Tungsten uptake kinetics and trophic transfer into a novel gastropod model

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Abstract

Previous investigations on the military relevant metal, tungsten (W), have paved the way for further exploration of its relevant environmental and biological pathways. This investigation presents the most robust known trophic transfer study of W between plant and animal models. Steady state of W in cabbage (*Brassica oleracae*) was reached at 114-days with a bioaccumulation factor (BAF) of 0.55. For the herbivorous snail *Otala lactea*, the steady state of W bioaccumulation from direct exposure to contaminated soil was reached in 23 days with a hepatopancreas BAF of 0.05. Trophic transfer of W bioaccumulation from consumption of W-grown cabbage resulted in significantly greater body burdens with steady state estimated at 5-days and a hepatopancreas BAF of 0.36. These results indicate that consumption of contaminated food is the most important pathway for W movement into the snail model, likely because cabbage is more bioavailable to the plant model. Investigations into how W is compartmentalized into the snail model found that higher concentrations were found in the hepatopancreas as compared to the rest of the snail. Chemical speciation showed a higher degree of polytungstates within the hepatopancreas in comparison to the rest of the body, which was predominantly monomeric species of tungsten. The pathways that W taken within the snail model reveal that there is substantial evidence that W is incorporated into the shell matrix during exposure and that the shell can be utilized as biomonitoring tool.

Introduction

Tungsten is ubiquitous in the environment at low concentrations [1,2]. It is found in naturally occurring chemical compounds and minerals such as scheelite ($CaWO_4$) and wolframite ((Mn, Fe) WO_4). Both of these minerals are formed by contact metamorphism, where inclusions of magma at relatively shallow depths and a large gradient of local heat are responsible for mineral formation, and can be found in granite as well as quartz and tin veins. Tungsten is largely lithophilic, though it does show some siderophilic tendencies that are very similar to uranium and thorium, and which have similar partitioning coefficients [3]. Robert Scheele first discovered tungsten in 1781, and its properties make it a unique element. Tungsten has one of the greatest densities (19.25 g/cm^3), after only uranium and platinum and melting points (3422°C), after only carbon on the periodic table. Of the metals, it has the lowest coefficient of thermal expansion. Strong covalent bonding is the main reason behind these properties, and its strong bonding ability greatly lends itself to alloying with other metals, dramatically increasing the alloy's toughness. The industrial usage of tungsten has grown dramatically since alloying with steel, to the point of now being ubiquitous in household settings. Tungsten is utilized in light bulbs, x-ray

tubes, SEM filaments, and welding equipment, and almost anywhere that there is high heat with a need for toughness [1,4]. Tungsten carbide alloys are commonly used in hand tools, but also for industrial applications such as mine drilling heads and grinding bits. Considering its weight and relative inertness, tungsten was considered a greener alternative to lead and is now found in fishing weights, darts, and golf clubs [1], but further investigations have shown W is not inert [5, 6].

The Department of Defense saw an opportunity to replace lead with tungsten during the Green Armament Technology Program (GATP, 1997) [1, 7, 8,2]. In 2002, nearly 180 million small arms rounds were produced and by 2005, with 120 million of these rounds used in training. At this time, several military installations suspended their use because tungsten was detected in adjacent groundwater [1,2]. Military usage did not begin with the Green Ammunition Program. Germany began production of tungsten carbide armor-piercing rounds for anti-armor weaponry during World War II. General Erwin Rommel's forces used, for the first time, the high velocity tungsten carbide penetrators against British tanks that nearly led to Germany's success in its North Africa Campaign [9]. After perfecting the technology a few years later, American forces used the penetrators against German forces with great success. The development of advanced armor systems in the 1960s made the previous penetrators obsolete, but improvements were made and again the tungsten carbide penetrators were used [10]. It was not until depleted uranium began to be utilized in the 1970s that tungsten carbide penetrators were phased out. By the 1990s, the concern over public health risks associated with depleted uranium usage led to the return of using tungsten carbide penetrators for many of these applications.

Investigations on the environmental implications of W were rare considering its relative inertness as an alloy. However, the USEPA has since listed W as an emerging contaminant [11]. Attempting to backfill knowledge to catch up to environmental exposures and usages of tungsten has been arduous [4,6,12,13, 14, 15, 16]. Thermodynamic data are elusive, but gains were made with respect to identification of the variability of the many tungsten species. While tungsten is commonly found in the environment as a tungstate anion (e.g. WO_4^{2-}), Bednar et al, [5] showed that it polymerizes with other anions to form complex poly- and heteropoly-tungstates with diverse geochemical and toxicological properties [17]. However, metallic tungsten is naturally thermodynamically stable, although its mobility and speciation varies when the system is not in equilibrium [1,18]. The development of new analytical processes and increased resolution allow for determination of how species are processed, accumulated, and transformed, and which of them may have an adverse effect on the environment and human health [5, 6,19].

This study provides a more robust follow-up investigation to Kennedy et al [20] and will explore the fundamentals of tungsten interactions between soil, plant, and gastropods. Further, this study provides the most robust known trophic transfer investigation of W between plant and animal models. Our objectives include determining the trophic transfer kinetics of W from soil to plant or gastropod and from plant to gastropod (trophic transfer), which will lead to a better understanding of how quickly tungsten accumulates in plant and animal tissue. Building upon the kinetics work, our intent was to discern where the greatest accumulation and compartmentalization (whole snail, hepatopancreas, mantle, and shell) of trophically transferred tungsten occurs within the gastropod, by chemical species and total tungsten. In addition, examination of which W species tends to be the most mobile and what

trophic pathway provides the highest tissue burdens. The use of this novel gastropod model allowed us to demonstrate tungsten integration into the shell matrix during normal growth and re-growth of damaged shell. These findings may ultimately result in use of snail shells as a useful biomonitoring tool in the environment.

