

Distribution of Heavy Metals and Their Age-related Changes in the Eastern Great White Egret, *Egretta alba modesta*, in Korea

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Abstract. Organ and tissue distribution of eight metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd, Hg) and their age-related changes were investigated in the chick and adult eastern great white egret, *Egretta alba modesta*, collected in Korea. High concentrations of the metals were found in the liver, kidney, feathers, bone, and skin; low values were found in the muscle and brain. A majority of the metal burdens in the chick and adult egrets existed in the muscle, bone, and feathers; about 50% of the Hg was in the feathers. The concentrations of Fe, Mn, Zn, and Cu in organs and tissues of the chicks characteristically changed with age, and their accumulations depended upon the metabolic turnover. In contrast, the concentrations of Pb, Ni, Cd, and Hg increased with age, suggesting that age or exposure time is a dominant factor. However, the younger stage of the downy chicks showed a rapid accumulation plateau of Pb, Ni, Cd, and Hg, and a dilution effect of these metal concentrations by increased body weight with age also was observed. Furthermore, rapid decreases of the body burden of Mn, Cu, Ni, Pb, Cd, and Hg were found in the fledgling birds, indicating that the metals were excreted via the feathers by moulting. These results indicate that consideration of the growth stage of organs and tissues is necessary for understanding the bioaccumulation processes and the toxicological criteria of the metals.

Many comparative studies have shown the effects of anthropogenic pollution on birds by toxic metals such as Pb, Cd, and Hg (Fimreite *et al.* 1970; Tansy and Roth 1970; Martin and Nickerson 1973; Ohi *et*

al. 1974; Jenkins 1975; Munoz *et al.* 1976; Getz *et al.* 1977; White *et al.* 1977; Hulse *et al.* 1980; Hutton and Goodman 1980). Their toxicological effects including mortality, flying and reproductive failures and other physiological toxicities also have been suggested (Bagley and Locke 1967; Anderlini *et al.* 1972; Longcore *et al.* 1974; Martin and Coughtrey 1975; Simpson *et al.* 1979; Eastin *et al.* 1983; Grue *et al.* 1984; Hoffman *et al.* 1984). Recently, some birds in Korea have experienced a reduction in numbers (Won 1975; Seong 1979), mainly due to destruction of the habitat by industrialization. Additionally, direct or indirect disturbances by people, as well as environmental pollution by agricultural chemicals containing organochlorine compounds and heavy metals are closely related to the decline of these species (Min *et al.* 1984a). For elucidating such toxicological effects of metals on birds, accumulation characteristics of metals in organs and tissues and their variations are warranted. Metal concentrations and distribution in birds may be influenced by several physiological and biological processes, such as feeding habits, growth or age, reproduction, moulting, and migration. In particular, the growth stage or age of a bird is one of the causes of large variations, and several authors have shown that Cd, Pb, and Hg are accumulated in certain tissues of birds with growth (Haarakangas *et al.* 1974; Ojanen *et al.* 1975; Furness and Hutton 1979; Osborn 1979; Hulse *et al.* 1980; Cheney *et al.* 1981). However, a detailed analysis of organ and tissue distribution of metals and their variations with age in relation to developmental processes of organ and tissue has yet to be presented.

The eastern great white egret (EGWE), *Egretta alba modesta*, is relatively a large and long-life species, which migrates for breeding to Korea, China, and Japan late in March or early in April,

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Table 1. Metal concentrations^a (mean \pm SD, $\mu\text{g/wet g}$) in various organs and tissues and whole body of the chick and adult egret, *Egretta alba modesta*

Organ & tissue	Growth stage	Age (day)	(N)	Fe	Mn	Zn	Cu
Brain	Downy young	1-9	(-)	—	—	—	—
		12-24	(9)	24.6 \pm 2.10 ^a	0.20 \pm 0.05 ^a	12.0 \pm 2.03 ^a	2.08 \pm 0.10 ^a
	Prefledgling	30-45	(6)	25.3 \pm 2.98 ^a	0.20 \pm 0.04 ^a	23.1 \pm 6.23 ^b	2.10 \pm 0.13 ^a
	Fledgling	55-70	(4)	26.1 \pm 5.32 ^a	0.22 \pm 0.09 ^a	14.8 \pm 3.29 ^a	2.01 \pm 0.15 ^a
	Chick mean			24.7 \pm 6.93 ^a	0.21 \pm 0.11 ^a	13.4 \pm 4.31 ^a	2.02 \pm 0.18 ^a
Liver	Downy young	1-9	(6)	103 \pm 35.6 ^a	1.52 \pm 0.16 ^a	25.0 \pm 8.83 ^a	6.11 \pm 0.17 ^a
		12-24	(9)	76.1 \pm 4.21 ^b	2.26 \pm 0.84 ^b	32.3 \pm 3.89 ^b	10.9 \pm 6.49 ^b
	Prefledgling	30-45	(6)	369 \pm 305 ^c	2.98 \pm 0.32 ^c	58.3 \pm 15.2 ^c	11.0 \pm 3.74 ^b
	Fledgling	55-70	(4)	389 \pm 83.4 ^c	3.79 \pm 0.25 ^d	52.1 \pm 32.6 ^d	3.92 \pm 0.41 ^c
	Chick mean			182 \pm 182 ^d	2.65 \pm 0.73 ^c	31.2 \pm 19.0 ^b	9.24 \pm 5.04 ^b
Kidney	Downy young	1-9	(6)	—	—	—	—
		12-24	(9)	53.6 \pm 4.02 ^a	0.52 \pm 0.12 ^a	19.1 \pm 4.18 ^a	1.75 \pm 0.58 ^a
	Prefledgling	30-45	(6)	115 \pm 63.2 ^b	0.98 \pm 0.15 ^b	25.0 \pm 9.85 ^b	2.20 \pm 0.83 ^b
	Fledgling	55-70	(4)	67.5 \pm 19.8 ^c	1.10 \pm 0.10 ^c	13.9 \pm 1.31 ^c	2.70 \pm 1.10 ^c
	Chick mean			84.7 \pm 30.7 ^d	0.90 \pm 0.22 ^b	20.1 \pm 4.12 ^d	2.37 \pm 0.55 ^b
Muscle	Downy young	1-9	(6)	28.7 \pm 6.51 ^a	1.13 \pm 0.03 ^a	18.3 \pm 3.18 ^a	0.61 \pm 0.02 ^a
		12-24	(9)	30.0 \pm 3.19 ^a	0.30 \pm 0.08 ^b	21.3 \pm 6.05 ^a	1.04 \pm 0.18 ^b
	Prefledgling	30-45	(6)	33.4 \pm 3.69 ^b	0.21 \pm 0.05 ^c	34.1 \pm 12.0 ^b	2.54 \pm 0.45 ^c
	Fledgling	55-70	(4)	37.3 \pm 10.7 ^c	0.20 \pm 0.05 ^c	30.9 \pm 7.07 ^b	1.64 \pm 0.69 ^d
	Chick mean			31.6 \pm 4.92 ^a	0.37 \pm 0.30 ^b	25.3 \pm 9.18 ^a	1.44 \pm 0.75 ^d
Skin	Downy young	1-9	(-)	—	—	—	—
		12-24	(9)	25.8 \pm 3.67 ^a	0.70 \pm 0.17 ^a	17.8 \pm 4.74 ^a	1.51 \pm 0.66 ^a
	Prefledgling	30-45	(6)	38.4 \pm 19.3 ^b	1.12 \pm 0.20 ^b	30.5 \pm 13.4 ^b	4.05 \pm 1.73 ^b
	Fledgling	55-70	(4)	29.1 \pm 11.9 ^c	1.00 \pm 0.08 ^b	30.2 \pm 2.47 ^b	3.23 \pm 1.36 ^c
	Chick mean			29.9 \pm 11.2 ^c	0.86 \pm 0.25 ^a	23.2 \pm 9.42 ^c	2.48 \pm 1.52 ^d
Bone	Downy young	1-9	(6)	25.2 \pm 5.30 ^a	1.43 \pm 0.20 ^a	54.3 \pm 3.85 ^a	0.39 \pm 0.10 ^a
		12-24	(9)	39.9 \pm 13.1 ^b	1.67 \pm 0.45 ^b	72.1 \pm 14.4 ^b	0.77 \pm 0.15 ^b
	Prefledgling	30-45	(6)	45.5 \pm 21.1 ^c	2.11 \pm 0.30 ^c	99.1 \pm 17.0 ^c	0.72 \pm 0.27 ^b
	Fledgling	55-70	(4)	52.4 \pm 20.6 ^d	3.03 \pm 0.90 ^d	115 \pm 35.6 ^d	0.74 \pm 0.03 ^b
	Chick mean			43.2 \pm 15.2 ^c	1.99 \pm 0.63 ^b	85.9 \pm 23.1 ^c	0.75 \pm 0.16 ^b
Feather	Downy young	1-9	(6)	3.20 \pm 0.32 ^a	0.05 \pm 0.02 ^a	25.2 \pm 2.50 ^a	0.35 \pm 0.10 ^a
		12-24	(9)	26.0 \pm 6.85 ^b	1.06 \pm 0.45 ^b	49.1 \pm 7.60 ^b	2.91 \pm 0.81 ^b
	Prefledgling	30-45	(6)	51.4 \pm 21.5 ^c	4.18 \pm 3.27 ^c	85.0 \pm 18.9 ^c	8.30 \pm 5.32 ^c
	Fledgling	55-70	(4)	13.0 \pm 1.70 ^d	1.58 \pm 0.50 ^d	76.4 \pm 0.21 ^d	4.78 \pm 0.32 ^d
	Chick mean			31.4 \pm 17.4 ^b	2.03 \pm 2.07 ^e	63.3 \pm 19.5 ^e	4.71 \pm 3.45 ^d
Whole body	Downy young	1-9	(6)	30.4 \pm 7.40 ^a	1.59 \pm 0.15 ^a	15.1 \pm 1.47 ^a	1.42 \pm 0.36 ^a
		12-24	(9)	38.4 \pm 5.48 ^b	0.90 \pm 0.16 ^b	31.0 \pm 3.71 ^b	1.89 \pm 0.44 ^b
	Prefledgling	30-45	(6)	47.2 \pm 5.54 ^c	1.46 \pm 0.46 ^a	52.2 \pm 4.45 ^c	2.67 \pm 0.74 ^c
	Fledgling	55-70	(4)	55.7 \pm 6.37 ^d	1.25 \pm 0.10 ^c	56.1 \pm 4.45 ^d	1.76 \pm 0.34 ^b
	Chick mean			38.3 \pm 8.41 ^b	1.25 \pm 0.37 ^c	33.3 \pm 16.9 ^b	1.93 \pm 0.67 ^b
Adult			(5)	69.2 \pm 9.85 ^e	1.82 \pm 0.60 ^d	57.2 \pm 5.74 ^d	2.77 \pm 0.31 ^c

