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Trace Metal Concentrations in Nile Tilapia (*Oreochromis niloticus*) in Three Catchments, Sri Lanka

G. Allinson · S. A. Salzman · N. Turoczy · M. Nishikawa · U. S. Amarasinghe · K. G. S. Nirbadha · S. S. De Silva

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Abstract Samples of the muscle and liver of the Nile tilapia (*Oreochromis niloticus*) were obtained from a single reservoir in each of three Sri Lankan catchments (Kaudulla, Rajanganaya, and Udawalawe reservoirs in the Mahaweli, Kala Oya, and Walawe Ganga river basins, respectively) in 2002. The concentrations of 12 elements were consistently detected in the tilapia muscle and liver (Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, P, Sr and Zn). However, a three factorial principal components analysis suggested that there were no differences in the metal profiles (range of elements and concentration) of the fish obtained from any of the three reservoirs, although the chemistries of each tissue (muscle and liver) were different. Metal concentrations were below WHO and Food Standards Australia and New Zealand guideline values, and substantial quantities of tilapia would need to be consumed each week on a regular basis to exceed intake limits (e.g. more than 1.5 kg to exceed intake lits for Cu), suggesting consumption of tilapia from these reservoirs poses little risk to human health.

Keywords Sri Lanka · Artisanal fisheries · Tilapia · Metals · Reservoirs

G. Allinson (✉)
Future Farming Systems Research Division,
Department of Primary Industries Queenscliff Centre,
PO Box 114, Queenscliff, VIC 3225, Australia
e-mail: graeme.allinson@dpi.vic.gov.au

S. A. Salzman
School of Information Systems, Deakin University,
PO Box 423, Warrnambool, VIC 3280, Australia

N. Turoczy · S. S. De Silva
School of Ecology and Environment, Deakin University,
PO Box 423, Warrnambool, VIC 3280, Australia

M. Nishikawa
Laboratory for Intellectual Fundamentals for Environmental Studies, National Institute for Environmental Studies,
16-2 Onogawa, Tsukuba, Ibaraki 305, Japan

U. S. Amarasinghe · K. G. S. Nirbadha
Department of Zoology, University of Kelaniya, Kelaniya 11600, Sri Lanka

S. S. De Silva
Network of Aquaculture Centres in Asia-Pacific, PO Box 1040,
Kasetsart Post Office, Bangkok 10903, Thailand

Reservoir fish are the main source of animal protein for rural people living in the dry zone of Sri Lanka (annual precipitation less than 1870 mm; Amarasinghe and De Silva 1999), and a common feature of this fishery is the dominance in the landings of exotic cichlids, including the Nile tilapia, *Oreochromi niloticus*. Fish assimilate metals by ingestion of particulate material suspended in water, ingestion of food, ion exchange of dissolved metals across lipophilic membranes e.g. the gills, and adsorption on tissue and membrane surfaces. There is, however, a paucity of studies on metals in freshwater fish in Sri Lanka, and consequently limited understanding of the human health risks associated with consumption of reservoir fish.

In one of only two previous studies evaluating metal concentrations in Sri Lankan reservoirs, Duncan et al. (1993) evaluated the concentrations of Cu, Zn, Pb, and Cd in the sediment of an the ancient reservoir (Parakrama Samudra), but did not correlate sediment concentrations with those in local fish. Allinson et al. (2002) determined the concentration of sixteen elements in samples of the Mozambique tilapia (*Oreochromis mossambicus*) obtained from five reservoirs (Badagiriya, Chandrikawewa, Kiribbanara, Meegahajandura and Ridiyagama in two

catchments (Malala Oya and Walawe Ganga)) in southern Sri Lanka in 1998. A number of elements (As, Ca, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Sr and Zn) were consistently detected in the muscle and liver tissue (Cd was detected in only a few liver samples), with the data suggesting that the populations of tilapia (and consequently their human consumers) in the reservoirs may be exposed to different regimes of metals, possibly associated with different catchment land-use patterns.

