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Elevated Trace Element Concentrations in Southern Toads, *Bufo terrestris*, Exposed to Coal Combustion Waste

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Abstract. A number of recent studies have linked developmental, physiological, and behavioral abnormalities in amphibians to coal combustion wastes (coal ash). Few studies, however, have determined trace element concentrations in amphibians exposed to coal ash. In the current study we compare total body concentrations of 20 trace elements in adult southern toads, *Bufo terrestris*, inhabiting coal ash settling basins with toads that were not exposed to the combustion wastes (reference). In addition, we document the accumulation of trace elements in toads transplanted from reference sites to field enclosures in an ash settling basin for 7 and 12 weeks. Arsenic, selenium, and vanadium levels were significantly elevated in toads captured at the ash-contaminated site in comparison to toads from the reference site. All three of these elements were also significantly elevated in toads exposed to the contaminated habitat for only 7 weeks. Our study suggests that adult anurans can bioaccumulate particularly high levels of selenium and may be useful bioindicators in agricultural and coal ash-impacted habitats.

In recent years, much concern has focused on the potential role that xenobiotics may play in the global decline of amphibian populations (Carey and Bryant 1995). Although there is much speculation on the causes of these possible declines, there have been few systematic studies determining the factors involved (Dunson *et al.* 1992; Carey and Bryant 1995). Of the many global sources of pollution, coal combustion wastes (coal ash) have recently been linked to numerous sublethal anomalies in amphibians. Coal ash effluent has been shown to cause developmental deformities of the oral region in bullfrog tadpoles (Rowe *et al.* 1996, 1998a). In addition, the waste alters the tadpoles' standard metabolic rates, grazing success, and ability to avoid predators (Rowe *et al.* 1996, 1998b; Raimondo *et al.* 1998).

Few studies, however, have examined the impact of coal combustion wastes on adult anurans. Adult southern toads, *Bufo terrestris*, transplanted to a coal ash–polluted site (ash basins) exhibit increased standard metabolic rates similar to the elevations observed by Rowe *et al.* (1998b) in bullfrog tadpoles (Hopkins *et al.* unpublished data). In addition, male toads inhabiting the polluted site were recently shown to have abnormally high circulating androgen and corticosterone levels (Hopkins *et al.* 1997). The same study indicated that toads transplanted from reference sites to the ash basin exhibited a pronounced adrenal stress response (Hopkins *et al.* 1997).

Despite evidence that coal combustion wastes adversely affect amphibians, few studies document tissue levels of trace elements in amphibians inhabiting coal ash–impacted sites. Guthrie and Cherry (1979) provide evidence that ranid tadpoles inhabiting a coal ash basin have elevated levels of numerous trace elements including selenium; however, this study did not make comparisons with tadpoles inhabiting unpolluted sites. In a more recent study, Rowe *et al.* (1996) reported that bullfrog tadpoles, *Rana catesbeiana*, inhabiting coal ash basins had significantly elevated tissue levels of As, Ba, Cd, Cr, and Se when compared to tadpoles from unimpacted sites. Based on 96-h LC50 data, Herfenist *et al.* (1989) ranked these elements, with the exception of barium, as four of the most toxic known substances to developing amphibians. Only one study to date, however, has examined concentrations of these trace elements in adult amphibians (Furr *et al.* 1979). Furr *et al.* (1979) indicated that bronze frog tadpoles (*Rana clamitans*) and adult newts (*Notophthalmus viridescens*) inhabiting a coal ash–contaminated site had elevated tissue levels of selenium in comparison to conspecifics at a reference site.

By the year 2000 it is estimated that 120 million tons of coal ash will be produced annually in the United States by coal-burning power plants (US EPA 1988). Due to an increased reliance on coal combustion as a global power source, the waste product's ubiquity alone makes it critical for future study. In light of the recent studies indicating that coal ash can have a profound impact on the development, physiology, and behavior of anurans, we sought to determine the whole body content of trace elements in adult anurans inhabiting a coal ash–impacted site. We also transplanted adult anurans to the polluted site for 7 and 12 weeks to determine if significant accumulation of trace elements would occur over a relatively short period of exposure.