then returns to the Philippines, Indonesia, and Malaysia for wintering late in September or early in October (Min *et al.* 1984a; Min and Shiraishi 1984). As the EGWE is relatively high in the trophic level of the food web, it may be useful as an "indicator species" for environmental pollution of heavy

metals and other chemicals. Additionally, this species is important for understanding the bioaccumulation phenomenon of pollutants. Min *et al.* (1984a) examined the biometry of growth and food habits of young of the EGWE in Korea and reported that the development of organs and tissues,

Table 1. (cont'd)

Organ & tissue	Growth stage	Age (day)	(N)	Ni	Pb	Cd ^b	Hg
Brain	Downy young	1-9	(-)	—	—	—	—
		12-24	(9)	0.03 ± 0.01 ^a	0.05 ± 0.01 ^a	<1.0 ^a	<0.005 ^a
	Prefledgling	30-45	(6)	0.06 ± 0.02 ^b	0.10 ± 0.03 ^b	<1.0 ^a	0.05 ± 0.01 ^b
	Fledgling	55-70	(4)	0.06 ± 0.04 ^b	0.06 ± 0.05 ^a	<1.0 ^a	0.05 ± 0.01 ^b
	Chick mean		(5)	0.05 ± 0.03 ^b	0.08 ± 0.05 ^b	1.0 ± 1.0 ^b	0.05 ± 0.04 ^b
Liver	Downy young	1-9	(6)	0.10 ± 0.01 ^a	0.25 ± 0.05 ^a	14.0 ± 8.0 ^a	0.26 ± 0.03 ^a
		12-24	(9)	0.05 ± 0.01 ^b	0.08 ± 0.02 ^b	3.0 ± 1.0 ^b	0.15 ± 0.03 ^b
	Prefledgling	30-45	(6)	0.05 ± 0.01 ^b	0.28 ± 0.23 ^a	17.5 ± 11.0 ^c	0.84 ± 0.93 ^c
	Fledgling	55-70	(4)	0.05 ± 0.04 ^b	0.10 ± 0.01 ^b	2.0 ± 1.0 ^b	0.44 ± 0.04 ^d
	Chick mean		(5)	0.06 ± 0.02 ^b	0.16 ± 0.14 ^c	12.0 ± 13.0 ^a	0.36 ± 0.47 ^c
Kidney	Downy young	1-9	(6)	—	—	5.7 ± 3.0 ^a	—
		12-24	(9)	0.04 ± 0.01 ^a	0.07 ± 0.02 ^a	3.0 ± 2.0 ^b	0.10 ± 0.05 ^a
	Prefledgling	30-45	(6)	0.06 ± 0.01 ^b	0.38 ± 0.12 ^b	25.0 ± 30.0 ^c	0.46 ± 0.41 ^b
	Fledgling	55-70	(4)	0.04 ± 0.01 ^a	0.11 ± 0.01 ^c	30.0 ± 10.0 ^d	0.22 ± 0.02 ^c
	Chick mean		(5)	0.06 ± 0.02 ^b	0.18 ± 0.09 ^d	15.0 ± 27.0 ^e	0.28 ± 0.36 ^d
Muscle	Downy young	1-9	(6)	0.14 ± 0.02 ^a	0.17 ± 0.01 ^a	4.0 ± 1.0 ^a	0.06 ± 0.03 ^a
		12-24	(9)	0.06 ± 0.01 ^b	0.07 ± 0.03 ^b	3.0 ± 1.0 ^a	0.04 ± 0.01 ^b
	Prefledgling	30-45	(6)	0.09 ± 0.04 ^c	0.14 ± 0.06 ^a	7.0 ± 2.0 ^b	0.11 ± 0.09 ^c
	Fledgling	55-70	(4)	0.05 ± 0.01 ^d	0.06 ± 0.01 ^b	4.0 ± 2.0 ^a	0.08 ± 0.02 ^a
	Chick mean		(5)	0.08 ± 0.03 ^c	0.10 ± 0.05 ^c	4.0 ± 2.0 ^a	0.06 ± 0.05 ^a
Skin	Downy young	1-9	(-)	—	—	—	—
		12-24	(9)	0.11 ± 0.03 ^a	0.16 ± 0.04 ^a	5.0 ± 2.0 ^a	0.06 ± 0.02 ^a
	Prefledgling	30-45	(6)	0.22 ± 0.15 ^b	0.50 ± 0.18 ^b	9.0 ± 8.0 ^b	0.39 ± 0.38 ^b
	Fledgling	55-70	(4)	0.15 ± 0.04 ^a	0.15 ± 0.02 ^a	13.0 ± 3.0 ^c	0.10 ± 0.01 ^c
	Chick mean		(5)	0.15 ± 0.09 ^a	0.15 ± 0.10 ^a	7.0 ± 5.0 ^b	0.21 ± 0.28 ^d
Bone	Downy young	1-9	(6)	0.14 ± 0.09 ^a	0.32 ± 0.05 ^a	3.0 ± 2.0 ^a	0.04 ± 0.01 ^a
		12-24	(9)	0.07 ± 0.02 ^b	0.17 ± 0.05 ^b	3.0 ± 1.0 ^a	0.04 ± 0.01 ^a
	Prefledgling	30-45	(6)	0.08 ± 0.04 ^b	0.27 ± 0.09 ^c	9.0 ± 2.0 ^b	0.11 ± 0.06 ^b
	Fledgling	55-70	(4)	0.11 ± 0.01 ^c	0.14 ± 0.06 ^b	6.0 ± 2.0 ^c	0.03 ± 0.01 ^a
	Chick mean		(5)	0.08 ± 0.03 ^b	0.19 ± 0.07 ^b	9.0 ± 4.0 ^b	0.06 ± 0.04 ^c
Feather	Downy young	1-9	(6)	0.12 ± 0.13 ^a	0.23 ± 0.08 ^c	15.0 ± 2.0 ^d	0.23 ± 0.05 ^d
		12-24	(9)	0.12 ± 0.13 ^a	0.09 ± 0.01 ^a	2.0 ± 1.0 ^a	0.13 ± 0.03 ^a
	Prefledgling	30-45	(6)	0.14 ± 0.05 ^a	0.15 ± 0.06 ^b	7.0 ± 2.0 ^b	0.19 ± 0.03 ^b
	Fledgling	55-70	(4)	0.26 ± 0.09 ^b	0.56 ± 0.19 ^c	19.0 ± 7.0 ^c	1.19 ± 1.20 ^c
	Chick mean		(5)	0.17 ± 0.02 ^a	0.33 ± 0.05 ^d	14.0 ± 4.0 ^d	0.61 ± 0.07 ^d
Whole body	Downy young	1-9	(6)	0.17 ± 0.09 ^a	0.30 ± 0.20 ^d	13.0 ± 16.0 ^d	0.54 ± 0.71 ^d
		12-24	(9)	1.14 ± 0.31 ^c	1.44 ± 0.42 ^e	24.0 ± 8.0 ^e	2.65 ± 0.95 ^e
	Prefledgling	30-45	(6)	0.08 ± 0.01 ^a	0.16 ± 0.03 ^a	6.0 ± 2.0 ^a	0.07 ± 0.03 ^a
	Fledgling	55-70	(4)	0.05 ± 0.01 ^b	0.09 ± 0.01 ^b	3.0 ± 1.0 ^b	0.04 ± 0.01 ^b
	Chick mean		(5)	0.11 ± 0.04 ^c	0.24 ± 0.11 ^c	11.0 ± 5.0 ^c	0.23 ± 0.14 ^c
Adult			(4)	0.08 ± 0.01 ^a	0.11 ± 0.05 ^d	6.0 ± 2.0 ^a	0.13 ± 0.03 ^d
			(5)	0.08 ± 0.03 ^a	0.14 ± 0.07 ^a	6.0 ± 6.0 ^a	0.10 ± 0.10 ^d
			(5)	0.17 ± 0.05 ^d	0.24 ± 0.07 ^c	10.0 ± 2.0 ^c	0.55 ± 0.16 ^e

