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Accumulation and Depuration of Trace Metals in Southern Toads, *Bufo terrestris*, Exposed to Coal Combustion Waste

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Abstract Accumulation and depuration of metals by an organism are underrepresented in the literature. We collected southern toads (*Bufo terrestris*) from coal by-product (ash)-contaminated and uncontaminated sites to examine metal concentrations over time. Toads were placed in four exposure regimes, then sacrificed periodically over a 5-month period, and whole-body metal levels were measured. Toads exposed to ash accumulated significant concentrations of metals. Metal concentrations changed throughout the experiment, and profiles of accumulation and depuration differed depending on the metal and exposure regime. Ash-exposed toads exhibited elevated levels of 11 of 18 metals measured. Increases ranged from 47.5% for Pb to more than 5000% for As. Eight of 18 metals did not change in control toads, while 10 of 18 metals decreased in toads removed from ash, ranging from −25% for Co to −96% for Tl. Seven metals that decreased in toads removed from ash did not change in control toads.

In recent decades, industry has released many harmful toxicants into the soil, air, and water. One group of toxicants, in particular, is trace metals, which are becoming ubiquitous components of the environment. Smelting plants, mines, and coal-burning power plants all produce waste effluent containing trace metals (Nriagu 1988). Due to our reliance on fossil fuels for producing electricity, the amount of coal by-products produced yearly in the United Stated has increased greatly. For example, 71.2 million tons of coal by-product, coal fly ash, was produced in 2001 alone (EPA 2004). Coal fly ash contains numerous trace metals (e.g., arsenic, cadmium, lead, selenium, and chromium) known to cause physiological problems. The fly ash is often pumped into aquatic settling basins, where the trace metals become available for bioaccumulation (Rowe et al. 2002).

Many vertebrates from trace metal-polluted areas contain significantly higher concentrations of metals than animals from reference sites. Most studies have examined fish or birds and compared body metal levels in polluted vs. reference areas (e.g., Diaz et al. 1994; Hernandez et al. 1999; Moiseenko and Kudryavtseva 2001; Olsvik et al. 2000). While these types of studies are important, values often obtained for body metal levels rely on a single-time-point measurement. Studies that do examine the timeline of uptake and subsequent excretion of environmentally relevant levels of trace metals have been conducted under laboratory conditions (e.g., Hill 1984; McGreer et al. 2000; Perez-Coll et al. 1997; Vogiatzis and Lounmbourdis 1997; White and Finely 1978, Pustamante et al. 2004). There are no field studies that examine the uptake of metals over time from vertebrates exposed to environmental pollution. Additionally, studies on the extent of recovery from long-term exposure and excretion of trace metals are also underrepresented in the literature.

Furthermore, most studies characterizing body levels of pollutants focus on species that are relatively site specific. There are almost no studies that examine vertebrates that

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may only utilize a polluted area for a portion of the year, such as migratory vertebrates. Sedentary vertebrates lend themselves to studies investigating toxicant levels at a single point in time. However, migratory vertebrates may require multiple measures within the population to accurately understand the dynamics of whole-body metal levels. Thus, data need to be gathered to determine the profile of metal accumulation and the time line of depuration from metal exposure.

Southern toads (*Bufo terrestris*) are found throughout the southeastern United States. Their habitats include a wide range of areas, which include upland and lowland forests as well as areas around coal fly ash collection basins. In the spring, toads migrate into breeding sites, such as ash basins. After breeding, they move out of the basin itself but stay nearby until late summer or early fall. Toads usually do not show site fidelity, (i.e., breeding sites for individual toads change from year to year (Dickerson 1969)). Toads could enter the basin in April or May, accumulate metals throughout the breeding season, then leave the basin area. This study examines changes in body metal concentrations in a captive southern toad population over time during experimental exposure to, and removal from, coal fly ash sediment.

Materials and Methods

Experimental Approach

Male southern toads were captured by hand at the Savannah River Site, Aiken, SC, USA, in early spring (March and April 2002; $n = 168$). Toads were collected from either the ash basin area associated with a coal-powered steam generation plant ($n = 84$) or an uncontaminated control site, ~15 km from an ash basin ($n = 84$). These ash basins contain elevated levels of numerous metals including Al, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, Ni, Pb, Rb, Se, Sr, Rl, U, V, and Zn (Rowe et al. 2002). Both ash and control sediment were tested at the end of the experiment. The mass of each toad was measured on a digital balance (0.01-g accuracy). Immediately following collection and measurements, three individuals from each site were euthanized by immersion in an aqueous solution of MS222 (300 ppm) (Andrews 1993).

Housing and Experimental Design

Within 2 days of capture, toads were transported to Auburn University, Auburn, AL, USA, and transferred into mesocosms (208-L [55-gal] Rubbermaid [Fairlawn, OH, USA] containers with screen lids). The bottom of each mesocosm was covered with70% sediment (coal fly ash collected from the capture site or control sand) and 30% water, by area. The water and sediment averaged 6 cm in depth. Toads were placed in a mesocosm with a sediment type equivalent to the type on which they were captured. There were 60 total mesocosms (30 ash and 30 sand controls), each housing three toads. Sediment was covered with pine straw (5 cm deep) and a 10 × 25-cm piece of pine bark was added for shelter. All mesocosms were located outdoors under a shade-cloth tent and subject to ambient conditions.