Materials and Methods

Chemicals and soils

Test materials utilized were previously described in Kennedy et al [20]. An aged spiked soil was generated by mixing a Grenada Loring silt loam soil [previously described 6, 13,21] with 7000 mg/kg tungsten [21], then aged for 4 years in 55 gal drums to reach W species equilibrium. A 500 mg/kg W soil for plant and animal exposures was created by thoroughly homogenizing clean silt loam Grenada-Loring soil with the 7000 mg/kg aged tungsten Grenada-Loring soil created a test soil with a nominal tungsten concentration of 500 mg/kg. This concentration represents the highest concentration that did not impose deleterious effects to exposed species [20]. Soil was neutralized with 0.250 M NaOH (VWR) by adding 450 ml of solution to 3 kg batches of soil, mixing by hand and then sieved through a U.S. Standard #5 mesh to disperse clumps and thoroughly homogenize the soil. Neutralization was required to avoid pH-induced inhibition of plant growth and avoidance by selected gastropod. NaOH was utilized to neutralize the soil in these exposures rather than the $CaCO_3$ used previously in Kennedy et al [20] to avoid Ca^{2+} binding W, which could alter its bioavailability.

Plant and gastropod uptake kinetics experiments

Stonehead Cabbage (*Brassica oleracae*) seeds (Guerney Seed and Nursery, Greendale, IN, USA) were sown in clean Grenada-Loring control soil and the aged tungsten spiked Grenada-Loring soil. Growth facility and equipment were as previously described [20]. Concisely, growth chambers, lighting, and humidifiers were utilized to maintain ideal environmental conditions for plant growth throughout this exposure. Four seeds were sown per 1.145 kg of soil in 16.5 cm pots (AZE0650G, Grower's Supply, Salem, OR) per soil type (clean and aged W). This experiment consisted of six replicates per exposure per planting period (n=6, C=2) with each planting period separated by approximately 2 weeks. Collection of leaves from one plant per replicate occurred on days 10, 18, 35, and 53 to assess tissue weight and tungsten concentration. On day 35, three whole plants were sampled to assess total tissue growth (excluding roots) and tungsten concentrations. On day 67, the remaining whole plants were sampled, total tissue masses (excluding roots) recorded, and tungsten concentrations analyzed. Leaf and whole plant samples were rinsed with reverse osmosis (RO) water, dried, weighed, vacuum sealed using custom cut 28 cm wide bags (Food Saver, model V3020) and stored at -80ºC (REVCO, Model ULT 1386-5-A35, Kendra Laboratory Products, Asheville NC) prior to chemical analysis.

The gastropod *Otala lactea* (Carolina Biological Supply, Burlington, NC, USA) was exposed to three different treatment regimens over 21 days (n=5): control (clean soil and clean food), soil (aged W-spiked soil), and cabbage (clean soil and cabbage grown in W-spiked aged soil.) Reverse Osmosis (RO) water (192 ml) was used to moisten 650 g soil of each replicate to 75% water-holding capacity, and the interior

of 16x13x13 cm replicate tanks was subsequently lined with soil. Exposure to the control regimen occurred in three replicates and all sampling occurred on day 0 and 21. Exposure to the soil regime occurred over 21 days with 3 replicates per time point at 0, 3, 7, 14, and 21 days. Exposure to the cabbage regimen occurred over 21 days with 3 replicates per time point at 0, 3, 7, 14, and 21 days. These exposures occurred concurrently. The experiment was performed in environmental rooms (Darwin, St. Louis, MO, USA) in order to maintain humidity, temperature, and light cycle (80%, 25 ± 1 °C, 16h:8h light/dark respectively). Soil moisture was maintained by manually misting the exposure tanks twice daily with RO water. Depending on exposure regimen rations of clean and W grown cabbage (1 g cabbage per snail), were provided twice weekly. Cabbage rations were available to snails with the remainder removed after 24 hours of feeding. At the completion of the test, snails were removed from treatments to allow purging of any consumed food. At the completion of the test the snails were placed in a freezer (-20 ºC, Kenmore, Model 363.78162891) prior to dissection. Dissection occurred by separating the hepatopancreas from the rest of the snail and then homogenizing the samples with a handheld tissue homogenizer (Omni, Kennesaw, GA, USA). After homogenization, the samples were frozen again (-20 ºC) before chemical analysis.

Soil and Trophic Transfer experiments

Mirroring the exposure regimens from uptake kinetics, *O. lactea* was tested for 28-days in control, soil, and cabbage treatments, each consisting of five experimental replicates containing five individuals. Soil was hydrated at the rate of 192 ml RO water to 650g soil for each replicate (to 75% water holding capacity) and then used to coat the interior of 16x13x13 cm tanks. In addition to the five snails, three additional snails per exposure in three of the replicates had a section of their shell removed by cutting a window (~1 cm^2) into the outer whirl of the shell with a Dremal 300 rotary diamond blade (model EZ545), to eliminate potential W-blade contamination. To regulate environmental parameters, the exposures were run in a Darwin environmental chamber (Model KB030, St. Louis, MO, USA). Feeding rations were given twice a week as in the kinetic test. During the removal of the residual cabbage, fecal material was removed and stored for analysis. At the completion of the 28-day exposure, snails were purged for 24 hours, frozen and later dissected, as previously described. The mantle was also removed from one snail per replicate and four of them were composited and homogenized, while the rest of those bodies were discarded and the remaining mantle was prepared for synchrotron analysis. Re-growth shell windows were removed by a Dremal rotary diamond blade and then prepared for synchrotron analysis.

Dermal exposure to tungsten species

To determine the dermal-only bioavailability of tungstate and polytungstate species to gastropods, an elutriate of the 7000 mg/kg soil was prepared as per American Society for Testing and Materials Guide E 1391 (22) and USEPA-U.S. Army Corps of Engineers (USEPA-USACOE 1998). In a ratio, (1:4) soil to dechlorinated tap water, the mixture is rigorously aerated for 30 minutes, allowed to settle for 1 hour, and then the supernatant was filtered to 0.45 µm (HAWP04700, membrane, Millipore, Billerica, MA, US). Utilizing similar methods to the dermal exposure described in Kennedy et al (20), two concentrations were made by saturating microfiber clothes in the W elutriate and a control, consisting

of dechlorinated water. In each exposure (n=5) and three snails per replicate, feeding rations were given as per previous exposures for 7 days. To compensate for evaporative loss, 6 ml of RO water maintained moisture content without increasing W concentration. The saturated cloth was replaced with a freshly W-spiked cloth on the fourth day of exposure. Exposures occurred in Darwin environmental rooms to maintain ambient parameters. Upon completion, snails were frozen (-20 ºC) and then prepared for analysis by dissection and separation of whole snail from hepatopancreas. Samples of elutriate, saturated cloth, whole snail, and hepatopancreas were preserved for analysis by freezing (-20 ºC).

