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Assessment of Food Safety of Imported Fish Available in Iraqi Markets Using ICP-OES for Determination of Heavy Metals and Biochemical Indicators

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
﴿وَأَنْ لَّيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَى
(39) وَأَنْ سَغِيهٖ سَوْفَ يُرَى﴾

صدقَ اللهُ العليُّ العظيم



Dedication

To my dear father,

The role model who instilled in me a love for ambition and giving. You are the source of my determination, the secret to my persistence, my primary support, my pride.

To my dear mother,

My first teacher, my strength and stability, my refuge and sanctuary. To the one who overwhelmed me with her prayers, whose grace—after God—was the reason for everything I have achieved and attained.

To my brothers,

In whom I see an extension of myself, those upon whom I have leaned, and who have been my support in weakness and my joy in success.

To my sister,

***My refuge in
sorrow and my
soulmate. To my
friends, my pillar
of support...***

To everyone who has been an aid and a source of support along this path...

I dedicate the fruits of my success to you.

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Acknowledgment

In the Name of God, the Most Gracious, the Most Merciful.

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Summary

Imported fish products must meet safety and quality standards to protect public health in Iraq. This present study gives a complete assessment of the nutritional and toxicological status of 39 different imported fish in Iraqi markets of Babylon, Najaf and Diwaniyah; canned species (tuna, sardines) and frozen species (tilapia, red-bellied pacu, common carp, salmon, Nile tilapia, golden pompano and mackerel). Concentrations of heavy metals (Pb, Cd, Zn, Ni) were measured in 117 commercial samples, by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) in triplicate technical replicates. Proximate analysis of protein, fat and ash contents and evaluation of oxidative stress indicators (MDA, SOD) were also carried out. Multivariate statistical analyses were performed, including principal component analysis (PCA) and Pearson correlation.

Results showed variation in the concentrations of heavy metals with lead concentrations ranging from 5.60 ppb in canned tuna to 131.20 ppb in frozen salmon, while maximum cadmium was 6.765 ppb. Nutritional analysis revealed that frozen fish had higher and more stable protein content ($25.8 \pm 0.6\%$) than canned products ($21.5 \pm 1.2\%$). The fat content was influenced by the method of preservation and the medium of packaging. The ash contents ranged from 2.4-2.5%. Protein was negatively correlated ($r = -0.911$, $P < 0.01$) with fat and ($r = -0.659$, $P < 0.01$) with ash, which reflects structural and physiological changes associated with processing and storage.

There were significant differences in oxidative stress indicators between species and preservation methods. MDA ranged from 15.2–74.7 nmol/g and SOD ranged from 214.8–344.0 units/g in canned tuna, 17.8–68.9 nmol/g and 212.8–418.8 units/g in canned sardines, 22.5–80.9 nmol/g and 208.5–275.4 units/g in frozen fish ($P < 0.001$). PCA clustered the samples into three groups, group A, characterized by high fat content and moderate metal levels, included canned products; group B, characterized by high zinc and nickel content, included benthic and freshwater species such as common carp, Nile tilapia and red-bellied pacu; group C, characterized by high protein content and accumulation of lead and cadmium, included predatory marine species such as Sea Bream and mackerel.

Results showed that accumulation of heavy metals is related to ecological niche, trophic level and preservation method, while nutritional quality and oxidative stability are affected by species characteristics, thermal processing and storage conditions. The study emphasizes the need to couple heavy metal assessment with nutritional and oxidative stress parameters for the overall safety appraisal of imported fish. It is recommended to monitor certain species, assess packaging materials, and apply multivariate risk assessment models to safeguard public health and maintain the quality of Iraq's imported fish supply chain.

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List of Abbreviation

Abbreviation	Full Term
PUFAs	Polyunsaturated Fatty Acids
FAO	Food and Agriculture Organization
WHO	World Health Organization
Cd	Cadmium
Pb	Lead
Ni	Nickel
Zn	Zinc
SOD	Superoxide Dismutase
MDA	Malondialdehyde
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
ANOVA	Analysis of Variance
LSD	Least Significant Difference
ROS	Reactive Oxygen Species
ATP	Adenosine Triphosphate
TMAO	Trimethylamine Oxide
TMA	Trimethylamine
BSA	Bovine Serum Albumin
DIW	Deionized Water
UV-Vis	Ultraviolet-Visible Spectrophotometry
FA	Fatty Acids

Abbreviation	Full Term
AOAC	Association of Official Analytical Chemists
COSQC	Central Organization for Standardization and Quality Control
IQS	Iraqi Quality Standards
TBARS	Thiobarbituric Acid Reactive Substances
PCA	Principal Component Analysis

Chapter One

Introduction



Introduction

Fish are globally recognized as a major dietary component for humans, providing high-quality, easily digestible protein, essential polyunsaturated fatty acids (PUFAs), vitamins and vital minerals (Mohanty, Sahu, & Mohanty, 2019; FAO, 2024; Boyd et al., 2022). These are vital biomolecules for somatic growth, regulation of the immune system, metabolism and overall human health. Their consumption is a major contributor to the world's dietary protein intake and nutritional security. This is particularly the case in areas such as Iraq, where the combined effect of ecological stress on local river systems (Tigris and Euphrates), variable local aquaculture yields, and rapid population growth, does not meet the increasing domestic demand (Tacon & Metian, 2013; Maulu, Gichuki, & Mwangi, 2021). Thus, the dependence on imported aquatic products has increased dramatically, and imported fish has become a key commodity to meet the nutritional requirements and maintain the economic stability of the country. The Iraqi consumer market places a central and indispensable role on imported fish products. This includes processed products such as canned tuna and sardines and a broad range of frozen fish. Year-round availability, extended shelf life and economic accessibility ensure consistent consumer access to protein-rich foods regardless of seasonal local shortages (Hassoun, Chtourou, & Mrabet, 2020; Wang, Zhao, & Li, 2022). However, the complexities of complex, transnational supply chains render these imported products vulnerable to multiple risks. These fish, from the point of harvest in potentially polluted international waters, through the rigorous processes of freezing, canning and lengthy shipping, may be exposed to conditions that severely compromise their safety and nutritional integrity, requiring rigorous and continuous biomonitoring to protect public health (FAO, 2024).

One of the most important food safety concerns is the silent threat of chemical contamination with heavy metals, as well as the biochemical decay during processing and storage (Olatunde & Benjakul, 2018). Persistent inorganic pollutants like Cadmium (Cd), Lead (Pb), Nickel (Ni) and Zinc (Zn) are heavy metals which biomagnify through dietary intake and accumulate in fish tissues via direct uptake through gills. The global anthropogenic activities such as industrial discharge, agricultural runoff, and marine pollution is the contamination source (Ali, Khan, & Ilahi, 2019; Kumar, Singh, Sharma, & Gupta, 2024).

Several studies at the regional level during the last few years have reported the existence of residues of heavy metals in fish that are consumed in Iraq and neighboring countries, often at levels that are close to or above the maximum permissible limits as defined by international regulatory agencies (Al-Sammarrai, 2021; Al-Khafaji & Hassan, 2022). These alarming results point to an urgent public health problem and the absolute necessity to conduct routine advanced spectral analyses of imported fish in the local Iraqi markets to prevent possible health hazards in the diet and to provide regulatory authorities with evidence-based data.

Quality assessment is an essential additional dimension in order to fully understand the extent of cellular damage and nutritional loss through oxidative stress markers. Fish lipids are highly susceptible to oxidation due to the high degree of unsaturation, especially in the presence of heavy metals that act as pro-oxidants and physical stressors during handling, freezing, thawing and long-term storage (Ali, Khan, & Ilahi, 2019; Kumar, Singh, Sharma, & Gupta, 2024).

1.2. Aims of the Study

The primary aim of this research is to evaluate the safety and quality of selected imported fish products in Iraqi markets, with specific objectives to:

To determine concentrations of selected toxic and trace heavy metals (Cadmium (Cd), Lead (Pb), Nickel (Ni) and Zinc (Zn)) in various imported fish products (canned Tuna, canned Sardines, frozen species) by using advanced spectral analysis.

To determine the total protein, lipid and ash contents of the selected fish products to assess their proximate biochemical composition and thus their true nutritional value.

To assess oxidative stress and cellular degeneration in fish tissues by estimation of enzymatic activity of Superoxide Dismutase (SOD) and estimation of Malondialdehyde (MDA) as a terminal marker of lipid peroxidation.

To statistically compare the levels of heavy metals, nutritional profiles and oxidative stress biomarkers between heavily processed canned fish and frozen raw fish species

Chapter Two

Literature Review



Chapter Two

Literature Review

2.1 Fish Species Adopted in the Study

The fish species investigated in the present study represent a diverse group of imported seafood products available in local markets, including canned fish products (tuna and sardines) and frozen marine and freshwater species (sea bream, salmon, mackerel, Nile tilapia, common carp, red-bellied pacu, and golden pompano). These species differ considerably in their ecological habitats, trophic positions, feeding behaviours, lipid composition, and physiological characteristics. Such biological differences are important because they influence nutritional composition, susceptibility to oxidative deterioration, and patterns of heavy-metal accumulation in fish tissues. Therefore, interpretation of seafood quality requires consideration of species-specific characteristics in addition to analytical measurements of contaminants and biochemical parameters (Ahmed et al., 2022; Feng et al., 2020; Khawar et al., 2024).

Fish occupy different ecological positions within aquatic ecosystems, resulting in variation in contaminant exposure pathways. Predatory marine species may accumulate certain contaminants through dietary transfer, whereas freshwater and sediment-associated species may experience greater exposure through contaminated water, sediments, and feed sources. Consequently, differences in heavy-metal concentrations among fish species should be interpreted according to habitat, feeding strategy, trophic level, and environmental conditions rather than processing method alone (Feng et al., 2020; Garnero et al., 2018; Kumar et al., 2024).

In addition to their toxicological importance, fish species differ significantly in nutritional composition. Fish muscle provides high-quality protein, essential amino acids, omega-3 polyunsaturated fatty acids, vitamins, and minerals. However, protein and lipid contents vary according to species, habitat, diet, season, age, and production system. Therefore, proximate composition analysis is essential for evaluating nutritional quality and comparing commercially available fish products (Mohanty et al., 2022; Santos et al., 2023).

2.1.1 Tuna

Tuna species belong to the family **Scombridae** and represent some of the most commercially important marine fish worldwide. Their high economic value is related to their desirable nutritional characteristics, including high-quality protein and significant amounts of long-chain omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These characteristics make tuna an important source of essential nutrients in human diets (Mohanty et al., 2022; Santos et al., 2023).

From an ecological perspective, tuna species are highly migratory marine predators occupying relatively high trophic positions. Their predatory feeding behaviour increases exposure to contaminants transferred through marine food webs. The accumulation of trace elements in tuna depends on multiple factors, including geographical origin, environmental contamination, age, size, dietary habits, and physiological regulation mechanisms (Feng et al., 2020; Arisekar et al., 2024).

Although mercury is traditionally considered the primary contaminant associated with tuna consumption, other trace elements including lead (Pb), cadmium (Cd), nickel (Ni), and zinc (Zn) may also be detected depending on environmental conditions and anthropogenic contamination sources. Therefore, multi-element analysis provides a broader evaluation of tuna safety compared with assessment of a single contaminant (Arisekar et al., 2024; Kumar et al., 2024).

Canning is the most common preservation method for tuna because it provides extended shelf life, convenient storage, and wide commercial distribution. However, thermal sterilization, storage duration, and packing medium may influence lipid stability, protein characteristics, and oxidative quality. Therefore, evaluation of canned tuna requires consideration of both the original biological characteristics of the fish and the effects of processing conditions (Aubourg, 2023; Mozzon et al., 2024).

2.1.2 Sardines

Sardines are small pelagic fish belonging mainly to the family **Clupeidae** and are widely consumed because of their affordability, nutritional value, and availability as canned products. Compared with large predatory fish, sardines occupy a lower trophic position, which may reduce biomagnification potential for some contaminants. However, their coastal and pelagic habitats may expose them to pollutants originating from industrial discharge, agricultural runoff, and urban activities (Šimat et al., 2020; Arisekar et al., 2024).

Sardines are nutritionally valuable because they contain high-quality protein, minerals, and considerable amounts of omega-3 polyunsaturated fatty acids. Nevertheless, the high proportion of unsaturated lipids increases susceptibility to oxidative deterioration during storage and processing. Lipid oxidation may result in the formation of secondary oxidation products such as malondialdehyde (MDA), which can negatively affect sensory quality and nutritional stability (Aubourg, 2023; Ayala et al., 2014).

The quality of canned sardines depends on several factors, including raw material quality, thermal processing conditions, storage period, packaging integrity, and type of packing medium. Heat treatment may modify protein structure and influence lipid oxidation, while the packing medium can alter the final lipid composition and oxidative behaviour of the product (Bouriga et al., 2022; Mozzon et al., 2024).

Because sardines are frequently consumed seafood products, monitoring of toxic metals such as Pb, Cd, and Ni remains important for consumer safety. The combination of elemental analysis with nutritional and oxidative biomarkers provides a more comprehensive evaluation of canned sardine quality than contaminant measurement alone (Arisekar et al., 2024).

2.1.3 Sea Bream (*Sparus aurata*)

Sea bream represents an economically important marine fish group widely consumed in international markets because of its favourable nutritional characteristics, including high-quality protein, essential amino acids, and valuable lipid components. Species such as gilthead sea bream (*Sparus aurata*) are important commercial species in both wild fisheries and aquaculture systems, and their nutritional composition is influenced by environmental conditions, feeding regime, and production practices (Mohanty et al., 2022).

The accumulation of trace elements in sea bream is affected by habitat characteristics, feeding behaviour, and environmental contamination levels. As marine fish interact continuously with surrounding water and prey organisms, metals may enter their tissues through both dietary and waterborne pathways. Recent studies have demonstrated that metal accumulation varies considerably among fish species according to ecological characteristics and geographical origin, highlighting the importance of species-specific evaluation during seafood safety assessment (Almafrachi et al., 2025; Feng et al., 2020).

Because sea bream is frequently marketed as frozen seafood, evaluation of its quality requires consideration of both contaminant accumulation and storage-related biochemical changes. Freezing may preserve nutritional value but prolonged storage can influence protein stability and lipid oxidation, particularly in species containing appreciable amounts of unsaturated fatty acids (Du et al., 2022).

2.1.4 Red-Bellied Pacu (*Piaractus brachypomus*)

Red-bellied pacu (*Piaractus brachypomus*) is a freshwater omnivorous fish species belonging to the family Serrasalminidae. It has become an important aquaculture species because of its rapid growth, adaptability to different environmental conditions, and acceptance by consumers. Unlike marine predatory fish, pacu occupies a lower trophic position and has a feeding behaviour based on plant material, fruits, and aquatic organisms, which influences both nutritional composition and contaminant exposure patterns.

Freshwater fish species are particularly influenced by local environmental conditions because rivers and lakes may receive contaminants from agricultural runoff, domestic wastewater, and industrial activities. Heavy metals may accumulate in freshwater organisms through direct absorption from water, ingestion of contaminated food, and interaction with sediments. Therefore, metal

concentrations in pacu should be interpreted according to environmental conditions and production systems rather than species characteristics alone (Garnero et al., 2018; Zahran et al., 2025).

2.1.5 Common Carp (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*) is one of the most widely distributed freshwater fish species worldwide and represents an important aquaculture species because of its tolerance to environmental variation and high production capacity. It contributes significantly to global freshwater fish consumption and provides an accessible source of animal protein.

The ecological behaviour of common carp contributes to its exposure to sediment-associated contaminants because it frequently feeds near the bottom of aquatic environments. Sediments may act as reservoirs for heavy metals, allowing their transfer to aquatic organisms. Consequently, feeding behaviour and habitat preference are important factors affecting metal accumulation patterns in carp tissues (Garnero et al., 2018; Oros, 2025).

The nutritional composition of common carp varies according to environmental conditions, feed composition, and aquaculture practices. Therefore, assessment of protein, lipid, and mineral composition together with elemental analysis provides a more comprehensive evaluation of carp quality and safety.

2.1.6 Salmon (*Salmo salar*)

Salmon represents one of the most valuable commercial fish groups worldwide because of its high nutritional quality and consumer demand. Atlantic salmon (*Salmo salar*) is particularly recognized for its high protein content and abundance of long-chain omega-3 polyunsaturated fatty acids, especially EPA and DHA, which contribute to cardiovascular and neurological health benefits (Mohanty et al., 2022).

The high lipid content of salmon provides important nutritional advantages but also increases susceptibility to lipid oxidation during storage. Oxidative deterioration of salmon lipids may result in formation of secondary oxidation products, including malondialdehyde (MDA), which can influence flavour, nutritional quality, and shelf stability (Ayala et al., 2014; Aubourg, 2023).

Salmon may accumulate environmental contaminants through dietary exposure because of its position within aquatic food webs. However, accumulation patterns depend on multiple factors, including production system, feed composition, environmental exposure, and biological characteristics. Therefore, monitoring of toxic metals remains important even in aquaculture-derived salmon products (Almafrachi et al., 2025).

2.1.7 Nile Tilapia (*Oreochromis niloticus*)

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater aquaculture species worldwide due to its rapid growth, environmental tolerance, and economic importance. It represents a major source of affordable animal protein, particularly in regions where access to marine fish is limited.

The nutritional characteristics of tilapia are influenced by feed composition, farming conditions, and environmental parameters. Compared with fatty marine species, tilapia generally contains lower lipid levels but provides high-quality protein and essential minerals. Differences between wild and cultured tilapia demonstrate the influence of production systems on elemental profiles and nutritional composition (Simukoko et al., 2022).

Because tilapia inhabits freshwater environments, it may be exposed to metals originating from contaminated water, sediments, and aquaculture inputs. Recent studies have shown that freshwater fish species exhibit species-specific patterns of metal accumulation depending on environmental exposure and physiological regulation mechanisms (Zahran et al., 2025).

2.1.8 Golden Pompano (*Trachinotus blochii*)

Golden pompano (*Trachinotus ovatus*) is a commercially valuable marine fish species appreciated for its taste, nutritional quality, and market value. It contains high-quality proteins and beneficial lipid components, making it an important seafood product.

As with other marine fish species, the accumulation of trace metals in golden pompano depends on habitat characteristics, environmental contamination, trophic position, and feeding behaviour. Marine ecosystems affected by anthropogenic activities may facilitate transfer of metals into aquatic food chains, resulting in measurable accumulation in edible tissues (Oros, 2025).

2.1.9 Mackerel (*Scomber scombrus*)

Mackerel belongs to the family (*Scombridae* and) is widely consumed because of its high nutritional value, particularly its abundance of omega-3 fatty acids. Similar to tuna, mackerel is an active pelagic species with high metabolic requirements and a lipid-rich muscle composition.

The high proportion of polyunsaturated fatty acids increases nutritional value but also increases susceptibility to oxidative deterioration during storage and processing. Therefore, oxidative stability is an important quality parameter in frozen and canned mackerel products (Aubourg, 2023; Prego et al., 2022).

Mackerel may accumulate trace elements from marine environments through dietary transfer and environmental exposure. Recent evidence indicates that metal accumulation varies among marine fish species according to habitat, trophic characteristics, and environmental conditions (Oros, 2025; Almafrachi et al., 2025).

2.2 Heavy Metal Toxicity and Biochemical Mechanisms

Heavy metals are among the most persistent environmental contaminants affecting aquatic ecosystems because they cannot be degraded through biological or chemical processes. Their persistence allows continuous interaction with aquatic organisms and facilitates accumulation within food chains. Anthropogenic activities, including industrial discharge, agricultural runoff, mining operations, urban wastewater, and atmospheric deposition, represent major sources of heavy-metal contamination in aquatic environments (Ali et al., 2019; Kumar et al., 2024).

Fish are considered important bioindicators of aquatic pollution because they accumulate contaminants from surrounding water, sediments, and dietary sources. The degree of metal accumulation depends on several factors, including metal concentration and chemical form, exposure duration, water characteristics, feeding behaviour, trophic position, and species-specific detoxification mechanisms (Zhang et al., 2023; Zahran et al., 2025).

Heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), and zinc (Zn) differ in their biological significance and toxicological effects. Lead and cadmium are classified as non-essential toxic metals because they have no known physiological role and may cause harmful effects even at relatively low exposure levels. Nickel and zinc have physiological functions at trace concentrations; however, excessive exposure may result in oxidative damage and disruption of cellular homeostasis (EFSA CONTAM Panel, 2020; WHO, 2023).

2.2.1 Lead (Pb) Toxicity

Lead is a widely distributed environmental contaminant originating from industrial activities, mining, combustion processes, batteries, and contaminated wastewater. Due to its persistence and limited biological degradation, lead can accumulate in aquatic environments and enter food chains through uptake by aquatic organisms (Ali et al., 2019).

Following absorption, lead can interfere with cellular metabolism through several mechanisms, including inhibition of antioxidant enzymes, disruption of essential mineral metabolism, and induction of reactive oxygen species (ROS). Lead has a high affinity for sulfhydryl groups in proteins, allowing interaction with enzymes involved in cellular defence and metabolism (Kumar et al., 2024).

Oxidative stress represents one of the major mechanisms of lead toxicity. Lead exposure may increase ROS production and decrease antioxidant capacity, resulting in lipid peroxidation, protein oxidation, and DNA damage. In fish, these effects are commonly reflected by alterations in antioxidant enzymes such as superoxide dismutase (SOD) and increased levels of lipid oxidation products such as malondialdehyde (MDA) (Zahran et al., 2025).

From a human-health perspective, lead is considered a cumulative toxicant affecting multiple organ systems. Chronic exposure has been associated with neurological impairment, renal dysfunction, cardiovascular effects, and developmental toxicity, particularly among children and pregnant women (WHO, 2023).

2.2.2 Cadmium (Cd) Toxicity

Cadmium is a highly toxic non-essential metal introduced into aquatic ecosystems through industrial discharge, mining activities, phosphate fertilizers, and urban contamination. It has a long biological half-life and may accumulate in organisms exposed to contaminated environments (Ali et al., 2019).

Unlike metals that directly participate in redox reactions, cadmium induces oxidative stress indirectly by disrupting mitochondrial function, reducing antioxidant molecule availability, and interfering with cellular defence mechanisms. Increased ROS generation caused by cadmium exposure contributes to membrane lipid oxidation, protein modification, and cellular injury (Kumar et al., 2024).

A major biological response to cadmium exposure is the induction of metallothionein proteins. These cysteine-rich proteins bind metals and contribute to cellular detoxification. However, prolonged or excessive exposure may exceed this protective mechanism, resulting in increased free cadmium ions and oxidative damage (Zhang et al., 2023).

Cadmium accumulation is particularly concerning because of its ability to affect kidney function and bone metabolism after chronic exposure. Therefore, monitoring cadmium levels in edible fish tissues remains an important component of seafood safety assessment (EFSA CONTAM Panel, 2020).

2.2.3 Nickel (Ni) Toxicity

Nickel is a naturally occurring element present in aquatic environments through geological processes and anthropogenic activities. Although nickel participates in certain biological processes at trace concentrations, excessive exposure may produce toxic effects in aquatic organisms and humans (EFSA CONTAM Panel, 2020).

Nickel toxicity is associated mainly with oxidative stress, inflammatory responses, and mitochondrial dysfunction. Excess nickel may increase ROS production and modify antioxidant enzyme activities, leading to cellular damage and altered physiological functions in exposed organisms (Kumar et al., 2024).

In fish, nickel accumulation depends on environmental availability, water chemistry, sediment contamination, and biological regulation mechanisms. Therefore, evaluation of nickel together with other metals provides a more comprehensive assessment of contaminant exposure because combined metal interactions may influence toxicological responses (Zahran et al., 2025).

2.2.4 Zinc (Zn): Biological Importance and Toxicity

Zinc is an essential trace element required for numerous physiological processes, including enzyme activity, immune function, DNA synthesis, and antioxidant defence. Unlike lead and cadmium, zinc has an important biological role and is actively regulated by organisms (Ali et al., 2019).

However, excessive zinc exposure may disrupt cellular homeostasis and interfere with absorption and metabolism of other essential elements. High zinc concentrations may affect enzyme activity, induce oxidative imbalance, and cause physiological stress in aquatic organisms (Kumar et al., 2024).

Therefore, zinc assessment requires careful interpretation because it represents both a nutritional component and a potential contaminant depending on concentration. In fish products, zinc evaluation should consider its contribution to nutritional value as well as possible effects of excessive exposure.

2.2.5 Heavy Metals and Reactive Oxygen Species (ROS)

Reactive oxygen species are chemically reactive molecules generated naturally during aerobic metabolism. At physiological levels, ROS participate in cellular signalling and immune responses; however, excessive production results in oxidative stress and cellular damage (Rosa et al., 2021).

