivore; eats mostly leaves and stems, some seeds; in winter feeds mostly on tree bark and cambium
Home range	0.05 - 0.4 ha	5-25 ha (but may be very restricted during winter)
Habitat	Shrub steppe, prefers sandy soils, underground burrows	Conifer and hardwood forest, also riparian, often arboreal
Hibernation?	Yes	No

2.0 Methods

Sampling areas were selected based on the presence of contaminated groundwater plumes along the shorelines of the Columbia River, (Figure 1) and sample results reported during prior sampling events that showed uptake and/or accumulation of Sr^{90} and trace metals in vegetation and mammals (DOE/RL-2006-26; DOE/RL-2007-21 Rev. 0). A multi-incremental sampling design was used to help obtain sample results that were representative of each general area.

Mammalian tissue sampling - Personnel searched selected areas for porcupines and for sign that porcupines had been feeding in the area. Once porcupines were located in a desired sampling area, they were euthanized using approved methods (AVMA 2007). All tissue samples were collected in the field using pre-cleaned equipment. Samples removed from porcupines for chemical analysis were quills, bone, and liver. Samples of the testicular tissue, liver, and kidney were also collected and fixed in formalin for possible future histopathological interpretation (Cromey 2004). At least 11 grams of quills (approximately 440 quills) were collected from the tail region of the porcupine in order to reach the required sample masses for Sr^{90} and trace metals analysis. For Sr^{90} analysis, the Standard Operating Procedure GL-RAD-A-004 Rev 14 was used while Standard Operating Procedures GL-MA-E-013 REV# 20, GL-MA-E-009 REV# 21 and GL-MAE- 014 REV# 24 were used for trace metals analyses.

Vegetation tissue sampling – Between six and ten bark/cambium sub-samples (each weighing approximately 20-30 grams) were collected from each porcupine collection location. Samples were only taken from trees that had signs of porcupine herbivory. The bark was collected within 150 meters (492 feet) of the porcupine collection point. The sub-samples were combined to form a single sample that represented the area. The tree bark was sampled using a vegetable peeler. Samplers attempted to remove bark to a depth similar to that removed by the porcupines, typically 1-4 millimeters (0.04-0.16 inches) (Figure 2). Care was taken to leave enough bark on the trees and limbs to avoid "girdling" and killing the trees. Tree species sampled included white mulberry, Siberian elm (*Ulmus pumila*), and honey locust (*Gleditsia triacanthos*).

Samples were subsequently shipped to selected laboratories for radiological and chemical analysis. All samples designated for chemical or radiological analysis (i.e. bones, liver, quills, bark) were rinsed thoroughly with deionized water prior to being placed in sample containers. Analyses performed on the respective tissue types are described in Table 2. Analytes in the metals analysis were aluminum, cadmium, chromium, copper, lead, manganese, selenium, silver, uranium, and zinc.

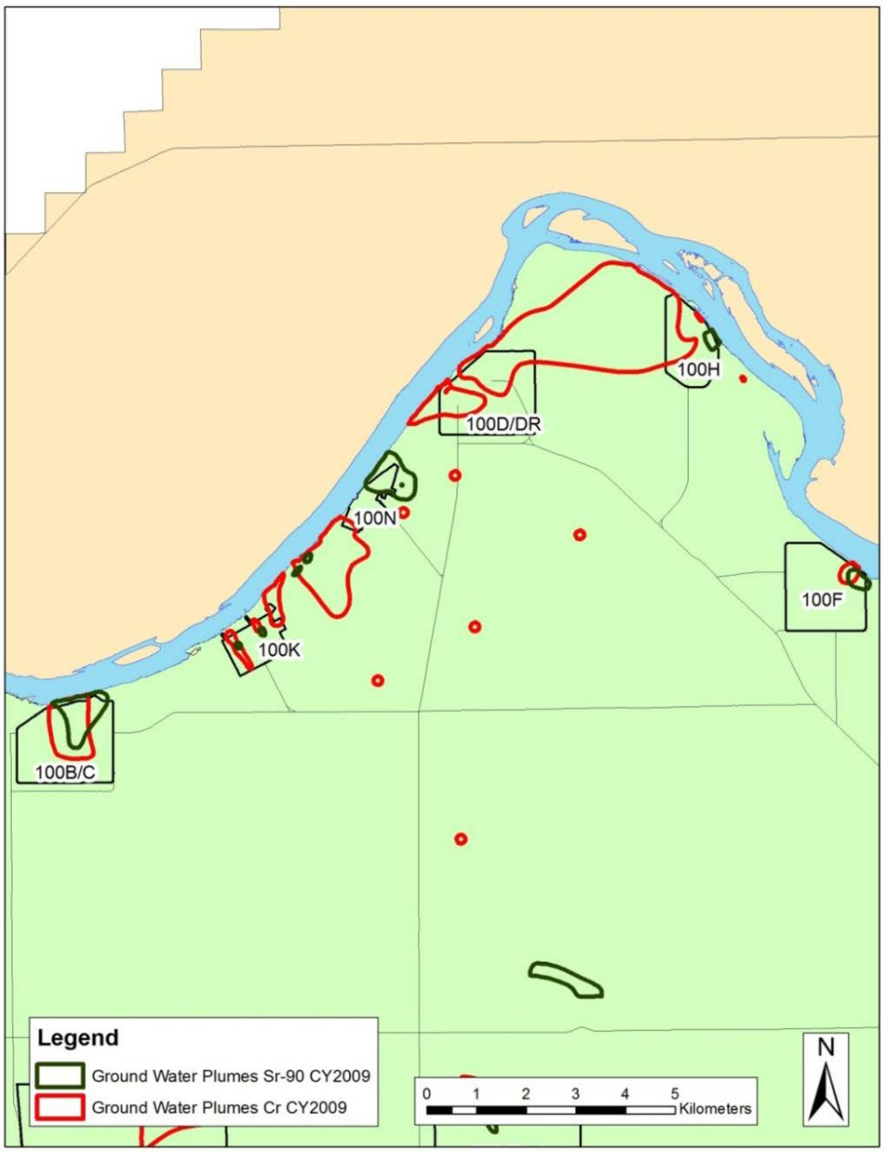

Figure 1. Vegetation and Mammal Sampling Areas were Selected Along the Shoreline of the Columbia River where Groundwater Contamination Plumes Existed