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Materials and Methods

Polluted Site Description

A coal-burning electric power plant with its associated coal ash settling basins (33°11′N, 081°44′E) is located on the United States Department of Energy Savannah River Site, a National Environmental Research Park near Aiken, South Carolina. Sluiced coal ash is pumped from the plant into a primary settling basin (15 ha). The surface water flows from the primary basin into a smaller secondary basin (6 ha) and then into a 2-ha swamp before reaching Beaver Damn Creek, a tributary of the Savannah River. Previous studies indicate that the water and sediment in the ash basin habitat have elevated levels of a number of trace elements including Cd, As, Se, Cr, Cu, and Ba. (Cherry and Guthrie 1977; Alberts *et al.* 1985; Rowe *et al.* 1996).

Sample Collection and Analysis

Adult male toads, *B. terrestris*, were concurrently captured at the polluted site and at reference sites (mean mass = 16.4 and 15.4 g, respectively) between 6 July and 25 August 1996. Toads at the polluted site were captured along the roadbed between the ash basins and the ash-basin swamp. The reference sites are located within 13 km of the polluted site and have historically remained unimpacted by coal combustion waste. Toads captured at the polluted site were immediately transported to the laboratory, where they were allowed to void gastrointestinal contents for 7 days. Toads were then sacrificed and frozen for later analysis. Toads captured at reference sites were transported to outdoor enclosures either at the polluted site or at another undisturbed reference site (33°18′N, 081°39′E) (n = 8 enclosure/site).

The enclosures were constructed of a PVC (polyvinyl chloride) pipe frame and surrounded by galvanized mesh hardware cloth. Within the enclosures, toads were in direct contact with the sediment and were able to feed on naturally occurring prey items moving freely through the 1.5-cm openings in the hardware cloth. The primary focus of these experiments was to evaluate the endocrine stress response of toads to coal ash effluent (see Hopkins *et al.* 1997). Therefore, toads at both sites were supplementally fed uncontaminated crickets once a week in order to remove food limitation as a potential stressor. Toads remained in the enclosures at the reference site for 12 weeks and at the ash basin for 7 and 12 weeks. They were then transported to the laboratory, held for 7 days to void gut contents, and frozen for later analysis.

Three sediment samples were taken near the enclosures at the ash basin and the reference site. Sediment samples and toads were freeze-dried and homogenized before being sent to either the University of Georgia Crop and Soil Sciences Department (Athens, GA) or the Skidaway Institute of Oceanography (Savannah, GA) for analysis. A subset of toads from each of the four treatment groups (field collected ash basin, +7 week ash basin, +12 week ash basin, and +12 week reference) were sent to both laboratories. Samples at each laboratory were digested with HNO_3/H_2O_2 before being analyzed using inductively coupled plasma mass spectroscopy (US EPA 1994). Comparable element concentrations were obtained from each laboratory with low variability between labs. Sediment samples were analyzed for 20 trace elements (see Table 1). Toads (whole body) were analyzed for seven of the most common contaminants associated with coal ash pollution; As, Ba, Cd, Cr, Cu, Se, and Pb (US EPA 1988; Carlson and Adriano 1993). In addition, a subset of individuals from each site was analyzed for 13 other trace elements (see Table 2).

Statistics

Whole body tissue concentrations and sediment concentrations of individual trace elements were tested for normality and homoscedasticity using the Shapiro-Wilk and Hartley's test, respectively (Neter *et al.* 1990; Minitab 1996). Data were log-transformed prior to analysis. Comparisons of mean sediment concentrations from the two sites were made using *t* tests. Comparison of trace element concentrations among the four treatment groups were made using one-way analysis of variance (ANOVA). A sequential Bonferroni adjustment (Rice 1989) was used separately for sediment and tissue samples in order to adjust critical values downward since 20 elements were repeatedly compared from the same samples. Following ANOVA, a Student Newman Keuls test was utilized to identify significantly different groups.

