Technical Memorandum 36

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Brennan, K.G., Noller, B.N., LeGras, C., Morton, S.R. & Dostine, P.L. (1991). Heavy metals in waterbirds from the Magela Creek flood plain, Alligator Rivers Region, Northern Territory, Australia. Technical Memorandum 36, Supervising Scientist for the Alligator Rivers Region.

Baseline information is provided on the pre-mining levels of cadmium, chromium, copper, lead, manganese, nickel and zinc in the primary feathers, pectoral muscle, and liver of 22 species of waterbirds that occur commonly on the Magela Creek flood plain. The ranges of mean metal concentrations (geometric mean, μg/g dry weight) in liver were: cadmium 0.013-1.3 μg/g (black-winged stilt [species with highest mean cadmium concentration in liver]), copper 51-2500 μg/g (green pygmy goose), lead 0.015-0.43 μg/g (comb-crested jacana), manganese 19-61 μg/g (little pied cormorant) and zinc 260-630 μg/g (wandering whistling duck). The Pacific black duck had the highest total metal content in liver.

Metal concentrations in the three tissues were not equivalent in any species, but the overall pattern of variation was similar for all species for each metal. Concentrations in feathers were lower than for liver (see above) but higher than in muscle. No quantitive relationship was found between metal levels in different tissues, nor was any pattern of variation found between species - related to sexual, seaonal or dietary factors. The tissue and organ concentrations of most metals were comparable with, or lower than, metal levels reported from waterfowl in 'pristine' environments elsewhere. Cadmium and lead levels were very low but zinc concentrations were found to be high relative to other literature values. The problems of determining the sources of accumulated metals in waterbirds are briefly discussed.

Feathers have proved poor predictors of metal levels in either muscle or liver, furthermore it has been clearly demonstrated that concentrations in feathers may not reflect the degree of physiological exposure. So the use of feathers as monitors of metals in waterbirds is very limited.

The waterfowl species utilised as food by Aboriginal people contained metal levels either within prescribed NH & MRC food standards or, where they exceeded them (i.e. copper in green pygmy goose liver), the possibility of a health risk to humans is considered remote.

1 INTRODUCTION

1.1 Background

The Ranger Uranium mine lies within, but is excluded from, Kakadu National Park in the Alligator Rivers Region of the Northern Territory, Australia. The Magela Creek flood plain lies immediately downstream of the Ranger Uranium mine and is adjacent to the presently undeveloped Jabiluka uranium and gold deposit. Extraction of uranium during the mining process can mobilise other metals such as lead and cadmium which are disposed of in tailings. If these wastes escaped, either accidentally or through poorly planned releases, they would be washed into the Magela Creek flood plain and stored in it for long periods, with severe environmental consequences (Hart et al. 1986).

The Magela Creek wetland is also part of Kakadu National Park. It forms part of an extensive system of flood plains throughout the Alligator Rivers Region, which collectively support large and diverse floral and faunal assemblages. These wetlands were granted World Heritage status in 1987. One of the striking biological features of the flood plains is the waterbird population; huge flocks gather at the end of the Dry season, when water and food resources elsewhere are diminished. It is believed that the flood plains of Kakadu provide important refuge areas critical to the conservation of waterfowl throughout the Top End of the Northern Territory (Morton et al. 1989).

The Park also supports several resident groups of Aboriginal people who lead a semi-traditional lifestyle using the flood plains as a source of traditional foods; these foods include the waterbirds, in particular the ducks and geese. Aboriginal groups living adjacent to the Magela flood plain have expressed concern about the safety of these foods now that a mine operates nearby.

1.2 Birds as monitors of environmental contamination

It is difficult to use waterbirds as monitors of environmental change. However well individual species may accumulate metals in any organ or tissue, the inherent mobility of most species makes it almost impossible to determine just where the metals were accumulated without using labelled precursors. In Australia waterbirds undertake massive, cyclical population shifts throughout the whole of the Alligator Rivers Region, if not the entire Top End of the Northern Territory (Morton et al. 1989), in response to seasonal abundances and shortages of food. To establish which species are resident in particular areas would first involve a large banding program and sampling would necessarily involve destruction of some birds; this may not be viewed favourably by either governmental authorites or the public.

1.3 Metal selection

A number of metals have been identified as associated with uranium mining or milling (Fox et al. 1977, Noller et al. 1985); these would be of concern if transferred to surrounding environments. The metals chosen for this study are all known to be present in mine/milling wastes at elevated levels relative to background; some are also known to be bio-accumulated in birds.

1.4 Tissue/organ selection

In birds the liver is considered to be the organ to give the best indication of change in exposure, via uptake from food, to heavy metals; abnormal increases in metal concentrations in the liver indicate potential adverse effects on health (Roberts 1981). In the Magela Creek situation it is particularly relevant that the liver from waterbirds is also eaten preferentially by Aboriginal people, although muscle is the predominant tissue consumed.

Muscle is not generally recognised as a site of accumulation of heavy metals in birds and by determining levels in muscle it should be possible to allay fears regarding contamination of traditional foods. Feather samples were collected with a view to developing a nondestructive monitoring procedure for the future. The study examined the possibility that metal levels in feathers might be related in a predictable way, to those in liver or muscle.

1.5 Toxicology

The only detailed report of toxic metal levels in Australian waterfowl concerns Bool Lagoon in South Australia, where birds showed signs of acute lead poisoning due to ingestion of lead shot (Koh & Harper 1989). In international literature comprehensive data exist on several metals in a variety of species of wild birds in polluted situations and of birds in laboratory-bred experimental populations. Lead, cadmium, copper and zinc are most frequently reported whereas very little information has been published on the levels of chromium, manganese, and nickel in waterbirds.

Cadmium

Cadmium is known to accumulate throughout the lives of wild animals irrespective of the dose to which they are exposed (Woolf et al. 1982). Kidney and liver tissues store about 90% of the total body burden. In these organs the cadmium binds to a low molecular weight protein, metallothionein (Friberg et al. 1974, Cherian & Goyer 1978, Webb 1979). Cadmium uptake occurs via digestive absorption through the intestinal mucosa - it is dose-dependent; however, expression of toxic effects can depend on the levels of dietary zinc. Animals with zinc deficient diets develop cadmium toxosis at much lower levels of dietary exposure than those on diets with normal or zinc supplemented diets (Friberg et al. 1974, DiGuilio & Scanlon 1984*b*). Cadmium is not transferred into bird's eggs (White & Finley 1978). Scheuhammer (1987) suggests that the liver is the best single organ for monitoring exposure of birds to cadmium because it stores about half the body burden and does not show toxic effects.

Chromium

Chromium is an essential micro-nutrient, important in the metabolism of insulin. Gastro-intestinal dietary uptake in animals other than birds varies from less than 1% to 6% (Langard & Norseth 1979).

Copper

Copper is an essential micro-nutrient and not accumulated under normal circumstances in birds; however, individual birds can accumulate very high tissue concentrations without any apparent deterioration of physiological condition. Honda et al. (1985) showed that a relatively high proportion of the total body copper burden in eastern great white egrets was in the feathers.

Lead

Lead has been the most extensively studied heavy metal in birds. Smelting processes and petro-chemical emissions have led to widespread, low level pollution of many habitats; sport shooting of waterfowl using lead shot is also of increasing concern. The ingestion by ducks and geese of spent pellets along with food can quickly cause lead poisoning or death and has been a recognised problem for nearly a hundred years (Grinnell 1894).

Lead is absorbed by animals as soluble salts with food. Intake of lead in birds is also associated with uptake of calcium and accumulation in female birds is higher than in males during the breeding season when calcium absorption is increased for eggshell production

(Taylor 1970). Inside the body, lead is primarily deposited with calcium in bones but also concentrates in liver and kidney tissues. Dietary lead concentrations of less than 100 μg/g wet do not usually cause detectable effects (Scheuhammer 1987) although sublethal effects of lead have been identified in birds with liver concentrations as little as 1 - 2 μg/g dry (Edens et al. 1976, Dieter 1979).

Manganese

Manganese is an essential micro-nutrient and the few studies reported suggest that it can be efficiently metabolised in birds without noticeable physiological disruption. Studies by Custer et al. (1986) and Howarth et al. (1981), showed that manganese levels in populations of terns from polluted and control sites were not significantly different. Hulse et al. (1980) found that laughing gulls and cattle egrets with high levels of dietary manganese had low tissue concentrations. Honda et al. (1985) found almost half the total body burden in eastern great white egrets in the feathers and it has been suggested that excretion takes place with feathers during moult cycles.

Nickel

Little is known about the mechanisms of uptake, effects, or fate of ingested nickel in birds although it appears to be an essential micro-nutrient (Nriagu 1980). Honda et al. (1985) found about 60% of the total body burden to be in feathers and so its probable excretion during moult cycles was suggested. This was confirmed by Rose & Parker (1982), who noted that mean nickel concentrations in the primary feathers of ruffed grouse increased from 0.8 μg/g dry when new to 2.9 μg/g dry just prior to moulting.

Zinc

Zinc is an essential element. Increasing dietary exposure to zinc does not usually result in accumulation in tissues, as efficient homeostatic processes control its uptake and excretion. Although the mechanisms are incompletely understood, zinc has also been shown to be effective in supressing toxic effects of cadmium (Sandstead 1977) and some of the symptoms of lead poisoning (Thawley et al. 1978).

1.6 Objectives

The objectives of this work were: to determine the naturally occurring levels of cadmium, chromium, copper, lead, manganese, nickel and zinc in the primary feathers, pectoral muscle and liver of some of the common species of waterbirds inhabiting the Magela Creek flood plain; to investigate possible sexual, seasonal and annual differences between heavy metal levels in individual species; to examine differences in metal levels between species and species groups formed on the basis of dietary similarity; to discuss the toxicology of these metals in relation to both the birds and people who may eat them.

Comparison with other studies is difficult because there are no others reporting natural background metal concentrations in the tissues of Australian waterbirds; the species studied in other, mostly temperate, Northern Hemisphere countries have differed from those examined here. Comparative discussions have therefore been restricted to studies focussing on waterfowl ecologically similar to the birds studied here and which were collected from sites regarded as pristine.

2 MATERIALS AND METHODS

2.1 Field collection

Between 1981 and 1984 some 700 waterbirds were collected from the Magela Creek flood plain for dietary analysis. Two hundred and thirty five (representing 22 of the common species) were analysed for heavy metal concentrations in their primary feathers, pectoral muscle and liver. Most samples were collected within 2 years of the beginning of mining by Ranger; so, as no discharges of wastes had occurred the results are representative of naturally occurring, pre-mining levels. Most specimens were taken with a 12 gauge shotgun using #4 stainless steel shot cartridges; however, some birds, collected at the beginning of the study, were shot with a standard lead shot, and a few individuals were collected using a high powered .22 rifle with standard ammunition. The essential characteristics of the different types of ammunition used are tabulated in Table 1.

Carcasses were chilled in the field before being individually bagged in food-grade polyethylene tubing and stored frozen at the laboratory. Information recorded for each bird included sample site, time and date of collection, weight, estimated age, sex, reproductive condition, moult scores from wings and tail and various measurements for morphometric evaluation.

2.2 Preparation and determination

After each bird was thawed its right wing primary feathers, the right pectoral muscle and the liver were removed and stored in heat-sealed plastic bags. For several species it was not possible to submit a complete set of samples for analysis. Prior to chemical analysis, feathers were ground in a Cullatti DCFH48 mill while pectoral muscle and liver were homogenised (Janke and Kunke 'Ika Werk' Ultraturrax). All samples were then dried at 80°C and digested in nitric acid. Two drops of double-distilled kerosene was added to each digestion to prevent frothing.

Metal concentrations in the digest solutions were determined by either flame atomic absorption spectrometry (AAS) using a Varian Model 1475 AAS or graphite furnace AAS (GFAAS) using a Perkin Elmer Model 5000 AAS, Model 500 HGA and Model AS40 Auto-sampler. Both instruments had double beam optics and were equipped with automatic deuterium lamp background correction. Accuracy and precision of results were checked by: (a) comparison with results from parallel digestions of US NBS Standard Reference Materials SRM 1566 'Oyster Tissue', SRM 1577 'Bovine Liver' and IAEA H4 'Animal Muscle', and (b) determining metals in a composite sample of each tissue by direct calibration and standard additions to evaluate possible matrix interference. All metals were determined using the direct calibration technique except for cadmium in bird muscle digest solutions.

Table 1. Metal constituents of shotgun pellets and rifle bullets[c] used to collect samples. Values are parts per million unless otherwise stated.

Element	Shotgun (stainless steel)	Shotgun (lead)	0.22 calibre bullet (lead)
Cadmium[b]	<0.5	<0.5	<0.5
Chromium[a]	300	<20	<20
Copper[a]	330	105	14%
Lead[a]	<100	87%	79%
Manganese[a]	4800	<20	<20
Nickel[a]	170	<20	<20
Zinc[a]	35	36	2.1%

[a] = determined by ICP-OES

[b] = determined by ICP-MS

[c] = all measurements performed by Analabs WA.

2.3 Analyses

Metal concentration data for each sample were initially scrutinised for anomalous values; concentration data lying outside 3 s.d. of the mean were rejected as such data could not be distinguished from contamination including shot fragments. Because of the possibility of water loss while in storage all wet weight metal levels for liver and muscle were converted to $\mu g/g$ dry weights ($\mu g/g$ dry). The mean proportions (mean ± S.E.) of water (as a percentage), obtained from a subsample of 5 species, in feather, muscle and liver were 3.7 ± 0.1, 72.1 ± 0.1 and 74 ± 0.1 respectively.

To equalise variances and normalise the predominance of sample distributions showing lognormal distributions, all data were transformed to natural logarithms for statistical analyses. Geometric means (GM) and data ranges are presented; arithmetic means (AM) are also given to facilitate comparison with other studies. Differences between groups of several means were determined by single factor analysis of variance (ANOVA); if variances were shown to be homogeneous using the Fmax ($p < 0.05$) test (Sokal & Rohlf 1981) to test for significance. The Newman-Keul or SNK multiple range test, corrected for unequal sample sizes (Zar 1974) was used to identify individual differences between means. If variances were not homogenous, then the nonparametric Kruskal- Wallis ANOVA by ranks was employed (Conover 1980) and differences between means established using the multiple comparison procedure described by Conover (1980). When pairs of means were compared, either the parametric Students-t Test or the nonparametric Wilcoxon Rank-sum Test was used after testing for equivalence of variances. No group mean derived from less than 5 samples was used in comparative tests. In all instances, significance was determined at $p < 0.05$.

For many comparisons the 22 species were divided into four groups based on dietary similarity. Details of these groups are listed below.

Diet 1.