^a The metal concentrations in muscle, bone and feather were calculated from the weight of positions of each tissue and its metal concentration. Means within each metal not followed by the same letter are significantly different ($p < 0.05$) (one-way analysis of variance of the concentration)

^b ng/wet g

as well as food intake varied widely with growth. We have already reported the detailed distribution of heavy metals in organs and tissues of the

EGWE, and the nature of the organ(s) of birds to be selected for ecological comparison was also discussed (Honda *et al.* 1985).

This report describes the concentration levels of metals (Fe, Mn, Zn, Cu, Ni, Pb, Cd, Hg) in various organs and tissues of the EGWE collected in Korea and age-related changes related to the developmental processes of the organs and tissues.

Materials and Methods

Field examinations of EGWE were carried out at the breeding site covering an area of 1.2 ha on a hill near a small village, 2 km away from the Cheonan City, Chungcheongnando, central Korea, which is far from industrial areas. Three adult EGWEs were captured by slingshot every month from April to August 1981, and three chicks of the age group 2–5 days were captured from different nests. Collecting the EGWE in larger numbers was avoided since protection and management of this species is mandatory. The age estimation of adult egrets was made by the color and shape of the beaks. After weighing, the specimens were immediately frozen at -20°C until subject to necropsy and other measurements.

Twenty five chicks and 5 adult males of age 2 yr collected from April to August were selected for metal analysis. The remainder of the specimens were shared for preservation in the museum and for the analysis of organochlorine compounds (Min *et al.* 1984b). The specimens selected for metal analysis were dissected, and the bone, skin, feathers, liver, kidney, brain, heart, lungs, and muscle were weighed separately. The bone samples of sternum, humerus, cervical vertebrae, femur, and tibia were analyzed after carefully removing the adhering muscles and ligaments. The surface of the bone samples was gently washed with distilled water, dried by filter paper and weighed before analysis. The muscles from the pectoral and femoral regions were weighed separately. Three types of feathers were collected: the remiges, coverts, and abdominals. The feather samples were rinsed thoroughly in tap water, distilled water, and acetone, and dried at 20°C . For moisture determination, all the samples were dried at 80°C for 12 hr. The lipid content was measured by Soxhlet ether extraction.

For the analysis of Fe, Mn, Zn, Cu, Ni, Pb, and Cd, the dried tissues except bones were homogenized, and 1- to 10-g of samples were digested to a transparent solution with a mixture of nitric, perchloric, and sulfuric acids in a Kjeldahl flask (200 ml volume). The bone samples were digested in a nitric and perchloric acid mixture. The resultant solutions were diluted to a known volume with deionized water and transferred to acid-washed test tubes with Teflon screw-caps. The concentrations of Fe, Mn, and Zn were determined by flame atomic absorption spectrophotometer (Model Shimadzu AA-670). Cu, Ni, Pb, and Cd were measured after methyl isobutyl ketone-diethyl dithiocarbamate treatment by flame atomic absorption spectrophotometry (Honda *et al.* 1982). Corrections for background interference were made by running appropriate blanks. The accuracy of this method has been determined in this laboratory by spiking bones and other tissues of dolphin with known amounts of Fe, Mn, Zn, Cu, Ni, Pb, and Cd with recoveries of 90.0 to 99.9%.

Mercury was determined by cold vapor atomic absorption spectrophotometry (Honda *et al.* 1983). It consisted of the mineralization of samples with a nitric, perchloric, and sulfuric acid mixture in a flask equipped with a Liebig condenser and followed by potassium permanganate (KMnO_4) digestion. The excess of KMnO_4 was reduced with a 20% hydroxylamine hydrochloride solution and the mercury to Hg^0 with tin(II)chloride.

Determinations were made with a Shimadzu AA-670 spectrophotometer, and the limit of quantification for Hg was 0.005 ppm on a wet-weight basis.

