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Trace element content of fish feed and bluegill sunfish (*Lepomis macrochirus*) from aquaculture and wild source in Missouri

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Abstract

Trace element content of fish feed and bluegill sunfish muscles (*Lepomis macrochirus*) from aquaculture and natural pond in Missouri were determined using the inductively coupled-plasma optical emission spectrometer (ICP-OES) and the direct mercury analyzer (DMA). Dietary intake rates of trace elements were estimated. Dogfish muscle (DORM-2) and lobster hepatopancreas (TORT-2) reference standards were used in trace element recovery and method validations. The average elemental concentrations (mg/kg diet, dry wt.) of fish feed were: As 1.81, Cd 2.37, Co 0.10, Cr 1.42, Cu 8.0, Fe 404, Mn 35.9, Ni 0.51, Pb 9.16, Se 1.71, Sn 20.7, V 0.09, Zn 118 and Hg 0.07. The mean elemental concentrations (μg/kg wet wt.) in bluegill muscles from both aquaculture and wild (in parenthesis) sources were: As 0.36 (0.06), Cd 0.28 (0.01), Co 0.0 (0.0), Cr 0.52 (0.05), Cu 0.38 (0.18), Fe 17.5 (2.43), Mn 0.18 (0.24), Ni 0.18 (0.04), Pb 1.03 (0.04), Se 0.34 (0.30), Sn 0.66 (0.42), V 0.02 (0.01), Zn 6.97 (9.13) and Hg 0.06 (0.24). Kruskal–Wallis chi square indicated significant differences in As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, V, Zn and Hg ($P < 0.001$), Se ($P < 0.01$) and Mn ($P < 0.05$) across the sampling locations. Dietary intake rates, estimated from weekly consumption of 228 g of aquaculture and wild bluegills, posed no health risks for approximately 85% of all samples.

Keywords: Aquaculture; Fish feed; Bluegill (*Lepomis macrochirus*); Trace element; Reference dose (RfD); Provisional tolerable weekly intake (PTWI)

1. Introduction

The global contribution of fish as a source of protein is high, ranging from 10% to 15% of the human food basket across the world (Wilson, Corraze, & Kaushik, 2007). Wild fish contributes to the global fish supplies, but this source is limited in some regions due to degraded ecosystems or over fishing. Aquaculture contribution to the global fish production is on the increase in many countries as human population increases. Statistics suggest that aquaculture production increased from 12 million tonnes in 1986 to 34 million tonnes in 1996 with output valued at US$47 billion (Dar, 1999). Fish consumption has increased in importance in various regions of the world and the past decade has recorded more interest in the quality of fish and fishery products (Çelik & Oehlenschläger, 2007; Fabris, Turoczy, & Stagnitti, 2006; Ikem & Egiebor, 2005; Kojadinovic, Potier, Le Corre, Cosson, & Bustamante, 2007). Fish provide omega−3 ($n-3$) fatty acids and essential elements necessary for adequate human nutrition. Omega−3 ($n-3$) fatty acids are particularly beneficial to both heart health (Ruxton, Calder, Reed, & Simpson, 2005) and those at high risk or suffering cardiovascular disease (Domingo, 2007).

Trace elements such as manganese, cobalt, iron, nickel, vanadium, copper, zinc and selenium, are considered essential (FAO, 2004) for fish development but toxicities may manifest at high concentrations. Non-essential elements in fish are unregulated and they perform no biological roles (Kojadinovic et al., 2007). The United States agency for toxic substances and disease registry (ATSDR) classified mercury, lead, cadmium, and arsenic as potentially toxic to human health due to their known or suspected toxicity (ATSDR, 2006).

The bluegill sunfish (*Lepomis macrochirus*) is widely caught from freshwaters by anglers and is presently not

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commercially available in local seafood markets or supermarket chains. The bluegill is the most abundant sunfish, and widespread introductions have increased its range in North America, Europe and South Africa. The diet of bluegill sunfish includes insects, crustaceans, mollusks and small fish. Sunfish have been key components in farm ponds throughout the United States. They are usually caught during leisure by anglers, stocked in ponds as forage fish, and used in toxicology research (Morris & Mischke, 2003).

Lincoln University of Missouri aquaculture research farm is funded by the United States Department of Agriculture (USDA) to research and grow bluegills for possible commercialization in future. For the first time, a preliminary assessment of the concentrations of trace elements in the muscles of bluegills from Lincoln University aquaculture research facilities and the potential risks to humans were evaluated. The objectives of this study were: (i) to determine the concentrations of trace elements in fish feed used at Lincoln University aquaculture facility and evaluate its toxicity; (ii) to determine the concentrations of trace elements in edible bluegill muscles from aquaculture ponds and wild source in Missouri and compare with regulatory thresholds; and (iii) to estimate dietary intake rates of trace elements from aquaculture and wild bluegill sunfishes and the potential human health risks from a single weekly consumption of 8 oz (228 g) of fish muscle. Bluegill muscles were assessed for trace elements toxicity since they are usually the most consumed part by humans.

