Bio-Detoxification of Toxins

Herbal and Probiotic Approacbes

Ranging from Food Safety to Infant Health

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Tuğba DEMİR

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Biodetoxification of Toxins: Herbal and Probiotic Approaches Ranging from Food Safety to Infant Health

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PREFACE

Chemical contaminants emerging at various stages of the food chain have evolved into an invisible yet profound public health concern in modern societies. This book addresses major risk categories ranging from heavy metals and mycotoxins to antibiotic residues and process contaminants together with their toxicological impacts and, in particular, natural detoxification strategies. Plant-derived compounds and probiotic microorganisms, acting as “natural barriers,” are not merely topics of laboratory research but increasingly stand out as feasible and sustainable solutions in real-world applications.

It is hoped that this work will serve both as a scientific reference and a decision-support framework for researchers, policy makers, and practitioners in the field of food safety.

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Tuğba DEMİR

Table of Contents

Biodetoxification of Toxins: Herbal and Probiotic Approaches Ranging from Food Safety to Infant Health 1

PREFACE 3

CHAPTER 1: 8

Natural Detoxification Approaches in Food Safety: From Plant Extracts to Probiotic Adsorbents 8

1.Introduction 8

Figure 1. Overview of Natural Detoxification Approaches in Food Safety: Plant Extracts and Probiotic Adsorbents 10

1.1. Natural Detoxification of Heavy Metals 10

1.1.1. Plant-Derived Detoxification Strategies 11

1.2. Natural Detoxification of Mycotoxins 16

1.2.1. Plant-Based Detoxification Strategies 17

1.3. Natural Mitigation of Antibiotic Residues 23

1.3.1. Plant-Based and Natural Adsorbent Approaches 23

1.4. Natural Mitigation of Acrylamide Formation 29

1.4.1. Plant-Derived Strategies for Acrylamide Mitigation 29

1.4.2. Probiotic and Enzymatic Approaches 32

1.4.3. Applications and Practical Constraints 34

2. Conclusion 36

3. References 39

CHAPTER 2: 42

Biodetoxification Capacities of *Lactobacillus* Species 42

1.Introduction 42

2. Mycotoxins (AFB_1 and AFM_1) and *Lactobacillus* Species 44

2.1. AFM_1 Removal Capacity of *Lactobacillus* and Its Practical Significance in Dairy Products 45

3. Heavy Metals (Pb, Cd, Zn, Ni) and *Lactobacillus* Species..... 46

3.1. Species-Specific Variability in Heavy Metal Binding by *Lactobacillus*..... 47

3.2. Species-Specific Affinity of *Lactobacillus* Toward Different Heavy Metals 49

4. Polycyclic Aromatic Hydrocarbons (PAHs) and *Lactobacillus* Species..... 49

4.1. Influence of pH and Environmental Factors on PAH Binding by *Lactobacillus*..... 50

4.2. Mechanistic Insights into PAH Detoxification by *Lactobacillus*..... 51

5. Biodetoxification Mechanisms: Binding and Biotransformation 54

6. Variability in Cell Wall Components and Reversibility of Adsorption 55

7. Impacts on Human Health 55

7.1. Protective Effects Against Heavy Metals and Immunomodulatory Benefits 56

8. Future Research Directions and Biotechnological Advances... 60

9. Conclusion..... 61

10. References 62

CHAPTER 3: 65

Toxicokinetics of Heavy Metals in Infants..... 65

1. Introduction: 65

The Unique Toxicological Vulnerability of the Pediatric Population 65

Figure 1. Heavy Metal Toxicokinetics in Infants (ADME Process) 66

1.1. Scope and Definition of Critical Toxic Metals (Lead, Cadmium, Inorganic Arsenic, Mercury) 66

1.2. Etiology of Dietary Exposure: Sources and Regulatory Concerns..... 67

1.3. Ontogenic Differences in Infants Affecting Toxicokinetics (Physiological Context) 68

2. Bioavailability and Gastrointestinal Absorption Mechanisms..... 69

2.1. Speciation and Bioavailability: Why Chemical Form Matters 70

2.2. Uptake Mechanisms via Enterocytes: Essential Nutrient Competition 70

2.3. Immature Gastrointestinal Barrier and Permeability..... 71

2.4. The Role of the Infant Gut Microbiota 71

3. Distribution Kinetics and Accumulation in Target Organs 72

3.1. General Distribution Dynamics: Tissue Tropism 72

3.2. Neurodevelopmental Risk: Blood-Brain Barrier (BBB) Crossing 73

3.3. Distribution in Developing Tissues: Bone, Kidney, and Liver Reserves..... 73

Bone sequestration (Lead). 73

4. Metabolism and Intracellular Detoxification Pathways..... 74

4.1. Biotransformation of Heavy Metals (Example: Arsenic Methylation)..... 74

4.2. Intracellular Binding Proteins: The Role of Metallothionein (MT) in Sequestration 75

4.3. Oxidative Stress as a Central Toxic Mechanism..... 75

5. Elimination and Excretion in the Developing Infant..... 76

5.1. Renal Elimination (Urinary Excretion)..... 76

5.2. Biliary and Fecal Elimination..... 77

6. Quantitative Modeling and Risk Assessment 77

6.1. Principles of Physiologically Based Pharmacokinetic (PBPK) Modeling in Pediatrics 78

6.2. Application of PBPK Models to Estimate Heavy Metal Exposure 78

6.3. Limitations and Regulatory Context 79

7. Conclusion, Mitigation Strategies, and Regulatory Recommendations 79

7.1. Synthesis of Bioavailability and Toxicokinetic Differences in Infants 79

7.2. Public Health Implications and Nutritional Mitigation Strategies 81

7.3. Current Regulatory Frameworks and Future Action Levels 82

8. References 84

CHAPTER 1:

Natural Detoxification Approaches in Food Safety: From Plant Extracts to Probiotic Adsorbents

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1.Introduction

One of the major challenges in food safety is the unintended presence of chemical contaminants in food products. Heavy metals, mycotoxins, antibiotic residues and acrylamide not only compromise product safety and quality but also pose serious long-term risks to human health. These contaminants may enter the food chain through multiple routes: heavy metals can accumulate in soil and water due to environmental pollution and subsequently transfer to plant- and animal-derived foods; mycotoxins are secondary metabolites produced by filamentous fungi during storage and frequently occur in commodities such as cereals and nuts; antibiotic residues may remain in meat, milk, eggs or honey as a consequence of veterinary drug use; and acrylamide is a process-induced toxicant formed in carbohydrate-rich foods exposed to high temperatures. Many of these contaminants exhibit toxic, genotoxic or carcinogenic properties and are associated with immune dysregulation, neurotoxicity and reproductive impairment. Chronic exposure to heavy metals such as cadmium and lead, for example, has been linked to

immunosuppression, endocrine disturbances and neurological dysfunction (Mehrandish et al., 2019). Likewise, aflatoxins are recognized as potent hepatocarcinogens (Liu et al., 2022). Antibiotic residues, even at low doses, can elicit allergic reactions or contribute to antimicrobial resistance, while acrylamide is monitored closely in processed foods due to its documented neurotoxic and suspected carcinogenic effects (Liu et al., 2015).

Conventional strategies employed to reduce food contaminants include the use of chemical binders, physical separation techniques, and the adjustment of processing parameters. For example, chemical chelating agents may be administered in cases of heavy metal intoxication; clay minerals or activated carbon are widely applied as adsorbent additives in the feed industry to mitigate mycotoxins; and mitigation of acrylamide formation can be attempted by lowering frying/baking temperatures or by employing industrial enzymes such as asparaginase. However, these approaches have notable limitations: chelation therapy carries the risk of adverse effects, classical adsorbents may non-selectively remove valuable nutrients, and enzyme-based interventions are often costly. These limitations have intensified interest in natural detoxification strategies. Plant-derived compounds (e.g., polyphenols, dietary fibers, essential oils) and probiotic microorganisms (particularly lactic acid bacteria such as *Lactobacillus* spp. and beneficial yeasts such as *Saccharomyces* spp.) have been extensively investigated for their ability to neutralize foodborne toxicants. These natural agents act through multiple mechanisms, including chemical binding, biotransformation into less toxic derivatives, inhibition of intestinal absorption, or enhancement of excretion. Importantly, such biological strategies often provide targeted detoxification without damaging nutrients or introducing synthetic additives (Liu et al., 2022). In this chapter, we provide an in-depth examination of natural detoxification strategies relevant to food safety. Focusing on four major contaminant classes heavy metals, mycotoxins, antibiotic residues, and acrylamide we discuss how these toxicants can be neutralized using plant-derived compounds and probiotic-based adsorbents. For each contaminant, we address the underlying mechanisms of natural detoxification, the influence of environmental and processing conditions on efficacy, and practical applications in food processing and human nutrition. We also critically evaluate the potential limitations and implementation

challenges of these strategies. All arguments are grounded in current scientific evidence with particular emphasis on peer-reviewed studies and reviews published in recent years. In the context of food safety, natural detoxification encompasses a broad spectrum of approaches, ranging from the antioxidant and toxin-binding capabilities of plant extracts to the microbial adsorption properties of probiotic adsorbents (Figure 1).

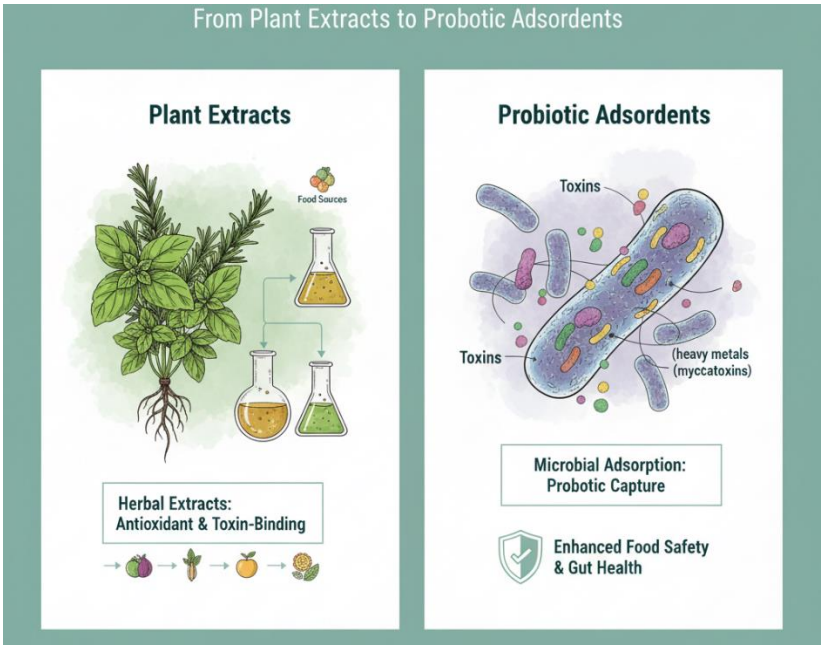

Figure 1. Overview of Natural Detoxification Approaches in Food Safety: Plant Extracts and Probiotic Adsorbents

1.1. Natural Detoxification of Heavy Metals

Heavy metals are elements that tend to accumulate in the human body and exert toxic effects when exposure exceeds certain thresholds (e.g., lead, cadmium, mercury, arsenic). Contamination of foods with heavy metals typically originates from environmental pollution and is observed in both plant- and animal-derived products. Chronic ingestion of heavy metals has been associated with severe health outcomes

including neurological impairment, renal and hepatic dysfunction, immunosuppression, and carcinogenesis (Mehrandish et al., 2019). Consequently, either reducing the levels of heavy metals in foods or immobilizing them in the gastrointestinal tract to prevent systemic absorption is of critical importance. The following sections examine plant-derived compounds and probiotic microorganisms as natural strategies for heavy metal detoxification.

1.1.1. Plant-Derived Detoxification Strategies

A growing body of evidence indicates that certain medicinal and aromatic plants exert protective or remedial effects against heavy metal toxicity due to their phytochemical constituents. Many plant-derived compounds act as natural chelating agents that bind metal ions and form insoluble complexes. For instance, garlic (*Allium sativum*) is rich in organosulfur compounds (e.g., alliin, allicin and their derivatives), which have been shown to bind lead, cadmium and mercury, thereby reducing their tissue accumulation. Experimental studies have demonstrated that regular garlic intake lowers hepatic, renal and testicular metal burden in exposed animals and increases metal excretion via urine and feces. This protective action is attributed to both the metal-binding capacity of sulfur-rich molecules and the concurrent enhancement of antioxidant defense. Similarly, sulfur-containing foods such as onion and broccoli have been reported to facilitate the elimination of toxic metals including arsenic, mercury and lead (Mehrandish et al., 2019).

Another major class of plant-based detoxification agents is polyphenolic compounds. Flavonoids and phenolic acids, abundantly found in polyphenol-rich foods such as green tea (Demir et al., 2017), grapes, turmeric, artichoke and grape seed, possess multiple hydroxyl and carboxyl groups capable of forming complexes with heavy metal ions. Experimental evidence indicates that green tea catechins and grape seed extract can chelate cadmium and lead, thereby reducing their intestinal absorption (Zhai et al., 2019; Arun et al., 2021). Beyond chelation, polyphenols mitigate metal-induced toxicity through their strong antioxidant capacity, attenuating oxidative stress generated by metal ions at the cellular level and consequently limiting downstream biochemical damage (Arun et al., 2021). In addition, milk thistle

(*Silybum marianum*), whose active component is silymarin, has been shown to protect the liver against heavy metal injury by reducing cadmium accumulation and associated hepatic damage through combined antioxidant and metal-binding actions (Mehrandish et al., 2019). Coriander (*Coriandrum sativum*) leaves are also traditionally regarded as "metal-cleansing"; although some studies suggest that coriander extract can lower tissue lead levels in exposed animals, its efficacy appears dose-dependent and generally weaker than garlic or silymarin, making it more suitable as an adjunctive approach rather than a primary intervention (Mehrandish et al., 2019).

Dietary fibers and natural polymers also play an important role in plant-based heavy metal detoxification. Both insoluble and soluble fibers can bind metal ions in the gastrointestinal tract and thereby limit their absorption. Adequate intake of fiber-rich foods such as whole grains and fruits has been reported to reduce body metal burden by sequestering metals in the intestine and promoting their fecal excretion (Mehrandish et al., 2019). In addition, fibers may act as a functional chelation therapy by preventing the reabsorption of metal ions through interruption of the enterohepatic cycle. Among polysaccharides, citrus-derived pectin and alginate extracted from marine algae show particularly high affinity for metal ions. Supplementation with alginate has been shown to accelerate the elimination of metals such as strontium, cobalt and cadmium by forming stable complexes (Mehrandish et al., 2019). Likewise, chlorophyll derivatives (e.g., chlorophyllin) can bind planar toxins such as aflatoxin as well as certain metal–porphyrin structures, which is why the consumption of leafy green vegetables is considered beneficial in metal detoxification (Mehrandish et al., 2019).

In summary, a wide range of plant-derived agents including garlic, onion, coriander, milk thistle, turmeric, ginkgo, green tea, as well as natural fibers such as pectin and alginate can either reduce intestinal absorption of heavy metals or enhance their elimination. Regular consumption of such plant-based agents has been emphasized as an effective means of lowering metal exposure, often with fewer adverse effects than synthetic chelators and with the added benefit of antioxidant and anti-inflammatory properties (Mehrandish et al., 2019).

Probiotic-Based Adsorbents and Bioremediation

Live probiotic microorganisms particularly lactic acid bacteria and beneficial yeasts have emerged as promising biological tools for mitigating the bioavailability of heavy metals in food and along the gastrointestinal tract. The cell walls of certain *Lactobacillus* and *Bifidobacterium* strains contain high levels of peptidoglycan, teichoic acids and polysaccharides capable of binding metal ions through surface adsorption. Through this biosorption mechanism, probiotic cells can trap cadmium, lead and arsenic, thereby limiting their translocation across the intestinal epithelium. Indeed, in vitro studies have demonstrated that various *Lactobacillus* strains can remove substantial amounts of heavy metals from solution (Daisley et al., 2019). For example, the probiotic strain *Lactobacillus rhamnosus* GR-1 was shown to retain cadmium and lead ions on its surface over 48 h and, in a Caco-2 intestinal epithelium model, substantially reduce apical-to-basolateral transport of these metals (Daisley et al., 2019). The same work also reported that increasing the external metal concentration led to saturation of binding sites, leaving a fraction of unbound metal available for absorption consistent with the reversible and surface-dependent nature of biosorption and its sensitivity to physicochemical factors such as pH and ionic strength (Daisley et al., 2019). Despite this reversibility, probiotic cells may still provide meaningful protective effects in vivo by functioning as a transient biological barrier that delays or reduces metal uptake in the gastrointestinal tract (Chen et al., 2022; Zhai et al., 2019).