Results and Discussion

Organ and Tissue Distribution of Metals

Table 1 shows the mean concentrations of metals in organs and tissues of the chick and adult EGWEs. In general, high concentrations in the adults were found in the liver, kidney, skin, bone, and feathers, and low concentrations in the brain and muscle. These distribution patterns agreed with those in many other bird species (Tatsukawa *et al.* 1974; Finley and Stendell 1978; Furness and Hutton 1979; Osborn *et al.* 1979; Cheney *et al.* 1981; Nicholson 1981; Delbeke *et al.* 1984). In particular, a low concentration of metals in the brain may be explained by the "blood-brain barrier" (Okada 1978), although the mechanism is not clear. But their tissue distribution showed some discrepancies and metal-specific accumulations. The concentrations of Fe, Mn, Cu, Cd, and Hg were relatively high in the liver and kidney, but not with Zn, Ni, and Pb. A relatively high accumulation of Cu was observed in the brain and feathers and it has been reported that Cu in the brain is associated with Cu-protein as albuminopretein-I and -II (Kimura and Araki 1981). Furthermore, high accumulations of Mn, Zn, Ni, Pb, and Hg were found in the hard tissues such as bone, skin, and feathers. High accumulations of these metals are found in hard tissues of upland and sea birds (Tatsukawa *et al.* 1974; Kitamura *et al.* 1976; Osborn *et al.* 1979; Hulse *et al.* 1980; Cheney *et al.* 1981). According to Underwood (1971), Zn and Pb are stored in bone with ossification and Mn, Zn, and Ni are related to the pigmentation of feathers. However, the chemical forms of these metals in the hard tissues are not adequately understood.

The distribution patterns of the metals in organs and tissues in the adult birds were similar to those found in EGWE chicks. In the case of the chicks, Cu in liver, and Pb and Ni in the soft tissues such as brain, liver, and kidney showed comparatively high concentrations when compared to the adults.

Organ and tissue burdens of the metals were calculated from the weight of organs and tissues and their metal concentrations, and the results are expressed as percentage of organ and tissue burden to total body burden (Table 2).

In adult EGWE, relatively high percentages of the metal burdens in the whole body generally were present in the feathers, which comprised only

Table 2. Percentages (mean \pm SD) of organ and tissue burdens to total body burdens of the metals in the chick and adult egrets, *Egretta alba modesta*

	Weight organs & tissues	Fe	Mn	Zn	Cu
Chick (N = 25)					
Brain ^a	1.02 \pm 0.48	0.43 \pm 0.13	0.22 \pm 0.09	0.31 \pm 0.05	0.87 \pm 0.45
Liver	5.05 \pm 2.01	16.0 \pm 5.36	9.77 \pm 4.39	3.13 \pm 1.07	20.4 \pm 14.9
Kidney ^a	1.57 \pm 0.68	2.38 \pm 0.88	0.91 \pm 0.25	0.62 \pm 0.41	1.71 \pm 1.00
Muscle	35.0 \pm 3.85	31.6 \pm 4.39	29.4 \pm 4.99	27.2 \pm 3.84	23.3 \pm 5.93
Bone	15.9 \pm 7.54	20.7 \pm 6.80	26.0 \pm 13.9	44.5 \pm 4.44	9.98 \pm 4.72
Skin ^a	9.18 \pm 1.95	7.03 \pm 1.87	7.07 \pm 2.01	4.96 \pm 1.22	9.88 \pm 1.11
Feathers	9.30 \pm 4.46	9.69 \pm 6.44	14.4 \pm 24.2	19.7 \pm 5.06	21.0 \pm 14.7
Adult (N = 5)					
Brain	0.53 \pm 0.05	0.22 \pm 0.08	0.96 \pm 1.83	0.16 \pm 0.02	0.49 \pm 0.10
Liver	2.74 \pm 0.41	9.60 \pm 2.13	5.88 \pm 1.91	1.92 \pm 0.37	8.08 \pm 2.33
Kidney	0.84 \pm 0.17	1.56 \pm 0.40	0.82 \pm 0.28	0.35 \pm 0.05	0.90 \pm 0.23
Muscle	42.1 \pm 2.49	30.6 \pm 2.54	11.0 \pm 3.29	26.0 \pm 1.90	35.3 \pm 3.09
Bone	22.4 \pm 2.62	17.0 \pm 1.52	30.2 \pm 3.90	40.1 \pm 1.40	5.10 \pm 1.86
Skin	6.47 \pm 0.69	10.7 \pm 1.68	11.1 \pm 6.45	4.99 \pm 0.80	9.97 \pm 3.68
Feathers	9.89 \pm 0.81	12.6 \pm 4.48	35.4 \pm 13.3	19.2 \pm 1.79	34.4 \pm 2.87

Table 2. Percentages (mean \pm SD) of organ and tissue burdens to total body burdens of the metals in the chick and adult egrets, *Egretta alba modesta*

	Ni	Pb	Cd	Hg
Chick (N = 25)				
Brain ^a	0.80 \pm 0.31	0.83 \pm 0.46	1.12 \pm 0.80	0.58 \pm 0.14
Liver	2.01 \pm 1.23	2.63 \pm 2.10	4.77 \pm 1.55	9.30 \pm 4.79
Kidney ^a	0.76 \pm 0.42	0.88 \pm 0.36	2.05 \pm 0.99	2.06 \pm 0.75
Muscle	29.1 \pm 5.17	19.8 \pm 11.0	19.7 \pm 6.80	19.8 \pm 7.82
Bone	20.9 \pm 12.4	22.7 \pm 5.48	22.2 \pm 8.79	7.55 \pm 1.07
Skin ^a	16.6 \pm 8.62	11.6 \pm 6.32	15.4 \pm 12.5	7.55 \pm 2.33
Feathers	24.2 \pm 9.13	36.1 \pm 21.3	21.9 \pm 15.8	48.3 \pm 12.4
Adult (N = 5)				
Brain	0.11 \pm 0.08	0.13 \pm 0.09	0.13 \pm 0.11	0.47 \pm 0.28
Liver	0.52 \pm 0.29	0.70 \pm 0.43	19.6 \pm 1.67	7.00 \pm 2.64
Kidney	0.16 \pm 0.16	0.29 \pm 0.07	14.6 \pm 1.67	1.20 \pm 0.43
Muscle	8.45 \pm 3.75	9.28 \pm 4.61	15.3 \pm 2.88	20.2 \pm 7.55
Bone	16.8 \pm 11.8	22.8 \pm 6.97	12.1 \pm 7.28	7.96 \pm 1.73
Skin	8.30 \pm 4.64	4.89 \pm 0.98	7.54 \pm 5.07	5.57 \pm 2.62
Feathers	44.3 \pm 7.74	48.0 \pm 10.0	22.3 \pm 6.24	55.8 \pm 12.7

^a Samples analyzed N = 19

about 10% of the body weight, especially with Hg, Ni, Pb, and Mn. This means that a majority of these metals in a bird's body is excreted by moulting. Cd and Cu burdens in liver and Cd in kidney were also comparatively high. In contrast to the adults, the chicks showed relatively high burdens of the metals in the muscle and liver, and very low burdens of Mn and Ni in the feathers. Among the soft tissues of the chick, hepatic Cu was at a comparatively high level, while hepatic and renal Cd showed relatively low levels. These observations suggest that accumulation of metals vary with developmental processes of organs and tissues, and the details are discussed in a later section.