In the present study, we revisited the potential risk to human health in Sri Lanka through consumption of reservoir fish through a survey of metal concentrations in the Nile tilapia (*Oreochromis niloticus*) obtained from three Sri Lankan reservoirs in three catchments in 2002. The concentrations of sixteen elements (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr and Zn) were determined, and, where relevant, compared to the maximum values permitted by the Australian Food Standards Code, and the human health implications for heavy consumers of this food are discussed.

Materials and Methods

Nile tilapia (*Oreochromis niloticus*) were obtained from three reservoirs in three Sri Lankan catchments (Kaudulla, Rajanganaya, and Udawalawe reservoirs in the Mahaweli, Kala Oya, and Walawe Ganga river basins, respectively) in 2002. These reservoirs were selected for ease of access and also to cover a wide range of size, age and catchment features. In all instances, fish samples were obtained from commercial landings and brought to the laboratory in ice. In the laboratory, the total length and body weight were determined. Thereafter, the fish were gutted, and the liver removed and weighed. Individual fish were filleted, and from the middle of the right fillet a block of muscle, devoid of skin and bone, was taken. Muscle and liver samples were oven dried at 80°C to a constant weight, and stored in air-tight vials until further analysis. Fish were divided into four length categories (17–20, 20–22, 22–23 and 23+ cm, respectively), and pooled within categories. The samples were ground to a powder using a mortar and pestle.

For metals other than mercury, tissue samples were digested in open vessels using a mixture of nitric acid/perchloric acid. Fish samples, and quality control samples (blanks and certified reference material (CRM), specifically National Institute of Standards and Technology (NIST) CRM No. 1566a Oyster Tissue, and NIST CRM No. 1577b Bovine Liver) were digested in six batches of 12–15 samples, with two CRM samples and a reagent blank digested with each batch (the oyster tissue CRM was digested with tilapia muscle samples, bovine liver CRM with tilapia liver samples, respectively). Deionised water having a resistivity of at least 18 MΩ cm^{-1} was produced by passing singly distilled water through a Milli-Q Water Purification System. In short, nitric acid (68% w/v Ultrapure analytical reagent grade; Tama Chemicals Co. Ltd, Kawasaki City, Japan; 5 mL) and perchloric acid (70% w/v Ultrapure analytical reagent grade; Tama Chemicals Co. Ltd, Kawasaki City, Japan; 1 mL) were added to the sample (approximately 50 mg accurately weighed into a 50 mL glass beaker), and the mixture heated gently on a hot-plate for 2 h. When the reagent blanks were processed, nitric acid quantities were adjusted to maintain similar treatment volumes in samples and blanks. The acid volume was allowed to decrease by ~90% during heating, although the mixtures were not heated to dryness. The digests were allowed to cool to room temperature, following which a small (~1 mL) aliquot of nitric acid (1% w/v) was added, and the solution filtered through 0.2 μm cellulose acetate disposable syringe filters (Sartorius CE Minisart RC15, Sartorius, Germany). The digest volume was then quantitatively made up to 6 mL with nitric acid (1% w/v) prior to analysis.

Digests were analysed using two inductively coupled plasma atomic emission spectrophotometers. Calcium (Ca), Cd, Cu, Fe, K, Mg, Mn, Na, Sr, and Zn concentrations were measured using an IRIS ICP-61E (Thermo Jarrell Ash, Japan), with the following analytical wavelengths monitored: Ca, 393.366; Cd, 228.802; Cu, 324.754; Fe, 238.204; K, 796.896; Mg, 279.553; Mn, 257.61; Na, 588.995; Ni, 231.604; Sr, 407.771; and Zn, 213.856 nm, respectively. Aluminium (Al), As, P, Pb and Sn concentrations were determined using a Spectroflame-EOP (Spectro Analytical Instruments, USA), with the following analytical wavelengths monitored: Al, 396.152; As, 189.04; P, 178.29; Pb, 168.22; and Sn, 189.98 nm, respectively.