De ovo maternal transfer

Snail cultures were performed in the presence of tungsten-contaminated cabbage in order to demonstrate maternal transfer to shell and uniform distribution of tungsten to growing young. Two cultures of *O. lactea* were maintained in 37 L aquaria by placing 50:50 mix (by volume) of Magic Worm Bedding (Carolina, Cat# 141684) and clean sand to the depth of a 16.5 cm pot and hydrated to 75% water holding capacity. Propagation ratios for tank dimensions indicated fourteen snails for best results (23). To maintain humidity and temperature with a reversed light cycle, in order to observe activity during the day while it was dark in their enclosure, Darwin environmental chambers housed the cultures. A Misting System (Big Apple Herpetological, Hauppauge, NY, USA) maintained moisture within the aquaria. One culture was fed clean-grown matured Stonehead Cabbage and the exposed culture was fed matured Stonehead Cabbage grown in tungsten-spiked soil. Placing entire cabbage plant, (plant, roots, soil and pot), within the aquaria allowed feeding until consumption of the entire head had occurred. To prevent snails from consuming and touching the soil surface, pea gravel covered the bare soil in the pots. When offspring hatched from egg masses laid in the bedding and sand mix, a sample was taken to ensure that only maternal transfer of tungsten occurred. After approximately 400% growth a second sample was taken. Samples were frozen and then desiccated in a 60 ºC drying oven (Fisher Scientific, Isotemp Oven, 655G) overnight, sonicated for 10 minutes (Branson, 8510, Danbury, CT, USA) to remove any remaining tissue, and dried again in the drying oven in order to prepare for synchrotron analysis.

Chemical Analytical methods

Chemical analyses of soil and tissue samples are similar to Kennedy et al (20). Concisely, soil and tissue water extracts were prepared for tungsten speciation according to Bednar et al (5, 6). The extracts were then analyzed by HPLC-ICP-MS methods (5). Total tungsten determination was achieved after acid digestion extraction with analysis by ICP-AES and ICP-MS (24,25).

Synchrotron methods

Soft tissue fixation was started with 10% neutral-buffered formalin up to 24 hours. A dehydration process followed to remove free and bound water from tissues efficiently at a series of 70%, 90% and 100% ethanol (VWR, Suwannee, GA). Then, to remove the residual ethanol the tissue was placed in Xylene (VWR). The final step, embedding, involves infiltrating the tissue with the embedding agent, paraffin wax (paraplast, VWR), at 56ºC and sectioning the tissues on an Olympus microtome to 10-15 microns, followed by mounting on mylar film slides. Soft tissue (hepatopancreas, cabbage leaf) was

sectioned at 20-50 µm using a microtome (Olympus Cut 4060, Olympus America, Lake Success, NY, USA). Shells were sectioned using a diamond blade model EZ545 on a Dremel 300 Series prior to mounting on diglycidyl ether resin and nonylphenol hardener (Allied High Tech epoxy, Rancho Dominguez, CA, USA) and cut in cross-section with a Buehler Isomet Low Speed Saw to expose the fragile new growth and old growth shell. Tissue samples were mounted onto 3 µm thick mylar film. Tungsten was calibrated to the LIII-edge of 10207 eV using a W foil. X-ray Fluorescence (XRF) data were generated at the microprobe beamline (2-3) at the Stanford Synchrotron Radiation Lightsource (SSRL, Stanford University, Menlo Park, CA). Beam size on the sample was approximately 2 µm x 2 µm using Pt-coated Kirkpatrick-Baez focusing optics. X-rays were selected using a water cooled Si(111) double crystal monochromator. XRF data were collected using a single element Si Vortex detector. The Mylar film and resin were analyzed for total metal content to minimize contamination of the W signal. Samples were prepared directly before arrival at the synchrotron facilities. Due to an overlap with the zinc Kα1 line and the W LIII emission lines, the XRF maps were collected both above and below the W edge. Data collected were analyzed using the SMAK microtoolkit.

Statistical analysis

All statistical comparisons and determinations of data normality (Kolmogorov-Smirnov test) and homogeneity (Levene's test) were performed using SigmaStat v3.5 or SigmaPlot v10 software (SSPS, Chicago, IL, USA). One-way, two-way (factors: exposure regimen vs. tissue compartment) and three-way (factors: exposure regimen vs. tissue compartment vs. treatment (control vs. W)) ANOVAs were performed to determine statistically significant differences ($\alpha = 0.05$). If data failed normality or homogeneity tests, $\log_{10}$ transformations were performed. Uptake and elimination rate kinetics in addition to the time (in days) required for acquisition of 95% steady state body burdens (i.e., stable tissue concentrations) were calculated according to a non-linear one component-uptake model and associated equations were provided in ASTM[26]. While the one-compartment model appeared to have application to the cabbage, a caveat must be made that the cabbage violated the model assumption of minimal growth during the exposure period. Bioaccumulation Factors (BAF) were calculated as the ratio of the measured concentration of W in tissue to the concentration of W within the exposure medium.

Results and Discussion

Soil Analysis

Total measured tungsten present in the clean Grenada-Loring soil control was 2.37 ± 0.21 mg/kg, which is in the range of normal background levels [1, 2]. For all tungsten-spiked soil exposures, the nominal 500 mg/kg mix was 547 ± 34 mg/kg. Soil species were 90% mono-tungstate and 10% poly-tungstate as compared to previous reporting of 37% mono-tungstates and 63% poly-tungstates [20], suggesting variation between neutralization methods may alter species equilibrium distribution and thus bioavailability.

Kinetic Comparisons

Separate kinetic bioaccumulation experiments determined steady state tissue residues and the time to reach steady state (in days) for cabbage exposed to contaminated soil, snails exposed to contaminated soil and snails fed contaminated cabbage (trophic transfer). Time-course sampling of cabbage plants over a 65 day exposure period to tungsten contaminated soil resulted in successful modeling of uptake (k_u) and elimination rate (k_e) constants of 0.015 ± 0.03 g/g/d ($p < 0.05$) and 0.03 ± 0.01 1/t ($p = 0.07$), respectively (Figure 1a). Populating the one compartment uptake model [26] with these rate constants and the measured soil concentration yielded a modeled steady state concentration of 287 mg/kg reached after 114-day exposure. The resulting steady state BAF was 0.55, which was comparable to a BAF range of 0.53 – 0.72 for *B. oleracae* previously reported by Kennedy et al [20], suggesting that there was no substantial difference in total measureable W bioavailability with $CaCO_3$ vs. NaOH soil neutralization methods.