Herbivores: magpie goose *Anseranus semipalmata*, green pygmy goose *Nettapus pulchellus*, wandering whistling duck *Dendrocygna arcuata*, plumed whistling duck *Dendrocygna eytoni* and purple swamphen *Porphyrio porphyrio.*

Diet 2.

Omnivores: masked lapwing *Vanellus miles*, comb-crested jacana *Irediparra gallinacea*, and Pacific black duck *Anas superciliosa.*

Diet 3.

Essentially Invertebrate-eaters: pink-eared duck *Malacorhynchus membranaceus*, radjah shelduck *Tadorna radjah*, black-winged stilt *Himantopus himantopus*, pied heron *Ardea picata*, whiskered tern *Chlidonias hybrida*, intermediate egret *Egretta intermedia*, glossy ibis *Plegadis falcinellus* and straw-necked ibis *Threskiornis spinicollis.*

Diet 4.

Mainly eaters of aquatic vertebrates (eg. fish, snakes, amphibians): little black cormorant *Phalacrocorax sulcirostris*, little pied cormorant *Phalacrocorax melanoleucos*, great egret *Egretta alba*, little egret *Egretta garzetta*, darter *Anhinga melanogaster* and the rufous night-heron *Nycticorax caledonicus.*

Some data were grouped as samples by season. The Alligator Rivers Region has a monsoonal climate with essentially all of the rain falling in the Wet season from December until March, and the Dry season from June to September. Years were measured from

December to November (i.e. from wet season to wet season) so that data for analysis from the seasonal year 1982 could have been collected between December 1981 and November 1982.

3 RESULTS

3.1 Interspecific and trophic variation

Because of small sample sizes ($n < 5$) the following species comparisons could not be carried out: plumed whistling duck (muscle and liver/all metals); purple swamphen (all tissues/all metals); pink-eared duck (all tissues/all metals); glossy ibis (muscle and liver/all metals); straw-necked ibis (muscle and liver/all metals); great egret (muscle and liver/all metals, feather/Cr, Pb and Mn), and radjah shelduck (liver/all metals).

The metal concentrations in the samples from most species showed high variability. Some of this variation probably reflected intraspecific differences due to sex, season and year of collection (Table 2); nevertheless, all ANOVA's showed significant differences among species of mean metal concentrations in feathers, pectoral muscle and liver (Table 3).

3.2 Intraspecific variation

It was possible to test for differences in mean tissue metal concentrations due to either sex, season of collection or year of collection in only 7 species. Sample sizes (n) of less than 10 were common for many species ($n < 10$ occurred in 54% of the species for feathers, 77% of species for muscle and 86% of species for liver); further division of these small samples was considered statistically useless. Furthermore when sample sizes were large they were often highly unbalanced between sexes, seasons and years so opportunities for comparative tests were again limited. Table 2 shows the distribution patterns of the samples for each species according to season and year of collection.

Mean lead levels in pectoral muscle from female green pygmy geese collected in the Wet season were significantly higher than in males over the same period (Table 4B). Male comb-crested jacanas had higher mean concentrations of cadmium and manganese in their feathers than had females (Table 4E). No differences in mean metal concentrations between sexes were found for magpie goose feathers and muscle, green pygmy goose feathers, whiskered tern feathers or masked lapwing feathers (Table 4A,B,C,D).

It was possible to test only magpie goose feathers and pectoral muscle for seasonal differences (Table 4A). No significant differences were found between mean metal levels in goose feathers sampled early in the Dry season (May-June) when compared with those sampled early in the Wet season (November). However, magpie goose muscle collected early in the Dry season had a significantly higher mean lead level than that from early in the Wet season. The opposite was true of zinc and manganese. Mean concentrations of each of these metals in muscle was significantly greater in magpie geese from early in the Wet season than those from early in the Dry season.

Mean metal concentrations in darter feathers (Table 4F), intermediate egret feathers (Table 4G) and whiskered tern feathers and muscle (Table 4C) were tested for differences between years. No significant differences were found for the metals sampled in either whiskered tern muscle or darter feathers. There were, however, significant differences for most of the metals in whiskered tern and intermediate egret feathers, but no consistent pattern of differences between the two species was apparent. Whereas mean concentrations of copper, zinc, chromium and nickel in feathers were higher in 1984 than in 1981 in tern feathers, the same was true of egret feathers only for copper and zinc. Concentrations of chromium and nickel in egret feathers showed the opposite pattern, being significantly higher in 1981 than in 1984. Mean cadmium concentrations in whiskered tern feathers were significantly higher in 1981 than in 1984 and there was a greater concentration of lead in

Table 2. Distribution of tissue samples analysed of each species, by year and season of collection

Species	Season	Feather '81	Feather '82	Feather '83	Feather '84	Muscle '81	Muscle '82	Muscle '83	Muscle '84	Liver '81	Liver '82	Liver '83	Liver '84
HERBIVORES													
Magpie goose	wet	7	0	0	0	1	0	0	0	0	0	0	0
	dry	9	10	2	0	8	9	2	0	0	4	2	0
Green Pygmy Goose	wet	2	18	1	0	0	12	1	0	0	9	1	0
	dry	3	0	0	0	0	0	0	0	0	0	0	0
Wandering whistling duck	wet	0	9	0	0	0	6	0	0	0	3	0	0
	dry	1	0	3	0	1	0	3	0	0	0	3	0
Plumed whistling duck	wet	4	1	0	0	0	1	0	0	0	0	0	0
	dry	0	0	1	0	0	0	1	0	0	0	1	0
Purple swamphen	one sample of feathers, date not known												
OMNIVORES													
Masked lapwing	wet	7	0	0	2	0	0	0	2	0	0	0	2
	dry	0	5	2	0	0	3	2	0	0	3	2	0
Comb-crested jacana	wet	3	0	0	5	0	0	0	5	0	0	0	5
	dry	0	5	0	0	0	3	0	0	0	3	0	0
Pacific black duck	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	1	2	7	0	0	1	7	0	0	0	7	0
INVERTEBRATE-EATERS													
Pink-eared duck	wet	1	0	0	0	0	0	0	0	0	0	0	0
	dry	0	0	0	0	0	0	0	0	0	0	0	0
Radjah shelduck	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	11	0	0	0	8	0	0	0	2	0	0	0
Black-winged stilt	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	1	0	7	0	0	0	7	0	0	0	7	0
Pied heron	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	0	4	4	0	0	1	4	0	0	1	4	0
Whiskered tern	wet	10	0	0	6	0	0	0	6	0	0	0	6
	dry	0	5	0	0	0	5	0	0	0	4	0	0
Intermediate egret	wet	5	0	0	6	0	0	0	6	0	0	0	6
	dry	0	4	0	0	0	4	0	0	0	4	0	0
Glossy ibis	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	0	2	7	0	0	1	3	0	0	0	3	0
Straw-necked ibis	wet	0	0	0	1	0	0	0	0	0	0	0	0
	dry	0	0	5	0	0	0	0	0	0	0	0	0
VERTEBRATE-EATERS													
Little black cormorant	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	0	0	6	0	0	0	6	0	0	0	6	0
Little pied cormorant	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	0	2	5	0	0	2	5	0	0	1	5	0
Great egret	wet	3	0	1	1	0	0	1	1	0	0	0	1
	dry	0	0	0	0	0	0	0	0	0	0	0	0
Little egret	wet	1	0	1	2	0	0	1	2	0	0	1	2
	dry	0	4	0	0	0	3	0	0	0	4	0	0
Darter	wet	0	0	0	0	0	0	0	0	0	0	0	0
	dry	0	6	5	0	0	4	5	0	0	1	5	0
Rufous night heron	wet	0	0	4	0	0	0	4	0	0	0	2	0
	dry	0	1	4	0	0	1	4	0	0	0	4	0

Table 3. Metal relationships between tissues - all significant regressions

Metal	Regression	df	r^2	P
ALL BIRDS				
Cd	Liver with muscle	96	0.0468	0.0324
Cu	Muscle with feather	149	0.0634	0.0018
	Liver with feather	100	0.0531	0.0204
	Liver with muscle	103	0.0930	0.0016
Pb	Liver with feather	105	0.1158	0.0004
	Liver with muscle	107	0.2151	0.0000
DIETARY GROUPS				
Herbivores				
Cu	Liver with feather	20	0.229	0.028
Zn	Liver with muscle	21	0.443	0.0007
Invertebrate-eaters				
Cd	Liver with muscle	30	0.232	0.006
Pb	Liver with feather	34	0.146	0.023
	Liver with muscle	34	0.219	0.004
Zn	Liver with muscle	34	0.224	0.004
Omnivores				
Cd	Liver with muscle	18	0.315	0.012
Cu	Liver with feather	18	0.302	0.014
	Liver with muscle	18	0.421	0.0026
Pb	Liver with muscle	20	0.192	0.046
Mn	Liver with muscle	19	0.261	0.021
Vertebrate-eaters				
Cu	Liver with muscle	27	0.525	0.000
Pb	Liver with muscle	29	0.264	0.003
SPECIES				
Green Pygmy Goose				
Cu	Liver with feather	7	0.5015	0.0493
Mn	Liver with muscle	8	0.5897	0.0157
Wandering Whistling Duck				
Cd	Liver with muscle	4	0.7925	0.0429
Masked Lapwing				
Zn	Liver with muscle	6	0.8109	0.0057
Black Duck				
Cd	Muscle with feather	6	0.8403	0.0037
Radjah Shelduck				
Pb	Muscle with feather	7	0.8315	0.0016
Black-winged Stilt				
Mn	Liver with muscle	6	0.6076	0.0388
Whiskered Tern				
Mn	Liver with muscle	9	0.6671	0.0039
Little Black Cormorant				
Cu	Liver with muscle	5	0.6925	0.0399
Mn	Muscle with feather	5	0.6744	0.0451
Darter				
Cd	Liver with feather	5	0.7069	0.0360

Table 4. Statistical tests for differences between sexes, seasons or years in levels of heavy metals in waterbirds of the Magela Creek flood plain

Tissue Type (Conditions)	Comparison	Cadmium	Chromium	Copper	Lead	Manganese	Nickel	Zinc
A. MAGPIE GOOSE:								
Feather (years and seasons pooled)	Between sexes	$t(25) = 0.655$[a] $p > 0.50$	$Z = 0.00$[b] $p = 1.0$	$t(26) = 0.194$ $p > 0.75$	$t(26) = 0.481$ $p > 0.50$	$t(26) = 0.072$ $p > 0.90$	$Z = -0.508$ $p > 0.50$	$t(26) = -0.875$ $p > 0.25$
Feather (sexes and years pooled)	May-June and November	$t(25) = 1.95$ $p > 0.05$	$Z = -1.17$ $p > 0.10$	$t(26) = -1.16$ $p > 0.25$	$Z = -0.564$ $p > 0.5$	$Z = -0.423$ $p > 0.50$	$t(26) = -1.26$ $p > 0.10$	$t(26) = -1.30$ $p > 0.10$
Muscle (years and seasons pooled)	Between sexes	$t(17) = 0.882$ $p > 0.25$	deleted[d]	$t(18) = -0.399$ $p > 0.50$	$Z = -0.792$ $p > 0.05$	$t(18) = 0.561$ $p > 0.50$	deleted[d]	$t(18) = 0.367$ $p > 0.5$
Muscle (sexes and years pooled)	May-June and November	$t(17) = -0.220$ $p > 0.75$	deleted[d]	$t(18) = 1.60$ $p > 0.1$	$t(18) = 3.37$ $p < 0.01$ * early dry > early wet	$Z = -2.07$ $p < 0.05$ * early dry < early wet	deleted[d]	$t(18) = -2.95$ $p < 0.01$ * early dry < early wet
B. GREEN PYGMY GOOSE:								
Feather (years pooled)	Between sexes in wet season	$t(21) = 0.247$[a] $p > 0.75$	$t(21) = -1.82$ $p > 0.05$	$t(21) = -1.23$ $p > 0.10$	$Z = -0.782$[b] $p > 0.25$	$t(22) = 0.820$ $p > 0.25$	$t(20) = -2.06$ $p > 0.05$	$Z = -1.51$ $p > 0.10$
Muscle (years pooled)	Between sexes in wet season	$t(11) = 1.20$	deleted[d]	$t(11) = 1.00$ $p > 0.25$ females > males	$t(11) = -3.05$ $p < 0.05$ *	$Z = -1.71$ $p > 0.05$	deleted[d]	$Z = -1.21$ $p > 0.10$
C. WHISKERED TERN:								
Feather (years and seasons pooled)	Between sexes	$t(19) = -0.486$[a] $p > 0.50$	$t(16) = -1.19$ $p > 0.10$	$Z = -1.69$[b] $p > 0.05$	$t(18) = -0.128$ $p > 0.75$	$t(19) = 0.354$ $p > 0.50$	$t(19) = -0.017$ $p > 0.95$	$t(19) = 0.299$ $p > 0.75$
Feather (sexes pooled)	Between years 1981, 1982, 1984 in early dry seasons	$F(2,18)$[c] $= 10.07$ $p < 0.05$ * 1981 > 1982 = 1984	$F(2,15) = 19.28$ $p < 0.001$ ** 1981 < 1982 = 1984	$F(2,16) = 21.19$ $p < 0.001$ ** 1981 = 1982 < 1984	$F(2,17) = 10.56$ $p < 0.05$ * 1981 = 1984 > 1982	$F(2,18) = 12.78$ $p < 001$ ** 1981 < 1982 = 1984	$F(2,18) = 8.17$ $p < 0.05$ * 1981 < 1982 = 1984	$F(2,18) = 12.94$ $p < 0.001$ ** 1981 < 1982 = 1984

Table 4 cont/d.

