NATURAL SCIENCE AND MATHEMATICS IN THEORY AND PRACTICE

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TABLE OF CONTENTS

Chapter 1

Emergence of Fluorescent and Colorimetric Probes in Food Safety Assessments

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Ensuring food safety is vital, as food is one of the most basic physiological needs of humans. It is essential that food reaches consumers without any loss of nutritional value and without posing physical, chemical, or biological risks. To address these risks, necessary precautions are taken at every stage, including cultivating safe food products, preparing them for domestic and international markets, processing, consumption, and storage. These measures follow globally established guidelines set by various authorities (Fung et al., 2018; TKB, 2004). Numerous national and international organizations, such as the US Food and Drug Administration (FDA), World Health Organization (WHO), Food and Agriculture Organization (FAO), and European Food Safety Authority (EFSA), prioritize food safety and enforce stringent regulations (Alshannaq & Yu, 2017).

Food is recognized as one of the areas most vulnerable to the intentional or accidental use of toxic agents for various purposes (Karatepe & Ekerbiçer, 2018). Environmental conditions, the rapid growth of the global population, irregular dietary habits, and other factors can also pose significant threats to food safety and increase associated risks (Fung et al., 2018).

1. Sources of Food Safety Threats

Threats to food safety can be broadly classified into two categories: biological and chemical origins. Among these, biological risks represent the most significant and dangerous contributors to foodborne spoilage. Pathogenic bacteria—such as *Clostridium botulinum*, *E. coli*, *Salmonella*, *Listeria monocytogenes*, and *Bacillus cereus*—can be transmitted through raw/processed food products, human or animal feces, dust, or soil (Shaw, 2018). Additionally, yeasts and molds commonly found in foods like cereals, cheese, and bread, as well as toxic components naturally present in food and controversial genetically modified organisms (GMOs), also pose serious threats to food safety. Some of these microorganisms are pathogenic and can cause food poisoning. The primary sources of microbial contamination include airborne particles, food packaging, animal feed, and the devices and materials used during food production (Vaillant et al., 2005).

Another significant risk factor that can contaminate food at any stage before it reaches the table is chemical contamination. Chemical contaminants, which may contribute to chronic diseases, can either occur naturally within the food's original structure or transfer from packaging materials used during production. Examples include non-food-grade chemical additives such as colorants, emulsifiers, thickeners, flavor enhancers, preservatives, and pesticide residues, all of which have been detected in food products. (*Food Additives*, 2002; Martins et al., 2021; Musarurwa et al., 2019).

1.1. Heavy Metals: A Major Concern for Food Safety

Heavy metals pose a critical problem due to their persistence, non-biodegradability, and tendency to accumulate in biological systems, warranting special attention. While some heavy metals, like copper (Cu), are essential nutrients—acting as cofactors for various enzymes—elevated levels of copper can lead to severe health issues. This is particularly critical for individuals with Wilson disease, where proper regulation of dietary and environmental copper intake is necessary to prevent harmful effects. In agriculture, Bordeaux mixture (a combination of copper sulfate and calcium hydroxide) is still widely used by wine growers as an effective insecticide and fungicide (Shaw, 2018). As a result, screening wine samples for copper contamination is considered essential, especially in industrial wine production, to ensure safety and quality of the end product. The sources of heavy metals may include volcanic eruptions (e.g., cadmium, Cd), catalytic converters of motor vehicles (e.g., palladium, Pd), and improper industrial waste management. These sources can lead to the contamination of agricultural crops grown in affected areas with toxic metals, posing significant risks to food safety and human health. For example, when heavy metal concentrations in fish tissues exceed the maximum permissible limits, they can pose serious health risks to humans. Metals such as Cd, chromium (Cr), mercury (Hg), and lead (Pb) can cause serious damage to the nervous system, kidneys, and liver. Consequently, elevated levels of heavy metals in fish or other foods can have severe adverse effects on human health (Habib et al., 2024). In certain food samples, the levels of heavy metals such as Pb, Cd, arsenic (As), Hg, and Cu have been reported to exceed those found in average food samples (Hassan et al., 2024). A very recent study conducted in Turkey examined heavy metal concentrations in various tissues of two fish species, *Squalius pursakensis* and *Cyprinus carpio* (Kaçar, 2024). High concentrations of as much as 10 heavy metals—Cu, iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn), As, Cd, cobalt (Co), Cr, and Pb—were detected in the muscle, gills, and liver of both fish species.

1.2. Inorganic anions

The safety of our food is also threatened by several inorganic anions, including (bi)sulfites (HSO_3^-/SO_3^{2-}), nitrites (NO_2^-), nitrates (NO_3^-), cyanides (CN^-), and others (Duan et al., 2021). Sulfur dioxide, the gaseous form of aqueous bisulfite solutions, is widely used in the wine industry. It is both naturally produced by fermentation yeasts during wine production and externally added to enhance and control the aroma, taste, and color of wines. The dried fruit industry, particularly for products like apricots and figs, also heavily relies on sulfur dioxide or sulfites

to enhance food color and extend shelf life. Table sugar is another product where sulfites may be present. However, sulfites are toxic at high concentrations and can trigger severe reactions in asthmatic individuals, with extreme cases reportedly leading to death. As a result, the use of sulfites is strictly regulated by authorities and must be closely monitored to ensure safety (Işık et al., 2019).

The nitrite ion is a critical anion used to prevent microbial contamination in processed foods such as bacon, sausage, sucuk, and ham, particularly against the risk of Clostridium botulinum. However, nitrites also pose a potential threat to drinkable water resources, often stemming from nitrate fertilizers. Nitrate is converted to nitrite in the gut through bacterial reduction or in the soil via microbial processes. The nitrite thus formed can very efficiently react with dietary secondary amines to form potentially carcinogenic nitrosamines (Beard & Swager, 2021). Therefore, the presence of nitrate/nitrite pairs in foods should be carefully regulated and monitored to minimize health risks.

1.3. Neutral (In)Organic Compounds

The chemical contaminats can include both inorganic and organic neutral compounds, such as hydrazine (H_2NNH_2), hydrogen sulfide (H_2S), formaldehyde (HCOH) and benzenethiol (PhSH), among many others. These toxic substances should be closely monitored usually at parts per million (ppm) levels in food samples. Alongside chemical contaminants, biogenic amines such as putrescine and cadaverine can form as a result of microbial spoilage. To ensure consumer safety and accurately determine the expiry dates of packaged meat or fish products, the detection of these biogenic amines is crucial (Duan et al., 2021).

Additionally, poor personal hygiene practices among food handlers and preparers pose significant risks to both personal and public health. Simple measures, such as proper handwashing and the provision of adequate washing facilities, can effectively prevent many foodborne illnesses.

2.1. Traditional Analytical Techniques in Food Safety

Food safety is a critical global concern, with foodborne illnesses posing significant threats to public health and economic stability (Garcia et al., 2020; Todd, 2020). Chromatography remains one of the key analytical tools used to monitor contaminants and ensure compliance with safety standards in the food industry. Traditional techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), along with advanced versions like nano-LC, have long been regarded as the gold standard for detecting contaminants and ensuring food quality (Fedorenko & Bartkevics, 2023; L. Liu et al., 2020; Malik et al., 2010; Núñez & Lucci, 2020).

Traditional chromatography methods, such as GC, HPLC, UHPLC, nano-LC, and LC, have been pivotal in identifying and quantifying food contaminants, including pesticides, mycotoxins, and veterinary drug residues (Alcántara-Durán et al., 2018; He & Aga, 2019; Zhao et al., 2020). Among these, gas chromatography (GC) is widely used for analyzing volatile and semi-volatile compounds in food samples. Its popularity arises from its high resolution and sensitivity, which make it ideal for detecting pesticides and environmental contaminants (Lehotay & Hajšlová, 2002). It provides high sensitivity and resolution, enabling the detection of trace levels of contaminants. Additionally, its versatility allows for the analysis of various matrices, including liquids and gases. Coupling with mass spectrometry (GC-MS) further enhances its specificity and the accurate identification of compounds (Beale et al., 2018; Pico et al., 2020).

Similarly, HPLC is another predominant technique in food safety analysis, particularly effective for detecting non-volatile and thermally unstable compounds such as mycotoxins and pharmaceutical residues (Danezis et al., 2016). Recently, an HPLC-FLD method has been utilized for the simultaneous detection of multiple mycotoxins, including aflatoxins (AFs) and ochratoxin A (OTA), in various food products such as peanut butter, maize cereal products, ginseng, and ginger. (Chan et al., 2004); AFs, OTA, and ZEA in cereal grains, rice, and rye (Rahmani et al., 2010); AFs, OTA, ZEA, and DON in corn (Ofitserova et al., 2009). Among the advantages of HPLC are its wide application range for both polar and non-polar analytes, simplified sample preparation compared to GC—reducing time and labor—and compatibility with various detectors, such as UV-Vis and fluorescence, which enhances its analytical versatility (LaCourse & LaCourse, 2023; Swartz, 2010). However, despite their many advantages, these techniques also have notable disadvantages, including: 1) Complex sample preparation processes, which can be time-consuming and costly. 2) The need for skilled operators and significant maintenance to ensure optimal performance. 3) Lower resolution compared to GC for certain analytes, potentially leading to overlapping peaks. 4) High solvent consumption, which increases both operational costs and environmental impact. 5) Extensive method development requirements for analyzing complex matrices. These limitations highlight the need for ongoing innovation and optimization in analytical techniques for food safety. Due to these challenges, modern approaches to food safety have emerged in recent years, blending traditional practices with advancements in science and technology. These innovations aim to improve the prevention, detection, and response to foodborne illnesses more effectively (Ramírez-Coronel et al., 2024; Zhang, Yue, et al., 2023). This chapter explores

the principles, applications, and advantages of these techniques analyses in food safety, highlighting their roles in enhancing real-time monitoring and ensuring consumer protection.

2.2. Fluorescent and Colorimetric Probes in Food Safety

Fluorescent and colorimetric probes have emerged as transformative tools for detecting chemical contaminants, pathogens, and toxins in food (Das & Mishra, 2022; Yuan et al., 2023; Hua et al., 2021). While colorimetric probes, such as litmus paper and pH indicators, have been used for ready chemical analysis for over a century, fluorescent probes have gained significant attention only in the past half-century (Tsien, 1980). Their prominence has grown particularly at the turn of this millennium, largely due to their remarkable applications in biological contexts (de Silva et al., 1997). Despite their potential, it took some time for these analytical tools to gain widespread attention within the scientific community focused on food safety. A Web of Science analysis of the keywords "colorimetric probe and food safety" (Figure 1) and "fluorescent probe and food safety" (Figure 2) from 1990 to 2024 reveals that the potential of colorimetric and fluorescent probes was largely overlooked until the 2010s. However, an exponentially growing trend in their application becomes clearly noticeable after arround 2012.

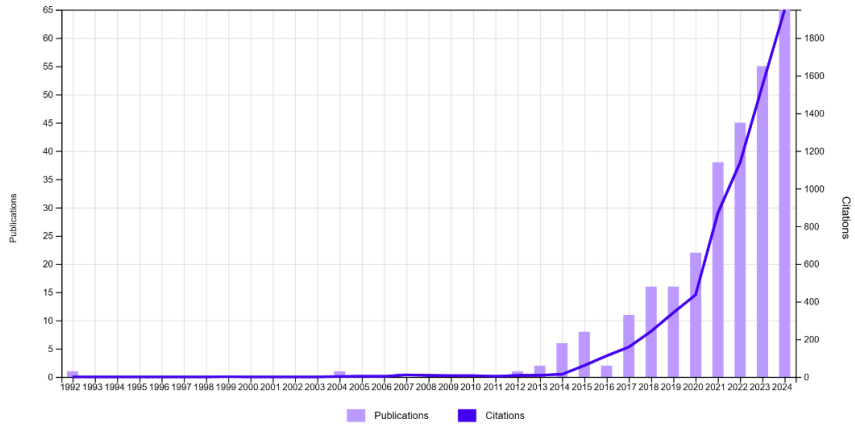

Figure 1. Web of Science analysis of the keywords "colorimetric probe and food safety" in topic search, illustrating the growing trend in research publications and citations over time.

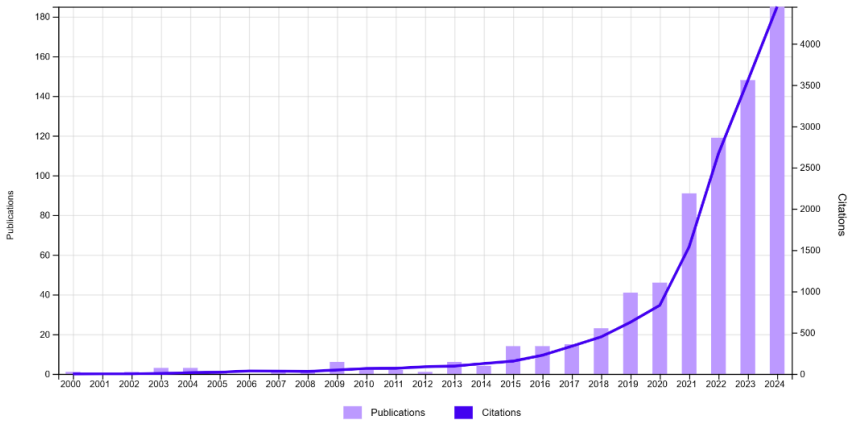

Figure 2. Web of Science analysis of the keywords "fluorescent probe and food safety" in topic search, illustrating the growing trend in research publications and citations over time.

Fluorescent probes operate by emitting fluorescence in response to either a chemical reaction or a non-covalent interaction, such as supramolecular assembly, with specific analytes of interest (Chang et al., 2012). Figure 3 illustrates the general working principle of sensing events using designer probes, employing either colorimetric or fluorescence-based detection mechanisms.

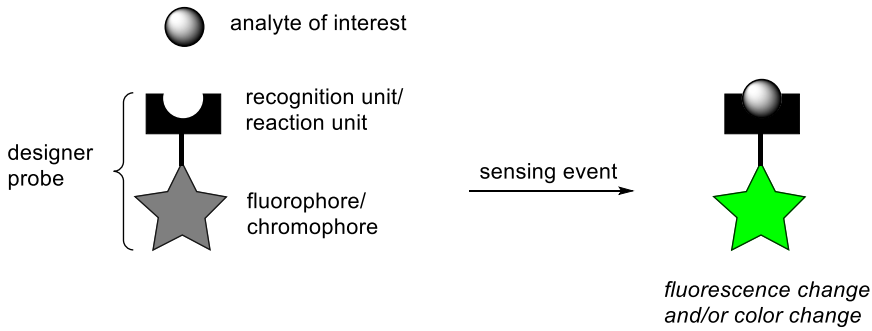

Figure 3. General working principle of fluorescent and colorimetric probes.

While the recognition event may involve a single reaction-based scenario (Isik et al., 2013), it can also include a combination of a reaction-based event and a non-covalent interaction, enabling more selective and discriminative sensing among potential competitors (Işık et al., 2014). Regardless of the specific mechanism, one essential principle remains: the presence of the analyte must induce electronic changes in the chromophore or fluorophore during the recognition process. These electronic changes, whether occurring directly on the chromophore/fluorophore or on an electronically decoupled part, result in distinct

electronic states in both the ground and excited states of the dye. This electronic reorganization is then detected using UV-vis absorption instruments or fluorescence spectrometers. The electron/energy transfer mechanisms that underlie typical sensing platforms commonly include, but are not limited to: 1) Photoinduced electron Transfer (PeT), 2) Fluorescence Resonance Energy Transfer (FRET), and 3) Internal Charge Transfer (ICT) (de Silva et al., 1997). These processes form the foundation of many sensing systems, facilitating precise and efficient detection of target analytes. The emission change in fluorescent probes can occur in an off-on, on-off, or ratiometric manner. In cases where the colorimetric reaction causes distinct color changes or the appearance/disappearance of colors, these probes can function as colorimetric sensors suitable for paper/test strips or badges, enabling naked-eye detection. This facilitates real-time, on-site detection with high specificity and sensitivity (Vinayaka & Thakur, 2010). The emission changes can be detected using fluorescence spectroscopy or imaging devices. The fluorophore or chromophore used in these probes may be a small organic molecule, quantum dots, nanoparticles or else. For example, quantum dot-based fluorescent probes have demonstrated promising applications in detecting heavy metals like lead and cadmium in food products (S.-Y. Chen et al., 2021; Lai et al., 2023). Similarly, fluorescent biosensors employing dual-readout probes based on red-emission carbon dots have been successfully used for nitrite detection in meat products (M. Yu et al., 2022).

Fluorescent probes are widely used to detect microbial contaminants like *E. coli* and *Salmonella* by targeting specific proteins or genetic markers (Bhardwaj et al., 2017; Zhang, Zhou, et al., 2023). Moreover they enable the rapid detection of harmful mycotoxins like aflatoxin in nuts, grains, and dairy products (Q. Chen et al., 2023; Sharma et al., 2018; Wu et al., 2019). Another area of application is detection heavy metals like lead and cadmium in beverages (Lai et al., 2023). The primary advantage of fluorescent probes lies in their rapid response time and ability to provide real-time results, making them ideal for use in point-of-care settings. Furthermore, advancements in portable fluorescence detection devices have expanded their applicability in remote or resource-limited regions. However, challenges such as the potential quenching of fluorescence in complex food matrices need to be addressed to enhance their accuracy and robustness (Chu et al., 2021).

Colorimetric analyses have gained attention due to their simplicity, low cost, and visual interpretability. These methods rely on visible color changes in the presence of specific analytes, enabling semi-quantitative and, in some cases, quantitative analysis without requiring sophisticated instrumentation. For

example, gold nanoparticles (AuNPs) have been widely applied in the detection of pesticides and antibiotics. Upon interaction with target analytes, these nanoparticles aggregate, leading to a color change that can be observed by the naked eye (Hua et al., 2021). Moreover, paper-based colorimetric sensors have emerged as a versatile platform for food safety applications. These sensors can be impregnated with reactive chemicals or enzymes that react with contaminants, producing distinct color changes. For instance, a study demonstrated the use of paper-based devices for detecting nitrite in processed meats (Guembe-García et al., 2022).

Organophosphate pesticides can be detected using enzyme-inhibition-based colorimetric assays. For example, acetylcholinesterase reacts with pesticides to produce a color change indicative of pesticide concentration (D.-M. Liu et al., 2021). Common adulterants like formalin in fish or starch in milk can be identified through colorimetric reactions (Nascimento et al., 2017; Rahman et al., 2023). These simple assays often involve inexpensive and readily available reagents. Gold nanoparticles functionalized with specific ligands are used to detect toxins. Upon interaction with the toxin, the nanoparticles aggregate, causing a visible color shift. Furthermore colorimetric sensors in smart packaging systems indicate pH changes and spoilage levels in perishable foods, like fresh meat and fish (Ahmed et al., 2018; Kuswandi et al., 2011).

3. Conclusion

Modern approaches, such as fluorescent probes and colorimetric analyses, are revolutionizing food safety by offering low-cost, rapid, accurate, and user-friendly alternatives to traditional methods. While these techniques have not yet fully replaced conventional approaches, they complement existing practices and show significant potential for widespread adoption in ensuring food quality and safety. In the context of fluorescent probes for food safety, the water solubility of probes deserves increasing attention, as most foods—particularly milk, beverages and drinkable water ilself—naturally contain water as a major component. By prioritizing water-soluble probes, the need for organic solvents can be bypassed, making these methods more practical and environmentally friendly. Additionally, considering the variety of chromophore/fluorophore units present in biological components, such as amino acids in proteins (e.g., tryptophan, tyrosine) and vitamins (e.g., retinol, riboflavin, pyridoxine), it is essential to shift the operating wavelengths to the red or near-infrared (NIR) region of the electromagnetic spectrum. This adjustment helps minimize interference from these naturally occurring substances. The integration of colorimetric patches into smart food packaging offers a promising solution for enhancing food safety in the

marketplace. Developing non-toxic, food-grade chromophores for detecting spoilage represents a significant advancement, not only in ensuring food safety but also in mitigating food waste by providing real-time spoilage indicators. Future research should focus on improving the stability, reproducibility, and multiplexing capabilities of these techniques. Moreover, integrating these technologies into portable devices, such as smartphones or handheld sensors, holds tremendous promise for revolutionizing food safety monitoring and making it more accessible.

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Chapter 2

Investigating the Role of Copula Functions for Different Associations

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1.Introduction:

One of the most fundamental topics that a researcher focuses on while conducting data analysis is the dependency structure within the data. The discovered dependency structure not only helps the researcher build an accurate model but also enables extracting valuable insights from the data. Linear, nonlinear, or multidimensional dependency structures can be observed within the data. Copula functions are among the primary methods that can be used to determine and measure the dependency structure statistically.

Copula functions are commonly applied in areas like finance, insurance, econometrics, health and biostatistics where modeling the relationships between variables is crucial for making predictions and decisions. Copulas allow us to model the marginal distributions and the dependence structure independently.

In investment data, relationships are observed in the tail parts, while in health data, a holistic positive dependency can be observed. On the other hand, researchers need to use different association models when working with two different vectors that are negatively related to each other. Spesific copula functions capture different association between the responses. The researcher should first identify the statistical relationships between the variables in the data, and then use models which are appropriate for the structure of the data.

This chapter aims to offer a helpful guide to practitioners interested in getting started with copula functions for dependence modeling. Defining the specific dependence relationships among random variables is emphasized through the chapter.

In this chapter, studies that use copula functions to model dependence structure are first categorized and summarized within their respective fields. In the second part, information is provided about the correlation coefficient and the rank correlation coefficients, which measure the dependence between random variables. In the third section, the definition and properties of the copula function are first explained. Subsequently, the attention is directed towards Archimedean copula functions, and information is provided about the types of dependencies that can be modeled using these functions. In the conclusion, the advantages of using bivariate Archimedean copula functions for different types of dependencies are discussed.

1.1 An Overview of the Applications of Copulas:

1.1.1. Finance:

Copulas are used to model the joint distribution of asset returns, portfolio risks, Value at Risk (VaR) and in simulating joint scenarios for stress testing financial systems. McNeil et al. (2015), authored a book on the quantitative risk management, focusing on the mathematical and statistical techniques used to assess and manage financial risk. They used the techniques for modeling how different assets or risks are interrelated, especially in terms of copulas.

1.1.2. Insurance:

Risks are often not independent in the insurance and copulas are used to model the joint distribution of multiple risk factors. Copulas help capture the dependence structure between the correlated risks, improving predictions for joint risk exposure.

One of the most important topics for those interested in dependency structures in insurance is the modeling of claim severity and frequency. Czado et al. (2012), proposed a joint copula model for modeling the number of claims and severity of the claims. Frees et al. (2016), used copula functions to model multivariate frequency - severity models with the insurance data.

1.1.3. Econometrics:

In econometrics, copula functions are used to study the relationships between economic variables, such as inflation, interest rates, GDP growth, and unemployment. Copulas are also applied to provide a flexible framework for modeling joint distributions and dependencies between multiple economic factors. Perez-Rodríguez et al. (2015) worked on employing different copulas to jointly model the time-varying dependence between the growth rates of GDP and tourism receipts, capturing their joint distribution.

Patton (2012) reviews how copula models can be used for economic time series to make predictions regarding dependence structure.

1.1.4. Health and Biostatistics:

Copulas are employed in survival analysis to model the dependence between the survival times of different groups or conditions, especially when dealing with censored data. Frees et al. (1996) and Carriere (2000) proposed alternative models with copula functions for modeling the dependence between the death times of couples.

Copulas are also used to model the joint distribution of multiple diseases or health conditions. Stöber et al. (2015) proposed a mixed model with copulas to investigate the comorbidity of chronic diseases in data from the elderly age group.

2. Association Between the Variables:

The relationship between variables can exist in different directions and with varying scales. Variables can be independent, positively dependent or negatively dependent. Not all copula functions are appropriate for modeling the different relationship between variables.

2.1. Correlation:

$\boldsymbol{\rho_{XY}}$ is a measure of linear dependence and the first measure of the relationship between two random variables is the correlation coefficient. The correlation coefficient between the variables of X and Y:

$\rho_{XY} = \frac{\mathrm{cov}[X,Y]}{\sigma_X \sigma_Y}$.

$\mathrm{Cov}[X,Y] = E[XY] - E[X]E[Y]$, σ_X and σ_Y:,the standard deviations of X and Y. ρ_{XY} is defined between (-1,+1). Independence of random variables occurs when $\rho_{XY} = 0$. (Trivedi and Zimmer, 2007)

2.2. Rank Correlation :

Kendall's tau and *Spearman's rho* are rank correlation coefficients.

Spearman's rho is defined for two random variables of X and Y:

$\rho_S(X,Y) = \rho(F_1(X), F_2(Y))$,

when ***Kendall's tau*** is defined as

$\rho_\tau(X,Y) = \Pr[(X_1 - X_2)(Y_1 - Y_2) > 0] - \Pr[(X_1 - X_2)(Y_1 - Y_2) < 0]$.

$\Pr[(X_1 - X_2)(Y_1 - Y_2) > 0]$, is known as the probability of concordance; $\Pr[(X_1 - X_2)(Y_1 - Y_2) < 0]$, is the probability of discordance in the previous equation.

When the random variables X and Y take small or large values simultaneously, it is referred to as concordance, whereas the tendency for one to take a small (large) value while the other takes a large (small) value is referred to as disconcordance. (Trivedi and Zimmer, 2007)

Kendall's tau and *Spearman's rho* can be described in terms of bivariate copulas:

$$\rho_S(X,Y) = 12\int_0^1\int_0^1 \{C(u_1,u_2) - u_1u_2\}du_1du_2$$

$$\rho_\tau(X,Y) = 4\int_0^1\int_0^1 C(u_1,u_2)dC(u_1,u_2) - 1$$

(Schweizer and Wolff, 1981).

3. Definition of the Copula:

The copula is fundamentally a multivariate distribution function with standard uniform marginal distributions. The copula is introduced by ***Sklar*** in 1959.

F is a d- dimensional distribution function with F_1 , F_2, …, F_d ; univariate marginal distribution functions, and C: $[0,1]^d \rightarrow [0,1]$ is a copula with uniform margins

$F(y) = C\big(F_1(y_1), \dots, F_d(y_d)\big)$, $y \in R^d$.

The ***Sklar's theorem*** states that any multivariate joint distribution can be expressed in terms of its marginal distributions and a copula function that defines the dependency structure.

The copula density is derived through differentiation for continuous case in the following equation.

$c(u_1, \dots, u_d) = \frac{\partial^2 C(u)}{\partial u_1 \dots \partial u_2}$, $u \in (0,1)^d$

(Joe, 2014).

Copula functions are generally divided into two categories: Elliptical and Archimedean copula families. An Archimedean copula is characterized by a generator function. Elliptical copulas, on the other hand, are copulas derived from multivariate elliptical distributions.

Frank, Clayton (Cook-Johnson), and Gumbel-Hougaard are the main types of Archimedean copulas. In Archimedean copulas, the dependence structure is controlled by the parameter $\boldsymbol{\alpha}$, the parameter of the copula. It is typically specified as a scalar measure of dependence for bivariate case, although can be a vector of parameters for higher dimensions.

Bivariate Archimedean copulas can model both positive and negative associations. Archimedean copulas with three or more dimensions can only work with positive association. The dependence structure for multivariate elliptical copulas is defined by the correlation matrix.

In this chapter, the discussion will continue by examining dependency structures through bivariate Archimedean copula functions with uniform margins.

3.1. Frank Copula:

Frank's (1979) copula allows for dependence which is controlled by the parameter α:

$$C_\alpha(u_1, u_2) = -\frac{1}{\alpha}\ln\left(1 + \frac{(e^{\alpha u_1}-1)(e^{\alpha u_2}-1)}{e^{\alpha}-1}\right).$$

The Frank copula is capable of capturing both positive and negative dependence. The dependence parameter is defined within (-∞,∞). As the value of the dependence parameter α increases, positive dependence rises.

3.2. Clayton Copula:

Clayton (1978) copula for bivariate case is defined in the following equation,

$$C_\alpha(u_1, u_2) = \left((u_1^{-\alpha} + u_2^{-\alpha} - 1\right)^{-\frac{1}{\alpha}},\ \alpha > 1.$$

The dependence parameter is defined within (1,∞) and positive dependence rises when the value of the dependence parameter α increases. The Clayton copula can not represent negative dependence.

