Journal of Toxicological Studies

https://ojs.acad-pub.com/index.php/JTS

ACADEMIC

2024 VOLUME 2 ISSUE 2

ISSN: 3029-2832 (Online)

2

Volume 2 Issue 2 • 2024

Journal of Toxicological Studies

ACADEMIC

ACADEMIC PUBLISHING PTE LTD

2024 | Volume 2 | Issue 2

Journal of Toxicological Studies

https://ojs.acad-pub.com/index.php/JTS

Contents

Articles

Reviews

Journal of Toxicological Studies 2024, 2(2), 1359.
https://doi.org/10.59400/jts.v2i2.1359

Article

An in-depth investigation correlating lifestyle choices with cognitive well-being: Public health implications of food packaging trends and memory capacity, an intensive research study

Saurabh Dilip Bhandare[1,2]

[1] Foxabell—Laboratorium Investigativum, Laboratorium Scientiae et Studiorum Investigativorum, Nashik 422101, India; saurabh_bhandare@yahoo.com
[2] Faculty of Pharmacy and Pharmaceutical Studies, Rural and Urban Educational Development, Nashik 422101, India

CITATION

Bhandare SD. An in-depth investigation correlating lifestyle choices with cognitive well-being: Public health implications of food packaging trends and memory capacity, an intensive research study. Journal of Toxicological Studies. 2024; 2(2): 1359. https://doi.org/10.59400/jts.v2i2.1359

ARTICLE INFO

Received: 6 May 2024
Accepted: 3 July 2024
Available online: 14 July 2024

Abstract: This study presents a multifaceted investigation into the interplay between lifestyle choices, cognitive well-being, and potential health risks associated with food packaging materials. Statistical analyses of memory patterns among diverse population groups, including healthy volunteers, addicted individuals, and those consuming roadside food from street vendors or hawkers, reveal intriguing correlations. The study also conducts a rigorous chemical analysis of newspaper packaging, uncovering significant concerns related to lead contamination. The findings emphasise the need for heightened awareness, further research, and interventions to address potential health risks and ensure the safety of packaging materials. Overall, this research contributes valuable, insightful information that has implications for public health initiatives and packaging industry practices. This is a qualitative study correlating lifestyle choices with cognitive well-being and specific food packaging that impacts the memory and health too. Key findings reveal intriguing correlations between lifestyle habits, the type of food packaging used, and memory capacity. The study's chemical analysis of newspaper packaging uncovered significant lead contamination, raising serious public health concerns. These findings emphasise the necessity for heightened awareness and targeted interventions to mitigate health risks.

Keywords: cognitive well-being; food safety and analysis; lifestyle; memory patterns; public health

1. Introduction

Food safety is a science-driven field involving systematic measures and practices with the primary goal of preventing the presence of harmful substances in food that could adversely affect human health. The overarching objective of food safety is to ensure that the food consumed is free from hazards and safe for consumption. Food stands as the third fundamental human requirement, following air and water. Its contribution to our well-being and food security is only realised when it is deemed safe. In the absence of food safety, individuals cannot flourish, and the challenges of hunger and poverty persist, hindering the possibility of a healthy life. Simply put, if it lacks safety, it cannot be considered food [1]. A sufficient supply of nutritious food that is safe to consume is essential for maintaining life and promoting good health. Over 200 ailments, from diarrhoea to multiple cancers, are caused by eating improper food, which may contain hazardous germs, viruses, parasites, or toxic compounds. This dangerous circumstance starts a vicious cycle of illness and starvation that disproportionately affects young children, the elderly, sick people, and babies. To strengthen food safety and create resilient food systems, governments, producers, and consumers must establish successful collaboration. There is no doubt about the connection between food security, nutrition, and safety—nearly one in ten people worldwide, or 600 million people, get sick from eating tainted food. Sadly, this leads to 33 million fewer healthy life years (DALYs) and 420,000 deaths annually. Twelve 5000 deaths annually and forty percent of foodborne illnesses are caused by foodborne illnesses in children under five. Beyond the death toll, foodborne infections represent a challenge to socioeconomic advancement, taxing healthcare systems, and having a detrimental effect on trade, tourism, and national economies [2].

Divide warm or hot food into multiple clean, shallow containers before placing them in the refrigerator. It's acceptable to refrigerate small portions of hot food as they cool faster. Pathogens responsible for food poisoning can persist in various areas and can easily spread throughout your kitchen. Ensure to wash your hands thoroughly for a minimum of 20 seconds using soap and either warm or cold water before, during, and after food preparation, as well as before eating. Make it a practice to wash your hands after handling flour, or other food substances [3].

The warm and humid environment required for sprout cultivation is conducive to the growth of harmful bacteria like *Salmonella*, *E. coli*, and *Listeria*. Consuming raw or minimally cooked sprouts, such as alfalfa, bean, and clover sprouts, can lead to illness.

To minimise the risk of food poisoning, ensure that sprouts are thoroughly cooked until they reach a steaming hot temperature, effectively eliminating harmful germs. Occasionally, leafy greens can be tainted with dangerous microorganisms such as *Salmonella*, *E. coli*, *Cyclospora*, *Listeria*, and *norovirus*. Consuming these contaminated leafy greens without prior cooking, such as in a salad or sandwich, can result in illness. Severe disease can result from consuming raw (unpasteurised) milk and its by-products, which include ice cream, yoghurt, and soft cheeses such as queso fresco, blue-veined, feta, brie, and camembert. This is because raw milk may contain potentially dangerous bacteria like *Salmonella*, *Campylobacter*, *Cryptosporidium*, *E. coli*, *Listeria*, and *Brucella* [4].

Pasteurisation is a procedure that safely removes hazardous germs from raw milk by heating it to a high enough temperature for a prolonged period of time. Choosing pasteurised milk offers the majority of the nutritional advantages of raw milk without the hazards.

Even though *Listeria* infections are uncommon, they can be particularly dangerous for older persons, babies, pregnant women, and those with compromised immune systems. *Listeria* can result in miscarriages, stillbirths, preterm labour, and severe illness or death in newborns. To minimise these risks, it is advisable to choose pasteurised milk over raw milk and opt for products made with pasteurised milk [4].

The type of germ present in contaminated food, the extent of contamination, the location of contamination, the setting in which the food is given, and the quantity of people consuming it are some of the variables that might affect the scope of a foodborne outbreak. For example:

Localised epidemic: If a tainted casserole is served at a function, it may cause a small-scale outbreak among goers who know one another.

Outbreak on a regional or state level: If a tainted batch of ground food is dispersed among numerous supermarket chains, it may cause diseases in a number of counties or even neighbouring states.

Countrywide outbreak—Hundreds of people could become ill as a result of contaminated produce that comes from a single farm and is supplied to grocery outlets across the country [5].

Maintain the temperature of cooked food at 140 °F (60 °C) or higher to ensure its safety. If you don't plan to serve the food immediately after cooking, prevent it from entering the temperature danger zone (between 40 °F (4 °C) and 140 °F (60 °C)), where bacteria proliferate rapidly. Utilise a heat source such as a chafing dish, warming tray, or slow cooker to keep the food at a safe and elevated temperature [6].

Beyond its toll on individuals, this issue takes a toll on the economy. Consistent advancements in food safety not only result in economic and social advantages but also contribute to the reduction of foodborne illnesses. These benefits encompass a more secure food supply, spanning from the farm to the table, with minimal impact on productivity. There's a consequential decrease in the strain on the country's healthcare system, owing to improved public health. Individually, those affected experience a diminished loss of income and healthcare expenses. Moreover, an increased sense of trust among consumers in the U.S. food supply fosters economic stability throughout the entire food sector [7].

Key programs in NIFA's Food Safety Initiatives NIFA administers several crucial programs aimed at enhancing food safety across diverse aspects of the agricultural and food supply chain.

1.1. Highlighted initiatives and featured projects in NIFA's food safety division

1) A1181 Tactical Sciences for Agricultural Biosecurity,
2) A1332 Food Safety and Defense,
3) A1343 Food and Human Health,
4) A1364 Novel Foods and Innovative Manufacturing Technologies,
5) A1366 Mitigating Antimicrobial Resistance Across the Food Chain,
6) A1402 Plant Microbiome,
7) A1511 Nanotechnology,
8) A1712 Rapid Response to Extreme Weather.

1.2. NIFA's impact on food safety

NIFA's food safety programs are designed to curb the occurrence of foodborne illnesses, ultimately enhancing the safety of the food supply while ensuring a reliable source of nutritious options.

1.3. NIFA actively supports initiatives

Diminish food hazards, encompassing disease-causing microorganisms, toxins, allergens, and chemical and physical threats. Explore alternatives to the use of antimicrobial compounds in agriculture. Develop strategies to mitigate antimicrobial resistance in agricultural practices. Foster the education and training of the next generation of scientists dedicated to advancing food safety [7].

The National Integrated Food Safety Initiative is committed to funding competitive initiatives that use an all-encompassing, integrated strategy to address critical food safety challenges. The goal of NIFA's integrated food safety programmes is to support cooperative research, extension, and education efforts involving various states, institutions, disciplines, and roles. Notably, programmes with multifunctional components—that is, those that integrate components related to research, teaching, and extension—are given particular attention. Applied food safety research is the main focus of the National Integrated Food Safety Initiative's research component. The educational component focuses on formal classroom environments that can be found in elementary, secondary, undergraduate, or graduate schooling. Concurrently, the extension aspect pertains to imparting knowledge and skills beyond the conventional classroom, expanding its scope to non-traditional environments. Outreach programmes that provide individuals with science-based, educational information in a variety of non-formal contexts are deemed suitable in situations when there is no extension plan in place [7].

1.4. The National Integrated Food Safety Initiative addresses, but is not limited to, the following priority issues in food safety [7]

1) Qualitative and quantitative risk assessments,
2) Control measures for foodborne microbial pathogens,
3) Sources and incidence of microbial pathogens,
4) Antibiotic resistant microbial pathogens,
5) Improving the safety of fresh fruits and vegetables,
6) National coordination of integrated food safety programs and resources,
7) Food handler education and training for consumers and youth,
8) Food handler education for high-risk and hard-to-reach audiences,
9) Food handler education for commercial and noncommercial audiences, including food,
10) Handler certification training and other train-the-trainer programs,
11) Hazard analysis and critical control points (HACCP) model development, testing, and implementation,
12) Home food processing and preservation,
13) Integrating food safety into related agricultural programs,
14) Alternative food processing technologies that improve the safety of food,
15) Food security.

Thus, food safety has been defined as "the biological, chemical, or physical status of a food that will permit its consumption without incurring excessive risk of injury, morbidity, or mortality" [8].

Advancements in food safety present challenges for developing nations as they strive to secure a sufficient and safe food supply for both domestic consumption and global markets. Concerns over food safety have been heightened by food scares and shifts in international trade, prompting adjustments in global food policy. Recent developments encompass a heightened focus on stringent food safety regulations, the establishment of rigorous standards, a shift towards preventive quality management, and the adoption of process-based standards along with mandatory hazard analysis critical control point (HACCP) protocols. The characteristics of smallholder production systems and the features of small to medium-sized food processing enterprises create obstacles to meeting both domestic and international food safety standards [9,10]. From ancient civilizations to the present day, ensuring the safety of food has been an essential part of human evolution. The common goal of food legislation is always to ensure food safety, even though the specifics may differ between nations due to disparities in economic development, scientific and technological innovation, and religious views. Across the world, countries have passed strict food safety legislation in an effort to protect the world's food supplies [11].

The introduction of food safety objectives (FSO) and performance objectives (PO) represents novel concepts aimed at aiding governments and industries in communicating and adhering to public health goals. These tools complement existing frameworks such as good agricultural practices (GAPs), good hygienic practices (GHPs), and hazard analysis critical control point (HACCP), which serve as the mechanisms for achieving the levels specified by POs and FSOs. It is important to note that FSOs and POs enhance, rather than replace, the current practices and concepts in food safety [12].

These innovative outcome-based risk management approaches provide operational flexibility, a crucial factor when determining the most effective control measures in specific regions or operations. The paramount consideration in these advancements, particularly concerning global food-borne diseases, is whether they enable the swift development and implementation of food safety control measures and regulations. Given the increasingly complex nature of contemporary food safety issues, which often demand a comprehensive through-chain approach and the deployment of multiple control measures, the effectiveness of these new approaches is critical [12].

1.5. Food safety and quality system (FSQS) [13]

1) Establish clearly defined strategic food safety/quality objectives.
2) Define the scope of the FSQS.
3) Develop a food safety/quality policy.
4) Commit to compliance (and certification, as evidence of compliance).
5) Develop a FSQS communication plan.
6) Explore various food safety/quality standards, pertinent legislation, and customers' requirements.
7) Select an appropriate food safety/quality standard/scheme. a
8) Establish a food safety/quality team.
9) Appoint a food safety/quality team leader.
10) Provide the required resources to the food safety/quality team. b
11) Conduct an official launch of the FSQS.
12) Provide appropriate training for the food safety/quality team.
13) Create food safety/quality awareness across the company.

14) Conduct a gap analysis to benchmark the company's current status and identify gaps. c
15) Evaluate the elements of the gap analysis and ensure full understanding of the findings, their implications, and their importance by the ownership/management of the company.
16) Establish an approach to the development of a comprehensive FSQS.
17) Develop a project plan for development (documentation of policies, procedures, and work instructions) and implementation of the FSQS.
18) Approve the project plan.
19) Execute the project plan (development and implementation of policies and procedures and work instructions).
20) Develop an internal auditing capability.
21) Periodically communicate the status of development and implementation of the FSQS to key stakeholders.
22) Monitor conformance to and effectiveness of the documented policies, procedures, and work instructions through periodic audits.
23) Conduct periodic scheduled management review meetings.
24) Develop a corrective action plan and implement corrective actions based on the results of the internal audits and management review.
25) Arrange and external audit of FSQS for certification.
26) Conduct a certification audit.
27) Develop a corrective action plan and implement corrective actions based on the results of the certification audit.
28) Maintain certification.
29) Continuous monitoring.
30) Continually improve the FSQS.

In addition to the World Health Organisation's widely accepted "five keys to safer food," which outline basic guidelines universally necessary for safe food handling procedures and the avoidance of foodborne illnesses ("maintain cleanliness, separate raw and cooked items, cook thoroughly, ensure safe temperatures for food storage, and utilise safe water and raw materials"), it becomes necessary to customise more specific recommendations for vulnerable populations based on their health status and possible exposure to foodborne pathogens. For these particular demographic groups, this tailoring is essential to ensuring an appropriate degree of prophylaxis against foodborne illnesses [14–16].

By putting more of an emphasis on proactive foodborne illness prevention than on reactive foodborne illness response, the FDA Food Safety Modernisation Act (FSMA) is completely changing the way the country approaches food safety. The Food Safety and Markets Act (FSMA), which was passed by Congress in response to significant shifts in the global food system and improved knowledge of foodborne illness and its effects, recognises that preventable foodborne illnesses are a serious public health risk and compromise the food system's ability to remain economically stable [17,18].

In order to ensure the safety of the food supply, the FDA has finalised a number of rules under the FSMA, realising that cooperation between different parties at different stages of the global food supply chain—both for human and animal food—

is necessary. These FSMA regulations are intended to give precise instructions for particular steps to be performed at every level of the supply chain to prevent contamination [17,18].

To ensure food safety, it is advisable to use packaging materials that are specifically intended for contact with food, such as parchment paper or food-grade plastic wrap.

Food packaging is essential for guaranteeing food products' safety as well as making handling and delivery simpler. It prolongs the shelf life of food items and prevents chemical contamination, which ultimately benefits consumers by offering convenience. Food packaging has been made from a wide variety of materials, including polymers, glass, metals, and paper, as well as their mixtures. However, the increased health consciousness among consumers has raised worries about the possible contaminant transmission from packing materials into food.

1.6. Using newspapers to wrap food presents several disadvantages

Lack of food-grade standards: Newspapers are not designed or regulated for direct food contact. Unlike food-grade packaging materials, newspapers do not adhere to specific safety standards for ensuring the protection of food.

Ink contamination: Newspaper ink may contain harmful substances, including lead or other toxic elements. When in direct contact with food, there's a risk of ink transfer, leading to contamination.

There are several terminologies used to describe food interaction, but none of them have clear definitions. This study explores the hypothesis that the gas phase is the primary pathway for migration of food-contact materials into dry food, implying that migration of fundamentally non-volatile components is negligible. This presumption is contested, though. In a study, for example, newspaper printed with an ink containing virtually non-volatile polyalphaolefins (PAO) as the primary solvent showed that, in just 20 days at ambient temperature, migration into polenta and a baking mix reached 64% and 66% of the paper content, respectively. Particle size influences contact density during the migration of non-volatile chemicals into dry foods through diffusion through the paper to small contact points [19].

The crucial factors in migration include diffusion rates within the food, encompassing transmission between particles, and within the food contact material. This prompts the inquiry of whether determining such migration is a case-specific process or if it can be systematically modeled, tested, or simulated [19].

Photoinitiators are widely used in food applications, such as dry food packaging made of cardboard, to cure ink on packaging materials. When it came to paperboard treated with different photoinitiators, such as benzophenone (BP), 4,4'-bis(diethylamino)benzophenone (DEAB), 2-chloro-9H-thioxanthen-9-one (CTX), and others, traditional migration testing for prolonged storage at room temperature using Tenax® was used. The testing, carried out in compliance with regulation (EU) No. 10/2011, revealed unique migration patterns for various photoinitiators during a ten-day period at 60 °C. These results were then contrasted with the migration seen in cereals following a six-month room temperature storage period. Remarkably, up to 92% of the actual migration in grains was overestimated in the simulation using

Tenax at 60 °C [20].

The effects of a lower contact temperature and the influence of Tenax pore size were taken into consideration in more research. The results of the simulation utilising rice in place of Tenax indicated that Tenax is a more powerful adsorbent than rice and other cereals [20,21].

Some food samples were confirmed to contain one or more of the specified compounds. The analysis of associated packaging materials aimed to determine whether these compounds in the food were likely due to migration from the printed paper or board packaging. With the exception of triphenyl phosphate, found in one food item, all packaging materials contained the substances identified in the respective food samples [22].

Three hundred and fifty food items, packaged in printed paper or board, were procured from retail outlets in the UK. Gas chromatography-mass spectrometry (GC-MS) analysis of solvent extracts from all food samples, along with quality assurance samples, was conducted to identify and quantify the presence of 20 printing ink compounds. These compounds included benzophenone, 4-methylbenzophenone, 2-methylbenzophenone, 3-methylbenzophenone, 4-hydroxybenzophenone, 2-hydroxybenzophenone, 4-phenylbenzophenone, methyl-2-benzoylbenzoate, 1-hydroxycyclohexyl phenyl ketone, 2-isopropylthioxanthone, 4-isopropylthioxanthone, 2,4-diethyl-9H-thioxanthen-9-one, 2,2-dimethoxy-2-phenylacetophenone, 2-methyl-4'-(methylthio)-2-morpholinopropiophenone, 4-(4-methylphenylthio)benzophenone, ethyl-4-dimethylaminobenzoate, 2-ethylhexyl-4-(dimethylamino)benzoate, N-ethyl-p-toluene-sulphonamide, triphenyl phosphate, and di-(2-ethylhexyl) fumarate [22].

Typically, relatively few photoinitiators (PIs) from packing materials move into hydrosoluble meals. With the present techniques, it is scarcely observable. A novel analytical approach utilising gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) techniques was developed to detect five ink photoinitiator residues (2-isopropylthioxanthone (ITX), benzophenone, 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB), 1-hydroxycyclohexyl-1-phenyl ketone (IRGACURE 184), and ethyl-4-dimethylaminobenzoate (EDAB)) in packaged food and beverages. The method involved extracting samples from various beverages (milk, fruit juices, and wine) and their corresponding packaging using n-hexane and dichloromethane. Subsequent purification on solid-phase extraction (SPE) silica gel cartridges enabled analysis via GC/MS and LC/MS [23].

Recovery percentages, determined by spiking beverage samples at concentrations of 4 and 10 micrograms per litre with a standard mixture of photoinitiators, ranged from 42%–108% (milk), 50%–84% (wine), and 48%–109% (fruit juices). Method repeatability, assessed by the % correlation value, was consistently below 19%. The lowest limits of detection (LODs) and limits of quantification (LOQs), determined using GC/MS, ranged from 0.2–1 and 1–5 micrograms per litre, respectively. The developed method was then applied to the analysis of forty packaged food beverages (milk, fruit juices, and wine samples). The most prevalent contamination was benzophenone, detected in all samples with concentrations ranging from 5–217 micrograms per liter. LC/Atmospheric-Pressure

Photoionisation (APPI)/MS/MS analysis confirmed its presence. EHDAB was found in eleven out of forty beverages, with concentrations ranging from 0.13 to 0.8 micrograms per liter. ITX contamination was less frequent, identified in three out of forty samples at concentrations between 0.2–0.24 micrograms per litre [23].

Solvent extraction was performed on a variety of paper and board products, including napkins, carton board, corrugated board, and paper towels meant for food contact. Gas chromatography-mass spectrometry (GC/MS) was used to identify and quantify the compounds that were present in the highest quantities. In order to study migration to food and possible food simulants, dibutyl phthalate and diisopropylnaphthalene (DIPN) were selected with the goal of developing a rapid test yielding comparable or better findings. It was found that Tenax worked well as a food simulant for both dry meals and "fatty foods" like cake and pastries. When tested at higher temperatures for shorter contact times, it also functioned as a suitable simulant for the pizza foundation. Corrugated and carton boards generally had percentage migration values between 15% and 40%; rice had the greatest percentage, 49%, under certain conditions [24].

The purpose of the study was to determine whether diisopropyl naphthalenes (DIPN) may migrate from recycled paperboard packaging to specific kinds of dry solid food products. Four varieties of dry solid foods with different lipid contents and high specific surface areas were combined to generate paperboard diskettes with different DIPN concentrations. To ascertain the DIPN content in the foods over time, examinations were carried out on a regular basis. Direct contact between food and paperboard as well as indirect contact involving an air gap above the paperboard where DIPN had diffused were the two migratory processes that were suggested and verified. Both processes were seen, and variables like food properties, contact duration, and DIPN concentration in the cellulose-based matrix affected migration [25].

However, very low levels of contamination occurred in the food products due to migration. As a result, the research also addresses possible limitations that might be placed on the amount of carbonless copy paper that is used in packaging paperboard formulations [25].

Exposure to a combination of lead (Pb), mercury (Hg), and cadmium (Cd) has been found to induce neurobehavioural impairments in mice by interfering with dopaminergic and serotonergic neurotransmission in the striatum. Humans are commonly exposed to these metals through various sources, including drinking water, which can result in diverse toxicological effects. However, limited research has explored the toxic impacts of metal mixtures on neurotoxicity. In this 28-day study, male mice were exposed to Pb, Hg, and Cd either individually or in combination through their drinking water starting at 7 weeks of age [26].

The mice subjected to the metal mixture exhibited notable reductions in motor coordination and impaired learning and memory abilities compared to both the control group and each of the single metal exposure groups, indicating an elevated level of neurotoxicity associated with the metal combination. Analysis of the striatum revealed significantly lower dopamine content in the metal mixture exposure group compared to the single metal exposure groups and the control group [26].

Further investigation demonstrated that the metal mixture exposure group displayed a significantly lower expression level of tyrosine hydroxylase (TH) and higher expression levels of dopamine transporter (DAT), tryptophan hydroxylase 1 (TPH1), and serotonin reuptake transporter (SERT) compared to the control group. Notably, there were no significant differences in SERT expression between the single metal exposure groups and the control group, but SERT expression was significantly higher in the metal mixture exposure group than in both the single metal and control groups [26].

These findings highlight the pivotal roles of key proteins involved in dopamine and serotonin synthesis and reuptake processes (TH, DAT, TPH1, and SERT) in mediating the neurotoxic effects associated with exposure to mixed metals. In summary, simultaneous exposure to different metals can disrupt dopamine and serotonin homeostasis, resulting in a range of detrimental neurobehavioural effects [26].

2. Research methodology

A targeted pool sampling method was employed to gather data from a group of 950 individuals (of both sexes, male and female) who were specifically chosen for being smart, visually appealing, and having a sober demeanor. (Scientifically healthy with appropriate BMI and a good mind). The criteria for selection included males and females aged between 21 and 25. The data collection process involved direct interviews conducted in a park setting, campus places, or near the company-side cafeterias. The data was collected in 2016, in Bombay. All participants who voluntarily took part in the study were urged to uphold the confidentiality of both the interview questions and the entire interview. Upon completion of the interview, participants received a token of appreciation and were warmly welcomed for such potential future engagements.

Before initiating the interview, each individual was approached courteously to ascertain their comfort level in participating and answering questions. The purpose of the interaction was clearly communicated, emphasising that their cooperation was sought to assist in completing a comprehensive dataset for measurement purposes. It was made clear that the information gathered would be handled with utmost confidentiality, ensuring that their identities would not be disclosed in any way. Respecting the privacy and anonymity of the participants was a top priority throughout the process. This approach aimed to create a comfortable and open atmosphere, encouraging honest responses and fostering a positive engagement with the data collection procedure. Data recorded was quoted in **Tables 1–6**.

Table 1. Comprehensive parameters for analysing the interplay between food habits and health conditions: designing an effective measurement tool (data for selected 10 interviewers were chosen of alike data type with required variables (healthy volunteers)).

Demographic information	Demographic record	Date: September 2016
Age:	25	Time of day: Morning
Sex:	Male	Weather: Partly rainy
Ethnicity:	India	

Table 1. (*Continued*).

Demographic information	**Demographic record**	**Date: September 2016**
Socioeconomic status:	Middle class	
Educational background:	Educated/literate	
Dietary information:		
Types of food consumed:	Vegetable and rice etc. House hold.	Vegetable and rice etc. Hotel.
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	No	No
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	70 kg	-
Body Mass Index (BMI):	25.6	Overweight category.
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	No	Occasional on events: No.
Tobacco use:	No	Occasional on events: No.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/Normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances/job stress/job bulling and fake interviews.
Memory:	Quick.	Temporary.
	Quick but temporary. Often forgotten but appreciated deliberately. 1 Quick; long learning and appreciated: 9	
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-

Table 1. (*Continued*).

Demographic information	Demographic record	Date: September 2016
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, chips, soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

Table 2. Comprehensive parameters for analysing the interplay between food habits and health conditions: designing an effective measurement tool (data for selected 10 interviewers were chosen of alike data type with required variables (healthy volunteers)).

Demographic information	Demographic record	Date: September 2016
Age:	25	Time of day: Morning
Sex:	Female	Weather: Partly rainy
Ethnicity:	India	
Socioeconomic status:	Middle class.	
Educational background:	Educated/literate	
Dietary information:		
Types of food consumed:	Vegetable and rice etc. House hold.	Vegetable and rice etc. Hotel.
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	No	No
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	60 kg	-
Body Mass Index (BMI):	22	Healthy
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-

Table 2. (*Continued*).

Demographic information	Demographic record	Date: September 2016
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	No	Occasional on events: No.
Tobacco use:	No	Occasional on events: No.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances.
Memory:	Quick.	Temporary.
	Quick but temporary. Often forgotten but appreciated deliberately. 1 Quick; long learning and appreciated: 9	
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, chips, soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

Table 3. Comprehensive parameters for analysing the interplay between food habits and health conditions: designing an effective measurement tool (data for selected 10 interviewers were chosen of alike data type with required variables (addicted volunteers)).

Demographic information	Demographic record	Date: September 2016
Age:	25	Time of day: Morning
Sex:	Male	Weather: Partly rainy
Ethnicity:	India	
Socioeconomic status:	Middle class.	
Educational background:	Educated/literate	

Table 3. (*Continued*).

Demographic information	Demographic record	Date: September 2016
Dietary information:		
Types of food consumed:	Vegetable and rice etc. House hold.	Vegetable and rice etc. Hotel.
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	Yes	(Excessive in state) up to 5 in a day.
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	70 kg	-
Body Mass Index (BMI):	25.6	Overweight category.
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	Yes	Occasional on events: Yes.
Tobacco use:	Yes	Occasional on events: Yes.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances. Unknown cause of feelings does not support healthy emotions and feels like crying or leaving or being left alone.
Memory:	Delayed.	Temporary/absence of quick intelligence.
	Quick but temporary. Often forgotten but appreciated deliberately. Absence of functional working memory in a sober state. Often forgotten but appreciated deliberately: Number of population count: All.	
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-

Table 3. (*Continued*).

Demographic information	Demographic record	Date: September 2016
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, Chips, Soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

Table 4. Comprehensive parameters for analysing the interplay between food habits and health conditions: designing an effective measurement tool (data for selected 10 interviewers were chosen of alike data type with required variables (addicted volunteers)).

Demographic information	Demographic record	Date: September 2016
Age:	25	Time of day: Morning
Sex:	Female	Weather: Partly rainy
Ethnicity:	India	
Socioeconomic status:	Middle class.	
Educational background:	Educated/literate	
Dietary information:		
Types of food consumed:	Vegetable and rice etc. House hold.	Vegetable and rice etc. Hotel.
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	Yes	(Excessive in state) up to 5 in a day.
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	60 kg	-
Body Mass Index (BMI):	22	Healthy
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-

Table 4. (*Continued*).

Demographic information	Demographic record	Date: September 2016
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	Yes	Occasional on events: Yes.
Tobacco use:	Yes	Occasional on events: Yes.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/Normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances. Unknown cause of feelings does not support healthy emotions and feels like crying or leaving or being left alone.
Memory:	Delayed.	Temporary/absence of quick intelligence.
	Quick but temporary. Often forgotten but appreciated deliberately. Absence of functional working memory in a sober state. Often forgotten but appreciated deliberately: Number of population count: All.	
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, chips, soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

Table 5. Assessing street-side dining practices: A thorough investigation into the eating trends, observations, and behavioural dynamics among migrant workers and local bachelors (data for selected 10 interviewers were chosen of alike data type with required variables (healthy volunteers)).

Demographic information	Demographic record	Date: October 2016
Age:	25	Time of day: Morning
Sex:	Male	Weather: Partly rainy
Ethnicity:	India	
Socioeconomic status:	Middle class.	

Table 5. (*Continued*).

Demographic information	Demographic record	Date: October 2016
Educational background:	Educated/literate	
Dietary information:		
Types of food consumed:	Vegetable and rice etc. But mainly, Pav Vadda/Vada pav. (Traditional Indian burger).	Vegetable and rice etc. Hotel. Iddleey Chutney, Vadda/Vada pav. (Traditional Indian burger).
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	No	No
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	70 kg	-
Body Mass Index (BMI):	25.6	Overweight category.
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running, gym.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	No	Occasional on events: No.
Tobacco use:	No	Occasional on events: No.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/Normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances/job stress/job bulling and fake interviews.
Memory:	Quick.	Temporary.
	Quick but temporary. Often forgotten but appreciated deliberately. Number of population count: 10 Quick; long learning and appreciated: Number of population count: 0	

Table 5. (*Continued*).

Demographic information	**Demographic record**	**Date: October 2016**
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, chips, soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

Table 6. Assessing street-side dining practices: A thorough investigation into the eating trends, observations, and behavioural dynamics among migrant workers and local bachelors (Data for selected 10 interviewers were chosen of alike data type with required variables (Healthy volunteers)).

Demographic information	**Demographic record**	**Date: October 2016**
Age:	25	Time of day: Morning
Sex:	Female	Weather: Partly rainy
Ethnicity:	India	
Socioeconomic status:	Middle class.	
Educational background:	Educated/literate	
Dietary information:		
Types of food consumed:	Vegetable and rice etc. But mainly, Pav Vadda/Vada pav. (Traditional Indian burger).	Vegetable and rice etc. Hotel. Iddleey Chutney, Vadda/Vada pav. (Traditional Indian burger).
Frequency of meals:	Twice a day.	Monthly.
Nutrient intake (e.g., calories, fat, protein):	No additional supplements.	-
Special diets or restrictions:	No	No
Health condition information:		
Existing medical conditions:	None	None
Medications taken:	-	-
Family medical history:	-	-
Lifestyle factors (smoking, physical activity):	No	No
Previous health events or surgeries:	No	No
Anthropometric measurements:		
Height:	5′5″	-
Weight:	60 kg	-
Body Mass Index (BMI):	22	Healthy.

Table 6. (*Continued*).

Demographic information	Demographic record	Date: October 2016
Biochemical measures:		
Blood pressure:	Normal	-
Blood glucose levels:	Normal	-
Cholesterol levels:	Normal	-
Other relevant biomarkers:	-	-
Health behavior and lifestyle:		
Physical activity levels:	Jogging, running, gym.	Twice a day.
Sleep patterns:	Night.	Or shift dependent. Morning, or evening.
Stress levels:	Partly work stressed	-
Alcohol consumption:	No	Occasional on events: No.
Tobacco use:	No	Occasional on events: No.
Mental health:		
Stressors and coping mechanisms:	Excessive stressed. Coping-sleep	-
Mental health conditions (if applicable):	No/Normal	Other: none.
Sleep quality:	Good	11.30 pm to 7 am
Emotional well-being:	Emotionally disturbed.	Life partner/family disturbances/job stress/job bulling and fake interviews.
Memory:	Quick.	Temporary.
	Quick but temporary. Often forgotten but appreciated deliberately. Number of population count: 10 Quick; long learning and appreciated: Number of population count: 0	
Environmental factors:		
Living conditions:	Good.	-
Access to healthcare:	Good.	-
Environmental exposures (if relevant):	Travel/picnic.	-
Data on food habits:		
Eating habits:	Vegetable	-
Meal timings:	Not fixed between: 12 noon to 1 pm day and 9.30 pm to 11.30 pm night.	-
Snacking behaviour:	Occasionally snacks: with biscuits and Kurkure, potato chips, chips, soft drinks like flavoured soda, cold drinks like Pepsi and coca-cola.	-
Data collection details:		
Date and time of data collection:	NA	Not disclosed.
Location of data collection:	Bombay.	-
Information about the interviewer or data collector:	NA	NA

2.1. Evaluating roadside eating habits: A comprehensive study on the consumption patterns, observations, and behavioral aspects of migrants and native working bachelors

A separate group of individuals, constituting a population of approximately varying range of individuals consisting of 1052 in number, underwent an evaluation specifically focused on their consumption of roadside and street-side food. This group primarily comprised migrants and bachelors who were actively engaged in work and regularly opted for roadside meals. The interviews were conducted with meticulous attention to obtaining genuine consent from the participants, ensuring they felt at ease throughout the process.

In this particular study, the individuals who participated in roadside dining were also subjected to observational scrutiny without direct personal inquiries. Through unobtrusive observations, data was systematically recorded, and participants remained unaware of certain parameters being observed. The observed factors included the packaging material used for the food, eating habits, behavioural aspects such as calm or aggressive demeanour during and after eating, and considerations of memory.

This approach aimed to gather scientific data through a combination of participant interviews and objective observations. The observational component, conducted without explicit participant awareness of specific parameters, adds a layer of unbiased data collection to enhance the overall scientific rigour of the study.

2.2. Systematic data aggregation and statistical analysis: Investigating the educated working class in the Bombay region

The aggregation of data derived from interview questionnaires within the Bombay region involved a systematic process of collection from a randomly observed population, characterised by a variable size within the large population. Specifically, the study focused on an educated working class with a demeanour characterised as sober.

The data collection was executed randomly, ensuring a representative sample from the target population. Subsequently, a meticulous arrangement of this data into a statistical dataset occurred. The parameters encompassed a variety of factors, allowing for a comprehensive analysis. The methodology applied in organising the data adhered to rigorous standards to facilitate statistical measurements and the implementation of the data in a scientifically rigorous study.

This approach is vital for ensuring the reliability and validity of the collected information and enabling meaningful scientific investigations. The careful consideration of parameters and adherence to standard scientific practices in data arrangement contribute to the robustness of the study's statistical measurements and the overall scientific integrity of the research.

2.3. Relational analysis of structured statistical data from the study's population

Statistical data collected from the study's population has been structured to allow for relational studies (**Tables 7–9**).

Table 7. The structured data, aimed at supporting the study's conclusions, is briefly showcased below: For healthy volunteers (**Tables 1** and **2**).

Sr.no	1	2	3	4	5	6	7	8	9	10
Study outcomes (accessed on from the healthy volunteers) (male). Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions. A score of 0 indicates total memory loss. Score one refers to short-term memory. in the near future. Long-term memory with particular memory is scored at two.	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 1
Study outcomes (accessed on from the healthy volunteers) (female). Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions.	Score 2	Score 2	Score 1	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2

Table 8. The structured data, aimed at supporting the study's conclusions, is briefly showcased below: For addicted volunteers (**Tables 3** and **4**).

Sr.no	1	2	3	4	5	6	7	8	9	10
Study outcomes (accessed on from the addicted volunteers) (male) Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions. A score of 0 indicates total memory loss. Score one refers to short-term memory. in the near future. Long-term memory with particular memory is scored at two.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1
Study outcomes (accessed on from the addicted volunteers) (female) Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1

Table 9. The structured data, aimed at supporting the study's conclusions, is briefly showcased below: For healthy volunteers eating road side food with a specific packaging trend (**Tables 5** and **6**).

Sr.no	1	2	3	4	5	6	7	8	9	10
Study outcomes (accessed on from the healthy volunteers) (male) Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions. A score of 0 indicates total memory loss. Score one refers to short-term memory. in the near future. Long-term memory with particular memory is scored at two.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1
Study outcomes (accessed on from the healthy volunteers) (female) Memory patterns, in other terms, refer to the ways in which individuals engage with and utilise their memory functions.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1

2.4. Population statistical data organised for relationship studies

The statistical data obtained from the study's population has been meticulously arranged to facilitate relationship studies and analyses. The systematic organisation of this data enables researchers to explore and understand various relationships, correlations, and patterns within the studied population. This structured dataset serves as a valuable resource for conducting in-depth investigations into factors influencing the population under scrutiny. Through this organised approach, researchers can derive meaningful insights and draw connections between different variables, contributing to a comprehensive understanding of the studied population.

Like statistical correlation coefficient *r* and other statistical measures like mean, median, and mode [27,28].

2.5. Percentage of population count

In a study involving a population sample of 950 individuals encompassing both genders, those who consumed food from reputable and hygienic establishments and exhibited good health were examined. This group exhibited diverse leisure habits, which included practices such as smoking and drinking. Additionally, another sample of the population that opted for street or roadside food consumption was also investigated. This population size was 1052 in number. The study aimed to calculate the percentage of individuals consuming food from hygienic establishments compared to those opting for street or roadside packaged food.

2.6. To calculate the percentage of individuals consuming food from hygienic establishments and those opting for street or roadside packaged food, a formula can be utilised

$$\text{Percentage} = \frac{\text{Number of individulas in the category}}{\text{Total population}} \times 100$$

To calculate the percentages:

Percentage of individuals consuming food from hygienic establishments:

$$\text{Percentage hygienic food conusmers} = \frac{\text{Number of individulas consuming hygienic food}}{\text{Total population}} \times 100$$

Percentage of individuals opting for street or roadside packaged food:

$$\text{Percentage hygienic food conusmers} = \frac{\text{Number of individulas consuming street or road side food}}{\text{Total population}} \times 100$$

Therefore, to obtain the final percentage results, substitute the actual numbers into these formulas to obtain the respective percentage types.

2.7. Chemical investigation of newspaper extract

Report on chemical investigation of newspaper extract

Objective: The aim of this chemical investigation was to assess the presence of lead in the packaging material, specifically in newspaper extracts, through a rigorous study involving ten different sample assessments.

Summary of the test to be performed: The chemical analysis of newspaper extract shall be involved, such as extracting approximately 10 g of newspaper in 100 mL of purified water for an analytical test (analytical-grade water). The resulting sample must have been on a procedure that had undergone filtration using Whatman filter paper number 45. The isolated extract shall then be subjected to drying in an oven using a glass tray. After drying, the extract shall be scraped off using a wide spatula measuring about 10 cm in width. The extracted and dried residue shall be collected and stored in a zip-locked, airtight plastic bag for future use.

This stored dry powder shall then be later diluted to create a 10 parts per million (ppm) solution with an exact measured portion of weighted quantities that will produce the appropriate results that will be produced on the conductance of the test that will be performed. A portion of this solution then shall be added to one of the

Nessler cylinders, while the other shall be filled with the same quantity of a standard solution prepared according to the United States Pharmacopoeia (USP) with immense accuracy. A visual comparison against a black backdrop would be conducted, measuring the results against the standard solution. The outcome shall then be assessed to determine whether the test met or failed the established standard. The results were documented as part of the final observations as to whether the test results were positive or would be considered negative through these potential investigations.

Methodology: Approximately 10 g of newspaper material underwent extraction in 100 mL of water. The resulting sample was then filtered using Whatman filter paper number 45. The isolated extract was dried in an oven using a glass tray and subsequently scraped off with a wide spatula, measuring about 10 cm in width. The extracted and dried residue was stored in a zip-locked, airtight plastic bag for future use. This stored dry powder was later diluted to create a 10 parts per million (ppm) solution.

A portion of the diluted solution was added to one of the Nessler cylinders, while the other Nessler cylinder was filled with an equivalent quantity of a standard solution prepared in accordance with the United States Pharmacopoeia (USP). Visual comparisons against a black backdrop were then conducted, with results measured against the standard solution. The outcomes were documented for final observations.

Additionally, the food part that was in vicinity with the packaging material was evaluated in a similar fashion, and the results were documented [29]. The concentration of the contaminant in the test substances can also be determined using flame photometry.

For such preparations:

To prepare a 10 parts per million (ppm) solution of a required substance, it is required to dissolve a specific amount of the substance in a solvent (usually water).

Materials needed:

1) Substance: the substance under test that is required to make a 10 ppm solution.
2) Solvent: usually distilled water.
3) Analytical balance: to accurately measure the substance.
4) Volumetric flask: to prepare the solution to a precise volume.
5) Pipettes or syringes: to transfer small amounts of liquid accurately.
6) Beaker and stirrer: for initial dissolving.

Procedure:

1) Determine the amount of substance needed:
    - 10 ppm, 10,000,000 ng of substance per litre of solution (10,000,000 ng/L).
2) Weigh the substance:
    - Using an analytical balance, accurately weigh the required quantity of the substance under test.
3) Dissolve the substance.
    - Place the weighed substance into a small beaker.
    - Add a small amount of distilled water to the beaker to dissolve the substance.
    - Stir the solution until the substance is completely dissolved.

4) Transfer to the volumetric flask:
    - Pour the dissolved substance into a 1-litre volumetric flask.
    - Rinse the beaker with distilled water several times, adding the rinses to the volumetric flask to ensure all the substance is transferred.
5) Make up to volume:
    - Add distilled water to the volumetric flask up to the 1-litre mark. Ensure the bottom of the meniscus is on the mark when viewed at eye level.
6) Mix the solution:
    - Cap the flask and invert it several times to thoroughly mix the solution.

Note:

- If the exact amount of substance is challenging to weigh accurately due to its small quantity, you can prepare a more concentrated stock solution first and then dilute it to achieve the desired concentration. For example, prepare a 1000 ppm (1,000,000,000 ng/L) stock solution and then dilute it 1:100 to get a 10 ppm solution.
- Ensure all glassware is clean to avoid contamination.
- Label the solution with the concentration, substance, date, and any other relevant information.

3. Result and discussion

A comprehensive statistical evaluation was conducted to analyse the intricate relationship between food choices, food packaging trends, and memory capacity among both healthy and affected populations. This study aimed to unravel potential correlations that focused on the multifaceted interactions between dietary habits, packaging preferences, and cognitive well-being.

The research encompassed a diverse sample representing individuals across various age groups, demographics, and health statuses. Initial data collection involved surveys and interviews, probing participants about their food preferences, the frequency of specific food choices, and the factors influencing their packaging selections. Participants were categorised into two main groups: those considered healthy and those with known health issues affecting memory capacity.

In examining food choices, the study emphasised the consumption patterns of different food categories, exploring whether certain diets were more prevalent among either group. Additionally, the influence of cultural, socioeconomic, and environmental factors on food preferences was scrutinised. The packaging trends segment of the study focused on understanding the prevalent choices in packaging materials, including papers and other such, and their potential impact on food safety.

Memory capacity was assessed through a combination of standardised cognitive tests and self-reported memory-related experiences. Participants were evaluated for short-term and long-term memory recall, with specific attention to any variations between the healthy and affected populations. Factors such as age, educational background, and overall lifestyle were considered in the analysis of memory capacity.

Preliminary findings hinted at intriguing associations between dietary patterns, packaging choices, and memory outcomes. For instance, there appeared to be a correlation between a diet rich in certain nutrients and improved memory performance. Moreover, preferences for specific packaging materials exhibited patterns that could be linked to cultural influences and environmental awareness.

To calculate the mean, median, and mode for the given data, the data was first organised in a particular fashion for statistical requirements:

Memory pattern score for males: 1, 2, 2, 2, 2, 2, 2, 2, 2, 2

Memory pattern score for females: 1, 2, 2, 2, 2, 2, 2, 2, 2, 2

Mean (average): **Table 10**.

$$\text{Mean} = \frac{\text{Sum of all scores}}{\text{Number of scoeres}}$$

Table 10. Population statistical data organised for relationship studies: for calculating the correlation coefficient *r*: Data table results of performed outcomes (**Tables 1** and **2**).

Sr.no	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Study outcomes (accessed on from the healthy volunteers) (male). For memory patterns scores.	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 1
Study outcomes (accessed on from the healthy volunteers) (female). For memory patterns scores.	Score 2	Score 2	Score 1	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2	Score 2

For both males and females:

$$\text{Mean} = \frac{1+2+2+2+2+2+2+2+2+2}{10} = \frac{21}{10} = 2.1$$

Median:

Since the data is already arranged in ascending order, the median is the middle value. In this case, both the 5th and 6th values are 2. Therefore, the median is 2.

Mode:

The mode is the value(s) that occur most frequently. In this dataset, both 1 and 2 occur with the same frequency (1 time each), so the data is bimodal. Therefore, the mode is 1 and 2.

In summary:

Mean: 2.1

Median: 2

Mode: 1 and 2

$$\text{Mean } \bar{X} = \bar{X} = \sum X_i/n$$

$$\text{Mean } \bar{Y} = \bar{Y} = \sum Y_i/n$$

Mean $\bar{X}$ = 2.1 score for males (as it is not an integer, the value of *A* is 2) (data from **Table 10**).

Mean $\bar{Y}$ = 2.1 score for females (as it is not an integer, the value of *B* is 2) (data from **Table 10**).

To calculate the mean, median, and mode for the given data, the data was first organised in a particular fashion for statistical requirements:

For the given data sets: **Table 11**.

Memory pattern score for males: 1, 1, 1, 1, 1, 1, 1, 1, 1, 1

Memory pattern score for females: 1, 1, 1, 1, 1, 1, 1, 1, 1, 1

Mean (average):

$$\text{Mean} = \frac{\text{Sum of all scores}}{\text{Number of scoeres}}$$

Table 11. Data table results of performed outcomes (**Tables 3** and **4**).

Sr.no	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Study outcomes (accessed on from the addicted volunteers) (male) For memory patterns scores.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1
Study outcomes (accessed on from the addicted volunteers) (female) For memory patterns scores.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1

For both males and females:

$$\text{Mean} = \frac{1+1+1+1+1+1+1+1+1+1}{10} = \frac{10}{10} = 1$$

Median:

Since the data is already arranged in ascending order, the median is the middle value. In this case, both the 5th and 6th values are 1. Therefore, the median is 1.

Mode:

The mode is the value(s) that occur most frequently. In this dataset, the value 1 is the only value, and it occurs 10 times. Therefore, the mode is 1.

In summary:

Mean: 1

Median: 1

Mode: 1

$$\text{Mean } \bar{X} = \bar{X} = \sum X_i/n$$

$$\text{Mean } \bar{Y} = \bar{Y} = \sum Y_i/n$$

Mean $\bar{X}$ = 1 score for males (as it is not an integer, the value of *A* is 1) (data from **Table 11**).

Mean $\bar{Y}$ = 1 score for females (as it is not an integer, the value of *B* is 1) (data from **Table 11**).

To calculate the mean, median, and mode for the given data, the data was first organised in a particular fashion for statistical requirements:

For the given data sets:

Memory pattern score for males: 1, 1, 1, 1, 1, 1, 1, 1, 1, 1

Memory pattern score for females: 1, 1, 1, 1, 1, 1, 1, 1, 1, 1

Mean (average): **Table 12**.

$$\text{Mean} = \frac{\text{Sum of all scores}}{\text{Number of scoeres}}$$

For both males and females:

$$Mean = \frac{1+1+1+1+1+1+1+1+1+1}{10} = \frac{10}{10} = 1$$

Median:

Since the data is already arranged in ascending order, the median is the middle value. In this case, both the 5th and 6th values are 1. Therefore, the median is 1.

Table 12. Data table results of performed outcomes (**Tables 5** and **6**).

Sr.no	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Study outcomes (accessed on from the healthy volunteers) (male) For memory patterns scores.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1
Study outcomes (accessed on from the healthy volunteers) (female) For memory patterns scores.	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1	Score 1

Mode:

The mode is the value(s) that occur most frequently. In this dataset, the value 1 is the only value, and it occurs 10 times. Therefore, the mode is 1.

In summary:

Mean: 1

Median: 1

Mode: 1

$$\text{Mean } \bar{X} = \bar{X} = \sum X_i/n$$

$$\text{Mean } \bar{Y} = \bar{Y} = \sum Y_i/n$$

Mean $\bar{X}$ = 1 score for males (as it is not an integer, the value of *A* is 1) (data from **Table 12**).

Mean $\bar{Y}$ = 1 score for females (as it is not an integer, the value of *B* is 1) (data from **Table 12**).

Percentage:

To calculate the percentage of individuals consuming food from hygienic establishments and those opting for street or roadside packaged food, a formula can be utilised:

$$\text{Percentage} = \frac{\text{Number of individulas in the category}}{\text{Total population}} \times 100$$

To calculate the percentages:

Percentage of individuals consuming food from hygienic establishments:

$$\text{Percentage hygienic food conusmers} = \frac{\text{Number of individulas consuming hygienic food}}{\text{Total population}} \times 100$$

Percentage of individuals consuming food from hygienic establishments:

$$\text{Percentage hygienic food conusmers} = \frac{950}{950 + 1052} \times 100$$

$$\text{Percentage hygienic food conusmers} = \frac{950}{2002} \times 100 \approx 47.55\%$$

Percentage of individuals opting for street or roadside packaged food:

$$\text{Percentage hygienic food conusmers} = \frac{\text{Number of individulas consuming street or road side food}}{\text{Total population}} \times 100$$

$$\text{Percentage hygienic food conusmers} = \frac{1052}{2002} \times 100 \approx 52.45\%$$

So, approximately 47.55% of the population consumes food from hygienic establishments, while 52.45% opts for street or roadside packaged food.