Heavy metals promote oxidative stress through multiple pathways, including mitochondrial dysfunction, inhibition of antioxidant enzymes, depletion of cellular antioxidants, and disruption of redox regulation. Lead, cadmium, and nickel are particularly associated with increased ROS production and oxidative injury (Kumar et al., 2024).

The susceptibility of fish to oxidative damage is increased because fish tissues contain high levels of polyunsaturated fatty acids. These fatty acids are highly vulnerable to ROS-mediated oxidation, leading to formation of lipid peroxidation products such as MDA (Ayala et al., 2014).

2.2.6 Antioxidant Defence and Superoxide Dismutase (SOD)

Fish possess complex antioxidant systems that protect cells against oxidative injury. These systems include enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants including glutathione (Rosa et al., 2021).

Superoxide dismutase represents the first enzymatic defence mechanism against oxidative stress. It converts superoxide radicals into hydrogen peroxide, which is subsequently detoxified by other antioxidant enzymes. Changes in SOD activity may indicate either adaptation to oxidative stress or impairment of antioxidant defence capacity (Rosa et al., 2021).

In environmental toxicology studies, increased SOD activity often reflects an adaptive response to elevated ROS production, whereas decreased activity may indicate antioxidant system exhaustion. Therefore, SOD should be interpreted together with other oxidative indicators such as MDA and metal concentrations (Kumar et al., 2024).

2.3 Nutritional Composition of Fish

Fish represents an important source of high-quality animal protein and essential nutrients, including long-chain omega-3 polyunsaturated fatty acids, vitamins, minerals, and bioactive compounds. The nutritional value of fish is influenced by several biological and environmental factors, including species, habitat, feeding behaviour, age, reproductive status, season, and production system. Therefore, nutritional composition varies considerably among fish species and must be evaluated when comparing different seafood products (Mohanty et al., 2022; Santos et al., 2023).

The fish species investigated in the present study include marine and freshwater species with different ecological characteristics. Marine fatty fish such as tuna, sardines, salmon, and mackerel generally contain higher lipid concentrations and greater amounts of omega-3 fatty acids, whereas many freshwater species such as tilapia and carp are often characterized by high protein content with variable lipid levels depending on diet and culture conditions (Ahmed et al., 2022; Khawar et al., 2024).

In addition to species variation, processing and preservation methods influence the final nutritional characteristics of fish products. Freezing, canning, storage duration, and packing medium may alter moisture distribution, lipid composition, protein characteristics, and oxidative stability. Therefore, evaluation of imported fish products requires consideration of both biological composition and post-harvest processing effects (Aubourg, 2023; Du et al., 2022).

2.3.1 Protein Content and Nutritional Importance

Protein is one of the most important nutritional components of fish muscle because it provides essential amino acids required for tissue growth, repair, enzymatic activity, and immune function. Fish proteins are considered highly digestible due to their favourable amino acid profile and relatively low connective tissue content compared with many terrestrial animal proteins (Mohanty et al., 2022).

Protein concentration differs among fish species according to physiological activity, feeding strategy, habitat, and environmental conditions. Highly active marine species such as tuna and salmon require continuous energy metabolism and muscular adaptation, which may contribute to differences in protein composition. Similarly, aquaculture species such as tilapia and carp may show variation in protein content depending on feed formulation and farming conditions (Ahmed et al., 2022).

Processing methods may influence the structural properties of fish proteins. Thermal treatment during canning causes protein denaturation and aggregation, resulting in changes in texture and water-holding capacity. However, properly controlled thermal processing generally preserves the nutritional availability of most amino acids (Boziaris, 2022).

During frozen storage, proteins may undergo gradual oxidation, aggregation, and degradation due to ice crystal formation and residual enzymatic activity. These changes may reduce functional properties and affect the sensory quality of frozen fish products (Du et al., 2022).

2.3.2 Lipid Content and Omega-3 Fatty Acids

Fish lipids are highly valued because they contain long-chain omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids contribute to cardiovascular health, regulation of inflammatory responses, and neurological development, making fatty fish an important component of a healthy diet (Mohanty et al., 2022).

The lipid content of fish varies widely among species. Pelagic migratory species such as tuna, salmon, sardines, and mackerel generally contain higher lipid levels because they require energy reserves for continuous swimming activity. In contrast, many freshwater species may contain lower lipid levels depending on environmental conditions and feeding systems (Santos et al., 2023).

Despite their nutritional benefits, high levels of polyunsaturated fatty acids increase susceptibility to oxidative deterioration. Lipid oxidation results in the formation of hydroperoxides and secondary oxidation products, including malondialdehyde (MDA), which can reduce nutritional quality and produce undesirable sensory changes (Ayala et al., 2014; Aubourg, 2023).

The oxidation rate of fish lipids is influenced by several factors, including temperature, oxygen exposure, storage duration, fatty acid composition, and the presence of catalytic metals. Heavy metals may contribute to oxidative deterioration by increasing reactive oxygen species generation and disturbing antioxidant defence mechanisms (Kumar et al., 2024).

2.3.3 Ash Content and Mineral Composition

Ash content represents the inorganic mineral fraction remaining after complete combustion of organic matter. In fish, minerals contribute to important physiological functions, including bone development, enzyme activity, osmotic regulation, and antioxidant defence mechanisms (Mohanty et al., 2022).

The mineral composition of fish varies according to species, habitat, diet, and environmental conditions. Marine fish may contain different mineral profiles compared with freshwater species because of differences in surrounding water chemistry and dietary sources. Similarly, aquaculture practices can influence mineral composition through feed formulation and water quality management (Ahmed et al., 2022).

However, total mineral content does not differentiate between beneficial essential elements and toxic contaminants. For example, zinc contributes to normal biological functions, whereas lead and cadmium represent undesirable contaminants. Therefore, ash determination should be complemented by specific elemental analysis techniques such as ICP-OES for accurate evaluation of nutritional and toxicological profiles (Thomas, 2021; Dragoev et al., 2024).

2.3.4 Relationship Between Nutritional Composition and Oxidative Stability

Nutritional composition and oxidative stability are closely interconnected in fish products. Species with high lipid content and abundant polyunsaturated fatty acids provide greater nutritional benefits but are also more vulnerable to oxidation during storage and processing. Therefore, evaluation of fish quality requires consideration of the balance between nutritional value and oxidative susceptibility (Aubourg, 2023).

Lipids and proteins may interact during oxidative deterioration. Lipid oxidation products can react with proteins, causing structural modifications and reducing nutritional quality. In addition, metals present naturally in fish tissues or acquired from environmental contamination may accelerate oxidative reactions through catalytic mechanisms (Kumar et al., 2024).

Consequently, assessment of proximate composition together with oxidative biomarkers provides a more comprehensive evaluation of imported fish products. Protein, lipid, and ash analysis describes nutritional quality, whereas MDA and SOD measurements provide information regarding oxidative stability and biochemical changes during storage and processing. (Mittakos et al., 2025).

2.4 Post-Mortem Changes in Fish

Following harvesting, fish muscle undergoes a series of biochemical and structural changes collectively known as post-mortem deterioration. These changes begin immediately after death because oxygen supply stops and physiological regulation mechanisms cease. The rate of deterioration depends on species characteristics, temperature control, handling procedures, microbial activity, and preservation method (Boziaris, 2022).

Fish are particularly susceptible to post-mortem deterioration because of their high moisture content, active endogenous enzymes, relatively low connective tissue content, and abundance of unsaturated lipids. These characteristics contribute to desirable nutritional properties but also accelerate quality loss during storage (Sveinsdóttir et al., 2021). The major post-mortem processes include ATP depletion, rigor mortis development, enzymatic autolysis, protein degradation, lipid hydrolysis, and oxidative deterioration. Understanding these processes is essential for interpreting quality changes in frozen and canned fish products. (Wang et al., 2026)

2.4.1 ATP Depletion and Rigor Mortis

Immediately after death, fish muscle shifts from aerobic metabolism to anaerobic glycolysis because oxygen delivery is interrupted. Glycogen is converted into lactic acid, causing a reduction in muscle pH. Simultaneously, ATP reserves gradually decline, resulting in the formation of rigor mortis due to permanent interaction between actin and myosin filaments (Boziaris, 2022).

The onset and duration of rigor mortis vary among fish species and are influenced by temperature, body size, nutritional condition, and stress before harvesting. Rapid temperature reduction after harvesting can delay deterioration processes and improve preservation quality (Sveinsdóttir et al., 2021).

2.4.2 Autolysis and Proteolytic Degradation

After rigor mortis, endogenous enzymes initiate autolytic degradation of muscle tissues. Proteolytic enzymes such as cathepsins and calpains break down structural proteins, resulting in muscle softening, reduced texture quality, and changes in water-holding capacity (Boziaris, 2022).

Freezing slows enzymatic activity but does not completely stop proteolysis. During prolonged frozen storage, residual enzyme activity and structural damage caused by ice crystals may contribute to protein degradation and quality deterioration (Du et al., 2022).

2.4.3 Lipid Oxidation During Fish Storage

Lipid oxidation is one of the most important biochemical processes responsible for deterioration of fish quality during storage. Fish lipids contain high proportions of polyunsaturated fatty acids, which contain multiple double bonds that are highly susceptible to oxidative attack. The oxidation process begins with free radical formation and proceeds through initiation, propagation, and

termination stages, producing primary oxidation products such as lipid hydroperoxides and secondary products including aldehydes and ketones (Ayala et al., 2014).

Among secondary lipid oxidation products, malondialdehyde (MDA) is one of the most commonly measured indicators because it reflects the extent of oxidative degradation of polyunsaturated fatty acids. Increased MDA levels are associated with deterioration of flavour, nutritional losses, and reduced acceptability of fish products. Therefore, MDA measurement is widely applied for evaluating oxidative stability in seafood products (Ayala et al., 2014; Suárez-Medina et al., 2024).

The rate of lipid oxidation in fish depends on several factors, including lipid composition, storage temperature, oxygen availability, processing conditions, and presence of pro-oxidant compounds. Transition metals such as iron and copper, as well as environmental contaminants including certain heavy metals, may accelerate oxidative reactions by promoting reactive oxygen species generation (Kumar et al., 2024).

Frozen storage decreases oxidation rates by reducing molecular mobility and enzymatic activity; however, oxidation is not completely prevented. Temperature fluctuations during storage may cause repeated partial thawing and refreezing, resulting in structural damage and increased exposure of lipids to oxygen and catalytic compounds (Du et al., 2022).

2.4.4 Freezing-Induced Structural Changes

Freezing is considered an effective preservation method for fish because it inhibits microbial growth and slows chemical reactions. However, freezing may cause structural modifications depending on freezing rate, storage duration, and

temperature stability. The formation of ice crystals represents the major mechanism responsible for physical damage to muscle tissue (Du et al., 2022).

Slow freezing results in formation of large extracellular ice crystals that disrupt muscle fibres and cellular membranes. During thawing, damaged tissues release intracellular fluids, leading to drip loss, reduction in water-holding capacity, and loss of soluble proteins and minerals. Rapid freezing minimizes these effects by producing smaller ice crystals and preserving muscle structure more effectively (Tian et al., 2022).

Prolonged frozen storage may also affect myofibrillar proteins through denaturation, aggregation, and oxidation. These changes contribute to reduced texture quality and altered functional properties of fish muscle. Therefore, maintenance of stable low temperatures throughout transportation and storage is essential for preserving frozen fish quality (Du et al., 2022).

2.4.5 Thermal Processing Effects During Canning

Canning involves sealing fish products in containers followed by thermal sterilization to eliminate pathogenic microorganisms and achieve commercial stability. Although this process provides long shelf life and improves food safety, exposure to high temperatures induces several biochemical changes in fish tissues (Boziaris, 2022).

Thermal treatment causes protein denaturation and structural rearrangement, which contribute to the characteristic soft texture of canned fish. The extent of these changes depends on temperature, processing time, fish species, and initial composition of muscle tissue (Boziaris, 2022).

Thermal sterilization also affects lipid oxidation and antioxidant activity. Although canned products are protected from oxygen because of hermetic sealing, high temperatures may accelerate certain oxidation reactions. Enzymatic antioxidants such as superoxide dismutase (SOD) are sensitive to heat and may undergo partial or complete inactivation during processing. Therefore, SOD measurements in canned fish should be interpreted as indicators of residual enzymatic stability rather than direct physiological responses (Aubourg, 2023).

The packing medium also influences the final quality of canned products. Fish packed in oil may show higher lipid content because of migration of external oil into the tissue, whereas brine or water-packed products may have different moisture and oxidative characteristics. Therefore, differences among canned fish products should consider both fish species and packing conditions (Mozzon et al., 2024).

2.5 Oxidative Stress Biomarkers in Fish Products

Oxidative stress represents an imbalance between reactive oxygen species production and antioxidant defence capacity. Under normal physiological conditions, reactive oxygen species are continuously generated during cellular metabolism and controlled by antioxidant systems. However, excessive ROS production may result in oxidative damage to lipids, proteins, and nucleic acids (Rosa et al., 2021).

In fish, oxidative stress can arise from multiple factors, including environmental pollutants, heavy-metal exposure, oxygen availability, storage conditions, and processing treatments. Because fish tissues contain high levels of polyunsaturated fatty acids, they are particularly vulnerable to oxidative damage (Kumar et al., 2024).

Oxidative biomarkers provide valuable information regarding biochemical deterioration and contaminant-induced stress. Among these biomarkers, malondialdehyde (MDA) reflects lipid peroxidation, whereas antioxidant enzymes such as superoxide dismutase (SOD) indicate the capacity of biological systems to neutralize oxidative damage (Rosa et al., 2021).

2.5.1 Malondialdehyde (MDA) as an Oxidative Deterioration Marker

Malondialdehyde is a secondary product generated during oxidation of polyunsaturated fatty acids. Because of its relative stability and relationship with lipid degradation, MDA is widely used as an indicator of oxidative deterioration in fish and seafood products (Ayala et al., 2014).

The level of MDA in fish products depends on lipid content, fatty acid composition, storage conditions, processing method, and presence of pro-oxidant factors. Fat-rich fish species such as salmon, sardines, tuna, and mackerel generally demonstrate greater susceptibility to lipid oxidation compared with lean fish species (Aubourg, 2023).

In the context of imported fish products, MDA analysis provides information regarding the oxidative history of the product. Elevated MDA concentrations may indicate prolonged storage, inadequate temperature control, high lipid susceptibility, or increased exposure to oxidative catalysts (Suárez-Medina et al., 2024).

2.5.2 Superoxide Dismutase (SOD) as an Antioxidant Biomarker

Superoxide dismutase is one of the primary enzymatic antioxidant defences responsible for converting superoxide radicals into hydrogen peroxide. This enzyme represents an essential component of cellular protection against oxidative injury (Rosa et al., 2021).

Changes in SOD activity may reflect alterations in oxidative status. Increased activity may indicate an adaptive response to elevated ROS production, whereas decreased activity may suggest exhaustion or inhibition of antioxidant defence mechanisms. Therefore, SOD results should always be interpreted together with oxidative damage indicators such as MDA and contaminant concentrations (Kumar et al., 2024).

In processed fish products, especially canned fish, thermal treatment may reduce enzymatic activity. Consequently, measured SOD levels represent the remaining antioxidant capacity after processing rather than the original physiological antioxidant response of living fish (Aubourg, 2023).

2.6 Imported Canned and Frozen Fish: Market and Preservation Dynamics

Fish products represent an important component of global food supply because they provide high-quality protein, essential fatty acids, vitamins, and minerals. International trade allows consumers to access diverse fish species throughout the year; however, imported products may differ in quality depending on origin, harvesting conditions, processing technology, transportation, storage, and regulatory control (FAO, 2024).

Imported fish products generally include frozen marine and freshwater species as well as canned products such as tuna and sardines. These products undergo different preservation strategies. Freezing relies on temperature reduction to inhibit microbial growth and slow biochemical reactions, whereas canning depends on thermal sterilization and hermetic sealing. Each preservation method produces specific effects on nutritional composition, oxidative stability, and contaminant behaviour (Du et al., 2022; Aubourg, 2023).

Quality assessment of imported fish therefore requires an integrated approach that considers environmental contamination before processing, chemical changes during preservation, packaging effects, and storage conditions. Combining heavy-metal analysis with nutritional composition and oxidative biomarkers provides a comprehensive evaluation of seafood quality and safety (Arisekar et al., 2024).

2.6.1 Freezing and Thawing Effects

Freezing is one of the most widely applied preservation techniques for fish products because it effectively reduces microbial growth and decreases enzymatic activity responsible for quality deterioration. By reducing temperature below the freezing point of water, metabolic activity of microorganisms is inhibited, allowing extended storage and distribution of fish products without significant microbial spoilage (Du et al., 2022).

However, freezing does not completely prevent biochemical deterioration. The quality changes occurring during frozen storage are mainly associated with ice crystal formation, protein denaturation, lipid oxidation, and changes in water distribution within muscle tissues. The extent of these changes depends strongly on freezing rate, storage temperature, duration of storage, and species characteristics (Tian et al., 2022).

The freezing rate represents an important determinant of fish quality. Slow freezing promotes the formation of large ice crystals, mainly in extracellular spaces, causing mechanical damage to muscle fibres and disruption of cellular membranes. During thawing, this damage results in increased drip loss, reduction in water-holding capacity, and leakage of soluble proteins, minerals, and other intracellular components (Du et al., 2022).

In contrast, rapid freezing produces smaller ice crystals and minimizes structural damage, thereby maintaining better muscle integrity and nutritional quality. Therefore, industrial freezing technologies aim to achieve rapid temperature reduction to preserve texture and reduce biochemical deterioration during storage (Tian et al., 2022).

Repeated freezing and thawing cycles represent an important factor affecting frozen fish quality. Temperature fluctuations during transportation or storage may result in partial thawing followed by refreezing, increasing cellular damage, accelerating protein degradation, and promoting lipid oxidation. Therefore, maintaining a continuous cold chain is essential for preserving frozen fish quality (Du et al., 2022).

2.6.2 Protein Changes During Frozen Storage

Fish muscle proteins are highly sensitive to freezing-related changes. During prolonged frozen storage, myofibrillar proteins may undergo denaturation, aggregation, and oxidation, resulting in reduced solubility and altered functional properties. These changes negatively affect texture, water retention, and consumer acceptability of frozen fish products (Tian et al., 2022).

Protein oxidation during frozen storage may occur through interaction with reactive oxygen species generated from lipid oxidation processes. Oxidized proteins may exhibit structural modification, reduced nutritional quality, and increased susceptibility to enzymatic degradation. Therefore, protein stability represents an important parameter for evaluating frozen fish quality (Du et al., 2022).

2.6.3 Canning and Thermal Sterilization Effects

Canning is a preservation method based on thermal sterilization of fish packed in sealed containers. The main objective of this process is to eliminate pathogenic microorganisms and prevent spoilage, allowing long-term storage without refrigeration. Although highly effective for food safety, thermal processing causes several chemical and structural modifications in fish tissues (Boziaris, 2022).

High temperatures during sterilization induce protein denaturation and aggregation, resulting in changes in muscle texture. These changes are responsible for the characteristic softness of canned fish products. The extent of protein modification depends on processing temperature, heating duration, fish species, and initial muscle composition (Boziaris, 2022).

Thermal processing also influences lipid stability. Although canned products are protected from oxygen by hermetic sealing, heating may accelerate certain oxidative reactions. This effect is particularly relevant for fatty fish species because their high polyunsaturated fatty acid content increases susceptibility to oxidation (Aubourg, 2023).

Antioxidant enzymes are also affected by thermal treatment. Enzymes such as superoxide dismutase (SOD) are sensitive to heat and may undergo partial or complete inactivation during sterilization. Therefore, SOD values measured in canned fish should be interpreted as indicators of remaining antioxidant activity after processing rather than direct indicators of biological oxidative stress (Aubourg, 2023).

2.6.4 Influence of Packing Medium on Canned Fish Quality

The packing medium is an important factor influencing the nutritional and oxidative characteristics of canned fish products. Commercial canned fish may be packed in vegetable oils, olive oil, brine, or water, and each medium produces different effects on moisture content, lipid composition, flavour, and oxidative stability (Mozzon et al., 2024).

Oil-packed products may demonstrate higher measured fat content because lipids from the packing medium can migrate into fish tissues during storage. Therefore, differences in lipid content among canned fish products should not be interpreted only as biological variation between species but should also consider the composition of the surrounding medium (Mozzon et al., 2024).

The packing medium may also influence oxidative reactions. Oils containing natural antioxidants may provide some protective effects, whereas highly unsaturated oils may increase susceptibility to oxidation. Consequently, evaluation of canned fish quality requires consideration of interactions between fish composition and packing environment (Aubourg, 2023).

2.6.5 Long-Term Storage and Oxidative Deterioration

Storage duration is an important factor affecting the quality of both frozen and canned fish products. Although preservation methods significantly slow deterioration processes, biochemical reactions may continue during storage and gradually influence nutritional and sensory characteristics (Du et al., 2022).

In frozen products, prolonged storage may result in slow lipid oxidation, protein modification, and structural damage caused by ice crystal formation. Temperature fluctuations accelerate these processes by increasing molecular mobility and promoting repeated freeze–thaw damage (Tian et al., 2022).

Canned fish products generally demonstrate greater stability because oxygen availability is limited by hermetic sealing. However, storage time, temperature, packaging integrity, and interactions between food components and packaging materials may influence long-term quality changes (Begum et al., 2023).

The determination of oxidative biomarkers such as MDA provides valuable information regarding the oxidative history of fish products. Elevated MDA concentrations may indicate prolonged storage, inadequate preservation conditions, high lipid susceptibility, or increased exposure to pro-oxidant factors (Suárez-Medina et al., 2024).

2.6.6 Effect of Processing on Heavy Metal Concentrations

Processing methods may influence measured heavy-metal concentrations in fish products; however, these changes do not always represent actual increases or decreases in total metal burden. Processing may alter moisture content, redistribute soluble components, and modify elemental concentrations when expressed on a wet-weight basis (Liao et al., 2020).

Cooking and thermal processing may influence both total metal concentration and gastrointestinal bioaccessibility. Liao et al. (2020) demonstrated that processing conditions affected the concentration and bioaccessibility of several elements, including cadmium, lead, nickel, and zinc, in seafood products.

Therefore, comparisons between frozen and canned fish should consider differences in processing history, moisture changes, storage conditions, and analytical expression basis. Total elemental concentration provides valuable information regarding contamination status; however, interpretation of potential

human exposure requires consideration of bioavailability and bioaccessibility factors (Liao et al., 2020; Marmelo et al., 2020).

2.7 Ecological Niches and Trophic Levels of the Investigated Species

The ecological characteristics of fish species represent major determinants of contaminant accumulation patterns. Habitat preference, trophic position, feeding behaviour, mobility, and physiological regulation mechanisms influence the pathways through which fish are exposed to environmental contaminants (Feng et al., 2020).

Predatory pelagic species such as tuna, salmon, and mackerel occupy relatively high trophic positions and may accumulate contaminants through dietary transfer. This process, known as biomagnification, describes the increase of contaminant concentrations through successive trophic levels. However, biomagnification varies among metals depending on their chemical properties, environmental availability, and biological regulation mechanisms (Oros, 2025).

Freshwater species such as carp and tilapia may experience different exposure pathways because of their interaction with sediments and freshwater environments. Sediments can act as reservoirs for metals, allowing gradual release and uptake by benthic-associated organisms. Therefore, feeding strategy and habitat preference should be considered when interpreting metal accumulation patterns (Garnero et al., 2018; Zahran et al., 2025).

2.8 Environmental Toxicity and Heavy Metal Bioaccumulation

Heavy metals represent one of the most important groups of aquatic environmental pollutants because of their persistence, toxicity, and ability to accumulate within biological systems. Unlike organic contaminants that may undergo degradation, metals remain in ecosystems and can circulate between water, sediments, and living organisms for extended periods. Consequently, aquatic organisms, particularly fish, represent important indicators for evaluating environmental contamination and potential human exposure through seafood consumption (Ali et al., 2019; Kumar et al., 2024).

Fish accumulate heavy metals through several exposure pathways, including direct absorption through gills, ingestion of contaminated food organisms, and contact with contaminated sediments. The relative contribution of each pathway depends on the chemical properties of the metal, environmental conditions, feeding behaviour, habitat preference, and physiological characteristics of the fish species (Zhang et al., 2023).

Following uptake, metals are transported through the bloodstream and distributed among different tissues. The biological response to metal exposure involves several defence mechanisms, including sequestration by metallothioneins, antioxidant enzyme activation, and cellular repair mechanisms. However, when exposure exceeds the detoxification capacity of the organism, metals may induce oxidative stress, inflammation, impaired physiological functions, and tissue damage (Zahran et al., 2025).