3.3. Gumbel-Hougaard Copula:

Gumbel (1960) copula is also named Hougaard (1986) copula for bivariate case is defined in the following equation,

$$C_\alpha(u_1, u_2) = \exp\left\{-[(-\ln u_1)^\alpha + (-\ln u_2)^\alpha]^{\frac{1}{\alpha}}\right\},\ \alpha \geq 1.$$

The dependence parameter is defined within [1,∞) . The Gumbel copula can not represent negative dependence.

Kendall's tau is expressed by the dependence parameter *(α)* for one parameter Archimedean copulas in the Table 1:

Table 1: ***Kendall's tau*** expressions for bivariate Archimedian Copula Functions

Copula	***Kendall's tau***
Frank	$1-\frac{4}{\alpha}\{D_1(-\alpha)-1\}$
Clayton	$\frac{\alpha}{\alpha+2}$
Gumbel-Hougaard Copula	$1-\alpha^{-1}$

The relationship between the dependence parameter and τ for the Frank copula is given by the Deybe function , [$D_k(x)=\frac{k}{x^k}\int_0^x \frac{t^x}{e^t-1}dt$], (Frees and Valdez, 1998).

The most important features of Archimedean copulas are their symmetry and scalability. Even though a closed-form solution for higher dimensions may not exist.

3.4. Specialized Copula Types for Boundaries:

The relationship between variables for boundaries will be examined here by expressing it with copula models. ***Independence, Fréchet-Höeffding upper bound***, and ***Fréchet-Höeffding lower bound*** are the special cases of the dependence structure. In this section, the boundaries are considered by copula functions for the two-dimensional case.

3.4.1. Independence copula:

Independece copula refers to the situation where random variables are independent.

C(u_1, u_2) = $u_1\ u_2$

3.4.2. Fréchet-Höeffding upper bound:

The Fréchet-Höeffding upper bound occurs when the two random variables are perfectly positively dependent. The Fréchet-Höeffding upper bound is given by:

$\mathrm{M}(u_1, u_2) = \min(u_1, u_2)$.

3.4.3. Fréchet-Höeffding ***lower bound:***

The Fréchet-Höeffding lower bound occurs when the two random variables are perfectly negatively dependent. The Fréchet-Höeffding lower bound is given by:

$$W(u_1, u_2) = \max(u_1 + u_2 - 1, 0).$$

It is not feasible to have three or more variables where every pair has a perfectly negative dependency. (Genest and Favre, 2007)

The Fréchet-Höeffding upper and lower bounds are used to model the extreme levels of dependence between random variables. They play a significant role in defining the range within which the dependence structure between random variables can move.

4. Discussion:

Copula functions play a critical role in modeling dependencies between random variables, offering flexibility and a powerful tool for analyzing joint distributions. This ability makes copulas particularly valuable in various fields such as finance, economics, health and biostatistics, where complex relationships between variables often arise. By separating the marginal distributions from the dependence structure, copulas allow for more accurate and detailed modeling of multi-dimensional data. Multi-dimensional copula functions are quite difficult to handle theoretically, and their complexity needs more sophisticated methods and computational tools. Working with copula functions for bivariate data and examining the relationship between two variables can be relatively more preferable in terms of mathematical manageability.

Copula functions do not focus exclusively on linear relationships; they can also represent non-linear and more complex dependency structures. When working with copula functions to model the dependency structure, the type and measure of the dependency between the random variables in the data should first be determined. The selection of a copula function that can capture the dependency between the random variables is crucial. This section emphasizes that the Kendall's tau, which is a rank correlation coefficient, can be used to measure the dependency between variables. The relationship between the dependency parameter in Archimedean copulas and the Kendall's tau value is important.

Bivariate Archimedean copula functions can be as a good starting point, when studying dependence for a bivariate model. The dependence between the variables will be represented by $\boldsymbol{\alpha}$, the dependence parameter of the copula function.

The Fréchet-Höeffding upper and lower bounds also provide a definitive range within which the direction and strength of dependence between random variables can change. These bounds help to establish the potential extremes of dependence, guiding the selection of copula functions.

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Chapter 3

Damage Detection in Honeycomb Panels Using Computer-Aided Tap Testing

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Abstract

Periodic control of honeycomb panels, which are used especially in sectors such as aviation, automotive and energy, is of vital importance both financially and morally. Non-destructive testing (NDT) methods detect damage to materials without damaging them. Computer Aided Tap Test (CATT), which is one of the non-destructive test methods, has proven to be a successful method in revealing the defects of honeycomb panels, which are widely used in aviation and spacecraft. The traditional tap test, which depends on human auditory perception, has been eliminated by producing quantitative data with the integration of accelerometers and electronic circuits used depending on the developing technology. In this study, after the development and working principles of the CATT method are given, sample applications showing how it is used to detect damage in some honeycomb panels are included. The CATT system provides detailed, high-resolution data by measuring contact times and stiffness values, allowing 2D and 3D images of the material under test to be created. Findings from the study reveal that CATT offers a fast, economical, and reliable approach to damage detection in complex materials, with potential applications extending to quality control in a variety of industries.

Keywords: Computer-Aided Tap Test, Damage, Honeycomb panel, Contact time, Stiffness.

INTRODUCTION

The reason for the deterioration of the structural integrity of a material is defined as discontinuity. Stratifications, cracks, forged folds, seams, foreign-matter residues, and pores are the main sources of contamination. Identifying discontinuities present in the material is important for the life and function of the part. Destructive material analysis is no longer preferred except under mandatory conditions because it damages the material, takes a long time, cannot be used in every material, and is not economical. Instead, non-destructive testing (NDT) methods that are cheaper, more economical, and give results in a short time are preferred.

Non-destructive testing has a wide range of applications, from medicine to many industries. Traditional non-destructive testing methods are constantly being improved to make them easier to interpret while at the same time providing more sensitive and reliable results. Non-destructive testing is used to test products at different stages of production without causing any damage to ensure maximum reliability. It is necessary to discover defective parts at the earliest possible stage in the production and service stages. In this approach, non-destructive tests should be applied in the production and service stages to ensure the highest reliability and lowest cost. With the development of technology, the importance of non-destructive testing methods has grown even more (Hellier, 2001).

Non-destructive testing is of vital importance, especially in the aviation, automotive, defense, and energy sectors, where huge investments have been made today. It is obvious how important it is to check the wings, propellers, engines, and outer coatings of any aircraft, whether for civil aviation or military purposes, the tanks of a nuclear power plant, wind energy turbines, and many parts used in cars before and during use in a sensitive, fast, and economical manner. For example, aircraft maintenance activities have a mandatory "must-have" place in aviation. These activities are controlled by civil aviation authorities at certain time intervals and are carried out under continuous inspection mechanisms. Aircraft maintenance work can be carried out in the hangar as well as on the apron. Non-destructive testing is of great importance for flight safety. Non-destructive testing is carried out before service, not only during maintenance work but also during the production of aircraft parts. Non-destructive testing is of great importance because it is directly related to the detection of damage to the aircraft. Therefore, aircraft maintenance work should be carried out in a timely and serious manner. Time is another important issue. Because aircraft whose maintenance work cannot be completed on time will not be able to take off, this creates great financial damage for the aircraft company.

Non-destructive testing methods are applied in different ways with various physical principles. The method to select is determined by the type of material to be examined and the type of error to be sought. Each method has advantages over the other and is usually complementary to each other. The methods applied in non-destructive testing can be summarized in Table 1 (Göktepe & Ege, 2004).

Table1. Nondestructive testing methods

1-Surface Methods	2-Volumetric Methods	3-Combined Methods
Visual Inspection	Radiographic Test	Tap Test
Magnetic Particle Test	Ultrasonic Test	Acoustic emission tests
Liquid Penetrant Test		Vacuum Test
Eddy current test		Leak Test
		Material Thickness Test
		Paint Thickness Test
		Hydrostatic Test
		Pneumatic Testing

In this study, information will be given about the "Computer Aided Tapt Test" and how this test is used to evaluate errors in honeycomb panels, especially in aircraft and spacecraft, and sample applications obtained by this method will be included.

COMPUTER-AIDED TAP TEST (CATT)

The tap test is a simple but effective method for detecting defects and damages in honeycomb panels whose inner core consists of metal or polymer composites (Hagemaier & Fassbender, 1978). This inexpensive method has been used as a non-destructive qualitative and subjective control technique in the field of application for many years (Hsu, Barnard, Peters, & Dayal, 1999). It is especially useful for inspecting honeycomb panels in vehicles used in the aerospace industry. In the tap test, the local hardness of the click-clicked part was determined by the sound produced by the click. In the tap test, a very weak sound with a longer contact time is obtained in an area with low hardness, whereas in the tap test in a hard area, a sound with a shorter contact time but a much higher intensity is obtained. The evaluation of these tap tests was based entirely on whether the ear and eye of the test taker were healthy. Recently, however, test devices have been developed that translate test results into numerical data using accelerometers and electronic circuits (Georgeson, Lea, & Hansen, 1996). Thanks to these devices, dependence on the human ear can be eliminated, and tap tests based on more objective quantitative data can be performed. As a result, when the tap test is systematically performed on a specific surface of honeycomb material, it is possible to obtain a "scanned image" of the clicked area with the

help of the contact times obtained (Adams, 1985; Hsu, 2008; Hsu, Peters, Fei, Barnard, & Dayal, 1999; Peters, Barnard, Hudelson, Simpson, & Hsu, 2000). As a result of the evaluation of these scan images, defects and damages in the tested materials can be detected.

Development of the CATT Technique

The manual tap test has been used for general inspection of metal, wood, and especially honeycomb panels for many years. However, in the tests performed in this way, it was not possible to store the test data, and the dependence on the human ear and eye caused results that varied from person to person. In parallel with technological developments that have occurred over time, there have been developments based on quantitative data that provide the opportunity to store data obtained from tap tests. The common aspect of the development of the tap test is that the human factor has been eliminated in terms of auditory function due to the electronic circuits and devices used. Today, three computer-based test systems have been developed that perform the tap test based on quantitative data by eliminating the human factor. The first of these is the Woodpecker developed by Mitsui Heavy Industries, the second is the Rapid Damage Diagnostic Instrument (RD3) developed by the Boeing company and transferred to Wichi-Tech, and the third was the computer-aided tap test (CATT) systems developed by Iowa State University (Figure1). Within the scope of this study, after the introduction of the CATT system, which was developed by Iowa State University researchers, it was explained how the tap test was performed, especially in materials such as honeycomb panels, and how the data obtained from this test were analyzed.

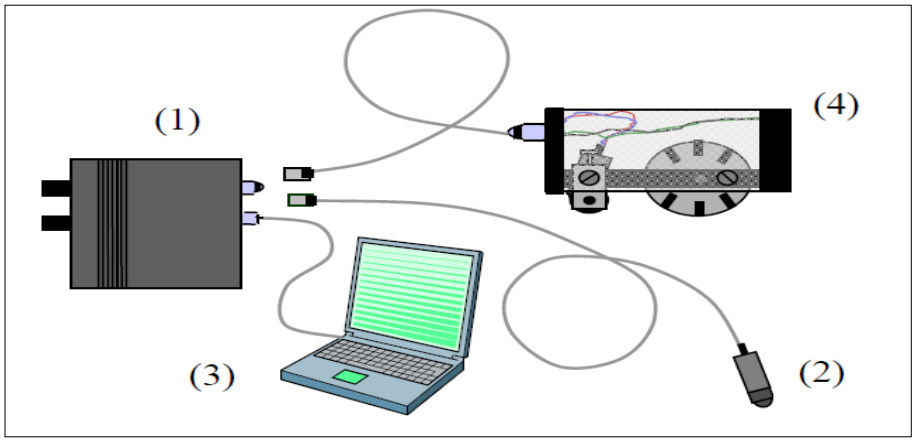

Figure 1. Components of the CATT system: (1) Electronic and power module, (2) Hand-tapping instrument, (3) Laptop computer and software, (4) Semiautomated tapping cart (ASI, 2000).

The CATT system was developed to obtain images of the scanned regions with the help of quantitative engineering data by eliminating the human factor in the impact test (Hsu, Barnard, et al., 1999). As a result of the evaluation of these images, we aimed to reveal the errors in the material examined. In the CATT system, an accelerometer with a known mass is used as the striking element. The electronic circuit in the system measures the contact time (τ) between the part of the accelerometer that touches the part under investigation and the material surface under investigation. The tap test is based on the motion of a mass-spring system that performs a simple harmonic motion. Studies have shown that there is a very close relationship between the time-dependent sinusoidal signal amplitude (A [Volt]) and the period of motion (T) of a mass-spring system that makes simple harmonic motion and the sinusoidal signal and contact time (τ) of the tap test.

When a round-tipped accelerometer was used as a striker in the tap test, the output signal of a tap was a voltage pulse, which also indicated the force-time relationship of the tap process. The signal in Figure 2 of the striking process is equal to half the period (T/2) of a sinusoidal signal.

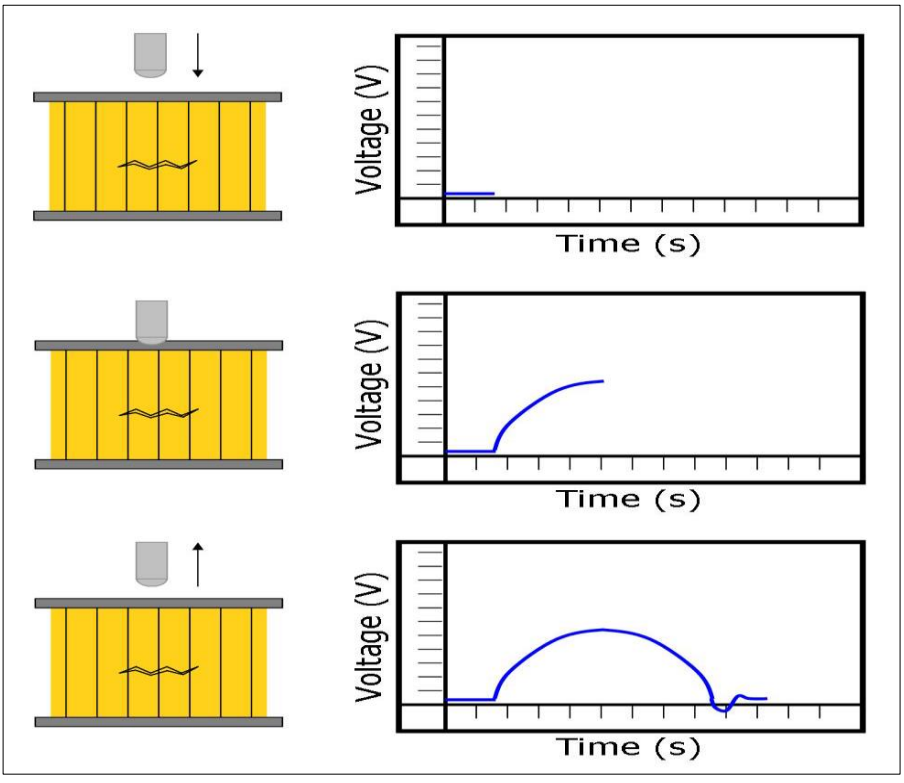

Figure 2. Accelerometer signal output during a tap motion(Foreman, 2008).

As can be seen in Figure 3, when a solid area of the material used in the tap test is hit, the amplitude of the sinusoidal pulse is large, and the contact time between the strike tip and the surface is short. However, when the damaged part of the material is hit, the amplitude of the sinusoidal pulse is lower, but the throw width, which indicates the contact time between the striking tip and the material surface, is greater. In conclusion, in the tap test, the amplitude of the half-sinusoidal signal obtained from a damaged area is smaller, but the contact time between the material and the striking tip is longer. The amplitude of the half-sinusoidal signal obtained from a solid material surface is larger, but the contact time between the material and the striking tip is much shorter.

Therefore, in the tap test, both the width and amplitude of the voltage pulse can be used. The signal width does not depend on the striking force, whereas the amplitude is highly dependent on the striking force. This is clearly seen from the signal images of the impact tests performed on both damaged and intact materials by applying different forces, as shown in Figure 4. In the tap test, the contact time between the tap tip and the material surface (i.e., the signal width) is the basic quantity of error detection.

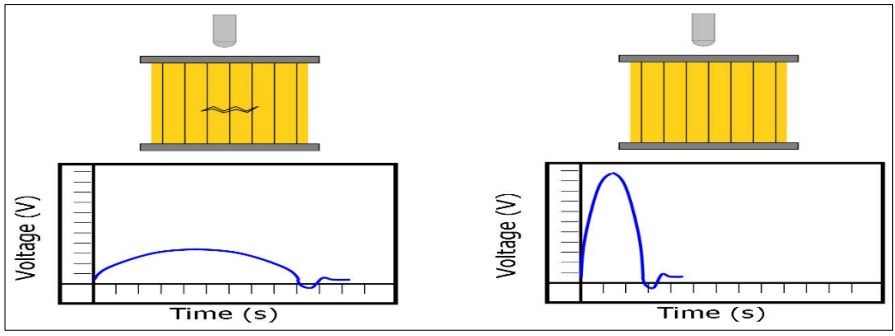

Figure 3. Effect of a flaw on the accelerometer's signal output during tap motion (Foreman, 2008).

As shown in Figure 4, the application of different forces only changed the amplitude of the obtained signal and did not affect the contact time. The analysis of contact stress is based on the Hertzian Theory of Contact (Hertz, 1896) in the 18th century, and the phenomenon of two substances colliding with each other has been the subject of many books (Goldsmith & Frasier, 1960).

In addition, recent studies have been conducted on tap test mechanics from the perspective of Non-Destructive Testing (Cawley & Adams, 1988; Cawley & Adams, 1989). As a result of these studies, the data obtained from many composite structures used in the tap test revealed that the tap process can be modeled as the movement of a mass-spring system with simple harmonic motion.

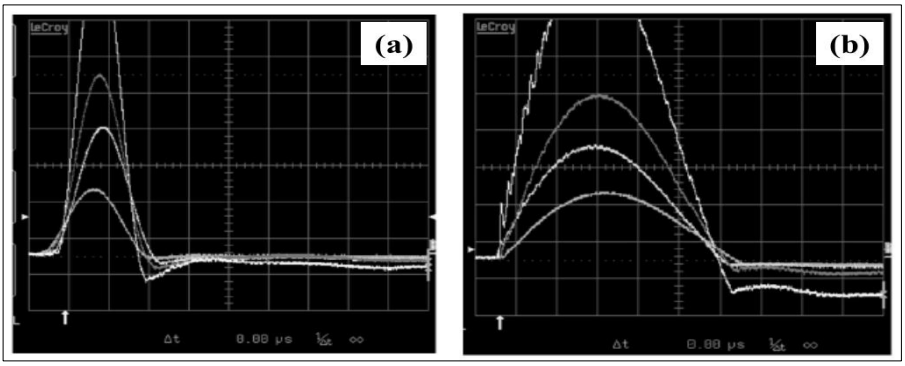

Figure 4. Output voltage of the accelerometer obtained at low, medium, and high power; (a) Intact material, (b) Damaged material (Hsu, 2009).

The data obtained from these studies revealed that the local hardness (spring constant[k]), which also gives information about the structure of the part being examined, can be calculated with the help of determining the contact time between the tap tip and the material being examined.

Grounded Mass-Spring Model

As can be seen in Figures 2 and 3, the output signal of the accelerometer during a tap motion corresponds to half a cycle of a sine signal. Therefore, a tap motion can be modeled as a simple harmonic motion made by a spring with constant k and mass m_T at its end. Therefore, half of the period (T/2) of a mass-spring system performing simple harmonic motion can be taken as the contact time (τ) obtained in a tap test (Hsu, Barnard, Peters, & Dayal, 2000).

$$\boldsymbol{\tau = \frac{1}{2f} = \frac{\pi}{\omega} = \pi\sqrt{\frac{m_T}{k}}} \qquad \textbf{(1)}$$

Here, k is the local hardness of the material being tested (spring constant). Equation (1) predicts that the graph drawn between $(\tau/\pi)^2$ and m_T will be directly proportional, and the slope of the graph should be 1/k. In fact, the data obtained from tests on various composite samples showed a linear relationship between $(\tau/\pi)^2$ and m_T, with the linear line approximately passing through the origin. This is because the local stiffness of a piece is an important physical quantity that provides information about the structure of the tested material. Once the mass of the tapper (m_T) and the contact time between the tapper's tip and tested material's surface (τ) are determined, the value can be easily calculated using equation (2).

$$\boldsymbol{k = \left(\frac{\pi}{\tau}\right)^2 m_T} \qquad \textbf{(2)}$$

By using the local stiffness value k (in Newtons/meter) for different parts of the material under investigation, a stiffness image can be drawn, revealing the structure of the sample under examination. An image of the wing of a Boeing 767 aircraft obtained from the tap test is shown in Figure 5.

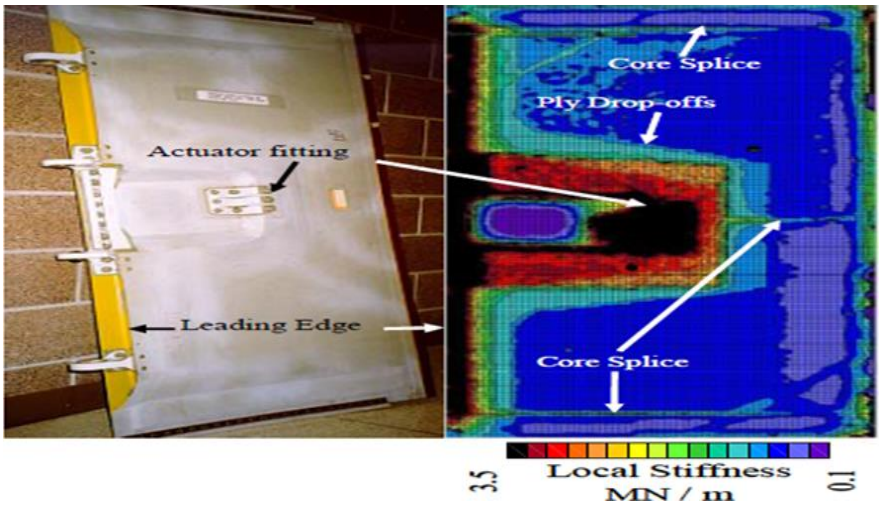

Figure 5. Impact test image of the wing of the Boeing 767 aircraft (Hsu, 2008).

Studies have shown that Computer-Aided Tap Test (CATT) can detect errors in different materials like ultrasonic air contact methods. This was evident in the images obtained from the tail section of a Delta Air Lines aircraft using the tap test and air-coupled ultrasonic method (Figure 6).

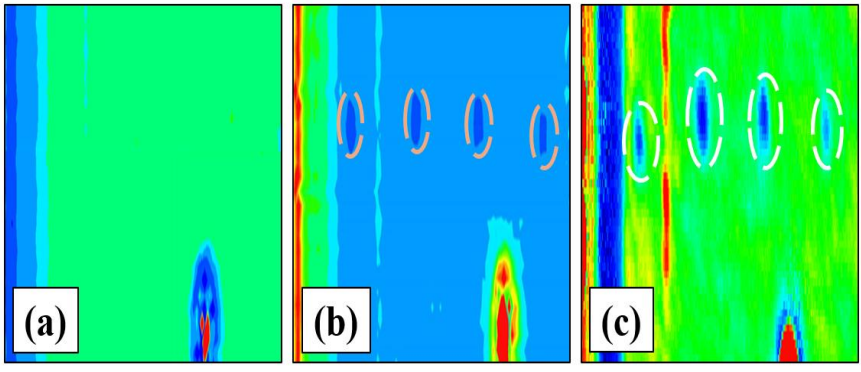

Figure 6. Images of the tail section of a Delta Air Lines aircraft: (a) Image of intact material obtained by the CATT system, (b) Image of damaged material obtained by the CATT system, (c) Image of damaged material obtained by the ultrasonic C-scanning method (Barnard, 2015).

DAMAGE ANALYSIS USING THE CATT

In this part of the study, we first explain how the tap test is performed with CATT on a material, how 2D contour images are obtained from the contact times and stiffness values obtained from the test, and then what kind of operations can be performed on the obtained contour images.

For the computer-aided tap test, first, the set up shown in Figure7 is prepared. The ASI CATTV5_3 program installed on the laptop is then opened, and a scanning window equivalent to the dimensions of the sample to be examined is opened. When opening this window, the unit of inches, cm, or mm and the feed step with the desired precision are selected (for example, 0.5; 1; 2 or 2.5 steps if cm is selected). Before the experiment began, the sample to be examined for the analysis report and the necessary information about the test were recorded in the system, and the experiment was started. In each striking operation, the contact time of the material surface and the tip of the accelerometer at the point of hit in the scan window were calculated in microseconds (μs) and appeared in different colors on the scan screen. When the scan is complete, the hitting process can be repeated at the desired points. After this repetition process, a window is obtained as shown in Figure 8.

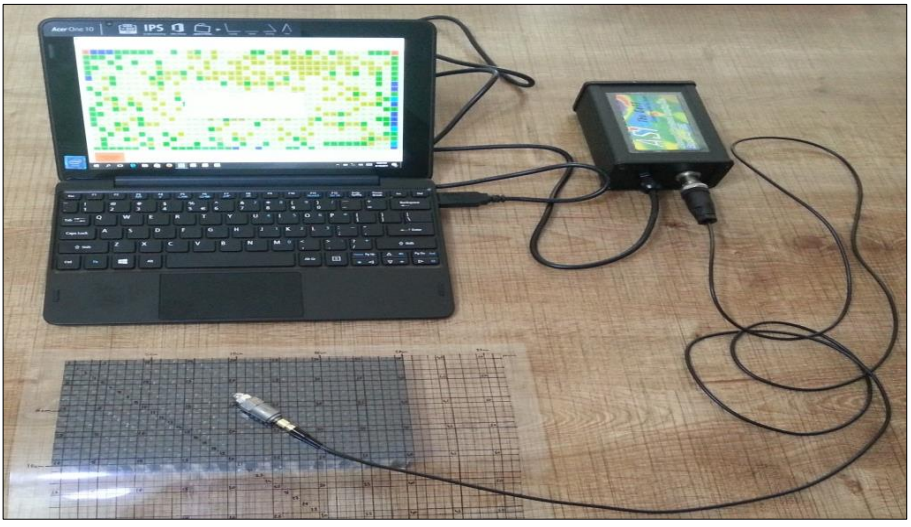

Figure 7. Computer-aided tap tester (CATT) setup

When the button in the lower left corner is pressed in Figure 8, a 2D contour image is obtained as in Figure 9, drawn according to the contact time. These contact time values can be converted into contour images of the local stiffness values in the CATTV5_3 program using equation (2). In addition, 3D images

based on contact time and stiffness data can be drawn using CATTV5_3 installed on the computer.