Figure 2. Honey Locust Tree Bark Sampling using Vegetable Peeler, with Signs of Porcupine Herbivory at Top of Photo

Table 2. Summary of Tissues Sampled and their Respective Disposition

Tissue Type	Metals	Sr-90	Histology
Porcupine Quill	X	X	
Porcupine Bone		X	
Porcupine Liver	X		X
Porcupine Kidney			X
Porcupine Testicle			X
Tree Bark	X	X	

3.0 Results

Three porcupines were located and sampled for Sr^{90} and trace metals during the CY2012 sampling effort (Figure 3). These samples were designated Porcupine 1, Porcupine 2, and Porcupine 4. Porcupine 3 was a duplicate sample taken from Porcupine 2. Three samples of tree bark, each consisting of multiple-incremental sub-samples collected from several trees found growing near (within 150m [492 feet]) each porcupine collection site, were also submitted for Sr^{90} and trace metals. The sample results are provided in the graphs in Attachment A. The frequency of detection for each analyte, by tissue type, is listed in Table 3.

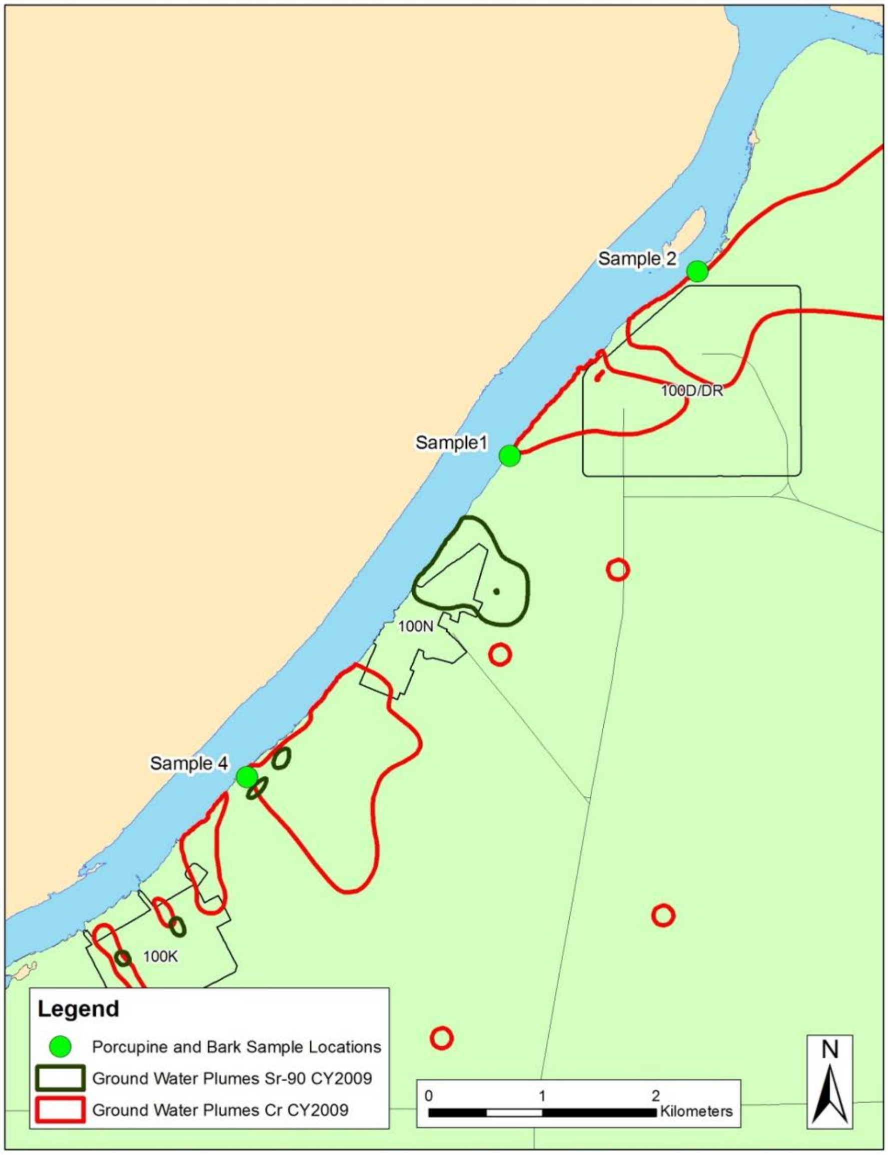

Figure 3. Porcupine and Bark Sample Locations on the Shoreline of the Columbia River

Table 3. Frequency of Detection (%) by Tissue Type for each Analyte

	Aluminum	Cadmium	Chromium	Copper	Lead	Manganese	Selenium	Silver	Uranium	Zinc	Sr^{90}
Bark	100	0	33	100	0	100	100	0	67	100	67
Liver	0	100	0	100	0	100	100	33	33	100	0
Quills	100	0	67	100	33	100	100	0	0	100	0
Bone	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	67

Strontium-90 was detected in both tree bark and porcupine bone collected from 2 of the 3 samples collected. Strontium-90 concentrations in samples of porcupine bone tissues ranged from below the analytical detection limit (0.05 pCi/g fresh wt.) up to 0.8pCi/g (fresh wt.). Concentration of Sr^{90} were also detected in 2 of the 3 tree bark samples and results ranged from below the analytical detection limit (0.05 pCi/g fresh wt.) up to (0.5 pCi/g fresh wt.). A positive relationship for Sr^{90} was observed between co-located samples of bark and bone (Figure 4).