2.8.1 Mechanisms of Heavy Metal Bioaccumulation

Bioaccumulation refers to the gradual increase in contaminant concentration within an organism when uptake from the environment exceeds elimination processes. In fish, bioaccumulation occurs through continuous exposure to metals present in water, sediments, and dietary sources. The extent of accumulation depends on exposure concentration, duration, metabolic activity, and species-specific regulation mechanisms (Kumar et al., 2024).

Essential metals such as zinc are actively regulated because they participate in biological processes, whereas non-essential metals such as lead and cadmium may accumulate progressively because organisms lack effective physiological functions for their removal. Long-term exposure may therefore result in increased tissue concentrations and potential toxic effects (EFSA CONTAM Panel, 2020).

Metallothioneins represent an important biological mechanism involved in metal regulation. These cysteine-rich proteins bind metals through sulfhydryl groups and reduce their reactivity. However, prolonged exposure may saturate this protective mechanism, allowing accumulation of free metal ions that contribute to oxidative damage and cellular dysfunction (Zhang et al., 2023).

2.8.2 Tissue-Specific Distribution of Heavy Metals

The distribution of heavy metals within fish tissues is not uniform and depends on the physiological function of each organ. Different tissues demonstrate different accumulation capacities according to their involvement in absorption, metabolism, detoxification, and elimination processes (Ali et al., 2019).

Gills represent one of the primary sites of metal uptake because they are continuously exposed to surrounding water and possess a large surface area for exchange processes. Dissolved metals may enter through gill membranes and subsequently be transported to internal organs. Therefore, gills are frequently used as indicators of aquatic contamination (Kumar et al., 2024).

The liver plays a central role in metal metabolism and detoxification. It accumulates metals through binding with metallothioneins and participates in regulating internal metal balance. However, excessive accumulation may impair hepatic function and induce oxidative stress through increased reactive oxygen species production (Zhang et al., 2023).

Kidneys are also important sites for metal accumulation because of their role in filtration and excretion. They may retain metals during attempts to eliminate toxic substances from circulation. Therefore, liver and kidney tissues generally contain higher metal concentrations compared with muscle tissue (Ali et al., 2019).

Although muscle usually contains lower concentrations of heavy metals compared with metabolically active organs, it represents the most important tissue from a food-safety perspective because it is the primary edible portion consumed by humans. Consequently, analysis of muscle tissue provides essential information for dietary exposure assessment and consumer-risk evaluation (Saha et al., 2016; EFSA CONTAM Panel, 2020).

2.8.3 Factors Influencing Heavy Metal Accumulation in Fish

Heavy-metal accumulation is influenced by multiple interacting factors, including environmental contamination, water chemistry, sediment characteristics, fish age, body size, trophic position, feeding behaviour, and physiological regulation. Therefore, differences among fish species cannot be explained solely by environmental contamination levels but require consideration of biological and ecological characteristics (Feng et al., 2020).

Environmental parameters such as pH, temperature, salinity, dissolved oxygen, and organic matter content influence metal solubility and availability. Changes in these conditions may increase or decrease the fraction of metals available for uptake by aquatic organisms (Garnero et al., 2018).

Age and body size may also influence accumulation because older fish have longer exposure periods. However, this relationship differs among metals because some essential elements are actively regulated, while non-essential metals may continue accumulating over time (Kumar et al., 2024).

2.9 Metal Migration from Packaging in Canned Fish Products

The safety of canned fish products depends not only on contamination acquired from the aquatic environment but also on possible chemical interactions between the food matrix and packaging materials. Commercial cans are commonly manufactured from tin-plated steel or aluminium with internal protective coatings designed to minimize direct contact between the metal container and food contents (Begum et al., 2023).

However, packaging coatings may deteriorate during prolonged storage, temperature fluctuations, mechanical damage, or exposure to acidic and saline food environments. Such conditions may increase the possibility of migration of certain elements from packaging materials into the fish product or packing medium (Begum et al., 2023).

Potentially migrating elements include lead, cadmium, nickel, and zinc. The extent of migration depends on several factors, including packaging composition, storage duration, food acidity, salt content, temperature, and integrity of the internal coating. Therefore, detected metal concentrations in canned fish may originate from both environmental accumulation before processing and packaging-related contamination after processing (Arisekar et al., 2024).

Long transportation periods associated with imported canned fish products make packaging evaluation particularly important. Products may remain in storage facilities or during international transportation for extended periods before reaching consumers. Therefore, assessment of canned fish safety should consider environmental exposure, processing conditions, packaging interactions, and storage history as interconnected factors (Begum et al., 2023).

2.10 Analytical Determination of Heavy Metals Using ICP-OES

Accurate determination of trace metals in fish products requires analytical techniques capable of detecting low concentrations within complex biological matrices. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is one of the widely used techniques for multi-element determination in food and environmental samples because of its high analytical capacity, reproducibility, and suitability for routine monitoring (Thomas, 2021).

ICP-OES is based on atomic emission principles. During analysis, prepared samples are introduced into an argon plasma operating at high temperature. The thermal energy of the plasma causes atomization and excitation of elemental species. When excited atoms return to lower energy states, they emit radiation at characteristic wavelengths. The intensity of emitted light is proportional to the concentration of the corresponding element in the sample (Thomas, 2021).

For fish analysis, samples are commonly subjected to acid digestion procedures to destroy organic components and release metals into solution. Effective digestion is essential because incomplete extraction may result in inaccurate estimation of elemental concentrations (Bozorgzadeh et al., 2021).

ICP-OES provides several advantages, including simultaneous detection of multiple elements, wide linear range, acceptable detection limits, and good precision. These characteristics make it suitable for determining Pb, Cd, Ni, and Zn concentrations in fish products and evaluating compliance with food-safety requirements (Dragoev et al., 2024; Abdul Mottaleb et al., 2025).

2.10.1 Major Element Concentration and Metal Speciation

The toxicological significance of metals is determined not only by their total concentration but also by their chemical form, solubility, and biological availability. Different chemical species of the same element may demonstrate different absorption rates, biological behaviour, and toxicity profiles. Therefore, total elemental concentration provides important information regarding contamination status but does not completely describe the potential biological effects after consumption (EFSA CONTAM Panel, 2020).

ICP-OES is primarily designed for determination of total elemental concentrations after complete digestion of the sample. Therefore, concentrations of Pb, Cd, Ni, and Zn obtained using ICP-OES represent total elemental content unless additional speciation procedures are performed. This distinction is important when interpreting analytical results in relation to human exposure because total concentration does not necessarily represent the fraction absorbed by the gastrointestinal tract (EFSA CONTAM Panel, 2020).

Metal speciation influences toxicity because chemical forms differ in stability, absorption, and interaction with biological molecules. For example, some metal complexes may be strongly bound to proteins and exhibit lower availability, whereas more soluble forms may become more accessible during digestion. Therefore, combining total elemental analysis with bioaccessibility studies provides a more comprehensive understanding of potential health risks associated with seafood consumption (Liao et al., 2020; Marmelo et al., 2020).

2.10.2 Method Validation and Quality Control for ICP-OES Analysis

Reliable analytical determination of heavy metals requires strict quality-control procedures throughout all stages of sample preparation and instrumental measurement. Errors may originate from contamination during digestion, incomplete extraction, calibration problems, matrix interference, or instrumental instability. Therefore, analytical validation is essential to ensure that measured concentrations accurately represent the composition of analysed fish samples (Cantwell, 2025).

Method validation evaluates whether an analytical procedure is suitable for its intended purpose. Important validation parameters include linearity, precision, accuracy, recovery, limit of detection (LOD), limit of quantification (LOQ), selectivity, and robustness. These parameters demonstrate the reliability and reproducibility of analytical measurements, particularly when determining trace concentrations of metals in complex biological matrices (Cantwell, 2025).

Recovery experiments are particularly important because digestion efficiency directly influences elemental determination. A validated method should achieve adequate recovery of target metals without introducing contamination or significant analytical bias. Certified reference materials are commonly used to verify analytical accuracy because they contain known concentrations of elements within matrices similar to the analysed samples (Bozorgzadeh et al., 2021).

Quality-control procedures should include reagent blanks, calibration verification, duplicate analyses, recovery tests, and certified reference materials. These procedures improve confidence in analytical results and ensure that reported concentrations are suitable for environmental monitoring and food-safety evaluation (ISO, 2017; Abdul Mottaleb et al., 2025).

2.11 Fish Importation in Iraq and National Quality Standards

Fish consumption in Iraq is supported by both domestic production and imported seafood products. Imported frozen and canned fish have become widely available because they provide affordable animal protein, extended shelf life, and access to species that may not be locally available. These products originate from different geographical regions and production systems, resulting in variation in environmental exposure, nutritional composition, and processing conditions (FAO, 2024).

The international seafood supply chain involves several stages, including harvesting, processing, packaging, transportation, and storage. Each stage may influence the final quality of fish products. Environmental contamination may occur before harvesting, whereas processing, packaging, and storage may introduce additional quality changes after production (Arisekar et al., 2024).

Food-safety assessment of imported fish products relies on international guidelines established by organizations such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Codex Alimentarius Commission. These frameworks provide maximum permissible levels for specific contaminants and establish principles for protecting consumers from chemical hazards in food products (Codex Alimentarius Commission, 2023).

For imported seafood, regulatory monitoring is particularly important because products may originate from regions with different environmental conditions, production systems, and food-control regulations. Therefore, continuous evaluation of heavy metals and other quality indicators is necessary to ensure that imported fish products comply with safety requirements.

2.11.1 Importance of Heavy Metal Monitoring in Imported Fish

Heavy-metal monitoring represents an essential component of seafood safety because fish consumption is one of the major dietary pathways for human exposure to environmental contaminants. Metals such as lead and cadmium are of particular concern because they are persistent, accumulate in biological tissues, and may produce adverse health effects after chronic exposure (WHO, 2023).

However, evaluation of seafood safety should not depend only on comparison with maximum permissible limits. A comprehensive assessment should consider species-specific accumulation patterns, consumption frequency, portion size, consumer characteristics, and possible interactions between multiple contaminants (EFSA CONTAM Panel, 2020).

Combining heavy-metal analysis with nutritional composition and oxidative biomarkers provides a broader evaluation of imported fish quality. Elemental analysis evaluates chemical safety, proximate composition determines nutritional value, and oxidative indicators provide information regarding deterioration processes during storage and processing.

2.12 Human Health Risk Assessment and Regulatory Frameworks

Fish consumption provides significant nutritional benefits; however, contaminated seafood may represent a potential source of exposure to toxic metals. The health impact of dietary exposure depends on contaminant concentration, consumption rate, body weight, exposure duration, and susceptibility of the consumer (EFSA CONTAM Panel, 2020).

Lead is considered a cumulative toxicant that affects several physiological systems, particularly the nervous system, kidneys, cardiovascular system, and developing tissues. Chronic exposure is associated with neurological impairment and developmental effects, especially among children and pregnant women (WHO, 2023).

Cadmium is another important food contaminant because of its long biological half-life and ability to accumulate mainly in kidney tissues. Chronic exposure may contribute to renal dysfunction and disturbances in bone metabolism. Nickel exposure may induce toxic effects at elevated levels, particularly among individuals with nickel sensitivity, whereas zinc represents an

essential element that becomes harmful only when excessive exposure occurs (EFSA CONTAM Panel, 2020).

Therefore, risk assessment of fish products requires evaluation of both toxic and essential elements. The interpretation of metal concentrations should consider nutritional contribution, potential toxicity, and long-term dietary exposure scenarios.

2.12.1 Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI)

The concentration of heavy metals measured in fish tissues represents the initial parameter for exposure assessment; however, potential human exposure depends on consumption behaviour and physiological characteristics. Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) are commonly applied approaches for converting contaminant concentrations into dietary exposure estimates (Saha et al., 2016; Simukoko et al., 2022).

EDI represents the estimated amount of contaminant ingested per day, whereas EWI provides an estimation of weekly exposure. These calculations incorporate parameters including metal concentration, consumption rate, and body weight, allowing comparison with established toxicological reference values (EFSA CONTAM Panel, 2020).

Dietary exposure assessment provides a more realistic interpretation of contamination data because it considers actual consumption scenarios rather than relying only on measured concentrations. This approach is particularly important for seafood products consumed regularly over long periods.

2.12.2 Target Hazard Quotient (THQ) and Hazard Index (HI)

The Target Hazard Quotient (THQ) is widely used to estimate potential non-carcinogenic risks associated with dietary exposure to contaminants. It compares estimated exposure levels with reference doses considered unlikely to cause adverse effects during lifetime exposure (Saha et al., 2016).

When multiple metals are present, individual hazard quotients can be combined to calculate the Hazard Index (HI). This approach provides an estimation of cumulative exposure from several contaminants simultaneously and allows comparison between different fish species and consumer scenarios (Simukoko et al., 2022).

Although THQ and HI are useful screening tools, they should not be interpreted as direct predictors of disease occurrence. Instead, they provide an indication of whether exposure levels require further toxicological evaluation and support comparisons among different seafood products (Ray et al., 2024).

2.12.3 Consumer Groups at Risk

The potential health effects of heavy metals differ among population groups because susceptibility depends on age, body weight, nutritional status, and physiological condition. Children, pregnant women, elderly individuals, and populations with frequent seafood consumption may represent groups with increased vulnerability (WHO, 2023).

Children are particularly sensitive to lead toxicity because of their developing nervous system and lower body weight. Even relatively low exposure levels may influence cognitive development and neurological function. Pregnant women and developing fetuses are also vulnerable because certain metals may

cross biological barriers and interfere with developmental processes (WHO, 2023).

Therefore, seafood risk assessment should consider not only average consumers but also vulnerable groups who may experience greater consequences from chronic exposure.

2.13 Fish Bioavailability and Bioaccessibility of Heavy Metals

The total concentration of heavy metals detected in fish tissues does not necessarily represent the actual amount available for absorption by humans after consumption. The toxicological impact of a contaminant depends not only on its concentration in food but also on its chemical form, release during digestion, absorption efficiency, metabolism, and distribution within the body. Therefore, bioaccessibility and bioavailability are important concepts in modern seafood safety assessment (Liao et al., 2020; Marmelo et al., 2020).

Bioaccessibility refers to the fraction of a contaminant released from the food matrix during gastrointestinal digestion and becoming available for intestinal absorption. In contrast, bioavailability represents the fraction that is absorbed into systemic circulation and becomes available to exert biological effects. Therefore, bioaccessibility represents an important intermediate factor between total contamination and actual human exposure (Liao et al., 2020).

The bioaccessibility of heavy metals in fish products depends on several factors, including chemical characteristics of the metal, food matrix composition, lipid and protein content, mineral interactions, and processing conditions. Metals strongly bound to proteins or structural components may demonstrate lower release during digestion, whereas soluble forms may exhibit greater potential availability (Marmelo et al., 2020).

Fish composition plays an important role in determining metal behaviour during digestion. Proteins, lipids, and minerals may interact with metals and influence their solubilization. Consequently, two fish products containing similar total concentrations of a metal may demonstrate different potential exposure levels because of differences in matrix composition and processing history (Liao et al., 2020).

2.13.1 Influence of Processing on Metal Bioaccessibility

Food processing may alter both the total concentration and gastrointestinal availability of heavy metals. Thermal processing, cooking, freezing, and other preservation methods can modify the structure of the food matrix and influence the release of metals during digestion (Liao et al., 2020).

Cooking may reduce or increase measured metal concentrations depending on the element, processing conditions, and changes in moisture content. Loss of water during heating may concentrate some elements, whereas transfer of soluble metals into cooking fluids may decrease measured concentrations in the edible portion (Liao et al., 2020).

Therefore, interpretation of elemental concentrations in processed fish requires consideration of processing history and analytical basis. Measurements obtained by ICP-OES after complete digestion represent total elemental concentrations and should not be directly interpreted as absorbed human exposure without considering bioaccessibility and bioavailability factors (Liao et al., 2020; Marmelo et al., 2020).

2.13.2 Importance of Bioaccessibility in Seafood Risk Assessment

Traditional seafood safety assessment commonly relies on comparing total metal concentrations with established maximum permissible limits. Although this approach is important for regulatory purposes, it may not fully represent actual human exposure because only a fraction of ingested metals may become available for absorption (EFSA CONTAM Panel, 2020).

The integration of bioaccessibility data with dietary exposure assessment provides a more realistic estimation of potential health risks. This approach is particularly relevant for seafood because fish species differ considerably in protein, lipid, mineral composition, and processing characteristics, all of which may influence metal release during digestion (Marmelo et al., 2020).

Therefore, combining total elemental determination, bioaccessibility evaluation, nutritional analysis, and oxidative biomarkers provides a more comprehensive framework for assessing the quality and safety of imported fish products.

2.14 Previous Studies

Previous studies have demonstrated that fish quality and safety are influenced by multiple interacting factors, including species characteristics, environmental exposure, ecological niche, processing conditions, and storage history. Heavy-metal concentrations in fish tissues vary considerably according to habitat, trophic position, feeding behaviour, and geographical origin (Feng et al., 2020).

Saha et al. (2016) evaluated heavy-metal contamination and associated health risks in edible fish species and demonstrated that metal accumulation was not uniform among species. Their findings emphasized the importance of considering biological and environmental differences when evaluating seafood safety.

Garnero et al. (2018) investigated metal accumulation patterns among freshwater fish species with different ecological characteristics. The study demonstrated that habitat preference and feeding behaviour influenced the distribution of metals among species, indicating that ecological factors should be considered during interpretation of elemental concentrations.

Feng et al. (2020) examined trace-metal accumulation among commercially important fish species and demonstrated significant variation associated with trophic level and habitat preference. The study also highlighted the usefulness of multivariate statistical analysis for identifying relationships between metal profiles and ecological characteristics.

Simukoko et al. (2022) compared wild and farmed Nile tilapia and reported differences in elemental composition between production systems. These differences were attributed to variation in environmental exposure, feed composition, and farming conditions. The study demonstrated that production origin represents an important factor influencing the chemical profile of fish products.

Processing-related changes have also been investigated in previous studies. Prego et al. (2022) evaluated the influence of canning and packing media on Atlantic mackerel and demonstrated that preservation methods affected fatty acid composition and nutritional characteristics. These findings indicate that

differences among canned fish products cannot be attributed solely to species variation but should also consider processing conditions.

Liao et al. (2020) investigated the influence of processing on concentrations and gastrointestinal bioaccessibility of metals in seafood. Their results demonstrated that cooking and processing conditions modified both elemental concentration and the fraction potentially available for absorption, emphasizing the importance of considering bioaccessibility in seafood risk assessment.

Recent studies have further demonstrated the importance of integrating contaminant analysis with health-risk assessment approaches. Almafrachi et al. (2025) evaluated heavy-metal bioaccumulation and health risks in commercial fish species and confirmed that accumulation patterns differed among species according to ecological and biological characteristics. Similarly, Ray et al. (2024) applied ICP-based metal determination combined with THQ and HI calculations to evaluate consumer risks associated with fish consumption.

2.14.1 Research Gap and Justification of the Present Study

Although previous investigations have provided valuable information regarding heavy-metal contamination, nutritional composition, ecological influences, and processing effects in fish products, most studies have focused on individual aspects of seafood quality rather than evaluating multiple parameters simultaneously.

Many studies have investigated heavy metals alone, while others have focused mainly on nutritional composition, lipid oxidation, or processing effects. Limited information is available regarding the simultaneous evaluation of toxic metals (Pb, Cd, Ni, and Zn), proximate composition, and oxidative biomarkers

such as malondialdehyde (MDA) and superoxide dismutase (SOD) in imported canned and frozen fish products.

The present study addresses this gap by providing an integrated evaluation of imported fish products available in local markets. The simultaneous assessment of elemental contamination, nutritional composition, and oxidative status provides a more comprehensive understanding of seafood quality and safety.

Furthermore, combining these parameters allows investigation of possible relationships between contaminant accumulation, nutritional characteristics, and oxidative deterioration. Such an approach may provide stronger evidence for evaluating imported fish products compared with studies based on a single analytical parameter.

Chapter Three

Materials and Methods



Chapter Three

Materials and Methods

3.1 Study Design and Sampling Strategy

The present study was designed as an analytical cross-sectional study conducted in Iraqi consumer markets to evaluate the nutritional composition, oxidative stress biomarkers, and heavy metal contamination of imported fish products available for consumption. Fish samples were randomly collected from wholesale and retail markets representing three Iraqi governorates: Babylon, Najaf, and Al-Diwaniyah, to provide a representative overview of imported fish products available in the Iraqi market.

A total of 39 primary fish samples representing different fish species, commercial brands, and preservation forms (canned and frozen products) were collected and analyzed, as summarized in Table 3.1. The collected samples included commercially available imported fish products commonly consumed by Iraqi consumers.

To ensure analytical accuracy and minimize experimental variability, all investigated parameters were measured in triplicate. Accordingly, a total of 117 analytical determinations were performed for all measured parameters ($n = 117$).

Sample collection was conducted in three consecutive batches at different time intervals. The first batch was collected in October 2025, the second in December 2025, and the third in January 2026. Samples were randomly selected from different markets within the selected governorates and immediately transported to the laboratory in insulated polystyrene boxes containing ice to maintain sample integrity and minimize enzymatic degradation and lipid oxidation during transportation.

Upon arrival at the laboratory, fish samples were properly labelled, prepared for storage, and preserved at -18°C in a freezer until further biochemical, oxidative, and elemental analyses were performed.

Table 3.1 Distribution, scientific nomenclature, and categorization of the imported fish samples collected from Iraqi consumer markets.

Sample ID	Common Name	Scientific Name	Form / Type	Predominant Origin	No. of Samples	No. of Technical Measurements
S01	Tuna	<i>Thunnus</i> / .spp <i>Scombridae</i>	Canned in) (oil/brine	/Thailand Vietnam Indone/Turkey /Iran/China/sia	25	75
S02	Sardine	<i>Sardina</i> <i>pilchardus</i>	Canned in) (oil/brine	India/Thailand Chi/Indonesia/ /na	7	21
S03	Sea Bream	<i>Sparus</i> <i>aurata</i>	Frozen	Turkey	1	3
S04	-Red bellied Pacu	<i>Piaractus</i> <i>brachypomus</i>	Frozen	Vietnam	1	3

S05	Common Carp	<i>Cyprinus carpio</i>	Frozen	Myanmar	1	3
S06	Salmon	<i>Salmo salar</i>	Frozen	Iran	1	3
S07	Nile Tilapia	<i>Oreochromis niloticus</i>	Frozen	Indonesia	1	3
S08	Golden Pompano	<i>Trachinotus blochii</i>	Frozen	Jordan	1	3
S09	Mackerel	<i>Scomber scombrus</i>	Frozen	China	1	3
Total					39	117

3.2 Preparation for Heavy Metal and Ash Analysis

The fish tissues were dried in an oven at 60°C to constant weight (AOAC, 2023) for the elemental and proximate composition analysis. The dried samples were powdered in a pre-cleaned ceramic mortar and pestle.

All sample preparations were carried out under strict contamination control to prevent cross contamination according to recent trace metal analysis protocols for biological matrices (Zhang et al., 2023).

3.3 Chemicals and Laboratory Equipment

All chemicals were of analytical grade for high analytical accuracy and low interference.

Ultrapure deionized water was used for all experiments.

The major reagents, laboratory apparatuses, specifications and applications used in this study are given in Table 3.2. The required analytical and preparative tools such as drying oven, analytical balance, muffle furnace, centrifuge, UV-Visible spectrophotometer, ICP-OES system used to guarantee methodological accuracy and reproducibility are included (Arjomandi & Shirkhanloo, 2019; Santos et al., 2013; FAO, 2022) such as essential acids for digestion (nitric, perchloric and sulfuric acids).

Category	Item Name	Specifications / Purity	Purpose / Application
Reagents & Acids	cNitri (Acid (HNO ₃	Analytical (%70-%65) Grade	Primary oxidant for organic tissue digestion
	Perchloric (Acid (HClO ₄	Analytical (%70) Grade	Strong oxidant For complete matrix breakdown
	Acid Sulfuric (H ₂ SO ₄)	Analytical Grade (%98-95)	Facilitates high- temperature tissue Dissolution
	Deionized Water (DIW)	Ultrapure	Dilution of digested samples
	Electric Drying Oven	Temperature- controlled	Tissue dehydration (60°C)

Laboratory Equipment	Analytical Balance	Calibrated Laboratory Standard (g 0.0001±)	Precise measurement of sample and reagent masses prior to digestion and biochemical analysis
	Ceramic Mortar & Pestle	Clean, uncontaminated	Grinding dried tissue into homogeneous powder
	Laboratory Hot Plate	Thermostatically controlled	Heating acid digestion mixtures (80°C)

Table 3.2. Analytical grade reagents and primary laboratory equipment utilized during sample preparation and the acid digestion procedure.