2. Materials and methods

2.1. Bluegill sampling

Native bluegill fingerlings (F1 generation) from Osage Fisheries, Missouri were raised to maturity (weight: 220 g) and crossed in a pond to produce another set of fingerlings (F2 generation) at Lincoln University aquaculture research facility. The F2 generation bluegills were then divided into two groups; one group was grown at Carver research farm and the other was raised at the in-door aquaculture research facility at Busby farm. Both groups were fed artificial feeds until maturity. Additional bluegill samples were collected for assessment from a wastewater holding pond receiving Carver aquaculture used water. Bluegill sunfish samples were caught with fish nets in October 2006. To also assess trace element content of bluegills from the wild, another set of samples were earlier caught using artificial bates from a rock quarry pond near McClung Park in Jefferson city, Missouri in October 2005. Overall, the number of bluegill samples collected for trace elements analysis and the sampling locations were as follows: Busby in-door tank ($n = 20$), Carver out-door pond ($n = 16$), wastewater pond ($n = 17$) and rock quarry pond ($n = 17$). The length and weight of all individuals were recorded immediately before removal of muscle samples for analysis. Edible muscle portions of bluegill samples were removed with a stainless steel knife and placed in coded zip loc bags. All coded samples were transported to the laboratory in coolers containing ice. Samples were frozen immediately at −80 °C in the laboratory. Fish feed pellets were also randomly collected from the feed bags at the aquaculture research facility for elemental analysis. Most of the aquaculture bluegill samples analyzed were males because most females were important in breeding of new fingerlings. Thus, trace elements variation with sex was not considered in this study.

2.2. Apparatus

An Ethos EZ microwave labstation (Milestone Inc., Shelton, CT 06484, USA) was used for all sample digestion. The Varian Vista PRO inductively coupled plasma-optical emission spectrometer (ICP-OES) (Varian Inc., Walnut Creek, CA 94598, USA) was used for the measurement of trace elements except mercury. The ICP-OES condition used for this study was as follows: view mode: axial; detector: charge array; RF power: 1.2 kW; gas: argon; plasma flow: 15 l/min; auxiliary flow: 1.5 l/min; nebulizer flow: 0.75 l/min; instrument stabilization delay: 15 s; pump rate: 15 rpm; sample uptake delay: 70 s; number of replicates: 3; read time: 5 s; read: peak height; rinse time: 30 s. The direct mercury analyzer (DMA) from Milestone Inc. was used for total mercury analysis. The operating condition for the mercury analysis was as reported in a previous experiment (Ikem & Egiebor, 2005).

2.3. Reagents, calibration standards and certified reference materials

Milli-Q water (resistivity of 18.2 MΩ) was used for sample dilutions, rinses and preparation of diluted standards. The reagents used were trace metal grade concentrated nitric acid and hydrogen peroxide (30%) from Fisher Scientific (St. Louis, Missouri, USA), and ICP tune and calibration solutions from SPEX Certiprep, Inc. (Metuchen, NJ, USA). Standard reference material (SRM 1640: trace elements in natural water) was purchased from the National Institute of Standards and Testing (NIST) Gaithersburg, MD 20899, USA. Other reference materials (DORM-2: dogfish muscle for trace metals and elemental species and TORT-2: lobster hepatopancreas for trace metals) were from the National Research Council, Halifax, Nova Scotia, Canada. The SRM samples were used in recovery and method validation experiments. All glassware and polyethylene containers in contact with sample digests were previously washed with metal-free soap, rinsed many times, soaked in 50% nitric acid for 24 h and finally rinsed with deionized water.

2.4. Bluegills processing and analyses

Fish samples were removed from the freezer and allowed to thaw at laboratory room temperature. Muscle samples of each bluegill collected were removed with the aid of a stainless knife previously washed in dilute nitric acid and rinsed with deionized water. A known sample portion (3 g of fish muscle; wet wt.) was digested in a microwave labstation with 15 ml of concentrated nitric acid and 2 ml of 30% hydrogen peroxide. The microwave digestion program used followed the five steps listed: 25–96 °C for 5 min at 1000 W; 96 °C for 5 min at 1000 W; 140 °C for 2 min at 1000 W; 140 °C for 2 min at 1000 W; 180 °C for 3 min at 1000 W and held at 180 °C for 5 min. Digests were allowed to cool and then quantitatively removed into cleaned 25 ml volumetric flasks and diluted to volume with deionized water. Digests were stored in previously washed 60 ml polyethylene bottles at 4 °C until used for elemental analysis with ICP. Reagent blanks and certified reference materials (DORM-2 and TORT-2) were also prepared according to the procedure used for the bluegill samples. The concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Cu), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), tin (Sn), vanadium (V) and zinc (Zn) in digests of bluegill muscles, fish feed and certified reference standards were measured after calibration of the ICP instrument with appropriately diluted mixed standard.

For the total mercury (Hg) determination in fish muscle and feed samples, DORM-2 and TORT-2 were used to calibrate the DMA and for method validation. To ensure that the quartz sample boats did not contain mercury that could present an erroneous result, each cleaned quartz boat was analyzed empty through the mercury analysis programme involving thermal decomposition, amalgamation and atomic absorption spectrophotometric measurement at 254 nm. Fish muscle samples were accurately weighed into analyzed empty quartz boats and placed on the DMA auto sampler for mercury analysis.

2.5. Statistical analysis

The Shapiro–Wilk test (Statistix 8 for Windows, Analytical Software, Tallahassee, FL 32317, USA) revealed that the analytical data obtained in this study were non-normally distributed. Therefore, Spearman correlation was used to evaluate the degree of associations of the variables and the Kruskal–Wallis non-parametric procedure set at P value of 0.05 was used to assess differences between trace element concentrations in fish muscles from four sampling sources. Significant levels of Kruskal–Wallis test are indicated by asterisks according to the probability ranges: $^{*}p < 0.05$, $^{**}0.01$, and $^{***}0.001$. Principal component analysis (PCA) was conducted with the Unscrambler version 9.2 software (Camo Inc., Woodbridge, NJ, USA) to see the relationship of the bluegills data across the sampled locations.