As previously reported [20], W concentrations in snail tissue were substantially lower relative to W in cabbage tissue. Within the snail, W was substantially more concentrated in the hepatopancreas relative to the rest of the snail tissue. Kinetic tissue data were successfully obtained for the hepatopancreas, yielding k_u and k_e values of 0.008 ± 0.002 g/g/d ($p = 0.03$) and 0.13 ± 0.05 1/t ($p = 0.09$), respectively. The relatively fast elimination rate is relatable to relatively fast elimination of W previously reported for rodents [27]. These rates were used to model a steady state concentration of 34 mg/kg after 23 days of exposure. The relatively low steady state BAF of 0.06 was similar to a range in hepatopancreas BAFs of 0.03 to 0.4 previously reported for *O. lactea* exposed to W contaminated soil (20). Attempts to model uptake and elimination rate kinetics for the rest of the snail body did not result in significant fits ($p = 0.16$ to 0.23); however, visual inspection of the uptake curve (Figure 1b) suggested that an approximate steady state concentration of 3.2 mg/kg (BAF = 0.01) was obtained after 14-days of exposure. Similarly, the kinetic model did not provide a significant fit to the dietary uptake of W into snails ($p = 0.15 – 0.43$). However, visual inspection of the uptake curves indicated a much higher steady state concentration (85.9 mg/kg) and faster time to steady state (5 days) for the snail hepatopancreas; the steady state concentration for the rest of the body was slightly lower than the hepatopancreas (36.7 mg/kg) (Figure1c). However, the higher steady state BAFs for the hepatopancreas and body of 0.36 and 0.15, respectively, indicated that the trophic transfer of W was a much more important uptake pathway for this herbivore relative to direct exposure to contaminated soil. Further, the ratio of W in the body relative to that in the hepatopancreas was much greater in the dietary exposure (43%) than in the soil exposure (9%), suggesting that W bioaccumulation thorough trophic transfer resulted is substantially greater assimilation efficiency.

Soil and Trophic Transfer

The total W concentration in the cabbage tissue grown in W-spiked soil was 212.83 ± 46 mg/kg. Total W in the mantle (12.8 mg/kg) and shell (1.25 ± 0.56 mg/kg) in feeding experiments relative to background shell concentrations from the control (< 0.1 mg/kg) provide a direct line of evidence that the trophic transfer pathway delivered W to the shell. Fecal data (not presented) were inconclusive due to contact with soil and likelihood of skewed results either through W transfer to soil or soil W to fecal material. There was virtually no difference in W-chemical species between soil and cabbage exposures for the

analyzed compartments (Figure 2a). Comparing tungsten tissue burdens for gastropods exposed to contaminated soil compared to contaminated food in the tissue compartments, there was a significantly ($p<0.001$) greater burdens in the trophic transfer relative to the soil exposure. (Figure 2b; α to β). While there was no significance ($p>0.05$) when comparing parallel tissue compartments (Figure 2b; A to A, B to B, C to C) between exposure types, there was significance ($p<0.001$) when comparing between compartments overall within the exposures (Figure 2b; A to B to C to A). Comparison of all compartments for the soil and cabbage exposures demonstrated significance ($p< 0.001$) for this test (Figure 2b, 1-6). There was no significance ($p>0.05$) in the difference in total concentrations between exposures for the mantle. It is worth noting that the average concentrations were nearly identical for the mantles in the soil and cabbage exposures, 12.2 and 12.8 mg/kg, respectively. Unfortunately, due to limited mantle tissue, speciation was not possible for this exposure. These values suggest that the mantle limits the W utilized for the shell matrix.

Dermal exposure

Total measurable tungsten in the cloth-test medium was 265.67 ± 30 mg/kg. Significantly more monomeric tungstates, relative to polytungstates, were detected in all tissue compartments (Figure 3). On average, there was no significant difference between monomeric and polytungstates, or between the elutriate water, saturated cloth, snail hepatopancreas, and the whole snail (Figure 3). Interestingly the whole body speciation analysis demonstrated absolutely no polytungstate present in any analyzed sample. This indicates that the dermal absorption of the available tungsten species is not as bioavailable in this type of exposure as they are within dietary related studies. In the four days between creating the elutriate and the second saturation of the cloth, there was a 5% increase in polytungstates in the elutriate liquid, which was not a significant change statistically, though it demonstrates that species redistribution of tungsten was occurring.

Culture

Integration of tungsten throughout the shell is visible via synchrotron analysis. It does not appear to have any particular arrangement and orientation within the structure. It is evident that W uptake occurs throughout growth of the snail and is fairly uniform (figure 4).

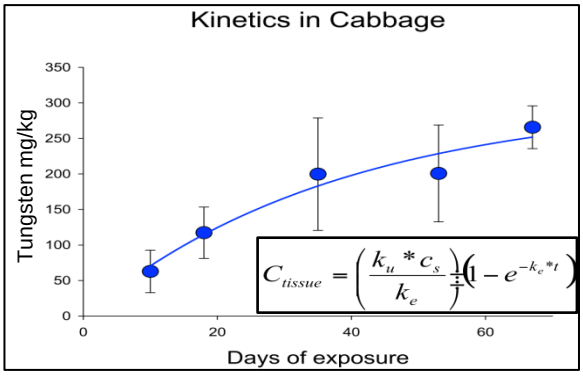
Building upon Kennedy et al [20] investigations of tungsten, this study answers the questions of uptake pathway kinetics, compartmental concentrations dependent on exposure medium, and relates the pathway of tungsten incorporation within the shell. Pathway kinetics demonstrates the rapid uptake and distribution of W within the different compartments of the snail. The analyzed compartments (whole snail, hepatopancreas, mantle, and shell) represent pathways and fate of tungsten once absorbed or consumed. The hepatopancreas is akin to a liver in mammals and all food is filtered through it before dispersal to other compartments or release of fecal material. The mantle is the pathway that the snail uses to exude a chemical matrix that grows and repairs the shell. The shell therefore would contain a lifetime of chemical constituents that the gastropod was exposed to or consumed, so the shell is similar to how bones accumulate elements in the human body. The whole snail compartment represents the rest of the snail tissue. While total tungsten and liquid speciation

analyses demonstrate the presence and ratios of tungsten in the different tissue compartments, synchrotron analysis confirms the distribution pathways of W (Figure 5a, b, c). Since the synchrotron analysis of juvenile snail shells demonstrates the full integration and distribution of W within the shells and the liquid chemistry of adult tissue and shell compartments corroborate the evidence that W is distributed via examined pathways, conclusions can be drawn that utilization of the snail shells as a biomonitoring tool is feasible. Future work to bring this into practical usage should include the development of lifecycle uptake rates to better discern how elements such as W are integrated over a lifetime of exposure.