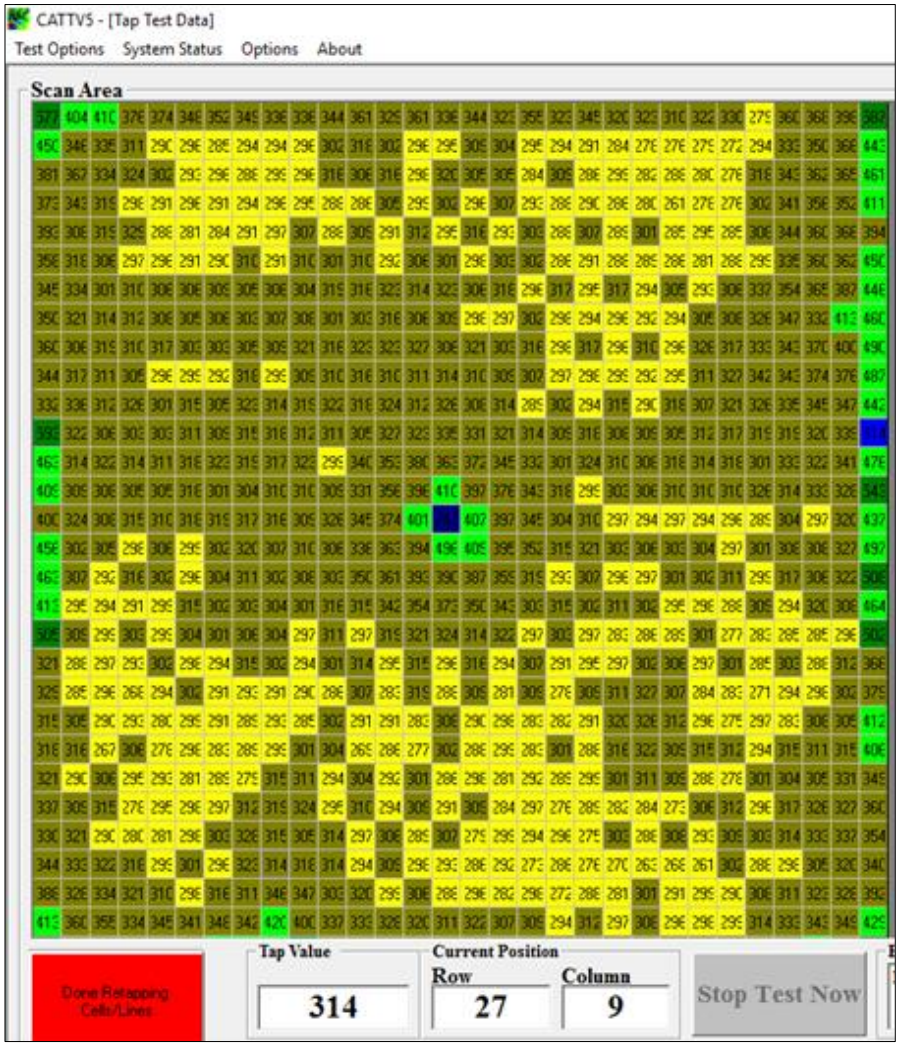

Figure 8. Tap-test screen obtained using the CATT system.

Figure 9 shows the 3D images obtained using the data for both contact time and stiffness values of the aluminum honeycomb panel. In Figure 9a, the pointed peaks that appear in the 3D image drawn with the data obtained from the contact times show the damaged areas in the material, while in the areas where there are pits showing the stiffness values at the lowest value seen in Figure 9b, these indicate the damaged areas in the material. Therefore, damaged areas that cannot be clearly detected from the 2D contour images drawn using the contact times

and stiffness values obtained because of the impact test can be easily detected with the help of the 3D images.

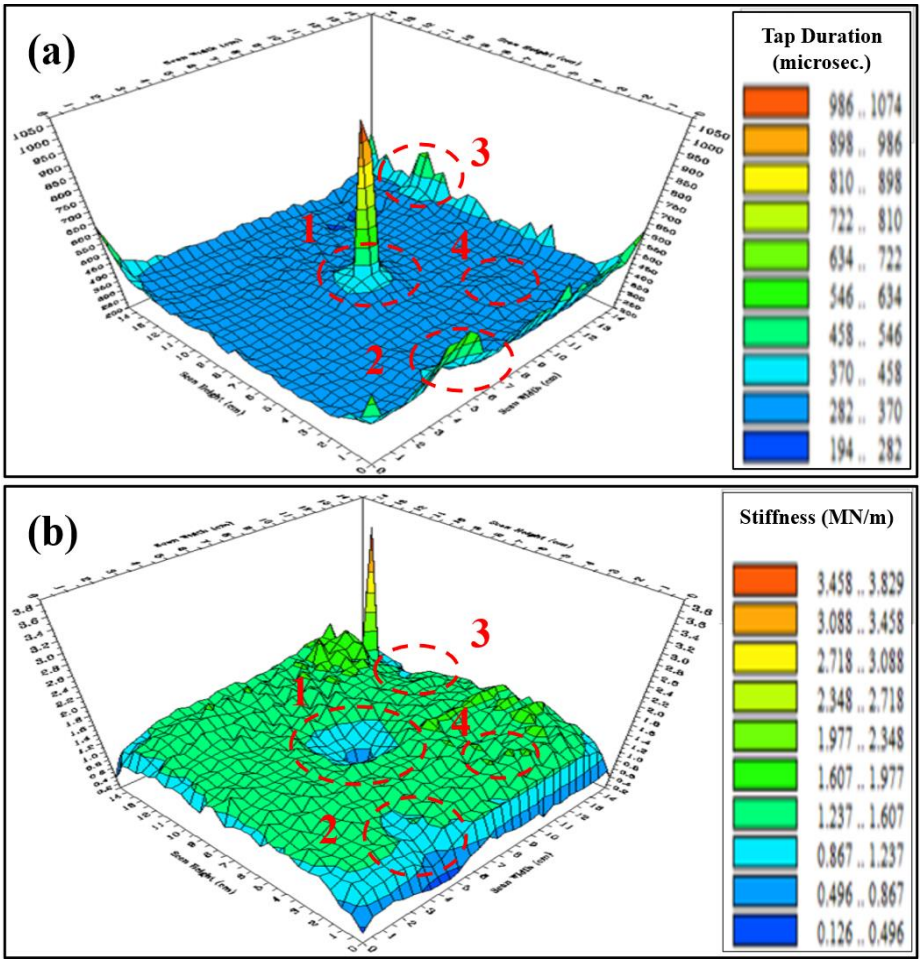

Figure 9. 3D images obtained with CATT: (a) contact time and (b) stiffness value.

With the help of the contact times and stiffness values obtained by the tap test, more detailed images of the material structure and the flaws detected in the material can be obtained by performing manual operations on the images obtained using the CATTV5_3 software (Figure 10).

The images in Figure 11 were obtained after a few manual operations on the 2D contour image shown in Figure 10. The images in Figure 11 were obtained by filtering after defining a threshold (for a certain contact time value or stiffness) that was considered an error in the material.

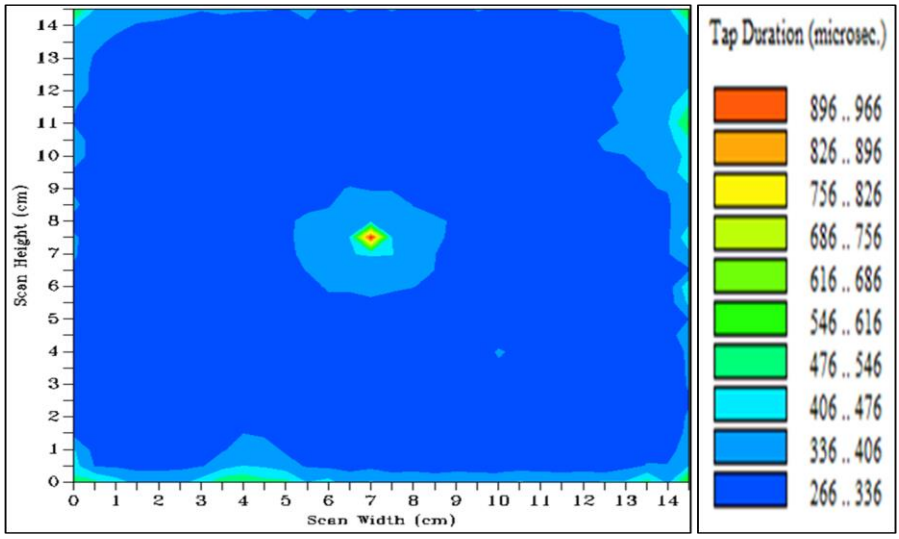

Figure 10. 2D contour plot of contact times in Figure 8.

For example, if the contact time is below the threshold value of 400 μs for contact time indicates robustness, points with a contact time above 400 μs will indicate damaged areas. Increased contact times above 400 μs will appear in different colors to indicate the extent of damage. As can be seen in Figure 11, the damaged areas and their sizes emerged more clearly after manual thresholding.

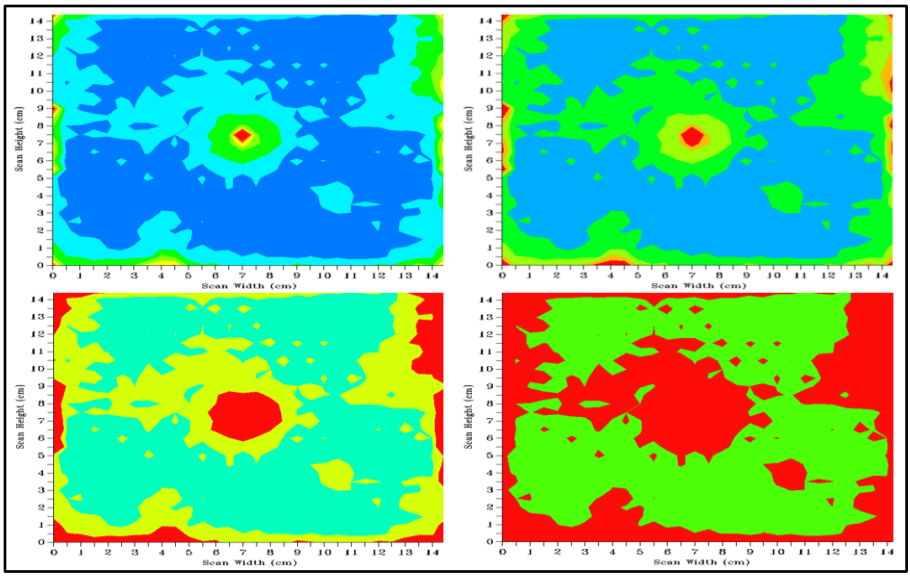

Figure 11. Images of damaged areas obtained after several manual operations on the 2D contour image in Figure 10.

CONCLUSION AND DISCUSSION

The Computer-Aided Tap Testing (CATT) represents a significant advancement over traditional manual tap testing, which is subjective and reliant on the human ear. By incorporating accelerometers and electronic circuits, CATT eliminates human error and provides more reliable, quantitative data for material analysis. The results presented in this study demonstrate the ability of CATT to detect damage in honeycomb structures with high precision, even in cases where traditional methods might fail to identify flaws. The system's capability to generate both 2D contour images and 3D models of material stiffness further enhances its utility in damage analysis. Thus, the CATT is a technique that allows more realistic information to be obtained by using quantitative data, such as contact time and stiffness, obtained from the tap test as an alternative to the situation based on the sensitivity of the human ear, which has been used since the early years of humankind. It is a very successful, fast, and economical method for detecting errors in honeycomb panel materials used in the space, aviation, and maritime sectors, especially due to their lightness. Product variety formed with developing technology in the manufacturing sector; It brings with it the understanding of unconditional assurance of products such as quality, technical safety, durability, and suitability for intended use. Since it aims to provide continuous and high-quality production, the importance of non-destructive testing methods in quality control applications is increasing. CATT application in the aerospace sector is particularly valuable, as honeycomb panels are extensively used in aircraft and spacecraft due to their lightweight and strong properties. The CATT method’s ability to rapidly assess the structural integrity of these components ensures both safety and cost-effectiveness, which is crucial in industries where time and resources are highly constrained. Additionally, the system's ability to store and analyze test data eliminates the variability introduced by human interpretation, providing consistent results that can be used for long-term monitoring and maintenance schedules.

When the relevant literature is examined, no studies have been conducted on CATT in Türkiye, but most have been conducted by American scientists. When the studies conducted abroad are examined, the CATT technique is seen to be mostly used in the quality control of sandwich honeycomb panel materials used in the space, aviation, and ship sectors. For example, Hsu et al. (2000) explained the physical foundations of CATT and explained how it can be applied to composite structures used in the aerospace industry as a quantitative imaging technique. Again, in another study, Foreman (2008) explained the basics of computer-aided tap testing and revealed its applicability in damage analysis of sandwich honeycomb panel structures. Barnard (2015) analyzed the damage

caused by 1,1.4, 2, 2.5, 3 and 4 J impacts on the honeycomb panels of aircraft by the CATT system and air-coupled ultrasonic method and compared the results obtained.

The data obtained in this study show that the CATT method can quite well detect damages in honeycomb panel and foamy structures, and even in the case of damage that cannot be detected, these damages can be detected by experts by performing manual operations on the data obtained with the CATT system. While CATT has been extensively studied and applied in the United States, its use in Türkiye and other countries remains limited. This study aims to bridge that gap, highlighting the advantages of CATT in industrial applications. The system's effectiveness in detecting defects such as cracks, voids, and delamination in honeycomb panels, which are critical for structural integrity, underscores its potential for widespread adoption in aerospace and related industries. Therefore, it is expected that this study will make an important contribution to the literature in terms of introducing CATT to scientists both in Türkiye and abroad.

In conclusion, the integration of quantitative data through CATT significantly improves the detection of material defects, contributing to better quality control, reduced maintenance costs, and enhanced safety. As non-destructive testing continues to evolve, methods like CATT that combine simplicity with precision will play an increasingly important role in ensuring the reliability and longevity of critical components across various sectors. Future studies should explore the further refinement of this method, its application in different material types, and its potential for integration into automated inspection systems for real-time damage detection.

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Chapter 4

Microplastic Pollution in Water Ecosystems: A Rising Issue

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Abstract

Plastics are widely used in various industries, including food packaging, water bottles, personal care products, healthcare materials, electronic devices, and the aviation sector. The low cost, durability, and versatility of plastics are the primary reasons for their extensive application. Plastic production is expected to increase at a rate nearly three times faster by 2060, which will lead to a significant rise in plastic waste, posing a substantial threat to the environment if no measures are taken. The degradation of plastics into microplastics over time is a major factor contributing to environmental pollution. Microplastics are small plastic particles, ranging from 0.1 μm to 5 mm in size, and composed of various materials. These microplastics are causing severe pollution in marine and terrestrial ecosystems, entering aquatic organisms and leading to biological impacts.

Consequently, plastic pollution, particularly the impact of microplastics on ecosystems, represents a significant environmental threat. Effective measures must be implemented to address this issue, with both governments and the public taking greater responsibility for reducing and recycling plastic waste.

Keywords: microplastic, pollution, aquatic ecosystem

Özet

Plastikler, gıda ambalajı, su şişeleri, kişisel bakım ürünleri, sağlık malzemeleri, elektronik cihazlar ve havacılık sektöründe yaygın olarak kullanılmaktadır. Plastiklerin düşük maliyetli, dayanıklı ve çok yönlü olmaları, onların geniş bir kullanım alanına sahip olmasının başlıca sebeplerindendir. Plastik üretimi (herhangi bir önlem alınmazsa) 2060 yılına kadar neredeyse üç katına çıkması beklenen bir hızla artmaktadır. Ancak, bu artış çevreye büyük bir tehdit oluşturan plastik atıklarının da çoğalmasına yol açmaktadır. Plastiklerin zamanla kırılarak mikroplastiklere dönüşmesi, çevre kirliliğini artıran önemli bir faktördür. Mikroplastikler, 0.1 μm ile 5 mm arasındaki boyutlarda olan ve farklı maddelerden oluşan küçük plastik parçacıklarıdır. Bu mikroplastikler, deniz ve kara ekosistemlerinde ciddi bir kirliliğe yol açmakta, sucul organizmaların vücutlarına girerek biyolojik etkiler yaratmaktadır.

Sonuç olarak, plastik kirliliği, özellikle mikroplastiklerin ekosistemler üzerindeki etkisi büyük bir çevresel tehdit oluşturmaktadır. Bu sorunun çözülmesi için etkili önlemler alınmalı, hem hükümetler hem de halk, plastik atıklarının azaltılması ve geri dönüştürülmesi konusunda daha fazla sorumluluk almalıdır.

Anahtar Kelimeler: mikroplastik, kirlilik, sucul ekosistem

1. Introduction

Plastics are widely utilized in various fields, such as food packaging, the production of water bottles, personal care items (PCPs), safety equipment, healthcare materials, electronic devices, and components for aviation (Khan et al., 2023). The extensive dependence on plastics can be attributed to their advantageous properties, including affordability, versatility, resilience, ability to form airtight seals, and ease of printing (Nielsen et al., 2020). In 2019, the global production of plastic was estimated at 376 million metric tons, of which approximately 133 million metric tons (35%) were allocated to single-use applications, predominantly for packaging purposes (Kan et al., 2023). Driven by rising demand, global plastic production is forecasted to almost triple between 2019 and 2060, growing from 460 million tonnes to 1,231 million tonnes per year (Schroder and Oyinlola, 2023) (Fig. 1). According to research, 80% of plastic waste in the oceans originates from land-based sources, while 20% is generated from marine-based activities such as fishing, aquaculture, and maritime operations (Andrady, 2011). Plastics, therefore, represent a significant ecological hazard. Larger plastic objects can break into tiny pieces when subjected to mechanical stresses and UV light (Khan et al., 2020). These tiny plastic particles, or MPs, are known to be emerging pollutants and are uncontrolled contaminants with sizes ranging from 0.1 μm to 5 mm (Prata et al., 2024). MPs vary in form, material, and structure and are small chemical particles (≤5 mm). Larger plastic objects, synthetic textiles, cosmetics, personal care goods, and the deliberate discharge of plastic pellets are the main causes of them (European Environment Agency, 2023).

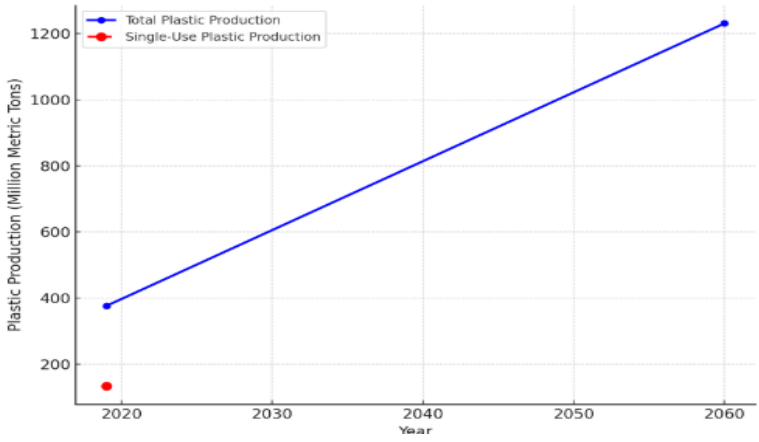

Fig.1. Global Plastic Production and Single-Use Plastic Production (2019 & Forecasted 2060) (Designed by the aurhors of current study).

Marine and terrestrial pollution associated with MPs has become a significant environmental issue (Sajid et al., 2022). MPs have been found in various environmental media, including soil, water (both freshwater and marine), sediment, and living organisms, primarily due to human activities such as industrial processes, agriculture, waste disposal, and textile manufacturing (Henderson and Green, 2020). Over the last century, global plastic production and consumption have surged dramatically. From 20 million tons in 1950, the global production reached approximately 460 million tons in 2019, bringing the total plastic produced to an estimated 8.3 billion tons (OSPAR, 2021). If this trend persists, the total plastic waste could rise to 26 billion tons, with around 12 billion tons projected to end up in the environment (Geyer et al., 2017). The amount of plastic waste in the world's marine environment is thought to be over 269,000 tons, and it is growing rapidly every year (Eriksen et al., 2014). Human consumption and the careless discharge of debris into rivers and streams are the primary causes of plastic pollution in the seas (Stoll et al., 2020). About 9 million tons of plastic pollution are produced each year by land-based sources, including 0.5 million tons from inland sources, 1.75 million tones from at-sea sources, and 0.95 million tons from MPs (<5 mm) (Jambeck et al., 2015). Just 1% of plastics stay on the ocean's surface, while 94% sink to the bottom and 5% wash up on shore (Eunomia, 2016). Effective measures must be put in place to reduce MP pollution, as land-based sources account for more than 80% of the total (Zhang et al., 2022). MPs, particularly non-surface polymers, can persist in the environment for centuries after they are released. It is widely acknowledged that all plastics that have found their way into the environment are still there, either in their whole or in pieces (Shen et al., 2020). Marine life is increasingly at risk from plastic waste because of its longer lifespan, which can lead to entanglement and ingestion. Food security, human health, and marine ecosystems are all greatly impacted by the environmental threats that MPs pose on a worldwide scale. Diverse physical, chemical, and biological impacts might arise from the accumulation of these microscopic particles in different habitats and their ingestion by aquatic organisms (FAO, 2023). Persistent organic pollutants (POPs), invasive species, and dangerous infections can also be transported by MPs (Liu et al., 2022a). Every year, people are exposed to between 39,000 and 52,000 microplastic particles through their diet (Cox et al., 2019). When extra exposure via inhalation, drinking water, and plastic utensils is taken into account, this figure can increase to 74,000 particles (Ziani et al., 2023; Kurniawan et al., 2024). Unpredictable consequences may result from MPs' danger to a variety of creatures in ecosystems, such as plankton, invertebrates, and vertebrates (Wu et

al., 2020). For governments, scientists, non-governmental groups, and the general public, pollution in marine ecosystems has grown to be a major and expanding environmental concern. It plays a role in climate change and habitat loss (Onyena et al., 2022). The loss of marine biodiversity impairs water quality, disrupts ecosystem equilibrium, and makes it harder for the ocean to recover back from disturbances. The most common, dangerous, and substantial marine pollution is plastic garbage, which makes up at least 85% of all marine debris (Lincoln et al., 2022). The safety of eating seafood has come under scrutiny in recent years because to worries about MPs' presence in the food chain (Pironti et al., 2021). The high levels of human exposure to microplastic pollution have been highlighted by the discovery of MPs in human organs, including blood, the placenta and deep lung tissues (Ragusa et al., 2021; Jenner et al., 2022; Yuan et al., 2022).

The influence of microplastic (MP) pollution on the aesthetics of urban, coastal, and marine habitats is another worrying aspect. This problem has spread around the world and has a negative impact on ecosystems and living things (Gautam et al., 2023). According to annual assessments of lakes, seas, and coasts, MP fragments are becoming more abundant and dispersed globally, while the average size of plastic particles is decreasing (Beiras and Schonemann, 2020).

Effective plastic management is essential for maintaining ecosystem health and achieving the 2030 Sustainable Development Goals (SDGs) (BM, 2020). Recent research has shown that plastics in marine environments hinder economic growth, leading to decreased tourism revenues, limited recreational opportunities, energy losses, damage to ocean ecosystems, increased invasions by non-native species, vessel damage, and health risks for the public (Sridharan et al., 2021; Watson et al., 2022). According to Beaumont et al. (2019), the annual economic impact of marine plastic pollution may be between $3,300 to $33,000 per ton. The United Nations has urged for action to reduce marine litter by 2025, citing the growing economic losses caused by this problem (Henderson and Green, 2020). A strategy to address marine waste made of plastic has also been endorsed by the United Nations Environment Assembly (UNEA), which urges prompt action and suggests steps to reduce the dangers to public health (UN, 2020).

Over 80% of the waste from the ocean is marine plastic pollution, an increasing problem that requires more aggressive measures (Lincoln et al., 2022). As a result, throughout the last ten years, financing and research into the causes of marine plastic waste have increased dramatically (Allen et al., 2022). Recycling, burning, and biodegradation are important methods for handling plastic waste (Shahnawaz et al., 2019). Despite the fact that many countries have developed comprehensive plans to lower MP pollution, the short-term measures

put in place at various levels have not been successful in reducing the harm that MPs inflict to the environment. According to Abbott and Sumaila (2019), it is crucial to assess current rules and regulations pertaining to the manufacturing, use, and recycling of plastic. Since plastic pollution is a worldwide problem that affects international waters as well, cross-border collaboration is essential, as are long-term fixes and multidisciplinary approaches to stop future degradation. Important steps to address this worldwide issue include enhancing life cycle management, enhancing wastewater treatment plant disposal procedures, educating consumers, and putting strong national and international legislation into place (Prata et al., 2019).

2. Classification of Microplastics

During polymerization, plastic materials acquire essential properties such as rigidity, flexibility, and malleability through chemical reactions. The selection of raw materials, manufacturing processes, and the addition of chemicals such as plasticizers (including BPA and BPS), adhesion promoters, flame retardants, and coloring agents all affect these properties. Determining the origins of plastics and evaluating the possible hazards they pose to the environment and human health depend heavily on their classification. In general, plastics fall into one of three primary categories: (i) size-based, (ii) composition-based, or (iii) shape-based.

2.1. Size-Based Microplastic Classification

The physical and chemical characteristics are closely associated with their size. They are typically classified as primary or secondary MPs.

2.1.1. Primary Microplastics

Primary MPs are pre-manufactured particles, often in granule or powder form, and are frequently used in cosmetics, textiles, and medical products (Yursever, 2018; Alvim et al., 2020). These particles are inadequately removed by wastewater treatment facilities, leading to significant environmental contamination.

2.1.2. Secondary Microplastics

Secondary MPs originate from the fragmentation of larger plastic items, subsequently dispersing into natural environments (Yursever, 2015; Yu et al., 2019).

2.2. Composition-Based Microplastic Classification

Microplastics are categorized into thermoplastics, elastomers, and thermosets depending on the nature of bonding within their molecular chains (e.g., linear, branched, cross-linked, or entangled structures) (Mohamadi, 2023).

2.2.1. Thermoplastic Polymers

The molecular architectures of thermoplastic polymers are mostly straight or somewhat branched, frequently displaying entangled and random chains. According to their chemical organization, these materials are further separated into amorphous and semi-crystalline categories (Luo et al., 2022). Polystyrene (PS), polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA), polycarbonate (PC), polybutylene terephthalate (PBT), and polyvinyl chloride (PVC) are a few examples. Thermoplastic polymers are used in the production of items such as toys, sports equipment, plastic bags, shampoo bottles, food storage containers, and beverage bottles (Proto Plastics, 2019).

2.2.2. Elastic Polymers

Elastic polymers are cross-linked by extensive covalent bonds, which make them insoluble, non-melting, and highly flexible. Their unique entropic chain configuration imparts elasticity. Applications of elastic polymers include toys, toothbrushes, medical tubing, sports equipment, tires, and adhesives (Kuraray Europe GmbH, 2021).

2.2.3. Thermosets

Thermosets exhibit a densely cross-linked molecular structure characterized by amorphous arrangements and high thermal resistance. These plastics are widely recognized for their electrical insulation and chemical resistance properties. Examples include polyurethane, urea-formaldehyde, and vinyl esters (Fan and Njuguna, 2016).

2.3. Shape-Based Microplastic Classification

Microplastics are further categorized based on their morphology into subgroups such as fibers, particles, films, and foams.

2.3.1. Microfibers

Microfibers, primarily derived from synthetic textiles like polyester, nylon, and acrylic, represent a distinct category of MPs. These fibers are released during washing or wearing and enter aquatic systems through wastewater. Research underscores that microfibers are among the most prevalent MPs found in the environment (Galvão et al., 2023).

2.3.2. Nurdles (Raw Plastic Pellets)

Nurdles, small plastic pellets with diameters below 5 mm, serve as precursors in producing plastic goods ranging from automobile components to packaging materials (Hammer et al., 2012). Their small size and translucent appearance render them susceptible to ingestion by marine organisms, which often mistake them for fish eggs (Sewwandi et al., 2023).

2.3.3. Particles

Plastic particles result from the degradation of plastic products, eventually forming micro- and nanoscale fragments indistinguishable from natural particles like sand grains (Zhang et al., 2023). Studies have revealed that certain marine organisms, such as microscopic crustaceans, can biochemically fragment MPs into smaller particles within 24–96 hours of ingestion (Hasegawa and Nakaoka, 2021).

2.3.4. Microbeads

Microbeads, typically composed of polyethylene, polystyrene, and polypropylene, are incorporated into various consumer products, including hand sanitizers, soaps, and shampoos, as cleansing or exfoliating agents. These particles, measuring less than 5 mm, exhibit diverse shapes and colors (Kumar et al., 2021). Due to their persistence and resistance to biodegradation, microbeads accumulate in aquatic environments and sediments, posing significant ecological and health risks. Their accumulation exacerbates marine life's and humans' potential toxicity (Miraj et al., 2021; Sallan et al., 2023). Examples of studies on microplastic determination conducted worldwide using various techniques in the last four years (Table 1.)

Table 1. Examples of studies on microplastic determination conducted worldwide using various techniques in the last four years (Soysal, 2024).