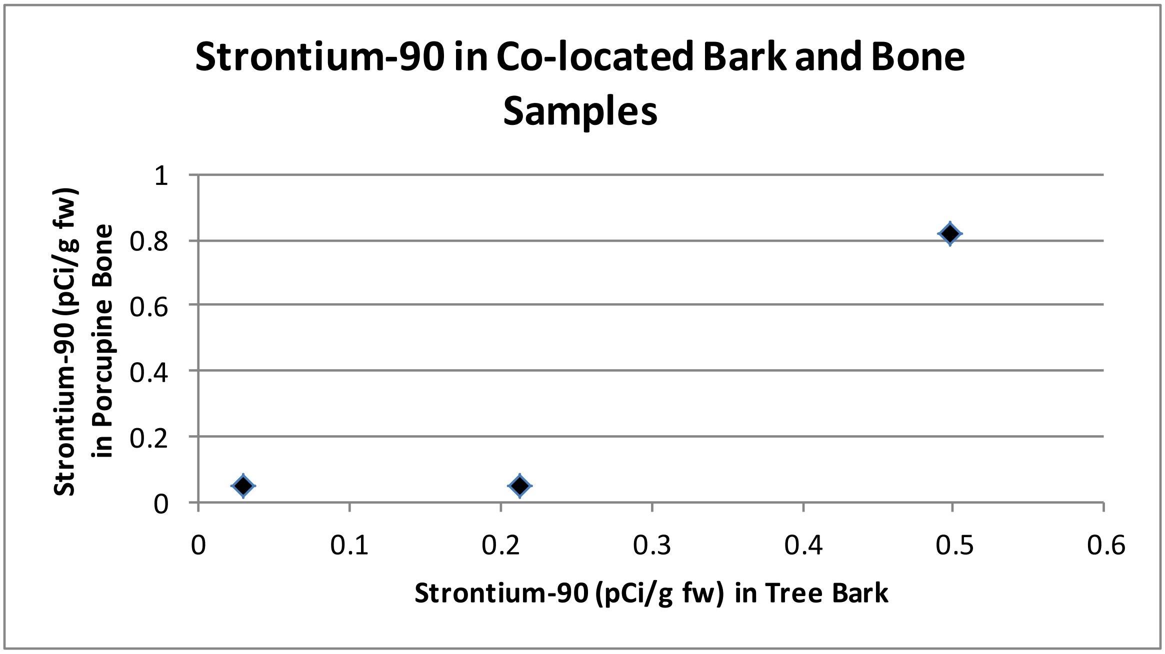

Figure 4. Strontium-90 Levels Observed in Co-located Porcupine Bone and Tree Bark Samples

4.0 Discussion

The goal of this assessment was to perform a preliminary assessment of the utility of using porcupines as sentinels for environmental contamination within the riparian zone of the Columbia River near the 100 Areas of the Hanford Site. Porcupines proved to be elusive and secretive creatures, making sampling at specific locations difficult and time consuming. Although signs of recent porcupine herbivory were prevalent in many of the target sampling areas, surveyors were unable to locate any during several day-time and night-time field surveys performed there. It appeared that porcupines seek shelter in brush and accumulated tumbleweeds (*Salsola kali*), and may only venture into trees to feed for short periods, followed by extended periods of inactivity. One example of this pattern was observed in the sampling 100-N Area. Although sign was prevalent, the trees along the 100-N Area shoreline were surveyed for porcupines on four separate days without locating one. However, surveyors were able to find a porcupine in this location during the only night-time survey. This location was of special interest due to the elevated levels of Sr^{90} in the groundwater near the 100-N Area, but the porcupine was never located during daylight hours when sampling was allowed. Porcupines in other areas did not appear to be more detectible during the night, so nighttime sampling was not explored further.

One porcupine was collected in an area with Sr^{90} contaminated groundwater (see Sample 4 in Figure 3). Strontium-90 was detected in both the tree bark and porcupine bone collected from this location, showing a likely pathway for this contaminant from the groundwater through the trees and to the porcupines. The corresponding levels reported in the bone and bark from this area were 0.82 pCi/g (bone) and 0.50 pCi/g (bark) but were not detectible in the quills (less than 0.05pCi/g.). Strontium-90 levels in the quills were expected to be a small percentage of the level in the bone. For example, Hopkins et al. 1963 found that the concentration of Sr^{90} in mammal hair (quills are modified hairs on porcupines) ranged from 0.02-0.1% of the body burden level of Sr^{90}. Thus, the resultant quill concentration from Porcupine 4 using the bone concentration observed, based on the range of values from Hopkins 1963, would be between 0.82E-03 and 0.16E-03. These hypothetical quill values are well below the analytical detection limit for this study. A correlation between the bone and hair levels may still be possible at higher environmental levels of Sr^{90} or by achieving lower detection limits. Further, there is limited practical utility of using porcupine quills to monitor porcupine uptake and exposure to Sr^{90} because of the relatively high sample mass requirements of the analytical process. The required minimum mass for the Sr^{90} analysis was 10 grams. Considering that there are approximately 40 quills per gram, it would be a significant effort (and potentially deleterious to the health of the individual) to remove 400 quills from a live porcupine in order to meet the minimum mass requirements for Sr^{90}. It was noted during this event that it took between 40 and 50 swipes from the back of the euthanized porcupine to attain the approximately 400 quills.

The data from this effort suggested that there may be a correlation between some metal levels observed in the quills and liver of porcupines (Attachment A). When metals such as copper, manganese, selenium and zinc were detected in the liver of an individual, they were also detected in the quills. The quill samples often contained higher levels than the liver samples for aluminum, chromium, selenium, manganese and zinc. This indicates that non-lethal quill collections could be used to assess accumulation of some trace metals in porcupines. Due to the low mass requirement for metals analysis (around one gram) it is reasonable that the required number of quills (~40) could be retrieved from a porcupine without difficulty. The study also confirms that uptake of some of Hanford Site contaminants of concern by woody riparian vegetation continues to occur along the 100 Area shorelines of the Columbia River.

Other sources of contaminants may be contributing to the porcupines' total tissue burdens in addition to riparian woody vegetation. These sources could include water, incidental soil ingestion, or other food types. In order to make a closer correlation between food intake and body burdens, samples taken directly from the gut could be useful. Although it was readily apparent that porcupines were feeding on the bark of woody plants (based on teeth marks left on several trees), this does not necessarily mean they did not consume other food items. Analysis of gut contents for food types and diet proportions could help to better correlate body burdens to plant tissue types.