The detoxification potential of probiotics against heavy metals is supported not only by in vitro findings but also by in vivo animal studies and preliminary human investigations. In rodent models, oral administration of specific *Lactobacillus* strains for example *L. plantarum* CCFM8661 has been shown to reduce circulating metal levels in exposed animals while increasing fecal excretion of the metals (Zhai et al., 2019). This effect has been partly attributed to the ability of probiotics to modulate bile acid metabolism and promote the re-secretion of metal–bile complexes into the intestinal lumen (Zhai et al., 2019). Other strains, such as *Lactobacillus casei* SYF-08, have attenuated liver and kidney injury in lead-exposed young mice, an effect linked to enhanced biliary elimination of lead and preservation of

intestinal barrier integrity (Chen et al., 2022). These findings suggest that probiotic-mediated detoxification may operate through a dual mechanism combining chemical immobilization of metals and host-level physiological modulation (Demir and Demir, 2021).

Limited but informative human data further support this concept. In a pilot trial conducted among pregnant women in Tanzania, regular consumption of *Lactobacillus rhamnosus*–containing probiotic yogurt prevented the gestational rise in blood mercury and arsenic levels observed in the control group, indicating that probiotic intake can stabilize systemic metal burden by limiting absorption or promoting excretion (Bisanz et al., 2014). Likewise, in a 12-week intervention among metal-exposed factory workers in China, daily intake of a probiotic dairy product containing *Pediococcus acidilactici* led to markedly greater reductions in blood copper and nickel concentrations compared with non-consumers, alongside lower markers of oxidative stress and inflammation (Feng et al., 2022). These observations highlight the potential of probiotics as an intervention strategy even under high-risk occupational exposure conditions.

The role of probiotic yeasts such as *Saccharomyces* spp. (e.g., baker's yeast and *S. boulardii*) in heavy metal detoxification has also been investigated. The cell wall of *Saccharomyces cerevisiae* is rich in mannans and β-glucans, which are capable of binding toxic molecules; although this yeast is primarily used in the feed industry to protect animals from mycotoxins, several reports indicate that it can also sequester cadmium and lead on its cell surface. In addition, it has been proposed that *Saccharomyces* yeasts may complex metals into less bioavailable forms during fermentation. In one study using the brewing yeast *S. pastorianus* A15, supplementation of the fermentation medium with defined concentrations of lead and cadmium resulted in a 15–30% reduction in bioavailable forms of the metals by the end of fermentation (Nathanail et al., 2016). Despite these observations, the mechanistic basis of metal transformation by yeasts remains insufficiently characterized. A potential advantage of probiotic yeasts over lactic acid bacteria is their intrinsic tolerance to antibiotics: *S. boulardii*, for instance, can survive concomitant antibiotic therapy, suggesting that it may persist even in contaminated gastrointestinal environments and

contribute to metal detoxification when bacterial probiotics would be compromised.

Evaluation and Limitations

Plant-based and probiotic-based strategies for heavy metal detoxification may be operationalized in food and nutrition practice in several ways. For instance, the incorporation of natural chelating botanicals (e.g., garlic extract, coriander leaf powder) into the diet may be advisable in regions where produce is at high risk of contamination. In high-risk populations such as mining workers or residents of industrialized areas, regular consumption of probiotic-enriched foods (yogurt, kefir, fermented vegetables) may reduce the chronic metal burden by limiting intestinal absorption. Similar principles are applied in animal nutrition: supplementation of livestock feed with yeast-derived cell wall preparations or lactic acid bacteria is used to mitigate the transfer of metal and mycotoxin residues to the animal, thereby protecting animal health while concomitantly reducing metal residues in edible products such as milk and meat.

However, several challenges exist regarding the efficacy and safety of these natural detoxification approaches. Botanical chelators typically require sustained and sufficiently high intake to exert a measurable effect; for example, garlic or coriander must be consumed in relatively large quantities over prolonged periods, which may be limited by sensory acceptability. High intake of polyphenols can, in some cases, reduce the absorption of essential minerals such as iron and zinc, underscoring the need for dose balance. The performance of probiotics is strain-specific not all bacteria or yeasts possess equal binding capacity and identifying the most effective strains and stabilizing them in food matrices remain critical technical questions. Furthermore, viable probiotic cells must survive passage through the gastrointestinal tract, which depends on storage conditions and tolerance to gastric acidity. The reversibility of biosorption introduces another uncertainty: pH shifts in the intestine may lead to desorption and re-release of bound metals. Consequently, current research increasingly investigates whether metal-binding probiotics are also capable of transforming metals into less toxic derivatives for instance, by reducing metal ions to nanostructures or converting them into volatile complexes rather than relying solely on reversible surface adsorption.

From a regulatory perspective, the integration of natural detoxification approaches requires careful consideration. The addition of plant extracts to foods may legally fall under the category of functional foods or dietary supplements, and thus trigger specific authorization procedures. Probiotic strains, in turn, must be recognized as safe by regulatory authorities (e.g., GRAS status) and must not carry transferable antibiotic resistance determinants. Despite these constraints, the current body of evidence indicates that plant-based chelators and probiotic adsorbents hold promise for mitigating heavy metal exposure. Combined interventions for example, co-administration of garlic extract with *Lactobacillus* supplementation may offer synergistic benefits. Future large-scale clinical trials will be essential to clarify their real-world effectiveness and to define optimal implementation protocols.

1.2. Natural Detoxification of Mycotoxins

Mycotoxins are toxic secondary metabolites produced by filamentous fungi, primarily *Aspergillus*, *Penicillium* and *Fusarium* species, and are commonly detected in food and feed. Aflatoxins, ochratoxin, patulin, fumonisins, zearalenone and trichothecenes (e.g., T-2 toxin and deoxynivalenol) are among the most prevalent groups (Demir and Ağaoğlu, 2023). These toxins typically occur in cereals, nuts, dried fruits, spices and, indirectly, in animal-derived products such as milk, where aflatoxin M_1 can appear following carry-over from contaminated feed. Mycotoxin exposure may cause acute intoxication at high doses, whereas chronic low-level exposure has been associated with immunosuppression, growth impairment, hepatic injury and carcinogenesis (Liu et al., 2022). Although the food industry employs preventive measures such as proper drying, storage and mold growth control complete prevention is not always feasible, making detoxification of contaminated products a critical need. Physical (e.g., sorting, washing, heat treatment) and chemical (e.g., ammonia treatment of aflatoxins) methods offer partial solutions, but biological detoxification strategies are increasingly favored because they can eliminate or neutralize toxins without compromising nutritional value. Natural approaches include the use of plant-derived constituents to adsorb mycotoxins or inhibit fungal growth, and probiotic

microorganisms capable of binding or biotransforming these toxins (Demir and Tutun, 2024).

1.2.1. Plant-Based Detoxification Strategies

Several botanical approaches have been proposed to mitigate mycotoxin exposure. One strategy relies on dietary fibers and phytochemicals that bind toxins and reduce their intestinal absorption. For example, chlorophyllin a semi-synthetic derivative of chlorophyll can form stable complexes with aflatoxin B_1 (AFB_1), thereby markedly preventing its uptake from the intestine. Human intervention trials conducted in high-risk regions reported that chlorophyllin supplementation decreased biomarkers linked to liver carcinogenesis, presumably by reducing AFB_1 absorption. Native chlorophyll in leafy greens may exert a similar protective role; indeed, populations with high consumption of dark green vegetables show lower aflatoxin metabolite levels, supporting the recommendation of leafy vegetables as a protective dietary measure in high-risk diets (Liu et al., 2022).

A second botanical strategy involves the use of antioxidant polyphenols to attenuate cellular damage caused by mycotoxins. Certain polyphenols can either directly react with mycotoxins, generating less toxic metabolites, or inhibit the growth of toxin-producing fungi. Curcumin, the principal bioactive compound in turmeric, has been shown to reduce aflatoxin-induced oxidative damage and DNA lesions in cell systems, and to attenuate hepatic toxicity in animal models. These effects are attributed to both the up-regulation of antioxidant responses and the suppression of aflatoxin metabolic activation. Likewise, resveratrol and epigallocatechin gallate have been reported to mitigate the cytotoxic effects of aflatoxin and ochratoxin A by inhibiting apoptosis-related signaling and inflammatory mediators (Ajmal et al., 2025; Liu et al., 2022). Thus, consumption of polyphenol-rich foods may serve as a supportive strategy to strengthen host defense when mycotoxin exposure cannot be fully avoided.

Use of Plant Extracts and Essential Oils for Mycotoxin Control

The application of plant extracts and essential oils to prevent mycotoxin formation in foods has gained considerable attention as a natural and innovative intervention. Many essential oils exert antifungal activities and can inhibit both fungal proliferation and mycotoxin biosynthesis. Oils derived from oregano, cinnamon, clove, rosemary and basil have been shown in laboratory studies to suppress the growth of *Aspergillus* and *Fusarium* species. These effects are largely attributed to bioactive constituents such as thymol, eugenol, cinnamaldehyde and linalool, which disrupt fungal cell membranes or downregulate toxin biosynthetic pathways. Among these, basil (*Ocimum*) oils have demonstrated particularly strong inhibitory effects against *Aspergillus flavus*, with some preparations completely blocking fungal growth and suppressing aflatoxin production (Ajmal et al., 2025). In that study, essential oil from *O. tenuiflorum* achieved full inhibition of *A. flavus* and reduced aflatoxin B_1 to below detectable levels at concentrations as low as 0.75 μL/mL (Ajmal et al., 2025). These findings support the potential of essential oils as natural alternatives to synthetic fungicides in food preservation. In practice, edible coatings and packaging films incorporating essential oils are being explored to prevent mold development during storage; for example, cinnamon oil–impregnated wrapping materials have been reported to delay mold growth on bread surfaces.

Another dimension of plant-based mycotoxin control is the use of fungal antagonists. Although this is technically a biological rather than a phytochemical approach, certain naturally occurring edible bacteria and yeasts can compete with mycotoxigenic fungi and suppress toxin formation. In controlled fermentations, reductions in mycotoxin levels have been observed as a consequence of microbial competition, acidification and metabolic degradation. For example, although *Aspergillus oryzae* is intentionally used during soy sauce and miso production, aflatoxins are absent in the final product, implying that fermentation conditions and coexisting microorganisms contribute to toxin degradation. Similarly, in sourdough fermentation, *Lactobacillus* species have been reported to partially degrade certain cereal-derived mycotoxins such as deoxynivalenol (DON), leading to measurable decreases in the final baked product. Because these biological

detoxification events occur intrinsically within food processing, they offer a practical solution without the need for external additives (Liu et al., 2022).

Detoxification by Probiotic Microorganisms

Probiotic bacteria and yeasts are increasingly investigated as biological scavengers against mycotoxins, both in food systems and within the gastrointestinal tract. Beneficial genera such as *Lactobacillus*, *Bifidobacterium* and *Propionibacterium*, as well as the yeast *Saccharomyces cerevisiae*, are capable of adsorbing mycotoxins onto their cell surfaces or biotransforming them enzymatically. Structural components of their cell envelope including glucans, mannans, peptidoglycan and teichoic acid can bind chemically distinct toxins such as aflatoxin B_1, ochratoxin A, zearalenone and fumonisin B_1. In vitro studies have reported that certain dairy-derived *Lactobacillus* and *Bifidobacterium* strains can bind AFB_1 at rates ranging from approximately 50–80%. Binding is typically rapid and mediated by weak interactions with carbohydrate and protein moieties on the cell surface, making the process potentially reversible; toxin release can occur when cells are washed with strong solvents. Nevertheless, the mere presence of viable microorganisms in the gastrointestinal tract allows toxins to adhere to microbial surfaces before absorption, which confers a physiologically relevant layer of protection. For example, supplementing animal feed with live or heat-killed *Lactobacillus* cultures has been shown to reduce the transfer of zearalenone from feed into systemic circulation, and similar strategies have partially reduced aflatoxin M_1 carry-over into milk. These findings collectively support the potential of probiotic adsorption as a practical detoxification approach (Liu et al., 2022, Demir and Tutun, 2024).

A further attractive attribute of probiotic microorganisms is their capacity to achieve true biochemical detoxification of mycotoxins rather than mere surface binding. Certain strains are able to enzymatically convert native toxins into less reactive metabolites. For example, several *Lactobacillus* and *Corynebacterium* strains have been shown to degrade trichothecenes such as deoxynivalenol and nivalenol via toxin-cleaving enzymes. Likewise, specific strains of *L. paracasei* and *L. plantarum* were reported to convert patulin into a less toxic glucosylated derivative through conjugation reactions. Yeasts also

participate in enzymatic detoxification; *Saccharomyces cerevisiae* can internalize zearalenone and subsequently open the lactone ring to yield metabolites with markedly reduced estrogenic potency. Evidence from brewery fermentations further indicates that *S. pastorianus* can partially biotransform Fusarium-derived toxins, resulting in reductions of approximately 15–30% during the course of fermentation. Although these reductions may appear modest, even partial biotransformation is highly valuable in realistic food systems where complete removal is rarely feasible and where cumulative exposure determines risk.

The efficacy of probiotics in mycotoxin detoxification can be utilized both as a preventive measure in food systems and as a protective strategy within the gastrointestinal tract. When feed or food batches carry a high mycotoxin risk, they can be subjected to fermentation with probiotic bacteria or yeast cultures to reduce toxin levels. A representative example is corn silage: silages fermented with *Lactobacillus* inoculants have been reported to contain lower concentrations of deoxynivalenol and zearalenone produced by *Fusarium* spp. compared with non-inoculated silages. In this context, lactic acid bacteria suppress fungal growth by acidifying the matrix while simultaneously adsorbing part of the toxin load.

In human nutrition, probiotic supplementation assists in binding ingested mycotoxins before they cross the intestinal barrier. In a field trial conducted in Kenya, children receiving probiotic powder containing *Lactobacillus rhamnosus* excreted significantly lower urinary levels of aflatoxin metabolites compared to the placebo group, an effect attributed to luminal binding of aflatoxin and its elimination through feces. The same study reported a concurrent trend toward improvement in liver injury biomarkers associated with aflatoxin exposure. Collectively, these findings support the feasibility of probiotic intervention as a biological detoxification strategy in both food matrices and the human gut (Liu et al., 2022).

The use of probiotic yeasts as mycotoxin binders is well established in the feed industry. Cell wall–enriched fractions of *Saccharomyces cerevisiae*, particularly beta-glucans and mannan oligosaccharides, are incorporated into commercial animal feeds as mycotoxin-binding additives. These compounds are able to structurally retain toxins such as aflatoxins, ochratoxin and fumonisins and thereby reduce their

intestinal absorption. In one study, dietary supplementation with purified yeast-cell-wall glucans markedly decreased the amount of AFB_1 that reached the liver in mice and attenuated its toxic effects. Nevertheless, the binding capacity of yeast cell walls is finite and may become insufficient when toxin concentrations are extremely high. These considerations highlight both the practical utility and the inherent limits of yeast-based binders as part of biological detoxification strategies (Liu et al., 2022).

Applications and Limitations

Plant-based and probiotic detoxification strategies can be integrated into both food processing and animal feeding practices. Before storage, cereals and nuts may be treated with natural antifungal agents to suppress mold growth and prevent toxin formation, and commercial botanical preservatives have already been developed for this purpose. Although the direct incorporation of essential oils into food is not always practical, their vapor-phase activity can be exploited; for example, dispersing plant volatiles in storage environments through a vaporizer has been shown to inhibit aflatoxin-producing fungi on grain. In food formulation, dietary fibers are considered not only for their ability to mitigate undesirable reactions such as acrylamide formation and lipid oxidation but also for their potential to reduce the intestinal bioaccessibility of mycotoxins by retaining them within the matrix during digestion. Such natural interventions are increasingly investigated as safer alternatives to synthetic binders (Ajmal et al., 2025; Khorshidian et al., 2020).