Table 1. Trace element content (ppm dry mass) of sediment samples from a coal ash–settling basin (Ash Basin) and from an unpolluted site (Reference Site)[a]

Element	Ash Basin	Reference Site	p Value
Ag	0.048 ± 0.003	0.007 ± 0.000	<0.0001
Al	15,789.40 ± 234.70	15,939.40 ± 530.00	0.83
As	39.638 ± 2.809	0.341 ± 0.008	<0.0001
Ba	83.80 ± 5.379	27.30 ± 0.350	<0.0001
Be	2.467 ± 0.115	0.576 ± 0.008	<0.0001
Cd	0.252 ± 0.011	0.032 ± 0.001	<0.0001
Co	6.424 ± 0.443	0.934 ± 0.007	<0.0001
Cr	10.87 ± 0.815	7.02 ± 0.186	<0.01
Cu	18.39 ± 1.310	4.04 ± 0.097	<0.0001
Mn	29.30 ± 2.415	22.80 ± 0.174	0.04
Mo	3.001 ± 0.181	0.055 ± 0.008	<0.0001
Ni	13.73 ± 0.957	4.11 ± 0.124	<0.0001
Pb	6.46 ± 0.500	4.22 ± 0.079	<0.01
Sb	0.226 ± 0.025	0.003 ± 0.001	<0.001
Se	4.383 ± 0.188	0.104 ± 0.005	<0.0001
Sr	55.82 ± 5.464	5.35 ± 0.192	<0.0001
Tl	0.705 ± 0.049	0.059 ± 0.001	<0.0001
U	1.001 ± 0.035	0.323 ± 0.006	<0.0001
V	28.77 ± 2.275	7.81 ± 0.102	<0.001
Zn	27.10 ± 1.362	15.9 ± 0.226	<0.0001

[a] Data are provided as mean ± standard error. Minimum critical value as determined by Bonferroni sequential adjustment is $p < 0.002$

Results and Discussion

Previous studies indicate that water in coal-ash settling basins is enriched with numerous trace elements (Guthrie and Cherry 1979). High concentrations of trace elements settle and concentrate in the sediment of these polluted habitats (Guthrie and Cherry 1979; Rowe *et al.* 1996). Indeed, we found that all measured elements, with the exception of aluminum and manganese, were significantly elevated in ash basin sediments in comparison to the reference sediment (Table 1). Although a number of elements including aluminum, cadmium, strontium, and thallium were elevated in toads inhabiting the polluted site, only arsenic, selenium, and vanadium were significantly elevated statistically in comparison to toads from reference sites (Table 2). Little is known about the effects of vanadium on wildlife (Willsky 1990; Kustin and Robinson 1995). Therefore, our discussion focuses on arsenic and selenium but future investigations will more rigorously examine the prevalence of lesser studied elements found to be elevated in amphibians from the ash-polluted habitat (Table 2).

Arsenic

In the United States alone, it is estimated that coal-fired power plants emit 3,000 tons of arsenic a year (US EPA 1980). Arsenic

Table 2. Total body trace element content (ppm dry mass) in adult male toads, *Bufo terrestris*

	Ash Basin (n = 10)	AB + 7 wk (n = 7)	AB + 12 wk (n = 6)	Ref + 12 wk (n = 6)	p Value
As	1.58 ± 0.27[a]	1.21 ± 0.26[a]	0.75 ± 0.04[a]	0.23 ± 0.05[b]	<0.0001*
Ba	133.64 ± 32.26	105.05 ± 11.92	71.27 ± 7.34	77.20 ± 11.25	0.09
Cd	0.27 ± 0.050	0.13 ± 0.02	0.15 ± 0.03	0.12 ± 0.02	0.04
Cr	1.87 ± 0.40	1.68 ± 0.34	1.23 ± 0.09	1.31 ± 0.24	0.54
Cu	29.50 ± 7.19	27.70 ± 4.38	22.80 ± 3.66	20.80 ± 1.67	0.87
Pb	0.70 ± 0.14	1.77 ± 0.27	1.48 ± 0.30	1.06 ± 0.09	0.14
Se	17.40 ± 3.32[c]	5.46 ± 0.68[b]	3.45 ± 0.12[ab]	2.10 ± 0.09[a]	<0.0001*
	Ash Basin (n = 7)	**AB + 7 wk (n = 3)**	**AB + 12 wk (n = 2)**	**Ref + 12 wk (n = 3)**	**p Value**
Ag	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.42
Al	140.10 ± 49.83	122.50 ± 35.85	70.80 ± 26.00	23.40 ± 9.18	0.05
Be	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.06
Co	0.27 ± 0.05	0.21 ± 0.01	0.18 ± 0.02	1.42 ± 0.85	0.05
Mn	59.80 ± 11.31	111.2 ± 5.89	49.80 ± 15.97	89.90 ± 25.16	0.14
Mo	0.27 ± 0.02	0.20 ± 0.02	0.23 ± 0.00	0.27 ± 0.07	0.51
Ni	2.34 ± 0.32	2.35 ± 0.19	2.07 ± 0.22	1.56 ± 0.76	0.28
Sb	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.23
Sr	386.70 ± 74.37	218.70 ± 25.00	178.30 ± 4.89	132.10 ± 13.92	0.04
Tl	0.12 ± 0.03	0.08 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.004
U	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.14
V	1.24 ± 0.21[a]	1.07 ± 0.07[a]	1.18 ± 0.07[a]	0.25 ± 0.02[b]	0.002*
Zn	99.20 ± 5.82	268.5 ± 21.78	257.3 ± 1.73	194.30 ± 93.38	0.008