After capture in April, toads were acclimated in mesocosms containing their native sediment for 1 month before being transferred to the experimental sediment. We began euthanizing toads for metal analysis 1 week after transfer. Toads were assigned to one of four groups ($n = 42$ per group). Toads captured at control (C) or ash (A) sites were then transferred to mesocosms containing control (e.g., $C \rightarrow C$, $A \rightarrow C$) or ash (e.g., $C \rightarrow A$, $A \rightarrow A$) sediments. Toads were fed weekly (~10 crickets per toad) with crickets raised on either a control diet of uncontaminated or contaminated (by coal fly ash; 50/50 mixture, by volume) dry cat food chow (The Meow Mix Company, Secaucus, NJ, USA), depending on the treatment sediment. Crickets fed a diet of cat food and ash had a significantly increased total-body load of metal (Ward and Mendonça 2006). There were two sampling periods in May, two in June, and one in September. These sampling periods where chosen to determine how quickly metals were accumulated. Three toads per group were sacrificed during each sampling period. All remaining toads were euthanized in October. The stomach contents of all sacrificed toads were removed prior to metal analysis. The MS222 solution contained no trace metals.

Metal Analysis

Toad carcasses were analyzed for 18 metals: Al, As, Ba, Cd, Co, Cr, Cs, Cu Fe, Ni, Pb, Rb, Se, Sr, Tl, U, V, and Zn. Whole-body samples were freeze-dried and ground in a Wiley mill using a no. 20 screen size. A subsample of each homogenized toad was weighed and digested with nitric acid using EPA method 3051 (microwave-assisted [model 81D; CEM Corp., Mathews, NC, USA] acid digestion of sediments, sludges, soils, and oils, using trace-metal-grade nitric acid). Digestion remains were then brought to volume with deionized water and analyzed with an intermittently coupled plasma-mass spectrometer (ICP-MS) (Perkin-Elmer 9000 ICP-MS, Wellesley, MA, USA). Quality control checks were analyzed on the ICP-MS every 15 to 20 samples and a standard reference material, as well as a blank, were tested every 10 samples for verification.

Statistics

The metal concentrations of toads (parts per million; ppm) were compared using ANOVAs, with significance set at

$P = 0.1$. Experimental group, time, and metal concentration were compared with a MANOVA. Statistics were calculated using Statview statistical package vs. 5.0.1 (SAS institute, Cary, NC, USA).

Results

Metal contents in the ICP-MS blanks were averaged and subtracted from measured metal values. Quality controls, which consisted of a sample with a known concentration (100 ppb) of each metal, were analyzed along with the unknowns. The measured values for the quality controls ranged from 75.85 ppb for Zn to 116.67 ppb for Sr, and averaged 102.3 ppb. Measured values for the standard reference materials were within one standard deviation of reported values.

Ash and control sediment analysis revealed that all of the 18 metals measured were elevated in ash sediment over control sediment (Table 1).

Body Metal Levels at Capture

At capture, 11 (Al, As, Cd, Co, Cr, Se, Sr, Tl, U, V, and Zn) of the 18 metals showed levels that were significantly elevated in ash toads compared to control toads (Al, $P = 0.03$; As, $P = 0.009$; Cd, $P = 0.06$; Co, $P < 0.0001$; Cr, $P = 0.1$; Se, $P = 0.05$; Sr, $P = 0.1$; Tl, $P = 0.002$; U, $P = 0.001$; V, $P = 0.003$; Zn, $P = 0.04$). Five metals (Ba, Cu, Fe, Ni, and Pb) did not differ significantly, and two (Cs, Rb) showed levels that were significantly lower in ash toads than in control toads (Cs, $P = 0.03$; Rb, $P = 0.02$).

Changes in Body Metal Levels Over Time

Our data indicate that there was a significant effect of both sampling time (Wilks' λ; $P < 0.0001$, df = 72, $F = 3.50$) and group ($P < 0.0001$, df = 54, $F = 3.47$) on metal concentrations, there was a significant group × month interaction on metal concentrations ($P < 0.0001$, df = 216, $F = 1.65$), and many of the metals were correlated (see Table 2).

We observed some general patterns in the 18 metals assayed in each group during the 5 months of the experiment. The majority of metals in the C → A group and A → A group (10 of 18) of toads increased. Six (Al, As, Cr, Co, Pb, U) of the eight metals that increased significantly over time in C → A toads also increased significantly in A → A toads. The majority of metals (12 of 18) measured in A → C toads decreased, and some (7 of 18) of the metals measured in C → C toads did not change. Nine (Al, Cd, Cu, Fe, Se, Sr, Tl, U, and Zn) of the 12 metals that decreased significantly in A → C toads did not change or decreased in C → C toads. Cr concentrations increased significantly in at least 1 month (May or June) throughout the experiment in every group, while Cd concentrations decreased significantly in at least 1 month (September or October) for every group (Table 2a–d).