One practical route for exploiting probiotics against food-borne mycotoxins is through their incorporation into functional foods. For individuals living in high-risk regions, probiotic fermented dairy products or probiotic capsules can be integrated into daily diets as a preventive measure, particularly in settings where aflatoxin exposure is linked to liver cancer risk. However, several variables determine the effectiveness of such interventions including the selection of the appropriate strain, sufficient dosing, sustained intake, and, critically, whether the administered microorganism truly binds the target toxin in vivo. The optimal binding organism may differ across toxin classes; for instance, *Lactobacillus rhamnosus* shows higher affinity toward aflatoxin B_1, whereas *L. plantarum* binds zearalenone more effectively.

This variability explains why commercial mycotoxin binders often contain mixed preparations of yeasts and bacteria to address multi-toxin contamination scenarios (Shetty & Jespersen, 2006; Khorshidian et al., 2020).

Natural detoxification strategies are nonetheless subject to limitations. First, their activity can be attenuated by the food matrix: real food systems are considerably more complex than laboratory media, which may hinder microbial access to toxins or deactivate active phytochemicals through matrix interactions. Second, these approaches rarely eliminate toxins completely; they primarily reduce or constrain exposure and therefore must be combined with upstream preventive measures. Third, probiotic cultures themselves may be inhibited at high toxin concentrations; for example, certain *Lactobacillus* strains lose viability in the presence of elevated deoxynivalenol levels, which directly compromises their detoxification capacity.

From a regulatory standpoint, the use of probiotics as food additives is restricted in most countries to strains that have been formally recognized as safe, and any health-related claims require substantiation. Claims referring specifically to “mycotoxin binding” are not yet authorized within the food sector, although certain feed additives are already registered as binding agents in the animal nutrition domain. Botanical extracts, by contrast, are typically approved under functional categories such as natural flavorings or preservatives. In the future, it is plausible that targeted functional ingredients designed for toxin detoxification for example, chlorophyll tablets or probiotic bars will reach the market, but their authorization will depend on evidence generated from robust clinical trials (Khorshidian et al., 2020).

In summary, plant-based agents (fibers, polyphenols and essential oils) and probiotic microorganisms constitute natural and safe tools for mitigating mycotoxin risk. These interventions can confer benefits at both the processing level by preventing or reducing contamination and at the consumer level, by reinforcing host defenses or binding ingested toxins before absorption. Compared with conventional approaches, they offer the advantages of minimal nutrient loss and no chemical residues. However, the most effective outcomes will be achieved when such natural strategies are implemented as components of an Integrated

Mycotoxin Management framework rather than as stand-alone measures.

1.3. Natural Mitigation of Antibiotic Residues

Antibiotics used in veterinary medicine and livestock production may enter the food chain in the form of residual parent compounds or metabolites. Residues are most commonly detected in the milk, meat, eggs and honey of animals exposed to prolonged or high-dose treatments. Although these residues may not exert overt acute toxicity in consumers, they can trigger allergic reactions and more critically can alter the human microbiota in ways that favor the selection of antibiotic-resistant bacteria. Continuous low-dose exposure has also been linked to dysbiosis and impairments in immune function. For these reasons, the reduction of antibiotic residues in foods is a priority both for public health and for antimicrobial resistance control. Conventional residue-prevention relies mainly on observing withdrawal periods prior to milking or slaughter, yet residues may persist when compliance is poor or when environmental contamination occurs. Physical and chemical removal techniques such as irradiation or ozonation have been investigated but risk damaging food quality. Accordingly, this section examines plant-based and microbial strategies for the natural detoxification of antibiotic residues as a safer alternative (He et al., 2024; Sardar et al., 2023).

1.3.1. Plant-Based and Natural Adsorbent Approaches

Although only a limited number of plant-derived compounds directly target antibiotic residues, several natural adsorbent materials and indirect botanical strategies may assist in their mitigation. Dietary fibers, clay minerals and carbon-based adsorbents can bind and neutralize antibiotics in the gastrointestinal tract in a similar manner to other chemicals. Activated carbon, which can be produced from plant by-products such as coconut shells, is a potent adsorbent capable of sequestering pharmaceuticals and toxins in aqueous media or within the digestive system; this same principle underlies its long-standing medical use in acute antibiotic overdose to block systemic absorption. By extension, residual antibiotics present in foods may also be trapped

and eliminated through dietary fibers or carbonized adsorbents before they are absorbed.

Experimental work further shows that natural clays such as bentonite and biopolymers such as chitosan can adsorb antibiotic molecules on their surfaces and thereby protect the intestinal microbiota. Bentonites have been reported to bind commonly used antimicrobials including tetracyclines and fluoroquinolones, reducing microbiota disruption in animal models. Likewise, chitosan a polysaccharide derived from crustacean shells or fungal cell walls has been shown to retain various antibiotics, including penicillin derivatives, in aqueous systems. A recent review highlighted that low-cost biosorbents obtained from materials such as microalgae, wood residues and plant leaves can remove antibiotics from contaminated water with efficiencies exceeding 90%, underscoring the environmental and practical utility of plant- and microbe-derived adsorbents for antibiotic residue mitigation (Sardar et al., 2023; Ajmal et al., 2025).

From a food safety perspective, mitigating the impact of antibiotic residues through plant-derived materials is a more indirect yet feasible strategy. For instance, supplementation of dairy products carrying a risk of β-lactam residues with *Rosmarinus officinalis* (rosemary) extract has been shown to accelerate the oxidative degradation of these drugs and to reduce their effective concentration, owing to the phenolic constituents of rosemary. In addition to exerting an antioxidant effect and protecting milk quality, the extract contributed to partial decomposition of the antibiotic molecules. Likewise, studies have explored the use of botanical extracts such as thyme or clove oils to degrade antibiotic residues in honey. Although techniques like supercritical fluid extraction can remove antibiotics directly from honey, such procedures are impractical at industrial scale. A more realistic preventive approach is to reduce antibiotic use in apiculture by replacing therapeutic antibiotics with plant-derived antimicrobials; treatments with thyme and propolis extracts have achieved comparable disease control in beehives without leaving detectable residues in honey. These findings collectively support the feasibility of using botanically derived agents to ameliorate antibiotic residue problems in food systems (Ajmal et al., 2025; Sardar et al., 2023).

Another innovative plant-based approach is the use of phytobiotics in animal feeds. The term "phytobiotic" refers to natural plant-derived products such as extracts or essential oils that are employed in animal husbandry to promote growth or prevent disease. In sectors such as poultry and livestock production, where antibiotic growth promoters were historically used, plant-based alternatives such as thyme oil, garlic powder, cinnamon extract and black seed oil are increasingly being adopted. These interventions have been shown not only to improve animal performance but also to reduce antibiotic usage, thereby minimizing the risk of residues in edible animal products. For example, a blend of garlic and thyme oils was reported to support broiler growth while simultaneously suppressing pathogens such as *Campylobacter* and coccidia, enabling antibiotic-free production. Although such practices do not constitute direct detoxification, they serve as upstream preventive strategies that ultimately lower antibiotic residue burden in foods and thus contribute meaningfully to food safety (Ajmal et al., 2025; Khorshidian et al., 2020).

Probiotic-Based Solutions

Probiotic microorganisms may contribute to the mitigation of antibiotic residues through two complementary mechanisms: first, certain strains possess enzymes capable of degrading antibiotics in vitro; second, probiotics can counteract the collateral damage of antibiotic therapy, thereby providing an indirect form of detoxification. A notable example is the probiotic yeast *Saccharomyces boulardii*, a transient colonizer of the human gut that is naturally resistant to most antibiotics and is widely used to prevent antibiotic-associated diarrhea. Recent work has demonstrated that this yeast can be further engineered to actively detoxify antibiotics in situ. In one proof-of-concept study, the gene encoding a β-lactamase enzyme was introduced into *S. boulardii*, enabling the yeast to hydrolyze amoxicillin-derived antibiotics within the intestine while remaining viable. This re-engineered probiotic acted as a "living biopharmaceutical," simultaneously colonizing the gut and enzymatically degrading residual antibiotic molecules, thereby preserving the intestinal microbiota (Demir and Demir, 2021). Although still experimental, this approach illustrates the potential of next-generation probiotics as targeted biotools for antibiotic detoxification (He et al., 2024; Sardar et al., 2023).

Although engineered strains represent the most explicit proof of concept, a limited number of naturally occurring probiotic candidates also possess the ability to metabolize antibiotics. Certain environmental *Bacillus* and *Pseudomonas* strains can utilize antibiotics such as penicillins or tetracyclines as carbon and energy sources, and safe variants of these organisms are being explored as next-generation probiotics. Likewise, members of the human gut microbiota such as *Eggerthella lenta* known for inactivating drugs like digoxin have been shown to modify macrolide antibiotics via analogous reductive mechanisms, thereby reducing their activity. In principle, controlled use of such organisms could be applied to degrade antibiotic residues in foods.

More widely documented, however, are studies describing the protective effects of conventional probiotics. For example, the inclusion of *Lactobacillus*-based probiotics in poultry feed attenuated the intestinal damage caused by oxytetracycline administration and resulted in lower measurable residue levels within the gut. A similar effect has been reported with *Lactobacillus rhamnosus GG*, which preserved intestinal barrier function in mice treated with β-lactam antibiotics and altered the absorption profile of the drug. These findings collectively suggest that probiotics may reduce the effective systemic burden of residual antibiotics indirectly by fortifying the intestinal barrier and modulating luminal exposure (He et al., 2024; Khorshidian et al., 2020).

Another major advantage of probiotics is their ability to counteract dysbiosis and its consequences following antibiotic exposure. Given that sub-residual levels of antibiotics in food may similarly disrupt the gut microbiota, probiotic supplementation can serve as a compensatory measure. For example, fish fed with diets containing low levels of enrofloxacin residues exhibited a decline in beneficial bacteria and an increase in pathogenic taxa within the gut microbiota; however, when a *Lactobacillus*-based probiotic was added to the feed, the microbial balance was preserved and growth performance was maintained (Demir and Demir, 2021). These findings indicate that probiotics are capable of neutralizing even sublethal effects of antibiotic residues on the intestinal ecosystem, thereby mitigating their downstream functional consequences (Khorshidian et al., 2020; Sardar et al., 2023).

Practical Applications and Challenges

Natural strategies to reduce antibiotic residues can be implemented at multiple stages of food production. In animal agriculture, probiotic- or phytobiotic-assisted feeding programs can reduce the need for therapeutic antibiotics. This approach has gained traction particularly in regions such as the European Union, where the use of antibiotics as growth promoters has been banned. A direct consequence of using fewer antibiotics at the farm level is a proportional reduction in residue risk in the resulting food products.

In the dairy sector, antibiotic residues interfere with the manufacture of fermented products for instance, residual antibiotics in raw milk inhibit starter cultures during yogurt production. For this reason, incoming milk is routinely screened, and contaminated batches are rejected. For borderline-positive batches, some manufacturers have explored enzymatic inactivation approaches. β-Lactamase enzymes are technically capable of hydrolyzing β-lactam antibiotics in milk, but the intentional addition of such enzymes to food poses regulatory barriers. A natural alternative is to exploit probiotic strains capable of producing low levels of antibiotic-degrading enzymes. Certain *Bifidobacterium* and *Lactobacillus* strains have been reported to exhibit mild β-lactamase activity, and yogurt fermented with such cultures showed no detectable residues of low-level penicillin derivatives in the final product. However, the use of these strains raises concerns regarding the potential presence or transferability of antibiotic resistance genes, necessitating a cautious regulatory and safety evaluation prior to application (Khorshidian et al., 2020; He et al., 2024).

There are inherent limitations in deploying plant-based or probiotic strategies against antibiotic residues. First, residue levels in food are typically extremely low (ppm–ppb range); capturing or degrading such trace quantities requires highly efficient systems. To bind even 1 ppb of residue, the required mass of an adsorbent might need to be many times higher, potentially impairing product quality. Second, antibiotic residues are chemically diverse; a single botanical agent or probiotic strain cannot neutralize all structural classes. Combination approaches may therefore be more effective for example, a functional food

containing both fiber and probiotics could simultaneously bind and metabolize residues, enabling broader protection. Third, safety constraints must be respected: botanical extracts should not introduce new toxicity at effective doses, and probiotic strains must not pose an infection risk or disseminate resistance determinants (Kabir, 2021).

Complete elimination of antibiotic residues is unlikely in practice; a more realistic objective is to minimize exposure and attenuate the biological impact of unavoidable residues. Natural detoxification strategies thus have value as supportive interventions. Growing consumer demand for "residue-free" and "naturally produced" foods is already motivating industry to invest in botanical and probiotic solutions. In the near future, targeted microbial strains engineered or selected for the degradation of unwanted chemicals in foods such as antibiotics or pesticides may enter application pipelines. Experimental work on probiotic-mediated pesticide detoxification has already been initiated, pointing to a shift in food safety paradigms toward the concept of *self-detoxifying foods*: foods or packaging materials incorporating natural agents that capture or neutralize contaminants before consumption (Halász et al., 2019).

In summary, natural interventions for mitigating antibiotic residues in the food chain can be broadly classified as direct and indirect strategies. Direct strategies include the binding of antibiotic molecules by natural adsorbents such as dietary fibers, clays, chitosan or activated carbon, as well as the enzymatic degradation of these compounds by selected probiotic strains or enzyme systems.
Indirect strategies involve reducing the initial need for antibiotics through the use of phytobiotics and probiotics in animal production, and supporting the gut microbiota to neutralize the biological effects of residual sub-therapeutic antibiotics. When applied in combination, these approaches hold significant potential not only to minimize the overall antibiotic burden in human foods, but also to mitigate the microbiota-mediated health risks associated with chronic low-level exposure.

1.4. Natural Mitigation of Acrylamide Formation

Acrylamide is a process contaminant predominantly formed in carbohydrate-rich foods subjected to high-temperature treatments such as frying, baking or roasting including potato chips, French fries, biscuits, roasted coffee, and bread crust. Its emergence as a food safety concern gained global attention in 2002, following the discovery of its widespread occurrence in processed foods. Acrylamide is generated via the Maillard reaction, arising from the interaction between free asparagine and reducing sugars at temperatures typically exceeding 120 °C. Toxicological evaluation has linked acrylamide exposure to neurotoxicity and carcinogenicity in experimental animals, and the International Agency for Research on Cancer has classified acrylamide as a Group 2A "probable human carcinogen." Regulatory actions followed, most notably in the European Union where Commission Regulation (EU) 2017/2158 established benchmark levels for selected high-risk food categories. Conventional mitigation strategies include reducing precursor concentrations (e.g., using low-asparagine potato cultivars), modifying thermal profiles (lower temperature–shorter duration), pre-treatments such as leaching to remove surface sugars or acidification prior to frying, and the enzymatic degradation of asparagine using L-asparaginase in dough systems. In parallel with these technological interventions, interest has grown in natural mitigation approaches based on plant-derived antioxidants and selected probiotic cultures, which may attenuate acrylamide generation or its downstream biological effects (Mesías & Morales, 2017; Zhang et al., 2021).

1.4.1. Plant-Derived Strategies for Acrylamide Mitigation

Plant-derived constituents can interfere with acrylamide formation at multiple stages, either by suppressing its generation during thermal processing or by reducing its bioaccessibility in the gastrointestinal tract. Among these, polyphenol-rich botanical extracts have attracted the greatest interest. In addition to their antioxidant capacity, polyphenols can chemically intercept reactive carbonyl intermediates of the Maillard reaction, thereby interrupting acrylamide-forming pathways. A comprehensive review by Liu et al. (2015) documented

that numerous plant polyphenols attenuate acrylamide formation under controlled heating conditions. Mechanistically, these phenolics can scavenge reactive carbonyls such as glycosylamines and 3-oxopropionamide key precursors in the acrylamide pathway or modulate the redox environment to limit secondary oxidative routes that fuel carbonyl stress (Mesías & Morales, 2017; Zhang et al., 2021).

For instance, biscuits supplemented with green tea extract exhibited significantly lower acrylamide concentrations after baking, an effect attributed to the trapping capacity of catechins (Sharma et al., 2014). Likewise, grape seed and rosemary extracts have been shown to reduce acrylamide levels by approximately 20–50% in fried potato products (Zhang et al., 2007; Gökmen et al., 2009). Specific phenolics such as chlorogenic acid and rutin have achieved up to ~50% inhibition in model systems, most likely by reacting with α,β-unsaturated carbonyls including acrolein and stabilizing them in non-reactive forms (Gökmen et al., 2009).