Tissue Type (Conditions)	Comparison	Cadmium	Chromium	Copper	Lead	Manganese	Nickel	Zinc
Muscle (sexes pooled)	Between 1982 and 1984 early dry seasons	not done[e]	deleted[d]	t(9) = 0.256 p > 0.75	t(9) = -0.249 p > 0.75	Z = -0.182 p > 0.75	deleted[d]	Z = -1.18 p > 0.10
D. MASKED LAPWING:								
Feather (seasons and years pooled)	Between sexes	t(13) = 0.092[a] p > 0.90	t(14) = -1.24 p > 0.10	t(14) = -0.215 p > 0.75	t(12) = -1.29 p > 0.10	t(14) = 0.852 p > 0.25	t(14) = -0.543 p > 0.50	t(13) = 0.522 p > 0.5
E. COMB-CRESTED JACANA:								
Feather (years and seasons pooled)	Between sexes	t(11) = 2.80[a] p < 0.05 * males > females	t(8) = -0.696 p > 0.50	Z = -0.857[b] p > 0.25	t(11) = 0.605 p > 0.50	t(11) = 2.56 p < 0.05 * males > females	t(11) = 1.50 p > 0.10	t(10) = -0.289 p > 0.75
F. DARTER:								
Feather (seasons and sexes pooled)	Between 1982 and 1983	t(8) = 1.07[a] p > 0.25	t(8) = 0.340 p > 0.50	Z = -0.522[b] p > 0.50	t(8) = -0.999 p > 0.25	t(8) = 0.462 p > 0.50	t(8) = 0.736 p > 0.25	t(8) = 0.163 p > 0.75
G. INTERMEDIATE EGRET:								
Feather (seasons and sexes pooled)	Between 1981 and 1984	t(9) = 1.70[a] p > 0.10	t(9) = 3.59 p < 0.05 * 1981 > 1984	t(9) = -3.04 p < 0.05 * 1981 < 1984	not done[e]	t(9) = 1.46 p > 0.10	t(9) = 3.92 p < 0.05 * 1981 > 1984	t(9) = -3.02 p < 0.05 1981 < 1984

[a] Comparison by Student 't' Test (degrees of freedom)
[b] Comparison by Wilcoxon Rank Sum Test
[c] Comparison by ANOVA. F(df, df)
[d] Chromium and Nickel Results deleted for muscle and liver tissues due to contamination
[e] Test not done due to small (n < 5) sample size

the feathers of the terns in 1982 than in either 1981 or 1984. The overall pattern of differences between mean metal concentrations in whiskered tern feathers during the 3 years is unclear.

3.3 Cadmium

Feathers (Table 5): The whiskered tern had the highest mean concentration of cadmium (0.069 μg/g dry). This was significantly higher than the mean values found for all other species except the darter, great egret, green pygmy goose and comb-crested jacana. There were no significant differences between mean cadmium concentrations for 14 of the 20 species tested with mean values ranging from 0.0045 to 0.021 μg/g dry. When grouped by diet the invertebrate-eating group had the highest mean concentration of cadmium (0.019 μg/g dry) and the aquatic vertebrate eaters the lowest (0.012 μg/g dry), but the differences were not statistically significant. Concentrations of cadmium in the samples of all species varied by a factor of 100 (from 0.0021 to 0.23 μg/g dry).

Table 5. Cadmium concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Whiskered Tern	21	0.069	0.089	0.014	0.012 - 0.23	a
Comb-crested Jacana	13	0.053	0.066	0.013	0.015 - 0.16	a b
Green Pygmy Goose	23	0.038	0.059	0.010	0.0021 - 0.18	a b c
Great Egret	5	0.033	0.037	0.0091	0.016 - 0.069	a b c d
Darter	11	0.026	0.030	0.0062	0.012 - 0.089	a b c d
Black-winged Stilt	8	0.021	0.031	0.0090	0.0054 - 0.078	b c d e
Intermediate Egret	15	0.019	0.021	0.0021	0.010 - 0.041	b c d e
Pacific Black Duck	10	0.018	0.032	0.012	0.0032 - 0.12	b c d e f
Straw-necked Ibis	6	0.013	0.028	0.016	0.0021 - 0.11	b c d e f
Plumed Whistling Duck	6	0.011	0.017	0.0055	0.0021 - 0.035	c d e f
Glossy Ibis	9	0.011	0.017	0.0055	0.0021 - 0.049	d e f
Magpie Goose	27	0.011	0.018	0.0030	0.0021 - 0.066	d e f
Little Egret	8	0.010	0.015	0.0046	0.0021 - 0.043	d e f
Little Pied Cormorant	7	0.0096	0.014	0.0047	0.0021 - 0.039	d e f
Pied Heron	8	0.0080	0.010	0.0022	0.0027 - 0.020	d e f
Wandering Whistling Duck	13	0.0080	0.017	0.0072	0.0027 - 0.095	d e f
Little Black Cormorant	6	0.0068	0.022	0.016	0.0021 - 0.098	d e f
Masked Lapwing	15	0.0061	0.012	0.0041	0.0021 - 0.047	d e f
Rufous Night Heron	9	0.0045	0.0074	0.0030	0.0021 - 0.030	e f
Radjah Shelduck	11	0.0042	0.0062	0.0020	0.0021 - 0.024	f
Pink-eared Duck*	1	0.087	0.087			
Purple Swamphen*	1	0.033	0.033			
Dietary Groups						
Invertebrate-eaters	79	0.019	0.038	0.0054	0.0021 - 0.23	a
Omnivores	38	0.017	0.036	0.0066	0.0021 - 0.17	a
Herbivores	70	0.015	0.031	0.0044	0.0021 - 0.18	a
Vertebrate-eaters	46	0.012	0.020	0.0031	0.0021 - 0.098	a

* species not included in the analysis due to small ($n < 5$) sample size

[1] species/trophic group not sharing same letter significantly different ($p < 0.05$)

Pectoral Muscle (Table 6): The mean cadmium concentration was highest (0.043 μg/g dry) in the black-winged stilt, but this differed significantly from only 4 other species. Cadmium concentration in all species ranged from 0.0005 to 0.22 μg/g dry. There were no significant differences in cadmium concentrations between dietary groups.

Table 6. Cadmium concentrations (μg/g dry) in muscle

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Black-winged Stilt	7	0.043	0.071	0.029	0.015 - 0.22	a
Comb-crested Jacana	8	0.028	0.036	0.011	0.013 - 0.093	a b
Whiskered Tern	9	0.024	0.046	0.017	0.0046 - 0.15	a b
Magpie Goose	19	0.023	0.037	0.0082	0.0064 - 0.12	a b
Masked Lapwing	7	0.022	0.034	0.015	0.0078 - 0.12	a b
Little Black Cormorant	6	0.021	0.023	0.0045	0.012 - 0.043	a b
Little Egret	6	0.021	0.030	0.012	0.0043 - 0.086	a b
Darter	9	0.019	0.025	0.0060	0.0050 - 0.054	a b
Rufous Night Heron	9	0.018	0.027	0.0070	0.0035 - 0.061	a b
Little Pied Cormorant	6	0.014	0.016	0.0041	0.0071 - 0.033	a b c
Radjah Shelduck	8	0.011	0.013	0.0031	0.0039 - 0.031	a b c
Pied Heron	5	0.0086	0.010	0.0028	0.0035 - 0.020	a b c
Intermediate Egret	10	0.0071	0.0074	0.0008	0.0039 - 0.014	b c
Wandering Whistling Duck	10	0.0068	0.010	0.0028	0.0012 - 0.032	b c
Pacific Black Duck	7	0.0063	0.0082	0.0021	0.0012 - 0.019	b c
Green Pygmy Goose	13	0.0047	0.0071	0.0014	0.0005 - 0.016	c
Plumed Whistling Duck*	2	0.0057	0.0071	0.0042	0.0029 - 0.011	
Purple Swamphen*	1	0.0075	0.0075			
Glossy Ibis*	4	0.043	0.044	0.0059	0.029 - 0.057	
Great Egret*	2	0.038	0.039	0.0044	0.034 - 0.043	
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	45	0.010	0.020	0.0040	0.0005 - 0.13	a
Omnivores	22	0.016	0.027	0.0065	0.0012 - 0.12	a
Invertebrate-eaters	43	0.016	0.031	0.0067	0.0035 - 0.22	a
Vertebrate-eaters	38	0.019	0.025	0.0029	0.0035 - 0.086	a

* species not included in the analysis due to small ($n < 5$) sample size

[1] species/trophic group not sharing same letter significantly different ($p < 0.05$)

Liver (Table 7): The black-winged stilt also had the highest mean concentration of cadmium (1.3 μg/g dry). This was significantly higher than in all other species except the Pacific black duck, whiskered tern and wandering whistling duck. The little black cormorant, the species with the lowest mean concentration of cadmium (0.013 μg/g dry), was significantly different from all but 4 species which had mean cadmium levels of 0.044 μg/g dry or less. The range of cadmium concentrations for all species was from 0.005 to 2.3 μg/g dry. No differences were found between dietary groups for cadmium concentrations.

Table 7. Cadmium concentrations (μg/g dry) in liver

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Black-winged Stilt	7	1.27	1.55	0.28	0.23 - 2.3	a
Pacific Black Duck	10	0.27	0.32	0.074	0.11 - 0.69	a b
Whiskered Tern	9	0.40	1.13	0.33	0.007 - 3.0	a b
Wandering Whistling Duck	6	0.23	0.36	0.098	0.020 - 0.65	a b c
Little Pied Cormorant	6	0.21	0.35	0.17	0.064 - 1.2	b c
Darter	5	0.16	0.26	0.15	0.053 - 0.88	b c
Magpie Goose	7	0.12	0.15	0.037	0.038 - 0.31	b c d
Green Pygmy Goose	9	0.089	0.14	0.046	0.022 - 0.46	c d e
Rufous Night Heron	6	0.071	0.13	0.052	0.005 - 0.31	c d e
Little Egret	7	0.076	0.45	0.27	0.007 - 1.5	c d e
Pied Heron	5	0.044	0.070	0.024	0.005 - 0.15	d e f
Intermediate Egret	8	0.037	0.070	0.033	0.007 - 0.29	e f
Masked Lapwing	7	0.039	0.051	0.016	0.018 - 0.13	e f
Comb-crested Jacana	7	0.021	0.022	0.003	0.019 - 0.045	f
Little Black Cormorant	5	0.013	0.023	0.014	0.005 - 0.080	f
Plumed Whistling Duck*	1	0.049	0.049			
Radjah Shelduck*	2	0.078	0.078	0.005	0.072 - 0.083	
Glossy Ibis*	3	1.2	1.3	0.46	0.73 - 2.2	
Great Egret*	1	0.005	0.005			
Purple Swamphen*	0					
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	23	0.12	0.20	0.038	0.020 - 0.65	a
Omnivores	21	0.061	0.13	0.038	0.018 - 0.69	a
Invertebrate-eaters	34	0.21	0.76	0.16	0.005 - 3.0	a
Vertebrate-eaters	30	0.078	0.25	0.077	0.005 - 1.5	a

* species not included in the analysis due to small ($n < 5$) sample size.
1 species/trophic group not sharing a common letter are significantly different ($p < 0.05$)

3.4 Chromium

Feathers (Table 8): Only about a half of the species, with chromium concentrations of less than 6.5 μg/g dry, were significantly different from the radjah shelduck which had the highest mean concentration of chromium (20 μg/g dry). The green pygmy goose had the lowest mean concentration of chromium (3.1 μg/g dry), but again this was only significantly different from about a half of the other species. The range of chromium concentrations recorded for all species was from 0.21 μg/g dry to 45 μg/g dry. No significant differences were found between the dietary groups.

Table 8. Chromium concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Radjah Shelduck	11	20	21	1.7	10 - 29	a
Darter	11	18	20	14	6.8 - 30	a
Magpie Goose	28	17	20	1.8	2.3 - 38	a
Straw-necked Ibis	6	15	17	3.3	6.5 - 30	a b
Plumed Whistling Duck	6	15	20	5.0	2.5 - 35	a b
Little Egret	8	13	17	3.9	2.3 - 33	a b
Pied Heron	8	12	21	5.8	1.6 - 45	a b
Intermediate Egret	15	9.8	14	3.0	2.7 - 42	a b
Masked Lapwing	16	9.7	12	1.8	2.4 - 27	a b
Little Black Cormorant	6	6.6	8.6	2.4	2.0 - 16	a b c
Wandering Whistling Duck	12	5.3	5.6	0.59	2.7 - 9.1	b c
Comb-crested Jacana	10	5.2	6.4	1.4	1.8 - 18	b c
Pacific Black Duck	5	4.9	6.2	1.8	1.2 - 12	b c
Glossy Ibis	9	4.8	6.7	1.7	0.58 - 18	b c
Whiskered Tern	18	4.0	4.9	0.80	1.5 - 13	c
Black-winged Stilt	7	3.6	6.4	2.0	0.21 - 14	c
Little Pied Cormorant	6	3.6	4.3	1.1	1.4 - 8.1	c
Green Pygmy Goose	23	3.1	3.6	0.43	1.8 - 8.9	c
Purple Swamphen*	1	11	11			
Pink-eared Duck*	1	8.1	8.1			
Rufous Night Heron*	4	6.8	7.7	1.9	2.9 - 12	
Great Egret*	4	6.6	8.2	3.5	4.5 - 19	
Dietary Groups						
Vertebrate-eaters	32	9.8	4.3	0.59	0.53 - 15	a
Herbivores	24	7.8	3.4	0.43	0.83 - 7.3	a
Invertebrate-eaters	37	7.8	5.2	1.0	0.45 - 28	a
Omnivores	22	7.1	4.4	0.60	0.24 - 9.5	a

* species not included in the analysis because of small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter are significantly different ($p < 0.05$).

3.5 Copper

Feathers (Table 9): The comb-crested jacana had a mean concentration of copper (36 μg/g dry), significantly higher than in any other species. There were no significant differences between 14 of the 20 species having mean copper concentrations between 11 μg/g dry and 18 μg/g dry. The lowest mean copper level was in the magpie goose (6.4 μg/g dry); this level was significantly lower than all other species except the wandering whistling duck (7.2 μg/g dry). In turn the wandering whistling duck had a mean copper concentration significantly lower than all but the radjah shelduck and the plumed whistling duck. These four species were among the many herbivorous waterbirds. The analysis of dietary groups confirmed that the herbivores had significantly less copper.

Table 9. Copper concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Jacana	13	36	38	3.5	12 - 57	a
Whiskered Tern	19	19	21	2.6	8.3 - 48	b
Little Black Cormorant	6	18	18	1.6	14 - 25	b c
Black-winged Stilt	8	18	18	1.3	12 - 23	b c
Intermediate Egret	15	17	18	1.1	13 - 29	b c
Little Egret	8	17	18	2.3	12 - 29	b c
Great Egret	5	17	18	1.9	11 - 22	b c d
Little Pied Cormorant	7	15	16	1.9	11 - 24	b c d
Masked Lapwing	16	15	15	0.84	8.9 - 22	b c d
Darter	11	13	14	0.91	8.3 - 18	c d
Glossy Ibis	9	13	14	1.6	8.6 - 20	c d
Green Pygmy Goose	23	13	13	0.75	9.2 - 23	c d
Pacific Black Duck	10	12	12	0.92	7.8 - 18	c d e
Pied Heron	8	12	12	0.43	9.6 - 13	c d e
Rufous Night Heron	9	11	12	0.81	8.9 - 15	c d e
Straw-necked Ibis	6	10	11	0.83	8.4 - 14	c d e
Plumed Whistling Duck	6	9.9	10	0.56	8.1 - 12	d e f
Radjah Shelduck	11	8.6	8.7	0.39	6.0 - 10	e f
Wandering Whistling Duck	13	7.2	7.6	0.72	5.2 - 13	f g
Magpie Goose	28	6.4	6.6	0.35	3.9 - 11	g
Purple Swamphen*	1	5.7	5.7			
Pink-eared Duck*	1	11	11			
Dietary Groups						
Omnivores	39	19	22	2.2	7.8 - 57	a
Vertebrate-eaters	46	15	15	0.70	8.3 - 29	a b
Invertebrate-eaters	77	14	16	0.86	6.0 - 48	b
Herbivores	71	8.5	9.2	0.46	3.9 - 23	c

* species not included in analysis due to small (n $<$ 5) sample size.