Changes in Whole-Body Metal Levels in the Control-to-Ash Group

In toads moved from a control site to ash sediment (C → A), nine metals (Al, As, Co, Cr, Pb, Tl, U, and V) were significantly elevated from capture in at least 1 month during the experiment (Al, $P_{\text{June}} = 0.01$; As, $P_{\text{June}} = 0.01$, $P_{\text{Sept}} = 0.04$, $P_{\text{Oct}} = 0.02$; Co, $P_{\text{June}} = 0.\ 1$, Cr, $P_{\text{May}} = 0.08$, $P_{\text{June}} = 0.03$; Pb, $P_{\text{Sept}} = 0.09$; Tl, $P_{\text{May}} = 0.01$, $P_{\text{June}} = 0.0003$, $P_{\text{Sept}} = 0.09$, $P_{\text{Oct}} = 0.02$; U, $P_{\text{June}} = 0.03$; and V, $P_{\text{June}} = 0.05$, $P_{\text{Oct}} = 0.09$ [P values in all cases indicate difference between noted month and capture]) (Fig. 1a). Sr behaved differently; the concentration rose after capture, although not significantly (from185 ± 1.13 to 331 ± 159; $P = 0.23$), but then decreased significantly from May to June ($P = 0.05$). Metals that increased significantly in C → A toads were elevated the first 2 months of exposure, May and June, and tended to stay elevated throughout the experiment. The concentrations of four metals decreased significantly from the time of capture (Cd, $P_{\text{June}} = 0.01$, $P_{\text{Sept}} = 0.05$, $P_{\text{Oct}} = 0.001$; Cs, $P_{\text{June}} = 0.005$, $p_{\text{Oct}} = 0.05$; Cu, $P_{\text{June}} = 0.001$, $P_{\text{Sept}} = 0.07$, $P_{\text{Oct}} = 0.01$; Rb, $P_{\text{June}} = 0.0006$, $P_{\text{Sept}} = 0.07$, $P_{\text{Oct}} = 0.02$). Five showed no significant changes in concentration throughout the experiment (Ba, Fe, Ni, Se, and Zn).

Changes in Whole-Body Metal Levels in the Ash-to-Ash Group

A → A toads had a similar pattern to C → A toads in that 10 metals increased significantly from the time of capture;

Table 1 Metal levels (mean ppm) in ash and control sediment

	Al	As	Ba	Cd	Co	Cr	Cs	Cu	Fe	Ni	Pb	Rb	Se	Sr	Tl	U	V	Zn
Ash	5011	41.32	387	0.15	5.24	11.45	3.0	29.83	8809	13.88	10.53	5.30	4.38	79.83	182	2.60	23.15	31.80
Control	214	1.83	8.49	0.02	0.17	1.64	0.08	1.56	886	0.58	2.09	0.35	0.08	1.22	4.3	0.07	0.88	7.18

Note: BDL, below detectable limit. Metals listed in alphabetical order

Table 2 Monthly-total body metal levels (mean ppm ± 1 SE) in ash-to-ash (a), control-to-ash (b), control-to-control (c), and control-to-ash (d) toads

Metal	Capture	May	June	September	October
(a) A → A toads					
Al	190.7 ± 35.7	324.9 ± 211.8	218.1 ± 53.3	748.2 ± 224.8	600.6 ± 106.7
As	2.95 ± 0.36	2.71 ± 0.60	1.65 ± 0.36	5.03 ± 0.90	4.77 ± 1.10
Ba	70.71 ± 11.63	84.51 ± 15.30	105.4 ± 27.2	101.1 ± 1.15	142.5 ± 22.1
Cd	0.126 ± 0.012	0.086 ± 0.026	0.049 ± 0.01	0.063 ± 0.009	0.069 ± 0.018
Co	0.31 ± 0.003	0.39 ± 0.08	0.31 ± 0.06	0.75 ± 0.14	4.43 ± 0.53
Cr	1.08 ± 0.20	1.91 ± 0.14	1.29 ± 0.31	2.83 ± 0.46	2.38 ± 0.26
Cs	0.053 ± 0.011	0.125 ± 0.041	0.116 ± 0.038	0.212 ± 0.025	0.132 ± 0.023
Cu	10.09 ± 1.90	9.65 ± 3.95	5.01 ± 0.86	6.98 ± 0.85	7.52 ± 0.81
Fe	502.0 ± 30.2	1063 ± 659	985.3 ± 328.0	2529 ± 542	1781 ± 618
Ni	0.30 ± 0.20	0.82 ± 0.40	0.18 ± 0.12	0.60 ± 0.60	0.55 ± 0.08
Pb	5.36 ± 0.24	20.86 ± 8.53	14.81 ± 5.92	8.46 ± 2.33	1.67 ± 0.16
Rb	14.06 ± 1.22	31.00 ± 2.30	30.46 ± 7.69	13.92 ± 1.59	14.06 ± 1.2
Se	8.57 ± 1.81	6.35 ± 1.45	1.98 ± 0.66	2.37 ± 0.24	3.67 ± 1.020
Sr	420.4 ± 93.0	462.3 ± 134.6	228.5 ± 53.7	214.7 ± 24.8	301.2 ± 77.5
Tl	0.232 ± 0.017	0.197 ± 0.045	0.123 ± 0.040	0.233 ± 0.034	0.190 ± 0.026
U	0.039 ± 0.001	0.048 ± 0.013	0.029 ± 0.008	0.119 ± 0.034	0.078 ± 0.012
V	1.61 ± 0.12	1.18 ± 0.34	0.76 ± 0.20	2.96 ± 0.74	1.49 ± 0.45
Zn	851.0 ± 86.7	181.4 ± 43.6	181.06 ± 41.1	258.0 ± 97.6	411.6 ± 39.5
(b) C → A toads					
Al	54.35 ± 31.50	366.0 ± 226.5	797.7 ± 202.2	877.8 ± 501.9	836.9 ± 378.8
As	0.106 ± 0.003	2.10 ± 0.78	3.74 ± 0.57	4.62 ± 0.92	7.68 ± 1.31
Ba	115.4 ± 24.0	70.71 ± 11.63	120.9 ± 28.8	97.7 ± 19.6	115.0 ± 15.5
Cd	0.198 ± 0.025	0.186 ± 0.077	0.083 ± 0.017	0.069 ± 0.018	0.050 ± 0.012
Co	0.175 ± 0.0003	0.464 ± 0.166	0.717 ± 0.155	0.852 ± 0.364	0.753 ± 0.239
Cr	0.388 ± 0.250	2.33 ± 0.54	3.07 ± 0.52	2.76 ± 1.26	2.72 ± 0.92
Cs	0.197 ± 0.042	0.194 ± 0.111	0.070 ± 0.012	0.079 ± 0.031	0.076 ± 0.015
Cu	14.60 ± 1.71	9.25 ± 2.65	6.16 ± 0.71	6.33 ± 1.44	7.08 ± 0.37
Fe	502.0 ± 30.2	1152 ± 687	2064 ± 545	2286 ± 980	2136 ± 913
Ni	0.148 ± 0.040	0.527 ± 0.339	0.651 ± 0.375	BDL	0.133 ± 0.133
Pb	5.48 ± 0.76	5.99 ± 0.48	5.93 ± 0.75	8.08 ± 0.29	8.64 ± 1.80
Rb	44.00 ± 9.09	42.48 ± 15.59	12.13 ± 1.29	12.43 ± 0.84	12.99 ± 0.75
Se	1.60 ± 0.52	4.60 ± 2.16	1.49 ± 0.16	1.54 ± 0.09	1.50 ± 0.14
Sr	185.5 ± 1.13	331.4 ± 159.1	130.9 ± 17.7	247.0 ± 49.1	228.2 ± 35.0
Tl	0.018 ± 0.0002	0.121 ± 0.013	0.208 ± 0.014	0.181 ± 0.052	0.223 ± 0.032
U	0.007 ± 0.003	0.068 ± 0.047	0.142 ± 0.036	0.162 ± 0.101	0.125 ± 0.050
V	0.068 ± 0.050	1.42 ± 0.97	3.13 ± 0.71	3.47 ± 1.94	2.95 ± 0.91
Zn	280.8 ± 164.7	160.7 ± 3.7	180.1 ± 42.9	150.2 ± 31.5	348.5 ± 129.7
(c) C → C toads					
Al	54.35 ± 31.50	200.5 ± 53.2	134.5 ± 38.4	238.7 ± 121.8	337.0 ± 124.8
As	0.106 ± 0.003	0.640 ± 0.348	0.636 ± 0.117	1.93 ± 1.30	1.79 ± 1.08
Ba	115.4 ± 24.0	75.10 ± 18.35	70.11 ± 10.56	86.06 ± 27.65	99.86 ± 30.02
Cd	0.198 ± 0.025	0.080 ± 0.017	0.044 ± 0.004	0.035 ± 0.007	0.047 ± 0.005
Co	0.175 ± 0.0003	0.501 ± 0.265	0.250 ± 0.016	0.315 ± 0.108	3.18 ± 0.35
Cr	0.388 ± 0.250	2.74 ± 0.74	2.57 ± 0.50	2.96 ± 0.65	1.51 ± 0.14
Cs	0.197 ± 0.042	0.060 ± 0.011	0.041 ± 0.005	0.028 ± 0.011	0.121 ± 0.032
Cu	14.60 ± 1.71	7.87 ± 1.67	4.54 ± 0.40	3.77 ± 0.12	6.49 ± 1.18
Fe	502.0 ± 30.2	468.2 ± 72.2	398.8 ± 31.9	572.6 ± 160.4	373.0 ± 38.4