It is important to note, however, that the effect of polyphenols is not invariably inhibitory. Under certain matrices and moisture regimes, highly tannin-rich extracts may lower water activity and inadvertently accelerate Maillard chemistry, leading to increased acrylamide formation (Liu et al., 2015). Therefore, the choice of extract and its dose must be carefully optimized. Overall, phenolics that combine high antioxidant capacity with demonstrated carbonyl-trapping ability such as extracts from green tea, grape seed, thyme and rosemary are considered the most robust candidates for natural acrylamide mitigation in thermally processed foods.

A second plant-derived avenue for acrylamide mitigation involves the use of dietary fibers and structurally complex carbohydrates. The presence of fibers in dough or batter can reduce acrylamide formation by competing for reactive precursors, altering the thermal and diffusional properties of the matrix, or shifting the chemical environment during heating. Passos et al. (2018) reported that pectic polysaccharides react with reducing sugars during baking, limiting their availability to react with asparagine. In the same study, the formation of organic acid by-products in the presence of pectin reduced pH, thereby decelerating acrylamide kinetics. More recent work on wheat-based cookies fortified with rye bran, inulin or resistant starch

demonstrated that fiber enrichment significantly lowers acrylamide levels post-baking (López-Ruiz et al., 2023). For instance, the addition of 5% soluble fiber such as pectin yielded ≈30% reduction in acrylamide without impairing product quality.

Several mechanisms may explain this reduction. First, fibers affect water-binding and heat distribution in the matrix, restricting the formation of local high-temperature zones favorable to acrylamide generation. Second, certain polysaccharides (e.g. arabinogalactan, carrageenan) can form non-covalent complexes with reducing sugars or asparagine, removing them from the Maillard pathway (Santana et al., 2017). Third, fibers can physically retain a fraction of the acrylamide formed, limiting its diffusion to the surface where it is typically quantified.

Beyond polymeric carbohydrates, naturally permissible inorganic and organic agents have also been used to moderate acrylamide formation. Açar et al. (2012) demonstrated that calcium carbonate added to cookie dough reduced acrylamide without compromising texture. Calcium ions may sequester asparagine through alternative reaction routes or bind nascent acrylamide. Similarly, the incorporation of food-grade organic acids such as citric or tartaric acid at 0.1–0.5% lowers pH and has been shown to reduce acrylamide formation by approximately 20–40% (Gökmen et al., 2007).

Natural antioxidant vitamins have also been investigated for their potential to limit acrylamide generation in heat-treated foods. Supplementation with α-tocopherol (vitamin E) or ascorbic acid (vitamin C) has been shown in some studies to reduce acrylamide levels in fried potato products (Becalski et al., 2004). Mechanistically, ascorbic acid can scavenge radicals produced during thermal processing and thereby suppress the acrolein-mediated branch of the acrylamide pathway, whereas tocopherol interrupts lipid-borne radical chain reactions, limiting both acrylamide and secondary oxidative by-products. The main constraint, however, is the thermal instability of these vitamins, which substantially reduces their functional persistence at typical frying or baking temperatures.

In summary, a strategically designed use of plant-derived constituents provides a feasible, label-friendly and mechanistically justified route to

attenuate acrylamide exposure without resorting to synthetic processing aids. Polyphenolic extracts can scavenge reactive carbonyl intermediates or divert oxidative pathways away from acrylamide precursors; dietary fibers can sequester reactants, modulate water mobility and alter heat transfer profiles; organic acids and calcium salts can lower system pH or chemically divert asparagine from the acrylamide-forming route; and certain vitamins can quench radical intermediates along the reaction cascade. Unlike conventional mitigation strategies that may compromise sensory quality or require regulatory approval, these nature-derived inputs can be integrated into existing formulations with minimal technological disruption and simultaneously provide additional nutritional or functional value appreciated by consumers. In practice, their deployment is most effective when embedded in a multi-layered mitigation concept not as a single standalone tactic but as part of an integrated risk-reduction framework that starts with raw material selection, continues with process optimization, and is reinforced by formulation-level natural modulators. Such convergence of technology, formulation and regulatory acceptance makes plant-based mitigation a realistic and scalable avenue for controlling acrylamide risk in modern food systems.

1.4.2. Probiotic and Enzymatic Approaches

Probiotic microorganisms have been investigated in relation to acrylamide mitigation in two principal capacities: (i) Pre-ingestion mitigation, by modifying the food matrix during processing and thereby suppressing acrylamide formation upstream; and (ii) Post-ingestion protection, by binding or biotransforming acrylamide within the gastrointestinal tract and reducing its mucosal absorption.

With respect to the first mechanism, the most intensively studied target is the enzymatic degradation of free asparagine the obligate amino acid precursor of acrylamide within the Maillard cascade. The enzyme L-asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and ammonia, thereby removing the limiting substrate for acrylamide generation. In industrial practice, L-asparaginase is typically sourced from Aspergillus niger or A. oryzae and has already been adopted as a processing aid in baked and fried products (Kaplan & Özler, 2019). More recently, screening efforts have identified probiotic strains with

intrinsic asparaginase activity, including selected Limosilactobacillus and Lactobacillus spp. Notably, specific strains of Lactobacillus casei and L. reuteri have been shown to rapidly hydrolyze asparagine in vitro, and their inoculation into bread dough resulted in post-baking acrylamide reductions exceeding 50% (Khorshidian et al., 2020). This observation is particularly striking, as it indicates that fermentation with an appropriate probiotic starter can endogenously generate L-asparaginase during processing, eliminating the need to add the enzyme exogenously. The consistently lower acrylamide levels in some traditional sourdough breads may, at least in part, be attributable to such endogenous microbial enzymatic activity. Current research is expanding the application of these L-asparaginase-producing strains to dairy desserts, fried-potato coatings, and other heat-processed matrices as natural acrylamide mitigation tools (Khorshidian et al., 2020).

The second potential role of probiotics in acrylamide mitigation relates to post-formation interaction with the compound, thereby reducing its bioavailability rather than preventing its synthesis. Experimental work has shown that certain Lactobacillus and Bifidobacterium strains are capable of decreasing the measurable concentration of acrylamide in model systems, with part of the compound later being detected in the microbial biomass. This behaviour is consistent with surface adsorption driven by cell-wall components such as lipoteichoic acids and associated proteins. In animal feeding experiments in which acrylamide was co-administered with probiotic cultures, less acrylamide reached the systemic circulation and higher amounts of acrylamide-derived metabolites were recovered in excreta, indicating that the compound had been retained within the gut lumen and eliminated rather than absorbed. Yeasts, particularly Saccharomyces cerevisiae, have demonstrated a similar capacity to bind acrylamide via cell-wall polysaccharides; in co-cultures of lactic acid bacteria and yeast, removal efficiencies approaching 50% have been reported. Although still at an early developmental stage, such binding-based detoxification is considered a plausible foundation for future functional foods aimed at reducing the internal burden of heat-derived processing contaminants.

Another dimension of probiotic-based mitigation involves pre-treatment fermentations performed before thermal processing. For

instance, briefly fermenting potato slices with lactic acid bacteria prior to frying can deplete acrylamide precursors and markedly suppress subsequent formation. In one investigation, potato slices fermented with *Lactobacillus plantarum* yielded almost ninety percent less acrylamide after frying compared with non-fermented controls, a reduction mechanistically consistent with microbial utilization of free asparagine and accumulation of organic acids capable of attenuating Maillard kinetics (Khorshidian et al., 2020). Such pre-fermentation concepts may be incorporated into both domestic cooking and industrial workflows for example, by briefly immersing starchy matrices in a probiotic inoculum before heat treatment.

Taken together, these findings indicate that probiotics and their enzymes constitute a promising dual-site approach for acrylamide mitigation: on the processing side via endogenous L-asparaginase production, and on the physiological side by lowering intestinal absorption through binding in the gut (Khorshidian et al., 2020). Relative to externally added industrial enzymes, the use of enzyme-producing probiotic cultures offers a more economical and label-neutral alternative. Although the evidence to date largely stems from in vitro and animal models, the consistency of the signal suggests that probiotic-assisted acrylamide control warrants further translational work toward human applications.

1.4.3. Applications and Practical Constraints

Implementing natural acrylamide-mitigation strategies in industrial food production requires careful balancing with sensory and quality constraints. High-level addition of polyphenolic or spice extracts can introduce undesirable color or flavor deviations; therefore, low yet effective doses must be optimized rather than maximized (Liu et al., 2015). Fiber enrichment may also modify product texture for instance, excessive fiber can harden bakery matrices yet enrichment levels around 3–5% have been reported to provide both acceptable sensory outcome and a meaningful reduction in acrylamide (López-Ruiz et al., 2023).

Probiotic or fermentation-based approaches typically introduce additional processing steps. In a large-scale potato-chip line, allocating

time and equipment for fermentation may disrupt the established process architecture. Even so, practical compromises such as integrating a short lactic pre-treatment at the marination stage or applying a fermentative culture as a pre-dip are conceptually feasible. Beyond process compatibility, consumer acceptance must also be considered, as fermented notes or slight acidity may not be desirable in all product categories.

With respect to the gastrointestinal binding of acrylamide by probiotics, this approach belongs more to the realm of nutrition- and consumer-level risk attenuation rather than a food-manufacturing intervention. In practical terms, the idea that "probiotic co-consumption may buffer exposure" would translate into a public-health style recommendation rather than a technological mandate. Although no official guidance currently endorses such co-intake, it is conceivable that, as evidence accumulates, awareness campaigns or targeted functional beverages could be positioned to accompany high-risk convenience foods.

From a regulatory perspective, acrylamide-mitigation strategies are typically issued as incentive-based guidance rather than binding requirements, as illustrated by sectoral documents in the EU that encourage low-impact natural controls (Liu et al., 2015). These guidance texts prioritize interventions such as pre-soaking potatoes, acidifying doughs, or enriching formulations with fiber or other natural modulators all of which implicitly promote plant-based and biological levers over synthetic additives.

In sum, there is no universal single solution to the acrylamide problem; mitigation is matrix-specific and often requires layered combinations. Nonetheless, botanical and biological strategies are increasingly positioned at the forefront because they offer toxicological benefit without undermining the "natural identity" of the food. For product reformulation, it is therefore rational to evaluate spice extracts, natural antioxidants and probiotic or enzymatic pre-treatments as components of an integrated mitigation logic, simultaneously ensuring legal compliance and consumer-aligned clean-label positioning.

2. Conclusion

Natural detoxification approaches represent a scientifically grounded and sustainability-aligned response to the rising burden of chemical contaminants in foods. The contaminants addressed in this chapter heavy metals, mycotoxins, antibiotic residues and acrylamide are heterogeneous in origin and chemistry, yet each can be attenuated to a meaningful extent by plant-derived compounds or probiotic microorganisms.

Plant-based strategies draw simultaneously from traditional dietary knowledge and contemporary mechanistic research. Bioactive compounds occurring in garlic, coriander, milk thistle, green tea, turmeric and related botanicals including organosulfur compounds, flavonoids and polyphenols have been shown to mitigate metal and mycotoxin toxicity by chelating ions, reducing intestinal absorption or accelerating elimination (Mehrandish et al., 2019). Likewise, polyphenols can modulate the carbonyl chemistry that drives acrylamide formation, whereas essential oils can suppress fungal growth and thereby interrupt mycotoxin biosynthesis at its source (Ajmal et al., 2025). Dietary fibers and natural adsorbents function as simple but effective physical–chemical barriers, retaining both inorganic and organic toxicants within the intestinal lumen and reducing their systemic delivery (Mehrandish et al., 2019). When such botanical levers are embedded into existing culinary and processing practices for instance via fermentation, herb/spice inclusion or fiber enrichment foods can become inherently safer without dependence on synthetic interventions.

Probiotic-based approaches exploit the detoxification capacity of living microorganisms. Lactic acid bacteria and *Saccharomyces* yeasts can immobilize heavy metals and mycotoxins on their cell walls or, in certain cases, biotransform these toxicants into less harmful derivatives through enzymatic activity (Liu et al., 2022; Shetty & Jespersen, 2006). *Lactobacillus* strains have been shown in animal and pilot human studies to bind cadmium or lead, limit their epithelial translocation and facilitate their excretion from the body (Zhai et al., 2019; Bisanz et al., 2014). Likewise, lactic acid bacteria and yeast have been reported to adsorb aflatoxin and zearalenone, lowering their bioavailability; in

some fermentations partial metabolic conversion further reduces toxicity (Nathanail et al., 2016; Shetty & Jespersen, 2006). In addition, the use of probiotic cultures as fermentation starters may help suppress acrylamide formation by depleting precursors or releasing L-asparaginase during dough or pre-fry fermentation (Khorshidian et al., 2020).

The effectiveness of natural detoxification ultimately depends on selecting the correct method or combination of methods for each contaminant context. For instance, in the case of chronic heavy metal exposure, co-consumption of sulfur-rich botanicals (such as garlic or onions) together with *Lactobacillus*-fermented dairy may provide synergistic protection. A cereal product with mold risk may be stabilized with essential oils while also undergoing lactic–yeast fermentation to minimize toxin development and bioaccessibility. Reducing antibiotic residues can begin at the farm level by replacing growth-promoting antibiotics with phytobiotics and probiotics, and be complemented at the consumer side by natural adsorbents such as fibers or chitosan in the food matrix (Sardar et al., 2023). For acrylamide, a layered, multi-barrier strategy combining low-asparagine raw materials with polyphenol or fiber enrichment and, where feasible, probiotic pre-fermentation is likely to provide the most robust reduction.

Although these nature-derived strategies are promising, their limitations must be acknowledged. Most demonstrations of efficacy originate from controlled laboratory systems; in real food matrices, constraints related to composition, processing and sensory acceptability can attenuate performance. Even so, within a risk-management paradigm in which partial reduction is preferable to no reduction, such interventions remain valuable. Importantly, the current shift in consumer preference toward clean-label and naturally processed foods is accelerating industrial interest in botanical and probiotic alternatives, with a notable decline in the use of synthetic additives in recent product reformulation trends (Liu et al., 2015).

Advances in biotechnology are likely to amplify this trajectory. Engineered or selectively screened probiotic strains capable of expressing detoxifying enzymes may be incorporated into foods or supplements to actively dismantle target toxicants (He et al., 2024). Likewise, by-products of sustainable agriculture such as algal biomass

or plant husks can be valorized as low-cost bio-adsorbents for environmental and food detoxification scenarios (Sardar et al., 2023). Such developments could simultaneously reduce waste and strengthen food-system safety.

Taken together, natural detoxification represents a complementary and integrative pillar in food safety. Botanicals and probiotics as long-standing dietary companions can assist in the management of unwanted chemicals without introducing new synthetic burdens. These strategies do not compete with conventional controls but rather extend and reinforce them. The most effective paradigm is dual: prevention of formation at the source and removal or neutralization once present. Natural agents can plausibly operate at both ends for example, essential oils preventing fungal toxin generation while probiotic cultures adsorb or transform residual molecules downstream.

Accumulating scientific evidence indicates that, when applied judiciously, natural detoxification can meaningfully attenuate food-related chemical risks. In light of the findings synthesized in this chapter, future research should prioritize the identification of optimal pairings of botanicals and probiotic strains for specific contaminant profiles and verify their effectiveness under physiologically relevant in vivo conditions. Achieving this would enable the issuance of actionable guidance for both industry and consumers, ultimately supporting the production of safer foods without compromising natural identity or technological feasibility.

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CHAPTER 2:

Biodetoxification Capacities of *Lactobacillus* Species

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1.Introduction

The *Lactobacillus* genus comprises numerous bacterial species commonly found in fermented foods and probiotic supplements, many of which are beneficial to human health. These bacteria have also attracted attention for their potential to neutralize harmful chemical contaminants through biodetoxification mechanisms. Toxins frequently detected in foods such as mycotoxins, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) cannot be easily removed by conventional thermal or chemical methods and therefore pose serious risks to human health (Choi et al., 2025; Owolabi et al., 2022). At this point, the ability of *Lactobacillus* species to bind or transform these toxins into less harmful forms offers a natural and reliable strategy to enhance food safety and reduce the intake of such substances by the human body.

This section provides an in-depth examination of the detoxification capacities of different *Lactobacillus* species such as *Lactobacillus acidophilus, L. kefiri,* and *L. fermentum* against mycotoxins (e.g., AFB_1 and AFM_1), heavy metals (Pb, Cd, Zn, Ni), and PAH compounds.