3. Result and discussion

3.1. Standard reference materials

The percentage trace element recoveries (Table 1) from the standard reference materials (DORM-2 and TORT-2) were close to the provided certified values by the manufacturers. Recoveries for DORM-2 and TORT-2 ranged from 80–116% and 78–102%, respectively. The measured values of SRM 1640 used as an ICP check solution were also close to the certified values reported by NIST (data not reported).

3.2. Trace elements in aquaculture fish feed

A summary of trace element concentrations (mg/kg dry wt.) in aquaculture fish feed analyzed in this study was compared with published values (Table 2). The average values obtained for Cd, Hg, Se, Cu and Pb in feeds analyzed in this study were lower than the respective maximum tolerable value (ppm) recommended by the Association of Feed Control Officials guidelines (AFCO) for Cd: 0.5, Hg: 2, Se: 2, Cu: 25 and Pb: 30 (Hanks, 2000). The mean values observed for As, Cu, Pb, Zn and Hg in feeds in this

Table 1
Elemental concentrations (mg/kg dry wt.) in standard reference materials (DORM-2 and TORT-2)

Element	DORM-2 (Dogfish muscle)			TORT-2 (Lobster hepatopancreas)		
	Certified value[a]	Our value[a]	Recovery (%)	Certified value[a]	Our value[a]	Recovery (%)
Arsenic	18.0 ± 1.1	16.1 ± 0.1	89	21.6 ± 1.8	18.4 ± 0.4	85
Cadmium	0.04 ± 0.01	0.05 ± 0.01	116	26.7 ± 0.6	22.9 ± 0.1	86
Cobalt	0.18 ± 0.03	0.18 ± 0.04	99	0.51 ± 0.09	0.52 ± 0.11	102
Chromium	34.7 ± 5.5	37.81 ± 2.39	109	0.77 ± 0.15	0.75 ± 0.00	97
Copper	2.34 ± 0.16	2.09 ± 0.15	89	106 ± 10	98.6 ± 0.02	93
Iron	142 ± 10	134 ± 4	95	105 ± 13	90.0 ± 0.5	86
Lead	0.065 ± 0.007	0.06 ± 0.01	92	0.35 ± 0.13	0.30 ± 0.01	86
Manganese	3.66 ± 0.34	3.26 ± 0.09	89	13.6 ± 1.2	11.6 ± 0.2	85
Mercury	4.64 ± 0.26	4.40 ± 0.10	95	0.27 ± 0.06	0.27 ± 0.01	100
Nickel	19.4 ± 3.1	15.4 ± 0.4	80	2.5 ± 0.2	1.95 ± 0.02	78
Selenium	np	na	na	5.63 ± 0.67	4.87 ± 0.35	87
Vanadium	np	na	na	1.64 ± 0.19	1.49 ± 0.02	91
Zinc	25.6 ± 2.3	23.9 ± 0.8	93	180 ± 6	163 ± 6	91

np: Not provided; na: not analyzed.

[a] Means ± standard deviation.

Table 2
Summary of trace element concentrations (mg/kg dry wt.) in 9 feed samples collected from aquaculture research facility

	Summary	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Sn	V	Zn	Hg
This study ($n = 9$)	Mean	1.8	0.2	0.1	1.4	7.95	403.8	35.85	0.51	9.16	1.7	2.1	0.09	118	0.07
	SD	0.3	0.1	0.3	0.2	1.62	32.49	2.71	0.06	4.87	0.6	0.6	0.04	8.5	0.00
Fish hatcheries[a]	Mean	2.62	0.39		1.50	10.54	353.9	84.3	2.35	0.78	2.48		2.07	142.76	0.03
	SD	1.37	0.21		0.74	5.43	134.9	45.9	1.39	1.11	0.68		1.78	42.36	0.03
Alam et al. (2002)	Mean	13.3	0.1	BDL[d]	1.23	6.39	260	72.4	1.08	BDL	5.40			70.0	
	SD	0.7	0.001		0.19	0.35	2	1.8	0.002		0.59			1.4	
*Norse LT 94[b]		14	0.19			4				0.09				80	0.08
*EU's upper limits[c]		15	2			25				10				250	0.5
AFCO			0.5			25				30	2				2

* Source: Moren et al. (2006). SD = standard deviation.

[a] Result of 55 fish feeds from 11 National fish hatcheries between October 2001 and 2003 (Maule et al. (2007)).

[b] Data in mg/kg diet, dry wt.

[c] Data in mg/kg diet, 88% dry wt; AFCO: Association of feed control officials (Hanks (2000)).