Table 2 continued

Metal	Capture	May	June	September	October
Ni	0.148 ± 0.040	0.921 ± 0.284	0.428 ± 0.115	0.506 ± 0.037	0.288 ± 0.037
Pb	5.48 ± 0.76	16.04 ± 3.56	31.73 ± 1.13	32.42 ± 2.04	1.48 ± 0.33
Rb	44.00 ± 9.09	15.18 ± 4.26	15.40 ± 1.85	7.60 ± 0.78	11.91 ± 1.32
Se	1.60 ± 0.52	0.975 ± 0.111	0.822 ± 0.125	0.937 ± 0.209	1.44 ± 0.40
Sr	185.5 ± 1.13	132.6 ± 37.6	117.8 ± 22.6	115.0 ± 48.0	129.1 ± 26.6
Tl	0.018 ± 0.0002	0.014 ± 0.001	0.015 ± 0.002	0.068 ± 0.055	0.017 ± 0.003
U	0.007 ± 0.003	0.022 ± 0.005	0.014 ± 0.002	0.036 ± 0.021	0.020 ± 0.006
V	0.068 ± 0.050	0.394 ± 0.119	0.401 ± 0.043	1.10 ± 0.66	0.120 ± 0.119
Zn	280.8 ± 164.7	193.3 ± 39.6	178.0 ± 17.5	273.2 ± 146.4	328.1 ± 42.4
(d) A → C toads					
Al	190.7 ± 35.7	192.4 ± 24.6	262.2 ± 89.9	78.76 ± 48.73	145.5 ± 43.0
As	2.95 ± 0.36	1.26 ± 0.07	0.892 ± 0.274	1.33 ± 0.45	0.939 ± 0.664
Ba	70.71 ± 11.63	83.54 ± 20.39	89.22 ± 26.31	104.7 ± 11.8	95.59 ± 11.87
Cd	0.126 ± 0.012	0.101 ± .050	0.095 ± 0.018	0.060 ± 0.022	0.059 ± 0.017
Co	0.31 ± 0.003	0.368 ± 0.016	0.361 ± 0.036	0.282 ± 0.070	0.232 ± 0.029
Cr	1.08 ± 0.20	1.75 ± 0.30	1.96 ± 0.38	1.99 ± 0.12	0.870 ± 0.104
Cs	0.053 ± 0.011	0.208 ± 0.078	0.103 ± 0.035	0.092 ± 0.005	0.074 ± 0.014
Cu	10.09 ± 1.90	6.38 ± 0.38	5.63 ± 0.32	3.64 ± 0.11	4.70 ± 0.51
Fe	502.0 ± 30.2	639.2 ± 102.5	475.0 ± 77.5	337.4 ± 63.0	364.0 ± 12.7
Ni	0.30 ± 0.20	0.467 ± 0.116	0.306 ± 0.043	0.632 ± 0.234	0.428 ± 0.163
Pb	5.36 ± 0.24	53.06 ± 38.44	13.96 ± 3.40	23.21 ± 6.12	10.57 ± 3.53
Rb	14.06 ± 1.22	38.24 ± 3.27	15.59 ± 0.55	9.04 ± 1.71	9.10 ± 1.03
Se	8.57 ± 1.81	4.83 ± 1.34	27.24 ± 12.66	1.50 ± 0.07	1.62 ± 0.19
Sr	420.4 ± 93.0	273.9 ± 19.0	759.6 ± 100.8	167.7 ± 37.9	163.0 ± 22.9
Tl	0.232 ± 0.017	0.128 ± 0.008	0.054 ± 0.016	0.013 ± 0.004	0.008 ± 0.001
U	0.039 ± 0.001	0.030 ± 0.003	0.022 ± 0.004	0.013 ± 0.005	0.010 ± 0.002
V	1.61 ± 0.12	0.918 ± 0.175	0.727 ± 0.056	0.206 ± 0.088	0.332 ± 0.091
Zn	851.0 ± 86.7	148.2 ± 3.0	156.5 ± 22.6	215.4 ± 32.1	283.2 ± 47.6