Furthermore, it discusses how these bacteria bind or biotransform toxins, the environmental and biological factors influencing detoxification efficiency, the impact of probiotic encapsulation technologies on this process, practical applications in food safety, their positive effects on human health, and future research directions. Several *Lactobacillus* strains exhibit the ability to bind or biotransform toxic compounds such as mycotoxins and heavy metals, thereby reducing intestinal absorption and systemic toxicity (Figure 1)

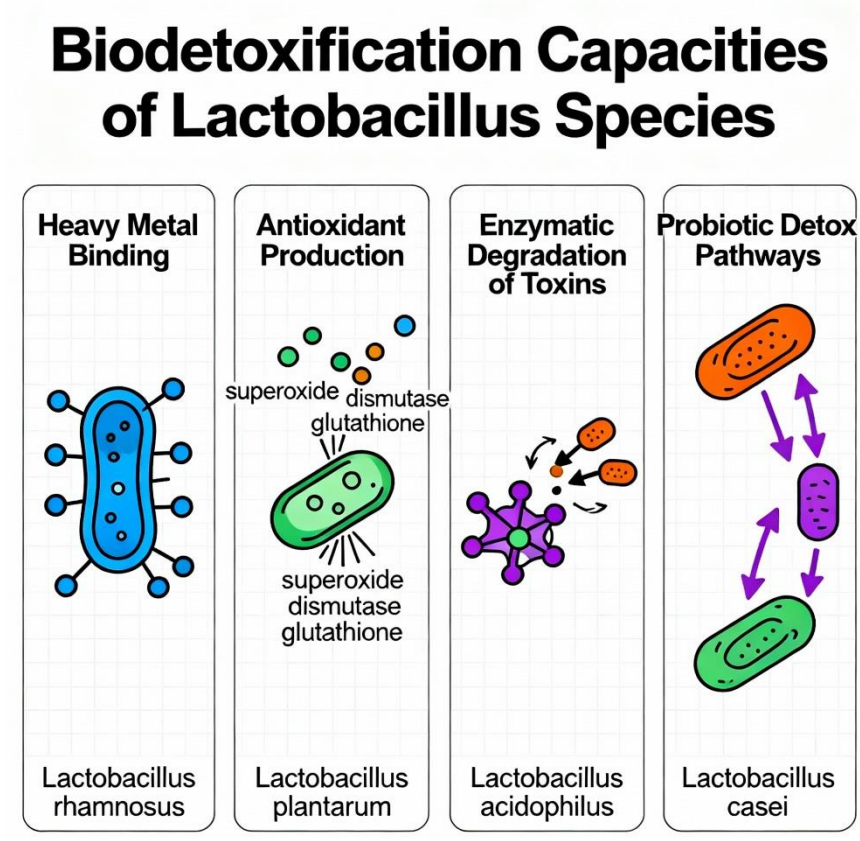

Figure 1. Biodetoxification Capacities of Lactobacillus Species

2. Mycotoxins (AFB_1 and AFM_1) and *Lactobacillus* Species

Aflatoxin B_1 (AFB_1) is one of the most toxic and widespread mycotoxins produced by molds such as Aspergillus flavus and A. parasiticus. Even at trace levels in food, it is strongly associated with the risk of hepatocellular carcinoma (liver cancer) (Choi et al., 2025; Owolabi et al., 2022). Aflatoxin M_1 (AFM_1), on the other hand, is a hydroxylated metabolite of AFB_1 formed in animals such as cows and excreted into milk; thus, it can be transmitted to humans through the consumption of milk and dairy products. The ability of *Lactobacillus* species to detoxify both AFB_1 and AFM_1 has been extensively studied as a promising approach to reducing human exposure to these toxins (Biniş and Demir, 2023, Demir et al., 2017).

Numerous *Lactobacillus* species can neutralize AFB_1 and AFM_1 through adsorption, a process largely mediated by the components of the bacterial cell wall (Owolabi et al., 2022). For instance, *Lactobacillus rhamnosus* GG has been shown to bind AFB_1 primarily via peptidoglycans in the cell wall, while cell wall proteins and lipids play a negligible role in this mechanism (Choi et al., 2025). In other species, such as *L. casei* Shirota and *L.* reuteri, teichoic acids alongside peptidoglycans also contribute to the binding of AFB_1 (Owolabi et al., 2022).

Due to this strong binding capacity, viable *Lactobacillus* cells can retain AFB_1 within the intestinal lumen, thereby reducing its absorption. Indeed, in human intervention studies, the inclusion of probiotic *Lactobacillus* strains significantly decreased AFB_1 biomarkers in body fluids (Choi et al., 2025). This finding indicates that probiotic *Lactobacillus* strains may mitigate AFB_1 toxicity by lowering its intestinal uptake and, consequently, the risk of liver cancer (Choi et al., 2025; Wacoo et al., 2020).

Species such as *L. acidophilus*, *L. casei*, *L. plantarum*, and *L. rhamnosus* are particularly noted for their high AFB_1-binding abilities. Both viable and heat-inactivated (dead) cells exhibit AFB_1 adsorption, highlighting that the mechanism primarily depends on the cell surface architecture rather than metabolic activity (Owolabi et al., 2022). For example, cell wall extracts of *L. rhamnosus* GG retain nearly the same AFB_1 binding capacity (~81%) as live cells, whereas extracellular polysaccharides (EPS) bind less than 1% of AFB_1 (Choi et al., 2025). This clearly demonstrates

that the key structural elements responsible for binding are the peptidoglycans and embedded polymers such as teichoic acids within the cell wall.

Moreover, differences in the composition and structure of teichoic acids influence binding efficiency across species. For example, the structural variations in teichoic acids between *L. casei* Shirota and *L. rhamnosus* GG may result in differences in their AFB_1-binding efficacy (Choi et al., 2025).

2.1. AFM_1 Removal Capacity of *Lactobacillus* and Its Practical Significance in Dairy Products

The capacity of *Lactobacillus* strains to remove AFM_1 is of particular practical importance in dairy products. Research has demonstrated that probiotic bacteria can substantially reduce AFM_1 concentrations in milk and fermented dairy products. For instance, when only conventional starter cultures such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are used in fermented milk products like yogurt or kefir, the reduction of AFM_1 is relatively limited (approximately 15-40%). In contrast, products supplemented with *L. acidophilus* have shown nearly complete (>95%) elimination of AFM_1 (Zareie et al., 2024).

Adibpour and colleagues reported that the inclusion of pure *L. acidophilus* culture at a concentration of 10^8 CFU/mL during fermentation resulted in over 99% AFM_1 removal in fermented milk (Zareie et al., 2024). Similarly, supplementation with *L. casei* achieved approximately 81% reduction of AFM_1 in milk (Zareie et al., 2024). Kefir culture presents a unique case: the mixed microbiota of kefir grains, which includes *L. kefiri*, exhibits a notably high AFM_1-binding capacity. In one study, the use of kefir starter culture alone reduced AFM_1 levels by about 85%, exceeding even the efficiency observed with *L. casei* -supplemented fermentation (Zareie et al., 2024). This suggests a synergistic effect between *Lactobacillus* strains and yeast species within the kefir microbiota in adsorbing AFM_1.

In general, traditional yogurt starter cultures alone show minimal capacity to eliminate AFM_1 (in some studies, reductions were negligible) (Zareie et al., 2024), whereas the addition of probiotic strains such as *L. acidophilus* or *L. casei* significantly enhances detoxification. Therefore, the presence

of *Lactobacillus* species plays a critical role in the biodetoxification of AFM_1 in dairy matrices.

Beyond physical adsorption, biotransformation mechanisms may also contribute to mycotoxin detoxification in certain cases. Although *Lactobacillus* strains generally lack specific enzymes capable of cleaving the chemical structure of aflatoxins, some species such as *L. plantarum* have been reported to degrade other mycotoxins (e.g., *Fusarium*-derived toxins) into less toxic metabolites (Nasrollahzadeh et al., 2022). Moreover, several *Lactobacillus* and related lactic acid bacteria species have been suggested to detoxify toxins like zearalenone and fumonisins through both cell wall interactions and enzymatic modifications (Nasrollahzadeh et al., 2022).

Nevertheless, in the case of aflatoxins, the predominant detoxification mechanism for the *Lactobacillus* genus is generally accepted to be reversible adsorption onto the bacterial cell wall (Owolabi et al., 2022). This binding primarily occurs through hydrophobic interactions, Van der Waals forces, and hydrogen bonding (Owolabi et al., 2022). Consequently, *Lactobacillus* species significantly lower the bioavailability of AFB_1 and AFM_1 by binding these toxins to their cell surfaces or incorporating them within their structures, thereby reducing their mutagenic and carcinogenic effects within both food systems and the human gastrointestinal tract (Table 1).

3. Heavy Metals (Pb, Cd, Zn, Ni) and *Lactobacillus* Species

Heavy metals are inorganic pollutants that can enter the food chain through environmental contamination and accumulate in living organisms, exerting toxic effects over time. Metals such as lead (Pb) and cadmium (Cd) are particularly known for their harmful impacts on the nervous system, kidneys, and liver, while zinc (Zn) and nickel (Ni), though essential trace elements, can cause toxicity when present at elevated levels. The ability of *Lactobacillus* species to bind heavy metals and thereby reduce their intestinal absorption has emerged as a promising biological approach for both food safety enhancement and protective dietary supplementation (Muhammad et al., 2021; Massoud & Zoghi, 2022).

The primary mechanism underlying the detoxification of heavy metals by *Lactobacillus* strains is biosorption, which involves the adherence of metal ions onto the bacterial cell wall surface. As Gram-positive bacteria, *Lactobacillus* species possess cell walls rich in peptidoglycans, teichoic acids, and various functional groups such as carboxyl, phosphate, hydroxyl, and amino groups. This chemically complex cell wall architecture enables strong electrostatic and coordination interactions with metal cations, promoting efficient sequestration and immobilization of toxic metals.

For instance, the *L. acidophilus* strain KLDS1.1003 has demonstrated an exceptionally high affinity for Pb^{2+} ions, with modeling studies indicating that it can sequester up to 430 mg/L of lead onto its surface (Muhammad et al., 2021). In vitro experiments conducted under near-neutral pH conditions (pH 6 -7) showed that *L. acidophilus* cells could rapidly adsorb Pb^{2+} ions to their surfaces, resulting in a substantial reduction in the concentration of soluble lead in the surrounding medium (Muhammad et al., 2021).

3.1. Species-Specific Variability in Heavy Metal Binding by *Lactobacillus*

The heavy metal binding capacity of *Lactobacillus* species varies considerably among strains. In a study on *L. acidophilus* ATCC 4356, the bacterium was found to effectively remove low concentrations (approximately 100 μg/L) of Pb^{2+} and Cd^{2+} ions from milk. Under optimal conditions (bacterial concentration of 10^{12} CFU and a 4-day contact period), *L. acidophilus* biomass eliminated approximately 80% of lead and 75% of cadmium from the milk matrix (Massoud et al., 2020). These findings demonstrate that *Lactobacillus* strains possess a tangible capacity to remove heavy metals even within real food systems.

Similarly, *L. plantarum* species have shown notable metal-binding performance. Experiments using a marine-derived strain, *L. plantarum* MF042018, revealed that this bacterium accumulated Pb^{2+} and Cd^{2+} ions on its cell surface, as evidenced by transmission electron microscopy (TEM) images showing dense metal precipitates coating the bacterial envelope (Ameen et al., 2020). Remarkably, in the same study, live *L.*

plantarum cells almost completely removed Ni^{2+}, Cr^{2+}, Cd^{2+}, and Pb^{2+} ions from industrial wastewater within only one hour of contact, achieving nearly 100% efficiency (Ameen et al., 2020). These striking results suggest that under favorable conditions, *Lactobacillus* biomass can achieve an exceptionally high metal removal capacity.

Lactobacillus fermentum also plays a role in metal detoxification. For instance, Halttunen and colleagues (2008) reported that lyophilized cells of *L. fermentum* ME3, when exposed to lead, exhibited pronounced Pb accumulation on their cell surfaces, confirming their strong affinity for Pb^{2+} ions. Probiotic species such as *L. rhamnosus* and *L. casei* have likewise been shown to rapidly adsorb Cd^{2+} and Pb^{2+} from aqueous environments, achieving maximum binding within the first hour, after which a slight decrease in removal efficiency was observed with prolonged exposure (Massoud & Zoghi, 2022). This pattern suggests that metal binding occurs rapidly and primarily at the surface level, reaching equilibrium as the available binding sites become saturated. Indeed, at higher initial metal concentrations (>50-100 ppm), the finite number of binding sites on the cell wall becomes saturated, limiting further ion attachment and consequently reducing overall removal percentages.

A critical determinant of the metal-binding capacity of *Lactobacillus* is the abundance of negatively charged functional groups on the cell surface. The surfaces of lactic acid bacteria are typically negatively charged, facilitating electrostatic attraction between the bacterial cell and positively charged metal cations such as Pb^{2+} and Cd^{2+} (Daisley et al., 2019). Carboxylate and phosphate groups on the cell wall can form strong complexes with “soft acid” metals like Pb^{2+} and Cd^{2+}, thereby immobilizing them. FTIR analyses have shown that upon Pb binding, significant spectral shifts occur in the amino, amide, phosphoryl, carboxyl, and hydroxyl functional groups of *L. acidophilus* cells and their microcapsule matrices, indicating that these groups directly participate in lead biosorption (Daisley et al., 2019). Similarly, adsorption studies on *L. plantarum* have revealed that Cd^{2+} and Pb^{2+} binding follows the Langmuir isotherm model, indicating a monolayer adsorption process characterized by a fixed number of high-affinity binding sites on the bacterial surface.

3.2. Species-Specific Affinity of *Lactobacillus* Toward Different Heavy Metals

Different *Lactobacillus* species exhibit varying affinities for distinct heavy metals. *L. bulgaricus* has been reported to efficiently remove Pb^{2+} and Cd^{2+} ions, achieving approximately 47% lead and 25% cadmium removal, whereas *L. casei* demonstrated comparatively lower binding efficiency in the same study. In contrast, *L. plantarum* and *L. brevis* species have shown the ability to tolerate and biosorb other metals such as nickel and chromium (Ameen et al., 2020). For example, a marine-derived *L. plantarum* strain (MF042018) was able to maintain normal growth even at high concentrations of Ni^{2+} and Cr^{2+} (up to 600 ppm), while simultaneously binding these metals at the cellular level (Ameen et al., 2020). Such adaptive capabilities suggest that these strains possess specific structural or compositional features within their cell walls that confer enhanced metal tolerance and binding ability.

In summary, *Lactobacillus* species are capable of binding and removing heavy metals such as lead, cadmium, zinc, and nickel from their environment (Table 1). This property implies that these bacteria can be utilized to reduce metal concentrations in contaminated food matrices (e.g., lead-contaminated milk or cadmium-tainted grains). Moreover, when consumed as probiotics, they may protect human and animal health by reducing intestinal absorption of heavy metals, as demonstrated by several *in vivo* studies (Nasrollahzadeh et al., 2022; Giri et al., 2024).

4. Polycyclic Aromatic Hydrocarbons (PAHs) and *Lactobacillus* Species

Polycyclic aromatic hydrocarbons (PAHs) are chemical contaminants formed during the incomplete combustion of organic materials and are commonly introduced into foods during processes such as smoking, frying, or grilling. Benzo[a]pyrene (BaP), one of the most studied PAHs, is a potent carcinogen and mutagen. Because PAHs are lipophilic and highly hydrophobic, they can accumulate in fatty foods and, upon ingestion,

induce long-term carcinogenic effects through DNA damage. Although the detoxification of PAHs by *Lactobacillus* species has been less extensively studied compared with other toxins, recent research indicates that these bacteria can also adsorb and remove PAHs from their surrounding environment (Qi et al., 2011).

The principal mechanism of PAH removal by *Lactobacillus* is adsorption, driven predominantly by hydrophobic interactions. PAH molecules such as BaP interact with hydrophobic regions on the bacterial cell surface, allowing them to adhere to the cells without being metabolized. In a study investigating the BaP-binding capacity of *L. plantarum* and *L. pentosus* strains, after 4 hours of incubation at 37 °C, *L. plantarum* 121 bound 65.9% of BaP, while *L. pentosus* ML32 bound 64.9% (Qi et al., 2011). These remarkably high binding rates indicate that LAB cells can effectively retain BaP on their surfaces even without internalizing it.