[1] species/trophic group not sharing common letter are significantly different ($p < 0.05$).

Pectoral Muscle (Table 10): The wandering whistling duck had a significantly higher concentration of copper (25 μg/g dry) than had all other species except the green pygmy goose, black-winged stilt and Pacific black duck. The intermediate egret had the lowest mean copper concentration (11 μg/g dry). This range of species means was one of the narrowest recorded from any analysis of other metals or tissue and organ types. The herbivorous waterbirds had significantly higher concentrations of copper in the muscle than all other trophic groups.

Table 10. Copper concentrations (μg/g dry) in muscle

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Wandering Whistling Duck	10	25	25	1.0	19 - 30	a
Pacific Black Duck	8	23	25	3.8	15 - 50	a b
Black-winged Stilt	7	19	19	1.1	16 - 24	a b
Green Pygmy Goose	23	19	20	1.4	14 - 29	a b c
Little Pied Cormorant	7	17	17	0.56	15 - 19	b c d
Magpie Goose	20	16	17	0.79	8.2 - 23	c d
Whiskered Tern	11	16	16	0.31	15 - 19	c d
Little Black Cormorant	8	16	16	0.38	15 - 18	c d
Radjah Shelduck	8	16	16	0.66	12 - 18	d
Rufous Night Heron	9	16	16	0.79	13 - 19	d e
Darter	9	15	16	0.93	12 - 19	d e
Masked Lapwing	7	14	14	0.51	11 - 16	e f
Comb-crested Jacana	8	13	13	1.2	8.2 - 20	e f
Little Egret	6	13	13	0.79	10 - 16	f
Pied Heron	5	13	13	0.62	11 - 14	f
Intermediate Egret	10	11	11	0.50	9.0 - 15	f
Plumed Whistling Duck*	2	16	16	1.4	14 - 17	
Purple Swamphen*	1	18	18			
Glossy Ibis*	4	13	13	0.47	11 - 14	
Great Egret*	2	11	11	1.4	9.7 - 13	
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	46	19	19	0.73	8.2 - 30	a
Vertebrate-eaters	39	15	15	0.40	9.7 - 19	b
Omnivores	23	16	18	1.8	8.2 - 50	b
Invertebrate-eaters	45	15	15	0.49	9.0 - 24	b

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Liver (Table 11): The green pygmy goose had significantly higher concentrations of copper (2500 μg/g dry) than all other species. The wandering whistling duck had the second highest mean copper concentration (640 μg/g dry); significantly lower than that of the green pygmy goose but significantly higher than all other species. There were no significant differences between 12 of the 15 species in the range, from comb-crested jacana up to the little egret. Overall, copper concentrations varied by a factor of 200 ranging from 32 to 6500 μg/g dry. The copper concentrations of the herbivores were significantly greater than the concentrations of all other dietary groups.

There were distinct contrasts between the rankings for species and dietary groups in feathers compared with muscle and liver. The comb-crested jacana had the highest mean feather concentration but had one of the lowest mean levels in both muscle and liver. Conversely, the wandering whistling duck ranked amongst the species with the lowest mean concentrations in feathers but had one of the highest mean copper levels in muscle and liver. Of the dietary groups, the herbivorous waterfowl had a significantly lower concentration in feathers yet had a significantly higher concentration than other groups in both liver and muscle.

Table 11. Copper concentrations (μg/g dry) in liver

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Green Pygmy Goose	10	2500	3600	730	200 - 6500	a
Wandering Whistling Duck	6	640	840	250	180 - 1800	b
Pacific Black Duck	7	280	340	58	53 - 530	c
Little Egret	5	180	240	80	69 - 500	c d
Pied Heron	5	120	130	42	73 - 300	c d
Intermediate Egret	10	97	100	9.1	57 - 150	d
Black-winged Stilt	7	89	130	67	61 - 530	d
Little Pied Cormorant	6	81	85	10	42 - 110	d
Whiskered Tern	10	77	81	8.8	53 - 140	d
Masked Lapwing	7	73	75	7.4	53 - 100	d
Rufous Night Heron	6	73	92	33	34 - 260	d
Darter	6	71	78	17	42 - 160	d
Magpie Goose	7	67	76	16	32 - 140	d
Little Black Cormorant	6	66	71	12	38 - 120	d
Comb-crested Jacana	6	51	54	10	38 - 100	d
Plumed Whistling Duck*	1	190	190			
Radjah Shelduck*	2	64	78	44	34 - 120	
Glossy Ibis*	3	39	40	3.2	35 - 46	
Great Egret*	1	340	340			
Purple Swamphen*	0					
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	24	580	1700	450	32 - 6500	a
Invertebrate-eaters	37	84	100	14	34 - 530	b
Omnivores	20	110	160	36	38 - 530	b
Vertebrate-eaters	30	85	120	20	34 - 500	b

* species not included in analysis due to small ($n < 5$) sample size.
1 species/trophic group not sharing common letter significantly different ($p < 0.05$).

3.6 Lead

Feathers (Table 12): The whiskered tern had the highest mean concentration of lead (3.2 μg/g dry), which was significantly higher than the mean levels in all other species except plumed whistling duck, straw-necked ibis, darter and comb-crested jacana. The rufous night heron had the lowest mean concentration of lead (0.28 μg/g dry), which was significantly lower than the means of all species except the pied heron, radjah shelduck and little black cormorant. Lead concentrations in all species varied by a factor of 200 (from 0.065 μg/g dry to 12 μg/g dry). The omnivores showed the highest lead level but were significantly different only from the vertebrate-eaters.

Table 12. Lead concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Whiskered Tern	20	3.2	4.3	0.76	0.60 - 12	a
Comb-crested Jacana	13	2.1	2.7	0.54	0.69 - 7.3	a b
Darter	11	1.6	2.1	0.56	0.45 - 6.5	a b c
Straw-necked Ibis	5	1.4	1.6	0.34	0.81 - 2.4	a b c
Plumed Whistling Duck	6	1.3	1.4	0.26	0.66 - 2.4	a b c
Little Pied Cormorant	7	0.99	1.1	0.17	0.54 - 1.5	b c d
Wandering Whistling Duck	13	1.0	1.2	0.26	0.47 - 3.8	c d
Little Egret	8	0.99	1.1	0.20	0.52 - 2.2	c d
Magpie Goose	28	1.0	1.6	0.32	0.25 - 6.1	c d
Pacific Black Duck	9	0.88	1.5	0.50	0.065 - 4.6	c d
Green Pygmy Goose	24	0.90	1.4	0.35	0.093 - 7.9	c d
Masked Lapwing	14	0.89	1.0	0.14	0.29 - 2.4	c d
Black-winged Stilt	8	0.76	1.5	0.51	0.30 - 4.1	c d e
Glossy Ibis	7	0.87	0.97	0.19	0.41 - 2.0	c d e
Intermediate Egret	13	0.73	0.77	0.11	0.25 - 1.6	d e
Little Black Cormorant	6	0.67	0.89	0.33	0.34 - 2.3	d e f
Radjah Shelduck	11	0.55	0.56	0.026	0.42 - 0.67	e f
Pied Heron	8	0.51	0.56	0.090	0.25 - 0.93	e f
Rufous Night Heron	9	0.28	0.31	0.050	0.10 - 0.60	f
Purple Swamphen*	1	0.42	0.42			
Pink-eared Duck*	1	3.1	3.1			
Great Egret*	4	0.43	1.0	0.28	0.39 - 1.8	
Dietary Groups						
Omnivores	36	1.2	1.8	0.27	0.065 - 7.3	a
Herbivores	72	0.99	1.4	0.18	0.093 - 7.9	a b
Invertebrate-eaters	73	1.1	1.9	0.28	0.25 - 12	a b
Vertebrate-eaters	45	0.80	1.1	0.17	0.10 - 6.5	b

* species not included in analysis due to small ($n < 5$) sample size.

[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Pectoral Muscle (Table 13): The darter (0.065 μg/g dry) and the little black cormorant (0.011 μg/g dry) respectively had the highest and lowest mean concentrations. The darter, however, was significantly different from only 6 species which had mean levels of less than 0.025 μg/g dry. Fewer than half the species, with mean levels of greater than 0.026 μg/g, were significantly different from the little black cormorant. The total range of concentrations was from 0.0035 to 0.17 μg/g. Omnivores had significantly higher concentrations than had species of the other dietary groups.

Table 13. Lead concentrations (μg/g dry) in muscle

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Darter	9	0.065	0.071	0.0098	0.030 - 0.11	a
Comb-crested Jacana	8	0.062	0.065	0.0082	0.043 - 0.11	a
Black-winged Stilt	7	0.060	0.064	0.0093	0.030 - 0.11	a b
Pacific Black Duck	8	0.053	0.065	0.017	0.025 - 0.17	a b c
Rufous Night Heron	9	0.049	0.055	0.0089	0.018 - 0.11	a b c d
Wandering Whistling Duck	10	0.035	0.038	0.0063	0.019 - 0.086	a b c d
Radjah Shelduck	8	0.034	0.043	0.012	0.013 - 0.10	a b c d
Masked Lapwing	7	0.034	0.040	0.011	0.014 - 0.10	a b c d
Magpie Goose	20	0.027	0.034	0.0054	0.0035 - 0.11	a b c d e
Pied Heron	5	0.025	0.027	0.0057	0.014 - 0.047	a b c d e
Whiskered Tern	11	0.023	0.028	0.0054	0.0078 - 0.072	b c d e
Green Pygmy Goose	13	0.023	0.030	0.0056	0.0035 - 0.065	b c d e
Intermediate Egret	10	0.020	0.023	0.0039	0.0075 - 0.054	c d e
Little Pied Cormorant	7	0.020	0.031	0.014	0.0078 - 0.11	c d e
Little Egret	6	0.015	0.017	0.0039	0.0071 - 0.032	d e
Little Black Cormorant	6	0.011	0.013	0.0038	0.0035 - 0.031	e
Plumed Whistling Duck*	2	0.046	0.051	0.021	0.029 - 0.072	
Purple Swamphen*	1	0.022	0.022			
Glossy Ibis*	4	0.044	0.058	0.027	0.017 - 0.14	
Great Egret*	2	0.076	0.079	0.022	0.057 - 0.10	
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Omnivores	23	0.049	0.0575	0.0075	0.014 - 0.17	a
Invertebrate-eaters	45	0.030	0.0379	0.0042	0.0075 - 0.14	b
Vertebrate-eaters	39	0.030	0.0430	0.0054	0.0035 - 0.11	b
Herbivores	46	0.028	0.0340	0.0032	0.0035 - 0.11	b

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Liver (Table 14): The comb-crested jacana had the highest mean concentration (0.43 μg/g dry). The little black cormorant, green pygmy goose, intermediate egret and little pied cormorant, with mean concentrations ranging from 0.015 to 0.020 μg/g dry, formed a distinct group with levels significantly lower than all other species. Lead concentrations in the liver samples from all species ranged from 0.005 to 2.0 μg/g dry. Omnivorous waterbirds had a significantly higher level than those in other dietary groups.

Mean concentrations in each organ and tissue were consistently highest in the comb-crested jacana, Pacific black duck, darter and black-winged stilt. Similarly, of the dietary groups, the omnivores (which included the Pacific black duck and the comb-crested jacana) also had the highest concentration in each organ/tissue.

Table 14. Lead concentrations (μg/g dry) in liver

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Comb-crested Jacana	8	0.43	0.56	0.17	0.18 - 1.4	a
Black-winged Stilt	7	0.42	0.64	0.25	0.16 - 2.0	a
Pacific Black Duck	7	0.37	0.40	0.055	0.18 - 0.57	a
Wandering Whistling Duck	6	0.23	0.29	0.085	0.095 - 0.65	a b
Darter	6	0.21	0.24	0.052	0.084 - 0.42	a b
Magpie Goose	7	0.16	0.18	0.033	0.064 - 0.32	a b
Whiskered Tern	10	0.13	0.14	0.015	0.072 - 0.20	b
Masked Lapwing	7	0.074	0.077	0.007	0.045 - 0.10	b
Little Egret	7	0.073	0.082	0.014	0.027 - 0.13	b
Rufous Night Heron	6	0.073	0.076	0.011	0.053 - 0.12	b
Pied Heron	5	0.072	0.077	0.012	0.034 - 0.10	b
Little Pied Cormorant	6	0.020	0.044	0.024	0.005 - 0.16	c
Intermediate Egret	10	0.018	0.031	0.010	0.005 - 0.10	c
Green Pygmy Goose	10	0.017	0.026	0.008	0.007 - 0.091	c
Little Black Cormorant	6	0.015	0.022	0.008	0.005 - 0.057	c
Plumed Whistling Duck*	1	0.24	0.24			
Radjah Shelduck*	2	0.092	0.093	0.013	0.080 - 0.11	
Glossy Ibis*	3	0.095	0.097	0.015	0.072 - 0.13	
Great Egret*	1	0.028	0.028			
Purple Swamphen*	0					
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Omnivores	22	0.23	0.35	0.074	0.045 - 1.4	a
Invertebrate-eaters	37	0.085	0.19	0.058	0.005 - 2.0	b
Herbivores	24	0.070	0.15	0.031	0.007 - 0.65	b
Vertebrate-eaters	31	0.051	0.090	0.017	0.005 - 0.42	b

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

3.7 Manganese

Feathers (Table 15): Mean manganese concentrations varied from 35 $\mu g/g$ dry in the darter to 6.0 $\mu g/g$ dry in the intermediate egret. Species with mean concentrations of less than 13 $\mu g/g$ dry differed significantly from the darter. Only 6 of the 19 species differed significantly from the intermediate egret. The total range of manganese concentrations measured in all birds was from 0.87 to 89 $\mu g/g$ dry. The invertebrate-eating group had significantly less manganese than all other groups and the vertebrate-eating group had the highest mean concentration of manganese.