Statistically, the mean of all the scores from **Tables 1** and **2**, and **Tables 3** and **4**, and **Table 5** and **6** or **Tables 1–6** were taken, and these means were logically subjected to a scientific outcome for the study results.

For instance, when comparing the memory pattern scores of males and females, the logical analysis involved identifying patterns or trends within the data. This could include observing whether there were consistent differences in memory scores between the two groups or if there were particular factors influencing the scores, such as age or educational background. By logically interpreting the statistical data, researchers could draw conclusions about the memory patterns in the studied population and potentially uncover insights into factors affecting memory performance.

So, Consequently, a logical analysis was applied to correlate the obtained results, drawing meaningful connections and inferences from the statistical data.

The mean value of memory like: Mean = 2, Mean = 1, Mean = 1 was observed from **Tables 1** and **2**, **Tables 3** and **4**, and **Tables 5** and **6**, respectively.

Report on chemical analysis of excerpt from newspaper

Results and findings: Upon careful study and assessment of ten different samples, the investigation revealed positive test results for the presence of lead in the packaging material, specifically in the newspaper extracts (**Figure 1**). The observations consistently indicated the existence of lead, highlighting a potential concern regarding the safety of using newspaper as a packaging material.

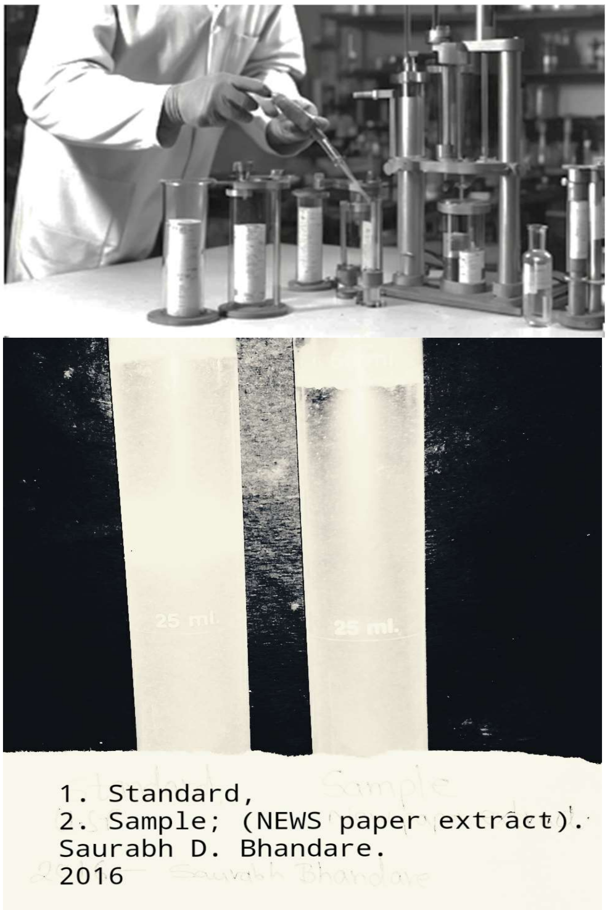


Figure 1. Lead test for NEWS paper packaging carried out compared with the standard as per USP and B.P.

Given the widespread use of newspapers in food packaging, the outcomes of this investigation emphasised the importance of meticulous scrutiny of packaging materials for potential contaminants or possible pollutants. It is imperative to conduct further research and enhance awareness to comprehensively understand the magnitude of this issue and implement necessary safeguards to ensure consumer safety.

Conducting additional studies and raising awareness are essential for determining the scope of this problem and putting the appropriate safeguards in place to protect consumers.

In my further investigation, an analogous evaluation was conducted on the portion of the food in close proximity to the packaging material, yielding positive results indicating the presence of lead in the examined sample.

4. Discussion

In conclusion, this statistical evaluation represents a significant step towards unravelling the intricate dynamics between food, packaging trends, and memory capacity. The results hold the potential to inform public health initiatives, guide packaging industry practices, and contribute to a deeper understanding of the intricate interplay between lifestyle choices and cognitive well-being. Further research and targeted interventions may be warranted to explore these associations in greater depth and ascertain their implications for individual and public health.

In order to investigate possible trends and causes, the mean scores for each category were examined.

The mean scores for all categories were analysed to explore potential patterns and causes. Upon logical examination of the statistical outcomes, it was observed that the mean value for healthy volunteers (**Tables 1** and **2**) was 2, indicating good memory retention. Conversely, for **Tables 3** and **4**, where individuals exhibited similar eating habits but also engaged in regular smoking and alcohol consumption, the mean value was 1, suggesting a shorter memory span at the given moment. Results from **Tables 5** and **6** indicated a mean value of 1, reflecting poor memory capacity that is the cause of packaging methods.

In summary, the findings from **Tables 1** and **2** suggest a well-functioning memory among the investigated population. **Tables 3** and **4** revealed that individuals with additional habits of smoking and alcohol consumption, despite sharing similar eating patterns with healthy volunteers, exhibited compromised memory and cognitive abilities. **Tables 5** and **6** highlighted that individuals consuming food from street vendors experienced memory loss due to lead-induced contamination in the packaging material, significantly impacting memory capacity and learning abilities.

5. Conclusion

In conclusion, this comprehensive research study fossicks through the intricate relationship between lifestyle choices, food packaging trends, and impact on memory capacity, focusing and alarming on critical public health implications. The research methodically assessed diverse populations, considering factors like dietary habits, packaging preferences, and cognitive well-being. Notably, the statistical analysis revealed distinct patterns among different groups.

The findings emphasise the significance of lifestyle choices, indicating that healthy volunteers exhibited a well-functioning memory, while those with additional habits of smoking and alcohol consumption showed compromised cognitive abilities. Additionally, individuals consuming street-side food faced memory loss due to lead-induced contamination in packaging material, significantly impacting their memory capacity.

The study emphasises the need for heightened awareness regarding food packaging materials, especially in the case of street vendors, and advocates for public health initiatives to address these concerns. The correlation between lifestyle choices and cognitive well-being serves as a crucial foundation for future research, offering insights that can inform policies, industry practices, and individual health interventions. The identification of potential risks associated with certain dietary and lifestyle patterns emphasises the importance of creating safeguards to ensure consumer safety. Overall, this study contributes valuable knowledge that extends beyond academia, with the potential to positively impact public health practices and enhance our understanding of the nuanced connections between lifestyle, food choices, and cognitive health.

6. Supporting data

Media 1: Saurabh D. Bhandare. Food safety NEWS. YouTube. Published online January 26, 2024.

Media 2. Saurabh D. Bhandare. food safety NEWS reports. YouTube. Published online January 28, 2024.

Media 3: Study of toxic substances. https://tinyurl.com/Study-of-toxic-substances

Conflict of interest: The author declares no conflict of interest.

References

1. Food and Agriculture Organization of the United Nations. Q&A on food safety. Available online: https://www.fao.org/food-safety/background/qa-on-food-safety/en/#:~:text=Food%20safety%20is%20a%20science,that%20is%20safe%20to%20eat (accessed on 7 July 2024).
2. World Health Organization. Food safety. Available online: https://www.who.int/news-room/fact-sheets/detail/food-safety (accessed on 7 July 2024).
3. Centers for Disease Control and Prevention. Four Steps to Food Safety: Clean, Separate, Cook, Chill. Available online: https://www.cdc.gov/foodsafety/keep-food-safe.html (accessed on 7 July 2024).
4. Centers for Disease Control and Prevention. Foods that can cause food poisoning. Available online: https://www.cdc.gov/foodsafety/foods-linked-illness.html (accessed on 7 July 2024).
5. Centers for Disease Control and Prevention. Current and past multistate foodborne outbreaks. Available online: https://www.cdc.gov/foodsafety/outbreaks/lists/index.html (accessed on 7 July 2024).
6. FoodSafety.gov. 4 steps to food safety. Available online: https://www.foodsafety.gov/keep-food-safe/4-steps-to-food-safety (accessed on 7 July 2024).
7. Nation Institute of Food and Agriculture. Food safety. Available online: https://www.nifa.usda.gov/topics/food-safety#:~:text=Importance%20of%20Food%20Safety,addition%20to%20reducing%20foodborne%20illnesse (accessed on 7 July 2024).

8. Keener L. Capacity building: Harmonization and achieving food safety. In: Boisrobert CE, Stjepanovic A, Oh S, Lelieveld HLM (editors). Ensuring Global Food Safety: Exploring Global Harmonization. Academic Press; 2010. pp. 139-149. doi: 10.1016/b978-0-12-374845-4.00008-4
9. Lamuka P. Public health measures: Challenges of developing countries in management of food safety. In: Motarjemi Y (editor). Encyclopedia of Food Safety. Academic Press; 2014. pp. 20-26. doi: 10.1016/b978-0-12-378612-8.00310-3
10. Centers for Disease Control and Prevention. Available online: https://www.cdc.gov/foodsafety/index.html (accessed on 7 July 2024).
11. Li OY, Shi X. Food safety regulations within countries of increasing global supplier impact. In: Martinovic A, Oh S, Lelieveld H (editors). Ensuring Global Food Safety: Exploring Global Harmonization, 2nd ed. Academic Press; 2022. pp. 151-158. doi: 10.1016/b978-0-12-816011-4.00026-4
12. International Commission on Microbiological Specifications for Foods. A simplified guide to understanding and using food safety objectives and performance objectives. In: Boisrobert CE, Stjepanovic A, Oh S, Lelieveld HLM (editors). Ensuring Global Food Safety: Exploring Global Harmonization. Academic Press; 2010. pp. 91-98. doi: 10.1016/b978-0-12-374845-4.00004-7
13. Gordon A, Kennedy H. Conclusions and lessons learned: steps for successful Food Safety and Quality System (FSQS) systems implementation. In: Gordon A (editor). Food Safety and Quality in Developing Countries. Academic Press; 2017. Volume 2. pp. 277-291. doi: 10.1016/b978-0-12-801226-0.00010-4
14. Rocourt J. Foodborne diseases: Foodborne diseases and vulnerable groups. In: Motarjemi Y (editor). Encyclopedia of Food Safety. Academic Press; 2014. pp. 323-331. doi: 10.1016/b978-0-12-378612-8.00080-9
15. Better Health Channel. Food safety and storage. Available online: https://www.betterhealth.vic.gov.au/health/healthyliving/food-safety-and-storag (accessed on 7 July 2024).
16. Australian Institute of Food Safety. What is Food Safety? Available online: https://blog.foodsafety.com.au/what-is-food-safety (accessed on 7 July 2024)
17. U.S. Food and Drug Administration. Food Safety Modernization Act (FSMA). Available online: https://www.fda.gov/food/guidance-regulation-food-and-dietary-supplements/food-safety-modernization-act-fsma (accessed on 7 July 2024).
18. U.S. Food and Drug Administration. FSMA final rule on Accredited Third-Party Certification. Available online: https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-final-rule-accredited-third-party-certification (accessed on 7 July 2024).
19. Eicher A, Biedermann M, Zurfluh M, et al. Migration by 'direct' or 'indirect' food contact? 'Dry' and 'wetting' foods? Experimental data for 'touching' contact of dry foods with paper and board. Food Additives & Contaminants: Part A. 2014; 32(1): 110-119. doi: 10.1080/19440049.2014.975753
20. Van Den Houwe K, Evrard C, Van Loco J, et al. Migration of photoinitiators from cardboard into dry food: evaluation of Tenax® as a food simulant. Food Additives & Contaminants: Part A. 2016; 33(5): 913-920. doi: 10.1080/19440049.2016.1179562
21. Van Den Houwe K, van de Velde S, Evrard C, et al. Evaluation of the migration of 15 photo-initiators from cardboard packaging into Tenax®using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Food Additives & Contaminants: Part A. 2014; 31(4): 767-775. doi: 10.1080/19440049.2014.886340
22. Bradley EL, Stratton JS, Leak J, et al. Printing ink compounds in foods: UK survey results. Food Additives and Contaminants: Part B. 2013; 6(2): 73-83. doi: 10.1080/19393210.2012.725774
23. Sagratini G, Caprioli G, Cristalli G, et al. Determination of ink photoinitiators in packaged beverages by gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry. Journal of Chromatography A. 2008; 1194(2): 213-220. doi: 10.1016/j.chroma.2008.04.057
24. Summerfield W, Cooper I. Investigation of migration from paper and board into food-development of methods for rapid testing. Food Additives and Contaminants. 2001; 18(1): 77-88. doi: 10.1080/02652030010004674
25. Boccacci Mariani M, Chiacchierini E, Gesumundo C. Potential migration of Diisopropyl naphthalenes from recycled paperboard packaging into dry foods. Food Additives and Contaminants. 1999; 16(5): 207-213. doi: 10.1080/026520399284073

26. Pyatha S, Kim H, Lee D, et al. Co-exposure to lead, mercury, and cadmium induces neurobehavioral impairments in mice by interfering with dopaminergic and serotonergic neurotransmission in the striatum. Frontiers in Public Health. 2023; 11. doi: 10.3389/fpubh.2023.1265864
27. Daniel WW. Biostatistics: A Foundation for Analysis in the Health Science, 7th ed. John Wiley and Sons; 2005.
28. Bolton S, Bon C. Pharmaceutical Statistics. In: Practical and Clinical Applications, 4th ed. Marcel Dekker; 2004.
29. United States Pharmacopeial Convention. Usp36-Nf31. Vol 1. United States of Pharmacopeia; 2012.

Journal of Toxicological Studies 2024, 2(2), 1326.
https://doi.org/10.59400/jts.v2i2.1326

Article

Ecotoxicity of 3D printing material polylactic acid (PLA) on sea urchin *Paracentrotus lividus*

Özlem Çakal Arslan*, Kaan Arslan, Başak Topçu

Department of Marine and Inland Water Science and Technology, Ege University, Bornova 35100, İzmir, Turkey
*** Corresponding author:** Özlem Çakal Arslan, ozlem.cakal@ege.edu.tr

CITATION

Çakal Arslan Ö, Arslan K, Topçu B. Ecotoxicity of 3D printing material polylactic acid (PLA) on sea urchin *Paracentrotus lividus*. Journal of Toxicological Studies. 2024; 2(2): 1326.
https://doi.org/10.59400/jts.v2i2.1326

ARTICLE INFO

Received: 25 April 2024
Accepted: 8 July 2024
Available online: 18 July 2024

Abstract: In this study, the ecotoxicity of 3D printing material [polylactic acid (PLA)] was investigated with marine echinoderms; sea urchin *Paracentrotus lividus*. To achieve this goal, (i) fertilization success, spermiyotoxicity, and embriyotoxicity exposed to PLA concentrations (0.001, 0.005, 0.01, 0.1, and 1 g/L) were assessed for 72 h. For this purpose, our study is important to make comprehensive evaluations to ensure the safety of bioplastic formulations and to take measures to regulate the use of additives. At the same time, the additive used to increase the durability of bioplastic materials will also allow us to understand the long-term effects on ecosystems, wildlife, and human health. Our aim is to minimize possible harm and ensure that the overall environmental impact of bioplastics remains positive.

Keywords: 3D printing; polylactic acid (PLA); sea urchin

1. Introduction

Today's studies aim to reduce overall plastic consumption and, at the same time, minimize its impact on ecosystems [1,2]. New methods are being developed to reduce plastic pollution, and the most striking of these is bioplastics, which are a sustainable alternative. Bio-based plastics are widely used as a replacement for traditional plastics in various applications such as packaging, automotive parts, and consumer goods, thus reducing greenhouse gas emissions from fossil fuels [3]. The use of 3D printers has been increasing rapidly, which are used with raw materials in the rapid manufacturing of devices. Because of this, it has enabled the mass introduction for use at different levels. 3D printers and bioplastics offer new opportunities for applications in fields such as medicine [4]. Biopolymers have attracted great attention in the fields of sustainable packaging, energy storage, biomedicine, and textiles [2]. Polylactic acid (PLA) is considered the most prominent bioplastic due to its physicochemical properties, low price, and cheapness. PLA has been reported as an environmentally friendly compound [4,5]. Although it is stated that it is biodegradable, biodegradation of PLA has not occurred at normal environmental conditions in the marine environment [2,6]. It is important to note that not all biodegradable plastics are suitable for all environments. Some require specific conditions, such as higher temperatures, to facilitate their breakdown [7]. In conclusion, bio-based and biodegradable plastics offer potential benefits for environmental sustainability compared to traditional petroleum-based plastics. However, it is essential to understand their properties and limitations properly and implement appropriate waste management practices to maximize their positive impact on reducing plastic pollution. Many studies focused on microalgae [8], mollusks, and fish [9,10] but no data available on marine echinoderm is still scarce. For this reason, the ecotoxicological effects of PLA on *Paracentrotus lividus* were determined. Investigations of hazardous effects on early developmental stages of aquatic organisms have great importance due to the protection of the natural population's health. The *P. lividus* sea urchin is found across various European waters and plays an important role in the conversation of marine ecosystems. Its life cycle, including the release of mature gametes directly into seawater and pelagic larval stages, makes it an important species for understanding the impacts of contaminants on marine environments [7]. Furthermore, this work delves into investigating the potential toxicity of commercial products 3D printing filament (PLA) shortly after their introduction to markets when they are released or disposed into seawater. Specifically focusing on PLA because of this material used by children in the school for education. This study employs *Paracentrotus lividus* as a model organism to examine the effects of these materials.

2. Materials and methods

Test mediums were prepared by adding the small piece (100 µm) PLA directly to sea water; 0.001, 0.005, 0.01, 0.1, and 1 g/L test concentrations. Test concentrations were selected as environmentally relevant concentrations. Control group were untreated negative controls (filtered natural seawater = FSW from the same area of sea urchins). 3x10-4M CdCl2 were used as a positive control. All treatments were tested in six replicates. Adult *Paracentrotus lividus* were collected from the Aegean Sea coast (Seferihisar, Turkey) by hand with gentle Bioassays were carried out as described previously by Arslan and Parlak [11]. For the spermyotoxicity test, 50 µL sperm cell suspensions were exposed to various PLA concentrations for 30 min before insemination. Changes in the fertilization success of exposed sperm were determined by scoring the percentage of fertilized eggs [11]. The embryotoxicity tests were carried out by adding the 1 mL fertilized egg suspension in FSW with increasing PLA concentrations throughout development (room temperature: 19 ± 2 ℃). Embryotoxicity was assessed on 72-h-old pluteus larvae according to morphological criteria defined by Arslan and Parlak [11]. A sample of 100 embryos was observed under a light microscope. Developmental defects were observed on living plutei, which were slowed down their mobilization in 10–4 M chromium sulfate, 72 h after fertilization.

Cytogenetic tests were carried out 6 h p-f, and the embryos were fixed in Carnoy's solution (ethanol, chloroform, acetic acid; 6:3:1 V: V: V). 24 h after fixation, absolute ethanol was renewed, and the samples were ready to be observed under a light microscope (1000×) with oil immersion. Mitotic activity (numbers of metaphase and anaphase) and chromosome aberrations (chromosome bridges, lagging chromosomes, multipolar spindles, free chromosome sets, fragmented chromosomes) were scored in each embryo, thus allowing to assess both quantitative endpoints and mitotic anomalies.

3. Statistical analysis

EPA Probit Analysis Program used for calculating LC/EC Values, Version 1.5. Dunnets tests were used to compare the differences in the frequency distribution of the evaluated parameters (N: normal plutei, R: retarded plutei, P1: skeletal malformations, P2: blocked gastrula or blastula, and D: dead) between the negative control (FSW) and the treatment groups by applying the logarithmic transformation to normalize distributions. Statistical comparisons were performed using a one-way ANOVA, and significant differences were detected with Tukey's [12] and Dunnett's multiple comparison test. The Statistica-6.0 computer programme was used in the data analysis [12].

4. Results and discussion

It was observed that sperm were exposed to PLA for 30 minutes, resulting in significant changes in their fertilization capacity (**Figure 1**). The fertilization rate was observed at 100% in the control group. At the first concentration of 0.001g-PLA/L no change was observed. It was determined that fertilization did not have a negative effect on this amount of PLA. The fertilized egg rate decreased to 92% at 0.005 g-PLA/L. This ratio decreased to 86.33% at 0.01 g-PLA/L in parallel with the increase in the amount of PLA and to 59% at the final concentration of 1 g-PLA, with a decrease of approximately 43% (**Figure 2**). The impact of PLA on fertilization was determined as EC50 = 0.49 g/L PLA by probit analyses **Table 1**. The scores of developmental defects of larvae showed that offspring quality was significantly decreased (**Figures 3** and **4**) at all concentrations tested (p < 0.0001). The EC50 value of PLA was estimated as 0.215 g/L for spermyotoxicity, as shown in **Table 1**. This result brings us to the conclusion that the PLA has less effects on fertilization success of sperms but extremely decreased offspring quality of exposed sperms, which became more important from the ecotoxicological point of view.

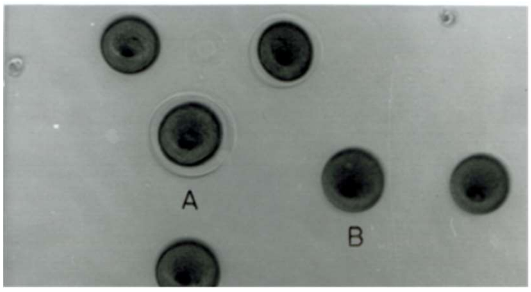


Figure 1. Effects of PLA on fertilization success (A: fertilized egg, B: non fertilized egg).

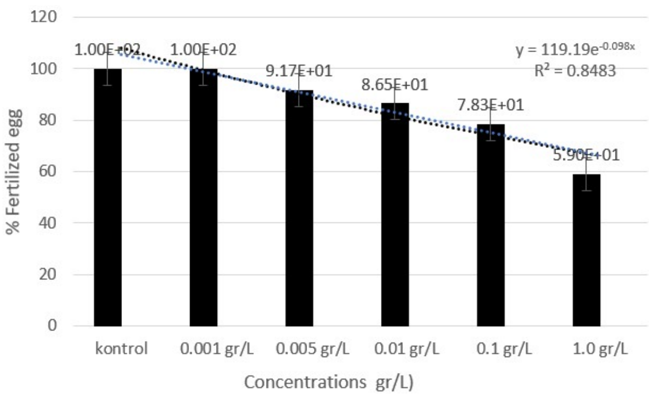


Figure 2. Effects of PLA on fertilization success.

Table 1. EC50/LC50 levels of PLA on sea urchin *P.lividus*.

Results of biotest	EC50/LC50 levels (g-PLA/L)
Fertilization success	0.49
Offspring quality	0.215
Embryotoxicity	0.087

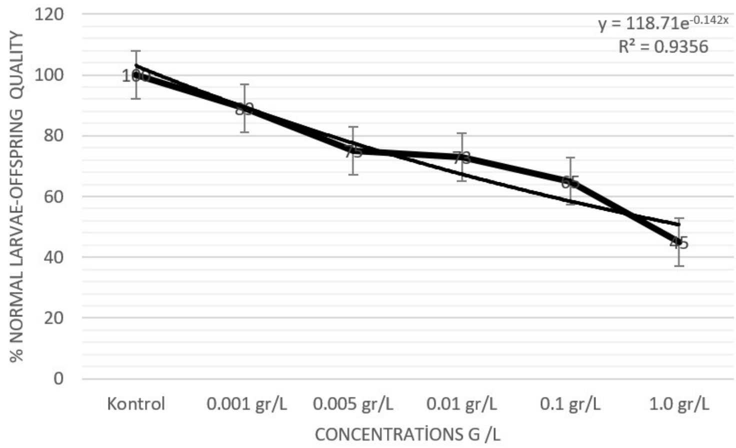


Figure 3. Spermyotoxicity after PLA exposure in *P. lividus* sea urchin sperm. Offspring quality percentage of *A. lixula* embriyos.

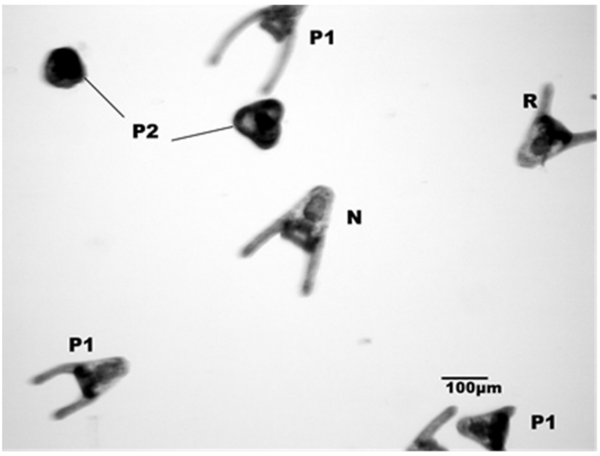


Figure 4. Developmental effects of PLA on sea urchin and normal pluteus. (N: Normal Plutei, P1; Skeletel deformaties, P2: blastula/gastrula blocked embryo, R: Retarded embryo).

Results of embryotoxicity tests: significant effects were observed at concentrations ranging from 0.001 to 1 g/L-PLA. The embryotoxicity tests show the classic dose-response curve indicating a decreased percentage of normal larvae development with increasing PLA concentrations (**Figure 5**). The impact of PLA on exposed embryos was estimated as EC50 0.087 g/L PLA concentration by probit analyses. According to the toxicity criteria of Arslan and Parlak [11] at 0.01 g-PLA/L, the normal pluteus frequency decreased by approximately 20% to 80%. In parallel with this decrease, the frequency of individuals with deformation in the skeletal system increased by 23%. It has been determined that this concentration is toxic according to the frequency of pluteus with developmental disorders [11].

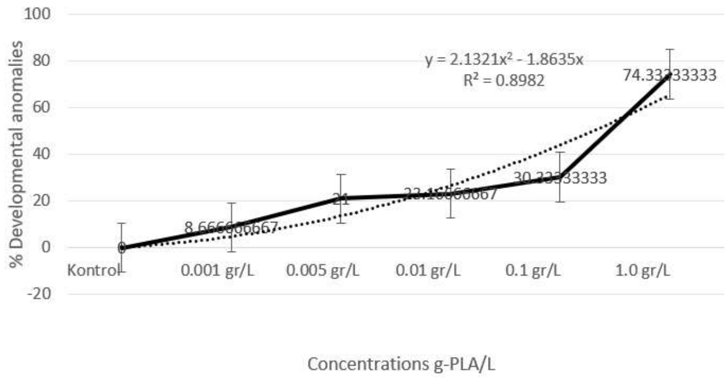


Figure 5. Embyotoxic effects of PLA on *P.lividus*.

The cytogenetic results for PLA are shown in **Figure 6**. As shown in **Figure 6**, the ratio of metaphase and anaphase was significantly decreased. Furthermore, mitotic activity in the embryos was inhibited at 0.01 g/L ($p < 0.05$) and 1 g/L-PLA ($p < 0.001$). **Figure 7** showed that the number of interphase embryos (IE) differed from 0.01 to 1 g-PLA/L. It is increased at high PLA concentrations. As shown in **Figures 6–8**, a significant difference was observed in average total mitotic aberrations in embryos exposed to 0.001 to 1 g-PLA/ L compared to controls.

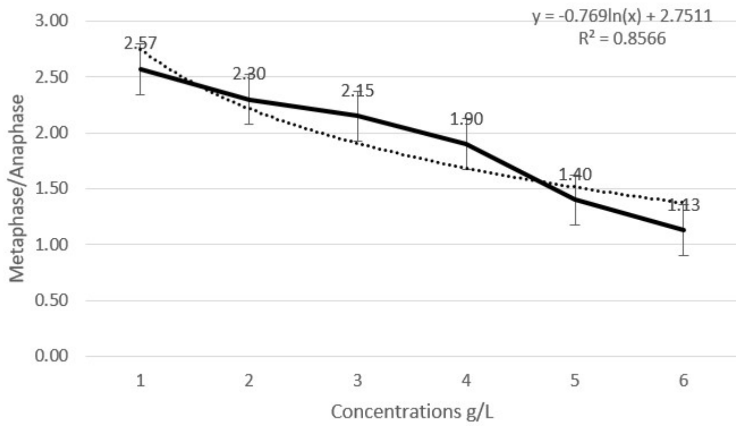


Figure 6. Cytogenetic toxicity of PLA on embryos. Metaphase/Anaphase ratio.

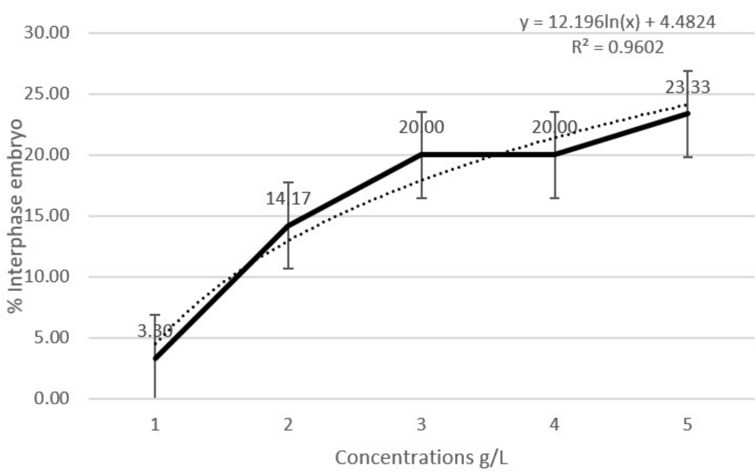


Figure 7. Cytogenetic toxicity after PLA exposure. Percentages of interphase embryos.

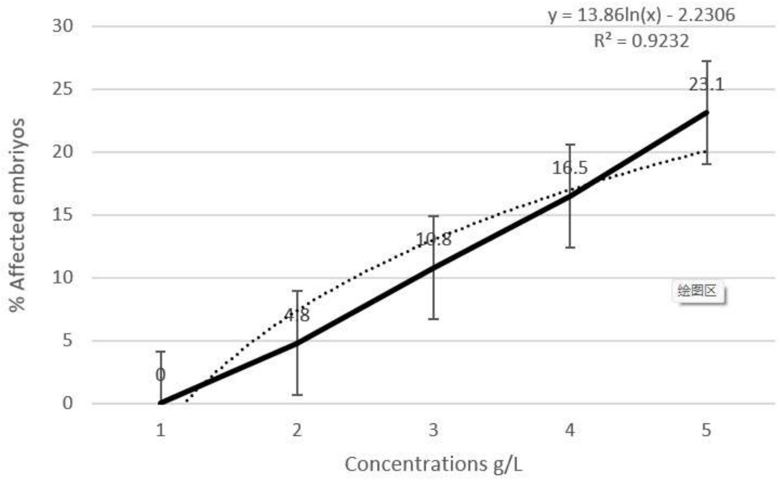


Figure 8. Cytogenetic toxicity after PLA exposure in *P. lividus* sea urchin embryos. Percentage of affected embryos (percent embryos having ≥1 mitotic aberrations).

3D polylactic acid (PLA)-based printers are increasing their use and popularity worldwide. However, this technology also causes environmental pollution, especially microplastic pollution in the aquatic environment [13]. Reported by Rodríguez-Hernandez et al. [14], the formation of nanoplastic pollution as a result of the cleaning process of the products taken from the 3D printer and their physicochemical characterization were reported. As a result of the study, they reported that nano-sized plastic particles easily enter the aquatic environment and that these residues aggregate around 1 mm on average in seawater. At the same time, researchers have stated that the resulting and clumping nanoplastics interact with pH and other positively charged pollutants, becoming an unexpected environmental problem and public health risk. Previous studies have reported that biodegradable microplastics (PLA), which are used extensively to reduce microplastic pollution, cause toxicity similar to microplastics. In the study conducted by Green [15], PLA potentially negatively affects the oyster Ostrea edulis as much as traditional microplastics. In addition, PLA has hazardous effects on the life and health of *Danio rerio*, *Mytilus edulis*, *Microcosmus exasperates*, and Daphnia magna. And also causes oxidative stress, reproductive problems, intestinal damage, and immunosuppression, have been reported [15–17].

It has been stated by many researchers that the PLA used in 3D printers is potentially toxic [18]. Many toxicity studies have shown that print parts and leachates of 3D printers are contaminated with *Daphnia magna* [19,20]. The aim of our study is to investigate the toxic effects of PLA on sea urchin *P. lividus* in both acute and chronic periods. Montalvão [6] reported in their study that although PLA is considered biodegradable due to its microbial origin, it almost does not decompose in aquatic environments. For this reason, ecotoxicity studies conducted in recent years have focused on the damages and risks that 3D printer raw materials may cause as a result of unconscious and incorrect use. The study by Bagheri et al. [2] reported the ecotoxicity of PLA on *Daphnia magna*. According to the result of the An et al. [21] study, the survival rate for *D. magna* declined to 52.4%, and the end of chronic exposure at 1 and 5 mg·L^{-1} PLA caused a decrease of offspring. This study contributes that biodegradable microplastics (PLA) have toxic effects on *D. magna,* which could be similar to conventional microplastics effects on aquatic organisms. When our results compare with An et al.'s [21] research, similar results were observed. In our results, PLA exhibited fertilization and normal development and also caused genetic hazards at sea urchin. In conclusion, previous studies and our study showed the importance of PLA contaminations. Balentine et al. [22] investigated the acute and chronic toxicity of 3D printer resin against *Ceriodaphnia dubia,* and as a result, it was reported that the LC50 value varied between 2.6 and 33 mg/L as a result of 48-hour acute toxicity tests. Researchers have also determined that 3D printing resin inhibits growth with IC25 values of 0.33 to 16 mg/L. Uribe-Echeverría and Beiras [23] tested the effects of a polyvinyl chloride (PVC) toy polylactic acid containers (PLA) and polylactic acid/polyhydroxyalkanoate 3D printing filament (PLA/PHA) using *Paracentrotus lividus* sea urchin larvae. As a result of their study, they reported that the PVC toy was very toxic, whereas PHB showed mild toxicity, even though it was considered a non-toxic polymer. Uribe-Echeverría and Beiras [23] exposed sea urchin embryos to the 3D printing material PLA and stated that, unlike our study, PLA containers and PLA/PHA filament were harmless to the larvae. The reason for this result is probably that the researchers used the materials diluted, whereas in our study we carried out the tests by adding them directly to the medium. It has been reported by several researchers that PLA is acutely toxic to algae. Li et al. [8] reported that PLA caused an inhibition of growth on *Skeletonoma costatum,* and they also concluded that the exposure of S. *costatum* to 0.1, 0.2, 0.3, and 0.5 mg/L PLA induced a significant reduction of Chl a content. A lack of information about the toxicity of PLA to the developmental stages of the sea urchin *P. lividus* was observed.

5. Conclusion

It can be concluded that PLA affects *P. lividus* during reproduction and embryonic developmental stages. As a result of biotests conducted with the PLA printing filament tested in this study, it was revealed that it negatively affected fertilization, sperm, embryos, and mitotic stages and revealed the need for the use of already commercialized, safe biobased and biodegradable products and attention in waste management.

Author contributions: Conceptualization, ÖÇA and KA; methodology, ÖÇA; software, ÖÇA and KA; validation, ÖÇA; formal analysis, ÖÇA; investigation, ÖÇA, KA and BT; resources, ÖÇA, KA and BT; data curation, ÖÇA and KA; writing—original draft preparation, ÖÇA; writing—review and editing, ÖÇA; visualization, KA and BT; supervision, ÖÇA; project administration, ÖÇA; funding acquisition, ÖÇA. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Aznar M, Ubeda S, Dreolin N, et al. Determination of non-volatile components of a biodegradable food packaging material based on polyester and polylactic acid (PLA) and its migration to food simulants. Journal of Chromatography A. 2019; 1583: 1-8. doi: 10.1016/j.chroma.2018.10.055
2. Bagheri AR, Laforsch C, Greiner A, et al. Fate of So-Called Biodegradable Polymers in Seawater and Freshwater. Global Challenges. 2017; 1(4). doi: 10.1002/gch2.201700048

3. European Bioplastics. Bioplastics market data. Available online: https://docs.european-bioplastics.org/publications/market_data/2022/Report_Bioplastics_Market_Data_2022_short_version.pdf (accessed on 1 April 2024).
4. Ikada Y, Tsuji H. Biodegradable polyesters for medical and ecological applications. Macromolecular Rapid Communications. 2000; 21(3): 117-132. doi: 10.1002/(SICI)1521-3927(20000201)21:3<117::AID-MARC117>3.0.CO;2-X
5. Chiulan I, Frone A, Brandabur C, et al. Recent Advances in 3D Printing of Aliphatic Polyesters. Bioengineering. 2017; 5(1): 2. doi: 10.3390/bioengineering5010002
6. Montalvão GR, Moshrefi-Torbati M, Hamilton A, et al. Behaviour of 3D printed PLA and PLA-PHA in marine environments. IOP Conference Series: Earth and Environmental Science. 2020; 424(1): 012013. doi: 10.1088/1755-1315/424/1/012013
7. Oliviero M, Tato T, Schiavo S, et al. Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus lividus. Environmental Pollution. 2019; 247: 706-715. doi: 10.1016/j.envpol.2019.01.098
8. Li X, Luo J, Zeng H, et al. Microplastics decrease the toxicity of sulfamethoxazole to marine algae (Skeletonema costatum) at the cellular and molecular levels. Science of The Total Environment. 2022; 824: 153855. doi: 10.1016/j.scitotenv.2022.153855
9. Pagter E, Frias J, Kavanagh F, et al. Differences in microplastic abundances within demersal communities highlight the importance of an ecosystem-based approach to microplastic monitoring. Marine Pollution Bulletin. 2020; 160: 111644. doi: 10.1016/j.marpolbul.2020.111644
10. Expósito N, Rovira J, Sierra J, et al. Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake. Marine Pollution Bulletin. 2022; 181: 113843. doi: 10.1016/j.marpolbul.2022.113843
11. Arslan OC, Parlak H. Embryotoxic effects of nonylphenol and octylphenol in sea urchin Arbacia lixula. Ecotoxicology. 2007; 16(6): 439-444. doi: 10.1007/s10646-007-0147-z
12. Hocking RR. Methods and Applications of Linear Models. Regression and the Analysis of Variance. Wiley; 1996.
13. Stephens B, Azimi P, El Orch Z, et al. Ultrafine particle emissions from desktop 3D printers. Atmospheric Environment. 2013; 79: 334-339. doi: 10.1016/j.atmosenv.2013.06.050
14. Rodríguez-Hernández AG, Muñoz-Tabares JA, Aguilar-Guzmán JC, et al. A novel and simple method for polyethylene terephthalate (PET) nanoparticle production. Environmental Science: Nano. 2019; 6(7): 2031-2036. doi: 10.1039/c9en00365g
15. Green DS. Effects of microplastics on European flat oysters, Ostrea edulis and their associated benthic communities. Environmental Pollution. 2016; 216: 95-103. doi: 10.1016/j.envpol.2016.05.043
16. Anderson G, Shenkar N. Potential effects of biodegradable single-use items in the sea: Polylactic acid (PLA) and solitary ascidians. Environmental Pollution. 2021; 268: 115364. doi: 10.1016/j.envpol.2020.115364
17. Zhang L, Huang C, Xu Y, et al. Synthesis and characterization of antibacterial polylactic acid film incorporated with cinnamaldehyde inclusions for fruit packaging. International Journal of Biological Macromolecules. 2020; 164: 4547-4555. doi: 10.1016/j.ijbiomac.2020.09.065
18. Mainwaring G, Foster JR, Lund V, Green T. Methyl methacrylate toxicity in rat nasal epithelium: studies of the mechanism of action and comparisons between species. Toxicology. 2001; 158(3):109-118. doi: 10.1016/S0300-483X(00)00332-2
19. Macdonald NP, Zhu F, Hall CJ, et al. Assessment of biocompatibility of 3D printed photopolymers using zebrafish embryo toxicity assays. Lab on a Chip. 2016; 16(2): 291-297. doi: 10.1039/c5lc01374g
20. Walpitagama M, Carve M, Douek AM, et al. Additives migrating from 3D-printed plastic induce developmental toxicity and neuro-behavioural alterations in early life zebrafish (Danio rerio). Aquatic Toxicology. 2019; 213: 105227. doi: 10.1016/j.aquatox.2019.105227
21. An G, Na J, Song J, et al. Chronic toxicity of biodegradable microplastic (Polylactic acid) to Daphnia magna: A comparison with polyethylene terephthalate. Aquatic Toxicology. 2024; 266: 106790. doi: 10.1016/j.aquatox.2023.106790
22. Ballentine M, Kennedy A, Melby N, et al. Acute and Chronic Toxicity of Uncured Resin Feedstocks for Vat Photopolymerization 3D Printing to a Cladoceran (*Ceriodaphnia Dubia*). Bulletin of Environmental Contamination and Toxicology. 2023; 110: 56. doi: 10.1007/s00128-023-03698-5
23. Uribe-Echeverría T, Beiras R. Acute toxicity of bioplastic leachates to Paracentrotus lividus sea urchin larvae. Marine Environmental Research. 2022; 176: 105605. doi: 10.1016/j.marenvres.2022.105605

Journal of Toxicological Studies 2024, 2(2), 1393.
https://doi.org/10.59400/jts.v2i2.1393

Article

Evaluation of the crude chitinases toxicity on the reproductive system of *Callosobruchus maculatus* (Coleoptera: Bruchidae)

Laribi-Habchi Hassiba[1,2,*], Amrouche Zoheir[1,3], Boudjamaa Nouara[4], Mohamed Mahmoud Fadhila[5], Medjdoub Ouafaa[2], Akroum-Amrouche Dahbia[6], Akroum Hamza[7]

[1] Laboratory of Functional Analysis of Chemical Process, University Saad Dahlab of Blida, Blida 09000, Algeria
[2] Loboratory of Energy Applications of Hydrogen, University Saad Dahlab of Blida, Blida 09000, Algeria
[3] Biological Department, Faculty of Nature and Life Science, University of Khemis Miliana, Ain-Defla 44003, Algeria
[4] Biological Department, Faculty of Natural and Life Sciences (NLS), University Saad Dahlab Blida, Blida 09000, Algeria
[5] Laboratory for the Protection and Valorization of Agro Biological Resources, Faculty of Natural and Life Sciences (NLS), University Saad Dahlab Blida, Blida 09000, Algeria
[6] Bioinformatics and Applied Microbiology and Bio Molecules Laboratory, Faculty of Sciences, M'Hamed Bougara University, Boumerdes 35000, Algeria
[7] Applied Automatic Laboratory, Faculty of Hydrocarbons and Chemistry, M'Hamed Bougara University, Boumerdes 35000, Algeria
*** Corresponding author:** Laribi-Habchi Hassiba, larhassiba@yahoo.fr

CITATION

Hassiba LH, Zoheir A, Nouara B, et al. Evaluation of the crude chitinases toxicity on the reproductive system of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Journal of Toxicological Studies. 2024; 2(2): 1393. https://doi.org/10.59400/jts.v2i2.1393

ARTICLE INFO

Received: 20 May 2024
Accepted: 22 July 2024
Available online: 3 August 2024

Abstract: Insecticides represent the most used control method against the insect pests of stored food products. But there are strains of insects that are resistant to these insecticides; this is the case of the beetle of the chickpea, *Callosobruchus maculatus*. The present study has for objective the evaluation of the biological activity of chitinolytic enzymes extracted from the fish offals *Scorpaena scrofa* (scorpion fish) on this beetle at different doses (3%, 6%, 9%, 12%, 15%, and 21%) prepared with the buffer solution. The results obtained from three treatments (T_1, T_2, and T_3) realized according to the dose of the crude enzyme and the time of exposure were compared with those of the controls and have shown a very significant efficiency of our crude enzyme on the reduction of the fertility rate of 100% by treating the couple (T_1) at the same time as to treat the male (T_3) and the female (T_2) separately with the strongest dose (21%) for 48 h of exposure. It would be thus desirable to demonstrate the activity of these enzymes in the real conditions of storage.

Keywords: chitinase; *Scorpaena scrofa*; bioinsecticide; stored foods; *Callosobruchus maculatus*

1. Introduction

The leguminous plant seeds represent the main source of interesting proteins as a supplement to cereal for human food [1,2]. The man always thought of protecting stored foodstuffs, and this protection has known enormous progress during the last century. The use of synthetic chemical insecticides represents the most widely used pest control method [3,4], but the development of resistance to the latter makes them more and more insensible to pesticides [5–8]. Compounds are often found in the form of residues in food and present risks for human health, animal health, and the environment [9,10]. The animal and bacterial reigns can present many possibilities: the use of enzymes with insecticidal properties in certain developing countries is told by plentiful literature [11,12]. In a concern of environmental respect and within the framework of sustainable development, it is advisable to reduce considerably the quantities of synthetic inputs of synthesis.

The efforts of researchers must therefore be directed towards the elaboration of alternative control strategies. These new approaches must be based on the joint use of biomolecules with insecticidal properties. The bacterial chitinases of certain insects have already been the object of some works showing their insecticidal potential [13–21]. *Chitinases* (E.C. 3.2.2.14) are glycosyl hydrolases that hydrolyze the chitin [10,22–24], which is a polymer of N-acetylglucosamine, the second compound of the living organism the most represented on the surface of the earth after cellulose [25]. Chitin is present in the shells of crustaceans, the cuticles of insects [26], in the walls of mushrooms (*Aspergillus nigers*, *Penicillium notatum*), and in certain microorganisms such as yeasts (*Sacharomyces cerevisiae*, *Candida albicans*) fungi [27–29].

Chitin is also found in a more minor way in squid feathers [30–32]. It constitutes the sclerified exoskeleton, or cuticle [10,33], and is also present in the trachea of the peritrophic membrane of the exochorion [9,21]. Chitin is a potential target for crop pest control. The use of chitinase extracted from the fish offal (scorpion fish) allows to confirm its potential effect against the populations of the chickpea beetle *Callosobruchus maculatus.* A study carried out by Laribi-Habchi et al. [21] has shown the efficiency of the chitinase on the mortality rate of the insect and its impact on the digestive tract. In this context, we were interested in the toxic effect of the enzyme on the male and female reproductive systems of the beetle.

2. Material and methods

2.1. Biological materiel

2.1.1. Marine biomass

The marine biomass used for our study is a fish (scorpion fish), from the family Scopaenidae and the species *Scorpaena scrofa*. The criteria of choice were based on the availability of this fish on the Algerian coast and on the study made preliminary by Laribi-Habchi et al. [10]. The offals were got back and transported in a cooler to the laboratory for the cleaning, grinding, and storage at −20 ℃ for the extraction of the chitinase enzyme.

2.1.2. Insect

The target insect in our experiment is the chickpea beetle *Callosobruchus maculatus* [34] from the family Bruchidae and the order Coleoptera. This species is cosmopolitan, with fast reproduction and infests stored economically important commodities (chickpea). The strain of *C. maculatus* was supplied by the laboratory of Agricultural and Forest Zoology of the National School of Agronomy of El Harrach—Algeria. Mass breeding of insects was carried out in the laboratory in 100 cm^3 glass jars closed by a mosquito net to ensure oxygenation conditions. These jars were kept in the dark in an oven at a temperature of 28 ℃ ± 2 ℃ and a relative humidity of 75% ± 5%. During our experimentation, we have worked on adult's males and females aged 0–15 min recovered by sieving.

2.1.3. Chickpeas

The used variety of chickpea is *Cicer arietinum*, not treated with conventional insecticide, which was provided by the Technical Institute of Great Cultivation of Oued Smar El Harrach—Algeria. The choice of this species depends on its availability in the Algerian market. 500 g of chickpeas were put in every jar in which were added the adults of opposite sex.

2.2. Preparation of the crude extract

The method of extraction of the crude chitinases was inspired by the experimental protocol of Laribi-Habchi et al. [26]. The samples were prepared by respecting the proportion 1/6 in weight of offal by volume of citrate (w/v) buffer solution, pH 5 (citric acid at 0.15 M and dissodic phosphate at 0.3 M). The reactive mixture was then centrifuged at 37 °C for 3 h at 120 rpm. At the end of the incubation, the macerated sample was filtered through a gauze cloth. The filtrate thus obtained was centrifuged at 6000 rpm for 30 min. The recovered supernatant constitutes the crude enzymatic extract.

2.3. Preparation of the natural substrate (colloidal chitin)

The colloidal chitin was prepared from the commercial chitin (Sigma) by the method of Robert and SelitreniKoff, modified by Lee et al. [35]. Dissolve 5 g of chitin powder in 60 mL of concentrated HCl at 4 °C. The mixture was stirred and then added to two liters of ethanol (95%), with a rapid stirring overnight at −20 °C. The precipitate recovered by centrifugation at 5000 rpm for 20 min at 4 °C was washed with sterile distilled water until neutrality (pH 7). The colloidal solution was thus prepared and stored at 4 °C for other applications (in this case the colloidal chitin has been lyophilized).

2.4. Chitinase activity test

The analytical method used has served to study the chitinase activity in the various doses of the crude extract prepared with the citrate buffer solution (3%, 6%, 9%, 12%, 15%, and 21%) (v/v). The reaction mixture consists of 0.5 mL of the mixed enzyme solution with 0.5 mL of 50 Mm buffer of 2-(N-morpholino) ethane sulfonic acid (buffer A) and supplemented with 2 Mm $CoSO_4$ at pH 5 containing 10 mg/mL chitin colloidal, incubated for 1 h at 37 °C. The mixture is boiled for 10 min, then cooled and centrifuged (30 min at 13,000 g) to eliminate non-degraded chitin. The total production of N-Acetyl-Glucosamine (NAG) was determined by the specific method for amino sugar [36]. Amino sugars are characterized by the Elson and Morgan reaction. When hot and in an alkaline environment, they cyclize in furan form and, by elimination of a water molecule, acquire a double bond. The product formed complexes in an acidic medium with paradimethyl aminobenzaldehyde to give a purplish color. Reading is compared with a standard curve prepared with a dilution series of NAG (0 to 10 mg/m).

2.5. Toxicity test

In order to estimate the toxicity of the enzyme studied, we calculated the fertility rate of the number of eggs laid and the viability rate of submerged adults, taking into account three factors: sex, the dose of the enzyme tested, and the duration of treatment. We impregnated the internal surface of the Petri dishes (including the cover) with 1 mL of each dose of crude chitinase extract (3%, 6%, 9%, 12%, 15%, and 21%) and 1 mL of the buffer solution (control). After drying the 14.5 cm Petri dishes, five pairs of *C. maculatus* insects aged 0 to 15 min were placed separately. The Petri dishes were incubated for 24 and 48 h at 27 °C. Several test cases were performed on the male and female reproductive systems (**Table 1**). For each dose, the tests were repeated five times. Adult bruchids that resisted the treatment were maintained on chickpea seeds until their death. After reproduction, the eggs laid were counted using a binocular microscope from the 5th day. The chickpeas were then placed in the rearing incubator until the adults emerged. Insects were removed from the petri dishes as they emerged from the chickpea seeds.