Method Used	Sample Type	Type of Microplastics Detected	Reference
Electron Microscopy	Water and soil sediments	Polyethylene, polyethylene-co-vinyl acetate, polyvinyl butyral, polyvinyl alcohol, etc.	Miserli et al., 2023
TGA-MS	Agricultural field atmosphere	Polystyrene	Penalver et al., 2021
Raman Microscopy	Water and food storage containers	Polypropylene, polyethylene terephthalate, polystyrene	Guo et al., 2024
Py-GC-MS	Human testes and semen samples	Polystyrene, polyethylene, polyvinyl chloride	Zao et al., 2023
Raman and Scanning Electron Microscopy	Atmospheric aerosol in the Antarctic region	Polystyrene, etc.	Marina-Montes et al., 2022
STXM	Environmental and food products	Polystyrene, polypropylene, etc.	Foetisch et al., 2022
NMR	Marine layer	Polystyrene	Papini et al., 2024

3. Ecotoxicological Effects of Microplastics

When abandoned, plastic waste can stay intact in the environment for many years because of its long lifetime and durability. Additionally, MPs have the ability to adsorb harmful organic substances such heavy metals, organochlorine insecticides, antibiotics, and endocrine disruptors (Corcaran, 2015). Toxic materials such as lead, copper, cadmium, phthalates, and bisphenol A are utilized in the manufacturing of plastics. The same heavy metals and hazardous materials are present in many smaller MPs that separate off these polymers for a variety of reasons. Numerous organic and inorganic hazardous chemicals found in water can be absorbed by microplastics and accumulated on their surfaces. These toxic substances on their surfaces can be transferred to living organisms. The most notable toxic effect of MPs is on aquatic ecosystems. Plastics constitute the majority of waste observed in water sources (Koelmans et al., 2019). Aquatic birds (such as albatrosses and seagulls) can ingest plastic particles while consuming food like fish, mussels, and squid. Marine turtles can also mistakenly ingest plastic bags, mistaking them for jellyfish, which they consume. A study by Nelms et al. (2018) found microplastic particles in the gastrointestinal tracts of caught fish species, highlighting the potential for these particles to transfer to predators (Karamanlioglu, 2017). Many aquatic organisms easily ingest MPs, as these particles are easily breakable, consumable, and resemble food in color and shape (Chamas, 2020).

The presence of MPs has been shown to reduce the chlorophyll content and photosynthetic activity of algae. Microplastics can also cause direct physical damage, reduce food consumption, increase osmotic pressure, and release toxic chemicals (Prata et al., 2019). If plastic particles entering the bodies of mammals are larger than 150 μm, they are not expected to be absorbed. However, if they are smaller than 150 μm, they can be absorbed in the lymph. If 100 μm, they can be absorbed in the vessels. If ≤20 μm, they can reach organs; if ≤0.1 μm, they can reach all organs, potentially crossing the blood-brain barrier and placenta.

Human activity can transport microplastics to environmental settings. In addition, the presence of MPs in freshwater environments, even in tap water, the air we breathe, soil, glaciers in polar regions, hard-to-reach mountain lakes, and deep-sea regions thousands of meters below the ocean surface, indicates that MPs can also be transported through natural processes (Yurtsever and Yurtsever; 2019). Given the extensive scale of this pollution, countries such as the United States and Sweden have implemented new regulations to limit plastic consumption. Although MPs are not directly targeted, restrictions have been placed on plastic bags that may contribute to microplastic formation in the environment, and shopping bags have been made subject to fees. Plastic pollution

has reached a level that cannot be addressed even through recycling indicates the scale of pollution caused by using single-use plastic products (Geyer et al., 2017)

Although recent studies have focused mainly on MPs in aquatic ecosystems, terrestrial ecosystems play a crucial role in transferring MPs to aquatic systems. This is because most microplastic use occurs in terrestrial ecosystems (He et al., 2018). For example, MPs entering the soil can be stored within the soil and may be transported elsewhere through erosion. Subsequently, they can break down due to other environmental factors and even infiltrate groundwater. Many organisms living in the soil can also absorb these leached MPs into their bodies. The movement of animals, such as moles and squirrels, can further transfer MPs to other areas (Duis and Coors, 2016).

4. Effects of Microplastics on Human Health

Inhalation, cutaneous absorption, and oral ingestion (drinking water, seafood, and other foods) are the three ways that microplastics can enter the human body. They can enter wounded skin, albeit this is less probable (Kosuth et al., 2018; Tang et al.,2021). People have been shown to eat microplastics in their everyday diet (salt, fish, squid, mussels, shrimp, and other seafood, sugar, and honey), water (tap and bottled), and drinks (soda) (Liu et al., 2021). An American adult and kid who drinks the usual quantity of these chemicals may be exposed to 81,000–123,000 MPs each year, according to a 2019 research by Cox and colleagues (Cox et al.,2019). A study by Sharme and Chatterjee (2017) suggested that the intake of MPs may cause mutations in human chromosomes and, in parallel, lead to infertility.

They further said that MPs could be a factor in obesity and perhaps cancer (Cox et al.,2019). Additionally, biological processes including tissue necrosis, oxidative stress, and cell apoptosis are adversely affected by microplastics. As a result, it is possible to propose that they might have carcinogenic consequences (Rafiee et al., 2018). A 2018 research by Schwabl et al. investigated for MPs in stool samples from people who regularly ate aquatic products in eight different countries: Austria, Finland, the UK, Italy, Japan, the Netherlands, Poland, and Russia. For every 10 grams of feces, the investigation detected 9–24 microplastic particles with sizes ranging from 50–500 μm (Leibman et al., 2018). It has been demonstrated that when cells come into contact with plastic particles, an immunological response is triggered, which results in inflammation. Due to their very small size, MPs can cause inflammation by interacting with different organisms after translocation events. Microplastics can accumulate on the skin, leading to various dermal issues (Yurtsever, 2018; Cox et al.,2019). Moreover, they can concentrate environmental pollutants from the atmosphere and

contribute to lung inflammation (Wang et al., 2016). Some studies have shown that MPs interact with microorganisms, causing harm to the human body and potentially forming a separate microbial habitat (Zhu et al., 2018).

Additionally, chemicals such as nonylphenol and bisphenol A, which may be present in MPs, can negatively affect hormones and disrupt the endocrine system. This can lead to impairments in brain development, issues in sexual development, and an increase in cancer, particularly breast and prostate cancer. Therefore, the proximity of the microplastic hazard and the potential adverse outcomes it may cause demonstrate the need for increased attention to this issue (Wright and Kelly, 2017).

5. Addressing Microplastic Pollution: Strategies and Technologies

Microplastic (MP) contamination is a complicated environmental problem that requires a variety of strategies to effectively mitigate. Based on their main goals, current technology solutions may be roughly divided into four categories: monitoring, removal, interception, and prevention. By encouraging sustainable patterns of consumption and manufacturing, enforcing laws against single-use plastics, and creating recyclable items, prevention techniques seek to reduce or eliminate plastic waste at its source. By building stormwater filters and sieves, improving waste collection and management systems, and involving communities and stakeholders in cleaning efforts, intercepting plastic waste aims to keep it out of waterways. Removal solutions, such as devices, nets, barriers, bioremediation agents, and biofilters, use mechanical or biological techniques to remove plastic waste from water bodies. To identify and evaluate the existence and effects of plastic pollution in aquatic habitats, monitoring methods depend on indicators, standardized procedures, citizen science projects, and remote sensing technologies (Al-Hazmi et al., 2024). Membrane filtration, which uses techniques including reverse osmosis, microfiltration, and ultrafiltration, is acknowledged as an efficient treatment strategy. While dynamic membranes have the benefits of accessibility and economical cleaning, ultrafiltration may effectively remove MPs without the need for extra processing (Lares et al., 2018; Ma et al., 2019). However, membrane filtration's effectiveness depends on the size of MPs and does not ensure total elimination (Golgoli et al., 2021). For bigger MPs, rapid sand filtration (RSF) works well; however, for smaller particles, its effectiveness decreases and is constrained by variables including adsorbent saturation (Chabi et al., 2024). Another effective technique for removing microplastics is adsorption, which makes use of processes including π-π interactions, electrostatic interactions, and hydrogen bonding. Depending on the pH, materials such as stacked zinc hydroxides can show up to 96% in deionized water (Rios et al., 2007;

Tiwari et al., 2020). To prevent secondary contamination, however, the toxicity, reusability, and biodegradability of adsorbents must be taken into account. Using processes like hydrophobicity and electrical interactions, magnetic separation uses magnetic nanoparticles as adsorbents to remove microplastics. According to studies, removal rates have above 90%; for example, nano-FeO_4 has average efficiencies ranging from 62.83% to 86.87% (Chorghe et al., 2017; Shi et al., 2022). Despite its promise, this technology has to be developed further before it can be used widely.

6. Conclusion

Every plastic item we discard into the environment can eventually break down into millions, or even billions, of microplastic particles. Regardless of how small these plastic particles are, they do not lose their polymer properties. Therefore, the most crucial issue to focus on is preventing the damage caused by MPs to the environment while they are still at their source. Achieving this will primarily depend on increasing our collective environmental awareness, sensitivity, and consciousness as a society. In our daily lives, we should choose environmentally friendly products in the cosmetics we use for personal care, the detergents and other cleaning products we use, textiles, and various consumer goods such as bags, shoes, and automobile tires. By revising our lifestyle, we should aim to use recyclable materials. Due to the environmental harm caused by MPs, certain developed countries (such as the United States, Sweden, etc.) have implemented restrictions on microplastic usage. However, there are very few studies on MPs and their environmental impact on our country, and no specific restrictions exist. The most recent action taken was in 2019, when the use of plastic bags was restricted. However, further studies on the effects of MPs on the environment and human health are necessary for our country, along with implementing appropriate measures and developing public awareness on this issue.

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Chapter 5

The Antimicrobial Activity Of Honey Bee Venom (*Apis Mellifera Carpathica*)

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Introduction

By the year 2050, one person is expected to die every three minutes from an antibiotic-resistant infection. This impressive prediction by the World Health Organization has made the discovery of new antimicrobial agents an urgent priority. Bee venom (apitoxin), which has been used for therapeutic purposes throughout human history, has become an important subject of research in modern medicine. The extensive history of its use, ranging from ancient Egyptian and Chinese medicine to the writings of Hippocrates and modern apitherapy practices, has clearly demonstrated the importance of this natural bioactive substance. In recent years, especially as antibiotic resistance has become a global health threat, interest in the antimicrobial properties of bee venom has intensified. The versatile therapeutic potential of bee venom was investigated by El-Seedi et al. (2020).

Antimicrobial resistance is currently considered one of the greatest threats to global healthcare systems. The report published by the World Health Organization (WHO) in 2020 predicts that antibiotic resistance could lead to more deaths than cancer-related deaths by 2050. Zolfagharian et al. (2016) emphasize that the number of bacteria developing resistance to conventional antibiotics is increasing, necessitating research into alternative antimicrobial agents. In this context, natural antimicrobial compounds are classified as a priority area of research in scientific studies due to their multiple mechanisms of action that prevent the development of resistance in bacteria.

Bee venom has been characterized as a complex mixture of bioactive components containing various peptides, enzymes, amino acids and other bioactive molecules. Studies by Ferreira Júnior et al. (2010) have shown that the composition of the venom is subject to seasonal variation. Melittin, identified as the most important component of bee venom, can be characterized as a potent antimicrobial peptide with a linear structure of 26 amino acids, accounting for 50-60% of the total dry weight. Phospholipase A2 (10-12%), an enzyme that degrades membrane phospholipids, has been shown to synergistically enhance the effect of melittin. In addition, apamin (2-3%) has been characterized as an 18 amino acid peptide with neurotoxic and anti-inflammatory properties. Using MALDI-TOF MS analysis, a powerful mass spectrometry technique widely used in biochemistry and analytical chemistry, Baracchi and Turillazzi (2010) demonstrated that the proportions of these components vary according to bee breed, geographical location and environmental factors.

It was found that the antimicrobial activity of bee venom is expressed through different mechanisms. Studies by Nam et al. (2003) have shown that melittin exerts its effect through the formation of pores in the bacterial cell membrane.

This effect is enhanced by the degradation of membrane phospholipids by phospholipase A2. This mechanism leads to an interruption of bacterial metabolic processes, which inhibits ATP synthesis and induces cell death. While this mechanism has proven to be more effective in Gram-positive bacteria, Gram-negative bacteria have different dynamics due to their outer membrane structure.

The anti-inflammatory properties of bee venom at the molecular level were described in detail in the study by Nam et al. (2003). The inflammatory response is modulated by the suppression of the production of proinflammatory cytokines by melittin and phospholipase A2. The potential of bee venom in the treatment of chronic inflammatory diseases such as rheumatoid arthritis has been elucidated through the inhibition of the enzyme COX-2 and the regulation of inflammatory mediators such as TNF-α.

The anti-cancer potential of bee venom was comprehensively discussed in the detailed review by Oršolić (2011). Melittin was shown to have selective cytotoxic effects on cancer cells, and these effects were attributed to differences in the membrane structure of cancer cells. Studies by Yaacoub et al. (2022) on HeLa cells have shown that melittin and phospholipase A_2 induce cancer cell death by activating apoptotic signaling pathways.

Innovative applications based on bee venom are being developed in nanotechnology. Nanoparticles based on melittin are used in systems for the targeted administration of drugs. The anticoagulant properties of bee venom and its potential for cardiovascular applications were investigated in studies by Zolfagharian et al. (2015).

From a safety and toxicological perspective, it was emphasized that bee venom should be evaluated as a bioactive substance that requires careful testing. Research by El Mehdi et al. (2021) has defined the therapeutic window by examining the dose-dependent toxicity profile of bee venom. While allergic reactions pose a significant safety risk, it was pointed out that this risk can be minimized by appropriate dosing and administration methods.

Apis mellifera carpathica Foti et al., 1965 (Hymenoptera: Apidae), identified as an ethnic group of the subspecies *Apis mellifera carnica* adapted to the mountainous regions of Central Europe, is characterized by a strong colony structure, resistance to diseases and a high honey production capacity (Kakhramanov vd. 2021). In this context, the antimicrobial potential of the venom of the bee breed *Apis mellifera carpathica* was evaluated in studies carried out. The microorganisms tested in these studies included the Gram-positive bacteria *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212, the Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and the yeast *Candida albicans* ATCC®10231. These

microorganisms are among the pathogens that frequently occur in hospital infections and are characterized by a high potential for the development of antibiotic resistance.

MATERIALS AND METHODS

This study was conducted in two phases between June and August 2022. In the first phase, bees of *Apis mellifera carpathica* Foti et al., 1965 (Hymenoptera: Apidae) ethnicity were procured from the Karabük Province Beekeepers Association, placed in hives, kept during the summer season and their apitoxin (bee venom) was collected. In the second phase, the antimicrobial efficacy of apitoxin against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* was investigated using the disk diffusion method.

Management of the bee colony

Apis mellifera carpathica colonies were placed in standard Langstroth hives facing south. The colonies were regularly maintained and the health status of the colonies was checked before apitoxin collection.

Figure 1. *Bee hive.*

Extraction of apitoxin

Apitoxin was extracted from June to August 2022 using a modified version of the electrostimulation method described by Benton et al. (1963). A glass plate

(20 × 30 cm) was fitted with a thin wire mesh at 0.5 cm intervals and connected to a low current 12V DC source. This device was placed in the hive, with each extraction session lasting 10 minutes. The procedure was repeated nine times at 10-day intervals. To minimize the risk of impurities and contamination, the glass plates were covered with stretch film before the procedure.

Figure 2: *Apparatus for the collection of bee venom*

The collected crystallized apitoxin was transported to the laboratory and mechanically scraped off with a sterile spatula. This apitoxin was dissolved in 0.9% NaCl solution at a concentration of 5 mg/mL and then passed through a membrane filter with a pore diameter of 0.22 μm for sterilization.

Test microorganisms and culture conditions

The following microorganisms were used for the antimicrobial activity tests: Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212; Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853; yeasts: *Candida albicans* ATCC 10231. These microorganisms were provided by the American Type Culture Collection (ATCC, Rockville, Maryland).

The bacterial strains were cultured with Mueller-Hinton broth and Mueller-Hinton agar (Merck). *Candida albicans* was propagated in Sabouraud Dextrose Agar (Difco) and Sabouraud Dextrose Broth (Oxoid) media. All media were sterilized in an autoclave (Nüve OT 4060) at 121°C and a pressure of 1.5 atm for 15 minutes.

Preparation of the apitoxin concentrations

The *Apis mellifera carpathica* apitoxin collected was weighed to 0.008 g using an analytical precision balance and transferred to Eppendorf tubes. A volume of 10 mL of sterile 0.9% NaCl was added to each tube and the solution was vortexed

for 10-12 seconds to homogenize it. Serial dilutions ranging from 10^0 to 10^{10} were then prepared from this solution (Patel et al., 2015).

Antimicrobial activity test

The antimicrobial activity of apitoxin was evaluated using the Kirby-Bauer disk diffusion method (CLSI, 2012). The microbial suspensions were adjusted to 0.5 McFarland standard (10^8 CFU/mL). A volume of 100 µL of the microorganism suspension was spread on the surface of Mueller Hinton Agar (for bacteria) or Sabouraud Dextrose Agar (for C. albicans). Sterile paper disks (6 mm diameter) were placed on the agar surface and 15 µL apitoxin solution (1 mg/mL) was applied to each disk. Trimethoprim-sulfamethoxazole (SXT) was used as a positive control through which a 37.6 mm zone of inhibition was created. Sterile 0.9% NaCl was used as a negative control. The bacterial plates were incubated at 37°C for 24 hours, while the fungal plates were incubated at 30°C for 48 hours.

Figure 3: *Petri dish with inhibition zones*

Measurement of the inhibition zones and statistical analysis

After incubation, the zones of inhibition were measured in millimeters using a digital caliper. All experiments were performed in three independent replicates and the results were expressed as arithmetic mean ± standard deviation.

Statistical analyzes were performed using IBM SPSS Statistics 20 software. Differences between groups were assessed using one-way analysis of variance (ANOVA) and Tukey's test (post hoc). A p-value of <0.05 was considered statistically significant.

RESULTS

The antimicrobial activity of *Apis mellifera carpathica* bee venom was investigated against five different microorganisms using the disk diffusion

method. The microorganisms used in this study were selected from strains commonly found in hospital infections and characterized by a high potential to develop antibiotic resistance. Each of the microorganisms tested showed different susceptibility profiles to different dilutions (10^0-10^{10}) of bee venom (Table 1).

Of the microorganisms tested, the strain *Escherichia coli* ATCC 25922 showed the highest sensitivity to bee venom. At the highest concentration (10^0), an inhibition zone of 14.6 mm was generated, and this effect was observed up to the 10^9 dilution (6.8 mm). These results indicate that the venom of A. m. *carpathica* has a strong antimicrobial potential, especially against Gram-negative bacteria. The high sensitivity of E. coli indicates that venom components such as melittin and phospholipase A2 effectively attack the Gram-negative bacterial cell wall.

Table 1: Inhibition zones produced by *Apis mellifera carpathica* venom at different dilutions (mm)

Microorganisms	**10^0**	**10^1**	**10^2**	**10^3**	**10^4**	**10^5**	**10^6**	**10^7**	**10^8**	**10^9**	**10^{10}**
E. coli	14.6	12.6	10.6	9.8	9.4	8.8	8.4	8.2	7.2	6.8	-
S. aureus	12.2	10.2	9.6	9.4	9.0	8.6	7.6	7.0	-	-	-
E. faecalis	12.6	11.0	10.2	9.0	8.4	8.0	7.8	7.2	7.0	6.6	-
P. aeruginosa	7.6	7.2	7.0	6.8	6.5	6.4	6.2	-	-	-	-
C. albicans	12.2	11.2	8.2	6.6	-	-	-	-	-	-	-

Enterococcus faecalis ATCC 29212 was identified as the second most sensitive microorganism to bee venom. An inhibition zone of 12.6 mm was generated at the initial concentration, which gradually decreased to 6.6 mm at a 10^9 dilution. This susceptibility profile of E. faecalis reflects the general susceptibility of Gram-positive bacteria to bee venom components.

The antimicrobial effect on *Staphylococcus aureus* ATCC 25923 was demonstrated by an inhibition zone of 12.2 mm at the initial concentration, and this effect persisted until the 10^7 dilution (7.0 mm).

Candida albicans ATCC 10231 exhibited a zone of inhibition of 12.2 mm at the initial concentration, but this decreased rapidly with increasing dilution, dropping to 6.6 mm at a dilution of 10^3. No zone of inhibition was observed at higher dilutions.

Pseudomonas aeruginosa ATCC 27853 showed the lowest sensitivity among the microorganisms tested. At the initial concentration, only a 7.6 mm zone of

inhibition was produced, with this effect persisting minimally up to a 10^6 dilution (6.2 mm).

The variation of inhibition zones in the dilution series shows a concentration-dependent antimicrobial activity profile. The inhibition zones observed for all microorganisms decreased with increasing dilution. This reduction pattern indicates that the antimicrobial effect of the bee venom is dose-dependent, with different minimum inhibitory concentrations for each microorganism.

The statistical analyzes showed that the differences in the susceptibility of the different microorganisms to bee venom were significant ($p<0.05$). Particularly noteworthy is the difference in susceptibility between E. coli and P. aeruginosa. These results show that the venom of A. m. *carpathica* has an organism-specific efficacy and a broad antimicrobial spectrum.

Sensitivity profiles and analysis of microorganisms

The most striking result was observed with the Gram-negative bacterial strain *Escherichia coli* ATCC 25922. The zone of inhibition of 14.6 mm generated at the initial concentration (10^0) represents the highest value among all microorganisms tested. This effect remained measurable at a dilution of 10^9 (6.8 mm), with a gradual decrease observed despite increasing dilution. These results indicate the strong and persistent effect of bee venom on E. coli in particular.

Among the Gram-positive bacteria, *Enterococcus faecalis* ATCC 29212 showed the second highest sensitivity with an initial zone of inhibition of 12.6 mm. Particularly noteworthy is the persistence of this effect up to the 10^9 dilution (6.6 mm). This continuity demonstrates the long-term sensitivity of E. faecalis to bee venom components. The initial zone of inhibition of 12.2 mm for *Staphylococcus aureus* ATCC 25923, which persists up to a dilution of 10^7 (7.0 mm), also confirms the general susceptibility of Gram-positive bacteria to bee venom.

The susceptibility profile of *Candida albicans* ATCC 10231, which was tested as a yeast pathogen, is particularly interesting. Although it initially showed a similar zone of inhibition (12.2 mm) to Gram-positive bacteria, this effect rapidly decreased with increasing dilution and disappeared completely after 10^3 dilution. These results indicate that the antifungal activity of bee venom is concentration-dependent and is stronger at higher concentrations.

Pseudomonas aeruginosa ATCC 27853 showed the lowest sensitivity among the microorganisms tested. The formation of an inhibition zone of only 7.6 mm even at the initial concentration and the minimal continuation of this effect (6.2 mm) up to the 10^6 dilution reflects the characteristic resistant structure of P.

aeruginosa. This limited effect can be explained by the complex outer membrane structure of P. aeruginosa and the presence of multiple drug efflux pumps.

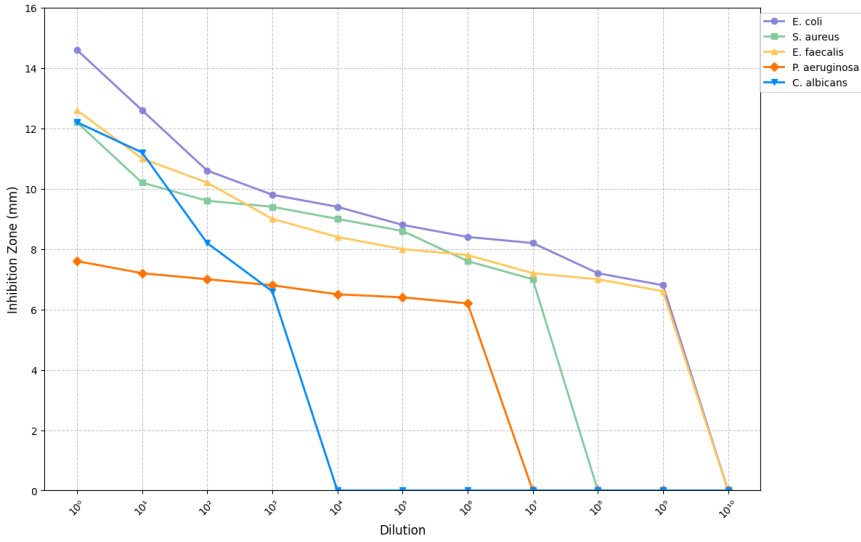

***Figure 4**: Inhibition zones of A. m. carpathica venom at different dilutions*

Statistical analysis

The results of one-way analysis of variance (ANOVA) and post hoc Tukey test showed that the differences in susceptibility between microorganisms were statistically significant ($p<0.05$). In particular, statistically significant differences were found between: E. coli and P. aeruginosa ($p<0.001$), Gram-positive bacteria and C. albicans ($p<0.01$) and E. coli and all other microorganisms ($p<0.01$).

These comprehensive results clearly demonstrate the organism-specific antimicrobial efficacy of A. m. *carpathica* bee venom. In particular, its strong effect on E. coli and its persistent activity against Gram-positive bacteria provide important data supporting the potential therapeutic applications of this natural bioactive substance.

DISCUSSION

Antimicrobial resistance is one of the greatest challenges facing modern medicine and necessitates the discovery and development of new and effective antimicrobial agents (El-Seedi et al., 2020). In this study, the antimicrobial activity of *Apis mellifera carpathica* bee venom against five different microorganisms was investigated and the results were analyzed in conjunction with existing findings from the literature.

In our study, the strain *Escherichia coli* ATCC 25922 was found to have the highest sensitivity to bee venom. The zone of inhibition of 14.6 mm obtained is

in agreement with the results of Zolfagharian et al. (2016). The synergistic effects of melittin and phospholipase A2 on the Gram-negative bacterial cell membrane explain this high efficacy (Yaacoub et al., 2022). In particular, the effects observed even at high dilutions (10^9, 6.8 mm) indicate that melittin has a mechanism that prevents the development of resistance.

The inhibition zones of 12.6 mm and 12.2 mm observed in *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923, respectively, demonstrate the general susceptibility of Gram-positive bacteria to bee venom. These results are consistent with the studies by Baracchi and Turillazzi (2010), who demonstrated the efficacy of venom components such as melittin and apamin on Gram-positive bacteria.

The antifungal activity observed with *Candida albicans* ATCC 10231 was effective at high concentrations, but decreased rapidly with increasing dilution. This result indicates that antifungal efficacy is concentration dependent and requires optimal dose determination (El Mehdi et al., 2021).

The lowest sensitivity was found for the strain *Pseudomonas aeruginosa* ATCC 27853. The resistant structure of this microorganism was associated with its outer membrane structure and the efficacy of several drug pumps (efflux pumps). This result is in line with the study by de Roodt et al. (2020), in which resistance profiles were investigated in the context of geographical differences.

The antimicrobial effect of bee venom is explained by the direct attack of its components on the cell wall of the microorganism and the interruption of metabolic processes (Nam et al., 2003). While melittin leads to an intracellular imbalance by forming pores in the bacterial membranes, phospholipase A2 enhances its effect by degrading membrane phospholipids. The results of Sciani et al. (2010) regarding the activity of melittin isoforms explain the organism-specific efficacy profiles observed in our study.

In this study, the effect of bee venom from A. m. *carpathica* was investigated especially on pathogens with a high potential to develop antibiotic resistance. The results showed that bee venom has significant antimicrobial potential against both Gram-positive and Gram-negative bacteria. Bee venom, which is highly effective against clinically important pathogens such as E. coli, is considered an important candidate for the development of new strategies against antibiotic resistance.

The results of Zolfagharian et al. (2016), who report that bee venom is as effective as gentamicin, are consistent with the high inhibition zone measurements in our study.

As Ferreira Júnior et al. (2010) emphasize that venom components may vary according to environmental and genetic factors, the geographical and genetic

characteristics of the bee venom samples used in this study should be taken into account.

In this study, the specific ratios of venom components (e.g. melittin and phospholipase A2) were not measured. This limitation has restricted the understanding of which components are responsible for the observed effect. In addition, comparative analyzes of time-dependent killing kinetics with standard antibiotics were not performed. Such analyzes are important for a more comprehensive evaluation of the therapeutic efficacy of bee venom.

Conclusions and recommendations

This study has shown that the bee venom of *Apis mellifera carpathica* has an organism-specific effect and significant efficacy against Gram-negative bacteria. Especially in the current era where antibiotic resistance is a serious threat, the evaluation of bee venom as an alternative antimicrobial agent is promising.