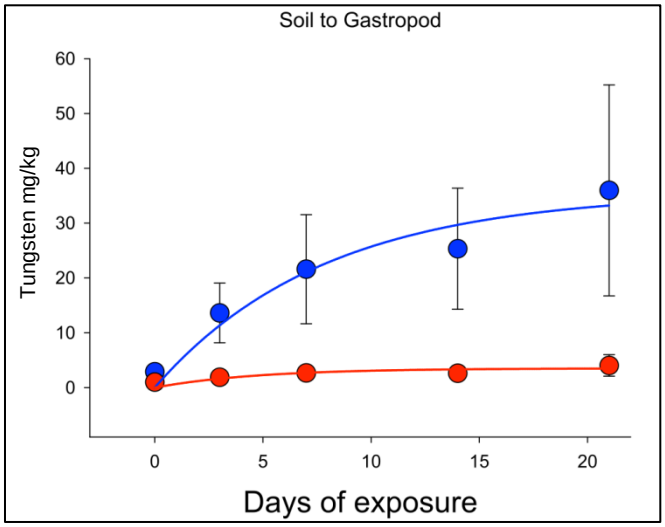
Figures:

Figure 1. Tungsten uptake kinetics from soil to cabbage (panel a), soil to gastropods (panel b) and cabbage to snails (panel c).

(a)



(b)



(c)

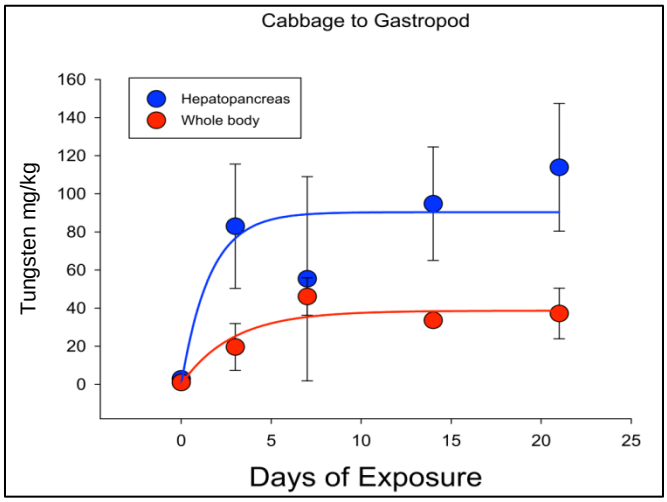

Cabbage to Gastropod
Hepatopancreas
Whole body
Tungsten mg/kg
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Days of Exposure

Figure 2a. Relative percentages of liquid-phase extracted monomer and polytungstates in snail hepatopancreas and the rest of the snail body

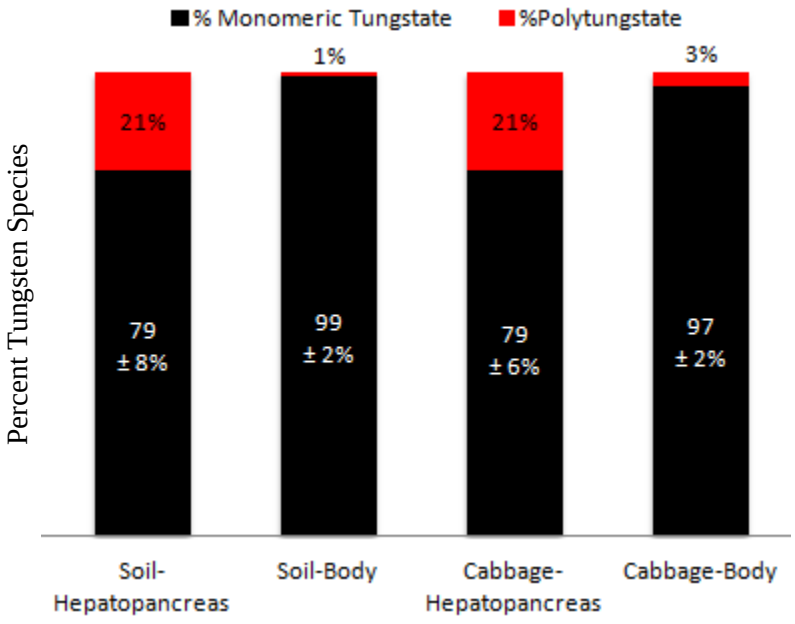


Figure 2b. Relative tissue residues in different gastropod compartments after exposure to contaminated soil or through trophic transfer. Alpha and beta indicate the entire exposure for soil and cabbage, respectively.