Table 1. Toxicity test of the crude chitinases on the reproductive system of the couple.

Treatment	Female	Male
T_1	T	T
T_2	T	NT
T_3	NT	T

NT: Untreated T: Treated.

Calculation methods:

- Preparation of dilutions: The tested doses were obtained by diluting a volume of the crude chitinase extract in 100 mL of the citrate buffer solution at 0.05 M.
- Fertility rate: The fertility rate was calculated by the following formula [37].

$$Tf = (\text{Number of laid eggs/Total number of laid eggs in the control}) \times 100 \quad (1)$$

- Reduction rate of the laying: The reduction rate of the laying is given by the following formula [37]:

$$R_r\ (\%) = (N_c - N_t)/N_c \times 100 \quad (2)$$

R_r: Reduction rate compared to the control (%).

N_c: Number of eggs in the control.

N_t: Number of eggs in the test.

- Viability rate: The adults which begin to emerge from the 30th day were regularly counted and removed from the boxes as they emerged from the seeds. The viability rate was calculated by the formula:

$$\text{Viability rate } (\%) = (\text{Number of emerged adults/Number of laid eggs}) \times 100 \quad (3)$$

2.6. Sampling of the female genital tract

The dissection was carried out under a binocular magnifying glass (magnification isotonic). We then make a tear incision using forceps on either side of the lateral part of the abdomen at the level of the sixth sternite, and then with the forceps we grasp the posterior part of the abdomen. Abdomen, and by slow, firm, and progressive traction, we extract the genital tract.

2.7. Statistic study

The results were analyzed by the analysis of variance (ANOVA) in order to determine the effect of three criteria (dose of the crude enzyme, time, and sex) on the fertility rate and the viability of the insect C. This test was realized by the software

Statistica version 7.

3. Results and discussion

3.1. Effect of toxicity on the reproductive system

3.1.1. Fertility rate

Figure 1 shows the fertility rate of three treatments realized according to the dose of the crude enzyme (chitinase) and exposure time. We noticed that the fertility rate decreases clearly and in a progressive way for T_1 with regard to T_2 and T_3, and this with an increase in the doses and as a function of time. The fertility rates for T_1 (49 and 31%), T_2 (62 and 42%), and T_3 (73 and 68%) were recorded with the lowest dose (3%) during 24 h and 48 h, respectively (**Figures 1a** and **1b**). Contrary to the strong dose (21%), the fertility rates for T_1 (3 and 0%), T_2 (3 and 1%) and T_3 (9 and 5%) were noted after 24 h and 48 h, respectively. According to these results, we noticed that the efficiency of the chitinase enzyme on the reduction of the fertility rate is 100% for a high dose during 48 h by treating the couple (T_1) at the same time as to treat the male (T_3) and the female (T_2) separately.

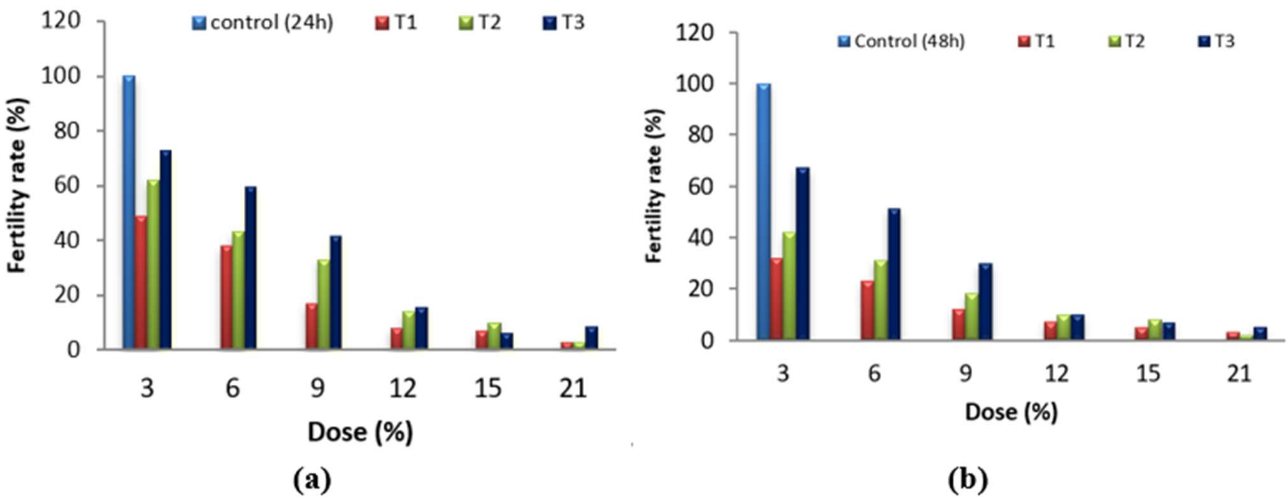


Figure 1. Fertility rate of three treatments according to the dose of the enzyme tested during **(a)** 24 h; and **(b)** 48 h.

3.1.2. Reduction rate

Figure 2 shows the reduction rate of egg-laying of the treated couple at a maximum dose (21%) as a function of the exposure time. The results showed that the reduction rate is important (100%) with T_1 with regard to T_2 (97.47%) and T_3 (93.27%) for a treatment time of 48 h. The effect of the crude enzyme has shown its toxicity effect on the reproductive system when the female and the male were treated at the same time.

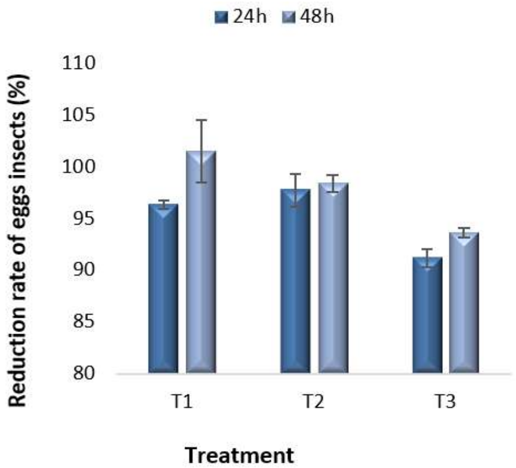
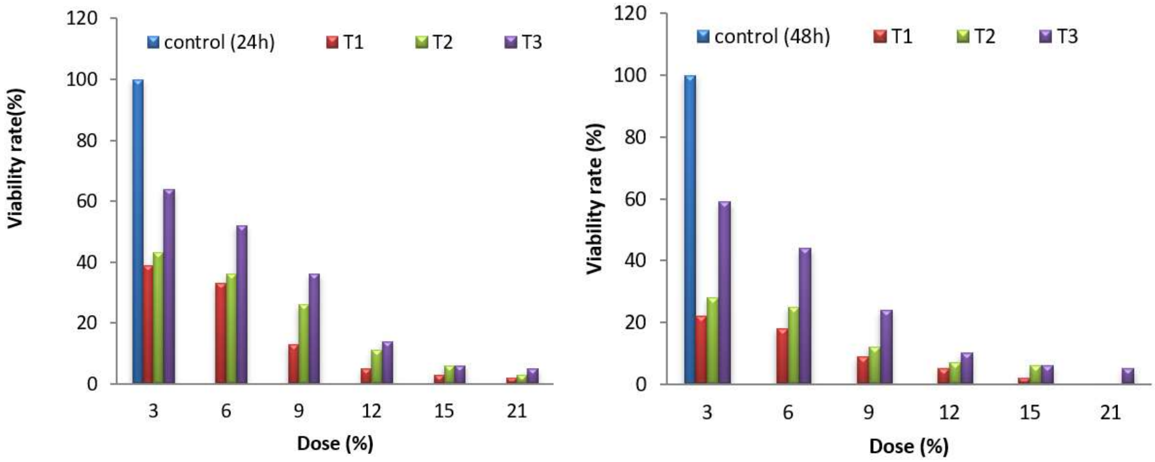


Figure 2. Reduction rate of the eggs-laying according to the maximal dose (21%) and time of treatment.

3.1.3. Viability rate

Figure 3 shows that the viability rate of the *C. maculatus* insect progressively decreases with an increase in dose and as a function of time. We have registered the same curve as the fertility rate. On the other hand, it is not the totality of the laid eggs that are emerged to give adults. The viability rates for T_1 (2 and 0%), T_2 (3 and 0%), and T_3 (5 and 5%) were recorded with the highest dose (21%) for 24 h and 48 h, respectively (**Figures 3a** and **3b**). According to these results, we noticed that the toxicity test has a significant impact on the female and male reproductive systems.



(a) **(b)**

Figure 3. Viability rate of three treatments according to the dose of the enzyme tested during **(a)** 24 h; and **(b)** 48 h.

This test seems interesting to us due to the fact that it has not been used in this area by other authors. The obtained results concerning the toxicity of the crude chitinases of the scorpion fish showed an effect on the reproductive system of the adult female insects according to the dose used and the time of exposure. This toxicity was probably due to a disturbance of the ovogenesis of the female insect by the high chitinase activity, which is 0.04 U/mg at the dose of 21%. The later caused the hydrolyze of the chitine of the ovarian membrane of the female genital system [38].

The toxicity was observed by Wiwat et al. [39] on the insect pest eggs of cabbage *Plutella xylostzlla* treated with crude chitinases extracted from the bacterial strain *Bacillus thuringiensis* ssp HD1 after 6 h of treatment. The toxicity was also observed by Laribi-Habchi et al. [40] on the insect pest *Callosobruchus maculates* with pure chitinase extracted from fish *Scorpeana scrofa* after 1 h of treatment. The works of Rishad et al. [41] have also shown that the chitinases extracted from the bacterial strain *Bacillus pumilus* MCB-7 have an insecticidal effect on the eggs of the insect pest of rice, *Scirpophagea incertulas*, with an inhibition rate of 68% after 12 days of treatment.

3.2. Microscopic observation of female reproductive system

Observation of the genital tract of females treated with the crude enzyme showed a physiological difference compared to the control (**Figure 4A**): deterioration of the genital tract with atrophy and an atretic appearance of the oocytes, visible through transparency (**Figure 4B**). We noticed that the oocytes, detectable by their yellowish-yellow charge, are no longer visible in the ovarioles. Indeed, the ovarian structure includes a protective cuticle based on sclerotized chitin, thus hardened by new bonds of chitino-protein fibers [38]. Therefore, the presence of chitin at the level of the ovarian membrane led to its degradation in the presence of chitinases at high doses, which overall led to the total deformation of the reproductive system (which confirms our results on fecundity and fertility, which are marked by a reduction in egg laying).

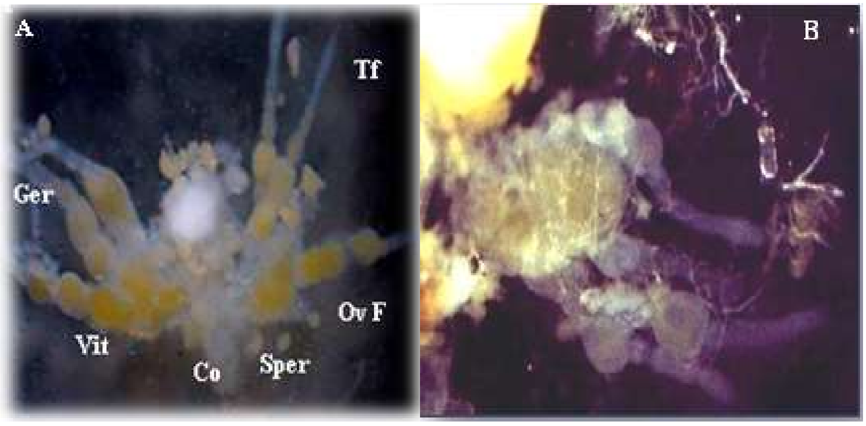


Figure 4. Optical microscopic observation G X 40 of the reproductive system of the female insect treated with the crude enzyme (21%) for **(B)** 48 h; and **(A)** control.
(OvF: ovarian follicles, Tf: terminal filament, Ger: germarium, Co: common oviduct, Sper: spermatheca, Vit: vitellarium).

3.3. Statistical study

Depending on the doses of the crude enzyme chitinase, the ANOVA showed a significant difference with $F = 6.66$ for $P = 0.00$. Depending on the duration of exposure, there is also a significant variation with $F = 9.38$ for $P = 0.00$.

Figure 5 shows a very important positive correlation between egg viability and the number of emerging insects for all treatments performed and dose-related to time. The dose factor is an important parameter which has a high impact on viability and production.

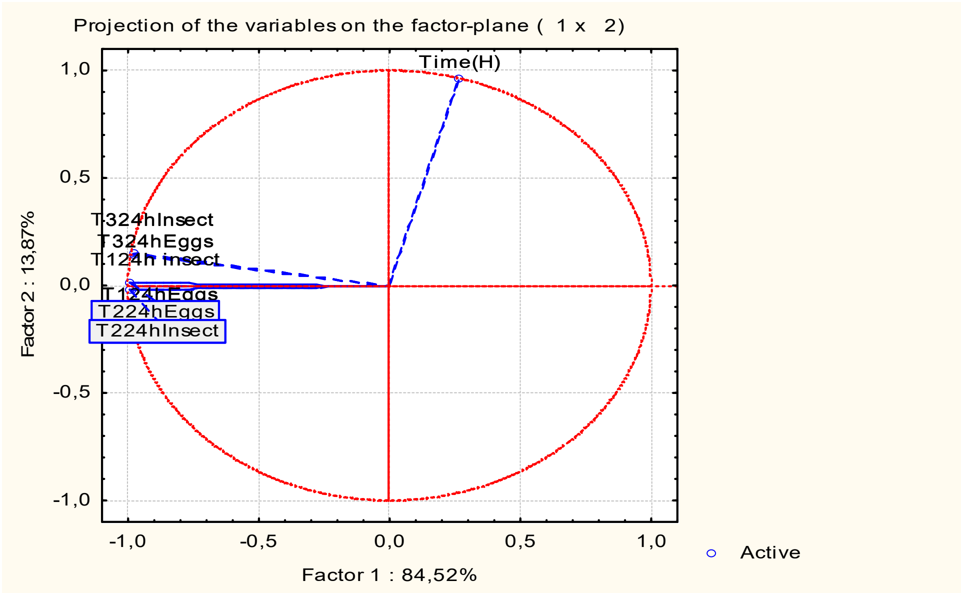


Figure 5. Principal component analysis (PCA) showing the correlation between biological variables (number of eggs and number of emerging insects) as a function of dose and treatment time.
P: Probability, F: Factor of fisher.

4. Conclusion

The use of chitinases capable of fighting harmful insects could constitute an alternative approach complementary to traditional insecticide treatment. The effectiveness of crude chitinases extracted from the offal of scorpion *scorpeana scrofa* has been demonstrated. In fact, they influence the population of harmful insects through a double action: toxicity acting on the reduction to the cancellation of female fertility as well as that of viability. Despite certainly encouraging results, the effectiveness of these enzymes still remains to be demonstrated in a real situation. Additional experiments are necessary, such as repeating these tests on other harmful insects, in order to confirm the effectiveness of these enzymes on a wide range of insects and also to understand and specify the mechanism of action of these enzymes responsible for this activity.

Author contributions: Conceptualization, LHH; methodology, LHH; software, BN, AAD, AH and MO; validation, BN and LHH; formal analysis, LHH and BN; investigation, LHH; resources, LHH; data curation, LHH and BN; writing—original draft preparation, LHH, BN, MMF and AZ; writing—review and editing, LHH; visualization, MMF; supervision, LHH; project administration, LHH; funding acquisition, LHH. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Kellouche A, Soltani N. Biological activity of five plant powders and one plant essential oil on *Callosobruchus maculatus* (F.) (French). International Journal of Tropical Insect Science. 2004; 24(2). doi: 10.1079/ijt200420

2. Malunga LN, Bar-El SD, Zinal E, et al. The potential use of chickpeas in development of infant follow-on formula. Nutrition Journal. 2014; 13(1). doi: 10.1186/1475-2891-13-8
3. Rasheed A, Saad Al J, Sun BF, et al. Efficacy of Various Plant Extracts and Synergism Against Domestic Species of Rice Weevil *Sitophilous Oryzae* (Curculionidae: Coleoptera). Polish Journal of Environmental Studies. 2024; 33(4): 1-12.
4. Chia XK, Hadibarata T, Kristanti RA, et al. The function of microbial enzymes in breaking down soil contaminated with pesticides: a review. Bioprocess and Biosystems Engineering. 2024; 47(5): 597-620. doi: 10.1007/s00449-024-02978-6
5. Benhalima H, Chaudhry MQ, Mills A, Price NR. Phosphine resistance in stored—product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research. 2004; 40: 241-249.
6. Kazemi F, Talebi AA, Fathipour Y, Farahani S. A comparative study on the effect of four leguminous species on biological and population growth parameters of *Callosobruchus maculatus* (F.) (col: Bruchidae). Advances in Environmental Biology. 2009; 3(3): 226-232.
7. Adegorite SO, Aladesida AA, John IB, kayode-Isola TM. Bioefficacy of *Chenopodium ambrosioides* L. (Chenopodiaceae) on adult *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). World Journal of Biology Pharmacy and Health Sciences. 2024; 17(2): 192-200. doi: 10.30574/wjbphs.2024.17.2.0039
8. Tengey TK, Alidu SM, Moro A, et al. Resistance to *Callosobruchus maculatus* among cowpea (*Vigna unguiculata* (L.) Walp) genotypes. Journal of Crop Improvement. 2023; 37: 309-322. doi: 10.1080/15427528.2022.2092573
9. Saguez J, Dubois F, Vincent C, et al. Differential aphicidal effects of chitinase inhibitors on the polyphagous homopteran *Myzus persicae* (Sulzer). Pest Management Science. 2006; 62(12): 1150-1154. doi: 10.1002/ps.1289
10. Laribi-Habchi H, Dziril M, Badis A, et al. Purification and Characterization of a Highly Thermostable Chitinase from the Stomach of the Red Scorpion fish *Scorpaena scrofa* with Bioinsecticidal Activity toward Cowpea Weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). Bioscience, Biotechnology, and Biochemistry. 2012; 76(9): 1733-1740. doi: 10.1271/bbb.120344
11. Mitsohiro U, Yukiko K, Aji S, et al. Purification and Characterization of Chitinase B from *Moderately Thermophilic Bacterium Ralstonia* sp. A-471. Bioscience, Biotechnology, and Biochemistry. 2005; 69(4): 842-844. doi: 10.1271/bbb.69.842
12. Chia XK, Hadibarata T, Ayu R, et al. The function of microbial enzymes in breaking down soil contaminated with pesticides: a review. Bioprocess and Biosystems Engineering. 2024; 47: 597-620.
13. Wang X, Ding X, Gopalakrishanan B, et al. Characterization of a 46 KDa insect chitinase from transgenic tobacco. Insect Biochemistry and Molecular Biology. 1996; 26: 1055-1064.
14. Lorito M, Woo SL, Fernandez IG, et al. Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. Proceedings of the National Academy of Sciences. 1998; 95(14): 7860-7865. doi: 10.1073/pnas.95.14.7860
15. Gongora CE, Wang S, Barbehenn RV, et al. Chitinolytic enzymes from *Streptomyces albidoflavus* expressed in tomato plants: effects on *Trichoplusia ni*. Entomologia Experimentalis et Applicata. 2001; 99(2): 193-204. doi: 10.1046/j.1570-7458.2001.00817
16. Carstens M, Vivier MA, Pretorius IS. The Saccharomyces cerevisiae chitinase, encoded by the CTSI-2 gene, confers antifungal activity against Botrytis cinerea to transgenic tobacco. Trangenic Research. 2003; 12(4): 497-508.
17. Fitches E, Wilkinson H, Bell H, et al. Cloning, expression and functional characterisation of chitinase from larvae of tomato moth (*Lacanobia oleracea*): a demonstration of the insecticidal activity of insect chitinase. Insect Biochemistry and Molecular Biology. 2004; 34: 1037-1050.
18. Saguez J, Hainez R, Cherqui A, et al. Unexpected effects of chitinases on the peach-potato aphid (*Myzus persicae* Sulzer) when delivred via transgenic potato plants (*Solanum tuberosum* Linné) and *in vitro*. Transgenic Research. 2005; 14(1): 57-67. doi: 10.1007/s11248-004-3100-4
19. Lawrence SD, Novak NG. Expression of Poplar Chitinase in Tomato Leads to Inhibition of Development in Colorado Potato Beetle. Biotechnology Letters. 2006; 28(8): 593-599. doi: 10.1007/s10529-006-0022-7
20. Fiandra L, Terracciano I, Fanti P, et al. A viral chitinase enhances oral activity of TMOF. Insect Biochemistry and Molecular Biology. 2010; 40(7): 533-540. doi: 10.1016/j.ibmb.2010.05.001
21. Laribi-Habchi H, Bouacem K, Allala F, et al. Characterization of chitinase from *Shewanella inventionis* HE3 with bio-insecticidal effect against granary weevil, *Sitophilus granarius* Linnaeus (Coleoptera: *Curculionidae*). Process Biochemistry. 2020; 97: 222-233. doi: 10.1016/j.procbio.2020.06.023
22. Dziril M, Grib H, Laribi-Habchi H, et al. Chitin oligomers and monomers production by coupling γ radiation and enzymatic

hydrolysis. Journal of Industrial and Engineering Chemistry. 2015; 26: 396-401. doi: 10.1016/j.jiec.2014.12.015
23. Bouacem K, Laribi-Habchi H, Mechri S, et al. Biochemical characterization of a novel thermostable chitinase from *Hydrogenophilus hirschii* strain KB-DZ44. International Journal of Biological Macromolecules. 2018; 106: 338-350. doi: 10.1016/j.ijbiomac.2018.08.026
24. Yahiaoui M, Laribi-Habchi H, Bouacem K, et al. Purification and biochemical characterization of a new organic solvent-tolerant chitinase from *Paenibacillus timonensis* strain LK-DZ15 isolated from the Djurdjura Mountains in Kabylia, Algeria. Carbohydrate Research. 2019; 483: 107747. doi: 10.1016/j.carres.2019.107747
25. Yuichiro K, Masaki K, Royoji M, et al. Structure of full-length class I chitinase from rice revealed by X-ray crystallography and small-angle X-ray scattering. Proteins: Structure, Function, and Bioinformatics. 2010; 78(10): 2295-2305. doi: 10.1002/prot.22742
26. Laribi-Habchi H, Biche M, Drouiche N, et al. Effectiveness of a biological insecticide derived from Scorpion fish offal against the chickpea weevil. Journal of Bioprocessing & Biotechniques. 2014; 2: 1-4. doi.org/10.1111/jfpp.12354
27. Neetu D, Rupinder T, Gurinder SH. Biotechnological aspects of chitinolytic enzymes: A review. Applied Microbiology and Biotechnology. 2006; 71(6): 773-782. doi: 10.1007/s00253-005-0183-7
28. Kondreddy A, Kottapalli S, Appa RP. A simple, rapid and yet less expensive method to detect chitinase in agarose plates. Journal of Biochemical and Biophysical Methods. 2007; 70(4): 683-684. doi: 10.1016/j.jbbm.2007.01.015
29. Baldrian P, Voříšková J, Dobiášová P, et al. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. Plant and Soil. 2010; 338(1-2): 111-125. doi: 10.1007/s11104-010-0324-3
30. Hamed I, Özogul F, Regenstein JM. Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. Trends in Food Science & Technology. 2016; 48: 40-50. doi: 10.1016/j.tifs.2015.11.007
31. Younes I, Rinaudo M. Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. Marine Drugs. 2015; 13(3): 1133-1174. doi: 10.3390/md13031133
32. Mechri S, Sellem I, Bouacem K, et al. A biological clean processing approach for the valorization of speckled shrimp *Metapenaeus monoceros* by-product as a source of bioactive compounds. Environmental Science and Pollution Research. 2020; 27(13): 15842-15855. doi: 10.1007/s11356-020-08076
33. Laribi-Habchi H, Biche M, Drouiche N, et al. Efficacy of Crude and Purified Chitinases (SsChi50) Extracted from Offal Red Scorpion Fish in Biological Control of Chickpea Weevil (*Callosobruchus maculatus* L.). Journal of Food Processing and Preservation. 2014; 39(6): 1355-1360. doi: 10.1111/jfpp.12354
34. Fabricius JC. The System of Entomology: Consisting of Insect Classes, Orders, Genera, Species, Additional Synonyms, Localities, Descriptions, Observations (Latin). Flensbvrgi et Lipsiae: In Officina Libraria Kortii; 1775.
35. Lee SC, Gepts PL, Whitaker JR. Protein Structures of Common Bean (*Phaseolus vulgaris*) α-Amylase Inhibitors. Journal of Agricultural and Food Chemistry. 2002; 50(22): 6618-6627. doi: 10.1021/jf020189t
36. Reissig JL, Strminger JL, Leloir LF. Amodified colorimetric method for the estimation of N-acetylamino sugars. The Journal of Biological Chemistry. 1955; 217: 959-967.
37. Mbata GN, Ekpendu OT. The insecticidal action of four botanicals [Mitracarpus scaber, Napoleona imperialis, Piper guineense, Diodia sarmentosa] against three storage beetles [*Callosobruchus maculatus*, Callosobruchus subinnotatus, Sitophilus]. Food and Agriculture Organization of the United Nations. 1992; 44(3): 723-733.
38. Nardon P. Ovogenesis and transmission of symbiotic bacteria in the weevil *Sitophilus oryzae* L. (Coleoptera: Curculionoidea) (French). Annales de la Société entomologique de France (NS). 2006; 42(2): 129-164. doi: 10.1080/00379271.2006.10700618
39. Wiwat C, Thaithanun S, Pantuwatana S, et al. Toxicity of Chitinase-Producing *Bacillus thuringiensis* ssp. *kurstaki* HD-1 (G) toward *Plutella xylostella*. Journal of Invertebrate Pathology. 2000; 76(4): 270-277. doi: 10.1006/jipa.2000.4976
40. Laribi-Habchi H, Bouanane-Darenfed A, Drouiche N, et al. Purification, characterization, and molecular cloning of an extracellular chitinase from *Bacillus licheniformis* stain LHH100 isolated from wastewater samples in Algeria. International Journal of Biological Macromolecules. 2015; 72: 1117-1128. doi: 10.1016/j.ijbiomac.2014.10.035
41. Rishad KS, Rebello S, Nathan VK, et al. Optimised production of chitinase from a novel mangrove isolate, *Bacillus pumilus* MCB-7 using response surface methodology. Biocatalysis and Agricultural Biotechnology. 2016; 5: 143-149. doi: 10.1016/j.bcab.2016.01.009

Journal of Toxicological Studies 2024, 2(2), 1423.
https://doi.org/10.59400/jts.v2i2.1423

Article

Microstructure of the dentate gyrus and spontaneous alternation behaviour of male Wistar rats following *Rauvolfia vomitoria* and *Gongronema latifolium* extracts administration

Moses B. Ekong*, Ini-Obong G. Essien, Cecilia K. Bassey, Ogechi M. Akanu

Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo 520003, Nigeria
*** Corresponding author:** Moses B. Ekong, mosesekong@uniuyo.edu.ng

CITATION

Ekong MB, Essien IOG, Bassey CK, Akanu OM. Microstructure of the dentate gyrus and spontaneous alternation behaviour of male Wistar rats following *Rauvolfia vomitoria* and *Gongronema latifolium* extracts administration. Journal of Toxicological Studies. 2024; 2(2): 1423. https://doi.org/10.59400/jts.v2i2.1423

ARTICLE INFO

Received: 2 June 2024
Accepted: 23 July 2024
Available online: 6 August 2024

Abstract: *Rauvolfia vomitoria* (RV) and *Gongronema latifolium* (GL) are medicinal plants used for the local treatment of various health issues. Their activities on the brain motivated this investigation on the histology and immunohistochemistry of the dentate gyrus and spontaneous alternation behaviour (SAB) of adult Wistar rats following RV root bark and GL leaf extract administrations. Twenty young adult Wistar rats (130–160 g) were assigned into four groups: Group 1 served as the control (5 mL/kg of distilled water placebo), while the test groups 2–4 were, respectively, singly administered 200 mg/kg of RV, 200 mg/kg of GL, and their combination. The administrations were oral and lasted for seven days. A T-maze SAB test was carried out, and the animals were sacrificed immediately after ketamine hydrochloride intraperitoneal anaesthesia. Serial sections of the hippocampal region from perfused rat brains were stained with Cresyl fast violet and immunolabelled with neuronal nuclei (NeuN) for neurons and glial fibrillary acidic protein (GFAP) for astrocytes. Results indicated that SAB was significantly ($p < 0.05$) lower in the test groups. Histologically, Nissl was less distributed in the RV and GL-only groups but not in the combined group, while there was less NeuN positivity in the RV group, with the GL and RV + GL groups not affected. There was less positive GFAP expression in individual RV and GL groups, but not in the RV + GL combined group, all compared with the control. In conclusion, the combination of RV and GL did not improve SAB but modulated Nissl, NeuN, and GFAP expression in the dentate gyrus.

Keywords: *Rauvolfia vomitoria*; *Gongronema latifolium*; Nissl substance; hippocampal region

1. Introduction

Folkloric treatment against diseases utilises medicinal plants, and this is rampant in Africa and Asia. Such diseases as malaria, diabetes, epilepsy, and depression disorders, among others, are known to be managed by medicinal plants [1–3]. However, an individual medicinal plant reported to be effective in addressing a particular disease condition can become toxic to body tissues and other metabolic processes [4–6]. As such, the use of a combination of these medicinal plants can help overcome the adverse challenge [7,8].

Two important medicinal plants with diverse medicinal properties utilised in folkloric treatment and in the present study are *Rauvolfia vomitoria* and *Gongronema latifolium* [2,7,9,10]. *Rauvolfia vomitoria* (*R. vomitoria*), also known as the swizzler stick or serpent wood, is found in Africa [5]. It is a shrub of the family Apocynaceae, whose roots, root bark, stem bark, and leaves are commonly used for herbal remedies [2]. Its phytochemical components include alkaloids, primarily reserpine, glycosides, polyphenols, and reducing sugars [11,12], and it is used in the management of fever, epilepsy, and hypertension, among other folkloric uses [12–14]. But even at these, there are reports of adverse effects of *R. vomitoria*, especially on the brain tissues [4,15,16] These adverse effects may erode the needed benefits derivable from the use of the plant material, especially in brain-related disease conditions. However, Ekong et al. [7,15,17] reported beneficial relationships with another medicinal plant, *Gongronema latifolium*, which this study seeks to explore.

Gongronema latifolium (*G. latifolium*) is also known as bush buck and is found in tropical Africa [18]. It is a climbing shrub of up to 5 m long belonging to the family Apocynaceae. It is an edible plant with a sharp, bitter, and sweet taste. The leaf, which is heart-shaped and highly nutritious, contains proteins, fatty acids, fibres, and elements such as sodium, potassium, calcium, copper, manganese, chromium, and selenium [18–20]. It is also made up of essential oils, alkaloids, saponins, tannins, amino acids, and vitamins [20]. *G. latifolium* is reported to have antimicrobial, cardioprotective, anti-inflammatory, antioxidant, antipyretic, hypotensive, and hypoglycaemic properties [21–24].

The effectiveness of *R. vomitoria* in combination with *G. latifolium* seems synergistic, as the cerebellum has been protected from the individual adverse effects of *R. vomitoria* [7,9,17], while still maintaining its useful properties. However, there is a report of non-effectiveness with the combination [25].

One critical target of most plant-based materials is the hippocampal formation, with the dentate gyrus being very important as it serves as a source of new cell formation to enhance memory [26–28]. The role of extraneous material on this region in memory activity cannot be overlooked, as some important medicinal plants with beneficial metabolic potential have been reported to cause adverse effects to the hippocampal formation [29]. These motivated this study on spontaneous alternation behaviour and the hippocampal formation histology in Wistar the rat following *R. vomitoria* and *G. latifolium* combination.

2. Materials and methods

2.1. Handling of the experimental animals

Twenty adult male Wistar rats weighing about 130–160 g were obtained from the Animal House Facility of the Faculty of Basic Medical Sciences, University of Uyo. The rats were acclimatised for two weeks at the animal house and were allowed normal rat chow (Vital Feed, Nigeria) and clean water ad libitum throughout the duration of the experiment. The rats were grouped into four groups of five rats each. Group 1 animals were the control, while groups 2–4 were the test groups. Each rat was handled within the shortest possible time and at the base of the tail, except during oral gavages and intraperitoneal injection.

2.2. Preparation of *R. vomitoria* and *G. latifolium* extracts

The *R. vomitoria* and *G. latifolium* plants were identified and authenticated in the Department of Botany and Ecological Studies of the University of Uyo, Nigeria. The roots of *R. vomitoria* and the leaves of *G. latifolium* were, respectively, obtained from local farms in Esit Eket and Ika in Akwa Ibom State, Nigeria. The barks of the roots of *R. vomitoria* were separated from the cambium for extraction, while the *G. latifolium* leaves were used for extraction. The plant parts were air-dried, crushed to a fine powder, and 75%–80% ethanol was used to macerate in a Soxhlet extractor. The extracts were concentrated by evaporation of the ethanol using a rotary evaporator, and the concentrates were dried in a Plus 11 Gallenkamp oven at 45–50 °C. The dry extracts obtained were stored in a refrigerator at 4 °C until used.

2.3. Experimental protocol

Group 1 rats (control) were administered 5 mL/kg body weight of distilled water placebo, while groups 2–4 were administered oral gavages of 200 mg/kg body weight of root bark extract of *R.* vomitoria [17], 200 mg/kg of leaf extract of *G. latifolium* [17], and a combination of 200 mg/kg body weight of root bark extract of *R. vomitoria* and 200 mg/kg leaf extract of *G. latifolium* [7,17], respectively, for seven days (**Table 1**), which was followed by the T-maze spontaneous alternation test.

Table 1. Schedule of administration for experimental groups.

Groups ($n = 5$)	Treatments	Duration of treatment (days)
1 (Control)	Distilled water (5 mL/kg)	7
2	200 mg/kg of *R. vomitoria*	7
3	200 mg/kg of *G. latifolium*	7
4	200 mg/kg of *R. vomitoria* + 200 mg/kg of *G. latifolium*	7

2.4. Spontaneous alternation test

The spontaneous alternation test was carried out on day 8 of the experiment. This test was conducted using the T-maze, which was made up of a start arm and two cross-arms. Each rat was placed in the start arm of the maze and allowed to freely choose between the right and left arms of the maze for a minute. Once a choice was made, the rat was prevented from leaving the chosen arm with a shutter door for 10 s and then placed in a holding cage for 10 min. If no choice was made, nothing was recorded for the trial. Four subsequent trials were carried out. A spontaneous alternation occurred when a rat chose a different arm of the maze from a previously visited one. Ethyl alcohol (70%) was used to clean the maze in-between trials. The spontaneous alternation results were recorded, analyzed, and percentage alternations were calculated [30,31].

2.5. Termination of the experiment

Immediately after the neurobehavioural test, the animals were anaesthetized with ketamine hydrochloride (50 mg/kg) and were then sacrificed by perfusion fixation with 10% buffered formalin. The abdomens of the animals were dissected through the diaphragm so as to access the heart, and 10% buffered formalin was transcardially perfused through the left ventricle. The skull was later excised, and the brains were removed and post-fixed in 10% buffered formalin for 48 h.

Whole brains were cryoprotected in a 30% sucrose solution overnight at 4 ℃, and sectioned at 40 µm on a freezing microtome at −30 ℃. Cut sections were picked up with a Carmel brush and floated on a well containing phosphate buffered saline (1 M, PBS at pH 7.35). The representative serial sections of the hippocampal formation were placed on glass slides and processed for Cresyl fast violet staining for Nissl substance and immunolabelled with anti-neuronal nuclei (NeuN) for neurons and glial fibrillary acidic protein (GFAP) for astrocytes.

For Cresyl violet staining, sections on slides were first incubated in a mixture of chloroform and methanol (1:1) for an hour and then stained in Cresyl fast violet solution for 30 min. They were then rinsed in distilled water and dehydrated in ascending grades of alcohol (50% to absolute), cleared in xylene, and mounted on Entellan.

Sections for immunohistochemistry were blocked with 3% hydrogen peroxide for 15 min, washed and incubated in 5% normal goat serum for an hour, and incubated in anti-NeuN and rabbit anti-GFAP in 1% normal goat serum and Triton-X overnight at room temperature (18 ℃). Sections were washed with PBS and incubated with goat anti-mouse for NEUN and goat anti-rabbit for GFAP for 2 h. Thereafter, they were washed in PBS and incubated in an avidin-biotin complex (1:1) for an hour. The sections were washed in PBS and incubated in diaminobenzedine (chromogen) to develop the staining. Sections were then washed in PBS, mounted on slides, dehydrated through ascending grades of alcohol (50% to absolute), cleared in xylene, and mounted on Entellan.

2.6. Statistical analyses

GraphPad Prism 5 was used to analyse data obtained from the spontaneous alternation test. Data were analysed using a repeated one-way analysis of variance (ANOVA), followed by a Tukey multiple comparison post hoc test to compare individual group means. A P value ≤ 0.05 was considered statistically significant. Data are presented as the mean ± standard error of the mean.

3. Results

3.1. Spontaneous alternation behaviour

The animals in the test groups administered only *R. vomitoria* (200 mg/kg) and *G. latifolium* (200 mg/kg), and their combination had significantly ($P < 0.05$) lower spontaneous alternation behaviour compared with the control. No spontaneous alternation ($p > 0.05$) was observed in the group administered *R. vomitoria* and *G. latifolium* combination compared with their individual groups (**Figure 1**).

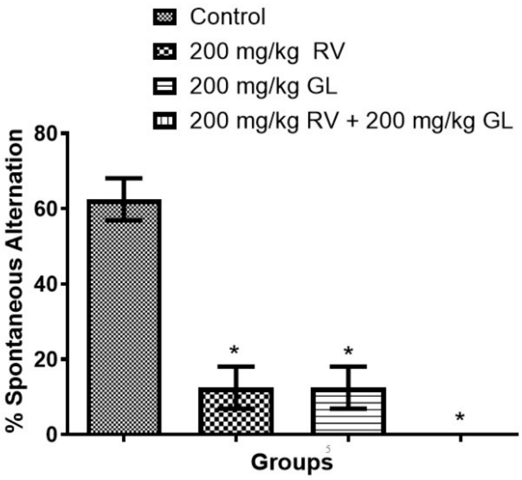


Figure 1. Spontaneous alternation behavioural test result.

Data are presented as the mean ± standard error of the mean, *n* = 5. ANOVA followed by a Tukey multiple comparison test * Significantly different from the control group at $P < 0.05$. RV = *R. vomitoria*, GL = *G. latifolium*.

3.2. Histology of the dentate gyrus

The hippocampal dentate gyrus of Wistar rats is made of three layers: from outside to inward, the molecular, granular, and polymorphic layers. In the control group, Nissl was well expressed and distributed in the small, sparse cells of the molecular layer. The granular layer consisted of a dense population of Nissl-expressed cells, while the polymorphic layer consisted of sparse Nissl-expressed cells (**Figure 2a**).

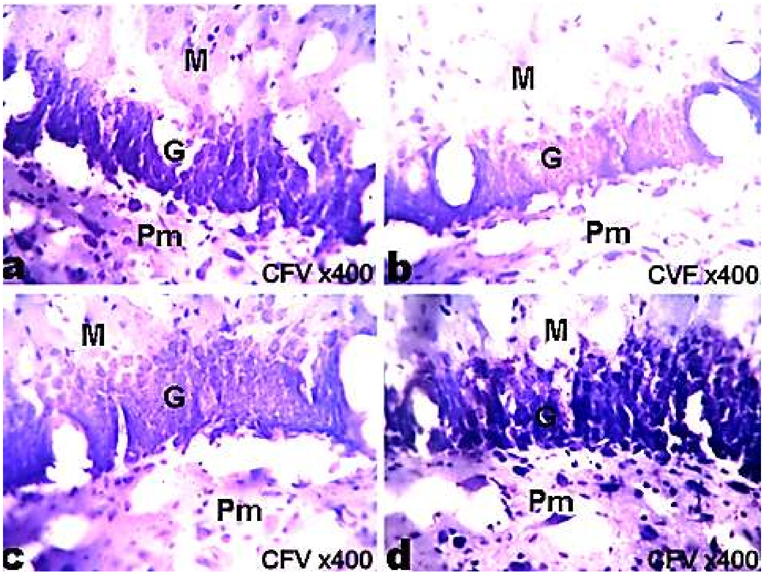


Figure 2. Representative sections of the dentate gyrus stained with Cresyl fast violet (CFV), indicating Nissl expression. Magnification: ×400. **(a)** The control group section showing well stained Nissl in the dentate gyrus layers; **(b)** the section of group 2 administered 200 mg/kg *R. vomitoria*; showing less-stained Nissl; **(c)** the section of group 3 administered 200 mg/kg *G. latifolium,* showing moderately stained Nissl; **(d)** the section of group 4 administered 200 mg/kg *R. vomitoria* and 200 mg/kg *G. latifolium* showing well stained Nissl.

Nissl—bluish.

In group 2, administered 200 mg/kg *R. vomitoria* only, Nissl was less stained compared with the control group (**Figure 2b**). In group 3, administered 200 mg/kg *G. latifolium*, Nissl was moderately stained compared with the control group (**Figure 2c**). In group 4, administered 200 mg/kg *R. vomitoria* and 200 mg/kg *G. latifolium*, Nissl was well stained compared with the control group (**Figure 2d**).

3.3. Immunohistochemistry

3.3.1. Neuronal Nuclei (NeuN)

The dentate gyrus of the control group showed normal deep expression of neuronal nuclei (NeuN) in the molecular, granular, and polymorphic layers (**Figure 3a**). In group 2, administered 200 mg/kg of *R. vomitoria*, NeuN was moderately expressed in the central granular cells, but the peripheral granular cells were well expressed compared with the control group (**Figure 3b**).

In group 3, administered 200 mg/kg of *G. latifolium*, NeuN expression appeared normal in the granular cells compared to the control group (**Figure 3c**). In group 4 administered 200 mg/kg *R. vomitoria* and 200 mg/kg *G. latifolium*, NeuN was moderately expressed in the granular cells and appeared like the control group (**Figure 3d**).

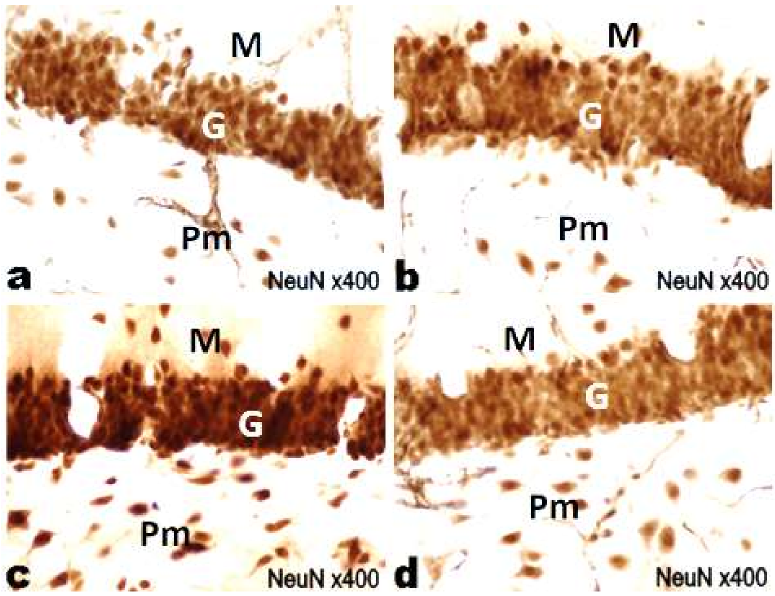


Figure 3. Representative sections of the hippocampal dentate gyrus immunolabelled with neuronal nuclei (NeuN), indicating neurons. Magnification: ×400. **(a)** The control group section showed the expression of neuronal nuclei (NeuN) in the soma of the granular cells throughout the molecular, granular, and polymorphic layers; **(b)** the section of group 2 administered 200 mg/kg *R. vomitoria*; NeuN expression was less in the soma of the pyramidal cells throughout the molecular, granular, and polymorphic layers; **(c)** the section of group 3 administered 200 mg/kg *G. latifolium, and* NeuN expression in the soma of the granular cells appeared like the control group; **(d)** the section of group 4 administered 200 mg/kg *R. vomitoria* and 200 mg/kg *G. latifolium in* combination, and NeuN expression in the soma of the granular cells appeared like the control group.
NeuN—brown.

3.3.2. Glial Fibrillary Acidic Protein (GFAP)

The section of the dentate gyrus of the control group animals showed the expression of GFAP within the astrocytes' soma and processes throughout the molecular, granular, and polymorphic layers (**Figure 4a**). In group 2, administered 200 mg/kg of *R. vomitoria*, GFAP expression was expressed in the processes but was less expressed in the soma of the astrocytes compared with the control group (**Figure 4b**).

In group 3, administered 200 mg/kg *G. latifolium*, GFAP expression was less in the astrocytes soma and processes compared with the control group. In group 4, administered 200 mg/kg of *R. vomitoria* and 200 mg/kg of *G. latifolium*, GFAP expression in the soma of the astrocyte cells appeared similar to the control group, while the processes were less expressed (**Figures 4c,d**).

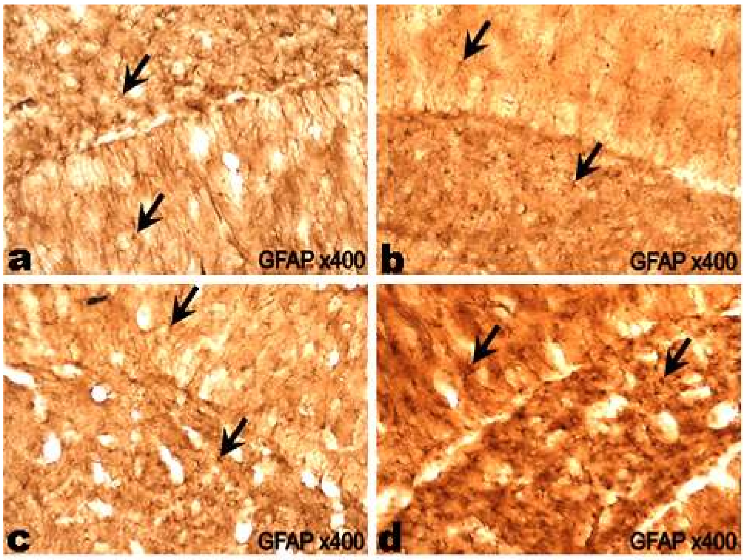


Figure 4. Representative sections of the hippocampal dentate gyrus immunolabelled with glial fibrillary acidic protein (GFAP) indicating astrocytes. Magnification: ×400. **(a)** The control group section showed the GFAP expression (arrows) within the astrocytes' soma and processes throughout the molecular, granular and polymorphic layers; **(b)** the section of group 2 administered 200 mg/kg *R. vomitoria* showing less expressed GFAP in the soma and processes of the astrocytes; **(c)** the section of group 3 administered 200 mg/kg *G. latifolium* showed less GFAP expression in the soma and processes of the astrocytes; **(d)** the section of group 4 administered 200 mg/kg *R. vomitoria* and 200 mg/kg *G. latifolium* combination, showing less expressed GFAP in the processes.
Result: GFAP—Astrocytes are brown.

4. Discussion

The present study investigated the microstructures of the dentate gyrus and spontaneous alternation behaviour of adult Wistar rats following *R. vomitoria* root bark and *G. latifolium* leaf extract administration. Results showed that spontaneous behaviour declined, along with Nissl, NeuN, and GFAP expressions.

To study the cognitive action of rats following *R. vomitoria* root bark and *G. latifolium* leaf extracts administration, the T-maze spontaneous alternation behaviour was carried out. This test is based on the willingness of rodents to explore a new environment, which in this case was the new arm of the maze instead of the previous visited one [30,31]. The ability of the rats to perform the spontaneous alternation in groups 2 and 3 administered only *R. vomitoria* root bark and *G. latifolium* leaf extracts, respectively, was significantly lower than that of the control group, implying a poor spontaneous alternation [31], associated with the extracts, and invariably impaired working memories [32,33]. *R. vomitoria* root bark extract sedates, usually resulting in poor motor and cognitive activities [4,15,16], which may have played out in the present study. Although *G. latifolium* leaf extract is not known to cause adverse behaviour [17,22], it is possible that the rats in the *G. latifolium* group were anxious, as this could have influenced their poor spontaneous alternation [34].

Group 4 rats administered a combination of *R. vomitoria* root bark and *G. latifolium* leaf extracts did not move from the point of entry in any direction in the maze, suggesting a sedative action of the combined extracts or their anxious state. It is possible that there was a synergistic action of *R. vomitoria* root bark and *G. latifolium*, which could have further worsened the sedative state of the rats. However, the present result is at variance with previous actions of *R. vomitoria* and *G. latifolium* combination, which improved cognition [8,35].

Spontaneous alternation is indicative of spatial memory [32], which the hippocampal formation regulates [28]. A part of the hippocampal formation essential for memory consolidation is the dentate gyrus. Its structure is vital for memory function [27]. In the present study, groups 2 and 3 administered only *R. vomitoria* root bark and *G. latifolium* leaf extracts, respectively, showed reduced Nissl expression, which was even less expressed in the *R. vomitoria* group. This implies that *R. vomitoria* and *G. latifolium* extract administration may have resulted in chromatolysis. Chromatolysis results from ribosomal protein degradation, which often precedes cellular degeneration [36]. *R. vomitoria* is reported in structural alterations of brain cells, although *G. latifolium* does not show such a major adverse effect [8–10,15]. The effect of the combined extracts in group 4 did not seem to affect the granular cell layer as the Nissl appeared well expressed, suggesting that the combination may have protected the dentate gyrus Nissl as previously reported in other brain areas [7–9]. Cells adapt to the environment to protect themselves from injury [37], and chromatolysis may have been a result of such.

Immunohistochemically, neurons can be identified with a neuron-specific nuclear protein, NeuN, expressed mostly by the mature ones [38]. Reduced expression of NeuN indicates loss of cell viability or antigenicity [39,40]. In the present study, NeuN expression reduced moderately in the group administered only *R. vomitoria* extract. This result aligns with the actions of *R. vomitoria* reported in cellular structural changes [4,8] and in the Nissl result of the present study. A report showed that dentate granule cells undergo morphological changes in response to excessive excitation or trauma [29], which may have also been applicable in the present study.