Future studies should focus on the following areas: In vitro and in vivo studies are needed to clarify the molecular mechanisms of venom components in detail. Standardization of venom preparations for therapeutic use and determination of clinical dose ranges are required. The investigation of synergistic combinations of bee venom with other antimicrobial agents could increase the therapeutic potential.

In conclusion, the potent antimicrobial effects of *Apis mellifera carpathica* bee venom and its suitability for clinical applications should be supported by more comprehensive and versatile research. A proper evaluation of this natural bioactive substance could provide an effective solution to the problem of antimicrobial resistance in modern medicine.

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Chapter 6

A view of Coherent Elastic Neutrino-Nucleus Scattering and beyond Standard Model: Neutrino Electromagnetic Properties

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1. Introduction

The coherent elastic neutrino-nucleus scattering (CEνNS) was first observed in 2017 by the COHERENT collaboration [1]. It is more than 40 years after the theoretical description of the process was proposed [2]. In the standard model (SM), CEνNS is a process where neutrinos scatter off a nucleus as a whole. It is explained by interactions of neutrinos and quarks via the neutral current. The cross section of the process is proportional to the neutron number squared. Its cross-section is larger than others interaction involving neutrinos at the same energy regime. Observing this process is difficult since its nuclear recoil energy located in the low keV scale, which need a sophisticated detector technology. The nuclear recoil energy is the only quantity of the process that can be observed.

In its first observation, COHERENT utilized neutrinos from pion decay at rest (π-DAR) at a Spallation Neutron Source (SNS). The collaboration used a CsI[Na] target as scintillating crystal detector in the first discovery and then advanced it using a liquid argon (LAr) target [3]. They recently updated the CsI analysis, improving statistics along with systematic of the experiment [4]. These milestones from COHERENT trigger many other scientific activities to investigate the CEνNS process. At the nuclear power reactor, the Dresden-II collaboration [5] reported compatible spectrum with CEνNS prediction. Other reactors such as CONUS [6] CONNIE [7] still trying to observe CEνNS events and able to extract upper limits compatible with the SM prediction so far. Significant role is played by direct detection (DD) dark matter (DM) experiments in measuring the process from astrophysical sources, such as the PandaX [8] and XENON [9]. The CEνNS significantly contributes in the experimental background since it has a similar prediction with the proposed DM candidate that generally called weakly interacting massive particle (WIMP). Both facilites recently reported their results on CEνNS signals triggered by ^{8}B solar neutrino flux [10, 11]. Moreover, there are also planned facilities that aim to detect CEνNS, for example NUCLEUS [12] and the European Spallation Source [13].

The CEνNS process provides a unique framework to examine SM predictions as well as to search for beyond the SM (BSM) physics. The process is used to study various topics such as the weak mixing angle measurement at low energy [14, 15], effective generalized interactions [16], the non-standard neutrino interactions (NSI) [17, 18], the light mediators [19, 20, 21], and the neutrino electromagnetic properties [22, 23]. Moreover, CEνNS provides useful information to extract nuclear structure, especially the nucleus neutron density distribution [24] which is an active area of research. The only signal of a CEνNS event in most experiments is a deposition of nuclear recoil energy. Future experiments are expected to detect simultaneously both nuclear recoil and angular

distribution with the advancement of detector technologies. This progress makes exploring CEνNS will be one of the rich tools in the future.

This work aims to review progress of the CEνNS process and effect of neutrino electromagnetic properties on it. We first explain formulation of CEνNS in the SM in Section 2. In Section 3, we describe the possible use of CEνNS to investigate neutrino electromagnetic properties. We present the available limits on neutrino electromagnetic properties in Section 4 and summarize results in Section 5.

2. Coherent Elastic Neutrino-Nucleus Scattering

The CEνNS process is well predicted in the framework of the SM. The process occurs as neutrinos with initial energy E_ν scatter off a nucleus target. The scattered nucleus carries a kinetic recoil energy T_{nr} in a few keV. The process is a pure quantum effect where the initial neutrino has a small enough energy so that it is unable to see the nucleon structure. This generally happens when the transfer momentum $\vec{q} \lesssim R$, where R represents the typical nuclear size. The process hence provides enhancement of cross-section in low energy nuclear recoil region.

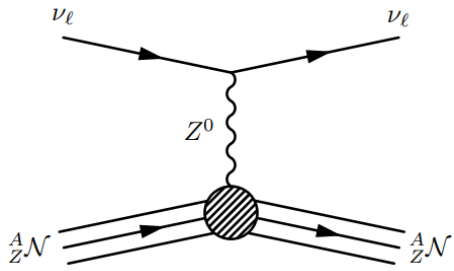

Figure 1. Representative Feynman diagram for CEνNS process in the SM.

The Feynman diagram of CEνNS in the SM is shown in Fig. 1. The factor ${}^A_Z N$ represents a nucleus with A nucleons (Z protons and N neutrons) and Z^0 is the SM neutral vector boson. The subscript ℓ denotes the neutrino flavor e, μ or τ. As neutrino interacts with the nucleus as a whole in CEνNS, the cross-section is much larger than those of other processes such as charged-current (CC) neutrino interactions or neutrino-induced neutron (NIN), inverse beta decay (IBD), and elastic scattering of ν_e on electrons [25]. The differential cross section for CEνNS in the SM with respect to the nuclear recoil energy is given by

$$\left[\frac{d\sigma}{dT_{nr}}\right]_{\text{SM}} = \frac{G_F^2 m_{\mathcal{N}}}{\pi} Q_{\text{SM}}^2 \left(1 - \frac{m_{\mathcal{N}} T_{nr}}{2E_\nu^2}\right) |F(|\vec{q}|^2)|^2. \quad (2.1)$$

where G_F represents the Fermi constant, m_N is the nucleus mass, and $F(|\vec{q}|^2)$ is the weak nuclear form factor. The weak nuclear charge is defined by $Q_{\rm SM} = g_V^p Z + g_V^n N$, where the vector couplings are given by $g_V^p = (1 - 4sin^2\theta_W)/2$ and $g_V^n = -1/2$ for proton and neutron, respectively. We use $sin^2\theta_W = 0.23863$ [26] for the weak-mixing angle, which is obtained at low-momentum transfer in the $\overline{\rm MS}$ scheme. The SM cross section of CEνNS is N^2 dependent due to the small prefactor associated with Z in $Q_{\rm SM}$, and flavor blind at the tree level. Note that the cross-section Eq. (2.1) is valid for both neutrino and anti-neutrino cases. The weak nuclear form factor $F(|\vec{q}|^2)$ contains the complex structure of the target nucleus. The form factor difference for proton and neutron is generally considered to be negligible, hence both form factors are taken to be equal, namely $F_p \simeq F_n \simeq F$.

Any deviation from the predicted SM CEνNS event rate may indicate new contributions to the interaction cross section, either by a change in the total event rate or by a change in the shape of the recoil spectrum. The CEνNS event rate is calculated in terms of nuclear recoil energy as

$$\frac{dR}{dT_{nr}} = N_T \int_{E_\nu^{\rm min}}^{E_\nu^{\rm max}} \frac{d\Phi(E_\nu)}{dE_\nu} \frac{d\sigma(E_\nu, T_{nr})}{dT_{nr}} dE_\nu, \tag{2.2}$$

where N_T is the number of nuclei in the detector per unit mass and $d\Phi(E_\nu)/dE_\nu$ is the neutrino flux per cm^2 per second from a particular source. The integration is taken from the minimum neutrino energy $E_\nu^{\rm min}$ to the maximum neutrino energy $E_\nu^{\rm max}$. The minimum neutrino energy is given by $E_\nu^{\rm min} = T_{nr}\left(1 + \sqrt{1 + 2m_{\mathcal{N}}/T_{nr}}\right)/2$. Note that in the detector, the observed energy is in the electron-equivalent recoil energy T_{ee}. To transform this into the nuclear recoil T_{nr} when calculating the predicted CEνNS rate, we need to use the following relation

$$T_{ee} = f_Q(T_{nr}) T_{nr}. \tag{2.3}$$

In this relation, f_Q represents the quenching factor. This dimensionless quantity is defined as the ratio of the ionization energy generated by nuclear recoils over the one generated by electron recoils of the same energy. In this conversion process, most of the initial energy is lost in dissipative processes and, thus, not accessible. The quenching factor has been extensively measured for nuclear recoils in a few tens of keV range where it follows the Lindhard parametrization [27]. It is further founded that the Lindhard quenching factor is well used for energy $T_{nr} \gtrsim 1$ keV [28]. However, for sub-keV nuclear recoils, the quenching factors are not well predicted by this model due to uncertainties in nuclear scattering. Precise measurements of this factor at these energies are still

lacking and the Lindhard theory is not valid anymore. Because of this, there are various quenching factors in the literature that could effect the measured results. For example, one proposes modification of the Lindhard theory [29] and other uses experimental data of detector calibration which called the Iron-filtered [30] quenching factor. Therefore, the determination of this quenching factor in this range is tremendously important to improve experimental sensitivity and to precisely measure the CEνNS signals in the future.

3. Beyond the Standard Model: Neutrino electromagnetic properties

Most of the experimentally known properties of neutrinos are consistent with the SM of electroweak interactions. The most important exception is that neutrinos have mass, as discovered in oscillation experiments, contrary to the SM prediction. Therefore, the SM must be extended to accommodate neutrino masses. Moreover, it has been well-known for quite many years that massive neutrinos should have electromagnetic properties. It was first mentioned by Pauli in 1930 when he postulated the existence of neutrino and discussed the possible occurrence of neutrino magnetic moment. Even though up to now there are no indications in favor of these properties, neither from terrestrial experiments nor from astrophysical observations, the electromagnetic properties of neutrinos are a popular issue related to neutrinos and this problem has been discussed many times in recent literature [22]. The electromagnetic properties of neutrino have potential to induce significant effects which can be probed in CEνNS framework.

Neutrino electromagnetic properties can play an important role since they are connected directly to the fundamentals of particle physics. These quantities can be used to distinguish whether neutrinos are Dirac or Majorana particles. The properties can also probe new physics that might exist beyond the standard theory.

Neutrino electromagnetic properties can be calculated though the effective one-photon coupling of neutrinos with the electromagnetic field, taking into account possible transitions between two different initial and final neutrinos (see Fig. 2). The matrix element of the electromagnetic current is written as

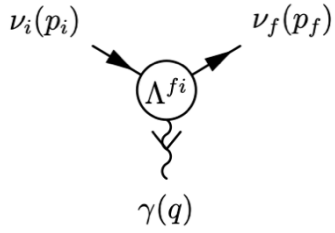

Figure 2. The effective vertex of the neutrino electromagnetic interaction

$$\langle \psi(p') | J_\mu^{em} | \psi(p) \rangle = \bar{u}(p') \Lambda_\mu(q, l) u(p), \tag{3.1}$$

where $\psi(p)$ is the initial fermion and $\psi(p')$ is the final fermion. The matrix element $\Lambda_\mu(q, l)$ between the spinors of the electromagnetic vertex function is a function of $q_\mu = p'_\mu - p_\mu$ and $l_\mu = p'_\mu + p_\mu$ and should be a Lorentz operator that satisfies the covariance requirement. In constructing the operator, recall that there are 16 linearly independent matrices that may be chosen, namely

$$1,\ \gamma_\mu,\ \gamma_5,\ \gamma_\mu\gamma_5,\ \sigma_{\mu\nu}. \tag{3.2}$$

Additionally, we can also use the metric tensor $g_{\mu\nu}$, the Levi-Civita anti-symmetric tensor $\epsilon_{\mu\nu\sigma\gamma}$, two vectors q_μ and l_μ. It is possible to reach the most general expression for the vertex [22]

$$\begin{aligned} \Lambda_\mu(q) = {} & f_1(q^2) q_\mu + f_2(q^2) q_\mu \gamma_5 + f_3(q^2) \gamma_\mu + f_4(q^2) \gamma_\mu \gamma_5 \\ & + f_5(q^2) \sigma q^\nu + f_6(q^2) \epsilon_{\mu\nu\sigma\gamma} \sigma^{\rho\gamma} q^\nu. \end{aligned} \tag{3.3}$$

Note that it is only now dependent on q. Since the current conservation and the electromagnetic gauge invariance need to be satisfied, we can further define the vertex function in terms of four form factors

$$\begin{aligned} \Lambda_\mu(q) = {} & f_Q(q^2) q_\mu + f_M(q^2) i\sigma_{\mu\nu} q^\nu + f_E(q^2) \sigma_{\mu\nu} q^\nu \gamma_5 \\ & + f_A(q^2) \left(q^2 \gamma_\mu - \gamma_\mu q^\nu \gamma_{\nu\gamma_5} \right). \end{aligned} \tag{3.4}$$

The right-hand side correspond to charge, dipole magnetic, dipole electric, and anapole neutrino form factors, respectively. We emphasize that the form factors are Lorentz invariant and depend on the only independent dynamical quantity q^2.

The form factors at zero momentum transfer, $q^2 = 0$, in any consistent theoretical model must be finite and gauge-independent. Hence, the form factor values at this point supply the static electromagnetic properties of the neutrino. The electromagnetic properties can be examined or measured in direct interaction with external electromagnetic fields. This is the case for charge, dipole magnetic, and electric neutrino form factors in the minimally extended SM, while the anapole form factor needs a further explanation since its toroidal interaction does not conserve the translation P and charge C parity. It is expected that these properties to contribute to the total cross-section of neutrino elastic scattering off electron, quark, or nuclei. In principle, these contributions can be investigated in scattering experiments at low energy such as CEνNS.

3.1 Neutrino magnetic moment

The dipole electric and magnetic moments (MM) are the most well studied and understood among the neutrino electromagnetic characteristics, which are defined by the relevant form factors at $q^2 = 0$; $\epsilon_\nu = f_E(0)$ and $\mu_\nu = f_M(0)$. For

a Dirac neutrino, the diagonal electric and magnetic moments in the minimally extended SM with right-handed neutrinos are derived [31] as

$$\epsilon_{ii} = 0, \quad \mu_{ii} = \frac{3e\, G_F m_i}{8\sqrt{2}\pi^2} \approx 3.2 \times 10^{-19} \mu_B \left(\frac{m_i}{1\ \mathrm{eV}}\right), \tag{3.5}$$

where μ_B is the Bohr magneton. According to (3.5), the value of the neutrino magnetic moment is very small. However, in many other BSM theories, the neutrino MM can reach values that are attractive for the next generation of terrestrial experiments and accessible for astrophysical observations.

The most sensitive investigation of neutrino electromagnetic properties can be achieved by direct scattering experiments at low energies. One such scattering process is the CEνNS process. The neutrino MM contribution to the CEνNS differential cross section is given by [32]

$$\left[\frac{d\sigma}{dT_{nr}}\right]_{MM} = \frac{\pi\alpha^2}{m_e^2} Z^2 |F(|\vec{q}|^2)|^2 \left|\frac{\mu_\nu}{\mu_B}\right|^2 \left(\frac{1}{T_{nr}} - \frac{1}{E_\nu}\right), \tag{3.6}$$

where α is the fine structure constant. This contribution is incoherently added to the SM CEνNS cross section.

3.2 Neutrino charge radius

Even if the electric charge of a neutrino is zero, the electric form factor $f_Q(q^2)$ could still involve nontrivial information on static properties of neutrino. The mean charge radius of a neutrino (CR) is defined by

$$\langle r_{\nu_\ell}^2 \rangle = 6 \left.\frac{df_Q(q^2)}{dq^2}\right|_{q^2=0}. \tag{3.7}$$

In the SM, the neutrino charge radius (CR) is theoretically generated by one-loop radiative corrections from the γ and Z boson mixing and also box diagrams involving W and Z bosons. Taking into account these corrections, the neutrino charge radius in the SM is given by [22]

$$\langle r_{\nu_\ell}^2 \rangle = \frac{G_F}{4\sqrt{2}\pi^2}\left[3 - 2\log\left(\frac{m_\ell^2}{m_W^2}\right)\right], \tag{3.8}$$

where the charge lepton mass is denoted as m_ℓ and the mass of W boson is denoted by m_W. The numerical values are

$$\begin{aligned} \langle r_{\nu_e}^2 \rangle &= -0.83 \times 10^{-32}\ \mathrm{cm}^2, \\ \langle r_{\nu_\mu}^2 \rangle &= -0.48 \times 10^{-32}\ \mathrm{cm}^2, \\ \langle r_{\nu_\tau}^2 \rangle &= -0.30 \times 10^{-32}\ \mathrm{cm}^2. \end{aligned} \tag{3.9}$$

Contribution of CR to the CEνNS is obtained by substituting [33]

$$Q_\ell^{CR} = \frac{\sqrt{2}\pi\alpha}{3G_F}\langle r_{\nu_\ell}^2\rangle \tag{3.10}$$

to $g_V^p \to g_V^p - Q_\ell^{CR}$ of the SM cross-section formula (2.1).

3.3 Neutrino electric charge

Neutrinos are constrained to be exactly neutral in the SM with only one generation by the cancellation of the quantum axial triangle anomalies. Instead, in the three-generation SM, two neutrinos could have opposite charges, and the third must be neutral. In particular, massive Dirac neutrinos can have diagonal neutrino charges.

The contribution of neutrino milli charge (MC) to the CEνNS is obtained by substituting $g_V^p \to g_V^p - Q_\ell^{MC}$ into SM cross-section formula (2.1). The MC contribution is given by [30]

$$Q_\ell^{MC} = \frac{2\sqrt{2}\pi\alpha}{G_F q^2} q_{\nu_\ell} \tag{3.11}$$

where the milli charge is denoted by q_{ν_ℓ}.

3.4 Neutrino anapole moment

Lastly, the neutrino can also carry anapole moment. Physically, it determines the correlation between the spin and charge distributions of neutrino has the same dimensions as that of the charge radius. The prediction for the neutrino anapole moment in SM can be expressed in terms of the neutrino charge radius [34]

$$a_{\nu_\ell} = -\frac{\langle r_{\nu_\ell}^2\rangle}{6}. \tag{3.12}$$

In the SM CEνNS cross-section, the anapole contribution is obtained by the substitution $g_V^p \to g_V^p - Q_\ell^A$ with

$$Q_\ell^A = -\frac{\sqrt{2}\pi\alpha}{18G_F} a_{\nu_\ell}. \tag{3.13}$$

Notice that, unlike neutrino milli charges, the anapole moment and the charge radius do not have a direct dependence on the inverse nuclear recoil energy and on the target mass. Their contributions are hence relatively less sensitive at low recoil energy.

4. Numerical Results

We present the predicted event rate from contributions of the neutrino magnetic moment, neutrino milli-charge, and neutrino charge-radius to the CEνNS in Fig. 3, Fig. 4, and Fig. 5, respectively. In these figures, the solid lines are for Ge and the dashed lines are for Xe targets. For the neutrino magnetic

moment, as benchmarks we set $\mu_{\nu_\ell} = 2 \times 10^{-10}\mu_B$ (square) and $\mu_{\nu_\ell} = 1 \times 10^{-9}\mu_B$ (circle). As for neutrino milli-charge, we set $q_{\nu_\ell} = 2 \times 10^{-9}e$ (square) and $q_{\nu_\ell} = 1 \times 10^{-8}e$ (circle). For neutrino charge-radius we set $\langle r_{\nu_\ell}^2 \rangle = 1 \times 10^{-32} cm^2$ (square) and $\langle r_{\nu_\ell}^2 \rangle = 1 \times 10^{-31} cm^2$ (circle).

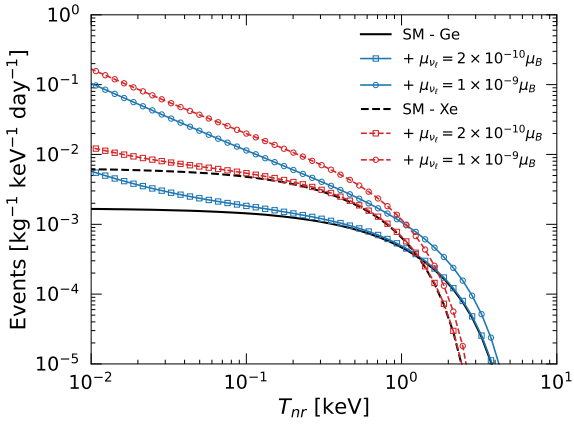

Figure 3. The predicted event rates of neutrino magnetic moment contributions to CEνNS for Ge and Xe targets.

We consider the ^{8}B solar neutrino flux with Ge and Xe targets. The heavier Xe target can be seen to have a higher contribution than the Ge target which is caused by the CEνNS which has a dependency on the number of neutron squares of the target nuclei. Regarding the benchmarks, the values are chosen to show the effects of each electromagnetic property of neutrino to the CEνNS process in the SM.

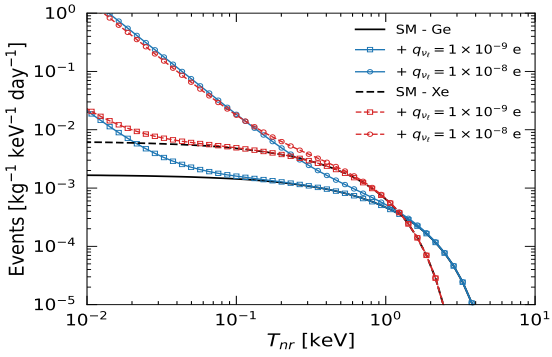

Figure 4. The predicted event rates of neutrino milli-charge contributions to CEνNS for Ge and Xe targets

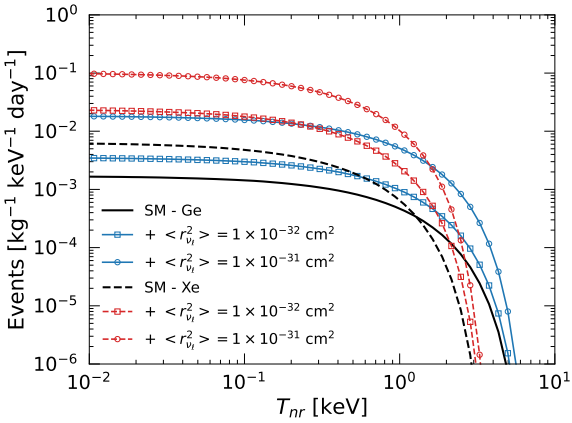

Figure 5. The predicted event rates of neutrino charge-radius contributions to CEνNS for Ge and Xe targets.

It is clear from these figures that the spectrum enhancements at low recoil energy in the case when neutrino MM and MC are present. In both MM and MC a pronounced enhancement of the event rate at low recoil energies due to the inverse dependency of T_{nr} from each electromagnetic property. In the MM, the $\mu_\nu = 2 \times 10^{-10}\mu_B$ show enhancements in the low energy region and it has no effect on the SM process in the high T_{nr} scale while the $\mu_\nu = 1 \times 10^{-9}\mu_B$ indicates an improved spectrum in all spectrum. A similar effect to the SM is shown in the MC for the benchmarks; prominent improvements are shown for $q_{\nu_\ell} = 1 \times 10^{-8}$ e than $q_{\nu_\ell} = 1 \times 10^{-9}e$ in the low energy region. As for the CR case, the constant contribution can be seen in all the energy ranges. This is anticipated from Eq. (3.10) which has no T_{nr} dependency. Regarding the benchmarks, the $\langle r^2_{\nu_\ell} \rangle = 1 \times 10^{-32}\text{cm}^{-2}$ and $\langle r^2_{\nu_\ell} \rangle = 1 \times 10^{-31}\text{cm}^{-2}$ have contributions to the SM case of each nuclear target for approximately half and an order of magnitude, respectively. All of these signify dark matter detectors with their low recoil energy thresholds ideal tools to investigate neutrino electromagnetic properties.

We next present various prominent limits of each neutrino electromagnetic property, obtained at 90% C.L. in Table 1. The results are from different kind of facilities, such as stopped-pion experiment of COHERENT derived in [35], neutrino beam laboratories of LSND [36] and CHARM-II [37], nuclear reactors of Dresden-II [5] and TEXONO [38], solar neutrino experiment of BOREXINO [39] derived in [40], as well as DD experiments of XENONnT [41], PandaX-4T [42], and LZ [43] derived in [44, 45].

Table 1. Upper limits on the neutrino electromagnetic properties from some experiments.

Properties	Experiment	Limit	Ref.
Magnetic moment (μ_B)	COHERENT	$\lvert\mu_{\nu_e}\rvert \lesssim 3.6 \times 10^{-9}$	[19]
	LSND	$\mu_{\nu_\mu} \lesssim 6.8 \times 10^{-10}$	[36]
	Dresden-II	$\lvert\mu_{\nu_e}\rvert \lesssim 2.1 \times 10^{-11}$	[35]
	BOREXINO	$\mu_{\nu_e} \lesssim 3.7 \times 10^{-11}$	[40]
	PandaX-4T	$\lvert\mu_{\nu_\ell}\rvert \lesssim 2.8 \times 10^{-11}$	[45]
	LZ	$\lvert\mu_{\nu_\ell}\rvert \lesssim 1.2 \times 10^{-11}$	[45]
	XENONnT	$\lvert\mu_{\nu_\ell}\rvert \lesssim 8.4 \times 10^{-12}$	[45]
Milli charge (e)	COHERENT	$q_{\nu_e} \in [-6.9, 5.6] \times 10^{-8}$	[19]
	Dresden-II	$\lvert q_{\nu_e}\rvert \lesssim 1.2 \times 10^{-11}$	[35]
	PandaX-4T	$-12.6 \times 10^{-13} < q_{\nu_e} < 16.4 \times 10^{-13}$	[45]
	LZ	$-3.0 \times 10^{-13} < q_{\nu_e} < 6.0 \times 10^{-13}$	[44]
	XENONnT	$-1.0 \times 10^{-13} < q_{\nu_e} < 6.0 \times 10^{-13}$	[44]
Charge Radius (cm^2)	PandaX-4T	$-134.5 \times 10^{-32} < \langle r_{\nu_e}^2 \rangle < 48.2 \times 10^{-32}$	[45]
	LZ	$-110.4 \times 10^{-32} < \langle r_{\nu_e}^2 \rangle < 26.4 \times 10^{-32}$	[45]
	Dresden-II	$\langle r_{\nu_e}^2 \rangle \in [-64.8, -42.4] \cup [-10.3, 12.2] \times 10^{-32}$	[35]
	XENONnT	$-93.4 \times 10^{-32} < \langle r_{\nu_e}^2 \rangle < 9.5 \times 10^{-32}$	[44]
	LSND	$-5.94 \times 10^{-32} < \langle r_{\nu_e}^2 \rangle < 8.28 \times 10^{-32}$	[36]
	TEXONO	$-4.2 \times 10^{-32} < \langle r_{\nu_e}^2 \rangle < 6.6 \times 10^{-32}$	[38]
	COHERENT	$\langle r_{\nu_e}^2 \rangle \in [-61.2, -48.2] \cup [-4.7, 2.2] \times 10^{-32}$	[19]
	CHARM-II	$\left\lvert \left\langle r_{\nu_\mu}^2 \right\rangle \right\rvert < 1.2 \times 10^{-32}$	[37]

It is observed that that results from dark matter DD facilities indicate the most stringent limits for MM and MC properties of neutrino. This behavior comes from the detector in the DD facilities that can probe low electronic recoils. Meanwhile, the limits for charge-radius are generally in the same order where the CHARM-II result indicates the most stringent limit among other facilities for now. Experimental advancement in the near future will be an interesting ground to update these neutrino electromagnetic properties.

5. Summary

We have discussed the CEνNS process in the SM and effect of neutrino electromagnetic properties on it. We have presented the standard differential cross-section of the process with the form factor effect and the role of the quenching factor. We have next explained the neutrino electromagnetic properties as new physics phenomena. These properties emerge as implications of non-zero

neutrino mass. The existence of them may be tested in low-energy experiments involving the CEνNS process. We have presented the predicted effects of these properties for some benchmarks utilizing solar neutrino flux with different targets. Then, we have provided various available limits on the neutrino electromagnetic properties in the literature. All neutrino electromagnetic properties have fairly stringent upper limits resulting from laboratory experiments or astrophysical observations. Results from DM direct detection experiments indicate stringent constraints due to their lower recoil energy thresholds, originated from the low energy of the detected neutrino. Finally, it is critical to continue experimental and theoretical studies of electromagnetic neutrino properties, which could open the door to new physics beyond the SM. Further astounding breakthroughs concerning this topic might be ahead in the upcoming years.