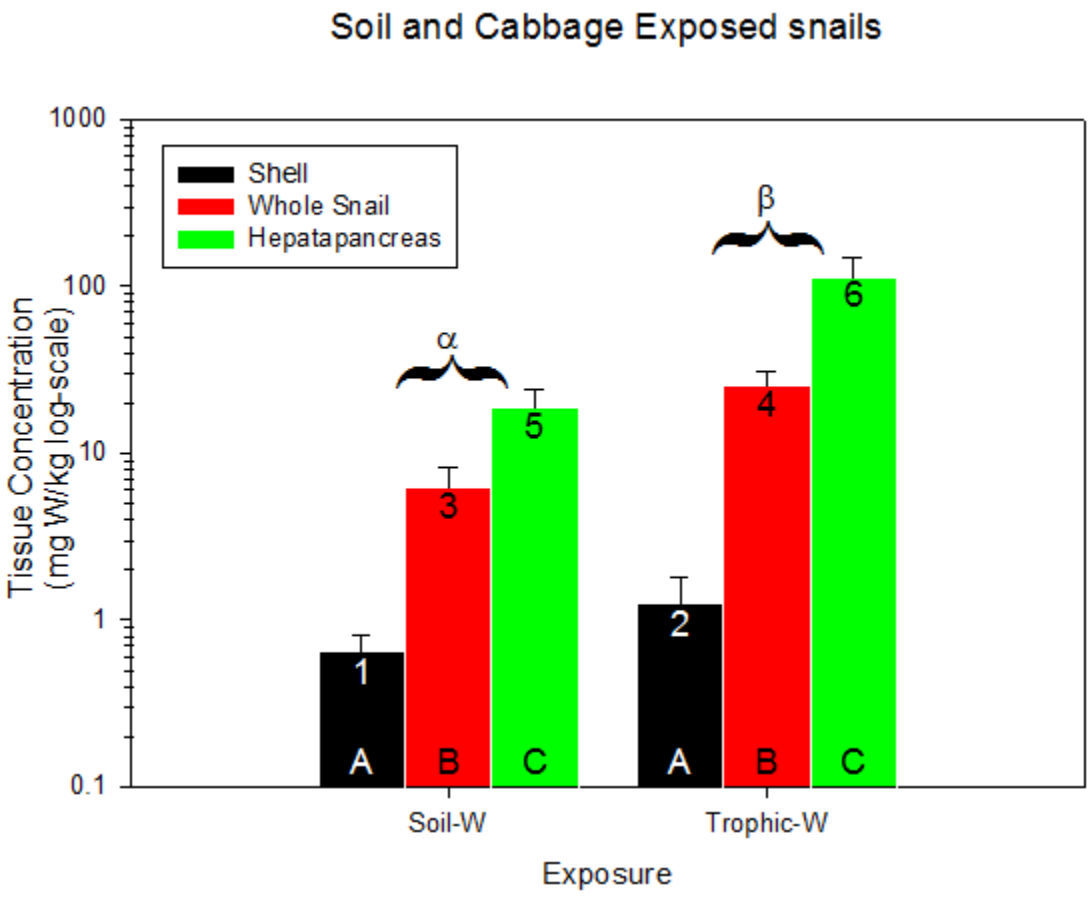


Figure 3. Relative percentages of liquid phase extracted monomeric and polytungstates in water, cloth, snail hepatopancreas and the rest of the snail body.

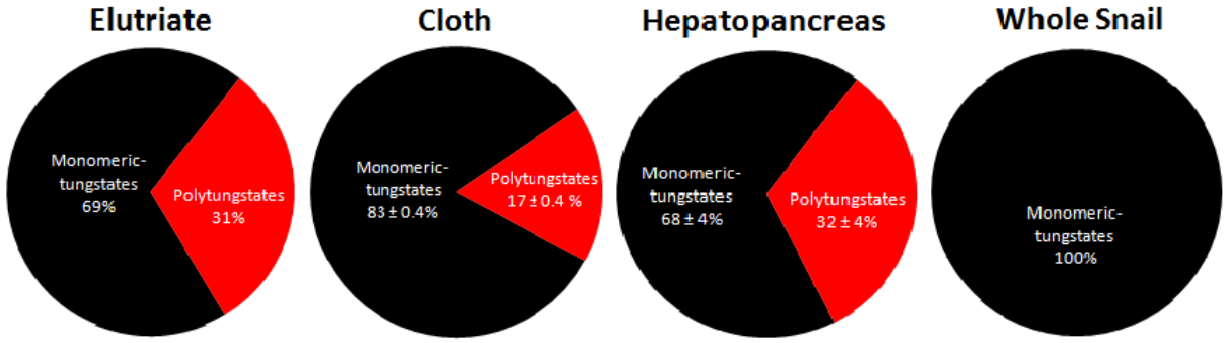


Figure 4. 3D tomography of juvenile snail shell from tungsten snail-culture. Warm colors indicate higher density of concentration of the indicated element. This map is of a whirl of a juvenile snail after approximately 400% growth from hatching.