The groups administered *G. latifolium* only showed positive NeuN that was well expressed in dentate gyrus, suggesting no adverse actions of the extract. *G. latifolium* administration is not known with adverse effects on most brain structures, and this could have been applicable in the present study. The groups administered *G. latifolium* in combination with *R. vomitoria* showed moderately reduced NeuN expression, indicating the potential of *G. latifolium* in protection against the adverse effect of *R. vomitoria.*

Immunohistochemically, astrocytes can be demonstrated with GFAP, an intermediate filament protein they mostly express, and an increased or decreased expression may indicate a trauma to the brain [41–43]. In the present study, GFAP was less expressed in the astrocytes' soma within the molecular, pyramidal, and polymorphic layers of the dentate gyrus of the group administered only *R. vomitoria*. This indicates a downregulation of the GFAP protein, which may be associated with degeneration. This is at variance with reports on the cerebellum from previous studies [7,9]. The group administered only *G. latifolium* also showed reduced GFAP expression, which may be a protective mechanism, as neurotoxicity is often not reported in *G. latifolium.* The group administered a combination of *G. latifolium* and *R. vomitoria* also showed reduced GFAP expression.

The dentate gyrus is the integral region of the hippocampal formation that contributes to the formation of new episodic memories, spontaneous exploration of novel environments, and other functions [44,45]. It is observed that destruction of the dentate gyrus cells leads to poor maze activities and memory impairment [26,46].

From the present findings, *R.* vomitoria elicited its action through the down-regulation of dentate gyrus structural proteins, including Nissl, NeuN, and GFAP, which affected neuronal and astrocyte viability and resulted in memory impairment. A combination with *G. latifolium* partially protected against these proteins down-regulation, as it improved Nissl and NeuN, suggesting antagonistic action. However, GFAP expression and memory did not improve, suggesting a synergistic action.

5. Conclusion

The results of the present study showed that the combination of *R. vomitoria* and *G. latifolium* could not reverse the individual effects of *R. vomitoria* and *G. latifolium* on spontaneous alternation but was able to show modulation of the dentate gyrus Nissl distribution and immunohistochemical expressions of NeuN and GFAP. These findings suggest a beneficial synergistic role for the *R. vomitoria* and *G. latifolium* combination on the dentate gyrus, but care should be taken as their administration is not free of adverse effects. Due to limited data, further analysis involving specific neurochemicals and proteins could identify the pharmacology of the *R. vomitoria* and *G. latifolium* combination in the dentate gyrus and other brain areas.

Author contributions: Conceptualization, MBE; methodology, MBE; software, MBE; validation, MBE and IOGE; formal analysis, MBE; investigation, MBE, CKB and OMA; resources, MBE, IOGE, CKB and OMA; data curation, MBE, IOGE, CKB and OMA; writing—original draft preparation, MBE and IOGE; writing—review and editing, MBE; visualization, MBE and IOGE; supervision, MBE; project administration, MBE; funding acquisition, MBE and CKB. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Adeleye OA, Femi-Oyewo MN, Bamiro OA, et al. Ethnomedicinal herbs in African traditional medicine with potential activity for the prevention, treatment, and management of coronavirus disease 2019. Future Journal of Pharmaceutical Sciences. 2021; 7(1). doi: 10.1186/s43094-021-00223-5
2. Beljanski M. Traditional use of *Rauwolfia vomitoria*: The Beljanski Foundation. Available online: https://www.beljanski.org/engl/press/traditional-use-of-rauwolfia-vomitoria (accessed on 25 July 2024).
3. Singh B, Singh B, Kishor A, et al. Exploring Plant-Based Ethnomedicine and Quantitative Ethnopharmacology: Medicinal Plants Utilized by the Population of Jasrota Hill in Western Himalaya. Sustainability. 2020; 12(18): 7526. doi: 10.3390/su12187526
4. Ekong MB, Peter AI, Edagha IA, et al. *Rauwolfia vomitoria* inhibits olfaction and modifies olfactory bulb cells. Brain Research Bulletin. 2016; 124: 206-213. doi: 10.1016/j.brainresbull.2016.05.008
5. Bisong SA, Abuo FE, Udefa AL, et al. Comparative Effects of Alkaloid and Saponin Fractions of *Rauwolfia vomitoria* on Social Behaviour and Depression in a CD1 Mouse Model of Memory Impairment. Archives of Current Research International. 2019; 16(1): 1-11. doi: 10.9734/acri/2019/v16i130083
6. Fang T, Xue Z sheng, Li J, et al. *Rauwolfia vomitoria* extract suppresses benign prostatic hyperplasia by reducing expression of androgen receptor and 5α-reductase in a rat model. Journal of Integrative Medicine. 2021; 19(3): 258-264. doi: 10.1016/j.joim.2020.12.002
7. Ekong MB, Ekpene UU, Nwakanma AA, et al. The combination of the extracts of *Rauwolfia vomitoria* and *Gongronema latifolium* show protective effects on the cerebellum. Synergy. 2017; 5: 29-34. doi: 10.1016/j.synres.2017.10.001
8. Nduohosewo IS, Ekong MB. Murine's amygdala microstructure and elevated plus maze activities following *R. vomitoria* root bark and *G. latifolium* leaf extracts administration. Anatomical Science International. 2020; 95(3): 342-355. doi: 10.1007/s12565-020-00527-1
9. B. Ekong M, A. Nwakanma A. Rauwolfia vomitoria and *Gongronema latifolium* extracts influences cerebellar cortex. Alzheimer's, Dementia & Cognitive Neurology. 2017; 1(3). doi: 10.15761/adcn.1000115
10. Ekong MB, Peter AI, Ekpene UU. Co-administration of *Rauwolfia vomitoria* with *Gongronema latifolium* or *Vernonia amygdalina* on spatial learning, memory, and some bio-molecules. Asian Journal of Medical Sciences. 2015; 7(1): 82-87. doi: 10.3126/ajms.v7i1.11156
11. Akpanabiatu MI. Effects of the biochemical interactions of vitamin A and E on the toxicity of root bark extract of *Rauwolfia vomitoria* (Apocynaceae) in Wistar albino rats. University of Calabar; 2006.
12. Chinonye II, Chijioke C, Iwuji CS, et al. Chemical and Medicinal Properties of *Rauwolfia vomitoria* (AFZEL) Harvested from the South Eastern Nigeria. Asian Journal of Chemical Sciences. 2021; 56-71. doi: 10.9734/ajocs/2021/v10i419103
13. Lobay D. Rauwolfia in the Treatment of Hypertension. Integr Med (Encinitas). 2015; 14(3): 40-6.
14. Fapojuwomi OA, Asinwa IO. Assessment of Medicinal Values of *Rauvolfia vomitoria* (Afzel) in Ibadan Municipality. Greener Journal of Medical Sciences. 2013; 3(2): 037-041. doi: 10.15580/gjms.2013.2.012013398
15. Ekong MB, Peter AI, Ekpene UU, et al. *Gongronema latifolium* Modulates *Rauwolfia vomitoria*-Induced Behaviour and Histomorphology of the Cerebral Cortex. International Journal of Morphology. 2015; 33(1): 77-84. doi: 10.4067/s0717-95022015000100013
16. Ekong M, Eluwa MA. Effect Of Aqueous Extract of *Rauwolfia Vomitoria* Root Bark on the Cytoarchitecture of the Cerebellum and Neurobehaviour of Adult Male Wistar Rats. The Internet Journal of Alternative Medicine. 2009; 6(2). doi: 10.5580/45
17. Ekong MB, Peter MD, Peter AI, et al. Cerebellar neurohistology and behavioural effects of *Gongronema latifolium* and *Rauwolfia vomitoria* in mice. Metabolic Brain Disease. 2013; 29(2): 521-527. doi: 10.1007/s11011-013-9453-8
18. Frederick Eleyinmi A, Sporns P, Bressler DC. Nutritional composition of *Gongronema latifolium* and *Vernonia amygdalina*. Nutrition & Food Science. 2008; 38(2): 99-109. doi: 10.1108/00346650810862975
19. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. Journal of Zhejiang University Science B. 2007; 8(5): 352-358. doi: 10.1631/jzus.2007.b0352

20. Alogun ME, Besong EE, Obimma JN, et al. *Gongronema Latifolium*: A Phytochemical, Nutritional and Pharmacological Review. Journal of Physiology and Pharmacology Advances. 2016; 6(1): 811. doi: 10.5455/jppa.1969123104000
21. Beshel JA, Beshel FN, Nku CU. *Gongronema Latifolium*: A Plant with Cardioprotective Potentials. International Journal of Trend in Scientific Research and Development. 2019; 3(2): 548-558. doi: 10.31142/ijtsrd21431
22. Al-Hindi B, Yusoff NA, Ahmad M, et al. Safety assessment of the ethanolic extract of *Gongronema latifolium* Benth. leaves: a 90-day oral toxicity study in Sprague Dawley rats. BMC Complementary and Alternative Medicine. 2019; 19(1). doi: 10.1186/s12906-019-2573-x
23. Okpala B. Benefits of *Gongronema latifolium* (utazi). Available online: https://www.globalfoodbook.com/benefitsofG.L. (accessed on 25 July 2024).
24. Beshel JA, Palacios J, Beshel FN, et al. Blood pressure-reducing activity of *Gongronema latifolium* Benth. (Apocynaeceae) and the identification of its main phytochemicals by UHPLC Q-Orbitrap mass spectrometry. Journal of Basic and Clinical Physiology and Pharmacology. 2019; 31(1). doi: 10.1515/jbcpp-2018-0178
25. Aquaisua AN, Mbadugha CC, Enobong IB, Ekong M. Effects of *Rauvolfia vomitoria* on the cerebellar histology, body and brain weights of albino wistar rats. Available online: https://www.researchgate.net/publication/330006420_Effects_of_rauvolfia_vomitoria_on_the_cerebellar_histology_body_and_brain_weights_of_albino_wistar_rats (accessed on 25 July 2024).
26. Xavier GF, Costa VCI. Progress in Neuro-Psychopharmacology and Biological Psychiatry. Science Direct. 2009; 33(5): 762-773. doi: 10.1016/j.pnpbp.2009.03.036
27. Aniol V, Manolova A, Gulyaeva N. Early Life Events and Maturation of the Dentate Gyrus: Implications for Neurons and Glial Cells. International Journal of Molecular Sciences. 2022; 23(8): 4261. doi: 10.3390/ijms23084261
28. Tsetsenis T, Broussard JI, Dani JA. Dopaminergic regulation of hippocampal plasticity, learning, and memory. Frontiers in Behavioral Neuroscience. 2023; 16. doi: 10.3389/fnbeh.2022.1092420
29. Takeda A, Tamano H. Is Vulnerability of the Dentate Gyrus to Aging and Amyloid-$\beta_{1–42}$ Neurotoxicity Linked with Modified Extracellular Zn^{2+} Dynamics? Biological and Pharmaceutical Bulletin. 2018; 41(7): 995-1000. doi: 10.1248/bpb.b17-00871
30. Mehrdad S. Learning and Memory Tests. Stanford Medicine Behavioral and Functional Neuroscience Laboratory; 2017.
31. Deacon RMJ, Rawlins JNP. T-maze alternation in the rodent. Nature Protocols. 2006; 1(1): 7-12. doi: 10.1038/nprot.2006.2
32. d'Isa R, Comi G, Leocani L. Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze. Scientific Reports. 2021; 11(1). doi: 10.1038/s41598-021-00402-7
33. Kim J, Kang H, Lee YB, et al. A quantitative analysis of spontaneous alternation behaviors on a Y-maze reveals adverse effects of acute social isolation on spatial working memory. Scientific Reports. 2023; 13(1). doi: 10.1038/s41598-023-41996-4
34. Lalonde R. The neurobiological basis of spontaneous alternation. Neurosci Biobehav Rev. 2002; 26(1): 91-104.
35. Ekong M, Ekpene U, Thompson F, et al. Effects of co-treatment of *Rauwolfia vomitoria* and *Gongronema latifolium* on neurobehaviour and the neurohistology of the cerebral cortex in mice. Internet Journal of Medical Update-EJOURNAL. 2015; 10(1): 3. doi: 10.4314/ijmu.v10i1.2
36. Moon LDF. Chromatolysis: Do injured axons regenerate poorly when ribonucleases attack rough endoplasmic reticulum, ribosomes and RNA? Developmental Neurobiology. 2018; 78(10): 1011-1024. doi: 10.1002/dneu.22625
37. Agozzino L, Balázsi G, Wang J, et al. How Do Cells Adapt? Stories Told in Landscapes. Annual Review of Chemical and Biomolecular Engineering. 2020; 11(1): 155-182. doi: 10.1146/annurev-chembioeng-011720-103410
38. Gusel'nikova VV, Korzhevskiy DE. NeuN As a Neuronal Nuclear Antigen and Neuron Differentiation Marker. Acta Naturae. 2015; 7(2): 42-47. doi: 10.32607/20758251-2015-7-2-42-47
39. Ünal-Çevik I, Kılınç M, Gürsoy-Özdemir Y, et al. Loss of NeuN immunoreactivity after cerebral ischemia does not indicate neuronal cell loss: a cautionary note. Brain Research. 2004; 1015(1-2): 169-174. doi: 10.1016/j.brainres.2004.04.032
40. Luijerink L, Waters KA, Machaalani R. Immunostaining for NeuN Does Not Show all Mature and Healthy Neurons in the Human and Pig Brain: Focus on the Hippocampus. Applied Immunohistochemistry & Molecular Morphology. 2021; 29(6): e46-e56. doi: 10.1097/pai.0000000000000925
41. Brenner M, Messing A. Regulation of GFAP Expression. ASN Neuro. 2021; 13: 175909142098120. doi: 10.1177/1759091420981206

42. Özevren H, Deveci E, Tuncer MC. The effect of rosmarinic acid on deformities occurring in brain tissue by craniectomy method. Histopathological evaluation of IBA-1 and GFAP expressions. Acta Cirúrgica Brasileira. 2020; 35(4). doi: 10.1590/s0102-865020200040000006
43. Lybeck A, Friberg H, Nielsen N, et al. Postanoxic electrographic status epilepticus and serum biomarkers of brain injury. Resuscitation. 2021; 158: 253-257. doi: 10.1016/j.resuscitation.2020.10.027
44. Saab BJ, Georgiou J, Nath A, et al. NCS-1 in the Dentate Gyrus Promotes Exploration, Synaptic Plasticity, and Rapid Acquisition of Spatial Memory. Neuron. 2009; 63(5): 643-656. doi: 10.1016/j.neuron.2009.08.014
45. Zhang J, Wei X, Zhang S, et al. Research on Network Model of Dentate Gyrus Based on Bionics. Journal of Healthcare Engineering. 2021; 2021: 1-12. doi: 10.1155/2021/4609741
46. Hainmueller T, Bartos M. Dentate gyrus circuits for encoding, retrieval and discrimination of episodic memories. Nature Reviews Neuroscience. 2020; 21(3): 153-168. doi: 10.1038/s41583-019-0260-z

Journal of Toxicological Studies 2024, 2(2), 1238.
https://doi.org/10.59400/jts.v2i2.1238

Article

A study on membrane enzyme Na^+-K^+-ATPase in lindane exposed fish, *Channa punctatus*

Aradhna Gupta, Bechan Sharma*

Department of Biochemistry, University of Allahabad, Allahabad 211002, India
*** Corresponding author:** Bechan Sharma, sharmabi@yahoo.com

CITATION

Gupta A, Sharma B. A study on membrane enzyme Na^+-K^+-ATPase in lindane exposed fish, *Channa punctatus*. Journal of Toxicological Studies. 2024; 2(2): 1238. https://doi.org/10.59400/jts.v2i2.1238

ARTICLE INFO

Received: 12 June 2024
Accepted: 4 August 2024
Available online: 17 August 2024

Abstract: Na^+-K^+-ATPase is a membrane-bound enzyme responsible for the transport of ions through the membrane and the immediate release of energy. This enzyme is known to be an early target for oxygen radical-induced damage to intact cells. Exposure of *C. punctatus* to subacute concentrations of lindane for 96 h caused a significant reduction in the activities of Na^+-K^+-ATPase in all the tissues of the fish tested, with the brain being maximally affected and the heart being the least affected organ at the highest concentration of lindane (0.1 mg/L). The effect of pesticides was concentration-dependent. The percent decrease in the activity of Na^+-K^+-ATPase in brain, gills, heart, kidney, liver, and muscle was found to be 36.7, 23.4, 19.2, 29, 22.9, and 29.7, respectively. The order of level of enzyme activity recorded was as follows: liver > gills > kidney > brain > muscle > heart in the control.

Keywords: lindane; Na-K-ATPase; organs

1. Introduction

The aquatic ecosystem is an open system exposed to all different kinds of pollutants, toxicants, and surfactants, thus polluting the aquatic environment. Their direct discharge without any pre-treatment either leads to large-scale destruction of aquatic life or accumulation in water, soil, or bioaccumulation in biotic aquata. Though the pollutants could be biodegradable or non-biodegradable, these pollutants sometimes decrease the rate of decay of biodegradables. Thus, the increase in the contaminants may allow their environmental persistence for a longer period. Excessive use of nitrate and phosphate fertilizers may lead to eutrophication, thereby reducing the amount of oxygen in the aquatic biota and increasing the biological oxygen demand of that water for a prolonged period. Industrial smokes, burning of wood, petroleum, and vehicle fumes all gave rise to gases like sulphur dioxide, nitrogen oxides, and carbon dioxide; lead and the particulate matter have all caused serious harm to the environment and humans [1–3]. Reports on the increased levels of heavy metals and pesticides and their effects on oxidative stress have been exhaustively documented [4–8]. Many bacteria like *Vibrio anguillarum*, *Aeromonas*, *Flavobacterium*, *Pseudomonas*, *Serratia*, and *Yersina*, etc. have been shown to grow in water, which has less oxygen, increased organic matter, and an unsuitable pH for aquatic life.

The presence of organic matter may be due to leakage from septic tanks or contamination by domestic sewage [9]. Aquatic life may suffer from diseases like fin rot, papilloma, hyper-neoplasia, gill diseases, etc. Gills are the main respiratory organs of fish; they regulate ion concentration and osmotic balance for survival in unfavorable concentrations [10]. A decrease in function of gills has been reported when exposed to a pH of water less than or more than 7 [11]. Lindane is an organochlorine, hydrophobic, and highly persistent pesticide. Due to its lipophilic nature, it gets easily bioaccumulated in aquatic organisms. Lindane is strongly adsorbed on soils that contain a large amount of organic matter. It can move downward by capillary action through the soil with water from rainfall or artificial irrigation. In the UV light, it undergoes rapid dichlorination or degradation to form pentachlorocyclohexenes and tetrachlorocyclohexenes. The fish are able to bioaccumulate due to direct exposure to chemicals in water and ingestion of contaminated food or prey [12]. Their accumulation in low concentration in aquatic animals generates warning signals about the environment. Ultimately, in the long run, when these fish are eaten by humans or animals, the pesticide residues accumulate in different organs of the exposed living systems and hence may pose serious health problems [13]. The pesticide and its residues are known to get strongly adsorbed on clays and sediments of surface water where several fish populations reside for feeding.

Sodium Potassium ATPases (Na^+-K^+-ATPase, EC 3.6. 3.9) are membrane-bound sulfhydryl-containing oligomeric enzymes whose function is critical for the maintenance of cell viability. It works as an electrogenic P-type pump involved in monovalent ion transport across membranes, utilizing the energy of ATP hydrolysis. It pumps 3 sodium outside (extracellular) and 2 potassium inside the cell membrane (intracellular) for 1 ATP. The concentration gradient created due to ion transport across the membranes helps in other secondary transports [14,15]. This pump has many diverse functions like contractions, signalling, homeostasis (osmoregulation), and cell-cell adhesions [16,17]. It also governs many physiological processes like reabsorption, filtration, pH, electrolyte osmotic regulation by kidneys [18], sperm motility [19], and action potential in neurons [20–23]. This pump consists of 3 subunits *α, β,* and FXYD. The α subunit is for ion transport (catalytic) and is under the influence of the *β* (auxiliary) and FXYD (tissue-specific) subunits [24]. The *β*2 subunit of Na^+-K^+-ATPase is responsible for regulation of egg development in *Aedes aegypti***,** the causative organism of many vector-borne diseases like yellow fever, zika, dengue, and chicken guinea. The reduced expression of mRNA of *β*2 Na^+-K^+-ATPase in the knockdown mice showed less egg formation, which directly reduced their population and so less spread of disease [25]. In humans, decreased heart functions or heart failures have been reported due to a decrease in its activity [26]. In plants, it helps in nutrient uptake, root development, stomatal regulation, and response to environmental stresses. It helps plants to maintain proper ion balance, participate in nutrient absorption, and regulate turgor pressure in cells. In the environment, it acts as a marker for organisms facing challenges such as varying salinity, ion concentrations, or osmotic stress. In the present study, the effect of subacute concentrations of lindane (0.025, 0.05, 0.1 mg/L) on the activity of Na^+-K^+-ATPase was assessed in order to evaluate the perturbations in the transport of ions in different fish tissues exposed for 96 h.

2. Materials and methods

2.1. Experimental design and exposure to lindane

The healthy and active fish of 30–40 gm with no signs of any diseases or external injury were equally distributed in four aquaria of 1 × 1 ft. The subacute concentrations of lindane (0.025, 0.05, 0.1 mg/L) prepared in acetone were used for the exposure of *C. punctatus* for 96 h. In the control group, an equal volume of acetone was added. All aquaria were constantly aerated during the period of exposure by the aerator, and the fish were fed properly. The water of all 4 aquaria was changed in 24 h and replenished with fresh lindane.

2.2. Preparation of cell-free extracts and biochemical assays

1) Protein determination: The quantitative estimation of total protein in various tissue extracts and solutions was done according to the known procedure [27]. The samples were homogenized 10% (w/v) in 0.05 m sodium phosphate buffer, pH 7.4, and centrifuged at 10,000 rpm for 10 min in a cold (4 °C) condition. The supernatants were collected in labelled vials, and volume was noted. Determination of protein was done using a Folin-Ciocalteau reagent. The bovine serum albumin (BSA) was used as a standard. A blank was prepared, which contained all reagents but no protein. The intensity of the blue color was measured colorimetrically at 620
2) Assay of Na^+-K^+-ATPase activity: The Na^+-K^+-ATPase activity was determined as inorganic phosphorus (Pi) production using the method of Svobaca and Mossinger [28] and Fiske and Subbarow [29]. The reaction mixture is given in **Table 1**.

Table 1. Process for assay of Na^+-K^+-ATPase activity.

10% (w/v) tissue homogenized in 0.25 m sucrose (pH 7.4)	
15 min centrifugation 12,000 rpm at 4 °C	
Supernatant collected	
1	**2**
0.2 mL of 200 mm KCl	
0.2 mL of 1 m NaCl	0.1 mL of 1000 mm $MgCl_2$
0.1 mL of 1000 mm $MgCl_2$	1.0 mL of 200 mm Tris buffer, pH 7.4
1.0 mL of 200 mm Tris buffer, pH 7.4	100–200 µg supernatant
0.2 mL distilled water	0.16 mL distilled water
100–200 µg supernatant	0.2 mL of 10 mm ouabain
Leave 5 min/35–37 °C	
0.2 mL of 25 mm ATP (di sodium salt)	0.2 mL of NaCl + 0.2 mL of 25 mm ATP
Leave 15 min/35–37 °C	
1 mL of 10%TCA	
3000 rpm/5 min	
Supernatant collected	
0.5 mL supernatant	
3.0 mL DW	
0.5 mL of 2.5% ammonium molybdate in 5N H_2SO_4	
0.2 mL of 1, 2, 3, 4 ANSA	
Vortexed/10 min	
Reading at 600 nm	
Difference between 1 and 2 gives activity of Na^+-K^+-ATPase in µmol Pi liberated/mg protein/h.	

ANSA = aminonaphthol sulphonic.

All reagents were obtained from Sigma Chemical Company, USA. The pesticide used in the experiments was from Rallis India Limited. The double-distilled water was used for all biochemical experiments.

2.3. Statistical analysis

The values were presented as means ± standard error of mean (SEM) of observed data of three to five replicates. GraphPad Prism version 3.0 (GraphPad Prism Software Inc., San Diego, CA, USA) was used to analyze the data. Results obtained from treated and control fish were compared using the Turkey H test.

3. Results and discussion

Effect of lindane on the activity of Na^+-K^+-ATPase in different organs of lindane exposed fish

The data demonstrated the highest activity of Na^+-K^+-ATPase to be present in liver (41.67 ± 0.35 units/mg protein) and lowest in heart (16.73 ± 0.11 units/mg protein) of the control fish. The activities of Na^+-K^+-ATPase in other fish tissues such as gills, kidney, brain, and muscle were recorded as 33.78 ± 0.23, 32.87 ± 0.19, 30.29 ± 0.07, and 21.29 ± 0.09 units/mg protein, respectively. The order of level of enzyme activity recorded in the control fish tissues was as follows: liver > gills > kidney > brain > muscle > heart (**Table 2, Figure 1**).

Table 2. Effect of lindane (mg/L) on the specific activity of Na^+-K^+-ATPase (units/mg protein) in different tissues of *C. punctatus* exposed for 96 h.

Lindane	Gills	Heart	Kidney	Liver	Muscle	Brain
0	33.78 ± 0.23	16.73 ± 0.11	32.87 ± 0.19	41.67 ± 0.35	21.29 ± 0.09	30.29 ± 0.07
0.025	30.59 ± 0.21 (–9.42)	15.69 ± 0.09 (–6.21)	29.79 ± 0.18 (–9.37)	39.27 ± 0.33 (–5.76)	19.17 ± 0.08 (–9.95)	25.31 ± 0.05 (–16.44)
0.05	28.67 ± 0.22 (–14.98)	14.87 ± 0.07 (–11.10)	25.66 ± 0.15 (–21.97)	37.09 ± 0.32 (–11.01)	17.15 ± 0.07 (–19.47)	22.11 ± 0.06 (–27.02)
0.1	25.87 ± 0.20 (–23.41)	13.52 ± 0.08 (–19.19)	23.35 ± 0.16 (–28.94)	32.13 ± 0.29 (–22.92)	14.97 ± 0.07 (–29.70)	19.17 ± 0.04 (–36.68)

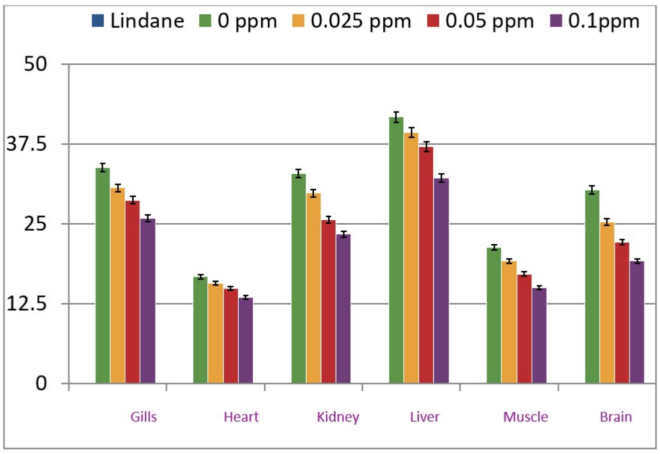


Figure 1. Lindane exposure at different concentrations (0, 0.025, 0.05, 0.1 ppm) on the specific activity of Na^+-K^+-ATPase (units/mg protein) in different tissues of *C. punctatus* exposed for 96 h.

Values are represented as µm of *Pi* released/h/mg wet weight of tissue. Each value represents the mean ± SEM of ten different observations. Values in parenthesis are percent change over control. The (–) sign represents a decrease over control. *h* represents time in hours. SEM = standard error of mean.

From the Turkeys HSD test all pairwise comparisons between different concentrations (0.025, 0.05, 0.1 mg/L) and control (0 mg/L) were statistically significant with $p < 0.05$. This indicates that there are significant differences in the mean values between the control and each of the concentrations, as well as between the different concentrations themselves for organs brain, muscle and liver. The non-significant differences were observed in the gills and kidney with 0.1 vs. 0.05mg/L and heart 0.05 vs. 0.025 mg/L (**Table 3**).

Table 3. Statistical inference of data based on pairwise comparisons between different concentrations of lindane (mg/L).

Lindane	Gills		Heart		Kidney		Liver		Muscle		Brain	
0.025 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.05 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.05 vs. 0.025	$P < 0.001$	S	$P = 0.34$	NS	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0.025	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0.05	$P = 0.52$	NS	$P < 0.001$	S	$P = 0.16$	NS	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S

S = Significant, NS = Not significant.

The treatment of the fish with three subacute concentrations of lindane displayed a marked decrease in the activity of Na^+-K^+-ATPase in all the tissues tested, i.e., the brain was maximally affected at all of these concentrations. At the lowest concentration of lindane (0.025 mg/L), the fish tissues such as gills, kidneys, and muscles showed the same level of percent inhibition (about 9%–10%) in the enzyme activity (**Table 2**).

At the highest concentration of the pesticide (0.1 mg/L), the brain of the fish exhibited a maximum decrease of 37% in the enzyme activity, and the heart indicated a minimum decrease of 19% after 96 h of treatment duration. The decrease in the enzyme activity was in a concentration-dependent manner. The percent decrease in the activity of Na^+-K^+-ATPase in brain, gills, heart, kidney, liver, and muscle was found to be 36.7, 23.4, 19.2, 29, 22.9, and 29.7, respectively. The data indicated that the gills and liver were affected by lindane to a similar level, showing about a 23% decrease in enzyme activity. Further, the kidney and muscle displayed almost the same level of enzyme inhibition to about 29% when the fish was treated with lindane at a 0.1 mg/L concentration for 96 h. The extent of inhibition in the enzyme activity from these fish tissues treated with the highest pesticide concentration (0.1 mg/L) was found in the following order: brain > muscle = kidney > gills = liver > heart (**Table 2**).

This enzyme is known to be an early target for oxygen radical-induced damage to intact cells [30,31]. Sharma observed a significant reduction of liver ATPase activity in *C. gaucha* upon ensdosulphan exposure [32]. Oruc et al. reported a reduction of liver activity in *Tillapia zilli* and *O. niloticus* and suggested that increased lipid peroxidation disturbed the anatomical integrity of the biomembrane and diminished its fluidity, leading to inhibition of activities of several membrane-bound enzymes, including Na^+-K^+-ATPase [33]. Very recently, cypermethrin at lethal (5.03 µg/L) and sublethal (1.02 µg/L) concentrations has been shown to cause significant alterations in the gills, liver, and muscle of the fish *Cirrhinus mrigala* [34].

Similar observations have been reported in *C. punctatus* exposed to pyrethroids [35] and *Clarias gariepinus* juvenile exposed to oxadiazon [35]. Increased activity in gills of silver catfish (*Rhamdia quelen)* was reported in water pH 9.0 and no significant change in kidney [36]. In a hypotonic environment, the kidneys of fish species excrete more dilute urine to maintain homeostasis [37], and in hypertonic they suffer from dehydration, and so they drink more sea water, excreting out extra salts [38,39]. At lethal concentration, the insecticide caused an increase in enzyme activity, whereas at sublethal concentration the enzyme activity decreased [40]. Maiti exposed the fish *Clarius batrachus* to 5.69 mg/L and 11.38 mg/L of chromium (lll) for 96 h and reported a decrease in activity in the brain [41–45]. This inactivity can lead to diverse alterations in the neurons, such as partial membrane depolarization, Ca^{+2} influx, altered neurotransmitter release, and even apoptosis, and all can be co-related to functional deficits in the brain [46].

4. Conclusions

The significance of sodium-potassium ATPase (Na^+-K^+-ATPase) in the environment is its role in maintaining cellular homeostasis both in plants and animals. It contributes to the adaptability and survival of organisms in diverse environmental conditions and serves as a sensitive indicator of environmental stress and toxicity. The exposure of the fish to varying subacute concentrations of lindane has caused significant perturbations in the activity of this enzyme in different organs of the fish tested. The results may serve as an indicator of pesticide contamination in water and better management of pesticide application in agricultural practices to secure environmental health.

Author contributions: Conceptualization, methodology and drafting, BS; performed experiment, AG. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors are grateful to University of Allahabad for providing facilities for carrying out the present work.

Conflict of interest: The authors declare no conflict of interest.

References

1. Gupta A, Rai DK, Pandey RS, et al. Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environmental Monitoring and Assessment. 2008; 157(1-4): 449-458. doi: 10.1007/s10661-008-0547-4
2. German AV, Zakonnov VV, Mamontov AA. Organochlorine compounds in bottom sediments, benthos, and fish in the volga pool of the Rybinsk Reservoir. Water Resources. 2010; 37(1): 84-88. doi: 10.1134/s0097807810010082
3. Gupta MA. A Case Study on the Bioaccumulated Organochlorines in Fish Related to their inhabitant Res. Rev. J Ecol. Environ. 2021; 9(5).

4. Gupta A, Sharma B. Evaluation of Levels of Phosphatases in the Lindane Exposed Fish, *Channa punctatus*. Journal of Biomedical Research & Environmental Sciences. 2023; 4(3): 555-561. doi: 10.37871/jbres1710
5. Jackson DA, Gardner DR. The effects of some organochlorine pesticide analogs on salmonid brain ATPases. Pesticide Biochemistry and Physiology. 1973; 2(4): 377-382. doi:10.1016/0048-3575(73)90049-7
6. Gupta A, Siddiqi NJ, Sharma B. Bioaccumulation and Biochemical Studies of Toxicants in Fish on AChE. Open J Pathol Toxicol Res. 2021; 1(1).
7. Gupta A, Sharma B. Acute Chronic Toxicity of Lindane in Channa punctatus. Open J Pathol Toxicol Res. 2021; 1(1).
8. Gupta A. An Evaluation of Lactate Dehydrogenase in the Lindane. International Journal of Animal Biotechnology and Applications. 2021; 7(1): 2455-7315.
9. Austin B. The effects of pollution on fish health. Journal of Applied Microbiology. 1998; 85(S1): 234S-242S. doi: 10.1111/j.1365-2672.1998.tb05303.x
10. Zimmer AM, Perry SF. Physiology and aquaculture: A review of ion and acid-base regulation by the gills of fishes. Fish and Fisheries. 2022; 23(4): 874-898. doi: 10.1111/faf.12659
11. Garcia Parra J, Baldisserotto B. Effect of Water pH and Hardness on Survival and Growth of Freshwater Teleosts. In: Baldisserotto B (editor). Fish Osmoregulation. Boca Raton; 2007. pp. 135-150. doi: 10.1201/b10994-6
12. Almeida CL, Aguiar LH, Moraes G. Effect of methyl parathion on the muscle and brain acetylcholinesterase activity of matrinxã (Brycon cephalus). Ciência Rural. 2005; 35(6): 1412-1416. doi: 10.1590/s0103-84782005000600029
13. Metcalf RL. Pesticides in Aquatic Environments. Springer US; 1977. doi: 10.1007/978-1-4684-2868-1
14. Lucu Č, Towle DW. Na^{++}/K^{+}-ATPase in gills of aquatic crustacea. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2003; 135(2): 195-214. doi: 10.1016/s1095-6433(03)00064-3
15. Thorsen K, Drengstig T, Ruoff P. Transepithelial glucose transport and Na^{+}/K^{+} homeostasis in enterocytes: An integrative model. American Journal of Physiology-Cell Physiology. 2014; 307(4): C320-C337. doi: 10.1152/ajpcell.00068.2013
16. Nie Y, Bai F, Chaudhry MA, et al. The Na/K-ATPase α1 and c-Src form signaling complex under native condition: A crosslinking approach. Scientific Reports. 2020; 10(1). doi: 10.1038/s41598-020-61920-4
17. Pivovarov AS, Calahorro F, Walker RJ. Na^{+}/K^{+}-pump and neurotransmitter membrane receptors. Invertebrate Neuroscience. 2018; 19(1). doi: 10.1007/s10158-018-0221-7
18. Mernissi G, Barlet-Bas C, Khadouri C, et al. Characterization and localization of ouabain-insensitive Na-dependent ATPase activities along the rat nephron. Biochim Biophys Acta. 1991; 1064(2): 205-211. doi: 10.1016/0005-2736(91)90303-P
19. Jimenez T, McDermott JP, Sánchez G, et al. Na/K-ATPase α4 isoform is essential for sperm fertility. Proceedings of the National Academy of Sciences. 2010; 108(2): 644-649. doi: 10.1073/pnas.1016902108
20. Clausen MV, Hilbers F, Poulsen H. The Structure and Function of the Na/K-ATPase Isoforms in Health and Disease. Frontiers in Physiology. 2017; 8. doi: 10.3389/fphys.2017.00371
21. Attwell D, Laughlin SB. An Energy Budget for Signaling in the Grey Matter of the Brain. Journal of Cerebral Blood Flow & Metabolism. 2001; 21(10): 1133-1145. doi: 10.1097/00004647-200110000-00001
22. Lei J, Nowbar S, Mariash CN, et al. Thyroid hormone stimulates Na-K-ATPase activity and its plasma membrane insertion in rat alveolar epithelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2003; 285(3): L762-L772. doi: 10.1152/ajplung.00376.2002
23. Pirahanchi Y, Jessu R, Aeddula NR. Physiology, Sodium Potassium Pump. Available online: https://www.ncbi.nlm.nih.gov/books/NBK537088/ (accessed on 13 June 2024).
24. Mercer RW, Biemesderfer D, Bliss DP, et al. Molecular cloning and immunological characterization of the gamma polypeptide, a small protein associated with the Na/K-ATPase. The Journal of cell biology. 1993; 121(3): 579-586. doi: 10.1083/jcb.121.3.579
25. Martinez NP, Pinch M, Kandel Y, et al. Knockdown of the Sodium/Potassium ATPase Subunit Beta 2 Reduces Egg Production in the Dengue Vector, Aedes aegypti. Insects. 2023; 14(1): 50. doi: 10.3390/insects14010050
26. Kjeldsen K. Myocardial Na/K-ATPase: Clinical aspects. Exp Clin Cardiol. 2003; 8(3): 131-133.
27. Lowry OH, Rosebrough NJ, Farr AI, Randall RJ. Protein measurement with Folin-Phenol reagent. J. Biol. Chem. 1951; 193: 265-275. doi: 10.1016/S0021-9258(19)52451-6
28. Svobaca P, Mossinger B. Catecholamine and brain microsomal Na^{+}/K^{+}-ATPase-1, protection against lipoperoxidative damages. Biochem. Pharmacol. 1981; 30: 427-432. doi: 10.1016/0006-2952(81)90626-2
29. Fiske CH, Subbarow Y. Colourimetric determination of phosphorous. J Biol. Chem. 1925; 66: 375-400. doi: 10.1016/S0021-

9258(18)84756-1
30. Kim MS, Akera T. O_2 free radicals: cause of ischemia-reperfusion injury to cardiac Na^+-K^+-ATPase. American Journal of Physiology-Heart and Circulatory Physiology. 1987; 252(2): H252-H257. doi: 10.1152/ajpheart.1987.252.2.h252
31. Kako K, Kato M, Matsuoka T, et al. Depression of membrane-bound Na^+-K^+-ATPase activity induced by free radicals and by ischemia of kidney. American Journal of Physiology-Cell Physiology. 1988; 254(2): C330-C337. doi: 10.1152/ajpcell.1988.254.2.c330
32. Sharma RM. Effect of endosulfan on adenosine triphosphatase (ATPase) activity in liver, kidney, and muscles of Channa gachua. Bulletin of Environmental Contamination and Toxicology. 1988; 41(3): 317-323. doi: 10.1007/bf01688873
33. Ozcan Oruc E, Uner N, Tamer L. Comparison of Na^+-K^+-ATPase Activities and Malondialdehyde Contents in Liver Tissue for Three Fish Species Exposed to Azinphosmethyl. Bulletin of Environmental Contamination and Toxicology. 2002; 69(2): 271-277. doi: 10.1007/s00128-002-0057-y
34. Prashanth MS, David M. Impact of Cypermethrin on Na^+-K^+, Ca^{2+} and Mg^{2+} ATPases in Indian Major Carp, Cirrhinus mrigala (Hamilton). Bulletin of Environmental Contamination and Toxicology. 2009; 84(1): 80-84. doi: 10.1007/s00128-009-9864-8
35. Kumar A, Sharma B, and Pandey RS. Toxicoogical assessment of the pyrethroids insecticides with special reference to cypermethrin and λ-cyalothrin in fresh water fishes. Int. J. Biol. Med Res. 2010; 1(4): 315-325.
36. Oluah NS, Mgbenka BO, Nwani CD, et al. Tissue-specific changes in Ca^{2+}-ATPase and Na^+/K^+-ATPase activities in freshwater African catfish Clarias gariepinus juvenile exposed to oxadiazon. The Journal of Basic and Applied Zoology. 2020; 81(1). doi: 10.1186/s41936-020-00186-8
37. Marx MTS, Souza C de F, Almeida APG, et al. Expression of Ion Transporters and Na^+/K^+-ATPase and H^+-ATPase Activities in the Gills and Kidney of Silver Catfish (*Rhamdia quelen*) Exposed to Different pHs. Fishes. 2022; 7(5): 261. doi: 10.3390/fishes7050261
38. Esbaugh AJ, Brix KV, Grosell M. Na^+/K^+-ATPase isoform switching in zebrafish during transition to dilute freshwater habitats. Proceedings of the Royal Society B: Biological Sciences. 2019; 286(1903): 20190630. doi: 10.1098/rspb.2019.0630
39. McCormick SD, Regish AM, Christensen AK. Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. Journal of Experimental Biology. 2009; 212(24): 3994-4001. doi: 10.1242/jeb.037275
40. Yang WK, Hsu AD, Kang CK, et al. Intestinal FXYD12 and sodium-potassium ATPase: A comparative study on two euryhaline medakas in response to salinity changes. PLOS ONE. 2018; 13(7): e0201252. doi: 10.1371/journal.pone.0201252
41. Maiti AK, Paul G, Maity B, et al. Chromium III Exposure Inhibits Brain Na^+/K^+-ATPase Activity of *Clarias batrachus* L. Involving Lipid Peroxidation and Deficient Mitochondrial Electron Transport Chain Activity. Bulletin of Environmental Contamination and Toxicology. 2009; 83(4): 479-483. doi: 10.1007/s00128-009-9827-0
42. Gupta A, Sharma B. A study on Transaminases in Lindane Exposed Fish C. *punctatus*. Journal of Biomedical Research & Environmental Sciences. 2023; 4(6): 1100-1107. doi: 10.37871/jbres1773
43. Gupta A, Sharma B. Oxidative stress biomarkers in a living cell. MOJ Toxicol. 2023; 7(1): 38-43. doi: 10.15406/mojt.2023.07.00176
44. Gupta A. A Study on Analysis of Water Quality of Two Rivers Ganges and Yamuna. J Waste Manage Xenobio. 2023; 6(3): 000191.
45. Gupta A, Sharma B. A review on water pollution by γHCH (lindane) and its removal using nanomaterials. Journal of Toxicological Studies. 2023; 1(1): 195. doi: 10.59400/jts.v1i1.195
46. Lijnen P, Hespel P, Lommelen G, et al. Intracellular sodium, potassium and magnesium concentration, ouabain sensitive rubidium uptake and sodium efflux and Na^+/K^+ cotransport activity in erythrocytes of normal male subjects studied on two occasions. Methods Find Exp Clin Pharmacol. 1986; 8: 525-533.

Journal of Toxicological Studies 2024, 2(2), 1562.
https://doi.org/10.59400/jts.v2i2.1562

Article

Efficiency of heat-treated sepiolite in the adsorption of Cd, Zn, and Co from aqueous solutions: A low-cost approach for wastewater treatment

Ramin SamieiFard[1,2,*], Ahmad Landi[2], Saeid Hojati[2], Nahid Pourreza[3]

[1] Department of Ecosystem Science & Management, Pennsylvania State University, University Park, PA 16802, USA
[2] Department of Soil science, Shahid Chamran University of Ahvaz, Ahvaz 6135783151, Iran
[3] Department of Chemistry, Shahid Chamran University of Ahvaz, Ahvaz 6135783151, Iran
*** Corresponding author:** Ramin SamieiFard, rks5845@psu.edu

CITATION

SamieiFard R, Landi A, Hojati S, Pourreza N. Efficiency of heat-treated sepiolite in the adsorption of Cd, Zn, and Co from aqueous solutions: A low-cost approach for wastewater treatment. Journal of Toxicological Studies. 2024; 2(2): 1562. https://doi.org/10.59400/jts.v2i2.1562

ARTICLE INFO

Received: 25 July 2024
Accepted: 24 September 2024
Available online: 30 September 2024

Abstract: This study investigated the adsorption of Cd, Co, and Zn ions onto unmodified and heat-treated sepiolite, focusing on the effect of contact time, initial pH, and heat pretreatments. Kinetic experiments were conducted in triplicate, and equilibrium experiments indicated that Co^{2+} had the highest adsorption preference, followed by Zn^{2+} and Cd^{2+}. The adsorption efficiency for Co^{2+} significantly increased with higher initial pH, whereas Zn^{2+} and Cd^{2+} showed optimal adsorption at lower pH levels. Heat-treated sepiolite at 250 °C exhibited a higher surface area and adsorption capacity in comparison with unmodified and 150 °C-treated sepiolite, which indicated the importance of heat pretreatment. The pseudo-second-order kinetic model better described the adsorption process, and it was confirmed chemisorption as the rate-limiting step. By increasing the contact time, adsorption rates enhanced, with equilibrium achieved within 480 min for all systems. Higher initial solute concentrations led to an increase in adsorption processes, with Co ions consistently showing higher adsorption efficiency in competitive multi-ionic solutions. Adsorption percentages varied with pH and thermal treatment, indicating the importance of these parameters in optimizing sepiolite's adsorption capacity for heavy metal removal.

Keywords: sepiolite; kinetic models; heavy metals; adsoption

1. Introduction

Ever-increasing industrialization brings about essential decisions to protect the environment and specifically conserve water resources by developing cost-effective wastewater treatment methods [1]. The application of clay and modified clays to remove toxic heavy metals from wastewater has attracted attention in recent decades [2,3]. Due to the high adsorption capacity of clays and their easy accessibility in nature, clay minerals have received more attention from researchers for adsorption experiments [4]. To improve their adsorption capacity, there are various methods like heat and acid treatment that are applied by many researchers [5]. The removal of toxic heavy metal ions and their lethal effects on the environment and industrial wastewater using different adsorbents has received more attention recently [6]. Sepiolite ($Si_{12}O_{30}Mg_8(OH)_4(H_2O)_4{\cdot}8H_2O$) is a natural, fibrous clay mineral with fine microporous channels [7]. The Sepiolite's high adsorption capacity suggests its effectiveness in removing heavy metals from polluted/waste waters. Sepiolite in aqueous suspensions can acquire a surface charge through the adsorption and desorption of potential-determining ions, particularly protons, on its surfaces [8]. Sepiolite's crystal-chemical features allow it to retain heavy metals through adsorption and/or cation exchange reactions [9].

The adsorption process can occur on the oxygen ions of the tetrahedral sheets, on the water molecules at the edges of the octahedral sheet, and on Si-OH (silanol groups) along the direction of the fibers, whereas cationic exchanges occur by substituting the solvated ions inside the channels and/or inside the octahedral on the edges of the channels [10,11]. This unique structure leads to the study of this mineral for many experimental applications, such as membranes for ultrafiltration, catalyst carriers [12–14], fillers in polymer composites [15], adsorbents for organic molecules and heavy metals [16], and inorganic templates [17].

Numerous studies have explored the efficient use of sepiolite, both natural (without heat pre-treatment) and modified (heat pre-treatment), for the removal of some selected heavy metals, including Cd, Zn, and Co, from industrial and municipal wastewater [18–20]. Specifically, the study assessed the effectiveness of both unmodified and heat-treated sepiolite in removing heavy metals from aqueous solutions, considering various factors such as solution pH, heavy metal concentration, and contact time (ranging from 5 to 2880 min). To gain a deeper understanding of the adsorption mechanism and kinetics, the experimental data was analyzed using the intraparticle diffusion, pseudo-first order, and pseudo-second order kinetic models. This analysis will help elucidate the rate-limiting step and the overall adsorption process involved in heavy metal removal by both unmodified and heat-pretreated sepiolite.

2. Material and methods

2.1. Materials

2.1.1. The sorbent

The sepiolite sample used in this study was obtained from a mine near the town of Fariman in the Khorasan region of Iran. All samples were ground using an electric mill, processed by a ball mill, and then sieved to obtain particle sizes smaller than 0.05 mm (<53 μm). By obtaining this size of sepiolite samples, all the sepiolite required for use as an adsorbent to remove Cd, Zn, and Co from aqueous solutions was saturated with calcium ions [21].

2.1.2. Chemicals

For the saturation process, a 0.5 M $CaCl_2$ solution was prepared using $CaCl_2$ from Merck, Darmstadt, Germany. 55.45 g of $CaCl_2$ dissolved in one liter of deionized distilled water to make this concentration of calcium. We ought to saturate all surfaces of this mineral with Ca^{2+} to start the sorption and desorption of heavy metals. So 5 g of sieved minerals were taken in centrifuge tubes, and 25 ml of $CaCl_2$ 0.5 M were added to them, and then the samples were shaken at 350 rpm for 30 min. After that, the suspensions were centrifuged at 3000 rpm for 10 min, and the limpid solutions of all these tubes were gathered to measure the EC. These steps were repeated until the EC of the clear solutions reached a stable value. Then the saturated samples in the extremity of tubes were gathered and were taken into the oven for 72 h at 75 °C to be dried completely, and then we started flouring and ball-milling again, same as sieving before, to obtain the 0.05 mm (<53 μm) sizes of mineral.

Sorption studies of cadmium (Cd), zinc (Zn), and cobalt (Co), along with the desorption of magnesium (Mg), were investigated using batch experiments. The adsorbent that is used in this study is prepared in three temperature treatments, including room temperature (22.5 ± 0.2 ℃), 150 ℃, and 250 ℃ heat pre-treatments. Sample pre-treatments of 150 ℃, and 250 ℃ of adsorbents were taken in an electrical kiln for 4 h. Since the kiln temperature was raised to the targeted warmth, the removal of the present (existent) zeolitic water, which was bound in the mineral structure, changed the adsorption capacity of this mineral for heavy metals in aqueous solutions. Therefore, the needed amounts of this mineral were taken in the kiln for 4 h at 150 ℃ and the same was done for the heat pre-treatment at 250 ℃. After this period, the kiln was cooled to room temperature, and the samples were extracted and stored in a desiccator to prevent the possible adsorption of air moisture on the surfaces of the heat-treated (modified) sepiolite [22].