Age-related Accumulations of Metals in the Whole Body

The average concentrations of metals in the whole bodies of the chick and adult EGWEs were calculated from the weight of organs and tissues and their metal concentrations, and statistical comparisons were made to ascertain relationships between the metal content and the growth stage. Based on age, growing feathers, and observations on standing, flapping and flying practices, each individual was grouped into five stages of growth, namely younger (1st–9th day) and older (12th–24th day) of downy young, pre fledgling, fledgling, and

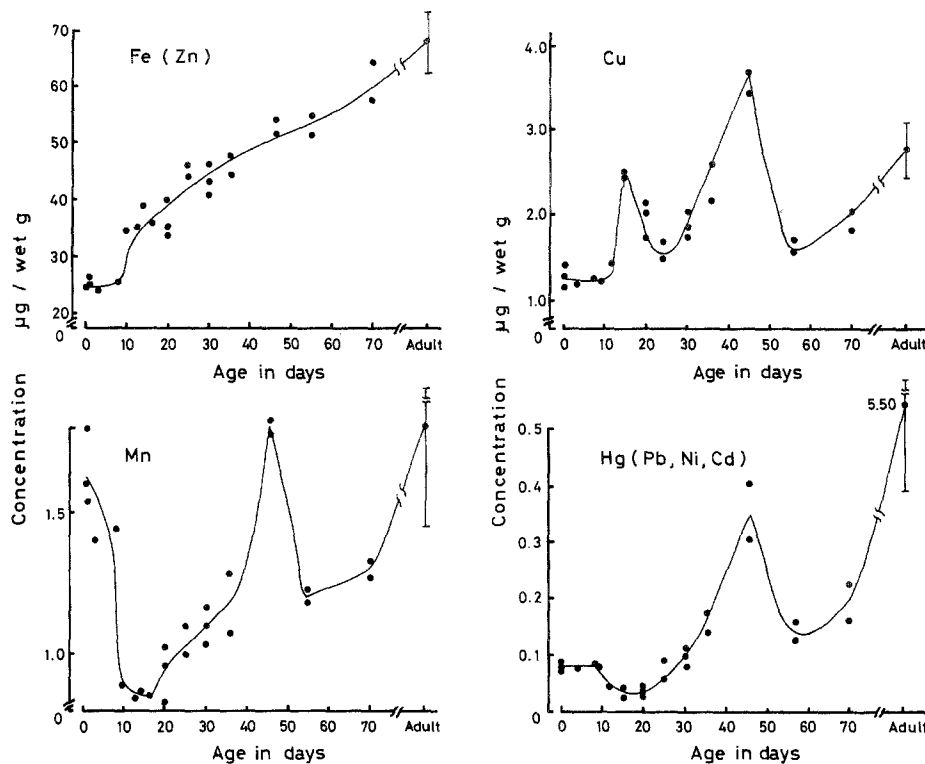


Fig. 1. Age-related changes of Fe, Mn, Cu and Hg concentrations in whole bodies of the chicks and adults of *Egretta alba modesta*. Zn showed a similar trend to Fe, and Pb, Ni, and Cd showed similar trends to Hg (indicated in brackets)

adult. One-way analysis of variance was made of the concentration for each metal. The data for both males and females were pooled.

The results (Table 1) indicated that the differences between growth stages were statistically significant for most of the metals. The adult egrets showed significantly higher concentrations of all the metals as compared to the chicks, indicating an accumulation of these metals with growth. Among growth stages of the chick, the lowest concentrations of Mn, Ni, Pb, Cd, and Hg were found in the downy young of 12th–24th day. Significant differences ($p < 0.01$ or 0.05) of the metals also were found between pre fledgling and fledgling: the former showed higher concentrations of most of the metals as compared to the latter, while the opposite was found with Fe and Zn. Furthermore, when the whole body concentrations and total burdens of metals were plotted against age, there was noted a change in the accumulation patterns. The age-related changes of concentrations and burdens of Fe, Cu, Mn, and Hg are shown in Figures 1 and 2.

Generally, the whole body concentrations of metals increased with age until the 45th day, corresponding to just before fledgling time, but afterward sudden decreases of the metal concentrations were found, and the decrease of the total body burdens of the metals indicated excretions of these metals.

In this connection, relatively high burdens of the metals in the pre fledgling (the 45th day) were found in the feathers; in particular, Hg in the feathers contributed more than 50% of the body burden (Table 2). Moreover, moulting was seen in the fledgling (the 55th day and the 70th day), and their total primary moult scores (according to Ashmole 1962) averaged 32 for the 55th day and 48 for the 70th day, respectively. These observations indicate that sudden decreases of the concentrations and burdens of Mn, Cu, Ni, Pb, Cd, and Hg in the fledgling birds were due to the excretion of these metals via moulting. In contrast, the burdens of Fe and Zn in the feathers were below 20% of the body burden (Table 2), and hence their excretions by moulting were also insignificant.

Furthermore, chicks during the period of hatching to the 45th day showed specific accumulations of the metals. A marked increase of all metal concentrations was observed through the period of 30th–45th day. The chicks during the period of 10th–15th day also showed relatively large increases of Fe, Zn, and Cu concentrations, while the concentrations of Ni, Pb, Cd, and Hg decreased. A rapid decrease was also noticed for Mn concentration from the hatching to 10th day (Figures 1 and 2).

With chicks, the increased rates of the body weight in the standing and flapping phases were

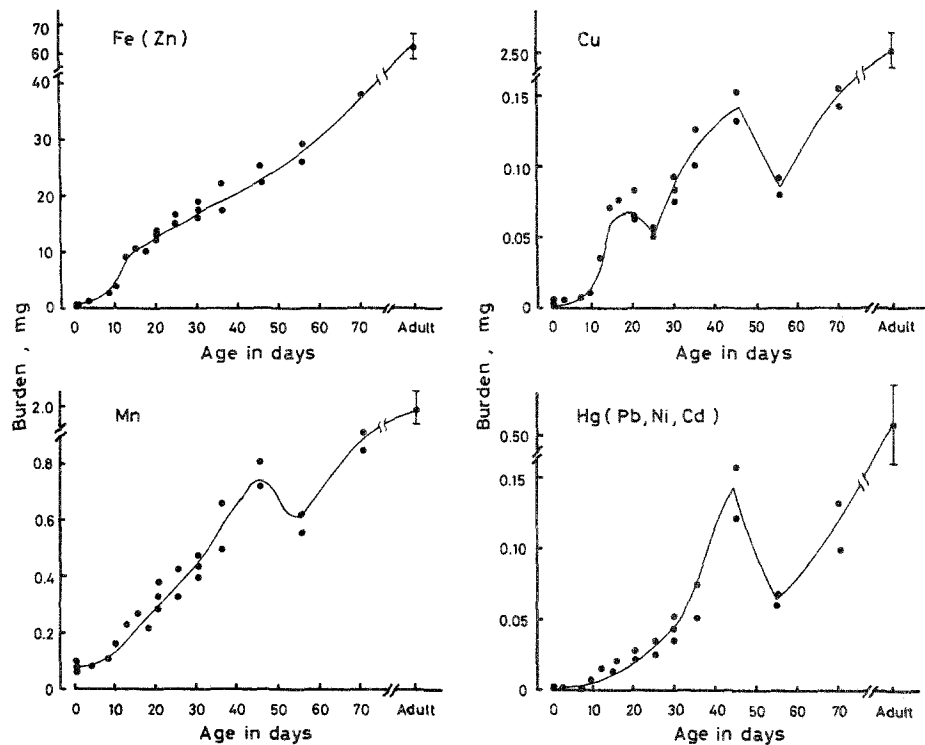


Fig. 2. Age-related changes of Fe, Mn, Cu and Hg burdens in whole bodies of the chicks and adults of *Egretta alba modesta*. Zn showed a similar trend to Fe, and Pb, Ni, and Cd showed similar trends to Hg (indicated in brackets)

generally much higher than those in the fledgling phase (Figure 3), and the food intake was higher up to the 40th day (the end of the flapping phase) compared with the later stages, as reported by Min *et al.* (1984a). Similar tendencies have been found with other bird species (Kuroda 1959, 1964a, 1964b; Diamond 1976; Round and Swann 1977; Cannings and Threlfall 1981; Grant 1982). However, observation showed that the increase of the body weight observed during the period of 19th–28th day was less notable than before the above period, which might be due to a greater expenditure of energy attributable to the standing practice, as seen in rapid decreases of the whole body lipid and moisture contents (Figure 4). After this period, the body weight increased during the period of 30th–37th day corresponding to flapping phase, and also the body lipid contents rapidly increased, along with continued decreases in moisture content. Moreover, the first flight of chicks was made at the 40th day, and a great consumption of energy for flapping and flying practices seems to be the reason for the decrease of both the body weight and the body lipid content during the period from the 37th–45th day. Observations on the biometric data of the chicks indicate that relatively large increases of Fe, Zn, and Cu concentrations during the period of the 10th–15th day were related to the rapid growth of

the body weight, and also that rapid decreases of Pb, Ni, Cd, and Hg concentrations noted during this period were due to a dilution by increased body weight. Marked increases of all the metal concentrations during the period of pre-fledgling can be explained by decreased body weight, and also a very slight change of Pb, Ni, Cd, and Hg burdens during the period of younger stage of the downy young suggests a relatively faster accumulation plateau of these metals compared to the other stages of the chick egret growth.