The same study also reported that BaP-binding efficiency is influenced by several factors, including incubation time, temperature, bacterial viability, pH, and the presence of ions such as Ca^{2+} and Mg^{2+} (Qi et al., 2011). For instance, pretreatment of bacterial cells with organic solvents (e.g., benzene) markedly reduced BaP adsorption capacity likely because benzene molecules occupy hydrophobic sites on the cell surface, thereby competing with BaP for binding.

4.1. Influence of pH and Environmental Factors on PAH Binding by *Lactobacillus*

The ambient pH plays a crucial role in the adsorption of PAHs by *Lactobacillus* species. According to the findings of Qi et a*L.* (2011), the highest binding efficiency of BaP by *L. plantarum* 121 and *L. pentosus* ML32 occurred at pH 4-5. This effect is likely attributable to the partial denaturation of cell surface proteins at lower pH values, which increases their hydrophobicity and/or neutralizes surface charges through protonation, thereby enhancing the affinity of hydrophobic PAH molecules for the bacterial surface (Owolabi et al., 2022).

Indeed, similar phenomena have been observed for aflatoxin binding, where exposure of LAB cells to acidic conditions improved their toxin-

binding capacity. The increased binding was attributed to conformational changes in surface proteins that exposed additional binding sites (Owolabi et al., 2022). A comparable mechanism is presumed for PAHs: during yogurt fermentation, as pH decreases, starter culture bacteria tend to adsorb higher amounts of PAH compounds (Qi et al., 2011). Abou-Arab and colleagues further demonstrated that the pH reduction during yogurt production facilitates the removal of certain PAH molecules through adsorption by starter cultures such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Qi et al., 2011).

Similarly, *L. bulgaricus* EMCC1102 has been reported to bind a range of PAH compounds (approximately 60-77% removal) and significantly decrease the total PAH content in cold-smoked meat products (Sultana et al., 2021). These findings collectively indicate that acidic environments and fermentation processes can enhance PAH removal efficiency through increased bacterial surface hydrophobicity and the exposure of additional binding domains.

Some *Lactobacillus* species have also been evaluated for their ability to simultaneously bind multiple PAH compounds. For instance, the PAH removal performance of *L. brevis* TD4 against four major PAHs benzo(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), and chrysene (Chr) was analyzed using Response Surface Methodology (RSM) (Chlebicz & Śliżewska, 2020). Multivariate statistical analysis revealed that *L. brevis* TD4 effectively removed all four PAHs, although binding efficiency varied depending on the compound. The study also identified several critical parameters influencing PAH adsorption, including pH (optimal ≈5), bacterial population density ($\geq 10^9$ CFU/mL), initial PAH concentration (~10 ppm), and contact time. Notably, cell density was the most influential factor higher bacterial populations provided more available binding surfaces, resulting in markedly greater overall adsorption efficiency. At 10^{11} CFU/mL, maximum removal was achieved for all tested PAHs, underscoring the importance of sufficient biomass in effective PAH detoxification.

4.2. Mechanistic Insights into PAH Detoxification by *Lactobacillus*

Although adsorption is the principal mechanism in PAH detoxification by *Lactobacillus*, possible contributions from microbial degradation have

also been explored. Since lactic acid bacteria are facultatively anaerobic and generally lack the enzymatic systems required to cleave complex aromatic rings, they tend to retain PAHs rather than biodegrade them. Nonetheless, it has been suggested that during lactic acid fermentation, the formation of reactive metabolites such as peroxides might induce partial chemical modification of PAH structures (Nasrollahzadeh et al., 2022).

While current evidence for enzymatic breakdown of PAHs by *Lactobacillus* remains limited, it is widely accepted that, unlike species from genera such as *Bacillus* or *Pseudomonas*, *Lactobacillus* primarily immobilizes PAHs on its cell surface instead of mineralizing them. Thus, the detoxification effect arises from physical sequestration rather than true biodegradation (Table 1).

In conclusion, *Lactobacillus* species exhibit significant potential to mitigate PAH contamination particularly carcinogenic compounds like benzo[a]pyrene found in smoked or grilled foods by adsorbing these molecules onto their cell surfaces. This process can reduce the total PAH content in foods, thereby limiting their gastrointestinal absorption and ultimately contributing to the long-term reduction of PAH-associated carcinogenic risks in humans.

Table 1. Representative data on the binding/detoxification capacities of selected *Lactobacillus* species against various toxins.

Toxin	Representative *Lactobacillus* species (strain)	Detoxification mechanism	Removal efficiency (conditions)
Aflatoxin B_1 (AFB_1)	*L. rhamnosus* GG, *L. casei* Shirota	Cell wall adsorption (peptidoglycan, teichoic acid interaction)	80-95% binding (in vitro, high cell density) (Choi et al., 2025); significant reduction of AFB_1 biomarkers in humans after probiotic intake (Choi et al., 2025)

Aflatoxin M_1 (AFM_1)	*L. acidophilus* (fermented milk culture)	Cell wall adsorption	>95% removal (fermented milk, 24 h, ~10^8 CFU/mL) (Zareie et al., 2024); ~85% reduction with kefir culture (Zareie et al., 2024)
Lead (Pb)	*L. acidophilus* ATCC 4356, KLDS1.1003	Cell surface biosorption (carboxyl and phosphate group interactions)	~80% removal (milk, 4 days, 10^{12} CFU) (Massoud et al., 2020); significant decrease in blood Pb levels in mice after microencapsulated probiotic treatment (Muhammad et al., 2021)
Cadmium (Cd)	*L. acidophilus* ATCC 4356	Cell surface biosorption	~75% removal (milk, 4 days, 10^{12} CFU) (Massoud et al., 2020)
Nickel (Ni)	*L. plantarum* MF042018 (marine isolate)	Cell surface biosorption	Near 100% removal (industrial wastewater, dialysis bag setup, 1 h) (Ameen et al., 2020)
PAH (Benzo[a] pyrene)	*L. plantarum* 121, *L. pentosus* ML32	Cell wall adsorption via hydrophobic interactions	~65% binding (MRS broth, pH 6.2, 37 °C, 4 h) (Qi et al., 2011); enhanced binding at optimal pH 4-5 (Qi et al., 2011)
PAH mixture	*L. brevis* TD4	Cell wall adsorption	Significant removal of four PAH compounds; highest binding achieved at pH ≈ 5, 10 ppm initial concentration, and≥10^9 CFU/mL

			(Chlebicz & Śliżewska, 2020)

5. Biodetoxification Mechanisms: Binding and Biotransformation

The fundamental mechanisms underlying toxin detoxification by *Lactobacillus* species can be broadly categorized into two main pathways: (i) adsorptive binding, and (ii) biotransformation, in which the chemical structure of the toxin is modified, though this occurs less frequently. Most studies indicate that *Lactobacillus* strains neutralize both mycotoxins and heavy metals primarily through cell wall-mediated surface adsorption (Owolabi et al., 2022; Nasrollahzadeh et al., 2022). The bacterial cell wall, typical of Gram-positive organisms, consists of a thick peptidoglycan layer decorated with teichoic acids, lipoteichoic acids, proteins, and polysaccharides, all of which contribute functional moieties capable of interacting with toxic molecules (Demir and Demir, 2021).

The peptidoglycan backbone, composed of *N-acetylglucosamine* and *N-acetylmuramic acid* linked by peptide chains, provides numerous functional groups that can physically adsorb toxins. Carboxyl ($-COO^-$) and phosphate (PO_4^-) residues serve as strong binding sites for metal cations, whereas amino and amide groups can interact with certain organic toxins, such as patulin. Spectroscopic analyses using *L. acidophilus* cells have confirmed that amino, phosphoryl, and carboxyl groups participate directly in lead binding (Daisley et al., 2019).

In the case of mycotoxins, adsorption primarily involves hydrogen bonding and van der Waals interactions between the toxin molecules and the polysaccharides or peptidoglycan chains of the cell wall (Pop et al., 2022). For instance, *L. rhamnosus* binds aflatoxin B_1 mainly via hydrogen bonds with peptidoglycans, while *L. reuteri* and *L. casei* interact not only with peptidoglycan but also with teichoic acid structures (Owolabi et al., 2022). Some hydrophobic mycotoxins, such as zearalenone, are believed to attach to surface proteins through hydrophobic and electrostatic interactions and may also penetrate the cell membrane to form complexes with intracellular proteins (Pop et al., 2022).

6. Variability in Cell Wall Components and Reversibility of Adsorption

The affinity of *Lactobacillus* cell wall components for toxins can vary considerably among species and even among strains. In *L. plantarum* and *L. pentosus*, for instance, the highest binding capacity toward fumonisin B_1 has been localized within the peptidoglycan fraction, whereas the lipid and protein fractions of the cell wall showed little or no involvement in toxin adsorption. Conversely, in certain cases, cell surface proteins play a more dominant role. For example, the binding of patulin by *L. casei* has been shown to depend largely on surface adhesion proteins. Treatments with protease and periodate significantly reduced patulin-binding capacity, while esterification increased it indicating that specific functional groups on surface proteins act as potential binding sites for this mycotoxin.

In summary, toxin binding in *Lactobacillus* involves multiple structural components peptidoglycans, teichoic acids, surface proteins, and exopolysaccharides and the predominant binding element differs depending on the particular toxin -bacterium interaction. Adsorptive binding is generally a reversible process, meaning that the toxin remains attached to the bacterial surface but can be released back into the surrounding medium unless the chemical characteristics of the binding sites are altered. Therefore, maintaining environmental conditions that favor strong and stable interactions is critical for achieving durable detoxification (as discussed in the following section). Moreover, because adsorption-based detoxification does not chemically alter the toxin molecule, the compound remains present but is immobilized in a non-toxic, bound state that can be safely eliminated from the body (Owolabi et al., 2022).

7. Impacts on Human Health

The detoxification capacity of *Lactobacillus* species exerts numerous direct and indirect health benefits in humans. By reducing exposure to foodborne toxins, these bacteria help prevent both acute and chronic disorders associated with such contaminants. When combined with the

intrinsic probiotic effects of *Lactobacillus* such as enhancing gut health, modulating the immune system, and maintaining intestinal barrier integrity an overall protective shield against toxic and pathogenic insults is formed.

One of the most notable benefits is the reduction of cancer risk through decreased exposure to carcinogenic and mutagenic compounds. For instance, aflatoxin B_1, a potent hepatocarcinogen, can be bound by probiotic *Lactobacillus* cells in the intestinal tract and subsequently eliminated without being absorbed (Choi et al., 2025). Human clinical trials have shown that individuals consuming probiotic supplements or dairy products enriched with *L. rhamnosus* GG or *L. casei* Shirota exhibited significantly lower levels of AFM_1-DNA adducts and aflatoxin -albumin biomarkers, which are indicators of aflatoxin exposure (Choi et al., 2025).

These findings suggest that the regular intake of probiotics could play a crucial preventive role in reducing aflatoxin-induced liver damage and, consequently, the development of hepatocellular carcinoma, especially in populations residing in high-risk regions (Choi et al., 2025; Wacoo et al., 2020). Notably, the World Health Organization (WHO) has estimated that 5 -30% of the global liver cancer burden may be attributed to aflatoxin exposure (Choi et al., 2025). Thus, the incorporation of probiotic *Lactobacillus* strains into the human diet can be regarded as a public health intervention aimed at mitigating carcinogenic risks in areas where aflatoxin contamination remains prevalent.

7.1. Protective Effects Against Heavy Metals and Immunomodulatory Benefits

Another significant health benefit of *Lactobacillus* species lies in their ability to mitigate the toxic effects of heavy metals in the human body. Metals such as lead (Pb) and cadmium (Cd) are known to cause severe physiological damage upon accumulation, including bone marrow suppression, neurodevelopmental delay, kidney injury, and immune dysfunction (Massoud & Zoghi, 2022). Probiotic *Lactobacillus* strains can bind these metals within the intestinal lumen, thereby reducing their bioavailability and systemic absorption.

Clinical and experimental studies have demonstrated that probiotic supplementation can significantly influence metal kinetics in vivo. For example, a probiotic mixture containing *L. rhamnosus* and *L. plantarum* was shown to increase urinary lead excretion and decrease blood lead concentrations in children exposed to lead-contaminated environments. Similarly, animal experiments have revealed that groups receiving probiotics exhibited lower tissue accumulation of heavy metals, enhanced antioxidant enzyme activity, and reduced inflammatory responses compared to control groups (Giri et al., 2024; Muhammad et al., 2021).

In lead-exposed mice treated with *L. acidophilus*, researchers observed less structural damage in liver and kidney tissues and lower tissue lead levels than in non-supplemented animals (Muhammad et al., 2021). These findings provide direct evidence that probiotic-mediated metal detoxification can protect vital organs. Furthermore, this protective mechanism involves the attenuation of oxidative stress probiotics have been reported to sustain high levels of antioxidants such as glutathione, while suppressing the production of proinflammatory cytokines induced by heavy metal exposure (Massoud & Zoghi, 2022).

The immunomodulatory properties of probiotic *Lactobacillus* species are equally noteworthy. In addition to counteracting the immunosuppressive effects of toxic compounds, these bacteria actively enhance host immune defenses through multiple mechanisms. Chronic exposure to aflatoxins, for instance, is known to suppress antibody production and increase susceptibility to infections. Probiotics such as *L. rhamnosus* can reverse these effects by activating immune cells within the intestinal mucosa and by producing anti-inflammatory metabolites such as short-chain fatty acids (Pop et al., 2022).

In cellular studies, *L. rhamnosus* GG was shown to inhibit NF-κB signaling and downregulate the expression of proinflammatory cytokines such as IL-1β in aflatoxin-exposed cultures, thereby suppressing toxin-induced inflammation. These experimental findings suggest that probiotics may protect against the immune-disruptive and proinflammatory effects of aflatoxin exposure. Clinical evidence also supports this observation: individuals receiving probiotic supplementation display lower circulating inflammatory markers and experience symptomatic improvements in certain allergic and inflammatory conditions. Hence, probiotic

Lactobacillus strains contribute not only to detoxification but also to immune homeostasis, alleviating chronic inflammation and oxidative stress associated with toxin exposure.

Another critical aspect of *Lactobacillus* function is its ability to strengthen the intestinal barrier, which plays a key role in preventing toxin absorption and systemic inflammation. Mycotoxins particularly deoxynivalenol (DON) can damage intestinal epithelial cells, increasing permeability and leading to the so-called "leaky gut" condition, which facilitates the passage of toxins and pathogens into the bloodstream. *Lactobacillus* strains counteract this effect by upregulating tight junction proteins, thereby reinforcing the intestinal wall and reducing the translocation of harmful compounds (Choi et al., 2025). For instance, in animal models, *L. casei* Shirota has been shown to protect intestinal mucosa and prevent the systemic absorption of aflatoxins (Choi et al., 2025). This barrier-strengthening effect not only limits toxin uptake but also helps block the invasion of harmful microorganisms, thereby promoting overall gut and systemic health. Collectively, these mechanisms highlight the broad protective role of probiotic *Lactobacillus* in reducing overall toxicity and enhancing physiological resilience. The following key health outcomes have been consistently reported:

Reduction in Cancer Risk: By lowering exposure to carcinogenic compounds such as aflatoxins and PAHs, *Lactobacillus* contributes to the prevention of liver and colorectal cancers (Choi et al., 2025). Furthermore, certain species can bind dietary mutagens such as nitrosamines and heterocyclic amines, suggesting a possible reduction in colon cancer risk associated with these genotoxic compounds (Demir et al., 2025).

Organ-Protective Effects: Continuous exposure to toxins imposes heavy metabolic stress on detoxification organs such as the liver and kidneys. Probiotics alleviate this burden by reducing toxin load and oxidative damage. In aflatoxin-exposed animals, probiotic supplementation resulted in lower serum ALT and AST levels, reduced hepatic lipid accumulation, and less necrotic damage indicating protection against chronic toxin-induced injury.

Support for Growth and Development: Chronic aflatoxin exposure during childhood has been linked to stunted growth and low body weight (Wacoo et al., 2020). Intervention studies in Africa have shown that children consuming probiotic-fermented foods exhibited better growth trajectories and lower aflatoxin biomarkers compared with control groups. These findings suggest that probiotics may indirectly promote growth and nutritional well-being in vulnerable populations.