Mercury concentrations were determined after vaporisation of tissue samples (tilapia muscle and liver, and CRM (NIST CRM No. 1566a Oyster Tissue)) and reagent blanks in the vaporisation chamber of a Mercury Atomiser MA-1S (Nippon Instruments, Japan) connected to an external Mercury Detector MD-1 (Nippon Instruments, Japan).

Instrument limits of determination (LOD) were: Sr, 0.004; Cd, Mg, Mn, 0.005; Cu, Ni, P, 0.01; Ca, Hg, Sn, Zn, 0.02; As, Fe, 0.03; Pb, 0.06; Al, 0.2; and K, Na, 1 mg L-1, respectively. The method detection limits (MDL) were based on instrumental limits of determination (LOD), which in turn are based on mean ± 10 standard deviations for the digested blanks (American Public Health Association 1995), and were 120 × LOD values.

All statistical analysis was conducted using Microsoft Excel, SPSS version 14.0 for windows (SPSS Inc, Chicago), or XLStatistics 2008. For assessment of differences between independent groups, where the assumptions associated with the application of parametric statistical

methodologies were met, overall differences were identified using a one way analysis of variance, with subsequent post hoc analysis conducted using t-tests. Where assumptions were not met, the nonparametric analogue to the ANOVA, the Kruscal–Wallis rank test was used, with subsequent post hoc analysis conducted using the Mann–Whitney test. In some instances, data transformation using the natural log was successful, yet according to Levenes test, the homogeneity of variance assumption was violated. Due to this uncertainty in similarity between population variance equivalence, and some small sample sizes, the Games Howell test was used for subsequent post hoc multiple comparisons. Regression and correlation was used to assess for concentration changes with changes in fish length. Factor Analysis was also used as a data reduction technique to identify if there were any subsets of the dependent variables. Principal Components was the extraction method used.

Results and Discussion

The concentrations of 23 elements were certified by the NIST in one or both of the CRMs used, namely Ag, As, Ca, Cd, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Mo, Na, P, Pb, Rb, S, Se, Sr, and Zn. However, of these, Ag, Cl, Co, Cr, I, Mo, P, Rb, S, and Se were not measured. Analysis of the certified reference materials found most trace elements to be within 25% of expected values (75–120% recovery; Table 1), with the exception of Fe in NIST CRM No. 1566a (67%), and As and Sr in NIST CRM No. 1577b (>200%).

The concentrations of 12 elements were consistently detected in the tilapia muscle and liver (Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, P, Sr and Zn; Table 2). Values quoted are on a wet weight basis, and have not been corrected for analyte recoveries from certified reference materials. Values quoted, therefore, represent a conservative evaluation of metal concentrations. The need to pool between six and ten individual fish samples in each size class in order to obtain enough sample for the acid digestions unfortunately meant that the survey was essentially an un-replicated basket survey. That said, by considering each size class as a sample, we were able to determine that there were no statistically significant correlations between tilapia length and metal concentration in muscle tissue for any of the three reservoirs ($p > 0.05$). This was to be expected, since freshwater fin fish are known to maintain constant internal metal concentrations, with the result that concentrations of essential elements do not increase with age (Chapman et al. 1996). There was, however, a significant linear relationship between fish length and calcium concentration in the liver ($p < 0.000$), i.e. as fish get longer, the concentration of calcium in the liver decreases. A three factorial principal components analysis suggested that there were no differences in the metal profiles (range of elements and concentration) of the fish obtained from any of the three reservoirs, although the chemistries of each tissue (muscle and liver) were different.

Table 1 Summary of observed and certified metal concentrations in the CRMs used in this study