Organ and Tissue Accumulations of Metals with Age

The results (Table 1) indicate that most of the metals examined in various organs and tissues were significantly ($p < 0.01$ or 0.05) higher in the adults than in the chicks, but Ni and Pb in brain, muscle, liver, kidney and skin, and Cu in liver showed lower concentrations in the adults. Furthermore, differences of the metal concentrations by growth stages of the chicks were statistically significant for most of the examined organs and tissues. The metal concentrations were plotted against age to determine if there was a relationship between the metal content and the developmental process of organs and tissues.

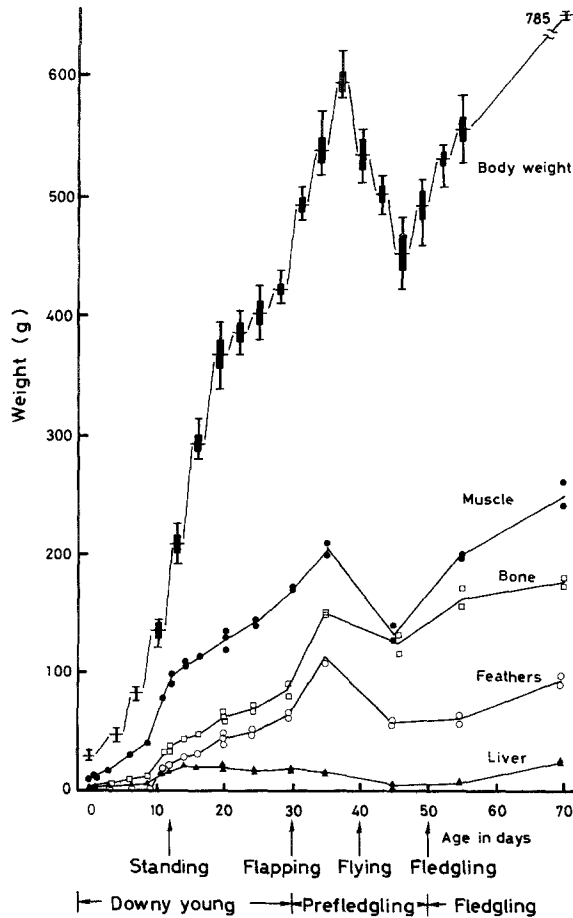


Fig. 3. Changes in body weight and various organs and tissues weights in the chicks of *Egretta alba modesta*. The vertical line, horizontal line and block indicate the range, mean, and standard error, respectively

Although the age-related accumulations of Fe, Mn, Zn and Cu in the muscle, liver, bone and feathers were metal- and organ-specific (Figures 5 and 6), they could be grouped conveniently into seven patterns. The results obtained from the brain, kidney, and skin were not discussed, because of lack of data on the younger stage of the downy young.

One of the age-related accumulations, which was found with Fe in the liver and with Zn and Cu in the muscle, is as follows: their concentrations were considerably higher in the chicks soon after hatching, decreased through the downy young periods, and were followed by an increase with age until the 45th day. With Fe, hepatic Fe consists mainly as hemosiderin and ferritin and it is also supplied to other tissues according to the requirements of the respective tissue. Since the downy young EGWEs showed rapid increases of organ and tissue weights (Figure 3), the age-related changes of Fe concentration in the liver can be explained mainly by accumulation and redistribution of hepatic Fe with the developmental processes.

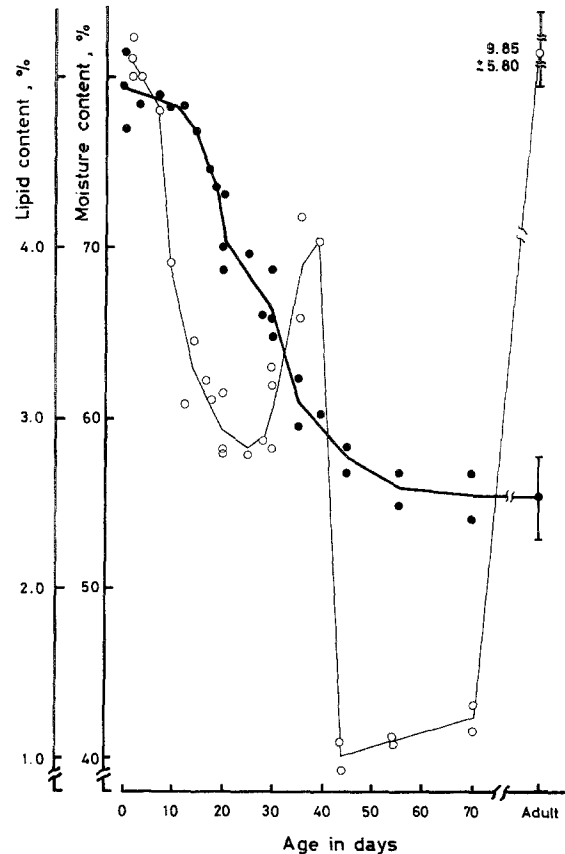


Fig. 4. Age-related changes of whole body lipid (○) and moisture contents (●) in the chicks and adults of *Egretta alba modesta*. The vertical line, shown in adult egret, indicates standard deviation

The age trends with Fe, Mn, Zn, and Cu in the feathers showed an increased concentrations with age during the period of 10th–45th day corresponding to the growing feathers, and followed by decreased concentrations. The increased metal concentrations with growth of feathers indicate that the metals participate in the keratinization process. However, relatively high accumulations of Fe, Mn, Zn, and Cu were also found in the period of 10th–15th day. The feathers during this time contained relatively high moisture and blood contents, increasing Fe, Mn, Zn, and Cu concentrations rapidly.

The age-related accumulation, with Fe in the muscle and with Zn and Mn in the bone, showed increased concentrations with age throughout all the chick periods. It is known that muscular Fe is present mainly as myoglobin-Fe (Mb-Fe) (Comar and Bronner 1982). Mb content of the muscle also increases with growth of muscle (Underwood 1971) and the linear increase of Fe concentration in the muscle can be accounted for by an accumulation of muscular Mb-Fe. Furthermore, according to our previous study, the concentrations of Mn and Zn in several positions of the chick bone correlated posi-