[d] BDL: Below detection limit.

study were below the European Union (EU) upper tolerable limits but Cd was not comparable. Also the mean values observed for As, Cr, Cu, Mn, Ni, Se, V and Zn in feeds in this study were lower than the corresponding values reported for feeds from the National fish hatcheries (Maule, Gannam, & Davis, 2007). Furthermore, the mean values for As, Mn, Ni and Se in this study were also consistently lower than the corresponding values reported for other feeds (Alam et al., 2002). Fish feeds are made from various diverse sources and differences in their element content may be influenced by varying fortifications by manufacturers. Artificial feed, in particular, is known to contain contaminants (Maule et al., 2007; Moren et al., 2006) often associated with raw materials used in feed production and feed fortification materials. Therefore, feeds may contain varying amounts of toxic elements that can potentially affect fish health and also contribute to biomagnification of metals in fish representing various trophic levels in an ecosystem.

3.3. Trace elements in aquaculture and wild bluegill sunfishes

The average values obtained for aquaculture and wild bluegill samples are given in Table 3. The average values of As, Cd, Cr, Cu, Fe, Ni, Pb, Sn and V in muscles of bluegills from Busby farm were higher than the values obtained for muscles from other sample locations. Fig. 1 shows the representation (total variance loading of 96%) of the analytical data after mean normalization across the sample locations on two principal components (PC1 and PC2). The score plot revealed that the trace element content of bluegill samples from Busby aquaculture facility were generally different from values obtained for Carver, wastewater and rock quarry ponds. Also differences in the trace element content of bluegill muscles across the sample locations were evaluated using the Kruskal–Wallis Chi Square approximation method (one-way nonparametric analysis of variance). Results showed that the Kruskal–Wallis chi square values obtained for As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, V, Zn and Hg ($P < 0.001$) and Se ($P < 0.01$) and Mn ($P < 0.05$) in muscles were significantly different across the sites studied. Further tests with the Kruskal–Wallis all-pair wise comparisons ($\alpha = 0.05$) revealed that Cd, Cr, Fe, Sn and Pb values of the muscle samples from Busby farm were significantly different from the corresponding values reported for Carver farm, wastewater and rock quarry ponds. The levels of Cu and Co in the Busby muscles were higher than the levels observed for the rock quarry muscles and also the Ni contents in Busby muscles were significantly higher ($P < 0.05$) than values observed for Carver and rock quarry fish samples. However, the As, Zn and Hg contents of muscles from the rock quarry and wastewater ponds, on the one hand, and Busby and Carver, on the other, were found to be similar ($\alpha = 0.05$). The vanadium values obtained for muscles from Busby and wastewater sites were also similar. Differences in batches of feeds, feeding habits, quantity of feed or food consumed, age of fish and differing abilities to detoxify materials may have contributed to the variability observed between the sampling locations. The concentration of a trace element in fish is influenced by metal bioavailability, uptake and toxicokinetics (Burger et al., 2001; Spry & Wiener, 1991).

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. Excretion of metals occurs via the feces, urine, and respiratory membranes. The distribution of metals between the different fish tissues depends on the mode of exposure, i.e., dietary and/or aqueous exposure, and can serve as a pollution indicator (Alam et al., 2002).

The sequence of trace elements in muscles analyzed varied with sampling locations but zinc and iron were the highest in the sampling areas studied. The trends observed for fish muscles were as follows: Zn > Fe > Sn >

Table 3
Average elemental concentrations (mg/kg, wet wt.) in muscles of aquaculture and wild bluegills

Element	Aquaculture bluegills Carver pond ($n = 16$)	Aquaculture bluegills Busby in-door ($n = 20$)	Aquaculture bluegills Carver & Busby combined ($n = 36$)	Wild bluegills Waste water pond ($n = 7$)	Wild bluegills Rock quarry pond ($n = 17$)	Kruskal–Wallis χ^2 Degree of freedom: 3
Sex	Male/female	Male/female	Male/female	Male/female	Male/female	–
Length[a]	17.7 ± 4.4	20.8 ± 2.5 (NA)	19.3 ± 3.7	17.0 ± 0.5	14.5 ± 0.8	–
Weight[b]	171 ± 119	267 ± 110 (NA)	225 ± 123	77.4 ± 16.2	88.7 ± 17.4	–
As	0.26 ± 0.15	0.44 ± 0.16 (0.6 ± 0.19)	0.36 ± 0.18	0.03 ± 0.04	0.06 ± 0.03	48.84 (0.0000)***
Cd	0.00 ± 0.00	0.50 ± 1.13 (1.81 ± 4.45)	0.28 ± 0.87	0.00 ± 0.00	0.01 ± 0.03	47.38 (0.0000)***
Co	0.00 ± 0.00	0.00 ± 0.00 (1.97 ± 8.12)	0.00 ± 0.00	0.13 ± 0.42	0.00 ± 0.00	24.52 (0.0000)***
Cr	0.02 ± 0.02	0.93 ± 0.50 (26.33 ± 89.6)	0.52 ± 0.59	0.11 ± 0.18	0.05 ± 0.02	57.32 (0.0000)***
Cu	0.26 ± 0.11	0.48 ± 0.41 (1.51 ± 0.70)	0.38 ± 0.33	0.27 ± 0.12	0.18 ± 0.10	23.16 (0.0000)***
Fe	1.83 ± 0.77	30.1 ± 14.2 (82.0 ± 52.2)	17.54 ± 17.70	6.38 ± 4.66	2.43 ± 0.67	56.62 (0.0000)***
Mn	0.14 ± 0.04	0.20 ± 0.06 (0.72 ± 0.29)	0.18 ± 0.06	0.16 ± 0.06	0.24 ± 0.29	8.80 (0.0321)*
Ni	0.05 ± 0.05	0.28 ± 0.50 (0.17 ± 0.20)	0.18 ± 0.39	0.09 ± 0.06	0.04 ± 0.03	21.05 (0.0001)***
Pb	0.04 ± 0.05	1.83 ± 0.92 (8.78 ± 13.15)	1.03 ± 1.13	0.31 ± 0.86	0.04 ± 0.02	42.7 (0.0000)***
Se	0.30 ± 0.11	0.38 ± 0.29 (0.88 ± 0.24)	0.34 ± 0.23	0.19 ± 0.12	0.30 ± 0.07	13.18 (0.0000)**
Sn	0.36 ± 0.41	0.90 ± 0.26 (1.13 ± 0.93)	0.66 ± 0.43	0.51 ± 0.27	0.42 ± 0.15	26.39 (0.0043)***
V	0.00 ± 0.00	0.03 ± 0.02 (0.10 ± 0.06)	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	35.63 (0.0000)***
Zn	6.24 ± 0.75	7.56 ± 5.29 (19.80 ± 6.60)	6.97 ± 3.98	10.59 ± 2.13	9.13 ± 1.61	32.89 (0.0000)***
Hg	0.04 ± 0.02	0.07 ± 0.01 (0.03 ± 0.01)	0.06 ± 0.02	0.19 ± 0.09	0.24 ± 0.13	53.91 (0.0000)***