Element	NIST CRM #166a, Oyster Tissue		NIST CRM #1577b, Bovine Liver	
	Certified value	Observed value (n = 15)	Certified value	Observed value (n = 12)
	(weight % dry weight)			
Ca	0.196	0.144 ± 0.019	0.012	0.014 ± 0.005
K	0.79	0.713 ± 0.145	0.994	0.816 ± 0.165
Mg	0.118	0.093 ± 0.018	0.06	0.051 ± 0.009
Na	0.417	0.363 ± 0.052	0.242	0.205 ± 0.024
	(mg kg^{-1} dry weight)			
As	14	12.2 ± 3.5		
Cd	4.15	4.4 ± 1.1	0.5	n.d
Co	0.57	n.d	0.57	n.d
Cr	1.43	n.d	1.43	n.d
Cu	66.3	62 ± 11	160	151 ± 23
Fe	539	363 ± 43	184	150 ± 22
Hg	0.064	0.061 ± 0.008		
Mn	12.3	9.7 ± 1.3	10.5	8.6 ± 1.2
Sr	11.1	9.5 ± 1.1	0.14	0.69 ± 1.6
Zn	830	766 ± 98	127	111 ± 16

n.d, not determined

Table 2 Summary of metal concentrations in muscle and liver of *O. niloticus* (all As <limits of determination (LOD); n.d. not determined)

Catchment	Reservoir	Fish length (cm)	Metal concentration (mg kg^{-1} wet weight)											
		MDL	Ca 0.6	Cd 0.15	Cu 0.30	Fe 0.9	Hg 0.005	K 30.0	Mg 0.2	Mn 0.15	Na 30.0	P 0.3	Sr 0.1	Zn 0.6
			Muscle											
Mahaweli	Kaudulla	17–20	248	<LOD	0.67	5.3	n.d.	4125	265	0.59	353	2005	0.5	4.1
		20–22	200	0.2	1.12	2.8	n.d.	3250	225	0.53	370	1808	0.4	4.0
		22–23	218	<<LOD	0.88	2.7	n.d.	3450	253	0.48	410	1933	0.4	5.2
		23+	140	<LOD	1.22	1.9	n.d.	3475	241	0.54	438	1690	0.2	4.0
Kala Oya	Rajanganaya	20–22	133	<LOD	0.83	3.3	0.006	4750	313	<LOD	443	2150	0.3	5.7
		22–23	143	<LOD	0.96	7.7	0.007	4750	315	0.18	460	2100	0.4	5.3
		23+	190	<LOD	0.48	4.0	0.007	4625	305	<LOD	400	2078	0.4	4.8
Walawe Ganga	Udawalawe	15–17	222	<LOD	0.77	4.1	0.017	3850	270	0.54	408	1768	0.4	4.7
		17–20	205	<LOD	0.97	6.4	0.019	3875	273	0.60	360	1955	0.4	4.0
		20–22	190	<LOD	2.13	8.9	0.018	3675	258	0.80	408	1730	0.3	4.2
		22–23	130	<LOD	2.33	5.4	0.025	4100	238	0.58	340	1870	0.2	4.6
		23+	178	<LOD	0.80	5.8	0.016	4000	253	0.59	328	1948	0.4	4.7
			Liver											
Mahaweli	Kaudulla	17–20	350	0.78	153	265	0.018	1385	93	11.2	1003	1833	0.5	18.3
		20–22	250	0.32	188	342	0.015	1510	123	18.3	1178	1940	0.6	19.2
		22–23	210	0.30	147	233	0.012	1495	101	13.0	1135	1865	0.5	18.5
		23+	203	0.32	183	248	0.014	1478	98	13.8	1168	1723	0.5	16.6
Kala Oya	Rajanganaya	20–22	250	<LOD	45	207	0.011	2355	200	7.1	1475	2675	1.8	23.3
		22–23	183	<LOD	68	322	0.015	2485	164	7.6	1528	2625	1.0	24.1
		23+	145	<LOD	65	350	0.009	2460	158	11.4	1680	2600	0.9	21.6
Walawe Ganga	Udawalawe	15–17	n.d.	n.d.	n.d.	n.d.	0.032	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		17–20	275	<LOD	488	837	0.043	1910	159	30.9	1413	2385	1.1	23.7
		20–22	208	0.74	733	1525	0.045	1632	162	42.7	1377	2385	0.8	27.6
		22–23	228	1.14	440	562	0.076	1550	177	30.8	1553	2498	0.8	28.1
		23+	125	0.93	645	785	0.046	2675	159	16.7	1210	2415	0.4	27.3