Table 15. Manganese concentrations ($\mu g/g$ dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Darter	11	35	38	5.6	13 - 81	a
Little Pied Cormorant	7	32	37	8.8	19 - 70	a b
Comb-crested Jacana	13	24	27	3.7	13 - 63	a b
Rufous Night Heron	9	23	31	8.1	11 - 89	a b c
Straw-necked Ibis	6	21	24	4.5	9.4 - 39	a b c d
Magpie Goose	28	19	25	3.2	2.2 - 27	a b c d
Pied Heron	8	16	17	2.7	6.3 - 27	a b c d e
Masked Lapwing	16	15	18	2.6	5.8 - 38	a b c d e
Little Black Cormorant	6	14	14	1.8	7.2 - 20	a b c d e
Wandering Whistling Duck	13	12	21	5.4	1.6 - 56	b c d e
Plumed Whistling Duck	6	12	12	0.91	8.3 - 14	b c d e
Radjah Shelduck	11	9.4	11	2.1	5.3 - 30	c d e
Green Pygmy Goose	24	8.6	10	1.3	2.2 - 27	d e
Black-winged Stilt	8	8.5	8.9	0.85	3.6 - 11	d e
Glossy Ibis	9	8.4	9.5	1.9	4.4 - 23	d e
Little Egret	8	7.9	8.5	1.2	3.3 - 14	d e
Whiskered Tern	21	6.9	8.6	1.4	2.7 - 24	e
Pacific Black Duck	10	6.0	11	4.1	1.2 - 42	e
Intermediate Egret	15	6.0	7.8	1.5	0.87 - 25	e
Purple Swamphen*	1	42	42			
Pink-eared Duck*	1	4.8	4.8			
Great Egret*	4	4.9	6.0	1.6	1.4 - 9.2	
Dietary Groups						
Vertebrate-eaters	45	18	25	3.2	1.4 - 89	a
Omnivores	39	14	19	2.1	1.2 - 63	a b
Herbivores	72	13	18	1.8	1.6 - 78	b
Invertebrate-eaters	79	8.6	11	0.87	0.87 - 39	c

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Pectoral Muscle (Table 16): Although the range of the mean concentrations in the species examined was quite narrow, the whiskered tern ranked highest (4.8 μg/g dry), and was significantly higher than all other species except the wandering whistling duck. Intermediate egrets had the lowest mean level (1.5 μg/g dry), which was significantly lower than that of any other species except the little egret. Most species had mean levels ranging from 2.0 to 3.0 μg/g dry. The range of concentrations recorded from all species was from 1.1 to 16 μg/g dry. There were no significant differences between the dietary groups.

Table 16. Manganese concentrations (μg/g dry) in muscle

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Whiskered Tern	11	4.8	5.4	1.1	3.3 - 16	a
Wandering Whistling Duck	10	3.8	3.9	0.18	3.2 - 5.0	a b
Rufous Night Heron	9	3.0	3.0	0.15	2.2 - 4.0	b c
Little Black Cormorant	6	2.6	2.6	0.17	2.0 - 3.2	c d
Pied Heron	5	2.5	2.6	0.20	2.1 - 3.3	c d e
Pacific Black Duck	8	2.4	2.4	0.11	1.9 - 3.0	d e
Black-winged Stilt	7	2.4	2.4	0.15	1.7 - 3.0	d e
Radjah Shelduck	8	2.4	2.5	0.16	2.0 - 3.2	d e
Green Pygmy Goose	13	2.3	2.4	0.17	1.1 - 3.4	d e
Comb-crested Jacana	8	2.3	2.4	0.12	2.0 - 2.8	d e f
Magpie Goose	20	2.4	2.6	0.34	1.6 - 9.0	d e f
Darter	9	2.2	2.2	0.07	1.9 - 2.6	e f g
Masked Lapwing	7	2.0	2.1	0.25	1.4 - 3.3	f g
Little Pied Cormorant	7	2.0	2.1	0.13	1.6 - 2.7	f g
Little Egret	6	1.9	1.9	0.11	1.5 - 2.3	g h
Intermediate Egret	10	1.5	1.5	0.05	1.3 - 1.8	h
Plumed Whistling Duck*	2	2.4	2.5	0.34	2.1 - 2.8	
Purple Swamphen*	1	2.4	2.4			
Glossy Ibis*	4	1.7	1.7	0.12	1.4 - 1.9	
Great Egret*	2	2.0	2.0	0.39	1.6 - 2.4	
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	46	2.7	2.83	0.18	1.14 - 9.0	a
Omnivores	23	2.3	2.30	0.09	1.36 - 3.3	a
Invertebrate-eaters	45	2.5	2.90	0.33	1.32 - 16	a
Vertebrate-eaters	39	2.3	2.38	0.08	1.46 - 3.9	a

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Liver (Table 17): Very few significant differences in concentrations were evident between species. Thirteen of the 15 species had mean concentrations of greater than 30 μg/g dry and did not differ significantly from the little pied cormorant, ranked highest, with a concentration of 61 μg/g dry. The pied heron was ranked lowest and had a mean level of 19 μg/g dry which was significantly lower than in all other species except the wandering whistling duck. The total range of concentrations in the samples was from 8.8 to 110 μg/g dry. No significant differences were evident between the dietary groups.

Table 17. Manganese concentrations (μg/g dry) in liver

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Little Pied Cormorant	6	61	64	9.8	38 - 110	a
Comb-crested Jacana	7	60	63	6.8	29 - 80	a
Black-winged Stilt	7	58	61	7.1	42 - 88	a
Darter	6	55	59	9.0	28 - 88	a
Pacific Black Duck	7	53	57	7.2	26 - 76	a
Green Pygmy Goose	10	48	50	4.5	29 - 73	a
Little Black Cormorant	6	47	47	2.3	42 - 57	a
Intermediate Egret	10	45	47	4.4	34 - 73	a
Whiskered Tern	10	45	51	5.7	8.8 - 76	a
Rufous Night Heron	6	44	48	7.6	23 - 69	a
Little Egret	7	41	43	4.4	26 - 57	a
Masked Lapwing	7	41	43	4.2	29 - 69	a
Magpie Goose	7	34	35	3.3	24 - 50	a b
Wandering Whistling Duck	6	28	29	4.6	23 - 19	b c
Pied Heron	5	19	20	1.3	18 - 25	c
Plumed Whistling Duck*	1	14	14			
Radjah Shelduck*	2	44	47	15	32 - 61	
Glossy Ibis*	3	30	30	3.6	23 - 34	
Great Egret*	1	53	53			
Purple Swamphen*	0					
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Omnivores	21	51	54	4.0	26 - 80	a
Vertebrate-eaters	32	49	52	3.2	23 - 110	a
Invertebrate-eaters	37	41	46	3.2	8.8 - 88	a
Herbivores	24	36	39	3.1	14 - 73	a

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

3.8 Nickel

Feathers (Table 18): Only 4 species, with mean nickel concentrations of less than 2.0 μg/g dry, were significantly different from the plumed whistling duck, which had the highest mean level (6.5 μg/g dry). There were also only a few species significantly different from the black-winged stilt, the species with the lowest level (0.99 μg/g dry). The range of concentrations from all species was from 0.12 to 36 μg/g dry. There were no significant differences between the dietary groups.

Table 18. Nickel concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Plumed Whistling Duck	6	6.5	8.9	2.5	1.4 - 16	a
Radjah Shelduck	11	6.4	7.0	0.97	2.7 - 13	a
Darter	11	6.1	7.0	1.1	2.0 - 12	a
Straw-necked Ibis	6	6.0	6.4	1.2	4.2 - 12	a
Magpie Goose	28	5.1	6.5	0.69	0.23 - 14	a b
Pacific Black Duck	10	4.7	9.0	3.4	0.69 - 36	a b c
Pied Heron	7	4.4	6.8	2.2	0.84 - 18	a b c d
Little Egret	8	3.9	5.2	1.2	1.0 - 10	a b c d
Rufous Night Heron	6	3.7	5.5	2.1	0.94 - 13	a b c d
Masked Lapwing	16	3.6	4.6	0.84	0.94 - 14	a b c d
Whiskered Tern	21	3.1	4.6	1.1	0.67 - 21	a b c d
Wandering Whistling Duck	13	3.0	3.6	0.74	1.1 - 10	a b c d
Great Egret	5	2.9	3.7	1.3	1.2 - 8.3	a b c d
Comb-crested Jacana	13	2.8	3.2	0.46	1.2 - 6.7	a b c d
Intermediate Egret	15	2.6	4.0	0.98	0.53 - 13	a b c d
Little Pied Cormorant	7	2.4	2.6	0.48	1.3 - 5.0	a b c d
Little Black Cormorant	6	1.8	2.8	1.4	0.75 - 9.3	b c d
Green Pygmy Goose	22	1.4	1.8	0.32	0.16 - 6.5	c d
Glossy Ibis	9	1.3	1.7	0.46	0.65 - 4.9	c d
Black-winged Stilt	7	0.99	2.1	0.73	0.12 - 4.8	d
Purple Swamphen*	1	2.0	2.0			
Pink-eared Duck*	1	2.2	2.2			
Dietary Groups						
Omnivores	22	3.6	5.3	0.98	0.69 - 36	a
Vertebrate-eaters	32	3.5	4.8	0.55	0.75 - 13	a
Herbivores	24	3.1	4.6	0.47	0.16 - 16	a
Invertebrate-eaters	37	2.9	4.6	0.48	0.12 - 21	a

* species not included in analysis due to small (n < 5) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

3.9 Zinc

Feathers (Table 19): The pied heron had the highest concentration (190 μg/g dry), this was significantly higher than in 9 other species with mean concentrations of less than 160 μg/g dry. The great egret had the lowest mean concentration (110 μg/g dry) which was significantly lower than for all species except the darter, magpie goose and plumed whistling duck. Concentrations ranged from 43 to 460 μg/g dry. The invertebrate-eating and omnivorous groups had significantly more zinc in feathers than the vertebrate-eaters or herbivores.

Table 19. Zinc concentrations (μg/g dry) in feathers

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Pied Heron	8	190	200	15	160 - 280	a
Little Pied Cormorant	7	190	190	11	140 - 240	a
Radjah Shelduck	11	190	190	8.0	140 - 240	a
Whiskered Tern	21	210	260	20	130 - 460	a
Rufous Night Heron	9	180	190	14	140 - 270	a b
Black-winged Stilt	8	180	190	14	140 - 240	a b
Masked Lapwing	15	180	180	11	140 - 250	a b
Straw-necked Ibis	6	190	210	47	150 - 440	a b c
Pacific Black Duck	10	180	190	23	130 - 360	a b c
Wandering Whistling Duck	13	170	180	17	130 - 330	a b c
Little Black Cormorant	6	160	160	14	150 - 230	a b c d
Comb-crested Jacana	12	160	190	30	55 - 400	b c d
Green Pygmy Goose	23	150	160	5.7	130 - 270	c d
Intermediate Egret	15	140	150	7.1	110 - 210	d e
Glossy Ibis	9	120	130	13	43 - 170	d e
Little Egret	8	140	140	7.7	120 - 180	d e
Darter	11	130	130	11	100 - 210	e f
Magpie Goose	28	130	130	3.6	94 - 180	e f
Plumed Whistling Duck	6	125	130	4.6	110 - 140	e f
Great Egret	5	110	110	7.3	97 - 140	f
Purple Swamphen*	1	140	140			
Pink-eared Duck*	1	100	100			
Dietary Groups						
Invertebrate-eaters	79	170	180	7.8	43 - 460	a
Omnivores	37	170	190	12	55 - 400	a
Vertebrate-eaters	46	150	160	6.1	97 - 270	b
Herbivores	71	140	150	4.5	94 - 330	b

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Pectoral Muscle (Table 20): Magpie geese had the highest mean concentration (190 µg/g dry), and the green pygmy goose the lowest (97 µg/g dry). Although these species were significantly different from each other, very few differences between the mean levels in other species were found. Concentrations in all species ranged from 54 to 350 µg/g dry. The omnivores had significantly less zinc in muscle than the other dietary groups.

Table 20. Zinc concentrations (µg/g dry) in muscle

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Magpie Goose	20	190	190	6.0	130 - 270	a
Wandering Whistling Duck	10	190	190	8.5	150 - 230	a b
Rufous Night Heron	9	180	180	7.1	150 - 220	a b
Little Pied Cormorant	7	180	180	11	150 - 240	a b
Whiskered Tern	11	180	180	9.1	150 - 250	a b
Little Black Cormorant	6	170	180	8.9	140 - 200	a b c
Radjah Shelduck	8	170	180	25	130 - 350	a b c
Little Egret	6	160	160	5.0	150 - 180	a b c
Pied Heron	5	160	160	11	140 - 190	a b c d
Intermediate Egret	10	160	160	16	75 - 270	a b c d
Darter	9	140	140	4.2	120 - 160	b c d
Black-winged Stilt	7	130	130	11	79 - 180	c d e
Masked Lapwing	7	120	130	5.9	100 - 150	c d e
Pacific Black Duck	8	120	120	4.9	100 - 140	d e
Comb-crested Jacana	8	97	100	9.1	54 - 120	e
Green Pygmy Goose	13	97	100	7.1	50 - 140	e
Plumed Whistling Duck*	2	170	180	39	140 - 220	
Purple swamphen*	1	270	270			
Glossy Ibis*	4	120	120	5.1	110 - 130	
Great Egret*	2	190	190	39	150 - 230	
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Vertebrate-eaters	39	170	170	4.1	120 - 240	a
Herbivores	46	160	170	7.7	50 - 270	a
Invertebrate-eaters	45	150	160	6.9	75 - 350	a
Omnivores	23	110	110	4.4	54 - 150	b

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing common letter significantly different ($p < 0.05$).

Liver (Table 21): The wandering whistling duck had the highest mean concentration (630 μg/g dry) which was significantly different from 7 other species having mean levels of less than 400 μg/g dry. The whiskered tern had the lowest mean level (260 μg/g dry), and 9 species with concentrations greater than 400 μg/g dry differed significantly from it. The range of concentrations for individual samples from all birds was from 130 to 920 μg/g dry. No significant differences between the dietary groups were evident.

Table 21. Zinc concentrations (μg/g dry) in liver

Species	N	Geometric mean	Arithmetic mean	S.E.	Range	Differences[1]
Wandering Whistling Duck	6	630	650	67	480 - 800	a
Darter	6	560	570	41	420 - 730	a b
Pacific Black Duck	7	550	600	88	250 - 880	a b
Magpie Goose	7	550	560	36	460 - 690	a b
Black-winged Stilt	7	550	580	79	370 - 920	a b
Little Pied Cormorant	6	500	500	41	420 - 690	a b c
Intermediate Egret	10	470	500	69	310 - 950	a b c
Comb-crested Jacana	7	450	480	58	210 - 610	a b c d
Little Egret	7	420	430	26	340 - 530	a b c d
Green Pygmy Goose	10	360	370	22	280 - 500	b c d e
Masked Lapwing	7	340	350	27	240 - 460	b c d e
Little Black Cormorant	6	320	320	16	270 - 360	c d e
Pied Heron	5	300	310	24	250 - 370	c d e
Rufous Night Heron	6	290	290	10	260 - 320	d e
Whiskered Tern	10	260	270	21	130 - 350	e
Plumed Whistling Duck*	1	310	310			
Radjah Shelduck*	2	420	420	38	380 - 460	
Glossy Ibis*	3	370	370	26	330 - 420	
Great Egret*	1	340	340			
Purple Swamphen*	0					
Pink-eared Duck*	0					
Straw-necked Ibis*	0					
Dietary Groups						
Herbivores	24	470	490	33	280 - 800	a
Omnivores	21	440	470	41	210 - 880	a
Invertebrate-eaters	37	370	410		130 - 950	a
Vertebrate-eaters	32	400	420	22	260 - 730	a

* species not included in analysis due to small ($n < 5$) sample size.
[1] species/trophic group not sharing a common letter are significantly different ($p < 0.05$).