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Chapter 7

Important σ-Donor Ligands Used in Platinum Chemistry

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Introduction

The first known use of platinum was in ancient Egypt, when impure local platinum was unknowingly used by the masters of the time. It is also known that the Indians of Ecuador used it before the Spanish conquest to make ornaments, jewellery and small items. The definition of platinum as a metal in Europe began in 1736, when it was observed in Colombia by the Spanish astronomer and naval officer A. de Ulloa, who described it as an impossib-le-to-work metal, A. de Ulloa named the platinum he found in the gold mines "platina" (Spanish for small silver) [1], [2]. It is commonly recognized that platinum has been essential to the advancement of several scientific fields. Although platinum may exist in a variety of oxidation states ranging from zero to six, the most prevalent ones are +2 and +4, especially in coordination chemistry [3].

Sigma-donor ligands, as used in coordination chemistry, are ligands that contribute electron density to a metal center via a sigma bond, usually using lone pairs of electrons from atoms such as oxygen, nitrogen, sulphur or phosphorus. Because it can form stable coordination bonds with these ligands and has a relatively high electron density, platinum (Pt), a transition metal, is very reactive with them. These ligands are of great importance in platinum complex chemistry, mainly because of their distinct steric and electronic properties, which affect the general behavior, stability and reactivity of the complexes in a wide variety of chemical reactions. The importance of sigma donor ligands in platinum complexes can be considered from a number of perspectives, including their function in drug design, catalytic applications and coordination behavior. The importance of sigma donor ligands in platinum complexes can be analyzed from a number of perspectives, including their role in coordination behavior, drug design and catalytic applications [4,5]. The following types of sigma donor ligands are commonly found in platinum complexes:

1. Phosphines

One of the most common sigma donors in platinum chemistry is the phosphorus ligand (PR_3). The single electron pair of the phosphorus atom effectively donates to platinum, stabilizing the metal center and increasing the reactivity of the complex for catalytic reactions [6,7]

Phosphine ligands are monodentate, neutral ligands with the generic formula PR_3, where R can be any of a variety of substituents including mixed, aryl or alkyl groups. The ability to modify electronic and steric properties by choosing the right substituents accounts for their adaptability. For example, alkyl groups that donate electrons increase σ-donation, whereas aryl groups that extract electrons decrease it. Bulky substituents cause steric hindrance, which can have a major

effect on the shape and reactivity of the metal complex. For example, the proportion of s in the phosphorus lone pair decreases as the angle between the substituents increases. Bond lengths and angles can also be affected by changes in the electronegativity of an atom. As a result, it is difficult to make a pure distinction between steric and electronic effects. However, the parameters stretching frequencies (v) and cone angles (Ɵ) can be used to make a useful and practical distinction. Phosphine-containing platinum(II) complexes, such as $[PtCl_2(PR_3)_2]$, have a square planar shape characteristic of d^8 metal centers. The stability of the cis and trans isomers in the complexes is influenced by the steric and electronic properties of the PR_3 ligands. In catalytic and medicinal chemistry, cis-$[PtCl_2(PR_3)_2]$ is very important [8]. Strong electron-donating properties of trialkylphosphines, such as PMe_3, encourage oxidative addition processes. Triarylphosphines, such as PPh_3, stabilize complexes by π-backbonding interactions despite being weaker σ-donors. The character of the donor orbital S increases in the following order $PF_3 > P(OR)_3 > PR_3$. The -donor orbital (lone pair) is left with a greater proportion of phosphorus 3s character because the more electronegative substituents use a greater proportion of the available phosphorus 3p orbitals in bonding.

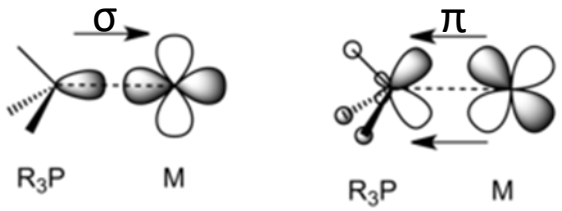

Figure 1. σ-bonding for R_3P–M and π-back bonding for M–R_3P

Phosphine ligands are also π-acceptors. The phosphor-carbon (P-C) σ*-antibonding orbitals overlap with the full metal orbitals, resulting in their π acidity (**Figure 1.**). Compared to alkyl phosphines, aryl and fluorophosphines are more potent π acceptors. Similar to the carbonyl ligand, trifluorophosphine (PF_3) is a potent π-acid with binding properties [9]. Although d-orbitals on phosphorus are now recognised to be irrelevant to bonding, early research suggested that phosphine ligands use 3d orbitals to form metal-phosphorus (M-P) pi-bonds. Because phosphines with electronegative substituents have lower σ*-orbital energies, phosphorus trifluoride is a particularly good π-acceptor [10, 11].

Complexes of phosphorus and platinum (P-Pt) are of great importance in the field of catalysis because of their remarkable activity, selectivity and adaptability. The binding of platinum centers to phosphorus ligands, including phosphines (PR_3), produces highly tunable complexes capable of catalysing a wide range of

reactions. Platinum complexes, such as $Pt(PPh_3)_4$ or other phosphine-platinum complexes, also act as catalysts in reactions such as hydrosilylation, hydroformylation and hydrogenation. The versatility of the phosphine ligand in changing the electronic environment of platinum leads to highly selective catalytic processes. The catalytic activity and selectivity of square planar Pt(II) complexes in the hydrosilylation of 1-alkenes can be regulated by modifying the steric and electronic properties of neutral and anionic ligands (e.g., donor-acceptor ability of neutral ligands) [12].

Similar to 1-heptene hydrosilylation, phosphine ligands also influence the properties of platinum catalysts in the siloxane systems considered: As the -donor ability of the ligands increases, the induction time and the hydrosilylation selectivity increase in the order $Ph_3P < PhMe_2P < Bu_3P$. The reaction rate decreases slightly but changes insignificantly in the following order: cis-$[Pt(PMe_2Ph)_2Cl_2]$ > cis-$[Pt(PPh_3)_2Cl_2]$ > cis-$[Pt(PBu_3)_2Cl_2]$ [13].

2. Amines

Amines (NR_3) form stable platinum complexes when the nitrogen provides electron density from its lone pair. The trigonal pyramidal structure with bond angles of 109.5° is the result of sp^3 hybridisation in the central nitrogen. Whether the amine is primary, secondary or tertiary and any substituents on the amine group determine the amount of electron donation. Despite their tendency to be weaker sigma donors than phosphines, amines are still very important in tuning the electrical environment of platinum complexes [14]. Primary amines (RNH_2) contribute electron density via the nitrogen lone pair. In catalytic cycles, their ability to stabilize platinum intermediates is critical for processes such as hydrogenation and carbon-carbon bond formation. The inductive effects of the alkyl groups attached to the nitrogen make secondary and tertiary amines (R_2NH, R_3N) often more electron donating than primary amines. Platinum complexes can benefit from improved stability and reactivity influenced by secondary and tertiary amines. They are efficient catalysts in a variety of processes, including hydrogenation, C-H activation, and cross-coupling, due to their ability to modify the electrical properties of platinum complexes by donating electron density to the platinum core [15].

The resonance stability of the π-electron systems and the steric repulsion of protons from nearby phenyl rings are the two opposing forces that determine the molecular geometry of triphenylamine (TPA) derivatives, a family of adaptable redox-active compounds that have attracted particular interest due to their encouraging hole transport properties. Compounds containing nitrogen, including

amines, enamines and imines, are useful and important to the pharmaceutical and speciality chemical industries [16].

Platinum complexes are particularly important in the fight against cancer. In 1969, Rosenberg and his colleagues discovered the anti-tumour activity of $PtCl_2(NH_3)_2$, commonly known as 'cisplatin' (**Figure 2.**), which revived interest in platinum complexes and led to the study of a significant number of structures in platinum chemistry. Platinum complexes with amine ligands target cancer cells by binding to their DNA and preventing them from multiplying. The effectiveness of cisplatin in treating a range of malignancies, including lung, bladder, ovarian and testicular tumours, has rekindled interest in the therapeutic uses of platinum compounds. DNA-damaging platinum-based anticancer drugs such as carbocationin and cisplatin have become mainstays of clinical chemotherapy regimens [17]. As a result, second- and third-generation platinum drugs, such as oxaliplatin and carboplatin (**Figure 2.**), have been developed to reduce side effects and increase efficacy. The disciplines of medicinal and inorganic chemistry have also benefited greatly from these investigations. In conclusion, the development of platinum and other transition metal-based compounds with higher anticancer effects has significantly improved cancer treatment [18-20].

3. Carboxylates and Phosphates

Carboxylates, like acetate (CH_3COO-), are ligands that can bind through the oxygen atoms as either bidentate or monodentate. By contributing electron density to the empty orbitals of the metal, carboxylates, which are sigma donors, stabilize platinum complexes. While they are less efficient as electron donors than neutral ligands like ammonia, they are nonetheless useful in mediating catalytic reactions like olefin polymerization and hydroformylation. Carboxylate ligands are also commonly used to add pi-interactions and sigma-donating properties to platinum complexes [21].

Carboxylates, as opposed to carbonate ligands, can enhance hydrolytic stability in platinum(IV) complexes, for instance. The regulated release of active medications in platinum-based chemotherapies may depend on this stability [22].

Research indicates that Pt(IV) complexes with two extra coordination sites provide more synthetic flexibility. The Pt(IV)-estrogen complex, which is created by conjugating an estrogen derivative onto a trans-Pt(IV) carboxylate structure via a succinate linker, has been demonstrated by Lippard et al. to sensitize estrogen receptor(+) human tumor cells to treatment. Additionally, Lippard suggested using trans-Pt(IV) carboxylate as a design framework for adding functional groups to compounds based on platinum. Because trans-Pt(IV) carboxylates have been shown to be quickly reduced by biological reducing agents like glutathione

under physiological settings, releasing functionalized ligands and producing cytotoxic Pt(II) species, this is an appealing synthetic approach from a design standpoint. Importantly, satraplatin, the prototype Pt(IV) complex, is still undergoing rigorous clinical evaluation after entering Phase III clinical trials in 2001 as an effective oral anticancer agent for the treatment of hormone-refractory prostate cancer [23].

Phosphates are also polydentate ligands with many oxygen atoms available for coordination, such as PO_4^{3-} or HPO_4^{2-}. By contributing significantly to the electron density, phosphates enhance the electron-rich nature of platinum complexes and provide a strong framework. This property is beneficial in catalytic processes that require a lot of electrons, such as CO_2 activation or hydrogenation. Furthermore, the carboxylate groups provide stability and tunability while allowing activation via photoreduction to release platinum(II) species, which are highly reactive. This concept is being explored in anticancer therapies [24].

Recent studies have shown that phosphates or carboxylates added to phosphine ligands improve the stability and electron-donating capacity of platinum complexes, thereby increasing their catalytic activity. These ligands can influence the conformation of the complex and often help to stabilize platinum in different oxidation states, including Pt(II) and Pt(IV). The ligands also stabilize reactive intermediates and promote bond activation by increasing the electron density at the platinum center. This property is important for the efficient hydrosylation of difficult substrates [22, 25].

3. Thiols and Thiolates

The strong bond between the sulphur atom and platinum gives thiols (R-SH) and thiolates (RS-) special sigma donor properties. Compared to oxygen and nitrogen, sulfur has a greater atomic radius and is softer, which enables these ligands to bind with platinum centers more selectively and frequently resulting in increased complex stability. Through the lone pair of the sulphur atom, both thiols and thiolates donate electron density, but because of the negative charge on the sulphur, thiolates (RS-) often donate more electron density than thiols. As a result, the platinum center becomes more nucleophilic, which promotes certain catalytic processes such as alkylation, hydrogenation and dehydrogenation. Depending on which alkyl group (R) is bonded to sulfur, thiols and thiolates can cause steric hindrance. This steric effect has the potential to change platinum's coordination geometry, which might affect its catalytic activity in selective C-H activation processes, for example [26].

Depending on their size, Brønsted basicity, and electrical properties, phosphorothiolato ligands interact as soft-soft chelates with a wide variety of donors.

Because of this distinction, phosphinothiolato species are distinct heterotopic ligands. These complexes are important for imaging and radiotherapy, hydrodesulfurization (HDS) and modeling of sulphur-containing metalloproteins [27].

4. Halides

Although often thought of as basic ligands, halides such as iodide, chloride and bromide are essential sigma donors in a variety of platinum complexes. By providing electron density to the platinum center, halide ligands stabilize reactive intermediates in catalytic cycles. William Christopher Zeise, a Danish scientist, made a revolutionary discovery in 1827 while studying the interaction between platinum and ethylene. He saw a yellow precipitate, which he later identified as a complex consisting of an ethylene molecule bound to a trichloridoplatinate(II) ($PtCl_3$) moiety - the first known organometallic compound, also known as Zeise's salt (**Figure 2.**)[28]. Catalytic methods are used in olefin polymerisation to convert monomeric olefins into polymers. The understanding of how olefins coordinate to metal centers was made possible by platinum halide complexes, such as Zeise's salt ($[PtCl_3(C_2H_4)]$-), which paved the way for the development of sophisticated polymerisation catalysts [29].

Platinum halide complexes are widely used in olefin hydroformylation processes [30]. The hydroformylation or oxo process produces aldehydes by adding a formyl group (CHO) and a hydrogen atom to an olefin. Platinum halide complexes, particularly in combination with tin halides, have been investigated as catalysts for this reaction. In their patent, John E. Poist et al. highlights the potential of platinum-tin systems in hydroformylation by describing a hydroformylation catalyst comprising a quaternary ammonium compound and a ligand-stabilized complex of platinum dihalide and tin halide [31].

The hydrosilylation process can also be carried out more easily, at lower temperatures and with higher yields using transition metal catalysts. Speier demonstrated the remarkable efficacy of chloroplatinic acid, H_2PtCl_6, commonly referred to as Speier's catalyst, as a precursor to hydrosilylation catalysts in 1957 [32]. A platinum(0) species produced by HSiR3-induced reduction of H_2PtCl_6 was thought to be the first step in the catalytic hydrosilation reaction cycle. In summary, platinum halide complexes are essential for hydroformylation and olefin polymerization and research is underway to improve their catalytic properties for use in industry [33,34].

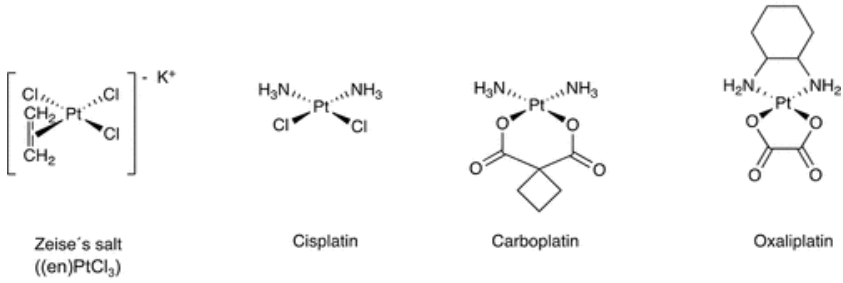

Figure 2. Zeise's Salt and Platinum(II)-Based Medicines (Cisplatin, Carbo-platin, Oxaliplatin)

In conclusion, novel platinum-based anticancer agents can still be found today by transforming platinum complexes according to traditional structure-activity relationships. It is no longer only possible to construct novel platinum complexes using the basic NH_3 as the carrier ligand, as the modification of the carrier ligand is the easiest change to discover new derivatives compared to alternative techniques [17]. The importance of σ-donor ligands in platinum chemistry is also emphasized by their role in stability, reactivity, bioactivity, and catalytic activity. The future development of environmentally friendly catalytic processes and new uses for platinum complexes will be possible through the creation of more complex and multifunctional types of these ligands. It is thought that these platinum compounds, which have a very important place in the field of coordination chemistry, will provide important scientific and economic benefits by increasing their therapeutic and industrial applications.

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Chapter 8

A Report on the Chlorine-based Compound Vapour Sensing Properties of Electrospun Polyacrylonitrile (PAN)-based Nanofibre-Coated Quartz Crystal Microbalances

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ABSTRACT

In recent years, the rapid growth of technology has made life easier for people. However, it has also been extremely harmful to human health and the environment. This includes air pollution and harmful solvents used in industry. In particular, exposure to the vapours of these solvents in the environment can cause serious health problems. Therefore, the detection of hazardous gases has become very important, and the choice of sensor material used to detect them is also very important. It has been reported in the literature that macrocyclic compounds are preferred as sensor materials in chemical sensing applications due to their various properties. This study presents i) the preparation of nanofibres (by electrospinning method) and morphological images of polyacrylonitrile (PAN) and polyacrylonitrile/polypyrrole (PAN/PPy) materials, ii) the comparison of the responses of PAN and PAN/PPy nanofibres prepared on quartz crystal to some chlorine-based noxious gases previously reported in the literature. As a result, the effect of the PPy material on the sensor performance is analysed.

Keywords: PAN/PPy; mass sensitive sensor; hazardous vapors.

1. INTRODUCTION

The environment consists of a social, biological, chemical, physical and economic environment in which humans and living things maintain their interrelationships throughout life. The negative effects of the development of technology, population growth and industrialisation cause pollution of the environment and natural resources (Li vd., 2020:6364). This pollution is increasing day by day. Pollution of air, water and soil is harmful to animals and plants as well as to human health through the food we eat (Woellner vd., 2018:1704679). Air pollution has been properly addressed and monitored around the world for the past 30 years. However, it appears that air pollution is still not at safe levels. Fuels used for domestic heating, motor vehicles and especially industrial plants are the main sources of air pollution worldwide. Air pollution is on the increase day by day as a result of regional development, urban growth and excessive energy consumption. The most vulnerable to air pollution are the elderly, pregnant women, children and people with chronic illnesses. The risk of death or serious illness is particularly high in children, whose lungs are not fully developed. This is because they breathe faster, have a weaker immune system and spend more time outdoors (Akal, 2013:112; Çomunoğlu vd., 2009:33; Kotb vd., 2013:411).

Industrial activities, which are increasing with the development of technology, play an important role among the causes of air pollution. In addition, Volatile Organic Compounds (VOCs) are emitted into the atmosphere from industrial, agricultural, petrochemical, and industrial gases. The toxicity, the concentration and the duration of exposure to VOCs have an impact on human health. While exposure to low doses of VOCs causes acute and chronic health problems, exposure to high concentrations causes coma and death. It is therefore essential that VOCs are detected in order to avoid harming living organisms in their environment. Optical and mass change sensitive surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) techniques are used to detect these harmful vapours. Compared to the SPR system, the QCM system can receive systemic signals for thicker samples. Therefore, QCM measurements can be carried out on samples with a thickness in the micro and nano range. Depending on the mass sensitivity, all measurements are recorded as frequency variations in the QCM system. In chemical sensor applications, the interaction between the sensor material and the noxious gas is recorded in the QCM system as a time-dependent frequency changes (Acikbas vd., 2015:99; Acikbas vd., 2016:470; Acikbas vd., 2016:18; Büyükkabasakal vd., 2019:9097).

In the field of chemical sensing, the choice of sensor material is as important as the choice of technique. Besides macrocyclic compounds such as calixarenes,

phthalocyanines, pillararenes and dextrins, polymer-based materials have established themselves as the most preferred sensing materials. Polyacrylonitrile (PAN) is an organic polymer with a semi-crystalline structure, featuring a nitrile functional group attached to a polyethylene backbone. It is characterized by its high carbon content (Sada vd., 2014). Acting as a hydrogen bond acceptor, the nitrile group creates a significant dipole moment between nitrogen and carbon. This ensures the formation of a strong electrostatic attraction. As a result, the polymer chains experience intermolecular forces that contribute to the material's high strength and resistance.

PAN's superior properties include flexibility, chemical stability, high carbon yield, and high melting point (Awad vd., 2021:410). In addition, PAN-based carbon fibres offer superior strength relative to fibres made from other precursors like rayon and pitch. This is due to the carbon yield and high melting point of PAN. (Rahaman vd., 2011:32). It also remains thermally stable up to 300 ºC (Versney vd., 1998:145). Due to their unique superior mechanical properties, thermal stability and good solvent resistance, PANs are widely used in a variety of polymer applications. (Hashni vd., 2019:1) Polypyrrole (PPy) is a promising conducting polymer because of its high conductivity and easy synthesis and nanofibrous materials containing PPy generate a conducting network structure capable of reaching metallic electrical conductivities (Trevino vd., 2021: 138). When gas or vapor is adsorbed, PPy behaves as a p-type semiconductor, and the interaction causes a change in its conductivity (Bazzaoui vd., 2007:43); (Bhat vd., 2003:88); (Lin vd., 2003:94).

The disadvantages of PPy include its poor solubility and its brittle mechanical behavior. To solve these problems, (Amani vd., 2021: 28), PPy is blended with PAN which offers excellent stability, hydrophilicity, and adhesion properties to the PAN/PPy blend (Peng vd., 2017:184).

2. VOLATILE ALIPHATIC COMPOUNDS

Aliphatic or aromatic hydrocarbons with a high vapour pressure, a low solubility in water and a boiling point of up to 260 °C are referred to as volatile organic compounds. These compounds may cause drowsiness, headache, fatigue and nervous system disorders when inhaled for short periods in enclosed spaces and at low concentrations (Su ve Hu, 2018:1234); (Hirota vd., 2004:1185). Long-term inhalation of low concentrations may cause chronic respiratory disease, asthma or cancer. It is now recognised that these compounds not only reduce indoor air quality, but also outdoor air quality and cause significant damage to the atmosphere as a result of their increasing use in industry, manufacturing and cosmetics.

It has been measured that the volatile organic compounds found or used in the indoor environment are below the odour threshold and are 5 times higher than the volatile organic compounds found in the outdoor environment (Wallace vd., 1991; Alyüz ve Veli, 2006). For this reason, the detection of these compounds is important for the prevention of VOCs in indoor environments. In recent years, there have been experimental studies in the literature to investigate the sensor properties of materials that can help detect gaseous or organic volatiles in indoor environments that we cannot detect by odour. Among these harmful volatile organic compounds, information on some chlorine-based aliphatic compounds is given below.

A common laboratory solvent, **chloroform's** main use today is in the production of chlorodifluoromethane, an important precursor for tetrafluoroethylene. This is because it is relatively unreactive, miscible with most organic liquids, non-volatile and non-flammable. It is used as a solvent in the pharmaceutical industry, in the manufacture of paints and in pesticides. As a reagent, chloroform provides the dichlorocarbon (CCl_2) group and is preferred in organic separation and purification processes, in the joining process in plastics production and as the main material in the production of Teflon (non-stick). Because of its anaesthetic properties, chloroform was used as an anaesthetic in surgery and dentistry when it was first discovered, and as a chemical weapon in the First World War.

The **dichloromethane** (DCM) compound, also known as methylene chloride, is an organic compound with the chemical formula CH_2Cl_2. DCM, which is widely used as a solvent, is a volatile, colourless liquid. It has a vapour pressure of 46.6 kPa at 20°C, is not soluble in water and is mixable with a wide range of organic solvents. Natural sources of DCM include marine sources, macroalgae, wetlands and volcanoes, but the majority of dichloromethane in the environment is the result of industrial emissions. It is used as a paint stripper and degreaser, in the food industry to decaffeinate coffee and chocolate, and in the production of hop extracts and other flavourings. DCM is chemically used to weld some plastics together, for example to seal the housing of electricity meters. It is often sold as the main ingredient in plastic welding adhesives. It is also widely used by hobbyists in model making to assemble plastic components. It is used in the garment printing industry to remove heat-sealed garment transfers and in civil engineering materials testing, particularly as a solvent for testing bituminous materials (Karlık, 2004).

Carbon tetrachloride (CCl_4), also known as tetrachloromethane, is a chlorinated hydrocarbon compound. CCl_4 is a colourless, water insoluble substance with a pleasant odour that can be detected by smell at low levels and

has a vapour pressure of 11.94 kPa at 20 °C. Due to its pleasant odor, it is used in the production of incense, dry cleaning, fire extinguishers, to dissolve non-polar compounds and oils, and as an intermediate compound in the production of refrigerant fluorochlorocarbons. However, since the 1980s, due to environmental concerns and the realisation that it causes significant damage to the ozone layer, the production of carbon tetrachloride and the use of chlorofluorocarbons derived from carbon tetrachloride have been rapidly reduced and continue to be reduced. KTK can be fatal to humans if 3-5 ml is ingested orally. It can enter the body through the digestive tract, skin and respiratory tract. Once in the body, it is distributed to all organs and tissues. It accumulates mainly in fatty tissues. CTC poisoning causes central nervous system depression. Accordingly, symptoms such as headache, dizziness, weakness, ataxia, tremor, unconscious speech, memory loss, drowsiness, loss of consciousness, optic nerve damage and hearing loss are observed (Kitiz, 2011).

3. ELECTROSPUN POLYACRYLONITRILE (PAN)-BASED NANOFIBRE

Preparation of PAN-based Electrolysis Solutions

In order to provide information on the preparation process of PAN-based electrospinning solutions, the solution preparation of PAN/PPy material is described below. PAN is added to DMF as a solvent and PPy is added to DMF to give a total polymer ratio of 10% to prepare the PAN/PPy solution. PAN in DMF is first stirred with a magnetic stirrer at 60 °C until a clear colour is obtained. After mixing the two solutions, the two polymers are further mixed at 60 °C for 24 hours. The optimum PAN:PPy ratio has been determined by means of preliminary experiments and scanning electron microscope images (Ince Yardımcı vd., 2020).

Electrospinning process

First, the prepared solution was filled into a 5 ml plastic syringe. The distance between the needle of the syringe and the grounded collector plate was adjusted to 15 cm. A metal plate covered with aluminium foil was used as a collector for the preparation of PAN/PPy nanofibre membranes. In order to obtain nanofibres in the droplet of polymer solution at the tip of the needle, a single-needle electrospinning setup was used and operated at a voltage of 15 kV. The high voltage is applied to the metal needle of the syringe placed on the syringe pump, which can feed the solution with a precision of μL/min, creating an electric field and collecting the nanofibres on the grounded aluminium foil-coated plate (Ince

Yardimci vd., 2019). The PAN/PPy nanofibre production setup by electrospinning method is given in Figure 1.

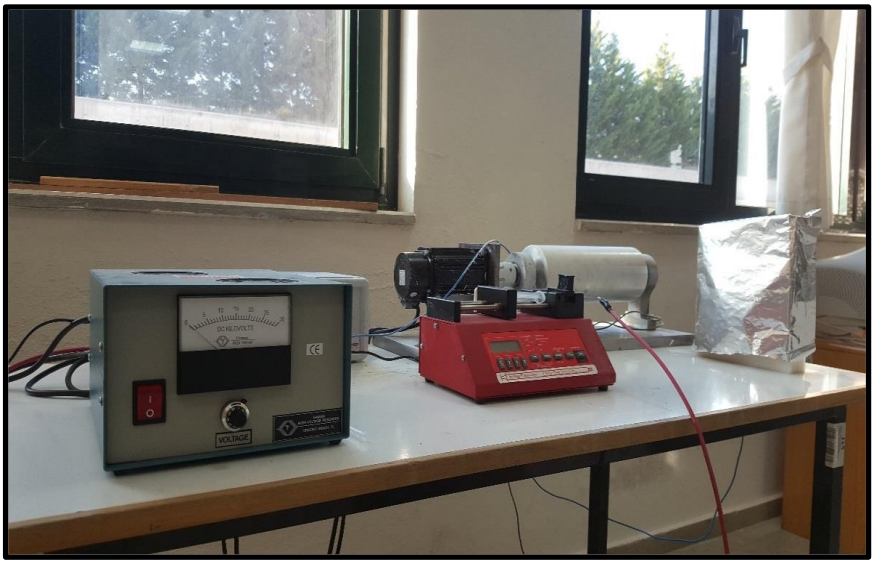

Figure 1. The PAN/PPy nanofibre production setup by electrospinning method.