Sri Lanka has one of the highest densities of reservoirs in the world, and continues to develop reservoir fisheries in the country's dry zone. There is clear evidence from reservoirs in temperate regions that Hg concentrations in the water column and sediments, and thence fish, rise after impoundment. Temperate reservoir fish tend to have relatively high levels of Hg, e.g. up to about 0.5 mg kg^{-1} wet weight, although the Hg concentrations are observed to stabilise at a slightly lower level as the reservoir ages (Rosenberg et al. 1995; Heckey et al. 1992). The Hg concentrations observed in this study (Table 2) are broadly consistent with those reported in *O. mossambicus* obtained from five reservoirs (Badagiriya, Chandrikawewa, Kiribbanarawewa, Meegahajandura and Ridiyagama) in southern Sri Lanka in 1998 (Allinson et al. 2002), and with those reported by Yingcharoen and Bodlay (1993) in four fish species from three reservoirs in Thailand (0.06–0.59, <0.01–0.12, 0.02–0.63, and <0.01–0.08 mg kg^{-1} wet weight in *Pristolepis* (herbivorous), *Puntioplites* (omnivorous), *Hampala* (predatory) and *Morulius* (herbivorous) species, respectively). However, in this study there were no correlations between fish tissue Hg concentration and reservoir age, suggesting that reservoir Hg concentrations have already stabilised, an observation that is perhaps not too surprising given the age of some of the impoundments, e.g. Kaudulla and Minnneriya were first constructed in 273 and 276, respectively (reconstructed 1958, 1903), while Kiriibbanara Udawalawe are the youngest (having been constructed in 1969).

To the best of the authors' knowledge the Sri Lankan government has not established maximum permitted levels for metals in fish. Consequently, guideline values set by the WHO/FAO Joint Expert Committee on Food Additives (JECFA), and Food Standards Australia and New Zealand (FSANZ) have been used to assess the human health risk from consumption of fish taken from the reservoirs investigated. For instance, UN FAO Codex levels for metals in fish are 0.5 mg kg^{-1} and 1.0 mg kg^{-1} wet weight methylmercury in non-predatory and predatory fish, respectively, while FSANZ has established maximum

Table 3 Summary of dietary intakes for measured elements when consuming 60 g (the Sri Lanka daily average consumption of fish (Anon 1999)) of tilapia from southern Sri Lanka reservoirs and comparison with food standards

Element	Standards			This study[a]	
	JECFA (mg day^{-1})	FSANZ RDI (mg day^{-1})	FSANZ ML [GEL] (mg kg^{-1})	Conc. (mg kg^{-1})[b]	Intake (mg day^{-1})[c]
As	0.12[d]		2		
Ca		800		92	5.5
Cu	30[e]	3	[5]	3.2	0.2
Fe		12		7.4	0.4
Hg			1	0.004	0.0003
Mg		320		137	8.2
Mn		5		0.4	0.02
Pb	3.6		0.5		
Zn	1000	12	[130]	2.6	0.2

JECFA, WHO/FAO Joint Expert Committee on Food Additives; FSANZ, Food Standards Australia and New Zealand; RDI, Recommended Daily Intake; ML [GEL], Maximum Level [Generally Expected Level] in fish; [a] Pooled *Oreochromis nilioticus* data; [b] Estimated whole fish concentration assuming liver and muscle represent 1 and 50% of wet-weight of whole fish, respectively, and converting to wet weight concentrations by dividing dry weight concentrations in these tissues (Table 2) by 4; [c] Calculated from a weekly intake of 420 g tilapia; [d] Calculated from JECFA provisional tolerable weekly intakes (PWTI); [e] Calculated from JECFA provisional maximum tolerable daily intake (PMTDI)

levels (ML) for several metals in fish (inorganic-As, Cu, Hg, and Pb; FSANZ 2006; Table 3). Generally expected levels (GELs) have also been established by FSANZ to complement MLs, and although not legally enforceable, they do provide a benchmark against which to measure contaminant levels in organisms (FSANZ 2005). Of the three elements for which FSANZ has established MLs (As, Hg, Pb), Pb was not determined in this study, while As concentrations must have been less than 3.75 mg kg^{-1} (our method detection limits) in these animals, since the concentrations of this element in the digests was <LOD (Note: this still leaves the possibility that As concentrations are higher than ML (1 mg kg^{-1}). Mercury concentrations were found to be well below FSANZ ML (Table 3). Zinc and Cu concentrations in the tilapia were well below their respective GELs.