4 DISCUSSION

4.1 Analytical data

Data on chromium and nickel concentrations in pectoral muscle and liver are not reported because the samples were contaminated by chrome-plated steel from the homogeniser.

Feathers ground in the Cullatti mill (also steel) did not result in Cr or Ni contamination. Manganese contamination from either the steel homogeniser or the mill was not evident. Reference materials were analysed as received, and not treated with the homogeniser or the mill, contamination was deduced on the basis of results from another study (Noller unpublished data) using freshwater mussel samples which had been processed using the same 'Ika Werk' Ultraturrax homogeniser.

The accuracy and precision of the determinations of all other metals in all other tissues and organs was satisfactory. Zinc determinations were least precise but are within the quality standards usually accepted for this metal. Analytical data for cadmium and lead are the most accurate and are highly precise. Quality control data are discussed in Appendix 2. Uranium determination was not readily available at the time this work was undertaken and was not included.

4.2 Intraspecific, interspecific and trophic group variation

The levels of metals, and their patterns of variability, found in the tissues and organs of waterfowl from the Magela Creek flood plain were generally comparable with those reported from waterfowl elsewhere in uncontaminated situations. Cadmium and lead, the two metals for which no physiological requirement has been demonstrated, were present in very low concentrations compared with results from other studies. Unexpectedly, the mean zinc concentrations in each tissue and organ examined for each species greatly exceeded other published data. The authors have no explanation for this result.

As most waterfowl in the region are highly mobile it would be impossible to determine precisely where accumulated metals may have originated. An alternative stategy to destructive sampling of waterbird populations is to identify the main pathways through which waterbirds may acquire metals and then to monitor these food sources. An examination of the diets of the birds in this study having the highest liver concentrations of the various metals indicates which food items contribute most to a bird's metal uptake. These results are summarised in Tables 22, 23, 24 and 25.

4.3 Cadmium

DiGuilio & Scanlon (1984*a*) reported mean concentrations of cadmium in liver from 13 species of wild ducks of fresh and brackish waters. Species means ranged from 3.1 to 0.64 μg/g dry. Only one of their freshwater species had a mean level of greater than 2.0 μg/g dry. Parslow et al. (1982), who also analysed duck liver, found mean cadmium levels in 8 species of less than 2.0 μg/g dry. Hall & Fisher (1985) measured cadmium levels in the feathers of 6 species of ducks. Mean concentrations ranged from 0.09 to 0.58 μ/g dry. There are no reports of cadmium concetntrations in muscle from ducks.

Cattle egrets *Bubulcus ibis* have been reported with mean cadmium levels in muscle of 0.064 μg/g dry and 0.089 μg/g dry (Hulse et al. 1980, Cheney et al. 1981 respectively). The liver concentrations of cadmium in these birds were 0.42 μg/g dry and 0.44 μg/g dry respectively. Cheney et al. (1981) reported mean cadmium concentrations in muscle and liver from Louisiana herons *Hydranassa tricolor* of 0.068 μg/g dry and 0.29 μg/g dry respectively. For black-crowned night heron *Nycticorax nycticorax* livers, concentrations of cadmium ranging from the detection limit to 0.64 μg/g dry were reported by Custer & Mulhern (1983). Honda et al. (1985) reported that eastern great white egrets, *Egretta alba*

Table 22. Species ordered from maximum to minimum by total metal content in liver

Species	Dietary group	Liver concentration ranking 1 = highest Cd	Cu	Pb	Mn	Zn	Overall ranking
Pacific black duck	Omni	3	3	3	5	3	1
Black-winged stilt	Invt	1	7	2	3	5	2
Wandering whistling duck	Herb	4	2	4	14	1	3
Darter	Vert	6	12	5	4	2	4
Little pied cormorant	Vert	8	5	12	1	6	5
Green pygmy goose	Herb	8	1	14	6	10	6
Comb-crested jacana	Omni	14	15	1	2	8	7
Little egret	Vert	9	4	9	11	9	8
Whiskered tern	Invt	2	9	7	9	15	9
Magpie goose	Herb	7	13	6	13	4	10
Intermediate egret	Invt	13	6	13	8	7	11
Masked lapwing	Omni	12	10	8	12	11	12
Rufous night heron	Vert	10	11	10	10	14	13
Pied heron	Invt	11	5	11	15	13	14
Little black cormorant	Vert	15	14	15	7	12	15

Table 23. Species of each dietary group showing the highest liver concentration of five metals

Metal	Species showing highest liver concentration	Dietary Group	Liver Concentration (μg/g dw) GM	AM	Range
Cadmium	Black-winged stilt	Invt	1.3	1.5	0.23 - 2.33
	Black duck	Omni	0.27	0.32	0.11 - 0.69
	Wandering whistling duck	Herb	0.23	0.36	0.02 - 0.64
	Little pied cormorant	Vert	0.21	0.35	0.06 - 1.18
Copper	Green pygmy goose	Herb	2500	3600	200 - 6500
	Black duck	Omni	280	340	53 - 530
	Little egret	Vert	180	240	69 - 500
	Pied heron	Invt	120	130	73 - 300
Lead	Comb-crested jacana	Omni	0.42	0.56	0.18 - 1.4
	Black-winged stilt	Invt	0.42	0.64	0.16 - 2.0
	Wandering whistling duck	Herb	0.23	0.28	0.10 - 0.65
	Darter	Vert	0.20	0.24	0.08 - 0.42
Manganese	Little pied cormorant	Vert	61	64	38 - 110
	Comb-crested jacana	Omni	60	63	29 - 80
	Black-winged stilt	Invt	58	61	42 - 88
	Green pygmy goose	Herb	48	50	29 - 73
Zinc	Wandering whistling duck	Herb	630	650	380 - 800
	Darter	Vert	560	570	420 - 730
	Black duck	Omni	550	600	250 - 880
	Black-winged stilt	Invt	550	580	370 - 920

Table 24. Summary of tests for differences in metal concentrations between tissues in each diet group

Dietary Group	Cadmium	Copper	Lead	Manganese	Zinc
Herbivores	F[a](2,130)=31.4 P << 0.001 Liver > muscle = feather	F(2,133)=242 P << 0.001 Liver > muscle = feather	F(2,134)=219 P << 0.001 Feather > liver > muscle	F(2,134)=135 P << 0.001 Liver > feather > muscle	F(2,133)=151 P << 0.001 Liver > muscle = feather
Invertebrate-eaters	F(2,143)=34.9 P << 0.001 Liver > muscle = feather	F(2,146)=284 P << 0.001 Liver > muscle = feather	F(2,142)=192 P << 0.001 Feather > liver > muscle	F(2,148)=187 P << 0.001 Liver > feather > muscle	F(2,148)=66.0 P << 0.001 Liver > muscle = feather
Omnivores	F(2,78)=8.52 P < 0.001 Liver > muscle = feather	F(2,79)=62.0 P << 0.001 Liver > muscle = feather	F(2,78)=103 P << 0.001 Feather > liver > muscle	F(2,80)=123 P << 0.001 Liver > feather > muscle	F(2,78)=90.9 P << 0.001 Liver > muscle = feather
Vertebrate-eaters	F(2,108)=22.7 P << 0.001 Liver > muscle = feather	F(2,109)=212 P << 0.001 Liver > muscle = feather	F(2,106)=123 P << 0.001 Feather > liver > muscle	F(2,106)=313 P << 0.001 Liver > feather > muscle	F(2,111)=174 P << 0.001 Liver > muscle = feather

[a] ANOVA F(df,df)

Table 25. Summary of regression analyses of relationships between metal levels in feathers, muscle and liver

	Total regressions	Total significant ($p < 0.05$)	Distribution of r^2's of significant regressions 0-0.1	0.1-0.25	0.25-0.50	0.50-0.75	>0.75
All Birds							
Muscle with feather	5	1	1				
Liver with feather	5	2	1	1			
Liver with muscle	5	3	2	1			
Total	**15**	**6**	**4**	**2**			
Dietary Groups							
Muscle with feather	20	0					
Liver with feather	20	3		2	1		
Liver with muscle	20	10		4	5	1	
Total	**60**	**13**		**6**	**6**	**1**	
Species							
Muscle with feather	75	3				1	2
Liver with feather	65	2			1	1	
Liver with muscle	65	6			1	3	2
Total	**205**	**11**			**2**	**5**	**4**

modesta contained mean cadmium levels of up to 0.05 μg/g dry in feathers and 0.007 μg/g dry in pectoral muscle.

Our findings are mostly comparable to these results. For each of our duck species, however, mean cadmium concentrations for both feathers and liver were below the ranges of means found in other studies. In a recent review, Scheuhammer (1987) suggested that liver cadmium concentrations below 3 μg/g dry are indicative of normal environmental exposure in most adult, wild, freshwater duck species.

4.4 Chromium

Very little has been published on chromium levels in birds. Hall & Fisher (1985) recorded mean chromium levels in feathers ranging from 0.2 to 5.5 μg/g dry from 6 species of freshwater ducks and only one of their species exceeded 1 μg/g dry. The mean chromium levels we found exceed most of these by a factor of about 5. The significance of this is unclear given the paucity of published information.

4.5 Copper

Copper levels in liver show considerable variability both within and between species. Eight of the 9 duck species studied by Parslow et al. (1982) had mean concentrations in liver of less than 120 μg/g dry. The pochard *Aythya ferina* though, showed a mean of 600 μg/g dry with individuals as high as 1200 μg/g dry. Parslow et al. (1982) reported that mean liver copper concentrations decreased as the amount of animal food in the diet of the birds increased. Nearly all of the species of ducks examined by DiGuilio & Scanlon (1984*a*) had less than 100 μg/g dry of copper in liver but the mean for the ring-necked duck *Aythya collaris* was over 260 μg/g dry. Copper concentrations in the liver samples of their species varied typically from just a few μg/g dry to several hundred μg/g dry. Custer & Mulhern (1983) found a mean copper level in the livers of black-crowned night herons of 257 μg/g dry and cite a sample of mute swans, *Cygnus olor*, from an uncontaminated area in which

30% of the birds had liver copper concentrations exceeding 1000 μg/g wet (=several thousand μg/g dry). Custer et al. (1986) found a mean copper level in the livers of common terns, *Sterna hirundo*, of 29 μg/g dry and livers from brown pelicans, *Pelecanus occidentalis*, averaged 24 μg/g dry (Ohlendorf et al. 1985). The only mean copper level from muscle known to us is 3.7 μg/g wet in the eastern great white egret (Honda et al. 1985). Mean copper levels in the feathers of waterfowl are typically less than 50 μg/g dry (Hall & Fisher 1985, Honda et al. 1985, Parker 1985).

Our results displayed trends in variability similar to those recorded in the literature. Most species had mean liver levels below 100 μg/g dry, a few showed mean copper concentrations of several hundred μg/g dry, and green pygmy geese had a mean liver concentration of over 3500 μg/g dry wt. There was also great variation amongst individuals within each species. We confirmed that the herbivorous waterfowl had significantly more copper in liver than the other dietary groups. Of particular interest were the copper concentrations in the livers of the green pygmy goose and the wandering whistling duck. Both these species feed in deep water upon the seeds of macrophytes. Parslow et al. (1982) reported that wheat and barley seed contained high levels of copper compared to the other plant foods eaten by the ducks in their study. Other reseachers have usually performed metal analyses only on whole plants if attempts to describe levels of environmental exposure have been undertaken (White et al. 1979, DiGuilio & Scanlon 1985). The copper concentrations we found in muscle and feathers were comparable with levels found in other, albeit limited, studies.

4.6 Lead

Eighty-six percent of the 287 liver samples from 9 species of waterfowl examined by Parslow et al. (1982) contained lead concentrations of less than 0.6 μg/g dry. White et al. (1979) and Bagley & Locke (1967) each investigated lead levels in liver in canvasbacks *Aythya valisineria* and recorded mean concentrations of 0.14 - 0.25 μg/g wet and 0.5 μg/g wet respectively. Mean lead concentrations in the livers of other ducks and geese studied by Bagley & Locke (1967) ranged from 0.5 to 1.5 μg/g wet. For ducks and geese a background lead concentration in muscle of 0.082 μg/g wet is reported for the mottled duck, *Anas fulvigula* (Montalbano et al. 1983). Hall & Fisher (1985) recorded mean lead levels in the feathers of 6 species of ducks ranging from 0.2 to 22 μg/g dry.

For other types of waterfowl, egrets and herons typically had less than 3.0 μg/g dry of lead in liver (Bagley & Locke 1967, Hulse et al. 1980, Cheney et al. 1981, and Custer & Mulhern 1983); less than 0.5 μg/g dry lead in muscle (Hulse et al. 1980, Cheney et al. 1981 and Honda et al. 1985) and less than 3.0 μg/g dry in feathers (Honda et al. 1985). Ohlendorf et al. (1985) studied brown pelicans in which they found mean lead levels of 0.74 μg/g dry and 0.9 μg/g dry in liver and feathers respectively.

The mean tissue concentrations of lead in our study are among the lowest ever to have been reported in waterfowl, a fact presumably attributable to the remoteness of the Alligator Rivers Region from sources of industrial pollution and also to its history of relative isolation from recreational shooting. For adult birds, mean concentrations of lead in liver ranging from 0.5 to 5.0 μg/g dry are considered normal from uncontaminated areas (Scheuhammer 1987).

4.7 Manganese

The literature on manganese levels in birds is exceedingly sparse. Custer et al. (1986) and Hulse et al. (1980) examined manganese concentrations in the livers of common terns, and cattle egrets and laughing gulls *Larus atricilla* respectively. Concentrations of less than 20 μg/g dry were found. Levels in muscle for the cattle egret were less than 2.0 μg/g dry. This result is similar to that of Honda et al. (1985), who reported manganese concentrations in muscle for the eastern great white egret. Mean manganese concentrations in feathers of less than 30 μg/g dry were reported by Hall & Fisher (1985) for 6 species of duck, and by Honda et al. (1985) for the eastern great white egret.

There are too few published data on manganese levels in muscle and liver in birds for a meaningful comparison to be made. Our results from manganese levels in feathers are similar to those reported in the literature above.