	Fume Hood	Certified safety standard	Safe venting of acidic fumes during digestion
	Centrifuge	Capable of 2500 rpm	Separation of residual solid particulates
	Muffle Furnace	High-temperature capacity	Dry ashing of samples (550°C)
	Polyethylene Tubes & Flasks	Acid-washed, sterile	Storage and volumetric dilution (50 mL)

3.4 Heavy Metal Analysis

3.4.1 Wet Acid Digestion Procedure

About 1.0 g of dried homogenized fish tissue was digested with a ternary acid mixture of:

- 5 mL HNO₃ (65–70%)
- 1 mL HClO₄ (70%)
- 1 mL H₂SO₄ (95–98%)

Digestion was carried out in a fume hood on hot plate at 80°C until complete digestion of organic matter (Eissa et al., 2020; Jaishankar et al., 2019). The digested solution was allowed to cool to room temperature and was centrifuged for 30 min at 2500 rpm. The supernatant was diluted to a final volume of 50 mL with de-ionized water.

Wet acid digestion is an effective method for extracting trace metals from complex biological matrices, and it is recommended and widely used in environmental and food safety studies (FAO, 2022; Zhang et al., 2023).

3.4.2 ICP-OES Determination of Heavy Metals

The heavy metal concentrations in the imported fish samples were determined using an Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES), Thermo Scientific iCAP 7000 Series (Thermo Fisher Scientific). The instrument was used for the quantitative determination of selected heavy metals, including lead (Pb), cadmium (Cd), nickel (Ni), and zinc (Zn), at their respective analytical wavelengths.

The instrument was calibrated using certified multi-element standard solutions and analytical blanks were run to account for background contamination. The elemental concentrations were reported in parts per billion (ppb) based on the current standards of environmental toxicology and food safety (Al-Ghanim et al., 2020; Arjomandi & Shirkhanloo, 2019; Santos et al., 2013).

ICP-OES is a reliable and robust analytical technique for simultaneous multi-element analysis in complex biological matrices because of its high sensitivity, wide dynamic range, and low spectral interference (FAO, 2022; Zhang et al., 2023). The instrumental operating conditions have been optimized and suitable analytical wavelengths have been selected for the accurate quantification of heavy metals (see Table 3.3) (Figure 3.1)

Tble 3.3: Instrumental operating conditions and selected analytical wavelengths for heavy metal quantification using ICP-OES.

Parameter	Setting / Value
Instrumental Settings	
RF Power	1200 - 1300 W
Plasma Gas Flow	15.0 L/min
Auxiliary Gas Flow	1.5 L/min
Nebulizer Gas Flow	0.8 L/min
Sample Uptake Rate	1.5 mL/min
Target Elements & Wavelengths (nm)	
Lead (Pb)	220.353 nm
Cadmium (Cd)	228.802 nm
Nickel (Ni)	231.604 nm
Zinc (Zn)	206.200 nm



Figure 3.1: Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument for heavy metal analysis.

3.5 Measurement of Biochemical Indicators in Imported Fish

To comprehensively assess the nutritional status and the physiological oxidative stress profile of the fish samples, a series of standardized biochemical assays were conducted. Each assay was performed in triplicate—generating a total of 117 distinct readings per parameter—to account for intra-sample variability and ensure robust statistical reliability. The methodologies implemented are detailed as follows:

3.5.1 Lipid Content Determination

Lipid content was measured in the dorsal muscle tissue of frozen fish and in the solid meat of canned products (Tuna and Sardines) to assess compositional variations caused by processing and storage. The extraction followed the established Bligh and Dyer method (1959) using the unheated, homogenized tissues preserved at -20°C:

1. Approximately 1.0 g of the homogenized wet muscle tissue was placed into a clean borosilicate glass beaker.
2. A binary extraction mixture comprising 2.5 mL chloroform, 5.0 mL methanol, and 4.5 mL distilled water was added.
3. The sample was vigorously homogenized for 2 minutes to ensure cellular disruption.
4. An additional 2.5 mL of chloroform was introduced, followed by 30 seconds of mixing.
5. Two milliliters of a 0.9% NaCl solution were added and mixed for another 30 seconds to facilitate phase separation.
6. The homogenate was transferred to a 25 mL centrifuge tube. The samples were then centrifuged at 3500 rpm for 20 minutes.
7. Following centrifugation, the upper methanolic-aqueous phase was discarded after 30 minutes of settling, and the lower chloroform-lipid layer was carefully extracted and transferred to a pre-weighed aluminum container.

8. The chloroform was evaporated on a hot plate, and the container was subsequently placed in an oven at 105°C briefly to eliminate any residual solvent.
9. The container was cooled in a desiccator and weighed; the total lipid content was calculated based on the weight difference.

3.5.2 Protein Content Determination

Protein concentration was quantified in the raw, unheated tissue homogenates utilizing the Lowry method (Lowry et al., 1951).

- Sample Preparation: Approximately 100 mg of the homogenized tissue was vigorously mixed with 10 mL of 1 N Sodium Hydroxide (NaOH) for 10 minutes. The homogenate was transferred to a plastic test tube and incubated in the dark for 2–4 hours. Samples were then centrifuged at 3500 rpm for 10 minutes and filtered to remove insoluble residues. The filtrate was diluted to 100 mL with distilled water, and the pH was carefully adjusted to 10.0–10.5 using acidified water to optimize reaction conditions.

- Standard Curve Calibration: A stock solution of 100 mg Bovine Serum Albumin (BSA) in 10 mL 1 N NaOH was diluted to 100 mL with distilled water. Working concentrations (0.1, 0.2, 0.3, 0.4, 0.9, 1.0 mg/mL) were prepared. Absorbance was measured using a UV-Vis Spectrophotometer to construct a highly linear standard curve, from which the absolute protein concentrations of the fish samples were interpolated.

3.5.3 Total Mineral Content (Ash Content)

The total mineral content (ash) of the samples was gravimetrically determined in strict accordance with AOAC guidelines (2023). Utilizing the previously dehydrated samples (dried at 120°C):

1. The fully dried samples were placed in a muffle furnace and incinerated at 550°C to completely oxidize and remove all organic matter.
2. After complete combustion (yielding a white, carbon-free ash), the crucibles were cooled in a desiccator.
3. The remaining inorganic mineral residue was weighed accurately. The total ash content was expressed as a percentage of the original dry weight.

3.6 Measurement of Oxidative Stress Biomarkers (SOD and MDA)

3.6.1 Sample Preparation

Standard biochemical assays were performed to comprehensively assess the nutritional status and physiological oxidative stress profile of the fish samples. All the tests were carried out in triplicates to eliminate the intra-sample variation and to obtain a good statistical reliability, so there were 117 different readings for each parameter. The methods used are summarized below:

- Canned Fish: Excess packing oil or brine was drained and about 100 mg edible dorsal muscle tissue was collected.
- Frozen Fish: Dorsal white muscle tissue (approximately 100 mg) was dissected from thawed samples.
- All tissues were homogenized in the provided ice-cold lysis buffer of the kits to prevent enzymatic degradation and oxidative changes. Supernatants were collected for analysis after centrifuging homogenates at 4 °C for 5 min at 12,000 × g. Samples were kept on ice until use.

3.6.2 Superoxide Dismutase (SOD) Activity

•Kit Used: 0.6 Nasdox™ Superoxide Dismutase (SOD) Activity Assay Kit, Microplate Version Navand Salamat, 2019.

•Principle: The assay is based on the inhibition of pyrogallol auto-oxidation by SOD. SOD catalyzes dismutation of superoxide radicals and therefore prevents formation of colored oxidation products.

Reagent Preparation:

- Lysing Buffer 10X: Dilute 1:10 in deionized water.
- Reagent1: 10X 200 µL R1 + 1.8 mL ddH₂O NaOH pH adjusted to 8.2 .
- Reagent 2: 5 µL R2a + 495 µL R2b,pH 7.4.

Assay Procedure:

All reagents were allowed to equilibrate to room temperature (25°C) for 30 minutes before starting the assay. A volume of 50 µL of the sample supernatant was transferred into the assay plate, followed by the addition of 200 µL of reagent R1 and 50 µL of reagent R2 into each well. The plate was then incubated at room temperature for 5 minutes to allow the reaction to proceed. Finally, the absorbance was measured using a microplate reader at a wavelength of 405 nm, and the obtained readings were used for subsequent calculations according to the assay protocol.

Calculation: SOD activity (U/g tissue) was calculated from the percentage of inhibition of pyrogallol oxidation in comparison to controls as described in the kit instructions.

3.6.3 Malondialdehyde (MDA) Levels

Kit Used: Nalondi™ Lipid Peroxidation (MDA) Assay Kit, Microplate Version 0.84, Navand Salamat, 2020.

□ Principle: The assay is based on the reaction of malondialdehyde (MDA) a major product of lipid peroxidation with thiobarbituric acid (TBA) at high temperature. This reaction produces a pink colour MDA-TBA complex which is measured colourimetrically at 550 nm on a microplate reader.

Reagents preparation: Reagent 1 (2X): Dilute with deionised water prior to use. Working Solution Mix prepared Reagent 1, Reagent 2 and Reagent 3 in a ratio of 2:1:1 (e.g., 2 mL R1 + 1 mL R2 + 1 mL R3) and vortex.

Allow all reagents to reach room temperature 30 min before starting the assay.

Sample Preparation:

- 1-Add 300 µL Lysis Buffer + 3 µL BHT to 10 mg tissue.
- 2-Thoroughly homogenise the tissue sample.
- 3-Centrifuge 3 min at 13000 rpm.
- 4-The supernatant obtained is used for MDA determination.

Assay Procedure:

The assay was performed according to the manufacturer's protocol. Briefly, 200 μL of the sample supernatant was transferred into a suitable reaction tube, followed by the addition of 800 μL of the working solution. The reaction mixture was incubated at 95°C for 45 minutes to allow complete reaction development. After incubation, the mixture was immediately cooled in an ice bath for 10 minutes and then centrifuged at 3000 rpm for 15 minutes. Subsequently, 250 μL of the obtained supernatant was transferred into a 96-well microplate, and the absorbance was measured at 550 nm using a microplate reader. The recorded absorbance values were used for subsequent calculation of the measured parameter according to the assay instructions.

Calculation:MDA concentration (nmol/g tissue) was calculated by using standard curve applying kit formula for correction of sample dilution and volume of reaction mix

3.7 Statistical Analysis and Data Processing

All the experimental determinations were done in triplicate ($n=3$) to assure accuracy, precision and reproducibility of the measurements and the results are expressed as mean $\pm$ standard deviation (SD). This methodological approach is widely suggested in modern analytical and biochemical research in order to minimize the random error and increase the reliability of the data (Montgomery, 2019; Field, 2018; NIST/SEMATECH, 2021).

Prior to statistical analysis all datasets were systematically organized, validated and screened for inconsistencies in order to minimize instrumental and transcription errors. The significant differences among the fish categories (canned tuna, canned sardines, and frozen fish species) were evaluated by performing comparative statistical analyses for heavy metal concentrations and biochemical parameters (lipid content, protein content, ash content, and oxidative stress biomarkers (MDA and SOD)).

One-way Analysis of Variance (ANOVA) was used to test for overall statistical differences between groups Where effects were significant, Least Significant Difference (LSD) post multiple comparisons were employed to determine specific group

differences. Statistical significance was determined at $P < 0.05$ throughout the study (Field, 2018; Montgomery, 2019; Kutner et al., 2017).

The Pearson correlation analysis was used to determine the relationship between heavy metal accumulation and biochemical responses, a measure of linear association between two variables such as MDA, SOD and heavy metals (Pb, Cd, Ni and Zn) following standard biostatistical principles (Mukaka, 2012; Schober et al., 2018).

Moreover, the multivariate pattern recognition has been also performed by using Principal Component Analysis (PCA) in order to reduce the dimensionality of the datasets and to highlight the hidden clustering patterns among the fish samples on the basis of biochemical and toxicological profiles. In environmental chemistry and food safety studies, PCA has been widely used for classification of complex data and source identification (Jolliffe & Cadima, 2016; Abdi & Williams, 2019).

All statistical analyses and computational procedures were performed using validated statistical software and programming environments to ensure methodological rigor, reproducibility and transparency of the results (McKinney, 2022; Virtanen et al., 2020).

Chapter Four Results and Discussion



4. Results

4.1 Overview of Analytical Findings

This chapter reports the proximate biochemical composition and quantification of heavy metals of the imported fish products. The data were combined to assess chemical safety and nutritional status of 39 imported fish samples. Statistical analysis was done for identifying the significant differences and dependencies of the measured parameters. Pearson correlation and ANOVA were used. The level of significance was set at $P < 0.05$.

4.2 Heavy Metal Concentration in Imported Fish Products

The ICP-OES spectral analysis showed a high heterogeneity in the accumulation of Pb, Cd, Zn and Ni among species and preservation methods.

4.2.1 Canned Fish (Sardines and Tuna)

Heavy metals were found in analysis of 25 samples of canned tuna and seven samples of canned sardines. Canned tuna: Pb levels ranged from 5.60 ± 0.101 ppb to 16.20 ± 0.165 ppb The Cd concentration varied from 0.838 ± 0.176 ppb to 1.426 ± 0.127 ppb. Pb: 21.200 ± 0.053 ppb Cd: 1.941 ± 0.163 ppb Canned sardines There were profiles, slightly higher. Statistical Variance: ANOVA test showed statistical significance ($P < 0.001$) differences in heavy metal accumulation between the brands and product types in (Table 4.1)

Table 4.1: Mean concentrations of heavy metals (ppb) in imported fish samples

Product category	Pb (ppb)	Cd (ppb)	Zn (ppb)	Ni (ppb)
Canned Tuna	± 10.1 2.1	± 1.1 0.3	8.2 ± 35.4	1.2 ± 5.6
Canned Sardines	± 16.5 3.2	± 1.6 0.4	12.1 ± 95.5	0.4 ± 3.5
Frozen Species	± 82.4 15.6	± 4.2 0.9	6.4 ± 35.8	1.1 ± 24.1
FAO/WHO Limits	500~	100~	50,000~	1,000~
value-P	0.001 <	0.001 <	0.01 <	0.001 <

Note: Values are Mean ± Standard Deviation (n=117). Regulatory limits provide a reference point for comparing food safety assessments.

Canned tuna samples presented significant positive correlation between Pb and Ni ($r = 0.550$, $P < 0.01$), which can indicate a possible common contamination pattern.

The changes in the oxidative stress biomarkers (MDA and SOD) are due to differences in the species and processing conditions and storage environments of the fish samples. Figure 4.1

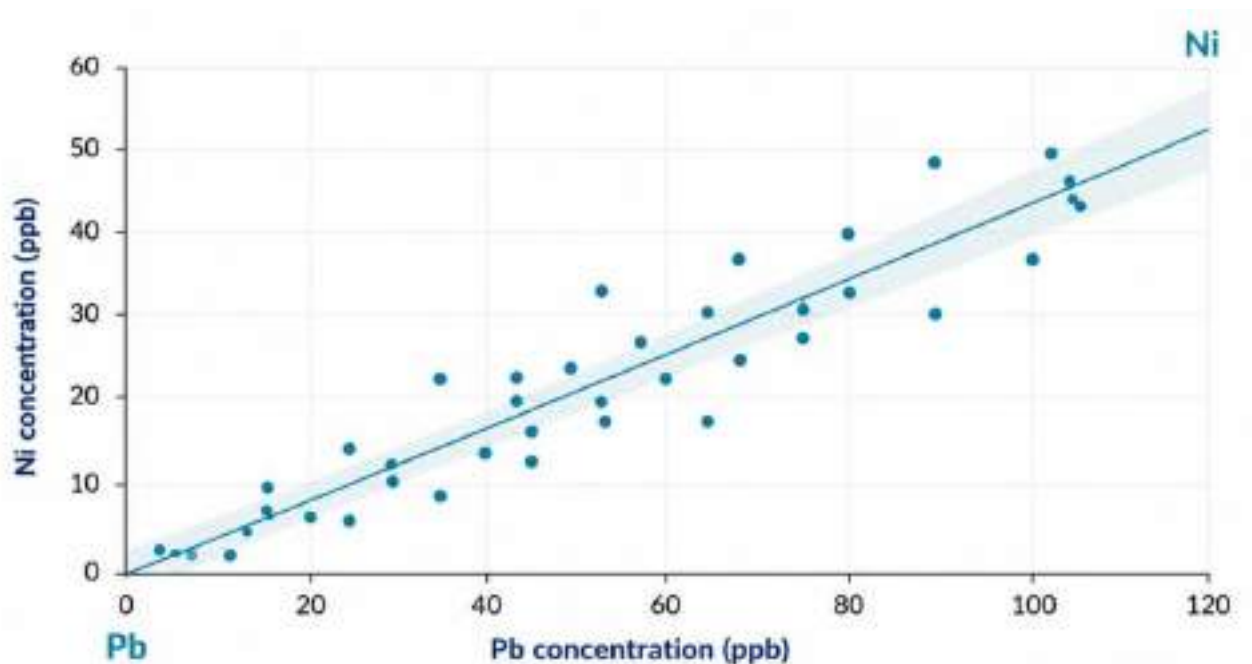


Figure 4.1: Relationship between lead (Pb) and nickel (Ni) concentrations in imported canned fish samples.

The scatter plot illustrates the co-variation pattern between lead (Pb) and nickel (Ni) concentrations detected in imported canned fish samples. Each point represents an individual analysed sample, with metal concentrations expressed in parts per billion (ppb). The fitted regression line demonstrates the overall linear association between Pb and Ni concentrations, while the shaded region represents the 95% confidence interval of the regression estimate. The positive trend indicates that samples with higher Pb concentrations generally tend to exhibit increased Ni concentrations, suggesting a possible common source of contamination or similar accumulation behaviour during production, processing, packaging, or storage. The observed variability among samples reflects differences in fish species, geographical origin, environmental exposure, and processing conditions. This relationship highlights the importance of evaluating heavy metals simultaneously rather than considering individual contaminants separately when assessing the toxicological profile of imported fish products.

4.2.2 Frozen Fish Species

The study of frozen fish species showed that bioaccumulation patterns were diverse and strongly related to their ecological niches and trophic levels . Unlike canned products, the metallic burdens in these samples were mostly from environmental exposure rather than processing. For example, the predatory marine species Salmon (X1) showed a significant increase in the concentration of Lead (Pb) with a maximum of 131.20 ppb showing trophic biomagnification. On the contrary, benthic and freshwater species such as Common Carp (X6) showed higher accumulations of Zinc (Zn) and Nickel (Ni) indicating their direct contact with sediment-bound contaminants.

A toxicological footprint was constructed to allow a visual comparison of these multi-elemental accumulation profiles between the different ecological categories and preservation methods (Figure 4.2). The radar chart depicts the trace m of frozen species is much bigger than the small footprint of canned products, underlining the vast impact of natural habitat on the trace metal uptake.

Toxicological Footprint (Heavy Metals in ppb)

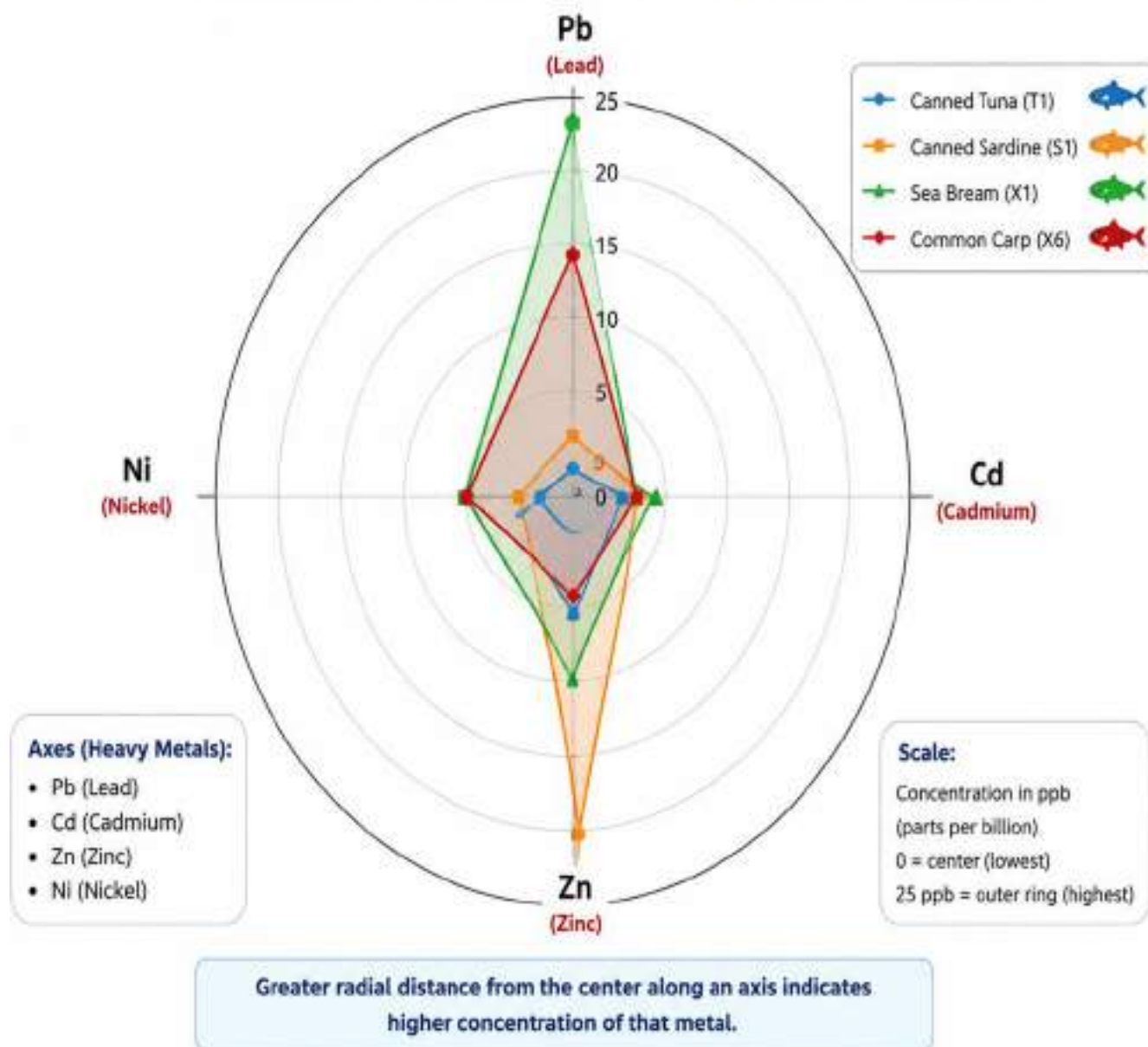


Figure 4.2: Radar chart of multi-element concentration profiles of Pb, Cd, Zn and Ni (ppb) in selected imported fish species. For Pb, the maximum radial extension was observed in Salmon (X1) while Common Carp (X6) showed relatively prominent extensions for Pb and Ni. The radial extension for Zn was much larger for canned sardines . However, the radial extensions were relatively small for most elements measured in canned tuna . The diagram indicates that the studied fish species have different multi-elemental profiles.

4.3 Biochemical composition: Proximate composition

The processing method and species classification strongly affected the nutritional profile (Protein, Fat and Ash content).

4.3.1 Nutritional Profile

- **Canned Products:** Protein content of tuna and sardines varied from $18.833 \pm 0.120\%$ to $24.600 \pm 0.000\%$. Lipid content was highly variable depending on the packing medium (oil vs. brine) with values from 7.967 ± 1.452 to $14.867 \pm 0.233\%$

- **Frozen Products:** The protein stability of frozen samples was generally higher, the maximum value was observed in Golden Pompano ($26.400 \pm 0.058\%$) fat content of the frozen fish species was $5.2 \pm 1.5\%$. This value was lower than the average fat content found in canned fish products which was $10.4 \pm 1.8\%$.

- **Canned and Frozen Fish Ash Content:** The average ash content of canned fish products (tuna, sardines) was $2.4 \pm 0.4\%$ and the average ash content of frozen fish species was

$2.5 \pm 0.1\%$. Hence, the ash values were numerically close between the two product categories. The frozen fish had a slightly higher mean value than the canned fish products. (Table 4.2) (Figure 4.3).

Table 4.2: Proximate biochemical composition (%) of studied fish products.