Data shown are arithmetic means with standard deviations of trace elements in bluegill muscles and liver (in parentheses) samples from Busby research farm. Also in parentheses are Kruskal–Wallis Chi Square P values.$^{*}\chi^2$ significant at the 0.05 level.$^{**}\chi^2$ significant at the 0.01 level.$^{***}\chi^2$ significant at the 0.001 level.

[a] Unit in cm.

[b] Unit in g; NA: not available.

Se > As = Cu > Mn > Ni > Hg = Pb > Cr > Cd = Co = V (Carver pond); Fe > Zn > Pb > Cr > Sn > Cd > Cu > As > Se > Ni > Mn > Hg > V > Co (Busby in-door tanks); Zn > Fe > Sn > Pb > Cu > Se > Hg > Mn > Co > Cr > Ni > As > V > Cd (wastewater pond); Zn > Fe > Sn > Pb > Cu > Se > Hg > Mn > Co > Cr > Ni > As > V > Cd (rock quarry pond).

Spearman correlations were conducted to ascertain the degree of associations of the variables with one another. There were no significant correlations ($P < 0.001$) either between fish length or fish weight with the trace elements analyzed in Busby bluegill muscles. A similar finding was published earlier (Afonso, Lourenço, Dias, Nunes, & Castro, 2007). However, positive correlations ($P < 0.001$) were observed between age and length of fish ($r^2 = 0.77$), and fish weight and length ($r^2 = 0.93$). For the Busby bluegill muscles, significant positive correlations (r^2 ranged from 0.4–0.72) were observed between several elements: Hg and V ($P < 0.05$) and Cu ($P < 0.05$); Zn and Cd ($P < 0.05$), Cu ($P < 0.05$), Mn ($P < 0.001$) and Ni ($P < 0.05$); Fe and Pb ($P < 0.01$); correlations also occured between Cd and Ni ($P < 0.05$), Sn ($P < 0.05$) and V ($P < 0.05$). For Carver bluegill samples, arsenic correlated positively ($r^2 > 0.72$, $P < 0.01$) with length and weight of fish. Other positive correlations noted were between Cd and Cr, Pb and V (r^2 ranged from 0.63–0.72, $P < 0.01$), and between Ni and Sn ($r^2 > 0.51$, $P < 0.05$). Furthermore, positive correlations between Fe and Ni ($r^2 > 0.68$, $P < 0.01$) and between Pb and Sn ($r^2 > 0.76$, $P < 0.001$) were also observed. Chromium associated positively with Ni, Pb and Sn (r^2 ranged from 0.82–0.93, $P < 0.001$) and also with V and Fe ($r^2 > 0.66$, $P < 0.01$). For the rock quarry bluegill muscles, strongest positive associations were observed between Cr and Co and Cu ($r^2 > 0.75$, $P < 0.001$) and between Co and Cu ($r^2 = 0.77$, $P < 0.001$). Other significant positive correlations observed were between Pb and Sn and Ni ($r^2 > 0.61$, $P < 0.01$), Sn and V ($r^2 = 0.62$, $P < 0.01$), and between Cu and V and Ni

