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DETERMINATION OF TRACE HEAVY METALS IN FISH SPECIES BY DIFFERENT ANALYTICAL TECHNIQUES

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ABSTRACT

Heavy metal pollution is a serious environmental problem. The presence of such metals in different areas of an ecosystem subsequently leads to the contamination of consumable products such as dietary and processed materials. Accurate monitoring of metal concentrations in various fish samples is of importance in order to minimize health hazards resulting from exposure to such toxic substances. For this purpose, it is essential to have a general understanding of the basic principles for different methods of elemental analysis. This article provides an overview of the most sensitive techniques of elemental analysis such as atomic absorption, ICP-OES mass spectrometry, Gas chromatography, and other techniques.

INTRODUCTION

Heavy metals, with their high density and toxicity at low concentrations, pose significant health risks to humans. While the body requires trace elements like Calcium and Magnesium for health, heavy metals such as Lead, Copper, Cadmium, and Zinc are toxic and can lead to various health issues including impaired kidney and liver function, decreased cognitive abilities, reproductive impairments, hypertension, neurological changes, and even cancer.

Heavy metals enter the food web primarily through water or organisms, potentially accumulating in edible fish. Fish, a vital part of many diets due to its nutritional value, also serve as indicators of heavy metal contamination in aquatic systems. Their varied ecological roles, metabolisms, and habitats make them sensitive to environmental pollution levels.

The intake of heavy metals by fish depends on factors such as salinity, pollution levels, diet, and sediment composition. Marine fish, being directly exposed to water and sediment contaminants, can accumulate heavy metals, which poses risks to human health upon consumption.

Understanding heavy metal contamination in marine fish is crucial for food safety and environmental health. Monitoring and regulating heavy metal levels in aquatic environments are essential to mitigate health risks associated with fish consumption and preserve ecosystem integrity.

1.1 SOURCES OF HEAVY METAL POLLUTION

Heavy metal pollution originates from diverse sources including mining, industrial, domestic, urban, and agricultural activities, as well as atmospheric emissions and petroleum-related industries. Specific activities like copper melting, nuclear fuel preparation, and electroplating contribute to the release of cadmium, lead, and zinc into the environment. As fish age and feed, these metals are absorbed into their tissues, where they can accumulate over time.

In natural waters, metals exist in soluble or particulate forms, with labile compounds posing the greatest risk to fish. These compounds include various ionic forms with different availability to fish. Field studies have shown that fish can accumulate significant amounts of metals without experiencing mortality, with different metals accumulating in fish tissues at varying rates. Generally, higher environmental metal concentrations result in increased uptake and accumulation in fish.

1.2 BIOACCUMULATION OF HEAVY METALS

Metal accumulation in fish follows a pattern, with iron, zinc, lead, copper, cadmium, and mercury being commonly observed. The extent of accumulation varies among fish species and is influenced by factors such as pollution levels and species-specific behaviors. Understanding these dynamics is crucial for assessing environmental health and managing heavy metal contamination in aquatic ecosystems.

2. ANALYTICAL METHODS

2.1 INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

ICP-MS, utilizing inductively coupled plasma, efficiently detects metals and non-metals in liquid samples, including different isotopes of elements. Concentrations of Cd, Cu, Mn, and Zn were assessed in muscle, liver, and gills of Arius thalassinus and Johnius belangeri from Kapar and Mersing, Peninsular Malaysia. Results showed Zn and Cd had the highest and lowest concentrations respectively in all tissues. A. thalassinus exhibited higher metal levels than J. belangeri. However, none of the metal concentrations in muscles exceeded standard guidelines, indicating no health hazards for consumers.

2.2 GAS CHROMATOGRAPHY (GC)

In chromatographic analysis, a mixture's components partition between a stationary phase and a mobile phase, with interactions varying based on properties and structures. Methyl mercury, extracted as bromide from fish and sediment and chloride from water, undergoes a cleanup procedure converting it to iodide for gas chromatographic analysis. Recoveries averaged 88.5% for water, 95.5% for perch, and 96.3% for sediments. When applied to polluted river sediments, a correlation between total and methyl mercury concentrations was established in the 0-10 µg/g range, but failed for samples with high inorganic mercury levels.

2.3 ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Atomic Absorption Spectroscopy (AAS) was utilized to quantify heavy metal concentrations in eight popular cultured fish species from Dhaka, Bangladesh, revealing no health risks based on estimated daily intake (EDI) except for arsenic, which posed a cancer risk. Two wet destruction methods were compared for sample dissolution in tuna (Euthynnus affinis), with the HNO_3, H_2SO_4, $HClO_4$ (1:2:1) mixture showing greater efficiency. AAS measurements indicated heavy metal levels within permissible limits in tuna, with the standard addition method yielding more precise results. Additionally, a Zeeman Mercury analyzer facilitated rapid mercury determination in fish, with most samples below recommended levels by FAO/WHO.

2.4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY (ICP-OES)

ICP-OES, utilizing argon plasma to excite atoms and ions, enables the quantitative determination of elements in samples. By emitting photons at specific wavelengths during the transition from excited to ground states, the technique accurately measures elemental concentrations. In Beymelek Lagoon, heavy metal accumulation ratios in various tissues of multiple fish species were investigated, with concentrations found to be lower than other contaminated Mediterranean regions of Turkey. In another study, liver samples from farmed or wild fish were analyzed for multiple elements, revealing higher concentrations in liver compared to muscle, with Cd and Pb levels exceeding European Commission regulations in a significant portion of samples. However, Hg and As levels remained below legislated limits across all samples.