Note: BDL, below detectable limit. Metals listed in alphabetical order

the increase however, generally occurred in the fourth month of exposure (Al, $P_{\mathrm{Sept}} = 0.07$, $P_{\mathrm{Oct}} = 0.08$; As, $P_{\mathrm{June}} = 0.06$, $P_{\mathrm{Sept}} = 0.1$; Ba, $P_{\mathrm{Sept}} = 0.03$; Co, $P_{\mathrm{Sept}} = 0.03$, $P_{\mathrm{Oct}} = 0.002$; Cr, $P_{\mathrm{May}} = 0.01$, $P_{\mathrm{Sept}} = 0.02$, $P_{\mathrm{Oct}} = 0.04$; Cs, $P_{\mathrm{Sept}} = 0.004$; Fe, $P_{\mathrm{Sept}} = 0.02$; Pb, $P_{\mathrm{Oct}} < 0.0001$; Rb, $P_{\mathrm{May}} = 0.0006$, $P_{\mathrm{June}} = 0.009$; and U, $P_{\mathrm{Sept}} = 0.08$). By October, the metal concentrations for both groups (C → A, A → A) were essentially equal for most metals (Figs. 1 and 2). However, unlike in C → A toads, the increase in the concentrations of these metals occurred in September, rather than May or June (Figs. 1 and 2). Six metals showed a significant decline in concentrations (Cd, $P_{\mathrm{June}} = 0.002$, $P_{\mathrm{Sept}} = 0.01$; Se, $P_{\mathrm{June}} = 0.004$, $P_{\mathrm{Sept}} = 0.03$, $P_{\mathrm{Oct}} = 0.05$; Sr, $P_{\mathrm{June}} = 0.09$, $P_{\mathrm{Sept}} = 0.1$; Tl, $P_{\mathrm{June}} = 0.1$; V, $P_{\mathrm{June}} = 0.03$; and Zn, $P_{\mathrm{May}} = 0.0002$, $P_{\mathrm{June}} < 0.0001$, $P_{\mathrm{Sept}} = 0.01$, $P_{\mathrm{Oct}} = 0.0003$), while Ni and Cu did not change significantly.

Changes in Whole-Body Metal Levels in the Ash-to-Control Group

In animals removed from ash environments (A → C), most metals decreased in concentration over time from capture. As, Tl, U, V, and Zn concentrations decreased significantly from capture at all time points (May, June, September, and October: P values ranged from 0.06 to < 0.0001, with the largest differences in October). Al ($P_{\mathrm{Sept}} = 0.1$), Cd ($P_{\mathrm{Sept}} = 0.06$, $P_{\mathrm{Oct}} = 0.03$), Co ($P_{\mathrm{May}} = 0.03$, $P_{\mathrm{Oct}} = 0.09$), Cu ($P_{\mathrm{Sept}} = 0.03$, $P_{\mathrm{Oct}} = 0.01$), Fe ($P_{\mathrm{Sept}} = 0.08$, $P_{\mathrm{Oct}} = 0.003$), Se ($P_{\mathrm{Sept}} = 0.02$, $P_{\mathrm{Oct}} = 0.002$), and Sr ($P_{\mathrm{June}} = 0.06$, $P_{\mathrm{Sept}} = 0.07$, $P_{\mathrm{Oct}} = 0.01$) concentrations also decreased from capture (Fig. 3b). The concentrations of three metals in A → C toads increased significantly from capture in at least 1 month, Cr ($P_{\mathrm{Sept}} = 0.02$), Cs ($P_{\mathrm{Sept}} = 0.03$), and Pb ($P_{\mathrm{May}} = 0.05$, $P_{\mathrm{June}} = 0.0007$,