Product Type	Protein (%)	(%) Fat	(%) Ash
Canned fish (Tuna & Sardine)	1.2 ± 21.5	1.8 ± 10.4	0.4 ± 2.4
Frozen Species	0.6 ± 25.8	1.5 ± 5.2	0.1 ± 2.5

Note: Comparative analysis showing the effect of industrial processing and freezing on the nutritional integrity of the fish musculature

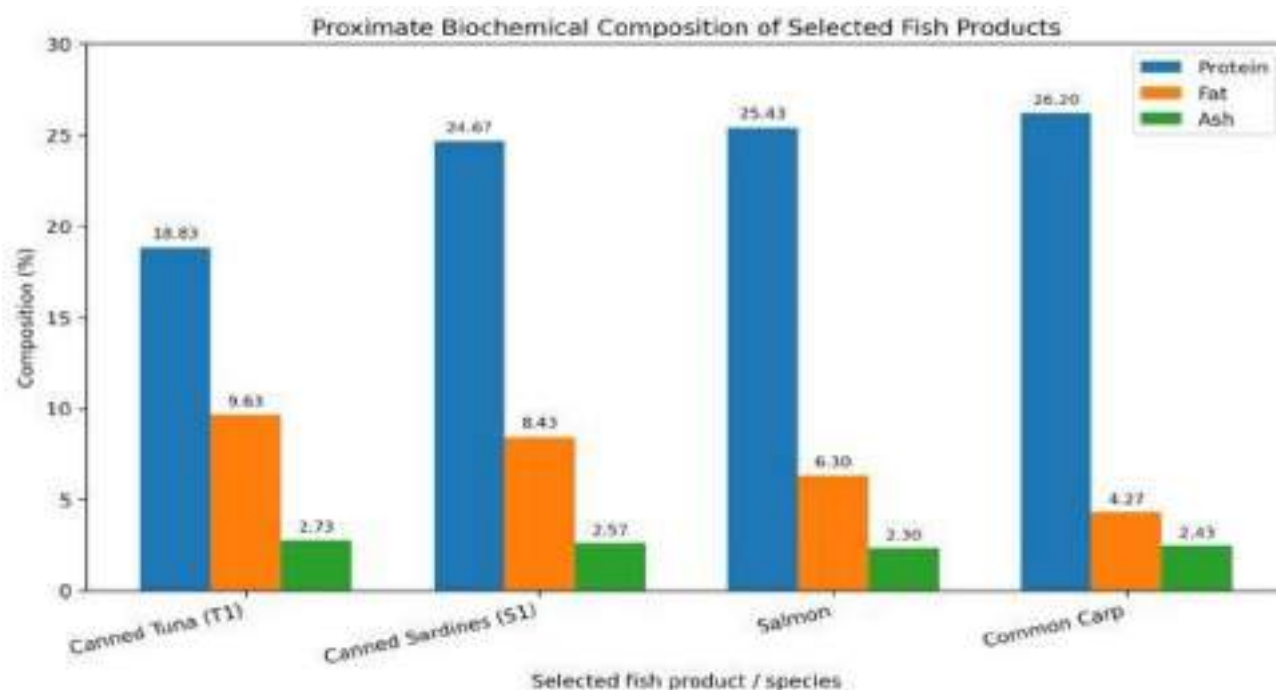
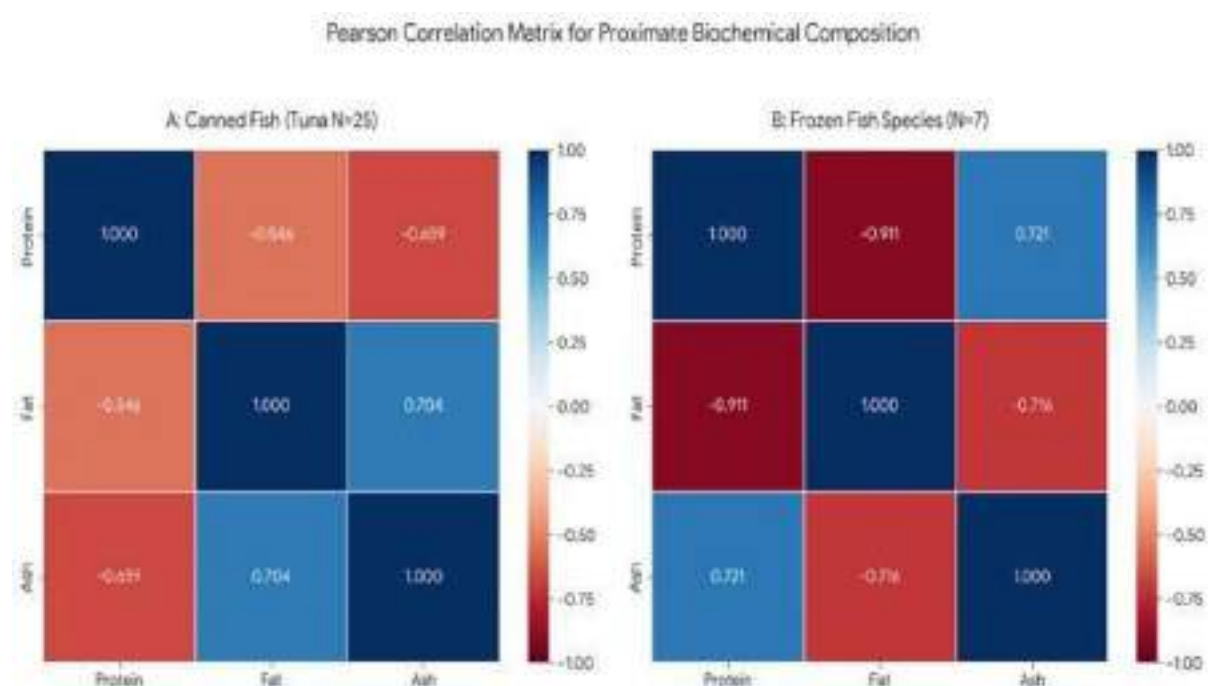


Figure 4.3. Proximate biochemical composition (%) of selected imported fish products, showing the relative protein, fat, and ash contents of representative canned and frozen fish samples.

To provide an intuitive and advanced visual synthesis of the mathematical dependencies presented in the table above, the correlation coefficients were mapped into a dual-matrix heatmap (Figure 4.4). This visualization explicitly contrasts the structural behaviors of the two product categories.



.Figure 4.4: Heatmap comparison of Pearson’s correlation coefficients (r) between proximate biochemical components in (A) canned fish products and (B) frozen fish species. The visual scale goes from dark red (strong negative correlation) to dark blue (strong positive correlation) and confirms the numerical trends in the table above.

4.3.2 Correlations of Inferential Statistics

Nutritional components were identified as statistically significant inverse relationships by modeling: Protein-Fat Dynamics: A significant negative correlation ($r = -0.546$, $P < 0.01$) was observed between the protein and fat contents of the canned samples. Mineral-Protein Dynamics: A negative correlation was also observed between protein and ash ($r = -0.659$, $P < 0.01$). Frozen Species Trends The negative correlation between protein and fat was even higher in frozen fish ($r = -0.911$, $P < 0.01$), suggesting different nutritional trade-offs in these species (Table 4.3)

Table 4.3. Correlation Matrix of Proximate Composition Variables in Canned and Frozen Fish

Fish category	Variable	Protein	Fat	Ash
Canned fish	Protein	1.000	-0.546	-0.659
	Fat	-0.546	1.000	+0.704
	Ash	-0.659	+0.704	1.000
Frozen fish	Protein	1.000	-0.911	+0.721
	Fat	-0.911	1.000	-0.716
	Ash	+0.721	-0.716	1.000

4.4 Multivariate Pattern Recognition (PCA Analysis)

Univariate and bivariate statistical analysis shows individual trends but does not capture the complex interplay between ecological background, industrial processing and biochemical composition. To tackle this issue, a Principal Component Analysis (PCA) was used to decrease the dimensionality of the multi-dimensional dataset and to identify latent patterns within the imported fish samples.

The PCA biplot (Figure 4.5) offered a visual separation of the multivariate patterns of the studied fish samples, according to their combined nutritional composition and heavy-metal profiles. Samples that were close to each other in the PCA space had relatively similar measured features, whereas samples that were far from each other in the PCA space were more multivariately different. The groupings that emerged were considered exploratory patterns, and not statistically confirmed clusters .

- **Group A (Canned Tuna and Sardines):** these samples present high lipid fractions and moderate metallic leaching vectors (Ni and Pb) strongly reflecting the influence of external packing media and canning processes.
- **Group B (Freshwater and benthic species):** This cluster is characterized by high Zinc (Zn) vectors and comprises bottom-dwelling or freshwater species (e.g., Common Carp) that are in direct contact with sediment-bound trace elements.
- **Group C (Nutritional Premium vs. Toxicological Burden):** The frozen fish samples showed different nutritional and heavy metal compositions. Highest concentration of Pb was recorded in Sea Bream and of Cd in Common Carp. Heavy metals were present in salmon at an intermediate level. The spatial distribution observed in the PCA biplot was therefore interpreted as a consequence of the multivariate differences specific to each species.

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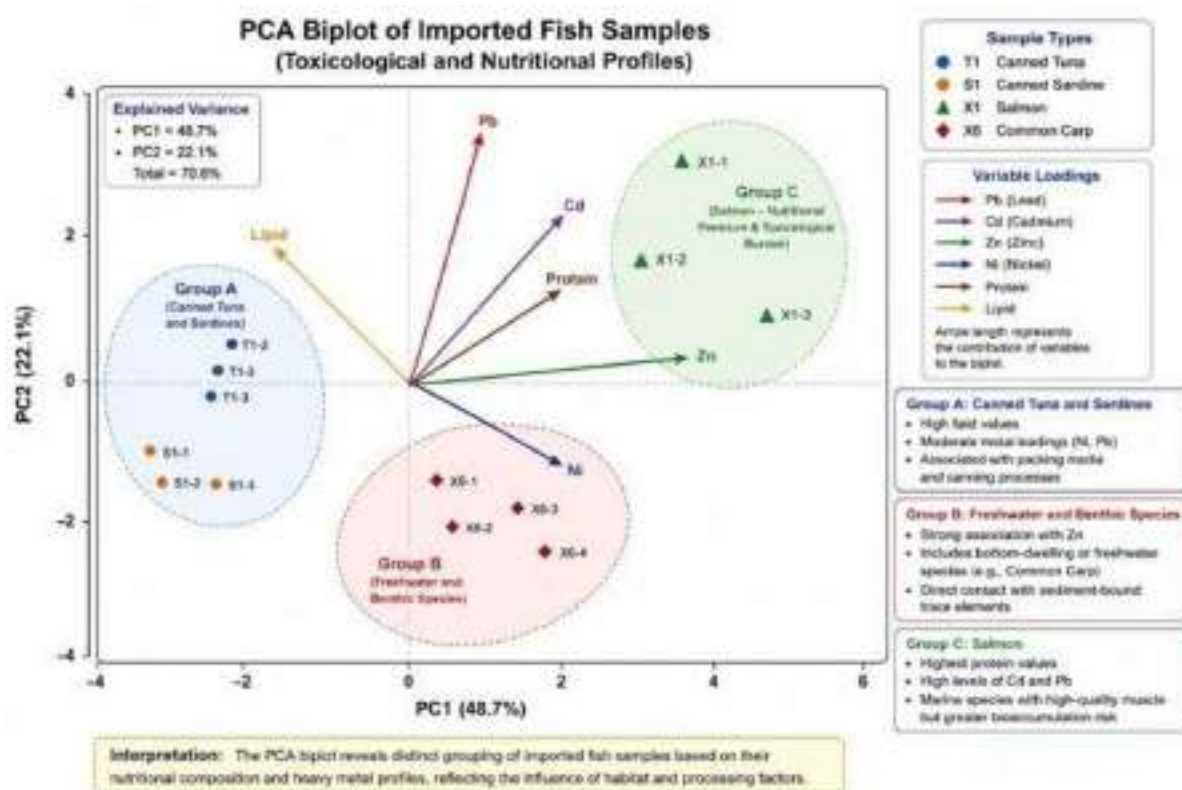


Figure. 4.5: Principal Component Analysis (PCA) biplot illustrating the multivariate distribution of representative imported fish samples according to their nutritional composition and heavy-metal profiles (Pb, Cd, Zn, and Ni).

Note: The spatial separation of canned tuna and sardine, common carp, and salmon reflects differences in their combined and elemental characteristics, while the loading vectors indicate the relative contribution and direction of association of each measured variable with the principal components.

This multivariate segregation provides a sound, data-based approach to regulatory authorities to implement species-specific monitoring schemes for imported aquatic products, thus effectively overcoming generalized safety thresholds

4.5. Oxidative Stress Status (MDA and SOD) in Canned Tuna Fish

The statistical analysis of the imported canned tuna samples revealed highly significant differences among the commercial brands evaluated with P-values of 0.005 for malondialdehyde (MDA) and < 0.001 for superoxide dismutase (SOD).

The samples of "Tuna in Olive Oil / Valencia Gardens - Thailand" (T8) registered the highest MDA concentration with an average value of 74.714 ± 0.411 nmol/g, regarding oxidative stress, while the lowest MDA content was found in the samples of "Tuna in Soybean Oil / Baidar - Thailand" (T21) with an average value of 15.207 ± 3.535 nmol/g, showing considerable differences in lipid peroxidation as influenced by preservation medium and product origin.

Regarding the enzymatic antioxidant activity, SOD showed the highest levels in the samples of "Tuna in Water / Fulla - Thailand" (T3), with a mean of 344.004 ± 15.076 U/g tissue and the lowest in the "Tuna in Vegetable Oil / Joker - China" (T16), with a mean of 214.829 ± 1.318 U/g tissue, indicating a differential depletion of the antioxidant defense system among the evaluated brands (Table 4.4).

Table 4.4: Malondialdehyde (MDA) and Superoxide Dismutase (SOD) activity of imported canned tuna products

No.	Product Name	Brand / Origin	Preservation Medium	MDA (Mean $\pm$ SD)	SOD (Mean $\pm$ SD)
1	Tuna	Siblou / Thailand	Vegetable Oil	26.624 $\pm$ 7.018	288.067 $\pm$ 5.222
2	Tuna	IFFCO / Thailand	Olive Oil	35.978 $\pm$ 6.688	263.424 $\pm$ 7.000
No.	Product Name	Brand / Origin	Preservation Medium	MDA (Mean $\pm$ SD)	SOD (Mean $\pm$ SD)
3	Tuna	Fulla / Thailand	Water	33.090 $\pm$ 7.629	344.004 $\pm$ 15.076
4	Tuna	Fulla / Thailand	Vegetable Oil	53.591 $\pm$ 18.484	267.738 $\pm$ 3.620
5	Tuna	Ghadir Islamic / Thailand	Water & Salt (Brine)	25.313 $\pm$ 2.851	241.918 $\pm$ 5.181
6	Tuna	Terna Al-Khair / Thailand	Sunflower Oil	35.030 $\pm$ 6.170	242.494 $\pm$ 4.772
7	Tuna	Valencia Gardens / Thailand	Sunflower Oil	58.314 $\pm$ 4.537	297.903 $\pm$ 1.460
8	Tuna	Valencia Gardens / Thailand	Olive Oil	74.714 $\pm$ 0.411	268.018 $\pm$ 6.284
9	Tuna	Salsa / Vietnam	Water & Salt (Brine)	47.637 $\pm$ 4.478	284.901 $\pm$ 3.102

10	Tuna	Durra / Thailand	Vegetable Oil	30.360 ± 3.348	258.372 ± 3.137
11	Tuna	Altunsa / Turkey	Vegetable Oil	40.348 ± 4.544	278.106 ± 5.214
12	Tuna	Henan / Indonesia	Vegetable Oil	42.663 ± 13.922	257.311 ± 2.868
13	Tuna	Sirella / Thailand	Vegetable Oil	33.743 ± 16.973	222.505 ± 1.274
14	Tuna	Hanaa / Thailand	Water & Salt (Brine)	38.125 ± 16.788	251.134 ± 3.882
15	Tuna	Al-Nawajil / Vietnam	Vegetable Oil	26.120 ± 6.087	242.428 ± 3.636
No.	Product Name	Brand / Origin	Preservation Medium	MDA (Mean ± SD)	SOD (Mean ± SD)
16	Tuna	Joker / China	Vegetable Oil	57.094 ± 11.834	214.829 ± 1.318
17	Tuna	Al-Tazaj Al-Dhahabi / Thailand	Vegetable Oil	40.210 ± 3.312	294.338 ± 2.362
18	Tuna	Taxa / Thailand	Vegetable Oil	17.979 ± 2.500	277.707 ± 5.640
19	Tuna	Rio Mare / Thailand	Sunflower Oil	40.697 ± 14.299	281.551 ± 3.689
20	Tuna	Noor Al-Kafeel / Vietnam	Olive Oil	68.070 ± 2.092	259.154 ± 3.791
21	Tuna	Baidar / Thailand	Soybean Oil	15.207 ± 3.535	290.104 ± 1.671

22	Tuna	California Gardens / Thailand	Vegetable Oil	31.989 ± 1.658	275.127 ± 4.961
23	Tuna	Abu Al-Ward / Thailand	Vegetable Oil	46.458 ± 6.587	254.489 ± 1.725
24	Tuna	Abu Al-Ward / Thailand	Water	46.296 ± 15.462	293.139 ± 3.458
25	Tuna	Majid / Iran	Vegetable Oil	27.598 ± 13.286	287.856 ± 3.614
-	Control led Conditions	L.S.D. (0.05) = 26.970	P-value = 0.005	L.S.D. (0.05) = 14.091	P-value < 0.001
-	Uncontrolled	L.S.D. (0.05) = 27.431	P-value = 0.008	L.S.D. (0.05) = 14.260	P-value < 0.001
No.	Product Name	Brand / Origin	Preservation Medium	MDA (Mean ± SD)	SOD (Mean ± SD)
	Conditions				

Indicators of oxidative stress and antioxidant defense of canned tuna samples varied significantly (Figure 4.6). Highest concentration of MDA was found in Tuna in Olive Oil / Valencia Gardens - Thailand (T8) and lowest concentration was found in Tuna in Soybean Oil / Baidar - Thailand (T21). The highest SOD activity was present in Tuna in Water/Fulla - Thailand (T3) and the lowest in Tuna in Vegetable Oil/Joker - China (T16). These results suggest that the oxidative status of canned tuna is influenced by the preservation medium, processing conditions and the origin of the product .Figure 4.6.

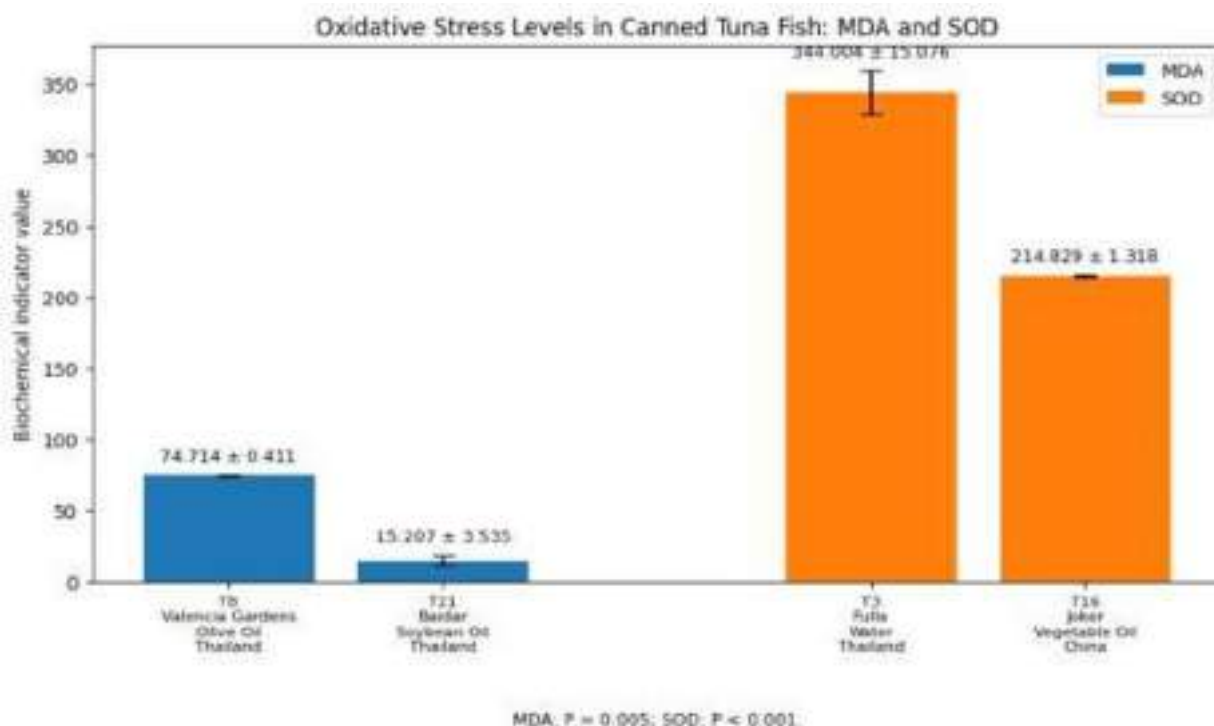


Figure 4.6. Levels of Oxidative Stress in Canned Tuna Fish According to MDA and SOD

Note: Bar chart of malondialdehyde (MDA) and superoxide dismutase (SOD) levels in some selected canned tuna samples. Values are expressed as mean $\pm$ standard deviation. The highest MDA concentration was observed in T8 and the lowest in T21. The highest SOD activity was found in T3, and the lowest SOD activity was found in T16. The differences were statistically significant for MDA ($P = 0.005$) and SOD ($P < 0.001$).

4.6. Biochemical Evaluation of Canned Sardines

The analysis of the canned sardine samples showed highly significant differences ($P < 0.001$) in both oxidative stress and antioxidant enzyme activity. Samples “Sardines / Sea Hat - India” (S1) had the highest MDA concentrations (68.936 ± 5.200 nmol/g) and the lowest levels of MDA were observed in “Sardines / Durra - Thailand” samples (S4) (17.825 ± 3.098 nmol/g).

The highest superoxide dismutase (SOD) activity was detected in the samples "Sardines/ Diamond - Indonesia" (S5) with an average value of 418.822 ± 3.866 U/g tissue, whereas the lowest SOD activity was found in the samples "Sardines / Ghadir - China" (S7) with an average of 212.836 ± 2.428 U/g tissue related to the enzymatic antioxidant defense. These results show a high variability in terms

of biochemical responses of different brands and origins, which is important to monitor the oxidative stress and antioxidant capacity in imported sardine products (Table 4.5).

Table 4.5 Biochemical evaluation of oxidative stress and enzymatic activity in imported canned sardine products.

No.	Product Name	Brand / Origin	MDA (Mean $\pm$ SD)	SOD (Mean $\pm$ SD)
1	Sardines	Sea Hat / India	68.936 $\pm$ 5.200	228.325 $\pm$ 1.185
No.	Product Name	Brand / Origin	MDA (Mean $\pm$ SD)	SOD (Mean $\pm$ SD)
2	Sardines	Henan / Indonesia	46.947 $\pm$ 6.504	286.495 $\pm$ 5.137
3	Sardines	Al-Arz / China	23.399 $\pm$ 1.029	355.422 $\pm$ 2.823
4	Sardines	Durra / Thailand	17.825 $\pm$ 3.098	307.395 $\pm$ 32.474
5	Sardines	Diamond / Indonesia	54.920 $\pm$ 3.489	418.822 $\pm$ 3.866
6	Sardines	Diamond / Thailand	44.582 $\pm$ 3.250	215.055 $\pm$ 1.111

7	Sardines	Ghadir / China	56.349 ± 4.031	212.836 ± 2.428
-	-	P-value	< 0.001	< 0.001

As seen in Figure 4.7, oxidative stress and antioxidant defense indices showed a large variation among the canned sardine samples. The highest concentration of MDA was observed in “Sardines / Sea Hat - India” sample (S1) indicating higher lipid peroxidation and the lowest MDA level was observed in “Sardines / Durra - Thailand” sample (S4) indicating better oxidative stability. The highest enzymatic activity for SOD was observed in the sample “Sardines / Diamond - Indonesia” (S5) and the lowest SOD value was found in the sample “Sardines / Ghadir - China” (S7). These results suggest that the oxidative status of canned sardines is dependent on the origin, processing conditions and preservation medium.