4.8 Nickel

Mean nickel concentrations in feathers ranged from 0.2 to 4.0 μg/g dry among 5 of the 6 species of duck examined by Hall & Fisher (1985), and the results from other studies are similar (Ranta et al. 1978, Scanlon et al. 1979, Rose & Parker 1982, Honda et al. 1985, Parker 1985). Only the gadwall *Anas strepera* showed a mean feather nickel concentration (17 μg/g dry) above this range (Hall & Fisher 1985).

All of the species we examined had nickel concentrations in feathers comparable to those reported above. It is of interest to note however, that Ranta et al. (1978) examined the feathers of mallards *Anas platyrhynchos* and black ducks *Anas rubripes* in an area contaminated by nickel fallout and reported a mean feather nickel level of 5.3 μg/g dry. So in some respects many of our species had nickel levels more characteristic of those from this contaminated area overseas. There are clearly insufficient data to allow a meaningful comparison.

4.9 Zinc

Parslow et al. (1982) and DiGuilio & Scanlon (1984*a*) collectively examined zinc levels in 496 liver samples from 19 species of freshwater ducks. Mean concentrations for each species ranged from 100 μg/g to 200 μg/g dry. The mean zinc concentration in liver of canvasbacks studied by White et al. (1979) also fell within this range (41 μg/g wet, about 150 μg/g dry), as did the level found by White & Cromartie (1985) in the livers of green-winged teal *Anas crecca* (31 μg/g wet, about 110 μg/g dry). No zinc levels in muscle from any ducks or geese were found in the literature, and only one record of zinc in feathers (Ranta et al. 1978) reported a mean of 120 μg/g dry in mallards and black ducks. Among other waterfowl, young black-crowned night herons had mean liver concentrations ranging from 500 to 650 μg/g dry (Custer & Mulhern 1983). Other studies report liver levels within the same range as found for the ducks. These included the brown pelican (Ohlendorf et al. 1985), green-backed heron *Butorides striatus* (Neithammer et al. 1985), American avocet *Recurvirostra americana* and black-necked stilt *Himantopus mexicanus* (White & Cromartie (1985), and American coot *Fulica americana* (White et al. 1986). Honda et al. (1985) found mean zinc levels in the muscle of the eastern great white egret to be 21 μg/g wet (about 77 μg/g dry). Feather levels ranged between 100 and 130 μg/g dry. Brown pelicans had a mean feather concentration of 67 μg/g dry.

Compared to the literature, the zinc levels found in this study were high. The lowest of our species means for zinc in both liver and muscle were higher than any from the literature except that for immature black-crowned night herons. This result is unexpected given that environmental concentrations of zinc in the Alligator Rivers Region are low (Hart et al. 1981, Morley et al. 1984). Furthermore, the tissue concentrations are elevated for all species spanning a full spectrum of dietary positions. Even in areas known to be contaminated by zinc (Hutton & Goodman 1980, Custer et al. 1986) liver levels in birds do not exceed 240 μg/g dry.

4.10 Metal concentrations in feathers - use as predictors

Monitoring heavy metal levels in birds through the use of feather samples is an attractive option because the bird does not need to be killed to obtain a sample. This is an important consideration especially when samples are needed from birds within National Parks. To assess the worth of feathers as a monitor of metals in birds it is first necessary to establish which metals accumulate in feathers and secondly, important to find out whether feather metal levels can be quantitatively related to levels in either liver or muscle.

Mean metal concentrations in the feathers, muscle and liver were not equivalent for any species. There appeared to be pronounced differences in concentrations between tissues in each species; furthermore, the pattern of variation between tissues appeared the same for all species for each metal. All species were grouped by dietary similarity and tested to establish the significance of this pattern of tissue variation. Table 24 presents a summary of the results of these tests. Cadmium, copper, manganese and zinc concentrations were significantly higher in liver than in either muscle or feathers. The lead concentration in feathers was significantly higher than in liver which in turn had concentrations significantly higher than muscle. Overall, metal concentrations in feathers were lower than those for liver but higher than for muscle. For lead, it appears that feathers are the best substance to sample.

Linear regressions were used to establish whether the metal levels in feathers could be predictably related to those in either muscle or liver. Relationships between muscle and liver were also tested. For each metal the birds were: (i) treated as a single group; (ii) grouped by dietary similarity; and (iii) grouped by species. The results of the 280 regressions are summarised in Table 25. Details of all significant ($p<0.05$) regressions are presented in Tables 3 and 4. Only about 7% of all the regressions involving feather with either muscle or liver were significant and few of these had any practical predictive capacity. Significant regressions between feathers and the other tissues occurred most often (about 30%) when the birds were treated as single group . However, these represented little more than broad correlations, as in most cases less than 10% of the total variation was explained. In contrast, when the birds were grouped by species, typically with small sample sizes, only 5 out of 140 regressions (3.5%) between feathers and either muscle or liver were significant. Far more of the regressions between muscle and liver were significant (about 21% overall).

It is apparent that metal levels in feathers could not be used with confidence to predict the metal levels in either muscle or liver. Furthermore, lead levels in feathers can be used as an example to highlight one of the major drawbacks of using feathers to monitor metal levels: that of not knowing precisely where a metal may have come from. Goede & de Bruin (1984) showed that metals can be deposited in bird's feathers (i) during feather growth through internal deposition, (ii) through contamination by a bird's secretions during preening, and (iii) through contamination by contact with the environment. They concluded, using results from wading birds, that most lead in feathers was deposited during preening. Feathers immersed in lead-contaminated water did not acquire lead but, as has been found for copper and nickel (Ranta et al. 1978), lead may be adsorbed onto feathers from the air. Lead levels in feathers may also vary with age as well as in different parts of the feather (Doi & Fukuyama 1983, Dmowski 1984, Fisher & Hall 1984, Honda et al. 1985). Unless each of these factors can be accounted for then the interpretation of the results from a program of monitoring using feathers would be extremely difficult. In conclusion, metal levels in feathers may not reflect the degree of physiological exposure that a bird has been subject to; their use as monitors of metals in waterbirds is therefore limited.

4.11 Birds as food - human health risks

The Magela Creek flood plain remains an area throughout which Aboriginal people with traditional ties to the region may hunt. Waterfowl figure prominently in their diets during some seasons. The magpie goose is probably the most actively sought-after of the waterbirds but plumed and wandering whistling ducks, green pygmy geese, Pacific black ducks, grey teal and radjah shelducks are also abundant and may be taken.

It was expected that metal levels in all tissues would fall comfortably within the recommended national food standards prescribed for meat and offal products (National Health & Medical Research Council, 1986). This, in general, was true.

Perhaps the two most important metals are lead and cadmium. Of all the metals tested these are the only two that are not essential micro-nutrients. They tend to accumulate in humans throughout life or have very long retention times once ingested. The concentrations of lead and cadmium were exceptionally low in both liver and muscle when compared with national food standards.

There are no specific standards developed to cover the levels of chromium, nickel and manganese in foodstuffs in Australia. Furthermore, there have not been any reports of toxic effects in humans or other animals from these metals related to ingestion of food (Roberts 1981). A far greater literature describes the effects of diets deficient in them. Chromium related conditions are rare in nature and have only been found in people working in enclosed industrial situations where inhalation of chromium dust leads to lung disorders (Langard & Norseth 1979).

The zinc levels in the muscle of ducks and geese were well within the standard for 'other foods' (150 $\mu g/g$ wet or about 520 $\mu g/g$ dry), although zinc levels in the liver of almost all ducks marginally exceeded it. Dietary exposure of up to 1000 $\mu g/g$ wet of zinc is permitted for oyster tissue and all liver concentrations in this study were well below this. Zinc is a readily metabolised essential element, and again medical conditions in humans related to deficiencies rather than of excesses dominate the literature (Roberts 1981).

Comparison of the copper levels in liver and muscle with the food standards gave an unexpected result. Muscle was within the standards (100 $\mu g/g$ wet or about 370 $\mu g/g$ dry), but the mean copper level in the liver of both the green pygmy goose and the wandering whistling duck greatly exceeded it. The mean copper concentration for green pygmy goose liver was more than 10 times greater than the maximum recommended. In practical terms this result is almost certainly inconsequential. Green pygmy goose livers have probably been eaten in traditional aboriginal diets for generations without sign of toxic effects. Roberts (1981) states the fatal dose of copper as 200 mg/kg body weight. To acquire this a young child (about 20 kg weight) would have to consume a wet weight of pygmy goose liver of about 3.5 kg, or the livers from about 35 geese. This would need to be consumed in a single sitting as elevated copper levels quickly fall when the intake is ceased (Piscator 1979). A sustained dietary exposure to copper such as this would be extremely unlikely unless other dietary items also proved to contain similarly high copper levels. Copper toxosis has only rarely been seen in humans, invariably as a result of industrial accidents in confined spaces where inhalation of copper-rich dust has occurred, or due to attempted suicide by ingestion of copper sulphate solutions (Piscator 1979).

Species of waterfowl hunted for food by traditional Aboriginal residents in the region proved to be safe to eat when metal concentrations in pectoral muscle were compared to food standards prescribed by national health authorities. However, copper concentrations in liver from two species, the green pygmy goose and wandering whistling duck, exceeded recommended standards by a factor greater than 10. It is unlikely that eating the liver of either of these species could result in reduced health unless it could be shown that most items in the total diet also had high copper levels.

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APPENDIX 1

Determinations for seven metals in standard reference materials and bulk bird tissue samples.

Table 26. Determination of cadmium, chromium, copper, lead, manganese, nickel and zinc in standard reference materials including some comparisons of direct calibrations and standard addition techniques

Metal	Technique	Certificate Value	n	Mean ± SD	Precision (% RD)	Accuracy (% RE)
1. NBS SRM 1566 'Oyster Tissue'						
Lead	Graphite Furnace AAS					
(μg/g dry wt)	Direct	0.48 ± 0.04	4	0.46 ± 0.05	11	4.2
Nickel	Graphite Furnace AAS					
(μg/g dry wt)	Direct	1.03 ± 0.19	8	1.0 ± 0.04	3.5	1.0
	Std Addn		8	0.89 ± 0.12	13	14
Chromium	Graphite Furnace AAS					
(μg/g dry wt)	Direct	0.69 ± 0.27	8	0.29 ± 0.02	6.9	43
	Std Addn		8	0.31 ± 0.03	10	41
Manganese	Graphite Furnace AAS	17.5 ± 1.2	5	20 ± 3	15	20
(μg/g dry wt)	Direct					
2. NBS SRM 1577 'Bovine Liver'						
Lead	Graphite Furnace AAS					
(μg/g dry wt)	Direct	0.34 ± 0.08	41	0.46 ± 0.10	22	35
Cadmium	Graphite Furnace AAS					
(μg/g dry wt)	Direct	0.27 ± 0.04	8	0.27 ± 0.01	3.7	< 0.1
Copper	Graphite Furnace AAS					
(μg/g dry wt)	Direct	193 ± 10	7	180 ± 2.4	1.3	6.7
	Std Addn		7	210 ± 4.4	2.1	7.8
	Flame AAS					
	Direct		14	190 ± 18	9.4	3.6
Manganese	Graphite Furnace AAS					
(μg/g dry wt)	Direct	10.3 ± 1.0	9	12 ± 1	8.3	20
	Flame AAS					
	Direct		18	8.6 ± 0.9	11	17

Table 26 cont/d.

Metal	Technique	Certificate Value	n	Mean ± SD	Precision (% RD)	Accuracy (% RE)
Zinc (μg/g dry wt)	Flame AAS Direct	130 ± 13	16	130 ± 5	4.0	0.77
3. IAEA H-4 Animal Muscle						
Copper (μg/g)	Graphite Furnace AAS Direct	3.96 ± 0.35	20	3.1 ± 0.3	9.7	22
	Flame AAS Direct		5	2.8 ± 0.2	7.1	27
Manganese (μg/g)	Graphite Furnace AAS Direct	0.52 ± 0.04	6	0.53 ± 0.04	8.3	8.0
	Flame AAS Direct		5	0.48 ± 0.13	27	7.7
Zinc (μg/g)	Flame AAS Direct	86 ± 4	10	79 ± 7	8.9	8.1

Note: n = number of determinations
RSD = relative standard deviation
RE = relative error
Direct = direct calibration technique
Std Addn = standard addition technique

Table 27. Determination of cadmium, chromium, copper, lead, manganese, nickel and zinc in bulk bird tissue samples including comparison of direct calibration and standard addition techniques

		Feather $\mu g/g$ dry wt	Liver $\mu g/g$ wet wt	Muscle Tissue $\mu g/g$ wet wt
A. Graphite Furnace AAS				
Cadmium	Direct	0.021 ± 0.002 (n = 9)	0.061 ± 0.002 (n = 10)	0.0027 ± 0.0004 (n = 10)
	Std Addn	0.019 ± 0.012 (n = 7)	0.063 ± 0.043 (n = 6)	0.0037 ± 0.0007 (n = 10)
Chromium	Direct	17 ± 0.5 (n = 10)	1.5 ± 0.04 (n = 10)	3.5 ± 0.28 (n = 10)
	Std Addn	16 ± 2.9 (n = 9)	1.3 ± 0.12 (n = 9)	2.7 ± 0.35 (n = 9)
Copper	Direct	6.9 ± 0.60 (n = 5)	-	5.1 ± 0.1 (n = 5)
	Std Addn	6.4 ± 0.40 (n = 5)	-	5.7 ± 0.37 (n = 10)
Lead	Direct	0.70 ± 0.11 (n = 23)	0.043 ± 0.016 (n = 23)	0.0068 ± 0.0006 (n = 9)
	Std Addn	0.62 ± 0.09 (n = 5)	0.049 ± 0.010 (n = 5)	0.018 ± 0.015 (n = 5)
Manganese	Direct	-	-	1.2 ± 0.06 (n = 10)
	Std Addn	-	-	0.92 ± 0.11 (n = 9)
Nickel	Direct	5.0 ± 0.20 (n = 10)	2.1 ± 0.04 (n = 10)	4.1 ± 0.19 (n = 10)
	Std Addn	3.8 ± 0.23 (n = 10)	1.3 ± 0.27 (n = 9)	3.4 ± 0.60 (n = 9)
B. Flame AAS				
Copper	Direct	5.9 ± 0.7 (n = 6)	34 ± 0.3 (n = 3)	-
	Std Addn	4.1 ± 2.7 (n = 4)	34 ± 5 (n = 3)	-
Manganese	Direct	13 ± 3 (n = 5)	2.8 ± 0.03 (n = 5)	-
	Std Addn	15 ± 1.0 (n = 6)	2.9 ± 0.6 (n = 3)	-
Zinc	Direct	120 ± 5 (n = 5)	31 ± 0.3 (n = 3)	12 ± 0.9 (n = 6)
	Std Addn	81 ± 33 (n = 4)	38 ± 3 (n = 3)	14 ± 0.95 (n = 4)

Note: Dry/wet ratios for the bulk bird tissues were as follows:
feather 0.889, liver 0.770 and muscle tissue 0.279

APPENDIX 2

Sample preparation, technique details and quality control data

Methods of Preparation

Preparation of homogenised samples

Feathers were ground using a Cullatti DCFH48 Mill. Samples were fed into the grinder without the sieve plate for an initial size reduction and the ground material was collected in plastic tubes. These plastic tubes were rinsed with 10% nitric acid solution and high purity water prior to use. Samples were then fed into the grinder with a medium sieve plate in position and transferred to sterile 100 mL plastic containers. Feather pieces were pushed from the hopper into the grinding chamber using an acid washed polyethylene rod. The grinder was cleaned using acid-washed brushes and compressed air to blow out any smaller pieces. Between samples a feather from the following sample was passed through the grinder. The sieve plate was acid washed after each sample. Periodically the grinder was disassembled during sample preparation and all components excepting the actual grinding body were acid washed, dried and re-assembled.