Although uptake of Sr^{90} by porcupines was demonstrated in this assessment, all concentrations reported for bark, quills, and liver were below the values listed in Table 8-9 of DOE-RL-2007-21 Rev.0 "Summary of Lowest Observable Adverse Effect Level (LOEL) Based Tier 1 Refined Ecological Preliminary Remediation Goals for Wildlife". Based on this limited sampling effort, it does not appear that porcupines feeding on woody vegetation within the 100 Area shorelines of the Columbia River were experiencing greater exposure than was estimated for their guild (riparian herbivorous mammal) in the River Corridor Baseline Risk Assessment (RCBRA) (DOE/RL-2007-21 Rev. 0). Additional sampling of bark in areas with the highest known groundwater contaminant concentrations could show whether porcupines consume materials with higher contaminant loads than were observed during this study. Due to the relatively low levels of contaminants observed in the tissues of the porcupines, when compared to the LOELs described in the RCBRA and other documents, the testicular tissue, liver, and kidney were not submitted for histological interpretation, but rather were archived for possible future assessments.

5.0 References

American Veterinary Medical Association. 2007. American Veterinary Medical Association's Guidelines on Euthanasia. http://www.avma.org/issues/animal_welfare/euthanasia.pdf. Accessed February 26, 2013.

Beyer, N.B. and J.P. Meador. 2011. *Environmental Contaminants in Biota Interpreting Tissue Concentrations*, 2nd Edition. Boca Raton, Florida: CRC Press.

Cromey, D. W. 2004. *Formaldehyde Fixatives. Cell Biology & Anatomy Histology Service Lab, University of Arizona, Tucson, Arizona*. http://swehsc.pharmacy.arizona.edu/micro/formaldehyde. Accessed February 26, 2013.

Fitzner, R.E. and R.H. Gray. 1991. Status, Distribution, and Ecology of Wildlife on the U.S. DOE Hanford Site: A historical overview of Research Activities. *J. of Env. Mon. and Assessment*. V 18, pp 173-202.

DOE/RL-2006-26. 2007. *Aquatic and Riparian Receptor Impact Information for the 100-NR-2 Groundwater Operable Unit*, Rev. 0, U.S. Department of Energy, Richland Operations Office, Richland, Washington.

DOE/RL-2007-21 Rev. 0. 2012. *River Corridor Baseline Risk Assessment Volume I: Ecological Risk Assessment*. U.S. Department of Energy, Richland Operations Office, Richland, Washington. http://www.washingtonclosure.com/projects/environmental_protection/mission_completion/project_library/. Accessed March 12, 2013.

GL-RAD-A-004. 2012. *Standard Operating Procedure for the Determination of Sr 89/90 in Water, Soil, Milk, Filters, Vegetation, and Tissues*, Rev 14. Gel Laboratories, Charleston, South Carolina.

Patton, G.W., B. L. Tiller, E.J. Antonio, T.M. Poston, and S.P. VanVerst. 2002. *Survey of Radiological and Chemical Contaminants in the Near-Shore Environment at the Hanford Site 300 Area*. PNNL-13692, Rev.1. Pacific Northwest National Laboratory, Richland, Washington. http://www.pnl.gov/main/publications/external/technical_reports/PNNL-13692Rev1.pdf. Accessed March 12, 2013.

Tenneson, C., and L. W., Oring. 1985. Winter Food Preferences of Porcupines. *The Journal of Wildlife Management* 49: 28-33. http://www.jstor.org/stable/3801835. Accessed February 26, 2013.

Tiller, B.L, L.E. Eberhardt, and T.M. Poston. 1995. *Location Analysis and Strontium-90 Concentrations in Deer Antlers on the Hanford Site*. PNL-10711. Pacific Northwest Laboratory, Richland, Washington

Tiller, B.L. and T.M. Poston. 2000. Mule Deer Antlers as Biomonitors of Strontium-90 on the Hanford Site. *J. of Environmental Radioactivity*: V47, pp 29-44.

ATTACHMENT A. Calendar Year 2012 Porcupine and Woody Vegetation Analytical Sample Results

Note: results that were recorded as non-detects were plotted as zero to make sample results easier to view. When applicable, the method detection limit (MDL) was also plotted on the graphs.

Figure 1. Strontium-90 Concentrations Measured in Riparian Tree Bark and Porcupine Bone and Quill Samples Collected during 2012

