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Concentrations of copper, zinc and manganese in Tree Sparrow (*Passer montanus***) at Jixi, Heilongjiang Province, China**

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Abstract: Concentrations of copper (Cu), zinc (Zn) and manganese (Mn) were measured in four tissues of Tree Sparrow (*Passer montanus*) from three sites in Heilongjiang province, China, during four seasons. Among the four measured tissues (primary feathers, liver, heart, and pectoral muscle), the highest concentrations were found in feathers (Zn) or liver (Cu and Mn), and the lowest concentrations were in muscle, except Cu. For Mn, mean concentrations of the birds from the three study sites were, in decreasing order: Didao mining area (DMA) > urban district of Jixi (UDJ) > Phoenix Mountain national nature reserve (PMR, the reference site), but not for Zn and Cu. There were significant differences between metal concentrations at the three sites, but differences were not significant for Mn (in muscle and feathers) and Cu (in feathers). For most elements, the mean concentrations were greatest in summer.

Keywords: copper; manganese; mining; tree sparrow; zinc; Jixi

Introduction

In recent decades, birds have been recognized as bio-indicators of environmental contamination because birds are abundant, widely distributed, have long lifespans, and feed at different trophic levels, being often the top consumers (Burger 1993; Muralidharan et al. 2004; Rothschild and Duffy 2005; Pan et al. 2008; Lopes et al. 2010). Most of these studies focused on raptors and waterbirds (Kim et al. 1996; Jager et al. 1996; Wayland

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et al. 1999; Goutner et al. 2001). However, many of these species are migratory, feeding over a wide geographical area. This makes it difficult to determine where contaminants were acquired (Costa et al. 2011).

 Few studies have looked at the effects of metals on passerine species in terrestrial ecosystems (Dauwe et al. 2003; Beyer et al*.* 2004; Nam et al. 2004; Dauwe et al. 2005). Tree Sparrow (*Passer montanus*) is ubiquitous and abundant, mainly non-migratory, and has limited mobility throughout the year, foraging over small ranges (only about $7,600 \text{ m}^2$). This implies that any detected contamination would be sourced from a limited area, making the species suitable for identifying local pollution sources and impacts (Pan et al. 2003; Nam and Lee 2006).

The objectives of this study were to sample tissues of Tree Sparrow to determine trace metal concentrations of copper (Cu), zinc (Zn) and manganese (Mn); to evaluate the concentrations of the three metals in the primary feathers, liver, heart, and pectoral muscle; to compare the seasonal and locational differences among four seasons and three sites of Didao mining area (DMA), urban district of Jixi (UDJ) and Phoenix Mountain national nature reserve (PMR, the reference site) in Heilongjiang Province, China.

Materials and methods

Collection and field sampling

A total of 110 adult Tree Sparrows were collected at three study sites, DMA, UDJ, and PMR. PMR was designated the reference site because of its presumed limited availability of anthropogenic sources of metals. All three sites were located in Heilongjiang Province, China and bird collection took place during winter (2009-2010) and autumn (2010) (Table 1).

 Didao mining area (45°27′48" N, 130°50′29" E) is located northwest of the urban district of Jixi, about 15 km from the Jixi urban center. The samples from this site were collected in the center of the Didao urban district. Phoenix Mountain national nature reserve (45°03′40″N, 131°15′00″E) is located in Jidong County, about 28 km southeast of Jixi urban district. The PMR area was relatively undisturbed with forest cover over 94.6%.

 All birds were captured by mist netting and after securing legal permission. Each specimen was euthanized by ether, then weighed, stored in plastic bags, and kept at -20°C for dissection and analysis.

Table 1. Collected individual numbers in four seasons from DMA (Didao mining Area), UDJ (urban district of Jixi) and PMR (Phoenix Mountain nature reserve)

	Winter, 2009-2010	Spring, 2010	Summer, 2010	Autumn, 2010	Total
DMA		10	9	8	34
UDJ	8	8	10	9	35
PMR	8	11	12	10	41
Total	23	29	31	27	110

Laboratory analysis

Before use, all glassware was washed with distilled water, soaked in nitric acid (30%) overnight, rinsed in deionized water, and air-dried. All birds were weighed and dissected within a few hours of death. Samples of pectoral muscle, heart, liver, and primary feathers were removed from each bird. Feathers were washed vigorously three times with deionized water and acetone, and interior tissues were washed with deionized water. After washing, specimens were air dried at 70°C overnight. Each sample was homogenized with the aid of porcelain pestle and mortar, and then weighed to the nearest 0.1 mg, dried until no further weight loss (Deng 2007). A sample was weighed to 0.1−0.2 g, then dissolved in 1 ml of mixed acid (volume ratio of concentrated nitric acid and perchloric acid was 4:1). The mixtures were then heated at 160°C for about 4 h until the solution was colorless and clear. After cooling, each mixture was poured into a numbered container and topped up to 10 ml with stirred water (Pan 2008). All elements were analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (VARIAN AA-240).