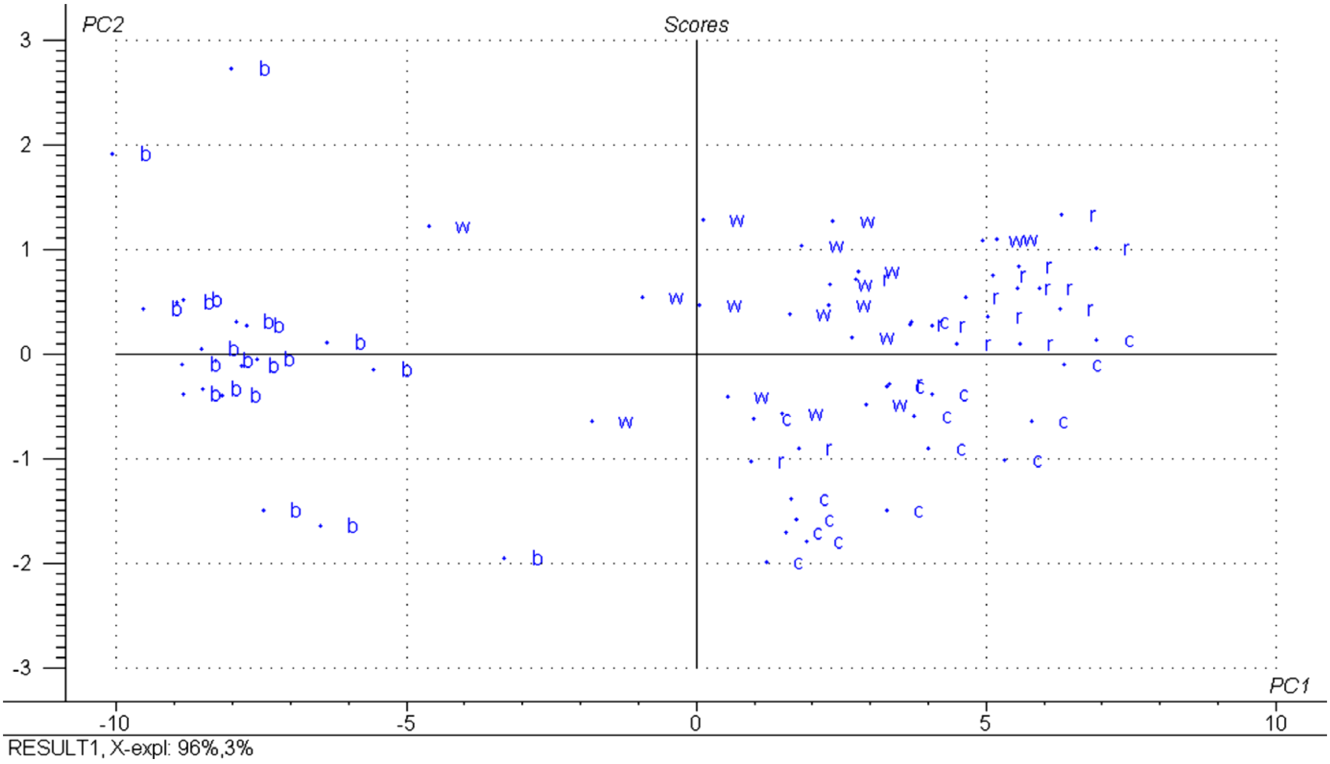

Fig. 1. Score loading plot of all data set after normalization on the principal components (b: Busby, c: Carver, w: Wastewater and r: Rock quarry bluegill samples).

($r^2 = 0.59$, $P < 0.01$). Trace elements found in bluegill muscles from the wastewater pond also showed strong and moderate correlations with one another in some cases. The strongest positive correlations ($r^2 = 0.76$; $P < 0.001$) were between Co and Cr, and between Cu and Cr and Cu. Other moderate relationships observed ($r^2 > 0.58$; $P < 0.01$) were between Cr and Pb and V, Cu and Ni and V, fish weight and Cr, Ni and Pb, Pb and Sn, and Sn and V.

Liver samples of bluegills from most of the sampled areas were not analyzed but evaluation of Busby livers indicated that, except for Hg and Ni, the average values observed for As, Cr, Cd, Co, Cu, Fe, Mn, Pb, Se, Sn, V and Zn were higher than the corresponding values obtained for Busby bluegill muscles (Table 3). However, the Wilcoxon rank sum test indicated no significant difference ($\alpha = 0.01$) between Busby liver and muscle samples for As, Cr, Co, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, V and Zn. Mean trace element values observed for Busby liver samples were in the order: Fe > Cr > Zn > Pb > Co > Cd > Cu > Sn > Se > Mn > As > Ni > V > Hg. The liver is the site of detoxification of materials and higher levels of contaminants in liver relative to muscles (liver: muscle ratio > 1) have been published (Afonso et al., 2007; Agusa et al., 2007). Regulatory thresholds for liver are currently not available and there is no health threat if it is never consumed by humans.

The concentration of Zn in liver was positively correlated with Cu, Cr, Sn, Mn and Ni ($r^2 > 0.72$, $P < 0.01$). Also Fe was associated with Mn ($r^2 = 0.60$, $P < 0.01$), Ni ($r^2 = 0.88$, $P < 0.001$) and Cu ($r^2 = 0.72$, $P < 0.001$). Lead also exhibited positive correlations with Be ($r^2 = 0.48$, $P < 0.05$), Ni ($r^2 = 0.69$, $P < 0.001$), Cu ($r^2 = 0.51$, $P < 0.05$), Cr ($r^2 = 0.65$, $P < 0.001$), Sn ($r^2 = 0.59$, $P < 0.01$) and Mn ($r^2 = 0.53$, $P < 0.05$). Total mercury also correlated with Se ($r^2 = 0.40$; $P < 0.05$) and selenium, known to play a role in mercury detoxification (Burger et al., 2001). The correlations of trace elements in fish tissues observed may be related to the elemental regulation which is affected by metabolic activity (higher in young individuals), environmental conditions and physiological needs (Kojadinovic et al., 2007).

