Instrumental Detection Of Microplastics In Fish And Their Harmful Effects On Human Health

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Abstract
Microplastics, defined as plastic particles smaller than 5 mm, pose serious environmental and human health risks. These tiny plastics originate from primary sources, such as cosmetics and industrial products, or secondary sources, where larger plastics degrade due to environmental factors. Microplastics are difficult to remove from the environment and are often ingested by marine organisms, leading to ecological and health consequences. Their ingestion can cause injuries, metabolic disruptions, and immune or neurotoxic effects in marine life and humans through the food chain. Detection methods include microscopy (SEM), spectroscopy (FT-IR, ATR), and thermal analysis (TGA, Py-GC-MS). Density separation and filtration techniques help extract microplastics, while tissue digestion methods using chemicals like KOH and H_2O_2 isolate them from biological samples. These pollutants can act as carriers for harmful chemicals and microbes, increasing their toxicity. Human exposure through seafood consumption varies by region, raising concerns about long term health effects, including oxidative stress, metabolic disorders, and inflammation.

Key Words – Fish, Microplastics, μFTIR, Scanning Electron Microscopy (SEM) etc.

Introduction

Microplastics mean plastic with a length or diameter of less than 5mm (1). It can reduce the health and productivity of marine ecosystems because it is so fine that it is difficult to collect and it can be mistaken and eaten by marine organisms, causing human damage through the consumption of fish (2). Plastic pollution is a global problem that continues to threaten human health and the environment. Until now, the management of plastic waste has focused mainly on large size plastic materials. Plastic containers discharged from domestic households are collected and used for recycling, but most of them are large plastic products visible to the naked eye. However, poorly collected plastics have been exposed to the environment for a long time and are decomposed into many microplastics by photolysis by ultraviolet light or by physical stimulus (3). The problem of environmental pollution caused by such small broken microplastics is socially increased seriously. In addition, microplastics from personal hygiene products, cosmetics, and tire wear are causing more serious environmental pollution (4). Microplastics, along with the flow of wind and sewage, pollute faraway seas, causing serious environmental risks (5).

Microplastics

Microplastics are so small that they are dumped into the ocean without being filtered out of sewage treatment facilities and marine organisms are misunderstood by marine organisms as food containing adsorbable or additive contaminants, causing marine ecological disturbances. The easier it is to be ingested, the smaller the microplastics. Ingestion of microplastics has been reported to cause physical injuries to marine organisms, changes in eating behavior, growth and decreased fertility. Microplastics can be consumed at all levels of nutrition through the food chain. In other words, fish and shellfish intake can eventually reach the human body. (6.)
Examples of different particles classifications are labeled

1. Primary microplastics.
2. Secondary microplastics.

Plastics can be carved into small pieces by environmental factors, and can be changed to numerous microplastics (7). Microplastics mean less than 5 mm of plastic and can be classified into primary microplastics and secondary microplastics according to the expected source. Primary microplastics are intentionally small plastics from the time of production, and have been used in cosmetics, industrial abrasives, toothpaste, cleaning products, detergents, whole body exfoliants, face washes, and tooth pastes for decades. It also includes resin pellets, which are used as raw materials for

the production of various kinds of plastic products. Secondary microplastics are polymers that are purpose fully or naturally micronized during the use, consumption, and disposal of plastics. Primary microplastics are plastics that are larger when they are manufactured. Not only physical pressures but also photochemical processes, such as light, can produce secondary microplastics (8).

Primary Microplastics

Primary microplastics are plastics that are released directly into the environment in the form of small particulates. Primary microplastics are manufactured as micro beads, capsules, fibers or pellets. Examples include raw resin pellets used in the production of plastic, industrial scrubbers used for abrasive blast cleaning, microfibers used in textiles, and microbeads used in cosmetics and personal care goods. A plastic particle used as a raw material for household goods (drugs, detergents, cleansers) or emitted from tire wear, synthetic fiber laundry and so on.

Table -1 Classification of Plastic Debris is mainly According to their Sizes.