To create multi-ion solutions, the following metal concentrations were used: 20 ppm Cd, 20 ppm Co, and 20 ppm Zn for the 60-ppm solution; 80 ppm Cd, 80 ppm Co, and 80 ppm Zn for the 240-ppm solution; and 160 ppm Cd, 160 ppm Co, and 160 ppm Zn for the 480-ppm solution. The materials used to make these solutions were $CdCl_{2\cdot 2½}H_2O$ obtained from BDH Company (England) and $CoCl_2$ and $ZnCl_2$ from Merck, Darmstadt, Germany. To make the desired solutions with high punctuality, at first, 1000 ppm concentrations of each of the heavy metals as a stock solution in a single component solution were made. To prepare 1000 ppm solutions of Cd, Co, and Zn, 2.031 g of $CdCl_{2\cdot 2½}$H2O, 2.084 g of $ZnCl_2$, and 2.203 g of $CoCl_2$ were weighed, and each was dissolved in 1 liter of deionized water to obtain 1000 ppm solutions of these heavy metals. All solutions were made by using deionized water. In the presence of some Zn^{+2} complexes, such as the zincate complex [Zn $(OH)_4{}^{2-}$], we had to reduce the stock solution pH by using the HCl 0.5N to prevent the deposition of those complexes and to get a sleek stock solution. This was due to the low solubility of $ZnCl_2$ in deionized water, and the Ksp of Zn^{2+} is close to 1.2×10^{-17}. Finally, the produced multi-component solutions were conducted at pH 4 and pH 5. For adjusting the solutions at these pHs, if necessary, HCl 0.2N and NaOH 0.2N were used [23].

2.2. Methods

2.2.1. Batch experiments

A batch experiment is a simple method commonly used to assess the adsorptive capacities of natural and modified sorbents. Besides the ease of using this technique, it helps to provide important information about the efficiency of the sorbent in removing adsorptive species under static conditions [24]. Thus, batch techniques are widely used for environmental studies, especially for the removal of heavy metals from municipal and industrial wastewater [25].

To start this part of the experiments, 0.3 g of adsorbent was precisely weighted for each unmodified and the heat-pretreated sepiolite into polyethylene containers, then 30 cc of multi-component solutions were added to them (1:100 solid to liquid ratio) with three repetitions for each anticipated temperature treatment at the three specified concentrations: 60 ppm, 240 ppm, and 480 ppm, comprising adequate amounts of cadmium, cobalt, and zinc. All the above-mentioned stages were conducted for eleven contact times, including: 5, 10, 20, 30, 60, 120, 240, 480, 720, 1440, and 2880 min.

2.2.2. Kinetic experiments

Kinetic experiments were done by mixing 0.3 g of weighted samples from modified and unmodified sepiolite by heat treatments in polyethylene containers at three repetitions with 30 cc of multi-component solutions. So, we had 27 suspension containers for each contact time, which included 9 containers for adsorbents without heat treatment, 9 containers for the heat treatment of 150 °C, and even 9 containers for the heat treatment of 250 °C. Total sorption and desorption processes by eleven contact times, including 5, 10, 20, 30, 60, 120, 240, 480, 720, 1440, and 2880 min, were investigated. So, we had 298 suspension containers for the kinetic experiments. Each of the 27 containers related to each experimental contact time was shaken at an agitation speed of 350 rpm in the shaker for any of the mentioned contact times. Then these suspensions were centrifuged for about 10 min at 3000 rpm to separate the adsorbent from multi-ionic solutions, and after that, the clear solutions were passed through filter paper, poured into new containers, and prepared for atomic adsorption readings (model: SavantAA-GBC). Several kinetic models are available to investigate the adsorption kinetic mechanisms and potential rate-controlling steps such as chemical reactions and mass transport processes [26].

The adsorbed metal concentrations were determined by the difference between the initial and final concentrations in the aqueous solutions after the adsorbing process, using the equation below.

$$Q_e = \frac{C_0 - C_e}{m} . V \quad (1)$$

where the Q_e is the amount of metal absorbed per mass unit of adsorbent. The C_0 and C_e are the initial and equilibrium concentrations ($mg.kg^{-1}$), respectively, m is the mass of the adsorbent (g) and V is the volume of the solution (L) [27].

In this experiment three kinetics models including Pseudo-first and Pseudo-second order model and Intraparticle diffusion model have been investigated to examine the controlling mechanism of the adsorption processes and to understand the behavior of the adsorbent and to test the experimental data.

The Pseudo-first order model that was presented by Lagergren, is expressed as follows:

$$\frac{d_q}{d_t} = k_1(q_e - q_t) \quad (2)$$

where the q_e and q_t are the amounts of adsorbed ions onto the sepiolite at equilibrium and at any time t, respectively. The K_1 is the rate constant of first-order adsorption [28].

The linear form of this equation is expressed as follows:

$$ln(q_e - q_t) = lnq_e - k_i . t \quad (3)$$

The plots of ln (q_e–q_t) against t, give a linear relationship from which k_1 and q_e values can be determined from the slope and intercept of this equation, respectively [29].

The pseudo-second order model was also used to describe the sorption of metal ions. The equation for the reaction is expressed as follows:

$$\frac{d_q}{d_t} = k_2(q_e - q_t)^2 \tag{4}$$

where k_2 is rate constant for pseudo-second-order adsorption (g.mg^{-1} min). q_e and q_t are the sorption capacity (mg/g) of ions at equilibrium and at a time t, respectively [30].

Also, the linear form of this equation is represented as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 . q_e} + \frac{t}{q_e} \tag{5}$$

The plots of t/q_t against t give a linear relationship in which k_1 and q_e values can be calculated from the slope and intercept of this equation, respectively [31].

The sorption data was also analyzed in terms of Intraparticle diffusion mechanism that can be described as following Equation (6):

$$q_t = k_i . \sqrt{t} + C \tag{6}$$

where q_t (mg. g^{-1}) is the concentration of cations absorbed at time t by the adsorbent, and k_i (mg. g. t$^{-0.5}$) is the intraparticle rate constant [31].

3. Result and discussion

This study delves into the molecular structure and composition of sepiolite, a mineral characterized by its unique fibrous arrangement. Sepiolite's structure consists of alternating sheets of tetrahedra and octahedra, forming a network of channels that house coordinated water molecules bonded to magnesium centers. Additionally, zeolitic water resides within the mineral's porous channels (**Figure 1a**).

Transmission Electron Microscopy (TEM) images revealed the elongated, needle-like morphology of sepiolite, confirming its fibrous crystal structure and any potential defects (**Figure 1b**). X-ray Diffraction (XRD) patterns further validated the mineral's phase identity, providing insights into its crystalline arrangement and purity (**Figure 1c**). Energy Dispersive X-ray Spectroscopy (EDAX) analysis confirmed the presence of magnesium, silicon, and other key elements within the sepiolite structure (**Figure 1d**).

By combining TEM imaging, XRD, and EDAX, this comprehensive study provides a detailed understanding of sepiolite's structural and compositional characteristics. This knowledge is essential for evaluating its adsorption properties and exploring its potential applications.

The kinetic experiments were done in triplicate under the same conditions for each heat-treated sepiolite and for any of the contact times studied. The remaining concentrations of metal ions in the aqueous phase were computed from the averages of experimentally determined adsorbate concentrations after adsorption, using Equation (1). These values were then used in the analysis of data and for graph fitting by some kinetic models.

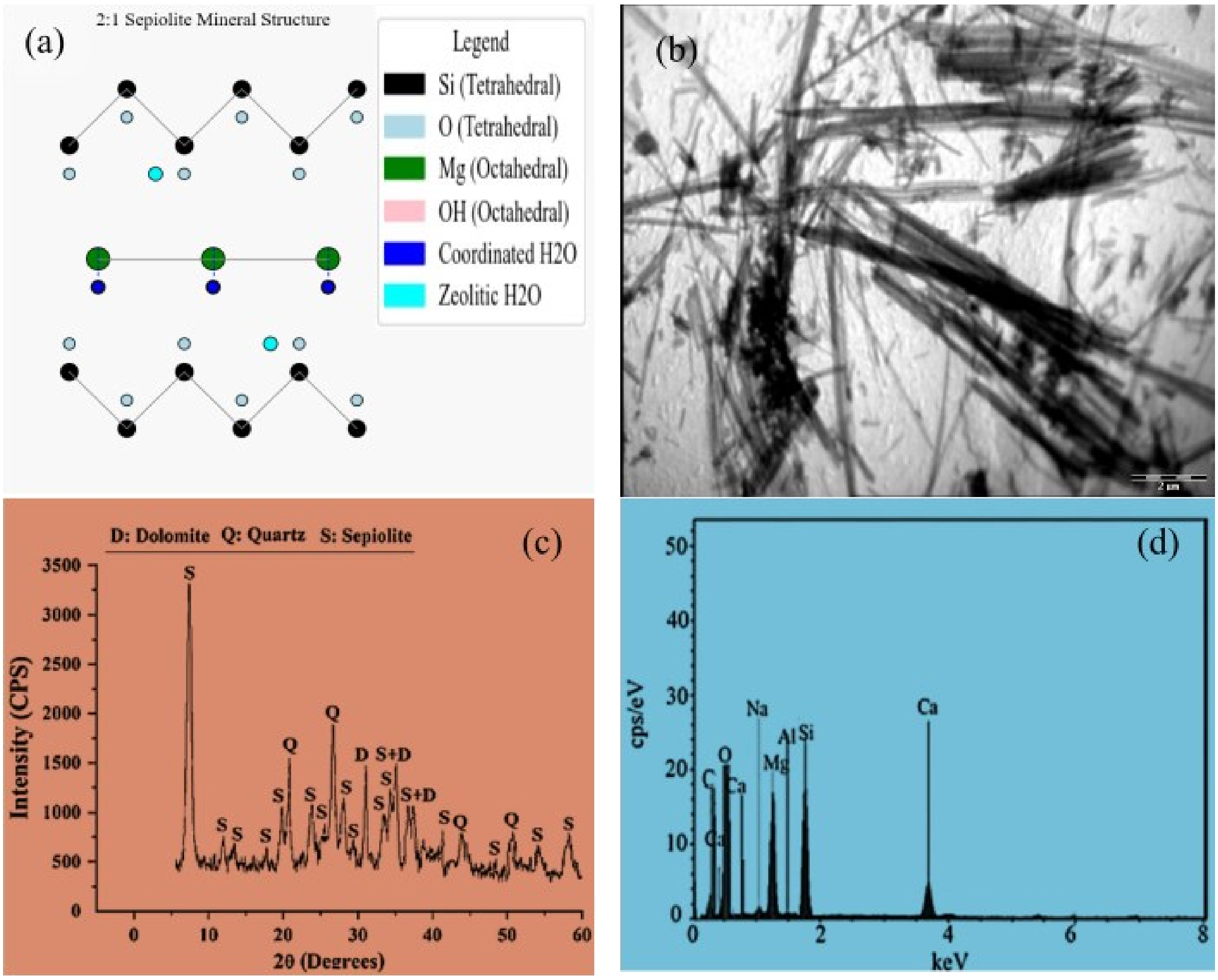
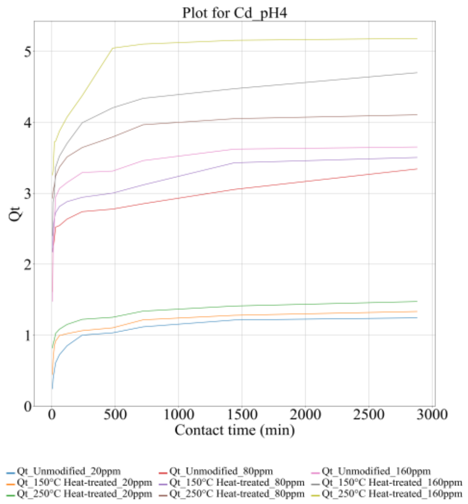
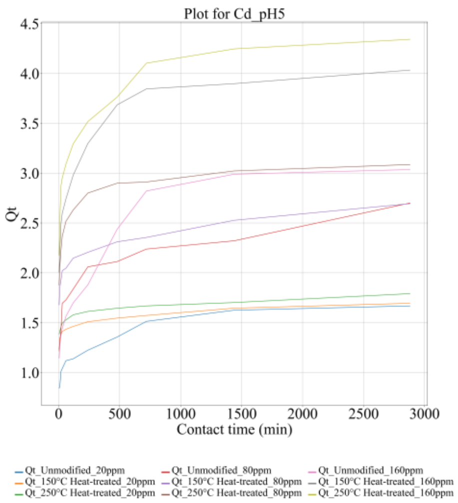


Figure 1. (a) 2:1 mineral structure; **(b)** TEM images; **(c)** XRD pattern; **(d)** EDAX spectrum of applied sepiolite.

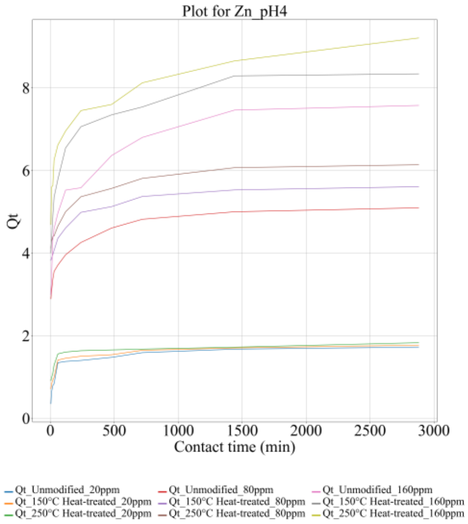
Equilibrium experiments were done, and the kinetic curves obtained for the sorption of Cd, Co, and Zn by sepiolite are presented. **Figure 2** shows the heavy metal adsorption on unmodified and modified (heat-treated) sepiolite samples in various solution concentrations and different pH values. Also, **Figure 3** shows the adsorption percentages of the investigated heavy metals on unmodified and modified sepiolite samples. The number of milligrams absorbed per gram of unmodified and heat-treated sepiolite as an adsorbent versus the number of milligrams at equilibrium per volume of solution is illustrated for Cd, Co, and Zn cations. Equilibrium was strongly favorable for Co^{2+}. According to the equilibrium kinetics, the selectivity series is $Co^{2+} > Zn^{2+} > Cd^{2+}$. The adsorption capacity of natural and heat-treated sepiolite was higher for cobalt than for the other two cations in all experiments and both studied pH solutions. It could be attributed to the atomic and adsorbing properties of cobalt that this element formed fewer complexes in multi-component solutions at pH 4 and 5 than other existing elements. And the adsorbing properties of natural and modified sepiolite refer to the crystalline structure and morphology of this mineral to substituting the existing cations in liquid phase with the leached Mg^{2+} from octahedral sheets under acidic conditions [32,33].



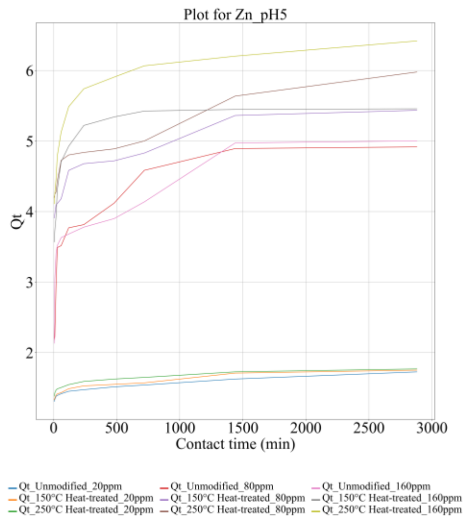
(a)



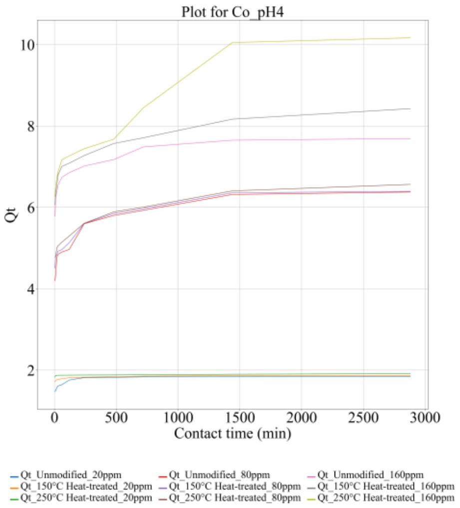
(b)



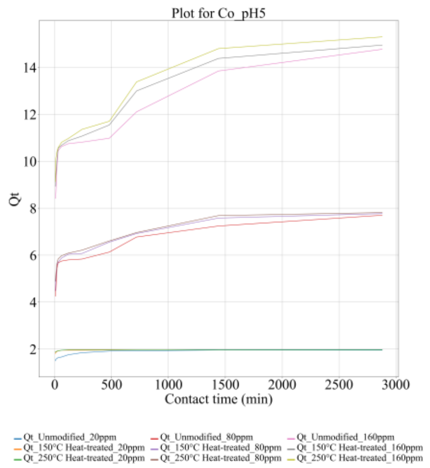
(c)



(d)



(e)



(f)

Figure 2. Heavy metal adsorption on unmodified and modified sepiolite samples under different solution concentrations and pH values: **(a)** Cd at pH 4; **(b)** Cd at pH 5; **(c)** Zn at pH 4; **(d)** Zn at pH 5; **(e)** Co at pH 4; **(f)** Co at pH 5.

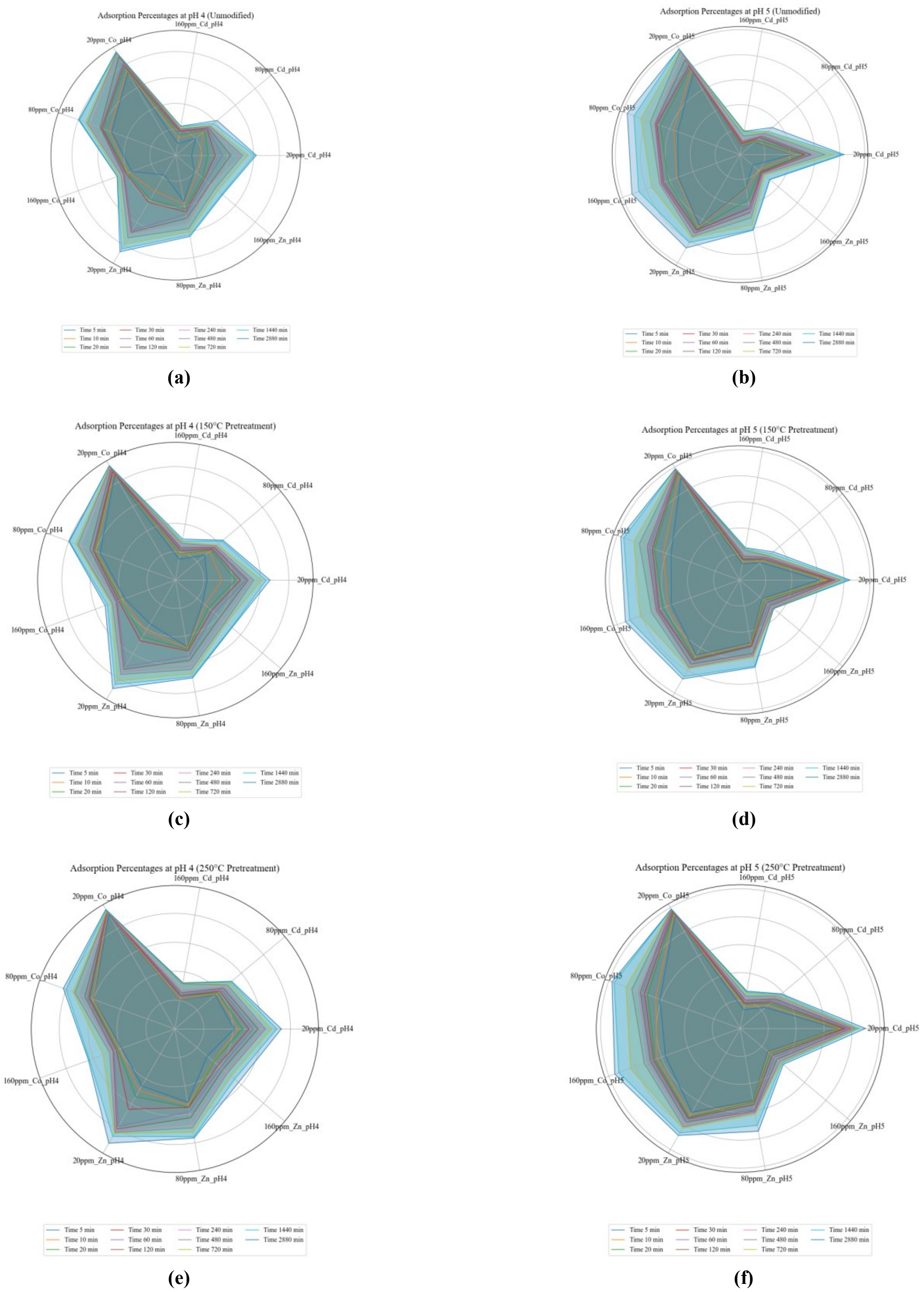


Figure 3. Adsorption percentages of the investigated heavy metals on unmodified and modified sepiolite samples: **(a)** Unmodified sepiolite at pH 4; **(b)** Unmodified sepiolite at pH 5; **(c)** 150°C heat-pretreated sepiolite at pH 4; **(d)** 150°C heat-pretreated sepiolite at pH 5; **(e)** 250°C heat-pretreated sepiolite at pH 4; **(f)** 250°C heat-pretreated sepiolite at pH 5.

3.1. Effect of initial pH value

Adsorption usually directly depends on the amounts of H^+ and OH^- in solutions. The pH values have an important effect on the surface charge of an adsorbent, such as protonation and deprotonation of surface functional groups, and affect the interaction between chemical species of metal ions present in the solution [34]. A decrease in pH value increased the competition between H^+ and other cationic species for adsorptive sites on the surface and edges of sepiolite. On the other hand, interaction of positive charges with sepiolite at lower pHs should be decreased [35]. As can be seen in **Figures 1** and **2**, the efficiency removal of cobalt ions increased considerably when the pH value of initial solutions increased from 4 to 5 in all concentrations of multi-component studied solutions, and it occurs for Zn and Cd in multi-component solutions at concentrations less than 60 ppm. Although the adsorption of zinc and cadmium was decreased by increasing the initial pH values, it could justify that pH 4 was not the optimum adsorption pH value for these two elements. Similarly, Guerra et al. [36] have investigated the absorption of arsenic (V) on sepiolite and modified sepiolite in the pH range of 1.0–8.0. They reported that the maximum values of adsorption of arsenic ions were observed in pH < 4. Factors such as concentration, metal recovery procedures, the formation of soluble metal complexes, and subsequently their stability in aqueous solutions are considerably affected by changes in pH values. In this study, it's been clearly demonstrated that the surface charge of the adsorbent can be modified by charging the pH of the solution, and this parameter affected the chemical species in the solution [37]. The reason for diminished adsorption capacity of zinc and cadmium by natural and heat-treated sepiolite at pH 5 in comparison with pH 4 at the high concentrations is the positively charged cationic species that are present in solution and the competition between the excess OH groups in multi-component solutions. On the top side, higher acid concentrations suppress the hydrolysis of the Zn and Cd ions (See **Figures 1** and **2**). The analysis of contact time and Q_t data across multiple pH values and heavy metal concentrations revealed consistent trends in the behavior of the measured parameters. Each plot represents a clear relationship between contact time and the quantity of substance adsorbed (Q_t), with varying slopes and intercepts indicating different kinetic behaviors across the datasets. This consistency across plots facilitates straightforward comparisons between different concentrations and various heat treatments, highlighting any outliers or anomalies that warrant further investigation.

The observed trends suggested that the adsorption kinetics follow a predictable pattern, with most investigated batch systems and displaying a linear or near-linear relationship between contact time and Q_t. This indicates that the adsorption process reaches equilibrium within the studied time frames. Future studies should focus on extending the contact time to explore the possibility of reaching a new equilibrium or identifying any potential secondary kinetics that may occur beyond the observed timeframe. Additionally, replicating this analysis with different substances (e.g., various clay minerals or organo-minerals) could validate the general applicability of the observed trends and further our knowledge of the adsorption mechanisms.

3.2. The effect of heat pre-treatment

The effect of heat pre-treatment on sepiolite was investigated, and it was observed that the specific surface area of sepiolite samples that were heated before application as an adsorbent dominantly increased. Sepiolite heated at 250 ℃ exhibits a larger surface area compared to sepiolite heated at 150 ℃. As observed across all systems, the adsorption capacity of natural and heat-treated sepiolite follows the order: SEP 250 ℃ > SEP 150 ℃ > natural SEP. Natural sepiolite contains a significant amount of water in its structure, which is located mostly in channels and is known in terms of zeolitic water [38]. This kind of water can be considered free since it's exposed to heat treatment. By heat treating this mineral, the zeolitic water will be lost, and the specific surface area of this mineral should be increased in comparison to natural sepiolite [39]. The adsorption capacity of heat-treated sepiolite was considerably greater than that of natural sepiolite, e.g., up to 90% of Co ions in the 60 ppm multi-component solutions adsorbed by heat-treated minerals at both pHs 4 and 5 during a short time, and the needed time for adsorbing this amount of Co by natural sepiolite at the same conditions was about 48 h.

Table 1. the results of kinetic adsorption models by natural sepiolite in multi-component solutions at pH 4 and 5.

Temperature treatment	**pH**	**Concentration (ppm)**	**Element**	**Intraparticle diffusion**			**Pseudo-first order**			**Pseudo-second order**		
				K_i	C	R^2	K_1	Q_e	R^2	K^2	Q_e	R^2
22.5 ± 0.2	4	20	Cd	0.018	0.5	0.752	0.001	0.584	0.907	0.021	1.256	0.999
	4	20	Co	0.006	1.151	0.623	0.001	0.149	0.658	0.223	1.845	1
	4	20	Zn	0.022	0.811	0.638	0.001	0.674	0.851	0.027	1.730	0.999
	4	80	Cd	0.023	2.199	0.728	0.00	1.144	0.887	0.026	3.311	0.996
	4	80	Co	0.042	4.546	0.853	0.00	0.054	0.894	0.044	6.410	0.999
	4	80	Zn	0.042	3.289	0.826	0.001	1.496	0.862	0.039	5.128	0.999
	4	160	Cd	0.029	2.461	0.543	0.001	0.854	0.873	0.053	3.663	0.999
	4	160	Co	0.032	6.304	0.794	0.001	1.141	0.921	0.081	7.752	0.999
	4	160	Zn	0.084	3.965	0.821	0.001	3.062	0.945	0.024	7.633	0.998
Temperature treatment	pH	Concentration (ppm)	Element	Intraparticle diffusion			Pseudo-first order			Pseudo-second order		
				K_i	C	R^2	K_1	Q_e	R^2	K^2	Q_e	R^2
22.5 ± 0.2	5	20	Cd	0.016	0.931	0.895	0.002	0.733	0.996	0.023	1.680	0.998
	5	20	Co	0.009	1.601	0.750	0.002	0.216	0.566	0.125	1.960	1
	5	20	Zn	0.007	1.341	0.968	0.00	0.414	0.976	0.037	1.718	0.998
	5	80	Cd	0.025	1.432	0.875	0.00	1.200	0.940	0.018	2.660	0.993
	5	80	Co	0.057	4.925	0.847	0.001	2.609	0.969	0.024	7.692	0.997
	5	80	Zn	0.05	2.814	0.755	0.001	1.670	0.893	0.029	4.975	0.998
	5	160	Cd	0.041	1.26	0.890	0.001	1.499	0.872	0.016	3.096	0.997
	5	160	Co	0.112	9.078	0.908	0.00	5.596	0.977	0.019	14.705	0.995
	5	160	Zn	0.043	2.997	0.783	0.001	1.831	0.866	0.021	5.050	0.996

Table 1 shows the statistical analysis for the kinetic adsorption results of unmodified sepiolite in multi-ionic solutions at pH 4 and 5. The adsorption of Cd, Co,

and Zn was evaluated at three different concentrations (20 ppm, 80 ppm, and 160 ppm) and analyzed using the mentioned kinetic models, including intraparticle diffusion, pseudo-first order, and pseudo-second order. At pH 4, the pseudo-second-order model provided the highest R^2 values across all concentrations and ions, indicating that this model best describes the adsorption process. For instance, at 20 ppm, the R^2 values for Cd, Co, and Zn were 0.999, 1, and 0.999, respectively. This suggests that the rate-limiting step might involve chemisorption. Similarly, at pH 5, the pseudo-second-order model again showed superior fitting, with R^2 values consistently close to 1. For instance, at 80 ppm, the R^2 values for Cd, Co, and Zn were 0.998, 0.998, and 0.998, respectively. The intraparticle diffusion model, while not the best fit, indicated that intraparticle diffusion might also play a role, especially for higher concentrations.

Table 2. the results of kinetic adsorption models by 150 °C heat treated sepiolite in multi-component solutions at pH 4 and 5.

Temperature treatment	**pH**	**Concentration (ppm)**	**Element**	**Intraparticle diffusion**			**Pseudo-first order**			**Pseudo-second order**		
				k_i	C	R^2	K_1	Q_e	R^2	K_2	Q_e	R^2
150 °C heat treated	4	20	Cd	0.013	0.758	0.688	0.001	0.478	0.902	0.027	1.335	0.998
	4	20	Co	0.002	1.757	0.752	0.001	0.080	0.774	0.365	1.866	1
	4	20	Zn	0.018	0.990	0.674	0.001	0.608	0.798	0.033	1.770	0.999
	4	80	Cd	0.021	2.516	0.833	0.001	0.905	0.943	0.035	3.508	0.998
	4	80	Co	0.038	4.736	0.890	0.001	1.445	0.940	0.048	6.451	0.999
	4	80	Zn	0.037	4.008	0.833	0.001	1.298	0.912	0.051	5.618	0.999
	4	160	Cd	0.039	3.013	0.771	0.00	1.464	0.855	0.036	4.695	0.999
	4	160	Co	0.042	6.450	0.895	0.00	1.816	0.965	0.044	8.475	0.999
	4	160	Zn	0.082	4.891	0.788	0.001	2.821	0.921	0.033	8.403	0.999
Temperature treatment	pH	Concentration (ppm)	Element	Intraparticle diffusion			Pseudo-first order			Pseudo-second order		
				k_i	C	R^2	K_1	Q_e	R^2	K_2	Q_e	R^2
150 °C heat treated	5	20	Cd	0.007	1.342	0.831	0.001	0.320	0.966	0.053	1.692	0.999
	5	20	Co	0.009	1.601	0.750	0.001	0.050	0.663	0.695	1.976	1
	5	20	Zn	0.008	1.363	0.944	0.00	0.378	0.959	0.042	1.751	0.999
	5	80	Cd	0.017	1.864	0.899	0.001	0.786	0.976	0.030	2.680	0.998
	5	80	Co	0.056	5.156	0.869	0.001	2.375	0.955	0.029	7.812	0.998
	5	80	Zn	0.03	4.00	0.916	0.001	1.324	0.952	0.037	5.464	0.998
	5	160	Cd	0.039	2.395	0.806	0.00	1.388	0.788	0.035	4.048	0.999
	5	160	Co	0.113	9.435	0.938	0.001	5.296	0.970	0.021	15.151	0.997
	5	160	Zn	0.035	4.125	0.627	0.002	0.774	0.828	0.120	5.494	1

Tables 2 and **3** present the kinetic adsorption results of heat-treated sepiolite, respectively, for 150 °C and 250 °C. The pseudo-second-order model continued to exhibit the highest R^2 values, indicating its suitability in describing the adsorption kinetics of heat-treated sepiolite. At pH 4 and 80 ppm, the R^2 values for Cd, Co, and Zn were 0.998, 0.999, and 0.999, respectively, for sepiolite treated at 150°C. Similarly, for heat-treated sepiolite at 250 °C, the R^2 values were 0.999 for Cd, Co, and Zn,

confirming the dominance of the pseudo-second-order kinetics. Interestingly, the heat treatment seemed to enhance the adsorption capacity (Q_e) of sepiolite, specifically by increasing the heat treatment temperature and by increasing the concentrations. For example, at 160 ppm and pH 4, the Q_e values for Cd increased from 3.663 mg. g^{-1} in natural sepiolite to 5.236 mg. g^{-1} in sepiolite treated at 250 °C. This enhancement suggests that heat treatment modifies the sepiolite structure, potentially increasing the number of active sites available for contaminant adsorption. The intraparticle diffusion model's k_i values also increased with heat treatment, indicating improved diffusion rates within the sepiolite matrix. Overall, these results highlighted the significant impact of heat treatment and pH on the adsorption kinetics of heavy metals by Iranian sepiolite.

Table 3. the results of kinetic adsorption models by 250 °C heat treated sepiolite in multi-component solutions at pH 4 and 5.

Temperature treatment	**pH**	**Concentration (ppm)**	**Element**	**Intraparticle diffusion**			**Psudo-first order**			**Psudo-second order**		
				k_i	C	R^2	K_1	Q_e	R^2	K_2	Q_e	R^2
	4	20	Cd	0.012	0.935	0.842	0.001	0.476	0.926	0.031	1.473	0.998
	4	20	Co	0.001	1.859	0.655	0.00	0.054	0.894	0.344	1.912	1
	4	20	Zn	0.015	1.186	0.602	0.001	0.495	0.787	0.041	1.818	0.999
	4	80	Cd	0.023	3.121	0.832	0.001	0.809	0.926	0.061	4.115	0.999
250 °C heat treated	4	80	Co	0.036	4.867	0.935	0.001	1.567	0.988	0.04	6.579	0.999
	4	80	Zn	0.041	4.347	0.859	0.001	1.496	0.896	0.046	6.173	0.999
	4	160	Cd	0.040	3.560	0.785	0.001	1.120	0.792	0.057	5.236	0.999
	4	160	Co	0.078	6.337	0.944	0.001	3.807	0.952	0.019	10.309	0.995
	4	160	Zn	0.077	5.634	0.853	0.00	3.560	0.890	0.026	9.174	0.998
Temperature treatment	pH	Concentration (ppm)	Element	Intraparticle diffusion			Psudo-first order			Psudo-second order		
				k_i	C	R^2	K_1	Q_e	R^2	K_2	Q_e	R_2
	5	20	Cd	0.007	1.446	0.873	0.00	0.339	0.916	0.054	1.782	0.999
	5	20	Co	0.001	1.915	0.467	0.001	0.026	0.573	1.566	1.976	1
	5	20	Zn	0.007	1.440	0.910	0.00	0.317	0.938	0.058	1.770	0.999
	5	80	Cd	0.020	2.221	0.722	0.001	0.687	0.833	0.057	3.096	0.999
250 °C heat treated	5	80	Co	0.054	5.328	0.900	0.001	2.279	0.939	0.030	7.874	0.998
	5	80	Zn	0.033	4.243	0.944	0.00	2.063	0.951	0.026	5.952	0.997
	5	160	Cd	0.038	2.681	0.819	0.001	1.378	0.903	0.033	4.367	0.999
	5	160	Co	0.115	9.686	0.940	0.00	5.425	0.959	0.022	15.384	0.997
	5	160	Zn	0.043	4.571	0.764	0.00	1.586	0.813	0.050	6.410	0.999

3.3. Effect of contact time

The effect of contact time on the adsorption of Cd, Co, and Zn was analyzed kinetically over a range of 5 min to 48 h. The parameter of contact time is an important factor because it can reflect the adsorption kinetics of an adsorbent for a given initial concentration of the adsorbate [40]. The experimental data showed that by lapsing the contact time from 5 min to 48 h, the adsorption of heavy metals on natural and heat-treated sepiolite samples increased. Although the sorption equilibrium for each system was different from the others, the equilibrium was achieved within the initial 480 min (**Figures 1** and **2**). clearly indicates that the sorption of Cd, Co, and Zn at different concentrations in all systems increased instantly at the initial stage of the adsorption process and then increased gradually until the equilibrium was reached and after that remained constant. Similar results have been reported in research done by Bektas et al. [41], when they studied the removal of Lead from aqueous solutions by natural sepiolite [41]. Shirvani et al., indicated that by increasing contact time between the mineral adsorbent and heavy metals, the adsorption was increased [42].

3.4. Effect of initial solute concentration

The removal amounts of Cd, Co and Zn were calculated in term of q_t and the value of q_t against the contact times for all multi-component solutions showed that by increasing the initial concentrations from 60 ppm to 240 ppm and then to 480 ppm, the amounts of adsorbed heavy metals on adsorbent increased. It was clear that, due to the high adsorption capacity of sepiolite, by increasing the solute concentrations, the sorption equilibrium will be reached at higher qt values. However, by increasing concentrations in initial solutions, the interactions between cationic species with each other and competitions for adsorption sites will be increased. Similar results have been reported by other researchers [43].

3.5. The effect of heavy metals competition in aqueous solutions

Experimental data showed that the adsorption of Cobalt ions by natural and heat-treated sepiolite at all concentrations and for both investigated pH solutions was greater than the adsorption of Cd and Zn. The reason for this difference must be attributed to the atomic characteristic of Co and to the adsorption properties of sepiolite for adsorbing cations in multi-component solutions [44]. The Co with atomic number of 27, has smaller cationic size compared to Zn and Cd, with atomic numbers of 30 and 48, respectively. Under the same conditions, the quantity of absorbed heavy metal by natural and modified sepiolite layer increases for smaller cations [45]. This increase can be supported by this hypothesis that small cations are adsorbed in channels and on the exchangeable structural sites of the sepiolite [46]. Similar results have been reported by Brigatti et al. [47], where they studied the removal of heavy metals Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} by sepiolite and they showed that the amount of heavy metal sorbed by sepiolite layer increases for smaller cations [47]. Also, Kocaoba and Akyuz, studied the effect of pH, concentration, stirring time, stirring speed and sorption amount on adsorption, thus they indicated that the adsorption of Co by natural sepiolite was more than Ni, at all those factors [48].

3.6. Adsorption percentages

As can be seen in **Figure 3**, the adsorption experiments conducted under various conditions revealed distinct adsorption efficiencies for the sepiolite mineral. At pH 4, the unmodified sepiolite showed varying adsorption percentages for Cd, Co, and Zn, demonstrating a gradual increase over time. The treatment at 150 ℃ significantly enhanced the adsorption capacity, particularly for Co and Zn, indicating a possible modification of the mineral's surface properties that facilitated better adsorption. The 250 °C pretreatment, however, showed a different trend, with the adsorption percentages for Cd remaining relatively high while those for Co and Zn plateaued, suggesting that higher temperatures might lead to structural changes in the sepiolite, affecting its adsorption behavior differently for each metal. These results underscore the importance of both pH and thermal pretreatment in optimizing the adsorption efficiency of sepiolite for heavy metal removal.

The varying adsorption efficiencies observed under different conditions highlight the complex interplay between pH, temperature, and the mineral's adsorption capacity. The increased efficiency at pH 4 and 150 °C suggests that moderate thermal activation enhances the active sites available for adsorption, possibly by increasing the surface area or altering the surface chemistry to favor heavy metal interaction. However, the plateau observed at 250 °C indicates a potential limit to thermal activation, beyond which structural integrity might be compromised, reducing the number of effective adsorption sites. This finding aligns with previous studies that suggest optimal adsorption conditions are specific to the type of heavy metal and the physicochemical properties of the adsorbent [49]. Future research could further elucidate the mechanisms behind these observations, providing deeper insights into optimizing adsorption processes for environmental remediation.

3.7. Kinetic analysis

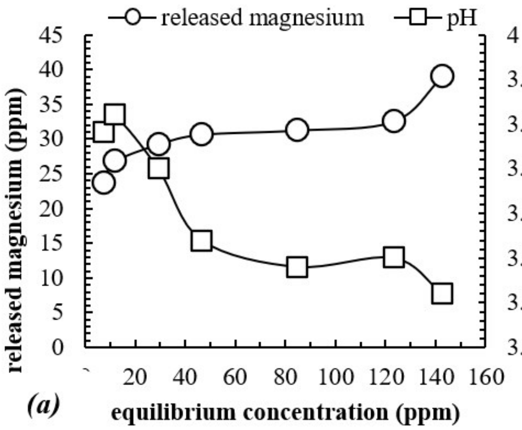
Sorption data have been interpreted via Pseudo-first and Pseudo-second order and the Intra-particle diffusion kinetic models. From the testing of plots which, q_t versus $t^{1/2}$, ln (q_e−q_t) versus t and (t/q_t) versus *t*, the rate constants k_i, k_1, and k_2, and correlation coefficients are calculated in **Tables 1–3** for the unmodified, 150 °C, and 250 °C heat treated sepiolite, respectively and a good correlation coefficient (R^2) obtained for the pseudo-second order kinetic model. It was reported that the Pseudo-second order model with the ($R^2 \geq 0.999$) yields the best linearity model than the Pseudo-first and the Intra-particle diffusion model as R^2 values are compared [30,50].

The adsorption kinetic data were analyzed by an intraparticle diffusion model to determine the rate and the quantity of adsorbing cations in the adsorption process. Furthermore, the Intra-particle diffusion kinetic model suggests that the adsorption process could be controlled by the internal diffusion with a partial effect of the external diffusion [51]. The fitted data via this model showed correlation coefficients mostly less than 0.9 and revealed the fact that the Intraparticle diffusion model couldn't adequately support the adsorption process in these experiments. Similar results reported by Dada et al. [52], which investigated the parameters for modeling the kinetics with statistical validity, desorption and adsorption of Cu (II) ions onto zerovalent iron nanoparticles [52]. Also, Wang et al. [53] demonstrated that Interparticle diffusion model is not the controlling factor in determining the kinetics of Cd adsorption on clay minerals [53]. Also from the comparison data of Pseudo-first and the Pseudo-second order models, this fact clarified that all investigated parameters in the Pseudo-second order including adsorption capacity at time t (Q_e), the rate constant (k_2) and correlation coefficient (R^2) were more than adsorption capacity at time t (Q_e), the rate constant (k_1) and correlation coefficient (R^2) in Pseudo-first order which indicated that the adsorption kinetic process was well supported by the Pseudo-second order model. Other scientists reported that the adsorption from liquid solutions has been extensively studied, with a wealth of experimental data focusing on the adsorption capabilities of various materials towards different solutes. While many studies include both equilibrium and kinetic data, a common practice involves fitting the kinetic data to empirical models. Among these, the pseudo-first and pseudo-second order models are widely used, particularly in their linearized forms. Even though the pseudo-second order model often achieves excellent fitting quality, it lacks a physical basis and is inconsistent with the understanding that diffusion processes govern adsorption kinetics [54]. The present study demonstrates, through examples and analysis, that the apparent success of the Pseudo-second order model in fitting data is better compared to the two other employed kinetic models.

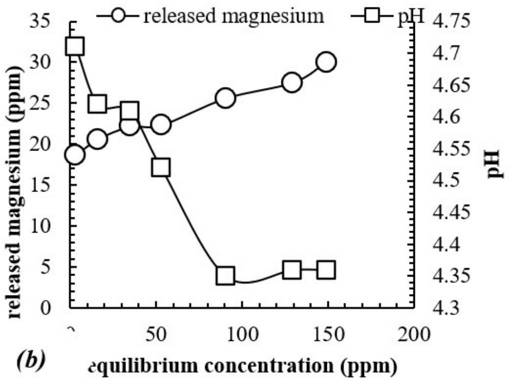
3.8. Released magnesium

In this study, the release of Mg^{2+} from the sepiolite structures into the solutions was investigated, along with the pH changes in the final solutions. Atomic adsorption measurements indicated that more Mg^{2+} was released from unmodified sepiolite compared to heat-treated samples, with minerals heated at 150 °C releasing more Mg^{2+} than those heated at 250 °C. These findings suggest cation exchange between the investigated heavy metals and structural Mg^{2+}. The differences in released magnesium quantities are due to the increased surface area and adsorption capacity of heated samples in comparison with natural ones. The magnesium at the edges of the octahedral sheets in 250 °C heated minerals, influenced by zeolitic water and increased surface area, was less than in 150 °C heated and unmodified mineral samples. Therefore, magnesium at the edges of octahedral sheets in natural sepiolite exchanged more with existing cationic ions in experimental solutions. Lazarevic et al. [35] reported that acid-treatment on sepiolite led to magnesium leaching from octahedral sheets and exchange with lead (Pb) ions in solutions. Miura et al. [33] observed that thermal, acid, and base treatments degraded sepiolite's fibrous morphology, with high-temperature or long-term treatments and acid treatments increasing the sepiolite surface area.

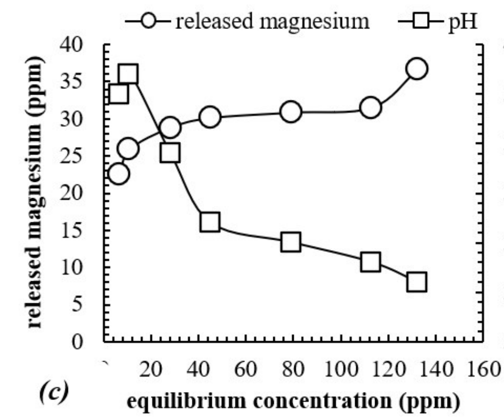
Figures 3 shows the changes in pH values and released magnesium corresponding to heavy metal concentrations. At low equilibrium concentrations, the acidic content of the solutions, the freedom effect of solute ions, and the high adsorption ability of sepiolite resulted in high Mg^{2+} release, which interacted with OH^- ions, brought about decreasing °C the final pH of the solution. Since the initial heavy metal concentrations increased, pH values decreased, and magnesium release increased smoothly. Continued increases in heavy metal concentrations led to significant Mg^{2+} release and pH decreases due to the exchangeable effect of magnesium with the investigated positive charge ions (See **Figure 4a–f**).



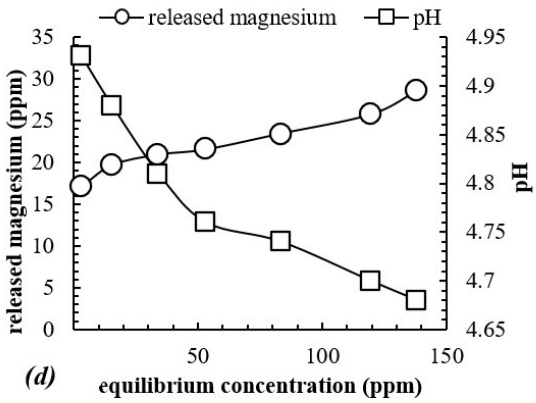
(a)



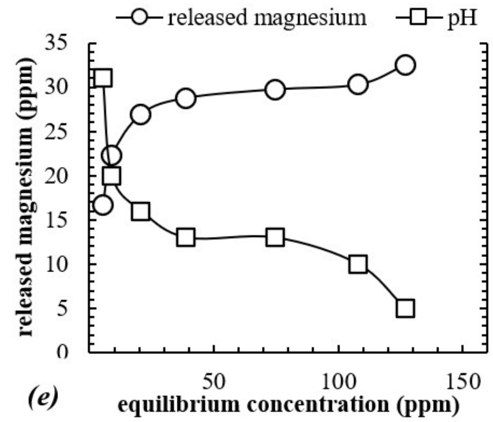
(b)



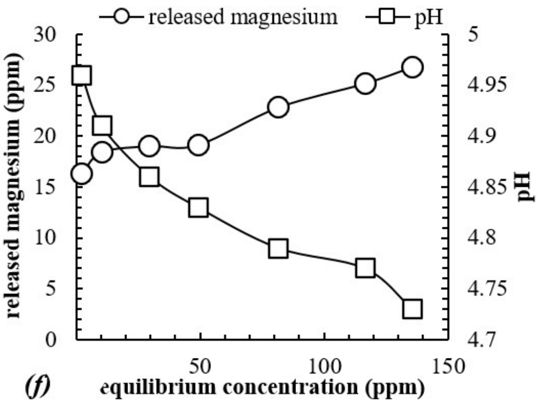
(c)



(d)



(e)



(f)

Figure 4. The changes in pH values and released magnesium correspond to heavy metal concentrations. **(a)** Natural sepiolite in solution with pH 4 values; **(b)** Natural sepiolite in solution with pH 5 values; **(c)** heat pretreated sepiolite at 150 ℃ in solution with pH 4 values; **(d)** heat pretreated sepiolite at 150 ℃ in solution with pH 5 values; **(e)** heat pretreated sepiolite at 250 ℃ in solution with pH 4 values; **(f)** heat pretreated sepiolite at 250 ℃ in solution with pH 5 values.

The ability to regenerate and reuse sepiolite adsorbent is crucial for its sustainability and economic viability in real-world applications. While this study focused on sepiolite's capacity to remove Cd, Co, and Zn ions, the thermal pretreatment at 250 °C that enhanced its adsorption also opens the door for efficient regeneration. Previous research has shown that heat-treated absorbents can often be restored through simple thermal or chemical processes, which release bound ions and reactivate adsorption sites [55]. Given sepiolite's stability at moderate temperatures, it's likely that the material can be regenerated and reused multiple times without significant performance loss. Although, according to the natural condition of sepiolite constitution, the mineral's original structure will be changed but the adsorption capacity for future uses will remain.

Future studies should explore the regeneration potential of heat-treated sepiolite through desorption experiments. These experiments should assess both the adsorbent's efficiency over multiple cycles and its long-term structural integrity. Such investigations are essential for confirming sepiolite's suitability for large-scale environmental remediation as a sustainable adsorbent. On the other hand, given the abundant and low-cost availability of sepiolite in Iran, these methods and minerals present viable options for use in wastewater treatment.

4. Conclusion

The present study successfully indicates the enhanced adsorption capacities of heat-treated sepiolite for the removal of contaminant heavy metals from wastewaters. The findings indicated that heat pretreatment at 250 °C considerably increased the potential surface area and adsorption efficiency of sepiolite, specifically for Co^{2+} ions. The pseudo-second order kinetic model best fitted the experimental data and suggested chemisorption as the primary adsorption mechanism. The effect of pH on adsorption efficiency showed the importance of optimizing solution conditions for effective heavy metal removal. These understandings can inform low-cost and sustainable approaches for wastewater treatment, to provide readily available and easily modified natural adsorbents like sepiolite. Moreover, future research can focus on exploring the long-term stability of heat-treated sepiolite in various environmental conditions and its scalability for industrial applications.

Although the present study primarily focused on Cd, Co, and Zn, the kinetic experiments were performed in multi-ionic solutions that reflect real-world conditions. The influence of additional ions, including H^+ and OH^- at varying pH levels, was considered, particularly in the analysis of adsorption behavior. However, it would be acknowledged that a more comprehensive investigation involving a wider range of competing ions (e.g., Na^+, Ca^{2+}, Mg^{2+}) could provide deeper insights into competitive adsorption dynamics. Future work should extend these findings by examining the impact of these common ions in multi-component solutions to better understand their role in metal ion adsorption efficiency and adsorption site competition. This would provide a more complete understanding of the adsorption mechanisms in complex environmental matrices.