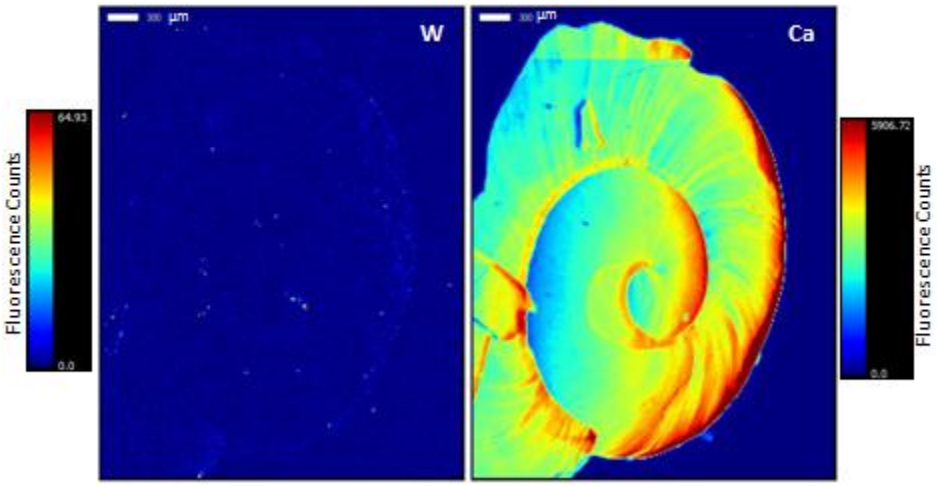
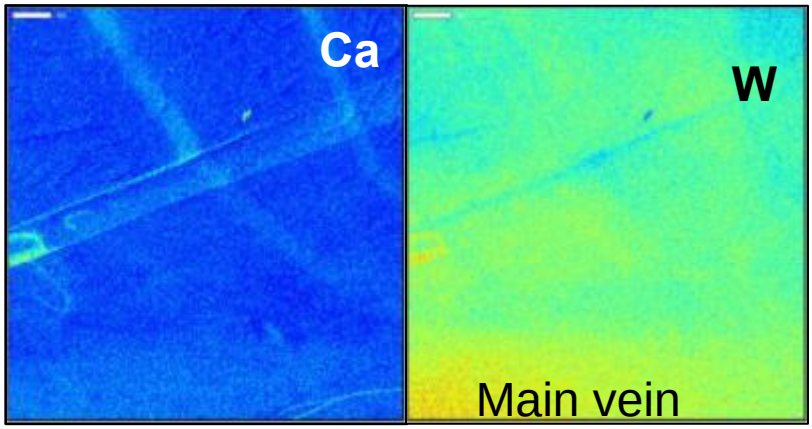
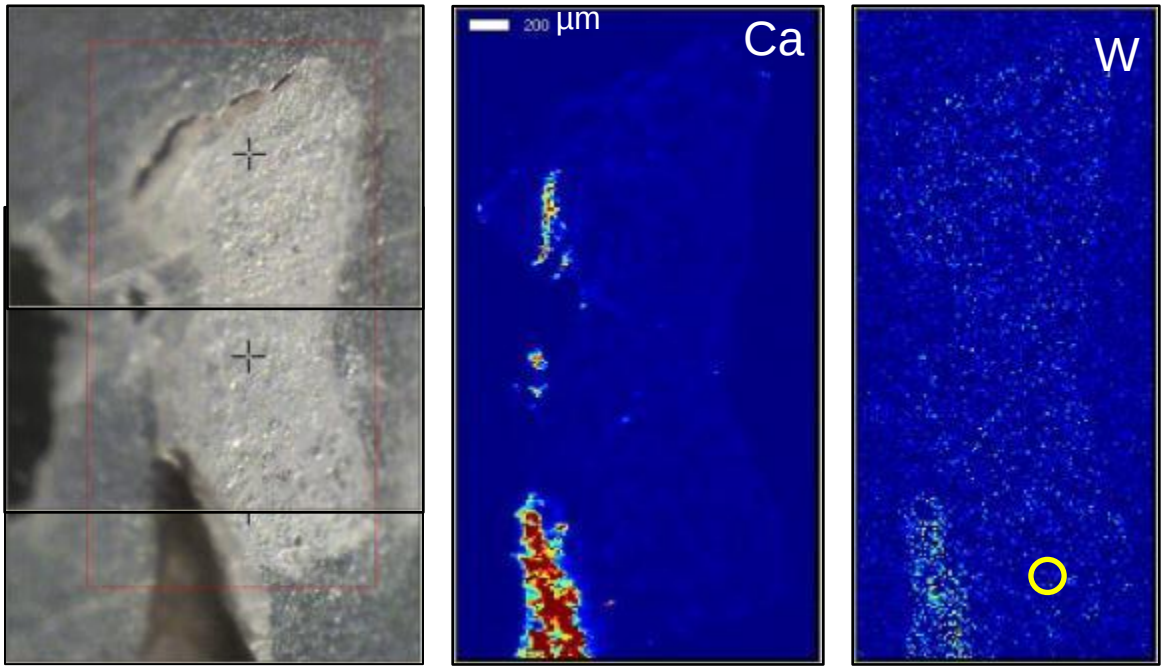


Figure 5a,b,c. Synchrotron mapping of Cabbage, regrowth of shell matrix, and mantle. Warm colors of red and yellow indicate higher concentrations of indicated elements (Ca and W). 5a indicates that W is distributed across the entire leaf structure, with a high ratio located in the central leaf vein. 5b displays the W dispersal through the mantle. 5c indicates that there is W integration into the shell matrix on the outside of the new growth, but there is also a thin layer of W matrix being integrated in the interior of the shell.

(a)



(b)



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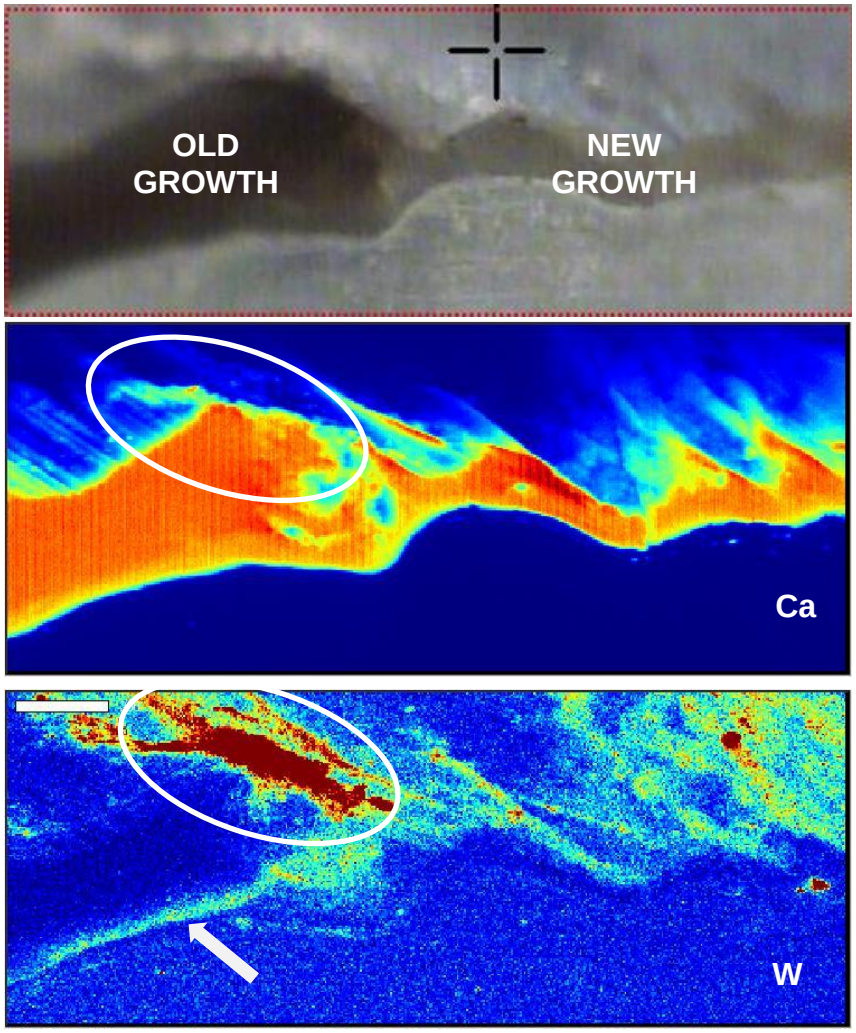
(c)



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357 # References

[1] Koutsospyros A., Braida W., Christodoulatos C., Dermatas D., Strigul N., 2006. A review of tungsten: From environmental obscurity to scrutiny. Journal of Hazardous Materials, 136, 1-19

[2] Clausen, J.L.; Korte, N. Environmental fate of tungsten from military use. Sci. Total Environ. 2009, 407,2887-2893

[3] Moynier F., Yin Q., Irisawa K., Boyet M., Jacobsen B., and Rosing M.T., Coupled ^{182}W-^{142}Nd constraint for early Earth differentiation. Proceedings of the National Academy of Sciences of the United States of America. 2010 June 15; 107(24): 10810–10814

[4] Strigul, N.; Koutsospyros, A.; Arienti, P.; Christodoulatos, C.; Dermatas, D.; Braida, W. Effects of tungsten on environmental systems. Chemosphere 2005, 61, 248-258.