Plastic debris classifications	Diameter sizes	References
Classification 1.		
Micro-debris	< 5mm	Carpenter and Smith, (1972); Colton et al. (1974); Arthur et al. (2009); Thompson et al. (2004). Claessens et al. (2011); Galgani et al. (2015); JRCEC, (2013); Thompson (2015).
Meso-debris	5 – 20mm	
Macro-debris	> 20mm	
Mega-debris	> 100mm	
Classification 2		
Microplastics	1 – 5mm	Cole et al. (2011); Galgani et al. (2015); Gigault et al. (2018).
Macroplastics	5 – 10 mm	
Megaplastics	10 – 20mm	
Plastics	>20mm	
Classification 3		
Nanoplastics	1 – 1000 nm and < 20 (μm)	
Small microplastics	20 μm– 1mm	
Large microplastics	1 – 5mm	
Mesoplastics	5 – 25mm	
Macroplastics	> 25mm	

Secondary Microplastics

Large plastic (pat bottles, plastic bags, fishing nets) have been exposed to the marine environment and have been carved into small pieces. Secondary microplastics which are formed by larger plastic are decomposed. Plastic is broken down into millions of tiny fragments by ultraviolet radiation, wind, and wave action. A good example of secondary microplastics is a piece of polystyrene (PS) that breaks to the fish and chip boxes at the beach. If they are not properly disposed of, these plastic chips end up in the ocean. It was also analyzed that human intake 37 microplastics per year from the edible salt, which did not have a fatal effect on the human body, but it was concluded that accumulation of microplastics products from marine ecosystems could gradually increase and threaten human health.

Human Health Risks Associated with the Ingestion of Microplastics

Although microplastics pollution is not a recent phenomenon, identifying the risks connected to microplastics is a growing issue. Risk is typically defined as the result of multiplying the hazard that an agent represents by the duration of exposure. Microplastics offer risks due to their physical properties (such as size, shape, and surface), purposefully added additives used in the plastic production process, environmental resilience, and capacity to absorb infections and chemical pollutants and concentrate them along the food chain (9). The human diet is known to contain significant amounts of microplastics from fishery products. Therefore, if they are polluted, eating them could be harmful to human health (10, 11). The intake of microplastics, based on the consumption of three species of fish that are caught and frequently consumed (Dicentrachus labrax, Trachurus, and Scomber colias), varies from 112 to 842 particles per year according to the EUMOFA (European Market Observatory for Fisheries and Aquaculture Products) and NOAA.(12) Furthermore, it is important to take into account that consumption of bivalves and the level of microplastics pollution varied significantly from one nation to the next (13-17), resulting in varying annual levels of microplastics ingestion in various nations. The amount of microplastics to which individuals are really exposed, which serves as a crucial measure for measuring the consequences on human health, cannot, however, be determined from the information we now have(18).

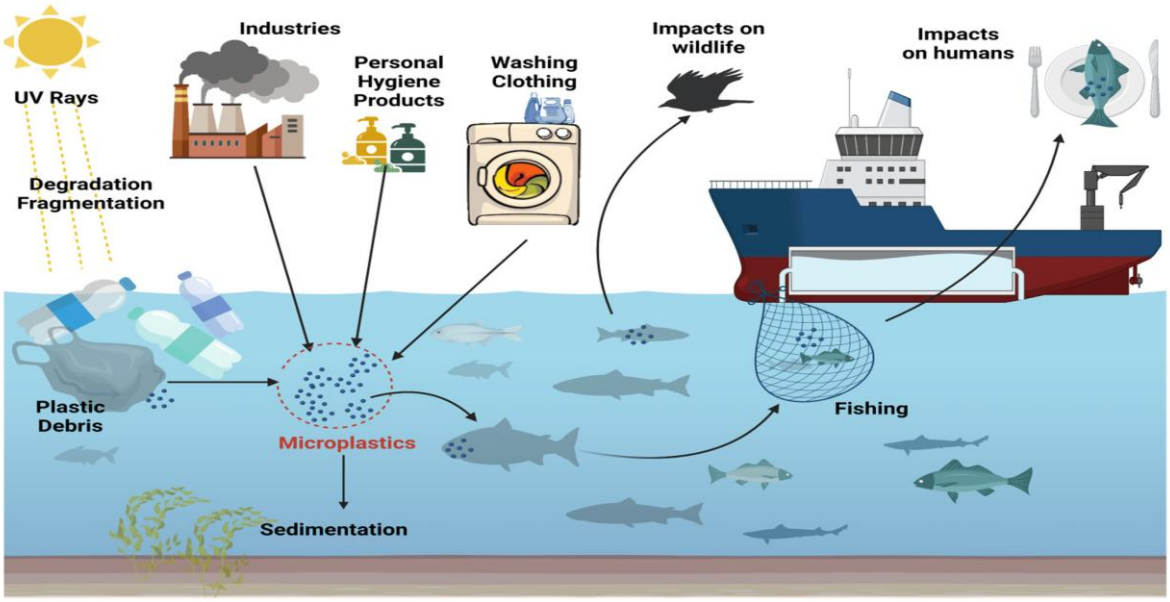

Figure-1 shows the Microplastic Pathway from the Aquatic Ecosystem to the Human Body.