Liver and muscle tissues were extracted using sterile techniques, weighed and placed in food-grade polyethylene tubing, heat-sealed and frozen. Prior to homogenisation the plastic bags were cut using a sterile surgical blade and samples were removed using acid washed Teflon forceps. Samples were placed in acid washed polycarbonate vials and homogenised using a Janke and Kunke 'Ika Werk' Ultraturrax homogeniser. The homogeniser spindle was washed under a jet of high purity water from a wash bottle and remaining fragments of tissue in the spindle were removed using a pair of fine pointed stainless steel forceps. The spindle was then washed in Decon 90 detergent solution, rinsed with high purity water, 10% nitric acid solution, further high purity water and dried by hand with filter paper before use with tissues. A section of bench was reserved for the tissue homogenisation in a room requiring entry through two doors. The surface of the bench was covered with Benchkote (Whatman) with the plastic surface up. The surface was cleaned, successively, with detergent solution, high purity water, 10% nitric acid solution and finally high purity water before and between sample homogenisations. PVC gloves with no talc were worn and washed externally in a similar manner to the Benchkote surface by persons undertaking this activity.

Preparation of sample digest solutions

Homogenised undried tissues were weighed into clean 20 mL pyrex test tubes with 10 mL calibration marks. Typical sample weights used were 0.25 g for feathers, and 0.5-1.0 g for liver and muscle tissues, respectively as received. Feathers were weighed into tared pyrex test tubes and each sub-sample in each test tube was dried in an oven at 80°C overnight, cooled in a dessicator and then reweighed. The weight difference was used to calculate the wet/dry tissue ratio. It was anticipated that the wet/dry tissue ratio would be more variable between samples of bird feathers than for either liver or muscle tissue samples. Accordingly, an average wet/dry tissue ratio was used for liver and muscle tissues using the weight difference following drying at 80°C of bulk tissue samples from a variety of birds.

One mL nitric acid, BDH Aristar grade, and 1 drop of double-distilled kerosene was added to each test tube plus sample. Blanks were treated similarly. The test tubes were covered with a piece of Nescofilm and allowed to stand overnight. The following day test tubes were placed in a heated aluminium block set to 90°C and the contents were allowed to reflux gently. Following sufficient digestion, the test tubes were removed, allowed to cool and the volume was made up to the 10 mL mark with high purity water (Millipore, Super Q grade). The contents of each test tube were mixed using a vortex mixing, transferred to clean 15 mL polypropylene tubes with caps and stored frozen.

All apparatus was soaked overnight in Decon 90 solution following use, rinsed with high purity water and soaked in 10% nitric acid solution one additional day before further use.

Chemical Analysis

The concentrations of various metals in tissue digest solutions were determined by either flame atomic absorption spectrometry (AAS) using a Varian Model 1475 AAS or graphite furnace AAS using a Perkin Elmer Model 5000 AAS, Model 500 HGA and Model AS40 Auto-sampler. Both AAS instruments had double beam optics and were equipped with automatic deuterium lamp background correctors.

Initial screening of metal concentrations in bulk tissue digests was undertaken by flame AAS to establish which elements were present at concentrations too low for determination by flame AAS. Elements at concentrations lower than flame AAS detection limits were determind by graphite furnace AAS. A summary showing metal, tissue, choice of AAS technique and if digest solution was diluted during analysis is shown in Table 28.

The analytical conditions for flame and graphite furnace AAS are given in Tables 29, 30 and 31. Some relevant details are summarised here. Background correction was applied to all flame and graphite furnace AAS measurements. Graphite furnace AAS heating parameters were developed for each combination of metal and sample matrix. Cadmium, chromium, copper, manganese and nickel were determined using standard pyrolytically coated graphite tubes whereas lead was determined using the L'vov platform inserted into a grooved uncoated graphite tube. Use of the L'vov platform for lead determination overcomes the classic chemical and spectral interferences associated with non-isothermal conditions generated by atomisation of lead off the walls of the standard graphite tube (Slavin & Manning 1980).

Working standard solutions for AAS determinations were prepared from BDH 'stock standard solutions for AAS' by serial dilution and were all acidified to the same concentration as sample digests with BDH Aristar grade nitric acid. Sample digest solutions were diluted to appropriate concentration ranges when required.

Accuracy and precision of results were checked by comparison with results from parallel digestions of US NBS Standard Reference Materials SRM 1566 'Oyster Tissue', SRM 1577 'Bovine Liver' and IAEA H4 'Animal Muscle'. These reference materials were not treated with either the homogeniser or the grinding mill that other samples were pre-processed with.

A comparison of direct calibration and standard addition determinations was undertaken for flame and graphite furnace AAS determinations of metals in standard reference materials and bulk samples of bird feather, liver and muscle tissues to establish if any interferences arose from the sample matrix of the various bird tissues. For purposes of methodology it is noted that all metals were determined using the direct calibration technique excepting for cadmium in bird muscle tissue digest solutions.

Reference

Slavin, W. & Manning, D.C. (1980). The L'vov platform for furnace atomic absorption analysis. *Spectrochim Acta* **35B**, 701-714.

Table 28. Choice of AAS technique for metal determinations in bird tissues including details of dilution

Metal	Feather	Liver	Muscle Tissue
Cadmium	Graphite Furnace (Diluted 1:10)	Graphite Furnace (Undiluted)	Graphite Furnace (Undiluted)
Chromium	Graphite Furnace (Undiluted)	Graphite Furnace (Undiluted)	Graphite Furnace (Undiluted)
Copper	Flame (Undiluted)	Flame (Undiluted)	Graphite Furnace (Undiluted)
Lead	Graphite Furnace (Diluted 1:10)	Graphite Furnace (Diluted 1:10)	Graphite Furnace (Undiluted)
Manganese	Flame (Undiluted)	Flame (Undiluted)	Graphite Furnace (Diluted 1:10)
Nickel	Graphite Furnace (Diluted 1:10)	Graphite Furnace (Diluted 1:10)	Graphite Furnace (Diluted 1:10)
Zinc	Flame (Undiluted)	Flame (Undiluted)	Flame (Undiluted)

Table 29. Flame AAS determinations of copper, manganese and zinc in bird tissue digest solutions

Setting or Condition	Copper	Manganese[a]	Zinc[a]
Wave length (nm)	324.7	279.5	213.9
Slit (nm)	0.5	0.2	0.5
Integration Time (sec)	3	3	3
Scale Expansion	x 1	x 1	x 1
Hollow Cathode Lamp Current (mA)	4.0	5.0	5.0
Double Beam in use?	Yes	Yes	Yes
Deuterium Background Correction in use?	Yes	Yes	Yes
Standards (mg/L)	0,0.5,1.0,2.5	0,0.5,1.0,2.0	0,1,2,5
Flame	Air/acetylene lean	Air/acetylene lean	Air/acetylene lean

Note a : Burner rotated if concentration too high.

Table 30. Graphite furnace AAS (HGA 500) heating parameters

Step	1 (DRY)	2 (ASH)	3 (ATOMIZE)	4 (CLEAN)
a) Cadmium - Bird feather, liver and muscle tissue digest solutions				
Temperature (°C)	110	450	1700	2700
Ramp Time (sec)	10	5	0	1
Hold Time (sec)	30	10	2	2
Internal Gas Flow (Arbitrary units)	300	300	0	300
b) Chromium - Bird feather, liver and muscle tissue digest solutions				
Temperature (°C)	110	1200	2700	2800
Ramp Time (sec)	10	10	0	1
Hold Time (sec)	20	10	5	2
Internal Gas Flow (Arbitrary Units)	300	300	50	300
c) Copper - Bird feather and muscle tissue digest solutions				
Temperature (°C)	110	900	2300	2700
Ramp Time (sec)	20	5	0	1
Hold Time (sec)	10	10	2	2
Internal Gas Flow (Arbitrary Units)	300	300	50	300

Step	1 (DRY)	2 (DRY)	3 (ASH)	4 (ASH)	5 (ATOMIZE)	6 (CLEAN)	7 (COOL)
d) Lead - Bird feather, liver and muscle tissue digest solutions							
Temperature (°C)	90	120	250	550	1700	2700	20
Ramp Time (sec)	10	10	20	5	0	1	1
Hold Time (sec)	5	20	10	30	6	4	10
Internal Gas Flow (Arbitrary)	300	300	300	300	0	300	300

Step	1 (DRY)	2 (ASH)	3 (ATOMIZE)	4 (CLEAN)
e) Manganese - Bird muscle tissue digest solutions				
Temperature (°C)	110	1100	2700	2800
Ramp Time (sec)	2	2	0	1
Hold Time (sec)	20	20	5	2
Internal Gas Flow (Arbitrary Units)	300	300	50	300
f) Nickel - Bird feather liver and muscle tissue digest solutions				
Temperature (°C)	110	1000	2700	2800
Ramp Time (sec)	10	10	0	1
Hold Time (sec)	30	10	5	2
Internal Gas Flow (Arbitrary Units)	300	300	50	300

Table 31. Settings and conditions for graphite furnace AAS. Determinations of cadmium, chromium, copper, lead, manganese and nickel in bird digest solutions

Setting or condition	Cadmium	Chromium	Copper	Lead	Manganese	Nickel
Wave length (nm)	228.8	357.9	217.9	283.3	279.5	232.0
Slit (low) (nm)	0.7	0.7	0.7	0.7	0.7	0.2
Read time (sec)	5	5	5	5	5	5
Kind of lamp	Electrode less Discharge Lamp	Hollow Cathode	Hollow Cathode	Electrode less Discharge Lamp	Hollow Cathode	Hollow Cathode
Lamp current (mA) or power (watts)	6 watts	25 mA	15mA	11 watts	10mA	25mA
Double beam in use?	Yes	Yes	Yes	Yes	Yes	Yes
Deuterium background corrector in use?	Yes	Yes	Yes	Yes	Yes	Yes
Inert shield gas	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen
Standards (μg/L)	0.0.15, 0.30, 0.45	0, 2, 12, 25	0, 2, 12, 25	0, 1, 2, 4	0, 5, 10, 20	0,15,30,45
Sample volume (μL)	50	15	20	40	10	30
Measure peak height/area	Height	Height	Height	Area	Height	Height

Quality Control Data

The precision and accuracy of bird digest data was assessed by examining the analytical results for various standard reference materials and bulk bird tissues. Precision was calculated as relative standard deviation (% RSD) and accuracy as relative error (% RE), the percentage difference from the certified result.

Satisfactory precision and accuracy was achieved for all metals in standard reference materials excepting for chromium. This element gave good precision but poor accuracy attributed to losses during the digestion step. Other aspects observed were a deterioration of precision and accuracy at lower concentration for copper, better precision and accuracy for lead in U.S. N.B.S. 'Oyster Tissue' compared with U.S. N.B.S. 'Bovine Liver' (more typical of bird tissue) and better precision for manganese in U.S. N.B.S. 'Bovine Liver' compared with U.S. N.B.S. 'Oyster Tissue'. Thus methodology appeared to give satisfactory results with standard reference materials but was further examined to determine if any unusual matrix effects arose in bird tissue digests.

A comparison of results for the determination of metals in composite bird tissues by both direct calibration and standard additions technique was made. No significant difference was observed for lead in any of the three tissues and other metal-tissue combinations excepting for the following:

i) cadmium in muscle tissue was higher by the standard addition technique and was determined by the standard additions technique;

ii) chromium and copper in muscle tissue were higher by the standard additions technique;

iii) manganese in muscle tissue was lower by the standard additions technique;

iv) nickel in feather tissue was lower by the standard additions technique; and

v) zinc in feather tissue was lower by the standard additions technique but the precision of this technique was very poor.

The differences in metal concentration between direct calibration and standard additions techniques, respectively, for copper and manganese in muscle tissue and nickel and zinc in feather tissue were further examined to see if any of the observed differences in metal concentrations were of significance to bird tissue data treatment. For each metal-tissue combination, the range of sample metal concentration far exceeded the concentration difference between direct calibration and standard additions technique results. Thus it was considered satisfactory to use direct calibration metal concentration data.

On the basis of results from another study (Noller unpubl. data), using freshwater mussel samples which had been processed using the 'Ika Werk' homogeniser, it was concluded that chromium and nickel contamination may have occurred in muscle and liver samples These results are not reported.

SUPERVISING SCIENTIST FOR THE ALLIGATOR RIVERS REGION

RESEARCH PUBLICATIONS

Alligator Rivers Region Research Institute Research Report 1983-84
Alligator Rivers Region Research Institute Annual Research Summary 1984-85
Alligator Rivers Region Research Institute Annual Research Summary 1985-86
Alligator Rivers Region Research Institute Annual Research Summary 1986-87
Alligator Rivers Region Research Institute Annual Research Summary 1987-88
Alligator Rivers Region Research Institute Annual Research Summary 1988-89
Alligator Rivers Region Research Institute Annual Research Summary 1989-90
Alligator Rivers Region Research Institute Annual Research Summary 1990-91 (in press)

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RR 3	A limnological survey of the Alligator Rivers Region. I. Diatoms (Bacillariophyceae) of the Region. August 1983 (pb, mf - 160 pp.)	D.P. Thomas
	*A limnological survey of the Alligator Rivers Region. II. Freshwater algae, exclusive of diatoms. 1986 (pb, mf - 176 pp.)	H.U. Ling & P.A. Tyler
RR 4	*Ecological studies on the freshwater fishes of the Alligator Rivers Region, Northern Territory. Volume I. Outline of the study, summary, conclusions and recommendations. 1986 (pb, mf - 63 pp.)	K.A. Bishop, S.A. Allen, D.A. Pollard & M.G. Cook
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	Ecological studies on the freshwater fishes of the Alligator Rivers Region, Northern Territory. Volume III. Autocology. (in press)	K.A. Bishop, S.A. Allen, D.A. Pollard & M.G. Cook
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