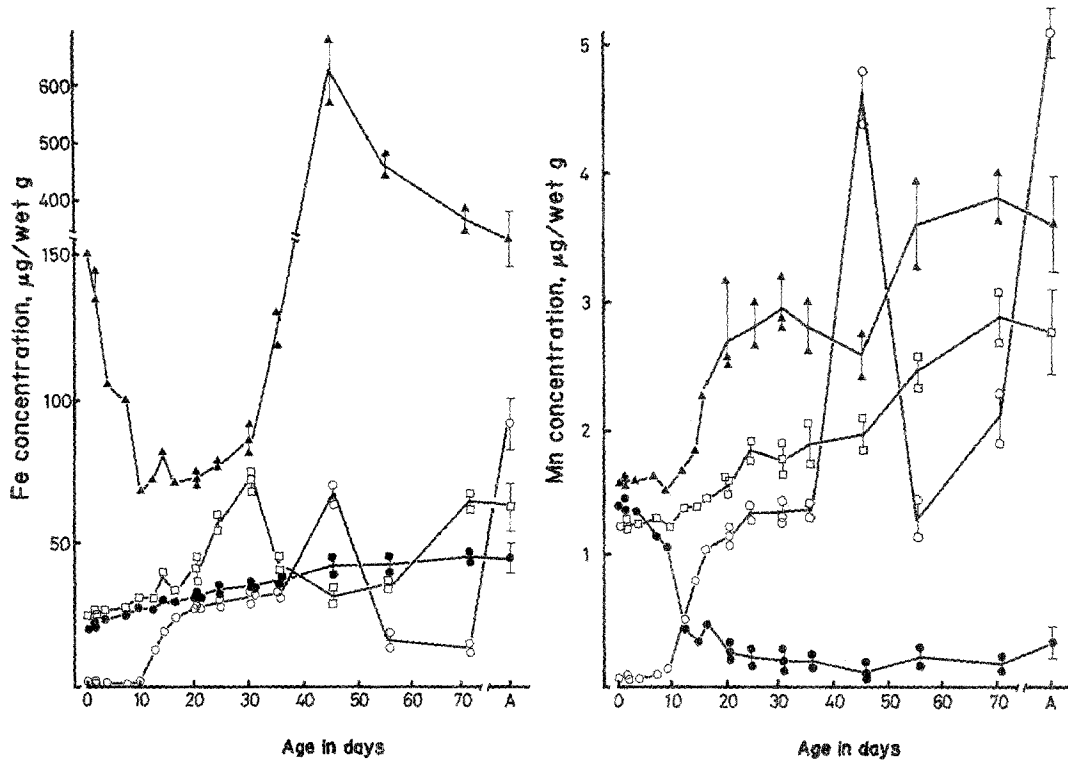


Fig. 5. Age-related changes of Fe and Mn concentrations of liver (▲), muscle (●), feathers (○) and bone (□) of the chicks and adults of *Egretta alba modesta*. A, adult (mean ± standard deviation)

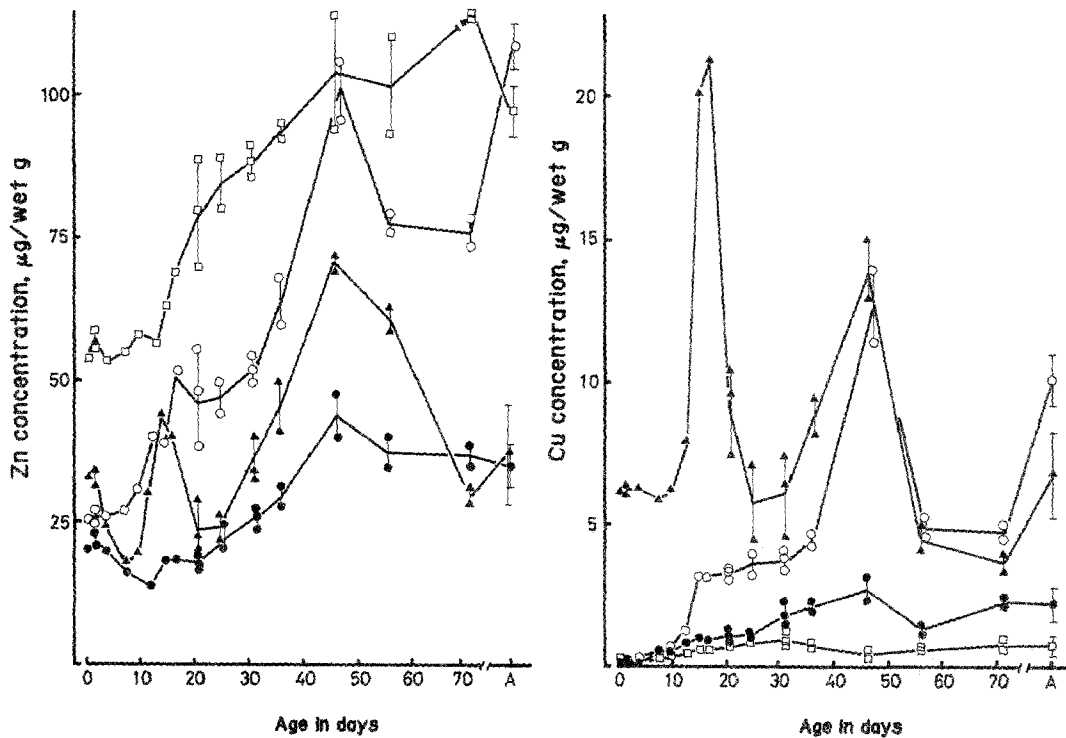


Fig. 6. Age-related changes of Zn and Cu concentrations of liver (▲), muscle (●), feathers (○) and bone (□) of the chicks and adults of *Egretta alba modesta*. A, adult (mean ± standard deviation)

tively with the concentration of Ca in the bone (Honda *et al.* 1985). Therefore, an accumulation of Mn and Zn in the chick bone also can be explained in relation to ossification.

The Fe and Cu of bone, increased with age during the period of downy young, decreased during the period of pre fledgling and fledgling, followed by a gradual increase with age up to the adult

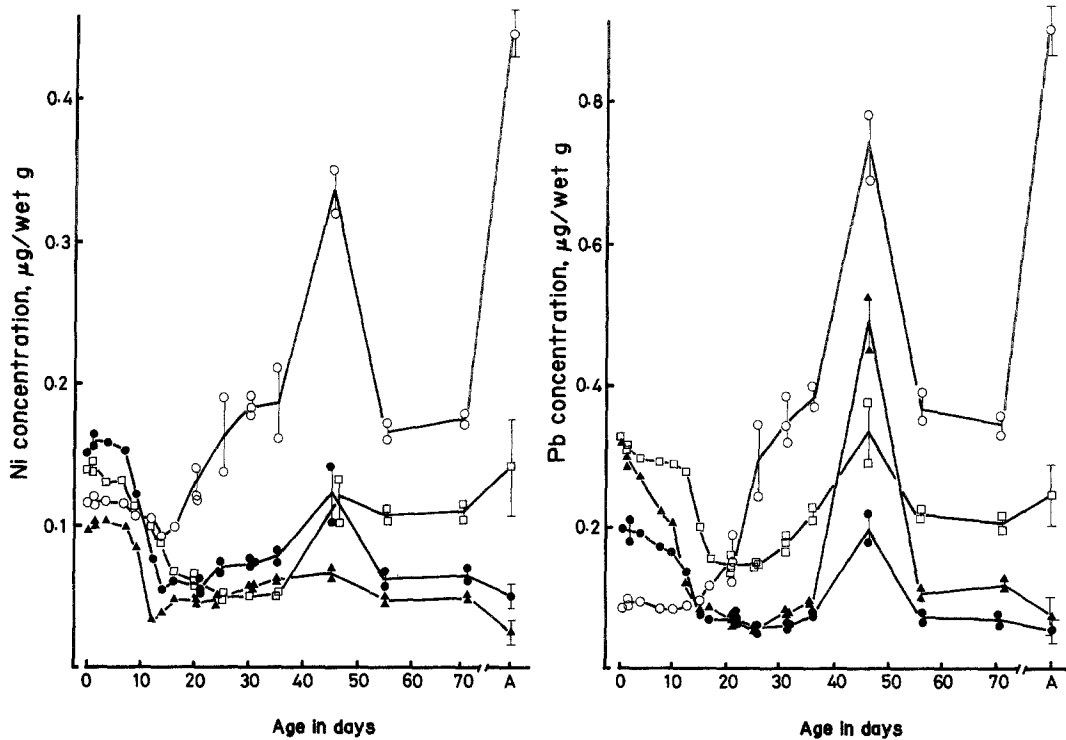


Fig. 7. Age-related changes of Ni and Pb concentrations of liver (▲), muscle (●), feathers (○) and bone (□) of the chicks and adults of *Egretta alba modesta*. A, adult (mean \pm standard deviation).

level. Visual observation also showed that the bone became mildly red during nestling (the 15th day) and reached a bright red at the 30th day, which probably means an increase of blood content in bone marrow. This may account for the increase of Fe and Cu concentrations in the bone.