Author contributions: Conceptualization, RS and SH; methodology, SH and NP; software, SH; validation, AL, SH and NP; formal analysis, AL and SH; investigation, RS; resources, SH; data curation, AL; writing—original draft preparation, RS;

writing—review and editing, AL; visualization, RS and SH; supervision, AL; project administration, RS; funding acquisition, AL. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Sherugar P, Padaki M, Naik NS, et al. Biomass-derived versatile activated carbon removes both heavy metals and dye molecules from wastewater with near-unity efficiency: Mechanism and kinetics. Chemosphere. 2022; 287: 132085. doi: 10.1016/j.chemosphere.2021.132085
2. Zhang T, Wang W, Zhao Y, et al. Removal of heavy metals and dyes by clay-based adsorbents: From natural clays to 1D and 2D nano-composites. Chemical Engineering Journal. 2021; 420: 127574. doi: 10.1016/j.cej.2020.127574
3. Azha SF, Shahadat M, Ismail S, et al. Prospect of clay-based flexible adsorbent coatings as cleaner production technique in wastewater treatment, challenges, and issues: A review. Journal of the Taiwan Institute of Chemical Engineers. 2021; 120: 178-206. doi: 10.1016/j.jtice.2021.03.018
4. Ding L, Azimi G. Impact of particle size and associated minerals on rare earth desorption and incorporation mechanisms in a South American ion-adsorption clay. Scientific Reports. 2024; 14(1). doi: 10.1038/s41598-024-67134-2
5. Pellenz L, de Oliveira CRS, da Silva Júnior AH, et al. A comprehensive guide for characterization of adsorbent materials. Separation and Purification Technology. 2023; 305: 122435. doi: 10.1016/j.seppur.2022.122435
6. Qasem NAA, Mohammed RH, Lawal DU. Removal of heavy metal ions from wastewater: a comprehensive and critical review. npj Clean Water. 2021; 4(1). doi: 10.1038/s41545-021-00127-0
7. Mazloom Jalali A, Afshar Taromi F, Atai M, et al. Effect of reaction conditions on silanisation of sepiolite nanoparticles. Journal of Experimental Nanoscience. 2016; 11(15): 1171-1183. doi: 10.1080/17458080.2016.1200147
8. Yao Y, Feng Y, Li H, et al. Investigation of the adsorption performance and mechanism of multi-source mineral composite calcination materials on heavy metal ions. Desalination. 2024; 586: 117847. doi: 10.1016/j.desal.2024.117847
9. Hamid Y, Tang L, Hussain B, et al. Sepiolite clay: A review of its applications to immobilize toxic metals in contaminated soils and its implications in soil–plant system. Environmental Technology & Innovation. 2021; 23: 101598. doi: 10.1016/j.eti.2021.101598
10. Liu L, Zhang C, Jiang W, et al. Understanding the sorption behaviors of heavy metal ions in the interlayer and nanopore of montmorillonite: A molecular dynamics study. Journal of Hazardous Materials. 2021; 416: 125976. doi: 10.1016/j.jhazmat.2021.125976
11. Deng L, Miyatani K, Suehara M, et al. Ion-exchange mechanisms and interfacial reaction kinetics during aqueous corrosion of sodium silicate glasses. npj Materials Degradation. 2021; 5(1). doi: 10.1038/s41529-021-00159-4
12. Ruiz AI, Ruiz-García C, Ruiz-Hitzky E. From old to new inorganic materials for advanced applications: The paradigmatic example of the sepiolite clay mineral. Applied Clay Science. 2023; 235: 106874. doi: 10.1016/j.clay.2023.106874
13. Anderson JA, Daza L, Damyanova S, et al. Hydrogenation of styrene over nickel/sepiolite catalysts. Applied Catalysis A: General. 1994; 113(1): 75-88. doi: 10.1016/0926-860X(94)80242-4
14. Bautista FM, Luna D, Luque J, et al. Gas-phase selective oxidation of chloro- and methoxy-substituted toluenes on TiO2–Sepiolite supported vanadium oxides. Applied Catalysis A: General. 2009; 352(1-2): 251-258. doi: 10.1016/j.apcata.2008.10.015
15. Khan ZI, Habib U, Mohamad ZB, et al. Mechanical and thermal properties of sepiolite strengthened thermoplastic polymer nanocomposites: A comprehensive review. Alexandria Engineering Journal. 2022; 61(2): 975-990. doi: 10.1016/j.aej.2021.06.015
16. Wang Z, Liao L, Hursthouse A, et al. Sepiolite-Based Adsorbents for the Removal of Potentially Toxic Elements from Water: A Strategic Review for the Case of Environmental Contamination in Hunan, China. International Journal of Environmental Research and Public Health. 2018; 15(8): 1653. doi: 10.3390/ijerph15081653
17. Zhou J, Jiang W, Peng J, et al. An environmentally friendly sepiolite/Cu_2O/Cu ternary composite as anode material for Li-ion batteries. Ionics. 2022; 28(3): 1091-1098. doi: 10.1007/s11581-021-04432-z
18. Zhou J, Wang Z, Alcântara ACS, et al. Study of the adsorption mechanisms of NH3, H2S and SO2 on sepiolite using molecular dynamics simulations. Clay Minerals. 2022; 58(1): 1-6. doi: 10.1180/clm.2022.22

19. Yeniyol M. Characterization of two forms of sepiolite and related Mg-rich clay minerals from Yenidoğ an (Sivrihisar, Turkey). Clay Minerals. 2014; 49(1): 91-108. doi: 10.1180/claymin.2014.049.1.08
20. Christidis GE, Athanasakis N, Marinakis D. Rheological properties of magnesium bentonite and sepiolite suspensions after dynamic ageing at high temperatures. Clay Minerals. 2024; 59(2): 113-126. doi: 10.1180/clm.2024.11
21. Samieifard R, Landi A, Pourreza N. Adsorption of Cd, Co and Zn from multi-ionic solutions onto Iranian sepiolite isotherms. Central Asian journal of environmental science and technology innovation. 2021; 2: 102-118.
22. Tian G, Han G, Wang F, et al. Sepiolite Nanomaterials: Structure, Properties and Functional Applications. Nanomaterials from Clay Minerals; 2019.
23. Mahmoud MR, Rashad GM, Metwally E, et al. Adsorptive removal of 134 Cs + , 60 Co 2+ and 152+154 Eu 3+ radionuclides from aqueous solutions using sepiolite: Single and multi-component systems. Applied Clay Science. 2017; 141: 72-80. doi: 10.1016/j.clay.2016.12.021
24. Aktar J. Batch adsorption process in water treatment. Intelligent Environmental Data Monitoring for Pollution Management; 2021.
25. Vidu R, Matei E, Predescu AM, et al. Removal of Heavy Metals from Wastewaters: A Challenge from Current Treatment Methods to Nanotechnology Applications. Toxics. 2020; 8(4): 101. doi: 10.3390/toxics8040101
26. Saleh TA. Kinetic models and thermodynamics of adsorption processes: classification. Surface Science of Adsorbents and Nanoadsorbents; 2022.
27. Söğüt EG, Gülcan M. Adsorption: basics, properties, and classification. Adsorption through Advanced Nanoscale Materials; 2023.
28. Wang J, Guo X. Adsorption kinetic models: Physical meanings, applications, and solving methods. Journal of Hazardous Materials. 2020; 390: 122156. doi: 10.1016/j.jhazmat.2020.122156
29. Kanagalakshmi M, Devi SG, Ananthi P, Pius A. Adsorption Isotherms and Kinetic Models. In: Carbon Nanomaterials and their Composites as Adsorbents. Cham: Springer International Publishing; 2024. pp. 135-154.
30. Revellame ED, Fortela DL, Sharp W, et al. Adsorption kinetic modeling using pseudo-first order and pseudo-second order rate laws: A review. Cleaner Engineering and Technology. 2020; 1: 100032. doi: 10.1016/j.clet.2020.100032
31. Hu Q, Ma S, He Z, et al. A revisit on intraparticle diffusion models with analytical solutions: Underlying assumption, application scope and solving method. Journal of Water Process Engineering. 2024; 60: 105241. doi: 10.1016/j.jwpe.2024.105241
32. de Oca-Palma RM, Solache-Ríos M, Jiménez-Reyes M, et al. Adsorption of cobalt by using inorganic components of sediment samples from water bodies. International Journal of Sediment Research. 2021; 36(4): 524-531.
33. Miura A, Nakazawa K, Takei T, et al. Acid-, base-, and heat-induced degradation behavior of Chinese sepiolite. Ceramics International. 2012; 38(6): 4677-4684. doi: 10.1016/j.ceramint.2012.02.050
34. Song S, Li B, eds. Adsorption at Natural Minerals/Water Interfaces. Springer International Publishing; 2021.
35. Lazarević S, Janković-Častvan I, Potkonjak B, et al. Removal of Co2+ ions from aqueous solutions using iron-functionalized sepiolite. Chemical Engineering and Processing: Process Intensification. 2012; 55: 40-47. doi: 10.1016/j.cep.2012.01.004
36. Guerra DL, Batista AC, Corrêa da costa PC, et al. Adsorption of arsenic ions on Brazilian sepiolite: Effect of contact time, pH, concentration, and calorimetric investigation. Journal of Colloid and Interface Science. 2010; 346(1): 178-187. doi: 10.1016/j.jcis.2010.02.041
37. Kosmulski M. The pH dependent surface charging and points of zero charge. IX. Update. Advances in Colloid and Interface Science. 2021; 296: 102519. doi: 10.1016/j.cis.2021.102519
38. Tian L, Wang L, Wang K, et al. The Preparation and Properties of Porous Sepiolite Ceramics. Scientific Reports. 2019; 9(1). doi: 10.1038/s41598-019-43918-9
39. Derbe T, Temesgen S, Bitew M. A Short Review on Synthesis, Characterization, and Applications of Zeolites. Fedel M, ed. Advances in Materials Science and Engineering. 2021; 2021(1). doi: 10.1155/2021/6637898
40. Murphy OP, Vashishtha M, Palanisamy P, et al. A Review on the Adsorption Isotherms and Design Calculations for the Optimization of Adsorbent Mass and Contact Time. ACS Omega. 2023; 8(20): 17407-17430. doi: 10.1021/acsomega.2c08155
41. Bektaş N, Ağım BA, Kara S. Kinetic and equilibrium studies in removing lead ions from aqueous solutions by natural sepiolite. Journal of Hazardous Materials. 2004; 112(1-2): 115-122. doi: 10.1016/j.jhazmat.2004.04.015

42. Shirvani M, Shariatmadari H, Kalbasi M. Kinetics of cadmium desorption from fibrous silicate clay minerals: Influence of organic ligands and aging. Applied Clay Science. 2007; 37(1-2): 175-184. doi: 10.1016/j.clay.2006.12.010
43. Lei X, Lian Q, Zhang X, et al. A review of PFAS adsorption from aqueous solutions: Current approaches, engineering applications, challenges, and opportunities. Environmental Pollution. 2023; 321: 121138. doi: 10.1016/j.envpol.2023.121138
44. Ding J, Wei Z, Li F, et al. Atomic high-spin cobalt(II) center for highly selective electrochemical CO reduction to CH3OH. Nature Communications. 2023; 14(1). doi: 10.1038/s41467-023-42307-1
45. Velarde L, Nabavi MS, Escalera E, et al. Adsorption of heavy metals on natural zeolites: A review. Chemosphere. 2023; 328: 138508. doi: 10.1016/j.chemosphere.2023.138508
46. Rao F, Li Z, Garcia RE. Adsorption of Cations on Minerals. In: Adsorption at Natural Minerals/Water Interfaces. Springer Nature Link; 2021.
47. Brigatti MF, Franchini GC, Medici L, et al. Behaviour of sepiolite in Co2+ Cu2+ and Cd2+ removal from a simulated pollutant solution. Annali di chimica. 1998; 88: 461-470.
48. Kocaoba S, Akyuz T. Effects of conditioning of sepiolite prior to cobalt and nickel removal. Desalination. 2005; 181(1-3): 313-318. doi: 10.1016/j.desal.2005.04.010
49. Healy C, Patil KM, Wilson BH, et al. The thermal stability of metal-organic frameworks. Coordination Chemistry Reviews. 2020; 419: 213388. doi: 10.1016/j.ccr.2020.213388
50. Ezzati R. Derivation of Pseudo-First-Order, Pseudo-Second-Order and Modified Pseudo-First-Order rate equations from Langmuir and Freundlich isotherms for adsorption. Chemical Engineering Journal. 2020; 392: 123705. doi: 10.1016/j.cej.2019.123705
51. Wang J, Guo X. Rethinking of the intraparticle diffusion adsorption kinetics model: Interpretation, solving methods and applications. Chemosphere. 2022; 309: 136732. doi: 10.1016/j.chemosphere.2022.136732
52. Dada AO, Adekola FA, Odebunmi EO, et al. Two–three parameters isotherm modeling, kinetics with statistical validity, desorption and thermodynamic studies of adsorption of Cu(II) ions onto zerovalent iron nanoparticles. Scientific Reports. 2021; 11(1). doi: 10.1038/s41598-021-95090-8
53. Wang W, Chen H, Wang A. Adsorption characteristics of Cd(II) from aqueous solution onto activated palygorskite. Separation and Purification Technology. 2007; 55(2): 157-164. doi: 10.1016/j.seppur.2006.11.015
54. Kostoglou M, Karapantsios TD. Why Is the Linearized Form of Pseudo-Second Order Adsorption Kinetic Model So Successful in Fitting Batch Adsorption Experimental Data? Colloids and Interfaces. 2022; 6(4): 55. doi: 10.3390/colloids6040055
55. Vakili M, Cagnetta G, Deng S, et al. Regeneration of exhausted adsorbents after PFAS adsorption: A critical review. Journal of Hazardous Materials. 2024; 471: 134429. doi: 10.1016/j.jhazmat.2024.134429

Journal of Toxicological Studies 2024, 2(2), 1252.
https://doi.org/10.59400/jts.v2i2.1252

Review

Understanding and role of gut microbiota on drug response and toxicity

Sriram Seshadri*, Devanshi Gajjar, Aneri Joshi, Zeel Bhatia, Sunny Kumar

Institute of Science, Nirma University, S G Highway, Ahmedabad 382481, India
*** Corresponding author:** Sriram Seshadri, sriram.seshadri@nirmauni.ac.in

CITATION

Seshadri S, Gajjar D, Joshi A, et al. Understanding and role of Gut Microbiota on drug response and toxicity. Journal of Toxicological Studies. 2024; 2(2): 1252. https://doi.org/10.59400/jts.v2i2.1252

ARTICLE INFO

Received: 30 March 2024
Accepted: 16 June 2024
Available online: 2 July 2024

Abstract: The gut microbiota affects human health profoundly, and evidence is mounting that it can cause, worsen, or resolve illnesses. Particularly in drug-induced toxicity, its role in diverse toxicological reactions has garnered attention recently. Drugs may interact directly or indirectly, through the gut flora, whether or not they are taken orally, changing the toxicity. Current research focuses mainly on the one-way effect of xenobiotics on the makeup and activities of gut microbes, which leads to altered homeostasis. However, there are two-way interactions between the gut microbiota and xenobiotics, and it is important to consider how the gut microbiota affects xenobiotics, particularly medications. Therefore, we emphasise the microbiome, microbial metabolites, and microbial enzymes in this review to emphasise how the gut microbiota affects medication toxicity. To aid in the identification of micro-biologic targets and processes linked to drug toxicity, we establish connections between medications, the microbiome, microbial enzymes or metabolites, drug metabolites, and host toxicological reactions. In addition, a summary and discussion of contemporary mainstream approaches to controlling medication toxicity by microbiota targeting are provided.

Keywords: gut microflora; inflammation; drug detoxification; metabolites; immune response

1. Introduction

In recent decades, the role of the microbiome in disease pathogenesis in various vital as well as other organs of the body has attracted attention. Its accumulative weight is equivalent to that of the liver, and its vast numbers surpass those of human cells by magnitudes, suggesting that it has enormous potential for controlling human health and illness. The microbiota, which is considered an essential microorganism system, is found in nearly every bodily niche and is primarily colonised in the gastrointestinal, urogenital, eyes, skin, and airways. The bulk of the microbiota's habitats are found in the gut. They are categorised as viruses, bacteria, fungus, archaea, and several other microorganisms [1].

The majority of intestinal microorganisms are prokaryotes, with Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes accounting for more than 90% of the gut microbiota [2]. Diverse taxa have diverse functions for their gut flora. A dysregulated Firmicutes/Bacteroidetes ratio is frequently interpreted as an indication of gut dysbiosis. Several phyla play a crucial role in maintaining host homeostasis. The Firmicutes/Bacteroidetes ratio is linked to metabolic disorders [3].

Certain enzymes, including *β-glucuronidase*, *β-glucosidase*, and *β-galactosidase*, can be secreted by a particular gut microbiota. Additionally, it can produce metabolites, such as lipids, bile acids, vitamins, and amino acids, which are all heavily involved in both the pathogenesis and maintenance of health. Given that the gut microbiota is thought to be a key regulator of human health [4–8].

Research has shown that the intestinal microbiota has a role in the aetiology of diseases in the brain, liver, kidney, gastrointestinal tract [9,10], and other areas of the human body. Treatments such as food modification, FMT, and pre-/pro-/synbiotic supplementation have been used in both human and animal trials [11]. Terms like the gut-liver axis, gut-brain axis, gut-kidney axis, etc. are derived from the interactions between the gut and other organs, and each is made possible via unique routes [12].

However, modifications in one of these axes may trigger modifications in another, leading to a changed microbiome. To put it another way, the gut acts as a link between several human systems. For example, the gut-liver-kidney and gut-liver-brain axis are proposed as important regulators in the pathophysiology of chronic kidney disease [13] and hepatic encephalopathy (HE) [14], respectively.

Throughout the process of developing and using drugs, adverse drug responses might happen. Significant drug toxicity in preclinical and clinical trials results in the discontinuation of the medication's development; nevertheless, significant drug toxicity in marketed pharmaceuticals causes fatalities and eventually leads to the removal of the drug [15]. Therefore, it is possible to decrease compound attrition during medication development and safeguard patient safety by precisely anticipating and preventing headache, delirium, psychosis, and drug toxicity.

Differences in host toxicological reactions to certain medications remain poorly understood, despite the ever-expanding understanding of processes via which pharmaceuticals are metabolised, absorbed, distributed, and removed alongside advances in pharmacogenetics and pharmacogenomics. A new area, i.e., pharmacomicrobiomics, aims to understand how interindividual differences in the microbiome influence the toxicity and effectiveness of drugs [16].

From the standpoint of the microbiome, which is now understood to play critical roles in drug metabolism [17,18], pharmacokinetics [19,20], efficacy, and toxicity, it offers a fresh perspective on explaining variability in drug outcomes [21]. These days, evidence-based medicine emphasises the use of pharmacogenetics and pharmacogenomics to interpret patient variances in drug responses [22]. Additionally, as the human gut microbiota expresses many more genes than the host, it should also be highlighted how drugs affect patient outcomes [23].

Drug-metabolizing enzymes regulate the metabolism of both foreign and endogenous drugs. Drugs often lose their pharmacological activity through metabolic change, producing highly water-soluble metabolites that are easily eliminated. Therefore, metabolising enzymes play a very important role. Controlling drug PK is critical. Characterization of enzymes involved in human drug metabolism is crucial for preventing severe adverse effects.

In the present review, we are trying to understand the role of microbiome on drug toxicity and therapeutic alternatives to exploiting them.

1.1. Role of microbial enzymes in drug toxicity

There is growing evidence to show that the metabolic repertoire of microorganisms is greater than that of human cells [24]. Drug transformation is mediated by the microbiota, which also triggers a variety of chemical processes. The majority of drugs are affected by hydrolysis and reduction [25]. Drugs are exposed to the microbiota either directly or by biliary excretion, regardless of how they are administered [26]. Drugs may undergo microbial changes that change their pharmacokinetic characteristics, activate prodrugs, cause unwanted side effects, or reduce their effectiveness. By means of chemical transformation, the microbial enzymes can either exacerbate or provide relief from the harmful reactions that medications elicit.

1.2. Microbial role in drug activation

The most prevalent metabolic route in the gastrointestinal system is hydrolysis, which is mostly catalysed by microbial proteases, glycosidases, and sulfatases. In addition to producing products like glucose and sulphates to promote microbiological development, these activities often release smaller molecules for further metabolism [24]. However, they can also activate medications and change their toxicity. The gut microbiota is the only source of the hydrolytic enzyme *β-glucosidase*. Aglycones are formed when it releases glucose from glucosides; some of these aglycones are even more hazardous than the glucosides they are associated with. This mechanism is particularly prevalent in the digestion of phytomedicine components by gut microbes.

The main bioactive ingredient in Armeniacae semen, amygdalin, is degraded by the microbial *β-glucosidase* to produce glucose and mandelonitrile, the latter of which is poisonous when amygdalin is present [27]. Similar to this, geniposide, a significant bioactive ingredient found in many phytomedicines such as *Gardenia jasminoides Elli*, *Eucommia ulmoides Oliv.*, and *Rehmannia glutinosa Libosch*. [28], is broken down by microbial *β-glucosidase* into its aglycone genipin [29], and genipin is assumed to be the cause of geniposide-induced hepatotoxicity [30]. By eliminating gut microorganisms, antibiotic therapy may significantly reduce the microbial enzyme and impede the genipin formative process [31].

Numerous enzymes, some of which are exclusively microbial, such as nitroreductases, azoreductases, alkene reductases, and sulfoxide reductases, facilitate the reductive conversions that the gut microbiome mediates [32]. A decrease in gut microbiota chemicals results in changes to their polarity, bioavailability, and action. While the microbiome and the host both express nitroreductases, gut microbial nitroreductases are a class of enzymes that significantly impact medication toxicity. Nitrazepam is a kind of nitrobenzodiazepines that are metabolised into 7-aminonitrazepam, the metabolite that causes nitrazepam-induced teratogenicity, via nitroductases that are generated by the liver and microbiota [33].

Antibiotic therapy, however, significantly reduced malformation and almost eliminated 7-aminonitrazepam synthesis, indicating a direct relationship between nitrazepam-induced teratogenicity and its nitroreduction by gut microorganisms [33]. Another study that used N-nitrosodiethylamine as the substrate verified the metabolic action of nitroreductases to generate toxicity [34]. The gut microbial azoreductase cuts its azide bond reductively to produce sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA), the former of which is in charge of sulfasalazine's adverse effects [35]. Even though azoreductase metabolises ipsalazide and balsalazide, two analogues of sulfasalazine, their toxicity is eliminated by the altered structure of SP [36].

1.3. Microbial role in drug reactivation

The metabolism of xenobiotics in the gut microbial community frequently promotes microbial development by providing nutrition and energy generation, even as the host metabolism helps eliminate xenobiotics from the body [24]. Notably, the gut microbiota frequently opposes or reverses host-performed chemical changes, changing the pharmacokinetic and pharmacodynamic characteristics of xenobiotics. The action of microbial *β-glucuronidases* on medicines that are reabsorbed and metabolised in the gut through enterohepatic circulation embodies this well.

A hydrolase often found in bodily fluids, microbiota, and mammalian tissues is *β-glucuronidase*. Numerous harmful drugs are detoxified in the liver by UDP-glucuronosyltransferases (UGTs); however, the metabolites conjugated with glucuronic acid are reabsorbed in the gut, where they are converted back into their poisonous precursors by gut microbial *β-glucuronidases*. The first drug for Alzheimer's disease to be licensed was tacrine, but it was taken off the market because of significant pharmacokinetic variation [37] and the erratic hepatotoxicity that resulted [38]. According to a recent thorough investigation, rats react to tacrine in diverse ways. Strong responders show larger levels of tacrine exposure, increased deglucuronidation capacities, and an abundance of *β-glucuronidase*. The transportation and metabolic routes of drugs within the host are crucial for their reactivation by microbial *β-glucuronidases*.

One of the most widely used anticancer drugs for colon cancer treatment, iminotecan, can be fatally hazardous to at least 36% of patients, the majority of whom have mucositis, diarrhoea, and other gastrointestinal side effects [39]. The liver and the gut microbiota in humans both metabolise iminotecan. Liver carboxylesterases first convert it to bioactive SN-38, and then hepatic UGTs conjugate it with glucuronic acid to form SN-38G. This is subsequently subjected to gut bacterial *β-glucuronidases* to regenerate SN-38, which is also a toxin that causes severe diarrhoea and damage to intestinal epithelial cells [40]. It has been noted that the gut bacterial *β-glucuronidases* play a crucial role in reducing irinotecan-induced gastrointestinal toxicity. The suppression of intestinal bacterial *β-glucuronidases* has been shown to be efficient in reducing irinotecan toxicity and boosting anticancer activity and is thought to be a predictive biomarker of irinotecan-triggered diarrhoea severity [41–44].

Furthermore, inhibiting bacterial *β-glucuronidases* with distinct origins and structures produced quite different results: inhibiting *β-glucuronidases* derived from Firmicutes and Proteobacterium alleviates irinotecan-induced diarrhoea in mice, whereas inhibiting *β-glucuronidases* derived from Bacteroidetes does not, indicating functional diversity in orthologous enzymes of the gut microbiota [45]. Interestingly, the liver and gut microbiota of mice also use the same metabolic route to break down mycophenolate mofetil (MMF) in conjunction with irinotecan. It functions as a prodrug that is hydrolyzed to mycophenolic acid (MPA) to provide effectiveness, and hepatic enzymes then further convert it to glucuronized MPA (MPAG).

The majority of MPAG is eliminated by urine, but 10% of it enters the digestive system and is converted back into MPA by the gut microbial βglucuronidase. This buildup of MPA in the colon is linked to MMF-induced colonic inflammation [46]. Additionally, MMF increases the production of active β-glucuronidase, which aggravates its deleterious effect on the gastrointestinal tract and can be remedied with antibiotics [47].

It has been shown that inhibiting *β-glucuronidase* activity is an effective way to relieve associated drug toxicities, and it is a promising target for the creation of new treatments. Certain natural compounds, like quercetin, have the ability to block *β-glucuronidase* as well as cause the gut microbiota to produce protective metabolites [48]. Unfortunately, the poor pharmacokinetic profile of currently available *β-glucuronidase* inhibitors limits their clinical use [49]. Nevertheless, *β-glucuronidase* might be a useful target for reducing medication toxicity.

Microbial metabolism of food and endogenous substances has an indirect effect on important host hepatic enzymes that contribute significantly to drug metabolism. For example, Phase I hepatic enzymes, which comprise the cytochrome P450s (CYPs) superfamily and flavin-containing monooxygenases (FMOs), account for 80% of the oxidative metabolism of routinely used drugs. Phase II hepatic enzymes, such as glutathione S-transferases (GST), sulfotransferases (SULTs), and uridine diphosphate-glucuronosyltransferases (UGTs), play critical roles in drug detoxification and removal from the body. The gut microbiota's metabolism of uremic solutes, bile acids, and steroid hormones influences the expression and activity of these enzymes; these microbiome-drug interactions can have negative effects for patients taking medicines that are substrates for these enzymes. Microbiota-produced uremic solute indoxyl sulphate reduces CYP3A4 expression, lowering CYP3A4-mediated metabolic clearance of a wide variety of medications, including erythromycin, nimodipine, and verapamil.

1.4. Role of microbiome on drug inactivation

The inactivation of drugs by microbial metabolism has different effects than the activation and reactivation that often worsen drug toxicity. Through chemical alteration, the microbiome-mediated inactivation lowers medication toxicity while simultaneously increasing adverse effects and decreasing treatment effectiveness. One of the main drugs used to treat Parkinson's disease is levodopa. Reduced effectiveness in the brain and heightened adverse effects in the peripheral tissues and an increased dose schedule of levodopa treatment in Parkinson's disease may be explained by the gut microbiome's inactivation of levodopa [50]. By concurrently giving levodopa and carbidopa, abd AADC inhibitor *(S)-α-Fluoromethyltyrosine*—a substance that suppresses Enterococcus faecali and Eggerthella lenta—blocks the microbial metabolism of levodopa and enhances its bioavailability [51]. This suggests that a useful strategy for managing microbial inactivation-induced reduced effectiveness and increased toxicity might be the targeted suppression of gut microbiota that takes part in drug metabolism. Similarly, Eubacterium lenta inactivates digoxin, a nature-derived cardiac glycoside used to treat arrhythmia and heart failure, in the stomach to generate 20R-dihydrodigoxin [52].

Digoxin needs the unsaturated lactone ring structurally to elicit its therapeutic actions; however, the microbiota reduces it, which results in inactivation. The microbial metabolism of digoxin results in variable toxicity because of its limited therapeutic window and the notable individual variability in gut flora. Despite its identified function in the metabolism of digoxin, Eubacterium lenta is also present in individuals lacking the excretion of 20R-dihydrodigoxin [53]. Digoxin's varying toxic effects and pharmacokinetic characteristics among the population are better understood thanks to the discovery of cardiac glycoside reductase (CGR) in its metabolism. It also provides ways for lowering metabolism and, as a result, regulating digoxin's toxicity [53].

On the other hand, by breaking down harmful drugs into less hazardous metabolites, the microbiome-initiated drug inactivation also serves as detoxification. The anticancer drug doxorubicin has side effects that include vomiting, diarrhoea, hair loss, and even cardiotoxicity. It has been shown that Raoultella planticola functions as an inactivator of doxorubicin by deglycosylating it to produce the less harmful metabolites 7-deoxydoxorubicinol and 7-deoxydoxorubicinolone [54].

This suggests that depending on the toxicity of metabolites following deglycolation, pharmacological activation or inactivation results from microbial metabolism-induced deglycosylation [28,30,54]. Furthermore, mitochondrial inactivation detoxifies arsenic, a hazardous pollutant linked to a number of illnesses such as diabetes, heart disease, and multiorgan malignancies. When assessing arsenic biotransformation and toxicity, the monomethylarsonic acid/dimethylarsinic acid (MMA/DMA) ratio is thought to be a biomarker. In general, pentavalent arsenic species are less dangerous than trivalent arsenic ones. Growing data indicates that the metabolism and toxicity of arsenic are significantly influenced by the microbiota [55].

By promoting arsenic methylation and lowering the MMA/DMA ratio, the gut microbiota lessens the toxicity of arsenic [56]. Due to its capacity to encode methyltransferase, supplementary Faecalibacterium prausnitzii offers protection against arsenic poisoning, even if antibiotic therapy and a germ-free state enhance it [57]. Given that the microbiome plays a role in the metabolism of arsenic and the toxicity that results, it is possible to reduce drug toxicity by using microbial inactivation of xenobiotics to aid in detoxification.

Even though fecal microflora or accessible separated species were used by many researchers to study the impact of microbial metabolism on drug toxicity prior to the 1980s, this field is still largely unknown despite the development of high-throughput techniques in recent years.

Microbial incubation can reveal the effects of *β-glucosidase* and other enzymes that are only produced by the gut microbiota. However, the host and gut microbiota overlap many metabolic pathways, making it difficult to distinguish the role of the microbes in drug metabolism from that of the host. In addition, systematic studies on microbial metabolism are limited by the individual differences in gut microbial diversity and function. The only current mainstream approaches are germ-free animal models and nonspecific antibiotics.

Periodically, the metabolic profiles of medicines in conventional, gnotobiotic, and germ-free animal models are compared in an effort to disentangle the role of gut microbes on drug pharmacokinetics and toxicity from the host [23]. Disentangling the roles of the host and microbiome in drug metabolism and hazardous effects is more challenging though, as the germ-free condition may cause changes in the host's metabolic characteristics. However, a deeper comprehension of the particular species and enzymes involved in drug metabolism may help with dosage and medication selection.

1.5. Regulating the drug toxicity via nuclear receptors

Nuclear transcriptional factors are important regulators of the production of phase II enzymes and transporters, as well as CYP450s. To improve the clearance, they serve as sensors of xenobiotics and harmful metabolites of endogenous metabolism. Microbiota-nucleus transcription factor connections are studied using germ-free animal models, which provide valuable insights. The expression levels of PXR and CAR in germ-free mice are considerably less than in the particular pathogen-free mice, which causes CYP450 expression to be reduced as a result [58]. Contradictory findings have been noted in another study, indicating that germ-free mice exhibit greater levels of PXR and CAR in conjunction with CYPs such as CYP2A4, CYP2B13, CYP2C38, and CYP4A14 [59]. Different studies may yield different results due to variations in the expression of nuclear regulators during different growth stages [60]. However, it has been determined that the gut microbiota is involved in controlling nuclear regulator expression, which in turn controls the expression of metabolic enzymes and transporters. The finding that compounds originating from the gut microbiota, particularly microbial products of aromatic metabolism, function as either agonists or antagonists of PXR and AHR, confirms this [61].

AHR and PXR ligands have been found to be associated with a number of microbial tryptophan catabolites, including skatole, indole, tryptamine, and a series of indolyl-3-(lactate, pyruvate, acrylate, propionate, acetate, aldehyde, acetamide, ethanol) [62]. It is commonly known that these microbial tryptophan catabolites stimulate the intestinal mucosa's innate immunity and cause quick inflammatory reactions by stimulating or opposing epithelial nuclear receptors [63].

A fresh approach to drug development has been suggested in the form of microbial metabolite imitation. Strong PXR agonists, such as indole and indole 3 propionate, are designed to lessen colitis by enhancing the expression of CYP3A4 and multidrug resistance 1 (MDR1) and preventing NF-κB activation [64].

While a large body of research has shown how the gut microbiota may influence how host metabolic enzymes are expressed, very few have examined the impact of certain xenobiotics on metabolic alterations and the ensuing harmful effects. It has been discovered recently that AHR activation reduces intestinal toxicity caused by chemotherapy by progressively blocking the tryptophan-kynurenine-kynurenic acid axis metabolism [65]. In addition, it has been discovered that indole 3-propionic acid, via PXR activation, protects against gastrointestinal tract injuries and radiation-induced hematopoietic system damage [66]. These investigations suggest that by activating nuclear factors, it is possible to use gut microbial metabolites and their analogues to reduce drug-induced gastrointestinal toxicity.

Nuclear regulators are thought to be implicated in xenobiotic toxicity by controlling endogenous metabolism, as these nuclear factors also take part in endogenous metabolism, and xenobiotics might cause harm by altering the host metabolome. Myopathy is a side effect of statins, a family of medications intended to control blood cholesterol and lower the risk of heart disease. They also raise the risk of type 2 diabetes mellitus. It is thought that changes in cholesterol and glucose metabolism as well as PXR-dependent gut dysbiosis are responsible for the adverse effects of statins [67,68].

The most often used non-nucleoside reverse transcriptase inhibitor, efavirenz, has been linked to hepatic steatosis and dyslipidemia side effects. The fundamental mechanism is that efavirenz effectively activates PXR, which in turn controls important hepatic lipogenic genes. This leads to enhanced hepatic cell lipid absorption and cholesterol production [69]. One of the main defense mechanisms against oxidative stress brought on by xenobiotic toxicity is the activation of nuclear factor erythroid-derived 2-like 2 (Nrf2). Under normal circumstances, Nrf2 is attached to Kelch-like ECH associating protein 1 (Keap1) in the cytoplasm. However, in times of high oxidative stress, Nrf2 is released, translocates to the nucleus, and binds to the antioxidant response element (ARE), inducing the activation of defense genes [70].

Lactobacilli activates hepatic Nrf2, which reduces APAP-induced hepatotoxicity [71]. The in vitro activation of Nrf2 by Lactobacilli-derived 5-methoxyindoleacetic acid (5-IAA) is comparable to the impact of oral Lactobacilli administration, suggesting that Lactobacilli activate Nrf2 by 5-IAA secretion [71]. Additionally, the gut microbiota controls the circadian liver transcriptome and detoxification pattern, resulting in APAP-induced hepatotoxicity that varies during the day and is more severe at night [72].

The gut microbiota modulates drug toxicity through metabolic intervention and host defense activation. It does this by exerting significant impacts on nuclear factors. But the majority of what we now know about the microbiome's impact on nuclear factors is very limited, and it hardly touches on the effects it may have on xenobiotic metabolism and toxicity. Further understanding of how the microbiota affects nuclear factors to modify medication toxicity is needed.

1.6. Regulation of drug toxicity via host metabolism

Numerous metabolites that the microbiome produces contribute to the physiology of the host; these metabolites' production, roles, and mechanisms of action have all been thoroughly studied in other places [5,6]. The microbial metabolites, exemplified by bile acids and SCFAs, play a significant role in the host's metabolism of endogenous chemicals and have been linked to a number of metabolic disorders, such as obesity, hyperglycemia, and nonalcoholic fatty liver disease (NAFLD) [73,74]. Apart from their typical roles in influencing health and illnesses, microbiological metabolites take part in xenobiotics' host metabolism, which controls their harmful and metabolic effects.

The interaction between 5-fluorouracil (5-FU) and sorivudine is a classic example of how adverse drug-drug interactions can result from microbial metabolites interfering with the host drug metabolism. 5-FU is used to treat colon cancer, yet adverse effects to the mucosa, such as mucositis and diarrhoea, are commonly reported. Hepatic dihydropyrimidine dehydrogenase (DPD) is primarily responsible for detoxifying the human body; its suppression leads to the buildup of 5 FU and subsequently exacerbates negative consequences.

By vieing for the host's metabolic enzymes, the microbiome produces both endogenous and external metabolites and indirectly contributes to the metabolism of the host. While the activation of nuclear receptors caused by changes in the microbiota can result in a variety of alterations related to drug metabolism and toxicity, the competition of microbial metabolites with host enzymes often increases drug toxicity by impeding host detoxification. Despite the increasing interest in comprehending the interaction between the microbiome and host metabolism, more research is needed to clearly connect medication metabolism with microbial changes to host metabolic enzymes.

1.7. Modulation of drug toxicity via regulating the immune response

The effects of the microbiome on drug metabolism and immunity form a major basis for the postulated involvement of the microbiome in modifying drug toxicity [75]. In contrast to the impact of direct microbial metabolism on medication toxicity, a diverse array of chemicals and receptors modulate the immune-regulated drug toxicity associated with the microbiome. Drugs can directly harm the host and trigger immunological responses, but some can produce toxicity that is mediated via the interplay between the immune system and the microbiota.

1.8. Interplay between the microbiome and immune system

The immune system regulates the preservation of gut homeostasis, but the microbiome is essential to the growth and development of key elements of the host's innate and adaptive immune systems. On the other hand, a compromised immune system can also result in gut dysbiosis, which can increase pathogenic and/or Gram-negative bacteria and metabolites, disturb the epithelial barrier, and make the system more susceptible to infections. Gut dysbiosis can also promote inflammation and oxidative stress. The intestinal mucosa serves as an interface for two-way communication between the microbiome and the host immune system, as well as a natural barrier against pathogenic infection and commensal infiltration from the gut [76].

There are several chemicals and receptors that facilitate the reciprocal exchange of information between the host immune system and the microbiome. Intestinal paneth cells, which constitute one of the phylogenetically ancient components of the innate immune system, generate antimicrobial peptides, or AMPs. Multiple interactions between intestinal AMPs and the microbiota alter its structure [77]. In addition, pattern recognition receptors (PRRs) like TLRs and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) play a major role in mediating the crosstalk between the microbiome and the host innate immune system. These

PRRs identify pathogen-associated molecular patterns (PAMPs) and trigger the innate immune system in response [78,79].

Bacteroides fragilis produces a microbial compound called polysaccharide A (PSA), which is recognised by TLR2/1 and C-type lectin-like receptor. This recognition activates the anti-inflammatory arm of the phosphoinositide 3 kinase (PI3K) pathway, which in turn trains CD4+ Tregs to produce the immunomodulatory cytokine IL-10. Primary response protein 88 (MyD88) of myeloid differentiation functions as an adapter for inflammatory signalling pathways that are downstream of TLR and interleukin-1 (IL-1) receptor family members [80]. Its absence is linked to changed microbiota configuration and a lack of sensitivity to microbial ligands for TLR4 [81].

After identifying pathogenic bacteria or metabolites, certain PRRs combine to form inflammasomes. These inflammasomes then trigger inflammatory caspases, which release cytokines and cause pyroptotic cell death, as was previously discussed [82]. It is well known that inflammatory proteins, such as NLRP3 and NOD-pyrin domain-containing 6 (NLRP6), reversely control the makeup of microbes and preserve intestinal homeostasis [83,84]. The microbiome has the ability to modify innate immune responses, which are governed by immune cells like macrophages and natural killer (NK) T cells. This can impact the functionality of immunological organs like the liver [85,86]. The microbiome affects the innate immune system, but it also plays a role in the control of the host's adaptive immune system.

It is shown that the IgA antibody response, which mediates the preservation of gut homeostasis, may be activated by commensal bacteria [87]. Furthermore, microbial metabolites represented by SCFAs have a significant role in host adaptive immunity via suppressing the growth of proinflammatory Th17 cells and promoting the development of Treg cells [88] and anti-inflammatory forkhead box protein P3 (Foxp3) [89,90]. Strong evidence of the connection between the microbiota and human immune system has been found in a recent human study that compared the effects of immunomodulatory drugs with those of the microbiota. The study found that the microbiota affects systemic immune cell dynamics over time [91].

2. Challenges and limitations

Pharmacomicrobiomics is a rapidly evolving field that studies the complex relationship between gut bacteria and medication responses. While there have been considerable improvements in these areas, however, there are still various issues in this area: (1) Standardised procedures are lacking for sample collection, sequencing, and data processing in pharmacognostic investigations.

This makes it difficult to compare study data and produce clinical practice guidelines. (2) The gut microbiota is a dynamic ecosystem influenced by factors such as nutrition, lifestyle, and genetics. Distinguishing between the impact of these variables and drug-induced microbiome alterations can be challenging. (3) Individual Variability in Drug Response (IVDR): The gut microbiota varies significantly between individuals, which might have an influence on drug-microbiota interactions. (4) Limited knowledge of mechanisms: Although there is evidence of drug-microbiota interactions, the mechanisms remain unclear. It is unknown how interactions change across medications and people. (5) Therapeutic translation: While pharmacomicrobiomics has intriguing therapeutic applications, several challenges must be addressed before it can be implemented in practice. Developing microbiota-based biomarkers for treatment response requires extensive validation research and regulatory approval. (6) Ethical issues: Personalised medicine, including the use of microbiome data, requires ethical considerations. Concerns include privacy, data sharing, and potential discrimination based on microbial traits. (7) Small sample numbers in pharmacomicrobiomics investigations might impair statistical power and generalizability of findings. More extensive research is needed to validate and build on preliminary findings. (8) Population diversity: Pharmacomicrobiomics research has mostly focused on Western populations, resulting in a lack of variation in ethnicity, location, and lifestyle characteristics. This reduces our understanding of how drug-microbiota interactions may differ between communities. (9) Confounding variables: Diet, age, gender, and environmental exposures might affect gut flora, making it difficult to discriminate between medication effects and other factors. To address these challenges, researchers, clinicians, and industry partners should collaborate to develop standardised methodologies, improve understanding of drug-microbiota interactions, and translate findings into safe and effective clinical practice.

3. Conclusion

The complex relationship between drugs and the microbiota has a significant impact on the toxicity. Drugs have the potential to change the makeup and function of microbes, which could lead to toxicity because of increased risk factors brought on by dysbiosis in the gut; however, the microbiome also produces enzymes and metabolites that are involved in drug metabolism and host detoxification patterns, which could change the toxic effects of drugs. In addition, the microbiota influences immunological responses that can be used to mitigate drug toxicity.

To understand the relationship between the microbiome and drug toxicity systemically, the microbial components that affect drug toxicity may be linked as follows: drug, microbiome, microbial enzymes/metabolites, drug metabolites, host toxicant responses. The ultimate objective is to identify the microbial species that cause the interaction between medication toxicity and the microbiome and to use strategies to target them in order to minimise drug toxicity. Currently, pro-, pre-, and synbiotic supplementation, FMT, and dietary manipulation are the methods for reducing medication toxicity by focusing on the microbiome; none of these methods is drug-specific.

Targeting specific microbial species, enzymes, or metabolites can improve drug toxicity, as seen in the successful manipulation of levodopa's peripheral toxicity by targeting a microbial enzyme. To do this, though, we must completely understand the microbial drug metabolism pathways and the processes behind drug toxicity. We can only take advantage of the microbiome's amazing potential to lessen toxicity and increase the effectiveness of medications by developing a thorough grasp of these mechanisms (**Figure 1**).

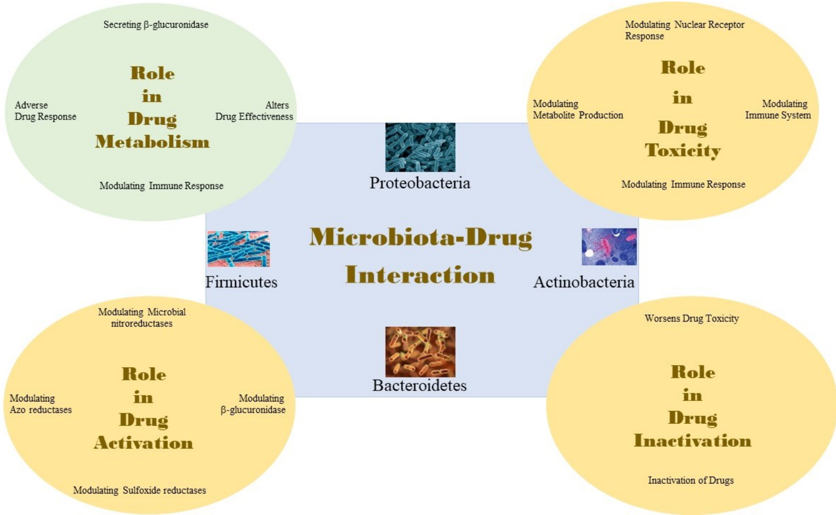


Figure 1. Drug-Microbiota interaction and role of microbiota on drug metabolism.

Precision medicine is hindered by the heterogeneity of the human microbiota, the rise of multi-drug-resistant bacteria, and the impact of various medications on microbial pathways. Nonetheless, precision medicine remains the perfect possibility for future theranostics, with a complete understanding of the role of the microbiota on IVDR permitting stratification of patients based on recognised biomarkers, microbiota types, and metabotypes.

Acknowledgments: We would like to thank Nirma University, Ahmedabad, India for providing Junior Research Fellowship (JRF) to Ms. Devanshi Gajjar, Senior Research Fellowship (SRF) to Ms. Zeel Bhatia and SHODH fellowship, Government of Gujarat, India to Mr. Sunny Kumar.

Conflict of interest: The authors declare no conflict of interest.