Toxicity of Microplastics

Depending on the level of exposure and the individual's vulnerability, microplastics are thought to be potentially damaging to organisms. They can, in fact, lead to cytotoxicity, oxidative stress, and translocation to other tissues. However, microplastics can cause chronic flogosis, cell proliferation, necrosis, and immune cell damage in creatures exposed to them over an extended period of time. Microplastics can also act as carriers for microbes and release substances (both organic and inorganic) found in their matrix or previously absorbed by the environment (19).

Inflammation and Cytotoxicity

Reactive oxygen species (ROS) produced during the inflammatory response and oxidizing species that were previously adsorbed on the surfaces of microplastics can both lead to oxidative stress (20). They also contain ROS, which are byproducts of the production and polymerization of plastics (21). Zebrafish (Danio rerio) have shown signs of oxidative damage following microplastic exposure (22, 24). However, it has been noted that polypropylene (PP) prosthesis in humans cause an inflammatory reaction and the release of ROS, which may result in rejection (25). Particle toxicity, oxidative stress, and inflammation all contribute to cytotoxicity. Numerous investigations have demonstrated that contact with microplastics may have harmful effects.

Disorders of the Metabolism and Energy Balance

Microplastics may affect human metabolism in a way that is comparable to what has been seen in marine organisms. They can modify metabolic enzymes, alter nutritional intake, and alter energy expenditure. However, given the low exposure concentrations, higher energy needs, and more sophisticated human metabolism compared to the creatures examined, the discovery of these effects in humans may be constrained. Microplastics Translocation to the Circulatory System and Remote Tissues Microplastics can act locally in the colon after exposure or spread to other organs via the circulatory system. Once inside blood vessels, microplastics can lead to pulmonary hypertension, vascular occlusions, blood cell Cytotoxicity by internalisation, and a generalised inflammatory response. Microplastics can also lead to persistent inflammation, poor organ function, and an elevated risk of cancer when they are absorbed into distant tissues (26, 27).

Immune System Disorders

Depending on their dispersion and the host response, microplastics might cause local or systemic immunological reactions after exposure. Particularly in those who are genetically predisposed, mere environmental exposure to microplastics is enough to impair immune function and result in autoimmune disorders or immune suppression. Additionally, some revealed that mussels exposed to microplastics showed immune suppression and immune response regulation.

Neurotoxicity

Neuronal behaviour and function may be impacted by microplastics. Particularly, it has been noted that these particles can block acetylcholinesterase, create oxidative stress with higher levels of lipid peroxidation, and induce anaerobic energy production pathways in the brain of sea bass (Dicentrarchus labrax) (28). Swimming ability, which is used as a behavioural indicator, also suffered from the same species' negative effects. However, very little is now known regarding the potential role of microplastics in human neurotoxicity (29).