[5] Bednar A.J. ; Boyd R.E.; Jones W.T.; McGrath C.J.; Johnson D.R.; Chappell M.A.; Ringelberg D.B. Investigations of tungsten mobility in soil using column tests. Chemosphere, 2009, 75, 1049-1056

[6] Johnson, D.R.; Inouye, L.S.; Bednar, A.J.; Clarke, J.U.; Winfield, L.E.; Boyd, R.E.; Ang, C.Y.; Goss, J. W bioavailability and toxicity in sunflowers (Helianthus annuus L.) Land Contam. Reclam., 2009, 17, 141-151.

[7] Felt, D.; Larson, S.; Griggs, C.; Nestler, C.; Wynter, M. Relationship of surface changes to metal leaching from tungsten composite shot exposed to three different soil types. Chemosphere 2011, 83, 955-962.

[8] Interstate Technology and Regulatory Council. Characterization and remediation of soils at closed small arms firing ranges. Report prepared by Interstate Tecnology and Regulatory Council Small Arms Firing Range Team. 2003.

[9] Li, K.C. and Yu Wang, C. Tungsten: Its-History, Geology, Ore-Dressing, Metallurgy, Chemistry, Analysis, Applications, and Economics, Reinhold Publishing Corporation; New York, 1955

[10] Davitt R.P. A Comparison of the Advantages and Disadvantages of Depleted Uranium and Tungsten Alloy as Penetrator Materials. Technical Report, U.S. Army Armament Research and Development Command, Dover, NJ, 1980

[11] U.S. EPA Emerging Contaminant- Tungsten 2009 Accessed from: http://www.clu-in.org/download/contaminantfocus/epa505f09007.pdf

[12] Babish, J.G.; Stoewsand, G.S.; Furr, A.K.; Parkinson, T.F.; Bache, C.A.; Gutenmann, W.H.; Wszolek, P.C.; Lisk, D.J. Elemental and polychlorinated biphenyl content of tissues and intestinal aryl hydrocarbon hyroxylase activity of Guinea pigs fed cabbage grown on municipal sewage sludge. J. Agric. Food Chem. 1979, 27, 399-402.

[13] Inouye, L.S.; Jones, R.P.; Bednar, A.J. Tungsten effects on survival, growth, and reproduction in the earthworm, Eisenia fetida. Environ. Toxicol. Chem. 2006, 25, 763-768

[14] Strigul, N.; Galdun, C.; Vaccari, L.; Ryan, T.; Braida, W.; Christodoulatos, C. Influence of speciation on tungsten toxicity. Desalination, 2009**,** 248, 869-879.

[15] Butler, A.D.; Medina, V.F.; Larson, S.; Nestler, C. Uptake of lead and tungsten in Cyperus esculentus in a small-arms range simulation. Land Contam. Reclam. 2009, 17, 153-159.

[16] Bamford, J.E.; Butler, A.D.; Heim, K.E.; Pittinger, C.A.; Lemus, R.; Staveley, J.P.; Lee, K.B.; Venezia, C.; Pardus, M.J. Toxicity of sodium tungstate to earthworms, oats, radish, and lettuce. Environ. Toxicol. Chem. 2011, 30, 2312-2318.

[17] Bednar, A. J., Kirgan, R. A., Johnson, D. R., Russell, A. L., Hayes, C. A., & McGrath, C. J. (2009). The use of SEC-ICP-MS and direct infusion ESI-MS for the investigation of polymeric tungsten compounds. Land contamination reclamation, 17, 1-9.

[18] Seiler R.L., Stollenwerk K.G., Garbarino J.R., 2005. Factors controlling tungsten concentrations in ground water, Carson Desert, Nevada. Applied Geochemistry, 20, 423-41

[19] Bednar. A.J.; Inouye, L.S.; Mirecki J.E.; Ringelberg D.B.; Larson, S.L. Determination of tungsten, molybdenum, and phosphorus oxyanions by HPLC-ICP-MS, Talanta, 2007, 72, 1828-32

[20] Kennedy, A. J., Johnson, D. R., Seiter, J. M., Lindsay, J. H., Boyd, R. E., Bednar, A. J., & Allison, P. (2012). Tungsten Toxicity, Bioaccumulation and Compartmentalization into Organisms Representing Two Trophic Levels. Environmental Science & Technology.

[21] Bednar, A.J., Jones, W.T., Boyd, R.E., Ringelberg, D.B., Larson, S.L., 2008. Geochemical parameters influencing tungsten mobility in soils. Journal of Environmental Quality 37, 229–233.

[22] American Society for Testing and Materials (ASTM). 2008. Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates. Method E1391 − 03. West Conshohocken, PA.

[23] Cobbinah, J.R (1993). Snail farming in West Africa. A practical guide: CTA publication, 126

[24] Bednar, A.J.; Jones, W.T.; Chappell, M.A.; Johnson, D.R; Ringelberg, D.B. A modified acid digestion procedure for extraction of tungsten from soil. Talanta 2010, 80, 1257-1263.

[25] Bednar, A.J.; Griggs, C.S.; Hill, F.C. Addendum to "A modified acid digestion procedure for extraction of tungsten from soil", Talanta, 2010, 82, 1627-28.

[26] American Society for Testing and Materials (ASTM). 2000. Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates. Method E 1688-00a. West Conshohocken, PA.

[27] Sheppard, P. R.; Speakman, R. J.; Ridenour, G.; Glascock, M. D.; Farris, C.; Witten, M. L. Spatial patterns of tungsten and cobalt in surface dust of Fallon, NV. Environ. Beochem. Health 2007, 29, 405−412