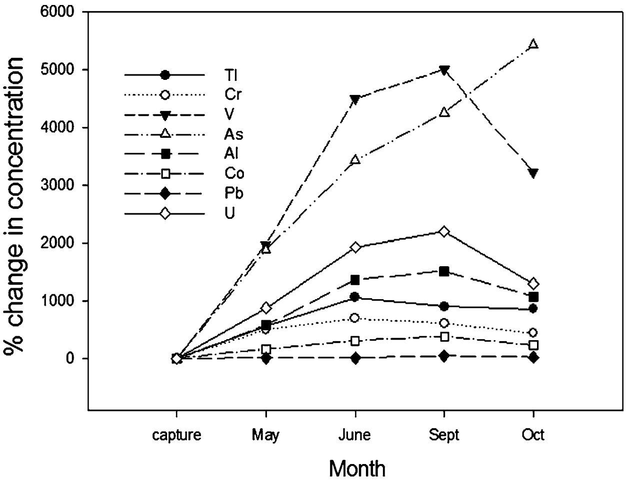

Fig. 1 Percentage change in monthly averages of eight metals (Al, As, Co, Cr, Pb, Tl, U, and V) that increased significantly over a 5-month period (April to October) in C → A toads. Percentage changes are presented without standard errors. Exposure occurred at the beginning of May and the first sample was taken at the end of May

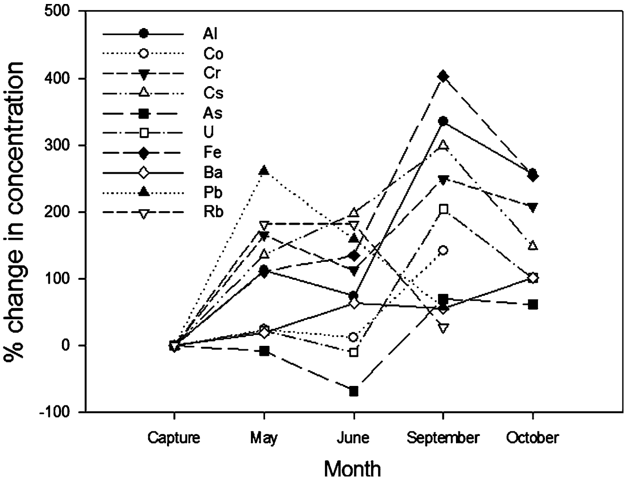

Fig. 2 Percentage change in monthly averages of 10 metals (Al, As, Ba, Co, Cr, Cs, Fe, Pb, Rb, and U) that increased significantly over a 5-month period (April to October) in A → A toads. Concentrations are presented without standard errors

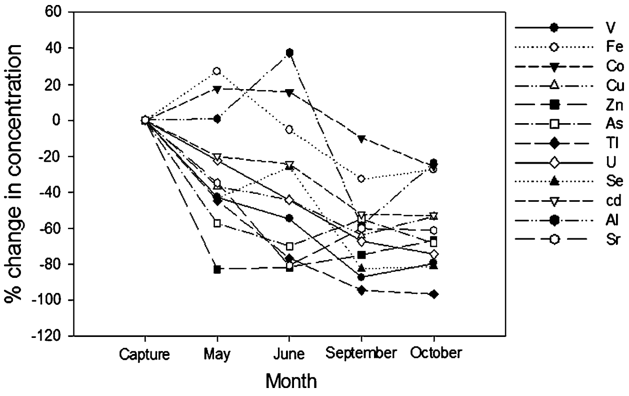

Fig. 3 Percentage change in monthly averages of 12 metals (Al, As, Cd, Co, Cu, Fe, Se, Sr, Tl, U, V, and Zn) that decreased significantly over a 5-month period (April to October) in A → C toads. Percentage changes are presented without standard errors. Toads were removed from ash at the beginning of May and the first sample was taken at the end of May

$P_{\mathrm{Sept}} = 0.0002$, $P_{\mathrm{Oct}} < 0.0001$). Rubidium increased significantly in June ($P = 0.001$) and then decreased significantly in September and October ($P = 0.009$ and $P = 0.001$ respectively). Additionally, concentrations of Ba and Ni showed no change.

Whole-Body Metal Levels in the Control Group

The concentrations of seven metals (Al, Ba, Fe, Sr, Tl, U, and Zn) in C → C toads did not change significantly from capture in any month during the experiment. The concentrations of As ($P_{\mathrm{June}} = 0.04$), Ni ($P_{\mathrm{Sept}} = 0.008$), Se ($P_{\mathrm{May}} = 0.08$), and V ($P_{\mathrm{June}} = 0.02$) showed no significant change in 4 of the 5 months. As, Ni, and V concentrations increased significantly in their respective months, while Se decreased significantly. Cd, Cu, and Rb concentrations decreased significantly in all months compared with the controls (P's ranging from 0.1 to < 0.0001). Cs ($P_{\mathrm{May}} = 0.001$, $P_{\mathrm{June}} = 0.02$, $P_{\mathrm{Sept}} = 0.02$) decreased significantly in 3 of 5 months, and Co ($P_{\mathrm{June}} = 0.04$, $P_{\mathrm{Oct}} = 0.004$), Cr ($P_{\mathrm{June}} = 0.05$, $P_{\mathrm{Sept}} = 0.06$, $P_{\mathrm{Oct}} = 0.008$), and Pb ($P_{\mathrm{June}} = 0.0005$, $P_{\mathrm{Sept}} = 0.002$, $P_{\mathrm{Oct}} = 0.0005$), increased significantly.

Discussion

Toads initially captured in the ash basin had metal levels significantly elevated above those in toads captured at control sites. Additionally, toads that were moved from a control environment to an ash environment (C → A) accumulated significant amounts of eight trace metals (Al, As, Co, Cr, Pb, Tl, U, and V) within 1 month of exposure, equivalent to or surpassing those in field-captured ash toads by May.