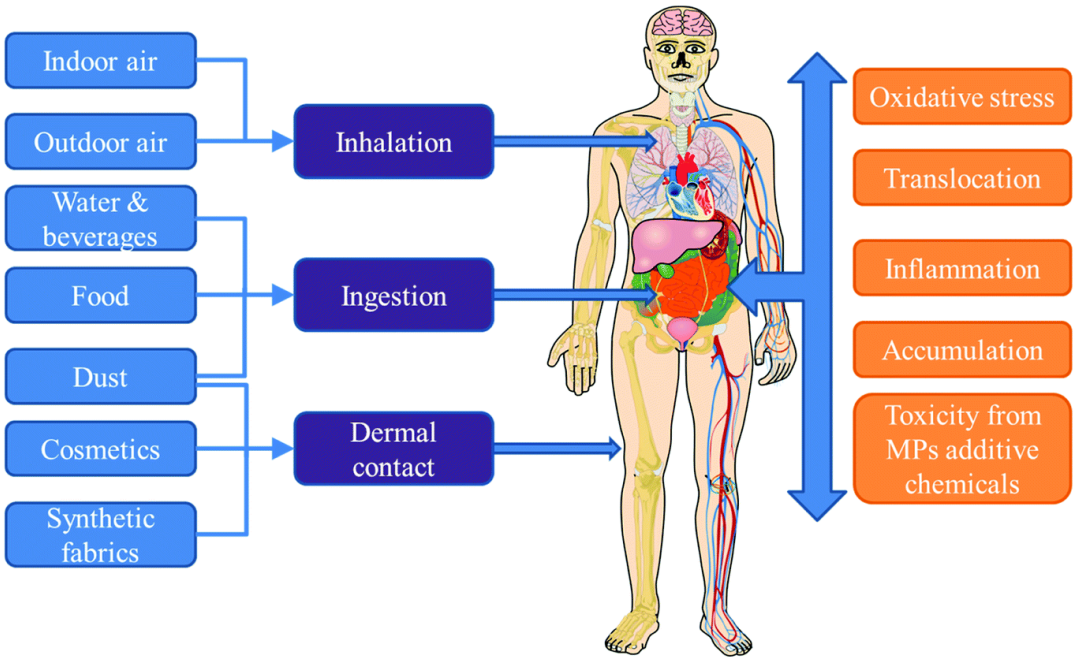

Figure 2 shows the Entry of Microplastics into the Human Body and its Toxic Effects.

Microplastics Detection Methods

Scientific techniques are required to quantify and distinguish microplastics in order to more thoroughly understand the effects of microplastics on the ecosystem. Microplastics are heterogeneously mixed in size, shape, color, specific gravity, and chemical components and require analysis techniques to separate and distinguish plastic components (30). Monitoring microplastics in various bio environments can provide fundamental scientific data to identify contamination levels, gradients, and degrees of exposure of organisms to plastics. In microbiological testing or sampling of the environment, the analysis process for microplastics consists of extraction, separation, identification, quantification, and classification. It is very difficult to distinguish microplastics using one analysis method because microplastics are composed of various sizes, shapes, and polymer types. Thus, it analyzes microplastics by mixing two or more types of analytical techniques. In general, microplastics analysis uses a microscopy method to observe the structural, physical, and morphologic properties of a surface and uses a spectroscopy method to observe the chemical components of the microplastics (31).

Microscopy Methods

Structural properties and morphology studies of microplastics have been analyzed using optical microscopes based on color, size, and shape. Microscopy is used to analyze microplastics of various sizes ranging from hundreds of microns to tens of nanometers in size. Scanning electron microscopy (SEM) can provide very sharp and high magnification images of materials such as plastics. It is very useful for distinguishing microplastics from common organic matter through high resolution images of particle surfaces. By using energy-dispersive X-ray spectroscopy (EDS) together, the elemental composition of the same material can be determined (32). To improve the mechanical and thermal properties of ordinary microplastics, organic additives are combined with them. More recently, inorganic additives have also been introduced to the process to enhance flame retardancy, antioxidant, process ability, and optical properties. In addition to distinguishing polymers in waste plastics, pyrolysis gas chromatography mass spectrometry (Pys-GC/MS) is used in the microscope to identify organic and inorganic additives, increasing the accuracy of component analysis. Through Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS), it is possible to measure the content of the fine plastic particles together with the shape and inorganic type. Using the Pys-GC/MS method, inorganic (TiO2) nanoparticles were analysed in addition to the organic chemicals (diethyl phthalate, benzaldehyde, and 2,4-di-tert-butylphenol) found in the microplastics. (33). Recently, a novel optical microscope technique called a hyperspectral imaging system (HIS) has been employed to separate and analyse microplastics. The HIS method has a narrow spectrum composed of tens to hundreds of spatial pixels from visible light to infrared light. As a result, it is very effective in analyzing chemical components of individual spatial pixels according to spectral information (34).