"Ash Basin" = field-captured toads from polluted site; "AB + 7 wk" and "AB + 12 wk" = toads transplanted from a reference site to polluted site for 7 and 12 weeks, respectively; "Ref + 12 wk" = toads transplanted from a reference site to another reference site for 12 weeks
Data are provided as mean ± standard error
* Denotes significant differences. Trace element levels sharing common superscripts do not differ significantly from one another
Minimum critical value as determined by Bonferroni sequential adjustment is $p < 0.002$

levels in the ash basin sediments were more than 100 times higher than the levels found in the reference sediment (Table 1). There was almost a sevenfold difference in As levels between adult toads captured in the ash basin and those from the reference sites (Table 2). Adult toads transplanted to the ash basin exhibited significant increases in whole body arsenic content after only 7 weeks of exposure to the contaminated site and levels remained significantly elevated after 12 weeks (Table 2). Recall that the transplanted toads at each site were supplementally fed uncontaminated crickets. Due to this feeding regime, trace element levels provided in our study following 7 and 12 weeks of exposure are highly conservative because some food consumed at the ash basin was not contaminated with coal-derived trace elements.

Few studies have examined arsenic levels in amphibians (Byrne *et al.* 1975; Furr *et al.* 1979; Rowe *et al.* 1996). Byrne *et al.* (1975) reported relatively low arsenic levels in the liver of various amphibians from a mercury-contaminated site, but there was no evidence that the sampling sites were contaminated with anything but Hg. On the other hand, Furr *et al.* (1979) found no difference in arsenic levels in tadpoles (*R. clamitans*) and adult newts (*N. viridescens*) inhabiting a coal ash–impacted site when compared to conspecifics from a reference site. In the most recent study, Rowe *et al.* (1996) found that bullfrog tadpoles from the ash basins had very high whole body concentrations of arsenic (48.9 ppm). The levels of arsenic reported by Rowe *et al.* (1996) are considerably higher than the levels we found in adult toads inhabiting the same coal ash basins.

To date, no studies have evaluated the relationship between life stage differences in amphibians and trace element accumulation within a species. Thus, only interspecific comparisons can be made regarding the differential accumulation of trace elements in tadpoles (bullfrogs) (Rowe *et al.* 1996) and adults (toads; current study). Anuran tadpoles feed in surface sediments, where they are likely to receive the greatest exposure to trace elements in polluted aquatic ecosystems (Hall and Mulhern 1984; Birdsall *et al.* 1986; Lemly 1987; Ohlendorf *et al.* 1988). Adult anurans, on the other hand, maintain contact with the sediments but do not typically ingest large quantities of the sediments while feeding. Species-specific traits, feeding habits, and the ability of adult anurans to move in and out of the most highly contaminated sediments likely explains much of the observed differences in the accumulation of trace elements.

Selenium

Although selenium is an essential trace element, it has been linked to a number of toxic effects at slightly higher levels in a variety of organisms (Browne and Dumont 1979, 1980; Hoffman and Heinz 1988; Ohlendorf *et al.* 1989; Hamilton *et al.* 1990; Lemly 1993b; Stanley *et al.* 1994). Selenium levels in the ash basin sediment were significantly elevated in comparison to the reference sediment (Table 1). In contrast to arsenic, which was not accumulated to levels greater than the concentrations in the sediment, toads accumulated very high levels of selenium. Whole body selenium concentrations were significantly higher in ash basin toads (17.40 ppm) than in reference toads (2.10 ppm) and were four times as high as concentrations in the polluted sediment (Table 2). The levels of selenium in toads from the polluted site are likely hazardous, given the fact that selenium concentrations of only 10 ppm are known to cause reproductive failure in other vertebrates (Gillespie and Baumann 1986; Lemly 1993a).