The remaining trends are as follows: the hepatic Mn, the muscular Mn and the hepatic Cu and Zn. The concentration of Mn in the liver increased rapidly during the period of 10th–15th day, subsequently constant until the 45th day and decreased suddenly at fledgling time. This trend was in contrast to that of the hepatic Fe concentration. It is known that Mn and Fe act antagonistically and Mn inhibits gestational absorption of Fe in the rat and rabbit (Yamane *et al.* 1980). In the present study, the reverse trend of both metals in the liver also suggest an antagonistic action of Mn to hepatic Fe accumulation. The concentration of Mn in the muscle was the highest in the chick soon after hatching, decreased with age until about the 15th day; thereafter, it remained constant. Furthermore, the hepatic Cu and Zn showed two peaks in concentration, and each peak was found during the 10th–15th day period (rapid increase of liver weight, Figure 3) and on the 45th day. These two peaks also correspond to the beginning of the first moulting and just before the second moulting, respectively.

Several workers reported that the high concentrations of Cu and Zn in the liver during moulting and/or just before moulting were found in some bird species, such as the house sparrow, *P. domestica*, and starling, *S. vulgaris* (Haarakangas *et al.* 1974; Osborn 1979). Moulting requires a great energy expenditure, as the weight of the plumage increases by approximately 70% (Barnett 1970), and keratin is produced to the extent of about 5% of the body weight; in addition, the decrease of insulation at the beginning of moult leads to increased metabolism (King and Farner 1967). At this time, thyroid activity begins to rise (King and Farner 1967) and correlates positively with the progress of the moult (Kendeigh and Wallin 1966; Voitkevich 1966; Ling 1972). Both processes increase the activity of cytochrome oxidase, which is known to be a cuproenzyme (Frieden 1968). Copper is also essential for pigmentation (Voitkevich 1966) and it is assumed that a copper-containing enzyme participates in the keratinization process (Braun-Falco 1958). The feathers of EGWE contained comparatively large amounts of Cu, as mentioned above. The liver acts as the main reserve of Cu (Evans *et al.* 1970; Owen and Orvis 1971; Hyvarinen 1972) and thus the high content of Cu in the liver is possible in light of the above.

According to Supplee *et al.* (1958), Zn is required by poultry for normal feather development and Zn

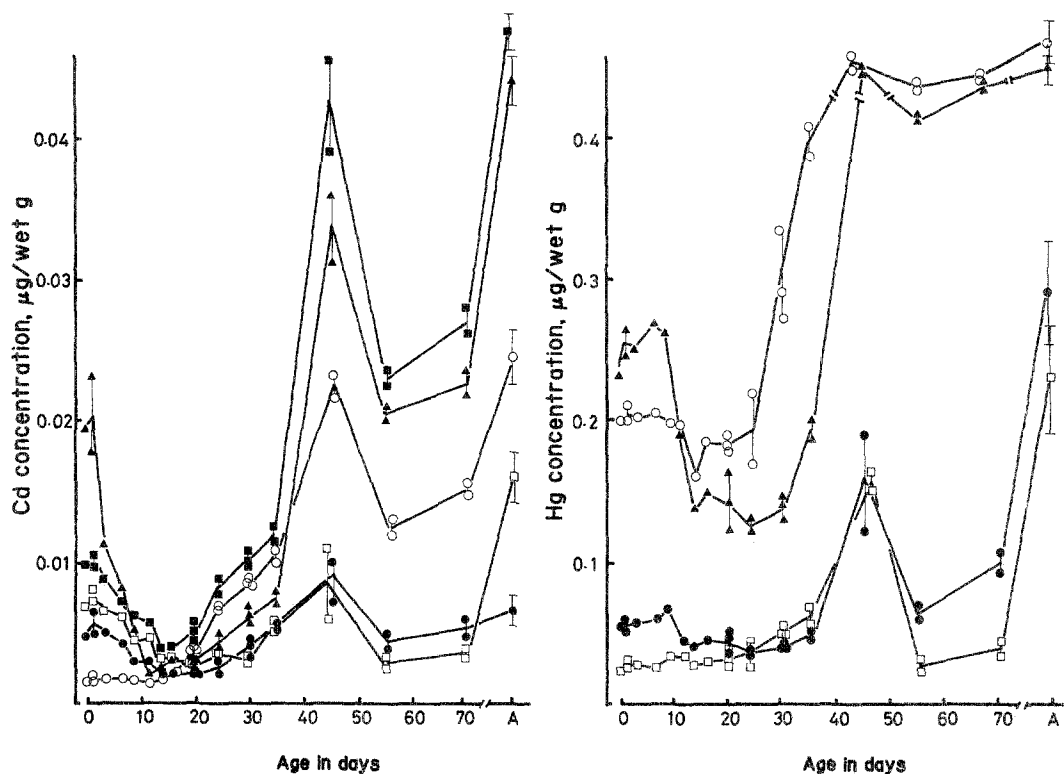


Fig. 8. Age-related changes of Cd and Hg concentrations of liver (▲), muscle (●), kidney (■), feathers (○) and bone (□) of the chicks and adults of *Egretta alba modesta*. A, adult (mean \pm standard deviation)

deficiency is manifested in a frayed feather condition (Sunde 1972). Sunde (1972) also indicated that the period when a large amount of Zn must be present in poultry lasts one week around the onset of the moulting. As mentioned earlier, the content of Zn in the liver correlates positively with high protein synthesis periods and the feathers of the egret also contain Zn, in an average of 127 $\mu\text{g/wet g}$ at the 45th day period. The concentrations of Zn in the liver in both the 10th–15th day and 45th day, at the beginning and just before moulting, respectively, were double that found in the livers during the other chick periods.

In contrast to Fe, Mn, Zn, and Cu, depending on the metabolic turnover, the accumulations of Ni, Pb, Cd and Hg in the organs and tissues increased with age (Figures 7 and 8). This indicates that age or exposure time is a dominant factor for metal accumulations in birds. However, decreases of Ni, Pb, Cd, and Hg concentrations in the organs and tissues were found in the downy young, due to dilution by increased body weight, but not significant in the feathers. Marked decreases of the concentrations of Ni, Pb, Cd, and Hg in all the tissues also were observed in the chicks at fledgling time, especially Hg. These decreases can be explained by moulting, as mentioned above. Furthermore,

changes of organ and tissue distribution of Ni, Pb, Cd and Hg in the chick body were found between the younger stage of downy young and the older ones: *e.g.*, the former (hatching–10th day) showed higher concentrations of Pb and Hg in the liver than in the feathers, and also a higher concentration of Cd in the liver than in the kidney.

The results indicate that consideration of the growth stage of organs and tissues is essential for understanding the bioaccumulation processes and also for the ecotoxicological effects of the metals in birds. In particular, the fact that the EGWE showed a fast uptake and accumulation of Ni, Pb, Cd, and Hg in the younger stage of the downy young, and rapid increases of the concentrations of these metals in the organs and tissues of the pre fledglings indicate that these two growth stages of the egret chicks may be critical for toxicities of the metals. Furthermore, excreting metals by moulting is a good protection system for birds against toxic metals, and should be considered when trying to understand the bioaccumulation process of metals in birds.

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