Spectroscopy Methods

Quantitative analysis of chemical properties and composition of microplastics has been studied using spectroscopy. By measuring the absorbance according to the wavelength by irradiating the sample with infrared rays and then determining the location and intensity of the absorption band, the Fourier transform infrared (FT-IR) method can analyse chemical bonding of substances both qualitatively and quantitatively. Carbon based polymers can be easily analyzed by FT-IR and are useful for identifying organic and inorganic materials from plastics because the corresponding spectra is generated according to the composition of the bond (35). When the attenuated total reflection (ATR) mode is used, a

stable absorption wavelength region can be obtained even in microplastics with irregular surfaces, unlike the reflection mode (36). In addition, micro FT-IR is used to identify and distinguish irregularly shaped microplastics by directly implementing images. Optical microscopes have the advantage of being able to distinguish microplastics faster and easier than FT-IR. However, when using an optical microscope, it is difficult to accurately distinguish non-plastics from plastics when the sample size is 1 mm or less, so it is possible to misinterpret the concentration and type of microplastics. Spectroscopy using FT-IR enables accurate measurement of microplastics as small as 50 micro millimeters or less, enabling analysis of the type and concentration of polymers contained in plastics (37). Fourier transform micro-infrared spectroscopy (μFT-IR) and environmental scanning electron microscopy-EDS (ESEM-EDS) enable the chemical mapping of microplastics surfaces, resulting in particle composition (main atomic composition of polymer) and morphology (38).

Thermal Analysis

Thermal analysis is used to distinguish microplastics by measuring changes in the physical and chemical properties of a polymer according to the thermal stability of a material. Differential scanning calorimeter (DSC) measures the difference in heat flow from a sample by applying the same temperature program to a sample and an inert reference in a furnace to measure the amount of energy. Qualitative characteristics of the sample can be confirmed through information proceeding. Thermo gravimetric analysis (TGA) measures the change in mass of a sample as a function of time or temperature by applying a temperature program to the sample. Majewsky researchers used TGA-DSC to analyze the mass and concentration of polymers by distinguishing seven different plastics from microplastic samples taken from the ocean (39). TGA is used in conjunction with thermal desorption gas chromatography mass spectrometry (TDS-GC-MS) to provide very useful information for the detection of PE in microplastics. Py-GC-MS measures the mass of a sample that is approximately 200 times higher than TDS-GCMS of an advantage in that it can accurately measure a large amount of non-uniform microplastic Additionally, because the TDS-GC-MS approach makes qualitative and quantitative analysis of microplastic samples collected in a variety of contexts rapid and simple, the PE present in the sample PE, PP, PS can be precisely separated and screened (40).

Density Separation and Filtration

Density separation was performed using zinc chloride solution ($ZnCl_2$) with a density of 1.7 g cm^3 to remove inorganic debris and allow extraction of the heavier polymers (41). Every time, a fresh ZnCl2 solution was made and filtered through 0.7 m glass microfiber filters before usage. At the end of the last digestion cycle and after filtration through a 38 μm sieve, samples were rinsed three times with $ZnCl_2$ instead of ultrapure water, to prevent a change in density. Samples were first poured into small beakers and then into 100 ml glass separation funnels previously rinsed three times with $ZnCl_2$, kept closed with lids and left to settle for a minimum of 15 h, after which 2/3 of the solution was drained out through the valve(42). To remove the percentage greater than 100 m, the residual solution was poured onto a sieve stack with a 100 m sieve on top and a 38 m sieve below. To make sure that all of the particles were moved from the separation funnels to the sieves, the separation funnels were washed three times with ultrapure water. The samples obtained on the 38 m sieve were then placed into tiny glass beakers after being thoroughly cleaned with a lot of ultrapure water to remove the $ZnCl_2$. $ZnCl_2$ residues were occasionally still visible in solution, though. Two drops of HCl acid were added to the sample with a glass pipette to dissolve any remaining salts in order to prevent any interference with the spectrum collection. For the ensuing FT-IR analysis, the samples were immediately vacuum-filtered using a 13 mm glass filter holder equipped with a 25 mm silver membrane filter. Following filtration, the silver filters were dried for more than 15 hours in a 50 °C oven before being placed in small Petri dishes and kept in the dark.