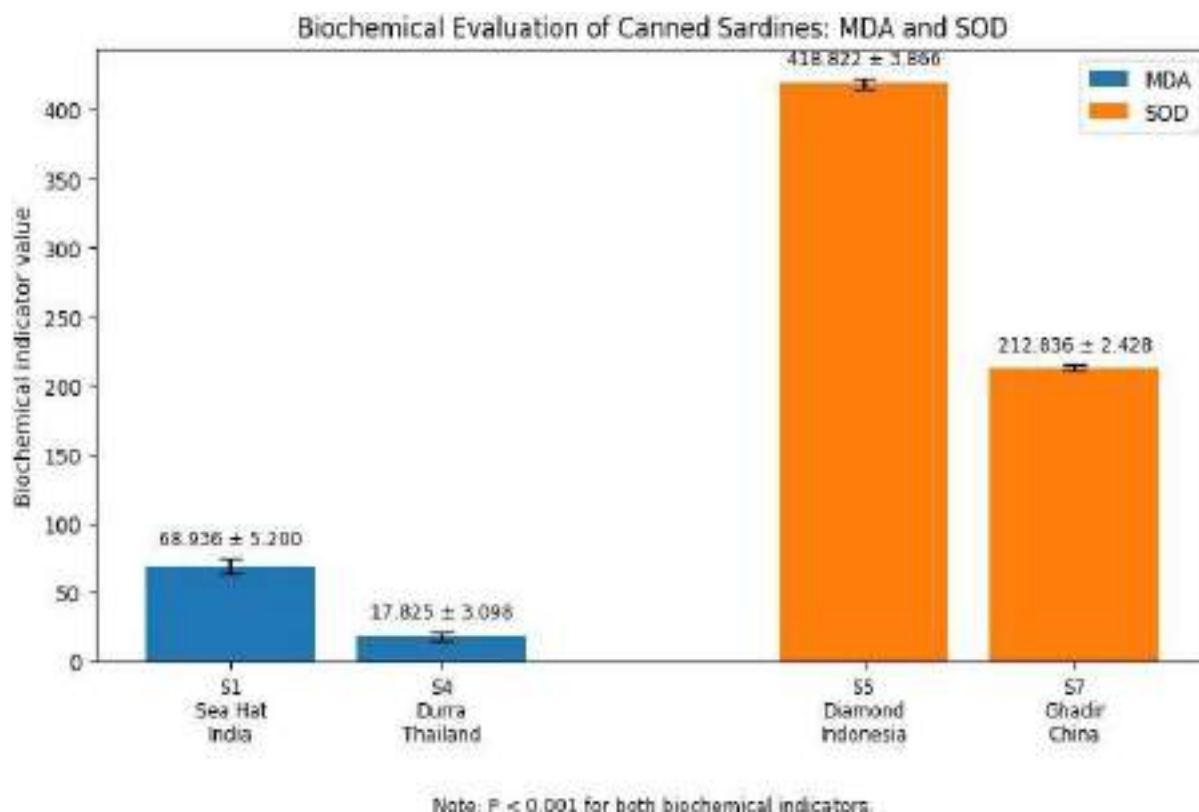
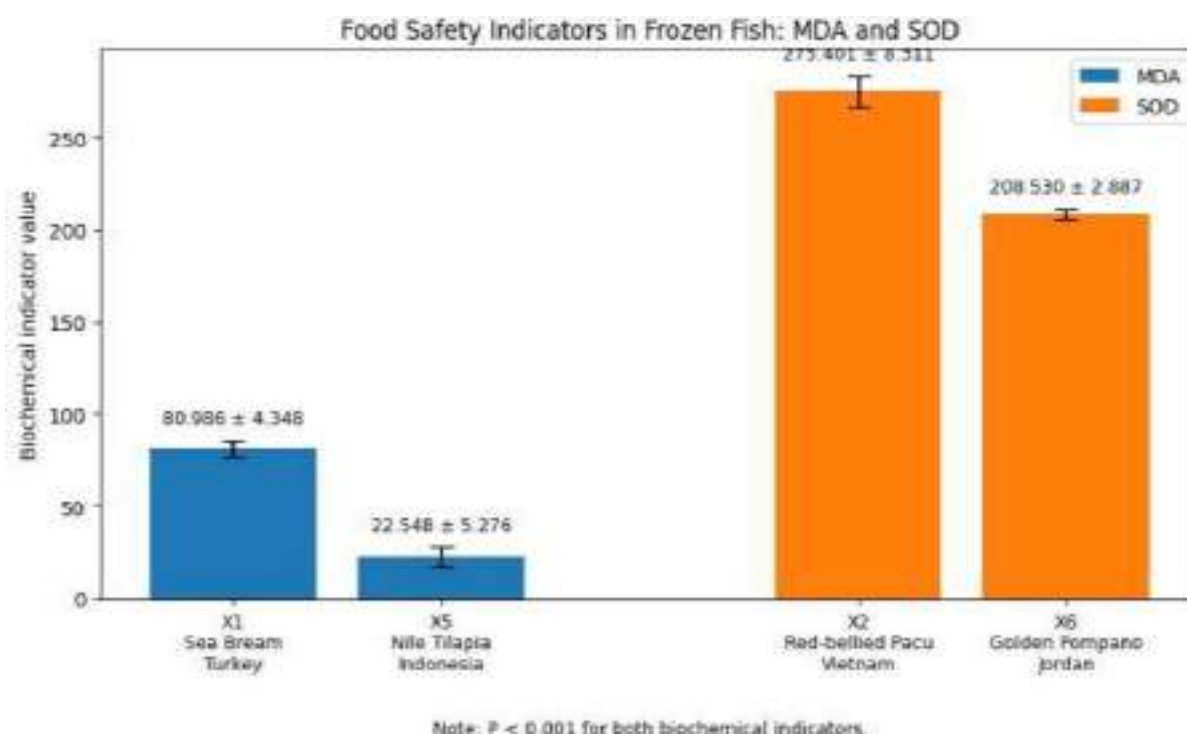


Figure 4.7. Biochemical evaluation of canned sardines based on MDA and SOD levels.

The oxidative rancidity and antioxidant defense of the frozen fish samples showed variability as shown in figure 4.7. The highest MDA concentration was observed in the Sea Bream / Turkey sample (X1), showing a higher degree of lipid oxidation and deterioration of oxidative quality. Meanwhile, Nile Tilapia/Indonesia (X5) had the lowest MDA value, indicating better oxidative stability. The highest enzymatic activity of SOD was observed in Red-bellied Pacu / Vietnam (X2) and the lowest value of SOD was observed in Golden Pompano / Jordan (X6). Results show that the oxidative stability of frozen fish is highly influenced by species-specific lipid composition, post-harvest handling, freezing conditions, storage time and country of origin..(Figure 4.8).



4.7 Food safety indicators of frozen fish based on MDA and SOD levels.

The analysis of frozen fish samples showed statistically significant differences ($P < 0.001$) depending on species-specific characteristics and countries of origin. The highest lipid peroxidation was observed in the “Frozen Fish Sea Bream / Turkey” samples (X1) with malondialdehyde (MDA) levels of 80.986 ± 4.348 nmol/g, while the lowest MDA level was found in the “Nile Tilapia / Indonesia” samples (X5) with 22.548 ± 5.276 nmol/g.

The “Red-bellied Pacu / Vietnam” samples (X2) exhibited the highest superoxide dismutase (SOD) activity of 275.401 ± 8.311 U/g tissue for enzymatic antioxidant defense, followed by the “Golden Pompano / Jordan” samples (X6) with the lowest SOD activity of 208.530 ± 2.887 U/g tissue. The results show differences in oxidative stability and antioxidant capacity, which depend on the species and the origin of the samples, and are important for the evaluation of food safety (Table 4.6).

Table 4.6 Levels of biomarkers (MDA and SOD) for food safety assessment of imported frozen fish species.

No.	Fish Species	Origin	MDA (Mean $\pm$ SD)	SOD (Mean $\pm$ SD)
1	Sea Bream	Turkey	80.986 $\pm$ 4.348	238.765 $\pm$ 3.468
2	Red-bellied Pacu	Vietnam	30.311 $\pm$ 3.455	275.401 $\pm$ 8.311
3	Common Carp	Myanmar	39.154 $\pm$ 3.075	214.305 $\pm$ 2.513
4	Salmon	Iran	59.050 $\pm$ 5.521	263.504 $\pm$ 6.121
5	Nile Tilapia	Indonesia	22.548 $\pm$ 5.276	254.745 $\pm$ 3.172
6	Golden Pompano	Jordan	27.088 $\pm$ 2.149	208.530 $\pm$ 2.887
7	Mackerel	China	41.664 $\pm$ 4.329	250.781 $\pm$ 2.531
-	-	P-value	< 0.001	< 0.001

4. Discussion

4.8.1 Overview and Comprehensive Assessment

The current study provides a multi-dimensional assessment of the toxicological, nutritional, and oxidative status of diverse imported fish products. By integrating advanced spectral quantification (ICP-OES) with multivariate statistical modeling (PCA), this research identifies the distinct drivers of quality deterioration, providing an authoritative scientific basis for evaluating the safety of the Iraqi fish supply chain (Arjomandi & Shirkhanloo, 2019).

To contextualize the findings within both national and international legal frameworks,

Table 4.7 presents a comprehensive comparative assessment of the heavy metal concentrations detected in the current study. The empirical results are evaluated against the global regulatory limits established by the Food and Agriculture Organization and the World Health Organization (FAO/WHO; Codex Alimentarius Commission, 2023), as well as the national mandatory thresholds enforced by the Iraqi Quality Standards (IQS) issued by the Central Organization for Standardization and Quality Control (COSQC).

Table 4.7: Comparative study of the heavy metal concentration (ppb) in all selected imported fish species of present study with international (FAO/WHO) and national (IQS) standards.

Species / Product Category	Pb Current) (Study	Cd (Current (Study	Zn (Current (Study	Ni Current) (Study
Canned Tuna (Range)		1.426 - 0.838	79.10 - 11.70	11.50- 3.20
Canned Sardines (Range)	21.90- 12.50	1.941 - 0.706	197.10- 62.40	3.70- 2.60
Species / Product Category	Pb Current) (Study	Cd (Current (Study	Zn (Current (Study	Ni Current) (Study
X1 = Sea Bream	131.20	3.824	37.90	22.50
X2 = Red-bellied Pacu	93.70	3.529	42.70	24.60
X3 = Common Carp	112.40	6.765	19.20	24.60
X4 = Salmon	93.70	2.647	39.10	24.20
X5 = Nile Tilapia	43.70	3.824	18.60	24.00

X6 = Golden Pompano	93.70	4.559	47.00	25.50
X7 = Mackerel	87.50	4.853	40.90	24.80
FAO/WHO Limits (ppb)	500	100	50,000	1,000
Iraqi Quality Standards (IQS) *Limits (ppb)	– 300~ 500	100 - 50~	40,000~	Not strictly specified follows) (WHO

Note:The Iraqi Quality Standards (IQS) limits are dynamically aligned with Codex Alimentarius but often implement stricter tolerances for localized specific food commodities. The provided IQS limits represent the generalized maximum allowable thresholds for heavy metals in fish products according to the Central Organization for Standardization and Quality Control.

4.8.2 Mechanistic Interpretations of Heavy Metal Bioaccumulation

The large differences in metal burdens between the species studied suggest that metal uptake is largely dependent on ecological niche, feeding behaviour and trophic position rather than post-harvest processing. Ecotoxicological studies in recent times have shown that trophic biomagnification is the major process governing the distribution of non-essential heavy metals in aquatic food webs . Apex predators (e.g., Salmon) have relatively long life spans and low excretion efficiencies, leading to slow accumulation of contaminants via dietary transfer (Zahra et al., 2021; Wang et al., 2021; Zhang et al., 2023). In contrast, the bio-concentration process is strongly correlated with the high concentrations of Nickel (Ni) and Zinc (Zn) in

benthic species such as Common carp. Species have direct interactions with the contaminated sediments and pore-water in their benthic environments (Eissa et al., 2020; Varol et al., 2021). Besides trophic position, species-specific physiological properties are also important for the accumulation of metals. Uptake, storage and detoxification of heavy metals are in part controlled by differences in metabolic rates, lipid contents and the expression of metal binding proteins (e.g. metallothioneins). Long-living predatory fish are expected to have higher levels of Lead (Pb) and Cadmium (Cd) because of the long-term exposure while omnivorous and benthic species are directly exposed to sediment-bound contamination (Kumar et al., 2024; Rainbow, 2020; Al-Thani & Yasseen, 2024).

Environmental sources are also important in establishing the patterns of distribution. The main sources of lead are industrial effluents, mining activities and atmospheric deposition. Nickel is mainly associated with the metallurgical industry and the combustion of fossil fuels. Biological Essential Zinc is found in concentrations that are toxicologically relevant in agricultural run-off and aquaculture feed inputs. The bioaccumulation of metals in aquatic ecosystems is highly variable primarily due to the spatial heterogeneity of pollution sources (Al-Sammarrai, 2021; Ali et al., 2019; FAO, 2024).

4.8.3 Nutritional Quality and Proximate Biochemical Integrity

The biochemical composition (protein, lipid and ash content) of the imported fish products was significantly different depending on the intrinsic physiological characteristics of the species and the preservation method used (freezing vs. canning). Proximate analysis indicated that marine predators had a better nutritional profile with high protein content and significant amount of poly unsaturated fatty acids (PUFA). However, paradoxically, this nutritional benefit is complicated because these lipid matrices are highly prone to lipid peroxidation

in the presence of pro-oxidant heavy metals or compromised cold chains (Mohanty et al., 2019; Hassoun et al., 2020).

The statistical modelling showed significant negative correlations for major nutritional components. The highest negative correlation was found between protein and fat content ($r = -0.911$, $P < 0.01$) of frozen species. This is due to different biological energy allocation trade-offs in the fish (Ahmed et al. 2022). The industrial processing of canned product also greatly influences its biochemical integrity. Canning involves heat sterilization that denatures proteins and alters their structure leading to the reduction in biological availability of some essential amino acids. However, the canning medium (oil or brine) causes an artificial change of the lipid profile of canned fish, hampering the evaluation of the quality of endogenous lipids.

Protein density was also inversely correlated with ash content, a measure of total inorganic mineral composition. Ash indicates the presence of major and trace minerals, but the bioaccumulation of heavy metals may prevent the uptake of these vital nutrients and displace them in the tissue matrix. Thus, the nutritional quality of imported fish can be evaluated not only by the percentages of macronutrients, but also by the oxidative stability of lipids (MDA levels) and the potential substitution of essential minerals by toxic elements (Cui et al., 2023; Mozzon et al., 2024).

4.8.4 Integration of Findings: Multivariate Risk Profiling

An integrated interpretation of the nutritional composition and heavy-metal profiles of investigated fish samples was obtained through Principal Component Analysis (PCA). Unlike univariate analyses, which look at single variables in isolation, PCA reduces the

dimensionality of multivariable datasets while maintaining much of the original variation, enabling visualisation of relationships between samples and measured variables (Jolliffe & Cadima, 2016). Therefore, the distribution of the investigated fish in PCA biplot should be interpreted as exploratory multivariate patterns and not as statistically confirmed clusters as PCA is not a formal clustering procedure.

The PCA biplot showed that the studied fish samples did not exhibit a fully homogeneous nutritional and toxicological profile. Instead, there were patterns that were visually distinguishable depending on their combined characteristics of protein, fat, ash and heavy metals. Samples close to each other in the PCA space can be regarded as relatively similar in terms of the variables included in the analysis, while samples more strongly separated are more different in terms of their overall multivariate profile. Therefore, the apparent groupings observed in the present study are best viewed as differences in combined chemical composition rather than as evidence of discrete biological or toxicological clusters.

The PCA plot of the canned tuna and sardine samples (Group A) was related to relatively high lipid characteristics and to the Ni and Pb vectors. The association with lipid-related variables may in part reflect differences in preservation and packing media. Experimental studies have shown that the type of packing medium, such as water, brine, sunflower oil, and olive oil, and canning process may affect the fatty-acid composition of canned fish products (Prego et al., 2022). This evidence, although collected with a different species of fish, supports the interpretation that nutritional variation among canned fish products may be due to processing and preservation conditions.

However, the association of canned samples with Pb and Ni in the present PCA does not mean that there is a direct evidence of the origin of these metals from canned material or packing medium. PCA identifies statistical relationships between measured variables, but does not identify the source of contamination. Differences in metal levels in fish may result from a combination of factors including the environment of the harvesting site, species-specific bioaccumulation, feeding ecology, geographic origin, processing and other production factors (Feng et al., 2020; Garnero et al., 2018). Thus the similarity of the Pb and Ni vectors to the canned samples indicates an association in the present dataset, but does not prove migration or leaching of metals from the cans.

For the group B (freshwater and benthic-associated fish), the PCA showed a higher association with the Zn vector, especially for species such as Common Carp. This could be an indication of ecological and physiological differences between fish species. Zinc is a trace element that is crucial for numerous metabolic processes and that is biologically regulated . Therefore, variations in Zn concentrations should not be automatically interpreted as contamination. Trace-metal accumulation reported in fish has varied considerably between species with trophic level and habitat preference accounting for some of the differences in elemental concentrations. Fish associated with bottom habitats may also display different metal profiles than pelagic species (Feng et al., 2020).

Differences in the accumulation of elements have also been reported among freshwater fish species with different feeding strategies and habitat preferences, which emphasises the importance of ecological characteristics for the interpretation of metal burdens in fish (Garnero et al., 2018). Therefore, the correlation between Common Carp and Zn in present PCA may be related to a mixture of species-specific metabolism, feeding behaviour, habitat preference and environmental exposure rather than a single pollution origin (Feng et al., 2020; Garnero et al., 2018).

However, the potential contribution from sediment-associated exposure should be interpreted with caution. Trace metal concentrations may differ between fish species that live in or are near sediments and pelagic species, but the relative contribution of water, sediment, and dietary exposure varies with metal and species (Feng et al., 2020). The PCA association cannot demonstrate that sediment was the direct source of the observed Zn or other metals because sediment and water from the original harvesting locations of the imported fish were not analysed simultaneously in the present study.

In the frozen fish species (Group C), the pattern of PCA was determined by differences in both nutritional composition and heavy-metal concentrations. In the present results, the highest concentration of Pb was recorded in Sea Bream while the highest concentration of Cd was recorded in Common Carp. Salmon, however, showed intermediate concentrations of the investigated heavy metals. The results show that the separation of frozen species in the PCA space can not be explained by a single toxicological variable. But each species filled its place according to its joint nutritional and elemental characteristics.

The observed species-specific variation is consistent with previous evidence for a substantial interspecific variability in the accumulation of Pb, Cd, Zn and other trace metals. Such differences may be a function of trophic characteristics, habitat preference, feeding behaviour, physiological regulation, and environmental exposure (Feng et al., 2020; Garnero et al., 2018). The relatively high Pb concentration in Sea Bream and the high Cd concentration in Common Carp may be due to species biological differences, habitat, food sources, geographic origin and previous environmental exposure. However, these factors were not independently measured in the present study, and should thus be taken as plausible explanations rather than proven causal mechanisms.

Crucially, the position of salmon in the PCA should not be interpreted as evidence of a higher toxicological burden or of Pb biomagnification, as salmon showed intermediate heavy-metal concentrations in the present dataset. Moreover, trophic transfer of metals does not always lead to progressive biomagnification at higher trophic levels. Data from aquatic food webs (Verstijnen et al., 2024) show that some environmental conditions may lead to decreasing concentrations of Cd, Cu, Pb and Zn at higher trophic positions, rather than consistent biomagnification. Thus, a relatively high concentration of Pb in a certain fish species should not be interpreted as biomagnification without direct evidence based on measurements at the trophic level, stable-isotope analysis or assessment of the food web (Verstijnen et al., 2024).

Therefore, the higher Pb concentration in Sea Bream from the present study is more properly considered as a species-specific effect rather than trophic biomagnification.

Likewise, elevated Cd concentrations in Common Carp cannot be explained solely by benthic exposure without direct measurements of environmental matrices. Especially for imported fish, this conservative interpretation is particularly relevant, because the final chemical profile of the marketed product may be influenced by geographical origin, capture environment, species ecology, processing and storage (Feng et al., 2020; Garnero et al., 2018).

The PCA overall complemented the univariate and correlation analyses by showing that nutritional composition and heavy-metal burden varied simultaneously among the investigated fish samples. The positions of protein, fat, ash, Pb, Cd, Zn and Ni vectors indicate which variables contributed to the multivariate differentiation of samples and the relative positions of fish species represent their combined measured characteristics. Thus PCA provides an integrated representation of nutritional and toxicological variability that may not be apparent when each variable is considered individually (Jolliffe & Cadima, 2016).

The PCA results also indicate that wide categories, such as canned, freshwater, benthic-associated or frozen fish, do not completely explain the observed variance. Instead, the chemical signature of each sample seems to be the result of a combination of species-specific and product-related features. Processing and packing media might contribute to the nutritional differences between canned products (Prego et al., 2022), and feeding ecology, habitat preference, physiological regulation and environmental exposure could contribute to the differences in trace-metal accumulation between fish species (Feng et al., 2020; Garnero et al., 2018). However, the present study did not test these potential determinants independently and hence they cannot be regarded as confirmed causal factors.

To summarise, PCA should be considered as an exploratory multivariate tool summarising the overall structure of the dataset and pointing out similarities and differences between fish samples (Jolliffe & Cadima, 2016). The biplot shows groupings visible to the eye, suggesting the presence of distinct nutritional and elemental profiles, but they should not be considered statistically confirmed clusters unless an independent clustering analysis

is performed. The results suggest therefore that the nutritional profile and heavy-metal load of the studied fish are species- and product-specific and are better assessed by an integrated multivariate approach rather than being ascribed to an individual ecological, processing or toxicological factor.

4.8.5 Implications for Public Health and Food Safety

The comparison of the determined heavy metal loads with the limits set by the FAO / WHO and Codex Alimentarius shows a clear duality of the current food safety assessment. The levels of toxic metals such as Cadmium and Lead were below the maximum permissible levels as per international standards. But meeting the standards does not resolve public health issues. Heavy metals are persistent in environment, non-biodegradable and strongly tend to bioaccumulate in biological systems. Therefore, long-term dietary exposure to sublethal levels can cause cumulative toxic effects over time (EFSA, 2021; Ali et al., 2019).

Lead (Pb) is of particular concern because it is a known cumulative neurotoxin and there is scientific consensus that there is no safe level of exposure. There is evidence to support an association between chronic low-level exposure and neurodevelopmental impairment, cognitive deficits, hypertension and cardiovascular dysfunction . The risk for vulnerable populations, particularly children and pregnant women, is increased many-fold due to increased rates of intestinal absorption and the ability of Lead to cross the placental and blood–brain barriers (Lanphear

et al., 2018; WHO, 2024; Zheng et al., 2024). Similarly, Cadmium (Cd) has a very long biological half-life and is a major toxicological hazard. Its chronic consumption in the diet leads to progressive accumulation in the renal cortex and hence nephrotoxicity and demineralization of bone (Al-Thani & Yasseen, 2024; Satarug et al., 2019).

Another important implication is trace metal migration in canned seafood products. Some of the higher levels found in canned samples may be due to further leaching of metals from internal polymeric coatings or tinplate packaging, especially in cases of long-term storage or where the integrity of the can has been compromised. Packaging contamination emphasizes that the toxicological load of processed foods is not only inherent in the raw fish material but is synergistically enhanced by the preservation technology (Kowalska et al., 2020; Begum et al., 2023).

These results demonstrate a clear trade-off between preservation efficiency and nutritional integrity from a nutritional standpoint. Canning provides commercial sterility and long shelf life, but the thermal treatment required may change lipid profiles, decrease heat-sensitive bioactive compounds and reduce the activity of endogenous antioxidant enzymes like SOD (Mozzon et al., 2024; FAO, 2024).

These results justify the urgent need to abandon isolated maximum residue limits in favour of more inclusive, exposure-based monitoring systems from a regulatory point of view. Cumulative exposure assessments, including multiple contaminants, specific consumption frequencies, packaging migration vectors and oxidative stability of the products should be included in modern food safety protocols. The development of these effective and data-driven surveillance systems is critical to safeguard the public health and safety of the imported seafood supply chain in Iraq (Codex Alimentarius Commission, 2023; EFSA, 2021; WHO, 2023)

4.8.6 Biochemical and Oxidative Stability Assessment of Imported Fish Products

The present results showed a different change of oxidative stress markers (MDA, SOD) in canned and frozen fish samples. These results suggest the complex interplay of industrial, environmental and biological factors in the definition of the quality of imported

seafood products. The very significant differences ($P < 0.001$) suggest that they are not random, but the results of some mechanisms such as processing techniques, storage conditions, lipid composition of fish species and environmental exposure before and after harvesting. This is in agreement with recent literature on oxidative stress in marine food system (Tchounwou et al., 2012; FAO, 2022).