Statistical analysis

Statistical analyses were carried out using SPSS version 17.0. One-way analysis of variance (ANOVA) was applied to test the differences in metal concentrations between samples from different sites.

Results

Locational differences

Metal concentrations at DMA ranked highest in six of 12 categories (3 metals x 4 tissue types), and higher than at PMR in 10 of 12 categories. Metal concentrations at UDJ were greater than at the reference site at PMR in nine of 12 categories. Metal concentrations at PMR ranked lowest in nine of 12 categories.

 Concentrations of most elements differed significantly be- $\textcircled{2}$ Springer

tween DMA, UDJ, and PMR, but concentrations of Mn (in muscle) and Cu (in feathers) did not differ between DMA and UDJ. Mn (in feathers) did not differ between UDJ and PMR (Table 2). Concentrations of Cu (in muscle, liver and heart) and Zn (in muscle, liver and feathers) at DMA were less than at UDJ. Concentrations of Cu (in muscle) and Zn (in heart and liver) at UDJ were lower than at PMR (Table 2).

Distribution pattern in tissues

The greatest concentrations of metals were generally found in primary feathers or liver of Tree Sparrow, the least were in pectoral muscle. The highest levels of Cu and Mn were accumulated in the liver, and Zn was most concentrated in feathers. Muscle contained the lowest metal concentrations, except for Mn and Cu at PMR, where the lowest levels were in the heart (Table 2).

Table 2. Trace metal concentrations (µg/g, dry weight, Mean±SD) and ranges in tissues of Tree Sparrow from DMA, UDJ and PMR (all seasons combined)

Tissue	Area	Mn	Cu	Zn
Pectoral muscle	DMA	3.381 ± 1.237 a	5.149±1.985 a	26.751 ± 7.590 a
		1.124-6.827	2.044-9.933	13.676-42.360
	UDJ	3.090 \pm 1.012 a	5.791 \pm 2.358 a	32.028 ± 8.381 b
		0.732-5.356	2.290-12.627	15.342-46.774
	PMR	$2.405 \pm 1.117 b$	6.947 ± 2.517 b	22.498 ± 5.775 c
		1.039-5.388	2.840-13.281	12.516-38.746
Heart	DMA	14.608 ± 5.955 a	9.140 ± 3.069 a	50.151 ± 16.162 a
		5.682-29.216	3.341-14.420	24.476-84.346
	UDJ	5.553 ± 4.233 b	10.016 ± 4.998 a	41.140 \pm 15.268 b
		0.837-15.284	2.374-20.360	20.007-83.510
	PMR	2.289 ± 0.915 c	4.699 ± 1.763 b	43.186 ± 11.621 b
		0.41-4.394	2.054-9.746	21.570-68.433
Liver	DMA	28.977 ± 8.840 a	22.693 ± 7.987 a	65.026 ± 12.417 a
		13.958-49.092	9.137-42.483	34.058-100.371
	UDJ	$18.631 \pm 7.512 b$	25.561 ± 8.467 a	74.257±31.280 a
		7.375-34.620	9.381-48.392	29.657-136.573
	PMR	11.718±4.422 c	12.181 ± 3.867 b	82.903±19.860 b
		3.572-22.290	5.284-21.209	40.092-135.770
Primary feathers	DMA	24.231 ± 8.461 a	16.109±4.828 a	112.331 ± 28.390 a
		9.356-44.282	7.843-28.548	63.464-173.563
	UDJ	11.705 ± 4.481 b	14.642 ± 4.443 a	$126.973 \pm 30.692 b$
		4.963-20.550	5.556-21.302	65.382-205.511
	PMR	10.072 ± 5.665 b	11.068 ± 6.155 b	83.404 ± 20.343 c
		1.305-21.282	2.012-25.307	47.829-132.677

Note: Means sharing the same letter are not significantly different among areas (*p*>0.05).

Seasonal differences

Concentrations of Mn, Cu and Zn from all of sites were highest in summer (Figs. 1−3).

Figs. 1−**3 Mean concentrations of trace metal (µg/g, dry weight, means) from DMA, UDJ and PMR in winter (2009-2010); spring, summer and autumn (2010) (all tissues combined)**

Discussion

Spatial variations

One objective of our study was to determine if there were locational differences in heavy metal concentrations in the tissues of Tree Sparrow among three habitats. Since mining areas are exposed to high levels of pollution, we expected that Tree Sparrows at DMA would have significantly higher concentrations of metals than other areas. Indeed, for manganese, there were significant differences among the three sampling sites and the ranking of mean manganese concentrations showed a consistent pattern: Highest at DMA, intermediate at UDJ, and lowest at PMR. However, contrary to our expectations, there were no significant spatial differences in the levels of zinc and copper; they were even slightly greater in UDJ or/and PMR compared to in DMA, especially for zinc levels in liver and copper in muscle.