2.5 HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROSCOPY (HGAAS)

In the hydride technique a continuous flow system involves the reaction of acidified aqueous samples with a reducing agent such as sodium borohydride to generate volatile hydride, which is then transported to the heated quartz cell (atomizer) along the optical axis of the conventional AAS by mean of an argon gas. In the quartz cell the hydrides are converted to gaseous metalloid analyte atoms in the path of a source lamp and a signal is generated by measuring the amount of light absorbed. HGAAS technique is restricted to certain elements.

HGAAS was applied for the determination of As in canned tuna fish from the Persian Gulf area of Iran. As concentration varied from 0.0369 to 0.2618 with an average value of 0.128 ppm. The hydride method was used was developed for the determination of arsenic in sea fish. Mean concentrations were 0.039, 0.036, and 0.075 ppm, respectively.

2.6 COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY (CVAAS)

Cold Vapor Atomic Absorption Spectrometry (CVAAS) is a primary technique for mercury analysis, utilizing a peristaltic pump to transport samples and stannous chloride into a Gas Liquid Separator (GLS) where mercury vapor is released by introducing pure, dry gas (typically argon). The vapor is carried into an atomic absorption optical cell, where it absorbs light at 253.7 nm in proportion to its concentration. This flameless procedure allows for mercury detection at room temperature, with results showing mercury concentrations in fish samples from Ghana ranging from 0.004 to 0.122 ppm and from 0.721 to 1.41 ppm in fish from Karachi, Pakistan. Mercury was also detected in natural remedies and pharmaceuticals, with concentrations ranging from 0.51 to 2.35 ppm. Another method utilizing CVAAS, involving a mixture of HNO_3, $HClO_4$, and H_2SO_4 for complete oxidation of organic tissue, revealed mercury concentrations in fish from Ghana below the WHO limit of 0.5 µg g^{-1} wet weight, suggesting no significant mercury exposure through fish consumption.

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2.7 NEUTRON ACTIVATION ANALYSIS (NAA)

Neutron Activation Analysis (NAA) is an analytical procedure used to detect trace elements in various matrices. In this technique, samples are exposed to a neutron flow, generating radioactive isotopes for the elements of interest. As these isotopes decay, they emit delayed gamma rays with characteristic energies, allowing for quantitative measurement of element concentrations. NAA was applied to determine heavy metal concentrations in green tiger shrimp and blue crab from the Persian Gulf, revealing concentrations ranging from 1.91 to 25.43 ppm for Mn, 21.38 to 8.28 ppm for As, 0.15 to 0.40 ppm for Co, 62.87 to 288 ppm for Fe, and 66.64 to 68.73 ppm for Zn. Additionally, NAA detected V, As, Cd, Cr, Cu, Fe, Mn, Ni, and Zn in fish samples from an irrigation facility in Ghana, with concentrations ranging from 0.02 to 45.95 ppm.

2.8 DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRY (DPASV)

DPSAV (differential pulse stripping anodic voltammetry) is an electrochemical technique used for determining lead (Pb) and cadmium (Cd) contents in fish muscle samples. In this method, the analyte is first electroplated onto the working electrode and then removed or 'stripped' by applying an oxidizing potential. The cell current is measured as a function of time and potential between the indicator and reference electrodes. Lead and cadmium concentrations were determined in 17 commercially used fish species from the South Egypt River Nile (Aswan) after lyophilization, milling, and decomposition with mixed acid (HNO_3 + $HClO_4$). The accuracy was validated using certified reference material CRM No. 422. Results indicate that fish from the Egypt River Nile area are a good source of these essential elements, demonstrating the effectiveness of the DPSAV method as an analytical routine for such samples.

3. SUMMARY OF ANALYTICAL METHODS

Table 1: Summary of Analytical methods

HM	ICP-MS	GC	AAS	ICP-OES	HGAAS	CVAAS	NAA	DPASV
Cd	✓		✓				✓	✓
Cu	✓		✓	✓			✓	
Zn	✓		✓	✓			✓	
Mn	✓			✓			✓	
Hg		✓	✓	✓		✓		
Pb			✓	✓				✓
Cr			✓	✓			✓	
As			✓	✓	✓		✓	
Fe				✓			✓	
Mg				✓				
Ni				✓			✓	
Co				✓			✓	
B				✓				
V							✓	
Ba				✓				
Sr				✓				

4. CONCLUSION

- The heavy metals like Cd, Cu, Zn , Mn etc. concentration in different fish species have been determined using different analytical techniques. Therefore, regular monitoring of heavy metal concentrations in fish tissue is necessary.
- Although the concentrations of heavy metals in the fish and water column were detected in low concentrations, the potential for metal toxicity danger may become more severe in the future depending upon the extent of industrial and domestic wastewater influx into the water bodies due to human activities in the adjacent areas.
- To develop a healthy freshwater fishing industry and to prevent heavy metal risks to human health, the water standards and concentrations of heavy metals in the water column and fish should be monitored regularly.
- An extensive range of analytical methods are available for trace elemental analysis in different species of fishes, a number of which have been discussed in this review.
- The type of sample and concentration range of heavy metal influence the selection of the technique to be used.
- Overall it was found that Inductively coupled plasma- optical emission spectroscopy technique, could provide not only analysis of wide range of heavy metals but also ensure immense reliability by exhibiting good precision and accuracy at trace level.
- Thus, this study can be used to determine the level of trace heavy metals in fish species by different analytical techniques and therefore be useful in the future for the people who consume it.

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