3.4. Comparison of trace element concentrations in bluegill muscles with International standards

Evidence of toxicity of bluegill muscles was evaluated by comparing observed values in this study with various regulatory thresholds (Table 4). Mercury toxicity causes growth deficits and affects fish organs (Burger et al., 2001). In humans, mercury is toxic to the developing fetus and considered a possible carcinogen. Cadmium may induce kidney dysfunction and reproductive damage. Lead affects intelligence quotient in children and causes cardiovascular disease in adults (Ikem & Egiebor, 2005). Arsenic and Cu levels in this study were below the Australia New Zealand food standards (Australia New Zealand Food Authority., 1998) of 1.0 and 10 mg/kg, respectively. Also, the Se levels observed in this study were below the recommended 2 mg/kg in fish muscle (Burger & Gochfeld, 2005) and the US EPA draft selenium whole body criterion of 7.9 μg/g dry

Table 4
Percentages of samples in this study that exceeded the regulatory upper limits for fish muscle

Element	MAFF (1995) (mg/kg)	Other Standards (mg/kg)	Aquaculture bluegills Carver pond: ($n = 16$)	Aquaculture bluegills Busby in-door: ($n = 20$)	Wild bluegills Wastewater pond ($n = 17$)	Wild bluegills Rock quarry pond ($n = 17$)
Hg	0.5	–	0	0	0	6
As	–	1.0[a]	0	0	0	0
Cr	–	0.1[b]	0	100	12	6
Cd	0.2	–	0	30	0	0
Pb	2.0	0.5[c]	0	100	6	0
Sn	–	250[d]	0	0	0	0
Se	–	2[e]	0	0	0	0
Cu	20	10[a]	0	0	0	0
Zn	50	30[c]	0	0	0	0

[a] Australia standard (Australia New Zealand Food Authority (1998)).
[b] Brazil standard (Tarley et al. (2001)).
[c] FAO (1983).
[d] WHO (1996).
[e] Burger and Gochfeld (2005).

wt. (US EPA, 2004). However, mercury (1 sample) and chromium (1 sample) in muscles from rock quarry pond exceeded the MAFF (MAFF, 1995) value of 0.5 mg/kg Hg and the Brazil standard of 0.1 mg/kg Cr (Tarley, Coltro, Matsushita, & de Souza, 2001). Lead and chromium were more apparent in fish muscles from Busby in-door tanks. The Cr (20 samples), Pb (20 samples) and Cd (6 samples) contents of Busby muscles exceeded the respective limits of 0.1, 0.5 and 0.2 mg/kg. For the wastewater bluegill muscles, Cr (2 samples) and Pb (1 sample) were above the MAFF and FAO (FAO, 1983) limits for fish muscle. The Carver bluegill muscles generally posed no risks to humans since all the samples were within the safety limits provided in Table 4 for As, Cd, Cr, Cu, Pb, Se, Sn, Zn and Hg. The Carver bluegill muscle samples generally had smaller elemental concentrations than had the Busby muscle samples, probably because of the feeding practices (infrequent feeding) and uneaten feed pellets were often trapped in Carver pond sediment. Busby bluegill samples were fed periodically by feeding machines.

3.5. Comparison of data from bluegill muscles with published values

Data on trace elements in muscles of bluegills are limited and, where data are available, the unit of reporting results may be on a dry weight basis with no conversion factors provided. Moisture variations across various fish species may affect derivation of a common conversion factor. A comparison of trace element contents of fish muscles across geographical areas is important but considerable variations may exist across fish species because of their nature and habits. In this study, it is best to compare our values specifically with published data on bluegills (*Lepomis Microchirus*) because of differences that may exist across trophic levels.

The average Hg levels obtained in this study for bluegill muscles from the wastewater (0.19 mg/kg wet wt.) and rock quarry (0.24 mg/kg wet wt.) ponds were slightly higher than the average values (above: 0.13, along: 0.14, and below: 0.16 mg/kg wet wt.) reported for bluegill muscles from Savannah River, USA (Burger et al., 2001). However, the mean Hg levels for aquaculture bluegill muscles in this study (Carver: 0.04 and Busby ponds: 0.07 mg/kg wet wt.) were below the corresponding values for bluegills from Savannah River and the English estuary (mean: 0.32 mg/kg wet wt.; Collings, Johnson & Leah, 1996). Also, the mean Se levels in muscles in this study (range of means: 0.19–0.38 mg/kg wet wt.) were generally below the average (0.41 mg/kg wet wt.) reported for fish muscles from Savannah River. Irrespective of the fish collection sites, the mean values for As, Cu, Cr, Pb, Se and Zn in this study were consistently lower than the corresponding average values in mg/kg dry wt. (As: < 3.75 Cd: < 0.10; Cu: < 1.5, Cr: 1.3, Pb: < 1.25, Se: < 3.75, and Zn: 36) reported for whole body samples (bluegill fishes) from small streams at US EPA reference sites in the Mid Atlantic region (US EPA, 2001). However, the average results (0.5 mg/kg wet wt.) reported for Cd and Pb (1.83 mg/kg wet wt.) in Busby muscle samples were five times and one and a half times higher than the corresponding US EPA reference value.