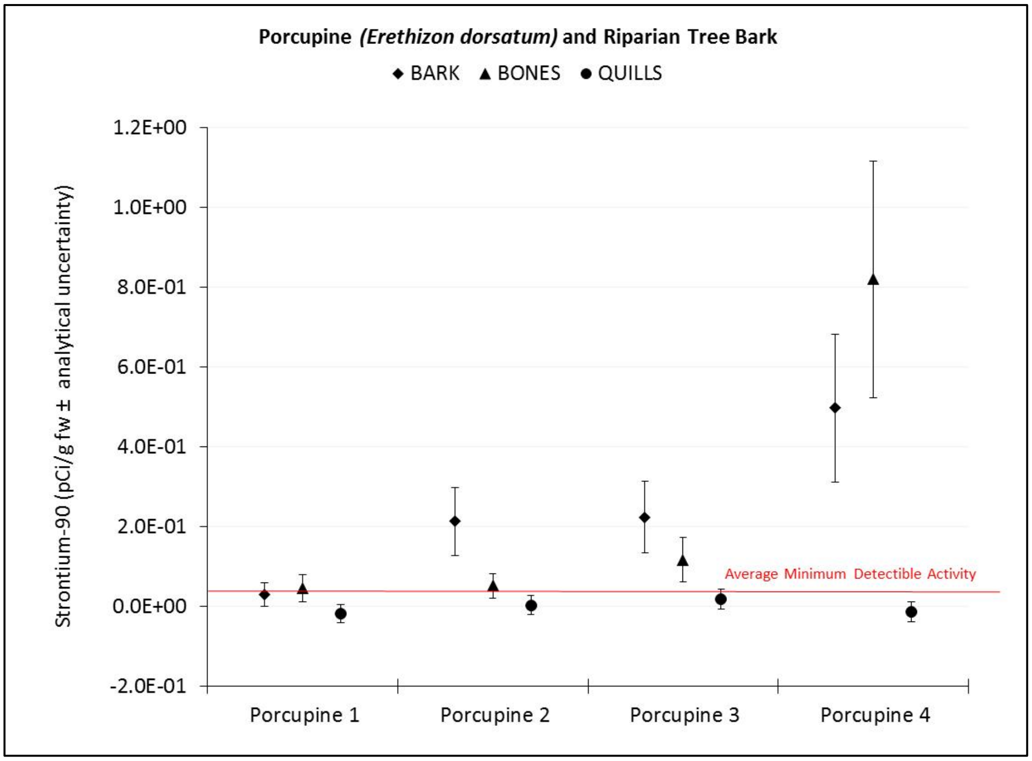

Figure 2. Aluminum Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

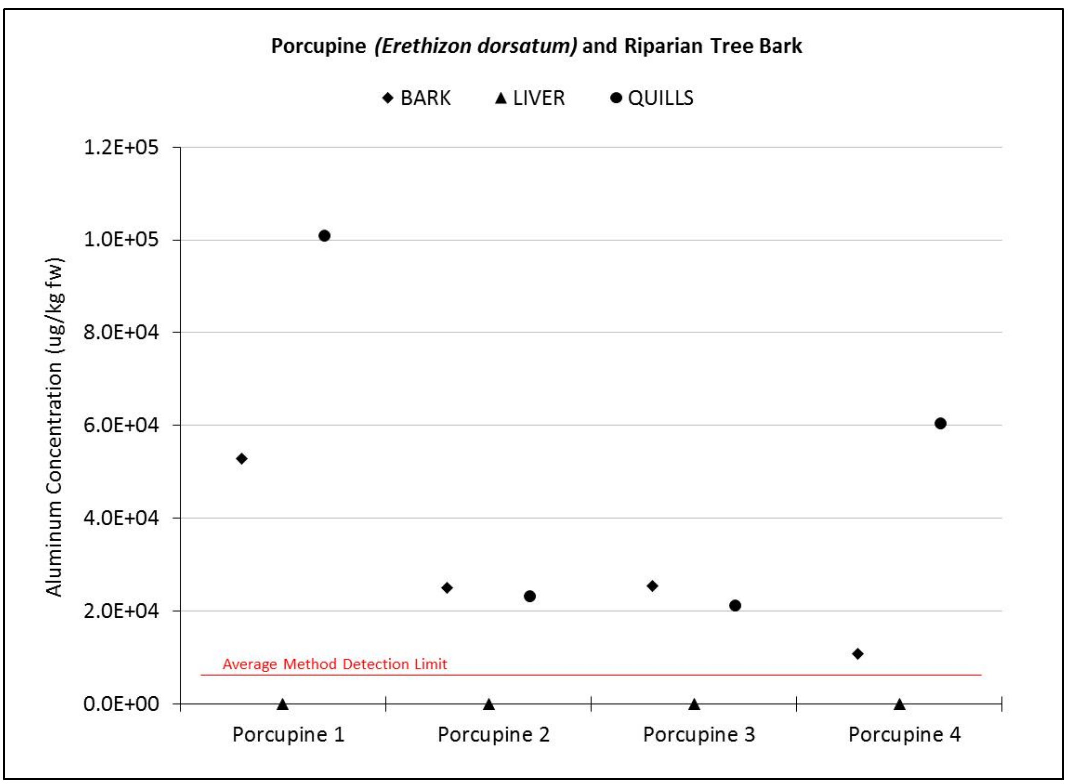

Figure 3. Cadmium Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

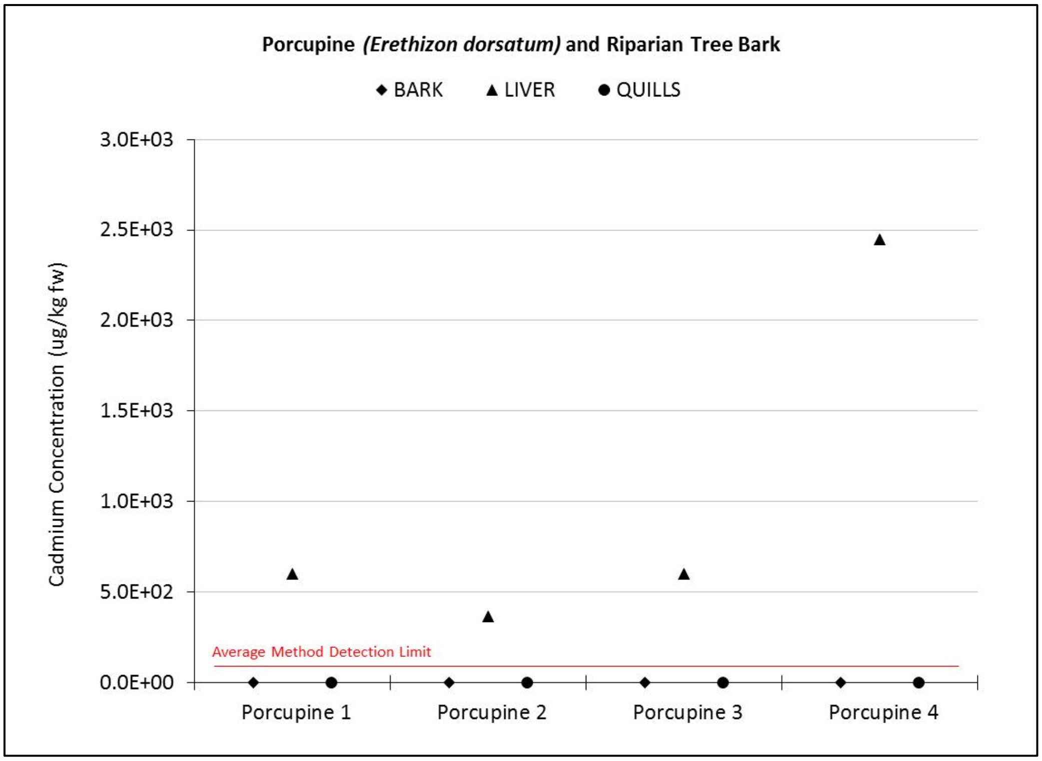