References

1. Leung C, Rivera L, Furness JB, et al. The role of the gut microbiota in NAFLD. Nature Reviews Gastroenterology & Hepatology. 2016; 13(7): 412-425. doi: 10.1038/nrgastro.2016.85
2. Bäckhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. Science. 2005; 307(80): 1915-1920. doi: 10.1126/science.1104816
3. Ley RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444: 1022-1023. doi: 10.1038/4441022a
4. Seshadri S. Microbial Communication via Quorum Sensing: Influence and Alteration of Gut Ecosystem. JSM Biochemistry and Molecular Biology. 2017; 4(1): 1020-1023.
5. Mandaliya DK, Seshadri S. Short Chain Fatty Acids, pancreatic dysfunction and type 2 diabetes. Pancreatology. 2019; 19(2): 280-284. doi: 10.1016/j.pan.2019.01.021
6. Mandaliya DK, Patel S, Seshadri S. The combinatorial effect of acetate and propionate on high fat diet induced diabetic inflammation or metaflammation and T cell polarization. Inflammation 2021; 44: 68-79. doi: 10.1007/s10753-020-01309-7.
7. Patel S, Seshadri S, Dalai S. Gut microbiome and type 2 diabetes. Progress in Molecular Biology and Translational Science. 2022; 191(1): 175-185. doi: 10.1016/bs.pmbts.2022.06.029
8. Kumar S, Bhatia Z, Seshadri S. Mapping the Microbial Metabolites in Metabolic Disorder with Special Reference to Type-2 Diabetes. In: Kothari V, Kumar P, Ray S (editors). Probiotics, Prebiotics, Synbiotics, and Postbiotics: Human Microbiome and Human Health. Springer Press; 2023. pp. 67-80. doi: 10.1007/978-981-99-1463-0_4
9. Nishida A, Inoue R, Inatomi O, et al. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clinical Journal of

Gastroenterology. 2018; 11: 1-10. doi: 10.1007/s12328-017-0813-5
10. Gorkiewicz G, Moschen A. Gut microbiome: a new player in gastrointestinal disease. Virchows Archiv. 2018; 472: 159-172. doi: 10.1007/s00428-017-2277-x
11. Wiest R, Albillos A, Trauner M, et al. Targeting the gut-liver axis in liver disease. Journal of Hepatology. 2017; 67(5): 1084-1103. doi: 10.1016/j.jhep.2017.05.007
12. Bhatia Z, Kumar S, Seshadri S. Exploring the Unexplored Arena: Butyrate as a Dual Communicator in Gut-Brain Axis. In: Kothari V, Kumar P, Ray S (editors). Probiotics, Prebiotics, Synbiotics, and Postbiotics: Human Microbiome and Human Health. Springer Press; 2023. pp. 153-164. doi: 10.1007/978-981-99-1463-0_9
13. Yang T, Richards EM, Pepine CJ, et al. The gut microbiota and the brain-gut-kidney axis in hypertension and chronic kidney disease. Nature Reviews Nephrology. 2018; 14: 442-456. doi: 10.1038/s41581-018-0018-2
14. Mancini A, Campagna F, Amodio P, et al. Gut: Liver: brain axis: the microbial challenge in the hepatic encephalopathy. Food & Function. 2018; 9(3): 1373-1388. doi: 10.1039/c7fo01528c
15. Stevens JL, Baker TK. The future of drug safety testing: expanding the view and narrowing the focus. Drug Discovery Today. 2009; 14: 162-167. doi: 10.1016/j.drudis.2008.11.009
16. Lam KN, Alexander M, Turnbaugh PJ. Precision medicine goes microscopic: engineering the microbiome to improve drug outcomes. Cell Host Microbe. 2019; 26(1): 22-34. doi: 10.1016/j.chom.2019.06.011
17. Li H, He J, Jia W. The influence of gut microbiota on drug metabolism and toxicity. Expert Opinion on Drug Metabolism and Toxicology. 2016; 12: 31-40. doi: 10.1517/17425255.2016.1121234
18. Collins SL, Patterson AD. The gut microbiome: an orchestrator of xenobiotic metabolism. Acta Pharmaceutica Sinica B 2020; 10: 19-32. doi: 10.1016/j.apsb.2019.12.001
19. Zhang J, Zhang J, Wang R. Gut microbiota modulates drug pharmacokinetics. Drug Metabolism Reviews. 2018; 50: 357-368. doi: 10.1080/03602532.2018.1497647
20. Li Y, Meng Q, Yang M, et al. Current trends in drug metabolism and pharmacokinetics. Acta Pharmaceutica Sinica B. 2019; 9: 1113-1144. doi: 10.1016/j.apsb.2019.10.001
21. Yip LY, Aw CC, Lee SH, et al. The liver-gut microbiota axis modulates hepatotoxicity of tacrine in the rat. Hepatology. 2017; 67(1): 282-295. doi: 10.1002/hep.29327
22. Relling MV, Evans WE. Pharmacogenomics in the clinic. Nature. 2015; 526(7573): 343-350. doi: 10.1038/nature15817
23. Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, et al. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. Science. 2019; 363(6427). doi: 10.1126/science.aat9931
24. Koppel N, Maini Rekdal V, Balskus EP. Chemical transformation of xenobiotics by the human gut microbiota. Science. 2017; 356(6344). doi: 10.1126/science.aag2770
25. Wilson ID, Nicholson JK. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Translational Research. 2017; 179: 204-222. doi: 10.1016/j.trsl.2016.08.002
26. Clarke G, Sandhu KV, Griffin BT, et al. Gut Reactions: Breaking Down Xenobiotic-Microbiome Interactions. France CP, ed. Pharmacological Reviews. 2019; 71(2): 198-224. doi: 10.1124/pr.118.015768
27. Carter JH, McLafferty MA, Goldman P. Role of the gastrointestinal microflora in amygdalin (laetrile)-induced cyanide toxicity. Biochemical Pharmacology. 1980; 29(3): 301-304. doi: 10.1016/0006-2952(80)90504-3
28. Zhou YX, Zhang RQ, Rahman K, et al. Diverse Pharmacological Activities and Potential Medicinal Benefits of Geniposide. Evidence-Based Complementary and Alternative Medicine. 2019; 2019: 1-15. doi: 10.1155/2019/4925682
29. Akao T, Kobashi K, Aburada M. Enzymic Studies on the Animal and Intestinal Bacterial Metabolism of Geniposide. Biological and Pharmaceutical Bulletin. 1994; 17(12): 1573-1576. doi: 10.1248/bpb.17.1573
30. Kang MJ, Khanal T, Kim HG, et al. Role of metabolism by human intestinal microflora in geniposide-induced toxicity in HepG2 cells. Archives of Pharmacal Research. 2012; 35(4): 733-738. doi: 10.1007/s12272-012-0418-y
31. Jin MJ, Kim IS, Kim DH, et al. Effects of Intestinal Microbiota on the Bioavailability of Geniposide in Rats. Journal of Agricultural and Food Chemistry. 2014; 62(40): 9632-9636. doi: 10.1021/jf502557f
32. Rafii F, Cerniglia CE. Reduction of azo dyes and nitroaromatic compounds by bacterial enzymes from the human intestinal tract. Environmental Health Perspectives. 1995; 103(suppl 5): 17-19. doi: 10.1289/ehp.95103s417
33. Takeno S, Sakai T. Involvement of the intestinal microflora in nitrazepam-induced teratogenicity in rats and its relationship to nitroreduction. Teratology. 1991; 44(2): 209-214. doi: 10.1002/tera.1420440209
34. Aiub CAF, Mazzei JL, Pinto LFR, et al. Evaluation of nitroreductase and acetyltransferase participation in

N-nitrosodiethylamine genotoxicity. Chemico-Biological Interactions. 2006; 161: 146-154. doi: 10.1016/j.cbi.2006.03.012
35. Das KM, Eastwood MA, McManus JPA, et al. The metabolism of salicylazosulphapyridine in ulcerative colitis: I The relationship between metabolites and the response to treatment in inpatients. Gut. 1973; 14(8): 631-636. doi: 10.1136/gut.14.8.631
36. Chan RP, Pope DJ, Gilbert AP, et al. Studies of two novel sulfasalazine analogs, ipsalazide and balsalazide. Digestive Diseases and Sciences. 1983; 28(7): 609-615. doi: 10.1007/bf01299921
37. Jarrott B. Tacrine: In vivo veritas. Pharmacological Research. 2017; 116: 29-31. doi: 10.1016/j.phrs.2016.12.033
38. National Institute of Diabetes and Digestive and Kidney Diseases. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
39. Encarnação JC, Pires AS, Amaral RA, et al. Butyrate, a dietary fiber derivative that improves irinotecan effect in colon cancer cells. The Journal of Nutritional Biochemistry. 2018; 56: 183-192. doi: 10.1016/j.jnutbio.2018.02.018
40. Yang W, Wei B, Yan R. Amoxapine Demonstrates Incomplete Inhibition of β-Glucuronidase Activity from Human Gut Microbiota. SLAS Discovery. 2018; 23(1): 76-83. doi: 10.1177/2472555217725264
41. Chamseddine AN, Ducreux M, Armand JP, et al. Intestinal bacterial β-glucuronidase as a possible predictive biomarker of irinotecan-induced diarrhea severity. Pharmacology & Therapeutics. 2019; 199: 1-15.
42. Roberts AB, Wallace BD, Venkatesh MK, et al. Molecular Insights into Microbialβ-Glucuronidase Inhibition to Abrogate CPT-11 Toxicity. Molecular Pharmacology. 2013; 84(2): 208-217. doi: 10.1124/mol.113.085852
43. Wallace BD, Wang H, Lane KT, et al. Alleviating Cancer Drug Toxicity by Inhibiting a Bacterial Enzyme. Science. 2010; 330(6005): 831-835. doi: 10.1126/science.1191175
44. Bhatt AP, Pellock SJ, Biernat KA, et al. Targeted inhibition of gut bacterial β-glucuronidase activity enhances anticancer drug efficacy. Proceedings of the National Academy of Sciences. 2020; 117(13): 7374-7381. doi: 10.1073/pnas.1918095117
45. Wallace BD, Roberts AB, Pollet RM, et al. Structure and Inhibition of Microbiome β-Glucuronidases Essential to the Alleviation of Cancer Drug Toxicity. Chemistry & Biology. 2015; 22(9): 1238-1249. doi: 10.1016/j.chembiol.2015.08.005
46. Lamba V, Sangkuhl K, Sanghavi K, et al. PharmGKB summary. Pharmacogenetics and Genomics. 2014; 24(1): 73-79. doi: 10.1097/fpc.0000000000000010
47. Taylor MR, Flannigan KL, Rahim H, et al. Vancomycin relieves mycophenolate mofetil-induced gastrointestinal toxicity by eliminating gut bacterial β-glucuronidase activity. Science Advances. 2019; 5(8). doi: 10.1126/sciadv.aax2358
48. Weng ZM, Wang P, Ge GB, et al. Structure-activity relationships of flavonoids as natural inhibitors against E. coli β-glucuronidase. Food and Chemical Toxicology. 2017; 109: 975-983. doi: 10.1016/j.fct.2017.03.042
49. Awolade P, Cele N, Kerru N, et al. Therapeutic significance of β-glucuronidase activity and its inhibitors: A review. European Journal of Medicinal Chemistry. 2020; 187: 111921. doi: 10.1016/j.ejmech.2019.111921
50. van Kessel SP, Frye AK, El-Gendy AO, et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. Nature Communications. 2019; 10(1). doi: 10.1038/s41467-019-08294-y
51. Maini Rekdal V, Bess EN, Bisanz JE, et al. Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. Science. 2019; 364(6445). doi: 10.1126/science.aau6323
52. Dobkin JF, Saha JR, Butler VP, et al. Digoxin-Inactivating Bacteria: Identification in Human Gut Flora. Science. 1983; 220(4594): 325-327. doi: 10.1126/science.6836275
53. Haiser HJ, Gootenberg DB, Chatman K, et al. Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium Eggerthella lenta. Science. 2013; 341(6143): 295-298. doi: 10.1126/science.1235872
54. Yan A, Culp E, Perry J, et al. Transformation of the Anticancer Drug Doxorubicin in the Human Gut Microbiome. ACS Infectious Diseases. 2017; 4(1): 68-76. doi: 10.1021/acsinfecdis.7b00166
55. Isokpehi RD, Udensi UK, Simmons SS, et al. Evaluative Profiling of Arsenic Sensing and Regulatory Systems in the Human Microbiome Project Genomes. Microbiology Insights. 2014; 7: 25-34. doi: 10.4137/mbi.s18076
56. Chi L, Xue J, Tu P, et al. Gut microbiome disruption altered the biotransformation and liver toxicity of arsenic in mice. Archives of Toxicology. 2018; 93(1): 25-35. doi: 10.1007/s00204-018-2332-7
57. Coryell M, McAlpine M, Pinkham NV, et al. The gut microbiome is required for full protection against acute arsenic toxicity in mouse models. Nature Communications 2018; 9: 5424.
58. Toda T, Saito N, Ikarashi N, et al. Intestinal flora induces the expression of Cyp3a in the mouse liver. Xenobiotica. 2009; 39(4): 323-334. doi: 10.1080/00498250802651984
59. Björkholm B, Bok CM, Lundin A, et al. Intestinal Microbiota Regulate Xenobiotic Metabolism in the Liver. PLoS ONE.

2009; 4(9): e6958. doi: 10.1371/journal.pone.0006958
60. Selwyn FP, Cheng SL, Bammler TK, et al. Developmental Regulation of Drug-Processing Genes in Livers of Germ-Free Mice. Toxicological Sciences. 2015; 147(1): 84-103. doi: 10.1093/toxsci/kfv110
61. Illés P, Krasulová K, Vyhlídalová B, et al. Indole microbial intestinal metabolites expand the repertoire of ligands and agonists of the human pregnane X receptor. Toxicological Letters. 2020; 334: 87-93. doi: 10.1016/j.toxlet.2020.09.015
62. Dvořák Z, Sokol H, Mani S. Drug Mimicry: Promiscuous Receptors PXR and AhR, and Microbial Metabolite Interactions in the Intestine. Trends in Pharmacological Sciences. 2020; 41(12): 900-908. doi: 10.1016/j.tips.2020.09.013
63. Zhang J, Zhu S, Ma N, et al. Metabolites of microbiota response to tryptophan and intestinal mucosal immunity: A therapeutic target to control intestinal inflammation. Medicinal Research Reviews. 2020; 41(2): 1061-1088. doi: 10.1002/med.21752
64. Dvořák Z, Kopp F, Costello CM, et al. Targeting the pregnane X receptor using microbial metabolite mimicry. EMBO Molecular Medicine. 2020; 12(4). doi: 10.15252/emmm.201911621
65. Wang D, Li D, Zhang Y, et al. Functional metabolomics reveal the role of AHR/GPR35 mediated kynurenic acid gradient sensing in chemotherapy-induced intestinal damage. Acta Pharmaceutica Sinica B. 2021; 11(3): 763-780. doi: 10.1016/j.apsb.2020.07.017
66. Xiao H wen, Cui M, Li Y, et al. Gut microbiota-derived indole 3-propionic acid protects against radiation toxicity via retaining acyl-CoA-binding protein. Microbiome. 2020; 8(1). doi: 10.1186/s40168-020-00845-6
67. Caparrós-Martín JA, Lareu RR, Ramsay JP, et al. Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. Microbiome. 2017; 5(1). doi: 10.1186/s40168-017-0312-4
68. Seshadri S, Rapaka N, Prajapati B, et al. Statins exacerbate glucose intolerance and hyperglycemia in a high sucrose fed rodent model. Scientific Reports. 2019; 9(1). doi: 10.1038/s41598-019-45369-8
69. Gwag T, Meng Z, Sui Y, et al. Non-nucleoside reverse transcriptase inhibitor efavirenz activates PXR to induce hypercholesterolemia and hepatic steatosis. Journal of Hepatology. 2019; 70: 930-940. doi: 10.1016/j.jhep.2018.12.038
70. Kansanen E, Kuosmanen SM, Leinonen H, et al. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. Redox Biology. 2013; 1(1): 45-49. doi: 10.1016/j.redox.2012.10.001
71. Saeedi BJ, Liu KH, Owens JA, et al. Gut-Resident Lactobacilli Activate Hepatic Nrf2 and Protect Against Oxidative Liver Injury. Cell Metabolism. 2020; 31(5): 956-968. doi: 10.1016/j.cmet.2020.03.006
72. Thaiss CA, Levy M, Korem T, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. Cell. 2016; 167(6): 1495-1510. doi: 10.1016/j.cell.2016.11.003
73. Bridgeman SC, Northrop W, Melton PE, et al. Butyrate generated by gut microbiota and its therapeutic role in metabolic syndrome. Pharmacological Research. 2020; 160: 105174. doi: 10.1016/j.phrs.2020.105174
74. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nature Reviews Gastroenterology & Hepatology. 2017; 15(2): 111-128. doi: 10.1038/nrgastro.2017.119
75. Garcia-Cortes M, Robles-Diaz M, Stephens C, et al. Drug induced liver injury: an update. Archives of Toxicology. 2020; 94(10): 3381-3407. doi: 10.1007/s00204-020-02885-1
76. Mowat AMcI. To respond or not to respond — a personal perspective of intestinal tolerance. Nature Reviews Immunology. 2018; 18(6): 405-415. doi: 10.1038/s41577-018-0002-x
77. Sun D, Bai R, Zhou W, et al. Angiogenin maintains gut microbe homeostasis by balancing α-Proteobacteria and Lachnospiraceae. Gut. 2020; 70(4): 666-676. doi: 10.1136/gutjnl-2019-320135
78. Chu H, Mazmanian SK. Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nature Immunology. 2013; 14(7): 668-675. doi: 10.1038/ni.2635
79. Prajapati B, Jena P, Rajput P, et al. Understanding and Modulating the Toll Like Receptors (TLRs) and NOD Like Receptors (NLRs) Cross Talk in Type 2 Diabetes. Current Diabetes Reviews. 2014; 10(3): 190-200. doi: 10.2174/1573399810666140515112609
80. Deguine J, Barton GM. MyD88: a central player in innate immune signaling. F1000Prime Reports. 2014; 6. doi: 10.12703/p6-97
81. Kawai T, Adachi O, Ogawa T, et al. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 1999; 11: 115-122. doi: 10.1016/S1074-7613(00)80086-2
82. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nature Reviews Immunology. 2016; 16(7): 407-420. doi: 10.1038/nri.2016.58

83. Elinav E, Strowig T, Kau AL, et al. NLRP6 Inflammasome Regulates Colonic Microbial Ecology and Risk for Colitis. Cell. 2011; 145(5): 745-757. doi: 10.1016/j.cell.2011.04.022
84. Seo SU, Kamada N, Muñoz-Planillo R, et al. Distinct Commensals Induce Interleukin-1β via NLRP3 Inflammasome in Inflammatory Monocytes to Promote Intestinal Inflammation in Response to Injury. Immunity. 2015; 42(4): 744-755. doi: 10.1016/j.immuni.2015.03.004
85. Krishnan S, Ding Y, Saedi N, et al. Gut Microbiota-Derived Tryptophan Metabolites Modulate Inflammatory Response in Hepatocytes and Macrophages. Cell Reports. 2018; 23(4): 1099-1111. doi: 10.1016/j.celrep.2018.03.109
86. Wei Y, Zeng B, Chen J, et al. Enterogenous bacterial glycolipids are required for the generation of natural killer T cells mediated liver injury. Scientific Reports. 2016; 6(1). doi: 10.1038/srep36365
87. Peterson DA, McNulty NP, Guruge JL, et al. IgA Response to Symbiotic Bacteria as a Mediator of Gut Homeostasis. Cell Host & Microbe. 2007; 2(5): 328-339. doi: 10.1016/j.chom.2007.09.013
88. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504(7480): 446-450. doi: 10.1038/nature12721
89. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504(7480): 451-455. doi: 10.1038/nature12726
90. Singh N, Gurav A, Sivaprakasam S, et al. Activation of Gpr109a, Receptor for Niacin and the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis. Immunity. 2014; 40(1): 128-139. doi: 10.1016/j.immuni.2013.12.007
91. Schluter J, Peled JU, Taylor BP, et al. The gut microbiota is associated with immune cell dynamics in humans. Nature. 2020; 588(7837): 303-307. doi: 10.1038/s41586-020-2971-8

Journal of Toxicological Studies 2024, 2(2), 1486.
https://doi.org/10.59400/jts.v2i2.1486

Review

Is acetylsalicylic acid use in cats contraindicated or limited indicated?

Said Cafer, Bengü Bilgiç, Mehmet Erman Or*

Department of Internal Medicine, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul 34315, Turkey
* **Corresponding author:** Bengü Bilgiç; bengu.bilgic@iuc.edu.tr

CITATION

Cafer S, Bilgiç B, Or ME. Is acetylsalicylic acid use in cats contraindicated or limited indicated?. Journal of Toxicological Studies. 2024; 2(2): 1486. https://doi.org/10.59400/jts.v2i2.1486

ARTICLE INFO

Received: 29 June 2024
Accepted: 14 September 2024
Available online: 23 September 2024

Abstract: Acetylsalic acid, (Aspirin®) is a non-steroidal anti-inflammatory drug (NSAID) widely used in human and veterinary medicine, especially for its analgesic and antithrombotic effects, mainly in the prevention of cardiovascular complications and in the treatment of various diseases. Aspirin® can not be metabolized in cats because they do not have the enzyme glucuronyl transferase. For this reason, it has a long half-life and a narrow dose range. High dose administration in cats may cause serious toxicity in the liver. Acetylsalicylic acid is known to cause gastric ulcers associated with decreased prostaglandin levels. For these reasons, it is considered toxic to cats. But it also has antipyretic, analgesic, anti-inflammatory and antithrombotic properties. It is indicated for use alone or in combination with other antithrombotic drugs in the treatment and prophylaxis of thrombus formation resulting from cardiovascular diseases. This review aims to examine the indicated and contraindicated areas of use of Aspirin®, which is widely considered toxic in cats.

Keywords: aspirin; NSAID's; ulcer; salicylate; thromboembolism; toxicity

1. Introduction

Acetylsalicylic also known as Aspirin®, is widely used in human and veterinary medicine [1]. Aspirin® was initially used only for its analgesic effects, but over time its antipyretic, anti-inflammatory and antithrombotic effects were also discovered. Today, its use in human medicine is quite common, but its use in cats is controversial [2].

Aspirin® has been widely thought to be toxic in cats for years, as reported in many studies, but studies are being conducted to examine whether it can be used in low doses due to its anti-inflammatory and antithrombotic effects in patients with pain, fever, and cardiovascular diseases. Its effectiveness has been reported and suggested to be indicated, especially in cardiovascular diseases and the prevention of arterial thromboembolism [3]. It is also claimed that the use of Aspirin® prolongs life in geriatric patients because it reduces the risk of various diseases [4]. However, cats are more sensitive to Aspirin® than humans and dogs [5]. The half-life of Aspirin® is longer in cats than in other animals (especially dogs) [6]. The reason for this is thought to be the slowness of glucuronidation [7].

As a result of the combination of a toxic substance with glucuronic acid, it transforms that drug into a form that is less toxic than original substance, more water soluble and more easily excreted from the body. This physiological situation is called glucuronidation. However, glucuronidation in the cat body is very slow than other animals and incomplete. For this reason, the elimination of Aspirin® from the cat body is very slow. This increases the half-life of Aspirin® in the cat body [8]. On the other hand, it is thought that one of the reasons for the slow clearance of Aspirin® from the body may be due to the weak glycine conjugation. However, it has many side effects in cats and, at higher doses, in humans or dogs [9].

It has been mentioned in numerous literature over the years that Aspirin® causes gastric hyperemia and ulceration in dogs and rabbits. In the years following its discovery, similar situations were observed with intravenously administered Aspirin®. This has shown that Aspirin® can damage the gastric epithelium even if it does not come into contact with the stomach mucosa [10]. For this reason, studies were carried out to produce a drug that has as many indications and effects as Aspirin®, but has fewer side effects on the gastrointestinal tract. As a result of these studies, the active ingredient ibuprofen was discovered in 1961 [2]. Despite the toxicity of Aspirin®, especially in cats, Aspirin® is still considered indicated in some diseases today, even if different medications are used. Therefore, in this review study, a perspective on limited indications versus contraindications is examined.

2. History of aspirin

Aspirin® is one of the most widely used drugs today. It has been the most interesting drug throughout history. Its history dates back to 3500 years ago. It was used by Sumerians and Egyptians for joint diseases. During these periods, it was obtained as extracts from the willow tree (especially its bark) and these extracts were applied topically [11]. Later, it was recorded that it was used during the times of Hippocrates and Galen, the famous scientists of the period, and it was used for analgesic purposes in a geography extending from South Africa to North America [12]. It is mentioned that it is used especially for joint pains and headaches during these periods. Later, in the third century, the use and effect of salicylates spread to the Far East [11].

In the 18th century, the willow tree was mentioned as "The Bark of an English Tree" by Lord Edward Stone. Lord mentioned its analgesic properties and suggested its use in the treatment of malaria [13]. In the following century, it was studied for its commercial form and its extract was obtained and put on the market [14]. The commercial form of acetylsalicylic acid spread throughout the world soon after it was produced and became widespread immediately. It was used by famous people during these periods. State leaders were also among these people. Even famous writers mentioned Aspirin® in their books and works. This fame made the drug itself the subject of textbooks. As the years progressed, it began to be produced in different ways by different manufacturers. However, information about the mechanism of action was very limited [11].

In the 1900s, Aspirin® and other NSAIDs began to be investigated in more detail, and a number of studies were conducted only in the later years of the 20th century [11]. In these studies, prostaglandins were studied for their mechanism of action. One of the most important of these was made by Sir John Robert Vane. The study was published in 1971 and focused on the mechanisms of action of aspirin-like drugs. The study mentioned, but was not limited to, the antipyretic, anti-inflammatory and analgesic effects of anti-inflammatory drugs. In addition, its bronchoconstrictor effects were also mentioned. In addition to the mechanism of action, gastrointestinal effects were mentioned as side effects. In this regard, peptic ulcers have been mentioned [15]. This study shed great light on the unknown mechanism of action of Aspirin® [11]. Despite such side effects, different drugs such as ibuprofen have also been discovered [2].

In the following years, the mechanism of action of Aspirin® became much clear and its antithrombotic effects began to be mentioned. This situation has set an important example for the uses of Aspirin® other than its anti-inflammatory properties. It has been shown that it can be used and even indicated in the treatment of cardiovascular diseases [11].

Today, it is known that Aspirin® is indicated for the treatment of many diseases and also has many side effects. Studies are progressing further than the mechanism of action, towards indication areas and reducing side effects. For this purpose, the aim is to determine appropriate dose ranges, determine the lowest effective dose ranges throughout the disease and use them correctly. Studies are being conducted on the use of Aspirin® in many areas beyond anti-inflammatory processes, from cardiovascular diseases to cancer treatment. In modern days, it is known as the most widely used drug in the world [11].

3. Mechanism of action

Although Aspirin® is a weaker analgesic than strong analgesics, it is a very effective analgesic for mild and moderate pain [4]. It is an ester of acetic acid hydrolyzed to salicylic acid by esterases found in the gastrointestinal tract and liver and is a weak acid [2].

Aspirin® is absorbed from the stomach and duodenum because it is lipophilic [12]. The absorption of the drug depends on many factors such as stomach pH and food intake [16]. It inhibits the biosynthesis of thromboxane A2 (TXA2) and other prostaglandins. TXA2 is a prostanoid produced by the oxidation of arachidonic acid produced from membrane phospholipids. Arachidonic acid, on the other hand, plays a role in the production of prostaglandin H2 (PGH2) and TXA2 through its cyclooxygenase (COX) activity [14]. They permanently inhibit COX function. Finally, they are cleared from the body by conjugation with glucuronic acid and glycine [17]. They are excreted from the body through the kidneys, and the excretion rate may vary depending on the urine pH [16]. As the urine pH increases, their excretion from the urine increases, and while they are excreted from the urine, they also reduce the pH of the urine [18].

Aspirin® is a phenol compound and it can not be adequately glucuronidated in cats due to glucuronyl transferase deficiency. However, dogs may tolerate Aspirin® better [2]. It is also suitable for use in cattle and goats. They are not preferred in newborns because biotransformation is insufficient and urinary excretion is limited. The absorption of enteric-coated Aspirin® is thought to be low [18].

4. Contraindications

Aspirin® may increase bleeding times and lead to anemia due to its antiplatelet effect [19]. Additionally, it should be used with caution in patients with respiratory diseases as it may cause oxidative damage and asthma [20]. Aspirin® causes blood pH to increase due to hyperventilation and associated CO_2 loss. This may result in respiratory alkalosis. The kidneys may cause metabolic acidosis by secreting bases such as sodium bicarbonate to reduce the increasing pH. Aspirin® causes blood pH to increase due to hyperventilation and associated CO_2 loss. This may result in respiratory alkalosis. The kidneys may cause metabolic acidosis by secreting bases such as sodium bicarbonate to reduce the increasing pH [19]. In addition, due to its undesirable effects such as gastrointestinal ulcers, its therapeutic index is very narrow and therefore it should be used with extreme caution. Compared to Aspirin®, the use of clopridogrel, which has fewer side effects in terms of its antithrombotic effect, is preferred. However, its combined use with Aspirin® has been reported to cause gastrointestinal side effects in humans [6].

Aspirin® can cause serious toxicities in the liver of cats [21]. Almost all NSAIDs can cause stomach damage. Most NSAIDs are acidic and damage the gastric mucosa. NSAIDs may reduce mucosal proliferation. However, whether mucosal proliferation can cause gastropathy is still a matter of debate. In particular, the local toxic properties of Aspirin® are known. Aspirin® causes fever, respiratory alkalosis, metabolic acidosis, stomach irritation and liver necrosis. Ataxia and seizures may occur at high doses. Additionally, electrolyte disorders and depression may occur [17]. At the same time, Aspirin® is assumed to be a hemolytic agent and may cause hypoprothrombinemia [21]. In human studies, it has been reported that Aspirin® may cause acute renal failure [22]. It can cause deterioration of kidney function in people with chronic kidney disease (CKD). CKD, the most common kidney disease in cats, is especially common in older cats and is irreversible. As a result of the disease, there is a decrease in kidney functions. Since it is a progressive disease, the aim is to slow the progression of the disease. The disease may also cause thrombosis. For this reason, its use is not recommended, especially in geriatric patients and patients with CKD [6]. Also NSAIDs can cause severe organ damage [16].

The reason why it causes stomach ulcers is thought to be related to the decrease in mucosal prostaglandin levels due to inactivation of the prostaglandin system [23]. It is thought that a barrier disorder occurs in the back diffusion of hydrogen ions of Aspirin®, which is absorbed through nonionic diffusion. As a result, lesions and injuries may occur due to the back diffusion of hydrogen ions into the mucosa. However, these hypotheses can not explain the gastric lesions formed in Aspirin® applications outside the gastrointestinal tract [24].

Aspirin® reduces prostaglandin formation and increases leukotriene formation, which has inflammatory effects. This makes the stomach non-resistant to simple ulcerogenic agents. It is also deacetylated with salicylate immediately after inhibition of COX activity. Aspirin® and salicylate are toxic to mucosal epithelial cells. Salicylate disrupts mucosal barrier function, reduces ATP and accelerates proton loss [20]. Aspirin® can also cause mucosal damage in the intestine [25].

Especially its use with corticosteroids or other NSAIDs increases the risk of stomach ulcers. For this reason, it should not be used with other NSAIDs [16]. Renal excretion may be delayed with some drugs, such as furosemide. In addition, drug effectiveness decreases when used together with Furosemide in heart diseases. It has been reported that it reduces or prevents the effectiveness of spironolactone [18].

In a study conducted in the past years, it was reported that intravenous Aspirin® administration alone did not cause stomach ulcers, but its combined use with histamine caused stomach ulcers [26]. In a similar study, it was reported that intravenous histamine and intravenous Aspirin® caused stomach ulcers equally in cats [27]. Additionally, the risk of erosion increases if there is ongoing bleeding in the stomach [28]. For this reason, it is recommended to take Aspirin® with or after a meal on a full stomach and use it together with stomach protectors such as proton pump inhibitors or H2-receptor antagonists [25].

It has been suggested that Aspirin® may cause hypoplasia in the bone marrow. In a study, severe anemia and Heinz bodies in red blood cells were observed in some cats treated with Aspirin®, and the cause of the anemia was thought to be due to gastrointestinal tract bleeding [21]. It is unsafe to use in pregnant animals and has teratogenic effects. It can also delay birth. In addition, there is a risk of bleeding in long-term use, it can pass through the placenta and decrease to fetal levels and even pass into milk [18].

NSAIDs should not be used in cats with sensitivity, anemia, stomach ulcers, asthma, and organ failure such as the liver, as they are eliminated from the body as a result of liver metabolism. In addition, it should be used with extreme caution in creatures that are dehydrated or have other abnormal clinical findings [16].

Gastric lavage, symptomatic and supportive treatment are applied for treatment of toxicity [17]. In fact, some veterinarians do not recommend prescribing Aspirin® to cats, even in low doses [21]. It should be used with caution, especially in elderly cats and cats with kidney or liver disease [12]. In addition, although its use in the treatment of pain, fever and inflammation is not clear enough, reports of toxicosis due to Aspirin® are very few [17,18].

5. Indications

NSAIDs are used specifically to treat inflammation. The concept of inflammation is the reaction of the tissue due to damage. This may be caused by microorganisms such as bacteria and viruses, but also by physical factors such as heat or trauma. In such cases, the analgesic properties of aspirin are used. However, aspirin prevents the body temperature from rising as a result of the inflamed area, due to its antipyretic properties [16]. It is recommended to use it in combination with opioids for pain management in cats. At the same time, its anti-inflammatory effect is used in the treatment of arthritis. The many therapeutic effects of aspirin are very advantageous for veterinary medicine [16].

It has been reported that the increase in body temperature caused by bacterial pyrogen was reduced from 40.2 °C to 39.30 °C with Aspirin® and the prostoglandin-like activity in the cerebrospinal fluid was reduced [29]. Pain management is a big problem in cats. Because cats are extremely sensitive to most drugs and most drugs are toxic to cats. However, Aspirin® can be used for short periods of time [30]. Its analgesic, anti-inflammatory and antithrombotic effective dose is administered enterally every 48–72 h [18].

Nowadays, its use in cats has become widespread, especially due to its antithrombotic effect [17]. It can be used in the treatment and even prevention of thrombosis that occurs as a result of heart and brain diseases [25]. It is also recommended to prevent thrombosis in diseases such as cardiomyopathy and heartworm [17]. By inhibiting the COX enzyme, NSAIDs suppress the production of TXA2, which is the activator of platelet aggregation [1].

Thromboembolism may occur as a result of cardiac, inflammatory, metabolic or neoplastic processes [31]. While in the systemic circulation, thrombi can occlude arterial segments and alter tissue perfusion. Treatment options are limited and relapses are common [32]. Aspirin® permanently inhibits the COX activities of PGH synthase-1 and PGH synthase-2 [31]. There are three different cyclooxygenase enzymes: COX-1, COX-2 and COX-3. While COX-1 plays a role in cell communication, COX-2 plays a role in the production of prostanoids responsible for inflammatory reactions in inflamed areas. As a result of acetylsalicylic acid inhibiting COX-2, analgesic and anti-inflammatory effects are observed. COX-3 plays a role in pain and increase in body temperature, especially in humans [16]. COX-1 and COX-2 catalyze the conversion of arachidonic acid to PGH2, the precursor of TXA2. TXA2 induces platelet aggregation and vasoconstriction. As a result, platelet function increases. Vascular endothelial cells convert PGH2 into prostacyclin (PGI2). PGI2 inhibits platelet aggregation and induces vasodilation. TXA2 is produced by platelets via COX-1. PGI2 is produced by the vascular system with COX-1 and COX-2. Aspirin® also creates permanent defects in TXA2 synthesis (**Figure 1**) [31].

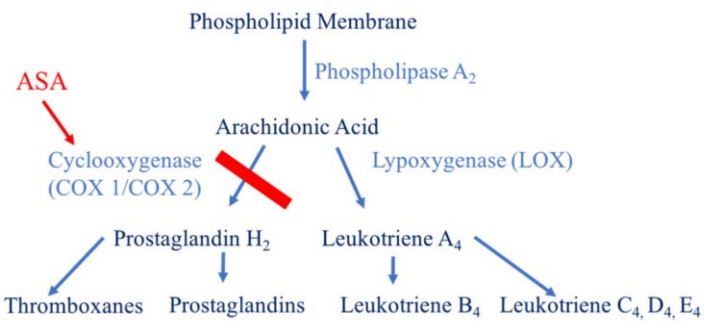


Figure 1. As a result of the binding of acetylsalicylic acid to COX-1 and COX-2, it causes the inhibition of protsaglandins and, as a result, platelet aggregation. ASA: Acetylsalicylic asid [33].

Hypertrophic cardiomyopathy is the most common disease that causes heart failure in cats. While the disease fundamentally changes the functioning of the heart, it also changes the level of trace elements. While copper levels are high in male cats, a study on canine dilated cardiomyopathy showed significant changes in selenium levels depending on age [34,35]. It has been suggested that platelets may be hyperreactive in cats with hypertrophic cardiomyopathy (HCM). On the other hand, it is suggested that although Aspirin® can not completely prevent platelet aggregation, it may limit it to some extent [1]. Aortic thromboembolism may develop in cats with HCM. The most important factors in this risk factor are left atrial enlargement and associated weakness of function. For this reason, the use of clopidogrel is recommended in cats with HCM and at risk of developing aortic thromboembolism. Clopidogrel is constantly spit out by cats due to its bad taste and also does not completely eliminate the risk of aortic thromboembolism. For this reason, it is recommended to use it in combination with another antithrombotic drug, especially aspirin [36]. Aspirin® may be considered as a treatment option in cats with left atrial enlargement. For this purpose, it is aimed to reduce platelet function [37]. However, Aspirin® can not be used alone as an antithrombotic agent in cats with cardiomyopathy [38]. It has been reported that the combination of clopidogrel and Aspirin® may be effective in the treatment of arterial thromboembolism [39]. Additionally, there are opinions arguing that clopidogrel is much more effective and better tolerated than Aspirin® [32]. Platelet levels are important in determining the risk of thrombus in cats with HCM. The decrease in platelet count increases the number of platelets and a hypercoagulative state occurs. Although the use of acetylsalicylic acid appears to be indicated in cats at risk of thrombus, further studies are recommended to evaluate the risk of thrombus in cats with HCM, particularly based on blood parameters such as platelets [40].

In some studies, it has been reported that Aspirin® increases the survival rate after endotoxin applications [41]. Other studies have also reported that Aspirin® inhibits acute vascular responses to endotoxins in cats [42]. In human cancer studies, the anti-cancer activity of Aspirin® has been suggested. Accordingly, it is thought that Aspirin®'s antithrombotic and anti-inflammatory effects may prevent cancer [43]. However, the platelet inhibition effectiveness of Aspirin® is still a matter of debate [37]. Aspirin also has pro-apoptotic effects. It destroys cancer stem cells and also inhibits the genes that cause cancer development. In this regard, it induces the arrest of G0/G1 cell cycle phases [44].

Clarence et al. (1990) examined the effect of Aspirin® in cats experimentally transplanted with heartworms, but no satisfactory response was obtained. However, as a result of the study, less arteriosclerosis was observed [45]. According to some studies, Aspirin® increases the absorption of ascorbic acid from the intestines, while other studies have observed that its excretion in the urine increases [46]. It has also been reported to suppress the development of lung lesions [41].

Aspirin® is recommended at a dose of 10–20 mg/kg twice a day for dogs and once every other day for cats. Toxicosis may not be observed in cats at doses of 25 mg/kg every other day [6]. In a study conducted on this subject, a clinical improvement of nearly 50% was reported with the use of Aspirin® at a dose of 25 mg/kg every three days in cats with thromboembolism [47]. The clinical efficacy of lower doses is still a matter of debate [6].

In one study, 35 cats received Aspirin® at doses ranging from low doses up to 200mg/kg, resulting in mucosal lesions, including ulcers. However, the frequency of lesions did not change in cats given low doses. Additionally, as a result of applications at a dose of 200 mg/kg, a decrease in body weight, anorexia and even death was observed. In the same study, death was observed even at relatively lower doses of 50mg/kg, and bronchopneumonia was found on necropsy [24].

6. Safer use of acetylsalicylic acid

Although acetylsalicylic acid is widely used in human and veterinary medicine, it has many side effects. The most discussed side effect in the literature is gastrointestinal mucosal damage. This carries the risk of damaging the gastric mucosa even at very low doses in cats and humans. For this reason, its administration to those at risk of bleeding, those who have previously had a stomach ulcer, and geriatric patients is limited [44]. Despite all this, there are many indications in both human and veterinary medicine. Therefore, using aspirin in safer doses or the production of a safer Aspirin® model is of great importance for human and veterinary medicine. Studies on this subject are not yet sufficient.

Despite the side effects of Aspirin®, various studies have been conducted, especially in human medicine. These studies were generally conducted for gastrointestinal effects. For this reason, instead of bolus administration, low-dose infusion applications were tried and as a result, a high degree of platelet selectivity was obtained. However, it has the potential to cause inflammation in the gastrointestinal tract. It has also been suggested that Aspirin® is absorbed through the skin as a result of transdermal applications and may reduce gastrointestinal side effects [48]. In addition, simultaneous use with proton pump inhibitors or H2 receptor antagonists is recommended [44].

A method developed against gastrointestinal side effects is the use of enteric-coated Aspirin tablets. It is thought that this type of Aspirin® tablets may cause less damage to the gastrointestinal mucosa. It has also been suggested that enteric-coated tablets will cause less erosion in long-term use [14]. It has also been reported that the cardiovascular prophylactic effect of enteric-coated Aspirin tablets remains sufficient [49]. However, TXA2 inhibition of enteric-coated Aspirin tablets is much lower than standard Aspirin tablets. Moreover, although it does not damage the gastric mucosa, it may cause mucosal erosion in the small intestine. This brings into focus the discussion that enteric-coated tablets have clinically significant improvements [50].

In one study, the prostaglandin analog misoprostol, which is used to prevent gastrointestinal lesions resulting from the use of NSAIDs in humans, was tested on dogs. It has been suggested that misoprostol inhibition of stomach acid production may be effective against the gastrointestinal side effects of NSAIDs. As a result, it has been reported that if misoprostol is given to dogs treated with aspirin, it can prevent the development of gastroduodenal lesions [51].

As a result, methods to reduce the gastrointestinal side effects of Aspirin include avoiding long-term use and simultaneous use of other anticoagulants or steroidal drugs. In addition to medications, the use of NSAIDs with alcohol or in the presence of bacterial infection increases the risk of gastrointestinal, mucosal damage and even bleeding. It is recommended to use it together with stomach protectors such as proton pump inhibitors or H2-antagonists [52]. Despite the negative effects of aspirin-induced COX inhibition, prostaglandin-inducing agents can be used or external prostaglandin application can be made [44]. Apart from these, the mechanisms associated with a safer form of aspirin are still under investigation. Discovery of these forms or mechanisms will be a ray of hope for many future treatments, especially in cats.

7. Conclusions

Acetylsalicylic acid is a non-steroidal anti-inflammatory drug used in a wide range of areas, from simple pain management to the treatment of complex cardiovascular complications. While it has a wide range of uses, especially in human medicine, its use is limited in cat practice because it can not be glucuronidated sufficiently. It is especially known to cause stomach ulcers. However, despite its contraindications, there are many indication areas in cats. It is possible to benefit from its antithrombotic effects in inflammatory and heart diseases. However, it should be used with caution due to its side effects. The effectiveness of aspirin needs to be studied further. We believe that if studies are carried out to reduce the toxic effects of the drug in cats, it will find use in the treatment of many diseases with correct dosing and use in the right situations.

Conflict of interest: The authors declare no conflict of interest.

References

1. Cathcart CJ, Brainard BM, Reynolds LR, et al. Lack of inhibitory effect of acetylsalicylic acid and meloxicam on whole blood platelet aggregation in cats. Journal of Veterinary Emergency and Critical Care 2011; 22(1): 99-106. https://doi.org/10.1111/j.1476-4431.2011.00681.x.
2. Khan SA, McLean MK. Toxicology of frequently encountered nonsteroidal anti-inflammatory drugs in dogs and cats. Veterinary Clinics of North America Small Animal Practice 2012; 42(2): 289-306. https://doi.org/10.1016/j.cvsm.2012.01.003.
3. Fuentes VL, Abbott J, Chetboul V, et al. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. Journal of veterinary internal medicine 2020; 34(3): 1062-1077. https://doi.org/10.1111/jvim.15745.
4. Vane JR and Botting RM. The mechanism of action of aspirin. Thrombosis research 2003; 110(5-6): 255-258. https://doi.org/10.1016/s0049-3848(03)00379-7.
5. Albo AG, Nebbia C. Incidence of Poisonings in Domestic Carnivores in Italy. LVII Annual Meeting of the Italian Society for Veterin Ary Sciences (SISVET); 2003 Ischia; Veterinary Research Communications; 2004. p.83-88.
6. De Santis F, Boari A, Dondi F, et al. Drug-Dosing Adjustment in Dogs and Cats with Chronic Kidney Disease. Animals 2022; 12(3): 1-29. https://doi.org/10.3390/ani12030262.
7. Lautz LS, Jeddi MZ, Girolami F, et al. Metabolism and pharmacokinetics of pharmaceuticals in cats (Felix sylvestris catus) and implications for the risk assessment of feed additives and contaminants. Toxicology Letters 2020; 338: 114-127. https://doi.org/10.1016/j.toxlet.2020.11.014.
8. Shrestha B, Reed JM, Starks PT, et al. Evolution of a Major Drug Metabolizing Enzyme Defect in the Domestic Cat and Other Felidae: Phylogenetic Timing and the Role of Hypercarnivory. PloS ONE 2011; 6(3): 1-11 . https://doi.org/10.1371/journal.pone.0018046.
9. Court MH. Feline drug metabolism and disposition: pharmacokinetic evidence for species differences and molecular mechanisms. The Veterinary clinics of North America Small animal practice 2013; 43(5): 1039–1054. https://doi.org/10.1016/j.cvsm.2013.05.002.
10. Lynch A, Shaw H and Milton GW. Effect of aspirin on gastric secretion. GUT 1964; 5(3): 230-236. https://doi.org/10.1136/gut.5.3.230.
11. Montinari MR, Minelli S, De Caterina R. The first 3500 years of aspirin history from its roots – A concise summary. Vascular Pharmacology 2018; 113: 1-8. https://doi.org/10.1016/j.vph.2018.10.008
12. Gupta RC. Veterinary Toxicology: Basic and Clinical Principals, 3rd ed. London, UK: Academic Press of Elsevier; 2018.
13. Stone E. XXXII. An account of the success of the bark of the willow in the cure of agues. In a letter to the Right Honourable George Earl of Macclesfield, President of R. S. from the Rev. Mr. Edward Stone, of Chipping-Norton in Oxfordshire. Philosophical Transactions of the Royal Society of London 1763; 53: 195-200. https://doi.org/10.1098/rstl.1763.0033
14. Awtry EH, Loscalzo J. Aspirin. Circulation 2000; 101(10): 1206-1218. https://doi.org/10.1161/01.CIR.101.10.1206.
15. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature: New biology, 1971; 231(25): 232-235. https://doi.org/10.1038/newbio231232a0
16. Karademir Ü, Boyacıoğlu M. Nonsteroidal Antiinflamatuvar İlaçların Kedi ve Köpeklerde Etkili ve Güvenli Kullanımı. Journal of Faculty of Veterinary Medicine, Erciyes University 2014; 11(2): 137-143.

17. Lascelles BDX, Court MH, Hardie EM, et al. Nonsteroidal anti-inflammatory drugs in cats: a review. Veterinary Anaesthesia and Analgesia 2007; 34: 228-250. https://doi.org/10.1111/j.1467-2995.2006.00322.x.
18. Maddison JE, Page SW and Church DB. Small Animal Clinical Pharmacology, 2nd ed. Edinburgh: Saunders Elsevier; 2008.
19. Whalan JE. A Toxicologist's Guide to Clinical Pathology in Animals: Hematology, Clinical Chemistry, Urinalysis, Switzerland: Springer International; 2015.
20. Wallig MA, Haschek WM, Rousseaux CG, et al. Fundamentals of Toxicologic Pathology, 3rd ed. London: Academic Press, Elsevier; 2018.
21. Penny RH, Carlisle CH, Prescott CW, et al. Effects of aspirin (acetylsalicylic acid) on the haemopoietic system of the cat. The British veterinary journal 1967; 123(4): 154-161. https://doi.org/10.1016/s0007-1935(17)40054-6.
22. D'Agati V. Does aspirin cause acute or chronic renal failure in experimental animals and in humans?. American Journal of Kidney Diseases 1996; 28(1): S24-S29. https://doi.org/10.1016/S0272-6386(96)90565-X.
23. Konturek SJ, Radecki T, Brzozowski T, et al. Aspirin-induced gastric ulcers in cats. Prevention by prostacyclin. Digestive diseases and sciences 1981; 26(11): 1003-1012. https://doi.org/10.1007/BF01314763.
24. Bugat R, Thompson MR, Aures D, et al. Gastric mucosal lesions produced by intravenous infusion of aspirin in cats. Gastroenterology 1976; 71(5): 754-759.
25. Satoh H, Amagease K and Takeuchi K. The role of food for the formation and prevention of gastrointestinal lesions induced by aspirin in cats. Digestive diseases and sciences 2013; 58(10): 2840-2849. https://doi.org/10.1007/s10620-013-2725-7.
26. Hansen DG, Aures D and Grossman MI. Histamine augments gastric ulceration produced by intravenous aspirin in cats. Gastroenterology 1978; 74(3): 540-543.
27. Hansen DF, Aures D and Grossman MI. Comparison of Intravenous and Intragastric Aspirin in Production of Antral Gastric Ulcers in Cats. Proceedings Of The Society For Experimental Biology And Medicine 1980; 164(4): 589-592. https://doi.org/10.3181/00379727-164-40921.
28. Svanes K, Leiknes KA, Varhaug JE, et al. Aspirin damage to ischemic gastric mucosa in shocked cats. Scandinavian journal of gastroenterology 1979; 14(5): 633-639. https://doi.org/10.3109/00365527909181402.
29. Feldberg W, Gupta KP, Milton AS, et al. Effect of pyrogen and antipyretics on prostaglandin activity in cisternal c.s.f. of unanaesthetized cats. The Journal of Physiology 1973; 234(2): 279-303. https://doi.org/10.1113/jphysiol.1973.sp010346.
30. Hardie EM. Management of Osteoarthritis in Cats. Veterinary Clinics of North America: Small Animal Practice 1997; 27(4): 945-953. https://doi.org/10.1016/S0195-5616(97)50088-X.
31. Lunsford KV, Mackin AJ. Thromboembolic therapies in dogs and cats: an evidence-based approach.T he Veterinary clinics of North America: Small animal practice 2007; 37(3): 579-609. https://doi.org/10.1016/j.cvsm.2007.01.010.
32. Hogan DF, Fox PR, Jacob K, et al. Secondary prevention of cardiogenic arterial thromboembolism in the cat: The double-blind, randomized, positive-controlled feline arterial thromboembolism; clopidogrel vs. aspirin trial (FAT CAT). Journal of veterinary cardiology: the official journal of the European Society of Veterinary Cardiology 2015; 17(1): S306-S317. https://doi.org/10.1016/j.jvc.2015.10.004.
33. Etscheidt J, Shahien A, Gainey M, et al. Review of Therapeutic Options for the Prevention of VTE in Total Joint Arthroplasty. Geriatrics 2020; 5(1): 1-15. https://doi.org/10.3390/geriatrics5010018.
34. Bilgic B, Tarhan D, Ekiz B, et al. The effect of pimobendan and enalapril use on blood serum trace element levels in dogs with myxomatous mitral valve disease. Journal of Trace Elements and Minerals 2023, 4: 1-8. https://doi.org/10.1016/j.vph.2018.10.008.
35. Sedláčková K, Száková J, Načeradská M, et al. Essential microelement (copper, selenium, zinc) status according to age and sex in healthy cats. Acta veterinaria Hungarica 2022; 70(4): 296-304. https://doi.org/10.1556/004.2022.00036.
36. Rishniw M. How much protection does clopridogrel provide to cats with hypertrophic cardiomyopathy?. Journal of the American Veterinary Medical Association 2024; 1-3. https://doi.org/10.2460/javma.24.04.0269.
37. Ho KH, Abrams-Ogg AC, Wood RD, et al. Assessment of platelet function in healthy cats in response to commonly prescribed antiplatelet drugs using three point-of-care platelet function tests. Journal of feline medicine and surgery 2017; 19(6): 638-647. https://doi.org/10.1177/1098612X16648182.
38. Blais MC, Bianco D, Goggs R, et al. Consensus on the Rational Use of Antithrombotics in Veterinary Critical Care (CURATIVE): Domain 3-Defining antithrombotic protocols. Journal of veterinary emergency and critical care 2019; 29(1): 60-74. https://doi.org/10.1111/vec.12795.