3.6. Health risks from consumption of aquaculture and wild bluegill sunfishes

The goal of Lincoln University is to produce high quality bluegills commercially for human consumption. Since toxic trace elements above tolerable limits for fish can result in health risks in humans, we attempted to evaluate the possible health threat from consumption of aquaculture and wild bluegills from Missouri. The United States Environmental Protection Agency (US EPA) provided reference dose (RfD) values in µg/kg body wt./day for several trace elements (US EPA, 2007a; US EPA, 2007b). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA, 2007b). Also, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended permissible tolerable weekly intakes (PTWI) of

Table 5
Dietary intake estimate from trace element mean value (μg/kg wet wt.) and consumption of 8 oz (0.228 kg) meal/week

Element	Aquaculture bluegills* Carver pond ($n = 16$)	Aquaculture bluegills* Busby in-door ($n = 20$)	Wild bluegills Wastewater pond ($n = 17$)	Wild bluegills Rock quarry pond ($n = 17$)	EPA chronic oral RfD (μg/kg/day)	Guideline[b] (μg/kg/week)	FAO/JECFA PTWI*
As	0.9	1.4	0.1	0.2	0.3[a]	2.1	15
Cd	0.0	1.6	0.0	0.0	1[a]	7	7
Co	0.0	0.0	0.4	0.0	10[c]	70	
Cr	0.0	3.0	0.4	0.2	3[a]	21	
Cu	0.8	1.6	0.9	0.6	40[d]	280	3500
Fe	6.0	98.0	20.8	7.9	700[e]	4900	5600
Mn	0.5	0.7	0.5	0.8	140[a]	980	
Ni	0.2	0.9	0.3	0.1	20[e]	140	
Pb	0.1	5.9	1.0	0.1	3.57[d]	25	25
Se	1.0	1.2	0.6	1.0	5[a]	35	
Sn	1.2	2.9	1.7	1.4	600[a]	4200	14000
V	0.0	0.1	0.1	0.0	1[a]	7	
Zn	20.3	24.6	34.5	29.7	300[a]	2100	7000
Hg	0.1	0.2	0.6	0.8	0.1[a]	0.7	5

* Unit: μg/kg bw; RfD: US EPA reference dose; PTWI: permissible tolerable weekly intakes (μg/kg bw).

[a] RfD obtained from the Integrated Risk Information Systems,US EPA (IRIS: http://www.epa.gov/iris) (US EPA (2007a, b))

[b] Guideline values were calculated from RfD values, average body weight (70 kg) and fish consumption duration (7 days).

[c] Draft- Alternate Concentration Limits (2007), EPA value not available.

[d] RfD for lead not available; we used JECFA 3.57 μg/kg bw per day value.

[e] EPA provisional *t* value.

As, Cd, Cu, Fe, Pb, Sn, Zn and Hg (JECFA, 2003). All bluegill muscle samples in this study were analyzed for total mercury but it was reported that over 95% of total mercury is in the form of methyl mercury (Agusa et al., 2007). Therefore, the total mercury values in this study were assumed to be mostly in the form of methyl mercury.

The PTWI values (Table 5) were used to compare the estimated dietary intakes of trace elements in this study. The RfDs were used to generate guideline values (weekly intake of an element per kg body weight of a 70 kg individual) for the trace elements analyzed. Dietary intakes of trace elements from bluegill muscles analyzed were estimated using average concentration values (Table 3), a single 8 oz (228 g) of fish consumption per week and average body weight of 70 kg. The estimated dietary intake values of trace elements from aquaculture and wild bluegills (Table 5) were mostly below the derived guidelines and the recommended PTWI values. However, the estimated dietary intakes of arsenic (15% of samples) and cadmium (10% of samples) in bluegill muscle samples from Busby in-door tanks exceeded the weekly guideline value of 2.1 and 7 μg/kg bw, respectively.

4. Conclusions

Fish feeds used at Lincoln University aquaculture facility had high concentrations of lead (average: 9.2 mg/kg dry wt., slightly below the EU guideline value of 10 ppm) which can potentially affect bluegills health and also result in bioaccumulation in tissues. The aquaculture research at Lincoln University is committed to growing quality bluegills that will be safe to eat. Therefore, future research of the aquaculture programme will include the evaluation of available feeds in the market for trace element toxicity and bioaccumulation in bluegill tissues. Provision of universal quality feed standards, verification of feed ingredients and sources, implementation of stringent quality controls by manufacturers during production of feeds and good record keeping may contribute to reduction in levels of toxic elements in fish feed but this may come at an extra cost to the aquaculture industry.

Lead and chromium levels were above their respective threshold limits in aquaculture bluegills from Busby farm and this could pose a threat to the health of humans if consumed. Kruskal–Wallis Chi Square approximation showed that the concentrations of As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, V, Zn and Hg ($P < 0.001$) and Se ($P < 0.01$) and Mn ($P < 0.05$) in muscle samples from Busby farm were significantly different from the values observed for other sampled locations. Therefore, aquaculture practices at Busby farm may need to be evaluated to reduce contaminants in fish tissues.

From the dietary intake estimates of trace elements for a 70 kg individual, we found that weekly consumptions of a single 8 oz (228 g) of bluegills from Carver, rock quarry and wastewater ponds were generally safe for humans. However, arsenic (15% of samples) and cadmium (10% of samples) concentrations in muscles of bluegills from Busby farm exceeded the weekly guideline value of 2.1 and 7 μg/kg bw, respectively. As the quantity of fish produced by the aquaculture industry increases, there is a need to safeguard the health of humans through reduction in level of toxic elements often associated with fish feeds. As a caution, individuals consuming fish should follow the EPA recommendations of eating not more than 6 oz (one average meal) per week for fish caught in local waters and eat up to 12 oz (2 average meals) a week of a variety of fish and shellfish that are known to be lower in mercury.

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