Selenium is primarily incorporated in vertebrate tissues via trophic transfer (Lemly 1987; Besser *et al.* 1993). Despite the fact that toads in the enclosures were supplementally fed uncontaminated crickets, toads remaining in the ash basin for only 7 weeks exhibited significantly elevated levels of selenium in comparison to toads at the reference site (Table 2). However, selenium levels in toads transplanted to the ash basin decreased after 12 weeks of exposure (Table 2). In fact, we observed slight decreases in several element concentrations between our 7-week and 12-week sample (Table 2). Due to the limitations created by our feeding regime, we cannot differentiate between natural decreases in element concentrations and artifacts created by supplemental feeding (*i.e.* seasonal changes in the proportion of supplemental to natural food sources).

Few other studies have examined selenium levels in amphibians inhabiting selenium contaminated habitats (Furr *et al.* 1979; Ohlendorf *et al.* 1988; Rowe *et al.* 1996). All of these studies, however, indicate that adult and larval amphibians in polluted habitats have elevated tissue concentrations of selenium. In the most recent investigations, Ohlendorf *et al.* (1988) found that adult bullfrogs inhabiting an area impacted by agricultural drainwater had accumulated selenium concentrations of 45.0 ppm in their livers. The sediment in this agricultural system contains up to 100 ppm of selenium, more than 20 times the levels we document in the coal ash–polluted sediment (Saiki and Lowe 1987). In the same coal ash–settling basins examined in the current study, Rowe *et al.* (1996) found that *R. catesbeiana* tadpoles had higher whole body concentrations of selenium than the concentrations we found in adult toads (25.70 ± 3.60 vs. 17.40 ± 3.32 ppm, respectively) and almost six times that of the polluted sediment. It is possible that the prolonged larval period of bullfrogs, which places them in contact with the water and surface sediments for more than a year, is responsible for such high selenium accumulation in this species.

Anurans as Bioindicators

Numerous studies have suggested that amphibians may be useful as bioindicators of harmful levels of xenobiotics in the environment (Cooke 1981; Hall and Mulhern 1984; Birdsall *et al.* 1986; Dunson *et al.* 1992). With increasing amounts of agricultural drainwater and coal ash effluent being globally produced every year, biomonitoring techniques are crucial for assessing the impact of arsenic, selenium, and other contaminants on wildlife as well as humans. Our study, in conjunction with others (Ohlendorf *et al.* 1988; Rowe *et al.* 1996) provides evidence that amphibians may be particularly useful in monitoring selenium concentrations within aquatic and terrestrial communities. Since selenium is known to bioaccumulate in the food web, monitoring the quantities of selenium entering different trophic levels can alert us to the potential consequences of current agricultural and industrial practices.

Many studies have examined the ability of other vertebrates to accumulate selenium including birds (Ohlendorf *et al.* 1986, 1990), fish (Saiki and Lowe 1987; Hothem and Ohlendorf 1989; Ogle and Knight 1989; Saiki *et al.* 1993) and mammals (Clark 1987; Clark *et al.* 1989). However, utilizing amphibians as biomonitors may have several distinct advantages. First, larval and adult amphibians are relatively spatially confined (compared to birds and mammals) making them useful in estimating the local movement of contaminants into adjacent habitats. Under conditions where other vertebrates may be logistically impractical for sampling, both adult and larval amphibians tend to be easily captured and often have high local densities. As illustrated in recent studies, some amphibian species can even be utilized in controlled transplant experiments to assess the impact of exposure to pollutants in the field (present study; Hopkins *et al.* 1997; Rowe *et al.* 1998a, 1998b). It is also likely that amphibians are more susceptible to certain pollutants than other vertebrates because they may breed, develop, feed, and overwinter in intimate contact with contaminated water and sediments (Dunson *et al.* 1992). In addition, adult and larval amphibians, which primarily feed on insects and plant material, respectively, provide information about the earliest stages of trophic transfer of contaminants to vertebrate populations. Future studies assessing the uptake of trace elements in biota should include both adult and larval anurans because they are an important trophic link to birds, mammals, reptiles, and predatory fish. The fact that many amphibian populations are apparently declining (Wyman 1990; Wake 1991) should provide further impetus for researchers to more closely examine the quantities of trace elements and other pollutants in these organisms.

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