Toads captured from the ash basin and maintained on ash sediment (A → A) continued to take up metals until October, showing significant increases (i.e., an order of magnitude) above levels at capture (April). Interestingly, A → A animals took 4 to 5 months longer to accumulate the same concentration of metals as C → A toads. C → A toads experienced significantly increased levels of eight trace metals within 1 month of exposure, surpassing metal levels of toads captured in the field. Toads removed from an ash environment and placed in a control environment (A → C) had significantly lower concentrations of metals (within control concentrations) after 1 month of capture, indicating that toads have the ability to rapidly excrete significant levels of trace metals (Table 2c and d).

These results indicate that whole-body metal loads of male toads, and most likely other organisms, in polluted environments are dynamic and metals are accumulated and excreted at different rates. For example Al, V, and As are taken up rapidly (within the first month) when a toad enters an ash environment, while Zn, Tl, and V are excreted within a month of removal from ash. Even organisms that are maintained in polluted environments experience large fluctuations in metal concentrations over time. For example, toads collected and maintained on ash exhibited a 107 % decrease in whole-body levels of Zn and a 1329% increase in Co.

The fact that metal levels are dynamic in A → A toads is not surprising; these toads were captured in their contaminated environment shortly after having migrated into the pond for breeding. Thus, levels, though already significantly elevated over those of controls, could, and did, increase as the toads ingested and absorbed more metals from its food and environment. Therefore, metal levels can change for more than 6 months after entering a polluted environment. Many metals (e.g., Al, Cr, V, Fe, As) increased or decreased by an order of magnitude throughout the course of this experiment. This information would be lost in a single-time-point measure as is usually obtained for most field studies (e.g., Diaz et al. 1994; Hernandez et al. 1999; Moiseenko and Kudryavtseva 2001; Olsvik et al. 2000).

It is important to understand the dynamic profile of each metal within an organism due to the fact that so many of the metals are known to cause physiological changes and have long-term effects. The concentrations of metals in these toads are such that they can produce results ranging from no observed effect to mortality. For example, 5 ppm of As can cause 24% mortality of larval bullfrogs (Birge and Just 1973); concentrations in our A → A toads ranged from 0.1 to 7.7 ppm from capture to the end of the experiment. The concentrations of metals found in these toads are enough to cause physiological changes, such as replacement of Zn in Zn fingers (Chang 1996), that may have long-term effects (e.g., Ward and Mendonça 2006; Hopkins et al. 1997, 1999a, b, 2002).

It is also interesting to note that the movements of metals into and out of the body do not necessarily move in a predictive manor. For example, in control-to-ash toads, four metals decreased over the exposure period, even though the sediment values indicate exposure. Although it is possible that these toads are losing metals that they accumulated at another site prior to immigrating into the breeding area and capture, it is also possible that these toads are depurating the metals. Of the four metals that decreased, Cd, Cu, and Cs are known either to be micronutrients or to mimic micronutrients. Vertebrates have evolved mechanisms to transport, chelate, and control body concentrations of metal micronutrients. These mechanisms include depuration via the intestine and kidney (Bridges and Zalups 2005), as well as chelation by metallothionein and melanin (Chang 1996). Increases in the three metals in ash-to-control toads are more troubling. Cr, Cs, and Pb were not found at elevated concentrations in either the crickets fed to the toads or the sediment.

Changes in environmental conditions (from capture to experimental) may also be affecting the movement of metals. The osmolality of the water (Rainbow and Black 2005), temperature (Alberts et al. 1985, Baines et al. 2005), interactions between metals (Franklin et al. 2005), pH (Spurgeon et al. 1985), and toad's previous exposure to metals (Rainbow et al. 2003; Ng and Wang 2004) can all play a part in determining what metals are bioavailable and how an animal takes up and depurates metals.

Our data indicate that toads exposed to trace metals, once removed from the polluted environment, can eliminate a large portion of metals—through the intestinal epithelium and the kidney (for review see Bridges and Zalups 2005), enough to return to within control levels. However, some metals did not decline significantly, indicating that the toads could emigrate from polluted areas with an elevated amount of metal. For example, Pb did not decrease significantly, possibly due to being stored in bone, and Cd is known to be sequestered and stored by metallothionein, which may lead to retention of the metal (Chang 1996). These toads have the potential to experience delayed, sublethal physiological effects. For example, many animals chronically exposed to mixtures of trace metals experience liver fibrosis or kidney necrosis (Ganser et al. 2003; Groten et al. 1994), which may cause metabolic or osmoregulatory dysfunction. Other animals may experience anomalous levels of reproductive and adrenal steroid hormones, indicating possible reproductive dysfunction (Clark et al. 1994; Hopkins et al. 1997). Onset and abatement of these symptoms and their association with chronic, yet dynamic, metal exposure are an understudied aspect of the toxicology literature.

This study illustrates that metal levels in organisms that migrate into or out of polluted environments are dynamic and that different metals behave differently in their bioaccumulation and excretion patterns. A single-point measurement can grossly over- or underestimate the concentration of metals in an organism, depending on when, in relation to migration, the measurement was taken. Because each metal can have a different effect on an animal's physiology, it is also important to understand how each metal changes throughout a season, which can represent a reproductive cycle, a migratory path, or an entire life history, and how it impacts an organism's performance at that stage of life.