Cardiovascular and Neurological Protection: By lowering the accumulation of heavy metals and toxins, probiotics can reduce long-term risks of hypertension, renal failure, and neurotoxicity. For instance, lead exposure is associated with decreased cognitive performance in children, whereas probiotic interventions that reduce blood lead concentrations may help protect against such neurodevelopmental impairments (Massoud & Zoghi, 2022).

Maintenance of Gut Microbiota Balance: Toxins can disrupt the intestinal microbial ecosystem by suppressing beneficial bacteria. High levels of aflatoxin B_1 have been shown to alter gut microbial composition, reducing populations of commensal bacteria (Choi et al., 2025). Probiotic supplementation can counteract this dysbiosis, fostering a resilient and detoxification-competent microbiota that acts as an "extra metabolic organ" to neutralize toxins. In this sense, *Lactobacillus* species not only directly bind and inactivate toxins but also promote a gut environment capable of sustaining long-term resistance to toxic stress.

Taken together, these findings demonstrate that probiotic *Lactobacillus* strains play a multifaceted role in human health protection. Their detoxification capacity constitutes a vital component of this effect, reducing dietary exposure to carcinogenic and toxic compounds and supporting long-term physiological well-being. Consequently, functional foods and dietary supplements enriched with *Lactobacillus* represent a strategic nutritional tool for safeguarding both individual and public health.

8. Future Research Directions and Biotechnological Advances

Significant progress has been made in understanding the biodetoxification potential of *Lactobacillus* species; however, several promising research directions remain open. Future studies and technological innovations are expected to focus on:

Discovery of potent strains: Screening naturally occurring and fermented food isolates may reveal novel *Lactobacillus* strains with exceptional toxin-binding or degradation abilities. Environmentally adapted strains such as those isolated from metal-rich sites often show high tolerance and adsorption capacity. Genetic improvement through mutation or metabolic engineering could further enhance these properties.

Enhanced enzymatic detoxification: Since adsorption alone does not destroy toxins, future approaches aim to equip *Lactobacillus* with detoxifying enzymes via genetic modification or synthetic biology. Recombinant strains capable of degrading aflatoxins or zearalenone are already being explored, marking a step toward multifunctional "detox probiotics."

Synergistic probiotic and synbiotic formulations: Combining strains with complementary mechanisms or pairing probiotics with prebiotic fibers could broaden detoxification efficiency. Optimal strain compatibility and formulation stability will be key to next-generation probiotic products.

Industrial and applied uses: Functional foods such as "aflatoxin-binding yogurts" or feed additives with probiotic-based detox agents represent potential commercial innovations. Similarly, probiotic-based biofilters and packaging materials capable of sequestering contaminants are emerging research areas.

Mechanistic and safety studies: Molecular modeling, genomic analysis, and CRISPR-based approaches will deepen our understanding of toxin-binding mechanisms. At the same time, long-term safety evaluations and regulatory frameworks will be essential to ensure consumer and environmental safety.

Overall, multidisciplinary collaboration among microbiology, biotechnology, and nutrition sciences will be vital to translate laboratory findings into real-world applications.

9. Conclusion

Lactobacillus species represent not only beneficial probiotics but also powerful natural detoxifiers capable of binding or transforming foodborne contaminants such as mycotoxins (AFB_1, AFM_1), heavy metals (Pb, Cd, Zn, Ni), and PAHs. Their detoxification capacity primarily driven by cell wall adsorption and occasionally by enzymatic conversion is influenced by pH, temperature, cell density, and food matrix conditions.

Encapsulation technologies further enhance their stability and functionality, enabling effective detoxification even in challenging environments. In foods such as milk and cereals, *Lactobacillus* has already proven capable of reducing toxin levels. For human health, these bacteria lower carcinogen exposure, protect vital organs, strengthen intestinal barriers, and modulate immune responses.

Looking ahead, the development of high-performance probiotic strains and innovative functional products will expand the biotechnological role of *Lactobacillus* as "living detoxification agents." Ultimately, these microscopic allies long recognized in food microbiology are now emerging as key defenders in the fight against foodborne toxicants, contributing to safer foods and healthier generations.

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CHAPTER 3:

Toxicokinetics of Heavy Metals in Infants

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1. Introduction:

The Unique Toxicological Vulnerability of the Pediatric Population

Dietary exposure to heavy metals has emerged as a major public health issue, with infants and young children representing the most vulnerable segment of the population (Bair, 2022). Rapid industrialization and persistent environmental pollution have intensified the burden of contaminants in the food supply, parallel to those found in air and water. Toxic heavy metals (THMs), although naturally present in the earth's crust, can enter the food chain through soil and irrigation water and may also be introduced during processing and packaging (Bair, 2022). This chapter aims to explore why infants are biologically more susceptible to these contaminants and to discuss how heavy metals behave once they enter the body, focusing on their absorption, distribution, metabolism, and excretion (ADME) processes. The toxicokinetic fate of dietary heavy metals in infants follows the classical ADME framework, yet with greater absorption and reduced metabolic and excretory capacity compared with adults (Figure 1).

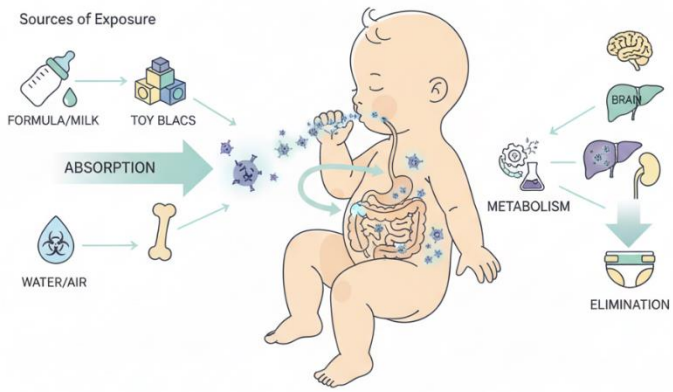

Figure 1. Heavy Metal Toxicokinetics in Infants (ADME Process)

1.1. Scope and Definition of Critical Toxic Metals (Lead, Cadmium, Inorganic Arsenic, Mercury)

This chapter gives primary attention to four toxic heavy metals that are most frequently detected in infant diets and are of highest toxicological concern: arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) (American Academy of Pediatrics, 2021). None of these elements serve any physiological or nutritional function in the human body (Jaishankar et al., 2014). Even at low doses, they are capable of inflicting damage on multiple organ systems (Balali-Mood et al., 2021). Evidence from recent analyses demonstrates that these metals are widespread in infant formulas and processed baby foods (Collado-López et al., 2025). In one scoping review, inorganic arsenic was present in all tested specialized formulas, while cadmium was detected in 91% of them (Collado-López et al., 2025). Among food categories, rice-based products pose a particularly high exposure risk for infants once complementary feeding begins beyond six months of age (American Academy of Pediatrics, 2021).

1.2. Etiology of Dietary Exposure: Sources and Regulatory Concerns

Heavy metals are present in soil naturally, but their concentrations in crops increase when pollutants are introduced into the environment. Rice is a notable example, as it accumulates considerably higher amounts of arsenic compared to other grains (American Academy of Pediatrics, 2021). Root vegetables and various processed purées for infants can also serve as contamination sources. The problem has been serious enough to trigger product recalls; baby foods were withdrawn from the market in both March and September 2025 due to elevated lead levels (American Academy of Pediatrics, 2021).

To mitigate this risk, regulatory bodies have begun tightening control measures. The U.S. Food and Drug Administration (FDA) launched the "Closer to Zero" initiative to progressively bring contaminant exposure in infant diets down to the lowest feasible levels (U.S. Food and Drug Administration, 2025a). These policies are motivated by the greater biological susceptibility of infants, whose lower body mass and immature organ systems heighten dose-related toxicity (U.S. Food and Drug Administration, 2025a). Importantly, it has been argued that even contaminant levels currently allowed by legislation such as the 20 µg/kg lead limit under EU Regulation 1881/2006 may still be unsafe for vulnerable populations, reinforcing the need for stricter sourcing and processing controls (Depa, 2019).

The consequences of early exposure are clinically significant. Documented outcomes include anemia, kidney damage, impaired reproductive function, and measurable reductions in IQ (Bair, 2022). Prenatal and early postnatal exposure is especially harmful to the developing brain, manifesting later as cognitive, behavioral, and learning impairments (da Silva et al., 2025). Evidence from systematic reviews also shows that cadmium exposure can adversely affect neurodevelopment even at low exposure levels (Chandravanshi et al., 2021).

1.3. Ontogenic Differences in Infants Affecting Toxicokinetics (Physiological Context)

Infants and young children are considerably more susceptible than adults to both carcinogenic and non-carcinogenic effects of toxic heavy metals (Bair, 2022). This vulnerability arises from the convergence of three biological determinants:

• Higher intake relative to body size. Infants consume up to three times more food per kilogram of body weight than adults (Bair, 2022). As a result, even when exposed to similar contaminant concentrations, the internal dose they receive is proportionally much greater.

• Physiological immaturity. Several organ systems that regulate toxicokinetic fate including the gastrointestinal tract, the blood–brain barrier, and the kidneys are not yet fully developed (Fadrowski & Furth, 2016). Immaturity in these systems increases absorption, reduces metabolic capacity, delays elimination, and ultimately elevates internal exposure levels (Bair, 2022).

• Exposure during critical developmental windows. Early-life exposure overlaps with periods of rapid neurodevelopment. Insults occurring during these windows may trigger lifelong health consequences. Prenatal exposure, in particular, has been associated with adverse neurodevelopmental outcomes (da Silva et al., 2025).

Toxicokinetic modeling reinforces that risk is not defined solely by contaminant concentration. High intake rates and age-dependent physiological parameters can convert levels considered “safe” for adults into toxic cumulative doses in infants due to fundamental kinetic differences.

These physiological differences and their effects on heavy metal toxicokinetics are summarized in Table 1.

Table 1. Developmental determinants of heavy metal bioavailability and retention in infants

Physiological Parameter	Ontogenic Status (vs. Adult)	Implication for Toxicokinetics
Food intake / body weight ratio	Significantly higher (up to 3×)	Increases internal dose of ingested metals (Bair, 2022)
Gastrointestinal absorption efficiency	Generally higher	Enhanced absorption due to higher transporter expression and higher permeability (Bair, 2022)
Blood–brain barrier integrity	Structurally and functionally immature	Facilitates CNS penetration of metals, especially lipophilic species (de Burbure et al., 2006)
Renal filtration and excretion rate	Reduced efficiency (immature GFR and tubular transport)	Slower clearance and increased bioaccumulation (Bair, 2022; Fadrowski & Furth, 2016)
Hepatic enzyme systems (metabolism)	Decreased or immature function	Altered detoxification capacity (e.g., impaired arsenic methylation) (Chandravanshi et al., 2021)

2. Bioavailability and Gastrointestinal Absorption Mechanisms

The extent to which dietary heavy metals reach systemic circulation is not a fixed property of the food matrix alone; it depends on multiple factors, including the chemical form of the metal, the integrity of the gastrointestinal barrier, and the availability of specific transport mechanisms. Because the gastrointestinal system of infants differs structurally and functionally from that of adults, the bioavailability of these metals is substantially altered in early life.

2.1. Speciation and Bioavailability: Why Chemical Form Matters

The toxic potential of a metal is strongly determined by its chemical speciation, which governs solubility, membrane passage, interaction with carrier proteins, and ultimately bioavailability.

Arsenic speciation

Arsenic in foods exists predominantly in two forms: inorganic arsenic (iAs) and organic species. The inorganic form commonly present in rice-based infant foods is the main toxicological concern (American Academy of Pediatrics, 2021). Organic arsenic, such as arsenobetaine in seafood, is far less harmful and is cleared rapidly. For both toxicological assessment and regulatory action, determining which species is present is essential.

Mercury speciation

The behavior of mercury in the body varies dramatically by species. Methylmercury (MeHg), typically acquired through fish consumption, is absorbed almost completely (95–100%) in the intestine. Owing to its lipophilic character, it crosses both the blood–brain barrier and the placenta with ease, which explains its pronounced neurotoxicity (Jaishankar et al., 2014). Inorganic mercury (iHg), by contrast, is absorbed to a much smaller extent (7–15%) and does not effectively enter the brain. Elemental mercury vapor (Hg^0), however, is efficiently absorbed via inhalation and translocates to the brain and fetus, making it among the most hazardous forms (Balali-Mood et al., 2021).

2.2. Uptake Mechanisms via Enterocytes: Essential Nutrient Competition

Many toxic heavy metals exploit physiological similarities with essential trace minerals such as iron, zinc, and calcium, allowing them to use nutrient transport systems for cellular entry (Balali-Mood et al.,

2021; Jaishankar et al., 2014). Divalent Metal Transporter-1 (DMT1), which mediates ferrous iron absorption in the duodenum, also facilitates intestinal uptake of lead and cadmium (Bair, 2022; Chandravanshi et al., 2021). Nutritional status modulates this process: iron deficiency upregulates DMT1 expression and inadvertently increases Pb and Cd absorption from the same diet (American Academy of Pediatrics, 2021; Bair, 2022). Arsenic may instead utilize phosphate transporters or aquaporin channels, while cadmium and lead can also enter via calcium channels when calcium intake is insufficient (Jaishankar et al., 2014; Zhu et al., 2024).

2.3. Immature Gastrointestinal Barrier and Permeability

In early life, the gastrointestinal barrier is anatomically and immunologically immature, resulting in greater intestinal permeability compared to adults (Fadrowski & Furth, 2016; Balali-Mood et al., 2021). The barrier comprises the mucus layer, intestinal epithelial cells, and mucosal immune components, all of which are still developing in infants. Experimental evidence shows that mercury can increase epithelial cell volume and membrane permeability without inducing cytotoxicity, thereby facilitating co-absorption of multiple heavy metals and enhancing synergistic toxicity risks (Balali-Mood et al., 2021; Zhu et al., 2024).

2.4. The Role of the Infant Gut Microbiota

The intestinal microbiota is now recognized as a key determinant in heavy metal toxicokinetics, influencing both absorption and downstream metabolic handling (Zhu et al., 2024; Mah.bioscientifica.com, n.d.). Heavy metals themselves induce dysbiosis, yet the altered microbiota can conversely modify the absorption efficiency and biotransformation of these metals, demonstrating a bidirectional host–contaminant interaction (Zhu et al., 2024).

Microbial communities can function as a biological barrier by limiting luminal metal bioavailability, modulating detoxification enzyme expression, and regulating local pH and oxidative balance (Balali-Mood et al., 2021; Zhu et al., 2024). Experimental data from animal models show that probiotic genera such as Lactobacillus and Bacillus can significantly reduce intestinal uptake of individual metals including Cd, Cu, and As (Zhu et al., 2024). Mechanistically, these bacteria strengthen intestinal tight junctions, preserve barrier integrity, and sustain microbial diversity all of which collectively reduce systemic exposure. This evidence positions the gut microbiota as a viable therapeutic target for attenuating heavy metal toxicity in early life (Demir and Demir, 2021).

3. Distribution Kinetics and Accumulation in Target Organs

Once absorbed, heavy metals are carried in systemic circulation primarily through binding to plasma proteins or erythrocytes, and then distributed to target tissues depending on their lipophilicity, ionic form, and affinity for intracellular carriers such as metallothioneins (Thirumoorthy et al., 2011; Yang et al., 2024). Patterns of tissue accumulation differ markedly between metals: some preferentially localize to neural tissue, whereas others concentrate in bone, liver, or kidneys, where they may persist for years and contribute to chronic toxicity (de Burbure et al., 2006; Rana et al., 2018).

3.1. General Distribution Dynamics: Tissue Tropism

Once in circulation, heavy metals are distributed according to their chemical characteristics and affinity for biological targets. Lead and cadmium frequently co-occur and display similar accumulation patterns, which suggests that they may share overlapping pathways in transport, detoxification, and excretion (de Burbure et al., 2006; Rana et al., 2018). In contrast, mercury and arsenic exhibit distinct kinetic behavior and tissue preferences due to differences in speciation, lipophilicity, and protein binding (Balali-Mood et al., 2021; Jaishankar et al., 2014).