The variation of malondialdehyde (MDA) levels in canned fish particularly in sardine samples is caused by the variation of peroxidation intensity of lipids due to the variation of intensity of thermal processing, type of packing oil and storage period . High levels in some samples indicated extensive oxidative degradation of polyunsaturated fatty acids. This might be due to the fact that thermal sterilization destroys the endogenous antioxidant defense systems in fish tissues and thus increases the susceptibility of lipids to oxidation. Moreover,

the type of oil used for canning, the quality of packaging, and the integrity of the metal container can also contribute to accelerate oxidative processes through pro-oxidant interactions, thereby promoting or reducing oxidative reactions depending on the type of oil used for canning (Aubourg, 2023; Kavuşan et al., 2024; Robertson, 2016).

The differences observed in the activity of the superoxide dismutase (SOD) in the sardine samples showed the level of damage on the enzymatic proteins caused by the heat and the storage conditions. Sterilization involves the use of high temperatures, which denatures the protein, destroying its tertiary structure and reducing enzymatic activity. Some samples presented a higher SOD activity, which could be associated to less severe processing conditions or better preservation of the enzymatic integrity. In other samples a significant decrease was observed, most likely due to critical protein degradation during severe thermal treatment or long-term storage. This is consistent with studies showing that antioxidant enzymes are very sensitive to thermal processing (Cheng et al., 2020).

The results obtained in frozen fish show that oxidative reactions do occur at low temperature storage, since freezing inhibits and does not prevent lipid oxidation. Some species such as sea bream showed higher levels of MDA that could be related to the higher content of polyunsaturated fatty acids more susceptible to oxidation. Freezing also causes the formation of ice crystals that can rupture cell walls, releasing lipids and enzymes and leading to increased exposure to oxidative reactions. Disruption of the cold chain during transportation or storage may encourage lipid degradation. Low natural levels of lipids and MDA in some species limit the availability of substrates for oxidation (FAO, 2022; Ma et al., 2024).

In the SOD activity, the antioxidant defence capacity of frozen fish differed among species . Some species with higher enzyme activity may have better internal defence system or physiological adaptation to environmental stress. Lower activity could be caused by post-harvest stress, enzyme instability or long-term storage effects. Species differences might also be an issue. Biological characteristics of the species. Recent studies (Hu et al.,, 2023) showed that the handling and storage time after harvesting has a high impact on the enzymatic activity in fish .

The results generally suggest that oxidative stress in imported fish is due not to a single factor but the complex interaction between environmental pollution with heavy metals which

promote the formation of reactive oxygen species, industrial processing methods such as canning and freezing which influence the stability of lipids and proteins, and biological characteristics of fish species and their position on the food chain. Moreover, the toxicity of heavy metals, particularly lead and cadmium, can exacerbate oxidative stress by generating reactive oxygen species and impairing antioxidant defense, as evidenced by research on metal toxicity (Jaishankar et al., 2014; Lushchak, 2016).

Results also highlight the importance to assess the quality and safety of imported fish not only by traditional chemical parameters, but also by oxidative stress biomarkers as sensitive indicators of subtle changes in food quality . Also one indicator is not enough for the overall picture. The health risk of eating seafood can be evaluated through chemical analysis, enzymatic activity, and nutritional evaluation.

Conclusions & Recommendations



4.9 Conclusions

1. The results showed that the level of heavy metals (Pb, Cd, Zn, Ni) was different among imported fish species. The distribution is not the main source of contamination but the type of fish and the way it is preserved.
2. Highest levels of Pb and Cd were found in frozen predatory fish species, suggesting biomagnification along the trophic levels.
3. The relatively high concentrations of zinc and nickel in benthic organisms such as common carp indicate that sediment-related contamination is an important route of exposure.
4. The canned fish had lower levels of heavy metals than the frozen fish, but the significant correlation between Pb and Ni suggested that the packaging materials could be a source of metal contamination.
5. The measured concentrations were within the permissible limits of FAO/WHO but may pose a cumulative health risk upon chronic exposure.
6. Proximate biochemical analysis showed significant differences in the nutritional composition of the product types. In general, the protein content was higher and more stable in frozen fish than in canned products.
7. There was a negative correlation between protein and fat and between protein and ash. Not only processing. This suggests physiological differences between species.
8. Antioxidant defense systems, lipid quality and oxidative stress markers (MDA and SOD) were significantly decreased with differences according to the type of product, origin and medium of conservation

9. PCA allowed for a clear separation of the fish samples according to their ecological origin and processing method, confirming the effect of the interaction of environmental and industrial factors in the fish quality and safety.
10. The integrated results support the use of multivariate approaches combining heavy metal contamination, nutritional composition, and oxidative status for safety evaluation of imported fish, rather than single-parameter evaluation.
11. Antioxidant defense systems, lipid quality and oxidative stress markers (MDA and SOD) were significantly decreased with differences according to the type of product, origin and medium of conservation.
12. PCA allowed for a clear separation of the fish samples according to their ecological origin and processing method, confirming the effect of the interaction of environmental and industrial factors in the fish quality and safety.
13. The integrated results support the use of multivariate approaches combining heavy metal contamination, nutritional composition, and oxidative status for safety evaluation of imported fish, rather than single-parameter evaluation.

4.10. Recommendations

1. Reinforce routine quality control of imported fish by continuous monitoring of heavy metals in all product categories.
2. Establish maximum levels by trophic level (predatory, benthic, pelagic) rather than standard maximums.
3. Better control of packaging materials and (ex-)post tests of migration of metals from containers to food.
4. Add toxicological parameters and nutritional quality parameters (protein, fat, ash) to the official protocols for assessment of food safety.
5. Adopt models for cumulative risk assessment of lifetime dietary exposure to heavy metals in preference to threshold based compliance.
6. Improved control of freezing, canning, and storage conditions is recommended to reduce oxidative stress and lipid degradation in imported fish products, thereby preserving nutritional quality and enhancing the safety of final products. Establish a national database to track heavy metal contamination levels in imported fish, broken down by species, country of origin and method of preservation.
7. Increase awareness of the public on the risks of frequent consumption of fish species with high bioaccumulation of heavy metals.
8. Promote the use of advanced preservation technologies to minimize the loss of nutrients and oxidation byproducts.

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APPENDICES

Laboratory and Statistical Data Tables



APPENDIX A SAMPLE IDENTIFICATION

No.	Sample code	Product category	Product / species	Brand / origin
1	T1	Canned tuna	Tuna in vegetable oil	Seablo / Thailand
2	T2	Canned tuna	Tuna in olive oil	Afco / Thailand
3	T3	Canned tuna	Tuna in water	Fella / Thailand
4	T4	Canned tuna	Tuna in vegetable oil	Fella / Thailand
5	T5	Canned tuna	Tuna in water and salt	Ghadeer Al-Islamia / Thailand
6	T6	Canned tuna	Tuna in sunflower oil	Terna Al-Khair / Thailand
7	T7	Canned tuna	Tuna in sunflower oil	Valencia Gardens / Thailand
8	T8	Canned tuna	Tuna in olive oil	Valencia Gardens / Thailand
9	T9	Canned tuna	Tuna in water and salt	Salsa / Vietnam
10	T10	Canned tuna	Tuna in vegetable oil	Durra / Thailand
11	T11	Canned tuna	Tuna in vegetable oil	Altunsa / Turkey
12	T12	Canned tuna	Tuna in vegetable oil	Henan / Indonesia
13	T13	Canned tuna	Tuna in vegetable oil	Cerella / Thailand
14	T14	Canned tuna	Tuna in water and salt	Hanaa / Thailand
15	T15	Canned tuna	Tuna in vegetable oil	Al-Nawajil / Vietnam
16	T16	Canned tuna	Tuna in vegetable oil	Joker / China
17	T17	Canned tuna	Tuna in vegetable oil	Golden Fresh / Thailand
18	T18	Canned tuna	Tuna in vegetable oil	Taksa / Thailand
19	T19	Canned tuna	Tuna in sunflower oil	Rio Mare / Thailand

20	T20	Canned tuna	Tuna in olive oil	Noor Al-Kafeel / Vietnam
21	T21	Canned tuna	Tuna in soybean oil	Baidar / Thailand
22	T22	Canned tuna	Tuna in vegetable oil	California Garden / Thailand
23	T23	Canned tuna	Tuna in vegetable oil	Abu Al-Ward / Thailand
24	T24	Canned tuna	Tuna in water	Abu Al-Ward / Thailand
25	T25	Canned tuna	Tuna in vegetable oil	Majeed / Iran
26	S1	Canned sardine	Sardine	Sea Hat / India
27	S2	Canned sardine	Sardine	Henan / Indonesia
28	S3	Canned sardine	Sardine	Al-Arz / China
29	S4	Canned sardine	Sardine	Durra / Thailand
30	S5	Canned sardine	Sardine	Diamond / Indonesia
31	S6	Canned sardine	Sardine	Diamond / Thailand
32	S7	Canned sardine	Sardine	Ghadeer / China
33	X1	Frozen fish	Sea Bream	Turkey
34	X2	Frozen fish	Red-bellied Pacu	Vietnam
35	X3	Frozen fish	Common Carp	Myanmar
36	X4	Frozen fish	Salmon	Iran
37	X5	Frozen fish	Nile Tilapia	Indonesia

No.	Sample code	Product category	Product / species	Brand / origin
38	X6	Frozen fish	Golden Pompano	Jordan
39	X7	Frozen fish	Mackerel	China

Note. T1-T25 = canned tuna samples; S1-S7 = canned sardine samples; X1-X7 = frozen fish samples.

APPENDIX B

PROXIMATE COMPOSITION RESULTS

Note. Protein, fat, and ash values are presented exactly as reported in the source as mean $\pm$ reported error; the source table does not state the measurement unit.

Table B1. Canned tuna: reported proximate composition

Cod e	Product / species	Brand / origin	Protein	Fat	Ash	Product category
T1	Tuna in vegetable oil	Seablo Thailand	18.833 $\pm$ 0.120	9.633 $\pm$ 0.845	2.733 $\pm$ 0.033	Canned tuna
T2	Tuna in olive oil	Afco / Thailand	19.333 $\pm$ 0.088	10.433 $\pm$ 1.634	2.867 $\pm$ 0.033	Canned tuna
T3	Tuna in water	Fella / Thailand	19.700 $\pm$ 0.058	10.433 $\pm$ 1.384	2.967 $\pm$ 0.033	Canned tuna
T4	Tuna in vegetable oil	Fella / Thailand	20.100 $\pm$ 0.115	12.267 $\pm$ 0.570	3.033 $\pm$ 0.033	Canned tuna

T5	Tuna in water and salt	Ghadeer Al-Islamia / Thailand	20.500 ± 0.058	11.867 ± 0.088	3.100 ± 0.000	± Canned tuna
T6	Tuna in sunflower oil	Terna Al-Khair / Thailand	20.867 ± 0.067	12.867 ± 0.623	3.200 ± 0.000	± Canned tuna
T7	Tuna in sunflower oil	Valencia Gardens / Thailand	21.733 ± 0.203	14.867 ± 0.233	3.400 ± 0.058	± Canned tuna
T8	Tuna in olive oil	Valencia Gardens / Thailand	22.867 ± 0.033	9.267 ± 1.659	2.000 ± 0.058	± Canned tuna
T9	Tuna in water and salt	Salsa / Vietnam	23.033 ± 0.033	11.367 ± 0.491	2.067 ± 0.267	± Canned tuna
T10	Tuna in vegetable oil	Durra / Thailand	23.133 ± 0.033	9.600 ± 1.044	2.100 ± 0.200	± Canned tuna
T11	Tuna in vegetable oil	Altunsa / Turkey	23.367 ± 0.033	10.033 ± 0.706	2.033 ± 0.088	± Canned tuna
T12	Tuna in vegetable oil	Henan / Indonesia	23.433 ± 0.033	9.967 ± 0.674	2.600 ± 0.289	± Canned tuna
T13	Tuna in vegetable oil	Cerella / Thailand	23.533 ± 0.033	9.733 ± 0.578	2.267 ± 0.267	± Canned tuna
T14	Tuna in water and salt	Hanaa / Thailand	23.633 ± 0.033	9.567 ± 0.176	1.933 ± 0.120	± Canned tuna
T15	Tuna in vegetable oil	Al-Nawajil / Vietnam	23.700 ± 0.000	9.667 ± 0.273	1.967 ± 0.033	± Canned tuna
T16	Tuna in vegetable oil	Joker / China	23.800 ± 0.000	9.833 ± 0.926	2.133 ± 0.145	± Canned tuna
T17	Tuna in vegetable oil	Golden Fresh / Thailand	23.967 ± 0.033	9.233 ± 0.088	2.133 ± 0.033	± Canned tuna

T18	Tuna in vegetable oil	Taksa / Thailand	24.033 ± 0.033	9.033 ± 0.240	± 2.367 ± 0.088	±Canned tuna
T19	Tuna in sunflower oil	Rio Mare / Thailand	24.100 ± 0.000	9.067 ± 0.536	± 2.200 ± 0.058	±Canned tuna
T20	Tuna in olive oil	Noor Al-Kafeel / Vietnam	24.100 ± 0.000	9.100 ± 0.416	± 2.300 ± 0.208	±Canned tuna
T21	Tuna in soybean oil	Baidar / Thailand	24.267 ± 0.033	9.500 ± 0.651	± 2.067 ± 0.033	±Canned tuna
T22	Tuna in vegetable oil	California Garden / Thailand	24.300 ± 0.000	8.700 ± 1.002	± 2.567 ± 0.133	±Canned tuna
T23	Tuna in vegetable oil	Abu Al-Ward / Thailand	24.367 ± 0.033	9.567 ± 0.874	± 2.667 ± 0.418	± Canned tuna
T24	Tuna in water	Abu Al-Ward / Thailand	24.533 ± 0.033	7.967 ± 1.452	± 2.300 ± 0.100	±Canned tuna
T25	Tuna in vegetable oil	Majeed / Iran	24.600 ± 0.000	9.800 ± 0.557	± 2.633 ± 0.353	± Canned tuna

Table B2. Canned sardine: reported proximate composition

Cod e	Product species	/	Brand / origin	Protein	Fat	Ash	Pro duct cate gory
S1	Sardine		Sea Hat / India	24.667 ± 0.033	8.433 ± 0.120	± 2.567 ± 0.418	±Canned sardine
S2	Sardine		Henan / Indonesia	24.700 ± 0.000	9.700 ± 0.416	± 2.433 ± 0.273	±Canned sardine
S3	Sardine		Al-Arz / China	24.800 ±	9.367	± 2.300	±Canned

				0.000	0.731	0.115	sardine
S4	Sardine		Durra / Thailand	24.900 ± 0.000	8.500 ± 0.153	± 2.267 ± 0.067	± Canned sardine
S5	Sardine		Diamond	/25.033 ± 0.033	± 5.767 ± 1.560	± 2.533 ± 0.186	± Canned sardine
S6	Sardine		Diamond	/25.100 ± 0.000	± 8.633 ± 1.396	± 2.600 ± 0.208	± Canned sardine
			Thailand				
Code	Product	/	Brand / origin	Protein	Fat	Ash	Pro duct cate gory
S7	Sardine		Ghadeer / China	25.233 ± 0.033	± 6.533 ± 2.042	± 2.567 ± 0.219	± Canned sardine

Table B3. Frozen fish: reported proximate composition

Code	Product	/	Brand / origin	Protein	Fat	Ash	Pro duct cate gory
X1	Sea Bream		Turkey	25.433 ± 0.033	± 6.300 ± 1.850	± 2.300 ± 0.058	± Frozen fish
X2	Red-bellied Pacu		Vietnam	25.633 ± 0.033	± 5.967 ± 1.841	± 2.400 ± 0.100	± Frozen fish
X3	Common Carp		Myanmar	25.700 ± 0.000	± 5.800 ± 1.808	± 2.500 ± 0.058	± Frozen fish
X4	Salmon		Iran	25.833	± 4.500	± 2.533	± Frozen

			0.033	2.501	0.033	fish
X5	Nile Tilapia	Indonesia	25.967	± 5.300	± 2.533	± Frozen
			0.033	3.001	0.033	fish
X6	Golden Pompano	Jordan	26.200	± 4.267	± 2.433	± Frozen
			0.058	2.070	0.033	fish
X7	Mackerel	China	26.400	±1.933	±2.600	± Frozen
			0.058	0.186	0.058	fish

APPENDIX C HEAVY-METAL RESULTS

Note: Concentrations are in ppb as explicitly stated in the supplied heavy-metal result tables. Values are shown as reported mean ± error

Code	Product / species	Brand / origin	Pb (ppb)	Cd (ppb)	Zn (ppb)	Ni (ppb)
T1	Tuna in vegetable oil	Seablo / Thailand	8.700	±0.838	±45.600	± 3.200
			0.143	0.176	0.045	0.207
T2	Tuna in olive oil	Afco / Thailand	8.100	±0.971	± 60.000	±6.800
			0.267	0.072	0.003	0.145
T3	Tuna in water	Fella / Thailand	15.600	±0.868	±37.800	±9.700
			0.025	0.114	0.072	0.116
T4	Tuna in vegetable oil	Fella / Thailand	16.200	± 1.221	± 73.400	± 11.500
			0.165	0.067	0.086	0.003
T5	Tuna in water and salt	Ghadeer Al-Islamia / Thailand	8.100	±0.897	± 25.200	± 6.400
			0.170	0.038	0.018	0.219
T6	Tuna in sunflower oil	Terna Al-Khair / Thailand	14.400	± 1.206	± 34.500	± 19.200
			0.195	0.102	0.121	0.169
T7	Tuna in sunflower	Valencia Gardens /	14.400	± 1.338	± 34.100	± 9.100

	oil	Thailand		0.089	0.284	0.014	0.184	
T8	Tuna in olive oil	Valencia Gardens / Thailand		11.900	± 1.426	± 12.000	±4.800	±
				0.040	0.127	0.024	0.093	
T9	Tuna in water and salt	Salsa / Vietnam		13.100	± 1.206	± 37.300	±3.900	±
				0.024	0.083	0.108	0.025	
T10	Tuna in vegetable oil	Durra / Thailand		13.100	± 1.044	± 31.300	±6.700	±
				0.016	0.027	0.106	0.178	
T11	Tuna in vegetable oil	Altunsa / Turkey		15.600	± 1.235	± 14.400	± 7.200	±
				0.067	0.166	0.111	0.012	
T12	Tuna in vegetable oil	Henan / Indonesia		5.600	± 1.368	± 15.400	±4.800	±
				0.101	0.010	0.010	0.090	
T13	Tuna in vegetable oil	Cerella / Thailand		6.900	± 1.059	±11.700	±3.700	±
				0.019	0.151	0.057	0.130	
T14	Tuna in water and salt	Hanaa / Thailand		6.200	± 1.426	±36.800	± 5.400	±
				0.087	0.021	0.104	0.053	
T15	Tuna in vegetable oil	Al-Nawajil / Vietnam		7.500	± 1.059	±27.700	± 5.100	±
				0.045	0.004	0.004	0.223	
T16	Tuna in vegetable oil	Joker / China		10.000	±0.853	±19.600	± 4.400	±
				0.151	0.041	0.082	0.053	
T17	Tuna in vegetable oil	Golden Fresh / Thailand		8.100	± 1.074	±25.900	± 4.200	±
				0.016	0.092	0.113	0.079	
T18	Tuna in vegetable oil	Taksa / Thailand		10.600	± 1.309	± 46.200	± 5.500	±
				0.053	0.016	0.001	0.210	
T19	Tuna in sunflower oil	Rio Mare / Thailand		10.000	± 1.103	± 19.000	± 3.300	±
				0.063	0.112	0.110	0.179	
T20	Tuna in olive oil	Noor Al-Kafeel / Vietnam		8.100	± 1.250	±31.700	±4.600	±
				0.105	0.021	0.157	0.031	

T21	Tuna in soybean oil	Baidar / Thailand	10.000 ± 1.000 ± 39.100 ± 5.200 ± 0.107 0.134 0.077 0.025
T22	Tuna in vegetable oil	California Garden / Thailand	12.500 ± 1.132 ± 28.100 ±5.600 ± 0.036 0.090 0.071 0.106
T23	Tuna in vegetable oil	Abu Al-Ward / Thailand	13.700 ± 1.279 ± 79.100 ± 5.300 ± 0.063 0.053 0.004 0.010
T24	Tuna in water	Abu Al-Ward / Thailand	10.000 ± 1.000 ±53.700 ±5.900 ± 0.040 0.015 0.049 0.143
T25	Tuna in vegetable oil	Majeed / Iran	13.100 ±0.941 ± 20.400 ± 5.400 ± 0.014 0.074 0.045 0.055

Table C2. Canned sardine: heavy-metal concentrations

Code	Product species	/	Brand origin	/	Pb (ppb)	Cd (ppb)	Zn (ppb)	Ni (ppb)	
S1	Sardine		Sea Hat / India		15.000 ±1.838 ± 93.900 ± 3.300 ± 0.112 0.022 0.110 0.139				
S2	Sardine		Henan / Indonesia		16.900 ± 1.074 ± 62.400 ± 3.300 ± 0.113 0.083 0.120 0.015				
S3	Sardine		Al-Arz / China		12.500 ±0.706 ± 197.100 ±2.600 ± 0.034 0.024 0.134 0.119				
S4	Sardine		Durra / Thailand		21.200 ±1.941 ± 74.100 ±3.600 ± 0.053 0.163 0.187 0.122				
S5	Sardine		Diamond / Indonesia		21.900 ± 1.353 ±103.800 ± 3.500 ± 0.078 0.094 0.215 0.176				
S6	Sardine		Diamond / Thailand		13.100 ± 1.265 ±103.800 ±3.600 ± 0.010 0.021 0.165 0.153				

S7	Sardine	Ghadeer	/	15.000	±1.926	± 83.800	±3.700	±
		China		0.015	0.137	0.125	0.100	

Table C3. Frozen fish: heavy-metal concentrations

Code	Product species	/	Brand origin	/	Pb (ppb)	Cd (ppb)	Zn (ppb)	Ni (ppb)
X1	Sea Bream		Turkey		131.200	±3.824	± 37.900	± 22.500 ±
					0.262	0.195	0.059	0.129
X2	Red-bellied Pacu		Vietnam		93.700	± 3.529	±42.700	±24.600 ±
					0.155	0.085	0.125	0.213
X3	Common Carp		Myanmar		112.400	±6.765	± 47.000	±24.600 ±
					0.184	0.038	0.006	0.106
X4	Salmon		Iran		93.700	±2.647	± 39.100	± 25.500 ±
					0.046	0.209	0.111	0.061
X5	Nile Tilapia		Indonesia		43.700	±3.824	± 18.600	± 24.000 ±
					0.127	0.115	0.099	0.067
X6	Golden Pompano		Jordan		93.700	± 4.559	± 19.200	± 24.200 ±
					0.136	0.051	0.069	0.019
X7	Mackerel		China		87.500	±4.853	±40.900	±24.800 ±
					0.098	0.122	0.061	0.043

APPENDIX D

MDA AND SOD RESULTS

Note: MDA and SOD values are presented exactly as reported in the supplied file as mean ± reported error. Measurement units are not stated in the source table.

