

**Studies on the occurrence of nutritionally  
and toxicologically significant metals in  
selected marine edible fish and their  
availability in *in vitro* hydrolysates.**

by

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significant metals in selected marine edible fish and their  
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**Thesis submitted to the University of Sri Jayewardenepura  
for the award of the Degree of Doctor of Philosophy in Food  
Science on 2009.**

## DECLARATION

The work described in this thesis was carried out by me under the supervision of Prof. Arthur Bamunuarachchi, Professor of Applied Chemistry, Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura and Prof. K.K.D.S. Ranaweera, Head/Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree.

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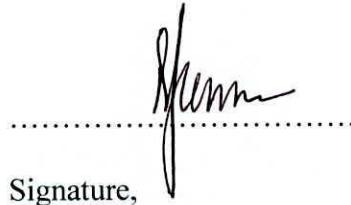
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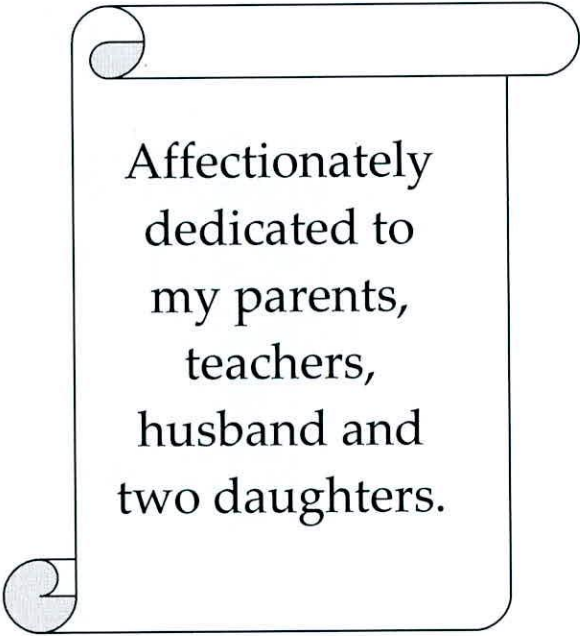
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Affectionately  
dedicated to  
my parents,  
teachers,  
husband and  
two daughters.



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## LIST OF ABBREVIATIONS

PTWI - Provisional tolerable weekly intake

AAS- Atomic Absorption Spectrometry

X-RF - X-ray fluorescence spectrometry

DW - dry weight

µg/g – micrograms per gram (ppm)

AED - Atomic Emission Detector

ANOVA - Analysis of variance

PTDI - Provisional tolerable daily intake

ATSDR - Agency for Toxic substances and Disease Registry

AOAC - Official methods of Analysis of Association of Official Analytical Chemists

FAO - Food and Agricultural Organization

WHO - World Health Organization

USEPA - United States Environmental Protection Agency

EC - European Communities

Fe - Iron

Zn - Zinc

K - Potassium

Cu - Copper

Mg - Magnesium

Ca - Calcium

Cl - Chloride

Cr - Chromium

Mn - Manganese

Br - Bromine

Rb - Rubidium

Sr - Strontium

Br - Bromine

Rb - Rubidium

Sr - Strontium

Ti - Titanium

Cd - Cadmium

Hg - Mercury

As - Arsenic

Pb - Lead

Ni - Nickel



**Studies on the occurrence of nutritionally and toxicologically significant metals in selected marine edible fish and their availability in *in vitro* hydrolysates.**

**Indira Wickramasinghe**

**ABSTRACT**

Fish is considered to be a rich source of minerals as well as proteins. Fifteen (15) popular fish species were used to study the nutritionally and toxicologically significant metals by using Atomic Absorption Spectroscopy (AAS) and X-ray fluorescence techniques (XRF). In raw fish, Fe, Zn, Cu, Br, Mn, Cr, K, Ca, Na, Mg, Cl, Rb, Sr, Ti, Ni, Hg, As, Cd and Pb contents were estimated on dry weight basis and expressed as  $\mu\text{g}$  metal / g of dried tissue. As bioavailability of these metals are more crucial a comparative *in vitro* assay of bioavailability of minerals in raw fish hydrolysates of 10 fish species from the above 15 was carried out. *Sardinella melanura*, *Sardinella albella*, *Exocoetus volitans*, *Carnax* spp., *Stolephorus commersoni*, *Anchovy commersonii*, *Selar* spp. and *Leiognathus* spp. were used for the studies with boiled fish. Cooking methods including steaming, pressure cooking, boiling, frying and drying were applied to *S. melanura* and *Amblygaster* spp. in order to determine the effect of cooking on *in vitro* bioavailability of metals. The effect of digestion time on metal released was investigated by subjecting the samples to digestion at different times. The levels of Fe, Zn, Cu, Br, Mn, Cr, K, Ca, Na, Mg, Cl, Rb, Sr, Ti, Ni, Cd, and Pb in raw fish were 2.1- 11.08, 0.9 – 275, 0.2- 9.33, 0.3 – 30.1, 0.6- 28.7, 0.0- 2.1, 578.3 – 13553, 11.9- 80516.2, 12879- 71502, 28493 –171429, 98.3 - 24902.4, 0.1 –2.8, 0.2- 232.4, 3.0-41.8, 4.4 -7.4, 0.0 –1.9 and 0.7- 10.7  $\mu\text{g}$  /g respectively. Hg and As were not detected. The *in vitro* metal availability of raw fish hydrolysates was found to be lower than of that of the fish flesh.

The percentage of *in vitro* Fe availability in boiled *S. melanura*, *Carnax*, *S. albella* and *E. volitans*, *A. commersonii*, *Selar* spp, *Leiognathus* spp and in *S. commersoni* were 20%, 36%, 23% and 28% 55%, 55%, 42% and 40% respectively. In *S. melanura* and *Amblygaster* spp. the metal contents were ( $p < 0.05$ ) found to be increased than raw fish with cooking except in few instances. *Amblygaster* spp. subjected to boiling, steaming and pressure cooking had higher percentages of *in vitro* Fe, Zn, Cu, Mg, Na and Pb availability compared with *S. melanura*. The percentages of the above metals ranged from 51-75 %, 13-27%, 47-91%, 44-59%, 62-70%, 9–60% respectively. The highest mean *in vitro* available Fe content, the highest *in vitro* available Fe percentage (77%) and the highest *in vitro* available Cu percentage (73%) were observed in the boiled fish. Drying of fish increased the *in vitro* available Pb, Na and Zn percentage. Boiling of fish increased the *in vitro* available Pb, Mg, Cu and Fe percentage in *S. melanura*. In *Amblygaster* spp. Fe, Zn and Cu decreased with drying at 65<sup>0</sup>C. Frying of fish increased the *in vitro* available Cu percentage. Pressure cooking of fish increased the *in vitro* available Fe and Zn percentage. Drying at higher temperatures lowered the *in vitro* available Fe and Zn content and increased the Pb content of dried fish in both *S. melanura* and in *Amblygaster* sp. It can be concluded that boiling, steaming and pressure cooking are the best cooking methods for *S. melanura* and *Amblygaster* spp as far as they retain least amounts of heavy metals. The normal digestion time was found to be sufficient enough to provide *in vitro* available forms and the extended digestion time had not significant effect on the release of metals.

Therefore it can be concluded that fish studied can be a good source of nutritionally significant metals and these species contain within the permissible range of metals concerned. As cooking methods studied reduce toxicologically significant metals' *in vitro* bioavailability, these cooking methods can be considered to be effective.