39. Borgeat K, Wright J, Garrod O, et al. Arterial thromboembolism in 250 cats in general practice: 2004-2012. Journal of veterinary internal medicine 2014; 28(1): 102-108. https://doi.org/10.1111/jvim.12249.
40. Çolakoğlu EÇ, Alihosseini H, Haydardedeoğlu AE. Hipertrofik Kardiyomyopatili Kedilerde Trombosit ve Ortalama Trombosit Hacmi Düzeyleri Erken Emboli Oluşumunu Belirlemede Kullanılabilir mi?. Harran Üniversitesi Veteriner Fakültesi Dergisi 2018; 7(1): 84-88. https://doi.org/10.31196/huvfd.470810.
41. Greenway CV, Murthy VS. Mesenteric vasoconstriction after endotoxin administration in cats pretreated with aspirin. British journal of pharmacology 1971; 43(2): 259-269.
42. Hall RC, Hodge RL, Irvine R, et al. The effect of aspirin on the response to endotoxin. The Australian journal of experimental biology and medical science 1972; 50(5): 589-601. https://doi.org/10.1038/icb.1972.52.
43. Riciotti E, Wangensteen KJ, FitzGerald GA. Aspirin in hepatocellular carcinoma. Cancer Research 2021; 81(14): 3751-3761. https://doi.org/10.1158/0008-5472.CAN-21-0758.
44. Kim MY, Han YM, Park JM, et al. GI-Safer Aspirin: Sometimes Sugar Coating Helps. Digestive diseases and sciences 2016; 61(8): 2151-2153. https://doi.org/10.1007/s10620-016-4236-9.
45. Rawlings CA, Farrell RL, Mahood RM. Morphologic changes in the lungs of cats experimentally infected with Dirofilaria immitis: Response to aspirin. Journal of veterinary internal medicine 1990; 4(6): 292-300. https://doi.org/10.1111/j.1939-1676.1990.tb03126.x.
46. Daş N, Nebioğlu S. Vitamin C aspirin interactions in laboratory animals. Journal of clinical pharmacy and therapeutics 1992; 17(6): 343-346. https://doi.org/10.1111/j.1365-2710.1992.tb01315.x
47. Dion PD. Feline aortic thromboemboli and the potential utility of thrombolytic therapy with tissue plasminogen activator. The Veterinary clinics of North America. Small animal practice 1988; 18(1): 79-86. https://doi.org/10.1016/s0195-5616(88)50008-6.
48. Keimowitz RM, Pulvermacher G, Mayo G, et al. Transdermal modification of platelet function. A dermal aspirin preparation selectively inhibits platelet cyclooxygenase and preserves prostacyclin biosynthesis. Circulation 1993; 88(2): 556-561. https://doi.org/10.1161/01.cir.88.2.556
49. Hawthorne AB, Mahida YR, Cole AT, et al. Aspirin-induced gastric mucosal damage: prevention by enteric-coating and relation to prostaglandin synthesis. British journal of clinical pharmacology 1991; 32(1): 77-83. https://doi.org/10.1111/j.1365-2125.1991.tb05616.x.
50. Clerici B, Cattaneo M. Pharmacological Efficacy and Gastrointestinal Safety of Different Aspirin Formulations for Cardiovascular Prevention: A Narrative Review. Journal of cardiovascular development and disease 2023; 10(4): 1-11. https://doi.org/10.3390/jcdd10040137.
51. Johnston SA, Leib MS, Forrester SD, et al. The effect of misoprostol on aspirin-induced gastroduodenal lesions in dogs. Journal of veterinary internal medicine 1995; 9(1): 32-38. https://doi.org/10.1111/j.1939-1676.1995.tb03269.x.
52. Tarnawski AS, Caves TC. Aspirin in the XXI century: its major clinical impact, novel mechanisms of action, and new safer formulations. Gastroenterology 2004; 127(1): 341-343. https://doi.org/10.1053/j.gastro.2004.05.037

Journal of Toxicological Studies 2024, 2(2), 1667.
https://doi.org/10.59400/jts.v2i2.1667

Review

The impact of pesticides: Assessing residue persistence, environmental contamination, and human health risks

Aline Viancelli*, Caroline Comelli, Cheila Maria Nogara, Vanessa De Araujo, William Michelon

Universidade do Contestado, Concórdia 89711-330, Brazil
*** Corresponding author:** Aline Viancelli, alinevbortoli@gmail.com

CITATION

Viancelli A, Comelli C, Nogara CM, et al. The impact of pesticides: Assessing residue persistence, environmental contamination, and human health risks. Journal of Toxicological Studies. 2024; 2(2): 1667.
https://doi.org/10.59400/jts.v2i2.1667

ARTICLE INFO

Received: 29 August 2024
Accepted: 21 October 2024
Available online: 29 October 2024

Abstract: The intensification of agricultural practices to meet global food demand has led to extensive pesticide use, which poses significant challenges for food safety, environmental health, and human well-being. This narrative review provides a comprehensive analysis of the global use of pesticides in agriculture, focusing on the persistence of pesticide residues in food crops, their environmental impacts, and the associated health risks. Historically, pesticides have been integral to agricultural productivity, but their adverse effects have become increasingly clear. Notably, pesticide residues in food can pose serious health risks, particularly to vulnerable populations such as children and pregnant women. This review also discusses regional disparities in pesticide-related health outcomes, with a focus on Brazil. The findings underscore the urgent need for sustainable pest management practices, including organic farming and improved regulatory measures, to mitigate the adverse effects of pesticide use. By integrating these strategies, a more balanced and sustainable agricultural system can be achieved, safeguarding both human health and environmental quality.

Keywords: pesticide residues; environmental health; sustainable agriculture

1. Introduction

The intensification of agricultural practices, driven by the need to meet growing global food demand, has led to the extensive application of pesticides [1]. Pesticides have become an integral component of modern agriculture, applied to protect crops from pests, weeds, and diseases by killing or restricting the population expansion of some organisms [2]. While these pesticides have undoubtedly contributed to increased agricultural productivity, their widespread use raises serious concerns regarding their residues in food, environmental contamination, and potential health risks to humans [3,4].

The persistence of pesticide residues in vegetables and other food products poses a direct threat to consumer health. Numerous studies have documented the presence of these residues in market-ready products, often exceeding recommended safety levels [5–8]. Exposure can lead to adverse health effects, particularly in vulnerable populations such as children, pregnant women, and agricultural workers [9]. Beyond direct human exposure, pesticides also have significant environmental implications, contaminating soil, water, and non-target organisms, further complicating the assessment of their safety and long-term impacts [10–13].

Given the increasing awareness of their harmful effects on both the environment and human health, this review is significant as it provides a comprehensive assessment of the global use of pesticides, their persistence in food crops, and the associated health and environmental risks. By highlighting the alarming prevalence of pesticide residues and their potential impacts on vulnerable populations, this work aims to raise awareness among stakeholders, including policymakers and the agricultural community, about the critical need for safer pesticide management practices. Ultimately, this review serves as a call to action for adopting sustainable agricultural practices that safeguard human health and the environment.

2. Global use of pesticides in agriculture

The use of pesticides in agriculture has a long history that dates back to ancient civilizations where natural substances like sulfur, arsenic, and plant extracts were employed to protect crops from pests and diseases [14]. However, the advent of synthetic chemical pesticides in the mid-20th century marked a turning point in agricultural practices worldwide. The introduction of compounds such as DDT (dichlorodiphenyl-trichloroethane) and organophosphates revolutionized crop protection, leading to significant increases in agricultural productivity [15–17]. These chemicals quickly became the cornerstone of pest management strategies, allowing farmers to control various pests with efficacy. However, in 1962, Rachel Carson published the book "Silent Spring", highlighting the harmful effects of DDT on the environment and human health, which made some states prohibit the use of DDT [18].

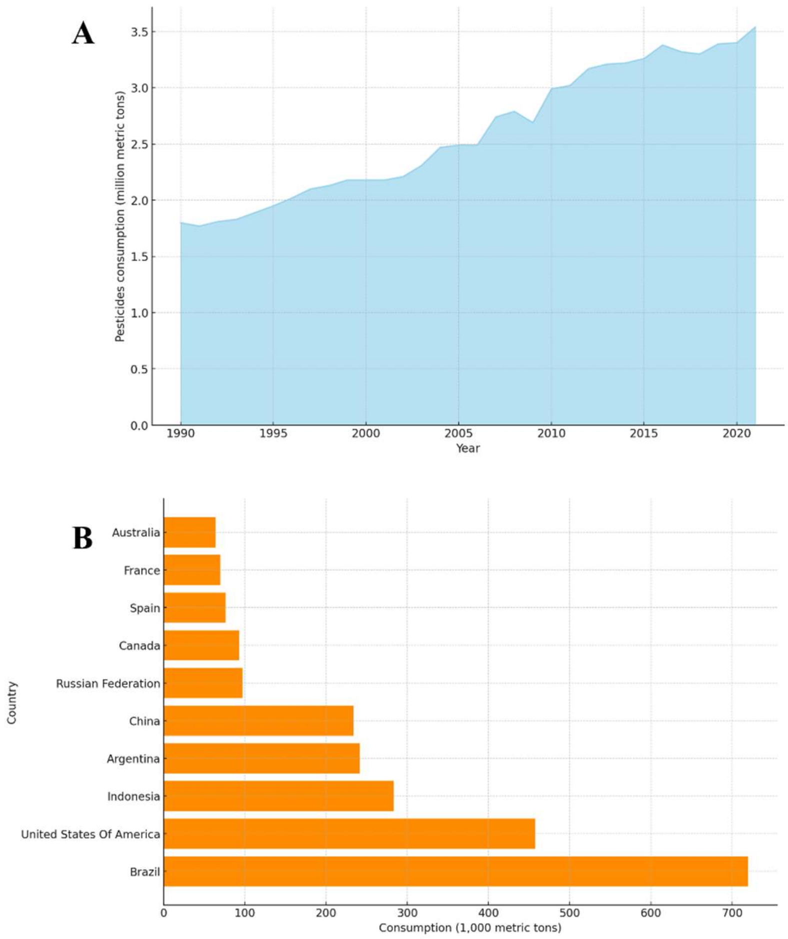


Figure 1. Pesticide consumption worldwide. **(A)** and country ranking of consumption of pesticides; **(B)** from 1990 to 2021.

The Green Revolution of the 1960s further accelerated the global adoption of other pesticides, such as high-yield crop varieties, coupled with the intensive use of agrochemicals, which were promoted to combat food insecurity in developing countries [14]. This period saw a dramatic increase in pesticide production and usage, particularly in countries with rapidly expanding agricultural sectors [19].

In recent decades, the global pesticide market has continued to grow (**Figure 1A**) [20]. Brazil, the United States, and Indonesia were the biggest consumers of pesticides (**Figure 1B**). Brazil applied 719,507 tons of pesticides in 2021, followed by the United States (457,385 tons) and Indonesia (283,297 tons). Herbicides, insecticides, and fungicides remain the most commonly used classes of pesticides, with glyphosate, in particular, becoming the most widely applied herbicide globally [21].

Considering the vast quantities of pesticides applied annually, it is important to evaluate their potential impacts on living organisms across all environmental compartments.

A study by Maggi et al. [22] mapped the global indexes of pesticide hazards, population exposure, and human intake dose in 133 nations. All the indexes showed hotspots in the Americas, Asia, and Europe; however, it is worth highlighting that in Europe (EU27), the legislation is strict on pesticide use. The study also revealed that 1.7 billion people live near pesticide application areas with pesticide loads above 100 kg km-2 year-1, 2.3 billion exceed the acceptable pesticide intake, and 15% of them exceed by 10 fold.

The study by Tang et al. [23] reported that 64% of global agricultural land is at risk of pesticide pollution involving more than one active ingredient. Additionally, 31% of this land is at high risk, meaning pesticide residues exceed no-effect concentrations by three orders of magnitude. Among the high-risk areas, 34% are located in regions of high biodiversity, 5% in water-scarce areas, and 19% in low- and lower-middle-income countries. Watersheds in South Africa, China, India, Australia, and Argentina were identified as high-concern regions due to high pesticide pollution risk, rich biodiversity, and water scarcity. This study stands out by evaluating multiple active ingredients and integrating risks across different environmental compartments on a global scale.

The widespread use of pesticides continues to raise significant concerns regarding environmental pollution and public health risks worldwide. These findings underscore the urgent need for more stringent regulations, better management practices, and global collaboration to mitigate the impacts of pesticide pollution and safeguard both ecosystems and human populations.

3. Pesticide residue persistence in vegetables

Pesticide residues in food crops, particularly vegetables, are a significant concern due to their potential impacts on human health [24]. The persistence of these residues is influenced by a complex interplay of factors that determine the length of time that a pesticide remains in or on the plant after application [25,26]. Every year, the Environmental Working Group (EWG), a nonprofit organization from the USA, publishes a list of the 12 vegetables with the most pesticide residues [27]. In 2024, the vegetables were: strawberries (1°), spinach (2°), kale, collard and mustard greens (3°), grapes (4°), peaches (5°), pears (6°), nectarines (7°), apples (8°), bell and hot peppers (9°), cherries (10°), blueberries (11°), and green beans (12°) [27].

One of the primary factors influencing pesticide residue persistence is the chemical nature of the pesticide itself. Pesticides can vary widely in their stability, solubility, and volatility, which directly affect their behavior in the environment and within the plant. For instance, highly lipophilic pesticides tend to accumulate in the waxy cuticle of plant leaves, leading to longer persistence. In contrast, hydrophilic pesticides may be more readily washed off by rain or irrigation; however, they can also be absorbed more easily by the plant's roots and transported throughout its tissues [28].

The mode of application influenced the residue levels. Foliar applications, in which pesticides are sprayed directly onto the leaves, often result in higher residue levels on the surface of the vegetable. A study conducted by Juraske et al. [29] evaluated the application of the insecticide imidacloprid in tomatoes, and the results showed that the total residues were up to five times higher in plants treated by foliar spray application than by soil irrigation.

In contrast, systemic pesticides, which are absorbed by the plant and translocated throughout its tissues, can lead to residues within the plant itself [30]. The timing of the application relative to the harvest period is another key factor; pesticides applied closer to harvest are more likely to leave residues that persist until the crop reaches the consumer [29].

Environmental conditions such as temperature, humidity, and sunlight also significantly impact pesticide residue persistence [31]. Higher temperatures can accelerate the degradation of some pesticides, reducing their persistence, while others may become more stable under these conditions. Ultraviolet (UV) radiation from sunlight can break down certain pesticide molecules, but some compounds may degrade into more toxic byproducts, complicating residue management [31]. Soil composition and microbial activity further influence the degradation or persistence of pesticides, particularly for those absorbed through the roots [32].

Vegetable characteristics, such as the type of crop, growth stage, and presence of protective structures like leaves or husks, also affect residue persistence [33]. Leafy vegetables, for example, often have higher residue levels due to their large surface area and exposure to pesticide sprays [24]. Root vegetables, on the other hand, may accumulate residues differently because pesticides in the soil can be absorbed directly by the roots [34].

4. Pesticide contamination of soil and water

When pesticides are applied to crops, a portion inevitably reaches the soil, where it can either be absorbed by plants, leach into groundwater, or run off into surface waters such as rivers, lakes, and streams [34]. The extent of soil and water contamination depends on various factors, including the chemical properties of the pesticide, soil composition, climate conditions, and agricultural practices [35].

In soil, pesticides can persist for a long time depending on their degradation rates, which are influenced by factors such as temperature, moisture, and microbial activity [36]. Some pesticides, particularly those with high persistence, pose a risk of accumulation over time, affecting soil health and fertility [37]. Additionally, pesticides can bind to soil particles and be transported by erosion, further spreading contamination to adjacent ecosystems [35].

A study by Silva et al. [38] investigated the contamination by pesticide residues in soil collected from 11 countries members of the European Union. The results showed that 80% of the tested soils were contaminated by pesticide residues in a total of 166 different pesticide combinations [38].

Water resources are particularly vulnerable to pesticide contamination from runoff from agricultural fields [39]. A study by Andrade et al. [40] evaluated river water before and after rainfall during the pesticide application in agricultural regions of Argentina. The results showed that pesticide concentrations in the rivers increased immediately after rainfall events. However, the authors also observed that pesticide concentrations can change depending on the crop life cycle, the pesticide solubility, the area slope, and the percentage of riparian forest [40].

This contamination poses risks not only to aquatic ecosystems but also to human populations that use these water sources for drinking and irrigation [1]. Pesticides in water can persist in the environment, sometimes forming harmful byproducts that exacerbate their toxic effects [41]. The contamination of water resources by pesticides is a critical concern because it can lead to the bioaccumulation of toxic substances in aquatic organisms, which may then be transferred up the food chain, ultimately affecting human health [1].

Clasen et al. [42] evaluated the bioaccumulation of five pesticides during fish growth in consortium with rice. The results showed that after 100 days of exposure, the pesticides lambda-cyhalothrin and tebuconazole bioaccumulated in carp muscles. Additionally, pesticides induce oxidative stress on fish, indicating the potential risk to other organisms [42].

The ecological consequences of pesticide accumulation underscore the need for sustainable pest management practices that minimize environmental contamination and protect biodiversity. Strategies such as integrated pest management, organic farming, and the development of less persistent and targeted pesticides are essential for reducing the environmental impact of pesticide use and ensuring the health of ecosystems [43].

5. Effects on non-target species and biodiversity

Pesticides are designed to target specific pests, but their effects often extend to non-target species, including beneficial insects, plants, and animals. This non-selectivity can have severe consequences for biodiversity, disrupting ecological balances and leading to the decline of important species [44]. For example, pollinators such as bees and butterflies, which are important for the reproduction of many plants, can be affected by pesticide exposure, leading to declines in their populations [45,46]. Similarly, predators and parasitoids that naturally control pest populations can be adversely affected, reducing the effectiveness of biological pest control and potentially leading to pest outbreaks [47].

Aquatic ecosystems are particularly sensitive to pesticide contamination. Fish, amphibians, and aquatic invertebrates can be directly affected by pesticide exposure, leading to reduced survival, reproductive failure, and altered behavior [1]. The loss of these species can disrupt food webs and cause cascading effects throughout the ecosystem. Downing et al. [48] observed the impact of pesticides in a mesocosmic aquatic ecosystem, where zooplankton richness, diversity, abundance, and oxygen concentrations decreased and remained with significant differences even after 40 days.

6. Pesticide occurrence in the atmosphere

The atmospheric presence of pesticides has become an increasingly critical issue [49]. Pesticides can enter the atmosphere through various pathways, including volatilization during and after application, drift during aerial spraying, and atmospheric deposition from agricultural fields [50]. Once in the atmosphere, these chemicals can be transported over considerable distances, often affecting ecosystems and communities far from their original localization [51].

Recent studies have detected a range of pesticides in air samples collected from rural and urban areas [49,52]. For instance, volatile organic compounds such as chlorpyrifos and diazinon have been identified in the air, even in regions with no direct agricultural activity [53]. This phenomenon raises significant concerns about the potential for human exposure to these toxic substances, particularly among populations living near agricultural fields [54].

Factors influencing the volatilization and atmospheric transport of pesticides include temperature, humidity, and wind speed [50]. Higher temperatures can enhance volatilization rates, increasing the likelihood of pesticide dispersal into the air. Additionally, wind can facilitate the movement of pesticide particles, thereby contributing to their spread over large areas [50].

Atmospheric deposition is another important mechanism through which pesticides can pollute land and water resources. Pesticides in the atmosphere can be removed by precipitation, which leads to the contamination of soil and surface water [55]. This process, often referred to as “pesticide rain”, can lead to the accumulation of harmful chemicals in ecosystems, affecting plant and animal life [52]. For example, studies have shown that pesticides can be found in rainwater collected in agricultural regions, raising concerns about the long-term effects of pesticides on soil health and water quality [56,57].

A previous study identified the widespread presence of organochlorine pesticides in the air of urban areas in Southeast Brazil, particularly in the regions of Rio de Janeiro and São Paulo [58]. The most prevalent organochlorine pesticides detected were Σ-HCH and Σ-DDT, both still registered for domestic sanitation purposes in Brazil. Concentrations of these pollutants tended to be higher during the summer months [58]. This seasonal fluctuation suggests that environmental conditions, such as increased temperatures, may enhance the volatilization and dispersion of these chemicals, further elevating the exposure risk in urban areas. A key aspect of the research was the assessment of health risks, specifically cancer risks associated with inhaling organochlorine pesticides. The findings indicated that individuals living in the studied regions face increased cancer risks, with infants and children showing the highest vulnerability [58].

Another study conducted in both urban and rural areas of São Paulo, Brazil, evaluated pesticides associated with particles measuring 2.5 micrometers or smaller. The findings revealed that in rural areas, the most frequently detected pesticides were λ-cyhalothrin, kresoxim-methyl, and atrazine. In contrast, permethrin and malathion were most commonly found in urban and industrial locations [59].

The impact of atmospheric pesticide occurrence is complex. Understanding the dynamics of pesticide occurrence in the atmosphere is essential for developing effective monitoring and regulatory strategies aimed at protecting public health and the environment.

7. Human health risks from pesticide exposure

The initial enthusiasm for these "miracle" chemicals soon gave way to concerns about their environmental and health impacts [60]. Pesticide exposure can cause acute and chronic health effects, depending on the level, duration, and frequency of exposure. Acute exposure typically occurs through direct contact with pesticides during application, accidental ingestion, or inhalation. The symptoms of acute pesticide poisoning can range from mild, such as headaches, and nausea, to severe, including respiratory distress, convulsions, and even death in extreme cases [61].

Organophosphates and carbamates, which are commonly used insecticides, are notorious for their acute toxicity, as they inhibit acetylcholinesterase, an enzyme essential for normal nervous system function [62]. As a consequence, the patient presents with muscle paralysis affecting particularly upper-limb muscles, neck flexors, and cranial nerves some 24–96 h after organophosphates exposure and is often associated with the development of respiratory failure [62].

Chronic exposure to pesticides, often resulting from prolonged, low-level exposure, can lead to various long-term health issues. These conditions may include respiratory problems, skin disorders, and chronic neurological conditions [61]. Chronic pesticide exposure has been associated with reproductive and developmental effects, such as reduced fertility, congenital disabilities, and developmental delays in children [63]. The latency period between exposure and the manifestation of chronic conditions can make it challenging to establish direct causal relationships, further complicating risk assessment and management.

Considering that Brazil is the largest consumer of pesticides, below is reported data from intoxication by pesticides, which most frequently occurs by contact during agricultural practices. **Figure 2** provides data on intoxication cases around Brazilian states over the years 2013 to 2023 reported by the Health Ministry—DATASUS system (https://datasus.saude.gov.br/).

It is possible to conclude that states such as Minas Gerais, São Paulo, and Paraná consistently show the highest notification rates across all years. These states are major economic centers with significant agricultural and industrial activities, which could explain their higher levels of reporting. The infrastructure and resources available in these states may contribute to better data collection and reporting mechanisms. In contrast, states such as Acre, Amapá, and Roraima show significantly lower notification rates. These regions are characterized by lower population densities and more remote locations, which could hinder data collection and reporting. The low notification rates may not accurately reflect reality but rather highlight the challenges in monitoring and reporting in these areas.

The observed disparities suggest the need for more equitable distribution of resources and efforts to improve data collection in underreported regions. Enhancing monitoring infrastructure in less developed states could lead to more accurate and comprehensive data.

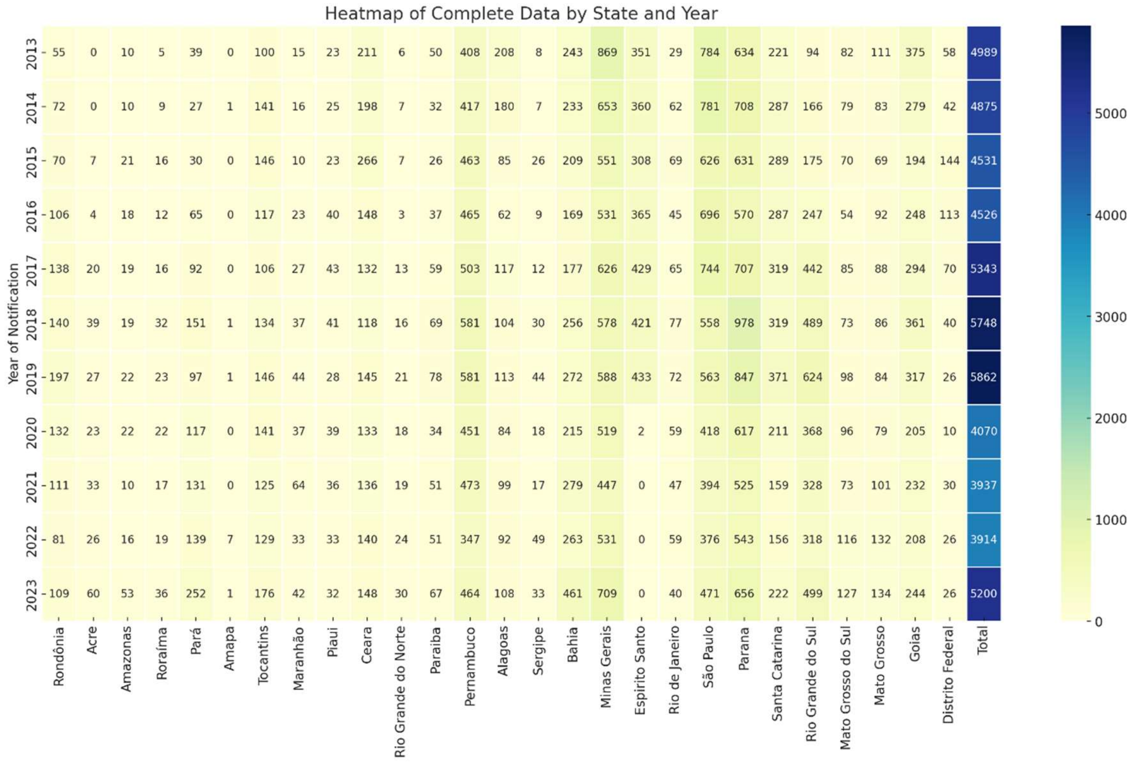


Figure 2. Occurrence of notification by intoxication with pesticides used in agriculture reported in Brazilian States from 2013 to 2023. Source: DATASUS.

Figure 3 illustrates deaths across Brazil from 2013 to 2023. The analysis reveals significant regional disparities that highlight the varying impacts of health-related challenges across the country. Pernambuco, in the Northeast region, stands out with the highest number of deaths, accounting for 382 fatalities. In the southeast region, São Paulo and Minas Gerais have reported 189 and 172 deaths, respectively. The Southern region, particularly Paraná, has a high number of deaths, with 231 fatalities reported. In contrast, the Northern and Central-Western regions, while showing lower absolute numbers of deaths, reflect concerning trends in states like Rondônia (89 deaths) and Goiás (45 deaths).

The high mortality rate could be attributed to a combination of factors, including socioeconomic conditions, healthcare infrastructure, or poor careful practices during pesticide application [64]. In any event, this concentration of deaths underscores the need for targeted public health interventions to address the underlying causes.

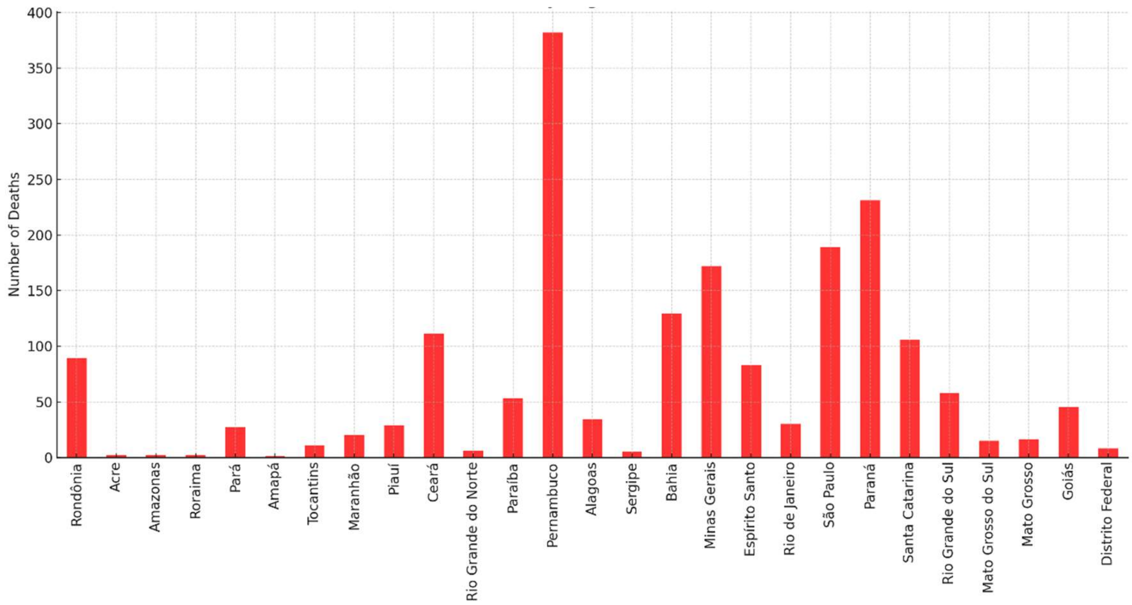


Figure 3. Occurrence of deaths caused by intoxication by pesticides of agricultural use in Brazil, from 2013 to 2023.

Agricultural workers, pesticide applicators, and residents of rural areas near agricultural fields are among the most at-risk groups due to their frequent and direct contact with pesticides [65]. Occupational exposure to pesticides can be particularly hazardous because workers may be exposed to concentrated forms of these chemicals, often without adequate protective measures [66]. Studies indicate that agricultural workers are at a high risk of various chronic conditions, including cancer, mental, neurological, endocrine, renal, auditory, respiratory, and autoimmune diseases. Additionally, they are susceptible to subclinical effects, such as genetic damage and biochemical alterations, as well as clinical signs and symptoms of acute intoxication. These studies also highlight several limitations, particularly in the assessment of exposure and outcomes, as well as in study design and sampling [67,68].

In their study, Pignati et al. [69] reported that pesticide workers from Mato Grosso State (Brazil) were 20 to 49 years old, often lacked formal qualifications, and were employed as machine operators, mechanics, and agricultural workers. Additionally, they live less than 500 meters from agricultural fields and handle herbicides. Additionally, Pluth et al. [70] reported a higher incidence of cancer in people living in rural areas with extensive use of pesticides.

Children are another vulnerable population because of their developing bodies and behaviors that increase their exposure risk, such as playing on treated surfaces or consuming residues from food. Their metabolic pathways are also less developed, making them less capable of detoxifying and eliminating pesticides from their bodies [71]. Pregnant women are similarly at risk because pesticide exposure during pregnancy can affect fetal development, leading to adverse birth outcomes such as low birth weight, preterm birth, and congenital disabilities [72].

8. Strategies to reduce pesticide risks

Organic and sustainable farming practices play an important role in reducing pesticide risks by minimizing or eliminating the use of synthetic pesticides and promoting ecological balance in agricultural systems [73]. In Brazil, the Fazenda Malunga, located in the Federal District of Brazil, serves as a model for sustainable agriculture through its production of organic vegetables. Established in the 1980s, the farm has become a leader in pesticide-free food production, supplying fresh produce to various markets across the Central-West region of Brazil. The farms' practices emphasize environmental stewardship, social equity, and economic viability, aligning with the principles of organic farming. Furthermore, Fazenda Malunga contributes to environmental education by offering guided tours that educate visitors about organic agriculture and sustainable practices [74].

One of the primary benefits of organic farming is the reduction in pesticide residues in food and in the environment, which directly translates into lower health risks for consumers and reduced environmental contamination. By fostering natural pest control mechanisms, such as the presence of beneficial insects and microorganisms, organic farming reduces the reliance on chemical interventions. Additionally, organic practices often lead to improved soil health, which can enhance crop resilience to pests and diseases, further decreasing the need for pesticides.

The widespread use of pesticides in modern agriculture, while instrumental in enhancing crop yields and combating pests, presents significant challenges related to food safety, environmental contamination, and human health. The persistence of pesticide residues in food and the environment poses direct health risks to consumers, especially vulnerable groups. The widespread contamination of soil and water resources further complicates the issue, resulting in ecosystem degradation and potential long-term impacts on biodiversity.

Addressing pesticide contamination necessitates not only the implementation of regulatory measures but also the exploration of innovative remediation techniques. Among these, the use of magnetic graphene oxide (MGO) has gained significant attention owing to its superior adsorption properties and ease of separation from contaminated media [75]. MGO exhibits a high surface area and functional groups that enhance its affinity for various pesticide molecules, enabling its effective removal from both water and soil matrices. The incorporation of magnetic nanoparticles into graphene oxide facilitates the rapid separation of the adsorbent using an external magnetic field, significantly reducing the time and labor associated with traditional filtration methods [76].

Recent studies have demonstrated the efficacy of MGO in adsorbing common agricultural pesticides, such as glyphosate and organophosphates [77], with its adsorption capacities surpassing those of conventional adsorbents. Furthermore, the functionalization of MGO can be tailored to enhance its selectivity for specific pesticide classes, improving its overall efficiency in real-world applications.

In addition to MGO, alternative methods, such as phytoremediation, which utilizes specific plant species to uptake and detoxify pesticides from contaminated soils, show promise. Plants like sunflowers have been identified as effective pesticide residue accumulators, enabling their safe disposal through harvesting [78]. Furthermore, advanced oxidation processes, including ozonation and photocatalysis, use reactive oxygen species to degrade pesticide contaminants into less harmful byproducts, thus enhancing the safety of agricultural runoff [79].

Collectively, these innovative approaches, including the use of magnetic graphene oxide, provide strategies for addressing pesticide residues. They not only mitigate the environmental and health impacts of pesticide use but also contribute to the development of sustainable agricultural practices. The integration of these technologies could serve as a vital component of comprehensive pesticide management programs, ensuring the safety of food systems and the protection of ecological health.

Addressing these concerns requires a multidisciplinary approach that includes the adoption of sustainable agricultural practices, such as organic farming. Efforts to improve pesticide management, enforce safety regulations, and enhance public awareness are essential to mitigate the adverse effects associated with pesticide use. By integrating these strategies, we can work toward a more balanced and sustainable agricultural system that protects both human health and the environment.

Conflict of interest: The author declares no conflict of interest.

References

1. Hayes TB, Hansen M. From silent spring to silent night: Agrochemicals and the anthropocene. Science of the Anthropocene. 2017; 5: 57. doi: 10.1525/elementa.246
2. Aftab T. Emerging Contaminants and Plants. Springer International Publishing; 2023. doi: 10.1007/978-3-031-22269-6
3. Narenderan ST, Meyyanathan SN, Babu B. Review of pesticide residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques. Food Research International. 2020; 133: 109141. doi: 10.1016/j.foodres.2020.109141
4. Rani L, Thapa K, Kanojia N, et al. An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production. 2021; 283: 124657. doi: 10.1016/j.jclepro.2020.124657
5. Chawla P, Kaushik R, Shiva Swaraj VJ, et al. Organophosphorus pesticides residues in food and their colorimetric detection. Environmental Nanotechnology, Monitoring & Management. 2018; 10: 292-307. doi: 10.1016/j.enmm.2018.07.013
6. Khan N, Yaqub G, Hafeez T, et al. Assessment of Health Risk due to Pesticide Residues in Fruits, Vegetables, Soil, and Water. Journal of Chemistry. 2020; 2020: 1-7. doi: 10.1155/2020/5497952
7. Mac Loughlin TM, Peluso ML, Etchegoyen MA, et al. Pesticide residues in fruits and vegetables of the Argentine domestic market: Occurrence and quality. Food Control. 2018; 93: 129-138. doi: 10.1016/j.foodcont.2018.05.041
8. Philippe V, Neveen A, Marwa A, et al. Occurrence of pesticide residues in fruits and vegetables for the Eastern Mediterranean Region and potential impact on public health. Food Control. 2021; 119: 107457. doi: 10.1016/j.foodcont.2020.107457
9. Mehmood Y, Arshad M, Kaechele H, et al. Pesticide residues, health risks, and vegetable farmers' risk perceptions in Punjab, Pakistan. Human and Ecological Risk Assessment: An International Journal. 2020; 27(3): 846-864. doi: 10.1080/10807039.2020.1776591
10. Agrawal A, Pandey RS, Sharma B. Water Pollution with Special Reference to Pesticide Contamination in India. Journal of Water Resource and Protection. 2010; 02(05): 432-448. doi: 10.4236/jwarp.2010.25050
11. Raheem WS, Niamah A. Contamination methods of milk with pesticides residues and veterinary drugs. IOP Conference Series: Earth and Environmental Science. 2021; 877(1): 012003. doi: 10.1088/1755-1315/877/1/012003
12. Shukla S, Mostaghimi S, Shanholt VO, et al. A County-Level Assessment of Ground Water Contamination by Pesticides. Groundwater Monitoring & Remediation. 2000; 20(1): 104-119. doi: 10.1111/j.1745-6592.2000.tb00257.x
13. Vischetti C, Casucci C, De Bernardi A, et al. Sub-lethal effects of pesticides on the DNA of soil organisms as early ecotoxicological biomarkers. Frontiers in Microbiology. 2020; 11: 1892.
14. Abubakar Y, Tijjani H, Egbuna C, et al. Pesticides, History, and Classification. Natural Remedies for Pest, Disease and Weed Control. Published online 2020: 29-42. doi: 10.1016/b978-0-12-819304-4.00003-8
15. Metcalf RL. Century of DDT. Journal of Agricultural and Food Chemistry. 1973; 21(4): 511-519. doi: 10.1021/jf60188a040

16. Tudi M, Daniel Ruan H, Wang L, et al. Agriculture Development, Pesticide Application and Its Impact on the Environment. International Journal of Environmental Research and Public Health. 2021; 18(3): 1112. doi: 10.3390/ijerph18031112
17. Zhang Q, Xia Z, Wu M, et al. Human health risk assessment of DDTs and HCHs through dietary exposure in Nanjing, China. Chemosphere. 2017; 177: 211-216. doi: 10.1016/j.chemosphere.2017.03.003
18. Carson R. Silent Spring. Houghton Mifflin Harcourt; 1962.
19. Pimentel D. Green revolution agriculture and chemical hazards. Science of the Total Environment. 1996; 188: S86-S98.
20. Ritchie H, Roser M, Rosado P. Pesticides. Available online: https://ourworldindata.org/pesticides (accessed on 23 October 2024).
21. Maggi F, la Cecilia D, Tang FHM, et al. The global environmental hazard of glyphosate use. Science of The Total Environment. 2020; 717: 137167. doi: 10.1016/j.scitotenv.2020.137167
22. Maggi F, Tang FHM, Black AJ, et al. The pesticide health risk index - An application to the world's countries. Science of The Total Environment. 2021; 801: 149731. doi: 10.1016/j.scitotenv.2021.149731
23. Tang FHM, Lenzen M, McBratney A, et al. Risk of pesticide pollution at the global scale. Nature Geoscience. 2021; 14(4): 206-210. doi: 10.1038/s41561-021-00712-5
24. Ngabirano H, Birungi G. Pesticide residues in vegetables produced in rural south-western Uganda. Food Chemistry. 2022; 370: 130972. doi: 10.1016/j.foodchem.2021.130972
25. Ellgehausen H, Guth JA, Esser HO. Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food chains. Ecotoxicology and Environmental Safety. 1980; 4(2): 134-157.
26. Mrema EJ, Rubino FM, Brambilla G, et al. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology. 2013; 307: 74-88. doi: 10.1016/j.tox.2012.11.015
27. EWG. EWG's 2024 Shopper's Guide to Pesticides in Produce™. Available online: https://www.ewg.org/foodnews/dirty-dozen.php (accessed on 23 October 2024).
28. Krähmer H, Walter H, Jeschke P, et al. What makes a molecule a pre- or a post-herbicide – how valuable are physicochemical parameters for their design? Pest Management Science. 2021; 77(11): 4863-4873. doi: 10.1002/ps.6535
29. Juraske R, Castells F, Vijay A, et al. Uptake and persistence of pesticides in plants: Measurements and model estimates for imidacloprid after foliar and soil application. Journal of Hazardous Materials. 2009; 165(1-3): 683-689. doi: 10.1016/j.jhazmat.2008.10.043
30. Mendes KF, Da Silva AA. Applied Weed and Herbicide Science. Springer International Publishing; 2022. doi: 10.1007/978-3-031-01938-8
31. Yigit N, Velioglu YS. Effects of processing and storage on pesticide residues in foods. Critical Reviews in Food Science and Nutrition. 2019; 60(21): 3622-3641. doi: 10.1080/10408398.2019.1702501
32. Tarla DN, Erickson LE, Hettiarachchi GM, et al. Phytoremediation and Bioremediation of Pesticide-Contaminated Soil. Applied Sciences. 2020; 10(4): 1217. doi: 10.3390/app10041217
33. Liu Q, Liu Y, Dong F, et al. Uptake kinetics and accumulation of pesticides in wheat (Triticum aestivum L.): Impact of chemical and plant properties. Environmental Pollution. 2021; 275: 116637. doi: 10.1016/j.envpol.2021.116637
34. Ju C, Dong S, Zhang H, et al. Subcellular distribution governing accumulation and translocation of pesticides in wheat (Triticum aestivum L.). Chemosphere. 2020; 248: 126024. doi: 10.1016/j.chemosphere.2020.126024
35. Srivastav AL. Chemical fertilizers and pesticides: role in groundwater contamination. Agrochemicals Detection, Treatment and Remediation. Published online 2020: 143-159. doi: 10.1016/b978-0-08-103017-2.00006-4
36. Aslam S, Iqbal A, Lafolie F, et al. Mulch of plant residues at the soil surface impact the leaching and persistence of pesticides: A modelling study from soil columns. Journal of Contaminant Hydrology. 2018; 214: 54-64. doi: 10.1016/j.jconhyd.2018.05.008
37. Sabzevari S, Hofman J. A worldwide review of currently used pesticides' monitoring in agricultural soils. Science of The Total Environment. 2022; 812: 152344. doi: 10.1016/j.scitotenv.2021.152344
38. Silva V, Mol HGJ, Zomer P, et al. Pesticide residues in European agricultural soils – A hidden reality unfolded. Science of The Total Environment. 2019; 653: 1532-1545. doi: 10.1016/j.scitotenv.2018.10.441
39. Malla MA, Gupta S, Dubey A, et al. Contamination of groundwater resources by pesticides. Contamination of Water. Published online 2021: 99-107. doi: 10.1016/b978-0-12-824058-8.00023-2

40. Andrade VS, Gutierrez MF, Regaldo L, et al. Influence of rainfall and seasonal crop practices on nutrient and pesticide runoff from soybean dominated agricultural areas in Pampean streams, Argentina. Science of The Total Environment. 2021; 788: 147676. doi: 10.1016/j.scitotenv.2021.147676
41. Dara D, Drabovich AP. Assessment of risks, implications, and opportunities of waterborne neurotoxic pesticides. Journal of Environmental Sciences. 2023; 125: 735-741. doi: 10.1016/j.jes.2022.03.033
42. Clasen B, Loro VL, Murussi CR, et al. Bioaccumulation and oxidative stress caused by pesticides in Cyprinus carpio reared in a rice-fish system. Science of The Total Environment. 2018; 626: 737-743. doi: 10.1016/j.scitotenv.2018.01.154
43. Deguine JP, Aubertot JN, Flor RJ, et al. Integrated pest management: good intentions, hard realities. A review. Agronomy for Sustainable Development. 2021; 41(3). doi: 10.1007/s13593-021-00689-w
44. Serrão JE, Plata-Rueda A, Martínez LC, et al. Side-effects of pesticides on non-target insects in agriculture: a mini-review. The Science of Nature. 2022; 109(2). doi: 10.1007/s00114-022-01788-8
45. Nath R, Singh H, Mukherjee S. Insect pollinators decline: an emerging concern of Anthropocene epoch. Journal of Apicultural Research. 2022; 62(1): 23-38. doi: 10.1080/00218839.2022.2088931
46. Stuligross C, Williams NM. Past insecticide exposure reduces bee reproduction and population growth rate. Proceedings of the National Academy of Sciences. 2021; 118(48). doi: 10.1073/pnas.2109909118
47. Sánchez-Bayo F. Indirect Effect of Pesticides on Insects and Other Arthropods. Toxics. 2021; 9(8): 177. doi: 10.3390/toxics9080177
48. Downing AL, DeVanna KM, Rubeck-Schurtz CN, et al. Community and ecosystem responses to a pulsed pesticide disturbance in freshwater ecosystems. Ecotoxicology. 2008; 17(6): 539-548. doi: 10.1007/s10646-008-0211-3
49. Guida Y, Pozo K, de Carvalho GO, et al. Occurrence of pyrethroids in the atmosphere of urban areas of Southeastern Brazil: Inhalation exposure and health risk assessment. Environmental Pollution. 2021; 290: 118020. doi: 10.1016/j.envpol.2021.118020
50. Boonupara T, Udomkun P, Khan E, et al. Airborne Pesticides from Agricultural Practices: A Critical Review of Pathways, Influencing Factors, and Human Health Implications. Toxics. 2023; 11(10): 858. doi: 10.3390/toxics11100858
51. Fu J, Fu K, Chen Y, et al. Long-Range Transport, Trophic Transfer, and Ecological Risks of Organophosphate Esters in Remote Areas. Environmental Science & Technology. 2021; 55(15): 10192-10209. doi: 10.1021/acs.est.0c08822
52. Capella R, Guida Y, Loretto D, et al. Occurrence of legacy organochlorine pesticides in small mammals from two mountainous National Parks in southeastern Brazil. Emerging Contaminants. 2023; 9(2): 100211. doi: 10.1016/j.emcon.2023.100211
53. Zhao M, Wu J, Figueiredo DM, et al. Spatial-temporal distribution and potential risk of pesticides in ambient air in the North China Plain. Environment International. 2023; 182: 108342. doi: 10.1016/j.envint.2023.108342
54. Dereumeaux C, Fillol C, Quenel P, et al. Pesticide exposures for residents living close to agricultural lands: A review. Environment International. 2020; 134: 105210. doi: 10.1016/j.envint.2019.105210
55. Cui S, Fu Y, Zhou B, et al. Transfer characteristic of fluorine from atmospheric dry deposition, fertilizers, pesticides, and phosphogypsum into soil. Chemosphere. 2021; 278: 130432. doi: 10.1016/j.chemosphere.2021.130432
56. Hamers T, Smit MGD, Murk AJ, Koeman JH. Biological and chemical analysis of the toxic potency of pesticides in rainwater. Chemosphere. 2001; 45(4-5): 609-624. doi: 10.1016/S0045-6535(01)00017-0
57. Kumari B, Madan VK, Kathpal TS. Pesticide residues in rain water from Hisar, India. Environmental Monitoring and Assessment. 2007; 133(1-3): 467-471. doi: 10.1007/s10661-006-9601-2
58. Guida Y, de Carvalho GO, Capella R, et al. Atmospheric Occurrence of Organochlorine Pesticides and Inhalation Cancer Risk in Urban Areas at Southeast Brazil. Environmental Pollution. 2021; 271: 116359. doi: 10.1016/j.envpol.2020.116359
59. Yera AMB, Vasconcellos PC. Pesticides in the atmosphere of urban sites with different characteristics. Process Safety and Environmental Protection. 2021; 156: 559-567. doi: 10.1016/j.psep.2021.10.049
60. Karunarathne A, Gunnell D, Konradsen F, et al. How many premature deaths from pesticide suicide have occurred since the agricultural Green Revolution? Clinical Toxicology. 2019; 58(4): 227-232. doi: 10.1080/15563650.2019.1662433
61. Ye M, Beach J, Martin J, et al. Occupational Pesticide Exposures and Respiratory Health. International Journal of Environmental Research and Public Health. 2013; 10(12): 6442-6471. doi: 10.3390/ijerph10126442
62. Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. Handbook of Clinical Neurology. 2015; 131: 149-168.

63. Frazier LM. Reproductive disorders associated with pesticide exposure. Journal of Agromedicine. 2007; 12(1): 27-37. doi: 10.1300/J096v12n01_04
64. Buralli RJ, Ribeiro H, Leão RS, et al. Knowledge, attitudes and practices of the Brazilian family farmers on exposure to pesticides. Saude e Sociedade. 2021, 30: e210103.
65. Tudi M, Li H, Li H, et al. Exposure Routes and Health Risks Associated with Pesticide Application. Toxics. 2022; 10(6): 335. doi: 10.3390/toxics10060335
66. Damalas C, Koutroubas S. Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention. Toxics. 2016; 4(1): 1. doi: 10.3390/toxics4010001
67. Nogueira FAM, Szwarcwald CL, Damacena GN. Exposure to pesticides and health problems in rural workers: what does the literature reveal? (Portuguese). Revista Brasileira de Saúde Ocupacional. 2020; 45. doi: 10.1590/2317-6369000041118
68. Ristow LP, Battisti IDE, Stumm EMF, et al. Factors related to the occupational health of farmers exposed to pesticides (Portuguese). Saúde e Sociedade. 2020; 29(2). doi: 10.1590/s0104-12902020180984
69. Pignati WA, Soares MR, de Lara SS, et al. Exposure to pesticides, self-reported health conditions and Popular Health Surveillance in municipalities in Mato Grosso (Portuguese). Saúde em Debate. 2022; 46(2): 45-61. doi: 10.1590/0103-11042022e203
70. Pluth TB, Zanini LAG, Battisti IDE, et al. Epidemiological profile of cancer patients from an area with high pesticide use. Saúde em Debate. 2020; 44(127): 1005-1017. doi: 10.1590/0103-1104202012705
71. Buralli RJ, Dultra AF, Ribeiro H. Respiratory and Allergic Effects in Children Exposed to Pesticides—A Systematic Review. International Journal of Environmental Research and Public Health. 2020; 17(8): 2740. doi: 10.3390/ijerph17082740
72. Enderle I, Costet N, Cognez N, et al. Prenatal exposure to pesticides and risk of preeclampsia among pregnant women: Results from the ELFE cohort. Environmental Research. 2021; 197: 111048. doi: 10.1016/j.envres.2021.111048
73. Gamage A, Gangahagedara R, Gamage J, et al. Role of organic farming for achieving sustainability in agriculture. Farming System. 2023; 1(1): 100005. doi: 10.1016/j.farsys.2023.100005
74. Madureira JG. Overcoming obstacles and innovation: a case study of Fazenda Malunga (Portuguese). Universidade de Brasília; 2009.
75. Bruckmann FS, Schnorr C, Oviedo LR, et al. Adsorption and Photocatalytic Degradation of Pesticides into Nanocomposites: A Review. Molecules. 2022; 27(19): 6261. doi: 10.3390/molecules27196261
76. da Silva Bruckmann F, Rhoden CRB. Applications of magnetic graphene oxide in water decontamination. Analytical Applications of Graphene Oxide. Published online 2024: 687-703. doi: 10.1016/bs.coac.2023.10.002
77. Bouzidi M, Alwadai N, Al Huwayz M, et al. Efficient removal of organophosphate insecticide employing magnetic chitosan-derivatives. International Journal of Biological Macromolecules. 2024; 279: 134992. doi: 10.1016/j.ijbiomac.2024.134992
78. Mitton FM, Gonzalez M, Monserrat JM, et al. Potential use of edible crops in the phytoremediation of endosulfan residues in soil. Chemosphere. 2016; 148: 300-306. doi: 10.1016/j.chemosphere.2016.01.028
79. Malakootian M, Shahesmaeili A, Faraji M, et al. Advanced oxidation processes for the removal of organophosphorus pesticides in aqueous matrices: A systematic review and meta-analysis. Process Safety and Environmental Protection. 2020; 134: 292-307. doi: 10.1016/j.psep.2019.12.004

Academic Publishing Pte. Ltd.

Add: 73 Upper Paya Lebar Road, #07-02B-01, Centro Bianco, Singapore 534818
Tel: +65 83184869
E-mail: editorial_office@acad-pub.com
Web: http://ojs.acad-pub.com/