SEM results of PAN and PAN/PPy nanofibers

Advanced imaging techniques are used to characterise the nano-sized fibres produced by electrolysis. One of the most important techniques preferred for morphological characterization of nanofibers is scanning electron microscopy (SEM). SEM images of PAN and PAN/PPy polymer nanofibers are shown in Figure 2. In order to take SEM images, Au was first coated on them to provide conductivity. Images of the samples were taken with a secondary electron detector under a voltage of 20 kV.

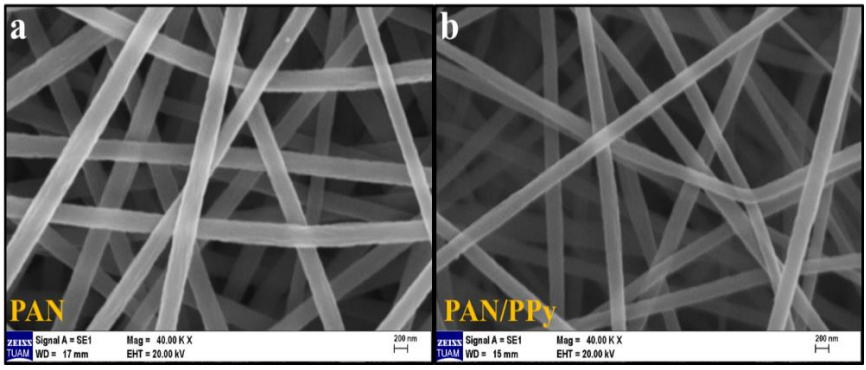

Figure 2. SEM results of (a) PAN and (b) PAN/PPy nanofibers.

4. QUARTZ CRYSTAL MICROBALANCE (QCM) MEASUREMENT SYSTEMS

The QCM is a high frequency method that is sensitive to surface mass and is used in a wide range of sensor applications. The basis of this technique is the determination of changes in resonant frequency (Δf) caused by adsorbed layers on sensor surfaces. Prior to 1950, qualitative definitions were employed to describe the frequency shift, denoted as Δf. Later researchers began to study the phenomenon in more detail because of the need to monitor small mass variations. It was first reported in 1960 that the geometrical dimensions of the quartz layer and the thickness of the electrodes affect the resonant frequency of a quartz crystal. As a result, the manufacturers prepared quartz crystals with a resonant frequency that was higher than the desired value and then controlled the frequency by adjusting the thickness of the quartz electrodes present (Acikbas, 2015:99; Acikbas, 2017:77; Halay vd., 2019:2521).

The quartz crystal microbalance system is sensitive to changes in mass. It is used in a wide range of sensor applications. Its technique, founded upon the piezoelectric principle, is a straightforward and highly resolute mass sensitive methodology with a vast quantification range. The device is an electromechanical resonator. It converts electrical energy into mechanical energy. The process is facilitated by electrodes that are coated with a sensing materials (Acikbas vd., 2016:470; Durmaz vd., 2020:4670). The setup of QCM is given in Figure 3.

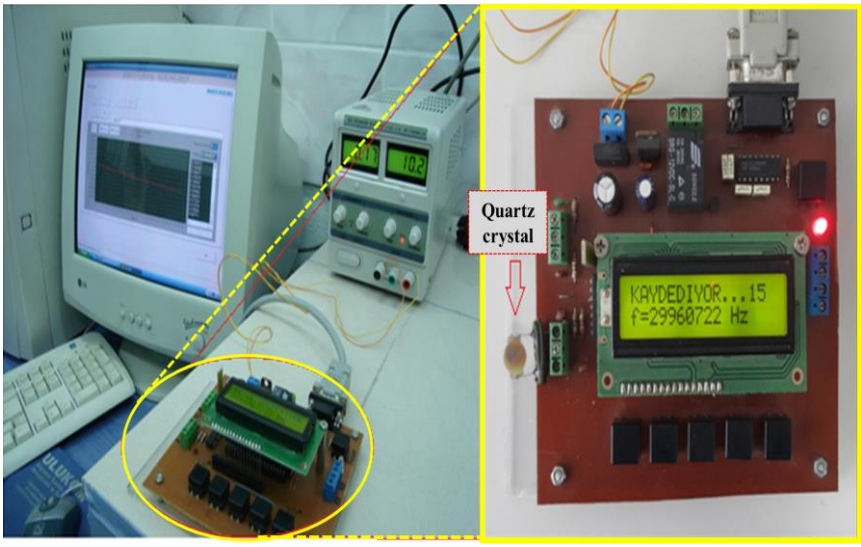

Figure 3. The setup of QCM system.

Gas Kinetic Measurements via QCM Technique

In the ideal gas kinetic study graph shown in Figure 4, A represents vapour and B represents air. There is air in the atmosphere between 0 and 120 seconds. At 120 seconds, saturated organic vapour (A) was released into the environment, kept in the environment for 2 minutes and the resonance frequency was measured. The frequency shift of the QCM rises quickly due to the introduction of harmful organic vapour molecules into the atmosphere. During this critical period, it is observed that the frequency changes rapidly to different values once the harmful vapors are inhaled into the surrounding area. This situation can be explained as follows: The sensor materials reacts very quickly to harmful organic vapours. The frequency will approximate an absolute value after showing a variable value. During this time, the frequency remains constant. This indicates that the sensor materials are interacting with harmful organic vapours. For 120 seconds, the organic vapour stayed in the surrounding area. To allow the changes in the crystal's resonant frequency to return to their previous values, fresh air (B) was introduced into the environment for 240 seconds.

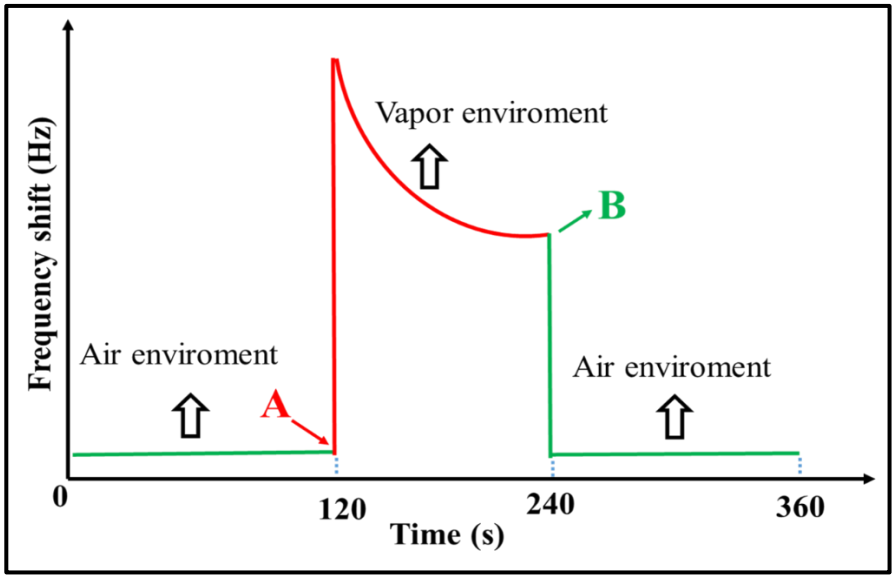

Figure 4. A schematic diagram of the gas kinetic measurements.

In Figure 5 the sensitivity of the PAN and PAN/PPy nanofibre coated QCM sensors to chloroform vapour is plotted as a time dependent frequency variation. The PAN/PPy based QCM sensor shows a difference of about 2 Hz compared to the PAN based QCM sensor, but it is understood that both sensors have a very close sensitivity to chloroform vapour.

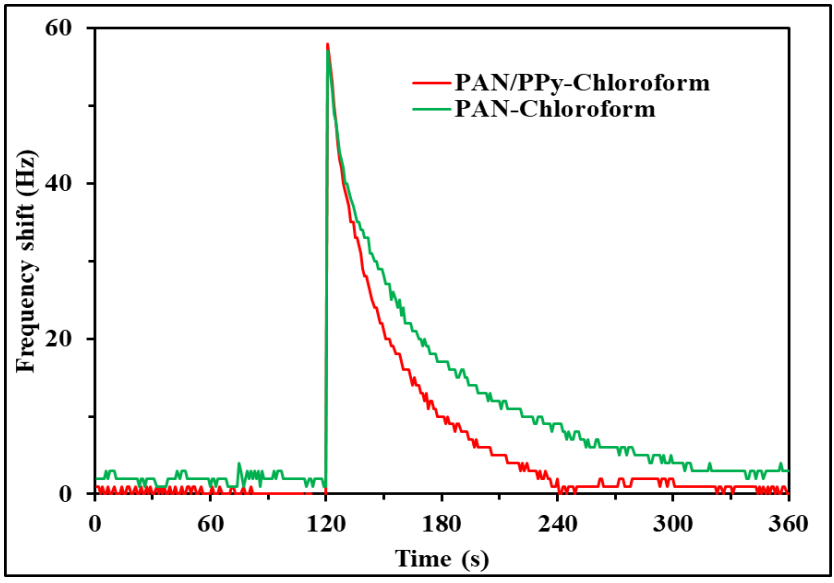

Figure 5. Measurement of the response of PAN and PAN/PPy nanofibre sensors to chloroform vapour.

In Figure 6, the sensitivity of PAN and PAN/PPy nanofibre coated QCM sensors to dichloromethane vapour was plotted as a time dependent frequency variation. It was observed that the PAN/PPy based QCM sensor produced a difference of approximately 41 Hz compared to the PAN based QCM sensor. As a result, it was observed that the PAN/PPy based QCM sensor was more sensitive to DCM vapour. Similarly, Figure 7 shows the sensitivity of these QCM sensors to carbon tetrachloride vapour. It was observed that the PAN/PPy based QCM sensor produced a difference of approximately 23 Hz compared to the PAN based QCM sensor. Consequently, it is observed that the PAN/PPy based QCM sensor is more sensitive to carbon tetrachloride vapour. The performance of the PAN and PAN/PPy coated QCM sensors against the vapours of dichloromethane, chloroform and carbon tetrachloride is given in Table 1.

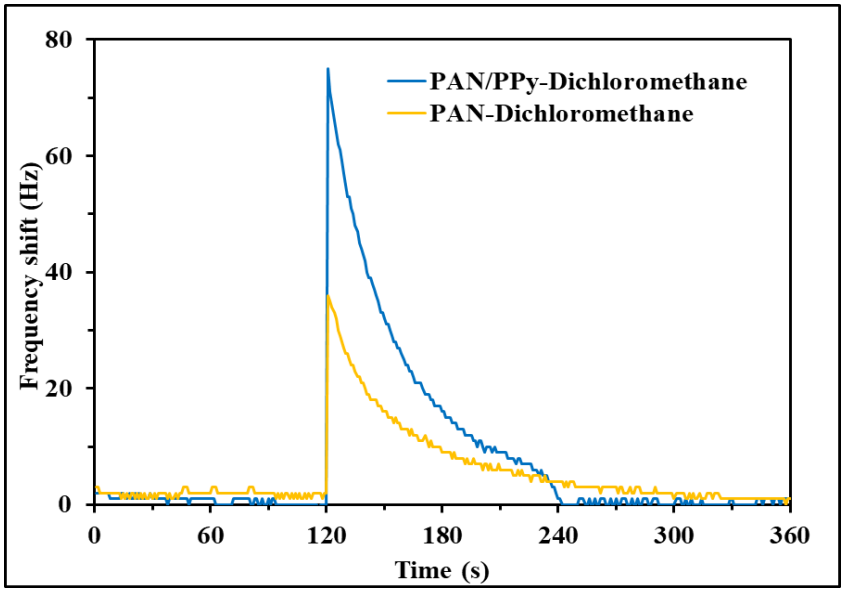

Figure 6. Measurement of the response of PAN and PAN/PPy nanofibre sensors to dichloromethane vapour.

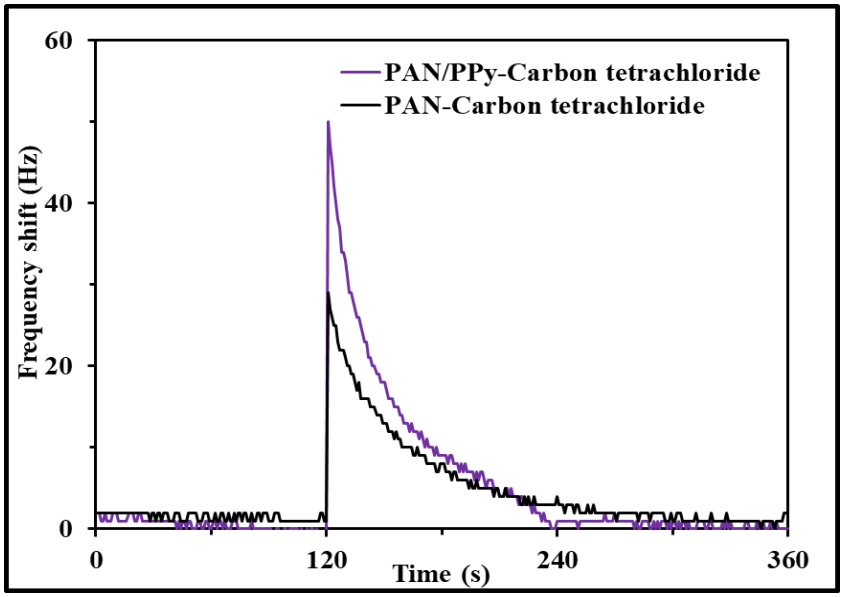

Figure 7. Measurement of the response of PAN and PAN/PPy nanofibre sensors to carbon tetrachloride vapour.

Table 1. The sensor performances of PAN-based QCM nanofibers.

Produced sensor type	VOCs	Response (Frequency change, Hz)	Ref.
PAN-based QCM sensor	Dichloromethane Chloroform Carbon tetrachloride	34 57 28	(Ince Yardimci vd., 2022:173)
PAN/PPy-based QCM sensor	Dichloromethane Chloroform Carbon tetrachloride	75 59 51	(Yagmurcukardes vd., 2023:1869)

5. CONCLUSIONS

The comparison of the performance of PAN and PAN/PPy nanofibre-based QCM sensors against chlorine-based noxious vapours, which have been introduced in the literature in previous studies, is presented in this study. The preparation of PAN and PAN/PPy nanofibres by electrospinning method and the morphological properties of these nanofibres are presented with SEM results. The sensitivity values (as frequency change) of the PAN nanofibre-based QCM sensor to dichloromethane, chloroform and carbon tetrachloride vapours were recorded as 34, 57, and 28 Hz, respectively. Similarly, the sensitivity values (as frequency change) of the PAN/PPy nanofibre-based QCM sensor to dichloromethane, chloroform and carbon tetrachloride vapours were recorded as 75, 59, and 51 Hz, respectively. As a result, it is suggested that the addition of PPy to PAN polymer increases the efficiency of the QCM chemical sensor and sheds light on the development of PAN nanofibre-based chemical sensors.

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Chapter 9

Oxidative Stress, Mitochondrial Nutrition and Cellular Health

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Introduction

Why has the topic of nutrition still not reached a conclusion after so many years? Healthy eating is such a popular subject that new books are published on it every month, and it remains a constant topic of discussion both globally and in Turkey. Countless studies have been conducted on the link between diseases and poor nutrition, with new ones emerging every day. Every day, new publications explore the relationship between nutrition and diseases. Conferences, documentaries, and films on healthy eating are also being produced. I have attended numerous conferences and events both domestically and internationally to stay up-to-date with the latest information in nutrition and medical sciences. However, what I've observed is that despite the latest technological advancements, new discoveries, and numerous interesting scientific publications, nutrition still remains a top research topic abroad as well. It seems that the desired answers have yet to be fully found there either.

Nutrition, especially mitochondrial nutrition, alkaline diets, and the topic of free radicals, has become one of the most researched subjects in recent years. Every day, health authorities are highlighting the role of nutrition in the pathogenesis of various diseases, the importance of choosing the right fuel, and the impact of the waste products produced by the foods we consume. In particular, the effect of nutrition-related radicals on today's most challenging diseases in medicine, such as diabetes mellitus, cancer, cardiovascular diseases, and aging, has sparked significant interest. New findings on these topics are being added regularly.

In my opinion, the seeming contradictions or differences in information are related to the level at which the issue is being approached. If you look at nutrition from the perspective of weight and obesity, you're gathering information from one particular level. If you're focusing on reducing diseases related to organs like the heart, liver, brain, or intestines, your attention will be drawn to publications and presentations at that level. However, if you approach nutrition from a cellular perspective, or even from the subatomic level within the cell, you encounter an entirely different approach to nutrition. In essence, we tend to find the answers we are looking for, depending on what we are seeking.

In this presentation, my main goal, while respecting the time limitations, is to clear up any confusion you may have, and to help you organize the scattered information you've gathered into a more understandable and structured algorithm. If we do not realize that our greatest power in reversing aging and reducing diseases lies in knowing what bite we put into our mouths, it means we do not know why we eat.

What does nutrition mean?

Nutrition means the burning of food with oxygen, which results in the formation of some ash. This ash is harmful to us; it is waste, garbage. Although we may appear to be getting sick or aging due to other reasons, this is just the broader view of the picture. The real scenario is at the atomic level, and there are some basic common denominators.

Every food that is considered nutrition for our stomach might not be nutrition for our cells. Today, we will focus a bit on the cellular foundations of nutrition. Therefore, it would not be wrong to say that the most important organelles to protect in the body are the cell membranes and the mitochondrial membranes. They are even more important than the heart and brain. If you think this is an exaggeration, you're mistaken. These organs are also made up of cells, and their cells use the same energy metabolism. In fact, because these organs require more energy, they have more mitochondria than other cells.

Thus, these organs are the ones that suffer most from waste, meaning free radicals, in the process of energy production.

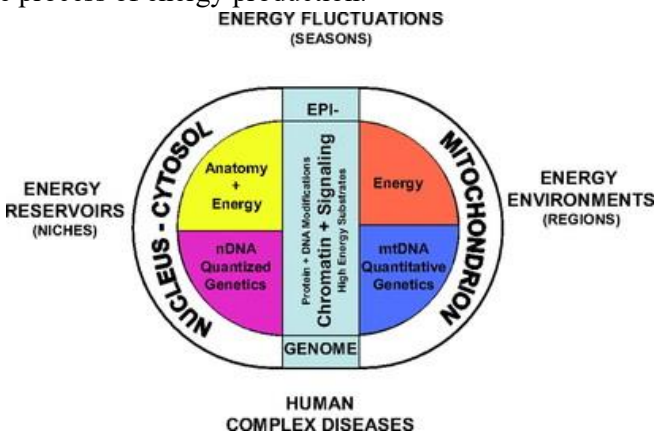

Figure 1. Energy Conversion in Human (https://www.soma-psiche.org/bioenergetic.html)

Let's take a look at the questions we need to find answers to within ourselves:

- Are you aware of your biological rhythm?
- What is intermittent fasting?
- What is the secret to being a biologically superior human?
- Where is the engine in our body, and what is the right fuel for this engine?
- Does time seem to pass quickly or slowly for you?
- Do you know that our daily food choices are the biggest cause of diseases that will affect us today, 7 years from now, and even 70 years later?
- Do you know if the food you eat gives you energy?

- Do you know when and what to eat?
- And do you know exactly why you're eating?
- How is the relationship between nutrition and diseases established?
- Is there a connection between nutrition and longevity?
- Are your cellular cycles regular?

Nutrition means the burning of food with oxygen, and as a result, the formation of a certain amount of ash. This ash is harmful to us, it's waste, it's trash. Even though it may seem like we get sick or age due to other reasons, this is just the broader view of the picture. The real scenery is at the atomic level. And there are some basic common factors. Not every food that is good for our stomach may be good for our cells. Today, we will focus a bit on the cellular foundations of nutrition. That's why it wouldn't be wrong to say that the most important organelles to protect in the body are the cell membrane and the mitochondrial membranes. They are even more important than the heart and brain. If you think I'm exaggerating the situation, no, I'm not. These organs are made up of cells, and their cells also use the same energy metabolism. In fact, because they need more energy, they have more mitochondria than other cells. Therefore, these organs are where the real battle with waste, i.e., free radicals, takes place in terms of energy.

Everything is included in the circadian rhythm

Our biorhythm is cyclical. It is NOT fixed. It starts, ends, and starts again. Other living beings on Earth also have biological internal clocks that are in sync with daily and yearly cycles. I see the terms 'cycle' and 'flow' as the essence of life. Time is a flow, and the morning-afternoon-evening, night-day, summer-winter, and years that make up time are all cycles. The fundamental event that

creates this cycle is the existence of the sun. Day means sunlight. Whether there are clouds outside or we never leave the house doesn't matter; our internal biological clocks recognize day and night by the wavelength of light. All the cells in our body have biological clock receptors. And this is why we call the adjustment of our biological clock to the external sunlight **circadian rhythm**. Everything is part of the circadian cycle. Hormones are secreted in a circadian rhythm, enzymes become active or inactive in a circadian pattern. Eating and drinking, mood, and even physical strength are all regulated by these rhythms. It should be no surprise that records broken in the Olympics align with these rhythms. The number of Olympic records set during the early afternoon, when the human body's physical circadian rhythm peaks, is higher. In essence, nutrition should also be circadian. Metabolic processes occur at different speeds throughout the day.Body temperature is circadian. Digestion of food is

circadian.The cleaning of old cells is circadian. DNA repair can be done well or poorly depending on the circadian rhythm.Production processes are done at one time, repairs are done at another time. Both do not happen at the same time.

We should eat according to the circadian rhythm; this should be our main starting point. When it comes to nutrition, the first thing that comes to mind is our troublemaker, the insulin hormone. The organ that produces insulin, which regulates blood sugar, is the pancreas, and its function, like that of other organs, is circadian. That is, it works more at certain times of the day and less at others. For example, in the early hours of the day, the circadian receptors in the pancreas recognize that it's morning and are better at regulating blood sugar. However, as the day progresses, the pancreas' biological clock changes. By the evening, its ability to regulate blood sugar decreases. Instead of converting glucose into energy by allowing it to enter the cells, a system focused on storing it as fat takes over. In daily life, you may observe that you gain weight as you eat more in the evening. The science behind this is circadian rhythm medicine.

Yes, we all know that in order to live, we must breathe and eat. But why? Not just us, animals, plants, and even bacteria eat as well. We cannot survive in any other way. A baby begins its journey from the fusion of two cells, and for 9 months and 10 days, while growing from a single cell into a weight of 3.5 kilos in the mother's womb, it is nourished by the mother. That single initial cell, by the time it weighs 3.5 kilos, multiplies into trillions of cells. So, our first need for food is to provide the energy and raw materials required to increase the number of our cells and enable our growth.

What I want to emphasize here is the importance of answering the question, "Why do we eat?" at such a fundamental level, starting from the formation of our being as a single cell. We ate to grow. NOW, as adults, we eat to maintain the balance of our body. This balance is called homeostasis. The purpose of all the cells in our body is to maintain this healthy balance for as long as possible. When we get injured, our body wants to heal quickly. If we catch an infection, our immune system immediately works to overcome it.

Apart from movement, we also obtain the raw materials and energy required to think, sleep, and carry out tasks from our food. So, why, despite eating three meals a day, do many of us still feel low on energy and tired? What does this mean? Maybe we don't really know why we're eating. Because if we did, we would make the right choices and find the correct energy. If we’re not only questioning our fatigue but also attributing our increasing illnesses over the years to fate or bad luck, it means we still don’t know why we’re eating.

Food - Mitochondria – Energy

In the matter of eating and drinking, the place the topic always comes back to is mitochondria... The primary job of mitochondria is to provide energy, and therefore, they become more active or passive depending on the speed of metabolism and the energy demand. Depending on the energy needs at any given time of the day, their numbers can increase or decrease. When energy demand is low, they shift into repair mode. Studies have shown that in experimental animals removed from their daily circadian rhythm, the efficiency of mitochondria in energy production decreases. The food-mıtochondrıa-energy trıad is an inseparable trio, but it would still be a mistake to think that we eat just to produce energy.

Look, the human body is a very powerful biological machine. It is a biological machine made up of billions of cells and dozens of organs, designed to be flawless. This biological machine wants to operate with minimum energy, maximum efficiency, and minimum waste. This logic is the desired performance even in the simplest of machines. This is where the key point comes into play. Let's emphasize again: an ideal machine should operate on the principles of minimum fuel, maximum efficiency, minimum waste, minimum wear and tear, and maximum longevity!! The same is true for the human machine.

If we were really machines, we would be getting our working energy from being plugged into an outlet. But we are not plugged in, so how are we going to run efficiently? Our electricity comes from food! Energy comes from there. In cars, we obtain energy by burning gasoline, and in our bodies, we obtain energy by burning food. To burn something means to react it with oxygen. The place in our body where this heat is generated is the mitochondria. Every bite of food we put into our mouth undergoes hundreds of complex processes, traveling a long path, and finally reaching our energy factories where it will be converted into energy. These factories are the mitochondria. The job of these energy plants is to further break down the food that has been digested and turned into molecules, releasing the energy contained within. Mitochondria make up 10% of the total weight of the human body. If the body weight is 80 kg, that means we have 8 kg of mitochondria in our energy system. The heart is 50% mitochondria, the brain is 25% mitochondria.

General Information about Mitochondria

Mitochondria are membrane-bound organelles found in the cytoplasm of eukaryotic cells. Often referred to as the "powerhouses" of the cell, their main function is to produce energy in the form of adenosine triphosphate (ATP)

through cellular respiration. This energy is essential for the cell to perform its various functions.

Mitochondria are unique in several ways. One of the most notable features is that they have their own DNA (mtDNA), which is separate from the nuclear DNA of the cell. This mitochondrial DNA is inherited maternally, meaning it is passed down from the mother to her offspring. Unlike other cellular organelles, mitochondria can replicate independently of the cell, and they also have a double membrane structure, with the inner membrane being highly folded into structures called cristae, which increase the surface area for energy production.

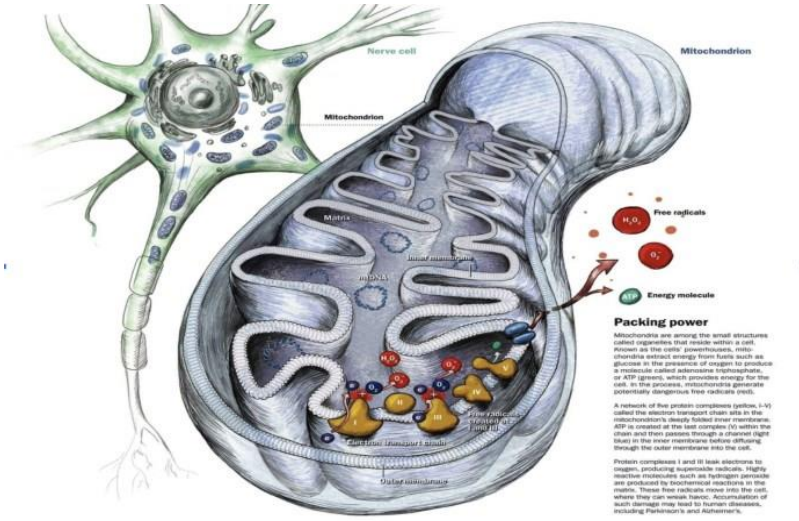

Figure 2. General structure of mitocondria
(https://static.wixstatic.com/media/c45f84_beee5ddd856d470e821444d2e9feedb3.jpg)

In addition to ATP production, mitochondria play important roles in other cellular processes, including regulation of the cell cycle, cell growth, and apoptosis (programmed cell death). They are also involved in calcium storage, the production of reactive oxygen species (ROS), and the maintenance of cellular homeostasis.

Mitochondria are found in every cell and are inherited only from the mother. The egg from the mother contains thousands of mitochondria—more than 100,000, in fact. Sperm, on the other hand, contains almost no mitochondria, and mitochondria are not passed down from the father. Everyone inherits their mitochondria from their mother, grandmother, great-grandmother, and so on, carrying the mitochondrial lineage of women back through generations. It is widely accepted that the ancestors of mitochondria were single-celled bacteria, a theory known as symbiosis. Mitochondria share many physical features with

these bacteria. They also have their own DNA, which is referred to as mtDNA. The presence of mtDNA is crucial to understanding the exceptional capabilities of humans. While our nuclear DNA is well-known and often the focus, mtDNA deserves equal importance, though in recent years it has often been overshadowed. When investigating the causes of diseases, mtDNA—rather than nuclear DNA—should be the first point of focus. In fact, many diseases have been linked to dysfunctions in mitochondria or mtDNA. I will list a few diseases below where mitochondrial or mtDNA issues have been identified: Type 2 diabetes, cancer, dementia, neurodegenerative brain diseases, obesity, fibromyalgia, polycystic ovary syndrome, migraine headaches, vision loss, thyroid problems, even drooping eyelids. We could add more, but I think this list is enough, because I trust you can see the logic here: Wherever there are cells, they have a function, and to perform that function, they need energy. If there is not enough energy, the function will falter or fail. In short, the performance of cells depends on the performance of mitochondria. Similarly, the performance of organs depends on mitochondria. Ultimately, all diseases are related in some way to mitochondria. So when we talk about general health, we are essentially talking about mitochondria.