Figure 4. Total Chromium Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

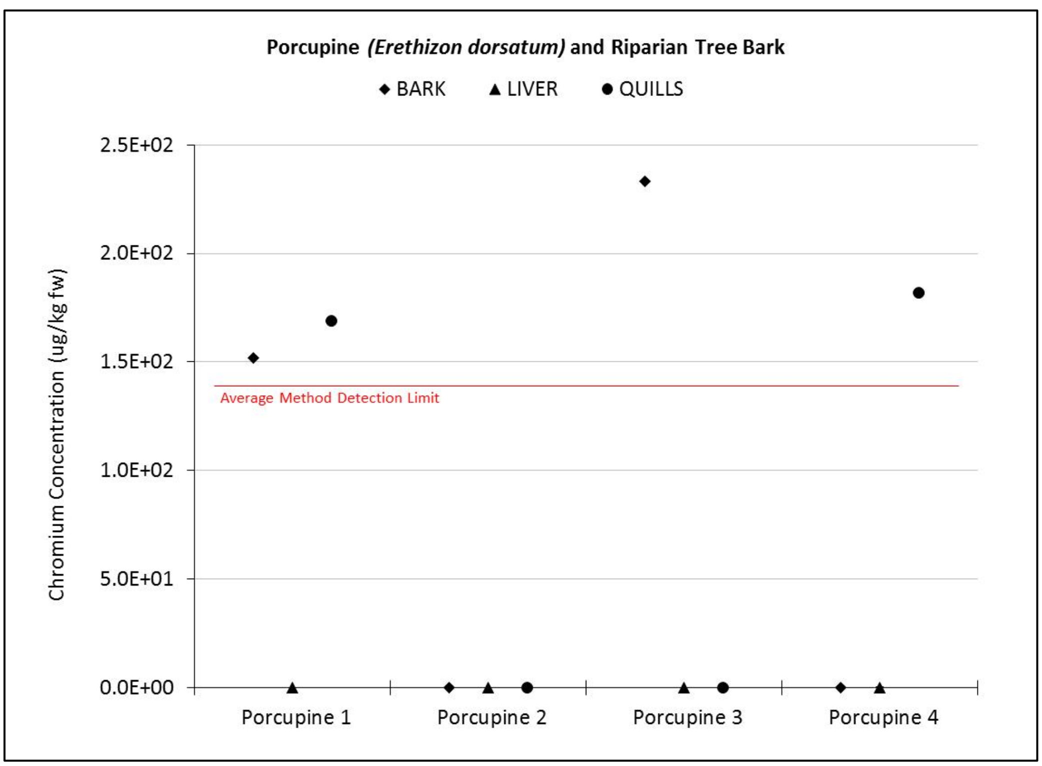

Figure 5. Copper Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

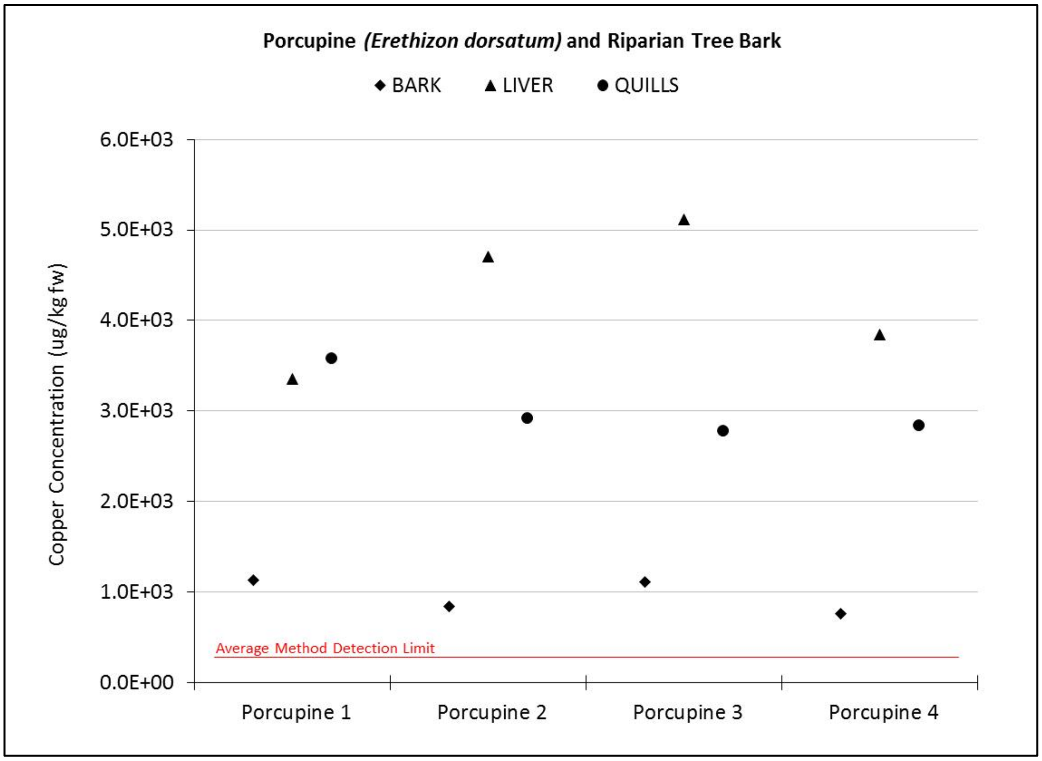

Figure 6. Lead Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

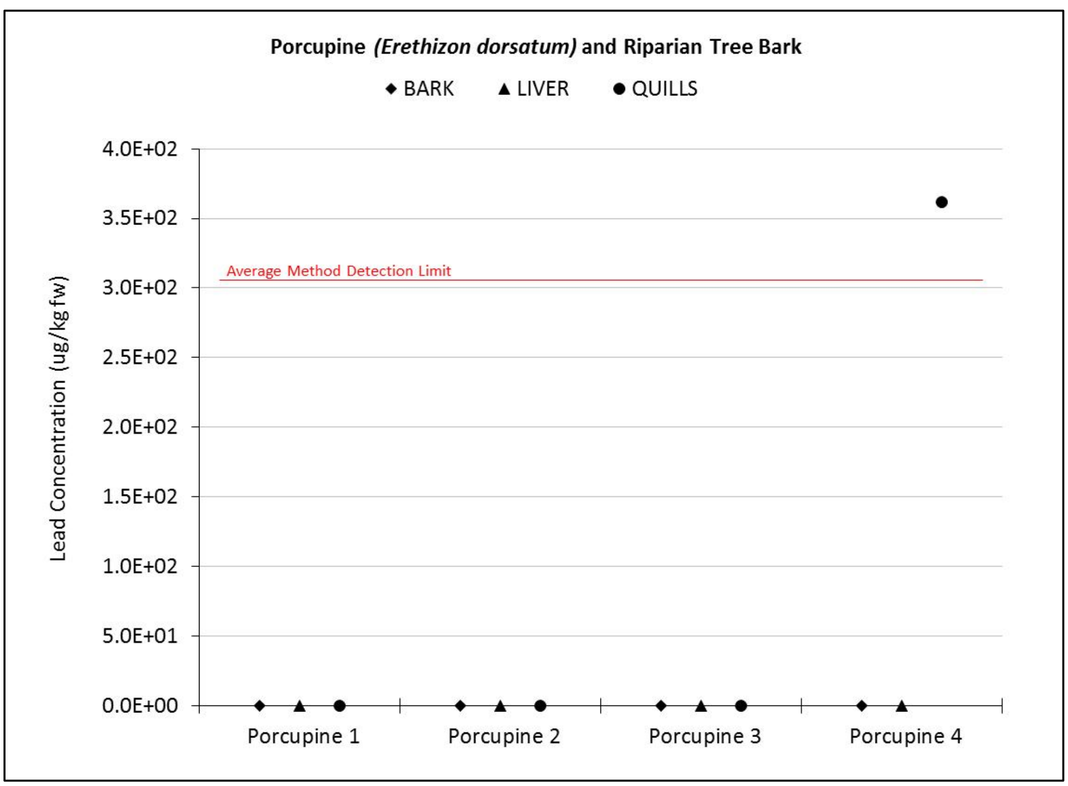