Table D1. Canned tuna: MDA and SOD biomarker results

Code	Product / species	Brand / origin	MDA	SOD	
T1	Tuna in vegetable oil	Seablo / Thailand	26.624 ± 7.018	288.067	±
				5.222	
T2	Tuna in olive oil	Afco / Thailand	35.978 ± 6.688	263.424	±
				7.000	
T3	Tuna in water	Fella / Thailand	33.090	± 344.004	±
			7.629	15.076	
T4	Tuna in vegetable oil	Fella / Thailand	53.591	±267.738	±
			18.484	3.620	
T5	Tuna in water and salt	Ghadeer Al-Islamia / Thailand	25.313	±241.918	±
			2.851	5.181	
T6	Tuna in sunflower oil	Terna Al-Khair / Thailand	35.030	± 242.494	±
			6.170	4.772	
T7	Tuna in sunflower oil	Valencia Gardens / Thailand	58.314	±297.903	±
			4.537	1.460	
T8	Tuna in olive oil	Valencia Gardens / Thailand	74.714 ± 0.411	268.018	±
				6.284	
T9	Tuna in water and salt	Salsa / Vietnam	47.637 ± 4.478	284.901	±
				3.102	
T10	Tuna in vegetable oil	Durra / Thailand	30.360	± 258.372	±
			3.348	3.137	
T11	Tuna in vegetable oil	Altunsa / Turkey	40.348	± 278.106	±
			4.544	5.214	
T12	Tuna in vegetable oil	Henan / Indonesia	42.663	± 257.311	±

					13.922		2.868	
T13	Tuna in vegetable oil	Cerella / Thailand			33.743	±	222.505	±
					16.973		1.274	
T14	Tuna in water and salt	Hanaa / Thailand			38.125	±	251.134	±
					16.788		3.882	
T15	Tuna in vegetable oil	Al-Nawajil / Vietnam			26.120	±	242.428	±
					6.087		3.636	
T16	Tuna in vegetable oil	Joker / China			57.094	±	214.829	±
					11.834		1.318	
T17	Tuna in vegetable oil	Golden Fresh / Thailand			40.210	±	294.338	±
					3.312		2.362	
T18	Tuna in vegetable oil	Taksa / Thailand			17.979 ± 2.500		277.707	±
							5.640	
T19	Tuna in sunflower oil	Rio Mare / Thailand			40.697	±	281.551	±
					14.299		3.689	
Code	Product / species	Brand / origin			MDA	SOD		
T20	Tuna in olive oil	Noor	Al-Kafeel	/	68.070	±	259.154	±
		Vietnam			2.092		3.791	
T21	Tuna in soybean oil	Baidar / Thailand			15.207	±	290.104	±
					3.535		1.671	
T22	Tuna in vegetable oil	California	Garden	/	31.989 ± 1.658		275.127	±
		Thailand					4.961	
T23	Tuna in vegetable oil	Abu Al-Ward / Thailand			46.458	±	254.489	±
					6.587		1.725	
T24	Tuna in water	Abu Al-Ward / Thailand			46.296	±	293.139	±
					15.462		3.458	

T25	Tuna in vegetable oil	Majeed / Iran	27.598 13.286	$\pm 287.856 \pm 3.614$
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Table D2. Canned sardine: MDA and SOD biomarker results

Code	Product / species	Brand / origin	MDA	SOD
S1	Sardine	Sea Hat / India	68.936 ± 5.200	228.325 ± 1.185
S2	Sardine	Henan / Indonesia	46.947 ± 6.504	286.495 ± 5.137
S3	Sardine	Al-Arz / China	23.399 ± 1.029	355.422 ± 2.823
S4	Sardine	Durra / Thailand	17.825 ± 3.098	307.395 ± 32.474
S5	Sardine	Diamond / Indonesia	54.920 ± 3.489	418.822 ± 3.866
S6	Sardine	Diamond / Thailand	44.582 ± 3.250	215.055 ± 1.111
S7	Sardine	Ghadeer / China	56.349 ± 4.031	$\pm 212.836 \pm 2.428$

Table D3. Frozen fish: MDA and SOD biomarker results

Code	Product / species	Brand / origin	MDA	SOD
X1	Sea Bream	Turkey	80.986 ± 4.348	238.765 ± 3.468
X2	Red-bellied Pacu	Vietnam	30.311 ± 3.455	275.401 ± 8.311
X3	Common Carp	Myanmar	39.154 ± 3.075	214.305 ± 2.513

X4	Salmon	Iran	59.050 5.521	± 263.504 6.121	±
X5	Nile Tilapia	Indonesia	22.548 5.276	±254.745 ± 3.172	
X6	Golden Pompano	Jordan	27.088 2.149	± 208.530 2.887	±
X7	Mackerel	China	41.664 ± 4.329	250.781 ± 2.531	

APPENDIX ERAW TRIPLICATE MDA AND SOD READINGS

Note: Each sample contains three replicate readings. The replicate values are presented without recalculation or alteration.

Table E1. Canned tuna: raw triplicate MDA and SOD readings.

Code	Repl icate	Product / species	Brand / origin	Product category	MDA	SOD
T1	1	Tuna in vegetable oil	Seablo / Thailand	Canned tuna	17.03	285.28
T1	2	Tuna in vegetable oil	Seablo / Thailand	Canned tuna	22.55	280.74
T1	3	Tuna in vegetable oil	Seablo / Thailand	Canned tuna	40.29	298.18
T2	1	Tuna in olive oil	Afco / Thailand	Canned tuna	33.55	276.60
T2	2	Tuna in olive oil	Afco / Thailand	Canned tuna	25.80	252.73
T2	3	Tuna in olive oil	Afco / Thailand	Canned tuna	48.58	260.95
T3	1	Tuna in water	Fella / Thailand	Canned tuna	22.70	337.23
T3	2	Tuna in water	Fella / Thailand	Canned tuna	28.61	372.84

T3	3	Tuna in water	Fella / Thailand	Canned tuna	47.96	321.94
T4	1	Tuna in vegetable oil	Fella / Thailand	Canned tuna	89.53	260.90
T4	2	Tuna in vegetable oil	Fella / Thailand	Canned tuna	28.10	269.10
T4	3	Tuna in vegetable oil	Fella / Thailand	Canned tuna	43.14	273.22
T5	1	Tuna in water and salt	Ghadeer Islamia / Thailand	Al- Canned tuna	25.03	247.52

Code	Replicate	Product / species	Brand origin	/Product category	MDA	SOD
T5	2	Tuna in water and salt	Ghadeer Islamia Thailand	Al-Canned /tuna	20.52	246.67
T5	3	Tuna in water and salt	Ghadeer Islamia Thailand	Al-Canned /tuna	30.39	231.57
T6	1	Tuna in sunflower oil	Terna Khair Thailand	Al-Canned /tuna	47.23	234.90
T6	2	Tuna in sunflower oil	Terna Khair Thailand	Al-Canned /tuna	27.35	241.29
T6	3	Tuna in sunflower oil	Terna Khair Thailand	Al-Canned /tuna	30.51	251.30
T7	1	Tuna in sunflower	Valencia	Canned	57.31	297.76

		oil	Gardens Thailand	/tuna		
T7	2	Tuna in sunflower oil	Valencia Gardens Thailand	Canned /tuna	51.01	300.50
T7	3	Tuna in sunflower oil	Valencia Gardens Thailand	Canned /tuna	66.63	295.45
T8	1	Tuna in olive oil	Valencia Gardens Thailand	Canned /tuna	75.40	256.40
T8	2	Tuna in olive oil	Valencia Gardens Thailand	Canned /tuna	74.77	269.68
T8	3	Tuna in olive oil	Valencia Gardens Thailand	Canned /tuna	73.97	277.97
T9	1	Tuna in water and salt	Salsa Vietnam	/Canned tuna	44.85	279.32
T9	2	Tuna in water and salt	Salsa Vietnam	/Canned tuna	41.66	285.33
T9	3	Tuna in water and salt	Salsa Vietnam	/Canned tuna	56.40	290.05
T10	1	Tuna in vegetable oil	Durra Thailand	/Canned tuna	36.79	262.50
T10	2	Tuna in vegetable oil	Durra Thailand	/Canned tuna	28.76	260.40

T10	3	Tuna in vegetable oil	Durra Thailand	/Canned tuna	25.53	252.22
T11	1	Tuna in vegetable oil	Altunsa Turkey	/Canned tuna	48.76	288.49
T11	2	Tuna in vegetable oil	Altunsa Turkey	/Canned tuna	39.12	272.05
T11	3	Tuna in vegetable oil	Altunsa Turkey	/Canned tuna	33.17	273.78
T12	1	Tuna in vegetable oil	Henan Indonesia	/Canned tuna	36.02	257.57
T12	2	Tuna in vegetable oil	Henan Indonesia	/Canned tuna	22.57	252.22
T12	3	Tuna in vegetable oil	Henan Indonesia	/Canned tuna	69.40	262.14
T13	1	Tuna in vegetable oil	Cerella Thailand	/Canned tuna	59.34	224.61
T13	2	Tuna in vegetable oil	Cerella Thailand	/Canned tuna	40.24	220.21
T13	3	Tuna in vegetable oil	Cerella Thailand	/Canned tuna	1.64	222.69
T14	1	Tuna in water and salt	Hanaa Thailand	/Canned tuna	30.42	248.24
T14	2	Tuna in water and salt	Hanaa Thailand	/Canned tuna	70.28	258.82
T14	3	Tuna in water and salt	Hanaa Thailand	/Canned tuna	13.68	246.34

T15	1	Tuna in vegetable oil	Al-Nawajil /Canned Vietnam tuna	16.18	249.26
T15	2	Tuna in vegetable oil	Al-Nawajil /Canned Vietnam tuna	37.18	236.86
T15	3	Tuna in vegetable oil	Al-Nawajil /Canned Vietnam tuna	25.00	241.17
T16	1	Tuna in vegetable oil	Joker /Canned China tuna	35.26	216.91
T16	2	Tuna in vegetable oil	Joker /Canned China tuna	60.10	212.39
T16	3	Tuna in vegetable oil	Joker /Canned China tuna	75.92	215.19
T17	1	Tuna in vegetable oil	Golden FreshCanned / Thailand tuna	36.17	289.66
T17	2	Tuna in vegetable oil	Golden FreshCanned / Thailand tuna	37.68	297.26
T17	3	Tuna in vegetable oil	Golden FreshCanned / Thailand tuna	46.78	296.10
T18	1	Tuna in vegetable oil	Taksa /Canned Thailand tuna	18.18	288.88
T18	2	Tuna in vegetable oil	Taksa /Canned Thailand tuna	22.20	270.76
T18	3	Tuna in vegetable oil	Taksa /Canned Thailand tuna	13.55	273.48
T19	1	Tuna in sunflower oil	Rio Mare /Canned Thailand tuna	31.55	286.91

T19	2	Tuna in sunflower oil	Rio Mare /Canned Thailand tuna	68.74	274.48
T19	3	Tuna in sunflower oil	Rio Mare /Canned Thailand tuna	21.80	283.26
T20	1	Tuna in olive oil	Noor Al-Canned Kafeel /tuna Vietnam	72.22	261.96

Code	Repl icate	Product species	/	Brand / origin	Product category	MDA	SOD
T20	2	Tuna in olive oil		Noor Al-Kafeel Vietnam	/Canned tuna	65.51	263.85
T20	3	Tuna in olive oil		Noor Al-Kafeel Vietnam	/Canned tuna	66.49	251.65
T21	1	Tuna in soybean oil		Baidar / Thailand	Canned tuna	22.05	292.78
T21	2	Tuna in soybean oil		Baidar / Thailand	Canned tuna	10.26	287.03
T21	3	Tuna in soybean oil		Baidar / Thailand	Canned tuna	13.31	290.51
T22	1	Tuna in vegetable oil		California Garden Thailand	/Canned tuna	29.72	284.20
T22	2	Tuna in vegetable oil		California Garden Thailand	/Canned tuna	35.22	267.11
T22	3	Tuna in vegetable oil		California Garden Thailand	/Canned tuna	31.03	274.07
T23	1	Tuna in vegetable oil		Abu Al-Ward Thailand	/Canned tuna	33.29	252.18

T23	2	Tuna vegetable oil	in	Abu Al-Ward Thailand	/Canned tuna	53.25	253.42
T23	3	Tuna vegetable oil	in	Abu Al-Ward Thailand	/Canned tuna	52.83	257.86
T24	1	Tuna in water		Abu Al-Ward Thailand	/Canned tuna	22.62	297.33
T24	2	Tuna in water		Abu Al-Ward Thailand	/Canned tuna	40.90	286.28
T24	3	Tuna in water		Abu Al-Ward Thailand	/Canned tuna	75.36	295.80
T25	1	Tuna vegetable oil	in	Majeed / Iran	Canned tuna	49.15	293.18
T25	2	Tuna vegetable oil	in	Majeed / Iran	Canned tuna	3.36	289.43
T25	3	Tuna vegetable oil	in	Majeed / Iran	Canned tuna	30.28	280.96

Table E2. Canned sardine: raw triplicate MDA and SOD readings

Code	Repl icate	Product / species	Brand / origin	Product category	MDA	SOD
S1	1	Sardine	Sea Hat / India	Canned sardine	65.22	229.10
S1	2	Sardine	Sea Hat / India	Canned sardine	79.21	226.00
S1	3	Sardine	Sea Hat / India	Canned sardine	62.38	229.88

S2	1	Sardine	Henan / Indonesia	Canned sardine	45.16 278.48
S2	2	Sardine	Henan / Indonesia	Canned sardine	36.68 296.07
S2	3	Sardine	Henan / Indonesia	Canned sardine	59.00 284.93
S3	1	Sardine	Al-Arz / China	Canned sardine	21.41 350.71
S3	2	Sardine	Al-Arz / China	Canned sardine	24.86 360.47
S3	3	Sardine	Al-Arz / China	Canned sardine	23.93 355.09
S4	1	Sardine	Durra / Thailand	Canned sardine	22.70 372.34
S4	2	Sardine	Durra / Thailand	Canned sardine	12.08 274.55
S4	3	Sardine	Durra / Thailand	Canned sardine	18.69 275.29
S5	1	Sardine	Diamond Indonesia	/ Canned sardine	58.05 412.06
S5	2	Sardine	Diamond Indonesia	/ Canned sardine	47.95 425.45
S5	3	Sardine	Diamond Indonesia	/ Canned sardine	58.76 418.96
S6	1	Sardine	Diamond / Thailand	Canned sardine	47.26 212.91

S6	2	Sardine	Diamond / Thailand	Canned sardine	38.11	215.61
S6	3	Sardine	Diamond / Thailand	Canned sardine	48.37	216.64
S7	1	Sardine	Ghadeer / China	Canned sardine	50.91	209.56
S7	2	Sardine	Ghadeer / China	Canned sardine	53.91	217.58
S7	3	Sardine	Ghadeer / China	Canned sardine	64.22	211.36

Table E3. Frozen fish: raw triplicate MDA and SOD readings

Code	Repl icate	Product species	/	Brand / origin	Product category	MDA	SOD
X1	1	Sea Bream		Turkey	Frozen fish	81.79	237.20
Code	Replicate	Product species	/	Brand / origin	Product category	MDA	SOD
X1	2	Sea Bream		Turkey	Frozen fish	73.09	233.70
X1	3	Sea Bream		Turkey	Frozen fish	88.08	245.40
X2	1	Red- bellied Pacu		Vietnam	Frozen fish	34.82	277.46
X2	2	Red- bellied		Vietnam	Frozen fish	23.52	288.65

		Pacu				
X2	3	Red-bellied Pacu	Vietnam	Frozen fish	32.59	260.09
X3	1	Common Carp	Myanmar	Frozen fish	34.04	214.58
X3	2	Common Carp	Myanmar	Frozen fish	38.75	218.52
X3	3	Common Carp	Myanmar	Frozen fish	44.67	209.82
X4	1	Salmon	Iran	Frozen fish	55.47	262.23
X4	2	Salmon	Iran	Frozen fish	51.79	274.68
X4	3	Salmon	Iran	Frozen fish	69.89	253.60
X5	1	Nile Tilapia	Indonesia	Frozen fish	28.62	251.86
X5	2	Nile Tilapia	Indonesia	Frozen fish	12.04	261.08
X5	3	Nile Tilapia	Indonesia	Frozen fish	26.99	251.30
X6	1	Golden Pompano	Jordan	Frozen fish	27.85	212.76
X6	2	Golden Pompano	Jordan	Frozen fish	23.04	203.01
X6	3	Golden Pompano	Jordan	Frozen fish	30.37	209.81
X7	1	Mackere	China	Frozen fish	49.07	248.69

		1				
X7	2	Mackere	China	Frozen fish	34.07	255.82
		1				
X7	3	Mackere	China	Frozen fish	41.85	247.83
		1				

APPENDIX F PEARSON CORRELATION RESULTS

Note: * significant at $P < 0.05$; ** significant at $P < 0.01$; NS = not statistically significant. Correlations are two-tailed; each unique variable pair is presented once.

Table F1. Canned tuna: pairwise Pearson correlations among heavy metals and proximate-composition variables

Variable 1	Variable 2	Pearson r	P-value (2-tailed)	(2-N)	Significance
Pb	Cd	0.066	0.754	25	NS
Pb	Zn	0.264	0.202	25	NS
Pb	Ni	0.550**	0.004	25	**
Pb	Protein	-0.185	0.377	25	NS
Pb	Fat	0.408*	0.043	25	*
Pb	Ash	0.346	0.090	25	NS
Cd	Zn	0.005	0.981	25	NS
Cd	Ni	0.112	0.593	25	NS
Cd	Protein	0.292	0.157	25	NS
Cd	Fat	0.142	0.498	25	NS
Cd	Ash	-0.121	0.566	25	NS

Zn	Ni	0.239	0.250	25	NS
Zn	Protein	-0.302	0.143	25	NS
Zn	Fat	0.113	0.589	25	NS
Zn	Ash	0.359	0.078	25	NS
Ni	Protein	-0.449*	0.024	25	*

Variable 1	Variable 2	Pearson r	P-value tailed)	(2- N	Significance
Ni	Fat	0.627**	0.001	25	**
Ni	Ash	0.613**	0.001	25	**
Protein	Fat	-0.546**	0.005	25	**
Protein	Ash	-0.659**	0.000	25	**
Fat	Ash	0.704**	0.000	25	**

Table F2. Canned sardine: pairwise Pearson correlations among heavy metals and proximate-composition

variables					
Variable 1	Variable 2	Pearson r	P-value tailed)	(2- N	Significance
Pb	Cd	0.371	0.413	7	NS
Pb	Zn	-0.488	0.266	7	NS
Pb	Ni	0.439	0.324	7	NS
Pb	Protein	0.044	0.926	7	NS
Pb	Fat	-0.466	0.292	7	NS
Pb	Ash	-0.223	0.630	7	NS
Cd	Zn	-0.647	0.116	7	NS
Cd	Ni	0.740	0.057	7	NS

Cd	Protein	0.278	0.546	7	NS
Cd	Fat	-0.444	0.319	7	NS
Cd	Ash	0.241	0.602	7	NS
Zn	Ni	-0.823*	0.023	7	*
Zn	Protein	-0.093	0.843	7	NS
Zn	Fat	0.177	0.704	7	NS
Zn	Ash	-0.310	0.499	7	NS
Ni	Protein	0.590	0.163	7	NS
Ni	Fat	-0.529	0.222	7	NS
Ni	Ash	0.493	0.261	7	NS
Protein	Fat	-0.679	0.093	7	NS
Protein	Ash	0.397	0.378	7	NS
Fat	Ash	-0.467	0.291	7	NS

Table F3. Frozen fish: pairwise Pearson correlations among heavy metals and proximate-composition

variables					
Variable 1	Variable 2	Pearson r	P-value	(2- N	Significance
			tailed)		
Pb	Cd	0.232	0.617	7	NS
Pb	Zn	0.609	0.146	7	NS
Pb	Ni	-0.334	0.464	7	NS
Pb	Protein	-0.514	0.238	7	NS
Pb	Fat	0.282	0.540	7	NS
Pb	Ash	-0.605	0.150	7	NS
Cd	Zn	0.251	0.587	7	NS

Cd	Ni	-0.031	0.948	7	NS
Cd	Protein	0.139	0.766	7	NS
Cd	Fat	-0.052	0.912	7	NS
Variable 1	Variable 2	Pearson r	P-value (2-tailed)	(2-N)	Significance
Cd	Ash	0.173	0.711	7	NS
Zn	Ni	0.236	0.611	7	NS
Zn	Protein	-0.390	0.387	7	NS
Zn	Fat	0.072	0.878	7	NS
Zn	Ash	-0.015	0.974	7	NS
Ni	Protein	0.438	0.325	7	NS
Ni	Fat	-0.472	0.285	7	NS
Ni	Ash	0.745	0.055	7	NS
Protein	Fat	-0.911**	0.004	7	**
Protein	Ash	0.721	0.068	7	NS
Fat	Ash	-0.716	0.070	7	NS

Appendix G
Fish Samples and Experimental Materials



Figure G1: Canned tuna fish sample used in the study.



Figure G2. Fresh whole fish sample of sardine used in the present study.



Figure G3: Sea Bream (*Sparus aurata*)



Figure G4 : Red-bellied Pacu (*Piaractus brachipomus*)



Figure G5 : Common Carp (*Cyprinus carpio*)



Figure G6: Salmon (*Salmo salar*)



Figure G7 : Nile Tilapia (*Oreochromis niloticus*)



Figure G8 : Golden Pompano (*Trachinotus blochii*)



Figure G9: Mackerel (*Scomber scombrus*)



Figure G10: Prepared extracts used for oxidative stress biomarker analysis.



Figure G11: Collection and labeling of fish samples before laboratory analysis

الخلاصة



الملخص:

يجب أن تستوفي المنتجات السمكية المستوردة معايير السلامة والجودة لحماية الصحة العامة في العراق. تهدف الدراسة الحالية إلى تقديم تقييم شامل للحالة الغذائية والسّمية لـ 39 نوعاً مختلفاً من الأسماك المستوردة المتداولة في الأسواق العراقية في محافظات بابل والنجف والديوانية، وشملت الأنواع المعلبة (التونة والسردين) والأنواع المجمدة (البطي، والباكو أحمر البطن، والكارب الشائع، والسلمون، والبلطي النيلي، والبومبانو الذهبي، والماكريل). وتم قياس تراكيز المعادن الثقيلة (Pb, Cd, Zn, Ni) في 117 عينة تجارية باستخدام تقنية مطيافية الانبعاث البصري بالبلازما المقترنة حثياً (ICP-OES)، وبثلاثة مكررات تقنية. كما أُجري التحليل التقريبي لمحتويات البروتين والدهون والرماد، فضلاً عن تقييم مؤشرات الإجهاد التأكسدي (MDA, SOD). وتم إجراء تحليلات إحصائية متعددة المتغيرات، شملت تحليل المكونات الرئيسية (PCA) ومعامل ارتباط بيرسون.

أظهرت النتائج وجود تباين في تراكيز المعادن الثقيلة؛ إذ تراوحت تراكيز الرصاص بين 5.60 جزء بالليون (ppb) في التونة المعلبة و 131.20 جزء بالليون في السلمون المجمد، في حين بلغ الحد الأقصى للكاديوم 6.765 جزء بالليون. وأظهر التحليل الغذائي أن الأسماك المجمدة تمتلك محتوى بروتينياً أعلى وأكثر استقراراً، إذ بلغ $25.8 \pm 0.6\%$ مقارنةً بالمنتجات المعلبة التي بلغت $21.5 \pm 1.2\%$. كما تأثر محتوى الدهون بطريقة الحفظ ووسط التعبئة، بينما تراوح محتوى الرماد بين $2.4-2.5\%$ وارتبط البروتين ارتباطاً سلبياً مع الدهون ($r = -0.659$, $P < 0.01$)، مما يعكس التغيرات التركيبية والفسيولوجية المرتبطة بعمليات التصنيع والتخزين.

كما أظهرت النتائج وجود فروق معنوية في مؤشرات الإجهاد التأكسدي بين الأنواع المختلفة وطرائق الحفظ. وتراوح MDA بين 15.2–74.7 نانومول/غم، بينما تراوح SOD بين 214.8–344.0 وحدة/غم في التونة المعلبة، وبين 17.8–68.9 نانومول/غم و 212.8–418.8 وحدة/غم في السردين المقلب، في حين تراوح في الأسماك المجمدة بين 22.5–80.9 نانومول/غم و 208.5–275.4 وحدة/غم، مع وجود فروق معنوية عالية ($P < 0.001$).

وأظهر تحليل المكونات الرئيسية (PCA) تجميع العينات في ثلاث مجموعات؛ تميزت المجموعة A بارتفاع محتوى الدهون ومستويات متوسطة من المعادن، وضمت المنتجات المعلبة. أما المجموعة B فتميزت بارتفاع محتوى الزنك والنيكل، وضمت الأنواع القاعية وأسماك المياه العذبة، مثل الكارب الشائع والبلطي النيلي والباكو أحمر البطن. في حين تميزت المجموعة C بارتفاع محتوى البروتين وتراكم الرصاص والكاديوم، وضمت الأنواع البحرية المفترسة، مثل الدنيس والماكريل. وأظهرت النتائج أن تراكم المعادن الثقيلة يرتبط بالموطن البيئي والنمط الغذائي والمستوى الغذائي للنوع السمكي، فضلاً عن طريقة الحفظ، في حين تتأثر الجودة الغذائية والاستقرار التأكسدي بخصائص النوع السمكي، والمعالجة الحرارية، وظروف التخزين. وتؤكد الدراسة أهمية الجمع بين تقييم المعادن الثقيلة والمؤشرات الغذائية ومؤشرات الإجهاد التأكسدي عند إجراء تقييم شامل لسلامة الأسماك المستوردة.

وتوصي الدراسة بضرورة مراقبة بعض الأنواع السمكية بصورة دورية، وتقييم مواد التعبئة والتغليف، وتطبيق نماذج متعددة المتغيرات لتقييم المخاطر؛ وذلك بهدف حماية الصحة العامة والحفاظ على جودة سلسلة تجهيز الأسماك المستوردة في العراق.



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تقييم السلامة الغذائية للأسماك المستوردة المتداولة في الأسواق
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ICP-OES
لتحديد تراكيز المعادن الثقيلة ودراسة المؤشرات الكيميائية الحيوية

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درجة الماجستير في علوم البيئة

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