Feeding is never a 100% profitable endeavor. No matter what you eat, you will always suffer some harm in the process. This is a law of nature. As living beings, the "waste" created from nourishment will accumulate over the years. Aging itself is the result of this accumulation of "waste." We are born into the world to trade immortality for life. Our eating habits should be focused on minimum harm and maximum gain. Maximum gain means maximum energy efficiency. This energy will be used for repair, growth, and movement. Minimum harm means minimizing the waste products, or "trash," created by energy metabolism. This, in turn, leads to minimum disease and maximum lifespan. In other words, if we have an energy production system that operates at maximum efficiency, we will have maximum lifespan. The only barriers to eternal life are energy scarcity and waste. However, no matter how healthy we eat, we will inevitably experience some degree of radical damage—free radical damage. Since nutrition involves the burning of food with oxygen, it is wise to anticipate that, just like any substance burned with oxygen, there will be some ash produced as a result of this process. This is where the complexity begins: the ash is harmful, it is waste, it is trash. The biochemical name for this ash is "free radical." Free radicals are, in essence, anarchists. The issue of oxidant systems and free radicals is closely connected to nutrition, energy production, and diseases. After all, everything that happens to us stems from these radicals.

The Relationship Between Mitochondria and Oxygen

Mitochondria are the energy production centers of our cells, and they are directly linked to oxygen. The primary function of mitochondria is energy production, which heavily relies on oxygen. This relationship is critical for the survival and proper functioning of cells.

Mitochondria are the powerhouses of the cell, and their primary role is to produce energy through a process known as cellular respiration. This process requires oxygen. Without oxygen, mitochondria cannot effectively produce the energy (ATP) that cells need to function properly.

Oxygen is essential for the mitochondria because it is the final electron acceptor in the electron transport chain, which takes place in the inner membrane of the mitochondrion. During this process, energy is produced by transferring electrons through a series of proteins. Oxygen combines with electrons and hydrogen ions to form water, a byproduct of this energy production. This process is called oxidative phosphorylation, and it is how the majority of ATP is generated in our cells.

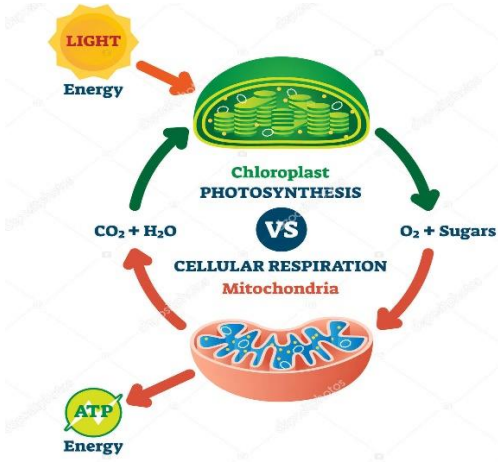

Figure 3. Photosynthesis and Cellular Respiration Interaction (https://depositphotos.com/tr/vector/chloroplast-vs-mitochondria-process-educational-scheme-vector-illustration-369855496.html)

When mitochondria are not functioning properly, a condition called **hypoxia** (lack of oxygen) can occur in tissues, leading to various health issues. Inadequate mitochondrial function can reduce the efficiency of oxygen use in the cells, contributing to chronic low oxygen levels, even if we breathe normally. This situation is sometimes referred to as **pseudohypoxia**, where the cells experience oxygen deprivation, leading to a range of metabolic and health problems.

Therefore, mitochondrial health is crucial for optimal oxygen utilization in the body. If mitochondria are damaged or malfunctioning, it directly affects the body's ability to produce energy efficiently, resulting in fatigue, cellular dysfunction, and various diseases. Maintaining healthy mitochondria is key to proper oxygen utilization, energy production, and overall health.

The ones that truly breathe are our mitochondria. Mitochondria cannot produce energy without oxygen. The importance of breathing is equal to the importance of mitochondrial health. When mitochondria do not function properly, tissues experience a lack of oxygen, known as hypoxia. If mitochondria are not healthy, then the breath we take is not truly effective. Due to poor nutrition and unhealthy lifestyles, we are damaging our mitochondria. As a result, the utilization of oxygen in our cells becomes inefficient. In fact, we are all constantly experiencing low-grade chronic hypoxia—essentially, we are in a state of slow suffocation without realizing it.

When we talk about calories in relation to weight, it is again dependent on mitochondrial function. Well-functioning mitochondria produce good energy. If mitochondria are not working well, the food we consume is converted into weight instead of energy, even if we don't overeat. Looking at the prevalence of obesity in our country, just like in the world, it is clear that we are missing something.

In the medical field, even in a narrow scope, there is a growing awareness that the solution lies in maintaining mitochondrial health. The entire world is researching not only weight issues but also healthy living and longevity through the lens of mitochondria. In nearly all diseases, the common factor is the health of the mitochondria. New methods for disease prevention are increasingly focused on protecting mitochondri.

How do we nourish our mitochondria?

The reason we eat is to nourish our mitochondria. We should eat in a way that enhances the performance of our energy-producing machines, the mitochondria. This is a fundamental biological rule, and there are no exceptions. If we don't accept this without debate, we risk underestimating the importance of the issue. The phrase "We must feed our mitochondria" expresses our power to control not only all diseases and aging but even cancer.

In the world and the universe, everything that exists as "matter" is made of the same building blocks: atoms. We, plants, animals, insects, air, meteorites, and everything else are made of atoms. In the structure of an atom, as you might remember from high school chemistry, there are protons (positively charged) and neutrons (neutral) in the nucleus. Surrounding the nucleus are electrons, which have a negative charge and orbit in the electron cloud. The positive and negative

charges attract each other, and without this attraction, we could not exist as matter, and we would dissipate in space.

In stable atoms, the number of protons always equals the number of electrons, and we like them to stay that way because they don't cause any harm. But for life to exist, atoms must come together and interact through chemical reactions to create new substances. For example, H2O (water) is formed when two hydrogen atoms and one oxygen atom bond together.

Matter and atoms in the universe can transform into each other, and everything is still made of atoms. When we eat food, we transform the atoms in the food into energy within our cells. This is the purpose of nutrition: to take the latent energy in the materialized form of food and use it. When we eat plants, we convert the solar energy that the plant stored into usable energy. This is what we call nutrition.

Energy Production in Mitochondria - The Furnaces Where Food is Burned with Oxygen: Mitochondria

Ninety percent of the body's energy needs are supplied by the mitochondria. This is where food ultimately ends up. We can think of mitochondria as the digestive system of the cell. They accept food in its final form and release its subatomic energy. In fact, we can think of them like a furnace. They burn food with oxygen, producing energy and heat. This process is called cellular respiration because it involves oxygen. Essentially, the mitochondria are the "lungs" of the cell. Cellular respiration is the process in which oxygen and food transform into energy in the mitochondria.

If we can't survive without breathing for more than 3-4 minutes, we should understand from this that without oxygen, energy cannot be produced in the mitochondria. This means that when our mitochondria stop working, we would die within three minutes. In movies, when the heart monitor in intensive care flattens, turning from a zigzag heart rate pattern to a straight line, that is the moment when the mitochondria in the heart fail to produce energy, and the heart muscle, unable to generate energy, can no longer pump blood.

Mitochondria are tiny organelles, but they have an incredible work capacity. Despite their small size, imagine them as a hydroelectric power plant in your mind. In the mitochondria, when we burn food, the energy signals produced generate free oxygen radicals instead of ash and smoke. For every 100 oxygen atoms used in the burning process, 1-2 of them are converted into free radicals.

There are two basic requirements for energy production in mitochondria: fuel and oxygen to burn that fuel. The burning of food inside us is no different from the burning of coal in a fireplace. Coal stores energy. When it burns with oxygen, that energy is released. The "coals" from food also burn in the mitochondrial

furnace to be converted into energy. The energy produced is synthesized as ATP, a currency of energy. When needed, this energy can be "exchanged" and used. Mitochondria have specific preferences when it comes to burning food for energy. They can obtain energy from three types of nutrients: glucose, fat, and protein. However, their preference order is first glucose, then fat, and lastly protein. No matter what we eat, the electrons within the food are ultimately what get converted into energy. Whether it's bread, grapes, or yogurt, the result is the same — the only difference is the waste products produced. Our complex digestive system and processing organs, such as the stomach and liver, work to convert the food we consume into a form that can be ultimately funneled into a structure called NADH. We don't burn the apple itself in the mitochondria; we burn the electrons that come from the apple. The hidden energy within food is broken down, and this energy accumulates in the hydrogen atoms, denoted by the H in NADH. This is the final form in which the energy from food is stored.

You might not be familiar with these chemical abbreviations, but they were introduced by scientists interested in longevity as part of "vitamin support", and they have already become part of our daily lives. NADH is an electron carrier, meaning that while the apple looks like food, the energy inside it is actually hidden in the electrons that are stored in the bonds between the carbon atoms of the apple.

Glucose is the preferred fuel because it is an easy-to-burn fuel. Look at your plate, and in your next meal, the foods that turn easily into glucose are the easiest to burn. Other foods are only burned if glucose is insufficient or unavailable. However, the fact that glucose is the preferred fuel comes with its own set of problems, as glucose is, in fact, a cheap and dirty fuel!

When glucose is low or absent, the second choice is fat, which is a fuel that healthy cells can easily burn. Cells that can switch between burning glucose and fat, depending on need, are the healthiest cells. A healthy cell should be able to perform this metabolic switch immediately, based on necessity. As we age, or if we often consume glucose or have various diseases, this metabolic switch becomes more difficult to perform. In cancer, this switch is almost non-existent. Cancer cells prefer not to perform the metabolic shift. In terms of food preference, cancer cells give first priority to glucose, second to protein, and never to fat, as they cannot burn fat.

How Food is Converted into Energy in Our Body

Food undergoes several stages of digestion and metabolism in our body before it is converted into usable energy. Here's a general overview of how this process works:

1. **Ingestion and Digestion:**

The process begins when we consume food. Enzymes in our mouth, stomach, and intestines break down the food into its basic components: carbohydrates, fats, and proteins.

Carbohydrates are broken down into glucose (sugar), fats into fatty acids and glycerol, and proteins into amino acids.

2. **Absorption:**

These broken-down components are absorbed into the bloodstream through the walls of the small intestine.

Glucose enters the bloodstream and circulates throughout the body, providing immediate energy to cells.

Fatty acids and glycerol are carried by the lymphatic system to cells that can store or use them for energy.

Amino acids are used by the body for building proteins or converted into energy when needed.

3. **Energy Production in Cells:**

The glucose, fatty acids, and sometimes amino acids are transported into the cells, where they are used to produce energy.

Inside the cells, mitochondria act as the "power plants." Through cellular respiration, mitochondria convert glucose and fatty acids into ATP (adenosine triphosphate), the primary energy currency of the body.

4. **Cellular Respiration:**

Glycolysis: Glucose is first broken down in the cytoplasm into pyruvate, generating a small amount of ATP.

Citric Acid Cycle (Krebs Cycle): Pyruvate is further broken down in the mitochondria, releasing more energy.

Electron Transport Chain: Oxygen is used in the mitochondria to convert high-energy electrons into ATP. This stage produces the majority of the ATP used by the body.

5. **Energy Storage and Use:**

Any excess energy from food is stored in the form of glycogen in muscles and the liver or as fat in adipose tissue.

When the body needs energy, such as during exercise or fasting, these stores are converted back into glucose or fatty acids for use by the cells.

In summary, food is broken down into its basic components, absorbed into the bloodstream, and transported to the cells. In the mitochondria of the cells, glucose and fats are converted into ATP, providing the energy needed for the body to function.

When mitochondria cannot be used, the backup energy model employed for a short period is glycolysis. In cancer and chronic diseases, long-term reliance on glycolysis is actually the cause of these diseases. Yes, cancer cells prefer to rely continuously on glycolysis. They do not want to use the pathway that leads to the mitochondria. However, healthy cells that are not cancerous do not prefer to use this pathway for long periods because it produces less energy, and the cells cannot perform their normal functions with that limited energy. Cancer cells, on the other hand, are not performing their normal functions anyway. They are just trying to survive. This is why cancer cells prefer to use glycolysis.

The short-term nature of glycolysis is fine, but its continuation into the mitochondria is essential for health. The only advantage of glycolysis for the body is that it is fast, but it is an anaerobic (oxygen-lacking) process. This is exactly why, due to its speed, cancer cells thrive on glycolysis. You may have heard that the final product of pyruvate in glycolysis is lactic acid, or lactate.

What do you know about lactate? Lactate lowers the cell's pH to an acidic level. However, cells have an ideal pH range at which all the enzymes that catalyze cellular processes work efficiently. In general, the body prefers an alkaline pH of around 8 to 8.5 for metabolism. But when there is waste removal, such as in urine excretion, the body tends to favor an acidic pH for those breakdown reactions. As lactate production increases, the pH becomes more acidic, which is incompatible with life for the cell. Consequently, lactate is expelled from the cell, and the liver attempts to clear it.

Cancer cells, due to their persistent reliance on glycolysis, produce excessive amounts of lactic acid, which they then expel to avoid cell death. The extracellular environment becomes so acidic that even the immune system cannot detect the cancer cells, and chemotherapy drugs have difficulty approaching the cancer cells because of the acidic "mantle" surrounding them.

If pyruvate can enter the mitochondria, oxidative phosphorylation begins. Here, we find the final molecular forms of food, which are NADH and FADH2. These molecules are produced through a cycle in the mitochondria and exist in their current forms as NAD and FADH. When we eat, a hydrogen atom is added, and they acquire their final forms. These are electron carriers that transport electrons from the food via an electron transport chain to produce ATP. No matter what food we eat, this is what will eventually happen.

In the next stage, we will witness the interaction between the electrons and protons inside the hydrogen atom. Hydrogen is the most abundant atom in the universe, as you know, and the simplest. It consists of one proton and one electron. It carries the energy from the food.

So far, we have broken down glucose, and now we are going to break down hydrogen. Yes, the process of energy production is essentially about splitting atoms, which is quite a complex and laborious task. Now, our work takes place in the mitochondrial membrane.

How is an electron used?

On the inner mitochondrial membrane, there is an electron transport chain (ETC). As the name suggests, the electron transport chain is where the food's electrons are used. It's not called a glucose transport chain or a fat transport chain—it's an electron transport chain. The key to food lies in the electrons that will eventually reach this chain. Without a doubt, the most important metabolic action in our body happens here. Let's take a closer look. This region has a folded structure, greatly increasing its surface area, thus creating an enormous energy-producing space. We call this folded structure the "crista," and cells with higher energy production have more cristae. For example, the number of cristae in your heart cells is three times higher than in your liver cells.

If we compare this chain to a train system, it has five different stations, which we refer to as "complexes." The goal of these stations is to extract the energy from the electrons coming from food. They carry out their tasks sequentially. The electrons move from one station to the next at a certain speed and flow. Here's a thought: in a microcosm, everything either rotates or flows, and in the macrocosm (the universe), everything rotates and flows—nothing stands still. The electrons inside atoms continuously flow to one another, light flows, time flows, day flows, night flows. There is nothing that remains still. The moment we stop, it's the moment we lose the battle against entropy. This is why it’s important to stay in the cycle of movement!

Now, where do the electrons stop or seem to stop? They reach the fourth station, and in the fifth and final station, ATP is formed. In terms of nutrition and diseases, the first station, Complex I, is the most important. This is because the first station is where the NADH carrying electrons from glucose enters the chain. The electrons coming from carbohydrates always enter the ETC via NADH. Then, the journey of the hydrogen atom begins: the electrons and protons separate. The whole story is about the separation of positive and negative charges.

All these processes should flow at a certain speed, in a circular pattern, and never stop. The ATP motor must continuously spin. The TCA cycle must continuously rotate, and NAD/NADH must keep turning into each other. The goal of all these cycles is to maintain a steady flow of electrons in the electron transport chain (ETC). If the flow is fast and balanced, we remain young! If the ETC is shorter, the flow increases even further. However, if the ETC lengthens due to

poor nutrition, unhealthy lifestyle, or sedentary behavior, the speed decreases. If the chain is lengthened by just one angstrom, the speed drops by 10%, energy production decreases, free radical production increases, and we age. In short, as the ETC slows down, we age.

Athletes use cold therapy to shorten the length of the ETC and speed up the quantum tunneling flow. This helps in proper energy production and regeneration. This is why cold after exercise feels energizing.

Yes, look at our energy system. All the eating, drinking, digestion, and other processes are done to produce ATP. ATP holds energy in the phosphate group (denoted by the "P" letter). When energy is needed, the three phosphates are broken down into two phosphates, then one phosphate, to be used for energy throughout the body.

As the phosphates decrease, the molecule's name changes. ATP levels serve as a communication tool for the entire body. Whether or not there is energy available can be determined by the ATP levels. This is very decisive for metabolism. Should we burn fat or store it? The decision is made based on the ATP-ADP-AMP ratios. Like a battery, if ATP is abundant, the battery is fully charged; if ADP is high, the battery is three-quarters charged; and if AMP is high, the battery is nearly empty. At station 5 (complex 5), there is the ATP motor. Each time it spins, it adds a phosphate group to the ADP molecule, creating ATP. In the end, the energy from the food is stored in the phosphate group, represented by the letter "P." This is how the chemical energy from food is converted into usable energy.

More energy from less food : Electron Transport Chain (ETZ)

Producing a lot of energy from a small amount of food is one of the extremely efficient energy production processes of mitochondria. This process is particularly related to how nutrients are processed in the cell and how mitochondria convert these nutrients into energy. The energy carriers, NADH and $FADH_2$, derived from glucose or fats, generate energy through a system called the electron transport chain (ETC) located in the inner membrane of the mitochondria. Here's how the process works:

- NADH and $FADH_2$ transfer their electrons to the electron transport chain (ETC).
- As the electrons move along this chain, they lose some of their energy.
- This lost energy is used to pump protons (H^+) into the intermembrane space of the mitochondria.
- The proton gradient created by the protons is used by the ATP synthase enzyme to produce ATP.

The important point here is that even when food intake is low, the cell can still produce energy efficiently because these processes are highly effective. When necessary, the cell can continue energy production by burning fats. In other words, when our food intake is low, mitochondria can still produce high-efficiency energy by burning fatty acids.

This "energy efficiency" of mitochondria is called metabolic flexibility. This refers to the cell's ability to switch its energy production pathways based on the type of nutrient available. If there is insufficient glucose, the cell begins to burn fats. In this case, mitochondria quickly adapt to use fatty acids to produce energy.

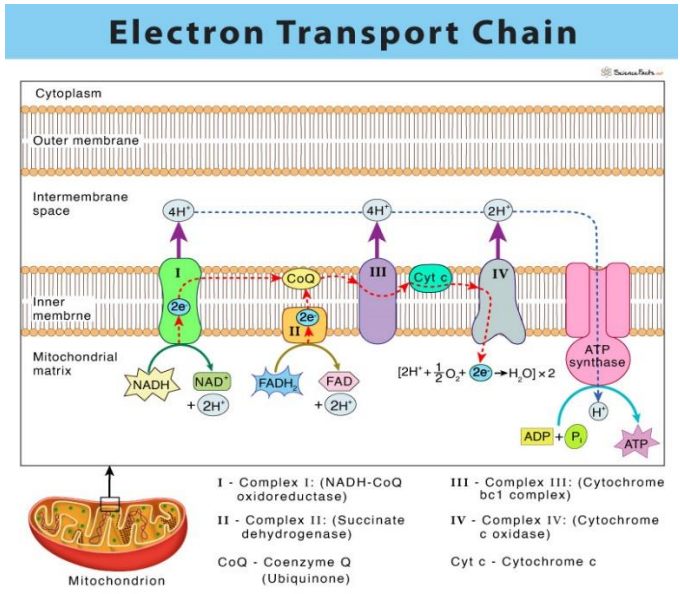

Figure 4. Electron transport chain in human (https://www.sciencefacts.net/electron-transport-chain.html)

Restricted feeding can improve mitochondrial efficiency. Not getting enough nutrients signals the body to become more efficient in energy production. In this case, mitochondria work more effectively to utilize available nutrients and produce energy. Moreover, restricted feeding and low-carbohydrate diets encourage mitochondria to produce energy from fats, which can help maintain high energy levels for longer periods.

In summary, mitochondria can produce energy very efficiently even with limited food intake. The fundamental mechanisms behind this include the conversion of fatty acids and glucose into energy through processes like oxidative phosphorylation and the citric acid cycle within mitochondria. This energy

production is crucial for increasing cellular efficiency, and mitochondria play a critical role in these energy-generating processes. Therefore, producing a lot of energy from little food is related to the body's ability to use nutrients in the most efficient way, and this efficiency depends on the effectiveness of mitochondria.

In order to understand how unhealthy foods make us sick, we first need to understand how mitochondria get damaged. Because all diseases start at the cellular level. The main cause of mitochondrial damage is the electron transport chain (ETC), which produces an excess of free radicals during the process of extracting energy from food. The ETC machine is never 100% efficient. Even under ideal conditions, there is always a 3-4% leakage. This leakage is called free radicals. However, when the system can no longer compensate for this leakage, and the percentage of leakage exceeds the capacity to neutralize it, this becomes the foundation of diseases and unhealthiness. All chronic degenerative diseases and even aging itself occur in conjunction with an increase in free radicals and damaged mitochondria**.**

The Importance of Diet and Mitochondria in Free Radical Formation:

Diet and mitochondria play a crucial role in the formation of free radicals. When we consume food, particularly foods that are high in sugar, unhealthy fats, and processed ingredients, they can increase the production of free radicals in the mitochondria. Mitochondria are responsible for energy production through oxidative phosphorylation, but this process is not perfectly efficient. A small portion of the energy generated is lost as free radicals, which are highly reactive molecules that can damage cells and tissues.

Unhealthy diets, particularly those rich in refined sugars, trans fats, and artificial additives, can overwhelm the mitochondria's ability to manage this excess of free radicals. As a result, these free radicals cause oxidative stress, leading to cellular damage. Over time, this damage accumulates, contributing to the development of chronic diseases, accelerated aging, and mitochondrial dysfunction.

On the other hand, a balanced diet rich in antioxidants (such as fruits, vegetables, and healthy fats) can help mitigate the production of free radicals and support mitochondrial health. By providing the right nutrients, we can enhance the mitochondria’s ability to produce energy efficiently, reducing oxidative damage and promoting overall health.

The largest source of free radicals in the body is the mitochondrial electron transport chain (ETC). The most free radicals are produced at the first station of the ETC. Unfortunately, this is also the most commonly used pathway. Most

carbohydrates, especially simple carbohydrates, primarily use this pathway. After eating, this pathway is heavily activated when consuming these carbs.

The second station of the ETC does not produce free radicals. If carbohydrates are not consumed, fats enter the system through the second station and are converted into energy. This means that in the absence of carbohydrates and during fasting, the body uses its stored fat for energy via the second station. From this, we can understand how free radical production decreases during fasting.

Furthermore, in the evening, mitochondria slow down the first complex (Complex 1) because they do not want to burn carbohydrates. Complex 1 is most active between 09:00 and 14:22 and works minimally after 17:00. It is important to note that the activity of the second complex (station 2) indicates that the body has started burning fats. The control of all other complexes is within the mitochondria, while the regulation of station 2 is controlled by the nucleus, specifically by the DNA. The genes responsible for longevity, known as SIRT genes, are activated by the activity of this complex in the DNA.

The relationship between food and disease

The relationship between food and disease is crucial to understanding how our health is influenced by what we eat. Our diet plays a significant role in either promoting or preventing various diseases. The types of food we consume can directly affect the functioning of our cells, including the mitochondria, which are responsible for energy production.

When we eat nutrient-dense, whole foods, we provide our bodies with the necessary vitamins, minerals, and antioxidants needed to support cellular health, reduce oxidative stress, and fight off harmful free radicals. On the other hand, consuming highly processed, unhealthy foods—such as those high in refined sugars, unhealthy fats, and artificial additives—can lead to an increase in free radicals and inflammation, both of which contribute to chronic diseases.

For instance, diets high in processed carbohydrates, sugars, and unhealthy fats can disrupt mitochondrial function, leading to increased oxidative stress and mitochondrial damage. Over time, this damage can contribute to the development of chronic conditions like diabetes, cardiovascular diseases, and even cancer.

In contrast, certain foods, particularly those rich in antioxidants, healthy fats, and essential nutrients, help support mitochondrial function and reduce inflammation, promoting long-term health and preventing disease. Thus, the food we eat can either fuel disease or enhance our body's natural defense systems, making diet an essential factor in disease prevention and overall wellness.

So, what happens if we can't use the ATP that's produced and the cycle gets disrupted?

ATP production stops immediately, but stopping ATP production doesn't solve the problem. Because if we're still eating, electrons are still entering the Electron Transport Chain (ETC), but they can't be converted into ATP. As a result, the electrons accumulate in the ETC but can't flow properly and start to back up. The energy inside them can't be extracted. Normally, the energy comes from the electrons that pump protons across the mitochondrial membrane. But without energy, the protons stay on the same side of the membrane. The charge difference between the two sides of the membrane decreases. The electrical voltage across the membrane drops, and this voltage is critical for the cell's function. Logically, if the end of the chain isn't flowing, the start of the chain won't be working either. Since ATP is not needed, pyruvate can no longer send energy to the mitochondria and instead gets stored as triglycerides, or fat.

In other words, if there is too much ATP and it isn't used, the system signals to start storing what was consumed.

Conclusion

If the mitochondrial membrane is damaged due to free radical leakage, electrons cannot be obtained from the electron transport chain (ETC). If the ETC doesn't function, it won't be possible to produce energy through the oxygen-dependent pathway. In this case, pyruvate produced from glucose will be converted into lactic acid.To survive, the cell will prefer to use glucose—its only source of anaerobic energy—far more intensively.The accumulation of acidity leads to damage, and the cell's DNA is also affected. Ultimately, the cell begins to proliferate uncontrollably. The development of diseases certainly doesn’t happen overnight. The damage we’ve discussed accumulates within the cells over years, and only later does it manifest as diseases in organs. Therefore, serious illnesses arise from the accumulation of poorly functioning cells as we age. For example, cancer is more common in older age because cancer cells are essentially just "smart" cells trying to survive under harsh conditions. To avoid death, they push the limits so much that, instead of functioning like a developed human cell, they resort to fermentation—basically, decay—to produce energy. The root cause of all of this is the increased damage from free radicals.

Photosynthesis in plants and oxidative phosphorylation in humans' ETZ are exactly the opposite processes. Simply put, we, through plants and the animals that eat them, consume sunlight to gain energy. We derive the power to resist the entropy of the universe from the sun. Energy is necessary to maintain order and prevent chaos. Since our true energy source is the sun, we cannot derive life energy from processed foods that lack sunlight.

Free radicals oxidize the membranes. They rust them. When the membrane rusts, it becomes a poor membrane, and since energy production itself takes place on the membrane, energy production is also impaired. When free radical damage occurs in the matrix, ATP production slows down. In this case, energy is depleted. Which organ would want a poorly functioning mitochondrion? It's better to eliminate malfunctioning mitochondria. New ones should take their place. However, the process of mitochondrial renewal becomes more difficult as we age. In youth, when there are more mitochondria and more energy, we can easily perform these tasks. But as we age, we won't have enough energy or mitochondria to perform them. In fact, by the age of 90, a person's mitochondria have decreased by 50% compared to when they were 20. Fewer mitochondria mean less energy. Less energy means less work, less repair. Without renewal, we either have poorly functioning cells or are forced to settle for less energy than before. Therefore, as we age, we need to take better care of our mitochondria, protect them from free radical damage, and understand what to eat, how to eat, when to eat, and how much to eat.

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