Sri Lankans consume an average of 21.0 kg of fish per person per year (Anon 1999). Contamination of fish is therefore of particular concern to both the fisheries industries and general public. The daily intake of metals via consumption of tilapia is below the JECFA provisional maximum tolerable intakes when using the standard assumption that a 60 g portion is consumed each day. Indeed, consumption of at least 900 g of tilapia per day on a regular basis would be required to exceed the FSANZ recommended dietary intake limits for Cu (although this is increased substantially if the liver is not consumed), and at least 3.6 kilograms per day to exceed intake limits for Zn. This is considered highly unlikely for all but very heavy consumers of tilapia. The FSANZ has also established recommended daily intakes (RDIs) for a number of other elements (Table 3). Dietary intake from consumption of 60 g of the tilapia investigated in this study would, however, provide negligible amounts of Ca, Fe, Mg, and Mn (FSANZ 2005). These results suggest that, in short, the tilapia (*O. niloticus*) appear uncontaminated by metals, and no adverse health affects are anticipated from moderate consumption of these fish, whatever the source.

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References

Allinson G, Nishikawa M, De Silva SS, Laurenson LJB, De Silva K (2002) Observations on metal concentrations in tilapia (*Oreochromis mossambicus*) in reservoirs of south Sri Lanka. Ecotoxicol Environ Saf 51:197–202. doi:10.1006/eesa.2001.2112

Amarasinghe US, De Silva SS (1999) Sri Lankan reservoir fishery: a case for introduction of a co-management strategy. Fisheries Manage Ecol 6:387–399. doi:10.1046/j.1365-2400.1999.00170.x

American Public Health Association (1995) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association, Washington DC

Anonymous 1999. Sri Lanka Fisheries Year Book (1999) Socio-economic and Marketing research Division, National Aquatic Resources research and Development Agency, Colombo, Sri Lanka, 62 pp

Chapman PM, Allen HE, Godtfredsen K, Z'Graggen MN (1996) Evaluation of bioaccumulation factors in regulating metals. Environ Sci Technol 30:448A–452A

Duncan A, Gunatilaka A, Schiemer F (1993) Limnological aspects of landscape management in Sri Lanka. In: Erdelen W, Preu C, Ishwaran N, Madduma Bandara CM (eds) Ecology and landscape management in Sri Lanka. Margraf Scientific Books, Weikersheim, pp 381–395

FSANZ (2005) Generally Expected Levels (GELs) for metal contaminants. Additional Guidelines to Maximum Levels in Standard 1.4.1 – Contaminants and Natural Toxicants. Food Standards Australia New Zealand. January 2005. http://www.foodstandards.gov.au/_srcfiles/GELs_0801.pdf. Last Accessed May 2006

FSANZ (2006) Australia New Zealand Food Standards Code, Standard 1.4.1 – Contaminants and Natural Toxicants. Food Standards Australia New Zealand. http://www.foodstandards.gov.au/foodstandardscode. Last Accessed May 2006

Hecky RE, Ramsey DJ, Bodaly RA, Strange NE (1992) Increased methyl mercury contamination in fish in newly formed freshwater reservoirs. In: Suzuki T, Imura A, Clarkson TW (eds) Advances in mercury toxicology. Plenum Press, New York, pp 33–52

Rosenberg DM, Bodlay RA, Usher PJ (1995) Environmental and social impact of large scale hydro-electric development: who is listening? Global Environ Chang 5:127–148. doi:10.1016/0959-3780(95)00018-J

Yingcharoen D, Bodlay RA (1993) Elevated mercury levels in fish resulting from reservoir flooding in Thailand. Asian Fisheries Sci 6:73–80