 Hogstad (1996) found no significant difference in liver concentrations of zinc and copper between industrial and rural areas for juvenile Tits or Bullfinch from central Norway. In contrast, Dauwe (2000) reported significantly higher levels of heavy metals in excrement of Great and Blue Tit from Belgium at the polluted site. Moreover, his results showed that the feathers of Great Tit chicks contained significantly lower levels of zinc at the polluted site than at the reference site. Copper and zinc concentrations in feathers of Blue Tit nestlings were not significantly different at the two sites. These findings were consistent with the results of Costa (2011) who reported that feather concentrations of copper and zinc in Great Tit nestlings were not significantly different between an industrial area and a forest area in Portugal.

 Zinc, copper, and manganese are known to be essential elements. If dietary intake remains below toxic levels, the body might be expected to conserve them (Kim 2009). The concentration of heavy metals in tissues depends on the stage of development, physiological status, and the amount of metals taken up from the habitat (Tuormaa 2000; van Eeden 2003). The transfer of essential elements, such as zinc, in the food web is modified by homeostatic mechanisms in organisms, which strive to keep levels physiologically adequate.

Synergistic and/or antagonistic behaviours of those essential

elements with respect to some non-essential ones (lead and cadmium) are important issues to be considered (Tuormaa 2000; Kalisińska et al. 2007). O'Flaherty (1998) showed that the uptake of non-essential metals such as lead inhibits uptake of essential metals such as zinc and copper. The waste minerals and industrial exhaust gases emitted by the combustion of coal contains high concentrations of lead. The elevated concentrations of lead at the pollution sites, according to this theory, probably explains why tissues contained no significantly higher, and in some cases even slightly lower levels of zinc and copper at the pollution sites in this study.

Seasonal variations

Studies on seasonal variations of trace metal levels in birds are few in comparison to those focusing on other variations (e.g. interspecific, gender, and age variations). O'Hogstad (1996) reported that the heavy metal concentration in the liver of juvenile and adult tits (from Central Norway) in late winter was higher for Cu and lower for Zn than in October-December (but the sample size was small). Gómez (2004) found in most of 14 waterfowl species from Spain, liver zinc levels tended to decrease slightly, whereas liver arsenic levels tended to increase during summer. Cadmium and lead concentrations in liver showed a general increase during winter, and cadmium and lead concentrations in some species showed a statistically significant increase. Our results suggest that for most elements, mean concentrations were highest in summer.

 Birds were generally exposed to heavy metals mainly by ingestion of food, drinking and by geophagy, eating soil to obtain essential nutrients such as sulfur and phosphorus (Malik and Zeb 2009). Food was one of the most important sources of elements in tissues. Tree Sparrows are omnivorous birds, and the foods that they consume vary by season. Foods of the adults consisted mainly of seeds and cereals when available. However, Lepidopteran insects (e.g. Cabbage Butterfly) become the main food source for adult Tree Sparrows during summer and for the nestlings, which are parasitic in cruciferous plants (e.g. cabbage) and feed on its leaves. According to contaminant flow theory in food chains, increased levels of trace metals might be found in the birds feeding on insects, rather than those feeding on plants.

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Distribution pattern in tissues

In the present study, the highest concentrations of metals were generally found in primary feathers or liver, the lowest were in pectoral muscle. This result is in accordance with the results of Deng (2007) who showed the highest concentrations of zinc and manganese to be in feathers, copper in liver; and lowest concentrations of zinc and manganese were detected in pectoral muscle. Lowest concentrations of copper were in feathers of Great Tit and Greenfinch from Beijing, China. Torres (2010) reported the concentrations of copper, manganese, and zinc were highest in feathers of Feral Pigeon from Spain.

Feathers of birds as bioindicators

Bird feathers have been used for metal biomonitoring since the 1960s (Burger and Gochfeld 1993), and they have been shown to be suitable indicators of heavy metal pollution (Dauwe et al. 2000; Janssens et al. 2002). This method did not involve the sacrifice of birds and provides effective metal quantification, so feathers could be collected from live birds and particularly appropriate for rare and endangered species (Malik 2009).

 Although in most cases adult birds were used to monitor, for metals might accumulate with the age of the organism, much previous studies have reported that the use of nestling passerines had several advantages (Furness 1993). Nestlings are restricted to the nest, and they will be fed with food items collected close to the nest site (Dauwe et al. 2000). Therefore, the heavy metal contamination will originate from a restricted parental foraging area around the nest (Furness 1993). In addition, contamination will be accumulated in the short period of nestling growth (Dauwe et al. 2000).

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