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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 $-80\ ^{\circ}C$ 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