Figure 7. Manganese Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

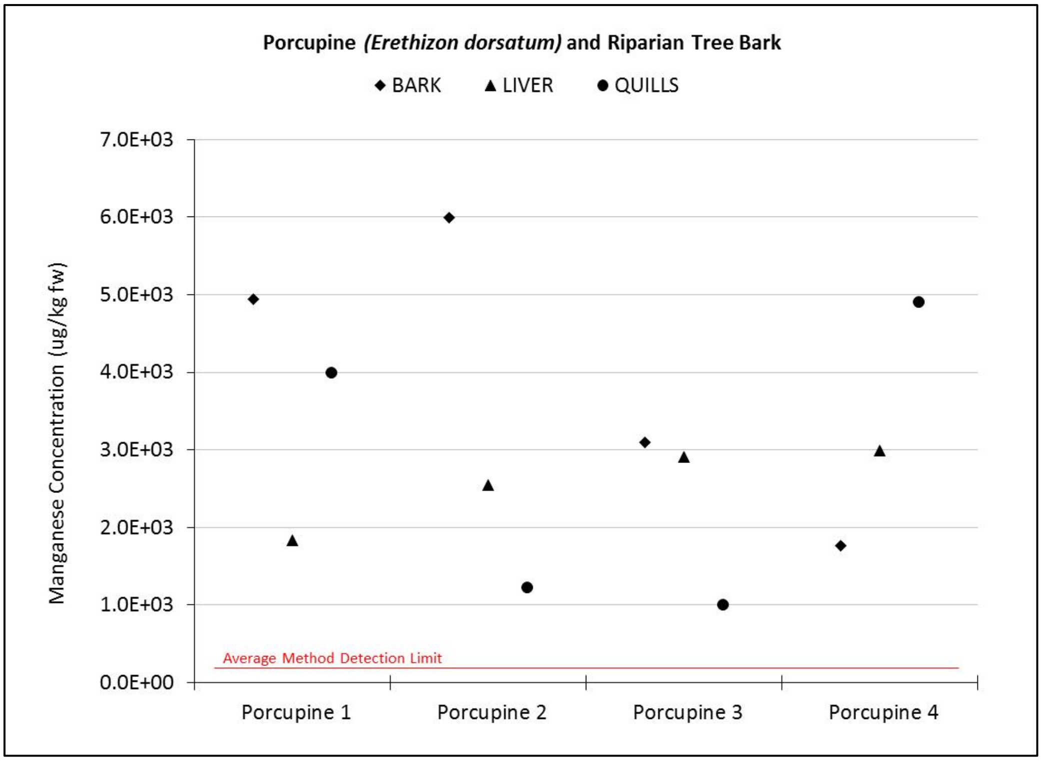

Figure 8. Selenium Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

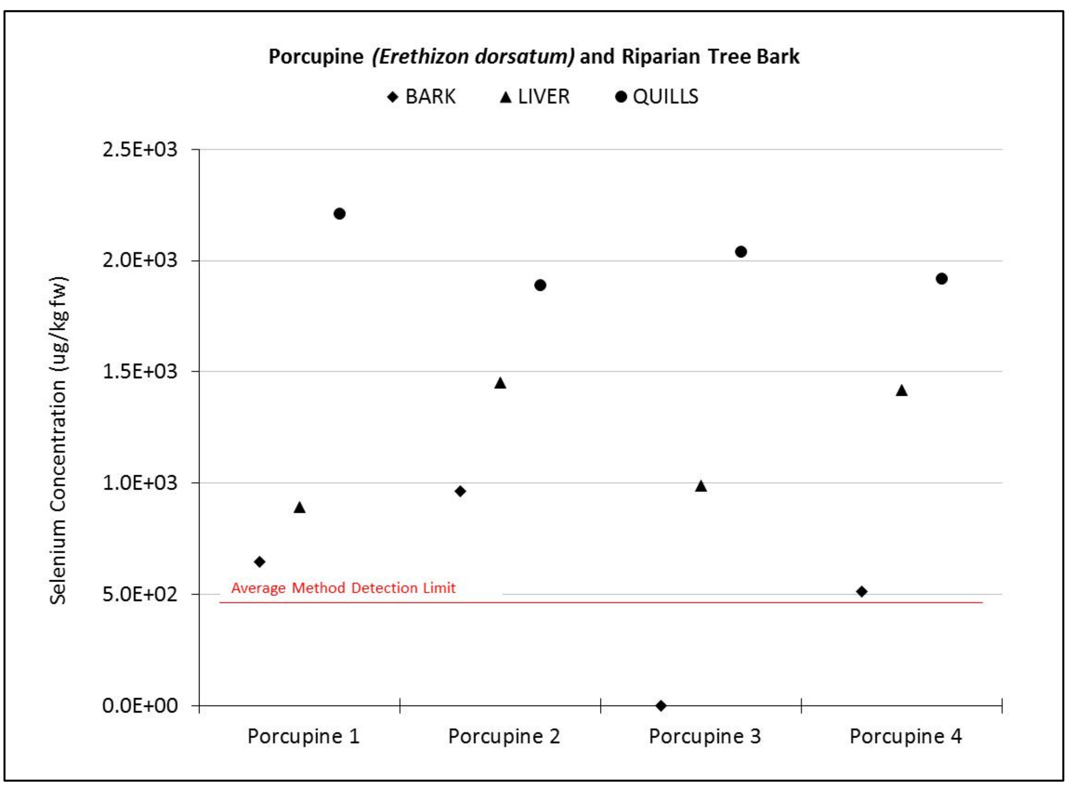

Figure 9. Silver Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