3.2. Neurodevelopmental Risk: Blood-Brain Barrier (BBB) Crossing

Among all toxic endpoints, the brain is the most consequential target in infancy because exposure occurs during a period of intense neural growth and synaptic organization. Even low-level exposure during these windows has been associated with long-term deficits in cognition, learning, behavior, and executive function (da Silva et al., 2025; Chandravanshi et al., 2021). The neonatal BBB is structurally and functionally immature, allowing greater permeability compared to adults (de Burbure et al., 2006; Balali-Mood et al., 2021). The neurotoxic potential therefore differs by metal species:

• Methylmercury (MeHg) Because of its lipophilic nature and ability to hijack amino acid transporters, MeHg crosses both the BBB and placenta efficiently, leading to pronounced brain accumulation and severe neurotoxicity (Balali-Mood et al., 2021; Jaishankar et al., 2014).

• Lead (Pb) Lead accumulates in the developing brain and has been repeatedly linked to impaired cognitive and motor development, even at sub-threshold exposure levels (da Silva et al., 2025; Bair, 2022). From a toxicokinetic lens, cumulative exposure over time during neurodevelopment not just peak döşe determines the magnitude of irreversible neurological injury (da Silva et al., 2025).

3.3. Distribution in Developing Tissues: Bone, Kidney, and Liver Reserves

Bone sequestration (Lead).

Lead closely mimics calcium in ionic behavior and is therefore incorporated into the mineral matrix of growing bone, forming a long-term endogenous reservoir (de Burbure et al., 2006). During physiologic periods of elevated bone turnover such as adolescence,

pregnancy, or prolonged lactation this stored lead can be remobilized into circulation, resulting in chronic low-grade internal exposure even in the absence of ongoing dietary intake (Waisberg et al., 2006; Depa, 2019).

Renal accumulation (Cadmium and inorganic mercury).

Because the kidney is a major route of metal excretion, it is also a principal site of deposition for several toxic elements (Rana et al., 2018; Fadrowski & Furth, 2016). Cadmium and inorganic mercury accumulate preferentially in the renal cortex, where they drive progressive nephrotoxicity through oxidative injury and transporter-mediated retention (Rana et al., 2018; Sabolíc, 2006). Cadmium is of particular concern because its biological half-life can extend for decades, meaning that exposures occurring in infancy add to a lifelong internal burden and increase long-term toxic risk (Chandravanshi et al., 2021; Bair, 2022).

4. Metabolism and Intracellular Detoxification Pathways

The toxic impact of heavy metals is not defined solely by the absorbed dose, but also by how efficiently the body can biotransform, sequester, or eliminate them. In infants, this defensive capacity is inherently limited due to the immaturity of hepatic and enzymatic systems responsible for detoxification (Fadrowski & Furth, 2016; Su et al., 2025). As a result, even moderate exposures may produce disproportionately higher systemic toxicity compared to adults.

4.1. Biotransformation of Heavy Metals (Example: Arsenic Methylation)

Inorganic arsenic (iAs) is metabolized primarily in the liver through sequential methylation, producing monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Su et al., 2025). Although methylation is typically regarded as a detoxification route that enhances urinary elimination, some intermediary products particularly MMA are themselves highly reactive and cytotoxic. In infancy, the immaturity of

hepatic enzyme systems, including cytochrome P450 isoforms, may alter both the rate and completeness of arsenic methylation. As a result, infants may generate a larger fraction of toxic intermediates or experience delayed clearance relative to adults (Su et al., 2025).

4.2. Intracellular Binding Proteins: The Role of Metallothionein (MT) in Sequestration

Metallothioneins are low-molecular-weight, cysteine-rich proteins with a high affinity for both essential (e.g., Zn, Cu) and toxic metals (e.g., Cd, Hg), binding them within distinct structural clusters (Yang et al., 2024). The primary detoxification function of MT is to sequester toxic cations (Cd^{2+}, Hg^{2+}) intracellularly, thereby neutralizing their reactivity and promoting their eventual elimination (Thirumoorthy et al., 2011). MT synthesis can be induced by exposure to zinc or cadmium; however, inadequate intake of essential metals may reduce MT inducibility, impairing defense against toxic loads during infancy.

Cadmium is a potent MT inducer, and the resulting Cd–MT complexes are preferentially transported to and retained within the kidney (Rana et al., 2018). Ironically, this protective sequestration contributes to the long biological half-life and persistent renal accumulation of cadmium, increasing the risk of chronic nephrotoxicity over the life course.

4.3. Oxidative Stress as a Central Toxic Mechanism

A major unifying mechanism underlying the toxicity of lead, cadmium, mercury, and arsenic is their capacity to generate reactive oxygen species (ROS) and disrupt redox homeostasis (Balali-Mood et al., 2021). Excess ROS initiates a cascade of biochemical injury, including lipid peroxidation, protein oxidation, mitochondrial dysfunction, and ultimately apoptosis or necrotic cell death. These redox disturbances are further amplified when heavy metals inactivate antioxidant enzymes or suppress endogenous defense pathways (Balali-Mood et al., 2021). Mercury and arsenic have a particularly strong affinity for sulfhydryl

groups, allowing them to directly bind and disable key metabolic and antioxidant proteins, thereby exacerbating oxidative injury.

5. Elimination and Excretion in the Developing Infant

A key reason why infants experience greater internal accumulation of heavy metals is the immaturity of their elimination systems. Even when exposure levels are similar to those seen in adults, the reduced capacity to excrete contaminants prolongs biological residence time and increases the likelihood of bioaccumulation.

5.1. Renal Elimination (Urinary Excretion)

The kidney is the principal route of excretion for inorganic forms of lead, cadmium, mercury, and arsenic metabolites, which also makes it one of the most vulnerable organs to metal-induced damage (Fadrowski & Furth, 2016). In early life, renal clearance is inherently inefficient due to low glomerular filtration rate (GFR) and immature tubular transport systems. GFR is physiologically depressed at birth and rises gradually over the first year of life, which delays the elimination of absorbed metals during this highly sensitive window (Fadrowski & Furth, 2016). As a consequence, infants retain metals for longer durations, increasing the cumulative internal dose and amplifying future risk. The renal proximal tubule is the major site of intracellular metal accumulation, where transport proteins such as DMT1 facilitate the reabsorption not only of essential micronutrients but also of toxic cations including Cd^{2+} and Pb^{2+} (Sabolíc, 2006; Rana et al., 2018). Persistent uptake into tubular cells results in intracellular contamination and triggers a spectrum of toxic effects, including oxidative stress, tubular dysfunction, and proteinuria (Fadrowski & Furth, 2016; Balali-Mood et al., 2021). These injuries further diminish renal excretory capacity, thereby establishing a self-perpetuating cycle in which reduced clearance promotes additional accumulation and sustained nephrotoxicity.

5.2. Biliary and Fecal Elimination

In addition to renal pathways, gastrointestinal excretion via biliary and pancreatic secretions represents a secondary route of elimination for heavy metals. Lipophilic forms such as methylmercury (MeHg) may be eliminated predominantly through bile, with up to 90% of the absorbed dose excreted via feces under certain conditions (Balali-Mood et al., 2021). However, MeHg is also subject to enterohepatic recirculation, which prolongs its residence time in the body and delays final elimination, especially in early life when metabolic capacity is limited (Jaishankar et al., 2014).

5.3. Physiological Half-Lives and Potential for Long-Term Retention

Because infants display both high gastrointestinal absorption and low excretory capacity, even modest chronic exposures can result in substantial internal accumulation. Metals that deposit in bone or renal tissue persist for prolonged periods due to long biological half-lives cadmium, for example, may remain in the body for decades (Depa, 2019; Rana et al., 2018). The immaturity of glomerular filtration and tubular transport during infancy is therefore a key kinetic determinant of lifetime metal burden. Reduced elimination amplifies total exposure over time, such that the cumulative area under the curve (AUC) of tissue dose is markedly higher in infants than in adults exposed to the same concentration (Fadrowski & Furth, 2016).

6. Quantitative Modeling and Risk Assessment

Infants constitute the most physiologically immature and thus the most vulnerable age group with respect to chemical exposure, including dietary heavy metals. Their anatomical and functional differences arising from developmental changes that alter absorption, distribution, metabolism, and excretion (ADME) require the use of quantitative approaches that reflect age-specific biology rather than extrapolations from adult data (Yang et al., 2023). For this reason, risk assessment in

early life increasingly relies on mechanistic and physiologically informed models rather than conventional toxicological assumptions (Demir and Ağaoğlu, 2023; Demir et al., 2024).

6.1. Principles of Physiologically Based Pharmacokinetic (PBPK) Modeling in Pediatrics

Classical pharmacokinetic frameworks are largely inadequate for infants because they assume static physiology and cannot incorporate the rapid ontogenic changes that characterize early development (Yang et al., 2023). PBPK models overcome this limitation by integrating age-dependent physiological parameters such as organ volumes, blood perfusion rates, and renal maturation with chemical-specific properties to estimate tissue-level exposure as a function of time (Yang et al., 2023). These models are particularly valuable in populations where empirical trials are restricted or impossible, such as neonates, enabling prediction of neurodevelopmental toxicity and optimization of exposure thresholds without direct experimental dosing (Hattis et al., 2002). By explicitly accounting for maternal transfer, breastfeeding dynamics, and infant physiology, PBPK modeling provides a more realistic and ethically acceptable basis for regulatory decision-making in infants.

6.2. Application of PBPK Models to Estimate Heavy Metal Exposure

PBPK frameworks are widely applied to quantify fetal exposure through maternal transfer and postnatal exposure through breast milk, infant formula, or complementary feeding (Hattis et al., 2002). These models can incorporate competitive interactions at shared transporters, as demonstrated in kinetic simulations developed for iodide and perchlorate (Hattis et al., 2002). Despite their utility, model performance is constrained by gaps in developmental data particularly organ size trajectories, age-specific blood flow patterns, and ontogenic

enzyme expression. Strengthening these physiological datasets is essential for improving predictive confidence. Given the non-linear and rapidly evolving physiology of infants, PBPK is currently regarded as the most advanced and mechanistically appropriate approach for translating exposure into risk-based decision thresholds.

6.3. Limitations and Regulatory Context

In regulatory applications, PBPK outputs are typically integrated with Internal Reference Levels (IRLs) to derive Action Levels (ALs) for contaminants in infant foods. The FDA's recently proposed limits for lead 10 ppb in purées and 20 ppb in dry cereals reflect an effort to align permissible levels with toxicokinetic evidence rather than historical feasibility (U.S. Food and Drug Administration, 2025b). Nonetheless, regulatory gaps and flexible standards allow heavy metals to persist in commercially marketed infant foods (Bair, 2022). To achieve the objectives of the "Closer to Zero" initiative, standards must be iteratively tightened, especially in high-risk categories such as rice-based infant products (U.S. Food and Drug Administration, 2025a). As summarized in Table 2, infants exhibit distinct toxicokinetic characteristics for priority heavy metals, including higher intestinal absorption, lower biliary excretion efficiency, and prolonged tissue retention compared with adults (Bair, 2022; Smith et al., 2021).

7. Conclusion, Mitigation Strategies, and Regulatory Recommendations

7.1. Synthesis of Bioavailability and Toxicokinetic Differences in Infants

The toxicokinetics of heavy metals in infancy reflect a convergence of physiological attributes that convert exposure into disproportionately high internal burden (Bair, 2022). Three determinants are central: (i) a higher intake of food and fluids per unit body weight; (ii) an enhanced

intestinal absorption capacity particularly via DMT1 upregulation in nutritionally deficient children; and (iii) a reduced ability to eliminate absorbed metals due to immature renal function. This combination means that even low concentrations in food can yield clinically meaningful levels of internal exposure, heightening the risk of irreversible neurodevelopmental injury during early life (da Silva et al., 2025). Impaired renal clearance (characterized by low GFR and inefficient tubular handling) prolongs systemic retention and accelerates tissue accumulation, especially for metals with known long-term storage depots such as lead in bone and cadmium in the kidney (Bair, 2022). Importantly, toxicokinetic susceptibility is further modified by nutritional status: deficiencies in iron and other essential minerals upregulate shared transport pathways, increasing systemic uptake of Pb and Cd. Thus, mitigation strategies should not be restricted to reducing contaminant presence in foods, but must also include optimization of nutritional adequacy as a biological countermeasure.

Table 2. Toxicokinetic profiles of priority heavy metals in infants

Metal	Lead (Pb)	Cadmium (Cd)	Arsenic (As)	Mercury (Hg)
Primary Dietary Form	Inorganic Pb	Inorganic Cd	Inorganic arsenic (iAs)	MeHg; Inorganic Hg (iHg)
Dominant Absorption Mechanism	DMT1; Ca^{2+} transport channels	DMT1 (Fe^{2+} competition); Passive diffusion	Phosphate transporters; Aquaporin channels	MeHg: amino acid transporters; iHg: low GI absorption
Primary Detoxification / Handling Pathway	Secondary MT binding; Bone sequestration	Metallothionein binding and sequestration	Hepatic methylation (biotransformation to MMA/DMA)	MT binding; Glutathione conjugation
Major Sites of Accumulation	Bone, developing brain, kidney	Renal cortex, liver	Skin, hair, liver, kidney	MeHg: brain; iHg: kidney

Critical Neurotoxic Mechanism	Direct penetration across immature BBB (de Burbure et al., 2006)	Indirect neurotoxicity via oxidative stress and prenatal exposure (Chandravanshi et al., 2021)	Oxidative stress–mediated behavioral and developmental effects (Su et al., 2025)	Efficient placental/BBB transport of MeHg (Balali-Mood et al., 2021)
Long-Term Retention Concern	Bone stores act as a chronic internal reservoir, mobilized later in life	Extremely long renal half-life (decades) leading to lifelong accumulation	Clearance capacity depends on methylation efficiency; retention increases when metabolism is immature	MeHg mainly excreted via bile; iHg via kidney prolonged enterohepatic cycling delays removal

7.2. Public Health Implications and Nutritional Mitigation Strategies

Public health guidance aimed at reducing infant exposure to dietary metals should be interpreted within the framework of toxicokinetic vulnerability (American Academy of Pediatrics, 2021). Several practical strategies are supported by current evidence:

• Dietary diversification rather than reliance on a single staple. Rotating grain sources particularly substituting rice-based products, which accumulate more arsenic, with alternative cereals helps reduce chronic intake from a single contaminated food source (American Academy of Pediatrics, 2021).

• Correction of micronutrient deficiencies as a toxicological intervention. Adequate iron and zinc status lowers effective bioavailability of lead and cadmium by downregulating shared

transporters such as DMT1. Nutritional adequacy therefore functions as an indirect but mechanistically grounded protective factor.

• Control of environmental and waterborne sources. Tap water may contribute arsenic (well water) or lead (old plumbing systems). Identifying and mitigating such sources, as well as selecting low-mercury fish species, is essential to limiting cumulative exposure (American Academy of Pediatrics, 2021).

• Breastfeeding when feasible. Compared with some commercial formulas, breastfeeding is associated with lower exposure to contaminants and is recommended when physiologically and socially possible (American Academy of Pediatrics, 2021).

7.3. Current Regulatory Frameworks and Future Action Levels

> Evidence indicates that even contaminant levels currently permitted under existing legal standards may still confer long-term health risks in sensitive pediatric populations due to their cumulative prevalence and bioaccumulation potential (Depa, 2019). This underscores the need for continuous regulatory refinement aimed at further lowering exposure thresholds.

• Ontogeny-informed regulatory limits.

Future action levels should be derived using PBPK-based frameworks that incorporate age-dependent physiology and food-category specificity, reflecting the dynamic toxicokinetic profile of infants (U.S. Food and Drug Administration, 2025a). The FDA's "Closer to Zero" initiative represents a meaningful transition toward science-driven, developmentally appropriate standards.

• Control at the raw material and manufacturing stage.

Given persistent findings of lead and cadmium in infant products, stricter sourcing controls, improved processing oversight, and enhanced

blending safety criteria are essential to preventing contamination before it reaches the retail stage.

• Biological mitigation via microbiome-based strategies.

Preclinical evidence showing that commensal bacteria can inhibit intestinal metal absorption suggests that probiotic approaches may represent a viable adjunct intervention to reduce systemic uptake in high-exposure contexts (Zhu et al., 2024). In summary, the health risk posed by dietary heavy metals in infancy is driven not merely by their concentration in food products, but by the unique biological amplification processes inherent to pediatric toxicokinetics. Effective protection of this highly vulnerable population therefore requires a dual strategy: stringent regulatory control to minimize contaminant entry into the food chain, and targeted nutritional and physiological interventions to reduce susceptibility at the host level.

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