Tissue Digestion

When using the dissection method, undesired organic debris and contaminants on microplastics are a problem that can be solved using a variety of chemical treatments. Alkaline, oxidative, acidic, or enzymatic substances can be found in the digestive tract. By first digesting the entire mussel or fish individual or target organ, these drugs frequently allow the dissection phase to be skipped. Animal tissues can be broken down with potassium hydroxide (KOH) and other alkaline substances by hydrolyzing and denaturing proteins. Several marine biota have been observed to digest well when exposed to KOH at a 10% concentration (43-45). In comparison to other digesting chemicals including sodium hydroxide (NaOH), nitric acid (HNO_3), and hydrochloric acid (HCl) (46, 47). KOH's harm to microplastics is rather low. In KOH, digestion typically takes days to weeks, although it can be sped up at high temperatures. Examined and advised the use of KOH at 40 °C, however they found that increasing the temperature to 60 °C resulted in a lower particle recovery rate of polyethylene terephthalate, whose surface damage was visible. In order to recover microplastics from mussels and fish, hydrogen peroxide (H_2O_2) is a frequently employed oxidising agent. At room temperature, tissue digestion in 30% H_2O_2 can be finished in less than a week. However, H_2O_2 and biomass can react vigorously and produce foam, which could result in sample loss (48, 49). Thus, it is crucial to regularly monitor the development of foam while utilising H_2O_2 for tissue digestion. However, H_2O_2 can be a useful addition to enhance the ability of alkaline chemicals like KOH to extract microplastics from samples of marine biological systems. For the soft tissue of oysters, a mixed solution of 30% H_2O_2 and 10% KOH (1:9) has been suggested (50). For a variety of reasons, including the removal of microplastics from marine species (51, 52), HNO_3 is a characteristic acid utilised in tissue digestion. In comparison to other digestive chemicals like HCl, NaOH, and H_2O_2, complete digestion of mussel soft tissue in HNO3

can be accomplished in less than 24 hours. The tissue digesting process has been accelerated to less than two hours by heating at temperatures above 80°C (53, 55). Although HNO_3 is undoubtedly effective at breaking down tissues, care should be taken to prevent damage to microplastics because acidic solutions have the potential to cause deformation, coloration, or dissolution of materials like nylon and polypropylene Proteinase K and lipase, among other enzymes, can be utilised as a biochemical strategy to degrade biomass and speed up the extraction of microplastics (56)..

Conclusion

Microplastics, defined as plastic particles smaller than 5mm, pose a significant environmental and human health threat. These particles originate from both primary sources (such as microbeads in personal care products and synthetic fibers from textiles) and secondary sources (degraded larger plastics exposed to environmental factors). Due to their minute size, microplastics evade filtration systems and accumulate in marine ecosystems, leading to severe ecological disruptions. Marine organisms ingest these particles, mistaking them for food, which in turn introduces microplastics into the human food chain through seafood consumption. The impact of microplastics on human health is an emerging concern. Their potential to cause oxidative stress, cytotoxicity, immune suppression, metabolic disorders, and even neurotoxicity. Their ability to absorb and transport pollutants further amplifies their risk, making them vectors for hazardous chemicals and microbial pathogens. While studies confirm microplastics' presence in human consumed seafood, the long term health effects remain uncertain due to varying exposure levels and differences in metabolism across species. To better understand and mitigate microplastic pollution, various detection and analytical techniques have been developed. Microscopy methods, including scanning electron microscopy (SEM) and hyperspectral imaging, help in structural identification, while spectroscopy methods such as Fourier transform infrared (FT-IR) enable precise chemical analysis. Thermal analysis techniques, including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), assist in characterizing plastic polymers. Additionally, density separation and tissue digestion methods help isolate microplastics from complex environmental and biological samples. Overall, microplastic pollution is a growing global issue that requires urgent action. Enhanced waste management, reduction in plastic usage, and improved regulatory frameworks are necessary to mitigate their impact. Focus on developing efficient removal technologies and understanding long term health risks associated with microplastic exposure.

Abbreviation

PE	**-**	**Polyethylene**
PS	**-**	**Polystyrene**
PP	**-**	**Polypropylene**
ROS	**-**	**Reactive Oxygen Species**
SEM	**-**	**Scanning Electron Microscopy**
EDS	**-**	**Energy Dispersive X- Ray Spectroscopy**
Pry-GC/MS	**-**	**Pyrolysis Gas Chromatic Mass Spectroscopy**
HIS	**-**	**Hyper spectral Imaging System**
FTIR	**-**	**Fourier Transform Infrared**
ATR	**-**	**Attenuated total Reflection**
TAG	**-**	**Thermo Gravimetric Analysis**
TDS	**-**	**Thermal Desorption**
EUMOFA	**-**	**European Market Observatory for Fisheries and Aquaculture Products**
NOAA	**-**	**National Oceanic and Atmospheric Administration**

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