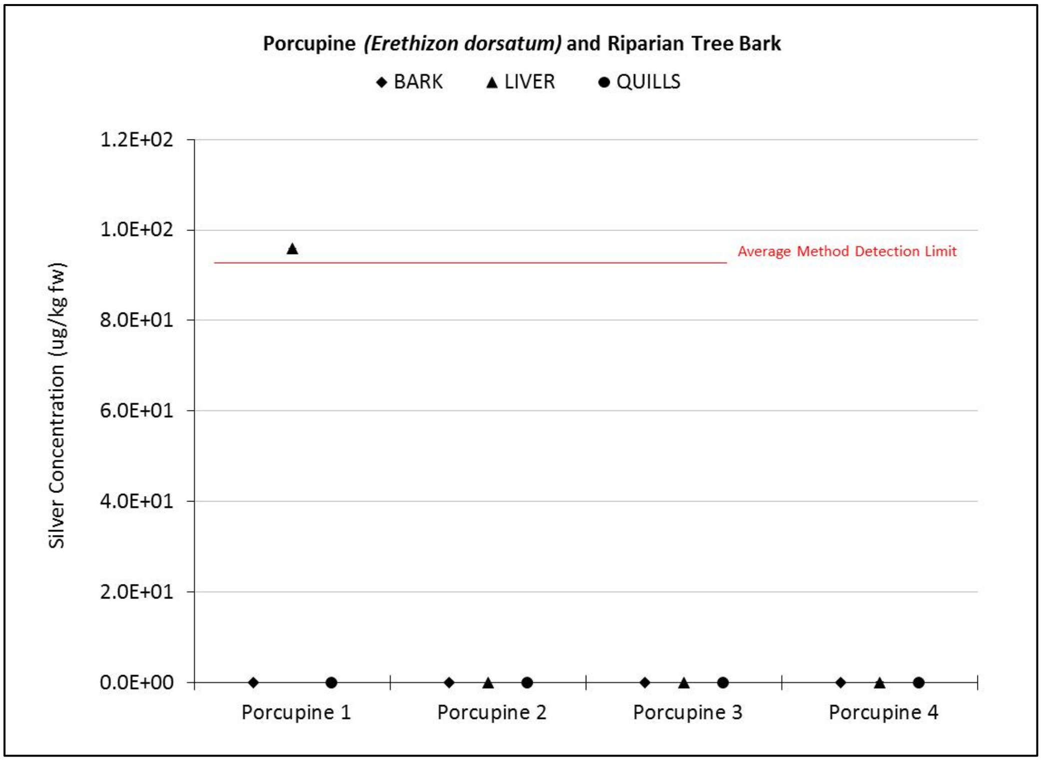

Figure 10. Uranium Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012

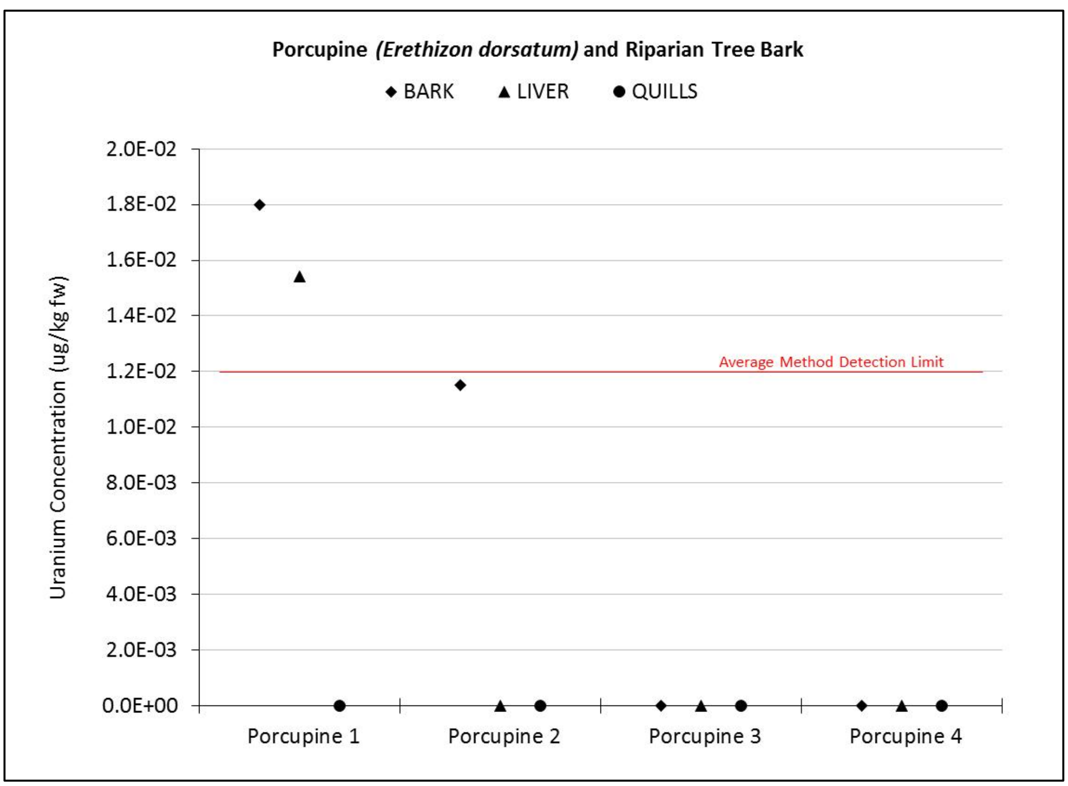

Figure 11. Zinc Concentrations Measured in Riparian Tree Bark and Porcupine Liver and Quill Samples Collected during 2012