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References

Alberts JJ, Newman MC, Evans DW (1985) Seasonal variation of trace elements in dissolved and suspended loads for coal ash ponds and pond effluents. Water Air Soil Pollut 26:111–128

Andrews EJ (1993) 1993 report of the AVMA panel on euthanasia. J AmVet Med Assoc 202:229–249

Baines SB, Fisher NS, Kinney EL (2005) Influence of temperature on dietary metal uptake in Artic and temperate mussels. Mar Ecol Prog Ser 289:201–213

Birge WJ, Just JJ (1973) Sensitivity of vertebrate embryos to heavy metals as a criterion of water quality. Water Resources Research Institute, University of Kentucky, Lexington

Bridges CC, Zalups RK (2005) Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharm 204:274–308

Bustamante P, Teyssié J-L, Danis B, Fowler SW, Miramand P, Cotret O, Warnau M (2004) Uptake, transfer and distribution of silver and covalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. Mar Ecol Prog Ser 269:185–195

Chang LW (ed) (1996) Toxicology of metals. Lewis, Boca Raton, FL

Clark JT, Jimenez B, Evans SL, Barrow R, Winfree M, Mrotek JJ (1994) Cadmium-induced sexual dysfunction does not involve increased hepatic metabolism of testosterone nor increased circulating levels of corticosterone. Physiol Behav 56:975–981

Diaz C, Galindok L, Farcia Montelongo F (1994) Distribution of metals in some fishes from Santa Cruz de Tenerife, Canary Islands. Bull Environ Contam Toxicol 52:374–381

Dickerson MC (1969) The frog book. Dover, New York

EPA (2004) Coal fly ash. Available at: http://www.epa.gov/epaoswer/non-hw/recycle/jtr/comm/cfa.htm. Accessed February 9, 2005

Franklin NM, Glover CN, Nicol JA, Wood CM (2005) Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. Environ Toxicol Chem 24:2954–2964

Ganser LR, Hopkins W, O'Neil L, Hasse S, Roe JH, Sever DM (2003) Liver histopathology of the southern water snake, *Nerodia fasciata fasciata*, following chronic exposure to trace element-contaminated prey from a coal ash disposal site. J Herpatol 37:219–226

Groten JP, Koeman JH, van Nesselrooij JH, Luten JB, van Fenetener Vlissingen JM, Stenhuis WS, van Bladeren RJ (1994) Comparison of renal toxicity after long-term oral administration of cadmium chloride and cadmium-metallothionein in rats. Fundam Appl Toxicol 23:544–552

Hernandez LM, Gomara B, Fernandez M, Jimenes B, Gonzalez MJ, Baos R, Hiraldo F, Ferrer M, Benito V, Suner MA, Devesa V, Munoz O, Montoro R (1999) Accumulation of heavy metals and As in wetland bird in the area around Donana National Park affected by the Aznalcollar toxic spill. Sci Total Environ 242:293–308

Hill EF (1984) Subchronic mercury exposure in *Conurnix* and a method of hazard evaluation. Environ Toxicol Chem 3:489–502

Hopkins WA, Mendonça MT, Congdon JD (1997) Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. Gen Comp Endocrinol 108:237–246

Hopkins WA, Rowe CL, Congdon JD (1999a) Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata)* exposed to coal combustion wastes. Environ Toxicol Chem 18:1258–1263

Hopkins WA, Mendonça MT, Congdon JD (1999b) Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris)* exposed to coal combustion wastes. Comp Biochem Physiol C122:191–196

Hopkins WA, Roe JH, Snodgrass JW, Staub BP, Jackson BP, Congdon JD (2002) Effects of chronic dietary exposure to trace elements on banded water snakes (*Nerodia fasciata*). Environ Toxicol Chem 21:906–913

McGreer JC, Szebedinsky C, McDonald DG, Wood CM (2000) Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout. 1: Iono-regulatory disturbance and metabolic costs. Aquat Toxicol 50:231–243

Moiseenko TI, Kudryavtseva LP (2001) Trace metal accumulation and fish pathologies in areas affected by mining and metallurgical enterprises in the Kola Region, Russia. Environ Poll 114:285–297

Ng TYT, Wang XW (2004) Detoxification and effects of Ag, Cd, and Zn pre-exposure on metal uptake kinetics in the clam *Ruditapes philippinarum*. Mar Ecol Prog Ser 268:161–172

Nriagu JO (1988) A silent epidemic of environmental metal poisoning? Environ Pollut 50:139–161

Olsvik PA, Gundersen P, Andersen RA, Zachariassen KE (2000) Metal accumulation and metallothionein in two populations of brown trout, *Salmo trutta*, exposed to different natural water environments during a run-off episode. Aquat Toxicol 50:301–316

Perez-Coll CS, Herkovits J, Fridman O, Daniel P, D'Eramo JL (1997) Metallothioneins and cadmium uptake by the liver in *Bufo arenarum*. Environ Pollut 97:311–315

Rainbow PS, Black WH (2005) Physiochemistry or physiology: cadmium uptake and effects of salinity and osmolality in three crabs of different ecologies. Mar Eco Prog Ser 286:217–229

Rainbow PS, Blackmore G, Wang WX (2003) Effects of previous field-exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*. Mar Ecol Prog Ser 259:201–213

Rowe CL, Hopkins WA, Congdon JD (2002) Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. Environ Monitor Assess 80:207–276

Spurgeon DJ, Lofts S, Hankard PK, Toal M, McLellan D, Fishwick S, Svedsen C (1985) Effect of pH on metal speciation and resulting meal uptake and toxicity for earthworms. Environ Toxiol Chem 25:788–796

Vogiatzis AK, Loumbourdis NS (1997) Uptake, tissue distribution, and depuration of cadmium (Cd) in the frog *Rana ridibunda*. Bull Environ Contam Toxicol 59:770–776

Ward CK, Mendonça MT (2006) Chronic exposure to coal fly ash causes minimal changes in corticosterone and testosterone concentrations in male southern toads, *Bufo terrestris*. Arch Environ Toxicol Chem 51:263–269

White DH, Finley MT (1978) Uptake and retention of dietary cadmium in mallard ducks. Environ Res 17:53–59